NESTING SITE AND NEST ARCHITECTURE OF WALLACEA ENDEMIC STINGLESS BEE SPECIES *Tetragonula* cf. *biroi* AND *Wallacetrigona incisa* OF INDONESIA

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ABSTRACT

Generally, stingless bees have distinct geographical distribution and nesting sites, inhabiting tree cavities, rock crevices, land, anthills, and termite nests. Although they have been identified base on their unique morphological characteristics, their nest architecture could be utilized as a potential species identification tool however, inadequate research has been conducted so far. Belonging to the Wallacea species group, *Tetragonula cf. biroi* and *Wallacetrigona incisa* share similar brood cell forms. This study was conducted to measure the nesting areas and nest architecture of *T.*cf. *biroi* and *W. incisa* in the Bone regency, the North Luwu, and the South Sulawesi region. The nesting area data was collected by interviewing the local bee breeders and wild bee hunters of the forest. The research included the measurement of the nest entrance, the structure of the food and brood cells, and other nest parts. Observation and measurements of nest characteristics was carried out on five nest architectural forms of *T.*cf. *biroi* and one nest architectural form of *W. incisa*. Ten colonies were observed in each nest shape. The research finding revealed that *T.*cf. *biroi* nested on the tree trunk, roof, stone, and land. On the other hand, the nest entrance of *W. incisa* was hard and thick in texture and dominated by black color. The brood cell form of *T.*cf. *biroi* was found variably in either spiral, semispiral, and irregular shape, surrounded by soft and slight involucrum. The brood cell form of *W. incisa* was found only in spiral form, with thick and stiff involucrum. In both species, the honey pot was located adjoining the nest entrance. The pollen pot was situated behind or under the honey pot. In both species, the nest architecture is determined by microclimatic factors, composite dammar, colony age, and natural antagonists.

Keywords: Nesting site, Nest Architecture, Wallacea Species, *Tetragonula* cf. *biroi*, *Wallacetrigona incisa*

ABSTRAK

Lebah kelulut mempunyai taburan sebaran dan tempat bersarang tertentu. Secara umum, ia bersarang di dalam rongga batang, celah batu, tanah, sarang semut dan rayap. Pengecaman spesies lebah kelulut pada umumnya menggunakan karakter morfologi tubuh. Struktur sarang dapat digunakan sebagai salah satu ciri penting dalam pengecaman spesies, namun belum banyak kajian telah dilakukan. Lebah kelulut *Tetragonula cf. biroi* dan *Wallacetrigona incisa* merupakan spesies endemik kawasan Wallacea yang memiliki persamaan pada bentuk sel anak. Kajian ini bertujuan untuk mengkaji tempat bersarang dan struktur sarang *T.* cf. *biroi* dan *W. incisa* di Kabupaten Bone dan Luwu Utara, Sulawesi Selatan. Informasi tempat bersarang diperoleh melalui temuramah terhadap penternak dan pemburu lebah liar di hutan. Penelitian struktur sarang meliputi pengukuran pintu masuk, susunan dan ukuran sel anakan sel-sel makanan, dan bagian sarang lainnya. Pengamatan dan pengukuran sarang dilakukan ke atas lima bentuk sarang *T.* cf. *biroi* dan satu bentuk sarang *W. incisa.* Masing-masing sepuluh koloni diperhatikan pada tiap bentuk sarang. Hasil kajian menunjukkan *T.* cf. *biroi* bersarang pada batang pohon, akar, batu dan tanah. Sedangkan pada *W. incisa*, ditemukan tekstur pintu masuk yang keras dan tebal, dan didominasi warna hitam. Variasi bentuk sel anakan *T.* cf. *biroi* ditemukan spiral, semi spiral, dan tidak beraturan, dikelilingi lapisan involucrum lunak dan tipis. Bentuk sel anakan *W. incisa* yang ditemukan hanya berbentuk spiral, dengan lapisan involukrum tebal dan kaku. Pada kedua spesies, pot madu terletak paling dekat dengan pintu masuk sarang. Pot debunga berada berlekatan dengan pot madu, dan berada di belakang atau dibagian bawah pot madu. Properti struktur sarang pada kedua spesies adalah berbeza dan dipengaruhi oleh faktor iklim mikro, resin penyusun, umur koloni, dan musuh semulajadi.

Kata kunci: Struktur sarang, spesies Wallacea, tempat bersarang, *Tetragonula* cf. *biroi*, *Wallacetrigona incisa*

INTRODUCTION

Commonly found in both tropical and subtropical regions, stingless bees (Hymenoptera*:* Apidae) have been reported to mainly prevail in the tropical part of the world (Kerr & Maule 1964) with approximately 400 species, out of which 50 are known to belong to the Southeast Asia region (Inoue et al. 1984; Sakagami et al. 1989). Also referred to as Meliponini, its distribution has been categorized into four tropical regional groups, namely Neotropical, Afrotropical, Australasian, and Indo-Malayan (Michener 2007; Rasmussen & Cameron 2010). Indonesia categorized under the Indo-Malayan tropical spread region, comprises of 46 species of stingless bees from 10 different genera widely distributed throughout the island (Kahono et al. 2018; Rasmussen 2008; Rasmussen & Cameron 2010). Among the 10 genera, genus *Tetragonula* is dispersed across various regions of Indonesia namely Sumatera, Java, Borneo, Celebes, Ambon, Mullocas, and Irian Jaya (Kahono et al. 2018; Rasmussen & Cameron 2010).

Meliponini prefer to build nests in cavities of living and dead tree trunks, in rock crevices, bamboo cavities, and in the ground (Kerr & Maule 1964; Roubik 2006). The nests are composed of beeswax and plant resin mixture known as cerumen or propolis (Dollin et al. 1997; Rasmussen 2008). Characteristics including the layout and thickness of pollen and honey pots, the shape and type of brood cell, and the model of the nest entrance to the nest can categorize the stingless bee species (Farisya & Kumara 2013; Rasmussen 2008). Ndungu et al. (2019) also reported that the attributes of the nest could be the key to the identification of the genus *Hypotrigona* (Apidae: Meliponini). However, in the case of the genus *Geotrigona* located in Brazil, the similarity in nest structure could not be used as a sole identification tool due to the resemblance in the nest structures between different species in the genus (Barbossa et al. 2013). Generally, in identifying species of bee propolis, the morphological and architectural elements of the nest are used. Identification based on nest traits is rarely used. One of the endemic species of the genus *Tetragonula* which inhabits the Wallacea region (Celebes and its surroundings), is *T. biroi.* The characteristics of several *Tetragonula* species in Australia has been reported by Dollin et al. (1997) and Dollin et al. (2015), but that of species *T.* cf. *biroi* has not been described yet. The research conducted by Suriawanto et al. (2017) in Celebes also did not explain the nest architecture, hence the diversified nest structure of *T.* cf. *biroi* requires further studies. In addition, another endemic species named *W. incisa* inhabits the Celebes region at an altitude of 800–2200 asl (Rasmussen & Cameron 2010). Unlike *T.* cf. *biroi*, the nest entrance to *W. incisa*’s nest inhabiting the North Luwu region was narrow longitudinal slit with thick and hard resin clusters (Sayusti et al. 2020). However, stem cells form of both *W. incisa* and *T.* cf. *biroi* looks vastly similar. Therefore, further research is required to analyze the differences in the nest shape of *W. incisa* and *T. cf. biroi*. This study explores the variations in the shape and size of the nests of *T.* cf. *biroi* and *W. incisa* in the South Sulawesi region of Indonesia.

MATERIALS AND METHODS

Time and Location

Observations were conducted on the *T.* cf. *biroi* species inhabiting in the Bontocani district, the Bone regency (5° 02’49.1 “S 119°54’42.0” E), the Baebunta district, the North Luwu regency (2°33’01.5“S 120° 17’45.0” E) and the South Sulawesi Province, Indonesia. Observations of *W. incisa* were carried out in the Rongkong District, and the North Luwu regency (2°25’37.7“S 119°49’08.5” E/1600 asl). The study was conducted during the period of November 2019 to January 2020 and July 2020.

Observation and Measurement of the Natural Nest of *T.* cf. *biroi* and *W. incisa*

Natural nests were observed on the living trees, and parts of trees that have been collected by beekeepers. Observation of the living trees as the nest sites was carried out in their natural settings in the vicinity of the study location based on information provided by the breeders and the bee hunters of the forest. The data recorded included local names of the trees, number of nests per tree, and its height from the ground level, a diameter of nest entrance (mm), and the length of the external nest entrance (mm). The natural nest sites retained from the new nest and previously collected by the breeders was also observed. Natural nests collected by breeders are used as objects for measuring the external and internal nest structures.

Measurement of the External Entrance of the Nest of *T.* cf. *biroi* and *W. incisa*
Observations and nest measurements of nests were carried out on each of ten colonies in five different nest architectural forms of *T.* cf. *biroi,* and one nest of *W. incisa* which had been transferred to an artificial nest. According to Barbossa et al. (2013), the architecture and arrangement of the observed nests start from the end of the nest entrance funnel to the internal part of the nest.

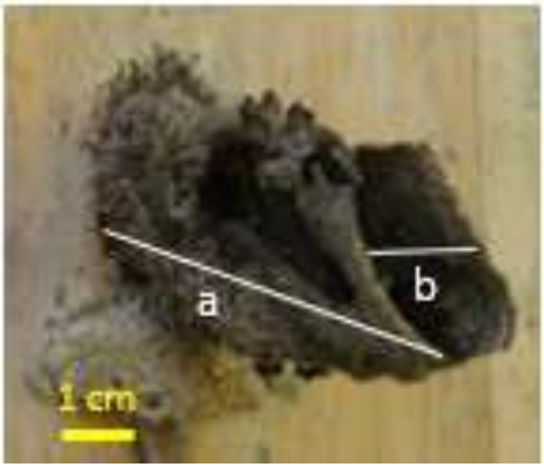

Figure 1. Nest entrance measurement; a) length, b) diameter

The external structure measured included the diameter of the nest entrance. The shape, ornamentation, and color of the nest entrance was also observed. The nest entrance's estimated length was measured from the tip to the base of the funnel visible at the front of the nest (Figure 1). Measurements were made by stretching the rope following the funnel groove and measuring the length of the rope using calipers.

Measurement of the Internal Part of the Nest of *T.* cf. *biroi* and *W. incisa*
On the internal side, brood cells were observed and measured (diameter per cell, height per cell, total diameter, total height, and cell arrangement); honey pot and pollen pot data were also collected (diameter per cell, height per cell, shape, and arrangement of the cells). The volume of honey was calculated in each of the 20 pots per colony. Honey in each pot was taken using a 20 ml syringe. The results are recorded and averaged as the volume of honey/pot. The pollen in each of the 20 closed cells was taken using a spatula. The pollen was weighed using an analytical balance with an accuracy of 0.01 g.

RESULTS

Nest Structure
Measurement of natural nest
The *T.* cf. *biroi* in the Bontocani and the Baebunta Districts were found nesting in cavities of live or dead tree trunks, roots or base of trees, cavities in the soil, and in the rock gaps. Based on observations, the tree trunks used as nests by stingless bees are taken by beekeepers and kept in their homes (Figure 2).

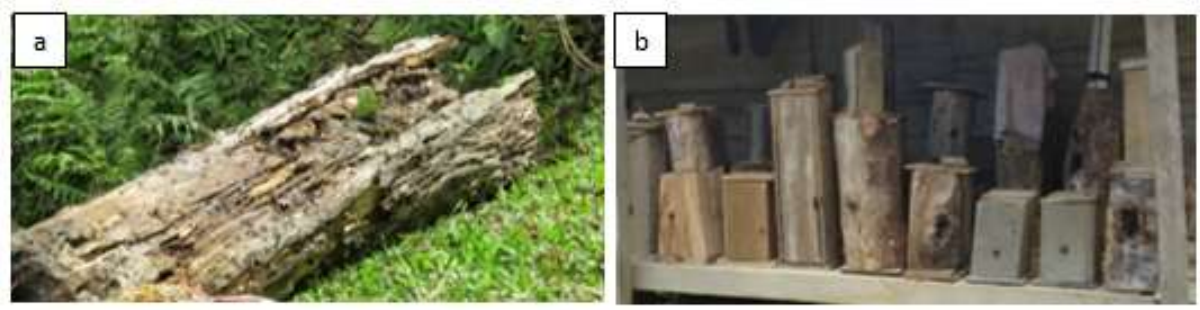

Figure 2. Natural nest sites that have been collected by the beekeeper: (a) *W. incisa*, (b) *T.* cf. *biroi*

The living tree species recognized as nest sites were *Cassia fistula* (Fabaceae), *Lanneacoromandelica* (Anacardiaceae), *Syzigiumaquaeum* (Myrtaceae), *Theobroma cacao* (Malvaceae), *Ficussubulata* (Moraceae), *Aleuritesmoluccana* (Euphorbiaceae), *Shorealeprosula* (Dipterocarpaceae), *Canariumindicum* (Burseraceae). In natural nest sites, the nest entrance was small (<1 mm diameter) and faint, the external funnel was coequally short (0–1 mm). The number of nests per tree varied (1–4 nests) (Table 1). Based on observations, more than one stingless bee colony was also discovered in the nest of the living tree. However, it is not certain whether they are the same species or not.

Wallacetrigona incisa bees were found nesting in the cavity of large tree trunks, not excessively rotten or eaten by termites with a tree height until nine meters, the diameter of nest entrance were >1 mm, and the length of external funnel were >4 mm. The tree species recognized as nest sites included the *Shorea leprosulameranti* tree (Dipterocarpaceae), the *Lithocarpus sundaicus* oak tree (Fagaceae), and the *Pigafetta elata* (Arecaceae) (Table 1).

Table 1. Natural nest sites for *T*. cf. *biroi* and *W. incisa* in South Sulawesi

Bee species	**Nest place**	**n**	**Tree name**		**The height of the nest above ground (m)**	**number of nests per tree**	**Diameter of nest entrance (mm)**	**Length of an external entrance (mm)**
			Local	**Scientific**				
T. cf. *biroi*	Tree	2	*Raja*	*Cassia fistula* (Fabaceae)	2–9	3-4	0.75±0.07	0.47±0.1
		2	*Kalimbajo*	*Lannea coromandelica* (Anacardiaceae)	1–3	2-4	0.74±0.05	0.67±0.03
		2	*Jambu mete*	*Anacardium occidentale* (Anacardiaceae)	2–5	1-3	0.67±0.03	0.38±0.07
		1	*Jambu air*	*Syzygium aqueum* (Myrtaceae)	2–4	2	0.6	0.9
		3	*Coklat*	*Theobroma cacao* (Malvaceae)	1–2	2-4	0.55±0.21	0.26±0.9
		2	*Kajuara*	*Ficus subulata* (Moraceae)	2–5	1	0.71±0.14	0.39±0.24
		2	*Kamiri*	*Aleurites moluccana* (Euphorbiaceae)	3–6	3-5	0.62±0.10	1.05±0.35
		2	*Meranti/Londong*	*Shorea leprosula* (Dipterocarpaceae)	2–7	1-3	0.55±0.06	0.37±0.06
		3	*Kenari*	*Canarium indicum* (Burseraceae)		2-3	0.81±0.07	0.25±0.07
	Tree roots	1	*Kenari*	*Canarium indicum* (Burseraceae)	<1	1	0.78	0.32±0.1
		2	*Meranti*	*Shorea leprosula* (Dipterocarpaceae)	<1	1	0.82±0.08	0.56±0.03
	Stone		-					
	In the Soil		-					
W. incisa	Tree	3	*Londong*	*Shorea leprosula* (Dipterocarpaceae)	4–8	3-4	1.37±0.09	4.6±1.51
		3	*Palli'*	*Lithocarpus sundaicus* (Fagaceae)	4–9	2-5	1.29±0.05	4.4±0.28
		4	*Wanga*	*Pigafetta elata* (Arecaceae)	5–8	1-3	1.43±0.15	4.9±0.42

Composition of the Nest Structure

The nest structure arrangement of *T.* cf. *biroi* and *W. incisa* generally are identical to other stingless bee species, namely the nest entrances, the food pots, and the brood cells. On the external side, the nest structure arrangement of these two species starts from the nest entrance funnel with various sizes and colors. At the base of the funnel, a resin ornament can be seen scattered on the walls of the fifteen wooden boxes where the nest is located.

In the internal part of *T. cf. biroi*'s nest, a short funnel is connected to the external nest entrance (Figure 3a-b). However, the internal nest entrance funnels connected to the external nest entrance were absent in these colonies (Figure 3e).

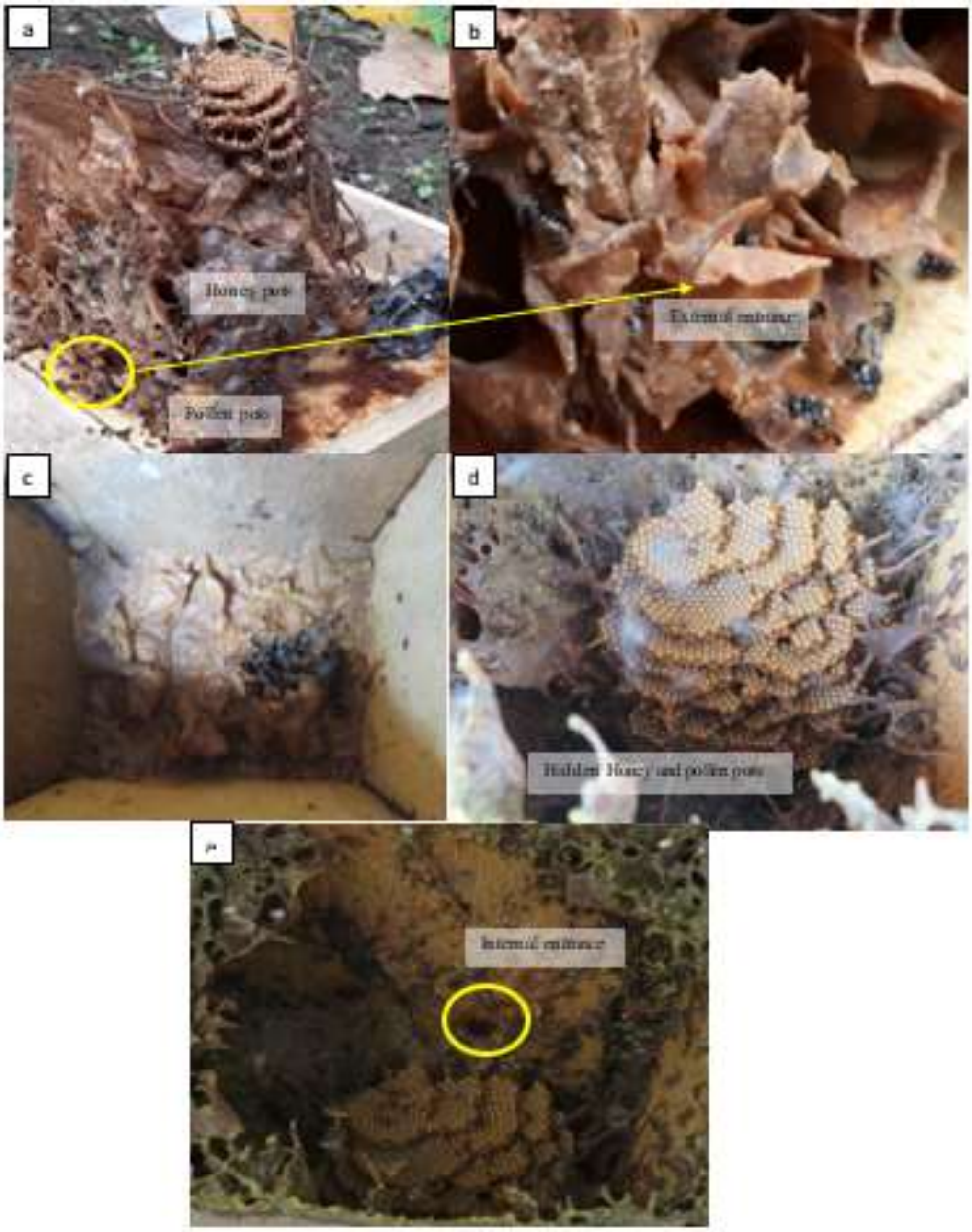

Figure 3. Structure arrangement nest of T. cf. biroi: (a, b, e) internal nest entrance, (a, d) location of pollen and honey pots, (c) Inv: involucre covering the upper surface of the nest

It was also found that the internal parts were densely packed and full; there were only empty spaces between the pillars (Figures 4a-f). The honey and pollen pots were the closest to the nest entrance, while the brood cells were the farthest from the nest entrance. The position of the food pot with the brood cell was separated by an involucre arranged in layers. Dark brown pillars were also established supporting the brood cells and the food pots to stick to the wooden surfaces. Figure 5. The nest arrangement of *W. Incisa*; (Ine) internal nest entrances; (Bc1) first brood cell; (Bc2) second brood cell; (FP) food pots.

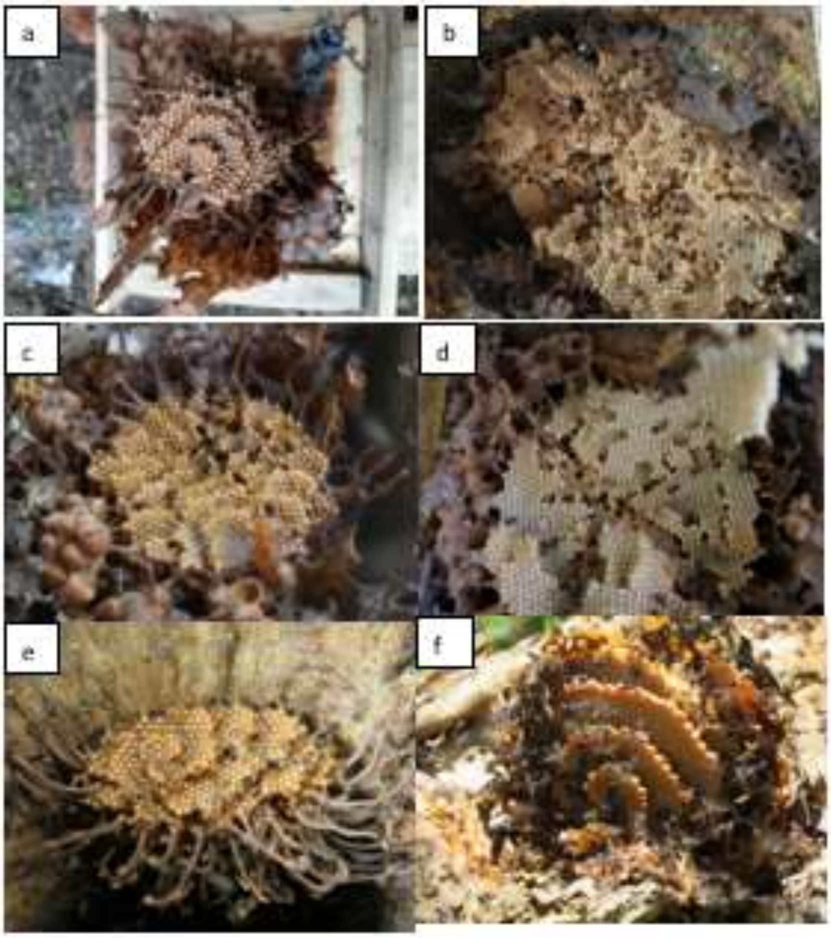

Figure 4. Variation of stingless bee comb structure in South Sulawesi: (a–e) *T.* cf. *biroi* in Bone and in North Luwu; (f) *W. incisa* in North Luwu

On the other hand, the internal nest entrance of the *W. incisa* emerged as multilayered and was composed of a solid mass of resin (Figure 5a).

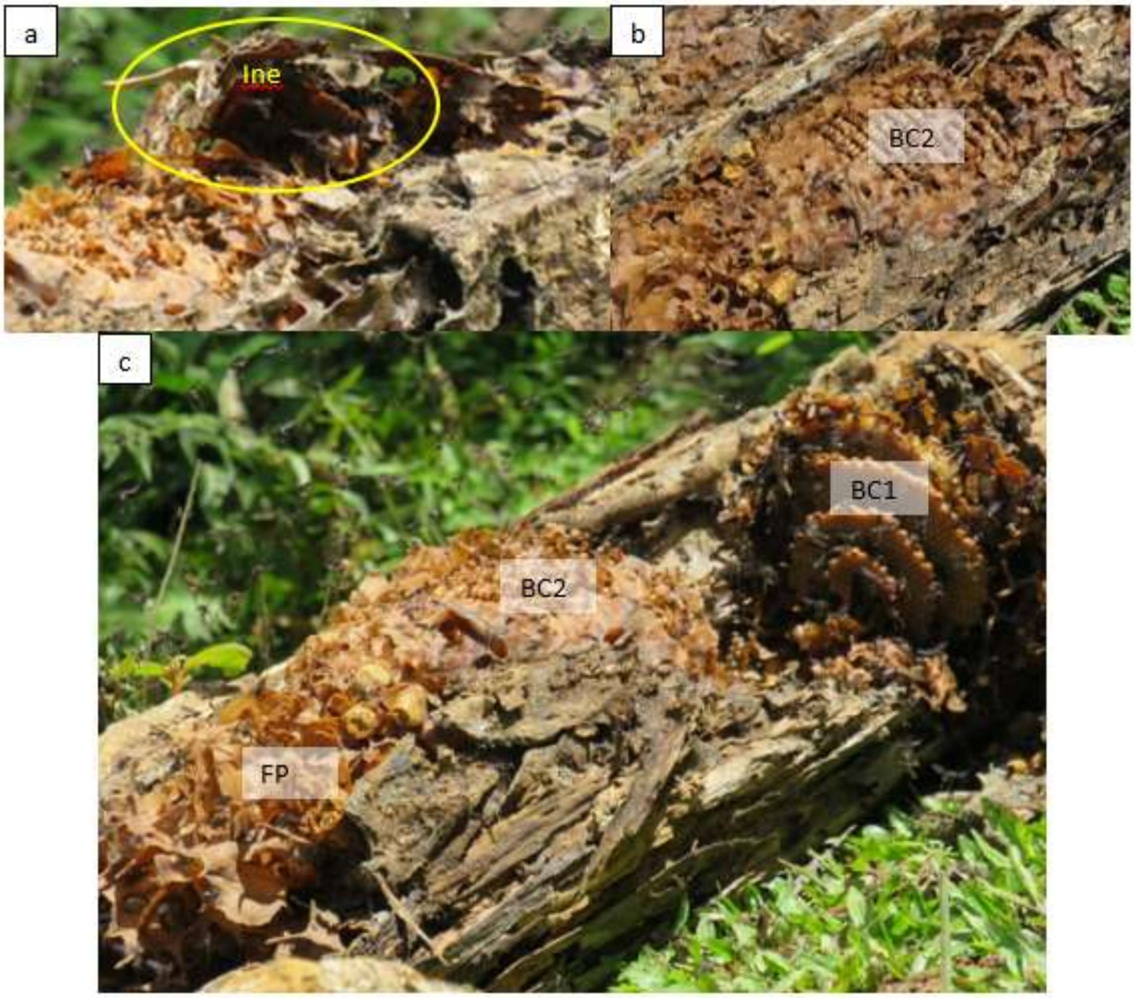

Figure 5. The nest arrangement of *W. incisa*; (Ine) internal nest entrances; (Bc1) first brood cell; (Bc2) second brood cell; (FP) food pots

Alike *T.* cf. *biroi*, honey and pollen pots were also adjoining the nest entrance. The food pot and some brood cells were indistinguishable due to the thin involucre covering it. In the other brood cell, they were seen to be separated and farthest from the nest entrance (Figure 3a). There were also visible pillars that connected and supported the brood cells and the food pots in the nest of *W. incisa* (Figure 5).

External Part Nest Entrance

The study results revealed that the nest entrance of *T.* cf. *biroi* has diversified shapes, sizes, and colors. Most of the nests found had circular, elliptical, wavy circular mouths and a hollow shape with a funnel narrowed in the middle. The color of the funnel also varied; there were black, dark brown, light brown, yellowish black, to bright yellow. Ornamentation in the form of a hardened resin was also found around the funnel base in some of the observed nests. The data disclosed a collection of resin similar in texture to the nest entrance funnel on the floor near the nest. The length of the funnel of *T.* cf. *biroi* ranged from 0.59 to 4.93 mm, and the diameter of the funnel ranged from 0.74 to 2.53 mm. In *W. incisa*, the length of the funnel ranged from 4.48 to 7.18 mm, and the diameter of the funnel was between 1.11 and 2.22 mm (Table 2). In each funnel mouth, 4–6 individual bees were found guarding the nest all day.

Brood Cell

Based on the measurements of brood or incubation cells, the diameter per cell of *T.* cf. *biroi* ranged from 1.08 to 2.23 mm, and the total diameter ranged between 7.53 and 19.57 cm. The height per brood cell ranged from 0.12 to 2.54 mm, and the total height recorded was between 11.08 and 22.61 cm. In *W. incisa*, the diameter per cell was between 4.96 and 5.7 mm; with a total diameter ranging between 23.1 and 27.3 cm; height per brood cell ranging between 2.96 and 3.41 mm; and a total height ranging between 32.78 and 35.22 cm (Table 2).

Variation in the cell shape of the *T.* cf. *biroi* was spiral (rounded comb shape and arranged in an upward spiral) (Figure 4a), irregular (Figure 4b), semispiral (comb shape looked square and hexagonal, arranged neatly scrolled sideways) (Figure 4c), semispiral (the comb shape looked triangular, and is placed in a circle and piled up) (Figure 4d), and semispiral (rounded comb shape, curved upward and sideways), connected and supported by short pillars attached to the wooden box (Figure 4e). Young brood cells were dark brown in color, while other brood cells were old and about to hatch, with light brown color.

Among the brood cells (prospective worker bees), there were queen cells with a diameter per cell in *T.* cf. *biroi*, ranging between 3.36 and 5.17 mm, and the diameter of the queen cells of *W. incisa* ranged from 6.47 to 8.03 mm or 2–3 times larger than the cells of prospective worker bees. Total 5 to 8 queen candidate cells were found in each colony, arranged within individual cell.

Honey and Pollen Pots

The honey pot was located at the front, adjoining the nest entrance. The pollen pot was attached to the honey pot and was situated at the back or bottom of the honey pot. In the spiral nest form (Figure 3a), the pollen and honey pots are surrounded by an involucre that was dark brown colored and layered. Based on the measurements, the pollen and honey pot's sizes were found identical in individual colonies. There was no difference in the size of pollen pots and honey pots at any observation location. Pollen pots and honey pots were found round equipped with a tip at the mouth that naturally changes sizes and closes when the pot begins to fill with honey or pollen.

Based on the honey volume and pollen weight measurements, the average honey volume per cell at the *T.* cf. *biroi* was recorded between 2.91 and 4.12 ml, and the pollen weight per cell was ranged between 1.19 and 2.62 g. The honey volume per cell in *W. incisa* was between 5.61 and 7.35 ml, and the pollen weight per cell ranged from 4.12 to 5.44 g. The total volume of honey of *T.* cf. *biroi* per colony on average was 800–1000 ml/2 months. Meanwhile, the volume of *W. incisa* honey per colony reached 2000–2500 ml/2 months. The taste of honey of *T.* cf. *biroi* in all locations was dominated by a sour taste, while *W. incisa*'s honey tasted bitter.

Table 2. Variation in the size of *T.* cf. *biroi* and *W. incisa*

Measured Character		**Unit**	**n**	***T.* cf. *biroi***					***W. incisa***
				Shape 1	**Shape 2**	**Shape 3**	**Shape 4**	**Shape 5**	**Shape 1**
Nest entrance (external)	Hole diameter (measured horizontally)	mm	10	2.13 ± 0.30	1.87 ± 0.15	1.28 ± 0.32	1.51 ± 1.02	0.89 ± 0.56	1.66 ± 0.56
	Hole diameter (measured vertically)	mm	10	3.42 ± 0.61	2.95 ± 0.59	2.5 ± 0.40	2.70 ± 1.01	1.76 ± 0.35	3.12 ± 0.11
	Nest entrance length	mm	10	3.56 ± 1.27	1.78 ± 1.19	3.16 ± 1.77	2.43 ± 1.09	2.16 ± 1.11	5.83 ± 1.35
	Nest hole shape and ornamentation	-	10						

									 
	Color	-	10	Light brown, dark brown, brownish yellow, light yellow, gray, brownish black					Black, dark brown, light brown
Brood Cell	Diameter per cell	mm	20	1.66 ± 0.57	2.01 ± 0.11	1.25 ± 0.17	1.36 ± 0.13	1.41 ± 0,21	5.33 ± 0.37
	Total diameter	cm	10	17.08 ± 1.15	10.16 ± 1.12	15.32 ± 4.15	11.12 ± 3.15	10.15 ± 2.62	25.21 ± 2.11
	Height per cell	mm	20	2.18 ± 0.13	2.13 ± 0.21	1.33 ± 1.21	1.51 ± 0.14	1.50 ± 0.11	3.53 ± 0.57
	Total height	cm	10	20.80 ± 1.60	19.03 ± 1.60	19.51 ± 3.10	15.33 ± 3.21	14.25 ± 3.17	34 ± 1.22
	cell arrangement shape		10	Spiral	Pill up	Semispiral	double decker	Spiral	Spiral
Queen cells	Diameter per cell	mm	20	4.70 ± 0.47	3.88 ± 0.22	4.01 ± 0.21	4.31 ± 0.55	3.79 ± 0.43	7.25 ± 0.78
	Height per cell	mm	20	5.27 ± 0.41	3.91 ± 0.32	4.68 ± 0.61	5.22 ± 0.28	4.05 ± 0.32	8.18 ± 0.44
	Number of cells per colony	unit	20	4–6	5–7	5–7	4–7	4–7	7–11
Pollen pot	Diameter per cell	mm	20	12.63 ± 0.51	10.34 ± 1.11	9.41 ± 0.15	8.19 ± 0.26	7.18 ± 0.24	18.22 ± 0.31
	Height per cell	mm	20	14.51 ± 0.92	12.31 ± 1.12	10.13 ± 0.21	9.71 ± 0.42	7.23 ± 0.22	19.11 ± 1.17
	Weight per cell	g	20	1.56 ± 0.33	2.23 ± 0.12	1.86 ± 0.67	2.17 ± 0.45	2.01 ± 0.24	4.78 ± 0.66
	Cell shape	-	10	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical
	Arrangement of cells	-	10	Pill Up	Pill Up	Pill Up	Pill Up	Pill Up	Pill Up

Honey pot	Diameter per cell	mm	20	12.26 ± 0.95	11.34 ± 0.21	9.21 ± 0.12	10.14 ± 0.20	8.21 ± 0.52	18.16 ± 0.18
	Height per cell	mm	20	12.91 ± 0.37	10.21 ± 0.13	10.11 ± 0.13	10.65 ± 0.32	9.12 ± 0.31	19.02 ± 0.77
	Volume per cell	ml	20	3.45 ± 0.39	3.76 ± 0.32	4.01 ± 0.11	3.76 ± 0.12	3.19 ± 0.28	6.48 ± 0.87
	Cell shape	-	10	Round	Round	Round	Round	Round	Round
	Cells arrangement	-	10	Pill Up	Pill Up	Pill Up	Pill Up	Pill Up	Pill Up

DISCUSSION

Stingless bees generally build nests in cavities (Eltz et al. 2002). The shape of the cavity determines the composition of the nest including the distribution of pollen, nectar, and larva storage cells (Alain et al. 2013). Stingless bees prefer building a nest in tree trunks, dead wood, rock gaps, brick walls, anthill of the tree, traces of anthill in the ground, empty nests attached to tree branches, and bamboo (Barbossa et al. 2013). The primary material used to build nests is resin retrieved from the tree trunks (Rasmussen 2008). Unlike *Apis*, the stingless bee group does not use wax to build a nest, nor do they use water to cool the nest's contents (Roubik 2006).

In contrast to *Apis*, which actively conducts thermoregulation to regulate the temperature in the nest to keep it optimal, the group of stingless bees regulates heat through brood cells. The brood cell area is the thermal core that stores the heat through the involucrum. The involucrum layer surrounding the brood cell retains the heat in the nest by sustaining the interior temperature (Engels et al. 1995; Roubik 2006). The brood cells of *W. incisa* and *T.cf. biroi* are spiral-shaped equivalent to *Trigona ventralis flavibasis,* which are also surrounded by a stratified involucrum (Chinh et al. 2005). However, the construction and presence of the involucrum layer at each location differ in accordance with the external temperature conditions, namely the soil temperature variance and the local environment (Lima et al. 2010; Kerr & Maule 1964; Wille 1983). However, in *Trigona fulviventric* colonies, the involucrum layer was absent even when it detected low soil temperature differential in the nest sites. Several species that do not have an involucrum have also been reported in species with clustered brood cells (Barbossa et al. 2013). A nest of T. *compress,* located in the Kalimantan region was found with no involucre separating the cutlets from the feed storage pots (Sakagami et al. 1989). Involucre, which coats the brood cells of *T. ventralis flavibasis* in Vietnam, has been reported to become thicker during winter (Sakagami & Yamane 1984). Low homeostatic temperatures can be a limiting factor in the geographic distribution of stingless bees outside the tropics, consequently influencing the nest's site selection (Chinh et al. 2005). However, we found different conditions in the observations of *W. incisa* retrieved from South Sulawesi region. Even though, situated at an altitude >800 mamsl with ambient temperatures reaching 16℃ in the early morning hours, the involucre in the brood cells is thinner and consists of only one layer compared to *T.* cf. *biroi*. The involucre texture of *W. incisa* was also softer than *T.* cf. biroi, which tends to be stiff and inflexible. A thin single-layer involucre was also reported in the species of Australia namely *Papua Plebeia australis* and *P. cincta*. These features are referred to as primitive characters due to their long-lasting adaptive patterns, thinning, and even loss of the involucrum (Michener 1961). The existence of *W. incisa* as a species endemic to the tropics allows constant acclimatization to the environment.

However, the shape of the *T.* cf. *biroi* stingless bees found in South Sulawesi is still uncertain. The five contrasting shapes of brood cells are presumed to be a member of *T.* cf. *biroi* or *biroi* group species. According to Franck et al. (2004), the nest architecture attributes are a significant part but not abundantly used to identify the species. A research conducted by Franck et al. (2004) on the *Carbonaria* species group revealed diversification in the comb shapes between individual species group. The neatly arranged spiral shape of the *T. carbonaria* brood cells was the most developed nest architecture among other species. Meanwhile, the irregularly arranged brood cells shape is assumed to be a characteristic of the descendent of the *Carbonaria* group found in the Southeast Asia region (Michener 1961; Sakagami et al. 1983; Starr & Sakagami 1987).

The stingless bee nest consists of a nest entrance, food storage pots, and brood cells. The pollen and nectar (honey) collected by worker bees are stored in food storage pots in the nest, while the resin is used to build the nest. Honey and pollen are stored in individual pots along with additional secondary reserve, when the number and types of plants around the nest are reduced (Roubik 2006). Food storage pots come in various sizes and shapes, including round, oval, and cone. The shape of the nest also varies, including, spiral, stacked, layered, and clustered. The nest entrance not only acts as the primary defense of the colony against natural antagonists, but also assists in physicochemical regulations. Nest entrance properties (shape, color, size, and texture) vary and are determined by microclimatic factors, constituent resins, colony age, and natural predators (Roubik 2006). Conventionally, the long sticky nest entrance funnel serves as a ventilator, oxygenating through the air in the ground. It also performs as the first line of defense against natural enemies penetrating the nest.

The study concluded diverse variations in the sizes of nest entrance funnels of species *T. cf. biroi* and *W. incisa*. Sayusti et al. (2020) also reported high variations in the nest entrance of stingless bee nests in South and West Sulawesi region. Differential nest entrance is affiliated to the types of natural predators around the nest. To illustrate, species *W. incisa*, design their nest entrances that are thick and unsusceptible to attacks caused by mountain rats and other types of natural enemies found large in the vicinity of the mountains. Similarly, species *T.* cf. *biroi*, known for its thinner, stickier, and softer funnel, acclimatizes its funnel design in accordance with the type of natural enemies when situated in the lowlands. The sticky composition of the nest entrance funnel made from the resin assists in trapping tiny organisms such as beetles, flies, ants and others from penetrating further inside the nest (Roubik 2006; Lehmberg et al. 2008). However, in some cases, natural predators such as Nitidulidae have managed to penetrate the securities at the bee nest entrance due its ability to move quickly to evade the aggregation of worker bees (Wenning 2001). Resin and its residues strategically placed near the nest entrance funnel not only assists in reducing susceptibility to natural predators, but it also inhibits the weed growth blocking entry into the nest. (Wille 1983; Barbossa et al. 2013).

In addition, the resin composition aids to guide the worker bees to return to the nest (Lacerda et al. 1998). In the genus *Tetragonula*, the external nest entrance funnel for the nest cannot be identified as the genus, the species material. In the Sumatra region, the data established that *Trigona (Tetragonula) laeviceps* has primarily no external and internal nest entrance funnels; however, another observation at the same location found *T. laeviceps* creating a funnel at its external nest entrance (Sakagami et al. 1983).

CONCLUSIONS

The research established an eminent diversification in the nest entrances and the shape of brood cells of *T. cf. biroi* in the Baebunta district, the Bontocani district and the South Sulawesi region of Indonesia. On the other hand, *W. incisa* was discovered with only one type-spiral brood cell form. The nest entrance of *W. incisa* also only consisted of one distinctive type which was thick and hard in texture. Natural antagonists of both species influenced the type of nest entrance in both species.

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