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RESEARCH ARTICLE

Nesting sites characteristics of stingless bees (Hymenoptera: Apidae) in Central Sulawesi, Indonesia

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Abstract: Stingless bees (Hymenoptera: Apidae) is eusocial insects that live together in a colony. This research was aimed to study the nesting site characteristics of stingless bees in the settlement areas at Central Sulawesi, Indonesia. The nesting sites were observed by purposive sampling method from July 2015 to January 2016. Four species belong to genus *Tetragonula* were found, namely *T. fuscobalteata*, *T. biroi*, *T. sapiens*, and *T. laeviceps*. Two spesies, *T. biroi* and *T. sapiens* are the new record in Sulawesi island. The highest abundance of stingless bees colony was *T. fuscobalteata* (92.26%), followed by *T. biroi* (4.17%), *T. sapiens* (2.98%), and *T. laeviceps* (0.59%). Nesting sites of *T. fuscobalteata* were found in the stone, brick wall, wooden wall, bamboo, and iron cavities, *T. biroi* in the wooden wall, stone, and brick wall cavities, *T. sapiens* in stone cavities, while *T. laeviceps* in wooden walls.

Key words: Nesting sites, Stingless bees, Tetragonula, Settlement area, Central Sulawesi.

Introduction

The diversity of stingless bees vary among different ecosystems. The most diverse commonly is found in the natural forest and gradually reduce in secondary forest, utilized forest, and settlement area, respectively (Boontop *et al.* 2008; Salim *et al.* 2012; Kelly *et al.* 2014; Syafrizal *et al.* 2014; Rahman *et al.* 2015). Generally, lowland ecosystem has more diverse of stingless bees than highland ecosystem (Sakagami & Inoue 1989; Salim *et al.* 2012). In the settlement areas, nests of stingless bees usually are found in the part of buildings and houses, i.e., roof and wooden wall cavities.

The nesting sites, nest structure, and characteristics of nest entrance are characters can be used for identification of stingless bees species. The nest entrance of stingless bees varied in shape, length, and colour (Syafrizal *et al.* 2014; Kelly *et al.* 2014). For examples, nest entrance of *Heterotrigona itama* is funnel shape, whereas, in *Geniotrigona thoracica* is round mount-like shape (Kelly *et al.* 2014). The common structure of stingless bees nests consists of entrance tunnels, brood cells, food storages (honey and pollen cells), cerumen and batumen layers (Sakagami *et al.* 1983; Starr & Sakagami 1987; Michener 2007; Boongird 2011; Erniwati 2013).

Sulawesi island has a high degree of endemicism of plants and animals. Honey bee, *Apis nigrocincta* is endemic species in Sulawesi (Hadisoesilo 2001; Engel 2012). The other endemic of stingless bee species in Sulawesi is *Geniotrigona incisa* that found in Central Sulawesi forest in the altitude more than 900 m above sea level (Sakagami & Inoue 1989). Central Sulawesi has different land use, such as plantation, agriculture, secondary forest, primary forest, and settlement areas that favorable for nesting and foraging sites of stingless bees. Until now, bee keeping of stingless bees in Sulawesi is very limited. People prefer to keep honey bee, *Apis cerana* for honey production rather than stingless bees. The nesting sites of stingless bees in the settlement areas in Central Sulawesi were described in this study.

Material and methods

Study Sites

The study was conducted from July 2015 to January 2016 in Watumaeta, Lore Utara Subdistric, Poso Regency (01°23'57,1" S, 120°19'30,0" E; 1.200 m asl), Pakuli, Gumbasa Subdistric, Sigi Regency (01°13'51,0" S, 119°56'43,2" E; 130 m asl), and Purwosari, Torue Subdistric, Parigi Moutong Regency (00°58'31,0" S, 120°19'04,8" E; 10 m asl). Observations of stingless bees were conducted in settlement areas in Watumaeta village (about 20.000 ha), Pakuli village (about 502 ha) and Purwosari village (about 94 ha), respectively.

Observation of Stingless Bee Nesting Sites

Observations of stingless bee nesting sites were carried out in 90 days, consist of 30 days in Watumaeta (July 2015), 30 days in Pakuli (August 2015), and 30 days in Purwosari (September 2015) using purposive sampling method (Tongco 2007). The nest characters observed were location of nesting sites, nest-entrance measurements (length, diameter, height from ground surface), shapes (funnel, mount-like, or round-ringed), colour (brown, black, or light brown), and rigidity (soft or hard) (Kelly *et al.* 2014).

Collection, Preservation, and Identification of Stingless Bee specimens

Collection of workers stingless bee of each colony were conducted by using sweep net. The specimens collected (10 individuals of each colony) were put into a bottle with 90% ethanol. Stingless bee specimens were preserved in the laboratory of Entomology, Zoology Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Bogor, Indonesia. The stingless bee specimens were identified based on Sakagami (1978), Sakagami & Inoue (1987), Dollin *et al.* (1997), Sakagami *et al.* (1990), and Smith (2012). All specimens were also verified with specimen collections of the Museum Zoologicum Bogoriense (MZB), Bogor, Indonesia.

Data Analysis

Statistical analysis of one-way ANOVA and Tukey's test were used to compare the diameter, length, and height of the nest entrance from the ground surface among three species of

Tetragonula. All the analysis used software *Paleontological Statistics* (PAST) ver. 1.89 (Hammer *et al.*, 2009).

Results

Species and Nesting Sites of Stingless Bees

A total of 168 colonies which belong to four species of stingless bees were found in this study. Three, ninety three, and seventy two colonies were found in Watumaeta, Pakuli, and Purwosari, respectively. Four species of stingless bee were identified, namely *Tetragonula fuscobalteata*, *T. laeviceps*, *T. biroi*, and *T. sapiens*. Two stingless bee species were recorded in each vilage. *Tetragonula fuscobalteata* and *T. sapiens* were found in Pakuli, *T. laeviceps* and *T. biroi* were found in Watumaeta, while *T. fuscobalteata* and *T. biroi* were collected in Purwosari.

The workers of each bee species have varied morphological characteristics (Fig 1). *Tetragonula fuscobalteata* has 3.47-3.54 mm body length, blackish-brown of body color, mesoscutum with six longitudinal hair bands, each band separated by five conspicuous glabrous areas (Fig. 1A). *Tetragonula laeviceps* has 3.40-3.43 mm body length, black body color, metasoma with brown color, first and second tergum are pale, while fourth and fifth tergum are dark. Mesoscutum is hairy, vertex is blackish and not banded, and anterior hind tibia is hairy (Fig. 1B). Workers of *T. biroi* had 4.00 - 4,17 mm body length, body color predominantly black, abdomen is brownish, clypeus and tegula are dark, mesoscutum and mesoscutellum with black hairs, malar space clearly separate the mandible and eyes (Fig. 1C). Workers of *T. sapiens* had 3.69-3.80 mm body length, black body color, metasoma is brown, first and second tergum are blackish-brown, and blackish in the apical. Mesoscutum consisted of glabrous areas and anterior hind tibia with dark to blackish-brown hairs, while in the posterior is brownish-yellow (Fig. 1D).

The highest number of colonies found was *T. fuscobalteata* (155 colonies), followed by *T. biroi* (7 colonies), *T. sapiens* (5 colonies), and *T. laeviceps* (1 colony). The nesting sites of *Tetragonula* were found in the parts of the houses, such as stone, brick, wooden, bamboo, and iron cavities. The highest number of *T. fuscobalteata* colonies was found in wooden wall (74 colonies), followed by stone cavity (40 colonies), brick wall (31 colonies), bamboo (6 colonies), and iron cavity (4 colonies). The nesting sites of *T. biroi* were found in the wooden wall (3 colonies), stone cavity (2 colonies), and brick wall (2 colonies). While, nesting sites of *T. sapiens* were found in stone cavity (5 colonies) and nesting site of *T. laeviceps* was found in wooden wall (1 colony) (Table 1).

Characteristics of nest entrance

Characteristics of nest entrance are properties that can be used to identify species of stingless bees. In average, the diameter and length of nest entrance are 1.86 ± 1.40 cm and 2.23 ± 2.52 cm in *T. biroi*, 2.18 ± 1.27 cm and 1.88 ± 0.95 cm in *T. sapiens*, and 1.24 ± 0.49 cm and 3.70 ± 3.88 cm in *T. fuscobalteata*. The diameter and the height of nest entrance of *T. sapiens* and *T. fuscobalteata* were significanly different (p=0.002 and p= 0.009), but the length of nest entrance among three species of Tetragonula was not significanly different (p>0.05) (Table 2). Unfortunately, there was no data available of the diameter and length of nest entrance from the ground surface occured in *T. laeviceps* (321.00 cm),



Figure 1. Worker adults of *Tetragonula* species. A, *Tetragonula* fuscobalteata; B, T. laeviceps; C, T. biroi; D, T. sapiens.

Tabel 1. Number of colony of stingless bees found in different nesting sites in Central Sulawesi										
		Number of colony found (colony)								
Species	Brick wall	Stone cavity	Bamboo	Iron cavity	Wooden wall	Total	Percentage (%)			
Tetragonula biroi	2	2	0	0	3	7	4.17			
Tetragonula fuscobalteata	31	40	6	4	74	155	92.26			
Tetragonula sapiens	0	5	0	0	0	5	2.98			
Tetragonula laeviceps	0	0	0	0	1	1	0.59			
Total	33	47	6	4	78	168	100			

followed by *T. fuscobalteata* (116.90 \pm 67.54 cm), *T. biroi* (56.64 \pm 38.08 cm), and *T. sapiens* (1.88 \pm 4.71 cm) (Table 2).

The shape, color, and rigidity of nest entrance varied in each species of *Tetragonula*. Characteristics of nest entrance of *T. biroi* are a funnel shape, mount-like, and round-ringed, color are black and light-brown, and soft rigidity. Nest entrance of *T. sapiens* is characterized by mount-like shape, round-ringed, black in color, and soft rigidity. Funnel shape, black and light-brown in color, and soft rigidity are characters of nest entrance of *T. fuscobalteata*, while, brown in color and hard rigidity are characters of nest entrance of *T. laeviceps* (Table 2).

four species of Tetr	Diameter (cm)			Length (cm)			Height from ground surface (cm)			Shape	Color	Rigi dity
	Mean	Range	St.dev	Mea n	Range	St.dev	Mean	Range	St.dev			
T. biroi (n=7)	1.86 ^{ab}	0.1-3.6	1.40	2.23ª	0.1-7.0	2.52	56.64ª	20-130	38.08	F, R, M	Bl, Lb	S
T. sapiens $(n=5)$	2.18 ^a	0.9-4.2	1.27	1.88 ^a	0.5-3.0	0.95	23.8ª	18-31	4.71	M, R	Bl	S
T. fuscobalteata (n=156)	1.24 ^b	0.1-3.6	0.49	3.7ª	0.1-25	3.88	116.9 ^b	0.95-300	67.54	F	Br, Lb	S
T. laeviceps $(n=1)$	na	na	na	na	na	na	321	na	na	na	Br	Н

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Note: St.dev= standart deviation, F=funnel (tube-like), M=mount-like, R=round-ringed, Br=brown, Bl=black, Lb=light brown, S=soft, H=hard, na=data not available. Different letter(s) in the same column indicated significant difference among means based Tukey's test.



Figure 2. Nest entrance of Tetragonula species. A, T. fuscobalteata; B, T. laeviceps; C, T. biroi; D, T. sapiens.

Discussions

All species stingless bees found in this study belong to the genus *Tetragonula*. The genus is small body size (Sakagami 1978; Smith 2012) and distributed in wide range in the world. Genus *Tetragonula* has been reported in the Asia continental and Sri Lanka (Sakagami 1978), Indian subcontinent (Rasmussen 2013), Southeast Asia (Sakagami & Inoue 1985), Thailand (Schwarz 1939; Sakagami *et al.* 1985; Michener & Boongird 2004; Klakasikorn *et al.* 2005; Boontop *et al.* 2008; Boongird 2011), Vietnam (Chinh *et al.* 2005), Peninsular Malaysia (Rasmussen & Michener 2010; Salim *et al.* 2012), Philippines (Starr & Sakagami 1987), and Indonesia (Erniwati 2013). In Indonesia, genus *Tetragonula* has been reported in Sumatra (Sakagami *et al.* 1990), Samarinda-East Kalimantan (Syafrizal *et al.* 2014), Sulawesi (Schwarz 1939), Maluku and Irian Jaya (Dollin *et al.* 1997).

Four species, *T. fuscobalteata, T. laeviceps, T. biroi* and *T. sapiens* were collected in the study and the most abundant colony was *T. fuscobalteata*, followed by *T. biroi, T. sapiens*, and *T. laeviceps*. Previous study showed that in Sulawesi islands were reported three species of stingless bee, namely *T. fuscobalteata* (Schwarz 1939), *Geniotrigona insica* as endemic species in Sulawesi (Sakagami & Inoue 1989), and *T. laeviceps* (Erniwati 2013). *Tetragonula biroi* had been reported in the Philippines (Oceanic Islands) and New Guinea (Starr & Sakagami 1987; Dollin *et al.* 1997). While, *T. sapiens* had been reported in Indonesia (Maluku), Philippines, New Guinea, and Australia (Dollin *et al.* 1997; Rasmussen 2008; Smith 2012). Both species, *T. biroi* and *T. sapiens* found in this study were the new record from Sulawesi islands. The common species found in Sulawesi is *T. fuscobalteata*. The species was distributed in the Southeast Asia (Thailand, Cambodia, peninsular Malaysia, Borneo, Sumatra, Sulawesi, Palau Island, Caroline Island, and Philippines (Palawan and Oceanic Islands) (Rasmussen 2008; Smith 2012).

In Sulawesi, nest of *Tetragonula* commonly were found in the part of the houses, such as wooden wall, brick, and stone cavities. Nesting sites of Tetragonula in the settlement areas had been reported, such as T. laeviceps, T. minangkabau (Sakagami et al. 1983; Erniwati 2013), T. fuscobalteata, and T. sapiens (Starr & Sakagami 1987). Nest of Tetragonula also were found in forest areas, such as dry dipterocarp forest, upper mixed deciduous forest, lower mixed deciduous forest, and dry evergreen forest in Thailand (Boontop et al. 2008), dipterocarp reserve forest in Peninsular Malaysia (Salim et al. 2012), and Lempake Education Forest in east Kalimantan (Syafrizal et al. 2014). Their nests also are adaptable in open forests and grasslands (Inoue et al. 1984). These species often built their nest in the hollow trunk as well as on termite and ants nests (Michener 1974; Sakagami 1982). Results showed that nests of T. fuscobalteata were highest in the wooden wall (74 colonies) than in the stone cavity, brick wall, bamboo, and iron cavity. While, all nests of *T. sapiens* found (5 colonies) in stone cavity and one colony of T. laeviceps was found in wooden wall. The nesting sites of T. biroi were found in the wooden wall, stone cavities, and brick walls. In the Philippines, Starr & Sakagami (1987) reported 26 colonies of T. fuscobalteta and 7 colonies of T. sapiens nest in bamboo cavities. As reported by Sakagami et al. (1983), nests of T. laeviceps can be found in some parts of houses and the nests also were found in hollow of tree with diameter 30-50 cm (Chinh et al. 2005; Kelly et al. 2014). In Karnataka, India, T. iridipennis build its nest around the manmade structures, such as residential, educational areas, and road sides parks that made by different substratum (brick walls, rock crevices, pillars, metallic sheath, and water pipes) (Pavithra et al. 2013).

The shape, color and rigidity of nest entrance of *Tetragonula* varied between species (Fig. 2). Nest entrance properties of stingless bees related to many factors, such as age of nest, microclimate, predators, parasites, and symbionts (Roubik 2006). The size of nest

entrance of stingles bees also varied (Kelly *et al.* 2014). Starr & Sakagami (1987) stated that the nest entrance of *T. sapiens* and *T. fuscobalteata* are tube-like with many variations of texture, diameter, and length. Variation of nest entrances related to defense and foraging activities of stingless bees (Biesmeijer *et al.* 2005).

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