

Mycorrhizal Association of Terrestrial Orchids of Cycloops Nature Reserve, Jayapura

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ABSTRACT

Study on exploration of mycorrhizal association of terrestrial orchid of Cycloops Nature Reserve, Jayapura was done. The aims of this study were to collect terrestrial orchid and to isolate orchid mycorrhiza associated with it. Survey method was used in this study. Isolation of orchid mycorrhiza was based on modified methods of Masuhara and Katsuya (1989). The result showed that there were 10 species of terrestrial orchid in this area. Eleven orchid mycorrhizal fungi were isolated from five terrestrial orchids. Among them, 6 isolates were associated with *Geodorum* sp. From the seventeen mycorrhizal fungi, 3 isolates were identified, namely *Rhizoctonia* sp., *Tulasnella* sp., and *Ceratophthora* sp, while the last fourteen isolates have not been identified yet. Mostly, each isolate has a specific orchid host, except species G (sp. G) which associated with *Phaius* sp. and *Plocoglottis* sp.

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Key words: terrestrial orchid, orchid mycorrhiza, Mt. Cycloops Nature Reserve, Jayapura.

INTRODUCTION

Papua contains very high level of plant diversity. It may have at least 20.000-25.000 species of vascular plants including orchids. 3000 species of orchids are found in Papua, mostly are epiphytic. The exploration of orchids in Papua remains uncompleted due to a complicated geographic mosaic. Some orchid species have restricted ranges as a consequence of the complex geologic history of the island and its numerous barriers to dispersals. One area which should be explored is Cycloops Nature Reserve (Cagar Alam Pegunungan Cycloops, CAPC). It is totally 22.520 ha (SK Mentan No.05/KPTS/UM/1978).

All orchids, epiphytic or terrestrial and autotrophic or heterotrophic heavily dependent on fungi for their existence. It is different with the symbiosis between plant and fungi called AM (arbuscular mycorrhizal), and also ECM (ectomycorrhizal). The role of the fungi is unique, which known to serve as orchids mycorrhiza. Terrestrial orchids, in their habitats require the presence of suitable fungi in the living cells of the plant embryo and development of multicellular absorptive structures in order to develop and mature successfully (Currah et al., 1990)

Many researches have been done for mycorrhizal of terrestrial orchids in temperate area, but only a few in tropical area. Mycorrhizal fungi are associated with root systems of more than 90% of terrestrial plant species in a mutual symbiosis. In nature, all orchids utilize endomycorrhizal fungi to initiate seed germination and seedling development. The availability of each fungi, therefore, is an absolute requirement of orchid life cycle. The orchids-fungus symbiosis is initiated when orchid seeds are infected by a suitable fungus (Arditti, 1982; Rasmussen, 1995)

Orchids inoculated by fungi isolated from other plant did not show any positive effect on seedling development (Agustini and Kirenius, 2002). *Dendrobium* seedling inoculated with orchid-mycorrhiza isolated from other orchids shown a better growth than non inoculated one (Agustini, 2003). Most of orchid-mycorrhiza is endomycorrhiza. Fungi associate with photosynthetic orchid mostly belong to subdivision Basidiomycotinae, class Hymenomycetes, genus *Rhizoctonia*. Otero et al. (2002) reported that among 9 species of Puerto Rican orchids there were 108 *Rhizoctonia* like fungi which belong to *Tulasnella*, *Ceratobasidium*, and *Thanatephorus*.

Studies by Taylor and Bruns (1997) and Taylor et al. (2004) shown that 17 to 22 fungi species of Russulaceae are associated with *Corallorhiza maculata*. *Limodorum abortivum*, an orchid grown in Mediterranean is also associated with fungi belong to family Russulaceae (Girlanda et al., 2006). Russulaceae is basidiomycetes-ectomycorrhizal.

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Orchids of CAPC still remain as one of the least studied (Pokja Cycloops, 2003), therefore some areas such as surrounding Jayapura city, 6 species of terrestrial-orchids were found (Numberi, 2005). Study for the rest of CAPC areas is needed. Because of virtually lack of knowledge in the biodiversity of the mycorrhizal fungi of tropical Orchidaceae, the distribution and identification of fungi from variety of terrestrial orchids were examined. The objectives were to make an inventory of terrestrial-orchids of CPAC area, and to isolate and identify the naturally occurring mycorrhizal fungi of terrestrial orchids from various habitats.

MATERIALS AND METHODS

The status of orchids-mycorrhiza

Survey on status of orchids-mycorrhiza was done due to determine whether any infection of fungi in the living cells of the roots of the developing orchids or not. Healthy roots of terrestrial-orchids were collected from CPAC areas, namely: (i) UNCEN Campus at Waena (Kamp Walker and Buper), (ii) Sentani (Kemiri and Kampung Harapan), and (iii) downtown city of Jayapura. They were taken, wrapped in tissue paper, put in plastics bag and bring to the laboratory.

Collection and identification of terrestrial orchids

Sample of terrestrial orchids were collected from various locations of CPAC areas. They were collected ex situ on sterile media due to collect fungi from the orchids. The samples were identified at Botany Laboratory, Cenderawasih University, Jayapura. Some references like Becker and Bakhuizen van den Brink (1963, 1965, 1968); Comber (1990); Segerback (1992); Schuiteman (1995); Mahyar and Sadili (2003); Banks (2004) were used.

Fungal isolation

The root segment taken from root tip was washed with distilled water to remove any soil. For surface sterilization they were treated with ethanol 70% for 30 second. The roots were then cut into transverse section about 300 µm thick and observed for the presence of hyphal coil (polotons) on a glass slide under a stereo-microscope in sterile condition. Then they were culture onto potato dextrose agar (PDA) media in petridishes, three pieces in each petridish. (Irawati, 2006; pers. comm). Mycorrhizal fungi were isolated using a modification of Masuhara and Katsuya Methods (Manoch and Lohsomboon, 1991). After 1-2 days incubation, the hyphae will grow. If it shows any differences in shape and pattern of growth, then they will be isolated and culture on PDA in order to separate the variety of the fungi. Pure cultures were maintained on PDA slant.

Fungal identification

Macroscopic features examined were colony growth pattern, color, and mycelia formation. Fungal

growth rate was measured from the colony on PDA. For microscopic examination, fertile hyphae were mounted in sterile water on a microscopic slide, covered with a cover slip, and examine under light microscope. Hyphal and monilioid cells were measured. Literatures used in the fungal identification were Sharma et al. (2003) and Athipunyakom et al. (2004).

RESULTS AND DISCUSSION

Terrestrial orchid species

Ten terrestrial orchids were found in this study. Among the tenth species, 3 are found in campus areas, seven others are found in Sentani areas (Table 1).

Table 1. The location of terrestrial orchids of CPAC

No. of collection	Name of species	Location
WAE-01	<i>Spathoglottis plicata</i> L.	Campus
WAE-02		Campus (Buper)
SEN-01		Sentani (Kemiri)
SEN-02		Sentani (Harapan)
WAE-03	<i>Phaius tankervilleae</i>	Campus
WAE-04		Campus
WAE-05	<i>Geodorum</i> sp.	Campus
SEN-10		Sentani (Harapan)
SEN-04	<i>Calanthe</i> sp.1	Sentani (Harapan)
SEN-05	<i>Calanthe</i> sp.2	Sentani (Harapan)
SEN-06	<i>Plocoglottis</i> sp.1	Sentani (Harapan)
SEN-07	<i>Plocoglottis</i> sp.2	Sentani (Harapan)
SEN-08	<i>Plocoglottis</i> sp.3	Sentani (Harapan)
SEN-09	<i>Paphiopedilum violascens</i> Schltr.	Sentani (Harapan)
JAP-06	<i>Macodes petola</i>	Jayapura

The result is slightly different with Numberi's studied on 2005. She found 6 species of terrestrial orchids growth at city of Jayapura areas including campus surrounding areas. 4 species namely *Spathoglottis* sp., *Phaius* sp., *Geodorum* sp., and *Macodes* sp. are found in Jayapura surrounding areas.

Two of them *Spathoglottis* sp. and *Geodorum* sp. were found in broader areas of study. *Spathoglottis* sp. can be found in many habitats; from open areas to shading areas in forest either secondary or tertiary. The variety of habitat of *Spathoglottis* sp. make the flowers have different colors which were identified by Numberi (2005) as different species. It is stated in some literatures that wide ranges of habitat might caused the variety of color of flower.

Among the tenth species, some mycorrhizal fungi has been isolated just from 8 species, fungi from the 2 species remains unisolated. The results of the present study showed that one orchid was associated with a number of mycorrhizal fungi, for example *Geodorum densiflorum* associated with 6 mycorrhizal fungi; *Plocoglottis* sp.3 associated with 3 mycorrhizal

Table 2. Mycorrhizal fungi isolated from terrestrial orchids of CPAC.

No. of collection	Species	Mycorrhizal fungi	Habitat	Location	Isolate characteristics
WAE-01	<i>Spathoglottis</i> sp.	<i>Rhizoctonia</i> sp.	Open areas	Campus	On PDA, colony growth slow, 4,5 cm in diameter after 4 days incubation, thin mycelia, hyalin, globose, hyphae septate.
		<i>Tulasnella</i> sp.	Open areas	Campus	On PDA colony growth slowly but faster than <i>Rhizoctonia</i> sp., mycelium white dense, hyphae branches. Diameter 5,5 cm after 4 days incubation. Hyphae septate, rare, some of hyphae have branches, upright angles thicken. Conidia globe to ellipsoidal.
WAE-05	<i>Geodorum densiflorum</i>	Sp.A *)	Shading/ canopy areas	Campus	On PDA colony growth rapidly, mycelia dense, hyphae branches rarely, end of hyphae bearing abundant conidia, globe.
		Sp.B *)	Shading/ canopy areas	Campus	On PDA colony growth slowly, thin mycelia, hyphae branching often at nearly upright angles but more commonly at 40-60°
		<i>Ceratorhiza</i> sp.	Shading/ canopy areas	Campus	On PDA colony growth slowly, mycelia thin, hyphae branching, end of hyphae bearing abundant moniloid cells with typical mode of attachment in a chain 5-6 globes.
		Sp.D *)	Shading/ canopy areas	Campus	On PDA colony growth more slowly, dense, circle growth, black-white. Mycelia thin, septate, abundant globulus oil. Fungi isolated from terrestrial orchid
		Sp.E *)	Shading/ canopy areas	Campus	On PDA colony growth slow, circle growth, black and white. Concentric zonation, mycelia dense, septate dense, a few number of globulus oil.
		Sp.F *)	Shading/ canopy areas	Campus	On PDA colony growth slowly. Mycelia hyaline, branched, single globulus. A few number of coil.
WAE-03	<i>Phaius</i> sp.	Sp.G *)	Shading areas, next to creek.	Campus	On PDA colony growth rapidly. Hyphae dense, branches, peloton. Hyphae septate, multinucleate, a few number of globulus.
SEN-04	<i>Calanthe</i> sp.1	Sp.H *)	Secondary forest	Harapan, Sentani	On PDA colony growth rapidly, densely hyphae, branches. Characteristics globulus with stem on right and left of main hyphae.
SEN-05	<i>Calanthe</i> sp.2	Sp.J *)	Secondary forest	Harapan, Sentani	On PDA colony growth rapidly, white until optimal growth, turn on black color. Diameter 1 cm after 1 day incubation, and 5.5 cm after 5 days of incubation. Hyphae dense, globulus branches.
SEN-06	<i>Plocoglottis</i> sp.1	Sp.I *)	Secondary forest	Harapan, Sentani	On PDA colony growth rapidly, dense, parallel growth hyphae, on tip of hyphae abundant granule.
SEN-07	<i>Plocoglottis</i> sp.2	unisolated	Secondary forest	Harapan, - Sentani	
SEN-08	<i>Plocoglottis</i> sp.3	Sp.G *)	Secondary forest	Harapan, Sentani	On PDA colony growth rapidly. Hyphae dense, branches, peloton. Hyphae septate, multinucleate, a few number of globulus.
		Sp.K *)	Secondary forest	Harapan, Sentani	On PDA colony growth rapidly, white, dense at center, hyphae thin, branching upright, smaller branches loosely arrange. Bearing abundant globulus, especially at the tip of growing hyphae.
		Sp.L *)	Secondary forest	Harapan, Sentani	On PDA colony growth slowly after 15 days of incubation the color turn in black, concentric zonation, dense and thin alternately. Mycelia growth fast and dense. Abundant granular.
SEN-09	<i>Phapiopedilum violascens</i> Schltr.	Sp.M *)	Secondary forest	Harapan, Sentani	On PDA colony growth rapidly, white, with dense aerial mycelium in the centre. Hyphae septate, dense, granular rare. Host: <i>Paphiopedilum violascens</i> Schltr.
		Sp.N *)	Secondary forest	Harapan, Sentani	On PDA colony growth rapidly, white, hyphae dense, branching densely, with abundant globular. Host: <i>Paphiopedilum violascens</i> Schltr.
JAP-06	<i>Macodes</i> sp.	unisolated	Secondary forest	Jayapura -	

Note: *) unidentified.

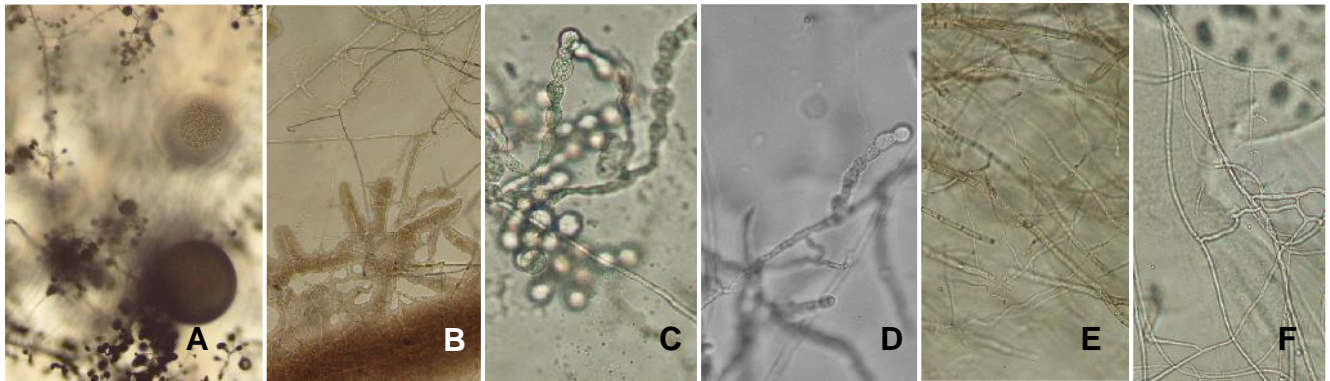


Figure 1. Six isolates taken from the root of terrestrial orchid *Geodorum* sp.: A. Sp.A, B. Sp.B, C. *Ceratorhiza* sp., D. Sp.D, E. Sp.E, and F. Sp.F (400x).

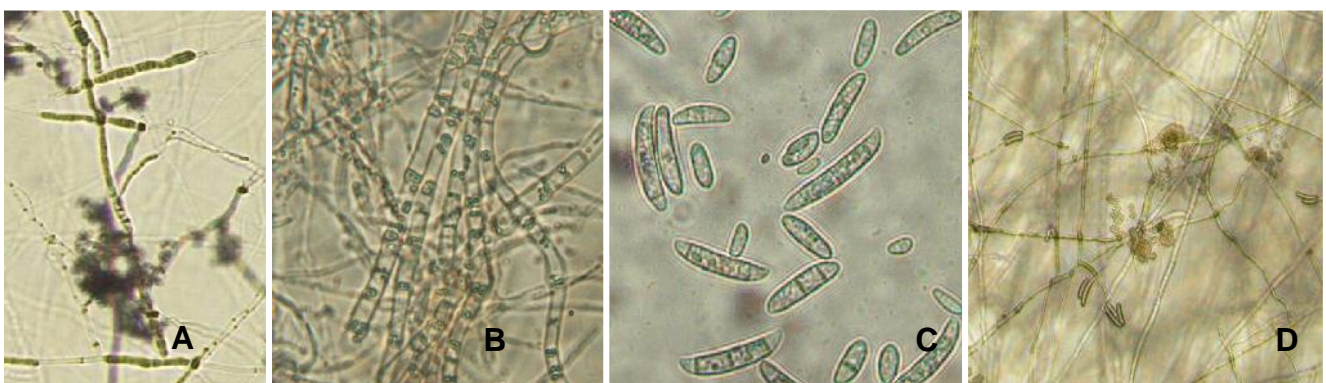


Figure 2. Reproductive portion of mycorrhiza. A. Hyphae of *Rhizoctonia* sp. 4 days on PDA. 400 x. B. Hyphae Sp.G taken from *Phaius* sp., septate, multinucleate, globulus oil, 1000x. C. Conidia of *Tulasnella* sp., 400 x. D. Hyphae supporting conidiospores, 100x.

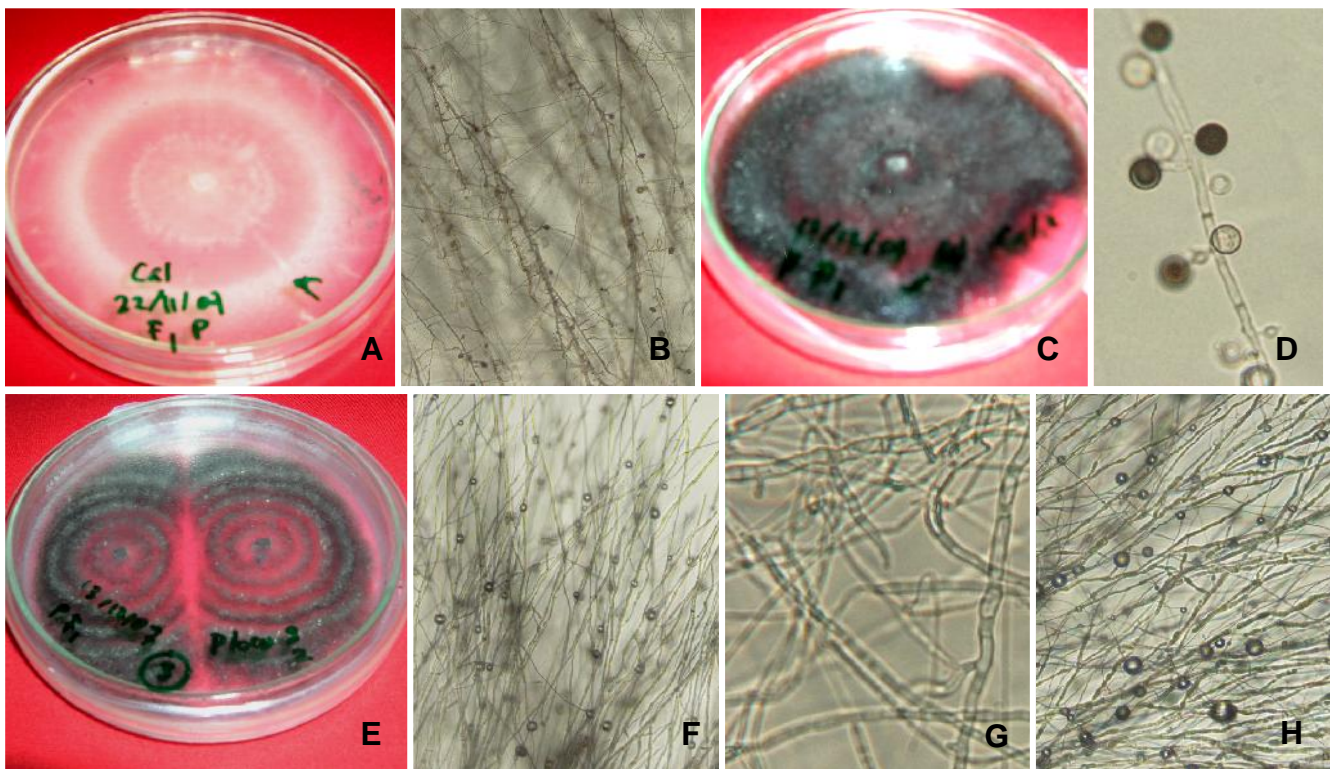


Figure 3. Colony of mycorrhiza. A. Sp.I: colony on PDA showing concentric zonation, 100x; B. Sp.I: abundant granule on tip of hyphae, 100x; C. Sp.J: colony on PDA; D. Sp.J: branches hyphae with globulus, 400x; E. Sp.L: colony on PDA showing concentric zonation and black in color after 15 days incubation; F. Sp.L: hyphae with abundant granule, 100x; G. Sp.M: dense and septate hyphae with few granule, 1000x; H. Sp.N: dense and branching hyphae with abundant granule, 100x.

fungi (sp.G, sp.K, and sp.L); *Spathoglottis* and *Paphiopedilum violascens* associated with 2 mycorrhizal fungi each. The study also found that a number of mycorrhizal fungi were associated with a single orchid host, for instance *Rhizoctonia* sp. and *Tulasnella* sp. are associated with *Spathoglottis* whereas Mycorrhizal fungi sp.M and sp.N are associated with *Paphiopedilum violascens* (Figure 3). The other orchids are associated with single fungus. This study indicated that mycorrhiza sp.G was isolated from *Phaius* sp. and also from *Plocoglottis* sp.3.

This study showed that from the two orchids *Plocoglottis* sp.2 and *Macodes* sp., mycorrhizal fungi remained unisolated. There are 17 mycorrhizal fungi isolated from 8 orchids of CPAC areas (Table 2). Athipunyakom et al. (2004) did a similar study, and found 14 mycorrhizal fungi from eleven terrestrial orchids in Thailand, among them are *Rhizoctonia globularis*, *Ceratrhiza* sp., and *Tulasnella* sp.

Association of mycorrhiza and terrestrial orchid

The results of the study shown that a number of mycorrhizal fungi were associated with only one orchid. There were six mycorrhizal fungi associated with *Geodorum* sp. (Figure 1). *Rhizoctonia* sp. and *Tulasnella* sp. are symbiont of *Spathoglottis*. (Figure 2). According to Athipunyakom et al. (2004), *Tulasnella* sp. is also associated with *Cymbidium tracyanum*, in root of *Calanthe* sp. was found fungi *Epulorhiza* and *Ceratrhiza*, while in this study *Ceratrhiza* fungi were isolated from *Geodorum* sp. Fungi sp.G are associated with both *Phaius* and *Plocoglottis* sp.3. Kristiansen et al. (2004) also found a similar situation that many fungi were associated with one specific orchid.

Almost all mycorrhiza associated with terrestrial orchid is *Rhizoctonia* including anamorphic of *Tulasnella*, *Ceratobasidium*, and *Thanatephorus* (Otero et al., 2002; Bonnardeaux et al., 2007). Fungi known to be associated with *Spathoglottis* sp. is *Rhizoctonia*, similar to study done by Tan et al. (1998) using terrestrial orchid *Spathoglottis plicata*. He inoculated *Rhizoctonia* AM9 on the media to germinate seed. The study showed that there is important role of the fungi on *Spathoglottis plicata* seed germination. Hayakawa et al. (1999) showed different results, there was no significant effect of the isolate on in vitro seed germination.

In contrast, this study indicated that one fungi merely associated with a single orchid host. *Calanthe* sp.1 and Sp.H and *Calanthe* sp.2 and Sp.J which both remained unidentified (Table 2). Study of symbiosis of fungi and orchid is extremely unique, mainly *Pterostylis*, *Caladenia* and *Thelymitra*. These fungi grow in very close habitat but they have different host, namely *Ceratobasidium*, *Sebacina* and *Tulasnella* (Andersen and Rasmussen, 1996). The Fungi *Rhizoctonia* is known as a symbiont of *Spathoglottis* sp, and is found in *Pinus radiata* root as

well. Furthermore, Bidartondo et al. (2004), reported that orchids grow under canopy can be associated both with fungi mycorrhiza and trees at the surrounding habitat. Fungi which are associated with orchid are basidiomycetes. Some of them are saprophytic, ectomycorrhiza, and plant parasitic (Rasmussen, 2004), while Yamato et al. (2005) said that achlorophilous *Epipogium roseum* also known associated with *Coprinus* which is saprophytic fungi.

The specific relationship between fungi and orchid also reported by Rasmussen (2004). The specificity was found from taxon species to subtribe (Warcup, 1981). Photosynthetic orchids showed a high specificity in association of mycorrhizal-orchids (Shefferson et al., 2005). Specificity possibly leads to high rates of orchid seed germination and a more efficient physiological association when the interaction is fully functional (Bonnardeaux et al., 2007). Mycoheterotrophic orchid *Corallorhiza maculata* was associated with twenty two fungi belong to family Russulaceae which is ectomycorrhizal in plants (Taylor et al., 2004).

In this study, transversal root segments of terrestrial orchids were used as a source of mycorrhizal fungi, this method were also used by Shan et al. (2002) and Bonnardeaux et al. (2007). Other isolates source of orchids-fungi were hyphal coils (peloton) taken from longitudinal sections of roots (Athipunyakom et al., 2004; Bonnardeaux et al., 2007).

CONCLUSION

Ten terrestrial orchids were found in Cagar Alam Pegunungan Cycloops, Jayapura. From eight of the ten orchids there were seventeen mycorrhizal orchids, three of them are identified as *Rhizoctonia* sp., *Tulasnella* sp., and *Ceratrhiza* sp. Dari Among them six fungi isolated from a single orchid *Geodorum* sp. In this study the specificity was found in most mycorrhizal-fungi except Sp.G which found in two different terrestrial orchid species namely *Phaius* sp. and *Plocoglottis* sp.

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