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Morphological Characteristics and Nest Structure of Stingless Bee (*Heterotrigona itama*) from Different Meliponiculture Practices

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Abstract. Exploration of *Heterotrigona itama* biology characteristics besides being valuable for health and biodiversity also beneficial as well as an environmental bioindicator. This research aims to evaluate morphology characteristics and nest structure of stingless bee *Heterotrigona itama* from three different meliponiculture practices (MP1:meliponiculture in the back yard; MP2:meliponiculture in the rubber garden and MP3: meliponiculture in the side yard) in Banjar Regency, Indonesia. The parameters studied are morphology, nest design, colony structure, and environmental suitability. One-way ANOVA at a 95% confidence level was used to analyze differences between parameters. The results show *Heterotrigona itama* body size average from rubber gardens is bigger (7.11±0.60 mm) than the side yard (6.79±0.34 mm) and back yard (6.64±0.46 mm). The degree of difference is visible in the fore leg ($\alpha=0.00<0.05$) and hind leg ($\alpha=0.004<0.05$). The nest structure in MP3 was significantly different than MP1 and MP2, especially in terms of funnel length ($p=0.007<0.05$) and nest height from the ground ($p=0.000<0.05$). Environmental conditions MP3 (temperature 31.69±1.93 0C, humidity 60.70±11.2 % and water source) is more supportive for meliponiculture than MP2 (disturbed by household waste) and MP3 polluted by chicken farms. Meliponicultures practices based on various locations and environmental characteristics influence several parameters of Heterotrigona itama morphology and nest structure.

Keywords: characteristics; *Heterotrigona itama*; meliponiculture; morphology; nest structure

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INTRODUCTION

Beekeeping has been carried out throughout the world from ancient times until now because making a nest does not require electricity so it can be placed in any location (Girotti et al., 2020). This practice plays a fundamental role in the maintenance of biodiversity and food security (Adler et al., 2023). In the last decades, the production of medicinal honey, propolis, and its derivatives from stingless beekeeping has been increasingly developed in Indonesia (Tamizi et al., 2020). For this reason, Indonesia needs to develop beekeeping by improving existing natural ecosystems and artificial green environments as food sources and also promoting native stingless bees (Kahono et al., 2018). It has been practiced for centuries by pre-colonial populations and has been gaining adepts in recent years as an alternative for conservation that promotes sustainability (Barbiéri & Francoy, 2020).

Meliponiculture refers to the practice of rearing stingless bees (Apidae: Meliponini)for various purposes, such as honey production, pollination, and conservation. This social bee (>500 species) with a pantropical distribution distribution spanning South and Central America, Africa, India, Australia, and Asia (Bueno et al., 2023). Meliponiculture is an ecological perspective to save species from extinction (Syafrizal, Kusuma, et al., 2020), because it encompasses the interrelationship between different elements of ecosystems and is therefore ideal for analyzing and questioning the impact of human actions (Maya et al., 2023). Meliponiculture is generally done with a topping system, by placing stup boxes on top of Wooden logs containing bee colonies (Askary et al., 2022). In Banjar Regency of South Kalimantan Province, Indonesia meliponiculture is also experiencing rapid development and winning the first best award in the category of Farmer Economic Institutions, especially for honey bee cultivation (Setiawan, 2021).

Heterotrigona itama are a species of stingless bees that are commonly studied in Meliponiculture, and have a natural habitat in Kalimantan (Borneo island) (Wahyuningtyas et al., 2021). It was a favorite among farmers because of the quality/quantity of the honey and the easy of cultivation (Syafrizal, Ramadhan, et al., 2020). This species also stands as a captivating subject in the realm of entomology. It also offers a nuanced exploration of their intricate colony structure and nest characteristics. But the identification is difficult because of the cryptic species phenomenon, and morphological similarities between species (Azizi et al., 2020). The main factors in colony characteristics of the *H. itama* can be signed as colony size and the overall nest structure. In terms of colony size, this species is highly social in which the colony members are subjected to labor division where a queen functions as the reproductive caste (Tamizi et al., 2020). These colonies are relatively small compared to other bee species, but they typically consist of a few hundred to a few thousand worker bees. The number of worker bees can vary depending on factors such as the availability of resources and the age of the colony, so it is estimated that the average worker bee will adapt its body to environmental conditions (Sauthier et al., 2017).

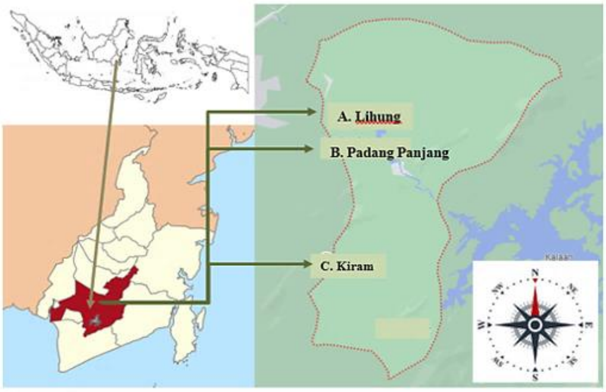
The nest structure of *Heterotrigona itama* colonies can also vary depending on the Meliponicultures employed. Three common nests are used in Meliponiculture, namely: 1) traditional log hives, 2) wooden box hives, and 3) modern hive designs. Traditional wooden hives in the form of hollow wood are used as nesting places for stingless bees, while wooden box hives are man-made structures designed to mimic natural nesting conditions. Modern hive designs often incorporate innovative features to enhance beekeeping practices and colony management (Askary et al., 2022). Hollow tree trunks were the initial colony hives with diverse diameters, farmers added wooden boxes on the top to accommodate the bee colony. Artificial hives were made of box-shaped pieces covered by plastic to control harvesting (Syafrizal et al., 2020).

This research aims to evaluate colony characteristics and nest structure of *Heterotrigona itama* from three meliponicultures practices, especially that install in the side yard, back yard, and rubber garden in Banjar Regency of South Kalimantan, Indonesia. This research is very important, apart from helping to develop agricultural cultivation as a livelihood for the population and also as preserving the agricultural landscape which is the supporting capacity for tourism (Peraturan Daerah Kabupaten Banjar Nomor 07 Tahun 2009, 2009). The study of variations in colony organization and nest attributes of stingless bees reared in Meliponicultures is an excellent mediator for exploring the complexity of the relationship between humans and bees. It's also a great way to demonstrate how biodiversity supports the development of ecosystem services through learning and culture (Maya et al., 2023).

METHODS

Study Site

This research was carried out from August – October 2023 in three meliponicultures practices in Banjar Regency of Kalimantan Selatan Province, Indonesia. Selecting a location for meliponiculture requires careful consideration because environmental conditions affect the health and productivity of honey bees (Harianja et al., 2023). Three villages were chosen as pilot projects for the development of Farmer Economic Institutions in Banjar Regency, Indonesia, namely 1) Lihung Village for meliponiculture development in backyards or MP1. 2) Padang Panjang Village for meliponiculture development in rubber plantations or MP2, and 3) Padang Panjang Village for meliponiculture development in side yards or MP3 (Figure 1). MP1 is located behind the beekeeper's house with a cultivation area of around 500 m2 and 25 colonies of *H.itama*. MP2 is located about 1 km from the beekeeper's house in a rubber plantation with a cultivation area of 9.800 m2 and a larger number of *H.itama* colonies around 75 nests. MP3 is located side the beekeeper's yard with a cultivation area of about 300 m2 with 30 *H.itama* colonies. The purposive sampling method was used for data collection. 10 colonies were selected from each meliponiculture practice based on suggestions from stingless beekeepers. Dependent variables are morphology characteristics, nest structures, and environmental conditions.



Sites (Village)	Coordinates	Meliponiculture practices	
Lihung	3°26'09.3"S 114°55'37.1"E	Back yards	MP1
Padang Panjang	3°28'33.3"S 114°55'21.4"E	Rubber garden	MP2
Kiram	3°32'58.7"S 114°55'34.6"E	Side yards	MP3

Figure 1. Map showing the location of Banjar Regency in Borneo, Indonesia (inset), sampling location of *Heterotrigona itama* from Meliponicultures (private collection, 2023).

Procedures

Morphological analysis. Samples were taken from each nest which must agreed upon by the stingless beekeeper (Saufi & Thevan, 2015). Stingless bee capture is carried out by placing an individual plastic bag (12 cm in width and 25 cm in length) in front of the nest hole (Lamerkabel et al., 2021). The collected samples were then put into a glass bottle with 70% ethanol, then pinned and oven-dried at 40°C overnight (Kelly et al., 2014). The specimens were identified in the Laboratory of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, Indonesia. Morphological analysis is limited to the fourteen parameters: body length, head length and width, antenna length, eye length, and width, fore wing length and width, hind wing length and width, fore leg and hind leg, antenna shape, and body color. The specimens were identified with the taxonomy keys provided by (Engel et al., 2023), (Trianto & Purwanto, 2020), and (Azmi et al., 2019). Morphometric measurements were performed using a Stereo Swift Microscope type SM95-SM90CL with an Optilab viewer and Image Raster software.

External nest characteristic. The external nest (hive) of stingless bees can be identified by measuring the dimensions of the wooden log, topping, in-out funnels, and the distance of the funnel from the ground (Pangestika et al., 2020). The external nest characteristics, including the height of the tree trunk, the trunk circumference at the top and bottom, and the height of the entrance tube from the bottom, were measured together (Kelly et al., 2014)

Nest entrance characteristic. The nest entrance is a tunnel for colony members to leave and return. The shape, diameter, width, and height of their openings; the shape and length of their external entrance; and their ornamentation and color were used to characterize nest entrances (Purwanto et al., 2022). The dimensions (width and height) and length of the entrance tunnel above the nest to the ground level were measured using a measuring tape. A digital caliper was used to measure the height of the external entrance above the ground, the diameter of the entrance, and the thickness of the tube (Hora et al., 2023).

Internal nest characteristic. The internal nest characteristics and differentiation in stingless bees can be seen from the arrangement, position, and size (Sriwahyuni et al., 2023). The variations in nest size within the species could be related to many factors, such as microclimate, predators, and the foraging activities of the species. Understanding the nesting habitat and substrate preferences, not only helps to characterize the species but also helps the development and providing important information for sustainable colony management (Hora et al., 2023).

Brood Cell Characteristics. The shape of brood combs and storage pots, their arrangements and colors, as well as their placements in the nest, were carefully examined and recorded, particularly for the ground nesting one. Brood comb diameter, comb thickness, pillar height, pillar thickness, worker cell depth, and width were analyzed. To get the average width of a single cell, the widths of 10 cells were measured and the result was divided by 10. This close examination was conducted only for 14 colonies, as the method is destructive (Hora et al., 2023).

Environmental Condition. Temperature and humidity availability are measured to determine environmental suitability for Meliponicultures. Temperature control within the beehive is critical to stingless bee survival (Ali et al., 2021). The Digital Anemometer model AS816+ is used to measure the temperature, while the Digital Hygrometer model CX-0726 measures humidity.

Data analysis. The morphology, colony characteristics, and nest structure of *Hetrorotrigona itama* from each Meliponiculture were described (Pangestika et al., 2020). One-way ANOVA tests at a 95% confidence level were used to identify significant differences between quantitative data. The next step was to use the Tukey HSD test to compare all pairs of treatment means.

RESULTS AND DISCUSSION

Morphological characteristics. Figure 2. Explain about *Heterotrigona itama* morphology characteristics from meliponiculture practices in Banjar Regency. Fig.2A From a lateral view this stingless bee is predominantly black, head with a flagellomere segment that is blackish, black clypeus, long malar space, mandible: one weak tooth, thorax is black, mesonotum is wholly black, coarser, and covered with long setae at anterior (Azizi et al., 2020). Fig.2B Frons are fully covered with fine white hair and are slightly thick white hairs approaching the clypeus, scutellum is short, reaching back only to the metanotum. thorax (lateral): short mesoscutellum and thorax (dorsal): mesoscutum has no hair pattern, abdomen: smooth and shiny propodeum. Fig.2C. Wing: The fore wing has sub-marginal cells. The fore wing color is uniform, wing venation is dark brown and semi-transparent, and the hind wing with seven hamuli. Fig.2D. Leg: hind basitarsus elliptical disc and hind tibia plumose hair. The posterior fringe of the hind tibia is simple (unbranched). An elliptical disk on the inner basitarsus is absent (Purwanto et al., 2022).

Figure 2. *Heterotrigona itama* morphology characteristics; A. Lateral view; B. Frons; C. Fore wing and Hind wing; D. Foreleg and hind leg; Scale bar: 5 mm (private collection, 2023).

Based on the morphological identification it was known the stingless bees from meliponiculture practices in Banjar Regency is *Hetrorotrigona* itama. The body color of the worker bee is predominantly black. Thorax is black. The mesonotum is wholly black, coarser, and covered with long setae at the anterior. Scutellum is short, reaching back only to metanotum. The abdomen is uniformly black from tergites 1-6 (Purwanto et al., 2022). The forewings and hindwings are black, wing venation is dark brown and semi-transparent. Hind tibiae and basitarsi are entirely black. The number of hamuli is 7 per hindwing (Trianto & Purwanto, 2020).

Table 1 below explains there are five morphometric characteristics of *H. itama* from MP2 (Meliponiculture in the rubber garden) is larger than *H itama* from MP1 (Meliponiculture in the back yard) and MP3 (Meliponiculture in the side yard). These five parameters include body length (mean at 7.11±0.60 mm), head length (mean at 2.43±0.21 mm), antenna length (mean at 3.10±0.30 mm), fore wing width (mean at 2.07±0.29) and hind wing width (mean at 1.13±0.21). There are also ten parameters of *H. itama* morphometric characteristics from MP1 (meliponiculture in the back yard) is bigger than *H.itama* from MP1 (meliponiculture in the rubber garden) and MP1 (meliponiculture in the side yard). These ten morphometrics parameters are: 1) head width: 2.08 ± 0.22 mm, 2) eye length:1.49 ± 0.11 mm, 3) eye width:0.62 ± 0.09 mm, 4) gena width:0.60±0.08 mm, 5) thorax length: 2.06±0.21 mm, 6) thorax width at 1.88±0.27 mm, 7) abdomen width at 3.32±0.26

mm, 8) fore wing length at 5.99±0.49 mm, 9) hind wing length at 4.07±0.36 mm, and 10) hind leg at 6.54 ± 0.92 mm.

From the Anova test, it shows that the average length of fore legs (p = 0.000; p <0.05) and hind legs (p = 0.002; p<0.05) from three meliponiculture sites were significantly different. Multiple Comparison tests using Tukey HSD concluded that the foreleg length is different (p=0.000) between MP1 (Meliponiculture in the backyard) and MP2 (Meliponiculture in the rubber garden). The length of hind legs is different (p=0.001) between MP1 (Meliponiculture in the back yard) and MP3 (Meliponiculture in the side yard). The gena width is different (p=0.029) between MP1 (Meliponiculture in the backyard) and MP3 (Meliponiculture in the side yard).

Table 1. Morphometry characteristic of *Heterotrigona itama* (mm) N=30

Morphometric Parameters	MP1		MP2		MP3		Anova
	Range	Mean ±SD	Range	Mean±SD	Range	Mean ±SD	
Body Length	6.03-7.23	6.79 ±0.34	6.22-8.11	7.11±0.6	5.2-7.22	6.64 ±0.46	0.096
Head Length	2.11-2.63	2.38 ±0.18	2.11-2.73	2.43±0.21	2.24-2.42	2.34±0.08	0.447
Head Width	1.73-2.35	2.08±0.22	1.12-2.33	1.78±0.42	1.74-2.35	2.08±0.28	0.070
Antenna Length	2.42-3.22	2.95±0.29	2.53-3.52	3.1±0.3	2.42-3.11	2.82±0.24	0.105
Eye Length	1.22-1.62	1.49±0.11	1.24-1.53	1.45±0.11	1.22-1.54	1.43±0.11	0.541
Eye Width	0.50-0.71	0.62±0.09	0.41-1.11	0.6±0.21	0.43-0.62	0.54±0.06	0.355
Gena Width	0.41-0.63	0.5±0.08	0.42-0.72	0.55±0.1	0.48-0.70	0.6±0.08	*0.037**
Thorax Length	1.63-2.22	2.06±0.21	1.74-2.15	1.96±0.15	1.68-2.12	1.94±0.16	0.268
Thorax Width	1.51-2.24	1.88±0.27	1.62-2.32	1.84±0.27	1.60-2.12	1.81±0.23	0.826
Abdomen Length	1.88-2.32	2.03±0.11	1.68-2.41	2.03±0.25	1.68-2.21	2.21±0.2	0.374
Abdomen Width	2.71-3.52	3.32±0.26	2.51-3.80	3.18±0.42	2.52-3.82	3.2±0.43	0.658
Fore wing Length	5.08-6.45	5.99±0.49	5.21-7.34	5.97±0.61	5.32-6.32	5.7±0.37	0.362
Fore wing Width	1.53-2.21	1.89±0.24	1.61-2.53	2.07±0.29	1.65-2.14	1.98±0.18	0.269
Hind wing Length	3.32-4.52	4.07±0.36	3.62-4.32	4.05±0.24	3.36-4.20	3.92±0.31	0.522
Hind wing Width	0.81-1.12	0.95±0.11	0.82-1.51	1.13±0.21	0.78-1.12	0.99±0.15	0.053
Foreleg Length	3.62-7.32	5.36±1.07	5.09-5.51	5.29±0.17	6.53-7.44	6.95±0.32	*0.000**
Hind Leg Length	5.21-8.11	6.54±0.92	4.71-7.51	5.92±0.79	4.23-6.32	5.15±0.58	*0.002**

Morphometric Parameters	**Multiple Comparison**	**Tukey HSD**
Fore leg length	MP1 – MP2	0.000*
Hind leg length	MP1 – MP3	0.001*
Gena width	MP1 – MP3	0.029*

Note: MP1:meliponiculture in the back yard; MP2:meliponiculture in the rubber garden; MP3:meliponiculture in the side yard; SD: Standard Deviation. * significant difference between (p<= 0.050)

Stingless bee (*H. itama*) morphological characteristics based on (Saifullizan et al., 2021) research study in Terengganu, Malaysia has a body length of 4,860–7,823 mm, head length of 1,179-2,152 mm, head width at 1.743-2,557 mm, and thorax width at 1,809-2,329 mm. Higher values were obtained for body length (5.82-8.11 mm) and head length at 2.11-2.73 mm when compared with this study results. Meanwhile, a smaller value was obtained for head width (1.12–2.35 mm), and thorax width (1.51–2.32) mm.

However, based on research (Azizi et al., 2020), it was found the body length of *H. itama* in Belitung was smaller than in this study (5.04 ± 0.35 mm). Based on Purwanto et al., (2022) research study from meliponiculture in South Kalimantan shows a body length of 6.1±0.2 mm, width of the gena at 0.405±0.002 mm and width of the eye at 0.689±0.001 mm. When compared with this study results, higher values were obtained for body length at 6.68±0.98 mm, the gena width at 0.58±0.14 mm, and the eye width at 0.57±0.12 mm.

H. itama has two pairs of wings (fore wings and hind wings) attached to the mesothorax and metathorax respectively. Based on (Azmi et al., 2019) research study, the wing size of *H. itama* is 4.24±1.03 mm. When compared with the results of this study, higher values were obtained for the fore wing length (5.89±0.50 mm); the fore wing width (1.98±0.24 mm); hind wing length (4.01±0.30 mm), and the hind wing width (1.02 ± 0.17 mm). Leg parts of *H. itama* are covered with sensillum (a sense organ in insects, typically consisting of a receptor organ in the integument connected to sensory neurons). A research study by Azmi et al., (2019) from Tasik Kenyir, Terengganu found the average leg length of stingless bees was 4.25±0.05 mm. Higher values can obtained from this study where the front leg length is 5.86 ± 1.00 mm and the hind leg length is 5.87 ± 0.94 mm.

External nest characteristics. Stingless bee cultivation for honey production, especially the *Heterotrigona item* is relatively new in Malaysia and Indonesia (Tamizi et al., 2020). Generally, cultivating stingless bees is carried out in a nest in a box or topping to make management easier (De Carvalho et al., 2014). Hollow tree trunks type are usually used by the aboveground nesting stingless bees to build their nests (Assefa et al., 2021), and this traditional method of meliponiculture is preferred by most farmers (Syafrizal,et al., 2020). The first step to cultivate is removing the original part of the tree trunk or branch cultivation nest (Cortopassi-laurino et al., 2020), then stingless bee colonies in wooden logs moved to the stop box or topping (Istikowati et al., 2019). Figure 3. A shows *Heterotrigona itama* external nest characteristics from three meliponiculture practices in Banjar Regency, Indonesia. Fig 3. B Wooden log with the entrance nest. Fig 3.C. Topping as the storage box for brood cells and honey or pollen pot.

Figure 3. A. Stingless bee of *Heterotrigona itama* nest design; B. Wooden log or hollow tree trunks for nest cultivation; C. Wooden topping. Scale bar: 5 cm (private collection, 2023).

Table 2 below explains the *Heterotrigona itama* nest structure from three meliponiculture practices in Banjar Regency. Four parameters were measured of nest structure, namely: wood log volume, topping volume, nest height to the ground, and distance between nest (m). The largest wooden log size was found in MP2 (Meliponiculture in the rubber garden) at 38.88±24.39 liters. The largest topping volume comes from MP1 (Meliponiculture in the backyard) at 9.92-21.85 liters. The farthest nest height from the ground was found in MP2 (Meliponiculture in the rubber garden) at 35.00-86.00 cm. Anova test showed differences in wooden log volume (p=0.000), and nest height from the ground (p=0.000). Multiple comparisons test using Tukey HSD showed a significant difference in wooden log volume between MP1 and MP2 (p=0.000), and also between MP2 and MP3 (p=0.018).

Table 2. External nest characteristics of *Heterotrigona itama*

Parameters	MP1		MP2		MP3		Anova
	Range	**Mean±SD**	**Range**	**Mean±SD**	**Range**	**Mean±SD**	
Wooden log volume (Liters)	3.18-13.85	6.91±2.79	7.96-66.87	38.88±24.39	7.12-32.24	19.44±7.54	0.000
Topping volume (Liters)	9.92-21.85	15.65±3.75	8.62-18.48	14.13±2.98	12.25-17.32	15.28±1.64	0.488
Nest height to the ground (cm)	30.00-58.00	42.8±8.64	35.00-86.00	60.8±17.61	5.00-53.00	23.6±15.05	0.000
Distance between nest (m)	2.30-4.00	3.38±0.56	7.00-60.00	18.60±15.76	2.00-4.00	2.80±0.63	0.001

Parameters	Multiple Comparison	Tukey HSD
Wooden log volume	MP1 – MP2	0.000*
Nest height to the ground	MP1 – MP3	0.001*
Distance between nest	MP1 – MP2	0.002*
Distance between nest	MP2 – MP3	0.002*

Note: MP1:meliponiculture in the back yard; MP2:meliponiculture in the rubber garden; MP3:meliponiculture in the side yard; SD: Standard Deviation. * significant difference between (p<= 0.050)

Beekeeping for stingless bees is generally done with a topping system, by placing stup boxes on top of wooden logs containing bee colonies with a door hole on one side for storing honey (Harjanto et al., 2020). The stup developed in a single stup model. The stup box is made of dry wood planks free of chemicals and odorless. The wood planks are selected based on the level of durability. Board thickness measures ± 1.5 cm – 2 cm, with dimensions that can be adjusted as needed. The connecting hole with ±2-3 cm in diameter will be glued using propolis and leave a small space for bees' entry and exit (Askary et al., 2022). Many of the hives stay closed and sealed by using the propolis made by the bees (Fisher, 2008). From this research, it was discovered that all meliponiculture practices still use topping systems with wooden logs type, namely: Coconut (*Cocos nucifera*), rubber (*Hevea brasiliensis*), jackfruit (*Artocarpus heterophyllus*), cempedak (*Artocarpus integer*) and meranti (*Shorea* Sp.). The beekeepers in the rubber garden have succeeded in dividing the colony, but remain using wooden logs.

For meliponiculture purposes, based on (Askary et al., 2022) the wooden logs were cut ±1 meter on the right and left sides and the colony can still be seen. If the average diameter of a forest log is around 40 cm, the volume will reach 125.6 liters. When compared with the observation results, the wooden log size from meliponiculture in this research is relatively small with a volume proportion is < 31.2% from 125.6 liters. The highest average wooden log volume was found in MP2 (Meliponiculture in the rubber gardens) at an average value of 38.88±24.39 liters. The topping size needs to be the right size, if too small the propolis and honey will melt, but if it is too big then the mold can grow quickly. It is known that bees associate with a variety of symbiotic fungi that can influence their behavior and health (Rutkowski et al., 2023). The optimal size of the topping is dependent on stingless bee species, but typically these volumes range wooden a of box about 1 - 4 liters. (Fisher, 2008). Recommended size of stup or topping based on Askary et al (2022) opinion is 30 x 40 x 5 cm, or with a volume of around 6 liters. However, in this study, various topping sizes exceeded this size. The largest average topping size was found from MP1 (Meliponiculture in the backyard) with an average volume of about 21.45±3.08 liters.

Stingless bee colonies not only breed when they divide to produce new colonies, but they also reproduce their principal nest structures and contents (Roubik, 2020). Nesting substrates, nest architecture, and nest biology data are of utmost importance for future designing suitable bee hives for keeping both species and also to conserve the natural nesting sites for their future utilization in pollination (Hora et al., 2023). The average nest height from the ground according to Pujirahayu et al., (2020) in the Halu Oleo University Campus Forest Area is around 3.4 m. When compared with this study's results, turns out the lower value. The highest average value can be found in MP2 (meliponiculture in the rubber gardens) at 60.80 ± 17.61 cm. Aside from materials for nest construction (resin, nectar, and pollen), bees also diligently collect water, for temperature control in colonies (Michener, 2007).

Nest entrance characteristic. Nest existence is indicated by a building that functions as the entrance to the nest. It not only serves as a pathway for entry and exit but also serves as a marker for the nest (Febrianti et al., 2020). The nest entrance is a specific characteristic (Sriwahyuni et al., 2023) and tingless bees construct a unique, funnel-shaped entrance that resolves an evolutionary conflict between foraging efficiency and defense (Shackleton et al., 2019). Figure 4 explains the nest entrance ornamentation of *H. itama* from three meliponiculture practices in Banjar Regency, Indonesia. Fig 4.A Funnel-shaped entrance with spreading of propolis. Fig. 4. B Funnel-shaped entrance with a droplet of propolis. Fig. 4. C Cylindrical tube-shaped entrance with irregular ridges.

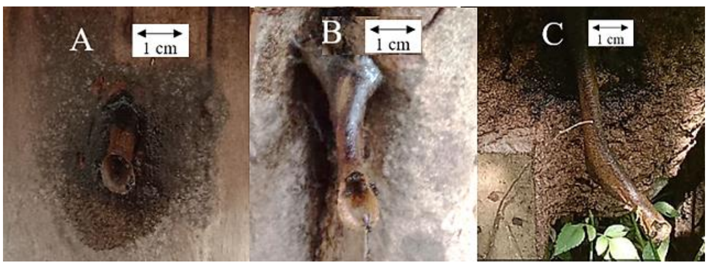


Figure 4. Nest entrance ornamentation of *Heterotrigona itama*: A. Funnel-shaped entrance with the spread of propolis, B. Droplet of propolis, and C. Cylindrical tube with Irregular ridges. Scale bar: 1 cm (private collection, 2023).

Table 3 below explains the *Heterotrigona itama* nest entrance characteristics from three meliponiculture practices in Banjar Regency, Indonesia. The nest entrance types of *H. itama* species are funnel and cylindrical tubes (Purwanto et al., 2022). From Table 3 known that the largest entrance nest size was found in MP3 (Meliponiculture in the side yard) at 1.59 ± 0.45 cm and length at 16.95 ± 14.50 cm. The ANOVA test showed a significance value for entrance length (p=0.007), but not for entrance diameter.

Table 3. The mean and standard deviation of *Heterotrigona itama* nest entrance characteristics

Num	Parameters	Unit	MP1	MP2	MP3
1.	Entrance diameter (cm)	Range	0.40-2.10	1.10-1.70	1.10-2.20
		Mean±SD	1.38±0.40	1.36±0.26	1.59±0.45
		Anova	0.469		
		Tukey HSD	None		
2.	Entrance length (cm)	Range	0.50-8.00	2.00-15.0	16.95±14.50
		Mean±SD	3.90±2.39	6.65±4.50	16.95±14.50
		Anova	0.007*		
		Tukey HSD	MP3-MP1 (p=0.008*), MP3-MP2 (p=0.039*)		

Note: MP1:meliponiculture in the back yard; MP2:meliponiculture in the rubber garden; MP3:meliponiculture in the side yard; SD: Standard Deviation. * significant difference between (p<= 0.050)

The large outer entrance allows many foragers to pass while the narrow inner entrance requires few guards to defend. This structure has given rise to remarkable behavior in returning foragers, who appear to approach the nest entrance at high speed and 'crash' head first into the entrance (Shackleton et al., 2019). The observation revealed that the funnel entrance shape is more prevalent in meliponiculture practices in Banjar Regency. There was no cylindrical tube entrance shape with irregular ridges in MP1 (meliponiculture in the back yard), but in MP3 (meliponiculture in the side yard) can be found in quite large numbers. Purwanto et al., (2022) said that the entrance of *H. itama* is large with a diameter of 1.59 - 2.25 cm and a length of 1.74 - 2.50 cm, and it is surrounded by soft and hard propolis. When compared with the results of this study, the entrance diameter from all meliponicultures practices is lower with an average value of 0.20 - 2.20 cm.

Based on Sayusti et al., (2021), there are six types of entrance openings, i.e.: 1) irregular, 2) round, 3) oval, 4) ellipse, 5) triangle, and 6) longitudinal slit. Then three types of entrance shapes i.e.: 1) funnel, 2) cylindrical tube, and 3) slit. The last is five types of propolis ornamentation types i.e.: 1) irregular ridges, 2) spread, 30 mounds, 4) lamellate, and 5) droplets. Purwanto et al., (2022) also said that a cylindrical tube opening was found in Hulu Sungai Selatan District, South Kalimantan, which consists of one ornamentation type, an enlargement mount of propolis with black color.

Based on Table 4, almost entrance shape meliponiculture practices in the funnel form. The highest value, namely: 100% from MP1(Meliponiculture in the back yard); 70% from MP2 (Meliponiculture in the rubber garden), and 50% from MP3 (Meliponiculture in the side yard). For entrance ornamentation, the spread of propolis is mostly (±70%) found in MP1; droplets of propolis are evenly found in all meliponiculture practices, and irregular ridges are most commonly (±50%) found in MP3. For entrance color, only brown and dark brown dominate with a range value between 40-60%. For entrance rigidity, it shows the balance between soft and hard with a ratio of 40:60, 60:40, and 50:50.

Table 4. Entrance types of *Heterotrigona itama* from meliponiculture practices

Num	Parameters	Unit	MP1 (%)	MP2 (%)	MP3 (%)
1.	Entrance Shape	Funnel	100	70	50
		Cylindrical tube	0	30	50
2.	Entrance Ornamentation	Spread of propolis	70	40	30
		Droplet of propolis	30	30	20
		Irregular ridges	0	30	50
3.	Entrance Color	Brown	40	60	50
		Light brown	20	0	0
		Dark brown	40	40	50
4.	Entrance Rigidity	Soft	60	40	50
		Hard	40	60	50

Note: MP1:meliponiculture in the back yard; MP2:meliponiculture in the rubber garden; MP3:meliponiculture in the side yard; SD: Standard Deviation. * significant difference between (p<= 0.050).

Internal nest characteristics. The nest internal shape of stingless bees can be used as a biological indicator of colony strength. According to beekeepers in Banjar Regency, Indonesia, stingless bee colonies can be categorized into three groups: weak, strong, and super, depending on their internal nest condition. Weak colonies are characterized by a small number and activity in the nest. A strong colony is characterized by the large number and activity in the nest. Meanwhile, super colonies are characterized by a very large number and activity of bees, exceeding strong colonies.

Figure 5 shows Various forms of *Heterotrigona itama* internal nests from meliponiculture practice in Banjar Regency, Indonesia. Fig.5A. Super colony nest with brood cells arrangement surrounded by honey and pollen pots and root-like structure of cerumen. Fig.5B. Super colony nest surrounded by honey and pollen pot arrangement and covered by involucrum and batumen or the external involucrum (Shanahan & Spivak, 2021). Fig.5C. Strong colony nest arrangement, with honey and pollen pots and covered by a root-like structure of cerumen. Fig.5D Strong colony nest arrangement, with honey and pollen pots and covered by involucrum and batumen. Fig.5E Weak colony nest arrangement, with a little honey and pollen pot. Fig.5E Weak colony nest arrangement, with a little cerumen, and batumen used to cover nest gaps.

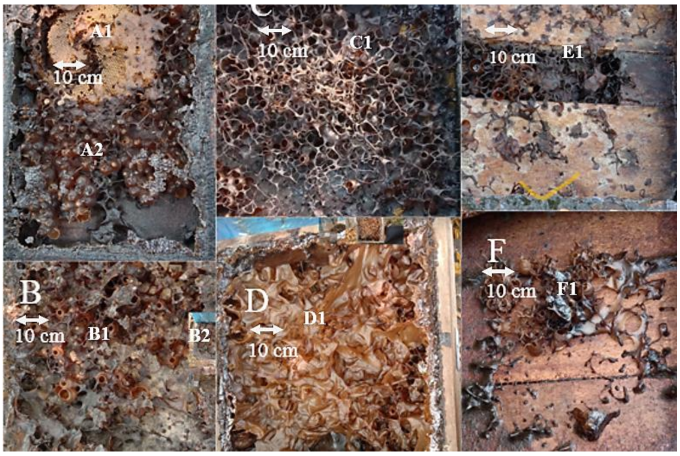


Figure 5. Various forms of *Heterotrigona itama* internal nest from meliponiculture practice in Banjar Regency, Indonesia: A1.Brood cells; A2&B1.Honey and pollen pots; B2.Batumen or nest wall; C1.Root-like structure of cerumen; D1. Involucrum as multiple layers of cerumen; E1. Honey and pollen pots in the connecting hole; F1.; Pot honey and pollen are separated in small amounts. Scale bar: 10 cm (private collection, 2023).

A horizontal model is normally used for stingless bee species that build honey and pollen pots next to the brood clusters (Rattanawannee & Duangphakdee, 2020). This nest is a room where a collection of eggs-larva-pupae (called brood cells), honey pots, and pollen pots are placed (Harjanto et al., 2020). *H. itama* internal nests are built from a mixture of wax and resin that consists of nest entrance, cerumen, batumen, involucrum, storage pots, and brood cells (Pangestika et al., 2020). Cerumen is the mixture of wax and droplets of fresh resin to construct storage pots and brood cells. The multiple layers of cerumen by a soft sheath around the brood chamber are called the involucrum (Achyani & Wicandra, 2019), it also makes a roots-like structure to trap the predators and parasites (Michener, 2007). It can be thick or thin as the plates or layers enclosing the whole nest or nest walls called batumen consisting of one or multiple layers of wax mixed with resin or mud (Vamshikrishna et al., 2020). When a cerumen sheet is formed into a cylinder as an entrance tube, it can be pulled and closed at its apex to exclude an intruder (Roubik, 2020).

Brood Cell Characteristics. Stingless bees are considered domesticated species because they are being reared for their products such as honey, propolis, bee bread (which is the pollen and other ingredients stored in the brood cells), and bee wax (Azmi et al., 2019). most stingless bees are cavity nesters and some of them arrange their brood cells in combs, others build brood cells in clusters (Michener, 2007). Figure 6 explains about horizontally layered comb of the *H. itama* nest with brood cells from meliponiculture practice in Banjar Regency, Indonesia. Fig. 6A. *H. itama* brood cells arrangement with long columnar pillars**.** Fig. 6B. The brood cells are covered with involucrum and pillars and also for queen eggs; Fig. 6C. The queen eggs above the brood cell.

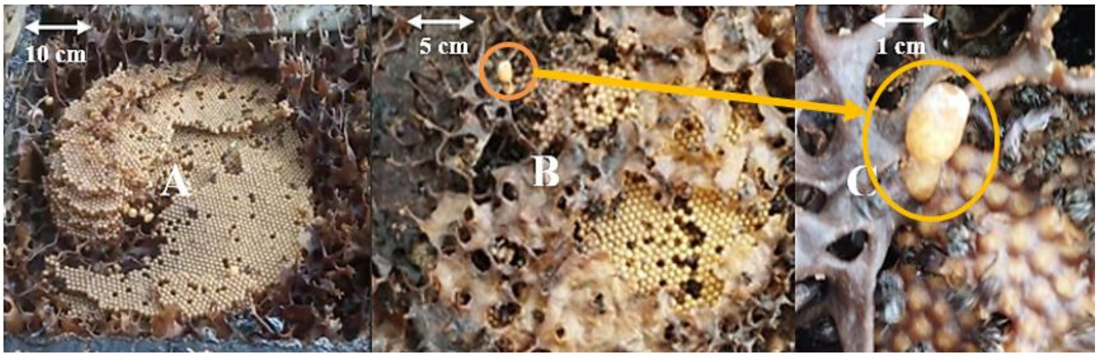


Figure 6. A. Horizontal layered comb of *H. itama* brood cells; B. Brood cells are covered with involucrum and pillars and also for queen eggs; C. The queen eggs are ready to hatch. Scale bar: 5 cm (private collection, 2023).

The oval-shaped storage pots for honey and bee-bread (pollen) are built with cerumen. Brood cells are arranged horizontally in most species or a cluster (Kwapong et al., 2010), and horizontal layered comb brood cells are for *H. itama* (Purwanto et al., 2022). Observations around the nests of five *H. itama* colonies in Aceh Besar District by (Sriwahyuni et al., 2023) showed the presence of batumen in the nests, and involucrum was also found around the brood cells. From this research, we get the same thing with observations results of Sriwahyuni et al., (2023) that *H. itama* from the Great Forest Park in Aceh Besar Regency, Indonesia have varying architectural patterns related to brood cells, pollen pots, and honey pots arrangements.

An architectural pattern of the *H. itama* nest from this research also has a varied arrangement of brood cells, pollen, and honey pots. Honey and pollen pots are generally located at the topping (top and separate), while the brood cells are in the cavity of a tree trunk. Sakagami et al., (1983) said that brood cells of Trigona were arranged definitely in horizontal combs, and each connected with pillars and not spirally continuous. Based on Purwanto et al., (2022) research results, the brood cells of *H. itama* are arranged in clusters with long columnar pillars (0.50-0.51 cm and 0.32-0.33 cm) that connect the brood cells and hive substrate.

Environmental Conditions. *H. itama* stingless bee cultivation not only reveals the secrets of this important pollinator but also provides a unique meeting point between natural influences and human intervention. In this case, the differences in location and nectar sources will influence the honey's physicochemical characteristics (Saputra et al., 2021). The study of variations in colony organization and nest attributes of stingless bees reared in Meliponicultures is an excellent mediator for exploring the complexity of the relationship between humans and bees. It's also a great way to reveal how biodiversity supports the development of ecosystem services through learning and culture (Maya et al., 2023). Conducting research in Meliponicultures involving *Heterotrigona itama* is very useful for saving ecosystem functions and services (Barbiéri & Francoy, 2020). It is critical for biodiversity conservation and the protection of ecosystem services caused by habitat loss, land conversion, and chemical use (Romanelli et al., 2015).

One of the benefits generated by meliponiculture is the maintenance of ecosystem services, since, in the keeping of stingless bees, meliponists maintain a large number of pollinating agents responsible for pollination service (Barbiéri & Francoy, 2020). Beekeepers may encounter several obstacles that may result in colony failure and under-production. These problems can be attributed to a variety of factors such as surrounding temperature, surrounding humidity, and predators (Ali et al., 2021). Table 5 below explains the environmental conditions (temperature and humidity from meliponiculture practices in Banjar Regency, Indonesia. The highest average temperature can be found in MP2 (Meliponiculture in the rubber garden) at 35.79±1 ^{0}C, and the lowest in MP3 (Meliponiculture in the side yard) at the value 31.69±1.93. Otherwise, the highest average of humidity can be found in MP3 (Meliponiculture in the side yard) at 60.70±11.21 %, and the lowest in MP2 (Meliponiculture in the rubber garden) at the value of 50.40±8.86 %. Multiple comparisons test using Tukey HSD showed a significant difference in temperature between MP1 and MP2 (p=0.027*), also between MP2 and MP3 (p=0.000*). A significant difference in humidity was found between MP2 and MP3 (p=0.033*).

Table 5. Environmental conditions at meliponiculture practices in Banjar Regency, Indonesia

	Units	**MP1**	**MP2**	**MP3**
Temperature (^{0}C)	Range	30.30-37.90	34.50-38.10	29.80-34.50
	Mean±SD	33.52±2.33	35.79±1.00	31.69±1.93
	Anova	0.000		
	Tukey HSD	MP1-MP2 (0.027), MP2-MP3 (0.000)		
Humidity %	Range	55-66	41-65	43-76
	Mean±SD	59.20±4.39	50.40±8.86	60.70±11.21
	Anova	0.027		
	Tukey HSD	MP2-MP3 (0.033)		

Note : MP1: meliponiculture in the back yard; MP2:meliponiculture in the rubber garden; MP3:meliponiculture in the side yard; SD: Standard Deviation. * shows a significant difference between (p<= 0.050).

The environmental conditions are the main factors that influence stingless bee development and productivity and significantly will support stingless bee productivity (Salatnaya et al., 2020). A study result by Salatnaya et al., (2020) showed that the environment influenced the daily activities of stingless bees that went out and bees into the hives The worker bees started the activity at 06:00 at the temperature was 23.08 °C, with humidity 70%, and light intensity still low at 183 lux. The observational results indicate that the environmental conditions with a temperature range of 29.80-34.50 °C and humidity range of 43-76 % are more favorable for meliponiculture.

The novelty of the research lies in its species-specific focus, relevance to meliponiculture practices, conservation implications, contributions to ecosystem services knowledge, applicability to beekeeping, and potential for inspiring biotechnological advancements. The interdisciplinary nature of the study enhances its significance and potential impact across various domains. This research can have a significant impact on the advancement of scientific knowledge. For the socio-economic aspect, local farmers may benefit from this and wild stingless bee nests may not be overexploited (Assefa et al., 2021). By promoting sustainable stingless beekeeping, society can achieve positive environmental development and outcomes.

CONCLUSION

Meliponiculture practices of *Heterotrigona itama* in Banjar Regency influence several parameters of morphology, external nest, nest entrance, internal nest, and also brood cell characteristics. For morphometric characters, there are differences in matters in the average of fore legs length (p = 0.000) and hind legs length (p = 0.002; p<0.05). For external nests, there are several differences in terms of wooden log volume (0.000;p<0.05), nest height to the ground (0.000; p<0.05), and distance between nests (0.001;p <0.05). There is a difference in terms of entrance length (0.007; p<0.05), but not for entrance diameter. There a various forms of *Heterotrigona itama* internal nests from meliponiculture practice. Horizontal layered comb of *H. itama* nest covered with brood cells arranged with long columnar pillars and covered with involucrum and pillars. Environmental conditions suitability (eg temperature ±29^{0}C, humidity ± 68%, and river as a water source) in the side yard is more supportive for meliponiculture. For future research, we should examine the environmental pollution impact on meliponiculture practice in the study area. It is necessary to develop the performance of stingless bees (*Heterotrigona itama*) as a potential bioindicator of pollution.

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