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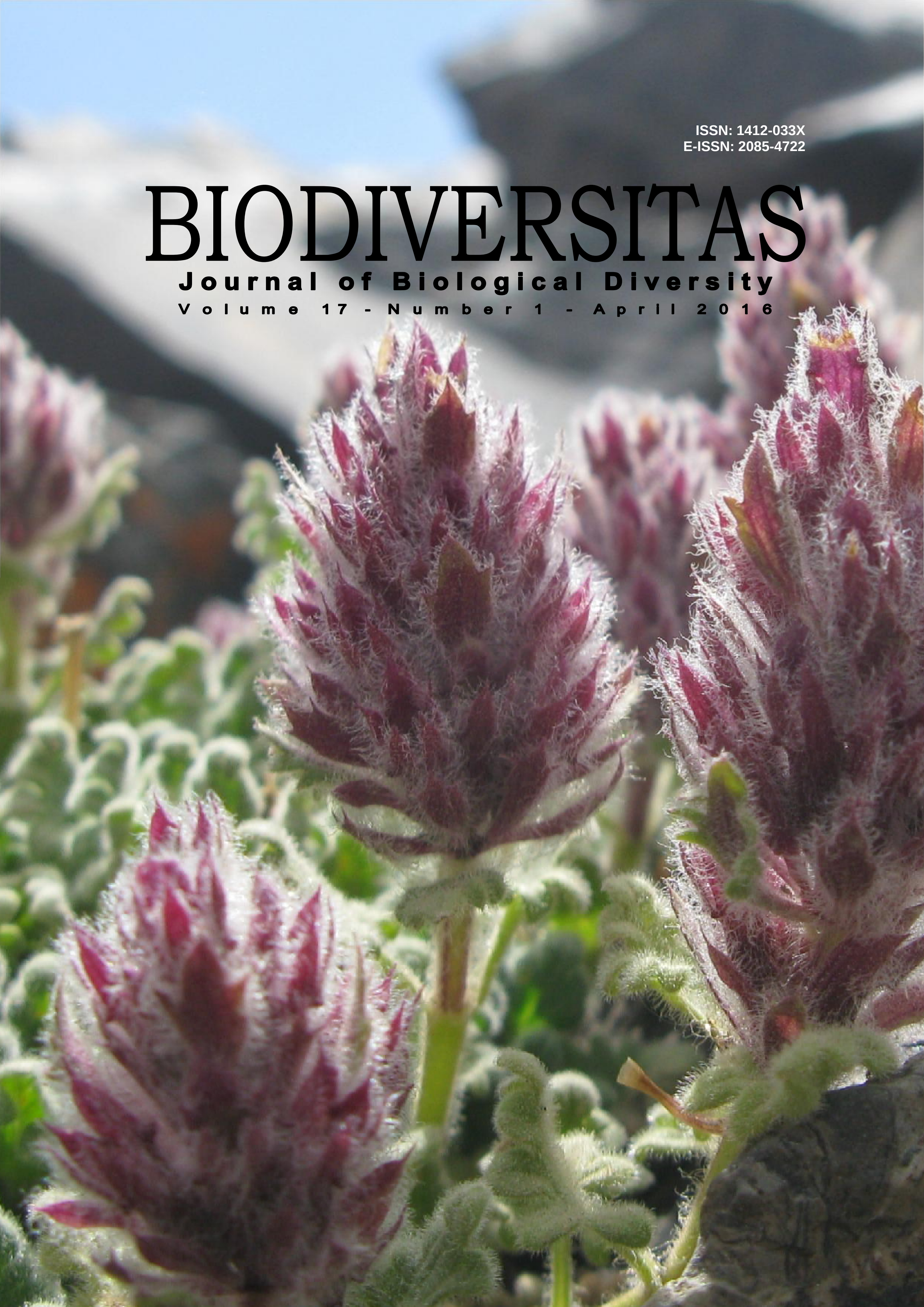

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Evaluation of vegetation types in the West Zagros (Beiranshahr region as a case study), in Lorestan Province, Iran

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Abstract. *Farouji AE, Khodayari H. 2016. Evaluation of vegetation types in the West Zagros (Beiranshahr region as a case study), in Lorestan Province, Iran. Biodiversita 17: 1-10.* The accurate identification of plant communities and their distribution are classical tools for mapping and classification of plants. Plants are strongly sensitive against changing environmental conditions. Moreover, Iran has a special position in Asia which has been creating a diverse ecosystem. Close floristic studies are necessary for precise determination of diversity and uniformity. To determine plant species in different vegetation types in Beiran shahr region, modified multi-scale Whittaker plots were constructed. This studied area was about 20000 ha and located in Northwest to Southwest of Iran. Eighty-nine plots were made in different longitude and latitudes. The minimum and maximum heights were 1101 and 2489 m asl., respectively. Within each modified multi-scale plots, each species was explored. Of 608 species, 498 and 97 are belonging to dicotyledons and monocotyledons, and remained 13 species were Pteridophytes. As measured, Therophyte and Champhyte are the dominant and recessive form of life in studied area. Based on species of each plot and subplots, mean Jaccard's coefficient, number of unique species and mean unique species/plot were estimated for each vegetation type. It is obvious that there is a clear correlation between Slopes of the species-log area curve and Mean number of unique species per plot.

Keywords: Beiranshahr, Iran, life form, vegetation type, Northwest, Zagros

INTRODUCTION

To determine all layers of vegetation in an ecosystem not only forests but also all parts of an area must be considered. Ecosystems have different parts in the world such as mountains (Lindenmayer et al. 2006). In fact, the accurate identification of plant communities and their distribution are classical tools for mapping and classification of plants (Tiner 1999). Iran has a special position in Asia which has been creating a diverse ecosystem (Alvarez-Rogel et al. 2006). Close floristic studies are necessary for precise determination of diversity. According to these aims, some protected areas are created for more environmental conservation (Lindenmayer and Franklin 2002). Biology conservation has a precise discipline in nature and will disturb if the conservation biologist doesn't make a right decision in different environments (May 1984). It should be noted that mentioned factors are not enough for conservation biology and it need more aspects like distribution of each species either rare or abundant, earth position whether polluted or not and, etc (Lombard et al. 1997). It should be noticed that the diversity of many urban areas are less than the natural ones because of some disturbance which caused by people. As a result, the geographic diversity and ecological complexity will be decreased soon (Marsh 1984). Ethical basics have a huge effect on conservation goals (Passmore 1974).

It should be considered that, conservation biology is based on systematic, ecology and many other biological fields which paid attention to all organisms in biotic world (Forman 1995). Unfortunately, conservation biology is noticed as a limited principle between biodiversity conservation and economic development (Daly and Cobb 1989). Investigators used various indices to estimate species diversity. Moreover, Whittaker method, sample both tree and herb strata instantly, is a common procedure that the herb-layer plots nested within reticular plots (Shmida 1984). Such plots have different sizes according to plant dimension. Therefore, species-area curves could be constructed. Such curves transparent many significant things like diversity, richness and all differences in each vegetation type. Beside this, log (species)-log (area) curves could be good coefficients of determination of vegetation types. To investigate ecosystems which need more watchful conservation, it was decided to use multi-scale Whittaker plots in West and Northwest of Iran. As a geological view, the studied area which situated in the West of Iran, has a very special status, this means that it include Sanandaj-Sirjan metamorphic zone (North of Boroujerd to South of Doroud). The remaining part belongs to Zagros Mountains. These two bodies don't have similar morphologies, for instance; Rocks are metamorphic In Sanandaj-Sirjan region, but sedimentary rocks are dominant in Zagros area.

The most important functional properties of Zagros vegetative area is protection and conservation issues (Chong and Stohlgren 2007). Briefly, the importance of vegetation as well as ecosystems accentuates conservation discussion (Magurran 2004). Eventually, we can say that each species considered as a renewable resource that must be examined (Coppolillo et al. 2004).

Zagros Mountain is stretched along Northwest to Southwest of Iran. It comprises about 10 provinces, more than 50 cities, important rivers, and a strategic place for agriculture and livestock (Fattahi 2001). Furthermore, over 885750 hectares of Lorestan Province cover with vegetation (total of 32 percent). The aims of this study were evaluation of vegetation types, life forms (Phanerophytes, Chameophytes, Semi-Cryptophytes, Cryptophytes and Therophytes), Chorotypes, determination of unique species and environmental conservation status of plant species in Beiranshahr region, located in Lorestan Province (middle Zagros), Iran.

MATERIALS AND METHODS

Study area

The study area (Beiranshahr, formerly called Chaghalvandi) is about 20,000 ha and located in the northwest of Khorramabad, Lorestan Province, middle part of Zagros Mountain, Iran (33°64′ N, 48°56′ E) (Figure 1).

Procedures

To investigate topoclimatic gradients which constitute various vegetation types, several plots (Eighty-nine) were constructed. The minimum and maximum heights above sea level were 1101 and 2489 m, respectively. Based on floristic composition of vegetation types in recent investigations, the reticulated modified multi-scale Whittaker plot includes a 200 m^2 plot which consists of one 25 m² sub-plot, centrally. In the opposite corner of chief plot and around the inside of plot perimeter, two 5 m² and ten 0.5 m² sub-plots were constructed, respectively. The size of the plots depends upon vegetation types. For instance; if there is an herbal floristic composition, the size of these modified multi-scale Whittaker could be declined. Therefore, the area of main plot and subplots will 200 m², 25, 2.5 and 0.5 m², correspondingly (Figure 2).Total of eighty-nine modified multi-scale Whittaker plots were set up in 5 vegetation types utilizing random sampling. Within each modified multi-scale plots, each species was explored. Based on species of each plot and subplots, mean Jaccard's coefficient, species area evaluation (slope, r² and c), number of unique species (species that was only recorded from one vegetation type), mean unique species/ plot and species-area lognormal curves were estimated for each vegetation type using Excel 2013 and PAST ver. 2.17.

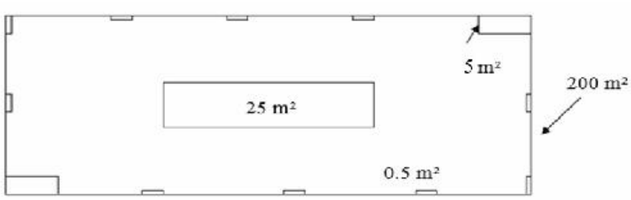

Figure 2. 200 m² modified multi- scale Whittaker plots and sub- plots in investigated regions in Beiran shahr region, Lorestan Province, Iran.

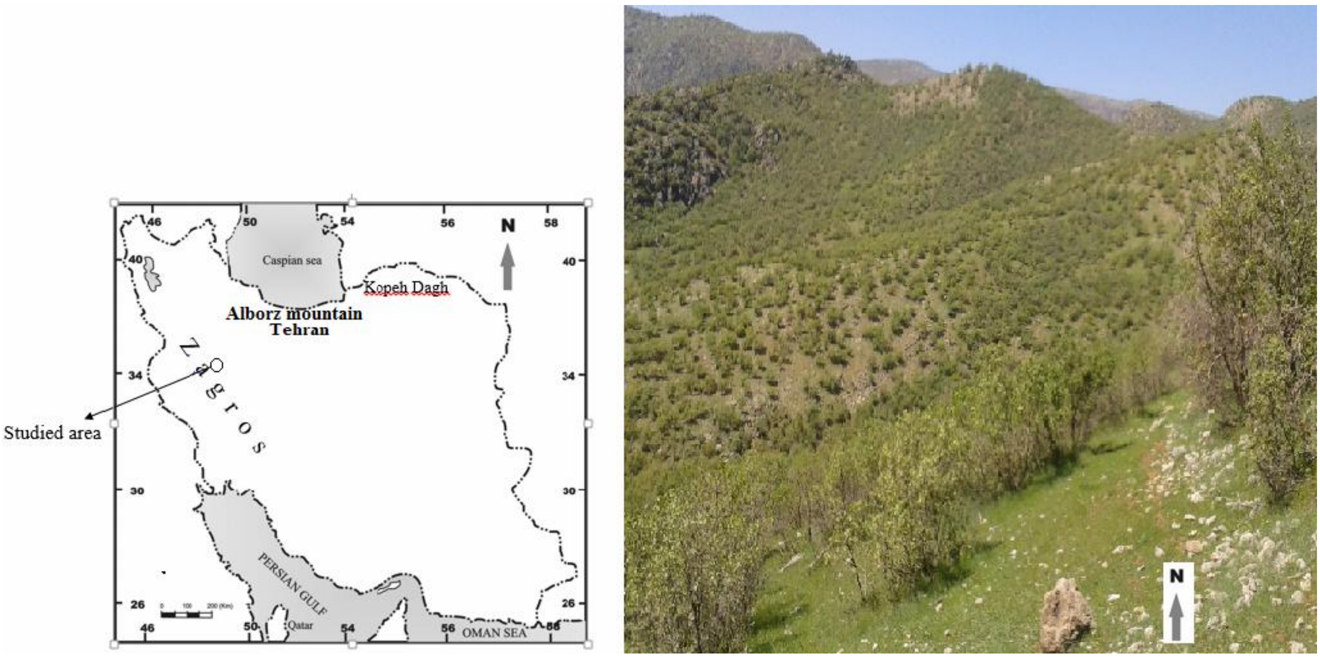

Figure 1. The Map of Iran (left) and studied area (Beiranshahr) in Zagros Mountain at Lorestan Province, Iran

Data analysis

In addition, some species were transferred to Lorestan University Herbarium to negotiate stages of identification (Raunkiaer 1934). Then, Chorotype and life form of each species was carefully investigated (some species listed in Table 5) (Stohlgren et al. 1997a, b; Chong and Stohlgren 2007). On the other hand, an index of overlap between lists, Jaccard's coefficient, was used to correct the slope of the species-log (area) curves. Jaccard's coefficient is defined as (Krebs 1999): J= A / (A + B + C)

A is the number of species which occurred in both plots, B is the species just on the first plot, and C is the species on second plot but not on the first one (Krebs 1999). Jaccard's coefficient is useful for comprising all plots within a vegetation type. The threatened species list was extracted from Red data book of Iran (Jalili and Jamzad 1999).

RESULTS AND DISCUSSION

Results

According to collected species for all vegetation types in eighty-nine modified multi-scale Whittaker plots, 608 species belonging to 334 genera and 74 families (Table 5) were identified (Mozafarian 2000). Of 608 species, 498 and 97 are belonging to dicotyledons and monocotyledons, orderly, and remained 13 species were Pteridophytes. Totally, Asteraceae (99.16%), Papilionaceae (57.9%), and Poaceae (54.8%) families have the most species in all studied area. Generally, the most genera are *Centaurea* (14), *Astragalus* (12) and *Trifolium* (10) respectively in five vegetation types. The major types of these districts are (i) Annual and perennial herbs including many families such as Asteraceae, Poaceae, Apiaceae and Brassicaceae (53, 32, 28 and 26 genera, orderly) which made the major part of vegetation type 1. According to evidences, it can be predicted that biodiversity have been declining over time because of severe livestock grazing (Helms 1998). In this layer, the most abundant species owned by *Centaurea*, *Astragalus, Trifolium*, respectively. (ii) Higher elevations transparent some more character species (*Eryngium caucasicum* Trautv., *Muscari neglectum* Guss. ex Ten., *Scilla arenaria* Baker, *Achillea wilhelmsii* C. Koch, *Anthemis odontostephana* Boiss., *Centaurea virgata* subsp. *squarrosa* (Boiss.) Gugler). Obviously, each layer involve the species of former layer, but with different abundances (iii) Lamiaceae, Orchidaceae and Rosaceae families have seen more in the third layer. (iv) Gradually, shrubs and trees (*Astragalus gossypinus* Fisch., *Astragalus microcephalus* Boiss., *Prunus arabica* (Olivier) Meikle, *Prunus cerasus* L., *Prunus orientalis* (Mill.) Koehne) have noticed and (5) in the fifth layer trees (as mentioned in the fourth layer) are dominated. Actually, many factors such as temperature, elevation, soil structure, and grazing degree were varied from area to area. The curve equations showed that, in the slope of species-area curves, the slope of Type 1 (0.7499) and Type 4 (0.2) had the most and least ranges (Table 1). The Type 1 had the lowest and Type 4 and 5 had the highest adjusted r^2 among others. Based on Jaccard's coefficient, the third and fifth layer of vegetation types are the most and least heterogeneous areas (Table 1). According to Table 2, the maximum and minimum number of unique species were observed in first and forth layer. Moreover, the mean number of species in each vegetation type was calculated (Table 3). Thus, most effort in conservation management must be devoted to forth layer and the others need less attention based upon total score of ranking (Margules and Pressey 2000).

Discussion

The authors noticed that plant life forms depend on many factors such as genetic and environmental properties, elevation from sea, climate, soil texture, and plant distribution (Lindenmayer et al. 2008). The life form of each ecosystem represents the adaptation of each plant in various climates and each layer has its own effect on environmental structure and function, but Gilliam believe that the role of herbaceous layer is more (Gilliam 2007). As measured, Therophyte and Champhyte are the dominant and recessive form of life in studied area, respectively. The number of other life forms (Hemicryptophyte, Cryptophyte, Phanerophyte) are among these ranges. Therophyte high frequency can be due to abundant destruction in this region (different process such as road construction, building manufacturing, traffic, grazing and etc). Chamaephytes such as the spiny *Astragalus* indicate their importance in soil stabilization and erosion control. In any case, climate and life forms shows close relationships between each other (Chou et al. 2000). It should be noticed that many phytosociologists are concerned about environmental conservation and the dilemma of threatening species. Based on this research each ecologist faced with detection of areas which need serious attention. Geophytes are rare in investigated region because these plants are intolerance to dry and arid climate. It should be noted that some species such as *Astragalus* demonstrate more attendance by increasing height. Moreover, because of the limited topographic variation, plant distribution was uniform (Giriraj et al. 2008).

To identify rare species, researchers use many criteria such as length of life, life forms, geographical distribution and plants utilization. Existence of endangered or vulnerable species could be a sign of degradation in this region (Myers et al. 2000). The number of endangered species resounds the significance of conservation biology in each vegetation type and normally it used as a standard for conservation management (Table 4). According to Table 5, it is obvious that there is a clear correlation between Slopes of species-log area curve and Mean number of unique species per plot. Because of similar sample unit and sampling area for all vegetation types, the number of unique species are trustful. However, this result is accordance with Pilehvar et al. (2010) research in central Zagros forests. Heterogeneity can be estimated by Jaccard's coefficient. Obviously, more species were find in more heterogenous areas. As a result, mean number of unique species per plot can be compared with others and necessary conservation requirements will be specified for each vegetation type in studied area. Absolutely, vegetation type 3 has the most heterogeneity according to Jaccard's coefficient, but the most ranking belonging to Type 1 (Table 5).

Table 1. species-area curves and mean Jaccard's coefficient for five vegetation types in Beiran shahr, Lorestan Province, Iran.

Vegetation type	Species area			Jaccard 's coefficient
	Slope	c	r^2	
Type 1	0.7499	0.0578	0.9098	0.14
Type 2	0.3306	0.4839	0.9483	0.70
Type 3	0.4566	1.378	0.9733	0.10
Type 4	0.2	5.4	1	0.50
Type 5	0.4	2	1	0.90

Table 2 number of unique species and mean unique species/ plot in each vegetation type.

Vegetation type	Mean number of species in each vegetation type	Number of unique species in each vegetation type	Mean number of unique species/ plot
Type 1	158.3	51	7.6
Type 2	108.9	18	3.1
Type 3	112	31	5.1
Type 4	78.6	14	2.9
Type 5	90.2	27	4.9

Table 3. The list of threatened species in each studied layer (vegetation type). Note: LR: lower risk; EN: endangered; DD: data deficient

Scientific name	Type 1	Type 2	Type 3	Type 4	Type 5
Mentha longifolia (L.) L.				LR	LR
Tanacetum polycephalum Sch. Bip.	LR			LR	LR
Jurinea carduiformis (Jaub. & Spach.) Boiss.	LR		LR	LR	LR
Cousinia cylindracea Boiss.		LR	LR	LR	LR
Pilostyles haussknechtii Boiss.	DD	DD			
Prangos uloptera DC.		EN	EN	EN	EN
Ferulago contracta Boiss. & Hausskn.			LR	LR	LR
Dianthus austroiranicus Lemperg				LR	LR
Cousinia khorramabadensis Bornm.	DD	DD			
Sameraria stylophora Boiss.			LR	LR	LR
Fraxinus rotundifolia Mill.			LR	LR	LR
Amygdalus haussknechtii (C. K. Schnider) Bornm.		LR	LR	LR	LR
Gallium kurdicum Boiss. and Hohen.	DD	DD			
Bunium luristanicum Rech. f.	DD	DD	DD		
Eryngium creticum Lam.	DD	DD	DD		
Stachys persepolitana Boiss.		LR	LR	LR	LR
Total	7	9	10	11	11

Table 4. Ranking measurement and absolute value for each vegetation type

Vegetation type	Mean number of species	Number of threatened species	Slopes of the species-log area curve	Jaccard's coefficient	Mean number of unique species per plot	Total score of ranking
Type 1	158.3 (5)	7 (1)	0.7499 (5)	0.14 (4)	7.6 (5)	20
Type 2	108.9 (3)	9 (2)	0.4 (3)	0.70 (2)	4.9 (3)	13
Type 3	112 (4)	10 (3)	0.4566 (4)	0.10 (5)	5.1 (4)	19
Type 4	78.6 (1)	11 (4)	0.2 (1)	0.50 (3)	2.9 (1)	10
Type 5	90.2 (2)	11 (4)	0.3306 (2)	0.90 (1)	3.1 (2)	11

Table 5. Floristic list of some characteristic species in Beiran shahr, Lorestan Province, Iran (ES: Euro-Siberian, IT: Irano-Turanian, M: Mediterranean, Cosm: Cosmopolitan (Reshinger, 1963-2001)

Taxa	Species No.	Genus No.	Life form	Chorotype
Adianthaceae	1			
Adiantum capillus-veneris L.		1	Hemicryptophyte	Cosm
Amaranthaceae	5			
Amaranthus albus L.		4	Therophyte	IT
Amaranthus blitoides S. Watson			Therophyte	IT
Gomphrena brachystylis F. Muell.		1	Therophyte	IT
Amaryllidaceae	8			
Allium jesdianum Boiss. & Buhse			Cryptophyte	IT
Narcissus tazetta L.		1	Cryptophyte	IT
Apiaceae/Umbeliferae	44			
Bunium luristanicum Rech. f.		3		IT
Bunium paucifolium DC.			Cryptophyte	IT
Bunium rectangulum Boiss. & Hausskn.			Cryptophyte	IT
Bupleurum falcatum subsp. cernuum (Ten.) Arcang.		1	Hemicryptophyte	IT
Chaerophyllum macropodum Boiss.		1	Hemicryptophyte	IT
Daucus broteroi Ten.		1	Therophyte	ES, IT, M
Dorema aucheri Boiss.		1	Therophyte	IT, M, ES
Eryngium billardieri Delile.		6	Hemicryptophyte	IT, M
Eryngium caucasicum Trautv.			Hemicryptophyte	IT, M
Eryngium creticum Lam.			Hemicryptophyte	IT, M
Eryngium glomeratum Lam.			Hemicryptophyte	IT, M
Eryngium noeanum Boiss.			Hemicryptophyte	IT
Eryngium thyrsoideum Boiss.			Therophyte	IT, M
Prangos uloptera DC.		1	Hemicryptophyte	IT, M
Pycnocycla flabellifolia Boiss.		1	Hemicryptophyte	IT
Korshinskia assyriaca (Freyn & Bornm.) Pimenov & Kljuykov		1	Hemicryptophyte	IT
Scandix iberica M. Bieb		2	Therophyte	IT
Scandix pecten-veneris L.			Therophyte	IT
Smyrniopsis aucheri Boiss.		1	Hemicryptophyte	IT
Smyrnium cordifolium Boiss.		1	Hemicryptophyte	IT, ES
Turgenia latifolia (L.) Hoffm.		1	Therophyte	IT, M, ES
Zosimia absinthifolia Link		1	Therophyte	IT
Asparagaceae	10			
Leopoldia comosa (L.) Parl.		2	Cryptophyte	IT, M
Leopoldia tenuiflora (Tausch) Heldr.			Cryptophyte	IT, M
Muscari neglectum Guss. ex Ten.		1	Cryptophyte	IT, M
Ornithogalum brachystachys C. Koch		2	Cryptophyte	IT, M
Ornithogalum persicum Hausskn. ex Bornm.			Cryptophyte	IT, M
Pseudomuscari inconstrictum (Rech.f.) Garbari		1	Cryptophyte	IT, M, ES
Scilla arenaria Baker		1	Cryptophyte	IT, M
Zagrosia persica (Hausskn.) Speta		1	Cryptophyte	IT
Asteraceae/Compositae	99			
Achillea aleppica DC.		3	Hemicryptophyte	IT
Achillea arabica Kotschy			Hemicryptophyte	IT
Achillea wilhelmsii K. Koch			Cryptophyte	IT, M, ES
Anthemis cota Sibth. & Sm.		6	Hemicryptophyte	IT, M
Anthemis cotula L.			Hemicryptophyte	IT, M
Anthemis haussknechtii Boiss. & Reut.			Therophyte	IT
Anthemis microcephala (Schrenk) B.Fedtsch.			Hemicryptophyte	IT, M
Anthemis odontostephana Boiss.			Hemicryptophyte	IT, M
Anthemis pseudocotula Boiss.			Hemicryptophyte	IT, M
Artemisia haussknechtii Boiss.		2	Hemicryptophyte	IT
Artemisia vulgaris Burm. F.			Therophyte	IT
Centaurea amadanensis Sch. Bip.		14	Therophyte	IT, M, ES
Centaurea behen L.			Therophyte	IT
Centaurea bruguierana subsp. *belangeriana* (DC.) Bornm.			Therophyte	IT
Centaurea elymaitica Mozaff.			Therophyte	IT
Centaurea hyalolepis Boiss.			Therophyte	IT
Centaurea iberica Trevir. ex Spreng.			Therophyte	IT, M, ES
Centaurea intricata Boiss.			Therophyte	IT

Centaurea irritans Wagenitz			Therophyte	IT
Centaurea koeieana Bornm.			Therophyte	IT
Centaurea minor Willd. ex Spreng.			Therophyte	IT
Centaurea paradoxa Mozaff.			Therophyte	IT, M, ES
Centaurea solstitialis L.			Therophyte	IT, ES
Centaurea virgata subsp. *squarrosa* (Boiss.) Gugler			Hemicryptophyte	IT, ES
Chardinia orientalis (L.) Kuntze		1	Therophyte	IT
Cichorium intybus L.		2	Therophyte	Cosmo
Cichorium pumilum Jacq.			Therophyte	IT, M
Cirsium sorocephalum Fisch. & C. A. Mey.		2	Hemicryptophyte	IT
Cousinia haussknechtii C.Winkl.			Hemicryptophyte	IT
Cousinia jacobsii Rech. f.			Hemicryptophyte	IT
Cousinia stenocephala Boiss.			Hemicryptophyte	IT
Echinops kermanshahanicus Mozaff.		5	Hemicryptophyte	IT
Echinops mosulensis Rech. f.			Hemicryptophyte	IT
Echinops pachyphyllus Rech. f.			Hemicryptophyte	IT
Echinops quercifolius Freyn.			Hemicryptophyte	IT
Echinops viscidulus Mozaff.			Hemicryptophyte	IT
Gundelia tournefortii L.		1	Hemicryptophyte	IT
Inula britannica L.		1	Hemicryptophyte	IT
Jurinea carduiformis (Jaub. & Spach) Boiss.		1	Therophyte	IT
Lactuca tuberosa Jacq.			Hemicryptophyte	IT, M
Onopordum carduchorum Bornm. & Beauverd		2	Therophyte	IT
Onopordum heteracanthum C. A. Mey.			Hemicryptophyte	IT
Sonchus asper (L.) Hill		2	Hemicryptophyte	IT, ES
Sonchus oleraceus (L.) L.			Hemicryptophyte	Cosm
Tanacetum polycephalum Sch. Bip.		1	Hemicryptophyte	IT, M, ES
Taraxacum sonchoides (D. Don) Sch. Bip.		2	Hemicryptophyte	IT
Taraxacum wallichii DC.			Hemicryptophyte	IT
Tragopogon buphthalmoides (DC.) Boiss.		3	Hemicryptophyte	IT
Tragopogon porrifolius subsp. *longirostris* (Sch. Bip.) Greuter			Hemicryptophyte	IT
Tragopogon vaginatus Ownbey & Rech. F.			Hemicryptophyte	IT
Biebersteiniaceae	1			
Biebersteinia multifida DC.		1	Cryptophyte	IT
Boraginaceae	19			
Anchusa azurea Mill.		2	Hemicryptophyte	IT, ES
Anchusa strigosa Banks & Sol.			Hemicryptophyte	IT, ES
Echium italicum L.		1	Therophyte	IT, ES
Heliotropium europaeum L.		3	Hemicryptophyte	IT, ES
Heliotropium noeanum Boiss.			Hemicryptophyte	IT
Heliotropium supinum L.			Hemicryptophyte	IT
Myosotis arvensis (L.) Hill		2	Therophyte	IT
Myosotis refracta Boiss.			Therophyte	IT, M
Onosma asperrima Bornm.		7	Hemicryptophyte	IT
Onosma bulbotrichum DC.			Hemicryptophyte	IT
Onosma dasytrichum Boiss.			Hemicryptophyte	IT
Onosma haussknechtii Bornm.			Hemicryptophyte	IT
Onosma microcarpum DC.			Hemicryptophyte	IT
Onosma rostellatum Lehm.			Hemicryptophyte	IT
Onosma sericeum Willd.			Hemicryptophyte	IT
Brassicaceae (Cruciferae)	28			
Alyssum bracteatum Boiss. & Buhse		1	Hemicryptophyte	IT
Brassica nigra (L.) K. Koch		1	Therophyte	M
Capsella bursa-pastoris (L.) Medik.		1	Therophyte	P1
Descurainia sophia (L.) Webb ex Prantl		1	Therophyte	Cosm
Lepidium draba L.		2	Therophyte	IT
Lepidium latifolium L.			Therophyte	IT
Sameraria stylophora Boiss.		1	Therophyte	IT
Sisymbrium officinale (L.) Scop.			Therophyte	IT, ES
Caryophyllaceae	21			
Acanthophyllum caespitosum Boiss.		2	Cryptophyte	IT
Acanthophyllum mucronatum C. A. Mey.			Cryptophyte	IT
Arenaria leptoclados Boiss.		1	Hemicryptophyte	
Cerastium dichotomum subsp. *inflatum* Cullen		1	Therophyte	IT, M
Dianthus austroiranicus Lemperg		3	Hemicryptophyte	IT

Dianthus macranthoides Hausskn. ex Bornm.			Hemicryptophyte	IT
Dianthus orientalis Adams			Hemicryptophyte	IT
Gypsophila elymaitica Mozaff.		3	Hemicryptophyte	IT
Gypsophila pallida Stapf			Hemicryptophyte	IT
Silene chaetodonta Boiss.		4	Hemicryptophyte	IT
Silene conoidea L.			Therophyte	Cosm
Silene odontopetala Fenzl.			Therophyte	IT, M
Silene microsperma Fenzl.			Hemicryptophyte	IT, M, ES
Stellaria alaschanica Y. Z. Zhao		1	Hemicryptophyte	IT
Chenopodiaceae/ Amaranthaceae	5			
Atriplex lasiantha Boiss.		1	Therophyte	IT
Salsola canescens (Moq.) Boiss.		1	Therophyte	IT
Colchicaceae	2			
Colchicum Kotschyi Boiss.		2	Cryptophyte	IT
Colchicum persicum Baker.			Cryptophyte	IT
Cyperaceae	7			
Cyperus eremicus Kukkunen.		4	Hemicryptophyte	IT
Cyperus longus L.			Hemicryptophyte	IT
Euphorbiaceae	9			
Euphorbia aleppica L.		8	Therophyte	IT, M
Euphorbia helioscopia L.			Therophyte	IT
Geraniaceae	4			
Geranium tuberosum L.			Cryptophyte	IT
Iridaceae	5			
Gladiolus atroviolaceus Boiss.		2	Cryptophyte	IT
Gladiolus italicus Mill.			Cryptophyte	IT, M
Iris × germanica L.		1	Cryptophyte	IT
Ixioliriaceae	1			
Ixiolirion tataricum (Pall.) Schult. & Schult. f.		1	Cryptophyte	IT
Juncaceae	4			
Juncus inflexus L.			Hemicryptophyte	Cosm.
Juncus maritimus Lam.			Hemicryptophyte	Cosm.
Lamiaceae (Labiatae)	43			
Eremostachys macrophylla Montbr. & Auch.		1	Hemicryptophyte	IT
Lamium album L.		2	Therophyte	IT
Lamium amplexicaule L.			Therophyte	IT
Marrubium astracanicum Jacq.		3	Hemicryptophyte	IT
Marrubium cuneatum Banks & Sol.		2	Hemicryptophyte	IT
Marrubium vulgare L.			Hemicryptophyte	IT, M
Nepeta meyeri Benth.		4	Therophyte	IT
Nepeta petraea Benth.			Therophyte	IT
Phlomis Bruguieri Desf.		3	Hemicryptophyte	IT
Phlomis kurdica Rech. f.			Hemicryptophyte	IT
Phlomis olivieri Benth.			Hemicryptophyte	IT
Salvia indica L.			Hemicryptophyte	IT
Salvia limbata C. A. Mey.			Hemicryptophyte	IT
Salvia multicaulis Vahl			Hemicryptophyte	IT
Salvia palaestina Benth.			Hemicryptophyte	IT
Salvia persepolitana Boiss.			Hemicryptophyte	IT
Salvia sclarea L.			Hemicryptophyte	IT
Satureja bachtiarica Bunge		2	Hemicryptophyte	IT
Satureja khuzistanica Jamzad			Hemicryptophyte	IT
Stachys benthamiana Boiss.		6	Therophyte	IT
Stachys kermanshahensis Rech. F.			Therophyte	IT
Stachys kurdica Boiss. & Hohen.			Hemicryptophyte	IT
Stachys lavandulifolia Vahl			Hemicryptophyte	IT
Stachys multicaulis Benth.			Hemicryptophyte	IT
Stachys persepolitana Boiss.			Hemicryptophyte	IT
Teucrium oliverianum Ging. ex. Benth.		5	Therophyte	IT
Teucrium orientale L.			Hemicryptophyte	IT
Teucrium polium L.			Hemicryptophyte	Cosm.
Teucrium pumilum Loefl. ex L.			Hemicryptophyte	Cosm.
Teucrium scordium L.			Therophyte	IT

Thymus eriocalix (Ronniger) Jalas		1	Therophyte	IT
Vitex agnus-castus L.		1	Phanerophyte	M
Ziziphora capitata L.		3	Hemicryptophyte	IT
Ziziphora clinopodioides Lam.			Hemicryptophyte	IT
Ziziphora tenuior L.			Therophyte	IT
Liliaceae	7			
Gagea gageoides (Zucc.) Vved.		2	Cryptophyte	Cosm.
Gagea reticulata (Pall.) Schult. & Schult. f.			Cryptophyte	Cosm.
Tulipa Montana Lindl.		2	Cryptophyte	IT
Tulipa uniflora (L.) Besser ex Baker			Cryptophyte	IT
Malvaceae	6			
Alcea angulata (Freyn & Sint.) ex Iljain		2	Hemicryptophyte	IT
Alcea kurdica (Schlest.) Alef			Hemicryptophyte	IT
Malva neglecta Wallr.		3	Hemicryptophyte	IT, M, ES
Malva nicaeensis All.			Therophyte	IT, M
Orchidaceae	3			
Orchis adenocheila Czerniak.		3	Hemicryptophyte	IT
Orchis anatolica Boiss.			Hemicryptophyte	IT
Papaveraceae	6			
Corydalis rupestris Kotschy.		1	Therophyte	
Glaucium corniculatum (L.) Curtis		1	Therophyte	IT, M
Papaver dubium L.		3	Therophyte	IT, M, ES
Papaver macrostomum Boiss. & A. Huet			Therophyte	IT
Papaver rhoeas L.			Therophyte	IT, M
Roemeria refracta DC.		1	Therophyte	ES
Papilionaceae/Leguminosae	57			
Alhagi persarum Boiss. & Buhse		1	Hemicryptophyte	IT, M
Astragalus adscendens Boiss. & Hausskn.		12	Champhyte	IT
Astragalus baba-alliar Parsa			Champhyte	IT
Astragalus bodeanus Fisch.			Champhyte	IT
Astragalus campylorhynchus Fisch. and C. Mey.			Champhyte	IT
Astragalus ecbatanus Bunge.			Champhyte	IT
Astragalus gossypina (Fisch.) Podlech			Champhyte	IT
Astragalus microcephalus Willd.			Champhyte	IT, M, ES
Astragalus neomozaffarianii Maassoumi			Hemicryptophyte	IT
Astragalus rhodosemius Boiss. & Hausskn.			Hemicryptophyte	IT, ES
Astragalus rytidocarpus Ledeb.			Hemicryptophyte	IT
Astragalus scorpioides Willd.			Hemicryptophyte	IT
Astragalus verus Olivier			Champhyte	IT
Glycyrrhiza glabra L.		1	Hemicryptophyte	IT, M, ES
Hymenocarpos circinnatus (L.) Savi		1	Therophyte	M
Lathyrus cicera L.		4	Therophyte	IT,M
Lathyrus hirsutus L.			Therophyte	IT
Lotus corniculatus L.		1	Therophyte	IT
Medicago crassipes (Boiss.) E. Small		8	Therophyte	IT, ES, M
Medicago laciniata (L.) Mill.			Therophyte	IT, ES, M
Medicago persica (Boiss.) E. Small			Therophyte	IT
Medicago polymorpha L.			Therophyte	Cosm.
Trifolium alexandrium L.		10	Hemicryptophyte	IT, M
Trifolium bullatum Boiss. & Hausskn.			Hemicryptophyte	IT, M
Trifolium campestre Schreb.			Hemicryptophyte	IT, M, ES
Trifolium echinatum M. Bieb.			Therophyte	IT, ES
Trifolium fragiferum L.			Therophyte	IT, ES
Trifolium grandiflorum schreb.			Therophyte	IT, ES
Trifolium lappaceum L.			Therophyte	IT, ES
Trifolium nigrescens subsp. petrisavii (Clementi) Holmboe.			Therophyte	M
Trifolium purpureum Loisel.			Therophyte	IT, M
Trifolium repens L.			Therophyte	IT
Trigonella spruneriana Boiss.		1	Therophyte	IT
Trigonella stellata Forssk.		1	Therophyte	IT
Vicia sativa subsp. *amphicarpa* (Dorthes) Asch.		4	Therophyte	IT, ES, M
Vicia sativa L.			Therophyte	IT, M, ES
Vicia villosa Roth			Therophyte	IT, ES

Poaceae/Gramineae	54			
Aegilops tauschii Coss.		3	Therophyte	IT, M
Aegilops triuncialis L.			Therophyte	IT, M
Aegilops umbellulata Zhuk.			Therophyte	IT
Agropyron trichophorum (Link.) K. Richter.		1	Cryptophyte	ES, M
Avena fatua L.			Therophyte	IT, M
Bromus danthoniae Trin.		6	Therophyte	IT
Bromus japonicus Thunb.			Therophyte	IT
Bromus sericeus Ten.			Therophyte	IT, M
Bromus sterilis L.			Therophyte	IT, M
Bromus tectorum L.			Therophyte	IT, M
Bromus tomentellus Boiss.			Therophyte	IT, M
Carex phacota Spreng.		1	Hemicryptophyte	IT
Hordeum brevisubulatum (Trin.) Link		5		IT, M
Hordeum bulbosum L.			Cryptophyte	IT, M
Hordeum murinum subsp. *glucum* (Steud.) Tzvelev			Therophyte	IT, M
Hordeum spontaneum K. Koch.			Therophyte	IT, M
Lolium rigidum Gaudin.			Therophyte	IT, M
Phleum iranicum Bornm. & Gauba		1	Champhyte	IT
Phragmites australis (Cav.) Trin. ex Steud.		1	Champhyte	IT, ES, M
Poa annua L.		3	Hemicryptophyte	Cosm.
Stipa hohenackeriana Trin. & Rupr.			Hemicryptophyte	IT
Taeniatherum caput-medusae (L.) Nevski.		1	Therophyte	IT,M
Polygonaceae	8			
Rumex dentatus L.		2	Therophyte	IT
Rumex ephedroides Bornm.			Hemicryptophyte	IT, M, ES
Ranunculaceae	12			
Adonis microcarpa DC.			Therophyte	M
Anemone biflora DC.		3	Cryptophyte	IT, M
Anemone coronaria L.			Cryptophyte	IT, M
Anemone elongata D. Don.			Cryptophyte	IT, M
Consolida orientalis (J. Gay.) Schrodinger		1	Therophyte	IT
Delphinium ambiguum L.		1	Therophyte	IT
Ranunculus asiaticus L.		3	Therophyte	IT, M
Ranunculus millefolius Banks & Sol.				IT
Ranunculus oxyspermus Willd.			Therophyte	IT
Rosaceae	20			
Amygdalus haussknechtii (C.K. Schnider) Bornm.		2	Phanerophyte	IT
Amygdalus lycoides Spach			Phanerophyte	IT
Cotoneaster morulus Pojark.		1	Phanerophyte	IT
Crataegus atrosanguinea Pojark.		4	Phanerophyte	IT, ES
Crataegus meyeri Pojark.			Phanerophyte	IT, ES
Prunus arabica (Olivier) Meikle		5	Phanerophyte	IT
Prunus cerasus L.			Phanerophyte	IT
Prunus mahaleb L.			Phanerophyte	IT
Prunus microcarpa C. A. Mey.			Phanerophyte	IT, M
Prunus orientalis (Mill.) Koehne			Phanerophyte	IT
Pyrus glabra Boiss.		2	Phanerophyte	IT
Pyrus syriaca Boiss.			Phanerophyte	IT, M
Rosa berberifolia Pall.		3	Phanerophyte	IT, M
Rosa canina L.			Phanerophyte	IT, ES, M
Rosa elymaitica Boiss. & Hausskn. ex Boiss.			Phanerophyte	IT, ES
Rubus anatolicus Focke		2	Phanerophyte	IT
Rubus caesius L.			Champhyte	IT, ES
Sanguisorba minor Scop.		1	Hemicryptophyte	IT, ES, M
Rubiaceae	10			
Galium humifusum M. Bieb.			Hemicryptophyte	IT, M, ES
Galium kurdicum Boiss. & Hohen.			Hemicryptophyte	IT, M, ES
Galium parisiense L.			Hemicryptophyte	IT, M, ES
Galium setaceum Lam.			Hemicryptophyte	IT
Galium tricornutum Dandy			Hemicryptophyte	IT
Galium verum L.			Hemicryptophyte	IT
Scrophulariaceae	7			
Scrophularia alpestris J. Gay ex Benth.		3	Hemicryptophyte	IT
Scrophularia atrata Pennell.			Hemicryptophyte	IT

Scrophularia striata Boiss.		1	Hemicryptophyte	IT
Verbascum agrimoniifolium Huber-Morath		3	Hemicryptophyte	IT
Solanaceae	4			
Hyoscyamus tenuicaulis Schönb.-Tem.		1	Hemicryptophyte	IT
Solanum americanum Mill.		2	Hemicryptophyte	IT, ES, M
Solanum luteum Mill.			Hemicryptophyte	IT, ES, M
Urticaceae	3			
Urtica dioica L.		2	Hemicryptophyte	IT, ES
Urtica pilulifera L.			Therophyte	IT, M, ES
Violaceae	1			
Viola modesta House		1	Therophyte	IT, M
Zygophyllaceae	1			
Tribulus terrestris L.	0	1	Therophyte	IT, ES, M

REFERENCES

Alvarez-Rogel J, Martinez-Sanches JJ, Blazquez LC, Semitiel CMM. 2006. A conceptual model of salt marsh plant distribution in coastal dunes of Southeastern Spain. Wetlands 26: 703-717.

Chong, GW, Stohlgren TJ. 2007. Species-area cureves indicate the importance of habitats contributions to regional biodiversity. Ecol Indic 7: 387-395.

Chou C, Chen T, Liao C, Peng C. 2000. Long-term ecological research in the Yuan gang Lake Forest ecosystem. Vegetation composition and analysis. Bot Bull Acad Sin 41: 61-72.

Coppolillo P, Gomez H, Maisels F, Wallace R. 2004. Selection criteria for suites of landscape species as a basis for site-based conservation, Biological Conservation. 115: 419-430.

Daly HE, Cobb JBJr. 1989. For the common good: redirecting the economy toward community, the environment, and a sustainable future. Beacon Press, Boston, MA.

Fattahi M. 2001. Degradation West forests of Iran, Research Institute forests and rangelands Of Iran.

Forman RTT. 1995. Land Mosaics: The Ecology of Landscapes and Regions. Cambridge University Press, Cambridge, UK.

Giriraj A, Murthy MSR, Ramesh BR. 2008 Vegetation Composition, Structure and Patterns of Diversity: A Case Study from the Tropical Wet Evergreen Forests of the Western Ghats, India. Edin J Bot 65: 1-22.

Helms JA. 1998. The dictionary of Forestry. The Section of American Forests and CABI Publishing, New York.

Jalili A, Jamzad Z. 1999 Red Data Book of Iran. Research institute of forest and rangelands, Tehran.

Krebs CJ. 1999. Eclolgical Methodology. 2nd ed. Benjamin Cummings, Reading, UK.

Lindenmayer DB, Franklin JF. 2002. Conserving Forest Biodiversity: A Comprehensive Multiscaled Approach. Island Press, Washington, DC.

Lindenmayer DB, Franklin JF, Fischer J. 2006. General management principals and a checklist of strategies to guide forest biodiversity conservation. Boil Conserv 131: 433-445.

Lindenmayer DB, Margules CR, Botkin D. 2008. The focal species approach and landscape; Management of protected areas and conservation of biodiversity in Iran, International Journal of Environmental Studies Restoration: a critique. Conserv Biol 16: 338-345.

Lombard AT, Cowling, RM, Pressey RL. Mustart PJ. 1997. Reserve selection in a species-rich and fragmented landscape on Agulhas plain, South Africa. Conserv Biol 11 (5): 1101-1116.

Magurran AE. 2004. Measuring Biological Diversity. (Periodical style-Accepted for publication), Blackwell Publishing, London, UK.

Margules CR, Pressey RL. 2000. Systematic conservation planning. Nature. 405: 242-53.

Marsh RM. 1984. An overview: real and apparent patterns in community structure. In: Strong DRJr, Simberloff D, Abele LG, Thistle AB (eds). Ecological Communities, Conceptual Issues and the Evidence. Princeton University Press, Princeton, NJ.

May RM. 1984. An overview: real and apparent patterns in community structure. In: Strong DRJr, Simberloff D, Abele LG, Thistle AB (eds). Ecological Communities, Conceptual Issues and the Evidence. Princeton University Press, Princeton, NJ.

Mozafarian M. 2000. Plant Systmatics. Amirkabir Press, Tehran.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature. 403: 853-858.

Passmore J. 1974. Man's Responsibility for Nature: Ecological Problems and Western Traditions. Duckworth, London.

Pilehvar B, Veiskarami G, Taheri Akaber K, Soosani J. 2010. Relative contribution of vegetation types to regional biodiversity in Central Zagros forests of Iran. Biodiv Conserv 19: 3361-3374.

Raunkiaer C. 1934. The Life Forms of Plants and Statistical Plant Geography. Clarendon Press, Oxford.

Shmida A. 1984. Whittaker's plant diversity sampling method. Isr J Bot 33: 41-46.

Stohlgren TJ, Chong CW, Kalkhan MA, Schell LD. 1997a. Rapid assessment of plant diversity patterns: a methodology for landscapes. Environ Monit Assess 48: 25-43.

Stohlgren TJ, Coughenour MB, Chong GW, Binkley D, Kalkhan MA, Schell LD, Buckley DJ, Berry JK. 1997b. Analysis of plant diversity. Landsc Ecol 12: 155-170.

Tiner RW. 1999. Wetlands Indicators. A Guide to Wetland Identification, Delineation, Classification, and Mapping. Lewis Publishers, CRC Press, Boca Raton, FL.

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Floristic changes at Khersan Glacier Territory, Alamkuh Mountain, Central Alborz, North of Iran

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[1]Department of Biology, Science and Research Branch, Islamic Azad University, Hesarak-1477893855, Tehran. Iran, ♥email: nejadsattari_t@yahoo.com
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Abstract. *Kavousi K, Nejadsattari T, Asri Y, Ejtehadi H, Khavari-Nejad RA. 2016. Floristic changes at Khersan Glacier Territory, Alamkuh Mountain, Central Alborz, North of Iran. Biodiversitas 17: 11-15.* Extensive investigation in subnival-nival area around Khersan glacier moraine introduced 71 vascular plant species. From this list 43 species have been listed in Noroozi et al. (2011) in " subnival-nival vascular plant species of Iran : a unique high mountain flora and its threat from climate warming" and the others are new for subnival-nival area of Iran. Among this plant list 31 species had introduced with Kotschy (1861a,b), Bornmuller (1906), Melchior (1937), Klein (1982), European researchers and the other is named for the first time from Khersan glacier territory. Many species such as *Astragalus macrosemius*, *Pseudocamelina kleinii*, *Crepis multicaulis* subsp. *congesta*, *Didymophysa fedtschenkoana* and *Draba melanopus* due to glacier condition have very sensitive habitat, vulnerable and only gathered from restrict area with conservation value. Vegetation change happened in many nival and subnival areas with upward movement in the same habitat and movement from lower altitude at alpine towards summit in subnival and nival. *Carex oreophila*, *Campanula stevenii*, *Bromus brachystachyus*, *Oxytropis immersa*, *Erigeron uniflorus, Trachydium pauciradiatum, Scorzonera radicosa* and some other species are surprisingly movement to subnival area and many nival and subnival species such as *Didymophysa aucheri, Didymophysa fedtschenkoana, Dracocephalum aucheri* and *Arabis caucasica* have come significantly upward in nival. The movement is different in all side of Khersan glacier moraine in north, south and the east (beside moraine tongue) slops and limited with presence of soil natural generation and other ecological remarks. Limitation for soil generation starts at different altitude in northern, southern and eastern slopes of Khersan glacier valley. This study examined changes of flora in Khersan glacier territory during recent decades according to extensive data gathering, full list of Khersan glacier territory introduced *Barbarea stricta, Draba melanopus, Pseudocamelina kleinii, Crepis multicaulis* subsp. *congesta* as new report for flora of Iran and flora Iranica area.

Keywords: Alamkuh, alpine, glacier territory, Khersan, moraine, subnival-nival

INTRODUCTION

Alamkuh in Mazandaran province is the second high after Damavand peak in Iran which is protected as natural national monument with Iranian Department of Environment (Figure 1.A). There are 13 active glacier such as Haft-khan with 7 western glacial valley, Marji-kash with an eastern active moraine and parallel with Khersan valley, 4 Nordic moraines towards Sarchal valley, and Khersan glacier with an active longest and widest glacier in these series. Hasarchal is a plain at the base of Khersan glacier valley which surrounded by mountain series in north such as Alamkuh and Marji-kash, Khersan, Shane-kuh, Menar and Lashkarag in the south side of glacier moraine valley.

Khersan peak with 4620 m lead to Shane-kuh, Menar, Lashkarag in the east, Khersan glacier in the southeast and Alam peak and Marji-kash glacier in the western location. Biodiversity and abundance of native species is given high values to this location as a centre of biodiversity for plant species. In this paper Khersan glacier has been studied and presented with more detailed information with new plant record from Iran and Khersan glacier territories. Important location and study area of Khersan glacier shows in the Figure 1.B.

Botanical studies in this area was conducted in 1843 by Theodor Kotschy and after Kotschy (1861a,b), Bornmuller (1906), did gathering extensive in the area, the information that is given in the Flora Iranica. Melchior (1937) after in depth studies in Hasarchal has provided a great deal of taxonomic information. Klein (1982) completed Bornmuller (1906) information with an extensive data gathering in Alborz Mountains and has provided botanical and phytosociological data from Hasarchal and Khersan glacier territories.

The result of all previous study identified 31 plant species from nival and subnival area of Khersan glacier territories and Hasarchal around Khersan moraine. This data is the basis for judgment and interpreting the theory of plant movement and shift from lower altitude to highlands during the recent decades. According to recent studies since 2013, 71 plant species have been identified in this area. Of the 71 species 40 species are reported for the first time from Khersan glacier territory and 4 plant species are reported for the first time from Iran. This study focuses on the botanical condition in all sides of Khersan glacier territory during the recent year and suggests floristic changes such as movement, elimination and plant species up warding.

MATERIALS AND METHODS

Landsat ETM + has been used in this study to identify normal range of Khersan glacier and it changes and study area, GPS and fixed prefabricated quadrate (2m x 2m) has been used to record the location information for studies and comparing between present and past. European researcher collected information such as Kotschy (1861a,b) and Bornmuller (1906), including the location and their species nomenclature is used to replicate the recent studies and extensive field visit during 2013 to 2015 conducted this work studies.

Studies begin from the lower pilot in altitude 3800 m asl. towards the mountains in three access road around Khersan moraine with 38 quadrate in north, south and east slopes during 2013 to 2015. Quadrate size is determined based on standard curve for square size and number of species and fixed for each altitude. Plant species gathered from 38 square (12 in north and 15 in west, 11 in south and east) has been named with literatures from Komarov (1939), Assadi et al. (1988-2010), Rechinger (1963-2010), Breckle (2007), and Noroozi et al. (2008, 2010a,b, 2011, 2013), and has been updated according to extensive data gathering. Comparative study has done according to recent field visit and data which had been gathered with Kotschy (1861a,b), Klein (1982), Bornmuller (1906), Melchior (1937) and Rechinger (1963-2010) since 1843 for determine present or absent of plant species, biological characteristics changes, upward movement and etc.

RESULTS AND DISCUSSION

Results

The Table 1 is set for discussion present the species gathered from study area during 2013-2015. It lists the plant species collected since 1843 in Khersan glacier territory and has been compared with the last documents such as Noroozi et al. (2010a, 2011, 2013) about subnival-nival vascular plant of Iran, Flora Iranica and recent studies which is demonstrated in Khersan moraine column. Each row in the table shows the history of plant species since 1843 to the last gathered in summer 2015.

The list shows there are 71 vascular plant species in margin of Khersan moraine glacier from 4435 m asl. in the northern slope to lower elevation in borderline of subnival in 3900 m asl. around Khersan territory. About 50% of this plant list is endemic in Iran and 6 of those are mono-regional and have only known from one locality in Iran. This number is much more than of previous reports since 1982 with 31 species and this studies added 40 plant species to Khersan glacier territories. A group of these added species come upward from lower elevation and much of it dependent to appropriate collection during the past three years with authors.

According the plant species list *Barbarea stricta*, *Crepis multicaulis* subsp. *congesta*, *Didymophysa fedtschenkoana*, *Draba melanopus* recorded for the first time from Iran. These species have very specific habit due to Khersan glacier territory and with very restricted distribution. Many species of list as above new records and some other species with low habit and endangered location affected by human activities classified in Critically Endangered (CR) class according to IUCN Red List Categories and Criteria (IUCN 2001) and need to conservation and protection program.

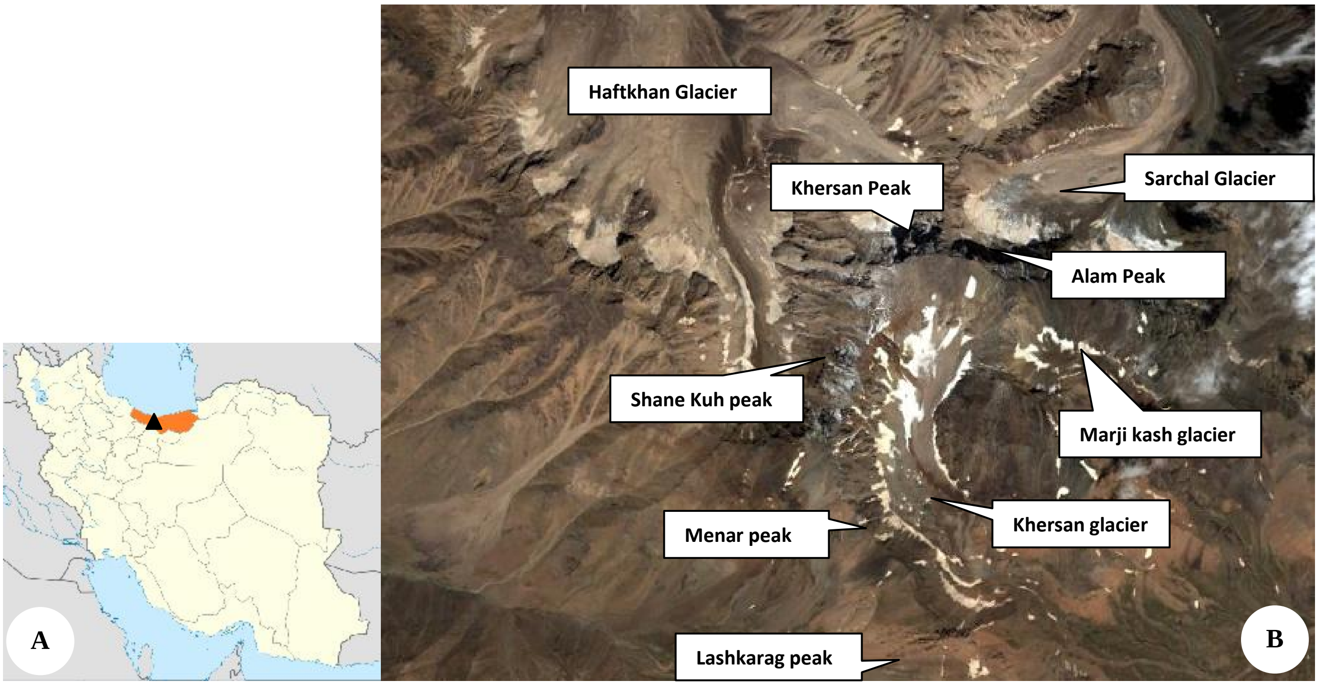

Figure 1. A. Alamkuh, Mazandaran province, Iran (▲), B. Highland condition with main peaks in Khersan glacier territory

Table 1. Alphabetical list for plant species in study area and it's history

Species	Family	Endemic	Mono-regional	Noroozi et al. (2011)	Collection reports Rechinger (1963-2010) Other localities in Iran	Collection reports Rechinger (1963-2010) Khersan moraine	This study (2013-2015)
Acantholimon demavendicum	Plumbaginaceae	*		*	*		*
Achillea aucheri	Asteraceae	*		*	*		*
Achillea millefolia subsp. *elbursensis*	Asteraceae	*			*		*
Allium capitellatum	Alliaceae	*		*	*		*
Alopecurus textilis	Poaceae			*	*		*
Arabis caucasica	Brassicaceae			*	*	*	*
Artemisia melanopsis	Asteraceae	*		*	*	*	*
Asperula glomerata subsp. *bracteata*	Rubiaceae	*		*	*		*
Astragalus aurea	Fabaceae				*		*
Astragalus macrosemius	Fabaceae	*	*	*		*	*
Astragalus monanthemus	Fabaceae	*			*	*	
Asyneuma amplexicaule	Campanulaceae	*			*	*	
Barbarea stricta	Brassicaceae		*				*
Bromus brachystachyus	Poaceae				*		*
Campanula stevenii	Campanulaceae	*			*		*
Carex oreophila	Cyperaceae				*		*
Catabrosa aquatica	Poaceae				*		*
Catabrosella parviflora	Poaceae			*	*		*
Cerastium cerastoides	Caryophyllaceae			*	*		*
Cousinia alferedii	Asteraceae	*			*		*
Crepis heterotricha subsp. *lobata*	Asteraceae	*			*		*
Crepis multicaulis subsp. *congesta*	Asteraceae		*				*
Crepis multicaulis subsp. *multicaulis*	Asteraceae			*	*		*
Cystopteris fragilis	Woodsiaceae			*			*
Didymophysa aucheri	Brassicaceae	*		*	*		*
Didymophysa fedtschenkoana	Brassicaceae		*		*		*
Draba melanopus	Brassicaceae		*				*
Draba pulchella	Brassicaceae	*		*	*	*	*
Draba siliquosa	Brassicaceae			*	*	*	*
Dracocephalum aucheri	Lamiaceae	*		*	*	*	*
Erigeron uniflorus subsp. *elbursensis*	Asteraceae	*		*	*	*	*
Erysimum nanum	Brassicaceae	*			*	*	*
Festuca ovina	Poaceae				*		*
Ficaria kochii	Ranunculaceae				*	*	*
Gagea soleimani	Alliaceae	*		*	*	*	*
Gallium aucheri	Rubiaceae	*		*	*	*	*
Gnaphallium supinum	Asteraceae				*		*
Gentiana pontica	Gentianaceae			*	*	*	*
Helichrysum plicatum	Asteraceae				*		*
Lepescheniella persica	Boraginaceae	*		*	*		*
Minuartia lineata	Caryophyllaceae	*		*	*		*
Oxyria digyna	Polygonaceae			*	*	*	*
Oxytropis hirsutiscula	Fabaceae				*		*
Oxytropis immersa	Fabaceae			*	*	*	*
Oxytropis takhti-soleimanii	Fabaceae	*		*	*		*
Paraquilegia caespitosa	Ranunculaceae	*		*	*		
Pedicularis caucasica	Scrophulariaceae			*	*	*	*
Pedicularis sibthorpii	Scrophulariaceae				*		
Plantago atrata subsp. *spadicea*	Plantaginaceae			*	*	*	*
Poa bulbosa	Poaceae				*		*
Polygonum serpyllaceum	Polygonaceae			*	*	*	*
Potentilla argaea	Rosaceae			*	*	*	*
Potentilla aucheriana	Rosaceae	*		*	*	*	*
Potentilla polyschista	Rosaceae	*		*	*	*	*
Pseudocamelina kleinii	Brassicaceae	*	*			*	*
Ranunculus crymophyllus	Ranunculaceae	*		*	*	*	*
Ranunculus polyschista	Ranunculaceae				*		*
Saxifraga iranica	Saxifragaceae	*		*	*		*
Scorzonera radicosa	Asteraceae			*	*	*	*
Scutellaria glecomoides	Lamiaceae			*	*		

Scutellaria pinnatifida	Lamiaceae			*		*
Senecio vulcanicus	Asteraceae	*	*	*	*	*
Tarxacum crepidiforme	Asteraceae		*	*	*	*
Thymus caucasica	Lamiaceae			*		*
Trachydium depressum subsp. *depressum*	Apiaceae		*	*	*	*
Trachydium pauciradiatum	Apiaceae	*		*	*	*
Tragopogon kotschyi	Asteraceae	*		*	*	
Veronica aucheri	Scrophulariaceae	*	*	*		*
Veronica gaubae	Scrophulariaceae	*		*		*
Veronica kurdica	Scrophulariaceae	*	*	*		*
Veronica paederotae	Scrophulariaceae	*	*	*	*	*

Note: * = present

Entering livestock around Khersan glacier margin above 3800 m asl. in the recent year, camping at Hasarchal more than of capacity, medicinal plant collection, consecutive changes of climbing rout toward peaks that makes mechanical erosion, and some other human activities and due to these activities affect extremely on Critically Endangered (CR) plant species habitat and should be controlled and preventing.

Discussion

In the glacial valley to the accommodation camp at 3800 m asl., 71 plant species were collected, 35 species equivalent 50% are native to Iran. Two species *Pseudocamelina kleinii, Astragalus macrosemius* grow only in this area throughout the world and those are located on an very small area in the southern part of Khersan moraine. In addition to the above plant *Draba melanopus, Crepis multicaulis* subsp. *congesta* has only known from eastern area of Khersan moraine and *Barbarea congesta* has only distributed around major spring east of Khersan moraine with very restricted and vulnerable habitat.

The extensive survey since 2013 in Khersan territory has not collected again *Astragalus monanthemus, Asyneuma amplexicaule, Paraquilegia caespitosa* and *Tragopogon kotschyi* from this area and it seems these species had disappeared. Plant species *Astragalus aurea, Achillea aucheri, Achillea millefolia* subsp. *elbursensis, Asperula glomerata* subsp. *bracteata, Acantholimon demavendicum, Alopecurus textilis, Allium capitellatum, Cerastium cerastoides, Carex oreophila, Catabrosa aquatica, Crepis heterotricha* subsp. *lobata, Crepis multicaulis* subsp. *multicaulis, Crepis multicaulis* subsp. *congesta, Campanula stevenii, Cystopteris fragilis, Catabrosella parviflora, Didymophysa aucheri, Scutellaria glecomoides, Scutellaria pinnatifida, Draba melanopsis, Bromus brachystachyus, Festuca ovina, Gnaphallium supinum, Helichrysum plicatum, Lepescheniella persica, Minuartia lineata, Poa bulbosa, Didymophysa fedtschenkoana, Barbarea stricta, Oxytropis hirsutiscula, Oxytropis takhti-soleimanii, Ranunculus polyschista, Saxifraga iranica, Thymus caucasica, Veronica gaubae, Veronica aucheriana* and *Veronica kurdica* gathered for the first time from Khersan glacier territory. Many plant species such as *Astragalus aurea, Astragalus monanthemus, Achillea millefolia* subsp. *elbursensis, Catabrosa aquatica, Campanula stevenii, Draba melanopsis, Bromus brachystachyus, Erysimum nanum, Festuca ovina, Gnaphallium supinum, Helichrysum plicatum, Pedicularis sibthorpii, Poa bulbosa, Pseudocamelina kleinii, Ranunculus polyschista, Scutellaria pinnatifida, Trachydium pauciradiatum, Thymus caucasica, Veronica gaubae and Ficaria kochii* has not included by Noroozi et al. (2010a, 2011, 2013) in the previous list of subnival and nival area of Iran while these species are growing in the highlands around Khersan glacier valley and probability these species growing in the other place around Alamkuh too. *Pseudocamelina kleinii* has gathered from Khersan moraine margin by Klein (1971) after Iranian flora of Brassicaceae has published. It has mono regional location in the world with low amount of population less than 20 and grows at altitude between 4119 and 4127 m asl.

Collecting of *Carex oreophila, Poa bulbosa, Catabrosa aquatica, Campanula stevenii, Bromus brachystachyus, Oxytropis immersa, Erigeron uniflorus* subsp. *elbursensis, Trachydium pauciradiatum, Scorzonera radicosa* and many species of veronica in subnival-nival area which are naturally belong to alpine area proof entering Alpine species at the nival and subnival, phenomena which illustrate the occupation and disturbing of summit with invasive plants. During 2013-2015 *Draba melanopus* from the Khesan glacier tongue, *Crepis multicaulis* subsp. *congesta* on calcareous conglomerate at the southern area of moraine and *Didymophysa fedtschenkoana* in volcanic margin of Khersan between Alamkuh and Marji-kash peak has been collected for the first time. These thrice species added to actual Iranian subnival and nival plant species no mentioned with Noroozi et al. (2011).

In subnival-nival area place to live are limited and some late comer such as *Cousinia alferedii, Festuca ovina, Poa bulbosa* and *Alopecurus textilis* that have higher survival chance for living, reduce grow of the threatened species. *Pseudocamelina kleinii* has a low population is at risk of global threat. In addition the current growth point of this plant in Iran have also been collected from Azad-kuh in 1974 at altitude 3880 m asl. Central Alborz. Now the situation was not known and in the recent studies species has not been collected again. This species observed in Khersan glacier valley for the first time in 2013 and its habit and ecological condition has monitored in 2014 and 2015.

Plant species such as *Pseudocamelina kleinii, Arabis caucasica, Dracocephalum aucheri, Didymophysa aucheri* shows altitude displacement and do not affect the entry of alpine invasive species but the displacement height with existing of soil in summit restricted. Many species didn't moved upward and log in alpine and sub alpine invasive plants species changed their habitat and will be destroy gradually.

The area is highly impacted by human activities and local livestock. Livestock disturb highlands ecosystem regulation and native people uses rare plant medicinal and edible and made pressure on natural ecosystem. Upward shift of grasses such as *Poa*, *Festuca* and *Alopecurus* species with wind, human, animal skins and livestock increase population and network nest of snow vole around of *Astragalus aurea* roots and some other similar plants, gradually, ice influencing around the root canal which made by mature Voles, plant roots freezing and dead after two or three winter. Livestock have main role in seed displacement and actually it improved by human activities. *Pseudocamelina kleinii, Astragalus macrosemius* with one address in the world and very restricted population classified as Critically Endangered (CR) in IUCN (2001) conservation categories and need to effective protection plan and monitoring program.

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REFERENCES

Assadi M, Maassoumi AA, Khatamsaz M, Mozaffarian V (eds.). 1988-2010. Flora of Iran. Vols 1-66. Research Institute of Forests and Rangelands Publications, Tehran. [Persian]

Bornmuller J. 1906. Beitrage zur Flora der Elburzgebirge Nord-Persiens, Bull Herb Boiss. Geneve. 6: 605-620,765-780.

Breckle SW. 2007. Flora and vegetation of Afghanistan. Basic Appl. Dryland Res. 1 (2): 155-194.

Ghelichnia H. 2014. Flora and vegetation of Mt. Damavand in Iran Phytologia Balcanica 20 (2-3): 257-265.

IUCN. 2001. IUCN Red List Categories and Criteria, Version 3.1. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland, and Cambridge, United Kingdom.

Klein JK. 1982. Les groupments chinphilesde l' Alborz central (Iran). Comparasion avec lears homologues d' Asia centrale. Phytocoenologia 10: 463-485.

Komarov VL. 1939. Flora of USSR, Botanical Institute of the Academy of Sciences of the U.S.S.R, Izdatel'stvo Akademii Nauk SSSR Moskva-Leningrad. 8: 276-237.

Kotschy T. 1861a. Die Vegetation des westlichen Elbrus in Nordpersien. Osterr Bot Zeitschr 11 (4): 105-117.

Kotschy T. 1861b. Der westliche Elbrus bei Teheran. Mitt. der K. K. Oester Geogr Gesell Wien 5: 65-110.

Melchior H. 1937. Zur pflanzengeographie ddes Elburs-Gebirges in Nord-Iran. Sber Ges Naturf Freunde Berl 1937: 55-71.

Noroozi J, Ajani Y, Nordenstam B. 2010a. A new annual species of *Senecio* (Compositae-Senecioneae) from subnival zone of southern Iran with comments on phytogeographical aspects of the area. Comp Newsl 48: 4-23.

Noroozi J, Akhani H, Breckle SW. 2008. Biodiversity and phytogeography of the alpine flora of Iran. Biodiv Conserv 17 (2): 493-521.

Noroozi J, Akhani H, Willner W. 2010b. Phytosociological and ecological study of the high alpine vegetation of Tuchal Mountains (Central Alborz, Iran). Phytocoenologia 40 (4): 293-32.

Noroozi J, Pauli H, Grabhen G, Breckle SW. 2011.The subnival-nival vascular plant species of Iran: a unique high-mountain flora and it threat from climate. Biodiv Conserv 20: 1319-1338.

Noroozi J, Willner W, Pauli H, Grabherr G. 2013. Phytosociology and ecology study of the high-alpine to subnival scree vegetation of N and NW Iran (Alborz and Azerbaijan Mts.). Appl Veg Sci 17 (1): 142-161.

Rechinger KH. (ed) (1963-2010) Flora Iranica, vols 1-178. Akad. Druck-u. Verlagsanstalt Graz.

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Molecular phylogeny of *Acer monspessulanum* L. subspecies from Iran inferred using the ITS region of nuclear ribosomal DNA

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Abstract. *Khademi H, Mehregan I, Assadi M, Nejadsatari T, Zarre S. 2015. Molecular phylogeny of* Acer monspessulanum *L. subspecies from Iran inferred using the ITS region of nuclear ribosomal DNA. Biodiversitas 17: 16-23.* This study was carried out on the *Acer monspessulanum* complex growing wild in Iran. Internal transcribed spacer (ITS) sequences for 75 samples representing five different subspecies of *Acer monspessulanum* were analyzed. Beside this, 86 previously published ITS sequences from GenBank were used to test the monophyly of the complex worldwide. Phylogenetic analyses were conducted using Bayesian inference and maximum parsimony. The results indicate that most samples of *A. monspessulanum* species from Iran were part of a monophyletic clade with 8 samples of *A. ibericum* from Georgia, *A. hyrcanum* from Iran and one of *A. sempervirens* from Greece (PP= 1; BS= 79%). Our results indicate that use of morphological characteristics coupled with molecular data will be most effective.

Keywords: Biogeography, maple, phylogeny, Sapindaceae

INTRODUCTION

The genus *Acer* L. is a member of Sapindaceae that mainly grows in tropical and subtropical regions. This genus is one of the most diverse tree genera in the Northern Hemisphere with approximately 129 species and is the largest tree genus of the northern hemisphere besides *Quercus* (Fang 1966; Grimm et al. 2006). A number of characteristics are shared among all members of *Acer*. The arrangement of the two winged pericarps (samaras) in the fruits ranges from subparallel to diverging at about a right angle.

A number of characters that are beneficial for identification of species in the field may have evolved independently (lobe shape, margin of lobes). These traits may be highly variable within species like pubescence of lower leaf surface (Grimm et al. 2007). Several different florescence types, including racemes, panicles, corymbs and spikes, occur in this genus. These variations make infrageneric divisions very difficult. Species delimitation and phylogenic relationships within the genus *Acer* are also very controversial (Kholie 1967; Judd et al. 2002). Fang (1966) proposed a different system in which the genus was divided into two subgenera, mainly on the basis of simple versus compound leaves (Koidzumi 1911). In Ogata's system (Ogata 1967), the genus was classified into 26 sections (Momotani 1962). In 1970, Murray published his monograph of the Aceraceae with 7 subgenera, 24 sections and 35 series within *Acer* (Murray 1970). Ogata's system was essentially followed by Xu (1966), with some additions and amendments. More recently de Jong (1994) recognized only 19 series in 16 sections, providing a quite different arrangement from those of other authors (Pax 1902; Xu 1966, 1998; Ogata 1967; Xu et al. 2008). Some researchers discussed the infrageneric phylogenetic relationships in the genus by analyzing gross morphology, seed proteins, fossils and geographic distributions, but the conclusions were not in consensus (Momotani 1962; Rechinger 1969; Pax 1985, 1986; Wolfe and Yanai 1987; Thorne 1992).

Acer monspessulanum is a medium-sized deciduous tree or densely branched shrub that grows to a height of 10-15 m (rarely to 20 m) (Fontaine 2011).The trunk is up to 75 cm diameter, with smooth, dark grey bark on young trees, becoming finely fissured on old trees. Among similar maples is most easily distinguished by its small three-lobed leaves, 3-6 cm long and 3-7 cm wide, glossy dark green, sometimes a bit leathery, and with a smooth margin, with a 2-5 cm petiole. The leaves fall very late in autumn, typically in November. The flowers are produced in spring, in pendulous, yellow to white corymbs 2-3 cm long. The samaras are 2-3 cm long with rounded nutlets (Rushforth 1999; van Gelderen and van Gelderen 1999).

Acer monspessulanum from *Acer* section (van Gelderen et al. 1994) has distinct small, 3-lobed leaves, while its close relatives *A. hyrcanum* and *A. opalus* normally have 5-lobed leaves. *Acer ibericum* displays dimorphic leaves that are 5-lobed in juvenile plants and sucker shoots, and 3-lobed in older plants (Grimm et al. 2007). *Acer monspessulanum* fossils are fairly common in Late Miocene and Pliocene floras from southern Europe and south-western Asia (Kvacek et al. 2002; Sachse 2004).

The ITS is highly variable nuclear region suitable for phylogenetic reconstruction of closely related taxa. The utility of this marker has already been investigated in other plant groups including trees, e.g. *Acer* (Tian et al. 2002; Grimm et al. 2006; Grimm et al. 2007) and *Crataegus* (Zarrei et al. 2014, 2015) and bulbs (Zarrei et al. 2009).

The aim of this study is to clarify taxonomy and to delimit *Acer monspessulanum* subspecies that grow in Iran using ITS marker and comparing results with morphological traits.

MATERIALS AND METHODS

Plant samples

The ITS sequencing was performed on 75 individuals from 15 populations of *Acer monspessulanum* distributed in Iran. Population name, localities, altitude, and herbarium number for each population are shown in Table 1. The plant specimens were identified in the Department of Biology, Science and Research Branch of Islamic Azad University in Tehran, by the aid of local and regional Floras, and voucher specimens of the plants with numbers 14821-14835 were deposited in the IAUH (Table 1). The specimens were collected during July and December 2014. Wherever possible, five trees from at least 50 m distant from each other were sampled randomly from each population. Fresh leaves were collected and kept in 50 CC falcon tubes, filled with Silica Gel, for the purpose of drying them (Chase and Hill 1991). The leaves were then used as a DNA extraction source.

DNA extractions and ITS amplification

Total DNA was extracted following a modified CTAB protocol of Doyle and Doyle (1990) using the DNeasy Plant Mini kit (Qiagen, Germany). We amplified the Internal Transcribed Spacer region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA using primer combinations 18S (forward primer 5'-CCT TMT CAT YTA GAG GAA GGA G-3') and 28S (reverse primer 5'-CCG CTT ATT KAT ATG CTT AAA-3'). The PCR protocol for ITS region included: 34 cycles of 18 seconds denaturation (94°C), 30 seconds annealing (53°C), and 60 seconds elongation (72° C), with two additional minutes elongation (Gaskin and Schaal 2003). The quality of PCR products was checked by electrophoresis on a 1.0% agarose gel and then visualized under UV light.

Table 1. List of Acer monspessulanum subspecies investigated in our analysis and their morphological characters and locality in Iran (small= up to 2×2 cm, large=2-3 × 3.5-4 cm)

Taxon	Locality with herbarium numbers and GenBank accession numbers	Major features of morphological traits (Rechinger 1969)
A. monspessulanum ssp. *turcomanicum* (Pojark.) Rech. f.	Iran: Khorasan Shomali, 45 km N of Shirvan, Golul-Sarani, 2302 m, Basiri 14823 (IAUH)	Leaves: large Loculus inside: hairy Loculus outside: sparsely hairy
A. monspessulanum ssp. *ibericum* (M.B.) Yaltirik	Iran: Azarbayejan Sharghi, Kaleybar, Arasbaran forest, Venigh, 1070 m, Masoud, 14821 (IAUH), KT587662	Leaves: large Loculus inside: hairy Loculus outside: glabrous Lower surface midrib: glabrous
A. monspessulanum ssp. *ibericum* (M.B.) Yaltirik	Iran: Azarbayejansharghi, Kaleybar, Arasbaran forest, Tuali, 850 m, Masoud, 14822 (IAUH), KT587663	
A. monspessulanum ssp. *Ibericum* (M.B.) Yaltirik	Iran: Golestan, Gorgan, Golestan National Park, 677 m, Khademi, 14833 (IAUH), KT587665	
A. monspessulanum ssp. *ibericum* (M.B.) Yaltirik	Iran: Mazandaran, Amol, Haraz road, Chelav, 737 m, Khademi, 14834 (IAUH), KT587661	
A. monspessulanum ssp. *assyriacum* (Pojark.) Rech.	Iran: Kordestan, Mariwan, Mohhamadeh village toward Benavechele, 1550 m, Khademi, 14828 (IAUH), KT587655	Leaves: large Loculus inside: hairy Loculus outside: glabrous Lower surface midrib: sparsely hairy
A. monspessulanum ssp. *assyriacum* (Pojark.) Rech.	Iran: Kordestan, Mariwan, Mohhamadeh village toward Benavechele, 1510 m, Khademi, 14829 (IAUH), KT587653	
A. monspessulanum ssp. *assyriacum* (Pojark.) Rech.	Iran: Kermanshah, Jawanroud toward Salas, 1585 m, Khademi, 14832 (IAUH), KT587654	
A. monspessulanum ssp. *cinerascens* (Boiss.) Yaltirik	Iran: Fars, Marwdasht, Jahanabad village, 1756 m, Khademi, 14824 (IAUH), KT587656	Leaves: small Loculus inside: densely hairy Loculus outside: glabrous
A. monspessulanum ssp. *cinerascens* (Boiss.) Yaltirik	Iran: Fars, Marwdasht, Bizjan village, Dorodzan Dam, 1715 m, Khademi, 14825 (IAUH), KT587657	
A. monspessulanum ssp. *cinerascens* (Boiss.) Yaltirik	Iran: Fars, Marwdasht, Chav road, 1823 m, Khademi, 14826 (IAUH). KT587658	
A. monspessulanum ssp. *cinerascens* (Boiss.) Yaltirik	Iran: Fars, Bayza. Tang Tir forest, 1632 m, Khademi, 14827 (IAUH), KT587659	
A. monspessulanum ssp. *cinerascens* (Boiss.) Yaltirik	Iran: Kohgiloye-va-Boir Ahmad, Gachsaran, Gachsaran, 15 km to Choram, After Abrigoon, Deel neck, 1600 m, Mehrgan, 14835 (IAUH)	
A. monspessulanum ssp. *persicum* (Pojark.) Rech.	Iran: Kerman, 25 km from Dalfard toward Jiroft, 980 m, Meyjani, 14830 (IAUH)	Leaves: small Loculus inside: glabrous Loculus outside: sparsely hairy
A. monspessulanum ssp. *persicum* (Pojark.) Rech.	Iran: Kerman, Meyjan, 1218 m, Meyjani, 14831 (IAUH), KT587664	

Table 2. List of taxa used in our analysis with their GenBank accession numbers.

Taxon	Region	GenBank accession numbers
A. hyrcanum ssp. *hyrcanum*	Iran	AY605305
A. hyrcanum ssp. *hyrcanum*	Iran	AY605306
A. hyrcanum ssp. *hyrcanum*	Iran	DQ366129
A. hyrcanum ssp. *hyrcanum*	Iran	DQ366130
A. ibericum	Georgia	AM238352
A. ibericum	Georgia	AM238353
A. ibericum	Georgia	AM238354
A. ibericum	Georgia	AY605307
A. ibericum	Georgia	AY605308
A. ibericum	Georgia	AY605309
A. ibericum	Georgia	AY605310
A. ibericum	Georgia	AY605311
A. ibericum	Georgia	AY605312
A. ibericum	Georgia	AY605313
A. ibericum	Georgia	AY605314
A. monspessulanum	France	AM238407
A. monspessulanum	France	AM238408
A. monspessulanum	France	AM238409
A. monspessulanum	France	AM238410
A. monspessulanum	France	AM238411
A. monspessulanum	France	AM238412
A. monspessulanum	France	AM238413
A. monspessulanum	France	AM238414
A. monspessulanum	France	AM238415
A. monspessulanum	France	AM238416
A. monspessulanum	Bulgaria	AM238423
A. monspessulanum	Bulgaria	AM238424
A. monspessulanum	Bulgaria	AM238425
A. monspessulanum	Bulgaria	AM238426
A. monspessulanum ssp. *monspessulanum*	France	AM238355
A. monspessulanum ssp. *monspessulanum*	France	AM238357
A. monspessulanum ssp. *monspessulanum*	France	AM238358
A. monspessulanum ssp. *monspessulanum*	France	AM238359
A. monspessulanum ssp. *monspessulanum*	France	AM238361
A. monspessulanum ssp. *monspessulanum*	France	AM238362
A. monspessulanum ssp. *monspessulanum*	France	AM238363
A. monspessulanum ssp. *monspessulanum*	France	AM238364
A. monspessulanum ssp. *monspessulanum*	France	AM238365
A. monspessulanum ssp. *monspessulanum*	France	AM238366
A. monspessulanum ssp. *monspessulanum*	France	AM238367
A. monspessulanum ssp. *monspessulanum*	France	AM238368
A. monspessulanum ssp. *monspessulanum*	France	AM238369
A. monspessulanum ssp. *monspessulanum*	France	AM238370
A. monspessulanum ssp. *monspessulanum*	France	AM238371
A. monspessulanum ssp. *monspessulanum*	France	AM238373
A. monspessulanum ssp. *monspessulanum*	Spain	AM238374
A. monspessulanum ssp. *monspessulanum*	Spain	AM238375
A. monspessulanum ssp. *monspessulanum*	Spain	AM238376
A. monspessulanum ssp. *monspessulanum*	France	AM238377
A. monspessulanum ssp. *monspessulanum*	France	AM238378
A. monspessulanum ssp. *monspessulanum*	France	AM238379
A. monspessulanum ssp. *monspessulanum*	France	AM238380
A. monspessulanum ssp. *monspessulanum*	France	AM238381
A. monspessulanum ssp. *monspessulanum*	France	AM238382
A. monspessulanum ssp. *monspessulanum*	France	AM238383
A. monspessulanum ssp. *monspessulanum*	France	AM238384
A. monspessulanum ssp. *monspessulanum*	France	AM238385
A. monspessulanum ssp. *monspessulanum*	France	AM238386
A. monspessulanum ssp. *monspessulanum*	France	AM238387
A. monspessulanum ssp. *monspessulanum*	France	AM238388
A. monspessulanum ssp. *monspessulanum*	France	AM238391
A. monspessulanum ssp. *monspessulanum*	France	AM238393
A. monspessulanum ssp. *monspessulanum*	France	AM238394
A. monspessulanum ssp. *monspessulanum*	France	AM238395
A. monspessulanum ssp. *monspessulanum*	France	AM238396
A. monspessulanum ssp. *monspessulanum*	France	AM238397
A. monspessulanum ssp. *monspessulanum*	France	AM238398
A. monspessulanum ssp. *monspessulanum*	France	AM238399
A. monspessulanum ssp. *monspessulanum*	Germany	AM238401
A. monspessulanum ssp. *monspessulanum*	Germany	AM238402
A. monspessulanum ssp. *monspessulanum*	Spain	AY605315
A. monspessulanum ssp. *monspessulanum*	Spain	AY605316
A. monspessulanum ssp. *monspessulanum*	Spain	AY605317
A. monspessulanum ssp. *monspessulanum*	France	AY605318
A. monspessulanum ssp. *monspessulanum*	France	AY605319
A. monspessulanum ssp. *monspessulanum*	France	AY605320
A. monspessulanum ssp. *monspessulanum*	France	AY605321
A. monspessulanum ssp. *monspessulanum*	France	DQ366124
A. monspessulanum ssp. *monspessulanum*	France	DQ366125
A. monspessulanum ssp. *monspessulanum*	France	DQ366126
A. monspessulanum ssp. *monspessulanum*	France	DQ366127
A. monspessulanum ssp. *monspessulanum*	France	DQ366128
A. obtusifolium	Cyprus	AM238327
A. obtusifolium	Cyprus	AM238328
A. obtusifolium	Cyprus	AM238329
A. obtusifolium	Cyprus	AM238330
A. obtusifolium	Syria	AM238331
A. obtusifolium	Syria	AM238332
A. opalus	France	AM238417
A. opalus	France	AM238418
A. opalus	France	AM238419
A. opalus	France	AM238420
A. opalus	France	AM238421
A. opalus	France	AM238422
A. sempervirens	Greece	AM238334
A. sempervirens	Greece	AM238335
A. sempervirens	Greece	AM238336
A. sempervirens	Greece	AM238337
A. sempervirens	Greece	AM238340
A. sempervirens	Greece	AM238341
A. sempervirens	Greece	AM238342
A. sempervirens	Greece	AM238343
A. sempervirens	Greece	AM238344
A. sempervirens	Greece	AM238348
A. sempervirens	Greece	AM238349
A. sempervirens	Greece	AM238350
A. sempervirens	Greece	AM238351
A. sempervirens	Greece	AY605349
A. sempervirens	Greece	AY605350
A. sempervirens	Greece	AY605351
A. sempervirens	Greece	AY605352
A. sempervirens	Greece	AY605353
A. sempervirens	Greece	DQ366122
A. sempervirens	Greece	DQ366123
A. velutinum	Iran	AY605361

Note: ssp. = subsp. = sub species

Phylogenetic analyses

Phylogenetic reconstructions were performed with 15 samples from each of 75 accessions (15 populations) belonging to five subspecies of *Acer monspessulanum* from Iran (Table 1). In addition, we used the ITS sequence of 86 accessions of *Acer* from GenBank. List of non-Iranian taxa used in our analysis with GenBank accession numbers are shown in Table 2. We used ITS sequences of *Acer velutinum* Boiss. from GenBank as the outgroup based on the earlier studies including Grimm et al. (2006) (Table 2).

The 3' region of the 18S rDNA, the 5' region of the 26S rDNA, and the whole ITS1-5.8S rDNA-ITS2 region were sequenced for all the taxa, and these were compared to sequences produced for other maples. Forward and reverse sequences were visually compared and edited, and then aligned using Sequencher 4 software (Gene Codes Corporation, Ann Arbor, MI, USA). In addition to our sequences, 86 ITS sequences from other taxa were taken from GenBank (Table 2). All ITS sequences were assembled and aligned using MacClade 4 (Maddison and Maddison 2005). The parsimony analyses were performed using PAUP*4.0b10 (Swofford 2002), with the following options: heuristic search with 1,000 random-addition-sequence replicates; tree bisection-reconnection (TBR) branch swapping; saving all most parsimonious trees. Character state changes were treated as equally weighted. Relative clade support was estimated using 1,000 bootstrap replicates in PAUP* via full heuristic searches and simple taxon addition. Clades with a bootstrap value of 50% or more were considered as robustly supported nodes. The consistency index (CI) and retention index (RI) were calculated to assess the amount of homoplasy present in the data. The best-fitting substitution model (TrN+I) was determined under the Akaike Information Criterion (AIC; Akaike 1974) using Modeltest 3.7 (Posada and Crandall 1998). The Bayesian analysis (BA) of the ITS datasets were performed using MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). TrN+I is a transitional model with six rates. For the ITS dataset, the TrN+I model was chosen. The amount of proportion of invariable sites (I) was 0.6732.

RESULTS AND DISCUSSION

The data set of the ITS region included 675 characters, 43 of them parsimony informative. Strict consensus tree (length of 138 steps, consistency index (CI) = 0.703, retention index (RI) = 0.904) is shown in Figure 1. Figure 2 shows tree from Bayesian analysis using MrBayes. All sampled species of Iranian *Acer* were part of a monophyletic clade with Posterior Probability (PP) = 1 and Bootstrap Support (BS) = 79% (Clade N; Figure 1). Since Iranian *A. monspessulanum* origin are from Mediterranean (Rechinger 1969), we compare other studies that have involved North Africa samples, and found that European, North Africa and Asia Minor samples are in one clade as Acer core clade with BS 75% and PP 0.96 (Grimm et al. 2007).

Our maximum parsimony results (Figure 1) indicate that Iranian *A. monspessulanum* subspecies (13 populations) in clade N are closely related to eight *A. ibericum* samples from Georgia, one sample of *A. hyrcanum* from Iran and two samples of *A. sempervirens* from Greece (pp= 1, BS= 79). This agrees with results reported by Grimm et al. (2007) where different taxa of one of one group fall into three lineages. In their results, *Acer monspessulanum* and *A. ibericum* + *A. hyrcanum* group together in the graph and long proximal edges indicate that they are most closely related. Clones of *A. monspessulanum* are distinct and placed near the center of the graph (Grimm et al. 2007).

Clade O that includes six specimens of *A. ibericum* from Georgia with one specimen of *A. monspessulanum* subsp. *turcomanicum* from Iran and one specimen of *A. sempervirens* from Greece (PP= 0.79; BS= 62%) has proved Rechinger results about Iranian *Acer* origin. Clade M comprises 4 species of *A. monspessulanum* from Bulgaria with PP= 0.8 and BS= 77%. Clades M and N together are in clade C (PP= 0.95). This close relation between Iranian and Bulgarian *Acer monspessulanum* species samples support this notion that they have an origin in Mediterranean region (Rechinger 1969). Clade L has two species from France, one *A. monspessulanum* and *A. monspessulanum* ssp. *monspessulanum.* Clade K include clade L with another *A. monspessulanum* ssp. *monspessulanum* from France (PP= 0.84). Clade J includes clades K and L with four taxa of *A. monspessulanum* ssp. *monspessulanum* from France and Spain. Clade I have three *A. monspessulanum* ssp. *monspessulanum* from France with PP= 0.91. Clade H include 17 *A. monspessulanum* ssp. *monspessulanum* with *A. opalus*, all from France (PP= 0.97). Each clades of G and F has two *A. monspessulanum* ssp. *monspessulanum* from France with PP= 1 and BS= 80%. Clade E comprise clades F, G, H, I, J, K and L from France and Spain. Clade D has only one species *A. monspessulanum* from France that with clade E are in clade B with BS= 50%. Clade P that has one *A. velutinum* from Iran, consider as out-group in our analysis. Data analysis indicates that the classification of species according presence or absence of hairs in inner or outer surface of loculus is a true morphological characteristic for delimitation of subspecies in *Acer monspessulanum*.

The observed polytomies in clades E and N indicated that these taxa are taxonomically closely related and there were not enough time passed since divergence from their ancestral taxa (Zarrei et al. 2009). More divergent markers, i.e. low-copy nuclear genes, could potentially resolve these branches.

Based on our results using Bayesian analysis, some well resolved clades were present (Figure 2). Clade A comprise two subclades, E and F. Clade E includes four *A. monspessulanum* specimens from Bulgaria and clade F includes 27 taxa (PP= 1), that 13 of them are *A. monspessulanum* subspecies from Iran and others are *A. ibericum* (from Georgia), *A. sempervirens* (from Greece) and *A. hyrcanum* subsp. *hyrcanum* (from Iran), this clade proved Mediterranean origin of Iranian *Acer* (clade F; Figure 2). One of our taxa (*A. monspessulanum* subsp. *ibericum*) placed in clade L with five *A. ibericum* from Georgia and an *Acer sempervirens* from Greece with high support (PP= 0.79). This placement indicates that *A. monspessulanum* and *A. sempervirens* are closely related together (Grimm et al. 2007).

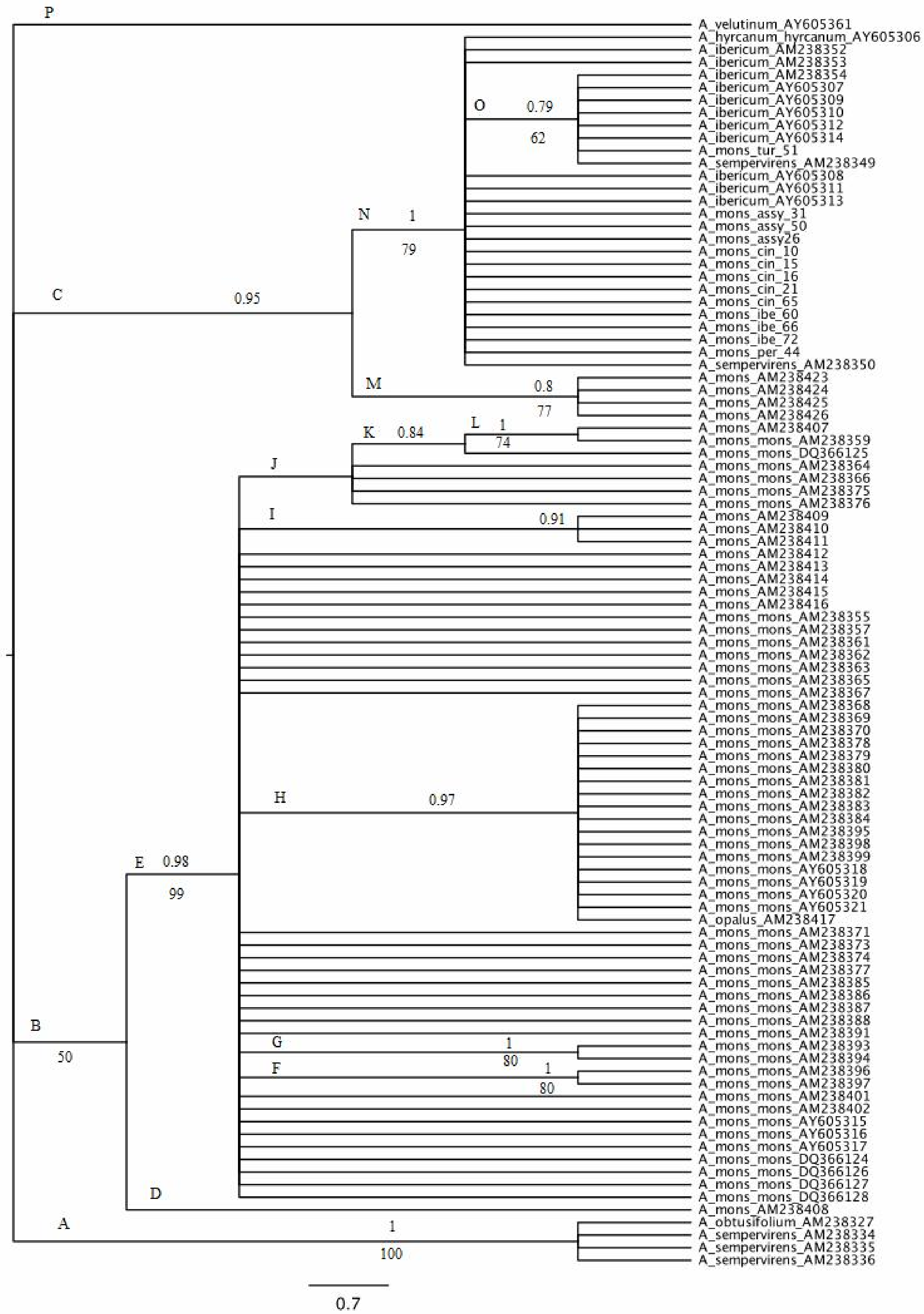

Figure 1. Phylogenetic relationships of 13 samples of *Acer* from Iran, on the basis of the analysis of internal transcribed spacer (ITS) sequences. Numbers above branches are Bayesian posterior probabilities. Numbers below branches are bootstrap values

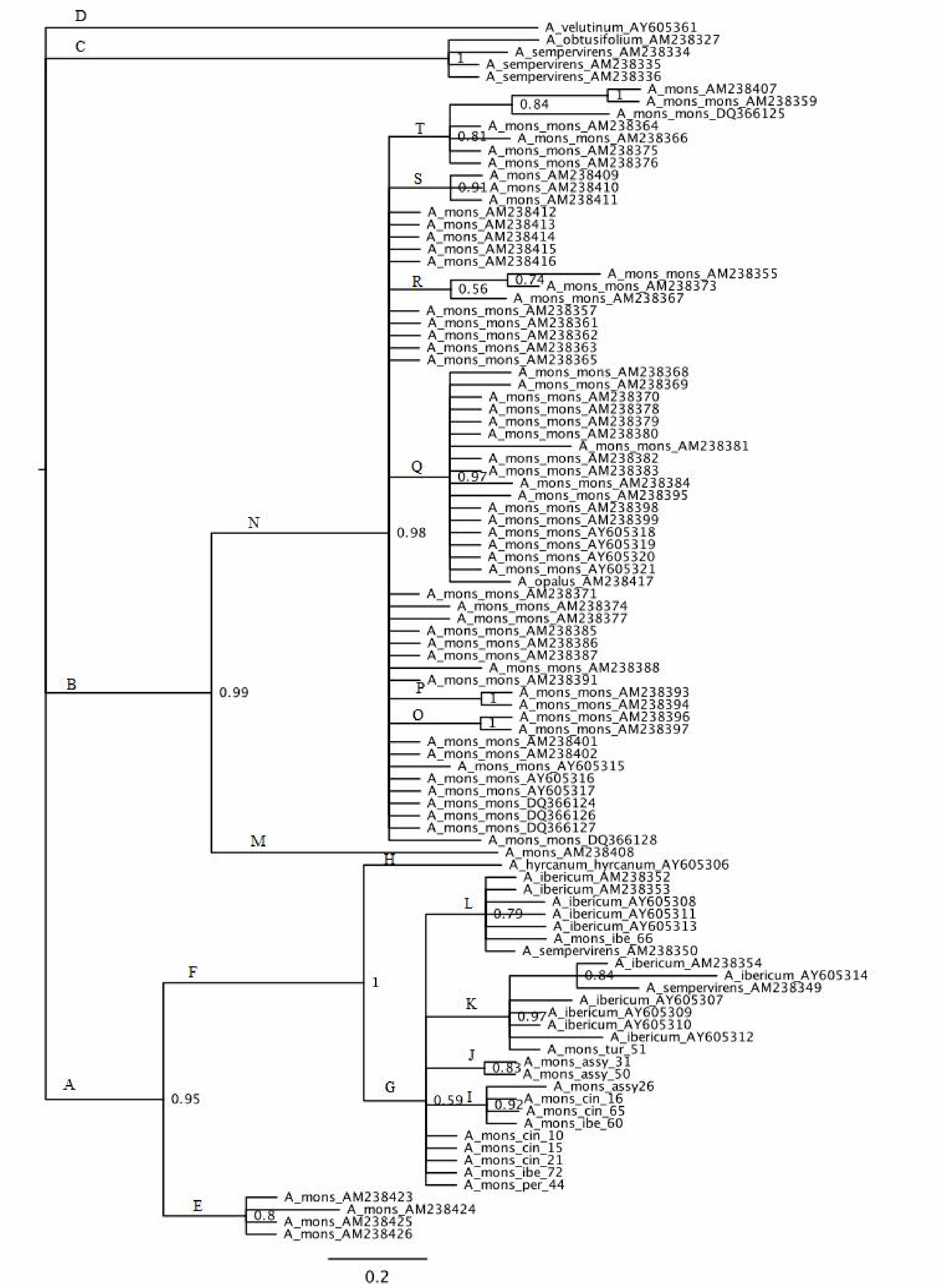

Figure 2. Bayesian tree. Numbers on nodes represent posterior probability values

Clade B (Figure 2) have two subclades M and N (PP= 0.99) that include European *Acer monspessulanum* (from France, Spain and Germany). Clades G and N show polytomies since comprise species their morphology are closely related together.

Clade C (Figure 2) have four taxa, three of them are *A. sempervirens* from Greece and one *A. obtusifolium* from Cyprus (PP= 1) that in Grimm et al. (2007) study In Europe, their distribution ranges from northern, cool-temperate latitudes (southern Sweden, *A. pseudoplatanus* L., naturalized) throughout central, western, and south-eastern Europe (*A. hyrcanum* Fischer & Meyer, *A. opalus* P. Miller, *A. pseudoplatanus*) to the subtropical Mediterranean (*A. monspessulanum* L., *A. opalus*, *A. heldreichii* Orphanides ex Boissier, *A. sempervirens* L., and *A. obtusifolium* Sibthorp & Smith), and with an eastward expansion to Asia Minor, the Caucasus, and Iran (*A. trautvetteri* Medvedev, *A. hyrcanum*, *A. ibericum* Bieberstein ex Willdenow, *A. monspessulanum*, *A. pseudoplatanus*, and *A. velutinum* Boissier) (Grimm et al. 2007).

Subspecies geographical distribution show that *assyriacum* subspecies from Kordestan and Kermanshah provinces are near together and support with Bayesian analysis (PP= 0.82). *A. monspessulanum* subsp. *ibericum* collected from Arasbaran forest (Azarbayejansharghi province) placed in clade L (Figure 2) with *A. ibericum* from Georgia (PP = 0.79) that from geographical distribution approach is justifiable. All Iranian *Acer monspessulanum* in the present survey made a clade with two *A. sempervirens* from Greece and eleven *A. ibericum* from Georgia and one *A. hyrcanum* subsp. *hyrcanum* from Iran with high Bayesian support (PP= 1).

Because ITS results couldn't delimitate on subspecies level we used from morphological traits. Some morphological features are important for identification of subspecies *A. monspessulanum*, such as size of the leaves, lower surface midrib hair and loculus inside and outside base on presence or absence of hair. One of the most important characteristic traits for distinguish between some subspecies of *A. monspessulanum*, is presence or absence of hair inside and outside their loculus. Based on the size of the leaves we have two groups, (i) small (up to 2×2 cm) that has two subspecies (ssp. *persicum* and ssp. *cinarescens*) and (ii) large (2-3×3.5-4 cm) with three subspecies (ssp. *turcomanicum*, ssp. *assyriacum* and ssp. *ibericum*). Lower surface midrib hair separate only two subspecies of *A. monspessulanum*, one of them is glabrous (ssp. *ibericum*) and the other is sparsely hairy (ssp. *assyriacum*), so this trait is not a proper discriminative factor for other three subspecies. Loculus outside hair has two state, (i) glabrous (ssp. *turcomanicum*, ssp. *persicum*) and (ii) sparsely hairy (ssp. *ibericum*, ssp. *assyriacum*, ssp. *cinarescens*). We have three group base on loculus inside hair, (i) hairy (ssp. *turcomanicum*, ssp. *ibericum*, ssp. *assyriacum*), (ii) densely hairy (ssp. *cinarescens*) and (iii) glabrous (ssp. *persicum*) (Wolfe and Yanai 1987), so the most important and discriminative character to detect subsp. *persicum* from subsp. *cinarescens* is loculus inside hair.

The internal transcribed spacer of the nuclear region (ITS) is a widely used molecular marker for reconstruction of evolutionary patterns in plant kingdom. It has been used both in the higher taxonomic level (i.e. family) as well as lower even below the species rank (Zarrei et al. 2014). Our results indicate that this marker could be potentially valuable in delineating subspecies boundaries in maple species. The limiting factor is that this marker is not well diverged in some groups. A more divergent molecular marker such as low copy nuclear genes and intergenic nuclear spacers could potentially be helpful. Based on our experiences working on *Acer* and results of others studies on tree taxa (Zarrei et al. 2014); we suggest combining our ITS DNA sequences with additionally markers to increase the power of our phylogenetic analysis and improve resolution of unresolved clades. Such strategies have been applied before (see Zarrei et al. 2015). The implication of next generation sequencing data has been already proven in revolving systematic problem with closely related species (Liston et al. 2015).

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REFERENCES

Akaike H. 1974. A new look at the statistical model identification. IEEE Trans Automat Contr 19, 718-723.

APG [Angiosperm Phylogeny Group]. 1998. An ordinal classification for the families Ann Mo Bot Gard 85: 531-553.

Chase MW, Hills HG. 1991. Silica gel: an ideal desiccant for preserving field-collected leaves for use in molecular studies. Taxon 40: 215-220.

De Jong PC. 1994. Taxonomy and reproductive biology of maples. Timber Press, Portland, USA.

Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Fang W. 1966. Rivisio Taxorum Aceracearum Sinicarum. Acta Phytotax Sin 11: 139-189.

Fontaine C. 2011. Fleurs De France (French Edition), Nabu Press, French.

Gaskin JF, Schaal BA. 2003. Molecular phylogenetic investigation of US invasive *Tamarix*. Syst Bot 28 (1): 86-95.

Grimm GW, Denk T, Hemleben V. 2007. Evolutionary history and systematics of *Acer* section *Acer*-a case study of low-level phylogenetics. Plant Systematics and Evolution. 267: 215-253.

Grimm GW, Renner SS, Stamatakis A, Hemleben V. 2006. A Nuclear Ribosomal DNA Phylogeny of *Acer* Inferred with Maximum Likelihood, Splits Graphs, and Motif Analysis of 606 sequeces. Evol Bioinform 2: 7-22.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754-755.

Judd WS, Campbell CS, Kellogg EA, Donoghue MJ. 2002. Plant Systematics: A Phylogenetic Approach, 2nd ed. Sinauer Associates, Sunderland, MA.

Kholie DK. 1967. Les chênes de. L`Iran. [Ph.D. Dissertation]. Universite de Montpellier, Montpellier, France [France]

Koidzumi G. 1911. Revisio Averacearum Japonicarum. J Coll Sci Imp Univ Tokyo 32: 1-75.

Kvacek Z, Velitzelos D, Velitzelos E. 2002 Late Miocene Flora of Vegora, Macedonia, N Greece. Korali, Athens.

Liston A, Weitemier K, Kronmiller B, Zarrei M, Dickinson T. 2015. Using Hyb-Seq data to test phylogenetic hypotheses in hawthorns (*Crataegus*, Rosaceae). Botany 2015, Botanical Society of America. Edmonton, AB.

Maddison DR, Maddison WP. 2005. MacClade 4: Analysis of phylogeny and character evolution. Version 4. http://macclade.org.

Momotani Y. 1962. Taxonomic study of the genus *Acer*, with special refrence to the seed protein. Mem Coll Sci Uni Kyoto Ser. B. (29): 177-189.

Murray E. 1970. A monograph of the *Acer*. Pennsylvania Pennsylvania State University.

Ogata K. 1967. A systematic Study of the genus *Acer*. Bull Tokyo Univ Forest. 63: 89-206.

Pax F. 1902. Aceraceae. Engler's Pflanzenreich IV. 163: 1-89.

Pax F. 1985. Monogeraphic der Gattung *Acer*. Bot Jahrb Engler. 6: 287-373.

Pax F. 1986. Monogeraphic der Gattung *Acer*. Bot Jahrb Engler. 7: 177-263.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14 (9): 817-818.

Rechinger KH. 1969. Flora Iranica, Aceraceae. Vol 61. Graza, Austria: Akademische Druck-u. Verlagasanstalt.

Rushforth KD. 1999. Trees of Britain and Europe. HarperCollins, London.

Sachse M. 2004. Die Neogene Mega-und Mikro-flora von Makrilia auf Kreta und ihre Aussagen zur Klima-und Vegetationsgeschichte des o¨stlichen Mittelmeergebietes. Flora Tertiaria Mediterranea 6: 1-323.

Swofford DL. 2002. Phylogenetic analysis using parsimony (PAUP). Ver. 4. Sinauer Associated Sunderland, MA.

Thorne R. 1992. An updated phylogenetic classification of the flowering plants. Aliso 13: 365-389.

Tian X, Guo ZH, Li DZ. 2002. Phylogeny of Aceraceae Based on ITS and *trn* L-F Data Sets. Acta Botanica Sinica 44 (6): 714-724.

van Gelderen CJ, van Gelderen DM. 1999. Maples for Gardens: A Color Encyclopedia. Timber Press, Inc., Portland.

van Gelderen DM, de Jong PC, Oterdoom HJ. 1994. Maples of the World. Timber Press, Portland.

Wolfe JA, Yanai T. 1987. Systematics, Phylogeny and distribution pf *Acer* (Maples) in the Cenozoic of Western North America. J Fac Sci, Ser. IV. 22: 1-246.

Xu T, Chen Y, de Jong PC, Oterdoom HJ, Chang CS. 2008. Aceraceae.In: Flora of China. Vol. 11. Flora of China Editorial Committee. Science Press, Beijing.

Xu T. 1966. A new system of *Acer*. Acta Bot Yunnan 18: 277-292.

Xu T. 1998. The systematic evolution and distribution of the genus *Acer*. Acta Bot Yunnan 20: 383-393.

Zarrei M, Stefanović S, Dickinson TA. 2014. Reticulate evolution in North American black-fruited hawthorns (*Crataegus* section *Douglasia*; Rosaceae): evidence from nuclear ITS2 and plastid sequences. Ann Bot 114: 253-269.

Zarrei M, Talent N, Kuzmina M et al. 2015. DNA barcodes from four loci provide poor resolution of taxonomic groups in the genus *Crataegus*. AoB Plants 7: plv045. DOI: 10.1093/aobpla/plv045

Zarrei M, Wilkin P, Fay MF, Ingrouille MJ, Zarre S, Chase MW. 2009. Molecular systematics of *Gagea* and *Lloydia* (Liliaceae; Liliales): implications of analyses of nuclear ribosomal and plastid sequences for infrageneric classification. Ann Bot 104: 125-142.

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Determination of appropriate grid dimension and sampling plot size for assessment of woody species diversity in Zagros Forest, Iran

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Abstract.*Zohrevandi AA, Pourbabaei H, Akhavan R, Bonyad AE. 2015. Determination of appropriate grid dimension and sampling plot size for assessment of woody species diversity in Zagros Forest, Iran. Biodiversitas 17: 24-30.* This research was conducted to determine the most suitable grid (dimensions for sampling) and sampling plot size for assessment of woody species diversity in protected Zagros forests, west of Iran. Sampling was carried out using circular sample plots with areas of 1000 m^2, 1500 m^2 and 2000 m^2 for 9 grid sizes in 200ha forest area. Importance-value curve which fitted by a Lognormal Distribution Model was plotted using Species Importance Value index (SIV). After determining the diversity indices such as Hills (N_0, N_1, N_2) and Alatalo's evenness (E_5) in each plot and assigning each sampling time, $E\%^2$x T criterion was calculated in order to compare the methods. Results of hundred percent inventory and sampling in forests showed that the accuracy of sampling in 1000m^2plot size is higher than 2000 m^2 ones. According to the Importance-value curve, accuracy and $E\%^2$x T criterion, the 150 m x 400 m grid size and sampling plot size with an area of 1000 m^2 was proposed as the most appropriate one to estimate woody species diversity in Iranian Zagros Forests.

Key words: Importance-value curve, inventory grid, sampling plot size, woody species diversity, Zagros forests

INTRODUCTION

Zagros forests in west of Iran are severely degraded and consequently their basic structure and complexity in identifying forest communities have changed (Khanlari 2006). This issue leads to poor soil condition and loss of woody species diversity in forests. Diversity is a prerequisite for understanding the patterns and processes of forest ecosystems which described well the forest structure in quantitative terms (Aguirre et al. 2003). Diversity indices are important input variables for restructuring of forest (Hasenauer and Pommerening 2006; Pommerening and Stoyan 2008). With increase human population and demands for resources and habitats, destructive human pressure on nature will increase and which is the beginning of the destruction of biodiversity (Lund et al. 2004). Measuring woody species diversity helps us to calculate the economic consequences of diversity destruction (Buongiorno et al. 1994; Kant 2002). Biodiversity increases the reproductive capacity and ability of adaptation to changes in forest ecosystems (Macneely 2002). Studying the patterns of species diversity helps us to understand the mechanisms that create diversity in a society (Wang et al. 2008). In a study in New Zealand by means of species level curve, it showed that sampling in 500 m^2 plot size is the most appropriate area for studying diversity of plant species (Neldner 2008). Alijanpour et al. (2009) in their study for comparison of woody plants diversity in protected and non-protected areas of Arasbaran forests used an inventory grid with 300 m x 150 m. They concluded that protection-based management increase woody species diversity in forest biomasses of Arasbaran forests. Recent studies about diversity patterns focused on tropical forests that have too much plant species (He et al. 1996; Hubbell et al. 1999; de Oliveira and Mori 1999; Condit et al. 2006).For example, in a 52-hectareplot area in Borneo and a 25-hectareplot area in Ecuador, there were 1175 and 1104 species, respectively (Wright 2002). In contrast, the 4.2 x 10^6 km^2 of temperate forests in Europe, north of America and Asia support only 1166 tree species (Latham and Ricklefs 1993).

In other words, the diversity of a small area in tropical forests is comparable to the diversity of tree species in the North Temperate Zone. Although ecologist studies rely on a better understanding of species diversity in moderate temperature forests, few studies focus on the spatial distribution of species diversity and the extent in which these patterns are influenced by environmental and spatial factors (Legendre and Fortin 1989; Legendre 1993). In general, number of species and their distribution (Two components which form species diversity), are estimated based on sampling of population at an extended level. Obviously, a large sample size need high cost and time, and small sample size leads to lack of accuracy of estimations. It is always tried to select the best possible sample size in the existing information framework with respect to the time, cost, and accuracy (Amidi 2006). Accuracy of estimation increases with extending the sample size (Nilsson 2002). The plot size also is a crucial factor in determining the species diversity of studied area. In inventory discussions, clarity of goals and application of results in accordance with the goal or other predefined and predetermined purposes are the basic principles in sampling (Zobeiry 2002). Hence, inventory methods are precisely selected with regard to the goal of inventory, forest structure, and available facilities. Wang et al. (2008) studied 25 hectares of Chang-bye forests in China and used plot size of 10 m x 10 m. Then, the areas of samples were doubled until, were covered the whole of 25hectares. In this research, the estimation of species richness, abundance of species, and Shannon diversity index were used to assess the patterns of species diversity.

The aim of current study is determining the appropriate network and sampling plot size for estimating the shrub and tree species diversity in the protected Zagros Forests in west of Iran (Zagros oak forests). Eventually with the highest accuracy and lowest cost of sampling (an acceptable combination of accuracy and cost) the diversity of woody species was studied in Zagros forests.

MATERIALS AND METHODS

The study area

The study area is a part of Ghalajeh forests located in the southwestern part of Kermanshah province, west of Iran (33°99´ N, 46°3´E) (Figure 1).The altitude ranges from 1450 to 1950 m asl., stretched at the geographical direction of northeast to southwest. Annual average of precipitation and temperature are 516.7 mm and 12.8°C, respectively. According to the Emberger climatic, Ghalajeh forest has mountainous sub-humid cold climate (Zohrevandi 2012). Stone types include limestone and marl which belong to the tertiary (Oligo-Miocene) period. The depth of soil is medium and its texture is heavy.

Data collection

According to a Digital Elevation Model (DEM), 9 inventory grids with dimensions of 100 m x 200 m, 150 m x 200 m, 200 m x 200 m, 100 m x 400 m, 150 m x 400 m, 400 m x 200 m, 100 m x 600 m, 150 m x 600 m, and 200 m x 600 m were designed. Sampling procedure was the systematic random method. Data was collected in circular sample plots with areas of 1000 m^2, 1500 m^2 and 2000 m^2.

In each plot, the type of woody species (trees and shrubs) were identified, counted and the two perpendicular diameters of canopy of each tree and shrub were calculated and recorded along with the coordinates of the plot center.

Data analysis

Species abundance distribution

Importance-value curve is plotted using Species Importance Value index (SIV).

Species Importance Value

Species importance value (SIV) was calculated for all species using relative frequency, relative density and dominance values for woody species. The following formulas were used for each calculation (Maingi and Marsh 2006; Adam et al. 2007):

Relative frequency = Number of plots that contain a species x 100 / Number of all plots

Relative density = Individuals number of a species in all plots x 100 / Total individuals number of species in all plots

Relative dominance = Total basal area of a species in all plots x 100 / Total basal area of all species in all plots

Diversity indices

The following indices are used to determine the variety of woody species in the study area. Hill (1973) has introduced a series of indices that are known as Hill numbers. The Hill numbers provide a better ecological interpretation than other indices. These numbers measure the effective number of species in the sample.

$$H' = \sum_{i=1}^{s} (p_i)(Log_2 p_i)$$

where:

H´= Information content of sample (bits/individual) or index of species diversity

S = Number of species

p_i = Proportion of total sample belonging to i^{th} species

Figure 1. The geographical location of the study area in Ghalajeh forest, Province of Kermanshah, Iran

The Shannon-Wiener index may be expressed in another form (MacArthur 1965) in units of numbers of species as

$N_1 = e^{H'}$

Where:
e = 2.71828 (base of natural logs)
H´= Shannon-Wiener function (calculated with base e logs)
N_1= Number of equally common species which would produce the same diversity as H´

$D = \sum p_i^2$

Where:
D = Simpson's index
p_i = Proportion of species i in the community

$$\frac{1}{D} = \frac{1}{\sum p_i^2}$$

Where:
1/D = Simpson's reciprocal index (= Hill's N_2)
p_i = Proportion of species i in the community

Hill (1973) called this reciprocal N_2.

Evenness

$$E_5 = \frac{N_2 - 1}{N_1 - 1}$$

E_5: This index is known as the modified index of Hill which is known as Alatalo index. Alatalo (1981) showed that when a species becomes very dominant, E_5 closes to zero. This index is not affected by species richness.

Inventory time cost

The necessary time for measuring every plot includes the time of measuring the intended features of the trees of every sample as well as time of movement from one plot to the next one. The total time is calculated by T_i= (n_i x ta_i) + (n_i x tb_i), where T_i is the total time of inventory of i method, $n_i$is the number of plot of *i* method, ta_i is the average time taken for measuring the trees of each plot in i method and tb_i is the average time taken for movement from one plot to the next (adjacent) plot in i method (Heidari et al. 2007). It should be pointed that since the routes of all samples were equal, the average time of moving from one plot to another was considered the same. As a result, the time of going from one plot to the other one was removed from time estimation. Accordingly, the relation Ti = (n_i x ta_i) was used to calculate time in every sampling method.

Selection criterion

Following formula is used to calculate the precision of inventory.

$$E\% = \pm t x S_{\bar{x}}\%$$

Where:
E% =Precision of inventory or the percentage of inventory error
t =Statistic of t-student table
S =Percentage of standard error

The best sampling grid was determined by$E\%^2$ x T criterion, where T is the total time of sampling in each method (Heidari et al. 2007).

Hundred percent inventories

Firstly to assess the data accuracy, hundred percent inventories was done in 40ha of the forest then N_0, N_1, N_2andE_5 diversity indices were determined 4.55, 2.28, 1.86, 0.59, respectively. Data analysis was done using GIS, SPSS, Past and Excel software.

RESULTS AND DISCUSSION

Trees and shrubs species

In current study the following species of trees and shrubs were studied: *Quercus brantii* Linddl., *Acer cinerascens* Boiss., *Pistacia atlantica* Desf., *Crataegus azarolus* L., *Cornus australis* C.A.Mey., *Lonicera persica* Jauh. & Spach., *Cerasus microcarpa* (C.A.Mey.) Boiss., *Amygdalus orientalis* Duh., and *Pyru scommunis* L.

Species abundance distribution

Importance-value curve was plotted using Species Importance Value index (Figure 2). Means and standard error was determined for diversity indices through 27 different sampling methods (Tables 1and 2).Table 1 shows that estimating the richness index N_0 and diversity indices N_1,and N_2 using different sampling grids and different surfaces of plot size, have no significant differences. Table 2 shows that the standard error for estimating the richness index N_0 and diversity indices N_1, and N_2 in different sampling grids, have no significant differences, but increasing of grid dimension (Lowering the number of plot), leads to increase standard error. ANOVA was performed to measure the estimation mean of diversity indices in grid dimension and sampling plot size(Table 3).

The variance analysis ofE_5 index in different sampling methods showed that there is a significant difference (95% level) between 1000 m^2 and 2000 m^2 plot size.

Comparing different methods of sampling

Different grid dimension and plot size were compared for estimation of woody species diversity using $E\%^2$x T criterion.

Regarding Table 5, is the least value of $E\%^2$x T criterion belongs to sampling grid of 150 m x 400 m with 1000 m^2 plot size.

Table 1. The estimated mean of diversity indices (N_0, N_1, N_2, and E_5) for 9 grid dimensions (Left-hand column) and 3 plot sizes (1000 m^2, 1500 m^2, and 2000 m^2)

Statistical parameters	**Mean**											
Plot size (m^2)	**1000**				**1500**				**2000**			
Diversity indices Network(m)	N_0	N_1	N_2	E_5	N_0	N_1	N_2	E_5	N_0	N_1	N_2	E_5
100 x 200	4.4	2.2	1.8	0.58	4.5	2.3	1.9	0.59	4.6	2.4	1.9	0.63
150 x 200	4.5	2.3	1.9	0.59	4.6	2.4	1.9	0.65	4.8	2.5	2	0.67
200 x 200	4.7	2.3	1.9	0.55	4.8	2.4	1.9	0.62	4.9	2.5	2	0.64
100 x 400	4.5	2.2	1.8	0.60	4.6	2.3	1.9	0.59	4.7	2.4	1.9	0.61
150 x 400	4.5	2.2	1.8	0.59	4.6	2.3	1.9	0.60	4.8	2.4	2	0.62
200 x 400	5	2.4	1.9	0.57	5	2.5	2	0.60	5.1	2.5	2	0.62
100 x 600	4.3	2.2	1.8	0.58	4.4	2.3	1.9	0.57	4.5	2.4	2	0.65
150 x 600	4.5	2.4	1.9	0.61	4.6	2.5	2	0.70	4.7	2.6	2.1	0.72
200 x 600	4.5	2.4	1.9	0.54	4.5	2.5	2	0.67	4.6	2.5	2	0.69

Table 2. The standard error of estimating of diversity indices (N_0, N_1, N_2, and E_5) for 9 grid dimensions (Left-hand column) and 3 plot sizes (1000 m^2, 1500 m^2, and 2000 m^2)

Statistical parameters	**Standard error $(S_{\bar{x}})$**														
Plot size (m^2)	**1000**					**1500**					**2000**				
Diversity Indices Network (m)	N_0	N_1	N_2	E_5	**Mean**	N_0	N_1	N_2	E_5	**Mean**	N_0	N_1	N_2	E_5	**Mean**
100 x 200	0.15	0.09	0.08	0.02	0.08	0.14	0.09	0.07	0.02	0.08	0.13	0.09	0.07	0.02	0.08
150 x 200	0.19	0.10	0.08	0.03	0.10	0.17	0.11	0.09	0.03	0.10	0.17	0.11	0.09	0.03	0.10
200 x 200	0.20	0.14	0.11	0.03	0.12	0.20	0.13	0.11	0.03	0.12	0.20	0.13	0.11	0.03	0.12
100 x 400	0.21	0.13	0.10	0.04	0.12	0.19	0.12	0.10	0.02	0.11	0.18	0.12	0.10	0.02	0.10
150 x 400	0.25	0.14	0.11	0.03	0.13	0.24	0.14	0.11	0.03	0.13	0.20	0.14	0.12	0.03	0.12
200 x 400	0.28	0.19	0.15	0.03	0.16	0.26	0.18	0.14	0.03	0.15	0.23	0.18	0.15	0.03	0.15
100 x 600	0.28	0.16	0.13	0.05	0.15	0.26	0.16	0.13	0.04	0.15	0.25	0.16	0.13	0.05	0.15
150 x 600	0.32	0.15	0.11	0.04	0.15	0.31	0.16	0.12	0.06	0.16	0.30	0.16	0.12	0.06	0.16
200 x 600	0.40	0.24	0.19	0.04	0.22	0.40	0.25	0.20	0.08	0.23	0.38	0.24	0.19	0.08	0.22

Table 3.ANOVA for comparing the estimated mean diversity indices in 27 sampling methods

Indices	**Source of variation**	**df**	**Sum of squares**	**Mean square**	**F**	**Sig.**
N_0	Net	8	2.180	0.272	0.293	0.965^{ns}
	Size	2	0.783	0.392	0.421	0.659^{ns}
	Net x Size	16	0.078	0.005	0.005	1^{ns}
N_1	Net	8	0.554	0.069	0.117	0.998^{ns}
	Size	2	0.444	0.222	0.374	0.689^{ns}
	Net x Size	16	0.007	0	0.001	1^{ns}
N_2	Net	8	0.310	0.039	0.108	0.999
	Size	2	0.305	0.153	0.424	0.656
	Net x Size	16	0.007	0	0.001	1
E_5	Net	8	0.087	0.011	0.624	0.754^{ns}
	Size	2	0.111	0.056	3.190	0.049^{*}
	Net x Size	16	0.072	0.004	0.258	0.998

Note: ns: not significantly different*: significantly different

Table 5. $E\%^2$ x T criterion for comparing different modes of samplingfor the estimation of woody species diversity

Parameters Plot size (m^2) Network (m)	N	The time taken for each plot(minute) 1000 t	1500 t	2000 t	T=Nxt (Minute) 1000	1500	2000	$E\%^2$xT 1000	1500	2000
100 x 200	90	25	36	47	2250	3240	4230	$(7.95)^2$ x 2250 = 142206	$(7)^2$ x 3240 = 158760	$(6.95)^2$ x 4230 = 204319
150 x 200	60	25	36	47	1500	2160	2820	$(8.85)^2$ x 1500 = 117484	$(8.65)^2$ x 2160 = 161617	$(8.32)^2$ x 2820 = 195207
200 x 200	50	25	36	47	1250	1800	2350	$(10.67)^2$ x 1250 = 142311	$(10.37)^2$ x 1800 = 193566	$(10.25)^2$ x 2350 = 246897
100 x 400	45	25	36	47	1125	1620	2115	$(11.57)^2$ x 1125 = 150598	$(9.5)^2$ x 1620 = 146205	$(8.67)^2$ x 2115 = 158982
150 x 400	30	25	36	47	750	1080	1410	$(11.75)^2$ x 750 = 103547	$(11.12)^2$ x 1080 = 133547	$(10.45)^2$ x 1410 = 153975
200 x 400	25	25	36	47	625	900	1175	$(14)^2$ x 625 = 122500	$(12.25)^2$ x 900 = 135056	$(11.87)^2$ x 1175 = 165554
100 x 600	36	25	36	47	900	1296	1692	$(14.75)^2$ x 900 = 195806	$(13.25)^2$ x 1296 = 227529	$(12.75)^2$ x 1692 = 275056
150 x 600	24	25	36	47	600	864	1128	$(13.25)^2$ x 600 = 105337	$(14.25)^2$ x 864 = 175446	$(13.75)^2$ x 1128 = 213262
200 x 600	20	25	36	47	500	720	940	$(20)^2$ x 500 = 200000	$(21.25)^2$ x 720 = 325125	$(19.75)^2$ x 940 = 366659

Note: ta_i: The average needed time for measuring the trees of every plot in i method; $T_i = n_i \times ta_i$; $E\% = \pm t x S_{\bar{x}}\%$; $E\%^2$ x T: criterion for comparing different modes of sampling

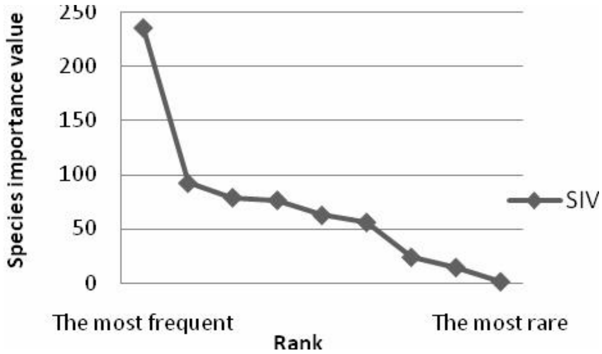

Figure 2. Rank-abundance curve

Table 4. Duncan test for 3 plot sizes (for evenness index)

Size	N	Subset	
		1	2
1000 m^2	27	0.56	-
1500 m^2	27	0.62	0.62
2000 m^2	27	-	0.64

Discussion

The first Part of species abundance distribution curve indicates that the large areas of the Zagros forests was dominated by *Q. brantii* that are the main species of this area forests which cover around 72% of total canopy of trees and shrubs. Destructive human pressure on nature has led to poor soil condition and loss of woody species diversity in forests. Zagros forests in west of Iran are severely degraded and consequently their basic structure and complexity in identifying forest communities have been changed. This issue leads to poor soil condition and loss of woody species diversity in forests (Khanlari 2006). The second part of the curve has a low slope that shows diversity and higher evenness. These areas include valleys and stone walls that are less subject to degradation. The third part of the curve shows that there are three rare species in the habitats. This species due to environmental features have been deployed in specific areas of habitat.

Results of hundred percent inventory and samplings in the forest showed that the accuracy of sampling with 1000 m^2 plot size is higher than sampling with 2000 m^2 plot size. Variance analysis ofN_0 index showed that there is no significant difference between variable sampling grids and areas of plot.

Species abundance distribution curve shows that 5woody species gather in more suitable areas which may be the result of group behavior, heterogeneous environment, restoring methods (Pourbabaei 2010). The forests with this distribution pattern of tree species have lower sampling accuracy and higher costs. In such cases, increase the number of plots (reducing the grid dimension) is effective in increasing the accuracy of the sampling. The number of plot for sampling depends on the homogeneity and heterogeneity of under studied stands (Zobeiry 2002).

He et al. (1996) studied 50 hectares of rain forests of Malaysia and investigated different areas of sampling for estimation Shannon diversity index and illustration of species-area curve. They found that sampling design is effective on the estimation and illustration of diversity curves especially species-area. Wang et al. (2008) studied species diversity models in a part of moderate-temperature forests in China. They estimated Shannon diversity index, species richness and species abundance using geostatistics and the diversity curves. They concluded that sampling design is effective on studying the diversity pattern of study area.

In sampling the transparency of purpose and using the results of such purpose is a key rule (Zobeiry 2002).So the sampling method should be selected based on the target of sampling, forest structure and available tools. If our aim is the comparison of plant diversity in two different areas, diversity estimation will suffice with equal sampling methods and don't need to more accurate sampling methods and spending higher costs. Etemad et al. (2014) used sampling plots at the dimensions of 10 m x 10 m, 15 m x 15 m, 20 m x 20 m, and 40 m x 40 m for estimation of trees diversity in northern Zagros forests. They compared the diversity indices with hundred percent inventory data using $E\%^2$x T criterion, and concluded that square methods with dimension of 40 m x 40 m, and 20 m x 20 m were the best sizes for determining the density and canopy cover diversity of trees. Saber (1993) used the grid dimensions of150 mx400 m and circular sample plots with an area of 1000m^2 for estimating the canopy trees of Zagros Forest.

In conclusion, according to the Importance-value curve, accuracy and $E\%^2$xT criterion, the 150 m x 400 m grid size and sampling plot size with an area of 1000 m^2 was proposed as the most appropriate one to estimate woody species diversity in Iranian Zagros Forests.

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REFERENCES

Adam JH, Mahmud AM, Muslim NE. 2007. Cluster analysis on floristic and forest structure of hilly lowland forest in Lak Kawi, Sabah of Malaysia. Intl J Bot 3 (4): 351-358.

Aguirre O, Hui G, Gadow KV, Jimenez J. 2003. An analysis of spatial forest structure using neighbourhood-based variables.For Ecol Manag 183: 137-145.

Alatalo R. V. 1981. Problems in themeasurement of evenness in ecology. Oikos 37: 199-204.

Alijanpour A, Eshaghi Rad J, Banej Shafiei A. 2009. Comparison of woody plants diversity in protected and non-protected areas of Arasbaran forests. Iranian J For Poplar Res 17 (1): 125-133.

Amidi A. 2006. Sampling Theory and Applications. University Publication Center. Tehran University, Tehran.

Buongiorno J, Dahir S, Lu HC, Lin CR. 1994. Tree size diversity and economic returns in uneven-aged forest stands. For Sci 40: 83-103.

Condit R, Ashton P, Bunyavejchewin et al. 2006. The importance of demographic niches to tree diversity. Science 313: 98-101.

De Oliveira AA, Mori SA. 1999. A central Amazonian terra firme forest. I. High tree species richness on poor soils. Biodiv Conserv 8: 1219-1244.

Etemad S, Zobeiry M, Namiranian M, Ghahramany L. 2014. Determine the most appropriate surface sampling method for estimating spiral tree diversity in the forests of northern Zagros. Proceedings of the Second National Conference on Science Student Forests. Tehran University, Karaj, 7-8 May 2014.

Hasenauer H. 2006. Sustainable Forest Management. Growth Models for Europe. Springer, Berlin.

He F, Legendre P, LaFrankie JV. 1996. Spatial pattern of diversity in a tropical rain forest in Malaysia. J Biogeogr 23: 57-74.

Heidari RH, Zobeiri M, Namiranian M, Sobhani H. 2007. Application of T-square sampling method in Zagros forests. Iranian J For Poplar Res 15 (1): 32-42.

Hill MO. 1973. Diversity and evenness. A unifying notation and its consequences. Ecology 54: 427-432.

Hubbell SP, Foster RB, O'Brien ST et al. 1999. Light-gap disturbance, recruitment limitation, and tree diversity in a Neotropical forest. Science 283: 68-71.

Kant S. 2002. The marginal cost structural diversity of mixed uneven-aged hard maple forests. Canadian J For Res 32: 616-628.

Khanlari D. 2006.Iranian Forestry and Silvicultural. Published by Application of Agricultural Sciences, Tehran.

Latham RE, Ricklefs RE. 1993. Continental comparisons of temperate-zone tree species diversity. In: Ricklefs RE, Schluter D. (eds). Species Diversity in Ecological Communities. University of Chicago Press, Chicago.

Legendre P, Fortin MJ. 1989. Spatial pattern and ecological analysis. Vegetation 80: 107-138.

Legendre P. 1993. Spatial autocorrelation: Trouble or new paradigm? Ecology 74: 1659-1673.

Lund HG, Dallmeier F, Alonso A. 2004.Biodiversity in Forest. Elsevier, Amsterdam.

Macneely JA. 2002. Forest biodiversity at the ecosystem level: where do people fit in? Unasylva J 53 (209): 10-15.

Maingi JK, Marsh SE. 2006. Composition, structure, and regeneration patterns in a gallery forest along the Tana River near Bura, Kenya. For Ecol Manag 236: 211-228.

Neldner VJ, Butler DW. 2008. Is 500m^2 an effective plot size to sample floristic diversity for Queensland's vegetation. Cunninghamia10 (4): 513-519.

Nilsson M. 2002. Deriving nationwide estimates of forest variables for Sweden using Land sat ETM + and field data. University of Agricultural Science, Umea, Sweden.

Pommerening A, Stoyan D. 2006. Edge-correction needs in estimating indices of spatial forest structure. Canadian J For Res 36: 1723-1739.

Pommerening A, Stoyan D. 2008. Reconstructing spatial tree point patterns from nearest neighbour summary statistics measured in small sub windows. Canadian J For Res 38: 1110-1122.

Pourbabaei H. 2010. Statistical Ecology (translation). Guilan University, Someahsara, Iran. [Persian]

Saber NM. 1993. Measurement of appropriate characteristics in inventory of protective forests of Zagros South-Fars Province. [M.Sc. Thesis].Tehran University, Tehran. [Persian].

Wang X, Hao Z, Ye J, Zhang J, Li B, Yao X. 2008.Spatial pattern of diversity in an old-growth temperate forest in Northeastern China. Acta Oecologica 33: 345-354.

Wright SJ. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. Oecologia 130: 1-14.

Zobeiry M. 2002. Forest Biometry. Tehran University, Tehran.

Zohrevandi AA. 2012. Site demands of Manna Oak (*Quercus brantti*) trees in Kermanshah Province. J Nat Ecosyst Iran 2: 52-62.

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Short communication: Algal leaf spot associated with *Cephaleuros virescens* (Trentepohliales, Ulvophyceae) on *Nephelium lappaceum* in Thailand

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Abstract. *Sunpapao A, Pitaloka MK, Arikit S. 2015. Algal leaf spot associated with* Cephaleuros virescens *(Trentepohliales, Ulvophyceae) on* Nephelium lappaceum *in Thailand. Biodiversitas 17: 31-35.* Algal leaf spot disease of *Nephelium lappaceum* (rambutan) was observed in southern Thailand. The algae were isolated on Bold's basal medium (BBM) and identified based on appearance of the lesions, algal morphology and molecular properties. Characteristics of the filamentous thallus cells, sporangiophores, sporangia, gametes and zoospores were clarified. A portion of the 18S small subunit rRNA was amplified to validate the morphological identification by sequence similarity. To summarize the main results, the plant parasite causing algal leaf spot was identified as *Cephaleuros virescens*, and in sequencing-based phylogenetic analysis the *Cephaleuros* PSU-R5.1 isolate from rambutan grouped with the algae in genus *Cephaleuros*. This confirms *C. virescens* as a causal organism of algal leaf spot disease on rambutan in southern Thailand.

Key words: Green algae, leaf spot, morphology, *Nephelium lappaceum*, rRNA

INTRODUCTION

Rambutan (*Nephelium lappaceum* Linn.) is a large-sized, evergreen tree belonging to the family Sapindaceae. It produces numerous fruits with a protuberant hairy surface. Rambutan is mostly cultivated in Southeast Asia and in other tropical areas. In Thailand, most rambutan cultivation is in the southern province of Surat Thani. Algal leaf spot disease is among the minor diseases commonly found in rambutan trees, exacerbated by the moist environment and long periods of high rainfall. Rambutan is subject to attack by several plant-parasitic organisms, some of which can be severe. This algal disease can damage plant leaves, fruits and stems, and cause economic loss. Rambutan fruits are well known to possess several health benefiting components. The most common algal parasites of woody plants belong to the genus *Cephaleuros*.

Cephaleuros species are filamentous green algae widely known as a parasite of higher plants. The genus belongs to the division of aquatic green algae (Chlorophyta), class Ulvophyceae, order Trentepohliales and family Trentepohliaceae (Guiry and Guiry 2015). They are aerial and need free water to germinate (Suto and Ohtani 2009). The genus *Cephaleuros* has been known to cause diseases with morphological characteristics similar to those caused by fungi. The disease caused by this alga manifests as bright orange spots on leaves and stems, similar to rust fungi (Mann and Hutchinson 1907). Algae in this genus are widespread in tropical and subtropical areas causing damage on leaves, young stems and fruits of several host plants (Alfieri 1969). *Cephaleuros* has a relatively simple structure, but its classification based on morphology is complex (López-Bautista et al. 2006).

Though *Cephaleuros* species occur on the leaves of numerous economical plants (Brooks, 2004), *C. virescens* is the most frequently reported. The disease caused by this genus has been recorded in Hawaii (Rindi et al. 2005), Japan (Suto and Ohtani 2009), Florida (Marlatt and Campbell 1980; Marlatt and Alfieri 1981), America (Brooks 2003), Africa (Rindi et al. 2006) and Panama (Rindi et al. 2008). In Thailand, *Cephaleuros solutus* was the first to be reported as a causal agent of algal leaf spot disease (Pitaloka et al. 2014). Based on morphology and identification of its 18S small subunit rRNA, the algal leaf spot disease on Para rubber (*Hevea brasiliensis*) was attributed to *C. virescens* (Pitaloka et al. 2015), and on acacia (*Acacia auriculiformis*) it was attributed to *C. diffusus* (Sunpapao and Pitaloka 2015). Algal leaf spot is considered a minor disease in Thailand, so studies on the biology, ecology, distribution, taxonomy and pathogenicity of *Cephaleuros* are rare. Therefore, the aim of this research was to isolate and identify the species of *Cephaleuros* on rambutan based on its morphology and molecular analysis.

MATERIALS AND METHODS

Morphological characters

Thirty algal leaf spot samples (*n*=30) of rambutan were collected on August, 2014 from the Pest Management field, Faculty of Natural Resources, Prince of Songkla University, Thailand. Algal thalli were removed from fresh leaves and preliminary observed under stereomicroscope. The shape, size, color and growth of algal thalli and their lesions were described and photographed. Taxonomic characters, including gametangia, gametes, zoosporangia, zoospores and setae were evaluated using the dichotomous key of Thompson and Wujek (1997). Algal specimens were deposited in the culture collection of the Pest Management Department, at the Prince of Songkla University, Thailand.

Algal culture, isolation and DNA extraction

Algal cultures were obtained based on the methods of Suto and Ohtani (2011). Leaves with fresh thalli were washed under running water for one hour, wiped with sterile cotton wool, dipped in 70% ethanol and rinsed with sterile distilled water. After that, small thalli (2-3 mm) were removed with a sterile razor blade and placed on Bold's basal medium (BBM; Bischoff and Bold 1963; Andersen 2005), and incubated at 20°C in a light: dark cycle of 12:12 hours, for two to six months. Algal colonies were then scraped from the BBM medium with a glass slide and put into a microcentrifuge tube. DNA was extracted using the cetyl trimethyl ammonium bromide (CTAB) method following a prior report (Kollar et al. 1990).

Amplification of 18S nuclear small subunit rRNA by PCR

The extracted DNA was amplified with PCR using universal primers for a conserved sequence of 18S nuclear small subunit rRNA. The primer pairs were: PNS1-forward (5' CCAAGCTTGAATTCGTAGTCATATGCTTGTC 3') (Hibbett 1996) and NS41-reverse (5' CCCGTGTTGAGTCAAATTA 3'). The PCR used a final 50-µl reaction volume containing 10 pmol of each primer, 2x DreamTaq Green PCR Master Mix (Thermo Scientific), and 50 ng of template DNA. An initial denaturation step for 3 min at 95°C was followed by 35 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 50°C, and extension for 1 min at 72°C, with a final extension step of 10 min at 72°C. The PCR products were visualized by agarose gel electrophoresis. The 1 kb GeneRuler DNA ladder (Thermo Scientific) was used as DNA marker.

DNA sequencing and phylogenetic analyses

The PCR products of the 18S nuclear small subunit rRNA gene region were bidirectionally sequenced at the Scientific Equipment Center, Prince of Songkla University by automated DNA sequencing with ABI Prism 377 (Applied Biosystems, USA), using the same primers as in the PCR reaction. Phylogenetic analysis of the 18S rRNA sequences from the *Cephaleuros* samples included known *Cephaleuros* sequences from the GenBank database for comparison. The phylogenetic and molecular evolutionary analyses were conducted using CLUSTAL W and the software package MEGA6.

RESULTS AND DISCUSSION

Cephaleuros virescens Kunze ex. E. M. Fries

Synonyms: *Mycoidea parasitica* Cunningham, *Phylactidium tropicum* Möbius, *Cephaleuros albidus* Karsten, *Cephaleuros laevis* Karsten, *Cephaleuros mycoidea* Karsten, *Cephaleuros candelabrum* Schmidle, *Cephaleuros pulvinatus* Schmidle, *Chrooderma endophytica* F.E. Fritsch, *Phycopeltis hawaiiensis* J.W. King, and *Cephaleuros virescens* f. sessilis Islam (Guiry and Guiry 2015).

Host: *Nephelium lappaceum* Linn.

Fresh thalli or lesions appeared separately or in small groups on the leaves of rambutan in late August 2014 when rain was frequent and the weather was warm. The symptoms were orange to brown small circular scurf on the leaves, approximately 1-4 mm in diameter (Figure 1.A-1.B). Transverse sectioning demonstrated that the thalli were subcuticular with subepidermal growth through leaf tissue, and caused necrosis of the epidermal cells (Fig 1.E). Filamentous cells of the algae were long and cylindrical, 95.5-147.5 (mean, 85.5) µm long × 17.5-37.5 (24.5) µm wide, with length/width (L/W) ratio of 1: 3-7 (Figure 1.D). Setae were short filaments of two to five cells, 17.5-50 (27.5) µm long × 2.5-7.5 (5.5) µm wide.

Sporangiophores developed from thalli on the upper leaf surface. They were cylindrical, three to five cells, erect, solitary, 252-430 (320.5) µm long × 10-20 (17) µm wide (Figure 1.C). Head cells developed terminally and produced four sporangia, each on a sporangiate lateral (Figure 1.C). Sporangia were elliptical, 20-30 (25.5) µm long × 15-22.5 (18.5) µm wide (Figure 1.C). Gametangia were spherical to elliptical, 37.5-50 (45) µm long × 30-37.5 (32.5) µm wide and produced beneath the cuticle (Figure 1.D). Gametes were spheroidal, 7-10 (5.5) µm long × 5-7 (6) µm wide with flagella 13-17 µm long. Zoospores were elliptical, 10-13 (11.5) µm long × 5-7 (6.5) µm wide with flagella 16-22 µm long. Based on these results and using the monograph of Thompson and Wujek (1997), the thirty samples of the algae causing leaf spot disease on rambutan were identified as *Cephaleuros virescens*. We also compared the distinguishing characteristics of *C. virescens* and *C. diffusus* (Table 1).

Fresh thalli cultured on BBM grew slowly, producing 3-to 5-mm diameter greenish colonies of tufted filaments in two to six months. To confirm the morphological identification, one isolate (PSU-R5.1) was selected for molecular analysis. PCR was conducted to amplify a portion of 18S rRNA using the PNS1 and NS41 primer pair. The nucleotide sequence analysis of the *Cephaleuros* 18S rRNA using BLAST search revealed that our partial sequence was 1,052 bases long. This nucleotide sequence was compared to known *Cephaleuros* spp. and other algal genera in the NCBI (the National Center for Biotechnology Information) databases and deposited in GenBank with accession number (AB971690). A 98% sequence identity

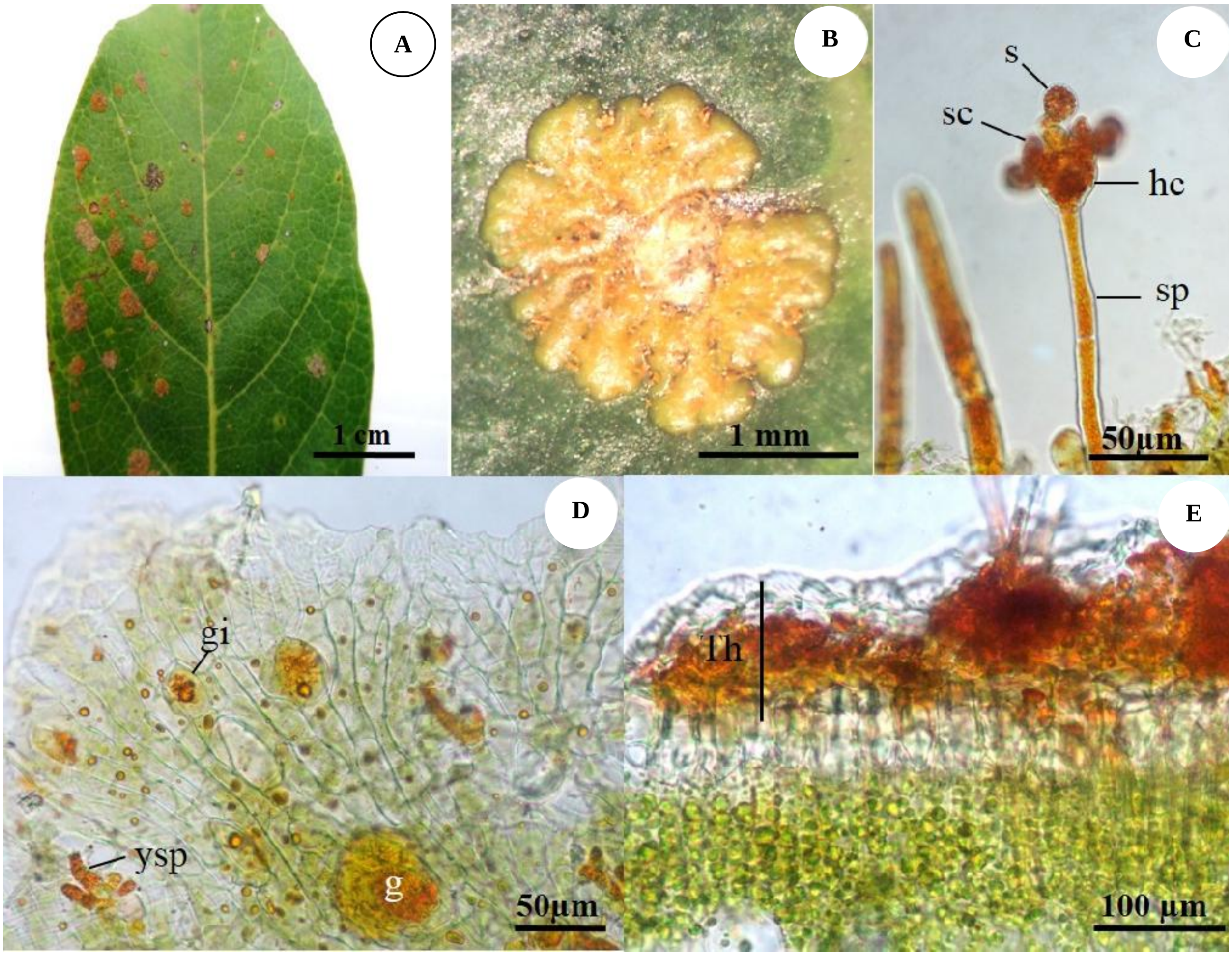

Figure 1. A. Leaf spot lesions caused by *Cephaleuros virescens* on the upper leaf surface of rambutan, B. entire, lobed thallus, C. erect sporangiophores of *C. virescens*, each with four sporangia laterals consisting of a suffultory cell and attached sporangium, D. entire closed-ramulate thallus with young sporangiophore (ysp), gametangia initial (gi) and gametangia (g), E. transverse section of a rambutan leaf showing the thallus (Th) of *C. virescens* and associated necrotic tissue

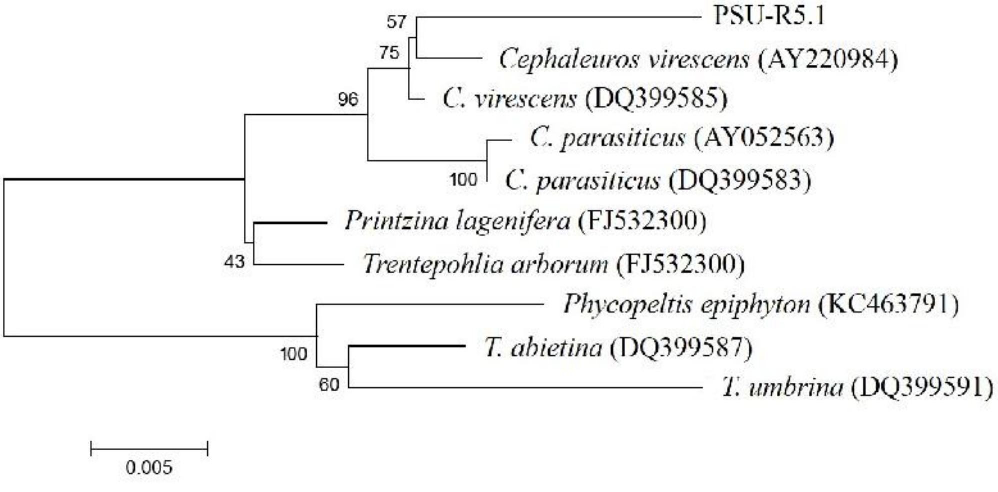

Figure 2. Phylogenetic tree based on 18S rRNA sequences of *Cephaleuros* (PSU-R5.1) shows closely related *Cephaleuros* cases with less similarity to other cases. The tree was constructed with the neighbor-joining method, and the GenBank accession numbers are presented in parentheses

Table 1. Comparison of phenotypic characteristics between *Cephaleuros virescens* (PSU-R5.1) and *C. diffusus*

Characters		***Cephaleuros virescens* (PSU-R5.1)**	***Cephaleuros diffusus* (Sunpapao & Pitaloka 2015)**
Host		*Nephelium lappaceum*	*Acacia auriculiformis*
Thalli	Shape	Raised spot, circular disk	Raised spot
	Diameter (mm)	1-4	1-5
	Growth habit	Pseudoparenchymatous	Open filamentous
Filamentous cells	Shape	Long cylindrical	Short cylindrical to irregular
	Length × wide (μm)	95.5-147.5 (85.5) × 17.5-37.5 (24.5)	7.5-42.5 (30.5) × 5-17.5 (12.5)
	L/W ratio	3-7	1-6
	Branching manner	Equal dichotomous	Dichotomous
Setae	Shape	Cylindrical filament	Cylindrical filament
	Length × wide (μm)	17.5-50 (27.5) × 2.5-7.5 (5.5)	16.5-280 (150) × 5-7.5 (5.5)
Gametangia	Shape	Elliptical	Spherical
	Length × wide (μm)	37.5-50 (45) × 30-37.5 (32.5)	12.5-32.5 (17.5) × 12.5-22.5 (20.5)
Gametes	Shape	Spheroidal	Obconic to spherical
	Length × wide (μm)	7-10 (5.5) × 5-7 (6)	5-10 (7.5) × 5-7.5 (6)
Sporangiophores	Length × wide (μm)	252-430 (320.5) × 10-20 (17)	250-440 (350.5) × 10-12.5 (10.5)
	Head cell placement	Terminal	Terminal
Sporangia	Shape	Elliptical	Spherical to ellipsoid
	Length × wide (μm)	20-30 (25.5) × 15-22.5 (18.5)	12.5-27.5 (15.5) ×10-20 (12.5)
Zoospores	Shape	Elliptical	Obovoid
	Length × wide (μm)	10-13 (11.5) × 5-7 (6.5)	10-16.25 (13.5) × 5-8.75 (7.5)
Lesions	Discoloration	Absent	Absent

confirmed the isolate as a member of the *Cephaleuros* genus. A phylogenetic analysis of sequences imported from GenBank indicated that Thai isolate PSU-R5.1 was closely related to the genus *Cephaleuros*, but well separated from the other species (Figure 2).

In this study, the organism isolated from rambutan leaf spot was characterized based on its morphological characteristics and molecular properties. Algae in genus *Cephaleuros* have reportedly caused diseases in numerous plant species in various habitats (Jourbert and Rijikenberge 1971; Marlatt and Alfieri 1981; Chapman and Good 1983; Holcomb 1986; Thompson and Wujek 1997; Suto and Ohtani 2009; Pitaloka et al. 2015; Sunpapao and Pitaloka 2015). The algae were consistently found growing beneath the host cuticle. The tissue beneath thalli was necrotic, and no necrotic spots were observed without thalli. Cross-sections through thalli suggested the plant tissue necrosis was spreading from the thalli into healthy tissues. However, the lesions were rather small and caused only minor damage to *Nephelium lappaceum*. Even small algal spots can cause a yield reduction if there are enough of them to reduce photosynthesis or trigger premature leaf senescence.

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REFERENCES

Alfieri SA. 1969. The green scurf disease caused by *Cephaleuros virescens* Kunze. Plant Pathol Circ 78: 1-2.

Andersen RA (ed). 2005. Algal Culturing Techniques. Elsevier Academic Press, Amsterdam.

Bischoff HW, Bold HC. 1963. Phycological studies. IV. Some Soil Algae from Enhanted Rock and Related Algal Species. University of Texas Publication, Dallas.

Brooks F. 2004. Plant-parasitic algae (Chlorophyta: Trentepohliales) in America Samoa. Pacific Sci 58 (3): 419-428.

Chapman RL, Good BH. 1983. Subaerial symbiotic green algae. Interaction with vascular plant hosts. In: Goff LJ. (ed.) Algal Symbiosis: A Continuum of Interaction Strategies. Cambridge University Press, Cambridge.

Guiry MD, Guiry GM. 2015. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org [access: November, 2015]

Hibbet DS. 1996. Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi. Mol Biol Evol 13: 903-917.

Holcomb GE, Van SR, Buckley JB. 1998. First report of *Cephaleuros virescens* in Arkansas and its occurrence on cultivated blackberry in Arkansas and Louisiana. Plant Dis 82: 263.

Holcomb GE. 1986. Hosts of the parasitic alga *Cephaleuros virescens* in Louisiana and new host records for the Continental United States. Plant Dis 70: 1080-1083.

Joubert JJ, Rijkenberg FHJ. 1971. Parasitic green algae. Ann Rev Phytopathol 9: 45-64.

Kollar A, Seemuler E, Bonnet F, Saillard C, Bove JM. 1990. Isolation of the DNA of various plant pathogenic mycoplasma like organisms from infected plants. Phytopathology 80: 233-237.

López-Bautista JM, Fabio R, Guiry MD. 2006. Molecular systematics of the subaerial green algal order Trentepohliales: an assessment based on morphological and molecular data. Intl J Syst Evol Microbiol 56: 1709-1715.

Mann HH, Hutchinson CM. 1907. *Cephaleuros virescens* Kunze: the 'Red rust' of tea. Mem Dept Agric India Bot 1: 1-33.

Marlatt RB, Alfieri SA. 1981. Host of *Cephaleuros*, A parasitic alga in Florida. Proc Fl State Hort Soc 94: 311-317.

Marlatt RB, Campbell CW. 1980. Susceptibility of *Psidium guajava* selections to injury by *Cephaleuros* sp. Plant Dis 64 (11): 1010-1011.
Pitaloka MK, Petcharat V, Arikit S, Sunpapao A. 2015. *Cephaleuros virescens*, the cause of an algal leaf spot on Para rubber in Thailand. Australasian Plant Dis Not 10: 1-4.
Pitaloka MK, Petcharat V, Sunpapao A. 2014. *Cephaleuros solutus* Karsten, as a causal agent of durian (*Durio zibethinus* Murray) algal leaf spot disease in Thailand. Khon Kaen Agric J 42 (Suppl.3): 644-648.
Rindi F, Guiry MD, López-Bautista JM. 2006. New records of Trentepohliales (Ulvophyceae, Chlorophyta) from Africa. Nova Hedwigia 83: 431-449.
Rindi F, Lam DW, López-Bautista JM. 2008. Trentepohliales (Ulvophyceae, Chlorophyta) from Panama. Nova Hedwigia 87: 421-444.
Rindi R, Sherwood AR, Guiry MD. 2005. Taxonomy and distribution of *Trentepohlia* and *Printzina* (Trentepohliales, Chlorophyta) in the Hawaiian Islands. Phycologia 44: 270-284.
Sunpapao A, Pitaloka MK. 2015. A new record of plant parasitic green algae, *Cephaleuros diffusus* (Trentepohliaceae, Chlorophyta) on *Acacia auriculiformis* hosts in Thailand. Biodiversitas 16 (2): 116-120.
Suto Y, Ohtani S. 2009. Morphology and taxonomy of five *Cephaleuros* species (Trentepohliaceae, Chlorophyta) from Japan, including three new species. Phycologia 48 (4): 213-236.
Suto Y, Ohtani S. 2011. Morphological features and chromosome numbers in culture of five *Cephaleuros* species (Trentepohliaceae, Chlorophyta) from Japan. Phycol Res 59: 42-51.
Thompson RH, Wujek DE. 1997. Trentepohliales *Cephaleuros*, Phycopeltis and Stomatochroon, Morphology, Taxonomy and Ecology.1st ed. Enfield Publishing and Distribution, USA.

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Temporal variability in macroinvertebrates diversity patterns and their relation with environmental factors

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Abstract. *Gerami MH, Patimar R, Negarestan H, Jafarian H, Mortazavi MS. 2015. Temporal variability in macroinvertebrates diversity patterns and their relation with environmental factors. Biodiversitas 17: 36-43.* Seasonal changes are the most important factor in temporal variability of macroinvertebrates communities in marine benthic zone. Realizing the pattern of these changes are the key attributes to maintain benthic resources. For this purpose this study aimed to investigate temporal variability of macroinvertebrates diversity patterns in different seasons in Hormozgan province waters, Persian Gulf. Five sites were identified and sampling was carried out randomly at three places with three replicate in each site from autumn 2014 to spring 2015. Physiochemical properties of water were recorded in each sampling site. Total of 19 macroinvertebrate orders were identified form four seasons in all sites. Results showed that Polychaeta were dominant taxa in all seasons and maximum diversity of benthic macroinvertebrates exhibitedin winter. According to evenness index (E1), abundances of species were most balanced in spring. Analysis of weight and density revealed that the species diversity and mean weight of macroinvertebrates had maximum in winter and minimum in summer. Macroinvertebrate community structure was similar in the spring and summer and partly discriminated from remaining two seasons. SIMPER analysis confirmed these dissimilarities and revealed that Foraminifera, Gastropoda and Polychaeta have three major contributions in dissimilarities between seasons. According to BIO-ENV analysis, oxygen and chlorophyll a were the best variables (r = 0.7143) explaining changes in the abundance over time of the benthic fauna under study. On the contrary, eight orders (Amphipoda, Secernentea, Cumacea, Euphausiacea, Gastropoda, Isopoda, Anthozoa and Sagittoidea) did not show any convergence with environmental factors in this study.

Keywords: Macroinvertebrate assemblages, diversity indices, seasonal changes, Persian Gulf

INTRODUCTION

Macrobenthic invertebrates are key components in the functioning of coastal and marine ecosystems and also play a significant role in marine benthic food chain (Lu 2005). In addition, macrofauna of marine sediments play an important role in ecosystem processes such as dispersion and burial, pollutant metabolism, nutrient cycling, and secondary production (Snelgrove 1998). Therefore, they can be employed as ecological indicators to provide synoptic information about the state of ecosystems, and help in developmental planning and decision-making processes (Marques et al. 2009).

The aquatic organisms are exposed to anthropogenic disturbances and natural changes in their habitats, which makes them react in different ways (Saghali et al. 2013; Nouri et al. 2008). Macrobenthic invertebrates'structure is affected by environmental factors such as temperature, pH, dissolved oxygen and pollution (Saghali et al. 2013; Sharma and Rawat 2009). Seasonal changes in these factors cause variation in rate of supply of organic matter and consequently affect the spatial and temporal distribution of marine organisms, such as macrobenthic communities (Bachelet et al. 2000; Erftemeijer and Herman 1994).

Seasonal changes in macrobenthic communities in aquatic environment have been studied by many researchers. Chapman and Brinkhurst (1981) declared that seasonal movements of subtidal benthic invertebrates in the Fraser River estuary were affected by runoff and salt-wedge seasonal changes. These changes were most apparent in the oligochaetes and polychaetes. Morrisey et al. (1992) reported significant temporal variation of soft-sediment benthos ranging in length from days to months in Botany Bay, New South Wales, Australia.Furthermore, Livingston (1987) found variations in the abundance and composition of infauna at scales from weeks to years.

Consideration of the diverse environmental factors that might potentially cause variation in macroinvertebrate abundance over long-short period would suggest that seasonal or monthly fluctuations are common. In addition, revealing the relationship between macroinvertebrates and environmental factors could describe seasonal changes more accurately. As a consequence, any spatial heterogeneity in the macroinvertebrates is likely to be interpreted as an effect of time, not the effect of pollution, for example. This process shows the importance of studying seasonal changes.

The aim of this study is to investigate seasonal variation in macroinvertebrates diversity in a part of Persian Gulf and their relation to environmental factors. The initial step for such a survey is the identification of organisms living in the aquatic ecosystem (Niemi and McDonald 2004). The results have relevance to studies of macroinvertebrates of soft sediments other parts of Persian Gulf (or elsewhere with the same conditions) and also to the sampling of other marine habitats and other variables, such as pollutants.

MATERIALS AND METHODS

Due to soft sediment and high nutrients enrichment, Hormozgan province waters are a favorablehabitat for macroinvertebratesin Persian Gulf (Pourjomeh et al. 2014). Five sampling sites were identified and selected (Figure 1). Stations were chosen to give broad geographic coverage of the most divers habitats of macroinvertebrates in Hormozgan province waters, Iran. Due to patchy distribution of macroinvertebrates, sampling was done randomly at three places with three replicate, in each site and 180 samples were analyzed during the study. Samples were taken by Van veen Grab with 0.0256 m^2 cross section. Sampling was performed seasonally, from autumn 2014 to spring 2015. Physiochemical properties of water were recorded by CTD device in each site. Samples were sieved through 0.5 mm mesh and the remaining was fixed and preserved by 97% ethanol and transferred to laboratory for further analysis. Organism were stained by Rose Bengal and sorted to major taxonomic groups. Macroinvertebrates were identified by illustrated keys such as: Fauchald (1997), Sterrer (1986), Bosch et al. (1995) and Debruyne (2003). Samples were weighted with 0.0001 gr accuracy and wet weight was determined as biomass measurement.

The following biodiversity indices were employed to compare seasons:

Shannon-Wiener Index (Shannon and Weaver 1963): This index is based on the Information Theory. It assumes that individuals are sampled at random, out of an "indefinitely large" community, and that all the species are represented in the sample and can be estimated according to the algorithm:

$$H' = -\sum P_i \log_2 P_i$$

Where P_i is the proportion of individuals belonging to species i in the sample. The index can usually take values between 0 and 5, and maximal values above 5 bits/individual are very rare (Marques et al. 2009). For instance, Molvær et al. (1997) established the following relation between the Shannon-Wiener Index values and the different levels of ecological quality (Table 1), in accordance with that recommended by the Water Framework Directive (WFD, 2000/60/CE).

Table 1. Categories considered as a function of Shannon-Wiener Index values.

Classification	Shannon-Wiener value
High status	>4 bits/individual
Good status	4-3 bits/individual
Moderate status	3-2 bits/ individual
Poor status	2-1 bits/ individual
Bad status	1-0 bits/ individual

Simpson Index (Simpson 1949):

$$\lambda = \sum P_i^2$$

Where P_i is the proportion of individuals from species i in the community. The Simpson Index may vary from 0 to 1, it has no dimensions and, in the same way, higher values correspond to lower diversity.

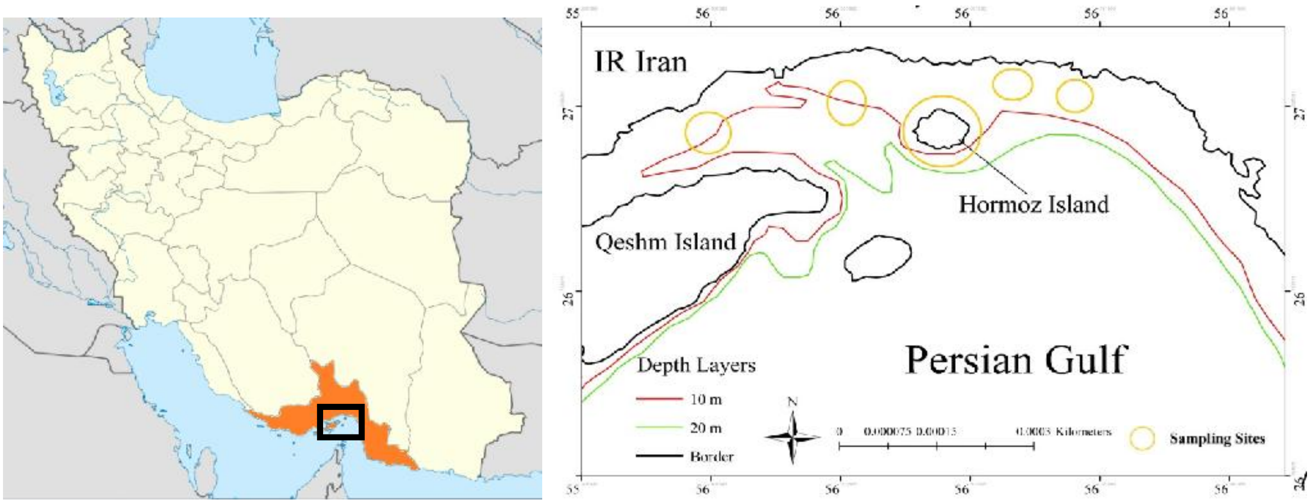

Figure 1. Location of the sampling sites at Hormozgan province waters, Persian Gulf

According to H' the following diversity indices are available (Ludwig and Reynolds 1988; Gerami et al. 2014):

$$N1 = e^{H'}$$
$$N2 = 1/\lambda$$

N1 and N2 show effective numbers of species with common species in population. Lower values shows incompatible conditions for taxa.

Margalef Index (Margalef 1969): The Margalef Index quantifies diversity by relating specific richness to the total number of individuals.

$$M = \frac{(S-1)}{\log_e N}$$

Where S = number of species and N = total number of individuals. According to Margalef (1969) higher level of the index represents better environmental conditions in ecosystem.

Pielou Evenness Index (Pielou 1969): This index is a measure of how evenly distributed abundance is among the species that exist in a community and estimated by following equations:

$$E1 = H'/Ln\,(S)$$
$$E2 = e^{H'}/S$$
$$E3 = e^{H'} - 1/S - 1$$
$$E4 = N2/N1$$
$$E5 = (N2-1)/(N1-1)$$

For E1, E2 and E3 the values may vary from 0 to 1, where 1 represents a community with perfect evenness, and decreases to zero as the relative abundances of the species diverge from evenness. E4 and E5 demonstrate dominance in community. Higher values of E4 and with lower values of E5 show harsh condition due to tendency of dominant species to increase.

One way ANOVA test was performed for assessing significant differences between environmental factors in different seasons. In addition, normality test (Chi-square test) was done before analysis. The non-parametric multidimensional-scaling (nMDS) and analysis of similarities (ANOSIM) were used to examine the spatial patterns of macroinvertebrate assemblages. For parametric analyses the abundance data were square-root transformed to reduce heteroscedasticity before running similarity matrix. Bray-Curtis coefficient was used as the measure of similarity. The level of significance was calculated by means 999 permutations between groups. BIO-ENV analysis was used to find the best subset of environmental variables and community-development pattern. In addition, Canonical correspondence analysis (CCA) was applied to extracts major gradients among combinations of macrobenthic assemblages and environmental variables. Canonical discriminant analysis (CDA) was used to assess differences between seasonal environmental parameters. All the analyses were carried out with R statistical packages (Version 3.13) and vegan package were used to run MDS, ANOSIM, BIO-ENV, CCA and CDA analysis (Oksanen et al. 2015)

RESULTS AND DISCUSSION

Physico-chemical parameters

The mean values of physico-chemical parameters at Hormozgan province waters were represented in Table 2. One way ANOVA analysis showed that there was a significant difference in mean temperature, Oxygen, pH and Chlorophyll a between seasons, while this difference in salinity was not significant (Table 2, Figure 2). Normality test showed that distribution of data was not normal ($p<0.05$).

Diversity indices

Total of 21 macroinvertebrate orders were identified from four seasons in all sites. Results showed that Polychaeta were dominant taxa in all seasons (Figure 3-6). Results of diversity indices showed that maximum diversity of benthic macroinvertebrates was found in winter. In addition, evenness indices (E1) had its maximum in spring (Table 2). Analysis of weight and density reveled that maximum species diversity and species density occurred in winterwhile these amounts were minimum at summer (Figure 7). Canonical analysis of all orders in all seasons showed that winter was separated from all seasons.

ANOSIM analysis revealed that there was a significant difference between benthic community structures (R = 0.125, P = 0.001). However, R value showed that different between environmental data was not strong. nMDS showed that summer and spring were similar in community structure. Moreover, results indicated that winter and autumn were partly discriminated in macrobenthic community structure from other two seasons (Figure 8). In addition, SIMPER analysis confirmed these dissimilarities and revealed that Foraminifera, Gastropoda and Polychaeta have three major contributions in dissimilarities between seasons (Table 3).

Table 2. The mean values (mean ± SE) of physiochemical properties of Hormozgan province waters, Persian Gulf (2014-2015)

Season	Temperature (°C)	Salinity (psu)	Oxygen (ppm)	pH	Chlorophyll a (mg/m^3)
Spring	31.83±1.22^a	37.47±0.13^a	4.04±0.89^a	8.4±0.00^a	0.91±0.26^a
Summer	33.35±0.26^b	37.27±0.06^a	5.17±0.04^b	8.19±0.00^b	0.61±0.04^b
Autumn	27.45±2.33^c	38.17±0.35^a	5.17±0.40^b	8.26±0.00^c	1.45±0.23^c
Winter	22.11±1.83^d	36.14±1.37^a	5.70±0.35^c	8.19±0.00^d	0.952±0.13^d

Note: Different uppercase letters indicating significant difference in columns (ANOVA, P<0.05).

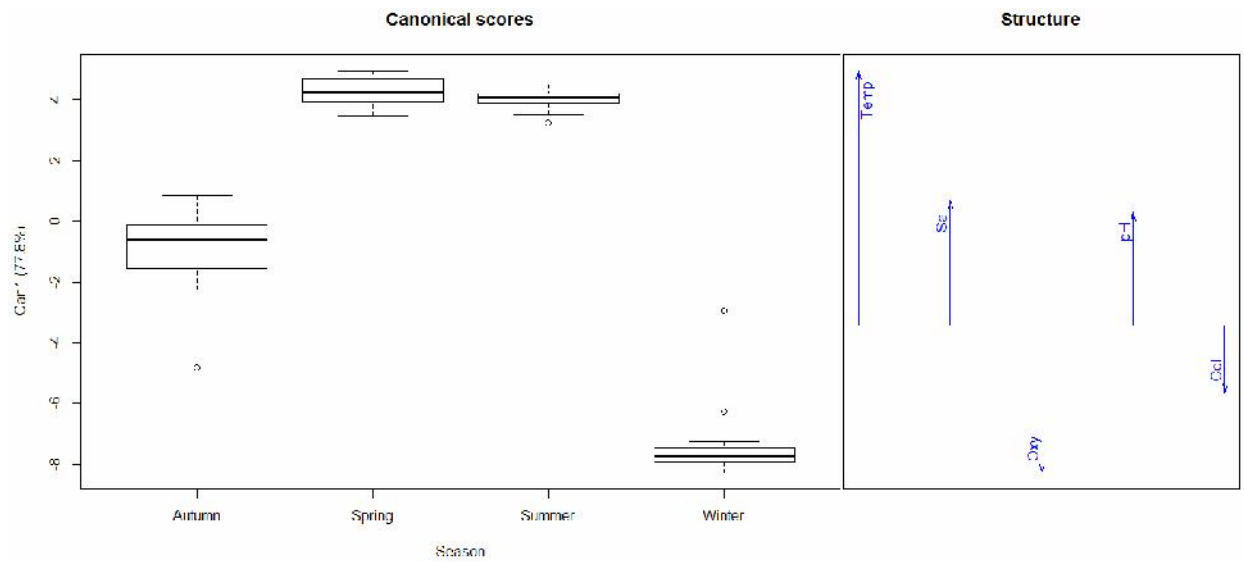

Figure 2. Canonical discriminant analysis plot for physiochemical properties of water in Hormozgan province waters. Note: Sal: Salinity, Coll: Chlorophyll a, Oxy: Oxygen, Temp: Temperature

Table2. Seasonal changes in diversity indices of benthic macroinvertebrates of Hormozgan province waters, Persian Gulf

Season	H'	M	λ	N1	N2	E1	E2	E3	E4	E5
Winter	1.762	3.353	0.234	5.826	5.960	0.598	0.307	0.268	0.733	0.677
Spring	1.713	3.023	0.287	5.542	3.472	0.633	0.369	0.324	0.626	0.549
Summer	1.515	2.736	0.193	4.551	5.161	0.590	0.350	0.295	1.133	1.171
Autumn	1.512	3.537	0.208	3.166	4.795	0.406	0.186	0.135	1.513	1.761

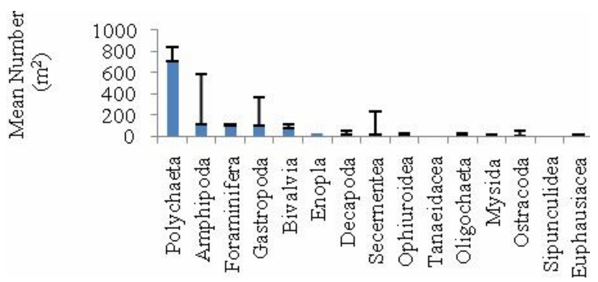

Figure 3. Mean frequency of benthic macroinvertebrates of Hormozgan province waters in autumn

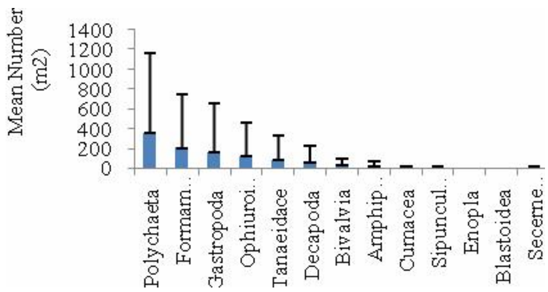

Figure 5. Mean frequency of benthic macroinvertebrates of Hormozgan province waters in autumn summer

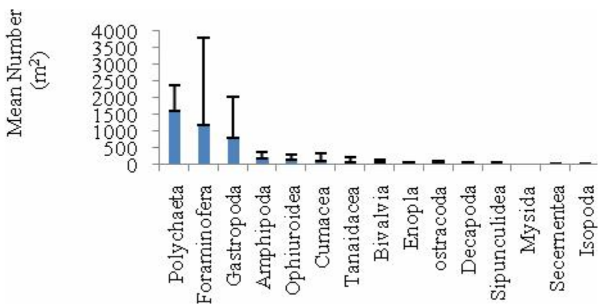

Figure 4. Mean frequency of benthic macroinvertebrates of Hormozgan province waters in autumn spring

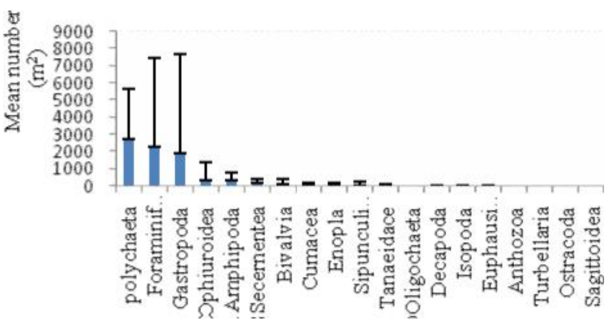

Figure 6. Mean frequency of benthic macroinvertebrates of Hormozgan province waters in winter

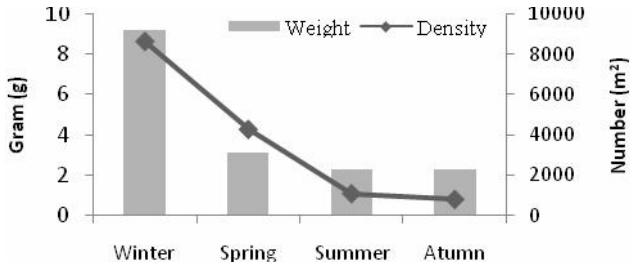

Figure 7. Weight and density of benthic macroinvertebrates of Hormozgan province waters

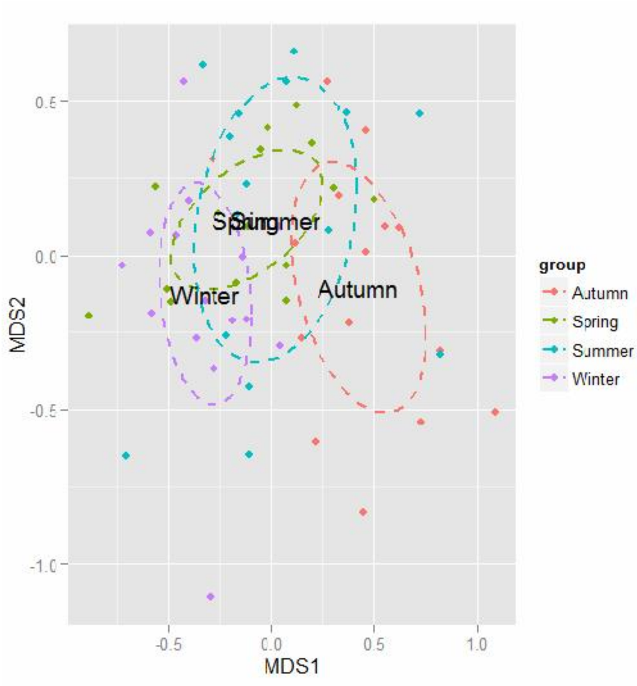

Figure 8. Two-dimensional nMDS ordination plots of the benthic community during different seasons in Persian Gulf. (stress = 0.3)

Table 3. BIO-ENV analysis for various sets of Spearman's correlations between environmental variables and abundances of taxa.

Environmental factors	Size	Correlation
Oxy	1	0.4857
Oxy Col	2	**0.7143**
Sal Oxy Col	3	0.5429
Sal Oxy pH Col	4	0.4286
Temp Sal Oxy pH Col	5	0.0857

Note: * Sal: Salinity, Col: Chlorophyll a, Oxy: Oxygen, Temp: Temperature

According to BIO-ENV analysis, oxygen and chlorophyll a, were the best variables (r = 0.7143) for explaining changes in the abundance over time of the benthic fauna under study (Table 4). However, Amphipoda, Secernentea, Cumacea, Euphausiacea, Gastropda, Isopoda, Anthozoa and Sagittoidea did not show any convergence with environmental factors in this study. Moreover; Blastoidea, Foraminifera, Sipunculidea and Decapoda showed convergence with oxygen and temperature, and Bivalvia, Enopla, Oligochaeta, Ophiurida, Ostracoda, Turbellaria, Polychaeta, Tanaeidace and Mysida showed convergence with pH, chlorophyll a and salinity in CCA analysis (Figure 9).

Table 4. SIMPER analysis of dissimilarity among seasons based on taxa abundance

Taxa	% contribution Winter vs. Autumn	Winter vs. Summer	Winter vs. Spring	Autumn vs. Summer	Autumn vs. Spring	Summer vs. Spring
Amphipoda	6.48	6.01	4.97	7.47	6.92	5.93
Anthozoa	-	-	-	-	-	-
Bivalvia	5.57	4.62	3.96	4.35	4.27	3.90
Sagittoidea	-	-	-	-	-	-
Cumacea	5.13	4.14	3.93		4.51	4.20
Decapoda	2.49	3.21	2.24	5.23	2.24	3.40
Euphausiacea	-	-	-	-	-	-
Foraminifera	20.96	21.30	24.36	**25.05**	**25.57**	**26.55**
Gastropoda	**24.10**	**24.24**	**26.13**	15.35	22.60	22.73
Isopoda	-	-	-	-	-	-
Secernentea	4.65	4.19	4.06	-	-	-
Enopla	3.12		2.72	4.78	3.46	2.95
Oligochaeta	-	-	-	-	-	-
Ophiurida	4.44	6.11	5.86	8.07	5.89	7.59
Ostracoda	-	-	-	-	-	-
Turbellaria	-	-	-	-	-	-
Polychaeta	12.38	12.62	9.17	14.07	10.72	10.31
Sipunculidea	-	-	-	-	-	-
Tanaeidace	2.27	3.93	3.93	5.98	4.05	5.34
Mysida	-	-	-	-	-	-
Blastoidea	-	-	-	-	-	-
Average	61.63	59.03	49.22	57.78	57.34	54.49

Note: Bold numbers shows maximum contribution in columns

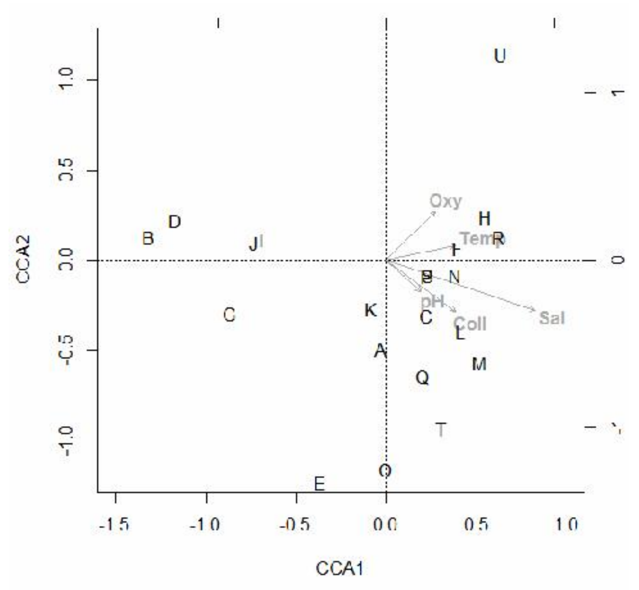

Figure 9. CCA plot of environmental variables and macroinvertebrates (A = Amphipoda, B = Anthozoa, C = Bivalvia, D = Sagittoidea, E = Cumacea, F = Decapoda, G = Euphausiacea, H = Foraminifera, I = Gastropda, J = Isopoda, K = Secernentea, L = Enopla, M = Oligochaeta, N = Ophiurida, O = Ostracoda, P = Turbellaria, Q = Polychaeta, R = Sipunculidea, S = Tanaeidace, T = Mysida, U = Blastoidea, Sal: Salinity, Coll: Chlorophyll a, Oxy: Oxygen, Temp: Temperature)

Discussion

Persian Gulf is an important place in terms of fisheries. Commercial fisheries (especially shrimp fisheries) are preformed every year in fishing seasons (Gerami et al. 2013). Macrobenthos as secondary producers, play an important role in feeding of the fish and shrimp in aquatic ecosystems (Dolbeth et al. 2003). However, due to limited studies on macrobenthic communities of the Persian Gulf, data for comparison of seasonal changes and diversity pattern is scarce.

In the present study, measured physico-chemical parameters were significantly different among seasons. However, summer and spring overlapped in Canonical discriminant analysis (Figure 2). Actually, two climatological regimes have been described for Persian Gulf area: short cold spring and long warm summer (Alijani 1998; Akbari and Masoudian 2009). However, results indicated three seasons in this study (spring-summer, autumn, and winter). This combination of seasons was also observed in nMDS analysis (Figure 8). This analysis showed that macroinvertebrates had similar abundance and pattern in spring and summer while dissimilarities in winter and autumn was higher than in other seasons.

In this study, Polychaeta was the most abundant taxa in all seasons which is not surprising giving the fact that this group is dominant taxa in all brackish aquatic ecosystems such as estuaries or lagoons. Results indicate that the most dominant taxa in terms of abundance were Polychaeta, Foraminifera and Gastropoda, respectively. Other taxa exhibited high abundance fluctuations during seasons and had not high contribution in dissimilarities between seasons (Table 3). Cusson and Bourget (2003) used published data of 15 major marine ecology journals (from 1970 to 1999) and declared that the major taxonomic groups in global patterns of macroinvertebrate inhabiting marine ecosystems were bivalves (36%), polychaetes (20%), amphipods (15%), gastropods (7%) and echinoderms (5%). Foraminifera were not included among dominant taxa in this perspective; although in this study they were abundant in all seasons. There is an apparent conflict in consideration of Foraminifera as a meio- or macro-benthos from the past to the present (Mare 1942; Zobrist and Coull 1992; Gooday et al. 1995; Bett 2014). Bridges et al. (1994) stated that Polychaeta are the most opportunist species with more flexible response to environmental condition than other macroinvertebrates. Chapman and Brinkhurst (1981) declared that Polychaeta comprised over 25% of the total taxa collected and over 60% of the individuals collected in the Fraser River estuary. Furthermore, Salen-Picard and Arlhac (2002) stated that Polychaeta numerically dominated the Calcasieu Estuary macrobenthic communities, and were the dominant species of surface-deposit feeders throughout the estuary. In addition, Ansari et al. (1986), Mohammed (1995) and Kumar (2001) reported Polychaeta, Gastropoda, and Crustacea as dominant taxa in their studies.

The values of H' at different seasons varied from 1.512 to 1.762. According to Table 1 and analyzing the diversity, evenness and dominance indices, macroinvertebrates community had poor health status in all seasons. Many researchers reported that Persian Gulf experienced high risk of various types of environmental degradation and harsh conditions for marine communities due to anthropogenic pressure. Sale et al. (2011) reported that artisanal fisheries, hydrocarbon pollution, wastewater and desalination have substantial negative impacts on marine ecosystems in Persian Gulf and Carpenter et al. (1997) found that Crustacea, Mollusca, and Echinodermata are depauperate in Persian Gulf in comparison with Gulf of Oman.

Temporal variation in benthic community is mostly driven by changes in temperature, which affects salinity, dissolved oxygen and pH of sea water (Amini-Yekta et al. 2013). Grey (1981) stated that seasonal changes of physical and chemical environmental variables are the most probable reason causing temporal patterns of benthic assemblages. According to diversity indices, in comparison with other seasons, winter had better ecological condition in taxa diversity. However, according to dominance and evenness indices, ecological condition was in poor status in winter. Therefore, it could be suggested that species diversity was higher in winter, but opportunist species dominated in the community of macroinvertebrates. Table 2 shows higher amounts of oxygen, lower temperature and high amounts of chlorophyll ain winter. Better physic-chemical conditions of water for macroinvertebrates in winter were reported by others in Persian Gulf (Tabatabaie et al. 2009; Yekta et al. 2013; Allesi et al. 1999; Johns et al. 2003; Nabavi et al. 2011). In addition, Discriminant analysis distinguished winter macrobenthic assemblage from other seasons and showed no significant differences between spring and autumn assemblages (Figure 2, Table 2). Values of diversity indices decreased in spring but the evenness indices improved from winter. It was suggested that due to continuing suitable condition from winter to spring, non-opportunist species reproducedgradually and increased the evenness of community. Values of diversity and evenness indices decreased in summer, while dominant index was in appropriate condition. Table 2 indicates that shortage of chlorophyll a and oxygen, together with high values of pH caused harsh living conditions for macroinvertebrates in summer. In autumn, by changes in physicochemical properties of water; diversity and evenness indices improved in macroinvertebrates community.

Figure 7 shows seasonal changes in biomass (weight and density) of macroinvertebrates in Homrozgan province waters. Results revealed that maximum biomass of macroinvertebrates in this region occur in winter, while minimum was found in summer. It is thus hypothesized that winter have better condition for macroinvertebrates growth in Hormozgan province waters or their species spawning and recruitment may occur in late autumn or early winter. Gaughan and Potter (1995) declared that high values of species richness and density of zooplankton in Wilson Inlet was found in summer because of recruitment and spawning season. This finding was also confirmed for polychaete *Ceratonereis aequisetis* in Broke Inlet in south-eastern Australian estuaries (Glasby 1986). However, high seasonal differences in taxa richness, density and diversity that were detected in this study must partly reflect the fact that some of the taxa spawn seasonally and cannot tolerate a harsh conditions or seasonal changes in environmental factors such as temperature, salinity, oxygen or chlorophyll a. In addition, changes between weight and density of macroinvertebrates are due to differences in sizes.Such as, high values of density with decrease in weight represent small sizes of macroinvertebrates.

BIO-ENV analysis revealed that macroinvertebrates community had best correlation with oxygen and chlorophyll a (Table 4). Therefore, seasonal variation between sites could be explained by these factors. It is well known that oxygen availability is a major factor influencing the composition of macrobenthic communities. Furthermore, dissolved oxygen levels depend primarily on the relative magnitudes of photosynthetic oxygen production and total plankton respiration (Steel 1980). Plankton is the main source of chlorophyll a in marine ecosystems; therefore, oxygen-plankton relationship could be described as oxygen-chlorophyll relationship. However, Kunlasak et al. (2013) studied the relationships of dissolved oxygen with chlorophyll a and concluded that the relationships between chlorophyll a, and dissolved oxygen is complex, being affected by season, nutrient inputs and elevation. In addition, Kuhnt et al. (2013) suggested that the pore density of some benthic foraminiferal species is controlled by bottom-water oxygen content. However, they declared that this relation is species-specific. In this respect, CCA analysis revealed that there was not specific relationship between all the taxa and environmental factors in this study (Figure 9). Amphipoda, Secernentea, Cumacea, Isopoda, Anthozoa and Sagittoidea did not show any relationship with environmental factors. This phenomenon was more important for Gastropoda as this taxon had high contribution in between-season dissimilarity (Table 3). Euphausiacea showed negative correlation with oxygen and temperature and Gastropoda showed strong negative relation to salinity and little weaker, but still quite strong, negative respond to pH values and amount of chlorophyll a.

In conclusion, this study showed that macroinvertebrates in Persian Gulf have strong temporal variation among seasons. This variation was affected by changes in physico-chemical properties of water especially oxygen and chlorophyll a. In addition, diversity and evenness indices revealed that macroinvertebrates ecological conditions is poor in this region and needs to be improved. These finding could be useful in furthers studies for habitat management and monitoring programs.

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REFERENCES

Akbari T, Masoudian SA. 2009. Regionalization of temperature regions of Iran. Geog Environ Plan Sea J 33 (1): 59-74. [Persian]

Alijani B. 1998. Natural seasons in Iran. J Geog Res 35: 21-33 [Persian, abstract available in English].

Allesi CA, Hunt HD, Bower SA. 1999. Hydrographic data from the U.S. naval oceanographic office: Persian Gulf, southern Red Sea, and Arabian Sea 1923-1996 [Technical Report]. Woods Hole Oceanographic Institution, Washington DC.

Amini-Yekta F, Kiabi B, AshjaArdalan A, Shokri M. 2013. Temporal Variation in Rocky Intertidal Gastropods of the Qeshm Island in the Persian Gulf. J Persian Gulf 4 (13): 9-18.

Ansari ZA, Ingole BS, Banerjee G, Parulekar AH. 1986. Spatial and temporal changes in benthic macrofauna from Mandovi and Zuari estuaries of Marmugoa harbor, Goa (central west coast of India). Ind J Mar Sci 23 (4): 225-231.

Bachelet G, De Montaudouin X, Auby I, Labourg PJ. 2000. Seasonal changes in macrophyte and macrozoobenthos assemblages in three coastal lagoons under varying degrees of eutrophication. ICES J Mar. Sci 57 (5): 1495-1506.

Bett BJ. 2014. Macroecology and meiobenthos: Reply to Warwick (2014). Mar EcolProgSer 505: 299-302.

Bosch T, Dance SP, Goolenbeek R, Oliver PG. 1995. Seashells of Eastern Arabia. Motivate Publishing, UAE.

Bridges TS, Levin LA, Cabrera D, Plaia G. 1994. Effects of sediment amended with sewage, algae, or hydrocarbons on growth and reproduction in two opportunistic polychaetes. J Exper Mar Bio Ecol 177 (1): 99-119.

Carpenter KE, Krupp F, Jones DA, Zajonz U. 1997. FAO species identification field guide for fishery purposes: The living marine resources of Kuwait, Eastern Saudi Arabia, Bahrain, Qatar, and the United Arab Emirates. Food and Agriculture Organization of the United Nations, Rome.

Chapman PM, Brinkhurst RO. 1981. Seasonal changes in interstitial salinities and seasonal movements of subtidal benthic invertebrates in the Fraser River estuary, B.C. Estuar Coast Shelf Sci 12 (1): 49-66.

Cusson M, Bourget E. 2005. Global patterns of macroinvertebrate production in marine benthic habitats. Mar Ecol Prog Ser 297: 1-14.

Debruyne RH. 2003. The complete encyclopedia of shells. Rebo Publisher, Lisse Netherlands.

Dolbeth M, Pardal MA, Lillebø AI, Azeiteiro U, Marques JC. 2003. Short-and long-term effects of eutrophication on the secondary production of an intertidal macrobenthic community. Mar Biol 143 (6): 1229-1238.

Erftemeijer PL, Herman PM. 1994. Seasonal changes in environmental variables, biomass, production and nutrient contents in two contrasting tropical intertidal seagrass beds in South Sulawesi, Indon Oecologia 99 (1-2): 45-59.

Fauchald K. 1977. The polychaete worms, definition and keys to the order, families and genera.Natural History Museum of Los Angeles in conjunction with the Allan Hanccock Foundation, University of Southern Califotnia, USA.

Gaughan DJ, Potter IC. 1995. Composition, distribution and seasonal abundance of zooplankton in a shallow, seasonally closed estuary in temperate Australia. Estuar Coast Shelf Sci 41: 117-135.

Gerami MH, Dastbaz M, Patimar R. 2014. Gill-net efficiency on fish catch based on economical and biological diversity indices. Iran J Ichthyol 1 (2): 121-128.

Gerami MH, Ghorbani R, Paighambari SY, Momeni M. 2013. Reproductive season, maturation size (LM50) and sex ratio of *Metapenaeus affinis* (Decapoda: Penaeidae) in Hormozgan shrimp fishing grounds, south of Iran. Int J Aqua Biol 1 (2): 48-54

Glasby CJ. 1986. Population structure and reproductive biology of *Ceratonereislimnetica* (Polychaeta: Nereididae) at Lower Portland, Hawkesbury River, Australia. Mar Biol 90: 589-595.

Gooday AJ, Carstens M, Thiel H. 1995. Micro- and nanoforaminifera from abyssal Northeast Atlantic sediments: a preliminary report. Int Rev Ges Hydro 80: 361-383.

Grey JS. 1981. The ecology of marine sediments. Cambridge University Press, London.

Johns WE, Yao F, Olson DB, Josey SA, Grist JP, Smeed DA. 2003. Observations of seasonal exchange through the Straits of Hormuz and the inferred heat and freshwater budgets of the Persian Gulf. JGeoph Res 108 (C12): 3391, DOI:10.1029/2003JC001881.

Kuhnt T, Friedrich O, Schmiedl G, Milker Y, Mackensen A, Lückge A. 2013. Relationship between pore density and bottom-water oxygen content in benthic Foraminifera. Deep-Sea Res. Pt I 76: 85-95.

Kumar RS. 2001. Intertidal zonation and seasonality of benthos in a tropical mangrove. Int J Ecol Environ Sci 27: 199-208.

Kunlasak K, Chitmanat C, Whangchai N, Promya J, and Lebel L. 2013.Relationships of Dissolved Oxygen with Chlorophyll a and Phytoplankton Composition in Tilapia Ponds. Int J Geo 4: 46-53.

Livingston RJ. 1987. Field sampling in estuaries: the relationship of scale to variability. Estuar 10: 194-207.

Lu L. 2005. The relationship between soft bottom macrobenthic communities and environmental variables in Singaporean waters.Mar Poll Bull 51: 1034-1040.

Ludwig JA, Reynolds JF. 1988. Statistical Ecology: a Primer on Methods and Computing. John Wiley and Sons. New York.

Mare MF. 1942. A study of a marine benthic community with special reference to the micro-organisms. J Mar Biol Assoc UK 25:517-554.

Margalef R. 1969. Perspectives in Ecological Theory. The University of Chicago Press, Chicago.

Marques JC, Salas F, Patricio J, Teixeira H, Neto JM. 2009. Ecological Indicators for Coastal and Estuarine Environmental Assessment: A User Guide. WIT Press, Southampton UK.

Mohammed SZ. 1995. Observation on the benthic macrofauna of the soft sediment on western side of the Arabian Gulf (ROPME sea area) with respect to 1991Gulf war oil spill. Ind J Mar Sci 24: 147-152.

Molvær J, Knutzen J, Magnusson J, Rygg B, Skei J, Sørensen J. 1997. Classification of environmental quality in Fjords and Coastal Waters. SFT Guidelines 97 (03): 36 p

Morrisey DJ, Underwood AJ, Howitt L, Stark JS. 1992. Temporal variation in soft-sediment benthos. J Exper Mar Biol Ecol 164 (2): 233-245.

Nabavi SMB, Salari-Aliabadi MA, Shamoradi AR, Vazirizadeh A, Arebi I. 2011. Ecological assessment of intertidal ecosystems in Khark Island (Persian Gulf) using community structure of macrobentic bivalves. World J Fish Mar Sci 3 (6): 559-563.

Niemi GJ, McDonald ME. 2004. Application of ecological indicators. Annu. Rev Ecol Evol Sys 35: 89-111.

Nouri J, Karbassi AR, Mirkia S. 2008. Spatial distribution and assemblage structure of macrobenthos in a tidal creek in relation to industrial activities. Intl J Environ Sci Tech 6: 651-662.

Oksanen J, Blanchet FG, Kindt R, Legendre P,Minchin, PR, O'Hara RB, Simpson GL, Solymos P, M. Stevens MHM, Wagner H. 2015. vegan: Community EcologyPackage. R package version 2.3-0.http://CRAN.R-project.org/package = vegan

Pielou EC. 1969. An Introduction to Mathematical Ecology. Wiley Interscience, New York.

Pourjomeh F, Hakim Elahi M, Rezai H, Amini N. 2014. The Distribution and Abundance of Macrobenthic Invertebrates in the Hormozgan Province, the Persian Gulf. J Persian Gulf 5 (15): 25-32.

Saghali M, Baqraf R, Hosseini SA, Patimar R. 2013. Benthic community structure in the Gorgan Bay (Southeast of the Caspian Sea, Iran): Correlation to water physiochemical factors and heavy metal concentration of sediment. Int J Aqua Biol 1 (5): 245-253.

Sale PF, Feary DA, Burt JA, Bauman AG, Cavalcante GH, Drouillard KG, Kjerfve B, Marquis E, Trick CG, Usseglio P, Van Lavieren H. 2011). The growing need for sustainable ecological management of marine communities of the Persian Gulf. Ambio 40 (1): 4-17.

Salen-Picard C, Arlhac D. 2002. Long-term changes in a Mediterranean benthic community: Relationships between the polychaete assemblages and hydrological variations of the Rhône River. Estuar 25 (6): 1121-1130.

Shannon CE, Weaver W. 1963. The Mathematical Theory of Communication. University of Illinois Press, Chicago.

Sharma RC, Rawat JS. 2009. Monitoring of aquatic macroinvertebrates as bioandicator for assessing the health of wetlands: A case study in the Central Himalayas, India. Ecol Indic 9: 118-128.

Simpson EH. 1949. Measurement of diversity. Nature: 163: 688

Snelgrove PVR. 1998. The biodiversity of macrofaunal organisms in marine sediments. Biodiv Conserv 7: 1123-1132.

Steel JA. 1980. Phytoplankton Models," In: Le Cren ED, Lowe-McConnell RH (eds.). Functioning of Freshwater Ecosystems, Cambridge University Press, Cambridge.

Sterrer W. 1986. Marine Fauna and Flora of Bermuda A systematic guide to the identification of marine organisms, A Wiley-Interscience Publication John Wiley and Sons, New York.

Tabatabaie T, Amiri F, Pazira A. 2009 Monitoring structure and biodiversity of benthic macrofauna community as pollution index on Mossa and Ghanam creek. J Fish 3 (4): 29-40. [Persian]

Warwick RM, Uncles RJ. 1980. Distribution of benthic macrofauna associations in the Bristol Channel in relation to tidal stress. Mar EcolProgSer 3: 97-103.

Zobrist EC, Coull BC. 1992. Meiobenthic interactions with macrobenthic larvae and Juveniles: an experimental assessment of the meiofaunal bottleneck. Mar Ecol ProgSer 88: 1-8.

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Status of coastal forests of the Northern Sumatra in 2005 (after 2004's tsunami catastrophe)

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Abstract. *Onrizal, Mansor M. 2015. Status of coastal forests of the Northern Sumatra in 2005 (after 2004's tsunami catastrophe). Biodiversitas 17: 44-54.* The first intensive ecological study of coastal vegetation including mangrove, littoral and peat swamp forests after the 2004 tsunami catastrophe in Northern Sumatra was conducted from January to December 2005 where 16 sampling sites along 2960km coastline in Northern Sumatra were selected. In each site, one quadrate of 100 m x 100 m was established and divided into 10 m x 10 m subplots where all standing trees of ≥ 2 cm diameter at breast height (DBH) were identified to species level and measured. Overall 54,871 standing trees were recorded in 16 sites comprising 84 species in 65 genera and 37 families. Mangrove trees *Rhizophora apiculata* and *R. mucronata* were widely distributed and are dominant in most of the sampling sites. This indicated that these species have stronger resilient compared to other species. The highest value of Shannon-Wiener index of species and Evenness index of species was 3.03 and 0.85, respectively. It means that some sites were rich in biodiversity which harbors various species of plants. Subsequently, undisturbed coastal forests including mangroves, littoral forests and peat swamp forests characterized by dense stands, mixed species and structures play an important role in coastal protection against tsunami. Therefore, the coastal vegetation is needed to conserve the biodiversity and to maintain the production capacity as part of sustainable and longlasting vegetation bioshield.

Keywords: Biodiversity, bioshield, resilient species, natural hazard, integrated coastal forest management system

INTRODUCTION

On 26th December 2004, a mega-thrust earthquake of magnitude ranges from 9.1 to 9.3 on Richter scale occurred off the northwestern coast of Northern Sumatra, Indonesia (Bilham 2005; Chen et al.2005; Lay et al. 2005; Ghobarah et al. 2006; Subarya et al. 2006; Chlieh et al. 2007). This huge earthquake triggered giant tsunami waves, which the combined destructive impact of the earthquake and the tsunami was enormous to the coastlines and its inhabitants lining coastal shores of the Indian Ocean, both Asia and Africa. The earthquake and tsunami not only caused human fatalities and hardship. They also caused destruction of the coastal vegetation and natural resources.

To date, there are limited publications on Northern Sumatra coastal vegetation particularly in peer-reviewed journals before the 26th December 2004 Indian Ocean tsunami disaster. Whitten et al. (1997) provided information and accounts on ecology of Sumatra, however the facts related to the ecology of Northern Sumatra coastal vegetation was still lacking information. According to Kartawinata (1990, 2005), the ecological studies of natural vegetation in Northern Sumatra are rare with only four studies were conducted in last six decades. All of them are in tropical lowland forests which three of them located at Gunung Leuser National Park (GLNP), and one of them was at Batang Gadis National Park (BGNP).

Northern Sumatra coastal studies increased after the tsunami catastrophic; however most of them are about coastal geo-morphological studies focusing on earthquake and tsunami. Ammon et al.(2005), Bilham (2005), Borrero (2005a, 2005b), Borrero et al. (2006a, 2006b), Lay et al. (2005), Subarya et al. (2006), Natawidjaja and Triyoso (2007), Kayanne et al. (2007), Kusuma et al. (2008) and Meilianda et al. (2010) studied on the impact of tsunami and earthquake on coastal deformation and destruction. Campbell et al. (2007) and Hagan et al. (2007) studied the impact of tsunami and earthquake on coral reef in Northern Sumatra. Descriptive study on Aceh coastal impact and recovery from tsunami was done by Wong (2009). Some articles on coastal vegetation and resource destructions by tsunami mainly based on remote sensing and geographic information system. Chen et al. (2005), Shofiyati et al. (2005), Iverson and Prasad (2007), and Liew et al. (2010) provided such examples. It should be noted that coastal vegetation studies in Northern Sumatra were very limited, both before and after the Indian Ocean tsunami disaster. Consequently, the ecology of coastal vegetation in Northern Sumatra is mostly unknown.

The aim of this study is to assess the coastal vegetation communities and diversity at affected areas by tsunami in Northern Sumatra.

MATERIALS AND METHOD

Study sites

The 16 study sites have been established from January to December 2005 (Table 1) along 2960-km coastline in Northern Sumatra (Figure 1). One sample plot 100 m x 100 m (1 ha) was established in each study site. Each plot was divided into 10 m x 10 m subplots; therefore, there are 100 subplots within each sample plot.

Data collection

The most effective and acceptable method to study and quantify species diversity and vegetation communities is plot sampling method (e.g. Condit et al. 1996, Shimida 1984). All trees (or woody plant) greater than 2 cm DBH were identified and measured. The tree diameter was measured (a) at 20 cm above the highest prop-roots for *Rhizophora* species, (b) whereas for tree when the stem forked below 130 cm, individual 'branches' in a clump were treated as separate stems, or (c) at 1.3 m above ground level (diameter at breast-height; DBH) for tree species without stilt roots, (c) except for mangrove palm of *Nypa fruticans*, which the diameter was by measuring the diameter of clump.

Table 1. Study sites of coastal vegetation after the 2004 tsunami

Site code	Location	Coastal region	Forest type
S01	Deah Gelumpang, Banda Aceh	North coast	Mangroves
S02	Gampong Jawa, Banda Aceh	North coast	Mangroves
S03	Neuhen, Aceh Besar	North coast	Mangroves
S04	Ujung Batee, Aceh Besar	North coast	Littoral forests
S05	Lhok Nga, Aceh Besar	West coast	Littoral forests
S06	Lhok Bubon, Aceh Barat	West coast	Mangroves
S07	Rawa Singkil, Aceh Singkil	West coast	Peat swamp forests
S08	Rawa Singkil, Aceh Singkil	West coast	Mangroves
S09	Tabuyung, Madina	West coast	Mangroves
S10	Pasar Lahewa, Nias Utara	Offshore	Mangroves
S11	Teluk Belukar, Gunung Sitoli	Offshore	Mangroves
S12	Sirombu, Nias Barat	Offshore	Agroforests
S13	Sirombu, Nias Barat	Offshore	Mangroves
S14	Kuala Pekanbaro, Sigli	East coast	Mangroves
S15	Kuala Keureutou, Aceh Utara	East coast	Agroforests
S16	Jaring Halus, Langkat	East Coast	Mangroves

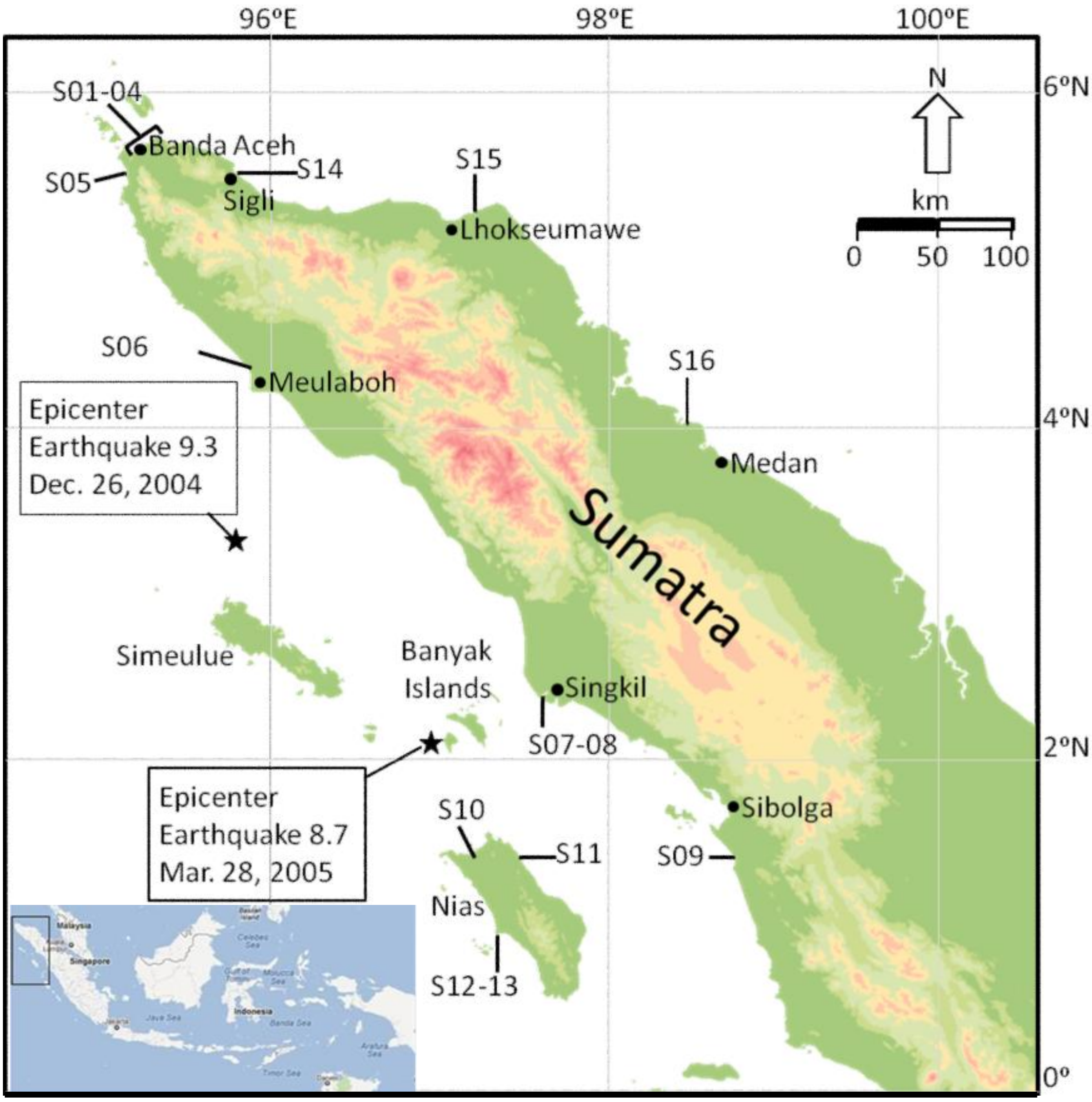

Figure 1. Distribution of study sites along Northern Sumatra coasts

Data analysis

Stem density (individuals/ha), basal area (m^2/ha) and Importance Value Indices (IVI) were calculated for each sampling sites. To determine the dominant species (in term of phytosociological position in a vegetation community), the IVI from Curtis and McIntosh (1951) and Magurran (2004) was adopted. The IVI of each species for tree stage was calculated by summing the relative density, relative frequency and relative dominance. From the data collected, a species diversity analysis using statistical software MVSP (Multi Variate Statistical Programme) ver. 13.3d by Kovach Computing Services (2002) was conducted.

Stem density of coastal vegetation (individual/ha) of 16 study sites was used to distinguish the community structure by hierarchical clustering performed using the MVSP program ver. 13.3d by Kovach Computing Services (2002) following UPGMA method. In this case, to know the species indicator of each vegetation group, the IVI were calculated for each groups.

RESULTS AND DISCUSSION

Taxonomic composition

A total of 54,871 standing trees (i.e., 47,723 of DBH 2 to < 10 cm, and 7,148 DBH ≥ 10 cm) were recorded in 16 sites in Northern Sumatra coast immediately after the 2004 tsunami encompassing 84 species in 65 genera and 37 families. The species are indicated as persistent species and surviving against the natural catastrophic.

In term of number of species, the family of Rhizoporaceae (8.33% of total species) was recorded as family with largest number of species, followed by Arecaceae (7.14%), Euphorbiaceae, Lauraceae, and Malvaceae (each 5.95%). The families with highest individual count in 16 study sites were Rhizophoraceae (82.86%), followed by Arecaceae (7.43%) and Meliaceae (2.55%). The densities of other family have only less than 2%.

The species of *Rhizophora apiculata* was recorded as widest distribution (10 sampling sites or 62.5% of total sampling sites), following by *Rhizophora mucronata* (8 sampling sites or 50.0% of total sampling sites) and *Xylocarpus granatum* (7 sampling sites or 43.8% of total sampling sites). The others (74 species) were recorded at limited distribution that varied from 1 to 2 sampling sites.

Important Value Index

The dominant species varied between 16 study sites (Table 2). The mangrove tree *Rhizophora apiculata* was recorded as dominant species at 5 sites (31.25% of total sites), i.e., S08-11 and S16. Subsequently, the mangrove palm *Nypa fruticans* dominated three sites (18.75% of total sites), i.e., S02, S06, and S13. Three species, i.e., mangrove tree *Rhizophora mucronata,* littoral tree *Casuarina equisetifolia* and agroforest tree *Cocos nucifera* were found to be dominant in 2 sites. Lastly, the mangrove tree *Rhizophora stylosa* and peat swamp tree *Tetramerista glabra* were recorded as dominant species at each one site. The co-dominant species also varied between sites.

Diversity indices

The value of diversity indices has been listed in Table 3. The highest value for richness (*R*) was recorded from S07 (peat swamp forests at Singkil swamps, 6.74) and the lowest value was recorded from S05 (littoral forests, 0.00) where this site contained only one species immediately after tsunami disaster, namely *Casuarina equisetifolia*. For diversity (*H'*), the highest value was also recorded in S07 (3.03), followed by S11 (1.49), S08 (1.45) and S16 (1.36). The lowest value for diversity (*H'*) was recorded also in S05 (0.00). Subsequently, the highest value for evenness (*E*) was recorded at S16 (0.85), followed by S07 (0.76), S01 (0.73) and S11 (0.72), while the lowest for *E* value was recorded at S05 (0.00). The highest value for richness (*R*) and diversity (*H'*) recorded in S07, a peat swamp forests, compared to other sites, which was probably due to the naturally the forest type was richer compared to mangroves and littoral forest in the same size of plot.

Vegetation community

The result of cluster analysis allowed for a floristically (cluster group and indicator species) and ecologically (habitat/vegetation type) sound scheme of six main vegetation groups (A-F) of the 16 study sites. A UPGMA dendrogram is shown in Figure 2. Bray Curtis's coefficients for species dissimilarities are more than 0.8 between groups suggesting that one group contain many different species compared to other group, as shown in Figure 2.

The Group A contains three sites, namely S01, S03, and S14 and represented of mangrove forests along the affected coast by tsunami in Northern Sumatra. Two sites of them were situated at North Coast, and remaining site was in East Coast. Before tsunami, all sites were degraded mangroves surrounding the aquaculture ponds which the ponds were developed with conversion of mangrove forests. This group contains nine species, which the species indicator was *Rhizophora mucronata* having mean density and IVI were 115.7 individual/ha and 133.5%, respectively. The second species indicator in this group was *R. stylosa*, which the mean density and IVI were 112.7 individual/ha and 73.3% (Table 4). Therefore, this group is represented by *R. mucronata*-*R. stylosa* communities.

The Group B contains five sites, namely S08, S09, S10, S11 and S16. They represented of the healthy mangrove forests along the affected coast by tsunami in Northern Sumatra. Two sites (S08 and S09) were situated in the West Coast, two sites were situated in the Offshore (S10 and S11), and remaining site was in the East Coast (S16). Prior to tsunami, all sites were healthy mangroves and they were low or without disturbance both natural and anthropogenic factors. This group contains 18 species, which the species indicator was *Rhizophora apiculata* having mean density and IVI were 5,426.2 individual/ha and 140.4%, respectively. The IVI of others species was lower than 40%. In addition, *Dolichandrone spathacea* was recorded as the lowest IVI, i.e., 0.1% and it mean density was only 0.6 individual/ha (Table 4). Therefore, this group is represented by *R. apiculata* communities.

The Group C contains three sites, namely S02, S06 and S13. They represented of the landward zone of mangrove forests along the affected coast by tsunami in Northern Sumatra. Each site was distributed in North Coast (S02), West Coast (S06) and Offshore Coast (S13). Before tsunami, sea ward and mid ward of the mangrove forests were mostly converted to aquaculture ponds. This group contains nine species, which the species indicator was *Nypa fruticans* with mean density and IVI of 1,116.7 individual/ha and 58.9%, respectively. The IVI of others species was lower than 10%. In addition, *Xylocarpus granatum* and *Ceriops tagal* was recorded with the lowest IVI, i.e., 0.3% and its mean density was only 0.3 individual/ha (Table 4). Therefore, this group is represented by *N. fruticans* communities.

The Group D contains two sites, namely S04 and S05. They represented of the littoral forests along the affected coast by tsunami in Northern Sumatra. Site S04 was situated in the North Coast, and remaining site was in the West Coast. Prior to tsunami, the sites were as recreational areas. This group contains three species, where the species indicator was *Casuarina equisetifolia* having mean density and IVI were 173.0 individual/ha and 273.8%, respectively. The IVI of others species were only 19.4% for *Gliricidia sepium* and 6.8% for *Pterocarpus indicus* (Table 4). Therefore, this group is represented by *Casuarina equisetifolia* communities.

Table 2. List of dominant and co-dominant species at each sampling site of Northern Sumatra coast immediately after the 2004 tsunami disaster

Study site	Dominant species		Co-dominant species	
	Species	IVI (%)	Species	IVI (%)
S01	*Rhizophora mucronata*	173.4	*Xylocarpus granatum*	62.3
S02	*Nypa fruticans*	282.7	*Oncosperma tigillarium*	3.3
S03	*Rhizophora stylosa*	144.9	*Avicennia marina*	90.6
S04	*Casuarina equisetifolia*	269.3	*Gliricidia sepium*	22.6
S05	*Casuarina equisetifolia*	300.0	(None)	-
S06	*Nypa fruticans*	285.6	*Excoecaria agallocha*	4.2
S07	*Tetramerista glabra*	66.8	*Syzygium pycnanthum*	27.2
S08	*Rhizophora apiculata*	139.0	*Sonneratia caseolaris*	33.1
S09	*Rhizophora apiculata*	109.6	*Bruguiera parviflora*	80.1
S10	*Rhizophora apiculata*	207.2	*Bruguiera sexangula*	49.5
S11	*Rhizophora apiculata*	97.2	*Bruguiera sexangula*	79.2
S12	*Cocos nucifera*	282.3	*Arenga pinnata*	6.5
S13	*Nypa fruticans*	297.2	*Rhizophora apiculata*	1.4
S14	*Rhizophora mucronata*	248.6	*Avicennia marina*	33.6
S15	*Cocos nucifera*	278.9	*Mangifera indica*	6.7
S16	*Rhizophora apiculata*	134.0	*Xylocarpus granatum*	71.4

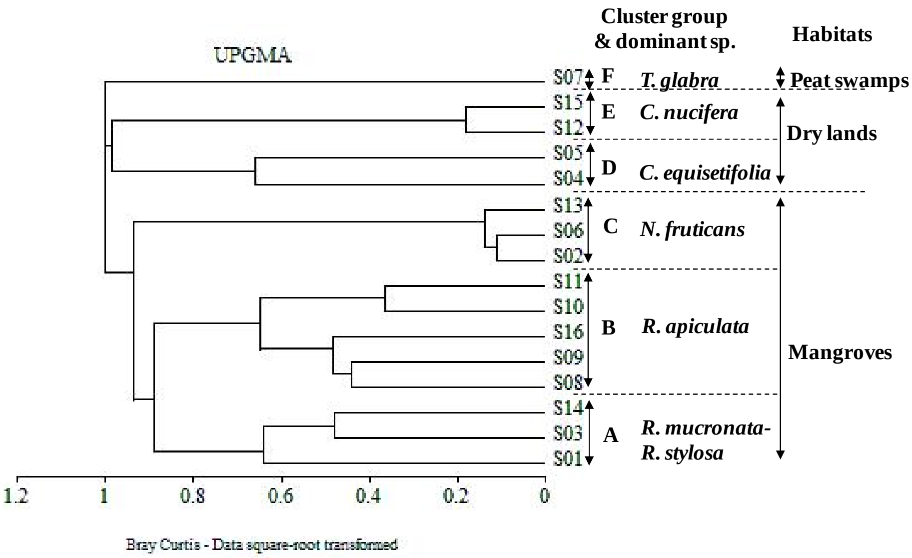

Figure 2. Dendrogram generated by cluster analysis of the 16 coastal forests investigated showing the species dissimilarities between the sampling sites. Six groups and three habitats can be distinguished; group (A) contains 3 sites of the mangrove forests, (B) contains five sites of the mangrove forests, (C) contains three sites of the mangrove forests, (D) contains two sites of coastal dry land vegetation, (E) contains two sites of coastal fry land vegetation, and (F) contains one site of peat swamp forests. See Table 1 for site acronyms.

Table 3. The value of indices in each sampling site

Study sites	Richness S	Richness R	H'	E	Vegetation type
S01	6	1.01	0.73	1.31	Mangroves
S02	7	0.84	0.04	0.09	Mangroves
S03	4	0.51	0.69	0.96	Mangroves
S04	3	0.34	0.33	0.36	Littoral forest
S05	1	0.00	0.00	0.00	Littoral forest
S06	6	0.70	0.05	0.08	Mangroves
S07	54	6.74	0.76	3.03	Peat swamp
S08	15	1.74	0.53	1.45	Mangroves
S09	7	0.76	0.68	1.33	Mangroves
S10	5	0.41	0.33	0.53	Mangroves
S11	8	0.70	0.72	1.49	Mangroves
S12	5	0.69	0.13	0.21	Agroforest
S13	3	0.29	0.03	0.04	Mangroves
S14	3	0.39	0.33	0.36	Mangroves
S15	6	0.98	0.13	0.24	Agroforest
S16	5	0.53	0.85	1.36	Mangroves

Note: S = species number, R = species richness index, H' = Shannon diversity index, E= evenness index

The Group E contains two sites, namely S12 and S15. They represented of the agroforest along the affected coast by tsunami in Northern Sumatra. Site S12 was situated in the Offshore, and remaining site was in the East Coast. Prior to tsunami, the sites were former littoral forests converted by inhabitant to coconut agroforest. This group contains eight species, which the species indicator was *Cocos nucifera*having mean density and important value index (IVI) were 335.0 individual/ha and 280.6%, respectively. The IVI of others species were lower than 10% (Table 4). Therefore, this group is represented by *Cocos nucifera*community.

The Group F contains only one site, namely S07. This site represented of the peat swamp forests along the affected coast by tsunami in Northern Sumatra, especially in the West Coast. Before tsunami, the site was low impact by human activities. This group contains 54 species, where the species indicator was *Tetramerista glabra*having mean density and important value index (IVI) of 621.0 ind./ha and 66.8%, respectively. The IVI of others species was about 10% or less (Table 4). In addition, there are four species which their density was only 1 ind./ha, namely *Garcinia celebica*, *Litsea resinosa*, *Mangifera griffithii*, and *Terminalia foetidissima*. The four latest species, i.e., *G. celebica*, *L. resinosa*, *M. griffithii*, and *T. foetidissima* were categorized as very rare species in the area. Therefore, this group is represented by *Tetramerista glabra* communities.

Discussion

Mangrove forests

According to Tomlinson (1986), Kusmana et al. (1992) and Whitten et al. (1997), the family of Rhizophoraceae represents the most common in mangrove forests, including in Sumatra. The analysis of tree flora immediately after the 2004 tsunami in 16 sites of affected area by tsunami in Northern Sumatra showed also the largest family was Rhizophoraceae both number of species and abundance

Table 4. Mean density (*D*, individual/ha), basal area (*BA*, m^2/ha), frequency (*F*, %) and important species index (*IVI*, %) of each member of Group A to F

Species	D	BA	F	IVI
Group A				
Rhizophora mucronata	115.7	0.750	14.0	133.5
Rhizophora stylosa	122.7	0.230	5.0	73.3
Avicennia marina	24.3	0.381	4.7	47.7
Xylocarpus granatum	12.0	0.065	2.7	17.2
Rhizophora apiculata	5.7	0.075	1.3	11.1
Avicenia officinalis	0.7	0.061	0.7	6.3
Thespesia populnea	6.7	0.015	0.3	4.3
Sonneratia alba	0.7	0.026	0.7	4.1
Excoecaria agallocha	2.7	0.008	0.3	2.6
Group B				
Rhizophora apiculata	5426.2	9.286	85.2	140.4
Bruguiera parviflora	1177.2	1.770	34.2	35.8
Bruguiera sexangula	1428.6	1.608	28	35.1
Xylocarpus granatum	257.4	2.101	31.8	26.7
Bruguiera gymnorrhiza	524.0	1.401	25.6	23.4
Rhizophora mucronata	386.4	0.931	9.8	12.9
Sonneratia caseolaris	22.4	1.098	2.8	7.0
Excoecaria agallocha	57.2	0.391	8	5.9
Aegiceras corniculatum	73.4	0.266	3.6	3.6
Heritiera littoralis	19.8	0.203	3.6	2.7
Nypa fruticans	5.2	0.436	0.6	2.5
Avicennia officinalis	3.0	0.139	1.2	1.2
Sonneratia alba	3.0	0.046	2	1.1
Avicennia marina	1.4	0.085	0.8	0.8
Oncosperma tigillarium	0.8	0.008	0.6	0.3
Ceriops tagal	7.4	0.006	0.2	0.2
Hibiscus tiliaceus	1.4	0.018	0.2	0.2
Dolichandone spathacea	0.6	0.009	0.2	0.1
Group C				
Nypa fruticans	1116.7	40.628	100.0	290.3
Rhizophora apiculata	3.0	0.094	2.0	2.3
Excoecaria agallocha	1.3	0.058	1.3	1.5
Sonneratia caseolaris	1.7	0.043	1.3	1.5
Avicennia marina	1.7	0.038	1.3	1.5
Rhizophora mucronata	1.3	0.017	1.3	1.4
Oncosperma tigillarium	1.7	0.056	0.7	0.9
Xylocarpus granatum	0.3	0.003	0.3	0.3
Ceriops tagal	0.3	0.001	0.3	0.3
Group D				
Casuarina equisetifolia	173.0	28.655	39.5	273.8
Gliricidia sepium	14.5	0.331	5	19.4
Pterocarpus indicus	2.5	0.043	2.5	6.8
Group E				
Cocos nucifera	335.0	23.607	99.0	280.6
Mangifera indica	3.0	0.560	2.5	5.4
Pandanus tectorius	4.5	0.026	3.5	4.6
Arenga pinnata	2.5	0.069	2.5	3.3
Sterculia foetida	0.5	0.340	0.5	2.0
Metroxylon sagu	1.5	0.212	0.5	1.7
Manilkara kauki	1.0	0.040	1.0	1.4
Gliricidia sepium	1.0	0.004	1.0	1.2
Group F				
Tetramerista glabra	621	21.380	98	66.8
Syzygium pycnanthum	296	4.319	82	27.2
Dactylocladus stenostachys	193	4.282	75	22.4
Gluta wallichii	99	5.590	43	17.0
Horsfieldia glabra	111	4.521	41	15.7
Shorea seminis	93	4.698	38	14.9
Litsea gracilipes	115	1.824	44	12.2
Shorea sp.	106	1.999	45	12.2
Sandoricum beccarianum	77	2.955	37	11.6
Litsea mappacea	111	0.957	43	10.6

within this family compared to other families. In addition, Rhizophoraceae was also mostly dominant in 8 sampling sites (72.7%) of 11 sampling sites in mangrove forests, where *R. apiculata* was dominant in 5 sampling sites, followed by *R. mucronata* (2 sampling sites) and *R. stylosa* (1 sampling site).

Rhizophora apiculata and *R. mucronata* as member of Rhizophoraceae family were also recorded as widest distribution and most dominant in several sampling sites. This indicated that these species have stronger ability compared to other species to defense against tsunami disaster. In this study, *Rhizophora* spp. was the strongest species as compared to other genera of mangroves. This is in concordance with the finding by Yanagisawa et al. (2010). The dense structures of prop roots of a *Rhizophora* tree that extending all around (Jayatissa et al. 2002) have contributed to the resistance of tsunami flow even in the soft ground of tidal flat. Meanwhile, other genera without prop roots were easily uprooted. Based on field survey in Sri Lanka and Andaman coast, Tanaka et al. (2007) reported that *Rhizophora apiculata* and *Rhizophora mucronata* were especially effective in providing protection from tsunami damage due to their complex aerial root structure. Similar findings were also reported by Dahdouh-Guebas et al. (2005) for mangroves in Sri Lanka, Kathiresan and Rajendran (2005) for mangroves in India, Yanagisawa et al. (2009a, 2009b) for mangrove in Thailand after the 2004 Indian Ocean tsunami. The previous study by Mazda *et al* (1997) found that the effect of the drag force on *Rhizophora* spp. by the wave was higher compared to *Kandelia candel* because *Kandelia candel* has no pneumatophores.

Baba (2004) and Dahdouh-Guebas et al. (2005) reported that other true mangrove representatives, such as *Sonneratia* spp., the stem of which can measure several meters in circumference which has wide prop or knee roots, also stood firm against the ocean surge. This study also found the large *Sonneratia alba* succeeded against tsunami (Figure 3).

According to Chapman (1976) and Tomascik et al. (1997), along estuarine creeks and in bays and lagoons, stilt-root forming *Rhizophora* spp. are normally the main colonizers. Under pristine, natural conditions, distinct zones with different mangrove associations can frequently be observed along gently sloping, accreting shores. These reflect the degree of tidal inundation but also the level of salinity in estuarine environments. Kusmana and Watanabe (1991) stated that *Avicennia* species and *Sonneratia alba*, generally, which occur seaward and genera of *Rhizophora* and *Bruguiera* that exist generally mid and landward. This is due to the fact that the aerial stilt roots of the *Avicennia* spp. and *Sonneratia alba* are more tolerant than pneumatophores of the *Rhizophora* spp. and *Bruguiera* spp. to long periods of submergence by flood water (Kathiresan and Bingham 2001).

The mangrove palm, *Nypa fruticans* was recorded as dominant species in 3 sampling sites of the 11 sampling sites in mangrove forests along the Northern Sumatra coast. The zone of *Nypa fruticans* was situated at land ward zone of mangroves, while the mid and sea ward zones of mangroves surrounding areas were converted to aquaculture ponds before tsunami disaster. According to Lugo and Snedaker (1974), Chapman (1976), Tomlinson (1986), Kusmana and Watanabe (1991), Laumonier (1997), Tomascik et al. (1997), Whitten et al. (1997) and Duke et al.(1998), *Nypa fruticans* commonly grow at the upper (land ward) zones reached only by spring tides (1-20 flooding per month). Similar with this result in Northern Sumatra coasts, Dahdouh-Guebas et al. (2005) also reported that *Nypa fruticans* colony was thriving well and were by its rhizomatous stem allowed new young leaves to emerge less than a month after the tsunami impact.

Species richness in each sampling sites of mangrove forests less than 12 months after tsunami varied from 3 to 15 species. There were 5 sampling sites containing species richness less than 5 species in 1 ha plots. Subsequently, 5 sampling sites contain 6-10 species in 1 ha plot. Only 1 sampling site contains more than 10 species. The species richness in some sampling sites was mostly lower than other mangroves in Indonesia compiled by Kusmana et al. (1992), i.e., between 8 to 14 species. The species richness was only for commercial tree species with dbh more than 10 cm. This is probably because most of mangrove forest areas in Northern Sumatra were converted into ponds and other uses prior to tsunami. Based on field research by Satyanarayana et al. (2010) in the Kelantan Delta, Malaysia, the mangroves in the areas were ecologically sensitive to anthropogenic perturbation, including the intense aquaculture trade. Therefore, mangrove plant was not only loss by land clearing for aquaculture ponds, but remaining mangrove plant was continually treated by pollution of aquaculture activities.

According to Dahdouh-Guebas (2006), mangrove forest exhibits a unique biodiversity with uncommon adaptations such as vivipary in trees (young plants develop while still attached to the parental tree). Mangroves are adapted to intertidal environmental conditions such as high-energy tidal action, high salt concentrations, and low levels of oxygen (hypoxia). In addition, Cochard et al. (2008) stated that resilience of a mangrove ecosystem is likely to be influenced by factors such rates of tree regeneration and seedling recruitment, and renewed sedimentation reversing soil losses during the hazardous event.

According to Cochard et al. (2008), unlike the exposed coasts in temperate zones, tropical ecosystems include habitats such as offshore barrier reefs, dense mangrove forests and high sand dunes stabilized by beach forest. As well as providing important natural resources for many communities, these ecosystems may represent an important insurance against tsunami hazards; but it is essential to properly evaluate the actual utility of these ‘‘insurance’’ assets.

Figure 3. Large stand of *Sonneratia alba* in Lhok Mee, East Coast of Northern Sumatra stood firm against the 2004 tsunami. Most of the trees have more than 1 m in dbh.

Figure 4. Sumatran orangutan populations stay in peat swamp forests in Singkil swamps. Some part of the forests were affected by tsunami, and the other hand the forests stood firm against the 2004 tsunami, and have capability in decreasing the impact of tsunami on coastal areas behind the forests.

Figure 5. The coastal belt of peat swamp forests wiped out in several sites in the West Coast of Northern Sumatra few years after the 2004 tsunami disaster. When tsunami struck, the peat swamp forests were functionally as barrier. Large areas of green belt were erased and loss. Therefore, an integrated approach was needed in term of economic and ecological uses, including natural hazard preparedness.

Peat swamp forests

A one ha sampling site of peat swamp forests (PSF) in West Coast of Northern Sumatra having 54 species of tree with dbh 2 cm and more. The species richness was higher than species richness of PSF at Pekan Forest Reserve, Pahang, Malaysia, i.e., 49 species reported by Hamzah et al. (2009). The species richness of this study was also higher than PSF in Riau, Indonesia, i.e., 43 species reported by Istomo (2002, 2006). On the other hand, the species richness value was lower than PSF at Selangor, Malaysia, i.e., 103 species reported by Ibrahim and Lepun (2004).

Based on this result, the PSF in Singkil swamp has high diversity, which the Shannon diversity and Richness Margalef indices reached 3.03 and 6.74, respectively. It indicated the PSF has rich plant species, which was important to support the nutrient cycle and food web surrounding the areas, including human and wildlife.

There was relatively limited research conducted on PSF in Sumatra, some of them were by Giesen et al. (1992), Laumonier (1997), Whitten et al. (1997), Istomo (2002, 2006), Giesen (2004). Giesen et al. (1992) stated that the PSF at Singkil swamps was the last remaining pristine PSF until 1992 in coastal areas of Northern Sumatra. The PSF in Singkil swamps represent the tropical lowland forests in Leuser ecosystem, main habitat of some endangered species, such as Sumatran orangutan (*Pongo abelii*), Sumatran tiger (*Panthera tigris sumatrae*) and Sumatran elephant (*Elephan maximus*). Some individuals and nests of Sumatran orangutan were recorded during this field work in Singkil swamps (Figure 4). If the forest become degraded and fragmented, the endangered species become extinct.

Along the west coast of Northern Sumatra between Tabuyung and Kuala Cangkoang, between Kuala Baru Singkil and Trumon, and between Lhok Kruet and Blangpidie, PSFs were the original dominant vegetation types. Except in between Kuala Baru Singkil and Trumon, large areas of PSFs have been logged and converted to various types of cultivated land, including oil palm plantations, predominantly in recent years. The large and healthy PSFs were mostly found in Singkil swamps were status as conservation forests and also managed by Aceh traditional forest management system, *Panglima Uteun*.

Similar with this observation result, Giesen et al. (1992), Rijksen et al. (1997) reported that the PSF in western coast of Northern Sumatra have probably formed behind coastal sand ridges in waterlogged conditions; this type of peatswamp has also been described as ‘‘shallow freshwater swamp’’ and ‘‘fringe aquaculture swamp”, for example PSFs in Singkil swamp. Subsequently, Whitten et al. (1997) explained that some peat swamp forests may also occupy coastal areas that were initially reclaimed by mangroves, these being replaced subsequently by freshwater peat vegetation during the course of succession; these types were most extensive on the east coast of Sumatra, while this research found the similar case in Singkil swamps which the areas received more fresh water input from large river (Giesen et al., 1992, Rijksen et al., 1997), one of them was Alas river.

According to Mansor (2004), Page (2004), Rieley (2004), the PSF has significant role, ecological, economical and social aspects. Mansor (2004) stated that the PSF was a significant habitat for rare and endangered species. As described previously, PSF in Singkil swamps was as main habitat for Sumatran orangutan and Sumatran tiger recorded as critically endangered species in IUCN red list. According to Wich et al. (2008), PSF in Singkil swamps were habitat about 1,660 population of Sumatran orangutan, and were recorded as the second highest population in the world.

Page (2004) stated that, PSF, as a forest, contributes to microclimate stabilization and to maintain of regional and global biodiversity; it also provides a range of economically important timber and non-timber product, including barks, resins and rattans. Rijksen et al. (1997) informed that the forests in Singkil swamps have important role in supporting the fishery production of estuarine which the production was approximately 360,000 ton per year. On the other hand, anthropogenic disturbance as impact of develop tropical peat swamps for short-term gain is increasing, whilst their long-time environmental importance is being ignored. Large areas of PSF in West Coast of Northern Sumatra were converted to oil palm plantations (Figure 5), including in affected area by tsunami soon after the tsunami disaster. It indicated the lack concerned of policy maker in land use setting. Therefore, an emphasis was needed for integrated approaches to the environmentally sustainable management of peat swamps incorporating principles of wise multiple uses.

Coastal dry land vegetation

Most of low land areas in Northern Sumatra were modified. Some of them were degraded and fragmented, including coastal dry land vegetation. Based on this research, littoral forests in affected area by tsunami contain only 1 to 3 species in 1 ha plots which the forest floor vegetation was totally dead or swept out by tsunami. It was probably due to (i) change in microhabitat as impact of tsunami flood which most of the plants in dry land coasts were non salt tolerance and/or (ii) the species richness of trees was also low prior to tsunami as impact of human disturbance. According to Onrizal and Kusmana (2004), the tree species number of littoral forests in Rambut Island, Jakarta was 22 species. Subsequently, Mansor and Othman (2003) recorded 109 plant species in coastal forest of Pantai Acheh Forest Reserve (PAFR), Penang, Malaysia, which species number varied from 17 species to 27 species in each quadrate measuring 10m x 10m. Therefore, the plant species in affected area by tsunami in Northern Sumatra was very lower than slightly disturbed or undisturbed coastal vegetation.

The other type in coastal dry land was agroforest dominated by coconut. Based on this study, the agroforest contains 5-6 species in 1 ha plots. The Richness Margalef index was between 0.3 and 0.7, and the evenness was between 0.21 and 0.24. It indicated the vegetation community was poor and one species were very dominant.

All regeneration stage of trees, shrubs, herbaceous and grasses were killed due to tsunami. This is indicated the understory plants in coastal dry land were limited capacity against salinization due to tsunami flood.

There have been relatively little research conducted on littoral forests in Indonesia, particularly Sumatra. Some of them are Mahmud (1991), Mardiastuti (1992), Imanuddin (1999), Ayat (2002), and Onrizal and Kusmana (2004). All publications were from littoral forest in Rambut Island, Jakarta. According to the publications, littoral forests were important as habitats of water birds and migratory birds. Most of the tree canopies were used as nesting places and other activities of these birds.

Some large trees, such as *Cocos nucifera*, *Casuarina equisetifolia* and *Sterculia foetida* have stood firm against tsunami. The species also thrive well after tsunami, however the capacity to decrease tsunami impact was low probably due to the low density of stands, therefore many gap was present in agroforest and littoral forest. Based on Tanaka (2009), Thuy et al. (2009), the presence of an open gap in a forest could intensify the force of the tsunami by channeling them into the gap. In addition, Tanaka (2009) stated that floating debris from broken trees can also damaging the surrounding buildings and hurting the people.

Casuarina equisetifolia has widest distribution in coastal dry land coast of Northern Sumatra immediately after tsunami disaster. Orwa et al. (2009) informed that *C. equisetifolia* is commonly confined to a narrow strip adjacent to sandy coasts, rarely extending inland to lower hills. This tree was found on sand dunes, in sands alongside estuaries and behind fore-dunes and gentle slopes near the sea. It may be at the leading edge of dune vegetation, subject to salt spray and inundation with seawater at extremely high tides. The species tolerates both calcareous and slightly alkaline soils but is intolerant of prolonged water-logging and may fail on poor sands where the subsoil moisture conditions are unsatisfactory.

Many studies have revealed that these demerits can be overcome with proper planning and management of mangroves and coastal forests, and that coastal vegetation has a significant potential to mitigate damage in constructed areas and save human lives by acting as buffer zones during extreme natural events. However, many coastal vegetation including mangroves and littoral forests were degraded and fragmented by anthropogenic disturbance, making coastal areas increasingly vulnerable to tsunamis and other natural disasters. Tanaka (2009) and Samarakoon et al. (2013) explained that the effectiveness of vegetation also changes with the age and structure of the forest. Subsequently, Tanaka et al. (2011) and Samarakoon et al. (2013) proposed *Pandanus odoratissimus* as the front vegetation layer of *Casuarina equisetifolia* stands to reduce the disadvantages of the open gaps in existing forests in dry land coasts.

According to Tanaka et al. (2011), the effectiveness of coastal dry land vegetation (littoral forests) against tsunami were as follow (1) multiple rows were considered more effective than single or double rows of density of vegetation established, (2) plant species were monoculture less effective than mixed species, (3) low density less effective than high density, (4) front-line species were more effective with complex aerial root structure, (5) multi-layer vegetation structure was more effective than single-layer. In addition, the effectiveness could be more effective with continuous maintenance of coastal vegetation and high community participation.

Some of coastal forests have rich in biodiversity which harbors various species of plants, including as habitat of critically endangered species, such as Sumatran orangutan and Sumatran tiger. Subsequently, undisturbed coastal forests including mangroves, littoral forests and peat swamp forests characterized by dense stands, mixed species and structures play an important role in coastal protection against tsunami. Therefore, the coastal vegetation is needed to conserve the biodiversity and to maintain the production capacity as part of sustainable and longlasting vegetation bioshield. Mansor (2003) stated that local people know more about the plants and animals in their own surroundings. Perhaps the local knowledge especially from the old folks should not be cast aside, and their participation should also be encouraged.

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REFERENCES

Ammon CJ, Ji C, Thio HK, Robinson D, Ni S, Hjorleifsdottir V, Kanamori H, Lay T, Das S, Helmberger D, Ichinose G, Polet P, Wald D. 2005. Rupture process of the 2004 Sumatra-Andaman earthquake. Science 308 (5725): 1133-1139.

Ayat A. 2002. Bad Behavior of Bluwok bird (*Mycteria cinerea* Raffles) in Pulau Rambut Wildlife Sactuary. [Hon. Thesis]. Faculty of Forestry, bogor Agricultural University. Bogor. [Indonesian]

Baba S. 2004. Keynote presentation: what we can do for mangroves. In: Vanucci M. (ed.) Mangrove management and conservation workshop, Okinawa, Japan, 2000. United Nations University Press, Tokyo

Bilham R. 2005. A flying start, then a slow slip. Science 308 (5725): 1126-1127.

Borrero JC. 2005a. Field data and satellite imagery of tsunami effects in Banda Aceh. Science 308 (5728): 1596-1596.

Borrero JC. 2005b. Field survey of northern Sumatra and Banda Aceh, Indonesia after the tsunami and earthquake of 26 December 2004. Seismol Res Lett 76 (3): 312-320.

Borrero JC, Synolakis CE, Fritz H. 2006a. Northern Sumatra field survey after the December 2004 great Sumatra earthquake and Indian Ocean tsunami. Earthquake Spectra 22 (S3): 93-104.

Borrero JC, Sieh K, Chlieh, M, Synolakis CE. 2006b. Tsunami inundation modeling for western Sumatra. Proc Natl Acad Sci USA 103 (52): 9673-19677.

Campbell SJ, Pratchett MS, Anggoro AW, Ardiwijaya RL, Fadli N, Herdiana Y, Kartawijaya T, Mahyiddin D, Mukminin A, Pardede ST, Rudi E, Siregar AM, Baird AH. 2007. Disturbance to coral reefs in Aceh, Northern Sumatra: impacts of the Sumatra-Andaman tsunami and pre-tsunami degradation. Atoll Res Bull 544: 55-78.

Chapman VJ. 1976. Mangrove Vegetation. J. Cramer, Vaduz.

Chen P, Liew SC, Kwoh LK. 2005. Tsunami damage assessment using high resolution satellite imagery: a case study of Aceh, Indonesia. IEEE Intl Symp Geosci Rem Sens 2:1405-1408.

Chlieh M, Avouac JP, Hjorleifsdottir V, Song TRA, Ji C, Sieh K, Sladen A, Hebert H, Prawirodirdjo L, Bock Y, Galetzka J. 2007. Coseismic slip and afterslip of the great Mw 9.15 Sumatra-Andaman earthquake of 2004. Bull Seismol Soc Amer 97 (1A): S152-S173.

Cochard R, Ranamukhaarachchi SL, Shivakoti GP, Shipin OV, Edwards PJ, Seeland KT. 2008. The 2004 tsunami in Aceh and Southern Thailand: a review on coastal ecosystems, wave hazards and vulnerability. Persp Pl Ecol Evol Syst 10 (1): 3-40.

Condit R, Pitman N, Leigh EG, Chave J, Terborgh J, Foster RB, Nunez P, Aguilar S, Valencia R, Villa G, Muller-Landau HC, Losos E, Hubbell SP. 2002. Beta-diversity in tropical forest trees. Science 295 (5555): 666-669.

Curtis JT, McIntosh RP. 1951. An upland forest continuum in the prairie-forest border region of Wisconsin. Ecology 32 (3): 476-496.

Dahdouh-Guebas F. 2006. Mangrove forests and tsunami protection. In: 2006 McGraw-Hill Yearbook of Science and Technology. McGraw-Hill Professional, New York.

Dahdouh-Guebas F, Jayatissa LP, Di Nitto D, Bosire JO, Lo Seen D, Koedam N. 2005. How effective were mangroves as a defence against the recent tsunami? Curr Biol 15 (12): R443-R447.

Duke NC, Ball MC, Ellison JC. 1998. Factors influencing biodiversity and distributional gradients in mangroves. Global Ecol Biogeogr 7: 27-47.

Ghobarah A, Saatcioglu M, Nistor I. 2006. The impact of the 26 December 2004 earthquake and tsunami on structures and infrastructure. Engineer Struct 28 (2): 312-326.

Giesen WBJT. 2004. Causes of peat swamp forest degradation in Berbak NP, Indonesia, and recommendations for restoration. Water for Food and Ecosystems Programme Project on:‘‘Promoting the river basin and ecosystem approach for sustainable management of SE Asian lowland peat swamp forests: case study Air Hitam Laut River basin, Jambi Province, Sumatra, Indonesia’’. ARCADIS Euroconsult, Arnhem.

Giesen W, van Balen B, Sukotjo, Siregar P. 1992. Singkil Barat Swamps (Aceh). In: Giesen W, van Balen B (eds) Several short surveys or Sumatra wetland: notes and observations. PHPA/AWB Sumatra Wetland Project Report No. 26.PHPA/AWB, Bogor.

Hagan AB, Foster R, Perera N, Gunawan CA, Silaban I, Yaha Y, Manuputty Y, Hazam I,Hodgson G. 2007. Tsunami impacts in Aceh Province and North Sumatra, Indonesia. Atoll Res Bull 544: 37-54.

Hamzah KA, Ismail P, Kassim AR, Hassan CH, Akeng G, Said NM. 2009. Ecological Characteristics of a Gonystylus bancanus-rich Area in Pekan Forest Reserve, Pahang, Malaysia. Trop Life Sci Res20 (2): 15-27.

Ibrahim FH, Lepun P. 2004. Tree species composition of Kuala Langat North peat swamp forest, Selangor. In: Mansor M, Ali A, Rieley J, Ahmad AH, Mansor A (eds) TropicalPeat Swamps: Safe-guarding a Global Natural Resource. Universiti Sains Malaysia, Pulau Pinang.

Imanuddin. 1999. Some Aspect of Nesting and Growth of Wilwo bird (*Mycteria cinerea*Raffles) in Pulau Rambut Wildlife Sanctuary, Jakarta. [Hon. Thesis]. Faculty of Forestry, Bogor Agricultural University, Bogor. [Indonesian]

Istomo. 2002. Phosphorous and Calcium Content and its Distribution on Soil and Peat-swamp Forest Plant: Study Case in Bagan Forest Management Unit area, Rokan Hilir District, Riau. [Dissertation]. Bogor Agricultural University, Bogor. [Indonesian]

Istomo. 2006. Phosphorous and Calcium Content on Soil and Biomass of Peat-swamp Forest in Forest Concessions Area of PT. Diamond Raya Timber, Bagan Siapi-api, Riau Province. Jurnal Manajemen Hutan Tropika 12 (3): 40-57. [Indonesian]

Iverson LR, Prasad AM. 2007. Using landscape analysis to assess and model tsunami damage in Aceh province, Sumatra. Landscape Ecol 22 (3): 323-331.

Jayatissa LP, Dahdouh-Guebas F, Koedam N. 2002. A review of the floral composition and distribution of mangroves in Sri Lanka. Bot J Linn Soc 138 (1): 29-43.

Kartawinata K. 1990. A review of natural vegetation studies in Malesia, with special reference to Indonesia. In: Baas P, Kalkman K, Geesink R (eds) The Plant Diversity of Malesia. Springer, Netherlands.

Kartawinata K. 2005. Six decades of natural vegetation studies in Indonesia. Six Decades of Science and Scientists in Indonesia. Naturindo, Bogor.

Kathiresan K, Bingham BL. 2001. Biology of mangroves and mangrove ecosystems. Adv Mar Biol 40: 81-251.

Kathiresan K, Rajendran N. 2005. Coastal mangrove forests mitigated tsunami. Estuar Coast Shelf Sci 65: 601-606.

Kayanne H, Ikeda Y, Echigo T, Shishikura M, Kamataki T, Satake K, Malik JN, Basir SR, Chakrabortty GK, Roy AKG. 2007. Coseismic and postseismic creep in the Andaman Islands associated with the 2004 Sumatra-Andaman earthquake. Geophys Res Lett 34:L01310.

Kovach Computing Services. 2002. Multivariate Statistical Package. Version 3.13d. (MVSP). http://www.kovcomp.com.

Kusmana C, Abe K, Watanabe H. 1992.Species composition and structure of a mangrove forest in East Sumatra, Indonesia. Indonesian J Trop Agric 3 (2): 67-77.

Kusmana C, Watanabe H. 1991. Zonation pattern of a mangrove forest in Riau, eastern Sumatra, Indonesia. Rimba Indonesia 24 (3-4): 13-18.

Kusuma MSB, Adityawan MB, Farid M. 2008. Modeling two dimension inundation flow generated by tsunami propagation in Banda Aceh city. In: Proceedings of International Conference on Earthquake Engineering and Disaster Mitigation, Banda Aceh.

Laumonier Y. 1997. The vegetation and physiography of Sumatra. Kluwer, Dordrecht, Netherlands.

Lay T, Kanamori H, Ammon CJ, Nettles M, Ward SN, Aster RC, Beck SL, Bilek SL, Brudzinski MR, Bhutler R, DeShon HR, Ekstrom G, Satake K, Sipkin S. 2005. The great Sumatra-Andaman earthquake of 26 December 2004. Science 308 (5725): 1127-1133.

Liew SC, Gupta A, Wong PP, Kwoh, LK. 2010. Recovery from a large tsunami mapped over time: the Aceh coast, Sumatra. Geomorphology 114 (4): 520-529.

Lugo AE, Snedaker SC. 1974. The ecology of mangroves. Ann Rev Ecol Syst5: 39-64.

Magurran AE. 2004. Measuring Biological Diversity. Blackwell, Oxford.

Mahmud A. 1991. Abundance and Distribution Pattern of Merandai Birds in Pulau Rambut Nature Reserve. Faculty of Forestry, bogor Agricultural University. Bogor. [Indonesian]

Mansor A, Othman AS. 2003. The diversity and species composition of forest vegetation in Pantai Acheh Forest Reserve, Penang, Malaysia. In: Chai LK (ed.) Pantai Acheh Forest Reserve: the Case for a State Park.Penerbit Universiti Sains Malaysia, Pulau Pinang.

Mansor M. 2003. The conservation of Pantai Acheh Forest Reserve; the Universiti Sains Malaysia’s involvement. In: Chai LK (ed.) Pantai Acheh Forest Reserve: the Case for a State Park. Penerbit Universiti Sains Malaysia, Pulau Pinang.

Mansor M. 2004. The uniqueness of tropical peat swamp ecosystem: a significant habitat for rare and endangered species. In: Mansor M, Ali A, Rieley J, Ahmad AH, Mansor A (eds) Tropical Peat Swamps: Safe-guarding a Global Natural Resource. Universiti Sains Malaysia, Pulau Pinang.

Mardiastuti A. 1992. Habitat and Nest-site Characteristics of Waterbirds Indonesia Pulau Rambut Nature Reserve, Jakarta Bay, Indonesia.[Dissertation]. Michigan State University, Michigan.

Mazda Y, Magi M, Kogo M, Hong, PN. 1997a. Mangroves as a coastal protection from waves in the Tong King delta, Vietnam. Mangroves and Salt Marshes 1 (2): 127-135.

Meilianda E, Dohmen-Janssen CM, Maathuis BHP, Hulscher SJMH, Mulder JPM. 2010. Short-term morphological responses and developments of Banda Aceh coast, Sumatra Island, Indonesia after the tsunami on 26 December 2004. Mar Geol 275 (1): 96-109.

Natawidjaja DH, Triyoso W. 2007. The Sumatran Fault Zone—from Source to Hazard. J Earthquake Tsunami 1 (1): 21-47.

Onrizal, Kusmana C. 2004. Ecological studies on littoral forest in Pulau Rambut Wildlife Reserve, Jakarta bay. Jurnal Komunikasi Penelitian 16: 77-83.

Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. 2009. Agroforestree Database: a tree reference and selection guide version 4.0. http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp

Page SE. 2004. The natural resource functions of tropical peatlands. In: Mansor M, Ali A, Rieley J, Ahmad AH, Mansor A (eds) Tropical Peat Swamps: Safe-guarding a Global Natural Resource. Universiti Sains Malaysia, Pulau Pinang.

Rieley JO. 2004. Lowland tropical peatlands of Southeast Asia: importance, impacts and prospects. In: Mansor M, Ali A, Rieley J, Ahmad AH, Mansor A (eds) Tropical Peat Swamps: Safe-guarding a Global Natural Resource. Universiti Sains Malaysia, Pulau Pinang.

Rijksen HD, Diemont WH, Griffith M. 1997. The Singkil swamp: the kidneys of the Leuser ecosystem in Aceh, Sumatra, Indonesia. In: Rieley JO, Page SE (eds) Biodiversity and Sustainability of Tropical Peatlands.Samara Publishing, London.

Samarakoon MB, Tanaka N, Iimura K. 2013. Improvement of effectiveness of existing *Casuarina equisetifolia* forests in mitigating tsunami damage. J Environ Manag 114: 105-114.

Satyanarayana B, Idris IF, Mohamad KA, Husain ML, Shazili NA, Dahdouh-Guebas F. 2010. Mangrove species distribution and abundance in relation to local environmental settings: a case-study at Tumpat, Kelantan Delta, east coast of peninsular Malaysia. Botanica Marina 53 (1): 79-88.
Shmida A. 1984. Whittaker's plant diversity sampling method. Israel J Bot 33: 41-46.
Shofiyati R, Dimyati RD, Kristijono A, Wahyunto. 2005. Tsunami effect in Nanggroe Aceh Darussalam and North Sumatra Provinces, Indonesia. Asian J Geoinform 5 (3): 100-111.
Subarya C, Chlieh M, Prawirodirdjo L, Avouac JP, Bock Y, Sieh K, Meltzner AJ, Natawidjaja DH, McCaffrey R. 2006. Plate-boundary deformation associated with the great Sumatra-Andaman earthquake. Nature 440 (7080): 46-51.
Tanaka N. 2009. Vegetation bioshields for tsunami mitigation: review of effectiveness, limitations, construction, and sustainable management. Lands Ecol Eng 5 (1): 71-79.
Tanaka N, Jinadasa KBSN, Mowjood MIM, Fasly MSM. 2011. Coastal vegetation planting projects for tsunami disaster mitigation: effectiveness evaluation of new establishments. Lands Ecol Eng 7: 127-135.
Tanaka N, Sasaki Y, Mowjood MIM, Jinadasa KBSN, Homchuen S. 2007. Coastal vegetation structures and their functions in tsunami protection: experience of the recent Indian Ocean tsunami. Lands Ecol Eng 3 (1): 33-45.
Thuy NB, Tanimoto K, Tanaka N, Harada K, Iimura K. 2009. Effect of open gap in coastal forest on tsunami run-up-investigations by experiment and numerical simulation. Ocean Eng 36 (15): 1258-1269.
Tomascik T, Mah AJ, Nontji A, Moosa MK. 1997. The Ecology of the Indonesian Seas. Periplus, Singapore.
Tomlinson PB. 1986. The Botany of Mangroves. Cambridge University Press, Cambridge.
Whitten T, Damanik SJ, Anwar J, Hisyam N. 1997. The Ecology of Sumatra. Periplus, Singapore.
Wich S,Meijaard E, Marshall JA, Husson S, Ancrenaz M, Lacy RC,van SchaikCP, Sugardjito J,Simorangkir T, Traylor-Holzer K, Doughty M,Supriatna J, Dennis R, Gumal M, Knott CD,Singleton I. 2008. Distribution and conservation status of the orang-utan (*Pongo* spp.) on Borneo and Sumatra: how many remain? Oryx 42 (03): 329-339.
Wong PP. 2009. Impacts and recovery from a large tsunami: coasts of Aceh. Polish J Environ Stud 18 (1): 5-16.
Yanagisawa H, Koshimura S, Goto K, Miyagi T, Imamura F, Ruangrassamee A, Tanavud C. 2009a. The reduction effects of mangrove forest on a tsunami based on field surveys at Pakarang Cape, Thailand and numerical analysis. Estuar Coast Shelf Sci 81 (1): 27-37.
Yanagisawa H, Koshimura S, Goto K, Miyagi T, Imamura F, Ruangrassamee A, Tanavud C. 2009b. Damage to mangrove forest by 2004 tsunami at Pakarang Cape and Namkem, Thailand. Polish J Environ Stud 18 (1): 35-42.
Yanagisawa H, Koshimura S, Miyagi T, Imamura F. 2010. Tsunami damage reduction performance of a mangrove forest in Banda Aceh, Indonesia inferred from field data and a numerical model. J Geophys Res 115 (C06032): 1-11.

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The diversity and distribution of Holothuroidea in shallow waters of Baluran National Park, Indonesia

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Abstract. *Siddiq AM, Atmowidi T, Qayim I. 2015. The diversity and distribution of Holothuroidea in shallow waters of Baluran National Park, Indonesia. Biodiversitas 17: 55-60.* A study of the diversity and distribution of sea cucumber (Holothuroidea) in shallow waters at Baluran National Park, East Java, Indonesia was carried out from July until September 2015. The method used in this study was systematic transect in low tide condition. Samples were collected by hands at intertidal sites. Identification of sea cucumber species based on morphological ossicles. Twenty one species of Holothuroidea belonging two orders and four families were found in this study. The most dominant family found was Holothuriidae (16 species), followed by Stichopodidae (2 species), Synaptidae (2 species), and Chiridotidae (1 spesies). Four species (*Holothuria olivacea*, *H. verrucosa, Labidodemas rugosum*, and *Chiridota smirnovi*) are new record for Java waters and one species (*H. papillifera*) is a new record for Indonesian waters. Two morphospecies (*H*. aff. *macroperona* and *Stichopus* cf. *monotuberculatus*) need reconfirmation to species level. The highest abundance species of Holothuroidea was found at under rock with 15 species. Whereas, the highest number of individuals was found in seagrass areas with 5457 individuals. *H. atra* has extensive habitat distribution, such as seagrass, macroalgae, coral reef, dead coral, sand, and under rock.

Key words: Baluran National Park, distribution, diversity, Holothuroidea, shallow waters

INTRODUCTION

Sea cucumbers (Holothuroidea: Echinodermata) are found in various substrates and depths (Sluiter 1901; Samyn et al. 2006; Woo et al. 2013). Approximately, 300 species of sea cucumber were reported in the shallow to deep sea of Indonesian waters (Sluiter 1901; Clark and Rowe 1971; Massin 1996, 1999; Massin and Tomascik 1996; Purwati and Wirawati 2012). Diversity of Holothuroidea in Indonesian waters has been reported, such as Wirawati et al. (2007), Yusron (2006, 2007, 2009a, b, 2010, 2012), Setyastuti (2009), Yusron and Susetiono (2006, 2010), Purwati and Wirawati (2008, 2009, 2011, 2012), Selanno et al. (2014). In marine ecosystem, Holothuroidea help the process of decomposition of organic matter in sediments and produce nutrients in the food chain (Bakus 1973).

Some species of Holothuroidea have a high economic value, known as Trepang or Beche-de-Mer. In Asian tropical and sub-tropical waters, *Actinopyga*, *Bohadschia*, *Holothuria*, and *Stichopus* are exploited as food and medicinal product industry. Whereas, in temperate waters of Japan, *Apostichopus japonicus* is the most captured by fishery (Conand and Muthiga 2007). Some species of Holothuroidea are listed into Appendix II CITES (Convention on the International Trade in Endangered Species) (Polidoro et al. 2011), because the population of the species declines every year in the world, including in the Indonesian waters (Purwati 2005; Setyastuti and Purwati 2015).

Recently, 11 species of sea cucumbers are reported as new records in Johor strait and 10 species are potentially as new species (Woo et al. 2014; Ong and Wong 2015). Kamarudin et al. (2010) also stated that approximately 80 species of sea cucumbers are found in Malaysian waters. The diversity of sea cucumber in Indonesian waters has been studied only in some areas, while exploitations of some species that have economic value occur continuously (Purwati and Wirawati 2009). Expeditions of Holothuroidea in Indonesian waters, including international expeditions, were conducted in the eastern of Indonesia (Sluiter 1901; Massin 1996; Massin 1999). Study of sea cucumber in national parks in Indonesia is still limited.

Baluran National Park (BNP) is a conservation area located in the Situbondo district, East Java, Indonesia. Geographically, this park is directly adjacent to two open waters, Bali and Madura straits. BNP is located in the northern coast of Java, which has a variety of substrates and diverse marine habitat, such as sand, mud, rocks, dead coral, seagrass, macroalgae and coral reefs. This paper describes the diversity, distribution, and habitats of each Holothuroidea in shallow waters of BNP.

MATERIALS AND METHODS

Study sites

The study was conducted from July to September 2015 in shallow waters of BNP. Samplings of Holothuroidea were taken in low tide condition in three locations, i.e. Bama, Air Karang, and Bilik (Figure 1). **Bama** (100,000 m^2, 7^{o}50'72.1"S, 114^{o}27'80.4"E) was dominated by the substrate of sand and dead coral and covered by several types of seagrass and macroalgae. Coral reefs were found in the intertidal edge of the area. **Air Karang** (112,000 m^2, 7^{o}47'04.8"S, 114^{o}25'190"E) was dominated by coral reef and rocks with sand substrates, muddy-sand, and dead coral. Whereas, **Bilik** (60,000 m^2, 7^{o}45'28.1"S, 114^{o}22'75.5"E) was dominated by rocks with a substrate of sand and mud. Dead corals as well as coral reefs were found in the intertidal edge of the area.

Observation of Holothuroidea diversity

The method used to observe Holothuroidea in each location was transect at low tide condition. Observations of sea cucumbers were conducted in transect those set up in intertidal sites. We recorded the number of individuals and their coordinates of each sea cucumber found by Global Positioning System (GPS), as well as the characters of the habitats (coral reef, dead coral, macroalgae, seagrass, under rock, and sand). Specimens of Holothuroidea collected were anaesthetized by 7.5% Magnesium Chloride solution and then preserved in 70% denatured ethanol.

Identification of Holothuroidea specimens

Identification of species Holothuroidea was conducted based on morphological ossicles using Clark (1938), Cherbonnier (1952), Cherbonnier (1988), Clark and Rowe (1971), Massin (1996), Massin (1999), Samyn and Massin (2003). Technique for ossicles observation based on Purcell et al. (2012). Ossicles observations were carried out using Leica DMRBE microscope. All specimens of Holothuroidea were deposited in the Oceanography Research Centre, Jakarta, Indonesia.

Data analysis

Diversity of Holothuroidea was analyzed using Shannon-Wiener index (H'), evenness index (J'), and species dominant (D) performed by PAST software (http://folk.uio.no/ohammer/past) version 2.17c. Distribution pattern of Holothuroidea was constructed by mapping of each coordinate points of Holothuroidea in their habitats. Data of Holothuroidea coordinates and their habitats were exported into shape file format and processed by QGIS software (http://qgis.org/). Basic maps were obtained from Indonesian Geospatial Agency (http://www.bakosurtanal.go.id/peta-rupabumi/).

RESULTS AND DISCUSSION

The diversity of Holothuroidea

Twenty one species of Holothuroidea belonging two orders (Aspidochirotida and Apodida), four families, and eight genera were found in this study. The family Holothuriidae (16 species) has highest species richness, followed by Stichopodidae (2 species) and Synaptidae (2 species), and Chiridotidae (1 species) (Table 1). The highest number of individuals found in BNP was *Holothuria atra* (9935 individuals). The species is found in various substrates, such as seagrass, macroalgae areas, dead coral, coral reef, rock, and sand. Species *Chiridota smirnovi* was found at under rock with lowest abundance. In Bama, dominant species found was *H. atra* (9875 individuals), whereas in Air Karang and Bilik were *Opheodesoma grisea* (835 and 198 individuals), respectively. The highest diversity of Holothuroidea was found in Bilik (S=15, H'=1.335, J'=0.506), followed by Air

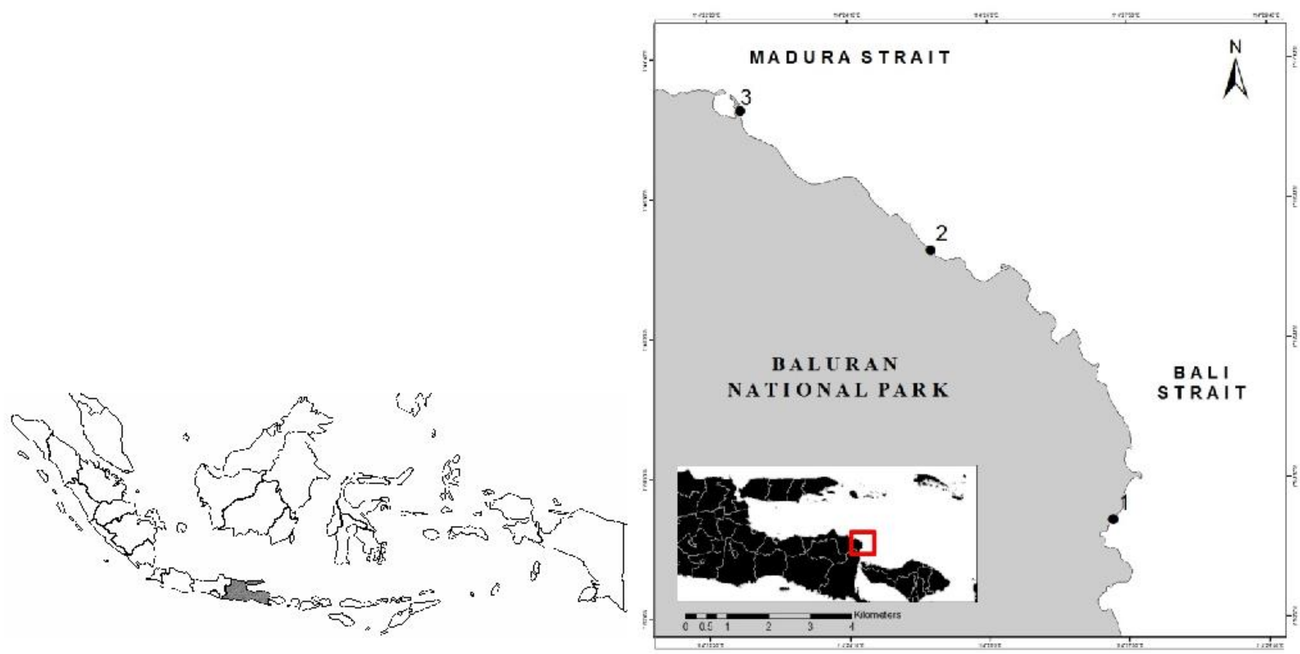

Figure 1. Study sites in shallow waters of Baluran National Park, East Java, Indonesia: 1. Bama, 2. Air Karang, 3. Bilik

Karang (S=13, H'=0.666, J'=0.259), and Bama (S=9, H'=0.146, J'=0.066). In contrast, the highest dominance was found in Bama (D=0.951), followed by Air Karang (D=0.761), and Bilik (D=0.404), respectively (Table 1).

Spatial distribution pattern of Holothuroidea

Distribution of Holothuroidea related to habitat composition (Figure 2). Holothuriidae and Synaptidae were found in Air Karang, Bama, and Bilik. Stichopodidae was found in Air Karang and Bilik, while Chiridotidae in Bilik. Family Holothuriidae dominated in all habitats, such as macroalgae, seagrass, coral reef, dead coral, under rock, and sand. In contrast, Chiridotidae has limited habitat at under rock. Commonly, Stichopodidae is found at under rock, while Synaptidae is found in seagrass and macroalgae. Only few individuals of Stichopodidae and Synaptidae have been found in dead coral.

Habitat preference of Holothuroidea

From 21 species Holothuroidea found in shallow waters of BNP, seven species (33%) were found at under rock. Fourteen species (67%) were found at more than one habitat, i.e., coral reef, dead coral, macroalgae, sand, and seagrass. The highest abundance of Holothuroidea is found at under rock (15 species), followed by dead coral (10 species), sand (9 species), seagrass (7 species), macroalgae (5 species), and coral reef (2 species). In contrast, the highest number of individuals of Holothuroidea was found in seagrass (5457 individuals), followed by sand (2658 individuals), coral reef (1014 individuals), dead coral (998 individuals), macroalgae (920 individuals), and at under rock (194 individuals) (Figure 3).

Discussion

Based on this study, diversity of Holothuroidea in shallow waters at BNP is higher than at Moti Island (8 species; Yusron 2007), Minahasa (8 species; Yusron 2009), West Lombok (14 species; Purwati and Wirawati 2009), Prigi Bay (7 species; Purwati and Wirawati 2012), South China Sea, Sulu Sea and Sulawesi Sea (12 species; Woo et al. 2013), but lower than in Central Moluccas (22 species; Selanno 2014), Ambon (53 species; Massin 1996) and Spermonde Archipelago (56 species; Massin 1999). Four species (*H. olivacea*, *H. verrucosa*, *L. rugosum*, and *C.*

Tabel 1.Number of individuals and species of Holothuroidea collected in shallow waters at Baluran National Park

Order: Family Species	Number of individuals				Habitat
	AK	BM	BL	Total	
Aspidochirotida: Holothuriidae					
Actinopyga echinites Jaeger, 1833	0	0	3	3	DC, SG
Bohadschia marmorata Jaeger, 1833	0	2	2	4	CR, DC, MA
B. similis Semper, 1868	2	0	2	4	DC, MA, SG
Holothuria (Selekonthuria) erinaceus Semper, 1868	0	3	0	3	UR
H. (Halodeima) atra Jaeger, 1833	15	9875	45	9935	CR, DC, MA, SD, SG, UR
H. (Mertensiothuria) hilla Lesson, 1830	4	0	39	43	SG, UR
H. (Mertensiothuria) leucospilota Brandt, 1835	14	16	8	38	DC, UR
H. (Mertensiothuria) papillifera Heding inMortensen, 1938	0	0	2	2	UR
H. (Thymiosycia) impatiens Forskal, 1775	26	2	1	29	UR
H. (Thymiosycia) aff. *Macroperona* Clark, 1938	0	0	7	7	DC, UR
H. (Stauropora) fuscocinerea Jaeger, 1833	10	0	0	10	DC, UR
H. (Stauropora) olivacea Ludwig, 1835	8	0	0	8	UR
H. (Lessonothuria) pardalis Salenka, 1867	0	4	3	7	SD, UR
H. (Lessonothuria) verrucosa Salenka, 1867	4	0	0	4	UR
H. (Metriatyla) scabra Jaeger, 1833	7	4	0	11	SD, SG
Labidodemas rugosum Ludwig, 1875	0	0	4	4	UR
Aspidochirotida: Stichopodidae					
Stichopus cf. *monotuberculatus* Quoy & Gaimard, 1833	4	0	5	9	DC, UR
S. quadrifasciatus Massin, 1999	2	0	0	2	DC, UR
Apodida: Synaptidae					
Opheodesoma grisea Semper, 1868	835	121	198	1154	DC, MA, SG
Synapta maculata Chamisso & Eisenhardt, 1821	28	102	6	136	SG, MA
Apodida:Chiridotidae					
Chiridota smirnovi Massin, 1996	0	0	1	1	UR
Number of individual	959	10129	326	11414	
Number of species	13	9	15		
Shannon-wiener index (H')	0.666	0.146	1.335		
Evenness index (J')	0.259	0.066	0.506		
Dominance index (D)	0.761	0.951	0.404		

Reference: Clark (1938); Cherbonnier (1952, 1988); Massin (1996, 1999); Samyn and Massin (2003). Note: AK=Air Karang, BM=Bama, BL=Bilik, CR=coral reef, DC=dead coral, MA=macroalgae, SG=seagrass, SD=sand, UR=under rock

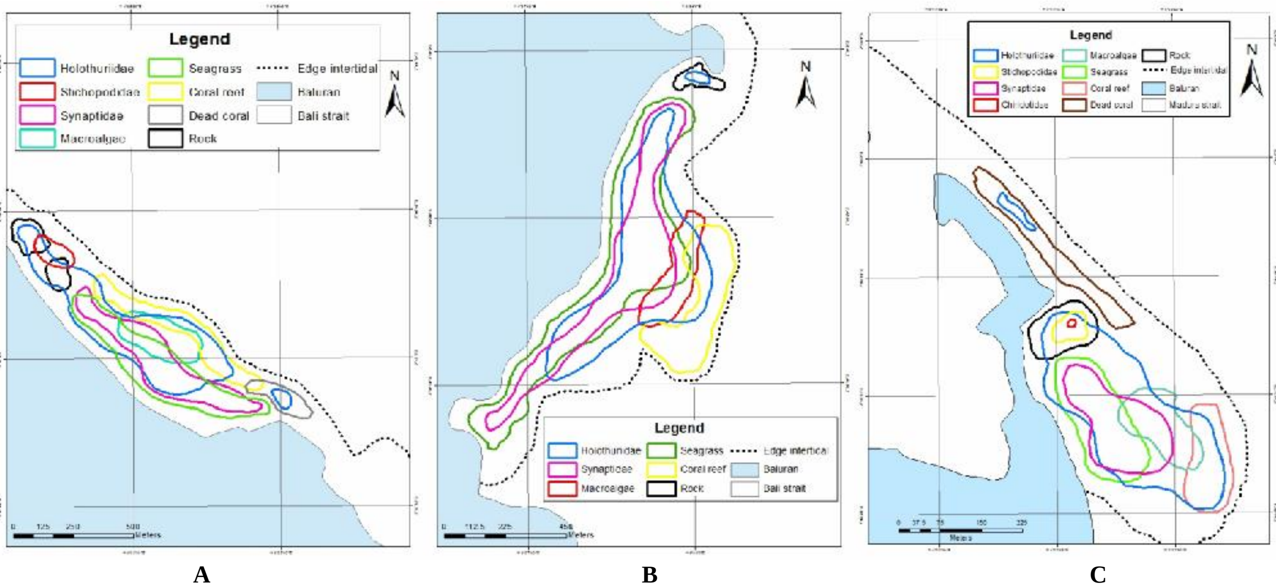

Figure 2. Spatial distribution of Holothuroidea species in shallow waters at Baluran National Park: A. Air Karang, B. Bama, and C. Bilik

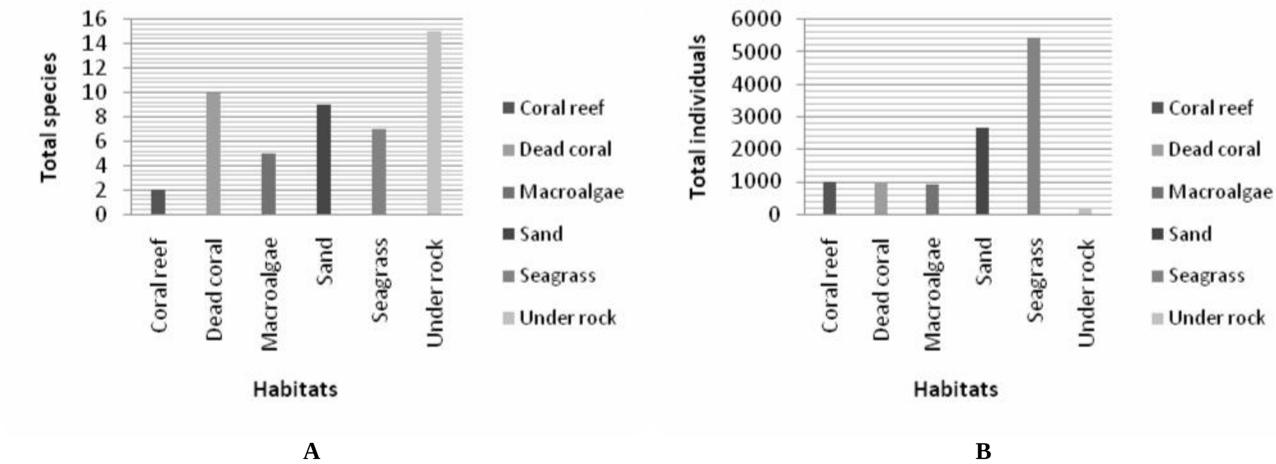

Figure 3. Number of species (A) and individuals (B) of Holothuroidea found at five habitats in BNP

smirnovi) are new record in Java waters. Previously, the species were reported in Sulawesi, Nusa Tenggara, and Irian Jaya waters (Sluiter 1901; Massin 1996; Massin 1999; Purwati and Wirawati 2011). One species (*H. papillifera*) is a new record in Indonesia. Previously, the species was reported in the Red Sea (Gardaqha, Abu Sadaf, and Abu Fanadir) (Samyn and Massin 2003). Two morphospecies (*H.* aff. *macroperona* and *S.* cf. *monotuberculatus*) need reconfirmation to species level. Based on ossicles morphology, *H.* aff. *macroperona* is similar with *H. macroperona*. This species is only distributed in Western Australia (Clark 1938). *S.* cf. *monotuberculatus* is similar with *S. monotuberculatus* (Cherbonnier 1952) and the species widely distributed across the tropical Indo-West Pasific (Massin et al. 2002).

Distribution of sea cucumber was influenced by microhabitat. Woodby et al. (2000) reported that some sea cucumbers prefer harder substrate, such as sand, rocks and dead coral related to their locomotion. Based on this study, approximately 71.4% species of Holothuroidea (family Chiridotidae, Stichopodidae, and some species of Holothuroiidae) was found at under rocks around the reef. Purwati and Wirawati (2012) also reported in Prigi Bay, six species of sea cucumbers (*H. atra*, *H. erinaceus*, *H. mactanensis*, *Afrocucumis africana*, *Chiridota* sp., and *Polycheira rufescens*) were found at under rock and some individuals in sand-boulders. In shallow waters of BNP, dead coral and sand are the habitat prefered by Holothuroidea. The similar result also was reported by Woo et al. (2013), in South China Sea, Sulu Sea, and Sulawesi Sea, about 83% sea cucumber belonging family Holothuriidae, Synaptidae, and Stichopodidae prefered dead coral and sand as their habitat.

The highest number of individuals was found in seagrass areas, while the lowest at under rock. *H. atra* has a highest individual number, mainly in seagrass areas. It is showed by high dominance index with density 5-15 individuals/m^2. In the tropical area, *H. atra* has a density 5-35 individuals/m^2 (Bakus 1973). Totally, 9935 individuals of the species are found in shallow waters of BNP. When low tide conditions, this species immerse themselves in the sand substrates. Some individuals were found with sand grain attached in their body. *H. atra* is the common species from Indonesian waters (Yusron 2006, 2007, 2009; Purwati and Wirawati 2009, 2012) and Indo Pasific area (Massin 1999; Jontila 2014). In BNP, *C. smirnovi* is only found one individu. Previously, the species was reported in Ambon water (Massin 1996).

Eighteen species of order Aspidochirotida (*A. echinites*, *B. marmorata*, *B. similis*, *H. erinaceus*, *H. atra*, *H. hilla*, *H. leucospilota*, *H. papillifera*, *H. impatiens*, *H.* aff. *macroperona, H. fuscocinerea*, *H. olivacea, H. pardalis*, *H. verrucosa*, *H. scabra*, *L. rugosum*, *S.* cf. *monotuberculatus*, and *S. quadrifasciatus*) dominated in shallow water of BNP. Domination of order Aspidochirotida also was reported in Western Indian Ocean with 28 species and some species are new records (Conand et al. 2010). While, order Apodida in BNP has lower species richness (*O. grisea*, *Synapta maculata*, and *C. smirnovi*). The species were found in seagrass, macroalgae, dead coral, and under rock. *S. maculata* (Synaptidae) occupied in seagrass areas (Yusron 2006; Yusron and Susetiono 2006). Color variations of the species was found in this study. *O. grisea* has more than three color variations. Purwati and Wirawati (2008) also reported *O. grisea* from Timor Island, East Nusa Tenggara has three color variations. Massin (1999) stated that color variations of *O. grisea* depend on the nature of sediment type.

Marine conditions of Baluran National Park as a conservation area, can not ensure the existence of Holothuroidea from over exploitation. Many fishermen are periodically enters in this area, especially in Air Karang, and take some Holothuroidea that have high economic value. Approximately, 58 species of Holothuroidea in the world are listed as the fisheries commodities (Conand and Muthiga 2007; Choo 2008; Purcell et al. 2012), and 27 species of them are found in Indonesian waters (Purwati 2005; Setyastuti and Purwati 2015). Some species found in the study, such as *A. echinites*, *B. marmorata*, *B. similis*, *H. atra*, *H. hilla*, *H. impatiens*, *H. leucospilota*, *H. scabra* and *S. quadrifasciatus* were reported as fisheries commodities. Conand et al. (2014) reported population of *H. scabra* decrease and the species is listed as endangered category of Red List Threatened Species (IUCN 2015). *H. scabra* is endangered aquatic species in Indonesia (Ubaidillah et al. 2013). Cultivation or aquaculture of some species that have high commercial value, such as *H. scabra* (Conand and Muthiga 2007; Leopold et al. 2015) can be conducted in Baluran National Park. In addition, sustainable expeditions of sea cucumber are needed in Indonesian waters, especially in deep sea waters.

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REFERENCES

Bakus GJ. 1973. The biology and ecology of tropical holothurians. in: Q.A. Jones and R. Endean (eds). Biology and geology of coral reefs. Vol. I. Academic Press, London.

Cherbonnier G. 1952. Les Holothuries de Quoy et Gaimard. Memorie de l 'Institut royal des sciences naturelles de Belgique 2 serie 44: 1-50.

Cherbonnier G. 1988. Echinodermes: Holothurides. Faune de Madagascar 70: 1-292.

Choo PS. 2008. Population status, fisheries and trade of sea cucumbers in Asia. In V. Toral-Granda, A. Lovatelli, M. Vasconcellos (eds). Sea cucumbers. A global review of fisheries and trade. FAO Fish Aquacult Tech 516: 81-118.

Clark HL. 1938. Echinoderms from Australia. Printed For The Museum, Cambridge.

Clark AM, Rowe FWE. 1971. Monograph of shallow-water Indo-West Pacific Echinoderms. Trustees of The British Museum Zoology, London.

Conand C, Muthiga NA. 2007. Commercial Sea cucumbers: a review for the Western Indian Ocean. WIOMSA 5: 66.

Conand C, Michonneau F, Paulay G, Bruggemann H. 2010. Diversity of the Holothuroidea fauna (Echinodermata) at La Reunion (Western Indian ocean). WIOJMS 9 (2): 145-12.

Conand C, Polidoro B, Mercier A, Gamboa R, Hamel JF, Purcell S. 2014. The IUCN Red List assessment of aspidochirotid sea cucumbers and its implications. SPC Beche-de-mer Info Bull 35: 3-7.

Jontila JBS, Balisco RAT, Matillano JA. 2014. The sea cucumbers (Holothuroidea) of Palawan, Philippines. AACL Bioflux 7 (3): 194-206.

Kamarudin KR, Rehan AM, Hashim R, Usup G. 2010. Review An update on diversity of sea cucumbers (Echinodrmata: Holothuroidea) in Malaysia. Malay Nat J 62 (3): 315-334.

Leopold M, Ham J, Kaku R, Gereva S, Raubani J, Moenteapo Z. 2015. Spatial sea cucumber management in Vanuatu and New Caledonia. SPC Beche-de-mer Info Bull 35: 3-18.

Massin C, Tomascik T. 1996. Two new Holothurians (Echinodermata: Holothuroidea) from an Anchialine Lagoon of an uplifted atoll, Kakaban Island, East kalimantan, Indonesia. Raffles Bull Zool 44 (1): 157-172.

Massin C. 1996. Results of the Rumphius Biohistorical Expedition to Ambon (1990). Part 4. The Holothuroidea (Echinodermata) collected during the Rumphius Biohistorical Expedition. Zool Verh 307: 1-53.

Massin C. 1999. Reef dwelling Holothuroidea (Echinodermata) of the Spermonde Archipelago (South-West Sulawesi, Indonesia). Zool Verh 329: 1-144.

Massin C, Zulfigar Y, Tan Shau Hwai A, Rizal Boss SZ. 2002. The genus Stichopus (Echinodermata: Hoothuroidea) from the Johor Marine Park (Malaysia) with the description of two new species. Biologie 72: 73-99.

Ong JY, Wong HPS. 2015. Sea cucumbers (Echinodermata: Holothuroidea) from Johor Straits, Singapore. Raffles Bull Zool 31: 273-291.

Polidoro B, Tognelli M, Harwell H, Elfes C, Cepeda A, Gonzalez-Maya JF, Zarate-Charry DA, Alvarado JJ, Benavides M, Conand C, Ortiz EP, Gamboa R, Hamel JF, Mercier A, Purcell S, Toral-Granda V. 2011. IUCN Red List workshop for sea cucumbers. SPC Beche-de-mer Info Bull 34: 65.

Purcell SW, Samyn Y, Conand C. 2012. Commercially important sea cucumbers of the world. FAO Species Catalogue for Fishery Purposes, Rome.

Purwati P. 2005. Teripang Indonesia: Composition species and fisheries history. Oseana 30 (2): 11-18. [Indonesian]
Purwati P, Wirawati I. 2008. Synaptidae (Echinodermata: Apodidae) from Lamun Elnusa, Timor Island, East Nusa Tenggara. Oldi 34 (3): 371-384. [Indonesian]
Purwati P, Wirawati I. 2009. Holothuriidae (Echinodermata, Holothuroidea, Aspidochirotida) of shallow waters of West Lombok Part I. Genera Holothuria. J Oseanolgi 2 (1/2): 1-25. [Indonesian]
Purwati P, Wirawati I. 2011. Holothuriidae (Echinodermata, Holothuroidea, Aspidochirotida) of shallow waters of West Lombok Part II. Genera Actinopyga, Bohadschia, Labidodemas, Pearsonothuria. J Oseanologi 3: 1-10. [Indonesian]
Purwati P, Wirawati I. 2012. Sea cucumbers of Teluk Prigi, Southern Coast of Java Province. Oldi 38 (2): 241-254.
Samyn Y, Massin C. 2003. The holothurians subgenus Mertensiothuria (Aspidochirotida: Holothuriidae) revisited. J Nat Hist 37 (20): 2487-2519.
Samyn Y, Vandenspiegel, Massin C. 2006. Taxonomie des Holothuries des Comoros. Abc Taxa 1: 130.
Selanno DAJ, Natan YL, Uneputty PrA, Lewerissa YA. 2014. Ecological Study of Sea Cucumber Central Moluccas. IOSR-JAVS 7(1): 21-28.
Setyastuti A. 2009. Sea Cucumber (Echinodermata: Holothuroidea: Stichopodidae, Holothuriidae, Synaptidae) of West Seram, Maluku, Indonesia. Oldi 35: 369-396.
Setyastuti A, Purwati P. 2015. Speciest list of Indonesian trepang. SPC Beche-de-mer Info Bull 35: 19-25.
Sluiter CPh. 1901. Die Holothurien der Siboga Expedition. Siboga Exped 44: 1-142.
Ubaidillah R, Marwoto RM, Hadiaty RK, Fahmi, Wowor D, Mumpuni, Pratiwi, R, Tjakrawidjaja AH, Mudhiono, Hartati ST, Heryanto, Riyanto A, Mujiono N. 2013. Endangered of aquatic biota in Indonesia. Direktorat Konservasi Kawasan and Jenis Ikan, Ditjen Kelautan, Pesisir, and Pulau-Pulau Kecil, Kementerian Kelautan and Perikanan, Lembaga Ilmu Pengetahuan Indonesia, Jakarta. [Indonesian]
Wirawati I, Setyastuti A, Purwati P. 2007. Sea cucumber members of Famili Stichopodidae (Aspidochirotida, Holothuroidea, Echinodermata) collection of Puslit Oseanografi Lembaga Ilmu Pengetahuan Indonesia Jakarta. Oldi 33: 355-380. [Indonesian]
Woo SP, Yasin Z. Ismail SH, Tan SH. 2013. The Distribution and Diversity of Sea Cucumbers In The Coral Reefs Of The South China Sea, Sulu Sea, and Sulawesi Sea. Deep-Sea Res II 96: 13-18.
Woo SP, Teh CP, Norhanis MR, Nithiyaa N, Amelia-ng PF, Zulfigar Y, Tan SH. 2014. Sea cucumber species of the Merambong Shoal with notes on the distribution and habitat of the dominant species. Malay Nat J 66: 139-145.
Woodby D, Smiley S, Larson R. 2000. Depth and habitat distribution of Parastichopus californicus near Sitka, Alaska. Alaska Fish Res Bull 7: 22-32.
Yusron E. 2006. Echinodermata in Saleh Bay, Sumbawa, West Nusa Tenggara. Oldi 40: 43-52. [Indonesian]
Yusron E, Susetiono. 2006. Composition of speceis Echinodermata in Tanjung Pai Padaido waters, Biak Numfor-Papua. J Fish Sci 8 (27): 282-289.
Yusron E. 2007.Resources of Teripang (Holothuroidea) in Moti Island, North Maluku. Oldi 33: 111-121. [Indonesian]
Yusron E. 2009a. The diversity of Echinodermata in Kuta Bay, West Nusa Tenggara. Oldi 13: 45-49. [Indonesian]
Yusron E. 2009b. Biodiversity of Echinodermata Fauna in Selat Lembeh Waters, Bitung-North Sulawesi. Oldi 35 (2): 217-229. [Indonesian]
Yusron E. 2010. The diversity of Echinodermata in Likupang Waters, North Minahasa, Noth Sulawesi. Ilmu Kelautan 15 (2): 85-90. [Indonesian]
Yusron E, Susetiono. 2010. The diversity of Echinodermata in Ternate Waters, North Maluku. Oldi 36 (3): 293-307. [Indonesian]
Yusron E. 2012. Biodiversity of Echinoderms in Talise Water, North Minahasa. Bawal 4(3): 185-193. [Indonesian]

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Ethnobotany of *Canarium* plant species used by Tobelo Dalam (Togutil) ethnic community of Halmahera Island, Indonesia

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Abstract. *Tamalene MN, Al-Muhdhar MHI, Suarsini E, Rahman F, Hasan S. 2016. Ethnobotany of Canarium plant species used by Tobelo Dalam (Togutil) ethnic community of Halmahera Island, Indonesia. Biodiversitas 17: 61-69.* Tobelo Dalam (Togutil) ethnic group has been using local plants for years; one of them is *Canarium*. The ethnic are nomads and live in conservation forests. Data on ethnobotanical knowledge was collected through interview technique with "work in the wood" method. There were three types of informant: main informants, key informants, and recommended informants. Main informants were chosen through purposive sampling technique while key informants and recommended informants were chosen through snowball sampling technique. The informants in this study were grouped based on their age: 14 children (5-11 years), 18 teenagers (12-25 years), 13 adults (26-45 years), nine elder (46-65 years) and three old age (≥ 65 years). The result of fidelity level analysis (FL%) indicated that all age groups had FL value of 100% in utilizing walnut as local food. Regarding the use of skin exocarp the result was as follow: children (FL: 28.57%), teenagers (FL: 77.77%), adults (FL: 69.23%), and elder and old age (FL: 100%). Whereas, the use of Shell endocarp among the age groups was as follow: children (FL: 14.28%), teenagers (FL: 66.66%), adults (FL: 46.15%), elder (FL: 33.33%), and old age (FL: 100%). *Canarium* bark had value of FL% in children (FL: 35.71%), teenagers (FL: 61.11%), adults (FL: 92.3%), elder (FL: 33.33%), and old age (FL: 100%). The use of resin by the groups was children (FL: 50%), teenagers (FL: 83.33%), adults (FL: 92.3%), elder and old age (FL: 100%). The use of *Canarium* root among the groups was children (FL: 14.28%), teenagers (FL: 61.11%), adults (FL: 92.3%), elder (FL: 33.33%), and old age (FL: 100%). The use of plant's trunk was as follow: children (FL: 50%), teenagers (FL: 77.77%), adults, elder, and old age (FL: 100%).The research indicated that walnut had high utility value. All parts of the plant (root, wood, bark, and resin) were exploited for economic, health and cultural interest.

Keywords: Ethnobotany, Tobelo Dalam, Togutil, *Canarium*, Halmahera

INTRODUCTION

Local communities have knowledge of plants having ecological, economic, medical and cultural benefits. The knowledge is inherited from generation to generation through word of mouth. Local communities have a tradition in managing, utilizing and protecting local plants wisely. Ethnobotany studies the relationship between human's culture and local plants' use. Plants can be used as food, fabric, natural dye, medicine, and materials for cultural and religious rituals (Mathias 2004; Kim 2007).

Martin (2001) defined ethnobotany as a study of the utilization and preservation of plants by local society. Hun (2007) stated that ethnobotany studies the use of leaves, flowers, roots, barks, fruits and resin as herbal medicine. Ethnobotany is a study of the relationship between human and plant and the use of plant in society, technological manipulation, nomenclature, and agricultural system (Richard 2008). Ethnobotany helps to clarify the differences between how society utilizes forest products and preserves the nature (Sara et al. 2009). Hurrell and Albuquerque and de Medeiros (2013) stated that ethnobotany study leads to ecological system and plant as a religious symbol in every culture. Therefore, based on the definition of ethnobotany, study on the utilization of plants by human to fulfill their need such as food, health and culture is an important study in ethnobotany science and it has been going on for long.

Local society uses plants in their surrounding area to fulfill their necessities. Plants used by local society as food is always an important component in providing vitamins and nutrition for their bodies, such as vitamin C, A, calcium, and fiber (Arnason et al. 1981; Kuhnlein and Turner 1991; Marles et al. 2000). Plants also play a crucial role in a cultural society since they are used in traditional healing treatment. The society takes advantage of plants to fix bones' injury and other general health complaints. Wild plants consumed by local society come from various types of plant, such as trees, bushes, ferns, mosses or fungus. Local society has holistic approaches to healing various diseases, which are physical and emotional approaches. Cultural values, beliefs, and rituals, as well as the roles of family and other society members are the components needed to support the healing process (Andre et al. 2006). Ethnobotany can be used as a tool to document local society knowledge of the use of plants. People in villages have been benefited from plants to support their living. Various plants are used for food, medicine, building materials, traditional ceremonies, culture, dye, and so on. All society groups in compliance with their area characteristics and custom has a dependency on various plants at least for food. In this modern era, only hundreds kinds of plant are known as food sources; however, there are thousands of plants used by various ethnic groups worldwide (Uryadarma 2008).

Besides being used in traditional healing treatment, plants also serve as food sources, fabric, materials for construction, sources of energy, dye, fragrances, toxic, decorative or ornament, materials for cultural and religion rituals, medicine, rope/wrap, handicrafts, pesticides, and cosmetics. In addition, they can be used as signs of water resources, natural disaster, and season changes (Tamalene 2015). Local society still uses local plants as the sources of food despite the availability of modern food. Local wisdom and local culture are applied wisely, such as plants are utilized as firewood to boil water and to cook. Even some plants can be used as medicine, dye, and contain nutrition which can fulfill human needs for energy.

One of the local plants being used for years by Tobelo Dalam (Togutil) ethnic group in Halmahera is walnut plant (*Canarium*). The ethnic lives as nomads in conservation forests and takes advantages of *Canarium* as source of food, medicine, and construction. Their local knowledge about plants has been applied and well-preserved for years. Walnut plant has been used as the main source of food (walnut grains), resin, logs, and materials for ceremonial and spiritual activities (McClatcheyet al. 2011). *Canarium ovatum* (walnut plant) has long been popular on Halmahera Island in North Maluku province. Recently, the plant has been claimed as endemic to the eastern part of Indonesia. Therefore, local knowledge of *Canarium* needs to be recorded to establish the sustainability of local plants information for the sake of conservation.

Canarium is Burseraceaefamily, and it is a common plant in Indonesia. It exists in other countries as well, such as Africa, South Nigeria, Madagascar, South China, India, Philippine, and Southern part of Asia (Antanonius 2014). The genus *Canarium* contains approximately 77 species. Those species are spread in tropical area of Africa and Indo-Malaysia (Monteiro et al. 2006). *Canarium vulgare* is a native plant of East Malaysia, Papua, Papua New Guinea (Morobe), Alor, Nusa Tenggara Timur (Sunda Kecil Islands) and Moluccas (endemic to Ambon). *Canarium* can also be found in India, Sri Lanka and other tropical places. *C. indicum* is the famous species in Indonesia and grows well in the area of eastern Indonesia, such as Maluku and Southeast Sulawesi (Coronel 1996). Not only in Indonesia but *canarium* is also planted and spread in various Asian countries such as Malaysia, Thailand and Philippine. Philippine is a country that mostly cultivates *Canarium ovatum* Engl (Chaplin and Poa 1988). Walnut is rich in bioactive compound having high contribution in human health. Compounds containing in walnut are able to reduce generative diseases such as, cholesterol, hypertension, Diabetes, and cataract. Bioactive compounds containing in walnut fruits or seeds are phenolic, carotenoid, phytosterol, and tocopherol. The concentration of the compounds depends primarily on the variety and age of fruits and seeds (Djarkasi et al. 2011). The tree bark of local *Canarium* can be used for construction materials and musical instruments (Tesoro and Aday 1990). Walnut plant is beneficial for health and has high economic use value particularly for its seed that processed to be eaten and sold (Roberto 1996). The plant produces 4.5 ton of seeds per year (Thompson and Evans 2014).

Canarium, which has been utilized by various local ethnic groups for years, is a local knowledge worth knowing. This can give significant contribution to sustainable development in the future. To reach this ideal condition of development, however, support from ethnic groups who live in a particular place, either they are categorized as modern, traditional, or remote, is a necessity. Mostly, local communities who live in the village have maintained genetic resources in their areas. They have cultivated local plants to maintain a sustainable biodiversity for thousands of years. It has been proven that these practices can improve and promote biodiversity locally and can help keep the ecosystem healthy and balanced. Nevertheless, the contribution of local society in preserving and sustaining biodiversity is beyond their roles as the manager of natural resources. Their skills and techniques give invaluable information for a global community. The availability of local food in natural ecosystem has been unable to fulfill the inhabitants' needs. Changes have been brought to the ecosystem by human. Therefore, local biodiversity needs to be preserved to prevent it from extinction. Ethnobotanical study on *Canarium* (walnuts) in Halmahera is one of the efforts to reveal economic, ecological, medical, and cultural benefits of the plants. The results of this study is expected to give a contribution to the development of science and technology as well as to explore the potentials of tropical plants found in Halmahera Island in Indonesia and further to be used as the foundation of sustainable conservation in empowering local community in Halmahera.

MATERIALS AND METHODS

Study area

Data of this ethnobotanical study were collected in April 2014-April 2015, from Halmahera Island (Tobelo, Akelamo Pumlanga, Tayawi), Indonesia. Figure 1 shows map of the study area. Surveys were done in the areas that chosen based on its accessibility and availability of the remote ethnic group of Tobelo Dalam (Togutil). The surveys were done to gain information about the use of walnut plants. In-depth interviews were conducted to 57 local informants (36 males and 21 females). The informants were grouped based on their age: 14 children (5-11 years), 18 teenagers (12-25 years), 13 adults (26-45 years), 9 elder (46-65 years) and 3oldage (≥ 65 years).

Data were collected from three groups of informants: main informants, key informants, and recommended informants. Main informants were chosen through purposive sampling technique; whereas, the last two groups

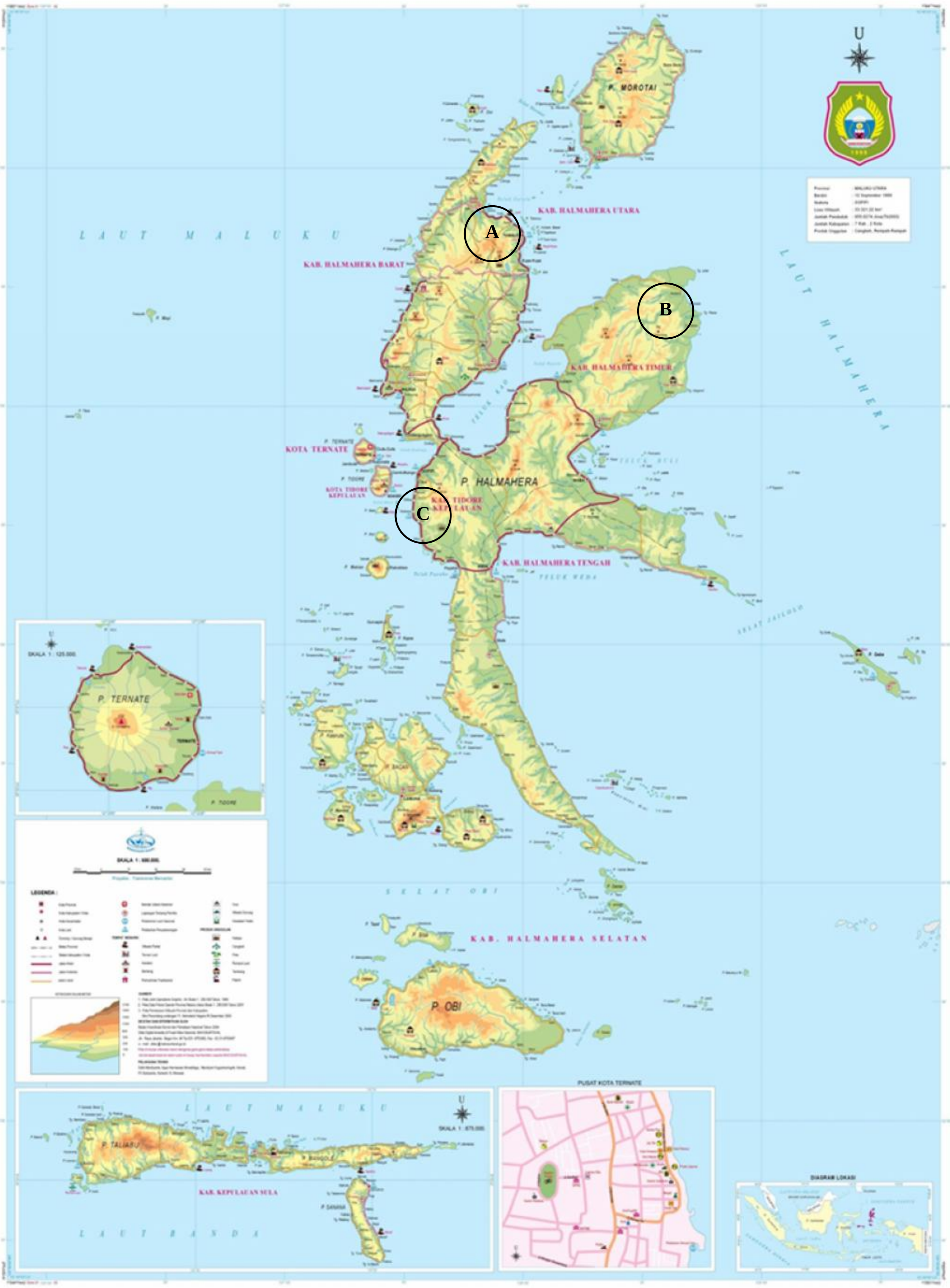

Figure 1. Location of the study conducted in Halmahera, North Maluku Province, Indonesia: A. Tobelo, B. Akelamo Pumlanga, C. Tayawi

of informants were chosen through snowball sampling technique. Information obtained from the main informants determined the presence of other informants and this technique was done continuously until homogenous data was able to answer the research problems. There were three main informants who are the areas' chieftains. However, there was no specific number of key informants and recommended informants since they were determined only to complement the data based on the purpose of this study. Data of this study were collected from interviews with "*work in the wood*" method to find out types of *Canarium* plants used by the Tobelo Dalam (Togutil) ethnic group.

Data analysis

Descriptive statistical analysis was employed to determine which parts of plants mostly used in relation to their benefits observed from economic, ecological, medical, and cultural aspects.

The frequency of parts of *Canarium* plants used was evaluated through the level of participants' responses based on parts of plant using the following formula:

$F = (S/N) * 100;$

S: number of informants who provided positive responses towards the parts of plants used; N: number of informants. Formula explained by Monteiro et al. (2006) was used to recognize the agreement level among the informants on which parts of plants used as well as how to utilize them.

Fidelity level(FL) which was measured to know the specific purpose of using particular parts of plants was calculated using the formula suggested by Friedman et al. (1986):

$FL (\%) = (n/N) * 100$

Where n is the number of informants for a specific use, and N istotal number of informants.

RESULTS AND DISCUSSION

There were three species of *Canarium* used by community of Tobelo Dalam (Togutil) ethnic i.e.: *Canarium decumanum* (Figure 2), *C. indicum* (Figure 3), and *C. vulgare* (Figure 4). The local name of *Canarium* is *Hiburu*. The fruit flesh (Seed kernel [Cotyledons]) of *C. decumanum* and *C.vulgare* is fairly thick. *C. indicum* is smaller with grayish yellow exocarp and white fruit flesh (mesocarp). The surface of inner skin of *C. decumanum* is uneven; whereas *C. indicum* and *C. vulgare* has smooth and even surface. The three species have 2-3 seeds (endocarp). *Canarium* fruit contained oil used for health such as for massage therapy, aromatherapy therapy, and cosmetic materials. The wood could be used as materials for house construction. Tree trunk of *Canarium* is upright and gray. The tree bark releases resin when it peeled. The resin is white and sticky at first and then it turns into pale yellow. The resin has soft texture, whitish color and aromatic odor (Figure 5A, B and). The threes are fruiting in March to November. The fruit of *C. decumanum, C. indicum* and *C. vulgare* has seeds encased in hard shell (endocarp) with flesh that can be eaten raw. The oil contained in the seed could be extracted as a substitute for coconut oil. The hard skin of *Canarium* was used as fuel to substitute firewood. Tree trunk of *C. decumanum, C. indicum* and *C. vulgare* species was used for house construction, and the leaves were boiled for traditional medicine to expedite menstruation in women.

Ethnobotanical uses of *Canarium* species

The result of frequency analysis (F) on *Canarium* related to its use showed that Tobelo Dalam (Togutil) ethnic group use *Canarium* for economic, ecological, health and cultural reasons. The values of F observed from the economic use aspects of *Canarium* include; (i) construction materials, (ii) food sources and (iii) medicine. Knowledge of economic values of *Canarium* as construction materials was found in 15.78% of children, 83.33% of teenagers, 100% of adults, elder, and old age. In general, all groups of age use *Canarium* as food sources (100%). *Canarium* was also sold as medicine to the local community. Knowledge of the use of *Canarium* as medicine to support the economy of Tobelo Dalam (Togutil) ethnic group was found in 42.85% of children, 66.66% of teenagers, and 100% of adults, elder, and old age (Figure 6).

Canarium is also functioned to save water, preserve animals habitat, and prevent flood. Tobelo Dalam (Togutil) ethnic group had knowledge of *Canarium* observed from the ecological aspect. The result of frequency (F) analysis on knowledge of how *Canarium* can be used to preserve water is as follows; 35.71% of children, and 100% of teenagers, adults, elder, and old age. Knowledge of how *Canarium* can be used to preserve animal's habitat was found in 78.57% of children and 100% of teenagers, adults, elder, and old age. Whereas, knowledge of how it can prevent flood was found in 35.71% of children, 88.88% of teenagers, and 100% of adults, elder and old age (Figure 7).

Canarium is beneficial for health especially as aroma-therapy oil, massage oil, and cosmetics. Knowledge of this health function was found in 15.78% of children, 100% of adults, elder, and old age. There were 71.42% of children and 100% of teenagers, adults, elder, and old age who had knowledge about *Canarium* functioned as massage oil. Whereas, there were 14.28% of children, 61.11% of teenagers, 46.15% of adults,33.33% of elder and 66.66% of old age who had knowledge of *Canarium* functioned as cosmetics (Figure 8).

Ethnobotanical use of *Canarium* as materials for religious rituals was found in 14.28% of children, 61.11% of teenagers, 46.15% of adults, 33.33% of elder, and 66.66% of old age. While knowledge of *Canarium* used in cultural rituals was found in 35.71% of children, 100% of teenagers, adults, elder, and old age.

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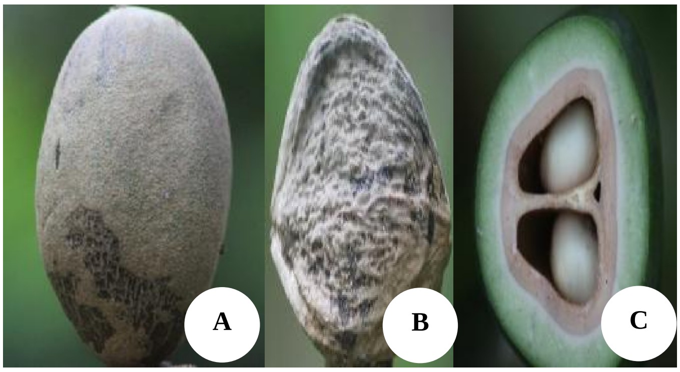

Figure 2.*Canarium decumanum*.A. Fruit, B.Seed, C. Cross section of fruit (with seed kernel or cotyledons)

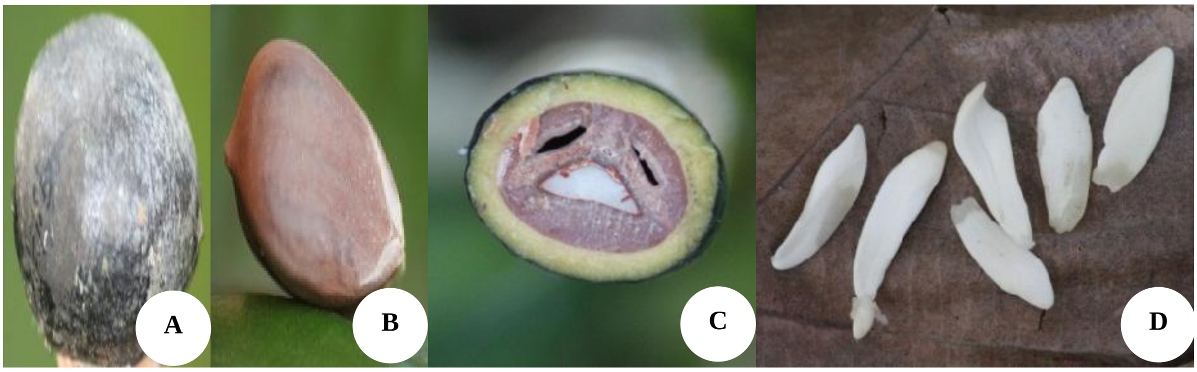

Figure 3.*Canariumindicum*.A. Fruit, B.Seed,C. Cross section of fruit,D.Seed kernel (cotyledons)

Figure 4.*Canarium vulgare*.A.Seed, B.Nuts(seed coat), C. Cross section of fruit, D.Seed kernel (cotyledons)

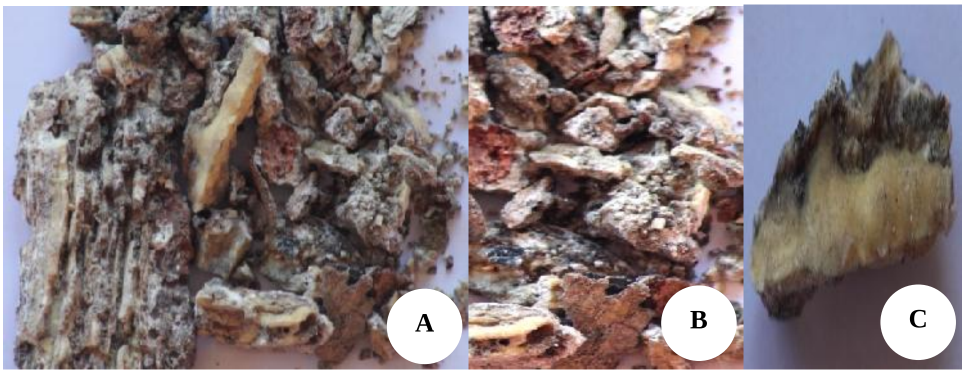

Figure 5.A. Resin of *Canarium indicum*, B. Resin of *C. vulgare*, C. Resin of *C. decumanum*

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Local knowledge of *Canarium* parts' functions

Tobelo Dalam (Togutil) ethnic group use *Canarium* parts: fruit, skin exocarp, shell endocarp, bark, sop, root, and stem, to fulfill their daily needs. Parts of *Canarium* plants give direct benefit to the people because they have high use-value observed from the economic, ecological (such as for water reserve, animal habitat and to prevent flood), medical, and cultural aspects. The result of *fidelity level* (FL) analysis (Figure 10) proves that all age groups had 100% of FL in utilizing *Canarium* as local food source.

The FL of skin exocarp use can be described as follows: 28.57% among children, 77.77% among teenagers, 69.23% among adults, 100% among elder and old age. The FL of

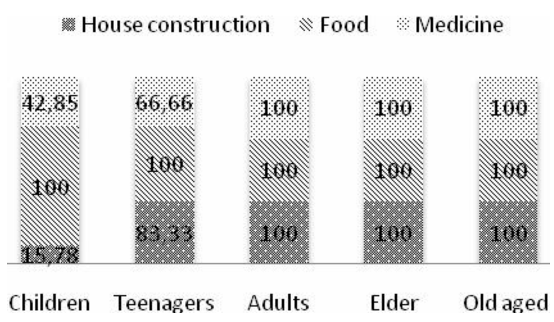

Figure 6. Ethnobotanical use of *Canarium* in economy based on age groups

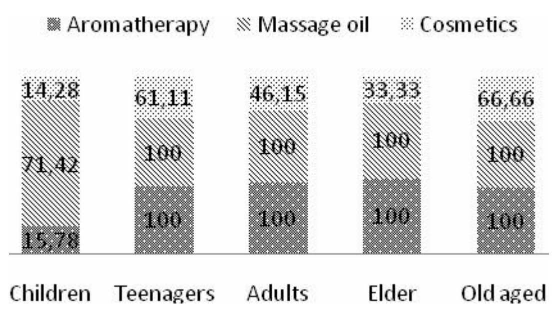

Figure 8. Ethnobotanical use of *Canarium* in medicine based on age groups

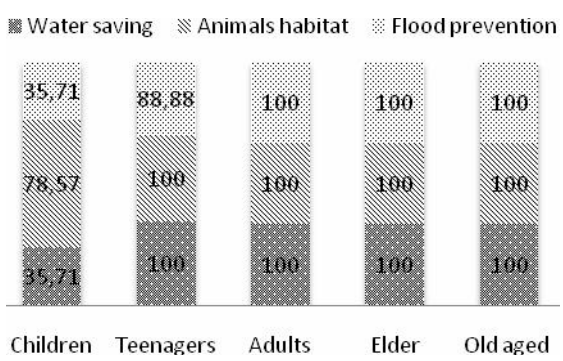

Figure 7. Ethnobotanical use of*Canarium* in ecology based on age groups

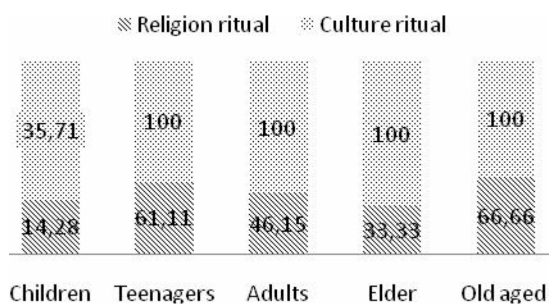

Figure 9. Ethnobotanical use of *Canarium* in rituals based on age groups

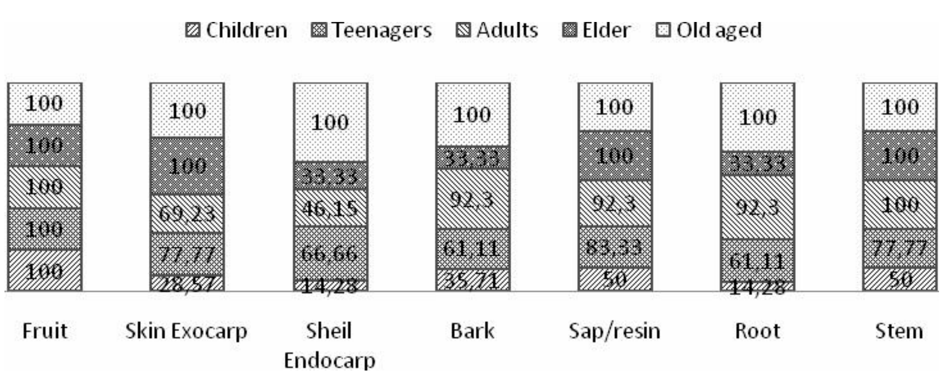

Figure 10. FidelityLevel (FL) of ethnobotanical use of *Canarium*

shell endocarp used can be described as follows: 14.28% among children, 66.66% among teenagers, 46.15% among adults, 33.33% among elder and 100% old age. The FL of *Canarium* bark used is described as follows: 35.71% among children, 61.11% among teenagers, 92.3% among adults, 33.33% among elder and 100% among old age. The FL values of sap/resin and roots part among group age were as follows: children (resin: 50%, roots: 14.28%), teenager (resin: 83.33%, roots: 61.11%),adults (resin and roots: 92.3%), elder (resin: 100%, roots: 33.33%),old age (resin and roots: 100%). The last is that FL of *Canarium* trunk can be described as follows: 50% of children, 77.77% of teenagers, and 100% of adults, elder, and old age. This study had depicted that walnut plants were beneficial and they had high use-value. Each part of the plants served many advantages to local people. All parts of the plants (root, wood, bark, sap) were exploited to fulfill the economic, health, and culture needs.

The results of the study revealed that children, teenagers, adults, elder, and old age had local knowledge of *Canarium* use-value and the knowledge had been preserved up to now. In every generation, the knowledge was obtained through direct experiences without having to attend any formal education. Local knowledge had been perceived as a system built on some subsystems that need one another. One of the subsystems' important components was humans. They, therefore, play role in protecting local resources especially plants that gave direct benefit to them. The overview of local knowledge of *Canarium* is illustrated by Figure 11.

Discussion

Based on the study result, it can be concluded that Tobelo Dalam (Togutil) ethnic group knew that *Canarium* plants are multifunctional in the aspect of economy, ecology, health, and culture. Therefore, they protect the plants through *ex situ* conservation. In addition, it is recommended for pregnant women to consume the *Canarium* fruit. It is believed that the fruit can keep the babies healthy when they are still in their mother's wombs. Roposi (1994) reported that *Canarium indicum* contains protein 14.2 g/100g, carbohydrate 5.5g/100g, calcium 119 mg/100g, and oil 74.9g/100mg. *Canarium* fruit or root can be used as sources of energy since they contain nutrition and vitamins (Terashima and Ichikawa 2003). *Canarium* fruit has become traditional food for local people for thousand years (Wissink 1994). The fruit is eaten raw to boost their energy (Smith 1991).

Henderson (1994) recorded that the economic use-value of *Canarium* could improve the consistent prosperity of local community through conservation. *Canarium* has the potential to increase income of local people by selling *Canarium* fruit products and producing oil for cosmetic industries (Varghese and Ticktin 2008; Pauku 2010). *Canarium* wood can be used as materials for home framing and musical instrumentals (Tesoro and Aday 1990; Menna at al. 2012). The wood can also be used to make key holders that very attractive and popular among local and international tourists in Indonesia (Gonzalez and Bunoan1947; Coronel1966). Resin powder is given orally to treat rheumatism, fever, cough, asthma, epilepsy, chronic skin disorders, syphilis, and hernia and also helps to improve skin (Meena at al. 2012).*Canarium* species are used extensively in the application of traditional medicine to treat bronchitis, catarrh, extreme coughing, aged, damaged or Injured skin and generalized stress (Schwab 2007). Walnut contains phenolic compounds from different chemical properties, including flavonoids, phenolic acids and tannins (Djarkasi et al. 2011). *Canarium indicum* has brought very useful value to society. *Canarium* can be used as food products that can be consumed, *Canarium* fruits contain fiber, and they can be used as fuel, or a pole house, and drugs. Resin powder is beneficial to cure rheumatic, fever, cough, asthma, epilepsy, skin chronic disturbance, syphilis, hernia and help to improve skin (Augustine and Krishnan 2006). In addition, *Canarium* species is also functioned for soil stabilization (Evans 1999; Elevitch 2006).

Remote tribe living in the forest area in Halmahera island-Indonesia had deep knowledge of source of native plants. Most of native fruits were collected from the forest. Fruits of wild plants such as *Canarium* were their everyday food; its fruit is an exotic fruit in Halmahera Island. *Canarium* is the most preferred fruit by children, teenagers, adults, the elderly, and seniors. Commercialization of walnut was carried out by certain groups to supplement their family income. *Canarium* is a local plant, which has an important role in the regeneration of forest vegetation on the Halmahera Island. Local ecological knowledge of Tobelo Dalam (Togutil) related to *Canarium* conservation could positively affect local biodiversity. Furusawa et al. (2014) reported that behaviors, such as giving respect to forest reserves and semi-domestication of some species can contribute to the effort of preserving local biodiversity especially local endemic species. *Canarium* plant also serves ecologically to withstand high winds (Tesoro and Aday 1990).

Canarium fruits were used by Tobelo Dalam (Togutil) as skin protection. This fruit has long been exploited for aromatherapy, cosmetics and massage products. The resin is used for dry skin (Athar and Nasir 2005). The resin is used differently to coat the ship, make a torch and tonic (Bradshaw 2013). *Canarium* species are functioned as panacea (panacea of witchcraft, couth) (Jiofack et al. 2009). *Canarium* resin is used as incense in religion and cultural ceremony (Agustine 2006). *Canarium* species is used by local people as food source (the seed), resin, and wood which can be used in healing ceremony, religion ceremony, rituals and wars (McClatchy et al. 2006). Local people use *Canarium* resin for prayer rituals(Varghese and Ticktin 2008).

This study has provided crucial information about ethnobotanical knowledge of Tobelo Dalam (Togutil) ethnic group found in Halmahera, Indonesia. *Canarium decumanum*, *C. indicum* and *C. vulgare* had been used economically, ecologically, medically, and culturally. It means that the plants are multifunctional. Local knowledge of *Canarium* by the age group showed that the elder and the old aged had the highest level of knowledge in the use of *Canarium*. Children, teenagers, and adults knew the

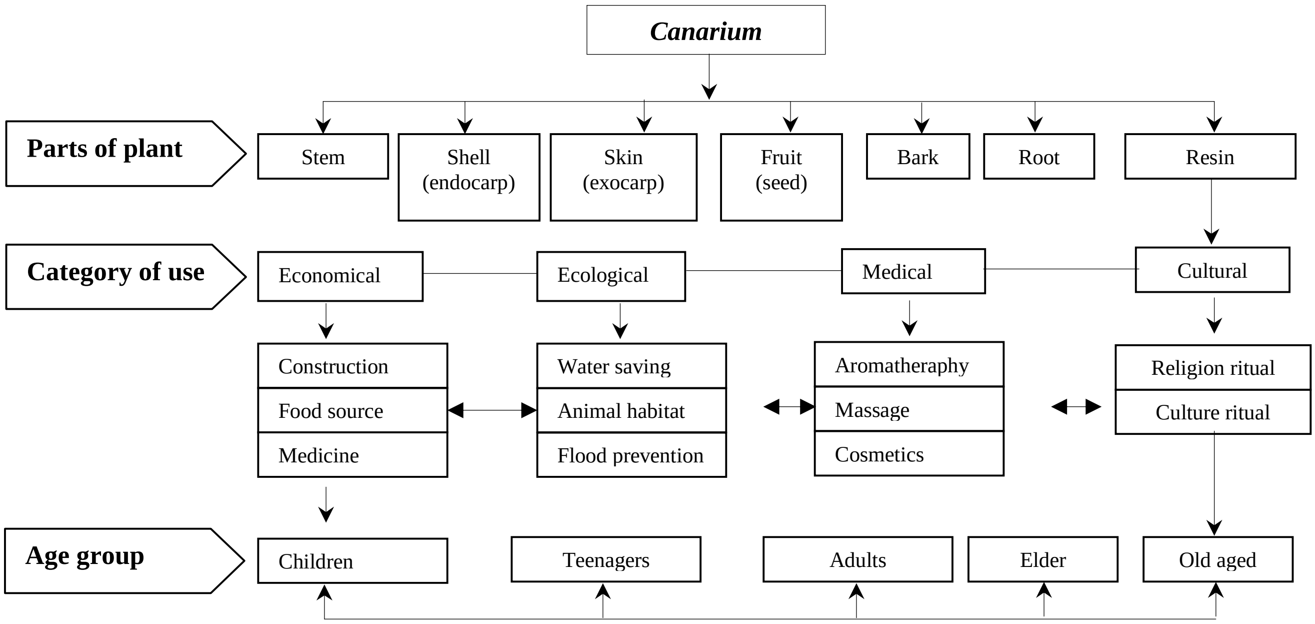

Figure 11. Local knowledge of *Canarium* use in Tobelo Dalam (Togutil) ethnic group

benefits of *Canarium* from everyday life experience gained from the elder and the old age. Socio-cultural study helped to promote *Canarium* species as one of the plants that has high use value for local communities. This study contributes to the development of science and technology as well as explores the potential of tropical plants on the Halmahera Island, Indonesia that will serve as the basis for sustainable conservation to empower local communities.

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REFERENCES

Albuquerque UP, de Medeiros PM. 2013. What is evolutionary ethnobiology? Ethnobiol Conserv 6: 38-47.

Andre A, Karst A, Turner N. 2006. Arctic and Subarctic Plants. In: UbelakerDH (ed) Handbook of North American Indians. Vol 3. Environment, Origins, and Population. Smithsonian Institution, Washington, DC.

Antanonius. 2014. Walnut plant. Academiaedu 6: 12. www.academia.edu.com

Arnason T, Hebda RJ, Johns T..1981. Use of plants for food and medicine by native peoples of Eastern Canada. Canadian J Bot 59: 21-25.

Athar, Nasir MS. 2005. Taxonomic perspective of plant species yielding vegetable oils used in cosmetics and skin care products. African J Biotechnol 4: 36-44.

Augustine J, Krishnan PG. 2006. Status of the black dammar tree (*Canarium strictum* Roxb) in Periyar Tiger Reserve, Kerala and the uses of black dammar. Indian Forester 132: 1329-1335.

Augustine JPG. 2006. Krishnan, Status of the black dammar tree (*Canarium strictum* Roxb) in Periyar Tiger Reserve, Kerala and the uses of black dammar. Indian Forester10: 1329-1335.

Bradshaw F. 2013. Chemical characterisation of museum-curated ethnographic resins from Australia and New Guinea used as adhesives, medicines and narcotics. Heritage Sci 36: 2-14

Chaplin GE,Poa D. 1988. Indications of the Potential Fruit and Kernel Yield of *Canarium indicum* and *C. salomonense* in Western Province, Solomon Islands. Forest Research Note No. 19-6/88. Honiara, Forestry Division, Solomon Islands.

Coronel RE. 1966. Let us save our dying pili industry. Philipp Farms Gard 3 (12): 16- 17, 28.

Coronel RE. 1996. Pili Nut *Canarium ovatum* Engl. International Plant Genetic Resources Institute. Rome, Italy.

Elevitch CR. 2006. Traditional Trees of Pacific Islands: Their Culture, Environment and Use. Permanent Agriculture Resources, Hawaii, USA.

Evans B. 1999. Edible Nut Trees in Solomon Islands: A variety collection of *Canarium*, *Terminalia* and *Barringtonia*. ACIAR Technical Report No. 44. Canberra, Australia.

Friedman J, Yaniv Z, Dafni A, Palewitch D. 1986. A preliminary classification of the healing potential of medicinal plants, based on a rational analysis of an ethno pharmacological field survey among Bedouins in the Negev Desert, Israel. J Ethnopharmacol16: 275-87.

Furusawa T, Sirikolo MQ, Sasaoka M, Ohtsuka R. 2014. Interaction between forest biodiversity and people's use of forest resources in Roviana, Solomon Islands: implications for biocultural conservation under socioeconomic changes. J Ethnobiol Ethnomed 10: 3-20.

Gonzalez LG, Bunoan JC. 1947. Variability of pili trees grown in the College of Agriculture. Philippines Agric 3: 60-65.

Djarkasi GSS, Nurali EJN, Sumual MF, Lalujan LE. 2011. Analysis of bioactive compound in *Canarium* nut (*Canarium indicum* L). USAID-Texas A and M University & Sam Ratulangi University, Manado.

Henderson M. 1994.The Role of Galip Nut *Canarium indicum* in Forest Conservation in East New Britain, Papua New Guinea. Proceedings of a workshop 31 October - 4 November 1994 Le Lagon Resort, Port Vila, Vanuatu.

Hunn ES. 2007. Ethnobiology in four phases. J Ethnobiol 27: 1-10.

Jiofack T, Ayissi C, Fokunang N, Guedje V, Kemeuze. 2009. Ethnobotany and phytomedicine of the upper Nyong valley forest in Cameroon. African J Pharm Pharmacol 4: 144-150.

Kim JY. 2007. Ethnobotany. United States of America, Chelsea House An imprint of Infobase Publishing.

Kuhnlein HV, Turner NJ. 1991.Traditional plant foods of Canadian Indigenous Peoples, nutrition, botany and use. In: Katz S (ed). Food and Nutrition in History and Anthropology Vol. 8.Gordon and Breach Science Publishers, Philadelphia.

Langenheim JH. 2003. Plant Resins: chemistry, evolution, ecology, and ethnobotany. Timber Press, Cambridge.

Lim TK. 2012. *Canarium vulgare*. Edible Medicinal and Non-Medicinal Plants (Fruit). Vol l .(Book). Springer, The Netherlands.

Marles RJ, Clavelle C, Monteleone L, Tays ND, Burns. 2000. Aboriginal Plant Use in Canada’s Northwest Boreal Forest. Natural Resources Canada. Vancouver, British Columbia..

Martin GJ. 2001. Ethnobiology and ethnoecology. In: Levin SA (ed). The Global Diversity Foundation, Morocco. Encyclopedia of Biodiversity. New York: Elsevier.

Mathias E. 2004. Ethnoveterinary medicine: Harnessing its potential. Vet Bull 74: 27-37

McClatchey W, Qusa M, Sirikolo B, Kaleveke L, Pitanapi C.2006. Differential conservation of two species of *Canarium* (Burseraceae) among the Babatana and Ririo of Lauru (Choiseul), Solomon Islands. Econ Bot 60: 212-226.

Meena D, Binaibabu N, Doss J. 2012. Prospects for the critically endangered Medicinally important species, *Canarium strictum* Roxb. A review. Intl J Conserv Sci 3: 231-237.

Monteiro JM, Albuquerque UP, LinsNeto EMF, Araùjo EL, Amorim ELC. 2006. Use patterns and knowledge of medicinal species among two rural communities in Brazil’s Semi-Arid Northeastern Region. J Ethnopharmacol 105: 173-86.

Pauku RL, Andrew J, Lowe Roger RB, Leakey. 2010. Domestication of indigenous fruit and nut trees for agroforestry in the Solomon Islands. For Tree Livelihoods 19: 269-287.

Richard ES. 2008. Ethnobotany. Encyclopaedia of the History of Science, Technology, and Medicine in Non-Western Cultures. Springer-Verlag Berlin.

Roberto E, Coronel. 1996. Pili Nut *Canarium ovatum* Engl. Internatioal Plant Genetic Resources Institute (IPGRI). Rome, Italy.

Roposi N. 1994. Research and development on edible nut tree crops in Solomon Islands. Proceedings of a Workshop, 31 October - 4 November 1994, Le Lagon Resort, Port Vila, Vanuatu.

Sara R, Van W, Stephen. 2009. Plant Uses: California Native American Uses of California Plants Ethnobotany. UC Santa Cruz Arboretum. California.

Schwab W. 2007.Genetic engineering of plants and microbial cells for flavour production. In: Berger RG (ed). Fragrance and Flavours: Chemistry, Bioprocessing and Sustainability, 1st ed.; Springer, Leipzig, Germany.

Smith NM. 1991. Ethnobotanical Field Notes from the Northern Territory, Australia. J Adelaide Bot Gard 14: 1-65.

Tamalene MN, Al Muhdhar MHI, Robo T. 2015. Climate changes mitigate anticipation strategy based on local wisdom - a study of Tobelo Dalam (Togutil) in Halmahera Island, Indonesia. J Biol Earth Sci 5: 40-48.

Terashima H, Ichikawa MA. 2003. Comparative ethnobotany of the Mbuti and Efe Hunter-gatherers in the Ituri forest, Democratic Republic of Congo. African Stud Monogr 24: 1-168.

Tesoro FO, Aday JU. 1990. Properties and uses of some Philippine woods. Forest Products Research and Development Inst. Los Baños, Laguna, The Philippines.

Thompson LAJ, Evans B. 2004. *Canarium indicum* var. *indicum* and *C. harveyi* (*Canarium* nut) species profiles for pacific Island Agroforestry. Versi 1.1.In: Elevitch CR (ed). Species Profiles for Pacific Island Agroforestry; Permanent Agriculture Resources (PAR): Holualoa, HI, USA.Uryadarma. 2008. Teaching Materials of Ethnobotany. Department of Biology Education, FMNS, UNY. Yogyakarta.[Indonesian]

Varghese A, Ticktin T. 2008. Regional variation in non-timber forest product harvest strategies, trade, and ecological impacts: the case of black dammar (*Canarium strictum* Roxb.) use and conservation in the Nilgiri Biosphere Reserve, India. EcolSoc13: 11-19.

McClatchey W, Sirikolo MQB Jr, Kaleveke L, Pitanapi C. 2006. Differential Conservation of Two Species of Canarium (Burseraceae) among the Babatana and Ririo of Lauru (Choiseul), Solomon Islands. Econ Bot 60 (3): 212-226.

Wissink D. 1994.Galip*Canarium indicum* as a cash crop in West New Britain, Papua New Guinea: Experiences of the Kandrian Gloucester Integrated Development Project. Proceedings of a Workshop 31 October-4 November 1994 Le Lagon Resort, Port Vila, Vanuatu.

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Dominance and diversity studies of tree species in lesser Himalayan forest of Uttarakhand, India

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Abstract. *Bisht AS, Bhatt AB. 2016. Dominance and diversity studies of tree species in lesser Himalayan forest of Uttarakhand, India. Biodiversitas 17: 70-77.* For the present investigation single mountain, approach was applied. This is a supplement the basic approach and extends the gradients further downward in to the forest belt. Vegetational analysis of nine stands covering all the four aspects of the study site of Pauri Garhwal district of Uttarakhand, India has been undertaken. In seven trees, species were encountered. East aspect is characteristics by highest density of *Cupressus torulosa* while west aspect comprised of *Cedrus deodara*, *Myrica esculenta*, *Pinus. roxbugrhii*, *Quercus leucotrichophora* and *Rhododendron arboreum,* i.e.high diversity with low dominance *Cupressus torulosa* and *Cedrus deodara* dominated the north aspect. In general, influence of higher anthropogenic pressure on *Quercus* species is an important factor for leads to gradual replacement of oak species by *Pinus roxburghii* in all the aspects.

Keywords: Diversity, Lesser Himalaya, niche width, regeneration

INTRODUCTION

Northwest Himalaya is a distinct Himalayan region with a chrematistic climate, geology and flora. The floristic diversity is manifested through different phyto-climatic and topographic regimes. The floral diversity is fascinating because of species richness and diverse community structure. The diversity has occurred in time and space due to a number of ecological changes, often resulting in speciation, isolation, competition, etc. (Gaur et al 2003).

Lesser Himalaya is the central part of Himalaya extending between Siwaliks in south and Great Himalaya in the north and range from 20-60 km in width. The mountain peak rang between 1200-1300 m highs where as the valleys range between 400-1200 m. The district Garhwal in northwest Himalaya though represents rich biodiversity, has remained neglected by the past explorers.

The pervasive influence of man in Himalayan forest and heavy dependence of Himalayan agriculture on an "*energy subsidy*" from the forest (Pandey and Singh 1984) inextricably bind the welfare if Himalayan people to that of the uncultivated ecosystem around them. Accurate measure of the degree of degradation and its effect upon future productivity and quality of lode in the Himalayas is difficult task. Shortage of abiotic resources in the habitat or an excess of a condition unfavorable for function is function known as stress. 'Stress' refer to same environmental situation that often produce changes in organism or ecosystems that we consider being undesirable for example, reduced productivity, an impoverished flora, or an unbalanced species composition stress affects the structure and composition of complex vegetation. Adverse climate, repeated fire, pollution, ionizing radiation (Woodwell 1970) including local folk (Wiart 1983) are the major mean causing stresses on Himalayan forest.

In any community diversity decrease with increasing stress resulting disappearance of most sensitive species first, then large woody plants and finally all higher plant (mostly woody). Mostly this change occur in natural system as across timberline (Arno and Hammerly 1984), with reduced nutrients (Westman 1975) and where stress has been imposed by man as around metal smelters producing SO_2 (Amiro and Courtin 1981). Mostly is observed that the species, which are important for fodder and fuel purpose, are completely eliminated in highly disturbed near settlement sites while unusable species viz. weeds (*Eupatorium adenophrum* and *Parthenium hysterophorum*) and shrubs (species of *Berberis* and *Rubus*) are frequently distributed near villages.

Damage to individual plants or to forests is accompanied or followed by damage to the productive potential of the land. In areas where stress can be recognized from the vegetation, direct observation of properties of the soil may allow one to estimate the likelihood of permanent damage to its productive capacity. Much nutrient loss may occur in product removal (fodder forest floor litter and dung) during burning or dissolved in run off water and is difficult to detect. However, there can be easily detectable soil losses with out occurrence of the gullies and landslide (Pandey et al 2000).

During the past century, there had been rapid depletion in forest area in whole of the Himalayan region in general and Uttarakhand in particular. The forests of the Lesser Himalayan zone are experiencing the problem of enormous damage to the biological diversity. The factor responsible for the depletion in biodiversity may be attributed to the settkenent of villages between 1000 to 2000 m asl. Most of the needs of villagers are fulfilled from forest, which result in latter's degradation.

MATERIALS AND METHODS

Study area

For the present investigation a survey of various summits in district of Pauri Garhwal, Uttarakhand, India, its proximity to site, latitudinal gradient, slope, aspects and other congenial region. The Pauri district lies between Lat.29^0 47'-30^0 13'N and Long. 78^0 18'-79^0 10'E. The study sites exhibited an elevation range from 1800 to 2250 m (Figure 1). Field research was conducted in two series, i.e. October 2010 to March 2011 and October 2011 to March 2012.

Geomorphology

District of Pauri Garhwal is one of the thirteen district of Uttarakhand extended in lesser Himalayan zone and known for the hill station. The district is one of the most fascinating segments of the Himalaya, stretches from the Ramganga river that separates Pauti-Kumaun boarder in the east and to the Ganga demarcating the western boarder. Physiographically the study site having undulating topography with gentle slopes in southern and South-Western direction (Bisht and Sharma 2014).

Meteorological aspects

The rainfall pattern in study area is monsoon dependant. The south-east monsoon commences towards the end of June and it rains until mid of September. Northeast monsoon causes occasional winter showering during December to February. The mean monthly rainfall fluctuated between 22.5 cm to 430 cm in 2 year. In November of both sampling years, there were no rains. The mean maximum rainfall with maximum number of rainy days in a month was reported in June to September in one or both sampling years (Rawat 2003).

Niche breath

Niche breath of the ith species was estimated by the following formula (Levins 1968).

$$Bi = \frac{1}{\Sigma j Pij^2} = \frac{(\Sigma j Nij)^2}{\Sigma j Nij^2} = \frac{Yi2}{\Sigma j Nij^2}$$

$$Bi = \Sigma j \ Pij \log Pij$$

Where,

Nij = Total number of individual of the ith species in the resource state.

Yi = Total number of individuals of the ith species over all resource state.

Pij = Nij/Y = Proportion of the individuals of ith species which is associated with resource state j.

Both the measures are maximized when the species, distributed uniformly over the other resource states. They minimized when the species are associated with only one of the resources state. The measures Bi and Bi' are inverse of Simpson's (1949) measure of concentration and Shannon-Wiener formula (Shannon-Wiener 1949) for formation or uncertainty.

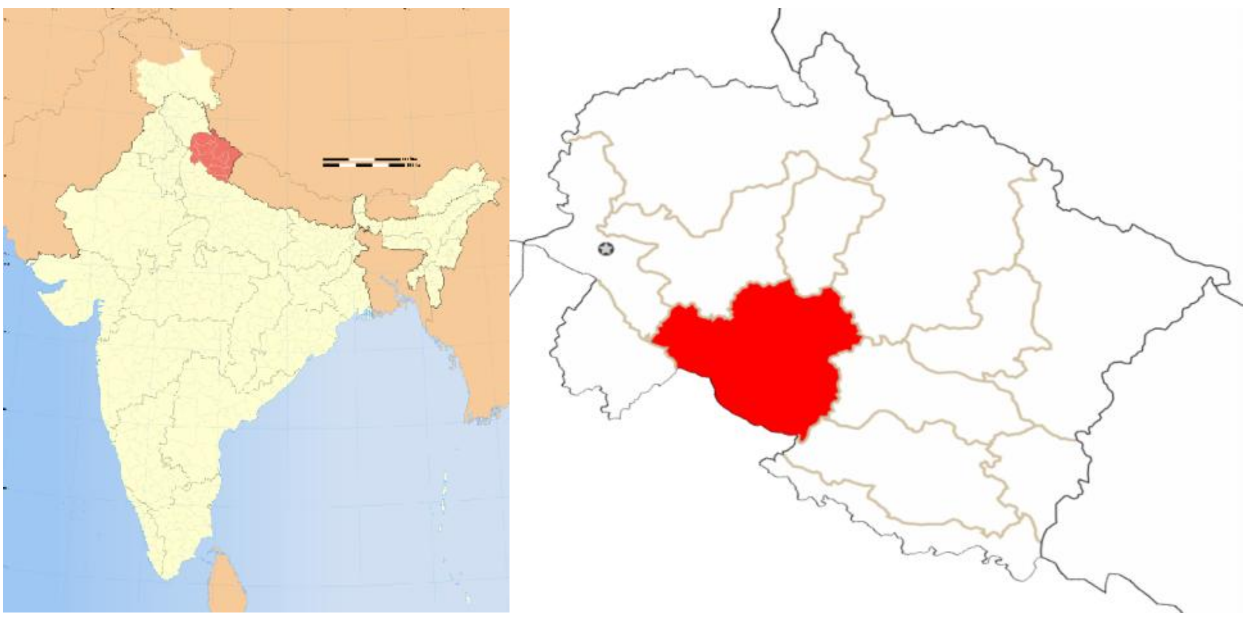

Figure 1. Study area in District of Pauri Garhwal, Uttarakhand, India

Niche overlap

The niche overlap between species i and h was calculated by the following formula (Colwell and Futuyma 1971).

C_{ih} = 1-1/2 Σ_j [P_{ij}-P_{hj}]

Where P_{ij} = N_{ij}/Y_i = Proportion of the individuals of i^{th} species which is associated with resource state j and P_{hj} = N_{hj}/ Y corresponding to a second species h.

The measure has a minimum value of 0, when species I and h share no resource among the resource states. The niche measures (1), (2) and (3) are absolute and are good estimators provider the resources states are all equally distinct. Otherwise, the measures are measures are liable to yield misleading results (Sai and Budholia 1986).

RESULTS AND DISCUSSION

Quantitative studies: Primary features

As stated in methodology there were nine stands where the qualitative and quantitative studies were undertaken. Because of quantitative analysis, the vegetation has been studies in terms of mean frequency, density, total basal cover (TBC) and important value index (IVI) of all species. The observations are presented stands wise in Table 1.

Secondary features

On the basis of primary data collected directly from the field, the derived attributes have also been worked out for two consecutive year and presented stand wise in Table 2. the features include concentration of dominance generally diversity index, alpha diversity and evenness value of species. Likewise the secondary parameters also include the beta diversity of all the three strata along various stands of the investigation site. The result are presented in Table 3.

Niche width

Niche width measures the degree of specialization of a species as its ability to exploit an environmental range in space and to maintain its population in different environments. In present investigation the niche breadth (Bi and Bi') of each species if tree were measured separately at all aspects (east, south, north and west) including submit top (stand 1^{st}). The niche breath of species was measured aspect wise. Lower and upper elevational stand of each aspect including summit top were considered for computing the niche width of species (Table 4).

Quercus lecotricophora appeared to possess maximum niche breadth at north (Bi=2.48, bi' 0.429) and east (Bi= 1.95. Bi'=0.295) aspect while at south and west aspect it turn out in to 3^{rd} position. *P. wallichiana* had broader niche at east aspect (Bi= 1.80, Bi'= 0.276) while at other aspect it covered minimum niche breath at south aspect (Bi=2.67, Bi'=0.453), the niche breath declined simultaneously at west (Bi=2.38, Bi'=0.410) and north aspect (Bi= 2.0, Bi'=0.31) followed by west (Bi=1.95, Bi'=0.295) and north (Bi=1.54, Bi'=0.233) and least at east aspect (Bi= 1.22, Bi'=0.141). *L. ovalifolia* had broader niche at south aspect while at other aspects it have minimum value (Bi = 1.64, Bi'=0.252) aspect but least at south aspect. *C. deodara* was the only tree species with maximum niche breath at east aspect and it take minimum value at all other aspects.

Niche overlap (Chi)

The niche overlap value for species of tree on the east south, north and west slopes of the forest of Pauri were calculated separately and presented on Tables 4. Data presented in Table 4 clearly indicate that at east aspect *P. roxburghii* vs *R. arboreum* exhibited maximum niche overlap (Chi = 0.900) followed by *C. deodara* vs *C. torulosa* (Chi= 0.889). Except *C. torulosa*, *C.dedara* shared minimum resources with other associated species at east slope (Stand 1^{st}-3^{rd}). *M. esculenta, P. roxburghii, P. wallichiana, Q. lehotrochophora* and *R. arboreum* shared huge products of each other as compared to *C. deodara* and *C. torulosa.*

All plant species occur in a limited range of habitat and with in this range, most of them are more abundant around their particular environmental optimum in the absence of competition (Ter Braak and Prenrice 1988). Species components pf communities thus change along environmental gradients, the replacement and separation of species in the environment depend on variation of resources along these gradients (Pichett 1980).

The Himalayan forest are most productive than the forest of other temperate region with similar rainfall amount, possibly because of a long season of relatively constant favorable temperature and the moderating effect of the mountains during cold winter (Mani 1974). Relatively high wood nutrient concentration produces nutrient accumulation in forest biomass that is relatively higher compared to temperate forest (Singh et al. 1985). It is suggested that forest with multilayer canopy and well-developed forest floor would be more productive of soil and water in comparison to a forest having thin layers. Profile diagrams can also be usefully employed in vegetation of lower height to illustrate the relationship between topography and drainage of an area (Kershaw 1973).

The presence of old oak trees and newly developed chir pine patches in the study ate indicates the presence of original oak forest in the area, The study showed the much anthropogenic disturbances in all stands. In a complex Himalayan forest ecosystem chronic from disturbances exists in which people remove only a small fraction of forest biomass in the form of grazing, lopping, surface burning and litter removal at a given time (Khera et al. 2001). These disturbances are affecting the stability of ecosystem and retarding the success ional process in the area. Both natural and human caused disturbances are considered since vegetation responses do not distinguish them between natural and human activities.

The present investigation on vegetational analysis is based upon the comparison of different aspect (N, E, S and W direction) from the summit top (Stand 1^{st}). In total 7 species were encountered during whole of the study period at all stands. The finding revels that the high density of *C.*

Table 1. Mean frequency, density, total basal cover (TBC) and important value index (IVI)

Name of species	Freq. 2010-2011	Freq. 2011-2012	Density 2010-2011	Density 2011-2012	TBC 2010-2011	TBC 2011-2012	IVI 2010-2011	IVI 2011-2012
Stand I								
Cupressus torulosa	30.00	23.33	1.4	0.8	5.68	3.23	79.35	57.24
Lyonia ovalifolia	10	*	0.1	*	0.36	*	9.56	*
Myrica esculenta	10	*	0.2	*	0.14	*	8.87	*
Pinus wallichiana	46.67	36.67	1.3	0.7	4.52	5.95	80.51	102.26
Quercus leucotrichophora	73.33	56.67	2.1	1.5	5.13	4.34	118.45	118.17
Rhododendron arboretum	13.33	16.67	0.2	0.2	0.26	0.52	12.59	22.32
Stand II								
Cedrus deodara	90	80	3.7	2.2	19	15.23	110.1	96.95
Cupressus torulosa	100	100	8.7	7.0	36.84	41.19	187.83	203.05
Pinus roxburghii	10	*	0.1	*	0.1	*	6.22	*
Stand III								
Cedrus deodara	33	13	0.8	0.2	3.96	1.06	32.52	15.90
Cupressus torulosa	27	27	0.5	0.4	1.69	1.67	21.53	28.14
Myrica esculenta	*	40	*	0.7	*	1.51	*	36.77
Pinus roxburghii	53	40	1.0	0.7	6.22	2.95	51.07	45.74
Pinus wallichiana	43	27	0.5	0.3	4.77	1.87	34.52	26.67
Quercus luecotrichophora	83	70	2.6	2.7	6.99	6.94	87.43	115.8
Rhododendron arboreum	53	40	2.3	1.2	10.45	3.03	72.93	55.49
Stand IV								
Cedrus deodara	20	13.3	0.3	0.2	1.38	0.62	21.3	14.52
Lyonia ovalifolia	*	20	*	0.1	*	0.16	*	8.94
Myrica esculenta	30	25	0.4	0.4	0.94	0.63	26.28	29.62
Pinus roxburghii	100	86.70	5.5	5.3	32.8	28.17	235.72	242.63
Quercus leucotrichophora	20	15	0.3	0.3	0.3	0.38	16.7	15.38
Stand V								
Lyonia ovalifolia	*	13.3	*	0.1	*	0.15	*	10.94
Myrica esculenta	15	23.3	0.2	0.3	00.3	0.41	10.19	20.13
Pinus roxburghii	100	100	5	3.4	29.13	20.16	196.68	218.71
Quercus lecotricophora	66.7	33.3	1.7	0.9	3.87	2.28	65.62	47.70
Rhododendron arboreum	40	10	0.6	0.1	1.6	0.19	30.90	7.53
Stand VI								
Cedrus deodara	*	15	*	0.2	*	0.41	*	14.45
Cupressus torulosa	20	30	0.2	0.7	0.64	1.53	20.04	37.39
Myrica esculenta	20	20	0.2	0.2	0.3	0.28	16.90	17.74
Pinus roxburghii	93.33	83.33	3.5	3.6	18.11	76.02	209.17	220.92
Quercus leucotrichophora	50	26.67	0.9	0.3	1.24	2.12	53.89	26.72
Stand VII								
Myrica esculenta	10.00	53.33	0.1	0.9	0.18	1.69	6.5	84.8
Pinus roxburghii	33.33	50	0.9	1.1	5.38	3.9	141.38	112.61
Quercus leucotrichophora	56.67	43.33	1.3	1.3	3.71	2.8	156.45	102.58
Stand VIII								
Cedrus deodara	13.33	20	0.3	0.4	1.45	2.09	19.08	34.09
Cupressus torulosa	20	20	0.4	0.2	1.99	0.99	25.3	21.56
Myrica esculenta	50	63.33	1.0	1.0	2.67	1.61	52.66	62.46
Pinus roxburghii	63.33	63.33	1.5	1.0	2.62	6.21	87.29	102.44
Quercus leucotrichophora	33.33	30	1.0	1.3	2.46	1.16	44.72	32.23
Rhododendron arboreum	63.33	43.33	1.3	0.4	3.95	1.4	70.96	47.23
Stand IX								
Myrica esculenta	50	*	0.8	*	1.63	*	69.4	*
Pinus roxburghii	43.33	33.33	1.5	*	4.21	2.18	113.53	159.53
Quercus leucotrichophora	33.33	10.00	0.7	*	1.44	0.43	54.76	34.14
Rhododendron arboreum	36.67	23.33	1.1	*	3.03	1.13	85.44	129.09

Note: * = absent

Table 2. Concentration of dominance (cd), general diversity index (H), alpha diversity and evenness value of tree species in different season and year at all stands includes gamma diversity

	1st Field research					2nd Field research				
	Oct 10	**Jan 11**	**Mar 11**	**Mean**	**SD**	**Oct 11**	**Jan 12**	**Mar 12**	**Mean**	**SD**
Stand I										
Cd	0.28	0.30	0.34	0.31	0.02	0.30	0.31	0.39	0.33	0.04
H	1.40	1.32	1.18	1.30	0.09	1.27	1.24	1.09	1.20	0.08
Alpha	6.00	5.00	4.00	5.00	0.82	4.00	4.00	4.00	4.00	0.00
Evenness	1.79	1.89	1.97	1.88	0.07	2.11	2.07	1.80	1.99	0.14
Stand II										
Cd	0.50	0.52	0.56	0.53	0.02	0.58	0.55	0.57	0.57	0.01
H	0.75	0.67	0.63	0.69	0.05	0.61	0.95	0.62	0.73	0.16
Alpha	3.00	2.00	2.00	2.33	0.47	2.00	2.00	2.00	2.00	0.00
Evenness	1.58	2.23	2.10	197	0.28	2.04	3.16	2.07	2.42	0.52
Stand III										
Cd	0.19	0.22	0.23	0.21	0.02	0.17	0.25	0.29	0.24	0.05
H	1.72	1.61	1.64	1.67	0.05	1.83	2.24	1.47	1.85	0.31
Alpha	6.00	6.00	6.00	6.00	0.00	7.00	6.00	6.00	6.33	0.47
Evenness	2.21	2.07	2.10	2.13	0.06	2.17	2.88	1.89	2.13	0.42
Stand IV										
Cd	0.63	0.62	0.65	0.63	0.01	0.68	0.64	0.69	0.67	0.02
H	0.76	0.77	0.73	0.75	0.02	0.67	0.78	0.58	0.68	0.08
Alpha	4.00	4.00	4.00	4.00	0.00	4.00	5.00	3.00	4.00	0.82
Evenness	1.26	1.27	1.20	1.24	0.03	1.11	1.12	1.22	1.15	0.05
Stand V										
Cd	0.51	0.52	0.45	0.49	0.03	0.54	0.53	0.63	0.57	0.04
H	0.91	0.89	0.93	0.91	0.02	0.82	0.96	0.74	0.84	0.09
Alpha	4.00	4.00	3.00	3.67	0.47	4.00	5.00	4.00	4.33	0.47
Evenness	1.52	1.48	1.94	1.64	0.21	1.37	1.37	1.23	1.32	0.06
Stand VI										
Cd	0.43	0.60	0.58	0.54	0.08	0.51	0.42	0.79	0.57	0.16
H	1.04	0.79	0.82	0.89	0.11	1.00	1.15	0.43	0.86	0.31
Alpha	4.00	4.00	4.00	4.00	0.00	5.00	5.00	3.00	4.33	0.94
Evenness	1.73	1.32	1.37	1.47	0.19	1.44	1.65	0.90	1.33	0.31
Stand VII										
Cd	0.54	0.55	0.51	0.53	0.02	0.34	0.34	0.35	0.34	0.00
H	0.72	0.64	0.68	0.68	0.04	1.09	1.09	1.08	1.09	0.01
Alpha	3.00	2.00	2.00	2.33	0.47	3.00	3.00	3.00	3.00	0.00
Evenness	1.52	2.12	2.26	1.97	0.32	2.29	2.29	2.26	2.28	0.01
Stand VIII										
Cd	0.20	0.20	0.21	0.20	0.00	0.20	0.24	0.22	0.22	0.02
H	1.68	1.68	1.66	1.67	0.01	1.70	1.58	1.66	1.65	0.05
Alpha	6.00	6.00	6.00	6.00	0.00	6.00	6.00	6.00	6.00	0.00
Evenness	2.16	2.16	2.13	2.15	0.02	2.18	2.03	2.14	2.11	0.07
Stand IX										
Cd	0.39	0.26	0.26	0.30	0.06	0.52	0.51	0.50	0.51	0.01
H	1.01	1.37	1.37	1.25	0.17	0.83	0.69	0.69	0.74	0.07
Alpha	3.00	4.00	3.00	3.33	0.47	3.00	2.00	2.00	2.33	0.47
Evenness	2.11	2.28	2.86	2.42	0.32	1.75	2.29	2.30	2.11	0.26

torulasa forest (87.0 trees 100m-^{2}) was observed on the east (Cooler)aspect and the lower zone (stand 2nd) where it was found associated with *C. deodara* only. The lowest density of *C. torulosa* (2.0 trees m^{-2}) was observed on the west aspect (stand 8th) where it was associated with *C. deodara, M. esculenta, P. roxurghii, Q. leucotrichophora* and *R. arboreum* which is supported by the fact that high diversity of plants decreases the dominance if species. In the south aspect (stands 4th and 5th) this species was completely absent. *C. torulosa* emerged as a co-dominant

Table 4. Niche overlap between species of trees at all aspects

S.N.	Name of species	1 Cd	2 Ct	3 Lo	4 Me	5 Pr	6 Pw	7 Ql	8 Ra
East aspect									
1	*Cedrus deodara*	*							
2	*Cupressus torulosa*	0.889	*						
3	*Lyonia ovalifolia*	0.000	0.112	*					
4	*Myrica esculenta*	0.152	0.152	0.22	*				
5	*Pinus roxburghii*	0.252	0.141	0.00	0.778	*			
6	*Pinus wallichiana*	0.152	0.152	0.667	0.556	0.333	*		
7	*Qurecus leucotrichophora*	0.152	0.152	0.420	0.802	0.58	0.753	*	
8	*Rhododendron arboreium*	0.152	0.141	1.00	0.878	0.900	0.433	0.68	*
South aspect									
1	*Cedrus deodara*	*							
2	*Cupressus torulosa*	0.000	*						
3	*Lyonia ovalifolia*	0.600	0.200	*					
4	*Myrica esculenta*	0.500	0.250	0.900	*				
5	*Pinus roxburghii*	0.497	0.000	0.679	0.729	*			
6	*Pinus wallichiana*	0.000	1.000	2.000	0.250	0.000	*		
7	*Qurecus leucotrichophora*	0.091	0.636	0.491	0.591	0.364	0.606	*	
8	*Rhododendron arboreum*	0.000	0.333	0.400	0.500	0.521	0.333	0.606	*
North aspect									
1	*Cedrus deodara*	*							
2	*Cupressus torulosa*	0.267	*						
3	*Lyonia ovalifolia*	0.000	0.365	*					
4	*Myrica esculenta*	0.182	0.003	0.182	*				
5	*Pinus roxburghii*	0.773	0.407	0.000	0.409	*			
6	*Pinus wallichiana*	0.000	0.367	1.000	0.182	0.000	*		
7	*Qurecus leucotrichophora*	0.150	0.675	0.525	0.657	0.377	0.525	*	
8	*Rhododendron arboreum*	0.000	0.733	1.000	0.182	0.000	1.000	0.525	*
West aspect									
1	*Cedrus deodara*	*							
2	*Cupressus torulosa*	0.214	*						
3	*Lyonia ovalifolia*	0.000	0.786	*					
4	*Myrica esculenta*	0.500	0.314	0.100	*				
5	*Pinus roxburghii*	0.583	0.214	0.000	0.900	*			
6	*Pinus wallichiana*	0.000	0.786	1.000	0.100	0.00	*		
7	*Qurecus leucotrichophora*	0.206	0.824	0.618	0.618	0.383	0.618	*	
8	*Rhododendron arboreium*	0.524	0.310	0.095	0.095	0.905	0.095	0.478	*

Table 3. Beta diversity matrices for species

Stands	1	2	3	4	5	6	7	8	9
1	*								
2	0.00	*							
3	1.24	0.95	*						
4	0.73	0.53	1.03	*					
5	1.10	0.00	1.03	1.20	*				
6	0.73	1.07	1.37	1.20	0.80	*			
7	1.10	0.00	0.95	1.07	1.07	1.07	*		
8	1.00	1.00	1.55	1.10	1.10	1.47	1.00	*	
9	0.83	0.00	1.18	0.90	1.35	0.90	1.17	1.25	*

species at stand 2nd (east aspect) it had maximum density (37.0 trees 100 m^{-2}), TBC (19.0 m^2) and frequency (90%) in this stand (2nd) followed by sites having west and north aspect. In the north aspect the presence of *C. torulosa* and *C. deodara* was due to the plantation program undertaken by the forest department. The forest of *C. deodara* can be literally attributed to edaphic and topographical conditions. The development of deodar forest is associated with residual soil formation (Joshi et al. 1983) being the east face, the low insolations and high moisture condition of slope further promoter its growth. *Q. leucotricophora* was found in all stands except stand 2nd. It is dominated in the summit top (Stand 1st) having maximum IVI value (118.45). Further it is more frequent in the upper elevation of east and north face, while in the west aspect its occupied small TBC (0.30-0.38 m^2 100 m^{-2}) and exhibits least density (3.0 trees 100 m^{-2}) as compared to *P. roxburghii*. Due the fact that the lower stands of north and south aspect were dominated by chir pine while the east and west aspects comprise *C. deodara*, *C. torulosa*, *R. arboreum*, etc. in the upper elevation of these aspects were dominated by *P. roxburghii*. Influence the higher

Table 4. Niche breath in all aspects

S.N.	Name of species	Bi	Bi'
East aspect			
1	*Cedrus deodara*	1.35	0.185
2	*Cupressus tolurosa*	1.36	0.223
3	*Lyonia ovalifolia*	1.00	0.00
4	*Myrica esculanta*	1.53	0.23
5	*Pinus roxburghii*	1.22	0.141
6	*Pinus wallichiana*	1.80	0.276
South aspect			
1	*Cedrus deodara*	1.00	0.00
2	*Cupressus torulosa*	1.00	0.00
3	*Myrica esculenta*	2.67	0.452
4	*Pinus roxburghii*	2.00	0.301
5	*Pinus wallichiana*	1.00	0.00
6	*Quercus luechotrichophora*	2.05	0.373
7	*Rhododendron arboreum*	1.80	0.276
North aspect			
1	*Cedrus deodara*	1.00	0.00
2	*Cupressus torulosa*	1.64	0.252
3	*Lyonia ovalifolia*	1.00	0.00
4	*Myrica esculenta*	2.12	0.394
5	*Pinus roxburghii*	1.54	0.233
6	*Pinus wallichiana*	1.00	0.00
7	*Quercus leucotrichophora*	2.48	0.429
8	*Rhododendron arboreum*	1.00	0.00
West aspect			
1	*Cedrus deodara*	1.00	0.00
2	*Cupressus torulosa*	1.51	0.226
3	*Lyonia ovalifolia*	1.00	0.000
4	*Myrica esculenta*	2.38	0.410
5	*Pinus roxburghii*	1.95	0.295
6	*Pinus wallichiana*	1.00	0.000
7	*Quercus leucotrichophora*	2.20	0.404
8	*Rhododendron arboreium*	2.33	0.404
Landscape level			
1	*Cedrus deodara*	2.020	0.451
2	*Cupressus torulosa*	1.560	0.333
3	*Lyonia ovalifolia*	2.270	0.414
4	*Myrica esculenta*	6.080	0.831
5	*Pinus roxburghii*	5.340	0.785
6	*Pinus wallichiana*	1.800	0.276
7	*Quercus leucotrichophora*	5.320	0.804
8	*Rhododendron arboreum*	3.500	0.604

anthropogenic pressure on this species us another important cause for presence of lower number of both small and large oak tree in the study area. Large number of tree are chopped and lopped for fodder, fuel purpose and log for construction work, resulting more open canopy which provide for favorable environmental condition for the invasion of secondary species. Chettri et al. (2002) observed that in both open canopy and closed canopy forest, reduced value for IVI and basal area for species that are preferred for fire wood. The chir pine (*P. roxburghii*) enjoyed as a dominant species at all aspect completely absent at the summit (stand 1st) with highest elevation (2300 m asl). As compared to other stands, it was less common in the east aspect as *Pinus* grows more rapidly in the drier area as compared to cool moist areas. This situation is comparable with the studies done by Singh and Singh (1992) and Singh et al.(1997) on Kumaun Himalaya and Sharma and Baduni (2000) in the moist temperate forest of Garhwal Himalaya and Sundriyal and Sharma (1996), Chetrii et al(2002) on Sikkim Himalaya. Summit top (stand 1st) possess the maximum tree species richness and represents the trees of all other aspect (except *P. roxburghii*), supported the fat that higher altitudes promotes heterogeneity. Summit point itself gives the idea of general vegetation pattern. In its east aspect was *C. deodara*, *C. torulosa* and *R. arboreum*. The south facing slope of this stands was burned by the forest fire but the dry tree of *C.torulosa*, *C. deodara* and *Q. leucotrichophora* give an idea about the closed forest patch in the past.

Bhandari et al. (1998), Ghidiyal et al. (1998), Bankoti and Tewari (2001), Khera et al. (2001) etc. workers reported the similar pattern of species diversity in distributed forest of Central Himalaya with special reference to aspect and altitude. The present finding for diversity index falls well with in the range of other temperate forests. Monk (1967) and Risser and Rice (1971) obtained 2.3 as the highest value for diversity index for temperate vegetation including forest trees. Barun (1950) reported species diversity between 1.69 and 3.40 in an eastern deciduous forest vegetation including herbs at North America. Baduni and Sharma (1997) reported diversity index value up to 1.70 for moist temperate forest of Garhwal Himalaya. On the other hand, tropical forest including vegetation of Savana indicate higher diversity index as calculated by Knight (1975) for young (H= 5.06) and old (H= 5.40) stands.

A second major component of diversity is evenness or equitability in the apportionment of individuals among the species. The evenness varied between 1.26 (south aspect) to 3.16 (east aspect). Moist cooler conditions, moderate soil temperature and lower degree of human disturbance are the main factor for the equal share of individuals among species at east aspect. The individual of species in south and north aspects were not equally distributed due to the microclimatic difference and varying anthropogenic disturbance.

The analysis of niche relationship in natural communities is of considerable interest. The way in which species with in ecological communities partitions available resources among themselves is a major determinant of the diversity of co-existing species (MacArthur 1958). All else being equal, a community, with more resources sharing, or greater niche overlap, will clearly support more species than one with less niche overlap (Pianka 1974).

REFERENCES

Amiro BD, Courtin GM. 1981. Pattern of vegetation in the vicinity of an industrially disturbed ecosystem, Sudbury, Ontario. Canadian J Bot 59: 1623-1639.

Arno SF, Hammerly RP. 1984. Timberline. Mountain and Arctic forest frontiers. Mountaineers, Seattle, Washington.

Baduni NP, Sharma CM. 1997. Flexibility-fitness-compromise of the trees in moist temperate forest of Garhwal Himalaya. Ann For 5 (2): 126-135.

Bankoti NS, Tewari LM. 2001. Analysis of forest vegetation at and around Soni-Binsar area in Kumaun Himalaya. In: Pande PC, Samant SS (ed.). Plant Diversity of the Himalaya. Gyanodaya Prakashan, Nainital.

Bhandari BS, Metha JP, Tiwari SC. 1998. Woody vegetation structure along an altitudinal gradient in a part of Garhwal Himalaya. J Hill Res 11 (1): 26-31.

Bisht AS, Sharma KD. 2014. Plants utilization by the communities of Bharsar and adjoining area of Pauri Garhwal District, Uttarakhand, India. Biodiversitas 15 (1): 94-100

Braun EL. 2000. The ecology of the forest of Eastern North America, their development, composition and distribution. Deciduous forest of Eastern North America. McGraw Hill, New York.

Chettri NE, Klabya DC, Sundriayal RC. 2002. Impact of firewood extraction on tree structure, regeneration and woody biomass productivity in a trekking corridor of the Sikkim Himalaya. Mountain Res Dev 22 (2): 150-158.

Colwell RK, Futuyma DJ. 1971. On the measurement of niche breadth and overlap. Ecology 52: 567-576.

Connell JH, Orias E. 1964. The ecological regulation of species diversity. Amer Nat 98: 399-414.

Gaur UN, Raturi GP, Bhatt AB. 2003. Quantitative response of vegetation in glacial moraine of Central Himalaya. The Environmentalist (23): 237-247.

Ghidiyal S, Baduni NP, Khanduri VP, Sharma CM. 1998. Community structure and composition of oak forests, along altitudinal gradients in Garhwal Himalaya, Indian J For 21 (3): 242-247.

Joshi SC, Joshi DR, Dani DD. 1983. Kumaun Himalaya- A Geographical Perspective on Resource Development. Gyanodaya Prakashan, Nainital.

Kershaw KK. 1973. Quantitative and Dynamic Plant Ecology. 2nd ed. ELBS and Edward Arnold (Publ.) Ltd. London.

Khera N, Kumar A, Ram J, Tewari A. 2001. Plant biodiversity assessment in relation to disturbances in mid-elevational forests of Central Himalaya, India. Trop Ecol 42 (1): 83-95.

Knight DH. 1975. A phytosociological analysis of species rich tropical forest on Barro Colorado Island, Panama. Ecol Monogr 45: 259-284.

Levins R. 1968. Evolution of changing environments: Some theoretical explorations. Amer Nat 147: 784-812.

MacArthur RH. 1958. Population ecology of some warblers of Northeastern coniferous forests. Ecology 39 (4): 599-619.

Mani MS. 1974. Ecology and Biogeography of High Altitudes. Insects. Junk Publishers. The Hague.

Monk CD. 1967. Tree species diversity in the eastern deciduous forest with particular reference to North Central Florida. Am Nat 101: 173-187.

Pandey AN. Singh JS. 1984. Mechanism of ecosystem recovery: A case study from Kumaun Himalaya. Reclam Reveg Res 3: 271-292

Pandey N, Nautiyal BP, Bhatt AB. 2000. Studies on vegetation analysis, plant form and biological spectrum of an alpine zone of north-west Himalaya. Trop Ecol, 40: 163-166

Pianka ER. 1974. Niche overlap and diffuse competition. Proc Natl Acad Sci USA 71: 2141-2145.

Pichett STA. 1980. Non equilibrium co existence of plants. Bull Theor Bot Club 107: 238-248.

Rawat J. 2003. Completion, Niche and Diversity patterns along an altitudinal gradient in a part of lesser Himalaya in Uttaranchal. [Dissertation]. HNB Garhwal University, Srinagar, India

Risser PG, Rice EL. 1971. Diversity in tree species in Oklahoma upland forest. Ecology 52: 876-880.

Sai VS, Budholia SS. 1986. Niche measurement of tree species in central India forest. Trop Ecol 27: 76-84.

Shannon CE, Wiener W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, IL.

Sharma CM, Baduni NP. 2000. Effect of aspect on the structure of some natural stands of *Abies pindrow* in Himalayan moist temperate forest. The Environmentalist 20: 309-317.

Simpson EH. 1949. Measurement of diversity. Nature 163: 688.

Singh JS, Singh SP 1992. Forests of Himalaya: Structure, functioning and impact of Man. Gyanodaya Prakashan, Nainital, India.

Singh JS, Tiwari AK, Saxena AK. 1985. Himalayan forest. A net source of carbon to the atmosphere. Environ Conserv 12: 67-69.

Singh SP, Rawat YS, Garkoti SC. 1997. Failure of broun oak (*Quercus semicarpifolia*) to regenerate in Central Himalaya: A case of environmental semi surprise. Curr Sci 73 (4): 371-374.

Sundriyal RC, Sharma E. 1996. Anthropogenic pressure on tree structure and biomass in the temperate forest of Mamlay Watershed in Sikkim. For Ecol Manag 81 (1-3): 113-134.

Ter Braak CJF, Prenrice IC. 1988. A theory of gradient analysis. Adv Ecol Res 18: 271-317.

Westman WE. 1975. Edaphic climax pattern of the pygmy forest region of California. Ecol Monogr 45: 109-135.

Wiart J. 1983. Ecosystem Villageois Traditionnel en Himalaya Nepalais: l Producton Forestiere Suffit-elle aux Besoins de la Population? [Dissertation]. Universite Scientifique et Medicale de Grenoble, France.

Woodwell GM. 1970. Effect of pollution on the structure and physiology of ecosystem. Science 168: 429-433.

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Cellulolytic and hemicellulolytic bacteria from the gut of *Oryctes rhinoceros* larvae

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Abstract. *Sari SLA, Pangastuti A, Susilowati A, Purwoko Tj, Mahajoeno E, Hidayat W, Mardhena I, Panuntun DF, Kurniawati D, Anitasari R. 2016. Cellulolytic and hemicellulolytic bacteria from the gut of* Oryctes rhinoceros *larvae. Biodiversitas 17: 78-83.* Lignocellulose is very potential as raw material for biofuel production because it is cheap, abundant and renewable. The main carbohydrate constituents of lignocellulosic material are cellulose and hemicelluloses (a group of heteropolymers that includes xylans and mannans). The most important process in bioethanol production from lignocellulose is the bioconversion of polysaccharides into fermentable sugar. Enzymatic hydrolysis has been developed because it is the more environmentally approach. Since the cost of hydrolytic enzyme production is the major problem of the process, many type of research has been focused on lowering the cost of enzyme production, including screening for organisms with a novel enzyme. This present study was conducted to isolate and screen of the cellulolytic and hemicellulolytic Bacteria from the gut of *Oryctes rhinoceros* L. larvae. The 3rd instars were used in this research. The research succeeded to isolate 11 bacterial isolates from the gut of *O. rhinoceros* larvae. The screening result demonstrated that bacterial isolates had cellulolytic (63.6% of total isolates), xylanolytic (72.7% of total isolates), and mannanolytic (100% of total isolates) activity. Based on the 16S rDNA sequence, 10 isolates were classified into *Bacillus* and only 1 isolate was classified into *Citrobacter*. The GOR2 which was closely related to *Bacillus pumilus* vit bac1 has the highest cellulolytic and xylanolytic activities. The isolate with the highest mannanolytic activity was the GOR7 which was closely related to *Bacillus aryabhattai* strain IHB B 6821.

Keywords: Cellulolytic, hemicellulolytic, lignocellulosic, *Oryctes rhinoceros*

INTRODUCTION

Increasing worldwide demand for energy, fuel oil depletion, continuously price increasing of crude oil and environment issues such as global warming and pollution encourage intensive investigation to develop an alternative renewable energy **(**Dashtban et al. 2009). Bioethanol is an alternative energy source that can be potentially developed to replace petroleum. It can be utilized as transportation fuels with little change to current technology, renewable and reduce green house gas emissions (Carere et al. 2008).

Bioethanol can be produced from the organic material which contains sugar, starch, or lignocellulose. Lignocellulose is the main component of plant cell wall. It is very potential to be developed as a substrate for bioethanol production since it is very cheap, abundant, and renewable. Structurally, it is formed of three main polymers, namely: cellulose (a homopolymer of D-glucosyl residues), hemicelluloses (a group of heteropolymers that includes xylans and mannans), and lignin (a complex polyphenolic polymer). The main polysaccharides of lignocelluloses, celluloses and hemicelluloses (mannan, and xylan), can be hydrolyzed to yield fermentable sugars, which can then be fermented to generate bioethanol (Gong et al. 1999; Cheng and Timilsina 2011).

Polysaccharides hydrolysis can be performed either thermochemically using acid and heat or biologically using hydrolytic enzymes. Enzymatic hydrolysis possesses several advantages such as its efficiency is quite high, its byproduct can be controlled, its process does not need expensive instruments, and its energy need is quite low (Badger 2002). However, the enzymatic digestion of native plant cell walls is inefficient, presenting a considerable barrier to cost-effective biofuel production (Lacayo et al. 2013). Much research efforts have been focused on lowering the cost of enzyme production. Various ways are done start from microbial exploration as a potential enzyme source, strain and enzyme engineering, process engineering, including substrate selection, cultivation condition and bioreactor design (Badger 2002; Howard et al. 2003).

Cellulase and hemicellulase are hydrolytic enzymes which play an important role in lignocellulose hydrolysis. Cellulase is a group of enzymes that degrade cellulose, a major component of lignocellulose. Components of cellulase systems were classified based on their mode of catalytic action into three major types: endoglucanase (EC 3.2.1.4), exoglucanase or Cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Lynd et al. 2002; Dashtban et al. 2009). Mannanase and xylanase are the key enzymes which have roles in hemicellulose hydrolysis.

Polysaccharide hydrolysis is the key process of herbivorous insect feed digestion (Shi et al. 2011). The herbivorous insect can utilize lignocellulose as its energy source. Its ability is supported by the presence of microbes in its gut, which produce hydrolytic enzymes (Suh et al. 2003). *Rhinoceros* beetles (*Oryctes rhinoceros* L.) is a herbivorous insect belong to the Coleopteran order (Scarabaeidae family) (Bedford 1974; Lavelle et al. 1997; Swamy and Deesh 2011). The *O. rhinoceros* larvae growth in a pile of decaying vegetation and using the residue from organic material as feed (Schmaedick 2005). This research was conducted to isolate and screen cellulolytic, hemicellulolytic (xylanolytic and mannanolytic) bacteria from the gut of *O. rhinoceros* larvae.

MATERIALS AND METHODS

Organism

Oryctes rhinoceros larvae were collected from rice stalk composting field in Srumbung, Magelang, Central Java, Indonesia. The 3rd instars were used in this research**.**

Media

Media for isolating bacteria from *O. rhinoceros* gut was Lauria-Bertani Agar (LA) with composition: 1% tryptone, 0.5% yeast extract, 0.5% NaCl, and 1% agar. Regeneration of bacteria used Lauria-Bertani medium. Screening of cellulolityc bacteria used minimal media (Berg's et al. 1972) with the addition of 10 g L^{-1} carboxymethyl cellulose (CMC) sodium salt low viscosity (Sigma-Aldrich) for cellulolytic, Locus bean gum for mannolytic and 1% beech wood xylan (Sigma-Aldrich) for xylanolytic and pH was adjusted to 7. Composition of minimal medium (in g/100 mL) was 0.2 g $NaNO_3$, 0.05 g $MgSO_4$, 0.005 g K_2HPO_4, 1 mg $FeSO_4$, 2 mg $CaCl_2$, 0.2 mg $MnSO_4$, and 2% agar.

Isolation of bacteria from the gut of *O. rhinoceros* larvae

The 3rd instars were used in this research. The instars were cleaned externally with 95% ethanol and dissected. The entire digestive tract was aseptically isolated in a UV laminar flow hood and homogenized for 5 minutes in sterile NaCl (0.85%) solution then incubated for 30 minutes at 37ºC. Isolation of bacteria was done by dilution plate method. The supernatant was serially diluted 10^3-10^6. After serial dilution, 0.1 mL of solution was taken using sterile micropipette and plated on LA medium. Incubation was done at 37^{o}C for 48 hours.

Screening for cellulolytic, xylanolytic and mannolytic bacteria

Bacterial isolates were grown in LB medium with the addition of 1% carbon source at 37^{o}C for 24 hours. Screening for hydrolytic activity was done by inoculating 0.5 µl inoculums into screening medium and incubated at 37°C for 48 hours. The clear zone around colony showed cellulolytic, xylanolytic or mannolytic activity. The clear zone was made become clearer by coloring with Congo red for cellulolytic and xylanolytic (Wood 1980) and Iodin for mannanolytic.

Identification of bacteria

Bacterial isolates were grown in LB medium at 37°C for 24 h. Cultures were centrifuged at 10,000× g for 1 min, and the supernatant was removed. DNA extraction was performed using a Presto™ Mini gDNA Bacteria Kit (Genaid, Taiwan) according to the manufacturer's instructions. Bacterial universal primers 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-CCCGGGAACGTATTCACCGC-3') were used to amplify the 16S rDNA from genomic DNA (Marchesi et al. 1998). Polymerase chain reaction (PCR) was performed in a Thermo Cycler (Aplied Biosystem). The amplification was performed as follows: initial denaturation for 5 min at 94°C, 35 cycles each of denaturation for 15s at 94°C, annealing for 15s at 55°C, and elongation for 15s at 72°C, and a final extension for 7 min at 72°C. PCR product purification and sequencing were done by 1st BASE (Singapore). Sequences of 16S rDN were compared to the 16S rDNA sequences available in the Gen Bank data base using the BLAST program at National Center for Biotechnology Information http: //www.ncbi.nlm.nih.gov/

Phylogeneticanalysis

Phylogenetic analysis was performed using the neighbor-joining method with MEGA 6.0. (Tamura et al. 2013). To statistically evaluate the branching, bootstrap analysis was carried out with data resampled 1000 times

RESULTSAND DISCUSSION

Isolation and screening of cellulolytic bacteria

The research succeeded to isolate 11 bacterial isolates from the gut of *O. rhinoceros* larvae. The screening result based on the formation of a clear zone around the colony demonstrated that bacterial isolates have cellulolytic (63.6%), xylanolytic (72.7%), and mannanolytic (100%) activity (Table 1). Based on the clear zone diameter, it was observed that the isolates have varied ability in hydrolyzing the carbon source in the forms of CMC, Xylan, and mannan. The clear zones diameter ranges between 0.97-3.03 cm on CMC media, 0.17-4 cm on xylan media, and

Table 1. Screening result of cellulolytic and hemicellulolytic bacteria from the gut of *O. rhinoceros* larvae

Code of isolates	Clear zone diameter (cm)		
	Xylan medium	Mannan medium	CMC medium
GOR1	1.17	1.91	3.01
GOR2	**4.00**	1.32	**3.03**
GOR3	0.17	2.61	1.87
GOR4	1.67	2.51	-
GOR5	1.60	2.53	-
GOR6	-	0.55	2.73
GOR7	2.50	**3.48**	2.90
GOR8	2.67	2.36	1.77
GOR9	2.33	1.04	0.97
GOR10	-	2.83	-
GOR11	-	3.39	-

Table 2. Identity of the cellulolytic and hemicellulolytic bacteria isolated from the gut of *O. rhinoceros* larvae based on 16S rDNA sequences

Code of isolates	Accession	E Value	Identity	Strain of closest match
GOR 1	KF135465.1	0.0	99%	*Bacillus subtilis* strain JK1316S
GOR 2	KC845305.1	0.0	99%	*Bacillus pumilus* strain Vit Bac1
GOR 3	KJ534434.1	0.0	99%	*Bacillus cereus* strain LD147
GOR 4	KJ534462.1	0.0	99%	*Bacillus megaterium* strain RD30
GOR 5	JF512478.1	0.0	99%	*Bacillus thuringiensis* strain Pak2310
GOR 6	KF933659.1	0.0	99%	*Bacillus aquimaris* strain HNS62
GOR 7	KF668459.1	0.0	99%	*Bacillus aryabhattai* strain IHB B 6821
GOR 8	KJ572277.1	0.0	99%	*Bacillus cereus* strain L-3
GOR 9	CP000822.1	0.0	99%	*Citrobacter koseri* ATCC BAA-895
GOR 10	FJ189762.1	0.0	99%	*Bacillus clausii* strain MSB08
GOR 11	JF512478.1	0.0	99%	*Bacillus thuringiensis*strain Pak2310

0.55-3.48 cm on mannan media. The highest cellulolytic and xylalolytic activity was demonstrated by GOR2 while the highest mannanolytic activity was displayed by GOR7.

Identification of bacteria

The gram staining results reveal that almost all bacterial isolates were rod-shaped gram-positive, except for GOR9, which was short rod-shaped gram-negative. All isolates were then identified based on the16S rDNA sequences. The 16S rDNA amplicons of some isolates using bacterial universal primers 63F and 1387R showed in Figure 1. Based on the BLAST analysis (Table 2.), all bacteria had a partial sequence similar to the database from the Gene Bank, having the level of similarity of over 99%.

Phylogeneticanalysis

Phylogenetic analyses placed the 11 isolates in the 2 groups: *Bacillus* and *Citrobacter* (Figure 2). *Bacillus* was the dominant group, including 10 isolates. Branching off this unknown group from *Bacteroides cellulolyticus* strain CRE2 was supported by a 100% bootstrap value.

Discussion

The ability to degrade cellulose and hemicellulose can be measured based on clear zone diameter (Figure 3). The isolate ability on making a clear zone showed that this isolate can hydrolyze polysaccharide as a carbon source or produced hydrolytic enzymes which were secreted to the growth medium. These enzymes degrade β-1,4-glycosidic bond in CMC, xylan and mannan. Nevertheless, clear zone diameters were qualitatively reproducible with the plate overlay technique. Theclear zone is made become clearer by coloring with Congo red and Iodin which strongly interacted with β-1,4-glikosidik bound (Wood 1980; Teather and Wood 1982).

Based on clear zone diameter, it was determined that the potential isolates for cellulase and xylanase producer were GOR2 that was closely related to *Bacillus pumilus* vit bac1. *Bacillus pulmilus* have been reported as cellulase (Kotchoni et al. 2006) and xylanase (Lamid 2006) producer. Several *Bacillus* have been reported to have cellulolytic activity such as *B. Brevis* (Singh and Kumar 1998), *B. pumilus*, *B. amyoliquefaciens* DL-3 (Lee et al. 2008), and *B. subtilis* YJ1 (Yin et al. 2010); and xylanolytic activity, such as *B. subtilis* (Heck et al. 2002), *B. altitudinis* (Adhyaru et al. 2014), and *B. cereus* (Mandal et al. 2012). *Bacillus* is the most dominant bacterium used in enzymes industry because of its ability to produce and secrete an amount of extracellular enzymes (Rastogi et al. 2010).

The bacterial isolate with the highest mannanolytic activity was GOR7, that was closely related to *Bacillus aryabhattai*. *Bacillus aryabhattai* have been isolated by Ray et al. (2012) from the rhizosphere region of *Lemna* sp. from the East Kolkata wetlands. *Bacillus aryabhattai* have the properties of withstanding Cr^{3+} exposure, salinity, stress, and also withstanding high UV exposure. *Bacillus aryabhattai* was also discovered as producer of L-asparaginase (Singh and Srivastava 2014) and protease (Sharma et al. 2014).

The result of the research showed that all isolates had cellulolytic or hemicellulolytic activities. Cellulolytic and hemicellulolytic bacteria are found in most herbivorous insects' gut such as termite (Kuhnigk and Konig 1997),

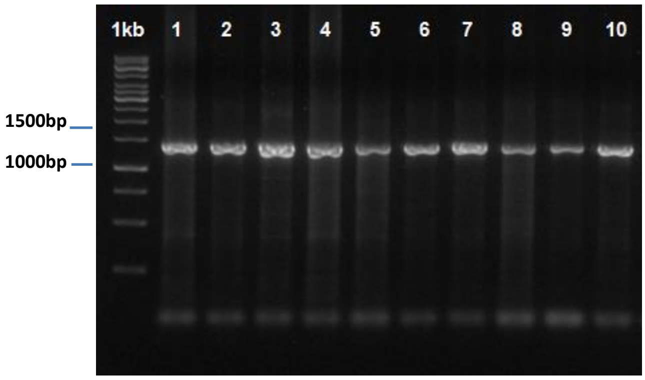

Figure 1. Bacterial 16S rDNA PCR products using Bacterial Universal Primer 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-CCCGGGAACGTATTCACCGC-3'). The following DNA templates were used for PCR (by lane): 1, GOR1; 2, GOR2; 3, GOR3; 4, GOR4; 5, GOR5; 6, GOR6; 7, GOR7; 8, GOR8; 9, GOR9; 10, GOR10

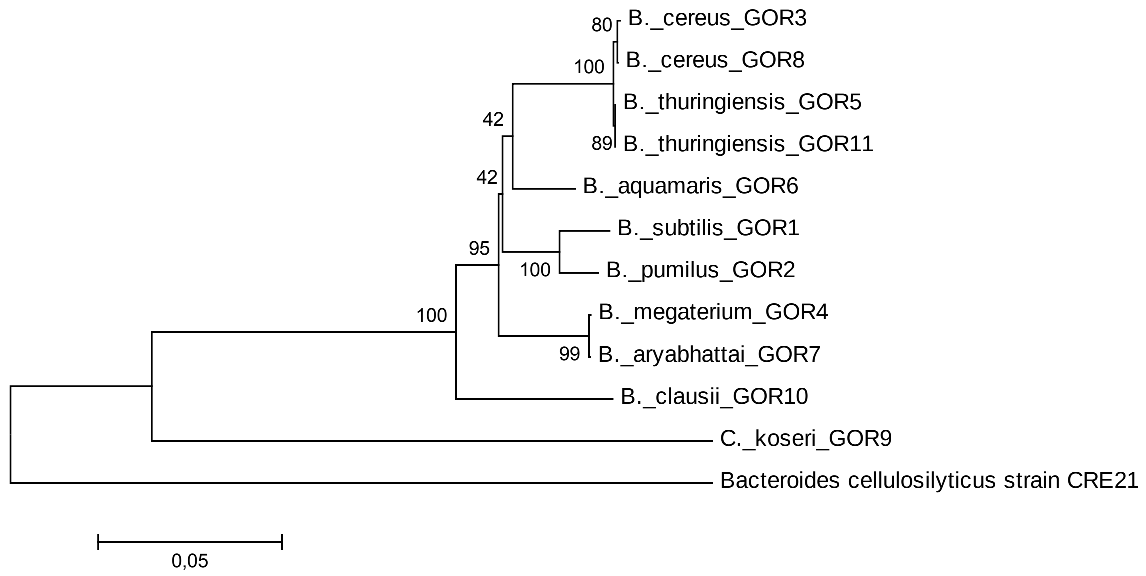

Figure 2.16S rDNA-based dendrogram showing phylogenetic relationships of cellulolytic and hemicellulolytic bacteria from the gut of *O. rhinoceros* larvae (shown with GOR as a code of isolate) to members of the cellulolytic bacteria from Gen Bank data base. Bootstrap values (n=1000 replicates) of ≥49% are reported as percentages. The scale bar represents the number of changes per nucleotide position

A **B** **C**

Figure 3. Isolate GOR2 on CMC medium (A) and *beech wood xylan* medium (B) after coloring with 1% Congo red and GOR7 on locust bean gum medium after coloring by 0.5 N Iodin (C). The clear zone surrounding a colony indicates hydrolitic activity

Bombix mori larvae (Anand et al. 2010), and *Holotrichia parallela* larvae (Huang et al. 2012). Insects such as *Reticulitermes flavipes* (Zhou et al. 2008), *Anoplophora glabripennis* (Geibet al. 2010), *Tenebrio molitor* (Ferreira et al. 2001), and *Pachnoda marginata* (Cazemier et al. 2003) can degrade plant biomass with the help of microorganism in their gut. The presence of bacteria in the gut of herbivorous insect has an important role in feed hydrolysis. The result of the research demonstrates that the gut of *O. rhinoceros* larvae is an attractive source for the study of novel cellulolytic, xylanolytic and mannanolytic microorganisms and enzymes that are useful for lignocellulose degradation.

The result of the identification shows that 10 out of 11 isolates were classified into *Bacillus*. *Bacillus* is also discovered in the digestive tract of *Holotrichia parallela* (Coleoptera: Scarabaeidae) during the second and third instars (Huang and Zhang 2013) and *Pachnoda* spp. (Coleoptera: Scarabaeidae) (Andert et al. 2010). *Bacillus* can survive in quite high ranges of temperature and pH such as in the gut of Lepidoptera larvae. Lepidoptera larvae have the average temperature around 37^0 C and the alkaline pH so that it is optimum for the growth of *Bacillus* (Broderick, 2003).The bacterial composition in insect's digestive tract also related to the types of feeds (Broderick, 2004).

In conclusion, the research succeeded to isolate 11 bacterial isolates from the gut of *O. rhinoceros* larvae. The screening result demonstrated that bacterial isolates have cellulolytic (63.6% of total isolates), xylanolytic (72.7% of total isolates), and mannanolytic (100% of total isolates) activity. Based on the 16S rDNA sequence, 10 isolates were classified into the *Bacillus* and only 1 isolate was classified into *Citrobacter*. The GOR2 which was closely related to *Bacillus pumilus* vit bac1 has the highest cellulolytic and xylanolytic activities. The isolate with the highest mannanolytic activity was the GOR7 was closely related to *Bacillus aryabhattai* strain IHB B 6821.

REFERENCES

Adhyaru DN, Bhatt NS, Modi HA. 2014. Enhanced production of cellulase-free, thermo-alkali-solvent-stable xylanase from *Bacillus altitudinis* DHN8, its characterization and application in sorghum straw saccharification. Biocatalys Agric Biotechnol 3: 182-190.

Anand AAP, Vennison SJ, Sankar SG, Prabhu DIG, Vasan PT, Raghuraman T, Geoffrey CJ, Vendan SE. 2010. Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin and starch and their impact on digestion. J Insect Sci 10: 1-20

Andert J, Marten A, Brandl R, Brune A. 2010. Inter-and intraspecific comparison of the bacterial assemblages in the hind gut of humivorous scarab beetle larvae (*Pachnoda* spp.). FEMS Microbiol Ecol 74: 439-449.

Badger PC. 2002. Ethanol From Cellulose: A General Review. In Janick J, Whipkey A (eds.). Trends in new crops and new uses. ASHS Press, Alexandria, VA

Bedford GO. 1974. Description of the larvae of some *Rhinoceros* beetles (Coleoptera, Scarabaeidae, Dynastinae) associated with coconut palms in New Guinea. Bull Entomol Res 63: 445-472

Berg B, von Hofsten B, Peterson G. 1972. Growth and Cellulase Formation of *Cellvibrio fulfus*. J Appl Bacteriol 35 (2): 201-14.

Broderick NA, Goodman RM, Handelsman J, Raffa KF. 2003. Effect of host diet and insect source on synergy of gypsy moth (Lepidoptera: Lymantriidae) mortality to *Bacillus thuringiensis* subsp. kurstaki by zwitter-micin A. Environ Entomol 32: 387-391

Broderick NA, Raffa KF, Goodman RM, Handelsman J. 2004. Census of the Bacterial Community of the Gypsy Moth Larval Midgut by Using Culturing and Culture-Independent Methods. Appl Environ Microbiol 70 (1): 293-300.

Carere CR, Sparling R, Cicek N, Levin DB. 2008. Third generation biofuels via direct cellulose fermentation. Intl J Mol Sci 9: 1342-1360

Cazemier AE, Verdoes JC, Reubsaet FA, Hackstein JH, van der Drift C, Op den Camp HJ. 2003. *Promicromonospora pachnoda* sp. nov., a member of the (hemi) cellulolytic hindgut flora of larvae of the scarab beetle *Pachnoda marginata*. Antonie Van Leeuwenhoek 83: 135-148

Cheng JJ, Timilsina GR. 2011. Status and barriers of advanced biofuel technologies: A review. Renew Energ 36: 3541-3549

Dashtban M, Schraft H, Qin W. 2009. Fungal bioconversion of lignocellulosic residues: Opportunities & perspectives. Intl J Biol Sci 3: 378-395

Ferreira AHP, Marana SR, Terra WR, Ferreira C. 2001. Purification, molecular cloning, and properties of a beta-glycosidase isolated from midgut lumen of *Tenebrio molitor* (Coleoptera) larvae. Insect Biochem and Mol Biol 31: 1065-1076

Geib SM, Tien M, Hoover K. 2010. Identification of proteins involved in lignocellulose degradation using in gel zymogram analysis combined with Mass Spectroscopy-based peptide analysis of gut proteins from larval Asian plonghorned beetles, *Anoplophora glabripennis*. Insect Sci 17: 253-264.

Gong CS, Cao NJ, Du J, Tsao GT. 1999. Ethanol production from renewable resources. Advances in Biochemical Engineering/ Biotechnology 65. Springer-Verlag, Berlin.

Heck JX, Hertz PF, Ayub MAZ. 2002. Cellulase and xylanase production by isolated amazon *Bacillus* strains using soybean industrial residue based solid-state cultivation. Braz J Microbiol 33: 213-218.

Howard RL, Abotsi E, Jansen van Rensburg EL, Howard S. 2003. Lignocellulose biotechnology: Issues of bioconversion and enzyme production. African J Biotechnol 2 (12): 602-619.

Huang S, Sheng P, Zhang H. 2012. Isolation and identification of cellulolytic bacteria from the gut of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae). Intl J Mol Sci 13: 2563-2577.

Huang S, Zhang H. 2013. The impact of environmental heterogeneity and life stage on the hindgut microbiota of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae). PLoS ONE 8 (2): e57169. DOI: 10.1371/journal.pone.0057169.

Kotchoni SO, Gachomo EW, Omafuvbe BO, Shonukan OO. 2006. Purification and biochemical characterization of carboxymethyl cellulase (CMCase) from a catabolite repression insensitive mutant of *Bacillus pumilus*. Intl J Agric Biol 8: 286-292

Kuhnigk T, Konig H. 1997. Degradation of dimeric lignin model compounds by aerobic bacteria isolated from the hindgut of xylophagous termites. J Basic Microbiol 37: 205-211.

Lacayo CI, Hwang MS, Ding S-Y, Thelen MP. 2013. Lignin depletion enhances the digestibility of cellulose in cultured xylem cells. PLoS ONE 8 (7): e68266. DOI: 10.1371/journal.pone.0068266.

Lamid M, Chuzaemi S, Puspaningsih NNT, Kusmartono. 2006. Inoculation of xilanolitic bacterial rumen origin an effort to improve the nutritional value of rice straw. Jurnal Protein 14 (2): 122-128. [Indonesian]

Lavelle P, Bignell D, Lepage M, Wolters V, Roger P, Ineson P, Heal OW, Dhillion S. 1997. Soil function in a changing world: the Role of invertebrate ecosystem engineers. Eur J Soil Biol 33: 159-193.

Lee Y-J, Kim B-K, Lee B-H, Jo K-I, Lee N-K, Chung C-H, Lee Y-C, Lee J-W. 2008. Purification and characterization of cellulase produced by *Bacillus amyoliquefaciens* DL-3 utilizing rice hull. Bioresour Technol 99: 378-386.

Lynd LR,Weimer PJ,van Zyl WH, Pretorius IS. 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol Mol Biol Rev 66: 506-577.

Mandal A, Kar S, Mohapatra PKD, Maity C, Pati BR, Mondal KC. 2012. Regulation of xylanase biosynthesis in *Bacillus cereus* BSA1. Appl Biochem Biotechnol167 (5): 1052-1060.

Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, Wade WG. 1998. Design and evaluation of useful Bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl Environ Microbiol 11: 375-382.

Rastogi G, Bhalla A, Adhikari A, Bischoff KM, Hughes SR, Christopher LP, Sani RK. 2010. Characterization of thermostable cellulases produced by *Bacillus* and *Geobacillus* strains. Bioresour Technol 101 (22): 8798-8806.

Ray S, Datta R, Bhadra P, Chaudhuri B, Mitra AK. 2012. From space to earth: *Bacillus aryabhattai* found in the Indian Subcontinent. BioSci Discov 3 (1): 138-145.

Schmaedick M. 2005.Coconut *Rhinoceros* Beetle. Pests and Diseases of American Samoa (8). Division of Community and Natural Resources, American Samoa Community College, USA.

Sharma KM, Kumar R, Vats S, Gupta A. 2014. Production, partial purification and characterization of alkaline protease from *Bacillus aryabhattai* K3. Intl J Adv in Pharm Biol Chem 3 (2): 290-298.

Shi W, Ding S-Y, Yuan JS. 2011. Comparison of insect gut cellulase and xylanase activity across different insect species with distinct food sources. Bioenerg Res 4: 1-10.

Singh Y, Srivastava SK. 2014. Performance improvement of *Bacillus aryabhattai* ITBHU02 for high-throughput production of a tumor-inhibitory L-asparaginase using a kinetic model based approach. J Chem Technol Biotechnol 89: 117-127.

Suh S-O, Marshall CJ, McHugh JV, Blackwell M. 2003. WoodIngestion by Passalid Beetles in the Presence of Xylose-fermenting Gut Yeasts. Mol Ecol 12: 3137-314.

Swamy BN, Deesh AD. 2011. Management of Coconut Rhinoceros Beetle (*Oryctes rhinoceros*) in Fiji. Technical Bulletin Ministry of Primary Industries, Fiji.

Tamura K, Stecher G, Petersen D, Filipski A, Kumar S. 2013. MEGA6: Moleculer Evolutionary Genetics Analysis versi 6.0. Mol Biol Evol 30: 2725-2729.

Teather RM, Wood PJ. 1982. Use of congo red-polysaccharide interaction in enumeration and characterizetion of cellulolytic bacteria from the bovine rumen. Appl Environ Microbiol 43 (2): 777-780.

Wood PJ. 1980. Specificity in the interaction of direct dyes with polysaccharides. Carbohydrate Res 85: 271-287.

Yin L-J, Lin H-H, Xiao Z-R. 2010. Purification and Characterization of a cellulase from *Bacillus subtilis* YJ1. J Mar Sci Technol 18 (3): 466-471

Zhou J, Wang Y-H, Chu J, Zhuang Y-P, Zhang S-L,Yin P. 2008. Identification and purification of the main components of cellulases from a mutant strain of *Trichoderma viride* T100-14. Bioresour Technol 99: 6826-6833.

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Mitigation of mercury contamination through the acceleration of vegetation succession

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Abstract. *Ekyastuti W, Faridah E, Sumardi, Setiadi Y. 2016. Mitigation of mercury contamination through the acceleration of vegetation succession. Biodiversitas 17: 84-89.* The success of the restoration of the tailings ex-gold mining through the succession is highly dependent on the ability of plants to grow and adapt to the troubled land. Restoration through natural succession takes a very long time. Therefore, human intervention is required to accelerate the succession. The purpose of this research was to improve the effectiveness of mitigation of mercury contamination through the acceleration of vegetation succession. This research has been carried out in a greenhouse using an experiment with a completely randomized design. There are 8 treatment consists of four indigenous species (*Dillenia excelsa, Melastoma affine, Cinnamomum porrectum* and *Casuarina junghuhniana*) grown alone (one species) and collective (more than one species) in the tailing media with a mercury content of 20 ppm. The results showed that the planting collectively have a mutually supportive interaction, so that increased the plant growth. In addition, collective planting two or four different species of plants, and the *D. excelsa* itself could decrease the concentration of mercury in the tailing. The acceleration of vegetation succession through the right choice of plants species and planting collectively, capable to increasing the potential of mitigation of mercury contamination in the tailings.

Keywords: Ex-gold mining succession, mitigation of mercury contamination, tailing

INTRODUCTION

Rehabilitation of the tailing areas of ex community gold mining in West Kalimantan, have been urged to do. One phase of the rehabilitation activities is revegetation. Through revegetation activities are expected to not only lower the mercury contamination in the tailings, but also improve the local micro-climate conditions. Revegetation can be done artificially by humans and naturally through the process of succession. The success of the improvement of the tailings of ex community gold mining through a succession are highly dependent on the ability of plants to grow and adapt to the troubled land, so the use of restoration techniques in revegetation is one right choice (Ekyastuti and Roslinda 2015). Restoration is an effort to repair or restore the condition of the damaged area by forming the structure and function close to the original condition (RECA Project 2014). Restoration through natural succession has been proven to reduce the levels of mercury contamination in the tailings, but the process takes a very long time. Previous research has found that the succession of the tailing areas of ex gold mining running very slow. In the tailing areas of ex community gold mining that have been abandoned for five years is still dominated by groundstorey and shrubs (Ekyastuti and Roslinda 2015). Therefore, human intervention is required to accelerate the succession. Human intervention can be done through increasing revegetation activities deliberately in the tailing areas of ex gold mining.

The use of indigenous species is preferred in re-vegetation activities which refers to the restoration techniques. This meant that the goal to forming the structure and function close to the original condition can be achieved. In addition, the use of indigenous species can also maximize the success of replanting, due to the suitability of species where they grow. Secondary forests around the tailing areas of ex community gold mining in West Kalimantan has excellent potential in providing a source of indigenous species for revegetation. Ekyastuti and Roslinda (2015) research in the tailing areas of ex gold mining found 10 indigenous species in Mandor location and 18 indigenous species in Menjalin location. This indicates that the use of indigenous species for revegetation is feasible in both locations. Potential and equal opportunities is also happening in mined land in other places.

Considering the succession in the first five years of the tailing areas are still dominated by groundstorey and shrubs, as previously described, it is necessary to know the role of species of these plants to improve revegetation success. Population groundstorey and shrubs are also thought to have a role in reducing mercury (or other pollutants) on the ground. However, there is no information that conveys the formulation or planting utilizing this groundstorey and shrubs. Therefore, this study aims to improve the effectiveness of mitigation of mercury contamination through the acceleration of vegetation succession. Acceleration of the vegetation succession is done by using some indigenous species elected.

MATERIALS AND METHODS

Research has been carried out in a greenhouse using an experiment with a completely randomized design. As an experimental material were four indigenous species elected refers Ekyastuti et al. (2016), which consists of two species of shrubs that was *Dillenia excelsa* and *Melastoma affine* and two species of woody plant that was *Cinnamomum porrectum* and *Casuarina junghuniana* (Figure 1). Selection of species was based on the ease of the species found in the study site and easy too propagation. Furthermore, the treatment consists of eight levels (planting with species grown alone (one species) and collective (more than one species)), and each treatment was repeated five times. As a plant growth media were tailings of ex gold mining + mature compost 1:1 (v:v) (Ekamawanti and Ekyastuti 2010), with a mercury content 20 ppm. It refers to Ekyastuti et al. (2016) that with the mercury content 20 ppm, these four species of plants still can grow well. Mercury added to the media using techniques of Rabie (2005).

Analysis of variance is done using statistical software program SAS 13 for data growth of plants that collected during the study, namely: (a) the increase of height (cm), (b) the increase of diameter (mm), (c) increase the number of leaves, (d) the ratio of roots and shoots, and (e) the total dry weight of the plant. At the end of the study was also carried out analysis of total mercury content in the roots, shoots and media (ppm) in the Laboratory of Baristand Pontianak using standard SNI 06-6992.2 2004. The data will be used as baseline data to calculate: bioconcentration factor, translocation factor and tolerance index following the technique of Rabie (2005).

RESULTS AND DISCUSSION

Results

Plant growth as a manifestation of the advancement of succession

In general, the growth of four indigenous species are in a good condition and healthy. Found as many as 10% of the crop showed symptoms of mercury poisoning but only until early symptoms of poisoning that are leaves turn yellowing in the bud and leaf edge become browning (Figure 2), and plants still can grow normally until the end of the study. Indigenous species that show symptoms of mercury poisoning are three individual of *D. excelsa* and three individual of *C. porrectum*. The results showed that at all levels of treatment, except in the number of leaves D. excelsa which planted individually, there were no differences in plant growth (Table 1).

The content of mercury in the media and plant tissue

Six months after planting, the results of the analysis of mercury in the tailings media vary considerably (Table 2). The decrease of mercury content in the media are from 20 ppm to 1.46 ppm until <0.002 ppm. Another fact that derived from this data is the discovery of a tendency that the collective planting (more than two species) and planting *D. excelsa* singly, faster to decrease of mercury content in the media than other treatments. The controls are the same medium but without the plant, that is used as a comparison to see the effect of plants in reducing mercury in the media. Mercury concentrations in control media showed the highest value (1.46 ppm), but not much different from the media of *C. junghuniana* that planted singly (1.44 ppm).

Mercury analyzes were also performed on plant tissue at the end of the study (Table 3). At all indigenous species, a different treatment does not cause the differences in mercury concentrations in the plant tissues. However, there is a tendency that *C. junghuhniana* and *D. excelsa* has a higher ability to accumulate mercury, compared with two other species (Figure 3). Meanwhile, for the ANOVA among the treatments (that is the sum of all the mercury content of the plants in each treatment) was significantly different. In order to track the differences among the treatments, we carried out further testing using DMRT (Table 4).

Based on the results of DMRT can be explained that the mercury content in plant tissue on collective planting of four species found the highest, followed by the collective planting of two species of woody plant, then the collective planting of two species of shrubs and planting single of *C. junghuhniana* and *D. excelsa*. Between *M. affine* and *C. porrectum* that grown singly did not show differences in mercury content in the plant tissues, and have the lowest content of mercury in the plant tissues.

Bioconcentration factor, translocation factor and tolerance index

Bioconcentration factor, translocation factor and tolerance index are measured to determine the distribution of mercury in the media and in the plant, as well as the ability of plants to face the presence of mercury contamination (Table 5). Bioconcentration factors are measured to determine where mercury accumulated, in plant tissue or in the media. The result showed that the majority of plants, except *C. junghuhniana* and *C. porrectum* that planted alone, have a value of bioconcentration factor > 1. This means that the accumulation of mercury generally occurs in the plant tissue, not in the media (Rabie 2005). Based on this data, *C. junghuhniana* and *C. porrectum* (woody plant) does not have the ability of mercury accumulation in plant tissues when planted alone. However, if they are planted collectively with other species, these plants have the ability of mercury accumulation in plant tissue. This suggests that the relationships formed among species of these plants is a co-founding or support each other to absorb mercury. The same trend also occurred in the group of herbaceous plants (*D. excelsa* and *M. affine*).

Figure 1. Indigenous species were planted. A. *D. excelsa,* B. *M. affine,* C. *C. porrectum,* D. *C. junghuniana,* and E. Experimental planting in the greenhouse

Figure 2.A-B. Early symptoms of mercury poisoning that appears on *D. excelsa*

Table 1. Results of the analysis of a varian (Anova) of plant growth

Variable of growth	Indigenous species	Average	P-value	Anova
The increase of height (cm)	*D. excelca*	2.00	0.178	n.s.
	M. affine	1.09	0.369	n.s.
	C. porrectum	1.11	0.362	n.s.
	C. junghuniana	0.25	0.786	n.s.
The increase of diameter (mm)	*D. excelca*	0.40	0.679	n.s.
	M. affine	2.67	0.110	n.s.
	C. porrectum	0.75	0.493	n.s.
	C. junghuniana	2.00	0.178	n.s.
Increase the number of leaves	*D. excelca*	0.93	0.420	n.s.
	M. affine	2.00	0.178	n.s.
	C. porrectum	4.96	0.027	*) s
	C. junghuniana	0.63	0.548	n.s.
The total dry weight of the plant (g)	*D. excelca*	2.66	0.110	n.s.
	M. affine	0.61	0.560	n.s.
	C. porrectum	2.11	0.164	n.s.
	C. junghuniana	0.99	0.399	n.s.
The ratio of roots and shoots	*D. excelca*	2.06	0.170	n.s.
	M. affine	0.79	0.476	n.s.
	C. porrectum	0.55	0.593	n.s.
	C. junghuniana	0.26	0.776	n.s.

Note: ns = non significant; *) s = significant

Table 2. Concentration of total mercury in the media six months after treatment

Treatment	Concentration of total mercury in the media (ppm)
Control	1.46
D. excelca (De)	< 0.002
M. affine (Ma)	0.278
C. porrectum (Cp)	0.758
C. junghuniana (Cj)	1.44
De + Ma	< 0.002
Cp + Cj	< 0.002
De + Ma + Cp + Cj	< 0.002

Table 3. ANOVA of mercury content in plant tissue

Indigenous species	Average of mercury (ppm)	P-value	Anova
Among plants:			
D. excelca	1.89	0.295	n.s.
M. affine	0.40	0.400	n.s.
C. porrectum	0.40	0.400	n.s.
C. junghuniana	2.85	0.203	n.s.
Among the treatments (7 planting)	3.56	0.0001	**) v.s.

Note: ns = non significant; **) v.s = very significant

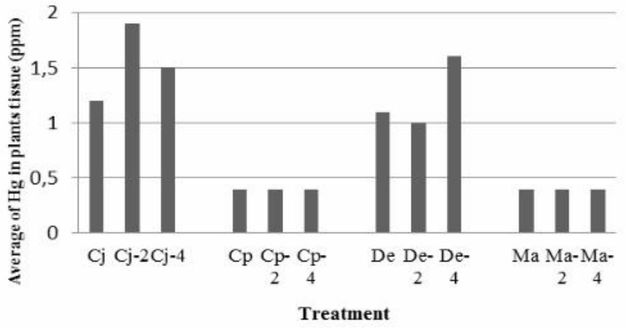

Figure 3. The average of mercury content in plant tissue (ppm) Note: Cj = *C. junghuhniana*, Cp = *C. porrectum*, De = *D. excelca*, Ma = *M. affine*; 2 = collectively planted two different species; 4 = collectively planted four different species

Table 4. DMRT of mercury content in plant tissue

Treatment	Average of mercury content (ppm)	DMRT
De + Ma + Cp + Cj	3.85	a
Cp + Cj	2.30	b
De + Ma	1.35	c
C. Junghuhniana (Cj)	1.20	cd
D. excelca (De)	1.10	cd
M. affine (Ma)	0.40	e
C. Porrectum (Cp)	0.40	e

Description: Mean followed by the same letter on DMRT showed no significantly different results

Table 5. Bioconcentration factor, translocation factor and tolerance index

Indigenous species	Bioconcen-tration Factor	Trans-location Factor	Tolerance Indexs (%)
D. excelca	578.95	0.22	148.79
M. affine	1.44	1.00	133.56
C. porrectum	0.53	1.00	97.06
C. junghuniana	0.83	0.17	113.04
D. excelca-2	526.32	0.27	119.74
M. affine-2	210.53	1.00	145.83
C. porrectum-2	210.53	1.00	65.52
C. junghuniana-2	1000.00	0.12	183.33
D. excelca-4	842.11	0.15	216.79
M. affine-4	210.53	1.00	180.00
C. porrectum-4	210.53	1.00	164.29
C. junghuniana-4	789.47	0.15	171.43

Description: -2 = collectively planted two different indigenous species and -4 = collectively planted four different indigenous species

The value of translocation factor is measured to determine in what part the mercury accumulated in plants, whether in the roots or shoots. If the value of the translocation factor ≥ 1 means that mercury accumulated in the shoots, whereas when the value < 1 means that the mercury accumulated in the roots (Rabie 2005). The value of factor translocation of four indigenous species (Table 5) showed that *C. junghuhniana* and *D. excelsa* accumulate mercury in the roots, while *C. porrectum* and *M. affine* accumulate mercury in the shoots. This phenomenon does not change in plants grown singly (alone) and collectively.

The value of tolerance index is used to see the level of tolerance of plants to mercury. If the value of the tolerance index <30% = low (intolerant), 31-70% = moderate tolerance and > 71% = high tolerance (tolerant). From Table 5 it is known that except the *C. porrectum* that grow together with *C. junghuhniana*, three other indigenous species have tolerance index 97.06% - 216.79%. This means that almost all species of plants that used in this study have a high tolerance for mercury. Another phenomenon is detected that the collective planting four indigenous species together always show the highest level of tolerance than the other treatments.

Discussion

The growth of the plant as a manifestation of success in addressing the problem of mercury

The success or failure of plants to grow normally, describes the ability or the failure of plants to exploiting the potential of media in order to supporting and overcoming the limitations. The areas of ex gold mining (tailings) have many limitations as a medium for plant growth. This media belongs to the criteria of degraded land because the nutrient content is very low, there is no organic matter, acidic to very acidic pH, CEC is very low, and contains of mercury (Ekamawanti and Ekyastuti 2010). Furthermore, it also made clear that these limitations can be minimized by the addition of mature compost in the ratio 1: 1 (v:v). Therefore, in this study we used tailings + mature compost as a plant growth media with a ratio 1: 1 (v:v). The result is a better fertility rate, making it feasible to be used as a medium to plant growth. This is evidenced from the plant growth response that normal.

At the end of this study, the concentration of mercury in the media become much lower ranged between <0.002 ppm to 1.46 ppm. The decrease is due to several reasons, including: absorbed and accumulated by plants or evaporates into the atmosphere due to volatilization (Wang 2004). Mercury uptake by plants can occur through a process of phytoextraction or phytostabilization (Sarma 2011). Decreasing the concentration of mercury in the soil is an indicator of the occurrence of metal binding process by roots exudates. In many cases, soil microorganisms have a very important role in assisting this process of decline of heavy metals in the soil (Prasetyawati 2009). Low concentrations of mercury in the media will be followed by the successful plant growth. This condition proves that the four indigenous species used in this study has the ability to accumulation of mercury. This statement is supported by the bioconcentration factor in most of the plants in all treatments > 1, which means the mercury accumulated in the plant tissue.

Vegetative growth as the response of plants to the site conditions showed that most plants can grow well and healthy. Although not differ in terms of growth and total mercury concentrations in plant tissue, there is a tendency that the planting collectively have a better plant growth if compared with the planting singly. The same trend is also seen in its ability to accumulate mercury in plant tissue. This is thought to be caused by the rapid reduction of mercury in large amounts, because absorption is done jointly with another individual. So the opportunity to grow better is higher. This statement is supported by the results of the analysis of mercury in the media. Planting collectively is causing the mercury content in the media to be much lower at <0.002 ppm, while the planting singly is 0.278 to 1.46 ppm. Except *D. excelsa* that planted singly, the mercury content is <0.002 ppm. This indicates that specific to *D. excelsa*, not only planted collectively but also planted singly capable to lowering the concentration of mercury in the media <0.002 ppm. Safety threshold of mercury in the soil according to the Decree of the Minister of Environment No. 202 of 2004 is 0.005 ppm.

Based on the value of the translocation factor, it is known that in *C. junghuhniana* and *D. excelsa* accumulate mercury in the roots, while *C. porrectum* and *M. affine* accumulate mercury in the shoots. Thus, the process of remediation of mercury by *C. junghuhniana* and *D. excelsa* is phytostabilization, while *C. porrectum* and *M. affine* is phytoextraction (Fulekar et al. 2009; Sarma 2011).

In most plants are tolerant of heavy metals, the remediation process is done through phytostabilization. This is because the process phytostabilization cause heavy metals (mercury) substituted into the tissues of plants and will soon be accumulated in a safe place. A safe place is generally at the root vacuoles. Thus, the process of photosynthesis is not inhibited because mercury does not enter into the leaf tissue. Conversely, if the remediation of mercury through phytoextraction process, the mercury will be up to the leaf tissue so that the process of photosynthesis to be blocked. This disorder occurs through the inhibition of the light reaction and the dark reaction, caused by the substitution of the central atom of chlorophyll and magnesium by mercury (Patra and Sharma 2000; Wang 2004). Therefore, the plants which remediate mercury through phytoextraction process tend to be lower tolerance to mercury. This causes the visually growth *C. junghuhniana* and *D. excelsa* much better than *C. porrectum* and *M. affine*.

Opportunity of increase in mitigation of mercury contamination through the acceleration of vegetation succession

Mitigation of mercury contamination biologically using a plant is called phytoremediation. Phytoremediation is a techniques of mercury reduction which the most inexpensive and safe for the environment (Sarma 2011; Project RECA 2014). Many researches have been carried out on phytoremediation. As a result, today it has been reported there are about 500 species of plants from 101 families, which have the ability to remediate heavy metals (Sarma 2011). However, only a few were specifically reported to remediate mercury. The ability of the plant as a phytoremediator is specific to the kind of heavy metal

(metal-specific) (Ward and Singh 2004; Palapa 2009; Sarma 2011). Therefore, plant species are able to remediate lead may not be able to remediate mercury as well.

C. junghuhniana and *C. porrectum* (woody plant) and *D.excelsa* and *M. affine* (shrubs) are four indigenous species that used in this study. They have the ability as phytoremediator mercury. This study can prove that the four indigenous species are able to grow well in the tailing media containing mercury 20 ppm. In addition, the four indigenous species are also able to accumulate mercury in plant tissue with diverse abilities and different places (plant tissue). When compared with the planting singly, planting collectively have a tendency to grow better and decrease the concentration of mercury in the media much faster (<0.002 ppm). This is evident from the results of the analysis of mercury content in the plant tissue. Planting collectively four indigenous species (3.85 ppm) and two indigenous species of woody plant (2.30 ppm), significantly has a higher ability to absorb mercury compared to collective planting two species of shrubs (1.35 ppm) and planting singly (0.4 to 1.2 ppm). However based on the value of tolerance index, all these plants are tolerant of mercury with the category of moderate to high. This proves that the four species of these plants are phytoremediator mercury (as previous explanation) and can be used to remediate mercury from the soil.

Four indigenous species used in this study is a local species, obtained from the remaining forest in the Nature Reserve in Mandor (West Kalimantan), a place where the community gold mining is taking place. The natural seedlings of the four species of plants are found in abundance in this area. This indicates that the remaining forest surrounding the community gold mining have a good potential as a source of seed.

Naturally, in the tailing areas of ex community gold mining will be occurred the succession. However, from a previous study showed that the succession process in the tailing ex community gold mining run very slowly. The succession after lasting more than five years is still dominated by groundstorey and shrubs (Ekyastuti and Roslinda 2015). So to speed up the repairs required active action of people who deliberately make the succession process run faster (acceleration). Based on the explanations that have been presented previously, it can be concluded that the chances are very large to increase the mitigation of mercury contamination through the acceleration of the vegetation succession. At gold mining areas in Mandor, West Kalimantan, the acceleration by utilizing *D. excelca*, *M. affine*, *C. porrectum*, and *C. junghuhniana* which planted collectively are highly recommended for revegetation purposes simultaneously mitigate mercury contamination in the tailings. However, this research is still a basic research. Further fieldwork studies are to be continued on stable surfaces established on waste stockpiles where particular species combinations would be planted.

From this study obtained results that: (i) the planting of four indigenous species namely *D. excelca*, *M. affine, C. porrectum,* and *C. junghuhniana* collectively cause interactions mutually support each other so that mutual benefit, (ii) planting collectively of two or four species plants, and planting *D. excelsa* singly capable to lowering the concentration of mercury in the media until <0.002 ppm, and (iii) plant tissues where the accumulation of mercury in *C. junghuhniana* and *D. excelsa* are in the roots (phytostabilization), whereas in *C. porrectum* and *M. affine* are in the shoots (phytoextraction). Based on the summary of the results can be concluded that the opportunities for improving mitigation of mercury contamination through vegetation succession acceleration is very great.

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REFERENCES

Ekamawanti HA, Ekyastuti W. 2010. Effectiveness Test of Microbial Rhizosphere Isolates on Growth of Some species of Plants in Tailings Media Contaminated by Mercury. Faculty of Forestry, Tanjungpura University. Pontianak. [Indonesian]

Ekyastuti W, Roslinda E. 2015. The potency of indigenous species as a mercury phytoremediator on ex-illegal gold mining reclamation. Proceedings of the 6th International Symposium of IWoRS, Medan 12-13 November 2014. [Indonesian]

Ekyastuti W, Faridah E, Sumardi, Setiadi Y. 2016. Increased tolerance for mercury of indigenous plants with arbuscular mycorrhizal fungi. Proseding Seminar Nasional Silvikultur III, Bogor 19-20 Agustus 2015. [Indonesian].

Fulekar MH, Singh A, Bhaduri AM. 2008. Genetic engineering strategies for enhancing phytoremediation of heavy metals. African J Biotechnol 8 (4): 529-535.

Minister of Environment Decree No. 202 Year 2004 Regarding Waste Water Standard for Gold and Copper Mine Activities and/or Businesses. [Indonesian]

Palapa TM. 2009. Bioremediation of mercury by aquatic plants as an alternative waste management community gold mines Agritek 17 (5): 1-8. [Indonesian]

Patra M, Sharma A. 2000. Mercury toxicity in plants. Bot Rev 66 (3): 379-422.

Prasetyawati ET. 2009. Rhizosphere Bacteria as Reducing Mercury and Biological Agents. UPN Press Surabaya. [Indonesian]

Rabie GH. 2005. Contribution of Arbuscular Mychorrizal Fungus to Red Kidney and Wheat Plants Tolerance Grown in Heavy Metal-Polluted Soil. African J Biotecnol 4 (4): 332-345.

RECA Project. 2014. Restoration Procedures Guidelines on Protected Areas. Project on Capacity Building for Restoration of Ecosystems in Conservasion Areas. JICA & PHKA, Jakarta. [Indonesian]

Sarma H. 2011. Metal hyperaccumulation in plant: A review focusing on phytoremediation technology. J Environ Sci Technol 4 (2): 118-138.

Wang Y. 2004. Phytoremediation of Mercury by Terrestrial Plants. [Ph.D. Dissertation]. Department of Botany, Stockholm University, Sweden.

Ward OP, Singh A. 2004. Soil bioremediation and phytoremediation-An overview. In: Singh A, Ward OP (eds). Applied Bioremediation and Phytoremediation. Springer, Berlin.

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Diversity and the role of yeast in spontaneous cocoa bean fermentation from Southeast Sulawesi, Indonesia

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Abstract. *Jamili, Yanti NA, Susilowati PE. 2016. Diversity and the role of yeast in spontaneous cocoa bean fermentation from Southeast Sulawesi, Indonesia. Biodiversitas 17: 90-95.* Yeast is one of the microbial group which is role in the process of cocoa spontaneously fermentation. The objective of this study was to determinate and to know the diversity of yeast that role on cocoa bean fermentation. Yeast was isolated by pour plate method from cocoa bean that was naturally fermented by a cocoa farmer in Kolaka District, Southeast Sulawesi using yeast mannitol agar (YMA) media. Yeast was characterized and identified using phenotypic characters based on numeric-phenetic analysis. Yeast isolates applied to cocoa bean to determine its role in cocoa bean fermentation. The result was obtained seven isolates the dominant yeast during cocoa bean fermentation in Kolaka District, Southeast Sulawesi. The result of numerical-phenetic analysis based on phenotypic characters to seven yeast isolates showed that 1 isolates (Klk1) identical with *Candida krusei*. Three isolates (Klk4, Klk5 and Klk7) identical with *Candida tropicalis,* one isolate (Klk2) identical with *Saccharomycopsis fibuligera,* one isolate (Klk3) identical with *Kloeckera* sp. and one isolate (Klk6) identical with *Saccharomyces cerevisiae.* The result also showed that fermentation of cocoa with seeding of yeast inoculums served to increase the quality of cocoa beans than spontaneous fermentation. Therefore, the seven yeast isolates potentially be used as an inoculum to improve the cocoa quality.

Keywords: Cocoa bean, diversity, Southeast Sulawesi, spontaneous fermentation, yeast

INTRODUCTION

Fermentation is one of the important processes to improve the quality of the cocoa bean. However, the majority of cocoa farmers in Indonesia, especially in Southeast Sulawesi is still less interested in doing the fermentation of cocoa beans, since for processing cocoa beans through a fermentation process takes a long time which is five to six days. Fermentation of cocoa beans undertaken by cocoa farmers is a spontaneous fermentation. Spontaneous fermentation is fermentation utilizing natural microorganisms in the environment and proliferate spontaneously because the environment suitable for growth.

Fermentation of cocoa beans involves the role of microorganisms in outlining of polyphenolic compounds, proteins, and sugars contained in cocoa beans through the action of the enzyme produced by such microorganisms so that the fermentation of cocoa beans will undergo physical and chemical changes. The groups of microorganisms involved in the spontaneous fermentation of cocoa are yeast, acetic acid bacteria and lactic acid bacteria (Ardhana and Fleet 2003; Jespersen et al. 2005; Guehi et al. 2010; Pereira et al. 2012). Yeast is one of the microorganisms that play a role in the fermentation of cocoa beans to break down sucrose, glucose, and fructose into ethanol. In addition, the yeast also serves to degrade the pulp with the help of pectinolytic enzymes during fermentation of cocoa beans (Jespersen et al. 2005; Ho et al. 2014).

The diversity of yeasts in the fermentation of cocoa beans has been reported by several researchers earlier. Ardhana and Fleet (2003) found the yeast isolates *Kloeckera apis, Saccharomyces cerevisiae* and *Candida tropicalis* who have contributed in the fermentation of cocoa beans in three plantations in Central Java, Indonesia. Alwi (2009) reported some type of yeast of the genus *Saccharomyces, Candida, Debaryomyces* and *Rhodotorula* that dominate the fermentation of cocoa beans in three districts in Central Sulawesi. Sidarsyah (2005) also was isolate yeast of the genus *Saccharomyces, Endomycopsis* and *Hanseniaspora* of origin cocoa beans Ranomee to plantations in the Southeast Sulawesi. Daniel et al. (2009) also reported a yeast diversity of spontaneous fermentation of cocoa in Ghana is dominated by *Pichia kudriavzevii* (*Issatchenkia orientalis*), *Saccharomyces cerevisiae* and *Hanseniaspora opuntiae*. However, these yeasts, yet unknown role in improving the quality of cocoa beans.

The involvement of yeast in the fermentation process of cocoa beans contributes to determining the quality of cocoa beans product. However, not much is reviewing the role of yeast that is obtained from the fermentation of cocoa beans spontaneously to quality cocoa beans (Ardhana and Fleet 2003;Alwi 2009;Daniel et al. 2009;Pereira et al. 2012;Mahazar, et al. 2015). Therefore, the exploration of the role of yeast in the fermentation of cocoa beans needs to be done.

MATERIALS AND METHODS

Isolation of yeast from the fermentation of cocoa bean

The source of yeast isolates was obtained from samples of cocoa beans that have been fermented spontaneously by farmers in Kolaka District, Southeast Sulawesi, Indonesia. Isolation of yeast was done by pour plate method using yeast mannitol media agar (YMA) (Alwi 2009). A total of10g sample suspended in 10 mL of 0.85% NaCl solution and made serial dilutions. One mL of the suspension was inoculated into a sterile petridish and was added YMA media. Furthermore, the petridish was incubated at a temperature of 37°Cfor 48 hours. Purified colonies were grown on YMA media. Pure culture yeast obtained, was inoculated into the slant YMA media and was stored at 4°C.

Characterization and identification of yeast isolate

Characterization and identification of yeast isolates are based on phenotypic characters which include the characters of the colony and cell morphology, physiological and biochemical. Characterization of yeast isolates carried out based on the guidelines used Kirsop et al. (1984).Identification of yeasts was done based on numeric-phenetic analysis using the program Multi-variate statistical package (MVSP) version 3.1 to determine similarity between strains. Similarity value determined using the Simple Matching Coefficient (SSM) method and the classification is done using algorithms UPGMA (Unweighted Pair Group Method with arithmetic Averages).

Application of yeast isolates on fermentation of cocoa bean

Fermentation of cocoa beans is done on a laboratory scale using fresh cocoa bean varieties Forastero. Cocoa pod obtained from cocoa plantations in Kolaka District, Southeast Sulawesi. Cocoa pod skin surface is chemically sterilized using a solution of detergent and phenol while cocoa beans that will be fermented sterilized by U.V. radiation overnight (Jamili et al. 2014).

A total of 1kg of cocoa beans was fermented in a plastic box. Yeast inoculum was grown for 24 hours at Potato dextrose broth (PDB) +1% extract of cocoa pulp. A total of 10% (v/w) of inoculum containing 10^6 CFU/mL were inoculated in cocoa beans. Fermented cocoa beans without yeast inoculum (spontaneous fermentation) are performed as a comparison. Cocoa fermentation was done at room temperature for five days and sampling at the beginning of fermentation (0), 3 and 5 days of fermentation to measure parameters of quality cocoa beans in accordance with national standards of Indonesia (SNI 2008), which include the levels of unfermented beans and fat content. The method of measuring the levels of unfermented beans according to those described in SNI (2008) and measuring the fat content is done with the Soxhlet method (AOAC 2005).

RESULTS AND DISCUSSION

Yeast isolates from fermented cocoa bean

Yeast isolates were found in cocoa beans fermented naturally/spontaneously for three days by cocoa farmers in the village of Konaweha, Samaturu sub-district Kolaka District, Southeast Sulawesi was as much as seven isolates. Some yeast isolates obtained from the fermentation of cocoa beans are listed in Table1.The result in Table 1 showed that yeast was obtained from cocoa bean fermentation at the first day was as much as four isolates, whereas at the second day is two isolates and the third day only one isolate. These results showed that the amount of yeast isolates in the cocoa fermentation at 1st day (± 24 hours) higher than the yeast isolates were obtained at 2nd and 3rd day of fermentation. This indicates that the yeast acts on the 1st day of fermentation of cocoa bean so that the type more than on the 2nd and 3rd days. The results are consistent with research conducted by Ardhana and Fleet (2003) which states that the yeast at most the number and the type found in 24-36 hours after fermentation of cocoa because of their role in the process of degradation the various types of sugar that contained in the cocoa bean pulp.

Environmental conditions during cocoa fermentation process such as temperature, also be one cause of the decline in the number of types of yeast during the fermentation process. The result of temperature measurements during spontaneous fermentation of cocoa beans was done by farmers in Kolaka, after one day was 33^{o}C, on the second day was 36,5^{o}C and the third dayreach42^{o}C. The high temperature on the third day of fermentation of cocoa causing some type of yeast can not survive. According to Pereira et al. (2012), the range of optimum temperature for yeast in general is 25^{o}C-35^{o}C. Ardhana and Fleet (2003) also found that there are only a few yeasts were able to survive at high temperatures (>40°C), such as *Saccharomyces cerevisiae* and *Candida tropicalis*.

Identification of yeast isolates

Seven yeast isolates were identified based on similar phenotypic characters with eight reference strains, namely *Saccharomyces cerevisiae, Saccharomyces bayanus, Candida tropicalis, Candida krusei, Candida lambica, Saccharomycopsis fibuligera, Saccharomycopsis fermentant* and *Kloeckera* sp. Eight reference strains of yeasts are selected based on the resemblance of character with yeast isolates were isolated and the species of yeast that are often found in fermented cocoa. Phenotypic characters were analyzed by systematic numeric-phenetic is as much as31 characters (Table 2). Numerical systematic analysis results based on phenotypic characters yeast isolates and reference strains with 3.1 MVSP program are visualized in the form dendrogram listed in Figure1.

Table 1. Result of selection yeast isolates from fermented cocoa bean

Time fermentation of cocoa bean (days)	Number of yeast isolate
1	4
2	2
3	1

Table 2. Phenotypic characters of yeast isolates and reference strains

Characters	yeast isolates and reference strains														
	Klk1	**Klk2**	**Klk3**	**Klk4**	**Klk5**	**Klk6**	**Klk7**	***Candida krusei***	***C. tropicalis***	***C.lambica***	***Saccharomyces cerevisiae***	***S.bayanus***	***Saccharomycopsis fermentant***	***Saccharomycopsis fibuligera***	***Kloeckera* sp.**
Colonies morphology															
Shape															
Circular	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
Irregular	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Edge															
Entire	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
Undulate	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Color															
White	+	+	-	+	-	-	+	+	-	-	-	-	-	+	-
Beige	-	-	+	-	+	+	-	-	+	+	+	+	+	-	+
Elevation															
Low convex	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+
Convex	+	-	-	+	+	+	+	+	+	+	+	+	-	+	-
Structure in															
Opaque	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cell morphology															
Shape															
Ovale	-	-	-	-	+	+	-	-	+	-	+	+	-	-	-
Round	+	+	-	+	-	-	+	+	-	+	-	-	+	+	-
Elliptical	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Vegetative reproduction															
Type of budding															
Bipolar	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+
Multipolar	+	-	-	+	+	+	+	+	+	+	+	+	-	-	-
Pseudomycelium	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-
Sexual/asexual spores															
Ascospore	-	+	+	-	-	+	-	-	-	-	+	+	+	+	+
Chlamydospore	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-
Biochemical															
Urea hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Carbohydrate fermentation															
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	-	-	+	+	+	+	+	+	-	+	+	-	+	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	-	+	+	+	+	-	+	-	+	+	-	+	-
Assimilation															
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	-	+	+	+	+	+	+	-	+	+	-	+	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	-	+	+	+	+	-	+	-	+	+	-	+	-
Ethanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Physiological															
Temperature Tolerance (^{o}C)															
37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose tolerance of 50%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: characters for reference strains according to Kirsop et al. (1984) and NCYC (2016).

Dendrogram based on similarity values listed in Figure 1, shows there are 3 clusters are formed. Cluster I formed four sub-clusters with similarity value of 83.3%, which shows the group consisting of strains of the genus *Candida* that included isolates Klk1, *C. krusei, C. tropicalis*, *C. lambica*, isolates Klk5, isolates Klk7 and isolates Klk4. Sub cluster 1 consists of isolates Klk1 and *C. krusei* with a similarity value of 86.7%. This indicates that Klk1 isolates identical with *C. krusei*, the only difference being the shape of the colony and the ability to ferment maltose (Table 2).

Sub-cluster 2 consists of *C. tropicalis* and isolates Klk5 with a similarity value of 100%. This indicates that isolates Klk5 is a member of the species of *C. tropicalis* because all the phenotypic characters are compared, are similar. Sub Cluster 3 consists isolates Klk4 and isolates Klk7 has a similarity value of 100%, but the two isolates has similarities with *C. tropicalis* with the similarity value of 93.3%. This indicates that the two isolates were identical to *C. tropicalis*, the only difference from the structure of colony morphology and shape of the cells (Table 2). Sub cluster 4 only consists of *C. lambica* and it is joined with all strains in the cluster I with 83% similarity values.

Cluster II consists of isolates Klk3 and *Kloeckera* sp. with a similarity value of 100% (Figure 1). This indicates that isolates Klk3 is a member of the species *Kloeckera*. Characters of Klk3isolatesthat can ferment glucose, but it can not ferment sucrose, lactose and maltose according to the key characters of the species *Kloeckera* (Kirsop et al. 1984).

Cluster III formed three sub-clusters that show the group comprising the genus *Saccharomyces* and *Saccharomycopsis* (Figure 1). Sub-cluster 1 consisted of isolates Klk6 and *S. cerevisiae* with a similarity value of 100% and *S. bayanus* joined by second strains with a similarity value of 96.7% (Figure 1). This indicates that isolates Klk6 is a member of the species *S. cerevisiae*. Characters of Klk6 isolates that reproduce by budding multipolar and not forming pseudomycelium, are consistent with the key character of the species *S. cerevisiae* (Kirsop et al. 1984), thus strengthening the identity of the isolates Klk6 is a member of that species. Sub-cluster 2 consists of isolates Klk2, *Sachharomycopsis fibuligera* and *Saccharomycopsis fermentant*. Klk2 isolates and *S. fibuligera* have a similarity value of 93.3%. Sub Cluster 3 consist of *S. fermentant* joining both sub clusters 1 and 2 with a similarity value of 74%. Isolates Klk2 possibilities are members of the species *S. fibuligera* because isolates Klk2 reproduce by budding bipolar and does not form pseudomiselium and the character similar with the character *S. fibuligera* (Kirsop et al. 1984). These three main clusters are then joined with a similarity value of 58.3%.

The diversity of yeast were obtained in this study showed similar results with the diversity of yeast from fermented cocoa beans in East Java, Indonesia plantation found by Ardhana and Fleet(2003), there are of the species *Kloeckera*, *S. cerevisiae* and *C. tropicalis*. Species of yeast that is found by Alwi (2009) from the cocoa bean origin Central Sulawesi, namely from the genera *Saccharomyces*, *Candida*, *Debaryomyces*, and *Hanseniaspora*. In addition, in some countries such as Brazil, Ghana and Malaysia was found yeast of the genus *Saccharomyces* and *Candida* were dominant in the cocoa fermentation (Schwan and Wheals 2004; Daniel et al. 2009). This indicates that the yeast of the genus *Saccharomyces* and *Candida* are indigenous yeasts in the cocoa fermentation.

The role of yeast isolates on the cocoa fermentation

The role of the seven yeast isolates to improve the quality of cocoa beans in accordance with the standards of quality based on cut test results to determine of unfermented cocoa beans levels and fat content. Characteristics of the quality of cocoa beans are fermented using yeast isolates listed in Table 3.

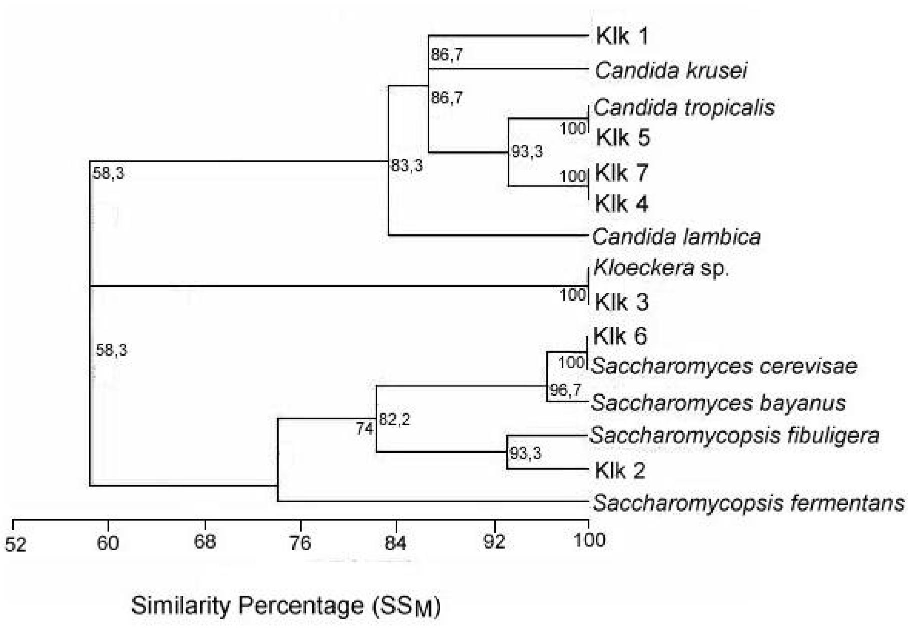

Figure 1. The dendrogram showing the phenetic relationship between 7 local yeast isolates and 8representatives of the yeasts species based on *simple matching coefficient* (SS_M) analysis and *unweighted pair-group method with arithmetic average* (UPGMA) algorithm based on phenotypic characters.

Table3showsthat the seven yeast isolates role in improving the quality of cocoa beans. Based on the levels of unfermented beans (Table 3) showed that the fermentation process, either fermented with the addition of yeast inoculum and spontaneous fermentation (without the addition of inoculum) can reduce levels of unfermented beans to 5-0% after five days of fermentation. This indicates that the fermentation process can improve the quality of cocoa beans up to the quality I (max. 3%) and quality II (max.8%) accordance with National Standard of Indonesia (SNI 2008). The addition of several inoculum yeasts (Klk3, Klk4 and Klk6) even produce cocoa beans that fulfilling the quality standards II within three days of fermentation and after five days of fermentation has fulfilled the quality standard I, while cocoa beans are fermented naturally only fulfilling quality standards II after five days of fermentation. Therefore, the local yeast isolates an important role in improving the quality of cocoa beans. It indicated that yeast inoculum in the fermentation can improve the quality of cocoa bean with reduced unfermented beans and enhanced fermented beans (entirely brown in color). This result consistent with research was done by Ho et al. (2014) was found beans fermented with yeast growth were fully brown in color and gave chocolate with typical characters which were clearly preferred by sensory panels. Therefore, yeast growth and activity were essential for cocoa bean fermentation and the development of chocolate characteristics.

The addition of yeast inoculum in the fermentation of cocoa beans can also increase the fat content of cocoa so that fulfilling the quality standards in accordance with SNI (2009), i.e. ≥ 48%. Table 3 shows that the addition of some of inoculum yeasts (Klk2, Klk4, Klk5, Klk6, and Klk7) in fermentation, able to produce cocoa beans with a fat content that fulfilling the quality SNI (2009) within 3 days of fermentation whereas spontaneous fermentation (without addition inoculum) has a fat content of less than standard quality up to the fifth day of fermentation. Joel et al. (2013) stated that the higher the percentage of fat content, hence the higher also quality of cocoa beans because fat is the most expensive component of cocoa beans. High-fat content in cocoa beans causes the cocoa is not easy rancid because the fat of cocoa contains polyphenols that act as antioxidants which can prevent rancidity and good for human health (Hii et al. 2009). Fat Cocoa contains one molecule bound triglycerides, oleic acid, palmitic and stearic cause cocoa easy to melt and give a distinctive flavor to the chocolate so that cocoa can be used in the manufacture of sweets or confectionery (Towaha et al. 2012).

Based on the observations result on cocoa beans were obtained after the fermentation using yeast inoculum (Table 3), showed that the addition of yeast inoculum role in the process of cocoa fermentation and can improve the quality of cocoa beans. The addition of yeast inoculums specially isolates Klk6 can also shorten the fermentation time from 5 days to 3dayswith the quality of cocoa beans fulfilling the SNI (2009).

Table 3. The characteristics of quality cocoa bean were fermented using yeast isolates for five days

Isolate code	Fermentation time (day)	Characteristics			
		Unfermented bean (%)		Fat content (%)	
		Sample	Quality standard[1]	Sample	Quality standard[2]
Klk1	0	80		43.18	
	3	10		45.93	
	5	5		50.55	
Klk2	0	80		44.30	
	3	15		48.70	
	5	0		50.05	
Klk3	0	85		44.46	
	3	5		46.07	
	5	0		52.67	
Klk4	0	85	I: max. 3%	44.67	
	3	7		48.04	
	5	3	II: max. 8%	50.71	≥ 48
Klk5	0	85		45.03	
	3	10	III: max. 20%	48.35	
	5	3		53.03	
Klk6	0	80		46.16	
	3	5		50.35	
	5	0		53.03	
Klk7	0	75		44.63	
	3	10		49.60	
	5	5		51.24	
Kontrol[3]	0	80		42.18	
	3	10		45.77	
	5	5		45.73	

Note: [1]SNI (2008); [2]SNI (2009); [3]Fermentation without yeast inoculum

In conclusion, diversity of seven yeast isolates from cocoa beans fermented spontaneously in Kolaka District, Southeast Sulawesi is quite high, which is identical to the five species of yeast, namely *Candida krusei, Candida tropicalis, Saccharomycopsis fibuligera*, *Kloeckera* sp. and *Saccharomyces cerevisiae*. The seven yeasts isolates role in the process of cocoa beans fermentation and can improve the quality of cocoa beans in a shorter time compared with spontaneous fermentation.

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REFERENCES

Alwi M. 2009. Characterization of yeast lived in the cocoa fruit in Southeast Sulawesi. Biocelebes 3 (1): 51-58. [Indonesian]

AOAC. 2005. Official Methods of Analysis of AOAC International. 18th ed. AOAC International, Gaithersburg, MD.

Ardhana MM, Fleet HG. 2003. The microbial ecology of cocoa bean fermentations in Indonesia. Intl J Food Microbiol 86 (1): 87-99.

Daniel HM, Vrancken G, Takrama JF, Camu N, De Vos P, De Vuyst L. 2009. Yeast diversity of Ghanaian cocoa bean heap fermentations. FEMS yeast Res 9: 774-783.

Guehi TS, Koffi KPB, Dabonne S. 2010. Spontaneous cocoa bean heap fermentation: Influence of the duration and turning on the quality of raw cocoa. World Acad Sci Eng Technol 46: 118-123.

Hii CL, Law CL, Suzannah S, Misnawi, Cloke M. 2009. Polyphenols in cocoa (*Theobroma cacao* L.). Asian J Food Agro-ind 2: 702-722.

Ho VTT, Zhao J, Fleet G. 2014. Yeast are essential for cocoa bean fermentation. Intl J Food Microbiol 174: 72-87.

Jamili, Yanti NA, Susilowati PE. 2014. Enhancement of cocoa quality by the indigenous yeast *Candida tropicalis* KLK4 through cocoa bean fermentation. J Adv Biotechnol 4 (1): 327-335.

Jespersen L, Nielsen DS, Hønholt S, Jakobsen M. 2005. Occurrence and diversity of yeasts involved in fermentation of West African cocoa beans. FEMS Yeast Res 5: 441-453.

Joel N, Pius B, Deborah A, Chris U. 2013. Production and quality evaluation of cocoa products (plain cocoa powder and chocolate).AmerJ Food Nutr 3 (1): 31-38.

Kirsop B, Painting K, Henry J. 1984. Yeast: Their Identification. Thailand Institute of Scientific and Technology Research, Bangkok.

Mahazar NH, Sufian NF, Meor Hussin AS, Norhayati H, Mathawan M, Rukayadi Y. 2015. *Candida* sp. as a starter culture for cocoa (*Theobroma cacao* L.) beans fermentation. Intl Food Res J 22 (5): 1783-1787.

NCYC [National Collection Yeast Cultures]. 2016. The National Collection yeast Cultures. Institute of Food Research, UK. http://www.ncyc.co.uk. [January 1, 2016]

Pereira GVM, Miguel MGCP, Ramos CL, Schwan RF. 2012. Microbiological and physicochemical characterization of small-scale cocoa fermentations and screening of yeast and bacteria strains to develop a defined starter culture. Appl Environ Microbiol 78: 5395-5405.

SchwanRF, WhealsAE. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. Crit Rev Food Sci Nutr 44 (4): 205-221.

Sidarsyah. 2005. Characterization of Microorganisms in the Cocoa Beans (*Theobroma cacao* L.). [Hon. Thesis]. Halu Oleo University, Kendari. [Indonesian]

SNI [Indonesian National Standard]. 2008. Cocoa Beans 2323, 2008. [Indonesian]

SNI [Indonesian National Standard]. 2009. Mass Cocoa Beans 3749, 2009. [Indonesian]

Towaha J, Anggraini DA, Rubiyo. 2012. Performance of cocoa beans quality and their derivative products at various levels of fermentation: A case study in Tabanan, Bali. Pelita Perkebunan 28 (3): 166-183. [Indonesian]

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Proof of *Acacia nilotica* stand expansion in Bekol Savanna, Baluran National Park, East Java, Indonesia through remote sensing and field observations

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Abstract. *Sutomo, van Etten E, Wahab L. 2016. Proof of* Acacia nilotica *stand expansion in Bekol Savanna, Baluran National Park, East Java, Indonesia through remote sensing and field observations. Biodiversitas 17: 96-101.* One of woody species that is known to inhabit certain savanna ecosystems is *Acacia nilotica.* The *Acacia nilotica* tree is widespread in the northern savannah regions, and its range extends from Mali to Sudan and Egypt. *Acacia nilotica* was first introduced to Java Island in 1850. It then spread to Bali, East Nusa Tenggara, Timor and Papua. Found in grasslands, savanna is reported as important colonizer at Baluran National Park in East Java and Wasur National Park Papua. We conducted Vegetation analysis in three areas of the Baluran Savanna namely: Grazed, burned and unburnt. Our observation result analysis showed that in terms of the three most important tree species in all of the sites that we sampled (grazed, burnt and unburnt savannas) *Acacia nilotica* appeared in each of these sites. The values however, vary between sites. *Acacia nilotica* Importance Value Index is highest in the unburnt savanna, with IVI reaching almost 250. The unburnt site is actually a burnt site but with moderate age or time since fire (approximately 6-7 years), whereas the burnt site is savanna with relatively young age/time since fire (few months to 1 year). We also conducted GIS analysis using Satellite Images (October 2013 and October 2014) to pick up changes in Bekol savanna. Result showed that expansion of *A. nilotica* stand occurred towards the savanna. Over dominance of the woody species *A. nilotica* could shift the savanna into another ecosystem state, i.e. secondary forest.

Keywords: *Acacia nilotica*, Baluran National Park, expansion, remote sensing, vegetation analysis

INTRODUCTION

Before the year 1928, A.H. Loe de Boer, from the Dutch Colonial, had areas of agriculture concession at Labuhan Merak and Mount Mesigit, Baluran. Since then, he had always interested in big mammals and thought that the areas will play important roles in conserving these animals. Following Indonesia independence, the Ministry of Agriculture also stated Baluran as game reserve area with decree No. SK/II/1962. Later on in 1980, the area then stated as a national park, the Baluran National Park with an areas of 25,000 ha. Baluran is known for its vast areas of savanna and is famous as the “Africa van Java” (Baluran National Park 2010).

Savanna is a term to define ecosystem in tropical and subtropical that typical of displaying a continuous herbaceous cover of C_4 grasses that has different patterns based on seasonality in which it is related to water, and where woody plants are also of important values but with sparse patterns and no closed canopy (Frost et al. 1986). Tropical savannas cover over 20% of the Earth surface, with the largest coverage is in Africa, Australia and South America, and just only approximately 10% occur in India and Southeast Asia (Bond and Wilgen 1996; Werner 1991).

Other savannas ecosystem have also been intensively studied such as eucalypt savanna woodlands in Northern Australia (Burrows et al. 1991; Werner 1991), and the Miombo woodland of Southern Africa (Isango 2007). Indonesian savanna however remain somewhat unfamiliar to the scholarly with very few studies have been done. One of the foremost early studies on Indonesian flora was of Backer and van den Brink (1963) and van Steenis (1972). Mountain flora of Indonesia especially in Java Island was what van Steenis specialized. In his report, even in early 1900, grasslands on Java Mountains are already common. Fire, according to van Steenis (1972) was presumed as the major factor that drive the existence of grasslands on Java mountains especially in East Java which is subject to dryer dry season, lower precipitation compare to other parts of the island.

One of woody species that is known to inhabit certain savanna ecosystem is *Acacia nilotica* (Figure 1). *Acacia nilotica* is known to be abundant originally in Africa (Brenan 1983) and has been scantly studied. In a study by Skowno (1999) in Hluhluwe Game Reserve, South African savanna, *A. nilotica* was described in terms of their quantitative structure and distribution. In Australia, this species is dominant in Queensland where it has been declared as weeds, and only a few are found in Western Australia, New South Wales, Adelaide and Northern Territory (Reynolds and Carter 1990). In Australian savanna ecosystem’s study by Radford (2001), *A. nilotica*

Figure 1. Sketch of *Acacia nilotica* subsp. *indica* from Baluran National Park (Illustrated by M. Sumerta, Bali Botanical Garden, Indonesia)

is acknowledged to have negative impacts on savannas. *A. nilotica* can be threatening to savannas as its adult trees are apparently fire tolerant and can form thorny thicket formation (Burrows et al. 1991).

Baluran savanna has also been introduced with the *A. nilotica* in the late 1960, where its original purpose was to create fire break to prevent fire to spread from Baluran Savanna to the adjacent teak forest. However today *A. nilotica* has spread rapidly and threatening the existence of Baluran Savanna and it has been observed to cause changes in ecosystem from open savannas to some extent a closed canopy of *A. nilotica* in some areas (Barata 2000; Djufri 2004). This condition could put large mammals of Baluran savanna such as barking deer (*Muntiacus muntjak*), sambar deer (*Cervus unicolor*) and banteng (*Bos javanicus*) in endangered due to the loss of browsing and grazing fields (Sabarno 2002).

This research objective are to provide up-dated information regarding the state of this exotic species *A. nilotica* in Bekol Savanna Baluran National Park and search for evidences of expansion of *A. nilotica* stand at the Bekol Savanna through field observation and Geographical Information System (GIS) application.

MATERIALS AND METHODS

Baluran National Park located in Banyuputih sub-District, belongs to Situbondo District in East Java Province, Indonesia (Coordinates: 7°50′S 114°22′E). Baluran National Park borders are adjacent to: On the north it is bordered with Madura Strait, on the east is Bali Strait, on the south is Bajulmati River, Wonorejo Village and on the west is with Klokoran River, Sumberanyar Village. The park is a rough circle, with the extinct volcano, Baluran, at its center. Baluran has a relatively dry climate and mainly consists of savanna (40%), as well as lowland forests, mangrove forests and hills, with Mount Baluran (1,247m) as its highest peak. According to Schmidt and Fergusson classification, Baluran National Park (BNP) has type F dry climate with temperature ranging 27.2-30.9ºC, relative humidity 77%, and wind speed average 7 knots. Rainy season in November to April, dry season is in April to October. Highest precipitation usually is in December to January. Baluran savanna has alluvial soil type. In dry season or drought the soil will crack and the deep of the crack could reach up to 80 cm.

We used vegetation analysis method by using quadrate plot to sample vegetation following Kent (2011) as seen in the Figure 2. Quantitative information including above-ground biomass and floristic composition obtained from each sites namely: burned, unburnt and grazed. Each site we created 10 plots for observation and so in total there were 30 plots all together, randomly located. All plant species in each plot identified (Indriyanto 2006). Field herbarium created so that the plants can be more easily recognized to species level in the subsequent field work, which demands rapid identification in the field. The identification assisted by Herbarium Baliensis of the Indonesian Institute of Sciences. Plant identification also make used of flora books such as Backer and Bakhuizen van den Brink (1963), van Steenis (1972), Soerjani et al. (1986), and Whitten et al. (1996). A diagram profile was also created by using a transect with 60 m long and 7 m wide and captured the woody habitus as well as sapling, seedling and grasses in the boundary area between the *A. nilotica* stand and the Bekol Savanna of Baluran National Park. Vegetation analysis was then conducted to calculate the value of Important Index (IVI) for tree habitus. Ordination analysis for tree species composition in the sampling plots was conducted using Non metric Multidimensional Scaling (NMDS) in the PRIMER V.5 Software (Clarke and Gorley 2005).

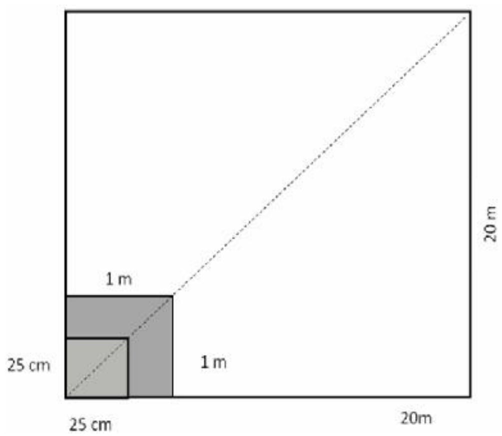

Figure 2. Shape and size of sampling plot (Kent and Coker 1992), 20 x 20 m^2 to observe and measure woody sapling and basal area. 1 x 1 m^2 to observe non woody species and 25 x 25 cm^2 for their biomass

Satellite images of the Bekol savanna in 2013 were obtained online using Universal Map Downloader (UMD) with highest level of pixel category to allow high standard images. The spatial data was download from Geo-Eye (Google Earth) using the UMD application.GeoEye-1/Digital Globe (Google Earth) has a resolution of 0.41-meter on panchromatic and 1.65-meter on multispectral imagery (Setiabudi et al. 2013). To cover Bekol savanna and the surrounding, many such small GeoEye-1 images were constructed by the UMD and stored in JPEG files to be merged and integrated into one mosaic of GeoEye-1 image. Finally the mosaic image was rectified or geo-referenced using coordinate data from Google Earth. The data were projected into WGS 1984-UTM, Zone_49S. In this way a detailed vegetative map was developed. The identification of *A. nilotica* stand is based on prior knowledge of the plant from the preliminary field observation in 2013. The interpretation result and up-date data was also checked through ground-truth observation in the field in 2014. Ground/field observation in 2014 was then compared with 2013 satellite image and was displayed using ARC-MAP software from ARC GIS.

RESULTS AND DISCUSSION

Our observation result analysis showed that in terms of the three most important tree species (described in Importance Value Index/IVI) in all of the sites that we sampled (grazed, burnt and unburnt savannas) *A. nilotica* appeared in each of these sites (Figure 3). The values however, vary between sites. *A. nilotica* IVI is highest in the unburnt savanna, with IVI reaching almost 250. The unburnt site is actually a burnt site but with moderate age or time since fire (approximately 6-7 years), whereas the burnt site is savanna with relatively young age/time since fire (few months to 1 year). It is assumed that *A. nilotica* pods grazed by buffalo and spread the seeds to many areas of savanna including the burnt areas. Once it was experienced fire, the heat assisted in scarification process of the seeds and speed up the germination. When there was no subsequent fire for significant period of time, these seeds grow and become mature and dominating the unburnt site. In terms of species diversity, all of these sites have low species diversity (around 0.8) as measured by Shannon and Wiener and Simpson Index (Table 1). This is perhaps, due to over dominance of certain species, as can be seen from the next graph.

Ordination analysis (Figure 4) shows that the grazed and ungrazed sites were not as different in terms of the species composition as shown by the close position between the dots that represent the two sites and sometimes these dots were mixed. Whereas when we look at the burn site, we notice that the dot that represent the burn site is clearly separated from the other two sites although the points of the burn area were also has different pattern, some are clumped and some are scattered. These results imply that it is difficult to find a site that is purely ungrazed as animals move around in such a dynamic pace. We can only assume that site/plots that have no traces of scats/stools or remains of the herbivore to be the ungrazed site, however we know now that it is not adequate. Therefore the ungrazed and grazed sites have did not separate very well and imply that these sites has similarity

Table 1. Species diversity in three sampling sites

	Index Shannon	Index Simpson
Un-grazed	0.83080091	0.437944506
Grazed	0.845667704	0.447737829
Burnt	0.745068622	0.45753436

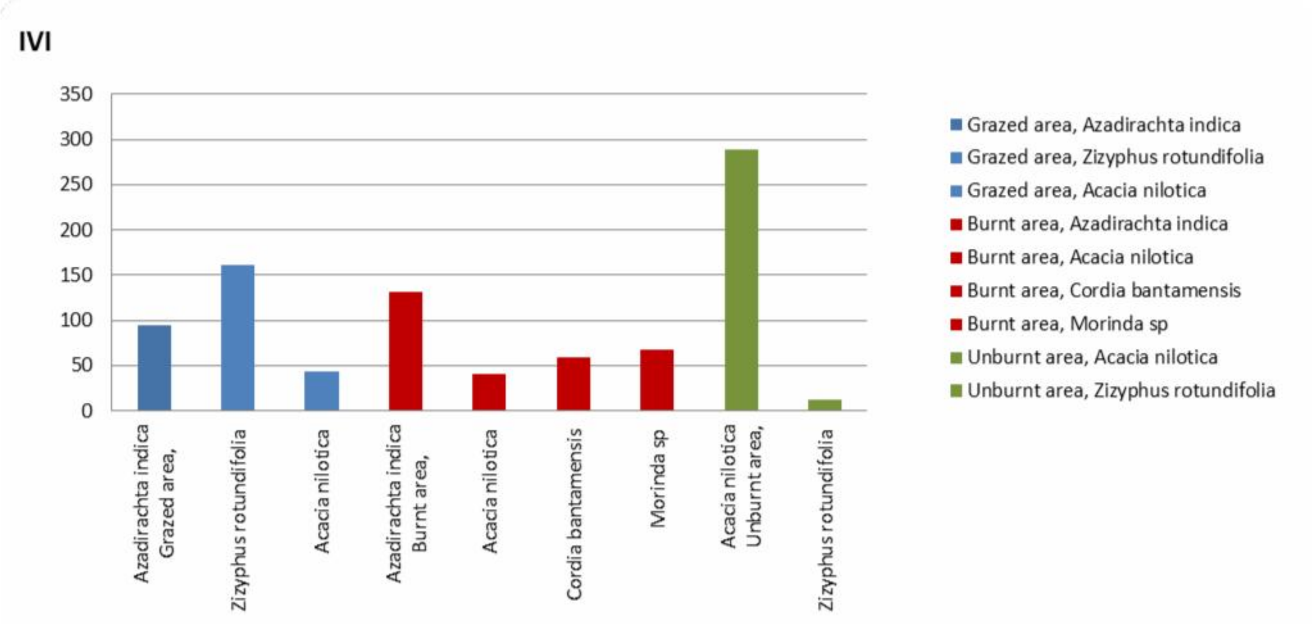

Figure 3. Importance Value Index analysis for tree habits at the sampling sites in Bekol Savanna, Baluran National Park, East Java, Indonesia

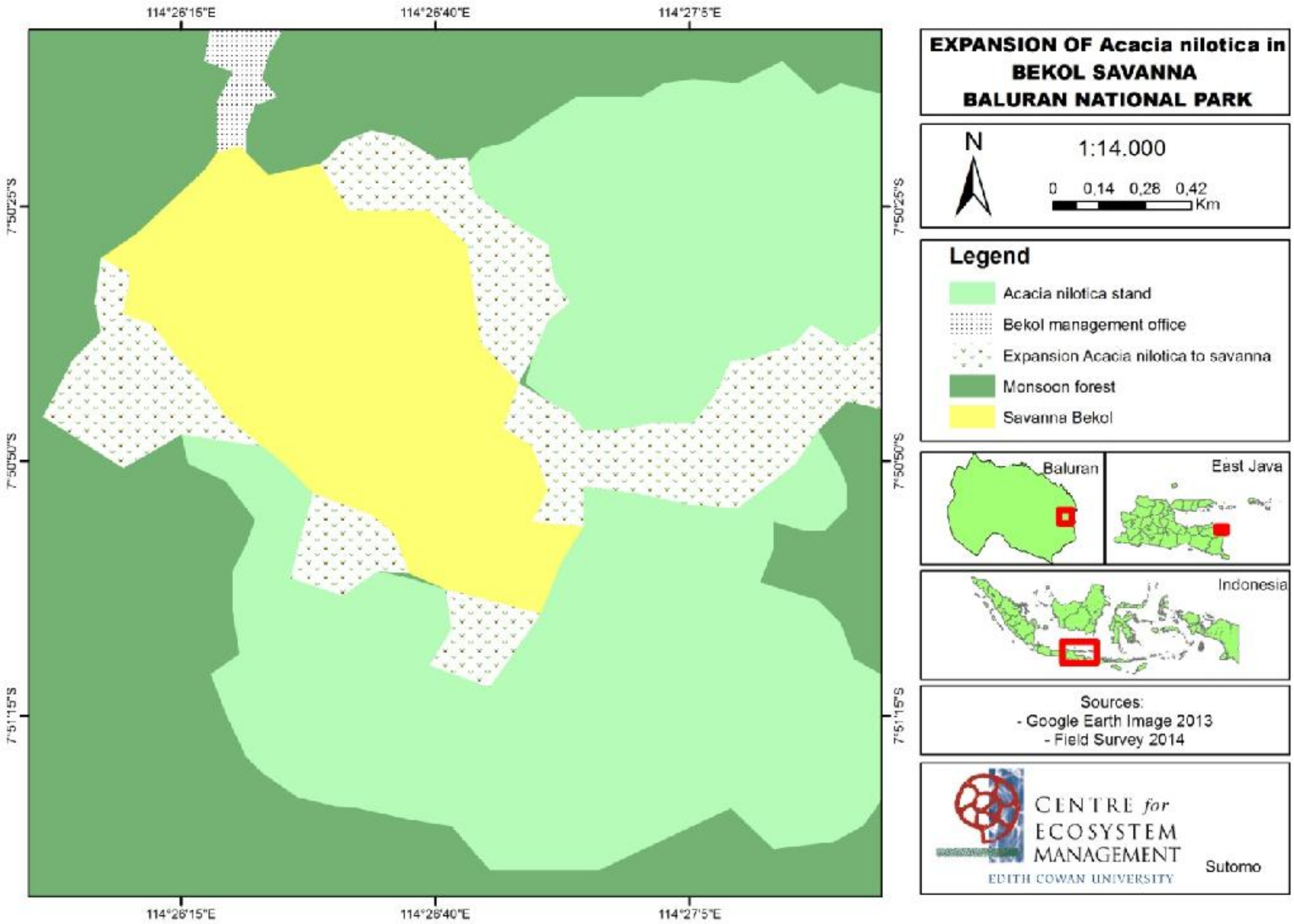

Figure 5. Result from GIS analysis and ground check showing expansion of *Acacia nilotica* stand at Bekol Savanna in 2014, Baluran National Park, East Java, Indonesia

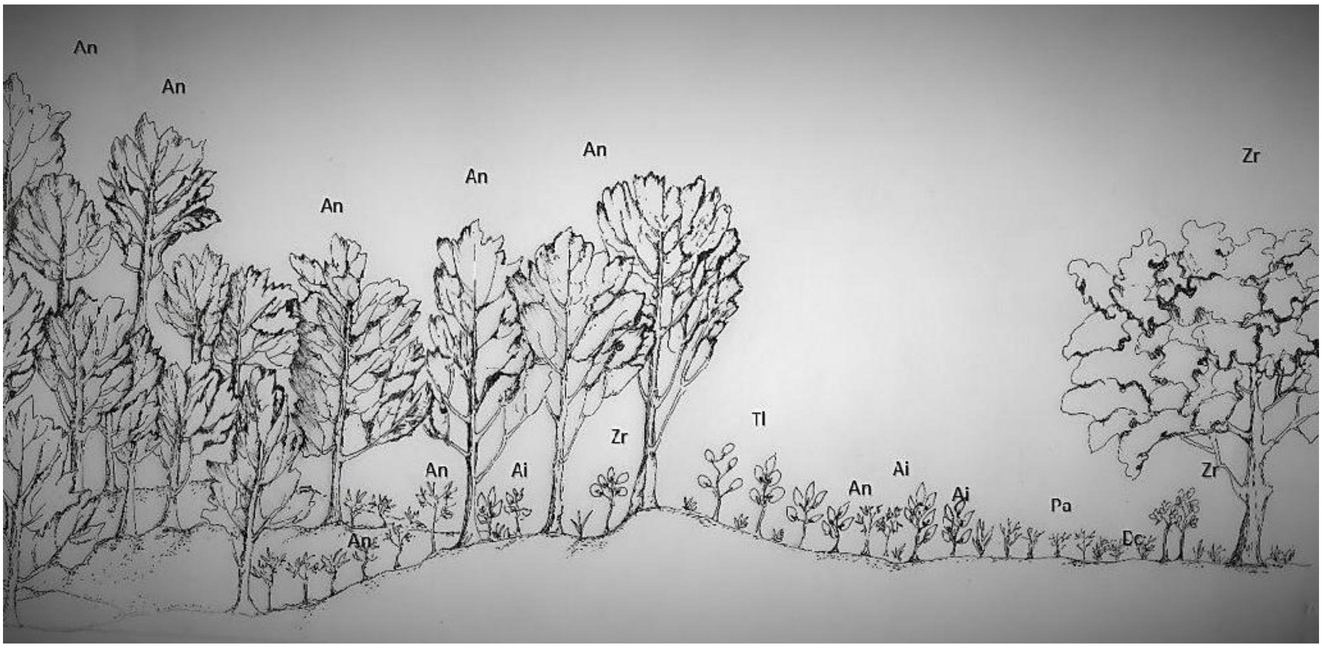

Figure 6. A profile diagram of the boundary area between the *A. nilotica* stand and Bekol Savanna, Baluran National Park, East Java, Indonesia. Notes: An = *Acacia nilotica*, Ai = *Azadirachta indica*, Zr = *Ziziphus rotundifolia*, Pa = *Polytrias amaura*, Dc = *Dichantium coricosum*, Tl = *Thespesia lampas*

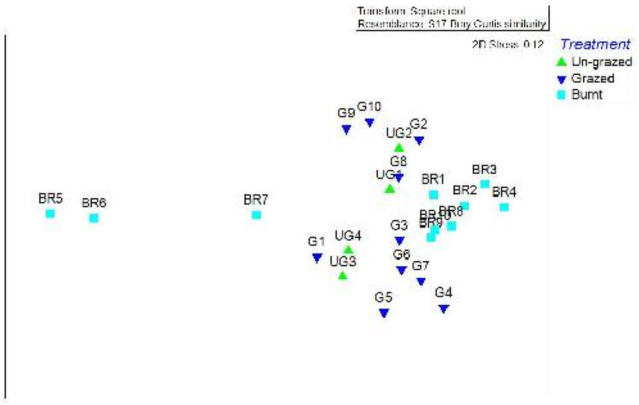

Figure 4. Result from NMDS ordination of sites in terms of their species composition at Baluran National Park, East Java, Indonesia

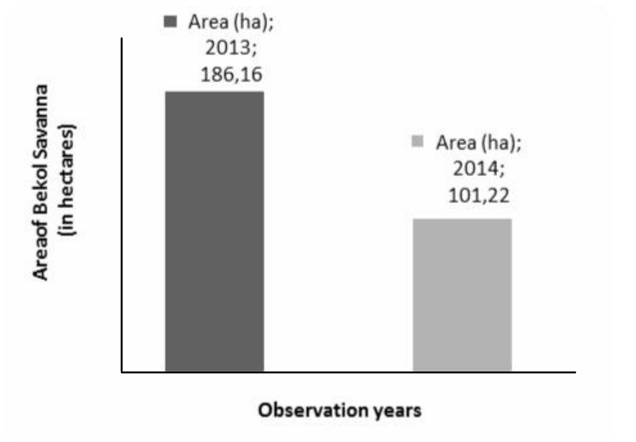

Figure 7. Decrease in area. Savanna Bekol, Baluran National Park, East Java, Indonesia from 2013-2014

in terms of their species composition. The burn site however was quite distinct in terms of species composition with the grazed and ungrazed sites. Burning create such a catastrophic condition in such a short time. Burning modified the environmental factors/microclimate in the area and thus changes the species composition.

Spatial analysis of the satellite images showed distinct patterns of expansion of *A. nilotica* stand. Older stand clumped together and form a dense dark green block like a carpet on the South and northeast of the Bekol Savanna. Meanwhile a younger stand identified by a lighter green color with a sparse density pattern that located at the front of each older stand (Figure 5). A profile diagram from field observation (Figure 6) revealed that these expansion areas were mainly comprises of species of younger *A. nilotica* in sapling sizes and also in seedling form. Beside *A. nilotica*, this area also mixed with other species but in less dominance namely *Azadirachta indica*, *Ziziphus rotundifolia*, *Thespesia lampas*, *Polytrias amaura* and *Dichanthium coricosum*. In just only 1 year there was a decrease in areas of the Bekol Savanna. As many as approximately 85 ha savanna areas have converted to *A. nilotica* stand (Figure 7).

Vegetation analysis and GIS application can be applied in combination as demonstrated in the present paper. *A. nilotica* survive and strive in burned, unburnt, grazed and ungrazed areas of the Bekol Savanna in Baluran National Park. We know now that *A. nilotica* is aggressively invading Baluran savanna, but, what are the factors that encourage the spread and the domination of *A. nilotica* in the savanna? This question needs further research into seed ecology of *A. nilotica* and its dispersal agent, which will be addressed in our next paper.

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REFERENCES

Backer CA, van den Brink Jr RCB. 1963. Flora of Java. The Rijksherbarium, Leiden.

Baluran National Park. 2010. Baluran National Park, A Piece of Africa in Java. Center for Baluran National Park, Banyuwangi. [Indonesian]

Barata UW. 2000. Biomass, composition and classification of below grown plant communities in the stands of *Acacia nilotica* in Baluran National Park, East Java. Faculty of Forestry. Universitas Gadjah Mada, Yogyakarta. [Indonesian]

Bond WJ, Wilgen BWv. 1996. Fire and Plants. Chapman & Hall, London.

Brenan JPM. 1983. Manual on Taxonomy of *Acacia* species: Present Taxonomy of Four Species of *Acacia* (*A. albida, A. senegal, A. nilotica, A. tortilis*). United Nations FAO, Rome.

Burrows WH, Carter JO, Scanlan JC, Anderson ER. 1991. Management of savannas for livestock production in North-East Australia: Contrast across the tree-grass continuum. In: Werner PA (ed). Savanna Ecology and Management. Blackwell, London.

Clarke KR, Gorley RN. 2005. PRIMER: Plymouth Routines In Multivariate Ecological Research. PRIMER-E Ltd., Plymouth.

Djufri. 2004. *Acacia nilotica* (L.) Willd. ex Del. and problematical in Baluran National Park, East Java. Biodiversitas 5: 96-104.

Frost PGH., Menaut JC, Walker BH, Medina E, Solbrig OT. 1986. Responses of savannas to stress and disturbance. In: Scholes RJ, Walker BH (eds), An African Savanna: Synthesis of the Nylsvley Study. Cambridge University Press, Cambridge.

Indriyanto. 2006. Forest Ecology. Penerbit Bumi Aksara, Jakarta. [Indonesian]

Isango JA. 2007. Stand Structure and Tree Species Composition of Tanzania Miombo Woodlands: A Case Study from Miombo Woodlands of Community Based Forest Management in Iringa District. Management of Indigenous Tree Species for Ecosystem Restoration and Wood Production in Semi-Arid Miombo Woodlands in Eastern Africa. MITMIOMBO, Tanzania.

Kent M. 2011. Vegetation Description and Data Analysis: A Practical Approach. John Wiley & Sons, New York.

Kent M, Coker P. 1992. Vegetation Description and Analysis: A Practical Approach. John Wiley & Sons, New York.

Radford IJ, Nicholas MD, Brown JR. 2001. Impact of prescribed burning on *Acacia nilotica* seed banks and seedlings in the Astrebla grasslands of Northern Australia. J Arid Environ 49: 795-807.
Reynolds JA, Carter JO. 1990. Woody weeds in central western Queensland. Proceedings 6th Biennial Conference, Australian Rangelands Society, Carnarvon, Perth.
Sabarno MY. 2002. Baluran Nasional Park Savanna. Biodiversitas 3: 207-212. [Indonesian]
Setiabudi, Tjitrosoediro S, Mawardi I, Bachri S. 2013. Invasion of *Acacia nilotica* into savannas inside Baluran National Park, East Java, Indonesia. In: Bakar B, Kurniadi D, Tjitrosoediro S. (eds). Proceedings of the 24th Asian-Pacific Weed Science Society Conference, Bandung, Indonesia, October 22-25, 2013.
Skowno ALM, Bond WJ, Balfour D. 1999. Secondary succession in *Acacia nilotica* (L.) savanna in the Hluhluwe Game Reserve, South Africa. Plant Ecol 145: 1-9.
Soerjani M, Kostermans AJGH, Tjitrosoepomo G. 1986. Weeds of rice in Indonesia Balai Pustaka, Jakarta. [Indonesian]
Van Steenis CGGJ. 1972. The Mountain Flora of Java. E.J Brill, Leiden.
Werner PA. 1991. Savanna Ecology and Management: Australian Perspective and Intercontinental Comparisons. Blackwell, London.
Whitten T, Soeriaatmadja RE, Afiff SA. 1996. The Ecology of Indonesia series volume II: The Ecology of Java and Bali. Periplus, Hongkong.

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Tropical peatland tree-species diversity altered by forest degradation

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Abstract. *Astiani D. 2016. Tropical peatland tree-species diversity altered by forest degradation. Biodiversitas 17: 102-109.* Indonesian experienced relatively high deforestation and degradation. The forests degradation could bring the forests into a temporary or might be permanent destruction not only in forest vegetation density and structure, but also in species composition. A study had been carried out to examined the impact of peatland forests degradation on their species diversity composition in Ketapang, West Kalimantan peatland forest. Stratified random sampling was used to distinguished forest degradation class (low, intermediate and high degradation levels) based on the differences in spectra image and confirmed with field checking by measuring forest canopy opening to measure the degradation levels. Six to twelve of a 20x100m plots were established to sample tree structure and composition distributed along peatland landscape. All trees species diameter >5cm was registered an species identified. Results indicated that tree diversity was significantly reduce due to forest degradation, in low, intermediate, and high degraded forest were 82, 72, and 48 consecutively. Forest degradation is not only resulted more than 50% of important species loss in high degraded peatland forest but also reducing ~40% tree abundance. Ten species were found in high degraded forest, e.g., *Calophylum inophyllum*, *Cyathocalyx biovulatus*, *Neoscortechinia kingii*, and *Eugenia cerina,* were not present in low degraded one. The species composition and abundance shifting due to forest degradation should be considered on peatland forest management to hinder permanent species loss.

Keywords: species abundance, species composition, tree species loss

INTRODUCTION

Tropical forest is regarded as one of the largest biodiversity which has rich array of plants, animals, and microbial life form and plays an important role in ecological system due to it large area of coverage, which are important to maintain their function in biosphere (Dirzo and Raven 2003). It also provides habitat biodiversity, hydrology regulator, and carbon storage and the ecosystem recently deserve special attention because of continuing considerable destruction (Celine et al. 2013). While the tropical forests are facing the environmental problem, many aspects of the issue had been overlooked until recently. The tropical forest degradation was not only clearing large area of forests (i.e. with selective logging)-but the altering and replacing of old growth forest also happened spreadly (Foley et al. 2007).

Forest and non forest term are not adequate for describing forest in a landscape, yet until recently it is used to draw the condition of tropical forest. Recent forest ecosystem is further dynamic and a complex system because they experience logging, opening landscape, regrowth, mortality which result more complex mosaic of intact rain forest and recovering secondary forest (Nepstad et al. 1999; Cardille and Foley 2003).

Indonesian rainforest plays an important role in as home to third most extensive humid tropical rainforest and account for 2.3% of global forest cover (FAO 2010). It contains high floral and faunal biodiversity (Ministry of Forestry 2011), yet about 27% of Indonesian population depends directly on these forests for their livelihood. Consequently, the forest experience relatively high deforestation and degradation. The deforestation rates of intact forest in Southeast Asian tropical peatlands-concentrated in Sumatra and Kalimantan Indonesia-has been reported as 3.4% y^{-1} from 1990-2010 (Miettinen et al. 2011; Achard et al. 2002).Similar to the global condition, tropical forests in Indonesia are in a lot of pressure mainly due to anthropogenic activities such as logging causing forest disturbances and degradation (Margono et al. 2012).

Land cover change pressures and rapid deforestation and degradation were also occurred in West Kalimantan peatland forests. The forest type extends about 1.58 million ha in this province however only around 45% of the forest is still remaining with variable forest conditions. The forests degradation may alter the forests into a temporary or might be permanent destruction in forest vegetation density and structure, species composition (Lambin 1999; Grainger 1993) and the condition could lead to reduce their productivities and ecological role in the landscape.

In addition, land use change will also impact effects and alter terrestrial ecosystem processes (Miettinen et al. 2011).Thus, land conversion has and will likely continue to alter the emissions of greenhouse gases (IPCC 2011; Carlson et al. 2012, 2013). However, the impacts of forest degradation on peatland forest in-situ condition such as micro climates and forest in state condition is not clearly stated and how this condition affect the process within the forest changes matrix is interesting to be investigated. The occurrence of various forms of peatland conversion and degradation is well known. Yet the impacts of forest degradation on forest dynamics is also not clear. To investigate the impacts on the alteration of forest tree composition and their dynamics, a study had been carried out to examine the impact of peatland forests degradation on their species diversity composition in Ketapang, West Kalimantan peatland forest.

MATERIALS ANDMETHODS

Study sites

This study was conducted in an *ombrotrophic*, peatland in Riam Berasap and Tulak of Ketapang District, West Kalimantan, Indonesia (110^0 6' 0" E to 110^0 18' 0" E and 1^0 21' 0" S to 1^0 31' 0" S ~ 4 m asl.; Figure 1; in Astiani 2012). Peat depth range 2-7.5 m. Mean annual rainfall was 2892 mm ± 17 mm with 172 ± 8.2 rain days per year (compiled from Ketapang airport, Rahadi Usman weather station, West Kalimantan; in 200-2014). This area had been low-impacted logged 8-10 year ago and naturally regrowth. However, the forest coverage and condition was left in various conditions in term of their canopy coverage and tree density. The peatland forest in this area were distributed to four blocks: Riam Berasap (RB), Manjau (MJ), Marsela (MS), and Tulak (TL) Peat Forest. These areas were chosen based on the indication of peat land area derived from satellite image interpretation and soil map.

From our peat surveys and studies in West Kalimantan (Astiani 2014), we determined that this study area was highly representative of West Kalimantan coastal-peatland areas as the overwhelming majority of coastal peat areas had been selectively-logged and transported with 'sepeda' or 'kuda-kuda' system along the skilled road (Figure 2.A and 2.B).

Sampling approaches for collecting data

Forest tree biodiversity was conducted following Astiani (2012) survey using stratified sampling based on the differences in spectra of Land Sat Image according to the land cover change or forest cover types. Based on overlaying of Landover Map 2009 and SRTM Spectra with 90 meter Resolution, this area is classified based on the canopy closures and land cover types in the area were grouped into: low, intermediate, and high degraded peatland forest. Field checkings were conducted to measure tree canopy opening using Densiometer measurement.

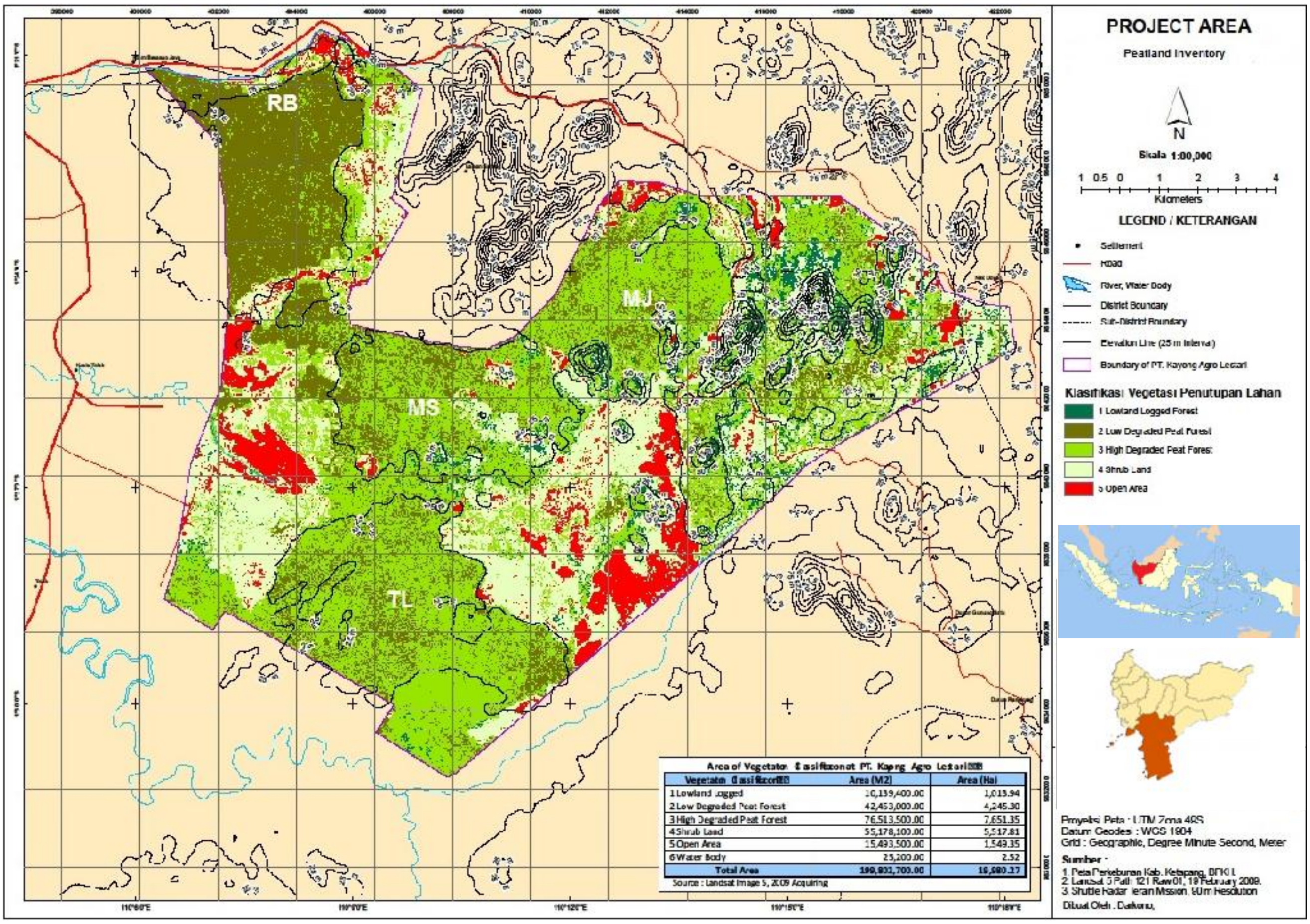

Figure 1. Study area map in Riam Berasap (RB) and Tulak (TL) of Ketapang, West Kalimantan, Indonesia

Figure 2. Low-impacted logging (A) and wood transportation using 'sepeda' (B) and 'gerobak' (C) on Ketapang, West Kalimantan peatland forest

Figure 3.A-B.Forest tree identification and measurement in peatland of Ketapang, West Kalimantan, Indonesia

Figure 4. Low (A), intermediate (B), and high (C) degradad peatland forest on Ketapang, West Kalimantan, Indonesia

Within each land cover stratification (low, intermediate, and high degraded forest), concurrently with carbon stock survey team works (Astiani et al. 2016, data not showed), we purposively measured 9-12 plots of a 20m x 100m size all tree species diameter >5cm. In each plot we measured: tree diameter >20cm within 20m x 100m area and tree diameter >5cm>20cm within 5m x 40m nested plot. Throughout each plot, we measured tree diameters, tree heights, and identified tree species local and scientific names. Some species found which not well identified in the field were made herbarium and brought to LIPI and other labs to have species identified.

Data analysis

Throughout the estimation of species structure and composition, data are presented as mean and standard error (SE) in selected intervals unless otherwise noted. Analysis of variance ANOVA was used and then Pairwise comparisons (Tukey Procedures) was tested among peatland degradation levels (low, intermediate, high degraded forest) in term of basal area, tree density etc.

RESULTS AND DISCUSSION

Forest stratification description

In the entire forest area of peatland in Riam Berasap, 108 species from 39 families were found. The land cover stratification was Low, Intermediate, and Low Degraded Peat Forest. Each land cover and canopy opening of forest stratification could be described as follows and visualized in Figure 4, and the peatland forest structure is drawn in Figure 5 and 6.

Low Degraded Peat Forest (LDPF). This type of forest is covered by low impacted logged-over peat forest, mainly old secondary peat forest that had occurred at least more than 10 year ago. Peat forest is in good succession, indicated by sufficient level of young trees and they formed 'J-shaped' as an indicator of balance uneven structure of trees. There are 80 tree species listed that compose this forest type. In general, for the whole area forest cover are dominated by Jungkang (*Pouteria malaccensis*), Nyatoh daun lebar (*Palaquium coclearifoium*), Nyatoh punjok (*Palaquium pseudorostratum*), Ubah, Perepat (*Sonneratia alba*), and Nyatoh beras (*Palaquium ridleyii*). Low Degraded Peat Forest dominated by trees (diameter >20 cm) and poles (diameter 5-<20cm) with stem density 258.9 ± 30.6 trees/ha and 340.7 ± 62.9 tree/ha consecutively. Canopy gaps assessment resulted they ranged from close to medium opened canopy, and showed very good succession of younger and smaller trees growth. Mean tree basal area was 22.6 ± 2.0 m^2/ha (N=14)

Intermediate Degraded Peat Forest (IDPF). This forest mainly secondary peat forest, that recently (less than 5-6 year ago) disturbed by logging. Dominant tree species in intermediate was similar to low degraded one such as mentioned above, yet they had less stem density with opened to rather opened canopy. The stem densities for tree diameter >20 cm and 5-<20cm were 199.5 ± 23.1 and 358.0 ± 21.3 consecutively. The trees diameter >20 cm quantity is approximately four fifth of the LDPF, yet the stem density of smaller trees is not different, only a bit higher than LDPF. The basal area was also less than LDPF (19.6 ± 2.3 m^2/ha (N = 11). The species composition was altered their dominance which prominent species registered such as Jonger (*Ploiarium alternifolium*), Kelentit Nyamuk (*Mangifera swintonioides*), Kumpang perawas (*Gymnacranthera contracta*), Nyatoh Banir (*Palaquium ridleyii*), Unang-unang (*Polyathia sumatrana*) and Leban Paya (*Porterandia anisophyllea*).

High Degraded Peat Forest (HDPF). These forests were mainly high degraded secondary peat forest that recently open or burned scatter and found interspaced among the other two canopy cover conditions. Vegetation was dominated by a few tree, pole, and saplings level tree growth and shrubs ferns, grasses were found scattered. The stem density was 130.8 ± ± 22.1 and 372.9 ±46.2 for tree diameter >20 cm and 5-<20cm. Its mean basal area was 13.2 ± 2.0 m^2/ha (N = 7). Some prominent species found were Jungkang (*Pouteria malaccensis*), Leban Tikus (*Vitex secundiflora*), Mentepis (*Calophylum inophyllum*), Keminting hutan (*Polyalthia glauca*), and Bintik (*Elaeocarpus graffithii*).

When we compared tree density among forest condition, it shown that tree diameter >20cm were significantly reduced their density in high degraded forest compared to low degraded one, while intermediate stage was a transition state between the low and high degraded forest. Smaller trees were relatively similar density (Figure 5).Difference in stand density affected their basal areas when compared amongst them. Reduction in basal area on high degradation forest reached 41,3% for tree diameter >20cm, yet increased ~33% for tree diameter 5-<20cm (Figure 6).

Tree species diversity among forest degradation levels

Resultsshowed that forest degradation had significant impacts on tree diversity in tropical peatland of Riam Berasap. Higher level of forest degradation reduced tree diversity variously and significantly influenced the existing peatland forest. For larger trees (diameter >20cm), there were found82, 72, and 48 tree species in low, intermediate, and high forest degradation respectively, while in smaller trees, in similar order of degradation,there were 61, 53, and 28 tree species Figure 4. demonstrated that high degraded peatland forest decreased tree species diversity of 14% from low to intermediate and 43% from low to high degraded one.

Some species were lost in high degraded forest, and yet some other species emerged, while others survived among the tree degradation levels (Figure 5, Table 1). There were 45 species that found in low degraded forest that were not present in high degraded one. The most prominent species losses were some species of Nyatoh i.e. Nyatoh beras daun lebar (*Palaquium coclearifoium*), Nyatoh punyok (*P. pseudorostratum*), Nyatoh Babi (*P. xanthochynum*), Nyatoh Beras (*P. ridleyii*), Ubah (*Syzigium spicata*), Bintangur (*Calophyllum hosei* Ridley), Kayu Cin (*Nageia wallichiana* (Presl.) O.K.) and other important species where found abundant in low degraded peatland. On the other hand, 10 species were found in high degraded forest such as Mentepis (*Calophylum inophyllum*), Mengkasai (*Cyathocalyx biovulatus*), Ilas (*Neoscortechinia kingii* King), Gelam tikus (*Eugenia cerina* Endl.) were not available in low degraded one.

Viewing from forest ecology side, Shannon-Winner Index (Diversity Index) of trees among the three degradation levels were consider high (3.63, 3.32, and 3.21) consecutively for low, intermediate and high degraded peatland forest. It is indicated that among the three forest coverage levels they were all high in tree species diversity. Tree species in each forest condition has relatively diverse in tree abundance especially when low and high degraded forest compared, that shown from their Eveness Index which were 0.63 and 0.43.When analyzing their Similarity Index, their species composition were relatively shifted. The value were 41.5%, 34.3%, and 30.1%respectively when contrasting low vs intermediate, low vs high, and intermediate vs high degraded peatland forest. Those values described that between the 2 forest condition they have <50% tree species in common.

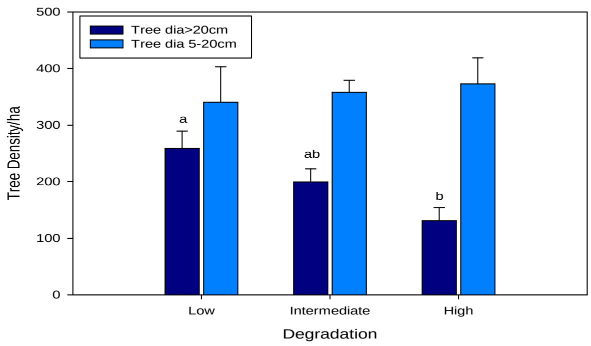

Figure 5. Peatland forest tree density among degradation levels

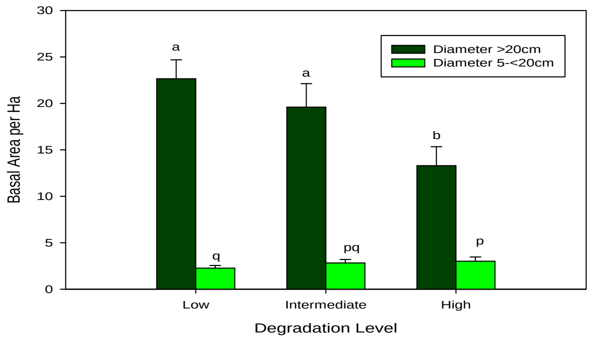

Figure 6. Tree basal area at various level of peatland forest degradation

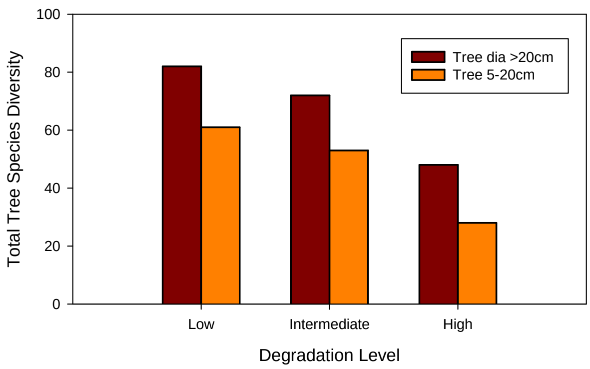

Figure 7. Total tree species diversity found among degradation level in peatland forest of Riam Berasap, Ketapang, West Kalimantan, Indonesia

Discussion

Simply, forest ecosystem classification is based on their type of habitat (i.e. tropical peatland forest is forest that is growing on peatland).However, forest ecosystem itself contribute to abundant and complicated roles in nature. There are variety of ecosystem function derived from tropical forest such as their roles in biogeochemical cycles, biological diversity, carbon sequester, improving welfare for people, opportunities on research, recreation and other ecosystem services (Putz and Redford 2010) and forest type description solely will mislead those ecosystem function.

Tropical peatland forest is one of vegetation ecosystem type which maintain enormous roles in their landscapes. Recent condition and on going pressure on this ecosystem type could have devastating impacts on biodiversity of forest. The problems that are fundamental for maintaining and monitoring biodiversity is not yet appropriate at present even though several technique has been established to monitor forest such as land cover mapping and monitoring, either on local, regional, and continental scale (Eva et al 2010; Margono et al 2012). Our concerns on peatland forest conservation in West Kalimantan led us to study the impact of forest degradation and revealed the facts of peatland tree biodiversity influenced by the anthropological degradation. These results acknowledge and clarify exact impacts of forest degradation (even though this area was practiced by low impact logging before) to the future peatland forest structure and species composition.

Forest structure is related to physical arrangement, intermixing size and composition distribution and other components in the forest that are related to ecological function and processes that also encompasses species composition and basal area (McElhinney et al. 2005; Husch et al. 2002; Hansen et al. 2001). Results show that forest degradation altered forest tree density and forest basal area per hectare although this forest has been abandoned for about 10 years. The density of larger trees was decreased, while younger/smaller trees were increased when the degradation level rose. This result supported our previous finding in peatland forest of Kubu Raya West Kalimantan (Astiani 2014). Reducing larger trees in higher level of degradation, gave more opportunity to younger trees due to optimal gap size produced (Curran et al. 1999). Prescott et al. (2003) and Denslow et al. 1998 mentioned that large gap size enhance N mineralization and phosphorus availability that elevate their concentration available N and P for enhancing tree growth. Increasing canopy gap size has been shown to impart greater microclimate change on in the forest floor (Asbjornsen et al. 2004; Proe et al. 2001; Barton et al. 1989).These results imply that in tropical peatlands, forest degradation and land cover change-with corresponding alterations of soil microclimate (e.g., temperature, CO_2, light, humidity)-will influence forest growth and dynamics.

The shifts also occurred on species composition. Species diversity was reduced when forest degradation increased. Moreover, some new species found their new sites in highly degraded forest. Although all those forest cover levels have relatively high in diversity, the species diversity shiftings are circumstances which need to be considered. The alteration of tree species composition could permanently cause the extinction of several important tree species along the peatland landscape. This post-logging dynamic in high degraded forest diminished more than one third species previously present in low degraded one. Most of species lost were high economic and conservation value timber/wood when logged, leaving less valuable tree species in high degraded forest. In term of tree species conservation, a lot of important tree species in tropical peatland (e.g., *Gonystylus bancanus, Shorea belangeran, Shorea teijsmanniana, Pouteria* spp., *Palaquium* spp. etc.) could be endangered when forest degradation continued.

Table 1. Species composition and tree density among forest degradation levels in peatland of Ketapang, West Kalimantan, Indonesia

Species	Local name	Tree density/Ha		
		Low	**Intermediate**	**High**
Actinodaphne sphaerocarpa (Bl.) Nees.	MedangAsam	0.6	1	0
*Adenanthera pavonina*Lam.	Empahong	0	1	0
Aglaia rubiginosa Blume.	Parak	0.6	1	0
Alangium longiflorum Merr.	Mengkapas	11.1	0	1.7
Alangium sp.	Mengkapas	0.6	0	0.8
Alseodaphne coreasea Nees	Medang Pasir	0	0	0.8
Alstonia spatulata Blume	Pelaik Pipit	2.8	0	0
Blumeodendron takbrai (Blume) Kurz	Mengkajang	0.6	0	0
Buchaniana arborescens Blume	Mate udang	2.8	0	0
Calophyllum hosei Ridley	Bintangur	5.6	4.5	0
Calophylum inophyllum Lam.	Mentepis	0	2.5	4.2
Calophylum schlerophyllum Lam.	Bintangor	0.6	0	0
Camnosperma squamatum Ridl.	Terentang Putih	2.2	0	0
Cantleya corniculata (Becc) Howard.	Bedaru	8.3	4	0.8
Choriophyllum malayanum Bth..	Ubah Merah	0	0.5	0.8
Cratoxylum glaucum Korth.	Gerunggang	2.2	1	0.8
Cyathocalyx biovulatus Boerl	Mengkasai	0	0	2.5
Dactylocladus stenotachys Oliv.	Mentibu	8.3	0.5	0.8
Dillenia pulchella (Jack) Gilg.	Simpur Laki	1.1	1.5	0
Diospyros bantamensis Kds.et Val. ex Bakh.	Kayu Malam dl	0	3	0
Diospyros maingayi (Hiern.) Bakh.	Kayu Malam	1.1	1	0
Dipterocarpus bornensis Slooten.	Keruing Paya	0	0	0.8
Durio carinatus (Mast).	Durian Burung	0.6	0	1.7
Dyera costulata Hook.f.	Jelutung	3.9	1	0.8
Elaeocarpusmastersii King	Mentanang	0.6	0	0
Elaeocarpus griffithii A. Gray	Mempening	1.7	1.5	0.8
Elaeocarpus petiolatus (Jack) Wallich ex Steudel.	Pangal	2.2	2	0.8
Elaeocarpus sp.	Mencubok	0	1.5	0
Eugenia cerina Endl	Gelam Tikus	0	1.5	0.8
Eugenia spicata Lam.	Ubah	11.1	1	0
Eugenia sp.	Kelempit	0	0	0.8
Ganua mottleyana Pierre ex Dubard	Ketiau	1.7	0.5	0
Garcinia cf. bancana Miq.	Manggis Hutan	0	0.5	0.8
Garcinia parvifolia (Miq.) Miq.	Asam Kandis	1.7	2.5	0
Gluta wallichii (Hook.f.) Ding Hou	Meransing	0	1	0
Gonystylus bancanus (Miq.) Kurz	Ramin	3.9	0.5	0.8
Gonystylus hankenbergii Diels.	Ramin Buaya	0.6	0	0
Gymnacranthera contracta Warb.	Kumpang perawe	0	9	0
Gymnacranthera sp.	Kumpang	2.8	0.5	3.3
Ilex cymosa Blume	Mensire	0	1	0
Ilex cf hypoglauca (Mig.) Loes.	Rawe Aek	1.7	0	0
Knema cinerea Warb.	Mendarahan1	1.1	0	0
Knema kunleri Warb.	Mendarahan 2	1.1	0	0
Koompasia malaccensis (Maingay) Benth.	Kempas	4.4	0.5	3.3
Litsea elliptica Blume.	Medang sp.1	0.6	0	0
Litsea gracilipes Hook.f.	Medang Lendir	0.6	0	0
Litsea grandis 1 (Wall ex Nees) Hook.f.	Medang Kelincir	0.6	0	0
Litsea nidularis Gamble	Medang Keladi	2.2	0	0
Litsea resinosa Blume	Medang Perawas	0.6	0	0
Litsea rufo-fusca Kosterm.	Medang sp2	0	0	0.8
Litsea sp.	Medang kunyit	1.1	0	0
Litsea turfosa Kosterm.	Medang mali	0	0.5	0
Macaranga caladiifolia Beccari	Garung	0	1	0
Macaranga pruinosa (Miq.) Muell. Arg.	Mahang	0	1	0
Madhuca mottleyana (de Vr.) Baeh.	Nyatoh Ketiau	2.2	0	2.5
Magnolia bintulensis (A. Agostini) Noot.)	Medang limau	2.2	0.5	0
Magnolia sp.	Medang Kuning	0.6	0	0.8
Mangifera longipetiolata King.	Rerawe Babi	0	5	0
Mangifera swintonioides Kosterm.	Kelentit Nyamuk	3.3	9.5	0
Mezzetia leptopada Hk. f. & Th.	Keminting d kecil	1.1	0	0
Mezzetia parviflora Becc.	Mempisang	1.7	1	1.7
Mezzetia umbelata Becc.	Keminting d besar	1.1	0	0

Nageia wallichiana (Presl.) O.K.	Kayu Cin	4.4	0	0
Neoscortechinia kingii King	Ilas	0	0.5	1.7
Nephelium maingayi Hiern	Rambutan Hutan	2.2	0.5	0
Notaphoebe umbeliflura Blume	Medang Bulu	0	0.5	0
Palaquium coclearifoium Boerl.	Nyatoh dl	23.9	1.5	0
Palaquium lanceolata Blanco	Nyatoh dk	0	0.5	0
Palaquium leicocarpum Boerl.	Nyatoh Cermai	0.6	0	0
Palaquium pseudorostratum H.J.Lam	Nyatoh Punjok	22.8	1	0
Palaquium ridleyi King & Gamble	Nyatoh Beras /Banir	7.2	8.5	1.6
Palaquium ridleyi King & Gamble	Nyatoh Beras	14.4	1	0
Palaquium xanthochynum Pierre.	Nyatoh Babi	11.7	4.5	0
Parkia singularis Miq. subsp. *borneensis*	Petai Hutan	1.1	0.5	1.7
Ploiarium alternatifolium (Vahl) Melch.	Jonger	2.8	46	0
Polyalthia glauca (Hassk.) Boerl.	Keminting Hutan	6.1	3	3.3
Polyathia sumatrana (Miq.) Kurz.	Unang-unang	1.7	7.5	0
Pometia pinnata J.R. & G. Forst.	Kasai	0.6	1.5	0
Porterandiaanisophylla (Jack ex Roxb.) Ridl	Leban Paya	5.6	6	2.5
Pouteria malaccensis (Clarke) Baehni	Nyatoh Jungkang	40.6	0.5	22.5
Pouteria obovata (R.Br.) Baehni	Nyatoh duduk	3.4	0.5	0
Pternandra galeata (Korth.) Ridl.	Meransik	2.8	0	0.8
Santiria laevigata Blume forma *glabrifolia* H.J.Lam	Asam Rawe	0	0	0.8
Shorea belangeran (Korth.) Burck.	Belangir	0.6	0	0
Shorea parvifolia Dyer.	Meranti	0	1.5	0
Shorea parvistipulata Heim.	Meranti Rawa	0.6	0	1.7
Shorea teijsmanniana Dyer ex Brandis	Meranti Batu	0	1	0
*Shorea uliginosa*Foxw	Meranti Bunga	0	3	0
Sindora leiocarpa Backer ex K. Heyne	Sindur	1.1	0	0
Sonneratia alba Seem.	Perepat	21.1	0.5	0.8
Stemonurus scorpioides Becc.	Mempasir dl	0.6	1	0.8
Stemonurus secundiflorus Blume	Mempasir dk	1.7	0.5	1.7
Sterculia lychnophora Hance	Semangkok	3.9	2	0
Syzygium havilandii (Merr.) Merr. & L.M.Perry	Ubah Bentan	3.9	0.5	0
Syzygium lineatum (DC.) Merr.& L.M. Perry	Ubah Jangkar	0.6	0	0
Syzygium sp.	Ubah Bunga	0	4	0
Syzygium zollingerianum (Miq.) Ams.	Ubah Jambu	0.6	0	0.8
Tabernaemontana macrocarpa Jack	Bintik	1.7	2	3.3
Tetractomia tetrandra Craib.	Ubah Putih	5	1.5	0.8
Tetramerista glabra Miq.	Punak	7.2	0.5	0.8
*Teysmaniadendron*sp.	Nyatoh sp.1	6.1	5.5	1.7
Tristaniopsis cf merguensis (Griff.) Peter G.Wilson & J.T.Waterh.	Pelawan	1.1	0	1.7
Vatica mangapachoi Blanko	Resak	0.6	0	0.8
*Vitex secundiflora*Hallier f.	Leban Tikus	5	0.5	8.3
Xanthophyllum ellpticum Korth. ex Miq.	Menjalin	1.1	0.5	0
Xylopia coryfolia (Griseb.) King & Robins.	Angin-angin	0	0.5	0
*Xylopia fusca*Maingay ex Hook. f. & Thomson	Bahang	0	1	0

Furthermore, there are numerous hydrological and ecological functions of tropical peatlands ranging from regulation of water flow to providing refuge for endangered animal species (Rieley and Page 2005). The increasing scarcity of available resources in mineral soils, advanced land conversion technology and continuously rising demand for forest and agricultural products have led to a rapid increase in peatland conversion and degradation. Escalating rates of logging, drainage, fires, conversion to plantations and expansion of small-holder dominated mosaic landscape have occurred since the 1980sand continued until recent time. These activities disturb ecosystem functions invariably, both directly because of altering forest dynamics, reduction of living biomass and acceleration in peat oxidation (Hooijer et al. 2006, 2010; Couwenberg et al. 2010) and indirectly by making the ecosystems more vulnerable to yearly fire activity (Siegert et al. 2001; Page et al. 2002). The species composition and abundance shifting due to forest degradation should be considered on peatland forest management to hinder permanent species loss.

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REFERENCES

Asbjornsen H, Ashton MS, Vogt DJ, Palacios S. 2004.Effects of habitat fragmentation on the buffering capacity of edge environments in a seasonally dry tropical oak forest ecosystem in Oaxaca Mexico. Agric Ecosys Environ103: 481-495.

Achard F, Eva HD, Stibig HJ. 2002. Determination of deforestation rate of world's humid tropical forest. Science 297: 999-1002.

Astiani D. 2012. Carbon assessment in PT Kayong Agro Lestari (KAL) Kabupaten Ketapang, West Kalimantan. Assessment Report. Flora Fauna International-Indonesia Programme. Jakarta

Astiani D. 2014.Bornean Peatlands: Forest Dynamics, Land Use and Carbon Flux. [Ph.D. Dissertation]. Yale University, New Haven, USA.

Barton AM, Fetcher N, Redheads. 1989. The relationship between treefall gap size and light flux in Neotropical rain forest in Costa Rica. J Trop Ecol 5: 437-439.

Cardilee J, Folet JA. 2001. Agricultural landuse change in Brazilian Amazon between 1980 and 1995: evidence from integrated satellite and census data. Remote Sens Environ 87: 551-562.

Carlson KM, Curran LM, Ratnasari D. Pittman AM, Soares-Filho BS, Asner GP, Trigg SN, Gaveau DA, Lawrence D, Rodrigues HO. 2012. Committed carbon emissions, deforestation, and community land conversion from oilpalm plantation expansion in West Kalimantan, Indonesia. Proc Natl Acad Sci USA 109: 7559-7564.

Carlson KM, Curran LM, Asner GP, Pittman AM, Trigg SN, Adeney JM. 2013. Carbon emissions from forest conversion by Kalimantan oil palm plantations. Nat Clim Ch 3 (3): 283-287.

Celine E, Philippe M, Astrid V, Catherine B, Musampa C, Pierre D. 2013. National forest cover change in Congo Basin: deforestation, reforestation, degradation and regeneration for the years 1990, 2000 and 2005.Glob Ch Biol 19:1173-1187.

Curran LM, Caniago I, Paoli GD, Astiani D, Kusneti M, Leighton M, Nirarita E, Haeruman H. 1999. Impact of El Niño and logging canopy tree recruitment in Borneo. Science 286:2184-2188.

Couwenberg J, Dommain R, Joosten H. 2010.Greenhouse gas fluxes from tropical peatland in South-East Asia. Glob Ch Biol 16: 1715-1732.

Curran LM, Caniago I, Paoli GD, Astiani D, Kusneti M, Leighton M, Nirarita E, Haeruman H. 1999. Impact of El Niño and logging on canopy tree recruitment in Borneo. Science 286: 2184-2188.

Denslow J, Ellison AM, Sanford SE.1998.Tree fall gap size effects on above and below ground processes on tropical wet forest. J Ecol 86: 597-609.

Dirzo R, Raven PH. 2003. Global state of biodiversity and loss. Ann Rev Environ Resour 28: 137-167.

Eva H, Carboni S, Achard F, Stach N, Durieux L, Faure J-F, Mollicone D. 2010. Monitoring forest areas from continental to territorial levels using a sample of medium spatial resolution satellite imagery. ISPRSJ Photogram Remote Sens 65: 191-197.

FAO [Food and Agricultural Organization]. 2010. Global forest resources assessment 2010 country report. Indonesia Forest Resources Assessment (FRA) 2010/095, UNFAO, Rome.

Foley JA, Asner GP, Costa MH, Coe MT, De Fries R, Gibbs HK, Howard EA, Olson S, Patz J, Ramankutty N, Snyder P. 2007. Forest Degradation and loss of ecosystem goods and services in the Amazon Basin. Ecol Environ 5 (1): 25-32.

Grainger A. 1993. Controlling Tropical Deforestation. Earthscan, London.

Hooijer A, Silvius M, Wösten H, Page SE. 2006. PEAT-CO_2, Assessment of CO_2 emissions from drained peatlands in SE Asia. Delft Hydraulics Rep Q 3943.

Hoojiers A, Page S, Canadell G, Silvius M, Kwadijk J, Wosten H, Jauhiainen J. 2010. Current and future CO_2 emissions from drained peatlands in Southeast Asia. Biogeosciences 7: 1505-1514.

IPCC [Intergovernmental Panel on Climate Change]. 2011. Summary for Policymakers. In:Edenhofer O, Pichs-Madruga R, Sokona Y, Seyboth K, Matschoss P, Kadner S, Zwickel T, Eickemeier P, Hansen G, Schlomer S, von Stechow C (eds). IPCC Special Report on Renewable Energy Sources and Climate Change Mitigation. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Hansen AJ, Neilson RP, Dale VH, Flather CH, Iverson LR, Currie DJ, Shafer S, Cook R, Bartlein PJ. 2001. Global change in forest: Response of species, communities and biomass.Bioscience 51 (9): 765-779.

Husch B, Beer WT, Kershaw AJ. 2002. Forest Mensuration. 4th ed. John Wiley and Sons, London.

Lambin EF.1999. Monitoring forest degradation in tropical region by remote sensing: some methodological issues. Glob Ecol Biogeogr 8: 191-198.

McElhinney C, Gibbons P, Brack C, Bauhas J. 2005. Forest and woodland stand structural complexity: its definition and measurement. For Ecol Manag 218 (3): 1-24

Margono BA, Turubanova S, Zhuravleva, Potapov P, Tyukavina A, Baccuni A, Goetz S, Hansen MS. 2012. Mapping and monitoring deforestation andforest degradation in Sumatra (Indonesia) using Landsat time series data sets from 1990 to 2010. Environ Res Lett 7 (034010): 1-16.

Ministry of Forestry of Indonesia. 2011.Land Cover Recalculation of Indonesia in 2009/2010. Planning Agency, Department of Forestry Indonesia, Jakarta. [Indonesian]

Miettinen J, Shi C, Liew SC. 2011. Influence of peatland and land cover distribution on fire regimes in insular Southeast Asia. Reg Environ Ch 11: 191-201.

Nepstad DC, Verissimo A, Alenca A. 1999. Large scale impoverishment of Amazonian forest by logging and fire. Nature 398: 505-508.

Page SE, Siegert F, Rieley JO, Boehm HDV, Jaya A, Limin S.2002. The amount of carbon released from peat and forest fires in Indonesia in 1997. Nature 420: 61-65.

Prescott CE, Hope GD, Blevin LL. 2003. Effect of gap size on litter decomposition and soil nitrate concentrations in a high spruce-fir forest. Can J For Res 33: 2210-2220.

Proe MF, Griffiths JH, McKay HM. 2001.Effect of whole tree harvesting on microclimate during establishment of second rotation forestry. Agric For Meteorol 110: 1410-154.

Putz FE, Redford KH. 2010. The importance of defining forest: tropical forest degradation, deforestation, long-term phase shifts, and further transitions. Biotropica 42 (1): 10-20.

Rieley JO, Page SE. 2005.Wise Use of Tropical Peatland: Focus of Southeast Asia, ALTERRA-Wagenigen University and Research Center and the EU INCO-STRAPEAT and RESTORPEAT Partnership, Wageningen.

Siegert F, Rueckee G, Hinrich A, Hoffman AA. 2001. Increase damage from fires in logged forest during droughts cause by El Niño. Nature 414: 437-440.

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Morphological responses, sensitivity and tolerance indices of four tropical trees species to drought and waterlogging

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Abstract. *Yulianti, Sudrajat DJ. 2016. Morphological responses, sensitivity and tolerance indices of four tropical trees species to drought and waterlogging. Biodiversitas 17: 110-115.* Indonesia's climate model predicts an increase in threat of drought and waterlogging in several areas. However, knowledge on variations between tropical forest tree species, i.e. *Anthocephalus macrophyllus, Anthocephalus cadamba, Fagraea fragrans*, and *Magnolia champaca* to drought and waterlogging stress are still limited, though they are relevant to future development of forest ecosystem. The objective of this research is to investigate the adaptability of four potential tree species to drought and waterlogging stress in a controlled greenhouse. The results showed that the adaptive responses of four tropical tree species were different, according to the results, all species were more tolerant to waterlogging than to drought stress, which can be observed from morphological, seedling survival, stress tolerant and stress sensitivity indices. The waterlogging resistant in four species followed the order of: *A. cadamba* > *F. fragrans* > *A. macrophyllus*, and > *M. champaca*, while for the drought resistant species followed the order: *A. macrophyllus* > *A. cadamba* > *F. fragrans* and > *M. champaca.*

Keywords: Adaptability, seedling, stress, tropical trees

INTRODUCTION

Indonesia, is an archipelago country lies in the equator area, is considered as a susceptible region to climate changes. Rainfall pattern changes, increase of water sea level and temperature, and extreme weather events are some serious impacts of climate changes occurring in Indonesia. According to Policy Synthetic Team of Ministry of Agriculture (2008), climate change will cause the following: (a) Indonesia will undergo temperature increase, the rate of which is lower than the temperature in subtropical areas; (b) South Indonesia area is predicted to undergo rainfall decrease enhancing the drought risks, while in North Indonesia, the rainfall intensity will increase, thus rising the frequency and intensity of floods.

Drought and flood or waterlogging limits the potential range of many species by affecting seedling survival, growth and development potential of plants. They showed significant effects at the initial stages of plant growth (e.g., during the first year of cultivation) (Kozlowski 1997; Dunisch et al. 2003) and endangered plants survival. The effects of water stress have been reported for a large number of angiosperms and gymnosperms, resulting considerable changes in plant physiology, morphology and overall biochemical processes (Cernusak et al. 2007; Chaves et al. 2008; Cordeiro et al. 2009; Ditmarova et al. 2009; Yang and Miao 2010; Li et al. 2011; Xioling et al. 2011). Water stress is a major growth-limiting factor highlighting the need for selecting drought resistant species for successful plantations (Ky-Dembele et al. 2010). To promote the successful current-year tree seedlings settlement, the key to understand is how they are adapted to drought and waterlogging, a critical condition for silviculture activities of several potential tree species.

However, very little is known about species variations in adaptability to drought and waterlogging stresses in tropical forest trees species. Some tropical tree species showed ability to satisfactorily tolerate or postpone drought such as *Swietenia macrophylla* (Cordeiro et al. 2009), *Garcinia kola* and *Garcinia afzelii* (Peprah et al. 2009). In other study, Rao et al. (2008) reported that seedling height and dry biomass of *Albizzia lebbek*, *Dalbergia sissoo, Leucaena leucocephala*, and *Shorea robusta* decreased at very high stress. The drought resistant in different species followed the order of: *L. leucocephala* > *T. grandis* > *D. sissoo* > *S. robusta* and > *A. lebbek*. Adaptability study of *Anthocephalus cadamba* to drought and waterlogging showed that the species were more resistant to waterlogging than to drought stress (Sudrajat et al. 2015).

Therefore, comprehensive knowledge about species responses on drought and waterlogging can become a reference in the development of forest trees species transfer guideline (Wang et al. 1989), selection of adaptive species, and also as a key of adaptation strategy on climate change (Millar et al. 2007). The hypothesis in response to drought and waterlogging stress, there is a large variation among tropical trees species expressed in growth, stress sensitivity and stress tolerance indices.

The objective of this study was to investigate the morphological responses, sensitivity and tolerance indices of *Anthocephalus macrophyllus*, *Anthocephalus cadamba*, *Fagraea fragrans*, and *Magnolia champaca* seedlings to drought and water logging stresses in a controlled greenhouse.

MATERIALS AND METHODS

Materials

The studied species were *A. macrophyllus*, *A. cadamba*, *F. fragrans*, and *M. champaca* (Table 1). The seeds of the species were collected from 10 dominant trees per species. The seeds from individual trees were equally sampled by weight, and bulked by species for the experiment.

Seedling preparation, experimental design and parameter measurement

A. macrophyllus, *A. cadamba*, *F. fragrans*, and *M. champaca* seedlings were tested in a controlled condition in the greenhouse to identify their adaptability on different water stress conditions. For each species, 60 normal seedlings (± 3 cm in height) were randomly taken from sowing boxes and planted in pots (18.5 cm in diameter x 16 cm in height). There were 20 seedling pots planted for each treatment. At the early stage of growing, seedlings were placed in an optimal condition in nursery and after 2 months, they were moved to the greenhouse. During the experiment, the average day and night temperatures in the greenhouse were set at 34° C and 29° C, respectively, and the relative humidity ranged from 60 to 75 %. The treatments of water stress condition were done after 1 month of seedling in the greenhouse.

A completely randomized block design was used with factorial combinations of water stress [well-water supply (control), 3-5 cm-water logging (W_L), watered every 2 days with 25% field capacity (W_{25})] and species (4 species). Five seedlings were randomly assigned accordingly to each of the twelve experimental treatment units and arranged randomly in each of the four blocks (5 seedlings × 4 species × 3 irrigation regimes × 4 blocks). There were a total of 240 seedlings pots or 60 seedlings per species. The soil volumetric water content for control and drought treatments were maintained at 32.8 ± 2.8% and 19.8 ± 1.4% and W_{25}, respectively. 120 days after the initial treatment, the experiment was terminated.

Height (SH) and root collar diameter (RCD) of seedlings were recorded prior to and at the end of the experiment. The growth of seedling height and diameter resulted from reduction of the final measurement with the first measurement. The number of leaves was counted in all plants. Seedling biomass was measured by harvesting roots, stems, and leaves. Roots were elutriated with water to remove soil. Roots, stems, and leaves were dried in a drying oven at 70° C for 48 h and weighed to ±0.0001 g.

Data analysis

The data were analyzed with ANOVA to test the effect on the water stress and populations on the morphological, anatomical and physiological variables. Duncan's multiple range test at a significance level of $p<0.05$ was used to compare the significant differences in the means. The statistical analysis was performed applying SAS 9.1 for windows. To compare the adaptability among species, stress tolerance index (STI) and stress susceptibility index (SSI) were analyzed on the growth parameters (seedling height and root collar diameter). STI was analyzed using formula (Fernandez 1992):

$$STI = (ypi \times ysi) / YP^2$$

while SSI was analyzed using formula (Fischer and Maurer 1978):

$$SSI = 1- (ysi / ypi) / SI, \text{ while } SI = 1- (YS / YP)$$

Where: ysi, ypi, YS, and YP were each species's parameter under stress and non-stress conditions, parameter mean of all species under stress and non-stress conditions, respectively.

RESULTS AND DISCUSSIONS

There were no seedlings died in the control and WL treatments for *F. fragrans*, *A. macrophyllus*, and *A. cadamba*, while in the $W_{25\%}$ treatment, 7 seedlings of *F. fragrans*, 6 seedlings of *A. macrophyllus*, and 8 seedlings of *A. cadamba* were observed to have died at the end of the experiment. The died seedlings on *M. champaca* were more occurred on the all treatments then died seedlings on the other species, i.e. 1seedling in the control, 12 seedlings in the water logging stress, and 14 seedlings in the drought stress (Figure 1).

In our experiment, significant differences among four species (*A. macrophyllus*, *A. cadamba*, *F. fragrans*, and *M. champaca*) were observed in the growth rate (seedling height, root collar diameter and biomass) under drought and waterlogging regimes. The seedling growth on the control (well-water supply) of 4 species was relatively not significant difference (Figure 2) and showed the highest growth. Drought and waterlogging stresses caused significant changes in seedling height and root collar diameter on all species. The lowest growth occurred on drought treatment followed by the growth on the waterlogging treatment. *M. champaca* had lower height and root collar diameter growth both on drought and waterlogging treatments then the other species. On the other hand, *F. fragrans* and *A. macrophyllus* had the highest root collar diameter under the WL treatment (Figure 3). The seedling survival and growth status under drought and waterlogging stress can be regarded as one of the important indices in plant tolerance (Vreugdenhil et al. 2006; Mommer et al. 2006; Li et al. 2011).

Table 1. Geographic origin of the investigated species

Species, family	Location	Latitude	Longitude	Altitude (m asl.)
Fagraea fragrans, Loganiaceae	Ogan Komering Ilir,South Sumatra	04^0 30' S	$104^0$02'E	25
Anthocephalus macrophyllus, Rubiaceae	Bolaang Mongondow, North Sulawesi	00^o39' N	124^o13'E	100
Magnolia champaca, Magnoliaceae	Lahat, South Sumatra	03^o 54' S	103^o 07' E	650
Anthocephalus cadamba, Rubiaceae	Parangloe, Gowa, South Sulawesi	05°14'S	119°35'E	119

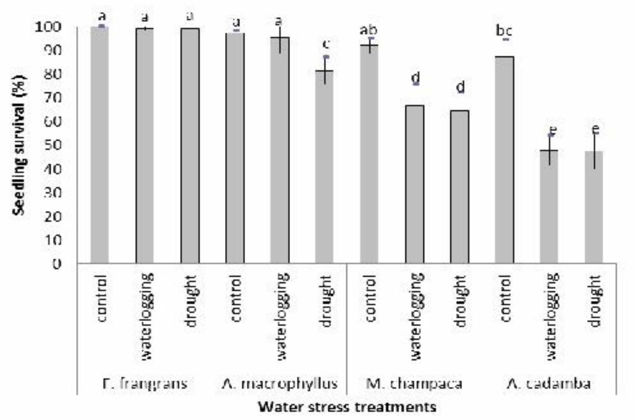

Figure 1. Survival percentage of 4 species under drought and waterlogging stresses at 4 months old

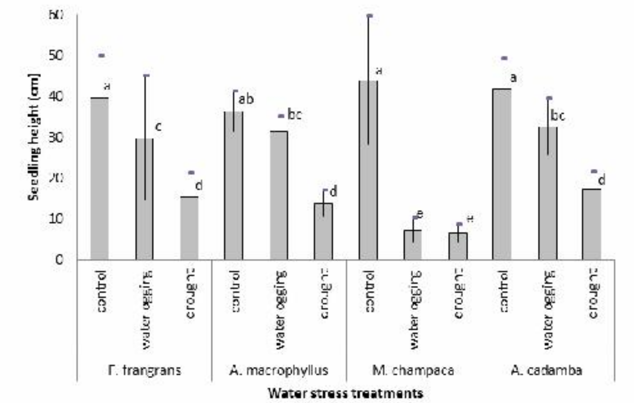

Figure 2. Seedling height of 4 trees species under drought and waterlogging stresses at 4 months old

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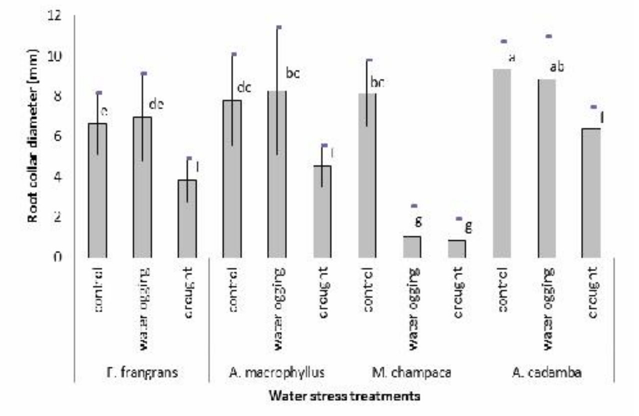

Figure 3. Seedling root collar diameter of 4 trees species under drought and waterlogging stresses at 4 months old

Total biomass, leaf number and leaf area were generally decreased in water stress (Table 2). A reduction biomass by drought stress was higher than waterlogging stress in all species. During water stress, the leaf number and leaf area per plant of white jabon significantly decreased except for leaf area of *F. fragrans* due to the leaf size of the species is very small so the changes on the leaf size is not significantly detected. Leaf number and leaf area were affected adversely in both seedling height and root collar diameter of all species. Reduction in leaf number and leaf area by water stress is an important cause of the reduced growth through reduction in photosynthesis (Rucker et al. 1995). The reduction in leaf area under water stress may be associated with the decline in the cell enlargement (Shao et al. 2008). The cell size reduction in leaf occurred as a result of turgidity being necessary for cell expansion. The cell size reduction is reasonably interpreted as a tolerance mechanism of the leaf to maintain tissue turgidity for the seedlings.

A reduction growth rate by drought stress was higher than by waterlogging stress in all species. The growth parameters reductions of four species were higher in drought stress indicated that the species were more tolerant to waterlogging than drought stress. In waterlogging and drought stresses, *M. champaca* showed the largest growth parameters reductions (Table 3). *F. fragrans* and *A. macrophyllus* had the negative reductions for root collar diameter due to root collar diameter in the waterlogging treatment was higher than in the other treatments. The similar result also occurred in waterlogged seedlings of *Carex lasiocarpa* and *C. limosa* (Lu 2011). According to Kozlowski (1997), waterlogging often affects xylem and phloem production. Waterlogged soils increased stem diameter growth more as a result of increasing bark thickening and stem hypertrophy, which then cause xylem increment. The increase in bark thickness was associated with accelerated proliferation of phloem parenchyma cells and large amounts of intercellular space in the phloem (Yamamoto and Kozlowski 1987).

To compare the adaptability of the stresses among species, stress sensitivity index (SSI) and stress tolerance index (STI) were used to observe the four trees species (Tables 4 and 5). SSI and STI were used to identify the resistant genotype (Yarnia et al. 2011; Anwar et al. 2011). STI can be used to identify genotypes that produces high yield under both stress and non-stress conditions (Fernandez 1992; Anwar et al. 2011). The species with low SSI and high STI can be considered to be more tolerant species among the all tested species (Olaoye et al. 2009). According to Fischer and Maurer (1978) and Badami and Amzeri (2011), the species were categorized as tolerant if SSI < 0.5, medium tolerant if 0.5 < SSI < 1, and sensitive if SSI > 1. While for STI, grouping of tolerant species followed criteria of Doreste et al. (1979) and Susanto and Sundari (2011), i.e. very tolerant species if STI > 0.898, tolerant if 0.74 < STI < 0.898, medium tolerant if 0.41 < STI <0.74, sensitive if 0.25 < STI < 0.41, and very sensitive if STI ≤ 0.25.Based on the SSI and STI on the root collar diameter of the waterlogged seedlings, *F. frangrans,* and *A. cadamba* (SSI ≤0.5, STI > 0.74) could be categorized as tolerant species to waterlogging stress, but for drought stress, no species could be grouped as tolerant species. According to early result, the SSI of *A. cadamba* was lowest on waterlogging stress, but highest on drought stress (Sudrajat 2015). Adaptability of *F. frangrans*

Table 2. Seedling leaf characteristics and biomass of 4 trees species under drought and waterlogging stresses at 4 months old

Treatment (species*watering regime)		**Leaf number**	**Leaf area**	**Biomass total**
Fagraea fragrans	Control	23.5±6.1 a	24.72 ±1.18d	16.62 ± 1.41 a
	Waterlogging	16.3 ± 5.2 b	21.73 ±1.97d	15.06 ± 0.78 a
	Drought	11.5 ± 3.6 c	17.76 ±4.13d	5.06±1.37 b
Anthocephalus macrophyllus	Control	6.4 ± 1.2 e	132.58 ±2.06a	16.95 ± 0.30 a
	Waterlogging	5.5 ± 1.3 ef	97.87 ±1.89b	15.62 ± 0.39 a
	Drought	4.9 ± 1.0 ef	33.19 ±5.22d	4.43 ± 0.86 b
Magnolia champaca	Control	9.4 ± 2.0 d	68.61 ±2.27c	18.42 ± 0.19a
	Waterlogging	5.1 ± 2.1 ef	33.54 ±6.33d	4.84 ± 1.63b
	Drought	4.5 ± 1.5 f	16.27 ±2.74d	3.99 ± 0.44 b
Anthocephalus cadamba	Control	6.4 ± 1.8 e	142.90 ±3.18a	18.17 ± 0.19 a
	Waterlogging	5.5 ± 2.2 ef	103.88 ±4.01b	15.99 ± 0.45 a
	Drought	2.8 ± 1.1 g	42.81 ± 11.35d	3.70 ± 0.70 b
Means		7.89	61.32	11.56
Coefficient of variation (CV)		30.35	7.66	7.51
F test (species*watering regime)		18.85**	72.49**	43.09**

Note: Different letters in the same column indicate significant differences at P ≤ 0.05 between treatment and provenances in each parameter.

Table 3. Percentage decrease of seedling growth parameters under drought and waterlogging compared with control condition

Treatment (species*watering regime)		**Height (%)**	**Root collar diameter (%)**	**Leaf number (%)**	**Leaf area (%)**	**Biomass total (%)**
Fagraea fragrans	Control	0	0	0	0	0
	Waterlogging	25	-5	31	12	9
	Drought	61	42	51	28	70
Anthocephalus macrophyllus	Control	0	0	0	0	0
	Waterlogging	13	-6	14	26	8
	Drought	62	42	23	75	74
Magnolia champaca	Control	0	0	0	0	0
	Waterlogging	83	88	46	51	74
	Drought	85	90	52	76	78
Anthocephalus cadamba	Control	0	0	0	0	0
	Waterlogging	22	5	14	27	12
	Drought	59	31	56	70	80

Table 4. Stress sensitivity index of seedling height and root collar diameter of 4 trees species under drought and waterlogging stresses at 4 months old

Species	**Seedling height**		**Root collar diameter**	
	Waterlogging	**Drought**	**Waterlogging**	**Drought**
Fagraea fragrans	0.59	0.98	0.25	0.92
Anthocephalus macrophyllus	0.67	0.88	0.27	0.73
Magnolia champaca	2.22	1.22	4.09	1.56
Anthocephalus cadamba	0.36	0.89	0.24	0.74

Table 5. Stress tolerance index of seedling height and root collar diameter of 4 trees species under drought and waterlogging stresses at 4 months old

Species	**Seedling height**		**Root collar diameter**	
	Waterlogging	**Drought**	**Waterlogging**	**Drought**
Fagraea fragrans	0.83	0.34	1.30	0.65
Anthocephalus macrophyllus	0.72	0.38	0.73	0.56
Magnolia champaca	0.20	0.18	0.13	0.11
Anthocephalus cadamba	0.70	0.31	1.01	0.40

and *A. cadamba* seedlings to waterlogging is correlated with their natural habitats, generally distributed on the deep, moist, and alluvial sites and vice versa, the general condition of the natural habitat also affected the adaptation of seedlings that is less adapted to drought stress (Soerianegara and Lemmens 1993; Kartawinata 1994). The seedlings of *M. champaca* behaved the opposite way or there was higher SSI on both waterlogging and drought stresses indicating that the species is not adapted both for drought and waterlogged sites, this results is similar with the early results of SSI of *M. champaca* (Yulianti et al. 2015).

In waterlogging stress, SSI values of *A. cadamba* were lowest, followed by *F. fragrans, A. macrophyllus*, and *M. champaca*, while for the STI values, from the highest to the lowest, were *F. fragrans, A. macrophyllus, A. cadamba*, and *M. champaca*. In drought stress, SSI values of seedling height and root collar diameter of *A. macrophyllus* had the lowest, followed by *A. cadamba*, *F. fragrans*, and *M. champaca*. On the other hand, the highest SSI value in drought stress for seedling height was *A. macrophyllus*, and *M. champaca* had the highest STI both for seedling height and root collar diameter. The lower SSI and the higher STI showed better adaptability of the plants to the stresses (Blum et al. 1992). The waterlogging resistant in four species were in the order of: *A. cadamba* > *F. fragrans* > *A. macrophyllus*, and > *M. champaca*, while for the drought resistant species were in the order of: *A. macrophyllus* > *A. cadamba* > *F. fragrans* and > *M. champaca*.

The data from the current experiment indicated that all species tend to be more tolerance to water logging than to drought stress. Waterlogging tolerance is determined by the ability of a plant to grow and survive in soils with water content above field capacity (Rowe and Beardsell 1973). In contrast to water logging, the species in the seedling stage were very sensitive to drought. The more adaptive seedling of four species to waterlogging described the natural habitat of the evergreen tropical tree species generally distributed on the relatively higher humidity and moist sites. The range of response of evergreen species to different drought intensities indicated a lower degree of plasticity than that of deciduous trees (Ditmarova et al. 2010).

In conclusion, seedling height and root collar diameter growth of *A. macrophyllus*, *A. cadamba*, *F. fragrans*, and *M. champaca* were more affected by drought and waterlogging stress conditions. The species were more tolerant to waterlogging than drought stress, which can be observed from morphological, the absence of seedling under the waterlogging treatment that died during the experiment, stress tolerant index, and stress sensitivity index. The waterlogging resistant in four species followed the order of: *A. cadamba* > *F. fragrans* > *A. macrophyllus*, and > *M. champaca*, while for the drought resistant species followed the order of: *A. macrophyllus* > *A. cadamba* > *F. fragrans* and > *M. champaca*. *A. cadamba* and *F. fragrans* can be planted in temporary waterlogging sites, while for dry sites, *A. macrophyllus* is more adaptive. *M. champaca* is preferably not to be planted in waterlogged and dry sites due to its relatively low tolerance to drought and waterlogging stresses.

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REFERENCES

Anwar J, Subhani GM, Hussain M, Ahmad J. Hussain M, Munir M. 2011. Drought tolerance indices and theirs correlation with yield in exotic wheat genotypes. Pakistan J Bot 43 (3): 1527-1530.

Badami K, Amzeri A. 2011. Identification of drought tolerant somaclonal on maize populations resulted from in vitro selection with PEG. Agrovigor 4 (1): 7-13. [Indonesian]

Blum A, Golan G, Mayer J, Sinmena B, Obilana T. 1992. Comparative productivity and drought response of semi-tropical hybrids and open pollinated varieties of sorghum. J Agric Sci Cambridge 118: 29-36.

Cernusak LA, Aranda J, Marshall JD, Winter K. 2006. Large variation in whole-plant water-use efficiency among tropical tree species. New Phytologist 173: 294-305.

Cordeiro YEM, Pinheiro HA, dos Santos-Filho BG, Correa SS, Silva JRR, Dias-Filho MB. 2009. Physiological and morphological responses of young mahogany (*Swietenia macrophylla* King) plants to drought. For Ecol Manag 258: 1449-1455.

Ditmarova L, Kurjak D, Palmroth S, Kmet J, Strelcova K. 2010. Physiological responses of Norway spruce (*Picea abies*) seedlings to drought stress. Tree Physiol 30: 205-213.

Doreste SE, Arias C. Bellotti A. 1979. Field evaluations of cassava cultivars for resistance to tetranychid mites.p. 161-164. *In* T. Brekelbaum, A. Bellotti, J.C. Lozano (*Eds.*) Proceeding of Cassava Protection Workshop. Cali. Colombia 7-12 November 1977.

Dunisch O, Erbreich M, Eilers T. 2003. Water balance and water potentials of a monoculture and an enrichement plantation of *Carapa guianensis* Aubl. in the Central Amazon. For Ecol Manag 172: 355-367.

Fernandez GCJ. 1992. Effective selection criteria for assessing plant stress tolerance. In: Proceedings of the International Symposium on Adaptation of Vegetable and Other Food Crops in Temperature and Water Stress. Taiwan.

Fischer RA, Maurer R. 1978. Drought resistance in spring wheat cultivars: I. Grain yield responses. Austr J Agric Res 29: 897-912.

Kartawinata K. 1994. The use of secondary forest species in rehabilitation of degraded forest lands. J Trop For Sci 7 (1): 76-86.

Konzlowski TT. 1997. Responses of woody plants to flooding and salinity. Tree Physiol Monogr 1: 1-28.

Ky-Dembele C, Bayala J, Savadogo P, Tigabu M, Odén PC, Boussim IJ. 2010. Comparison of growth responses of *Khaya senegalensis* seedlings and stecklings to four irrigation regimes. Silva Fennica 44 (5): 787-798.

Li FL, Bao WK, Wu N. 2011. Morphological, anatomical and physiological responses of *Campylotropis polyantha* (Franch.) Schindl. seedlings to progressive water stress. Scientia Horticulturae 127: 436-443.

Lu W. 2011. Growth and morphological responses to water level variations in two *Carex* species from Sanjiang Plain, China. African J Agric Res 6 (1): 28-34.

Millar CI, Stephenson NL, Stephens SL. 2007. Climate change and forests of the future: managing in the face of uncertainty. Ecol Appl 17: 2145-2151.

Mommer M, Lenssen JPM, Huber H, Visser EJW, Kroon HD. 2006. Ecophysiological determinants of plants performance under flooding: a comparative study of seven plant families. J Ecol 94 (6): 1117-1129.

Olaoye G, Menkir A, Ajala SO, Jacob S. 2009. Evaluation of local maize (*Zea mays* L.) varieties from Burkina Faso as source of tolerance to drought. J Appl Biosci 17: 887-898

Peprah T, Kyereh B, Owusu KA, Adu-Bredu S. 2009. Drought tolerance of *Garcinia kola* and *Garcinia afzelii* at the seedlings stage. Ghana J For 25: 13-27.

Policy Synthetic Team. 2008. Impact of climate change on the agriculture, and anticipation strategy and adaptation technology. Development of Agriculture 1 (2): 138-140. [Indonesian]

Rao PB, Kaur A, Tewari A. 2008. Drought resistance in seedlings of five important tree species in Tarai region of Uttarakhand. Trop Ecol 49 (1): 43-52.

Rowe RN, Beardsell DV. 1973. Waterlogging of fruit trees. Hort Abstr 43: 533-548.

Rucker KS, Kevin CK, Holbrook CC, Hook JE. 1995. Identification of peanut genotypes with improved drought avoidance traits. Peanut Sci 22: 14-18.

Shao H-B, Chu L-Y, Jaleel CA, Zhao CX. 2008. Water-deficit stress-induced anatomical changes in higher plants. C R Biol 331: 215-225.

Soerianegara I, Lemmens RHMJ. 1993. Plant resources of South-East Asia 5 (1): Timber trees: major commercial timbers. Pudoc Scientific Publishers, Wageningen, NL.

Sudrajat DJ, Siregar IZ, Khumaida N, Siregar UJ, Mansur I. 2015.Adaptability of white jabon (*Anthocephalus cadamba* Miq.) seedling from 12 populations to drought and waterlogging. Agrivita J Agric Sci 37 (2): 130-143.

Sudrajat DJ. 2015. Population Diversity, Provenance Test, and Adaptability of Jabon (*Neoclamarckia cadamba* (Roxb.) Bosser). [Ph.D. Dissertation]. Bogor Agricultural University. Bogor. [Indonesian]

Susanto GWA, Sundari T. 2011. The Changes of Agronomy Characters of Soybean Germplasm under Shading Condition. Jurnal Agronomi Indonesia 39 (1): 1-6. [Indonesian]

Vreugdenhil SJ, Kramer K, Pelsma T. 2006. Effects of flooding-duration,-frequency and-depth on the presence of saplings of six woody species in north-west Europe. For Ecol Manag 236: 47-55.

Wang JR, Hawkins CDB, Letchford T. 1998. Photosynthesis, water and nitrogen use efficiencies of four paper birch (*Betula papyrifera*) populations grown under different soil moisture and nutrient regimes. For Ecol Manag 112: 233-244.

Xiaoling L, Ning L, Jin Y, Fuzhou Y, Faju Y, Fangqing C. 2011. Morphological and photosynthetic responses of riparian plant *Distylium chinense* seedlings to simulated autumn and winter flooding in three Gorges Reservoir Region of the Yangtze River, China. Acta Ecologica Sinica 31: 31-39.

Yamamoto F, Kozlowski TT. 1987. Effect of ethrel on growth and stem anatomy of *Pinus halepensis* seedlings. IAWA Bull New Ser 8: 11-19.

Yang F, Miao LF. 2010. Adaptive responses to progressive drought stress in two poplar species originating from different altitudes. Silva Fennica 44 (1): 23-37.

Yarnia M, Arabifard N, Khoei FR, Zandi P. 2011. Evaluation of drought tolerance indices among some winter rapeseed cultivars. African J Biotechnol 10 (53): 10914-10922.

Yulianti B, Rustam E, Megawati, Mindawati N. 2015. Growth respon of Bambang Lanang seedling (*Michelia champaca* L.) to stress condition. Jurnal Penelitian Hutan Tanaman 12 (3): 211-221.

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Comparative characterization of *Macaranga* species collected from secondary forests in East Kalimantan for biorefinery of unutilized fast growing wood

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Abstract. *Amirta R, Nafitri SI, Wulandari R, Yuliansyah, Suwinarti W, Candra KP, Watanabe T. 2016. Comparative characterization of* Macaranga *species collected from secondary forests in East Kalimantan for biorefinery of unutilized fast growing wood*. *Biodiversitas 17: 116-123.* Wood species for industrial forest plantation has been selected to produce construction wood materials, boards and papers, and unutilized fast growing wood as a source for biofuel production has been out of the scope for selection. *Macaranga* Thouars (Euphorbiaceae) is widely distributed in the tropics and importance of the genus has been recognized due to its high level of growth rate and adaptability to constitute forest ecosystem. However, potency of the genus as a source for bioethanol production has not been systematically studied. We herein first report differential properties of six *Macaranga* wood species collected in East Kalimantan, Indonesia, as a raw feedstock for enzymatic saccharification for bioethanol production. Among the wood species examined, the highest sugar yield 48.6% (weight of original wood basis), which corresponds to 315 mL ethanol/kg biomass, was obtained with 5.0% NaOH at 160ºC for *M. hypoleuca*. Significant differences in the sensitivity to alkaline concentration and temperature have been found among the species. A high sugar yield, 40.4% was obtained for *M. winkleri* with a low alkaline concentration, 3.5% NaOH at 150ºC, while *M. motleyana* gave the sugar yield 12.8% under the same condition. *M. motleyana* required a set of the conditions with higher NaOH concentration 5.0% and temperatures over 160ºC. The harsh condition with 5.0% NaOH at 170ºC promoted delignification of all the species but *M. hypoleuca* decreased the saccharification yield by raising the temperature from 160ºC to 170ºC, probably due to decomposition of carbohydrate cores. This temperature-dependent negative effect was not observed with 3.5% NaOH for *M. hypoleuca*. These results indicate that differences in the balance between disintegration effects and excess degradation of carbohydrates are different among the species and the variation should be taken into account on screening. Thus, we found a wide range of diversity in the susceptibility to alkaline pretreatment in the genus *Macaranga* and selected the wood species giving high productivity of fermentable sugars.

Keywords: Alkaline pretreatment, biorefinery, ethanol, *Macaranga*, wood biomass

INTRODUCTION

Recently, it has been recognized that conversion of abundant lignocellulosic biomass to biofuels presents a viable option for improving energy security and reducing greenhouse emissions. Biofuels are cleaner-burning than fossil fuels, and the short cycle of growing plants coupled with their burning emits much less CO_2 to the atmosphere. It has been reported that bioethanol from lignocellulosic biomass has the potential to reduce greenhouse gas emissions by 86% (Wang et al 2008).

As one of the countries which have abundant reserves of forest biomass and agricultural residues and expect future energy crisis, Indonesia government has declared to start production of fuels and energy from renewable sources. The government realizes that the biofuels and bioenergy industries will increase the amount of domestic supply of fuels with decrease in subsidy for promotion of the biofuels (Watanabe et al. 2008). Thus far, industrial forest plantation has been designed to produce construction wood materials, boards and papers, and potency of unutilized fast growing wood as a source for biofuel production has received much less attention. However, tropical rain forest includes a wide variety of wood species which has no values for the current industry but may have a great deal of potential for production of biofuels and chemicals.

From the view point of biodiversity and potential of the bioresources for sustainable society, WWF Indonesia pointed out importance of the tropical forests in Indonesia, particularly in Kayan Mentarang Forest, Malinau, East Kalimantan, which includes around 15,000 species of plants (Pio and D'Cruz 2005). The biodiversity value of the forest is the highest compared to other places on the earth. Forest in Kalimantan is characterized also by richness in endemic species. There are at least 6,000 endemic species of plants, including 155 dipterocarp trees species and more than 30 of the genus *Macaranga* and *Mallotus* (Euphorbiaceae) plants (Pio and D'Cruz 2005; Slik 2003, 2005).

Macaranga is known as one of the pioneer species classified in very fast growing species in the tropical forest ecosystem. Distribution of more than 300 of the genus *Macaranga* spreads in the southern part of Asia, Africa, Australia, and the South Pacific regions (Webster 1994). Although importance of *Macaranga* for biological diversity and its potential as a source for biorefinery can be expected, the biomass potency of *Macaranga* is not fully perceived benefits. The lack of information on the basic properties, function and suitability as the feedstock for the fuels and energy production, is believed as the main reason and barrier factor for utilization of this wood species. *Macaranga* has a wide variety of variations in its genus, and understanding of the properties of each species as a feedstock for biofuels is important for establishment of biorefinery.

Woody biomass, including many species in the genus *Macaranga* has a complex composite structure, and its efficient utilization requires exposure of cellulose and hemicelluloses from the cell walls coated with lignin. Lignin is a major factor limiting the degradation of lignocellulosics by microbial, physical and chemical pretreatments. To break the composite structure, various pretreatment methods such as microbial treatment using white-rot fungi, diluted and concentrated acids, steam explosion, microwave irradiation, milling, CO_2 explosion, ammonia fiber explosion (AFEX), autohydrolysis and alkaline treatments have been studied (Itoh et al. 2003, Nakamura and Godliving 2003; Amirta et al. 2006; Kumar et al. 2009a; Verma et al. 2010; Chiaramonty et al. 2012). In this study, we selected alkaline pretreatments for processing of 6 species of *Macaranga* wood collected in secondary forests in East Kalimantan, Indonesia. The susceptibility of the wood species to alkaline pretreatment for enzymatic saccharification is reported.

MATERIALS AND METHODS

Study area

The field observation and plant material including wood biomass was collected from Bukit Soeharto Education Forest of Mulawarman University located at Kutai Kertanegara District, East Kalimantan, Indonesia (116^{o}50'6.89"E-117^{o}9'53.81"E, 0^{o}38'43.38"S-1^{o}5'24.71"S). This education forest has an area of about 22,000 ha and annual temperature of 24-30^{o}C, while the daily temperatures fluctuate between 3-4^{o}C. The mean annual precipitation was 1921.3 mm, whereas the highest monthly rainfall was obtained in March and the lowest occurs in September amounted to 54.3 mm, respectively.

Wood material

Wood samples from six species of *Macaranga* with diameter about 10-20 cm and their leaves and branches were collected from Mulawarman University Education Forest located at Bukit Soeharto, Kutai Kertanegara, East Kalimantan, Indonesia. Leaves of wood samples were identified as *Macaranga conifera*, *M. gigantea*, *M. hypoleuca, M. motleyana, M. pearsonii* and *M. winkleri* in the Laboratory of Forest Dendrology, Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia. The wood samples were debarked, chipped and air dried up to approximately 12% moisture content (MC), and used throughout this study.

Alkaline pretreatment of woody biomass

Alkaline pretreatment of *Macaranga* wood was carried out for 60 min at 150º and 170ºC using a liquid-to-solid ratio 8:1 (w/w) and NaOH concentration between 3.5% and 5.0% based on dry weight of the woody biomass. The reactions were carried out using a rotary digester equipped with a controller for pressure, rotary speed and temperature. After the reaction, the pulp fraction was separated by filtration and washed extensively with tap water until neutral pH.

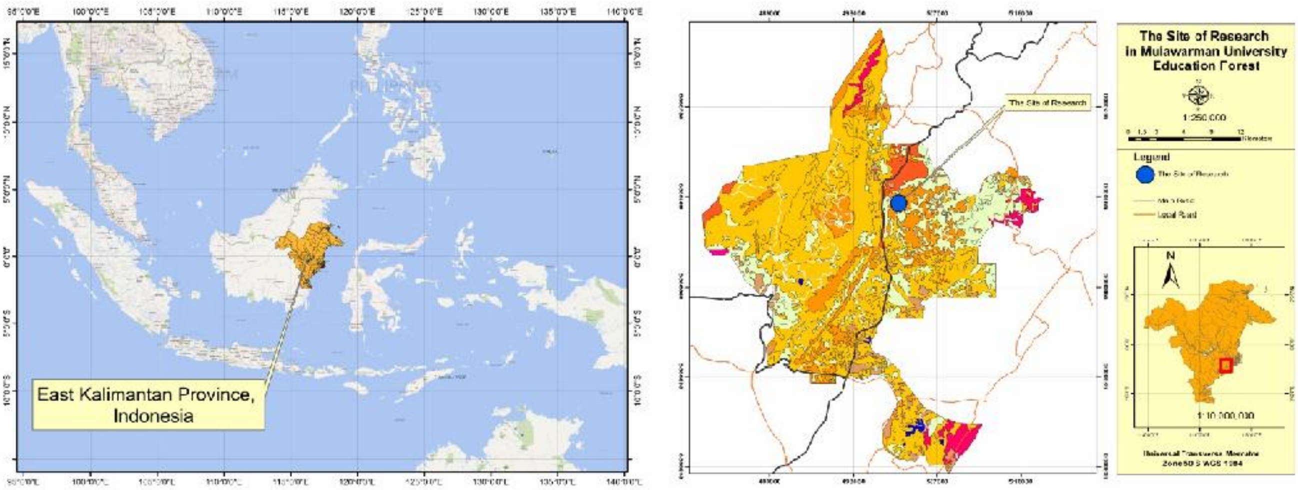

Figure 1. Sampling location at Bukit Soeharto Education Forest of Mulawarman University, Kutai Kertanegara, East Kalimantan, Indonesia

Wood component analysis

The Klason lignin content was determined by the TAPPI standard method (1998). The holocellulose and α-cellulose contents were determined according to Wise's chlorite method (Wise et al. 1946) and the TAPPI standard method (1988), respectively. The reducing sugar content was determined by the Somogyi-Nelson method (Somogyi 1952).

Enzyme activity

Filter paper unit activities were assayed in reaction mixture containing 50 mg (w/v) Whatman filter paper number 1, 50 mM tartrate buffer, pH 4.5 and the enzyme. After incubation at 50ºC for 30 min., the reducing sugars produced were determined by Somogy-Nelson method (Somogy 1952). One unit (U) of each enzyme activity is defined as the amount of enzyme, which produce 1 μmol reducing sugar as glucose in the reaction mixture per minute under above specific condition.

Saccharification of wood biomass

The wet pulp fraction was hydrolyzed with a commercial cellulose preparation, meicellase from *Trichoderma viride* (Meiji Seika Co., Ltd., Japan), 224 filter paper units (FPU)/g, β-glucosidase activity 264 IU/g). The cellulase enzyme loading was an 8-FPU/g substrate. Enzymatic hydrolysis was performed at a substrate concentration of 2% in 0.05 M sodium citrate buffer (pH 4.5) containing 0.02% sodium azide at 45ºC on a rotary shaker (NTS-4000C, Rikakikai, Japan) at 140 rpm for 48 h (Itoh et al. 2003). The saccharification ratio per pulp was calculated according to the NREL LAP-009 procedure (Brown and Torget 1996). The sugar yield per wood is based on the weight percentage of the reducing sugars to the original wood. The overall yield of sugars per wood is calculated by multiplying the saccharification ratio per pulp and the pulp yield. All enzymatic hydrolysis experiments were performed in triplicate.

Estimation of ethanol production from woody biomass

Potential ethanol production from *Macaranga* wood was estimated based on the amount of hexose sugar (HXTEL) in the lignocellulosic material obtained by enzymatic saccharification of the insoluble pulp fraction. Due to the high content of glucose in the pulp fraction, HXTEL was approximated by the amount of reducing sugars obtained from the pulp fraction (equation 1). The ethanol yields (ETOHBIO) based on the weight of original biomass was calculated from equation (2).

$$\text{HEXTEL} = \text{HEX} \times a \qquad \text{(mg/kg)} \qquad \text{eq.1}$$

$$\text{ETOHBIO} = \text{HXTEL} \times Ye.h/b \qquad \text{(mL/kg)} \qquad \text{eq.2}$$

Where HEX is the hexose (D-glucose) yield upon saccharification from hexosan (w/w, of original wood basis), a is the weight of substrate (1000 mg, 1kg), Ye.h is the theoretical ethanol yield from hexose (D-glucose) (0.511), and b is the ethanol density (0.789 kg/L) (Premjet et al. 2013-Modified).

RESULTS AND DISCUSSION

Lignocellulosic composition of *Macaranga*

The genus *Macaranga* widely distributes in tropical regions in the world with a wide range of diversity counting 300 different species. Since many years ago, the light density hardwood from a pioneer tree species of *Macaranga* was traditionally used as fuel wood material and to be a primary source of energy for the people living in the remote area and surrounding of forest area, particularly in Southeast Asia countries and India (Bhatt and Tomar 2002; Yuliansyah et al. 2012). Instead of fuelwood, utilization of *Macaranga* is still limited due to lack in natural physical strength as a construction material and short fiber morphology for paper production (Ang et al. 2009; Adi et al. 2014). However, the weak physical properties may contribute biorefinery process including production of biofuels through enzymatic saccharification and fermentation. We collected *Macaranga* species from the secondary forest in East Kalimantan and evaluated their properties as the source for sugar production by enzymatic saccharification, aiming at constituting a new biorefinery process of unutilized fast growing wood species in the tropics. Because the collected wood species is suitable to grow on the soil under the climate conditions of the collected area, selection of the wood species directly lead to the new forest plantation industry coupled with the biofuel production.

We characterized changes in the lignin content and susceptibility to enzymatic saccharification after alkaline pretreatment at temperatures between 150ºC and 170ºC with 3.5-5.0% NaOH. The pretreatment demonstrated that the alkaline pretreatment decreased the lignin content and gave a high level of sugar yields by enzymatic saccharification to the extent comparable to other hardwood and softwood species reported for bioethanol production (Zhao et al. 2008; Mirahmadi et al. 2010; Verma et al. 2010; Alvarez et al. 2013). The alkaline pretreatment effectively decreased the Klason lignin content and produced residual pulp fractions as a result of disintegration of the cell wall structure (Table 2 and 3). The removal of lignin is beneficial for enzymatic saccharification due to increased accessibility of hydrolases to cellulose and hemicelluloses and decrease in non-productive binding between lignin and the enzymes (Zhao et al. 2008; Alvarez et al. 2013; Rahikainen et al. 2013).

The highest decrease in lignin content was obtained when 5.0% NaOH was applied at 170ºC. Under this condition, decrease in Klason lignin content was from 27.6% to 4.8% (*M. hypoleuca*), 27.6% to 4.7% (*M. gigantea*), 30.8% to 5.2% (*M. motleyana*), 26.5% to 5.2% (*M. conifera*), 32.9% to 5.4% (*M. pearsonii*) and 28.7% to 3.1% (*M. winkleri*), (Table 3). As for *M. winkleri* 89% of the lignin was removed by the pretreatment. Although the pretreatment condition was suitable for delignification, the high temperature decreased the pulp yield owing to the decomposition of carbohydrates, resulted in the lower overall sugar yields (Table 4). For instance, when the reaction temperature was raised from 160ºC to 170ºC using 5.0% NaOH, the pulp yield decreased from 53.5% to

45.0% (*M. hypoleuca*), 50.3% to 44.5% (*M. motleyana*), 60.4% to 44.5% (*M. conifera*), 54.3% to 46.6% (*M. winkleri*), 56.1% to 49.5% (*M. pearsonii*) and 45.4 to 41.7% (*M. gigantea*) (Table 2).

The effects of alkali pretreatment on enzymatic saccharification was analyzed at two different concentrations of NaOH and three set of temperatures using a commercial cellulase from *T. viride*, meicelase (Table 4). The ethanol yields estimated by the sugar yield are tabulated in Figure 3. Neutral sugar composition of the pulp obtained using 3.5% and 5.0% NaOH at 160ºC for 60 min was analyzed by HPLC equipped with a fluorescence detector and post-labeling reactor (Figure 2). The pulp fraction processed at 3.5% and 5.0% of NaOH at 160^{o}C for 60 min contained 94.9% and 97.1% glucose as the major sugar with 5.1% and 2.9% xylose, respectively. The amount of arabinose and mannose were below the background level, indicating that the pulp fraction contained cellulose as a major carbohydrate component with a small amount of xylan. General trend for promotion of sugar yield by higher concentration of NaOH with the decreased amount of remaining lignin was found (Table 3 and 4), in accordance with the previous report by Taherzadeh and Karimi (2008); Wang et al. (2008); and Kumar et al. (2009b), who described that the hydrolizability of NaOH-treated hardwood increased with decrease in lignin content. However, we found exceptions of the theory and striking differences in the susceptibility to the concentration of NaOH among the *Macaranga* wood species examined.

High sugar yield, 40.4% (weight of original wood basis) was obtained for *M. winkleri* even at lower alkaline concentration, 3.5% NaOH at 150ºC. Increase in the NaOH concentration from 3.5% to 5.0% increased the sugar yield just by 3.2%. On the contrary, sugar yield of M. motleyana with 3.5% NaOH at 160ºC was only12.8%, while the pretreatment with 5.0% NaOH gave the sugar yield 45.3% at the same temperature. Thus, the higher concentration increased the sugar yield from *M. motleyana* by 3.5 times. This effect was not prominent at 150ºC for the same wood as found in the yields 12.8% and 14.6% at the NaOH concentration 3.5% and 5.0%, respectively. Thus, temperatures breaking the cell wall structures depends both wood species, and increase in the alkaline concentration below the breaking temperature is useless for the pretreatment. Thus, a wide range of diversity existed in the genus, *Macaranga* in terms of suitability to alkaline pretreatment for enzymatic saccharification and fermentation. Among the *Macaranga* wood species, *M. winkleri* can be highlighted by its high degradability caused by low concentration of NaOH at low temperature. In the series of *Macaranga* wood, *M. hypoleuca* gave the highest sugar yield 48.6% based on the weight of original wood with 5.0% NaOH at 160ºC. The sugar yield corresponds to 315 mL ethanol/kg of the original wood (Figure 3).

Table 1. Composition of original *Macaranga* wood

Wood species	Chemical component* (%)		
	Lignin	**Holocellulose**	**Cellulose**
M. hypoleuca	27.6 ± 0.2	73.0 ± 0.8	68.8 ± 0.5
M.gigantea	27.6 ± 0.7	71.0 ± 0.3	63.0 ± 0.1
M. motleyana	30.8 ± 0.4	72.2 ± 1.7	65.4 ± 0.3
M. conifera	26.5 ± 0.7	70.7 ± 0.3	61.0 ± 0.7
M. winkleri	28.7 ± 0.9	69.7 ± 0.2	63.2 ± 0.9
M. pearsonii	32.9 ± 1.1	70.7 ± 0.2	67.4 ± 1.1

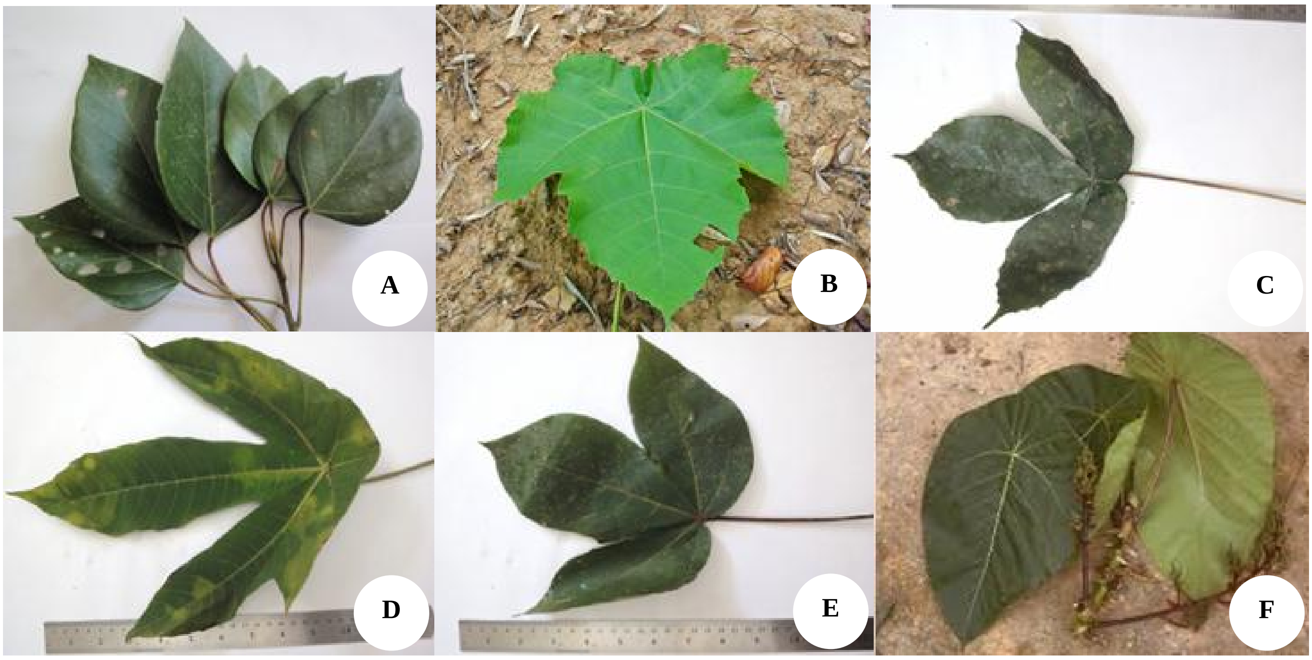

Figure 1. *Macaranga* plant species used in this research. A. *Macaranga conifera,* B. *M. gigantea,* C. *M. hypoleuca,* D. *M. motleyana,* E. *M. pearsonii* and F. *M. winkleri*

Table 2. Pulp yield of *Macaranga* wood pretreated with 3.5 and 5.0% of NaOH at 170°C for 60 min

Wood species	Pulp yield[a] (%)					
	150°C		160°C		170°C	
	3.5% NaOH	5.0% NaOH	3.5% NaOH	5.0% NaOH	3.5% NaOH	5.0% NaOH
M. hypoleuca	76.4[b]	72.1	71.4	53.5	66.0	45.0
M. gigantea	68.5[b]	60.7	61.2	45.4	49.8	41.7
M. motleyana	75.7[b]	66.6	71.3	50.3	56.8	44.5
M. conifera	74.5[b]	69.2	69.7	60.4	65.5	44.5
M. winkleri	69.2[b]	64.0	63.3	54.3	57.1	46.6
M. pearsonii	73.5[b]	71.7	68.1	56.1	56.7	49.5

Note: [a]Pulp yield based on the weight of original wood; [b]Pulp was not fibrillated completely

Table 3. Residual lignin content of *Macaranga* wood pretreated with 3.5% and 5% NaOH at 170°C for 60 min

Wood species	Lignin[a] (%)					
	150°C		160°C		170°C	
	3.5% NaOH	5.0% NaOH	3.5% NaOH	5.0% NaOH	3.5% NaOH	5.0% NaOH
M. hypoleuca	9.6 ± 0.2	9.2 ± 0.1	8.9 ± 0.1	5.7 ± 0.2	8.2 ± 0. 1	4.8 ± 0.1
M. gigantea	8.7 ± 0.3	7.6 ± 0.4	7.9 ± 0.2	5.3 ± 1.1	5.8 ± 0. 1	4.7 ± 0.1
M. motleyana	9.6 ± 0.1	8.7 ± 0.0	8.9 ± 0.1	5.8 ± 0.2	7.4 ± 1.2	5.2 ± 0.3
M. conifera	9.1 ± 0.2	8.6 ± 0. 3	9.1 ± 0.1	7.8 ± 0.1	8.4 ± 0.1	5.2 ± 0.1
M. winkleri	8.7 ± 0.1	7.9 ± 0.1	7.9 ± 0.3	6.2 ± 0.1	6.8 ± 0.0	3.1 ± 0.0
M. pearsonii	9.5 ± 0.1	9.2 ± 0.1	8.9 ± 0.1	7.1 ± 0.2	7.1 ± 0.2	5.4 ± 0.9

Note: [a] Klason lignin was determined based on the weight of original wood

Table 4. Enzymatic saccharification yields of *Macaranga* wood pretreated with 3.5% and 5% NaOH for 60 min

Wood species	Reaction temperature (°C)	Saccharification yield (%)			
		3.5% NaOH		5.0% NaOH	
		Based pulp[a]	Based wood[b]	Based pulp[a]	Based wood[b]
M. hypoleuca	150	27.0 ± 2.0	20.7 ± 4.5	41.8 ± 5.4	30.2 ± 3.0
M. gigantea		22.3 ± 1.7	15.3 ± 0.9	35.5 ± 2.4	21.5 ± 1.1
M. motleyana		16.9 ± 1.7	12.8 ± 2.9	21.9 ± 3.3	14.6 ± 0.9
M. conifera		28.1 ± 1.4	21.0 ± 2.3	58.1 ± 2.4	40.2 ± 3.5
M. winkleri		58.8 ± 0.9	40.4 ± 0.6	68.1 ± 0.7	43.6 ± 0.4
M. pearsonii		18.1 ± 0.9	13.3 ± 0.0	25.6 ± 3.9	18.3 ± 2.5
M. hypoleuca	160	31.1 ± 6.9	22.2 ± 4.4	91.0 ± 0. 7	48.6 ± 0.3
M. gigantea		26.5 ± 3.6	16.3 ± 2.0	83.1 ± 0.3	37.8 ± 0.1
M. motleyana		18.0 ± 6.0	12.8 ± 3.6	90.1 ± 1.7	45.3 ± 0.5
M. conifera		37.1 ± 1.0	25.8 ± 0.6	69.7 ± 5.7	42.1 ± 3.0
M. winkleri		58.4 ± 0.9	40.1 ± 1.6	80.2 ± 0.8	43.6 ± 0.4
M. pearsonii		33.0 ± 1.7	22.4 ± 1.1	54.4 ± 6.5	30.6 ± 3.4
M. hypoleuca	170	50.6 ± 5.0	33.4 ± 3.0	90.9 ± 0.8	40.9 ± 0.3
M. gigantea		53.0 ± 3.3	26.4 ± 1.2	81.4 ± 3.6	34.0 ± 0.2
M. motleyana		41.5 ± 6.3	23.6 ± 3.3	94.0 ± 3.9	41.8 ± 2.7
M. conifera		41.1 ± 1.9	26.9 ± 1.1	99.6 ± 5.1	44.4 ± 2.0
M. winkleri		71.8 ± 0.7	41.0 ± 0.5	96.9 ± 5.3	45.2 ± 2.2
M. pearsonii		51.3 ± 5.5	29.0 ± 3.0	63.3 ± 0.9	31.6 ± 0.4

Note: [a]Weight percentage of the pulp based on the weight of pulp fraction obtained. [b]Weight of pulp based on the weight of original wood

Macaranga hypoleuca was also sensitive to alkaline concentration like *M. motleyana* and it gave only 22.2% sugar yield at the same temperature 160ºC with 3.5% NaOH. The sugar yield is equivalent to 83 mL ethanol/kg of the original wood. Interestingly reducing sugar yield of *M. hypoleuca* decreased from 48.6% to 40.9% when the temperature was raised from 160ºC to 170ºC with 5.0% of NaOH whilst the same temperature increase with 3.5% NaOH increased the sugar yield from 22.2% to 33.4%. Under the high concentration of NaOH at 170ºC, degradation of carbohydrate core structures may become prominent over the range of disintegration effects of the

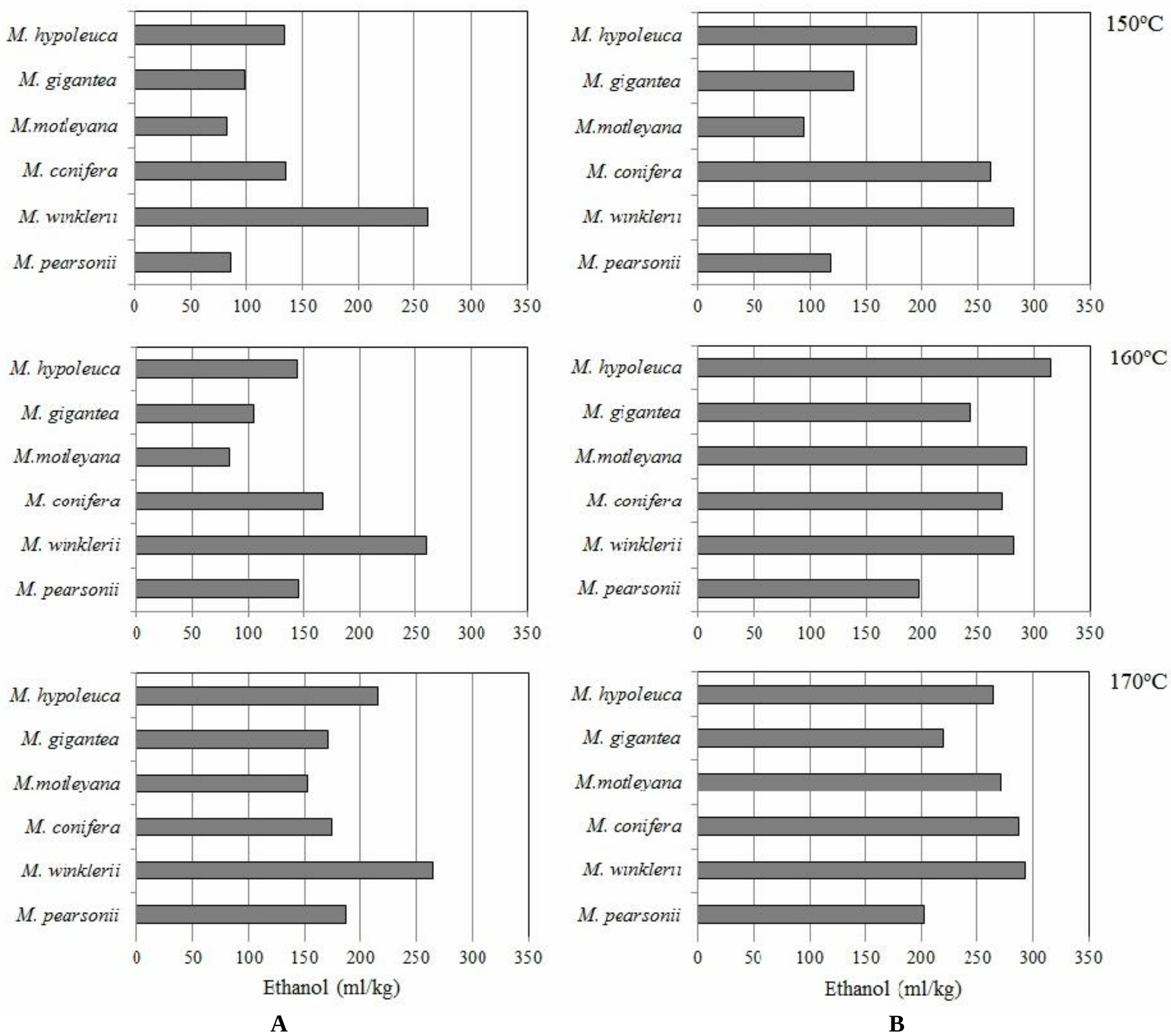

Figure 3. Estimated ethanol yields from *Macaranga* wood pretreated with 3.5% and 5% NaOH at 150^{o}C-170^{o}C for 60 min: A. 3.5% NaOH, and B. 5% NaOH. The values are expressed by the weight of original biomass

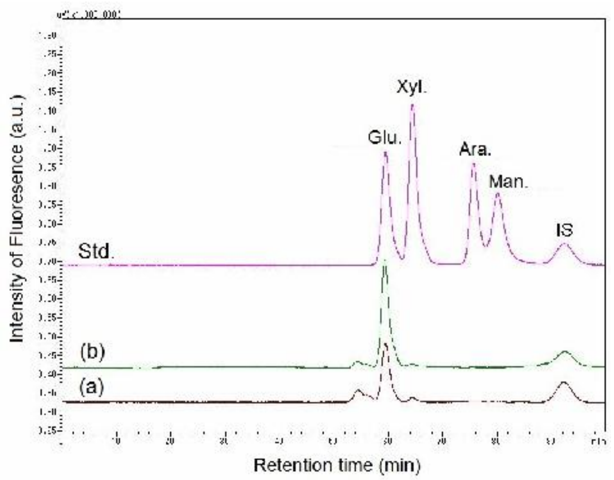

Figure 2. HPLC profile of the saccharified sugar obtained from *M. hypoleuca* by pretreatment with (a) 3.5% and (b) 5.0% of NaOH at 160ºC for 60 min

wood cell walls. This trend was also found for *M. gigantea* and *M. motleyana*. However, the theory was not true for *M. winkleri*, *M. conifera* and *M. pearsonii*. These wood species increased the sugar yield slightly with 5.0% NaOH by increasing the temperature from 160ºC to 170ºC. The effects of disintegration of the cell walls are prominent over the range of decomposition of the carbohydrate cores.

In this study a broad range of differences in adaptability to enzymatic saccharification for ethanol fermentation was found in the genus *Macaranga*. This phenomenon inline with the previous results reported. Biomass properties vary and are commonly associated with plant species (Avelin et al. 2014). *Macaranga hypoleuca* and *M. winkleri* are attractive for their high conversion efficiency and susceptibility to the pretreatment. *Macaranga motleyana* and *M. conifera* are also suitable for the bioethanol production while *M. pearsonii* lacks in the suitability for the conversion. *Macaranga gigantea, M. hypoleuca*, and *M. winkleri* have been used as a secondary firewood species by local people in East and North Kalimantan Provinces, instead of the higher density wood species such as *Vitex pinnata, Nephelium lappacelum, Blumeodendron kurzii* and *Dipterocarpus* sp. (Yuliansyah et al. 2012). The selected fast growing wood species belonging to *Macaranga* are the pioneer woods that usually grow after forest fire or opening area for the shifting cultivation. *Macaranga* was also reported growth sporadically on the gap of forest canopy, open area and degraded land (Slik et al. 2003). Instead of firewood, *Macaranga* was also traditionally used by Dayak people in East Kalimantan as the natural plant indicator to determine the end of the recovery period of forest land after ground fire or shifting cultivation activities. The present study gives a new role in the *Macaranga* as a resource for biorefinery. Design of the sustainable cycle including forest plantation of the *Macaranga* wood and other wood species, and their conversion into fuels and chemicals will activate the local economy in the tropics with concomitant contribution to the global environment.

Finally, our finding suggested that tropical wood biomass *Macaranga*, particularly *M. hypoleuca* is a promising material in term of pulp yield, and sugar production and potentially used as the feedstock or raw material for the lignocellulosic bioethanol production and chemical in the near future. Even, further investigation required to explore and find more attractive tropical fast growing wood plant species that available in the tropical rain forest, particularly in East Kalimantan, and also Indonesia in combination with the more effective pretreatment processes. Last but not least, based on our knowledge this is the first paper that report on the potential saccharified sugar and estimation of ethanol production from *Macaranga* wood biomass as far.

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REFERENCES

Adi DS, Risanto L, Damayanti R, Rullyati S, Dewi LM, Susanti R, Dwianto W, Hermiati E, Watanabe T. 2014. Exploration of unutilized fast growing wood species from secondary forest in Central Kalimantan: Study on the fiber characteristic and wood density. Procedia Environ Sci 20: 321-327.

Amirta R, Tanabe T, Watanabe T, Honda Y, Kuwahara M, Watanabe T. 2006. Methane fermentation of Japanese cedar wood pretreated with a white rot fungus, *Ceriporiopsis subvermispora*. Biotechnology 123: 71-77.

Ang A, Ashaari Z, Bakar ES, Sahri MH. 2009. Enhancing the properties of Mahang (*Macaranga* spp.) wood through acrylic treatment in combination with crosslinker. Mod Appl Sci 11: 2-10.

Alvarez CE, Miranda JL, Castro MR, Verdín GP, Pérez MAR, Hernández IC. 2013. Alkaline pretreatment of Mexican pine residues for bioethanol production. African J Agric 12 (31): 4956-4965.

Avelin A, Skvarila J, Aulin R, Odlare M, Dahlquist E. 2014. Forest biomass for bioenergy production-comparison of different forest species. Energy Procedia 61: 1820-1823.

Bhatt BP, Tomar JMS. 2002. Firewood properties of some Indian mountain tree and shrub species. Biomass Bioenerg 23: 257-260.

Brown L, Torget R. 1996. Chemical Analysis and Testing Task, Laboratory Analytical Procedure, Enzymatic Saccharification Lignocellulosic Biomass. NREL Laboratory Analytical Procedure #009, National Renewable Energy Laboratory, US Department of Energy, Washington DC.

Chiaramonti D, Prussi M, Ferrero S, Oriani L, Ottonello P, Torre P, Cherchi F. 2012. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. Biomass Bioenerg 46: 25-35.

Itoh H, Wada M, Honda Y, Kuwahara M, Watanabe, T. 2003. Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech Wood by ethanolysis and white rot fungi. J Biotechnol 103: 273-280.

Kumar P, Barret DM, Delwiche MJ, Stroeve P. 2009a. Methods for pretreatment of lignocellulosic biomass efficient hydrolysis and biofuel production. Industr Eng Chem Res 48: 3713-3729.

Kumar R, Wyman CE. 2009b. Effects of cellulose and xylanase enzymes on the deconstruction of solids from pre-treatment of poplar by leading technologies. Biotechnol Progr 25: 302-314.

Mirahmadi K, Kabir MM, Jeihanipour A, Karimi K, Taherzadeh MM. 2010. Alkaline pretreatment of Spruce and Birch to improve bioethanol and biogas production. Bioresources 5 (2): 928-938.

Nakamura Y, Godliving M. 2003. Anaerobic fermentation of woody biomass treated by various methods. Biotechnol Bioproc Eng 8: 179-182.

Pio D, D'Cruz R. 2005. WWF: Borneo's Lost World: Newly Discovered Species on Borneo. WWF Indonesia, Jakarta.

Premjet S, Pumira B, Premjet D. 2013. Determining the potential of inedible weed biomass for bio-energy and ethanol production. Bioresources 8: 701-716.

Rahikainen JL, Evans JD, Mikander S, Kalliola A, Puranen T, Tamminen T, Marjamaa K, Kruus K. 2013 Cellulase-lignin interactions—The role of carbohydrate-binding module and pH in non-productive binding. Enz Microb Technol 53: 315-321.

Slik JWF, Keßler PJA, Welzen PCV. 2003. *Macaranga* and *Mallotus* species (Euphorbiaceae) as indicators for disturbance in the mixed lowland dipterocarp forest of East Kalimantan (Indonesia). Ecol Indic 2: 311-324.

Slik JWF. 2005. Assessing tropical lowland forest disturbance using plant morphological and ecological attributes. Forest Ecol. and Manag. 205: 241-250.

Somogyi M. 1952. Notes on sugar determination. J Biol Chem 195: 19-23.

Taherzadeh MJ, Karimi K. 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Intl J Mol Sci 9: 1621-1651.

TAPPI. 1998a. Acid-insoluble lignin in wood and pulp. Technical Association of the Pulp and Paper Industry, T 222 om-98. Technical Association of the Pulp and Paper Industry, New York.

TAPPI. 1988b. Alpha-, beta-and gamma cellulose in pulp and wood. Technical Association of the Pulp and Paper Industry, T 203 om-93. Technical Association of the Pulp and Paper Industry, New York.

Verma P, Watanabe T, Honda Y, Watanabe T. 2010. Microwave-assisted pretreatment of woody biomass with ammonium molybdate activated by H_2O_2. Bioresour Technol 102: 3941-3945.

Wang Z, Keshwani DR, Redding AP, Cheng JJ. 2008. Alkaline pretreatment of coastal Bermuda grass for bioethanol production (Paper No. 084013). Proceedings of the ASABE Annual International Meeting, June 29-July 2, 2008, Providence, RI.

Watanabe T, Watanabe T and Amirta R. 2008. Lignocellulosic Biorefinery for Sustainable Society in Southeast Asia. Proceeding of the 1st Kyoto-LIPI-Southeast Asian Forum, Jakarta.

Webster GL. 1994. Classification of the Euphorbiaceae. Ann Miss Bot Gard 81: 3-32.

Wise LE, Murphy M, D'Addieco AA. 1946. Chlorite holocellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. Paper Trade J 122 (2): 35-43.

Yuliansyah, Kuspradini H, Amirta R, Muladi S. 2012. Characterization and preference analysis of fifteen tropical firewood species in East Kalimantan. Proceeding of the 6th Korea-Thailand-Indonesia Joint Symposium on Biomass Utilization and Renewable Energy, Seoul.

Zhao R, Bean SR, Ioerger BP, Wang D, Boyle DL. 2008. Impact of mashing on sorghum proteins and its relationship to ethanol fermentation. J Agric Food Chem 56 (3): 946-953.

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Evaluation of soybean genotypes for resistance to rust disease (*Phakopsora pachyrhizi*)

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Abstract. *Sulistyo A, Sumartini. 2016. Evaluation of soybean genotypes for resistance to rust disease (*Phakopsora pachyrhizi*). Biodiversitas 17: 124-128.* Pest and disease are one of limiting factor in soybean cultivation in Indonesia. One of the diseases that can reduce soybean production is rust diseases caused by *Phakopsora pachyrhizi*. The use of resistant varieties can reduce yield losses due to this disease. The aim of this study was to evaluate the resistance of soybean genotypes to rust disease and to study the interaction between agronomic traits with rust disease on soybean genotypes. This study was conducted at a screen house of the Indonesian Legumes and Tuber Crops Research Institute (ILETRI) in Malang, Indonesia from April to July 2015. A total of 10 soybean genotypes consist of eight lines and two varieties (Argomulyo and Grobogan) was evaluated for rust resistance to rust disease. The eight lines tested were a progeny of a cross between offspring of IAC 100 (resistant to rust disease) with high yielding soybean varieties (Argomulyo and Grobogan). The experiment was arranged as randomized completely block design with four replications. Each genotype planted in five plastic pots for each replicate. Three weeks after planting, all plants were inoculated with rust disease. Inoculation was done by spraying a suspension of spores (spore density of 10^4 spore mL^{-1}) to the surface of leaves. Observations were carried out on rust disease severity based on the method of International Working Group on Soybean Rust rating system, days to flowering, plant height, number of branches, number of fertile nodes, number of pods, and seed weight per plant. The results showed that all genotypes classified as moderately resistant to rust disease. In this study, days to flowering and plant height influence the development of rust disease severity. There are three lines that have seed weight per plant significantly heavier than Argomulyo (4.97 g) and Grobogan (4.30 g), namely K/I100//B63///G-7 (6.55 g), K/I100//B63///G-8 (6.15 g), and I100/B54//A-5 (5.85 g). The high value of the scales of seed weight per plant for the three lines is supported by high-performance plants with a lot of number of fertile nodes and pods. These three soybean genotypes potentially serve as genetic material to develop high yielding soybean varieties and resistant to rust disease.

Key words: disease severity, *Glycine max*, IWGSR, selection, soybean lines
Abbreviations: DAP (Days after planting), PDL (Position of diseased leaves), DL (Density of lesions/cm^2), DE (Disease existence), TI (Type of infection), RC (Reaction criteria), R (Resistance), MR (Moderately resistant)

INTRODUCTION

Soybean is one of the staple food commodities and a major source of vegetable protein in Indonesia. These commodities have a strategic value which ranks third after rice and maize because every day it is consumed by most people in Indonesia with an average consumption rate of 8.12 kg per year (Sudaryanto and Swastika 2007). Domestic soybean demand will continue to increase in line with the rise of Indonesia population. However, domestic soybean productions are only able to fulfill a small portion (35%) of the needs of soybean, and the remaining is obtained through imports (Direktorat Akabi 2013). The dependence of Indonesia on soybean import will be a serious threat on food security in the future (Supadi 2009). One solution to overcome this problem is to increase domestic soybean production.

One of the obstacles to increase soybean production in Indonesia is an infection of rust disease caused by the fungus, *Phakopsora pachyrhizi*. Yield losses due to this disease range from 28% in Argentina (Formento 2008) and 75% in Brazil (Yorinori et al. 2005). In Indonesia, the rust diseases found in soybean production centers in Sumatra, Java, Bali, West Nusa Tenggara, Kalimantan and Sulawesi (Semangun 2008). According to Huang and Wu (2009), there is no single method that is capable of controlling crop diseases satisfactory, but a combination of effective control measures may enhance protection of crops from diseases as well as reduce production inputs for crops. Sumartini (2010) states that environmentally-friendly control methods of rust disease include planting a soybean resistant variety, application of botanical fungicide made of oil clove, and use of antagonistic bacteria (*Bacillus*) as well as antagonistic fungi (*Verticillium*).

Most soybean crop improvement programs seek to develop rust resistant and high yielding varieties. The first step that must be done is to get the source of resistance genes. According to Tukamuhabwa and Maphosa (2010), recently there are six race-specific genes have been identified, namely Rpp1, Rpp2, Rpp3, Rpp4, Rpp5 and Rpp? (Hyuuga). In addition, there is also recessive gene have been reported to participate in controlling on soybean resistance to rust disease (Calvo et al. 2008).

Research on evaluation of soybean genotypes for resistance to rust disease has been carried out in several programs. One of the activities of screening germplasm in large quantities has been done in the USA by Miles et al. (2006). More than 16,000 soybean accessions of the USDA germplasm collection have been evaluated for resistance to soybean rust disease and acquired approximately 805 accessions that showing a low mean visual severity or the presence of a red-brown reaction. Other researchers have also reported the results of screening of its genetic material in Vietnam (Pham et al. 2009; Pham et al. 2010) and Nigeria (Twizeyimana et al. 2008). The aim of this study was to evaluate the resistance of soybean genotypes to rust disease and to study the interaction between agronomic traits with rust disease on soybean genotypes.

MATERIALS AND METHODS

Plant material and experimental design

A total of ten soybean genotypes, consisting of eight lines and two varieties (Argomulyo and Grobogan) were tested for resistance to rust disease. The eight lines tested were obtained from the cross between the descendants of IAC 100 with high yielding soybean varieties (Argomulyo and Grobogan). Martins and Juliatti (2014) found that IAC 100 has a partial resistance to rust disease. The experiment was conducted at a screen house of Indonesian Legumes and Tuber Crops Research Institute (ILETRI) in Malang, Indonesia from April to July 2015. Each genotype were planted in 20 plastic pots and laid out as a randomized completely block design with four replications, each replicate consist of five plastic pots.

Preparation of spore suspension and inoculation of rust disease

Spore suspension was prepared in the laboratory one day before inoculation. Spore density used was 10^4 mL^{-1}. Infected plants naturally in the field were used as a source of inoculums. Infected leaves were taken to the lab to be incubated at 100% humidity condition for 24 hours. Afterward, the spores were taken using a brush and diluting with distilled water. Furthermore, spore suspension was homogenized by using Tween-20 two drops per liter. Spore suspension obtained was used for inoculation of all the genotypes tested. Inoculation was done on a three-week-old plant by spraying the spore suspension to the surface of the leaves. At three-week-old plant, soybeans have formed 3-4 trifoliolate leaf. According to Pham et al. (2009), the inoculation may be applied at the second or third trifoliolate leaf. Additionally, inoculating on a three-week-old plants aim to ensure that the rust disease symptoms can appear before the plant flowering (R2 phase).

Observations and data analysis

Observations were carried out on rust disease severity, days to flowering, plant height, number of branches, number of fertile nodes, number of pods, and seed weight per plant. The observation of rust disease severity based on the method of International Working Group on Soybean Rust (IWGSR) rating system and carried out at two and three weeks after inoculation. According to Shanmugasundaram (1977) this method uses a system of the three-digit score to categorize soybean resistance against rust disease. The first digit indicates the top position of diseased leaves (1 = bottom third of the canopy, 2 = middle third of the canopy, 3 = upper third of the canopy). The second digit indicates the density of rust lesions on the most diseased leaves (1 = no lesions, 2 = 1-8 lesions/cm^2, 3 = 9-16 lesions/cm^2, 4 = more than 16 lesions/cm^2). The third digit indicates the type of infection (1 = no pustule, 2 = no spores in pustules, 3 = pustules with spore). The data obtained was statistically analyzed using PKBT-STAT 1.0 software, except for rust disease severity. The category rust disease resistance based on a three-digit scoring system shown in Table 1.

RESULTS AND DISCUSSION

Rust disease severity

The results showed that the incubation period of rust disease (time from inoculation until the appearance of disease symptoms) was between 7 to 14 days for all the genotypes tested (data not shown). The reaction of soybean genotypes to rust disease on the first observation showed that all the tested soybean genotypes classified as resistant to rust diseases (Table 2). In the following week, there was an increase reaction to the rust disease that causes a change in resistance reaction of all soybean genotypes. In the second assessment all soybean genotypes categorized as moderately resistant to rust disease (Table 2).

Yield components

Analysis of variance showed that there were significant differences almost for all characters were observed, except number of branches which were not significantly different among genotypes tested (Table 3). The varieties of Argomulyo and Grobogan categorized as early flowering genotypes, 33 days after planting (DAP) for each variety. There was only one soybean line among eight lines which has days to flowering similar to both varieties, namely K/I100//B63///G-7. Two soybean lines, namely K/I100//B63///G-8 and I100/B54//A-5 has the highest performance, with a plant height of each line were 49.50 and 48.25 cm, respectively. The soybean line I100/B54//A-5 also has a lot of number of fertile nodes (16.00), followed by I100/K8//A-1 with the number of fertile nodes as much as 15.50.

Table 1. Relationship between disease reactions and IWGSR ratings for soybean rust

Disease reaction	IWGSR rating
Immune	111
Resistance	122, 123, 132, 133, 222, 223
Moderately resistant	142, 143, 232, 233, 242, 243, 322, 323
Moderately susceptible	332, 333
Susceptible	343

Table 2. Disease reactions and resistance criteria of 10 soybean genotypes to rust diseases

Genotypes	First observation						Second observation					
	PDL	DL	DE	TI	Score	RC	PDL	DL	DE	TI	Score	RC
I100/K8//A-1	1	10	3	2	132	R	2	15	3	3	233	MR
I100/K8//A-2	1	7	2	2	122	R	2	11	3	3	233	MR
I100/K14//G-3	1	10	3	2	132	R	2	12	3	3	233	MR
I100/K14//G-4	1	10	3	2	132	R	2	11	3	3	233	MR
I100/B54//A-5	1	9	3	2	132	R	2	11	3	3	233	MR
I100/B54//A-6	1	7	2	2	122	R	2	11	3	3	233	MR
K/I100//B63///G-7	1	7	2	2	122	R	2	14	3	3	233	MR
K/I100//B63///G-8	1	6	2	2	122	R	2	10	3	3	233	MR
Argomulyo	1	11	3	2	132	R	2	12	3	3	233	MR
Grobogan	1	10	3	2	132	R	2	12	3	3	233	MR

Note: PDL = Position of diseased leaves, DL = Density of lesions/cm^2, DE = Disease existence, TI = Type of infection, RC = Reaction criteria, R = Resistance, MR = Moderately Resistant

Table 3. Yield and its components of 10 soybean genotypes in rust disease inoculated conditions at screen house

Genotypes	Days to flowering (DAP)	Plant height (cm)	Number of branches	Number of fertile nodes	Number of pods	Seed weight per plant (g)
I100/K8//A-1	38.00^{a}	44.50^{abcd}	2.75	15.50^{ab}	33.00^{a}	4.70^{cd}
I100/K8//A-2	38.50^{a}	42.75^{bcde}	2.00	12.00^{cde}	23.50^{bc}	3.88^{d}
I100/K14//G-3	34.75^{b}	39.00^{de}	1.75	11.25^{de}	25.00^{bc}	4.35^{cd}
I100/K14//G-4	34.25^{b}	39.75^{de}	2.25	12.75^{bcde}	24.00^{bc}	4.22^{cd}
I100/B54//A-5	36.75^{ab}	48.25^{ab}	3.00	16.00^{a}	30.75^{ab}	5.85^{ab}
I100/B54//A-6	38.75^{a}	46.25^{abc}	2.75	14.25^{abcd}	27.00^{abc}	4.30^{cd}
K/I100//B63///G-7	33.00^{c}	47.25^{abc}	2.25	14.50^{abc}	32.75^{a}	6.55^{a}
K/I100//B63///G-8	34.50^{b}	49.50^{a}	2.25	14.00^{abcde}	30.50^{ab}	6.15^{a}
Argomulyo	33.00^{c}	38.00^{e}	2.25	11.00^{e}	22.75^{c}	4.97^{bc}
Grobogan	33.00^{c}	42.00^{cde}	2.00	13.75^{abcde}	27.25^{abc}	4.30^{cd}

Note: The numbers in the same column followed by the same letter are not significantly different by LSD at 5%

In the character of yield, there were four soybean lines with number of pods significantly higher than Argomulyo and Grobogan varieties (Table 3). The four soybean lines are I100/K8//A-1 (33.00), I100/B54//A-5 (30.75), K/I100//B63///G-7 (32.75) and K/I100//B63///G-8 (30.50). The last three soybean lines were genotypes with grain yield per plant higher when compared to other genotypes. The soybean line K/I100//B63///G-7 produces seed with a weight of 6.55 g per plant, while K/I100//B63///G-8 and I100/B54//A-5 successively produce seeds with a weight of 6.15 and 5.85 per plant.

Discussion

Rust disease symptoms in this study started to appear since 7 to 14 days after inoculation (data not shown). The incubation period in the present study a little longer when compared with the results of other research in Africa. Twizeyimana et al. (2007) found that in Nigeria it took 5 to 7 days after inoculation to lesion of rust disease appear on the surface of leaves. Meanwhile, Maphosa et al. (2013) reported that the incubation period of rust disease in Uganda began to be seen since 4 to 5 days after inoculation. This means that the isolates of rust fungus from Africa are more virulent compared with isolates from Indonesia, and or soybean genotypes from Indonesia are more resistant than soybean genotypes from Africa.

The differences of incubation period that found in this research caused by the differences in environmental factors as well as genetic differences of the plant material used. The optimal environmental conditions required for spore germination and disease perpetuation, i.e.: high relative humidity of at least 85% (Twizeyimana and Hartman 2010) for 14 hours (Nunkumar et al. 2009), and a temperature between 17 and 28°C (Bonde et al. 2007; Del Ponte and Esker 2008). In this study, the lowest relative humidity that recorded during the research was 56%, and the highest was 81%. While the recorded temperature ranges between 22 and 31°C, the environmental condition like this theoretically can not support the spores of *P. pachyrhizi* to germinate and grow to the maximum.

Although the incubation periods of rust disease in present study longer when compared with the results from Africa, the inoculation of rust disease that has been done is able to bring up the different reactions of soybean genotypes tested. The reaction differences seen in the number of lesions between one genotype to other genotype were observed. Lesions of rust disease that appears varies between genotype and within genotype, ranging from 2 lesions cm^{-2} (in line I100/K14//G-3 and K/I100//B63///G-7) to 22 lesions cm^{-2} (line I100/K8//A-1) on the first observation, and from 4 lesions cm^{-2} (in line I100/B54//A-6, K/I100//B63///G-7 and K/I100//B63///G-8) to 25 lesions

cm^{-2} (line I100/K8//A-1) on the second assessment (data not shown). Differences in the reaction of genotypes tested are also found in other studies (Pham et al. 2009; Twizeyimana et al. 2008). Pham et al. (2010) stated that genotypes with non-characterized genes for resistance may be useful for host plant resistance studies and breeding soybeans for rust resistance.

The reaction of soybean genotypes with resistance against rust diseases showed that all of the genotypes classified as resistant on the first observation while at the second assessment all of the genotypes categorized as moderately resistant. The different resistance reaction between the first observation and second assessment is caused by spores of the rust disease require time to germinate and formed the new spores. According to Yang (2002), after an infection has occurred, it takes 5 to 7 days to produce uredinia by urediniospores and 10 to 20 days to produce a new generation of spores. This difference gives guidance for soybean breeders to determine the appropriate time to conduct the selection. Sulistyo and Sumartini (2015) found that there are differences in heritability of rust disease severity on observation of one, two and three weeks after inoculation.

The emergence of rust diseases on the various phases of the development of soybean will determine how much yield loss will occur. Kumudini et al. (2008) found that if the rust disease began to occur at the R2 growth stage (full flowering phase), it would cause yield losses up to 66-68%, meanwhile, when it started at the R5 growth stages (seed filling phase), it will cause yield losses reach 35-39%. In this research, a soybean genotype with early flowering can avoid a large yield loss. The mechanism was shown by line K/I100//B63///G-7 and K/I100//B63///G-8. Both of these soybean lines flowering at 33 and 34 DAP (respectively), had the highest seed yield per plant (6.55 and 6.15 g, respectively) compared with other lines. In contrast, the line I100/K8//A-1, I100/K8//A-2 and I100/B54//A-6 were flowering at about 38 DAP, had a weight of seeds per plant (4.70, 3.88 and 4.30 g, respectively) were significantly lower than the two previous line.

Plant height in this study appears to be one of the factors that will determine differences in the severity of rust disease on soybean genotypes tested. Correlation analysis showed that there is a significant negative correlation ($r = -0.753$, $P < 0.05$) and negative correlation ($r = -0.077$, $P > 0.05$) between plant height with the number of rust lesions in the first and second observation, respectively. It means that the higher a plant, then the fewer rust disease lesions. This is not surprising because *P. pachyrhizi* does not have an active mechanism for spreading the spores. According to Isard et al. (2005), wind seems to be critical factors for spread out spores and lifting them out of the canopy. Thus, it takes quite much wind to spread the spores of rust on soybean genotypes with appearances tall plants.

Rust disease in present research did not seem to affect the character of other yield components, such as the number of branches, the number of fertile nodes and the number of pods. However, the three characters have an influence on seed yield per plant. According to Oz et al. (2009) number of pods per plant had significant correlations with seed yield and gave direct positive effect. Valencia-Ramirez and Ligarreto-Moreno (2012) found a similar result with the addition character i.e. the number of nodes per plant. Malik et al. (2007) suggested that number of pods can be considered as selection criteria in improving the bean yield of soybean genotypes.

The evaluation of resistance of soybean genotypes against rust disease in this study showed that no soybean genotype classified as immune or resistant genotype to rust disease. The whole genotypes tested were categorized as moderately resistant genotype. Days to flowering and plant height influence the development of rust disease severity. Three of the eight lines produce seeds with the seed weight per plant heavier than Argomulyo and Grobogan varieties, namely K/I100//B63///G-7, K/I100//B63///G-8 and I100/B54//A-5. Characteristics of the three genotypes, among other the performance of plants is high with a lot of number of fertile nodes and number of pods.

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REFERENCES

Bonde MR, Berner DK, Nester SE, Frederick RD. 2007. Effects of temperature on urediniospore germination, germ tube growth, and initiation of infection in soybean by *Phakopsora* isolates. Phytopathol 97 (8): 997-1003

Calvo ES, Kiihl RAS, Garcia A, Harada A, Hiromoto DM. 2008. Two major recessive genes conferring soybean rust resistance. Crop Sci 48 (4): 1350-1354.

Del Ponte EM, Esker PD. 2008. Meteorological factors and asian soybean rust epidemics-a systems approach and implications for risk assessment. Sci Agric 65: 88-97.

Direktorat Akabi. 2013. Soybean production met only 35%. http://tanamanpangan.pertanian.go.id/akabi/berita-142-produksi-kedelai-baru-terpenuhi-35-persen.html [Indonesian]

Formento AN. 2008. Epidemiology of Asian soybean rust (*Phakopsora pachyrhizi*), range of hosts and management in the Pampa region of Argentina. In: Suenaga K, Kudo H, Oshio S (Eds). Comprehensive studies on the development of sustainable soybean production technology in South America. Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan, Working Report No. 51.

Huang HC, Wu MT. 2009. Plant disease management in the era of energy conservation. Plant Pathol Bull 18: 1-12.

Isard SA, Gage SH, Comtois P, Russo JM. 2005. Principles of the atmospheric pathway for invasive species applied to soybean rust. Bio Sci 55 (10): 851-861.

Kumudini S, Godoy CV, Board JE, Omielan J, Tollenaar M. 2008. Mechanisms involved in soybean rust-induced yield reduction. Crop Sci 48: 2334-2342.

Malik MFA, Ashraf M, Qureshi AS, Ghafoor A. 2007. Assessment of genetic variability, correlation and path analyses for yield and its components in soybean. Pak J Bot 39 (2): 405-413

Maphosa M, Talwan H, Tukamuhabwa P. 2013. Assessment of comparative virulence and resistance in soybean using field isolates of soybean rust. J Agric Sci 5 (5): 249-257.

Martins JAS, Juliatti FC. 2014. Genetic control of partial resistance to Asian soybean rust. Acta Sci Agron 36 (1): 11-17.

Miles MR, Frederick RD, Hartman GL. 2006. Evaluation of soybean germplasm for resistance to *Phakopsora pachyrhizi*. Plant Health Progress 10. DOI: 10.1094/PHP-2006-0104-01-RS.
Nunkumar A, Caldwell PM, Pretorius ZA. 2009. Development of *Phakopsora pachyrhizi* on soybean at controlled temperature, relative humidity and moisture periods. S A J Plant Soil 26 (4): 225-230.
Oz M, Karasu A, Goksoy AT, Turan ZM. 2009. Interrelationship of agronomical characteristic in soybean (*Glycine max*) grown in different environments. Intr J Agric Biol 11: 85-88.
Pham TA, Hill CB, Miles MR, Nguyen BT, Vu TT, Vuong TD, VanToai TT, Nguyen HT, Hartman GL. 2010. Evaluation of soybean for resistance to soybean rust in Vietnam. Field Crop Res 117: 131-138.
Pham TA, Miles MR, Frederick RD, Hill CB, Hartman GL. 2009. Differential responses of resistant soybean entries to isolates of *Phakopsora pachyrhizi*. Plant Dis 93 (3): 224-228.
Semangun H. 2008. Crops Diseases in Indonesia. Gadjah Mada University Press, Yogyakarta. [Indonesian]
Shanmugasundaram. 1977. The International Working Group on Soybean Rust and Its Proposed Soybean Rust Rating System. Workshop on Rust of Soybean. The Problem and Research Needs. Manila, Philippines. 28 Feb-4 March 1977.
Sudaryanto T, Swastika DKS. 2007. Economic soybean in Indonesia. Forum Agro Ekonomi 12 (3): 1-27. [Indonesian]
Sulistyo A, Sumartini. 2015. Heritability of rust disease resistance in F_3-F_4 soybean populations using variance component analysis and parent-offspring regression method. Poster no. 2 presented at The 13th SABRAO Congress and International Conference, 14-16 September 2015, Bogor, Indonesia
Sumartini. 2010. Soybean rust disease and how to control environmentally friendly. J Litbang Pertanian 29 (3): 107-112. [Indonesian]
Supadi. 2009. The impact of sustainable soybean imports to food security. Analisis Kebijakan Pertanian 7 (1): 87-102. [Indonesian]
Tukamuhabwa P, Marphosa M. 2010. State of Knowledge on Breeding for Durable Resistance to Soybean Rust Disease in the Developing World. FAO, Rome.
Twizeyimana M, Hartman GL. 2010. Culturing *Phakopsora pachyrhizi* on detached leaves and urediniospore survival at different temperatures and relative humidities. Plant Dis 94 (12): 1453-1460.
Twizeyimana M, Ojiambo PS, Ikotun T, Ladipo JL, Hartman GL, Bandyopadhyay R. 2008. Evaluation of soybean germplasm for resistance to soybean rust (*Phakopsora pachyrhizi*) in Nigeria. Plant Dis 92 (6): 947-952.
Twizeyimana M, Ojiambo PS, Ikotun T, Paul C, Hartman GL, Bandyopadhyay R. 2007. Comparison of field, greenhouse, and detached-leaf evaluation of soybean germplasm for resistance to *Phakopsora pachyrhizi*. Plant Dis 91 (9): 1161-1169.
Valencia-Ramirez RA, Ligarreto-Moreno GA. 2012. Phenotypic correlation and path analysis for yield in soybean (*Glycine max* (L.) Merril). Acta Agron 61 (4): 322-332.
Yang XB. 2002. Soybean rust: Epidemiology and Management. In Anais do II Congresso Brasileiro de Soja e Mercosoja 2002. Foz do Iguazú. PR. Brasil
Yorinori JT, Paiva WM, Frederick RD, Costamilan LM, Bertagnolli PF, Hartman GE, Godoy CV, Nunes J Jr. 2005. Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001 to 2003. Plant Dis 89 (6): 675-677.

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Cowpea Mild Mottle Virus (CpMMV) infection and its effect to performance of South Korean soybean varieties

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Abstract. *Sutrisno, Kuswantoro H. 2016. Cowpea Mild Mottle Virus (CpMMV) infection and its effect to performance of South Korean soybean varieties. Biodiversitas 17: 129-133.* Cowpea Mild Mottle Virus (CpMMV) is a very detrimental disease to soybean. This virus infection can cause yield reduction up to 56%. Introduction varieties can be served as alternative material in breeding program to develop resistant variety. This study aimed to evaluate the response of introduction soybean varieties from South Korea to the CpMMV infection. Ten South Korean soybean varieties and two check Indonesian soybean varieties were grown at ILETRI from August to November 2012. CpMMV infection was carried out by natural infection using whitefly (*Bemisia tabaci*) transmission. The results showed that the South Korean variety of Daemang-2 had lower leave malformation level than two Indonesian varieties. Varieties of Cheongja-3, Daemang, Daehwang, Daewon, Danweon, Geomjeong-3 Geonjeongsaeol produced grain yield that equal to Anjasmoro variety. Daepung was the most resistant to CpMMV than other varieties, but having low grain yield. Daemang-2 was the most tolerant to CpMMV, because it still able to produce high grain yield although showing high leaf infection. These two varieties could be used as gene sources for resistant/tolerant to CpMMV in soybean breeding program.

Keywords: Agronomic performance, *Bemisia tabaci*, CpMMV, Korean soybean, yield potential

INTRODUCTION

As long as last decade, demand of soybean in Indonesia increases but national production decreases every year. For instance, in 2005, 2010, and 2015, soybean production 808.353, 907.031, and 982.967 ton whereas consumption level achieved 1.89, 2.65, and 2.6 million ton. As a consequence, Indonesian government had to import as much as 1.08, 1.74, and 1.6 million ton to fulfill this demand (BPS 2015; Faostat 2015). There are many aspects that cause low soybean production in Indonesia. One of the aspects is the limiting soybean production by pest and disease. The high level of whitefly (*Bemisia tabaci*) population is a major constraint in soybean cultivation (Marwoto et al. 2011). This pest is a viral vector of Cowpea Mild Mottle Virus (CpMMV) that attacks the leaf tissue (Laguna et al. 2006). The infected leaves gradually become malformations which ultimately inhibits the growth and yield. The impact of the damage caused by these pests can reduce yields up to 11-56% (Akin 2003).

Various attempts to obtain whitefly resistant variety have be done by exploration, selection of local varieties, crossing (Barmawi et al. 2012), as well as the introduction of new varieties from other country. Introduction of new varieties is often done to increase the diversity of genetic resources. The present of the wide genetic resources diversity is expected to be able to improve varieties for high yielding or pests and diseases resistances. To broaden the diversity of genetic resources, we can introduce soybean germplasm from areas with rich in soybean landraces and wild relative such as China, Japan and Korea.

Adaptation of a plant in new habitat is very important. Therefore, adaptation test is intended to determine plant characteristics expressed in the new area. The performance of the plants generally changes when grown in the new area (Li et al. 2014). If an introduced variety can grow and maintain its characters well, this introduced variety can be used as a new materials for further breeding program based on the superior traits of the variety such as high yielding, large grain size, and pest and disease resistance.

Adaptation test of South Korea introduced varieties that performed in 2012 showed that those varieties have a shorter plant height and lower grain yield than the Indonesian varieties (Kuswantoro et al. 2014). However, the introduction varieties have larger grain size than the Indonesian varieties. This larger grain size trait is expected to be superior traits that can be inherited through a crossing and allows plant genetic improvement for high yielding through large grain size improvement. In addition, other characters such as a resistance to pest and disease are also needed. Some studies reported that whitefly infestation by transmission virus CpMMV causes many types of physiological obstacles such as photosynthesis and enzyme activity inhibition (Zhang and Wen 2008) decreasing chlorophyll leaf index, plant height, number of branches (Jindal et al. 2009), number of pods, (Barmawi et al. 2012), grain size, grain yield (Akin 2003), and acceleration of defoliation (senescence) (Jindal et al. 2009). These physiological obstructions will greatly affect the quality of the grain yield of the genotype. However each genotype has a different response or resistance level to the whitefly infestation or CpMMV infection. It leads the pest and or disease resistance level of each variety should be tested. This study aimed to evaluate the response of introduction soybean varieties from South Korea to the CpMMV infection.

MATERIALS AND METHODS

The experiment was conducted in Indonesian Legume and Tuber Crops Research Institute (ILETRI), Malang, East Java, Indonesia, since August until November 2012. The tested varieties consist of ten soybean varieties that were introduced from South Korea, i.e. Cheongdu-1, Cheongja-3, Daehwang, Daemang, Daemang-2, Daepung, Daewon, Danweon, Geomjeong-3, Geonjeongsaeol, and two Indonesian soybean varieties i.e. Detam-1 and Anjasmoro). The design was arranged in Randomized Completely Block Design (RCBD) with three replications. Each experimental unit consisted of three polybag with two plants per polybag. Plants were fertilized using NPK fertilizer (16-16-16) at a dose of 2 g/polybag that was applied at seven days after planting (DAP). Watering was given every three days with one liter of water for each polybag. Natural whitefly infestation since 21 DAP until maturing age was used for CpMMV resistance level test. Population of whitefly was controlled with pesticide in order to keep the population between 50 - 100 individual per clump. Plant were harvested when the leaves were fallen, pods color start to be brown, and the pods were dry. Growth component were observed on leaf diseased score, flowering age, harvested age, plant height, number of branches, number of reproductive nodes, pod length, pod width, number of filled pods, number of empty pods, number of grains per plant, percentage of abnormal grains, 100 grains weight and grain weight per plant. The level of infected leaf score was calculated by the following method (Zubaidah et al. 2010):

$$I = \frac{\sum (n.v)}{N.Z} \; x\, 100$$

Where:
I = the infestation intensity per plant (%)
n = number of infected leaves on certain score
v = score category at certain leaf
N = number of observed leaves per plant
Z = the highest score category

RESULTS AND DISCUSSION

The results showed that preference levels of whitefly against all tested varieties that indicated by a leaves score were quite diverse. Performance of agronomic characters and infected level by CpMMV were different among all tested varieties. The level of susceptibility of varieties maybe caused by genetic different.

Flowering ages of introduction varieties were shorter than Indonesian varieties ranging from 4 to 6 DAP. Four introduction varieties, i.e. Cheongja-3, Daehwang, Daemang-2, and Geonjeongsaeol, had equivalent flowering age to variety of Detam-1, while other introduction varieties had longer flowering age. Anjasmoro had the longest flowering age 33 DAP (Table 1). Flowering age of introduction varieties of this study was generally shorter than flowering age in the previous study that was not infested by whitefly. Flowering age in this study was shorter one to two days than previous study except Geonjeongsaeol that had four days longer (Kuswantoro et al. 2014), This fact indicates that whitefly infestation could affect flowering time. Infected of CpMMV through whitefly caused metabolism disturbance, and finally encourage plant to faster flowering. This stress basically effect availability and activity of gibberellin hormone (Hamayun et al. 2010) or gibberellin, abscisic acid, and brassinosteroids (Domagalska et al. 2010).

Table 1. Agronomical characters of South Korean introduced soybean varieties that infected by CpMMV, Malang, East Java, Indonesia 2012

Varieties	Flowering age (DAP)	Harvesting age (DAP)	Plant height (cm)	Number of branch	Number of reproductive nodes	Number of filled pods*)	Number of unfilled pods**)
Anjasmoro	33.2 [a]	91.8 [a]	57.3 [a]	4.4 [ab]	20.3 [ab]	6.4 [ab]	1.8 [a]
Detam-1	31.9 [ab]	83.2 [a-c]	55.8 [a]	6.2 [a]	26.9 [a]	6.9 [a]	1.0 [b]
Cheongdu-1	27.0 [c]	74.3 [cd]	23.3 [bc]	3.0 [b]	8.3 [c]	3.6 [de]	1.2 [b]
Cheongja-3	28.1 [bc]	77.0 [b-d]	30.8 [b]	4.8 [ab]	9.0 [c]	4.0 [de]	1.3 [ab]
Daehwang	29.4 [a-c]	73.6 [cd]	26.5 [bc]	4.5 [ab]	9.8 [c]	3.5 [e]	1.2 [b]
Daemang	27.2 [c]	77.5 [b-d]	18.6 [c]	4.8 [ab]	9.7 [c]	4.1 [de]	1.2 [b]
Daemang-2	28.5 [bc]	85.3 [ab]	24.4 [bc]	3.8 [b]	12.9 [bc]	5.4 [bc]	1.3 [ab]
Daepung	25.6 [c]	70.4 [d]	22.0 [bc]	2.8 [b]	9.9 [c]	4.3 [c-e]	1.3 [ab]
Daewon	25.9 [c]	77.3 [b-d]	26.3 [bc]	3.5 [b]	9.5 [c]	4.1 [de]	0.6 [b]
Danweon	27.1 [c]	72.7 [cd]	20.4 [bc]	4.1 [b]	14.7 [bc]	4.8 [cd]	1.3 [ab]
Geomjeong-3	27.7 [c]	72.6 [cd]	24.7 [bc]	4.4 [ab]	11.3 [c]	3.8 [de]	1.3 [ab]
Geonjeongsaeol	29.0 [bc]	73.6 [cd]	21.1 [bc]	4.6 [ab]	10.6 [c]	4.2 [c-e]	1.1 [b]
CV	4.63	4.61	13.6	16.4	19.8	8.92	13.1

Note: *) 1× sqrt transformation; **) 2× sqrt transformation. Values followed by the same letter in the same column do not different according to LSD α 0.05

Table 2. Infected leaves score and agronomical characters of South Korean introduced soybean varieties, Malang, East Java, Indonesia, 2012

Varietas	Infected leaf score (%)	Pod length (cm)	Pod width (cm)	Number of seed per plant	Weight of 100 seeds	Abnormal seed (%)	Grain yield per plant (g)
Anjasmoro	50.8	3.7 [a]	0.9 [a]	77.2 [ab]	10.7 [c]	50.2	8.2 [bc]
Detam-1	47.3	3.9 [a]	0.9 [a]	103.7 [a]	12.2 [c]	4.3	12.2 [a]
Cheongdu-1	30.0	4.5 [a]	1.0 [a]	26.6 [ef]	15.3 [bc]	22.1	3.9 [d]
Cheongja-3	52.2	4.3 [a]	1.2 [a]	22.1 [f]	23.9 [a]	32.0	5.5 [cd]
Daehwang	40.3	4.4 [a]	1.2 [a]	22.4 [f]	25.9 [a]	36.7	6.5 [b-d]
Daemang	44.8	4.3 [a]	1.1 [a]	29.3 [d-f]	19.9 [ab]	16.6	5.3 [cd]
Daemang-2	51.5	4.0 [a]	1.0 [a]	56.4 [bc]	19.8 [ab]	17.5	9.4 [ab]
Daepung	22.2	3.9 [a]	0.9 [a]	43.8 [c-e]	11.3 [c]	6.7	4.1 [d]
Daewon	43.3	3.8 [a]	1.3 [a]	28.2 [d-f]	23.8 [a]	14.7	6.1 [b-d]
Danweon	33.1	4.2 [a]	1.0 [a]	48.8 [b-d]	14.5 [bc]	8.3	7.1 [b-d]
Geomjeong-3	46.5	4.3 [a]	1.2 [a]	24.7 [ef]	23.2 [a]	28.4	6.5 [b-d]
Geonjeongsaeol	43.3	3.9 [a]	1.2 [a]	28.7 [d-f]	23.5 [a]	13.2	6.7 [b-d]
CV	50.8	6.61	14.7	9.58	13.1	20.7	19

Note: Value that followed by the same letter did not different according to LSD α 0.05

Harvesting ages of introduction varieties were shorter than Indonesian varieties except variety of Daemang-2. Harvesting age of introduction varieties ranged from 72 to 85 DAP, while Indonesian varieties ranged between 83-92 DAP. Anjasmoro variety had the longest generative phase compared to other tested varieties, followed by Daemang-2 and Detam-1 with 58, 57, and 49 days respectively (Table 1). Daemang-2 that had long generative phase (since flowering until harvesting time) also had longer and maximum pod filling phase. Longer flowering time caused longer harvesting age. Hakim (2012) stated that the lowering date is closely linked to harvest age. Harvesting age in this study seems to be shorter than previous study with harvesting age of 74-84 DAP (Kuswantoro et al. 2014). For example, varieties of Cheongja-3 and Daemang with the most severe infection had harvesting time 5 days shorter than when they were grown in normal condition without whitefly infection, namely 83 and 82 DAP. The level of whitefly infestation that affects plant growth and development may be influence the speed up of harvesting age. Zhang (2008) concluded that offensive of *Bemisia tabaci* inhibits antioxidant enzymes activities that finally increase senescence or defoliation and accelerates plant death.

Plant height of introduction varieties was lower than Indonesian varieties. In general, the Indonesian varieties had double plant height than introduction varieties. The highest plant height of introduction varieties was achieved by Cheongja-3, while the lowest was achieved by Daemang i.e. 30 and 18 cm respectively (Table 1). Infection of CpMMV caused abnormality in plant growth lead hampering plant growth. Other studies stated that virus which spread by whitefly causes lower plant height (Jindal et al. 2009). Some varieties extremely decreased plant height, while others slightly decreased. For instance, variety of Cheongja-3 decreased 1 cm while Daemang decreased 3 cm. The declining of plant height is not always in line with the level of CpMMV infection, but also by genetic factor. For example on the similar leaves score and number of abnormal grain, the declining plant height of the tested genotypes was not similar.

Branches numbers of several introduction varieties were equivalent to Anjasmoro variety but lower than Detam-1 variety. The number of branches in introduction varieties was quite a lot compared to Indonesian varieties despite having shorter plant (Table 1). A large number of branches likely due to stunted plant growth so that vegetative growth directed to establishment of branches. Infestation of CpMMV detained plant growth but likely did not directly affected number of branches. This evidenced with number of branches in this study were equal to varieties when grown normally (Kuswantoro et al. 2014). However, other study stated that whitefly infection diminished branches number (Jindal et al. 2009). The difference occur, probably due to each genotype has different response to whitefly and CpMMV infestations. In this research, number of branches was closely linked to performance of the plant height (Hakim 2012).

The numbers of reproductive nodes of Indonesian varieties were double than the introduction varieties. Reproductive nodes number of introduction varieties of Danweon, Daemang-2, and Geomjeong-3 were 14.7, 12.9, and 11.3 respectively (Table 1). Number of reproductive nodes seemed very affected by plant height and number of branches. Varieties that had higher plant height and more branches tend to generate more reproductive node number. Judge (2012) stated that plant height and branch positively associated with the number of nodes. Higher infection of CpMMV may reduce the number of nodes that produce pods. For instance, varieties of Cheongja-3 and Daemang which looked susceptible to CpMMV infection produced fewer amount of reproductive nodes than Daemang-2. Compared to previous study, reduction of reproductive node number of Cheongja-3 and Daemang was higher than Daemang-2 where it was more resistant to CpMMV infection and had no decreasing reproductive nodes number (Kuswantoro et al. 2014).

Infection of CpMMV diminished both establishment of filled pods and unfilled pods. The moderate resistance variety to whitefly, Daemang-2, produced more number of pods than other introduction varieties. Performance of pods number seems more diverse than previous study (Kuswantoro 2014). This may be affected by plant response to CpMMV. Barmawi et al. (2012) suggested that CpMMV infection that transmitted by whitefly decrease the number of pods. Number of pods of Indonesian varieties seemed more than introduction varieties. Both of Daemang-2 and Danweon varieties produced the most pods than other introduction varieties.

Pods length and width or pods size of all tested varieties did not different but the number of grains, 100 grains weight and grain yield were different on each variety. The amount of grains of Indonesian varieties was higher than introduction varieties. Danweon and Daemang-2 varieties achieved the highest number of seed among tested introduction varieties. Number of grains in each of varieties affected by plant performance i.e. plant height, number of branches, and number of nodes. For example, Daemang-2 variety that has higher plant height, more number of productive nodes and number of filled pods had more number of seeds than others introduction varieties (Table 2). Similar finding also stated by Sarutayophat (2011) and Judge (2012) that the number of grains was positively correlated with its vegetative performance.

Introduction varieties achieved 100 grains weight or grain size larger than Indonesian varieties. Daehwang produced the largest grain size, followed by Choengja-3, Daewon, Geomjeong-3, Geonjeongsaeol, Daemang, and Daemang-2 varieties respectively. Daepung, Cheongdu-1 and Danweon varieties produced the smallest grain size and their sizes were similar to the Indonesian varieties (Table 2). Generally, level of CpMMV infection reduced weight of 100 seeds with varying in decline level. Infection of CpMMV causes photosynthesis rate and photosynthate distribution into seed is hindered and lead smaller grain size. According to Zhang and Wen (2008), infestation of whitefly that transmitted by CpMMV inhibits rate of photosynthesis and activity of antioxidant enzymes. Jindal et al. (2009) suggested that whitefly transmission decreased leaf chlorophyll index. That conclusion is equal to this finding that weight of 100 grains in Daepung and Daehwang only 10 to 25 g respectively whereas in the previous study that grown without whitefly infection reached 19.10 and 33.99 g. In this fact, whitefly infection caused decreasing yield until 33%.

Infection of CpMMV caused different leaf infection. Two Korean varieties i.e. Cheongja-3 and Daemang-2 had higher infected leaf percentage than Anjasmoro and Detam1 whereas other seven Korean varieties had lower infected leaf than Indonesian varieties. Cheongja-3 and Daemang-2 had the highest infection among all tested Korean varieties. However, Daepung had the lowest damage (Table 2). The level of leaf damage was likely affected by thickness and leaf trichomes. Other studies reported that density, length, and stiffness of trichomes affect whitefly preference to infect plant leaves (Taggar and Gill 2012).

CpMMV infection increased percentage of abnormal grain. Anjasmoro varieties seemed the most susceptible while Detam-1 showed the most resistant to CpMMV infection. Cheongja-3 and Daehwang had the highest percentage of grain damage among all Korean varieties while Daepung and Danweon had the lowest abnormal grain percentage. Difference in this abnormality is due to the different resistance of the varieties through the mechanism of metabolic, genetic, physic, as well as interaction among those factors. The level of susceptibility of introduction varieties to disease is caused by environmental factors that different with their original region. Introduction varieties have to adjust with two environmental stresses i.e. abiotic (environmental) and biotic (pest) stresses simultaneously (Mbeyagala et al. 2014).

Infection of CpMMV virus decreased crop yield with different level. The same result also was stated by Akin (2003) and Barmawi (2012) that CpMMV virus infection decrease soybean grain yields. Some varieties having different susceptibility could have similar yield reduction, while varieties with similar susceptibility showed similar yield reduction. Cheongdu-1 and Daemang-2, for example, were equivalent in decreasing yield although they had different susceptibility, whereas Daewon, Danweon, Geomjeong-3, and Geonjeongsaeol that had same susceptibility had different yield. Daewon was the most decreased varieties than their yield when grown in normal condition without CpMMV stressed (Kuswantoro et al. 2014). Grain yield of Indonesian varieties were higher than introduction varieties. However, Daemang-2, the introduction varieties, produced grain yield higher than Anjasmoro but lower than Detam-1. Some of introduction varieties such as Daewon, Danweon, Geomjeong-3, Geonjeongsaeol, and Daehwang had grain yield equivalent to Anjasmoro.

In conclusion, agronomical performance of South Korean introduction varieties that infected by CpMMV seemed to be lower than Indonesian varieties. Infection of CpMMV hampered plant height growth, decreased number of filled pods and grain yield, increased percentage of infected grain, and accelerating harvesting time. All Korean varieties were more resistant than Anjasmoro variety but more susceptible than Detam-1. Variety of Daepung having the lowest infection indicated that this variety was more resistant than other varieties. In the other hand, Daemang-2 seemed to be more tolerant to CpMMV, because although it had high infected leaf score but it was able to produce high grain yield. Therefore, Daepung and Daemang-2 could be used as gene sources in soybean breeding program for resistant/tolerant to CpMMV.

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REFFERENCES

Akin HM. 2003. Response of various soybean genotipes against Cowpea Mild Mottle Virus (CpMMV) infection. J Hama dan Penyakit Tumbuhan Tropika 3: 40-42. [Indonesian]

Barmawi M, Utomo SD, Akin HM, Ramli S. 2009. Evaluation of the resistance to Cowpea Mild Mottle Virus of 19 f1 population of soybean developed by dialel crosses. J Agrotropika 14: 81-85 [Indonesian]

BPS. 2015. Soybean Production year of 1993-2005. Central Bureau of Statistics, Jakarta. [Indonesian]

Cooper RL. 2003. A delayed flowering barrier to higher soybean yields. Field Crops Res 82: 27-35.

Domagalska MA, Sarnowska E, Nagy F, Davis SJ. 2010. Genetic analyses of interactions among gibberellin, abscisic acid, and brassinosteroids in the control of flowering time in *Arabidopsis thaliana*. PloS One 5: 14012-14012.

Faostat. 2015. Import soybean every year by Indonesia. faostat.fao.org /import/commodity by country. [2nd February 2015].

Hakim L. 2012. Yield components and morphological characters determining grain yield of soybean. J Penelitian Pertanian Tanaman Pangan 31: 173-179. [Indonesian]

Hamayun M, Khan SA, Khan AL, Shinwari ZK, Iqbal I, Sohn EY, Lee IJ. 2010. Effect of salt stress on growth attributes and endogenous growth hormones of soybean cultivar Hwangkeumkong. Pak J Bot 42: 3103-3112.

Hamayun M, Khan SA, Shinwari ZK, Khan AL, Ahmad N, Lee IJ. 2010. Effect of polyethylene glycol induced drought stress on physio-hormonal attributes of soybean. Pak J Bot 42: 977-986.

Jindal V, Dhaliwal GS, Dhawan AK, Dilawari VK. 2009. Mechanisms of resistance in cotton to whitefly (*Bemisia tabaci*): Tolerance. Phytoparasitica 37: 249-254.

Kuswantoro H, Sutrisno, Supeno A, Han WY, Lee PY, Cho YH, Baek IY. 2014. Performance of korean soybean varieties in Indonesia. Korean Soc Intl Agric J 26: 107-113

Laguna IG, Arneodo JD, Pardina PR, Fiorona M. 2006. Cowpea Mild Mottle Virus infecting soybean crops in Northwestern Argentina. Fitopatologia Brasileira 31: 317-317.

Li Q, Hu Y, Chen F, Wang J, Liu Z, Zhao Z. 2014. Environmental controls on cultivated soybean phenotypic traits across China. Agric Ecosyst Environ 192: 12-18.

Marwoto, Indriani FC, Sulistyo A. Hapsari RT. 2011. Diagnosis of whitefly *Bemissia tabaci* outbreaks in soybean plantation. (Case study of factors causing whitefly outbreaks in soybean in Muneng, Probolinggo). In: Wdjono A, Hermanto, Adie MM, Prayogo Y, Suharsono, Sholihin, Rahmiana AA, Nugrahaeni N, Saleh N, Kasno A, Subandi, Marwoto (eds). Proceeding of National Seminary on Biodiversity of Legumes and Tuber Crops. Indonesian Legumes and Tuber Crops Research Institute. Malang, East Java, 21st December 2009. [Indonesian]

Mbeyagala EK, Mukasa BS, Tukamuhabwa P, Bisikwa J. 2014. Evaluation of cowpea genotypes for virus resistance under natural conditions in Uganda. J Agric Sci 6: 176-187.

Sarutayophat T. 2011. Correlation and path coefficient analysis for yield and its components in vegetable soybean. Songklanakarin J Sci. Technol 34: 273-277.

Taggar GK, Gill RS. 2012. Preference of whitefly, *Bemisia tabaci*, towards black gram genotypes: Role of morphological leaf characteristics. Phytoparasitica 40: 461-474.

Zhang LL, Wen DZ. 2008. Photosynthesis, chlorophyll fluorescence, and antioxidant enzyme responses of invasive weed *Mikania micrantha* to *Bemisia tabaci* infestation. Photosynthetica 46: 457-462.

Zubaidah S, Kuswantoro H, Corebima AD, Saleh N. 2010. Developing method for determining the soybean resistance to CpMMV based foliar simptoms any recovery. Berk Penel Hayati 4: 47-52.

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Screening of elite black soybean lines for resistance to rust disease, *Phakopsora pachyrhizi*

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Abstract. *Krisnawati A, Gatut-Wahyu AS, Adie MM. 2016. Screening of elite black soybean lines for resistance to rust disease, Phakopsora pachyrhizi. Biodiversitas 17: 134-139.* Indonesian tropical climate is ideal for both of soybean growth and harmful disease development. Soybean rust, *Phakopsora pachyrhizi,* has been a serious disease in Indonesia and may have an impact on soybean production. The objective of the study was to evaluate the resistance of elite black soybean lines to rust disease. Agronomic characters (yield and yield component) were evaluated based on research conducted in 16 soybean production centers in Indonesia. The evaluation for rust resistance was conducted in Indonesian Legumes and Tuber Crops Research Institutes' greenhouse in 2011 using ten elite black soybean lines. The seed yield of ten black soybean lines grown in 16 locations ranged from 2.51-2.88 t/ha, with an average of 2.59 t/ha. The eight lines showed higher yield than check cultivar of Malika, whereas only one line (W9837 × Cikuray-66) had higher yield than check cultivar of Detam-1. Resistance reaction to rust disease fluctuates over time. Two elite lines of Cikuray × W9837-171 and Cikuray × W9837-184 showed a consistent resistance to *P. pachyrhizi*. W9837 × Cikuray-66 and check cultivar of Detam-1 consistently showed a moderately resistant. In the terms of agronomic aspects, W9837 × Cikuray-66, which has moderately resistant to *P. pachyrhizi*, produced the highest yield (2.88 t/ha). The both of resistant lines of Cikuray × W9837-171 and Cikuray × W9837-184 have early maturity, and medium seed size with yield 2.57 t/ha and 2.56 t/ha, respectively. The resistant and moderate lines with high yield, early maturing, and medium seed size found in this study playing an important role in the soybean varietal development program for rust resistance in Indonesia.

Keywords: Black soybean, *Glycine max,* resistance, rust disease, screening, yield

INTRODUCTION

Soybean rust, caused by *Phakopsora pachyrhizi*, has been a major disease limiting soybean production and has caused significant economic annual yield losses in Asia, Africa, and South America (Hartman et al. 2004; Levi 2004; Panthee et al. 2007; Jarvie 2009; Khanh et al. 2013). Soybean rust also has become one of the obstacles to increase soybean production in Indonesia due to the tropical climate (high temperature and humidity) providing suitable conditions for disease development, especially during the dry season.

Soybean rust becomes the most destructive foliar disease of soybean worldwide due to the widespread distribution and the potential for severe yield losses (Hartman et al. 2005). Soybean rust symptoms generally occur first on the leaves at the base of the plant and spread up to the canopy as the disease severity increases. Rust symptoms include presence of tan to dark brown or reddish brown lesions (Sinclair and Hartman 1999). An increase in leaf density will result in leaf yellowing, early leaf senescence, and yield losses (Tichagwa 2004). The heavy defoliation due to rust disease was affected the pod formation and pod filling (Yang et al. 1991).

Yield losses due to soybean rust can occur 100% depending on the weather conditions and degree of plant susceptibility (Kawuki et al. 2003a; Mueller et al. 2009). In southern Japan, yield losses were estimated at 15-40% (Bromfield 1976), whereas the yield losses 10-30% was common in Southern China and can be over 50% in severe epidemics (Bromfield et al. 1980). In Korea, yield losses in susceptible and tolerant cultivar were 68.7% and 22.3%, respectively (Shin and Tschanz 1986). In Indonesia, soybean rust has potential to reduce the soybean yield from 10% up to 90% (Sumartini 2010).

Resistance to soybean rust is manifested phenotypically by red-brown lesions and characterized by the three plant responses that have been shown to be associated with single dominant genes for soybean rust resistance, i.e. an immune response, reddish-brown lesions (or incomplete resistance), and the susceptible tan lesions (Bromfield, 1984). Six major resistance genes (Rpp1, Rpp2, Rpp3, Rpp4, Rpp5, and RppHyuuga) controlled the resistance to soybean rust have been identified with different resistance to a limited set of rust isolates (Hartman et al. 2005; Maphosa et al. 2012). Several strategies for controlling soybean rust have been applied, such as fungicide application, cultural and seed sanitation technique, and the use of resistant cultivar. The use of adapted soybean cultivars with resistance to *P. pachyrhizi* is cost-efficient ways and harmless to the environment (Goellner et al. 2009; Hassan et al. 2014; Yamanaka et al. 2011).

Singh and Schwartz (2011) stated that there are principal factors that play important role in the implementation of strategies and methods used for breeding for resistance to soybean rust that consist of: (i) the genetic distance between the cultivar to be improved and resistant donor germplasm, (ii) the availability of direct and indirect screening methods, (iii) the genetics of resistance, and (iv) the number of resistances and other traits to be improved. Furthermore, the choice of the selection method will depend on various factors including the breeding objective, genetic variability, available facility and infrastructures, and the personal skill of breeding team (Vishnyakova and Seferova 2013).

In Indonesia, the major emphasis in soybean varietal improvement (soybean breeding) is focused on producing high-yielding cultivar. Within 97 years (1918-2015), the Indonesian government has successfully released nine black soybean varieties (ILETRI 2014). The potential development of soybean as a raw material for industry is reflected in the last ten years, showed by increasing demands for black soybean, thus contributes to farmers' income. Black soybean in Indonesia is used as raw material for soy sauce industry, both in the domestic and large scale industries. Since soybean rust becomes serious disease in Indonesia, it is important to develop black soybean genotypes with high yield potential and less yield loss from soybean rust.

The objective of the study was to evaluate the resistance of elite black soybean lines to rust disease, *Phakopsora pachyrhizi*.

MATERIALS AND METHODS

The genotypes used in this study were black soybean lines derived from the crossing of parental lines W9837, MLG 3102, Cikuray (black soybean); and 100H (yellow seed coat). The research materials consist of eight black soybean promising lines (Cikuray × W9837-171, Cikuray × W9837-105, W9837 × Cikuray-66, W9837 × 100H-236, MLG 3102 x Cikuray-435, Cikuray x W9837-181, Cikuray x W9837-184, and W9837 × Cikuray-26), and two commercial check varieties (Detam-1 and Malika).

The yield trial was conducted in 16 soybean production centers in 2011 cropping season. A randomized complete block design with four replicates was arranged in each location. Each line was planted on 2.8 m × 4.5 m plot size, 40 cm × 15 cm plant distance, two plants per hill. Fertilizer of 50 kg/ha Urea, 100 kg/ha SP36 and 75 kg/ha KCl were applied before sowing time. Weed, insect and disease were controlled intensively. The parameters measured consists of yield and yield components: days to flowering (when 50% of the plant population have been flowering), days to maturity (calculated if 95% of the leaves have turned yellow), plant height (taken from average of 5 randomly sample plants in cm), 100-seeds weight (determined by randomly weighing 100 seeds in gram), and seed yield (randomly taken from the seed yield per plot and converted to t/ha). All the data collected were subjected to statistical analysis of variance (ANOVA) of mean performance of genotypes, locations, and their interaction. Least significant differences (LSDs) were determined at 5% probability level.

Evaluation of rust resistance was conducted in Indonesian Legumes and Tuber Crops Research Institutes (ILETRI)'s screen house in Malang, East Java, Indonesia in dry season 2011 using a completely randomized design. The first factor was inoculation treatment (without inoculation and with inoculation), and the second factor was 10 black soybean genotypes. Each genotype was planted in a plastic polybag (Φ =15 cm), two plants per polybag. At 30 days after planting, plants were inoculated with uredospore. The inoculation density was 10,000 spores per millimeter. Spore suspension was originated from naturally infected plants. Uredospore were harvested from the lower surface of infected leaves. Rust severity was determined at weekly intervals (38, 45 52, 69, and 76 days after planting) using IWGSR (International Working Group on Soybean Rust) rating system (Shanmugasundaram 1977).

The IWGSR rating system uses a three-digit rating score to record the rust severity (Table 1). The first digit denotes the upper bond position of most diseased leaves in the canopy of the plant. The second digit denotes the density of rust lesions on most of the diseased leaves. The third digit denotes the infection type on most of the diseased leaves. The rating scale of each digit is explained as Table 1, and relationship between disease reactions and IWGSR ratings for soybean rust is explained as Table 2 (Yang 1977).

Table 2. Relationship between disease reactions and IWGSR ratings for soybean rust

Disease reaction	IWGSR rating
Immune	111
Resistance	122, 123, 132, 133, 222, 223
Moderately resistant	142, 143, 232, 233, 242, 243, 322, 323
Moderately susceptible	332, 333
Susceptible	343

Table 1. The International Working Group on Soybean Rust (IWGSR) rating system

Parameter criteria	Score	Description
First digit: upper bond position of most diseased leaves	1	1 = bottom third of the leaf canopy of the plant
	2	2 = middle third of the leaf canopy of the plant
	3	3 = upper third of the leaf canopy of the plant
Second digit: density of rust lesions on most diseased leaves	1	1 = no lesion
	2	2 = light lesion density (<1-8 lesions per cm^2)
	3	3 = medium lesion density (9-16 lesions per cm^2)
	4	4 = heavy lesion density (>16 lesions per cm^2)
Third digit: infection type on most diseased leaves	1	1 = no pustules
	2	2 = non-sporulating pustules
	3	3 = sporulating pustules

RESULTS AND DISCUSSION

Yield and yield component

The combined analysis of variance was presented in Table 3. Genotype, location, and genotype by environment interaction were highly significant. The agronomic characters of ten black soybean lines in 16 environments are presented in Table 4. All the data of agronomic characters (yield and yield components) were of the average of 16 locations. The seed yield of ten black soybean lines grown in 16 locations ranged from 2.51-2.88 t/ha, with an average of 2.59 t/ha. The eight black soybean lines showed higher yield than check cultivar of Malika, whereas only one line (W9837 × Cikuray-66) had higher yield than check cultivar of Detam-1.

The yield component consists of days to flowering, days to maturity, plant height, seed weight, and seed yield. Days to flowering ranged from 33 to 35 days, days to maturity ranged from 74 to 82 days, plant height varied from 51.16 to 63.46 cm, seed weight ranged from 10.92 to 13.60 g/100 seeds, and seed yield ranged from 2.46 to 2.88 t/ha. In Indonesia, soybean maturing day classified as late maturity (> 90 days), medium maturity (80-90 days), and early maturity (<80 days). According to maturity classification, all elite lines showed early maturing day, except the check cultivars which showed medium maturity (Table 4). The earliest maturity (under 76 days) were mostly showed by lines derived from the crossing of Cikuray × W9837, i.e. Cikuray × W9837-171 (74 days), Cikuray × W9837-105 (75 days), Cikuray × W9837-184 (75 days), and Cikuray × W9837-181 (75 days).

The soybean seed size are categories into three: small (< 10 g/100 seeds), medium (10-14 g/100 seeds), and large size (> 14 g/100 seeds (PVT 2007). The seed weight of all lines was categorized as medium-seeded size (Table 4). The medium soybean seed size in Indonesia is desirable for tofu industry.

Table 3. The combined analysis of variance for black soybean yield in 16 locations. 2011.

Source	df	Sum of Square	Mean of Square
Location (L)	15	12.6600	0.8440**
Genotype (G)	9	7.5400	0.8380**
G × L	135	23.3800	0.1730**
Error	432	44.0700	0.1020
CV (%)	12.31		

Note: CV = coefficient of variation; ** = significant at p = 0.01

Table 4. Agronomic characters of 10 black soybean elite lines in 16 locations, 2011

Black soybean lines	Days to flowering	Days to maturity	Plant height (cm)	Seed weight (g)	Yield (t/ha)
Cikuray × W9837-171	34 d	74 g	57.55 b	11.22 de	2.57
Cikuray × W9837-105	34 e	75 f	58.28 b	10.98 ef	2.58
W9837 × Cikuray-66	34 e	75 ef	56.87 bc	11.75 c	2.88
W9837 × 100H-236	36 a	76 de	53.16 d	11.01 ef	2.54
MLG 3102 × Cikuray-435	33 f	76 cd	55.41 c	11.36 d	2.54
Cikuray × W9837-181	35 c	75 ef	57.49 b	10.92 f	2.64
Cikuray × W9837-184	35 cd	75 f	55.04 c	10.96 ef	2.56
W9837 × Cikuray-26	33 f	76 c	51.16 e	12.20 b	2.51
Detam-1	34 e	82 a	57.36 b	13.60 a	2.66
Malika	35 b	81 b	63.46 a	11.32 d	2.46
Average	34	77	56.58	11.53	2.59
LSD (5%)	0.31	0.41	1.89	0.32	0.96

Note: Value within the same column followed by the same letter are not significantly different at the 0.05 level according to LSD test

Table 5. Rust rating score of ten elite black soybean lines at five weekly intervals, 2014

Black soybean lines	Rating score according to IWGSR														
	38 DAP			45 DAP			52 DAP			69 DAP			76 DAP		
Cikuray × W9837-171	2	2	2	2	2	2	2	2	3	2	2	2	2	2	2
Cikuray × W9837-105	2	3	2	2	3	2	2	3	3	2	3	3	2	2	3
W9837 × Cikuray-66	2	2	2	2	2	2	2	3	2	2	3	3	2	3	3
W9837 × 100H-236	2	2	2	2	2	2	2	2	2	2	3	2	2	3	2
MLG 3102 × Cikuray-435	2	2	3	2	2	3	2	3	3	2	3	2	3	3	3
Cikuray × W9837-181	1	2	2	2	3	2	2	3	2	2	2	2	2	2	2
Cikuray × W9837-184	2	2	2	1	2	2	1	3	2	1	2	2	2	2	2
W9837 × Cikuray-26	2	3	3	2	2	3	2	2	3	2	2	2	2	3	2
Detam-1	2	2	2	2	3	3	2	3	3	2	3	3	2	3	3
Malika	2	2	2	2	3	3	3	3	3	3	3	3	3	4	3

Note: DAP = days after planting, IWGSR = International Working Group on Soybean Rust (Yang 1977)

Table 6. Black soybean resistance to rust at five weekly intervals, 2014

Black soybean lines	Resistance criteria according to IWGSR				
	38 DAP	45 DAP	52 DAP	69 DAP	76 DAP
Cikuray × W9837-171	R	R	R	R	R
Cikuray × W9837-105	MR	MR	MR	MR	R
W9837 × Cikuray-66	R	R	MR	MR	MR
W9837 × 100H-236	R	R	R	MR	MR
MLG3102×Cikuray-435	R	R	MR	MR	MS
Cikuray × W9837-181	R	MR	MR	R	R
Cikuray × W9837-184	R	R	R	R	R
W9837 × Cikuray-26	MR	R	R	R	MR
Detam-1	R	MR	MR	MR	MR
Malika	R	MR	MS	MS	S

Note: DAP = days after planting, IWGSR = International Working Group on Soybean Rust (Yang 1977). R = resistant, MR = moderately resistant, MS = moderately susceptible

The rust resistance evaluation

The rust severity of ten black soybean lines was determined based on rating score, and evaluated at weekly intervals, consist of 38, 45 52, 69, and 76 days after planting (Table 5). According to the resistance criteria (Yang 1977), the rust resistance of black soybean elite lines consists of resistant (R), moderately resistant (MR), and moderately susceptible (MS) (Table 6).

At 38 days after planting (DAP), most soybean lines showed resistant reactions to the disease. At 45 DAP, which plants in the pods formation, the number of resistant lines decreased. The next intervals of evaluation at 52 DAP (pod filling stage/R5), 69 DAP (full pod filling stage/R5-R6), and 76 DAP (full seed/R6) showed a gradually decreasing resistance.

Discussion

Black soybeans play important roles in the industrial sector in Indonesia. Besides used as raw material for producing soy sauce, soybean with black seed coat are known to have high levels of phenolic and anthocyanin, and also have antioxidant and anti-inflammatory effects (Ku et al. 2000; Astadi et al. 2009; Xu et al. 2012). The main focus of the new soybean cultivar development in Indonesia involves breeding for high potential yield with desired characteristics, such as high nutritional contents and tolerance or resistance to biotic and abiotic stresses.

Seed yield as a complex trait consists of components which serve as significant indicator for the seed yield of soybean. The combined analysis of variance indicated that treatments (genotype, location, and genotype by environment interaction) were highly significant, showing that genotypes responded differently to the environments used during the study.

The yield and yield component showed variability between locations. The check cultivar of Detam-1 (2.66 t/ha) had higher yield than Malika (2.46 t/ha). Detam-1 is Indonesian black soybean varieties with high protein content (up to 45.36%), and released in 2008. Malika released as black soybean variety in 2007. The elite line of W9837 × Cikuray-66 produced the highest yield (2.88 t/ha); it is higher than the two check cultivars of Detam-1 and Malika (2.66 t/ha and 2.46 t/ha, respectively). In addition to high yield, this line also has characteristics of early maturity and medium seed size.

Indonesia as the tropical country receives plentiful sunshine throughout the year. This is an ideal environment for both of growing soybeans and the development of soybean pests and disease. In addition, the soybean cultivation mostly in the dry season II (June-September), which the pest and disease incidences occurred in the highest frequency.

The resistance evaluation to rust disease showed variation between weekly intervals. Most of the soybean lines showed resistant reactions at 38 days after planting, except the two lines (Cikuray × W9837-105 and W9837 × Cikuray-26) which were moderately resistant. In Indonesia, the soybeans plant age at 38 DAP were mostly in the first reproductive (R1) growth stage (Adie and Krisnawati 2007). The R1 growth stage is beginning flowering (Fehr and Caviness 1977). In the pods formation stage (45 DAP), the number of resistant lines were decreased. A total of six lines showed resistance reaction, and the rest were moderately resistant. Furthermore, the next intervals of the evaluation showed a gradually decreasing resistance. There were four resistant elite lines for each week interval. According to Kumudini et al. (2008), this is reasonable since disease severity increased as reproductive development advanced. Ribeiro et al. (2007) also stated that during the pod filling stage (R5-R6) become the most critical time due to the greater increase of the disease attack and causing the significant yield losses. Furthermore, it has been reported that soybean yield is most sensitive to leaf defoliation injury at the R5 growth stage (Fehr and Caviness 1977; Fehr et al. 1981). The overall results showed that Cikuray × W9837-171 and Cikuray × W9837-184 showed a consistent resistance to *P. pachyrhizi*, whereas the check cultivar of Detam-1 and W9837 × Cikuray-66 showed a consistent moderate resistance at 52 DAPS, 69 DAP, and 76 DAP. The other check cultivar of Malika showed a moderately susceptible reaction in pod filling formation, and then showed decreasing resistance to susceptible in full seed/R6.

A key requirement in breeding efforts is the screening of plants for resistance to diverse pathogen populations to identify cultivars that are likely to withstand variable pathogen populations (Harman et al. 2005). Several screening trials have been conducted for identification of resistant or tolerance genotypes. In Brazil, Santa Rosa, FT-1 and Uniao were identified as resistant cultivars and all the varieties and germplasm from US were found to be susceptible during screening trials (Ribeiro et al. 1985). Kawuki et al. (2003b) screened soybean germplasm for rust resistance at the NaCRRI and found none of the screened materials to be immune to rust infection. A total of 8 accessions were resistant, 45 moderately susceptible, 31 susceptible and 112 very susceptible. Screening through artificial inoculation using segregating populations (F3) of two crosses involving two high yielding varieties JS335 and JS9305 (both susceptible to rust), and one germplasm line EC241780 (resistant to rust) were conducted in India. Six among the 62 progeny lines showed resistant or moderately resistant to rust (Shivakumar et al. 2011). Furthermore, screening trial against soybean rust under natural epiphytotic conditions at Barapani (Meghalaya, India) revealed that only two lines NRC 80 and MAUS 417 were moderately susceptible. There were no line or variety was in the moderately resistant or resistant category (Baiswar et al. 2012). The newest study in ten Indonesian soybean lines resulted that there were no line identified as resistant line (Sumartini and Kuswantoro 2014).

A high yielding varieties with rust resistance is one of pursued goal in Indonesian soybean breeding program. The both of resistant black soybean elite lines of Cikuray × W9837-171 and Cikuray × W9837-184 have agronomic characteristics of early maturity and medium seed size. These lines produced yield 2.57 t/ha and 2.56 t/ha, respectively; higher than check cultivar of Malika. The highest yield was produced by W9837 × Cikuray-66 (2.88 t/ha), which has moderately resistant to *P. pachyrhizi*. The resistant and moderate lines found in this study can be used as a source in breeding programs and/or can be developed to be a new black soybean cultivar with desirable characteristics.

To conclude, resistance reaction of black soybean elite lines to rust disease fluctuates over time. Two elite lines of Cikuray × W9837-171 and Cikuray × W9837-184 showed a consistent resistance to *P. pachyrhizi*, and two others (W9837 × Cikuray-66 and check cultivar of Detam-1) consistently showed a moderately resistant, respectively. Resistant and moderately resistant soybean lines with early maturity and medium seed size identified in this research may provide the gene source needed for future development of soybean cultivars with soybean rust resistance in Indonesia.

REFERENCES

Adie MM, Krisnawati A. 2007. Soybean Plant Biology. In: Sumarno, Suyamto, Widjono A, Hermanto, Kasi H (eds). Soybean, Production Technology and Development. Indonesian Agency for Agricultural Research and Development. Indonesia. [Indonesian].

Astadi IR, Astuti M, Santoso U, Nugraheni PS. 2009. In vitro antioxidant activity of anthocyanins of black soybean seed coat in human low density lipoprotein (LDL). Food Chemistry 112 (3): 659-663.

Baiswar P, Tiameren N, Upadhyay DN, Chandr S. 2012. Screening of varieties against soybean rust caused by *Phakopsora pachyrhizi* in Mid-hills of Meghalaya. Indian Journal of Hill Farming 25 (1): 17-20.

Bromfield KR. 1984. Soybean Rust. American Phytopathological Society, St Paul, Minnesota.

Bromfield KR, Melching JS, Kingsolver CH. 1980. Virulence and aggressiveness of *Phakopsora pachyrhizi* isolates causing soybean rust. Phytopathology 70: 17-21.

Bromfield KR. 1976. World soybean rust situation. In: Hill LD (ed). World Soybean Research. Cooperative Extension Service, Agricultural and Home Economics Experiment Station, Iowa State University, Ames, Iowa, USA.

Fehr WR, Caviness CE. 1977. Stages of soybean development. Iowa State University Cooperative. Extension Service, Special Report 80. Iowa State University, Ames, IA.

Fehr WR, Lawrence BK, Thompson TA. 1981. Critical stages of development for defoliation of soybean. Crop Sci 21: 259-262.

Goellner K, Loehrer M, Langenbach C, Conrath U, Koch E, Schaffrath U. 2009. *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust. Mol Plant Pathol 11: 169-177.

Hartman GL, Miles MR, Frederick RD. 2005. Breeding for resistance to soybean rust. Plant Dis 89: 664-666.

Hartman GL, Bonde MR, Miles MR, Frederick RD. 2004. Variation of Phakopsora pachyrhizi isolates on soybean. In: Proceedings of the VII World Soybean Research Conference. Foz do Iguass, P.R. Brazil, 29 February to 5 March 2004.

Hassan FSC, Fakheri B, Sattari A. 2014. Review: Breeding for resistance to soybean rust. Intl J Agric Crop Sci 7 (6): 322-328.

ILETRI [Indonesian Legumes and Tuber Crops Research Institutes]. 2012. Description of Legumes and Tuber Improved Varieties. ILETRI. Malang.

Jarvie JA. 2009. A review of soybean rust from a South African perspective. S A J Sci 105: 103-108.

Kawuki R, Adipala E, Tukamuhabwa P. 2003a. Yield loss associated with soya bean rust (*Phakopsora pachyrhizi* Syd.) in Uganda. J Phytopathol 151: 7-12.

Kawuki RS, Adipala E, Lamu J, Tukamuhabwa P. 2003b. Responding to the soybean rust epidemic. African Crop Sci j 4: 301-318.

Khanh TD, Anh TQ, Buu BC, Xuan TD. 2013. Applying molecular breeding to improve soybean rust resistance in Vietnamese elite soybean. Amer J Pl Sci 4: 1-6.

Ku JH, Moon J, Yoon H, Park K, Lee Y. 2000. Composition ratios of cyanidin-3-glucoside and delphinidin-3-glucoside in Korean soybean with black seed coat. In: Kyoko Saio, ed. 2000. Proceedings–Third The Japanese Society for Food Science and Technology. Korin Pub. Co. Ltd., Japan.

Kumudini S, Godoy CV, Board JE, Omielan J, Tollenaar M. 2008. Mechanisms involved in soybean rust-induced yield reduction. Crop Sci 48: 2334-2342.

Levy C. 2004. Epidemiology and chemical control of soybean rust in South Africa. Plant Dis 89: 669-674.

Maphosa M, Talwana H, Tukamuhabwa P. 2012. Enhancing soybean rust resistance through Rpp2, Rpp3 and Rpp4 pair wise gene pyramiding. African J Agric Res 7 (30): 4271-4277.

Mueller TA, Miles MR, Morel W, Marois JJ, Wright DL, Kemerait RC, Levy C, Hartman GL. 2009. Effect of fungicide and timing of application on soybean rust severity and yield. Plant Dis 93: 243-248.

Panthee DR, Yuan JS, Wright DL, Marois JJ, Mailhot D, Stewart JCN. 2007. Gene expression analysis in soybean in response to the causal agent of Asian soybean rust (*Phakopsora pachyrhizi* Sydow) in an early growth stage. Funct Integr Genom 7: 291-301.

PVT. 2007. Guidelines for the conduct of test for distinctness, homogeneity and stability. Centre of Plant Variety Protection and Agriculture Permit. Ministry of Agriculture Republic of Indonesia. Jakarta.

Ribeiro DV, Zambolim FXL, Chaves GM. 1985. Reaction of soybean cultivars to *Phakopsora pachyrhizi* Sydow. Soybean Rust Newslett 7: 10-13.

Ribeiro AS, Moreira JUV, Pierozzi PHB, Rachid BF, Toledo JFF, Arias CAA, Soares RM, Godoy CV. 2007. Genetic control of Asian rust in soybean. Euphytica 157: 15-25.

Shanmugasundaram S. 1977. The International working group on soybean rust and its proposed soybean rust rating system. In: Ford RW, Sinclair JB (eds) Rust of Soybean: The Problem and Research Needs. Intl Agric Publ INTSOY Series 12, University of Illinois, Urbana, Il.

Shin DC, Tschanz AT. 1986. Studies on physiological reactions of soybean cultivar tolerant and susceptible to rust (*Phakopsora pachyrhizi*). Korea J Crop Sci 31: 440-446.

Shivakumar M, Basavaraja GT, Salimath PM, Patil PV, Akshay T. 2011. Identification of rust resistant lines and their genetic variability and character association studies in soybean [*Glycine max* (L.) Merr.]. Indian J Genet Pl Breed 71 (3): 235-240.

Sinclair JB, Hartman GL. 1999. Soybean rust. In: Hartman GL, Sinclair JB, Rupe JC (ed). Compendium of Soybean Diseases, 4th ed. American Phytopathological Society, St. Paul, Minnesota.

Singh SP, Schwartz HF. 2011. Review: Breeding common bean for resistance to insect pests and nematodes. Can J Plant Sci 91: 239-250.

Soares RM, Godoy CV. 2007. Genetic control of Asian rust in soybean. Euphytica, 157: 15-25.

Sumartini, Kuswantoro H. 2014. Resistance evaluation of large seeded soybean lines to rust disease. The 4th Annual Basic Science International Conference. Batu, East Java, Indonesia.

Sumartini. 2010. Soybean rust disease and how to control environmentally friendly. Jurnal Litbang Pertanian 29 (3): 107-112. [Indonesian]

Tichagwa JS. 2004. Breeding for resistance to soybean rust in Zimbabwe. In: Moscardi F, Hoffmann-Campo CB, Saraiva OF, Galerani PR, Krzyzanowski FC, Carrão-Panizzi MC (eds). Proceedings of the VII World Soybean Research Conference, Embrapa, Londrina, Brasil.

Vishnyakova M, Seferova I. 2013. Soybean: A dawn to the legume world. The journal of the International Legume Society Issue 1: 1-7.

Xu L, Choi TH, Kim S, Kim SH, Chang HW, Choe M, Kwon SY, Hur JA, Shin SC, Chung JI, Kang D, Zhang D. 2011. Black soybean (*Glycine max* cv. Heugmi) seed coat extract suppresses TPA or UVB-induced COX-2 expression by blocking mitogen activated protein kinases pathway in mouse skin epithelial cells. Food Sci Biotechnol 20 (6): 1735-1741.

Yamanaka N, Lemos NG, Akamatsu H, Yamaoka Y, Silva DGC, Passianotto AL de L, Abdelnoor RV, Soares RM, Suenaga K. 2011. Soybean breeding materials useful for resistance to soybean rust in Brazil. Japan Agric Res Quart 45 (4): 385-395.

Yang CY. 1977. Soybean rust in eastern hemisphere. In: Ford RE, Sinclair JB (eds). Rust of Soybean, the Problem and Research Needs. Intsoy Series No. 12, University of Illinois, Urbana, IL.

Yang XB, Tchanz AT, Dowler WM, Wang TC. 1991. Development of yield loss models in relation to reductions of components of soybeans infected with *Phakopsora pachyrhizi*. Phytopathology 81: 1420-1426.

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The recovery of Tabunio Watershed through enrichment planting using ecologically and economically valuable species in South Kalimantan, Indonesia

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Abstract. *Kadir S, Badaruddin, Nurlina, Ridwan I, Rianawaty F. 2016. The recovery of Tabunio Watershed through enrichment planting using ecologically and economically valuable species in South Kalimantan, Indonesia. Biodiversitas 17: 140-147.* Watershed is a medium system where hydrological-biophysical processes as part of hydrological cycle and social, economic, and cultural events as the results of human intervention on natural resource occur. The increasing human population results in the increasing need of water that may reduce the availability of water, causing environmental degradation. The objectives of this study were to study the characteristics of Tabunio watershed and to give recommendation on the enrichment planting for the watershed recovery. This study used watershed ecological area approach and the data were analyzed spatially using GIS. The results showed that based on the characteristics of its components, Tabunio Watershed was categorized as moderately to very highly qualified for recovery. The recovery should be done through: (i) enrichment planting based on the land capability, land suitability and the legal function of the area, (ii) conservation of soil and water using vegetative and civil engineering methods, and (iii) issuance of regulations to increase the watershed's carrying capacity.

Keywords: South Kalimantan, Tabunio, watershed recovery, water resource sustainability

INTRODUCTION

Watershed is a medium system where hydrological-biophysical processes as part of hydrological cycle and social, economic, and cultural events as the results of human intervention on natural resource occur. The increasing human population results in the increasing need of water that may reduce the availability of water, causing environmental degradation. The deteriorating environmental quality has threatened the sustainability of human and other living creatures, so that serious and consistent environmental protection and management must be done by all stakeholders. The program for environmental protection and management for certain period of time should be written in the Planning for Environmental Protection and Management document as part of the Medium Term Development Plan (Act No. 32/2009 regarding the Environmental Protection and Management).

The vulnerability to flood in Tanah Laut District of South Kalimantan Province, Indonesia which is located in Tabunio Watershed increased from 2007 to 2010, with a total of 22 villages being flooded in 2010 (Balitbangda 2010). The area of degraded land in Tabunio Watershed is 19,109.89 ha or 31 % of the watershed area (BPDAS Barito 2013). The recovery of Tabunio Watershed (The Ministry of Forestry Decree No. P.60/MENHUT-II/2014) is expected to create sustainable productive land which functions ecologically optimally to ensure the environmental and hydrological stability and, at the same time, to increase the community's income.

The objective of this study was to know the characteristics of Tabunio Watershed in South Kalimantan Province, Indonesia in order to give recommendation for watershed recovery through enrichment planting of ecologically and economically valuable species. The results of this study can be used as reference for the efforts to meet the water demand in Tabunio Watershed.

MATERIALS AND METHODS

This study used watershed spatial approach in which the watershed area was divided into three parts: upstream, middle and downstream. The data were analyzed and presented using GIS. The results were recommendation for Tabunio Watershed recovery through enrichment planting. The method of research was based on the Decree of the Ministry of Forestry No. P.60/MENHUT-II/2014 regarding the Determination of Criteria for Watershed Classification. The parameters analyzed were land use, classes of land degradation, water regulation, legal function of the area, and land capability classes for enrichment planting.

The area of Tabunio Watershed is 62,558.56 ha, located in Tanah Laut District, South Kalimantan Province, Indonesia spread in 6 sub-districts. The upstream is 17,542.82 ha, the middle 13,038.44 ha and the downstream 31,977.30 ha. The location of study is given in Figure 1.

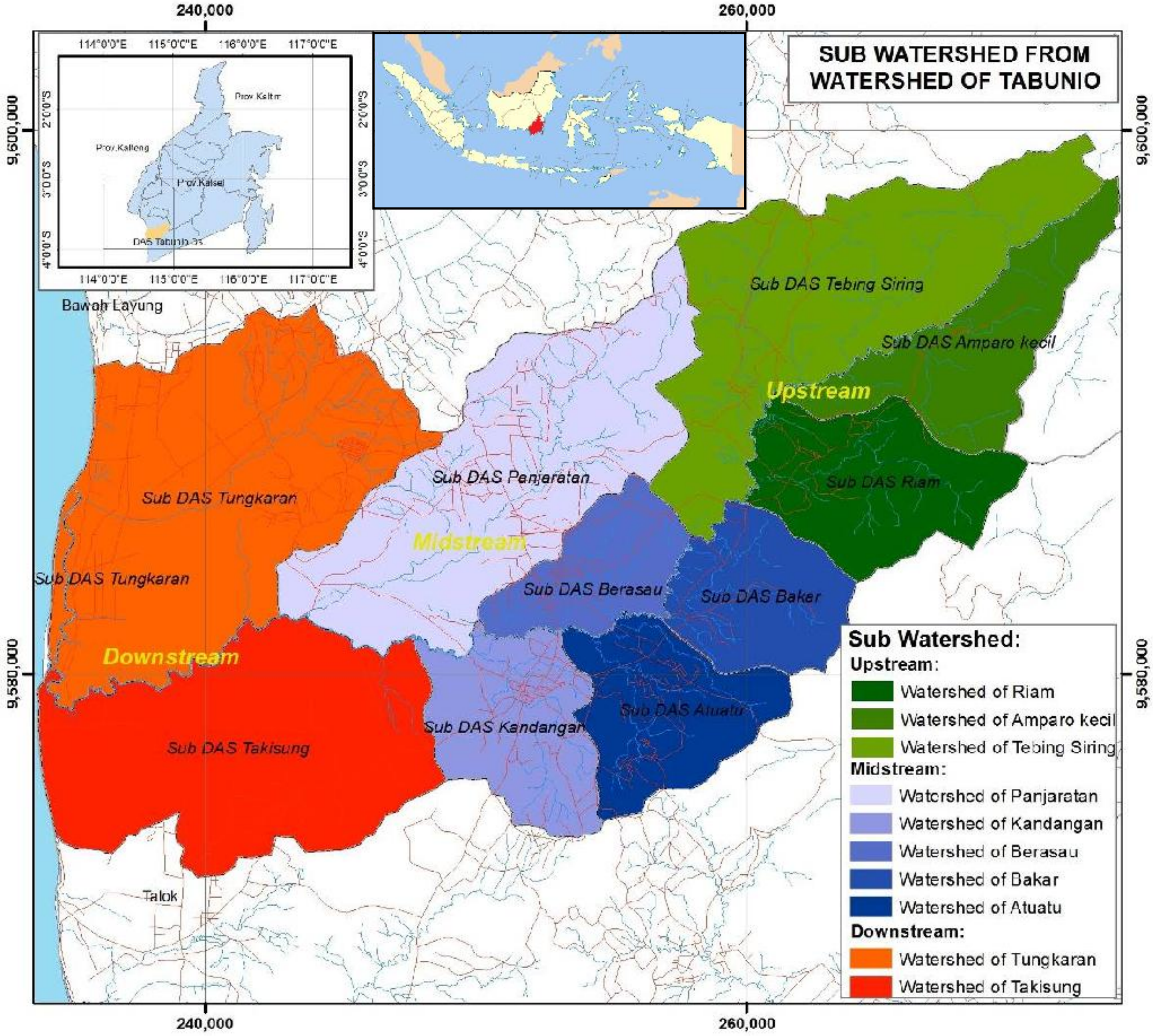

Figure 1. The map of Tabunio Watershed and its sub-watersheds in South Kalimantan Province, Indonesia

RESULTS AND DISCUSSION

The characteristics of Tabunio Watershed which are the main factors to be considered in watershed recovery are land use, land degradation classes, slope, hydrology, the legal function of the area, land capability classes, and plant species for enrichment planting.

Watershed characteristics

Land use

Based on the GIS analyses, there were 15 types of land use in the Tabunio Watershed (Table 1, Figure 2).

Table 1 shows that the permanent vegetation cover in Tabunio Watershed was 24,687.60 ha, or 39.46%, indicating that Tabunio Watershed was categorized as highly qualified for recovery in order to restore its carrying capacity.

Water infiltration is higher and the surface flow is lower in forest than in agriculture land, so vegetation cover should be increased in order to reduce the vulnerability to flood and to increase the community's prosperity (Kometa and Ebot 2012). Meng et al. (2011) stated that the expansion of rubber plantation had reduced the number of forest trees, so the expansion of rubber plantation must be controlled. Zhao et al. (2012) reported that the changes in land use and vegetation cover in watershed affected the surface flow. Liu and Chen (2012) state that the area of agriculture land (non forest) increase with the increasing population.

Classes of land degradation

The area of degraded land was determined by overlying land cover, erosion, slope and watershed management. The determination of degradation class was based on the Decree of Director General of Land Rehabilitation and Social Forestry, the Ministry of Forestry no. SK.167/V-SET/2004. The GIS was used for determination of land degradation class, analyses and presentation of spatial data. The area of degraded land is one of determining factors whether a certain watershed needs to be restored or maintained. The results of analyses are presented in Table 2 and Figure 3. Table 2 shows that the area of degraded and critically degraded land was 19,109.89 ha (30.55%). Based on the percentage of degraded land (30.55 %), Tabunio Watershed was categorized as highly qualified for recovery in order to increase its carrying capacity. The distribution of degraded and moderately degraded land is presented in Figure 3.

According to Kadir (2014), the Rehabilitation of Forest and Land is an effort to restore, maintain and improve the function of forest and land, so their carrying capacity, productivity and role as life supporting system can be maintained. Rueda (2010) says that conservation effort can reduce deforestation rate. In addition, Bukhari and Febryano (2008) state that the communities can practice traditional agroforestry system on degraded land based on local condition and wisdom.

Slope

Saud (2007) says that water flow increases with the increasing slope. Water, in the steep land, flows faster than in flat land. The results of slope analyses using GIS are presented in Table 3 and Figure 4. Table 3 shows that the slopes of 0-7% dominated the area of Tabunio Watershed (79.85%). Based on its dominant slope, the land is suitable for agriculture crops which can improve the prosperity of community in Tabunio Watershed.

Table 1. Types of land use in Tabunio Watershed, South Kalimantan, Indonesia

Land use types	Area (ha)			Total (ha)
	Upstream	Middle	Down-stream	
Secondary dry forest	2,343.74	201.08	-	2,544.82
Secondry mangrove	5.00	-	702.92	707.92
Plantation forest	274.85	4,487.91	832.07	5,594.83
Settlement	-	416.48	36.96	453.44
Plantation	512.69	20.65	-	533.34
Dry agriculture land	4,872.93	12,986.01	9,180.24	27,039.18
Dry agriculture land and shrub	4,347.61	1,900.35	2,275.88	8,523.84
Swamp	-	11.31	-	11.31
Field	-	984.13	5,420.88	6,405.01
Shrub/bush	2,971.28	1,320.77	3,014.86	7,306.91
Shrub/bush on swamp	-	-	9.30	9.30
Fish farming	-	-	349.52	349.52
Mining	821.47	321.02	-	1,142.49
Open land	1,393.30	202.99	213.53	1,809.82
Water body	-	82.78	44.08	126.86
Total	17,542.87	22,935.48	22,080.24	62,558.59

Table 2. The classes of land degradation in Tabunio Watershed, South Kalimantan, Indonesia

Classes of degradation	Area (ha)			Total (ha)
	Upstream	Middle	Down-stream	
Not degraded				
Potentially degraded	5,055.94	5,213.26	3,657.78	13,926.98
Moderately degraded	3,482.32	12,771.04	13,268.35	29,521.71
Degraded	6,634.49	4,861.22	5,154.11	16,649.82
Critically degraded	2,370.12	89.96	-	2,460.08
Total	17,542.87	22,935.48	22,080.24	62,558.59

Table 3. Slopes in Tabunio Watershed, South Kalimantan, Indonesia

Slopes	Area (ha)			Total (ha)
	Upstream	Middle	Down-steam	
0-2%	2,566.74	9,539.87	17,028.13	29,134.74
2-7%	7,967.23	8,959.05	3,892.70	20,818.98
7-14%	2,728.49	1,477.09	404.77	4,610.35
14-21%	1,457.51	876.84	302.30	2,636.65
> 21%	2,822.90	2,082.63	452.34	5,357.87
Total	17,542.87	22,935.48	22,080.24	62,558.59

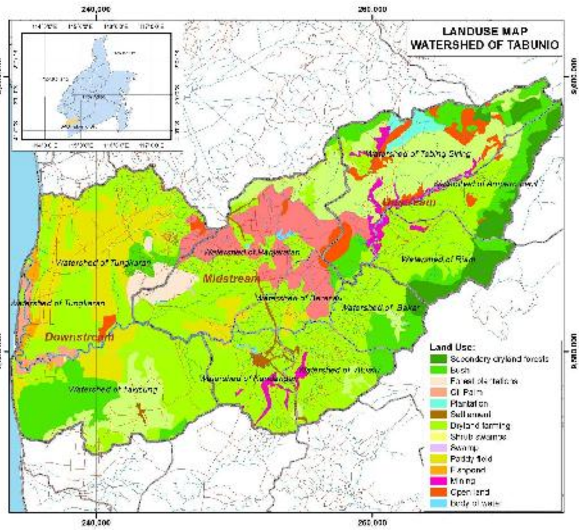

Figure 2. The map of land use in Tabunio Watershed, South Kalimantan, Indonesia

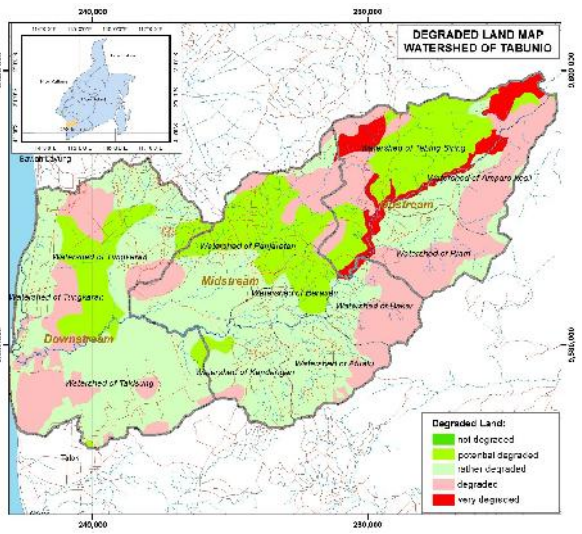

Figure 3. The map of degraded land in Tabunio Watershed, South Kalimantan, Indonesia

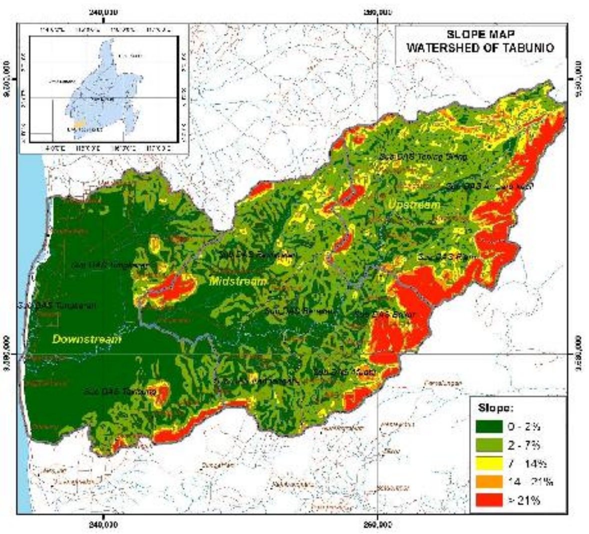

Figure 4. The map of slopes in Tabunio Watershed, South Kalimantan, Indonesia

The topography of a watershed affects the speed and volume of surface runoff. Steep slope results in high speed of surface runoff and low infiltration rate (Arsyad 2010). According to Asdak (2010), the upstream of watershed usually has steep slope and dense drainage, so that this part of watershed should be forested.

Hydrology

Flow regime coefficient (FRC)

Flow regime coefficient (FRC) is a number indicating the ratio of maximum water flow (Qmaks) with the dependable water flow (Qa) in a watershed/sub-watershed. The data of water flow from 1978 to 2000 from the Office of Public Work of South Kalimantan Province are presented in Table 4 and Figure 5.

Table 4. The average water flow (Q) in Tabunio Watershed, South Kalimantan, Indonesia within a period of 23 years (1978-2000)

Year	Water flow (m^3/second)			FRC
	Max	Min	Average	
1978	3.3	2.46	18.27	0.7
1979	9.5	2.89	15.58	2.4
1980	7.01	1.33	18.50	1.5
1981	45.14	2.42	14.94	12.1
1982	27.49	0.39	5.22	21.1
1983	40.24	0.99	10.39	15.5
1984	2.47	0.92	9.76	1.0
1985	5.7	0.99	8.64	2.6
1986	-	-	-	
1987	3.3	1.17	15.83	0.8
1988	3.34	0.68	13.83	1.0
1989	8.75	0.03	10.74	3.3
1990	4.99	0.89	12.50	1.6
1991	8.76	0.69	1.75	20.0
1992	36,5	0,92	11,68	12,5
1993	4.4	0.99	12.81	1.4
1994	40.42	8.15	9.92	16.3
1995	3.66	1.42	10.58	1.4
1996	1.49	0.6	11.65	0.5
1997	4.76	0.44	5.78	3.3
1998	9.54	1.23	13.10	2.9
1999	40.24	0.42	10.14	15.9
2000	8.67	0.5	9.93	3.5
Average	14.5	1.4	11.4	6.4

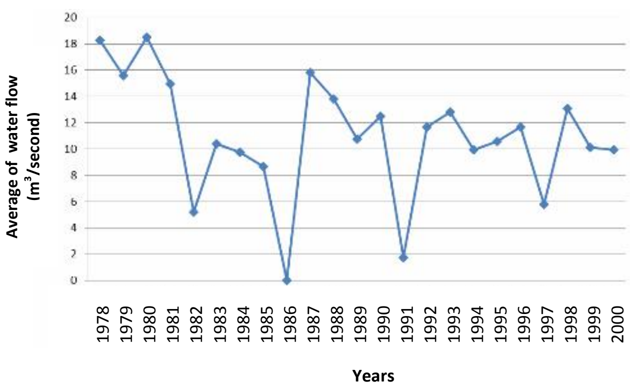

Figure 5. The average water flow in Tabunio Watershed, South Kalimantan, Indonesia

Table 4 shows that the maximum water flow within a period of 23 years (1978-2000), was 45.14 m^3/second, the dependable water flow was 2.86 m^3/second, so the Flow Regime Coeficient (FRC) was > 15.79, meaning that Tabunio Watershed was categorized as highly qualified for recovery. The water flow decreased from 1978 to 2000, and it was even lower in 2015 (from measurement).

Sajikumar and Remya (2015) have analyzed the effect of land use and cover on the characteristics of surface runoff and water flow from two watersheds in Kerala, India. Zhang et al. (2015) have given theoretical basis and technical support for land reclamation and conservation of water and soil in ecologically vulnerable mined land. Furthermore, Fox et al. (2012) analyzed the impact of land cover changes on the runoff and total water flow for a period between 1950 and 2003 in Mediterranean region of France, concluding that the change of land cover affected water flow.

Ma et al. (2010) say that flood control can be done by making water canals and creating artificial peak flood in order to discharge sediment and to increase the volume of water in the rivers. Polonskii and Solodovnikova (2009) recommended flood control by utilizing the water for hydro power.

Coefficient of flow

The annual flow coefficient is one of parameters to indicate the hydrological condition of a watershed. The coefficient was calculated from the average annual water flow (11.4 m^3/second), rainfall (1583.667 mm/year and area of Tabunio Watershed (62558.56 ha). The coefficient of flow in Tabunio Watershed was 0.36, categorized as moderately qualified for recovery.

Sediment load

The sediment load is one of parameters indicating the hydrological condition of a watershed, calculated from the concentration of sediment (0.0008123 gram/liter), average annual water flow (11.4 m^3/second) in Tabunio Watershed. The result was 29.20 ton/ha/year, so the Tabunio Watershed was categorized as very highly qualified for recovery.

Inappropriate land use and land ownership cause the loss of biodiversity and the loss of topsoil and its nutrient. Saygın et al. (2011) state that land degradation due to soil erosion is one of the most serious problems. Calvo-Cases and Arnau-Rosalen (2007) reported that potential erosion and surface runoff had increased significantly.

Arribas et al. (2003) reported the result of simulation showing that the impact of land degradation on the climate of the Iberian Peninsula depended on local factors, such as level of degradation and geographical location.

Flood

Flood, in this study, is defined as the spill of water from the rivers in Tabunio Watershed, inundating usually-dry areas, causing significant loss materially and immaterially to human and the environment. The data required for this study were flood frequency collected from reports and from direct observation. The data showed that within a year there could be 2-3 times of flooding, indicating that the watershed was categorized as highly qualified for recovery.

Cui et al. (2009) studied the Xiaoqinghe Watershed, China thoroughly to develop a design of river networks in order to reduce flood risk and at the same time to maintain the rivers at their natural condition. The results showed that the design increased connectivity and circuit during the period of low water flow, indicating that the water move faster, reducing the risk of flooding during high water flow.

To improve the success of recovery from flood, all tributaries must be seen as a functional ecosystem, so the recovery can be done integrally as a system. There must be a shift from a tactical approach toward a strategic approach in recovery of rivers and watersheds. Thomas (2014) predicted the possible response of flood frequency with the changes of river network complexity caused by urbanization within a watershed.

Legal function of the area

Forest area is a certain area designated by the government to be maintained as permanent forest (Act no 41, 1999 regarding Forestry). The forest area in Tabunio Watershed is categorized into several forest functions (Table 5). Each forest area has different physical conditions, topography, biodiversity and ecosystem types, so each area is designated to a certain forest function. Most area in Tabunio Watershed is non forest area (52,504.80 ha), while the forest area is only 4.031,82 ha.

Table 5 shows that sub-watersheds of Panjaratan and Tebing Siring have the largest protection forest, namely 1,563.89 ha and 682.90 ha respectively. The utilization of protection forest has the objective of improving the prosperity of rural community and at the same time to encourage community to maintain and improve the function of protection forest for the current and next generation through maintaining sustainable water regulation.

The main function of protection forest is to protect life support system through maintaining water regulation, flood protection, erosion control, sea water intrusion prevention, and soil fertility protection. Protection forest is located in the upstream of catchments area and along the river side, according to its function as water regulator as expected in the Act No. 41, 1999 regarding Forestry.

Changes of landuse in protection forest change the hydrological conditions in the river system, which in turn increase the risk of flooding in the urban areas. Therefore, a rational land use must be implemented in order to give maximum benefit and to minimize the negative impact of flood (Zhang and Wang 2007).

Land capability classes

The success of traditional gold mined land reclamation is determined by the availability of infrastructure and facilities for reclamation. In addition, the success of conservation of water and soil through vegetative method is determined by land capability class of the land unit.

The results showed that most of the land in Tabunio Watershed had land capability classes of III to VIII, indicating that the land could be used for: (i) limited agriculture, (ii) limited to intensive grazing, (iii) forest, and

(iv) nature reserve. Asdak (2010), Ruslan et al. (2013) and Kadir (2013) state that land use based on land capability may improve the function of watershed ecologically as water regulator and economically by increasing land productivity which in turn increases the community's income. The land capability classes in Tabunio sub-watersheds are presented in Table 6 and Figure 7.

Recovery of watershed

Based on the analyses of the characteristics of Tabunio Watershed, the recovery of watershed should be done through the following characteristics:

Enrichment planting for watershed recovery

Highland. Rehabilitation of forest and land must be conducted in the upstream area of the watershed, taking into account the land capability classes and land suitability and using economically and ecologically valuable tree species in order to increase the community's income to conserve the ecosystem. The non-forest area could be enriched with rubber trees, and the area of oil palm plantation must be controlled and it must be enriched with deep-rooted trees. The production forest area should be enriched with economically valuable forest tree species. The protection forest area should be maintained according to its function as water regulator through enrichment planting with forest trees (mahogany, teak, etc).

Lowland. Dry lowland and wetland should be enriched with *rengas*, *anglai* and other species which have high ecological and economic value and suitable to the habitat.

Civil engineering for watershed recovery

The actions needed include: (i) normalization of river previously used for traditional gold mining in order to increase the carrying capacity, normalization of water flow fluctuation and improvement of water flow speed; (ii) maintenance of river as close as possible to its natural condition both in rural and urban settlement; (iii) construction of infiltration wells, lakes and ponds for sedimentation of mining materials before the water flows into the main rivers; (iv) making of *biopori* in oil palm plantation for water and soil conservation by increasing water infiltration.

Regulations for watershed recovery

The activities include: (i) issuing government regulations (or bylaws) regarding the recovery of watershed carrying capacity, so it can function ecologically, socially and economically; (ii) issuing government regulation regarding the planting of deep-rooted along the river side and implementing the regulation in line with related regulations and laws (iii) Limiting the use of land for oil palm plantation only in downstream of Tabunio Watershed.

One of the Tabunio Watershed characteristics was its vegetation cover, which was 39.46%, of the watershed area, indicating that this watershed was categorized as highly qualified for recovery. The percentage of degraded land was 30.55 %, indicating that Tabunio Watershed was categorized as very highly qualified for recovery. The slope of the watershed is mostly 0-7%, indicating that the land is suitable for agriculture activities to increase the prosperity of people living in Tabunio Watershed. The Flow Regime Coefficient was > 15.79, meaning that Tabunio Watershed was categorized as highly qualified for recovery. The coefficient flow was 0.36, indicating the watershed was categorized as moderately qualified for recovery. The sediment load was 29.20 ton/ha/year, meaning that the watershed was categorized as very highly qualified for recovery. According to the people, flooding occurred 2 to 3 times a year, meaning that the watershed was categorized

Table 6. Land capability classes in each sub-watershed of Tabunio Watershed, South Kalimantan, Indonesia

Sub-watershed	Land capability classes	Area (ha)
Amparo kecil	IV e2	3,912.90
Atuatu	IV e2	3,676.86
Bakar	III d3o2, IV e2 and VII I5	3,161.36
Berasau	III d3o2 and IV e2	2,548.65
Kandangan	IV e2 and VIII I6	3,651.57
Panjaratan	III d3o2, IV e2 and VII I5	9.897,04
Riam	III d3o2, IV e2, VII I5 and VIII I6	4,288.91
Takisung	III d3o2 and V o4	9,775.09
Tebing Siring	IV e2, VII I5 and VIII I6	9,341.06
Tungkaran	III d3o2, IV e2 and V o4	12,305.15
Total		62,558.59

Table 5. The legal functions of area in Tabunio Watershed, South Kalimantan, Indonesia

Sub-watershed	Legal functions of the area				Total (ha)
	Protection forest	Production forest	Nature reserve	Non forest area	
Amparo kecil			3,041.11	871.79	3,912.90
Atuatu				3,676.86	3,676.86
Bakar		251.60		2,909.75	3,161.36
Berasau				2,548.65	2,548.65
Kandangan	70.55			3,581.02	3,651.57
Panjaratan	1,563.89			8,333.15	9,897.04
Riam		1,063.59	894.62	2,330.70	4,288.91
Takisung	206.86			9,568.23	9,775.09
Tebing Siring	682.90		2,086.25	6,571.91	9,341.06
Tungkaran	192.42			12.112,73	12.305,15
Total	2,716.62	1,315.20	6,021.97	52,504.80	62,558.59

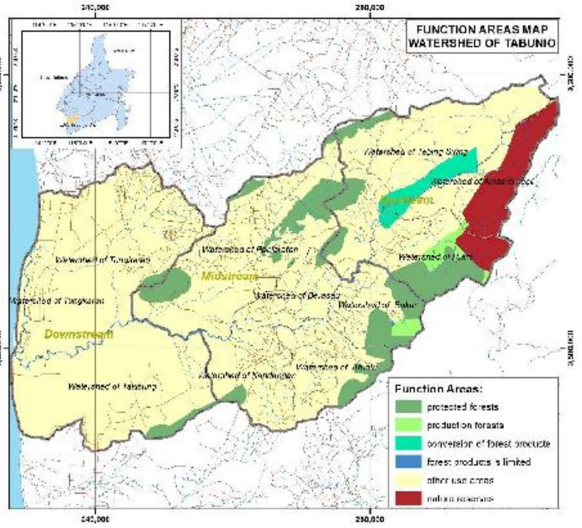

Figure 6. The legal functions of the area in Tabunio Watershed, South Kalimantan, Indonesia

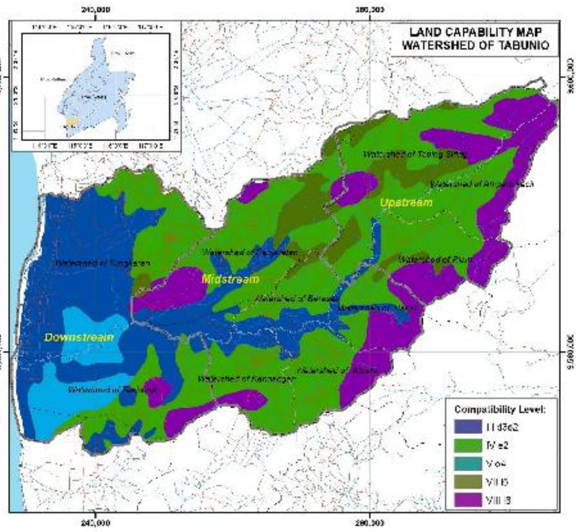

Figure 7. The map of land capability classes in Tabunio Watershed, South Kalimantan, Indonesia

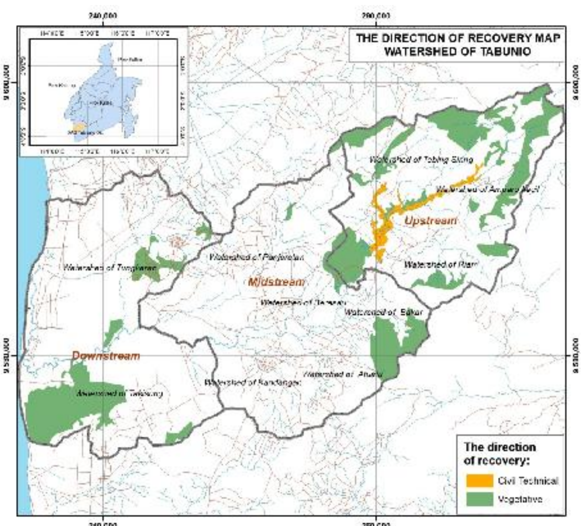

Figure 8. The map of recovery plan for Tabunio Watershed, South Kalimantan, Indonesia

as very highly qualified for recovery. Most of Tabunio watershed area is non-forest area, 52,504.80 ha, while the forest area is only 4,031.82 ha. The land capability classes in the watershed were mostly III to VIII. The use of water in the watershed was for vegetation, domestic and industries.

Based on the analyses of the watershed characteristics, recovery must be done through components of the watershed characteristics, using vegetative technique, civil engineering and related regulations. Watershed is an ecosystem which has several components and functions, whether in upstream, middle or downstream, so the watershed recovery must be conducted integrally, involving all stakeholders, all administrative areas for ecological and economic benefits.

REFERENCES

Arribas A, Gallardo C, Gaertner A, Castro M. 2003. Sensitivity of the Iberian Peninsula Climate to a land degradation. Clim Dyn 20 (5): 477-489.

Arsyad S. 2010. Conservation of Soil and Water. IPB Press. Bogor. [Indonesian]

Asdak C. 2010. Hydrology and Management of Watersheds. 4th ed (revised). Gadjah Mada University Press, Yogyakarta. [Indonesian]

Balitbangda. 2010. Master Plan of Flood Management in South Kalimantan. Center for Research and Regional Development of South Kalimantan Province, Banjarmasin. [Indonesian]

BPDAS Barito [Barito Watershed Management]. 2013. Updating of Spatial Data of Degraded Land in the Working Area of the Office of Barito Watershed Management. The Office of Barito Watershed Management, Banjarbaru. [Indonesian]

Bukhari, Febryano IB. 2008. Design of agroforestry on degraded land. A case study in Indrapuri Sub-district, Aceh Besar District. Jurnal Perennial 6 (1): 53-59. [Indonesian]

Chen P, Chen X. 2012. Spatio-temporal variation of flood vulnerability at the Poyang Lake Ecological Economic Zone, Jiangxi Province, China. Water Sci Technol 65 (7): 1332-1340.

Cui B, Wang C, Tao W, You Z. 2009. River channel network design for drought and flood control: A case study of Xiaoqinghe River basin, Jinan City, China. J Environ Manag 90 (11): 3675-3686.

Fox DM, Witz E, Blanc V, Soulié C, Navarro MP, Dervieux A. 2012. A case study of land cover change (1950-2003) and runoff in a Mediterranean catchment. Appl Geogr 32 (2): 810-821.

Indonesian Act no. 32/2009 regarding the Environmental Protection and Management. [Indonesian]

Indonesian Act no. 41, 1999 regarding Forestry. [Indonesian]

Kadir S, Rayes ML, Ruslan M, Kusuma Z. 2014. Watershed Management for Flood Control in Jaing Catchment Area, Negara Sub-watershed, South Kalimantan Province. [Ph.D. Dissertasion]. Graduate School, University of Brawijaya, Malang. [Indonesian]

Kometa SS, Ebot MAT. 2012. Watershed degradation in the Bamendjin Area of the North West Region of Cameroon and its implication for development. J Sustain Dev 5 (9): 75-84.

Liu Y, Chen Y. 2006. Impact of population growth and land-use change on water resources and ecosystems of the Arid Tarim River Basin in Western China. Intl J Sustain Dev World Ecol 13 (4): 295-305.

Ma Y, Li G, Ye S, Zhang Z, Zhao G, Li J, Zhou C. 2010. Response of the distributary channel of the Huanghe River estuary to water and sediment discharge regulation in 2007. Chinese J Oceanol Limnol 28 (6): 1362-1370.

Meng L-Z, Martin K, Weigel A, Liu J-X. 2011. Impact of rubber plantation on carabid beetle communities and species distribution in a changing tropical landscape (southern Yunnan, China). J Insect Conserv 16 (3): 423-432.

Ministry of Forestry Decree No. P.60/MENHUT-II/2014 regarding Determination of Criteria for Watershed Classification. [Indonesian]

Polonskii VF, Solodovnikova TY. 2009. Estimation of transformation of flood runoff hydrographs and water stages In the Lower Volga and its delta. Russian Meteorol Hydrol 34 (9): 618-627.
Rueda X. 2010. Understanding deforestation in the Southern Yucatán: Insights from a sub-regional, multi-temporal analysis. Region Environ Change 10 (3): 175-189.
Ruslan M, Kadir S, Sirang K. 2013. The Management of Barito Watershed. University of Lambung Mangkurat, Banjarmasin. [Indonesian]
Sajikumar N, Remya RS. 2015. Impact of land cover and land use change on runoff characteristics. J Environ Manag 161: 460-468.
Saud I. 2007. The Analyses of Flood Control in Pematusan area, Surabaya. Jurnal Aplikasi 3 (1): 1-10. [Indonesian]
Saygın SD, Basaran M, Ozcan AU, Dolarslan M, Timur OB, Yilman FE, Erpul G. 2011. Land degradation assessment by geo-spatially modeling different soil erodibility equations in a semi-arid catchment. Environ Monit Assess 180 (1-4): 201-215.
Thomas G. 2014. Improving restoration practice by deriving appropriate techniques from analysing the spatial organization of river networks. Limnologica-Ecol Manag Inland Waters 45: 50-60.
Zhang H, Wang X. 2007. Land-use dynamics and flood risk in the hinterland of the Pearl River Delta: The case of Foshan City. Intl J Sustain Dev World Ecol 14 (5): 485-92.
Zhang L, Wang J, Bai Z, Chunjuan L. 2015. Effects of vegetation on runoff and soil erosion on reclaimed land in an opencast coal-mine dump in a loess area. Catena 128: 44-53.
Zhao Y, Zhang K, Fu Y, and Zhang H. 2012. Examining land-use/land-cover change in the Lake Dianchi Watershed of the Yunnan-Guizhou Plateau of Southwest China with remote sensing and GIS techniques: 1974-2008. Intl J Environ Res Pub Health 9 (11): 3843-65.

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Anther culture of local upland rice varieties from East Kalimantan: effect of panicle cold pre-treatment and putrescine enriched medium

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Abstract. *Nurhasanah, Pratama AN, Sunaryo W. 2016. Anther culture of local upland rice varieties from East Kalimantan: effect of panicle cold pre-treatment and putrescine enriched medium. Biodiversitas 17: 148-153.* Anther culture has been evaluated as an effective method for homozygous plant production which is very important for the hybrid breeding program in rice. Low number of green plantlets is a main obstacle in the application of this technique. Culture conditions increasing anther culture efficiency can be applied to solve this problem especially for low regeneration frequency. This study aimed to investigate the effect of panicle cold pre-treatment and the addition of putrescine into culture medium to the anther culturability of local upland rice varieties originated from East Kalimantan. Two cold pre-treatment times, 8 and 10 days, at 4°C and anther culture medium with and without putrescine were examined in this research. The result showed that cold incubation time effect was a genotype-dependent in influencing the ability of rice microspores to develop into callus and green plantlet regeneration. The longer cold incubation time, 10 days, resulted in the highest number of callus, plantlet as well as green plantlets in Serai Gunung cultivar but the opposite result was found in Geragai cultivar. The effect of putrescine addition in the culture medium was also observed as a genotyped-dependent. It can either increase number of calli, plantlets and green plantlets or decrease them on a specific genotype.

Keywords: Anther culture, Panicle cold pre-treatment, Putrescine, East Kalimantan upland rice,
Abbreviations: IBA (Indole-3-butyric acid), MS (Murashige and Skoog medium), NAA (1-Naphthaleneacetic Acid)

INTRODUCTION

Hybrid rice technology is one of the breakthroughs to overcome increasing global demand for rice. Yield increasing of 15-20% was reported can be achieved through this technique (Virmani et al. 1997; Virmani and Kumar 2004). The development of hybrid varieties cannot be separated from the homozygous line production, which involves numerous cycles of selfing using conventional breeding method. In contrast to such technique, the application of anther culture technology can speed up the breeding process within one generation and significantly shorten the hybrid rice production.

Progress has been made to improve the in vitro androgenic response; nevertheless the application of this technique to rice breeding is still remain problem. Various factors influence culturability of anthers under in vitro condition (Silva 2010). Genotype, pre-culture condition and culture media affect the ability of microspore to form callus, and its regeneration to form shoots as well as green plantlets (Datta 2005; Hong and Rui-Zhen 2008; Silva 2010). High anther culture efficiency reflected by high number of green plantlet regeneration is actually the main objective of this method, and it is mainly influenced by the genetics of the donor plant (Yan et al. 1996; Yamagishi et al. 1998). Some genotypes included into *indica* rice subspecies, which have early anther necrosis, poor callus proliferation, and high albino plant regeneration, are known as less responsive varieties to anther culture (Chen et al. 2005; Balachandran et al. 1999, Silva 2010). The poor response of these varieties could be increased by pre-treatment of panicle and modification of nutritional and other supplemental requirements in rice anther culture media.

Stress to heat or cold treatment of the anthers before culture highly influences the induction of microspores to initiate sporophytic pathway in many plant species (Datta 2005). Low temperature shock or cold stress of spikes is the most widely used pre-treatment to improve androgenic response in cereals including barley (Kruczkowska et al. 2002; Haque and Islam 2014), wheat (Slama Ayed et al. 2010), rye (Mikołajczyk et al. 2012) and rice (Caushal et al. 2014). Cold pre-treatment can increase the culturability of anthers by the lack of synchronous development of the tapetum and microspore symmetric division of first pollen mitosis (Nitsch 1974) and delay of pollen and anther wall senescence (Sunderland 1978). Cold pre-treatment effect on rice anther culturability has been examined at different temperature and incubation time. The variation of cold temperature and duration influences the number of embryo/callus formation including development of green plants, and the effects were reported as a genotype-dependent (Trejo-Tapia, et al. 2002; Datta 2005; Khatun et al. 2012).

Effect of media (Herath et al. 2007) and its supplement compound, such as gelling agents (Lee and Lee 1995), carbon sources (Shahnewaz and Bari 2004; Hong and Rui-zhen 2008), amino acids and plant growth regulators (Herath et al. 2008; Lal et al. 2014) in rice anther culture has been evaluated in previous studies. Putrescine, a plant growth regulator included into polyamines group, was reported to increase green plantlet regeneration in rice anther culture. Its addition in rice anther culture media might improve the culturability of less responsive varieties.

Less is known about anther culturability of East Kalimantan local upland rice cultivars, since it has not been optimally exploited in plant breeding program yet, especially for hybrid breeding technology. Some superior varieties such as Mayas Kuning and Buyung, were observed as less responsive varieties (Nurhasanah et al. 2015). Some efforts should be done to increase anther culturability of these varieties. The present study was conducted to evaluate the effect of cold pre-treatment incubation time and putrescine addition in culture media to the anther culturability of local upland rice varieties originated from East Kalimantan, Indonesia.

MATERIALS AND METHODS

Plant material

Four local upland rice cultivars, originated from Kutai Kartanegara District of East Kalimantan, Indonesia were used in this study i.e. Buyung, Geragai, Mayas Kuning and Serai Gunung. The four varieties are classified as *indica* varieties based on their morphological characteristics. The mother plants were grown in green house as explant source plants.

Explant preparation and anther culture procedure

After harvested, panicles were wrapped using aluminum foil and incubated at ± 4 ºC for eight and ten days to evaluate the effect of cold pre-treatment time to rice anther culture. The general procedure for explant preparation and rice anther culture were conducted as explained in Nurhasanah et al. (2015). The effect of putrescine enriched medium to the anther culturability was evaluate by adding 0,1644 g L^{-1} putrescine to the callus induction medium (0,7% (w/v) agar solidified N6 medium supplemented with phytohormones, i.e. 0.5 mg L^{-1} Kinetin + 2 mg L^{-1} NAA and 60 g L^{-1} sucrose and incubated in dark condition (25 ± 2 ° C). Each petri dish is considered as one replication containing ±120 anthers from 20 spikelets.

Embryogenic calli were transferred in regeneration medium (0,7% (w/v) agar solidified N6 + 2 mg L^{-1} Kinetin + 0.5 mg L^{-1} NAA+ 40 g L^{-1} sucrose and placed in a light condition (25 ± 2 ° C). The regenerated green plantlets were transferred on root induction medium (0,7% (w/v) agar solidified MS + 0.5 mg L^{-1} IBA + 30 g L^{-1} sucrose.

Data analysis

Data were analyzed using analysis of variance (ANOVA). Prior to variance analysis, data were transformed using $\sqrt{X+0.5}$. The differences of mean values were analyzed using Duncan Multiple Range Test (DMRT) at α 5%.

RESULTS AND DISCUSSION

Anther culturability of East Kalimantan local upland varieties was evaluated from its androgenic response. In this study, microspores could develop into callus, and regenerate into green plantlets. Subsequently, the green plantlets were acclimatized and grown in green house (Figure 1).

Effect of panicle cold pre-treatment duration

Different cold pre-treatment incubation time of panicles at ± 4 ºC prior to anther inoculation resulted in various anther culturability. Cold incubation of eight days gave a better result than in the ten days, in Geragai variety. In this variety, the incubation time of eight days produced almost two fold of callus and eight fold of green plantlet number than that of in the incubation period of ten days. Contrary to that, in Serai Gunung the longer incubation time of ten days resulted in the highest number of callus and green plantlet formation, as well as anther culture efficiency (Table 1). Cold pre-treatment duration for ten days increased the number of callus and green plantlet formation for about three and five times, respectively than that of the eight days.

A number of studies have been conducted to evaluate the cold pre-treatment effect in rice anther culture (Trejo-Tapia et al. 2002; Sen et al. 2011; Khatun et al. 2012; Rukmini et al. 2013). Cold shock pre-treatment was reported increased the development of callus and green plantlet regeneration from microspore. It could enhance the androgenic response, since it delays the mitotic stages thereby synchronizing the stage of all microspore during pre-treatment. According to Kiviharju and Pehu (1998), some of the positive effects of cold pretreatment on callus induction included delaying of anther wall senescence, increasing of symmetric division of pollen grains and releasing of substances necessary for androgenesis, mainly amino acids and shockthermic proteins. The growth factors stimulating the embryogenesis of microspores can be optimally provided in an appropriate microenvironment during this process (Datta et al. 2005; Chen et al. 2005).

Statistically, different incubation times of eight and ten days had no significant effect to the number of callus, plantlet and green plantlet formation in this study (Table 2), since the incubation period tested in this study was not too different. However, it was observed that the effect of incubation time very depended on the genotype. The same cold incubation duration can either increase or decrease the rate of callus, plantlet and green plantlet production in different genotypes (Figure 2). Serai Gunung significantly produced the highest number of callus, plantlet as well as green plantlet regeneration than Geragai in incubation period of ten days (Table 3, Figure 1). It showed a genotype-dependent effect, verified by a significant interaction of genotype and cold incubation time in variance analysis results, especially for plantlet and green plantlet formation (Table 2).

Table 1. Effect of panicle cold pre-treatment duration to the anther culturability of East Kalimantan upland rice variety

Variety	Cold pretreatment	Callus+		Plantlet#		Green plantlet*		Anther culture eficiency**
		p	%	p	%	p	%	%
Geragai	8 days	65	5.42	49	75.38	23	46.94	1.92
	10 days	37	3.08	9	24.32	3	33.33	0.25
Serai Gunung	8 days	83	6.92	52	62.65	8	15.38	0.67
	10 days	220	18.33	128	58.18	41	32.03	3.42

Note: +Percentage of callus = (Σ of callus /Σ of anther inoculated (1200)) x100%. #Percentage of plantlet = (Σ of plantlet/Σ of callus) x100%. *Percentage of green plantlet = (Σ of green plantlet/Σ of total plantlet) x100%. **Percentage of anther culture efficiency = (Σ of green plantlet /Σ of anther inoculated) x 100%

Table 2. Variance analysis of the effect of genotype and panicle cold pre-treatment duration to the number of callus, plantlet and green plantlet

Source of variation	Number of callus	Number of plantlet	Number of green plantlet
Genotypes (G)	*	*	ns
Cold pre-treatment (C)	ns	ns	ns
G x C	ns	*	**

*Significant at p=0.05; **Significant at p=0.01; nsnot-significant

Table 3. Effect of panicle cold pre-treatment duration to the mean value of callus, plantlet and green plantlet formation

Genotype	Cold pre-treatment duration		Mean
	8 days	10 days	
Callus			
Geragai	6.50 a A	3.70 a A	5.10 A
Serai Gunung	8.30 a A	22.00 a B	15.15 B
Mean	7.40 a	12.85 a	
Plantlet			
Geragai	4.90 b A	0.90 a A	2.90 A
Serai Gunung	5.20 a A	12.80 a B	9.00 B
Mean	5.05 a	6.85 a	
Green plantlet			
Geragai	2.3 b A	0.3 a A	1.30 A
Serai Gunung	0.8 a A	4.1 b B	2.45 A
Mean	1.55 a	2.20 a	

Note: Data presented as mean value from ten replications; Different lowercase and capital letters show significant differences in the same row and column respectively, according to Duncan Multiple Range Test (DMRT) at α= 0.05

Table 4. Variance analysis of the effect of genotype and putrescine to the number of callus, plantlet and green plantlet

Source of variation	Number of callus	Number of plantlet	Number of green plantlet
Genotypes (G)	*	ns	ns
Putrescine (P)	ns	ns	ns
G x P	ns	ns	*

*Significant at p=0.05; **Significant at p=0.01; nsnot-significant

Table 5. Effect of putrescine addition in callus induction medium to the mean value of callus, plantlet and green plantlet formation

Genotype	Callus induction medium		Mean
	Without putrescine	With putrescine	
Callus			
Buyung	3.50 a A	2.00 a A	2.25 A
Geragai	7.50 b AB	3.17 a AB	5.34 AB
Mayas Kuning	2.17 a A	14.83 a B	8.50 AB
Serai Gunung	12.67 a B	7.33 a AB	10.00 B
Mean	6.46 a	6.83 a	
Plantlet			
Buyung	3.17 b A	2.00 a A	2.58 A
Geragai	5.67 b AB	2.00 a A	3.84 AB
Mayas Kuning	1.50 a A	7.67 a A	4.59 AB
Serai Gunung	8.00 a B	5.83 a A	6.92 B
Mean	4.58 a	4.38 a	
Green plantlet			
Buyung	0.17 a A	0.50 a A	0.33 A
Geragai	2.33 b B	0.50 a A	1.42 B
Mayas Kuning	0.17 a A	1.50 a A	0.83 AB
Serai Gunung	1.17 a AB	0.83 a A	1.00 AB
Mean	0.96 a	0.83 a	

Note: Remarks are the same as Table 3.

Table 6. Effect of putrescine enriched media to the anther culturability of East Kalimantan upland rice variety

Variety	Cold pretreatment	Callus+		Plantlet#		Green plantlet*		Anther culture efficiency**
		p	%	p	%	p	%	%
Buyung	No	21	2.92	17	80.95	1	5.88	0.14
	Yes	12	1.67	12	100.00	3	25.00	0.42
Geragai	No	45	6.25	34	75.56	14	45.16	1.94
	Yes	19	2.64	12	63.16	3	25.00	0.42
Mayas Kuning	No	13	1.81	9	69.23	1	11.11	0.14
	Yes	89	12.36	46	51.69	9	19.57	1.25
Serai Gunung	No	76	10.56	48	63.16	7	14.58	0.97
	Yes	44	6.11	35	79.55	5	4.29	0.69

Note: Remarks are the same as Table 1.

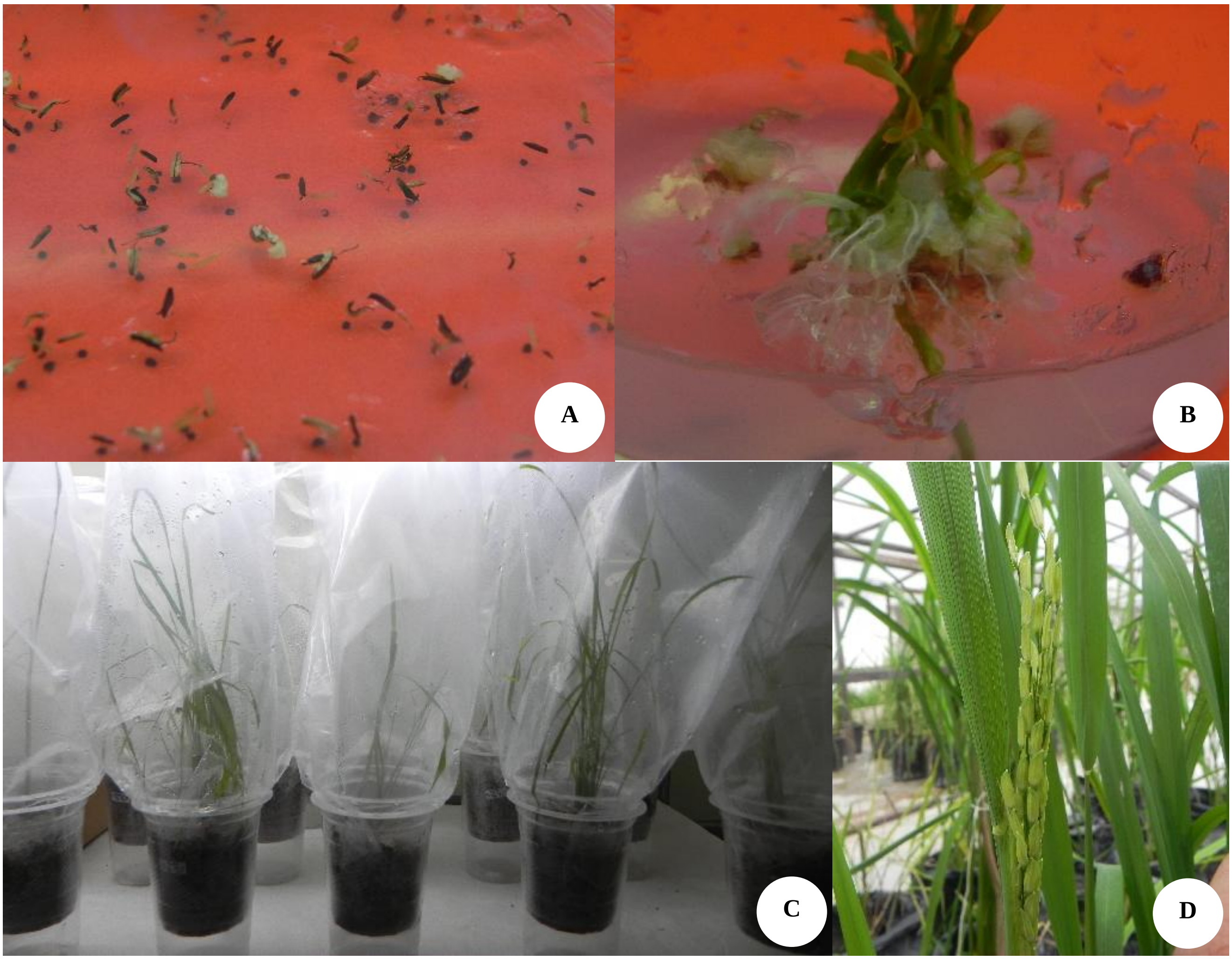

Figure 1. Anther culture of East Kalimantan upland rice varieties, A. Anther forming callus; B. Green plantlet; C. Acclimatization step; D. Fertile plants producing grains

Genotype-dependent effect of cold pre-treatment in rice anther culture, showing genotypic differences for anther culturability at different temperature and duration was also observed in previous studies (Trejo-Tapia, et al. 2002; Datta 2005; Khatun et al. 2012). The optimum temperature and incubation time of cold pre-treatment varied in different genotypes (Genovessi and Magill 1979; Rukmini et al. 2013; Kaushal et al. 2014). Therefore, optimation research to find the optimal temperature and incubation period for particular genotype is very important to increase rice anther culturability.

The presence of putrescine in anther culture medium

The effect of putrescine in anther culture medium did not significantly influence callus, plantlet and green plantlet formation based on analysis of variance (Table 4). Nevertheless, the effect of putrescine varied on different genotypes. The mean value of each genotype was significantly different in the presence and absence of putrescine based on DMRT test (Table 5).

In this study, the presence of putrescine in callus induction medium enhanced anther culturability of Mayas Kuning evaluated from the number of calli, plantlets and green plantlets (Table 6). Mayas Kuning, which was not responsive to anther culture according to previous study (Nurhasanah et al. 2015), showed a better response in putrescine enriched medium.

Putrescine is a naturally occurring low molecular weight polycation, that is obligate requirement for cell growth and sustenance. It has been implicated in many important cellular processes such as cell division, protein synthesis, DNA replication, and response to abiotic stress (Kakkar and Sawhney 2002). Putrescine can improve androgenesis and enhance embryo or callus formation from microspores by inhibiting early senescence of cultured anthers. Putrescine inhibits ethylene biosynthesis, a senescence inducer, because they compete for the same substrate S-adenosyl-methionine (SAM) in their biosynthetic pathway (Dewi et al. 2008).

Putrescine has been reported to increase green plantlet regeneration in rice anther culture (Sasmita 2007). In this current study, the total number of green plantlet could be elevated from one to nine in Mayas Kuning. Interestingly, putrescine enriched medium increased the number of green

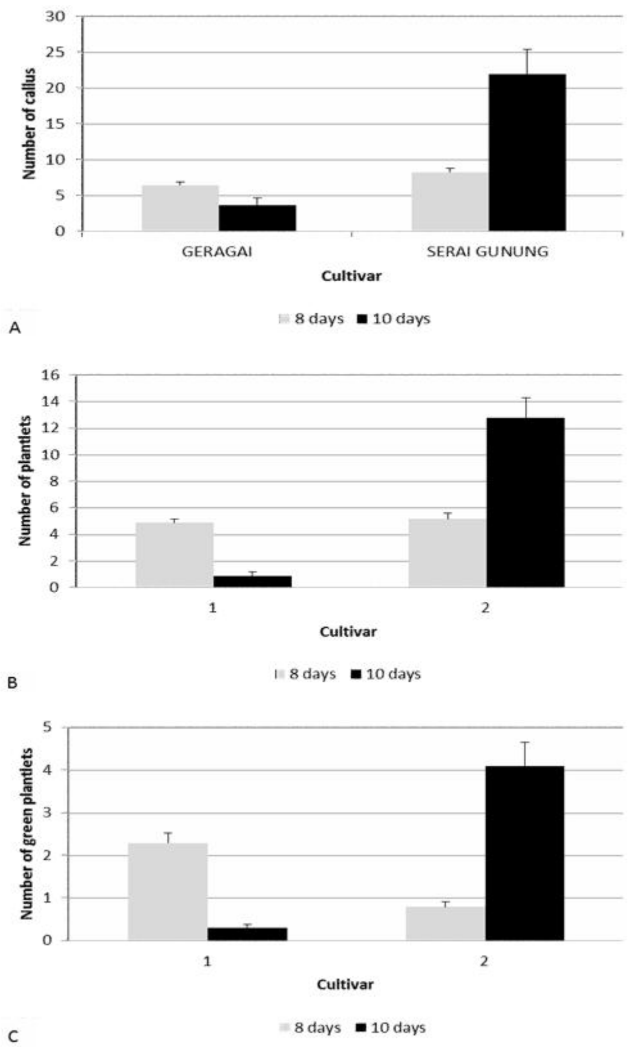

Figure 2. Effect of different cold incubation time of panicles to anther culture of East Kalimantan local upland rice varieties A. Number of callus; B. Number of plantlet; C. Number of green plantlet formation. (Data presented as mean value of original data from ten replications and the standard deviations).

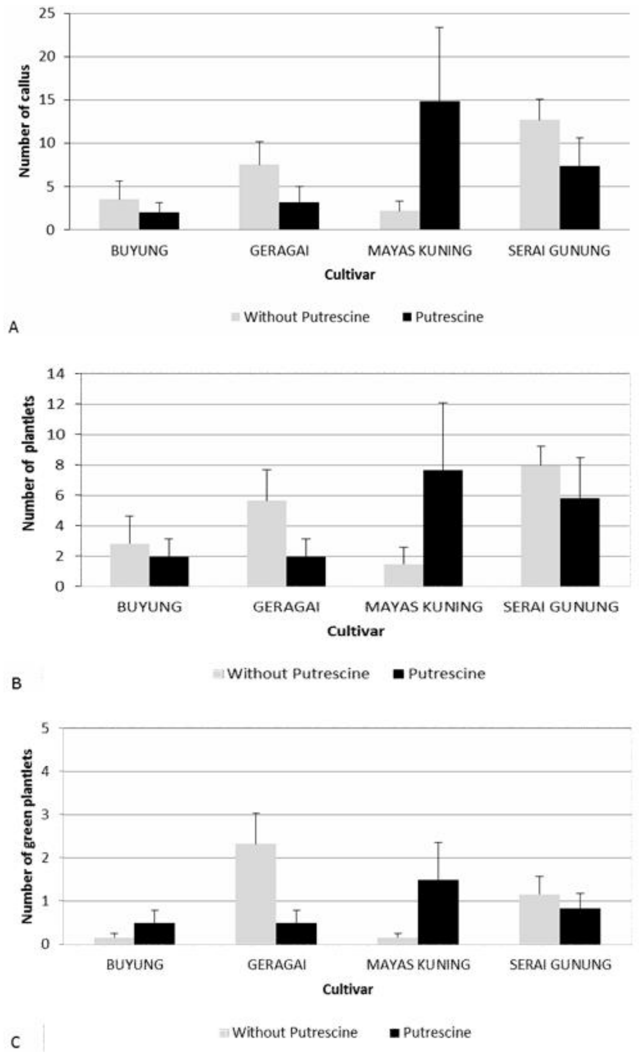

Figure 3. Effect of putrescine in callus induction medium to anther culture of East Kalimantan local upland rice varieties A. Number of callus; B. Number of plantlet; C. Number of green plantlet (Data presented as mean value of original data from six replications and the standard deviations).

plantlets in Buyung cultivar, although the number of callus and plantlet was lower than that of in the medium without putrescine. Therefore, the presence of putrescine in callus induction medium increases anther culture efficiency in Mayas Kuning and Buyung (Table 6).

The occurrence of a large proportion of albinos in anther culture limits the application of this technique to rice breeding program. The accumulation of ethylene in culture vessel might inhibit chlorophyll synthesis and chloroplast development that lead to albino plant formation (George and Sherrington 1984). A higher rate of ethylene production by anthers in *indica* compared to *japonica* types is also suggested as a reason for the poor response of these genotypes to anther culture (Dewi et al. 2008). Therefore, the use of anti-ethylene agent such as putrescine might prevent it and increase the green plantlet regeneration, especially in the improvement of anther culture in a recalcitrant genotype such as in subspecies *indica* which has early anther necrosis, poor callus proliferation, and high albino plant regeneration (Chen et al. 2005).

The effect of genotype x medium interaction, related to the presence or absence of putrescine, was observed in this research. The effect of putrescine was specific for each genotype, in which it can either increase the callus, plantlet and green plantlet regeneration, as found in Mayas Kuning and Buyung varieties, or decrease it on other genotypes (Table 6, Figure 3). The addition of putrescine in anther culture medium significantly reduced the ability of anther to form calli and plantlets as well as green plantlets regeneration in Geragai variety (Table 5). The presence of 0,1644 g L^{-1} of putrescine in callus induction medium decreased the number of callus for almost a half in Serai Gunung and two-thirds in Geragai. Furthermore, a drastic reduction of about 85% of green plantlets was exhibited by Geragai variety (Table 6).

A genotype x medium interaction was also observed by Talebi et al. (2007). A specific media requirement was needed for anther culture by different genotypes. In their study, high frequency of callus induction of a specific medium for a variety has low callusing frequency for another variety and vice versa, showing a genotype-dependent effect. The genotype-dependant effect of putrescine enriched medium in improving androgenesis was also reported in another study. Dewi et al. (2007) observed lower number of calli, callus forming shoots, and green plantlets in the presence than that of in the absence of putrescine in N6 callus induction medium in the F1 genotype from the cross of Taipei 309 x Asemandi. On the other hand, the contrary effect was showed by the F1 of the reciprocal cross.

The genotype-dependent effect of putrescine indicates that a general concentration of putrescine cannot be applied for all genotypes. Redha and Suleman et al. (2011), that studied the effect of exogenous application of polyamine on wheat anther culture, observed that the formation of embryo-like structure and green plantlet varied significantly among genotypes depend on the duration of pre-treatment of anthers using polyamines. These results appear to underscore that each genotype requires a certain concentration of putrescine to optimally improve its androgenic response and increase anther culture efficiency.

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REFERENCES

Balachandran SM, Sarma NP, Siddique EA. 1999. Inheritance of anther culture response in rice. Curr Sci 77 (7): 962-964.

Chen C-B, Xu Y-Y, Ma H, Chong K. 2005. Cell biological characterization of male meiosis and pollen development in Rice. Intl J Plant Biol 47 (6): 734-744.

Datta SK. 2005. Androgenic haploids: Factors controlling development and its application in crop improvement. Curr Sci 89 (11): 1870-1878.

Dewi IS, Purwoko BS, Aswidinnoor H, Somantri IH. 2007. Plant Regeneration in Rice Anther Culture: the Effect of Crosses and Putrescine Application. Bul Agron 35 (2): 6-12.

Dewi IS, Purwoko BS. 2008. Role of polyamines in inhibition of ethylene biosynthesis and their effects on rice anther culture development. Indonesian J Agric Sci 9 (2): 60-67

Genovesi D, Magill CW. 1979. Improved rate of callus and green plant production from rice anther culture following cold shock. Crop Sci 19 (5): 662-664.

George EF, Sherrington PD. 1984. Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories. Exegetic Ltd., England.

Haque M, Islam S. 2014. Application of cold pretreatment and optimisation of media for enhancement of anther culture response in two barley (*Hordeum vulgare* L.) genotypes derived from Bangladesh. Asia Pac J Mol Biol Biotechnol 22 (1): 127-136.

Herath HMI, Bandara DC, Samarajeewa PK, Wjesundara DSA 2008. The effect of plant growth regulators on anther culture response and plant regeneration in selected Sri Langkan indica rice varieties, japonica varieties and their inter sub-specific hybrids. Trop Agric Res 20: 243-250.

Hong C, Rui-zhen Q. 2008. Analysis of different effectors enhancing the anther culture ability of auto-tetraploid japonica rice. J Agric Sci Technol 10 (3): 90-96.

Kakkar RK, Sawhney VK. 2002. Polyamine research in plants-a changing perspective. Plant Physiol 116: 281-292

Kaushal L, Sharma R, Balachandran SM, Ulaganathan K, Shenoy V. 2014. Effect of cold pretreatment on improving anther culture response of rice (*Oryza sativa* L.). J Exp Biol Agric Sci 2: 233-242

Khatun R, Islam SMS, Ara, I, Tuteja, N, Bari MA. 2012. Effect of cold pretreatment and different media in improving anther culture response in rice (*Oryza sativa* L.) in Bangladesh. Indian J Biotech 11: 458-463.

Kiviharju E, Pehu E. 1998. The effect of cold and heat pretreatments on anther culture response of *Avena sativa* and *A. sterilis*. Plant Cell Tiss Org 54: 97-104.

Kruczkowska H, Pawlowska H, Skucinska B. 2002. Influence of anther pretreatment on the efficiency of androgenesis in barley. J Appl Genet 43 (3): 287-296

Lal,D, Shashidhar HE, Godwa PHR, Ashok TH. 2014. Callus induction and regeneration from in vitro anther culture of rice (*Oryza sativa* L.). Intl J Agric Environ Biotech 7 (2): 213-218

Lee JH, Lee SY. 1995. Effects of gelling agents and growth regulators on rice anther culture. Korean J Pl Tiss Cult 22 (91): 35-39.

Mikołajczyk S, Broda Z, Weigt D. 2012. The effect of cold temperature stress on the viability of rye (*Secale cereale* L.) microspores. BioTechnologia 93 (2): 139-142.

Nitsch C. 1974. Pollen culture-a new technique for mass production of haploid and homozygous plants. In: Kasha K (ed) Haploids in Higher Plants. Advances and Potential. Univ. Press, Guelph.

Nurhasanah, Pratama AN, Rusdiansyah, Sunaryo W. 2015. Effect of genotype and developmental stage of pollen on the success of anther culture of local upland rice varieties from East Kalimantan. Asian J Microbiol Biotech Env Sci 17 (2): 329-340.

Redha A, Suleman P. 2011. Effects of exogenous application of polyamines on wheat anther cultures. Pl Cell Tiss Org 105:345-353

Rukmini M, Rao GJN, Rao RN. 2013. Effect of cold pretreatment and phytohormones on anther culture efficiency of two indica rice (*Oryza sativa* L.) hybrids-Ajay and Rajlaxmi. J Exp Biol Agric Sci 1: 69-76.

Sasmita P. 2007. Applications of anther culture technique in rice plant breeding. Indonesian Research Center for Rice, Sukamandi, Subang.

Sen C, Singh RP, Singh MK, Singh HB. 2011. Effect of cold pretreatment on anther culture of boro rice hybrids. Intl J Plant Reprod Biol 3: 69-73.

Shahnewaz S and Bari MA. 2004. Effect of concentration of sucrose on the frequency of callus induction and plant regeneration in anther culture of rice (*Oryza sativa* L.). Pl Tiss Cult 14 (1): 37-43

Silva TD. 2010. Indica rice anther culture: can the impasse be surpassed? Pl Cell Tiss Org Cult 100 (1): 1-11.

Slama Ayed O, De Buyser J, Picard E, Trifa Y, Slim Amara H. 2010. Effect of pre-treatment on isolated microspores culture ability in durum wheat (*Triticum turgidum* subsp. *durum* Desf.). J Pl Breed Crop Sci 2 (2): 030-038.

Sunderland N. 1978. Strategies in the improvement of yields in anther culture. In: Proc. Symp.Plant Tissue Culture. Science Press, Beijing.

Talebi R, Rahemi MR, Arefi H, Nourozi M, Bagheri N. 2007. In vitro plant regeneration through anther culture of some Iranian local rice (*Oryza sativa* L.) varieties. Pakistan J Biol Sci 10 (12):2056-2060

Trejo-Tapia G, Amaya UM, Morales GS, Sánchez ADJ, Bonfil BM, Rodríguez-Monroy M, Jiménez-Aparicio A. 2002. The effects of cold-pretreatment, auxins and carbon source on anther culture of rice. Plant Cell Tiss Org 71: 41-46

Virmani SS, Kumar I. 2004. Development and use of hybrid rice technology to increase rice productivity in the tropics. IRRN 29 (1):10-18.

Virmani SS., Viraktamath BC, Casal CI, Toledo RS, Lopez MT, Manalo JO. 1997. Hybrid rice breeding manual. IRRI, Los Banos, Philippines.

Yamagishi M, Otani M, Higashi M, Fukuta Y, Fujui K, Yano M, Shimada T. 1998. Chromosomal regions controlling anther culturability in rice (*Oryza sativa* L.). Euphytica 103: 227-234.

Yan J, Xue Q, Zhu J. 1996. Genetic studies of anther culture ability in rice (*Oryza sativa* L.). Pl Cell Tiss Org 45:253-258.

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Karangwangi people's (South Cianjur, West Java, Indonesia) local knowledge of species, forest utilization and wildlife conservation

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Abstract. *Partasasmita R, Iskandar J. Malone N. 2015. Karangwangi people's (South Cianjur, West Java, Indonesia) local knowledge of species, forest utilization and wildlife conservation. Biodiversitas 17: 154-161.* In the past, many West Javan Villages had a high diversity of wildlife. Nowadays, however, the diversity of wildlife in these villages has tended to decrease rapidly. This is caused by many factors, including the loss or destruction of wildlife habitat destruction, intensive use of pesticides in the agricultural sector, and illegal hunting. Animal hunting is typically undertaken by villagers for various purposes, such as to fulfill household meet consumption, as well as for the capture and trading of pets. Traditionally, the use of wildlife by the Sundanese people of West Java is influenced by corpus (local knowledge) and cosmos (beliefs). As a result, the wild animals have been utilized within a sustainable system that enables wildlife conservation. Today, however, a lot of traditional knowledge or local knowledge of wildlife has eroded. This paper elucidates local knowledge of Karangwangi villagers of Subdstrict of Cidaun, District of Cianjur, Province of West Java, Indonesia on various species, utilization, and the conservation of wildlife. Methods used in this study are a combination of qualitative and quantitative techniques based on an ethnozoological approach. The result of study shows that the respondents recognize at least 45 species of wildlife consisting of 15 mammalian species, 21 species of Aves, and 9 species of herpetofauna. Based on the perception of respondents, those wildlife species have various socio-economic and cultural saliences (eg. household meat consumption, pets, trading, and appearing in mythology), as well as various ecological functions (e.g., crop pests, pest controls, and seed dispersal). Some cultural myths related with wildlife have been recorded and partly determine the utilization of wildlife by village people.

Keywords: Animal conservation, animal function, ethno-zoology, local knowledge, myth

INTRODUCTION

Indonesia is distinctive because it has a large number of islands and extremely high-levels of both biological and cultural diversity. In terms of biodiversity, more than 720 species of mammals, 1,599 species of birds, 723 species of reptiles, 385 species of amphibians, and 1,248 species of fish have been recorded (Sutrisno et al. 2014). Meanwhile, regarding cultural diversity, Indonesia has more than 30 ethnic groups with 655 mother languages. Indonesia ranks second of the total 25 countries in the world (after Papua New Guinea with 847 mother languages) with respect to its endemic local languages (Maffi 1999).

Historically, various ethnic groups in the world, including Indonesian ethnic groups had temporally deep, close relationships with wildlife (cf Jorgensen 1998, Iskandar 2012). Various wildlife, including mollusks, fish, amphibians, reptiles, birds, and mammals have some socio-economic and cultural functions (Alves 2012).

In the past, village people utilized various wildlife species (praxis) which is strongly embedded by corpus (knowledge) and cosmos (beliefs) (see Toledo 2000, Carlson and Maffi 2004). Local knowledge, indigenous knowledge, and traditional ecological knowledge have been widely discussed by many scholars (Ellen and Haris 2000). In the course of the 1990s, a major development was the growing recognition that one emergent property of ecosystems is that significant human presence and human resource use over time forms a biocultural system-a system jointly shaped by biological and cultural dynamics. Moreover, this in turn lead to the realization that local ecological knowledge, beliefs and practice have much to contribute to the conservation of biodiversity as well as to sustainable use of natural resources (Carlson and Maffi 2004). Based on this concept, some empirical examples can be provided in Indonesia and other countries. For example, an ethnobiological study of local rice varieties of swidden cultivation (*huma*) undertaken by Iskandar and Ellen (1999) revealed that at least 89 local rice varieties of the swidden system (*huma*) have traditionally been conserved by Orang Baduy through local knowledge and beliefs. In addition, culturally, the Tengger people abstain from killing animals, and they consume more vegetables, whole grain varieties, canna, taro, and cassava. Catapult tool was banned because it can kill birds (Batoro et al. 2012). Another example, the Iban community in Sarawak has traditionally prohibited hunting some animals, such as gibbon (*Hylobates* spp.), barking deer (*Muticus muntjak*), crocodile (*Crocodiulus porous*) and brahminy kite sea-eagle (*Haliastur indus*) due to their belief that animals consider as sacred animals (Horowitz 1998). As a result those animal species have been traditionally conserved by the local community.

Nowadays, however, the utilization of wildlife by many ethnic groups in Indonesian villages, including the Sundanese villages, is determined by economic factors instead of being based on local knowledge and cosmos. In other worlds, the sustainable use by local people or village people, based on local knowledge and beliefs, has tended to erode due to various factors including rapid cultural and socio-economic change. Moreover, various species of wild animals are freely and intensively hunted by many people for various purposes, including meet consumption, pet animal, and trading. The hunting of animals by local people out of economic necessity ignores some of the ecological benefits of wildlife (Alves and Souto 2015). Consequently, the diversity of wild animals has tended to decrease. In addition, destruction or loss of animal habitats and intensive use of pesticide in agriculture have caused additional decreases of wildlife diversity.

Based on ecological history, it has been recorded that some animals species became rare or extinct in local areas in Indonesia in the last few decades. For example, the Javan tiger (*Panthera tigris sondaica*) disappeared in Java in the 1980s (Whitten et al. 1999). Similarly, the Javanese lapwing (*Vanellus macropterus*, trulek jawa) was considered extinct in Java since the 1940s (Mackinnon et al. 1992). In addition, in 1991 the straw-headed bulbul (*Pycnonotus zeylanicus)* had been still found in Pananjung-Pangandaran Natural Reserves, but in 2005 have disappeared from this area (Partasasmita 2015). Similar events could likely be occurring in other places, especially in non-conservation areas.

In the past, the Village of Karangwangi, Sub-district of Cidaun, District of Cianjur, West Java had a rich diversity of animal species, due to the large size of forested wildlife habitats found in this village. In addition, in the neighboring village areas, the government establishes the nature conservation area of Bojonglarang Jayanti. Today, however, the wildlife diversity of Karangwangi Village is in decline due to many factors, such as the reduction of forests from conversion to other land use types, such as settlements and agricultural areas. In addition, socio-economic and cultural local people have rapidly changed due to the construction of a new road to connect the village area with the urban area of Cianjur and Bandung. As a result, an ethnozoological study to understand the utilization and conservation of wildlife by Karangwangi people is considered to be important. Hopefully, results of this study will provide information in relation to changes of the diversity of genetics, species, ecosystems, erosion, and utilization as a result of cultural changes, and progress practical information on the interlinked relationships within ecosystems (Rambo 1983). In this study, we focus on local knowledge of wildlife, specifically: (i) local knowledge on the various wildlife species in this area; and (ii) the people's perception of wildlife functions, both cultural/ socio-economic and ecological, including wildlife conservation based on the local people's mythology system.

MATERIALS AND METHODS

Study area

Geographically, the study area, Village of Karangwangi lies between 7° 25'-7° 30' S and 107° 23'-107° 25' E. Administratively, this village belongs to Sub-district (*kecamatan*) of Cidaun, District (*kabupaten*) of Cianjur, Province (*propinsi*) of West Java, Indonesia. The location of Karangwangi is approximately 120 km from the city of Bandung and approximately 70 km from the city of Cianjur. To reach this area by vehicle requires a travel time of 5-6 hours from Bandung and approximately 3-4 hours from Cianjur. Karangwangi Village is a relative remote area lies near the south coast of the Indian Ocean. The village is directly bordered by the nature conservation area of Bojonglarang Jayanti Natural Reserve. The north borders Cimaragang Village, extending east to Garut, and west to the Village of Cidamar. The southern boundary is the Indian Ocean. The most common livelihood of the Karangwangi people is recorded as subsistence farmers. Geographical location facilitates the development of the agricultural sector. Aproximately 2,000 hectares of land-use type in the Village of Karangwangi is recorded as the rain-fed agricultural land (Figure 1).

Procedure

The method used in this study is a combination of qualitative and quantitative, which is based on study ethnozoological or biological approach (cf. Martin 1995; Newing et al. 2011; Iskandar 2012). Quantitative methods were used in the form of a structured interview using a questionnaire for 91 respondents. Selection of respondents was using simple random technique determined by the formula of Lynch et al. (1974). Some techniques of collecting qualitative data were applied, namely observation and interviews. Additionally, we observed the diversity of wildlife in the surrounding villages and forests of Bojonglarang Jayanti Natural Reserve. Meanwhile, in-depth interviews with informants was purposively selected informants via snowball sampling, with attention to a diversity of informants was undertaken. Informants include wildlife hunters, informal community leaders, and the formal village leader and his staff.

Data analysis

The qualitative data were analyzed by means of cross-checking, summarizing, synthesizing, and narrated by descriptive analysis. Meanwhile, quantitative data from interviews with respondents was tabulated by simple statistic, namely by calculated percentages of respondent reply, and narrated by descriptive analysis (Newing et al. 2011).

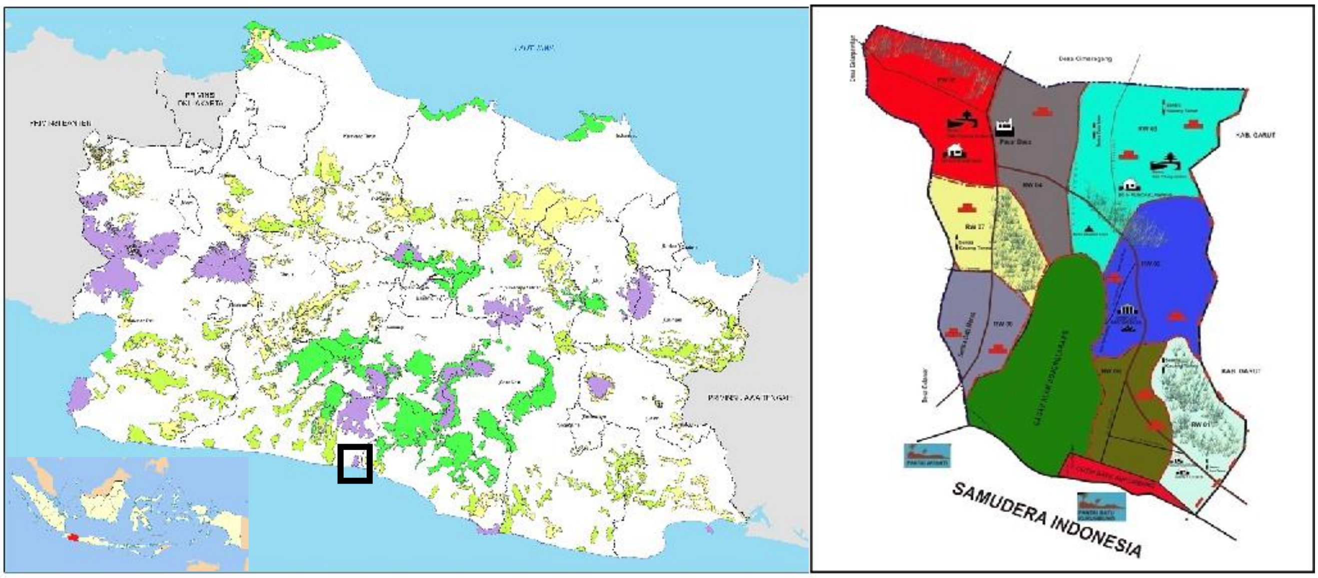

Figure 1. Research location, Karangwangi Village, Cianjur District, West Java, Indonesia

RESULTS AND DISCUSSION

The local knowledge on diversity of wild animals

The respondents of Karangwangi community recognize many wildlife species present in the environment both in the forest and around the settlement or hamlet (*kampung*). As many as 15 species of mammals, 21 species of birds, and 9 species of herpetofauna are known to the people (Table 1). The people are able to distinguish wildlife species based on morphological characters, habitats, and ecological role. Based on interviews with 91 respondents, we found that 100% of the respondents know that species of wildlife are in the forest, and 88% of respondents know the pig (wild boar, *Sus scrova*) as the wildlife, while only 1.1% could distinguish between biul and careuh; both are Asian palm civet (*Paradoxurus hermaproditus*).

Interviews revealed that many people commonly consider the wild boar to be a pest due to frequently entering villages and agricultural land causing damage to crops. In addition, people are able to describe the characteristics of wild boar that become pests as having a body length of about 1-1.5 meters and weighing up to 80 kg as adults. Moreover, based on respondent knowledge that wild boar body size is considered to be smaller in females than in males. The wild boar has grayish black hair, with some brown or reddish hair covering their bodies. Although the Village of Karangwangi is mountainous, wild boar are recognized as one of animals with a very quick running ability.

In addition, the respondents have accurate perception in identifying several other species of wildlife, such as biul and careuh, although there are characteristic distinctions as noted in the description received from the respondents; approximately 98.9% of respondents believed that biul and careuh are considered as animals. Biul has a characteristic reddish gray, while careuh is the mottled gray and black. Careuh sometimes eat fruit or sugar palm fruit (*Arenga pinnata* (Wurmb) Merr), but if biul go into the village they will steal chickens and eat them.

Karangwangi people have knowledge or familiarity with birds. For example, based on interview with respondents, it showed that 89.0% of respondents recognize several different bird species. One bird that is predominantly known by the respondents is the eagle (18.7%). The eagle easy to remember by respondents because the eagle is a large-sized bird, and can be distinguished by the clear color. Respondents recognize two types of eagles in the area of the village of Karangwangi. The first type is a black eagle or so-called ordinary heulang (*Ictinaetus malayensis*) and the second type is the white-bellied sea eagle or called as heulang bodas (*Haliaeetus leucogaster*). Black eagles nest in tall forest trees. Like the white-bellied sea eagle, they are active during the day. White-bellied sea eagle has a characteristic white feather. Food of white-bellied sea eagle is namely fish. In addition, the respondents generally understand about the nesting location of white-bellied sea eagle, i.e. around the forested edge of the nature reserve near beach.

However, people know the birds are believed to be related and considered mystical, but seem unable to describe this clearly (Table 1). For example, bird species which are strongly related with mythology are recognized as *Loklok* bird. The bird is considered the most mysterious bird, so nobody knows the precise species of the original form of this bird. But people believe in its existence and encounters are believed to be sightings of supernatural beings. Loklok can be transformed into various forms of birds like the peacock, changeable hawk-eagle (*Nisaetus cirrhatus*), hawks, crows and ayam brontok (Bangkok chicken, *Gallus gallus domesticus*). These birds are named with *loklok* because it has the sound hired as similar to *lok-lok-lok*. These birds are believed to bring misfortune or pain when his voice is heard in the village. As a result culturally, the people are prohibited to hunt or kill this bird. This bird has been traditionally conserved by local people. As a result, this kind of the traditional conservation has tended more effective compared to that of the governmental formal conservation (see Maffi 2004; Iskandar 2012).

Our results indicate that 90.1% of respondents recognized the different types of herpetofauna as wildlife. The herpetofauna that is most well-known to people (23.2%) is an Indo-Chinese rat snake (oray sawah, *Ptyas korros*), while the lowest was only 1.2% recognition of the tokay gecko (toke, *Gekko gecko*). This is because this animal is often found when people go to the fields. According to the respondents of Karangwangi, snake habitats are rice paddies. Snake scales of Indo-Chinese rat snake are recognized as black and white. The main prey item of this snake is frogs. This snake commonly nests in the holes of moist soil. The length is approximately 30-40 cm and is not venomous. The Indo-Chinese rat snake is active at night.

Although very few respondents who knew the tokay gecko categorized them as wild animals, respondents of Karangwangi community who knew this animal can describe clearly the tokay gecko (*Gekko gecko*). According to the respondents, a gecko is a reptile that slithers like a lizard. They have a size greater than lizards and have a gray color with slightly bluish skin with little red spots. Gecko is active at night and prey on small insects. Geckos prefer a special kind of habitat in trees of the forest, but the gecko can also be found in the houses of village people. Gecko vocalizations are pretty typical, with a very loud call that sounds like *gecko* repeated several times. Geckos are commonly captured and sold for use as traditional, medicinal materials.

Respondent knowledge on amphibians revealed that 12.1% recognize the various species of amphibian as wildlife. According to the respondents, there are three types of frogs. Firstly it is recognized that there are rice frogs or bancet (*Fejervarya limnonectes)*. Secondly, there is budug frog that respondents often call it as bangkong korodok (*Bufo melanotictus*). Naming bangkong korodok is based on it skin texture or as scabies (*budug)*. The last type of the frog is the well-known 'Common tree frog' or bancet kole.

Local people's perceptions on wildlife based upon ecological and socio-economic functions

People's perceptions of wildlife are strongly influenced by the local culture. In general, studies of ethnozoology have revealed that many indigenous peoples' beliefs are related to the use of animals, such as animals being used for traditional medicine as culturally practiced by the Malay and the Kubu community (Maryanto et al. 1993) and used as food and ritual of Tengger community (Baroto et al. 2012). Like the Malay, Kubu and Tengger communities, various animals have also played an important role for the community of Karangwangi Village, West Java.

Based on information from the respondents, in terms of the functions, the wild animals can be grouped into three categories, namely: (i) wildlife that play a role in ecological functions (e.g., seed dispersal, pest control, pest); (ii) wildlife that play a role in social-cultural functions (e.g., mystical, animals for ritual, a sign of the change of seasons); and (iii) wild animals that play an important role in the economic functioning of the community (e.g., food, traditional medicine, souvenirs) (Table 1; Figure 2.C).

The main source of livelihood of Karangwangi community is agriculture, so the presence of gardens (kebun) and perennial mixed-garden (talun) be important for fulfill daily household need of the community. Therefore, any damage of garden crops caused by wildlife can be considered as pests. The existence of these pests eliminated by various means such as by way of being hunted, killed with a knife or poisoned by using chemical toxic. Most pest animals have resided in the forest of nature conservation of Bojonglarang Jayanti. However, when the animal food supply has reduced in the forest and then wildlife have also attacked the crop gardens of the villagers for foraging.

Some wildlife has been considered an important role in the ecological, social and economic function for community of Karangwangi Village includes:

Figure 2. Wildlife species in Karangwangi Village, West Java, Indonesia: A. Wild boar in Bojonglarang Jayanti natural reservation; B. Cave swiftlet on swiftlet house; C. Wing feathers of White-bellied sea eagle-flesh removed to be eaten and wings for trading

Tabel 1. Various wildlife recorded in the Village of Karangwangi, West Java, Indonesia

Local name in Sundanese (Vernacular)	English name	Scientific name	Perception of respondents on wildlife		
			Ecological function	Social function	Economic function
Mamalia					
Bagong/Jadok	Wild boar	*Sus scrofa*	Pest	Myths	
Muka	Sunda slow loris	*Nyticebus caucang**		Myths	
Monyet	Crab-eating macaque	*Macaca fascicularis*	Pest		Traditional medicine
Lutung	Javan lutung	*Trachypithecus auratus**	Pest	Myths	Traditional medicine
Biul	Asian palm civet	*Paradoxurus hermaphroditus*	Pest		
Careuh	Asian palm civet	*Paradoxurus hermaphroditus*	Seed dispersal		
Meong congkok	Leopard cat	*Prionailurus bengalensis*	Pest		
Sero	Oriental small-clawed otter	*Amblonyx cinerea*	Pest		
Tikus	Rat	*Rattus rattus*	Pest		
Codot	Reusettus	*Reusettus amplexicaudatus*	Seed dispersal		
Kalong	Large flying fox	*Pteropus vampirus*	Seed dispersal		
Landak	Porcupine	*Histryx javanica**		Myths	Trading, traditional medicine
Peusing	Pangolin	*Manis javanica**		Myths	Trading, traditional medicine
Kancil	Java mouse-dee	*Tragulus javanicus**			
Mencek	Indian muntjac	*Muntiacus muntjak**			Meat for food consumption
Aves					
Heulang	Black eagle	*Ictinaetus malayensis**	Pest control		
Heulang bodas	White-bellied sea eagle	*Haliaeetus leucogaster**	Pest control		
Toed	Long-tailed shrike	*Lanius schach*	Pest control/ seed dispersal		Trading
Cikblek	Bar-winged prinia	*Prinia familiaris*	Pest control/ seed dispersal		Sale
Anis	Orange-headed thrush	*Zoothera citrina*	Pest control/ seed dispersal		Sale
Ungkut-ungkut	Coppersmith barbet	*Megalaima haemachepala*	Pest control/ seed dispersal		Sale
Perenjak	Common tailorbird	*Orthotomus sutorius*	Pest control/ seed dispersal		Sale
Piit	Javan munia	*Lonchura leucogastroides*	Pest		
Peking	Scaly-breasted munia	*Lonchura punctulata*	Pest		
Srigunting	Black drongo	*Dicrurus macrocercus*	Pest control		
Tikukur	Spotted dove	*Streptopelia chinensis*	Pest		Trading, traditional medicine
Perkutut	Zebra dove	*Geopelia striata*)	Pest		Trading, traditional medicine
Ekek	Red-breasted parakeet	*Psittacula alexandri*	Pest control/ seed dispersal		
Wallet	Cave swiftlet	*Collocalia linchi*	Pest control		Nest for trading
Caladi	Fulvous-breasted woodpecker	*Dendrocopos macei*	Pest insect control		-
Cakakak	Javan kingfisher	*Halcyon cyanoventris**	Pest		-
Cangehgar	Red jungle fowl	*Galus galus varius*	Seed dispersal		
Bueuk	Barn owl birds	*Tyto alba* javanica		Myths	
Uncuing	Rusty-breasted cuckoo	*Cacomantis sepulcralis*		Myths	
Gagak	Jungle crow	*Corvus macrorhynchos*		Myths	
Loklok		Unidentified		Myths	
Herpetofauna					
Ular sanca	Indian python	*Pithon morulus**	Pest		
Oray sendok	King kobra	*Ophiophagus hannah*	Pest control		Trading
Oray sawah	Indo-Chinese rat snake	*Ptyas korros*	Pest control		
Oray sapi	Radiated ratsnake	*Coelognathus radiate*	Pest control		
Oray kisi	Striped keelback	*Xenochrophis vittatus*	Pest control		
Oray kawat	Brahminy blind snake	*Ramphotyphlops braminus*	Pest control		
Toke	Tokay gecko	*Gekko gecko*	Pest control		Trading, traditional medicine
Bancet	Common pond frog	*Fejervarya limnonectes*	Pest control		For food consumption
Bancet kole	Common tree frog	*Polypedates leucomystax*	Pest control		
Bangkong	Javanese toad	*Bufo melanosticus*	Pest control		

Note: *) Protected animal in Indonesia based on UU No.5, 1990 and PP.27, 1999 (Noerdjito and Muryanto 2001). Source: interview with informants and field observations (2015)

Wild boar (*Sus scrofa*) is popularly known as animal crop pest in the agriculture of the Karangwangi community (F = 21.067; α = 0.05). This animal usually attack legumes, tubers, cassava and other crops. Because the wild boar has attacked food crops of plantation and agricultural area, it has considered as animal pest. The wild boar has frequently come to agricultural areas due to limitation of her habitat in the nature conservation forest of Bojonglarang Jayanti. In addition, the village garden areas are directed adjacent to the nature conservation forest area (Figure 1). Pig is also known as one of hunting animals for the local and non-local communities. For example, rural communities of Cimarel Village of West Bandung district have frequently undertaken pig hunting (Ind.: *berburu babi*) in the forest (PLN-LPPM Unpad 2014). Indeed, bearded pig (*Sus barbatus*) has been the most abundant mammals species caught by Penan and Kenyah who reside in Long Peliran, Kalimantan and contributing of all catches and 91% of all edible meat yielded during the study period (Puri 1997). Moreover, according to the respondents of Karangwangi community, pig was considered as a mythical animal in the past time. Pig was considered old animal can turn into very creepy animals, attacking and eating livestock of village community. Based on one of village elder informants of the Karangwangi villagers mentioned that the myths associated with wild boar commonly called *ragujik*. Recorded 62% of respondents of Karangwangi Village community mentioned that they knew the myth of the wild boar and 38% did not know the myth of the wild boar. Ragujik pig boar is a large, old, and be able to dig soil with deep enough. The pig stays in the cave, where people find the bones of wild boar. Respondents suspect that consuming wild boar is a pig ragujik.

Crab-eating macaque (*Macaca fascicularis*) and Javan langur or lutung (*Trachypithecus auratus*) are considered as pests, hunted for disrupting or damaging crops of Karangwangi Village community. Based on the respondents they believe that monkey is considered as a pest ($F = 13.853$; $\alpha = 0.05$), whereas the Javan langur considered by little a number of respondents as not pests ($F = 0.594$; $\alpha = 0.05$). Hunting monkeys and langurs is done by shot. Apart from being a pest, monkeys and langurs are hunted which its meat are culturally used for traditional medicine, particularly for an asthma medication medicine.

Not all wild animals that went into the gardens of the village community are considered as pest. But some of them are considered as beneficial animals for the village community. For example, the groups of migration butterflies which regularly move from west to south are usually used as an indicator of seasonal change and can be used as agricultural calendar to start planting crops in the agriculture area.

Asian palm civet or careuh (*Paradoxurus harmaproditus*) is recognized as a fruit-eating animals or palm seed eating. According to the respondents, civet in one hand is considered as pest, but on the other hand has the advantage of spreading seed plants including seeds of kawung or arenga palm seed (*Arenga pinnata* (Wurmb) Merr). Careuh can spread arenga palm seeds by eating the seeds and then released back through her feces. The droppings palm seeds then grow became palm trees. According to respondents mentioned that most arenga palm trees grown in the Karangwangi Village have predominantly been assisted by civet through seed dispersal of her feces. Because, the arenga palm seeds that grown by people have rarely succeeded. However, the respondents mentioned also that civet (*Biul*) is considered as chicken predator in the village ($F = 7.115$; $\alpha = 0.05$)

Orange-headed thrush or anis (*Zoothera citrina*) is recognized as a bird which has various foods, such as grain, insect and other invertebrates. As a result, this bird is considered as beneficial bird due to helping in spreading the plant seeds and pest control. In contrast, however, the villagers of Karangwangi hunt the orange-headed thrush for bird trading in urban bird markets. The bird has been intensively hunted by local people due to has high demand for pet animal and popularly as bird keeping in the cages and has been intensively involved in the contest bird song in a regular time in urban area, conducted in weekly, three monthly and annually, for the level of local, regional and national event, respectively. As a result, this bird has a high economic price and popularly trading in the urban bird markets (Iskandar and Iskandar 2015). Orange-headed thrush is usually sold in the village with price between Rp 800,000 and Rp 2,000,000. The price of this bird will increase more than two or three times, if it is sold in the urban bird markets. This bird is often found in the forest.

The local people of the Karangwangi catch also white-bellied sea eagle which taken flesh to be eaten and wings for trading. In addition, nest of cave swiftlet is traded by the community (Figure 1). Some people of the Karangwangi have involved in hunting animals and ignore some of the ecological benefits of wildlife because by trading wild animals people will get money which is needed for economic household income (cf. Alves and Souto 2015).

Bat (*Pteropus vampirus)* is recognized live in groups in and out of their nests at dusk to search for food at the night. Various fruits, especially sweet fruits and ripe, such as soursop and papaya are usually eaten by bats. Bats nesting is commonly found in large trees or caves in the forest. Based on interview, most respondents know that bats are recognized as animal seed dispersal function. Seeds are usually dispersed when bats eat irregularly scattered so that the seeds of the fruit they eat fall. In addition, bat is culturally used as an asthma drug medicine.

Cobra (*Ophiophagus hannah*) is considered by respondents as dangerous animal but they also realized that the cobra has a function as pest predator or a pest controller. In addition, some respondents culturally use cobra as drugs, including asthma, diabetes and liver disease. In order to trading cobra, special a middleman is found in the Village of Karangwangi.

Porcupine (*Hystryx javanica*) is often sought for their meat because it is believed to be useful as a drug. Based on respondent perception porcupine usually takes 40 kinds of leaves in one day. Therefore, the respondents hunt porcupine to take 40 leaves that have been eaten by porcupine is still stored in their stomach. Some people believe the leaves that have been eaten by porcupine can cure liver disease, diabetes and asthma. Like Karangwangi community, the Malay community and Kubu community also utilize porcupine which is used as traditional medicine (Maryanto et al. 1993). Similarly, rural communities of East Kalimantan and Java believe that porcupine meat is culturally used as traditional medicine that has medicinal properties value (Putra et al. 2008).

Pangolin or peusing (*Manis javanica*), considered as recorded as one of the protected animals in Indonesia and also protected internationally. According to the International Union for Conservation of Nature (IUCN) pangolin is categorized as Endangered Species, as well as listed in Appendix II of Convention on International Trade in Endangered Species of the Wild Fauna and Flora (CITES) (Soehartono and Mardiastuti 2003). Empirically, however, pangolin is usually hunted by villagers of Karangwangi to eat meat and scales used for trading. The scales of one individual of pangolin have price of Rp. 5,000, whereas a kg of pangolin is valued up to Rp. 200, 000. Pangolin is required a special technique for slaughtering. Before slaughtering, pangolin tongue should be pulled out; it is undertaken so that the pangolins quickly intervening die. Pangolin skin is culturally used as a treatment for skin diseases. This tradition similar that of practiced by the Malay and Kubu community, anteaters are culturally hunted for traditional medicine (Maryanto et al. 1994). Based on the respondent perception, body part of wild animals, such as pangolin is believed to be an antidote for certain diseases by the people of China, especially the scales and meat (Novriyanti 2010). In addition, some rural people of East Kalimantan believe that pangolin can be used as a tonic and food (Putra et al. 2008).

Sunda slow loris (*Nycticebus caucang*), recorded as protected animal both in national and international based on the IUCN category Endangered Species, as well as listed in Appendix I of CITES (Soerhartono and Mardiastuti 2003). The Sunda slow loris usually eats fruit and insects. Therefore, this animal is considered as seed dispersal and pest control. In addition, the Sunda slow loris has been known as mystical value in society. Like other ethnic Sundanese groups, the Sunda slow loris has been mythicized as associated with bad luck. Blood dripped of this animal on the ground face is strictly prohibited. Land affected by blood drop of Sunda slow loris will suffers from drought. As a result, blood from the Sunda slow loris has been frightening. Anyone who kills this animal, then the people who live near the site of the slaughter would become seriously ill, even up to 40 homes. Position slaughter advance also determine where misfortune will fall. For example, if the slaughter faces at east, people in the west will be affected by bad luck, and vice versa. In addition, this animal is also believed to be a tool or capital used by herbalists. Narrated by speakers on the practice of using bone shaman nosy advanced for their efforts. The bones were planted sector in the village to make a village the hospital. When people are sick they will be treated to the psychic and then the shaman would heal. Similarly, the village people of upper Cisokan believed that disturbing the Sunda slow loris will get this tradition is also found in the village community unlucky live (PLN-LPPM Unpad 2014). As a result this animal was culturally protected by local community, particularly in the past.

Barn owls or bueuk or koreak (*Tyto alba*) is known by residents as the animals come out at night (nocturnal), eating meats, such as snakes and rats, so it is considered as beneficial animal due to controlling crop pest, particularly rat. Some respondents also believed that when the barn owl sound is heard in the village, people suspect it is indicated that in the village will be found a woman pregnant without husband. This perception is predominantly found in many places of Sundanese village (Iskandar 2012). Another sign is when the barn owl sounds mean there are spirits around him and an omen of death. The presumption of the population against the messenger, and is associated with the occult into a separate spiritual values are developed within the Sundanese community (cf. Tidemann and Gosler 2010). Presumption or spiritual value becomes effective in order to protect the *Tyto alba* which is strongly embedded by culture (Soemarwoto 2004). Partasasmita et al. (2015) say that this perception is different from that of people who reside around the campus of Padjadjaran University at Jatinangor, Sumedang. Understanding of the population about the existence of a barn owl as a highly mystical and sinister cannot be separated. Stories that often arise when talking about barn owl including; barn owl birds are being sounded indicating the bird is being ridden by a demon; if there is the sound of birds, the hearing was lying then he should face down, otherwise it will stomach pain; barn owl birds often come out on Friday night because the devil is often out at night; If a barn owl bird continue to speak for several days in a house resident, then one of the family members in the house will die or sick. Unlike to the perception of the population, one of the scientific evidence relating to barn owl story as news carriers of disease or death in the population, caused by the bacterium *Salmonella* sp., not merely because of the presence of birds. Barn owl pellets or bird droppings that contain the bacteria *Salmonella* sp., can infect humans and cause salmonellosis symptoms are gastroenteritis (Masniari et al. 2005)

Rusty-breasted cuckoo (*Cacomantis spulclaris*) is considered mysterious *uncuing* mindless and just heard his voice has always been associated with mystical events or a catastrophe. The voice of uncuing means signifies the existence of death or the person who will be sick. In addition, voice of uncuing indicated as often associated birds always seized with unseen spirits.

Jungle crow (*Corpus macrorhynchos*) also believed by respondents that if this bird voice all the time in the village, it is indicated to bring bad luck or will be found to pass away person in the village. If the crows perched on the house means indicate the house was in the "*send*" something in the form of witchcraft or other occult sciences.

In conclusion, based on this study it can be concluded that: (i) the respondents of the Karangwangi community have recognized at least 45 species wildlife consisting of 15 mammalian species, 21 species of Aves, and 9 species of herpetofauna. Unlike biological classification (etic), the folk classification of the Karangwangi community (emic), the wildlife can be classified as color, voice, habitat, and role in the ecosystem; and (ii) based on the perceptions of the respondents, various wildlife of the Karangwangi have various socio-economic and cultural, ecological functions, including: household meat consumption, for keeping as pets, pest control, and seed dispersal. Some cultural myths related with wildlife have been recorded and determine the utilization patterns of wildlife by villagers.

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REFERENCES

Alves RRN, Souto WMS. 2015. Ethnozoology: a brief introduction. Ethnobiol Conserv 4 (1):1-13.

Alves RRN, Souton WMS. 2011. Ethnozoology in Brazil: current status and perspectives. Ethnomed 7 (22):1-18.

Alves RRN. 2012. Relationships between Fauna and People and The Role of Ethnozoology in Animal Conservation. Ethnobiol Conserv 1 (2): 1-57.

Batoro J, Setiadi D, Chikamawati T, Purwanto Y. 2012. Fauna knowledge (Ethnozoology) Tengger society in Bromo Tengger Semeru East Java. Biota 17 (1): 45-54.

Carlson TJS, Maffi L. 2004. Ethnobotany and Conservation of Biocultural Diversity, Advances in Economic Botany Series, Vol. 15. Botanical Garden Press. New York.

Ellen RF, Harris H. 2000. Introduction. In: Ellen RF, Parkes P, Bicker A. (eds). Indigenous Environmental Knowledge and its transformation: Critical Anthropological Perspective. Hardwood Academic Publishers, Amsterdam .

Frazier J. 2007. Sustainable use of wildlife: The view from archaeozoology. Nat Cons 15:163-173.

Horowizt DL. 1998. Integrating indigenous resource management with wildlife conservation: A case study of Batai Ai National Park, Sarawak, Malaysia. Human Ecol 26: 371-403.

Iskandar J, Iskandar BS. 2015. Benefit of various birds in the song-bird contest and its impact to bird conservation in nature: A case study in Bandung, West Java. Proceedings of the National Seminar on Biodiversity Society Indonesia. Gajah Mada University, 21 March 2015. [Indonesian]

Iskandar J, Ellen RF. 1999. In Situ Conservation of Rice Landraces Among the Baduy of West Java. Ethnobiology 19 (1): 97-125

Iskandar J. 2012. Etnobiology and Sustainable Development. [Research Report]. University of Padjadjaran, Sumedang. [Indonesian]

Jorgenson 1998. The impact of hunting on wildlife in the maya forest of Mexico. Timber, tourists and temples: conservation and development in the Maya forest of Belize, Guatemala and Mexico. Island press, Washington.

Lynch SJF, Hoelnsteiner M, Covar L. 1974. Data Gathering by Social Survey. Philippine Social Science Council, Quezon City.

Mackinnon J, Phillipps K, Balen BV. 1992. The birds in Sumatra, Java, Bali and Kalimantan. Center for Biology-LIPI. Bogor.

Maffi L. 1999. Linguistic diversity. In Posey D (ed). Cultural and Spiritual Values of Biodiversity. Intermediate Technology Publications, London.

Maffi L. 2004. Maintaining and restoring biocultural diversity: The evolution of a role for ethnobiology. In: Carlson TJS, Maffi L (eds). Ethnobotany and Conservation of Biocultural Diversity. Botanical Garden Press, New York.

Martin GJ. 1995. Ethnobotany: A Methods Manual. Chapman & Hall. London.

Maryanto I, Saim A, Danielsen F. 1994. Malay etnozoologi community survey and the Kubu tribe in the Province of Riau and Jambi: Animals and traditional medicine. In Sanbukt O, Wiriadinata H (eds). Rain Forest and Resource Management. Proceeding of the Norindra Seminar, Jakarta 25-26 May 1993.

Masniari P, Iyep K, Susan MN. 2005. Against *Salmonella* dangers of health. Proceedings of the National Workshop on Zoonotic Diseases. Center for Livestock, Bogor. 15 September 2005. [Indonesia]

Newing H, Eagle CM, Puri RK et al. 2011. Conducting Research in Conservation: Social Science Methods and Practice. Routledge, London.

Noerdjito M, Maryanto I. 2001. Biological Species Protected by Law in Indonesia. LIPI-Nature Conservancy, Cibinong-Bogor. [Indonesia]

Novriyanti 2010. Management pangolin (Manis javanica Desmarest 1822) in Habitat Insitu and Eksitu. [Thesis]. Bogor Agriculture Institute, Bogor. [Indoensia]

Partasasmita R. 2015. Biodiversity, Ecology and Conservation of Birds in Pananjung Pangandaran. [Research Report]. University of Padjadjaran, Sumedang. [Indonesian]

Partasasmita R, Muhamad GK, Iskandar J. 2015. Population, occupational and public knowledge about barn owl birds (*Tyto alba* javanica JF Gmelin 1788) at Padjadjaran University Campus of Jatinangor, Sumedang District. Procceding Seminar National Masy Biodiv Indon. Institute Technology Bandung, Bandung, 13 June 2015. [Indonesia]

PLN-LPPM Unpad. 2014. Biodiversity Management Plan Upper Cisokan Pumped Storage. [Research Report]. University of Padjadjaran, Sumedang.

Puri RK. 1997. Hunting Knowledge of the Penan Benalui of East Kalimantan, Indonesia. [Ph.D. Dissertation]. University of Hawaii, Honolulu, Hawaii.

Putra YAE, Masy"ud B, Ulfa M. 2008. Diversity of Medicinal Animals in Betung Kerihun National Park, West Kalimantan, Indonesia. Media Konservasi 13 (1): 8-15. [Indonesia]

Rambo AT. 1983. Conceptual Approaches to Human Ecology. [Research Report]. East-West Environment and Policy Institute, East-West Center, Honolulu, Hawaii.

Soehartono T, Mardiastuti A. 2003. The implementation of the CITES Convention in Indonesia. JICA, Jakarta

Soemarwoto O. 2004. Ecology, Environment and Development. Djambatan, Jakarta. [Indonesia]

Sutrisno H, Partomihardjo T, Triono T et al. 2014. Thematic Studies of Flora and Fauna in the Area of Hydropower Project "Upper Cisokan Pumped Storage" West Java. [Research Report]. Research Center for Biology, LIPI, Bogor. [Indonesian]

Tidemann S, Gosler A. 2010. Ethno-ornithology. Earthscan, London.

Toledo VM. 2000. Ethnoecology: A conceptual framework for the study of indigenous knowledge on nature. Seventh International Congress of Ethnobiology, Athens, Ga, 22-27 October 2000.

Whitten T, Soeriaatmadja RE, Afiff SA. 1999. Ecology of Java dan Bali. Periplus, Singapore.

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Phenetic analysis of the genera medicagoid *Trigonella*, *Medicago* and *Melilotus* (Fabaceae) on seed coat in Iran

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Abstract. *Khandani S, Assadi M, Nejadsatari T, Mehregan I. 2016. Phenetic analysis of the genera medicagoid* Trigonella, Medicago *and* Melilotus *(Fabaceae) on seed coat in Iran. Biodiversitas 17: 162-171.* Seed morphological characters of 11 species belonging to the Medicagoid *Trigonella* group from Iran were studied and compared with six species of *Trigonella* L. (non medicagoid), one species *Medicago* L. and *Melilotus* Mill. (Fabaceae), including general shape, size, color, surface shape and testa ornamentation patterns with Scanning Electron Microscope and Stereomicroscope. Phenetic relationships among the species were studied via important seed morphological data by PCA analysis and UPMGA method. Cluster analysis of seed morphology results showed that some of medicagoid *Trigonella* species clustered with non medicagoid species but majority of them joined together which is in agreement of some references. In conclusion, seed morphological characters show that most Iranian medicagoid *Trigonella* species are well separated from the other *Trigonella* species. Also, some of these characters are important in systematic discriminations of the taxa.

Keywords: Fabaceae, medicagoid, phenetic analysis, seed morphology, *Trigonella*

INTRODUCTION

Fabaceae is the third largest family of flowering plants with about 650 genera and 1000 species. Many of the species are important as the source of food, wood, raw material, fixation of atmospheric nitrogen (N_2) and etc. (Mirzaei et al. 2015b). *Trigonella* L. is a large genus with 135 species from the tribe *Trifolieae* family Fabaceae, and is distributed in dry regions with a Mediterranean climate, in West Asia, Europe, North and South Africa, North America and South Australia (Ceter et al. 2012).

The tribe *Trifolieae* consists of six genera, *Medicago* L. *Melilotus* Mill., *Ononis* L., *Parachetus* Buch-Ham., *Trifolium* L. and *Trigonella* L. The tribe was studied by Berchtold and Presl (1820) and characterized by having trifoliate leaves and stipules adnated to the petioles (Ranjbar and Hajmoradi 2012). Schulz (1901) put four genera *Melilotus*, *Trigonella*, *Factorouskya* and *Medicago* in the tribe *Trigonelleae* having seed with micropyle infra funiculum (Tia 2004). Small (1987) recognized *Melilotus*, *Trigonella* and *Medicago* a subtribe *Trigonellinae*. Members of this subtribe are united by the consistent presence of pinnately trifoliate leaves and high bootstrap support (99%) in analyses of *mat*K data (Steel et al. 2003).

In Flora Iranica (Rechinger 1984) the *Trigonella* genus is represented by 63 annual and perennial species in 12 sections. This taxon is known exhaling odor and is considerably useful for food and medicine (Ranjbar and Hajmoradi 2012). *Trigonella foenum-graecum* L. is known to be used beneficial health effects. (Srichamroen et al. 2011). Members of the Tribe *Trifolieae* bear trifoliate leaves and small seeds with developed radicle (Tia 2004). *Trigonella* species are hairy or glabrous annual herbs with pinnately trifoliolate leaves.

Leaflets are usually toothed, leafy stipules adnate to the petiole. Flowers are axillary, solitary or mainly in heads, spikes or short racemes, calyxis5-toothed, campanulate or sometimes tubular, regular or 2-lipped, petals are yellow or white, violet or blue. Fruit exerted from the calyx, straight or curved, linear to oblong or ovate or semilunar, with or without a beak. Seeds are tubercled or wrinkled rarely smooth (Huber-Morath 1970).

According to Steel et al. (2003), *Trigonella* and *Medicago* are well-supported clades and are sister to one another and according to Bena et al. (2001), they have proper relationship. Gazara et al. (2001) showed that the genera *Trifolium, Melilotus, Trigonella* and *Medicago* are closely related to each other, but according to Bena et al. (2001,) generic delimitation is problematic. Hyne (1966) reported intermediate species between *Trigonella* and *Melilotus* (Gazara et al. 2001). Some taxonomical studies for delimitation of *Trigonella* and *Medicago* such as floral characters, asymmetric leaves, phenolic variation, stigma morphology, pollen-ovule pattern and seed characters for discriminating among and between them were not successful (Bena 2001), but Small (1986) based on some floral adaptations transferred 23 *Trigonella* species to *Medicago* and named them medicagoid group (Gazara et al. 2001). These 23 species have taxonomic problem in the genus *Trigonella* and have been considered as belonging to the genus *Trigonella*. Baum (1968) introduced them as medicagoid species because of floral and seed structure similarities between them and *Medicago* species, but based on strong similarities between *Trigonella* and these species in the fruit appearance maintained them in the genus *Trigonella* (Bena 2001).

A molecular phylogeny supported the transfer of the medicagoid species from the genus *Trigonella* to *Medicago* (Ranjbar and Hajmoradi2 012). Analyses of nrDNA ITS and ETS data supports inclusion of the medicagoid *Trigonella* species in the genus *Medicago* (Steel 2003). Some authors have used the morphology, micromorphology and seed coat anatomy to identify *Trifolieae* species but they have seldom been used in the taxonomy of the group.

Lersten (1982) noted seed shapes, testa surface, testa color patterns, seed size, hilum and lens color to separate *Trifolieae* from *Vicieae*. Small and Brookers (1990) studied delimitation of *Medicago* from its close relatives in tribe *Trifolieae* subtribe *Trigonellinae* by Scanning Electron Microscope of seed surface in the genera *Medicago*, *Trigonella* and *Melilotus.* Tia (2004), studied some seed characters in thirty eight species distributed in tribe *Trifolieae*, which showed the genera *Trigonella* and *Melilotus* have variable characters and *Medicago* have characteristic seeds. Ceter et al. (2012) studied seed morphology of 37 taxa of *Trigonella* from various regions in Turkey with Scanning Electron Microscope and light microscope such as seed color, shape, size, weight and surface ornamentation pattern.

Salimpour et al. (2013) showed cluster analysis based on morphological characters of *Medicago* and *Trigonella* (*Bucerates*) species. In this study we use some seed characters to identify relationship between medicagoid species of *Trigonella* and the genera *Medicago* and *Melilotus.* Turki et al. (2013) studied seed morphology of 19 *Trigonella* L. Species such as *T. coerulescens* (M. Bienb.) Halacsy, *T. calliceras* Fisch. ex M. Bieb. and *T. monspeliaca* L. with light and Scanning Electron Microscope. They compared them in seed characters such as shape, hilum shape, position, seed coat pattern and evaluated their taxonomic significances.

The aim of this study is to evaluate seed morphological characters in the taxonomy of the genus *Trigonella*, especially *Trigonella* medicagoid and non medicagoid species.

MATERIALS AND METHODS

The materials collected from various regions of Iran during May and June 2014, and deposited specimens in TARI and IAUH Herbaria of Iran (Table 1). The collected samples were identified at the Department of Biology, Science and Research Branch, Islamic Azad University in Tehran and at the Research Institute of Forest and Rangelands by using various floras of Iran and adjacent countries. For Scanning Electron Microscopy studies, dry seeds were examined by Stereomicroscope and were photographed and then they were mounted directly on stubs by using adhesive tape and coated with gold by E5200 AUTO sputter coater for 15 minutes.

Testa ultrastructure surface ornamentations were assessed and photographed by Cam Scan MV2300 electron microscope. For each species we used nearly 10-20 numbers from each sample. Seeds were studied by Stereomicroscope DSC-H50 to assess morphological features and few of them were examined by Scanning Electron Microscope (SEM). Testa patterns terminology was based on Lersten (1982), Small and Brookers (1990), Pinar et al. (2009), Gunes et al. (2011). Ceter et al. (2012), Gunes F. (2013), Teixeira et al. (2013), Ozbek et al. (2014) and Mirzaei et al. (2015).

A total of 8 quantitive/qualitive characters related to seed morphology were studied in 21 taxa of *Trigonella* (6 species), medicagoid (11 species), *Medicago* (1 species) and *Melilotus* (1 species) (Table 2). For statistical analysis the qualitative characters encoded according to the multi-state method and related means were considered for quantitative characters. Phenetic analysis was carried out using SPSS Ver. 22 (Chicago, USA) and UPGMA method. Means of the characters were obtained by bootstrap analysis. Bootstrap result was based on 243-997 samples. Second analysis was done with principal component analysis (PCA) using SPSS software (Figure 6).

RESULTS AND DISCUSSION

Results

Microscopy observations showed that the studied medicagoid *Trigonella* taxa have variation in qualitative and quantitative characters (Table 3).

Seed size: seed sizes are very variable (Table 3). In medicagoid *Trigonella* group the largest seed belongs to *Trigonella monantha* (average 2. 35 mm in length and 0. 79 mm in width) and the smallest seeds observed in *Trigonella brachycarpa* (average 1.1 mm in length and 0.57 mm in width).

Seed shape and color: Seed shapes in medicagoid *Trigonella* species are oblong, ovoid, oblong-elliptic and seed color green and brown (Figures 1 and 2).

Testa texture: Testa textures in medicagoid group are two types creased and smooth (Figures 3 and 4).

Seed coat surface: By Scanning Electron Microscopy, several types of micro-ornamentation were observed on surface structures. The taxa have several ornamentation types that are reticulate, several types of verrucate such as foveolate-verrucate, aculate-verrucate, tuberculate-verrucate, reticulate-verrucate, rugolate-verrucate and rugolate (Table 3, Figures 3 and 4).

Table 2. Seed morphological characters and score of studied species

Morphological characters	Score
Mean seed length (mm)	0=1-2, 1=2.1-3, 2=3.1-4
Mean seed width (mm)	0=0.5-1, 1=1.1-1.5, 2=1.6-2
Seed shape	Oblong = 0, ovoid=1, oblong to another shapes=2
Seed color	Light brown=0, dark brown=1, green=2
Testa structure	Smooth=0, creased =1
Homogeneity of cell sizes	Uniform=0, irregular=1
Testa ornamentation	Verrucate=0, reticulate=1, rugulate=2
Hilum shape	Elliptic=0, circular=1

Table 1. Specimen examined of the genera medicagoid *Trigonella*, *Medicago* and *Melilotus* (Fabaceae) in Iran

Taxa	Location, collector (s) and herbarium number
Trigonella	
Sec. *Bucerates*	
T. arcuata C.A. Mey.	Iran: East Azerbaijan, 2 km from Jolfa to Siahrud, 770 m, Kasebi 14503 (IAUH)
T. arcuata C.A. Mey.	Iran: West Azerbaijan, Tabriz to Ahar, Khajeh Station, 1470 m, Kasebi 14502 (IAUH)
T. aurantiaca Boiss.	Iran: Khozestan, Hafttappeh, Chaghazanbil, 160 m, H. Foroughi 3277 (TARI)
T. crassipes Boiss.	Iran: Kurdestan, 54 km from Sanandaj to Kamiaran, Dolab, 1500 m Maroofi & Mansori & Sh-Naseri 5770 (TARI)
T. fischeriana Ser.	Iran: CharmahalBakhtiari, ShahreKord, Shalamzar, Mozaffarian 54602 (TARI)
T. macroglochin Durieu.	Iran: Quahak, 1900 m, Dini-Arazm 16865 (TARI)
T. monantha subsp. *monantha* C.A. Mey.	Iran: West Azerbaijan, Uromieh, 35 km Uromieh to Sero, 1700 m, Khandani 14514 (IAUH)
T. orthoceras Kar & Kir.	Iran: East Azerbaijan, Tabriz to Maraghe, 2 km from Police Station to Azarshahr, 1500m, Kasebi 14519 (IAUH)
T. orthoceras Kar & Kir.	Iran: West Azerbaijan, Uromieh, 35 km Uromieh to Sero, 1700 m, Khandani 14518 (IAUH)
T. persica Boiss.	Iran: CharmahalBakhtiari, roadfromLordegantoYasuj, Maymand, Margh-e-Chenar, 1750m Mozaffarian 54563 (TARI)
T. tenuis Fisch.	Iran: Ardebil to Khalkhal, Bahreman, 1800 m, Ranjbar & Hajmoradi 19601 (TARI)
T. uncinata Banks & Soland.	Iran: Lorestan, Road of Dorood to Pirabdollah, 1350 m, Hamzeh 71867 (TARI)
Sec. *Biebersteinianae*	
T. coerulescens (m. B).	Iran: Tehran, Chitgar, 8017 (IAUH)
Sec. *Callicerates*	
T. calliceras Fisch.	Iran: Azerbaijan, Arasbaran, Kalaibar to Hijrandust, 1350-1700m**,** Assadi & Maassoumi 20091 (TARI)
Sec. *Cylindrica*	
T. spruneriana Boiss.	Iran: Tehran, Lavizan, 8010 (IAUH)
Sec. *Elliptica*	
T. elliptica Boiss.	Iran: ChaharmahalBakhtiari, ShahreKord, 2400 m, Mozaffarian 54578 (TARI)
Sec. *Falcatulae*	
T. uncata Boiss & Noe.	Iran: Khuzestan, 7991 (IAUH).
Sec. *Lunatae*	
T. brachycarpa Fisch.	Iran: Azerbaijan, Arasbaran, protected area, between Asheghlow and Kalaleh, 400 m, Hamzeh & Asri 79167 (TARI)
Sec. *Reflexae*	
T. monspeliaca L.	Iran: Gorgan, East of Marvetappeh near Chazanghayeh, 300 m, Assadi & Maassoumi 55572 (TARI)
Sec. *Verae*	
T. turkmena M. Popov.	Iran: Esfehan, West Khansar, 2600m, Babakhanlo & Amin 16913 (TARI)
Medicago	
Sec. *Hymenocarpos*	
M. radiata Boss.	Iran: Kohgiloyeh Boirahmad, Mehregan & Yeganeh 13978 (IAUH)
Melilotus	
M. officinalis L.	Iran: 7571 (IAUH)

Homogeneity of cell sizes: All studied taxa have uniform homogeneity except *Trigonella arcuata* (EA) (Medicagoid) and *Trigonella calliceras* (non medicagoid) have irregular homogeneity cell sizes.

Hilum shape: All studied taxa have elliptic hilum shape except *Trigonella monantha*, *T. orthoceras* and *T. persica* (Medicagoid) and *T. coerulescens* (non medicagoid) that have circular hilum shape.

Morphological analysis: Morphological analysis of 21 taxa including six species of *Trigonella* non medicagoids, 11 species of *Trigonella* medicagoids, one species of *Medicago* and one species of *Melilotus* were analyzed based on 8 morphological seed characters. Figure 5 shows a phenogram with UPGMA method. The UPGMA phenogram showed the highest phonetic correlation (r> 0.60). Morphological analysis showed two main clusters, cluster A and cluster B. *Trigonella elliptica*, *Melilotus officinalis* and *Trigonella calliceras* form a separate group at a farther distance from the other species are placed in cluster A. Cluster B is divided into two groups that includes group B1 and B2, and group B2 includes group B2a and B2b.

Group B1 includes mostly medicagoid species except *Trigonella coerulescens*. In subcluster B2 *Trigonella spruneriana* and *T. turkmena* (non medicagoid) form a separate group and *T. aurantiaca*, *T. monspeliaca* and *T. crassipes* (Medicagoid) species are adjacent to *T. uncata* (non medicagoid). *Medicago radiate* is considered as an out group at a farther distance from the other species.

Table 3. Macro-and micro-morphological characters of seeds in medicagoid *Trigonella* species and some relatives from Iran

Taxa	Length (mm) Mean	Width (mm) Mean	Outline	Color	Testa texture	Homo-genity of cell sizes	Testa ornamentation	Hilum shape
Trigonella								
Sec. ***Bucerates***								
T. arcuata (EA)	1.17	0.64	Oblong	Light green	Creased	Irregular	Reticulate	Elliptic
T. arcuata (WA)	1.84	0.78	Oblong	Dark green	Creased	Uniform	Foveolate-verrucate	Elliptic
T. aurantiaca	1.41	0.58	Oblong-elliptic	Light brown	Smooth	Uniform	Aculate-verrucate	Elliptic
T. crassipes	1.72	0.77	Oblong-elliptic	Light brown	Smooth	Uniform	Reticulate-verrucate	Elliptic
T. fischeriana	1.25	0.68	Oblong-elliptic	Light brown	Smooth	Uniform	Tuberculate-verrucate	Elliptic
T. macroglochin	1.56	0.57	Oblong	Dark brown	Creased	Uniform	Rugolate-verrucate	Elliptic
T. monantha subsp. *monantha*	2.35	0.79	Oblong	Green	Creased	Uniform	Aculate-tuberculate-verrucate	Circular
T. orthoceras (EA)	1.28	0.62	Oblong	Green	Creased	Uniform	Reticulate	Circular
T. orthoceras (WA)	1.67	0.74	Oblong	Dark brown	Creased	Uniform	Aculate-tuberculate-verrucate	Circular
T. persica	1.95	0.69	Oblong	Dark brown	Creased	Uniform	Aculate-tuberculate-verrucate	Circular
T. tenuis	1.95	0.79	Oblong or oblong-elliptic	Dark brown	Creased	Uniform	Aculate-tuberculate-verrucate	Elliptic
Sec. ***Biebersteinianae***								
T. coerulescens	1.47	0.95	Ovoid	Dark brown	Creased	Uniform	Reticulate-verrucate	Circular
Sec. ***Callicerates***								
T. calliceras	1.77	1.82	Ovoid	Dark brown	Creased	Irregular	Reticulate-verrucate	Elliptic
Sec. ***Cylindrica***								
T. spruneriana	2.4	0.71	Allantoid-oblong	Light brown	Creased	Uniform	Aculate-reticulate-verrucate	Elliptic
Sec. ***Elliptica***								
T. elliptica	3.68	1.94	Elliptic-ovoid	Brown	Creased	Uniform	Reticulate-verrucate	Elliptic
Sec. ***Falcatulae***								
T. uncata	1.28	0.61	Oblong-elliptic	Brown	Smooth	Uniform	Reticulate-foveolate	Elliptic
Sec. ***Lunatae***								
T. brachycarpa	1.1	0.57	Ovoid	Light brown	Smooth	Uniform	Foveolate-verrucate	Elliptic
Sec. ***Reflexae***								
T. monspeliaca	1.12	0.67	Oblong-square	Dark brown	Creased	Uniform	Reticulate-verrucate	Elliptic
Sec. ***Verae***								
T. turkmena	2	0.63	Allantoid-oblong	Brown	Creased	Uniform	Aculate-reticulate-verrucate	Elliptic
Medicago								
Sec. ***Hymenocarpos***								
M. radiata	2	1.11	Oblong	Light brown	Smooth	Uniform	Rugulate	Elliptic
Melilotus								
M. officinalis	2.25	1.52	Ovoid	Dark brown	Smooth	Uniform	Aculate-tuberculate-verrucate	Elliptic

Note: EA: East Azerbaijan, WA: West Azerbaijan

Discussion

Studies on medicagoid *Trigonella* species including seed morphology, seed size, color, coat ornamentation, hilum shapes and homogeneity of cell sizes show relationship of medicagoid *Trigonella* species. Non medicagoid *Trigonella* species including *Medicago* and *Melilotus* species are grouped separately. Some seed morphological studies of Fabaceae taxa have been carried out from time to time (Salimpour et al. 2007; Al-Ghamdi et al. 2010; Salimpour et al. 2013; Gunes et al. 2011; Gunes 2013; Turki et al. 2013; Kahraman et al. 2014; Ozbek et al. 2014; Bianco et al. 2015; Mirzaei et al. 2015; Rodriues et al. 2015).

The results are summarized in Table 3 and Figures 1-4 using Stereomicroscope and SEM studies. These results revealed that the seed features variation may be used to discriminate medicagoid *Trigonella* species from the other taxa. Tai (2004) has noticed that the genus *Trigonella* have variable seed characters that can be used in the subscription of their species. We have noticed that the seed size in medicagoid *Trigonella* species is variable from smallest seed in *Trigonella brachycarpa* to largest seed in *Trigonella monantha* and a wide range of variety in other taxa, while Ceter et al. (2012) observed little differences in section *Lunatae* comparing to some other *Trigonella* sections. Turki et al. (2013) showed two types of seed size.

From Table 3 and Figures 1 to 4 can be resulted that the seed color in medicagoid *Trigonella* species are two types green and brown but in the other studied taxa are only brown. Shape of the seeds in studied species is four types. Ceter et al. (2012) introduced five types of color and three types of seed shapes. Turki et al. (2013) introduced four types of color and three types of seed shape. Seed coat microsculpture types in medicagoid *Trigonella* species

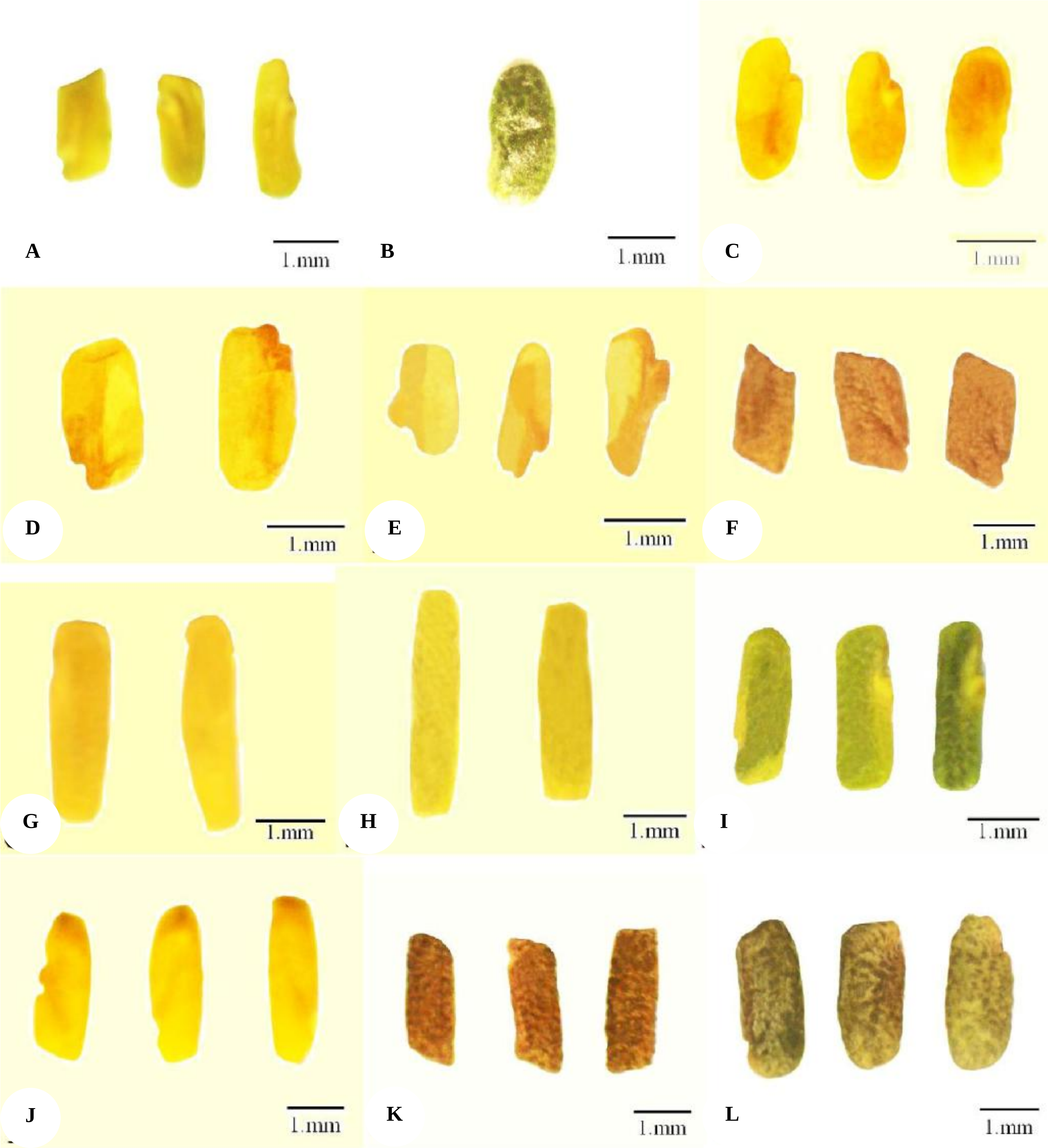

Figure 1. Stereomicroscope photograph of *Trigonella* seeds. A. *T. arcuata* (EA), B. *T. arcuata* (WA), C. *T. aurantiaca*, D. *T. crassipes*, E. *T. fischeriana*, F. *T. macroglochin*, G. *T. monantha* (EA), H. *T. monantha* subsp. *monantha* (WA), I. *T. orthoceras* (EA), J. *T. orthoceras* (WA), K. *T. persica*, L. *T. tenuis*.

were introduced by Small et al. (1990) as one type and by Ceter et al. (2012) as three types and Turki et al. (2013) as two types, while we observed two types, including reticulate and verrucate with secondary ornamentation (Table 3, Figures 3 and 4).

We introduce two 2 types of homogeneity cell size on seed surface and two types of hilum shapes in medicagoid *Trigonella* species, while Ceter et al. (2012) recorded one type for each character and Turki et al. (2013) recognized two types of hilum shape and six types for cell size shape. In this study all morphological characters of Stereomicroscope and SEM Studies used to demonstrate phenetic relationship of medicagoid *Trigonella* species with the other taxa.

Figure 2. Stereomicroscope photograph of *Trigonella*, *Medicago* and *Melilotus* seeds. A. *T. coerulescens*, B. *T. calliceras*, C. *T. spruneriana*, D. *T. elliptica*, E. *T. uncata*, F. *T. brachycarpa*, G. *T. monspeliaca*, H. *T. turkmena*, I. *Medicago radiata*, J. *Melilotus officinalis*

Salimpour et al. (2013) showed that based on cluster analysis of morphological characters two major groups including *Medicago* and *Trigonella* (*Bucerates*) species join together. Turki et al. (2013) based on numerical analysis indicated that *Trigonella coerulescens* and *T. calliceras* (non medicagoid) are closely related and placed them at a farther distance from *T. monspeliaca* (Medicagoid). In this study based on morphological seed characters mostly medicagoid *Trigonella* species are placed in a separate subcluster B1 and B2a (*Bucerates* species) but, non medicagoid species placed at a farther distance from the other species. *Medicago radiate* is placed at a separate cluster adjacent to *Bucerates* species, therefore our results agree with Salimpour et al. (2013) results.

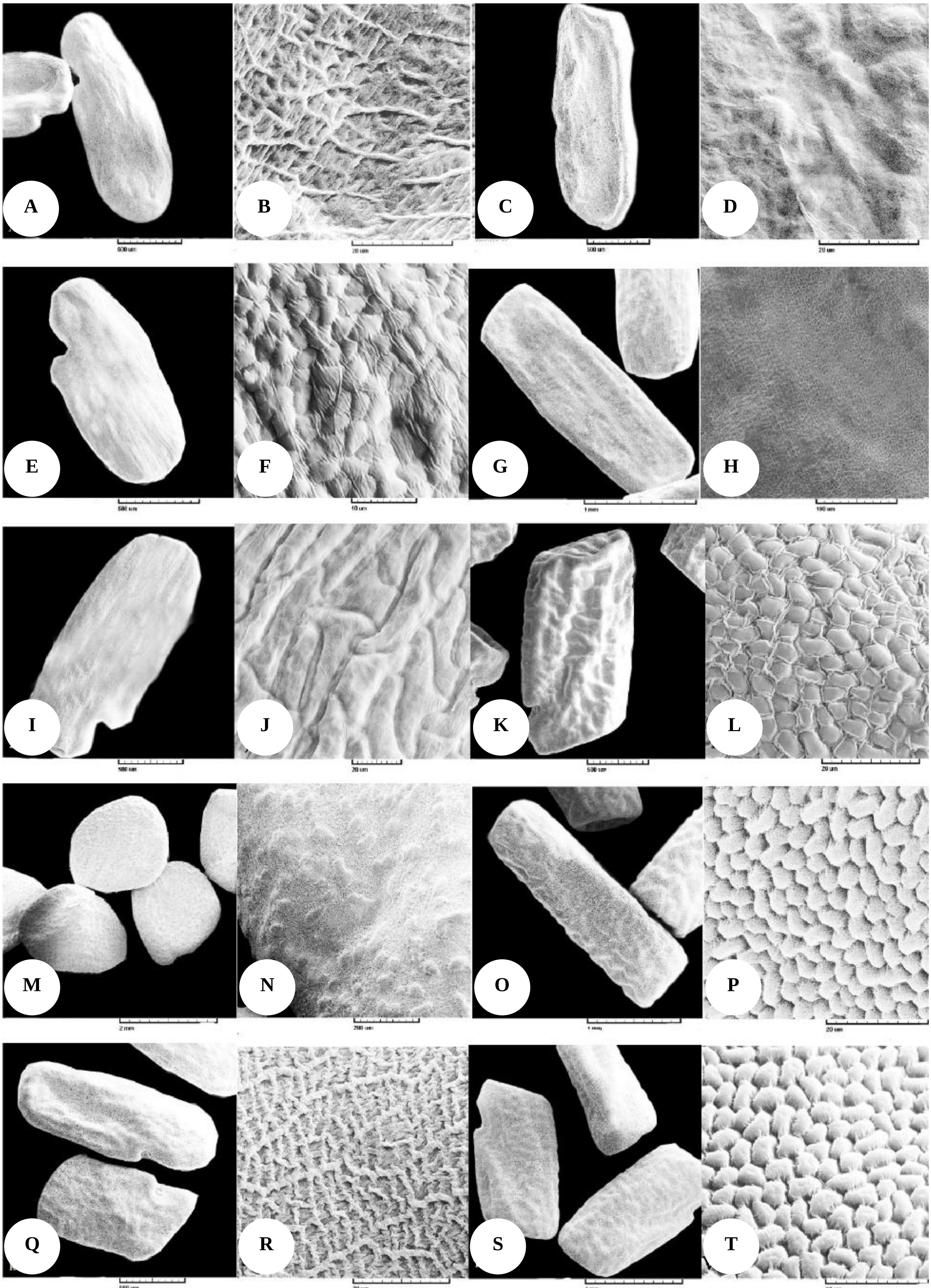

Figure 3. SEM microphotographs of *Trigonella* seeds. A-B. *T. arcuata* (EA), C-D. *T. arcuata* (WA), E-F. *T. aurantiaca*, G-H. *T. monantha*, I-J. *T. fischeriana*, K-L. *T. macroglochin*, M-N. *T. calliceras*, O-P. *T. monantha* subps. *monantha*, Q-R. *T. orthoceras* (EA), S-T. *T. orthoceras* (WA).

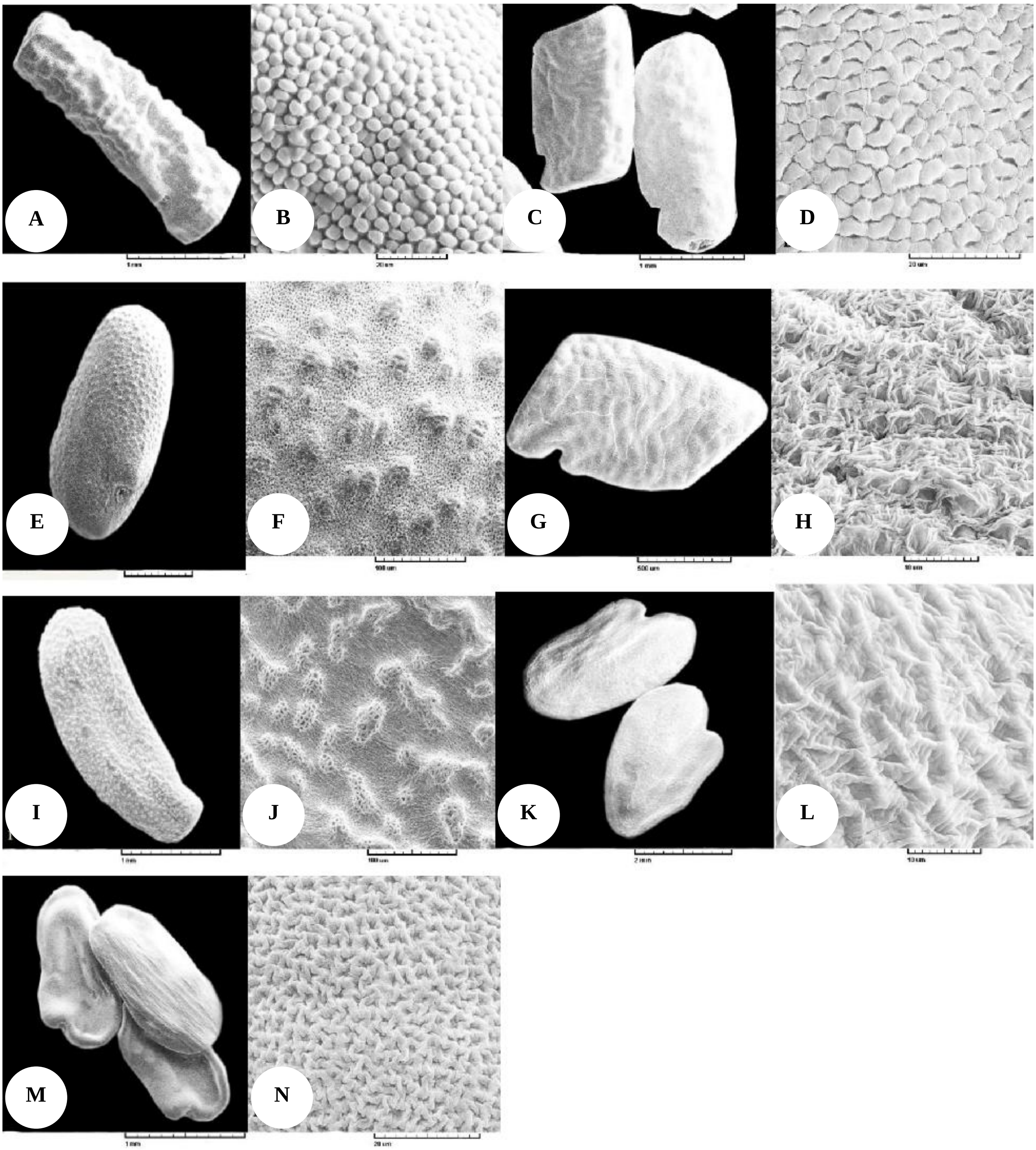

Figure 4. SEM Microphotographs of *Trigonella* seeds. A-B. *T. persica,* C-D. *T. tenuis*, E-F. *T. coerulescens*, G-H. *T. monspeliaca.,* I-J. *T. spruneriana*, K-L. *T. elliptica,* M-N. *T. uncata*

According to Figures 5 and 6 most of medicagoid *Trigonella* species are placed in subclusters B1 (except *T. coerulescens*) and B2a (except *T. uncata*) and are more closely related to each other than the non medicagoid species. *T. coerulescens* and *T. calliceras* are placed in two separate groups, so this result does not agree with Turki et al. (2013) results, but agree with them because of these two non medicagoid species are placed at a farther distance from *T. monspeliaca* medicagoid species. In subcluster B1 majority of *Bucerates* species and *T. brachycarpa* from *Lunatae* and in subcluster B2a two species from *Bucerates* section and *T. monspeliaca* from *Reflexae* section are nested together.

Result of PCA analysis showed that the first 3 components comprise about 63% of total variation. In the first component with about 26.3% of total variation, the seed shape, seed color, testa texture and hilum shape are the most variable characters (0.61>r> 0.68). In the second factor with about 20.5% of testa variance, mean width and seed homogeneity are most variable characters

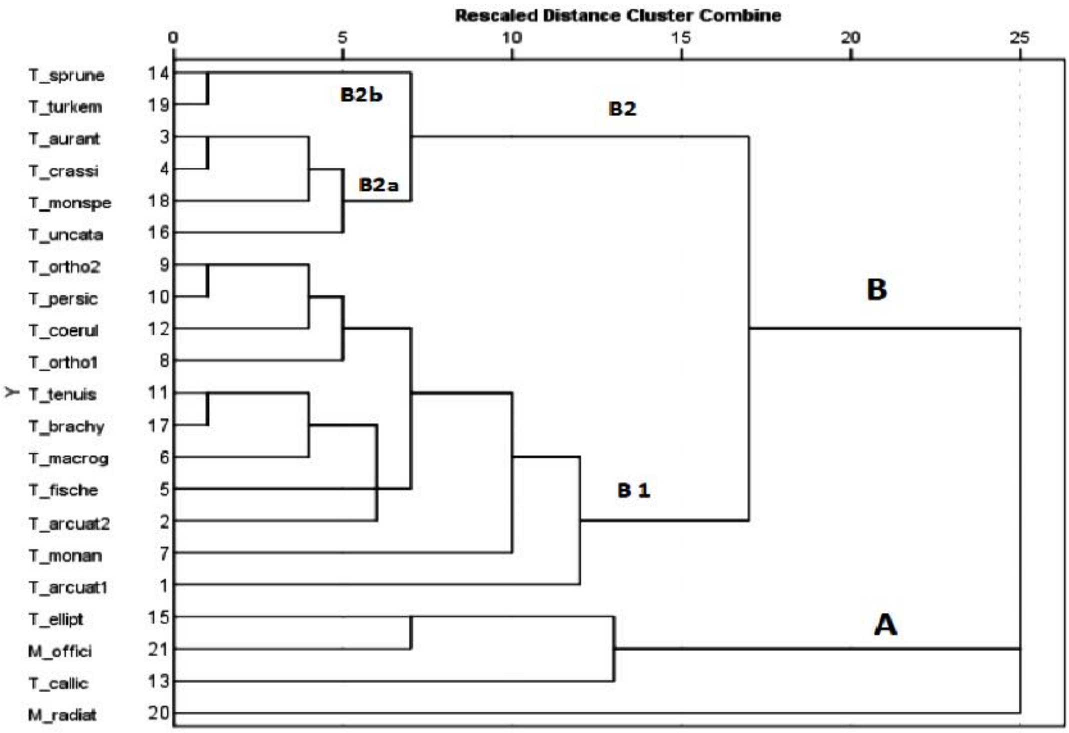

Figure 5. Phenogram based on morphological analyzing data of 22 taxa species (6 species of non medicagoid *Trigonella*, 11 species of medicagoid *Trigonella*, 1 species of *Medicago* and *Melilotus*). Note: T_arcuat: *Trigonella arcuata*, T_aurant: *T. aurantiaca*, T_crassi: *T. crassipes*, T_fische: *T. fischeriana*, T_macrog: *T. macroglochin*, T_monanth: *T. manantha*, T_ortho: *T. orthoceras*, T_persic: *T. persica*, T_coerul: *T. coerulescens*, T_callic: *T. calliceras*, T_ellipt: *T. elliptica*, T_brachy: *T. brachycarpa*, T_monspe: *T. monspeliaca*, T_turkem: *T. turkmena*, T_sprune: *T. spruneriana*, M_radiata: *Medicago radiata*, M_offici: *Melilotus officinalis*.

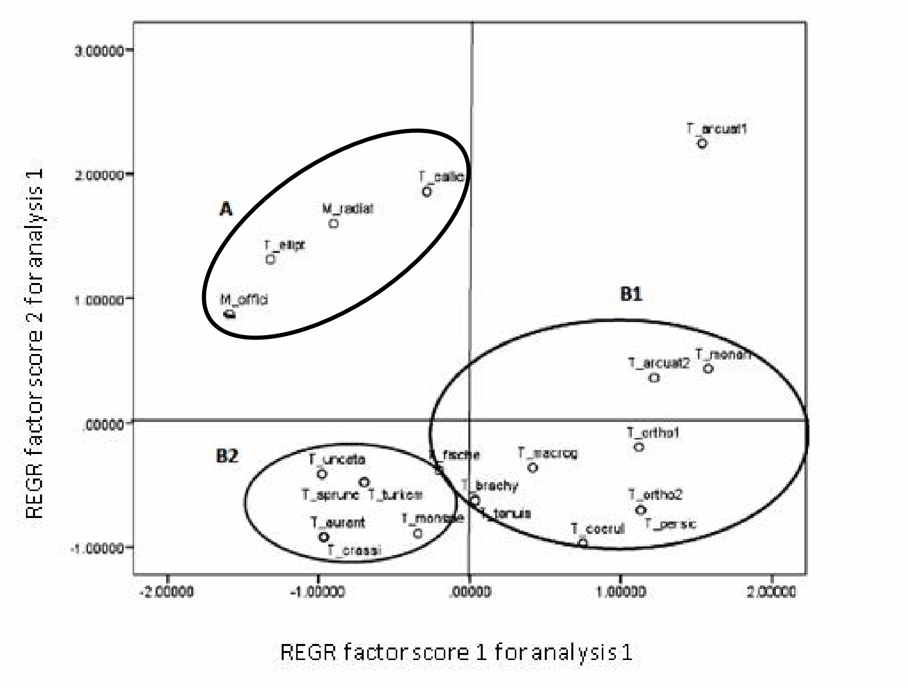

Figure 6. Relationship of all *Trigonella* species in Principal Component Analysis (PCA) based on seed morphological characters (abbreviations as Figure 5).

(0.66>r>0.68). In the third factor with about 16.2% of total variance testa ornamentation is the most variable character (r> 0.64). *T.arcuata1* and members of the group A, respectively, were diverse from members of the B1 and B2 groups by the most variable characters in the second factor. Also, the most variable characters in the first factor caused to separation of group B1 from B2. Result of PCA analysis confirmed phenogram result.

The results of this study agree with Flora Iranica (Rechinger 1984) which placed majority of medicagoid *Trigonella* species in *Bucerates* section, such as subclusters B1 and B2a based on seed morphology in our study. *T. monspeliaca* from *Reflexae* section and *T. brachycarpa* from *Lunatae* section grouped in medicagoid *Trigonella* species of *Bucerates* which is not in agreement of Flora Iranica treatment placing them in two separate sections from *Bucerates* section.

As a conclusion seed characters may be used as a useful tool in the taxonomy of the genus *Trigonella* but as Baum (1968) already pointed out more materials should be used in these studies.

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REFERENCES

Al-Ghamdi FA, Al-Zahrani RM. 2010. Seed morphology of some species of *Tephrosia*pers. (Fabaceae) from Saudi Arabia. Identification of species and systematic significance. Feddes Repertorium 121: 59-65.

Baum B. R. 1968. A Clarification of the generic limits of *Trigonella* and Medicago. Can J Bot 46: 741-749.

Bena G. 2001. Molecularphylogeny supports the morphologically based taxonomic transfer of the “Medicagoid” *Trigonella* species to the genus *Medicago* L. Plant Syst Evol 229: 217-236.

Bianco L M, Ferrer-Gallego P, Grillo O, Laguna E, Venora G, Bacchetta G. 2015. Seed image analysis provides evidence of taxonomic differentiation within the *Medicago* L. Sect. *Dendrotelis* (Fabaceae). Syst Biodivers 13 (5): 484-495.

Ceter T, PinarM, AkanH, EkiciM, AytacZ. 2012. Comparative seed morphology of *Trigonella* L. species (Leguminosae) in Turkey. Afr J Agric Res 7 (3): 509-522.

Gazara M, Kamel W, Haider A. 2001. Cladistic analysis of the genera: Trifolium, *Trigonella* and *Melilotus* (Fabaeeae ;Papilionaceae) in Egypt. Egypt J Biol 3: 161-170.

Gunes F, Cirpici A. 2011. Seed Characteristics and Testa Textures some Taxa of Genus *Lathyrus* L. (Fabaceae) from Turkey. Int J Agric Biol 13 (6): 1814-9596.

Gunes F. 2013. Seed characteristics and textures of *Pratensis*, *Orobon*, *Lathyrus*, *Orobastrum* and *Cicercula* srctions from *Lathyrus* (Fabaceae) in Turkey. Plant Syst Evol 299: 1935-1953

Huber-Morath A. 1970. *Trigonella* in P. H. Davis Flora of Turkey and the East Aegean Islands vol. 3: 452-482. Edinburgh.

Kahreman A, Chidire H, Dogan M. 2014. Anatomy, macro-and micromorphology *Lathyrus* sect. *Nissolia* (Fabaceae) and their taxonomic significance. Proc Nat Acad Sci, India Section B: Biol Sci 84: 407-417.

LerstenNR, Gunn CR. 1982. Testa characters in tribe *Vicieae* with notes about tribes *Abreae*, *Cicereae*, and *Trifolieae* (Fabaceae). U. S. Department of Agriculture Technical Bulletin. Number 1667: 1-40

Mirzaei L, Assadi M, Nejadsatari T, Mehregan I. 2015a. Seed surface Morphology in some Species of the *Colutea* L. (Fabaceae) in Iran. Adv Biores 6 (5): 105-109.

Mirzaei L, Assadi M, Nejadsatari T, Mehregan I. 2015b. Comparative seed and leaf micromorphology of *Colutea* species (Fabaceae) from Iran. Environ Exp Biol 13: 183-187.

Ozbek F, Ozbek MO, Ekici M. 2014. Morphological, anatomical, pollen and seed morphological properties of *Melilotus bicolour* Boiss & Balansa (Fabaeae) endemic to Turkey. Aust J Crop Sci 8 (4): 543-549.

Pinar MN, Duran A, Ceter T, Tug N. 2009. Pollen and seed Morphology of the Genus *Hesperis* L. (Brassicaceae) in Turkey. Turk J Bot 83-96.

Ranjbar M, Hajmoradi Z. 2012. Notes on *Medicago* sec *Lunatea* Boiss. and *Trigonella* sec. *Bucerates* Boiss. of the tribe *Trifolieae* (Fabaceae) with two new records from Iran. Iran J Bot 18 (2): 235-238.

Rechinger KH. 1984. *Trigonella.* In: Rechinger KH. Flora Iranica 157: 207-253. Graz.

Rodrigues RS. 2015. Note the morphology of *Entadapolyphylla* (Leguminoseae, Mimosoideae) and its taxonomic significance in E. sec. *Entadopsis*. Bol Mus Int de Roraima 9 (1): 8-11.

Salimpour F, Mostafavi F, Sharifnia F. 2007. Micromorphologic Study of the Seedof Genus *Trifolium*, section *Lotoideae* in Iran. Pak J Biol Sci 10 (3): 378-383.

SalimpourF, SafiedinM, Sharifnia F. 2013. Phylogenetic relationships between *Trigonella* species (*Bucerates* section) using ITS markers and morphological traits. J Bio Env Sci 3 (12): 116-124.

Small E, Brookes B, Lassen P. 1990. Circumscription of the genus *Medicago* (Leguminosae) by seed characters. Can J Bot 68: 613-629.

Srichamroen A, Vasanthan TH, Acharyas S, Basu T. 2011. Dry Milling improves the Aqueous Recovery of Galactomannas from *Fenugreek* seeds. Cur Nut Food Sci 7: 63-72.

Steele KP, Ickert-Bond M, Zarre S, Wojciechowski MF. 2010. Phylogeny and character evolution in *Medicago* (Leguminosae). Evidence from analyses of plastid *trn/matk* and nuclear GA30X1 sequences. Am J Bot 97: 1142-1155.

Steele KP, Wojciechowski MF. 2003. Phylogenetic analyses of tribes *Trifolieae* and Vicieae, based on sequences of the Plastid gene matk (Papilionoideae: Leguminosae). Advances in Legume Systematics, part 10, Higher Level Systematics. Royal Botanic Gardens, Kew.

Teixeira de QR, Azevedo de TAM, Lewis GP. 2013. Seed morphology: an addition to the taxonomy of *Tephrosia* (Leguminoseae, Paplionoideae, Millettieae) from South America. Plant Syst Evol 299: 459-470.

Tia WK. 2004. Tribe *Trifolieae*: Evidence from seed characters. Pak J Biol Sci 7 (7): 1287-1302.

Turki Z, El-Shayeb F, Abozeid A. 2013. Seed Morphology of Some *Trigonella* L. Species (Fabaceae) and its Taxonomic Significance. Intl J Sci Res 2319-7064.

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Ethnobotanical study of herbal medicine in Ranggawulung Urban Forest, Subang District, West Java, Indonesia

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Abstract. *Putri LSE, Dasumiati, Kristiyanto, Mardiansyah, Malik C, Leuvinadrie LP, Mulyono EA. Biodiversitas 17: 172-176.* Ethnobotanical study is the first time study done in Subang District, West Java, Indonesia focused in the surrounding area of Ranggawulung Urban Forest (RUF). This study is related to plants diversity in Ranggawulung urban forest which is under the management of PT. Pertamina EP Field Subang, Indonesia. The purpose of the study was to investigate and collect information from local people on the use of medicinal plants in Subang District, West Java, Indonesia. The field study was conducted from October-November 2015 in Subang District through deep personal interview and questionnaire then all information were written and documented. Based on the diversity index of Shannon-Wiener, RUF was categorized in high diversity (H'=3.64). The total number of plant species in RUF was 1655 species belonging to 179 species from 101 families and only 32 species used for traditional medicines among local peoples. The highest frequency of plant parts used were leaves (47%) and fruit (17%) and followed with other parts of trunk, root, tuber, latex, bark and seed. The form of decoction was the most frequently prepared and administered orally. It indicates that Skeleton-Muscular System Disorder (SMSD) and Endocrinal Disorder (ED) had the highest use reports which came from 19 species of plants belonging to 3 families (Moraceae, Meliaceae, and Myrtaceae) to heal diseases including diabetes and back pain/rheumatism. It can be stated that higher index of diversity in RUF did not directly affect the use of plants for traditional medicine. Socialization of herbal medicine used among local people has to be improved as there are many species of plants are available to heal many diseases surrounding Subang District.

Keywords: Ethnobotany, medicinal herbs, Ranggawulung Urban Forest, Subang District

INTRODUCTION

Indonesia is known as a country with a mega source of biodiversity which is distributed from western (Sabang region) to eastern part (Merauke region). There are approximately 30,000 species of plants, 9,600 species of which are medicinal plants (Decree of Minister of Health RI No. 381/Menkes/SK/III/2007). It has been identified that more than 1,800 of plant species exist and planted in the several forest formations, but it is only 940 plant species has been used by local people for traditional herbal medicine and only 300 species by drug industries (Indonesian Institute of Sciences 2014).

Along with public awareness of health enhancement and increasing prices of conventional medicinal, the use of traditional herbal medicine is not only more popular in Indonesia (Abdillah et al. 2014; Efremila et al. 2015) but also in Asian Pacific countries (Yabesh et al. 2014; Lone et al. 2012; Ali-Shtayeh et al. 2008) and Africa (Chekole et al. 2015); Maroyi 2013). Traditional herbal medicine has a comparative advantage because of a relatively lower cost, ease of use and minimal side effects than using synthetic and modern medicines. Some people also believe that this type of medicine can treat a variety of diseases, such as fever, dysentery, skin diseases, poison bites, wounds, ulcers, rheumatism (Yabesh et al. 2014; Sankaranarayanan et al. 2010; Soejarto et al. 2011).

Plant parts often used for the treatment of ailments are leaves, bark, stem, fruit and root or tubers. In District of Kerala-India, leaves are widely used as a medicinal ingredient (Yabesh et al. 2014). In addition, wild plant species are used as medicinal ingredients by local people in Maonan, Southwestern China (Hong et al. 2015).

Ranggawulung's urban forest (RUF), administratively managed by two government agencies which are Department of Forestry and Plantation and Department of Agriculture. The reserve is dominated by a high variety of plants including medicinal herbs (BPS Subang 2014). In 2012, It had approximately 4192 plant species belonging to 138 families (Centre for Environmental Studies UIN Jakarta 2012). Definitely, RUF has a high potential as a source of herbal medicine use. Unfortunately, current condition does not indicate optimal utilization of biodiversity by local people of surrounding RUF. This is due to lack of knowledge and information about the benefits of plant species in RUF and surrounding areas. Residents in communities surrounding RUF have not optimally used the plants for medicinal purposes. Therefore, this study aimed at making an inventory of potential medicinal plants in RUF and its surrounding utilized by local people to cure various diseases

MATERIALS AND METHODS

This research was carried out in the urban forest of Ranggawulung from October-November 2015. RUF is located in the district of Subang District, West Java, Indonesia with an area of 84 hectares at an altitude of 700 m above sea level (Figure 1). RUF has a strategic potential for providing ecosystem services to communities in Subang area. The level of rainfall in Subang is 1600-3000 mm/year. Subang District has unique topography divided into three types: mountain areas (in southern), hills areas (center), and lowland areas (in northern) (BPS Subang 2014).

The research method used included field observations, discussions and deeply personal interview with local community such as healers/herbal practitioners, ranger, farmers, homemakers and government staff. The informants used in this survey were 47 persons including 28 females, and 19 males were selected randomly between the ages of 31-75 years old in the region of western, eastern, southern and northern of RUF, approximately living less than 1 km in distance. The age of informants is an important parameter which can influence the level of knowledge about the use of plants for medicine (Hong et al. 2015).

Data collected from the informants regarding to the use of plants for herbal medicine were type of plants, parts of plants, the preparation and application of plants, and the kind of ailment. Those data were compiled and presented descriptively which were also discussed with the relation of plant diversity in RUF. All plants species were collected and identified directly in the field. For the name of plants species could not known were documented and identified by identification books (Soerjani et al. 1987; Priyadi et al. 2010; Djawarningsih 2011; Sabara 2011). The collection of plants sample was avoided to conserve the existence of them through destruction of plant parts.

RESULTS AND DISCUSSIONS

Plant biodiversity presented in RUF has the potential as herbal remedies which were categorized in high diversity (H'= 3.07 for shrub and H'= 3.64 for tree) based on Shannon-Wiener's index of diversity. It was found approximately 1655 plant species belonging to 179 genera from 101 families of which 32 species belonging to 12 families were used as traditional herbal medicine (Table 1). This is a huge number of medicinal plants species in RUF, compared to other urban forests in Asian and African Countries such as India and Ethiopia (Lone et al. 2012; Yabesh et al. 2014; Chekole et al. 2015).

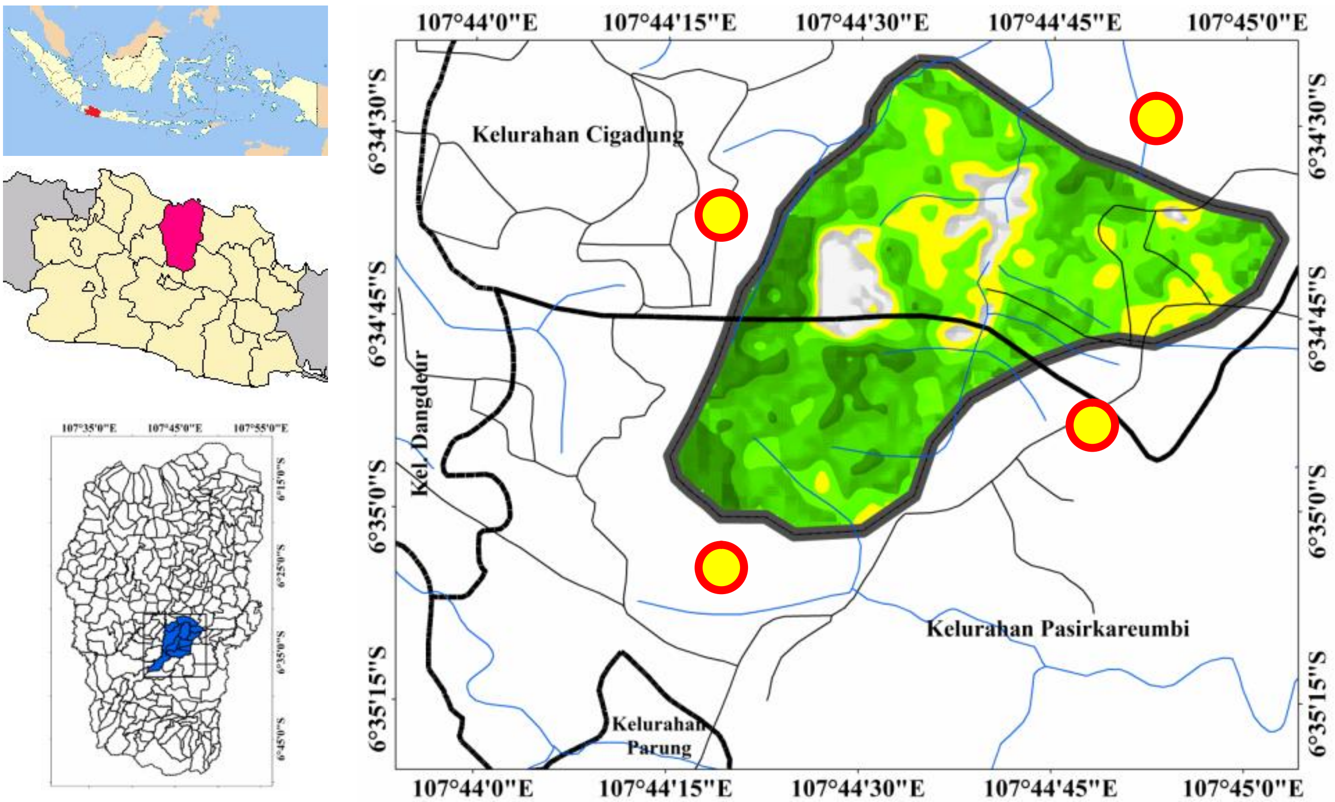

Figure 1. Map of Subang, West Java, Indonesia showing Ranggawulung's Urban Forest (RUF) and sampling sites of study (○)

Table 1. Use medicinal plant species found in the area of Ranggawulung's Urban Forest (RUF), Sudang, Indonesia

Botanical name	Family	Vernacular name	Parts used	Preparation	Application	Ailment category
Abelmoschus manihot (L.) Medik.	Malvaceae	Daun nedi/ Daun mujarab	Leaves	Juice mixed with water	Oral	Fever
Ageratum conyzoides L	Compositae	Babadotan	Leaves	Paste	Topical	Wound
Areca catechu L	Arecaceae	Jambe	Root	Decoction	Oral	Back pain/ rheumatism
Arenga pinnata Merr.	Arecaceae	Aren	Root	Decoction	Oral	Back pain/ rheumatism
Artocarpus altilis (Park.) Fsb.	Moraceae	Sukun	Fruit	Decoction	Oral	Heart disease
Artocarpus heterophyllus Lamk.	Moraceae	Nangka	Leaves	Decoction	Oral	Back pain/ rheumatism
Averrhoa carambola L.	Oxalidaceae	Belimbing	Fruit	Raw, Juice	Oral	Hypertension
Allium cepa L.	Amaryllidaceae	Bawang merah	Tuber	Raw mixed with water	Cleaning solution	Cataract
Capsicum frutescens L.	Solanaceae	Cengek	Leaves	Paste	Topical	Constipation
Ceiba pentandra Gaertn.	Malvaceae	Randu	Leaves	Decoction	Oral	Fever
Centella asiatica Urb.	Apiaceae	Antanan	Leaves	Decoction	Oral	Cough
Cocos nucifera L.	Arecaceae	Kelapa	Fruit	Baked and the water mixed with brown sugar	Oral	Urinary disease (urinary stones)
Costus spicatus Jacq.	Costaceae	Pacing	Trunk	Juice mixed with water	Oral, dripped	Diabetic, heartburn, eyes ache
Dracaena angustifolia (Medik.) Roxb.	Ruscaceae	Daun Suji	Leaves	Boiled	Oral	Hypertension
Graptophyllum pictum Griff.	Acanthaceae	Handeleum	Leaves	Decoction	Oral	Liver disease
Hibiscus tiliaceus L.	Malvaceae	Waru	Trunk	Liquid secretion	Dripped	Eyes ache
Imperata cylindrica Beauv.	Poaceae	Alang-alang	Root	Decoction	Oral	Body strength (increase stamina)
Leucaena leucocephala (Lamk.) de Wit.	Fabaceae	Petai Cina	Seed	Powder	Oral	Diabetic
Manihot utilissima Pohl.	Euphorbiaceae	Singkong	Leaves, Tuber	Juice mixed with brown sugar, raw	Oral	Diarrhea, anemic
Mimosa pigra L.	Leguminosae	Buset	Leaves, trunk	Decoction	Oral	Diabetic
Parkia speciosa Hassk. Pete	Fabaceae	Pete	Leaves	Paste	Topical	Ulcer
Persea americana Mill.	Lauraceae	Alpukat	Leaves	Decoction	Oral	Back pain/ rheumatism
Pterocarpus indica Willd.	Fabaceae	Angsana	Latex	Latex	Dripped	Tooth ache
Swietenia macrophylla King.	Meliaceae	Mahoni	Seed	Raw	Oral	Diabetic
Polyscias guilfoylei (W. Bull) L.H. Bailey.	Araliaceae	Kadondong lalap	Leaves	Boiled	Oral	Urinary disease (urinary stones)
Sauropus androgynus Merr.	Phyllanthaceae	Katuk	Leaves	Juice mixed with water	Oral	Breast milk production
Solanum nigrum L.	Solanaceae	Leunca	Fruit	Raw	Oral	Body strength (increase stamina)
Solanum torvum Swartz.	Solanaceae	Takokak	Fruit	Raw	Oral	Diabetic
Syzygium cumini (L.) Skeels	Myrtaceae	Jamblang	Bark	Powder	Oral	Diabetic
Syzygium polyanthum (Wight) Walpers.	Myrtaceae	Salam	Leaves	Decoction	Oral	Diabetic
Tamarindus indica L.	Fabaceae	Asem	Fruit	Paste	Topical	Eliminate bruises
Vernonia amygdalina Delile.	Compositae	Pohon Afrika	Leaves	Decoction	Oral	Back pain/ rheumatism

Recently, Forest Reserve Management supported by PT. Pertamina EP Field Subang had been involved in the critical role of RUF management providing medicinal plants started from 2010. The study of diversity in RUF was started from 2012 and was studied again in 2015 as monitoring activity. At 2012, diversity index was at value of H'=2.43 (Centre for Environmental Studies UIN Jakarta 2012) and based on this study the diversity index increased to a value of H'=3.64. However, the number of plant species that was 4192 species in 2012 decreased significantly to 153% at 2015 which found 1655 species in this study. The main cause of decreased plant species number was land use change into other uses such as rice field, crop plantation, recreational area and also destructive activities including sand mining and trees logging. This needs rapid intervention from regional government, including PT. Pertamina itself and also local community to conserve the ecosystem services provided by RUF.

The plant parts used for herbal medicine preparation were mostly leaves (47%) and fruits (17%) but other parts such as trunk, root, tuber, seed, bark and latex were also used (Figure 2). Ease of use and availability were the main reasons people took these parts to heal diseases. Back pain and diabetes were ailments mainly suffered by Ranggawulung's community at 18-19% (Figure 3). These were categorized into Skeleton-Muscular System Disorder (SMSD) and Endocrinal Disorder (ED). Those diseases were mostly treated from part of *Swietenia mahagoni* (seeds, raw form), *Artocarpus heterophyllus* (leaves, decoction form) and *Syzygium polyanthum* (leaves, decoction form).

Based on data of some research, it was proved that *Swietenia mahagoni, Artocarpus heterophyllus* and *Syzygium polyanthum* contained many important organic compounds such as flavonoids, saponins and alkaloid terpenoid and steroid (Sahgal et al. 2009, Linghuat 2008, Asaeli 2013). *Swietenia mahagoni* decreased blood glucose level in white mice with the doses of 250 mg/kg body weight (Suryani 2013). Asaeli (2009) also found anti-diabetic effect in the dose of 1.5 g/kg body weight in *Artocarpus heterophyllus*. Bay leaves (*Syzygium polyanthum*) were also reported by Taufiqurrohman (2015) to be a potential anti-diabetic drug. The use of these plants by people surrounding RUF collaborates with the possession of active ingredients as noted above.

The ailment type dominantly found among people surrounding RUF were Skeleton-Muscular System Disorder (SMSD) and Endocrinal Disorder (ED), General Health (GH) and Ear, Nose and Throat Problems (ENT) (Figure 4). These ailments were almost similar to those reported in India (Yabesh et al. 2014), Zimbabwe (Maroyi 2013) and Palestine (Ali-Shtayeh 2008). In Palestine and India, leaves were dominantly used while roots were mostly used in Zimbabwe. Regarding to knowledge of herbal medicine, people at Libo Kemkem district, Ethiopia was better than people at Subang district. People at Libo Kemkem district, Ethiopia mostly used plants to treat common ailment such as wound (*Cordia africana*) and stomachache (*Stephania abyssinica*) and also for serious ailments such as anthrax (*Stephania abyssinica, Adiantum capillus*) and cancer (*Clematis simensis*) (Chekole et al. 2015).

In Subang district, rather than using medicinal plants, people preferred to use conventional medicine from a local health center. The Indonesian government program of Health Card (*Kartu Sehat*) is one of the reasons why were more people preferred to go the health center than using traditional herbal medicine. This program is developed for poor people to get free health treatment and medication. The local people mostly poor felt better after taking conventional medication than herbal medicine as the effects appeared sooner than conventional medicine. BPJS (Badan Penyelenggara Jaminan Sosial/Social Security Administrator) is another program has developed to cover health problem including treatment in hospital. This program is a health insurance managed by state-owned enterprises with very low premium cost covered by the company (0.5% of minimum wage) where they work or paid individually for unemployment (IDR 40,000-60,000).

Using Health Card or BPJS card, local people gain free medical treatments from local health centers that exist in each village. This condition is good but the abundance of herbal plants in home garden and urban forest was another positive benefit taken by people as ecosystem services. In the future, it is hoped that herbal medicine can be used dominantly in local health center through intensive scientific analyzes of medicinal plants.

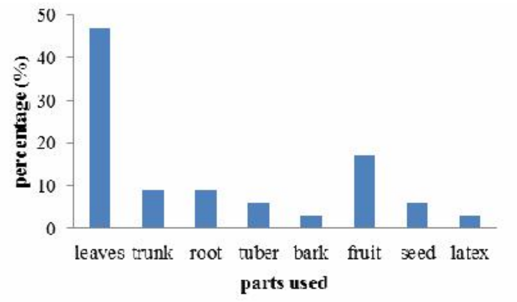

Figure 2. Percentage of plant parts used for the preparation of medicine

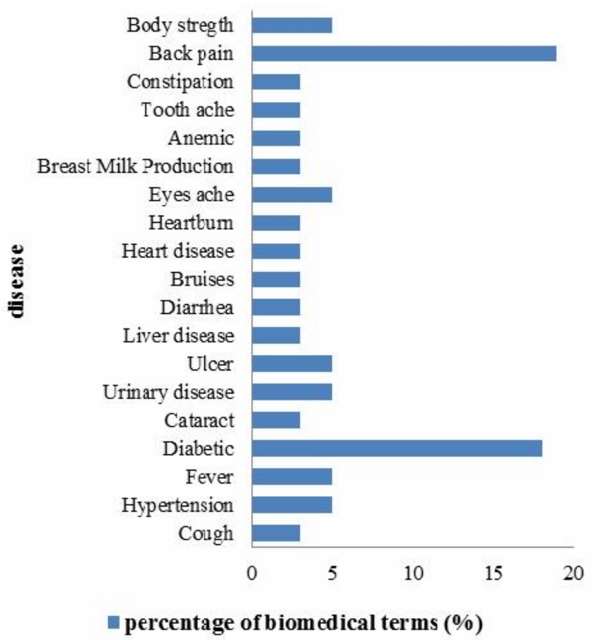

Figure 3. Percentage of health conditions suffered by people surrounding RUF

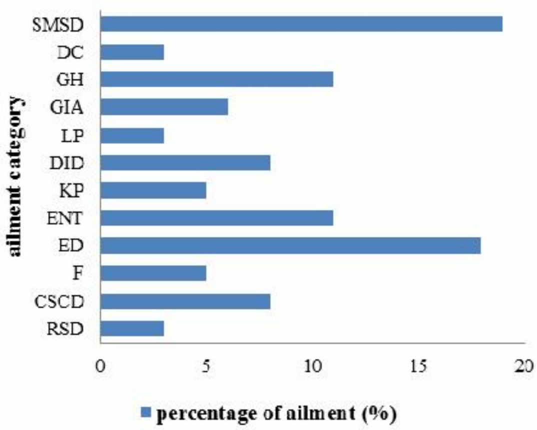

Figure 4. Percentage of ailment (SMSD: Skeleton-Muscular System Disorder, DC: Dental Care, GH: General Health, GIA: Gastro-Intestinal Ailment, LP: Liver Problem, DID: Dermatological Infection/Disease, KP: Kidney Problem, ENT: Ear, Nose, Throat Problems, ED: Endocrinal Disorder, F: Fever, CSCD: Circulatory System/Cardiovascular Disease, RSD: Respiratory System Disease)

The awareness of regional government and PT. Pertamina EP Field Subang as a stakeholder in the management of RUF and conservation of medicinal plants were good enough which was showed revealed in the planting program of medical plants in RUF. It had been demonstrated that the cultivated herbal plants including shrubs and trees dominantly existed in RUF at 58% which was planted by forest reserve management agency and also supported by PT. Pertamina field Subang, while a few was wild plants at 42%. This was similar at Kupwara District, Kashmir, India but the shrubs were dominantly used (Lone et al. 2012).

Some of the medicinal plants planted in RUF since 2012 had already grown significantly and included *Syzygium polyanthum* (2-3 cm dbh), *Acacia mangium* (4-5 cm dbh), *Alstonia scholaris* (2-12 cm dbh). Unfortunately, other species planted like *Parajubaea sunkaha, Gmelina arborea,* and *Cinnamomum verum* failed to grow due to destructive activities by local people and poor maintenance. This conservation of biodiversity of Subang district is aimed to avoid extinction of some plant species.

In conclusion, it was found 32 plant species are used for traditional medicines among local peoples surrounding Ranggawulung's Urban Forest. Leaves and fruit were mostly used plant parts used at 47% and 17% respectively, by trunk, root, tuber, latex, bark and seed. Decoction and oral administration were frequently used method of preparation and administration. Skeleton-Muscular System Disorder (SMSD) and Endocrinal Disorder (ED) were the ailments which had the highest use reports from 19 species of plants belonging to 3 families (Moraceae, Meliaceae, and Myrtaceae). Ailments in these categories included diabetes and back pain/rheumatism symptoms. Unfortunately, higher index of diversity in RUF (H'=3.64) and surrounding area particularly private garden or yard did not directly affect the use of plants for traditional medicine. Socialization of herbal medicine used among local people has to be improved as there are many species of plants available to heal many diseases surrounding Subang district. The role of PT. Pertamina as a stakeholder of RUF has to be enhanced regarding conservation through mitigation, monitoring and maintaining biodiversity in RUF so ecosystem equilibrium can be reached and sustained for a long period.

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REFERENCES

Abdillah S, Tambunan RM, Sinaga YM, Farida Y. 2014. Ethno-botanical survey of plants used in the traditional treatment of malaria in Sei Kepayang, shavings of North Sumatra. Asian Pacific J Trop Med 7 (Suppl 1): S104-S107.

Ali-Shtayeh MS, Jamous RM, Al-Shafie' JH et al. 2008. Traditional knowledge of wild edible plants used in Palestine (Northern West Bank): A comparative study. J Ethnobiol Ethnomed 4 (13): 1-13.

Asaeli HA. 2009. The effect of jackfruit leaf extract (*Artocarpus heterophyllus* Lamk.) to decrease blood glucose level in whiter male using glucose tolerance testing. [M.Sc.-Thesis]. Faculty of Pharmacy, Widya Mandala Catholic University, Surabaya. [Indonesian].

BPS Subang. 2014. Subang in Figure 2014. Central Bureau of Statistics of Subang District, Subang.

Centre for Environmental Studies UIN Jakarta. 2012. Report Study of Biodiversity and Biological Resources in Ranggawulung Urban Forest, Subang. Centre for Environmental Studies UIN Syarif Hidayatullah Jakarta and PT. Pertamina EP Java Regian Field Subang, Jakarta.

Chekole G, Asfaw Z, Kelbessa E. 2015. Ethnobotanical study of medicinal plants in the environs of Tara-Gedam and Amba remnant forests of Libo Kemkem District, the northwest Ethiopia. J Ethnobiol Ethnomed 11 (4): 1-38.

Decree of Minister of Health of the Republic of Indonesia Number: 381/Menkes/SK/III/2007, March 27, 2007: National Policy of Traditional Medicine in 2007

Djawarningsih T, Supriatna A, Amir M. 2011. Flora of Tukung Gede Mountain, Serang-Banten. LIPI Press, Jakarta.

Efremila, Wardenaar E, Sisillia L. 2015. Study on medicinal plants by Ethnic Dayak Tribe in the Kayu Tanam Village, Mandor Sub Regency, Landak Regency. Jurnal Hutan Lestari 3 (2): 234-246.

Hong L, Guo Z, Huang K, Wei S, Liu B, Meng S, Long C. 2015. Ethnobotanical study on medicinal plants used by Maonan people in China. J Ethnobiol Ethnomed 11 (32): 1-35.

Indonesian Institute of Sciences. 2014. Present Status of Indonesian Biodiversity. Indonesian Institute of Science, BAPPENAS and Department of Environment, Jakarta.

Linghuat R. 2008. The effect of Ethanol Extract in Mahogany Seed (*Swetenia mahagoni*) to Decrease Blood Glucose Level in White Mice. [M.Sc.-Thesis]. University of North Sumatra, Medan. [Indonesian].

Lone FA, Lone S, Aziz MA, Malla FA. 2012. Ethnobotanical studies in the tribal areas of district Kupwara, Kashmir, India. Intl J Pharma BioScie 3 (4): 399-411.

Maroyi A. 2013. Traditional use of medicinal plants in south-central Zimbabwe: a review and perspectives. J Ethnobiol Ethnomed 9 (31): 1-18.

Priyadi H, Takao G, Rahmawati I, Supriyanto B, Nursal IW and Rahman I. 2010. Five Hundreds Plant Species of Gunung Halimun Salak National Park, West Jawa: A Checklist Including Sundanese Names, Distribution and Use. Centre for International Forestry Research (CIFOR), Bogor.

Sabara E. 2011. A Protected of 100 Plants of Gede Pangrango Mountain. Green Radio and Gunung Gede Pangrango National Park, Cianjur. [Indonesian]

Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM. 2009. Antimicrobial activity of *Swietenia mahagoni* Crude 125 methanolic seed extract. Trop Biol 26: 274-279.

Sankaranarayanan S, Bama P, Ramachandran J, Kalaichelvan PT, Deccaraman M, Vijayalakshimi M, Dhamotharan R, Dananjeyan B, Bama SS. 2010. Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. J Med Pl Res 4 (12): 1089-1101.

Soerjani M, Kostermans AJGH, Tjitrosoepomo G. 1987. Weed of Rice in Indonesia. Balai Pustaka, Jakarta.

Suryani N, Endang TH, Aulanni'am. 2013. Effect of methanolic *Swietenia mahagoni* seed extracts in increasing insulin level, decreasing TNF-α expression and repairing pancreatic tissue damage on diabetic rat. J Med Brawijaya 27 (3): 137-145.

Taufiqurrohman. 2015. Indonesian bay leaves as antidiabetic for Type 2 Diabetes Mellitus. J Majority 4 (3): 101-108.

Yabesh JEM, Prabhu S, Vijayakumar S. 2014. An ethnobotanical study of medicinal plants used by traditional healers in silent valley of Kerala, India. J Ethnopharmacol 154: 774-789.

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Dayak Desa forest land use system as social capital to acquire forest management rights in West Kalimantan, Indonesia

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Abstract. *Roslinda E. 2016. Dayak Desa forest land use system as social capital to acquire forest management rights in West Kalimantan, Indonesia. Biodiversitas 17: 177-184.* People communities have an important role to play in biodiversity conservation. The ownership of land has often become a constraint on sustainable management of forest. The aim of this study was to explore the management system of forest land use in Dayak Desa in West Kalimantan, Indonesia. Data collection was undertaken through field observation, focus group discussion (FGD, and interviews with the community members. Dayak Desa has a several forest land use systems including *tawang semilas, tawang sebesai, tawang mersibung, tawang sepayan, tawang serimbak* and *bukit rentap* protection forest, which each system has its own function and utilization. Regardless of the system's differences, the forest land use systems have been supporting species diversity such as flora, fauna, and environmental services in Dayak Desa. This species diversity can support Dayak Desa community daily needs, regarding food, clothing, housing and other secondary needs. Forest utilization by Dayak Desa community is suitably paired to what can be produced by the land, which is equipped with their own rules. The role and the rule in managing, utilizing and protecting forest land use are Dayak Desa social capital. These are capital to acquire their rights to manage the forest.

Keywords: community, local knowledge, social capital

INTRODUCTION

Traditional communities have practiced conservation of forest land use. The term 'conservation' consists of the word 'con' (together) and 'servare' (keep/save) who have an understanding of the efforts to maintain or keep in wise use. The understanding of conservation is now translated as the wise use of nature resource or the management for the sustainable use. Conservation of forest land use should integrate local systems that adapt to changes for hundreds of years (Claus et al. 2010). It has been well-established that communities of natural resource users can play important roles in natural resource management (Shahabuddin and Madhu 2010). For example in Kasepuhan community of Western Java, their forest was divided into three categories of use, i.e. *leuweng geledegan*, *leuweng titipan*, and *leuweng sampalan* (Suharjito et al. 2008). Meanwhile community in Rantau Layung, East Kalimantan, their forest was classified into four subtypes of landscapes, i.e. *alas tuo*, *alas adat*, *alas nareng* and *alas mori* (Murniati et al. 2008). Examples of such features include effective monitoring and sanctioning processes, property rights, and enforcement mechanisms that limit access to an exhaustible resource.

The forest can be categorized as common-pool resource (CPRs). CPRs are natural resources for which it is hard to exclude potential users and which can be depleted through over-use (McKean 2000). Most CPRs in Indonesia are largely held under the common property. Common property resources belong to the community, and access rules are defined with respect to community membership. It is a system of shared private property with clear boundaries, rights and management and use rules, yet potential free-rider problems have to be surmounted for communities to organize collective action (McKean 2000).

Understanding the property rights systems is important as it is also based on understanding the local management of natural resources (Khumsri et al. 2009). The loose definition and careless usage of terms will be followed by a general misunderstanding of the various types of "property". There are essentially four types of property rights: open access, common property, private property and state ownership (Helberg 2001). Property rights regimes perform the functions of limiting utilization, coordinating users and responding to the changing of natural resource condition.

The protected forest areas in Indonesia are frequently surrounded by the local common property systems, and this situation has created an overlapping in the natural resources management. Hence, it is important to examine the function and contribution of the common property regimes towards the conservation. There has been limited research on common property regimes embedded with state property. Common property regimes in Ensaid Panjang communal, Kelam Permai Sub-district, Sintang District, West Kalimantan Province, Indonesia are suitable for the evaluation of such property right. The aim of this study was to explore the management system of forest land use in Dayak Desa, as a social capital to acquire the forest management rights.

MATERIALS AND METHODS

Conceptual framework

The main concept of this paper is social capital. Coleman (1988) stated social capital is defined by its function: "they all consist of some aspect of social structures, and they facilitate certain actions of actors-whether persons or corporate actors-within the structure." Since the 1990s, the concept of social capital has gathered an increased attention in the research regarding the CPRs' management and collective action, especially in relation to the sustainable use of natural resources and sustainable development (Ostrom 2000; Lethonen 2004).

Study area

This study was conducted in the Ensaid Panjang Village, Kelam Permai Sub-district, Sintang, West Kalimantan, Indonesia. The total area of the village is 4905.75 hectares. Ensaid Panjang Village is geographically located at N 00°04'01"-00°09'39" and E 111°39'49"-111°42'27". The Ensaid Panjang Village is located of about 27 km from the capital of Kelam Permai Sub-district, 58 km from Sintang (capital city of Sintang District) and 478 km from Pontianak (capital city of West Kalimantan Province). This location can be reached by four wheel drive vehicle for nine hours from Pontianak, or by plane taking two hours from Pontianak. The location of Ensaid Panjang Village can be seen in Figure 1.

Research methods

Survey methods were used in this research. The respondents were communities living in the Ensaid Panjang Village. There were three sub villages, i.e. Rentap Selatan, Ensaid Baru and Ensaid Panjang. In three sub-villages around the study sites there were 162 households. Interviews involved 30 respondents were selected by random sampling. The number of samples or respondents from each sub-villages was ten respondents.

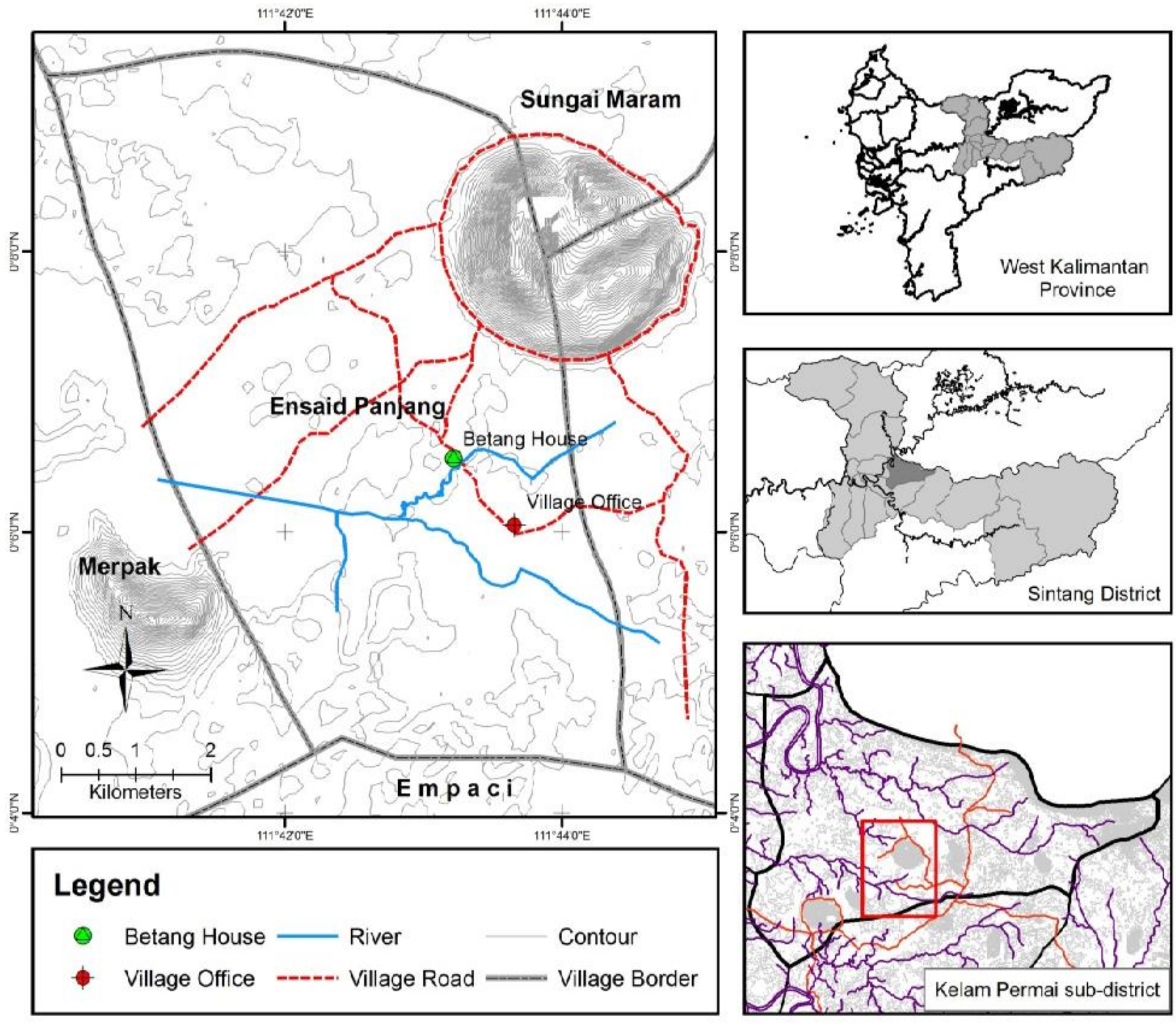

Figure 1. Location of Ensaid Panjang Village, Kelam Permai Sub-district, Sintang, West Kalimantan, Indonesia

Data collection

There were two types of data collected, i.e. primary and secondary data. The primary data were collected through personal interviews, general observation, and focused group discussion (FGD). Personal interviews were supposed to collect detailed information on local knowledge about forest land uses, and the utilization of forest land and natural resources. The general observation was conducted to describe the forest land use characteristics and natural resources. The observation was also done to cross check the information collected from the communities. The FGD was carried out basically to obtain general data from various people representing different groups in the community. A community meeting initialized the methods of data collection. The secondary data consisted of demography, education level, public facilities, and land use systems. The related literature was collected from several sources.

Community meeting

A community meeting was attended by most of the community members, comprising young and senior inhabitant, men and women, and traditional leaders. During the meeting, the participants were asked to discuss how they described the forest land uses around them. People defined six forest land used types i.e. bukit rentap, tawang mersibung, tawang sepayan, tawang semilas, tawang serimbak, and tawang sebesai.

Personal interviews

Personal interview was conducted with 30 selected households out of 162 total households using semi-structured questionnaires. The interview focused on local knowledge about forest land use and the utilization of forest land and natural resources. In addition, the interview was also accomplished with five key informants in the process to know how people manage their land and natural resources. The key informants were including the village head, customary leader, old villagers, and informal community leaders.

Focused group discussion (FGD)

The researcher facilitated FGD with the participated groups of villagers. Several topics were discussed including specific information about the importance of natural resources, forest land utilization, community's role and rules of forest land utilization.

Data analysis

The data from interview were tabulated and analyzed to obtain the general description of forest land use types and local knowledge of the community in Ensaid Panjang Village. The analysis includes two aspects. First, comparing the type of forest land use to obtain the conclusion whether there have any different characteristics in topography, covers area, types of forest, status of land, vegetation and animal, and function. Second, analyzing the results of data processing by using descriptive method, including literature about social capital as conceptual framework and literature about forest land use.

RESULTS AND DISCUSSION

People and livelihood

Ensaid Panjang Village has 162 households or 627 people with a population density of about one person/km^2 (Ensaid Panjang Village 2013). The dominant ethnic is Dayak Desa and most of them are indigenous people and live in the traditional longhouses. The majority of the community members have only completed their elementary school. There are also some community members who did not finish their elementary school and even some of them never gone to school at all. Customary law was still applied in the daily life as a guideline and rules to define what is right or wrong for the whole community. Communities depend on agricultural resources, and subsistence agricultural production became the family's main source of livelihood. They mostly work as shifting cultivators, rubber tappers, loggers, weaver (especially for women) and plantation workers. Most of the community members cultivated upland rice fields by shifting cultivation system for their daily consumption. They also practiced agroforestry in where they mixed the rice plantation with vegetables and fruit trees. The community members have left the area for a new rice field; it will become a fruit garden or agroforestry in the future. Besides, they also went to their garden for rubber tapping, hunting, fishing and collecting non-timber forest products (NTFP) such as rattan, fruits, vegetables, honey, *endas* (for mat), *senggang* (for webbing material), medicinal plant and dyed plant.

Weaving is one of the daily activities that they carried during their leisure time, especially for Dayak Desa women. Ensaid Panjang Village is a producer of weaving clothes. Weaving is a culture that inherited from past generation to recent generation. Currently, *ikat* weaving has been developed as a commodity that can be sold by the community.

Forest land use types

There are several types of land use in Ensaid Panjang Village. The distribution of land use is dominated by agricultural land, rubber and oil palm plantation, shrubs, protected forest, and swamp forest. In this paper, we just identified the forest land use. Ensaid Panjang Village has a forest covers of about 99.5 hectares. There are two types of forest i.e. dry land forest and swamp forest (local name: *tawang*). The identification of the forest land use types in Ensaid Panjang Village including their characteristics are described in Table 1.

Ensaid Panjang community identified six types of forest land. Bukit rentap is a forest which was declared as a protected forest by Decree of Minister of Forestry and Plantation No. 259/KPTS-II 2000 Date 23 August 2000. With facilitation by People Resource and Conservation Foundation (PRCF) Indonesia, bukit rentap has been appointed as forest village (*hutan desa*) in 2014. The forest village means the utilization of the forest is to be organized by Forest Village Management. Secondly, tawang mersibung is a forest area which was declared as the Community Conserved Area (CCA). The usage of tawang mersibung is organized according to customary law communally, located far from the settlement with a steep topography. This landuse cannot be exploited and converted into rice fields. Only NTFP can be utilized from this forest. Thirdly, tawang sepayan is a forest reserved for shifting cultivation area, located close to the settlement. Tawang semilas and tawang serimbak are the forest area that can be utilized by the community for their source of building materials, fire woods, craft materials, medicinal plants and dye plants, and places for fishing and hunting. Lastly, tawang sebesai is a forest that cannot be utilized by the community. There is no clear explanation on,-why tawang semilas was not utilized. Hence, based on information from community members, this forest that is believed to be a dangerous place or haunted area.

The trees in tawang semilas and tawang serimbak could only be logged for their subsistence or self-usage. Till now, this leader regulation is still being practiced by the community. Different from the bukit rentap, since management was carried out by the government, the timber production was extremely huge in this area. All of the forest area was infiltrated by water, but only bukit rentap provides source of clean water for community. Figure 2 shows the forested areas in Ensaid Panjang Village. Formerly, there was tawang sampur in Ensaid Panjang village (PRCF 2011). However, when this study was conducted, tawang sampur has been changed into farmland because of population growth.

All of forest land use system can support the Dayak Desa community needs, such as food, clothing, housing and other secondary needs. All participants indicated that vegetation in tawang sebesai, tawang mersibung, tawang serimbak, tawang semilas, and bukit rentap were dominated by tree and shrubs, except in tawang sepayan. Each species has its special use and can be fully utilized, but this utilization is guided by rules, norms and sanctions.

Local knowledge of forest utilization

Dayak Desa people have a close dependency toward their forest. It is not only limited to the utilization of timber and non-timber forest products, but also to the utilization of plant/animal species based on the suitability of its function. For example, plants that are used as building materials come from a specific species (see Table 2). When compared with Delyanet (2015), there are differences about number of plants that are used as building materials.

There are some important rules to use the timber for building materials. For example, belian (*Eusideroxylon zwageri*) is used for the first pole (*tiang mun*). For this usage, community must use lively belian. While for others usage, community should use a dead/lightning strike tree, especially durian (*Durio zibethinus*). Struck wood is considered as the best timber for building materials. Kumpang (*Horsfieldia polyspherula*) is used for the roof, because, it is believed to protect the occupant from evil spirit. Related to culture, in timber harvesting activities are always held an event *meramu*. Meramu was carried out in *tawang*. These were done by the elders with deep traditional and local knowledge.

Table 1. Identification of forest land use types in Ensaid Panjang Village, Kelam Permai Sub-district, Sintang, West Kalimantan, Indonesia

Land type	Topography	Covers (Ha)	Type of forest	Status of land	Vegetation/ animal	Function
Bukit rentap	Slope to steep and undulating	750.000	Dryland	Protected forest	Mixture of dipterocarps trees	Source of water, protected area
Tawang mersibung	Flat, undulating	53.983	Swamp	Other uses area	Trees, medicinal, ornamental, dyes plants and various types of animals	CCA, source for craft material and dye plants, place for hunting (especially *babi hutan*). Only non-timber forest product (NTFP) can be utilized.
Tawang sepayan	Flat, undulating	17.457	Swamp	Other uses area	Paddy, maize	Shifting cultivation area
Tawang semilas	Flat, undulating	42.778	Swamp	Other uses area	Trees, medicinal, ornamental, dyes plants and various types of animals	Source for building material, firewood, craft material, medicinal plant and dye plant, place for fishing and hunting
Tawang serimbak	Flat, undulating	67.012	Swamp	Other uses area	Trees, medicinal, ornamental, dyes plants and various types of animals	Source for building materials, fire woods, craft materials, medicinal plants and dye plants, place for hunting
Tawang sebesai	Flat, undulating	8.867	Swamp	Other uses area	Trees, medicinal, ornamental, dyes plants and various types of animals	Conservation area

Note: CCA: Community Conserved Area (area managed by customary law communally)

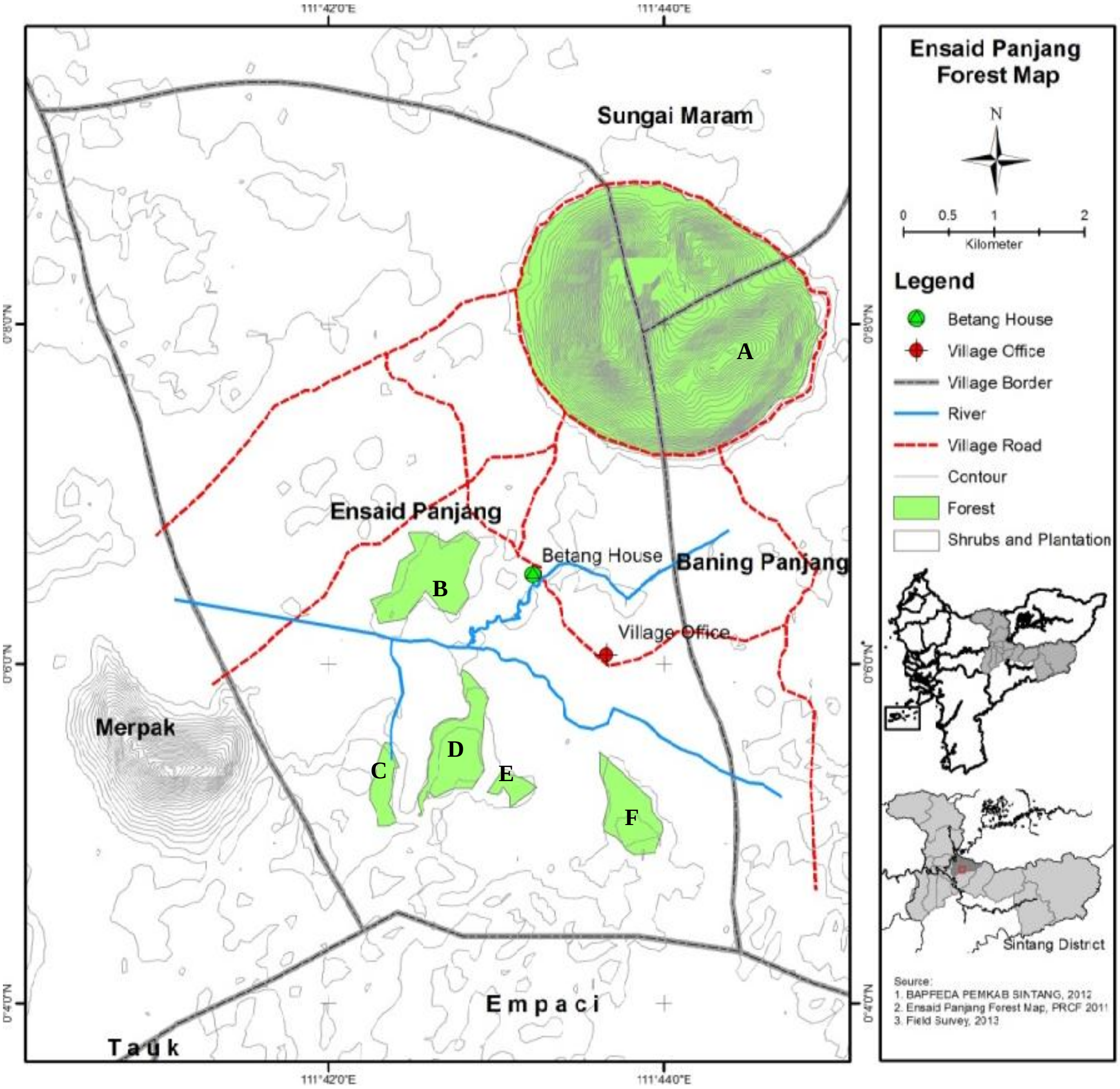

Figure 2. Map of Ensaid Panjang Village forest, Kelam Permai Sub-district, Sintang, West Kalimantan, Indonesia: A. Bukit rentap, B. Tawang serimbak, C. Tawang sepayan, D. Tawang mersibung, E. Tawang sebesai, F. Tawang semilas

Discussion

Dayak Desa people is one of the Dayak tribes who live in modern times but still have a visible state of primeval nature of their religious system. Dayak Desa community often mentions the name *Petara* (lord of the heaven), *Puyang Gana* (lord of the land) and *Raja Juata* (lord of the water). They believe the natural and supernatural is a distinct entity dimension. Supernatural beings have a major effect on human life and should be in harmony with nature. They always do traditional ceremonies to get permission from their lord when doing activities. The traditional ceremony, like *meramu*, is a form of communication and worships the unseen forces that have a higher position that determines life.

A leader in a *kampung* originally was *tumenggung*, however, recently there is a village head who administers a few kampung. Tumenggung is not under the authority of village head. The village head represents formal leader to handle administration while tumenggung is informal to handle social problems. The Catholic priest is also the spiritual leader besides tumenggung.

Dayak Desa people have their agricultural calendar that marked the harvest celebration with *nyelapat taun* ceremony that marks the ending of cultivating season activities. This calendar is a kind of computation time of harvesting heritage associated with rainy and dry seasons. Rice fields are very limited and are on a slope that is hard to apply with modern farming technology. Farm management is still traditional using organic systems, but some people have started using fertilizers and chemical pesticides.

The traditional education system has been altered by the presence of a formal education; people send their child minimal elementary school, where Ensaid Panjang Village has one elementary school. Accessibility and, its complement electricity are quite good though limited to an area on the edge of the highway. Communication from the area outside of the village is possible by the presence of a cell phone signal even though sometimes there is only have a weak signal available.

Table 2. Plant species used as a building material in Ensaid Panjang Village forest, Kelam Permai Sub-district, Sintang, West Kalimantan, Indonesia

Local vernacular name	Scientific name	Functions
Bambu	*Gigantochloa latifolia* Ridl	Temporary pole
Belian	*Eusideroxylon zwagery* Teijsm	First pole (*tiang mun*), stairs
Bengkal	*Albizia procera* Roxb	Floor
Durian	*Durio zibethinus* Murr	Floor
Durian burung	*Durio carinatus* Mast	Roof, floor
Empetir	*Copaifera pallustris* (Symington) Dewit	Roof, floor
Emperpat	*Combretocarpus rotundatus* (Miq.) Danser	Floor
Entemau	*Cratoxylum glaucum* Korth	Roof
Geronggang	*Cratoxylum arborescens* (Vahl) Blume	Roof
Jaung	*Nicolaia speciosa* Horan	Roof
Jengger	*Ploiarium alternifolium* (Vahl) Melch	Floor, wall of *ruai*
Kelampu'	*Sandoricum koetjape* Merr	Roof
Kelansau	*Dryobalanops oblongifolia* Dyer	Wall
Keleban	*Vitex pubescens* Vahl	Floor
Kumpang	*Horsfieldia polyspherula* (Hook.f.) J.Sinclair	Roof
Mabang	*Shorea pachyphylla* Ridl ex Sym	Roof, wall
Medang	*Dehaasia caesia* Blume	Floor
Melingkat	*Nepenthes ampullaria* Jack	Rope
Mengeris	*Kompassia malaccensis* Benth	Floor
Menyatu'	*Palaquium leiocarpum* Burl	Roof
Meranti	*Shorea* sp.	Roof, wall
Merbung	*Dactylocladus stenostachys* Oliv	Floor
Pelambabi	*Mangifera* sp.	Floor
Pendu'	*Polyalthia glauca* (Hassk.) Boerl	Rope
Ramin	*Gonystylus bancanus* (Miq.) Kurz	Floor
Rengas	*Gluta renghas* L.	Floor
Resak	*Dipterocarpus borneensis* Slooten	Roof
Terentang	*Campnosperma auriculata* Blume	Floor
Ubah	*Eugenia* sp.	Floor
Uwiantu'	*Calamus zonatus* Becc.	Rope

Table 3. Comparison of forest land use types in Ensaid Panjang Village, West Kalimantan, and Rantau Layung Village, East Kalimantan, Indonesia

Function	Ensaid Panjang	Rantau Layung (Murniati et al. 2008)
Source of water, protected area	Bukit rentap	Alas tuo
Community conserved area	Tawang mersibung	Alas adat
Shifting cultivation area	Tawang sepayan	Alas nareng
Source for community needs	Tawang semilas, tawang serimbak	Alas nareng
Conserved area	Tawang sebesai	Alas mori

Dayak Desa community is heavily dependent on the existence of natural resources for their livelihood but has no legal right in management of forest. Dayak Desa communities have an important role to play in biodiversity conservation, it is a social capital. The existing traditional rights of the local communities have been ignored, lead a claim about there are not capable of facing the recent condition. It is important to realize that local knowledge is not necessarily static, pristine, and culturally specific; it is dynamic and continuously evolving (Thomas et al. 2004). This change is influenced by cultural variation, rising population, market opportunities, and policy shifts. If biodiversity is to be maintained in the forest ecosystems, there is a need to recognize that these forest are present because of the actions of the local people who live in and around them (Berkes et al. 2000).

The forest land use types and system in Ensaid Panjang Village have created by local knowledge and have been practiced by the communities for a long time. This situation is similar to community in Rantau Layung. Their forest was classified into four sub types of landscapes, i.e. alas tuo, alas adat, alas nareng and alas mori (Murniati et al. 2008). Comparison between Ensaid Panjang dan Rantau Layung forest land uses can be seen in Table 3. The traditional community will contribute to the forest conservation if they receive benefit from the forest either directly or indirectly.

For common knowledge to function as an incentive mechanism or institutional arrangement for collective action, it is not enough to create it, yet it also need to be shared or diffused effectively among the members of the community (Ishihara, et al. 2009). It is different with the local community knowledge in which, it will automatically be internalized in the community's daily life. It can be taken as an incentive.

Dayak Desa people use a diversity of plants from diverse forest land use type. Based on Pei et al. (2009), this will help to maintain forest system generally in a good condition, impacting similar trend in species diversity as a whole. Sustainable utilization of non-timber is a form of interference at a medium level (intermediate) that impact sustainable maintains the level of biodiversity in the high category (Gueze 2011). In tawang mersibung they utilize some of the plant species in the forest to meet the needs of food, beverage, medicine, dyes, tools and crafts, rope materials, fodder and ornamental plants and fencing. In tawang semilas and tawang serimbak, they utilize some of the plant species in the forest to meet the needs of timber, firewood, food, beverage, medicine, dyes, tools and crafts, rope materials, fodder and ornamental plants and fencing. The numbers of crops that are cultivated Dayak Desa people in tawang sepayan, like paddy, maize, chili, etc. The use of plants is part of wisdom to survive by exploiting the diversity of species in the forest beside the cultivation of a limited number of species. Species diversity meets the needs of the number and quality of needs (Zhang et al. 2013).

Common rules, norms, and sanctions are the mutually agreed or handed-down norms of behavior that place group of interest above those of individuals. They give individuals the confidence to invest in collective or group activities, knowing that others will do similarly. People can take responsibility and ensure their rights are not infringed. Mutually-agreed sanctions ensure that those who break the rules know they will be punished.

Forest village (hutan desa) was a forest management model in Indonesia to accommodate the community knowledge. It is an institution. The institution is important enabling factors for effective governance of the forest commons. Institutions can be more specifically defined as a set of accepted social norms and rules in making decisions about resource use and these defined that controls the resource, how conflicts are resolved, and how the resource is being managed and exploited (Richards 1997). Indigenous groups offer alternative management perspectives and knowledge based on their time-tested management practices (Thomas et al. 2004). Strengthening local-level social institution is not by itself sufficient to institute effective co-management (Ticktin 2004). The role of government should be prepared to assist local people in their reconstruction of emerging knowledge systems and the adaptation of strategies for interacting with large-and global-scale political, economic realities (Agrawal 2007).

Dayak Desa community has practiced conservation based on the rule, role and inherited tradition. Conservation of local knowledge affects forest sustainability practiced by local people when they act as a subject in the management and benefits that sustain their needs. There were six forest land use types in Dayak Desa ethnic in Ensaid Panjang Village, where each system has its function and role. Dayak Desa forest land use systems are tawang semilas, tawang sebesai, tawang mersibung, tawang sepayan, tawang serimbak, and bukit rentap protection forest. Each system has its supporting biodiversity such as flora, fauna, and environmental services. Forest utilization by Dayak Desa community is appropriately paired to what can be produced by the land. This forest land use system supports almost Dayak Desa community needs, such as food, clothing, housing, and other secondary needs. The community conservation efforts generate as a local knowledge that is applicable from past generation to recent generation, which is equipped with their own rules. The rules contain what the communities must do to their resource management which also accompanied with sanctions.

In the remote areas, local knowledge such as the Dayak Desa knowledge related to conservation could be integrated into the activities of the protection, preservation and sustainable uses of natural resources. It ensures the sustainable conservation of forests in the long run as there are still many rural communities living within or at the fringe of forests in Indonesia. This local knowledge is Dayak Desa social capital to acquire their rights to manage the forest in forest village model. Indonesia Government should implement sustainable forest management by involving local communities as subjects who participated actively in managing the forest. Active involvement of local community based on existing local conservation rules, roles and tradition in forest management ensures sustainability of forest resources. Such a shift in forest management paradigm requires a change in national forest policy for effective implementation at local level.

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REFERENCES

Agrawal A. 2007. Forests, governance, and sustainability: common property theory and its contribution. Intl J Commons 1 (1): 111-136.

Berkes F, Colding J, Folke C. 2000. Rediscovery of traditional ecological knowledge as adaptive management. Ecol Appl 10: 1251-1262.

Claus CA, Chan KMA, Satterfield. 2010. The roles of people in conservation. In: Sodhi NS, Ehrlich PR (eds) Conservation Biology for All. Oxford University Press, Oxford.

Decree of the Minister of Forestry and Plantations No. 259 / KPTS-II / 2000 on Designation of Forest Areas and Regional Bodies in West Kalimantan Province Covering an area of 9,178,760 million (nine million and one hundred seventy-eight thousand and seven hundred sixty) hectares. [Indonesian]

Delyanet. 2015. Forest based Landscape Management for Cultural Landscape Sustainability of Dayak Desa in Ensaid Panjang, Sintang, West Kalimantan. [M.Sc.-Thesis]. Bogor Agricultural University, Bogor. [Indonesian]

Ensaid Panjang Village. 2013. Profile of Ensaid Panjang Village, Kelam Permai Subdistrict, Sintang District, West Kalimantan. Village Govt of Ensaid Panjang, Sintang [Indonesian]

Gueze M. 2011. Evaluation of tree diversity and utilization: the role of acculturation, a case study in the Bolivian Amazon [Ph.D.-Dissertation]. Universitat Autonoma de Barcelona, Barcelona.

Heltberg R. 2001. Determinants and impact of local institutions for common resources management. Environ Dev Econ 6: 183-208.

Ishihara H, Pascual U. 2009. Social capital in community level environmental governance: A critique. Ecol Econ 68: 1549-1562.

Lethonen M. 2004. The environmental-social interface of sustainable development: capacities, social capital, institution. Ecol Econ 49: 199-214.

McKean MA. 2000. Common property: what is it, what is it for, and what makes it work? In: Gibson CC, McKean MA, Ostrom E (eds) People and Forest: Communities, Institution and Governance. MIT Press, Cambridge, MA.

Murniati, Padmanaba M, Basuki I. 2008. Forest resources utilization value of the communities living in and around Gunung Lumut protection forest, East Kalimantan. J For Res 5 (2): 147-171

Ostrom E. 2000. Undersstanding social capital: learning from the analysis and experience of participation. In: Dasgupta P (eds) Social Capital: A Multifaceted Perspective. Worl Bank, Washington DC.

Pei SJ, Zhang G, Huai H. 2009. Application of traditional knowledge in forest management: ethnobotanical indicator of sustainable forest use. For Ecol Manag 257: 2017-2027.

PRCF [People Resource and Conservation Foundation]. 2011. Forest Profile in Ensaid Panjang Village. PRCF Indonesia, Pontianak. [Indonesian]

Shahabuddin G, Madhu R. 2010. Do community-conserved areas effectively conserve biological diversity? Global insight and the Indian context. Biol Conserv 143: 2926-2936.

Suharjito D, Saputro GE. 2008, Social capital in forest resources management in the Kasepuhan Community, South Banten. Jurnal Penelitian Sosial Ekonomi Hutan 5 (4): 317-335. [Indonesian]

Thomas E, Berkes F, Folke, Angelstam P, Crepin A, Niemela J. 2004. The Dynamics of Ecosystems, Biodiversity Management and Social Institution at High Northern Latitudes. Ambio 33 (6): 350-355.

Ticktin T. 2004. The ecological implication of harvesting non-timber forest products. J. of Applied Ecology 41: 11-21.

Zhang LL, Zhang Y, Wang L, Wang YH. 2013. An ethnobotanical study of traditional edible plants used by Naxi people in Nortwest Yunnan, China. Pl Div Res 35 (4): 479-486.

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The process of rehabilitation of mined forest lands toward degraded forest ecosystem recovery in Kalimantan, Indonesia

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Abstract. *Sudarmadji T, Hartati W. 2016. The process of rehabilitation of mined forest lands toward degraded forest ecosystem recovery in Kalimantan, Indonesia. Biodiversitas 17: 185-191.* An overview of mined forest lands at East and Central Kalimantan Indonesia was conducted to determine most important influencing factors supporting degraded forest ecosystem recovery. Consecutive stages of rehabilitation processes consist of reclamation-*backfilling*, *re-contouring*, *re-shaping*, *topsoils spreading*, and revegetation-*land preparation*, *planting*, *maintenance* covering minimum topsoils spreading, soil acidity, plant hole size, soil improvement application (*dolomite*, *organic-inorganic fertilizers*), vegetation planting (*plant species selection-quality and site matching-verified plant material sources*, *hardening-off*, *planting techniques*), and land management implementation. The potential degraded forest ecosystem recovery was shown by cover crops and fast growing species plant and undergrowths, survive primary species, decreasing surface run-off/overland flows following increasing soil infiltration capacities, decreasing soil erosion rate and it's erosion hazard, and an improved environments as habitat for invading wildlifes. The general characteristics of potential degraded forest ecosystem recovery after rehabilitation processes are: spread soil materials thickness > 70 cm, bulk density $\pm$ 1,2 g.ml^{-1}, soil acidity > 5,5, macro nutrients (N, P, K, Ca, Mg)-low to moderate, decreasing overland water flow following increasing soil infiltration capacity-moderate to high, decreasing soil erosion rate-very low to moderate, decreasing erosion hazard level-very slight to moderate, growing plants of fast growing species with significant layers and land cover, and growing interline planted primary species. Viewed from the ecological aspect, in the revegetated degraded forest lands wildlife such as insects, birds, reptiles, amphibians and small mammals were found feeding and also permanently living in ecosystem regeneration following gradual habitat improvement. The ecosystem status was identified as a progression towards degraded forest ecosystem recovery.

Keywords: Degraded forest, ecosystem, rehabilitation process, reclamation, revegetation

INTRODUCTION

Various natural resources utilization and environmental management must be able to minimize the negative impacts and retain the quality and it's sustainability for people welfare. For this reason, coal mining companies have an obligation to observe-monitor and manage potential emerging environmental impacts along with their mining operation. It has been widely known that coal mining operation causes a significant impact to the environment (Ibarra and Heras 2005). Degraded land is an area that either by natural causes or more by directs or indirect causes of human action, has been altered or modified from its natural state (Santamartaa et al. 2014).

It is therefore, environmental management must follow it's consecutive mining operation stages starting from clearing of the vegetation-topsoils striping and stockpiling-mining waste treatment, the coal mining process-land reclamation and revegetation of the disturbed site. It is also very clear that these activities determine efficient and rational coal utilization as non-renewable natural resources. However, coal mining operations as far as possible, must enhance a better life for mankind and achieve a brighter future. Environmental disturbances, especially forest lands degradation, must be seriously considered for the next generation who will utilize this land.

Coal mining operations contribute significant impacts both on and off-sites to the environment (Bradshaw 2002) resulting in heavy degraded lands and massively altered forest ecosystem. General features of mined lands are the dumping of overburden with disturbed soil, fragmented rocks mixed with coal fines without organic materials, bad water drainage, low soil water, compacted soils and with high soil temperatures (Datar and Mulligan 2011; Zhenqi et al. 2012). Such disturbed soil and overburden sites are unable to perform the main soils function to provide a plant growth media and conservation of water. Moreover, degraded lands are also characterized with bad drainage and low water holding capacities and highly compacted soils. To achieve good mining practices along with the many rules for coal mining operation, land rehabilitation practices must be carried out in order to achieve recovery of a mined forest lands (Siswanto et al. 2012). These rehabilitated lands would also be expected to be a productive land (Devathaa et al. 2015).

The open pit/cast method of coal mining operation which is commonly applied at Kalimantan Indonesia cause great and massive changing to the landform and therefore they need rehabilitation to recover and retain the environmental function capacity for supporting various rehabilitated ecosystems (Kilowasid et al. 2011). Specifically, mined lands suffer a drastic soil fertility deficit and a poor microclimate, with a huge increase of water flow and runoff causing a significant increase in soil erosion and sedimentation away from the disturbed site.

The rehabilitation processes require a specific knowledge and experiences with respect to soil formation and development, proper and practical techniques of mined land rehabilitation, plant species selection with appropriate site matching, and also planting techniques and vegetation maintenance after the rehabilitation process. Degraded forest lands rehabilitation has been carried out as an initial effort to restore altered ecosystem through reclamation activities of *backfilling, re-contouring, land leveling, re-shaping* and *topsoils spreading*, followed by revegetation process as such as *land preparation, planting,* and *maintenance* of the rehabilitated lands.

The main objective of this study was to identify the characteristics of mined forest lands for potential recovery after rehabilitation processes. The expected result of the study was to develop and/or improve the design of land rehabilitation processes enhancing degraded forest ecosystem recovery.

MATERIALS AND METHODS

Observation and fieldwork focused on several soil characteristics, water flows and infiltration, soil erosion and sedimentation, revegetation plants, wildlife (fauna), and ecosystem status were conducted. The study sites were six rehabilitated forest lands in Kalimantan, Indonesia, namely PT Berau Coal (BC), PT Kaltim Prima Coal (KPC), PT Trubaindo Coal Mining (TCM), PT Kitadin (KTD), and PT Kideco Jaya Agung (KJA), all situated in East Kalimantan, as well as PT Multi Tambangjaya Utama (MTU) in Central Kalimantan, covering reclamation processes (*backfilling, re-contouring, re-shaping, topsoils spreading*) and revegetation processes (*land preparation, planting, maintenance*), taking into account the consecutive stages of mined land rehabilitation.

Some minipits were selected as representatives of mined forest lands of different ages of vegetation to asses and diagnose the physical and chemical soil characteristics for indicating soil recovery and development with respect to the assessment of water flow and infiltration capacity.

Rainfall data and field observation of topographical condition, vegetation growth, land coverage density, soil and water conservation practices were used to estimate the potential soil erosion using Universal Soil Loss Equation (USLE) approach. Secondary data of biodiversity and habitat improvement studies at the same sites was used to construct scenario of degraded forest ecosystem recovery.

RESULTS AND DISCUSSION

Rehabilitation of ecosystem functioning

Forest succession is an ecosystem process in which the ecosystem change in the form of flora or fauna diversity is measured. Progressive succession is the normal sequential development of communities, from simple communities with few species and low productivity to the optimum sustainable in a given habitat or environment. Conversely, retrogression is a successional change usually from an existing climax community leading to a less diverse and less structurally complex community. It is usually triggered by an environmental factor. Primary succession is a succession in an area without any previous vegetation. Secondary succession is a succession that occurs in a degraded area with some remaining vegetation or an area where the vegetation has been disturbed. Progressive succession shows species increase whereas species decrease defines retrogressive succession. In the forested or vegetated areas seriously disturbed there might be complete failure in succession which means that the earlier condition or historical state could not be recovered.

One would expect the revegetation of mined-out lands to accelerate ecological processes to achieve the condition as of a pre-mining operation or even better. The main consideration in plant species selection is based on not only having high tolerance to the extremely degraded soil condition but also to the capacity of the plants to recover degraded ecosystem functioning (Zhenqi et al. 2012). However, there is a possibility that the tree plant species selected could not fulfill such expectations. Some species are tolerance of the extreme condition but less favorable for enhancing ecosystem recovery due to their intolerance to invading species and thus triggering a retrogressive succession. For these reason, it is important to understand that the potential of the natural vegetation to alternating the impact of the coal mining operation and at the same time also increase vegetation structure and composition following the rehabilitation process (Isahak et al. 2013; Humsa and Srivastava 2015).

Lands rehabilitation process

The main environmental principles for reducing land degradation are to maximize vegetation cover to prevent erosion, replace nutrients removed, and to put in place structures so as to reduce the speed and volumes of water flow over the soil (Morgan 1996; Blinkov et al. 2013; Gashaw et al. 2014; Dyguś 2015,).

The process of rehabilitation is studied in a consecutive activity of reclamation and revegetation in order to accelerate the recovery of degraded forest lands. Soils provide a plants growth medium with satisfactory aeration and drainage to ensure the development of a root system to absorb macro and micro nutrient. In this rehabilitation study, reclamation process assures sufficient thickness of soil through topsoils spreading and re-contouring, in order to control excessive water drainages. In order to achieve the improvement of soil aeration organic materials were added followed by immediate planting of land cover crops. Figure 1 shows the result of topsoils spreading at TCM, MTU and BC, while Figure 2 illustrates the initial planting at the same sites.

Based on the results of observation, field works and laboratory analysis; the recovery processes of mined forest lands are highly dependent on the determining factors and steps of rehabilitation which consist of reclamation and revegetation processes. Technically, the minimum standard required for mined lands rehabilitation are spreading of topsoils of a minimum of > 70 cm thickness, soil pH of > 5,5, planting holes with 40 x 40 x 40 cm in size, soil amelioration with dolomite, organic and/or chemical fertilizer application, vegetation planting and followed by intensive rehabilitated lands management. The summary of the general characteristics of mined forest lands potential recovery after rehabilitation works is shown in Table 1.

The ecosystem function of mined forest lands after rehabilitation processes shows a positive trend to be recovery as visually shown in Figure 3 to Figure 8.

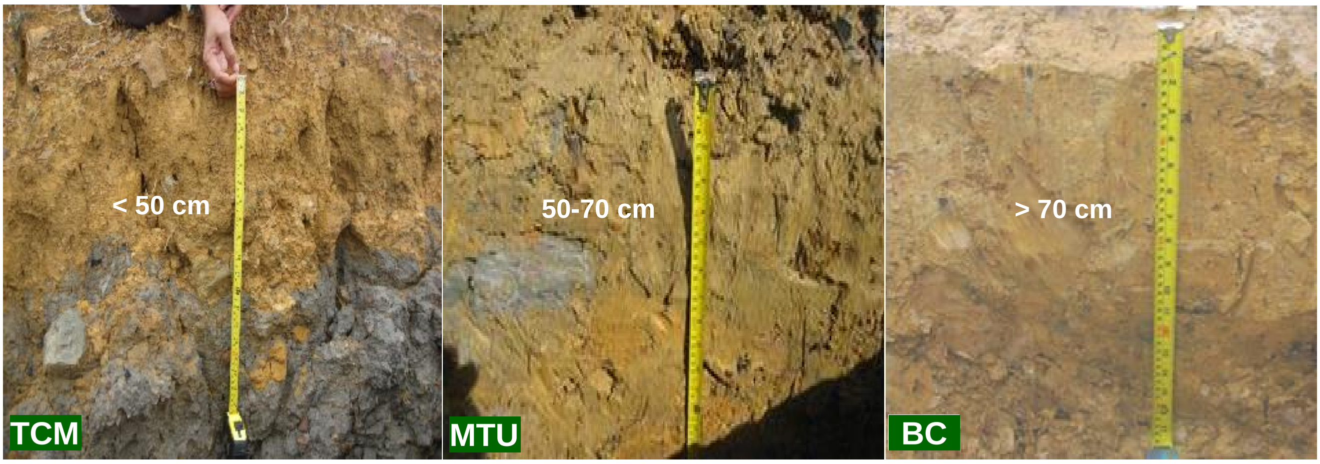

Figure1. Land Preparation) at Mined-out Lands of TCM (<50 cm), MTU (50-70 cm), and BC (>70cm)

Figure2. Planting after reclamation works at TCM (*fast growing species*), BC (*land cover crops*), and MTU (*land preparation-planting holes*)

Table 1. General characteristics of mined forest lands potential recovery after rehabilitation processes

Component	Parameter	Description
Soil physics	Soil materials thickness	$\geq$ 70 cm
	Bulk density	$\pm$ 1,25 g.ml^{-1}
Soil chemistry	Soil acidity (pH)	> 5,5
	Macro nutrients	Macro nutrients (N, P, K, Ca, Mg : Low to Moderate
Hydrology	Overland water flow	Decreasing in line with the increase of soil infiltration capacity: no significant detention of water
Erosion	Rate	Decreasing soil erosion rate: Class-Very low to Moderate, Hazard-Very slight to Moderate
Revegetation	Plants	Fast growing species form land coverage both of trees and herbs or grasses, interline planted primary species grow well
Wildlife	Fauna	Invading insects, birds, amphibians, reptiles, small mammals
Habitat	Improvement	Improved habitat: microclimate-air and soil temperature, relative humidity, solar radiation intensity, foods and coverage.
Ecosystem	Status	Prospective with coefficient of similarity 60-70%, completely developed food web, invading herbivores, carnivores, predators, but no top predator as yet.

Figure 3. Rehabilitated lands management at TCM (*fast growing species-4 years*)

Figure 4. Rehabilitated lands management at BC (*fast growing and primary species 10-12 years*)

Figure 5. Rehabilitated lands management at MTU (*fast growing species 4-5 years*)

Figure 6. Rehabilitated lands management at KTD (*fast growing and agriculture commodities 1-2 years*)

Figure 7. Rehabilitated lands Management at KPC (*fast growing, primary species > 10 years, palm oil, soil and water conservation structure*)

Figure 8. Rehabilitated lands management at KJA (*fast growing and primary species > 12 years*)

Mined forest lands recovery potential

After mining activities are complete, the topography and physical soil properties are reconstructed in an attempt to establish the foundation of a self-sustaining ecosystem (Arnold and Williams 2016). However, soil properties are still markedly different compared to unmined areas, including higher bulk density. Degraded lands initially showed soils disturbance especially that of soil structure and pores destruction. It was found important differences in hydrological functioning and erosion response of soils under different land uses and vegetation types (Ferreiraa et al. 2015). For this reason, degraded lands have to be assessed as recovery processes based on a soil characteristics approach (Agassi 1996). Analysis of erosion potential dynamics as a simple indicator of mined lands revegetation showed that to reach the status of low (L) and very low (VL) erosion rate require about 5 (five) years (Table 2).

Vegetation is the first biological component which is very important as primary producer providing nutrients, ground covers, clean air and habitats for various other life forms. The vegetation life form is a real measure of the soil quality (An et al. 2013), and also the wildlife. Therefore, soils, vegetation and wildlife are unseparable and strongly inter-dependent three components of rehabilitated habitat.

The seasonal emerging plants creeper species have an important role as an undergrowth land cover improve the microclimate, and also supply organic matter and enhance the return of mesofauna (invertebrates). Mesofauna return is closely related to vertebrate (reptiles and amphibians) recover, many as predator on the mesofauna. Therefore, revegetation with plants, whether planted or naturally emerging are very important in the mined forest lands recovery process.

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The individual presence and frequency of birds are an interesting and useful monitoring. Some changing trends in composition and population richness of bird species can be used as a bio-indicator of environmental changing. Similar with birds as bio-indicator of environment change, other animals such as butterflies, dragonflies, bumble bees, reptiles and amphibians can also be used as bio-indicators.

Soil erosion potential is influenced by several factors including vegetation development (Dantas et al. 2015). In order to decrease the risk of soil of erosion rehabilitated forest lands have to be intensively managed for least the first 5 (five) years especially in relation to land preparation and the intensity of vegetation maintenance. Land rehabilitation process must initially be introduced to control surface water flow (Lei et al. 2015). The plants grow and provide coverage to protect soil surfaces form excessive erosion.

Ecological potential recovery of mined forest lands

The intentional alteration of a site to establish a defined indigenous is a historic ecosystem. The goal of this process is to emulate the structure, functioning, diversity, and dynamics of the specified ecosystem (Aronson et al. 1993). With respect to the afore-mentioned, revegetated land recovery is made possible by reclamation process namely land preparation to make the land functional as a medium for plant biomass production. Post mining revegetated land management result in vegetation development of both vertical and horizontal cover.

In the early stages, stabilization of mined lands by growing a cover crop reduces soil erosion and gradually forms a better microclimate by decreasing air and soil temperatures and increasing air and soil humidities. The growth and development of vegetation supplies organic materials into the soils thus increasing soil fertility. In times, advance growth and development of vegetation contributes to the reduction of soil erosion potential and also forms a more favorable microclimate. These conditions attract various animals (wildlife) to feed on rehabilitated mined lands, and more over living and enhancing the regeneration of the ecosystems (Figure 9).

The invading wildlifes can be potentially used as bioindicators for assessing the progress of ecological recovery of degraded lands (Laurila et al. 2015). Also, interline planting by using primary species-dipterocarps species is a long-term investment to achieve more rapid recovery of mined lands. Cover crops and fast growing species planted in the early stages and rapidly reaching flowering and fruiting make an important contribution to bridging the growth developing of primary species to the ultimate goal of degraded forest lands rehabilitation. A higher diversity of invading wildlifes to feed, live and regenerate animals populations shows a clear direction and the steps of mined forest lands recovery that is required.

Table 2. Potential soil erosion dynamics following land coverage development at three sites

Sites[1]	Classification of soil erosion rate (classes of ton/ha/yr)[2]							
	Open	<2Yr	2-4Yr	4-6Yr	6-8Yr	8-10Yr	>10Yr	Original
SMO	(VH)	(H)	(L)	(L)	(VL)	(VL)	(VL)	(VL)
BMO	(VH)	(M)	(L)	(L)	(VL)	(VL)	(VL)	(VL)
LMO	(VH)	(H)	(H)	(VL)	(VL)	(VL)	(VL)	(VL)

[1]SMO, BMO, LMO: Sambarata, Binungan, Lati, [2]VR = Very Low (<15 $ton.ha^{-1}.yr^{-1}$), L = Low (15-60 $ton.ha^{-1}.yr^{-1}$), M = Moderate (60-180 $ton.ha^{-1}.yr^{-1}$), H = High (180-480 $ton.ha^{-1}.yr^{-1}$), VH = Very High (>480 $ton.ha^{-1}.yr^{-1}$)

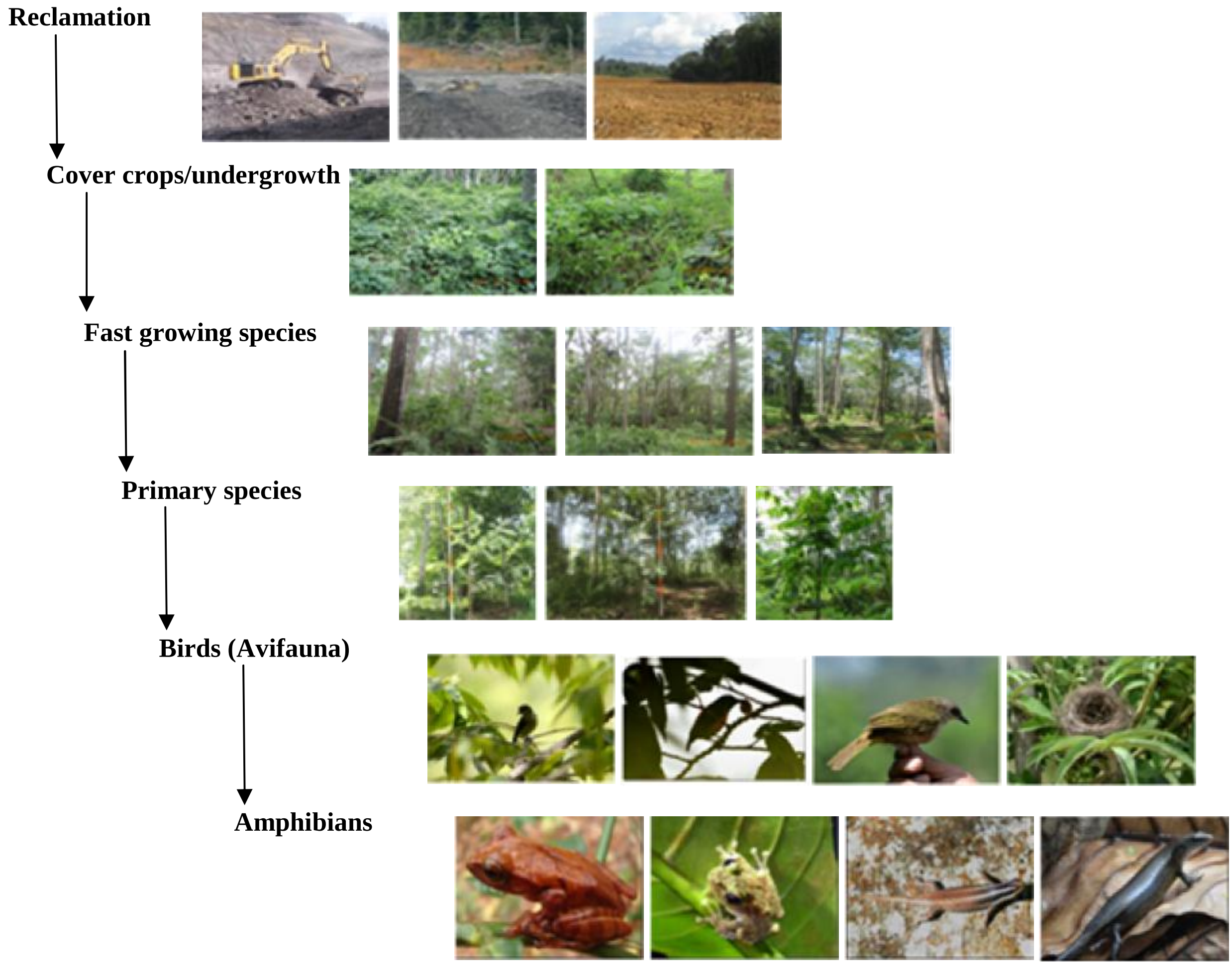

Figure 9. Steps in the rehabilitation processes and the recovery proccesses showing land coverage by vegetation and the invading wildlifes-birds (avifauna) and amphibians (herpetofauna)

To conclude, the most important factors and stages for land recovery are topsoils spreading (thickness and density), land preparation (planting hole, soil amendment/ amelioration materials), planting (plant materials, techniques), and rehabilitated forest lands management (replanting dead trees, maintenance, fertilization). The potential recovery of mined forest lands can be shown by revegetation plants (land cover crop and fast growing species grow combined with undergrowth and interlayered crown, primary species are interline planted, decreasing soil erosion rate (erosion hazard class: very low to moderate, erosion hazard level: very slight to moderate, decreasing overland flow increasing soil infiltration capacities), and invading animals (wildlife) feeding, multiplying and ecosystem regeneration. Mined forest land potential recovery could be assessed through soil characteristics, overland flows, infiltration capacity, soil erosion and sedimentation, revegetation plants, invading wildlife and ecosystem status. Degraded lands are continuously recovering, the mined lands being on the right track to be recovery in time, as indicated by interaction between ecosystem components of forest ecosystems, hydro-orological conditions, improved microclimate, and also invading wildlife for regeneration of the ecosystem. The applications of rehabilitation (*reclamation* and *revegetation*) processes have significantly enhanced the recovery of mined forest lands and were the most important basis for the improvement of degraded forest ecosystems.

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REFERENCES

Agasssi M. 1996. Soil erosion: Conservation and rehabilitation. Marcel Dekker, Inc. New York.

An SS, Darboux F, Cheng M. 2013. Revegetation as an efficient means of increasing soil aggregate stability on the Loess Plateau (China). Geoderma 209-210 (2013): 75-85.

Arnold S, Williams ER. 2016. Quantification of the Inevitable: The influence of soil macrofauna on soil water movement in rehabilitated open-cut mined lands. Soil 2: 41-48.

Aronson J, Fled C, LeFloc'h E, Ovalle C, Pontanier R. 1993. Restoration ecology: Restoration and rehabilitation of degraded ecosystems in arid and semi-arid lands. A View from the South. Restor Ecol 1 (1): 8-17.

Blinkov I, Kostadinov S, Marinov I. 2013. Comparison of erosion and erosion control works in Macedonia, Serbia and Bulgaria. Soil Water Conserv Res 1: 15-28.

Bradshaw AD. 2002. Introduction and philosophy of principles of restoration. In: Martin RP and Anthony JD (eds) Handbook of Ecological Restoration. Vol.1. Cambridge University Press, Cambridge.

Dantas de Paula M, Groeneveld J, Hutha A. 2015. Tropical forest degradation and recovery in fragmented landscapes-simulating changes in tree community, forest hydrology and carbon balance. Global Ecol Conserv 3: 664-677.

Datar A, Audet P, Mulligan D. 2011. Post-Mined Land Rehabilitation in India: Cataloguing Plants Species Used in Land Revegetation. Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, The University of Queensland, St. Lucia Campus, Brisbane, 4072 QLD, Australia.

Devathaa CP, Deshpandeb V, Renukaprasad MS. 2015. Estimation of soil loss using USLE model for Kulhan Watershed, Chattisgarh-A case study. Aquatic Procedia 4: 1429-1436.

Dyguś KH. 2015. The role of plants in experimental biological reclamation in a bed of furnace waste from coal based energy. Ecol Eng 16 (1): 8-22.

Ferreiraa V, Panagopoulosa T, Cakulab A, Andradea R, Arvelaa A. 2015. Predicting soil erosion after land use changes for irrigating agriculture in a large reservoir of Southern Portugal. Agriculture and Agricultural Science Procedia 4: 40-49.

Gashaw T, Bantider A, Silassie HG. 2014. Land degradation in Ethiopia: Causes, impacts and rehabilitation techniques. Environ Earth Sci 4 (9): 98-104.

Humsa TZ and Srivastava RK. 2015. Impact of rare earth mining and processing on soil and water environment at Chavara, Kollam, Kerala: A case study. Procedia Earth and Planetary Science 11: 566-581.

Ibarra JMN, de las Heras MM. 2005. Open-cast mining reclamation. In: Mansourian S, Vallauri D, Dudley N (eds). Forest Restoration in Landscapes: Beyond Planting Trees. Springer, New York.

Isahak A, Surif S, Sahani M, Gill A, Phang J. 2013. Environmental stewardship for gold mining in tropical regions. J Degrad Mining Lands Manag 1 (1): 37-42.

Kilowasid LMH, Herlina, Syaf H, Safuan LO, Tufaila M, Leomo S, Widiawan B. 2015. Engineering of soil biological quality from nickel mining stockpile using two earth worm ecological groups. J Degrad Mining Lands Manag 2 (3): 361-367.

Laurila-Panta M, Lehikoinenb A, Uusitaloc L, Venesjärvib R. 2015. How to value biodiversity in environmental management?. Ecol Indicat 55: 1-11.

Lei H, Peng Z, Yigang H, Yang Z. 2015. Vegetation succession and soil infiltration characteristics under different aged refuse dumps at the Heidaigou opencast coal mine. Global Ecol Conserv 4: 255-263.

Morgan RPC. 1996. Soil Erosion and Conservation, 2nd ed. Longman, London.

Santamartaa JC, Rodríguez-Martínb J, Merinoc C, Paz Arraizad M, López JV. 2014. Identification of degraded land in the Canary Islands; Tests and reviews. IERI Procedia 8: 77-82.

Siswanto B, Krisnayani BD, Utomo WH, Anderson CWN. 2012. Rehabilitation of artisanal gold mining land in West Lombok, Indonesia: Characterization of overburden and the surrounding soils. Geol Mining Res 4 (1): 1-7.

Zhenqi H, Peijun W, Jing L. 2012. Ecological restoration of abandoned mine land in China. Resour Ecol 3 (4): 1-7.

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Flowering, fruiting, seed germination and seedling growth of *Macaranga gigantea*

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Abstract. *Susanto D, Ruchiyat D, Sutisna M, Amirta R. Flowering, fruiting, seed germination and seedling growth of* Macaranga gigantea. *Biodiversitas 17: 192-199.* The stages of flower and fruit development of *Macaranga gigantea*, its seed germination behavior in nature and from fruit harvesting, as well as the process of raising its seedlings in the laboratory were studied to determine the potential for production of *M. gigantea* seedlings. Five reproductive trees of *M. gigantea* were chosen as sources of seed. To estimate natural germination rates, four sample plots of 1 x 1 m^2 in four cardinal directions below the crown of every tree were set up. In addition, dry and wet extraction processes were carried out to determine, which the more effective method for germination of *M. gigantea* seeds. The results showed that the time required from flower development to fruit ripening in *M. gigantea* flowers was 5-6 months. The flower buds initiated in the dry season (August 2011) and the fruits ripened in the rainy season (December 2011-January 2012). The seeds that fell under the parent tree germinated in approximately 24 days with an average seedling density of 75-267 per m^2. The germination in laboratory showed that the percentage and the rate of germination of the seeds extracted through the wet extraction process were higher than those extracted through the dry extraction process. The highest rate of germination (65%) is by the combination of seeds extracted through wet extraction process and grown on compost media. The relative growth rate of seedlings planted on mushroom spawn waste media was the highest rate (0.36 ±0.42%), followed by those planted on compost media (0.15±0.09%), top soil media (0.10±0.04%) and sand media (0.10±0.07%).

Keywords: Germination, *Macaranga gigantea*, pioneer species, seedling growth

INTRODUCTION

The giant mahang (*Macaranga gigantea*) has not been recognized as an important commodity with a high economic value. *M. gigantea* wood is light, soft and flimsy that it is not good for construction wood. However, Amirta (2010) reported that enzymatic hydrolysis process of *M. gigantea* wood produces the highest reducing sugar content (82.47% based on the weight of resultant pulp) compared to other types of fast growing woody species such as *Paraserianthes falcatria* and *Acacia mangium* and this plant has the potential to be used as raw material for bioethanol in the near future. Liquid bioethanol generated from wood is long lasting providing that the forest where it grows is preserved (sustainable) and it does not affect the production of food and the increasing price of food materials.

M. gigantea has not yet been cultivated and information about the stages of its flower and fruit development, seed germination in nature, as well as the process of raising its seedlings is still very limited. In order to have *M. gigantea* seedlings ready for cultivation, a source of seeds harvested from physiologically mature fruits is needed. Seed extraction is required to remove the seeds from other parts of the fruit (skin, flesh, wings, stalks), and if the seeds are not appropriately extracted, their germination viability will decrease.

In nature, *M. gigantea* plant is abundant in open mixed dipterocarp forests after extensive disturbances such as wood harvesting, forest fires, and shifting cultivation (Lawrence 2001; Silk et al. 2003; Lawrence 2005; Eichhorn 2006; Silk et al. 2008). After a great forest fire in East Kalimantan (1982-1983), *M. gigantea* and *M. triloba* simultaneously spread over and covered the canopy gaps of the destroyed forest, believed that seeds of *M. gigantea* and *M. triloba* might be buried in the soil before the forest fires and then they germinated immediately after the fires. However, Kiyono and Hastaniah (1997) reported that a careful observation indicated that *Austroeupatorium inulifolium* and some of wild bananas (*Musa* sp.) had been growing before the seedlings of *M. gigantea* and *M. triloba* grew and spread over the forest. They speculate that there were a considerable number of *M. gigantea* and *M. triloba* seed-trees that survived.

Seeds of *M. gigantea* were reported to have low water content of 8.23% and the seeds with low water content were commonly orthodox (Suita and Nurhasybi 2009). *M. gigantea* seeds resulting from dry extraction from mature fruits have low germination rates of 2-10%, but soaking the seeds into a solution of 0.2% potassium nitrate for 20 minutes before spreading on sand media can increase germination rates of seeds up to 20% (Mindawati et al. 2010).

It is necessary to conduct a study on the stages of flower and fruit development of *M. gigantea*, its seed germination behavior in nature, its seed germination rates from the fruit harvesting, as well as the process of raising its seedlings in the laboratory. From this reconstruction, it is expected that comprehensive information can be obtained so a way of preparing seeds and raising seedlings can be appropriately formulated in order to produce *M. gigantea* seedlings ready for cultivation.

MATERIALS AND METHODS

Study area

The research on flower and fruit development of *M. gigantea*, germination and seedlings growths in natural conditions was conducted in the Forest Education of Faculty of Forestry, Mulawarman University, East Kalimantan, Indonesia (inside Bukit Suharto Grand Forest Park, East Kalimantan). It was located between the coordinates of $0^o25'10''$-$0^o\ 25'24''$ South and $117^o\ 14'00''$-$117^o\ 14'14''$ East (Figure 1). The data obtained from 2003 to 2012 showed that the annual average rain fall was 2423 mm, and the highest annual rainfall was 2757.5 mm in 2008. The highest monthly rainfall was in April (288.3 mm) and the lowest was in August (115.3 mm). The wet season lasted for 9-12 months, while the dry season lasted for 0-3 months. The average monthly temperature was 27.5^oC and the average moisture was 82% (Anon. 2012). The research on seed germination from fruit harvesting and the growth of *M. gigantea* seedlings was conducted in the Plant Physiology Laboratory at the Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, East Kalimantan, Indonesia. The research was conducted from July 2011 to March 2012.

Procedures

The developmental stages of M. gigantea *flowers and fruits*

Observations were conducted on five *M. gigantea* trees which were blooming and growing on the sides of a street. The trunk diameter and the height of the *M. gigantea* trees were measured and the litter under the tree crowns was cleared. The observation of flowers started from the time when the flower buds initiated, to the time when the flowers blossomed (anthesis), until they wilted and then the stages of the fruit development were observed until the fruits were fully ripe (Aminah and Muharam 2009).

Seed germination and M. gigantea *seedlings below their parent trees*

Observations on the germination of seeds fallen under the tree crowns were conducted in 4 sample plots of 1 x 1 m^2 in the four cardinal directions of every *M. gigantea* parent tree. The time when the seeds initiated germination was recorded and the number of seedlings in every plot was calculated.

Fruits and seeds of M. gigantea

M. gigantea fruits were collected in bulk (mixed from the different trees) in December-January 2012. The fruits were picked using a hooked pole and a large plastic sheet was extended under the tree to place the fallen fruits. The fruit samples weighing a total of 3 kg (1 kg from each tree were peeled and the seed yields for every kilogram of fruits were weighed. One hundred fruits and seeds of *M. gigantea* were randomly chosen and every fruit/seed was weighed and then their diameters and lengths were measured to find the average weight and the size of the fruits and seeds (Suita and Nurhasybi 2009).

Seed extraction process

Seed extraction is to remove the seed from other parts of the fruit (peel, flesh, wings, and stalk). Dry extraction was done by drying the fruits in an open place for 3-4 days until the fruits were broken and the seeds were easily removed from the fruit. Sieving was done to separate the seeds from the fruit rinds (Kumara et al. 2000; Schmidt 2007; Suita and Nurhasybi 2009). Wet extraction was done by brooding the fruits into a gunny sack for a week (the fruits in the sack were watered every day to make them moist) until the outer skin of the fruit became soft. Then they were dried in the wind until the fruit peels were broken and the seeds were easily removed from the fruits. Sieving was done to separate the seeds from the fruit skin.

Seed selection

Seed selection was carried out to choose large and heavy seeds by soaking them in the water. The floating and the sinking seeds were separated. The seeds that sank in the water were collected and put into a laboratory test sieve (number 8; the size of the hole was 2.36 mm) and they were rubbed by hand. The seeds with the diameter of less than 2.36 mm would pass through the sieve, while the seeds of larger sizes would remain in the sieve and were used as the materials to investigate germination.

Seed germination after harvesting

This research applied a randomized design with two treatments as follows: (i) the different techniques of fruit extraction: wet versus dry extractions, (ii) different germination media: sands, topsoil, compost, and mushroom prawn waste. Twenty seeds was sown evenly on each the germination media. This was repeated three times and each round was observed for 60 days. The observed parameters include the percentage of the germination (G), the germination rate (GR), mean germination time (MGT), the germinating time of the first seed (GTFS) and the germinating time of the last seed (GTLS) were calculated according to the following formulas (based on Fariman et al. 2011; Mendes-Rodrigues et al. 2011):

$$G\,(\%) = \frac{\text{Number of germination seed}}{\text{Number of viable seeds initiated}} \times 100\%$$

$$GR = \sum_{n=1}^{n} (\text{Number germination since} - 1)/n$$

Where, N is the days

$$MGT = \frac{\sum Ti\ Ni}{S}$$

Where Ti is number of days after beginning of experiment, Ni the number of seeds germinated on day i, and S the total number of seeds germinated.

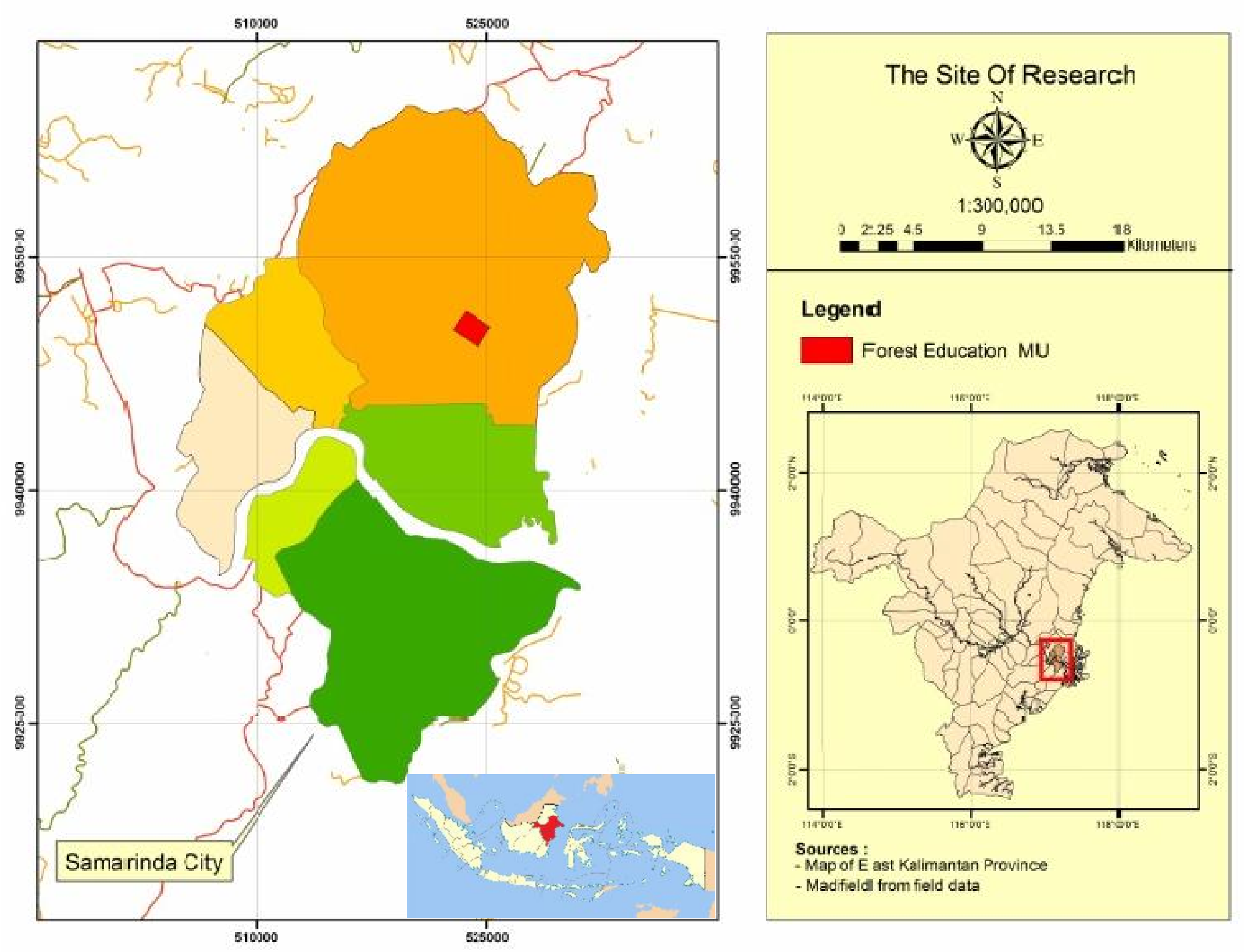

Figure 1. Map of study area in the Forest Education of Faculty of Forestry, Mulawarman University, East Kalimantan, Indonesia (inside Bukit Suharto Grand Forest Park, East Kalimantan)

The growth of M. gigantea *seedlings*

The seeds that germinated in the laboratory were put into polybags with a height of 30 cm and diameter of 15 cm. Each polybag was filled with different planting media: sands, topsoil, compost, and mushroom prawn waste media. The seedlings were kept in the green house and protected using a 50% shading net or paranet for a month. The height, number of leaves, wet mass, dry mass and the relative growth rate (RGR) were measured at the end of the experiment.

$$RGR = \frac{(\ln W2 - \ln W1)}{(T2 - T1)}$$

Where, W_1 and W_2 are plant dry weights at times t_1 and t_2.

Data analysis

The data obtained from the field observations were analyzed descriptively and correlation between diameters at breast height parent trees (X) with numbers of seedling in under the tree crowns (Y) was measured by simple linear correlation. The germination and seeding growth data from the laboratory experiments were analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at 95% level of significance.

RESULTS AND DISCUSSION

Developmental stages of *M. gigantea* flowers and fruits

The time from the initial flower buds to the ripe fruits took 5-6 months. The flower buds were initiated in August 2011 when the rainfall was in its lowest level of 106.1 mm (i.e., during the dry season). The outer floral envelopes of the flowers and the male flowers fell in September 2011; the young fruits developed from October to November 2011; and the fruits started to ripen in December 2011 when the rainfall was high 247.2 mm and this occurred until January 2012 when rainfall was highest 327.1 mm; during the rainy season (Figure 2). The flower and fruit developmental stage of *M. gigantea* is presented in Figure 3, while morphology of ripe fruit and seed is presented in Figure 4.

The seed germination and seedlings under the trees of *M. gigantea*

Ripe fruit was noticed on November 25th 2011. Seed on the ground under the *M. gigantea* trees were observed on January 8th 2012 (Table 1). Eventually, the average number of *M. gigantea* seedlings (with 2-4 leaves) found below their parent trees was 75-267 seedlings per meter per m^{-1} (Tables 4) and this was highly correlated with the diameter of their parent stems (r=0.823). *M. gigantea* fruits and seeds were small with an average diameter of 0.784 cm and 0.415 respectively. One kilogram of fresh fruits (without stalks) yielded 86.15 gram of seeds and to obtain 1 kg of seeds required 11.61 kg of fruits (Tables 5).

Seed germination of *M. gigantea* from the ripe fruit harvesting

The techniques of seed extraction, the types of planting media and interaction between the techniques of seed extraction and the types of planting media had statistically significant effects (p-value≤0.005) on the percentage of seed germination. The percentage of germination of seeds extracted through the wet extraction process was higher than those extracted through the dry extraction process. The highest percentage of germination 65% was in the combination wet extraction and a compost medium, respectively (Figure 5). The germination rate (GR), mean germination time (MGT), the germination time of the first

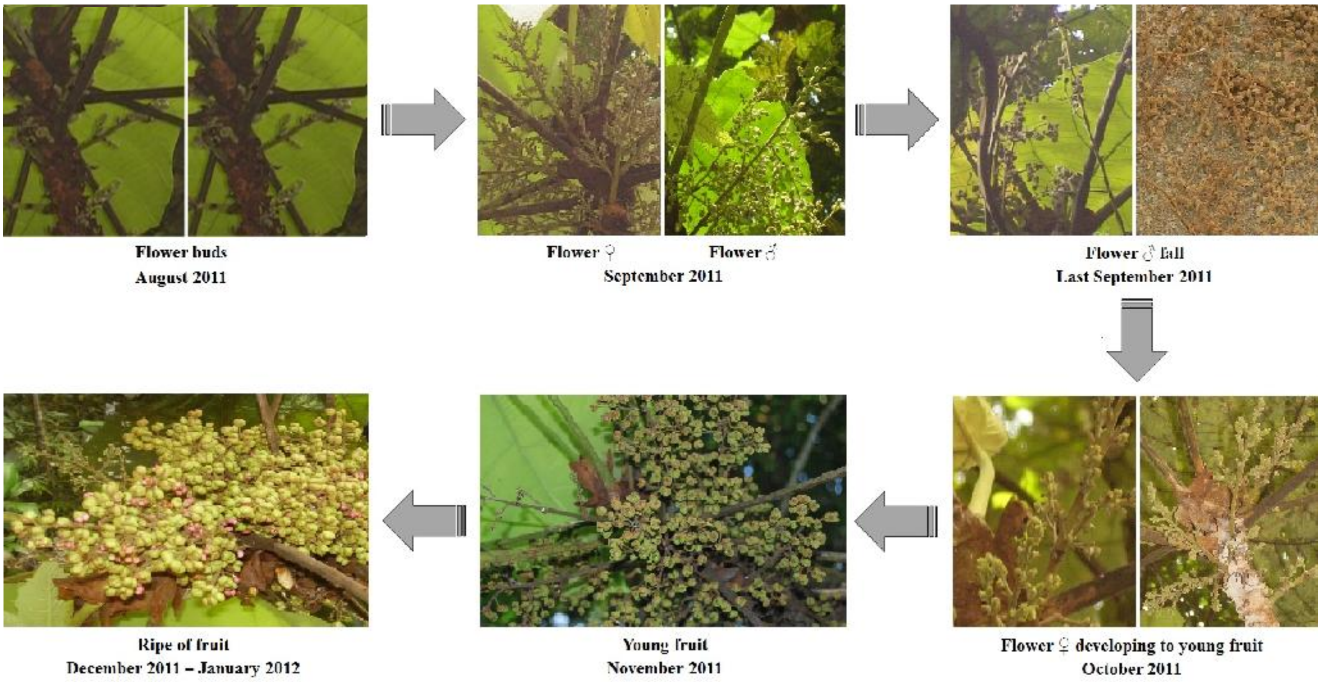

Figure 3. Developmental stages of *Macaranga gigantea* flowers and fruits

Figure 4. Morphology of fruits and seeds *Macaranga gigantea*: A. Ripe fruit, B. Seeds

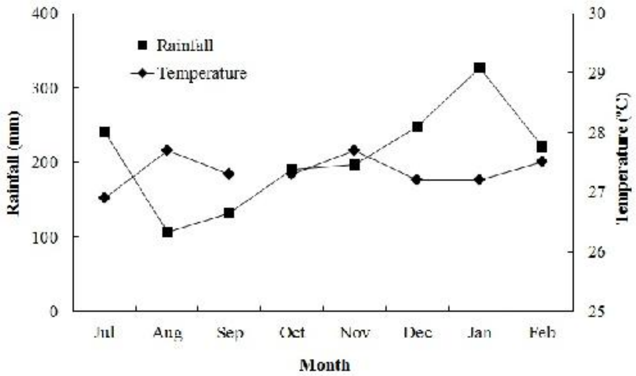

Figure 2. Mean temperature and rainfall in study region

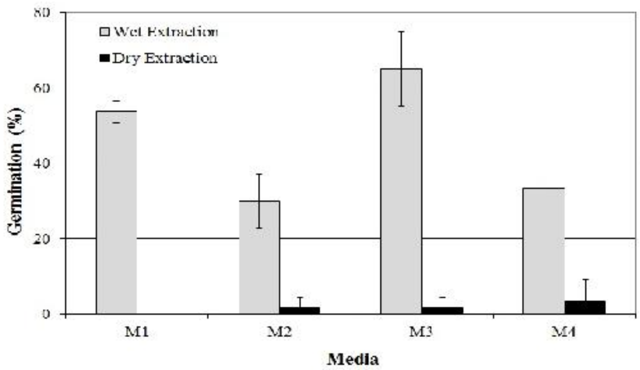

Figure 5. Effects of seed extraction method and media on seed germination (%) M1, sand media; M2, top soil media; M3, compost media; and M4, mushroom prawn waste media

Table 1. Time after fruits were fully ripe, seed germination and seedling growth under the mother trees

Germination stages	Date of obsevation								
	25-11-2011	5-12-2011	12-12-2011	19-12-2011	24-12-2011	02-01-2012	08-01-2012	15-01-2012	22-01-2012
Ripe fruit	■	■	■	■	■	■	■	■	■
Seed germination				■	■	■	■	■	■
Seedling (2-4 leaves)						■	■	■	■

Table 2. Influence of seed extraction and germination media on mean seed germination measurement (±SD)

Measurement	Treatments							
	WM1	WM2	WM3	WM4	DM1	DM2	DM3	DM4
GR (days)	0.89 ± 0.18	0.77 ± 0.31	1.22 ± 0.27	0.67 ± 0.13	0.00 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.11 ± 0.00
MGT (days)	13.67 ± 1.15	11.67 ± 1.10	11.97 ± 1.93	12.37 ± 1.52	15.00 ± 0.00	15.00 ± 0.00	15.00 ± 0.00	20.00 ± 0.00
GTFS (days)	7.67 ± 4.16	8.33 ± 1.15	7.67 ± 1.15	7.67 ± 2.31	0.00 ± 0.00	15 ± 0.00	15.00 ± 0.00	16.00 ± 0.00
GTLS (days)	19.00 ± 2.00	17.00 ± 2.65	17.33 ± 4.04	16.67 ± 1.53	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.00

Note: WM1, wet extractions + sand media; WM2, wet extractions + top soil media; WM3, wet extractions + compost media; and WM4, wet extractions + mushroom prawn waste media. DM1, dry extractions + sand media; DM2, dry extractions + top soil media; DM3, dry extractions + compost media; and DM4, dry extractions + mushroom prawn waste media. GR, germination rate; MGT (days), mean germination time; GTFS (days), germination time of the first seed; GTLS (days), germination time of the last seed.

Table 3. Influence of seed extraction and media on seedling growth after 1 month (mean±SD)

Treatment	Parameters				
	Heigh (cm)	Leaves	Fresh wight (g)	Dry weight (g)	RGR (%)
M1	20.83±1.89	5.00±1.00	0.40±0.13	0.08±0.03	0.10±0.07
M2	18.66±2.95	6.33±0.58	0.64±0.06	0.12±0.012	0.10±0.04
M3	22.20±2.89	6.33±1.16	0.71±0.17	0.12±0.012	0.15±0.09
M4	23.35±3.08	6.67±1.53	0.86±0.38	0.16±0.05	0.36±0.42

Note: M1 = sand media; M2 = top soil media; M3 = compost media; and M4 = mushroom prawn waste media; RGR = Relative growth rate

Table 4. Mean seedling measurement (±SD) under mother trees

DBH	∑ Number seedling (per 1 m^2)				
Mother trees (cm)	I	II	III	IV	Mean ± SD
20.05	108	115	116	110	112.25 ± 3.86
49.34	258	272	264	275	267.25 ± 7.72
28.65	78	74	77	72	75.25 ± 2.76
25.15	139	123	126	128	129.0 ± 6.98
26.42	107	99	117	115	109.5 ± 8.23

Table 5. Mean fruit and seed measurements and seed yields (±SD)

Measurement	Fruit	Seed	Ratio seed/ fruit (g/kg)
Long (cm)	0.54 ± 0.06	0.31 ± 0.03	86.15 ± 13.11
Diameter (cm)	0.78 ± 0.10	0.42 ± 0.05	
Weight (g)	0.21 ± 0.05	0.02 ± 0.00	

Note: DBH, diameter at breast height; I, II, III, IV, plot replicates seed (GTFS), and the germination time of the last seed (GTLS) of the seeds extracted through the wet extraction process were faster than those extracted through the dry extraction process (Table 2).

The growth of *M. gigantea* seedlings

Planting media had statistically significant effects (p-value≤0.005) on the growth of *M. gigantea* seedlings. The highest relative growth rate (RGR) of *M. gigantea* seedlings was found in the mushroom spawn waste media at the end of one month with a average height of 23.35±3.08 cm, 6.67±1.53 leaves, a wet mass of 0.86±0.38 kg, dry mass of 0.16±0.05 kg, and a relative growth rate of 0.36±0.42%, followed by the other planting media, such as compost, topsoil, and sands respectively (Table 3).

Discussion

The flower buds developed in August 2011 when rainfall reached the lowest point during the dry season at the research location. When rainfall was low, light intensity was higher. Davies and Aston (1999) previously reported that the episode of *Macaranga* reproduction in 1992 occurred during a great dry season at Lambir Hills National Park, Sarawak, Malaysia. Some reproductive trees require more intense light, lower fecundity in lower light levels, and lower growth rates than non reproductive trees, reflecting resource-limited reproduction. Fleming et al. (1985) and Bentos et al. (2008) also reported that the flowering of pioneer trees occurred at the end of the dry season and fruiting occurred at the beginning of the wet season. In addition, the phenology of most tropical plants depends mostly on rainfall (Stevenson et al. 2008).

This research revealed that the fruits were mature in December 2011-January 2012 when the intensity of rainfall reached the highest level during the wet season. On the other hand, Suita and Nurhasybi (2009) reported that the fruits of *M. gigantea* were ripe in February-April 2008 in Samboja, East Kalimantan. This difference was caused by some factors including the unpredictable change of climate between the wet season and the dry season in the research location at Samboja. In fact, the time for *Macaranga* to flower and to bear fruits is determined by the local climate conditions. Birds are the main seed dispersers of pioneer trees in tropical Asia, including a considerable number of important pioneer trees that belong to *Macaranga* genus (Corlett and Hau 2000).

Macaranga gigantea seeds that fall below the crown would germinate in less than 24 days provided that the fruits are ripe and fall into canopy gaps, such as those alongside of forest paths. At the time when the fruits were fully mature (January), rainfall at the research location was at its highest level (annual peak), causing the forest floor to be wet and humid. Kiyono and Hastaniah (1997) had reported the seeds of *M. triloba* and *M. gigantean*, sown on wet argillaceous sandy soil and exposed to sunlight 5 hours a day, germinated in 21 days after sowing. If the seeds were planted 8 cm under the soil surface and watered, the seeds germinated 59 days after they were sown. Previous research conducted by Daws et al. (2008) in Panama similarly found that the small seeds from pioneer trees germinated only in humid microsites such as in small canopy gaps with decreased risks of death due to drought. A study conducted by Raich and Khoon (1990) in Pulau Penang, Malaysia showed that the seeds of pioneer trees (including *M. gigantea*) that were moved from the forest shade to canopy openings or to logged-over forest clearing would germinate because of the increase in light irradiation, and soil and air temperature. The photoblastic response of some types of tropical rain forest seeds seems to be adaptations to the change of light quality that occurs as a result of canopy loss (Vazquez-Yanes and Orozco-Segovia 1990). However, higher proportions of germination were found under bright conditions in tree canopy gaps and there was no correlation between the percentage of germination and temperature change (Valio and Scarpa 2001).

Macaranga gigantea seeds can be categorized as small seeds with an average weight of 0.018 grams. The previous study by Pearson et al. (2002) in Panama showed that small seeds (<2 mg) have higher germination percentage to light than under dark condition. Small seeds from tropical pioneer trees will germinate in canopy gaps if the seeds land on microsites that are suitable for seedlings to grow. Phytochrome B controls the germination of seeds in small canopy gaps (Sugahara and Takaki 2004). A study conducted by Van Ulft (2004) in Guyana showed that gap size increased the success of germination of small seeds and dramatically decreased the success of germination of large seeds. In addition, Aud and Ferraz (2012) found that a decrease in seed size was followed by a decrease in light requirement and tolerance to temperature change. Garcia et al. (2005) stated that light is essential for germination under all temperature regimes. According Vazquez-Yanes and Orozco-Segovia (1992) the germination of seeds of all species is either partially or totally hampered if the seeds are covered with leaf litter and if the growth of seedlings is constrained by the litter on the soil surface.

Understanding the ecology of germination and the growth of seedlings is important not only for the knowledge about plant community processes and succession that it provides but also developmental strategies for the conservation of biodiversity and restoration tropical rain forests (Khurana and Singh 2001). The understanding of the germination of *M. gigantea* in nature can be applied for the purposes of cultivation and industrial forest plantations.

Wet seed extraction brought about higher percentages and rates of germination compared with dry seed extraction. Natural seed germination suggested that the fruits of *M. gigantea* are fully ripe during the rainy season so that seeds that fall on the humid forest floor will germinate immediately. Germination peak in the wet season when the soil fertility is high (Marques and Oliveira 2008). Dry seed extraction might decrease the seed water content and kill the embryo. Our finding are in line with the previous report by Suita and Nurhasybi (2009) where, the germination proportion of *M. gigantea* seeds from dry extraction was 2-10%. In addition, germination proportion increased up to 20% when the seed was soaked into 0.2% potassium nitrate solution for 20 minutes and then sown on sand media (Mindawati et al. 2010). Furthermore, Sao (2004) also reported that initial treatment by soaking the seeds in 10 mg/l of GA_3 hormone solution produced the best seed germination rates of *M. gigantea* up to 65%.

In research, wet seed extraction was found to be able to increase the germination proportion of *M. gigantea* seeds up to 65%, similar to an initial treatment by soaking the seeds in 10 mg/l of GA_3 hormone solution. However, the cost required for wet extraction method was cheaper than soaking in hormone solution.

The highest growth rate of *M. gigantea* seedlings was found in mushroom prawn waste planting media and in compost media. This may be because compost is derived from the decomposition of organic materials, contains a lot of nutrients, and is fairly loose. According to Bramasto (2008), a loose, porous medium is easily penetrated by roots growing from the seed and this kind of media has enough pores for water and air. A medium which is too dense will make it too difficult for roots to penetrate and cause water stagnation so that the conditions become too humid and the seedlings become decayed. Moreover, seed mass has a correlation with the rate of growth. Small seeds have a relatively low rate of growth (Arunachalam et al. 2004).

In conclusion, the development of *M. gigantea* flowers and fruits took 5-6 months. The seeds that fell below the crowns would germinate in less than 24 days. The percentage and rate of germination of seeds extracted through the wet extraction process was higher than those extracted through the dry extraction process. The highest growth rate of *M. gigantea* seedlings was found in the mushroom prawn waste media, followed by compost, topsoil, and sand media.

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REFERENCES

Aminah A, Muharam A. 2009. Flowers and fruit development stages Mindi (*Melia azedarach* L). Info Benih 13 (1): 7-13.

Amirta R. 2010. Potential utilization of tropical lignocellulosic biomass for biofuels-bioenergy production in East Kalimantan. JSPS Exchange Program for East Asian Young Researchers: "Fostering Program of Leading Young Scientists toward the Establishment of Humanosphere Science in East Asia", Shigaraki, Kyoto, Japan.

Anon. 2012. Meteorology, Climatology and Geophysics Station of Temindung Airport Samarinda, East Kalimantan. Indonesia.

Arunachalam A, Khan ML, Singh ND. 2003. Germination, growth and biomass accumulation as influenced by seed size in *Mesua ferrea* L. Turk J Bot 27: 343-348.

Aud FF, Ferraz IDK. 2012. Seed size influence on germination responses to light and temperature of seven pioneer tree species from the Central Amazon. Anais da Academia Brasileria de Ciencias. DOI: 10.1590/S0001-37652012000300018.

Bentos TV, Mesquita RCG, Williamson GB. 2008. Reproductive phenology of Central Amazon pioneer trees. J Trop Conserv Sci 1 (3): 186-203.

Bramasto Y. 2008. Techniques of forest plant seed treatment from harvesting fruits. Mitra Tanaman Hutan 3 (3): 131-140. [Indonesian]

Corlett RT, Hau BCH. 2000. Seed dispersal and forest restoration. In: Elliott S, Kerby J, Blakesley D, Hardwick K, Woods K, Anusamsuntom V (eds). Forest Restoration for Wildlife Conservation. International Tropical Timber Organization and The Forest Restoration Research, Chiang Mai University, Thailand.

Davies SJ, Ashton PS. 1999. Phenology and fecundity in 11 sympatric pioneer species of *Macaranga* (Euphorbiaceae) in Borneo. Amer J Bot 86 (12): 1786-1795.

Daws MI, Crabtree LM, Dalling JW, Mullins CE, Burslem DFRP. 2008. Germination responses to water potential in neotropical pioneer suggest large-seeded species take more risk. Ann Bot 102 (6): 945-951.

Eichhorn KAO. 2006. Plants diversity after rain-forest fires in Borneo. Blumea Suplement 18 Nationaal Herbarium Netherland, Universiteit Leiden branch, Leiden.

Fariman ZK, Azizi M, Noori S. 2011. Seed germination and dormancy breaking techniques for *Echinnacea purpurea* L. J Biol Environ Sci 5 (13): 7-10.

Fleming TH, Williams CF, Banaccorso FJ, Hernst LH. 1985. Phenology, seed dispersal, and colonization in *Muntingia calabora*, a neotropical pioneer tree. Amer J Bot 73 (3): 383-391.

Garcia X, Hong TD, Willis RH . 2005. Seed dormancy and germination of *Ficus lundellii* and tropical forest restoration. Tree Physiol 26: 81-85.

Khurana E, Singh JS. 2001. Ecology of tree seed an seedling: implications for tropical forest conservation and restoration. Curr Sci 6: 748-757.

Kiyono Y, Hastaniah. 1997. Slash and burn agriculture and succeeding vegetation in East Kalimantan. Appendix 1. PUSREHUT (The Tropical Rain Forest Research Center). Samarinda, Indonesia. Special Publication 6 : 125-129

Kumara AKKIU, Wickramasinghe U, Seneviratne R. 2000. Studies on dormancy and germination dynamics of *Macaranga peltalta* (Kanda), an early successional species. Proceeding of the Sixth Annual Forestry and Environment Symposium of the Department of Forestry and Environment, University of Sri Jayewardenepura, Peradeniya, 29-30 Desember 2000.

Lawrence D. 2001. Nitrogen and phosphorus enhances growth and luxury consumption of four secondary forest tree species in Borneo. Journal of Tropical Ecology 17: 859-869.

Lawrence D. 2005. Biomass accumulation after 10-200 years of shifting cultivation in Bornean rain forest. Ecology 86: 26-33.

Marques MCM, Oliveira PEAM. 2008. Seasonal rhythms of seed rain and seedling emergence in two tropical rain forest in Southern Brazil. Plant Biology 10: 596-603.

Mendes-Rodrigues C, Oliveira PE, Ranal MA. 2011. Seed germination and seedling growth of two *Pseudobombax* species (Malvaceae) with Contrasting Habitats from Brazilian Cerrado. Rev Biol Trop 59 (4): 1915-1925.

Mindawati N, Bogidarmanti R, Nuroniah HS, Kosassih AS, Suhartati, Rahmayanti S, Junaidi A, Rachmad E, Rochmayanto Y. 2010. Synthesis silviculture research of species alternatives for wood pulp production. Research and Development Center for Increasing Forest Productivity, Forest Research and Development Agency, Department of Forestry. Bogor. Indonesia.

Pearson TRH, Burslem DFRP, Mullins CE, Dalling JW. 2002. Germination ecology of neotropical pioneer : interacting effects of environmental conditions and seed size. Ecology 83 (10): 2798-2807.

Raich JW, Khoon GW. 1990. Effects of canopy openings on tree seed germination in a Malaysian dipterocarp forest. J Trop Ecol 6: 203-217.

Sao C. 2004. Germination and storage of *Dyera costulata* Hook. F. and *Macaranga gigantea* Mull.Agr seeds. [M.Sc.-Thesis]. Universiti Putra Malaysia, Selangor. [Malaysian].

Schmidt L. 2007. Tropical Forest Seed. Springer-Verlag, Berlin.

Slik FJW, Bernard CS, Van Beek M, Breman FC, Eichhorn KAO. 2008. Tree diversity, composition, forest structure and aboveground biomass dynamics after single and repeated fire in a Bornean rain forest. Oecologia 158: 579-588.

Slik FJW, Keßler PJA, Van Welzen C. 2003. *Macaranga* and *Mallotus* species (Euphorbiaceae) as indicators for disturbance in the mixed lowland dipterocarp forest of East Kalimantan (Indonesia). Ecol Indicat 2 (4): 311-324.

Stevenson PR, Castellanos ML, Cortes AI, Link A. 2008. Flowering patterns in seasonal tropical lowland forest in Western Amazonia. Biotropica 40: 559-567.
Sugahara VY, Takaki M. 2004. Effect of light and temperature on seed germination in Guajava (*Psidium guajava* L.-Myrtaceae). Seed Sci Technol 32: 759-764.
Suita E, Nurhasybi. 2009. Seed and plant propagation collection pioneers type *Macaranga* sp. for forest and land rehabilitation. Info Benih 13 (1): 170-175. [Indonesian]
Valio IFM, Scarpa FM. 2001. Germination of seeds of tropical pioneer under controlled and natural conditions. Rev Bras Bot 24 (1): 79-84.
Van Ulft LH. 2004. Effect of seed mass and gap size on seed fate of tropical rain forest tree species in Guyana. Plant Biol 6 (2): 214-221.
Vazquez-Yanes C, Orozco-Segovia A. 1990. Ecological significance of light controlled seed germination in two contrasting tropical habitats. Oecologia 83: 171-175.
Vazquez-Yanes C, Orozco-Segovia A. 1992. Effects of litter from a tropical rainforest on tree seed germination and establishment under controlled conditions. Tree Physiol 11: 391-400.

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Species diversity of cerambycid beetles at reclamation area of coal mining in Berau District, East Kalimantan, Indonesia

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Abstract. *Sugiarto, Boer C, Mardji D. 2016. Species diversity of cerambycid beetles at reclamation area of coal mining in Berau District, East Kalimantan, Indonesia. Biodiversitas 17: 200-207.* Longhorn beetles (Cerambycidae) are amongst the most popular beetle families and hence lots of research has been carried out on the family. However, the presence of this beetle in the reclamation area of PT Berau Coal has not been investigated yet. PT Berau Coal is a coal company that has long been operated. In the former mining areas have been planted with reclamation plants. The study was carried out to determine the cerambycid beetle species diversity in three sites of the reclamation area, mainly Lati (L), Sambarata (S) and Binungan (B). This is the first report of cerambycid beetles diversity at a reclamation area of PT Berau Coal. This study reveals a total of 16, 19 and 22 species with 100, 140 and 192 individuals respectively. Calculation with Simpson's diversity index (1-D) resulted in highly index of biodiversity, namely 0.90, 0.89 and 0.89 respectively. These highly index of diversity is likely due to varied types of vegetation and distance from natural forest to study sites is relatively close. There were seven species respectively dominant at Lati and Sambarata reclamation areas, while at Binungan were six species. Simpson's evenness index of Lati was the highest, followed by Sambarata and Binungan, that were 0.64, 0.53 and 0.41, respectively. This differences might be influenced by the ability of each beetle species to move to other habitats. Jaccard's similarity indices at the three sites were relatively same; they were L with S = 0.45, L with B = 0.46 and S with B = 0.46. The similarity index reflects similar environmental conditions of the three study sites. From the viewpoint of nature conservation, it is concluded that post-mining areas can play a key role in the conservation of beetle diversity since they are as new habitat for beetles species whose original habitats are now in critical condition due to human impact. An important task for future management of post-mining areas is to maintain successional processes and to prevent loss of habitat diversity through revegetation.

Keywords: Cerambycidae, Berau Coal, reclamation, species diversity

INTRODUCTION

Cerambycidae is the second largest family in order of Coleoptera with thousands of them are found almost worldwide, consisting of more than 35,000 species (Lawrence, 1982). Beetles in the family Cerambycidae are often called longhorned beetles because many species possess extremely long antennae, which may be as long or longer than the entire body. However, some species have short antennae, and may be confused with other families of beetles. Many longhorns are large and colorful, which has lead to the group being very popular among collectors. (Schiefer 2015).

Forest in East Kalimantan, the majority of natural forest is a habitat for many species of fauna including beetles of family Cerambycidae. The forest has been changed. Among others for the coal mining which is then planted with reclamation vegetation. The change in habitat resulted in conditions that are not suitable for insect life because of reduced food and changed in the microclimate, so that certain species that are not resistant to these conditions will be away to another place that is found to be appropriate.

This study was undertaken in the reclaimed area of post-mining of PT Berau Coal, where the company has been operated since 1983. The mining system made by this company is an open pit mine. Before the mining begins, land clearing needs to be done to remove vegetation. The soil is excavated to a certain depth to take coal while the woods of economic value are used by PT Inhutani. The site that has been completed mined, are back filled with soil originating from the next site which is being excavated. After mining at a site has been finished, the land is reclaimed around one year after the end of mining.

The species composition of cerambycids fauna depends on the geographical location, climate peculiarities and the vegetation of the region (Georgiev 2013). Establishment of forest plantation often has a negative impact on biodiversity of various types of organisms in many parts of the world because of its structure and function has changed between the natural forest and the plantation (Palik and Engstrom 1999). In Japan, one of the greatest consequence of habitat destruction is the conversion of natural and secondary forests to broadleaf plantations needles especially *Cryptomeria japonica* and *Chamaecyparis obstusa* (Hartley 2002). Disappearance of forested habitat and replacement of old forests with younger forests may have played a role in the decline; nine species were first collected after 1950, resulting in a net loss of 11 species (Mccorquodalel et al. 2007). Other studies revealed that beetles species diversity and their distributions are influenced by the change of habitat, such as habitat destruction and degradation (Baur et al. 2002), habitat fragmentation (Collinge et al. 2001), change of environment (Baselga 2008).

This study was conducted to determine the cerambycid beetles species diversity in the reclamation area, where such study was not conducted before.

MATERIALS AND METHODS

This study was undertaken in the reclamation area of PT Berau Coal, Berau District, East Kalimantan Province, Indonesia from March to August 2013. The study sites were located at Lati (117°35’25.17” E-2°16’49.88” N), Sambarata (117°24’29.34” E-2°10’2.13” N) and Binungan (117°25’58.93” E-2°2’51.86” N) (Figure 1).

The size of the reclaimed area up to the end of 2012 was 91.29 ha, 76.21 ha, and 48.30 ha, respectively. Based on the classification of Köppen, climate type in the study area is wet tropical (Af), while according to the classification of Schmidt and Fergusson the climate included to type A (very wet). The forest types are mainly classified as old secondary forest and plantation forest as reclamation plantation. There are more than 30 floral species of the lands available in the area including *Acacia mangium*, *Agathis*, *Anthocepallus cadamba*, *Areca catechu* (pinang), *Artocarpus integer* (cempedak), bamboo (bambu china), *Caliandra calothyrsus* (kaliandra), *Canna indica* (bunga kana), *Cassia siamea* (johar), *Citrus* sp. (jeruk), *Cupressus sempervirens* (cemara lilin), *Dimocarpus longan* (kelengkeng), *Dryobalanops* sp. (kapur), *Durio zibethinus* (durian), *Enterolobium cyclocarpum* (sengon buto), *Eugenia aquea* (jambu air), *Gliricidia sepium* (gamal), *Gmelina arborea*, *Hevea brasiliensis* (karet), *Hyophorbe lagenicaulis* (palem botol), *Lansium domesticum* (langsat), *Lavandula afficinalis* (lavender), *Macaranga hypoleuca* (mahang), *Mallotus paniculatus*, *Melaleuca leucadendron* (kayu putih), *Nephelium lappaceum* (rambutan), *Palaquium rostratum* (nyatoh), *Paraserianthes falcataria* (sengon laut), *Parkia speciosa* (petai), *Pterocarpus indicus* (angsana), *Salacca zalacca* (salak), *Shorea laevis* (bangkirai), *Shorea* sp. (meranti), *Spondias pinnata* (kedondong), *Swietenia macrophylla* (mahoni), *Terminalia catappa* (ketapang), *Theobroma cacao* (kakao) and *Vitex pubescens* (laban) (Berau Coal 2013).

To record the species of beetles, a plot measuring 20 m ò 100 m (0.2 ha) had been established in each site with the transect pattern (Figure 2).

Three malaise traps (Figure 3.A) at each site were set as described by MEM (2006) and three artocarpus (jackfruit) traps (Figure 3.B-C) were set as described by Makihara et al. (2011). They were used for capturing the beetles. Artocarpus trap was made by using bait consisted of

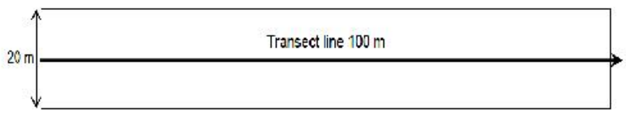

Figure 2. Beetles and vegetation monitoring plot

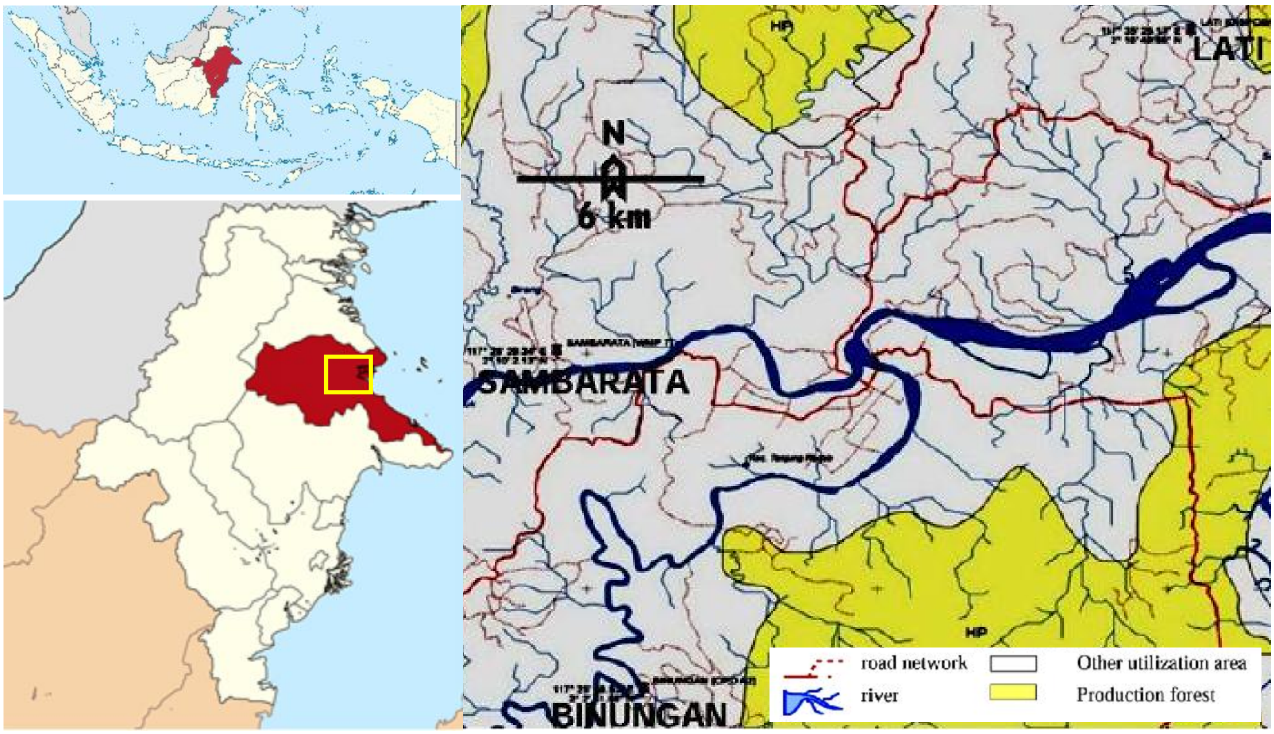

Figure 1. Study site at PT Berau Coal (Lati, Sambarata and Binungan) in Berau District, East Kalimantan, Indonesia

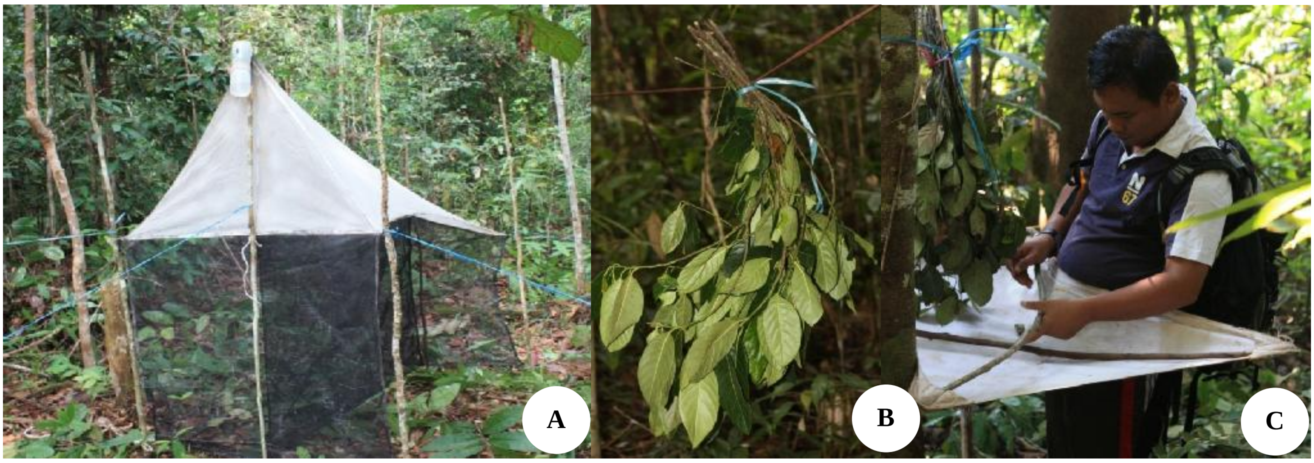

Figure 3. A. Malaise trap set under a light canopy. B. Artocarpus trap where the leafy twigs of jackfruit are tied together and hanged on a tree. C. Artocarpus trap is beaten on a sheet of cloth (beating method) for collecting beetles

several parts of the leafy twigs of jackfruit (*Artocarpus heterophyllus*), then tied into a single clump (Figure 3.B). After three days, artocarpus trap was pounded on a piece of cloth (beating method) for collecting beetles (Figure 3.C). Beetles entered in malaise trap were taken once a week and every time retrieval, old propylene glycol was replaced with a new one, while the beetles trapped in the artocarpus trap were taken every 3 days and when the leaves had withered, then replaced with a new one.

The gathered data were characteristics of beetles, namely: color of body, the size of the antennae, the size of the body and limbs. The primary source for identifications was Makihara (1999) and Makihara et al. (2000).

The Simpson's diversity index was applied to estimate beetle diversity with the following formula: $1 - D$. $D = \{\sum n(n-1)\}/\{N(N-1)\}$, where n = the total number of beetles of a particular species while N = the total number of beetles for all species (Doherty et al. 2011). The index values range between 0.0, which represents no diversity, and 1.0, which represents infinite diversity.

Dominant species of beetles are the most abundant of individuals of each species in a community and were calculated as a percentage. Dominant species were calculated according to Mühlenberg (1993) as follows: Dominant species = (number of individuals of certain species) / (sum of individuals of all species) × 100%. To determine the level of dominant of each species, a formula according to Jorgensen (1974) was used, where >5% is dominant, 2-5% is subdominant and 0-<2% is not dominant.

Simpson's evenness index was calculated according to Krebs (2014) as follows: $E = (1/D) / D_{max}$, where $D = \{\sum n(n-1)\} / \{N(N-1)\}$, where E = evenness index, D = Simpson's index, D_{max} = total species in the community. This index ranges from 0 (unequal distribution of all habitats) to 1 (equal distribution of all habitats).

Jaccard's similarity index was calculated according to Thada and Jaglan (2013) as follows: $S_J = |X \cap Y| / |X| + |Y| - |X \cap Y|$, where S_J is Jaccard index of similarity, *X* is the number of species found in the first site, *Y* is the number of species found in second site and $X \cap Y$ is the number of species shared by the two sites. The range of Jaccard index is 0 to 1, where larger values than 0 mean more similar.

RESULTS AND DISCUSSION

Species diversity

Species richness and individuals at the three sites were somewhat different, at Lati were 16 species with 100 individuals, at Sambarata 19 species with 140 individuals and at Binungan 22 species with 192 individuals (Table 1). There were total of 31 species captured at the three sites (Figure 4). Species diversity index at those three sites was almost same, at Lati 0.90, at Sambarata and Binungan were same, i.e 0.89.

The diversity index was relatively high. This indicated that those three sites were same appropriate for development of the beetles. The main factor that influenced the development was a diversity of vegetation. As above mentioned that there were more than 30 floral species of the lands available in the area at the end of 2012 and the size of revegetated area were 91.29 ha, 48.30 ha, and 76.21 ha, respectively. It is a good development of the area condition that revegetated area is providing diverse habitat that can support a large variety of beetles. The positive relationships between the numbers of longhorn beetle species and individuals with the number of tree species and individuals were reported by Meng et al. (2013) in a region of southern Yunnan, China.

Those species richness and individuals were included slightly when compared to natural forests. For example in the Education Forest of Mulawarman University at Bukit Soeharto were found 555 species of Cerambycidae beetle included in 6 subfamilies in after burned primary and secondary forests (Makihara 1999), at Bukit Bangkirai found 437 species (Makihara et al. 2000), while in the education forest of Unmul at Lempake there were found 45 species with 1744 individuals (Djatmiko 2005). Reclamation land is an area that has been converted from natural forest to artificial (plantation forest) so that the conditions have changed dramatically both physical and biological condition. Conversion of natural forests to other land use may result in loss of insect populations. However, so far there is no study about population of cerambycids beetle in the natural forest near the reclamation land. In this study, the species diversity index of Cerambycidae at the three study sites was high. This indicated that the sites conditions were appropriate for the development of Cerambycidae beetles, where the successive process of vegetation is going on.

There were 10 same species (32.3%) of 31 species present at three sites, that were *Acalolepta rusticatrix*, *Atimura bacillima*, *Epepeotes luscus*, *Nyctimenius ochraceovittata*, *Pterolophia annulitarsis*, *P. crassipes*, *P. melanura*, *Ropica marmorata sarawakiana*, *Sybra* (*Sybra*) *borneotica* and *Xenolea tomentosa*. This means that species were able to adapt at the three sites. The ability to adapt at the sites probably due to the structural similarity of the environment and availability of food, so might represent a suitable habitat, or the ability to fly and migration from one site to another is high.

The movement abilities of longhorn beetles vary greatly, from flightless species that may move 100 m in 10 days (Baur et al. 2002). *Nyctimenius ochraceovittata* was considered to be indicators for less disturbed forest, while *Atimura bacillima*, *Ropica marmorata* and *Xenolea tomentosa* were indicators for degraded forest (Noerdjito et al. 2010). Buchori et al. (2013) studied the importance of insect as bioindicator of reclamation success at Binungan reclamation area, and the result showed that insect diversity collected from reclamation area differed depends on age of reclamation area; the environmental factors such as change of pioneer plant age, vegetation diversity (tree and shrub) and soil chemistry (N total) affect insect diversity in reclamation area. They determined that ants can be potential bioindicator to assess reclamation success in the coal-mining area. More than 200 species of insects including moths, dragonflies, locust, flies and beetles are known to migrate over long distances by air (Meyer 2007). The distance from Lati to Sambarata is 22 km, Lati to Binungan 26 km and Sambarata to Binungan 11 km. The distance from natural forest to Lati reclaimed area is 2 km, to Sambarata 6 km, and to Binungan 3 km. Furthermore, these distances are relatively easy to achieve by the beetles within some days.

Table 1. Diversity, evenness, similarity indices and criteria of dominant of Cerambycidae beetle at reclamation area of PT Berau Coal, East Kalimantan, Indonesia

Species	**Lati (L)**		**Sambarata (S)**		**Binungan (B)**	
	n	**Criteria**	**n**	**Criteria**	**n**	**Criteria**
Acalolepta opposita (Pascoe)	2	SD	0		0	
Acalolepta rusticatrix (Fabricius)	20	D	4	SD	2	ND
Amechana nobilis Thomson	0		1	ND	0	
Astathes japonica (Thomson)	0		0		4	SD
Atimura bacillima Pascoe	8	D	15	D	14	D
Cereopsius sexnotatus Thomson	1	ND	1	ND	0	
Cleptometopus grossepunctatus Breuning	3	SD	0		1	ND
Epepeotes luscus (Fabricius)	10	D	3	SD	2	ND
Menesia vittata Pascoe	0		1	ND	0	
Mutatocoptops diversa (Pascoe)	0		0		1	ND
Myagrus vinosus (Pascoe)	2	SD	0		0	
Nyctimenius ochraceovittata (Auriv)	14	D	4	SD	7	SD
Pterolophia annulitarsis (Pascoe)	2	SD	24	D	31	D
Pterolophia crassipes (Wiedeman)	4	SD	12	D	34	D
Pterolophia melanura (Pascoe)	10	D	28	D	37	D
Rhaphipodus hopei (Waterhouse)	2	SD	0		0	
Rondibilis spinosula (Pascoe)	0		0		1	ND
Ropica angusticollis (Pascoe)	0		0		4	SD
Ropica marmorata sarawakiana Hayashi	3	SD	9	D	4	SD
Ropica nigrovittata (Breuning)	0		0		10	D
Ropica piperata Pascoe	0		2	ND	0	
Ropica sp. (Atlas: sp.2)	0		0		1	ND
Sybra (*Sybra*) *arator* Pascoe	0		1	ND	2	ND
Sybra (*Sybra*) *borneotica* Breuning	7	D	1	ND	4	SD
Sybra (*Sybra*) *lineolata* Breuning	2	SD	0		2	ND
Sybra (*Sybra*) *pulla* Breuning	0		4	SD	19	D
Sybra (*Sybra*) *umbratica* Pascoe	0		4	SD	0	
Sybra (*Sybra*) *cretifera* Pascoe	0		14	D	2	ND
Sybra (*Sybra*) *vitticolis* Breuning et de Jong	0		2	ND	0	
Xenolea tomentosa (Pascoe)	10	D	10	D	8	SD
Xystrocera globosa (Olivier)	0		0		2	ND
Sum (N)	100		140		192	
Diversity index		0.90		0.89		0.89
Evenness index		0.64		0.53		0.41

Note: D = dominant. SD = subdominant. ND = not dominant. Jaccard similarity index (S_J) L with S = 0.45. S_J L with B = 0.46. S_J S with B = 0.46

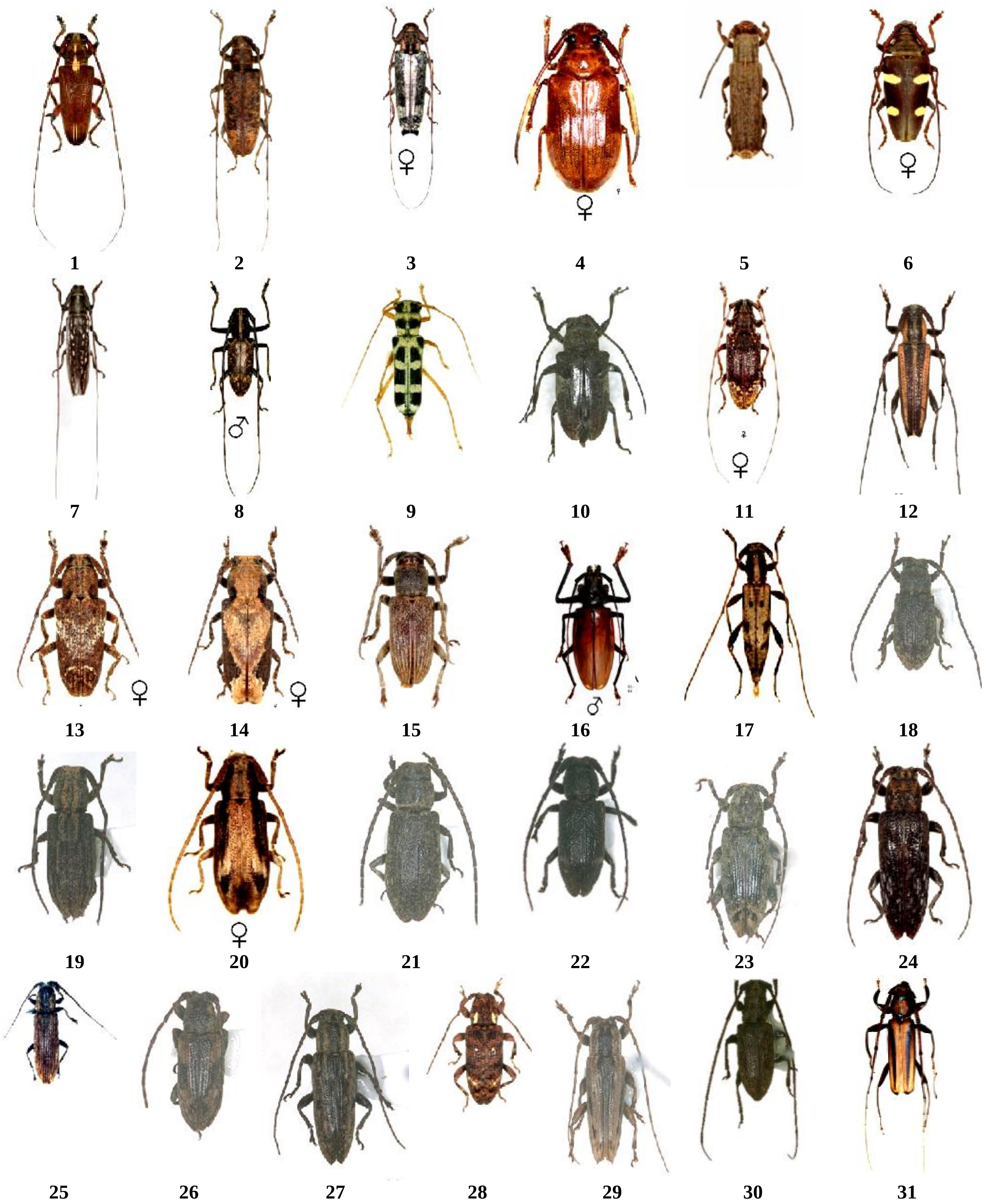

Figure 4. Cerambycidae beetles found in reclamation area of PT Berau Coal. Note: 1. *Acalolepta opposita* (Pascoe); 2. *Acalolepta rusticatrix* (Fabricius); 3. *Amaechana nobilis* Thomson; 4. *Astathes japonica* (Thomson). 5. *Atimura bacillima* Pascoe; 6. *Cereopsius sexnotatus* Thomson; 7. *Cleptmetopus grossepunctatus* Breuning; 8. *Epepeotes luscus* (Fabricius); 9. *Menesia vittata* Pascoe. 10. *Mutatocoptops alboapicalis* Pic; 11. *Myagrus vinosus* (Pascoe); 12. *Nyctimenius ochraceovittata* (Auriv.); 13. *Pterolophia annulitarsis* (Pascoe); 14. *Pterolophia crassipes* (Wiedeman); 15. *Pterolophia melanura* (Pascoe); 16 *Rhaphipodus hopei* (Waterhouse); 17. *Rondibilis spinosula* (Pascoe); 18. *Ropica angusticollis* (Pascoe); 19. *Ropica marmorata sarawakiana* Hayashi; 20. *Ropica nigravittata* (Breuning); 21. *Ropica piperata* Pascoe. 22. *Ropica* sp.; 23. *Sybra* (*Sybra*) *arator* Pascoe; 24. *Sybra* (*Sybra*) *borneotica* Breuning; 25.

Sybra (*Sybra*) *lineolata* Breuning; 26. *Sybra* (*Sybra*) *pulla* Breuning; 27. *Sybra* (*Sybra*) *umbratica* Pascoe; 28. *Sybra* (*Sybra*) *cretifera* Pascoe; 29. *Sybra* (*Sybra*) *vitticolis* Breuning et de Jong; 30. *Xenolea tomentosa* (Pascoe); 31. *Xystrococera globosa* (Olivier).

The company has successfully conducted reclamation and revegetation, as it can see now in Lati and Binungan various fauna species such as monkeys, deer, and Enggang birds have returned to nest just two years after the reclamation began (Berau Coal 2013). Smith et al. (2004) studied dispersal and spatiotemporal dynamics of the Asian longhorned beetle, *Anoplophora glabripennis* in Gansu Province of northern central China and resulted that 98% of beetles were recaptured within 920 m from a release point, whereas the median dispersal rate for all recaptured adults was 30 m/d; dispersal potential within the course of a season for males and gravid females was 2,394 and 2,644 m, respectively. Yarrow (2009) stated that habitat for any wild animal must provide cover (shelter) from weather and predators, food and water for nourishment, and space to obtain food, water, and to attract a mate. This criterion is met the conditions in the natural tropical rainforests such as in the study site. Many species in the rainforest, especially insects and fungi, have not yet been discovered by scientists; every year new species of mammals, birds, frogs, and reptiles are found in rainforests (Butler 2015). Forest actually contains most of the living species, particularly in the case of tropical forests where up to 90% of the planet's species live; tropical forests possess the highest level of biodiversity and therefore provide the biggest genes reservoir (EfB 2016). More than half of the world's estimated 10 million species of plants, animals and insects are living in the tropical rainforests. A 25-acre plot of rainforest in Borneo may contain more than 700 species of trees-a number equal to the total tree diversity of North America (PNF 2016).

Hence natural tropical rainforest is a species source and essential habitat for many living organisms. In contrast, the coal mining area is arid land with no vegetation. But, by reclaiming various species of vegetation, the area that was originally barren slowly turned into a nice area, thus attracting the animals to come in search of food, shelter and breeding. The animals came from a nice habitat (tropical rain forests) around the reclamation area. So here going on source-sink dynamics, where tropical rain forests as a source and reclamation area is a sink. The cause of the high diversity species index of longhorn beetle in three sites of reclamation areas likely due to environmental conditions suited for their lives, because the age of the reclamation plant at the time this study was conducted (the year 2013) was range from 2 to 11 years with an area of reclaimed quite extensive as those mentioned in materials and methods. Appropriate environmental conditions attract beetles from natural forests (source sites) to migrate to the reclamation area (sink sites). Migration in insects serves not only for escape from old habitats but also for reproduction and colonization in new ones (Dingle 1978). Weather seems to be an important factor for the majority of insect migrations. Insect migrations are usually completely confined to the lowest 2 km of the atmosphere, the planetary boundary layer (Drake and Farrow 1988). Williams et al. (2004) stated that adult beetles are prompted to move when the density of beetles in a given area reaches high levels. Individual beetles typically attack a single host tree but migrate to nearby host trees when beetle populations become too dense. Although Asian longhaired beetles can fly distances greater than 400 yards (0.37 km), migration often depends on the abundance of suitable host materials (USDA 2001). According to Bancroft and Smith (2005), the Asian longhorned beetle *Anoplophora glabrescent* move to another habitat when they are in search of a mate.

Foley and Holland (2010) predicted that the best cost surfaces for each species of beetle would reflect aspects of their ecology: *Neoclytus acuminatus acuminatus* would respond positively to urban areas as they provide additional wood resources such as stressed trees; *Neandra brunnea* would respond to urban and field areas, as they may contain damaged or stressed trees and utility poles; *Typocerus velutinus velutinus* would respond to agriculture areas as they contain both large areas of flowers that serve as adult feeding sites and some decaying wood for larval habitat; and *Urographis despectus* would respond to habitat only as it is a specialized larval feeder. Flight is one of the primary reasons that insects have been successful in nature; flight assists insects in the following ways: escaping from danger, finding food, locating mates and exploring for new places to live (Smithsonian 1999). Some common species of longhorn beetles can be categorized as open habitat species that prefer to live in open areas and bore into small branch while others as forest dwellers species or mature forest species living only in primary or very mature secondary forest depending on specific host plants (Noerdjito et al. 2010).

Species dominance

There were seven species respectively dominant at Lati and Sambarata reclamation area while at Binungan were six species. *Atimura bacillima* and *Pterolophia melanura* were dominant at the three sites while *P. annulitarsis* and *P. crassipes* were dominant at both sites Sambarata and Binungan, *Xenolea tomentosa* were dominant at both sites Lati and Sambarata. The dominant value comprises >5% of total individuals. The dominance of these species probably due to their high adaptability to revegetated area and the environment is suitable for breeding, resulting in a lot of the number of individuals. High numbers of ground beetles species and individuals had been captured directly after revegetation at Lusatian mining region (Germany); the dominant beetles were xerophilic species, known to prefer open sandy sites; catches in different plots were positively correlated with the amount of vegetation cover (Kielhorn et al. 1999). The number of herbivorous beetle species was positively correlated with the number of plant species present on the sites at reclaimed strip mines in Southwestern Wyoming. The trophic structure on reclaimed mine sites was dominated by omnivores, insect-carrion feeders, predators and fungivores, whereas the undisturbed site's beetle fauna was dominated by omnivores, herbivores and predators (Parmenter and Macmahon 1987). *P. melanura* and *P. anulitarsis* are common in all habitat types and thought to be habitat generalists (Noerdjito et al. 2010). A generalist species is able to thrive in a wide variety of environmental conditions and can make use of a variety of different resources and are thus often urban adapters (Douglas and James 2015).

There were eight subdominants (2-5%) and only one not dominant species (0-<2%) at Lati reclamation area; at Sambarata five subdominant and seven not dominant; at Binungan six subdominant and ten not dominant species. The criteria of dominance depend on the abundance of individuals of each species. The number of individuals in a population has never fixed the whole time. Births and immigration will increase the number of individual while the death and emigration reduced the number of individual.

Species evenness

The evenness index at Lati was 0.64, Sambarata 0.53 and Binungan 0.41. This shows that the number of individuals of each species of beetles at each site were distributed unevenly. The abundance of individuals each species at Lati were more evenly distributed than at Sambarata and at Sambarata were more evenly distributed than at Binungan. The uneven distribution of the study sites indicated there were some dominant species at the three sites, as above mentioned, there were seven species respectively dominant at Lati and Sambarata reclamation area while at Binungan were six species.

Species similarity

The similarity indices between Lati and Sambarata, Lati and Binungan, Sambarata and Binungan were relatively same, that were 0.45, 0.46 and 0.46, respectively. This means that the species of beetles at the three sites were similar, but the values of similarity index were low. These studies concluded that the low degree of similarity in the reclaimed areas were most likely due to the slow colonization by beetles, where this colonization depended on the succession of planted vegetation. In the Lusatian mining region (Germany), ground beetles species and individuals numbers were high directly after revegetation, while in control plots (not revegetated) were the lowest (Kielhorn et al. 1999). Species richness and diversity on reclaimed strip mines in Southwestern Wyoming showed an increase during the first 3 yr following revegetation, but then declined over the next 3 yr; the magnitude of the observed species richness and diversity trends may have been influenced by the presence or absence of topsoil on the sites (Parmenter and Macmahon 1987). At study sites, the mining process begins with land clearing, followed by topsoil removal, drilling and blasting for overburden removal, transporting overburden, extraction, and hauling of coal. Post-mining activities are backfilling, final disposal and after mining activities are completed, the revegetation of pits (Berau Coal 2013).

This study revealed that the species diversity of beetles at the three study sites (Lati, Sambarata and Binungan) was high, although the species richness in each of these sites is lower than in natural forests in other locations. However, the results of this study demonstrate the success of post-mining land reclamation by PT Berau Coal, in which the longer the diversity of the vegetation will be more and more, so the beetle species richness will also increase. Among the species of beetles, there are dominant, subdominant and not dominant. The spread of the number of individuals of each species of beetles is also uneven. This is related to differences in the nature of each species of beetles, good ability to adapt to their habitat as well as the ability to reproduce. Although the species richness, dominant species and the distribution of individuals number of each species are not the same, but the similarity of species of beetle is relatively the same at the three locations. This relates to the similarity of vegetation planted in three reclamation area. Replanting of post-mining areas is the company's obligation to restore damaged land to be returned to its original condition.

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REFERENCES

Bancroft J, Smith MT. 2005. Dispersal and influences on movement for *Anoplophora glabripennis* calculated from individual mark-recapture. Entomol Experiment Appl 116: 83-92.

Baselga A. 2008. Determinants of species richness, endemism and turnover in european longhorn beetles. Ecography 31: 263-271.

Baur B, Zschokke S, Coray A, Schläpfer M, Erhardt A. 2002. Habitat characteristics of the endangered flightless beetle *Dorcadion fuliginator* (Coleoptera: Cerambycidae): implications for conservation. Biol Conserv 105 (2): 133-142.

Berau Coal 2013. Company profile Berau Coal: Strategy for the brighter future. http://issuu.com/beraucoal/docs/company_profile_berau_coal_2013. [4 October 2015].

Buchori D, Rizali A, Rahayu GA, Sari A, Tabadepu H, Mansur I. 2013. Utilization of insects as bioindicator of reclamation success in Berau, East Kalimantan. Seameo Biotrop, Bogor.

Butler R. 2015. Why do rainforests have so many kinds of plants and animals? http://kids.mongabay.com/elementary/201.html. [29 February 2016].

Collinge SK, Holyoak M, Barr CB, Marty JT. 2001. Riparian habitat fragmentation and population persistence of the threatened valley Elderberry longhorn beetle in Central California. Biol Conserv 100: 103-113.

Dingle H (ed). 1978. Evolution of insect migration and diapause. Springer, New York.

Djatmiko R. 2005. Studies on diversity of longhorn beetles of Cerambycidae family in the effort of tourism development at Botanical Garden of Unmul, Samarinda. Master Thesis, Faculty of Forestry, Mulawarman University, Samarinda. [Indonesian].

Doherty JH, Harris C, Hartley L. 2011. Biological diversity: Calculating. Teaching issues and experiments in ecology (TIEE). www.esa.org/tiee/vol/v7/.../diversity.doc. [12 September 2013].

Douglas I, James P. 2015. Urban ecology: An introduction. Routledge, New York.

Drake VA, Farrow RA. 1988. The influence of atmospheric structure and motions on insect migration. Ann Rev Entomol 33: 183-210.

EfB. 2016. What are the benefits of forest and the consequences of deforestation? Environment for Beginners. http://www.environmenforbeginners.com/content/view/52/49/. [29 February 2016].

Foley CJ, Holland JD. 2010. Do flying beetles respond to human-dominated landscapes as complex mosaics or binary patterns? Landscape Online 16: 1-18. DOI: 10.3097/LO.200916.

Georgiev G, Doychev D. 2013. Contribution to the knowledge of cerambycid fauna (Coleoptera: Cerambycidae) of Belasitsa Mountain in Bulgaria. Silv Balcan 14 (1): 109-116.

Hartley MJ. 2002. Rationale and methods for conserving biodiversity in plantation forests. For Ecol Manag 155: 81-95.

Jorgensen OH. 1974. Result of IPA-censuses on danis farmland. Acta Ornithol 14: 310–321.

Kielhorn K–H, Keplin B, Hüttl RF. 1999. Ground beetle communities on eclaimed mine spoil: Effects of organic matter application and revegetation. Pl Soil 213 (1): 117-125.

Krebs CJ. 2014. Ecological methodology. 3rd ed. Addison-Wesley Educational Publishers, Inc., New York.

Lawrence JF. 1982. Coleoptera. In: Parker SP (ed.). Synopsis and classification of living organisms. Vol. 2. McGraw-Hill, New York.

Makihara H. 1999. Atlas of longicorn beetles in Bukit Soeharto Education Forest, Mulawarman University, East Kalimantan, Indonesia. Pusrehut Special Publication No. 7. Mulawarman University and Japan International Cooperation Agency (JICA), Jakarta.

Makihara H, Simbolon H, Sugiarto, Budi F, Bimaatmadja REI. 2000. Atlas of longicorn beetles in Bukit Bangkirai Forest. PT Inhutani I, East Kalimantan, Indonesia.

Makihara H, Sugiarto, Noerdjito WA. 2011. Longicorn beetles from Mts. Bromo in East Java, Indonesia, with descriptions of a new species and a new subspecies (Coleoptera; Cerambycidae). Bull FFPRI 10 (421): 251-261.

Mccorquodalel DB, Brown JM, Marshall SA. 2007. A decline in the number of long-horned wood boring beetle (Coleoptera: Cerambycidae) species in Ontario during the 20th century? J Ent Soc Ont 138: 107-135.

MEM [Mississippi Entomological Museum]. 2006. Malaise traps. Mississippi Entomological Museum. Mississippi Sate University. http://mississippientomologicalmuseum.org.msstate.edu/collecting.preparation.methods/Malaise.traps.htm. [22 January 2013].

Meng L-Z, Martin K, Weigel A, Yang X-D, Lortie CJ. 2013. Tree diversity mediates the distribution of longhorn beetles (Coleoptera: Cerambycidae) in a changing tropical landscape (Southern Yunnan, SW China). PLoS One 8 (11): 10.1371/annotation/bfe068c2-f5ce-4bc6-83d2-8fe47861ac03.

Meyer JR. 2007. Reason for success. https://www.cals.ncsu.edu/course/ent425/text01/success.html. [6 December 2015].

Mühlenberg, M. 1993. Freilandökologie. 3.Auflage. Quelle & Meyer Verlag, Heidelberg, Wiesbaden. [German].

Noerdjito WA, Makihara H, Sugiarto. 2010. Evaluation of various forest conditions based on longhorn beetles (Coleoptera: Cerambycidae) as bio-indicators in East Kalimantan. In: Fukuyama K, Oka T (eds) Results of a collaborative research in East Kalimantan. Proceedings of International Seminar on CDM Plantation and Biodiversity. Taisei Print Co., Hitachi, Japan.

Palik B, Engstrom RT. 1999. Species composition. In: Hunter Jr ML, (ed.). Maintaining Biodiversity in Forest Ecosystems. Cambridge University Press, New York.

Parmenter RR, Macmahon JA. 1987. Early successional patterns of arthropod recolonization on reclaimed strip mines in Southwestern Wyoming: The ground-dwelling beetle fauna (Coleoptera). Environ Entomol 16 (1): 168-177.

PNF 2016. The disappearing rainforest. The Pax Natura Foundation. http://www.paxnatura.org/RainforestPreservationBenefits.htm. [25 February 2016].

Schiefer TL. 2015. Cerambycidae of the Southeastern United States – Introduction. http://mississippientomologicalmuseum.org.msstate.edu/Researchtaxapages/Cerambycidae/Cerambycidaehome.html. [7 March 2016].

Smith MT, Tobin PC, Bancroft J, Li G, Gao R. 2004. Dispersal and spatiotemporal dynamics of Asian longhorned beetle (Coleoptera: Cerambycidae) in China. Environ Entomol 33 (2): 435-442.

Smithsonian. 1999. Encyclopedia Smithsonian: Insect flight. Information Sheet Number 19. http://www.si.edu/Encyclopedia_SI/nmnh/buginfo/insflght.htm. [2 February 2016].

Thada V, Jaglan V. 2013. Comparison of Jaccard, dice, cosine similarity coefficient to find best fitness value for web retrieved documents using genetic algorithm. Intl J Innov Engineer Technol 2 (4): 202-205.

USDA. 2001. Wanted: The Asian longhorned beetle. United States Department of Agriculture. Animal and Plant Health Inspection Service. Program Aid No. 1655., Washington DC.

Williams DW, Li G, Gao R. 2004. Tracking movements of individual *Anoplophora glabripennis* (Coleoptera: Cerambycidae) adults: application of harmonic radar. Environ Entomol 33 (3): 644-649.

Yarrow G. 2009. Habitat requirements of wildlife: food, water, cover and space. Clemson University Cooperative Extension's Forestry & Natural Resources. Department of Forestry & Natural Resources, Clemson, SC. http://www.clemson.edu/extension/natural_resources/wildlife/publications/fs14_habitat_requirements.html [25 February 2016]

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Length-weight relationship and population genetic of two marine gastropods species (Turbinidae: *Turbo sparverius* and *Turbo bruneus*) in the Bird Seascape Papua, Indonesia

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Abstract. *Saleky D, Setyobudiandi I, Toha HA, Takdir M, Madduppa HH. 2016. Length-weight relationship and population genetic of two marine gastropods species (Turbinidae:* Turbo sparverius *and* Turbo bruneus*) in the Bird Seascape Papua, Indonesia. Biodiversitas 17: 208-217.* *Turbo sparverius* and *Turbo bruneus* are herbivorous grazers gastropod that lived on the rocky intertidal area, which have a role in maintaining algae on the intertidal ecosystem. Local people in Papua have exploited them for food and souvenirs. Even though this exploitation might affect their genetic diversity and population, a study of the genetic structure of these species has not been previously reported. This study aimed to analyze the morphometric, genetic diversity, population structure and connectivity of *T. sparverius* and *T. bruneus* in coastal water of West Papua, Indonesia. The results showed that the growth pattern of *T. sparverius* and *T. bruneus* in all populations were negative allometric, which means that weight gain was slower than length. Haplotype diversity value of all population *T. sparverius* and *T. bruneus* were 0.657-0.705 and 0.739-0.816, respectively. In addition, the haplotype diversity of each population showed a high level of diversity. The genetic structure was found in all population of *T. sparverius* and *T. bruneus* with F_{ST} value-0.037-0.201 and 0.031, respectively. Population structure and phylogenetic tree showed the closeness of genetic due to gene flow between both *T. sparverius* and *T. bruneus*. Genetic distance value between populations *T. sparverius* and *T. bruneus* are very low were 0.002 and 0.003-0.004, respectively. High genetic similarity might occur due to condition and direction of current flow mediating of gene transport among population, and the similarity of habitats in each population.

Keywords: Coral triangle, *g*enetic diversity, morphometric, phylogenetic, *Turbo*

INTRODUCTION

The bird seascape of West Papua, including Cendrawasih Bay and Raja Ampat Archipelago, are located in the Coral Triangle region, which has the most diverse mollusks (Veron et al. 2009). *Turbo sparverius*, common name the corded turban, is a species of sea snail, gastropod marine mollusk in the family Turbinidae. The gastropods *Turbo* sp. or locally named "bia mata bulan", including *T. sparverius* and *T. bruneus* are herbivorous gastropods whose feed on algae living in the intertidal rocky area, often found in crevices of rocks or reef flat (Lee and Chao 2004; Quinones and Michel-Morvin 2006). *T. sparverius* and *T. bruneus* have a thick operculum with various coloration such as black, dark green, white or brownish (Dharma 2005). The operculum has a plate shape that necessary to protect the animals when they withdrawals their self into the shell (Quinones and Michel-Morvin 2006). Despite their functional role in the ecosystem, some of *Turbo* species such as *T. marmoratus, T. setosus* and *T. argyrostomus* became a target species at South Pacific (Kikutani et al. 2002). Also, *T. bruneus* have been exploited for their meets and shells in the Soccoro Archipelago, Mexico (Yamaguchi 1993; Quinones and Michel-Morvin 2006).

Shell's morphology has various shapes that contribute to species identification, classification and taxonomic information (Chiu et al. 2002; Moneva et al. 2012; Caill-Milly et al. 2012). The analysis of morphological characters such as the length-weight relationship of species is useful in marine organism management and also important to determine population condition (Turan 1999; Udo 2013). The difference of gastropod shell morphology are influenced by several factors such as substrate composition (Tan 2009), adaptation to waves exposure (Boulding et al.1999), pollution (Chiu et al. 2002; Urra et al. 2007), self-protection from of predator, and depth variation (Olabarria and Thurston 2003; Marquez et al. 2011). The length-weight relationship and condition factor have been broadly investigated in gastropod to obtain the index of the physical condition of populations and evaluate habitat quality (Albuquerque et al. 2009). Species identification based on shell's morphometric is quite difficult because morphology and color patterns of the shell are affected by the changes in environment factor (Mauro et al. 2003). Therefore, some gastropods identification based on genetic analysis are conducted to clarify the former founding (Marquez et al. 2011), and also other marine organisms (Madduppa et al. 2014; Prehadi et al. 2015; Sembiring et al. 2015; Jefri et al. 2015).

Genetic diversity becomes an important part of population (Hoffman et al. 2009) because genetic diversity gave information about the changes in nature and also in the monitoring of biodiversity and conservation (Schwartz et al. 2006). Genetic diversity defines the ability of the population to adapt to environment condition (Booy et al. 2000). More adapted species produced more genetic and morphological variation in response to environmental changes (Taylor and Aarssen 1988). Population with a high level of genetic diversity has more chance to survive (Bonde et al. 2012). Basic knowledge about genetic diversity is useful for conservation because low level of genetic diversity would increase the extinction risks (Jena et al. 2011).

Analysis of population structure aims to examine the dynamic of the natural populations (Hoffman et al. 2009). Life history traits and gene flow are strongly affected population structure (Storfer 1999; Hoffman et al. 2010) but tracking gene flow and larval dispersal in the marine environment are quite difficult (Weersing and Toonen 2009). Each organism has different dispersal pattern in response to various environmental and oceanographic conditions (Crandall et al. 2008). Habitat type, geography, and natural selection are also an important factor in shaping population structure and differentiation (Colson and Hughes 2004).

Genetic connectivity plays a central role in conservation because it helps maintain population and restoration from damages (Neel 2008), connectivity is also an important concern in almost all conservation plan (Luque et al. 2012). Benthic organism often shows a complex life cycle, and the connectivity among the population is maintained by individual transfer during pelagic larval phase (Cowen and Sponaugle 2009). Knowledge about the complexity of larval dispersal was critical in conservation of marine ecosystem because pelagic larval stage and larval dispersal together form the genetic structure of an organism (Avise 1998; Grantham et al. 2003; Madduppa et al. 2014).

The study of morphometric, genetic diversity, population structure and connectivity of *T. sparverius* and *T. bruneus* in West Papua has not been previously reported. These kinds of studies are necessary for marine management and conservation (Japaud et al. 2013). Therefore, this study aimed to analyze length-weight relationship, genetic diversity, population structure, and connectivity of *T. sparverius* and *T. bruneus* in West Papua.

MATERIALS AND METHODS

Studied species

Turbo is marine gastropod mollusk in the family Turbinidae (Williams 2007). *Turbo sparverius*, common name the corded turban, is a species of sea snail, characterized by their shell. The solid, imperforate shell has an ovate-conic shape with a dirty white or greenish and grows to a length of 75 mm. This marine gastropod species is distributed in the southwest Pacific and off the Philippines (Rosenberg 2015). *Turbo bruneus*, common name the Brown (Pacific) dwarf turban or the little burnt *Turbo*, has a length of the shell varies between 20 mm and 50 mm. This gastropod occurs in the Red Sea, in the Central Indo-Pacific, in the Western Pacific Ocean, off East India, the Philippines and off Western Australia (Rajagopal and Mookherjee 1978; Williams 2007).

Sample collection and morphometric measurement

A total of 179 specimens from four localities throughout the West Papua (Manokwari, Sorong, Raja Ampat and Teluk Wondama) were collected expeditions from October 2014 to January 2015 (Figure 1). *Turbo sparverius* were collected from Manokwari (72 specimens), Teluk Wondama (30), Sorong (3) and *T. bruneus* were collected from Raja Ampat (52), and Manokwari (22). The sample was collected from the rocky intertidal area during low tide. Indonesian Shells II (Dharma 1992) and Recent & Fossil Indonesian Shell (Dharma 2005) were used to identify samples morphologically. The total length and total weight of gastropod shell were measured for each sampled individual. Foot muscle tissues from *T. sparverius* and *T. bruneus* were collected, and preserved in 96% ethanol for subsequent analysis.

DNA extraction, amplification and sequencing

Genomic DNA for each sample was extracted using extraction kit (Qiagen kit. Cat No. 69504) or Chelex (Walsh et al. 1991). A fragment of mitochondrial Cytochrome Oxidase subunit-I gene (COI) was amplified using the following primer set: LCO1490 5’-GGTCAACAAATCATAAAGATATTGG-3’ and HCO2198 5’-TTAACTTCAGGGTGACAAAAAATCA-3’ (Folmer et al. 1994). Polymerase Chain Reaction (PCR) was conducted in 25 µL reaction volume containing 1-4 µL template DNA, 2.5 µL of 10x PCR buffer (Applied Biosystems), 2,5 µL dNTP (8 mM), 2 µL $MgCl_2$ (25 mM), 0.125 µL AmplyTaq Red™ (Applied Biosystems), 1.25 µL of each primer (10 mM), 1 µL 1x BSA, and 13.5 µL ddH_2O. PCR conditions were: initial denaturation at 94 °C for 15 s, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 45 s. The final extension step was conducted at 72 °C for 10 min. The quality of PCR products was assessed by agarose gel electrophoresis and ethidium bromide staining and visualized using UV transilluminator. All good PCR products were sent to Berkeley Sequencing Facility, USA.

Data analysis

Length-Weight Relationship of *T. sparverius* and *T. bruneus* were analyzed using linear regression. The length-weight relationship is the most widely used method for estimating biomass of benthic invertebrates. This analysis

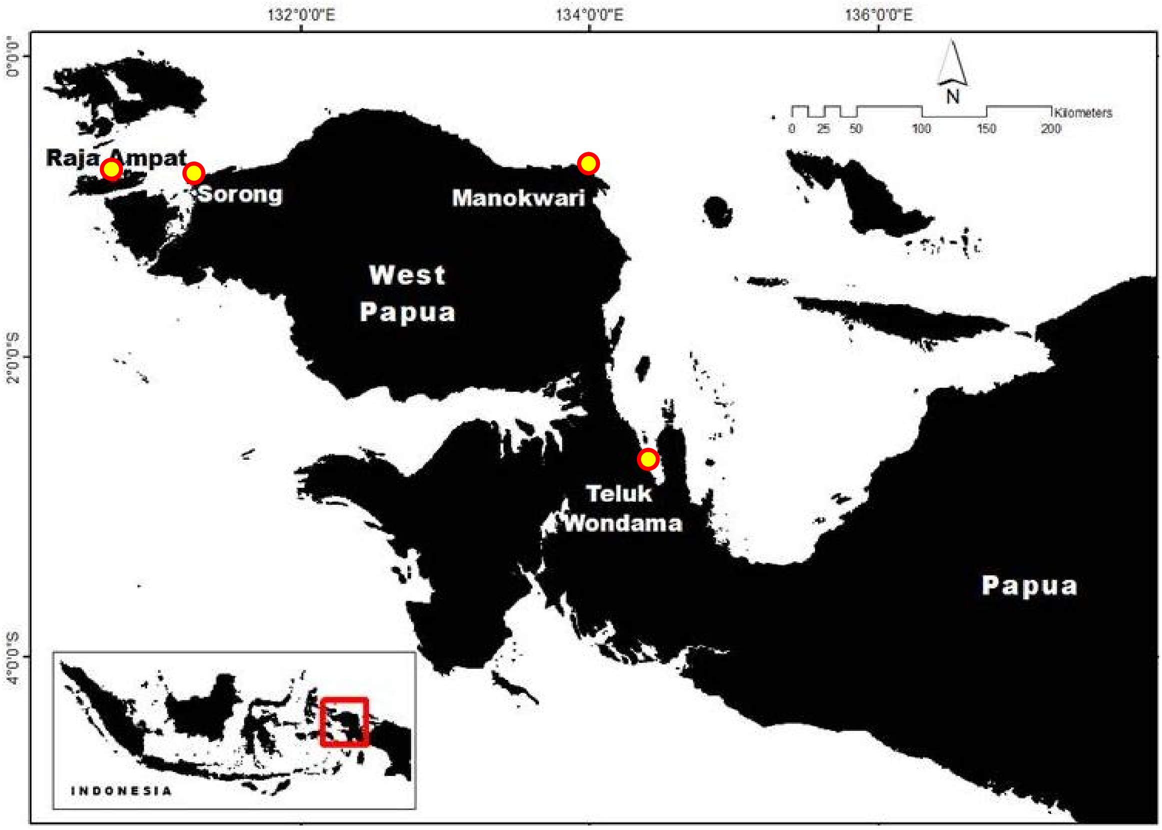

Figure 1. Sampling location (○) of *T. sparverius* dan *T. bruneus* in West Papua, Indonesia: Raja Ampat, Sorong, Manokwari, and Teluk Wondama, Papua, Indonesia

could presume whether there was a difference in size due to differences in environmental conditions (Schefler 1987; Benke et al. 1999). The length-weight relationships of these species were described by the following equation $W = aL^b$, where W is body weight (gram), L is body length (mm) and a and b are constant values (Effendie 1979). If b = 3 indicated an isometric growth, which means the length and weight increase equally. If b ≠ 3 indicated an allometric growth, which b < 3 indicated an increase in length was faster than weight, and b > indicated an increase in the weight was faster than the length (Effendie 1979).

Sequences were aligned and edited in Mega 6 (Tamura et al. 2013). Genetic distance (D) was calculated within and between populations. A Neighbour-Joining (NJ) tree was constructed in Mega 6 (Tamura et al. 2013) based on Kimura 2-parameter model, and 1,000 bootstrap replicates. Additional sequences data of *T. sparverius*, *T. bruneus*, and *T. setosus* were added from GeneBank (Table 1). *T. setosus* was utilized as an outgroup in phylogenetic reconstruction. DnaSP 5.10 (Rozaz et al. 2003) was used to analyzed a number of the haplotype (H), haplotype diversity (Hd) (Nei, 1987), and nucleotide diversity (π) (Lynch and Creasef 1990). Population differentiation was assessed with Fixation index (F_{st}) (Excoffier et al. 1992) using Arlequin 3.5 (Excoffier and Lischer 2009). Consider investigating the phylogenetic relationship among haplotype, a minimum spanning tree was constructed in Network 4.6.1 (http://www.fluxusengineering.com).

RESULTS AND DISCUSSION

Length-weight relationship

The growth pattern of *Turbo sparverius* and *Turbo bruneus* were presented in Table 2. The result from the length-weight calculation of the *T. sparverius* produced equation $W = 0.001L^{2.654}$ with $R^2 = 0.847$ (Manokwari), $w = 0.017L^{1.939}$ with $R^2 = 0.553$ (Teluk Wondama), and $W = 0.00017L^{3.091}$ with $R^2 = 0.996$ (Sorong). The analysis of total length and weight showed a linear relationship with the line equation Y = 2.4672x-2.6569 with $R^2 = 0.799$. Figure 2 presented the linear regression graph of the total length and weight of the *T. sparverius*. Based on obtained r^2 values, the contribution of shell length to weight was 84.7% (Manokwari), 55.3% (Teluk Wondama), 99.6 % (Sorong), and 79.9 % (all populations). These values suggested that body weight can be used to estimate the size of the shell length. The b values were less than 3 indicated the growth pattern of the *T. sparverius* was negative allometric, which means weight gain was slower than the length. *T. sparverius* from Sorong showed positive allometric growth pattern (b > 3), which means weight gain were faster than the length. This value could be due to low sample size (3 samples).

Length-weight relationship of *T. bruneus* described by equation $W = 0.00054L^{2.802}$ with $R^2 = 0.983$ (Raja Ampat) and $W = 0.05004L^{1,548}$ with $R^2 = 0.734$ (Manokwari). The analysis of total length and weight showed a linear relationship with the line equation y = 2.2954x-2.4604 with R^2 = 0.8724. Linear regression graph of the total length and weight of the *T. bruneus* was presented in Figure 2.

Based on obtained R^2 values, the contribution of shell length to weight was 98.30% (Raja Ampat), 73.4 % (Manokwari) and 87.2 % (all populations). These values also suggest that body weight can be used to estimate the size of the shell length.

Phylogenetic relationship

Phylogenetic analysis of *T. bruneus* resulted in two main clades with high bootstrap support (99). Clade 1 consisted of *T. bruneus* from West Papua and Clade 2 was *T. bruneus* from Malaysia. The close relationship between *T. bruneus* population from West Papua might be due to high sequence similarities. Phylogenetic tree of *T. sparverius* showed a group of all population into 2 main

Table 1. GenBank data information ot the *T. sparverius*, *T. bruneus* and *T. setosus*, included in this analysis, location and accesion number from National Center for Biotechnology Information (NCBI)

Species	Location	Acc. GenBank	References
Turbo bruneus	Sabah, Malaysia	AM403930	Williams 2007
Turbo bruneus	Sabah, Malaysia	AM403931	Williams 2007
Turbo sparverius	Longkang, Taiwan	AM403911	Williams 2007
Turbo setosus	Longkang, Taiwan	AM403910	Williams 2007

Tabel 2. Length-weight relationship of *T. sparverius* and *T. bruneus* showing the number of samples (n), constant values (a), the index of the growth values (b), correlation values (r), determinant values (R^2) and the growth pattern.

Species	Site	n	a	b	r	R^2	Growth pattern
T. sparverius	Sorong	3	0.00017	3.091	0.998	0.996	Positive allometric
	Manokwari	72	0.00104	2.654	0.921	0.847	Negative allometric
	Teluk Wondama	30	0.01678	1.939	0.744	0.553	Negative allometric
	All populations	105	0.0022	2.467	0.894	0.799	Negative allometric
T. bruneus	Raja Ampat	52	0.00054	2.802	0.992	0.983	Negative allometric
	Manokwari	22	0.05004	1.548	0.857	0.734	Negative allometric
	All populations	74	0.00346	2.295	0.934	0.872	Negative allometric

Table 4. Genetic distance (D) within and between population of *T. sparverius* and *T. bruneus*

Species	Genetic distance	Site	Manokwari	Teluk Wondama	Sorong
T. sparverius	Within population	Manokwari	0.002	-	-
		Teluk Wondama	-	0.002	-
		Sorong	-	-	0.002
	Between population	Manokwari	-	-	-
		Teluk Wondama	0.002	-	-
		Sorong	0.002	0.002	-
			Manokwari	**Raja Ampat**	
T. bruneus	Within population	Manokwari	0.004	-	
		Raja Ampat	-	0.003	
	Between population	Manokwari		-	
		Raja Ampat	0.003	-	

Table 5. Pairwise F_{ST} values of *T. sparverius* and *T. bruneus*

Species	Population	Manokwari	Teluk Wondama	Sorong
T. sparverius	Manokwari	-	-	-
	Teluk Wondama	0.037	-	-
	Sorong	0.201	0.146	-
T. bruneus		**Raja Ampat**	**Manokwari**	
	Raja Ampat	-	-	-
	Manokwari	0.031	-	-

clades, supported by high bootstrap values (99). Clade 1 composed of the population from Papua and clade 2 consisting of the population from Taiwan. Low gene flow possibly caused genetic differentiation between Papua and Taiwan due to distant geographic location. Overall, phylogenetic analysis revealed that all population of *T. sparverius* and *T. bruneus* lacked in genetic structuring indicated by low geographic partition between all samples (Figure 3). These were also supported by the low genetic distance between population (Table 4) and low F_{ST} values (Table 5).

Genetic diversity

A 656 bp fragment from Cytochrome Oxidase I (COI) was obtained from all samples (*T. sparverius* and *T. bruneus*) with a similarity of 98-99%. Similar fragment length was also found in family Turbinidae (Williams, 2007). In 656 bp DNA fragment of *T. sparverius* and *T. bruneus* there are many nucleotide difference (polymorphism) caused by point mutation. It is called point mutation because the mutation only occurs at a single nucleotide (Xiao et al. 2007). Transitions are substitution mutation between A and G (purines) or between C and T (pyrimidines), transversion occurs when nucleotide of purines changed to pyrimidines or vice versa (Graur 2003). There were five transition mutations found in *T. sparverius* consist of 1 synonymous substitution and four non-synonymous substitutions. The numbers of codons in *T. sparverius* were 218 with four mutated amino acids at codon position of 13, 35, 110, and 138. Meanwhile, in *T. bruneus*, 15 point mutations were found; consist of 14 transition mutations and one transversion mutation. The nucleotide substitution in *T. bruneus* consists of nine non-synonymous substitutions and six synonymous substitutions. The number of codons in *T. bruneus* was 218 with nine amino acid mutations at codon position of 51, 73, 132, 138, 168, 198, 201, 203, and 211. The occurrence of transition mutation are more often found than transversion mutation (Santos et al. 2003) and the transitional rate between pyrimidines (C and T) are higher than that between purines (A and G) (Castro et al. 1998). Mutations can occur in either somatic or germ-line cells. Somatic mutations are not inherited so that they can be disregarded in an evolutionary or genetic context (Graur 2003).

The number of haplotypes and various types of haplotype were influenced genetic diversity of a population (Akbar et al. 2014). A total number of haplotype found in *T. sparverius* and *T. bruneus* were 7 and 13 haplotypes, respectively (Table 3). *T. sparverius* from Manokwari had six haplotypes (20 samples), Teluk Wondama had four haplotypes (15 samples) and two haplotypes (3 samples) from Sorong. *T. bruneus* from Raja Ampat had eight haplotypes (17 samples) and Manokwari had six haplotypes (18 samples).

Each population showed a high level of haplotype diversity (Hd) ranged from 0.657 to 0.816 (Table 3). The highest haplotype diversity of the *T. sparverius* was found in Teluk Wondama (0.705) and the lowest was found in Manokwari (0.657). The highest haplotype diversity of the *T. bruneus* was found in Raja Ampat (0.816) and the lowest was found in Manokwari (0.739). Nucleotide diversity (π) of *T. sparverius* ranged from 0.0018 (Manokwari) to 0.0021 (Teluk Wondama and Sorong). Meanwhile, nucleotide diversities of *T. bruneus* ranged from 0.0027 (Raja Ampat) to 0.0037 (Manokwari). This study showed that nucleotide diversities of *T. sparverius* and *T. bruneus* were lower than other gastropod species such as *Lunnela gradulata* (π = 0.0046) (Chiu et al. 2013).

Table 3. Genetic diversity of *T. sparverius* and *T. bruneus* accessed from the number of the haplotype (Hn), haplotype diversity (Hd), and nucleotide diversity (π), N indicated the number of samples for each site

Species	Population	N	Genetic diversity		
			Hn	**Hd**	**π**
T. sparverius	Manokwari	20	6	0.657	0.0018
	Wondama Bay	15	4	0.705	0.0021
	Sorong	3	2	0.667	0.0021
	All populations	38	7	0.691	0.0020
T. bruneus	Raja ampat	17	8	0.816	0.0027
	Manokwari	18	6	0.739	0.0037
	All populations	35	13	0.785	0.0032

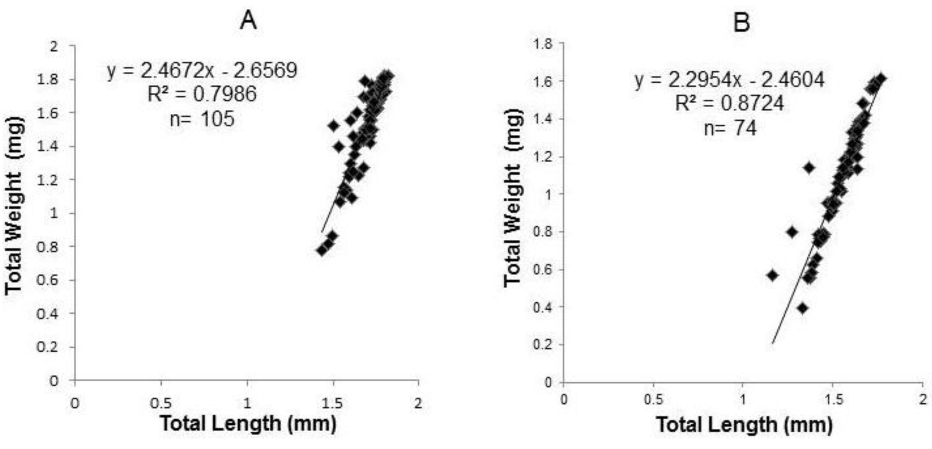

Figure 2. Linear regression of total length and weight from 105 individuals *T. sparverius* (A) and 74 individuals *T. bruneus* (B)

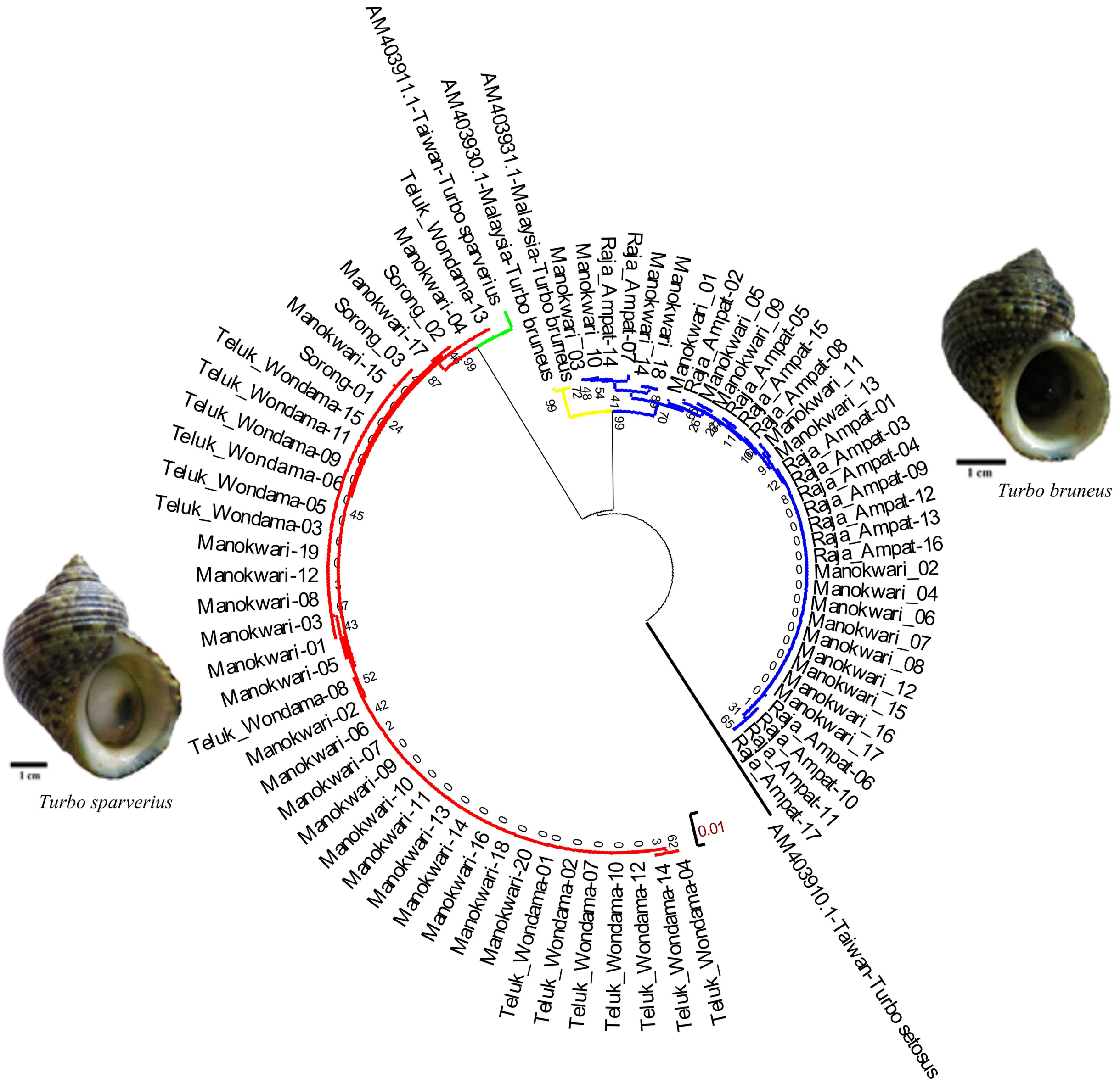

Figure 3. Phylogenetic tree of *T. sparverius* (39 sequences) and *T. bruneus* (37 sequences) using Neighbour-Joining (NJ) method with Kimura 2-parameter model and 1,000 bootstraps.

Population structure

Genetic distance within individuals of *T sparverius* and *T. bruneus* were 0.0-0,6 % and 0.0-1.1 %, respectively. Genetic distance between *T. sparverius* and *T. bruneus* was 10,6 %. Genetic distance within individual was less than 2% while genetic distance among species ranged from 7-12% (Jusmaldi et al. 2014). Meanwhile, according to Brown et al. (1982), the genetic distance among species ranged from 9-19%.

Genetic distance within and between population of the *T. sparverius* in Manokwari, Teluk Wondama, and Sorong was 0.002 (Table 4). Meanwhile, genetic distance within population of *T. bruneus* in Manokwari and Raja Ampat were 0.004 and 0.003, respectively. Genetic distance between populations of *T. bruneus* was 0.003 (Table 4).

Pairwise F_{ST} test showed low level of genetic differentiation with F_{ST} values ranged from-0.037 to 0.201 (Table 5). AMOVA indicated that P-value of *T. sparverius* and *T. bruneus* were 0.23 and 0.17, which mean these population showed non-significance genetic differentiation among population ($P > 0.05$).

Population connectivity

The highest number of haplotype of the *T. sparverius* was found in Manokwari (Figure 4). Among these populations, there were four shared haplotypes such as, H1, H2, H3, and H6. Private haplotypes were found in Teluk Wondama (H7) and Manokwari (H4 and H5). The haplotype network of the *T. bruneus* showed that Raja Ampat has the highest number of haplotype, and there was only one shared haplotype (H1) (Figure 4). Genetic distance and F_{ST} showed that all populations of *T. sparverius* and *T. bruneus* had close genetic relationship.

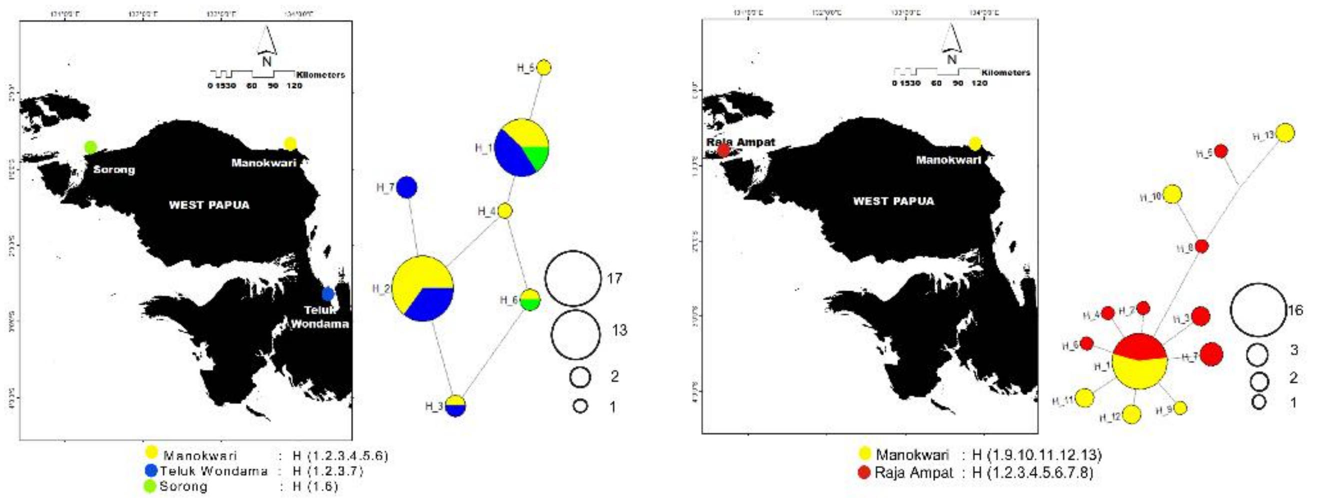

Figure 4. Haplotype network of the *T. sparverius* (left) and *T. bruneus* (right) from different locations in Papua. Each haplotype represents by a circle, whereas the size indicated the haplotype frequencies. Colors filling correspond to different sampling locations: Manokwari (yellow), Teluk Wondama (blue), Sorong (green)

Discussion

Length-weight relationship

The length-weight relationship are widely used in biological fisheries research for describing the change in the size of the individual, showing the growth pattern of an organism, obtaining the index of physical condition of populations and evaluate habitat quality (Gayon 2000; Albuquerque et al. 2009). This study showed that the growth pattern of *T. sparverius* and *T. bruneus* from West Papuan were negative allometric. The b values were less than 3, indicated the growth pattern of the *T. bruneus* was negative allometric, which means weight gain was slower than the length. A different result was obtained from the *T. sparverius* from Sorong which showed positive allometric growth pattern. *T. sparverius* were exploited by local people in Sorong and the sampling site was near the tourism object, these facts might be responsible for the low sample size found in this area. Negative allometric growth pattern means that weight gain was slower than the length. It is indicated that the collected individuals are the young individual. The Growth in younger individuals more focused on the growth of the shell so that the shell growth is faster than the growth in weight (Mulki et al. 2014). Differences of the growth patterns on the same species is caused by several factors such as the number of samples, sex differences, and other external factors such as environmental conditions suitable for the development of the growth of these species (Innal et al. 2015).

This study has a consistent result with Ramesh et al. (2009), which also found negative allometric growth pattern in *T. bruneus.* Various gastropod species also showed negative growth pattern such as *Lambis lambis* (Jaykhumar et al. 2011), *Achatina fulica* (Albuquerque et al. 2009), *Litorina* sp. (McKinney et al. 2004), and *Tympanotonus fuscatus* (Udo 2013). The morphometric variation found in mollusks seemingly triggered by various factor such as, tidal variation, food availability, changes in seasonality, and sexual maturity (Ramesh et al. 2009).

Population genetic

Genetic distance analysis showed that all population of *T. sparverius* and *T. bruneus* were closely related. Genetic similarities of these species are probably triggered by ocean currents that act as a medium for gene transfer among those species. Lin and Liu (2008) found genetic similarities may exist due to ocean currents, high larval dispersal, and an appropriate habitat condition. Genetic diversity of marine biota in North Papua probably affect by New Guinea Coastal Current (Kashino et al. 2007) because planktonic larval dispersal and ocean current influence genetic exchange between populations (Chiu et al. 2013). Differences in environmental condition resulted in morphological changes, anatomical, and phylogenetic of a population (Twindiko et al. 2013). According to Chiu et al. (2013), genetic diversity can be influenced by two factors such as over-exploitation and habitat condition. High genetic diversity of individual within the population, increase the ability of the population to respond to environmental changes or exploitation (Akbar et al. 2014). *T. sparverius* and *T. bruneus* are widely used by local people for food or merchandise, which could reduce the genetic diversity of these species.

Pairwise F_{ST} test showed a low level of genetic differentiation in the current study, implied that those populations were genetically similar. Similar results was found in *Echinolittorina ziczac* (Diaz-Ferguzon et al. 2011) and *Cittarium pica* (Diaz-Ferguzon et al. 2010) which showed low F_{ST} values ranged from-0.007 to 0.04, and from-0.07 to 0.106, respectively. They also found that adjacent populations tend to have a low-value F_{ST}, while the greater distances tend to have higher F_{ST} value (Diaz-Ferguzon et al. 2011). Genetic heterogeneity increases with increasing distance between populations (Wolf et al. 2000). *Coralliophila violacea* showed low average F_{ST} values (0.078), this is probably caused by the ability of larval dispersal and the pattern of ocean current (Lin and Liu 2008).

Gene flow and geographic isolation were affected by geographical distance and environment complexity. Genetic homogeneity of *T. sparverius* and *T. bruneus* seems to be caused by short distance between all sampling sites. Other possible explanation related to New Guinea Coastal Current (NGCC), which might facilitate larval dispersal across study areas. Genetic similarities might also exist due to the similarity of habitats in each population. Urra et al. (2003) found that gastropods originating from different habitat can have different both in genetic and morphology.

Shared haplotypes could be due to larval dispersal following ocean currents. *Turbo* sp. experienced a larval stage in its life cycle. *T. marmoratus* required 4 days to change from egg phase until larval settlement (Yamaguchi 1993). Dwiyono et al. (2001) revealed that the required time of *T. marmoratus* from the egg stage to become benthic organisms was about 60 hours. Such a long time larval duration provides an opportunity for this species to distribute widely via New Guinea Coastal Current (NGCC) (Kashino et al. 2007). New Guinea Coastal Current (NGCC) is a surface current that flows along the northern coast of Papua (Kuroda 2000). Genetic connectivity between populations of the benthic organism mainly occurred at pelagic larval stage (Cowen and Sponaugle 2009).

Analysis of genetic distance and F_{ST} showed that all populations of *T. sparverius* and *T. bruneus* had a close genetic relationship, possibly caused by the short distance between sampling sites (63-415 km) and geographic condition that are relatively open. Wyrtki (1961) found that circulation pattern in the northern coast of Papua has a strong seasonal variability, flowing continuously to the Philippines. Ocean current condition and pelagic larval stage were potential factors that play a role in the dispersal of organisms, for example, dispersal distance of *Gaimardia trapesina* could reach 1300-2000 km (Helmuth et al. 1994). Isolated population tends to have low population size and high extinction risk compared to connected populations (Noreen et al. 2009). Ecological isolation and geographic structure were also affected population genetic structure (Crispo and Chapman 2008). Adjacent populations usually showed more genetic similarities than distant populations (Palumbi 2003). Geographical isolation, population history, oceanographic condition, and ecological traits can lead to high variations in the genetic diversity among populations (Chiu et al. 2013; Silva et al. 2013). Similar microhabitat (rocky intertidal) contribute to the occurrence of the genes mixing between populations *T. sparverius* and *T. bruneus*. Albaina et al. (2012) stated that microhabitat was an important factor in the dispersal of species, even though these species possess similar life cycle and dispersal potential.

We conclude that growth pattern in all populations of *T. sparverius* and *T. bruneus* was negative allometric, which means that weight gain was slower than length. All populations showed high haplotype diversity and genetic diversity. Phylogenetic reconstruction and population structure analysis indicated that genetic homogeneity was dominant among the population of *T. sparverius* and *T. bruneus*, which possibly caused by gene flow between populations.

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REFERENCES

Akbar N, Zamani NP, Madduppa HH. 2014. Genetic diversity of yellowfin tuna (*Thunnus albacares*) from two populations in the Moluccas Sea, Indonesia. Depik 3: 65-73.

Albaina N, Olsen JL, Couceiro L, Ruiz JM, Barreiro R. 2012. Recent history of the European *Nassarius nitidus* (Gastropoda): phylogeographic evidence of glacial refugia and colonization pathways. Mar Biol 159: 1871-1884.

Albuquerque FS, Peso-Aguiar MC, Assuncao-Albuquerque MJT, Galvez L. 2009. Do climate variables and human density affect *Achatina fulica* (Bowditch) (Gastropoda: Pulmonata) shell length, total weight and condition factor?. Braz J Biol 69: 879-885.

Avise JC. 1998. The history and purview of phylogeography: A personal reflection. Mol Ecol 7: 371-379.

Benke AC, Huryn AD, Smock LA, Wallace JB.1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. JN Am Benthol Soc 18: 308-343.

Bonde RK, Mcguire PM, Hunter ME. 2012. A review of the key genetic tools to assist imperiled species conservation: analyzing West Indian manatee populations. JMATE 5: 1.

Booy G, Hendriks RJJ, Smulders MJM, Van Groenendael JM, Vosman B. 2000. Genetic diversity and the survival of populations. Plant Biol 2: 379-395.

Boulding EG, Holst M, Pilon V. 1999. Changes in selection on gastropod shell size and thickness with wave-exposure on Northeastern Pacific shores. J Exp Mar Biol Ecol 232: 217-239.

Brown WM, Prager EM, Wang A, Wilson AC. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. J Mol Evol 18: 225-39.

Caill-Milly N, Bru N, Mahe K, Borie C, D'amico F. 2012. Shell shape analysis and spatial allometry patterns of Manila clam (*Ruditapes philippinarum*) in a mesotidal coastal Lagoon. J Mar Biol. 2012: 11.

Castro JA, Picornell A, Ramon M. 1998. Mitochondrial DNA: a tool for populational genetics studies. Int Micro 1: 327-332.

Chiu Y, Bor H, Tan M, Lin H, Jean C. 2013. Phylogeography and genetic differentiation among populations of the Moon Turban Snail *Lunella granulata* Gmelin, 1791 (Gastropoda: Turbinidae). Int J Mol Sci 14: 9062-9079.

Chiu Y, Chen H, Lee S, Chen CA. 2002. Morphometric analysis of shell and operculum variations in the Viviparid Snail, *Cipangopaludina chinensis* (Mollusca: Gastropoda), in Taiwan. Zoo Stud 41: 321-331.

Colson I, Hughes RN. 2004. Rapid recovery of genetic diversity of dogwhelk (*Nucella lapillus* L.) populations after local extinction and recolonization contradicts predictions from life-history characteristics. Mol Ecol 13: 2223-2233.

Cowen RK, Sponaugle S. 2009. Larval Dispersal and Marine Population Connectivity. Ann Rev Mar Sci 1: 443-66.

Crandall ED, Frey MA, Grosberg RK, Barber PH. 2008. Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods. Mol Ecol 17: 611-626.

Crispo E, Chapman LJ. 2008. Population Genetic Structure Across Dissolved Oxygen Regimes In An African Cichlid Fish. Mol Ecol 17: 2134-2148.

Dharma B. 1992. Indonesian Shells II . Sarana Graha, Jakarta.

Dharma B. 2005. Recent & Fossil of Indonesian Shell. PT. Ikrar Mandiri Abadi, Indonesia.
Diaz-Ferguson E, Haney R, Wares J, Silliman B. 2010. Population genetics of a trochid gastropod broadens picture of Caribbean Sea Connectivity. PloS ONE 5: e12675. doi:10.1371/journal.pone.0012675
Diaz-Ferguson E, Haney RA, Wares JP, Silliman BR. 2011. Genetic structure and connectivity patterns of two Caribbean rocky intertidal gastropods. J Mollusc Stud 0: 1-7.
Dwiono SPA, Pradina, Makatipu PC. 2001. Spawning and seed production of the green snail (*Turbo marmoratus* L.) in Indonesia. SPC *Trochus* Information Bulletin vol B-January 2001.
Effendie MI. 1979. Method on Fish Biology. Yayasan Agromedia, IPB, Bogor. [Indonesian]
Excoffier L, Lischer H. 2009. Arlequin ver 3.5 user manual; an inegrated software package for population genetic data analysis. Institute of Bioinformatics, Swiss.
Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of moleculer variance inferred from metric distance among DNA haplotypes; application to human mitochondrial DNA restriction data. Genetics 131: 479-491.
Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of Mitochondrial Cytochrome c Oxidase Subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3: 294-299.
Gayon. 2000. History of the concept of allometry. Amer Zool 40: 748-758.
Grantham BA, Eckert GL, Shanks AL. 2003. Dispersal potential of marine invertebrates in Diverse habitats. Eco App 13: 108-116.
Graur D. 2003. Single-base Mutation. Nature Encyclopedia Of The Human Genome. Macmillan Publishers Ltd, New York.
Helmuth B, Veit RR, Holberton R. 1994. Long-distance dispersal of a subantarctic brooding bivalve *(Gaimardia trapesina)* by kelp-rafting. Mar Biol 120: 421-426.
Hoffman JI, Clarke A, Linse K, Peck LS. 2010. Effects of brooding and broadcasting reproductive modes On the population genetic structure of two Antarctic gastropod molluscs. Mar Biol 158: 287-296.
Hoffman JI, Dasmahapatra KK, Amos W, Phillips CD, Gelatt TS, Bickham JW. 2009. Contrasting patterns of genetic diversity at three different genetic markers in a marine mammal metapopulation. Mol Ecol doi: 10.1111/j.1365-294X.2009.04246.x.
Innal D, Ozdemir F, Dogangil B. 2015. Length-Weight relationships of *Oxynoemacheilus theophilii* (Teleostei: Nemacheilidae) from Turkey. Intl J Fish Aquacult Sci 2: 249-250.
Jaikumar M, Ramkumar R, Gunalan, Kanagu L. 2011. Length-weight relationship of *Lambis lambis* (Mollusc: Gastropoda) from Tuticorin coastal waters, Gulf of Mannar, Southeast Coast of India. J World App Sci 14: 207-209.
Japaud A, Fauvelot C, Bouchon C. 2013. Populations genetic study of the corals *Acropora palmata* and *Acropora cervicornis* of Guadeloupe (French West Indies) in view of their preservation. Proceedings of the 66 th Gulf and Caribbean Fisheries Institute. November 4-8, 2013 Corpus Christi, Texas USA.
Jefri E, Zamani NP, Subhan B, Madduppa HH. 2015. Molecular phylogeny inferred from mitochondrial DNA of the grouper *Epinephelus* spp. in Indonesia collected from local fish market. BIODIVERSITAS 16: 254-263.
Jena SN, Srivastava A, Singh UM, Roy S, Banerjee N, Rai KM, Singh SK, Kumar V, Chaudhary LB, Roy JK, Tuli R, Sawant SV. 2011. Analysis of genetic diversity, population structure and linkage disequilibrium in elite cotton (*Gossypium* L.) germplasm in India. Crop Past Sci 62: 859-875.
Jusmaldi, Duryadi D, Affandi R, Rahardjo MF, Gustiano R. 2014. DNA barcode of ornamental fish genus *Kryptopterus* of the Mahakam River in East Kalimantan. J Iktiologi Ind 14: 191-199. [Indonesian]
Kashino J, Ueki I, Kuroda Y, Purwandani A. 2007. Ocean variability north of New Guinea Derived from TRITON Buoy Data. J Ocean 63: 545-559.
Kikutani K, Ohba H, Yamakawa H. 2002. Distribution and Gut Contents of the Green Snail *Turbo marmoratus* in Tokunoshima Island, Ryukyus (Southern Japan). J Tokyo Univ Fish 88: 47-52.
Kuroda Y. 2000. Variability of the currents off the northern coast of New Guinea. J Ocean 56: 103-116.
Lee S, Chao S. 2004. Shallow-water marine shells from Kenting National Park, Taiwan. Coll Res 17: 33-57.
Lin T, Liu L. 2008. Low Levels of Genetic Differentiation among Populations of the Coral-Inhabiting Snail *Coralliophila violacea* (Gastropoda: Coralliophilidae) in Regions of the Kuroshio and South China Sea. Zoo Stud 47: 17-24.
Luque S, Saura S, Fortin M. 2012. Landscape connectivity analysis for conservation: insights from combining new methods with ecological and genetic data. Landscape Ecol 27: 153-157.
Lynch M, Creasef TJ. 1990. The analysis of population survey data on DNA sequence variation. Mol Biol Evol 7: 377-394.
Madduppa HH, Timm J, Kochzius M. 2014. Interspecific, spatial and temporal variability of self-recruitment in Anemone fishes. PLoS ONE 9: e90648. DOI:10.1371/journal.pone.0090648
Mamedov TG, Pienaar E, Whitney SE, Termaata JR, Carvill G, Goliath R, Subramanian A, Viljoen HJ. 2008. A fundamental study of the PCR amplification of GC-rich DNA templates. Comp Biol Chem 32: 452-457.
Marquez F, Gonzalez-Josea R, Bigatti G. 2011. Combined methods to detect pollution effects on shell shape and structure in Neogastropods. Ecol Ind 11: 248-254.
Mauro A, Arculeo M, Parrinello N. 2003. Morphological and molecular tools in identifying the Mediterranean limpets *Patella caerulea*, *Patella aspera* and *Patella rustica*. J Exp Mar Biol Eco 295: 131-143.
McKinney RA, Glatt SM, McWilliams SR. 2004. Allometric length-weight relationship for benthic prey of aquatic wildlife in coastal marine habitat. Wildlife Biol 10: 4.
Moneva CSO, Torres MAJ, Demayo CG. 2012. Relative Warp and Correlation Analysis based on Distances of the Morphological shell shape patterns among freshwater gastropods (Thiaridae: Cerithimorpha). AACL BIOFLUX 5: 3.
Mulki ABR, Suryono CA, Suprijanto J. 2014. Variations in the size of blood clams (*Anadara granosa*) in the coastal waters of Genuk Sub District, Semarang. J Mar Res 3 (2): 122-131. [Indonesian]
Neel MC. 2008. Patch connectivity and genetic diversity conservation in the federally endangered and narrowly endemic plant species *Astragalus albens* (Fabaceae). Biol Cons 141: 938-955.
Nei M. 1987. Molecular Evolutionary Genetics. Columbia Univ Press, New York.
Noreen AME, Harrison PL, Van Oppen MJH. 2009. Genetic diversity and connectivity in a brooding reef coral at the limit of its distribution. Proc R Soc B 276: 3927-3935.
Olabarria C, Thurston MH. 2003. Patterns of morphological variation of the deep-sea Gastropod *Troschelia berniciensis* (King, 1846) (Buccinidae) from the Northeastern Atlantic Ocean. J Moll Stud 70: 59-66.
Palumbi SR. 2003. Population Genetics, Demographic Connectivity, and the Design of marine reserves. Eco App 13: 46-158
Prehadi, Sembiring A, Kurniasih EM, Rahmad, Arafat D, Subhan B, Madduppa HH. 2015. DNA barcoding and phylogenetic reconstruction of shark species landed in Muncar fisheries landing site in comparison with Southern Java fishing port. Biodiversitas 16: 55-61.
Quinones OEH, Michel-Morvin JE. 2006. Population structure and accompanying biota of the snail *Turbo* (*Callopoma*) *funiculosus* (Gastropoda: Turbinidae), on Socorro Island, Revillagigedo Archipelago, Mexico. Rev. Biol Trop 54: 1079-1084.
Rajagopal AS, Mookherjee HP. 1978. Contributions to the molluscan fauna of India. Pt. I. Marine molluscs of the Coromandel Coast, Palk Strait and Gulf of Mannar-Gastropoda: Archaeogastropoda. Rec Zool Surv India 12: 1-48.
Ramesh R, Ravichandran S, Rameshkumar G. 2009. Analysis of age and growth rate of *Turbo bruneus*. World J Dairy Food Sci 4: 56-64.
Rosenberg G. 2015. *Turbo sparverius* Gmelin, 1791. In: MolluscaBase (2015). Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=413433 on 2015-12-17.
Rozas JJ, Sanchez-Delbarrio C, Messequer X, Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496-2497.
Santos S, Schneider H, Sampaio I. 2003. Genetic differentiation of *Macrodon ancylodon* (Sciaenidae, Perciformes) populations in Atlantic coastal waters of South America as revealed by mtDNA analysis. Gen Mol Biol 26: 151-161.
Schefler WC. 1987. Statistics for Biology, Pharmacy, Medicine and Related Sciences. Indonesian edition. Institut Teknologi Bandung, Bandung. [Indonesian]

Schwartz MK, Luikart G, Waples RS. 2006. Genetic monitoring as a promising tools for conservation and management. Tren Ecol Evol 22: 1.

Sembiring A, Pertiwi NPD, Mahardini A, Wulandari R, Kurniasih EM, Kuncoro AW, Cahyani NKD, Anggoro AW, Ulfa M, Madduppa H, Carpenter KE, Barber PH, Mahardika GN. 2015. DNA barcoding reveals targeted fisheries for endangered sharks in Indonesia. Fish Res 164: 130-134.

Silva SE, Silva IC, Madeira C, Sallema R, Paulo OS, Paula J. 2013. Genetic and morphological variation in two littorinid gastropods: evidence for recent population expansions along the East African coast. Biol J Linn Soc 108: 494-508.

Storfer A. 1999. Gene Flow and Endangered Species Translocations: a Topic Revisited. Biol Cons 87:173-180.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. Mega 5: Molecular Evolution Genetics Analysis Version 6.0. Mol Bio Evo 30: 2725-2729.

Tan CKW. 2009. Effects of trenching on shell size and density of *Turbo bruneus* (Gastropoda: Turbinidae) and *Monodonta labio* (Gastropoda: Trochidae) at Labrador Beach, Singapore. Nat Sing 2: 421-429.

Taylor DR, Aarssen LW. 1988. An interpretation of phenotypic plasticity in *Agropyron repens* (Gramminae). Am J Bot 75: 401-413.

Turan C.1999. A Note on the examination of morphometric differentiation among fish populations: the truss system. Trop J Zool 23: 259-263.

Twindiko AFS, Wijayanti DP, Ambariyanto. 2013. Phylogenetic studies coral fish Genus Pseudochromis and Pictichromis in the Indo-Pacific Waters. Bul Oseana Marina 2: 29-37. [Indonesian]

Udo PJ. 2013. Length-weight /girth relationship and condition factor of the periwinkle *Tympanotonus fuscatus,* (Cerithidae: Gastropoda) of the Cross River, Nigeria. Int J Fish Aqua Stud 1: 26-28.

Ulfa M, Madduppa H, Carpenter KE, Barber PH, Mahardika GH. 2015. DNA barcoding reveals targeted fisheries for endangered sharks in Indonesia. Fish Res 164: 130-134.

Urra A, Oliva D, Sepulveda M. 2007. Use of a morphometric analysis to differentiate *Adelomelon ancilla* and *Odontocymbiola magellanica* (Caenogastropoda: Volutidae) of Southern Chile. Zoo Stud 46: 253-261.

Veron JEN, Devantier LM, Turak E, Green AL, Kininmonth S, Stafford-Smith M, Peterson N. 2009. Delineating the coral triangle. Gal J Cor Reef Stud 11: 91-100.

Walsh PS, Metzger DA, Higuchi R. 1991. Chelex-100 as a medium for simple extraction of DNA for PCR based typing from forensic material. Biotechniques 10: 506-513.

Weersing K, Toonen RJ. 2009. Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393: 1-12.

Williams ST. 2007. Origins and diversification of Indo-West Pacific marine fauna: evolutionary history and biogeography of turban shells (Gastropoda, Turbinidae). Biol J Linn Soc 92: 573-592.

Wolf HD, Verhagen R, Backeljau T. 2000. Large scale population structure and gene flow in the planktonic developing periwinkle, *Littorina striata*, in Macaronesia (Mollusca: Gastropoda). J Exp Mar Biol Ecol 246: 69-83.

Wyrtki K. 1961. Physical oceanography of the Southeast Asian waters. Scientific results of marine investigations of the South China Sea and Gulf of Thailand 1959-1961. NAGA Rep. 2, Scripps Inst. Oceanogr., La Jolla, CA.

Xiao M, Gordon PM, Phong A, Ha C, Chan TF, Cai D, Selvin PR, Kwok Y. 2007. Determination of haplotype for single DNA molecules: A Method for single molecules barcoding. Hum Mut 0: 1-9.

Yamaguchi M. 1993. Green snail. In: A. Wrightand L. Hill. (eds). Nearshore marine resources of the South Pacific. SPC. Inshore Fish Res WP: 1-11.

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Responses of the *Arabidopsis KNOX* and Boron transport gene mutants against the deficiency and overdose of Boron nutrient

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Abstract. *Sunaryo W, Nurhasanah. 2016. Responses of the* Arabidopsis KNOX *and Boron transport gene mutants against the deficiency and overdose of Boron nutrient. Biodiversitas 17: 218-221.* Boron is a micro element essential to the plant. Deficiency or overdose of this element result in the stunted plant growth and leads to a decline in the quantity and quality of crop yield. This research was aimed to study the growth response of single homozygous mutant *KNOX* (*stm-GK*, $knat1^{bp\text{-}9}$) and Boron transport genes (*bor1-1, nip5; 1-1*) of *Arabidopsis thaliana* compared to wild type (*Col-0*) against Boron deficiency and toxicity stress in in vitro experiment. The sterile seeds were inoculated on the ½ MS medium containing 0 mg/L, 3.1 mg/L, 6.2 mg/L, 9.3 mg/L, and 12.4 mg/L H_3BO_3. Some seedling growth variables such as root length, leaf number and plant height were characterized to observe the response of plant against the treatments. Results showed that different concentration of Boron resulted in a significant effect to seedling growth of all mutants. The loss of *BOR1* gene function as Boron transporter at *bor1-1* mutant strains and loss of *NIP5;1* gene function as Protein Channel caused severe growth pressures in conditions of media without boron (deficiency). On the other hand, *stm-GK* and $knat1^{bp\text{-}9}$ mutants showed no significant growth differences compared to the wild type (*Col-0*) indicating that the *KNOX* genes does likely not related to the function of Boron transport genes.

Keywords: Boron transport genes, *KNOX* genes, Boron deficiency and overdose
Abbreviations: *KNOX* (*KNOTTED-like homeobox*), *KNAT (KNOTTED-like from Arabidopsis thaliana*), *stm* (*Shoot meristemless*), *Col* (*Columbia*), *NIP* (*NOD26-like Intrinsic Protein*), NASC (The Nottingham *Arabidopsis* Stock Centre)

INTRODUCTION

Boron is a micro element that is essential for the plants. Some studies reported that a deficiency of this element caused a decline in crop productivity in both quality and quantity (Miwa et al. 2010), while the excess of this element in the soil would be toxic to plants. Boron plays an important role in the structure and function of plant cell walls (Goldbach and Wimmer 2007). Boron deficiency is also reported to be a major problem in the production of agricultural crops causing stunted plant growth by inhibition of root growth, widening the leaves, and plant fertility (Dell and Huang 1997). Meanwhile, the excess Boron causes plants to show symptoms of poisoning with necrosis along the periphery of the leaf, decreased concentration of chlorophyll, and lower growth and fixation of CO_2/photosynthesis (Nable et al. 1997).

Boron concentration range between deficiency and toxicity is very narrow. Although, it was realized that overcoming Boron deficiency is easier than dealing with the excess Boron by fertilization, but the application of fertilizer can result in Boron poisoning. In addition to Boron fertilization, the application of such technology is very expensive and difficult. Therefore, the understanding of mechanisms of plants regulating the process of adaptation/tolerance to the condition of deficiency or excess/toxicity of boron is very important to deepen in terms of molecular physiology and to develop strategies that can be applied in the future of agriculture, plantation and forestry.

Boron had been known to be required in the structure and function of plant cell walls (O'Neill et al. 2004), in which the Boron constituent 2 Rhamnogalacturonan II molecules, plays a role as the entry of the polysaccharide pectin through Boratediol bond in the *Arabidopsis* plant cell wall (Kobayashi et al. 1996). Molecular mechanism of boron transport had been reported involving *BOR1* gene of *Arabidopsis thaliana* which is a Boron transporter (Noguchi et al. 1997). This gene is responsible for the response of plants to change Boron concentration on the plant root surface. Under deficiency condition, BOR1 responsible for boron loading in the xylem cells (xylem loading). Meanwhile, another gene, *NIP5;1* also plays an important role as a protein channel that mediates the uptake of boric acid under Boron deficiency condition (Takano et al. 2006). The function of *BOR1* and *NIP5;1* gene is the complement each other in an efficient transport of Boron in the deficiency conditions. The other gene, *NIP6;1* (the allele of *NIP5;1*) serves to facilitate and to be a channel for boric acid and is responsible for the distribution of Boron to the leaves (Tanaka et al. 2008).

Based on the report of Sunaryo (2010), the expression of genes associated with both primary and secondary cell wall formation in *Arabidopsis* was regulated and controlled by members of the *KNOX* gene family (*KNOTTED1-like homoebox*) i.e. *STM* (*SHOOT MERISTEMLESS*) and *KNAT1* (*KNOTTED1 -like homoebox in Arabidopsis thaliana 1*). In addition, *KNAT1* and *STM* were also the regulator of process in division and differentiation of cambial cells into xylem and phloem (Sunaryo and Fischer 2010). *STM* and *KNAT1* are important regulators to promote cell differentiation through repression of xylem fiber differentiation (Liebsch et al. 2014). Through a co-expression analysis, *STM/KNAT1* has been proven to also be a regulator of genes that play a important role in the formation of primary and secondary cell walls such as *IAA27* (auxin signaling), *ATHB-8* (xylem differentiation), *NST1* and *SND1* (xylem fiber cell identity), *SND2* (differentiation of xylem vessel element), *IRX1*, *IRX3*, *IRX5*, *COBL4* (cellulose biosynthesis), *PME61* (pectin biosynthesis), *GAUT12/IRX8* (hemicellulose biosynthesis) (Sunaryo 2010). As a transcription factor, *STM/KNAT1* gene plays an important role in the process, structure, and function of plant cell walls, including the structure and function of the cell wall in which the genes control the Boron transport and functions. Therefore, it is very important to study the association of *KNOX* (*STM* and *KNAT1*) with Boron transport genes in relation to the mechanism of plant tolerance against Boron deficiency and toxicity.

This article presented the growth responses of *KNOX* mutants (*stm-GK*, $knat1^{bp\text{-}9}$) and the Boron transport mutants (*bor1-1, nip5;1-1*) in *Arabidopsis thaliana* compared to the wild type (Wild Type; *Col-0*) against the Boron deficiency and toxicity stress via in vitro experiment.

MATERIALS AND METHODS

Searching Information of *KNOX* and Boron Transport Genes

To know and deepen the initial information about *KNOX (STM and KNAT1) and Boron transport (BOR1, NIP5;1,* and *NIP6;1*) genes especially their putative functions, we explored and collected the information from the publicly available website TAIR (www.arabidopsis.org). The locus, identity, insertion site of mutants, and the putative function data were collected to discover the regeneration and mutant selection method.

Plant material and explant preparation

The wild type and mutant seeds were supplied by Dr. Urs Fischer from Molecular Genetics Laboratory, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Sweden. The experiment was conducted at the Laboratory of Biotechnology, Faculty of Agriculture, Mulawarman University, East Kalimantan, Indonesia. Seeds of single homozygous mutants of *stm-GK*, $knat1^{bp\text{-}9}$, *bor1-1, nip5; 1-1*, and Wild Type *Col-0* were sterilized with 70% alcohol for 1 minute, Clorox 25% for 20 minutes and washed with sterile water 3 times for 1 minute each.

Media preparation

The sterilized seeds were inoculated in ½ MS medium (Murishage and Skoog) and treated by 0 mg/L, 3.1 mg/L, 6.2 mg/L, 9.3 mg/L, and 12.4 mg/L H_3BO_3. Each treatment was repeated 10 times and each bottle of media was inoculated by using 5 seeds. Furthermore, the bottles were incubated in the culture room with the light of 1000-3000 lux and temperature of 22 ± 2°C for germination and growth process.

Observation and data analysis

Some seedling growth variables such as root length, number of leaves and plant height were observed to look at plant growth responses to the treatments. Observations were made approximately one month after inoculation. Data were analyzed by analysis of variance (ANOVA) and if there was a noticeable/significant effect of the treatment, the differences between mean values will be analyzed using the Least Significant Difference test (LSD) at 5% level.

RESULTS AND DISCUSSION

Results of information search showed that almost all mutant lines were produced by insertion of T-DNA both at the exon and intron, except for $knat1^{bp\text{-}9}$ produced by inserting transposon d-SPM in the intron (Table 1). In Addition, the seeds were segregated lines therefore the homozygous mutant lines were done by screening seeds/seedlings in media containing kanamycin antibiotic (Table 1). Based on Sunaryo (2010) kanamycin concentration effective to screen the *Arabidopsis* mutant seeds containing T-DNA insertion was 35 mg/L kanamycin. Meanwhile, the mutant $knat1^{bp\text{-}9}$ and the wild strain (Wild Type/WT) propagated directly in the field because the seeds were homozygous (Venglat et al. 2002). The background (ecotype) of all strains was the same, *Columbia* (*Col-0*). The use of the same ecotype was to ensure that the difference responses in the experiment will not be caused by different background.

The preliminary information from previous studies compiled from TAIR website stated that BOR1 is a protein responsible for Boron transport in *Arabidopsis* plants exposed by Boron deficiency stress and NIP5;1 is a protein channel for borate acid transportation located in the outer membrane of root cells (Table 2). This protein serves to uptake of Boron in boron deficiency stress condition. KNAT1 and STM, as previously have also been researched by Sunaryo (2010), are KNOX proteins of homeobox gene family regulating the division and differentiation of meristem cells both in apical (buds) and lateral (cambium) meristems including the differentiation of secondary cell wall (Table 2). The same indication had been previously reported by Groover et al (2006) showing that the poplar *KNOX* gene *ARBORKNOX1* (*ARK1*) and *ARBORKNOX2* (*ARK2*), which are close homologues of the *Arabidopsis STM* and *KNAT1* respectively, involved in the regulation of differentiation in vascular development.

The growth of the *Arabidopsis* plant mutants under conditions of different Boron concentrations is presented at Tables 3, 4, and 5. Boron gave a significant effect on seedling growth of all tested strains of *Arabidopsis*. From the research it was clearly appeared that different concentrations of Boron resulted in the different growth

Table 1. Search results of the identity and structure of wild type and mutant plants used in the study

NASC code	Locus in genome	Gene name	No. SALK lines	Insertion site	Regeneration method	Background/ ecotype	Mutant name
N637958	At4g08150	*BP/KNAT1*	Not SALK Lines	Intron1 (transposon d-spm)	Homozygous	Col-0	*knat1^{bp-9}*
N409575	At1g62360	*STM*	Not SALK Lines	Intron1 (T-DNA)	Segregation	Col-0	*stm-GK*
N522077	At2g47160	*BOR1*	SALK_022077	Exon (T-DNA)	Segregation	Col-0	*bor1*
N656927	At4g10380	*NIP5;1*	SALK_122287C	Intron1 (T-DNA)	Segregation	Col-0	*nip5;1-1*
Colombia	-	-	-	-	Homozygous	Col-0	*Wild Type*

Table 2. The function of KNOX and transport boron genes in the *Arabidopsis* plant from literature search

Gene name	Putative function	Reference
BP/KNAT1	DNA binding, DNA-dependent, cell fate commitment, cell fate specification, determination of bilateral symmetry, floral whorl development, flower morphogenesis, meristem initiation, negative regulation of biological process, nucleus, organ development, organ morphogenesis, pattern specification process, polarity specification of adaxial/abaxial axis, protein binding, regulation of transcription, regulation of transcription, DNA-dependent, sequence-specific DNA binding, sequence-specific DNA binding transcription factor activity, xylem and phloem pattern formation.	(TAIR 2015)
STM	Class I knotted-like homeodomain protein that is required for shoot apical meristem (SAM) formation during embryogenesis. Protein binding, regulation of flower development, regulation of meristem structural organization, regulation of transcription, regulation of transcription, DNA-dependent, sequence-specific DNA binding, sequence-specific DNA binding transcription factor activity, specification of floral organ identity, stem cell maintenance, xylem and phloem pattern formation.	(TAIR 2015)
BOR1	Boron transporter. Protein accumulates in shoots and roots under conditions of boron deficiency and is degraded within several hours of restoring boron supply. Localized to the plasma membrane under B limitation, and to the cytoplasm after B application before degradation. Under high-boron is transported to the vacuole for degradation. Borate efflux transmembrane transporter activity, borate transmembrane transporter activity, borate transport, and response to boron-containing substance.	(TAIR 2015)
NIP5;1-1	Boric acid channel. Essential for efficient Boron uptake and plant development under Boron limitation. Localized preferentially in outer membrane domains of root cells. Borate transmembrane transporter activity, borate transport, borate uptake transmembrane transporter activity, cellular response to boron-containing substance levels, response to boron-containing substance, and water channel activity.	(TAIR 2015)

and responses (Tables 3, 4, and 5). On the condition of absence of Boron, plants were not able to grow perfectly even tended to be stunted indicated by the small number of leaves, short plant height and root length. In normal concentration of Boron, plants grew well, even when the Boron concentration was increased a half time, the plants could achieve the highest growth. The application of Boron at the highest level (12.4 mg/L) led to a decrease in plant growth (Tables 3, 4, and 5). It indicates that in the deficiency conditions (without Boron), plants were not able to grow well in all mutants or wild strains tested. Boron deficiency results in the cessation of root elongation, reduced leaf expansion and the loss of fertility (Loomis and Durst 1992; Marschner 1995; Dell and Huang 1997) and it most likely influences the cell elongation rather than cell division (Dell and Huang 1997).

In contrast to the condition, an overdose of Boron concentration caused problems in plant growth. Among all mutant strains tested, the deficiency conditions (without Boron), mutant strains *bor1;1* experienced a severe growth pressure, followed by *nip5;1-1*. While the *stm-GK* mutant strains and *knat1^{bp-9}* looked no different response compared to the wild type (*Col-0*) (Tables 1, 2, and 3). This indicates that the loss of gene function in *bor1;1* as Boron transporter gene and *nip5;1-1* as Protein Chanel for Borate ions on Boron deficiency conditions, has led to the loss of plant ability to tolerate the Boron deficiency. On the other hand, overdose of Boron has no effect on plant growth. The excess Boron causes a toxicity to plants shown by necrosis of marginal regions of leaves, decreased chlorophyll concentrations, reduced growth and decreased CO2 fixation (Nable et al. 1997).

The fact that there were no differences between the mutant strains of *knat1^{bp9}* and *stm-GK* with and wild type indicates that the *KNOX* genes is probably not related to the function of genes associated with *Boron transport*. However it is still too early to conclude, because testing at the level of the double mutant and gene expression test will prove this early preliminary results.

Table 3. Effect of Boron deficiency and overdose to the leave number of Boron transport (*bor1; 1* and *nip5;1-1*) and *KNOX* (*stm-GK* and *knat1*$^{bp-9}$) mutant strains of *Arabidopsis thaliana*

Boron concentration	Mutant strains				
	Col-0	***stm-GK***	***knat1*$^{bp-9}$**	***bor1; 1***	***nip5;1-1***
B0 (0 mg/L) (deficiency)	5.00 b	4.67 b	5.00 b	4.00 a	4.67 a
B1 (3.1 mg/L)	6.67 b	6.33 b	6.67 b	4.67 a	5.00 ab
B2 (6.2 mg/L) (normal)	7.00 b	6.67 b	7.33 b	5.67 a	5.67 a
B3 (9.3 mg/L)	8.33 c	7.67 b	7.67 b	6.33 a	7.00 ab
B4 (12.4 mg/L) (overdose)	6.67ab	7.33 b	7.33 b	6.00 a	6.67 ab

Note: The mean values followed by the same letter in the same row are not significantly different in LSD 5% test.

Table 4. Effect of Boron deficiency and overdose to the plant height (cm) of Boron transport (*bor1; 1* and *nip5;1-1*) and *KNOX* (*stm-GK* and *knat1*$^{bp-9}$) mutant strains of *Arabidopsis thaliana*

Boron concentration	Mutant strains				
	Col-0	***stm-GK***	***knat1*$^{bp-9}$**	***bor1; 1***	***nip5;1-1***
B0 (0 mg/L) (deficiency)	0.60 b	0.57 b	0.47 ab	0.37 a	0.50 ab
B1 (3.1 mg/L)	3.13 b	2.97 b	3.07 b	2.10 a	3.23 b
B2 (6.2 mg/L) (normal)	3.53 a	3.60 a	3.47 a	3.43 a	3.80 a
B3 (9.3 mg/L)	4.63 b	4.43 b	4.30 b	3.53 a	4.07 ab
B4 (12.4 mg/L) (overdose)	3.23 b	3.00 b	2.73 ab	2.17 a	2.20 a

Note: The mean values followed by the same letter in the same row are not significantly different in LSD 5% test.

Table 5. Effect of Boron deficiency and overdose to the root length (cm) of Boron transport (*bor1; 1* and *nip5;1-1*) and *KNOX* (*stm-GK* and *knat1*$^{bp-9}$) mutant strains of *Arabidopsis thaliana*

Boron concentration	Mutant strains				
	Col-0	***stm-GK***	***knat1*$^{bp-9}$**	***bor1; 1***	***nip5;1-1***
B0 (0 mg/L) (deficiency)	0.53ab	0.53 ab	0.63 b	0.43 a	0.53 ab
B1 (3.1 mg/L)	3.93 b	3.80 b	3.50 ab	2.73 a	3.30 ab
B2 (6.2 mg/L) (normal)	4.23 a	4.33 a	4.37 a	3.93 a	3.90 a
B3 (9.3 mg/L)	4.70 b	4.90 b	4.93 b	4.40 ab	3.60 a
B4 (12.4 mg/L) (overdose)	4.47 b	4.53 b	4.87 b	3.57 ab	3.13 a

Note: The mean values followed by the same letter in the same row are not significantly different in LSD 5% test.

To conclude, Boron concentrations gave significant effect on the growth of seedlings of all mutants observed. The loss of gene function of BOR 1 as Boron transporter and the loss of gene function of NIP5;1 as protein channel cause severe growth pressures in the absence of Boron conditions (deficiency). On the other hand, the *stm-GK* and *knat1*$^{bp-9}$ mutants look no different from compared to the a wild strain (*Col-0*) in the same conditions indicating that the *KNOX* genes are likely not related to the function of Boron transport genes. However, further experiment at molecular level (gene expression) is required to ascertain the fact of this result.

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REFERENCES

Dell B, Huang L. 1997. Physiological response of plants to low Boron. Plant Soil 193: 103-120.

Goldbach HE, Wimmer MA. 2007. Boron in plants and animals: Is there a role beyond cell-wall structure? J Pl Nutr Soil Sci 170: 39-48.

Groover AT, Mansfield SD, DiFazio SP, Dupper G, Fontana JR, Millar R, Wang Y. 2006. The *Populus* homeobox gene *ARBORKNOX1* reveals overlapping mechanisms regulating the shoot apical meristem and the vascular cambium. Plant Molecular Biology 61: 917-932.

Kobayashi M. 1996. Two chains of rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Pl Physiol 110: 1017-1020.

Liebsch D, Sunaryo W, Holmlund M, Norberg M, Zhang J, Hall HC, Helizon H, Jin X, Helariutta Y, Nilsson O, Polle A, Fischer U. 2014. Class I KNOX transcription factors promote differentiation of cambial derivatives into xylem fibers in the *Arabidopsis* hypocotyl. Development 141 (22): 4311-4319.

Loomis WD, Durst RW. 1992. Chemistry and biology of boron. Biofactors 3: 229-239.

Marschner H. 1995. Mineral Nutritin of Higher Plants, 2nd ed. Academic Press, San Diego.

Miwa K, Tanaka M, Kamiya T, Fujiwara T. 2010. Molecular mechanisms of Boron transport in plants: Involvement of *Arabidopsis NIP5;1* and *NIP6;1*. In: Thomas P, Jahn P, Gerd P, Bienert PG. MIPs and their Role in the Exchange of Metalloids. Landes Bioscience and Springer Science+Business Media, Berlin.

Nable RO, Banuelos GS, Paull JG. 1997. Boron toxicity. Plant Soil 193: 181-198.

Noguchi K, Yasumori M, Imai T. 1997. *Bor1-1*, an *Arabidopsis thaliana* mutant that requires a high level of boron. Pl Physiol 115: 901-906.

O'Neill MA, Ishii T, Albersheim P, Darvill AG. 2004. Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide. Ann Rev Pl Biol 55: 109-139.

Sunaryo W, Fischer U. 2010. In silico expression analysis of the *Arabidopsis KNAT1* gene and its homologs in poplar. In: Kharazipour AR, Schöpper C, Müller C, Euring M (eds.). Review of Forests, Wood Products and Wood Biotechnology of Iran and Germany - Part III. Universitätsdrucke Göttingen, Göttingen, Germany.

Sunaryo W. 2010. Secondary growth in the *Arabidopsis* hypocotyl. [Ph.D. Dissertation]. Cuvillier Verlag, Göttingen, Germany.

TAIR. 2015. The Arabidopsis Information Resource (TAIR). ABRC, Ohio State University, Columbus OH. www.arabidopsis.org [December 1, 2015]

Takano J, Wada M, Ludewig U. 2006. The *Arabidopsis* major intrinsic protein *NIP5;1* is essential for efficient boron uptake and plant development under Boron limitation. Plant Cell 2006 18: 1498-1509.

Tanaka M, Wallace IS, Takano J. 2008. *NIP6;1* is a boric acid channel for preferential transport of Boron to growing shoot tissues in *Arabidopsis*. Plant Cell 20: 2860-2875.

Venglat SP, Dumonceaux T, Rozwadowski K, Parnell L, Babic V, Keller W, Martienssen R, Selvaraj G, Datla R. 2002. The homeobox gene *BREVIPEDICELLUS is* a key regulator of inflorescence architecture in *Arabidopsis.* Proc Natl Acad Sci USA 99: 74730-74735.

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Stand damage due to mono-cable winch and bulldozer yarding in a selectively logged tropical forest

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Abstract. *Ruslim Y, Sihombing R, Liah Y. 2016. Stand damage due to mono-cable winch and bulldozer yarding in a selectively logged tropical forest. Biodiversitas 17: 222-228.* Timber yarding with bulldozers has substantial unwanted environmental impacts and degrades the quality residual stands. We contrasted the impacts of bulldozer yarding with yarding with a sled-mounted mono-cable winches equipped with 20 and 26 horsepower engines and 100 m of wire in a natural forest timber concession in East Kalimantan, Indonesia. We compared the two systems on the basis of productivity and stand damage in forests that were selectively logged at the same intensity on slopes ≤ 40% for bulldozer and for mono-cable yarding on slopes ≤55%. On slopes ≤40%, bulldozers yarded an average of 10.3 m^3 hm^{-1} $hour^{-1}$ to roadside log landings whereas mono-cable winch productivity was 7.8 m^3 hm^{-1} $hour^{-1.}$ In these areas, mono-cable winching caused 1.2%, 2.0%, 0.6%, and 27.0% less damage to seedlings, saplings, poles, and trees than bulldozer yarding. Our study demonstrates that conventional methods such as bulldozer skidding created damage at seedling, sapling, pole and tree levels of vegetation around 15.3%, 9.9%, 10.8% and 34.5% at slope ≤40%. Winch-yarding is rare in tropical forestry, but the low cost of the mono-cable system we tested ($4,000), its productivity of 20.9 m^3 day^{-1} for yarding distances that averaged 70.5 m, employment opportunities, and reduced environmental impacts indicate that such systems could make a major contribution to reduced-impact logging and promote local development.

Keywords: Kalimantan, log yarding, reduced-impact logging, selective logging, tropical forestry

INTRODUCTION

Sustainable forest management is a global objective for ensuring secure timber supply, protection of forest environmental services (e.g., flood buffering, carbon sequestration), protection of forest wildlife habitats, and protection of safe dwelling places of up to 1.6 billion forest-dependent people (Canadell and Raupach 2008; Chao 2012; FAO 2010; Putz et al. 2012). How to achieve true sustainability in forest management, however, or even how to define it, remains unclear (Sasaki and Putz 2009). What is clear is that most forests, and especially tropical forests, have not had the careful, long-term management required to protect their functional integrity (Edwards et al. 2014). Excessive residual damage to remaining forest stands because of poor planning of timber harvest processes and related infrastructure, and the use of unnecessarily large timber extraction equipment are important aspects of unsustainable forest management (Pinard and Putz 1996; Sist et al. 1998; Iskandar et al. 2006). Reducing residual damage, without incurring major additional costs, would benefit forest management, and increase financial returns from natural forest concessions. This, in turn, could reduce the likelihood that selectively logged natural forests are considered of little economic value, and would become more valuable when converted to mono-cultural crop plantations, such as *Acacia* spp. for pulp and paper production or *Elaeis guinensis* for palm oil.

The primary log skidding equipment used in logging exploitation activities in tropical forests is the bulldozer (Fredericksen and Pariona 2002; Pinard et al. 2000a). The use of bulldozers as timber removal equipment is thought to minimize the environmental impacts (soil compaction and canopy protection) in areas with moderate to heavily contoured topography. Especially following second or third rotation logging, however, remaining forest stands are often in poor ecological condition, because of skidding-related damage (Meijaard et al. 2005). Reducing damage requires the use of alternative removal equipment that match (or exceed) a bulldozer's capacity, but with reduced residual impacts. This includes helicopter yarding, an expensive method that also opens up forests not normally accessible to ground-based equipment (Putz et al. 2001), rubber-tired mini-skidders (Spinelli et al. 2012), and motor-winches (Escobar and García 2013).

We here focus on the use of motor-winches in reduced impact logging in Indonesian Borneo. These winches are thought to prevent residual damage to forests, because such mono-cable engines are stationed at a particular central point towards which logs are pulled using a sling or a cable. They thus require fewer skid-roads and tracks. A preliminary study by Ruslim (2011) showed that such a system using a 20 horsepower (HP) engine was able to remove 8 tons of logs per day with less damage to top soil and residual stands compared to bulldozer yarding. It also resulted in reduced environmental pollution due to reduced fuel consumption, in addition to the fact that it was less costly in operation and maintenance due to the involvement of the local community (Ruslim 2011). In this research study, we compared the performance and impacts of two mono-cable winches with 20 HP and 26 HP capacity and also compared with bulldozer, with regard to production capacity (in tons of timber extracted) and impact on residual tree stands after timber removal operation.

MATERIALS AND METHODS

Study area

The study was conducted in two forest concessions at production forest working area, i.e. Ratah Timber Company (114°55' E – 115°30' E;0°2'S – 0°15' N) in West Kutai district of East Kalimantan Province Indonesia and Belayan River Timber Company (115°30'21.60 E – 116°11'38.34" E; 0°32'35.16" N – 0°55' 35.16" N) in Kutai Kertanegara district, West Kutai District of East Kalimantan Province of Indonesia (Figure 1). The total area of Ratah Timber Company is 93,425 ha, which consists of a permanent production forest is 73,420 ha and limited production forest is 20,005 ha. The total area of Belayan River Timber Company is 97,500 ha, which consist of limited production forest. Based on the Schmidt-Ferguson's climate classification, the both forest concession holder belongs to type A climate where the average temperature varies from 22-27°C and average annual rainfall varies at 2,500-4,000 mm. Soil types of the study area include alluvial, latosols, podzolic, litosol, and regosol (Liah 2012). We implemented our study on log skidding using mono-cable winches at both company and bulldozer system only at Ratah Timber company.

Sample plots

To determine the residual stand damage as the result of skidding with mono-cable winch, we built three research plots with average slope below 40% and another three plots with average slope above 40%. We also built three plots with slope below ≤ 40% with similar extent (1 ha each) to determine the damage caused by felling and skidding with bulldozer.

Data collection

We analyzed the vegetation samples collected from both locations and measured forest degradation, which include assessment of forest damage and the depth of soil excavation as the result of mono-cable winch skidding and bulldozer skidding. Pre-logging forest inventory was conducted on seedling, sapling, poles and trees at the sample plots. The measured variables include tree species, diameter and number of trees. After the felling and skidding took place, we did another forest inventory on residual stands of all types of trees with a diameter ≥ 20 cm which were damaged by felling and skidding. The damages of residual stands were noted: (i) broken stems, (ii) broken crowns, (iii) bark scratched, (iv) fallen trees. Within the plots we counted the number of tree with diameter of 20 cm and above before and after harvesting, calculating residual impact.

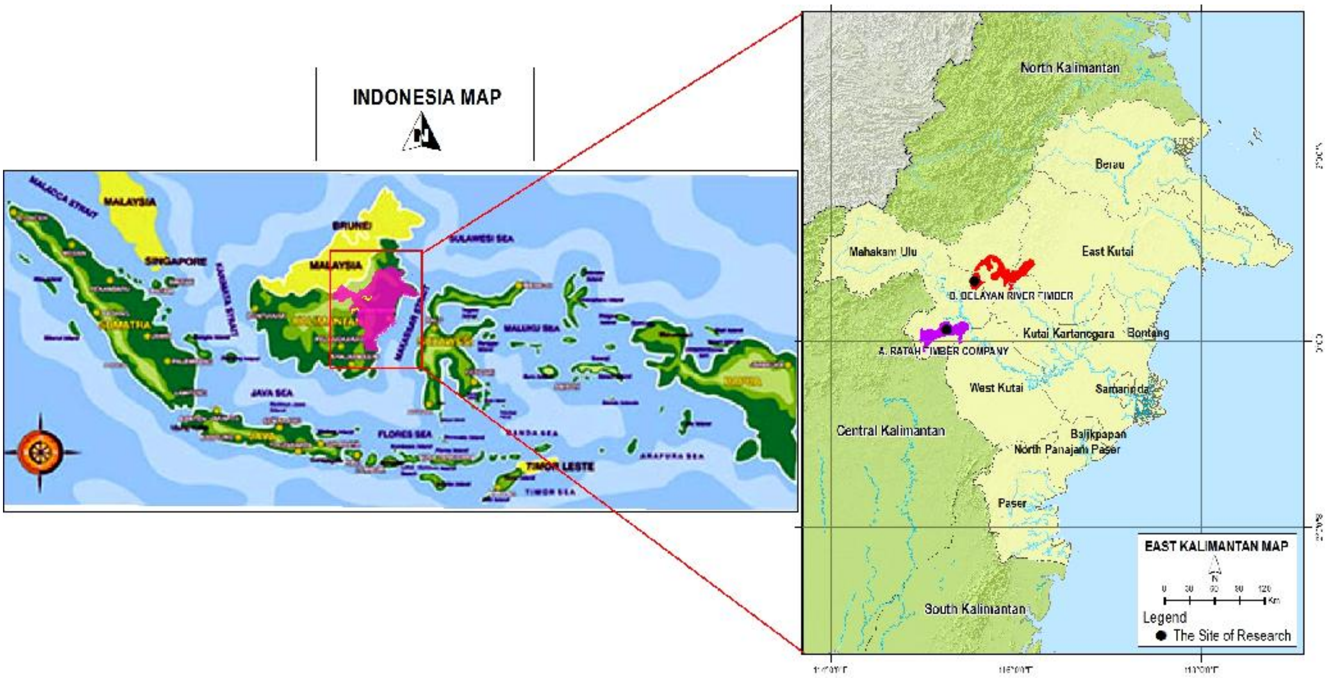

Figure 1. Study area (A) Ratah Timber Company (purple area), West Kutai and (B) Belayan River Timber Company (red area) in Kutai Kertanegara and West Kutai district of East Kalimantan, Indonesia

The time study was typical skidding operations begins with the identification of a set of functional elements comprising of the works cycle of the mono-cable winch machine and bulldozer skidding being evaluated. In performing time study observers watch of these elements as the machine works and note the duration of the event and any other factors that influence the performance of the machine. Mono-cable winch winches logs from stump to the skid trail and moves a maximum of three times to the secondary or main road. Bulldozer skid the logs from stump directly to the log landing. Data were collected continuously throughout each cycle from start to finish.

We estimated labor input using a continuous method in which we measured time allocated to (i) equipment preparation and engine start-up; (ii) access path clearance; (iii) machine fixation on trees or tree stands; (iv) sling directed to logs to be removed; (v) log leading point sharpening and sling fastened to logs using hooks; (vi) log stacking, and (vii) sling winching (sling adjustment, release and winding). These field data were supported by additional information, including trees maps from the company, and fuel consumption.

We estimated the skidding productivity using the Brown (1958) formula:

$$P = V/W$$

With P = removal productivity (m^3 $hour^{-1}$), V = volume removed per trip (m^3 $trip^{-1}$), and W = operating time (hours), i.e, the time needed for preparation, sling carrying to the log, hooking the sling onto the log, yarding, and sling release.

We calculated the volume of logs removed with the following formula:

$$V = \frac{1}{4} \pi d \ L$$

With V = Volume of logs removed (m^3); d = mean diameter (cm); and L = length of stem (cm).

RESULTS AND DISCUSSION

Forest stands before and after harvesting

Forest harvesting using the mono-cable winch in the area with slope less than 40% and area with slope more than 40% causes damage on the forest vegetation in all living phase, i.e. seedling, sapling, pole and tree. The number of damaged vegetation for seedling, sapling, pole and tree in the area with slope ≤ 40% due to felling operations are 21.0%, 20.6%, 18.9% and 24.5%, respectively. Due to skidding, the seedling, sapling, pole and tree damage were lost 14.1%, 7.9%, 1.2% and 7.6%, respectively. Species-wise, *Shorea johorensis* in all phases (e.g. seedling, sapling, pole and tree) is the one that was mostly damaged during felling and skidding. The only exception was for the poles during skidding, which left the most damage to *Shorea assamica*. The number of damaged vegetation for seedling, sapling, pole and tree in the area with slope more than 40% due to felling operations are 33.6%, 31.4%, 27.4% and 26.0% respectively. Due to skidding, the seedling, sapling, pole and tree were lost 14.1%, 1.9%, 1.7% and 8.0% respectively. Species-wise, *Shorea johorensis* and *Dipterocarpus* spp. in all phases (e.g. seedling, sapling, pole and tree) is the one that was mostly damaged during felling and skidding (Table 1).

Forest harvesting using the bulldozer in the area with slope less than 40 causes damage on the forest vegetation in all living phase, i.e. seedling, sapling, pole and tree. The number of damaged vegetation for seedling, sapling, pole and tree in the area with slope ≤ 40% due to felling operations are 9.3%, 7.2%, 8.6% and 10.9%, respectively. Due to skidding, the seedling, sapling, pole and tree were lost 15.3%, 9.9%, 10.8% and 34.5%, respectively. The tree species most damaged by felling and skidding at level poles and trees are *Dipterocarpus* spp and *Shorea johorensis* (Table 2).

Skidding labor input, timber volume, skidding distance and skidding productivity

The use of the larger (26 HP) engine reduced skidding time and increased log removal volume, and thus increased productivity. The skidding productivity using bulldozer is 10.3 m^3 hm^{-1} $hour^{-1}$, greater than mono-cable winch is in average 7.8 m^3 hm^{-1} $hour^{-1}$ (Table 3). The mono-cable winch system is more time consuming, more labor intensive and less productivity under the RIL method. This is reflected by results of the study that shows the reduced degree of destruction induced by removal as per the RIL procedure and efforts of releasing and un-releasing hooks where stem destruction induced by frictions became avoidable. Consequences of releasing and un-releasing hooks on site, however, was slower work performed by the mono-cable winch operator as he had to be more careful when doing his removal work, resulting in the reduced overall removal performance. On the other hand, stem destruction induced by removal was very small due to the slow friction with the stems.

Discussion

The analysis of collected data showed that by using a mono-cable winch with a winching process with distance between 30–100 meters , will be able to avoid damage to remaining trees. The result showed that around 8% of trees were destroyed by skidding operation with mono-cable winch and compared to bulldozer the damage to remaining stands reached 35% (Figures 2 and 3).

The skidding machines, winching distance are the important factors to influence on amount of ground based skidding to the residual stand in the forests. Directional felling is important techniques to reduce skidding damages to residual stand. Tavankar et al. (2012) reported with directional felling, trees were felled to reduce damage to the residual stand, to facilitate chocker hookups in preparation for skidding and to without creating unnecessary large forest disturbance. The main benefit of winching with mono-cable winch system was to reduced skidding damage around 26.9% (Figure 2a) compared to a bulldozer system (Figure 2b).

Table 1. Felling and skidding damage after logging with a mono-cable winch on slopes ≤ 40% and≥ 40% at Belayan River Company at ha^{-3}

	Felling damage		Skidding damage		Residual	Total
	(n)	(%)	(n)	(%)	stands (n)	(n)
			Slope ≤ 40%			
Seedling	73	21.0	49	14.1	225	347
Sapling	34	20.6	13	7.9	118	165
Pole	49	18.9	3	1.2	207	259
Tree	13	24.5	4	7.6	36	53
			Slope ≥ 40%			
Seedling	93	33.6	39	14.1	145	277
Sapling	49	31.4	3	1.9	104	156
Pole	65	27.7	4	1.7	166	235
Tree	13	26.0	4	8.0	33	50

Table 2. Felling and skidding damage after logging using bulldozer with slope ≤ 40% at Ratah Timber Company at ha^{-3}

	Felling damage		Skidding damage		Residual stands	Total
	(n)	(%)	(n)	(%)	(n)	(n)
Seedling	29	9.3	48	15.3	236	313
Sapling	8	7.2	11	9.9	92	111
Pole	8	8.6	10	10.8	75	93
Tree	6	10.9	19	34.6	20	55

Table 3. Average productivity of logs skidding using 20 HP and 26 HP engines with mono-cable winch and bulldozer on an inclination ≤ 40%

Skidding system	Operating time (hours)	Volume (m³)	Skidding distance (m)	Productivity (m³ hm^{-1}hour^{-1})
Mono-cable winch				
20 HP Engine	0.8	8.3	69	7.0
26 HP Engine	0.8	8.9	72	8.5
Bulldozer	1.2	7.6	153	10.3

The proportion of trees injured because of felling activity were bigger at Belayan River Company, it is dependent on height of the tree, the size of its crown and the topography. Mono-cable winch system mostly damaged *Shorea johorensis*, followed by *Shorea assamica*, *Shorea pinanga* and *Dipterocarpus* spp. respectively, while bulldozer system mostly damaged *Shorea laevis*, followed *Dipterocarpus* spp. Skidding with bulldozer has considerable impact on biodiversity conservation, forest structure and species composition. In average with mono-cable winch system were relatively small destruction towards forest floor induced by logs skidding as top soil is not even stripped down to 11 cm and 1 m width of skidding road. Another major impact of ground base skidding with bulldozer is removal of the top soil, very high destruction of forest soil during skidding, as soil is stripped down more than 30 cm and more than 4 m width of skidding road. Ruslim (2011) reported the application of the mono-cable winch system in reduced impact logging is an effort to reduce economical and environment damages when compared to conventional system of ground based skidding with bulldozer system. The mono-cable winch system was most efficient (operational cost) and reduced the soil damage by as much as 8% ha^{-1}.

Removal activities using a bulldozer requires sufficiently high cost per hour of operation due to its 8-hour work fuel consumption requirement, which can reach 250 L, in addition to high cost of parts replacement (Ruslim 2011). An experienced expert would require at least one full day to install the equipment depending on the site condition. It is all different when being compared to operating costs of a mono-cable winch with the lowest fuel consumption (5 L days^{-1}) for an eight hour operation resulting in a positive impact on global warming due to the reduced carbon emission induced by low fuel consumption. Bulldozer requires higher operating costs. This is because it needs more diesel fuel, i.e. in addition to more spare parts; it requires 250 liters for 8 hours of work. Healey et al (2000) also stated that the implementation of RIL will reduce carbon emissions and reduce other losses from harvesting activities.

Figure 2. A. The view of stand and soil damage after skidding with mono-cable winch, and B. with bulldozer

Figure 3. A. Small destruction towards forest floor induced by logs skidding as top soil with mono-cable winch, and B. very high destruction with bulldozer

This was backed up by previous research (Ruslim 2011), where, when viewed from the environmental, economic, engineering and even social aspects, the use of the mono-cable winch is an alternative that can be employed in the removal operation and is eligible to be used by the work area of forest timber products utilization business licensed concession holder (Table 3). Putz et al (2000) state lack of governmental incentives to change logging practices for not adopting RIL methods in the field.

Limbang Ganeca company has been using a bulldozer resulting in uncovered land in a conventional way 16.3% ha^{-1} (Ruslim et al. 2000). The application of RIL in Sabah, Malaysia has been able to reduce destruction of the remaining tree stands from 50% down to 28% when compared with conventional logging where the soil destruction has been able to reduce from 13% to 9% (Pinard et al. 2000b). Sist et al. (1998) state that using the RIL technique in Berau (East Kalimant**a**n) has been able to reduce destruction induced by logging down to 50% when compared with the conventional system. A study at Narkata Rimba Company (East Kalimantan) mentions that the degree of remaining tree stands induced by conventional harvesting system is 28–45% (Elias 2002). John et al. (1996) also state that a well-planned RIL application in the Amazon, managed to reduce destruction of the tree stands around 25–33%. The damage level of residual stands caused by tree cuttings with the selective cutting system was influenced by cutting intensity, forest harvesting techniques, and forest management types. Bertault and Sist (1997) reported that conventional logging by 87 m^3 ha^{-1} had damaged trees with a diameter > 10 cm as much as 40%, whereas the reduced-impact logging (RIL) techniques had caused damages of only 30.5%. Sist et al. (1998) reported that the cutting intensity of 8 trees ha^{-1} or less using the

Table 3. Comparison between environmental, economic, engineering and social aspects between a mono-cable winch and a bulldozer (Ruslim 2011)

Aspect	Variable	Skidding equipment	
		Mono-cable winch	**Bulldozer D7G**
Environment	Destruction to forest floor	Relatively small destruction towards forest floor induced by logs skidding as top soil is not even stripped down to 15 cm and 1 m width of skidding road.	Very high destruction of forest soil during skidding, as soil is stripped down more than 1 m and more than 4 m width of skidding road
	Soil erosion	Relatively small soil erosion	Very high soil erosion
Economical	Investment cost	Purchase price US$4,000 ,- unit^{-1} US$9.5 m^{3}	Purchase price . US$184,500 unit^{-1} US$16.5
	Skidding cost including felling	Skidding cost is 42% cheaper than that of bulldozer	
	Fuel consumption	Fuel consumption is 0.625 L hour^{-1}	Fuel consumption is 30 L hour^{--1}
	Productivity	Skidding capacity is on average 5 logs a day^{-1} with 100 m skidding distance	Skidding capacity is on average 10 logs a day^{-1} with 300 m skidding distance
Technical	Topography	Can be used on slopes up to 60%	Can be used on slopes up to 40%
	Cable length	Can skid logs with winching distance 100 m	Can skid with winching distance 32 m
	Skidding capacity	8-12 ton	15 ton
	Spare parts	Cheap, easy to order in Samarinda	Takes a long time to order (import)
Community impact	Work force (Chainsaw operator and mono-cable/bulldozer operator)	More employment from local people (5 people unit^{-1})	Commonly the employment is from Java and local people (4 people unit^{-1})

RIL techniques resulted in tree damages to 25%, while the conventional logging technique had caused damages to 50%. TNC (2010) reduced-impact logging method can directly decrease emissions by about 30-50% per unit of wood extracted. Furthermore, the requirements for special management of high conservation value forest HCVFs and other conservation zones provide greater carbon storage in those areas. Putz et al. (2008) stated that, the carbon lost due to harvesting through the conventional system using a bulldozer in Sabah Malaysia within a period of 30 years was 108 tons ha^{-1}, while the carbon lost using a bulldozer with RIL system was only 78 tons ha^{-1}. When compared to the conventional logging system, carbon stocks using RIL system will store more carbon reserves of 30 tons ha^{-1}. Bulldozer operation in logs removal will always bring negative impacts for the environment and forest ecosystems, such as the forest floor and canopy destruction. The application RIL is expected to reduce the negative impacts of logging operation using bulldozers in land clearing and forest canopy. The application of RIL method using bulldozers through intensive supervision produces a removal trail of around 5 m. Muhdi (2008) states that losses inflicted by removal using bulldozers results in destruction towards forest vegetation and soil physical condition, that is, soil compaction that will, in turn, destroy the soil structure. Efforts to reduce soil uncovering have been conducted by sharpening the log stems/edges (round shaped) to enable them to get through the standing trees during removal operations. This, in turn, will give positive impacts as the uncovered line using the mono-cable winch is much smaller than that using a bulldozer (sized just a log diameter wide). Expansion of the removal access road only occurs at the final stage located on the primary logging road, crossing or branching roads where all logs are stacked, up to five meters (Ruslim 2011). Using innovative equipment such as, mono-cable winch systems, that slides logs along the forest floor with long cables, reducing the damage to the soil and residual stands (TNC 2010).

In conclusion, Increase of the mono-cable winch engine capacity from 20 HP to 26 HP resulted in increasing of logs skidding productivity around 20.3%. Relatively small damages using mono-cable winch on forest floors induced by logs skidding on top soil and injured with bark scratched intensity for residual stands. Simple innovations on the utilization of used and waste material into bulldozer maybe applied as skidding machine. Uses of this technology are cost efficient, locally made and have environmental benefits. Future effective use of mono-cable winches implements cost effective ways for RIL. The use of timber products will only increase so the development of more effective ways for RIL is paramount to continued protection of diversity in tropical rain forests. With improvements from the mono-cable winch, improving productivity while reducing damage to the logging area, we have started on a better path to finding an amicable solution for conservationists and the needs of an ever growing population.

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REFERENCES

Bertault JG, Sist P. 1997. An experimental comparison of different harvesting intensities with reduced-impact and conventional logging in East Kalimantan, Indonesia. For Ecol Manag 94: 209-218.
Brown NC. 1958. Logging the principles and timber harvesting in the United States and Canada. John Wiley & Sons. Inc. New Work.
Canadell JG, Raupach MR. 2008. Managing forests for climate change mitigation. Science 320: 1456-1457.
Chao S. 2012. Forest people: Numbers across the world. Forest Peoples Programme, Moreton-in-Marsh, UK.
Edwards DP, Tobias JA, Sheil D, Meijaard E, Laurance WF. 2014. Maintaining ecosystem function and services in logged tropical forests. Trends Ecol Evol 29: 511-520.
Elias. 2002. Book I Reduced Impact Logging. Bogor. IPB Press.
Escobar FV, García GAR. 2013. Small and simple technology cable system for logging, Medellín, Colombia**.**
FAO. 2010. Global forest resources assessment 2010. Progress towards sustainable forest management. FAO Forest Paper 163. Food and Agricultural Organization of the United Nations, Rome, Italy.
Fredericksen TS, Pariona W. 2002. Effect of skidder disturbance on commercial tree regeneration in logging gaps in a Bolivian tropical forest. For Ecol Manag 171: 223-230.
Healey JR, Price C, Tay J. 2000. The cost of carbon retention by reduced impact logging. For Ecol Manag 139, 237-255.
Iskandar H, Snook LK, Toma T, MacDicken KG, Kanninen M. 2006. A comparison of damage due to logging under different forms of resource access in East Kalimantan, Indonesia. For Ecol Manag 237: 83-93.
John JS, Barreto P, Uhl C. 1996. Logging damage during planned and unplanned logging operations in the eastern Amazone. For Ecol Manag 89: 59-77.
Liah Y. 2012. Comparison of Impact Skidding with Mono-cable Winch with Bulldozer to Damage of Residual Stand. [Thesis]. Mulawarman University, Samarinda. [Indonesian].
Meijaard E, Sheil D, Nasi R, Augeri D, Rosenbaum B, Iskandar D, Setyawati T, Lammertink MJ, Rachmatika I, Wong A, Soehartono T, Stanley S, O'Brien T. 2005. Life after logging: reconciling wildlife conservation and production forestry in Indonesian Borneo. CIFOR, WCS and UNESCO, Bogor, Indonesia.
Muhdi. 2008**.** The impact of timber harvesting with reduced impact logging systems to the soil compaction in West Kalimantan. Kalimantan For J 13 (1): 42-45.
Pinard MA, Putz FE. 1996. Retaining forest biomass by reducing logging damage. Biotropica 28: 278-295.
Pinard MA, Barker MG, Tay J. 2000a. Soil disturbance and post-logging forest recovery on bulldozer paths in Sabah, Malaysia. For Ecol Manag 130: 213-225.
Pinard MA, Putz FE, Tay J. 2000b. Lessons learned from the implementation of reduced impact logging in hilly terrain in Sabah, Malaysia. Intl For Rev 2 (1): 33-39.
Putz FE, Dykstra DP, Heinrich R. 2000. Why poor logging practices persist in the tropics. Conserv Biol 14 (4): 951-956.
Putz FE, Sirot LK, Pinard MA. 2001. Tropical forest management and wildlife. Silvicultural effects on forest structure, fruit production, and locomotion of arboreal animals. In: Fimbel RA, Grajal A, Robinson JG (eds). The Cutting Edge: Conserving Wildlife in Logged Tropical Forest. Columbia University Press, New York.
Putz FE, Zuidema PA, Pinard MA, Boot RGA, Sayer JA, Sheil D, Sist P, Vanclay JK. 2008. Improved tropical forest management for carbon retention. PLoS Biol 6, 1368-1369.
Putz FE, Zuidema PA, Synnott T, Peña-Claros M, Pinard MA, Sheil D, Vanclay JK, Sist P, Gourlet-Fleury S, Griscom B, Palmer J, Zagt R. 2012. Sustaining conservation values in selectively logged tropical forests: the attained and the attainable. Conserv Lett 5 (4): 296-303.
Ruslim Y, Hinrichs A, Sulistioadi B. 2000. Study on implementation of reduced impact tractor logging. SFMP Document No. 01a.
Ruslim Y. 2011. Implementing reduced impact logging with mono-cable winch. J Trop For Manag 17 (3): 103-110.
Sasaki N, Putz FE. 2009. Critical need for new definitions of "forest" and "forest degradation" in global climate change agreements. Conserv Lett 2: 226-232.
Sist P, Nolan T, Bertault JG, Dykstra DP. 1998. Harvesting intensity versus sustainability in Indonesia. For Ecol Manag 108: 251-260.
Spinelli R, Magagnotti N, Relaño RL. 2012. An alternative skidding technology to the current use of crawler tractors in Alpine logging operations. J Clean Prod 31: 72-79.
Tavankar F, Majnounian B, Bonyad AE. 2012. Felling and winching damages to natural regeneration in the Hyrcanian forests of Iran. Intl J Agron Plant Prod 3 (8): 300-305.
TNC [The Nature Conservancy]. 2010. Improved forest management and REDD, Policy Brief, November 2010.

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Anatomical structure and antioxidant activity of red bulb plant (*Eleutherine americana*) on different plant age

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Abstract. *Kuntorini EM, Dewi M, Misrina. 2016. Anatomical structure and antioxidant activity of red bulb plant* (Eleutherine americana*) on different plant age. Biodiversitas 17*: *229-233. Eleutherine americana* Merr is an medicinal herb named "red bulb" or "bawang dayak". Red bulb plant is commonly used as anti-breast cancer in Kalimantan, which may be linked to their bioactive naphtoquinone-derivatives properties. The aim of this study was to characterize the anatomical structure and antioxidant activity in red bulb of different ages. The anatomical structure of bulb was fixed and prepared following paraffin embedding techniques. Measurement of antioxidant activity carried out using the DDPH method. The anatomical structure of red bulb showed that the P2 (12 week after planting (WAP)) bulb is thicker in the upper and lower epidermis, parenchyma, and vascular bundles than P1 (6 WAP) bulb. The result of antioxidant activity of P2 (12 WAP) with IC50 = 50.42 ppm have stronger antioxidant activity than the P1 (6 WAP) with IC_{50} = 93 ppm, but weaker than vitamin C (IC_{50} = 3.03 ppm) and BHT (IC50 = 5.52 ppm) as a control.

Keywords: Anatomical structure, antioxidant activity, *Eleutherine americana*, red bulb

INTRODUCTION

Red bulb plant or "bawang dayak" (*Eleutherine americana* Merr) grow in Kalimantan forest and has been used for traditional medicine (Galingging 2009). Bulbs of plant contain naphthoquinone group (elecanacine, eleutherine, elutherole, eleutherinone) (Hara et al. 1997; Alves et al. 2003; Nielsen and Wege 2006; Han et al. 2008), which is known has function as anti-cancer as well as antioxidant (Babula et al. 2009). Our previous research showed that bulb ethanol extract of red bulb from Banjarbaru has antioxidant activity of IC_{50} for 25.33 ppm. The same research reported the phytochemical screening red bulb contain triterpenoid and quinone (Kuntorini and Astuti 2010).

Triterpenoid and quinone are bioactive compounds present naturally in many plants. The formation process of bioactive compounds is complex, which involves interaction between biosynthetic processes, degradation, and can only be found in certain organisms or organism groups (Dewick 2002). The formation process highly depends on the physiological condition such as age and growth steps on different plants (Baikar and Malpathak 2010; Soetarno 1997). Research previously investigated the effect of ages and the leaf position on the quality and the quantity of Japanese mint oil production (Duriyaprapan and Britten 1982). The result showed the most of the oils and the main component (menthol) were synthesized for the first 2 weeks of the growth, yet it degraded after 2 weeks.

Bioactive compounds produced by plants as one of the ways to keep predators away (Dewick 2002). Plants do not always produce bioactive compound in each cell. Plants usually do biosynthetic on specific organs (Cseke et al. 2006), whereas the product can be accumulated inside the vacuole of the cell (Dixon 2001).

Our previous research showed the thickness of leaf mesophyll at the age of 6 and 12 weeks which were 52.3 µm and 66.9 µm respectively showed a marked difference, as well as on the bulb length showed that there was a difference at the age of 6 and 12 weeks which were 3.1 cm and 4.02 cm respectively. Through the observation of anatomical structure on the leaf, although there was a significant increase of the mesophyll thickness and the size of the constituent cells, it was not followed by the increase of naphthoquinone group content, whereas on bulb it was followed by the increase of the content of bioactive compound in naphthoquinone group. It can be assumed that naphthoquinone group was translocated to the bulb as the stockpiling organ (Kuntorini 2008). According to Babula *et. al.* (2009) the naphthoquinone compound is known to be highly toxic, commonly used as antimicrobial, antifungal, antiviral, anti parasite, and antioxidant.

However, despite the fact that red bulb is interesting from a medicinal and pharmacological point of view, the antioxidant activity on different plant ages uninvestigated. Therefore, the aim of this study was to investigate anatomical structure and the antioxidant activity of red bulb (*Eleutherine americana* Merr.) on different plant age.

MATERIALS AND METHODS

Nine hundreds of red bulbs with the diameter of 0.3-0.5 cm were planted in a 6 m x 1.5 m x 15 cm wooden container with the spacing of 10 cm x 10 cm. The soil was analyzed to show the N, P, and K content at the soil laboratory of Wetlands Agricultural Research Bureau (BALITTRA), Banjarbaru, South Kalimantan, Indonesia. The soil analysis result was used as a supporting data.

The bulb was taken after 6 weeks after planting (WAP) and 12 WAP. Five bulbs were collected in each harvesting time for study the anatomical structure, while 100 grams of gross weight of bulb were used to determine the anti-oxidant activity. Single-stain paraffin embedding method (Ruzin 1999) was applied in preparation of microscopic for examination of bulb slides included the thickness of paren-chyma tissue, the thickness of upper and lower epidermis tissue, and the diameter of vascular bundle on bulb.

Sample preparation for antioxidant analyses

Sample extraction of bulb used maceration method. Bulbs powder as much as 31 grams was put into 200 mL erlenmeyer and ethanol as much as 70 mL was added as a solvent. The extract was macerated for 24 hours at a room temperature. The extract was filtered using filtering paper after 24 hours maceration. The processes were repeated for three times. The first, the second, and the third filtrates were collected and evaporated using *rotary vacuum evaporator* until the thick extract was obtainable.

Antioxidant activity test

Antioxidant activity test was assessed using DPPH method. The concentrated extract of bulbs were weighed as much as 0.0085 grams as P1 sample (6 WAP) and 0.005 grams as P2 sample (12 WAP), then each of them was put into a beaker glass and dissolute using methanol. After that, the sample was put into the flask and methanol was added up to the marked sign, until the concentration of extract solution on P1 sample (6 WAP) reached 170 ppm and P2 sample (12 WAP) reached 100 ppm. Starting from concentration of 170 ppm, P1 sample (6 WAP) was then diluted to get the standard series with the concentration of 160 ppm, 120 ppm, 80 ppm, and 40 ppm. For concentration of 100 ppm, P1 sample (12 WAP) was diluted to 70 ppm, 50 ppm, 30 ppm, and 10 ppm. This dilution was done using methanol in a 10 ml measuring flask. It was then transferred into a beaker glass and was put into 1 ml of DPPH 1 mM, incubated for 30 minutes and later the absorption was measured on 515 nm wave lengths. As a positive control and for the comparison, ascorbic acid was used (concentration of 2, 3, 4 and 5 ppm) and BHT (concentration of 2, 4, 6 and 8 ppm) which was done by the same way as the extract (Andayani et al. 2008).

The antioxidant activity was estimated according to Andayani et al. (2008). The sample was determined by the amount of DPPH radical absorption resistance using % antioxidant power calculation in this formula:

$$\% \text{ of resistance} = \frac{(A\ blanko - A\ sampel)}{A\ blanko}\ 100\%$$

Where:

A Blank: DPPH radical absorption of 1 mM in methanol on 515 nm wave length

A sample: DPPH radical absorption of 1 mM treated as sample in methanol on 515 nm wave length

In DPPH method concept, antioxidant activity determination by looking at value of IC_{50} which is test compound concentration having antioxidant power to resist free radical is as much as 50 %. The value of IC_{50} was obtained using linier regression equation which stated the relation between sample concentration (test compound) and percentage of antioxidant power (Cholisoh and Utami 2008). The measurement of sample concentration in this research followed that concept, where the X value (concentration) has to be in the range % antioxidant power as much as 50%.

Phytochemical test

Phytochemical test was done as a qualitative support data. The test of some chemical compounds on the ethanol extract of bulb were done according to Harboren (1987) which consisted of steroid group, triterpenoid group, alkaloid group, flavonoid group, saponin group, tannin group and quinone group using appropriate reactant.

Data analysis

Qualitative data analysis was done in the phytochemical test, while quantitative data analysis was done for the measurement of parenchyma tissue thickness, upper and lower epidermis, as well as vascular bundle diameter using T Test. Antioxidant activity test was done by looking at the value of IC_{50}.

RESULTS AND DISCUSSION

Anatomical structure of red bulb

Anatomical structure of each layer of bulb cross section consisted of epidermis followed by parenchyma tissue and vascular bundle which were distributed randomly. Epidermis tissue consisted of upper surface (adaxial) and lower surface (abaxial), formed by cells with a thin wall, arranged closely each other in a small four-sided shape. According to Esau (1977) epidermis tissue functions as a cover for the tissue inside.

The observation showed that parenchyma tissue was formed by parenchyma cells in irregular hexagonal form as far as nearly round. According to Hidayat (1995) most parenchyma cells have a thin wall, yet some have a thicker wall. The most significant characteristic of parenchyma cells is that they can split and specialize to be a tissue with specific function. Parenchyma cells usually form the base tissue on plants, which is the reason why they are called as the base tissue (Fahn 1991). Moreover, parenchyma cells are known to play a role as food storage. The food can be found in vacuole.

On the observation of parenchyma cells of bulbs, amylum grain was found. Amylum is a food reserve most commonly found on plants, specifically inside the fruits, seeds, and roots (Hidayat 1995). On the red bulb layers, secretion cells were unavailable. It can be assumed that naphthoquinone group compound formation as a secondary metabolite takes place at cytosol and are stored inside the vacuole. Babula et al. (2005) pointed naphthoquinone group compound are usually stored inside the vacuole in the

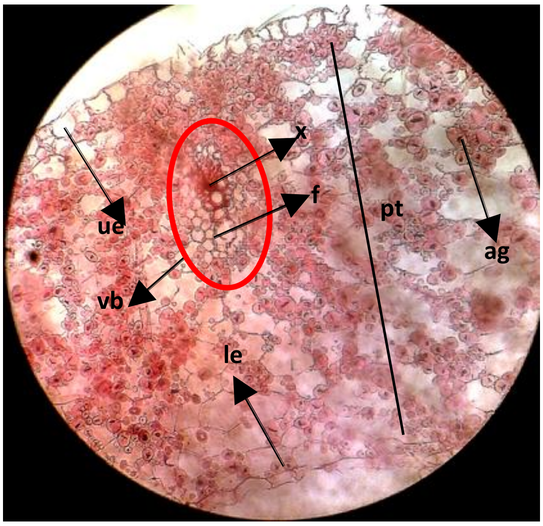

Figure 1. Cross section of Dayak onion bulbous layers at the age of 12 WAP (P2). Note: ue (upper epidermis), le (lower epidermis), vb (vascular bundle), x (xilem), f (floem),pt (parenchyma tissue), ba (amylum grain) 10x20 magnification

Table 1. The average of upper epidermis tissue thickness, lower epidermis tissue thickness, and vascular bundle diameter

Observation	**Tissue thickness** Upper epidermis (µm)	Lower epidermis (µm)	**Parenchyma (µm)**	**Vascular bundle diameter (µm)**
P1 (6 WAP)	17.30[a]	14.20[a]	290.50[a]	44.50[a]
P2 (12 WAP)	28.00[b]	17.60[b]	497.00[b]	73.80[b]

form of glycoside. Hidayat (1995) suggested most parenchyma cells contain tannin and mineral salt in a crystallized form.

Based on the result of T test on the observation of parenchyma tissue thickness, upper epidermis tissue thickness, lower epidermis tissue thickness, and vascular bundle diameter, it showed that there was a significant difference between P1 (6 WAP) and P2 (12 WAP) because the significant value on the T test was less than the value of α (0.025). The average anatomic measurement data of red bulb can be seen below

Description

The same number followed by the same letter in the same column showed that there was no significant difference on the T Test at test level α (0.025). The observation result of anatomical structure of bulb layers showed the thickness of parenchyma tissue was increased at P2 (12 WAP), and it also happened in the thickness of upper epidermis and lower epidermis tissue. On the vascular bundle structure of P1 (6 WAP), the number of the constituent cells was still low and smaller in size compared to P2 (12 WAP), thus influencing the size of vascular bundle diameter.

The growth of the plants was indicated by the increasing size because multicellular organisms grow from a zygote. The increasing number is not only about the volume, but also in weight, as well as in the number of cells, protoplasm, and the complexity. The growth and development process are the result of 3 basic phenomena at cellular level. The first step is the cellular fission: one of the fully-grown cells splits into two individual cells, which are not always similar from one to another. The second step is the development of the cells: The cells grow bigger in volume. The third step is the differentiation of cells: cells with certain volume are specialized in particular ways. Many kinds of cells split, grow, and specialize resulting in a wide variety of tissue and organs as well as into many kinds of plants (Salisbury and Ross 1995).

The growth of the cell itself is a process of absorbing liquids into the expanding vacuole, which later space out the cell wall. In that process, the growth power is the turgor pressure. The pressure inside the cell is caused by the mechanical resistance of cell wall against a tense. If the resistance is reduced, the wall loosens. The wall stretch causes decreasing pressure, reduced liquid potential, and increasing water gradient potential. Therefore, liquid comes into the cell causing the increasing size of the cell itself (Salisbury and Ross 1995).

In this research, the bulb development was visible at the age of 12 WAP (P2) on the thickness of parenchyma tissue, upper and lower epidermis, and the diameter of vascular bundle. Referring on the soil elements contained in the used soil, it was ascertainable that N was 0.126%, P was 0.041%, and K was 0.036%. Compared to the soil sample on the natural habitat in Kalimantan, It can be inferred that the NPK on this sample is higher than at the soil in the natural habitat in Kalimantan.

Based on the result of the research above, it showed the red bulb can grow better in the soil with higher soil elements as in the natural habitat Kalimantan as well as lower soil elements than the macro soil elements concentration which is considered as sufficient (0.1%) as mentioned before. According to Heyne (1987) red bulb are usually found in many places, growing freely between 600 and 1500 m above sea level, sometimes in a huge number at the grassy paths, between tea plants, quinine, and rubber trees. This wide spreading indicates that the contents of NPK are diverse or the range of the NPK is wide.

The amount of NPK needs is related to the needs of plants to grow well. If the NPK is insufficient, the growth will be interfered. The increasing amount of the thickness of upper and lower epidermis tissue, parenchyma tissue, and vascular bundle diameter on P2 (12 WAP) showed that the needs of NPK is sufficient (Lakitan 2004).

Antioxidant activity of red bulb extract

The result of the research showed the red bulb extract on P2 (12 WAP) had a value of IC_{50} as much as 50.42 ppm, while on P1 (6 WAP) the value of IC_{50} is as much as 93 ppm which means the antioxidant activity on P2 (12 WAP) is stronger than the antioxidant activity on P1 (6 WAP). This showed that the growth phase (seed age) according to

Soetarno (1997) has an effect on the secondary metabolite which has compound with antioxidant activity. Compared to the positive control of vitamin C and BHT, the sample had weaker antioxidant activity.

P1 (6 WAP) had weaker antioxidant activity than P2 (12 WAP). This might happen because at that seed age the plant was still in the early phase of growth. On P1 (6 WAP) vascular bundle was found having smaller cells in a very small number, resulting in a small number of the transported and piled accumulation of secondary metabolite. Besides, the observation of the anatomical structure showed that there was an increasing of parenchyma tissue thickness on P2 (12 WAP), whereas the increasing thickness is seen in the increasing size of the cells. According to Salisbury and Ross (1995) the increasing size of cells is caused by the expanding vacuole. According to Hidayat (1995) vacuole is the biggest organelle on mature plants, containing liquids and solvents. On red bulb secretion cells are unavailable, thus it can be assumed that the piled secondary metabolite takes process in the vacuole. Babula et al. (2005) suggested in the plants family of Plumbaginaceae, Juglandaceae, Ebenaceae, Boranginaceae, and Iridaceae, the contained secondary metabolite especially naphthoquinone is usually stored inside the vacuole. Red bulb is classified into the Iridaceae family.

The difference of antioxidant activity between the two observations was caused by the different concentration of the secondary metabolite. The more secondary metabolites lead to the stronger antioxidant activity. It can be estimated that P2 (12 WAP) had higher concentration of secondary metabolite than P1 (6 WAP), thus the antioxidant activity is stronger. This is supported by our previous research showed on red bulb planted inside a glass house and in an open space at the age of 4 WAP and 12 WAP, showed the increasing of secondary metabolite (bioactive) naphthoquinone on bulb at the age of 12 weeks (Kuntorini and Nugroho 2009). It was ascertainable that P1 (6 WAP) and P2 (12 WAP) had the same result on the secondary metabolite such as positive steroid, tannin, quinone, and flavonoid. In this research, phytochemical testing were done qualitatively, with the result that the detected secondary metabolite were based on the color change while reacting.

Some species showing the increasing compound of secondary metabolite along with the aging are *Cinnamomum camphora*, which its camphor is accumulated inside the old stems and *Blumea balsamifera* which is harvested when the leaves are old enough (Soetarno 1997).

The secondary metabolites were detected in this research was quinone. Our previous research showed that red bulb ethanol extract from Banjarbaru contains quinone based on the phytochemical screening (Kuntorini and Astuti (2010). Quinone very likely comes from the appropriate oxidized components of phenol, such as catechol (1.2 dihydroxybenzen) producing *ortho*-quinone and quinole (1.4 dihydroxybenzen) producing *para* quinone, thus quinone can be formed from phenol system which is produced through acetate or shikimate pathway. Chimaphiline, plumbagin, eleutherine are the plants known to have naphthoquinone and have been used as medicine and poison since prehistoric era. Naphthoquinone compounds are known to be toxic, commonly used as antimicrobial, antifungal, antiviral, antiparasite, and antioxidant (Babula et al. 2005; Babula et al. 2009).

Our previous research showed the value of IC_{50} (antioxidant activity) red bulb from Tanah Laut Regency containing naphthoquinone ranges from 29.18 to 51,11 ppm. In this research, the value of IC_{50} on P2 (12 WAP) was as much as 50.42 ppm, so the value of antioxidant activity is categorized within the range similar to in its natural habitat (Tanah Laut) (Kuntorini et al. 2011).

In this research, in addition to naphthoquinone compound, flavonoid compound was also detected. Flavonoid group has the ability to transform in order to produce higher activity compounds with antioxidant activity. Flavonoid is known as a good antioxidant because it has at least two hydroxyl clusters on *orto* and *para* (Andayani et al. 2008).

In conclusion, anatomical structure of bulbs layers on the thickness of upper and lower epidermis tissue, parenchyma tissue, and vascular bundle diameter is bigger on bulb at the age of 12 WAP. Bulb at the age of 12 WAP (IC_{50} = 50.42 ppm) has stronger antioxidant activity than at the age of 6 WAP (IC_{50} = 93 ppm), yet is weaker than vitamin C (IC_{50} = 3.03 ppm) and BHT (IC_{50} = 5.52 ppm) as the control.

REFERENCES

Alves TMA, Kloos H, Zani CL. 2003. Eleutherine a novel fungiside napthoquinon from *E. bulbosa* (Iridaceae). Mem Inst Oswaldo Cruz 98 (5): 709-712.

Andayani R, Maimunah, Lisawati Y. 2008. Determination of antioxidant activity, total phenolic content, and lycopene in tomatoes (*Solanum lycopersicum* L). Jurnal Sains dan Teknologi Farmasi 13 (1): 31-37. [Indonesian]

Babula P, Adam V, Havel L, Kizek R. 2009. Noteworthy secondary metabolites napthoquinones-their occurrence, pharmacological properties and analysis. Curr Pharmaceut Anal 5 (1): 47-68.

Babula P, Mikelova R, Potesil D, Adam V, Kizek R, Havel L, Sladky Z. 2005. Simultaneous determination of 1,4-naphtoquinone, lawsone, juglone and plumbagin by liquid chromatography with UV detection. Biomed Paper 149: 25-28.

Baikar S, Malpathak N. 2010. Secondary metabolites as DNA topoisomerase inhibitors: A new era towards designing of anticancer drugs. Pharmacog Rev 4 (7): 12-26.

Cholisoh Z, Utami W. 2008. Radical catcher activities of ethanol 70% extract of jengkol seed (*Archidendron jiringa*). Pharmacon 9 (1): 33-40. [Indonesian]

Cseke LJ, Kirakosyan A, Kaufman PB, Warber SL, Duke JA, Brielmann HL. 2006. Natural Products From Plants. 2nd ed. Taylor & Francis Group. United States of America.

Dewick PM. 2002. Medicinal Natural Product, A Biosynthetic Approach 2nd ed. John Wiley & Son, Inc., England.

Dixon RA. 2001. Natural products and plant disease resistance. Nature 411: 843-847.

Duriyaprapan S, Britten EJ. 1982. The effect of age and location of leaf on quantity and quality of Japanese mint oil production. J Exp Bot 33 (135): 810-814.

Esau K. 1977. Anatomy of Seed Plants. 2nd ed. John Wiley & Sons, Inc., New York.

Fahn A. l991. Plant Anatomy. 4th ed. Pergamon, New York.

Galingging RY. 2009. Bawang Dayak (*Eleutherine palmifolia*) as a multifunctional medicinal plants. Warta Penelitian dan Pengembangan 15 (3): 2-4. [Indonesian]

Han AR, Min HY, Nam JW, Lee NY, Wiryawan A, Suprapto W, Lee SK, Lee KR, Seo EK. 2008. Identification of a new naphthalene and Its derivatives from the bulb of *Eleutherine americana* with inhibitory activity on lipopolysaccharide-induced nitric oxide production. Chem Pharm Bull 56: 1314-1316.

Hara H, Maruyama N, Yamashita S, Hayashi Y, Lee KH, Bastow KF, Chairul C, Marumoto R, Imakura Y. 1997. Elecanacin, a novel new napthoquinon from the bulb of *Eleutherine americana.* Chem Pharm Bull 45 (10): 1714-1716.

Harborne JB. 1987. Phytochemical Methods. Chapman and Hall, London.

Heyne K. 1987. The Usefull Plants of Indonesia. Vol. 1. Forestry Research and Development Agency. Jakarta. [Indonesian]

Hidayat EB. 1995. Anatomy of Seed Plants. Penerbit ITB Bandung. [Indonesian]

Kuntorini EM, Astuti MD. 2010. Determination of the antioxidant activity of ethanol extract of Dayak onion (*Eleutherine americana* Merr.) bulbs. Sains dan Terapan Kimia 4 (1): 15-22. [Indonesian]

Kuntorini EM, Nugroho LH. 2009. Structural development and bioactive content of red bulb plant (*Eleutherine americana* Merr.): A traditional medicines for local Kalimantan People. Biodiversitas 11 (2): 102-106.

Kuntorini EM, Astuti MD, Puspita G. 2011. Anatomy structure, of bioactive constituens and antioxidant activity test of Dayak onion bulbus (*Eleutherine americana* Merr) from Takisung Sub-District, Tanah Laut District, South Kalimantan: Proceeding of National Seminary on Mathematics and Natural Sciences (Semirata BKS-PTN B). Lambung Mangkurat University, Banjarmasin 9-10 May 2011. [Indonesian]

Kuntorini EM. 2008. Bulbus and leaves development of Dayak onion (*Eleutherine americana* Merr.) and content of bioactive derived compounds naphtoquinon. [Thesis]. Program of Biology, School of Graduates, Gadjah Mada University, Yogyakarta. [Indonesian]

Lakitan B. 2004. Fundamentals of Plant Physiology. PT Raja Grafindo Persada. Jakarta. [Indonesian]

Nielsen LB, Wege D. 2006. The enantio-selective synthesis of elecanacin through an intramolecular naphtoquinone-vinyl ether photochemical cyclo-addition. Org Biomol Chem 4: 868-876.

Ruzin SE. 1999. Plant Microtecnique and Microscopy. Oxford University Press, Oxford.

Salisbury FB, Ross CW. 1992. Plant Physiology. 4th ed Wadsworth Publishing Co., London.

Soetarno S. 1997. Preparation of natural products extraction, in preparatif extraction and isolation of natural ingredients. Proceeding of National Seminary on Farmacy. Faculty of Mathematics and Natural Sciences, ITB, Bandung. [Indonesian]

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Shade tree species diversity and coffee productivity in Sumberjaya, West Lampung, Indonesia

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Abstract. *Evizal R, Sugiatno, Prasmatiwi FE, Nurmayasari I. 2016. Shade tree species diversity and coffee productivity in Sumberjaya, West Lampung, Indonesia. Biodiversitas 17*: *234-240.* Shade tree is an important variable that determines the productivity and sustainability of coffee plantation. In West Lampung, Indonesia coffee is grown on private land and on state land of Community Forest Program (CFP) using various types of shade trees. The research explored the diversity of shade trees and its influence on the productivity of coffee farms. The study area was one purposively sampled coffee farmer group in Sumberjaya District, West Lampung. We purposively chose one coffee farmer group. The group members' farms located in private land and in CFP land were sampled randomly, each consisted of 18 farms. From each farm, we observed a plot of 50 m x 50 m and interviewed the farmer who managed the farm. Data collected were on the species and the number of trees, farm age, coffee tree densities, and productivity of coffee in the last 3 years. Data analyses of important value, tree species diversity, correlation, and regression were performed. Shade trees found in coffee farms of Sumberjaya were 36 species, 10 species (28%) of them were legume trees. Technical shade trees that have high importance value were *Gliricidia sepium* and *Erythrina subumbrans.* Multi Purpose Trees Species (MPTS) widely planted were *Durio zibethinus* and *Parkia speciosa*. The wood trees with a high importance value in the CFP coffee farms were *Shorea javanica* and *Michelia champaca* while in private coffee farms were *Maesopsis eminii* and *Litsea* sp. Based on Shannon's index (H') and Simpson's dominance index (λ), a high diversity of shade tree species was found in CFP coffee farms at age ≥20 years. Shade trees with high dominance index had a positive effect on productivity of coffee and the percentage of MPTS had a negative effect. Whereas, the types of land tenure (private or CFP) and the abundance of shade trees did not affect the productivity of coffee.

Keywords: coffee, community forest, productivity, shade tree diversity

INTRODUCTION

Lampung Province is a center of the production of Indonesian Robusta coffee which is grown mainly in the mountainous region of Bukit Barisan (Philpott et al. 2008), including on private land and on the state land of Community Forest Program (CFP). As farmers participate in CFP must plant at least 400 trees per hectare, the CFP significantly increased planting of wood trees species and Multi Purpose Trees Species (MPTS) and also increased farmers' income (Pender et al. 2008).

Cultivating coffee under varieties of shade tree species is one of local wisdoms that have long been practiced in the District of Sumberjaya, West Lampung, Indonesia (Verbist et al. 2005). Understanding the diversity, characteristics and functions of shade trees as well as its strata is important in efforts to improve the sustainability of coffee agro-ecosystem (Mamani-Pati et al. 2012), and to improve biodiversity conservation (Lopez-Gomez et al. 2008) including to conserve native tree species (Tadesse et al. 2014).

Ecological functions of the shade trees in coffee agroforestry system are as environmental services, such as recycling nutrients (Lopez-Rodriguez et al. 2015), driving soil conservation (Lin and Richards 2007), improving growth, productivity, and quality of coffee (Bote and Struik 2011), and regulating biomass production (Evizal et al. 2009) including fire wood and timber as a source of alternative income (Shalene et al. 2014) and fodder for livestock production (Geta et al. 2014). Farmers realize those functions but decisions on shade trees management are more to keep the coffee production rather than reasons of environmental services (Cerdan et al. 2012). However, study on coffee agroforestry in Sumberjaya is important to support the conservation of water supply to the electric power plant of Way Besai (Pasha et al. 2012). Management of agroforestry systems in a sustainable manner requires conservation and proper management of MPTS strata (Tscharntke et al. 2011).

Shade trees in coffee plantations can be technical shade trees, wood trees, or MPTS. Determining the composition of shade trees is important to maintain the balance of the ecological functions and the coffee agro-ecosystem productivity (Tscharntke et al. 2011). Technical shade trees are legume trees planted on coffee plantations, not to harvest the yield but to provide shade for the coffee plants. In West Lampung, technical shade trees most widely grown are *Erythrina subumbrans* and *Gliricidia sepium* (Evizal et al. 2012).

CFP of coffee plantations in protected areas requires planting trees or MPTS that will affect the shade tree diversity and productivity of coffee plants under the shade. The research objectives were to explore the diversity of shade trees and its influence on the productivity of coffee plantations on private land and CFP land in District of Sumberjaya, West Lampung.

MATERIALS AND METHODS

Field study

We purposively chose one coffee farmer group in Sumberjaya District, West Lampung, Indonesia (Figure 1). The group members' farms located in private land and in CFP land were sampled randomly, each consisted of 18 farms. From each farm, we observed a plot of 50 m x 50 m and interviewed the farmer who managed the farm. Data collected were on the species and the number of the trees, farm age, coffee tree densities, and productivity of coffee in the last 3 years.

Data analysis

Data analyses of importance value, tree species diversity, correlation, and regression were performed. Analyses of Importance Value (IV) and diversity index are based on report of Sumantra et al. (2012). We calculated IV as sum of Relative Density and Relative Frequency and expressed diversity index based on the proportion (n/N) of individuals (n) of one particular species found (i) divided by total number of individuals found (N). The formula of Shannon-Wiener index of species diversity (H') is:

$$H' = -\sum_{i=1}^{n} \left[\frac{ni}{N}\right] \ln \left[\frac{ni}{N}\right]$$

We calculate Simpson's dominance index (λ) using formula (Morris et al. 2014):

$$\lambda = \sum_{i=1}^{n} \left[\frac{ni}{N}\right]^2$$

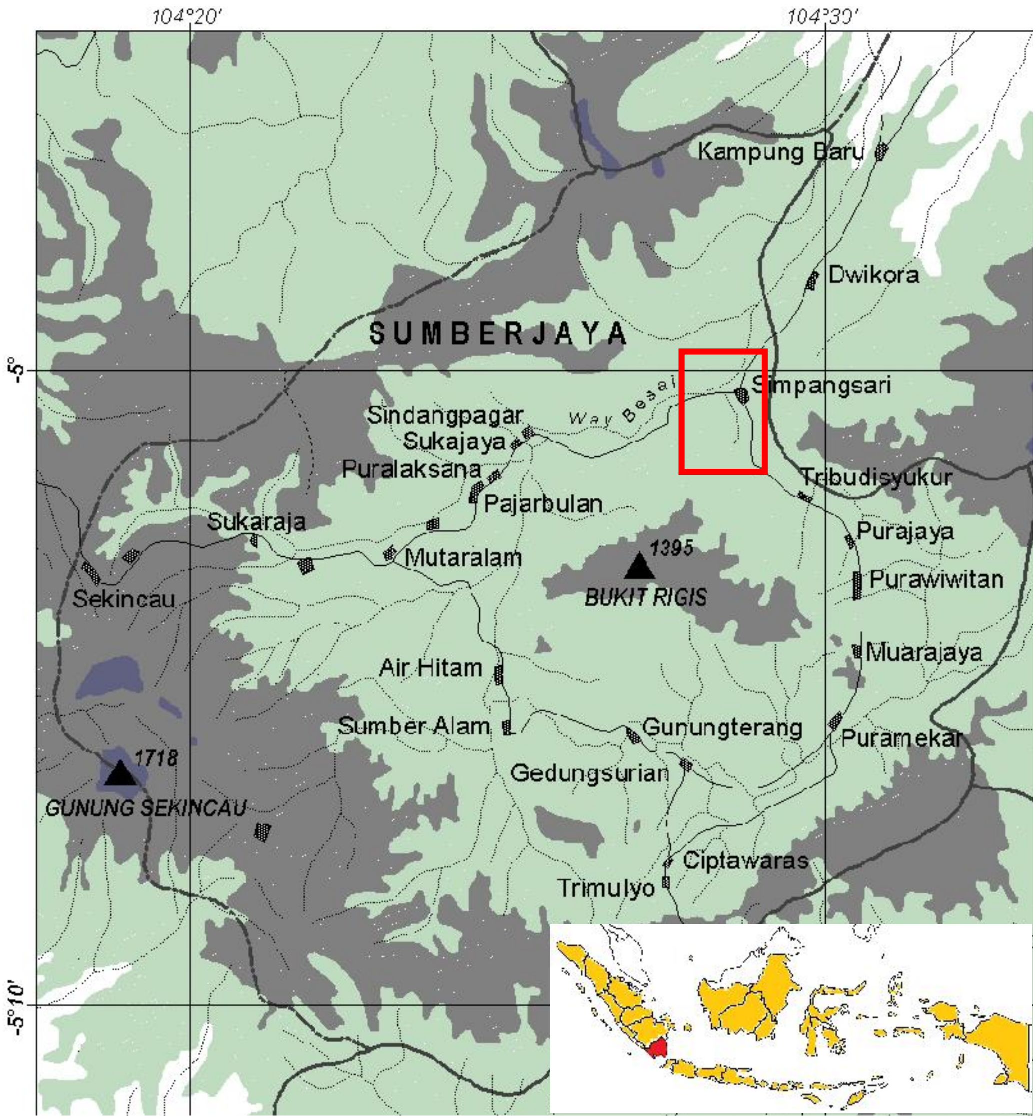

Figure 1. Study site in Sumberjaya, West Lampung, Indonesia (in red mark) (ICRAF in Pender et al. 2008)

RESULTS AND DISCUSSION

Importance value

Shade trees found in coffee farms of Sumberjaya were 36 species, 10 species (28%) of which were legume trees, namely *G. sepium* (gliricidia), *E. subumbrans* (coral trees), *Leucaena leucocephala* (lamtoro), *Dalbergia latifolia* (black rosewood), *Paraserianthes falcataria* (sengon), *Parkia speciosa* (petai), *Swietenia mahagoni* (mahogany), *Acacia* sp., *Archidendron pauciflorum*, and *Archidendron microcarpum*. CFP coffee farms had higher richness of shade trees with 31 species, whereas private coffee farms had 24 species. Comparing between coffee farms at age of <20 year and at age of ≥20 year, the density of shade trees increased from 97 to 160 trees in private coffee farms and from 276 to 350 trees in CFP coffee farms (Table 1). Technical shade trees species found in coffee farms were *G. sepium*, *E. subumbrans*, *L. leucocephala*, *D. latifolia*, and *P. falcataria*, but only *G. sepium* and *E. subumbrans* had high Importance Value. MPTS widely planted in coffee farms were *P. speciosa*, *Durio zibethinus* (durian), *Artocarpus heterophyllus* (jackfruit) and *Musa paradisiaca* (bananas). The wood trees with a high importance value in the CFP coffee farms were *Shorea javanica* and *Michelia champaca* while in private coffee farms were *Maesopsis eminii* and *Litsea* sp.

These results indicated that the shade trees commonly found in coffee farms in Sumberjaya were exotic species. Native tree species that had significant importance value in the CFP coffee farms were *D. zibethinus*, *Alstonia scholaris*, *Shorea* sp. and *S. javanica*. Trees of native species and exotic species were planted because it has economic value as an incentive (Ambinakudige and Sathish 2008), so that shaded coffee farms could serve as refugia for native tree species (Tadesse et al. 2014). In Sumberjaya, shade trees also functions as source of fodder from leaves of *G. sepium*, *E. subumbrans*, *P. falcataria*, *M. eminii*, *Litsea* sp., *M. champaca*, *A. heterophyllus*, *Artocarpus champeden* and *Persea americana*.

Tabel 1. Abundance and importance value (IV) of shade trees in private and CFP coffee farms

Tree species	Private (n=18)				CFP (n=18)			
	< 20 y		≥20 y		< 20 y		≥20 y	
	Tree ha^{-1}	IV	Tree ha^{-1}	IV	Tree ha^{-1}	IV	Tree ha^{-1}	IV
Gliricidia sepium	12.50	19.85	73.73	64.05	67.78	32.86	43.75	17.34
Erythrina subumbrans	6.67	13.80	14.54	21.09	7	8.78	9.75	7.62
Leucaena leucocephala	1.67	5.17	0	0	0	0	0.75	1.83
Dalbergia latifolia	0	0	0.27	2.17	0	0	7.75	7.05
Paraserianthes falcataria	0	0	0.91	2.57	0	0	0	0
Maesopsis eminii	12.5	23.30	6.64	10.15	0.22	2.164	1.25	1.97
Litsea sp.	14.5	21.92	3.45	6.16	3.33	3.29	0	0
Alstonia scholaris	0	0	8	6.99	0.67	2.32	25.62	12.16
Swietenia mahagoni	7.33	14.50	0	0	13	10.95	0	0
Michelia champaca	4	7.59	0	0	54.11	32.08	45.12	20.96
Tectona grandis	0	0	0	0	0	0	3.5	2.61
Shorea sp.	0	0	0	0	0	0	3.125	2.51
Cananga sp.	0	0	0	0	0	0	0.75	1.83
Toona sinensis	8.333	12.08	0	0	0	0	0	0
Acacia sp.	0	0	2.27	3.42	0	0	0	0
Shorea javanica	0	0	0	0	57.78	27.16	65	23.42
Parkia speciosa	3.333	6.90	7.73	12.83	18.44	19.17	21	14.07
Durio zibethinus	4.17	11.21	23.82	28.88	28.89	20.87	36.87	21.83
Artocarpus heterophyllus	2.33	12.76	6.09	13.8	4	7.70	7.12	10.10
Musa paradisiaca	11	21.74	6.27	11.92	2.22	2.89	6.25	5.01
Persea americana	0.33	7.24	0.73	4.45	2.78	5.17	5.75	8.09
Cinnamomum burmannii	0.50	3.97	0	0	0	0	5	3.04
Archidendron pauciflorum	0	0	4.54	4.84	8.22	13.39	1.87	3.76
Carica papaya	0.67	4.14	0	0	0	0	0	0
Areca catechu	4.667	8.28	0	0	0.89	2.40	5.12	4.69
Anacardium occidentale	2	5.52	0	0	0	0	0	0
Artocarpus communis	0	0	0	0	2.22	2.89	5	4.65
Aleurites moluccana	0	0	0	0	3.11	5.29	2.5	2.33
Mangifera indica	0	0	0.82	4.51	0	0	0.37	1.72
Artocarpus champeden	0	0	0.27	2.17	0	0	0.63	1.79
Hevea brasiliensis	0	0	0	0	0	0	42.5	13.76
Ceiba pentandra	0	0	0	0	0	0	0.75	1.83
Syzygium aromaticum	0	0	0	0	0	0	2.5	2.33
Gnetum gnemon	0	0	0	0	0	0	0.25	1.68
Archidendron microcarpum	0	0	0	0	1.11	0.40	0	0
Syzygium aqueum	0	0	0	0	0.56	0.20	0	0
Total	96.5	200	160.1	200	276.3	200	349.9	200

Tree composition

Types of shade trees consist of technical shade trees (legumes), wood trees, and MPTS. When comparing the composition of shade tree types, in the private coffee farms at age <20 years, the dominant shade trees are MPTS, especially bananas, whereas at age ≥20 years, the dominant trees are technical shade trees, especially *G. sepium*. This showed that in the initial opening of the coffee farms, farmers planted banana as a source of income and planted *E. subumbrans* as shade, then planted *G. sepium* trees while *E. subumbrans* grew old and died.

In the CFP coffee farms, the dominant shade trees are MPTS (Table 2). Cultivating coffee and MPTS in a protected area is legal under license of CFP. Farmers are allowed to harvest non-wood yield such as fruits, beverages, spices, resin, or latex to generate income. There is no incentive for farmers to plant wood trees in CFP land because farmers are not allowed to cut and harvest timber. Meanwhile, cultivating coffee and MPTS in state-owned forest of national park is illegal so that, as Phillpot et al. (2008) reported, there are more abundant MPTS in private coffee farm land than in illegal coffee farms of national park. In general, shaded coffee plantations have high number of tree species (Capitan et al. 2014) even more than in forest areas (Lopez-Gomez et al. 2008) that may have been disturbed.

The number of shade trees increases with the increasing of coffee tree age, which was shown by the ratio of shade trees to coffee tree (Table 2). Thus the carbon stocks of shaded coffee farms increases with the age of coffee and shade trees particularly in the farms at the age of 20 years and more. When the coffee trees grow larger and shade tree species increase in number and diversity, it will form a complex coffee agroforestry that shaded coffee plantations have a role in carbon sequestration (Goodall et al. 2014) and climate change mitigation (Mbow et al. 2014).

The private coffee farms of ≥20 years, with 51.8% of the shade trees are technical of legume shade trees, gave the highest productivity compared to the other types of coffee farms (Table 2). This indicated that the high coffee productivity was obtained when technical shade trees, especially legume tress, were established. Legume trees that serve moderate shade level, shed the leaves in the dry season which created conditions to encourage coffee flowering, and produced much litter biomass (Evizal et al. 2009).

Meanwhile according to farmers, some species of shade trees could harm the growth and productivity of coffee trees, especially those of MPTS including *Aleurites moluccana, Cinnamomum burmannii, Hevea brasiliensis, D. zibethinus* and those of wood trees including *M. eminii, Shorea* spp., *M. champaca*, and *Litsea* sp. Some studies reported that the dominant shade tree species affect the growth and productivity of coffee (Kufa and Burkhardt 2011; Ebisa 2014). Farmers classify the effect of shade trees on the coffee plants as hot, medium, and cool. To choose shade tree species, they consider the shape of the canopy, litter production, rooting properties (Cerdan et al. 2012), nitrogen fixation, and the harvest of fruit or wood. Preferred tree species will dominate the composition of coffee shade trees (Valencia et al. 2015).

At the private land, higher Shannon diversity index of shade tree species was found in coffee farms at age of <20 years, while at the CFP land, higher Shannon diversity index was found in coffee farms at age of ≥20 years (Table 3). However, the diversity indexes were still classified as a medium diversity. The diversity index of shade trees found in private coffee farms at age of ≥20 years was <1 and categorized as low diversity (Maridi et al. 2014). Related to Simpson's dominance index (λ) and species richness, in the private coffee farms, the older the coffee farms the lower the diversity index of shade trees. On the contrary, in CFP land, the older the coffee farms the higher the diversity index of shade trees. These results indicated that the composition of shade tree was dynamic according to the knowledge and local wisdom of farmers to sustain productivity of coffee farm (Soto-Pinto et al. 2007). Sustainable coffee plantations are not only determined by the high diversity of flora and fauna as ecological indicators (Moonen and Barberi 2008), but also by the coffee productivity as an economic indicator. As further analysis, the relationship between the diversity index of shade trees and coffee productivity was approximated by correlation and regression analysis as shown at Tables 4 and 5.

Coffee productivity

There was a negative correlation (r = -0.57) between the shade tree diversity (Shannon's index) and the coffee production in CFP land. This meant that a high diversity of shade tree species could lead to lower coffee productivity. The same meaning was indicated by positive correlation of dominance index (r = 0,58), that a high dominance of shade trees species could induce higher coffee productivity. Meanwhile the productivity of coffee in the private farms and the diversity of shade trees showed a weak correlation.

There was a fairly strong positive correlation between the productivity of coffee and some variables including the percentage of technical shade tree (r = 0.60) and the percentage of legume shade trees (r = 0.48) in CFP land, and the age of coffee farms (r = 0.52) in private land. However, the productivity of coffee was negatively correlated (r = - 0.53) with the percentage of timber shade tree in CFP land.

It is clear that age of coffee trees affects its productivity (Potvin et al. 2005) and the increasing age of the coffee will decrease the density of shade trees (Goodall et al. 2014) if not being replanted. Regarding to shade tree diversity, it has been reported that in Guatemala, by using four species of shade trees, the coffee production reached 925 kg ha-1, while in Peru that uses 17 species of shade trees, the coffee production was 386 kg ha-1 (Rice 2008). As the dominant shade tree, the genus *Inga* has been widely reported as legumes that enriches the soil due to the accumulation of biomass (Siles et al. 2010) and symbiosis with legume nodule bacteria. Therefore, the legume trees were widely used in the farm of organic coffee (Grossman et al. 2006).

Table 2. Shade tree composition and coffee productivity

Land tenure	Coffee age	Technical shade tree (% ha^{-1})	Wood trees (% ha^{-1})	MPTS (% ha^{-1})	Ratio shade/coffee (% ha^{-1})	Coffee productivity (00 kg ha^{-1})
Private	< 20 year	25.285	24.579	50.135	4.290	6.93
	≥20 year	51.867	18.575	29.556	7.424	11.06
CFP	< 20 year	27.201	23.934	48.864	14.193	7.47
	≥20 year	14.919	29.717	55.363	16.358	7.37
Average		32.73	23.50	43.76	10.86	8.52

Table 3. Diversity index of coffee shade tree species

Land tenure	Coffee age (year)	Shannon-Weiver Index (H’)	Simpson’s dominance Index (λ)	Species richness
Private	< 20 years	1.3052	0.3184	4.6666
	≥20 years	0.8229	0.4162	4.5454
CFP	< 20 years	1.1547	0.4242	5.5555
	≥20 years	1.4454	0.3510	7.7500

Table 4. Correlation among variables on coffee productivity in private and CFP farms

Variables		Coffee productivity	
		Private	CFP
Shade tree	Shannon’s index (H’)	-0.0177	-0.5688
	Dominance index (λ)	0.0721	0.5779
	Species richness	0.0455	-0.3870
	Abundance	0.0873	0.2646
	Technical shade tree (%)	0.1661	0.5955
	Wood tree (%)	0.1628	-0.5348
	MPTS (%)	-0.3960	-0.1479
	Legume tree abundance (%)	0.1306	0.4778
Coffee tree	Density (tree ha-1)	0.3483	0.2791
	Age (year)	0.5234	-0.0898

Table 5 presented the regression analysis of variables dominance index, the percentage of MPTS, type of land tenure, and the number of shade trees on the productivity of the coffee farms. Table 3-4 earlier showed that based on Shannon Index (H') and species richness, the highest diversity of shade trees was found in the CFP coffee farms aged ≥20 years. Moreover, in the CFP coffee farms, dominance index (λ) and the percentage of technical shade trees positively correlated to the coffee productivity. Likewise, Table 5 showed that the dominance index had a positive effect on productivity of coffee and the percentage MPTS had a negative effect. The type of land tenure (private or CFP) and the abundance of shade trees did not affect the productivity of coffee. In general, this indicates that the dominance of shade trees or technical shade trees such as *G. sepium*, *E. subumbrans*, *L. leucocephala*, *D. latifolia*, and *P. falcataria* had positive effect on the productivity of coffee. On the contrary, the higher the percentage of MPTS resulted in the lower the coffee productivity.

In private coffee farms the average density of shade trees was 135 trees ha^{-1}, and in the CFP coffee farms was 310 trees ha^{-1}. That could be included as medium density of shade trees based on farmers’ norm on new planting of coffee in farms in Sumberjaya Sub-district (density < 100 trees = low, 100-400 = medium, >400 = high). Rice (2008) reported that dominant shade of legume tree had a positive influence on coffee productivity. However, Ebisa (2014) reported that both legume and non-legume species of shade trees had less significant effect on the productivity of coffee. Shade trees could decrease or raise the productivity of coffee or could have no effect (Shalene et al. 2014)

Table 5. Regression analysis of some variables on coffee productivity

Variable	Coefficient	Std. Error	t calc.	Significance
Constantan	0.8584	0.2331	3.68254	0.00094
Dominance index (λ)	0.8104	0.4711	1.72037	0.09602*
% MPTS	-0.6018	0.3267	-1.84184	0.07575*
Land tenure (private vs CFP)	-0.2258	0.2027	-1.11397	0.27444
Shade trees abundance	0.0003	0.0007	0.34125	0.73538
R^2	0.263			
F calc.	2.581			
Significance of F calc.	0.058			

Note: * Significant at level α 10%

depending on the species of shade trees (Long et al. 2015), the density and diversity of shade trees (Schmitt et al. 2009), shade tree structures (Hernandez-Martinez et al. 2009), fertilization, variety and age of coffee (Potvin et al. 2005). The characteristics of trees that serve optimal shade, fertilize the soil, and provide additional products would affect the farmers in selecting the species of shade trees to plant in coffee farms (Kalanzi and Nansereko 2014).

In conclusion, shade trees found in coffee farms of Sumberjaya were 36 species, 10 species (28%) of which were legume trees. Technical shade trees that have high importance value were *G. sepium* and *E. subumbrans.* MPTS widely planted were *D. zibethinus* and *P. speciosa*. The wood trees with a high importance value in the CFP coffee farms were *S. javanica* and *M. champaca* while in private coffee farms were *M. eminii* and *Litsea* sp. Based on Shannon's index (H') and Simpson's dominance index (λ), a high diversity of shade trees species was found in CFP coffee farms at age ≥20 years. Shade trees with high dominance index had a positive effect on productivity of coffee, and the percentage of MPTS had a negative effect. Whereas, the type of land tenure (private or CFP) and the abundance of shade trees did not affect the productivity of coffee.

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REFERENCES

Ambinakudige S, Sathish BN. 2008. Comparing tree diversity and composition in coffee farms and sacred forests in the Western Ghats of India. Biodivers Conserv. DOI 10.1007/s10531-008-9502-5.

Bote AD, Struik PC. 2011. Effects of shade on growth, production and quality of coffee (*Coffea arabica*) in Ethiopia. J Hortic For 3: 336-341.

Capitan GC, Avila-Bello CH, Lopez-Mata L, Gonzales FL. 2014. Structure and tree diversity in traditional Popoluca coffee agroecosystem in the Los Tuxtlas Biosphere Reserve Mexico. Interciencia 39: 608-619.

Cerdan, CR, Rebolledo MC, Soto G, Rapidel B, Sinclair FL. 2012. Local knowledge of impacts of tree cover on ecosystem services in smallholder coffee production systems. Agric Sys 110: 119-130.

Ebisa L. 2014. Effect of dominant shade trees on coffee production in Manasibu District, West Oromia, Ethiopia. Sci Technol Art Res J 3: 18-22.

Evizal R, Tohari, Prijambada ID, Widada J, Widianto D. 2009. Biomass production of shade-grown coffee agroecosystems. Proceeding International Seminar on Sustainable Biomass Production and Utilization Challenges and Oppurtunities (ISOMASS). Bandar Lampung, 3-4 August 2009.

Evizal R, Tohari, Prijambada ID, Widada J, Widianto D. 2012. Soil bacterial diversity and productivity of coffee-shade tree agro-ecosystems. J Trop Soils 17: 181-187.

Geta T, Nigatu L, Animut G. 2014. Ecological and socio-economic importance of indigenous multipurpose fodder trees in three Districts of Wolayta Zone, Southern Ethiopia. J Biodivers Endanger Species 2 (4). http: //dx.doi.org/10.4172/2332-2543.1000136.

Goodall KE, Bacon CM, Mendez VE. 2014. Shade tree diversity, carbon sequestration, and epiphyte presence in coffee agroecosystems: A decade of smallholder management in San Ramon, Nicaragua. Agric Ecosyst Environ 199: 200-206.

Grossman JM, Sheaffer C, Wyse D, Bucciarelli B, Vance C, Graham PH. 2006. An assessment of nodulation and nitrogen fixation in inoculated *Inga oerstediana*, a nitrogen-fixing tree shading organically grown coffee in Chiapas, Mexico. Soil Biol Biochem 38: 769-784.

Hernandez-Martınez G, Manson RH, Hernandez AC. 2009. Quantitative classification of coffee agroecosystems spanning a range of production intensities in central Veracruz, Mexico. Agric Ecosyst Environ 134: 89-98.

Kalanzi F, Nansereko S. 2014. Exploration of farmers' tree species selection for coffee agroforestry in Bukomansimbi district of Uganda. Int J Res Land-use Sustain 1: 9-17.

Kufa T, Burkhardt MJ. 2011. Plant composition and growth of wild *Coffea arabica*: Implications for management and conservation of natural forest resources. Int J Biodivers Conserv 3: 131-141.

Lin BB, Richards PL. 2007. Soil random roughness and depression storage on coffee farms of varying shade levels. Agric Water Manag 92: 194-204.

Long NV, Ngoci NQ, Dung NN, Kristiansen P, Yunusa I, Fyfe C. 2015. The effects of shade tree types on light variation and Robusta coffee production. Engineering 7: 742-753.

Lopez-Gomez AM, Williams-Linera G, Manson RH. 2008. Tree species diversity and vegetation structure in shade coffee farms in Vercruz, Mexico. Agric Ecosyst Environ 124: 160-172.

Lopez-Rodriguez G, Sotomayur-Ramirez D, Amador JA, Schroder ED. 2015. Contribution of nitrogen from litter and soil mineralization to shade and sun coffee (Coffea arabica L.) agroecosystems. Trop Ecol 56: 155-167.

Mamani-Pati F, Clay DE, Clay SA, Smeltekop H, Yujra-Callata MA. 2012. The influence of strata on the nutrient recycling within a tropical certified organic coffee production system. ISRN Agron 2012: 1-8. DOI: 10.5402/2012/389290.

Maridi, Agustina P, Saputra A. 2014. Vegetation analysis of Samin watershed, Central Java as water and soil conservation efforts. Biodiversitas 15: 215-223.

Mbow C, Smith P, Skole D, Duguma L, Bustamante M. 2014. Achieving mitigation and adaptation to climate change through sustainable agroforestry practices in Africa. Curr Opin Environ Sustain 6: 8-14.

Moonen A, Barberi P. 2008. Functional biodiversity: An agroecosystem approach. Agric Ecosyst Environ 127: 7-21.

Morris EK, Caruso T, Buscot F, Fischer M, Hancock C , Maier TS, Meiners T, Muller C, Obermaier E, Prati D, Socher SA , Sonnemann I, Waschke N, Wubet T, Wurst S, Rillig MC. 2014. Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. Ecol Evol 4: 3514- 3524.

Pasha R, Asmawan T, Leimona B, Setiawan E, Wijaya CI. 2012. Commoditized or co-invested environmental services? Rewards for environmental services scheme: River Care program, Way Besai watershed, Lampung, Indonesia. Working Paper No. 148. Bogor, Indonesia: World Agroforestry Centre (ICRAF) Southeast Asia Regional Program. DOI: 10.5716/ WP12051.PDF.

Pender J, Suyanto, Kerr J, Kato E. 2008. Impacts of the Social Forestry Program in the Sumberjaya Watershed, West Lampung District of Sumatra, Indonesia. International Food Policy Research Institute. Washington DC.

Philpott SM, Bichier P, Rice RA, Greenberg R. 2008. Biodiversity conservation, yield, and alternative products in coffee agroecosystems in Sumatra, Indonesia. Biodivers Conserv 17: 1805-1820.

Potvin C, Owen CT, Melzi S, Beaucage P. 2005. Biodiversity and Modernization in Four Coffee-producing Villages of Mexico. Ecology and Society 10. www.ecologyandsociety.org/vol10/iss1 /art18/

Rice RA. 2008. Agricultural intensification within agroforestry: The case of coffee and wood products. Agric Ecosyst Environ 128: 212-218.

Schmitt CB, Senbeta F, Denich M, Preisinger H, Boehmer HJ. 2009. Wild coffee management and plant diversity in the montane rainforest of southwestern Ethiopia. Afr J Ecol 48: 78-86.

Shalene JHA, Bacon CM, Philpott SM, Mendez VE, Laderach P, Rice RA. 2014. Shade coffee: Update on a disappearing refuge for biodiversity. Bioscience 64: 416-428.

Siles P, Harmand J, Vaast P. 2010. Effects of *Inga densiflora* on the microclimate of coffee (*Coffea arabica* L.) and overall biomass under optimal growing conditions in Costa Rica. Agrofor Syst 78: 269-286.

Soto-Pinto L, Villalvazo-Lopez V, Jimenez-Ferrer G, Ramirez-Marcial N, Montoya G, Sinclair FL. 2007. The role of local knowledge in determining shade composition of multistrata coffee systems in Chiapas, Mexico. Biodivers Conserv 16: 419-436.

Sumantra IK, Ashari A, Wardiyati T, Suryanto A. 2012. Diversity of shade trees and their influence on the microclimate of agro-ecosystem and fruit production of Gulapasir Salak (*Salacca zalacca* var. Amboinensis) fruit. Int J Basic Appl Sci 12: 214-221.

Tadesse G, Zavaleta E, Shennan C. 2014. Coffee landscapes as refugia for native woody biodiversity as forest loss continues in southwest Ethiopia. Biol Conserv 169: 384-391.

Tscharntke T, Clough Y, Bhagwat SA, Buchori D, Faust H, Hertel D, Holscher D, Juhrbandt J, Kessler M, Perfecto I, Scherber C, Schroth G, Veldkamp E, Wanger TC. 2011. Multifunctional shade-tree management in tropical agroforestry landscapes - a review. J Appl Ecol 48: 619-629.

Valencia V, West P, Sterling EJ, Garcia-Barrios L, Naeem S. 2015. The use of farmers' knowledge in coffee agroforestry management: implications for the conservation of tree biodiversity. Ecosphere 6 (7): 122. http: //dx.doi.org/10.1890/ES14-00428.1

Verbist B, Putra AED, Budidarsono S. 2005. Factors driving land use change: Effects on watershed functions in a coffee agroforestry system in Lampung, Sumatra. Agric Syst 85: 254-270.

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Evaluating the level of sustainability of privately managed forest in Bogor, Indonesia

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Abstract. *Sukwika T, Darusman D, Kusmana C, Nurrochmad DR. 2016. Evaluating the level of sustainability of privately managed forest in Bogor, Indonesia. Biodiversitas 17: 241-248.* This study discusses the sustainability of small scale private forest in Bogor, Indonesia. It aims to determine the dimensions of sustainable private-forest and analyzing the sustainability index of privately managed forest. This study uses multidimensional scaling (MDS) to analyze the dimensions of sustainability, ranked from 0 (the lowest) to 10 (the highest), along with the support of *Rap-Pforest,* in order to assess the level of similarity and dissimilarity for each dimension. Using this scale from the sustainability index, this study estimates the level of sustainability of each dimension. After measuring each attribute's level of ordination RMS change on the X axis, we estimate the error's effect using Monte Carlo analysis. This study shows that the ecology as well as legal and institutional dimensions are moderately sustainable, with a sustainability index of 53.66% and 52.48%. However, the dimensions of economy, socio-culture, as well as accessibility and technology are less sustainable, with an index measurement of 41.62%, 47.02% and 47.56%, respectively. Based on those five sustainability dimensions, this study concludes that in average the level of sustainability of private-forest management in the Bogor is not sustainable (48.47%). We recommend that to improve the sustainability of small scale private forest management in Bogor, multiple stakeholders should be involved to development the most appropriate policy options.

Keywords Bogor, Monte Carlo analysis, private-forest, RMS, small scale forestry, sustainability index

INTRODUCTION

The total area of private-forests in Bogor is estimated to be 16,945 hectares, much less than the 74,521 acres of state state-owned forest (Distanhut 2014). According to the Distanhut (2008, 2014), the area of state forest of Bogor in 2008 was 79,437 hectares and decreased to 74,521 hectares in 2014. Meanwhile, the area of private-forest in Bogor was 11,379 hectares in 2008, and increased to 16,945 hectares in 2012.Increasing private-forest area does not automatically deliver economic and social benefits to proximate communities. In Bogor, people living around forests are still vulnerable to poverty. Distanhut (2012) reports that people who live around the forest are mostly poor, due to their reliance on (small-scale) farming and on-farm employment for living. On average, these households own between 0.25 and 1 hectares, including self-ownership and co-ownership (Andayani 2003; Birgantoro and Nurrochmat 2007; Kigenyi 2007; Plencovich 2014).The major contributors to continued poverty within these small-scale farming communities include low levels of technology and low market price. There is no exact planting time and harvesting schedule for private-forests. The basic capacity of extension workers, low level of access to information, and poor infrastructure also contribute to continued poverty in this area. All of these problems combine to contribute to stakeholders' low awareness on the sustainability issues (Darusman and Hardjanto 2006; Gunarso et al. 2007a).

Sustainable forest management is a way of using and caring for forests in order to maintain environmental, social, and economic values and benefits over time. "As a dynamic and evolving concept, it aims to maintain and enhance the economic, social and environmental value of all types of forests, for the benefit of present and future generations" (UN 2008; FAO 2010). Sustainable forest management is characterized by: (i) extent of forest resources; (ii) forest biological diversity; (iii) forest health and vitality; (iv) productive functions of forest resources; (v) protective functions of forest resources; (vi) socio-economic functions of forests; and (vii) legal, policy, and institutional framework and infrastructure (Prabhu et al. 1998; Levang 2002; Clarke 2006; Gunarso et al. 2007b; UN 2008; Nasi and Frost 2009; SCBD 2009; Kant 2010; Kant et al. 2013; Nurrochmat and Abdulah 2014).

Private-forest owners play a key role in sustaining forest ecosystems, enhancing rural development, and supplying resources to markets. Nevertheless, a significant lack of knowledge remains regarding private forest ownership (Schmithüsen and Hirsch 2010; Kant et al. 2013). Private-forest areas have an important role in the economic development in Bogor, but poor forest management has caused environmental damage. Factors causing deforestation in private-forest areas include activities performed by the inhabitants, increased population, and land conversion (Angelsen 1999; Kant and Lee 2004; Darusman and Wijayanto 2007; Gunarso et al. 2007a; Chakravarty et al. 2012).

In addition to poverty, other issues related to the private-forest degradation include relatively low levels of land ownership, low education, and lack of skills outside the agriculture and forestry sectors (Kusmana 2011; Zhang and Pearse 2011; Kant et al. 2013). This study discusses the obstacles that frequently prevent the sustainable management of private forests, including physical capital (Febriani et al. 2012), market incentives and private forest ownership (Kigenyi 2007; Wijayanto 2007; Kusmana 2011; Suryawati et al. 2011; Wolosin et al. 2012; Silas, 2014), and the contribution of market demand for timber products (Mutaqin 2008; Kant 2003, 2004; Kant and Berry 2005; Agrawal et al. 2013; Wollenberg 2014).

This study aims to assess the level of sustainability of private-forest in Bogor based on five key dimensions: ecology, economy, socio-culture, legality and institutional factors, as well as infrastructure and technology. It analyzes these dimensions using MDS (Multidimensional Scaling); to ascertain the sustainability index of privately managed forests in Bogor.

MATERIALS AND METHODS

This study was conducted within the District of Bogor, West Java Province, Indonesia from April 2015 to August 2015. Bogor has a total area of 298 thousand hectares, with 14.32% forest cover. This area consists of protected and production forest. Protected forest area is mostly located in the upland areas, and it serves as a water catchment area, while the production forest areas spread from lowlands to uplands.

Data collection

To detect the level of sustainability we use Multidimensional Scaling (MDS). MDS is a method of multivariate statistical analysis that determines the position of a concept based on similarity or dissimilarity to another principle or concept (Borg and Groenen; 1997; Groenen and van de Velden 2004; Groenen and Terada 2015). Yaoung (2009) indicates that MDS is a data analysis technique which displays conceptual similarity in the form of geometric images based on the Euclidean distance between concepts, based on questionnaire responses. This analysis occurs through several stages. (i) The determination of the private-forest sustainability dimensions of the Bogor District that includes five dimensions: ecological, economic, socio-cultural, legal and institutional, as well as infrastructure and technology. Each dimension is then measured using attributes scores (Pitcher and Preiksho 2001). (ii) The valuation of each attribute in an ordinal scale is based on sustainability criteria of each dimension. Expert respondents used *scientific judgment* to determine the attribute of each dimension. Experts scored the attributes of each dimension between 0 and 10 (Pitcher et al. 2013). (iii) Finally, this method is used to calculate the sustainability index and analyze the status of sustainability.

Through the MDS method, the position of the point of sustainability can be visualized through the horizontal and vertical axis. With rotation, the position of the point can be visualized on a horizontal axis with a rated value of the sustainability index score. Score estimation of each dimension is expressed from the lowest score (unsustainable) 0% to the best (sustainable) 100% (see Figure 1), and grouped into four categories namely; 0-25.00% (bad or unsustainable), 25.01-50.00% (less sustainable), 50.01-75.00% (fairly sustainable), and 75.01-100.00% (highly sustainable). The sustainability index includes the value of each dimension to describe the total level of sustainability (Pitcher 1999). Table 1 illustrates the index and rankings.

The index value of sustainability of each dimension can be visualized at the same time using a kite diagram. The symmetrical of kite diagram is determined by the sustainability index of each dimension (economic, social and culture, ecological, legal and institutional as well as infrastructure and technology). Further, kite diagrams display the value of sustainability index for each dimension.

Sensitivity analysis provides further information onthe MDS analysis and the private-forest sustainability index. Sensitivity analysis indicates which attributes contribute to the resources sustainability value. This sensitivity analysis used the attribute leveraging to assess the change in the analytical output from MDS. The effect of each attribute is observed in the change of root mean square (RMS), particularly on the x-axis for resources sustainability scale (Kavanagh 2001). The RMS formula is as follows:

$$RMS = \sqrt{\left[\frac{\sum_{i=1}^{n}\{Vf(i,1) - Vf(,1)\}^2}{n}\right]}$$

Vf (i1) = Value of MDS output (after rotation and flipping).
Vf (,1) = Median of MDS output in column-1.

Table 1. Sustainability status category of privately managed forest in Bogor District, Indonesia (Fauzi and Anna 2005)

Index value	Category
00.00-25.00	Poor (not continuous)
25.01-50.00	Less (less sustainable)
50.01-75.00	Enough (quite sustainable)
75.01-100.00	Good (very sustainable)

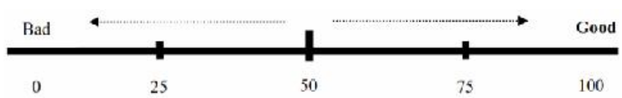

Figure 1. Rate value of sustainability index score of 0% (bad) to 100% (good)

Monte Carlo analysis evaluates the error effect by assessing the ordination. The effect of error can be caused by various conditions, including error in scoring due to imperfect understanding of the attribute or field condition, score variation from different opinion or valuation of the researcher, repeated MDS analytical processes, error in data input or missing data, iteration stability, and high stress value (acceptable stress value should be <25%) (Kavanagh and Pitcher 2004; Fauzi and Anna 2002).

Goodness of fit in MDS is indicated by the amount of S-Stress value, calculated based on the value of S and R^2. Lower stress values indicate a *good fit* while higher S values indicate the opposite. In the approach with *Rap-Pforest*, a good model contains a stress value less than 0.25 or S<0.25 (Fauzi and Anna 2005) and relatively better fitting models have an R^2 that approaches 1.0.

RESULTS AND DISCUSSION

Ecological dimension

Analysis from *Rap-Pforest* (Rapfish modification) shows that the sustainability index for the ecological dimension is 53.66 (Figure 2). This indicates that the ecological dimension is "quite sustainable". The main factors that contribute to the sustainability of this dimension include the potential of efficient land use by proximate communities (RMS=2.73), critical land conservation (RMS=2.20) and infrequent land conversion (RMS=2.33). These attributes contained the greatest values within the ecological dimension.

Economic dimension

This study indicates that the economic dimension has a value of 41.62 above the midpoint between unsustainable and sustainable (Figure 3). Thus, the economic dimension should be considered "less sustainable" based on the sustainability index value. Of the 17 attributes within the economic dimension, the results show wood productivity (RMS=2.37), incentive for farmer price of timber (RMS=1.61), monthly income of farmer (RMS=1.66) and middlemen (broker) (RMS=1.71) are the attributes that contribute most to the economic dimension.

Timber prices for private-forests are often determined by middlemen (*tengkulak*) and debt bondage (*ijon*). This represents a series of transactions that are not profitable for farmers.

Socio-culture dimension

This study indicates that the sustainability index on socio-culture dimension is 47.02 (Figure 4), and is thus considered "less sustainable". This score is driven by the inability for the forestry sector to sufficiently employ available human resources, and the related high levels of poverty. There are three attributes that are the most sensitive and should get the attention to increase the sustainability value of social and culture dimension, including: farmer participation for adding value (RMS=1.65), poverty (RMS=1.24), and employment opportunity (RMS=1.27).

Promoting community participation, increasing employment opportunities within the local forestry sector, and reducing poverty can increase the sustainability of the socio-culture dimension. Specifically, funding assistance and post-capture processing training can provide added value to timber. Increasing the added value from timber can directly increase farmer income, and reduce poverty within forest proximate communities.

Legal and institutional dimension

Figure 5 shows that legal and institutional dimension has a value of 52.48, indicating an index score of "quite sustainable". Of the 11 attributes on the legal and institutional dimension, the most sensitive attributes include microfinance institution (RMS=2.50), the number of forestry extension/counseling (RMS=2.59), government elucidation institution (RMS=2.32), and forestry extension/ counseling programs (RMS=2.67).

Of the 11 attributes that comprise the Legal and Institutional dimension, the number of forestry extension/ counseling of the private-forest management and forestry extension/counseling programs in the Bogor District are the most sensitive. In order to enhance the sustainability index, local government can increase the number of forestry extension organizations and/or agents in order to disseminate and implement more effective government forestry programs.

Accessibility and technology dimension

The value of sustainability index of the accessibility and technology dimension based on the *Rap-Pforest* analysis is 49.77 (Figure 6), categorized as "less sustainable". Leverage analysis indicates that of the 11 attributes in this dimension, four attributes contributed the most to the accessibility and technology dimension: the support of road infrastructure to public services (RMS=2.28), market information access (RMS=2.88), post-harvest processing of wood (RMS=3.13) and standardization of felling of trees (RMS=2.24).

Inter-village transport is limited. It depends upon the use of a private vehicle (commonly a motorbike), and roads are occasionally closed because of landslides or a lack of maintenance. In addition, knowledge of and access to information on post-harvest technology, important for ensuring the quality of timber and appropriate prices, is limited within communities that own private forests.

The overall result of the leverage analysis for the five dimensions generated 18 attributes that have substantial impacts on the management of sustainable private forests (Table 2). These values were selected based on their RMS (root mean square) value. These leverage factors are important for developing a model of sustainable private forest management policy.

Test of validity

The Monte Carlo test of validity indicates that the differences of average value of the two analyses are 0.60%. This means that the MDS analysis model is adequate for estimating the sustainability index value of the private-forest in the Bogor District. The small validity

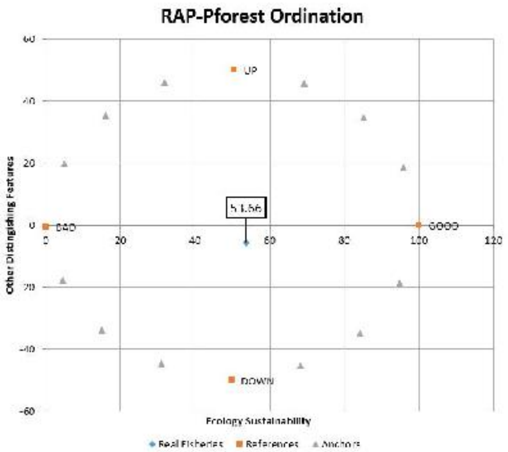

Figure 2. Ecological sustainability index in Bogor District, Indonesia

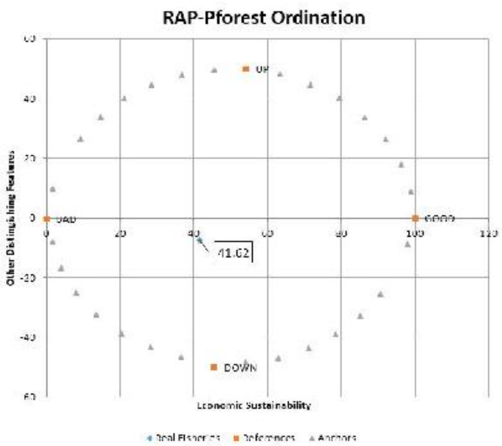

Figure 3. Economic sustainability index in Bogor District, Indonesia

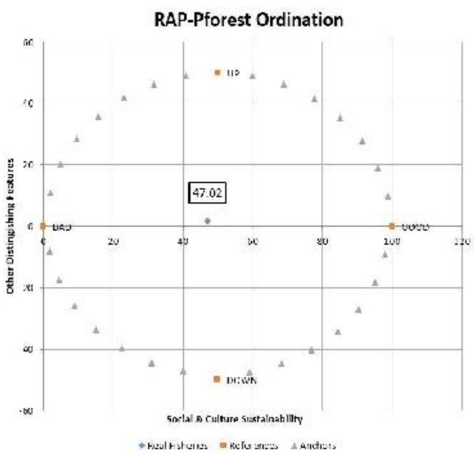

Figure 4. Social and culture sustainability index in Bogor District, Indonesia

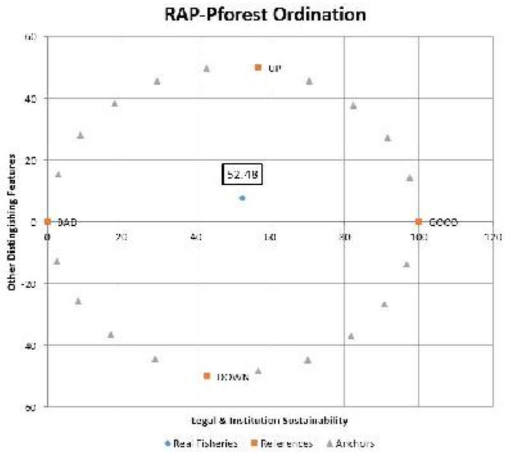

Figure 5. Legal and institutional sustainability index in Bogor District, Indonesia

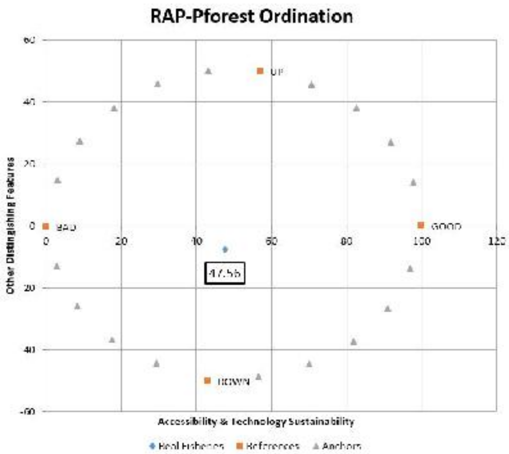

Figure 6. Accessibility and technology sustainability index in Bogor District, Indonesia

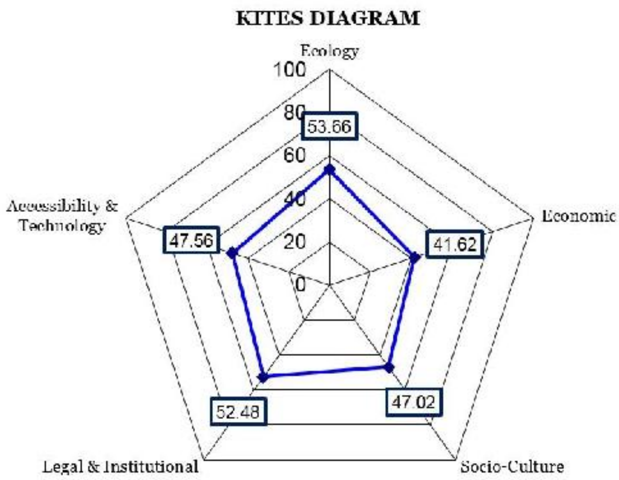

Figure 7. Kites diagram of the privately managed forest in Bogor District, Indonesia based on *Rap-Pforest* analysis result

Table 2. Sensitive attributes of sustainability of privately managed forest in Bogor District, Indonesia

Dimension	Attribute	RMS
Ecology	1. Land using efficiency by community	2.73
	2. Critical land conservation	2.20
	3. Land conversion	2.33
Economic	4. Wood productivity	2.37
	5. Incentive for farmer price	1.61
	6. Monthly income of farmer	1.66
	7. Broker (middlemen)	1.71
Socio-culture	8. Farmer participation for adding value	1,65
	9. Poverty (farm household)	1,24
	10. Employment opportunity	1.27
Legal and institutional	11. Microfinance institution	2.50
	12. The number of forestry extension/counseling	2.59
	13. Government extension/counseling institution	2.32
	14. Forestry extension/counseling programs	2.67
Accessibility and technology	15. Road infrastructure to public services	2.28
	16. Market information access	2.88
	17. Post-harvest processing of wood	3.13
	18. Standardization of felling of trees	2.24

Table 3. Difference of sustainability index value of *Rap-Pforest* in Bogor District, Indonesia and the Monte Carlo analysis

Dimension	Sustainability Index Value (%)			
	MDS	Monte Carlo (MC)	Difference (MDS-MC)	Difference (MDS-MC) %
Ecology	53.66	53.69	0.64	1.21
Economy	41.62	41.43	0.19	0.46
Socio-culture	47.02	46.67	0.35	1.75
Legal and institutional	52.48	52.26	0.22	0.42
Accessibility and technology	47.56	47.52	0.04	0.08
Average	48.47	48.18	0.29	0.60

Table 4. Stress value and the value of determination (R^2) *Rap-Pforest* result in Bogor District, Indonesia

Parameter	Dimension of				
	Ecology	Economy	Socio-culture	Legal and institutional	Accessibility and technology
Value of Index*	53.66	41.62	47.02	52.48	47.56
Value of Stress**	0.156	0.136	0.143	0.142	0.140
Value of R^2 ***	94.48	95.40	95.13	95.11	95.23
Number of Iteration	2.00	2.00	2.00	2.00	2.00

Note: *) Index value 50.01-75.00 is quite sustainable. **) Stress value < 0.25 is goodness of fit. ***) R^2 value 95% or > 80% is excellence contribution

value indicates the error from data acquisition and analysis is minimal, and does not jeopardize the results from this study (Fauzi et al. 2005).

Monte Carlo analysis can also be used as simulation methods to evaluate the impact of random error on the statistical analysis conducted for all dimensions (Pitcher and Preiksho 2001; Kavanagh and Pitcher 2004). Table 3 contains the results from MDS and Monte Carlo Analysis.

Test of accuracy

The accuracy test of the MDS analysis (good and fit) obtained the coefficient of determination (R^2) between 94.48%-95.40%. Since this value is larger than 80%, it is categorized as both good and fit (Kavanagh 2001). The stress value of 0.136 to 0.156, with a difference of 0.02, indicates the results obtained from MDS analysis is highly accurate (good and fit), and sufficient for assessing the private-forest sustainability index in Bogor, West Java (Fisheries 1999). Table 4 contains the stress value of the determination coefficient from the *Rap-Pforest* analysis.

Discussion

Based on the analysis of the sustainability index value for the five dimensions, Figure 7 illustrates the kites diagram from privately managed forests in the district of Bogor. Figure 7 demonstrates that economic, socio-culture, and accessibility and technology are "less sustainable", amounting to 41.62%, 47.02% and 47.56%, respectively. Two dimensions ecology as well as legal and institutional, are "quite sustainable", with sustainability indices of 53.66% and 52.48%, respectively. Based on the five sustainability indexes (see Table 3), the level of sustainability of privately managed forest in the Bogor is, overall, "less sustainable" (48.47%).

These five dimensions were calculated from expert opinion scores and bundled in sustainability dimensions. Within the dimension of ecology there are three leverage variables, one of which is efficiency of community-based land utilization (RMS value 2.73). Experts agree that farmers' behavior in land utilizing was very exploitative and less-organized (for instance, farmers used sporadic distances to cultivate agroforestry and inter-cropping). In the long term, this behavior can result in negative externalities for the land and ecosystem services surrounding forest areas. These negative ecological externalities should be considered in tandem with the poverty of farmers near forest areas (Guntoro 2011; Ingram et al. 2012).

Poverty and ecological degradation cannot be separated. Ecological degradation causes poverty, and poverty can increase ecological degradation (DeClerck 2006). In community forests, human-environment interactions are many (Abel and Stepp 2003; De Sherbinin et al. 2007). Thus, integrated and comprehensive poverty alleviation should not overlook ecological aspects. Farmers are not independent from forest ecosystems, as these systems provide environmental-based goods and services. Even welfare delivered mostly through markets often comes from ecosystem services (Wildenberg 2005; Schneider et al. 2010).

Within economic dimensions, the poverty of private-forest farmers (RMS value 1.24) was the main focus for the experts, as farmers' expenditures were three times that of their monthly income. In order to cover the monthly needs, the farmers rely on middlemen/broker (*tengkulak*) to buy their timber and non-timber production. Farmers sold sengon (*Albizia falcataria*) and kayu afrika (*Maesopsis eminii*). Farmers often sold these timber products at prices far below standard market valuation. Despite the profitability of timber markets elsewhere in Indonesia, the timber market in Bogor does not significantly contribute to the regional economy. [Data of BPS (2015) noted gross domestic product/GDP's forestry=1.89%].The availability of timber product in Bogor is purported to meet only local and regional needs. Based on information from interviews with key informants that despite this trend, there are remained many opportunities within the forestry sector. However, many who are able to work within this sector prefer manufacturing jobs or unemployment. Thus, private forests are managed mostly by the elderly. These results in a gap between their monthly expenses and income, and it contribute to the persistence of regional poverty. This is then also a concern in social dimension.

Experts agree that, for the accessibility and technology dimension, farmers do not use harvesting or processing technology, although they have some access to this technology (RMS value 2.24). According to data from this study, private forest managers employed brokers' services, such as logging, skidding, and transportation to the wood processing industry. The strong role of brokers has a negative impact on farmer's welfare, due to the reduction of timber prices (Nurrochmat et al. 2014). Despite promoting the community's involvement or participation in forest management, as well as reducing the role of broker (*tengkulak*), a private forestry policy system is necessary to develop the region's timber-based management and processing through an integrated system that involves community (society) and business (Sahide et al. 2015). Meanwhile, within the legal and institutional dimension, experts noted the lack of availability of extension workers (RMS value 2.59), as well as the uncertainty of their counseling schedule. The presence of extension workers is promotes the importance of ensuring economic, social and ecological sustainability.

The limited capacity for private forest farmers to implement planting and logging standards, and the current lack of community-level institution, have rendered private-forest utilization less successful and less sustainable. Meanwhile, the lack of available field officers to support farmers decreases the productivity private forests while the limited availability knowledge about processing technology, combined with minimal market access, have contributed to unfair timber pricing at the expense of farmer incomes.

Based on the five sustainability dimension that have been analyzed here, the private-forest management in the Bogor is "less sustainable", with the average sustainability index of 48.47<50. Focusing on the 18 sensitive attributes of sustainability of privately managed forest in Bogor (see Table 2), public and/or private programs should begin by monitoring the role of timber brokers.

To increase the sustainability index within the economic dimension, government and/or stakeholder intervention is needed to support a standard pricing for timber products, fertilizer, and insecticide. Further assistance to improve the empowerment of farmers' groups through a more productive planting program, farmers' timber-product price stabilization through a comprehensive partnership program on timber-product industrialization could also assist in the improvement of economic sustainability from private forests in Bogor.

To ensure sustainability of private forests in Bogor, it is necessary to (i) involve all stakeholders of society, businessmen and government in the management of private-forest resources in the Bogor, (ii) reduce the role of brokers by providing farmers information on timber prices and fair pricing incentives, and (iii) formulate and implement a strategy for development through multi-stakeholder engagement.

REFERENCES

Abel T, Stepp JR. 2003. A new ecosystems ecology for anthropology. Conserv Ecol 7 (3): 12.

Andayani W. 2003. The strategy for improving business efficiency of private forest. Jurnal Hutan Rakyat 5 (3): 17-29. [Indonesian]

Agrawal A, Cashore B, Hardin R, Shepherd G, Benson C, Miller D. 2013. Economic contributions of forests. United Nations Forum on Forests 20 March 2013. Istanbul, Turkey. Analysis of multiple forest values in Southern Ontario. For Pol Econ 6 (3-4): 215-227.
Angelsen A. 1999. Agricultural expansion and deforestation: modeling the impact of population, market forces and property rights. J Dev Econ 58: 185-218.
BPS [Badan Pusat Statistik]. 2015. Bogor District in Figure 2015. BPS, Bogor. [Indonesian]
Birgantoro BA, Nurrochmat DR. 2007. Utilization of forest resources by communities in KPH North Banyuwangi. Jurnal Manajemen Hutan Tropika 13 (3): 172-181.[Indonesian]
Borg I, Groenen PJF. 1997. Modern Multidimensional Scaling: Theory and Applications. Springer, New York.
Chakravarty S, Ghosh SK, Suresh CP, Dey AN, Shukla G. 2012. Deforestation: Causes, Effects and Control Strategies, Global Perspectives on Sustainable Forest Management. Clement A. Okia (ed.). InTech Europe, Rijeka, Croatia.
Clarke J. 2006. Trends in Forest Ownership, Forest Resources Tenure and Institutional Arrangements: Are They Contributing to Better Forest Management and Poverty Reduction? A Case Study From South Africa. FAO, Rome.
Darusman D, Hardjanto. 2006. Economic overview of private forest. Prosiding Seminar Hasil Penelitian Hasil Hutan 2006: 4-13. Departemen Kehutanan, Jakarta. [Indonesian]
Darusman D, Wijayanto N. 2007. The economics of private forest (Funding Scheme). Studium General dalam Pekan Hutan Rakyat II di Balai Penelitian Kehutanan Ciamis, 30 Oktober 2007. [Indonesian]
DeClerck F, Ingram JC, Rumbaitis del Rio C.2006. The role of ecological theory and practice in poverty alleviation and environmental conservation. Front Ecol 4: 533-540.
DeSherbinin A, Carr D, Cassels S, Jiang L. 2007. Population and environment. Ann Rev Environ Resour 32: 5.1-5.29.
Distanhut [Dinas Pertanian dan Kehutanan Kabupaten Bogor]. 2008. Annual Report of Agriculture and Forestry: A Monograph. Office of Agriculture and Forestry Bogor District, Bogor. [Indonesian]
Distanhut [Dinas Pertanian dan Kehutanan Kabupaten Bogor]. 2012. Annual Report of Agriculture and Forestry: A Monograph. Office of Agriculture and Forestry Bogor District, Bogor. [Indonesian]
Distanhut [Dinas Pertanian dan Kehutanan Kabupaten Bogor]. 2014. Annual Report of Agriculture and Forestry: A Monograph. Office of Agriculture and Forestry Bogor District, Bogor. [Indonesian]
FAO [Food and Agriculture Organization]. 2010. Global Forest Resources Assessment 2010. FAO, Rome.
Fauzi A, Anna Z. 2005. Modeling of Fisheries and Marine Resources. Gramedia. Jakarta [Indonesian]
Febriani D, Darusman D, Nurrochmat DR, Wijayanto N. 2012. Policy implementation strategy on Community Plantation Forest in Sarolangun District, Jambi. Jurnal Analisis Kebijakan Kehutanan. 9 (2): 81-95. [Indonesian]
Groenen PJF, Terada Y. 2015. Symbolic Multidimensional Scaling (No. EI 2015-15). www.repub.eur.nl/pub/78189/EI2015-15.pdf
Groenen PJF, van de Velden M. 2004. Multidimensional Scaling. (No. EI 2004-15). www.repub.eur.nl/pub/1274/ei200415.pdf
Gunarso P, Setyawati T, Sunderland T, Shackleton C (eds.). 2007a. Managing Forest Resources in a Decentralized Environment: Lessons Learnt from the Malinau Research Forest, East Kalimantan. Center for International Forestry Research (CIFOR), Bogor.
Gunarso P, Santosa KD, Shackleton C, Sunderland T, Campbell BM, Priyadi H, Levang P, Sheil D, Dounias E. 2007b. Towards Sustainable Forest Management and Improved Livelihoods In Tropical Forests: Lessons and Conclusions. CIFOR, Bogor.
Guntoro S. 2011. Time to Implement Techno-Ecological Agriculture. An Agricultural Future Model for Addressing Climate Change. PT Agromedia Pusaka, Jakarta [Indonesian]
Ingram JC, DeClerck F, Rumbaitis del Rio C (eds.). Integrating Ecology and Poverty Reduction: The Application of Ecology in Development Solutions. Springer, New York. DOI: 10.1007/978-1-4614-0186-5_6
Kant S, Wang S, Deegen P, Hostettler M, von Detten R, Howard T, Laband D, Montgomery C, Roberti N, Sekot W, Valatin G, Zhang D. 2013. New frontiers of forest economics. For Pol Econ 35: 1-8.
Kant S. 2010. Global Trends in Ownership and Tenure of Forest Resources and Timber Pricing. Professional Foresters Association, Ontario.
Kant S. 2003. Extending the boundaries of forest economics. For Pol Econ 5: 39-56.
Kant S. 2004. Economics of sustainable forest management. For Pol Econ 6: 197-203.
Kant S, Berry A. 2005. Economics. Sustainability, and Natural Resources: Economics of Sustainable Forest Management. Springer, Dordrecht.
Kant S, Lee S. 2004. A social choice approach to sustainable forest management: an analysis of multiple forest values in Northwestern Ontario. For Pol Econ 6: 215-227.
Kavanagh P. 2001, Rapid Appraisal of Fisheries (Rapfish) Project: Rapfish Software Description (for Microsoft Excel). University of British Columbia, Vancouver.
Kavanagh P, Pitcher TJ. 2004. Implementing Microsoft Excel Software for Rapfish: A Technique for the Rapid Appraisal of Fisheries Status. Tech. Rept. 12 (2). Fisheries Centre Research Reports, Vancouver.
Kigenyi FW. 2007. Trends In Forest Ownership, Forest Resources Tenure And Institutional Arrangements: Are They Contributing To Better Forest Management And Poverty Reduction? A Case Study From Uganda. FAO, Rome.
Kusmana C. 2011. Forest Resources and Forestry in Indonesia. For Sci Technol 7: 4, 155-160.
Levang P. 2002. People's Dependencies on Forests. In Technical Report, Phase I 1997-2001. ITTO Project PD 12/97 Rev. 1 (F)-Forest, Science and Sustainability: The Bulungan Model Forest. CIFOR, Bogor.
Marwa J, Purnomo H, Nurrochmat DR. 2010. Managing the Last Frontier of Indonesian Forest in Papua. IPB-AKECOP Korea, Bogor.
Muttaqin MZ. 2008. Good Governance in the Five Priority Policies of the Ministry of Forestry. Jurnal Analisis Kebijakan 5 (3): 143-151. [Indonesian]
Nasi R, Frost PGH. 2009. Sustainable forest management in the tropics: is everything in order but the patient still dying? Ecol Soc (2): 40.
Nurrochmat DR, Abdulah L. 2014. Utilizing forests, reducing emissions. Risalah Kebijakan Pertanian dan Lingkungan 1 (1): 18-23. [Indonesian]
Nurrochmat DR, Darusman D, Ruchjadi D. 2014. Reconstruction of the forestry tenure system. Risalah Kebijakan Pertanian dan Lingkungan 1 (1): 24-29. [Indonesian]
Plencovich MC. 2014. Voices from the forest: Integrating indigenous knowledge into sustainable upland farming. J Agric Educ Extens 20 (1): 147-149.
Pitcher TJ. 1999. Rapfish, a Rapid Appraisal Technique for Fisheries, and it Application to the Code of Conduct for Responsible Fisheries. University of British Columbia, Vancouver.
Pitcher TJ, Preiksho D. 2001. RAPFISH: a rapid appraisal technique to evaluate the sustainability status of fisheries. Fish Res 49: 255-270.
Pitcher TJ, Lam ME, Ainsworth C, Martindale A, Nakamura K, Perry RI, Ward T. 2013. Improvements to Rapfish: a rapid evaluation technique for fisheries integrating ecological and human dimensions. J Fish Biol 83: 865-889.
Prabhu R, Colfer C, Shepherd G. 1998. Criteria and Dimensions for Sustainable Forest Management: New Findings from CIFOR's Forest Management Unit Level Research. Rural Development Forestry Network, London.
Sahide MAK, Supratman S, Maryudi A, Giessen L. 2015. Forest Management Units Experimentation in Indonesia: Is it Supporting Devolution and Forest Management or Re-Centralisation?. Management of land use systems for enhanced food security-conflicts, controversies and resolutions. Tropentag, September 16-18, 2015 in Berlin.
SCBD [Secretariat of the Convention on Biological Diversity]. 2009. Sustainable Forest Management, Biodiversity and Livelihoods: A Good Practice Guide. Secretariat of the Convention on Biological Diversity, Montreal.
Schmithüsen F, Hirsch F. 2010. Private Forest Ownership In Europe. Geneva Timber and Forest Study Paper 26. Forestry and Timber Section, Geneva.
Schneider F, Jallis G, Martinez-Alier J. 2010. Crisis or opportunity? Economic degrowth for social equity and ecological sustainability. Introduction to this special issue. J Clean Prod 18: 511-518.
Silas SR. 2014. Reducing impact of land degradation in Tanzania: Do incentive market based mechanisms work for sustainable land management? J Sustain Dev 7 (6): 2014. DOI: 10.5539/jsd.v7n6p1
Subagyo PJ. 2006. Research Methods in Theoretical and Practical. Rineka Cipta, Jakarta. [Indonesian]
Suryawati SH, Soetarto E, Adrianto L, Purnomo AH. 2011. Identification resource management incentive systems at Laguna Segara Anakan.

Jurnal Kebijakan Sosial Ekonomi Kelautan dan Perikanan 1 (1):- [Indonesian]

UN [United Nations]. 2008. Resolution Adopted by the General Assembly 62/98: Non-legally Binding Instruments on All Types of Forests. www.daccessdds.un.org/doc/UNDOC/GEN/N07/469/65/PDF/N0746965.pdf

Wildenberg M. 2005. Ecology, Rituals and System-Dynamics. An attempt to Model the Socio-Ecological System of Trinket Island. Social Ecology Working Paper 80. Vienna.

Wollenberg E, Edmunds D, Buck L. 2001. Anticipating Change: Scenarios for Adaptive Forest Management Facility. CIFOR, Bogor.

Wolosin M, Elias P, Riddle A, Nguyen D. 2012. Forest Interventions with Economic Returns. Issue Paper November 2012. Climate Advisers, Washington, DC.

Young FY. 2009. Multidimensional Scaling (MDS). University of North Carolina, Chapel Hill.

Zhang D, Pearse PH. 2011. Forest Economics. UBC Press, Vancouver.

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The diversity of plant species, the types of plant uses and the estimate of carbon stock in agroforestry system in Harapan Makmur Village, Bengkulu, Indonesia

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Abstract. *Wiryono, Puteri VNU, Senoaji G. 2016. The diversity of plant species, the types of plant uses and the estimate of carbon stock in agroforestry system in Harapan Makmur Village, Bengkulu, Indonesia. Biodiversitas 17: 249-255.* Homegardens are a traditional form of agroforestry commonly found in rural areas in Indonesia, where a variety of agricultural crops and forest trees are grown in a mixed system. To some extent, the traditional homegardens resemble natural forest in vegetation structure and composition. The objective of this study was to know the diversity of plant species, the types of plant uses and the estimate of carbon stock in homegardens in, Harapan Makmur Village, Central Bengkulu District, Bengkulu Province, Indonesia. The field work was conducted in 2013. The data of uses were collected through interview, while data on vegetation were gathered from measurement. The data were analyzed qualitatively and quantitatively. The results showed that 101 species of plants were found in the homegardens, with a Shannon-Wiener diversity index of 0.99 for trees. The most dominant species of trees was *Hevea brasiliensis* Willd (rubber tree) with an importance value index of 127 %. For saplings and shrubs, rubber was also the most dominant with 169 individuals, while for herbs, *Agrostis* sp. was the most dominant species with an average coverage of 25.8 %. The community used plants for several purposes: 41 species for food, 21 for fire wood, 13 for ornamental plants, 11 for medicines, 7 for construction wood, 6 for shade trees, 2 for handy craft, 4 for hedge, 3 for forage, and 2 for coloring. Twenty three species were not used. The estimate of carbon stock in trees was 95.2 ton ha^{-1}.

Keywords: Agroforestry, homegardens, carbon stock, species diversity

INTRODUCTION

Having vast area of wet tropical forest, Indonesia is rich in biodiversity. However, large-scale forest exploitation which have been done for several decades has caused massive deforestation, which in turn can reduce biodiversity. Within the period of 2000-2009, the total area of deforestation in Indonesia was 15.6 million hectares (FWI 2011). Conversion of natural forest into oil palm plantation has been a driving force of deforestation in Indonesia, and most the oil palm plantation is located in Sumatra (Saxon and Roquemore 2011). Not only large plantation companies, but also farmers grow oil palm in their land. The farmers sell their oil palm fruit to the collectors who will sell the fruit to companies. Not only dry land, even rice field has been converted to oil palm plantation which threatens food security (Wildayana 2015).

Fortunately, some villagers still maintain agroforestry system in their homegardens. Agroforestry is a farming system, combining agricultural crops and forest trees. Agroforestry can also be defined as a dynamic, ecologically based, natural resources management system that, through the integration of trees on farms and in the agricultural landscape, diversifies and sustains production for increased social, economic and environmental benefits for land users at all levels (Mead 2004). It is a traditional farming system practiced in many parts of the world. In developing countries, this system has been practiced until today, but even in Europe it was a general practice until Middle Ages (King 1987). Agroforestry system may balance the goal of agricultural development and conservation of soil, water, regional climate and biodiversity (Schroth et al. 2004). The role of agroforestry in biodiversity conservation has been recognized, and recently, there have been a growing interest on the role of carbon sequestration in agroforestry system (Takimoto et al 2009, Kessler et al 2012).

The objective of this study was to know the diversity of plant species, the types of plant uses and the estimate of carbon stock in home gardens in Hamlet II of Harapan Makmur Village, in Pondok Kubang Sub-District, Central Bengkulu District, Bengkulu Province, Indonesia.

MATERIALS AND METHODS

Study area

This study was conducted in Hamlet II, Harapan Makmur Village, Pondok Kubang Sub-District, Central Bengkulu District, Bengkulu Province, Indonesia (Figure 1), from January to May 2013. Additional data were taken in early March 2016. Most of the residents are Javanese.

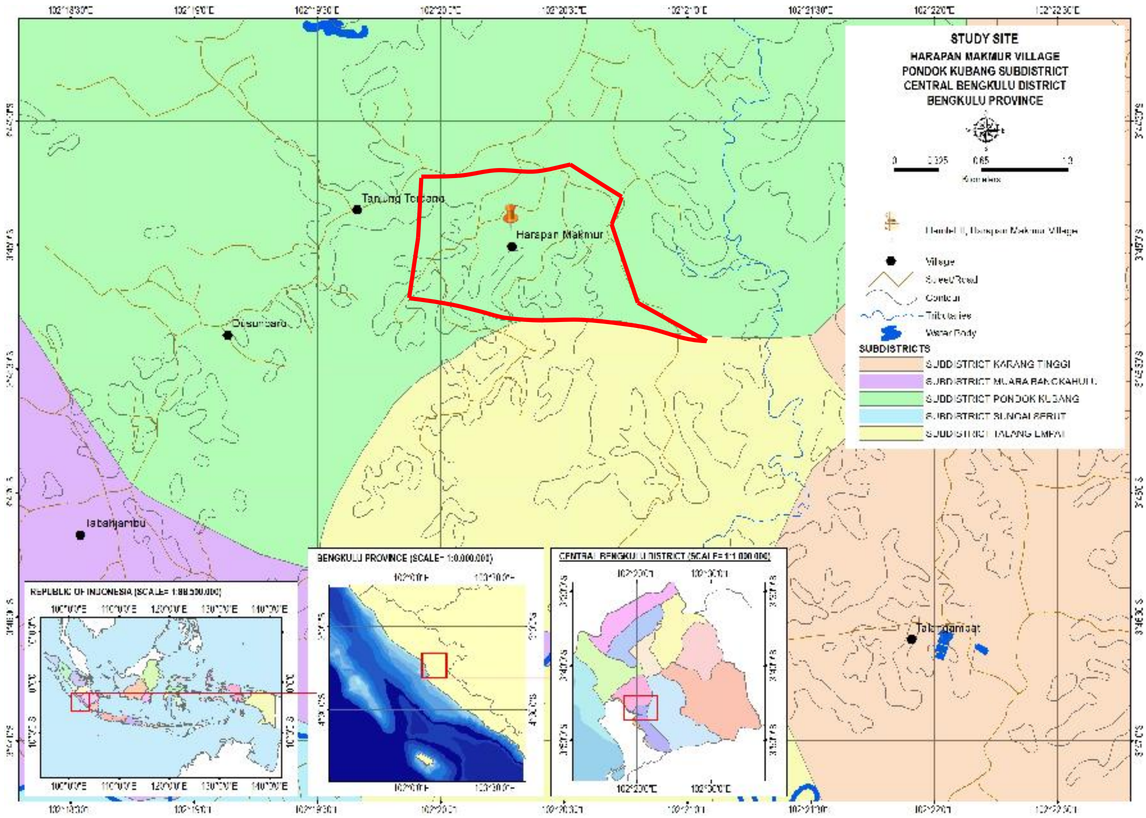

Figure 1. Study site in Harapan Makmur Village, Pondok Kubang Sub-District, Central Bengkulu District, Bengkulu Province, Indonesia

Sampling of vegetation

Sampling of vegetation was done in 23 homegardens, or 20% of the total homegardens. The plants were categorized into three categories based on its size and growth form: (i) tree: a woody plant having a diameter at breast height (dbh) ≥ 10 cm and height ≥ 3 m; (ii) sapling or shrub: woody plant with dbh < 10 cm and height < 3m; and (iii) herb: non-woody plant (it could be woody at old age). Coconut and oil palm were categorized as trees, while banana was considered a shrub. Every name of plant in the selected homegarden was recorded. To get data for quantitative measurement (diversity index, importance value index and carbon stock of trees, abundance of shrubs and average cover of herbs) for each home garden, 10 m x10 m quadrates were made for tree sampling with sampling intensity of 20%. Within each 10 m x10m quadrate, a 4 m x4m quadrate was made for sampling of shrubs and saplings, and a 1 m x1m quadrate was made for sampling of herbs.

The dbh (130 cm above the ground), local names, scientific names, number of individuals and height of trees in the 10 m x10m quadrates were recorded. For shrubs and saplings, only the local names, scientific names and number of individuals were recorded. For herbs, the local names, the scientific names and percentage of coverage were recorded. The data of plant uses were collected through interview with 23 respondents, 11 of whom were farmers, 10 farming laborers, 1 civil servant and 1 mechanic. Based on the education, 15 of respondents graduated from elementary school, 3 junior high school, 3 senior high school, 1 did not finish elementary school and 1 graduated from university.

Data analyses

Diversity index

Shannon-Wiener index of diversity was calculated for trees, with the following formula:

$$H = -\sum_{i=1}^{s} (pi)(\log pi)$$

Where:
H = species diversity index
s = number of species
Pi= proportion of species i= ni/N (number of trees of species i/total number of trees)
Mueller-Dombois and Ellenberg (1974)

Importance value index

Importance value index was calculated for each tree species, using this formula:

IV= relative density + relative frequency + relative dominance

Density

$$\text{Density of species i} = \frac{\textit{number of trees of species i}}{\textit{area of quadrats}}$$

$$\text{Relative density of species i} = \frac{\text{Density of species i}}{\text{Density of all species}} \times 100\ \%$$

Frequency

$$\text{Frequency of species i} = \frac{\text{number of quadrats where species i was found}}{\text{total quadrats}}$$

$$\text{Relative frequency of species i} = \frac{\text{Frequency of species i}}{\text{frequency of all species}} \times 100\ \%$$

Dominance

$$\text{Dominance of species i} = \frac{\text{Basal area of species i}}{\text{area of quadrats}}$$

$$\text{Relative dominance of species i} = \frac{\text{Dominance of species i}}{\text{Dominance of all species}} \times 100\ \%$$

Mueller-Dombois and Ellenberg (1974)

Carbon stock

Carbon stock was estimated for trees. The carbon stock was estimated indirectly through biomass estimate. The stored biomass was estimated using allometric equations summarized in Sari et al 2011 (Table 1) because these formula were the results of previous researches.

RESULTS AND DISCUSSION

Species diversity and dominance

A total of 101 plant species were found in Hamlet II of Harapan Makmur Village, 38 of which were trees with diameter $\geq$ 10 cm. Some plants were intentionally planted, and the others grew naturally. In another agroforestry system in the form of bamboo-tree gardens in West Java, Okubo et al (2010) found 42 planned and utilized species and 19 associated non-use species of plants with diameter > 10 cm. Also in West Java, Manurung et al (2008) found 52 tree species in *dudukuhan*, a traditional tree garden.

The Shannon-Wiener index of tree species diversity was 0.99. The Shannon-Wiener index value for home-gardens in this study was similar to the index values for dudukuhans in West Java. Dudukuhans value varied from 1.31 for mixed timber-fruit systems, to 1.18 for mixed fruit-timber-banana-annual crop systems, to 1.10 for fallow gardens (similar to secondary forests), to 0.44 for timber systems (Manurung et al 2008). Although agroforestry system has lower species diversity than natural forest (Kessler et al 2012), the plant diversity in agroforestry system is certainly higher than in single-species plantation. Amid the trend of conversion from agroforest into single species plantation in Indonesia (Feintrenie et al 2010) the attitude of Harapan Makmur villagers to maintain their agroforest is commendable.

Among trees, rubber tree, *Hevea brasiliensis* Willd, had the highest importance value index (127.0%), followed by oil palm tree or *Elaeis guineensis* L (28.4%), and coconut or *Cocos nucifera* L. (24.5%). Other trees had much lower importance value index (Table 2).

Table 1. Allometric equations to estimate carbon for some species of trees

Types of plants or species	Allometric equations
Tree with branches (in general)	AGB = $0.11\ \rho\ D^{2.62}$
Tree without branches	AGB = $\pi\ \rho\ H\ D^2/40$
Bamboo	AGB = $0.131\ D^{2.28}$
Paraserianthes falcataria	AGB = $0.0272\ D^{2.831}$
Tectona grandis	$0.0272\ D^{2.2227}$
Acacia mangium	$0.0528\ (D^2)^{1.3612}$
Swietenia macrophylla	$0.048\ D^{2.08}$

Note: D: diameter (cm), H: tree height (m) ; ρ: wood density (g cm^{-3}), the value of which for each species is taken from ICRAF 2013. For species which have no information of ρ, the value of ρ was assumed to be the same as that of another species having similar physical properties. The below ground biomass for mixed forest is about 0.37 of above ground biomass and the carbon stock is 0.47 of biomass (IPCC 2006).

Among shrubs and saplings, rubber plant also had the highest number of individuals, 159, followed by banana or *Musa* sp. with 51 individuals, robusta coffee (*Coffea canephora* Pierre ex A. Froehner), 41, and arabica coffee (*Coffea arabica* L.) 21. Other species had much lower values (Table 3).

Most trees and shrubs in homegardens do not grow naturally, but are intentionally planted, so their abundance reflects the preference of the owners. Economic consideration is one the main factors determining the selection of crops (Feintrenie et al 2010). The dominant tree and shrub species must have important economic or cultural values. Rubber tree, oil palm and coffee are the main crops found in people's plantation in Bengkulu because they have relatively high economic value. This finding was similar to study in homegarden systems in Lampung, another province of Sumatra (Roshetko et al 2002).

Traditionally, Bengkulu farmers have planted coffee and rubber trees, but in the last two decades, oil palm has been the most important plantation crop in Bengkulu, even in all Sumatra provinces. Sixty percent of oil palm in Indonesia is found in Sumatra (Pricewater Coopers 2010). Palm oil area in Indonesia grew 11.5 percent annually from 1997 to 2000, and by 15.8 percent annually from 2000 to 2007 (Saxon and Roquemore 2011). It is not surprising that the expansion of oil palm plantation is one of the main driving forces of deforestation in Sumatra. Not only plantation companies, but also individual farmers grow oil palm tree, who will then sell their oil palm fruit to companies.

The third most important tree species was coconut. This is a versatile plant. Most parts of this tree are usable, although the people of Harapan Makmur Village only used its leaves and fruit for food, beverage and handy craft. The coconut fruit has economic value, and the people of Harapan Makmur not only used the fruit for their own daily use, but also sold it in the market. In another study, in Batu Ampar Village, South Bengkulu District, Wiryono and Lipranto (2013) found coconut the most used species in the village.

Among shrubs, banana or *Musa* sp. was the second most abundant, after rubber plant. The people of Harapan Makmur consumed their own banana fruit and sold the fruit in the market. They also made handy craft from the banana leaves. Banana is one of the oldest cultivated plants on earth and is found in many parts of the world. It is also a versatile species. Its fruit contains much energy and vitamins, and most of its parts have medicinal values (Kumar et al 2012).

Among herbs, *Agrostis* sp had the highest average coverage (25.8), followed by *Imperata cylindrica* L. (17.0), *Dicranopsis linearis* (Burn.f.) Underw (13.6), lawn grass or *Axonopus compressus* (Sw) P. Beauv (12.0), *Eragrostis tenella* (L.) P. Beauv. Ex Roem et. Schult. (12), *Spigelia anthelmia* (10) (Table 4). Unlike trees and shrubs, most of the herbs were not intentionally planted and was not used, so some of them have no local names.

Type of uses

Out of 101 species of plants found in home gardens, 79 species were used by the people of Hamlet II of Harapan Makmur Village for their daily uses and for sale. In another study, villagers living near Bukit Daun Protected Forest Area, in Kepahiang District, Bengkulu Province used 95 species of plants from their land (Sunesi and Wiryono 2007). In Keramat Mulya, Soreang District, West Java Province, the villagers used only 42 plant species from their traditional bamboo-tree gardens (Okubo et al 2010). In Kabena Island, Southeast Celebes Province, villagers used 65 species of plants for their daily uses (Rahayu and Rugayah 2010).

The people of Hamlet II used 41 species of plants for food, 21 for firewood, 11 for medicines, 10 for ornamental plants, 7 for construction wood, 6 for beverage, 4 for hedge, 3 for forage, 3 for shade trees, 2 for handy craft, and 2 for coloring agent.

The most used part of plant for food was fruit (69%), followed by leaf (14%), tuber (8%), rhizome (6%) and stem (3%). The food consisted of three categories, namely fruit, vegetable and seasoning. Some of plants for food were consumed and some were sold. In Batu Ampar Village, South Bengkulu District, villagers used only 35 plant species for food (Wiryono and Lipranto 2013)

The fruits of plants were not only used as food, but also for beverage. No other parts of plants were used as beverage. Most of fruits were made into juice, except for coconut, robusta coffee (*Coffea canephora*) and arabica coffee (*Coffea arabica*). To give color on food, the people of Harapan Makmur Village used leaves of fragrant pandan (*Pandanus amaryllifolius)* to give green color and rhizomes of turmeric (*Curcuma domestica* Vall) give yellow color.

Homegarden is a source of firewood. Actually all trees and shrubs can be used as firewood, but the people of Harapan Makmur Village used only 21 species for firewood. Their selection of trees might be based on the easiness to get the wood and the quality of the wood as firewood. Both branches and trunk were used to firewood. The trunks of seven species were also used as construction wood. One of species for construction wood was *Tectona grandis* or teak, which has very high quality wood. This species was brought from Java. Some species were native species of Bengkulu, e.g. *Dysoxylum mollissimum* Blume and *Cinnamomum porrectum* Roxb.

Table 2. Five species of trees with the highest importance value index in the study area

Local names	Scientific names	Family	Rdo	RF	RDe	IVI
Karet	*Hevea brasiliensis* Willd	Euphorbiaceae	31.8	29.4	65.8	127.0
Sawit	*Elaeis guineensis* L	Palmaleceae	22.5	3.7	2.2	28.4
Kelapa	*Cocos nucifera* L	Arecaceae	7.8	12	4,6	24,5
Durian	*Durio zibethinus* Murray	Bombaceae	5.4	8.7	5.3	19.3
Kayu bawang	*Dysoxylum mollissimum* Blume	Burseraceae	3.0	9.4	5.0	17.4

Note: Rdo: relative dominance; RF: relative frequency; RDe: relative density; IVI: importance value index.

Table 3. Five species of saplings and shrubs with the highest number of individuals in the study area

Local names	Scientific names	Families	Number of individuals
Karet	*Hevea brasiliensis* Willd	Euphorbiaceae	159
Pisang	*Musa* sp.	Musaceae	51
Kopi robusta	*Coffea canephora* Pierre ex A.Froehner	Rubiaceae	41
Kopi arabika	*Coffea arabica* L.	Rubiaceae	21
Singkong	*Manihot utilissima* Pohl.	Euphorbiaceae	9

Table 4. Five species of herbs with the highest average cover in the study area

Local names	Scientific names	Families	Average coverage (%)
_	*Agrostis* sp.	Poaceae	25.8
Ilalang	*Imperata cylindrica* L.	Poaceae	17.0
Paku resam	*Dicranopsis linearis* (Burn.f.) Underw	Gleicheniaceae	13.6
Rumput kerbau	*Axonopus compressus* (Sw) P. Beauv	Poaceae	12.6
	Eragrostis tenella (L.) P. Beauv. Ex Roem et. Schult.	Poaceae	12.5

Table 5. The local names, scientific names, families and uses of plants found in the study area

Local names	Scientific names	Families	Parts of plants used	Types of uses
Used plants				
Gandarusa	*Justicia gandarussa* L	Achantaceae	ALL	MED, FENCE
Bayam	*Amaranthus spinosus* L	Amaranthaceae	LEAF	FOOD
Jeger ayam	*Celosia cristata* L	Amaranthaceae	ALL	ORN
Bawang daun	*Allium fistulosum* L	Amaryllidaceae	LEAF	FOOD
Bacang	*Mangifera foetida* Lour	Anacardiaceae	FRT, TRK, BRC	FOOD
Mangga	*Mangifera indica* L	Anacardiaceae	FRT, TRK, BRC	FOOD, BEV, FRW
Srikaya	*Annona muricata* L	Annonaceae	FRT	FOOD
Pulai hitam	*Alstonia angustiloba* Miq	Apocynaceae	TRK	FRW, CON
Tapak dara	*Catharanthus roseus* (L.) G.Don	Apocynaceae	ALL	ORN
Talas	*Colocasia esculenta* (L.) Schott	Araceae	TUB	FOOD
Pinang	*Areca catechu* L	Arecaceae	FRT, ALL	MED, FEN
Kelapa	*Cocos nucifera* L	Arecaceae	LEAF, FRT	FOOD, BEV, CRF
Sawit	*Elaeis guineensis* L	Arecaceae	FRT	FOR SALE
Salak	*Salacca zalacca* (Gaertner) Voss	Arecaceae	FRT	FOOD
Puding merah	*Cordyline fruticosa* L	Asparagaceae	ALL	ORN
Suji	*Dracaena angustifiola* Roxb	Asparagaceae	ALL	ORN
Lidah martua	*Sansevieria trifasciata* Prain	Asparagaceae	ALL	ORN
Kemangi	*Ocimum basilicum* L	Asteraceae	LEAF	MED, FOOD
Kalpataru	*Hurea crepitans* L.	Barringtoniaceae	ALL	SHADE
Kapuk	*Ceiba pentandra* (L.) Gaertn	Bombacaceae	BRC	FRW
Durian	*Durio zibethinus* Murray	Bombacaceae	FRT, TRK, BRC	FOOD, FRW, CON
Nanas	*Ananas comosus* (L.) Merr	Bromeliaceae	FRT	FOOD
Kayu bawang	*Dysoxylum mollissium* Blume	Burseraceae	TRK, BRC	FRW, FENCE, CON
Ganyong	*Canna discolor* Lindl.	Cannaceae	TUB	FOOD
Pepaya	*Carica papaya* L	Caricaceae	FRT, LEAF	MED, FOOD
Ubi rambat	*Ipomoea batatas* L	Convolvulaceae	TUB	FOOD
Gambas	*Luffa acutangula* (L.) Roxb	Cucurbitaceae	FRT	FOOD
Cemara kipas	*Thuja orientalis* L	Cupressaceae	ALL	ORN
Bunga euphorbia	*Euphorbia milii* Des Moul	Euphorbiaceae	ALL	ORN
Karet	*Hevea brasiliensis* Willd	Euphorbiaceae	SAP	FOR SALE
Jarak	*Jatropha curcas* L	Euphorbiaceae	SAP	MED
Singkong	*Manihot utilisima* Pohl	Euphorbiaceae	LEAF, TUB	FOOD
Katu	*Sauropus androgynus* (L.) Merr	Euphorbiaceae	LEAF	FOOD
Akasia	*Acacia mangium* Willd	Fabaceae	TRK, BRC	FRW, CON
Flamboyan	*Delonix regia* (Boj. ex Hook.) Raf	Fabaceae	TRK, BRC	FRW
Gamal	*Gliricidia sepium* (Jacq.) Kunth ex Walp	Fabaceae	TRK, LEAF	FEN, FOR
Lamtoro	*Leucaena leucocephala* Lamk	Fabaceae	TRK, BRC	FRW
Sengon	*Paraserianthes falcataria* L	Fabaceae	TRK	FRW
Jengkol	*Pithecellobium jiringa* Prain	Fabaceae	FRT, TRK, BRC	FOOD, FRW
Melinjo	*Gnetum gnemon* L	Gnetaceae	LEAF, FRT	FOOD
Manggis	*Garcinia mangostana* L	Guttiferae	FRT, TRK, BRC	FOOD, FRW
Jati	*Tectona grandis* Linn. F	Lamiaceae	TRK, BRC	FRW, CON
Kayu gadis	*Cinnamomum porrectum* Roxb	Lauraceae	TRK, BRC	FRW, CON
Alpokat	*Persea americana* Mill	Lauraceae	FRT, TRK, BRC	FOOD, BEV, FRW
Bunga sepatu	*Hibiscus rosa-sinensis* L	Malvaceae	ALL	ORN
Duku	*Lansium domesticum* (Osbeck) Sahni & Bennet var. *duku*	Meliaceae	FRT, TRK, BRC	FOOD, FRW
Mahoni	*Swietenia macrophylla* King	Meliaceae	TRK, BRC	FRW, CON
Nangka	*Artocarpus heterophyllus* Lam.	Moraceae	FRT, TRK, BRC, LEAF	FOOD, FRW, FOR
Sukun	*Artocarpus indicus* L. F	Moraceae	ALL	FOOD
Pisang	*Musa* sp.	Musaceae	LEAF, FRT	FOOD, CRF
Jambu biji	*Psidium guajava* L	Myrtaceae	BH, OBT	FOOD, FRW, SHD
Jambu air	*Syzygium aqueum* Burm. F	Myrtaceae	FRT, TRK, BRC	FOOD, FRW, SHD
Jambu bol	*Syzygium malaccense* (L.) Merr. & Perry	Myrtaceae	FRT	FOOD, SHD
Bunga pukul empat	*Mirabilis jalapa* L	Nyctaginaceae	ALL	ORN
Belimbing	*Averrhoa carambola* L	Oxalidaceae	FRT	FOOD
Pandan	*Pandanus amaryllifolius* Roxb	Pandanaceae	LEAF	FOOD, COL
Kacang tanah	*Arachis hypogeae* L	Papilionaceae	FRT	FOOD
Serai	*Andropogon nardus* L	Poaceae	TRK, LEAF	FOOD

Rumput kerbau	*Axonopus compressus* (Sw). P. Beauv	Poaceae	LEAF	FOR
Rumput jepang	*Zoysia japonica* Steud	Poaceae	ALL	ORN
Mawar	*Rosa* sp.	Rosaceae	ALL	ORN
Jabon	*Anthocephalus cadamba Miq*	Rubiaceae	TRK	FRW
Kopi arabika	*Coffea arabica* L	Rubiaceae	FRT, TRK, BRC	BEV, FRW
Kopi robusta	*Coffea canephora* Pierre ex A. Froehner	Rubiaceae	FRT, TRK, BRC	FOOD, FRW
Asoka	*Ixora coccinea* L	Rubiaceae	ALL	ORN
Mengkudu	*Morinda citrifolia* L	Rubiaceae	FRT	MED
Jeruk nipis	*Citrus aurantifolia* (Ch. & P.) Sw	Rutaceae	LEAF, FRT	FOOD, FRW, SHD
Rambutan	*Nephelium lappaceum* L	Sapindaceae	FRT, TRK, BRC	FOOD, FRW, SHD
Sawo	*Achras zapota* L	Sapotaceae	FRT	FOOD
Cabe besar	*Capsicum annuum* L	Solanaceae	FRT	FOOD
Cabe rawit	*Capsicum frutescens* L	Solonaceae	FRT	FOOD
Terong bulat	*Solanum blumei* L	Solanaceae	FRT	FOOD
Tomat	*Solanum lycopersicum* L	Solanaceae	FRT	FOOD
Rimbang	*Solanum torvum* Sw	Solanaceae	FRT	FOOD
Coklat	*Theobroma cacao* L	Sterculiaceae	FRT, TRK, BRC	FOOD, FRW
Mahkota dewa	*Phaleria macrocarpa* (Scheff.) Boerl	Thymelaeaceae	FRT	MED
Lidah buaya	*Aloe vera* (L.) Burm.f	Xanthorrhoeaceae	ALL	MED, ORN
Kunyit	*Curcuma domestica* Vall	Zingiberaceae	LEAF, RHI	FOOD, MED, COL
Jahe	*Zingiber officinale* Roscoe	Zingiberaceae	RHI	FOOD, MED
Non used plants				
Pegagan	*Centella asiatica* L	Apiaceae	_	NONE
Rumput sambora	*Ageratum* sp.	Asteraceae	_	NONE
Kerinyu	*Chromolaena odorata* L	Asteraceae	_	NONE
Batang babi	*Cyanthillium cinereum* (L.) H. Rob	Asteraceae	_	NONE
Jatong kuda	*Synedrella nodiflora* (L.) Gaertner	Asteraceae	_	NONE
Widelia	*Wedelia trilobata* (L.) Hitchc	Asteraceae	_	NONE
Rumput tapung	*Kyllinga nemoralis* L	Cyperaceae	_	NONE
Patikan kebo	*Chamaesyce hirta* (L.) Millsp	Euphorbiaceae	_	NONE
Meniran	*Phylantus amarus* L	Euphorbiaceae	_	NONE
Bunga telang	*Clitoria* sp.	Fabaceae	_	NONE
Putri malu	*Mimosa pudica* Duchass & Walp	Fabaceae	_	NONE
Paku resam	*Dicranopteris linearis* (Burm. f.) Underw	Gleicheniaceae	_	NONE
_	*Spigelia anthelmia* L	Loganiaceae	_	NONE
Harendong bulu	*Clidemia hirta* (L.) D. Don	Melastomaceae	_	NONE
Keduruk	*Melastoma malabathricum*	Melastomaceae	_	NONE
Sesuruhan	*Peperomia pellucida* L	Piperaceae	_	NONE
_	*Agrostis* sp.	Poaceae	_	NONE
_	*Eragrostis tenella* (L.) P. Beauv.ex Roem et.Schult	Poaceae	_	NONE
Ilalang	*Imperata cylindrica* L	Poaceae	_	NONE
Jagung	*Zea mays* ssp.. *Mays* L	Poaceae	_	NONE
Paku-pakuan	*Pteris vittata* L	Pteridaceae	_	NONE
Pecut kuda	*Stachytarpheta jamaicensis* (L). Vahl.	Verbenaceae	_	NONE

Eleven species of plants were used as traditional medicines. The most part of plant used as medicines was fruit (5 species), followed by leaf (4), rhizome (2) and sap (1). If the respondents for this study included a practicing traditional healer, the result might have been higher. In a village next to Harapan Makmur Village, the Tanjung Terdana Village, a traditional healer used 31 plant species for medicines (Nurliana 2011).

Homegardens in Hamlet II of Harapan Makmur Village usually had clear boundary from one another. The villagers used bricks, wood, wire or living plants or hedge as the border of home gardens. Four species of plants were used as living fence or hedge.

In the front yard, the people of Hamlet II planted ornamental plants and shade trees. Some species were planted as ornamental plants because of their bright flower, some for their characteristic crown and some for their characteristic leaves. The leaves of jackfruit *Artocarpus heterophyllus* Lamk and and quick stick *Gliricidia sepium* (Jacq.) Kunth ex Walp., lawn grass *Axonopus compressus* (Sw). P. Beauv were used as forage.

Carbon stock

The estimate of above ground tree biomass of trees was 147.8 ton ha^{-1}, below ground 54.7 ton ha^{-1}, and the total 202.5 ton ha^{-1}. With an average carbon stock estimated at

0.47 of biomass, the above ground carbon stock was 69.5 ton ha^{-1}, below ground carbon stock 25.7 ton ha^{-1} and total 95.2 ton ha^{-1}. The above ground biomass in the agroforestry system in Hamlet II was lower than that of Asian rain tropical broadleaf forest, which was 220 ton ha^{-1} (IPCC 2013), but higher than that in agroforestry system in Kalikunto watershed in Malang, East Java, which was 42.1 ton ha^{-1} (Hairiah et al. 2010).

To conclude, homegarden systems of Hamlet II, Harapan Makmur Village resembled tree garden systems in Lampung and West Java; maintained biodiversity, contained significant carbon stocks, and provided livelihood for the villagers.

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REFERENCES

Feintrenie L, Schwarze S, Levang P. 2010. Are local people conservationists? Analysis of transition dynamics from agroforests to monoculture plantations in Indonesia. Ecol Soc 15 (4): 37.

FWI [Forest Watch Indonesia]. 2011. The potret of Indonesian forest 2000-2009. Forest Watch Indonesia, Jakarta.

Hairiah K, Dewi S, Agus F, et al. 2010. Measuring Carbon Stocks Across Land Use Systems: A Manual. World Agroforestry Centre (ICRAF), SEA Regional Office, Bogor, Indonesia.

ICRAF. 2013. Wood Density. In: Tree Functional Attributes and Ecological Database. ICRAF, Bogor. http://db.worldagroforestry.org/wd [May 2013].

IPCC. 2006. IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, In: Eggleston HS, Buendia L, Miwa K, Ngara T, Tanabe K. (eds). IGES, Tokyo, Japan.

Kessler M, Hertel D, Jungkunst HF, Kluge J, Abrahamczyk S, et al. 2012. Can joint carbon and biodiversity management in tropical agroforestry landscapes be optimized? PLoS ONE 7 (10): e47192. doi:10.1371/journal.pone.0047192

King KFS.1987. The history of agroforestry. In: Steppler HA, Nair PKR (eds) Agroforesty, a Decade of Development. International Council for Research in Agroforetsry, Nairobi.

Kumar KPS, Bhowmik D, Duraivel S, Umadevi M. 2012. Traditional and medicinal uses of banana. J Pharm Phytochem 1 (3): 51-63.

Manurung GE, Roshetko JM, Budidarsono S, Kurniawan I. 2008. Dudukuhan tree farming systems in West Java: How to mobilize self-strengthening of community-based forest management? In: Snelder DJ, Lasco R (eds). Smallholder Tree Growing for Rural Development and Environmental Services, Springer, Berlin.

Mead DJ. 2004. Agroforestry. In: Forests and Forest Plants. Vol 1. Encyclopedia of Life Science Systems. EOLSS Publishers, Oxford UK.

Mueller-Dombois D, Ellenberg H. 1974. Aims and Methods in Vegetation Ecology. John Willey & Sons. New York.

Nurliana S 2011. The traditional knowledge of Lembak Tribe on the diversity of medicinal plants in two villages in Bengkulu. Seminar Proceedings on Conservation of Tropical Plants: The Current Condition and the Future Challenge. LIPI, Bogor. [Indonesian].

Okubo S, Parikesit, Harashina K, Muhammad D, et al. 2010. Traditional perennial crop-based agroforestry in West Java:the trade off between on-farm biodiversity and income. Agrofor Syst 80:17-31.

Pricewater Coopers. 2010. Palm Oil Plantation. Industry Landscape, Regulatory and Financial Review. Pricewater Coopers, Jakarta.

Rahayu M, Rugayah. 2010. Local knowledge and utilization of plants by local communities Kabaena Island, Southeast Sulawesi. Berita Biologi 10 (1): 67-75. [Indonesian]

Roshetko JM, Delaney M, Hairiah K, Purnomosidhi P. 2002. Carbon stocks in Indonesian homegarden systems: Can smallholder systems be targeted for increased carbon storage? Am J Altern Agric 17:138-148.

Sari RR, Hairiah K, Widianto, at al. 2011. Potency of natural forest and agroforestry as stock of carbon in Prigen Sub-district, Pasuruan. Proceedings of National Seminar Commemorating the 47th Anniversary of Faculty of Forestry, Gadjah Mada University, Yogyakarta. [Indonesian]

Saxon E, Roquemore 2011. Palm Oil. In: Boucher D, Elias P, Lininger K, et al. (eds.). The Root of the Problem. What is the Driving Force of Tropical Deforestation Today? Tropical Forest and Climate Initiative. Union of Concerned Scientists, Washington, DC.

Scrotch G, da Fonseca GAB, Harvey CA, et al. (eds). 2004. Agroforestry and Biodiversity in Tropical Landscape. Island Press, Washington DC.

Sunesi I, Wiryono. 2007. The diversity of plant species utilized by villagers living near protected forest in Kepahiang District, Bengkulu Province. Indonesian J Agric Sci, Special Edition (3): 432-439.

Takimoto A, Nair, VD, Nair PKR. 2009. Contribution of trees to soil carbon sequestration under agroforestry systems in the West African Sahel. Agrofor Syst 76:11-25.

Wildayana E. 2015. Formulating rice fields conversion control to oil palm plantations in tidal wetlands of South Sumatra, Indonesia. J Wetlands Environ Manag 3 (2): 72-78

Wiryono, Lipranto. 2013. The diversity of locally useful species Batu Ampar village near Bukit Raja Mandara protected forest area in South Bengkulu District. Jurnal Manusia dan Lingkungan 20 (2):119-127.

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Ethno-medicinal plants used for herbal medication of jaundice by the indigenous community of Tripura, India

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Abstract. *Deb D, Datta BK, Debbarma J, Deb S. 2016. Ethno-medicinal plants used for herbal medication of jaundice by the indigenous community of Tripura, India. Biodiversitas 17: 256-269*. The immemorial association of medicinal plants is highlighted in various utilization pattern by different indigenous community. An investigation was done for less known ethno-medicinal plants used for jaundice as well as some other ailments by the indigenous community of Tripura, Northeast India. The traditional utilization of plants for treatment of jaundice by the indigenous communities is not so well studied in the land locked hilly part of the country. Timely ethnobotanical survey was undertaken with the knowledge of the species mainly used for curing jaundice. Necessary specimens were collected and cross-checked with the existing literatures. A total of 50 ethno-medicinal plant species belonging to 37 families were documented used for the treatment of jaundice (45 plant species with an additional ingredient of 5 species) and other diseases. Mainly leaves and roots were preferred to prepare decoction, pills and paste etc. Most of the plant species are sources of different chemical constituents which further contribute in formulating drug for common use. The active biochemical compounds are investigated by thorough literature survey. There is need for further critical phytochemical analysis and investigation of new valid drugs. Immediate documentation of such valuable knowledge is necessary as we are gradually missing many precious traditional herbal formulations with increasing impacts of modernization.

Keywords: Antioxidant, herbal formulation, indigenous community, jaundice, phytochemical

INTRODUCTION

An indigenous community seems to hold the habitual knowledge of herbal remedies for different minor to chronic diseases. Such indigenous identity of the particular community is derived due to the immemorial association with their floral and faunal environment. This association has led to the use of many plants for food, fodder and medicine (Abbasi et al. 2009). The local folks are therefore dependent on various aspects of herbal formulations for curing different ailments (Annalakshmi et al. 2012). This traditional knowledge has been passing on verbally from one generation to another (Majumdar et al. 2006) which is equivalent to the irreversible loss of flora and fauna (Bora et al. 2012). However, there is always a chance to loss of this knowledge rather than leaving behind to next generations. Thus the customary approach to document the knowledge about the medicinal properties of plants for curing different diseases has become indispensable before it is lost perpetually.

Northeast region of India is considered as an abode of more than 50% of total ethnic communities present in the country with a unique folklore (WHO 2001). These ethnic people are living in remote areas with closest harmony to the adjacent forest. Their livelihood subsistence greatly depends on biotic resources available in that area (Deb et al. 2012). The indigenous community people are not well concerned about any modern medicinal facilities due to their restricted socio-economic condition. Therefore, the ethnic people from various parts of Northeast India are greatly inevitable with contingent on plant-based healing to their health care needs by indefinite cohorts (Majumdar and Datta 2007; Das et al. 2008).

The Northeastern part of India consists of half of the total flora of India (Lokho 2012) and Tripura also harbor varieties of medicinal plants (Singh et al. 1997; Majumdar et al. 2006; Majumdar and Datta 2007; Das et al. 2009; Das et al. 2012). The ethno-medicinal study is regarded as most feasible method for identifying new medicinal plants used for customary health care practices propagated by the ethnic communities (Annalakshmi et al. 2012). This is attracting the attention of several scientists to uphold direction to keen research towards the unearthing of several herbal remedies (Bhattacharya and Goel 1981; Karuppasmy 2007; Manikandan et al. 2009). Among them many workers throughout the globe have reported the usefulness of plants from several chronic to minor diseases like diabetes, dysentery, urinary disorder, jaundice etc. (Raman and Lau 1996; Matsuda et al. 2001; Abbasi et al. 2009; Zulfiker et al. 2010; Rahim et al. 2012; Annalakshmi et al. 2012; Karmakar et al. 2012; Marrufo et al. 2013; Doss and Anand 2014; Islam et al. 2015).

Jaundice is a disorder which occurs when the bilirubin content in the blood is excessive due to haemolysis (Annalakshmi et al. 2012; Rahim et al. 2012; Jayachandra and Devi 2012). It is a yellowish discoloration of the skin, sclera, eyeballs, nails and mucous membrane. It indicates disorder in liver or gall bladder; occurs mostly in the newborn babies as they do have the immature liver which cannot regulate the amount of bilirubin like an adult (Guyton 2005; Jayachandra and Devi 2012). It is one of the most common diseases in the less developed countries and in the Asian countries as well (Karmakar et al. 2012).

According to the oldest codified system of medicine from South India, the Siddha system of medicine illustrates jaundice as one of the pitha type of disease (Annalakshmi et al. 2012). However, in modern allopathic and homeopathic medicine there is no unique treatment for jaundice (Abbasi et al. 2009). Whereas, people relatively preferred the non-codified system like folk medicine prescribed by the rural communities. Although, different ethnobotany workers has mainly documented the less known herbal remedies curing jaundice along with other diversified diseases by the ethnic communities from Northeast India (Deb 1968; Singh et al. 1997; Kala 2005; Majumdar and Datta 2007; Sajem et al. 2008; Sikdar and Dutta 2008; Shil and Choudhury 2009; Das et al. 2009; Rai and Lalramnhinglova 2010; Das and Choudhury 2010; Sahu et al. 2011; Choudhury et al. 2012; Das et al. 2012; Das and Choudhury 2012; Jayachandra and Devi 2012; Dutta and Sharma 2013). However, to our acquaintance no sole systematic explorations on application of medicinal plants against jaundice have been made so far in Tripura. In this context, the present study is the first landmark with meticulous emphasis on less known medicinal plants used for jaundice by the indigenous communities of Tripura.

MATERIALS AND METHODS

Study site

The present study is the outcome of bioresource survey throughout the state of Tripura, India during 2013-2015. It is a hilly state in the northeastern part of India which lies between 22°56' to 24°32' N latitude and 90°09' to 92°20' E longitude. It is bordered internationally with Bangladesh in the North, West and South. Nationally bordered with two sister states Mizoram in the east and Assam in the northeast. The state has 19 ethnic communities distributed throughout the 8 districts. The survey conducted in the remote areas of community dwelling places like Kanchanpur, Gandacherra, Baramura, Twidu, Amarpur, Karbook, etc. (Figure 1). The major inhabitants of these areas were Tripuri, Jamatia, Halam, Santhal and nontribal community.

Data collection

The ethnobotanical data were collected by conducting a questionnaire survey and formal group discussions. In the survey, details of the species were asked which are mainly used for curing jaundice along with some other ailments. Vernacular names, parts used, composition and dosages were recorded to validate the curing process. Some precautions were also noted down for each use of the species composition. The details were carefully recorded. The species were identified by visiting in their natural growing sites. During the survey necessary specimens were collected to ensure the species identification. The botanical names along with author names were cross-checked with the help of IPNI website (http://www.ipni.org) and existing literatures (Deb 1981, 1983). Further, the phytochemical constituents of recorded plant species were also collected after thorough literature survey.

RESULTS AND DISCUSSION

Results

A total of 50 species were documented which are used in treating jaundice. The species are enumerated in a tabular form along with the scientific names, family

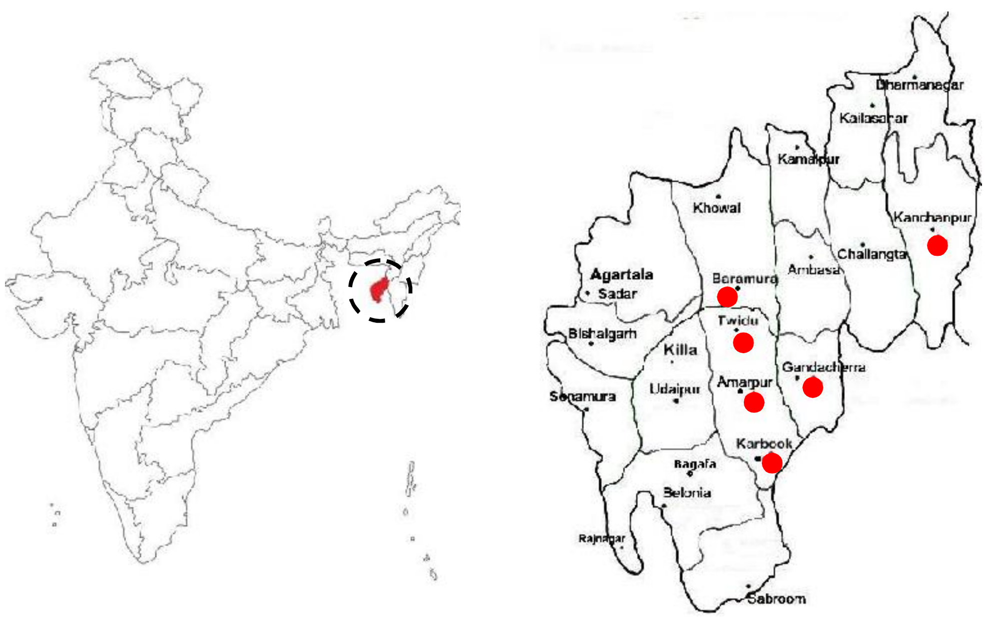

Figure 1. Study site in the state of Tripura, India (●)

names, vernacular names, parts used, composition, dosage, additional materials added and some recommended precautions (Table 1). The recorded genus and species were 46 and 45 respectively belonging to 37 families. The dominant family is Rutaceae contributed by 4 species, Acanthaceae (3 species) followed by Convolvulaceae, Cucurbitaceae and Mimosaceae with 2 species each. Whereas, the rest 32 families with single species each, which include Anacardiaceae, Apiaceae, Costaceae, Euphorbiaceae, Lamiaceae, Menispremaceae, Plumbaginaceae, etc. (Figure 2). The parts utilized for various ailments were whole plants, stems, leaves, fruits, barks and roots. The most widely used parts were leaf/twigs (38%) > roots/rhizome (18%), fruits (16%) > stems (11%) > barks (8%) > whole plant (7%) and 2% tuber. Figure 3 highlights the utilization pattern of plant parts with the percentage of uses.

The leaves are mostly taken in the form of decoction, raw, paste, powder and often juice is extracted for regular consumption. But even some are included in their daily diet by boiling for making curry. The species includes viz. *Azadirachta indica* A. Juss., *Asteracantha longifolia* Nees., *Cajanus cajan* (L.) Millsp., *Citrus limon* (L.) Osbeck, *Eclipta alba* L. ex B.D. Jacks, *Hibiscus surattensis* L., *Kalanchoe pinnata* Pers., *Mangifera indica* L., *Marsilea minuta* L., *Scoparia dulcis* L., *Typhonium trilobatum* (L.) Schott etc. Roots of *Citrus limon* (L.) Osbeck, *Glycosmis arborea* (Roxb.) DC., is utilized in the form of decoction. Often roots are also taken in the form of paste or powder form from species such as *Costus speciosa* (J. Koenig) Sm., *Entada phaseoloides* (L.) Merr., *Ichnocarpus frutescens* (L.) R. Br., *Plumgabo zeylanica* L., *Smilax zeylanica* L., *Solanum nigrum* L. and *Thunbergia grandiflora* Roxb. Most of the fruits are taken raw or when ripen (*Averrhoa carambola* L., *Garcinia pedunculata* Roxb. ex Buch.-Ham, *Phyllanthus nirruri* Roxb. ex Wall) and sometimes juice is extracted for consumption (*Citrus medica* L., *Momordica charantia* L.). The species whose barks are utilized are *Mangifera indica* L., *Micromelum integerrimum* (Roxb. ex DC.) Wight Arn. ex M. Roem; *Oroxylum indica* (L.) Benth. ex Kurz and *Ziziphus oenoplia* (L.) Mill. Bark paste of *Mangifera indica* L. is used to cure jaundice by rubbing against body before bath for 3-4 days, as the water color changes to orange, this practice is believed to cure jaundice as reported by local medical practitioners.

A total of 45 species out of 50 were recorded for treating jaundice including five species which are used as an additional ingredient added to prepare medicine. The additional species used includes *Allium sativum* L., *Allophyllus racemosa* L., *Cassia fistula* L., *Piper longum* L. and *Zingiber officinale* L. A number of ailments were recorded from this study other than jaundice. The key pointed ailments mentioned were worm (intestinal/ stomach), gum/tooth problem, anemia, dysentery, ophthalmic problem, kidney stone, diabetes, cough, skin disease, urinary disorder, snake bite and wound healing. During the treatment of jaundice, diet of the patient is highly taken care of and suggested to take food which keeps the body temperature cool. Therefore, oil rich foods were not included in diet. Generally high nutritious leafy green vegetables were advised to take.

The plant parts are taken in different forms as represented in Table 1. Decoction was mostly taken for all the parts utilized, followed by raw, paste, juice, powder, boil, crushed and cooked as food. These parts are taken either in a single or conjugated with some other additional materials like scented rice (*Oryza sativa* L.), *Coccinia grandis* (L.) Voigt, *Catharanthus roseous* L., salt, calcium hydroxide etc. either orally or is applied in case of wound healing or washing during gum or tooth ache.

The phytochemical constituents of species preferred for curing jaundice are assembled after critical review of literatures (Table 2). The identified compounds were known to have beneficial importance in medical science and also for industrial purposes.

The common constituents like carbohydrate, vitamin and minerals ensure about the utility of the material in regular diet. These phytochemicals provide pharmacological and therapeutic effects upon its use. Besides, these are also providing nutrition in our daily diet. Fruits of *Averrhoa carambola* L. is consumed during the occurrence of jaundice. It is indicated to contain a huge amount of antioxidant properties. These antioxidants significantly delays or prevents oxidation of oxidisable substrate that protects the body against damage caused by reactive oxygen. *Alocasia indica* (Roxb.) Schott. is well preferred

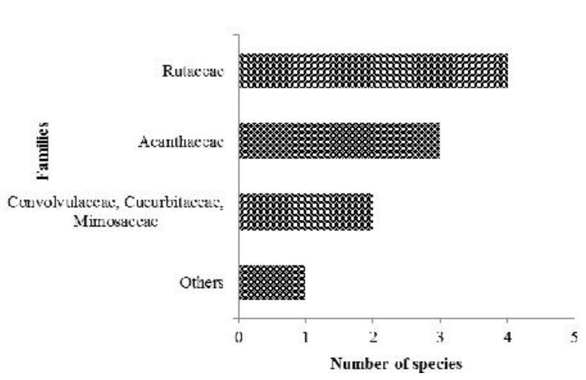

Figure 2. Taxonomic enumeration of the recorded plant species

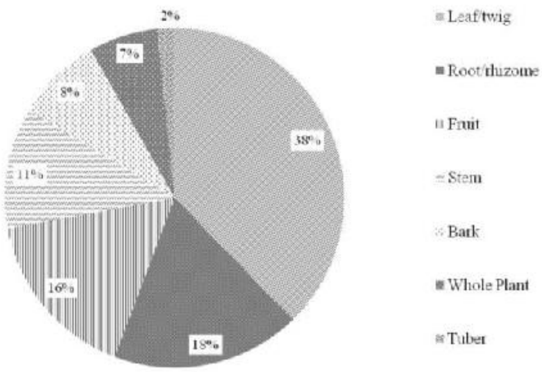

Figure 3. Utilization pattern of the recorded plant parts

vegetable of community's regular diet. Tubers of this plant have diagnostic effects due to the presence of compounds like phenolic acids and flavonoids. *Enhydra fluctuans* Lour. is another species, whose leaves are taken after boiling, contains sesquiterpene lactones. The other species which possess the same compounds are *Citrus medica* L., *Centella asiatica* (L.) Urb., *Coccinia grandis* (L.) Voigt, *Mangifera indica* L., *Neptunia prostrata* Baill., *Terminalia bellirica* (Gaertn.) Roxb. among others, listed for its beneficial chemical compounds. *Centella asiatica* (L.) Urb. is possessing an array of chemical constituents like asiatica, asiaticoside, betulinic acid, thankunic acid and isothankunic acid and many other beneficial active chemical component, which have cognitive enhancing effect, neuroprotective effect and influences oxidative stress parameters (Orhan 2012).

Discussion

The present study shows the preferences of many herbal medicines to cure varieties of ailments mainly jaundice, dysentery, intestinal worms, wound healing, ophthalmic problem, etc. An appraisal of other reports simplified that various species have some similarities across the communities in the same geographical region. Species like *Azadirachta indica* A. Juss., *Cajanus cajan* (L.) Millsp., *Carica papaya* L. *Centella asiatica* (L.) Urb. and *Oroxylum indicum* (L.) Benth. ex Kurz were found to be used against jaundice in *Manipuri* communities (Das and Choudhury 2012) and in *Darlong* communities (Deb et al. 2012). In the present study the use of species like *Cuscuta reflexa* Roxb. and *Glycosmis arborea* (Roxb.) DC. against jaundice are found similar with other studies (Majumdar et al. 2006; Das et al. 2009). This may be due to the inter-cultural homogeneity in the knowledge of the significant uses.

Many above and below ground parts were utilized in the form of decoction, food, paste, as fruit, powder, juice, crushed form, by soaking, by forming pills and others like brushing etc. The maximum parts utilized were above ground like leaves, stems and fruits which was found to be similar with the reports (Sen et al. 2011). Mishra et al. (2012) reported 15 species for curing jaundice out of 68 species from Tarai region of Uttar Pradesh.

Costus speciosus (J. Koenig) Sm. was recorded from the present study against jaundice. However, the same species was reported against kidney stone (Shankar et al. 2012) and diabetes (Choudhury et al. 2012). It shows the importance of the species irrespective of the location. In the present study, fruits of *Phyllanthus niruri* Roxb. ex Wall was recorded for treating jaundice which was similar with the others' findings (Sikdar and Dutta 2008; Sen et al. 2011; Das et al. 2012). The significant effects of the use of *Phyllanthus niruri* Roxb. ex Wall in treating jaundice has also been shown by chemical analysis (Jayachandra and Devi 2012). *Plumbago zeylanica* L. in the present study is found to be used in treating snake bite. But instead of this plant some other plant parts was reported for the same treatment (Lokho 2012). These differences may be due to the lack of knowledge and known effects of the same. It also signifies the same phytochemical properties of different species.

The listed plants have different kind of chemical constituents (Table 2). The components are different types of essential to secondary metabolites *viz.* vitamins, glycosides, tannins, flavonoids etc. It is assumed that these compounds are responsible for the various physiological activitites. Among the Vitamins, exact physiological role of Vitamin A is essential in vision. The main precursor of the rhodopsin is Vitamin A, as well as it boosts up immunity power (Igwenyi et al. 2011). Vitamin B (riboflavin) is essential for energy production and in its coenzyme forms (FMN and FAD) serves as hydrogen transport systems (Mayes 2000). Vitamin C, an antioxidant, facilitates wound healing, production of collagen, formation of red blood cells and boosts immune system (Monsen 2000). Some other vitamins act as an antioxidant and also plays a role in cellular respiration (Burtis and Ashwood 2003), coenzymes for various oxidoreductases. Besides, alkaloids are well known for their ability to inhibit pain.

The sugar molecules of many plant glycosides are often bound to poisons in order to move them from the body. The tannins prevent urinary tract infection by preventing bacteria from adhering to the walls (Igwenyi et al. 2011). Cholesterol is breakdown in the blood stream by the combine action of tannin and anthocyanins. Tannins also help build and strengthen collagen along with vitamin C (Okuda et al. 1991). During infections and microbial invasions in body saponins serve as natural antibiotics. It can also enhance the effectiveness of certain vaccines, lower cholesterol level and destroy lung & blood cancer tumor cells. Flavonoids have antioxidant activity, anti-allergic; prevent from inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins and tumors in biological systems (Okwu and Ndu 2006). Thus the presence of good amount of phytochemicals can provide and protect body's physiological process against different damaging effects (Igwenyi et al. 2011).

These chemical constituents are tested for several physiological disorders and as a whole these are screened out by pharmacological tests for specific and particular ailments (Raman and Lau 1996; Xie et al. 1998; Ito et al. 2004; Atangwho et al. 2009; Zulfiker et al. 2010; Orhan 2012; Islam et al. 2015). Besides, the present study reports the usefulness of these plants to prepare herbal formulation for jaundice. So there is a need for some specific phytochemical screening, whether these listed chemicals exactly perform to cure jaundice or not?

In conclusion, indigenous communities are habituated to live around the natural resources and utilizing the resources. This practice of plant product utilization is build up based on the trial and error method. Plants are the repository of potential medicinal values thus can be widely used for alleviating several health problems. New formulations of drugs are based on the local trials by the indigenous people. But due to the absence of the modern medicine and existence of the belief in the effect of the herbal drugs, people in the rural area still prefer traditional ways of curing. Species like *Ensete glacum* (Roxb.) Cheesm and *Thunbergia grandiflora* Roxb. are newly reported for their use in jaundice and hence require a necessary attention in finding the new chemical

Table 1: Enumeration of the recorded plant species with their scientific, vernacular and family names along with their parts used and dosages.

Species	Family name	Vernacular name	Part (s)	Composition	Dosage	Additional materials	Precautions
Alocasia indica (Roxb.) Schott.	Araceae	Kochu (B), Muitu (K)	Tuber	Tuber is boiled and taken as food	4-5 days during jaundice	-	-
Andrographis paniculata (Burm. f.) Wall. ex Nees	Acanthaceae	Chirata (K)	Whole Plant	Whole plant decoction	Administered 2-3 table spoon in a day for at least 5 days	Zinger (*Zingiber officinale* L.)	-
Asteracantha longifolia Nees.	Acanthaceae	Kulekhara (B)	Leaf	Leaf decoction or either eaten raw	Until cure	-	-
Averrhoa carambola L.	Oxalidaceae	Kamaranga (B/K)	Fruit	Fruit is advised to consume during jaundice	-	-	-
Azadirachta indica A. Juss.	Meliaceae	Neem (B/K)	Leaf	Dry leaves powder is taken with warm rice.	3-4 weeks	Warm rice (*Oryza sativa* L.)	-
Bacopa monnieri (L.) Pennell	Scrophulariaceae	Bramhi (B)	Leaf	Leaf juice is given until cure	10-20 ml daily	-	-
Cajanus cajan (L.) Millsp.	Papilionaceae	Arahar, Aroll (B), Khakleyng (J), Muimasing (K)	Leaf, Fruit	Decoction of leaf and also cooked Pulses are burnt with stem of *Mangifera indica*, leaf of *Musa paradisiaca* and the leg skin of chicken. A paste is prepared from the ashes after burning and applied on gum and tooth by *Jamatia*.	5-6 tea spoons in empty stomach	*Mangifera indica* L. (Thaichuk), *Musa paradisiaca* L. (Jay Thailwk), chicken leg skin	-
Carica papaya L.	Caricaceae	Kuwaifal (K)	Fruit	Eaten raw or even curry is prepared	Advised to take daily	-	Without oil, Garlic
Centella asiatica (L.) Urb.	Apiaceae	Thankuni, Adamoni (B), Ankhnaleng (H), Shamshota (K)	Whole plant	Whole plant parts crushed and decoction is given for curing jaundice. It is also eaten raw or is crushed and the extraction is taken with warm rice. Very popular vegetable item among the *Tripuri* tribe.	-	Warm rice	-
Citrus limon (L.) Osbeck	Rutaceae	Kagajilebu (B), Janbi (J)	Root, Leaf	The root decoction is very effective in curing hepatitis. 2-3 leaves rubbed with finger, are given for smelling to bring sense. Washing with the water of the boiled leaves during ophthalmic problem	Root decoction taken one cup for a month.	-	Root decoction taken in empty stomach. Wash properly after cooling.
Citrus medica L.	Rutaceae	Baranebu, Jamir (B), Jami (K)	Fruit	Decoction of ripe fruits is taken in more quantities.	-	-	Fruit must be ripened
Coccinia grandis (L.) Voigt	Cucurbitaceae	Thelakuchu (B) Tokhathaichumu (K)	Leaf	Leaf decoction	Taken for 2-3 teaspoon for 5 days	-	-

Cocos nucifera L.	Arecaceae	Narikol (B) Narikwra (K)	Fruit	Coconut milk, nutritive soft endosperm	Advice to take regularly	-	-
Costus speciosus (J. Koenig) Sm.	Costaceae	Kebuk (H), Kushtha, Keora (K)	Rhizome, Leaf, Root	Paste of rhizome taken during kidney stone. Taken as food. Leaves and root juice taken to cure diabetes	-	-	-
Cuscuta reflexa Roxb.	Convolvulaceae	Sarnalata (B), Ruiengte (H), Jirai (K)	Whole plant part	Decoction of the plant along with coconut water taken in severe jaundice. Stem is crushed, extracted, filtered and given to jaundice patient by *Halam.* Decoction is prescribed during cough and diabetes and also Plant scattered on the bed to prevent night bed urinate.	Decoction (1-2 cups) taken for 2 weeks at early morning along with 2-3 cups of coconut water. 40-50 gm of stem must be crushed and extracted.	*Cocos nucifera* L.	It is advised to drink the decoction facing the sun in standing position and later get heated up in sunlight for 5 minutes.
Enhydra fluctuans Lour.	Asteraceae	Helencha (B) Elencha (K)	Leaf	Boiled leaf	Boiled leaf and stem are taken with rice	-	No limits
Ensete glaucum (Roxb.) Cheesm	Musaceae	Chisau (L)	Pseudo-stem	Pseudo stem is eaten raw	Can take any time	-	-
Entada phaseoloides (L.) Merr.	Mimosaceae	Gila (B), Swkwi-bakhla (K)	Root bark	Paste of root bark is made and taken in very low quantity with water	-	-	-
Garcinia pedunculata Roxb. ex Buch.-Ham	Clusiaceae	Borthekera (K)	Fruit	Advised to take raw fruits during jaundice	-	-	-
Glycosmis arborea (Roxb.) DC.	Rutaceae	TuluthaPoka (K)	Root	Decoction	5-6 teaspoon of decoction is mixed with milk	Milk	Patients suffering with rheumatic pain must avoid
Hibiscus surattensis L.	Malvaceae	Sarba-ameli (B)	Leaf	Special type of curry is made by its tender leaves	-	-	-
Ichnocarpus frutescens (L.) R. Br.	Apocynaceae	Dugdhalata, Perialata (B), Soya lata (S)	Root, Bark	Juice of the mixture of old root barks of the plant and *Ziziphus oenoplia* (L.) Mill.	Juice taken twice a day after filtration along with 1-2 spoon sugar	Root bark of *Ziziphus oenoplia* (L.) Mill. (Banbadai)	The root bark must be old. Mixtures must be in equal ratio.
Ipomoea aquatica Forssk.	Convolvulaceae	Kamli (B)	Stem, Leaf	Plant parts are boiled to prepare dish	Consumed with rice	-	-

Mangifera indica L.	Anacardiaceae	Am (B), Thaaychuk (J), Thaichuk (K)	Bark, Young leaf, Stem	Bark paste is boiled and water used during bath during Jaundice *(Pallang)*. In this process few Calcium Hydro-oxide rubbed on the body and after few minutes that warm water slowly applied over the body. Then the water color quickly changes into orange color and is called *Pallang* by local medical practitioner. Soaked bark given in case of blood dysentery by *Jamatia*. Leaf is chewed to control obesity. Stem used as tooth brush to prevent dental diseases.	Bark soaked in water given in empty stomach.	Calcium Hydro-oxide *(Chun)*	Bark paste is boiled with water for about 10 minutes Bark is soaked overnight in a glass of water. Leaves and stem used must be young.
Marsilea minuta L.	Marsileaceae	Susni (H)	Leaf	Soup is prepared with its leaves and is included in regular for 15 days	-	-	-
Micromelum integerrimum (Roxb. ex DC.) Wight Arn. ex M. Roem.	Rutaceae	Bon Jamir (B), Karai (K)	Fruit, Bark	Fruits are taken during jaundice. Bark decoction to cure dysentery.	Decoction given in empty stomach	-	Fruits must be matured
Momordica charantia L.	Cucurbitaceae	Korola (B), Gangla (K)	Fruits, twigs	Extract of fruits and twigs prepared and taken in small quantity	-	-	-
Moringa oleifera Lam.	Moringaceae	Sajna (B/K/H)	Leaf, stem	Juice from crushed leaves and stems is taken	-	-	-
Neptunia prostrata Baill.	Mimosaceae	Panilajuk (B), Kharai (K)	Whole plant	Very good tonic	Given for 4-12 days regularly in empty stomach	-	-
Ocimum tenuiflorum L.	Lamiaceae	Tulsi (B)	Leaf	Decoction of leaf	Advice to take 1-2 tea spoon once in empty stomach	-	-
Oroxylum indicum (L.) Benth. ex Kurz	Bignoniaceae	Tokharung (K)	Bark	Decoction of bark is taken	Given orally for some days	-	Must be given in empty stomach
Pavetta indica L.	Rubiaceae	-	Root	Root decoction	Taken twice a day for 5-7 days	-	-
Phyllanthus niruri Roxb. ex Wall	Euphorbiaceae	Bon amlokhi	Fruit Leaf	Fruit is eaten raw Green leaves are spread over the infected area for soft feelings	Until cure	-	-
Plumbago zeylanica L.	Plumbaginaceae	Chita, Chitra (B), Jaundicea (S)	Leaf, Root	Leaf juice externally used in jaundice. Root paste applied on snake bite. Leaves tied over the wound.	-	-	Juice and paste must be used externally
Saccharum officinarum L.	Poaceae	Kussar (B) Kuruk (K)	Stem	Stem juice	It is advised to take regularly until cure	-	-

Schima wallichii (DC.) Choisy	Theaceae	Kanak, Makrisal (B), Chelauni (K)	Leaf	Decoction of the leaf crushed mixture along with *Cassia fistula* L. and *Solanum indicum* L.	Decoction taken with little salt	Salt, *Cassia fistula* L. (Sonal), *Solanum indicum* L.(Bon begun)	-
Scoparia dulcis L.	Scrophulariaceae	Naipungchewk (H)	Leaf, twigs	Extract is taken in empty stomach for a week	-	-	-
Smilax zeylanica L.	Smilacaceae	Komarialata, Koyargalata, Komarica (B), Ramlata (S), Koyarma (K)	Root, Leaf	Mixture of root powder along with bark powder of *Ziziphus oenoplia* (L.) (Banbadai) and *Streblus asper* (Harka) used to cure hepatitis, nephritic and blood dysentery. Root paste is boiled with goat milk given for sexual stimulant. Fresh leaves as fodder for cattle for high milk production.	Mixture given regularly in early morning for 2 weeks.	Bark of *Ziziphus oenoplia* (L.) Mill. (Banbadai) and *Streblus asper* (Harka) for the mixture.	Roots and barks must be dried. Mixture must be in the ratio of 3:2:1 in a warm water
Solanum nigrum L.	Solanaceae	Kakmachi (B), Rummunta (H)	Root	Powdered roots	2-3 tea spoon full mix with water advice to take at early morning	-	Must be given orally
Terminalia bellirica (Gaertn.) Roxb.	Combretaceae	Boira (K)	Fruit	Dried fruit dipped in water at least 2-3 hours before, then the water is taken.	Regularly	-	-
Thunbergia grandiflora Roxb.	Acanthaceae	Vako	Root	Root powder taken	Taken twice a day	-	-
Tinospora cordifolia Miers	Menispermiaceae	Paddakuruj, Gulancha, Ghamacilata (B), Duksha-sundari (K)	Stem	Stem is cut into small pieces and soaked and given in empty stomach to cure diabetic complains. Decoction of stem is used in liver complains and in vermifuges.	Soaked stem given in empty stomach.	-	Soaked in a glass of water overnight
Typhonium trilobatum (L.) Schott	Araceae	Kharkun (B)	Leaf	Leaves are used as vegetable and taken regularly with the meal	-	-	-
Vanda tessellata Hook. ex G. Don	Orchidaceae	Orchid, Rashna (B), Khumchuk (K)	Leaf	Mixture of the crushed leaf, along with root of *Carica papaya* L., *Allophylus racemosus* Sw. and little amount of snail flesh, few garlic and black pepper and small pills are made.	Pills to be taken in 2 hours for a week to cure jaundice	*Carica papaya* L. (Pepe), *Allophylus racemosus* Sw. (Choanti), snail flesh, garlic, black pepper	-
Ziziphus oenoplia (L.) Mill.	Rhamnaceae	Chiyakul, Bonbaroi (B), Brui, Chiyangbufang (S/ (K)	Bark	Grinded bark is taken 2 to 3 spoons daily with water.	-	-	Decoction of the bark must be taken and dry fruit can be used.

Note: B=Bengali, K=Kokborok, H=Halam, J=Jamatia, S=Santhal

Table 2: Enumeration of the recorded plant species used in jaundice with their active chemical constituents as reported by different workers

Species	Active chemical constituents
Allium sativum L.	Alanine, allicin, alliin, alliinase, allistatin-i, allistatin-ii, allixin, allyl-disulfide, allyl-methyl-disulfide, allyl-methyl-trisulfide, allyl-alpha-tocopherol, aniline; arachidonic-acid, arginine; beta-phellandrene, beta-tocopherol, biotin; caffeic-acid, choline, cis-ajoene, citral, cycloalliin, cystine, desgalactotigonin, gitonin, glutathione, glycerol-sulfoquinovoside, glycine, histidine, isobutyl-isothiocyanate, isoleucine, leucine, linalol, lysine, methionine, nicotinic-acid, p-coumaric-acid, phenylalanine, phosphatidyl-choline, phosphatidyl-ethanolamine, phosphatidyl-inositol, phosphatidyl-serine, proline, propene, propenethiol, scorodinin, scorodose, serine; thiamacornine, thiamamidine, threonine, tryptophan, tyrosinase, tyrosine, valine (http://www.mdidea.com/products/new/new00106.html).
Allophylus racemosus Sw.	β-sitosterol, phenacetamide, a chemical known for its antiulcer activity, flavonoid glycosides that are effective against ulcer (Rastogi and Mehrotra 1995).
Alocasia indica (Roxb.) Schott.	Protein, carbohydrate, ascorbic acid, alpha-tocopherol, nitric oxide, malonaldehyde, alkaloids, tannins, glycosides, saponins, flavonoids, β-hydroxyque brachamine, 10-dicarboxylic acid, 2, 4 α-dihydroxy-1-methyl-8 methylene, 14α-lactone, 1,2-benzenedicarboxylic acid, 9-hexadecenoic acid, dodecanoic acid, linoleic acid and its ester, oleic acid, ethyl oleate (Pal et al. 2014).
Andrographis paniculata (Burm. f.) Wall. ex Nees	Andrographolide, 14-deoxy-11-oxoandrographolide, 14-deoxy-11, 12-didehydroandropgrapholide, 14-deoxyandropgrapholide, neoandrographolide, andrographosterol, andrographane, andrographone, andrographosterin, andrograpanin, α-sitosterol, stigmasterol, andrographin, monohydroxy trimethyl flavones, dihydroxy-di-methoxyflavone, panicolin, andrographoneo, andrographoside, andropaniculosin A, andropani-culoside A (Niranjan et al. 2010; Hossain et al. 2014).
Asteracantha longifolia Nees.	Phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, alkaloids, enzymes, carbohydrates, flavonoids, terpenoids, vitamins (Doss and Anand 2014)
Averrhoa carambola L.	Ascorbic acid, protein, polyphenol oxidase, proanthocyanidins, epicatechin (Gheewala et al. 2012).
Azadirachta indica L.	Protein, Crude fibre, Fat, Manganese, Selenium, Zinc, Iron, Copper, Magnesium, Chromium, Flavonoids, Tannins, Saponins, Polyphenol, Alkaloids (Atangwho et al. 2009).
Bacopa monnieri (L.) Pennell	Baco saponin (A, B, C and D) (Srivastava et al. 2009).
Cajanus cajan (L.) Millsp.	Protein, amino acid, steroid, phenolic compound (Mohanty and Chourasia 2011).
Carica papaya L.	Protein, fat, fibre, carbohydrates, calcium, phosphorus, iron, vitamin C, thiamine, riboflavin, niacin, caroxene, amino acid, citric acids, molic acid (green fruits), volatile compounds: linalol, benzylisothiocynate, cis and trans 2, 6-dimethyl-3,6 expoxy-7 octen-2-ol. Alkaloid, α; carpaine, benzyl-β-d glucoside, 2-phenylethl-β-d-glucoside, 4-hydroxyl-phenyl-2 ethyl-b-d glucoside and four isomeric malonated benzyl-β-d glucosides (Nadkarni 1954; Bruneton et al. 1999).
Cassia fistula L.	Procyanidin B_2, biflavonoids, triflavonoids, rhein, rhein glucoside, sennoside (A & B), chrysophanol, physcion (Kaji et al. 1968; Mahesh et al. 1984; Kashiwada et al. 1996).
Centella asiatica (L.) Urb.	Asiatic acid, madecassic acid, asiaticoside, madecassoside, madasiatic acid, betulinic acid, thankunic acid, isothankunic acid (Orhan 2012); Flavonoid derivative s such as quercetin, kaempferol, patuletin, rutin, apigenin, castilliferol, castillicetin, and myricetin (Kuroda et al. 2001; Matsuda et al. 2001); centellose (Wang et al. 2014); cadinol, acetoxycentellinol, centellin, centellicin, and asiaticin (Siddiqui et al. 2007) sterols (Rumalla et al. 2010); phenolic acids (Yoshida et al. 2005).

Citrus limon (L.) Osbeck	Cymene, E-citral, DL-limonene, L-α-terpineol, β-pinene, α-terpinolene, terpinen-4-ol (Al-Jabri and Hossain 2014)
Citrus medica L.	Alkaloids, flavonoids, phenols, carbohydrates, glycosides (Negi et al. 2011)
Coccinia grandis (L.) Voigt	Sterols, tannins, flavonoids, proteins, amino acids, glycosides, phenols, saponins, alkaloids (Umamaheswari and Chatterjee 2008)
Cocos nucifera L.	Protein, total lipid (fat), carbohydrate, fiber, sucrose, glucose, fructose, mannitol, sorbitol, myo-inositol, scyllo-inositol (Yong et al. 2009)
Costus speciosus (J. Koenig) Sm.	Carbohydrates, vitamin C, vitamin E, flavonoids, phenols, glycosides, saponins, alkaloids and minerals like Zinc, Copper, Manganese, Selnenium and Iron (Jagtap and Satpute 2014).
Cuscuta reflexa Roxb.	Scoparone, melanettin, quercetin, hyperoside, phenolic compounds, caffeoylquinic acids (Prajapati et al. 2006)
Enhydra fluctuans Lour.	Sesquiterpene lactones (Husain 1992)
Ensete glaucum (Roxb.) Cheesm	The chemical constituents is not reported earlier for its root parts
Entada phaseoloides (L.) Merr.	Entada saponin (ES)-III (Okada et al 1987), chalcone glycosides 4′-*O*-(6″-*O*-galloyl-*β*-D-glucopyranosyl)-2′,4-dihydroxychalcone; 4′-*O*-(6″-*O*-galloyl-*β*-D-glucopyranosyl)-2′-hydroxy-4-methoxychalcone; 4′-*O*-*β*-D-glucopyranosyl-2′-hydroxy-4-methoxychalcone (Zhao et al. 2011)
Garcinia pedunculata Roxb. Ex Buch.-Ham	Protein, β carotene ,thiamine, riboflavin, ascorbic acid, calcium, phosphorus, magnesium, iron, sodium, potassium, copper, zinc, calcium (Islam et al. 2015)
Glycosmis arborea (Roxb.) DC.	Carbazole alkaloids, glybomines (A, B and C), arborinine, arborine (Ito et al. 2004)
Hibiscus surattensis L.	Monoterpenes, sesquiterpene compounds, β-caryophyllene, menthol, methyl salicylate, camphor, germacrene D, hexadecanoic acid, α-humulene, 1, 8-cineole, menthone (Ogundajo et al. 2014).
Ichnocarpus frutescens (L.) R. Br.	Flavonoids, polyphenols, anthocyanins and simple phenolic acids (Kumarappan et al.2012)
Ipomoea aquatica Forssk.	Protein, ash, fiber ,fat, iron, magnesium, calcium, phosphate, manganese, sulphate, nitrates, vitamins (A, B1, C and K), alkaloids, flavonoids, steroids, phenols, glycosides, β-carotene, saponins and tannins (Igwenyi et al. 2011)
Mangifera indica L.	Mangiferin (Nong et al. 2005); steroids, flavonoid, reducing sugar and cardiac glycosides anthraquinone, tannin (Aiyelaagbe and Osamudiamen 2009)
Marsilea minuta L.	Sodium, potassium, calcium, phosphorus, protein, β-carotene (Dewanji et al. 1993)
Micromelum integerrimum (Roxb. ex DC.) Wight Arn. ex M. Roem.	Coumarins as microintegerrin A, microintegerrin B, scopoletin, scopolin of leaf and bark. But chemical constituent is not available from roots (Wang et al. 2014)
Momordica charantia L.	Glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids (Raman and Lau 1996; http://www.raintree.com/bitmelon.htm), Vitamin C, Vitamin A, phosphorus and iron (http://momordica.allbio.org/). Several phytochemicals have been isolated such as momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, multiflorenol (Husain et al. 1994; Xie et al. 1998; Yuan et al. 1999; Parkash et al. 2002). These are reported in all parts of the plant (Murakami et al. 2001).
Moringa oleifera L.	Linalool, α-terpineol, p-vinylguaiacol, cis-dihydroagarofuran, eudesm-11-en-4-α,6α-diol, 1-octadecene, octadecane, 5-octadecin, n-hexadecanol, 1-eicosene, eicosane, n-octadecanol, heneicosane, cyclopentadecanol, 1-docosene, docosane, cis-9-eicosen-1-ol , tricosane, tetracosane, pentacosane, hexacosane, heptacosane, octacosane, nonacosane, triacontane, hexenyl propanoate, phenylethyl alcohol, pseudo phytol (Marrufo et al. 2013); calcium, magnesium, potassium, sodium, iron, zinc, copper (Valdez-Solana et al. 2015)

Neptunia prostrata Baill.	Glycosides, flavonoids, tannins, steroids, terpenoids, carbohydrate, protein, fat (Deb et al. 2013)
Ocimum tenuiflorum L.	Tannins, alkaloid, terpenoids, flavonoids, phlobatannins, glycosides, morphine, boldine, saponin, phlobatannins, steroid, ascorbic acid, carotenoids, phenols (Palla et al. 2012).
Oroxylum indicum (L.) Benth. ex Kurz	Baicalein (5,6,7-trihydroxyflavone) [Derivatives: Baicalein 6-glucuronide, baicalein 7-glucuronide, baicalein 7-0-β-gentiobioside, tetulin (6-glucoside of baicalein), 8,8”-bisbaicalein 1, baicalein-7-o-caffeate 2], oroxylin-a (5,7-dihydroxy-6-methoxyflavone), chrysin (5,7-dihydroxyflavone) (Raghu et al. 2013)
Pavetta indica L.	Tricyclin, benzaldehyde, sabinine, limonine, acetophenone, linalool, perilline, thujupsa-3-one, β-carryophylene, β-edudesmole, β-pinene, α terpenene, α humulene, α-guaiene (Prasad et al. 2010).
Phyllanthus niruri Roxb. ex Wall	Phyllanthine, hypophyllanthine, flavonoids, quercetin, astralgin, quercitrin, isoquercitrin, rutine, alkaloids (Prajapati et al. 2006).
Piper longum L.	Piperine, piplatin, piperlongumine, piperlonguminine, methyl-3,4,5-trimehoxycinnamate, guineensine, pipernonaline, pellitonine, piperanine (Mishra 2010; Chouhan et al. 2011)
Plumbago zeylanica L.	Plambagin, fatty acids and esters : palmitic acid,-essella acid (Do and Nguyen 1996), terpenoids (Gupta et al. 1998), taraxasterol (Rai et al. 2000), stigmasterol (Nile and Khobragade 2010), stigmasterol acetate, sitosterone (Gupta et al. 1995).
Saccharum officinarum L.	Abscisic acid, apigenin, glycoside, methyl lapigenin, arabinose, arunodin, benzoic acid, campesterol, coumarin, cylindrin, orientin, fructose, glactose, glucose, phytosterol, saccharans, schaftoside, sucrose, invert sugar, ether, tricin and vicenin (Prajapati et al. 2006).
Schima wallichii (DC.) Choisy	Alkaloids, flavonoids, cardiac glycosides, saponins, tannin, flobatannin (Lalrinzuali et al. 2015).
Scoparia dulcis L.	Alkaloid, Tannin, Carbohydrate, Glycoside (Zulfiker et al. 2010)
Smilax zeylanica L.	Alkaloid, polysterol, phenol, tannin, saponin, flavonoid, protein, amino acid (Madhavan et al. 2008).
Solanum nigrum L.	Alkaloids, saponins, terpenoids (Gogoi and Islam 2013; Djaafar et al. 2014).
Terminalia bellirica (Gaertn.) Roxb.	Glycosides, flavonoids, tannins, phenols, saponins, diterpenes, proteins, amino acids (Abraham et al. 2014).
Thunbergia grandiflora Roxb.	Not reported for its root pharmacognosical analysis.
Tinospora cordifolia Miers	Proteins, carbohydrates, phenols/tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids (Agarwala and Yadav 2011)
Typhonium trilobatum (L.) Schott	Flavanoids, alkaloids, saponins and tannins (Roy et al. 2012)
Vanda tessellata Hook. ex G. Don	Terpenoids, flavonoids, phenols, alkaloids, tannins, steroids, glycosides (Bhattacharjee et al. 2015).
Zingiber officinale L.	Protein, calcium, fat, phosphorous, polyphenols, soluble fibre, tannin, flavonoids insoluble fibre, zinc, carbohydrate, copper, vitamin c, manganese, carotenoids, chromium (Shirin and Prakash 2010).
Ziziphus oenoplia (L.) Mill.	Alkaloids, 7a-hydroxy-6-isopropenyl-3,3,3a,5,6a,10,10-11b,octamethyl-hexadecahydro-benzo[de]anthracene; 5-carboxylic acid identified from root bark (Prabhavathi and Vijayalakshmi 2015).

constituents. Therefore, proper and intensive inventorying of medicinal plants specific to certain health problems becomes necessary. The diminishing pattern of the knowledge system is in danger leading to more in-depth study across the genders and age to know the transfer system. It is necessary to carry the ethnobotanical study of these plants which would help in the preservation of their knowledge and can be utilized in the conservation of these potential plants. It however requires detail work initiated with inter-cultural study and representing about the status and usage of the same species in curing different ailments coupled with chemical analysis. In this context, activities of phytochemical against jaundice may forward an interesting theme for the future studies to screen out and testing their explicit function. Furthermore, pharmacological-ethnobotanical index of such plants may ascribe the indigenous community's knowledge to higher rates of validation that logically support erosion of traditional knowledge which really needs to be revitalized.

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REFERENCES

Abbasi AM, Khan MA, Ahmad M, Zafar M, Khan H, Muhammad N, et al. 2009. Medicinal plants used for the treatment of jaundice and hepatitis based on socio-economic documentation. African J Biotechnol 8 (8): 1643-1650.

Abraham A, Mathew L, Samuel S. 2014. Pharmacognostic studies of the fruits of *Terminalia bellirica* (Gaertn.) Roxb. J Pharmacogn Phytochem 3 (2): 45-52.

Agarwala M, Yadav RNS. 2011. Phytochemical analysis of some medicinal plants. J Phytol 3 (12): 10-14.

Aiyelaagbe OO, Osamudiamen PM. 2009. Phytochemical Screening for Active compounds in *Mangifera indica* leaves from Ibadan, Oyo State. Plant Sci Res 2 (1): 11-13.

Al-Jabri NN, Hossain MA. 2014. Comparative chemical composition and antimicrobial activity study of essential oils from two imported lemon fruits samples against pathogenic bacteria. Beni-Suef Univ J Basic Appl Sci 3(4): 247-253.

Annalakshmi R, Uma R, Chandran GS, Muneeswaran A. 2012. Common plants used in the treatment of jaundice in southern India as a natural remefier-A review. Indian J Drugs Dis 1 (2): 47-50.

Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GE. 2009. Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium*. African J Biotechnol 8 (18): 4685-4689.

Bhattacharjee B, Islam T, Rahman Z, Islam SMS. 2015. Antimicrobial activity and phytochemical screening of whole plant extracts of *Vanda tessellata* (Roxb.) Hook. ex. G. Don. World J Pharm Pharmaceut Sci 4 (1): 72-83.

Bhattacharya UC, Goel AK. 1981. A note on some plants found effective in treatment of jaundice (Hepatitis). J Econ Tax Bot 2: 157-159.

Bora A, Devi P, Borthakur SK. 2012. Phyto-remedies of jaundice-A traditional approach on Majuli, Special reference to Satra culture people, Assam. Asian J Plant Sci Res 2 (6): 664-669.

Bruneton J. 1999. *Carica papaya*. In Bruneton J (ed.). Pharmacognosy, Phytochemistry of Medicinal Plants. Tech Doc, Paris 2: 221-223.

Burtis CA, Ashwood ER. 2003. Tietz Fundamentals of Clinical Chemistry. 5th ed. Elsevier, New Delhi.

Choudhury S, Sharma P, Dutta Choudhury M, Dutt Sharma G. 2012. Ethnomedicinal plants used by Chorei tribes of Southern Assam, North Eastern India. Asian Pac J Trop Dis 2 (1):141-147.

Chouhan K, Solanki R, Patel A, Macwan C, Patel M. 2011. Phytochemical and therapeutic potentrial of *Piper longum* Linn.: a review. Intl J Res Ayurveda Pharm 2 (1): 157-161.

Das HB, Majumdar K, Datta BK, Ray D. 2009. Ethnobotanical uses of some plants by Tripuri and Reang tribes of Tripura. Nat Prod Radiance 8 (2): 172-180.

Das S, Choudhury MD. 2010. Plants used against gastro-intestinal disorders and as anti hemorrhagic by three tribes of North Tripura District, Tripura, India. Ethnobot Leafl 14: 467-478.

Das S, Choudhury MD. 2012. Ethnomedicinal uses of some traditional medicinal plants found in Tripura, India. J Med Pl Res 6 (35): 4908-4914.

Das S, Dutta Choudhury M, Mandal SC, Das Talukdar A. 2012. Traditional knowledge of ethnomedicinal hepatoprotective plants used by certain ethnic communities of Tripura state. Indian J Fundament Appl Life Sci 2 (1): 84-97.

Das S, Dutta Choudhury M. 2012. Ethnomedicinal uses of some traditional medicinal plants found in Tripura, India. J Med. Pl Res 6 (35): 4908-4914.

Das S, Sarma G, Barman S. 2008. Hepatoprotective activity of aqueous extract of fruit pulp of *Cassia fistula* (AFCF) against carbon tetrachloride (CCl4) induced liver damage in albino rats. J Clin and Diagn Res 2: 1133-1139.

Das T, Mishra SB, Saha D, Agarwal S. 2012. Ethnobotanical survey of medicinal plants used by ethnic and rural people in Eastern Sikkim Himalayan region. African J Basic Appl Sci 4 (1): 16-20.

De B, Debbarma T, Sen S, Chakraborty R. 2010. Tribal life in the environment and biodiversity of Tripura, India. Curr World Environ 5:59-66.

Deb D, Darlong L, Sarkar A, Roy M, Datta BK. 2012. Traditional ethno-medicinal plants use by the *Darlong* tribes in Tripura, Northeast India. Intl J Ayurvedic Herbal Med 2 (6): 954-966.

Deb DB. 1968. Medicinal plants of Tripura State. Indian For 94 (10): 753-765.

Deb DB. 1981. *Flora of Tripura State*. Vol. I. Today and Tomorrow Printers and Publisher, New Delhi.

Deb DB. 1983. *Flora of Tripura State*. Vol. II. Today and Tomorrow Printers and Publisher, New Delhi.

Deb PK, Das S, Nath Bhaumik K, Ghosh R, Ghosh TK, Bhakta T. 2013. Pharmacognostic & preliminary phytochemical investigations of *Neptunia prostrata* L. J Pharmacogn Phytochem 2 (3): 5-11.

Dewanji S, Matai L, Barik S SI, Nag A. 1993. Chemical composition of two semi-aquatic plants for food use. Pl Foods Human Nutr 44:11-16.

Djaafar Z, Ridha Ouahrani M. 2014. Phytochemical study of selected Medicinal plant, *Solanum nigrum*, the Algerian Desert. Intl Lett Chem Phys Astron 20: 25-30.

Do DR, Nguyen XD. 1996. Chemical constituents of *Plumbago zeylanica* Linn. Tap Chi Hoa Hoc 34: 67-70.

Doss A, Anand SP. 2014. Evaluation of anti-diabetic activity of methanol and aqueous extracts of *Asteracantha longifolia* (Linn.) Nees. J Pharmacol 8 (1): 1-5.

Dutta U, Sarma GC. 2013. Medicinal plants used by the local fringe communities of Chirang Reserve Forest, BTAD, Assam. Indian J Res 2 (2): 262-265.

Gheewala P, Kalaria P, Chakraborty M, Kamath JV. 2012. Phytochemical and Pharmacological profile of Averrhoa carambola Linn: An overview. Int Res J Pharm 3: 88-92.

Mohanty PK, Chourasia N. 2011. Preliminary phytochemical screening of *Cajanus cajan* Linn. Asian J Pharmaceut Technol 1 (2): 49-52.

Gogoi P, Islam M. 2012. Ethnomedicinal Study of *Solanum nigrum* L, and

S. myriacanthus Dunal used by tribals and non-tribals from Districts of Upper Assam, India. Asian J Exp Bio Sci 3 (1): 73-81.

Gupta A, Rai R, Siddiqui IR, Singh J. 1998. Two new triterpenoids from *Plumbago zeylanica*. Fitoterapia 69: 420-422.

Gupta MM, Verma RK, Gupta AP. 1995. A chemical investigation of *Plumbago zeylanica*. Curr Res Med Aromather Plants 17:161-164.

Guyton A, Hall J. 2005. Textbook of Medicinal Physiology. WB. Saunders, Philladelphia.

Hossain MS, Urbi Z, Sule A, Rahman KMH. 2014. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. Sci World J 2014: 1-28.

Husain A. 1992. Dictionary of Indian Medicinal Plants. Central Institute of Medicinal and Aromatic Plants, Lucknow.

Husain J, Tickle IJ, Wood SP. 1994. Crystal structure of momordin, a type I ribosome inactivating protein from the seeds of *Momordica charantia*. FEBS Lett 342: 154-158.

Igwenyi IO, Offor CE, Ajah DA, Nwankwo OC, Ukaomah JI, Aja PM. 2011. Chemical compositions of *Ipomoea aquatica* (green kangkong). Intl J Pharm Biol Sci 2 (4): 593-598.

Islam MZ, Hoque MM, Asif-Ul-Alam SM, Monalisa K. 2015. Chemical composition, Antioxidant capacities and storage stability of *Citrus macroptera* and *Garcinia pedunculata* fruits. Emir J Food Agric 27 (3): 275-282.

Ito C, Itoigawa M, Sato A, Hasan Choudhury M, Mohammad RA, Tokuda H, Mukainaka T, Nishino H, Furukawa H. 2004. Chemical constituents of *Glycosmis arborea*: Three new carbazole alkaloids and their biological activity. J Nat Prod 67: 1488-1491.

Jagtap S, Satpute R. 2014. Phytochemical screening and antioxidant activity of rhizome extracts of *Costus speciosus* (Koen.) J.E. Smith. J Artif Intell Res 3 (1): 40-47.

Jayachandra K, Devi VS. 2012. Development of herbal drugs in the treatment of jaundice-An overview. Intl J Ayurvedic Herb Med 2 (4): 636-645.

Kaji NN, Khorana ML, Sanghavi MM. 1968. Studies on *Cassia fistula* Linn. Indian J Pharm 30: 8-11.

Kala CP. 2005. Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. J Ethnobiol Ethnomed 1: 11-18.

Karmakar P, Islam MM, Kibria MG, Hossain MS, Sattar MM. 2012. Prevalence, belief and awareness of preferring traditional health care system in urban and rural people of Noakhali district, Bangladesh. Intl Curr Pharm J 1 (9): 229-234.

Karuppasmy S. 2007. Medicinal plants used by Paliyan tribes of Sirumalai hills of southern India. Nat Prod Radiance 6 (5): 436-442.

Kashiwada Y, Toshika K, Chen R, Nonaka G, Nishioka I. 1996. Tannins and related compounds. XCIII. Occurrence of enantiomeric proanthocyanidins in the Leguminosae plants, *Cassia fistula* L.; *Cassia javanica* L. Chem Pharm Bull 38: 888−893.

Kumarappan CT, Thilagam E, Mandal SC. 2012. Antioxidant activity of polyphenolic extracts of *Ichnocarpus frutescens*. Saudi J Biol Sci 19 (3):349-55. doi: 10.1016/j.sjbs.2012.04.004.

Kuroda M, Mimaki Y, Harada H, Sakagami H, Sashida Y. 2001. Five new triterpene glycosides from *Centella asiatica*. Nat Med 55 (3): 134-138.

Lalrinzuali K, Vabeiryureilai M and Ganesh Chandra J. 2015. Ethnomedicinal use and phytochemical analysis of selected medicinal plants of Mizoram, India. Trends Green Chem 1 (8): 1-9.

Lokho A. 2012. The folk medicinal plants of the Mao Naga in Manipur, North East India. Intl J Sci Res Publ 2 (6): 1-8.

Madhavan V, Hemalatha HT, Murali A, Yoganarasimhan SN. 2008. Antiepileptic activity of alcohol and aqueous extracts of roots and rhizomes of *Smilax zeylanica* Linn. Pharmacologyonline 3: 263-272.

Mahesh VK, Sharma R, Singh RS 1984. Anthraquinones and kaempferol from *Cassia fistula* species. J Nat Prod 47: 733-751.

Majumdar K, Datta BK. 2007. A study on ethnomedicinal usage of plants among the folklore herbalists and Tripuri medical practitioners: Part-II. Indian J Nat Prod Resour 6 (1): 66-73.

Majumdar K, Saha R, Datta BK, Bhakta T. 2006. Medicinal plants prescribed by different tribal and non-tribal medicine man of Tripura State. Indian J Trad Know 5 (4): 559-562.

Manikandan K, Rajendran K, Shanmugam S. 2009. Ethnomedicinal survey of medicinal plants for the treatment of diabetes & jaundice among the villagers of Sivagangai district Tamil nadu. J Ethnobot Leafl 13:189-94.

Marrufo T, Nazzaro F, Mancini E, Fratianni F, Coppola R, De Martino L et al. 2013. Chemical composition and biological activity of the essential oil from leaves of *Moringa oleifera* Lam. cultivated in Mozambique. Molecules 18: 10989-11000. DOI: 10.3390/molecules180910989.

Matsuda H, Morikawa T, Ueda H, Yoshikawa M. 2001. Medicinal foodstuffs. XXVII. Saponin constituents of gotu kola (2): structures of new ursane-and oleanane-type triterpene oligoglycosides, centellasaponins B, C, and D, from *Centella asiatica* cultivated in Sri Lanka. Chem Pharm Bull 49 (10):1368-1371.

Mayes PA. 2000. Harpers Biochemistry, 25th ed. McGraw Hill, New York.

Mishra D, Singh RK, Srivastava RK. 2012. Ethno-medicinal plants used to cure different diseases by rural folks and tribes of North Eastern Tarai districts of Uttar Pradesh, India. Res J Med Plants 6 (4): 286-299.

Mishra P. 2010. Isolation, spectroscopic characterization and computational modeling of chemical constituents of *Piper longum* natural product. Intl J Pharm Sci Rev Res 2 (2): 78-86.

Monsen ER. 2000. Dietary reference intake for antioxidant nutrients. J Am Diet Assoc 100: 637-640.

Murakami T, Emoto A, Matsuda H, Yoshikawa M. 2001. Medicinal foodstuffs. Part XXI. Structures of new cucurbitane-type triterpene glycosides, goyaglycosides-a,-b,-c,-d,-e,-f,-g, and-h and new oleanane-type triterpen saponins, goyasaponins I, II, and III, from the fresh fruit of Japanese *Momordica charantia* L. Chem Pharm Bull 49: 54-63.

Nadkarni KM. 1954. Indian material medica. Pop Pra Pvt Ltd, Bombay 1: 273-277.

Negi A, Joshi A, Juyal V, Kumar T. 2011. Antidiabetic and Hypolipidemic Activity of *Citrus medica* Linn. seed extract in streptozotocin induced diabetic rats. Pharmacog J 23 (12): 80-84.

Nile SH, Khobragade CN. 2010. Antioxidant activity and flavonoid derivatives of *Plumbago zeylanica*. J Nat Prod 3: 130-133.

Niranjan A, Tiwari SK, Lehri A. 2010. Biological activities of kalmegh (*Andrographis paniculata* Nees) and its active principles-a review. Indian J Nat Prod Resour 1 (2): 125-135.

Ogundajo AL, Ogunwande IA, Bolarinwa TM, Joseph OR, Flamini G. 2014. Essential oil of the leaves of *Hibiscus surattensis* L. from Nigeria. J Essent Oil Res 26 (2): 114-117.

Okada Y, Shibata S, Ikekaw T, Ana MJJ, Kamo O. 1987. Entada saponin-III, a saponin isolated from the bark of *Entada phaseoloides.* Phytochem 26 (10): 2789-2796.

Okuda T, Yoshiba T, Hatano T. 1991. Chemistry and biological activities of tannins in medicinal plants. J Sci Technol 5: 127-301.

Okwu DE, Ndu CU. 2006. Evaluation of the phytonutrients, mineral and vitamin contents of some varieties of yam. Intl J Mol Med Adv Sci 12 (2): 199-203.

Orhan IE. 2012. *Centella asiatica* (L.) Urban: from traditional medicine to modern medicine with neuroprotective potential. Hindawi Publishing Corporation 1-8. doi:10.1155/2012/946259

Pal S, Bhattacharjee A, Mukherjee S, Bhattacharya K, Khowala S. 2014. Antioxidant and hepatoprotective activity of ethanolic extract of *Alocasia indica* Tuber. Amer J Phytomed Clin Therapeut 2 (2): 191-208.

Palla R, Elumalai A, Eswaraiah MC, Kasarla R. 2012. A review on krishna tulsi, *Ocimum tenuiflorum* Linn. Intl J Res Ayurveda Pharm 3 (2): 291-293.

Parkash A, Ng TB, Tso WW. 2002. Purification and characterization of charantin, a napin-like ribosome-inactivating peptide from bitter gourd (*Momordica charantia*) seeds. J Peptide Res 59: 197-202.

Prabhavathi S, Vijayalakshmi S. 2015. New hydroxy carboxylic acid from the root bark of *Ziziphus oenoplia*. J Pharmacog Phytochem 3 (6): 150-152.

Prajapati ND, Purohit SS, Sharma AK, Kumar T. 2006. A Hand book of Medicinal Plants. 3rd edition. Agrobios Hindustan Printing Press, Jodhpur.

Prasad K, Moulekhi K, Bisht G. 2010. Chemical composition of the essential oils of *Pavetta indica* leaves. Res J Phytochem 2010: 1-4.

Raghu AV, George S, Krishna VR, Sindhu KK. 2013. Bioactive properties of phenolics present in *Oroxylum indicum*-A review. J Pharmacog Phytochem 2 (3): 23-27.

Rahim ZB, Rahman MM, Saha D, Hose SMZ, Paul S, Kader S. 2012. Ethnomedicinal plants used against jaundice in Bangladesh and its economical prospects. Bull Pharm Res 2 (2): 91-105.

Rai MK, Pandey AK, Acharya D. 2000. Ethno-medicinal plants used by Gond tribe of Bhanadehi, district Chhindwara, Madhya Pradesh. J Non-timber For Prod 7: 237-241.

Rai PK, Lalramnhinglova H. 2010. Lesser known ethnomedicinal plants of Mizoram, North East India: An Indo-Burma hotspot region. J Med Pl Res 4 (13): 1301-1307.

Raman A, Lau C. 1996. Anti-diabetic properties and phytochemistry of *Momordica charantia* L. (Cucurbitaceae). Phytomed 2: 349-362.

Rastogi RP, Mehrotra BN. 1995. Compendium of Indian Medicinal Plants. Vol 4. Publication and Information Directorate, New Delhi.

Roy SK, Mishra PK, Nandy S, Datta R, Chakroborty B. 2012. Potential wound healing activity of the different extract of *Typhonium trilobatum* in albino rats. Asian J Trop Biomed 2 (3): 1477-1486.

Rumalla CS, Ali Z, Weerasooriya AD, Smillie TJ, Khan IA. 2010. Two new triterpene glycosides from *Centella asiatica*. Planta Medica 76 (10): 1018-1021.

Sahu C, Pattnaik SK, Sahoo SL, Lenka SS, Dhal NK. 2011. Ethnobotanical study of medicinal plants in the coastal districts of Odisha. Curr Bot 2 (7): 17-20.

Sajem A, Gosai K. 2008. Ethnobotanical investigations among the *Lusai* tribes in North Cachar Hills district of Assam, Northeast India. Indian J Trad Know 9 (1): 108-113.

Sen S, Chakraborty R, De B, Devanna N. 2011. An ethnobotanical survey of medicinal plants used by ethnic people in West and South district of Tripura, India. J For Res 22 (3): 417-426.

Shankar R, Rawat MS, Majumdar R, Baruah D, Bharali BK. 2012. Medicinal plants used in traditional medicine in Mizoram. World J Sci Technol 2 (12): 42-45.

Shil S, Choudhury MD. 2009. Ethnomedicinal importance of Pteridophytes used by Reang tribe of Tripura, North East India. Ethnobot Leaflet 13: 634−643.

Shirin APR, Prakash J. 2010. Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). J Med Plants Res 4 (24): 2674-2679.

Siddiqui BS, Aslam H, Ali ST, Khan S, Begum S. 2007. Chemical constituents of *Centella asiatica*. J Asian Nat Prod Res 9 (4): 407-414.

Sikdar M, Dutta U. 2008. Traditional phytotherapy among the Nath People of Assam. Ethno-Med 2 (1): 39-45.

Singh HB, Hynniewta TM, Bora PJ. 1997. Ethno-Medico-Botanical studies in Tripura, India. Ethnobot 9: 56-58.

Srivastava S , Mishra N , Misra U. 2009. *Bacopa monniera*-a future perspective. Intl J Pharm Sci Drug Res 1 (3): 154-157.

Umamaheswari M, Chatterjee TK. 2008. In vitro antioxidant activities of the fractions of *Coccinia grandis* leaf extract. African J Trad Compl Altern Med 5 (1): 61-73.

Valdez-Solana MA, Mejía-García VY, Alfredo TV, Guadalupe GA, José SP, et al. 2015. Nutritional content and elemental and phytochemical analyses of *Moringa oleifera* grown in Mexico. J Chem. http://dx.doi.org/10.1155/2015/860381.

Wang ZY, He WJ, Zhou WB, Zeng GZ, Yin ZQ , Zhao SX, Tan NH. 2014. Two new phenylpropanoids from *Micromelum integerrimum*. Chin J Nat Med 2 (8): 619-622.

WHO. 2001. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO, Geneva, Switzerland.

Xie H, Huang S, Deng H, Wu Z, Ji A. 1998. Study on chemical components of *Momordica charantia*. Zhong Yao Cai 21: 458-459.

Yong JWH, Ge L, Fei YN, Tan SN. 2009. The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules 14: 5144-5164. doi:10.3390/molecules14125144.

Yoshida M, Fuchigami M, Nagao T, Okabe H, Matsunaga K, Takata J, Karube Y, Tsuchihashi R, Kinjo J, Mihashi K, Fujioka T. 2005. Antiproliferative constituents from umbelliferae plants VII. Active triterpenes and rosmarinic acid from *Centella asiatica*. Biol Pharm Bull 28: 173-175.

Yuan YR, He YN, Xiong JP, Xia ZX. 1999. Three-dimensional structure of beta-momorcharin at 2.55A resolution. Acta Crystallogr 55: 1144-1151.

Zhao Z, Jin J, Lin C, Zhu C, Liu Y, Lin A et al. 2011. Two new chalcone glycosides from the stems of *Entada phaseoloides.* Fitoterapia 82 (7): 1102-1105.

Zulfiker AHM, Rahman MM, Hossain MKamal, Hamid K, Mazumder MEH, Rana MS. 2010. In vivo analgesic activity of ethanolic extracts of two medicinal plants-*Scoparia dulcis* L. and *Ficus racemosa* Linn. Biol Med 2 (2): 42-48.

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Short Communication: Genetic identification of local pigs, and imported pigs (Landrace and Duroc) based on cytochrome b sequence analysis

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Abstract. *Hartatik T, Soewandi BDP, Volkandari SD, Tabun AC, Sumadi, Widodo. 2016. Genetic identification of local pigs, and imported pigs (Landrace and Duroc) based on cytochrome b sequence analysis. Biodiversitas 17: 270-274.* The aim of this study was to identify the genetics of local pigs and imported pigs (Landrace and Duroc) based on qualitative analysis. Thirty-eight pigs were used in this study and consisted of 11 local pigs (from Bali), six Landrace pigs and four Duroc pigs (from Malang), nine Landrace pigs (from Bali) and eight local pigs (from Kupang). Qualitative traits in pigs such as coat color, body shape (back shape, belly shape, and ears) and hair cover were observed. The cytochrome b (*Cyt* b) gene of mitochondrial DNA was analyzed using PCR-restriction fragment length polymorphism. The PCR analysis resulted in a 464 base pairs (bp) amplified band, and this was digested using *Taq*I restriction enzyme. The PCR-RFLP analysis resulted in two bands, 246 and 218 bp (monomorphic). The alignment analysis showed four points of single nucleotide polymorphisms. Bali and Kupang pigs had a specific pattern on exterior characteristics such as curved back shape, belly hanging shape, small ears and thick hair, and had many variations on coat color such as black, cream, spotted and mottled. The differences in coat color and body shape, and the corresponding mtDNA *Cyt* b sequence (with four SNPs) is a marker for genetic variation in pigs.

Keywords: Coat color, mtDNA, cytochrome b, qualitative analysis, genetic variation

INTRODUCTION

Indonesia has a great variety of domestic animals such as native chickens, ducks, goats, sheep, cattle and pigs. There are five species of local pig in Indonesia, *Sus barbatus* (Babi Berjanggut), *Sus celebenis* (Babi Sulawesi Berkutil), *Sus verrucous* (Babi Jawa Berkutil), *Sus scorfa* (Babi Alang-Alang) and *Babyroussa babyrussa* (Babirusa) (Rothschild et al. 2011). Genetic variations of local pigs in Indonesia are important to investigate. Since these pigs have important roles for cultural activities in the society, it is necessary to maintain their sustainability. The local Kupang pigs were used for traditional ceremonies such as weddings and religious ceremonies (Johns et al. 2010). For Bali cultural activities, pigs were used as the oblation for the religious activities. The oblation pig was a young intact boar up to eight or nine months old which had been fattened for five to six months (Soewandi 2013). The study of genetic diversity based on mitochondrial DNA (mtDNA) as a maternal line has been a reportedly useful tool as a molecular marker. Mitochondrial DNA is maternally inherited and experiences nucleotide changes faster than DNA (Brown et al. 1979), which makes mtDNA an ideal tool for studying population genetics (Bailey et al. 2000). Mitochondrial DNA is a useful genetic marker for both intra- and interspecies studies (Brown et al. 1979; Kikkawa et al. 1995).

Continental of wild boars and domestic pigs were clearly divided into eastern and western clades (Larson et al. 2005; Wu et al. 2007; Leutkemeier et al. 2010) using d-loop mitochondrial DNA. A previous study based on Single Nucleotide Polymorphism (SNP) revealed that population of wild boars from Near Eastern Asia (Turkey, Iran and Armenia) and Europe (Spain, Belgium and Russia) are genetically different (Manunza et al. 2013). Asian pig populations were comprised of three groups. One group is represented by Erhualian and Meishan breed, while the second represented by Lanyu pigs and the third represented by the Asian wild boars. The Asian domestic populations were derived from multiple Asian ancestral origins whereas the European domestic populations represent a single ancestral European lineage (Leutkemeier et al. 2010).

Most genetic studies on wild boars in East Asia were carried out using mtDNA sequence analysis, which revealed several subclades (Larson et al. 2005; Hongo et al. 2002; Cho et al. 2009; Ramayo et al. 2010; Ji et al. 2011; Larson et al. 2010). Previous studies based on both mtDNA and nuclear genes demonstrated no population substructures exists in neither wild boars nor domestic pigs in East Asia and showed a very high level of admixture between them (Ji et al. 2011). Korean wild boars were clearly clustered within Asian wild boar groups, sharing the same cluster with populations from Myanmar and Thailand (Cho et al. 2009) and the Vietnamese wild pig haplotype (Hongo et al. 2002). On the other hand, Larson et al. (2010) ascertained that wild boars in South Korea belong to groups unique within East Asia, and remain differentiated from domestic pigs.

Based on the mitochondrial DNA, the Vietnamese wild boars were clustered into two groups, group I was genetically distinct to Asian wild boars and group II that was genetically close to Asian wild boars. There are three types of haplotype in Vietnamese domestic pigs and two types of haplotypes in Vietnamese wild boars in Central Highland (Long et al. 2014). Chinese pig breeds were originally from the wild boars in the South China and the Yangtze River Region (Yu et al. 2013). The others studies showed that Chinese native pig breeds had a single origin (Lan and Shin 1993; Huang et al. 1999).

Study on the cytochrome b gene of indigenous pigs with PCR-SSCP methods had previously identified four SNPs located at positions 47 (T/C),49 (G/A), 52 (C/T) and 56 (G/A) and revealed the Asian origin of the Indigenous pigs (Ghungroo, Meghalaya local and Nagaland local) with A1 haplotype which revealed absence of mixed haplotype (Saikia et al. 2015). Other study in India, based on the analysis of sequence generated from the partial fragment (421 bp) mtDNA cytochrome b (*Cyt* b) gene exhibited unambiguous (>3%) genetic variation between Indian wild and domestic pigs. They observed nine forensically informative nucleotide sequence (FINS) variations between Indian wild and domestic pigs (Gupta et al. 2013).

This study was conducted to determine the genetic variation of local pigs (Bali and Kupang) compared with imported pigs (Landrace and Duroc) based on coat color, body shape and mtDNA *Cyt* b by using PCR-RFLP and sequence analysis.

MATERIALS AND METHODS

Samples and DNA extraction

Thirty-eight pigs were studied, consisting of 11 local pigs (Bali), eight local pigs (Kupang, NTT), 15 Landrace pigs and four Duroc pigs. Exterior characteristics of the pigs such as coat color, body shape (back shape, belly shape, and ears) and hair cover were observed. Blood and ear tissue samples were collected for DNA analysis. Blood samples were taken through jugular venipuncture and preserved in K_3EDTA solution tubes. Samples were stored frozen (-20 °C) until needed. The DNA from blood or ear tissue samples was extracted by using the standard SDS/Proteinase K modified method according to Sambrook et al. (1989).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

The 464 base pairs (bp) fragment of the mtDNA *Cyt* b gene was amplified by polymerase chain reaction (PCR) using forward and reverse primers according to Wolf et al. (1999): L14735 (5'-AAA AAC CAC CGT TGT TAT TCA ACTA-3') and H15149 (5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3'). Polymerase chain reaction was performed with a final volume of 20 µL of reaction mixture containing 1 µL of DNA sample (10-100 ng), 1 µL of each primer (10 pmol/ µL), 10 µL PCR KIT (Kappa, Biosystem), and 7 µL of double distilled water. The amplification process was performed using Thermocycler (Infinigen, TC-25/H) with the following conditions: initial denaturation at 94°C for 2 min, followed by 35 thermal cycles of denaturation at 95°C for 36 sec, annealing at 51°C for 73 sec, extension at 72°C for 84 sec and the final extension at 72°C for 3 min (Prado et al. 2005). The PCR product was visualized on 1% agarose gels buffered with 1X Tris-Boric-EDTA buffer (1XTBE), stained with ethidium bromide and visualised under ultraviolet (UV) light. The PCR-amplified DNA fragment of the *Cyt* b gene was digested using the *Taq*I restriction enzyme to identify genetic patterns. The total volume of digestion was 12 µL containing 3 µL PCR product, 0.2 µL *Taq*I (Fermentas) enzyme (1U), 1.2 µL Tango buffer and 7.6 µL aquabidest sterile. The enzymatic digestion was conducted at 65 °C for two hours by the *Taq*I enzyme. The digestion products were separated on 10% polyacrylamid gels in 1XTBE buffer and run with 50 V for three hours for separation of the DNA fragments. The bands were stained with ethidium bromide before visualization under UV light. The size of the amplified bands was compared with DNA marker ϕX174 DNA/*Bsu*RI (*Hae*III) (Fermentas).

Sequencing and analysis

A total volume of 30 µL for each PCR product and 10 µL *Cyt* b primer (10 pmol/µL) was prepared for sequencing. Sequencing the amplified bands of PCR products was performed by BioSM Macrogen (Korea). The DNA sequences were aligned by using BioEdit version 7.7 for identification of the single nucleotide polymorphism.

RESULTS AND DISCUSSION

The exterior characteristics of the pigs were determined based on expert judgment and included coat color and body shape. Landrace pigs had 100% white color while Duroc pigs had 100% reddish brown color. Bali and Kupang pigs had a huge diversity of color: 63.6% of Bali pigs had black coat color and 36.4% had mottled color, whereas 37.5% Kupang pigs had reddish brown color, 25% had cream color and 12.5% had spotted (white and black) color. Local pigs had specific characteristics of body shape at back and belly whereas imported pigs (Landrace and Duroc) had a straight body shape between back and belly. In addition, Bali and Kupang pigs had small, upright ears and thick hair. This is different from the Landrace and Duroc pigs that had sparse hair and big ears, with 50% having ears folded forward (Figure 1).

The specific DNA fragment of the mtDNA *Cyt* b gene in local pigs, Landrace pigs and Duroc pigs was amplified by using L14735 and H15149 primers. The PCR product of the mtDNA *Cyt* b gene was 464 bp (Figure 2a). The product size of PCR-RFLP using the *Taq*I enzyme showed the same restriction pattern. There were two fragments of DNA, 246 bp and 218 bp (Figure 2b), which indicates that the sample population was monomorphic. In total of 38 samples, one haplotype of cytochrome b sequence was observed based on PCR-RFLP.

The sequence analysis of the mtDNA *Cyt* b gene of local pigs (Bali), Landrace pigs and Duroc pigs was compared to the complete mtDNA of *Cyt* b gene database available at GenBank (DQ.534707.2/*Sus scrofa* breed Taoyuan; NC. 014692.1/*Sus scrofa taiwanensis*; NC.012095.1/*Sus scrofa domesticus*; and GQ. 338965.1/*Sus scrofa*). Based on mtDNA *Cyt* b sequence alignment analysis (Figure 3), we found four points of single nucleotide polymorphism (SNP) which changed the nucleotides from C to T and G to A. However the local, Landrace and Duroc pigs had a similar sequence of mtDNA *Cyt* b in this study.

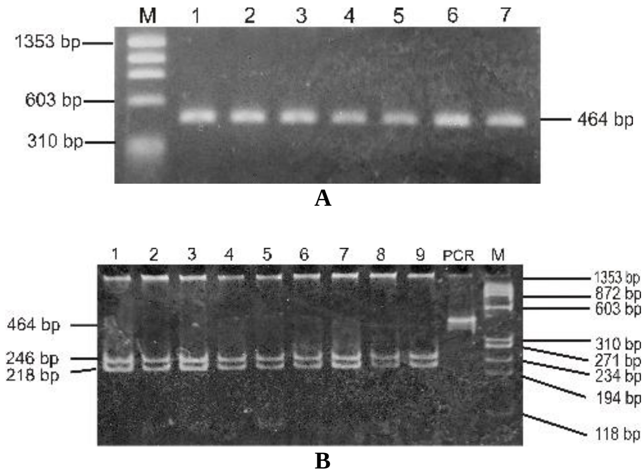

Figure 2. PCR product and RFLP with *Taq*I enzyme. A. PCR product of cyt b gene (464 bp); B. PCR-RFLP product using the *Taq*I enzyme: lanes 1-2: Duroc, lanes 3-5: Landrace, lanes 6-9: Bali pig, PCR: product, M: Marker ΦX174 DNA/*Bsu*RI (*Hae*III) (Fermentas).

A B C D

Figure 1. Coat color and body shape of pigs. A. Bali pig, B. Kupang pig, C. Duroc pig and D. Landrace pig

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DQ534707.2        TACACATCAGACACAACAACAGCTTTCTCATCAGTTACACACATCTGTCG 150
NC_014692.1       TACACATCAGACACAACAACAGCTTTCTCATCAGTTACACACATCTGTCG 150
LANDRACE (BALI)   TACACATCAGACACAACAACAGCTTTCTCATCAGTTACACACATCTGTCG 150
DUROC (MALANG)    TACACATCAGACACAACAACAGCTTTCTCATCAGTTACACACATCTGTCG 150
BALI (LOCAL)      TACACATCAGACACAACAACAGCTTTCTCATCAGTTACACACATCTGTCG 150
GQ338965.1        TACACATCAGACACAACAACAGCTTTCTCATCAGTTACACACATTTGTCG 150
NC_012095.1       TACACATCAGACACAACAACAGCTTTCTCATCAGTTACACACATTTGTCG 150
                  ******************************************** *****

DQ534707.2        AGACGTAAATTACGGATGAGTTATTCGCTACCTACATGCAAACGGAGCAT 200
NC_014692.1       AGACGTAAATTACGGATGAGTTATTCGCTACCTACATGCAAACGGAGCAT 200
LANDRACE (BALI)   AGACGTAAATTACGGATGAGTTATTCGCTACCTACATGCAAACGGAGCAT 200
DUROC (MALANG)    AGACGTAAATTACGGATGAGTTATTCGCTACCTACATGCAAACGGAGCAT 200
BALI (LOCAL)      AGACGTAAATTACGGATGAGTTATTCGCTACCTACATGCAAACGGAGCAT 200
GQ338965.1        AGACGTAAATTACGGATGAGTTATTCGCTATCTACATGCAAACGGAGCAT 200
NC_012095.1       AGACGTAAATTACGGATGAGTTATTCGCTATCTACATGCAAACGGAGCAT 200
                  ****************************** *******************

DQ534707.2        CCATGTTCTTTATTTGCCTATTCATCCACGTAGGCCGAGGCCTATACTAC 250
NC_014692.1       CCATGTTCTTTATTTGCCTATTCATCCACGTAGGCCGAGGCCTATACTAC 250
LANDRACE (BALI)   CCATGTTCTTTATTTGCCTATTCATCCACGTAGGCCGAGGCCTATACTAC 250
DUROC (MALANG)    CCATGTTCTTTATTTGCCTATTCATCCACGTAGGCCGAGGCCTATACTAC 250
BALI (LOCAL)      CCATGTTCTTTATTTGCCTATTCATCCACGTAGGCCGAGGCCTATACTAC 250
GQ338965.1        CCATATTCTTTATTTGCCTATTCATCCACGTAGGCCGAGGTCTATACTAC 250
NC_012095.1       CCATATTCTTTATTTGCCTATTCATCCACGTAGGCCGAGGTCTATACTAC 250
                  **** *********************************** *********
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Figure 3. Multiple sequence alignment of partial mtDNA cyt b in pigs

The sequenced DNA data showed that Bali pig ancestors were from China, which possibly were similar to the *Sus scrofa taiwanensis* ancestor. In four positions of SNPs, the same nucleotide between Bali pig and DQ.534707.2 (Breed Taoyuan) and NC. 014692.1 (Breed Taiwanesis) was observed (Figure 3). A previous study by Sihombing (1997) revealed that the Bali pig ancestor came from a cross between a second type of *Sus scorfa vittatus* and the South China pig. This finding supports our data that Bali pigs have a genetic similarity to *Sus scrofa taiwanensis* and the Taoyuan pig. The mitochondria of the pigs was divided into two types (Asian type and Europe type), Taoyuan pig included to Asian type (Chang et al. 2008).

A previous study by Clop et al. (2004) proved that Landrace and Duroc breeds had an Asian allele. It was likely due to an introduction process of Landrace and Duroc pigs with the Asian allele. Evidence showed that there was an introduction of Asian pig breeds to commercial pig breeds (Clop et al. 2004; Giuffra et al. 2000). The introduction processes did not result in any differences in their performances, rather their mtDNA showed high genetic similarity. Mitochondrial DNA has been widely used to unravel evolutionary studies, due to its greater diversity compared to nuclear DNA.

To conclude, local pigs, Landrace and Duroc pigs have the same sequence of cytochrome b gene. The sequence analysis of seven breed of pigs shows four position of single nucleotide polymorphism.

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REFERENCES

Bailey JF, Healy B, Jianlin H, Sherchand L, Pradhan SL, Tsendsuren T, Foggin, JM, Gaillard C, Steane D, Zakharov I, Bradley DG. 2002. Genetic variation of mitochondrial DNA within domestic yak populations. In: Jianlin H, Richards C, Hanotte O, McVeigh C, Rege JEO (eds.). Yak production in Central Asian highlands; Proceedings of the Third International Congress on Yak. Lhasa, PR China, 4-9 September 2000.

Brown WM, George MJr, Wilson AC. 1979. Rapid evolution of animal mitochondrial DNA. Proc Natl Acad Sci USA 76 (4): 1967-1971.

Chang WH, Chu HP, Jiang YN, Li SH, Wang Y, Chen CH, Chen KJ, Lin CY, Ju YT. 2008. Genetic variation and phylogenetics of Lanyu and exotic pig breeds in Taiwan analyzed by nineteen microsatellite markers. J Anim Sci 1-8.

Cho IC, Han SH, Fang M, Lee SS, Ko MS, Lee H, Lim HT, Yoo CK, Lee JH, Jeon JT. 2009. The robust phylogeny of Korean wild boar (*Sus scrofa coreanus*) using partial D-loop sequence of mtDNA. Mol Cells 28: 423-430.

Clop A, Amills M, Noguera JL, Fernandez A, Capote J, Ramon MM, Kelly L, Kijas JMH, Andersson L, Sanchez A. 2004. Estimating the frequency of Asian cytochrome B haplotypes in standard European and local Spanish pig breeds. Genet Selec Evol 36: 97-104.

Giuffra E, Kijas JMH, Amargoer V, Carlborg O, Jeon JT, Andersson L. 2000. The origin of the domestic pig: independent domestication and subsequent introgression. Genetics 154 (4): 1785–1791.

Gupta, S. K., A.Kumar, S.A. Hussain, Vipin, L. Singh. 2013. Cytochrome b based genetic differentiation of Indian wild pig (*Sus scrofa cristatus*) and domestic pig (*Sus scrofa domestica*) and its use in wildlife forensics. Sci Justice 53: 220-222

Hongo H, Ishiguro N, Watanobe T, Shigehara N, Anezaki T, Long VT, Binh DV, Tien NT, Nam NH. 2002. Variation in mitochondrial DNA of Vietnamese pigs: relationships with Asian domestic pigs and Ryukyu wild boars. Zoo Sci 19: 1329-1335.

Huang YF, Shi XW, Zhang YP. 1999. Mitochondrial genetic variation in Chinese pigs and wild boars. Biochem Genet 37: 335-343.

Ji Y-Q, Wu D-D, Wu G-S, Wang G-D, Zhang Y-P. 2011. Multi-locus analysis reveals a different pattern of genetic diversity for mitochondrial and nuclear DNA between wild and domestic pigs in East Asia. PLoS ONE 6: e26416.

Johns C, Cargill C, Patrick I, Geong M, Ly J. 2010. Commercial pig farming by small farmers in Nusa Tenggara Timur Indonesia. [Unpublished Report]. SADI-ACIAR, Canberra. [Indonesian]

Lan H, Shi L. 1993. The origin and genetic differentiation of native breeds of pigs in Southwest China: an approach from mitochondrial DNA polymorphism. Biochem Genet 31: 51-60.

Larson G, Dobney K, Albarella U, Fang M, Matisoo-Smith E, Robins J, Lowden S, Finlayson H, Brand T, Willerslev E, Rowley-Conwy P, Andersson L, Cooper A. 2005. Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. Science 307: 1618-1621.

Larson G, Liu R, Zhao X, Yuan J, Fuller D, Barton L, Dobney K, Fan Q, Gu Z,Liu X-H, Luo Y, Lv P, Andersson L, Li N. 2010. Patterns of East Asian pig domestication, migration, and turnover revealed by modern and ancient DNA. Proc Natl Acad Sci USA 107: 7686-7691.

Leutkemeier ER, Sodhi M, Schook LB, Malhi RS. 2010. Multiple Asian pig origins revealed through genomic analyses. Mol Phylogen Evol 54: 680-686.

Long LT, Mai NTP, Chung DC, Son HN, Si DM. 2014. The genetic relationship of Vietnamese pigs in central highlands assessed by cytochrome b. Open J Genet 4: 362-369.

Kikkawa Y, Amano T, Suzuki H. 1995. Analysis of genetic diversity of domestic cattle in east and Southeast Asia in terms of variations in restriction sites and sequences of mitochondrial DNA. Biochem Genet 33: 51-60.

Manunza A, Zidi A, Yeghoyan S, Balteanu VA, Carsai TC, Scherbakov O, Ramirez O, Castello A, Amills M, Mercade A. 2013. A high throughput genotyping approach reveals distinctive autosomal genetic signatures for European and Near eastern wild boar. Plos One 8 (2): e55891 doi: 10.1371/journal.pone.0055891.

Prado M, Calo P, Cepeda A, Barros-Velázquez J. 2005. Genetic evidence of an Asian background in heteroplasmic Iberian cattle (*Bos taurus*): effect on food authentication studies based on polymerase chain reaction-restriction fragment length polymorphism analysis. Electrophoresis 26: 2918-2926.

Ramayo Y, Shemeret'eva IN, Pérez-Enciso M. 2010. Mitochondrial DNA diversity in wild boar from the Primorsky Krai Region (East Russia). Anim Genet 42: 96-99.

Rothschild MF, Ruvinsky A. 2001. The Genetics of the Pig. CAB International, London.

Saikia J, Roy TC, Naskar1 S, Das B, Ferdoci AM. 2015. Molecular characterization of Cytochrome B gene in indigenous pig. Indian J Anim Res 49: 196-198.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular Cloning, A Laboratory Manual. Cold Spring Harbour Laboratory Press, New York.

Sihombing DTH. 1997. Pig Animal Science. UGM Press, Yogyakarta [Indonesian].

Soewandi BDP. 2013. Output estimation and identification growth hormone gene in pigs on Tabanan Regency Bali Province. [Thesis]. Gadjah Mada University, Yogyakarta. [Indonesian]

Wolf C, Rentsch J, Hubner P. 1999. PCR-RFLP analysis of mitochondrial DNA: a reliable method for species identification. J Agric Food Chem 47: 1350-1355.

Wu GS, Yao YG, Qu KX, Ding ZL, Palanichamy MG, Duan ZY, Li N, Chen YS, Zhang YP, Li H. 2007. Population phylogenomic analysis of mitochondrial DNA in wild boars and domestic pigs revealed multiple domestication events in East Asia. Gen Biol 8: R245.

Yu G, Xiang H, Wang J, Zhao X. 2013. The pylogenetic status of typical Chinese native pigs: analyzed by Asian and European pig mitochondrial genome sequences. J Anim Sci Biotechnol 4: 9.

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Review: The diversity of local cattle in Indonesia and the efforts to develop superior indigenous cattle breeds

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Abstract. *Sutarno, Setyawan AD. 2016. The diversity of local cattle in Indonesia and the efforts to develop superior indigenous cattle breeds. Biodiversitas 16: 275-295.* Cattle breeding are regarded indigenous to Indonesia. In the country, there are three types of cattle breeds: zebu (*Bos indicus*), Bali cattle (*Bos javanicus*), and taurine (*Bos taurus*). These breeds are farmed for their meat, milk, leather, and their power for agricultural work. Zebu was introduced by the Indians in the beginning of the first century. Bali cattle are indigenous breeds that have been domesticated from wild bantengs (*Bos javanicus*) in Java and Bali for hundreds of years. Several breeds of taurine were imported in early eighteenth century to be used as dairy cattle. Zebu and taurine are the major cattle breeds of the world; whereas in Indonesia, the major cattle breeds are Bali cattle, Ongole crossbred, and Madura cattle, which is a crossbred of the former two. Primary breeding between species in the genus *Bos* will result in sterile male and fertile female offspring. However, secondary breeding with a crossbred female will result in fertile offspring. In Indonesia, there are several local cattle breeds of zebu that have adapted to the local condition, for example Ongole crossbred, Aceh cattle, Pesisir cattle, Sumba Ongole, and, the less commonly found, Galekan cattle of Trenggalek. In addition, there are many hybrids between zebu and Bali cattle such as Madura cattle, Jabres cattle of Brebes, Rancah cattle of Ciamis, and Rambon cattle of Bondowoso, Banyuwangi, and the surrounding areas. A crossbreeding of zebu and taurine produces Grati dairy cattle. In 1970s, an Artificial Insemination program was conducted in a large scale using male cattle and semen from several breeds of zebu (Brahman, Brahman Cross) and taurine (particularly Simmental, Limousin, Holstein Friesians). The program resulted in more complex genetic mixes. Crossbreeding conducted directly in the field causes a concern since it may threaten the purity of the native species and decrease the cattle's potential for adaptation, reproduction, and productivity. It is better to conduct crossbreeding programs privately in research centers or corporate/large farmers, of which the result can be distributed to smaller farms. "Ongolization" program that was introduced in the early twentieth century should be a lesson to learn, because it had led to the extinction of Javanese cattle, while the produced offspring, the Ongole Crossbred, are considered unsatisfactory so that they still have to be crossbred with other species of cattle, particularly taurine.

Keywords: Bali cattle, crossbreeding, local cattle, taurine, zebu

INTRODUCTION

A cattle breeding is closely related to the religion, culture, and civilization of the people of Indonesia. There is no certain record on the history of cattle as livestock in Indonesia. However, it is predicted that cattle have been raised and bred for a very long time throughout the archipelago. On the inscription of the Kingdom of Kutai found in Muara Kaman, near Mahakam River, East Kalimantan from the fourth century, it is inscribed that Mulawarman, the king of Kutai, had generously given alms of 20,000 cows to Brahmin priests (Vogel 1918; Poerbatjaraka 1952). The Tugu inscription of Tarumanegara Kingdom that was found in North Jakarta from the fifth century mentioned that the King Purnawarman of Tarumanegara awarded 1,000 cows to Brahmin priests as a gratitude for the completion of the construction of a canal (Kern 1910; Poerbatjaraka 1952). The Dinaya inscription found in Malang, East Java from 760 AD stated that Gajayana, the king of Kanjuruhan (an escapee from the kingdom of Kalingga in Central Java), gave cows and other gifts to Brahmin priests (Bosch 1915/16; de Casparis 1941) (Figure 1). Considering a large number of cows given, it can be assumed that people had started breeding cattle during the period, although some historians doubt it (Poesponegoro and Notosusanto 2010). In Linggasutan Inscription (929 AD) from the period of King MPu Sindok of Medang, found in Malang, East Java, it is mentioned that types of animals that are taxed commodities in the market, where the minimum threshold of taxable cattle is 40 heads. Several other inscriptions show that the cow has an important position and so many are farmed, there is even an effort for the conversion of protected forest into grazing land (Poesponegoro and Notosusanto 2010).

The domestication of cattle is believed to have been introduced by traders from Kalinga Kingdom of India (now Odisha, Eastern India) since the first century and even before. In the first century, the people of Kalinga had traveled to Sri Lanka, Burma (Myanmar), Nicobar Islands, Malay Peninsula, Sumatra, Java, Bali, Borneo, mainland Southeast Asia, and China. In the next several centuries, a lot of large Indianized kingdoms were established in these states (Coedès 1968; Wheatley 1975; Keyes 1995; Lukas 2001). In the belief of Hinduism, cow is a sacred animal because it is the *vahanas* or vehicles of Hindu gods. Therefore, most Hindu temples are adorned with cow reliefs and sculptures (Bhattacharya 1977; Jha 2002). In Indonesia, sculptures of *Nandi* (bull) that are almost seen in Shiva Hindu temples are mostly precisely sculpted, indicating that the sculptor had seen an actual cow. Examples of accurate and proportional Nandi sculptures were found in Prambanan temple from 850 AD (Ariswara 1994). Nandi is also found in other Hindu temples, especially in Central Java, Yogyakarta and East Java, as well as in West Java (Cangkuang Temple)(Arifah 2013), South Sumatra (Bumiayu Temple) (Bottenberg 2010), Jambi (Muara Jambi) (Adam 1921), and South Kalimantan (Laras Temple). In Borobudur, a Buddhist temple, there is a carved relief of two cows ploughing a farm on the temple walls in the area of Kamadhatu (the base). The reliefs in Kamadhatu represent the daily life during the period of time in which the temple was built (Soekmono 1976). The images of cow in these temples are characterized by a hump on their shoulders, indicating that they are of the species of zebu (*Bos indicus*) from India. This type of cow is also known as the Javanese cattle, of which the offspring are called Ongole Crossbred (Peranakan Ongole) or Bengali (Benggala) cattle. However, in Sukuh temple, one of the last temples which were built during the era of Hinduism in Java from 14th century AD, there is a carved relief of a humpless cow (Asmadi et al. 2004; PNRI 2014). This is supposed to be the first record of the existence of Bali cattle (*Bos javanicus*) in Indonesia (Figure 2). Based on this timeline, Bali cattle may have been domesticated during the Majapahit Kingdom (1293 to around 1500), based in East Java.

The review aims to discuss the diversity of indigenous cattle in Indonesia that have adapted to the local climate and condition including the feed, the influence of foreign cattle genes on the quality of the native cattle, and the conservation efforts. This manuscript is complementary to Sutarno and Setyawan (2015).

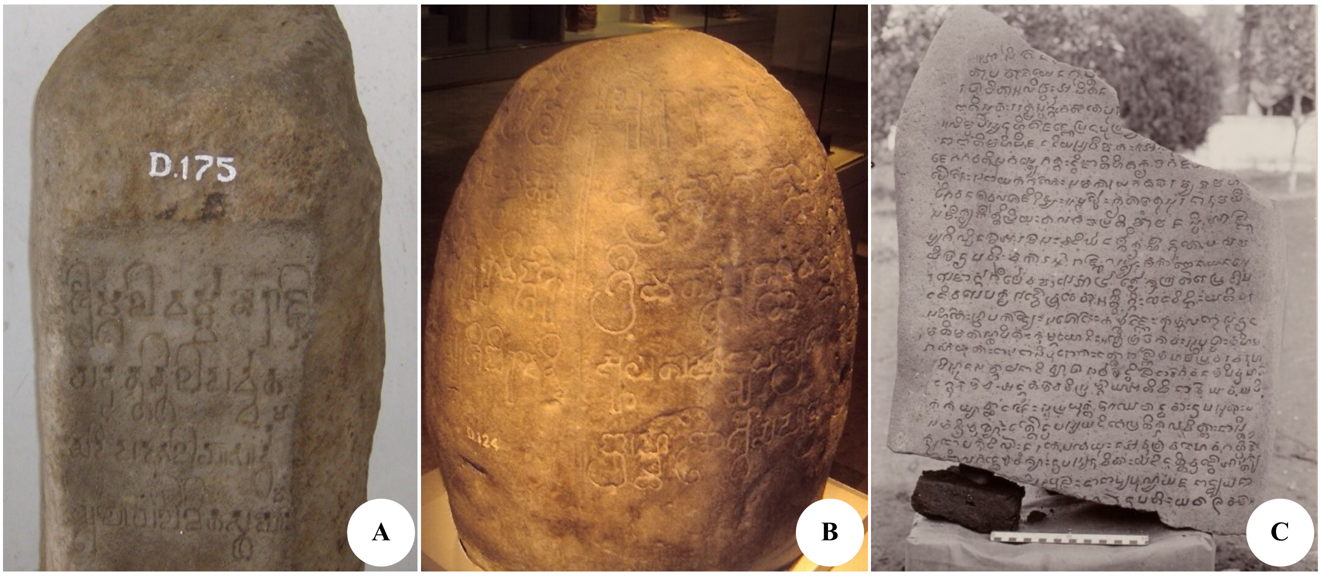


Figure 1. Stelae as the initial records of the presence of cattle in Indonesia. A. Muara Kaman stela, Kutai, East Kalimantan. B. Tugu stela, North Jakarta. C. Dinaya stela, Kanjuruhan, Malang, East Java. (from many sources)

Figure 2. Cow sculpture and ornaments on temples as the initial records of the presence of cattle in Indonesia. A. Nandi sculpture in Prambanan temple, Klaten (Zebu cattle). B. Carved relief of cows ploughing farm on the wall of Borobudur temple, Magelang (Zebu cattle). C. Bas-relief of a humpless cow (Bali cattle) in Sukuh temple, Karanganyar. All locations are in Central Java Province, Indonesia. (from many sources)

CATTLE DOMESTICATION WORLDWIDE

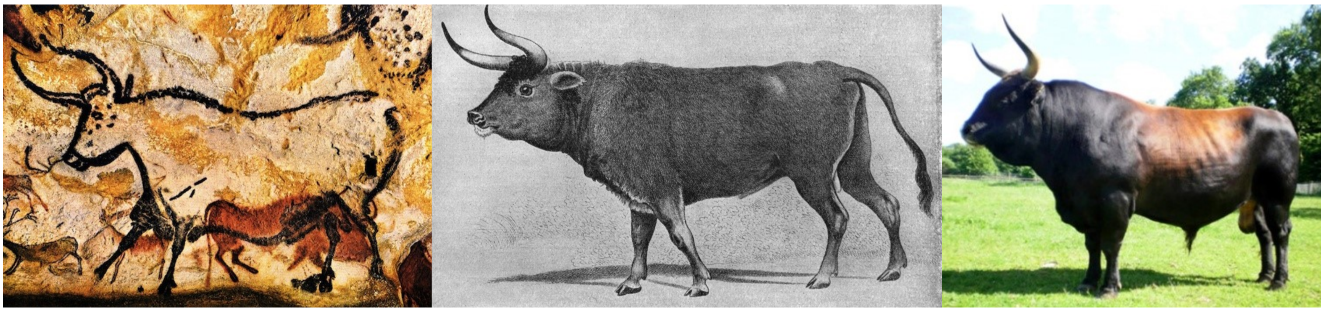
Throughout the history of human civilization, many types of cows have been domesticated (Lenstra et al. 1999, 2014), however, only taurine (*Bos taurus*) and zebu (*Bos indicus*) become the major cattle breeds of the world. Both types can be distinguished easily by the hump—zebu cattle are humped and taurine cattle are humpless. Both zebu and taurine descended from the wild Indian aurochs (*Bos primigenius*) that inhabited Asia, Europe, and North Africa at the end of the last glacial period (12,000 BP) (Felius et al. 2014) (Figure 3). Since the last species of aurochs died in Poland in 1627 (Rokosz 1995), the Europeans tried to preserve the existing species of taurine and zebu. Even though the wild aurochs are widely distributed around the world, the descendants, taurine and zebu cattle, are believed to be originated from two regions only.

Archaeological data indicates that taurine cattle were first domesticated between 10,300-10,800 BP in the western part of the border between Syria and Turkey (Helmer et al. 2005; Vigne 2011). Fossil remains of wild and domestic taurine cattle from that era were discovered in the region (Barker 1985; Zeder et al. 2006). It is estimated that around 80 female aurochs were the maternal ancestors of almost all present day taurine cattle (Van Vuure 2001). In another hand, Indus Valley, a desert ecoregion of southern Pakistan, is believed to be the center of origin of zebu domestication dating back to 8,000 BP (Ajmone-Marsan et al. 2010; Chen et al. 2010). Fossil remains of zebu cattle from that era were found in Mehrgarh, a proto-historic cultural site in Balochistan in the southwestern region of Pakistan (Jarrige et al. 2006). Based on the origin of domestication, the present day taurine cattle mostly live in sub-tropical regions, with Europe, North America, and Australia as the main producers. Zebu cattle have spread into tropical regions with the highest population being found in India, Africa, and Brazil. Both taurine and zebu have hundreds to thousands of breeds including crossbreds and hybrids.

Various attempts of cattle domestication are also found in Asia (Ho et al. 2008; Achilli et al. 2009). People in Tibet and its surrounding regions have domesticated yaks (*Bos grunnies*) that are able to adapt to highlands (Qiu et al. 2012) since around 4500 BP (Payne and Hodges 1997). The gayal or mithun (*Bos frontalis*) were domesticated from the gaur (*Bos gaurus*) or the Indian bison (Uzzaman et al. 2014) on the northeastern border of India, Bangladesh, and Myanmar (Mason 1988; Payne and Hodges 1997). In Indonesia, Bali cattle were domesticated from wild banteng (*Bos javanicus javanicus*) since around 5000 BP (Payne and Hodges 1997), and has become the most populous cattle after taurine and zebu cattle. Hybridization of these three breeds; yaks, gayal or mithun and Bali cattle, with taurine and zebu has produced many breeds with complex and unique mixtures that contribute to the diversity of cattle worldwide (Felius et al. 2014). Kouprey (*Bos sauveli*), a wild cattle that are found in Indo-china is a hybrid between local banteng (*Bos javanicus birmanicus*) and zebu (Hassanin and Ropiquet 2007) (Figure 4 and 5).

DIVERSITY AND DISTRIBUTION OF CATTLE IN INDONESIA

Bali cattle are an indigenous breed with the widest distribution and largest number of population in Indonesia. There are several other local cattle breeds in Indonesia, which are the direct descendants of the Indian zebu as well as crossbred between zebu and the Bali cattle (either directly or through the Madura cattle). Local breeds that are categorized as zebu are for example Pesisir cattle of West Sumatra, Aceh cattle of Aceh, and Sumba Ongole of the Sumba Island. In addition to the Madura cattle, other breeds that are intermediate between zebu and Bali cattle include the Jabres cattle of Brebes, the Rancah cattle of Ciamis and the surrounding areas, the Rambon cattle of Bondowoso and the surrounding areas, and the almost extinct Galekan of Trenggalek. Indonesia also has a crossbred between zebu and taurine, which is the Grati cattle or more popularly known as the Indonesian Holstein Friesians, an intermediate between a male Holstein Friesians and a female Ongole Crossbred.



A B C

Figure 3. Cattle domestication worldwide. A. Figure of aurochs on the wall of Lascaux cave, France (Ruspoli 1983), B. Aurochs by Charles Hamilton Smith (Pyle 1995), C. Tauros, one of the descendants of the extinct aurochs (Parkvall 2010)

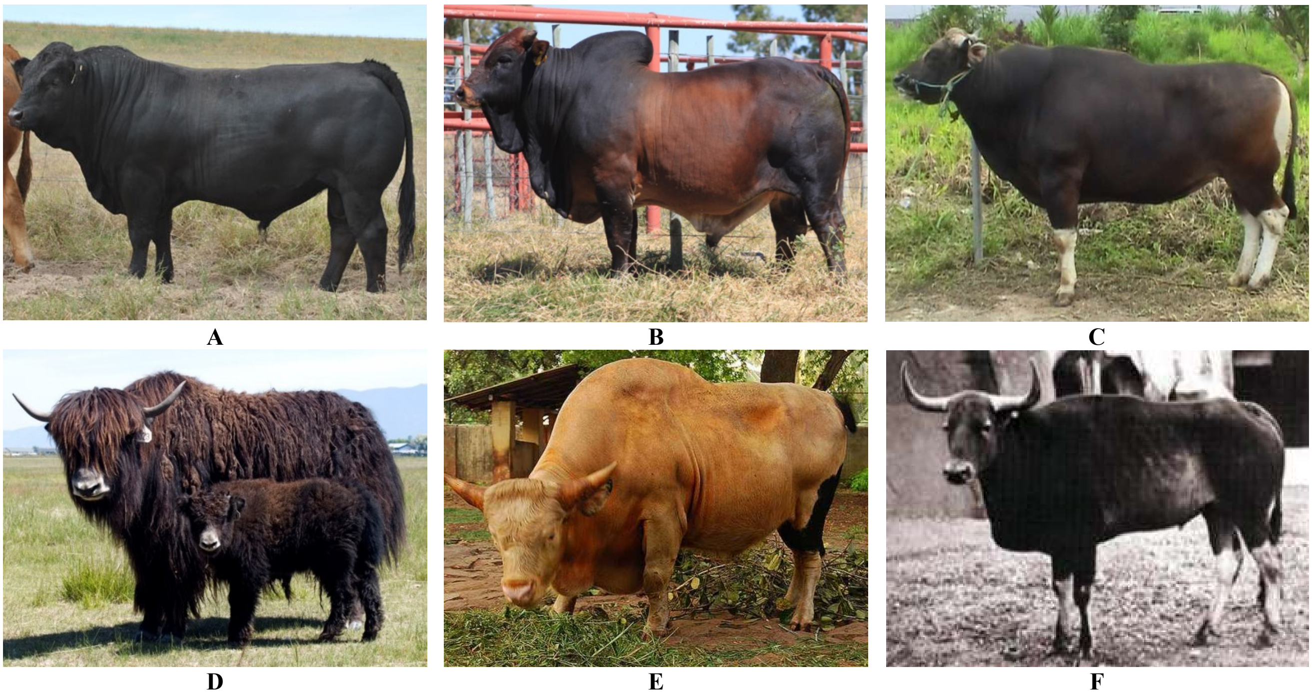


Figure 4. Cattle diversity in the world. A. European taurine cattle, B. Indian zebu cattle, C. Indonesian local Bali cattle, D. Tibetan yak, E. Northeast Indian gayal (gaur), F. Cambodian kouprey. (from many sources)

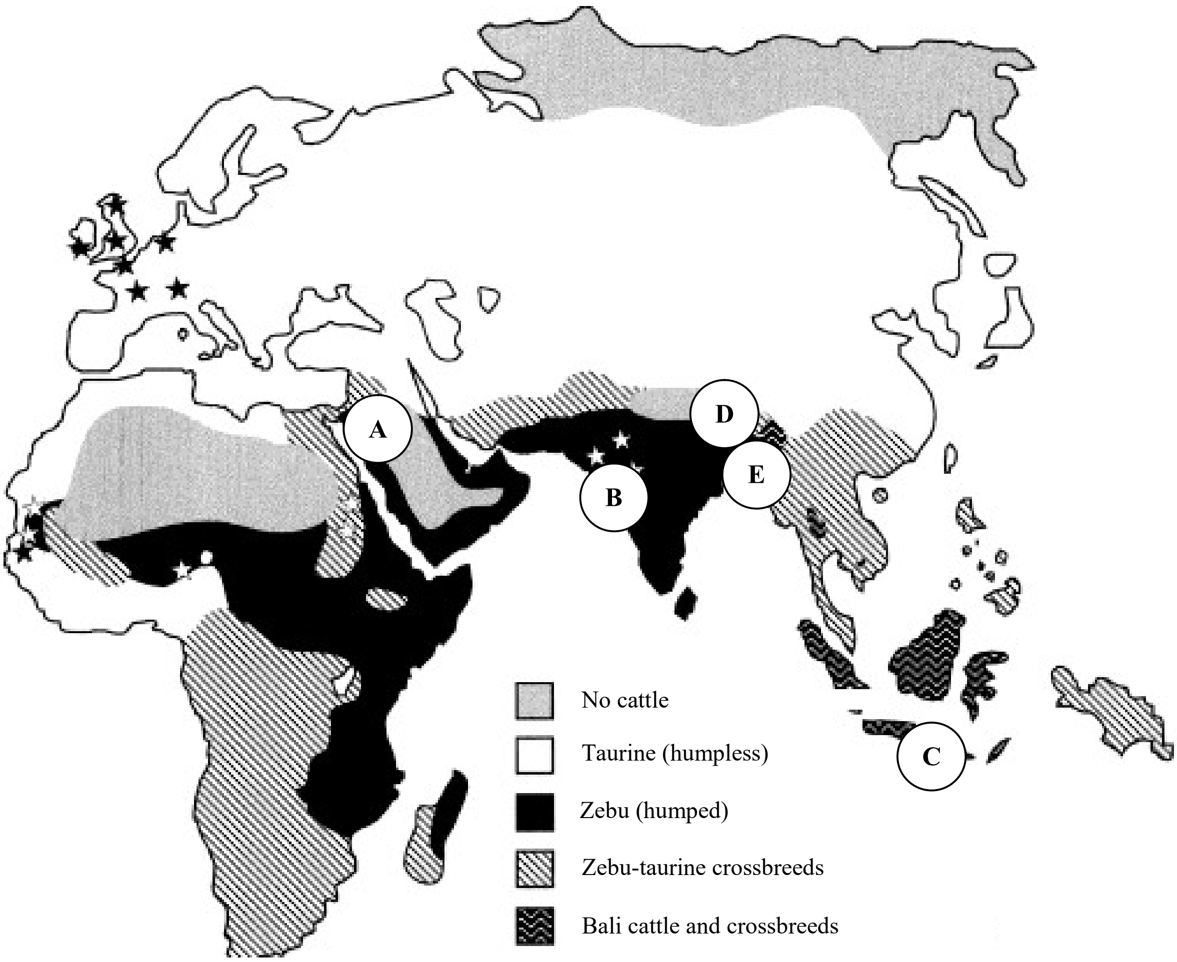


Figure 5. Distribution and domestication of cattle worldwide. Site domestication of A. Taurine in Mesopotamia, B. Zebu in Indus valley, C. Bali Cattle in Java or Bali, D. Yak in Tibetan Plateau, E. Gayal in northeast India and Bangladesh (Felius et al. 2014)

Bali cattle

Bali cattle are raised in all parts of the country except in Central Java, West Java, and in some other regions in which a lot of goats (*Capra hircus*) farms are found because goats can transmit the malignant catarrhal disease, a serious, often fatal, disease that affects many species of young Bali cattle. In Central Java and West Java, Ongole is the more dominant raised breed of cattle. This breed is descents of zebu that were imported from India a long time ago (Javanese cattle) and other zebus from various origins that were imported in a later period (particularly Ongole and Sumba Ongole). They have been introduced to and have adapted to the local climate for a long time, therefore they are considered indigenous to Indonesia. Ongole Crossbred (OC) cattle are the second most essential breed after the Bali cattle. Another indigenous breed is the Madura cattle, the crossbred of Bali cattle and zebu cattle (Javanese cattle or Sinhala cattle), that was first crossbred about 1500 years ago. In South Sulawesi, people started crossbreeding Ongole cattle in 1890.

The distribution of Bali cattle to all parts of Indonesia has been done for a long time. In 1912, Bali cattle were brought to Timor Island. In 1920-1940, Bali cattle were distributed again to Timor, this time also to Lombok, South Sulawesi, and South Kalimantan (Omerling 1957; Hardjosubroto 2004). A transmigration program peaked in 1970-1980, which relocated farmers in Java, Bali, West Nusa Tenggara, and East Nusa Tenggara to other islands with smaller number of population, especially Sumatra, Kalimantan, Sulawesi, and Papua. The program unexpectedly became a means of distributing Bali cattle throughout Indonesia because during the program Bali cattle were also moved to be bred and used as working animals. Bali cattle have relatively better productivity and endurance in their pure genetic condition without being crossed. Madura cattle are the outcome of successful crossbreeding between Bali cattle and zebu; however, their distribution is only limited to Madura Island and few regions in eastern East Java. In 1908, crossbreeding between Java cattle and male Bali cattle in East Java was initiated again, but was halted in 1921 due to the high death rate of Bali cattle and their offspring. Natural crossbreeding occurred in Trenggalek, Brebes, and Ciamis, however, the population number was limited and the productivity kept decreasing.

Zebu cattle

Before being substituted with the Ongole Crossbred, there had been attempts to breed Javanese cattle descending from the Indian zebu which was brought by traders during the period of Hindu and Islam kingdoms. These attempts were fully supported by the government that initiated a program to distribute a large number of male Javanese cattle to all areas in Java in 1905-1911. These cattle were distributed to regions in Central Java, East Java, and West Java to improve their quality. However, this program was stopped due to resistance from farmers who wanted stronger and bigger cattle for farm working; the characteristics that are owned by male cattle of other zebu breeds.

A century before, in 1806 and 1812, some breeds of Indian zebu (Mysore, Ongole, Hissar, Gujarat, and Gir) were brought by traders to East Java to be crossbred with Javanese cattle in order to produce good-quality offspring. The introduction of zebu was done again from 1878 before it was banned in 1897 due to the threat of rinder pest disease. In late nineteenth century, owners of big plantations in East Sumatra imported the Hissar breed of zebu as working cattle. In 1889, the local government of South Kedu castrated male Javanese cattle in a large scale, and only allowed female cattle to mate with zebu. Until today, Kebumen is one of the biggest producers of Ongole Crossbred calves in Indonesia. The same thing was done in Magetan, East Java, in 1890.

In 1905, the government allowed to import zebu, particularly the Hissar breed. Two years later, the government started to import Mysore cattle but it was not successful due to a relatively high death rate. In 1909, three zebu breeds—Ongole, Gujarat, and Hissar were imported from India. They were distributed to Java, Sumba, and Sumatra, where they successfully bred. In 1909-1911, considering that the Ongole developed most ideally in Java, the government imported a large number of Ongole, which were then, quarantined in Sumba Island together with the Gujarat cattle that were imported earlier. These breeds bred very impressively, producing the Sumba Ongole (SO) cattle. In 1915-1929, the SO cattle were distributed to Java through a program called “Ongolization”, in which male Javanese cattle were castrated and the female was mated with male SO. The program led to the extinction of the remaining Javanese cattle and the creation of a new species, the Ongole Crossbred (CO). In 1920-1940, the Ongole Crossbred was distributed from Java to Sumbawa Island, Sulawesi, West Kalimantan, and Sumatra.

Madura cattle

Development and distribution of Madura cattle have also been conducted since a long time ago. In 1891-1892, the local government of Pasuruan established a program to crossbreed Javanese cattle and Madura cattle. The program was conducted one more time in a larger scale in 1905, but was terminated in 1921 because it did not meet the expectation. In 1920-1940, Madura cattle were distributed to Flores and East Kalimantan; however, the breed was later substituted with Bali cattle that were brought later because the quality of Bali cattle was considered much better (Omerling 1957; Hardjosubroto 2004). In 1957, there was an attempt to improve the generic quality of Madura cattle by crossing them with the Red Danish dairy cattle. However, the offsprings were not very high in demand. In addition, in the last few decades, Madura cattle have been crossed with Simmental cattle, producing more popular offspring that are highly demanded by farmers particularly in Sumenep and the surroundings.

Taurine cattle

In Indonesia, the distribution of taurine cattle (*Bos taurus*) is more limited because they need highland and cool temperature to live and breed; so the more commonly raised breed are those that survive better in warm climate.

In Papua, there are many wide highland pastures, but limited infrastructure becomes a problem in developing cattle farms. Most taurine cattle in Indonesia are of Holstein Friesians breed, which is mostly raised in Java for their milk. In addition, in West Sumatra highlands, people raise Simmental taurine cattle in small scale. Recently, the number of crossbred species between Simmental and Limousin taurine with local breeds has been increasing, especially with Ongole Crossbred through artificial insemination (Pamungkas et al. 2012). However, this breed has not been naturalized very well since the gestation almost always happens through artificial insemination. Therefore, the existing population is only the first filial generation of offspring (F1).

The taurine breed that has been introduced since a long time ago is the FH dairy cattle or the offspring, which are only kept and raised in highlands. Dairy cattle have existed in Indonesia since 1786, but their breed remains unknown. In 1891-1892, several breeds of dairy cattle were imported from Australia (Hereford, Shorthorn, Ayrshire, and Jersey) and from Netherlands (Holstein Friesians) to Grati, Pasuruan. Crossbreeding between these imported breeds and local cattle particularly the Ongole Crossbred became the origin of the existence of the Grati dairy cattle (Sudono 1983; Sudono et al. 2003; Siregar 1995; Soehadji 2009). In late nineteenth century in Lembang, Bandung, a big cattle farm was established. It made Bandung popular as the main supplier of milk in Indonesia. In 1912, the government signed a contract with the farming company to buy all their young male cattle to be distributed to farmers around Bandung. In late nineteenth century in Jonggrangan, Klaten, there was also a dairy cattle farm that raised FH cattle. To increase the population of dairy cattle, a large number of male FH were mated with local cattle. The initial generation of offspring has high productivity of milk; however, in the next generations, the productivity kept decreasing. In 1939, a number of male FH have imported again to Grati to improve the quality of the dairy cattle. At the moment, the population of dairy cattle in Indonesia is about 350,000. The species mostly live in Bandung, Surakarta, and Malang. FA dairy cattle in Indonesia nowadays are mostly of pure genetic breed, because their milk production is quite satisfactory, even though there are also FH dairy cattle that are crossbred between male FH and female CO, such as the Grati.

In 1970, various breeds of superior alive cattle or their semen were introduced from Europe, the United States, Australia, and New Zealand, such as the Brahman, Brahman Cross, Simmental, and Limousine. They are considered very high-quality beef cattle because of their high rate of weight gain per day. However, they are not suitable for hot tropical areas, except the Brahman and Brahman Cross which are zebu descent. In Kebumen, the Brahman breed is mixed with OC and the offspring is named Madras, which is indigenous to the region. In 1990s, there was not enough domestic beef production, so that quantity of imported beef kept increasing day by day, mainly from Australia and the United States. In the end, young cattle—mostly the Brahman Cross and the Australian Commercial Cross (ACC) breeds—from Australia were imported to be raised and fed by a special diet to fatten them up. These breeds are crossbred between various breeds of taurine and zebu. In 2015, 650,000 ACC cattle were brought to Indonesian feedlots. Unfortunately, they only lived for 3-6 months in the feedlots before being slaughtered, and were never distributed to small farmers to be developed.

In addition to the Bali cattle, the native cattle breeds in Indonesia include the imported breed that has been raised in Indonesia for a long time and even has mixed genetically with the Bali cattle such as the Ongole Crossbred, Sumba Ongole, and Madura cattle. There are also Aceh, Pesisir, Jabres, Rancah, Rambon, Galekan, and Grati (FHI) cattle, as well as the imported cattle such as the Brahman, Brahman Cross, Simmental, Limousine, Holstein Friesians, and ACC cattle (Figure 6). The distribution of Indonesian local cattle is presented in Figure 7.

CATTLE POPULATION

Nowadays, the population of beef cattle in Indonesia is around 14.7 million (BPS 2014) and the population of dairy cattle is around 350,000. some local breeds such as Bali cattle (*Bos javanicus*), Ongole Crossbred (*Bos indicus*), and Madura cattle (*Bos indicus x Bos javanicus*) are the main source to meet the national demand of beef, even though their productivity and beef quality are not always excellent (Okumura et al. 2007). Holstein Friesians cattle are the main source of dairy products, which can only meet about 20% of the domestic demand. In addition, there are also local crossbreeds that are mostly mixed genetically among these breeds as well as between these breeds with taurine cattle that were imported later (Johari et al. 2007). Data from the Indonesian Directorate General of Livestock (DGLS 2010b) shows that local breeds of cattle in Indonesia consist of Bali cattle (33.73%), Ongole Crossbred (5.16%), and other local breeds (13.45%). Bali cattle and Ongole Crossbred are local breeds of beef cattle that have a particular strength, which make them be able to adapt very well and quickly to the surrounding environment in Indonesia, such as the climate, the availability of natural fodder and water, and the resistance to bacteria and parasites.

Most Indonesian cattle are found in Java, which is around 45% of all local cattle (35% are in East Java). Sumatra owns about 22%, Nusa Tenggara and Sulawesi each owns 13%, and the rests are raised in other islands. Bali cattle have been brought to most provinces in Indonesia because of their suitability and high adaptability to most agro-climatic zones. Other important breeds are the Ongole Crossbred, Madura cattle, and the Holstein Friesians dairy cattle (*Bos taurus*). The most popular breeds to be mixed with local breeds, especially Ongole Crossbred, are Simmental and Limousin. The offspring of this crossbreeding are highly preferable for fattening due to their high rate of weight gain despite the relatively high cost of production. Most cattle that are sold across islands will usually end up in Jakarta and West Java, as the main consumers (Sullivan and Diwyanto 2007).

Figure 6. The diversity of cattle breeds in Indonesia. A. Bali Cattle (Banteng), B. Madura cattle, C. Ongole Crossbred, D. Sumba Ongole, E. Aceh cattle, F. Pesisir cattle, G. Brahman cattle, H. Brahman Cross, I. Simmental bull, J. Simmental cow, K. Limousine bull, L. Limousine cow, M. Holstein Friesians bull, N. Holstein Friesians cow, O. ACC from North Australia. (from many sources)

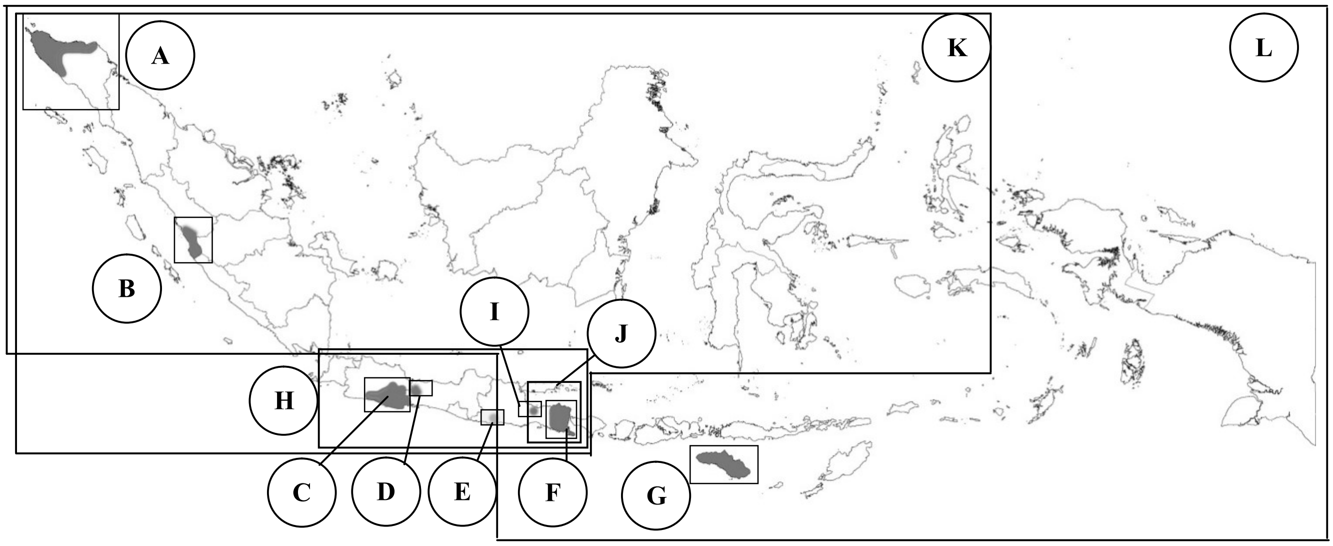


Figure 7. Distribution of Indonesian local cattle. A. Aceh cattle; B. Pesisir cattle; C. Rancah cattle; D. Jabres cattle; E. Galekan cattle; F. Rambon cattle; G. Sumba Ongole cattle; H. Holstein Friesian cattle; I. Grati cattle; J. Madura cattle; K. Peranakan Ongole cattle; L. Bali cattle (All parts of Indonesia except in Central Java, West Java and Banten) (Sutarno and Setyawan 2015)

Indonesia is one of the world's greatest importers of living cattle and beef. Import fits the gap between production and consumption; and the gap is projected to grow wider in the future. The production of beef in 2003 was 351,000 MT and the consumption was 418,000 MT. There was a 67,000 MT deficit which was filled by imports. In 2010, the production of beef was estimated to increase up to 362,000 MT and the consumption to 447,000 MT, resulting in 85,000 MT of deficit. In 2020, the deficit is expected to rise to 111,000 MT. The quantity import of living cattle is 428,000 in 2002, 374,000 in 2003, and 350,000 in 2006. In 2014, it reached 700,000 and in 2015 it reached 650,000. The main consumers of beef are Jakarta and West Java, where a large number of imported as well as local beef cattle are slaughtered (Sullivan and Diwyanto 2007).

The population of cattle has been decreasing in the last few years after the financial crisis that hit the country in late 1990s. The number of cattle declined after the crisis because the capability of importing cattle went down, which leads to an increase of local beef consumption, causing an increase in the number of slaughtered adult as well as young cattle. The rate of local cattle being slaughtered outnumbered their natural ability to reproduce, resulting in a decrease of breeding. To regain the quantity of cattle, the female cattle should not be slaughtered. However, in fact, around 40% of female cattle were slaughtered every year. Most beef cattle are raised by small farmers semi-intensively; and each farmer owns 2-3 cattle that are frequently sold because of their economic needs (Pamungkas et al. 2012). Unsustainable level of cattle sales contributes to a decrease in the number of population (Fordyce et al. 2002).

There are several inhibiting factors in developing cattle farming, including low genetic quality, limited availability of superior male species, limited ability of farmers in dealing with cattle breeding, and traditional maintenance system (Atmakusuma et al. 2014). Various attempts have been established by the government in order to increase the population of local cattle to develop them as the country's main source of beef, including reducing the slaughter of productive local cattle and introducing crossbreeding between local cattle using artificial insemination to more regions (DGLS 2010c). However, the program has lately become controversy because it is done directly in the field, which leads to uncontrollable genetic mixes and produces offspring with untested adaptability to the local climate, natural fodder, and disease.

MAJOR LOCAL CATTLE

In Indonesia, there are four local living genetic resources including Bali cattle, Ongole Crossbred, Madura cattle, and Aceh cattle (DGLSAH 2015). To maintain the genetic purity of the breeding of these breeds, the government has selected several islands to be the center for cattle conservation and breeding. Sapundi Island becomes the center for breeding Madura cattle, Nusa Penida for Bali cattle, and Raya (Rayeuk) Island for Aceh cattle. In addition, 18 districts are selected to be the center for local cattle breeding, particularly Bali cattle and Ongole Crossbred.

Furthermore, there are several breeds of cattle that have been recognized or are proposed to be recognized (DGLSAH 2015) as Indonesia's indigenous cattle, such as Sumba Ongole (SO), Pesisir, Jabres, Rambon, Galekan, and Rancah cattle. The conservation efforts gain a lot of challenges particularly due to increasing activities of artificial insemination using frozen semen from foreign cattle and increasing pressure from other local breeds.

Bali cattle

Bali cattle (*Bos javanicus*) are direct domestication of wild bantengs in Bali and Blambangan, East Java (MacHugh 1996; Verkaar et al. 2002; Martojo 2003; 2012; Hardjosubroto 2004). The first documentation of banteng was written by Schlegel and Muller in 1836 (Merkens 1926), which states that bantengs were found wild in small groups consisting of one male and several females and their offspring in forests in Java and Kalimantan (Borneo). Wild bantengs that were captured usually only survived up to 11 years; however, the Bali cattle can live to 20-25 years. Nowadays, wild bantengs still widely live in Java, but their existence is threatened, particularly due to habitat loss and illegal hunting (Duckworth and Hedges 1998; Pudyatmoko 2004; Steinmetz 2004). The genetic purity of bantengs is also debatable due to the possibility of hybridization with local cattle grazing in the forest (Wharton 1957; Tun Yin 1967; Hoogerwerf 1970). Meanwhile, genetic testing is not easy to conduct because of their characteristic that makes them difficult to approach to take DNA samples. The genetic purity of bantengs kept in zoos is debatable too.

At present, the popular and acceptable scientific name for bantengs is *Bos javanicus*, with three subspecies that are categorized based on their distribution areas: *Bos javanicus javanicus* (Javanese banteng) living in Java, *Bos javanicus lowii* (Kalimantan banteng) found in Kalimantan, and *Bos javanicus birmanicus* (Burma banteng) that are distributed through Southeast Asia mainland (Byers et al. 1995). Bali cattle descended from *Bos javanicus javanicus* and were given the scientific name of *Bos javanicus*. Domestication process is believed to have been conducted to the Burma banteng, even though the offspring is not purebred due to hybridization with other breeds of cattle. In Cambodia, Vietnam and Lao, people know local cattle, kouprey (Urbain 1937; Hoffmann 1986), it was believed to be the hybrid between wild banteng and zebu cattle (Galbreath et al. 2006; Hedges et al. 2007), of which the size is smaller than that of zebu cattle and have various colors (Galbreath et al. 2007). It was rated as "critically endangered" species and it may already be extinct (Timmins et al. 2008).

Bali cattle have been widely distributed throughout Indonesia, especially in Bali, Sulawesi, Java, Sumbawa, Timor, and Kalimantan (Entwistle and Lindsay 2003; Sutarno 2010). This breed is also found in North Australia and Malaysia (Toelihere 2003). Bali cattle live in North Australia as wild animals, descending from 20 cattle that were brought from Bali in 1849. Now, the population of wild Bali cattle in North Australia is about 8,000 to 10,000 (Bradshaw and Brook 2007). The genetic purity of these cattle is almost equivalent to that of the wild bantengs in Java, compared to the common Bali cattle that have supposedly received genetic mixing of zebu as well as taurine cattle. The characteristics of Bali cattle did not significantly change compared to their wild ancestors (Handiwirawan and Subandriyo 2004). Bantengs and Bali cattle differ physically in terms of size and behavior; male bantengs are way bigger and more aggressive (Martojo 2003, 2012). Bali cattle have not experienced rigorous selection as experienced by zebu and taurine. Studies on the genetic diversity of Bali cattle and bantengs are still limited (Kikkawa et al. 1995, 2003, Namikava 1981, Nijman et al. 2003, Verkaar et al. 2003). The studies are needed to understand the domestication process (Mohamad et al. 2012).

Bali cattle have large frame and solid muscle. The adult male can weigh up to 600-800 kilograms, and the adult female weighs around 500-600 kilograms (Martojo 2003). The young cattle are crimson red or reddish gold. As they grow, the females remain crimson, while the males change color into black at 12-18 months of age. Castrated adult male cattle will turn back into crimson red few months after castration. There are white spots on the legs, from the knees to the toes. The back of the pelvis is white-colored with clear oval-shaped line, and the tip of the tail is black. The black color on a castrated adult male will change into light brown, while the legs from the knees to the elbows down remain white (Williamson and Payne 1978). The skin is pigmented and smooth. The male cattle do not have withers; have a small dewlap and compact body. The head is wide and short with flat forehead and medium sized vertical ears. The horn of female cattle is short and small, while the male's horn is big and long, facing to the front top sides, sharp, with thin neck. The chest is deep and the legs are strong (Pane 1991; Susilorini 2010).

Bali cattle have high level of fertility; the reproduction efficiency is excellent because they can produce offspring every year and the life percentage of the calves is 80%. In addition, they can adapt well to tough condition, can live and grow in poor condition and hard, dry climate like in eastern Indonesia, can digest low-quality food, can survive with limited amount of food in dry season, and can easily recover to their best condition in normal season. They are also easy to maintain for any needs in agricultural system, as working animal as well as meat producing animal. The percentage of carcass is higher than that of zebu and taurine. The carcass and meat are of high-quality and meet the demands from the market because the meat is fatless, which make them have higher selling price. The leather is relatively thin but of excellent quality. The young castrated males and the adult males have standard, similar weight so that they are suitable to be transported to other islands or countries. However, it is important to consider potential negative impacts of genotype-environment interaction. Bali cattle are the most suitable breed on production systems with low input and high environmental stress, as widely practiced by millions of farmers in Indonesia (Williamson and Payne 1978; McCool 1992; Wirdahayati 1994; Copland 1996; Martojo 2003; Diwyanto and Praharani 2010; Susilorini 2010; Sutarno 2010).

However, Bali cattle also have weaknesses. Their growth is relatively slow and the production of milk is low, causing high calf mortality rate (Susilorini 2010). This breed is known to be resistant to diseases and parasites, but there are two fatal diseases that may put them at risks, malignant catarrhal disease with goats as carrier and Jembrana disease, a type of virus that attacks the brain

(Budiarso and Hardjosworo 1976). Malignant catarrhal disease becomes one of the primary causes of calf mortality, so Bali cattle are not raised in areas where goats are kept, such as in Central Java and West Java. Jembrana disease only affects Bali cattle that are indigenous to Bali, possibly due to long-term isolation of susceptible cattle with the intention of conservation instead caused inbreeding between cattle with decreasing immunity (Tenaya 2010).

Bali cattle are the ancestor of most local breeds in Indonesia. Even in Ongole Crossbred, which have relatively pure genetic, Bali cattle genes are also found. The same situation also happens to Pesisir cattle and Aceh cattle. Cattle breeds that undoubtedly have the genes of Bali cattle are Madura, Rambon, Galekan, Jabres, and Rancah cattle.

The genetic purity of Bali cattle began to be threatened; negative selection and hybridization degenerate the quality of the offspring. Negative selection occurred in Timor Island by sending high-quality cattle across islands and only leaving low-quality cattle for breeding. Consequently, after few generations, the cattle have very low weight and they are no longer profitable for trading across islands. Now, the island of Timor no longer becomes a major producer of Bali cattle. In addition, hybridization is conducted everywhere, even in Bali itself, particularly with Simmental taurine cattle through artificial insemination. During the reign of Klungkung Kingdom, the island of Bali was specially established as a region for developing Bali cattle, which prohibited any activity of importing other cattle breeds. This situation was still maintained in the colonial era. However, nowadays, Bali cattle are only specially kept and raised in Nusa Penida Island; in Bali Island, crossbreeding between Bali cattle and other cattle breeds is allowed.

The genetic purity of banteng as the ancestor of Bali cattle is also threatened due to hybridization. The utilization zones of national parks in Baluran, Alas Purwo, and Metu Betiri are used by the local residents for domestic cattle grazing, such as zebu, Bali cattle, Madura cattle, or Rambon, so that there is always possibility of crossbreeding between the cattle and wild bantengs, which may disrupt the wild banteng's genetic purity.

In 2000, the population of Bali cattle was approximately 2,300,000 with the main distribution in South Sulawesi (718,000), Bali (529,000), East Nusa Tenggara (443,000), West Nusa Tenggara (377,000), Southeast Sulawesi (300,000), and Lampung (255,000) (Talib et al. 2002).

Sumba Ongole and Ongole Crossbred (Peranakan Ongole)

Sumba Ongole cattle (*Bos indicus*) (SO) is local cattle from Indonesia. In the early of the 18^{th} century, the government introduced various cattle races such as Javanese cattle, Madura cattle, Balinese cattle, Gujarat cattle and Ongole cattle to Sumba Island. The cattle were sent to Sumba Island to be quarantined for breeding because they grew quite good in Java and to avoid the incidence of a disease. Evidently, Ongole cattle were able to grow and to adapt very well which made people of the island abandoned other livestock. In 1914, Sumba Island was assigned to be the center of Ongole cattle breeding which produced Sumba Ongole cattle (Hardjosubroto 2004). The assignment of Sumba Island as the center of Ongole cattle breeding is followed by the introduction of 42 male Ongole cattle, 496 female Ongole cattle, and 70 crossbreed cattle. Sumba island was able to send six male Ongole cattle to Java in 1915, 254 male Ongole cattle in 1919, and 829 male Ongole cattle in 1929 (Office of Veterinary East Sumba District 1989). SO cattle have been the pioneer of other Ongole cattle as the result of Ongole breeding program ("Ongolization") conducted in Java in 1915-1929 (Hardjosubroto 2004). SO cattle are considered as superior local beef cattle because they are reported of gaining weight almost 1.18 % per day, having the percentage of carcasses more than 50%, and beef production is 77% (Ngadiyono 1995). SO cattle work very good to cultivate land due to its big body.

Ongole Crossbred (*Bos indicus*) or Benggala is the result of genetic improvement of Javanese zebu cattle, which has been existed since the beginning of the first century AD, with Sumba Ongole from Sumba Island. At the beginning of the 19^{th} century, male and female Ongole cattle were imported from Madras, India to Java, Madura, and Sumba. Ongole cattle which were brought to Sumba Island breed Sumba Ongole (SO). This breed was then brought to Java and mated with Javanese cattle which produced Ongole Crossbred. Ongole Crossbred is famous as beef and working cattle because they are big, strong, tame and quiet, heat-tolerant, easy to adapt with environment, able to grow with limited food, having high reproduction, and having a good ability to go back to normal after giving birth.

Ongole Crossbred has white or grey colored fur, black tail and fur around the eyes, short curved shape of the head, short horn, long hanging ears, and a rather large belly. Male cattle has bright big eyes and circled by skin 1 cm from black eyes, big body and hump, short neck, long legs with strong vein, loose wattle hanging from the bottom of the head, and sometimes black splotches on his knee. Adult male cattle can weigh up to 600 kg and adult female cattle can weigh up to 450 kg. Weight gain ranges from 0.4-0.8 kg/day, but in unfavorable condition weight gain only reach 0.25 kg/day (Wiyatna et al. 2012).

Indonesia also develops other types of Zebu particularly Brahman and Brahman Cross races which are bigger than Ongole Crossbred. Pure Ongole Crossbred is difficult to find because many of them are crossed with Brahman cattle. The mating between Ongole Crossbred and Brahman produces fertile crossbreed called Ongole Crossbred due to its small size. Ongole Crossbred is sometimes called Benggala, referring to its origin in Bengal bay or Bengali, an area in East India. In Kebumen, Central Java, Ongole Crossbred is known as cow Madras referring to its original place. In this city, zebu cattle genetic improvement has been done long before *Ongolisasi* program so that Ongole Crossbred (Madras) in this area has similar quality as Ongole cattle (Utomo et al. 2015).

Madras Cow has convex and short face with round black or dark eyes, flat black snout, and backward-curved horns. Female cattle has longer horn and smaller root of horn, stand up ears which are able to move freely, white long sagging neck skin. It has also thick sag from the front splitting into two and folds. It will be straight and not break up when pulled. Female cattle also have long white umbilical, and the hair whorl is not parallel with the umbilical. Male cattle has wide forehead with slanted eyes, a hump which is big, upright, stand back, and not fall. New born calf will weigh over 28 kg. Ongole Crossbred population is estimated at 4.4 million and almost 90% or around 3.4 million are in Java (DGLS 2003).

Madura cattle

Madura cattle (*Bos javanicus x Bos indicus*) are a crossbreed between Balinese cattle and zebu cattle from India. However, history of the crossbreeding is not known for certain. One source states that the crossbreeding occurs in the middle of the first millennium AD (1500 years ago). Madura cattle also dominates northern coast of East Java wherein many Madurese immigrants live, proving that these immigrants brought the cattle. The area had undergone Javanese depopulation because of the war since the 15th to 18th century. Madura immigrants filled the abandoned land because the previous owners run to save themselves from the war. Madura cattle have a crimson or red brown color with white pattern in the back-bottom, small and short horn that lead outside. Uniformity within breeds is developed by people in Madura through selection.

Madura cattle grow well even though fodder quality is poor. It has high carcass with good quality of beef and good ability to adapt with tropical environment. Madura cattle are able to run quickly and used in a cattle race (*karapan sapi*). It is also used for a beauty contest due to its good body (*sapi sonok*). Cattle race requires high energy metabolism to gain physical strength, work of skeletal muscle, and emotional power (aggressiveness). On the contrary, *sapi sonok* needs high energy metabolism to hold the stretching of skeletal muscle and control the emotion (tamed). Cattle without those characteristics belong to beef cattle. In *Karapan sapi,* the cattle taking part in a race should have a very good performance and condition. The performance and condition are influenced by genetic and environmental factors including fodder and health. Madura culture of cattle selection should be controlled whether or not it influences variation of gene involved in energy metabolism. Gene characterization in energy metabolism is needed to improve the quality of Madura cattle within animal conservation (Siswijono et al. 2010; Febriana et al. 2015).

In colonial times, Madura Island was devoted to the development of Madura cattle that made the introduction of other race of cattle was forbidden. However, the development of other race of cattle was allowed at a later period. In 1957, crossbreeding between Madura cattle with Red Danish dairy cattle (*Bos taurus*) was carried out, but the result was less desirable. In the last decades, Madura cattle are crossed with Limousine (Siswijono et al. 2010) and Simmental through artificial insemination. The result of crossbreeding is highly on demand particularly in Sumenep and surrounding area. The process is carried out directly in the field and causes concern since it may change genetic composition of Madura cattle and influences its immune toward dry climate and limited food. Madura cattle were also introduced in Flores and East Kalimantan, but it failed to grow and replaced by Balinese Cattle. Sapundi Island is devoted to be the area of pure Madura cattle conservation so that it is able to avoid uncontrolled genetic changes. In 2002, total population of Madura cattle was about 900.000 (DGLS 2003).

MINOR LOCAL CATTLE

Indonesia has several kinds of superior local cattle such as Aceh cattle, Madura cattle, Javanese cattle, Grati cattle, and Pesisir cattle (Blakely and Bade 1998). Those cattle are known as local cattle. In some areas, local cattle are still desirable such as Aceh cattle. However, the high demand of the local cattle is not collateral to the production rate of the cattle. Indigenous cattle are domesticated cattle while local cattle are the offspring of several kinds of cattle that are able to adapt to certain location (Martojo 2003).

Aceh cattle are one of four kinds of Indonesian local cattle (Aceh, Pesisir, Madura, and Bali). Ongole Crossbred is also considered as Indonesian local cattle. The cattle are named after the area where the cattle are found that is Aceh, Pesisir, Java, Madura, and Bali. Aceh cattle are found in Aceh province, Pesisir Cattle are found in some part of North and West Sumatra, Madura cattle are found in Madura Island, and Bali cattle are found in Bali Island (Williamson and Payne 1978).

Although the production of indigenous cattle is lower than imported one, indigenous cattle are proven to be able to adapt to local environment including food, water availability, climate and disease (ILRI 1995; Noor 2004). Animals that are able to adapt to environment have better gene that regulates their production and reproduction when they are faced with environmental stress. Uncontrolled crossbreed between Aceh cattle and exotic cattle without considering the importance of indigenous cattle causes concern because it triggers the erosion of genetic resources and threatens the existence of this breed in the future. The loss of important genes in indigenous cattle that has been adapted to local environmental conditions will be a serious matter because the gene may not be replaced. This concern can be seen in the case of indigenous cattle extinction in India. Due to uncontrolled crossbreeding, those cattle came to extinction before they were identified properly, as reported by Sodhi et al. (2006). FAO (2000) warned that livestock are at risk of extinction, especially in developing country. This is due to new market demand, crossbreeding, change of breed, and agricultural mechanization.

Crossbreeding among local cattle or between local cattle and exotic cattle is conducted to improve genetic quality and cattle's productivity. Consequently genetic quality of local cattle decreased. Simple germplasm conservation by using a performance test makes exterior characteristics become important. In crossbreeding, originality of germplasm is the basic thing that must be considered.

Aceh cattle

Aceh cattle (*Bos indicus*) are small local cattle developed in Aceh (Martojo 2003; Dahlanuddin et al. 2003). They were first brought in by Indian traders in the past (Abdullah et al. 2007). Aceh cattle spread widely in Aceh area and are demanded as beef and working cattle. Most of local breeder use Aceh cattle to plow rice field. Aceh cattle business is conducted by local breeder and there has been no industrial cattle business (et al. 2007; Abdullah 2008).

Aceh cattle have a brownish red body (male) and crimson body (female), whitish color around the eyes, inner ears, and upper lips. Male cattle have a darker neck than the female, blackish brown back line, crimson back thighs, and light brown rump. The face and back shape are usually concave. The horns curved upward. The ears are small. The weight of male cattle is 253 ± 65 kg, female cattle 148 ± 37 kg; carcass percentage is 49-51%. Aceh cattle have a good ability in adaptation and in working as well as good immune. Aceh cattle are very productive; cattle breeder's fertility is 86-90%, birth rate is 65-85%, age of puberty is from 300-390 days, estrous cycle is from 18-20 days, and time for pregnancy is 275-282 days (Abdullah et al. 2007).

Pesisir cattle

Pesisir cattle (*Bos indicus*) are Indonesian local cattle which have the smallest body among other local cattle. Pesisir Cattle have small and short body, slender legs, small hump, and tame. Male cattle have short head, short and big neck, wide back of the neck, big, short, and round hump. Female cattle have rather long and thin head, tilt, short, and thin steering on the back, small horn (Saladin 1983). Adult male (4-6 years old) weighs only 160 kg (Adrial 2010). They have short horn pointing outwards like goat horns. The diversity of coat color is relatively high with single pattern. The color is grouped into five dominant colors namely crimson (34.3%), yellow (25.5%), brown (20%), black (10.9%), and white (9.3%) (Anwar 2004).

Pesisir Cattle have high reproduction efficiency (Sarbaini 2004), high birth rate; birth weight is 14-15 kg, the average of daily weight gain from birth to weaning is about 0.32 to 0.12 kg/day (Saladin 1983). Carcass percentage of Pesisir Cattle is 50.6%. This breed is able to survive in adverse environmental condition and poor greening. The ability to convert fibrous fodder into meat is high enough (Saladin 1983). Pesisir Cattle are raised traditionally relying on grasses in pasture, empty land, and rain drops, disease resistance and adaptability to a tropical environment (Hendri 2013). Improved feed quality can have a positive impact on the growth rate and carcass quality, even though it will increase the percentage of lean and fat (Khasrad and Ningrat 2010).

Most Pesisir Cattle are raised by farmers in South Pesisir District and few cattle are raised in Padang Pariaman and Agam districts, West Sumatra (Anwar 2004; Hosen 2006). The population of Pesisir Cattle in South Pesisir District continued to decline. Cattle population in 2009 was 91,777 and in 2010 was 93,881, but it has declined until 76.111 in 2011 (Office of Agriculture and Horticulture, Livestock and Plantation South Pesisir District 2012). The decline of Pesisir Cattle population is believed to be related to the extensive traditional maintenance system, the slaughtering of productive cattle, insufficient fodder, the decrease of pastureland and genetic quality (Adrial 2010). This is due to the slaughtering of cattle with good quality that left only cattle with below average of quality and performance.

Jabres cattle

Jabres cattle (*Bos javanicus x Bos indicus*) (Jabres= Jawa Brebes) are one of local cattle from Brebes, a city in Central Java. This breed is believed to be a crossbreed between Madura cattle or Balinese cattle with Ongole Crossbred. Jabres cattle grow very well in the plateau area of southern Brebes District. The area is at > 800m.a.s.l with average rainfall between 233-565 mm and the number of rainy days between 76-140 days per year. The ability of Jabres cattle to adapt with environment is high. It is able to consume low quality fodder, resistant to insect, and having a good ability for reproduction. Its ability to give birth is also high. One cattle are able to give birth until 15-20 times with a short birth spacing of 12 months and get pregnant again 45 days after giving birth. The average birth weight of Jabres cattle is 16 kg, male adult is between 195-269 kg and female is between 168-296 kg (Lestari 2012).

Jabres cattle have solid meat. Their carcass percentage can reach 52%, higher than the crossbreeding between Simmental and Ongole Crossbred. Besides, they have good quality of skin and can be working cattle. The colors may vary from brown, whitish brown, white, blackish brown and black. Male cattle are usually blackish brown until black and the female is usually brown. These breeds are humpless with slim, compact body shape and dense meat. Special feature that differentiates Jabres cattle with other cattle is its white rump and back leg, a stripe from the back until tail. Jabres cattle have similar characteristics to Balinese cattle, but Balinese cattle have white rump and leg contrast to its brownish red body. In Jabres cattle, the color gradation makes it having no visible boundary between the red brown and the white color. The cattle are able to survive in an extreme climate with limited supply of fodder. Jabres cattle have strong immune and are not susceptible to disease.

All Jabres cattle are raised by small farmers in traditional way. Most of them are taken care by *pangon* system, a system of grazing the cattle in forest or abandoned agricultural land from morning till evening. In the evening, cattle are put back in the barn and are fed by corn straw, rice straw, and grass. Mating system is still dominated by natural mating. Artificial insemination has not been done yet due to cost reason.

Rancah cattle

Rancah cattle (*Bos javanicus x Bos indicus*) are local cattle of southeastern of West Java, especially from Ciamis District (Indrijani et al. 2012). Rancah cattle have physical characteristics as Madura and Bali cattle. The females have no hump with relatively small body size, mostly red brick and white on the pelvis and the four lower legs (tarsus and carpus) with no clear restrictions. There is a stripe along the back with the older color of the dominant colors. Male cattle are similar to females, but mostly with darker body color. Some Rancah cattle male may experience changes in color from brick red to black according to sexual maturity (such as Bali cattle). Male cattle weigh on average of 240 kg and of 220 kg in females (Payne and Rollinson 1973; Huitema 1986). In Rancah, Ciamis, these cattle are relatively small compared to other cattle which are also kept by cattle breeders, such as PO, Simpo and Limpo (Derajat 2014). It can be pregnant again within 2.5-5 months after giving birth (Hilmia et al. 2013).

Rambon cattle

Rambon cattle (*Bos javanicus x Bos indicus*) are local cattle in the east of East Java particularly in Bondowoso, Situbondo, Jember, and Banyuwangi. There were three races of cattle in the past in this area. They were Ongole Crossbred, Madura cattle, and Balinese cattle. Ongole Crossbred was the new Javanese cattle that had been existed in Java since the beginning of the first century AD. Madura cattle were mainly brought conterminous with Madura movement after the conquest of Blambangan Hindu Kingdom in the sixteenth century. Besides being domesticated in Bali and introduced from Bali, Balinese cattle are believed to be domesticated from local wild bull. Wild bulls are still easily found in this area particularly in Alas Purwo National Park, Baluran National Park, and Betiri Meru National Park. Rambon cattle are natural crossbreed of three cattle races that make their genetic composition vary (Susilawati et al. 2002; Susilawati 2004).

Rambon cattle living in Situbondo and Bondowoso have features that are more dominant than Madura cattle and Ongole Crossbred. Rambon cattle in Jember and Banyuwangi have features that are more dominant than Balinese cattle and Ongole Crossbred (Susilawati et al. 2002; Susilawati 2004). It is related to geopolitical history in this area in the past. Demak and Mataram Islamic Kingdom repeatedly conquered this area, but they never built an effective local government. That is why this area is still controlled by Hindu Blambangan Kingdom or under the influence of Balinese Kings. In the middle of the sixteenth century, Mataram conquered this area with the help of Dutch colonial and Regent of Madura. This war caused depopulation in this area and then was inhabited by immigrants from Madura who mostly lived on the north coast. Consequently, Rambon cattle in the north tend to resemble Madura cattle and in the south resemble Balinese cattle.

Rambon cattle weigh about 300-400 kg. Those which live in Situbondo and Bondowoso have various dominant color of skin. They are crimson, brown red, raw red with no visible color boundary, white rump coat; long black tail; colors of leg coat may vary, clear white, white, and crimson; various shapes of the back, straight or curved with or without the back line; the direction of the horn may vary; some are with hump, some are not. Skins of Rambon cattle in Banyuwangi and Jember are dominated by crimson. They have thin dewlap, black back line, and white skin in the leg, white rump, horn, black fur in tail, and no hump (Susilawati et al. 2002; Susilawati 2004).

Galekan cattle

Galekan cattle (*Bos javanicus x Bos indicus*) are breed of Javanese and Balinese cattle that need to be preserved. Coat color is light brown, dark brown and darkish crimson. It has white or lights brown rump and dewlap with no visible boundary between the two. Its tail is long with black hair. It also has black eyes circle, the back with black straight line, hump, horn, and ears with black line. These breeds live in dry lowland.

Galekan cattle are local race of Trenggalek District, East Java. They belong to superior cattle. However, their existence is threatened by the development of Ongole Crossbred and other new cattle of artificial insemination. Frequent natural crossbreeding with Ongole Crossbred decreases the purity of its genetic and makes their breeds difficult to be identified as Galekan cattle. Nowadays, the number of pure superior Galekan cattle is estimated less than 20. These breeds live in Pringapus village, Dongko district, and Panggul village, Panggul district, Trenggalek District, East Java.

Grati cattle

Grati cattle are the only local dairy cattle that are still raised by people. In the beginning of the 20th century, Holstein Friesians (HF) dairy cattle were imported to develop dairy cattle in Indonesia. In 1939, 22 male of Holstein Friesians were imported from the Netherlands to be taken care in Grati, Pasuruan. Prior to Holstein Friesians are Shorthorn, Ayrshire, and Jersey that were imported from Australia. Crossbreeding between the imported cattle and the local cattle (Javanese or Madura cattle) produced new dairy cattle known as Grati. They are internationally recognized as Indonesian local dairy cattle (Payne 1970). This breed was mainly reared in the highland of Pasuruan and Malang (Pujon, Nongkojajar, Batu, and surrounding area) (AAK 1995). They are also known as Holstein Friesians crossbreed. In its development, Grati cattle are the result of crossbreeding of pure male HF and female Ongole Crossbred. These breeds are known as Indonesian Holstein Friesians. Unlike their predecessors that continue to decline in quality and abandoned by farmers, HF is still being developed until today, and the cement is also still being used for artificial insemination. Recently, old Grati cattle population is estimated less than 10.000, while the new Grati (Indonesian Holstein Friesians) has not been recorded (Sariubang 1992; DGLS 2003).

Grati cattle have similar color to Holstein Friesians cattle. They have black and white stripes fur but not as clear as Holstein Friesians. There is a white triangle on the forehead, the chest, and the lower abdomen. Their tail and leg are white with white, long, and straight head. They have small and short horn facing to the front. Their body's size and milk production are lower than Holstein Friesians'. In the beginning, Grati cattle were able to produce around 15 liters of milk per day, but because there was no further genetic improvement, nowadays, they are only able to produce 12.3 liters per day, with lactation period of 9 months. This breed can adapt to hot tropical environment, and easily controlled because it is tame and quiet. With intensive feed, their weight can be increased 0.9 kg per day (Darmono 1993; Yulianto and Saparinto 2010; Syarif and Harianto 2011).

EXOTIC CATTLE

In the 1970s, new type of zebu and taurine cattle were introduced in the form of frozen cement and live cattle. They were then crossed with local cattle. Zebu cattle that were mainly imported were Brahman and Brahman Cross, and from taurine were Simmental, Limousine, HF, and AAC. In Indonesia, there were also other foreign cattle but they were not much raised. ACC was generally put in cattle fattening farm and were not propagated to small farmers. In the beginning, artificial insemination was less successful because farmers were not used to raise cow intensively. Successful breeding is usually between zebu and zebu because the calves are able to live in hot climate and limited food (Martojo 2003). While farmer's knowledge improves, more crossbreeding between Ongole Crossbred and Simmental or Limousine using frozen cement are found. In the long term, there is some concern that genetic composition of Indonesian local cattle will change (Putro 2009).

Holstein Friesians

Holstein Friesians (HF) (*Bos taurus*) have been developed since the 13th century in the Netherlands (North Holland and Friesland) and North Germany (Schleswig-Holstein) to produce a type of cattle that are able to consume local grass. After a century, the breeding effort resulted in the best dairy cattle in the world with typical black and white color. These colors are preferable than the original brown of this race. In Indonesia, HF cattle were first imported from the Netherlands in the 19th century. However, the next import was coming from Australia, New Zealand, USA, Japan, and Canada. These cattle have a good ability to produce milk and meat, and good reproduction ability. They are mainly raised in the highland of Java Island; 700 meters above the sea level with temperature between 16 - 23° C and also moist and wet environment. However, they are also able to live in low land, 300 meters above the sea level with temperature 28 - 35° C and also moist and wet environment.

In Indonesia, the population of HF in 2002 is around 354.000 (DGLS 2003). This group mainly belongs to relatively pure HF because HF crossbreeding that was developed over the last 100 years has not been continued because the pure cattle produce more milk and meat. However, HF crossbreed, the result of crossbreeding between male HF with female Ongole Crossbred is still offered. This type of cattle was called Grati before being called as Indonesian HF. This breed has a high genetic quality obtained from the male parent and a high ability to adapt to tropical environment obtained from the female parent. HF has low birth weight (35 kg in average) but with rapid growth after birth. First ideal pregnancy is around 25 months with short calving interval around 12.6 months. Milk production is high with good production resistance due to high genetic quality and good adaptation to tropical environment.

Pure Holstein Friesian is generally black with white stripes but sometimes red with white stripes and with clear color boundary. The head is long, wide, and straight. The horn is short and curved facing toward (Sudono et al. 2003; Siregar 1995). This breed has wide mouth, wide open nostrils, strong jaw, clear eyes, medium ears, wide forehead, long and thin neck, good shoulders located on the chest wall and form a good joint with the body; strong and flat back with backbone that connected to each other very well. It also has long and wide steering on the back, quadrangular. Its nails are short with good sphere. The heel is low with flat palm. The udder is big and hanging down near the hind legs (Samad and Soeradji 1990).

Simmental

Simmental cattle (*Bos taurus*) originated in Simme valley, Switzerland. This breed is one of the oldest of all breeds of cattle in the world that survive until today. Simmental cattle have been domesticated since the thirteenth century and have contributed to the creation of other newer breeds. They are raised for milk, beef, and as working animals. Simmental cattle had been distributed to many places, for example to Italy in 1400s. However, large scale breeding started in 1960 in the United States. In 1972, Simmental cattle were exported to Australia and New Zealand. In 1985, Simmental cattle and their frozen semen arrived in Indonesia from those countries. Since Simmental cattle are subtropical animal, in Indonesia their pure breed are only raised for their semen in barns belonging to the government or large farmers in highlands. They have muscular and sturdy body, fast muscle growth, they produce high-quality low-fat beef carcass, and the weight of an adult cattle can exceed 1,000 kilograms. Simmental cattle are commonly black due to a selection in the U.S. in 1970-1980, however, the specific breed found in Indonesia have brownish yellow or red face, with white lower legs and white tail ends, similar to the pure breed. In Indonesia, Simmental cattle are mostly kept as beef cattle and widely crossbred with local cattle through artificial insemination, particularly with Ongole Crossbred, Madura cattle, and Bali cattle. In Java, artificial insemination is also conducted on Simmental cattle with Holstein Friesian cattle. From the crossbreeding, male offspring are much preferred due to their faster growth, while the female offspring do not grow very well and produce only a little milk. The artificial insemination is conducted directly on the field, so the offspring have not been adaptable to the local climate, food, and diseases. However, farmers seem to like the effort because the offspring are bigger and grow faster compared to other local breeds. By the age of 2.5 years old, a Simmental cow can weigh up to 1,000 kilograms. For this reason, artificial insemination is done continuously. In long term, this issue needs to be addressed because it is related to the genetic changes of Indonesian local cattle.

Limousin

Limousin cattle (*Bos taurus*) originated from the Limousin and Marche regions of France. The history of Limousin cattle is believed to begin in the sixteenth century; however, the first effort to ensure the breed's purity and improvement was done in 1886 by establishing the French Limousin Herd Book and the distribution to other parts of the world began in 1960s. Limousin cattle have large, long, and compact body, large chest, shallow ribs, and thick meat whose pattern is better than that of Simmental cattle. They have sharp eyes and strong legs. The males have curved horn and dark red, light brown, or greyish yellow body, except around the udders which are white and the lower legs and around the eyes which are lighter in coloration. Nowadays, people raise hornless black Limousin cattle. An adult male can weigh up to 1,400 kilograms and a female 850 kilograms. The most productive Limousin are at the age of 10 to 12. Their rate of weight gain is the highest among other breeds, which is 1.1 kilogram per day. Since they are originally from a subtropical region, Limousin cattle are only suitable for breeding on highlands with high precipitation. This breed is one of the most immune ones that are resistant to various diseases.

Limousin cattle around the world are widely hybridized with other cattle breeds. In Indonesia, their semen is used mainly for artificial insemination on local breeds, particularly the Ongole Crossbred and the Brahman cattle.

Brahman and Brahman Cross

The Brahman Cross cattle (*Bos indicus x Bos taurus*) are crossbreeds of Brahman breed of zebu with taurine in Australia. Brahman cattle were first domesticated in the United States from four breeds of zebu (Kankrej, Gujarat, Ongole, and Gir) which are imported from India since 1849. These breed have been resource of genes for several new breeds of cattle. The Brahman Cross have large, long, and deep body, have hump on the shoulders, and are loose-skinned with sagging skin from the lower jaw to the tip of the front part of the chest. The head is large and long with big hanging, pointed ears. They have large legs with thick and loose skin. The coloration highly varies, but commonly they are grayish white, even though many are black, brown, red, yellow, or striped. The Brahman Cross is one of the best beef cattle for breeding in lowlands due to their resistance to high temperature and endoparasites as well as ectoparasites (Banerjee 1978; Gunawan 2008).

Brahman cattle from the United States were exported to Australia in 1933 and frequently hybridized with cattle breeds that had previously existed in Australia, such as Hereford, Shorthorn, Santa Gertrudis, Droughmaster, Simmental, and Limousin. The offspring were then crossbred with Brahman cattle so that the genetic mix of the offspring is various, but the physical appearance and the features are similar to Brahman cattle because the Brahman cattle genes are more dominant (Banerjee 1978; Turner 1977; Friend and Bishop 1978). Brahman Cross have fairly good growth with daily weight gain of 1.0-1.8 kilogram, high carcass yield of 45-55%, and are resistant to various diseases, lice, and mites.

In 1973, Brahman Cross began to be imported to Sulawesi from Australia (Gunawan 2008). The cattle are mostly used for draft animals and are slaughtered when they get old, so they can only fulfill the demand of traditional market. In 1975, Brahman Crosses were imported to Sumba Island to improve the genetic quality of Sumba cattle. In 2000 and 2001 they were brought to Sumbawa and in 2006 they were distributed widely to many parts of Indonesia to support the acceleration to achieve self-sufficiency of beef. Nowadays, in Indonesia, cattle feedlots have become popular particularly in West Java, Banten, and Lampung. The breeders intensively fatten cattle especially the Brahman Cross. Ideal breeding for fattening is 60-70 days for female cattle and 80-90 days for male cattle, because if they are bred and fattened for too long, their growth will slow down and they will experience extensive fat marbling which is not preferred by local consumers.

Australian Commercial Cross (ACC)

The Australian Commercial Cross (ACC) cattle (*Bos indicus x Bos taurus*) do not have a very clear genetic background. These breeds are supposed to be the descendant of open crossbreeding on grazing lands between various types of cattle raised in North Australia and Queensland that have dry climate. On these grazing lands, many cattle breeds are found, such as the Brahman, Shorthorn, and Hereford (Beattie 1990), so that the ACC is believed to have been originated from a crossbreeding between the Brahman breed of zebu with Shorthorn and Hereford taurine (AMLC 1991; Ngadiyono 1995). However, unlike the Brahman Cross, the physical characteristics of ACC cattle are more similar to that of Shorthorn and Hereford, with shorter and more compact body, large head, small ears that are not hanging, no hump and wither, fur around the head, and color variation similar to Shorthorn and Hereford. These breeds are very good for fattening program because they can adapt easily to suboptimal environment like the Brahman do, and have fairly fast growth like the Shorthorn and Hereford. Young skinny ACC cattle can be fattened in a relatively short time (60 days) and will be very profitable because they gain ±1.61 kilograms per day with the fodder conversion of 8.22 compared to when they are fattened for a longer period (90 or 120 days) (Hafid and Hasnudin 1998). Together with the Brahman Cross, ACC cattle are the most favorite breed for large feedloter companies in Indonesia.

THE DEMAND FOR CATTLE BREEDING

Cattle play an important role in the development of human culture and civilization (Achaya 2002; Lodrick 2005). In addition to agricultural work and ceremonial traditions, cattle are also raised for the production of meat and milk because of their high protein and fat content (Baig et al. 2005). Cattle are raised worldwide as livestock for meat (beef and veal), as dairy animals for milk and other dairy products, and as working animals. Cattle accounts for the largest share of world meat production (Umar 2009), accounting for about 50% of meat demand worldwide, as well as 95% of milk demand and 85% of leather demand (Bappenas 2007). Cattle's ability to consume and digest high fiber materials in larger amount than other ruminants do leads to their relatively more rapid growth compared to other animals (Cheeke and Dierenfeld 2010; Purvis et al. 2011).

Beef cattle business in Indonesia is very potential, considering that the demand for beef is much higher than the supply. The needs for beef cattle increase sharply during two national holidays, the Eid al-Fitr and Eid al-Adha. Until the 1970s, Indonesia was one of the world's biggest beef exporters. However, in the next years, Indonesia became an importer. The amount of imported cattle and beef kept increasing from year to year, resulting in dependence on imported livestock products. Even in the last three years (from 2013 to 2015) there has always been chaos in the domestic market relating to the soaring price of beef. The increase of beef demand is caused by the population growth, the increase of living standard, the pattern changing in food consumption that have led to the increase of the consumption of animal-based foods, and the presence of expatriates who are used to consuming beef of certain quality (Mintert et al. 2001; Norton 2003; DGLS 2010a). In the next 10 years (2014-2024), the national demand for meat increased by almost 1.5% (Faculty of Animal Sciences of Gadjah Mada University 2014), while the world demand increased by almost 3% (OECD 2016).

The high demand of beef in Indonesia is not followed by improvement in quantity and quality of beef cattle (Khasrad and Ningrat 2010), because even high-quality young cows that have not yet reached the standard weight are also slaughtered. A study conducted by the Faculty of Animal Sciences of Bogor Agricultural University (2012) in ten provinces shows that the majority of local cattle that have been slaughtered have not reached the targeted slaughter weight. The financial crisis that hit Indonesia in late 1990s caused Rupiah exchange rate to fall sharply against USD and as a consequence, the government reduced the imports of cattle. This condition led to a significant increase in the slaughter of domestic cattle, which causes a decrease in the number of offspring in the next several years. The increased demand for beef is not only happened in Indonesia, but also in other large population countries such as China and the United States (Feuz 2009; Hoffman 2014; OECD 2016), thus increases environmental pressure, particularly for livestock feed, water and grazing areas as well as global warming (Machovina 2015).

The government of Indonesia has set a long-term policy to achieve beef self-sufficiency by increasing domestic production. The objective of the policy is to reduce reliance on imported beef and cattle, by at least 10 percent. However, the policy, which was introduced in 2000, has been unsuccessful. The same program was tried again in 2010 and once more in 2015 but still failed (DGLS 2010b; Mahbubi 2014). The government had imported live cattle and frozen semen from various types of cattle to increase domestic production. However, other factors such as the limited amount of land for grazing and limited fodder supplies have contributed to the failure.

Agricultural land in Indonesia that are mainly located in Java have been affected by residential and commercial development pressures (Fitriani 2005; Rohman and Hayati 2015; Wuryanta and Susanti 2015). Due to the decreasing area of agricultural land, there is not enough acreage to grow field crops to be used as forage, which affects the productivity of domestic cattle (Harianjogja.com 07/09/2014; Republika.co.id 14/01/2016). The decline of cattle population is also attributed to the slaughter rate that is much higher than the growth of the cattle population due to high demand of beef (Diwyanto 2011); even, productive beef cows and dairy cows are also slaughtered (Soejosopoetro 2011; Fauzi et al. 2013). It was banned by the government (Permentan No. 35/Permentan/ OT.140/7/2011), but the enforcement of these rules is not decisive.

Despite the limited area of land in Java, there are a lot of lands outside Java that can be used for cattle raising, for example lands under palm plantation (Aritonang 1986; Wirdateti et al. 2012) or cocoa plantation (Tanjung 2015), lands that have been deforested due to illegal logging (Santoso 2012), and abandoned mining sites (Diwyanto et al. 2009; Ali et al. 2014). In Papua, there are vast areas of unexploited lowland and highland pastures that can be used for raising tropical and sub-tropical cattle (Rajab 2009; Soltief 2009; Sa'adah 2013; Saiya 2013). These opportunities have never been considered seriously since there is no political policy and initiatives from the government. An estimation of the needs for beef in the next ten years is presented in Table 1.

Table1. Estimated amount of beef supply and consumption (2014-2024)

Year	Production (ton)	Consumption (ton)	Difference/ Deficiency (ton)	Fulfillment from domestic product (%)
2014	435,086.19	593,516.62	-158,430.42	73.31
2015	446,180.61	639,857.57	-193,676.96	69.73
2016	457,275.03	684,884.27	-227,609.23	66.77
2017	468,369.45	729,910.96	-261,541.51	64.17
2018	479,463.87	774,937.66	-295,473.79	61.87
2019	490,558.29	819,964.36	-329,406.06	59.83
2020	501,652.71	864,991.05	-363,338.34	58.00
2021	512,747.13	910,017.75	-397,270.62	56.34
2022	523,841.55	955,044.45	-431,202.90	54.85
2023	534,935.97	1,000,071.14	-465,135.17	53.49
2024	546,030.39	1,045,097.84	-499,067.45	52.25

Source: Faculty of Animal Sciences of Gadjah Mada University (2014)

THE DEVELOPMENT OF CATTLE INDUSTRY AND ITS CHALLENGES

The development of cattle industry in Indonesia has been done for thousands of years and is still being done until today to improve the quality and quantity of cattle. The quality of cattle is determined by genetic as well as environmental factors (climate, food, disease, etc.). Both can be manipulated in order to improve the quality of cattle; however, genetic factors play a more important role since they determine the rate of reproduction, meat or milk productivity, percentage of carcass, daily weight gain rate, food efficiency, immunity to climate and diseases, physical strength as working animals, and so on. Genetic studies have been done to give more understanding about the loss of genetic diversity due to inbreeding and its implication to the natural population. Genetic diversity conservation is important because it represents the evolution potential of a certain species (Frankham et al. 2002). One example of genetic studies of cattle in Indonesia that includes all types of cattle breed is conducted by Mohamad et al. (2009).

Nowadays, the main challenge in the sustainable use of cattle as a source of protein is the lack of information about the population of local cattle, their geographic distribution, and genetic characteristics (Long 2008). Phenotypic and genetic characteristics of cattle population are still limited (Hannotte and Jianlin 2005). The world's cattle population is mostly the descendant of two bovine species, zebu (humped) and taurine (humpless), which are scientifically called *Bos indicus* and *Bos taurus*. The history of the two breeds from their ancestor the wild aurochs has been traced through Mitochondrial DNA or mtDNA (Baig et al. 2005). Bali cattle (*Bos javanicus*) are the only breed of bovine that is significantly developed.

The diversity of cattle is developed through mutations, genetic shifts, and artificial selection of wild ancestor species in order to enable the cattle to grow and breed optimally in a local environment (Long 2008). Genetic studies are important to prevent a decline of cattle's genetic quality. Similar to wild species, the biggest threat for domesticated cattle is inbreeding and the loss of genetic variations. To ensure that a population can grow and breed continuously, the rate of genetic variation in the population must be determined. Genetic factors that affect the productivity and sustainability of cattle in the long term must be identified. Since genetic variation is often correlated with health, a decline in genetic variability may limit the ability of the population to respond to environmental changes such as climate change, disease, or parasites (Frankel and Soule 1981). Inbreeding causes a decline of genetic variation that leads to a decreasing immunity of the cattle in responding to environmental changes and disease. Inbreeding mostly occurs in small isolated populations that do not have the chance of getting new genes from outside. Cattle isolation in Bali that is done by preventing new genes to enter is believed to cause a decline in the cattle's immunity so that they can easily get infected with Jembrana disease, while other Bali cattle living outside the island do not get infected even though the population size is bigger.

On the other hand, new genes input through hybridization with other cattle breeds may threaten the purity and special characteristics of a certain species. During the colonial era, Madura Island was exclusive for raising Madura cattle and was closed for other types of cattle; and Bali was limited only for raising Bali cattle since the reign of the Klungkung Kingdom. However, today, the two islands become the target for improving cattle quality through artificial insemination using frozen semen from zebu (Brahman and Brahman cross) and taurine (Simmental, Limousin, etc.) cattle. The breeding conducted directly in the field started to become uncontrollable, so that the long-term effect is unpredictable. The development of Ongole Crossbred industry is a success story of cattle quality improvement in Indonesia, which has produced offspring that are highly adaptable to the climate, fodder, and diseases in Indonesia and are suitable as working animals. However, due to their low daily weight gain compared to other types of cattle, these cattle have become the target of hybridization. Crossbreeding between Madura cattle with various types of taurine dairy cattle shows failure because the offspring unexpectedly have low immunity and milk productivity, so that they can no longer be found in Indonesia.

The quality improvement through crossbreeding between the same breed of cattle, for example, Ongole Crossbred (*Bos indicus*) with Brahman (*Bos indicus*) or Brahman Cross (*Bos indicus x Bos taurus*), are generally successful. However, crossbreeding between different breeds is mostly unsuccessful. Even if it is successful, the quality of offspring will decrease after several generations. For example, a crossbreeding between Madura cattle (*Bos javanicus x Bos indicus*) with Red Danish (*Bos taurus*) produces offspring that grow and raise well and can adapt to the local environment. Therefore, there must be pure male semen available for hybridization. Another example is crossbreeding between Holstein Friesians cattle (*Bos taurus*) and Ongole Crossbred (*Bos indicus*) that produces Grati cattle, in which the quality of offspring decline after few generations, so that new Holstein Friesians semen is always available for breeding. The same case also happens to the crossbreed of Simpo and Limpo, in which the success rate of female offspring's pregnancy inseminated with Simmental or Limousin semen is lower compared to the crossbreeding with female Ongole Crossbred. However, in cases of beef cattle, farmers generally do not care about the long-term condition because the offspring are intended for being slaughtered instead of being raised, so that they need to provide frozen semen from pure breeders. Nowadays, most male cattle that are crossbred are of the Simmental and Limousin breed, and the female is Ongole Crossbred. However, there are also successful mating between female Bali cattle and male Madura cattle. In some cases, crossbreeding between different types of cattle can also produce high quality offspring after several generations, for example Madura, Brahman Cross, and ACC cattle. Before conservation and management efforts are done, it is very important to understand the rate of genetic variations in a certain cattle population.

DAFTAR PUSTAKA

AAK. 1995. Practical Guide to Cattle Farming. Kanisius, Yogyakarta. [Indonesian]

Abdullah MAN, Noor RR, Martojo H, Solihin DD, Handiwirawan E. 2007. Phenotypic diversity of Aceh cattle in Nanggroe Aceh Darussalam. J Indon Trop Anim Agric 32 (1): 11-21. [Indonesian]

Abdullah MAN. 2008. Genetic Characterization of Aceh cattle using Phenotypic Diversity Analysis on D-loop region of Mitochondrial DNA and DNA Microsatellite. [Ph.D. Dissertation]. School of Graduates. Bogor Agricultural University. Bogor. [Indonesian]

Achaya KT. 2002. A Historical Dictionary of Indian Food. Oxford University Press, Oxford.

Achilli A, Bonfiglio S, Olivieri A, Malusà A, Pala M, Kashani BH, et al. 2009. The multifaceted origin of taurine cattle reflected by the mitochondrial genome. PLoS ONE 4 (6): e5753. DOI: 10.1371/journal.pone.0005753

Adam, T., 1921: Oudheden te Djambi, In: Oudheidkundig Verlag, 194-197.

Adrial. 2010. Potential and development efforts of Pesisir cattle in West Sumatra. Jurnal Penelitian dan Pengembangan Pertanian 29 (2): 66-72. [Indonesian]

Ajmone-Marsan P, Garcia JF, Lenstra JA. 2010. On the origin of cattle: how aurochs became cattle and colonized the world. Evol Anthropol 19: 148-157.

Ali HM, Alam G, Syamsu JA, Salengke, Asja MA. 2014. Average Daily Gain, AST and Blood Nitrogen Urea (BUN) Responses of Bali Beef on Cocoa Waste Extract Supplement. J Adv Agric Technol 1 (1): 4-9.

AMLC [Australian Meat and Livestock Corporation]. 1991. A Workshop for tropical feedlot managers: An Introductory Workshop for Feedlot Managers in the Philippines. Australian Meat and Livestock Corporation, Perth Western, Australia.

Anwar. 2004. Diversity of External Character and Microsatellite DNA of Pesisir Cattle West Sumatra. [Ph.D. Dissertation]. School of Graduates. Bogor Agricultural University, Bogor. [Indonesian]

Arifah. 2013. Site Cangkuang: Meeting of Hindu and Islamic civilization. DikbuD 4 (2): 26-29. [Indonesian]

Ariswara. 1994. Prambanan. 4th ed. Intermasa, Jakarta.

Aritonang D. 1986. Palm oil plantations, a source of animal feed in Indonesia. Jurnal Litbang Pertanian 4 (4): 93-99. [Indonesian]

Asmadi S, Soemadi H. 2004. Sukuh Temple. C.V. Massa Baru, Surakarta [Indonesian]

Atmakusuma J, Harmini, Winandi R. 2014. Is it possible be realized meat self-sufficiency? Risalah Kebijakan Pertanian dan Lingkungan 1 (2): 105-109. [Indonesian]

Baig M, Beja-Pereira A, Mohammad R, Kulkarni K, Farah S, Luikart G. 2005. Phylogeography and origin of Indian domestic cattle. Curr Sci 89: 38-40.

Banerjee GC. 1978. Animal Husbandry. Oxford and IBH Publishing Co, New Delhi.

Bappenas. 2007. Animal Feed. Rural Community Economic Development Project-Bappenas, Jakarta. [Indonesian]

Barker G. 1985. Prehistoric Farming in Europe. Cambridge University Press, Cambridge, UK.

Bhattacharya G. 1977. Nandin and Vṛṣabha, Zeitschrift der Deutschen Morgenländischen Gesellschaft, Supplement III,2, XIX. Deutscher Orientalistentag, pp. 1543-1567.

Blakely J, Bade DH. 1998. The Science of Animal Husbandry. 8th ed. Prentice-Hall, Inc., New York.

Bosch FDK. 1915/16. De Sanskrit-inscriptie op den steen van Dinaja. TBG 57: 410-444

Bottenberg RW. 2010. Śriwijaya: Myth or Reality? [M.Sc. Thesis]. Leiden University, The Nederland.

BPS. 2014. Preliminary figures of 2013 Agricultural Census Results. BPS, Jakarta. st2013.bps.go.id [2 Oktober 2015] [Indonesian]

Bradshaw CJA, Brook BW. 2007. Ecological-economic models of sustainable harvest for an endangered but exotic megaherbivore in northern Australia. Nat Res Mod 20 (1): 129-156.

Budiarso IT, Hardjosworo S. 1976.Jembrana disease in Bali cattle. Aust Vet J 52 (2): 97.

Byers O, Hedges S, Seal US (eds.). 1995. Asian wild cattle conservation assessment and management plan workshop. Working document. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, Minnesota, USA.

Cheeke PR, Dierenfeld ES. 2010. Comparative Animal Nutrition and Metabolism. CABI, Wallingford, Oxfordshire, UK.

Chen S, Lin BZ, Baig M, Mitra B, Lopes RJ, Santos AM, Magee DA, Azevedo M, Tarroso P, Sasazaki S, et al. 2010. Zebu cattle are an exclusive legacy of the South Asia Neolithic. Mol Biol Evol 27: 1-6.

Coedes G. 1968. The Indianized States of Southeast Asia. Vella WF (ed). Cowing SB (transl.). Australian National University Press, Canberra.

Copland J. 1996. Bali Cattle: Origins in Indonesia. In: Wilcox GE, Soeharsono S, Darma DMN, Copland JW. ACIAR Proceeding. 75: 29-33.

Dahlanuddin DV, Liang TJB, Adams DB. 2003. An exploration of risk factors for bovine spongiform encephalopathy in ruminant production system in the tropics. Rev Sci Tech Off Int Epiz 22: 271-281.

Darmono. 1993. Management of Cattle Fattening Business. Kanisius, Yogyakarta. [Indonesian]

de Casparis JG. 1941. Nogmaals de Sanskrit-inscriptie op den steen van Dinojo. TBG 81: 499-514

DGLS [Director General of Livestock Services]. 2003. National Report on Animal Genetic Resources Indonesia; A Strategic Policy Document. Director General of Livestock Services, Jakarta. [Indonesian]

DGLS [Director General of Livestock Services]. 2010a. General Guidelines for Self-Sufficiency Beef Program in 2014. Director General of Livestock Services, Jakarta. [Indonesian]

DGLS [Director General of Livestock Services]. 2010b. Blue Print of Self-Sufficiency Beef Program in 2014. Director General of Livestock Services, Jakarta [Indonesian]

DGLS [Director General of Livestock Services]. 2010c. Technical Guidelines of PSDS Operational Activities. Director General of Livestock Services, Jakarta [Indonesian]

DGLSAH [Director General of Livestock Services and Animal Healts]. 2015. Guidelines for the Implementation of Strengthening Native/Local Cattle Breeding in Selected Island (Pulau Raya, Sapudi Island and Nusa Penida Island) and the Strengthening of Beef Cattle Breeding in Selected Districts (Siak, West Pasaman, South Lampung, Kebumen, Barito Kuala, Barru, Gunung Kidul, and Central Lombok). Director General of Livestock Services and Animal Health, Jakarta. [Indonesian]

Diwyanto K, Hasinah H, Nurhayati IS. 2009. Cattle breeding and development system of integrated rice plants, palm oil and cocoa. In: Integration System of Livestock and Crops: Rice-Palm Oil-Cocoa. LIPI Press, Jakarta.

Diwyanto K, Praharani L. 2010. Reproduction management and breeding strategies to improve productivity and quality of cattle.Proceeding of the Conservation and Improvement of World Indigenous Cattle. Bali, 3-4 September 2010.

Diwyanto K. 2011. Save productive cows. Sinar Tani 30: 2-4. [Indonesian]

Duckworth JW, Hedges S. 1998. Tracking Tigers: A Review of the Status of Tiger, Asian Elephant, Gaur, and Banteng in Vietnam, Lao, Cambodia, and Yunnan (China), with Recommendations for Future Conservation Action. WWF Indochina Programme, Hanoi, Vietnam.

Entwistle K, Lindsay DR (ed). 2003. Strategies to improve Bali cattle in eastern Indonesia. ACIAR Proceedings No. 110, Aciar, Canberra.

Faculty of Animal Sciences of Bogor Agricultural University. 2012. Activity Report Faculty of Animal Science, Bogor Agricultural University and the Directorate General of Livestock and Animal Health Ministry of Agriculture, Republic of Indonesia: Carcass Survey 2012. Bogor Agricultural University, Bogor.

Faculty of Animal Sciences of Gadjah Mada University. 2014. Roadmap of beef industry development in Indonesia. Seminar on Roadmap of Beef Cattle Industry in Indonesia. Faculty of Animal Sciences of Gadjah Mada University and APFINDO, Yogyakarta 9 Oktober 2014. [Indonesian]

FAO [Food and Agriculture Organization]. 2000. World Watch List for Domestic Animal Diversity. 3rd ed. FAO, Rome.

Fauzi RA, Sarwiyono, Setyowati E. 2013. Evaluation of slaughtered Friesian Holstein Crossbreed diary cows in productive age at Karangploso Subdistrict Malang. Faculty of Animal Husbandry, University of Brawijaya. Malang. [Indonesian]

Febriana A, Farajallah A, Perwitasari D. 2015. Indel incident simultaneously on the intron 7 Gen branched-chain α-keto acid dehydrogenase E1a (BCKDHA) in Madura cattle. Jurnal Ilmu Pertanian Indonesia 20 (2): 97-102. [Indonesian]

Felius M, Beerling ML, Buchanan DS, Theunissen B, Koolmees PA, Lenstra JA. 2014. On the history of cattle genetic resources. Diversity

6: 705-750.
Feuz D. 2009. Understanding Beef Demand: What factors change the demand for beef? Beef Feb 24, 2009. http://beefmagazine.com/sectors/retail/0225-understanding-beef-demand
Fitriani AA. 2005. Analysis of the Carrying Capacity of Agricultural Land and Population Pressures (Case Study of Districts in East Java Province). [Hon. Thesis]. Department of Economic Development, Faculty of Economics, Universitas Sebelas Maret Surakarta. [Indonesian]
Fordyce G, Panjaitan T, Muzani H, Poppi D. 2002. Management to facilitate genetic improvement of Bali cattle in Eastern Indonesia. In: Proceeding of an ACIAR Workshop on "Strategies to Improve Bali Cattle in Eastern Indonesia", Denpasar, Bali, Indonesia.
Frankel O, Soule M. 1981.Conservation and Evolution. Cambridge University Press, Cambridge.
Frankham R, Briscoe DA, Ballou JD. 2002. Introduction to conservation genetics. Cambridge University Press, New York, New York, USA.
Friend J, Bishop D. 1978. Cattle World. Blandford Press, Dorset.
Galbreath GJ, Mordacq JC, Weiler FH. 2007. An evolutionary conundrum involving kouprey and banteng: A response from Galbreath, Mordacq and Weiler. J Zool 271 (3): 253-254.
Galbreath GJ, Mordacq JC, Weiler FH. 2006. Genetically solving a zoological mystery: was the kouprey (*Bos sauveli*) a feral hybrid? J Zool 270 (4): 561-564
Gunawan (ed). 2008. Maintenance Instructions of Brahman Cross Cattle. BPTU Sembawa, Ditjen Peternakan, Palembang. [Indonesian]
Handiwirawan E, Subandriyo. 2004. Potential diversity of genetic resources of Bali cattle. Wartazoa 14 (3): 107-115. [Indonesian]
Hannotte O, Jianlin H. 2005. Genetic characterization of livestock populations and its use in conservation decision making. In: Ruane J, Sannino A (eds.). The Role of Biotechnology in Exploring and Protecting Genetic Resources. FAO, Rome.
Hardjosubroto W. 1994. Application of Cattle Breeding in the Field. Gramedia Widia Sarana, Jakarta. [Indonesian]
Hardjosubroto W. 2004. Alternative policy of sustainable management of genetic resources of local beef cattle in the local livestock breeding system. Wartazoa 14 (3): 93-97. [Indonesian]
Harianjogja.com. 07/09/2014. Yogyakarta Shortage of Land for Forage Crops. http://www.harianjogja.com/baca/2014/09/07/diy-kekurangan-lahan-untuk-tanaman-pakan-ternak-533752. [Indonesian]
Hassanin A, An J, Ropiquet A, Nguyen TT, Couloux A. 2013. Combining multiple autosomal introns for studying shallow phylogeny and taxonomy of Laurasiatherian mammals: Application to the tribe Bovini (Cetartiodactyla, Bovidae). Mol Phylogenet Evol 66: 766-775.
Hedges S, Groves CP, Duckworth JW, Meijaard E, Timmins RJ, Burton JA. 2007. Was the kouprey a feral hybrid? A response to Galbreath et al. (2006). J Zool 271 (3): 242-245
Helmer D, Gourichon L, Monchot H, Peters J, Saña Segui M. 2005. Identifying early domestic cattle from pre-pottery Neolithic sites on the middle Euphrates using sexual dimorphism. In: Vigne JD, Peters J, Helmer D (eds.). The First Steps of Animal Domestication.Oxbow Books, Oxford, UK.
Hendri Y. 2013. Development of Pesisir Cattle as a local bred of West Sumatra. J Litbang Pertanian 32 (1): 39-45 [Indonesian]
Hilmia N, Noor RR, Sumantri C, Gurnadi RE, Priyanto R. 2013. Productivity and genetic diversity of local cattle in Ciamis West Java. J Indon Trop Anim Agric 38 (1): 10-19.
Ho SY, Larson G, Edwards CJ, Heupink TH, Lakin KE, Holland PW, Shapiro B. 2008. Correlating Bayesian date estimates with climatic events and domestication using a bovine case study. Biol Lett 4: 370-374.
Hoffman B. 2014. How Increased Meat Consumption In China Changes Landscapes Across the Globe. Forbes Mar 26, 2014. http://www.forbes.com/sites/bethhoffman/2014/03/26/how-increased-meat-consumption-in-china-changes-landscapes-across-the-globe
Hoffmann RS. 1986. A new locality record for the kouprey from Viet Nam, and an archaeological record from China. Mammalia 50 (3), pp. 391-395.
Hoogerwerf A. 1970. Udjung Kulon—the Land of the Last Javan Rhinoceros. E. J. Brill, I.eiden. The Netherlands.
Huitema H. 1986. Animal Husbandry in the Tropics, Economic Meaning and Ability. Yayasan Obor Indonesia and PT Gramedia, Jakarta. [Indonesian]
ILRI [International Livestock Research Institute]. 1995. Global Agenda for Livestock Research. Proceedings of the Consultation for the South-East Asia Region. 10-13 May 1995 IRRI, Los Banos, The Philippines.
Jarrige JF. 2006. Mehrgarh Neolithic. Proceedings of the International Seminar on the "First Farmers in Global Perspective", Lucknow, India, 18-20 January 2006.
Jha DN. 2002. The Myth of the Holy Cow. Verso, London.
Johari S, Kurnianto E, Sutopo, Aminah S. 2007. Diversity of blood protein as biogenetic parameter in Java cattle. J Indon Trop Anim Agric 32 (2): 112-118. [Indonesian]
Kern H. 1910. Een woord in 'Sanskrit opschrift van Toegoe verbeterd. TBG 52: 123.
Keyes CF. 1995.The Golden Peninsula: Culture and Adaptation in Mainland Southeast Asia. SHAPS Library of Asian Studies, University of Hawai'i Press, Honolulu.
Khasrad, Ningrat RWS. 2010. Improving carcass quality of indigenous cattle of West Sumatera fed local feed resources. Pakistan J Nutr 9 (8): 822-826.
Kikkawa Y, Amano T, Suzuki H, 1995: Analysis of genetic diversity of domestic cattle in East and Southeast Asia in terms of variations in restriction sites and sequences of mitochondrial DNA. Biochem Genet 33 51-60.
Kikkawa Y, Takada T, Sutopo, Nomura K, Namikawa T, Yonekawa H, Amano T. 2003. Phylogenies using mtDNA and SRY provide evidence for male-mediated introgression in Asian domestic cattle. Anim Genet 34 96-101.
Lenstra JA, Bradley DG. 1999. Systematics and phylogeny of cattle. In: Fries R, Ruvinsky A (eds) The Genetics of Cattle. CAB International, Wallingford.
Lenstra JA, Theunissen B, Felius M. 2014. Domestic cattle and buffaloes. In: Melletti M, Burton J (eds). Ecology, Evolution and Behaviour of Wild Cattle: Implications for Conservation. Cambridge University Press, Cambridge, UK.
Lestari CMS. 2012. Exploration Potential of Jabres Cattle Production, as Local Beef with Method of In vivo and Non-invasive to the In situ and Ex situ Maintenance. [Ph.D. Dissertation]. School of Graduates, University of Diponegoro, Semarang. [Indonesian]
Lodrick DO. 2005. Symbol and sustenance: Cattle in South Asian culture. Dialectical Anthropol 29 (1): 61-84.
Long JA. 2008. Reproductive biotechnology and gene mapping: Tools for conserving rare breeds of livestock. Reprod Dom Anim 43: 83-88.
Lukas H. 2001. Theories of Indianisation exemplified by selected case studies from Indonesia (Insular Southeast Asia). Proceedings of Papers. "Sanskrit in Southeast Asia: The Harmonizing Factor of Cultures", International Sanskrit Conference, May 21-23, 2001. Sanskrit Studies Centre and Department of Oriental Languages, Silpakorn University (Mahachulalongkornrajavidyalaya Press), Bangkok.
Machovina B. 2015. The number thing each of us can do to protect biodiversity: Reducing our consumption of animal products can go a long way toward conserving endangered habitat around the world. http://ensia.com/voices/the-number-one-thing-each-of-us-can-do-to-protect-biodiversity/
MacHugh D.E. 1996. Molecular biogeography and genetic structure of domesticated cattle [Theses]. Department of Genetics.Trinity College, Univ. Dublin, Dublin.
Mahbubi A. 2014. Madura development program as a cattle island; the perspective of sustainable cattle supply chain management. Agronomika 3 (2): 98-109. [Indonesian]
Martojo H. 2003. Indigenous Bali Cattle: The Best Suited Cattle Breed for Sustainable Small Farms in Indonesia. Laboratory of Animal Breeding and Genetics, Faculty of Animal Science, Bogor Agricultural University, Bogor. [Indonesian]
Martojo H. 2012. Indigenous Bali Cattle is Most Suitable for Sustainable Small Farming in Indonesia. Reprod Domest Anim 47: 10-14.
Mason IL. 1988. World Dictionary of Livestock Breeds. 3rd edn. CAB International; Wallingford, CT, USA.
McCool C, 1992: Buffalo and Bali cattle-exploiting their reproductive behaviour and physiology. Trop Anim Health Prod 24: 165-172.
Merkens J. 1926. De Paarden en Runderteelt in Nederlandsch Indie. Veeartseijkundige Mededeeling. No. 51. Landsdrukkerij-Weltevreden, Nederland.
Mintert J, Schroeder TC, Marsh T. 2016. Factors Affecting Beef Demand. Kansas State University, Manhattan, KS
Mohamad K, Olsson M, Andersson G, Purwantara B, van Tol HTA, Rodriguez-Martinez H, Colenbrander B, Lenstra JA. 2012, The Origin of Indonesian Cattle and Conservation Genetics of the Bali Cattle. Breed Reprod Domest Anim 47: 18-20.

Mohamad K, Olsson M, van Tol HTA, Mikko S, Vlamings BH, Andersson G, Rodriguez-Martinez H, Purwantara BE, Paling RW, Colenbrander B, Lenstra JA. 2009. On the origin of Indonesian cattle. PLoS ONE 4 (5): e5490. http://doi.org/b65mtp

Namikawa T. 1981. Geographic distribution of bovine Hemoglobin-beta (Hbb) alleles and the phylogenetic analysis of the cattle in Eastern Asia. Z Tierzuchtg Zuchtgsbiol 98 151-159.

Ngadiono N. 1995. Growth and Qualities of Carcass and Beef of Sumba Ongole, Brahman Cross and the Australian Commercial Cross intensively reared at Various Cut Weight. [Ph.D. Dissertation]. Institut Pertanian Bogor, Bogor. [Indonesian]

Nijman IJ, Otsen M, Verkaar E.L.C, Ruijter C.D, Hanekamp E et al. 2003. Hybridization of banteng (*Bos javanicus*) and zebu (*Bos indicus*) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. Heredity 90: 10-16.

Noor RR. 2004. Genetics of Livestock. Penebar Swadaya, Jakarta.

Norton M. 2003. Factors affecting beef and cattle producer prices movements. Monthly Labor Review May 2005: 32-40.

OECD. 2016. Meat Consumption (indicator). Organization for Economic Co-operation and Development. DOI: 10.1787/fa290fd0-en

Office of Agriculture and Horticulture, Livestock and Plantation South Pesisir District. 2012. Report of the Office of Agriculture, Horticulture, Plantation and Animal Husbandry (Dipertahorbunnak), South Pesisir District, Painan. [Indonesian]

Office of Veterinary East Sumba District. 1989. Development Program of Sub-sector Livestock. Kabupaten Daerah Tingkat II Sumba Timur, Waingapu. [Indonesian]

Okumura T, Saito K, Sakuma H, Nade T, Nakayama S, Fujita K, Kawamura T. 2007. Intramuscular fat deposition in principal muscles from twenty-four to thirty months of age using identical twins of Japanese Black steers. J Anim Sci 85 (8): 1902-1907.

Omerling FJ. 1957. The Timor Problem. A Geographical Interpretation of an Underdeveloped.Island. 2nd ed. J.B. Wolters, Groningen, Netherlands.

Pamungkas D, Antari R, Mayberry DE, Poppi DP. 2012. A Growth Comparison of Ongole and European Cross Cattle kept by Smallholder Farmers in Indonesia. Proceeding of the The 15th Asian-Australian Animal Production (AAAP) Congress, Bangkok, Thailand, November 2012.

Pane I. 1991.Productivity and Breeding of Bali Cattle.Faculty of Animal Science, University of Hasanuddin, Ujung Pandang. [Indonesian]

Parkvall M. 2010. *Bos primigenius*, aurochs, Skånes djurpark, Höör, Sweden.

Payne WJA, Hodges J. 1997. Tropical Cattle: Origin, Breeding and Breeding Policies. Blackwell Science, Oxford, UK,

Payne WJA, Rollinson DHL. 1973. Bali cattle. World Anim Rev 7: 13-21.

Payne WJA. 1970. Cattle Production in the Tropics. Logman, London.

Permentan [Regulation of the Minister of Agriculture] No. 35/Permentan/OT.140/7/2011 on Animal Control of Female Productive Ruminant. [Indonesian]

PNRI. 2014. Temple Literature: Sukuh (Central Java). Perpustakaan Nasional Republik Indonesia, Jakarta [Indonesian]

Poerbatjaraka RMNg. 1952. Indonesia History I. Jambatan, Jakarta. [Indonesian]

Poesponegoro MD, Notosusanto N. 2010. Indonesia's National History II: Ancient Times. Balai Pustaka, Jakarta [Indonesian]

Pudyatmoko S. 2004. Does the Banteng (*Bos javanicus*) have a future in Java? Challenges of the conservation of a large herbivore in a densely populated island. Knowledge Marketplace Reports. The 3rd IUCN World Conservation Congress, Bangkok.

Purvis G, Downey L, Beever D, Doherty ML, Monahan FJ, Sheridan H, McMahon BJ. 2011. Development of a Sustainably-Competitive Agriculture. In: Lichtfouse E (ed.) Agroecology and Strategies for Climate Change: Sustainable Agriculture Reviews Volume 8. Springer, Netherlands.

Putro P. 2009. Crossbreeding Impact on the Reproduction of Parent Offspring, Clinical Study Results. Workshop on Crossbreeding Cattle in Indonesia: Applications and Implications for Development of Cattle in Indonesia, Lustrum VIII, Faculty of Animal Science, Gadjah Mada University, Yogyakarta 8 August 2009. [Indonesian]

Pyle CM. 1995. Update to: Some late sixteenth-century depictions of the aurochs (*Bos primigenius* Bojanus, extinct 1627): New evidence from Vatican MS Urb. Lat. 276. Arch Nat Hist 22 (3): 437-438

Qiu Q, Zhang G, Ma T, Qian W, Wang J et al. 2012. The yak genome and adaptation to life at high altitude. Nat Genet 44: 946-949.

Rajab. 2009. The Study on Development of Bali Cattle Breeding in Raja Ampat, West Papua Province. [M.Sc. Thesis]. Bogor Agricultural University, Bogor. [Indonesian]

Republika.co.id. 14/01/2016. Western Java Shortage of Land for Animal Feed. http://nasional.republika.co.id/berita/nasional/daerah/16/01/14/o0xwq3370-jabar-kekurangan-lahan-pakan-ternak [Indonesian]

Rohman MN, Hayati R. 2015. Analysis of Population Pressure of Agriculture in Central Java Province in 2020. Geo Image 4 (1): 1-8. [Indonesian]

Rokosz M. 1995. History of the Aurochs (*Bos taurus primigenius*) in Poland. Anim Genet Resour Inform 16: 5-12.

Ruspoli M. 1983. The Cave of Lascaux: The Final Photographic Record. Abrams, New York.

Sa'adah N. 2013. Cattle Ranch Development Planning in Kebar District, West Papua using Map Manager Land Use. [M.Sc. Thesis]. Bogor Agricultural University, Bogor. [Indonesian]

Saiya HV. 2013. Acclimatization of Ongole Crossbred and Bali cattle in Response to Weather Changes in Merauke Papua. [M.Sc. Thesis]. Bogor Agricultural University, Bogor. [Indonesian]

Saladin R. 1983. The Performance of Production and Reproduction Characteristics of South Pesisir Local Cattle of West Sumatra. [Ph.D. Dissertation]. School of Graduates. Bogor Agricultural University, Bogor. [Indonesian]

Samad MS, Soeradji.1990. General Animal Husbandry. CV. Yasaguna. Jakarta. [Indonesian]

Santoso T. 2012. Design of Land Development Model based on Agro-silvopasture in Lebong District. [M.Sc. Thesis]. Faculty of Forestry, Gadjah Mada University, Yogyakarta. [Indonesian]

Sarbaini. 2004. Study on Diversity of External Characteristics and DNA Microsatellite of Pesisir cattle, West Sumatra. [Ph.D. Dissertation]. School of Graduates, Bogor Agricultural University, Bogor. [Indonesian]

Sariubang M. 1992. Feedloting system of Grati cattle.Sub Research Center for Animal Production Grati, Pasuruan. [Indonesian]

Siregar SB. 1996. Maintenance of Dairy Cow Lactation in Lowlands areas. Center for Agricultural Research and Development, Ministry of Agriculture, Bogor. [Indonesian]

Siswijono SB, Subagiyo I, Nurgiartiningsih VMA, Kusmartono, Hartutik, Tadjuddin. 2010. Perception of Madurese community on crossbreeding program of Limousin and native Madura beef cattle in Madura Island of Indonesia. 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, August 1-6, 2010

Sodhi M, Mukesh M, Prakash B, Ahlawat SPS, Sobti RC. 2006. Microsatellite DNA typing for assessment of genetic variability in Tharparkar breed of Indian zebu (*Bos indicus*) cattle, a major breed of Rajasthan. Genetics 85: 165-170.

Soedono A. 1983. Developments Large Ruminant based on Dairy Cattle Breeding Science in Indonesia. Proceedings of the Scientific Meeting of Large Ruminant. Cisarua, 6-9 December 1982. Livestock Research and Development Center, Bogor. [Indonesian]

Soehadji. 2009. History of Dairy Industry Development. Directorate General of Agro and Chemical Industry, Ministry of Industry, Jakarta. [Indonesian]

Soejosopoetro B. 2011. The study of productive female cattle slaughters in Malang slaughterhouse. Jurnal Ternak Tropika 12 (1): 22-26. [Indonesian]

Soekmono. 1976. Chandi Borobudur: A Monument of Mankind. UNESCO Press, Paris.

Soltief MS. 2009. The Study on Beef Cattle Region in Raja Ampat, West Papua Province. [M.Sc. Thesis]. Bogor Agricultural University, Bogor. [Indonesian]

Sonbait LY, Santosa KA, Panjono. 2011. Evaluation of cattle development of cattle farming for the farmer groups under self-rooted institutions in the community of Manokwari District, West Papua. Buletin Peternakan 35 (3): 208-217. [Indonesian]

Steinmetz, R. 2004. Gaur (*Bos gaurus*) and banteng (*B. javanicus*) in the lowland forest mosaic of Xe Pian Protected Area, Lao P.D.R.: abundance, habitat use, and conservation. Mammalia 68: 141-157.

Sudono A, Rosdiana F, Setiawan BS. 2003. Intensive Dairy Cattle Farming. Agromedia Pustaka. Jakarta. [Indonesian]

Sullivan GM, Diwyanto K. 2007. A Value Chain Assessment of the Livestock Sector in Indonesia.United States Agency for International Development, Washington DC.

Susilawati T, Subagyo I, Budiarto A, Ciptadi G, Kuswati. 2002. Phenotype and Genotype Identification of Local Breed for Conservation and Improvement Strategies of Local Cattle Production in East Java. Faculty of Animal Husbandry, Brawijaya University, Malang and Office of Livestock Production, East Java Province, Surabaya. [Indonesian]

Susilawati T. 2004. Local Cattle of Indonesia.[Research Report]. Faculty of Animal Husbandry, Brawijaya University, Malang. [Indonesian]

Sutarno R. 1997. Various Ancient Temple in Indonesia. Dhahara Prize. Semarang. [Indonesian]

Sutarno, Setyawan AD. 2015. Genetic diversity of local and exotic cattle and their crossbreeding impact on the quality of Indonesian cattle. Biodiversitas 16 (2): 327-354

Sutarno. 2010. Genetic variation among Indonesian native cattle breeds based on polymorphisms analysis in the growth hormone loci and mitochondrial DNA. Biodiversitas 11: 1-5.

Syarif EK, Harianto B. 2011. Farming and Business of Dairy Cattle. AgroMedia Pustaka, Jakarta. [Indonesian]

Talib C, Entwistle K, Siregar A, Budiarti-Turner S, Lindsay D. 2002. Survey of population and production dynamics of Bali cattle and existing breeding programs in Indonesia. In: Proceeding of an ACIAR Workshop on Strategies to Improve Bali Cattle in Eastern Indonesia, Denpasar, Bali, Indonesia.

Tanjung FN. 2015. Analysis of beef cattle integration with Cocoa plants. [Hon. Thesis]. Andalas University, Padang. [Indonesian]

Tenaya IWM. 2010. Studies of the pathogenesis of Jembrana disease virus infection in *Bos javanicus* [Ph.D. Dissertation]. Murdoch University, Perth, Australia.

Timmins RJ, Hedges S, Duckworth JW. 2008. Bos sauveli. The IUCN Red List of Threatened Species 2008: e.T2890A9491262. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T2890A9491262.en.

Toelihere MR. 1981. Artificial Insemination in Livestock. P.T. Angkasa, Bandung. [Indonesian]

Tun Yin U. 1967.Wild Animals of Burma. Burma Forest Dept., Rangoon.

Turner HL. 1977. The Tropical Adaptation of Beef Cattle an Australian Study. In: Animal Breeding: Selected Articles from The World Animal Review. FAO Animal Production and Health Paper 1: 92-97.

Umar S. 2009. Potential of Oil Palm Plantations for Development Centre of Beef Cattle in Revitalizes and Accelerate Sustainable Development of Animal Husbandry. Inauguration of Professorship speech in the field of Animal Reproduction Science, Faculty of Agriculture, University of North Sumatra, Medan. [Indonesian]

Urbain A. 1937. Le kou-prey ou bœuf gris cambodgien. Bulletin de la Société Zoologique de France 62 (5): 305-307.

Utomo B, Oelviani R, Subiharta. 2015. Enhancing performance of weaned Ongole calf through management improvement using local resources. Pros Sem Nas Masy Biodiv Indon 1: 838-842. [Indonesian]

Uzzaman MR, Bhuiyan MSA, Edea Z, Kim KS. 2004. Semi-domesticated and Irreplaceable Genetic Resource Gayal (*Bos frontalis*) Needs Effective Genetic Conservation in Bangladesh: A Review. Asian Austr J Anim Sci 27 (9): 1368-1372

Van Vuure C. 2001. Retracing the Aurochs. Universität Paderborn, Höxter, Germany.

Verkaar ELC, Nijman IJ, Boutaga K, Lenstra JA. 2002. Differentiation of cattle species in beef by PCR-RFLP of mitochondrial and satellite DNA. Meat Sci. 60: 365-369.

Verkaar ELC, Vervaecke H, Roden C, Mendoza LR, Barwegen MW, Susilawati T, Nijman IJ, Lenstra JA. 2003. Paternally inherited markers in bovine hybrid populations. Heredity 91: 565-569.

Vigne JD. The origins of animal domestication and husbandry: A major change in the history of humanity and the biosphere. C R Biol 334: 171-181.

Vogel JP. 1918. The Yupa Inscriptions of King Mulavarman from Koetei (East Borneo). BKI 74: 167-232

Wharton, C. H. 1957. An ecological study of the kouprey, *Novibos sauveli*. Institute of Science Technology, Manila, Monograph 5: 1-111.

Wheatley P. 1975. Satyānrta in Suvarṇadvīpa: From Reciprocity to Redistribution in Ancient Southeast Asia. In: Sabloff J, Lamberg-Karlovsky CC (eds.) Ancient Civilisation and Trade. University of New Mexico Press, Albuquerque.

Williamson G, Payne WJA. 1980. An introduction to animal husbandry in the tropics. Tata McGraw-Hill Pub.Co.Ltd, New Delhi, India.

Wirdahayati RB. 1994. Reproductive characteristics and productivity of Bali and Ongole cattle in Nusa Tenggara, Indonesia. [Ph.D. Dissertation]. Department of Farm Animal Medicine and Production. University of Queensland, Brisbane, Australia.

Wirdateti, Semiadi G, Kurniati H, Dahruddin H, Fitriana YS. 2012. Integration Development of Cattle at Palm Oil Plantation in East Kalimantan. Research Center for Biology, Indonesian Institute of Sciences, Bogor. [Indonesian]

Wiyatna MF, Gurnadi E, Mudikdjo K. 2012. Productivity of Peranakan Ongole cattle on the community husbandry in Sumedang District. Jurnal Ilmu Peternakan 12 (2): 22-25. [Indonesian]

Wuryanta A, Susanti PD. 2015. Spatial analysis of population pressure on agricultural land in Keduang Sub-Watershed, Wonogiri District, Central Java. Jurnal Penelitian Sosial dan Ekonomi Kehutanan 12 (3): 149-162. [Indonesian]

Yulianto P, Saparinto C. 2010. Intensive Beef Cattle Fattening. Penebar Swadaya, Jakarta. [Indonesian]

Zeder MA, Emshwiller E, Smith BD, Bradley DG. 2006. Documenting domestication: The intersection of genetics and archaeology. Trends Genet 22: 139-155.

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Detection of plasmids and curing analysis in copper resistant bacteria *Acinetobacter* sp. IrC1, *Acinetobacter* sp. IrC2, and *Cupriavidus* sp. IrC4

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Abstract. *Irawati W, Yuwono T, Rusli A. 2016. Detection of plasmids and curing analysis in copper resistant bacteria Acinetobacter* sp. IrC1*, Acinetobacter* sp. IrC2, and *Cupriavidus* sp. IrC4. *Biodiversitas 17: 296-300. Acinetobacter* sp. IrC1, *Acinetobacter* sp. IrC2, and *Cupriavidus* sp. IrC4 were copper resistant bacteria. The aims of the study were to establish correlation between bacterial resistance and the presence of plasmid and to prove the presence of gene that encodes resistance to copper in plasmid. Plasmid curing was carried out by the addition of ethidium bromide, acridine orange, and SDS in Salt Base Solution broth medium. Detection of copper resistance gene in plasmid was carried out by PCR method using *CopA* primer. The study showed that plasmid isolation has been successfully performed in *Acinetobacter* sp. IrC1, *Acinetobacter* sp. IrC2, and *Cupriavidus* sp. IrC4. The size of plasmid was approximately more than 21 kb. The most effective curing treatment in *Acinetobacter* sp. IrC1 was 600-700 µg/ml ethidium bromide that reduced up to three times of copper resistance after curing treatment. Meanwhile, copper resistance in *Acinetobacter* sp. IrC2 and *Cupriadus* sp. IrC4 decreased four times after curing treatment using 150-200 µg/mL acridine orange and 3000-3500 µg/mL SDS, respectively. The decrease of copper resistance following plasmid curing treatment suggested that copper resistance gene was encoded by the plasmid. The amplification of *CopA* gene in the plasmid showed the presence of single band DNA with approximately 1.8 kb. The finding on copper resistance present in the plasmid may open a wider application of bacteria as copper bioremediation agent.

Keywords: Copper, curing, gene, plasmid, resistance
Abbreviations: Sodium dodecyl sulphate (SDS), Ethidium bromide (EtBr), Acridine orange (AO)

INTRODUCTION

The presence of toxic heavy metal contaminants in aqueous streams, arising from the discharge of untreated metal containing effluents into water bodies, is one of the most important environmental issues (Rehman et al. 2008). Exposures of toxic heavy metals make the cells of microorganisms develop resistance mechanisms and metal-ion homeostasis (Aspassia et al. 2007). Microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and become metal resistant (Prasenjit and Sumanthi 2005). Metal-tolerant bacteria could survive in these habitats and could be isolated and selected for their potential application in bioremediation of contaminated sites (Piotrowska-Seget et al. 2005).

Heavy metals are groups of pollutants, which are not biodegradable and tend to accumulate in living organisms (Kobya et al. 2005). Since heavy metal ions can not be degraded or modified, there are few mechanisms of bacteria for heavy-metal resistance systems such as binding to the cell surface, influx and efflux, accumulation, detoxification of toxic metals to less toxic form, the use of metallothionein protein, and combination of two or three mechanisms mentioned (Aspassia et al. 2007). The well-known copper resistant strain of *Pseudomonas syringae* pv. *tomato* is able to accumulate copper as resistance mechanism to copper. Molecular analysis of the copper resistance nature on *P. syringae* pv. *tomato* revealed an operon Copper (*Cop*) located in the plasmid, namely structural gene *CopA*, *CopB*, *CopC*, and *CopD* as well as regulator gene *CopR* and *CopS*. Each protein functioned specially but *CopA* protein was apparently the most responsible protein in the resistance of *P. syringae* pv. *tomato* (Cha and Cooksey 1991).

The genes conferring copper resistance in bacteria are usually present in plasmids or chromosome. Plasmid is one of the several environmental and genetic factors that carry the resistance property against a specific drug or number of drugs in bacteria. Curing of a cryptic plasmid from a bacterial strain is a method to substantiate the relationship between a genetic trait and carriage of that specific trait in the plasmid. Various methods involving chemical and physical agents have been developed to eliminate plasmid (Crosa et al. 1994).

Acinetobacter sp. IrC1, *Acinetobacter* sp. IrC2, and *Cupriavidus* sp. IrC4 were copper resistant bacteria isolated from activated sludge in an industrial wastewater treatment plant in Rungkut-Surabaya, Indonesia. Bacterial isolates showed resistance mechanism by copper accumulation inside cells (Irawati et al. 2012). The present study was a preliminary effort to observe the curing efficiencies of acridine orange, ethidium bromide, and SDS on copper resistant bacterial isolates to establish a correlation between plasmid elimination and subsequent loss of copper resistance, and also to prove the presence of the gene that encodes resistance to copper.

MATERIALS AND METHODS

Growth media

Bacteria were grown in Salt Base Solution (SBS) broth containing the following (per liter): K_2HPO_4 1.5 g; KH_2PO_4 0.5 g; $(NH4)_2SO_4$ 0.5 g; $Mg_2SO_4.7H_2O$ 0.2 g, supplemented with appropriate concentration of copper sulfate and in medium without copper. Cells were incubated at 37°C on a shaker 200 rpm. Growth was monitored by measuring optical density at 600 nm (Irawati et al. 2013).

Plasmid DNA isolation

Bacterial isolates were tested for the presence of plasmids using alkaline lysate method with slight modification (Sambrook et al 1989). Bacterial colony was inoculated into 5 mL SBS medium containing appropriate concentration of copper sulfate and incubated overnight on a shaker 150 rpm at 37°C. Overnight culture (1.5 mL) was transferred into a microfuge tube and centrifuged at 8,000g for 2 minutes. Bacterial pellets were resuspended in 100 µl of solution which contained 25 mM Tris-HCL, 10 mM EDTA, 50 mM glucose and 10 µl of 2 mg/mL lysozyme, then incubated on ice for 30 minutes. After incubation, 200 µl of solution II (2 N NaOH and 10% SDS) was added. The tube was mixed by inverting it for several times and then incubating it on ice for 10 min. A 150 µl 7.5 M potassium acetate solution was added to the tube and gently mixed by inverting the tube 6 times. It was then incubated on ice for 60 min, and centrifuged at 8,000g. The supernatant was transferred into a new microfuge tube and added with phenol (v/v), then gently mixed by inverting the tube several times and centrifuged at 8,000g. Top aqueous layer was transferred into a new microfuge tube and added with 1 mL ethanol absolute, mixed well, and stored at-20^{0}C for overnight. The aqueous was centrifuged at 8,000g, then ethanol absolute was discarded and washed with 1 mL of 70% ethanol. The pellet was dried and resuspended with 30 µl TE. Plasmid DNA was analyzed by electrophoresis on 1% (w/v) agarose gel in TAE buffer 0.5 µg/mL EtBr.

Plasmid curing

Plasmid was cured essentially as described by Miller (1972). Chemical agents used in this research were EtBr (500-800 µg/mL), AO (25-125 µg/mL), and SDS (500-5,000 µg/mL). Cells (approximately 0.1 mL of a 10^{-5} dilution of a fresh overnight) were inoculated into 5 mL SBS broth, pH 7.6, containing appropriate concentration of curing agents. In similar manner, a control culture without curing agents was prepared. The overnight cultures were plated by dilution on copper indicator plates and the single colonies were observed. The presence and absence of single colonies in medium containing 4 mM $CuSO_4$ were observed. Cured cells were tested to verify the loss of the plasmid and its resistance to copper by plasmid DNA isolation. The presence of plasmid was analyzed by electrophoresis on 1% (w/v) agarose gel in TAE buffer 0.5 µg/mL EtBr.

Amplification of copper resistance gene

Copper resistance gene was amplified as described by Rusli (2012). Amplification was carried out by using *CopA* primers encoding copper resistance in *Acinetobacter* sp. Amplification was accomplished by thermocycler with 1x PCR buffer with 4.5 mM $MgCl_2$, 200 µM each dNTP's (Promega), Taq polymerase 1U/µL (Promega), 0.1 µM forward primer of *CopA* (5'-TAG AGC AGA TGG CAA TGA ATC GCC CAT-3') and reverse primer of *CopA* (5'-AGT TGG AAG AGG GGG ATG AAG CTG TTA TTC-3'), 1 µL template DNA, and ddH_2O to a final volume of 60 µL. PCRs were performed with 10' initial denaturation at 95°C, 35 cycles of 1' denaturation at 95°C; 1' annealing at 64°C, 1,5'; and 1' extension at 72°C, 2' final extension at 72°C, and storage at 4°C. *CopA* gene was analyzed by 1% agarose gel electrophoresis by comparison with λ DNA/*Hind*III and 1kb markers. The *CopA* gene was analyzed by electrophoresis on a 1% (w/v) agarose gel in TAE buffer with 0.5 µg/mL EtBr.

RESULTS AND DISCUSSION

Plasmid DNA isolation

Isolation of plasmid DNA from bacterial isolates revealed the presences of single plasmid. The size of the plasmid was approximately more than 21 kb (Figure 1).

Plasmid isolation has been successfully performed in *Acinetobacter* sp. IrC1, *Acinetobacter* sp. IrC2, and *Cupriavidus* sp. IrC4. Plasmid are extra-chromosomal genetic elements that can range in size from several hundred base pairs to several thousand kilobases (Zholgharnein et al. 2007). Based on the illustrated sizes, the bacterial isolates had large plasmid size. Different plasmids vary considerably in their property to be cured, and not necessarily depending upon properties of specific plasmid (Zaman et al. 2010). Plasmid can be eliminated by curing agents which can be used to display the role of R-plasmid in drug resistance. The techniques used to promote curing include exposing the host strain to elevated temperatures, use of chemical agents such of intercalating dyes (acridine orange, ethidium bromide, SDS, thymidine starvation and exposure to UV radiation (Clowers 1972).

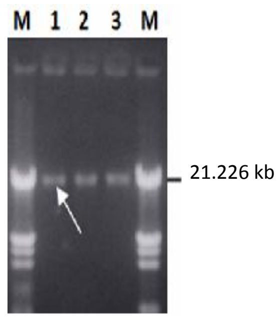

Figure 1. Plasmid DNA profile of (1) *Acinetobacter* sp. IrC1, (2) *Acinetobacter* sp. IrC2, and (3) *Cupriavidus* sp. IrC4. (M) Marker DNA lambda *Eco*R1/*Hind*III.

Plasmid curing

Plasmid curing in this study was carried out by AO, EtBr, and SDS. After curing treatment, each cured colonies of bacterial isolates was isolated and retested for copper resistance by growing in medium containing 4 mM $CuSO_4$. The effect of plasmid curing on the copper resistance of bacterial isolates was shown in Table 1.

Table 1 showed that plasmid curing by appropriate concentrations of curing agent resulted in colony inhibition so that there was no growth of colony on medium containing copper. The curing treatment was successfully done when cured cells which still allowed growth in medium containing the highest concentration of curing agent could not grow in medium supplemented with copper varied among plasmids of bacterial isolates. The effectiveness of each of bacterial isolates to curing agents plasmid of *Acinetobacter* sp. IrC1 could not be cured efficiently by 500 µg/mL SDS whereas *Acinetobacter* sp. IrC2 could not be cured efficiently by 250 µg/mL AO and SDS up to 2,000 µg/mL. Meanwhile, *Cupriavidus* sp. IrC4 could not be cured efficiently by treatment with 250 µg/mL EtBr and AO up to 225 µg/mL.

According to Horn and Korn (1979) as no universally effective curing agent has yet been identified, curing experiments are generally conducted on trial and error basis, both with respect to the choice of the curing agent and the culturing conditions used. Some curing agents works in a non-specific way by damaging and stressing out the cells, while some seem to act much selectively. Table 1 showed that the effectiveness of curing treatment in *Acinetobacter* sp. IrC1 was 600-700 µg/mL EtBr and 200 µg/mL AO. Meanwhile, the effectiveness of curing treatment in *Acinetobacter* sp. IrC2 and *Cupriavidus* sp. IrC4 were 150-200 µg/mL AO and 3000-3500 µg/mL SDS, respectively. The correlation between plasmid elimination and subsequent loss of copper resistance was shown in Table 2.

Table 2 showed that the plasmid elimination was accompanied by drastic changes in copper resistance in bacterial isolates. Copper resistance in *Acinetobacter* sp. IrC1 was reduced from 8 mM to 3 mM when cured by 700 µg/mL ethidium bromide and was reduced from 8 mM to 4 mM $CuSO_4$ when cured by 200 µg/mL acridine orange. Copper resistance of *Acinetobacter* sp. IrC2 was reduced from 9 mM to 2 mM after treatment with 200 µg/mL acridine orange, meanwhile copper resistance in *Cupriavidus* sp. IrC4 was reduced from 11 mM to 3 mM when it was cured by 3,500 µg/mL SDS.

The cured colonies were analyzed for the presence of plasmid DNA on agarose gels (Figure 2). It can be seen that there was difference of plasmid profile between cured strain and wild strain. The cured plasmid of *Acinetobacter* sp. IrC1 could not be detected clearly after treated by 200 µg/mL of acridine orange (lane 2 and 3). Meanwhile the cured plasmid of *Cupriavidus* sp. IrC4 was having the different migration with wild plasmid after cured by 3,500 µg/mL SDS (lane 11 and 12). It might be caused by intercalation between two bases of plasmid DNA by curing agent so that the plasmid could not be replicated well. This curing treatment accompanied by drastic changes in resistance of bacteria indicated that gene encoding copper resistance in *Acinetobacter* sp. IrC1 and *Cupriavidus* sp. IrC4 were located in plasmid.

Table 1. The effect of curing treatment on the growth of bacteria in medium containing 4 mM $CuSO_4$

Curing agent	(µg/mL)	Amount of colonies					
		Acinetobacter sp. IrC1		*Acinetobacter* sp. IrC2		*Cupriavidus* sp. IrC4	
		Without copper	With copper	Without copper	With copper	Without copper	With copper
Etidium bromida	250	> 100	> 100	> 100	> 100	> 100	> 100
	500	32	15	-	-	-	-
	600	21	-	-	-	-	-
	700	2	-	-	-	-	-
	800	-	-	-	-	-	-
Acridine orange	25	> 100	> 100	> 100	> 100	> 100	> 100
	50	> 100	> 100	> 100	33	> 100	> 100
	75	> 100	18	38	16	> 100	> 100
	100	> 100	15	27	8	> 100	> 100
	125	22	8	28	5	> 100	> 100
	150	23	5	8	-	> 100	> 100
	175	21	4	5	-	> 100	> 100
	200	2	-	4	-	> 100	> 100
	225	-	-	-	-	> 100	> 100
SDS	500	> 100	> 100	> 100	> 100	> 100	> 100
	1,000	-	-	> 100	> 100	> 100	> 100
	1,500	-	-	> 100	> 100	> 100	> 100
	2,000	-	-	> 100	> 100	> 100	> 100
	2,500	-	-	-	-	> 100	> 100
	3,000	-	-	-	-	52	-
	3,500	-	-	-	-	2	-
	4,000	-	-	-	-	-	-

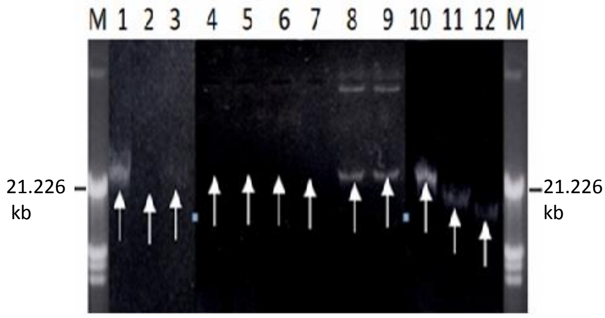

Figure 2. Plasmid DNA profile of bacterial isolates without curing and after curing treatment, (Lane 1: *Acinetobacter* sp. IrC1 plasmid DNA, 2 and 3: acridine orange cured, lane 8 and 9: *Acinetobacter* sp. IrC2 plasmid DNA, 4-7 acridine orange cured, lane 10: *Cupriavidus* sp. IrC4 plasmid DNA, 11-12 SDS cured). (M) Marker DNA lambda *Eco*R1/*Hind*III.

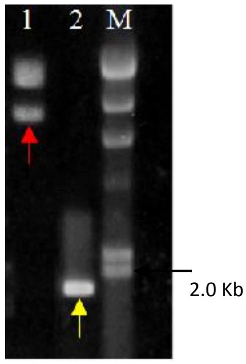

Figure 3. Plasmid and PCR product of *Acinetobacter* sp. IrC1. Note: 1: *Acinetobacter* sp. IrC1 plasmid, 2: *CopA* gene of *Acinetobacter* sp. IrC1 and M: lambda DNA/*Hind*III marker (Promega). Arrows indicate the presence of plasmid and *CopA* gene.

Table 2. The effect of plasmid curing on the copper resistance to isolates bacteria.

Treatment	**Concentration**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Acinetobacter sp. IrC1											
Without curing	+	+	+	+	+	+	+	+			
EtBr (700 µg/mL)	+	+	+	+							
A O (200 µg/mL)	+	+	+								
Acinetobacter sp. IrC2											
Without curing	+	+	+	+	+	+	+	+	+		
A O (200 µg/mL)	+	+									
Cupriavidus sp. IrC4											
Without curing	+	+	+	+	+	+	+	+	+	+	+
SDS 3500 µg/mL	+	+	+								

Note: (+) resistant to copper

Plasmid DNA profile of bacterial isolates without curing and after curing treatment can be seen at Figure 2. Figure 2 showed that the cured plasmid of *Acinetobacter* sp. IrC2 could not be detected by gel electrophoresis after treated by 200 µg/mL acridine orange (lane 4 and 7). Concomitant to this loss of plasmid, the cured cell became sensitive to copper. This clearly showed relationship between the loss plasmid DNA and the loss of copper resistance properties. Cured strain became sensitive to copper after curing treatment indicated the possible plasmid borne nature of gene encoding resistance to copper. The loss of copper resistance in cured strain may be because of mutation as a result of incubation in the presence of curing agent (Raja and Selvam 2009).

CopA gene amplification

The amplification of *CopA* gene in the plasmid showed the presence of single band DNA with approximately 1.8 kb similar to the length of *CopA* gene in *Pseudomonas syringae* pv. *tomato* (Figure 3).

CopA gene which has been successfully amplificated in plasmid of *Acinetobacter* sp. IrC1 indicated that copper resistance determinant of the bacterial isolate encoded in plasmid. It was previously reported that copper resistance mechanism of *Acinetobacter* sp. IrC1 was facilitated through the bioaccumulation of copper inside the cell which was marked by the alteration in the color of the colonies into blue in high concentration of copper similar to *Pseudomonas syringae* (Irawati et al. 2012). The *CopA* gene encodes a methionine-rich periplasmic protein capable of binding up to 11 copper atoms in *Pseudomonas syringae* pv. *tomato* (Cha and Cooksey 1991).

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REFERENCES

Alhaj NA, Shamsuddin MN, Zamri HF, Abdullah R. 2008. Extraction of essential oil from *Nigella sativa* using supercritical carbon dioxide: study of antibacterial activity. Am J Pharm Toxicol 3: 225-228.

Ali MR, Hasan RN, Mohammed AI, Abbas AA. 2010. Profilling and curing from *Shigella* spp. isolated from plasmid diarrheal patients. A J Microbiol 1 (1): 1-8.

Aspassia D, Chatzeifthimiou, Medina MC, Wang Y, Vetriani C, Barkay T. 2007. The isolation and initial characterization of mercury resistant chemolithotrophic thermophilic bacteria from mercury rich geothermal springs. Extremophiles 11: 469-479.

Andreaza R, Pieniz S, Okeke BC, Camarago FAO. 2011. Evaluation of copper-resistant bacteria from vineyard soils and mining waste for copper biosorption. Braz J Microbiol 42: 66-74.

Ansari FA, Khatoon H. 1997. Curing of R plasmids present in Gram negative bacteria isolated from poultry in Karachi by ethidium bromide. Pak J Pharm Sci 10 (2): 13-16.

Cha JS, Cooksey DA. 1991. Copper Resistance in *Pseudomonas syringae* mediated by Periplasmic and Outer Membrane Proteins. Proc Natl Acad Sci USA 88 (10): 8915-8919.

Crosa JH, Tolmasky ME, Actis LA, Falkow S. 1994. Plasmids. In: Gerhardt P (ed).Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, D.C.

Horn B, Korn D. 1969. Co-segregation of a sex factor with the *Escherichia coli* chromosome during curing by acridine orange. J Mol Biol 45: 385-395.

Irawati W, Yuwono T, Hartiko H, Soedarsono J. 2012. Molecular and physiological characterization of copper-resistant bacteria isolated from activated sludge in an industrial wastewater treatment plant in Rungkut-Surabaya, Indonesia. Microbiol J 6 (3): 107-116.

Irawati W, Yuwono T, Hartiko H, Soedarsono J. 2013. The potency of copper-resistant bacteria *Cupriavidus* sp. IrC4 isolated from industrial wastewater treatment plant in Rungkut-Surabaya as a bioremediation agent for heavy metals. International Conference on Biological Science Faculty of Biology, Gadjah Mada University, Yogyakarta.

Kobya M, Demirbas E, Senturk E, Ince M. 2005. Adsorption of heavy metal ions from aqueous solutions by activated carbon prepared from apricot stone. Bioresour Tech 96: 151-152.

Miller JH. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, New York.

Prasenjit B, Sumanthi S. 2005. Uptake of chromium by *Aspergillus foetidus*. J Mater Cycles Waste Manag 7: 88-92.

Piotrowska-Seget Z, Cynon N, Kozdroj J. 2005. Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. Appl Soil Ecol 28: 237-246.

Raja CE, Selvam GS. 2009. Plasmid profile and curing analysis of *Pseudomonas aeruginosa* as metal resistant. Intl J Environ Sci Tech 6 (2): 259-266.

Rehman A, Shakoori FR, Shakoori AR. 2008. Uptake of heavy metals by *Stylonychia mytilus* and its possible use in decontamination of industrial wastewater. World J Microbial Biotechnol 24: 47-53.

Rusli A. 2012. Isolation of *CopA* Gene Encoding Resistance *Acinetobacter* sp. IrC1 to Copper. [Thesis]. Universitas Pelita Harapan, Tangerang. [Indonesian]

Sambrook J, Fritisch EF, Miniatis T. 1989. Molecular Cloning. A laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, New York.

Zaman MA, Pasha MH, Akhter, MZ. 2010. Plasmid curing of *Escherichia coli* cells with ethidium bromide, sodium dodecyl sulfate and acridine orange. Bangladesh J Microbiol 27 (1): 28-31.

Zolgharnein H, Azmi MLM, Saad MZ, Mutalib AR, Mohamed CAR. 2007. Detection of plasmid in heavy metal resistance bacteria isolated from the Persian Gulf and enclosed industrial areas. Iranian J Biotech 5 (4): 232-239.

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Short Communication: Inventory of native orchids in Makki Sub-District, Lanny Jaya, Papua, Indonesia

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Abstract. *Agustini V, Zebua L, Wenda N. 2016. Inventory of native orchids in Makki Sub-District, Lanny Jaya, Papua, Indonesia. Biodiversitas 17: 301-305.* Orchids have been over exploitation since years ago. Some of orchids species are becoming threatened, endangered or even vanished that which may have not been found or discovered. Lanny Jaya, Papua, Indonesia is a new district located at latitude 03°57'08" S and longitude 138°25'05.02" E, covers an area of 2.248 km^2. The establishment of this new district gives impact to the plant habitat including orchids. The study was done in Makki Sub-District, Lanny Jaya, Papua, Indonesia. The present study is an inventory orchid species in Lanny Jaya by explorative method. There were 22 species (17 genera) consist of epiphytic, terrestrial and lithophytic orchids. Among them *Dendrobium subclausum* is the most abundance species in this area. With the combination of bright orange and yellow colors in sepal it can be seen throughout the year.

Keywords: Inventory, Lanny Jaya, orchids, Papua

INTRODUCTION

The Orchidaceae is one of the largest and most diverse families of flowering plants, consists 20,000-35,000 species which together occupy almost every kind of habitat on land New Guinea. Many orchids are terrestrials but most species are epiphytes that perch on, or hang suspended from, the trunks, branches and twigs of trees, mostly in tropical rainforests (Dressler 1981, 1993). Many epiphytes also grow on rocks while some plants grow in exclusively on them namely lithophytes or epilith. More than 70% of all orchid species are epiphytic (Gravendeel et al. 2004).

Papua occupies the western half of the island of New Guinea. It is divided administratively between the country of Papua New Guinea and the Indonesian Province of Papua. Papua New Guinea is a wonderful for orchids since it has many variations in climate and habitats from dry savanna and grasslands to high mist and moss forests, from lush wet coast to small orchid-covered islands encircled by reefs. Each distinct area has own types of orchids (Millar 1978). New Guinea is one of the richest places for orchids with up to 3500 species in 120-130 genera (O'Byrne 1994).

Lanny Jaya is a new district since 2008, formerly it is part of Jayawijaya district. This 2.248 km^2 area with around 1500-3000 m altitude, temperature 14-24^{0}C with rainfall 1900-2000 mm and also great variation of habitat like rainforest and tropical savanna woodlands is a suitable place for orchids. Lanny Jaya is divided into ten districts. Makki Sub-District is the biggest one which covers 150 km^2 areas. In wild most orchids live as epiphytes in the forest trees. A new administrative area, like Makki Sub-District has to build many public facilities such as road, government building and economic issues. These human activities will destroy the forest; the host plants of epiphyte orchids will be lost fast. The establishment for sure gives adverse impact to the orchids.

Study in some places like Pegunungan Bintang and Jayawijaya regencies shown that orchids population becoming extinct due to the rapid destruction of the remaining forest. Information about the orchids of Papua including this area is currently scattered in a small number of publications, and rather difficult to obtain in Indonesia especially in Papua (Lugrayasa 2004; Ungirwalu 2007; Agustini et al. 2012; Agustini et al. 2013; Agustini et al. 2015). The aim of this work is to inventory orchid's species in Makki Sub-District, Lanny Jaya, Papua, Indonesia through field exploration. This research intend to provide a basic tools to residents of the district to assist them recognizing and identifying the local orchids in the wild. And also make local people aware of the treasures that are still living in their forest so it will stimulate them in trying to preserve this forest.

MATERIALS AND METHODS

Study site

Lanny Jaya is a new district located at latitude 03^{0}57' 08''S and longitude 138 ^{0} 25'05,02'' E, which covers an area of 2.248 km^2. The study was conducted in Kemiri Kampong (150 km^2) Makki Sub-District, Papua, Indonesia which covers around 6,7% of total area of Lanny Jaya (Figures 1 and 2). The Kampong is the biggest one among the others nine. The study was done from April to September 2014. Some species which not identified were collected and grown in the green house for further identification.

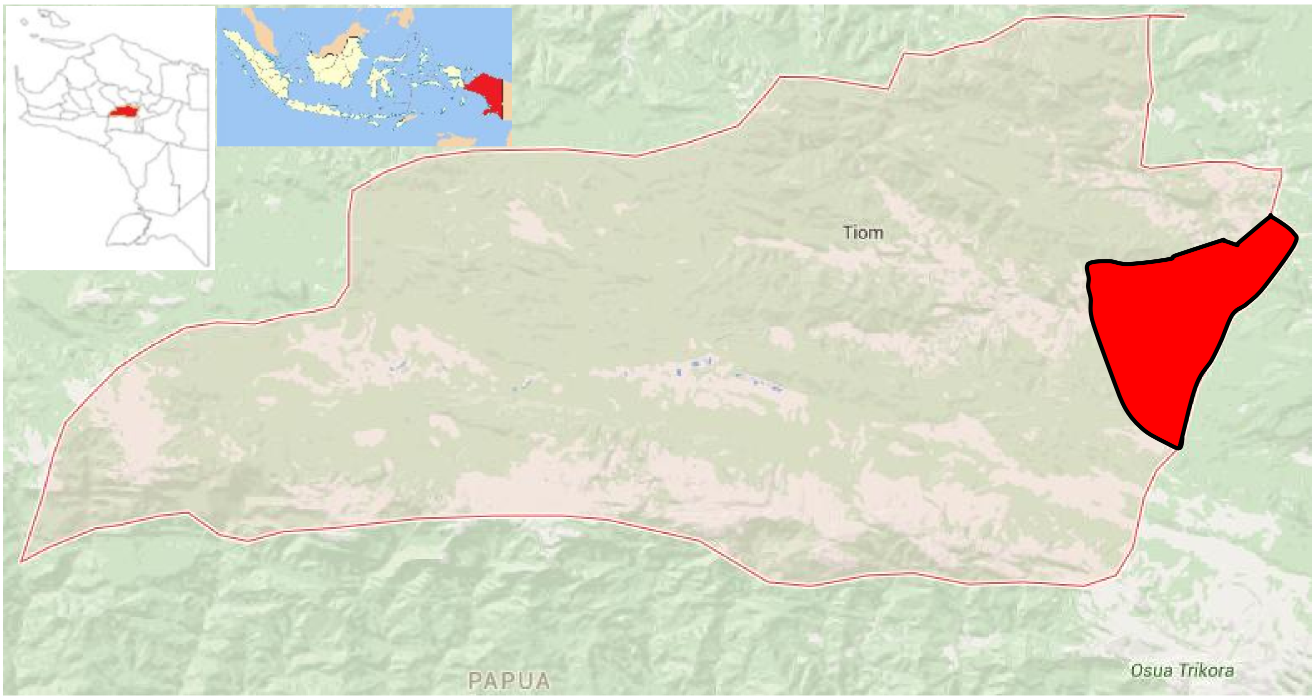

Figure 1. Location of Makki Sub-District, Lanny Jaya District, Papua, Indonesia

Figure 2. In the Makki forest where orchids can be found everywhere along aside the creek.

Procedures and data analysis

In order to assess the orchid flora, a good number of field explorations were made throughout the Makki Sub-District. If a species could not be immediately identified, either a photo was taken or a specimen was collected to facilitate later identification. For herbarium specimens one or two portions of the live plants were collected. For each species encountered, field notes were taken along with the voucher specimen following the standard technique (Jain and Rao 1977). The samples collections have been critically studied after their flowering. Live collections of different species also were grown and maintained in the net house of Biology Department, Faculty of Mathematics and Natural Sciences, Cenderawasih University, Jayapura, Papua, Indonesia for future molecular work.

Identification of the orchids was using literatures from Millar (1978), O'Byrne (1994), Schuiteman (1995), and Schuiteman and de Vogel (2002-2010).

RESULTS AND DISCUSSION

Results

The district is characterized by highly humid atmosphere around 80%, and abound rains with excessive wetness, and low temperature 14^{o}C minimum and 27^{o}C maximum which is responsible for the development of tropical rain forest. There are 22 taxa of orchids belonging to 22 species in 17 genera in Kemiri Kampong, Makki Sub-District. Of these 18 species are epiphytic, 3 species terrestrial, and 1 species lithophytic. (Table 1, Figure 3). Among those, *Dendrobium* is still the richest genus followed by *Bulbophyllum*. *Dendrobium* also found dominant in other parts of lowland and highland of Papua (Agustini et al. 2013; 2015) and New Guinea in general (Millar 1978).


A
B
C
D
2014.8.18 12:03
E
F
G
H
I
2014.8.24 14:28
J
K
L

Figure 3. Some flowers orchids of Makki Sub-District, Lanny Jaya District: A. *Arachnis flos-aeris* (L) Rchb.f.., B. *Bulbophyllum alticola* Schltr., C. *Calanthe rhodochylla* Schltr., D. *Bulbophyllum brachypetala* J.J.Sm. E. *Dendrobium subclausum* Rolfe., F. *D. brassii* T.M.Reeve & P.Woods., G. *D. erosum* (Blume) Lindl., H. *Mediocalcar bifolium* J.J.Sm., I. *Phaius tankervilleae* (Banks ex L'Herit) BL, J. *Diplocaulobium tipula* (J.J.Sm) Krzl., K. *Liparis* sp., L. *Epiblastus basalis* Schltr.

Table 1. List of species of Makki Sub-District, Lanny Jaya District, Papua, Indonesia

Genus	Species	Habit
Agrosthophyllum	*Agrosthophyllum* sp.	Epiphyte
Arachnis	*Arachnis flos-aeris* (L) Rchb.f.	Epiphyte
Bulbolphyllum	*Bulbolphyllum brachypetala* J.J.Sm.	Epiphyte
	Bulbophyllum alticola Schltr	Epiphyte
Calanthe	*Calanthe rhodochylla* Schltr	Terrestrial
Coelogyne	*Coelogyne asperata* Lindl	Epiphyte
Dendrobium	*Dendrobium finisterrae* Schltr	Epiphyte
	Dendrobium subclausum Rolfe	Epiphyte
	Dendrobium erosum (Blume) Lindl.	Epiphyte
	Dendrobium brassii T.M.Reeve & P.Wood	Epiphyte
Dendrochilum	*Dendrochilum longifolium* Rchb.f.	Epiphyte
Diplocaulobium	*Diplocaulobium tipula* (J.J.Sm) Krzl.	Epiphyte
Dockrillia	*Dockrillia* sp.	Epiphyte
Epiblastus	*Epiblastus basalis* Schltr	Terrestrial
Eria	*Eria* sp.	Epiphyte
Glomera	*Glomera compressa* J.J.Sm.	Epiphyte
Liparis	*Liparis* sp.	Lithophyte
Mediocalcar	*Mediocalcar bifolium* J.J.Sm.	Epiphyte
	Mediocalcar arfakense J.J.Sm.	Epiphyte
Oberonia	*Oberonia* sp.	Epiphyte
Phaius	*Phaius tankervilleae* (Banks ex L'Herit) Bl	Terrestrial
Robiquetia	*Robiquetia mooreana* (Rolfe) J.J.Sm.	Epiphyte

Discussion

It is now well known that biodiversity is being lost globally at a rate that is faster than at any previous time in history (Heywood and Watson 1995). In general Papua region is in a bit better position than most other places in Indonesia, large scale logging and agriculture including palm oil cultivation started relatively late, and so Papua still has vast amounts of untouched forest. It is good for orchids in wild, but no doubt that the situation will be changed soon since the development many new regencies last decade and still continue up to now. The situation is much worse for orchid species partly because most of them only survive in certain habitats Therefore, most orchid species are now considered to be at risk of extinction as a result, directly and indirectly, of human activities, and almost all of them are included in conservation lists.

These days numerous orchids species in Papua are being rare in wild land threatened with extinction because of degradation or even total destruction of their habitats. Its happened also in Cycloops Nature Reserve on terrestrial orchids status (Lugrayasa 2004; Agustini et al. 2008). The problem is particularly acute in region with a high orchids diversity like Papua, especially when pressure from collectors aggravates the problems.

In the case of the highland area of Papua, the establishment of new regencies and districts was done so fast which even worse that might faced by diversity of orchid species. More over, reducing some of the important tree species in the "reserve" forest which served as ideal habitat of orchids also happened now days, namely *Pandanus*, *Casuarina*, willo, kote and gii (local names).

Some genera are not to everybody's taste but these are important for the ecology and the botanic point of view. These species must have caused quite a stir among orchid botanist but the fact is that they all perished fairly quickly. So the laboratory in Biology Department, Cenderawasih University is focusing on developing the protocol for propagation of orchid species. This technique will help in conserving the rare species which are at the outmost threat for survivality.

In this investigation 17 genera and 22 orchid species were collected from Makki Sub-District, Lanny Jaya District in 6 months. Among them, which species and genera are becoming scarce and require special protections are difficult to tell. *Dendrobium*, 32 species, and *Bulbophyllum*, 10 species, are the two bigger species found in the study site. Some genera possess no horticultural value; some are high sough after by dealers and collectors but may be equally at risk because of exacting habitat requirements.

Some of the orchids can easily be found throughout Papua New Guinea island either high or low land. Furthermore, others can only be seen in high montane at an elevation 1200 m above sea level (asl.) and more. One species namely *Dendrobium subclausum* Rolfe is the most abundance species, not only in this study area but also in Habema, Jayawijaya at an elevation 3000 m asl. Although it can be locally abundant, if the habitat severely fragmented, it is undergoing a population decline for sure. There are no *D. subclausum* Rolfe in lowland areas. (Agustini et al. 2013; Panal et al. 2015).

Other species such as *Coelogyne asperata* Lindl, *Phaius tankervilleae* (Banks ex L'Herit) Bl. and others *Dendrobium* found in lowland area of Papua (Agustini et al. 2013). Among them *C. asperata* can be seen growing in almost all area in Indonesia (GBIF 2011), but become rare especially in Java and Sumatra due to forest fragmentation. *Robiquetia mooreana* (Rofle) J.J.Sm. is a species which distributed in Papua, Papua New Guinea and Solomon Islands. Study in population dynamics of some orchids *Dendrobium sinense* Tang & F.T. Wang (Hu et al. 2015), *Paphiopedilum appletonianum* (Gower) Rolfe (Chen et al. 2009) and *Phalaenopsis pulcherrima* (Lindl) J.J.Sm. (Hu et al. 2015) indicated that population of wild orchids were experiencing a rapid decline.

According to the list compiled by the International Union for Conservation of Nature (IUCN), loss of habitats like forest clearance for palm oil, farmland, cites, roads was the main cause of the rise of declining wild species including epiphytic orchids (Vrbek and Fiedler 1998; Riofrío et al. 2007; Yoder et al. 2010; de Moraes et al. 2015) and terrestrial (Yoder et al. 2010). The changing of size, shape, geographic isolation, and habitat condition may influence both structure and population of orchids in certain area. Furthermore fragmentation also threatens epiphytic orchids due to loss of pollinators, increased risk of desiccation and fire, and plants invasion.

There are still numerous undiscovered and unexplained patterns in the distribution of wild orchids in Lanny Jaya. The number of recorded species will almost certainly increase, with additional research and the opening up of many still inaccessible locations (Agustini et al. 2013). Recently, more work in orchids identification was done by molecular technique (Hidayat et al. 2013; Mytnik-Ejsmont et al. 2015; Deng et al. 2015; Ponert et al. 2016). The technique will help taxonomist to overcome the limitation of classical identification and to unsolved taxonomic problem both at species and genus levels such as genus *Hederorkis* (Mytnik-Ejsmont et al. 2015), *Polystachya* (Peraza-Flores et al. 2011), or genus into the subtribes (Ponert et al. 2016).

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REFERENCES

Agustini V, Sufaati S, Suharno. 2008. Terrestrial orchids in Mt. Cycloops Nature Reserve, Jayapura-Papua. The 9th New Guinea Biology Conference. Jayapura, 24-26 July 2008.

Agustini V, Sufaati S, Suharno. 2012. The diversity of orchid species in the forest area of Oksibil Sub-District, Pegunungan Bintang, Papua. Jurnal Biologi Papua 4 (1): 32-37. [Indonesian]

Agustini V, Sufaati S, Suharno. 2015. Orchids in the highlands of Habema, Nanggo Trikora Sub-District, Jayawijaya, Papua. Jurnal Biologi Papua 7 (1): 9-15. [Indonesian]

Agustini V, Suharyanto, Suharno, Dimara L, Sembay CD. 2013. The diversity of tropical orchids of South Papua. Jurnal Biologi Papua 5 (1): 1-9.

de Moraes CP, Souza-Leal T, da Silva Favetta MM, Sommer JR, Prezzi LE, Canonici TF, Junior OR. 2015. Floristic and ecology of Orchidaceae in semideciduous seasonal forest fragment in araras, São Paulo, Brazil. Rev Agro Amb 8 (3): 665-681.

Deng H, Zhang G.-Q, Liu Z.-J, Wang Y. 2015. A new species and a new combination of *Phalaenopsis* (Orchidaceae: Epidendroideae: Aeridinae): Evidence from morphological and DNA analysis. Phytotaxa 238 (3): 243-254.

Dressler RL. 1981. The orchids-natural history and classification. Harvard University Press, Cambridge.

Dressler RL. 1993. Field guide to the orchids of Costa Rica and Panama. Cornell University Press, Ithaca NY.

GBIF [Global Biodiversity Information Facility]. 2011. *Coelogyne asperata* Lindl. http://www.gbif.org/species/ 2811710.

Gravendeel B, Smithson A, Slik FJW, Schuiteman A. 2004. Epiphytism and pollinator specialization: drivers for orchid diversity. Phil Trans R Soc Land B 359: 1523-1535.

Heyword VH, Watson, RT. 1995. Global Biodiversity Assessment. Cambridge University Press. New York.

Hidayat T, Weston PH, Yukawa T, Ito M, Rice R. Phylogeny of subtribe Aeridinae (Orchidaceae) inferred from DNA sequences data: Advanced analyses including Australasian genera. J Teknol 59: 87-95.

Hu X.-Y, Zhu J, Song X.-Q, He R.-X. 2015. Orchid diversity in China's Hainan Island: Distribution and conservation. Collectanea Botanica 34: e007.

Jain SK, Rao, RR. 1977. A handbook of field and herbarium method. Today and Tomorrow's Printers & Publishers, New Delhi.

Lugrayasa IN. 2004. Conservation of natural orchids by communities around the Cycloops Nature Reserve area, Papua. National Seminar on Conservation and Utilization of Dry land Plant. LIPI, Bogor. [Indonesian]

Millar A. 1978. Orchids of Papua New Guinea: An introduction. Australian National University Press, Canberra, Australia.

Mytnik-Ejsmont J, Szlachetko DL, Baranow P, Jolliffe K, Górniak M. 2015. Phylogenetic placement and taxonomy of the genus *Hederorkis* (Orchidaceae). PLoS ONE 10 (4): e0122306.

O'Byrne. 1994. Lowland Orchids of Papua New Guinea. SNP, Singapore.

Panal CLT, Opiso JG, Opiso G. 2015. Conservation status of the Family Orchidaceae in Mt. Sinaka, Arakan, North Cotabato, Philippines. Biodiversitas 16 (2): 213-224.

Peraza-Flores LN, Fernandez-Concha GC, Romero-Gonzales GA. 2011. Taxonomic notes in America *Polystachya* (Orchidaceae): the identity of *P. foliosa* (Hook.) Rchb.f. and the reestablishment of *P. caracasana* Rchb.f. J Torrey Bot Soc 138 (4): 366-380.

Ponert J, Trávníček P, Vuong TB, Rybková R, Suda J. 2016. A new species of *Cleisostoma* (Orchidaceae) from the Hon Ba Nature Reserve in Vietnam: A multidisciplinary assessment. PLoS ONE 11(3): e0150631.

Riofrío L, Naranjo C, Iriondo JM, Torres E. 2007. Spatial structure of *Pleurothallis*, *Masdevallia*, *Lepanthes* and *Epidendrum* epiphytic orchids in a fragment of montane cloud forest in South Ecuador. Lankesteriana 7 (1-2): 102-106.

Schuiteman A, de Vogel EF. 2002-2010. Flora Malesiana: Orchid of New Guinea CD-ROMS series Vol. 1-6. National Herbarium Netherland. Netherland.

Schuiteman A. 1995. Key to the genera of Orchidaceae of New Guinea. Flora Malesiana Bul 11 (6): 401-424.

Ungirwalu A, Kilmaskosu MSE, Fatem SM. 2007. The diversity of orchid species in the mangrove forests of South Sorong. Beccariana 9 (1): 18-26. [Indonesian]

Vrbek M, Fiedler S. 1998. The distribution, degree of threat to and conservation of the orchids of Zumberak (Croatia). Nat Croat 7 (4): 291-305.

Yoder JA, Imfeld SM, Heydinger DJ, Hart CE, Collier MH, Gribbins KM, Zettler LW. 2010. Comparative water balance profiles of Orchidaceae seeds for epiphytic and terrestrial taxa endemic to North America. Plant Ecol 211: 7-17.

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Assessment of competition indices of an unlogged oriental beech mixed stand in Hyrcanian forests, Northern Iran

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Abstract. *Amiri M, Naghdi R. 2016. Assessment of competition indices of an unlogged oriental beech mixed stand in Hyrcanian forests, Northern Iran. Biodiversitas 17: 306-314.* Studying on structural dynamics of natural forest ecosystems is an important subject in close-to-nature silviculture and the pertaining tending operations. The objective of this research was to analyze the competition indices of the tree species and some structural characteristics in an unlogged mixed-beech natural forest stand located in Shastkalate forest, Northern Iran. To do so, the required data was collected from a 16ha (400×400 m^2) permanent plot established in 2006. The characteristics of the trees including species, DBH, total height, stem height, crown diameter, and the distance between the stems were measured and recorded. The results indicated that alder and wild service have the highest and lowest DBH, respectively, and also beech species comprises almost half of the basal area as well as the total volume of the stand trees. The results of competition effect on the distribution of the given species tree stems and also on low (≤30 cm), medium (35-50 cm), large (55-70), and very large (>75 cm) diameter classes showed that the competition existing among individual trees decreased as their respective distances, DBH, and crown area enhanced by the increase in distance, such that the highest (R^2_{adj} = 0.79) and the lowest (R^2_{adj} = 0.38)values of competition indices (e.g. Stand density and Relative Spacing index) were, respectively, observed in ≤30 cm and >75 cm diameter classes. The data of the present research, as well, indicated that a more appropriate competition index can be selected by increasing the number of the variables applied in competition indices such as considering the distance among stems, DBH, crown area, and height. As a whole, in the present study the distance-dependent competition indices (e.g. distance-weighted size ratios and Daniel et al) presented better results.

Keywords: Competition indicators, intact forest stands, Oriental beech, Caspian Forests

INTRODUCTION

One of the challenging subjects in ecology is the mechanisms by which plants influence on each other (Bazzaz 1996; Koocheki et al. 2005). The competition existing across the surface of vegetation is one of the significant mechanisms. Also, competition is a fundamental ecological process driving succession in a forest and effects on forest composition and stand structure (Akhavan et al. 2012). Understanding competition among tree species is especially important when management goal is to mimic the dynamics of natural ecosystems (Akhavan et al. 2012). This process arises when neighboring plants share limited resources, leading to a reduction in survivorship and/or growth rate (Clements 1929; Grime 1979; Begon et al. 1996; Oliver and Larson 1996; Avery and Burkhart 2002). For this reason, competition has long been known as a primary process governing population size, community structure and diversity (Oliver and Larson 1996; Newton and Jolliffe 1998; Simard and Sachs 2004; Simard and Zimonick 2005).

Also, intra- and inter-species tree competition is a critical factor effecting on forest succession. Based on the mentioned definition, competition is the interaction among individuals leading to a reduction in the survivorship and also an increase in mortality, growth rate and regeneration of competing individuals (Connell 1983; Schoener 1983; Goldberg 1987; Keddy 1989; Grace and Tilman 1990; Begon et al. 1996; Corral-Rivas et al. 2005). In the trend of succession, competition plays an important role in species substitution (Koocheki et al. 2005), and competitive dynamics between trees is ultimately a key factor in shaping forest stand evolution (Tillman 1982; Brand and Magnussen 1988). On the other hand, Disturbance is considered as a mechanism starting a succession and able to exert a long-term effect on stands growth and succession stages by changing competition dynamics amongst trees (Weber et al. 2008). In forest succession, competition is assumed to play a major role in species replacement. Disturbance is viewed as a mechanism for initiating succession (Bazzaz 1996), which can have a long-term effect on stand development and successional pathways (Oliver and Larson 1996) by changing competitive dynamics among trees. When predicting future forest development, reconstructing past disturbance regimes and identifying changes in competitive interactions are key issues. Understanding the dynamics of forest stands with strong past anthropogenic disturbances is particularly difficult because the different types of human impact typically vary in time and space (Webber et al. 2008; Amiri et al. 2013).

Akhavan et al. (2012) studied the application of bivariate Ripley's K-function for studying competition and spatial association in an intact oriental beech stands in the Kelardasht region, north of Iran. The results of their research showed that the association patterns varied among different size classes across different developmental stages, likely due to shade-tolerance features, seed dispersal limitation, and intra-specific competition of beech trees. This study highlighted the importance of competition in understanding the stand dynamics of beech forests across development stages. Corral-Rivas et al. (2005) and Daniel et al. (2008), respectively, evaluated the effect of competition on basal-area growth in Durango pine stand, Mexico and Norway spruce, Toronto, Italy. Employing the competition index dynamics in the past, Weber et al. (2008) carried out a research in mixed pine and oak stands of Alpine arid regions and Vallis valley in a 30-year period. In another study, Sagheb-Talebi and Shutz (2012) studied some criteria of density in the beech saplings of various forest associations growing in the sub-mountain region near Zurich, Swiss Central Plateau. Then, three collective criteria: (i) number of saplings (N.m^2), (ii) mean distance of saplings, and (iii) crown competition factor, and one individual criterion (growth space) were investigated within the sample plots. Their findings showed that the density of beech saplings was not homogenous. The number of saplings had wide amplitude varying between 2.5 and 54.8n.m^2, and the mean distance of saplings was between 14.5 and 68 cm. The crown competition factor varied between 1 (100%) and 5 (500%) indicating a five-fold overlapping in crown space of saplings. Also, Elahi et al. (2014) surveyed intraspecific competition of *Amygdalus orientalis* which was influenced by physiographic factors (slope, aspect and altitude) in the Semiroms Tang Khoshk forest reserve. Data showed that the competition index is substantially higher on western slopes. In addition, the competition index decreased on altitudes higher than 2200-2300m, as well along with increasing the slope.

The objective of this research is to analyze the competitive indices in a mixed broad-leaf beech stand in Hyrcanian forests, Iran. The following questions were presented as the research hypotheses: Does competition effect on the stand structure? How many categories are the competitive indices divided in to, and which indices' effect is more considerable on the properties of stand structure? Species competition is a critical factor in property variations of the existing trees structure in a beech mixed stand, especially the main species of stands including beech and hornbeam.

MATERIALS AND METHODS

Study site

This study was carried out in the 89.7ha natural unlogged oriental beech (*Fagus orientalis* L.) stand in compartment 32, district 1, located at Shast Kalateh Forest in the eastern Caspian region, North of Iran on 36° 43′ 27 ″ N, 54° 24′ 57″ E (Figure 1). Elevation of the study area varies from 820 to 960 m asl., with an average monthly temperature of 15.4°C, with maximum and minimum temperature in July (28.7°C) and February (8.71°C), respectively. Mean annual precipitation is 650mm. According to the De Martonne and Emberger classifications, the climate of the study area is cold and humid, having a temperate summer with short dry season. Stand total height is about 30 m, and the canopy cover varies between 60 and 100% (Habashi et al. 2007). Soil texture is loam to clay-loam with a pH of 5.5, and the soil is classified as brown type. Mean stand density and standing volume are 235ha^{-1} and 463m^{3}ha^{-1}, respectively (Anon. 1995, 2008). The compartment consists of a natural, mixed, uneven-aged deciduous old-growth forest dominated by shade-tolerant oriental beech with minor components of other broad-leaved species including hornbeam (*Carpinus betulus* L.), velvet and cappadocian maple (*Acer velutinum* Boiss. and *Acer cappadocicum*), Caucasian alder (*Alnus subcordata*), ironwood (*Parrotia persica*), date plum (*Diospyrus lotus*), and elm (*Ulmus glabra* Huds.). The compartment experienced very limited human intervention and disturbance and had no silvicultural activity in the last 50 years since forest management plans started in Iran. Therefore, this stand could be regarded as an example of an intact and unmanaged natural forest (Habashi et al. 2007).

Figure 1. Location of the study site in the Hyrcanian Forests, northern Iran

Living trees measurement

Selection of the 16ha (400×400m) permanent research plot in compartment 32, District 1 Shast Kalateh forest beech stand was made according to: (i) long period without management, that is about 50 years and (ii) the permanent plot should be homogeneous regarding to the slope and aspect. The plot was divided into 64 subplots of 50×50m. All living trees with a diameter at breast height (DBH) of ≥7.5 cm (Delfan-Abazari et al. 2004) were identified by species and their DBH, total height (m), and crown height (m) were measured within subplots. The position of the trees was determined by measuring their coordinates (distance and azimuth) in each subplot (Habashi et al. *20*07; Mataji et al. 2007; Akhavan et al. 2012). Distance and height measurements were made with Laser Distance Meter (Leica Disto D5). The vertical profile of the stand was divided into three height layers (lower, medium and upper) compared to the dominant height that reached 34m. All of the measured trees were assigned to one of the four diameter size classes: small size (dbh ≤32.5 cm), medium size (32.5< dbh ≤ 52.5), large size (52.5 < dbh ≤ 72.5), and very large size (dbh ≥72.5 cm) (Sagheb-Talebi and Schutz 2002; Sagheb-Talebi et al. 2005; Eslami et al. 2007).

Selection and measurement of competition indices in the forest stands

In general, several competitive factors have been used for species competitions that are evaluated based on variables type (Tome´ and Burkhart 1989; Begin and Dobbertin 1992; Wallace et al. 1998; Bachmann 1998; Corral-Rivas et al. 2005; Alvarez et al. 2003; Daniel et al. 2008). The archival journal publications clearly show that the use of competition indices has been extensively rising in the recent years. In order to understand competitive dynamics, several competition indices have been developed through time to assess the competitive intensity occurring on either the whole stand or the individual trees. Stand-level competition indices reflect the degree of tree crowding per unit area (Husch et al. 1982), allowing to compare competitive status in different stands. Individual-based competition reflects the local density of competitors interacting with an individual tree (Tome and Burkhart 1989).

The measures of density described previously are usually employed to determine the density of a stand in general or "on average". More specific measures of density have been developed to describe the degree of competition at a given point of tree in the stand. These measures have been referred to as either point density estimates or competition indices. The basic idea of these indices is to describe the degree to which growth resources (light, water, nutrients, and physical growing space) available to an individual tree are limited by neighboring trees. The increased interest in modeling individual tree growth and yield has produced a number of competition indices, which can be broadly classified into two categories including both the distance-independent and the distance-dependent indices (Corral-Rivas et al. 2005; Daniel et al. 2008).The stand density measures discussed by far are aimed at providing an estimate of the average completion level in stands. Point density measures attempt to quantify the competition level at a given point or tree in the stand (Avery and Burkhart. 2002).

Distance-independent indices

Distance-independent measures describe the competition status of a tree or class of tree relative to all trees in the stand. The main advantage of distance-independent indices is that time-consuming measures of the tree location are not required. The main disadvantage is that these indices measure a tree's status relative to average stand conditions rather than the immediate conditions surrounding the tree (Avery and Burkhart. 2002; Daniel et al. 2008).

Stand density index

The number of trees per unit land area can be used as another measure of stand density. At any age, there can be a wide range in the number of trees per unit land area, so that frequency by itself is of little value. For a useful descriptive measure of stand density, number of tree must be qualified by tree size. A useful measure of density for even-aged and uneven-aged stands based on number of trees is Reineke's stand density index (Reineke 1933). This stand density index is the number of trees per unit area that a stand would have at a standard average DBH. In the metric system the standard DBH is 10 cm. Reineke (1933) defined the stand density index relationship as (Eq. 1):

$$logN = blog\overline{D_Q} + a \quad (1)$$

Where,
N = stand density (trees per hectare)
$\overline{D}$ = quadratic mean diameter (diameter of tree of average basal area)

Reineke (1933) found that the b constant was -1.605 for several species, independent of site quality and age. Other investigators note that the linear relationship expressed by the equation holds for many species and that the slop (b) differ little, although the constant a (i.e., the intercept) varies considerably. The stand density index for any stand can be determined by plotting the position of the observed number of trees/ha and the quadratic mean DBH on the stand density. The stand density index is indicated by the closest line to the plotted point, which can be found by interpolation between the index lines. Alternatively, the stand density index can also be calculated from the formula (Eq. 2):

$$\log SDI = \log N - b\,.\left(\log \overline{D}_Q - \log \overline{D}_1\right) \quad (2)$$

Where,
SDI = Reineke's stand density index, N = number of trees per unit area
D_a = quadratic mean diameter (diameter of tree of average basal area)
D_I = standard diameter
logSDI = log N-b (log D_a-log D_I)

Relative spacing

Another expression of stand density is relative spacing. The average distance between trees divided by the average height of the dominant canopy has been termed relative spacing. In other words, RS_H is the average spacing between trees, assuming square spacing, divided by the average height of the dominant trees. Relative spacing (RS) is computed as (Eq. 3):

$$RS_H = \frac{A/_N}{H_D} \qquad (3)$$

Where,
A = unit area (ha)
N = number of trees per unit area (ha)
H_D = average height of dominant trees (m)

In the relative spacing index numbers will usually be round or decimal numbers such as 0.15 or 10, 11, or 12. Also, the RS index is higher when N is larger.

Basal area index

An example of a distance-independent index is the basal area index proposed by Glover and Hool (1979) (modified by Houtch et al. 2002 and Avery and Burkhart 2002):

$$G_i = \frac{\pi(\frac{D_i}{2})^2}{\pi\left[\left(\sum_{j=1}^{n} D_i/n\right)/2\right]^2} = \frac{D_i^2}{\bar{D}^2} \qquad (4)$$

Where,
G_i = basal area index for *i*th tree,
D_i = diameter of *i*th tree
$\bar{D}$ = mean plot or stand diameter

Crown Competition Factor

This index developed by Krajicek, Brinkman and Gingrich (1961), Crown competition factor (CCF) is a measure of stand density rather than of crown cover. CCF reflects the area available to the average tree in a stand relation to the maximum area it could use if it were open-grown. The compute CCF values, the crown-width/DBH relationship for open-grown trees of species of interest must be established (Avery and Burkhart 2002).

On the other hand, a measure of stand density, which in final form is similar to the tree-area ratio, although considerably different in derivation, is the crown competition factor (CCF) proposed by Krajicik et al. (1961). The CCF is considered independent of site quality and stand age and can be used in both even-and uneven-aged stand (Eq.5).

$$CCF = \frac{\frac{\sum_{i=1}^{n}\left(\pi.mcw_i^2\right)}{4}}{S} \qquad (5)$$

Where,
CCF = Crown competition factor,
mcw = maximum crown width
S = Crown area

Distance-dependent indices

Distance-dependent indices attempt to describe a tree's competitive status based on the immediate conditions surrounding the tree. Distance-dependent indices fall into three broad classes (Avery and Burkhart 2002): area overlap indices, distance-weighted size ratios, and area potentially available or polygons indices (Figure 2). These competition indices provide an estimate of the degree to which growth resources (e.g., light, water, nutrient, and physical growing space) may be limited by the number, size, and proximity of neighbors. The actual competition processes among trees are much more complex than can be described by a reasonably simple mathematical index. However, these indices have been found useful for predicting tree mortality and growth. A large number of distance-independent competition indices have been developed. These indices are described in three classes: (i) area-overlap measurement, (ii) distance-weighted size ratio indices, and (iii) area-available (or polygon) indices.

Area overlap indices

Area-overlap indices are based on the concept that there is a competition influence zone around each tree. Typically, this area over which the tree is assumed to compute for site resources is represented by a circle whose radius is a function of tree size. The area overlap competition index proposed by Gerrard (1969) is as follows: (Eq. 6)

$$CI_i = \frac{1}{A_i}\sum_{j=1}^{n} a_j \qquad (6)$$

Where,
CI_i = Competition index for subject tree i
A_i = area of competition circle for subject tree i
n = number of competition,
a_j = area of overlap of the *j*th competitor

The basic premise of Gerrard's index is that the competitive stress sustained by a tree is directly proportional to the overlapping area of its competition circle with those of its neighbors and inversely proportional to the area of its own competition circle. Area overlap indices are based on the idea that each tree has a potential area of influence over which it obtains or competes for site factors (Opie 1968). All trees whose areas of influence overlap with a subject tree's area of influence are considered competitors (Figure 2.A).

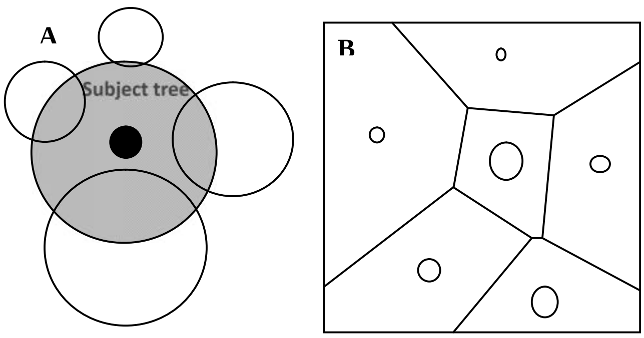

Figure 2. Examples of distance-dependent indices: A. Area overlap concept adapted from Clutter et al. 1983; B. Area potentially available or polygons adapted from Daniels et al. (2008) and Husch et al. (2002).

Distance-weighted size ratios

Distance-weighted size ratios are calculated as the sum of the ratios between the dimensions of each competitor and those of the subject tree, weighed by a function of inter tree distance (Tome and Burkhart 1989). An example is the Competition index proposed by Hegyi (1974).

$$G_i = \sum_{j=1}^{n} \frac{D_j / D_i}{L_{ij}} \quad (7)$$

Where,

C_i = Competition index for subject tree

D_j = diameter of *j*th competitor

D_i = diameter of subject tree

L_{ij} = distance from subject tree to *j*th competitorn = number of competitors

Hegyi (1974) defined n as the number of trees within a fixed radius of the subject tree. Daniels (1976) modified the index defining n as the number of trees within a fixed-angle gauge sweep.

The index of area potentially available

The mentioned index utilizes polygons created by the intersections of the perpendicular bisectors of the distance between a subject tree and its competitors (Figure 2.B). The polygon area, as calculated from the coordinates of the vertices, is the area potentially available for tree growth (Brown 1965). Moor et al. (1973) modified the index, so that the division was weighed by tree size (Eq. 8):

$$I_{ij} = \frac{D_i^2}{D_i^2 + D_j^2} L_{ij} \quad (8)$$

Where I_{ij} = distance from subject tree to weighed midpoint between subject tree and competitor

D_i = diameter of subject tree

Dj = diameter of *j*th competitor

L_{ij} = distance between subject tree and *j*th competitor

As mentioned above, numerous indices have been developed. Each index is derived based on a certain assumption about how the competitive process is manifested. Daniels (1976) suggests that the utility of an index be judged base on correlation with observed tree growth and computational simplicity. Several publications have investigated the efficacy of these measures for predicting individual tree growth (Alemdag. 1978; Noone and Bell 1980; Martin and Ek 1984; Daneils et al. 1986; Tome and Burkhart 1989; Biging and Dobbertin 1995; Avery and Burkhart 2002, Van Laar and Aka 2007).

RESULTS AND DISCUSSION

Quantitative characteristics of stand

The permanent plot consists of 10 species including beech, hornbeam, ironwood, alder, velvet maple, Cappadocia maple, lime, date plum, mountain elm, and wild service. The results of the present research indicate that alder and wild service have the highest and lowest DBH, respectively. Beech and ironwood with 116 and 77 stems, respectively, have the highest number per hectare, while Cappadocia maple, lime, mountain elm, and wild service each have only one stem per hectare (Table 1). Beech contains half of the basal area per hectare of the whole trees (17.2 m^2). Alder and lime with average heights of 31.3 m and 14.1 m are, respectively, the tallest and shortest among the whole stand trees. Beech and date plum have the highest and lowest crown area per hectare with 106.5 m^2 and 37.6 m^2, respectively. The least and most distance among tree stems refer to beech (4.5 m) and alder (69 m), and as for lime, mountain elm, wild servie, and Cappadocia maple, due to their small number per hectare, the average distance among trees was disregarded (Table 1).

Based on diameter classes, Table 2 exhibits the quantitative characteristics of trees in the studied permanent plot. Trees in diameter classes of <30 cm and 55-70 cm have, respectively, the largest and smallest number per hectare, whereas for the other quantitative characteristics of the studied stand including basal area, volume, average height, crown area, and average distance among stems, the very large diameter class >75 cm showed the highest values (Table 2).

Competition indices of stand

Based on the type of variables applied, the results obtained from the relation between basal area growth and competitive indices indicate that among the distance-independent indices, Reinke density and relative spacing indices own the highest R^2-adjust, respectively (Table 3). There is no statistically significant relation between the values obtained from the two mentioned indices and basal area growth. The results, also, show that among the distance-dependent indices, Daniel et al. (2008), Hegyi (1974), and Rouvinen and Kuuluvainen (1997) indices have the highest R^2-adjust.Thus, the above-mentioned indices have a lower square mean error, compared to the other indices. For the indices of crown area such as relative spacing, closed crown, and crown competitive coefficient, R^2-adjust, as well, increased by adding more variables; for instance, the value R^2-adjust is higher than those of the closed crown and crown competitive coefficient having a lower R^2-adjust and consequently a higher square mean error (Table 3).

The results presented in table 4 indicate that the R^2 and root mean square error values differ based on the type of variable and diameter classes used in different indices. As observed in table 4, the larger the number per hectare of trees in a stand is, the higher the R^2 value for the applied index is. It can, thus, be said that due to the large number per hectare of the stems, young and middle-aged diameter classes (35-50 cm and ≤30 cm) have a higher R^2 compared to 55-70 cm and >75 cm diameter classes. However, considering the type of the applied variable in the index, R^2 and root mean square error values of the given index are, as well, varied (Table 4).

Table 1.Structural characteristics of the living trees on the studied permanent research plot, based on species

Species name	DBH (cm)	Density (n.ha)	Basal area (m^2)	Height (m)	Volume (m^3)	Crown area (m^2.ha)	Average distance between of stem (m)
Beech	47	116	17.2	28.7	264	106.5	4.50
Horn beam	40.5	62	8.07	27.4	113.8	92.7	6.84
Ironwood	28	77	4.7	20.7	49	91.5	5.37
Date Plume	13.5	21	0.29	20.4	2.67	37.6	9.30
Velvet maple	48	9	1.6	28.5	25.8	94.6	15.75
Alder	69	3	0.94	31.3	15.8	86.7	25.85
Cappadocia maple	39	1.3	0.09	20.1	1.6	61.2	37.70
Lime tree	48	0.21	0.02	14.9	0.2	48.8	-
Elm	22	0.31	0.01	16.5	0.14	35.4	-
Wild service	12	0.06	0.003	15.2	0.09	48	-

Table 2. Main structural characteristics of the living trees on the studied permanent plot based on DBH classes

Diameter classes (cm)	DBH (cm)	Density (n.ha)	Basal area (m^2)	Height (m)	Volume (m^3)	Crown area (m^2.ha)	Average distance between of stem (m)
≤30	19.7	117.2	4.35	17.64	37.55	54.10	3.82
35-50	42.24	40.3	5.74	24.98	70.28	73.35	7.92
55-70	62.5	24.6	7.7	28.9	109.2	89.6	9.6
>75	107.34	25.8	16.34	36.3	253.3	187.8	12.56

Table 3. Analysis of competitive indices in the permanent plot based on structural characteristics

Competition index	Variable type	R^2_{adj}	RMSE	F-Value	P-Value
Stand density (Rienkeh Index)	n.ha, DBH	0.73	0.48	6.54	0.002
Relative Spacing index	Crown area, n.ha and Height	0.66	0.52	1.7	0.03
Basal area index	DBH	0.28	0.54	1.34	0.34
Crown Closure index	Plot size, Crown width	0.42	0.58	5.28	0.22
Crown Competition Factor	Maximum crown width, Plot size	0.49	0.47	3.9	0.04
Area overlap indices (Gerrard's index)	Crown area, Distance	0.347	0.46	1.27	0.52
Distance-weighted size ratios (Hegyi 1974; Rouvinen and Kuuluvainen1997)	DBH, Distance	0.57	0.49	2.82	0.023
area potentially available index (Brown 1965; Moor et al. (1973)	DBH, Distance	0.66	0.54	5.45	0.027
Alvarez et al. (2003)	Distance, Crown area	0.39	0.46	1.38	0.43
Daniel et al. (2008)	Distance, Crown area, Height	0.63	0.50	5.54	0.034

Table 4. Analysis of competitive indices in the permanent plot based on diameter classes

Diameters classes (cm)	≤30		35-50		55-70		75[	
Competition index	R^2_{adj}	RMSE	R^2_{adj}	RMSE	R^2_{adj}	RMSE	R^2_{adj}	RMSE
Stand density	0.79	0.54	0.61	0.53	0.48	0.54	0.38	0.61
Relative Spacing index	0.62	0.58	0.55	0.51	0.37	0.46	0.26	0.51
Basal area index	0.21	0.58	0.34	0.35	0.385	0.44	0.48	0.51
Crown Closure index	0.19	0.70	0.22	0.52	0.35	0.46	0.54	0.49
Crown Competition Factor	0.24	0.66	0.26	0.52	0.42	0.49	0.46	0.40
Area overlap indices (Gerrard's index)	0.34	0.45	0.38	0.51	0.46	0.49	0.56	0.50
Distance-weighted size ratios (Hegyi 1974; Rouvinen and Kuuluvainen 1997)	0.58	0.48	0.52	0.46	0.4	0.57	0.35	0.62
Index of area potentially available (Brown 1965; Moor et al. 1973)	0.53	0.50	0.46	0.58	0.38	0.59	0.29	0.64
Alvarez et al. (2003)	0.68	0.51	0.57	0.49	0.51	0.50	0.43	0.64
Daniel et al. (2008)	0.73	0.34	0.67	0.45	0.64	0.52	0.78	0.47

Discussion

The present study was carried out in a mixed-beech natural unlogged forest in the Hyrcanian broad-leaf forests. The existing differences for competition over the stand area were obtained by measuring the competition indices on the surface of the tree stems presenting an acceptable compatibility. Studying on competition and spatial structure of trees in a natural oriental beech stand located in Kelardasht (Northern Iran), Akavan et al. (2012) reported similar results. These findings are consistent with the results reported by Sagheb-Talebi and Shutz (2012) and Akhavan et al. (2012). On the other hand, the spatial structure existing in a natural ecosystem interpreted by ecology science can provide a number of indices to more appropriately determine and comprehend natural processes such as competition. In a previous study, Amiri et al. (2013) conducted a research on dynamics of structural characteristics beech mixed stand in the same region and reported the density and volumeof the stand to be 278n.ha^{-1} and 472 m^{2}ha^{-1}, respectively. Beech owned the highest volume of living trees (55.8%) and deadwood (62%) among other species. The present structure of natural and intact forests is the outcome of some complicated interactions among trees having a varied history combined with the effect of a number of factors namely site, climate, and natural distractions such as longevity, competition, storm, fire, pests and diseases, snow and frost, and flood (Akhavan et al. 2012). Information about the spatial patterns of trees having varied dimensions and competition conditions can be useful for regenerating the past structure of stand and interpreting the evolutionary stages forming the distribution pattern of trees. Based on the performed investigations, most of species in plant communities have a clump pattern (Denyslow 1980; Ludwig and Reynolds 1989; Nakashizoka 1989; Alavi et al. 2005; Mattaji et al. 2008). In another study on a permanent plot in the Shaskalateh forest of Gorgan, it was reported that the distribution pattern of most existing tree species including beech, hornbeam, and ironwood was, as well, clump. In some cases, the distribution pattern of date plum, in gaps created by the felling of large trees, is also considered as clump (Amiri 2013).

Hegyi (1974) and Lorimer (1983) analyzed the trend of variations in competition indices over the surface of stand, tree stems, and diameter classes. The results indicated that by the increase in tree size (due to increase in diameter, height, crown area, basal area, and the spacing between trees), competition stress initially increased but then reduced. This is possibly due to the unequal conditions of tree species in different diameter classes, such that in all diameter classes, the number of stems for beech and hornbeam is larger than that of the other existing species across the permanent plot under study. Since the major part of stand number per hectare is in diameter class<30 cm, the mentioned condition is more severe for all the measured tree stems having the given diameter class. It can, therefore, be said that due to the large number per hectare of stems, competition is more severe in the above-mentioned diameter class (Table 4).

To introduce the target tree, the DBH of existing trees in distance-independent indices was used. Since DBH is pertaining to basal area, it is expected that the mentioned indices have a strong relation with basal area. Thus, most of the competition indices in which DBH was influential showed a higher R^2. Root mean square error was higher in large and very large classes, compared to low and medium diameter ones, which is possibly due to their high variability. DBH is used in most competition indices (Hegyi 1974; Lorimer 1983). The spatial indices using DBH showed a good performance as a main prediction variable (e.g. Rouvinen and Kuuluvainen 1997) and were different with the other competition indices. The advantage of the given indices over the other ones is possibly due to the relation between the diameter growth of the target tree and its diameter. Holmes and Reed (1991) reported that such a correlation may be created as a result of competition pressure. However, the diameter of a tree is more dependent on its age and competition history than on the community status of tree in stand (Fox et al. 2007). When the distance-independent competition indices were used with regression relation and without competition index, they showed no statistically significant relation. The given results are corresponding with the results reported by Begin and Dobberrtin (1995) indicating that there was no significant relation between the models of diameter growth and height. Thus, the results of the present study showed that the main point is to use the type of variables in competition indices which determine the index effectiveness. This means that all the counted trees equally intervene in competition survey in spite of their size and closeness to one another. The results of distance-independent basal area and relative space exhibited more significant values. Since, none of the overlapping indices of area-effect showed a significant relation in terms of the growth model of basal area. The results of ratio-size indices introduce the application of DBH, along with other characteristics, as an effective distance-dependent index.

Since no human interventions have been yet reported to occur in the studied stand, a long-term period is required to study the growth and dynamics of forest structure. Furthermore, one of the important aims of establishing permanent plots in intact forests is to regularly monitor the characteristics of stand structure over time, which can be used as a precious tool when the competition amongst tree species is to be investigated. The results revealed that competition is an effective factor on the dynamics of forest stands structure, in particular on the immature stands of beech and hornbeam. Considering the distribution of number in diameter classes and the presence of inter- and intra-species competition in forest, it is recommended that the results of the present research be employed as a pattern for the other beech forest stands in the north of Iran and also implementing proper management operations. Therefore, the information derived from these untouched stands could be used as a key reference for future studies in terms of developing management programs, silvicultural interventions, and likewise plantation.

REFERENCES

Akhavan R, Sagheb-Talebi Kh. 2012. Application of bivariate Ripley's K-function for studying competition and spatial association of trees (Case study: intact Oriental beech stands in Kelardasht). Iranian J For Poplar Res 19 (4): 632-644.

Alavi SJ, Zahedi-Amiri Gh, Marvi-Mohajer MR, Noori Z. 2007. Physiographic factors associated with the spatial distribution of *Ulmus glabra* species in the forest research and education of Kheyrood-Kenar, Nowshahr. Iranian J Ecol 33 :93-100. [Persian]

Alemdag IS. 1978. Evaluation of some competition indices for the prediction of diameter increment in planted with spruce. Canadian Forest Service, Manage, Inst, Inf Rep FMR-X 108.

Alvarez Taboda, M F, Barrio, M, Gorgoso, F, Alvarez, JG. 2003. Influencia de la competecia en el crecimiento en seccion en *Pinus radiata* D. Don Invest Agrar Sist Rec For 12 (2): 25-35.

Amiri M. 2013. Dynamics of Structural Characteristics of a Natural Unlogged *Fagus orientalis* Lipsky Stand during a 5-year's Period in Shast-Kalate Forest, Gorgan, Iran. [Ph.D. Dissertation]. Gorgan University of Agricultural Sciences and Natural Resources, Gorgan. [Persian]

Amiri M, Rahmani R, Sagheb-Talebi Kh, Habashi H. 2013. Dynamics and structural characteristics of a natural unlogged oriental beech (*Fagus orientalis* Lipsky) stand during a 5-year period in ShastKalate Forest, Northern Iran. Intl J Environ Resour Res 1 (2): 107-129.

Avery T E, Burkhart H E. 2002. Forest Measurements. 5th ed. McGraw-Hill, New York.

Bazzaz FA. 1996. Plants in Changing Environments. Linking Physiological, Population and Community Ecology. Cambridge University Press, Cambridge, UK.

Begon GS, Dobbertin M. 1992. A comparison of distance dependent competition measured for height and basal area growth of individual conifer trees. Forest Sci 38: 695-720.

Begon M, Harper JL, Townsend C R. 1996. Ecology: Individuals, Populations, Communities. Blackwell Scientific, Oxford.

Biging GS, Dobbertin M. 1992. A comparison of distance-dependent competition measures for height and basal area growth of individual conifer trees. Forest Sci 38 (3): 695-720

Biging GS, Dobbertin M. 1995. Evaluation of competition indices in individual tree growth models. Forest Sci 41: 360-377.

Brand DG, Magnussen S. 1988. A symmetric, two-sided competition in even Aged monocoltures of redpine. Can J For Res 18: 901-910.

Burkhart HE, Tennent RB. 1977. Site index equations for radiate pine in New Zealand. NZ J For Sci 7: 408-418.

Clements FE. 1929. Plant Competition: An Analysis of Community Function.

Connell JH. 1983. On the prevalence and relative importance of interspecific competition :Evidence from field experiments. Am Nat 122: 661-696.

Corral Rivas JJ, Álvarez González JG, Aguirre O, Hernández FJ. 2005. The effect of competition on individual tree basal area growth in mature stands of *Pinus cooperi* Blanco in Durango (Mexico). European J For Res 124: 133-142.

Corral Rivas JJ, Alvarez Gonzalez JG, Ruız Gonzalez A, Gadow K. 2004. Compatible height and site index models for five pine species in El Salto, Durango Pine (Mexico). For Ecol Manag 201: 145-160.

Dani RF. 1976. Simple competition index and their correlation with annual Loblolly pine tree growth. Forest Sci 22: 454-466.

Daniele C, Giorgio V, Emanuele L, Renzo M. 2008. Analysis of interaspecific competition in two subalpine Norway spruce (*Picea abies*) stands in Paneveggio (Torento, Italy). For Ecol Manag 225: 651-659.

Delfan Abazari B, Sagheb-Talebi Kh, Namiranian M. 2004. Regeneration gaps and quantitative characteristics of seedlings in different development stages of undisturbed beech stands (Kelardasht, Northern Iran). Iranian J For Poplar Res 12 (2): 302-306. [Persian]

Denslow JS. 1980. Gap partitioning among tropical rainforest trees. Biotropica 12: 47-55.

Elahi M, Akbarinia M, Mohamadi G. 2014. Intraspecific competition of *Amygdalus orientalis* as influenced by physiographic factors. Iranian J For Poplar Res 22 (2): 204-215. [Persian]

Eslami AR, Sagheb-Talebi-Kh, Namiranian M. 2007. Determining of equilibrium state in uneven-aged oriental beech forests of Northern-Iran. Iranian J For Poplar Res 15 (2): 92-104. [Persian]

Fox JC, Bi H, Ades PK. 2007. Spatial dependence and individual-tree growth models: Characterising spatial dependence. For Ecol Manag 245: 10-19.

Gerrard DJ. 1969. Competition quotient: a new measure of the competition affecting individual forest trees. Mich Agri Expt Sat Res Bull 20: 1-30.

Goldberg DE. 1987. Neighborhood competition in an old field plant community. Ecology 68: 1211-1223.

Grace JB, Tillman D. 1990. Perspectives on Plant Competition. Academic Press, Inc. San Diego, CA.

Grime JP. 1979. Plant Strategies and Vegetation Process. Wiley, London.

Habashi H, Hosseini SM, Mohammadi J, Rahmani R. 2007. Stand structure and spatial pattern of trees in mixed Hyrcanian beech forests of Iran. Pakistan J Biol Sci 10 (8): 1205-1212.

Harper JL. 1977. Population Biology of Plants. Academic Press, London.

Hegyi F. 1974. A simulation model for managing Jack-Pine stand. In: Freisled J (ed) Growth Models for Tree and Stand Simulation. J Royal College of Forestry, Stockholm. Sweden.

Holmes MJ, Reed DD. 1991. Competition indices for mixed species northern hardwoods. Forest Sci 37: 1338-1340.

Husch B, Beers TW, Kershaw JA. 2003. Forest Mensuration. 4th ed. John Wiley and Sons, New York.

Husch B, Miller CI, Beers TW. 1982. Forest Mensuration. 3rd ed. John Wiley and Sons, New York.

IPCC. 2001. Climate Change, Impacts, Adaptations and Mitigation. The Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Jarvis P G. 1998. European Forests and Global Change. The Likely Impacts of Rising CO_2 and temperature. Cambridge University Press, Cambridge, UK.

Johnson EW. 1973. Relationship between point density measurement stand subsequent growth of southern pines. Auburn Univ, Ala Agr Expt Sta Bull 447: 1-109.

Keddy PA. 1989. Competition. Chapman and Hall, London.

Koocheki A, Zand E, Bannayan M, Rezvani-Moghaddam P, Mahdavi-Damghani A, Jami Al-Ahmadi M, Vesal SR. 2005. Plant Physiological Ecology. Ferdowsi University of Mashhad Press, Mashhad.

Krajicek JE, Brinkman KA, Gingrich SF. 1961. Crown competition-A measure of density. Forest Sci 7: 35-42.

Lorimer CG. 1983. Test of age-independent competition indices for individual trees in natural hardwood stands. For Ecol Manag 6: 343-360

MartinGL, Ek AR. 1984. A comparison of competition measures and growth models for predicting plantation red diameter and height growth. Forest Sci 30: 731-743.

Moore JA, Budelsky CA, Schlesinger RC. 1973. A new index representing individual tree competitive status. Canadian J For Res 3: 495-500.

Moore JA, Zhang L, Stuck D. 1996. Height-diameter equations for ten tree species in the Inland Northwest. West J Appl For 11: 132-137.

Nakashizuka T. 2001. Species coexistence in temperate, mixed deciduous forests. Trends Ecol Evol 16: 205-210.

Newton PF. 2003. Yield prediction errors of a stand density management program for black spruce and consequences for model improvement. Canadian J For Res 33 (3): 490-499.

Newton PF, Jolliffe PA. 1998. Assessing processes of intraspecific competition within spatially heterogeneous black spruce stands. Canadian J For Res 28: 259-275.

Noon CS, Bell JF. 1980. An evaluation of eight intertree competition indices. Oregon Stat Univ, Forest Research Lab Res, Note 66.

Oheimb VG, Westphal Ch, Tempel H, Hardtle W. 2005. Structural pattern of a near-natural beech forest (*Fagus sylvatica*) (Serrahn, North-east Germany). For Ecol Manag 212: 253-263.

Oliver CD, Larson BC. 1996. Forest Stands Dynamics. John Wiley and Sons, New York.

Opie JE. 1968. Predictability of individual tree growth using various definitions of competing basal area. Forest Service 14: 314-323.

Pukkala T, Kolstrom T. 1987. Competition indices and the prediction of radial growth in Scots pine. Silva Fenn 21: 55-67.

Reineke LH. 1933. Perfecting a stand-density index for Even-aged forests. J Agric Res 46: 627-638

Sagheb-Talebi Kh, Schütz JPh. 2002. The structure of natural oriental beech (*Fagusorientalis*) forests in the Caspian region of Iran and the potential for the application of the group selection system. Forestry 75 (4): 465-472.

Sagheb-Talebi Kh, Delfan Abazari B, Namiranian M. 2005. Regeneration process in natural uneven-aged Caspian beech forests. Swiss For J 156 (12): 477-480.
Sagheb-Talebi Kh, Schütz JPh. 2012. Some criteria of regeneration density in young beech populations. Caspian J Environ Sci 10 (1): 61-66.
Schoener TW. 1983. Field experiments on interspecific competition. Am Nat 122: 240-285.
Schroder J, Gadow KV. 1999. Testing a new competition index for Maritime pine in northwestern Spain. Canadian J For Res 29: 280-283.
Shugart HH. 1998. Terrestrial ecosystems in changing environments. Cambridge University Press, Cambridge.
Simard SW, Sachs DL. 2004. Assessment of interspecific competition using relative height and distance indices in an age sequence of seral interior cedar-hemlock forests in British Columbia. Canadian J For Res 34: 1228-1240.
Spurr SH. 1952. Forest Inventory. The Ronald Press Co, New York.
Tilman D. 1982. Resource competition and community structure. In: Monographs in Population Biology. Princeton University Press, Princeton NJ.
Tome M, Burkhart HE. 1989. Distance-dependent competition measures for predicting growth of individual trees. Forest Sci 35: 816-831.
Valles GAG, Torres RJM, Velazquez MA, Rodriguez FC.1998. Relacion de nueve indices de competecia con el crecimiento en diamtro de *Pinus cooperi* Blanco. Agrociencia 32 (3): 255-260.
Van Laar A, Akca A. 2007. Forest Mensuration. Springer, Dordrecht, Netherlands.
Weber P, Bugmann H, Fonti P, Rigling A. 2008. Using a retrospective dynamics competition index to reconstruct forest succession. For Ecol Manag 254: 96-106.

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The population of spotted cuscus (*Spilocuscus maculatus*) and its habitat carrying capacity in Numfor Island, Papua, Indonesia

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Abstract. *Sinery AS, Farida WR, Manusawai J. 2016. The population of spotted cuscus (*Spilocuscus maculatus*) and its habitat carrying capacity in Numfor Island, Papua, Indonesia. Biodiversitas 17: 315-321.* The purpose of this research was to study the population of spotted cuscus (*Spilocuscus maculatus*) and the carrying capacity of its habitat. The result showed that the cuscus population size in 105 ha research area was 17 individuals, with population density of 0.41 individuals/ha or each individual occupied 2.44 ha. Cuscus is the first consumer in a food chain system that consumes a variety of plants and plays an important role in the spread of plant seeds. Its birth rate is higher than its death rate despite its low reproduction rate. Fruit-bearing trees with diameter ≥ 10 cm as source of food for cuscus consisted of 13 species of 10 families with a diversity index of 2.43 (categorized as moderate). The productivity of cuscus food was 0.75 kg/season/ ha which indicated the limitations of the carrying capacity of cuscus' habitat so that the need of food was fulfilled from secondary forests and community gardens.

Keywords: Habitat, population, spotted cuscus, Numfor Island

INTRODUCTION

Cuscus population size in the wild is not well-know, but of the seven species of cuscus in Papua, *Spilocuscus maculatus* is thought to have large enough populations. According to Sinery et al. (2012), *Phalanger orientalis* dominates the natural forest area (0.37 ha/individual), especially in the open populations such as in Arfak Mountains, while *Phalanger gymnotis* and *S. maculatus*, each occupies only 0.10 ha/individual and 0.09 ha/individual. Its condition is influenced by species adaptation abilities to habitat and caused by low reproduction rate and habitat productivity to support the population of *S. maculatus* in Numfor Island.

Numfor Island with an area of 32,580.49 ha is one of the islands with relatively rich natural resources. Administratively, Numfor Island includes sub-districts of West Numfor, Orkeri, East Numfor, Poiru, and Bruyadori with a population of 9,732 people (2,105 households). The island has a relatively flat topography with altitudes ranging from 10 to 205 m asl (Sinery 2002). As one of the isolated regions, Numfor has biodiversity with a fairly high degree of endemicity, especially for the species of birds, so that the island has been called endemic bird areas (Petocz 1989). About 42 species of birds have been found in this island, including a black head parrot, king prawns, long-tailed starling, cockatoos, and bayan. Other wildlife, such as squirrels, bats and coconut crabs have been recorded too. The forest in this island stores various plant species which is home of various wildlife species and orchids, and the place for public hunting activities (Sinery 2015).

The fact shows that the rapid development and population growth encourages increasing forest clearing on this island. According to Sinery (2013), forest in Numfor has been cleared by the community for agriculture, plantation, building materials, and sources of energy (fire woods). Forest conversion in Numfor for agricultural activities, especially the green beans is large.

This study aimed to study the condition of the cuscus population and the carrying capacity of its habitat in the Island of Numfor.

MATERIALS AND METHODS

Area study

Sampling area was set in the middle of the villages of Namber, Kansai, and Yenmanu of Numfor Island, Papua, Indonesia with the area of 105 ha at 134°47'35''E and 0° 55'57''S. The duration of the study was 6 months, from September 2014 to February 2015. Monitoring began by making transects positioned perpendicular to baseline (highway 1000m), parallel to the coastline (crossing the contour line) from West to East in the West Numfor. The baseline was divided proportionally into six transects with an inter-transect distance of 200 m. The first transect was located at the starting point of the baseline (0 m) and the last transect was set at baseline endpoint (1000 m) with elevation 10-17 m asl. Each transect had a length of 3500 m with elevation 191-205 m and a width corresponding to minimum viewing distance (50 m). According to Sinery et al. (2012), the effective width of observation transect for dense forest types is 50 m (25m each side of transect), and we should use a narrower measure which is more effective in the observation of the population.

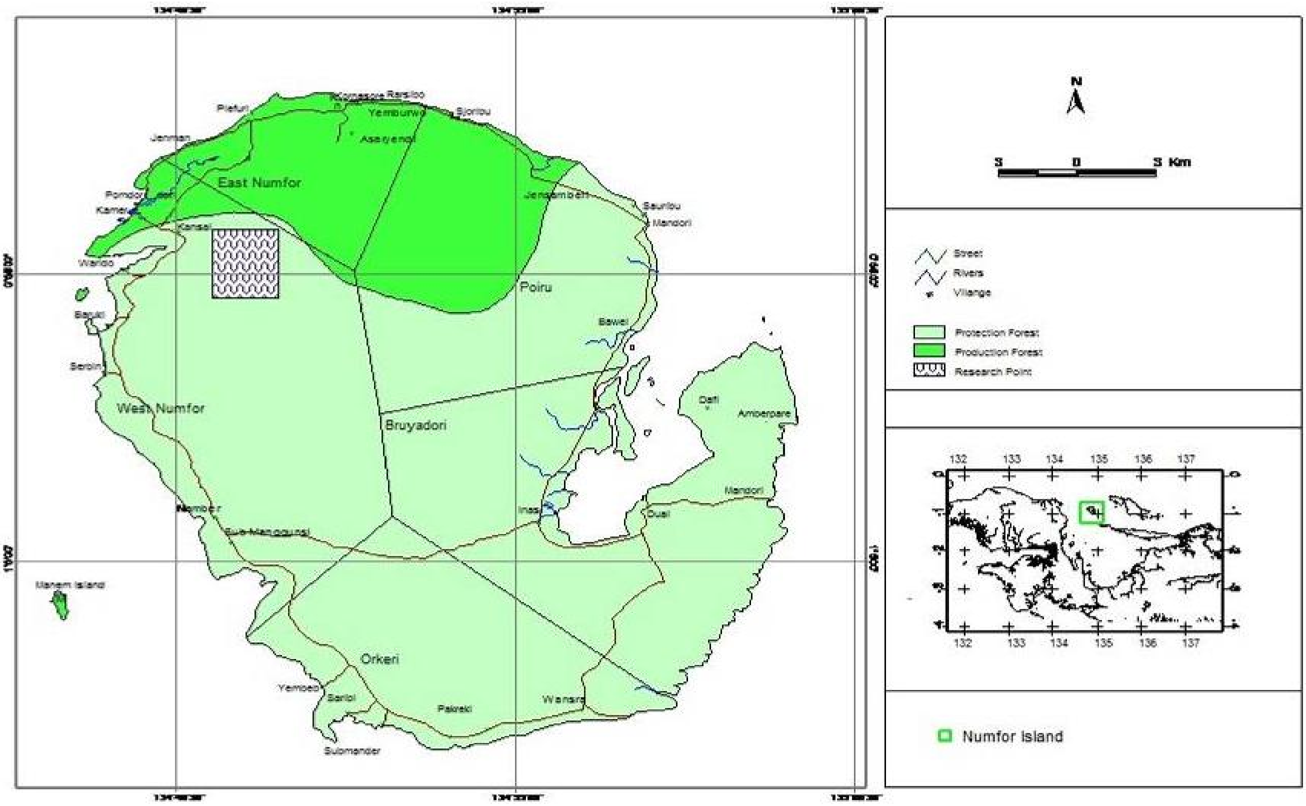

Figure 1. Map of study area in Namber, Kansai, and Yenmanu villages of Numfor Island, Papua, Indonesia

Procedures

The observations on cuscus was performed simultaneously by 6 groups and each group consisted of 3 people (1 taking note, 1 measuring the distance between the object and transect, and 1 identifying the species of cuscus). The monitoring on cuscus was done three times every two months. The survey was conducted in the night (19:00 to 05:00 CET). According to Sinery et al. (2012), daily activity cuscus occurs at night (7:00 p.m. to 05:00 hours CEST) and is influenced by the weather conditions (rain or not) and the moon.

Cuscus encountered was directly identified according to the morphological characters (color of the body, sex, and age), and the morphological measurements were only performed on adult males and females. The body weight of cuscus was expressed in grams (g) and length of organs in millimeters (mm). The observation was conducted in the home ranges of adult males and females, and started since the animal was found until it slept (morning). To facilitate the measurement of range area, every tree that becomes a displacement of cuscus was labeled yellow, and then GPS tracking during the day was done. Data from cuscus owner related to cuscus reproduction, age, disease, threats from poaching, and other information was collected through interviews.

For vegetation inventory, the method used was terraced path (line plot sampling) which included vegetation seedling (high ≤1.5 cm) plots of 2 x 2 m^2, saplings (high ≥1.5cm and diameter <10 cm) plots of 5 x 5 m^2, pole (diameter 10-19 cm) plots of 10 x 10 m^2, and trees (diameter ≥ 20 cm) plots of 20 x 20 m^2. The total transects was 6 with a length of 500 m, the distance between the lines was 200 m, and each transect consisted of 25 plots. The vegetation data was used to identify species of plants eaten by cuscus. The data collection for cuscus feed was done during the day, and this activity was also carried out at night simultaneously with cuscus population monitoring. Identification of plant species referred to the key identification of plants and other supporting literature with the help of the identifier of plant species from the Manokwari herbarium.

The production of fruit as food source for cuscus was estimated using in a single plot with the size of 50 x 50 m (0.25 ha). The estimation of fruit production was done by weighing the fruit located under the trees and expressed in kg/ m^2/season or kg/year. The processing of digital image data was done by analyzing digital image data from Google Maps (2013). The data were then overlaid with Land Cover Map of the region of the Numfor Island for potential forest conditions.

Data analysis

The data were analyzed using quantitative descriptive method and presented in the form of tabulation and images (charts, graphs, and photographs). The data needed, in accordance with the purposes of research, wew as follows: description of the species of cuscus, population conditions, home range, and ecological niches of cuscus. Cuscus morphological data, populations, home range, and ecological niches of cuscus were analyzed using tabulation. Cuscus population density was analyzed by using the equation according to Soegianto (1994), as follow.

$$N = \frac{n\,(2n-1)\,A}{2L\,\acute{O}\,r}$$

N = population density,
n = number of individuals encountered,
A = area of region (plot observations),
L = length of line/transect,
Ór = distance from the point where cuscus found to the line of transect

$$\text{Sampling eror} = \sqrt{Var(N)}$$

$$Var(N) = \left[\frac{n}{(2L/A\ \tau}\right]\left[[\frac{3n-2}{2(n-1)(2L/A\ \tau)} - 1]\right]$$

$$\tau = (2n-1)/\sum r$$

Vegetation conditions, including plants which produced cuscus feed and other plants, were analyzed to estimate the carrying capacity of the habitat. Analyzes were performed using the equation of Importance Value Index (IVI) according to Mueller-Dombois and Ellenberg (1974), as follows.

$$\text{Density} = \frac{\text{Species Individual Total Amount}}{\text{Sample Plot Wide Total Amount}}$$

$$\text{Relative Density} = \frac{\text{Species Density}}{\text{All Species Density}} \text{x100}$$

$$\text{Frequency} = \frac{\text{Plot Total Amount Found the Species}}{\text{All Sample Plot Total Amount}}$$

$$\text{Relative Frequency} = \frac{\text{Species Frequency}}{\text{All Species Frequency}} \text{x100}$$

$$\text{Dominancy} = \frac{\text{The Total Amount of Species Basic Field Wides}}{\text{All Sample Plot Wides}}$$

$$\text{Relative Dominancy} = \frac{\text{Species Dominancy}}{\text{All Species Dominancy}} \text{x100}$$

Importance Value Index (IVI) = Relative Density + Relative Frequency + Relative Dominance

To determine the species diversity of vegetation of which cuscus consumed, as an indicator of habitat's carrying capacity for cuscus population, researchers used species diversity index (H) with the equation according to Shannon and Wiener (1949) in Odum (1993):

$$H = -\sum \left[\frac{ni}{N}\right] \log \left[\frac{ni}{N}\right]$$

Where:
H = diversity index (Shannon index)
ni = Number of individuals of each type of feed
N = Number of individuals of all species feed

Cuscus feed productivity data were used to estimate the carrying capacity of the habitat based on the availability of feed as compared with need of the cuscus population in the study area.

RESULTS AND DISCUSSIONS

Residents know spotted cuscus in Numfor Island as "Rambab" (male) and "Mifan" (female). Based on the characteristics of the fur color, *S. maculatus* in Numfor consists of two variations, namely the overall white to dirty white (beige) cuscus and the spotted cuscus. According to Petocz (1994), Flannery (1994), Sinery (2002), there is only one species of cuscus in Island of Numfor, namely the regular spotted cuscus (*S. maculatus*) which covers two variations (spotted and non-spotted).

The body length (from anterior/head until inferior/tail) of *S. maculatus* male originating from this region ranges between 512 and 550 mm, while its body weight ranges between 4,200 and 5,520 grams. Length and weight of the female body range between 472 and 522 mm and 3,352 and 4,523 grams. Length and weight of the female body range between 472 and 522 mm and 3,352 and 4,523 grams. This research showed that *S. maculatus* located in Numfor have the morphological characters that are not much different from that of *S. maculatus* found in other areas in accordance with the results of previous studies. According to Flannery (1994) the body length of the male of this species is between 518 and 550 mm, while its body weight ranges between 2,300 and 6,000 gram and the length and weight of the female body range between 335 and 385 mm and 3.060 grams. According to Dimomonmau (2000) the body length the male of this species is between 495and 560 mm, while its body weight ranges between 3,700 and 4,500 gram and the length and weight of the female body range between 485 and 590 mm and 4.100 and 5.300 grams. Warmetan (2004) states the body length the male of this species is 520 mm, while its body weight is 4.000 gram and the length and weight of the female body range between 470 and 560 mm and 1.700 and 6.200 grams. According to Jandewoa (2005) the body length the male of this species is between 520 and 610 mm, while its body weight ranges between 3,600 and 4,500 gram and the length and weight of the female body range between 580 and 564 mm and 3.600 and 5.000 grams. The head of male animal is light brown, spreading from the base of the nose through inter parietal bone toward the back (posterior). The dorsal fur is white with light brown spots on the base of the head towards the back with the darker spot color (dark brown).

Figure 1. *Spilocuscus maculatus* from Numfor Island. A. Male/adult, B. Female/adult, C. Male/juvenile, D. Female/juvenile

This color spreads to the side body up to the outside of the legs and hands and ventral boundary. Ventral is light brown (beige) from below the head to the hairy tail's end. The head of female is light brown towards the back (posterior) and forming blackish brown color from the middle of the body (abdomen) to the base of the tail. This color spreads to the body side toward the ventral boundary up to the outside of the legs and arms. The ventral is light brown from under the head to the hairy tail's end with yellowish brown color around the baby bag.

Observational data in the first period, as shown in Table 1, were used as basic data in the cuscus population analysis, because in the calculation of the population, the researchers made no catching and marking (capture-recapture method) for all individuals encountered. Estimation of population was done through direct observation (encounter).

The small population size in the second and third observation period was allegedly due to adaptation to the availability of sources of feed, mainly in the form of fruit, because cuscus often walked to find alternative feed outside the forest as a substitute of the main feed. In addition, this animal allegedly has the instinct to learn from the experience, and thus tends to avoid areas that have been explored by humans. Cuscuses are solitary animals and sensitive to other activities that disturb their habitat, so that they will turn away and look for another safer place.

The structure of the population is tabulated by age class based on the category of growth that includes juvenile, adolescents and adults, as shown in Table 2.

From 17 cuscuses encountered, 9 (52.94%) were male and 8 (47.06%) female. The sex ratio of male and female cuscus was almost equal although the number of males was slightly higher. The cuscus population is quite good to be able to exist in the future because the sex ratio is sufficient for the marriage (breeding). It is known that cuscus is polygamous (change partners) with low reproductive ability.

Based on the number of offsprings produced in every reproductive period, researchers found that from two samples that were found both had 1 child, about 1 to 3-week old in the baby pouch. The body length of the cuscus juvenile ranged between 30 and 75 mm.

Based on these results it can be concluded that the average number of juvenile produced by cuscus is low and generally only one child in every reproductive period. According to Menzies (1991), Spilocuscus generally has low reproductive ability that is only one child in every reproduction period, so that its population is much lower than the genus of *Phalanger*.

In general, the number of individuals of adult cuscus is greater than the numbers of the adolescent and the juvenile. The presence of adult cuscus, both male and female, is a good condition for the conservation aspect because of its potential in the process of reproduction and regeneration of the population. Such conditions may occur when environmental components that affect the cuscus population can be controlled or maintained, such as danger from hunting, food availability, and space. Furthermore, the presence of adolescent and juvenile will be the deciding factor for the existence of the population, especially in the process of growth and development in the future.

Estimation of cuscus population density in study plots area of 105 ha was done through comparison of the number of individuals found in the range of distances between cuscus and transects. The cuscus population density is shown in Table 3.

Table 1. Number of individuals cuscus based on frequency observations

No	Sex	Individual	Amount
Frequency I	Male	9	17
	Female	8	
Frequency II	Male	5	9
	Female	4	
Frequency III	Male	4	7
	Female	3	

Table 2. Number of individuals and age structure of *S. maculatus* in Numfor Island, Papua

Age class	Sex		Individual	Percentage
	Male	Female	(ni)	(%)
Juvenile (<1.5 years)	1	1	2	11,76
Adolescent (1.5-3 years)	2	1	3	17,65
Adult (>3 years)	6	6	12	70,59
Total amount	9	8	17	100,00

Notes: Juvenile (neonatal/infant) = individual newborn (baby) including those not yet weaned (at the parent sac); adolescent = immature physically and sexually, already moving on its own but sometimes still with the parent; adult = had matured both physically and sexually and permanent dentition was complete.

Table 3. The population density of cuscus (*Spilocuscus maculatus*) in Numfor Island, Papua

Number transection	Total individual (n)	Cuscus distance from transection (m)	Population density
1	2	45	
2	6	105	
3	3	52	
4	1	25	
5	3	59	
6	2	36	
Total amount	17	322	0.41

Notes: Transection total long (L) = 21,000 m; transection wides (A) = 105 ha

The results (Table 3) shows that in the area of 105 ha there were 17 individuals cuscus with a population density of 0.41 individual/ ha or every individual had a control of an area of 2.44 ha. This population density was higher than that in the study of Febriadi (2012) which was 0.31 individuals/ha, as well as with population density of regular spotted cuscus in Arfak Mountains Nature Reserve (Sinery, et al. 2012). The situation was influenced by the concentration of individuals in the forest conditions which were still good in this region. Any increase in the number of individuals will be directly proportional to the total area required for activity. The control of space by the population of regularly spotted cuscus was an area of 322 m^2. The number was far greater than that of the population of regularly spotted cuscus in the area of Arfak Mountains Nature Reserve (Sinery et al. 2012). This fact indicates a positive correlation between the condition of space such as the availability of feed and cuscus populations. Monitoring results showed that the presence of cuscus was found more to the center of the area and concentrated in the western part of the island which had a fairly high it altitude with good condition e of vegetation.

The estimation results showed that this population density was higher than that found in the study by Febriadi (2012) which was 0.31 individuals/ha and that in Arfak Mountains according to Sinery et al. (2012). Based on these figures, assuming that the cuscus population spread evenly on the forest that was still good, the number of cuscus in Numfor for forest area that was still good, which was 11597.88 ha, was 4,811 individuals, with the lowest number of 4,689 individuals and the highest number of 4,933 individuals (sampling error/SE = 122).

The cuscus population in this forest is potential to be managed as one of the objects in the development of the area. From the aspect of sustainability, the population can be maintained in the future if there is no more hunting and forest conversion. According to Franklin (1980) at least, 50 individuals are required to maintain genetic diversity in captivity. The number is determined based on experience, that the stock of captive animals can be maintained when the loss of diversity is 2-3% per year, so the 50 individuals will only lost 1% of genetic diversity.

Consider these assumptions, the cuscus populations in Numfor Island is rated worrying in the future. The condition arises because in the wild cuscuses do more activities such as eating, mating, and other activities, but the limited food resources and increase in the rate of forest conversion and illegal hunting will lead to the decreasing population of this species.

The estimation minimum area of home range for each individual of cuscus was 2,000 m^2 and the maximum was 2,700 m^2, so that 17 individuals of cuscus who lived in this area will require a minimum of 86,000 m^2 of land (8.6 ha) and a maximum of 116,100 m^2 (11.61 ha). According to Sinery et al. (2012), the minimum home range area of individual cuscus in the open population such as in Arfak Mountains Nature Reserve is 1,225 m^2 and the maximum is 2,400 m^2. These conditions indicate that the carrying capacity of the habitat of cuscus in forested areas in Numfor Island is still good enough for the existing population. Modeling based on Soegianto's formula (1994), estimated that as many as 43 individuals of cuscus lived in the effective area in Numfor, Papua.

The development of these animals introduced is quite good and can spread to different areas, but the presence of high mountains caused the concentration of the population in some areas. These conditions do not become an obstacle in the growth and development of populations of native mammals such as cuscus, like conditions of cuscus populations in the island of Numfor.

Cuscus is a nocturnal animal that generally performs activities (foraging, mating, and playing) at night. The results of observation specifically to the example of male and female adult cuscuses from Numfor Island indicated that the active time of cuscuses in Numfor was the time at which cuscuses start doing the activity until they were back to rest/ hide, i.e., from 20:00 to 05:00 CET. The highest frequency of activity of cuscus was eating, followed by urination and defecation.

Time and frequency of eating *S. maculatus* were quite high because the proportion of the required amount of feed intake is in accordance with the size of the body, so that the amount of feed and meal times is relatively high. Based on the level of palatability, cuscus tends to consume the types of feed in the form of ripe fruit. Allegedly, in addition to having sweet taste, ripe fruit also contains much water to ease the digestion process.

Active time of cuscus in Numfor is influenced by rainy conditions and the moon. In both conditions, cuscus will usually do the activities. At the time of rain (at night), cuscus does not perform activities and generally rests and after rain cuscus started to do the activity again. This is because cuscus utilizes fresh feed material with high water content. In addition, when the light of the moon, cuscus rarely do activities, because it avoids other animal species (predators). Cuscus is usually found in the conditions after raining and when the moon shines brightly (Sinery et al. 2012). After the rain, cuscus feed themselves by eating part of the new shoots and performs other activities. In addition, when the moon shines brightly, cuscus takes advantage of moonlight to look for sources of feed and to find partners. Cuscuses are active at night and rest during the day in the grove, holes in the ground, or in the rock crevice. Sometimes this animal rests (sleeps), bends over and hugs branches or tree trunks which are not dense or open (Flannery 1994). The results showed that cuscus is generally found in certain types of location.

According to Sinery (2013), cuscus is a mammal that has a fairly small territory (4 individuals per ha of forest which is still good) and is polygamous with the life span which can reach 13 years, but in captivity, this animal can live up to 15 years. The distribution of age classes of cuscus is the same as in other mammals, which consists of juvenile class (< 1.5 years), adolescent class (1.5-3 years), and adult class (> 3 years). This animal has a low reproductive capacity, ie one parent only produces one child in every time period of reproduction, with the reproduction frequency of at least 1 time a year. According to Sinery et al. (2012), lower reproductive ability of cuscus is due to the long span of time of feeding or caring for the infant (in the baby bag), namely 6 months, despite having a relatively short gestation period (1.5-2 moon).

Under these conditions, the estimated population growth for the first year after the study is a minimum of 49 individuals, consisting of 43 beginning individuals and 6 new individuals, whereas in the second year the population will grow to 56 individuals consisting of 49 beginning individuals plus 7 new individuals. The population will be greatly developed in the next few years if the component of the environment are maintained or does not experience significant changes, including hunting.

During the execution of the study, researchers did not find mating activities between cuscuses observed. This situation is thought to be caused by the fact that the study was conducted during the dry season which was not mating season for cuscus; generally cuscuses perform mating activity during the rainy season. This is related with the productivity of the cuscus feed. In the rainy season, cuscus feed productivity is high; the feed was abundant, thus supporting the process of reproduction. From a number of cuscus which was found having 1-3 week old babies in their baby pouch/holster, it can be presumed that the mating season had ended. Sinery (2002) mentions that mating activities of cuscus will increase during the rainy season because the availability of food resources in the fruit season. Mating begins with the issuance of a male voice as a signal to females and other males which were in the area of territory. If there is reply (voice) from female cuscus, then the male cuscus will move closer to the female while making the sound. This process takes approximately 15-35 minutes before the mating. Monitoring result showed that at a distance up to 120 m, cuscus was still be able to communicate with each other by issuing sound.

The natural mortality rate of cuscus is lower than its birth rate. The condition is influenced by a relatively long life span of cuscus and also by the absence of natural predators that affect the population of this animal. Population changes will occur with the death of as many as 12 individual cuscuses from age class of adult, and population growth will take place on individual cuscuses from age classes of adolescent and juvenile which later will become mature individuals. Natural mortality of individual's cuscus does not significantly affect the presence of cuscus populations in this region if the other components of the environment can be maintained or does not experience significant changes, including hunting.

The forest area of Numfor Island has different level of plant vegetation. There are 31 species of trees found where first three species with highest important value index (IVI) are *Horsfieldia laevigata* (31.11%), *Canarium hirsutum* (23.99%) and *Palaquium amboinensis* (20.30%). At the poles, there are 27 species of plant where three species with highest IVI are *Horsfieldia laevigata* (21.66%), *Canarium hirsutum* (20.36%) and *Mallotus philippinensis* (19.44%). At the saplings, there are 24 species of plant where three species with highest IVI are *Palaquium lobianum* (33.29%), *Aglaia* sp. (23.45%) and *Lepiniopsis ternatensis* (23.39%). At the seedling, there are 25 species of plant where three species with highest IVI are *Horsfieldia laevigata* (19.52%), *Mallotus philippinensis* (14.20%) and

Pimelodendron amboinicum (11.88%) (The complete data is not shown).

Source of cuscus feed in the form of fruit-bearing plants (with a diameter of 10 cm or more) were found as many as 13 species of 10 families with a diversity index of 2.43 (moderate criteria) and productivity of 0,75 kg/ season/ ha. This situation illustrated the potential limitations of the feed so that the fulfillment of the feed shortage was done outside the forest area which was still good (secondary forest and community farm). The average of home range area for each of cuscus was 2000-2700 m^2 and the existing population required a minimum area of 9,622,024 m^2 area (962.20 ha) and maximum area of 12,989,732 m^2 (1298.97 ha) which indicated the presence of carrying capacity of habitat which was good enough. According to Saragih et al. (2010), there were 19 plant species identified as cuscus diets in natural habitat. They prefer fruits with astringent and sour taste as well as high crude fiber and low fat.

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REFERENCES

Dimomonmau P. 2000. Exploration of Cuscus at Moor Island Napan Weinami Sub-District, District of Nabire. [Hon. Thesis]. Faculty of Agriculture, University of Cenderawasih, Manokwari. [Indonesian].

Febriadi I. 2012. Study about Habitat and Estimation of Spotted Cuscus Population (*Spilocuscus maculatus*) at Numfor Island, Biak Numfor District. University of Mulawarman, Samarinda. [Indonesian].

Flannery T. 1994. Possums of the World. A Monograph of the Phalangeroidea. Geo Production Pty Ltd, Australia.

Frangklin IR 1980. Evalutionary change in small populations. In: Soule ME, Wilcox BA (eds). Conservation Biology and Evolutionary Ecological Perspective. Sinauer, Sanderland, MA.

Menzies JI. 1991. A Handbook of New Guinea Marsupials and Monotermes. Kristen Press Inc. Papua New Guinea, Madang

Mueller-Dombois D, Ellenberg H. 1974. Aims and Method of Vegetation Ecology. John Willey and Sons, Toronto.

Odum P. 1993. Fundamentals of Ecology. 3rd ed. Gajah Mada University Press, Yogyakarta [Indonesian].

Petocz RG. 1994. Terrestrial Mamalia of Irian Jaya. PT Gramedia Pustaka Utama, Jakarta. [Indonesian]

Saragih EW, Sadsoeitoeboen MJ, Pattiselanno F. 2010. The diet of spotted cuscus (*Spilocuscus maculatus*) in natural and captivity habitat. Nusantara Bioscience 2: 78-83.

Sinery A. 2002. Exploration of Cuscus at Numfor Island, District of Biak Numfor. [Hon. Thesis]. Faculty of Forestry, University of Papua, Manokwari. [Indonesian].

Sinery A. 2013. Management Strategies of Cuscus (*Spilocuscus maculatus*) on the Numfor Island, Papua Province. University of Mulawarman, Samarinda. [Indonesian].

Sinery A. 2015. Management Strategies of Cuscus on the Numfor Island. Deeppublish, Yogyakarta. [Indonesian]

Sinery A, Boer C, Farida W. 2012. The population condition and food availability of cuscus in Arfak Mountains Nature Reserve, West Papua. Biodiversitas 13 (2): 86-91

Soegianto. 1994. Quantitative Ecology: Analysis Methods of Population and Community. Usaha Nasional, Surabaya. [Indonesian]

Warmetan H. 2004. Exploration of Cuscus Species at Central Yapen Nature Reserve and its Surroundings, South Yapen Sub district, District of Yapen Waropen. [Hon. Thesis]. Faculty of Forestry, University of Papua, Manokwari. [Indonesian]

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Review: Climate-induced hydrological changes and the ecology of tropical freshwater biota

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Abstract. *Sunardi, Wiegleb G. 2016. Climate-induced hydrological changes and the ecology of tropical freshwater biota. Biodiversitas 17: 322-331.* Climate change is believed to pose adverse effects to biodiversity of aquatic systems, with no exception of those in tropical areas. However, next few decades species extinction is suggested as dark future as we lack researches uncovering how climate change threats the aquatic biota. Unluckily, the tropical freshwater systems are expected to suffer more severe impacts of climate change, from heavy floods or extended drought than do the boreal areas. A comprehensive understanding of biota' performance in face of climatic pressures, will guide the further necessary researches. This paper presents a review on the available researches addressing ecological effects of the most influential climatic parameter in tropic area, the hydrological regime, on freshwater biota. The research reveal that the extreme water fluctuations induced by climate change have negatively affected the performance of freshwater biota. In the next few decades, climate change seems to remain as one of the main threats for freshwater ecosystems, and is responsible for the lost of its biodiversity.

Keywords: Biota performance, climate change, drought, flood, tropical freshwater

INTRODUCTION

Global change has been shown and predicted to have major effects on biodiversity at global, regional, and local scales, although global change constitutes a number of different forms of anthropogenic impacts (Sala et al. 2000), including land use alterations, nitrogen deposition, and invasions of exotic species. In other words, changes in climate and climate variability would, somehow, significantly affect natural ecosystems, and may pose additional threats to the already-stressed ecosystems. Furthermore, the effect of future climate change on biodiversity has been predicted to be unprecedented as well, with 15-37% of terrestrial species possibly becoming extinct due to climate change alone in the next 50 years (Thomas et al. 2004), and a similarly dark future has been suggested for freshwater species in the next few decades (Xenopoulos et al. 2005).

Freshwater ecosystems are vulnerable to global change. Important global climate variables that are expected to change in the next decades with respect to freshwater habitat are air temperature and precipitation (Mitchell et al. 1990). Changes in these variables will affect water temperature, water quantity and water quality variables of freshwater environments which are the three primary linkages between climate and freshwater organisms (Regier and Meisner 1990).

Climate change pushes species out of their ecological synchrony and environmental landscape. This influences not only species distributions or community structure, but also the services they provide to ecosystems. Understanding how species' performances change along with the environmental gradients is worthwhile. This is particularly important in aquatic systems, where shifts in habitat quality associated with environmental perturbations threaten the integrity of aquatic biota (Strayer et al. 2004).

The magnitude of impacts and responses of aquatic ecosystems, however, differ between boreal and tropical area with regard to the global change. This is due to climatic variation between the two areas. In the tropics, the annual variation in air temperature is smaller, but there is a large and predictable annual variation in precipitation (Lowe-McConnell 1987). The seasonal precipitation cycle produces wide ranges in river flow rates and water levels, which directly alters the amount of freshwater habitat available for biota and indirectly alters many critical characteristics of that habitat (eg, O_2 levels, turbidity, food availability, etc.).

The increase in global temperature, nevertheless, will lead to a more vigorous hydrological cycle, with changes in precipitation and evapotranspiration rates regionally variable. Warming accelerates land-surface drying as heat goes into evaporation of moisture, and this increases the potential incidence and severity of droughts, which has been observed in many places worldwide (Dai et al. 2004). In tropical systems, evaporation and evapotranspiration often already exceed precipitation in the dry season (Irion and Junk 1997). In weather systems, convergence of increased water vapor leads to more intense precipitation and the risk of heavy rain and snow events, but may also lead to reductions in duration and/or frequency of rain events, given that total amounts do not change much (see Trenberth 2005). In such case, the tropical areas are expected to suffer more severe impacts of climate change, from heavy floods or extended drought than do the boreal areas.

Despite the increase of research on the topic, we lack a comprehensive understanding of the consequences of extreme precipitation fluctuation (as distinct from seasonal climate) on the ecology of tropical freshwater biota. The aim of the present article is to complement the existing information by reviewing current knowledge of climate change effects, paying particular attention on hydrological regime, on species' performances in tropical freshwater.

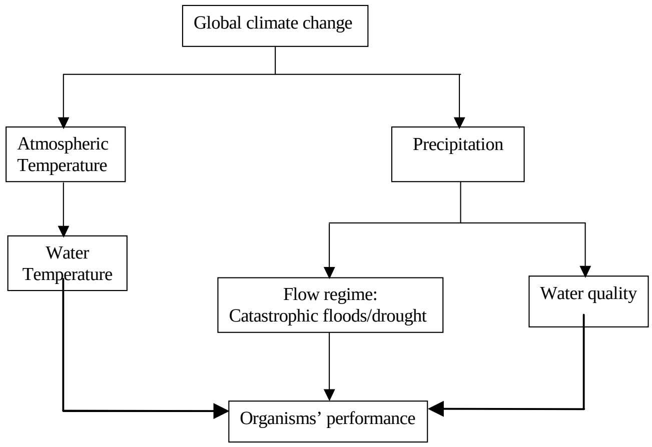

Figure 1. Key climate-related parameters affecting global freshwater organisms

KEY CLIMATE-RELATED PARAMETERS

Aquatic ecosystems are vulnerable to changes in quantity and quality of their water supply, and it is expected that climate change will have a pronounced effect on global freshwater through elevated temperature and alterations in hydrological regimes with great global variability. As a matter of fact, aquatic organisms have to deal with a wide variety of environmental factors simultaneously; however, temperature, water quantity and water quality are regarded as the most fundamental climate-related factors (Figure 1).

In tropical regions, fluctuations in rainfall often represent the strongest seasonal variation, and change the environment to an extent comparable to temperature in temperate areas (Jacobsen and Encalada 1998). Variation in rainfall that affects stream discharge is among the most important sources of natural disturbances (Taylor et al. 1996). Flow regimes range from spates or peak flows during the rainy season through to zero flow in the dry season. The shape and size of river channels, the distribution of riffle and pool habitats, and the stability of the substrate are all largely determined by the interaction between the flow regime and local geology and landform (Newbury and Gaboury 1993). In turn, this complex interaction between flows and physical habitat becomes the major determinant of the distribution, abundance, and diversity of stream and river organisms (Nilsson and Svedmark 2002).

It is reported that effects of climatic variability on hydrology can be particularly devastating, causing changes in water chemistry, stream size, water temperature, streambed structure, streambed substrate and stream flow (see Medeiros and Maltchik 2001; Starks et al. 2014). Hence, such environmental variation can dramatically alter the living conditions and aquatic habitats within the water, affecting much of the aquatic fauna inhabiting streams (Moyle and Vondracek 1985; Taylor and Warren 2001). Biota, in particular those living in vegetated riparian areas, respond differentially to water-level dynamics, either directly or indirectly. Direct effects on the biological communities include physical disturbance by wave activity. Indirect effects include the reworking of substrates (which can enhance or restrict colonization by vegetation, and which in turn depends on silt accumulation to establish roots), and alteration of habitats suitable for aquatic flora and fauna. Any significant changes in the water level of a lake will affect not only the physical processes, but also the biological productivity. Therefore, water level fluctuation might have an overriding effect on Lake Ecosystem health and integrity (Furey et al. 2006; Leira and Cantonati 2008).

In addition, ambient temperature is undoubtedly among the most important for ectotherm ontogeny because of its pervasive effects on biological rate processes, altering nearly all physiological functions (Deutsch et al. 2008; Kingsolver 2009). It has been known for more than a century that biochemical reaction rates, metabolic rates, and nearly all other rates of biological activity increase exponentially with temperature. In tropical area, however, seasonal temperature variability is small (Meisner and Shuter 1992), and it seems does not pose profound effects on organism' performance as does in boreal areas. Therefore, this paper will not deal with the effects of inclined temperature on freshwater organisms.

IMPACTS OF FLOOD ON BIOTA

Water flow in aquatic ecosystem, particularly lotic, is often subject to a high short-term variability. This short-term variability may be caused by high precipitation events in the catchment. Hydroperiod and flood frequency (Medley and Havel 2007), as well as high water flow (Godlewska et al. 2003) have been recognized as significant factors structuring communities.

This suggests that hydrology has a significant effect on both species richness and community structure of planktonic communities in rivers, streams, floodplain ponds and lakes. Flood events often act as disturbances that interrupt the succession in planktonic community (Mulyaert et al. 2001); even a short flood event may have effects on the planktonic ecosystem that last for weeks.

Nevertheless, the research showed that the responses of the planktonic communities differ from site to site showing its dependence to other biotic and abiotic factors. Some groups of plankton may respond positively or negatively, while some others do not show clear response; e.g. Chlorophyll a concentration and abundance of bacteria, oligotrich ciliates and crustacean zooplankton did not respond clearly to the flood event (Mulyaert and Vyverman 2006).

It is evident that flood waters transport large amounts of suspended solids and nutrients into lakes. An increase in the concentration of suspended matter in a lake leads to greater light attenuation and thus to a decrease in primary production (Lloyd et al. 1987). High concentrations of abiogenic turbidity can light-limit phytoplankton photosynthesis and therefore restrict biomass development (Holst and Dokulil 1987; Dokulil 1994). In most cases, an increase in nutrient input causes a consequent rapid increase in algal biomass. This is especially true of oligotrophic lakes (Thomas 1973). However, in several lakes, an impoverishment of the algal standing stock and a decrease in the phosphorus concentration are observed following events involving the discharge of suspended sediment into the lake, despite the fact that the turbid inflow transports a large load of particulate phosphorus into the lake (Sampl 1986). This is due to the phytoplankton is sedimented out after flood event, as the phytoplankters and suspended matter do coagulate efficiently (Elber and Schanz 1990), result in a decrease in primary production and phytoplankton biomass. Additionally, the negative responses can occur under condition of prolonged floods because the flood pulse can have dilution effects on nutrient, so that a significantly lower phytoplankton biomass is established (Keckeis et al. 2003; Mihaljević et al. 2009).

Grobbelaar (1992) and Dokulil (1994) suggest that the ratio of mixing to euphotic depth is one of the most important factors affecting overall productivity mainly in turbid waters. In these water bodies the aphotic portion is large compared to the euphotic zone and determines the relative time spent in the dark by the algae. The ratio of euphotic to mixing depth is considered to be the most important factor affecting overall productivity, while nutrients are of secondary importance only. Nutrients influence productivity only when a more favorable underwater light regime prevails, such as prior to the flooding. Energy available for phytoplankton growth is strongly regulated by underwater light availability which depends on the critical mixing depth, fluctuating light intensities and algal circulation patterns.

Godlewska et al. (2003) reported that shifts in phytoplankton distribution (from hypolimnion into the whole water column) and species composition were observed. In one case, this is because of high water flow eliminated large species of cladocerans and copepods, and favored development of rotifers; while in another case, plankton animals may concentrate at different depths in the water column before and after the flood because they are transported to different locations by water currents. Dirnberger and Threlkeld (1986) found that during the period of flooding most zooplankton populations declined and the distributions of remaining individuals deepened; greatest population losses occurred between 0 and 7m depths where most zooplankton had been concentrated prior to the flood. The changes in distribution may be results from search for the right compromise between two conflicting demands: to maximize feeding and to avoid predators (Gliwicz 1986).

On the other hand, floods can have positive impacts on planktonic communities. Dispersal among patches is important to the long-term viability of species in metapopulations, and flood connections can enhance the viability of certain species (Jenkins 1995). Immigrants with differential competitive ability can be introduced into communities which releases local communities from competitive exclusion, shifts local dynamics, and enhance long-term persistence (Leibold et al. 2004). Flooding can introduce new species (Havel et al. 2000), but intense flooding can wash out entire populations (Baranyi et al. 2002). Turbulence, which increases in high flood, can also have direct effects on reduced grazing rate (Miquelis et al. 1998) and food selectivity of zooplankton (Vanderploeg 1994).

In river, increase in discharge leads to increased drifts. Downstream invertebrate drift is a normal feature in lotic systems and facilitates the recolonization of denuded areas of a stream (Brittain and Eikeland 1988). However, disturbances notably flooding have an important role in regulating the distribution, abundance and coexistence of macroinvertebrate (Resh et al. 1988). Significant reductions in macroinvertebrate density have been recorded after scouring floods (Robinson et al. 2004). While moderate disturbance may encourage diversity in many systems (see Smith and Brown 2006). In regulated river reaches below dams, it was reported that sudden increases in flow can cause catastrophic downstream drift (Layzer et al. 1989).

The most frequently reported effect of sedimentation associated with floods is an increase in drift density (e.g., Doeg and Milledge 1991; Suren and Jowett 2001), which may account for the loss of individuals and species in response to a loss of suitable habitat and changes to the food web (Rabeni et al. 2005). In addition, high water flow can also be acute for invertebrate; Doeg and Koehn (1994) identified a reduction in total number of benthic macroinvertebrate taxa and abundance, after a flushing event that increased suspended solid concentration. Pruitt et al. (2001) reported that total suspended solids concentrations greater than 284 mg L^{-1} resulted in biological impairment of invertebrate communities, while a concentration of 58 mg L^{-1} or less during storm flow provided an adequate margin of safety and were protective of aquatic invertebrates. Variability in tolerance to suspended solid could be explained by sediment particle characteristics, water temperature, species differences and other stressor that might have synergistic effects (Bash et al. 2001). In addition, the degree of turbidity associated with flood events has been known to affect the response of benthic invertebrate to flood. Present study report that the magnitude of response of macroinvertebrate community to the flood is most severe in the non-turbid, upper main river and tributaries where benthos community is dominated by the most sensitive Ephemeroptera, Plecoptera and Trichoptera species (Miserendino 2009).

Sediment transport and deposition are processes that are a natural part of the stream environment and play a major role in structuring stream habitats. However, streams are vulnerable to increased sedimentation brought about by altered land uses in the surrounding catchments, with detrimental effects on benthic stream communities. The exacerbation of erosion and sedimentation may be particularly striking in the tropics (Newcombe and MacDonald 1991; Wood and Armitage 1997) where extreme climatic conditions can prevail and aquatic systems are increasingly under threat. However, ways of how sediment affects aquatic ecosystems are various depending on the shape, size and density of the particles; their potential for microbial colonization; the velocity, temperature, flow and turbulence of the water (Hellawell 1986); and the presence of associated factors, such as nutrients (Lemly 1982). Increased levels of sedimentation can bury macroinvertebrates and their habitats (Wood et al. 2001; Wood et al. 2005) leading to shifts in the structure of the habitat and its associated fauna (e.g., Ryder 1989).

Sedimentation has been shown to induce behavioral response of macroinvertebrate, where macroinvertebrates actively avoid substratum coated with excessive fine sediment (McClelland and Brusven 1980; Connolly and Pearson 2007). It is predicted that the upland fauna would be more sensitive to sedimentation because it would naturally experience lower exposure to sedimentation than the lowland fauna; such different response has been demonstrated in both, the mesocosm and in-situ experiments (Connolly and Pearson 2007). Fine sediment deposition can cause shifts in the community structure through the loss of sensitive species, particularly those requiring coarse substrata for attachment or feeding, and through increases in the abundance of burrowing animals, such as some Chironomidae and Oligochaeta (Hellawell 1986). Sedimentation can also affect the filter feeders, scrapers and collector through ingestion of inorganic when feeding, with a negative effect on nutrition and growth (Ryder 1989). Fine silt deposit trapped by periphyton can reduce photosynthesis (Yamada and Nakamura 2002), and thus algal availability to grazers (Donohue and Irvine 2004). There may also be indirect effects of sedimentation transmitted through top-down effects of predators, such as fish and crayfish (Schofield et al. 2004). However, several studies have shown that changes in abundance rather than diversity are commonly associated with sedimentation (e.g., Lenat et al. 1981, Wagener and LaPerriere 1985).

In large lenthic ecosystem, water level fluctuation plays a role more importantly than does flow regime. Such effects on ecosystems are very complex, and the biological effects in lakes are greatest in shallow water and littoral areas, where even small changes in water levels can result in the conversion of large areas of a standing-water environment in air exposed habitats (Leira and Cantonati 2008). The potential effects of lake-level changes have been judged by impacts at the physical level, i.e. transparency, sedimentation patterns, erosion; at the species level, i.e. target species, and by indicators at the ecosystem level, i.e. carrying capacity and biodiversity (Leira and Cantonati 2008). The fluctuation of water level can alter the lake morphometry and transform the characteristics of the sedimentation zone (erosion, transportation, accumulation; Håkanson 1977), thereby water-level drawdown enhances sediment erosion and has the potential to fundamentally change littoral sediment and biogeochemical characteristics (Furey et al. 2004). The water level fluctuation cause changes in the littoral area available for benthic macroinvertebrate. The lost of littoral vegetation due to inundation or the establishment of emergent species from seed during low water is always accompanied by changes in invertebrates and amphibians (Eulis et al. 2004).

The functioning of shallow lakes and floodplains is supposedly very sensitive to water level changes. Moreover, littoral plant communities in shallow lakes located in semi-arid to arid regions appear to be particularly susceptible to water-level fluctuations (Beklioglu et al. 2006). High water level can facilitate the expansion of submerged vegetation that from which some benthic invertebrates take benefits.

The effects of water level fluctuation on benthic macroinvertebrate are very subtle, both directly and indirectly (Leira and Cantonati 2008). Direct effects on invertebrates include changes in the structure and dynamic of taxa that cannot withstand dry periods which subsequently lead to a limiting of their distribution by low water levels (Rossa and Bonecker 2003; Bowers and de Szalay 2004. Meanwhile, indirect effects are generally through alteration of habitats (e.g. substrate composition, periphyton growth, resuspension versus sedimentation). Particularly important are those habitats with cobbles and macrophytes that provide an extensive suitable habitat for periphytic algae which are their major food source, egg-laying and tube building, and also provide a refuge from predation (Scheifhacken et al. 2007). However, different zooplankton groups seem to show different sensibility to water level and are distinctly affected by floods (Ortega-Mayagoitia et al. 2000).

Fishes are particularly susceptible to changes in environmental conditions. Flow plays a profound role in the lives of fish with critical life events linked to flow regime (see Bunn et al. 2002; Janáč et al. 2010). Numerous studies have shown that changes in stream flow associated with extreme variations in precipitation can alter fish communities and habitats. Many fish species display a preference for particular types of habitat such as pools, riffles, or backwater areas. While habitat structure is generally considered to be a good predictor of fish assemblage structure, so habitat instability associated with variations in stream flow will disturb the fish community residing within it (Gelwick et al. 2001). Therefore, sudden or long term variations in discharge, such as from extended droughts or large storms, can be particularly devastating, causing changes in water chemistry, stream size, water temperature, streambed structure, streambed substrate and stream flow (Medeiros and Maltchik 2001). Extreme discharge associated with storm events can dramatically alter channel morphology and benthic habitat, which may have significant effects on fish community. Such environmental alteration can dramatically alter the living conditions and aquatic habitats within the water, affecting much of the aquatic fauna inhabiting streams (Moyle and Vondracek 1985; Taylor and Warren 2001).

Crosa et al. (2009) reported that a large volume of sediment associated with reservoir flushing has decreased fish density and biomass; a greater mortality recorded for juveniles will likely result in long-term impairment of the age-structures fish populations. Juveniles' mortality was also reported due to flushing (Garric et al. 1990), while lower effect such as damage of gill epithelium was observed (Petz-Glechner et al. 2003). High level of sediment can cause mortality in fish (Newcombe and MacDonald 1991), in particular sensitive specimen (Lloyd 1987), whilst prolonged lower levels of suspended solids and turbidity is the cause of chronic sublethal effects, such as reduced weight since individuals are not able to feed efficiently (Sigler et al. 1984). Stream fish can become stranded on gravel bars or trapped in off-channel habitats during rapid flow decreases. Susceptibility to stranding is a function of behavioral response to changing flows, and this varies with species, body size, water temperature, time of year and day, substrate characteristics, and the rate of flow reductions (Bradford 1997). However, mature fish may be able to shift into temporarily suitable habitats to compensate for periodic reductions in the quality or availability of habitat (Bunt et al. 1999).

Many variables known to affect fish, including sediment load, pH, dissolved oxygen, and various nutrients, are frequently changed during increased flow associated with storm events (Winemiller et al. 2000, Ostrand and Wilde 2002). Winemiller et al. (2000) showed that diversity and abundance of freshwater fish populations positively correlate with total dissolved nitrogen, nutrient concentration, and food resources in the water. Gelwick et al. (2001) found positive correlations between common measures of assemblage structure (diversity and abundance) and dissolved oxygen and salinity. However, in other case, the degree of change in chemical composition observed was minor (Keaton et al. 2005).

On the other hand, extreme storm events that lead to flooding can introduce new species into assemblages and create new habitats (Winemiller et al. 2000), or increase availability of shelter and allochthonous food sources, and should provide water enrichment with nutrients carried from adjacent areas or present in flooded organic or inorganic material (Agostinho et al. 2004). Nevertheless, floods can dilute the aquatic biota by increasing water depth, reducing the availability of food resources, especially mobile ones. As a result, the hydrological cycle affect interspecific relations, particularly predation and competition. The flooding regime seems to favor piscivores, since floods are associated with the reproductive success of many of their prey species. However, due to their diluting effect, floods also reduce the density of prey species. In addition, increased shelter may also reduce prey availability (Luz-Agostinho et al. 2008; Janác et al. 2010).

DROUGHT AND FRESHWATER BIOTA

In contrast with the facts on flood effects, there have been relatively few studies of stream faunal dynamics after droughts (see Lake 2000). If floods amplify hydrological connectivity, conversely droughts in streams disrupt hydrological connectivity. With the onset of drought, falling water levels reduce the habitat availability for most aquatic biota, exposing the marginal areas (Stanley et al. 1997), breaking surface water contact between the stream and its riparian zone, and reducing the hydraulic heterogeneity of flow. Changing water levels are another stressor on lake and littoral communities. Water level fluctuations in lakes are dominant forces controlling the functioning of lacustrine ecosystems (Wilcox and Meeker 1992; Poff et al. 1997). It plays an important role in the lake's physical processes (e.g. the geomorphologic processes of erosion and sedimentation) (Leira and Cantonati 2008).With falling water levels, lentic habitats may increase in extent and new types of habitats may be created, that favor some species. As drying proceeds, the threshold of cessation of surface flow is reached.

Droughts can have direct and indirect impacts on stream biota. Direct impacts are those caused by loss of water and flow, and habitat reduction and reconfiguration, whereas indirect impacts are those associated with changes in phenomena such as interspecific interactions, especially predation and competition, and the nature of food resources. The direct and indirect impacts of drought can greatly reduce population densities, species richness and alter life-history schedules, species composition, patterns of abundance, type and strength of biotic interactions (predation and competition), food resources, trophic structure and ecosystem processes. Resh (1992) found that a severe drought has eliminated a population of the caddisfly, *Gumaga nigricula* (McL.).

Following water flow reduction, many aquatic biota cannot move and become trapped and concentrated in lingering pools (Boulton et al. 1992; Matthews 1998; Matthews and Marsh-Matthews 2003). Stream connectivity becomes differentially disrupted by the cessation of upstream-downstream longitudinal links, and the weakening of lateral links between the stream channel and riparian zone (this includes the flood plain) and vertical links between the surface, hyporheic zone and groundwater. In terms of available data on responses to drought in flowing waters, more information is available on invertebrates and fish than on micro-and macroalgae, macrophytes and riparian plants (e.g. Peterson 1996; Matthews 1998; Holmes 1999). During drought, flow may cease and some stretches of river consist of isolated pools, where biota become concentrated in pools and such pools may harbor very high densities of invertebrates (e.g. Boulton and Lake 1992; Miller and Golladay 1996) and of fish (e.g. Matthews 1998; Labbe and Fausch 2000; Matthews and Marsh-Matthews 2003). Different isolated pools may harbor different assemblages of biota and with time, such pools can diverge from each other in their community structure (Power et al. 1985; Meyerhoff and Lind 1987; Stanley et al. 1997). In reservoirs, during extreme drawdown events, the water quality changes significantly. In particular, drawdown events cause changes to nutrient dynamics and ultimately lead to periods of high algal biomass; in one case leading to the formation of a potentially toxic cyanobacterial bloom (Naselli-Flores 2003).

Physicochemical conditions shift rapidly with flow cessation and undoubtedly have adverse effects on the benthos. When flow decreases, the capacity of the stream to transport organic matter decreases and cause an increase in detritus coverage. Further, flow cessation lead to watercourse fragmentation into a series of isolated pools and caused an abrupt change in physicochemical conditions that imposed a threshold on the ecosystem (Acuna 2005). Organic matter or detritus and sediments are accumulated in pools, and reduce physical reaeration causing a decrease in DO concentrations and an increase in nutrient concentrations, typical responses to pool isolation (Stanley et al. 1997; Caruso 2002).

On the onset of drought, tolerant species can grow rapidly leading to a density peak soon after flow cessation, but this density peak dropped rapidly in response to changes caused by flow cessation (Boulton and Lake 1992). The most probable causes of these adverse effects are deoxygenation (Stanley et al. 1997; Labbe and Fausch 2000) and toxicity of certain leachates from leaf decomposition (Towns 1991; Boulton and Lake 1990, 1992; Chergui et al. 1997). As streams dry and the surface water shrinks to unshaded pools, the build-up of nutrients, high temperatures and solar radiation can precipitate blooms of algae (Freeman et al. 1994; Dahm et al. 2003; Winder et al. 2012). The algae may create large diel changes in oxygen concentration (Matthews 1998). As water flow and volumes decrease, water temperatures may start to rise (Acuna 2005) and can become lethal for aquatic biota such as fish (Matthews 1998). Simultaneously, especially in pools, deoxygenation may occur, threatening biota (Stanley et al. 1997; Labbe and Fausch 2000; Golladay et al. 2002).

Low discharge conditions during drought can limit habitat resources and mobility (Lohr and Fausch 1997) and can have marked effects on community composition, diversity, size structure of populations, spawning, and recruitment of fish (see Poff et al. 2001; Lake 2003; Ledger et al. 2012). Droughts also results in intense aggregations of fish and possible competition for food and/or space, because fish are confined to small areas and usually at considerably higher densities, thus potentially increasing competition. Poff and Ward (1989) considered that such biotic interactions contribute relatively little to community structure in rivers. However, during periods of low flow, and the attendant reduction of habitat area or volume, biotic interactions could become temporarily important (Cowx et al. 1984; Matthews 1988). Fish population structure can also be changed by drought (Resh et al. 2013), reducing spawning and recruitment (Cowx et al. 1984; Davies et al. 1988). Pires et al. (1999) note that some species are well adapted to natural droughts, however major native species are considered to be more sensitive to stream fragmentation and hydrological alteration (Parkin et al. 2014). In addition, habitat degradation and possibly the introduction of exotic species contribute to marked variability in species composition. Meanwhile, droughts may raise water temperature and reduce dissolved oxygen, imposing adverse physical conditions on fish in the pools.

CONCLUDING REMARKS

Research have regarded that tropical areas are expected to suffer more severe impacts of climate change, from heavy floods or extended drought. Such extreme fluctuation of water flow will, to some extent, threat the performance of tropical freshwater biota. Floods and drought can negatively affect the biota, both directly and indirectly, through the changing environment. Research suggested that the magnitude of hydrological changes may be acute to species, disturb reproduction or succession, change the distribution, and lastly restructure the biotic communities in rivers, streams, floodplain, ponds, and lakes.

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REFERENCES

AcunaV, Munõz I, Giorgi A, Omella M, Sabateri F, Sabateri S. 2005. Drought and postdrought recovery cycles in an intermittent Mediterranean stream: structural and functional aspects. J N Am Benthol Soc 24 (4): 919-933.

Agostinho AA, Gomes LC, Veri'ssimo SV, Okada EK. 2004. Flood regime, dam regulation and fish in the Upper Paraná River: effects on assemblage attributes, reproduction and recruitment. Rev Fish Biol Fish 14 (1): 11-19.

Alderdice DF. 1976. Some concepts and descriptions of physiological tolerance: rate-temperature curves of poikilotherms as transects of response surfaces. J Fish Res Bd Can 33: 299-307.

Allan JD, Palmer M, Poff NL. 2005. Climate change and freshwater ecosystems. In: Lovejoy TE, Hannah L (eds) Climate Change and Biodiversity. Yale University Press, New Haven, CT.

Baranyi C, Hein T, Holarek C, Keckeis S, Schiemer F. 2002. Zooplankton biomass and community structure in a Danube River floodplain system: effects of hydrology. Freshw Biol 47: 473-482.

Bash J, Berman C, Bolton S. 2001. Effects of turbidity and suspended solids on Salmonids. Report by Centre for Streamside Studies, University of Washington, Washington.

Beklioglu M, Altinayar G, Tan CO. 2006. Water level control over submerged macrophyte development in five shallow lakes of Mediterranean Turkey. Archive für Hydrobiologie 166: 535-556.

Bertahasa I, Dimitrioua E, Karaouzasa I, Laschoua S, Zacharias I. 2006. Climate change and agricultural pollution effects on the trophic status of a Mediterranean lake. Acta Hydrochim Hydrobiol 34: 349-359.

Boulton AJ, Lake PS. 1990. The ecology of two intermittent streams in Victoria, Australia. I. Multivariate analyses of physicochemical features. Freshw Biol 24: 123-141.

Boulton AJ Lake PS. 1992. The ecology of two intermittent streams in Victoria, Australia. III. Temporal changes in faunal composition. Freshw Biol 27: 123-138.

Bowers RW, de Szalay FA. 2004. Effects of hydrology on unionids (Unionidae) and zebra mussels (Dreissenidae) in a Lake Erie coastal wetland. Am Midl Nat 151: 286-300.

Boyd C, Tucker C. 1998. Pond aquaculture water quality management. Kluwer Academic Publishers, Norwell, MA.

Bradford MJ. 1997. An experimental study of stranding of juvenile salmonids on gravel bars and inside channels during rapid flow fluctuations. Regul Rivers Res Manag 13: 395-401.

Brett JR. 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am Zool 11: 99-113.

Brittain JE, Eikeland TJ. 1988. Invertebrate drift. Hydrobiologia 166: 77-93.

Bunt CM, Cooke SJ, Katopodis C, McKinley RS. 1999. Movement and summer habitat of brown trout (*Salmo trutta*) below a pulsed discharge hydroelectric generating station. Regul Rivers Res Manag 15: 395-403.

Bunn SE, Arthington AH. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environ Manag 30: 492-507.

Campagna CG, Cech Jr JJ. 1981. Gill ventilation and respiratory efficiency of Sacramento blackfish, *Orthodon microlepidotus*, in hypoxic environments. J Fish Biol 19: 581-591.

Caruso BS. 2002. Temporal and spatial patterns of extreme low flows and effects on stream ecosystems in Otago. N Z J Hydrol 257: 115-133.

Chellappa S, Câmara MR, Chellappa NT. 2004. Ecology of *Cichla monoculus* (Osteichthyes: Cichlidae) from a reservoir in the semi-arid region of Brazil. Hydrobiologia 504: 267-273.

Chergui H, Haddy L, Markaoui M, Pattee E. 1997. Impact of dead leaves leaching products on water oxygen content and on the survival of a gastropod. Acta Oecologica 18: 531-542.

Christensen JH, Christensen OB. 2003. Severe summertime flooding in Europe. Nature 421: 805-806.

Connolly NM, Pearson RG. 2007. The effect of fine sedimentation on tropical stream macroinvertebrate assemblages: a comparison using flow through artificial stream channels and recirculating mesocosms. Hydrobiologia 592: 423-438.

Cowx IG, Young WO, Hellawell JM. 1984. The influence of drought on the fish and invertebrate populations of an upland stream in Wales. Freshw Biol 14: 165-177.

Crosa G, Castelli E, Gentili G, Espa P. 2009. Effects of suspended sediments from reservoir flushing on fish and macroinvertebrates in an alpine stream. Aquat Sci 72 (1): 85-95.

Dahm C, Baker MA, Moore DI, Thibault JR. 2003. Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshw Biol 48: 1219-1231.

Dai AG, Trenberth KE, Qian T. 2004. A global dataset of Palmer Drought severity index for 1870-2002: relationship with soil moisture and effects of surface warming. J Hydrometeorol 5: 1117-1130.

Davies PE, Sloane RD, Andrew J. 1988. Effects of hydrological change and the cessation of stocking on a stream population of *Salmo trutta* L. Aust J Mar Freshw Res 39: 337-354.

Deutsch C., Tewksbury A., Huey JJ., Sheldon RB., Ghalambor CK., Haak D C., Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc Natl Acad Sci USA 105: 6668-6672.

Dirnberger JM, Threlkeld ST. 1986. Advective effects of a reservoir flood on zooplankton abundance and dispersion. Freshw Biol 16: 387-396.

Doeg TJ, Milledge GA. 1991. The effects of experimentally increasing suspended sediment concentrations on macroinvertebrate drift. Aust J Mar Freshw Res 42: 519-526.

Doeg TJ, Koehn JD. 1994. Effects of draining and desilting a small weir on downstream fish and macroinvertebrates. Regul Rivers Res Manag 9: 263-277.

Dokulil MT. 1984. Assessment of components controlling phytoplankton photosynthesis and bacterioplankton production in a shallow, alkaline, turbid lake (Neusiedler See, Austria). Intl Revue Ges Hydrobiol 69: 679-727.

Dokulil MT. 1994. Environmental control of phytoplankton productivity in turbulent turbid Systems. Hydrobiologia 289: 65-72.

Donohue I, Irvine K. 2004. Size-specific effects of increased sediment loads on gastropod communities in Lake Tanganyika, Africa. Hydrobiologia 522: 337-342.

Dudgeon D. 2003. The contribution of scientific information to the conservation and management of freshwater biodiversity in tropical Asia. Hydrobiologia 500: 295-314.

Elber F, Schanz F. 1990. The influence of a flood event on phytoplankton succession. Aquatic Sciences 52 (4): 330-344.

Euliss NH, Labaugh JW, Fredrickson LH, Mushet DM, Laubhan MRK, Swanson GA, Winter TC, Rosenberry DO, Nelson RD. 2004. The wetland continuum: a conceptual framework for interpreting biological studies. Wetlands 24: 448-458.

Ficke AD, Myrick CA, Hansen LJ. 2007. Potential impacts of global climate change on freshwater fisheries. Rev Fish Biol Fish 17: 581-613.

Flecker AS, Feifarek B. 1994. Disturbance and the temporal variability of invertebrate assemblages in two Andean streams. Freshw Biol 31: 131-142.

Franklin CE, Johnston IA, Crockford T, Kamunde C. 1995. Scaling of oxygen consumption of Lake Magadi tilapia, a fish living at 37^{o}C. J Fish Biol 46: 829-834.

Freeman C, Gresswell R, Guasch H, Hudson JA, Lock MA, Reynold B, Sabater S, Sabater F. 1994. The Role of drought in the impact of climatic-change on the Microbiota of peatland streams. Freshw Biol 32: 223-230.

Fry FEJ. 1971. The Effect of Environmental Factors on the Physiology of Fish. In: Hoar WS, Randall DJ (eds) Fish Physiology: Environmental Relations and Behavior. Academic Press, New York.

Furey PC, Nordin RN, Mazumder A. 2004. Water level drawdown affects physical and biogeochemical properties of littoral sediments of a reservoir and a natural lake. Lake Reserv Manag 20: 280-295.

Furey PC., Nordin RN., Mazumder A. 2006. Littoral benthic macroinvertebrates under contrasting drawdown in a reservoir and a natural lake. J N Am Benthol Soc 25: 19-31.

Garric J, Migeon B, Vindimian E. 1990. Lethal effects of drainning on brown trout. A predictive model based on field and laboratory studies. Water Res 24 (1): 59-65.

Gelwick FP, Akin S, Arrington DA, Winemiller KO. 2001. Fish assemblage structure in relation to environmental variation in a Texas gulf coastal wetland. Estuaries 24: 285-296.

Gibbons JW. 1976. Thermal alteration and the enhancement of species populations. In: Esch GW, McFarlane RW (eds) Thermal Ecology II Conference 750425. ERDA Symposium Series. Technical Information Center, United States Atomic Energy Commission, Oakridge, TN.

Gliwicz ZM. 1986. Predation and the evolution of vertical migration in zooplankton. Nature 320: 746-748.

Godlewska M, Mazurkiewicz-Boron G, Pociecha A, Wilk-Wozniak E, Jelonek M. 2003. Effects of flood on the functioning of the Dobczyce reservoir ecosystem. Hydrobiologia 504: 305-313.

Grobbelaar JU. 1992. Nutrients versus physical factors in determining the primary productivity of waters with high inorganic turbidity. Hydrobiologia 238: 177.

Groisman PY, Karl TR, Easterling DR, Knight RW, Jamason PF, Hennessy KJ, Suppiah R, Page CM, Wibig J, Fortuniak K, Razuvaev VN, Douglas A, Forland E, Zhai PM. 1999. Changes in the probability of heavy precipitation: important indicators of climate change. Climatic Change 42: 243-283.

Groisman PY, Knight RW, Karl TR, Easterling DR, Sun B, Lawrimore J. 2004. Contemporary changes of the hydrological cycle over the contiguous United States: trends. J Hydrometeorol 5: 64-85.

Groisman PY, Knight RW, Easterling DR, Karl TR, Hegerl GC, Razuvaev VN. 2005. Trends in intense precipitation in the climate record. J Clim 18: 1326-1350.

Håkanson L. 1977. Influence of wind, fetch, and water depth on distribution of sediments in lake Vanern, Sweden. Can J Earth Sci 14: 397-412.

Harem A, Kucklentz V. 1981. Effects of hydraulic load changes on the eutrophication of an alpine lake. Verb Internat Verein Limnol 21: 466-472.

Havel JE, Eisenbacher EM, Black AA. 2000. Diversity of crustacean zooplankton in riparian wetlands: colonization and egg banks. Aquat Ecol 34: 63-76.

Hellawell J. 1986. Biological Indicators of Freshwater Pollution and Environmental Management. Elsevier, London.

Holmes NTH. 1999. Recovery of headwater stream flora following the 1989-1992 groundwater drought. Hydrol Process 13: 341-354.

Holst I, Dokulil M. 1987. Die steuemden Faktoren der planktischen Primtrproduktion im Stauraum Altenworth an der Donau in Osterreich. 26. Arbeitstagung der IAD, Passau/Deutschland, Wiss. Kurzreferate: 133-137.

Huntington TG. 2006. Evidence for intensification of the global water cycle: review and synthesis. J Hydrol 319: 83-95.
Hynes HBN. 1970. The Ecology of Running Waters. Liverpool University Press, Liverpool, UK.
Intergovernmental Panel on Climate Change (IPCC). 2001. Climate change 2001: the scientific basis. Cambridge University Press, Oxford, UK.
Irion G, Junk WJ. 1997. The large Central Amazonian River floodplains near Manaus. In: Junk WJ (ed) The Central Amazon Foodplain: Ecology of a Pulsing System. Springer, Berlin.
Jacobsen D, Encalada A. 1998. The macroinvertebrate fauna of Ecuadorian Highland streams in wet and dry seasons. Archive Für Hydrobiologie 142 (1): 53-70.
Janáč M, Ondračková M, Jurajda P, Valová Z, Reichard M. 2010. Flood duration determines the reproduction success of fish in artificial oxbows in a floodplain of a Potamal river. Ecol Freshw Fish 19: 644-655.
Jenkins DG. 1995. Dispersal-limited zooplankton distribution and community composition in new ponds. Hydrobiologia 313/ 314: 15-20.
Keaton M, Haney D, Andersen CB. 2005. Impact of drought upon fish assemblage structure in two South Carolina Piedmont streams. Hydrobiologia 545: 209-223.
Keckeis S, Baranyi C, Hein T, Holarek C, Riedler P, Schiemer F. 2003. The significance of zooplankton grazing in a floodplain system of the River Danube. J Plankton Res 25: 243-253.
Kingsolver JG. 2009. The well-temperatured biologist. Am Nat 174:755-768.
Kitchell JF, Stewart DJ, Weininger D. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vireum vitreum*). J Fish Res Bd Can 34: 1922-1935.
Labbe TR, Fausch KD. 2000. Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. Ecol Appl 10: 1774-1791.
Lake PS. 2000. Disturbance patchiness and diversity in streams. J N Am Benthol Soc 19: 573-592.
Lake PS. 2003. Ecological effects of perturbation by drought in flowing waters. Freshw Biol 48: 1161-1172.
Layzer JB, Nehus TJ, Pennington W, Gore JA, Nestler JM. 1989. Seasonal variation in the composition of drift below a peaking hydroelectric project. Regul Rivers Res Manag 3: 305-317
Ledger ME, Brown LE, Edwards FK, Milner AM, Woodward G. 2012. Drought alters the structure and functioning of complex food webs. Nature Climate Change. Global Change Biol 17: 2288-2297.
Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzalez A. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecol Lett 7: 601-613.
Leira M, Cantonati M. 2008. Effects of water-level fluctuations on lakes: an annotated bibliography. Hydrobiologia 613: 171-184.
Lemly AD. 1982. Modification of benthic insect communities in polluted streams: combined effects of sedimentation and nutrient enrichment. Hydrobiologia 87: 229-245.
Lenat DR, Penrose DL, Eagleson KW. 1981. Variable effects of sediment addition on stream benthos. Hydrobiologia 79: 187-194.
Lloyd DS, Koenings JP, LaPerriere JD. 1987. Effects of turbidity in fresh waters of Alaska. N Am J Fish Manag 7: 18-33.
Lloyd D. 1987. Turbidity as a water quality standard for salmonid habitats in Alaska. North Am J Fish Manag 7: 34-45.
Lohr SC, Fausch KD. 1997. Multiscale analysis of natural variability in stream fish assemblages of a western Great Plains watershed. Copeia 1997: 706-724.
Lowe-McConnell RA. 1987. Ecological Studies in Tropical Fish Communities. Cambridge University Press, Cambridge.
Luz-Agostinho KDG, Agostinho AA, Gomes LC, Júlio-Jr HF. 2008. Influence of flood pulses on diet composition and trophic relationships among piscivorous fish in the upper Parana River floodplain. Hydrobiologia 607: 187-198.
Magnuson JJ. 2002. Future of adapting to climate change and variability. In: McGinn NA (ed) Fisheries in a Changing Climate. American Fisheries Society, Bethesda, MD.
Matthews WJ. 1998. Patterns in Freshwater Fish Ecology. Chapman & Hall, New York.
Matthews WJ, Marsh-Matthews E. 2003. Effects of drought on fish across axes of space, time and ecological complexity. Freshw Biol 48: 1232-1253.
McClelland WT, Brusven MA. 1980. Effects of sedimentation on the behaviour and distribution of riffle insects in a laboratory stream. Aquat Insects 2: 161-169.
Medeiros ESF, Maltchik L. 2001. Fish assemblage stability in an intermittently flowing stream from the Brazilian semiarid region. Austr Ecol 26: 156-164.
Medley KA, Havel JE. 2007. Hydrology and local environment factors influencing zooplankton communities in floodplain ponds. Wetlands 27 (4): 864-872.
Meisner JD. 1992. Assessing potential effects of global climate change on tropical freshwater fishes. Geojournal 28: 21-27.
Meyerhoff RD, Lind OT. 1987. Factors affecting the benthic community structure of a discontinuous stream in Guadalupe Mountains National Park, Texas. Internationale Revue der gesamten Hydrobiologie 72: 283-296.
Mihaljevic´ M, Stevic´ F, Horvatic´ J, Kutuzovic BH. 2009. Dual impact of the flood pulses on the phytoplankton assemblages in a Danubian floodplain lake (Kopački Rit Nature Park, Croatia). Hydrobiologia 618: 77-88.
Miller AM, Golladay SW. 1996. Effects of spates and drying on macroinvertebrate assemblages of an intermittent and perennial prairie stream. J N Am Benthol Soc 15: 670-689.
Miquelis A, Rougier C, Pourriot R. 1998. Impact of turbulence and turbidity on the grazing rate of the rotifer *Brachionus calyciflorus* (Pallas). Hydrobiologia 386: 203-211.
Miserendino ML. 2009. Effects of flow regulation, basin characteristics and land-use on macroinvertebrate communities in a large arid Patagonian river. Biodivers Conserv 18: 1921-1943.
Mitchell JFB, Manabe S, Meleshko V, Tokioka T. 1990. Equilibrium climate change-and its implications for the future. In: Houghton JT, Jenkins GJ, Ephraums JJ (eds) Climate Change. The IPCC Scientific Assessment, Cambridge University Press, Cambridge.
Mol J, Vandenberghe J, Kasse C. 2000. River response to variations of periglacial climate in mid-latitude Europe. Geomorphology 33: 131-148.
Moyle PB, Vondracek B. 1985. Persistence and structure of the fish assemblages in a small California stream. Ecology 66: 1-13
Moyle PB, Cech JJ. 2004. Fishes: An Introduction to Ichthyology. 5th ed. Prentice Hall, Englewood Cliffs, NJ
Muylaert K, Van Wichelen J, Sabbe K, Vyverman W. 2001. Effects of freshets on phytoplankton dynamics in a freshwater tidal estuary (Schelde, Belgium). Arch Hydrobiol 150 (2): 269-288.
Naselli-Flores L. 2003. Man-made lakes in Mediterranean semiarid climate: The strange case of Dr Deep Lake and Mr Shallow Lake. Hydrobiologia 506/509: 13-21.
Newbury R, Gaboury M. 1993. Exploration and rehabilitation of hydraulic habitats in streams using principles of fluvial behaviour. Freshw Biol 29: 195-210.
Newcombe CP, MacDonald DD. 1991. Effect of suspended sediments on aquatic ecosystems. J N Am Benthol Soc 11: 72-82.
Nicholls KH. 1998. El Nino, ice cover, and Great Lakes phosphorus: implications for climate warming. Limnol Oceanogr 43: 715-719.
Ortega-Mayagoitia E, Armengol X, Rojo C. 2000. Structure and dynamics of zooplankton in a semi-arid wetland, the National Park Las Tablas de Daimiel (Spain). Wetlands 20: 629-638.
Ostrand KG, Wilde GR. 2002. Seasonal and spatial variation in a prairie stream-fish assemblage. Ecol Freshw Fish 11 (3): 137-149.
Palmer TN, Räisänen J. 2002. Quantifying the risk of extreme seasonal precipitation events in a changing climate. Nature 415: 512-514.
Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. Ann Rev Ecol Evol Syst 37: 637-669.
Perkin JS, Gido KB, Costigan KH, Daniel MD, Johnson ER. 2014. Fragmentation and drying ratchet down Great Plains streamfish diversity. Aquat Conserv Mar Freshw Ecosyst. DOI: 10.1002/aqc.2501
Peterson CG. 1996. Response of benthic algal communities to natural physical disturbance. Pp. 375-402 In: Stevenson JR, Bothwell ML, Lowe RL (eds) Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego.
Petz-Glechner R, Petz W, Kainz E, Lapuch O. 2003. Die Auswirkungen von Stauraumspülungen auf Fische. Natur in Tirol-Ökologie und Wasserkraftnutzung. Amt der Tiroler Landesregierung-Abteilung Umweltschutz 12: 74-93.
Pires AM, Cowx IG, Coelho MM. 1999. Seasonal changes in fish community structure of intermittent streams in the middle reaches of the Guadiana basin, Portugal. J Fish Biol 54: 235-249.

Poff NL, Ward JV. 1989. Implications of stream flow variability and predictability for lotic community structure: a regional analysis of stream flow patterns. Can J Fish Aquat Sci 46: 1805-1818
Poff NL, Angermeier PL, Cooper SD. 2001. Fish diversity in streams and rivers. In: Sala OE, Chapin F, Huber-Sannwald E (eds) Global Biodiversity in a Changing Environment: Scenarios for the 21st Century. Springer, New York.
Poff NL, Allan JD, Bain MB. 1997. The natural flow regime: a paradigm for river conservation and restoration. Bioscience 47: 769-784.
Power ME, Matthews WJ, Stewart AJ. 1985. Grazing minnows, piscivorous bass and stream algae. Ecology 66: 1448-1456.
Pruitt AB, Melgaard DL, Flexner MC, Able AS. 2001. Chattooga River Watershed Ecological/Sedimentation Project. In: Proceedings of the Federal Interagency Sedimentation Conference, held 26-30 March in Reno, NV.
Rabeni CF, Doisy KE, Zweig LD. 2005. Stream invertebrate community functional responses to deposited sediment. Aquat Sci 6 (7): 395-402
Regier HA, Meisner JD. 1990. Anticipated effects of climate change on freshwater fishes and their habitat. Fisheries 15 (6): 10-l5.
Resh VH. 1992. Year-to-year changes in the age structure of a caddisfly population following loss and recovery of a Springbrook habitat. Ecography 15: 314-317.
Resh VH, Brown AV, Covich AP, Gurtz ME, Li HW, Minshall GW, Reice SR, Sheldon AL, Wallace JB, Wissmar RC. 1988. The role of disturbance in stream ecology. J N Am Benthol Soc 7: 433-455.
Resh VH, Bêche LA, Lawrence JE, Mazor RD, McElravy EP, O'Dowd AP, Rudnick D, Carlson SP. 2013. Long-term population and community patterns of benthic macroinvertebrates and fishes in Northern California Mediterranean-climate streams. Hydrobiologia 719: 93-118.
Reyjol Y, Lim P, Dauba F, Baran P, Belaud A. 2001. Role of temperature and flow regulation on the Salmoniform-Cypriniform transition. Arch Hydrobiol 152 (4): 567-582.
Robinson CT, Aebischer S, Uehlinger U. 2004. Immediate and habitat-specific responses of macroinvertebrates to sequential, experimental floods. J N Am Benthol Soc 23: 853-867.
Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA. 2003. Fingerprints of global warming on wild animals and plants. Nature 421: 57-60.
Rossa DC, Bonecker CC. 2003. Abundance of planktonic and non-planktonic rotifers in floodplain lakes of the Upper Paraná River floodplain. Amazoniana 17 (3-4): 567-581.
Ryder GI. 1989. Experimental Studies on the Effects of Fine Sediment on Lotic Invertebrates. [Ph.D. Dissertation]. Department of Zoology, University of Otago, Dunedin, New Zealand.
Sala OE, Chapin FS, Armesto JJ. 2000. Biodiversity-global biodiversity scenarios for the year 2100. Science 287: 1770-1774.
Sampl H. 1986. EinfluB von Nfihrstoffabschwemmung und Bodenerosion auf die Gew/issereutrophierung. Bundesministerium frir Land-und Fortwirtschaft, Wien.
Scheifhacken N, Fiek C, Rothhaupt KO. 2007. Complex spatial and temporal patterns of littoral benthic communities interacting with water level fluctuations and wind exposure in the littoral zone of a large lake. Fund Appl Limnol 169 (2): 115-129.
Schofield KA, Pringle CM, Meyer JL. 2004. Effects of increased bedload on algal-and detrital-based stream food webs: Experiemental manipulation of sediment and macroconsumers. Limnol Oceanogr 49 (4): 900-909.
Sigler JW, Bjornn TC, Everest FH. 1984. Effects of chronicturbidity on density and growth of steelheads and co salmon. T Am Fish Soc 113: 142-150.
Smith, MAK. 1991. Models of seasonal growth of the equatorial carp *Labeo dussumieri* in response to the river flood cycle. Environ Biol Fishes 31: 157-170.
Smith F, Brown AV. 2006. Effects of flow on meiofauna colonization in artificial streams and reference sites within the Illinois River, Arkansas. Hydrobiologia 571: 169-180.
Somero GN, Hofmann GE. 1997. Temperature thresholds for protein adaptation: when does temperature start to 'hurt'? In: Wood CM, McDonald DG (eds) Global Warming: Implications for Freshwater and Marine Fish. Cambridge University Press, Cambridge, UK.
Sorensen DL, McCarthy MM, Middlebrook EJ, Porcella DB. 1977. Suspended and Dissolved Solids Effects on Freshwater Biota: A Review. United States Environmental Protection Agency. 600/3-77-042.
Spooner DE, Vaughn CC. 2008. A trait-based approach to species' roles in stream ecosystems: climate change, community structure, and material cycling. Oecologia 158: 307-317.
Stanley EH, Fisher SG, Grimm NB. 1997. Ecosystem expansion and contraction in streams. BioScience 47: 427-435.
Starks E, Cooper R, Leavitt PR, Wissel B. 2014. Effects of drought and pluvial periods on fish and zooplankton communities in prairie lakes: systematic and asystematic responses. Global Change Biology 20: 1032-1042.
Strayer DL, Downing JA, Haag WR, King TL, Layzer JB, Newton TJ, Nichols S. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience 54: 429-439
Suren AM, Jowett IA. 2001. Effects of deposited sediment on invertebrate drift: an experimental study. New Zeal J Mar Fresh Res 35: 725-737
Taylor EW, Egginton G, Taylor SE, Butler PJ. 1997. Factors which may limit swimming performance at different temperatures. In: Wood CM, McDonald DG (eds) Global Warming: Implications for Freshwater and Marine Fsh. Cambridge University Press, Cambridge, UK.
Taylor CM, Warren ML. 2001. Dynamics in species composition of stream fish assemblages: environmental variability and nested subsets. Ecology 82: 2320-2330
Taylor CM, Winston MR, Matthews WJ. 1996. Temporal variation in tributary and mainstem fish assemblages in a Great Plains stream system. Copeia 1996: 280-289
Thomas EA. 1973. Phosphorus and eutrophication. In: Griffith EJ, Beeton A, Spencer JM, Mitchell DT (eds) Environmental Phosphorus Handbook. John Wiley & Sons, New York.
Thomas CD, Cameron A, Green RE. 2004. Extinction risk from climate change. Nature 427: 145-148
Towns DR. 1991. Ecology of leptocerid caddisfly larvae in an intermittent South Australian stream receiving Eucalyptus litter. Freshw Biol 25: 117-129.
Trenberth KE. 1998. Atmospheric moisture residence times and cycling: Implication for rainfall rates with climate change. Climate Change 36: 667-694.
Trenberth KE. 2005. Uncertainty in hurricanes and global warming, Science 308: 1753-1754.
Val AL, Almeida-Val VMF. 1995. Fishes of the Amazon and their environment: physiological and biochemical aspect. Springer, Berlin.
Van Der Kraak G, Pankhurst NW. 1997. Temperature effects on the reproductive performance of fish. In: Wood CM, McDonald DG (eds) Global Warming: Implications for Freshwater and Marine Fish. University Press, Cambridge, UK.
Vanderploeg HA. 1994. Zooplankton particle selection and feeding mechanisms. In: Wotton RS (ed) The Biology of Particles in Aquatic Systems. 2nd ed. Lewis Publishers, Boca Ratton.
Wagener SM, LaPerriere JD. 1985. Effects of placer mining on the invertebrate communities of interior Alaska streams. Freshw Invert Biol 4: 208-214.
Wang J, Tsai C. 2000. Effects of temperature on the deformity and sex differentiation of tilapia, *Oreochromis mossambicus*. J Exp Zool 286: 534-537.
Webb MAH, Van Eenennaam JP, Feist GW, Linares-Casenave J, Fitzpatrick MS, Schreck CB, Doroshov SI. 2001. Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed white sturgeon, Acipenser transmontanus. Aquaculture 201: 137-151.
Wilcox DA, Meeker JE. 1992. Implications for faunal habitat related to altered macrophyte structure in regulated lakes in northern Minnesota. Wetlands 12: 192-203.
Winder M, Berger SA, Lewandowska A, Aberle N, Lengfellner K, Sommer U, Diehl S. 2012. Spring phenological responses of marine and freshwater plankton to changing temperature and light conditions. Mar Biol. DOI 10.1007/s00227-012-1964-z
Winemiller KO, Tarim S, Shormann D, Cotner J. 2000. Fish assemblage structure in relation to environmental variation among Brazos River oxbow lakes. T Am Fish Soc 129: 451-468.
Wohlschlag DE, Cameron JN, Cech JJ. 1968. Seasonal changes in the respiratory metabolism of the pinfish (*Lagodon rhomboids*). Contr Mar Sci 13: 89-104.
Wood PJ Armitage PD. 1997. Biological effects of fine sediment in the lotic environment. Environ Manag 21: 203-217
Wood PJ, Vann AR, Wanless PJ. 2001. The response of *Melampophylax mucoreus* (Hagen) (Trichoptera: Limnephilidae) to rapid sedimentation. Hydrobiologia 455: 183-188

Wood PJ, Toone J, Greenwood MT, Armitage PD. 2005. The response of four lotic macroinvertebrate taxa to burial by sediments. Archive für Hydrobiologie 163: 145-162

Wotton RS. 1994. Particulate and dissolved organic matter as food. In: Wotton RS (ed) The Biology of Particles in Aquatic Systems. 2nd ed. Lewis Publishers, Boca Ratton.

Wotton RS. 1995. Temperature and lake-outlet communities. J Thermal Biol 20: 121-125

Xenopoulos MA, Lodge DM, Alcamo J, Märker M, Schulze K, van Vuuren DP. 2005) Scenarios of freshwater fish extinctions from climate change and water withdrawal. Glob Change Biol 11: 1557-1564

Yamada H., Nakamura F. 2002. Effect of fine sediment deposition and channel works on periphyton biomass in the Makomanai River, Northern Japan. River Res Appl 18: 481-493.

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Responses to environmental and socio-economic changes in the Karangwangi traditional agroforestry system, South Cianjur, West Java

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Abstract. *Iskandar J, Iskandar BS, Partasasmita R. 2016. Responses to environmental and socio-economic changes in the Karangwangi traditional agroforestry system, South Cianjur, West Java. Biodiversitas 17: 332-341.* In the past, the swidden agriculture system (*huma*) was dominant in village frontiers of West Java; including in the Karangwangi Village of Cidaun Sub-district, Cianjur District, West Java Province. Culturally, each Karangwangi household owned a right to cultivate upland rice (*huma*) by felling mature forest (*leuweung*). Moreover, the land was planted by upland rice and other annual crops, such as corn, cassava, cucumber, and various beans. After harvesting rice and other annual crops, the land was fallowed and transformed into secondary forest (*reuma*) through natural succession. Moreover, the mature secondary forest (*reuma kolot*) could be opened for rice planting again in the following year or fallowed for more than 3-5 years. People will shift to another piece of mature secondary forest for rice planting. Today, because of increasing population, decreasing forests, and increasing market economic penetration, the Karangwangi people have predominantly practiced the swidden farming in the non-forest instead of the forest. The forests had culturally been converted to traditional agroforestry systems, such as mixed-garden (*kebon tatangkalan*), and homegarden (*pekarangan*).This paper discusses the process of evolution of the swidden farming and innovatory cultural practice among the Karangwangi community, South Cianjur, West Java, in management of the traditional agroforestry systems which have been dynamically affected by ecological and socio-economic changes. Four parts are presented in this paper. In part one, we account for the Karangwangi people used to practice the swidden farming in the forest when the forest area that was still abundant. In part two, we discuss a focus on process of the evolution of the swidden farming, transformed to the traditional agroforestry systems In part three, we elaborate on some changes of the traditional agroforestry system and responses to environmental changes. In part four, it is summarized and inferred of the paper. Based on this study, it can be inferred that by developing innovatory cultural practice, Karangwangi people of South Cianjur, West Java have tended to success to evolve their swidden farming to new condition of the traditional agroforestry systems in sustainable way, despite population growth, the depletion of the forests, and intensive market economic penetration.

Keywords: Karangwangi Village, socio-economic changes, traditional agroforestry system

INTRODUCTION

Swidden agriculture, shifting cultivation, slash-and-burn cultivation or long fallow agriculture system which is called as *ladang* (Indonesia) or *huma* (Sundanese) was very popular in the frontier upland West Java and South Banten areas in ancient time (Haan 1912; Kools, 1935;Terra 1953; Geertz 1963; Iskandar 1998; Breeman 2014). Because at that time, the human population was low, forest was abundant, land was the cheap resource, labor was the expensive resource, and market economy non-existent. Gradually, however, as human population has become a more increase, forest has reduced, land has become a more expensive resource, *huma* has prohibited by the government, and increased market economic penetration to rural areas (cf. Kools 1935; Iskandar 1998). As a result, the swidden farming has decreased over time. Indeed, this oldest agriculture system has disappeared in many places in West Java and Banten, Indonesia. However, in some places, such as in Baduy area of South Banten, Kasepuhan area of South Sukabumi, and very refraction area of West Java relict swidden farming has still predominantly practiced by the local people (cf. Iskandar 1998). The Baduy and Kasepuhan have annually cultivated dry-land rice in the forest area. Like the Baduy and the Kasepuhan, the local people who reside in the frontier area of Karangwangi Village, Cidaun Sub-district, Cianjur, West Java, had predominantly practiced the swidden farming in the past. Nowadays, however, they have practiced the swidden farming in the dry land (*tegal* or *tegalan*) instead of in the forest because forests which had used to practiced swidden farming, has been converted to the traditional agroforestry systems, such as the homegarden (*pekarangan*) and mixed-garden (*kebon tatangkalan*), and rice field (*sawah*) (cf. Soemarwoto and Soemarwoto 1984, Iskandar and Iskandar 2011). Historically, the agroforestry is a new term which was introduced by western scholars in 1970s (Von Maydel 1985), but this indigenous agricultural system has been traditionally practiced for a long time in Java (Reijntjes et al. 1992; Iskandar and Iskandar 2011). The agroforestry may be defined “as a land-use system that resembles a forest in structure and combines the natural functions of forest with those for fulfilling the socio-economic needs of the people" (Soemarwoto and Soemarwoto 1984). In terms of management, the agroforestry may be defined "as sustainable management system for land that increases overall production, combines agricultural crops, tree crops and forest plants and/or animal simultaneously or sequentially, and applies management practices that are compatible with cultural patterns of the local population (Raintree and Warner 2015).

On the basis of ecological or environmental history, although the shifting cultivation system had been viewed as an environmentally destructive practice in the past, recently, it has changed, many scholars suggested that some ecological and socio-economic benefits of the swidden. The some benefits of the swidden, including as adapt to changing economic and ecological condition (Colffer et al. 2015), potential sequesters of carbon (van Noordwijk et al. 2015), enhance resilience in a changing climate (Garrity 2015), benefit of biodiversity conservation (Rerkasem et al. 2009; Sajise 2015), maintaining food plants and local rice varieties (Soedjito 2015), and the useful for rehabilitation of degraded lands (Kartawinata and Abdulhadi 2015).

Historically, most swidden farming in West Java had been evolved into the traditional agroforestry types, such as mixed-garden (*kebon tatangkalan*) and homegarden (cf. Soemarwoto and Soemarwoto 1984; Christanty et al. 1986; Iskandar and Iskandar 2011; Kosuke et al. 2013). The evolution of agriculture from the swidden farming system to the traditional agroforestry system because this has some benefits, such as to establish more permanent land right for village farmers with those rights can be transferred to future generation, and to create more sustainable production (cf. Rahman et al. 2016).

This paper discusses the process of evolution of the swidden farming and innovation cultural practice among the Karangwangi community, South Cianjur, West Java, in management of the traditional agroforestry systems which have been dynamically affected by ecological and socio-economic changes.

MATERIALS AND METHODS

Study sites

The present study was undertaken in the Village (*desa*) of Karangwangi, Sub-district (*kecamatan*) of Cidaun, District (*kabupaten*) of Sukabumi, Province (*provinsi*) of West Java, Indonesia. The village is located at approximately latitude 7°25'-7°30' S and longitude 107° 23'-107°25' E. It has the distance approximately 120 km from the city of Bandung and approximately 70 km from the town of Cianjur, with a travel time of 5-6 hours from the city of Bandung and approximately 3-4 km from the town of Cianjur. The Karangwangi Village is north bordering Cimaragang Village, east to Garut District, west to Cidamar Village and south by the Indonesian Ocean, and is directly bordered with the Nature Reserve (*Cagar Alam*) of Bojonglarang-Jayanti (Figure 1). The Karangwangi Village has a total area of about 2,300 hectares, which was inhabited by 5,587 people with 1,817 households in 2014 (Statistic of Karangwangi Village 2014). The main livelihood of the people recorded as farmer. Topographically, the Karangwangi consists of moderate to very steep slope hills, and at an altitude between 200-275 m above sea level (asl.). The village of Karangwangi area exhibit two main seasons: a dry season (*usum halodo*) and a wet season (*musim hujan* or *musim ngijih*). In general, from November to April, the south-west monsoon brings heavy rains, while from May to October the south-east monsoon brings drier weather.

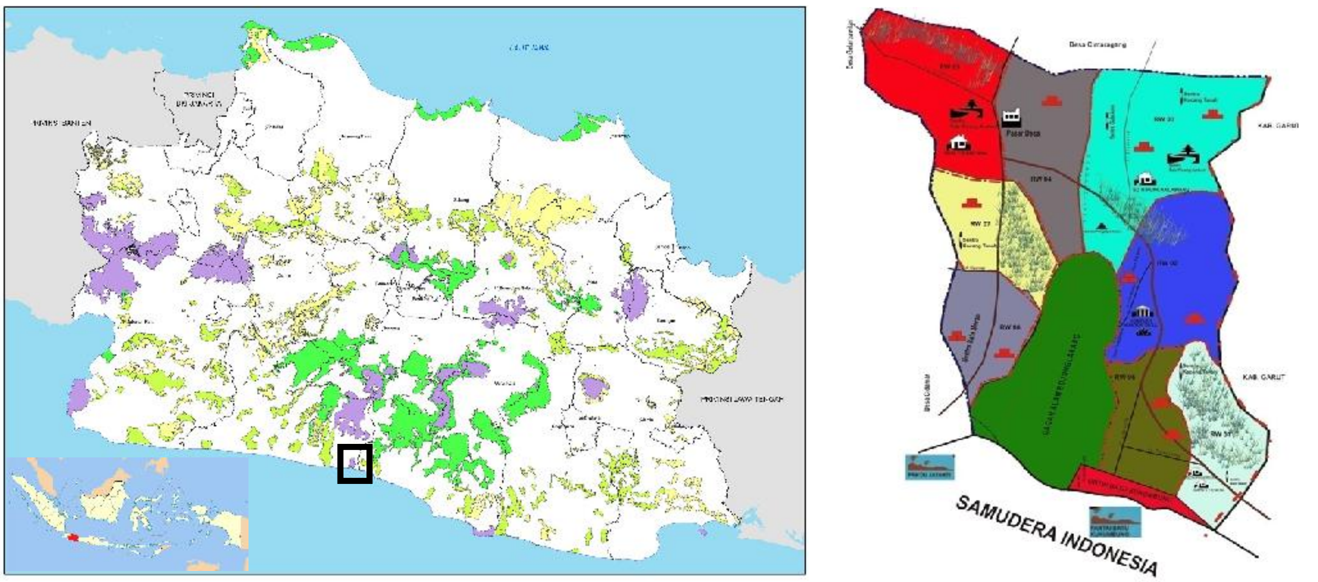

Figure 1. Research location, Karangwangi Village, South Cianjur, West Java, Indonesia

Procedure

The method used in this study was the qualitative which is based on ethnoecological approach (cf. Martin 1995; Cotton 1996; Cunningham 2001; Newing et al. 2011; Iskandar 2012). Several techniques were applied to collect data, i.e. semi-structure interview, observation, and participant observation. The semi-structure interview was undertaken by deep interview with competent informants (local experts), which is selected by purposive considered as experts, including old farmers, informal leaders, village staffs, and village middlemen. The deep interview was based on a prepared interview guide that consists of a list of questions or topics to be covered, particularly concerning the development of the traditional agroforestry systems in the Karangwangi Village. The observation was mainly applied to observe general condition of settlement and agricultural areas, various vegetation structure of traditional agroforestry systems, and village market activities, while participant observation that was conducted by researchers accompanied one or more farmers both to observe what they do and say, and also participate, to varying degree, in the activities being studied (cf. Newing et al. 2011). For example, we actively participated in harvesting various crops in the homegarden and mixed-garden, and processing and marketing of various crop productions, particularly fruits.

Data analysis

The data analysis involved cross-checking, summarizing and synthesizing from different sources, namely obtained from semi-interviews of informants, observations, participant observations, and secondary data of village and sub-district statistical data, and used to build up a narrative account (cf. Newing et al. 2011).

RESULTS AND DISCUSSION

Owing to a lack of numerical data, nothing definite can be said about the forest acreage and growth rate of the population in the Karangwangi in early-modern West Java after the Indonesian independent. The process of ecological and socio-economic changed is predominantly elucidated instead of the quantitative analyzed in the paper. This paper is presented in four parts. In part one, we account for the Karangwangi people used to practice the swidden farming in the forest when the forest area that was still abundant. In part two, with a focus on process of the evolution of the swidden farming, transformed to the traditional agroforestry systems; and part three, discuss on the traditional agroforestry and responses to environmental changes. In part four, summarized and concluded the paper.

The swidden cultivation in the forest

On the basis of ecological history, in the ancient time the frontier areas of West Java were predominantly covered by forests with owned relatively low human population (c.f. Lombard 1996; Geertz 1963; Breeman 2014). Therefore, to adapt with the local upland environment, Sundanese people who reside in the frontier villages, including people of Karangwangi Village, Cianjur District, West Java had developed the shifting cultivation system (*huma*) instead of cultivating the wet rice field cultivation (*sawah*). The *sawah* system was firstly introduced to upland areas of West Java in late of 1750 (Terra 1958; Geertz 1963).

In the past, almost all household of the Karangwangi Village and surrounding areas, including Cimaragang, and Cisewu culturally involved in practicing swidden farming in the forest. They opened the forest by moving from one places to another to practice of swidden farming. As mentioned by one of the informants that: "*Baheula mah Kuring pindah kadieu* (*Desa Karangwangi*) *ti keur bujangan keneh. Kuring ngilu ka pun Bapak pikeun ngagarap huma ti daerah Cisewu, terus pindah ka daerah Cimaragang terus weh pipindahan nepi ka tuntungna pindah kadieu. Baheula ge didieu masih keneh leuweung, terus loba keneh eurih jarangkung nepika nutupan jangjalaneun".* This can be translated as: " In the past, I moved to this place (Karangwangi Village) since I was bachelor. I came from Cisewu and moved to Cimaragang, and always moved, finally resided in this place. I always moved accompanied my father to practice the swidden farming (*huma*). At that time, in this place was predominantly covered by forest. In addition, the footpaths in the forest were covered by the tall grass of eurih (*Imperata cylindrica*)".

Each the village household has right to cultivate upland rice (*huma*) by felling mature secondary forest (*reuma kolot*) in different places, and planting in one or two successive years. Moreover, fallow land is not owned permanently and only perennial crops are inherited. Originally, after harvesting rice and other annual crops, fallowed land (*reuma*) could be cultivated by anyone, but with priority given to the previous users. Such rights disappear if rice is harvested and land returned to fallow. Therefore, each household usually moved as nomadic from one place to another to obtain fallowed land of sufficient maturity for swiddening. As a result, each household has the fallowed land between 5 to 8 plots of the fallowed land.

Culturally, to practice upland rice (*huma*), a piece of mature secondary forest (*reuma kolot*) was cleared and planted by upland rice and other annual crops, such as corn, cassava, cucumber, and various beans, strongly based on local knowledge (*corpus*) and *cosmos* or *belief* (cf. Toledo 2002). After harvesting rice and other annual crops, the land was fallowed and transformed into secondary forest (*reuma*) through natural succession. Moreover, the mature secondary forest (*reuma kolot*) could be opened for rice planting again in the following year or fallowed for more than 3-5 years. Each household will shift to another piece of mature secondary forest for rice planting. Thus, in ancient times, shifting cultivation was very popular in the upland of West Java, and status of *huma* land was considered as a tenure system. Each household of Karangwangi people usually moved from one place to other places to find appropriate mature secondary forest (*reuma kolot*) to practice shifting cultivation. If certain appropriate forest block had been found, this forest block has been given special signs (*diciri* or *disawen*), such as by tying shrubs (cf. Soepomo 1982). Thus, it means such forest block had been claimed by one family to be planted by rice and other annual crops. Moreover, forest vegetation in the signed forest block was opened in the dry season, and various vegetation biomasses were dried and burned, and then the land is readily planted by rice. In the beginning of the wet season, the land was planted by various local upland rice varieties, such as *pare sintung*, or popularly called as *pare buhun* or *pare jampang* and other annual crops, such as beans, including *kacang hiris* (*Cajanus cajan*). The forest land was planted by upland rice varieties and other annual crops are called *huma* (cf. Iskandar and Ellen 1999; Iskandar and Iskandar 2011). After planting rice approximately 5 month, the rice paddy was harvested and other annual crops was sequentially harvested between 1 and 6 months after harvesting rice depend on kind of crops. The *huma* land was recultivated in next year or fallowed between 3 and 5 years. The fallowed *huma* land less than one year that was still predominantly by straw (*jarami*) is called *jami*. The following year, the *jami* was replanted by upland rice or fallowed (*dipreikan* or *diperdiokeun*) that will developed to be form of the immature secondary forest (*reuma ngora*) with fallowed time 2-3 years, and mature secondary forest (*reuma kolot*) of fallowed time more than 3 years by natural succession. Before the fallowed secondary forests were readily recultivated the cultivator would move from one block to other forest block to practice the shifting cultivation (*huma*). Generally, the cultivators would be come back to recultivated the former fallowed secondary forest, after fallowing time more than between 3 and 5 years. Based on the farmer perception, the soil fertility of the fallowed mature secondary (*reuma kolot*) of fallowing time more than 3-5 years considered has been recovery. It is caused the top soil has been provided by some amount of litters over time during fallowed time and became compost (cf. Sanchez 1976; Okigbo 1984). Therefore, the secondary mature forest can be reopened to be replanted by upland rice and other annual crops. Culturally, the fallowed mature secondary forests might be recultivated by the former owners who the first opened the forest or may be cultivated by other farmers but they must get special permission from the former *huma* owners. Therefore, basically, the swidden system practiced by village people of Karangwangi had cycle, namely "the mature secondary forest (*reuma kolot*) – swidden field (*huma*) – immature secondary forest (*reuma ngora*) – mature secondary forest (*reuma kolot*) – swidden field (*huma*)" continuously over time.

During the cultivating rice (*ngahuma*) in the forest block, each household culturally built a farm house (*saung huma*) in his swidden field (*huma*). Some farmers between 3 and 5 usually built farm houses in located very closely one and each other's in a group. These farm houses would establish more permanent swidden and farm house grouping that is called *catihan*. Moreover, if one or two new farm permanent houses were built in the *catihan* and would develop to be *umbulan* (one or two permanent houses in a farm). Eventually, the *umbulan* would develop to be *babakan* (new hamlet consisting of houses). *Ampian* or *kampung* (hamlet consisting of 10-50 houses), and *desa* (consisting of some hamlets or administrative village) (cf. Iskandar 1998). At the present time many people who reside in the Karangwangi had originally came from neighboring villages, such as Cimaragang, Cianjur District and Cisewu, Garut District who predominantly used to swiddeners (*peladang* or *pahuma*) in the forest.

The evolution of the swidden farming

According to some informants, until the late of the twenties century, some people of Karangwangi Village had predominantly practiced the shifting cultivation or swidden cultivation in the forest due to still abundant forest area. In the past, the cultivation of dry rice was yearly undertaken by village farmers in once, only during the wet season. Various methods particularly environmental indicators were used by village farmers to start the swidden farming. For example, the appearance of position of *bintang kidang* (the belt of Orion) was usually used as indicators to determine the swidden farming activities. Culturally, the planting the upland rice of the swidden farming was begun when the *kidang* appears overhead (*nyuhun*). Another environmental indicator, the flowering and fruiting of the beurih (*Aporosa frutescens*; Family of Euphorbiacea), that the flowering of which usually synchronize with the beginning dry season. Conversely, the ripening and falling down of the fruits which usually synchronize with the beginning wet season. In addition to annual cycle of precipitation and various within it, Karangwangi also recognized a cycle of eight named years, which is called *windu*. The *windu* consists of *alip*, *he*, *jim awal*, *je*, *dal*, *be wau*, and *jim akhir* (cf. Iskandar 2007). Each named year in the cycle is said to exemplify special characteristic in relation to climate and the panting of appropriate crops which is mentioned by informants as follow (Table 1):

However, by the 1970s, the practice of swidden farming had been dramatically reduced due to various factors, such as increasing of human population, decreasing forest areas, and increasing market economic penetration to villages. Based on interview with the informants, the human population in the Karangwangi was still very low. They resided in the scatter hamlets which were closed to the swidden field. Administratively, the Karangwangi Village was established in 1984 and developed from the previous village, Cidaun Village. Since the early 1970s, the natural mature forest of the Karangwangi have disappeared due to converted to swidden field and fallowed lands. Indeed, the relic mature forest with total of 750 ha, was decreed by the national government as the nature conservation called Bojonglarang-Jayanti Nature Reserve. Nowadays, the total area of the Karangwangi was recorded 2,300.17 ha. The land use types can be divided into categorized, namely swidden (*huma*) 450 hectares, homegarden 1.8 hectares, mixed-garden 5 hectares, dry land (*tegalan*) 47 hectares, village people plantation (*perkebunan rakyat*) 5 hectares, semi-technical irrigation rice field (*irigasi setengah teknis*) 45 hectares, simple irrigation rice field (*irigasi sederhana*) 150 hectares, rain fed rice field (*sawah tadah hujan*) 760 hectares, and tidal rice field (*sawah pasang surut*) 12

Table 1. Names of year in a cycle of eight years (*windu*)

Name of year		Special characteristic	Which can be translated as
1.	*Alip*	*Halodo panjang, tapi pepelakan bakal sae*	The long dry season, but crops might be growing
2.	*He*	*Halodo henteu pati panjang, pepelakan kirang sae*	The dry season is not too long, crops might be growing not so well
3.	*Jim awal*	*Hujan seueur, pepelakan sae dina akhir taun*	A lot of rain in the wet season, crops might be growing well at the end of year
4.	*Je*	*Usum halodo, awal pepelkan sae tapi di ahir aya bencana, saperti banjir*	The dry season, crops may be growing well but in the end of year might be occurring disasters, such as flooding
5.	*Dal*	*Kirang hujan, pepelakan paparengan kadang-kadang sae atawa won*	Lack of the rining, crops sometimes might be growing well or might be not so well
6.	*Be*	*Seueur hujan, sae kanggo pepelakan*	A lot of raining and good for growing crops
7.	*Wau*	*Halodo pamjang tapi pepelakan sae*	The long dry seson and crops might be growing well
8.	*Jim ahir*	*Seueur hujan tapi pepelkan sae*	A lot of raining and crops might be growing well

hectares. In term of population, the total population of the Karangwangi was recorded 5,587 people and 5,672 people, in 2013 and 2014, respectively. The total population has dynamically changed by birth rate, death rate, and out-migration rate. The out-migration rate has been recorded rather high due to a lot of people migrated to Middle East as female (TKW = *Tenaga Kerja Wanita*; Indonesian women labor) and the male laborers. For example, 181 people consist of 14 males and 167 females were recorded in 2014 as the laborers (TKI = *Tenaga Kerja Indonesia;* Indonesian labor) in the Middle East countries (Karangwangi Village 2014).

By increasing human population, therefore, some fallowed secondary forests have been converted to dry land (*tegalan*) and usually cultivated by upland rice (*huma*) system and annual non-rice crops (*kebon*) system during the wet season. In addition, some areas of the *tegalan* have been cultivated into wet rice fields (*sawah*) system. Moreover, the wet rice fields system may be divided into some categories, namely irrigated rice fields (*sawah irigasi*) system and non-irrigated rice fields (*sawah non irigasi*) or rain fed system. Besides, some fallowed secondary forests and uplands (*tegalan*) have also been converted into the more permanent mixed-garden, planted by mixture of annual and perennial crops, which is locally called as *kebon bambu* (if bamboo trees are predominantly planted in garden), *kebon albasiah* (if albizia trees are dominant) *or kebon tatangkalan* (if various trees are dominant). It is also recognized with some local names in different places, such as *talun* in Soreang, South Bandung (Christanty et al. 1986; Mizuno et al. 2013), *kebon tatangkalan* in upper Citarum Watershed (Parikesit et al. 2004), *dudukuhan* in villages of Bogor (Manurung et al. 2005), or *dukuh lembur* in Baduy, South Banten (Iskandar 1998). This traditional agroforestry system is culturally predominantly planted by perennial crops, such as fruits and building materials. Sometimes, the *kebon tatangkalan* may be converted to *huma* (mainly panted by upland rice) and *kebon* (mainly planted by annual non-rice) during the wet season. Eventually, various land use types, dry land (*tegalan*), *reuma*, *kebon* and *kebon tatangkalan*, and *sawah* have been converted into settlement and homegarden, if in those land use types are constructed houses (Figure 2).

The *huma*, *kebon tatangkalan*, and *pekarangan* have predominantly planted by mixed of annual and perennial crops. Moreover, these traditional agroforestry systems have been culturally planted not only by rich species but also by local plant varieties (landraces). Since the *huma*, *kebon tatangkalan*, and *pekarangan* cultivated by various annual and perennial crops, the structure vegetation of these agricultural types similar to that of natural forest and we classified as the traditional agroforestry system. In this case, it means the land use type system that resembles a forest in structure and combines the natural or ecological functions of forest those for fulfilling the socio-economic and cultural needs of the people (cf. Soemarwoto and Soemarwoto 1984). The natural/ecological functions consist of hydrologic and erosion control, gene bank, microclimatic effects, enhance resilience enhance in changing climate, and potential carbon storage for reducing Emissions from the Deforestation and Forest Degradation (REDD), and animal habitats, particularly birds, insect and small mammals (cf. Soemarwoto and Soemarwoto 1984; Garrity 2015; van Noordwijk et al. 2015).Meanwhile, its socio-economic and cultural functions, include subsistence and commercial production, and socio-cultural functions. Subsistence productions of the traditional agroforestry system consists of additional staple food (taro, cassava, corn, etc.), vegetables, fruits, traditional medicines for curing of human and livestock health, and firewood and material buildings, whereas its commercial production, including fruits (orange, mango, sawo, banana, etc), and industrial materials (albizia woods, coconut, sugar palm, bamboos, etc.) (cf. Christanty et al. 1986; Jensen 1995; Okubo et al. 2010; Kosuke et al. 2013; Abdoellah et al. 2015). In addition, some crop production can fulfill various cultural functions, including aesthetical value, and the traditional ceremonies.

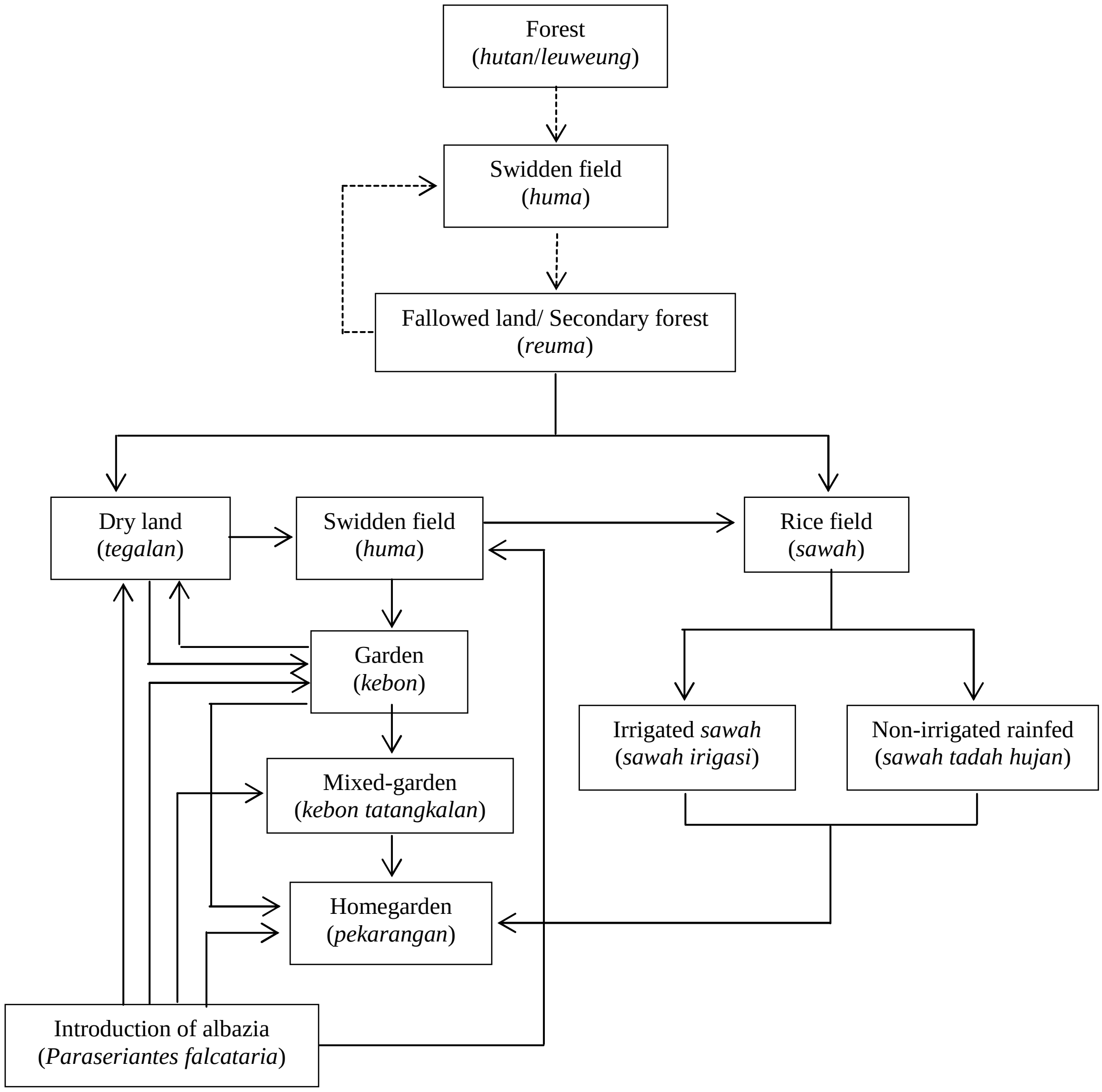

Figure 2. Evolution of the traditional agroforestry system derived from forest, and introduction of albizia (*Paraserianthes falcataria*) in the village of Karangwangi, Cianjur, West Java

Unlike in the past, nowadays the Karangwangi have practiced the swidden farming in the dry land (*tegalan*) instead of the forest area because the forests were converted to the traditional agroforestry, namely homegarden and mixed-garden and relict forest was converted to the Nature Reserve of Bojonglarang-Jayanti. The swidden farming system has been modified and adapted to local ecological and socio-economic condition. For example, the upland rice farming was normally cultivated by modern rice varieties mixed with corn varieties and beans. In addition, some inputs, such as seeds, pesticides, and industrial chemical fertilizers have been introduced from the market or urban area.

The traditional agroforestry systems and responses to environmental and economic changes

The last several decades, the traditional agroforestry systems of Karangwangi Village have been affected by various environmental and socio-economic changes. For example, a village road existed from Karangwangi to Cianjur by the Dutch to service plantation in the area. However, access to Karangwangi from the district of Cianjur was constructed by the early 2000s predominantly made of river stones. However, since 2009 the village road of Karangwangi has been upgraded by using asphalt. In addition, several bridges over the village road, including Cikawung and Cilaki Rivers have been built by permanent constructions. As a result, some vehicles now comfortably travel between the Karangwangi Village and Cianjur, Garut or Bandung urban. Since more easily access between Karangwangi and urban areas of Cianjur, Garut, and Bandung, the market economic system has intensively penetrated. For example, in the past the various agricultural productions of the agricultural productions, including the traditional agroforestry had been culturally traded in the village market. It has been recorded one village market in

Karangwangi, namely Pasar Desa Puncak Bayuning which is regularly operated weekly in Monday. In addition, some other village markets are also found in the Karangwangi adjacent areas, including Pasar Palabuhan Jayanti of Cidamar Village which is regularly weekly operated in each Sunday, Pasar Naringgul of Naringgul Village weekly operated in Sunday, Pasar Mangunjaya of Mangunjaya Village in Monday, Pasar Kertajati of Kertajati Village in Thursday and Thursday, and Pasar Ciujung of Mangunjaya Village in Friday. Nowadays, however, the number of small shops (*warung*) and permanent shops (*toko*) in the Karangwangi has increased. For instance, 97, 103, and 200 small shops and permanent shops were recorded in 2012, 2013 and 2014, respectively (Cidaun Sub-district 2015).

By rapid developing the transportation and communication, consequently, the agricultural land has increasingly demanded by urban people to establish commercial gardens or plantations, such as oil palm plantation. Indeed, the agricultural land price has dramatically increased. At the same time, some traditional agroforestry systems have also been predominantly converted to more commercial monoculture system, such as homogenization of Moluccan albizia (*Paraserianthes falcataria*).

Despite socio-economic and environmental events, including recent irregularly drought and the 2015 El Nino, the Karangwangi traditional agroforestry systems, including the *huma* system have been maintained and overall production levels have not been seriously affected compared to intensive wet rice field (*sawah*) farming in the lowland northern parts of West Java. It is caused of the Karangwangi people have developed some appropriate strategies, including development of the adoption of land certification, planting crop diversification; introduction of albizia; involving in petty trading; and diversification of off-farm job activities (cf. Iskandar 2007).

Land certification

As mentioned earlier that in the past, the Karangwangi culturally practiced the swidden in the forest which each household owned the swidden field and other swidden fallowed fields at the form of the secondary forest (*reuma*) in different fallowed times with have status as the tenure system. Today, however, most people of the Karangwangi people annually predominantly practice shifting cultivation (*ngahuma*) in non-forest areas, particularly by cultivating the upland field (*tegal*) and mixed-garden. Moreover, the dry fields (*tegal*) can be divided into two categories, namely the certified land (*tanah sertifikat*) and non-certified land (*tanah pangangonan* or grazing land). Recently, the dry lands have been predominantly certificated. For example, 2,780 plots which have total 360 hectares consist of 230 plots of 45 hectares and 2,550 plots of 315 hectares have been certified via the National Program and Redistribution, respectively. Meanwhile, the rest of the uplands have not been certified 500 plots of 350 hectares (Karangwangi Village 2014). The land certifications have tended to increase due to increasing of village human population parallel with the increase of food demand and development of agricultural technology, and increasing both land use intensity and labor intensity (cf. Boserup 1965; Raintree and Warner 2015). In other words, the people of Karangwangi have engaged in obtaining the land certification may be considered as strategy of people to get more secure on the land ownership system and to avoid social conflict on the land ownership in the near future. At this time, by improving the village road and more easily assess from the city, the land price has dramatically increased and many people of urban would like to buy the land for various purposes, including establishing the commercial plantations, such as oil palm plantation. Based on our study, we can predict that a lot of the agricultural land may be sold to outsiders and some social conflict on agricultural land conflict in the village may be occurred in the near future. Particularly, if the village leader and other stake holders have not consistently to maintain village lands that are bought by outsiders.

Planting crop diversification

Culturally, various traditional agroforestry types in Karangwangi, including the homegarden, mixed-garden, and swidden farming (*huma*) have been planted by a high diversity of plants. For example, based on survey 81 homegardens of Karangwangi Village, it was recorded 141 species of 61 families, with 62 per cent categorized as food materials (Aniya 2015). In addition, at least it was recorded 13 species of bamboo and 13 varieties (landraces) of banana (*Musa* sp.) in the traditional agroforestry of mixed-garden. Because the high species and varieties of local plant planted in various traditional agroforestry system; as a result it has been an important role as genetic sources which can be used for plant breeders to create new crop varieties in supporting the sustainable agricultural development to anticipate against future risk and uncertainty, caused of environmental stresses, including drought, flooding, and outbreak pests.

In addition, the biodiversity of plants of the traditional agroforestry systems have been an important to enhance the mitigation and adaptation of farmers and environmental and market economic changes. Generally, the high plant diversity planted in the traditional agroforestry systems has strongly related to stability of this traditional agroforestry on pest outbreak, drought, flooding, and economic changes (cf. Iskandar 1998; Iskandar 2007). For example, recent irregularly drought and the 2015 El Nino serious affected on rice production of the lowland rice field (*sawah*) farming of West Java. However, unlike the lowland sawah farming, the production of several types of the traditional agroforestry systems of Karangwangi, including homegarden and mixed-garden have severely reduced cased of drought. Empirically, during the dry season and drought time, the number of annual crops has decreased; but some perennial crop productions, such as bamboos, building material woods, such as albizia and jabon and various fruits, including sawo (*Manilkara zapota*), mangga (*Mangifera indica*), and alpuket (*Persea americana*) have not been not seriously damaged by drought. Conversely, by dry season and drought with full sun and full, might maximal produce fruits in the home gardens and mixed-gardens (cf. Iskandar 2007).

Introduction of albizia

Initially, Moluccan albizia or locally known as albasiah or sengon (*Paraserianthes falcataria*) and jabon (*Anthocephalus* sp.) were introduced in Karangwangi Village by the Forestry Office (*Dinas Kehutanan*) through the regreening program to rehabilitate the degradation forest and the former secondary fallowed land of the swidden system in 2000s. More recently, many people have popularly adopted these trees, particularly albizia trees planted in their upland fields (*tegalan*), swidden fields (*huma*) or mixed-gardens (*kebon tatangkalan*). Generally, the albizia trees have been predominantly planted in *tegalan* with monoculture system; whereas in the *huma* and *kebon tatangkalan* have been commonly planted with polyculture system (*tumpang sari*) which is strongly embedded by 'hybrid knowledge' (cf. Iskandar and Ellen 2000). Culturally, the albizia trees have been planted mixed with annual and perennial crops, such as upland rice (*Oryza sativa*), kacang hiris (*Cajanus cajan*), kacang tanah (*Arachys hipogaea*), cau (*Musa paradisiaca*), kapolaga (*Elettaria cardamomum*), and mahoni (*Swietenia mahagoni*). In the past, seeds of albizia were freely provided by the Forestry Office of Cianjur District (*Dinas Kehutanan*). Today, however, seeds of albizia are obtained from different sources, such as prepared by farmer themselves to make seedbed in the polybags, buy from other people at the same village who usually sell seeds by paying cash or credit, and buy from small shops in Sub-district of Cidaun and Rancabuaya. If the albizia trees are planted by intercropping with annual crops, they are commonly provided by various fertilizers, such Urea, NPK, Poskaan, and animal dung at least one time.

About between 5 and 7 years after planting, the albizia trees have been readily to harvest. The albizia wood production can be used for various household purposes and for commercial purposes. There are three model of harvesting albizia trees, particularly if the trees are going to sell. Firstly, all trees are totally sold by the owners to middlemen, and by paying cash before harvesting time, called as 'selling wholesale' (*jual borongan*). Secondly, the albizia trees are sold to buyers in cubic meter, selling the albizia trees that have been cut and special length measure between 130 cm and 160 cm, called as *jual cara palet*. It has price between Rp 670,000 and Rp 890,000 per m^3.Thirdly, the albizia trees are sold in the form of that has been processed in to the form of wood beams (*balok*) and planks (*papan*), which can be used for various purposes, such aschair furniture (*mebel*). It has price between Rp 1,500,000/m^3 and Rp 2,000,000/m^3.

Involving in petty trading

Productions of various traditional agroforestry systems, including *huma*, *pekarangan*, and *kebon tatangkalan* of the Karangwangi have been used both to fulfill household daily needs and some surpluses usually to sell to middle men and consumers in the weekly village markets. For example, various traditional agroforestry products, including gula aren (sugar palm, *Arenga pinnata*), cau, sawo, mangga, alpuket, petai (*Parkia speciosa*), and jengkol (*Archidendron pauciflorum*) are commonly sold in the weekly village markets, such as Pasar Desa Puncak Bayuning of Karangwangi Village and Pasar Palabuhan Jayanti of Cidamar village.

Today, in response to more intensive market economic penetration, many people in Karangwangi have been involving in petty trading as middlemen by trading various the traditional agroforestry products sold in the weekly markets (*pasar mingguan desa*) and village small shops (*warung*). In addition, some people have been involving in selling albizia seeds, albizia woods, bamboos, and fermented banana (*sele pisang*).

Diversification of off-farm jobs

Increasing people adoption of cultivating albizia in various traditional agroforestry systems have provided some benefits for local people, including in provide the off-farm job opportunity. For example, many people have involved in some jobs of harvesting albizia trees. Generally, albizia trees are cut by assisting some laborers, namely as woodcutter with chainsaw (*tukang nyenso*), carrying albizia logs, and transporting albizia logs. In addition, home industry in relation to process albizia woods for various products, such as table, chair, and cupboard. As a result, some people have involved as carpenters and construction workers.

Utilization of bamboos and bananas which are produced from the agroforestry systems has also provided some off-farm jobs for the local people. Generally, bamboo trees are commonly used to fulfill various people needs and to sell to middlemen. In addition, some people have engaged in the off-farm jobs in relation to bamboo production processes, including making various household utensil and handicrafts made of bamboo materials, such as bamboo wall (*bilik awi*), bamboo chair (*kursi bambu*), bamboo winnowing (*nyiru*), and bamboo basket (*cerangka*). Like bamboo, banana productions have generally used for fulfill households as food for home consumption. Some banana products, however, have also been industrially processed by households making the fermented banana (*pisang sale*). Moreover, the fermented bananas have been sold in villages and sent to urban markets of Cianjur and Bandung.

This paper has discussed on ecological or environmental historical of the shifting cultivation in West Java, particularly based on case study carried out in Karangwangi Village (cf. Boomgaard 1997). We begin describe the shifting cultivation system (*huma*) that was undertaken by the local people in the past. At that time, the local people farmed various local upland rice varieties and other annual crops in the forests which are strongly embedded by traditional local knowledge and cosmos (cf. Iskandar 1998; Toledo 2000; Iskandar 2007). The farming of *huma* practiced in the past may be considered as cultural adaptation and achieved extremely efficient and sustainable agricultural system of the frontier upland of West Java, when forest was still large, land was cheap resource, human population was low, labor was the expensive resource and economic market non existent. Gradually, however, as forest reduced, population increased, land has become a more expensive resource and market economy has intensively penetrated to rural areas, swidden agriculture has become less advantageous economically and less sustainable ecologically (cf. Bunch 2015). As a result, the traditional shifting cultivation system of the Karangwangi had developed into other traditional agroforestry systems, such as homegarden and mixed-garden and land tenure system have gradually changed to fixed ownership system by obtaining land certificate. In parallel with intensive market economic penetration to rural areas, commercial trees, namely albizia have been culturally adopted by the local people. This species has been culturally adopted due to provide some ecological and economic benefits. For example, the albizia is categorized as Leguminoceae family, capable improving soil fertility through nitrogen fixation, easily cultivated, provides for various needs, as well as yielding cash income (cf. Iskandar and Ellen 2000). Moreover, to adapt with ecological and socio-economic changes, such as forest and agricultural decrease, human population increase, and intensive market economic penetration increase, the Karangwangi people have developed some appropriate strategies, namely maintaining various traditional agroforestry systems and also various factor that can maintain their traditional agroforestry and their income, including adoption of land certification, planting crop diversification; involving in petty trading; and diversification of off-farm job activities.

In conclusion, by developing innovation cultural practice, Karangwangi people of South Cianjur, West Java have tended to success to evolve their shifting cultivation to new condition of the traditional agroforestry systems in sustainable way, despite population growth, the depletion of the forests, and intensive market economic penetration.

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REFERENCES

Abdoellah OS, Pariksit, Okubo S, Withaningsih S, Takeuchi K, Mizuno K. 2015. Perception of owners on the Roles and Future of Bamboo-Tree Gardens in the Agricultural Landscape of the Upper Citarum Basin, West Java-Indonesia. Agri Sci 6: 1333-1351

Aniya MU. 2015. Plant diversity studies and functions utilization courtyard in the Village Karangwangi Cidaun Sub-district of Cianjur District, West Java. [Research Report]. Padjadjaran University, Bandung [Indonesia].

Boomgaard P. 1997. Introducing environmental histories of Indonesia. In Boomgaard, Colombijn F, Henley D (eds) Paper Landscapes: Explorations in the environmental history of Indonesia. KITLV Press, Leiden.

Boserup E. 1965. The conditions of agricultural growth: the economics of agrarian change under population pressure. George Allen & Unwin Ltd., London

Breman J. 2014. Colonial Advantage of the Forced Labor System of the Plant Forced Priangan coffee in Java. Yayasan Pustaka Obor Indonesia, Jakarta. [Indonesia]

Bunch R. 2015. Learning from migratory agriculture around the world. In: Cairns MF (eds). Shifting Cultivation and Environmental Change: Indigenous People, Agriculture and Forest Conservation. Routhledge, London.

Bunch, R. 2015. Learning from migratory agriculture around the world. In: Cairns MF (eds). Shifting Cultivation and Environmental Change: Indigenous People, Agriculture and Forest Conservation. Routhledge, London.

Christanty L, Iskandar J, Abdoellah OS, Marten GG. 1986. Traditional agroforestry in West Java: The pekarangan (homegarden) and talun-kebun (annual-perennial rotation) cropping systems. In: Marten GG (eds). Traditional Agriculture in South East Asia: A Human Ecology Perspective. Westview Press, Boulder.

Cidaun Sub-district. 2015. Statistical data of Cidaun Sub-district 2015. Cidaun, Cianjur.

Colfer CJP, AlcornJB, Russell D. 2015. Swidden and fallows: Reflections on the global and local values of 'slash and burn'. In: Cairns MF (eds). Shifting Cultivation and Environmental Change: Indigenous People, Agriculture and Forest Conservation. Routhledge, London.

Cotton CM. 1996. Ethnobotany: Principles and applications. John Willey and Sons Ltd, London.

Cunningham AB. 2001. Applied Ethnobotany: People, World Plant Use and Conservation. Earthscan Publication, London.

Garrity D. 2015. Learning to cope with rapid change: Evergreen agriculture with rapid change: Evergreen agriculture transformations and insights between Africa and Asia. In: Cairns MF (eds). Shifting Cultivation and Environmental Change: Indigenous People, Agriculture and Forest Conservation. Routhledge, London.

Geertz C. 1963. Agricultural involution: The Processes of ecological change in Indonesia. University of California Press, Berkeley.

Iskandar J, Ellen FR. 1999. In Situ Conservation of rice landraces among the Baduy of West Java. J Ethnobiol 19 (1): 97-125.

Iskandar J, Ellen FR. 2000. The Contribution of *Paraserianthes* (*Albizia*) *falcataria* to sustainable swidden management practices among The Baduy of West Java. Human Ecol 28 (1): 1-17.

Iskandar J, Iskandar BS. 2011. Sundanese People Agroecosystem. Kiblat Utama Press, Bandung. [Indonesia]

Iskandar J. 1998. Swidden cultivation as a form of cultural identity: The Baduc case. [Dissertation]. University of Kent at Canterbury. UK

Iskandar J. 2007. Responses to environmental stress in the Baduy Swidden system, SouthBanten. In Ellen RF. (eds). Modern Crises and Traditional Strategies: Local Ecological Knowledge in Island Southeast Asia. Berghan Books, New York.

Iskandar J. 2012. Ethnobiological and Sustainable Development. [Research Report]. University of Padjadjaran, Sumedang. [Indonesian]

Jensen M. 1995. Woodfuel Productivity Systems in Asia: A Review of Current Knowledge. FAO, the United Nations, Bangkok.

Karangwangi Village 2014. Statistical Data of Karangwangi Village 2014. Karangwangi Village, Cianjur.

Kartawinata K, Abdulhadi R. 2015. Fallows and forest restoration. In: Cairns MF (eds). Shifting Cultivation and Environmental Change: Indigenous People, Agriculture and Forest Conservation. Routhledge, London.

Kools JF. 1935. Hoemas, Hoemablokken en Boschreserves in de residentie Bantam. H. Veenman & Zonen, Wageningen.

Kosuke M, Mugniesyah SS, Herianto SSet al. 2013. Talun-huma, Swidden Agriculture, and Rural Economy in West Java, Indonesia. Southeast Asia Stud Kyoto Univ 2 (2): 351-381.

Lombard, D. 1996. Nusa Jawa: Cross-cultural Study of Integrated History. Gramedia Pustaka, Jakarta. [Indonesia]

Manurung GES, Roshetko JM, Boedihasono S et al. 2005. Dudukuhan: traditional farming systems for poverty reduction. In Ploeg V, Masipiquena VD (eds). The Future of the Sierra Madre: Responding to Social and Ecological Changes. Proceeding of the 5th International Conference on Environmental and Development. CVPED, Golden Press, Tuguegararo.

Martin GJ. 1995. Ethnobotany: A Methods Manual. Chapman & Hall, London.

Newing H. 2011. Conducting Research in Conservation: A Social Science Perspective. Routledge, London.

Okigbo BN. 1984. Improve permanent systems as an alternative to shifting cultivation intermittence cultivation. In: Okigbo BN (eds). Improved Production Systems as an Alternative to Shifting Cultivation. FAO, Rome.

Okubo S, Parikesit, Harashina K et al. 2010. Traditional perennial crop-based groforestry in West Java: the tradeoff between on-farm biodiversity and income. Agrofor Syst 80: 17-31.

Parikesit, Takeuchi T, Tsunekawa A et al. 2004. Kebon tatangkalan: a disappearing agroforest in the Upper Citarum Wtershed, West Java, Indonesia. Agrofor Syst 63: 171-182.

Rahman SA, Jacobsen JB, Healey JR. 2016. Finding alternatives to swidden agriculture: does agroforestry improve livelihood options and reduce pressure on existing forest? Agroforest Syst. DOI 10.1007/s10457-016-9912-4

Raintree J. Warner K. 2015. Agroforestry pathways revisited: voices from the past. In: Cairns MF (eds). Shifting Cultivation and Environmental Change: Indigenous People, Agriculture and Forest Conservation. Routhledge, London.

Reijntjes C, Haverkort B, Waters-Bayer B. 1992. Farming for the future: An introduction to low-external-input and sustainable agriculture. The MacMillan Press Ltd, London.

Rerkasem K, Lawrence D, Padoch C et al. 2009. Consequences of swidden transition for crop and fallow biodiversity in Southeast Asia. Human Ecol 37: 347-360.
Sajise PE. 2015. Biodiversity and swidden agroecosystems: An analysis and some implications. In: Cairns MF (eds). Shifting Cultivation and Environmental Change: Indigenous People, Agriculture and Forest Conservation. Routhledge, London.
Sanchez PA. 1976. Properties and Management of Soil in the Tropics. Willey Interscience, NewYork.
Soedjito H. 2015. Shifting cultivators, curators of forests and conservators of biodiversity: The Dayak of East Kalimantan, Indonesia. In: Cairns MF (eds). Shifting Cultivation and Environmental Change: Indigenous People, Agriculture and Forest Conservation. Routhledge, London.
Soemarwoto O, Soemarwoto I. 1984. The Javanese rural ecosystem. In Rambo AT, Sajise PE (eds). An Introduction to Human Ecology Research on Agricultural Systems in Southeast Asia. East-West Environment and Policy Institute, Hawaii.
Soepomo. 1982. Customary Civil Law in West Java. Penerbit Djambatan Jakarta. Indonesian]
Terra GJA. 1953. The Distribution of mixed-gardening on Java. Landbouw 25: 163-223.
Terra GJA. 1958. Farm system in South-East Asia. Netherland J Agri Sci 6 (3): 1157-1182.
Toledo, V.M. 2002. Ethnoecology: A Conceptual Framework for the Study of Indigenous Knowledge of Nature. In J.R. Stepp, F.S. Wyndham, and R.K. Zarger (eds). Ethnobiology and Biocultural. The International Society of Ethnobiology, Georgia.
Van Noordwijk M, Minang PA, Hiriah K. 2015. Swidden Transitions: In an era of climate-change debate. In: Cairns M (eds). Shifting Cultivation and Environmental Change. Earthscan, New York, USA
Von Maydell, 1985.The contribution of agroforestry to World Forestry Development. Agrofor Syst 3: 83-90.

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Four newly recorded *Amanita* taxa from India

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Abstract. *Singh Y, Kaur M. 2016. Four newly recorded* Amanita *taxa from India*. *Biodiversitas 17: 342-348.* During the fungal forays in district Himachal Pradesh of North Western India, four unrecorded taxa of *Amanita* were collected. They are *Amanita flavoconia* var. *flavoconia*, *A. flavoconia* var. *inquinata*, *A. pilosella* f. *pilosella* and *A. porphyria*. *A. flavoconia* var. *flavoconia* is distinctive in having a brilliant yellow to yellow orange cap, with white lamellae and a stipe base turning light brownish on injury. Whereas. *A. flavoconia* var. *inquinata* possesses brownish orange to yellowish orange to orange yellow, subvicid pileus, having white to pastel yellow stipe, annulus grayish yellow, superior and volva forming broken rings of yellow patches around the bulb. *A. pilosella* f. *pilosella* unique in possessing grayish brown or brownish beige pileus having thick irregular warts and white lamellae edges. *A. porphyria* is represented by nonstriate pileus margin, off white stipe, decorated with grayish squamules, with violaceous tinge and has a marginate bulb, annulus is persistent, off white above and light grey below and volva is friable, as grey, cottony mass at the margin of the bulb.

Keywords: *Amanitaceae*, new record, India, taxonomy

INTRODUCTION

The genus *Amanita* belongs to the family Amanitaceae R. Heim ex. Pouzar. It comprises of both the edible as well as deadly poisonous species. It is known with 500 species worldwide (Kirk et al. 2008), but so far only 66 species are reported from India (Bhatt et al. 2003, Farook et al. 2013, Semwal et al. 2005, 2007, Vrinda et al. 2005). Very meager work has been done on the taxonomy of this genus in India. There are still many more species to be collected and identified. In this paper, four taxa viz. *A. flavoconia* var. *flavoconia* G. F. Atk., *A. flavoconia* var. *inquinata* Tulloss, Ovrebo & Halling, *A. pilosella* Corner & Bas and *A. porphyria* Alb. & Schwein.: Fr. belonging to subgenus *Lepidella* (E. J. Gilb.) Vesely emend. Corner & Bas and section *Valideae* (Fr.) Quél. are included, which have amyloid basidiospores, none appendiculate pileus margin, lacking a membranous, limbate to saccate volva. All these taxa recorded for the first time from India.

MATERIALS AND METHODS

The specimens were collected from Himachal Pradesh in North Western India with an altitude ranging from 1500 to 3647 m in temperate zones. The morphological details were recorded from fresh sporophores. The field characters pertaining to gross morphology, shape, color and size of the pileus, stipe and lamellae, presence or absence of annulus and volva were noted down in the field following Atri et al. (2005) and Kornerup and Wanscher (1978). The specimens were hot air dried and packed in cellophane paper bags containing 1-4 dichlorobenzene. The microscopic details were studied under compound microscope (Olympus) on 100x and 40x by cutting free hand sections of revived part of the dried specimen and staining them in 1% cotton blue or 2% congo red. The spores were studied from the spore print as well as from the crushed mounts of the lamellae and amyloid reaction were checked in Melzer's reagent. Statistics are based on 20-70 measured basidiospores. Abbreviations include Q' = the length: width ratio range as determined from all measured basidiospores. The dried specimens are deposited in the Herbarium of Botany Department, Punjabi University, Patiala, Punjab, India under accession numbers given as PUN for further reference.

RESULTS AND DISCUSSION

***Amanita flavoconia* var. *flavoconia* G. F. Atk.** *J. Mycol.*, 8 (3): 110, 1902.Figures 1.A, 1.B, and 2

Synonyms. *Amplariella flavoconia* (G. F. Atk.) E.-J. Gilbert. *Iconogr. Mycol. (Milan)* 27, suppl. (1): 78, 1940.

Venenarius flavoconius (G. F. Atk.) Murrill. *Lloydia* 11 (2): 101, 1948.

Sporophores 8.5-11.8 cm in height. Pileus 3.2-6.0 cm broad, companulate to convex; with or without an umbo; margin regular, none appendiculate, feebly striate; surface yellow ($2A_6$), light orange ($5A_5$), dark orange or deep orange ($5A_8$) at centre, deep yellow ($4A_8$) towards margins; covered with powdery, yellow volval remnants, more concentrated along margin; detersile; vicid; atomate; cuticle fully peeling; flesh white, unchanging, up to 0.1 cm thick; odor good like cucumber. Lamellae free to adnexed, subdistant to close, unequal, broad (up to 0.5 cm), yellowish white ($1\text{-}3A_2$); gill edges serrate, yellowish; lamellulae attenuate. Stipe central, 7.0-10.8 cm long, up to

1.2 cm broad above, up to 1.5 cm broad from centre and up to 2.5 cm broad at bulb, distinctly bulbous; white ($1A_1$) to pale yellow ($2A_3$), to light yellow ($4A_4$), with a whitish bulb; surface shiny, covered with pastel yellow ($3A_4$) fibrillose scales; solid; annulate, annulus skirt like, striated, pastel yellow ($3A_4$); volva in yellow concentric rings of floccose, cottony mass, detersile, easily lost while collecting; stipe base turns light brownish on injury.

Basidiospores [72/3/3] (5.6-) 6.4-9.6 (-10.4) × 4.8-7.2 (-8.8) µm (**L** = 6.4-8.8 µm; **L'** = 7.8 µm; **W** = 5.6-6.4 µm; **W'** = 6.0 µm; Q = 1.14-1.43 (-1.67); **Q'** = 1.30); subglobose, broadly ellipsoid to ellipsoid, occasionally elongate; amyloid, thin walled, hyaline, smooth; apiculate, apiculus up to 1.6 µm long. Basidia 19.2-48.0 x 6.4-9.6 µm, clavate, without clamp connections, tetrasterigmate, occasionally bisterigmate; sterigmata up to 7.2 µm long. Lamellae edge cells 12.8-27.2 x 6.4-22.4 µm, clavate to balloon shaped, grannular. Pileus cuticle hyphal, gelatinized, made up of sub-radially tangled, granular, septate 1.6-6.4 µm broad hyphae; pileus context made up of loosely interwoven, horizontally arranged, septate, granular, 3.2-12.8 µm broad hyphae; acrophysalides narrowly clavate, subglobose to ellipsoid, up to 64.0 µm broad. Hymenophoral trama bilateral divergent. Stipe cuticle made up of longitudinally and compactly arranged, thin walled, septate 1.6-8.0 µm broad hyphae; stipe context made up of loosely arranged, thin walled, 3.2-14.4 µm broad hyphae; acrophysalides thin walled, abundant up to 40.0 µm broad. Volval elements composed of thin walled, smooth, septate, 4.1-12.3 µm broad hyphae, intermixed with globose, subglobose, broadly ellipsoid to elongated, 20.4-102.2 x 16.4-73.6 µm inflated cells. Clamp connections absent throughout.

Material examined. India, Himachal Pradesh, Narkanda, Hattu peak (2,800 m), growing solitary under *Abies pindrow* in mixed coniferous and broad leaved forest, Yadwinder Singh and Munruchi Kaur, PUN 3845, August 14, 2007; Shimla, Faggu (2,600 m), growing scattered on humicolous soil, in the coniferous forest, Munruchi Kaur and Yadwinder Singh, PUN 3847, August 12, 2007; Shimla, Chadwick fall (1,800 m), growing solitary, under *Cedrus deodara* in mixed coniferous forest, Yadwinder Singh, PUN 6433, August 13, 2009.

Distribution and Ecology. *Amanita flavoconia* var. *flavoconia* was found growing solitary to gregarious in hardwood forest, swampy area of mixed deciduous forest and Northern Hemlock-Hardwood forest constituting *Quercus alba*, *Q. palustris*, *Acer*, *Fagus grandifolia*, *Liriodendron tulipifera*, *Tsuga canadensis*, *Betula allegheniensis*, *Carya ovata* and *Viburnum acerifolia* from New Jersey at 780 m-1,760 m altitude (Tulloss 2013a). Presently examined collections have been found growing solitary to scattered on humicolous soil (PUN 3847), under *Abies pindrow* (PUN 3845) and under *Cedrus deodara* (PUN 6433), in mixed coniferous and broad leaved forest or mixed coniferous forest at an altitude varying from 1,800-2,800 m in Mid August.

Discussion. The above examined collections completely match with the description given for *Amanita flavoconia* var. *flavoconia* by Jenkins (1982) and Tulloss et al. (2001). It is distinctive in having a brilliant yellow to yellow orange cap, with white lamellae and a stipe base turning light brownish on injury. Bhatt et al. (1988) and Kumar et al. (1990a, b) reported *A. flavoconia* from different parts of Himachal Pradesh, while Abraham and Kachroo (1989) and Pandotra (1997) reported this species from Jammu and Kashmir. Later, on the revision of collections of Himachal Pradesh and Jammu and Kashmir by Tulloss et al. (2001), these were found to be falling under *A. flavipes* S. Imai and later Bhatt et al. (2003) recommended the transfer of Indian specimens know under *A. flavoconia* to *A. flavipes.* The present collections belong to *A. flavoconia* as they differ from *A. flavipes* in having less robust sporophores with cap color varying in shades of yellow to deep orange and having serrate gill edges, the bulb changing to light brown on injury. *Amanita flavoconia* var. *flavoconia* is the first report from India.

***Amanita flavoconia* var. *inquinata* Tulloss, Ovrebo & Halling,** *Mem. New York Bot. Gard.*, 66: 30, 1992. Figures 1.C and 3

Synonym. *Amanita flavoconia* var. *sinapicolor* Tulloss, Ovrebo & Halling. *Mem. New York Bot. Gard.* 66: 34, 1992.

Sporophore up to 10.5 cm in height. Pileus up to 3.9 cm broad, flattened depressed; lacking umbo; margin regular, splitting at maturity; surface brownish orange ($6C_{5-6}$) at centre and yellowish orange to orange yellow ($5B_{6-7}$) towards margin, washed out due to rain; subvicid; glabrous; cuticle fully peeling; flesh white, unchanging, up to 0.2 cm thick; odor radish like. Lamellae free, subdistant, moderately broad (up to 0.4 cm), unequal, yellowish white ($4A_2$); gill edges serrate, yellowish white; lamellulae truncate. Stipe central, up to 9.5 cm long, up to 0.7 cm broad above, and up to 1.7 cm broad at base, with subglobose bulb at base, pastel yellow ($3A_4$) above, white at bulb, covered with yellowish scales; hollow; annulate, annulus superior, single, skirt like, membranous, collapsing on the stipe, hanging, grayish yellow ($4B_5$), striated above, smooth below; volva as yellow patches forming broken rings, around bulb, can easily lost while collecting.

Basidiospores [35/1/1] (5.6-) 6.4-9.6 (-11.2) × (4.8-) 5.6-7.2 (-8.0) µm (**L** =6.4-8.0 µm; **L**' = 8.0 µm; **W** = 5.6-7.2µm; **W'** = 6.4 µm; Q = (1.0-) 1.11-1.42 (-1.67); **Q'** = 1.27); subglobose to broadly ellipsoid to ellipsoid, infrequently globose, rarely elongated; amyloid, thin-walled, smooth, hyaline; apiculate, apiculus up to 1.6 µm long. Basidia 24.0-45 x 8.0-9.6 µm, clavate, inamyloid, without clamp connections; tetrasterigmate; sterigmata up to 6.4 µm. Pileus cuticle hyphal, made up of subradially intermingled, granular, septate 2.4-6.4 µm broad hyphae; pileus context made up of loosely interwoven, subradially to loosely interwoven, septate, granular, 3.2-11.2 µm broad hyphae; acrophysalides clavate to narrowly clavate, ellipsoid, up to 40.0 µm broad. Hymenophoral trama bilateral divergent. Stipe cuticle made up of longitudinally and compactly arranged, thin walled, septate 1.6-8.0 µm broad hyphae; stipe context made up of loosely interwoven, thin walled, 3.2-11.22 µm broad hyphae; acrophysalides abundant, narrowly clavate, up to 32.0 µm broad. Volval

Figure 1. A. *Amanita flavoconia* var. *flavoconia*. Carpophore growing in its natural habitat. B. Under side of cap showing yellowish white free lamellae with yellowish edges and distinctly bulbous stipe base having yellow, floccose volval remnants arranged in concentric rings. C. *Amanita flavoconia* var. *inquinata*. Carpophore showing cap brownish orange at centre and yellowish orange to orange yellow towards margin. D. *Amanita pilosella* f. *pilosella*. Carpophore growing in its natural habitat and showing cap surface grey to light grayish, decorated with fibrillose, squarros, flat, powdery, irregular warts. E. *Amanita porphyria*. Sporophore showing stipe with marginate bulb at the base. F. Cap surface dark brown at centre and light brown to brownish grey along margin and cuticle fully peeling

elements composed of thin walled, smooth, branched, septate, 2.0-6.1 µm broad hyphae, intermixed with subglobose, broadly ellipsoid to ellipsoid, clavate, elongated, subpyriform, 20.4-98.0 x 12.3-82.0 µm inflated cells arranged in chains. Clamp connections absent throughout.

Material examined. India**,** Himachal Pradesh, Narkanda, Hattu peak (2,800 m), growing solitary, on humicolous soil, in mixed coniferous forest, under *Picea smithiana*, Munruchi Kaur and Yadwinder Singh, PUN 6434, August 14, 2007.

Distribution and Ecology. Tulloss et al. (1992) reported *Amanita flavoconia* var. *inquinata* growing solitary to gregarious in loamy soil, under different species of *Quercus* in Colombia and Costa Rica at an altitude varying from 1,675-3,000 m, from México they collected this species from a mixed forest including *Pinus patula*, *Abies religiosa*, *Quercus* sp., *Arbutus xalapensis*, and *Baccharis conferta* at an altitude of 2,800 m. The present collection was found growing solitary, under *Picea smithiana* in mixed coniferous forest during mid August.

Discussion. In its macroscopic and microscopic details present collection matches well with *Amanita flavoconia* var. *inquinata* Tulloss, Ovrebo & Halling (Tulloss et al., 1992). In the field this variant is confusing with *A. flavoconia* var. *flavoconia*. *A. flavoconia* var. *flavoconia* differs from the present specimen in possessing brilliant yellow to yellow orange cap, with white lamellae and basidiospores broadly ellipsoid to ellipsoid, infrequently subglobose. The present collection is characterized in possessing brownish orange to yellowish orange to orange yellow, subvicid pileus, having white to pastel yellow stipe, with bulb at base, annulus grayish yellow, superior, collapsing on the stipe, while volva forming broken rings of yellow patches around the bulb, which are easily lost while collecting. *Amanita flavoconia* var. *inquinata* is the first report from India.

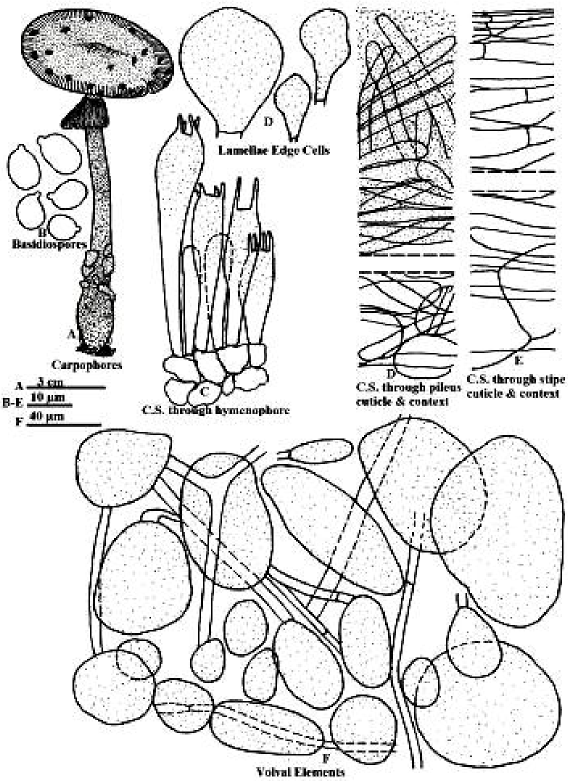

Figure 2. Internal details of *A. flavoconia* var. *flavoconia*

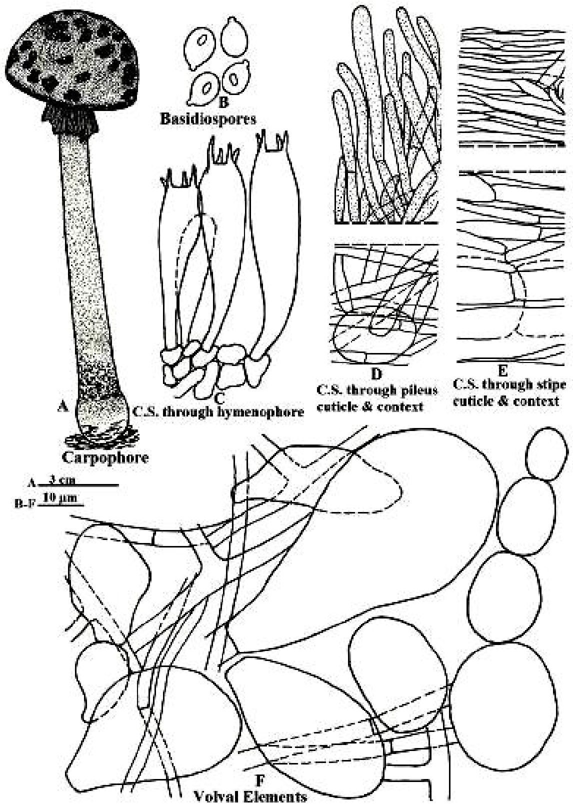

Figure 4. Internal details of *A. pilosella* f. *Pilosella*

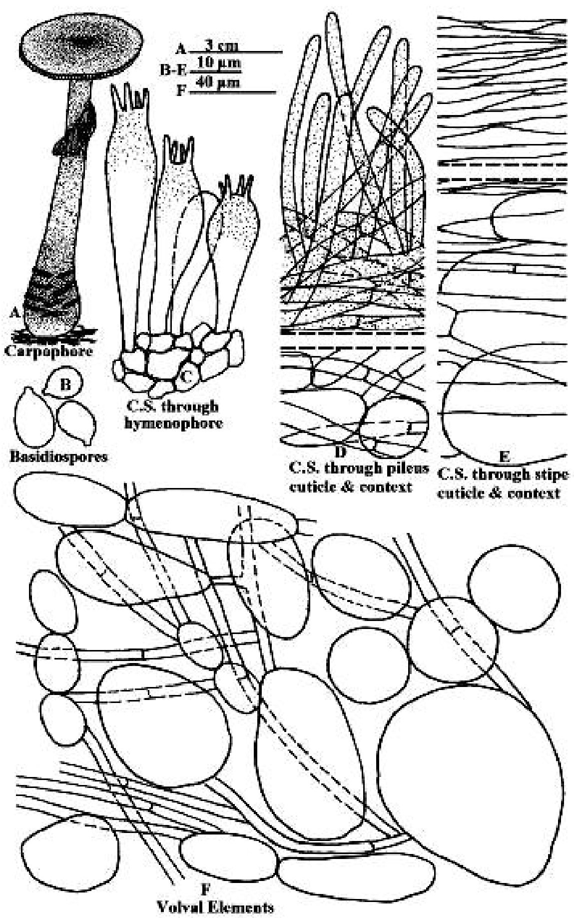

Figure 3. Internal details of *A. flavoconia* var. *inquinata*

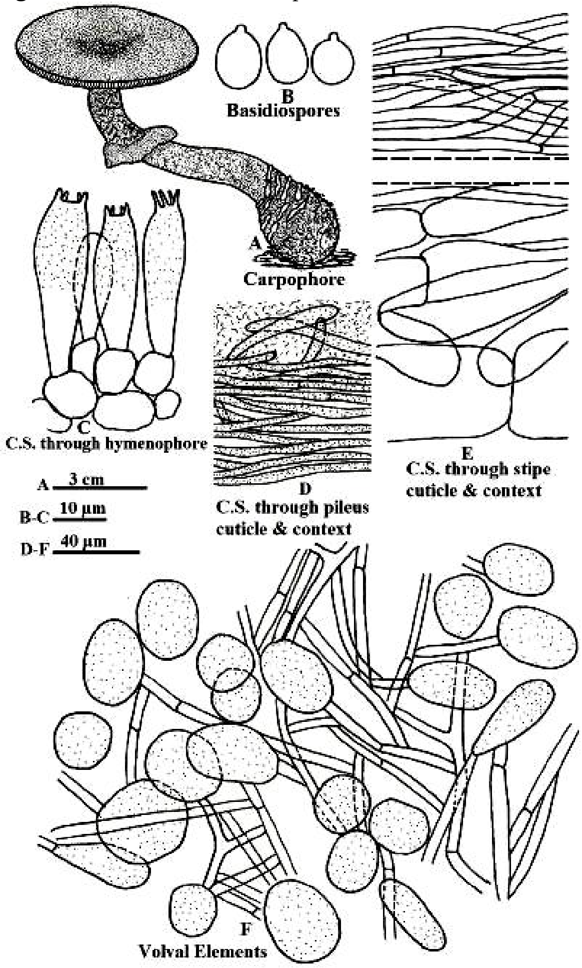

Figure 5. Internal details of *A. porphyria*

***Amanita pilosella* f. *pilosella* Corner & Bas.** *Persoonia*, 2: 267, 1962. Figures 1.D and 4

Sporophore up to 15.8 cm high. Pileus up to 5.0 cm wide, convex; lacking umbo; margin regular, very feebly striate along margins, surface grayish brown or brownish beige ($6E_3$), covered entirely, with grey to light grayish, fibrillose, squarros, flat, powdery, irregular warts; cuticle fully peeling; flesh white, unchanging, up to 0.3 cm thick; odor mild. Lamellae sinuate, close, broad (up to 0.5 cm); yellowish white ($2A_2$), unchanging; gill edges smooth; lamellulae attenuate. Stipe central, up to 15.0 cm long, up to 1.0 cm broad above, up to 1.8 cm broad at base, distinctly bulbous, surface white ($3A_1$), covered with grayish brown ($6F_3$), fibrillose to flocculose scales, unchanging; annulate, annulus single, grayish white ($1B_1$), superior, skirt like, pendulous, striated, light grey above and grey below; hollow; volva as few incomplete rings of grey powdery fibrillose scales at the margin of the bulb.

Basidiospores [25/1/1] 6.4-8.0 × (4.8) 5.6-6.4 µm (**L** = 6.4-8.0 µm; **L'** = 7.5 µm; **W** = 5.6-6.4 µm; **W'** = 5.9 µm; Q = (1.12-) 1.14-1.33 (-1.43); **Q'** = 1.27); broadly ellipsoid, infrequently ellipsoid, rarely subglobose; amyloid, hyaline, thin walled; guttulate; smooth; apiculate, apiculus up to 1.6 µm long. Basidia 33.6-45 x 8.0-11.2 µm, clavate, without any clamp connections, tetrasterigmate; sterigmata up to 6.4 µm long. Pileus cuticle hyphal, gelatinized, made up of horizontally septate hyphae giving rise a turf of thin walled, septate 1.6-4.0 µm broad granular hyphae, with brownish pigment; pilocystidia absent; pileus context composed of loosely interwoven, septate, thin walled, 3.2-9.6 µm broad hyphae; acrophysalides subpyriform to broadly clavate to clavate, up to 38.4 µm broad. Hymenophoral trama bilateral divergent. Stipe cuticle hyphal, made up of longitudinally and compactly tangled, septate, 1.6-6.4 µm broad hyphae; stipe context composed of loosely arranged, septate, thin walled, 3.2-12.8 µm broad hyphae; acrophysalides thin walled, abundant, up to 32.0 µm broad. Volval remnants composed of globose, ellipsoid, clavate, pyriform to subpyriform 16.0-70.4 x 11.2-37.0 µm broad inflated cells, abundant, single or terminal or in rows, intermixed with branched, thin walled, with brownish pigment, septate, 3.2-9.6 µm broad hyphae. Clamp connection absent throughout.

Material examined. India**,** Himachal Pradesh, Narkanda, Circuit house (2,900 m), growing solitary, in coniferous forest, among mosses and *Frageria*, under *Abies pindrow*, Yadwinder Singh and Munruchi Kaur, PUN 3846, August 13, 2007.

Distribution and Ecology. Corner and Bas (1962) collected *Amanita pilosella* f. *pilosella* growing solitary in Singapore and reported it to be very common in every rainy season. Yang (1997) found this species from China growing solitary or in small groups in a broad-leaved forest with dominant species of *Lithocarpus* and *Castanopsis*, at 1,000-2,000 m height.

Discussion. Corner and Bas (1962) proposed two forms of *Amanita pilosella* i.e. forma *pilosella* Corner & Bas and forma *atroconica* Corner & Bas based on size and form of volval remnants on cap surface and the color of lamellae edges. In forma *pilosella* pileus has thick irregular warts and white lamellae edges. While, in forma *atroconica* the cap has conical warts in the centre with regular patches along the margin, further in forma *atroconica* the gill edges are dark brown. *Amanita pilosella* f. *pilosella* is the first time report from India.

***Amanita porphyria* Alb. & Schwein**. *Fr. Consp. Fung.*, 142, 1805. Figures 1.E, 1.F and 5

Synonyms. *Agaricus porphyrius* (Alb. & Schwein.: Fr.) Fr. *Syst. Mycol.* 1: 14, 1821.

Venenarius porphyrius (Alb. & Schwein.: Fr.) Murrill. *Mycologia* 5: 81, 1913.

Amanitina porphyria (Alb. & Schwein.: Fr.) E.-J. Gilbert. *Iconogr. Mycol. (Milan)* 27, suppl. (1): 78, 1940.

Agaricus gracilis Schumach. *nom. illeg. Enumerat. Plant. Part. Saelland*. 2: 252, 1803.

Amanita recutita var. *gracilis* (Schumach.) Sacc. *Syll. Fung.* 5: 12, 1887.

Amanita porphyria [var. *recutita*] f. *gracilis* (Schumach.) E.-J. Gilbert. *Gen. Amanita* Persoon: 59, 1918.

Agaricus (*Amanita*) *porphyrius var. tenuior* Fr. *Hymenomyc. Eur.:* 19, 1874.

Amanita porphyria var. *tenuior* (Fr.) Sacc. *Syll. Fung.* 5: 11, 1887.

Amanita porphyria f. *tenuior* (Fr.) E.-J. Gilbert. *Gen. Amanita* Persoon: 57, 1918.

Amanita porphyria f. *tenuior* (Fr.) Killerm. *Denkschr. Bayer. Bot. Ges. Regensburg* 18 *(neue Folg.* 12): 5, 1931.

Amanita porphyria f. *tenuior* (Fr.) Veselý. *Ann. Mycol* 31 (4): 235, 1933.

Amanita tomentella Krombh. *Naturgetreue Abbild. Essbar. Schädl. Undverd. Schwäm.* 4: pl. 29, 1836.

Amanita recutita (Fr.) Bertillon in Dechambre. *Dict. Encycl. Sci. Médic.:* 500, 1866.

Amanita recutita (Fr.) Gillet. (February). *Tabl. Anal. Hymenomyc.:* 7, 1884.

Amanita porphyria var. *recutita* (Fr.) Quél. *Fl. Mycol.:* 308, 1888.

Amanita porphyria f. *recutita* (Fr.) E.-J. Gilbert. *Gen. Amanita* Persoon: 57, 1918.

Venenarius recutitus (Fr.) Murrill. *Mycologia* 5: 81, 1913.

Agaricus porphyrius var. *major* Fr. *Hymenomyc. Eur.:* 19, 1874.

Amanita porphyria var. *major* (Fr.) Sacc. *Syll. Fung.* 5: 11, 1887.

Amanita porphyria f. *major* (Fr.) E.-J. Gilbert. *Gen. Amanita* Persoon: 56, 1918.

Amanita porphyria f. purpurascens (Gillet) E.-J. Gilbert. *Gen. Amanita* Persoon: 56, 1918.

Amanita porphyria f. *purpurascens* (Gillet) Veselý. *Ann. Mycol.* 31 (4): 235, 1933.

Amanita porphyria f. *major* (Britzelm.) Killerm. *nom. illeg. Denkschr. Bayer. Bot. Ges. Regensburg* 18 (neue Folg. 12: 5, 1931.

Amanita porphyria var. *tenera* Boud. *Bull. Soc. Mycol. France* 18: 259, 1902.

Amanita porphyria f. tenera (Boud.) Veselý. *Ann. Mycol.* 31 (4): 236, 1933.

Sporophore up to 10.5 cm high. Pileus up to 6.0 cm wide, plano-convex, with depressed centre; lacking umbo; margin regular; nonappendiculate; splitting at maturity; surface dark brown ($7F_5$) at centre and light brown ($6D_4$) to brownish grey along margin; moist; atomate; glabrous; cuticle fully peeling; flesh up to 0.2 cm thick, white, unchanging; odor mild. Lamellae free, with a fine decurrent line on stipe; close; broad (up to 0.6 cm); creamy white, unchanging; gill edges smooth; lamellulae attenuate. Stipe central, up to 9.5 cm long, up to 0.8 cm broad above and up to 1.0 cm broad in middle, up to 1.1 cm broad near base, distinctly bulbous with marginate bulb up to 2.2 cm broad, off white background, decorated with grayish colored scales, with violaceous tinge; hollow; annulate, annulus single, attached, skirt like, off white above and light grey below; volva friable, present as grey, broken rings at the margin of the bulb.

Basidiospores (7.2-) 8.0-9.6 (-10.4) x 7.2-9.6 µm (**L** = 8.0-9.6 µm; **L'** = 8.4 µm; **W** = 7.2-8.0 µm; **W'** = 7.6 µm; Q = 1.0-1.11 (-1.30); **Q'** = 1.10); globose to subglobose, rarely broadly ellipsoid; amyloid, hyaline, thin walled; apiculate, apiculus up to 0.8 µm long. Basidia 24.0-32.0 x 7.2-9.6 µm, granular, inamyloid, clavate, without clamp connections, tetrasterigmate; sterigmata up to 4.0 µm long. Pileus cuticle hyphal, gelatinized, made up of sub-radially tangled, thin walled, granular, septate, 2.0-6.1 µm broad hyphae, pilocystidia absent; pileus context composed of radially to irregularly interwoven, septate, thin walled, 4.1-12.3 µm broad hyphae; acrophysalides thin walled, narrowly clavate to clavate, ellipsoid up to 41.0 µm broad. Hymenophoral trama bilateral divergent. Stipe cuticle hyphal, made up of longitudinally and compactly arranged, septate, 2.0-8.2 µm broad hyphae; stipe context made up of longitudinally and loosely arranged, septate, 4.1-16.4 µm broad hyphae; acrophysalides thin walled, abundant, up to 37.0 µm broad. Volval remnants on stipe base composed of irregularly arranged elements, inflated cells abundant, globose, subglobose, broadly ellipsoid to ellipsoid to elongated, 20.4-45.0 x 12.3-37.0 µm, thin walled, single, terminal or sometimes in rows of 2-3, intermixed with branched, thin walled, septate, abundant, 2.0-8.2 µm broad hyphae. Clamp connection absent throughout.

Material examined. India**,** Uttarakhand, Pigla Pani (2,500 m), growing solitary on soil in mixed broad leaved and coniferous forest, under *Pinus roxburghii*, Yadwinder Singh, PUN 6438, August 20, 2010.

Distribution and Ecology. *Amanita porphyria* was found growing solitary to subgregarious in *Pinus sylvestris* forest and in sand with *Arctoctaphylos*, *Castanopsis*, *Pinus*, *Quercus*, *Tsuga canadensis*, *T. heterophylla*, *F. grandifolia*, and *Acer* sp. from Norway, Switzerland, California, Vermont and Washington (Tulloss 2013b). According to *A. porphyria* is very common in the tree line forests of northern Europe.

Discussion. Macroscopic and microscopic details of the examined specimen matches with description of *Amanita porphyria* Alb. & Schwein.: Fr. provided by Tulloss (2013b). This species is characterized in possessing a distinctive nonstriate pileus margin, off white stipe, decorated with grayish squamules, with violaceous tinge and has a marginate bulb, annulus is persistent, skirt like, off white above and light grey below, whereas the volva is friable, present as grey, cottony mass at the margin of the bulb. In India, Adhikari and Bora (1989) reported *A. porphyria* from Uttarakhand, but according to Bhatt et al. (2003) Indian material may be misidentified and is possibly *A. pseudoporphyria* Hongo. The present collection was also compared with *A. pseudoporphyria*, but in *A. pseudoporphyria* the stipe is white and is covered with white fibrillose squamules and has a limbate volva with free limbs and basidiospores are broadly ellipsoid to ellipsoid. *Amanita porphyria* is the first time report from India.

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REFERENCES

Abraham SP, Kachroo JL. 1989. Larger fungi from Kashmir, India, VI. The genus *Amanita*. Micol Neotrop Apl 2: 41-51.

Adhikari RS, Bora SS. 1989. *Amanita porphyria*-A new record for India. Curr Sci 58: 90-91.

Atri NS, Kaur A, Kour H. 2005. Wild Mushrooms-Collection and Identification. In: Rai RD, Upadhyay RC, Sharma SR (eds). Frontiers in Mushroom Biotechnology. NRCM Chambaghat, Solan.

Bhatt RP, Kumar A, Lakhanpal TN. 1988. Fleshy fungi of North-Western Himalayas-VII. Indian J Mycol Pl Pathol 18: 143-148.

Bhatt RP, Tulloss RE, Semwal KC, Bhatt VK, Moncalvo J-M, Stephenson SL. 2003. Amanitaceae reported from India. A critically annotated checklist. Mycotaxon 88: 249-270.

Corner EJH, Bas C. 1962. The genus *Amanita* in Singapore and Malaya. Persoonia 2 (3): 241-304.

Gulden G, Jenssen KM, Stordal J. 1985. Arctic and Alpine Fungi 1. Soppkonsulenten, Oslo.

Jenkins DT. 1982. A study of *Amanita* types IV. Taxa described by GF Atkinson. Mycotaxon 14: 237-246.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth Bisby's Dictionary of Fungi. 10th ed. CABI, Wallingfod, UK.

Kornerup A, Wanscher JH. 1978. Methuen Handbook of Colors. 3rd ed.. Eyre Methuen, London.

Kumar A, Lakhanpal TN, Stephenson SL. 1990a. Ecological studies of some macrofungi in the northwestern Himalayas. Nova Hedwigia 50: 535-547.

Kumar A, Bhatt RP, Lakhanpal TN. 1990b. The Amanitaceae of India. Bishen Singh-Mehendra Pal Singh Publication, Dehra Dun.

Pandotra VR. 1997. Illustrated fungi of north India with special reference to J. and K. State. International Book Distributors, Dehra Dun.

Semwal KC, Bhatt RP, Upadhyay RC. 2005. The genus *Amanita* from Garhwal Himalayas region of India. Mushroom Res 14 (2): 50-55.

Semwal KC, Tulloss RE, Bhatt RP, Stephenson SL, Upadhyay RC. 2007. New records of *Amanita* from Garhwal Himalaya, India-*Amanita* section *Amanita*. Mycotaxon 101: 331-348.

Tulloss RE, Ovrebo CL, Halling RE. 1992. Studies on *Amanita* (Agaricales) from Andean Colombia. Mem New York Bot Gard 66: 1-46.

Tulloss RE, Iqbal SH, Khalid AN, Bhatt RP, Bhatt VK. 2001. Studies in *Amanita* (Amanitaceae) from Southern Asia. I. Some species of Pakistan's Northwest Frontier Province. Mycotaxon 77: 455-490.

Tulloss RE. 2013a. *Amanita flavoconia* var. *flavoconia*. Retrieved from:http://amanitaceae.org/*Amanita*+*flavoconia*+var.+*flavoconia*.
Tulloss RE. 2013b. *Amanita porphyria*. Retrieved from: http://amanitaceae.org/Amanita+porphyria.
Vrinda KB, Pradeep CK, Kumar SS. 2005. Occurrence of a lesser known edible *Amanita* in the Western Ghats of Kerala. Mushroom Res 14 (1): 5-8.
Yang ZL. 1997. Die *Amanita*-Arten von Südwestchina. Bibliotheca Mycologica 170: 1-240.

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Culturable fungal diversity associated with forest leaf litter from Bhandara District of Maharashtra, India

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Abstract. *Akare SM, Tagade WY, Warghat AR, Naryal A, Bhardwaj A. 2016. Culturable fungal diversity associated with forest leaf litter from Bhandara District of Maharashtra, India. Biodiversitas 17: 349-358.* Diversity of leaf litter fungi were carried out in 2010-11. Four samples were collected from Bampewada and Sakoli forest area of Bhandara District of Maharashtra, India. The Blotter paper method, Segment plate method and Serial dilution methods were used to assess the diversity of fungal species. Fungi were cultivated on potato dextrose agar, malt extract agar and czapek dox agar. A total of 31 species comprising 29 Ascomycetes and two Zygomycetes were recognized from 4 samples collected from Bampewada and Sakoli forest area. The most abundant group was Ascomycetes. The dominant species in both the forests were *A. flavus, A. niger, Aureobasidium* sp., *Fusarium oxysporum, Penicillium* sp.1, *Rhizopus stolonifer, A. caespitosus, Penicillium* sp.2, *A. nidulans, Helminthosporium* sp. and *Monodictys fluctuata.* While, common species were A. *flavus, A. fumigatus, A. nidulans, A. niger, Beltrania rhombica* and *Fusarium oxysporum*. The fungal diversity was higher in the Sakoli forest than that of Bampewada forest. Differences were observed in percentage occurrence of fungal species between two forest areas. The reason may be that the quality of litters, different microenvironments and other characteristics in the Sakoli forest provided more resources for fungi than in Bampewada forest.

Keywords: Decomposition, dominant species, fungal community, litter breakdown, vegetation

INTRODUCTION

Fungi play an important role in balancing ecological services, their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling and decomposing the dead organic matter present in soil and litter (Change and Miles 2004; Gates 2005). They are highly diverse in nature. Having a stable and estimate of taxonomic diversity for fungi is also necessary to enable fungi to be included in considerations of biodiversity conservation, land-use planning and management (Mueller and Schmit 2007).

The number of existing fungi worldwide has been estimated to 1.5 million species (Mueller et al. 2004). One-thirds of the fungal diversity of the globe exists in India and of this, only 50% are characterized yet (Manoharachary et al. 2005). Maharashtra is the third largest state of India next to Rajasthan and Madhya Pradesh covering an area of 307, 713 km^2. It lies at 18°57′36″N 72°49′12″E, and altitude ranges from 0-1800 m above mean sea level and the forests cover less than one fifth of the state and confined to the Western Ghats and eastern Vidarbha region. It receives an annual rain fall of about 4000 mm in the western region of Western Ghats and about 700-1250 mm in Vidarbha region brought by south west monsoon and the dry zone occurs in between western and Vidarbha region. The forests of Western Ghats and Vidarbha region are rich in mycobiota (Senthilarasu 2014).

Decomposition of fungi on the forest floor is a very complex phenomenon and achieved by different groups of microorganisms. The major component of the top soil consists of different parts of plant materials and immediately colonized by diverse groups of microorganisms as they fall on the soil surface and soon after, the processes of decomposition starts. Litter decomposition is also an important link in nutrient cycling of the forest (Grigal and McColl 1977). Litter is an important source of dead organic matter in terrestrial ecosystems, with inputs of tons of litter per year. Litter decomposition contributes directly to nutrient availability both for plant growth and ecosystem productivity. The studies on microorganisms suggest fungi to be the main contributors to leaf litter decomposition (Isidorov and Jdanova 2002). During the last few years various workers have developed interest to understand the nature of fungi both in forest and cultivated fields. The study on diversity of leaf litter fungi from various host plants were reported earlier (Saravanan 2013; Tokumasu et al. 1997). Some fungi were found to be common on leaf litter in previous studies, while many new fungal taxa have been described from decaying leaves and dead wood (Hughes 1989). Keeping the above facts in mind, the present study was focused on the isolation and identification of fungi associated with decomposition of forest leaf litter in

Bampewada and Sakoli area of Bhandara District of Maharashtra, India. Forest leaf-litter was selected for the present study because of the dense forest vegetation and its great economic value in Maharashtra, India.

MATERIALS AND METHODS

Study area

Present investigation embodies isolation and identification of fungi associated with forest leaf litter. The samples were collected from forest of Bampewada and Sakoli area of Bhandara District, Maharashtra State, India (Figure 1). For the collection of leaf litter samples, two places were selected namely, Bampewada and Sakoli forest (Figure 2). These two places are 53 km and 40 km away from Bhandara District of Maharashtra State. The forest areas are entirely dominated by Teak trees however; other tree species such as Babul, Ber, Palas, Shisham, Amla, Jamun, Arjun, Ain, Chichwa, Sewan, Dhaora, Tendu, Salai, Rohan, Mahua, Bija, Dhaman, etc. are also found. The climate in this area remains dry and hot throughout the year with the moderate rainfall from June to middle of October months. In each places two locations namely hilly region and open slopes were selected randomly for collections of leaf litter which were appropriate from ecological point of view (dry, hot and moderate rainfall during the monsoon). Collections were made before annual leaf fall starts particularly, in the first week of January. At the time of collections, partial decomposed leaves (for chances of presence of mycelium) were collected in a sterile plastic ziploc bags (ziplocs bags were sterilized by absolute alcohol) of size 13 cm × 10 cm and brought to the laboratory for fungal isolation.

Culture media

The following culture media were used for the isolation of fungi

Potato Dextrose Agar (Rawling 1933)

This culture medium is used for the isolation of fungi from forest leaf litter by serial dilution method. The compositions of Potato Dextrose Agar are as follows: Potatoes (200g, Dextrose (20g), Agar 17g (Distilled water (1000 mL).

Malt Extract Agar (Van der Walt & Yarrow 1984)

This culture medium is used for isolation of fungi by segment plate method. The compositions of Malt Extract Agar are as follows: Malt Extract (20 g), Agar (17 g), Distilled water (1000 mL).

Czapek Dox Agar (Raper & Thom 1949)

This culture medium is used for the isolation of fungi by segment plate method and pure culture of fungal colonies. The compositions of Czapek Dox Agar are as follows: $NaNO_3$ (3.00 g), K_2HPO_4 (1.00 g), $MgSO_4.7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4.7H_2O$ (0.01 g), Sucrose (30.00 g), Agar (15.00 g), Distilled water (1000 mL).

Methods for isolation

Moist chamber technique (*Blotter paper method*) (Fulzele 2002)

The collected samples were aseptically transferred to the sterile petriplate containing wet blotter papers and were kept at room temperature in the incubation chamber. Sterile distilled water was added at regular interval under aseptic condition to maintain blotter paper moist.

Direct plating method (*Segment plate method*) (Hutchinson and Richard 1921)

Randomly 4-5 segments of material nearly of size ½ to 1 inch were directly placed on culture petriplate containing sterile, cool, solid Malt Extract Agar medium at equidistance from one another and incubated at room temperature. The care was taken to avoid overcrowding of fungi in the plate.

Serial dilution methods (Hutchinson and Richard 1921)

1gm of material from collected samples was mixed with 10 mL sterile distilled water with continuous stirring for obtaining homogenous suspension of fungal spores. 1 mL of this homogenate was transferred to first dilution tube containing 9 mL sterile distilled water. The tube was shaken well to get homogenate and again dilution process was carried out in the same way till the dilution reached to 1: 100000 (10^{-5}). Afterwards, 1 mL of homogenate from each dilution tube was added to the sterile petriplate before pouring the sterile, cool, molten (approximately 45°C) PDA medium. The plates were rotated for a brief period to proper mixing of homogenate with the medium and then allowed to cool. Then the plates were kept in incubation chamber at the room temperature of 25-30° C. The colonies of fungi were counted per plates and were subcultured on the sterile solid, CDA medium. The Czapek Dox Agar medium was used for the growth of fungi Borkar (2014) and Fulzele (2002). The culture plates were observed at the interval of two days for a week and growing individual colonies were picked up and transferred to fresh sterile agar slants for further purification and identification.

Identification of fungi

Fungi were identified on the basis of their growth characteristics, morphological characteristics and ontogeny with the help of manuals, monographs and taxonomic papers of various authors (Ainsworth et al. 1972; Barnett & Hunter 1972; Sutton 1980; Von Arx 1981). Identification was based on morphological study examined under stereo, and compound microscopes (Olympus BX 50 F4, Japan and Axio Scope A, Carl Zeiss). Colony forming unit and occurrence of fungi were calculated as per the procedures described by Saksena (1955). Where Percentage of occurrence refers to the number of samplings in which a fungus was recorded out of the total number of samplings made during the period of study.

The Colony Forming Unit (CFU) of fungal species per gram of leaf litter were calculated as follows:

$$\text{CFU/gm} = \frac{\text{Average number of fungal colonies} \times \text{Dilution Factor}}{\text{Dry weight of sample}}$$

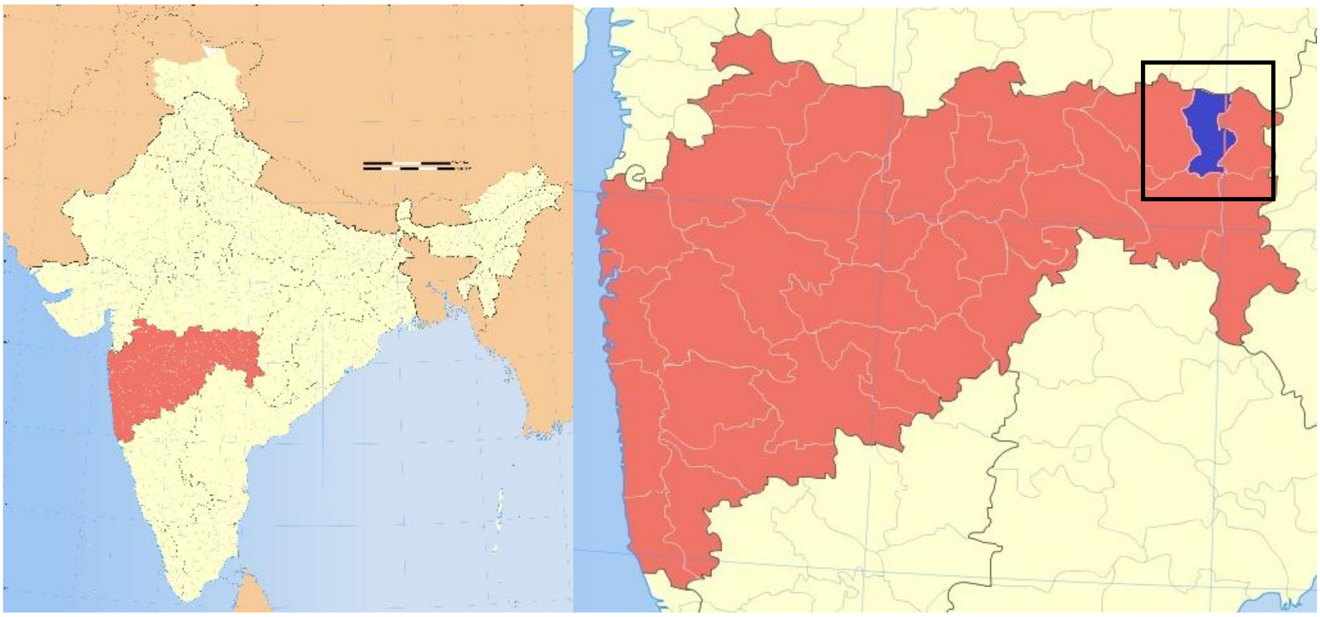

Figure 1. Location of the study area in Bampewada and Sakoli forests of Bhandara District (box), Maharashtra, India

Figure 2. Studied forest area. A. Bampewada, B. Sakoli, Bhandara district of Maharashtra, India

RESULTS AND DISCUSSION

Leaf litter fungi of Bampewada Forest

Twenty six species belonging to Seventeen genera of leaf litter fungi were isolated by Serial dilution method using Potato Dextrose Agar medium (Figure 3.C). Similarly 20 species belonging to 15 genera of leaf litter fungi isolated by Segment plate method using Malt Extract Agar and Czapek Dox Agar medium (Figure 3.B). While only 4 species belonging to 4 genera of leaf litter fungi were isolated by blotter paper method (Figure 3.A). Fungi namely *Alternaria* sp.1, *Alternaria* sp.2, *Aspergillus flavus, A. niger, A. ochraceus, Aureobasidium* sp., *Beltrania rhombica, Chaetomium* sp., *Curvularia* sp., *Fusarium oxysporum, Fusarium* sp.1, *Helminthosporium* sp., *Humicola* sp., *Penicillium* sp.1, *Penicillium* sp.2, *Pestalotia* sp., *Phoma* sp., *Stachybotris* sp., *Trichoderma* sp.1, *Trichoderma* sp.2 and *Rhizopus stolonifer* were isolated by serial dilution and segment plate method. Whereas *Aspergillus caespitosus, A. fumigatus, A. nidulans, Memnoniella* sp. and *Syncephalastrum* sp. were isolated by Serial dilution method and only *Memnoniella* sp., *Pestalotia* sp., *Stachybotris* sp. and *Trichoderma* sp.1 were isolated by Blotter paper method. While, *Monodictys fluctuata, Penicillium adametzi, Pithomyces* sp., *Torula* sp. and *Trichoderma* sp.3 were not identified from leaf litter fungi of Bampewada Forest by any of three method (Table 1). During the isolation of fungi it is found that *A. flavus, A. niger, Aureobasidium* sp., *Fusarium oxysporum, Penicillium* sp.1 and *Rhizopus stolonifer* were dominant and *Aureobasidium* sp.was highest colony forming fungi followed by *Fusarium oxysporum, A. flavus, A. niger, Rhizopus stolonifer* and *Penicillium* sp.1 in Bampewada forest area. Whereas, others showed least CFU (Table 2).

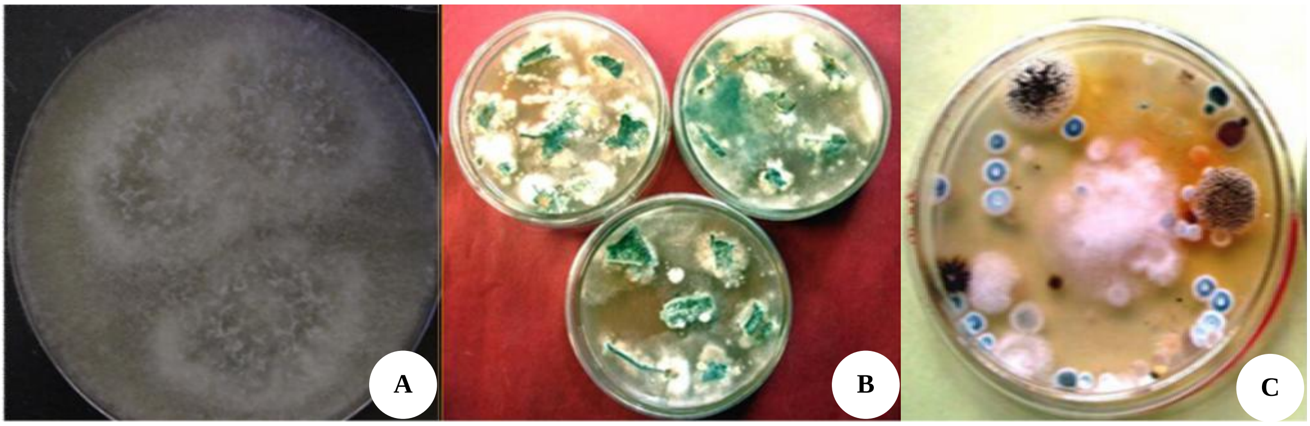

Figure 3. Isolation of fungi (samples of Bampewada and Sakoli forest area). A. Blotter paper method, B. Segment plate method, C. Serial dilution method

Table 1. Total number of fungal species isolated by different methods in selected forest area

Fungi identified	Bampewada Forest			Sakoli forest		
	A	B	C	A	B	C
Alternaria sp.1	-	+	+	-	+	+
Alternaria sp.2	-	+	+	-	-	-
Aspergillus caespitosus	-	-	+	-	-	+
Aspergillus flavus	-	+	+	+	+	+
Aspergillus fumigatus	-	-	+	-	+	+
Aspergillus nidulans	-	-	+	-	-	+
Aspergillus niger	-	+	+	-	+	+
Aspergillus ochraceus	-	+	+	-	+	+
Aureobasidium sp.	-	+	+	-	+	+
Beltrania rhombica	-	+	+	-	-	-
Chaetomium sp.	-	+	+	+	-	+
Curvularia sp.	-	+	+	-	+	+
Fusarium oxysporum	-	+	+	-	+	+
Fusarium sp.1	-	+	+	-	+	+
Helminthosporium sp.	-	+	+	-	+	+
Humicola sp.	-	+	+	-	-	-
Memnoniella sp.	+	-	+	+	-	+
Monodictys fluctuata	-	-	-	-	+	+
Penicillium adametzi	-	-	-	-	+	+
Penicillium sp.1	-	+	+	-	+	+
Penicillium sp.2	-	+	+	-	-	+
Pestalotia sp.	+	+	+	-	+	+
Phoma sp.	-	+	+	+	+	+
Pithomyces sp.	-	-	-	-	+	+
Stachybotrys sp.	+	+	+	+	-	+
Torula sp.	-	-	-	-	-	+
Trichoderma sp.1	+	+	+	+	+	+
Trichoderma sp.2	-	+	+	-	+	+
Trichoderma sp.3	-	-	-	-	+	+
Rhizopus stolonifer	-	+	+	-	+	+
Syncephalastrum sp.	-	-	+	-	+	+

Note: A. Blotter paper method, B. Segment plate method, C. Serial dilution method

Table 2. Colony Forming Unit (CFU) of fungal colonies at 10^{-4} concentration

Fungi isolates		Bampewada Forest		Sakoli Forest	
Fungi identified	Division	I	II	I	II
Alternaria sp.1	Ascomycota	4.17	-	-	-
Alternaria sp.2	Ascomycota	-	-	-	-
Aspergillus caespitosus	Ascomycota	-	-	6.67	11.1
Aspergillus flavus	Ascomycota	11.12	13.7	11.13	11.1
Aspergillus fumigatus	Ascomycota	-	5.88	4.46	5.57
Aspergillus nidulans	Ascomycota	6.95	-	13.23	-
Aspergillus niger	Ascomycota	13.87	9.82	13.23	11.32
Aspergillus ochraceus	Ascomycota	-	-	-	4.43
Aureobasidium sp.	Ascomycota	12.5	13.7	-	-
Beltrania rhombica	Ascomycota	3.12	-	3.46	3.23
Chaetomium sp.	Ascomycota	-	5.88	-	-
Curvularia sp.	Ascomycota	-	-	-	3.33
Fusarium oxysporum	Ascomycota	12.5	13.4	12.33	13.23
Fusarium sp.1	Ascomycota	4.17	-	4.46	2.23
Helminthosporium sp.	Ascomycota	-	-	6.67	5.57
Humicola sp.	Ascomycota	-	-	2.15	2.33
Memnoniella sp.	Ascomycota	2.5	3.2	-	-
Monodictys fluctuata	Ascomycota	-	11.25	12.21	-
Penicillium adametzi	Ascomycota	-	-	6.67	-
Penicillium sp.1	Ascomycota	11.12	-	-	10
Penicillium sp.2	Ascomycota	8.33	11.76	13.33	-
Pestalotia sp.	Ascomycota	5.54	-	-	-
Phoma sp.	Ascomycota	-	3.94	-	4.43
Pithomyces sp.	Ascomycota	-	-	-	2.23
Stachybotrys sp.	Ascomycota	-	1.32	-	1.59
Torula sp.	Ascomycota	-	-	-	4.43
Trichoderma sp.1	Ascomycota	-	-	-	2.23
Trichoderma sp.2	Ascomycota	-	3.94	-	-
Trichoderma sp.3	Ascomycota	-	-	-	-
Rhizopus stolonifer	Zygomycota	8.33	3.94	-	5.57
Syncephalastrum sp.	Zygomycota	-	5.88	-	-

Leaf litter fungi of Sakoli Forest

Twenty eight species belonging to 18 genera of leaf litter fungi were isolated by Serial dilution method using Potato Dextrose Agar medium. Whereas 21 species belonging to 14 genera of leaf litter fungi were isolated by Segment plate method using Malt Extract Agar and Czapek Dox Agar medium. While only 6 species belonging to 6 genera of fungi associated with leaf litter were isolated by

Blotter paper method. These are *Alternaria* sp.1, *A. flavus, A. fumigatus, A. niger, A. ochraceus, Aureobasidium* sp., *Curvularia* sp., *Fusarium oxysporum, Fusarium* sp.1, *Helminthosporium* sp., *Monodictys fluctuata, Penicillium adametzi, Penicillium* sp.1, *Pestalotia* sp., *Phoma* sp., *Pithomyces* sp., *Trichoderma* sp.1, *Trichoderma* sp.2, *Trichoderma* sp.3, *Rhizopus stolonifer* and *Stachybotris* sp. were isolated by serial dilution and segment plate method. *A. caespitosus, A. nidulans, Chaetomium* sp., *Memnoniella* sp., *Penicillium* sp.2, *Stachybotrys* sp., *Torula* sp. were also isolated by Serial dilution method. While, *A. flavus, Chaetomium* sp., *Memnoniella* sp. and *Stachybotrys* sp. were isolated by Blotter paper method. *Alternaria* sp.2, *Beltrania rhombica* and *Humicola* sp. were not identified from Leaf Litter Fungi of Sakoli Forest by any of three methods (Table 1). During the isolation of fungi it is found that *Fusarium oxysporum, A. niger, A. flavus, A. caespitosus, Penicillium* sp.2, *A. nidulans, Helminthosporium* sp. and *Monodictys fluctuata* were dominant and *Fusarium oxysporum* was found highest colony forming fungi followed by *A. niger, A. flavus, A. caespitosus, Penicillium* sp.2, *A. nidulans, Helminthosporium* sp. and *Monodictys fluctuata* in Sakoli forest area. Whereas others showed least CFU (Table 2). The collected specimens are shown in fig.4 and description recorded is as follows.

***Alternaria* sp.** Nees. Colonies effuse, gray, dark, blackish brown or thick brown; mycelium immersed or partly superficial, hyphae pale brown, conidiophores simple, irregularly or loosely branched, brown, solitary or in fascicles; conidia caternate or solitary, dry, typically ovoid or obclavate, often with beak, pale or mid olivaceous brown, smooth or verrucose, muriform, with transverse and frequently with oblique or longitudinal septa.

Aspergillus caespitosus Raper & Thom**.** Colonies growing slowly, mycelium largely submerged and extremely tough, tearing with difficulty, producing numerous dark green, hemispherical to loosely columnar heads in central colony areas, characterized particularly by clusters of irregular ovoid to elliptical and thick walled hulle cells, at first colourless, becoming reddish purplish in age, scattered unevenly or arranged in irregular concentric zones; reverse colourless changing to dark reddish purple with age, particularly beneath hulle masses; odour absent; conidial heads dark yellow green to green, generally radiate to loosely columnar, mostly 75-125 µm in diam; conidiophores straight or slightly sinuous, mostly 250-325 µm in length, 5-6.5 µm in diam, thick walled, smooth when young, roughened in age, tan to brown in colour, septate just below the vesicle; vesicle slightly elongate, the upper hemisphere loosely covered by phialides, lower half sterile and often cloured, 15-20 µm in diam; phialides beseriate; metulae 6.5-8.5 × 3.5-5 µm; phialides 6.5-8 × 3-4.5 µm, rarely larger; hulle cells abundant, thick walled, irregular; globose, spinulose, green, mostly 3.5-4.5 µm, rarely larger.

Aspergillus flavus Link. Colonies growing rapidly; conidial heads yellow when young, becoming dark yellow-green in age, in older cultures deep grey-green, reverse colourless to pale yellow brown; exudates inconspicuous, except in heavily sclerotial strains, red brown; conidial heads radiate, splitting into poorly defined columns, rarely exceeding 500-600 µm, with big conidial heads yellow in the centre, greenish towards periphery; conidiophores arising separately from the substratum, 0.5-1.5 mm long, with coarsely roughened, heavy walls, colourless, broadening gradually to vesicles, 10-65 µm in diam; metulae present predominantly, sometimes absent, only phialides (uniseriate or biseriate) arising on metulae or both types present sometimes in the same head, conidia globose to subglobose, conspicuously echinulate, yellowish green, 4.5-5.5 × 3.5-4.5 µm, sometimes elliptical when young infrequently remain so, 3.5-4.5 µm, up to 6 µm also.

Aspergillus fumigatus Fresen. Colonies spreading dull blue-green, velvety to floccose; rapidly, white at first becoming reverse colourless to varying in shades; conidial heads columnar, compact, often densely crowded, up to 400 × 50 µm; conidiophores short, smooth, light green, up to 300 µm in length and 5-8 µm in breath, septate, gradually enlarging into a flask shaped vesicle; vesicle bearing a single series of phialides; phialides closely packed, 6-8×2-3 µm; conidia globose to subglobose, green in mass, echinulate, sclerotia and cleistothecia absent.

Aspergillus nidulans Fennell & Raper. Colonies growing well at room temperature, dark cress green in some strains from abundant conidial heads, margins thin irregular; reverse purplish red to very dark in age; exudates lacking; conidial heads slightly larger than in typical representatives of the species; cleistothecia commonly 400 µm in diam, occasionally up to 450-500 µm; hulle cells thick walled, globose to subglobose, 1-25 µm; asci subglobose to ovate, 8 spored, ascospores lenticular, red orange in colour, with two prominent pleated equatorial crests about 1 µm wide and with convex surface conspicuously echinulate rather than smooth, 3.6-4.6×3.4-4.1 µm.

Aspergillus niger Tiegh. Colonies with abundant mycelium, conidial heads carbon black or sometimes deep brownish black; reverse colourless to pale yellow; exudates limited or lacking with minute droplets; conidial heads large and black, at first globose then radiate or splitting in well defined columns in age, up to 700-800 µm in diam; conidiophores arising directly from the substratum, smooth, non septate, thick walled, 1-2 mm × 15-20 µm; vesicles globose, walls thick, commonly 45-75 µm in diam, occasionally longer, beraring two series of fully packed phialides, brownish; metulae, rarely septate; phialides 7-10×3-3.5 µm; conidia globose, spinulose with colouring substance, black, 4-5 µm; globose to subglobose sclerotia produced in some strains, at first cream to buff, later vinaceous buff in age.

Aspergillus ochraceus Wilh. Colonies growing moderately, plane with a tough basal mycelium producing crowded conidial structures, light ochraceous buff to warm buff colour; reverse yellowish to greenish brown or reddish purple; exudates amber coloured in small droplets; conidial heads globose when young, typically adhering into two or three divergent compact columns in age, up to 750 µm diam; conidiophores light yellow to light brown 0.8-1.5 mm × 10-15 µm, walls thick; vesicles globose, thin walled, colourless, phialides biseriate; metulae varying in size greatly, phialides 7.5-10.5 × 2-3.7 µm; conidia globose or subglobose, delicately roughened or in some cases appearing smooth, 2.3-3.37 µm; sclerotia abundant, globose to subglobose, white to light pink when young, later vinaceous purplish, up to 1 mm in diam.

***Aureobasidium* sp.** Viala & Boyer. Colonies effuse, white or creamy, later becoming black at least in part and usually slimy; mycelium mostly immersed, variable in thickness; conidiophores branched, flexuous, at first hyaline, becoming mid to dark brown, smooth, thick walled; conidia aggregated in slimy mass, semi-endogenous, pleurogenous, simple, ellipsoidal or ovoid, colourless, smooth, aseptate, each completely encased in slimy coat; secondary conidia produced by yeast like budding of primary conidia; dark hyphal portions many act as chlamydospores or fragment like arthroconidia.

Beltrania rhombica Penzig. Colonies brown to black; mycelium immersed or superficial; Setae smooth, usually less than 200 µm long, occasionally longer, 4-6 µm near the base; conidiophores upto 130 µm long, 4-8 µm wide; separating cells ellipsoidal or obovoid, pale, 6-15 × 3-6 µm; conidiogenous cells integrated, terminal, polyblastic, denticulate; conidia spicate, biconic, symmetrical, proximal end V-shaped, 15-30 × 7-14 µm (without appendages); appendages 3-20 µm long, 2 µm wide at the base, tapering to a pointed tip.

***Chaetomium* sp.** Kunze & Schmidth. Mycelium thread like, thick or thin walled, septate, hyaline to yellowish-brown, septate; perithecia superficial, attached to the substrate by rhizoids, ostiolate, translucent to opaque to dark coloured, oval, subglobose, barrel shaped, clothed with dark setaeform or hair like appendages; hairs terminal and lateral, myceloid, stiff or coiled, branched or unbranched, smooth or ornamented; asci cylindrical, clavate to club shaped, 4-8 spored, unitunicate, hyaline, evanescent; paraphyses greatly reduced; ascospores unicellular, olive-brown to chocolate-brown, globose to subglobose to broadly lemon shaped, with a single germ pore, rounded to umbonate or faintly apiculate at one or both ends, column like mass of black ascospores liberating from ostiole; rarely producing aleurospores or chlyamydospores.

***Curvularia* sp.** Kunze & Schmidt**.** Colonies effuse, brown, grey or black, hairy, cottony or velvety; mycelium immersed in natural substrata; erect, black, cylindrical, sometimes branched, conidiophores indeterminate, continuing growth sympodially, straight or flexuous, often geniculate, sometimes later becoming intercalary, sympodial, cylindrical or occasionally swollen, cicatrized; conidia porospores, solitary, acropleurogenous, simple, often curved, clavate, ellipsoidal, broadly fusiform, obovoid or pyriform with 3 or more transverse septa, pale or dark brown, often with some cells (usually one end ones) paler than the other cells, sometimes with dark bands at the septa, smooth or verruculose; hilum distinctly protuberant in some species of scarcely or not at all protuberant in others.

Fusarium oxysporum Schlecht. Mycelium white or peach, but usually with a purple or violate tinge; reverse colourless to dark purple; conidiophores unbranched or sparsely branched, monophialidic; microconidia usually abundant, mostly 0-septate, oval ellipsoidal, kidney shaped or straight, produced on simple lateral phialides, solitary on free conidiophores never form in chains, 5-12 × 2.5-3.5 µm; macroconidia 2-5 septate, spindle to fusiform, curved or almost straight, pointed at both the ends, definite or weakly pedicellate, 27-60 × 3-5 µm; chlamydospores mostly terminal, globose smooth or roughened.

***Fusarium* sp.** Link. Mycelium extensive and cottony in culture, often with some tinge of pink, purple or yellow, in the mycelium or medium; conidiophores variable, slender and simple, or stout, short, branched irregularly or bearing a whorl of phialides, single or grouped into sporodochia; conidia (phialospores) hyaline, variable, principally of two kinds, often held in small moist heads; macroconidia several-celled slightly curved or bent at the pointed ends, microconidia 1-celled, oblong or slightly curved; parasitic on higher plants or saprophytic on decaying plant material.

***Helminthosporium* sp.** Link ex Fr. Mycelium dark, often in substrate; conidiophores single or clustered, tall, erect, brown, simple; conidia (porospores) develop laterally through pores beneath septa while apex of conidiophores is still growing whorls, single, subhyaline to brown, obclavate, phragmosporous, pseudoseptate, with prominent basal scar; parasitic or saprophytic.

***Humicola* sp.** Traaen. Colonies effuse, cottony, sometimes, funiculose, at first white, later grey, brown with age; mycelium superficial and immersed; intercalary chlamydospores sometimes present; conidiophores micro- or semi-macronematous, unbranched or irregularly branched, straight or flexuous, colourless to pale golden brown, smooth; conidia (aleuroconidia) solitary, dry, acrogenous, simple, typically spherical, occasionally obovoid or pyriform, pale to mid golden brown, 1-celled smooth; phialidic conidia may be catenate, small, colourless, smooth 1-celled.

***Memnoniella* sp.** Hohnel. Colonies effuse, black, velvety or powdery; mycelium partly superficial or immersed; conidiophores macronematous, mononematous, unbranched swollen at the apex, pale to mid grey, olivaceous or brown, smooth or minutely verruculose, often covered with dark granules; conidia acrogenous, catenate, spherical, hemispherical, grey dark brown or black, slightly flattened in one plane, smooth or verrucose.

Monodictys fluctuata S. Hughes. Colonies becoming dark grey with age; mycelium thin, hyaline and thick; conidiophores both terminal and lateral, light brown, 5.2-8 µm wide, flattened end bearing conidia of very variable shape and size; conidia dark brown, 2-14 celled, often constricted at the septa, at first smooth, later verruculose, roughly subspherical with straight or oblique septa, the size of the different types of conidia varies, up to 40 µm in diameter.

Penicillium adametzi Zaleski. Colonies growing 30-35 mm diameter on MEA in 7 days at 25^0 C, plane very deep, funiculose; mycelium white to cream coloured; conidiophores mostly arising from funicles, sometimes from aerial or surface hyphae; stipe short, smooth, mono-verticillate, non-vesiculate or slightly swollen apices; phialides usually in compact verticils of 5-8, ampulliform, 6-8 × 2-2.2 µm; conidia spheroidal, 1.8-2.5 µm diam, smooth to finely roughened, borne in short, poorly defined columns.

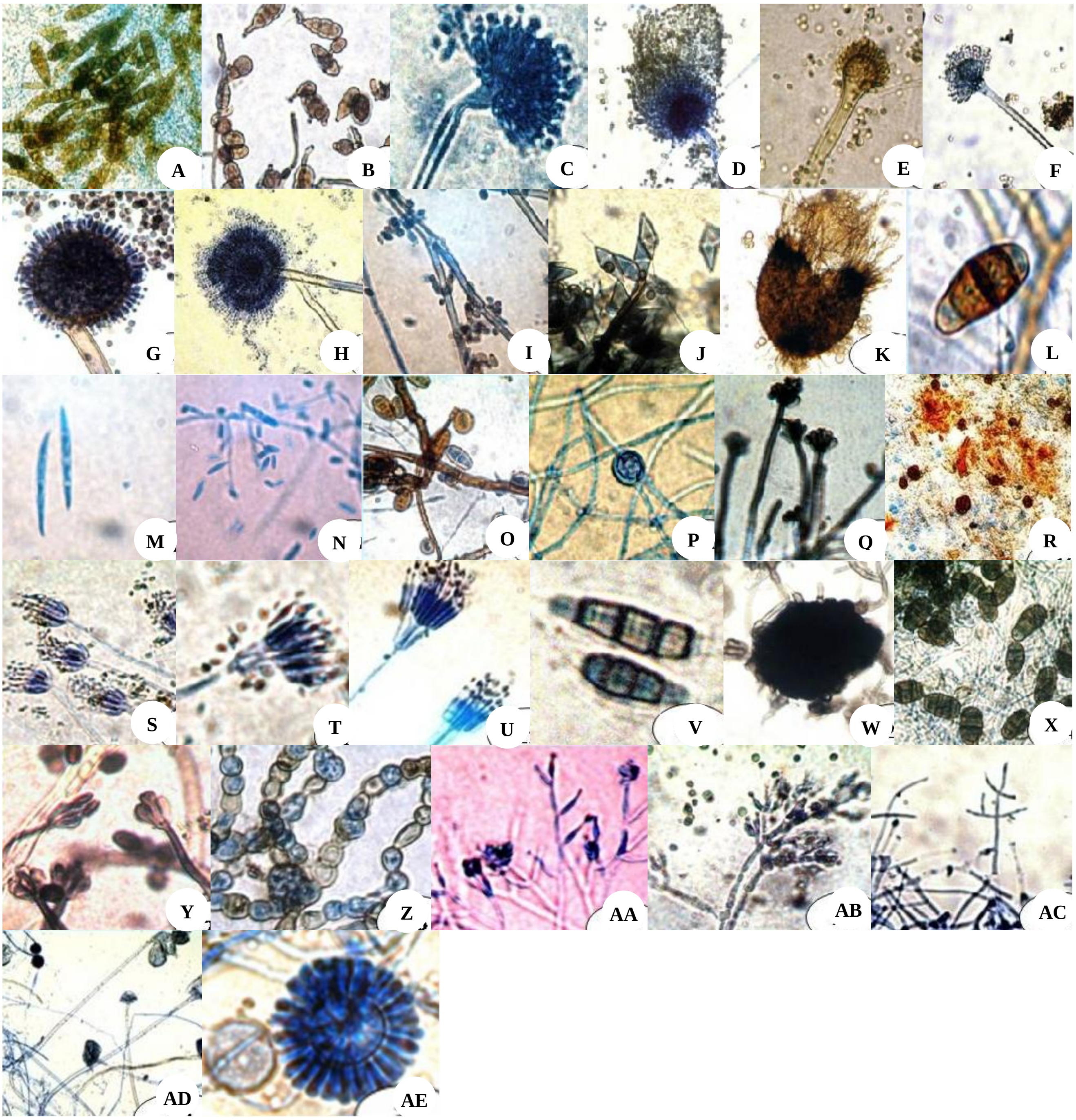

Figure 4. Schematic diagram of isolated fungi. **A.** *Alternaria* sp.1, **B**. *Alternaria* sp.2, **C.** *Aspergillus caespitosus***, D.** *Aspergillus flavus*, **E.** *Aspergillus fumigatus*, **F.** *Aspergillus nidulans*, **G.** *Aspergillus niger*, **H.** *Aspergillus ochraceus*, **I.** *Aureobasidium* sp., **J.** *Beltrania rhombica*, **K.** Chaetonium sp., **L.** *Curvularia* sp., **M**. *Fusarium oxysporum*, **N.** *Fusarium* sp.1, **O.** *Helminthosporium* sp., **P.** *Humicola* sp., **Q.** *Memnoniella* sp., **R.** *Monodictys fluctuata*, **S.** *Penicillium adametzi*, **T.** *Penicillium* sp.1, **U.** *Penicillium* sp.2, **V.** *Pestalotia* sp., **W.** *Phoma* sp., **X.** *Pithomyces* sp., **Y.** *Stachybotrys* sp., **Z.** *Torula* sp., **AA.** *Trichoderma* sp.1, **AB.** *Trichoderma* sp.2, **AC.** *Trichoderma* sp.3., **AD.** *Rhizopus stolonifer*, **AE.** *Syncephalastrum* sp.

***Penicillium* sp.** Link. Colonies variously coloured, usually blue-green, texture variable, zonate or azonate; vegetative hyphae creeping, septate; exudates present or absent; reverse uncoloured or variously coloured; conidiophores usually conspicuous, more or less erect, sometimes aggregated into synnemata, hyaline, rough or smooth, septate, sometimes branching at or near apex, branches divergent or adpressed to the main conidiophores axis, series of branches giving characteristics brush-like penicullus; phialides borne in groups directly at the apex of the conidiophores or branches of the conidiophores, ampulliform or acerose, hyaline; conidia borne in long chains, globose to ovoid, sometimes bacillar, hyaline to darkly pigmented, smooth or roughened; sclerotia produced in some species.

***Pestalotia* sp.** Steyaert. Colonies compact or effuse, buff, grayish brown, blackish brown or black; mycelium immersed, branched, septate, hyaline to pale brown; conidiophores hyaline, branched and septate at the base and above, cylindrical or lagerniform, formed from the upper cells of the pseudoparenchyma; conidia fusiform, straight or slightly curved, 4-euseptate, base simple or rarely with branched appendage, apical cell conic, hyaline, with 2 or more apical, simple or branched, spathulate or espathulate appendages, median cells brown, sometimes versicoloured, thick walled, smooth or verruculose.

***Phoma* sp.** Sacc. Colonies variable in form and growth; aerial mycelium white, grey-green, olivaceous or black often sectoring, zonate or azonate; mycelium immersed, branched septate hyaline or pale brown; reverse buff, yellow, saffron, reddish, greenish brown, dark brown; pycnidia unilocular, rarely multilocular, brown, globose, separate or aggregated, occasionally confluent mostly thin, 1-3 celled thick, pale to medium brown; ostioles single or occasionally confluent and multi-ostiolate, central, not papillate; conidiophores present few species only, then either filiform, septate and branched or short, irregularly branched and ramified respectively; conidia slimy, hyaline, aseptate or occasionally one septate, thin walled, often guttulate, ellipsoid, cylindrical, fusiform, pyriform or globose, smooth.

***Pithomyces* sp.** Berkeley & Broome. Colonies punctiform or effuse, yellow, olive green, brown or black; mycelium all or mostly superficial; conidiophores branched, straight or flexuous, subhyaline, pale olive to brown, smooth or verruculose; conidia solitary, dry, simple, ellipsoidal, clavate, limoniform, obovoid, oblong, rouded at ends, pyriform, detached through fracture of the denticle, a part or which often remains attached to the conidium of short pegs, straw coloured to blackish brown, smooth, echinulate or verruculose, up to 13-transversely septate, often with one or more oblique or longitudinal septa.

***Stachybotrys* sp.** Corda. Colonies effuse, usually black or blackish green; mycelium superficial, immersed; hyphae sometimes forming ropes; conidiophores macronematous, unbranched or cymosely branched, each stipe and branch straight or flexuous, colourless greey brown, olivaceous brown or black, smooth or verrucose; phialides forming in clustures and in succession, ellipsoidal or subclavate, hyaline or pigmented, determinate, usually with very small opening and no collarette; conidia aggregating in large, slimy often black and glistening heads, acrogenous, simple, cylindrical or oblong, rounded at one end or both ends, ellipsoidal, reniform or subsperical, gray, greenish, dark brown ± opaque, smooth or verrucose, covered with dark granules or longitudinal striations, or none, 1-celled, released in basipetal succession, new conidia arise after the release of mature conidia from phialide neck.

***Torula* sp.** Peersoon ex Fries. Colonies effuse, sometimes small and discrete, olive, brown, dark, blackish brown or black, often velvety; mycelium superficial and immersed; micronematous or semi-macronematous, unbranched or irregularly branched, straight or flexus, subhyaline to mid browm, smooth or verruculose; conidia (phragmoconidia) blastic, dry, in simple or branched chains arising from surface of upper half of the conidiogenous cells, cylindrical with rounded ends, ellipsoidal, subspherical, cylindrical or fusiform, brown or olivaceous brown, smooth, verruculose or echinulate, with 0-several transverse septa, breaking into phragmo-conidia.

***Trichoderma* sp.** Pers. Colonies growing rapidly or variable depending on species, turning from watery translucent smooth surface to floccose to compactly tufted pustules; Conidiophores hyaline, much branched, not verticillate; phialides single or in groups; conodia (philospores) hyaline, 1-celled, ovoid, borne in small terminal clusters; usually easily recognized by its rapid growth and green patches or cushions of conidia; saprophytic in soil or on wood.

Rhizopus stolonifer Ehrenberg. Colonies white at first, turning brownish black, stolons spreading, internodes brown, with well branched brown rhizoids at each node; sporangiophores in clusters of 3-10, unbranched, 0.4-4 mm long and 24-42 µm, white, becoming pale to dark brown at maturity; sporangia globose, hemispherical, granular, olivaceous, black, 100-300 µm, columella hemispherical, very often becoming pilate, 45-100 µm or bigger; sporangiospores irregular, round to oval, angular, straight, grey, striate, 9-12 × 7.5-8.1 µm; zygospores round to oval, 160-220 µm, exine brown-black, verrucose; clamydospores absent.

***Syncephalastrum* sp.** Schroet. Colonies fast growing, light to dark grey; mycelium wide spreading coenocytic, profusely branched; sporangiophores erect, lacking rhizoids or often produce adventitious rhizoides, branching irregular or ± racemosely branched, often curved with branches, each branch apically dilated to form globose vesicle; vesicle globose to ovoid, separating from sporangiophores by septum, bear merosporangia directly over their entire surface; merosporangia rod shaped, many spored without a basal cell, wall evanescent at maturity; sporangiospores uniseriately arranged, globose or ovoid, formed by simultaneous cell delimitation; zygospores globose, dark brown, rough with broad shallow pointed projections, formed in the aerial mycelium between nearly equal suspensors.

Discussion

Present investigation carried out for the isolation and identification of forest leaf litter fungi to study fungal diversity of Bampewada and Sakoli forest area in Bhandara district of Maharashtra state. Studies of fungal diversity of Bampewada and Sakoli forest area in Bhandara district have not been carried out until. Although, the fungal genus found in present study is very common in forest habitat throughout the world (Paulus et al. 2006; Turkoglu et al. 2007). Many fungal species were found in litters in different forest types (Yao et al. 1997). Fifty-seven taxa comprising of 18 ascomycetes fungi were identified with direct identification method in rain forest (Parungao et al. 2002). Seventy fungi were reported in the temperate forest of Japan (Osono et al. 2002). Similarly many workers isolated number of fungi from different decomposing matter. Warcup (1957) isolated *Fusarium* sp., *Penicillium* sp. and *Mucor* sp. during decay when soluble components of straw are available. Elkady et al (1981) isolated 37 sp. of mesophilic fungi belonging to 19 genera from 15 wheat straw samples by using dilution plate technique. Kiran, Usha and Garecha (1982) isolated *Helmithosporium* sp. and *Fusarium oxysporum* from mushroom compost. Likewise *Fusarium* sp., *Mucor* sp., *Penicillium* sp. and *Trichoderma* sp. were isolated by Harper and Lynch (1984).

In this study, total 31 fungi were found in two forests namely Bampewada and Sakoli. The results suggested that the diversity of fungi associated with forest leaf litter were varied with types and climate of the forests. Number of fungal species from Sakoli forest was more than that from the Bampewada forest (Table 2). The reason may be that the quality of litters, different microenvironments and other characteristics in the Sakoli forest provided more resources for fungi than in Bampewada forest. For the significant isolation of fungi more than one method should be applied (Ramesh and Chalannavar 1998). These conclusions also correlate with the present study. The isolated fungi have shown variability in their occurrence and number. During the study it was found that *Fusarium oxysporum* and *Trichoderma* sp.1 were more common in segment plate method. From this observation it is concluded that the abundance of fungal species are slightly different in the selected areas. The total number of fungal colonies observed and identified was more in the leaf litter of Sakoli forest. This phenomenon could be even found in different parts of a plant (i.e. leaves, leaf midribs and petioles) (Hyde et al. 2000). This indicated that some fungi may preferentially develop in certain tissue types (Huang et al. 1998).

The decomposition of plants began with the intrusion of the still growing frond by the fungal pathogens (Osono et al. 2002). After falling down, the leaf litter was then decomposed by a series of fungi. The investigation indicated that the fungi diversity in the same forest litter layer in Sakoli forest was notably higher than that in Bampewada forest, suggesting that fungi diversity was related with litter quality. The reason may be that the diverse litter quality between two types of forest communities decreased with the decomposing process (Tian et al. 2002), leading to fungi richness. Litter decomposition included a processed, the physical and biological complexity of litters generally increased, leading to an increase of decomposer diversity (Huang et al. 1995; Nazim et al. 2013). Most decomposers were of litter specific (Li et al. 2000). This may be due to the fact that the fungi have their own ecological characteristics to litters. These results were very similar to previous work carried out by many workers on isolation of leaf litter fungi and conclude that members of Zygomycetes and Ascomycetes play active role in the decomposition process. Borkar (2014) observed that most of the fungi isolated from degrading biomass are the members of ascomycetes where as very few fungi belongs to other groups like Zygomycetes and Basidiomycetes. Also, Mehrotra and Aneja (1979) reported similar observations when they isolated mycoflora of *Chenopodium* leaf litter. The highest number of fungal species could be detected by serial dilution method compared to other two methods which emphasize the importance of using a combination of several cultural methods for studying fungal diversity of decaying plant substrates.

In conclusion, the results of the study suggest that the diversity of fungi in Sakoli forest is higher than the Bampewada forest. All total 31 species belonging to 20 genera of leaf litter fungi were found in selected forest area i.e. Sakoli forest and Bampewada forest. Overall, greater diversity of fungi was observed in forest area and varied between the methods employed. Based on results, sampling design to capture the diversity of microfungi in Sakoli forest area should include litter of many different tree species or leaf types as well as samples should collect from different sites. For a single time point sample, many more species of microfungi were recovered from leaf litter using serial dilution method and segment plate method rather than Blotter paper method. This further emphasizes the need for assessment of fungal communities using a molecular and biochemical techniques.

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REFERENCES

Ainsworth GC, Sparrow FK, Sussan AS. 1972. The Fungi, an Advanced Treatise. Vol. IV A. Academic Press, New York.

Barnett HL, Hunter B. 1972. Illustrated Genera of Imperfect Fungi. 3^{ed}. Burgess Pub. Co., Minneapolis.

Borkar KM, Thakre RP. 2014. Effect of temperature, pH and substrates on CMCase enzyme activity of thermophilic fungus *Humicola* insolens. Intl J Life Sci A2: 91-94.

Change ST, Miles PG. 2004. Mushrooms cultivation, national value, medicinal effect and environmental impact. 2nd ed. CRC Press, New York.

Elkady IA, Abdel-Hafez SII, Moubasher MH. 1981. Survey on the cellulose decomposing fungi of wheat straw in Egypt. Mycopathologia 76: 59-64.

Fulzele AA, Thakre RP. 2002. The cellulolytic activity of some biodegrading fungi. Biotechnology of Microbes and Sustainable Utilization. Vedams eBooks (P) Ltd., New Delhi.

Gates GM, Ratkowsky DA, Grove SJ. 2005. A Comparision of macrofungi in young Silvicultural regeneration and mature forest at the Warra LTER siet in the southern forests of Tasmania. Tasforests 16: 127.

Grigal DF, McColl JG. 1977. Litter decomposition following forest fire in northeastern Minnesota. J Appl Ecol 14: 531-538.

Harper SHT, Lynch JM. 1984. Effects of microbial colonization of barley (*Hordeum vulgare* L.) roots on seedling growth. J Appl Bacteriol 57: 131-137.

Huang J, Chen L, Han X. 1998. Change of organic matter in the decomposing oak twigs in the temperate forest eco-systems. Acta Botanica Sinica 40: 362-369.

Huang J, Han X. 1995. A review on the biogeochemical cycling in forest ecosystems: Theories and methods. Chinese Bull Bot 12: 145-223.

Hughes SJ. 1989. New Zealand fungi 33, some new species and new record of dematiaceous hyphomycetes. N Z J Bot 27: 449-459.

Hutchinso HB, Richards EH. 1921. Artificial farmyard manure. J Ministry Agri 28: 398.
Hyde KD, Alias SA. 2000. Biodiversity and distribution of fungi associated with decomposing *Nypa fruticans*. Biodivers Conserv 9: 393-402.
Isidorov V, Jdanova M. 2002. Volatile organic compounds from leaves litter. Chemosphere 48: 975-979.
Li BO, Yang C, Lin P. 2000. Ecology. Higher Education Press, Beijing.
Manoharachary C, Sridhar K, Singh R, Adholeya, Suryanarayanan TS, Rawat S, Johri BN. 2005. Fungal biodiversity: Distribution, conservation and prospecting of fungi from India. Curr Sci 89: 58-71.
Mehrotra RS, Aneja KR. 1979. Microbial decomposition of *Chenopodium album* litter. Succession of decomposers. J Indian Bot Soc 58: 189-195.
Mueller, G. M. Bills, G. F.Foster, M. S. (eds) 2004. Biodiversity of fungi: inventory and monitoring methods. Elsevier, Amsterdam.
Mueller, G. M., Schmit, J.P. 2007. Fungal biodiversity: what do we know? What can we predict? Biodivers Conserv 16 (1): 1-5.
Nazim K, Ahmed M, Shaukat SS, Khan MU. 2013. Seasonal variation of litter accumulation and putrefaction with reference to decomposers in a mangrove forest in Karachi, Pakistan. Turk J Bot 37: 735-743.
Osono TS, Hobara S, Fujiwara K, et al. 2002. Abundance, diversity, and species composition of fungal communities in a temperate forest affected by excreta of the Great Cormorant *Phalacrocorax carbo*. Soil Biol Biochem 34: 1537-1547.
Parungao MM, Fryar SC, Hyde KD. 2002. Diversity of fungi on rainforest litter in North Queensland, Australia. Biodivers Conserv 11: 1185-1194.
Paulus B, Kanowski J, Gadek P, Hyde KD. 2006. Diversity and distribution of saprobic microfungi in leaf litter of an Australian tropical rainforest. Mycol Res 110 (12): 1441-1454.
Ramesh CH, Chalannavar RK. 1998. Preliminary studies on decomposition of leaf litter of *Citrus aurantium*: comparative evaluation of different isolation techniques. Indian Phytopathol 51 (4): 385-386.
Raper KB, Thom C. 1949. A Manual of Penicillia. Williams and Wilkins Company, Baltimore.
Rawling TE. 1933. Phytopathological and Botanical Research Methods. John Wiley and Sons, London.
Saksena SB. 1955. Ecological factors governing the distribution of microfungi in forest soil of Sagar. J Indian Bot Soc 34: 262-298.
Saravanan R, Sivakumar T. 2013. Biodiversity and Biodegradation potentials of Fungi isolated from Marine systems of East Coast of Tamil Nadu, India
Int J Curr Microbiol App Sci 2 (7): 192-201.
Senthilarasu G. 2014. Diversity of agarics (gilled mushrooms) of Maharashtra, India. Curr Res Environ Appl Mycol 4 (1): 58 78.
Sutton BC. 1980. The Coelomycetes, fungi imperfectii with pycnidia, ascervulii and stromata. CMI, Kew, England.
Tian X, Tateishi T. 2002. Relative roles of microorganisms and soil animals on needle litter decomposition in a subalpine coniferous forest. Acta Phytoecology Sinica 26: 257-263.
Tokumasu S, Tubaki K, Manoch L. 1997. Microfungal communities on decaying pine needles in Thailand. In: Janardhanan KK, Natarajan KR, Hawksworth DL. (eds.) Tropical Mycology. Science Publishers Inc, Enfield, New Hampshire.
Turkoglu A, Kanlik A, Gezer K. 2007. Macrofungi of Çameli District (Denizli-Turkey). Turk J Bot 31: 551-557.
Van der Walt JP, Yarrow D. 1984. Methods for the isolation, maintenance, classification and identification of yeast. In Kreger-van Rij NSJ (ed.). The Yeast: A Taxonomic Study. 3rd ed. Elsevier, Amsterdam.
Von Arx JA. 1981. The genera of fungi sporulating in pure culture. 3rd ed. J Cramer, Vaduz, Germany.
Warcup JH. 1957. Studies on the occurrence and activity of fungi in a wheat-field soil. Trans Br Mycol Soc 40: 237-262.
Yao T, Yang J. 1997. Microbial Ecology of litter and Soil in Forest. J Northwest For Coll 12 (4): 97-103. [Chinese]

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The roles of community fruit garden (*tembawang*) on maintaining forest structure, diversity and standing biomass allocation: an alternative effort on reducing carbon emission

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Abstract. *Astiani D, Ripin. 2016. The roles of community fruit garden (tembawang) on maintaining forest structure, diversity and standing biomass allocation: an alternative effort on reducing carbon emission. Biodiversitas 17: 359-365.* Fruit garden (*tembawang*) Cempedak Village in Sanggau West Kalimantan has been established by local community for their family mixed garden ~ >100 years. The families of 3rd generation were utilizing the tembawang for their needs of building materials, fire woods, rattan, vegetables, fruits, and traditionals medicine. It is important to study how this tembawang play their roles in maintaing forest structure, species diversity and stocking biomass. In 2014, we studied this area for exploring the vegetation composition and their ability to stock biomass of the tembawang. Stratified Random Sampling was applied to the 6.69 ha tembawang area, which devided into three major land cover patches (Mixed fruit garden, mixed rubber plants, and fruit garden mixed with *apik-Arenga undulatifolia* Becc palm). We surveyed and sampled vegetation using transect methods purposively chosen on each landcover patch, with sampling area consecutively for mixed fruit garden, mixed rubber plants, and mixed of fruit garden and *apik* were 1.52, 0.6, and 0.72 hectars. Results demonstrated that mixed fruit garden carried out higher tree diversity, density, basal area, and maintained the largest above ground biomass per hectar compared to the two other patches. From 97 vegetation spesies registered, it maintained 49 tree species of diameter >20cm and 51 tree species in the lower strature in the forest structure. Interestingly, *Durio zibethinus* Murr. was a dominant species on all landcover patches types and sinked the largest above ground living biomass. The choice of fruit species on tembawang determined the capability of the *tembawang* land to sequester and stock carbon in trees, because the trees were standing in tembawang for longer time compared to the one in production forest. This results show that, beside its multiple role for people community, tembawang provide other benefits to the nature in maintaining forest structure, diversity, and stocking large carbon in standing biomass.

Keywords: Above ground biomass, carbon stocks, lowland tropical forest, mixed fruit garden

INTRODUCTION

Rapid loss and degradation of humid tropical rain forests over recent years threatened the sustainability of forest in serving their functions to nature and surrounding people, which has direct need on forest for their daily life (Colfer et al. 1997). It had been long time ago, West Kalimantan dayak people established agricultural system that traditionally based on production of upland rice field. Upon the 2-3 times of growing rice, the land was planted with fruit trees, rubber, plant together with edible plant such as mushroom, fern, vegetables, medicinal herbs and other non timber forest products (Wulan et al. 2008; Potter 2012). Non timber forest product in the mixed fruit garden, so called *tembawang*, play an important role in rural livelihood strategies and contributes to sustained forested landscapes in various tropical areas (Jong, 2001). The mixed planted fruit trees and other then grown and maintain for many generation, and taken care of by communal families. The *tembawang* has also been used for their historical placed where their ancestors longhouse were built at the site.

Tembawang Cempedak Village is one sample of the mixed forest that was established in Tayan District Kabupaten Sanggau West Kalimantan. It was part of lowland tropical forest that has large range of species diversity. The alteration of natural forest to tembawang changed the natural species composition into mixed fruit garden and trees. Forest management should facilitate both the preservation of ecosystem function and the conservation of biodiversity, as well as supporting economic benefit (Lawrence et al. 2007; Potter et al. 2008). However, those mixed forest provide an array of benefits and goods for people communities such as supplying raw material for housing and building, fire woods, vegetables, fruits, traditional medicines and other uses. In fact, the forest is maintain for many years growing large trees especially fruit trees, bamboos, shrubs, and vegetables sustainably. The mixed vegetation demonstrated enhancing soil nutrition in tembawang (Lawrence et al. 2007).

Recent condition, on the other hand, these areas was also facing large pressures from other land uses which could destruct those valuable lands. Since there are large benefits for communities and the sustanable management they applied, it is urgent to maintain tembawang for local communities demonstrating their roles on preserving biodiversity, mitigating global warming, and protect them from the pressures. The study of the mixed forest species composition, structure, and their role in carbon allocation is important to describe this subsistent management practices in West Kalimantan rural communities.

MATERIALS AND METHODS

Study site

The area of research was in community fruit garden of Cempedak Village, Tayan Hilir, Sanggau District in West Kalimantan which located between 00º43'06"LU and 110º18,5'39'' BT, and 24 -140 m above sea level (Figure 1). The site was about 200 km from Pontianak (West Kalimantan Capital City) and can be reach ~ 2 hours with land transportation. From 2000-2013, mean annual rainfall was 3025 mm ± 52 mm with 182 ± 5 rain days per year (compiled from local Climate station 2000-2013) with mean temperature 29^{0}C. The soil of area was dominated by 'Red-Yellow Podsolic' Ultisols, an acidic, leached, light color surface layer soil, with topography dominated by flat area mixed with some hilly terrains which reached 30% slope.

People community was native Dayak Tobak ethnicity. Shifting cultivation and tree-crop management have continously practiced for over 100 years by people community in this region. The community fruit gardens were formerly started as practices of cultivation of upland rice within scattered areas, and followed by establihment of relatively longtime mixed fruit-forest garden management. The fruit garden supplied community's basic needs of families for 3-4 generation.

Vegetation analysis

Survey technique. Vegetation analysis was done with survey and vegetation inventory. First, we used *stratified random sampling* to group landcovers variation within the tembawang by horizontally surveying overall the area and discover that the tembawang had 4 main different land covers (i.e., upland rice field, mixed fruit garden, mixed

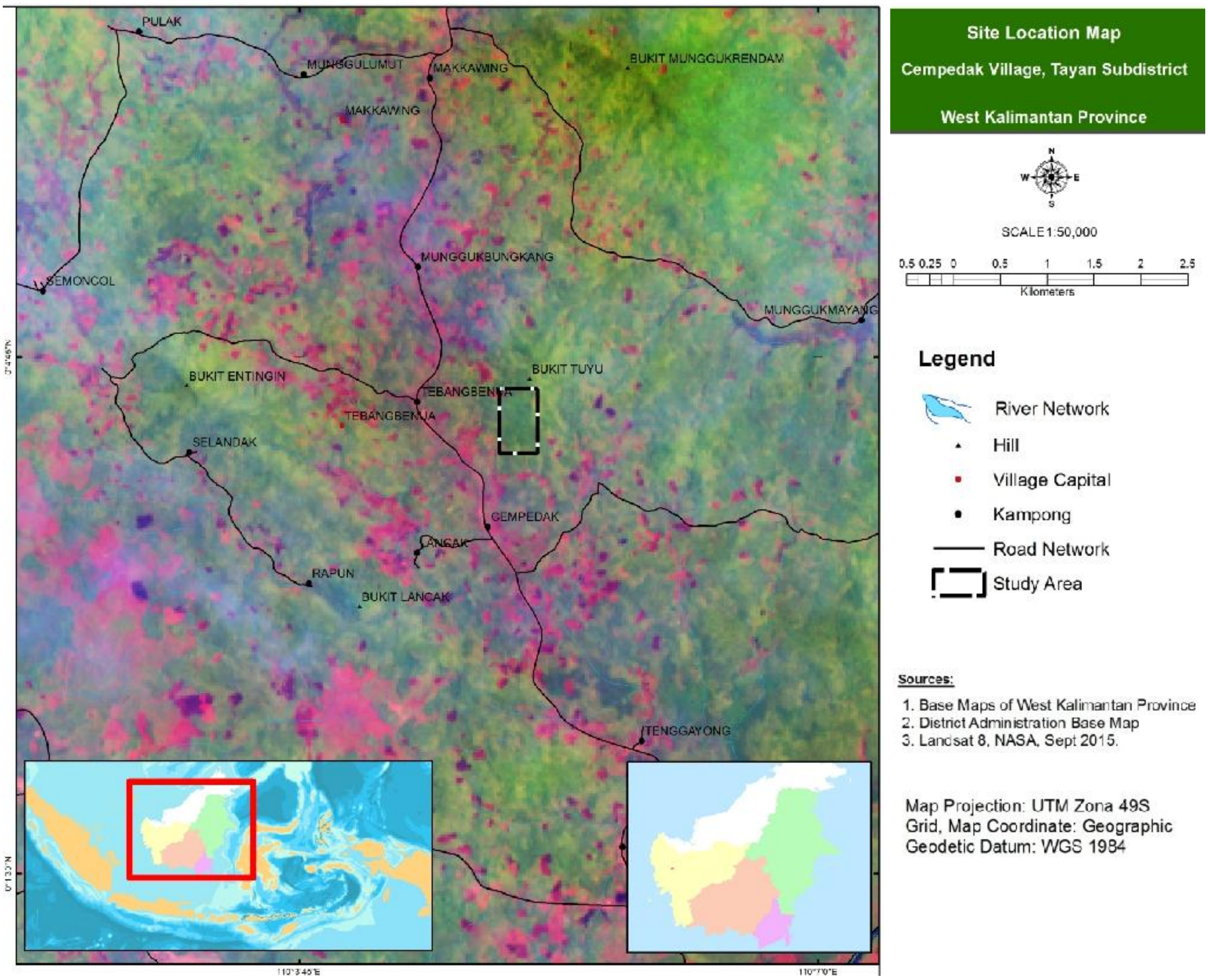

Figure 1. Research site in Cempedak Village, Sanggau District, West Kalimantan, Indonesia

rubber plants, and mixed of fruit garden and *apik* (*Arenga undulatifolia* Becc.). The *apik* was naturally growing plant on the hilly site which found abundant and grown among the fruit garden. The soil on this site was shallow and mixed with large rocks, with a dominant proportion of fruit trees, dominant rubber plant (*Hevea brasiliensis*) and *apik* plant (*Arenga undulatifolia*) as well as upland ricefield (this patch was not measured in this study).

Sampling technique. Since the area of each landcover patch varies, the sampling areas on each land cover type were also proportionally different. The sample area for mixed fruit garden, mixed rubber plants, and mixed of fruit garden and *apik* were 1.52, 0.6, and 0.72 hectars respectively. Based on our pre-survey the mixed fruit garden had higher vegetation variability and diversity, therefore, the sample transects were distributed wider and more than other 2 land covers, while mixed rubber plants and mixed *apik* forest were relatively more uniform. Transects in each landcover type were distributed sistematicaly within each area. Along each transect, 20m x 20m plots were established for tree diameter >20cm registration, and smaller nested plots were established to analyse smaller tree structure (10 m x 10 m for tree diameter 10-20cm, 5m x 5m for tree diameter 5-10cm, and 2m x 2m for seedlings, including groundstorey, small shrubs, and liana. Trees diameter >5 cm were assessed for their diameter, identified their species name, and collected their sample for scientific name. All tree diameters were assessed at breast height (DBH) using *phi band*. Smaller trees were disturbedly sampled with 1m x1m of 4-5 plots nested within their larger plots. The all species within the sample plots were harvested, weighted and sampled for water content and carbon analysis. All field works were executed by Tanjungpura University team and 4-5 local community workers

Data analysis

Within each landcover condition, data were separated based on tree/plant growing levels. Basal areas of trees on patches were calculated using the area of a circle formula (*Basal area of a tree* = $\pi / 4 \times DBH^2$). The cummulative basal area was presented in m^2 per hectar. Biomass of tree diameter >5cm biomass were estimated using Chave (2005) equation which involved tree wood-specific gravities. Each data was presented as total (species composition) or mean and standard error. The comparation among three each landcover was compared using multiple T Test.

RESULTS AND DISCUSSION

Vegetation structures

Each landcover patch demonstrate significantly in its own stature. More than 100 years old forest tree garden resulted an advance trees succession and growth compared to other two landcaover patches. More and larger trees standing resulted in higher basal area and higher persentage canopy cover which rated ~>80% yet lesser amount of trees in small trees compared to mixed rubber plants (Figure 2A and 2B). Figure 2C. demonstrate that mixed fruit garden with *apik* had less trees and the natural growth *apik* has relatively high density and dominated that stony, lesser topsoil land, while Figure 2D showed hilly rice field which hardly trees available. The ricefield patch was not measured in this study.

All three land cover patches demonstrated a balance composition in the forest stature shown by 'J-shape' in their logged graphics (Figure 3). However, mixed rubber plant and mixed fruit with *apik* land cover show much lower density. Seedling, sapling, pole, and tree density were respectively ranged between 40,000-50,000; 975-2,500; 234-300; and 131-176 trees per hectar. This structure represented the present of each forest stature. This results explained that the *tembawang* forest regeneration was distributed well in each landcover type, regardless what type was the landcover patch..

Based on tree basal area analysis, mixed fruit garden seems more eficient in accupying land per unit area. Observed basal area were 31.8, 14.1, and 13.2 m2 ha-1 respectively for mixed fruit garden, mixed rubber plants, and mixed fruit garden with *apik*. Figure 4. described how trees basal area per hectar of mixed fruit garden produced much higher (more than double) than the other land cover types, while mixed rubber and fruit mixed with *apik* showed relatively similar basal area. The basal arrea of mixed fruit garden was comparable to published value abd a bit higher than Chave et al. (2005) (26.35 m^2 ha^{-1}, Djulkono et al. (2010) (29.38 m^2 ha^{-1}), and Swamy et al. (2010) (29.42 m^2 ha^{-1}).

Species diversity on each land covers type

Mixed fruit garden tree species content showed significantly higher in tree species diversity, comparatively on each structural level (Figure 5). It maintain 49 tree species on diameter >20cm and 51 tree species in the lowest strature in the forest structure. Our previous analysis, there were 97 plant species from 37 family were found in the tembawang (Ripin et al. 2014). Our results indicated significant reduction of larger tree species diversity in mixed rubber (6 tree species) and mixed *apik* garden (7 tree species) compared to mixed fruit garden (49 tree species). The most prominent amount of species was showed in seedling and tree level of structure. The list of tree species is presented in Table 1. Mixed fruit garden was dominated with rambutan (*Nephelium lappaceum*), duku (*Lansium domesticum* var. duku), mahang (*Macaranga pruinosa* Muell. Arg), karet (*Hevea brasilliesis* Will ex A. Juss), durian (*Durio zibethinus* Murr). Mixed rubber plant was dominated by karet, duku, mahang, kepayang (*Scaphium macropodum* J. Beum), and durian while in Mixed Fruit Garden with *apik* was dominated with asam gandaria (*Bouea macrophylla* Griff.), asam kemantan (*Mangifera torquenda),* jengkol (*Pithecellobium jiringa* (Jack) Prain.), engkasai (*Pometia* sp.), and durian. Interestingly, each land cover type was dominated by almost similar species such as karet, mahang, and durian. This is indicated that those common species were significantly and economically important in fulfilling community daily needs.

Biomass allocation on landcover patches

Similar with tree basal area on each landcover patch, because more trees diameter >20 cm mean in mixed fruit garden patch, biomass allocation was also significantly different, however, the trend on mixed fruit garden with *apik* was lower than mixed rubber plants (Figure 6).

The biomass was estimated by using allometric equation that consider spesific wood density of each tree species. Aboveground standing biomass were 554.9 ±21.7, 200.8 ± 18.4, 97.7 ± 9.7 ton ha^{-1} respectively for mixed fruit garden, mixed rubber plant, and mixed fruit garden with *apik*. Mixed fruit garden biomass alocation reach This results show that eventhough the land accupation by vegetation was equal on mixed rubber and mixed fruit with *apik*, their biomass allocation was much depend on the vegetation species which was higher in mixed rubber plant.

Figure 2. The figures of (A) mixed fruit garden, (B) mixed rubber plants, (C) mixed fruit garden with *apik*, and (D) hilly rice-field

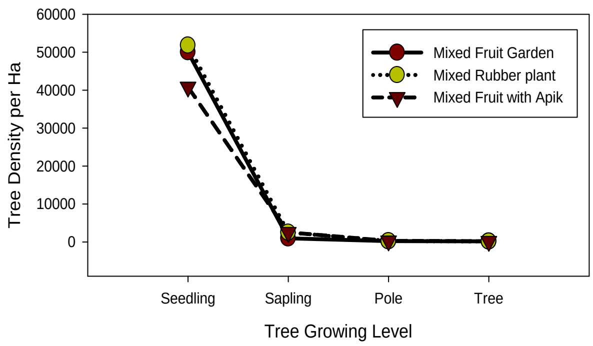

Figure 3. Tree structure distributions on each tembawang dominant land cover patches

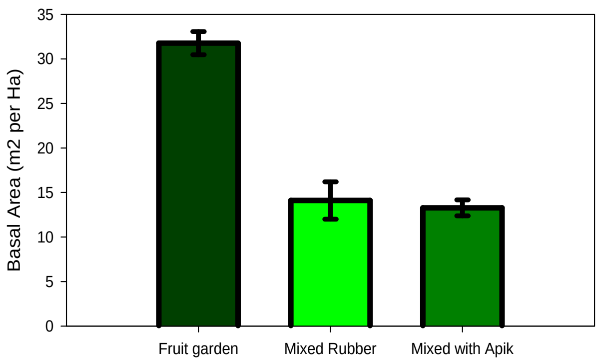

Figure 4. Basal Area distribution on each land cover patch

Table 1. Tree species diameter >20, basal area, and tree density found on each land cover patch of Tembawang Cempedak Village, Sanggau District, West Kalimantan, Indonesia

Nama jenis	Scientific names	LBD (m^2 ha^{-1})	Tree density (N ha^{-1})
Mixed fruit garden			
Asam gandaria	*Bouea macrophylla* Griff.	0,15	1
Asam kemantan	*Mangifera torquenda* Kosterm.	0,24	1
Asam mawang	*Xanthophyllum excelsum* Miq .	0,59	1
Belimbing darah	*Baccaurea angulata* Merr.	0,45	1
Benyalit/langer	*Xanthophyllum excelsum* Miq .	0,18	2
Cempedak	*Artocarpus teysmanni* Miq .	1,15	9
Ceriak	*Baccaurea sp* (Euphorbiaceae)	0,17	3
Dadap	*Erythrina variegata* L.	0,08	1
Duku	*Lansium domesticum var Aqueun* Corr	0,44	7
Durian	*Durio zibethinus* Murr.	14,49	44
Embacang	*Mangifera swintonioides*	0,19	1
Engkasai	*Pometia* glabra (Bl) Teijsm.	0,16	2
Entanak	*Pterocarpus* sp. (Fabaceae)	0,05	1
Jabon	*Antocephalus cadamba* Miq	0,12	1
Jengkol	*Pithecellobium jiringa* (Jack) Prain	0,84	1
Karet	*Hevea brasilliesis* Will ex A. Juss	0,73	13
Kayu Ara	*Ficus benyamina* Linn.	0,10	1
Kelampai	*Elateriospermum tapos* Bl.	0,17	1
Kelampai Tupai	*Blumeodendron tokbrai* Bl.	0,42	1
Kelawik/Teratung	*Durio oxleyanus* Griff.	0,79	1
Kemenyan	*Styrax benzoin* Dryand.	0,47	11
Kempas	*Koompassia malaccensis* Maing.	0,48	1
Ketup	*Lansium domesticum var. aquaeum*	0,19	7
Kubing	*Artocarpus* heterophyllus. Lam	0,21	3
Kumpang	*Gynnacranthera forbesii* Warb.	0,49	3
Lansat	*Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet	0,05	9
Leban	*Vitex pubescens* Vahl.	0,03	1
Manggis	*Garcinia mangostana* L.	2,68	1
Mentawa	*Artocarpus anisopyllus* Miq	0,90	12
Meranti batu	*Shorea palembanica* Miq.	0,06	3
Meranti padi	*Shorea leprosula* Miq .	0,48	1
Nyatoh	*Palaquium pseudocuneatum* H.J.L	0,11	4
Panting	*Aporosa arborea* Muell. Arg.	0,36	1
Peluntan	*Artocarpus rigidus* Bl.	0,43	2
Petai	*Parkia speciosa* Hassk.	0,08	3
Petai hutan	*Leucaena leucocephala* (Lam) de Wit	0,09	1
Rambai	*Baccaurea motleyana* Mull.Arg.	0,08	1
Sibau	*Nephelium uncinatum* Radlk. ex Leenh.	0,78	6
Sotol	*Sandoricum koetjape* (Burm. f.) Merr.	0,30	3
Tampui	*Baccaurea grifithii* Hookf.	0,25	3
Tengkw. rambai	*Shorea splendida* (de Vriese) P. Ashton	0,02	1
Tengkw. tungkul	*Shorea stenoptera* Burck	0,45	1
Terap	*Artocarpus elasticus* Reinw.	0,93	6
Terentang	*Campnosperma auriculata* Hook. F.	0,11	1
Ubah sp.1	*Eugenia albidiramea* Merr.	0,10	1
Ubah sp.2	*Syzigium kunstleri* (King) Bahadur & R.C.Gaur	0,12	1
	Total	32,20	176
Mixed rubber plants			
Durian	*Durio zibethinus* Murr.	4,07	6
Karet	*Hevea brasilliesis* Will ex A. Juss	5,57	88
Kelangkik	*Blumeodendron tokbrai* Bl.	0,28	6
Ketup	*Koompassia malaccensis* Maing.	0,57	6
Lansat	*Gynnacranthera forbesii* Warb.	0,22	6
Peluntan	*Aporosa arborea* Muell. Arg.	2,76	6
Sotol	*Sandoricum koetjape* (Burm. f.) Merr.	0,28	6
Tampui	*Baccaurea grifithii* Hookf.	0,36	6
	Total	14,11	131
Mixed forest with *apik*			
Asam Mawang Bulan	*Xanthophyllum excelsum* Miq .	0,63	8
Cempedak	*Artocarpus teysmanni* Miq .	0,67	8
Durian	*Durio zibethinus* Murr.	3,95	33
Karet	*Hevea brasilliesis* Will ex A. Juss	2,51	33
Kemenyan	*Styrax benzoin* Dryand.	2,35	33
Kepayang	*Scapium macropodum* J.Beum	1,64	8
Terap	*Artocarpus elasticus* Reinw.	1,53	33
	Total	13,27	158

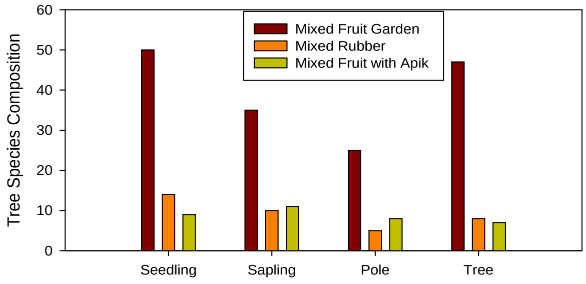

Figure 5. Tree species composition on each growing stage of three land cover in tembawang

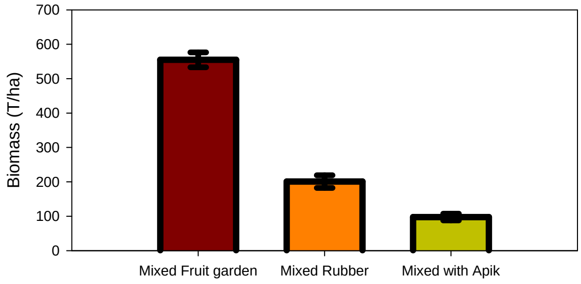

Figure 6. Biomass allocation of each land cover patch in tembawang

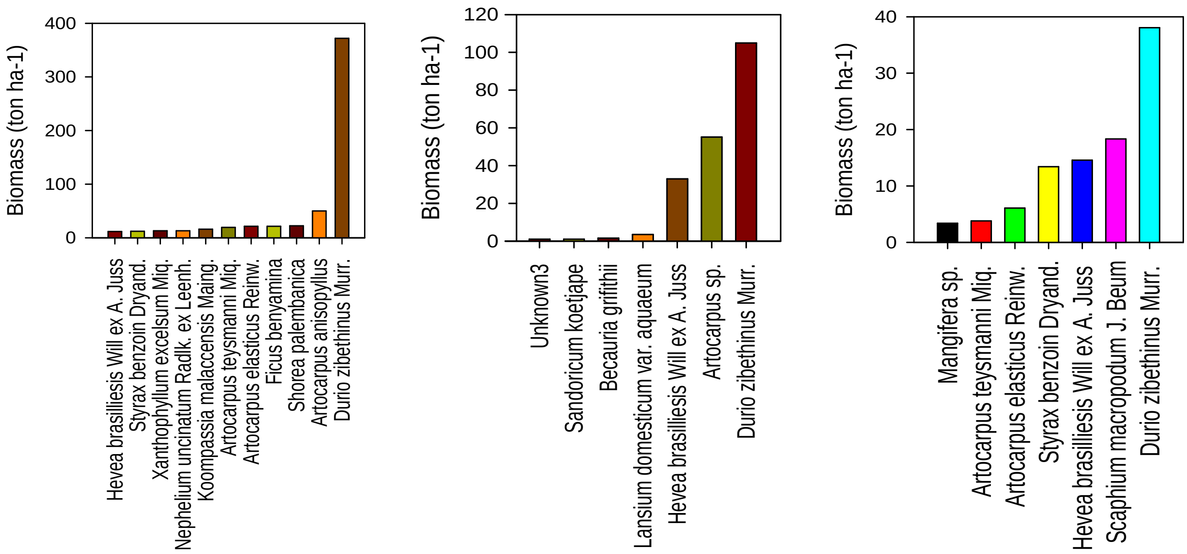

Figure 7. Tree species biomass allocation on three land cover patches: A. Mixed fruit garden, B. Mixed rubber plants, and C. Mixed fruit garden with *apik*

Each tree had their special ability in producing living biomass. Our further analysis on each tree biomas found that durian trees (*D. zibethinus* Murr.) allocated biomass significantly high in all land cover patches (Figure 7A, 7B, and 7C). This trend affected because most of durian trees in this area were old and mostly huge in tree diameter, which cause high total biomass allocation.

Discussion

We compared 3 land cover patches type of forest gardens, or *tembawang*, in Cempedak Village, Sanggau District, West Kalimantan, Indonesia, to to examine community forest in terms of diversity, structure, and biomass alocation. This information was acquired for 97 vegetation species inventoried in the *tembawang* forest gardens and its 3 varians of forest landcover-type patches. In particular tembawang with mixed fruit garden type were found to have higher species diversity, higher density, and higher biomass allocation compared to mixed rubber plant and mixed fruit garden with *apik*. mixed fruit garden also showed better successional stages and modes of dispersal as natural forests. Thus, this was emphasising the potentialof *tembawang* in conserving tree species.

Within two patches of land cover types (mixed rubber plant and mixed with *apik*), non-planted trees species of *tembawang* and natural forests also have practically been maintained, indicating that the management of these *tembawang* does not significantly discriminate between species with certain uses. However, we also identified two aspects that should be taken into account in considering the conservation role of *tembawang*. The species composition of the 3 patches showed significant difference, implying that efficient conservation in West Kalimantan ex-agricultural land areas. We found the reduction in species diversity of pathes in the tembawang were worse than the on impacted by forest degradation (Astiani 2016). We also found some species of the same ecological characteristics found in natural forests. Thus, even if *tembawang* are similar to natural forests in terms of numbers of species with different ecological characteristics, the composition of non-planted tree species in *tembawang* is not a random sample from natural forest, but over represents species that are useful and provided benefits for community daily needs.

Because the carbon stocks of tropical forest were uncertain (Houghton et al. 2001; Fearnside and Laurance 2003; Eva et al. 2010), this *tembawang* mixed fruit garden with the various kind of patches is important to refine and explain that the deforestation in tropical forest can not only explain the emission rate Houghton et al. (2005), yet on the other hand with tembawang management applied by rural communities in West Kalimantan also show how much carbon can be sinked for longer period of time. Viewed from *tembawang* ability to sink carbon, it is undoubtable that they sink large amount of above ground carbon especially within mixed fruit garden patches that reach ~300 ton/ha. The carbon maintained far larger than peatland forest that we assessed (Astiani et al. 2015). This results proof that tembawang at Cempedak Village provides an array of benefit, not only for local economic conservation, but also for biodiversity conservation and environment points.

In conclusions, West Kalimantan community fruit garden management was able on maintaining forest structure, diversity, and sinking a significant amount of carbon in forest biomass, fulfilling wide range of family needs to support their life as well as tending the sustainability of the forest for more than a hundred years. The choice of tree species determined the *tembawang* characteristics and their ability to sequestered and sink carbon. It is important to support this type of forest management since it demonstrated to benefit more for community life and forest conservation, especially in efforts on reducing carbon emission from forest degradation and deforestation activities. A scheme of financial award through carbon sequestration insentives program could be an alternative to enhance and maintain this type of forest management.

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REFERENCES

Achard F, Eva HD, Stibig HJ, Mayaux P, Gallego J, Richards T, Malingreau JP (2002) Determination of deforestation rates of the world's humid tropical forests. Science 297 (5583): 999-1002.

Astiani D, Mujiman, Rafiastanto A, Nurdiansyah E, Darkono, Hatta M. 2015. Impact of forest degradation on forest biomass and diversity of peatland forest. In: Hartono R, Iswanto KH, Hartini KS et al (eds) The Utilization of Biomass from Forest and Plantation for Environment Conservation Efforts. Prosiding Seminar Nasional MAPEKI XVII: 249-256.

Astiani D. 2016. Tropical peatland tree species diversity altered by forest degradation. Biodiversitas 17 (1): 102-109.

Colfer CJP, Peluso NL, Ching Sc. 1997. Beyond slash and burn: building on indigenous knowledge in managing Borneos tropical rain forests. . New York Botanical Garden Press, Bronx, NY, USA.

Chave J, Andalo C, Brown S, Cairns MA, Chambers JQ, Eamus D, Folster H, Fromard F, Higuchi N, Kira T, Lescure JP, Nelson BW, Ogawa H, Puig H, Riera B, Yakamura T. 2005. Tree allometry and improved estimartion of carbon stocks and balance in tropical forests. Oecologia 145: 87-99.

Djulkono MNK, Doucet JL, Nguembou CK, Lewis SL, Sonke B. 2010. Diversity and above ground biomass in three tropical forest types in Dja Biosphere Reserve Cameron. Afr J Ecol 48: 1053-1063.

Eva H, Carboni S, Achard F, Stach N, Durieux L, Faure J-F, Mollicone D. 2010. Monitoring forest areas from continental to territorial levels using a sample of medium spatial resolution satellite imagery. ISPRS J Photogrammetr Remote Sens, 65: 191-197.

Fearnside PM, Laurance WF. 2003. Comment on determination of deforestation rates of the world's humid tropical forest. Science 299: 1015a

Houghton RA. 2003. Revised estimates of the annual net flux of carbon to the atmosphere from change in landuse and land management 1850-2000. Tellus 55B: 378-390.

Houghton RA. 2005. Abovegroun biomass and the global carbon balance. Global Ch Biol 11: 945-958.

Jong WD. (2001. The rubber impacts on the forest landscaoe in Borneo. In: Angelsen A, Kaimowitz D (eds) Agricultural Technologies and Tropical Deforestation. CAB International Publishing. New York.

Jong WD, Noordwijk MV, Sirait M, Liswanti N. 2001. Farming secondary forests in Indonesia. J Trop For Sci 13 (4): 705-726.

Lawrence D, Astiani D, Karwur MS, Fiorentina I. 2007. Does tree diversity affect soil fertility? A crytical hypothesis and initial finding from various fallow management system in West Kalimantan Indonesia. Cairns M (ed) Voices from the Forest, Integrating Indigenous Knowledge into Sustainable Upland Farming: An RFF Press, Washington, DC, USA.

Marjokorpi A, Ruokolainen A. 2003. The role of traditional forest gardens in the conservation of tree species in West Kalimantan, Indonesia. Biodiv Conserv 12 (4): 799-822.

Mirjam AFR, Wiersum KF. 2005. The scope for improving rural livelihoods through non-timber forest products: an evolving research agenda. For Trees Livelihoods 15 (2): 129-148.

Potter L. 2012. Swidden, oil palm, and food security in West Kalimantan. Kasarinlan Philliphine J 3rd World Stud 26 (1-2): 252-263.

Swamy SL, Dutt CBS, Murthy MSR, Mishra ALKA, Bargau SS. 2010. Floristics and dry matter dynamics of tropical wet evergreen forest of Western Ghats India. Curr Sci 99: 353-364.

Wulan YC, Budidarsono S, Joshi L. 2008. Economic analysis of improved smallholder rubber agroforestry systems in West Kalimantan, Indonesia-implications for rubber development. ICRAF, Bogor Indonesia.

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Short Communication: *Fusarium* as endophyte of some terrestrial orchid from Papua, Indonesia

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Abstract. *Sufaati S, Agustini V, Suharno. 2016. Fusarium as endophyte of some terrestrial orchid from Papua, Indonesia. Biodiversitas 17: xxx.* The aim of the study was to identify endophytic fungi associated with the roots of terrestrial orchid *Phaius tankervilleae* (Banks) Blume, *Dendrobium lancifolium* A. Rich var. *papuanum* and *Calanthe triplicata* (Willem) Ames from Papua, Indonesia. The endophytic fungi were isolated from the transversal section of the orchid roots. Identification of the endophytes was carried out based on the morphological characters. The phylogenetic analysis of nucleotide sequences generated from ITS rDNA region of the endophytic fungi isolated from *P. tankervilleae* showed that those isolates were determined as *Fusarium solani*. This is the first report of *F. solani* found as endophyte of *P. tankervilleae* in Papua. While the ITS rDNA of *Fusarium* isolated from *C. triplicata* need to be sequenced for further identification.

Keywords: Endophyte, *Fusarium*, ITS rDNA, terrestrial orchid, Papua

INTRODUCTION

Endophytic fungi are one of fungal group that grow in the intracellular tissue healthy plants (Cabezas et al. 2012). Fungal endophyte is defined as all or part of their life cycle colonizing inter- and/or intra-cellular of healthy tissue of the host plant, usually does not cause symptoms (Zhao et al. 2010). The linkage between endophytic fungi and plant host system enables systematic work involving a variety of metabolic processes in plant cells. In addition, endophytic fungi also source of various antibacterial metabolites (Sugijanto et al. 2009; Sinaga et al. 2009), various types of enzymes (Kumala et al. 2007), anti-tumor/cancer (Tabudrayu and Jaspars 2005; Kumala et al. 2008; Tejesvi and Pirttila 2011), and other bioactive compounds for various purposes (Zhao et al. 2010), including biological control (Zimmerman and Vitousek 2012). It also increases resistance of plants to herbivores, pathogens and various abiotic stress (Diaz et al. 2012).

Environmental conditions affect the existence of endophytic fungi, in association with various types of host plant as phycobiont symbiotic (Zimmerman and Vitousek 2012; Aschehoug et al. 2012). Endophytic fungi association is not only symbiotic mutualism, but also parasitism depending on the condition of their host. Several fungi have shown this type of association, such as *Fusarium* spp. and *Colletotrichum* spp. (Redman et al. 2001). Several endophytic fungi are also capable to shift their lifestyle to saprobes once their host decayed, such as *Xylaria* spp. and *Diaporthe* spp. The remaining endophytic fungi are true endophytes, including many *mycelia sterilia* fungi (Redman et al. 2001; Aschehoug et al. 2012). Therefore, determination of the endophytic fungal identity is very important in studying their diversity and ecology.

However, several problematic in endophytic fungal studies were reported; include limitation in isolation method and accurate identification method to species level. The identification based on morphological characters can perform only to the level of genus of sporulating fungi. The identification to species level is still needed technical or other aids; given that the fungi have a high morphological similarity among them. Thus, the molecular identification is expected to provide more accurate information about the species name of endophytic fungi (Faeth and Fagan 2002). The nucleotide sequences providing virtually unlimited character for phylogenetic analysis (Diaz et al. 2010). An area that can be used to detect sequences of fungi is Internal Transcribed Spacer region (ITS) (Gomes et al. 2002). This area has a high variability nucleotide sequences for studies molecular systematic of fungi at the species level. Therefore, ITS sequences can also be used for studying and determining endophytic fungi because it shows high sequence heterogeneity.

Endophytic fungi also found in some Orchidaceae in several area of the world (Tupac and Otero 2006, Gezgin and Eltem 2009), both epiphytic (Ovando et al. 2005; Yuan et al. 2009) and terrestrial orchid (Chutima 2010). In Indonesia, however, little is known about the endophytic fungi associated with orchid. Suciatmih (2008) was able to isolate and identify the endophytic fungus from *Dendrobium crumenatum* Sw.

Papua is home of about 512 species of *Dendrobium* (Millar 1978). The terrestrial orchid *Phaius tankervilleae*, *Dendrobium lancifolium* A. Rich var. *Papuanum* and *Calanthe triplicata* (Willem) Ames are also common found in Papua (Agustini et al. 2013; Agustini and Sufaati 2014).

In addition, forming mycorrhizal symbiosis with fungi such as *Rhizoctonia*, they are also associated with a group of other fungi, such as endophyte. So far, there is few data on the presence of the endophytic fungi in the Papuan orchid (Agustini et al. 2009). Therefore, this study was done to identify endophytic fungi associated with the roots of terrestrial orchid *P. tankervilleae*, *D. lancifolium* var. *Papuanum* and *C. triplicata* from Papua, Indonesia.

MATERIALS AND METHODS

Collection site

Roots of *P. tankervilleae* were collected at Papua Province of Indonesia, namely: Ifar Gunung, Sentani, Jayapura District (August 2014), *D. lancifolium* var. *Papuanum* from Biak District (April 2015) and *C. triplicata* from Keerom District (April 2015) (Figure 1).

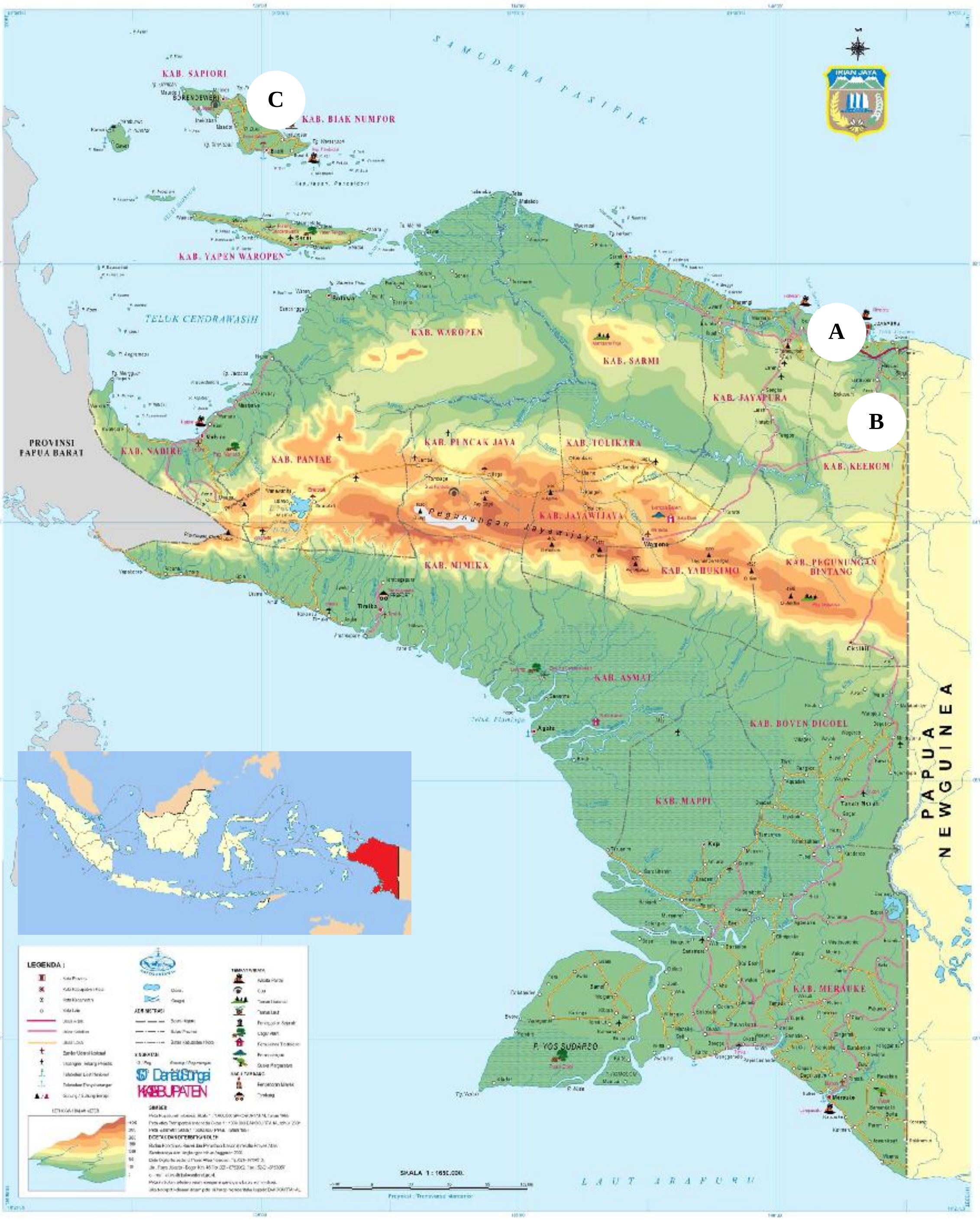

Figure 1. Location of root collection of *P. tankervilleae*: A. Ifar Gunung, Sentani, Jayapura, Papua Indonesia (2º55’69.78”S, 140º54’02.87”E), B. *Calanthe triplicata* (Willem) Ames from Keerom (2º50’03.60”S, 140º43’54.94”E) and C. *Dendrobium lancifolium* A. Rich var. *papuanum* from Biak (01º09’94.0”S, 136º10’95.5”E)

B

Isolation of endophytic fungi from plant roots

Isolation of fungi was done using a technique developed by Manoch and Lohsomboon (1991) with modification. Roots was washed to remove soil debris, and then cut ± 1 cm, followed by surface sterilization with 10% Clorox for 30 seconds, 70% alcohol for 1 min, and rinsed with distilled water three times. Sterilized root pieces were thinly sliced (200-300 µm) using a razor blade in a laminar air flow (LAF). About 1-3 pieces of the sliced roots were placed on the Fungal Isolating Medium (FIM) and incubated at 28°C in dark condition. Mycelia emerged after 1-2 days. Mycelia tips grow from the roots tissue were cut and transferred to another Potato Dextrose Agar (PDA) medium for purification. The morphological characteristics include color, diameter colonies, form (shape) and pattern of colony growth, and microscopic features such as hypha, spores or conidia were observed.

DNA isolation

Fungal mycelia from liquid medium (PDB) was vacuum filtered using a 0.22 µm sterile filter. Mycelia (wet weight ± 20 mg) was then transferred to the mortar and crushed using liquid nitrogen. Extraction process was conducted using CTAB method (Roger and Bendich 1994).

DNA amplification

DNA amplification of ITS rDNA region was done by Polymerase Chain Reaction (PCR) using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990; Diaz et al. 2012). PCR reaction was performed as follow: initial denaturation at a temperature of 95°C for 120 seconds, 35 cycles of denaturation at 95°C for 60 seconds, annealing at 55^{o}C for 60 seconds, extension at 72°C for 60 seconds, a final elongation of 72°C for 600 seconds. The quality of the PCR products was checked by agarose gel electrophoresis (1% agarose).

DNA sequencing

The amplicons were sent to 1st BASE, Malaysia for sequencing process. Sequences were compared with the homologous nucleotides sequence in GenBank database (NCBI) (www.ncbi.nlm.nih.gov/guide/sequence-analysis/). The most homologous sequences were retrieved from the GenBank for phylogenetic analysis.

Phylogenetic analysis

Multiple alignments were done using CLUSTALW in MEGA 6. Phylogenetic analysis was conducted using Neighbor Joining (NJ) method PAUP 4.0 (Swofford 1999). Clade robustness was assessed using bootstrap analysis using 1000 replications. The phylogenetic tree was refined using Tree Graph 2 software.

RESULTS AND DISCUSSION

Result

Endophytic fungi were successfully isolated from the root of terrestrial orchid *P. tankervilleae, D. lancifolium,* and *C. triplicata.* The orchid habitat of *P. tankervilleae* and *C. triplicata* is generally found in a protected area, is quite moist and not far from water source, while *D. lancifolium* was found in dry area in the road side (Figure 2).

Three isolates (IPAR2B, IPAR2C, and IPAR3) fungi isolated from the roots of *P. tankervilleae* show slightly different characteristics in morphology of colony, but they have a similar pattern of growth. The isolates grow rapidly, hyphae reach a diameter of 9 cm within 7 to 10 days. One isolate from *D. lancifolium* has hyaline mycelia that grew covering the edge of 9 cm diameter petridish in 8 days. The hyphae show distinctive septate under 1000x magnification. Fungi that isolated from *C. triplicata* (CalFus 1 and CalFus 4) also grow rapidly. The colony reach 9 cm diameter in day 10. The thin hyaline hyphae

Figure 2. Habitat and the morphology of *P. tankervilleae* (A-C), *D. lancifolium* (D-E), and *C. triplicata* (F).

Table 1. *Fusarium* isolates of *P. tankervilleae* and *D. lancifolium*, and *C. triplicata* from Papua

Orchid species	Location	Time collection	Isolate code	GenBank accession code	Closest species matches	Accession code	Sequence identity/ similarity (%)
Phaius tankervilleae	Jayapura	May 2014	IPAR2B*	KU842423	*Fusarium solani*	NRRL 28579T	100
			IPAR2C*	KU842422		MAFF 238538	
			IPAR3*	KU842424		CBS 132898	
Dendrobium lancifolium	Biak Island	April 2015	SIIID2C (1)**	KU842428	*Fusarium solani*	JF436948.1	99
Calanthe triplicata	Keerom	April 2015	CalFus 1***		na	na	na
			CalFus 4***		na	na	na

Note: * : DNA were sequence to construct the phylogenetic tree ; ** : DNA were sequence and submitted to a similarity search using BLASTn software ; ***: DNA was not isolated yet

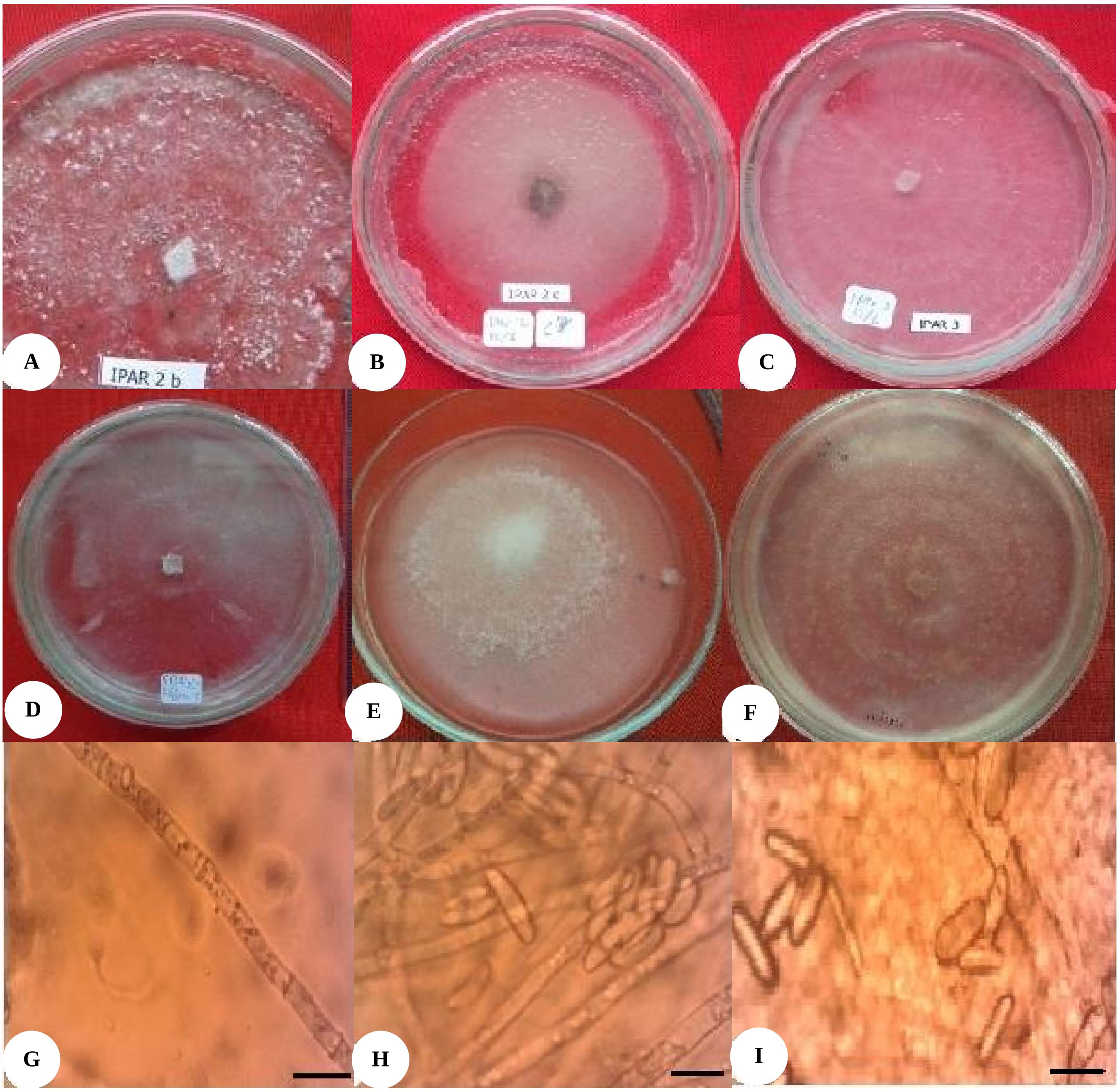

Figure 3. Morphology of *Fusarium* isolated from *P. tankervilleae* (A, B and C), *D. lancifolium* (D and G), and *C. triplicata* (E, F, H, I) (scale bar = 20 μm)

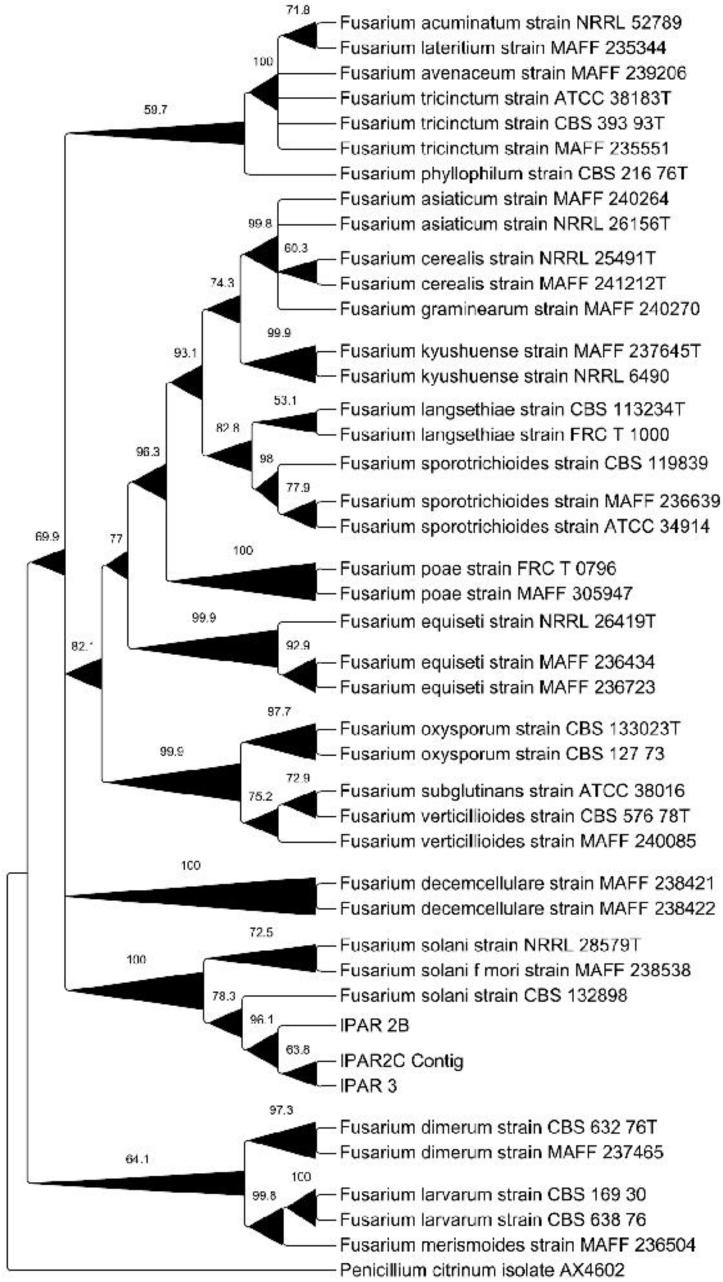

Figure 4. Phylogenetic tree of fungal endophyte of *P. tankervilleae* generated from NJ analysis with 1000 bootstrap replication

show concentric zone. Under the low power of the microscope, the curve shape conidia were able to observe (Figure 3).

Discussion

Identification based on colony and microscopic structure characteristics showed that the endophytic fungi isolated from the root of terrestrial orchid *P. tankervilleae*, *D. lancifolium*, and *C. triplicata* belong to *Fusarium* spp. These fungi is commonly found as endophytes in orchids and other hosts (Ma et al. 2015). Spore morphology is the important character of fusaria. Conidia are fusoid or curved with 0-1 septate. However, many isolation of *Fusarium* tend to grow with abundant mycelium without forming spores (Booth 1971). It is why the morphological identification make difficult to be done. Therefore, to determine the species of *Fusarium*, then we did molecular identification using the DNA sequence, especially for the isolate from *P. tankervilleae* and *D. lancifolium* that we could not find the conidia in the culture.

Based on the BLAST identification of the ITS nrDNA sequence, the *Fusarium* isolates from *P. tankervilleae* and *D. lancifolium* was confirmed as *Fusarium solani* (GenBank accession no NRRL 28579T, MAFF 238538, CBS 132898 and JF436948.1) with 99-100 % similarity (Table 1). Furthermore, the phylogenetic tree that was constructed using closely related species sequences generated from GenBank and *Penicillium citrinum* isolate

AX4602 as an outgroup showed that the sequences of fungal endophytes IPAR 2C, IPAR 3 and IPAR 2B which were isolated from *P. tankervilleae* are nested in the same clade with *Fusarium solani* strain CBS 132898, *Fusarium solani* f. *mori* strain MAFF 238538 and *F. solani* NRRL 28579T (Figure 3).

F. solani is one type of fungus that associated with many species of orchids, including the *Phalaenopsis* sp (Chung et al. 2011; Su et al. 2012). This fungus caused leaf yellowing on *Phalaenopsis* (Chung et al. 2011). The teleomorphic state of this fungus is *Nectria haematococca*, the causal agent of root rot disease in *Phalaenopsis* spp (Chung et al. 2011; Benyon et al. 1996). Besides *F. solani*, *F. oxysporum* and *F. proliferatum* also cause the same disease in *Dendrobium* (Latiffah et al. 2009).

F. solani may affect of 30–60 % seedling growth in *Phalaenopsis*. Infected seedling shows small leaves and yellow with black rotten spots or dots (Su et al. 2012). This fungus was also found in *Cymbidium* spp., *Oncidium* sp., *Dendrobium* sp. and *Cattleya* sp., but did not shows the symptom as in *Phalaenopsis* sp. (Chung et al. 2011). *Fusarium solani* are often associated with orchids and well-known as a virulent species, under optimal growth conditions, tend to asymptomatic endophyte rather than pathogens (Ma et al. 2015). Endophytic *Fusarium* promoted seed germination in *Cypripedium* and *Platanthera* orchid (Ma et al. 2015).

This preliminary study is the first report on the presence of *Fusarium* as endophyte in terrestrial orchid from Papua. Therefore, further study on re-inoculation test of *F. solani* in *P. tankervilleae, D. lancifolium*, and *C. triplicata* is necessary to investigate whether it has effect on both seed germination in axenic condition and seedling growth of that orchid. It is just the beginning steps in conserving the beautiful natural orchid in the New Guinea Island.

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REFERENCES

Agustini V, Sufaati S, Suharno. 2009. Mycorrhizal association of terrestrial orchids of Cycloops Nature Reserve, Jayapura. Biodiversitas 10 (4): 175-180.

Agustini V, Sufaati S. 2014. Orchid mycorrhiza on *Phaius tankervilleae* (Banks) Blume dari Jayapura. Proceeding of the National Seminar on Biology Indonesia, Jayapura 7-8 October 2014. [Indonesian]

Aschehoug ET, Metlen KL, Callaway RM, Newcombe G. 2012. Fungal endophytes directly increase the competitive effects of an invasive forb. Ecology 93 (1): 3-8.

Benyon F, Summerell BA, Burgess LW. 1996. Association of *Fusarium* species with root rot of *Cymbidium orchids.* Aust Pl Pathol 25: 226-228.

Booth C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute. Kew, Surrey, England.

Cabezas L, Calderon C, Medina LM, Bahamon I, Cardenas M, Bernal AJ, Gonzales A, Restrepo S. 2012. Characterization of celluloses of fungal endophytes isolated from *Espeletia* spp. J Microbiol 50 (6): 1009-1013.

Chung WC, Chen LW, Huang JH, Huang HC, Chung WH. 2011. A new 'forma specialis' of *Fusarium solani* causing leaf yellowing of Phalaenopsis. Plant Pathol 60: 244-252.

Chuttima R, Dell B, Vessabutr S, Busaban B, Lumyong S. 2011. Endhophytic fungi from *Pecteilis susannae* (L.) Rafin (Orchidaceae), a threatened terrestrial orchid in Thailand. Mycorrhiza 21: 221-229.

Diaz PL, Hennell JR, Sucher NJ. 2010. Genomic DNA extraction and barcoding of endophytic fungi. In*:* Plant DNA fingerprinting and barcoding: Methods and protocols, Methods in molecular Biology 862: 171-178.

Faeth SH, Fagan WF. 2002. Fungal endophyte: common host symbiots but uncommon mutualists. Integ Comp Biol 42: 360-368.

Gomes EA, Kasuya MCM, de Barros EG, Borges AC, Araujo EF. 2002. Polymophism in the internal transcribed spacer (ITS) of the ribosomal DNA of 26 isolates of ectomycorrhizal fungi. Genet Mol Biol 25 (4): 477-483.

Kumala S, Agustina E, Wahyudi P. 2007. Test of antimicrobial activity of secondary metabolites from trengguli (C*assia fistula* L.) endophytic fungi. Jurnal Bahan Alam Indonesia 6 (2): 46-48. [Indonesian]

Kumala S, Septisetyani EP, Meiyanto E. 2008. N-butanol fraction of endophytic fungi from Makassar fruit improving effect of doxorubusin apoptosis on MCF-7 cells. Majalah Farmasi Indonesia 20 (1): 42-47. [Indonesian]

Kumar S, Kaushik N. 2013. Endophytic fungi isolated from oil-seed crop *Jatropha curcas* produces oil and exhibit antifungal activity. PloS One 8 (2): e56202.

Latiffah Z, Hayati MZN, Baharuddin S, Maziah Z. 2009. Identification and pathogenicity of *Fusarium* species associated with root rot and stem rot of *Dendrobium*. Asian J Pl Pathol 3: 14-21.

Ma X, Kang J, Nonachaiyapoom S, Wen T, Hyde KD. 2015. Non-mycorrhizal endophytic fungi from orchids. Special section: Endophyte. Curr Sci 108: 1-16.

Manoch L, Lohsomboon P. 1992. Isolation of mycorrhizal fungi from orchid roots. In: SEAMEO BIOTROP-Chiang May University Regional Training Course on Biology and Technology of Mychorrhiza 2: 5.

Redman RS, Dunigan DD, Rodriguez RJ. 2001. Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? New Phytol 151 (3): 705-716.

Sinaga E, Noverita, Fitria D. 2009. Antibacterial activity of endophytic fungi isolated from galangal leaf (*Alpinia galanga* Sw.). Jurnal Farmasi Indonesia 4 (4): 161-170. [Indonesian]

Su JF, Lee YC, Chen CW, Hsieh T-F, Huang JH. 2011. Sheath and root rot of *Phalaenopsis* caused by *Fusarium solani***.** Acta Hort (ISHS) 878: 389-394.

Sugijanto NE, Putra H, Pritayuni F, Albathaty N, Zaini NC. 2009. Antibacterial activity of *Lecythophora* sp. extracts, an endophyte isolated from *Alyxia reinwardtii*. Berkala Penelitian Hayati 15: 37-44. [Indonesian]

Swofford DL. 1999. (Pre-Release) PAUP*: Pylogenetic Analysis Using Parsinony, version 4. Sinauer Associates. Sunderland, Mass.

Tabudrayu JN, Jaspars M. 2005. Anticancer activities of constituents of kava (*Piper methysticum*). South Pacific J Nat Sci 23: 26-29.

Tejesvi MV, Pirttila AM. 2011. Potential of tree endophytes as sources for new drug compounds. In*:* Pirttila AN, Frank AC (eds). Endophytes of Forest Trees. Springer, New York.

White T, Bruns T, Lee S. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White L (eds). PCR Protocols: A Guide to Methods and Applications. Academic Press Inc, New York.

Zhao J, Zhou L, Wang J, Shan T, Zhong L, Liu X, Gao X. 2010. Endophytic fungi for producing bioactive compounds originally from their host plants. Current Research, Technology and Education Topics in Applied Microbiology Biotechnology 567-575.

Zimmerman NB, Vitousek PM. 2012. Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. Proc Natl Acad Sci USA 109 (32): 13022-13027.

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Plants diversity of the deforested peat-swamp forest of Tripa, Indonesia

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Abstract. *Djufri, Wardiah, Muchlisin ZA. 2016. Plants diversity of the deforested peat-swamp forest of Tripa, Indonesia. Biodiversitas 17: 372-376.* Tripa peat swamp forest has been degraded due to human perturbation and resulted in decreasing the plant diversity. Currently, no report on plant diversity from this region in another hand the degradation is occurring continuously. Hence, the purpose of the present study was to evaluate the plants diversity of the Tripa peat swamp forest in Aceh Barat District, Indonesia. A quadratic method was employed in this study. Determination of the square area was carried out based on the curve of minimum area. The result showed that there were 41 species of herbs with diversity index ranging from 1.8785 to 2.4180 classified as low to moderate categories. A total of seven species of shrubs and 24 species of trees were found at the locations with diversity index (H') ranging from 1.5186 to 1.7496 and 2.1713 to 2.9133 respectively, indicating the diversity of shrubs was in a low category, while the diversity of tress was in the moderate level. It is concluded that the diversity index of herbs and shrubs were in a low category while the tree groups were in the medium category. According to the diversity index and direct observation of the Tripa peat swamp forest, this area had been degraded due to land conversion.

Keywords: Deforestation, flora, peat swamp forest, Nagan Raya, Aceh Barat Daya

INTRODUCTION

Peat swamp forest plays a significant role in protecting the land from the intrusion of sea water, abrasion and tsunami (Djufri 2004); this area prevents floods during rainy seasons and supply fresh water for agriculture and aquacultural activities and settlements. The peat swamp has an important role as water storages and important habitat for various species of flora and fauna. Tropical peat swamps typically contain a high biodiversity of plants and animals (Muchlisin et al. 2015). However, slight changes in the environmental circumstances of peat swamp have strong effects on both forest structure and species diversity (Eijk and Leenman 2004).

Tripa peat swamp is one of the wetland forests in Indonesia. This ecosystem is covered approximately 61,000 ha and about 75% of this area had been deforested intensively (PIU-SERT 2013; Muchlisin et al. 2015). This area is situated approximately 100-300 m from sea level. In addition, the average rainy days per month are 16 days with an average monthly rainfall reaching of 300.27 mm. The Tripa peat swamp forest is a unique habitat for Sumatran Orangutans (*Pongo abelii* Lesson), the endangered and prior to extinction animal; Sumatran Tigers (*Panthera tigris sumatrae* Linnaeus*)*, Bears (*Helarctos malayanus* Raffles)*)*, and Crocodiles (*Crocodylus porosus* Schneider) (YLI-AFEP 2008). It is also an important habitat for many species of plants.

Peat swamp has been known as a hostile and unsuitable area for agriculture with low economic value. However, the increase in human population and economic development lead to utilize and cultivate this area. Conversion of the forest into oil palm plantation or plantation of commercial timber species and other cultivated plants contribute not only in reducing plant and animal diversities, but also leading to decrease in water flows and influences susceptibility to fires during the dry season (Eijk and Leenman 2004; Posa et al. 2011). According to Setiadi (1998), the opening of peat swamp for palm oil plantations, settlements, and other purposes affected on the reduction of vegetation, animals, genetic diversity, disruption of aquatic habitat, deterioration of water quality due to the rise of pyrite level. Furthermore, the irresponsible utilization of peat swamp also generated negative impacts on the global warming and provides access to illegal logging.

Similar to other peat swamp areas in Indonesia, the peat swamp of Tripa is also threatened by land conversion for palm oil plantations, and settlements (Task Force REDD 2012), which potentially degrades the flora species in this important ecosystem. However, there is no information on the plant diversity of Tripa peat swamp forest. Information on the composition of vegetation is crucial to arrange a better conservation strategy. Hence, the paper presented the plant diversity of the Tripa peat swamp forest as a baseline data to plan a better conservation and monitoring strategies.

MATERIALS AND METHODS

The Tripa peat swamp is situated in Nagan Raya and Aceh Barat Daya Districts, Aceh Province, Indonesia. The sampling was focused in the peat swamp area at Pulo Kruet Village, Nagan Raya District (Figure 1). The study area was divided into five different stations with the total areas of 4.2 ha (or 10% from the total peat-swamp areas), namely: Western Peat-swamp Forest (WPSF), Eastern Peat-swamp Forest (EPSF), Northern Peat-swamp Forest (NPSF), Southern Peat-swamp Forest (SPSF), and Local Beaches (LB). Ten sampling points which covered approximately 50 m^2 were determined randomly on each sampling station (with a total of 500 m^2 on each sampling station). Determination of the number of squares was carried out using three series techniques while the squares area determination of the sample was based on the technique of minimum curve area (Barbour et al. 1999).

The transect techniques were utilized in the study to examine the changes in vegetation stratification according to the topography and elevation. The values of the Absolute Density (AD), Absolute Frequency (AF) and Absolute Dominancy (ADM) were examined following Ludwig and Reynold (1988). The introduced species of plants were identified according to Backer and Bakhuizen van den Brink (1968); and Soerjani et al. (1987).

The importance value (IV) of each species was calculated according to Cox (2001), while the Index of Shannon-Wiever's Species Diversity (H') was examined using the criteria according to Barbour et al. (1999) and Djufri (2002). The H' value was typically ranged from 0 to 7, where H'≤ 1 is for very low category, H'≥ 1-2 is for low category, H' ≥ 2-3 is for medium category, H'≥ 3-4 is for high category, and H' ≥ 4 is for very high category. In addition, the Species Evenness Index (e) was examined using the formula as described by Barbour et al. (1999), Djufri (2004), and Djufri et al. (2005).

RESULTS AND DISCUSSION

The herbaceous plants dominated the deforested peat swamp of Tripa. A total of 41 species of herbs belonging to 39 genera were found in the study area (Table 1). Generally, the species composition in this area was higher compared to other stations.

The average diversity indices (H') of herbaceous species observed in five locations in the area of palm oil plantations was ranged from 1.88 to 2.42. This indicates that the important value was in the low to medium categories.

The dominant shrub species at WPSF were *Cassia siamea* (IV = 90.79%) and *Vernonia cinerea* (IV = 95.60%), while EPSF was dominated by *C. siamea* (IV = 99.19%) and *Mimosa pigra* (IV = 62.06%). The *C. Casia siamea* (IV= 84.25%) and *V. cinerea* (IV = 87.65%) were also the predominant species at NPSF (Table 2). In addition, at SPSF and LB, *C. siamea* (IV = 95.87%), *Lantana camara* (IV = 64.36%), *Bixa orellana* (78.10%), and *M. pigra* (IV = 60.69%) were the most predominant species, respectively. The diversity indices of tree species in the forest ranged from 2.17 to 2.91 with an average of 2.40 indicating a moderate category.

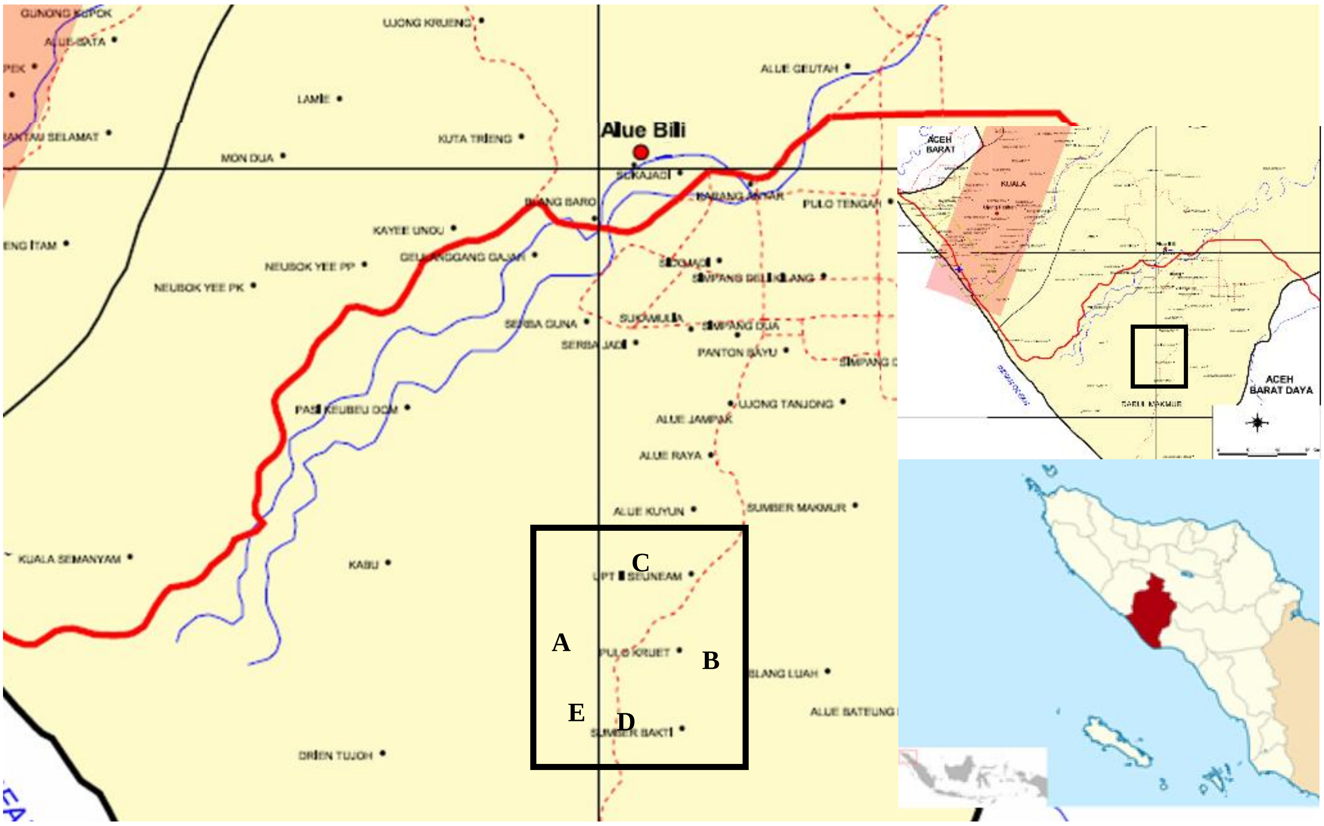

Figure 1. The study area (in red cycle): A. Western Peat swamp forest (WPSF), B. Eastern Peat Swamp Forest (EPSF), C. Northern Peat swamp forest (NPSF), D. Southern Peat swamp forest (SPSF), E. Local Beaches (LB)

The species of trees varied in the forest. There were 24 species of tree groups found in the study area (Table 3). In general, the compositions of Peat swamp forest in the village of Pulo Kruet were relatively similar to the peat swamp forests in other parts of Indonesia. The main tree species on site of WPSF were *Eugenia polyantha* (IV = 99.10%) and *Alstonia villosa* (IV= 90.60%). Whereas at EPSF, they were *E. polyantha* (IV = 79.07%) and *Acronychia porteri* (IV = 59.10%). At NPSF, they were *E. polyantha* (IV = 79.34%) and *Litsea cassiaefolia* (IV = 56.88%). Moreover, SPSF and LB were occupied by *Eugenia polyantha* (IV = 80.42%), *Litsea cassiaefolia* (IV = 55.13%), *Eugenia polyantha* (86.53%) and *Calophyllum spectabile* (IV = 54.66%) (Table 1). It is known that the environment plays an important role in vegetation diversity. Thus, this factor can change due to human activities, for example deforestation, open drainage, and fires that disturb the environmental condition (Hirano et al. 2007).

Table 1. Species composition, importance value (IV) and diversity index (H') of herbaceous stratification at deforested peat swamp forest of Tripa in the village of Pulo Kruet, Aceh, Indonesia

Species	Sampling location									
	WPSF		EPSF		NPSF		SPSF		LB	
	IV	H'	IV	H'	IV	H'	IV	H'	IV	H'
Eichhornia crassipes (Mart.) Solms	20.80	-0.19	79.19	-0.35	98.62	0.37	95.87	-0.36	44.24	-0.28
Panicum repens L.	24.20	-0.20	42.06	-0.28	-	-	-	-	60.69	-0.32
Hyptis capitata Jacq.	2.80	-0.04	3.19	-0.05	-	-	-	-	13.55	-0.14
Ipomoea *aquatica* Forsk.	95.60	-0.36	3.40	-0.05	-	-	-	-	-	-
Mimosa púdica L.	1.98	-0.03	4.76	-0.07	-	-	-	-	5.41	-0.07
Ageratum conyzoides L.	1.76	-0.03	6.13	-0.08	-	-	-	-	-	-
Cyperus pygmaeus Rottb.	5.44	-0.07	4.36	-0.07	2.20	-0.04	14.36	-0.15	30.24	-0.23
Desmodium heterophyllum Willd.	1.78	-0.03	1.80	-0.03	-	-	-	-	-	-
Desmodium triflorum (L.) DC	2.53	-0.04	2.95	-0.05	-	-	-	-	-	-
Coix lacrima-joby L.	0.76	-0.02	0.17	-0.01	-	-	-	-	-	-
Clitoria ternatea L.	3.07	-0.05	5.12	-0.07	-	-	-	-	-	-
Calopogonium muconoides Desv.	86.38	-0.36	49.77	-0.29	96.77	-0.37	97.55	-0.37	-	-
Emilia sonchifolia (L.) DC	4.24	-0.06	3.36	-0.05	-	-	-	-	9.57	-0.11
Eclipta prostrata (L.) L.	13.64	-0.14	7.49	-0.09	3.34	-0.05	21.56	-0.19	-	-
Passiflora foetida L.	9.22	-0.11	3.65	-0.05	-	-	-	-	-	-
Colocasia esculenta (L.) Schott	10.35	-0.12	37.65	-0.26	33.15	-0.24	-	-	-	-
Salvia splendens Sello	8.78	-0.10	7.07	-0.09	-	-	4.17	-0.06	-	-
Dactyloctenium aegyptium Richt.	5.43	-0.07	9.74	-0.11	-	-	-	-	-	-
Phyllanthus niruri L.	1.28	-0.02	1.54	-0.03	-	-	7.54	-0.09	-	-
Bidens pilosa L.	-	-	2.48	-0.04	-	-	4.48	-0.06	-	-
Leucas lavandulaefolia J.E. Smith	-	-	8.34	-0.10	4.61		7.29	-0.09	-	-
Euphorbia hirta L.	-	-	0.24	-0.01	-	-	-	-	-	-
Saccharum spontaneum L.	-	-	3.75	-0.05	21.44	-0.06	-	-	-	-
Sida rhombifolia L.	-	-	8.78	-0.10	-	-	8.66	-0.10	6.02	-0.08
Tridax procumbens L.	-	-	2.46	-0.04	-	-	-	-	-	-
Axonopus compressus Swartz.	-	-	0.55	-0.01	-	-	-	-	25.24	-0.21
Richardia brasiliensis Gomez.	-	-	-	-	0.53	-0.01	5.42	-0.07	-	-
Cyperus rotundus L.	-	-	-	-	3.29	-0.05	5.30	-0.07	-	-
Crotalaria striata L.	-	-	-	-	6.27	-0.08	-	-	-	-
Cyperus bulbosus Vahl.	-	-	-	-	0.47	-0.01	2.82	-0.04	-	-
Cleome rutidosperma DC.	-	-	-	-	0.09	-0.00	-	-	-	-
Physalis angulata L.	-	-	-	-	7.49	-0.09	7.82	-0.10	-	-
Synedrella nodiflora (L.) Gaertn	-	-	-	-	2.47	-0.04	1.99	-0.03	-	-
Commelina benghalensis L.	-	-	-	-	6.73	-0.09	3.36	-0.05	-	-
Solanum melongena L.	-	-	-	-	4.25	-0.06	3.71	-0.05	-	-
Urena lobata L.	-	-	-	-	0.88	-0.02	3.80	-0.05	-	-
Borreria laevis (Lamk) Griseb.	-	-	-	-	3.45	-0.05	-	-	-	-
Eleusine indica L.Gaertn.	-	-	-	-	1.62	-0.03	-	-	-	-
Dactyloctenium aegyptium Richt.	-	-	-	-	1.62	-0.03	-	-	-	-
Stachytarpheta indica (l..) Vahl.	-	-	-	-	-	-	4.30	-0.06	16.44	-0.16
Nephrolepis exaltata Schott	-	-	-	-	-	-	-	-	88.60	-0.36
Total	300	-2.05	300	-2.42	300	-1.88	300	-2.01	300	-1.97
Total	300	2.05	300	2.42	300	1.88	300	2.01	300	1.97

Note: WPSF = West of Peat swamp Forest, EPSF = Eastside Peat swamp Forest, NPSF = North side Peat swamp Forest, SPSF = Southern Peat swamp Forest, LB = Local Beach

Table 2. Species composition, importance value (IV) and diversity index (H') of shrub stratification at the Tripa peat swamp forest which was converted to oil palm plantations in the village of Pulo Kruet, Aceh, Indonesia

Species	Sampling location									
	WPSF		EPSF		NPSF		SPSF		LB	
	IV	H'	IV	H'	IV	H'	IV	H'	IV	H'
Cassia siamea Lmk	90.79	-0.36	99.19	-0.37	84.25	-0.36	95.87	-0.36	44.24	-0.28
Mimosa pigra L.	34.17	-0.25	62.06	-0.33	65.44	-0.33	43.12	-0.28	60.69	-0.32
Bixa orellana L.	12.80	-0.13	23.19	-0.20	-	-	23.44	-0.20	78.10	-0.35
Vernonia cinerea (L.) Less.	95.60	-0.36	33.40	-0.24	87.65	-0.36	41.31	-0.27	-	-
Melastoma malabatricum L.	21.98	-0.19	24.76	-0.21	27.46	-0.22	-	-	35.41	-0.25
Elaeocarpus edulis T. & B.	21.76	-0.19	23.04	-0.20	-	-	31.90	-0.24	44.32	-0.28
Lantana camara L.	22.90	-0.20	34.36	-0.25	35.20	-0.25	64.36	-0.33	37.24	-0.26
Total	300	-1.69	300	-1.79	300	-1.52	300	-1.68	300	-1.75
Total	300	1.69	300	1.79	300	1.52	300	1.68	300	1.75

Table 3. Species composition, importance value (IV) and diversity index (H') of tree stratification at the Tripa peat swamp forest in the village of Pulo Kruet, Aceh, Indonesia

Species	Sampling location									
	WPSF		EPSF		NPSF		SPSF		LB	
	IV	H'	IV	H'	IV	H'	IV	H'	IV	H'
Acronychia trifoliata Zoll.	30.79	-0.23	59.1	-0.32	24.53	-0.20	14.33	-0.20	24.35	-0.20
Adina polycephala Benth.	24.72	-0.21	42.06	-0.28	12.88	-0.14	22.08	-0.04	2.77	-0.04
Aglaia odorata Lour.	2.80	-0.04	3.19	-0.05	5.87	-0.08	9.77	-0.18	19.46	-0.18
Alstonia villosa Bl.	90.60	-0.36	3.4	-0.05	12.66	-0.13	4.07	-	-	-
Alstonia spatulata Bl.	11.98	-0.12	4.76	-0.07	20.35	-0.18	22.5	-	-	-
Anisoptera costata Korth.	1.76	-0.03	6.13	-0.08	0.77	-0.02	4.79	-	-	-
Antidesma bunius (L.) Spreng.	8.42	-0.10	4.36	-0.06	10.75	-0.12	10.51	-0.25	33.57	-0.25
Blumeodendron tokbrai (Bl.) Kurtz.	11.72	-0.13	1.8	-0.03	20.35	-0.18	23.35	-0.05	3.56	-0.05
Calophyllum inophyllum L.	12.50	-0.13	2.95	-0.05	10.2	-0.12	12.24	-0.19	22.29	-0.19
Calophyllum spectabile Willd.	4.66	-0.06	00.17	-0.01	14.71	-0.15	14.11	-0.31	54.66	-0.31
Campanumoea celebica Bl.	3.07	-0.05	5,12	-0.04	2.87	-0.10	8.87	-0.17	18.05	
Cinnamomum iners Reinw. ex. Bl.	6.38	-0.08	9.77	-0.11	3.66	-0.05	4.66	-0.15	14.92	-0.15
Cryptocarya costata Bl.	14.24	-0.14	3.36	-0.05	4.77	-0.07	4.12	-0.06	4.27	-0.06
Cryptocarya griffithiana Wight.	33.64	-0.25	7.49	-0.09	3.97	-0.06	5.71	-0.07	5.10	-0.07
Diospyros hasseltii Zoll.	29.22	-0.23	3.65	-0.05	-	-	-	-	-	-
Durio kutejensis (Hassk.) Becc.	30.35	-0.23	34.65	-0.25	-	-	-	-	-	-
Eugenia polyantha Wight.	99.10	-0.37	79.07	-0.35	79.34	-0.35	80.42	-0.36	86.53	-0.36
Hopea celebica Burck.	13.94	-0.14	9.04	-0.11	-	-	-	-	-	-
Knema cinerea (Poir.) Warb.	-	-	1.54	-0.03	-	-	-	-	-	-
Litsea angulata Bl.	-	-	2.4	-0.04	-	-	-	-	-	-
Litsea cassiaefolia Bl.	-	-	8.34	-0.10	56.88	-0.32	55.13	-0.09	7.13	-0.09
Macaranga semiglobosa J.J.S.	-	-	00.24	-0.01	-	-0.15	-	-	-	-
Palaquium javense Burck.	-	-	3.75	-0.06	15.44	-	3.34	-0.05	3.34	-0.05
Tristania conferta R. Br.	-	-	8.78	-0.10	-	-	-	-	-	-
Total	300	-2.91	300	-2.39	300	-2.35	300	-2.17	300	-2.17
Total	300	2.91	300	2.39	300	2.35	300	2.17	300	2.17

There were five predominant species found at the study locations, i.e. *Ipomoea aquatica, Eichhornia crassipes, Calopogonium muconoides, Panicum repens* and *Nephrolepis exaltata.* The first two species have grown rapidly in wet and humid areas while the last three species grow better in drier areas. Different kinds of forest provide markedly different habitat and support different species of wildlife. Moreover, different species have different responses to environmental factors that determine the survival and thriven species on certain regions (Nebel and Wright 1993).

The most of Tripa peat swamp forest areas have been converted to palm oil plantations. Conversion of the forest to oil palm plantations would alter its ecological functions for example, the increased frequency and severity of floods and forest fires during the last decade (Muchlisin et al. 2015). In addition, according to Wahyunto et al. (2005) the conversion of Tripa peat swamp forest to oil palm plantations has insignificant positive economic benefit for local people.

The peat swamp forest mainly functions in regulating the water flows and water storages. The peat area has an important function for water storage. These are because the peat swamp forest has the capability to absorb the water rapidly during the rainy season and discharge the water slowly during the dry season and therefore the forest guarantees water sustainability (Eijk and Leenman 2004).

The study revealed that *C. siamea* was a predominant species in all locations except at LB. Seven species of shrubs have been found in the post deforested peat swamp (Table 2). These species were invasive into the peat swamp forests of this region, for example, Eijk and Leenman (2004) reported that those species were not commonly found in the virgin peat swamp in Indonesia.

The ecology of peat swamp forest which was converted to palm oil plantations were dominated by shrub group. The value of diversity indices (H') of shrub species in five locations in the area of palm oil plantations (converted peat swamp) had the average Diversity Index values that ranged from 1.5186 to 1.7496, with the importance value at the low category. The average value of the species in the five locations was 1.6848 indicating low category (Table 2). It indicates that the value of the shrub species diversity in oil palm plantation area was low. Posa et al. (2011) stated that the majority of trees of peat swamp forest in the Southeast Asia are dipterocarp, with species composition of *Shorea albida* and *Shorea balangeran*. However, these species were not recorded during the study, probably due to the deforestation which are occurring in this area.

It is concluded that the diversity index of herbs and shrubs were in a low category, while the tree groups was in the medium category. According to the diversity index and direct observation of the Tripa peat swamp forest, this area has been degraded due to land conversion.

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REFERENCES

Backer CA, Bakhuizen v.d. Brink Jr. RC. 1968. Flora of Java (Spermatophyte Only) Vol. III. Nordhoff, Groningen.

Barbour MG, Burk JH, Pitts WD, Gilliam FS, Schwartz MW. 1999. Terrestrial Plant Ecology. 3rd ed. Benjamin Cummings, Menlo Park, CA.

Cox GW. 2002 Laboratory Manual of General Ecology. WM.C. Brown, New York.

Djufri. 2002. Determination of distribution pattern, association, and interaction of plant species in grassland of Baluran National Park, East Java. Biodiversitas 3 (1): 181-188 [Indonesian].

Djufri. 2004 Review: The effect of conversion of peatswamp forest into agriculture land. Enviro 4: 34-43 [Indonesian].

Djufri, Setiadi D, Guhardja E, Qayim I. 2005 Effect of stand density Acacia (*Acacia nilotica*) (L.) Willd. Del ex. Composition and diversity of lower plants in Savannah Baluran National Park East Java. J Environ Anal 2 (1): 135-145 [Indonesian].

Eijk PV, Leenman P. 2004 Regeneration of Fire Degraded Peatswamp Forest in Berbak National Park and Implementation in Replanting Programmes. Water for Food and Ecosystems Programme Project on Promoting the River Basin and Ecosystem Approach for Sustainable Management of SE Asian Lowland Peatswamp Forest, Case Study on Air Hitam Laut River Basin, Jambi Province, Indonesia. Alterra Green World Research, Wageningen.

Hirano H, Segah H, Harada T, Limin S, June T, Hirata R, Osaki M. 2007 Carbon Dioxide Balance of A Tropical Peat Swamp Forest in Kalimantan, Indonesia. Global Change Biology 13: 412-425.

Ludwig JA, Reynolds JF. 1988 Statistical Ecology. John Wiley and Sons Inc., New York.

Muchlisin ZA, Akyun Q, Rizka S, Fadli N, Sugianto S, Halim A, Siti-Azizah MN. 2015. Ichthyofauna of Tripa Peat Swamp Forest, Aceh province, Indonesia. Check List 11 (2): 1560

Nebel BJ, Wright RT. 1993. Environmental Science Fourth Edition. Prentice-Hall. Inc. Englewood Cliffs, New Jersey.

PIU SERT. 2013. A Scientific Study for Rehabilitation and Management of Tripa Peat Swamp Forest. Research Note of Stakeholders Workshop of Tripa Peat Swamp Forest Management, Banda Aceh February 19–20, 2013. Unsyiah – Satgas Redd/UKP4, Banda Aceh.

Posa, MRC, Wijedasa LS, Corlett LT. 2011 Biodiversity and conservation of tropical peatswamp forest. J BioScience 61 (1): 49-57.

Soerjani M, Koestarman AJGH, Tjitrosoepomo G. 1987. Weeds of Rice in Indonesia. Balai Pustaka. Jakarta.

Task Force REDD. 2012. Vision and missions of the Tripa peatswamp rehabilitation program. Initial Report of Satgas REDD/UKP4, Jakarta. [Indonesian].

Wahyunto, Ritung S, Suparto, Subagyo H. 2005. Distribution of Peat Swampt and Carbon Content in Sumatra and Kalimantan. Project of Climate Change, Forests and Peatlands in Indonesia. Wetlands International-Indonesia Programme and Wildlife Habitat Canada. Bogor. 21 pp

YLI-AFEP. 2008. Monitoring Report on Current Condition of Tripa Peat Swamp Forest, the Leuser Ecosystem. Program of Aceh Forest and Environment Project, Yayasan Leuser Internasional, Banda Aceh. [Indonesian]

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Short Communication: *Rhizoctonia*-like fungi isolated from roots of *Dendrobium lancifolium* var. *papuanum* and *Calanthe triplicata* in Papua, Indonesia

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Abstract. *Agustini V, Sufaati S, Suharno, Suwannasai N. 2016.* Rhizoctonia*-like fungi isolated from roots of* Dendrobium lancifolium *var.* papuanum *and* Calanthe triplicata *in Papua, Indonesia. Biodiversitas 17: 377-383.* The aim of this study was to isolate and identify *Rhizoctonia*-like fungi associated with the roots of the terrestrial orchids *Dendrobium lancifolium* A. Rich var. *papuanum* and *Calanthe triplicata* (Willem) Ames in Papua. The fungi were isolated from the transversal section of the orchid roots. Two isolates have been morphologically identified as genus *Rhizoctonia.* Further identification was carried out based on the analysis of nucleotide sequences generated from ITS and 28S rDNA. The results revealed that both isolates closed to *Ceratobasidium* sp. and the phylogenetic analysis confirmed that they were determined as *Rhizoctonia*-like fungi.

Key words: *Calanthe triplicata*, *Dendrobium lancifolium*, ITS, 28S rDNA, Papua, *Rhizoctonia*-like fungi

INTRODUCTION

Tropical orchids constitute the greater part of orchid diversity, than can be found in anywhere in the world (Atala et al. 2015). Orchid is one of the world's largest plant families and contains over 25000 species (Berga-Pana 2005; Tao et al. 2013). *Dendrobium* is orchid that commonly found in the eastern part of Indonesia such as Papua and Maluku (Agustini et al. 2013). Two biggest genera in Papua are *Bulbophyllum* (569 species) and *Dendrobium* (512 species) (Millar 1978). *Dendrobium lancifolium* A. Rich var. *papuanum* as well as *Calanthe triplicata* (Willem) Ames (Agustini et al. 2013) spread widely in Papua.

Mycorrhizal fungi have a unique role in the life cycle of orchids (Pandey et al. 2013; Perotto et al. 2014). In nature, orchid associated with mycorrhizal fungi has become very essential in seed germination because of their lack of endosperm and seedling growth that require nutrients from the outside (Ding et al. 2014; Perotto et al. 2014). Orchid mycorrhiza has a significant effect on the growth of plantlets life, vegetative and reproductive growth (Cheng et al. 2012; Perotto et al. 2014; Wang and Liu 2013).

Fungi associated with photosynthetic orchids are generally included in the subdivision Basidiomycota class Hymenomycetes, genus *Rhizoctonia.* Nine species of orchids that grow in Puerto Rico found it has association with as many as 108 *Rhizoctonia*-like fungi that includes *Tulasnella*, *Ceratobasidium* and *Thanatephorus* (Otero et al. 2002; Ding et al. 2014). As Taylor and Bruns (1997) and Taylor et al. (2004) showed, 17–22 species of fungi associated with orchid are family Russulaceae. Studies on *Limodorum abortivum,* common orchid growing in Mediterranean regions, showed that it associated with fungi of the family Russulaceae (Gurlanda et al. 2006).

Identification of the orchid mycorrhizal fungi is very crucial in studying that association. Morphological identification of mycorrhizal pure isolates using the characteristics like the color, form and pattern of colonies, can only reach the genus level, whereas to the species level is more appropriate with the aid of molecular techniques. This molecular approach is simple because it only requires a DNA sequence that is not too long. The short sequence likes ITS (internal transcribed spacer) between the small subunit (SSU) and large subunit (LSU) rDNA can be used for identification to the species level (Kristiansen et al. 2001; Diaz et al. 2012).

Agustini et al. (2009) reported that several species of orchids in Papua, including the orchid *Calanthe* sp., have symbiosis with mycorrhizal fungi. However, some of the mycorrhizal fungi are still unidentified yet. In order to know fungal identity, this study was conducted to identify *Rhizoctonia*-like fungi associated with the root of terrestrial orchid *Dendrobium lancifolium* var. *papuanum* and *C. triplicata* in Papua using classical morphological identification together with molecular approach.

MATERIALS AND METHODS

Collection site

Root samples of *D. lancifolium* were collected from Biak Island while *C. triplicata* from Keerom, Papua, Indonesia (Figure 1) in April, 2015.

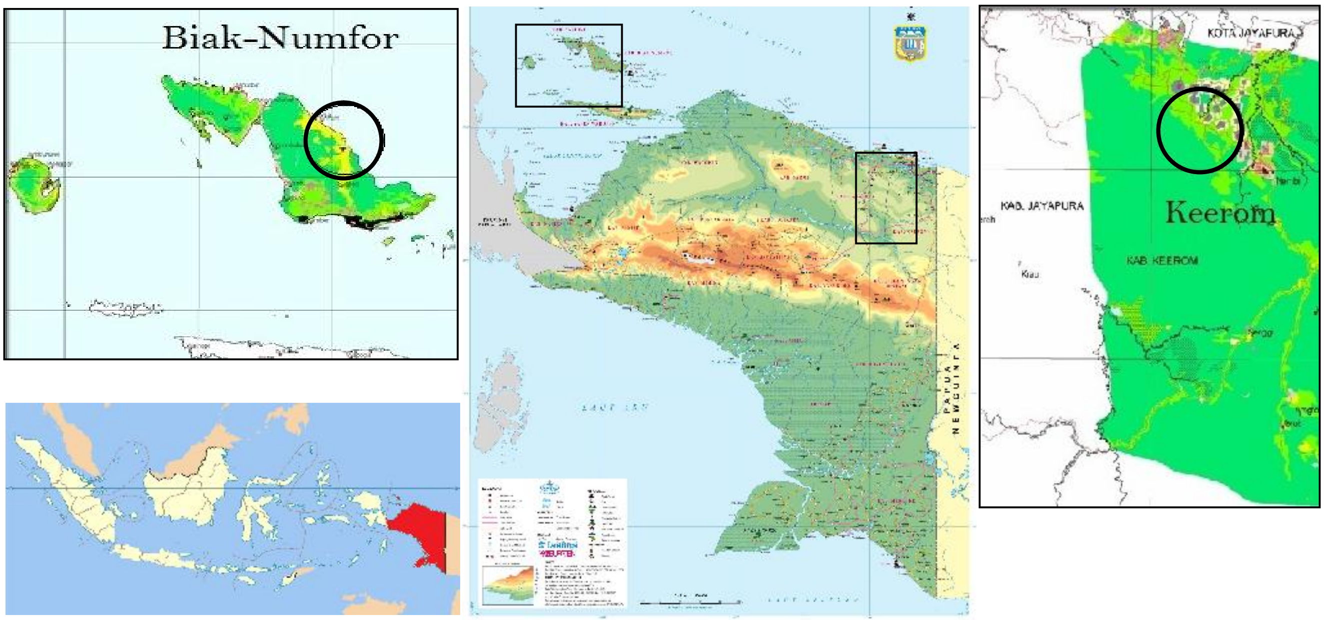

Figure 1. Locations of root collections of *D. lancifolium* var. *papuanum* from Biak (01º09'94.0"S, 136º10'95.5"E) and *C. triplicata* from Keerom (2º50'03.60"S, 140º43'54.94"E)

Fungal isolation

Isolation of fungi was conducted following Chutima et al. (2011) with modification. Orchid plant roots were cut at the root tip (about 2–5 cm from the root tip) and then sliced through the cross section. Roots were washed and cut ± 1 cm and sterilized with 70 % ethanol for 30 seconds, then with a solution of 95% alcohol : 5.25 % chlorox : distilled water (1:1:1 v/v/v) for 30 seconds, and rinsed with sterile distilled water three times. The sterilized roots were cut in thin slices ± 200-300 μm or 3-4 pieces in 1 mm. The presence of peloton (coil hyphae) in the root cortical cell was observed under the compound microscope at a magnification of 400 to 1000 x. The root pieces with peloton (hyphae coil) were grown on PDA and then incubated at a temperature of 28 ^{o}C on dark condition.

Morphological identification

Morphological identification was based mainly on the colony, the branching hyphae, conidia and other structures formed by fungi. Special characteristics formed by orchid mycorrhiza are the monilioid cell (Athipunyakom et al. 2004).

Molecular identification-DNA Sequence analysis

Genomic DNA was extracted from fresh mycelium by using DNA extraction kit (Favorgen). The ITS regions and partial ribosomal RNA gene of 28S were amplified by using ITS1/ITS4 (White et al. 1990) and LR0R/LR5 (Vilgalys and Hester 1990) primers respectively. The amplification was carried out in a BioRad thermocycler (USA) in a 50 μL reaction mixture containing 100 nM of template DNA, 0.5 mM of each primer, 10 mM of dNTP, 1.25 Unit of Top *Taq* DNA polymerase (Qiagen) and 1 x PCR buffer. The PCR cycles were run following the protocol: 94°C for 5 min initial denaturation; 35 cycles of 94°C for 1 min, 52°C (for ITS) or 55°C (for 28S rDNA) for 1 min, 72°C for 1 min; and 72°C for 10 min final extension (Suwannasai et al. 2013). The PCR products were purified following the QIAquick PCR Purification Kit protocol (Qiagen) and then sequenced at the 1st BASE laboratories Sdn Bhd (Malaysia). The sequences obtained were manually checked by using BioEdit program (Hall 1999) before analyzed by using BLASTN program to GenBank database (www.ncbi.nlm.nih.gov/BLAST/).

Phylogenetic analysis

DNA sequences of orchid mycorrhizal fungi were aligned to closely related sequences obtained from GenBank database by using MUSCLE program (Edgar 2004). The phylogenetic tree was analyzed based on maximum likelihood and Bayesian analysis using Mr. Bayes version 3.2.6 (Ronquist et al. 2012) with two independent runs of Markov Chain Monte Carlo chains with 1,000,000 generations sampling trees every 100th generations. A final standard deviation of <0.01 for the split frequency was interpreted to reflect convergence.

RESULTS AND DISCUSSION

Results

Two *Rhizoctonia*-like isolates, SIID3B2 and Cal8, were successfully cultured from roots of two terrestrial orchids, *D. lancifolium* and *C. triplicata* respectively (Table 1). The colonies of both isolates were white at first and became to pale yellow with flat or leathery in appearance. Both cultures were absent sporulation. The rapidly growing isolates reach a diameter of 9 cm within 7 to 10 days with concentric zone, hyaline mycelia has septate. Under 1000x magnification of the compound microscope show the presence of monilioid cells (Figure 2).

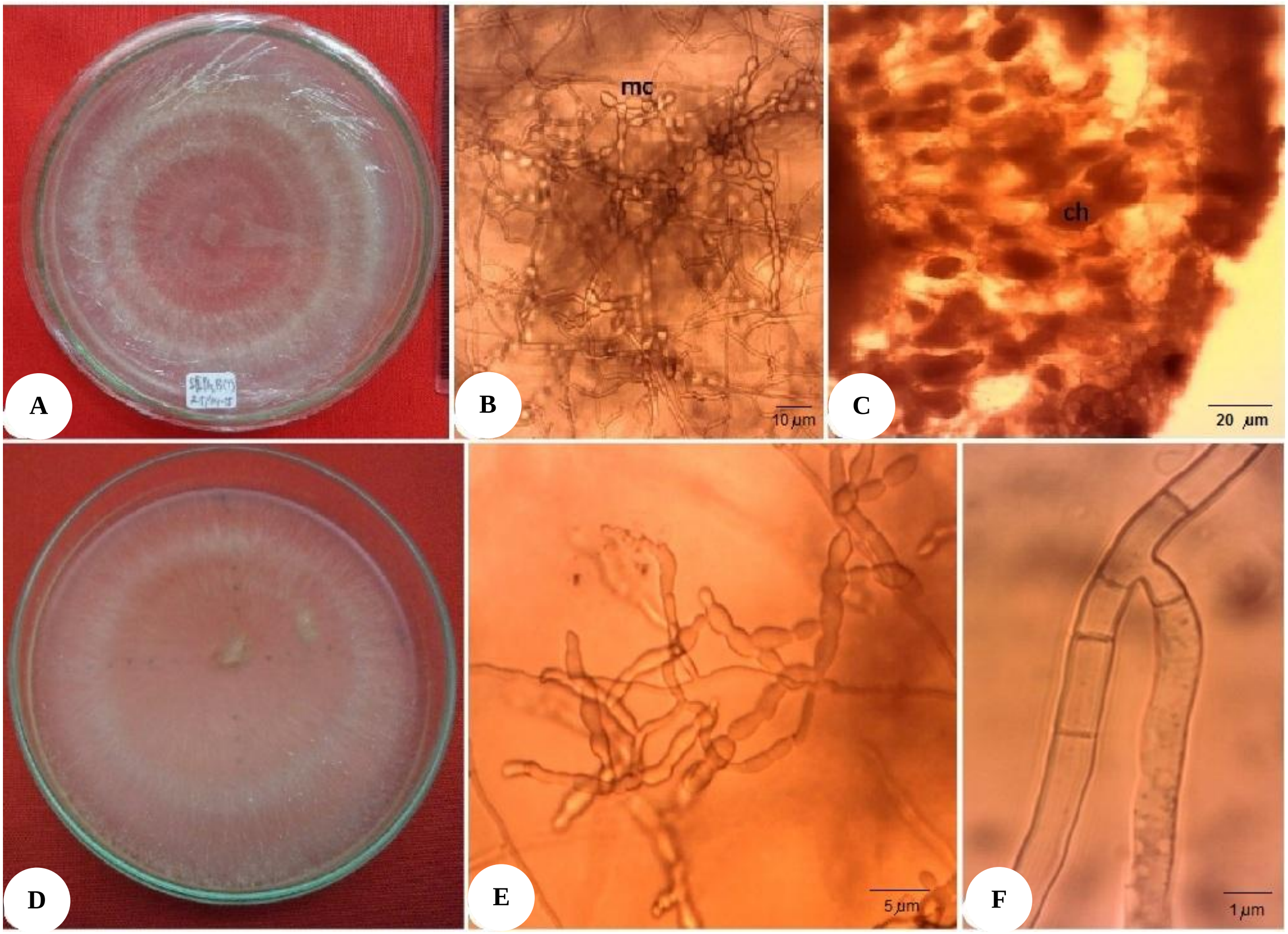

Figure 2. Isolate of *Rhizoctonia*-like fungi. a-c for the isolate SIID3B2. *D. lancifolium* on PDA, b. monilioid cells, c. hyphae-coil in the transversal root section of *D. lancifolium*. d–f for the isolate Cal 8 from *C. triplicata*: d. colony on PDA, e. monilioid cell and f. hyphae with sepatae; *mc*: monilioid cell, *h*: hyphae, *sh*: septate hyphae, *ch*: coil hyphae/peloton

Table 1. Orchid mycorrhiza associate with *Dendrobium lancifolium* and *Calanthe triplicata* from Papua, Indonesia

Orchid source	Location	Time collection	Isolate code	Cultural characteristics	Microscopic characteristics
Dendrobium lancifolium	Biak Island	April 2015	SIID3B2	Diameter of fungal colony reached 9 cm in day 8, concentric growth, white, aerial hyphae	Septate hyphae, globouse monilioid cells appear in day 14
Calanthe triplicata	Keerom	April 2015	Cal 8	Diameter of fungal colony is 9 cm in d 9, concentric growth, white, hyphae	Septate hyphae, irregular monilioid cells appear in day 7

Table 2. ITS and 28S rDNA sequences analysis of orchid mycorrhizal fungal isolates aerial SIID3B2 and Cal8 using BLAST program

Isolate code	BLAST results of ITS			BLAST results of 28S rDNA		
	Taxon	Accession no.	% similarity	Taxon	Accession no.	% similarity
SIID3B2	*Ceratobasidium* sp.	JX913817	90	*Ceratobasidium* sp.	AF354094	99
Cal8	*Ceratobasidium* sp.	JX913817	91	*Ceratobasidium* sp.	AF354094	99

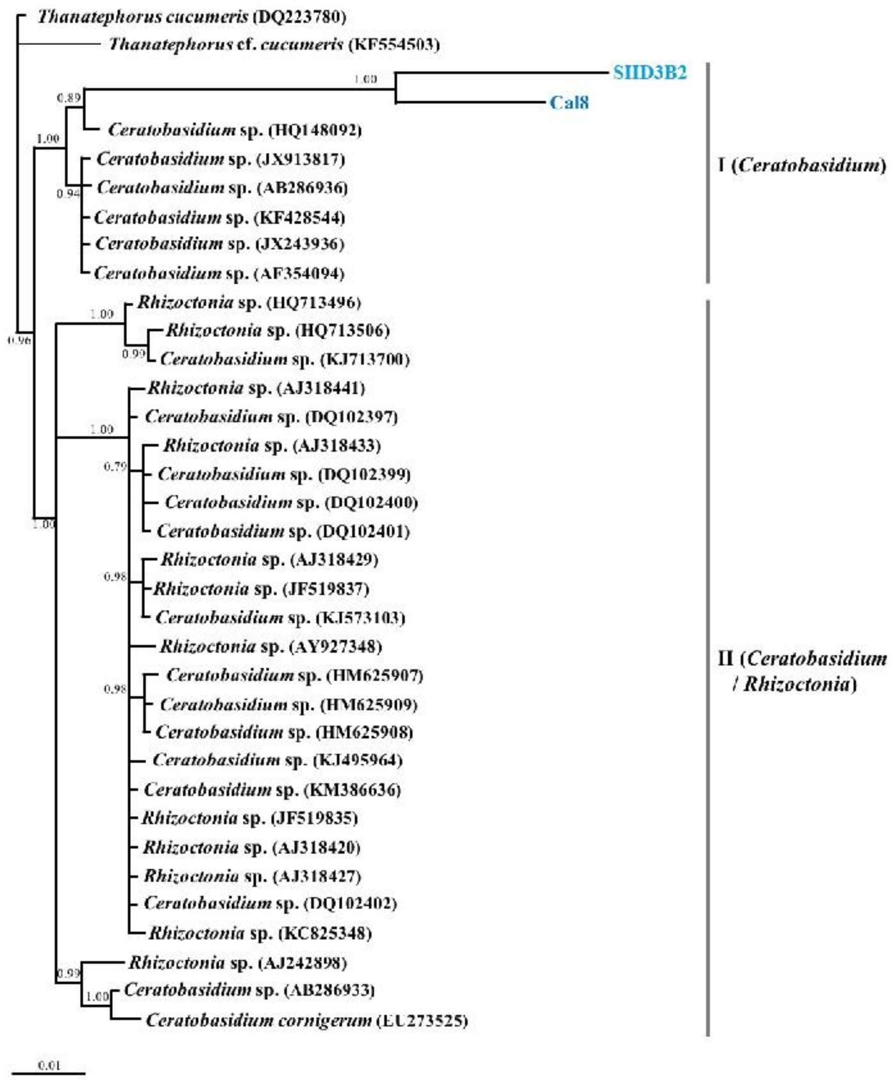

Figure 3. Phylogenetic tree of orchid mycorrhizal fungal isolates SIID3B2 and Cal8 and related species based on ITS rDNA sequences using Bayesian program. Number above branches represent their Bayesian posterior probabilities (500,000 generations). *Thanatephorus cucumeris* (DQ223780) and *T.* cf. *cucumeris* (KF554503) are out groups

ITS rDNA sequences of *Rhizoctonia*-like fungi, SIID3B2 and Cal8, were amplified and analyzed with BLAST against GenBank database. ITS amplicon sizes were 604 and 608 bp respectively. BLAST results of SIID3B2 and Cal8 revealed low similarity against nucleotide sequences from GenBank database (Table 2). They closed to *Ceratobasidium* sp. (JX913817) with 90% similarity for SIID3B2 and 91% similarity for Cal8. Due to the low similarity of ITS sequences, 28S rDNA sequences were then analyzed to confirm the genus and species identification. The amplified fragments of SIID3B2 and Cal8 resulted in 869 bp. BLAST identification of both isolates revealed 99% similarity to *Ceratobasidium* sp. (AF354094) (Table 2). The phylogenetic trees of both ITS and 28S rDNA sequences were then constructed with closely related species obtained from GenBank database (Figure 3, 4). ITS phylogenetic analysis was divided into two major clades. Clade I consisted of our orchid mycorrhizal isolates, SIIB3D2 and Cal8. They were grouped together with *Ceratobasidium* species with high bootstrap support. Although SIIB3D2 and Cal8 were placed in the same cluster, the pairwise comparison among them showed 93% similarity. This indicated that both isolates were different in species level. Clade II was a large group consisted of *Ceratobasidium* and *Rhizoctonia* species. The results obtained exhibited that SIIB3D2 and Cal8 isolates belong to *Ceratobasidium* sp. having *Rhizoctonia*-like anamorph. For 28S rDNA phylogenetic analysis, it contained three major clades, clade I (*Gloeophyllum* and *Griseoporia*), clade II (*Ceratobasidium* and *Thanatephorus*) and clade III (*Ceriporia*). The orchid mycorrhizal isolates of SIIB3D2 and Cal8 were placed in clade IIb which contained *Ceratobasidium* species. The results of both ITS and 28S rDNA analysis confirmed that SIIB3D2 and Cal8 belong to the same genus *Ceratobasidium* but they are different in species.

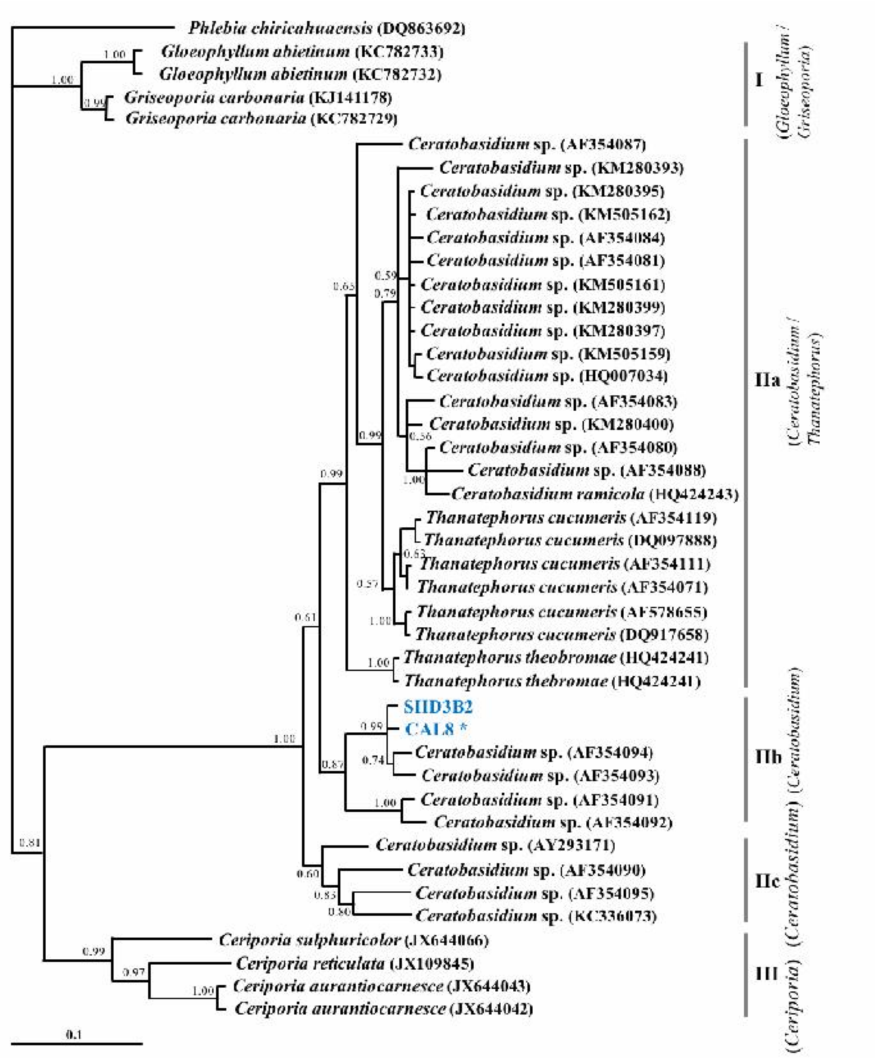

Figure 4. Phylogenetic tree of orchid mycorrhizal fungal isolates SIID3B2 and Cal8 and related species based on 28S rDNA sequences using Bayesian program. Number above branches represents their Bayesian posterior probabilities (500,000 generations). *Phlebia chiricahuaensis* (DQ863692) is an outgroup

Discussion

For morphological identification, mycelial color, number of nuclei per young vegetative hyphal cells, and the morphology of teleomorph can be used to differentiate the species of *Rhizoctonia*. The morphological character of the fungal isolates, such as the presence of monilioid cells (Figure 2), showed that they belong to *Rhizoctonia*-like fungi (Athipunyakom et al. 2004). The teleomorph of *Rhizoctonia* spp. belongs to class Hymenomycetes, sub-division Basidiomycota (Yang and Li 2012). On the other hand, the anamorph of *Rhizoctonia* are heterogeneous, for example, anamorph of *Ceratobasidium* was placed in new genus *Ceratorhiza* (Moore 1987), but many publications still used the name of *Rhizoctonia* (Yang and Li 2012).

The molecular technique using DNA sequences was used to confirm the *Rhizoctonia* that was isolated. Firstly, we used ITS rDNA sequences to analyze and resulted closely related to *Ceratobasidium* sp. (JX913817) with 90-91% similarity, which belong to Basidiomycota. The 28S rDNA sequences were then analyzed to confirm the identification of these orchid mycorrhizal fungi because of the low similarity of ITS sequences. BLAST results of 28S rDNA analysis were 99% similarity to *Ceratobasidium* sp. (AF354094) (Table 1). The phylogenetic trees of both ITS and 28S rDNA sequences analyzed in corporate representative sequences of various *Ceratobasidium* species supported fungal identification inferred from BLAST at the generic level. Although 28S rDNA sequences of both isolates were similar, their pairwise analyses were 93% similarity. Therefore, the orchid mycorrhizal isolates SIID3B2 and Cal8 belong to the same genus *Ceratobasidium* sp., but they are different in species. *Ceratobasidium* is a major genus of orchid mycorrhizal fungi which was originally incorporated in to the anamorphic form genus *Rhizoctonia* (Roberts 1999). Even though both isolates come from geographically different area, they are similar in genus level. Cal 8 was isolated from *C. triplicate* at the forest in Keerom, New Guinea mainland, while SIID3B2 was isolated from *D. lancifolium* in Biak, the small island in Pacific Ocean. Pereira et al. (2005) reported that some orchid from rain forest in Atlantic have association with *Rhizoctonia*-like fungi including genus *Ceratorhiza* and *Epulorhiza*. Zettler and Piskin (2011) found *Ceratorhiza* from protocorm, seedling and adult *Platanthera leucophaea*.

One interesting thing that is *D. lancifolium* was able to grow in hot (31^{o}C) and dry habitat (50%) along the road side. Presumably, this mycorrhizal association contributes to the survival of this orchid in this area. Mycorrhizal fungi may also be a key source of water for orchids. Water content in both the terrestrial orchid *Platanthera integrilabia* (Correll) Luer and the epiphytic *Epidendrum conopseum* R.Br. was higher for mycorrhizal seedlings than uncolonized controls (Yoder et al. 2000). Alexander et al. (1984) found that mycorrhizal *Goodyera repens* acquired 100 times more P than non-mycorrhizal controls. P and N (as glycine) transfer from fungus to plant was confirmed in radio-labeling experiments (Dearnaley 2007).

This study showed that molecular approach help in determining the orchid mycorrhizal fungi isolated from *D. lancifolium* and *C. tripicata* in Papua. For the future work, we still have to elucidate about the role of *Rhizoctonia* spp. in the growth-promoting of those terrestrial orchid, especially *D. lancifolium* for in situ conservation purpose.

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REFERENCES

Agustini V, Sufaati S, Suharno. 2009. Mycorrhizal association of terrestrial orchids of Cycloops Nature Reserve, Jayapura. Biodiversitas 10 (4): 175 -180.

Agustini V, Suharyanto, Suharno, Dimara L, Sembay CD. 2013. The diversity of tropical orchids of South Papua. Jurnal Biologi Papua 5 (1): 1-9.

Alexander C, Alexander IJ, Hadley G. 1984. Phosphate uptake bu Goodyera repens in relation to mycorrhizal infection. New Phytol 97: 391-400.

Atala C, Pereira G, Romero C, Muñoz-Tapia L, Vargas R, Suz LM. 2015. Orchidioid fungi of the form-genus *Rhizoctonia* associated with the roots of *Chloraea cuneata* Lindl. from Araucanía, Chile. Gayana Bot 72 (1): 145-148.

Athipunyakom P, Manoch L, Piluek C. 2004. Isolation and identification of mycorrhizal fungi from eleven terrestrial orchids. Nat Sci 38 (2): 216-228.

Berga-Pana H. 2005. Handbuch der orchideen-namen. Dictionary of Orchid Names. Dizionario dei nomi delle orchidee. Ulmer, Stuttgart.

Cheng SF, Yeh CH, Jan CH, Chang DCN. 2012. Growth and development of *Phaius tankervilleae (*Banks) Blume when inoculated with orchid mycorrhizal fungi. African J Agric Res 7 (42): 5644-5652.

Chutima R, Dell B, Vessabutr S, Busaban B, Lumyong S. 2011. Endhophytic fungi from *Pecteilis susannae* (L.) Rafin (Orchidaceae), a threatened terrestrial orchid in Thailand. Mycorrhiza 21: 221-229.

Dearnaley J. 2007. Further advances in orchid mycorrhizal research. Mycorrhiza 17 (6): 475-486.

Diaz A, Nellore A, Song JS. 2012. CHANCE: comprehensive software for quality control and validation of ChIP-seq data. Genome Biol 13 (10): R98. http://genomebiology.com/2012/13/10/R98.

Ding R, Chen X-H, Zhang L-J, Yu X-D, Qu B, Duan R, Xu YF. 2014. Identity and specificity of Rhizoctonia-like fungi from different populations of *Liparis japonica* (Orchidaceae) in Northeast China. PLoS ONE 9 (8): e105573. DOI:10.1371/journal.pone.0105573.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32 (5): 1792-1797.

Gurlanda M, Selosse MA, Cafasso D, Brilli F, Defline S, Fabbian R, Ghignone S, Pinelli P, Segreto R, Loreto F, Cozzolino S, Perotto S. 2006. Inefficient photosynthetis in the Mediterranean orchids *Limodorum abortivum* is mirrored by specific association to ectomycorrhizal Russulaceae. Mol Ecol 15 (2): 491-504.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95-98.

Jacquemyn H, Deja A, de Hert K, Bailarote BC, Lievens B. 2012. Variation in mycorrhizal associations with Tulasnelloid fungi among populations of five *Dactylorhiza* species. PLoS ONE 7 (8): e42212. DOI:10.1371/journal.pone.0042212.

Kristiansen KA, Taylor DL, Kjoller R, Ramussens HN, Rosendahl S. 2001. Identification of mycorrhizal fungi from single pelotons of *Dactylorhiza majalis* (Orchidaceae) using single-stand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences. Mol Ecol 10: 2089-2093.

Liu H, Luo Y, Liu H. 2010. Studies of mycorrhizal fungi of Chinese orchids and their role in orchid conservation in China—A review. Bot Rev 76: 241-262.

Luca A, Bellusci F, Pellegrino G. 2014. Interactions with mycorrhizal fungi in two closely related hybridizing orchid species. Acta Bot Croat 73 (1): 209-220.

Millar A. 1978. Orchid of Papua New Guinea: An introduction. Australian National University Press. Canberra, Australia.

Moore RT. 1987. The genera of *Rhizoctonia*-like fungi: *Asorhizoctonia*, *Ceratorhiza* gen. nov., *Epulorhiza* gen. nov., *Moniliopsis* and *Rhizoctonia*. Mycotaxon 29: 91-99.

Otero JT, Ackerman JD, Bayman P. 2002. Diversity and host specificity of endophytic Rhizoctonia-like fungi from tropical orchids. Am J Bot 89 (11): 1852-1858.

Pandey M, Sharma J, Taylor DL, Yadon VL. 2013. A narrowly endemic photosynthetic orchid is non-specific in its mycorrhizal associations. Mol Ecol. DOI: 10.1111/mec.12249.

Pereira OL, Kasuya MCM, Borges AC, de Araújo EF. 2005. Morphological and molecular characterization of mycorrhizal fungi isolated from neotropical orchids in Brazil. Can J Bot 83 (1): 54-65.

Perotto S, Rodda M, Benetti A, Sillo F, Ercole E, Rodda M, Girlanda M, Murat C, Balestrini R. 2014. Gene expression in mycorrhizal orchid protocorms suggests a friendly plant-fungus relationship. Planta 239: 1337-1349.

Roberts P. 1999. *Rhizoctonia*-forming fungi: a taxonomic guide. Royal Botanic Gardens, Kew.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hōhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. DOI: 10.1093/sysbio/sys029.

Suwannasai N, Martín MP, Phosri C, Sihanonth P, Whalley AJS, Spouge JL. 2013. Fungi in Thailand: A case study of the efficacy of an ITS barcode for automatically identifying species within the *Annulohypoxylon* and *Hypoxylon* genera. PLoS ONE 8 (2): e54529. DOI: 10.1371/journal.pone.0054529.

Tao G, Liu Z-Y, Liu F, Gao Y-H, Cai L. 2013. Endophytic *Colletotrichum* species from *Bletilla ochracea* (Orchidaceae), with descriptions of seven new speices. Fungal Div 61: 139-164.

Taylor DL, Bruns TD. 1997. Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. Proc Natl Acad Sci USA 94 (9): 4510-4515.

Taylor DL, Bruns TD, Hodges SA. 2004. Evidence for mycorrhizal races in a cheating orchid. Proc Biol Sci 271 (1534): 35-43.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172: 4239-4246.

Wang LK, Liu HX. 2013. Factors affecting the production and regeneration of protoplasts: the case of the mycorrhizal fungus

Tulasnella calospora from roots of orchids. For Sci Prac 15 (3): 186-195.
White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White YJ (eds.) PCR Protocols: A Guide to Methods and Application. Academic Press, San Diego, CA, USA.
Yang G, Li C. 2012. General description of *Rhizoctonia* species complex. In: Cumagun CJ (ed.) Plant Pathology. InTech. http://www.intechopen.com/books/plant-pathology/general-description-of-rhizocotonia-species-complex.
Yoder JA, Zettler LW, Stewart SL. 2000. Water requirements of terrestrial and epiphytic orchid seeds and seedlings, and evidence for water uptake by means of mycotrophy. Plant Sci 156: 145-150.
Zettler LW, Piskin KA. 2011. Mycorrhizal fungi from protocorms, seedlings and mature plants of the eastern Prairie fringed orchid, *Platanthera leucophae*a (Nutt.) Lindley: A comprehensive list to augment conservation. Am Midl Nat 166: 29-39.

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Diversity of *Selaginella* across altitudinal gradient of the tropical region

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Abstract. *Setyawan AD, Supriatna J, Darnaedi D, Rokhmatuloh, Sutarno, Sugiyarto. 2016. Diversity of* Selaginella *across altitudinal gradient of the tropical region. Biodiversitas 17: 384-400. Selaginella* prefers moist environment for its growth and requires water for fertilization; therefore it is often found flourish in plateau and hilly mountains with high rainfall. The aim of this research was to know species diversity of *Selaginella* along altitudinal gradient in the southern part of Java, Indonesia. The research was conducted between July 2007 and January 2014across the altitudinal region of Java, Indonesia, which includes the districts of Wonosobo, Purworejo, Kebumen, Banjarnegara and its border areas, with altitudes between 0 and 2500 m. asl. The research found 12 selaginellas species, 10 species collected from field research, namely: *S. aristata, S. ciliaris, S. intermedia, S. involvens, S. opaca, S. ornata, S. plana, S. repanda, S. remotifolia,* and *S. uncinata;* and two species only known from herbarium collection of Herbarium Bogoriense (BO), namely *S. singalanensis* and *S. willdenowii*. This research was the first publication of the *Selaginella* flora along altitudinal gradient from the coastal area to the high mountains of Central Java, Indonesia.

Keywords: diversity, Central Java, *Selaginella*, species, taxonomy

INTRODUCTION

Selaginellaceae Reinch. is a lycophyte family with only one genus namely *Selaginella* P. Beauv. (club moss, spike-moss). There are about 700-750 species of *Selaginella* in the world, which distributed cosmopolitan and pantropical in the tropics and sub-tropics regions (Tyron and Tyron 1982; Jermy 1990). In the islands of Southeast Asia (Malay Archipelago, Nusantara), there are more than 200 species of *Selaginella* (Camus 1997; Hassler and Swale 2002). Meanwhile, in Java, there are 25 species of *Selaginella* (Setyawan 2008). *Selaginella* is a difficult genus to be classified. A large number of *Selaginella* species are morphologically polymorphic and have high morphological similarity among them (Setyawan et al. 2012). This confusion led to almost every species having more than one name, such as *S. ornata* and *S. involvens*, which have high morphological variation and with more than 25 synonyms each (Hassler and Swale 2002).

Selaginella is a plant genus that prefers moist habitat and need water for fertilization. The southern part of Central Java has a diverse ecosystem which dominated by hilly area, ranging from the coastal areas to the middle and high mountains. Mountainous region with humid climate and abundant water sources throughout the year is a hotspot for its diversity. This species is likely to be the object of global climate change. *Selaginella* is widely distributed in shaded and moist places, although the distribution of each species can be limited depending on location and seasons. According to Setyawan et al. (2012), there were 10 species of *Selaginella* distributed from the coastal area to the montane rain forest in the southern part of Central Java. *S. plana* and *S. ciliaris* are important species for the lowland area, while *S. remotifolia* and *S. opaca* are important species for the highlands. Meanwhile, *S. ornata* and *S. aristata* are important species for the ecotone area. The presence of other species such as *S. involvens*, *S. intermedia*, *S. repanda* and *S. uncinata* are very limited and the distribution can not certainty be known. *S. uncinata* is an invasive species, presumably naturalized from ornamental plants. Setyawan et al. (2013) stated that the altitude of 1500 may be the upper limit for the distribution of *S. aristata, S. ciliaris, S. involvens, S. ornata, S. plana, S. singalanensis,* and *S. zollingeriana* in Java. Meanwhile, the altitude of 2100 m is probably the upper limit for the distribution of *S. opaca* and *S. remotifolia.*

Selaginella is useful for traditional medicine, especially for treating wounds, postpartum, menstrual disorders and body fit improvement (tonics). Biomedical research shows that this plant has potential as an anti-oxidant, anti-inflammatory, anti-cancer, antibacterial, etc (Setyawan 2011). *Selaginella* is also useful as an ornamental plant, handicraft materials, and vegetables (Winter and Jansen 2003; Setyawan 2009). *Selaginella* has a very diverse phytochemical content, such as: alkaloids, phenols (flavonoids, tannins, saponins), and terpenoids (triterpene, steroid) (Setyawan 2011; Chikmawati et al. 2012). These herbs also contain lignin, lignans, lignanosides, alkaloids, selaginellin, glycosides, glucosides, glycosyl flavones, and others. Some species contain certain natural products, such as ecdysteroid, essential oils and trehalose (Setyawan 2011).

Research on the flora of *Selaginella* along altitudinal gradient in the southern Central Java, Indonesia has never been done. However, it has been previously reported the altitudinal distribution of *Selaginella* in this region (Setyawan 2012). Meanwhile, previous research on the taxonomy of *Selaginella* in all over Java by Alston (1934a) has not been updated. This research aims to determine the flora of *Selaginella* along altitudinal gradient in the southern part of Central Java, Indonesia.

MATERIALS AND METHODS

Study areas

This research was conducted along altitudinal gradient in southern Central Java, Indonesia, known as historic area of Bagelen and surrounding areas. Administratively, the studied area mainly includes Wonosobo, Purworejo, Kebumen and Banjarnegara districts; since distribution of wildlife can not be restricted by administrative boundaries, the border region of neighboring district is also observed, i.e. part of Temanggung, Magelang, Kulonprogo, Banyumas, Purbalingga, and Pekalongan districts. All districts belong to Central Java Province, except for Kulonprogo that belongs to Yogyakarta Special Region (Figure 1). Geographically, this area is located between -7.1046° and -7.9033° (S) and between 109.3341° and 110.2298° (E), the total area of more than 4200 km^2, with a population of more than 4.5 million people. The altitude varies from 0 m in the southern coastal area to 3371 m. asl. at the top of Mt. Sumbing; the temperature is ranging between 24-34^{o}C, but in the Dieng Plateau and surrounding area it ranged from 12-20^{o}C; relative humidity is 70-94%, the average rainfall in the southern part is around 2300 mm, and in the northern hilly part is more than 4200 mm, with the highest rainfall is in the Garung sub-district, Wonosobo approximately 4802 mm (BPS Banjarnegara 2011, BPS Kebumen 2011, BPS Purworejo 2011, BPS Wonosobo 2011). This area has only two dry months in the north and 5-6 dry months in the south (Oldeman 1975). The main physiographic of this region are hilly mountains in the north consist of Dieng Plateau, highland of Mts. Sindoro and Sumbing, lowland area in the south along the coastal line, and Serayu Watersheds and South Serayu Mountains in the center. Soil types in the Dieng Plateau and southern lowlands are alluvial, the other highland areas are dominated by regosol or andosol soils, the southern lower mountains are dominated by latosol-andosol, complex-podzolic-latosol lithosol or regosol soils, whereas the karst region of Karangbolong has a complex mediterranian-regosol-grumosol soil (SRI 1960). More than half of the region's land is used for dry land farming, about a quarter is used for wet rice fields, the remainder is state forest, private plantations, rivers, lakes, developing areas (settlements, roads), etc (BPS Banjarnegara 2011, BPS Kebumen 2011, BPS Purworejo 2011, BPS Wonosobo 2011).Outside the forest, wild species inhabit abandoned places, such as river banks, edges of roads and irrigation canal, and fill in the sidelines of agricultural land, residential, grave and other built areas (Setyawan et al. 2012).

The field work was carried out of more than six years, between July 2007 and January 2014. Several massive surveys of *Selaginella* had been conducted, with altitude between 0 and 2500 m. asl., both in wet and dry seasons. In this study, most of the studied area has been developed, viz. paddy and dry fields, agroforestry (community forests), plantation and production forest, home garden, settlements, roads, water bodies and drainages, etc. Natural forest is only found in a limited size in the slope of Dieng Mountains, Mt. Sindoro and Mt. Sumbing, which periodically burned. This research is mainly conducted in shaded and humid places, especially in the hilly areas of Wonosobo which have higher annual rainfall (>4000 mm in 2011), one of the rainiest district in Java, but also along drier area in the southern coastal area.

Procedures

Almost all sites in the southern Central Java are anthropogenic area and influenced by human activities. *Selaginella* is generally found in abandoned places that are moist and shady, such as roadside cliffs, footpaths, tributaries cliffs, moist cliffs of forest, agroforests, and agricultural lands, etc. Some species can also grow in relatively open sites, such as pine forest (*Pinus merkusii*), the settlements and agricultural land. *Selaginella* rarely grows under a dense clump of herbs or shrubs; that place does not provide space and light for growth. All *Selaginella* species were recorded and collected as herbarium specimens and living collection for the experimental garden in Kejiwan, Wonosobo, Central Java (768 m. asl.). Both living plants and herbarium specimens were observed. Specimens of field collection were deposited at Herbarium Soloense (SO), Sebelas Maret University, Surakarta, Indonesia and some selected specimens were sent to Herbarium Bogoriense (BO), Indonesian Institute of Sciences, Cibinong-Bogor, Indonesia. A total of 440 herbarium specimens of *Selaginella* have been collected from the study site, and 18 historical herbarium specimens of BO from this area have also been observed. Since the existence of two species is only known from BO collections, the same plant species planted in the experimental garden is also observed, namely: *S. singalanensis* of Mt. Lawu, Central Java (ADS 467) and *S. willdenowii* of Mt Halimun Salak, West Java (ADS 460). Each herbarium specimen was unique, distinguished by location and time of collection. Data passport collected along with the specimens were used as standard for herbarium specimens. The specimens were identified by using several early literatures on *Selaginella* in Malay Archipelago, i.e. Alderwereld van Rosenburgh (1915a,b, 1916, 1917, 1918, 1920, 1922) and Alston (1934a, 1935a,b, 1937, 1940); and were compared with the specimens collection at BO, especially the specimens that had been determined by A.G.H. Alston before; and also by using several newest references from Malay Archipelago and adjacent area such as Wong (1982, 2010), Tsai and Shieh (1994), Li and Tan (2005), Chang et al. (2012), and Zhang et al. (2013), as well as previously study in Java such as Setyawan et al. (2012, 2013), Setyawan (2014), Setyawan (2015a,b), Setyawan et al. (2015a,b,c), and Setyawan and Sugiyarto (2015).

In addition to direct observations, the literatures were used to guide the preparation of description. Meanwhile, the synonyms and global distribution was mainly according to Hassler and Swale (2002) and Chang et al. (2012). Locality is according to the administrative division of sub-district level.

RESULTS AND DISCUSSION

Description

In this study, a total of 13 species of *Selaginella* have been identified along altitudinal gradient of the southern of Central Java, Indonesia. The 11 species was found from the field surveys, namely *S. aristata*, *S. ciliaris*, *S. intermedia*, *S. involvens*, *S. opaca*, *S. ornata*, *S. plana*, *S. remotifolia*, *S. repanda*, and *S. uncinata*. While, the two species were only found from the historic collection of Herbarium Bogoriense specimens, namely *S. singalanensis* and *S. willdenowii*. The altitudinal distribution of each species is showed in Figure 2A-L.

Selaginella is a rarely annual (*S. aristata*, *S. ciliaris*) or perennial herb; terrestrial, epiphythic (*S. involvens*, *S. repanda*), or occasionally epiphytic (*S. ciliaris*). Stems are leafy, slender, descending (*S. aristata*), creeping and rooting at intervals (*S. ciliaris*, *S. opaca*, *S. remotifolia*, *S. singalanensis*),ascending (*S. aristata*, *S. plana*), decumbent (*S. repanda*) or erect, roll up when dry (*S. involvens*), with or without branches on lower part, rooting near base, branching dichotomously, regularly or irregularly branched. Rhizomes rarely present (*S. involvens*, *S. repanda*).

Rhizophores are present or absent (*S. involvens*), geotropic, borne on dorsal (upper) or ventral (lower) side in axils of branches, throughout (creeping ones), or confined to base (*S. ornata*). Roots formed at tip of rhizophore, branched. Leaves are small, simple, with a single vein (exceptionally veins forked), always bearing an inconspicuous ligules in axil on adaxial side (only prominent in early development), monomorphic or dimorphic; vegetative leaves (tropophyll) are monomorphic-spirally arranged at basal main stem and dimorphic-4 lanes arranged on other parts (*S. involvens*, *S. plana*), or in most species dimorphic and usually arranged in 2 median (ventral) or upper side and 2 lateral (dorsal) or lower side rows on all branches (*S. ornata*, *S. singalanensis*); median leaves are usually smaller, and in different shape from the lateral leaves; axillary leaves are single borne at the forking of each branch, being somewhat different from other leaves. *Strobilus* (clusters of imbricating sporophylls) are usually terminal on the ends

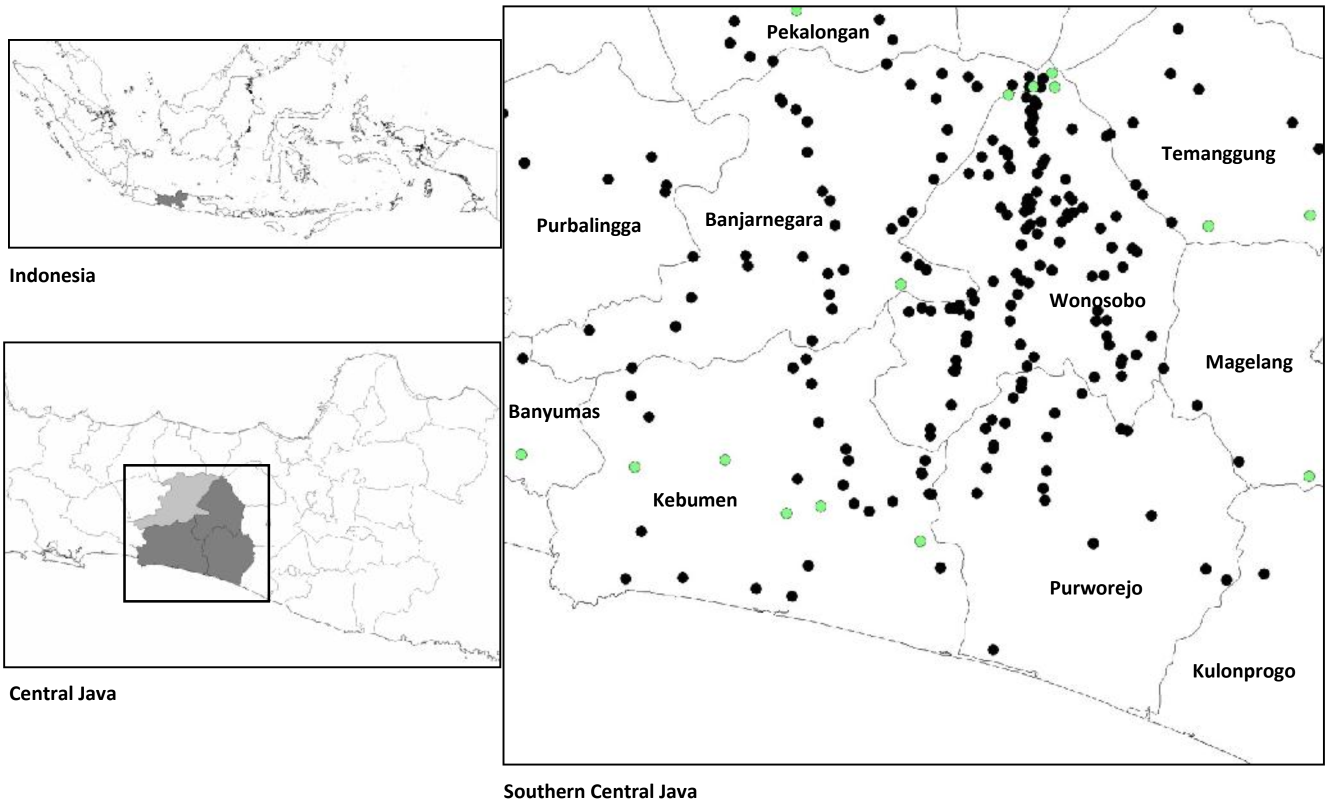

Figure 1. Study sites of selaginellas altitudinal diversity in the southern Central Java, Indonesia, namely: (i) Herbarium specimens from field collection (●), and (ii). Herbarium specimens from Herbarium Bogoriense (BO) (○)

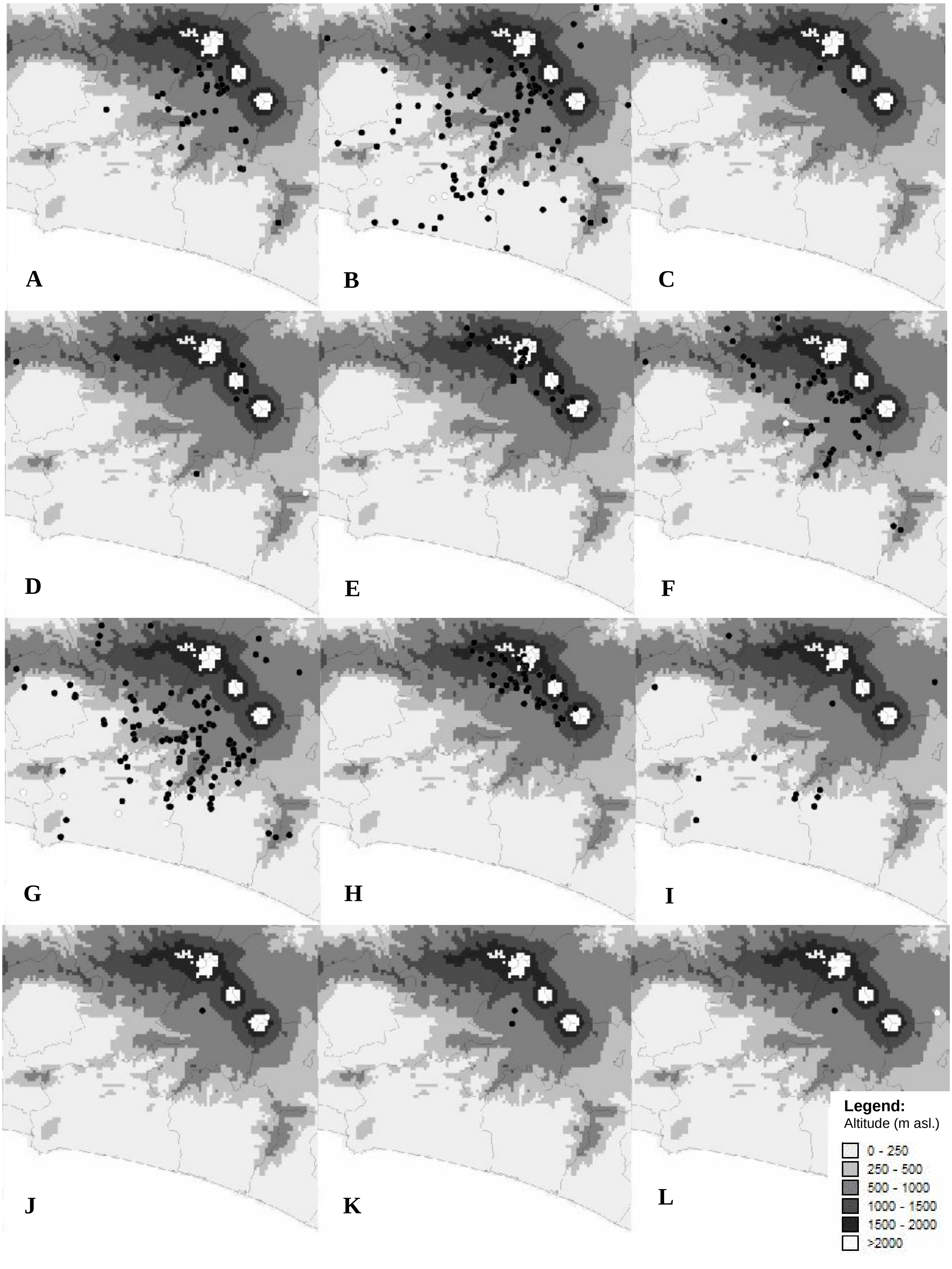

Figure 2. Species distribution of selaginellas in Southern Java and the surrounding; A. *S. aristata*, B. *S. ciliaris*, C. *S. intermedia*, D. *S. involvens*, E. *S. opaca*, F. *S. ornata*, G. *S. plana*, H. *S. remotifolia*, I. *S. repanda*, J. *S. singalanensis*, K. *S. uncinata*, and L. *S. willdenowii*

and sides of branches, cylindrical, tetragonal (*S. involvens*, *S. opaca*, *S. remotifolia*), flattened (*S. ciliaris*) or do not in compact strobilus (*S. aristata*, sometimes). Sporophylls (fertile leaves) are monomorphic or adjacently different, slightly or highly differentiated from vegetative leaves. Sporangia are short-stalked, solitary in an axil of sporophylls, opening by distal slits. Spores are of two types (heterosporous), megaspores tetrad (1-2-)4, large, commonly at the base of strobilus, microspores numerous (hundreds), minute; sporangia round or oval, opening by a transverse slit. White, black, brown, or reddish to yellowish red (*S. repanda*).

One genus and ca. 700-750 species: almost cosmopolitan, with its highest diversity in the tropics; 200 species in Malay Archipelago, 25 (5 endemic) species in Java, 13 species in southern Central Java.

Key to species

1. Stem (sub-)erect, rooting at base, or bearing rhizophores 2
 2. Stem shorter than 50 cm 3
  3. Stem fleshy .... *S. aristata*
  3. Stem fragile .... *S. ornata*
 2. Stem longer than 50 cm 4
  4. Stem fleshy .... *S. intermedia*
  4. Not so 5
   5. Stem hard, caulescent, easily broken .... *S. involvens*
   5. Stem tough .... *S. plana*
1. Stem mostly creeping, rooting at intervals 6
 6. Leaves with blue iridescence 7
 6. Not so 8
  7. Stem creeping, shorter than 1 m .... *S. uncinata*
  7. Stem creeping to scrambling (viselike), longer than 1 m .... *S. willdenowii*
   8. Stem shorter than 15 cm .... *S. ciliaris*
   8. Stem more than 15 cm 9
    9. Stem fleshy .... *S. opaca*
    9. Not so 10
     10. Leaves loosely arranged .... *S. remotifolia*
     10. Leaves imbricate 11
      11. Spores bright yellow........ *S. repanda*
      11. Not so .... *S. singalanensis*

Species description

Selaginella aristata Spring, Bull. Acad. Brux. 10: 232, no. 152 (1843) (Figure 3.A)

Synonym: *Lycopodioides leptophylla* (Baker) Kuntze, Revis. Gen. Pl. 2: 826 (1891); *Lycopodium philippense* Willd. ex Spreng., Syst. Veg. 4: 17 (1827); *Lycopodium remotifolium* Desv., Prodr. Fil., Ann. Soc. L. Paris, 6: 190, no. 155 (1827); *Selaginella aristata* var. *brevifolia* Hieron., Elmer's Leaflets Philip. Bot. 6: 2045 (1913); *Selaginella aristata* var. *kaudernii* Alderw., Bull. Jard. Bot. Buit. III, 5: 238 (1922); *Selaginella aristata* var. *obtusifolia* Hieron., Elmer's Leaflets Philip. Bot. 6: 2046 (1913); *Selaginella circinalis* var. *aristata* Presl., Bot. Bem. 153 (1845); *Selaginella cristata* Warb., Monsunia I: 125 (1900);*Selaginella hayata* Satake, Bot. Mag. (Tokyo) 48 (568): 261 (1934);*Selaginella leptophylla* Baker var wichurae (Warb.) Tagawa, Acta Phytotax. Geobot. 10 (3): 194 (1941); *Selaginella leptophylla* Baker, J. Bot. 23: 157 (1885); *Selaginella pickeringii* Hieron., Elmer's Leaflets Phil. Bot. 6: 2046 (1913); *Selaginella proniflora* auct. non (Lam.) Baker, Matsum. & Hayata, Enum. Pl. Formosa 554 (1906); *Selaginella satakeana* Koidz., Acta Phytotax. Geobot. 5: 40 (1936); *Selaginella stenostachya* Hayata, Icon. Pl. Formosan. 4: 129 (1914); *Selaginella wichurae* Warb., Monsunia I: 127 (1900).

Annual herb, small, fleshy, sub-erect, prostrate or ascending, caespitose, glabrous, fan-shaped, whitish green leaves with pink to brown stem, very fleshy; multiple branched at main stem, forming dendritic stem. *Stems* decumbent to ascending, multiple branched or dendritic, especially at the mature ones, ca. 4-21 cm long, 3-6 mm wide (including leaves). *Rhizophores* present at basal stem, originated from the ventral side of branching stem, ca. 1 mm in diam. *Leaves* (trophophylls) dimorphic, arranged in 4 lanes (2 lateral, 2 median), sparsely arranged at main stem but closely arranged at the branches, vein single; *lateral (ventral) leaves* lanceolate, oblong or ovate at main stem, lanceolate to falcate at branches, 2-3 mm long, 1-2 mm wide, apex acute or obtuse, asymmetrical, base subcordate or rounded, margin serrulate to subentire; *median (dorsal) leaves* smaller than the lateral ones, lanceolate to ovate, more or less symmetrically, 1.5-2 mm long, 0.5-1 mm wide, apex caudate to long tail-like, apices upward or bended back, base obtuse, margin serrulate, single vein reaching the apex; *axillary leaves* lanceolate, ovate or subcordate,1.5-3 mm long, 0.5-1.5 mm wide, apex obtuse, base rounded, margin serrulate, single vein nearly reaching the apex. *Strobilus* solitary, terminal, loosely, bisymmetrical, upper-plane sporophylls longer than lower-plane, up to 1 cm long.

Habitat and ecology: It widely grows on moist steep cliffs; rugged contours allow plenty of water seepage, humid and suitable for its growth. It was found on tributary cliff, small shaded river bank, the cliff and edge of irrigation canals, the cliff of water springs, river bridge, the cliff and edge of main roads, villages roads, footpath; on the steep cliff of acacia, albizia, and bamboo stands, on the dry field, agroforestry, pine and agathis forests; on the palawija crops, salak and vegetable fields; on the intensive agricultural field shaded by paranet; on the village cliffs and cemetery hills. It was only abundant in the rainy season, at altitude of 186-1209 m. asl.

Locality: Banjarnegara (Bawang, Madukara, Pagentan, Sigaluh), Purworejo (Kaligesing), Wonosobo (Garung, Kaliwiro, Kepil, Kertek, Mojotengah, Sapuran, Selomerto, Wadaslintang, Wonosobo-city).

Distribution: China (Guangxi, Guizhou, Henan, Hongkong, Sichuan, Yunan), India, Indonesia, Japan, Myanmar, Philippines, Sri Langka, Taiwan, Thailand, Vietnam. In Indonesia: Java, Moluccas (Buru, Halmahera, Seram, Ternate), Sulawesi.

Specimens observed: ADS 190, ADS 196, ADS 201, ADS 203, ADS 204, ADS 207, ADS 212, ADS 219, ADS 235, ADS 240, ADS 244, ADS 247, ADS 254, ADS 285,

ADS 301, ADS 307, ADS 309, ADS 317, ADS 332, ADS 343, ADS 344, ADS 351, ADS 353, ADS 358, ADS 364, ADS 367, ADS 370, ADS 396, ADS 398, ADS 405, ADS 410, ADS 521, ADS 534, ADS 537, ADS 541, ADS 554, ADS 740.

Note: It is a fleshy annual species that only abundant in the rainy season; cover-abundance in the rainy season can reach four times the dry season. In the dry season, it generally will only be remnants that led to death. This species generally co-habitation with *S. ciliaris*, especially in moist and shaded places, while in a very open place where *S. ciliaris* can still grow, *S. aristata* is unable to grow.

Selaginella ciliaris (Retz.) Spring, Bull. Acad. Brux. 10: 23 (1843) (Figure 3.B-C).

Basionym: *Lycopodium ciliare* Retz., Observ. Bot. 5: 32 (1789).

Synonym: *Lycopodioides ciliaris* (Retz.) Kuntze, Rev. Gen. Pl. 1: 826 (1891); *Lycopodioides depressa* (Sw.) Kuntze, Rev. Gen. Pl. 1: 826 (1891); *Lycopodioides exigua* (Spring) Kuntze, Rev. Gen. Pl. 1: 826 (1891); *Lycopodioides pumilio* (R. Br.) Kuntze, Rev. Gen. Pl. 1: 827 (1891); *Lycopodium belangeri* Bory, Bel. Voy. Bot. 2: 12 (1833 or 1834); *Lycopodium depressum* Sw., Schrad. Jour. Bot (1800) (2): 110. 1801; *Lycopodium pumilio* R. Br., Prodr. Fl. Nov. Holland 166 (1810); *Selaginella belangeri* (Bory) Spring, Monogr. Lyc. II: 242: 180 (1850); *Selaginella belangeri* f. *olivacea* Alderw., Mal. Fern Allies, 173 (1915); *Selaginella congregata* Alderw., Bull. Jard. Bot. Buit. III, 2: 179 (1920); *Selaginella depressa* (Sw.) Spring, Bull. Acad. Brux. 10: 234, no. 162 (1843); *Selaginella exigua* Spring, Mem. Acad. Sci. Belg. 24 (2): 238 (1850); *Selaginella papana* Alderw., Bull. Jard. Bot. Buit. III, 5: 235 (1922); *Selaginella proniflora* Baker, Jour. Bot. 22: 156, no. 260 (1885); *Selaginella pumilio* (R. Br.) Spring, Bull. Acad. Brux. 10: 232, no 143 (1843); *Selaginella tenera* sensu Narayanaswami & Carter, Mem. As. Soc. Beng. 7: 290 (1922); *Selaginella ujensis* Hieron., Bot. Jahrb. Engl. 44: 514 (1910); *Selaginella winkleri* Hieron., Bot. Jahrb. Engl. 44: 516 (1910); *Stachygynandrum ciliare* (Gmel.) Beauv., Prodr. Aetheog. 110 (1805); *Stachygynandrum depressum* (A. Br.) Carr., Cat. Afr. Pl. coll. by Dr. F. Welwitsch in (1853)-1861, 2 (2): 262. 1901; *Stachygynandrum depressum* var. *minus* (A. Br.) Carr., Cat. Afr. Pl. coll. by Dr. F. Welwitsch in (1853)-1861, 2 (2): 262. (1901).

Annual herb, small, shortly creeping, prostrate or ascending, sometimes fan-shaped, stramineous, 2-12.5 cm long, glabrous, angular or sulcate, rooting at intervals but mostly near base, sometimes in a clump, green, yellowish or dark brown. *Stems* decumbent, branched throughout without a significant main stem, 4-5 mm wide (including leaves). *Rhizophores* present at intervals but mostly near the base, originated from the ventral side in axils of branches, ca. 0.3 mm in diam. *Leaves* dimorphic, arranged in 4 lanes (2 lateral, 2 median), vein single; *lateral leaves* ovate to lanceolate, more or less symmetrical, 1.5-2mm long, 0.6-1 mm wide, apex acuminate or acute, base rounded or subcordate, margin serrulate or ciliate, single vein reaching the apex, keeled, pointing outwards; *median leaves* ovate to falcate, contiguous, nearly asymmetrical, 2-2.5 mm long, 0.6-1.5 mm wide, apex acute, aristate or cuspidate, base subcordate or rounded, margin serrulate but laciniate at basal part, pointing upwards, minutely toothed, ciliate, slightly carinate, midrib prominent, single vein reaching or nearly reaching the apex; *axillary leaves* lanceolate to ovate, equally sided (bisymmetrically) or slightly asymmetrical,1.5-2.5 mm long, 1-1.5 mm wide, single vein reaching or nearly reaching the apex, apex acute, base rounded to subcordate, exauriculate, ciliate, margin ciliate or laciniate at basal part and finely toothed or serrulate at apical part. *Strobilus* are solitary (or twin), terminal, compact, dorsiventrally complanate or flattened, up to ca. 0.5-2 cm long; sporophylls strongly dimorphic, spore greenish-yellowish orange.

Habitat and ecology: It is strongly influenced by growth season. Since it has small size and short roots, the plants die during the dry season and sprout new plantlets at the beginning of the rainy season (r-selection). Its habitat is the moist rocky slope, slightly open and shady places without excessive soil or humus, but it is sometimes also found on steep soil and flat ground rather open and moist. It is found in a variety of building walls and natural rocky cliffs, such as near water spring, small river, large and small irrigation canals, roadside ditch, fences and houses, ruins of abandoned housing, fish pond, tower, river bridge, cemetery and tombstones, terassering of fields, and roadside sandstones; around the footpath, dirt road, village road, main road, rice field dikes, fields of palawija, salak, cassava and vegetables, tea gardens, coffee plantations, agroforestry of albizia, forest of pine and agathis; intensive agricultural fields shaded by paranet; and the base of palm trees in the coastal areas. It is very often found in the cemetery. The locations with shaded large trees and deprived herbs and shrubs provide enough space for growth without significant competition. Setyawan (2011) found that this species is used as a tomb cover. It is found at altitudes of 11-1246 m. asl.

Locality: Banjarnegara (Banjarmangu, Banjarnegara-city, Bawang, Madukara, Mandiraja, Pagedongan, Pagentan, Pejawaran, Purwanegara, Sigaluh, Wanadadi), Banyumas (Somagede), Kebumen (Buayan, Bulus-pesantren, Karangsambung, Klirong, Mirit, Petanahan, Prembun, Gombong, Karanganyar, Kebumen-city), Kulonprogo (Girimulyo), Magelang (Salaman, Secang), Pekalongan (Paninggaran), Purbalingga (Kejobong, Karangmoncol), Purworejo (Bayan, Bener, Grabag, Kaligesing, Loano), Temanggung (Bejen, Jumo), Wonosobo (Garung, Kaliwiro, Kertek, Mojotengah, Selomerto, Wadaslintang, Wonosobo-city).

Distribution: N-Australia, S-China (Guangdong), India, Indonesia, Marianas, Micronesia, Myanmar, New Guinea, Palau Isl., Philippines, Sri Lanka, Solomons Isl., Taiwan, Thailand, Vietnam. In Indonesia: Java, Sulawesi, Moluccas (Ternate).

Specimens observed: ADS 78, ADS 91, ADS 176, ADS 182, ADS 191, ADS 194, ADS 200, ADS 202, ADS 205, ADS 206, ADS 211, ADS 213, ADS 215, ADS 218, ADS 236, ADS 239, ADS 243, ADS 248, ADS 250, ADS

255, ADS 258, ADS 284, ADS 302, ADS 306, ADS 310, ADS 314, ADS 316, ADS 319, ADS 320, ADS 324, ADS 327, ADS 331, ADS 333, ADS 342, ADS 345, ADS 349, ADS 354, ADS 359, ADS 363, ADS 366, ADS 369, ADS 372, ADS 375, ADS 376, ADS 395, ADS 401, ADS 404, ADS 407, ADS 409, ADS 414, ADS 433, ADS 445, ADS 516, ADS 522, ADS 524, ADS 525, ADS 526, ADS 527, ADS 528, ADS 529, ADS 530, ADS 531, ADS 532, ADS 533, ADS 540, ADS 549, ADS 574, ADS 616, ADS 617, ADS 618, ADS 619, ADS 620, ADS 621, ADS 622, ADS 623, ADS 734, ADS 738, ADS 742, ADS 743, ADS 744, ADS 1055, ADS 1059, ADS 1061, ADS 1063, ADS 1065, ADS 1070, ADS 1073, ADS 1074, ADS 1075, ADS 1076, ADS 1077, ADS 1083, ADS 1084, ADS 1085, ADS 1087, ADS 1098, ADS 1100, ADS 1129, ADS 1131, ADS 1134, ADS 1142, ADS 1405, ADS 1421, ADS 1425, ADS 1426, ADS 1428, ADS 1452, ADS 1456, ADS 1460, ADS 1463, Banjoemas 2219 (BO!), Banjoemas 2369 (BO!), Banjoemas 2436 (BO!), Banjoemas 2849 (BO!), R. Brinkman 75 (BO!), as well as ADS 303, ADS 311, ADS 346, ADS 350, ADS 360, ADS 446, ADS 1404, ADS 1406, and ADS 1419 that have broadly ovate to oblong leaves similar to *Selaginella devolii* H.M. Chang, P.F. Lu & W.L. Chiou, Blumea 56 (1): 21 (2011)

Note: It has a very diverse morphological characteristic. At the same place, two individuals can grow very different in shape, size and color; a brief observation will regard it as a distinct species. Figure 3.B shows two individuals of *S. ciliaris* growing side by side, but has a much different color, i.e. bright green and dark brown. Andrews (1990) has provided a clear description of some morphological variation of *Selaginella ciliaris* complex, which can be guided so as not separating this species without a clear and definite concept. It is also found a variant of *S. ciliaris* that have smaller size, more rounded leaves shape and tend to grow solitary, in the highland, that similar to *S. devolii*.

Selaginella intermedia (Blume) Spring, Bull. Acad. Brux. 10: 144 (1843) (Figure 3.D).

Synonym: *Lycopodioides atroviridis* (Wall.) Kuntze, Rev. Gen. Pl. 1: 825 (1891); *Lycopodioides plumea* (Spring) Kuntze, Rev. Gen. Pl. 1: 827 (1891); *Lycopodium atroviride* Hook. & Grev., Ic. Fil. 1: t. 39 (1831); *Lycopodium atro-viride* Wall., Cat., 6, no. 120 (1829); *Lycopodium cuspidatum* Hook., ms.; *Lycopodium furcatum* Roxb. ex Griff., Calc. Jour. Nat. Hist. 4: 475 (1844); *Lycopodium furcatum* Roxb. in Wall., Cat. 62, no. 120 (1829) [nomen]; *Lycopodium hymenophyllum* Roxb., ms.; *Lycopodium intermedium* Blume, Enum. Pl. Jav. 2: 269, no. 20 (1830); *Sclaginellabimarginata* Alderw., Bull. Jard. Bot. Buit. III, 5: 231 (1922); *Selaginella ascendens* Alderw., Bull. Jard. Bot. Buit. II, 11: 33 (1913); *Selaginella atrovirens* (Wall.) Spring, Flora, 21: 183 (1838); *Selaginella caudata* sensu F. von Muell., Descr. Notes Papuan PI. l (4): 75 (1876); *Selaginella consobrina* Alderw., Bull. Jard. Bot. Buit. III, 2: 178 (1920); *Selaginella cuprea* Ridl., Jour. Roy. As. Soc. Str. Br. 80: 152, no. 14 (1919); *Selaginella cuprea* var. *major* Ridl., Jour. Roy. Asiatic Soc., Str. Br., 80: 152 (1919); *Selaginella pentaphlebia* Alderw., Bull. Jard. Bot. Buit. III, 5: 228 (1922); *Selaginella plumea* f. *typica* Alderw., Mal. Fern Allies 102 (1915); *Selaginella plumea* Spring, Monogr. Lyc. II: 186 (136), no. 81 (1850); *Selaginella pseudovenulosa* Alderw., Bull. Jard. Bot. Buit. III, 5: 230 (1922); *Selaginella sibogana* Alderw., Bull. Jard. Bot. Buit. II, 11: 30 (1913); *Selaginella sibogana* f. *typica* Alderw., Bull. Jard. Bot. Buit. II, 16: 44 (1914); *Selaginella sibogana* var. *subbinervia* Alderw., Bull. Jard. Bot. Buit. II, 16: 44 (1914); *Selaginella similis* Kuhn, Forschungsr. Gazelle 4 (Bot.): 17 (1889); *Selaginella spurie-marginata* Alderw., Bull. Jard. Bot. Buit. III, 5: 231 (1922); *Selaginella trinervia* Spring, Bull. Acad. Brux. 10: 143, no. 62 (1843); *Selaginella vanvuureni* Alderw., Bull. Jard. Bot. Buit. II, 16: 44 (1914); *Selaginella venulosa* Alderw., Bull. Jard. Bot. Buit. II, 28: 47 (1918); *Selaginella wigmanii* Alderw., Bull. Jard. Bot. Buit. II, 11: 32 (1913); var. *dolichocentrus* K.M.Wong, Gard. Bull. Singapore 35 (2): 125 (1982) publ. 1983.

Perennial herb, suberect to ascending, glabrous, membranous, multiple branched at main stem, up to ca 70 cm long, pale green leaves. *Stems* suberect to ascending, cylindrical, glabrous, multiple branched toward apex, 3-5 mm wide (including leaves). *Rhizophores* present at basal stem, thick, cylindrical, originated from the ventral side of branching stem, ca. 1-1.5 mm in diam. *Leaves* dimorphic, arranged in 4 lanes (2 lateral, 2 median), sparsely arranged at main stem but closely arranged at the branches, vein single; *lateral leaves* oblong or ovate at main stem, lanceolate at branches, 4-5 mm long, 1.5-2.5 mm wide, apex acute or acuminate, asymmetrical, base rounded, margin dentate; *median leaves* smaller than the lateral ones, obovate, oblique at base, more or less symmetrically, 3-4 mm long, 1-2 mm wide, apex aristate, base rounded or cordate, margin dentate; *axillary leaves* lanceolate or ovate, 2-3 mm long, 1.5-2 mm wide, apex acute or obtuse, base rounded, margin dentate–denticulate, single vein nearly reaching the apex. *Strobilus* solitary, terminal, loosely, quadrangular, up to 5 cm long; sporophylls monomorphic.

Locality: Wonosobo (Mojotengah, Wonosobo-city)

Habitat and ecology: It was found on the steep cliff of small river banks and river banks shaded by lush of bamboo; at altitude of1012-1131m. asl.

Distribution: India (Kerala, Tamil Nadu), Indonesia, Malaysia (Peninsular, Mt. Kinabalu), Myanmar, Sri Lanka, Thailand, Vietnam,. In Indonesia: Java, Sumatra, Sulawesi.

Specimens observed: ADS 438, ADS 441, ADS 552, ADS 1104.

Note: This species is typical of the western Java highlands, and it is very rare in Central Java. In this study, it was only found two specimens in the southern slopes of Dieng mountains. In observation of BO collection, only one specimen that can be confirmed as *S. intermedia* of Central Java, i.e. from the northern slope of the Dieng mountain (Doro, Pekalongan; Docters v. Leeuwen 453, 7 June 1912). Not far from that place, it was also found this species (Paningaran, Pekalongan; ADS 1104, 28 August 2013).

Selaginella involvens (Sw.) Spring, Bull. Acad. Brux. 10: 136, no. 6 (1843) (Figure 3.E)

Basionym: *Lycopodioides involvens* (Sw.) Kuntze, Rev. Gen. Pl. 1: 826 (1891).

Synonym: *Lycopodioides pennula* (Desv.) Kuntze, Rev. Gen. Pl. 1: 827 (1891); *Lycopodium caulescens* Wallex Hook. & Grev., Enum. Fil. Hook. Bot. Misc. 2: 382 (1831); *Lycopodium circinale auct. non* L., Thunb., Fl. Jap. (Thunberg) 341 (1784); *Lycopodium involvens* Sw., Syn. Fil., 182: 50 (1806); *Lycopodium microstachyum* Desv. in Poir., Lamarck, Encycl. Suppl. 3: 554, no 97. [1813] 1814; *Selaginella bellula* Cesati, Atti Acad. Napoli, 7 (8): 36 (1876); *Selaginella caudispica* Alderw., Bull. Jard. Bot. Buit. II, 11: 35 (1913); *Selaginella caulescens* (Wall. ex Hook. & Grev.) Spring, Bull. Acad. Brux. 10 (1): 137 (1843); *Selaginella caulescens* f. *minor* Hieron., Bot. Tidsskr. 24: 114 (1901) [nomen; *Selaginella caulescens* f. *typica* Alderw., Mal. Fern Allies, 138 (1915); *Selaginella caulescens* var. *belulla* (Ces.) Hieron., Hedwigia 50: 4 (1910); *Selaginella caulescens* var. *brachypoda* Baker, Jour. Bot. 23: 24 (1885); *Selaginella caulescens* var. *gracilis* Bull., Cat. 164: 7 (1880); *Selaginella caulescens* var. *japonica* (Moore ex McNab) Baker, Jour. Bot. 23: 24 (1885); *Selaginella caulescens* var. *minor* Milde, Fil. Eur., 270 (1867); *Selaginella caulescens* var. *minor* Sandford, Man. Exot. Ferns & Selag., 258 (1882); *Selaginella caulescens* var. *subintegerrima* Spring, Monogr. Lyc. II: 159 (1850); *Selaginella homomorpha* Klotzsch ex Milde, Fil. Eur., 270 (1867); *Selaginella japonica* Hort., Proc. Roy. Hort. Soc. 4: 136 (1864); *Selaginella microstachya* (Desv.) Hieron., Elmer's Leaflets Phil. Bot. 6: 1988. (1913); *Selaginella microstachya* Warb., Monsunia 1: 104, 116, no. 62 (1900); *Selaginella pachystachys* Koidz., Acta Phytotax. Geobot. 4: 226 (1935); *Selaginella peltata* Presl; Bot. Bem. 152 (1844); *Selaginella pennula* (Desv.) Spring, Bull. Acad. Brux. 10: 137 (1843); *Selaginella pseudo-stauntoniana* Pamp., Nuovo Giorn. Bot. Ital N. S., 18: 103 (1911); *Selaginella warburgii* Hieron., Elmer's Leaflets Phil. Bot. 6: 1988. (1913).

Perennial herb, robust, erect or ascending, without branches on the lower half, with a creeping shallow stoloniferous rhizome, fan-shaped frond, terrestrial, epiphythic or xerophytic, up to ca. 60 cm tall, 3-4 cm wide (including leaves), yellowish green, rolling up when dry. *Stem* hard, but easily broken, two types, i.e.: erect main stem and creeping subterranean rhizome. Main stems branched from half upward, dendritic, fan-shaped, pinnately branched, stramineous, unbranched main stem 20-60 cm long, 1-1.5 mm in diam. with several dormant buds or leaves; in lower part, terete, not sulcate, glabrous; leafy main stem including leaves 4-6 mm wide at middle, ultimate branches 2-3 mm wide including leaves. *Rhizophores* restricted to creeping rhizomes, at intervals. *Leaves* on the rhizome scale like, monomorphic, ovate, ciliate, sessile, apex acute, apprised or recurved, colorless to pale yellow or brown; Leaves of the half basal main stem monomorphic, ovate, clasping, nearly asymmetrical, appressed, 1-2 mm long, 1-1.8 mm wide, apex acute to attenuate, base truncate, auriculate or not, margin serrate to serrulate but lacerate with spinose at the auricule, arose and long ciliate towards apex. Leaves on the branches dimorphic, arranged in 4 lanes (2 lateral, 2 median), vein single, reaching the apex; *lateral leaves* lanceolate to ovate, contiguous or overlapping, slightly ascending, asymmetrical, 1-2.5 mm long, 0.3-1.5 mm wide, slightly carinate, ciliate near base, apex attenuate or acuminate, base cuneate or oblique with auriculate, vein single always curved and pointing to abaxial side, having 2 significant grooves beside the vein, adaxial blade raised and forming two-main-vein, margin denticulate or laciniate but spinose at the auricule; *median leaves* ovate on the main stem but elliptical or laceolate to ovate on the top branch, asymmetrical, 1.5-3 mm long, 1-2.5 mm wide, apex acute, base rounded to subcordate, twisting to form miniature auricle at the base, single vein, obscure, 1-2 longitudinal groove (s) at the adaxial surface beside the vein of median leaves on the top branch, having 2-3 grooves at the abaxial surface on the top branch, 2 beside the vein and 1, less significant or absent, inside the midrib, margin entire to serrate, laciniate at most basal part of margin, concentrated spinose at the miniature-auricle base, minutely ciliate, pointing upwards; *axillary leaves* ovate to cordate on first forked site but lanceolate to ovate at following forked site, nearly symmetrical, 1-2.5 mm long, 0.5-1.5 mm wide, apex acute or attenuate, base subcordate or cordate, exauriculate, margin serrate but laciniate at basal part, minutely ciliate or denticulate. *Strobilus* are solitary, terminal, tetragonal, compact, up to 2 cm long; sporophylls monomorphic.

Habitat and ecology: It was found on the rocky steep cliff, the steep wall of small-river bank, the edge of irigation canal near tobacco fields, the cliff of rainwater canal near tea gardens and vegetable fields; at altitude of 351-1493 m. asl.

Locality: Banjarnegara (Kalibening), Purbalingga (Mrebet), Purworejo (Bruno), Temanggung (Ngadirejo, Parakan), Wonosobo (Kretek)

Distribution: Bhutan, Cambodia, China (common), India, Indonesia, Japan (Honshu, Shikoku, Kyushu, Ryukyu Isl.), Korea, Laos, Myanmar, Nepal, Palau Isl., Sri Lanka, Philippines, Taiwan, Thailand, Vietnam. In Indonesia: Java, Lesser Sunda Isl. (Flores), Kalimantan, Sulawesi.

Specimens observed: ADS 97, ADS 38, ADS 415, ADS 517, ADS 1095, ADS 1096, ADS 1116, ADS 1139, ADS 1443, ? (BO!).

Note: In the study site, *S. involvens* is collected in lowland until highland areas of rocky sites. In Central Java, it is also found in the highland of Mt. Merapi (Setyawan et al. 2012) and Mt. Lawu (Setyawan et al. 2013); and the lower mountain of Ngelanggeran (300-500 m) (Setyawan 2012).

Selaginella opaca Warb., Monsunia 1: 108, 122, no. 112 (1900) (Figure 3.F)

Synonym: *Selaginella cerebriformis* Alderw., Bull. Jard. Bot. Buit. II, 7: 32-33 (1912); *Selaginella mearnsii* Hieron., Elmer's Leaflets Phil. Bot. 6: 2018, no. 16 (1913); *Selaginella pervaga* Hieron., Elmer's Leaflets Phil. Bot. 6: 2021, no. 17 (1913).

Perennial fleshy herb, creeping, ascending, hanging, glabrous, up to ca. 2 m long, generally 0.5 m. *Stems* creeping to ascending, usually fertile branches alternate on long fleshy main stem, 40-80 (-200) cm long, 3-8cm wide (including leaves). *Rhizophores* at intervals stem, mostly near the base, originated from the dorsal side of stem, ca. 1-1.5 mm in diam. *Leaves* conspicuously dimorphic, but monomorphic on the main stem, oblong, asymmetrical, spaced farther apart than their width, midrib present. Leaves on the branches are dimorphic, arranged in 4 lanes (2 dorsal, 2 ventral), loosely arranged at long creeping stem but closely arranged at branches; *lateral leaves* ovate to oblong, asymmetrical, 2-5 mm long, 2-3 mm wide, apex acute, base rounded, margin serrulate to entire or minutely ciliate at the base, pointing outwards, imbricating at the ends of branches, vein single, obscure, not reaching the apex; *median leaves* ovate to oblong, asymmetrical, 1.5-3 mm long, 1-2 mm wide, apex caudate, base obliquely cordate or cordate, pointing upwards, imbricating at the ends of branches, margin serrulate or serrate, but entire at basal part, vein single not reaching the apex; *axillary leaves* ovate, entire, rounded or obtuse, symmetrical, 2.5-3.5 mm long, 1.5-2.5 mm wide, apex acute, base rounded, margin entire or serrulate at apical part. *Strobilus* solitary (rarely twin), terminal or lateral, tetragonal, up to more than 3.5 cm long; sporophylls monomorphic.

Locality: Banjarnegara (Batur, Wanayasa), Temanggung (Parakan), Pekalongan (Petungkriyono), Wonosobo (Garung, Kejajar, Kretek, Mojotengah).

Habitat and ecology: This species is typical of highland region. It is found on the banks of irrigation canals, water springs, creeks, roadside ditch, rainwater canals, river banks shaded by bamboo, waterfront of lake; in the fields of vegetables and medicinal plants, dry fields, and potato field; on the ground floor of pine forest, *Albisia lophanta* forest, and deforested areas; on the cliffs of roadsides village and main roads, vegetable fields and cemetery area; at altitude of993-2124m. asl.

Distribution: Indonesia, New Guinea, Philippines. In Indonesia: Java, Lombok, Moluccas (Ceram), Sumatra.

Specimens observed: ADS 76, ADS 84, ADS 86, ADS 88, ADS 89, ADS 171, ADS 181, ADS 183, ADS 189, ADS 193, ADS 199, ADS 208, ADS 216, ADS 224, ADS 238, ADS 246, ADS 253, ADS 283, ADS 305, ADS 308, ADS 339, ADS 340, ADS 347, ADS 355, ADS 357, ADS 362, ADS 373, ADS 374, ADS 399, ADS 402, ADS 411, ADS 434, ADS 437, ADS 442, ADS 444, ADS 520, ADS 538, ADS 542, ADS 543, ADS 550, ADS 1089, ADS 1091, ADS 1093, ADS 1094, ADS 1097, ADS 1099, ADS 1102, ADS 1119, ADS 1141, ADS 1408, ADS 1412, ADS 1415, ADS 1418, ADS 1422, ADS 1431, ADS 1433, ADS 1436, ADS 1438, ADS 1440, ADS 1445, ADS 1466, JA Lorzing 416 (BO!), R Brinkman 165 (BO!)

Note: This species is mostly cohabit with*S. remotifolia*; a habitat that covered by *S. opaca* is almost certainly covered by *S. remotifolia*, and vice versa. Both of these species can grow creeper, but *S. opaca* tend to ascending. *S. opaca* has a larger stem and juicy, while *S. remotifolia* has a small wiry stem with unlimited growth (when the main stem dies, the branches developing into a new individual). In the rainy season, the presence of *S. remotifolia* is more abundant due to its ability to grow in agricultural lands, while *S. opaca* is likely removed by the farmer. On the cliff of vegetable fields, a clump of this species can grow up to 2 m long and 1 m wide; they grow hanging on the cliffs of fields. In the Dieng plateau, it was also subjected to frost that caused the tops of branches bleach and die, but it will grow back new branches.

Selaginella ornata (Hook & Grev.) Spring, Bull. Acad. Brux. 10: 232 (1843) (Figure 3.G-H, Figure 4)

Basionym: *Lycopodium ornatum* Hook. & Grev., Enum. Fil. Hook. Bot. Misc. 3: 108 (1883).

Synonym: *Lycopodium atro-viride* Wall. ex Blume, Enum. Pl. Jav. 2: 269 (1830) (deser.) [non Wall. ex Hook. 1831]; *Selaginella balica* Alderw., Bull. Jard. Bot. Buit. III, 2: 181 (1920); *Selaginella blumei* Spring, Bull. Acad. Brux. 10. 143 (1843); *Selaginella brachystachya* var. *ornata* (Hook. & Grev.) Baker, Jour. Bot. 23: 180 (1885); *Selaginella duriuscula* Alston ex Knox; Trans. Bot. Soc. Edinb. 35: 269 (1950); *Selaginella fimbriata* Spring, Monogr. Lyc. II: 258, no. 198 (1850); *Selaginella fimbriata* var. *grandifolia* Alderw., Bull. Jard. Bot. Buit. III, 2: 183 (1920); *Selaginella fimbriata* var. *polyura* Warb., Monsunia 1: 110, 127 (1900); *Selaginella geniculata* var. *tomentosa* (Spring) Baker, Jour. Bot. 23: 121 (1885); *Selaginella javanica* Klotzsch ex Zoll., Syst. Verz. 1: 50 (1854); *Selaginella javanica* var. *gracilis* A. Br., ex Alderw., Bull. Jard. Bot. Buit. II, 7: 34 (1912); *Selaginella petrophila* Alderw., Bull. Jard. Bot. Buit. III, 5: 234 (1922); *Selaginella polita* Ridl., Jour. Fed. Mal. States Mus. 6: 202, no. 348 (1915); *Selaginella rabenavii* Hieron., Engl. & Prantl, Nat. Pfl. 1 (4): 694, no. 244. [1901] 1902; *Selaginella sclerophila* Alderw., Bull. Jard. Bot. Buit. III, 2: 182 (1920); *Selaginella subfimbriata* Alderw., Bull. Jard. Bot. Buit. II, 1: 26 (1911); *Selaginella subfimbriata* var. *koordersii* Alderw., Bull. Jard. Bot. Buit. III, 1: 26 (1911); *Selaginella subfimbriata* var. *polyura* (Warb.) Alderw., Bull. Jard. Bot. Buit II, 7: 34 (1912); *Selaginella subfimbricata* var. *backeri* Alderw., Bull. Jard. Bot. Buit. III. 1: 26 (1911); *Selaginella tomentosa* Spring, Monogr. Lyc. II: 231, no. 168 (1850); *Selaginella varians* Alderw., Bull. Jard. Bot. Buit. III, 5: 237 (1922); *Selaginella varians* f. *sciaphila* Alderw., Bull. Jard. Bot. Buit. III, 14: 237 (1922); *Selaginella xerophila* Alderw., Bull. Jard. Bot. Buit. III, 2: 182 (1920).

Perennial herb, creeping to ascending or sub erect, brittle, glabrous, 20-40 cm long, green to brownish (reddish) green. *Stems* oval, flattened, or sub quadrangular, sulcate or not, glabrous, branched from near base upward, green, stramineous or brownish, fragile, very easily broken,0.7-1.5 mm in diam. 1-3cm wide (including leaves). *Rhizophores* at intervals throughout length of creeping stem and branches, rarely on upper part, originated from ventral side in axils of branches, ca. 0.5-1 mm in diam, brownish or green. *Leaves* dimorphic, arranged in 4 lanes (2 dorsal, 2 ventral), densely arranged throughout the stem and imbricating at top of branches; *lateral leaves* oblong to oblong-falcate, denticulate to


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Figure 3. Species diversity of selaginellas in Southern Java and the surrounding; A. *S. aristata*, B. *S. ciliaris* (brown and green colors), C. *S. ciliaris*, D. *S. intermedia*, E. *S. involvens*, F. *S. opaca*, G. *S. ornata*, H. A variant of *S. ornata*, I. *S. plana*, J. *S. remotifolia*, K. *S. repanda*, L. *S. singalanensis*, M. *S. uncinata*, and N. *S. willdenowii*

Figure 4. A morphological variant of *S. ornata*; A. Habit. B. Adaxial stem, with lateral and median leaves. C. Abaxial stem, with lateral and axillary leaves. D. Lateral leaves. E. Median leaves. F. Axillary leaves. Note: Strobilus has not developed yet, but some leaves on the top branches clumped. Bar = 1 mm

dentate, exauriculate, asymmetrical, on main stem larger than on branches, distant or contiguous, spreading, 2-3 mm long, 1-2 mm wide, apex obtuse, acuminate or acute, and prickly tip, base rounded to truncate, basiscopic base decurrent, margin entire; acroscopic base rounded, overlapping stem and branches, margin denticulate in basal half, vein single not reaching the apex; *median leaves* contiguous or imbricate, ovate, denticulate to dentate, carinate, with arista often more than half the lamina length, asymmetrical, 1.5-3.5 mm long, 0.5-1.5 mm wide, apex acute or aristate, prickly tip, base obtuse or rounded, attenuate, vein single not reaching the apex, margin minutely denticulate to entire; *axillary leaves* ovate, lanceolate or subcordate, exauriculate, imbricating, asymmetrical, 1-1.5mm long, 0.5-1 mm wide, apex acute, base rounded, margin entire. *Strobilus* solitary or twins, terminal, compact, dorsiventrally complanate, bisymmetrical, upper-plane sporophylls longer than lower-plane sporophylls, up to more than 1 cm long; sporophylls strongly dimorphic, spores pale yellow to reddish brown.

Locality: Banjarnegara (Batur, Banjarmangu, Kalibening, Madukara, Pagentan, Sigaluh), Magelang (Kajoran), Pekalongan (Paninggaran), Purworejo (Kaligesing), Wonosobo (Garung, Kalikajar, Kaliwiro, Kepil, Kertek, Mojotengah, Sapuran, Selomerto, Watumalang, Wonosobo-city).

Habitat and ecology: It was found around the water springs and irrigation canals, river bank, small road ditch, footpath, vegetable and dry fields; in the pine forest, bamboo, albizia, guava and cloves fields; on the steep cliffs of vegetable fields, pine and agathis forest, rice fields, roadside of villages and main roads; creeks, small rivers and irrigation canals; on the cemetery hill; at altitude of222-1457 m. asl.

Distribution: Cambodia, India, Indonesia, Malaysia (Peninsular), Philippines, Thailand, Vietnam. In Indonesia:

Java, Kalimantan, Lesser Sunda Isl. (Bali, Flores, Lombok), Sumatra.

Specimens observed: ADS 76, ADS 77, ADS 84, ADS 86, ADS 88, ADS 89, ADS 164, ADS 174, ADS 176, ADS 187, ADS 191, ADS 196, ADS 205, ADS 213, ADS 221, ADS 235, ADS 243, ADS 249, ADS 279, ADS 300, ADS 303, ADS 334, ADS 335, ADS 342, ADS 350, ADS 352, ADS 357, ADS 368, ADS 369, ADS 394, ADS 397, ADS 406, ADS 429, ADS 432, ADS 437, ADS 439, ADS 514, ADS 531, ADS 535, ADS 536, ADS 542, ADS 1062, ADS 1064, ADS 1066, ADS 1067, ADS 1070, ADS 1072, ADS 1117, ADS 1120, CA Backer 21865 (BO!), Koorders 27142 (BO!).

Note: This species has a morphological appearance that is quite diverse and is generally characterized by a trunk which is very brittle. General appearance of *S. ornata* is brownish, but there are also variants of the green without brown nuances. In the study, *S. ornata* has a fairly wide distribution, ranging from the hilly area around the Wadaslintang reservoir in southern part of Wonosobo up to Dieng Mountains.

In this study, We have discovered a variant of *Selaginella* which the leaves (trophophylls) are round, either the lateral, median, or axillary leaves (apex long acuminate in this ones); it also occurs in the leaves at the tip of branches which expected to be developed into strobilus (sporophylls) (Figure 3.H and 4). The specimen is the only one, several intensive field surveys at the discovery site (Kaliwiro of Wonosobo District) and around was not able to find the same specimen, thus that the specimen was thought to be a variant of the species present at that location. The most similar species is *S. ornata*, although it was also found *S. aristata*, *S. ciliaris*, and *S. plana*.

Selaginella plana (Desv. ex Poir.) Hieron., Nat. Pflanzenfam. 1 (4): 703 (1901) (Figure 3.I)

Basionym: *Lycopodium planum* Desv. ex Poir., Lamarck, Encycl. Suppl. 3: 554, no. 98. [1813] 1814;

Synonym: *Lycopodium canaliculatum* var. *pallidius* Hook. & Arn., Bot. Beechey's Voy., 255 (1836)-1840; *Lycopodium durvillaei* Bory in Duprey, Voy. Bot. 1: (247, no. 12), t. 25 (1829) (excl. descr.); *Lycopodium nemorum* Desv., Ann. Soc. L. Paris, 6: 186, no. 83 (1827); *Lycopodium pellucidum* Desv. in Poir., Lamarck, Encycl. Suppl. 3: 552, no. 88. [1813] 1814; *Selaginella bellula* T. Moore, Gard. Chron. 11: 173, f. 25 (1879) [non Ces. 1876]; *Selaginella caudata* var. *durvillaei* (Bory) Spring, Monogr. Lyc. II: 141 (1850); *Selaginella caudata* var. *guichenotii* Spring, Monogr. Lyc. II: 140 (1850); *Selaginella durvillaei* (Bory) A. Br. ex Kuhn, Verh. K. K. Zool. Bot. Ges. Wine 19: 585, no. 131 (1869); *Selaginella guichenotii* (Spring) Hieron., Engl. & Prantl, Nat. Pfl. l (4): 701, no. 346. [1901] 1902; *Selaginella herpocaulos* var. *acuminata* Alderw., Bull. Jard. Bot. Buit. III, 2: 185 (1920); *Selaginella inaequalifolia* var. *perelegans* (Moore) Baker, Jour. Bot. 23: 4 (1885); *Selaginella perelegans* T. Moore, Gard. Chron. 11: 533 (1879).

Common name: Asian spike moss

Perennial herb, stout, sub erect to ascending with stoloniferous rhizome, without branches on the lower part, ascending from a subterranean trailing base, glabrous, fan-shaped, green. *Stems* suberect to ascending, branches on the upper half, up to 80-100 cm long, 3-10 cm wide (including leaves);subterranean stems (rhizome) shallowly radiating. *Rhizophores* present sometimes at the branching stem, originated from the dorsal side of stem at the branch site, ca. 1-1.5 mm in diam. *Leaves* on the lower part and main stem are monomorphic, well spaced, appressed, 1.5-3 mm long, 1-2 mm wide, upper part slightly spreading, ovate, asymmetrical, apex acuminate or acute, but rounded tip, margin translucent, entire. Leaves on the branches are dimorphic, arranged in 4 lanes (2 dorsal, 2 ventral), loosely arranged at lower stem but closely arranged at branches; *lateral leaves* are oblong to ovate, asymmetrical, 2-4.5 mm long, 2-3 mm wide, apex acuminate-acute to rounded, but rounded tip, sessile, vein single, obscure, not reaching the apex, base truncate and rounded, upper base with a spur-like lobe which overlaps the stem, margin transparent, entire; *median leaves* are ovate to oblong, asymmetrical, 1.5-3 mm long, 1-2 mm wide, apex acuminate-acute to obtuse-rounded, but rounded tip, sessile, vein single, obscure not reaching the apex, base truncate and rounded, margin transparent, entire; *axillary leaves* are ovate or obovate-oblong, asymmetrical, 2.5-3.5 mm long, 1.5-2.5 mm wide, apex acute to slightly acuminate, minutely ciliate, base rounded, margin entire. *Strobilus* are solitary, terminal, tetragonal, up to more than 3 cm long; sporophylls monomorphic.

Locality: Banjarnegara (Banjarmangu, Banjarnegara-city, Bawang, Kalibening, Madukara, Pagedongan, Pagentan, Sigaluh, Wanadadi), Kebumen (Buayan, Gombong, Karanggayam, Karangsambung, Kebumen-city, Prembun, Sempor), Kulonprogo (Girimulyo), Magelang (Kajoran), Pekalongan (Paninggaran), Purbalingga (Bobotsari, Karangmoncol, Rembang), Purworejo (Kaligesing), Temanggung (Bejen, Candiroto, Jumo, Kandangan), Wonosobo (Kaliwiro, Kepil, Kertek, Sapuran, Selomerto, Wadaslintang, Wonosobo-city)

Habitat and ecology: It was found around the water spring, riverside creeks and small rivers, small and large irrigation canals, fish pond, road ditch; roadside village and main road, home garden; dry fields and paddy fields; in the coffee plantations, salak, coconut, albizia, bamboo; in the pine, agathis and mixed forests; cliffs of the rivers bridges, water springs, creeks and small rivers, irrigation canals; rock cliffs and moist crevices of limestone, cliffs of the roadside villages and main street, cliff in the home garden, salak, acacia, albizia, bamboo, teak plantations, pine, agathis, mixed forests, dry fields vegetable and rice field; among the rocks and buildings around the tomb; on agricultural land, is considered a weed species that tend to be cleaned; at altitudes of 19-1020 m. asl.

Distribution: Indonesia, Malaysia (Peninsular). In Indonesia: Java, Lesser Sunda Isl. (Bali, Flores, Sumbawa, Solor, Timor), Sulawesi, Sumatra, Moluccas (Ambon, Banda, Buru, Ceram, Kei Isl., Ternate). Introduced to Asia: India, Philippines,Taiwan. Introduced to America: Barbados, Brazil, British Guyana, Colombia, Costa Rica, Dominica, Ecuador, Honduras, Jamaica, Martinique, Panama, Puerto Rico, USA (Florida), St. Kitts, St. Thomas, Trinidad. Introduced to Africa: Tanzania.

Specimens observed: ADS 74, ADS 75, ADS 79, ADS 80, ADS 81, ADS 82, ADS 98, ADS 99, ADS 100, ADS 169, ADS 170, ADS 172, ADS 173, ADS 174, ADS 175, ADS 177, ADS 178, ADS 179, ADS 184, ADS 185, ADS 209, ADS 210, ADS 214, ADS 217, ADS 225, ADS 226, ADS 237, ADS 241, ADS 242, ADS 245, ADS 251, ADS 252, ADS 312, ADS 313, ADS 315, ADS 318, ADS 321, ADS 325, ADS 326, ADS 334, ADS 341, ADS 348, ADS 361, ADS 365, ADS 368, ADS 371, ADS 377, ADS 397, ADS 400, ADS 403, ADS 406, ADS 408, ADS 523, ADS 535, ADS 539, ADS 736, ADS 737, ADS 739, ADS 741, ADS 1054, ADS 1056, ADS 1058, ADS 1060, ADS 1062, ADS 1064, ADS 1066, ADS 1068, ADS 1069, ADS 1071, ADS 1072, ADS 1078, ADS 1079, ADS 1082, ADS 1086, ADS 1088, ADS 1090, ADS 1092, ADS 1101, ADS 1103, ADS 1107, ADS 1118, ADS 1130, ADS 1132, ADS 1133, ADS 1135, ADS 1136, ADS 1137, ADS 1140, ADS 1424, ADS 1427, ADS 1429, ADS 1430, ADS 1432, ADS 1434, ADS 1439, ADS 1441, ADS 1444, ADS 1446, ADS 1447, ADS 1448, ADS 1451, ADS 1454, ADS 1455, ADS 1457, ADS 1458, ADS 1459, ADS 1461, ADS 1462, ADS 1464, ADS 1465, ADS 1467, Banjoemas 2117 (BO!), Banjoemas 2480 (BO!), R. Brinkman 28 (BO!), ? 790 (BO!).

Notes: It is originally low-lying selaginellas species that most widely spreading, and 1020 m is the highest point reached in the study area. To date, the highest point that can be reached in Java is 1150 m. asl. in Mt. Lawu (Setyawan et al. 2013) and 1200 m. asl. in Mt. Merapi (Setyawan et al. 2012).

Selaginella remotifolia Spring, Miq. Pl. Jungh. 3: 276, no. 5 (1854) (Figure 3.J)

Basionym: *Lycopodioides remotifolia* (Spring) H.S. Kung, Fl. Sichuanica 6: 65-67 (1988)

Synonym: *Selaginella involucrata* Warb., Monsunia 1: 102, 113, no. 28 (1900); *Selaginella japonica* Miq., Ann. Mus. Bot. Ludg. Bat. 3: 185 (1867); *Selaginella kelungensis* Hayata; Icon. Pl. Formosan 7: 97-98 (1918); *Selaginella kraussiana auct. non* (Kunze) A. Braun: French. & Sav., Enum. Pl. Jap. 2 (1): 200 (1877); *Selaginella remotifolia* Spring var. *japonica* (Miq.) Koidz., Acta Phytotax. Geobot. 4: 228 (1935); *Selaginella utchinensis* Koidz., Acta Phytotax. Geobot. 4: 229 (1935)

Perennial herb, wiry, creeping, glabrous, branched from near base upward, several fertile branches alternate on long main stem, not obviously articulate, up to 100 cm long, green to stramineous stem. *Stems* creeping, oval or terete, sulcate, stramineous, 0.5-1.5cm wide (including leaves). *Rhizophores* at the branching stem, throughout length of creeping stem and branches, originated from the dorsal side in axil of stem branches, ca. 0.5 mm in diam. *Leaves* on the main stem monomorphic, decussate, lanceolate, acuminate, asymmetrical, spaced farther apart than their width, midrib present. Leaves on the branches dimorphic, arranged in 4 lanes (2 dorsal, 2 ventral), loosely arranged at the long creeping main stem but closely arranged at branches, those on main stems slightly larger than those on branches; *lateral leaves* contiguous, lanceolate to ovate, distant or approximate, spreading, asymmetrical, 1.5-3 mm long, 1-2 mm wide, apex acute to acuminate, vein single, obscure not reaching the apex, base rounded, margin serrulate, entire or minutely ciliate, denticulate, pointing outwards, fertile branches erect; *median leaves* lanceolate to ovate, asymmetrical, 1.5-3 mm long, 0.5-1 mm wide, not carinate, apex attenuate acuminate, or caudate, base obliquely cordate or cuneate, uni-auriculate, approximate or imbricate at ends of branches, margin serrulate or serrate, but sub entire or minutely denticulate at abaxial medium and basal part, vein single; *axillary leaves* ovate, broadly ovate, entire, rounded or obtuse, symmetrical, 1.5-2.5 mm long, 1-1.5 mm wide, apex acute, base exauriculate, margin entire, slightly denticulate or loosely serrulate at apical part. *Strobilus* solitary, terminal or lateral, compact, tetragonal, up to more than 0.5-2 cm long; sporophylls monomorphic.

Locality: Banjarnegara (Batur, Pagentan, Pejawaran, Wanayasa), Pekalongan (Petungkriyono), Temanggung (Parakan), Wonosobo (Garung, Kejajar, Kretek, Mojotengah, Watumalang, Wonosobo-city)

Habitat and ecology: It was found on the banks and cliffs of creeks, small rivers, irrigation canals, ditch of the vegetable and tobacco fields and mixed forest, waterfront of lake, steep cliff of waterfall; on the intensive agricultural field shaded by paranet; the steep cliffs of dry field, on the edge of field and among vegetable plants, near the tea garden; in the agroforesty of albizia; *Paraserianthes lophantha* forest and deforested forests (reforestation); roadside and cliffs of the dirt road, villages road, and main roads, cliff near residential area; at altitudes of 1150 -2070 m. asl.

Distribution: China (common), Indonesia, Japan (Hokkaido, Honshu, Kyushu, Shikoku, Ryukyu Isl.), Korea, Malaysia, Myanmar, New Guinea, Philippines, Taiwan. In Indonesia: Java, Sumatra.

Specimens observed: ADS 21, ADS 23, ADS 40, ADS 42, ADS 83, ADS 85, ADS 87, ADS 90, ADS 93, ADS 94, ADS 95, ADS 96, ADS 180, ADS 192, ADS 198, ADS 256, ADS 257, ADS 259, ADS 260, ADS 261, ADS 263, ADS 264, ADS 282, ADS 300, ADS 304, ADS 352, ADS 356, ADS 412, ADS 416, ADS 436, ADS 439, ADS 443, ADS 518, ADS 544, ADS 547, ADS 553, ADS 555, ADS 556, ADS 558, ADS 559, ADS 562, ADS 565, ADS 566, ADS 1122, ADS 1124, ADS 1127, ADS 1125, ADS 1128, ADS 1057, ADS 1420, ADS 1409, ADS 1413, ADS 1416, Ruttner 276 (BO!), W Meijer 2755 (BO!).

Note: This species has the same habitat as *S. opaca*, but it is more easily found in farm fields, due to the small size and creeping stems that make it difficult to be eradicated and become weeds in the uplands; even it can compete with the ground grass.

Selaginella repanda (Desv. ex Poir.) Spring, Gaud. Voy. Bonite Bot. 1: 329 (1846) (Figure 3.K)

Basionym: *Lycopodium repandum* Desv. ex Poir.; Lamarck, Encycl. Suppl. 3: 558. [1813] 1814;

Synonym: *Lycopodioides barbata* (Spring ein. Baker) Kuntze, Rev. Gen. Pl. 1: 826 (1891); *Lycopodioides compta* (Hand.-Mazz.) H.S.Kung, Fl. Sichuanica 6: 76

(1988); *Lycopodium barbatum* Kaulf., Enum. Fil. 18 (1824); *Lycopodium plumosum* Dill., Hist. Musc., t. 66, f. 10 (1714); *Lycopodium radicatum* Hook. & Grev. Bot. Misc. 2: 397 (1831); *Lycopodium semicordatum* Wall. ex Hook. & Grev., Enum. Fil., Hook. Bot. Misc. 2: 396, no. 158 (1831); *Lycopodium tetragonostachyum* var. *major* Hook. & Grev., Enum. Fil., Hook. Bot. Misc. 2: 389 (1831); *Lycopodium tetragonostachyum* Wall., Cat. no. 124 (1829), ex Hook. & Grev., Enum. Fil., Hook. Bot. Misc. 2: 389, no. 129. (1831); *Sclaginella plumosa* var. *hamiltoni* Baker; Jour. Bot. 21: 145 (1883); *Selaginella barbata* (Kaulf.) Spring, Bull. Acad. Brux. 10: 226, no 101 (1843); *Selaginella ceratocaulos* Alderw., Bull. Jard. Bot. Buit. II, 16: 41 (1914); *Selaginella ceratocaulos* f. *madurensis* Alderw., Bull. Jard. Bot. Buit. II, 13: 434 (1914); *Selaginella ceratocaulos* f. *typica* Alderw., Mal. Fern Allies 239 (1915); *Selaginella ceratocaulos* var. *kangeanensis* Alderw., Bull. Jard. Bot. Buit. III, 5: 232 (1922); *Selaginella compta* Hand.-Mazz., Sumb. Sin. 6: 9 (1929); *Selaginella ganguliana* Dixit, Bull. Bot. Surv. India 26: A-I (1984) [fide Fraser-Jenkins (1997)]; *Selaginella henryi* Koidz., Fl. Symb. Orient. Asiat. 85 (1930); *Selaginella implexa* Scott, Jour. Agr. Hort. Soc. India N. S. 1 (2): 262 (1868); *Selaginella implexa* Scott, List Higher Crypt., 64 (1868); *Selaginella mongholica auct. non* Ruhr.: A. Henry, trans. Asiat, Soc, Japan 24 (Suppl.): 117 (1896); *Selaginella plumosa* f. *tetragonostachya* (Wall.) Haines, Bot. Brit. & For (Bot. Bih. Or.), p. 1224 (1924); *Selaginella plumosa* var. *radicata* (Hook. & Grev.) Warb., Monsunia I: 102 (1900); *Selaginella pyrrhopus* Spring, Monogr. Lyc. II: 114-115 (1850); *Selaginella radicata* (Hook. & Grev.) Spring, Mem. Acad. R. Sci. Belgique 24 (2): 114 (1850); *Selaginella subcaulencens auct. non* Hayata: Tak. Ito, Icon. Taiwan Pl. Suppl. Vol. 4 (1928); *Selaginella suberecta* Baker, Jour. Bot. 22: 245, no. 146 (1884); *Selaginella tetragonostachya* (Wall.) Spring, Bull. Acad. Brux. 10: 234, no. 163 (1843); *Selaginella vaginata* Spring, Mem. Acad. R. Sci. Belgique 24: 87 (1850).

Perennial (or annual) herb, terrestrial or epiphytic, ascending from decumbent base, ciliate, brownish (reddish) green leaves with reddish green to brown stem, scabrous; multiple branched at creeping stem, forming ascending dendritic, fan-shaped stem. *Stems* stramineous, oval or terete, 2-forked, consists of two types, i.e. creeping on the ground (vegetative), multiple branched, forming diffuse mats, up to ca. 45 cm long, 3-4 mm wide (including leaves); or decumbent to ascending (generative), dendritic, fan-shaped, especially at the mature ones, ca. 10-30 cm long, 4-5 mm wide (including leaves). *Rhizophores* present at creeping stem (rhizome), and sometimes also on base of ascending ones, originated from ventral side in axils of branching stem, ca. 1 mm in diam. *Leaves* dimorphic, those on main stem larger than those on branches, arranged in 4 lanes (2 lateral, 2 median), sparsely arranged at main stem but imbricate at the branches, vein single; *lateral leaves* asymmetrical, approximate, spreading, oblong-falcate, 2-3 mm long, 1-1.5 mm wide, apex acute, base rounded, margin ciliolate, minutely denticulate to apex, single vein; *median leaves* smaller than the lateral ones, asymmetrical, approximate, obliquely ovate at branches, 1-2 mm long, 0.5-1 mm wide, not carinate or slightly carinate, apex long acuminate to shortly aristate, base obliquely subcordate, not peltate, margin long ciliate (denticulate to apex), reflexed, parallel to axis; *axillary leaves* ovate or ovate-lanceolate, less or more symmetrical 2-3 mm long, 1-1.5 mm wide, apex acute or acuminate, base rounded-obtuse, exauriculate, margin ciliolate, single vein. *Strobilus* solitary, terminal, compact, sub tetragonal or sub-complanate, 2-6 mm long; sporophylls submonomorphic or sometimes dorsal sporophylls longer, spores orange-brown to bright yellow.

Locality: Kebumen (Buayan, Karanggayam, Prembun, Sempor), Purbalingga (Bobotsari)

Habitat and ecology: It is rare and usually found on the humid rocky cliffs, such as small river bank, moist crevices of limestone, sandstone cliffs of main road, roadsides and riverbanks shaded by bamboo, near ditch in the albizia fields; on the walls of cemetery and gravestones; at altitudes of 19-214 m. asl.

Distribution: Bhutan, Cambodia, China (common), India (Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Karnataka, Sikkim, Tamil Nadu), Indonesia, Laos, Malaysia (Perlis), Myanmar, Nepal, Philippines, Taiwan, Thailand, Vietnam. In Indonesia: Java, Lesser Sunda Isl. (Sumbawa, Timor), Sumatra.

Specimens observed: ADS 322, ADS 323, ADS 1053, ADS 1067, ADS 1080, ADS 1081, ADS 1138, ADS 1449, ADS 1450, and ADS 1453.

Note: In Java, It is thought to be typical species of rocky lowland to moderate hills, and dry climates (xerophytic).Spores are usually bright yellow, which distinguishes it from other selaginellas.

Selaginella singalanensis Hieron., Hedwigia 50: 18, no. 12 (1910) (Figure 3.L)

Synonym: *Selaginella flabelliformis* Alderw., Bull. Jard. Bot. Buit. III, 2: 181 (1920); *Selaginella junghuhniana* Spring, Miq. Pl. Jungh. III: 276, no. 7 (1854); *Selaginella modica* Alderw., Bull. Jard. Bot. Buit. Ill, 5: 236 (1922)

Perennial herb, creeping, tender, in humid environments, growing all year round but mostly rainy season, yellowish green in general appearance. *Stems* creeping, irregular lateral branches, rooting at intervals, attached to the ground, very soft and very thin, 20-25 cm long, 1-3cm wide (including leaves).*Rhizophores* at branching stem, originated from the dorsal side of stem at the branch site, ca. 0.5 mm in diam. *Leaves* are dimorphic, very soft, arranged in 4 lanes (2 dorsal, 2 ventral), densely arranged at thorough stem and imbricating at top of branches; *lateral leaves* are oblong, imbricating, asymmetrical, 1.5-2.5 mm long, 0.5-1.5 mm wide, apex acute, vein single not reaching the apex, base rounded, margin entire; *median leaves* oblong-ovate, dentate, exauriculate, imbricating, asymmetrical, 0.5-1.5 mm long, 0.5 mm wide, apex acute, base rounded, margin entire, vein single not reaching the apex; *axillary leaves* ovate, exauriculate, imbricating, asymmetrical, 0.5-1.5mm long, 0.5 mm wide, apex acute, base rounded, margin entire. *Strobilus* solitary, terminal, loosely, bisymmetrical, upper-plane, up to more than 1 cm long; upper-plane sporophylls longer than lower-plane.

Locality: Temanggung (Parakan)

Habitat and ecology: Locally not rare, also present somewhere else in the mountains. Prostate and at regular intervals rooting. The bottom lager is usually pressed to the ground. Usually often and regularly branching, 10- 30 cm long, often green, yellow or red brown, probably due to the drought. Very different [unreadable] spore careering parts which are not very visible; at altitude of1800 m. asl. (from the herbarium label of JA Lorzing 490, 18 June 1912 (BO!).

Distribution: Indonesia (Java, Sumatra)

Specimens observed: ADS 467, JA Lorzing 490 (BO!).

Selaginella uncinata (Desv. ex Poir.) Spring, Bull. Acad. Roy. Sci. Brux. 10: 141. 1843. (Figure 3.M)

Basionym: *Lycopodium uncinatum* Desv. ex Poir., Lamarck, Encycl. Suppl. 3: 558 (1814).

Synonym: *Lycopodioides uncinata* (Desv.) Kuntze, Rev. Gen. Pl. 1: 825 (1891); *Lycopodium aristatum* Roxb., Hort. Beng., 75 (1824) [nomen, non Hook. & Bak]; *Lycopodium caesium* Hort. ex Anon., Ann. Hort. Soc (1847): 361, descr; *Lycopodium dilatatum* Hook. & Grev., Enum. Fil., Hook. Bot. Misc. 2: 394, no 149 (1831); *Selaginella aristata* (Roxb.) Scott; List Higher Crypt., 64 (1868); *Selaginella caesia* Hort. ex A. Br., Ind. Sem. Hort. Berol. 23 (1860); *Selaginella caesia* Kunze, Linnaea 20: 2 (1847) [nom. nud.], *Selaginella caesia* var. *violacea* Hort. ex A. Br., Ind. Sem. Hort. Berol. 23 (1860); *Selaginella eurystachya* Warb., Monsunia 1: 105, 119, no. 79 (1900).

Common name: blue spike moss

Perennial herb, wiry, pliant, creeping, fan-shaped branching, up to ca. 75 cm long, forming diffuse mats. *Stems* are long-creeping, branched, 3-forked branching, flat or hanging, not articulate, glabrous with a wiry, pliant main stem, 1-1.5 mm diam., 4-5 cm wide (including leaves). *Rhizophores* are axillary at intervals, mostly at main stem base near to the ground, 0.3-0.5 mm diam. *Leaves* are dimorphic, delicate, papery, iridescent, green to metallic blue-green, single vein, arranged in 4 lanes (2 lateral, 2 median);*lateral leaves* are distant, ovate to oblong, asymmetrical, 2-3mm long, 1.5-2 mm wide, basiscopic base with small auricle, acroscopic base overlapping stem; base rounded or cordate, apex acute to obtuse, margins conspicuously transparent, entire, single vein reaching the apex, keeled; *median leaves* are ovate to lanceolate, asymmetrical, 2.5-4 mm long, 1.5-2 mm wide, base auriculate, apex acute to acuminate, margins transparent, entire, single vein; *axillary leaves* are present at branch forks, inserted at the ventral side of the stem, broadly ovate, more equally sided,3-4 mm long, 2-3 mm wide, single vein, base rounded to subcordate, apex acute to acuminate, margin transparent, entire, keeled. *Strobilus* is solitary, terminal, tetragonal, up to ca. 0.5-2 cm long and distinctly 4-keeled.

Habitat and ecology: It was only found once on the steep cliffs near the small river banks, covered/under the bushes; at altitude of 708 m. asl. In the experimental garden, it can grow well, forming a thick mass in the floor and walls, hanging on hanging pots, fill the empty floor between the potted plants, very suitable as land cover plant in moist and shaded places. It is very easy to grow and multiply and shows attractive metallic blue-green color in the shade and faded in sun exposed places; very suitable for ornamental plant.

Locality: Wonosobo (Wonosobo-city)

Distribution: China (common), NE-India, Japan, Vietnam. Introduced to India (West Bengal), Indonesia (Java), Paraguay, Taiwan, USA (Georgia, Louisiana, Florida).

Specimens observed: ADS 286, ADS 454, ADS 455.

Note: The discovery of *S. uncinata* in the capital district of Wonosobo is a new record for Central Java. The only *S. uncinata* sheets of Herbarium Bogoriense (BO) is the collection of Junghuhn no. 1239 (det. 1910) which may be obtained from the collections of the Bogor Botanical Gardens. The botanical garden has brought a variety of selaginellas from outside Java and abroad, but almost all the introduced selaginellas have died and have not been naturalized. However, in the upland botanical gardens near it, the Cibodas Botanical Gardens (1350 m), this species was found either planted or growing wild. The presence of *S. uncinata* in this city is presumably introduced as an ornamental plant from southern China, because the Chinese have lived in the city since hundreds of years ago (Setyawan 2012). Besides, the species of Cibodas Botanic Garden can easily be grown in the Depok city (100 m), indicating that it is not species specific to highlands (data not shown).

Selaginella willdenovii (Desv. ex Poir.) Baker; Gard. Chron., 783, 950 (1867) (Figure 3.N)

Basionym: *Lycopodium willdenovii* Desv. ex Poir., Lamarck, Encycl. Suppl. 3: 540, 552, no. 87. [1813] 1814.

Synonym: *Lycopodioides caespitosa* (BI.) Kuntze, Rev. Gen. Pl. 1: 826 (1891); *Lycopodioides willdenowii* (Desv.) Kuntze, Rev. Gen. Pl. 1: 827 (1891); *Lycopodium bicolor* Hort. ex Warburg, Monsunia, 1: 129 (1900); *Lycopodium caespitosum* Blume, Enum. Pl. Jav., 2: 270, no. 23 (1830); *Lycopodium laevigatum* Willd., Sp. Pl. 5: 45, no. 66 (1810); *Selaginella altissima* Kaulf. ex W. Lauche., Verz. August. Gart. 8 (1856) nomen; *Selaginella arborea* Hort. ex A. Br., App. Ind. Sem. Hort. Berol. 22 (1857); *Selaginella arborea* Hort. ex Dippel, Amtl. Ber. Deutsch. Naturf. 39 in Giessen (1864), 145, t. 4, f. 16. 1865; *Selaginella arborescens* Hort. ex Russow, Mem. Acad. Imp. Petersb. VII, 19: 176 (1873); *Selaginella caespitosa* (Blume) Spring, Bull. Acad. Brux. 10: 140, no. 33 (1843); *Selaginella denuana* Alderw., Bull. Jard. Bot. Buit. II, 16: 55 (1914); *Selaginella laevigata* (Willd.) Spring, Mart. Fl. Bras. l (2): 125, no. 13 (1840); *Selaginella uncinatum* var. *arborea* Mett., Fil. Hort. Lips. 124 (1856); *Selaginella willclenovii* var. *punctulata* Alderw., Mal. Ferns & Fern Allies, Suppl. I, Corn: 40 (1917); *Selaginella willdenovii* f. *typica* Alderw., Mal. Ferns & Fern Allies, Suppl. I, Corr.: 40 (1917); *Selaginella willdenovii* var. *caesia-arborea* (Hort.) Hieron. ex R. Bonap. Notes Pterid., 2: (1915).

Common name: Vine spike-moss

Perennial herb, very stout, pliant, viselike or shrub like, creeping, climbing, scandent, scrambling or hanging, fan-shaped frond, 3-4 m long (in experimental garden, regularly pruned, it can be longer); metallic blue-green iridescent leaves in shaded places. *Stems* are creeping, climbing, hanging, branched from near base upward, 4-5-forked branching, not articulate, glabrous with a large, stout main stem, stramineous or reddish, 2.5-8 mm diam., 50-80 cm wide (including frond leaves). *Rhizophores* borne on upper side or underside of stems throughout stem length, mostly at stem base or stem near to the ground, with some spine like protuberances at base (as well as at axes of stems), more rarely in the climbing or hanging ones, 3-4 mm diam. *Leaves* are conspicuously dimorphic, delicate, papery, iridescent, green to metallic blue-green, single vein, arranged in 4 lanes (2 lateral, 2 median), but somewhat spirally in stem with monomorphic leaves; *lateral leaves* distant or approximate, ovate to oblong-falcate, obviously larger than those on branches, asymmetrical, 3-5mm long, 2-3 mm wide (leaves on tertiary stems much smaller);basiscopic base rounded with small auricle, acroscopic base overlapping stem with whitish, long, downward-curving auricle; apex acute, rounded or obtuse, base asymmetrically rounded, margins conspicuously transparent (whitish and shiny when dry), entire, single vein, flattened; *median leaves* falcate-lanceolate or oblique-ovate, imbricate to overlapping at leaf apex, asymmetrical, those on main stems obviously larger than those on branches, 2-3 mm long, 1-2 mm wide, apex acute, acuminate or obtuse, base auriculate, outer auricle larger than inner, margins transparent, entire, single vein; *axillary leaves* present at branch forks, inserted at the dorsal side of the stem, obviously larger than those on branches, oblong, elliptic to broadly ovate, more equally sided,3-4 mm long, 2-3 mm wide, single vein, apex acute, base rounded to subcordate, biauriculate, margin serrulate. *Strobilus* solitary, terminal, compact, tetragonal, up to ca. 0.5-4 cm long; sporophylls monomorphic.

Locality: Temanggung (Temanggung-city)

Habitat and ecology: Shady slope of gorge among brushwood. Locally not rare. 450 m. asl. (from herbarium label of JA Lorzing 2510, March 1912 (BO!).

Distribution: Australia (Queensland), Cambodia, India (Arunachal Pradesh, Assam, Himalayas), Indonesia, Laos, Malaysia (Peninsular), Myanmar, Philippines, Singapore, Thailand, Vietnam. In Indonesia: Java, Lesser Sunda Isl., Sumatra. Introduced to: Brazil, Colombia, Jamaica, Panama, Puerto Rico, USA (Florida).

Specimens observed: ADS 460, JA Lorzing 2510 (BO!)

Note: The first published specific epithet is *willdenovii*, an error spelling, now, the accepted specific epithet is *willdenowii*, the full accepted name is *Selaginella willdenowii* (Desv. ex Poir.) Baker (ITIS 2016). Intensive field studies are currently unable to confirm the presence of *S. willdenowii* in the study site, although the herbarium records stated that the species is "locally not rare" in 1912. Extensive research across Java and observations of herbarium collections of BO indicate that the species is widely grown in the western part of Java, especially in the highlands of Bandung to Ujung Kulon (ADS, 2007-2013, pers. obs.). Environmental changes may have led to the scarcity of this species in Central Java. This species had also found on the southern slope of Mount Slamet, Central Java (ARS 103, 17 March 2004 (BO!). Transplant experiments in Wonosobo indicate that this species can grow well in the garden experiment, and showed blue-green iridescent that attractive as an ornamental plant, but during the five years of the experiment, there was no naturalized in the wild. The iridescence is caused by the effect of thin film interference filters in the leaf epidermis. The convex epidermal cells may focus light into a single, distal, large chloroplast, possibly adaptations for the improvement of photosynthetic efficiency at the forest floor level (Lee 1977).

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REFERENCES

Alderwereld van Rosenburgh CRWK van. 1915a. Malayan fern allies. Department of Agriculture, Industry, and Commerce. Batavia.

Alderwereld van Rosenburgh CRWK van. 1915b. New or interesting Malay ferns 7. Bull Jard Bot Buitenzorg 2 (20): 1-28.

Alderwereld van Rosenburgh CRWK van. 1916. New or interesting Malay ferns 8. Bull Jard Bot Buitenzorg 2 (23): 1-27.

Alderwereld van Rosenburgh CRWK van. 1917. New or interesting Malay ferns 9. Bull Jard Bot Buitenzorg 2 (24): 1-8.

Alderwereld van Rosenburgh CRWK van. 1918. New or interesting Malay ferns 10. Bull Jard Bot Buitenzorg 2 (28): 1-66.

Alderwereld van Rosenburgh CRWK van. 1920. New or interesting Malay ferns 11. Bull Jard Bot Buitenzorg 3 (2): 129-186.

Alderwereld van Rosenburgh CRWK van. 1922. New or interesting Malay ferns 12. Bull Jard Bot Buitenzorg 3 (5): 179-240.

Alston AHG. 1935a. The *Selaginella* of the Malay Islands: I. Java and the Lesser Sunda Islands. Bull Jard Bot Buitenzorg 3 (13): 432-442.

Alston AHG. 1935b. The Philippines species of *Selaginella.* Philippines J Sci 58: 359-383.

Alston AHG. 1937. The *Selaginella* of the Malay Islands: II. Sumatra. Bull Jard Bot Buitenzorg 3 (14): 175-186.

Alston AHG. 1940. The *Selaginella* of the Malay Islands: III. Celebes and the Moluccas. Bull Jard Bot Buitenzorg 3 (16): 343-350.

Alston, AHG. 1934. The genus *Selaginella* in the Malay Peninsula. Gard Bull Strait Settl 8: 41-62.

Andrews SB. 1990. Ferns of Queensland. Queensland Department of Primary Industry, Brisbane.

BPS Banjarnegara. 2011. Banjarnegara in Figures 2011. District of Banjarnegara and BPS Banjarnegara, Banjarnegara [Indonesian]

BPS Kebumen 2011. Kebumen in Figures 2011. District of Kebumen and BPS Kebumen, Kebumen [Indonesian]

BPS Purworejo 2011. Purworejo in Figures 2011. District of Purworejo and BPS Purworejo, Purworejo [Indonesian]

BPS Wonosobo 2011. Wonosobo in Figures 2011. District of Wonosobo and BPS Wonosobo, Wonosobo [Indonesian]

Camus JM. 1997. The genus *Selaginella* (Selaginellaceae) in Malesia. In Dransfield J (ed). Plant Diversity of Malesia III: 59-69.

Chang HM, Chiou WL, Wang JC. 2012. Flora of Taiwan, Selaginellaceae. Endemic Species Research Institute, Nantou, Taiwan.

Chikmawati T, Setyawan AD, Miftahudin. 2012. Phytochemical Composition of *Selaginella* spp. from Java Island Indonesia. Makara J Sci 16 (2): 129-133

Hassler M, Swale B. 2002. Checklist of World Ferns. http://homepages.caverock.net.nz/~bj/fern/

ITIS [Integrated Taxonomic Information System]. 2016. *Selaginella willdenowii* (Desv. ex Poir.) Baker; Taxonomic Serial No.: 505115. http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=505115

Jermy AC. 1990. Selaginellaceae. In: Kubitzki K, Kramer KU, Green PS (eds.). The Families and Genera of Vascular Plants, 1. Pteridophytes and Gymnosperms. Springer, Berlin.

Junghuhn F. 1855. Kaart van het Eiland Java-Topografische. A.J. Bogaerts Ausgabe, Germany.

Junghuhn F. 1860. Geographisch-Physikalische von Java-Topografische. Justus Perthes, Germany.

Lee DW. 1977. On iridescent plants. Gard Bull Singapore 30: 21-29

Li ZJ, Tan BC. 2005. A review of the species diversity of *Selaginella* in Fujian Province of China. Acta Phytotaxonomica Sinica 43 (1): 50-59

Oldeman LR., 1975. Agroclimatic map of Java & Madura. Contr. of Centra. Res. Inst. for Food Crops. Bogor.

Setyawan AD, Sugiyarto, Susilowati A, Widodo. 2015a. Diversity and distribution of *Selaginella* in the Province of Yogyakarta Special Region. Pros Sem Nas Masy Biodiv Indon 1: 987-992.

Setyawan AD, Sugiyarto, Susilowati A, Widodo. 2015b. Diversity of *Selaginella* in the karstic region of Sewu Mountains, Southern Java. Pros Sem Nas Masy Biodiv Indon 1: 1318-1323.

Setyawan AD, Sugiyarto, Widiastuti A. 2012. Species diversity of *Selaginella* in Mount Merapi National Park, the central part of Java. Proc Soc Indon Biodiv Intl Conf 1: 102-106

Setyawan AD, Sugiyarto, Widiastuti A. 2015c. Species diversity of *Selaginella* in the Dieng Plateau, Central Java. Pros Sem Nas Masy Biodiv Indon 1: 980-986.

Setyawan AD, Sugiyarto. 2015. Diversity of *Selaginella* in the Bromo Tengger Semeru National Park, East Java. Pros Sem Nas Masy Biodiv Indon 1: 1312-1317

Setyawan AD, Sutarno, Sugiyarto. 2013. Species diversity of *Selaginella* in Mount Lawu, Java, Indonesia. Biodiversitas 14 (1): 1-9.

Setyawan AD. 2008. Species richness and geographical distribution of Malesian *Selaginella.* The 8th Seminary and Congress of Indonesian Plant Taxonomy Association ("PTTI"), Cibinong Science Center, Bogor-Indonesia, 21-23 October 2008.

Setyawan AD. 2009. Traditionally utilization of *Selaginella;* field research and literature review. Nusantara Bioscience 1 (3): 146-158.

Setyawan AD. 2011. Natural products from Genus *Selaginella* (Selaginellaceae). Nusantara Bioscience 3 (1): 44-58.

Setyawan AD. 2012. Altitudinal distribution of *Selaginella* in the southern part of Central Java, Indonesia. Proc Soc Indon Biodiv Intl Conf 1: 153-157.

Setyawan AD. 2014. A new record of naturalized *Selaginella uncinata* (Desv.) Spring (Selaginellaceae) from Java, Indonesia. Biodiversitas 15: 261-268.

Setyawan AD. 2015a. Diversity and distribution of *Selaginella* in the southern West Java based on altitudinal gradient. Pros Sem Nas Masy Biodiv Indon 1: 490-495.

Setyawan AD. 2015b. Diversity of *Selaginella* in the Province of Banten, Western Java. Pros Sem Nas Masy Biodiv Indon 1: 1810-1815

SRI [Soil Research Institute]. 1960. Exploratory soil map of Java and Madura. Indonesian Ministry of Agriculture, Bogor

Tryon RM, Tryon AF. 1982. Fern and Allied Plants, with Special Reference to Tropical America. Springer, New York.

Tsai JL, Shieh WC. 1994. Selaginellaceae. In: Huang TC (ed) Flora of Taiwan. Vol. 1. 2nd ed. Department of Botany, National Taiwan University, Taipei

van Bemmelen RW. 1949. The geology of Indonesia. Martinus Nijhoff, The Hague.

Winter WP de, Jansen PCM. 2003. *Selaginella* Pal. Beauv. In: de Winter WP, Amoroso VB (eds). Plant resources of South-East Asia 15 (2) Cryptogams: ferns and fern allies. Backhuys, Leiden.

Wong KM. 1982. Critical observations on Peninsular Malaysian *Selaginella.* Gard Bull Sing 35 (2): 107-135

Wong KM. 2010. Selaginellaceae. In Parris BS, Kiew R, Chung RKC, Saw LG, Soepadmo E (eds) Flora of Peninsular4 Malaysia Series 1. Ferns and Lycophytes. Malayan Forest Records No. 48. FRIM Kepong, Selangor.

Zhang XC, Nooteboom HP, Kato M. 2013. Selaginellaceae. In: Wu ZY, Raven PH, Hong DY (eds.). Flora of China, Vol. 2–3 (Pteridophytes). Science Press, Beijing & Missouri Botanical Garden Press, St. Louis.

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Evaluation of vegetation types in the West Zagros (Beiranshahr region as a case study), in Lorestan Province, Iran 1-10
ATENA ESLAMI FAROUJI, HAMED KHODAYARI

Floristic changes at Khersan Glacier Territory, Alamkuh Mountain, Central Alborz, North of Iran 11-15
KOUROSH KAVOUSI, TAHER NEJADSATARI , YUNES ASRI, HAMID EJTEHADI, RAMEZAN ALI KHAVARI-NEJAD

Molecular phylogeny of *Acer monspessulanum* L. subspecies from Iran inferred using the ITS region of nuclear ribosomal DNA 16-23
HANIF KHADEMI, IRAJ MEHREGAN, MOSTAFA ASSADI, TAHER NEJADSATARI, SHAHIN ZARRE

Determination of appropriate grid dimension and sampling plot size for assessment of woody species diversity in Zagros Forest, Iran 24-30
ALI ASGHAR ZOHREVANDI, HASSAN POURBABAEI, REZA AKHAVAN, AMIR ESLAM BONYAD

Short communication:
Algal leaf spot associated with *Cephaleuros virescens* (Trentepohliales, Ulvophyceae) on *Nephelium lappaceum* in Thailand 31-35
ANURAG SUNPAPAO, MUTIARA K. PITALOKA , SIWARET ARIKIT

Temporal variability in macroinvertebrates diversity patterns and their relation with environmental factors 36-43
MOHAMMAD HASAN GERAMI, RAHMAN PATIMAR, HOSSEIN NEGARESTAN, HOJJATOLLAH JAFARIAN, MOHAMMAD SEDDIQ MORTAZAVI

Status of coastal forests of the Northern Sumatra in 2005 (after 2004's tsunami catastrophe) 44-54
ONRIZAL, MASHHOR MANSOR

The diversity and distribution of Holothuroidea in shallow waters of Baluran National Park, Indonesia 55-60
ARIF MOHAMMAD SIDDIQ, TRI ATMOWIDI, IBNUL QAYIM

Ethnobotany of *Canarium* plant species used by Tobelo Dalam (Togutil) ethnic community of Halmahera Island, Indonesia 61-69
M. NASIR TAMALENE, MIMIEN HENIE IRAWATI AL MUHDHAR, ENDANG SUARSINI, FATCHUR RAHMAN, SAID HASAN

Dominance and diversity studies of tree species in lesser Himalayan forest of Uttarakhand, India 70-77
A.S. BISHT, A.B. BHATT

Cellulolytic and hemicellulolytic bacteria from the gut of *Oryctes rhinoceros* larvae 78-83
SITI LUSI ARUM SARI, ARTINI PANGASTUTI, ARI SUSILOWATI, TJAHJADI PURWOKO, EDWI MAHAJOENO, WAHYU HIDAYAT, IKOW MARDHENA, DANIEL FAJAR PANUNTUN, DEWI KURNIAWATI, ROBIAH ANITASARI

Mitigation of mercury contamination through the acceleration of vegetation succession 84-89
WIWIK EKYASTUTI, ENY FARIDAH, SUMARDI, YADI SETIADI

Diversity and the role of yeast in spontaneous cocoa bean fermentation from Southeast Sulawesi, Indonesia 90-95
JAMILI, NUR ARFA YANTI, PRIMA ENDANG SUSILOWATI

Proof of *Acacia nilotica* stand expansion in Bekol Savanna, Baluran National Park, East Java, Indonesia through remote sensing and fieldobservations 96-101
SUTOMO, EDDIE VAN ETTEN, LUTHFI WAHAB

Tropical peatland tree-species diversity altered by forest degradation 102-109
DWI ASTIANI

Morphological responses, sensitivity and tolerance indices of four tropical trees species to drought and waterlogging 110-115
YULIANTI, DEDE J. SUDRAJAT

Comparative characterization of *Macaranga* species collected from secondary forests in East Kalimantan for biorefinery of unutilized fast growing wood 116-123
RUDIANTO AMIRTA, SYAFFIYA ISNU NAFITRI, RETNO WULANDARI, YULIANSYAH, WIWIN SUWINARTI, KRISHNA PURNAWAN CANDRA, TAKASHI WATANABE5

Evaluation of soybean genotypes for resistance to rust disease (*Phakopsora pachyrhizi*) 124-128
APRI SULISTYO, SUMARTINI

Cowpea Mild Mottle Virus (CpMMV) infection and its effect to performance of South Korean soybean varieties 129-133
SUTRISNO, HERU KUSWANTORO

Screening of elite black soybean lines for resistance to rust disease, Phakopsora pachyrhizi 134-139
AYDA KRISNAWATI, GATUT WAHYU A.S., M. MUCHLISH ADIE

The recovery of Tabunio Watershed through enrichment planting using ecologically and economically valuable species in South Kalimantan, Indonesia 140-147
SYARIFUDDIN KADIR, BADARUDDIN, NURLINA, ICHSAN RIDWAN, FONNY RIANAWATY

Anther culture of local upland rice varieties from East Kalimantan: effect of panicle cold pre-treatment and putrescine enriched medium 148-153
NURHASANAH, ANANDA N. PRATAMA, WIDI SUNARYO

Karangwangi people's (South Cianjur, West Java, Indonesia) local knowledge of species, forest utilization and wildlife conservation 154-161
RUHYAT PARTASASMITA, JOHAN ISKANDAR, NICHOLAS MALONE

Phenetic analysis of the genera Medicagoid *Trigonella*, *Medicago* and *Melilotus* (Fabaceae) on seed coat in Iran 162-171
SIMA KHANDANI, MOSTAFA ASSADI, TAHER NEJADSATARI, IRAJ MEHREGAN

Ethnobotanical study of herbal medicine in Ranggawulung Urban Forest, Subang District, West Java, Indonesia 172-176
LILY SURAYYA EKA PUTRI, DASUMIATI, KRISTIYANTO, MARDIANSYAH, CHAIRUL MALIK, LAKSMANA PUTRA LEUVINADRIE, EKA ADHI MULYONO

Dayak Desa forest landuse system as social capital to acquire forest management rights in West Kalimantan, Indonesia 177-184
EMI ROSLINDA

The process of rehabilitation of mined forest lands toward degraded forest ecosystem recovery in Kalimantan, Indonesia 185-191
TRIYONO SUDARMADJI, WAHJUNI HARTATI

Flowering, fruiting, seed germination and seedling growth of *Macaranga gigantea* 192-199
DWI SUSANTO, DADDY RUCHIYAT, MAMAN SUTISNA, RUDIANTO AMIRTA

Species diversity of cerambycid beetles at reclamation area of coal mining in Berau District, East Kalimantan, Indonesia 200-207
SUGIARTO, CHANDRADEWANA BOER, DJUMALI MARDJI

Length-weight relationship and population genetic of two marine gastropods species (Turbinidae: *Turbo sparverius* and *Turbo bruneus*) in the Bird Seascape Papua, Indonesia 208-217
DANDI SALEKY, ISDRAJAD SETYOBUDIANDI, HAMID A. TOHA, MUHAMMAD TAKDIR, HAWIS H. MADDUPPA

Responses of the *Arabidopsis KNOX* and Boron transport gene mutants against the deficiency and overdose of Boron nutrient 218-221
WIDI SUNARYO, NURHASANAH

Stand damage due to mono-cable winch and bulldozer yarding in a selectively logged tropical forest 222-228
YOSEP RUSLIM, RUSPITA SIHOMBING, YASON LIAH

Anatomical structure and antioxidant activity of red bulb plant (*Eleutherine americana*) on different plant age 229-233
EVI MINTOWATI KUNTORINI, MARIA DEWI, MISRINA

Shade tree species diversity and coffee productivity in Sumberjaya, West Lampung, Indonesia 234-240
RUSDI EVIZAL, SUGIATNO, FEMBRIARTI ERRY PRASMATIWI, INDAH NURMAYASARI

Evaluating the level of sustainablity of privately managed forest in Bogor, Indonesia 241-248
TATAN SUKWIKA, DUDUNG DARUSMAN, CECEP KUSMANA, DODIK RIDHO NURROCHMAT

The diversity of plant species, the types of plant uses and the estimate of carbon stock in agroforestry system in Harapan Makmur Village, Bengkulu, Indonesia 249-255
WIRYONO, VENNY NOVIA UTAMI PUTERI, GUNGGUNG SENOAJI

Ethno-medicinal plants used for herbal medication of jaundice by the indigenous community of Tripura, India 256-269
DIPANKAR DEB, B.K. DATTA, JABA DEBBARMA, SOURABH DEB

Short Communication: Genetic identification of local pigs, and imported pigs (Landrace and Duroc) based on cytochrome b sequence analysis 270-274
TETY HARTATIK, BAYU DEWANTORO PUTRO SOEWANDI, SLAMET DIAH VOLKANDARI, ARNOLD CHRISTIAN TABUN, SUMADI, WIDODO

Review: The diversity of local cattle in Indonesia and the efforts to develop superior indigenous cattle breeds 275-295
SUTARNO, AHMAD DWI SETYAWAN

Short Communication: Detection of plasmids and curing analysis in copper resistant bacteria *Acinetobacter sp.* IrC1, *Acinetobacter sp*. IrC2, and *Cupriavidus sp*. IrC4 296-300
WAHYU IRAWATI, TRIWIBOWO YUWONO, AMANDA RUSLI

Short Communication: Inventory of native orchids in Makki Sub-District, Lanny Jaya, Papua, Indonesia 301-305
VERENA AGUSTINI, LISYE I ZEBUA, NONANCE WENDA

Assessment of competition indices of an unlogged oriental beech mixed stand in Hyrcanian forests, Northern Iran 306-314
MOJTABA AMIRI, REZA NAGHDI

The population of spotted cuscus *(Spilocuscus maculatus)* and its habitat carrying capacity in Numfor Island, Papua, Indonesia 315-321
ANTON SILAS SINERY, WARTIKA ROSA FARIDA, JACOB MANUSAWAI

Review: Climate-induced hydrological changes and the ecology of tropical freshwater biota 322-331
SUNARDI, GERHARD WIEGLEB

Responses to environmental and socio-economic changes in the Karangwangi traditional agroforestry system, South Cianjur, West Java 332-341
JOHAN ISKANDAR, BUDIAWATI SUPANGKAT ISKANDAR, RUHYAT PARTASASMITA

Four newly recorded *Amanita* taxa from India 342-348
YADWINDER SINGH, MUNRUCHI KAUR

Culturable fungal diversity associated with forest leaf litter from Bhandara District of Maharashtra, India 349-358
SUNIL M. AKARE, WALAY Y. TAGADE, ASHISH R. WARGHAT, AVILEKH NARYAL, ASHWANI BHARDWAJ

The roles of community fruit garden (*tembawang*) on maintaining forest structure, diversity and standing biomass allocation: an alternative effort on reducing carbon emission 359-365
DWI ASTIANI, RIPIN

Short Communication: Fusarium as endophyte of some terrestrial orchid from Papua, Indonesia 366-371
SUPENI SUFAATI, VERENA AGUSTINI, SUHARNO

Plants diversity of the deforested peat-swamp forest of Tripa, Indonesia 372-376
DJUFRI, WARDIAH, ZAINAL A. MUCHLISIN

Short Communication: Rhizoctonia-like fungi isolated from roots of *Dendrobium lancifolium* var. *papuanum* and *Calanthe triplicata* in Papua, Indonesia 377-383
VERENA AGUSTINI, SUPENI SUFAATI, SUHARNO, NUTTIKA SUWANNASAI

Diversity of *Selaginella* across altitudinal gradient of the tropical region 384-400
AHMAD DWI SETYAWAN, JATNA SUPRIATNA, DEDY DARNAEDI, ROKHMATULOH, SUTARNO, SUGIYARTO

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Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50^{th} Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Sebelas Maret University, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon.[Dissertation]. Brawijaya University, Malang. [Indonesian]

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