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The effects of introduction of the Sulawesi Endemic Stingless Bee *Tetragonula* cf. *biroi* from Sulawesi to Java on foraging behavior, natural enemies, and their productivity

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Abstract. *Suhri AGMI, Soesilohadi RCH, Agus A, Kahono S. 2021. The effects of introduction of the Sulawesi Endemic Stingless Bee* Tetragonula *cf.* biroi *from Sulawesi to Java on foraging behavior, natural enemies, and their productivity. Biodiversitas 22: 5624-5632.* The existence of species in an environment has gone through a long adaptation process to the natural environment. This study aims to determine the behavior of bees, pollen types, natural enemies, and products of the Sulawesi endemic bee *Tetragonula* cf. *biroi* in its natural habitat and after being introduced to Java. This research was conducted in North Luwu and Bone District, South Sulawesi, Indonesia, as the native habitat. Purworejo, Magelang, and Bantul District in Java as an introduction site. The observation of foraging behavior was recorded by scan sampling method; pollen identification using the acetolysis method. Natural enemies were observed directly and through interviews with beekeepers. The results showed significant differences in the number of *T.* cf. *biroi* that returned to the nest between native and introduced habitats (*p value* < 0.05). Pollen collected by *T.* cf. *biroi* in Sulawesi were more varied in type. The woody plants and tall trees dominate the types of pollen they collect in Sulawesi. The pollen collected in Java was dominated by ornamental plants, intentionally grown as feed. The attack of Small hive beetle and black soldier flies larvae caused the colony of *T.* cf. *biroi* to die and not produce honey optimally. Another problem is the *T.* cf. *biroi* nest is taken over by the local bee *Tetragonula laeviceps.*

Keywords: Competition, endemic bees, introduction, local bees, meliponiculture

INTRODUCTION

Foraging activities of social bees are influenced by individual memory to react to stimuli, abiotic environmental factors, competition, and availability of food sources (Fidalgo and Kleinert 2010). The allocation of foragers pollen, nectar, and resin is determined by the availability of feed sources around the nest also on the brood's demand Honey bee and stingless bee colonies can allocate more pollen foragers if less pollen is available around the hive (do Nascimento and Nascimento 2012; Maia-Silva et al. 2016; Basari et al. 2018). Bees' foraging behavior depends on the abundance of food sources, colony size, abiotic factors, and communication skills within a colony (Sommeijer et al. 1983; Roubik 1989). Stingless bees are generalists and have the potential to be pollinators. Although generalists, worker bees show flower constancy to the main feed chosen. The feed chose plants with abundant pollen or nectar close to the nest (Ramalho 2004; Tepedino et al. 2016). A native bee colony in the natural environment has undergone a long adaptation and coevolution (Holway et al. 2002; Montalva et al. 2011). The adaptability of the colony in an environment to the type of feed plant, nesting site, and climatic conditions can affect the growth and productivity of the colony. Human activities carry the introduction of bees out of their original geographic distribution area. The adaptability of bees can change if a colony moves to a new environment (Macías-macías et al. 2011). Changes in habitat and natural food sources and colony movement to new habitats affect colony growth and productivity.

The major flora and fauna in Indonesia are grouped into three biogeographical regions, Indo-Malayan, Wallacea, and Indo-Australian (Lohman et al. 2011; Ali and Heaney 2021). *Tetragonula* cf. *biroi* is known as one of the native or endemic species of Sulawesi (Engel personal communication 2020). Despite its small size, this species has advantages in producing raw honey and propolis. This bee species can produce as much as two kg honey/colony/harvest (Rustan and Paimin personal communication 2020). Along with the increasing interest in meliponiculture in Indonesia, there have been colonies transfer of the bee species *T.* cf. *biroi* by introducing thousands of colonies to Java, Sumatra, and Kalimantan islands since 2018 until now. Colony adaptation in a new environment can be seen/expressed, among others, by the activity of bees foraging for food, plant species from which food sources are taken, natural enemies, and production of

T. cf. biroi in the new habitat. However, research on these bees has never been reported in their natural habitat in Sulawesi or their newly introduced habitat.

Preliminary research on bee species transferred to Java reveals that a variety of ecological issues in the new habitat hampers the colony growth and production of bee colonies, sometimes causing the death of the colony. This study aims to determine the foraging activities of bees, species of forage plants, natural enemies, and production of bees *T.* cf. *biroi* in its natural habitat in Sulawesi and in a new habitat in Java. This research helps in obtaining bee management strategies for the endemic species *T.* cf. *biroi*.

MATERIALS AND METHODS

Research site

We conducted this research in December 2019-February 2020. To investigate behaviour in the native habitat of *T.* cf. *biroi*, studies were conducted on the island of Sulawesi as the native habitat of *T.* cf. *biroi*, namely Baebunta Sub-district, North Luwu District (2°33'01.5", S 120°17'45.0" E) and Bontocani Sub-district, Bone District (5°02'49.1" S, 119°54'42.0" E) South Sulawesi, Indonesia.

On the Java island, as the introduction area (new environment) for *T.* cf. *biroi*: Candimulyo Sub-district, Magelang District, Central Java (7°30'04.5" S, 110°16'44.7" E), Bruno Sub-district, Purworejo District, Central Java, Indonesia (7°33'48.5" S, 109°55'30.8" E), and the area of Pajangan Sub-district, Bantul District, Yogyakarta, Indonesia (7°51'39.3" S, 110°18'58.7" E).

Behavioral observation (Foraging and daily activities)

We conducted the foraging activity of bees was determined by the number of bees entering the hive and the material brought by them. At each site, three colonies of *T.* cf. *biroi* were maintained and the observation was recorded by scan sampling method (Martin and Bateson 1993). Observation on nest entry behavior was carried out on sunny days for ten days per location every month. Daily activity of foragers was recorded from sunrise to sunset (4.30-17.55), with the number of observations recorded being three times/day. The number of worker bees that enter the hive carrying and not carrying pollen is counted using a hand counter for 15 minutes, with an interval of 10 minutes. Pollen-carrying bees were identified by the colorful pollen balls on the corbicula. The behavior of *T.* cf. *biroi* return to the nest was recorded using a Sony 5000 mirrorless camera.

Pollen collection and identification

Species of flowers visited by bees are known from the pollen that they will bring into the beehive. Bees that return to the hive carrying pollen balls in the corbiculae are caught with insect nets. The bees were released after the pollen ball was taken and stored in a flacon bottle containing glacial acetic acid. Slides of collected pollen were carried out using the acetolysis method (Erdtman 1972). Pollen identification was done using the Pollen Flora of Taiwan (Huang 1972) and Australian Pollen and Spores Atlas at http://apsa.anu.edu.au/ (APSA 2017).

Honey production measurements

Observation on honey production from five well-developed colonies of *T. cf. biroi* (diameter of brood cells 18-20 cm) at each study site and measured by the total volume of honey produced by the *T.* cf. *biroi* colony at each research site for three years since 2018-2020. The honey volume was measured according to the honey harvesting schedule depending on the conditions of the bee colony feasibility being harvested.

Natural enemies observation

Natural enemies were observed around the colony boxes of *T.* cf. *biroi*. The organisms found were photographed/videoed, then collected using tweezers and put into a flacon bottle containing 95% alcohol. The identification of beetle morphology refers to Ślipiński and Lawrence (2013). Interviews were also conducted with beekeepers and residents around the farm. The interview contains questions about the types of natural enemies that have been seen by the interviewer.

Measurements of physical environmental factors and data analysis

The abiotic parameters measured were humidity and air temperature using a thermo-hygrometer type HTC-2 One med, wind speed using an anemometer type AM-100 NSR, light intensity using a lux meter type smart sensor as803. Measurements were taken when the worker bees started leaving the hive (morning) until the bees stopped foraging (afternoon). Measurement of environmental parameters were recorded on 05.00, 07.00, 09.00, 11.00, 13.00, 15.00, and 17.00. The correlation between abiotic factors and foraging behavior was analyzed using Principal component analysis (PCA) in PAST3 software. Comparison of bee foraging activity in Sulawesi and Java was analyzed using ANOVA followed by Tukey's test with a 95% confidence interval.

RESULTS AND DISCUSSION

Bees foraging activities

The total number of worker bees looking for nectar and pollen in each observation area, in Bone District, 53303 individuals were observed entering the hive (25733 with pollen loads and 27570 without pollen loads); in North Luwu District, 33358 individuals were observed entering the hive (15438 with pollen loads and 17920 without pollen loads); in Bantul District, 14118 individuals were observed entering the hive (6542 with pollen loads and 7576 without pollen loads); in Purworejo District, 23500 individuals (11785 with pollen loads, 11715 without pollen loads); in Magelang District, 17515 individuals (7851 with pollen loads, 9664 without pollen loads). The data shows that the number of active individuals in and out of the nest is higher in the two natural habitats than in the introduced habitat (Table 1).

The average number of worker bees *T.* cf. *biroi* entering the nest for 15 minutes in Bone District was 795.89 ± 88.56 individuals, then followed by North Luwu 490.55 ± 69.45 individuals, Bantul 207.61 ± 60.90 individuals, Purworejo 345.58 ± 62.80 individuals, and Magelang 255.94 ± 60.37 individuals (Figure 1). These data indicate that worker bees entering the hive were higher at two locations in their natural habitat in Sulawesi than in the introduced habitat (Table 2).

Foraging activities in each colony in the native habitat and introduced habitat showed a significant difference in the number of bees returning to the hive with a p-value <0.05 (Bone vs. Bantul = 8.43E-16; Bone vs. Purworejo = 5.83E- 13; Bone vs. Magelang = 1.58E-14; Luwu vs. Bantul = 7.4E-06; Luwu vs. Purworejo = 0.008; Luwu vs. Magelang = 0.001). Meanwhile, in the introduced habitat, there was no significant difference in the number of bees returning to the hive (p-value > 0.05) (Bantul vs. Magelang = 0.2; Purworejo vs. Magelang = 0.4 (Table 1).

Daily activity foraging of *Tetragonula cf. biroi*

The pattern of foraging activity in the native habitat and introduced habitat showed a relatively similar trend, which was active in the morning to noon, and decreased in the afternoon (Figure 2). However, there are significant differences in the average number of worker bees entering and leaving the hive in Sulawesi and Java (Table 1).

Table 1. Number of worker bees returning to the hive carrying pollen and nectar in the original and introduced habitat

Locations	n	Average number of bees returning to the hive (from morning to afternoon)
Bone (native habitat)	5	795.89±88.56[a]
Luwu (native habitat)	5	490.55±69.45[b]
Bantul (introduced habitat)	5	207.61±60.90[c]
Purworejo (introduced habitat)	5	345.58±62.80[d]
Magelang (introduced habitat)	5	255.94±60.37[c.d.e]

Different letters in the same column show significant differences in the ANOVA test followed by the Tukey test (95% confidence interval)

Table 2. Total of worker bees return to the hive in native and introduced habitat

Locations	Total bees return to the hive	*p-value*	The average number of bees returning to the hive/15 minutes	*p-value*
Sulawesi Island	69739±1089.21[a]	1.39E-08	1162.31±544.6[a]	1.64E-11
Java Island	27312±438.66[b]		303.47±146.22[b]	

Different letters in the same column show significant differences in the ANOVA test followed by the Tukey test (95% confidence interval)

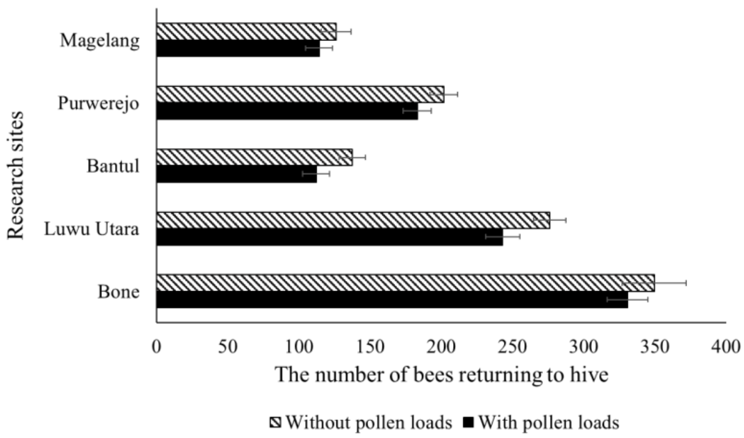

Figure 1. An average number of bees *Tetragonula cf. biroi* with pollen loads and without pollen loads every 15 minutes at five observation sites. Bars = standard deviation (n=5)

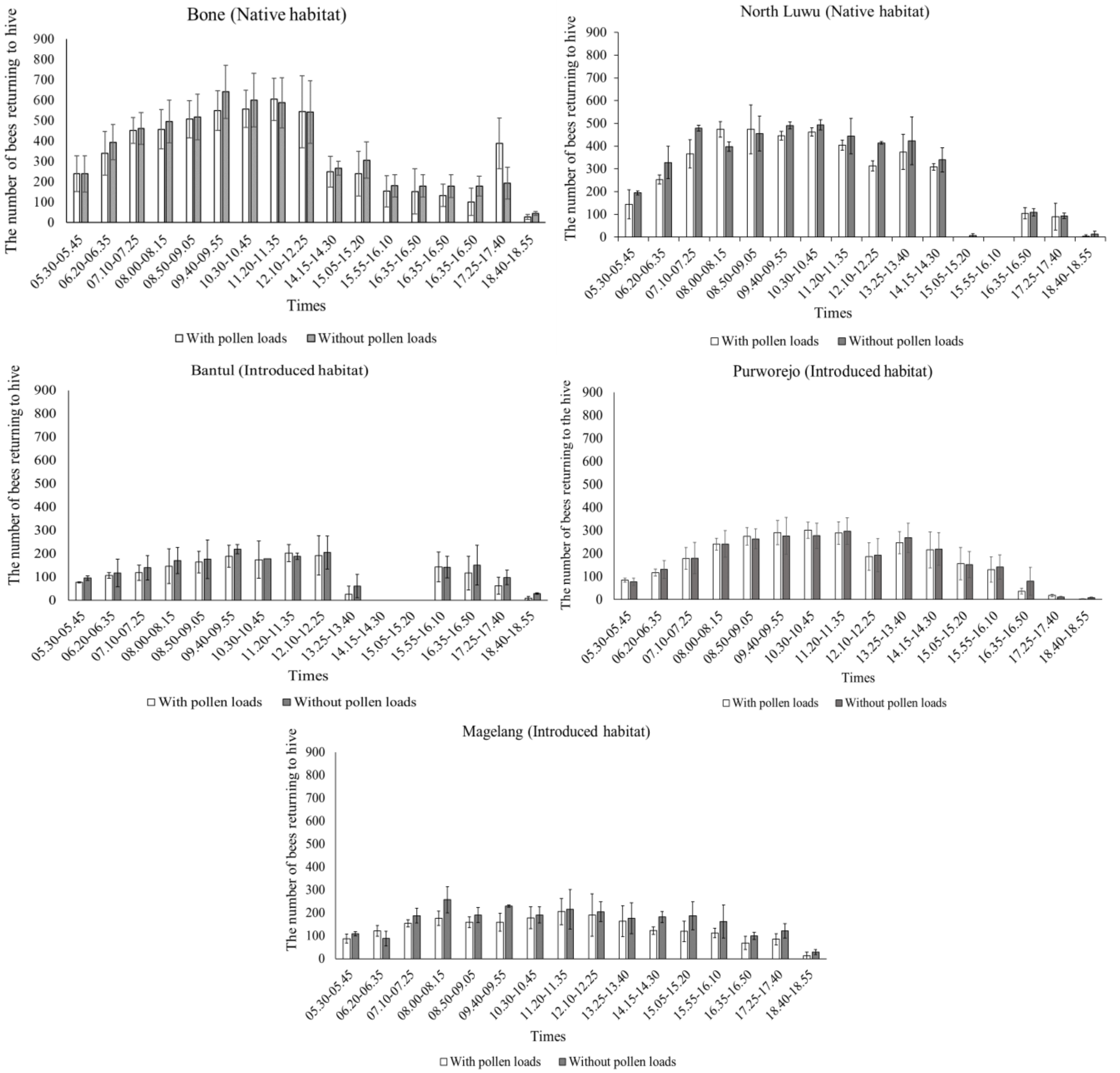

Figure 2. The average daily activity of the number of worker bees *Tetragonula cf. biroi* with pollen loads and without pollen loads from sunrise to sunset. Bar = standard deviation

Correlation of abiotic factors with the number of bees returning to the hive

The foraging pattern at the five research sites showed an increase in the number of bees returning to the nest from 06.20 to 12.25 (Figure 3). The number of worker bees *T.* cf. *biroi* outside the nest decreased at 13.25, then stopped their activities at 19.00, and the sun had set. *T.* cf. *biroi* actively foraging during the day with an ambient temperature range of 20ºC-30ºC. It had been observed that *T.* cf. *biroi* continue pollen foraging even during drizzle but the number was not significant. However, when it rains heavily, the activity of foragers completely ceases.

The number of bees leaving and entering the hive increased again when the rain stopped, with a minimum temperature of 23ºC and a minimum humidity of 68%. Temperature and light intensity (p-value <0.05) were the most influential abiotic factors on the number of foragers *T.* cf. *biroi*, while humidity and wind speed have no significant effect (p-value > 0.05) (Table 3).

Types of pollen collected by *Tetragonula cf. biroi*

From the morphological study of pollen brought into the hive, it had been found that 29 species of flowers belonging to 29 genera and 22 families were used as food sources for bees in their native habitat. There were 16 flower species from 16 genera and 14 families in the introduced habitat (Table 4).

Honey production

The mean minimum and maximum honey produced from *T.* cf. *biroi* during 2018 in south Sulawesi was 936±79 ml and 2024±129 ml respectivey. While in Java Island the production was somewhat less.

Natural enemies and competition between species

Natural enemies affect the development and survival of the bee colony of *T.* cf. *biroi* in native and introduced habitats (Figure 4).

In Bone District, Small hive beetles were found in two dead colonies of bees. In the Bantul District, Small hive beetles were found in three colonies of bees that had also died. In Magelang District, there were 150 colonies, and the remaining 20 colonies were attacked by Black soldier flies (black soldier fly) larvae and Small hive beetles on brood cells and food cells. In addition to beetle and fly attacks, the seizure of *T.* cf. *biroi* nests by *T. laeviceps* was also a factor in the damage of *T.* cf. *biroi* colonies in Magelang and Purworejo.

Table 3. Correlation of abiotic factors with foraging activity of *Tetragonula cf. biroi* in the native and introduced habitat

Parameter	Temperature (C)			Humidity (%)			Light intensity (x10 lux)			Wind velocity (m/s)		
	Max	Min	P-value	Max	Min	P-value	Max	Min	P-value	Max	Min	P-value
Native habitat	31	20	0.000007	98	68	0.55363	18880	121	0.008334	12.9	0	0.204484
Introduced habitat	34	24	0.052236	80	60	0.016554	3190	110	0.001682	1.7	0	0.221182

Table 4. Pollen collected by *Tetragonula cf. biroi* in native and introduced habitats

Family	Genus/species	Common names	Location (+ =present; - = absent)				
			Native habitat		Introduced habitat		
			Luwu Utara	Bone	Bantul	Purworejo	Magelang
Arecaceae	*Elaeis* sp.	Oil palm	+	-	-	-	-
	Borassus sp	Palm tree	-	+	-	-	-
	Areca vestiaria	Sunset palm	-	+	-	-	-
	Cocos nucifera	Coconut		+	-	-	-
Zingiberacea	*Etlingera elatior*	Torch ginger	+	-	-	-	-
Myrtaceae	*Xanthostemon* sp.	Golden penda	+	+	+	+	+
	Syzygium sp.	Guava	+	+	-	-	-
Polygonaceae	*Antigonon* sp.	Bee bush	+	+	+	+	+
Fabaceae	*Cassia* sp.	Cassias	+	-	-	-	-
Anacardiaceae	*Lannea coromandelica*	Indian ash tree	+	-	-	-	-
Malvaceae	*Theobroma cacao*	Kakao	+	+	-	-	-
	Urena lobata	Caesarweed	-	-	-	+	-
	Ceiba pentandra	White silk cotton tree	-	+	-	-	-
Moraceae	*Ficus subulata*	Ficus	+	-	-	-	-
Euphorbiaceae	*Aleurites moluccana*	Candlenut	+	-	-	-	-
	Claoxylon sp.	Laping budak	-	-	-	-	+
	Croton glandulosus	Tooth leaved croton	-	+	-	-	-
Ebenaceae	*Diospyros celebica*	Makassar ebony	+	-	-	-	-
Dypterocarpaceae	*Shorea leprosula*	Red meranti	+	-	-	-	-
Bignoniaceae	*Spathodea campanulata*	African tulip tree	+	-	-	-	-
Oxalidaceae	*Sarcotheca celebica*	Starfruit bajo	+	-	-	-	-
	Averrhoa carambola	Strafruit	-	+	+	-	+
	Oxalis barrelieri	Wood sorrel	-	-	-	+	+
Burceraceae	*Canarium indicum*	Galip nut	-	+	-	-	-
Fagaceae	*Lithocarpus*	Stone oaks	-	+	-	-	-
Dilleniaceae	*Dillenia celebica*	Jongi Tree	-	+	-	-	-
Asteraceae	*Ageratum* sp.	Floss flower	-	+	-	+	+
	Chromolaena	Siam weed	-	-	-	+	+
Fabaceae	*Tamarindus indica*	Tamarind	-	+	-	-	-
	Leucaena leucocephala	Lead tree, Horse tamarind	-	-	-	+	-
Muntingiaceae	*Muntingia calabura*	Jamaican cherry	-	+	-	-	-
Linderniaceae	*Picria fel-terrae*	Puguntano tree	-	-	-	+	-
Poaceae	*Zea mays*	Corn	-	-	-	+	-
Portulacaceae	*Portulaca*	Rose moss	+	+	+	+	+
Punicaceae	*Punica granatum*	Pomegranate	-	+	-	-	-
Passifloraceae	*Turnera subulata*	White buttercup	-	-	-	+	-
Rubiaceae	*Morinda citrifolia*	Indian mulberry	-	-	-	+	-
Lamiaceae	*Tectona grandis*	Teak	-	+	-	-	+
Anacardiaceae	*Bouea macrophylla*	Plum mango	-	-	-	+	-

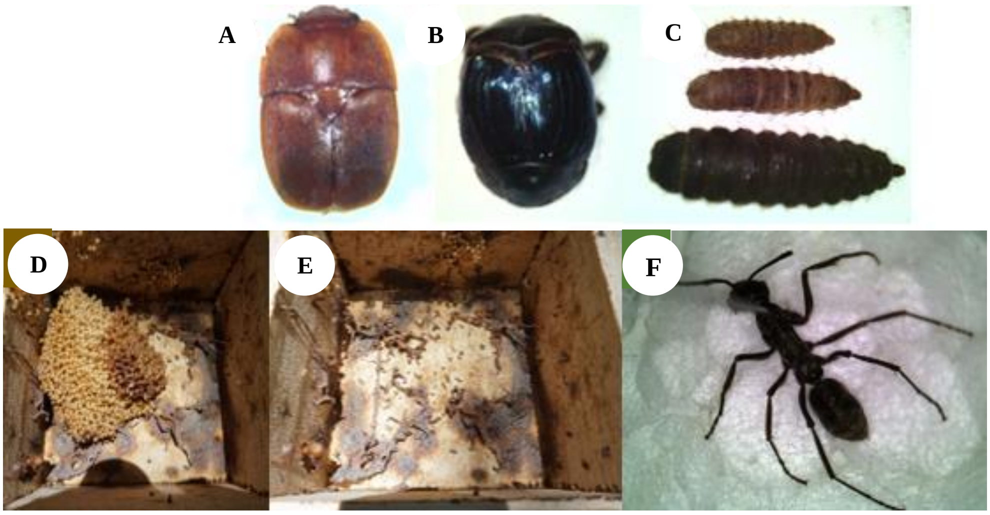

Figure 4. Natural enemies found in nests of *Tetragonula cf. biroi*: A. Nitidulidae; B. Scarabaeidae; C. Caterpillar of *Hermetia illucens*; D-E. Nests that have been taken over by *Tetragonula laeviceps*; F Formicidae

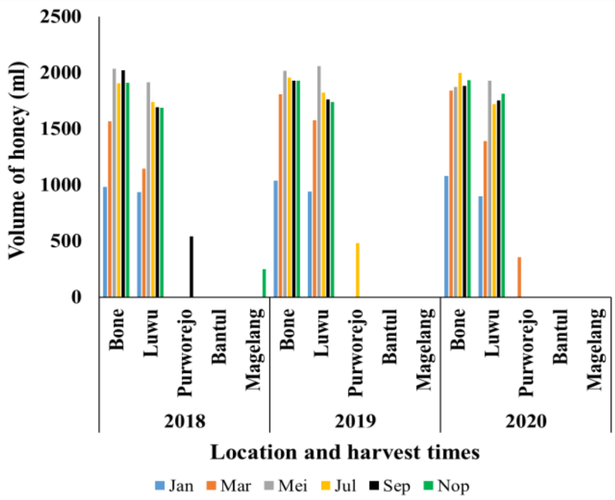

Figure 3. Honey production of *Tetragonula* cf. *biroi* in Sulawesi and Java for three years

Discussion

Bee foraging activity

Foraging activities of social bees are influenced by the colony's needs and physical environmental factors such as temperature, humidity, wind speed, and light intensity. (Wallace and Lee 2010; Keppner and Jarau 2016). Temperature and light intensity were the most influential factors on the foraging activity of *T.* cf. *biroi* at five locations (Table 2). Temperature is a significant factor that can explain the geographical distribution of insects as ectotherms (Andrewartha and Birch 1954; Cook and Breed 2013). The number of bees returning to the hive increases during the day, with the light intensity ranging from 17890 lux-328200 lux. Light helps bees navigate in search of food. High light intensity optimizes the forager's visual ability and helps to absorb heat into the body of the bees (Keppner and Jarau 2016; Chole et al. 2019). There was a decrease in bee activity in the afternoon or cloudy weather with light intensity <1000 lux. This condition occurs because of the possibility of a decrease in the visual ability of insects to fly when the light intensity is low. In the morning, the temperature affects the number of bees leaving the nest, while in the afternoon, the most influential factor is the intensity of light. Humidity and wind speed had no significant effect on the foraging activity of *T.* cf. *biroi* at the five observation locations. Wind speed can affect the flying activity of bees, but in this study, sometimes no wind was recorded, so it did not affect forager flying activity. The foraging activity of *T.* cf. *biroi* in Luwu and Bone are high in the morning (08.00-10.00) and the afternoon (13.00-14.00). However, in Bantul, Magelang, and Purworejo, there was no increase in the number of worker bees doing foraging in the morning and evening. In general, stingless bees have a peak foraging time in the morning and evening when biotic and abiotic factors around the nest are suitable (Layek and Karmakar 2018). Foraging activity of stingless bees was also influenced by season. Stingless bee *T. iridipennis* in India was reported to have high activity in July and lowest in December (Bharath et al. 2019). Stingless bee *Melipona asilvai* in Brazil was also said to have reduced foraging activity by 20 times during the rainy season (do Nascimento and Nascimento 2012).

Less number of forager *T.* cf. *biroi* in Bantul, Magelang, and Purworejo indicated the presence of factors inhibiting the activity of *T.* cf. *biroi* in a new environment. In general, *T.* cf. *biroi* can adapt to abiotic factors such as temperature, humidity, light intensity, and wind speed in a new environment because the conditions of abiotic factors in the native and introduced habitat are not significantly different. In addition, *T.* cf. *biroi* is a species with a high tolerance for these factors to live in the highlands to the lowlands. However, the activity and productivity of *T.* cf. the *biroi* were higher in Sulawesi than in Java. Possibly, the type of feed and natural enemies in Java affect its activities and products to be lower. Smaller and lighter-colored insects can retain heat for a shorter time and cool faster than larger and dark-colored insects (Halcroft et al. 2013; Vollet-Neto et al. 2014). Stingless bees that have a smaller body and light colors tend to forage in the sun with higher light intensity than stingless bees with large and dark colors (Pereboom and Beismeijer 2003).

Types of pollen collected

The stingless bee is a generalist bee to many plants (Atmowidi et al. 2018; Suhri et al. 2020). In general, the number of worker bees that carry pollen is less than bees that do not bring pollen back to the hive. Bees that do not get pollen back to the hive may carry nectar. Most bees that collect nectar do not carry pollen or carry only small amounts of pollen (less than 1µl) (Leonhardt et al. 2007). In addition, the amount of resin collected is also less than a quarter of the capacity of the corbicula if the bees carry nectar. On average, *Trigona fimbriata melanotricha* workers in Malaysia reported not bringing pollen back to the nest when the percentage of nectar collected was 80% from the total of material they brought to the hive. In the study, 24 worker bees returned to the hive carrying nectar, and none carried pollen on their legs. The amount of nectar in the stingless bee crop will decrease with the addition of pollen and resin material collected during foraging (Leonhardt et al. 2014). Stingless bees *T. collina* and *T. laeviceps* carry only <5% pollen in their limbs and carry 60% nectar from the total material they brought (Leonhardt et al. 2007). Stingless bees carry honey as their crop capacity, which is used as energy during foraging. Individually, stingless bees only collect one type of material in one trip foraging (Leonhardt et al. 2007; Hrncir et al. 2019), while honey bees can collect nectar and pollen/resin simultaneously in one trip foraging (Rana et al. 1997). Bees adjusted the pollen and nectar forager allocation in each colony to the availability of food sources around the nest (do Nascimento and Nascimento 2012; Saufi and Thevan 2015). Native plants can positively affect the quality and quantity of stingless bee honey production. The suitability of the availability of feed sources with the behavior of stingless bees in choosing a source of nectar affects honey production (Mohamad et al. 2020). Most pollen was collected by *T.* cf. *biroi* in the morning until noon and decreased in the afternoon. This pattern has also been reported in Brazil's *Melipona bicolor*, which collects pollen in the morning (do Nascimento and Nascimento 2012). Most other stingless bee species are diurnal animals (Nagamitsu and Inoue 2002; Macías-Macías et al. 2011). This condition is related to flower blooming time, temperature, and light intensity from morning to noon. Most flowers bloom in the morning, so that the availability of pollen and nectar is abundant in the morning (Roubik 1989; Fidalgo and Kleinert 2010).

The amount and type of pollen collected by *T.* cf. *biroi* depends on the available plants near the nest. At the five observation sites, not all flowering plants around the nest were visited by *T.* cf. *biroi*. Stingless bees show flower constancy to certain plants, usually to plants closest to the nest (Pangestika et al. 2017). Veiga et al. (2013) stated that there is a relationship between the body characteristics of the stingless bee with foraging activity and pollen collected.

Honey production

Foraging activity is a factor that affects colony productivity (do Nascimento and Nascimento 2012). Productivity *T.* cf. *biroi* in the introduced habitat is very low compared to the native habitat. Based on the results of interviews with farmers, the honey production of *T.* cf. *biroi* on the island of Java was not as good as its production in Sulawesi. In Purworejo, harvesting honey from *T.* cf. *biroi* just once in eight months with a maximum production of 500 ml/colony once harvested. In Bantul, *T.* cf. *biroi* has never been harvested since it was introduced in 2018.

Meanwhile, in Sulawesi, *T.* cf. *biroi* can be harvested once every two months, with a maximum production of 2030 ml/colony. The low productivity in the new environment could be influenced by the low frequency of *T.* cf. *biroi* back to the nest. The frequency of stingless bee activity returning to the nest is caused by two factors: the number of a forager in one colony and the frequency of foraging trips by individual forager (Nagamitsu and Inoue 2002). However, the foraging activity and the number of foragers can be different if the environmental conditions change. For example, there is a change in seasons and the types of feed available around the nest. In *Melipona asilvai*, colony productivity is also influenced by season. During the rainy season, the number of honey pots increased, and pollen pots decreased. While in the dry season, there was an increase in the number of pollen pots and a decrease of honey pots (do Nascimento and Nascimento 2012).

Natural enemies

The presence of natural enemies around the nest threatens the stingless bee colony (Hashim et al. 2017). Small hive beetle (Nitidulidae) in the beehive initially interferes with the growth of the bee population and eventually kills the bee colony (Halcroft et al. 2011). Small hive beetle is a pest that feeds on the cells of the bees and worker bees. These beetles destabilize the bee population by eating pollen, honey, eggs, and bee larvae and laying their eggs in bee brood cells (Ellis et al. 2004; Neumann and Hartel 2004). Small hive beetle reproduces successfully if the host bee population is weak. Small hive beetle attacks on a vulnerable population or a small population can cause the bee population to leave the hive (Ellis et al. 2003; Neumann et al. 2004). The behavior of Small hive beetle that attacks bee populations is also reinforced by the ability of adult beetles to survive in the hive for 48 days (Elzen et al. 1999; Ellis et al. 2002). The activity of worker bees in Java looking for pollen and nectar was not as active as *T.* cf. *biroi* in South Sulawesi. Small hive beetles were also more commonly found in *T.* cf. *biroi* hives in Java.

In addition to beetles, larvae of Black soldier fly were found in *T.*cf. *biroi* hives that have been broken. Black soldier fly larvae were a severe threat to the development of stingless bee colonies. Hashim et al. (2017) reported that Black soldier fly infected 225 colonies out of 305 colonies of *Heterotrigona itama,* and infected all of the colonies of *Geniotrigona thoracica* within one week in Malaysia. The smell of fermented honey triggers black female soldiers flies to lay their eggs in the stingless bee nest. Black soldier fly eggs are laid in pollen and honey pots because both are for black soldier fly larvae (Ivorra et al. 2020). in addition to small hive beetle and black soldier fly, ants were found in the *t.* cf. *biroi* in Bone. Duangphakdee et al. (2009) reported that *Oecophylla smaragdina* ants attack honey bees and stingless bees on infested flowers. However, ants are natural enemies of bees with low predation levels (Seeley 1983; Duangphakdee et al. 2009). Colony damage of *T.* cf. *biroi* in Java was not only caused by pests. In Magelang and Purworejo, the competition was noted, which could be due to limited food sources and nesting sites, through the struggle for nests of *T. cf. biroi* by the bee *T. laevice.* In Brazil, nest of *Tetragonisca angustula* was also taken over by *Lestrimelitta limao* (Wittmann 1984). *Trigona spinipes* was reported to attack the colony *Melipona rufiventris* in Brazil. The attack and seizure of interspecies stingless bee nests is a response to competition for food sources. *T. laeviceps* attacked and defeated the entire colony of *T.* cf. *biroi* in Java, and the nest was taken over by *T. laevicep*. This condition is influenced by the level of aggressiveness and colony size. With a greater number of attackers, a large colony size will carry out a lower level of aggression. At the same time, the colony with a small number of attackers will use a higher aggression power. The strategy of attacking at a lower level of aggression on a larger group scale can minimize individual injury risk (dos Santos et al. 2021). *T. laeviceps*, as a local bee in Java, has several colonies that dominate the beekeeping area, this successfully takes over the nests of *T.* cf. *biroi* as introduced bees.

To conclude, foraging activity *T.* cf. *biroi* decreased at the introduced habitat. In Bantul, Purworejo, and Magelang, the number of bees returning to the hive was significantly lower than Luwu and Bone. Due to the decline in foraging activity, honey production at the introduction site (new environment) has also greatly decreased. It has not even been harvested since 2018. The introduction of stingless bees is not recommended because it is not profitable for breeders and for local bees and introduced bees.

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