



# Morphology, Nest Characters, and Behavior: Taxonomic Aids to Facilitate Identification of *Tetragonula biroi* Friese (Hymenoptera: Apidae: Meliponini)

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**Abstract**—*Tetragonula biroi* Friese, the most popular stingless bee species in the Philippines, belongs to *Tetragonula*, a difficult genus to deal with due to the high character variability of workers. This paper provides a detailed description of the morphological characters, nest features, and behavioral characteristics of *T. biroi* to help facilitate its classification and identification. Proper identification of stingless bees is crucial in biodiversity conservation, genetic improvement, and pest management.

**Keywords**— genus *Tetragonula*, stingless bee identification, *Tetragonula biroi*, Philippines.

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## INTRODUCTION

While more than ten stingless bee species are recorded in the Philippines (Baltazar 1966), *Tetragonula biroi* Friese is the most widely cultivated for commercial beekeeping due to its sheer advantages. This species is used for pollination (Heard 1999, 2018; Roubik *et al.* 2018; Cervancia and Fajardo 2018), health (Suarez *et al.* 2021; Belina-Aldemita *et al.* 2019), melitourism (Nicolas *et al.* 2020; Hidalgo *et al.* 2022), and entrepreneurial purposes [Mostoles 2018; Hidalgo *et al.* 2020]. Known for its good characteristics, such as resistance to pests, resiliency, management simplicity, and, not to mention, abundance, it is the highly preferred species by stingless beekeepers. To date, most of the beekeepers use brood formation to distinguish *T. biroi* from other stingless bee species, whether cluster or spiral type. However, classifying stingless bees based on the arrangement of brood layers is inconclusive and often misleading of its identity.

Filipinos may regard *T. biroi*, locally known as "kiyot," "lukot," "kalulot," and "kiwot", the "Star of Philippine Meliponiculture" due to its extensive use in urban and peri-urban areas for beekeeping. It was described initially by Friese in 1898 with the Philippines as the type locality (Friese 1898). The type material was collected from the Philippines and New Guinea (Dollin *et al.* 1997). While this

species had already been described more than a century ago, its identity remains ambiguous due to insufficient diagnosis. The available information is limited only on the structure and size based on two syntypes (workers) deposited in the Zoologisches Museum der Humbolt-Universitat, Berlin (ZMHB) (Rasmussen 2008). Also, no holotype was designated.

This species belongs to the *carbonaria* group under the genus *Tetragonula*. In the Philippines, *Tetragonula* has three groups: *iridipennis*, *carbonaria*, and *laeviceps* (Nicolas 2013). In previous taxonomic work, *T. biroi* was distinguished from other *Tetragonula* species merely by the size of the malar area, the color of erect hairs on mesonotum and scutellum, and the length of the forewing, including tegula and hair bands on mesoscutum (Schwarz 1939). Recently, Nicolas (2013) introduced new characters of taxonomic value that would facilitate species separation in the *carbonaria* group. These taxonomic characters include the pilosity of malar area, wing clarity and iridescence, and pilosity and color of hairs in paraocular areas.

Stingless bee identification in the genus *Tetragonula*, particularly of workers, is challenging due to the insufficiency of stable and reliable morphological characters that would clearly distinguish one species from the other (Sakagami and Inoue 1985). Thus, in a morphologically conserved group like *Tetragonula*, it is vital to search for

more reliable characters, especially those that do not easily vary with changes in geographic location, environmental conditions, or those that do not easily get distorted in storage. Morphological data combined with information regarding nesting ecology, nest architecture, and behavior would be highly valuable (Nicolas 2013). However, little information is available on its taxonomy, particularly on tools or aids for species identification. Substantial data on body morphology, nest architecture, and behaviour is indeed necessary.

Insufficient knowledge on the morphology of a particular species due to poor description may hamper adequate comparisons between its congeners. One possible dilemma that may arise is the conspecific issue. Poorly described species may be treated as conspecific to their closest relatives. A substantial description is thus essential to avoid possible confusion in identifying species and preserving their taxonomic stability.

Nest architecture plays a vital role in classifying genera within the tribe Meliponini (Michener 2000). It is often used as a critical feature to solve taxonomic problems when morphological differentiation of species, due to close similarities in morphological and genetic composition, is difficult, or worst, quite impossible (Dollin *et al.* 1997; Michener 1961; Sakagami *et al.* 1983; Starr and Sakagami 1987). Such is particularly true in the case of three species of the Australian *carbonaria* group (*T. carbonaria* Smith, *T. hockingsi* Cockerell, and *T. mellipes* Friese), which are most readily distinguishable by the structural characteristics of their nests (Dollin *et al.* 1997; Michener 1961).

Morphological variations are not sufficient to differentiate species within the problematic genus *Tetragonula* successfully. Nest architecture characters are relevant criteria (Franck *et al.* 2004) that may help ease identification. In the Philippines, some authors have already provided a brief description of the nesting biology of *T. biroii*. However, no detailed description of the nest architecture of this species, both external and internal, yet exists.

Generally, this work aims to shed light on the ambiguity of *T. biroii*'s identity by characterizing their taxonomic attributes. Specifically, it seeks to: (1) provide a detailed description of the worker and queen morphology; (2) discuss the internal and external nest features thoroughly; and (3) describe the nesting ecology and defensive behavior of *T. biroii*.

By providing substantial presentation of the relevant data on *T. biroii*'s body morphology, nest characters, and behavior, this paper hopes to contribute to the faster identification of this species and differentiation from its conspecifics in the genus *Tetragonula*, particularly in the *carbonaria* group.

## MATERIALS AND METHODS

### *Nest Finding and Collection*

The study used the key informant approach in determining the location of feral colonies in Camarines Sur, Bicol, Philippines. This approach is the most reliable, as stated in the study of Nicolas (2013). Informants from the study sites provided information on the specific location of *T. biroii* colonies in the wild. This strategy has facilitated the process, reducing the finding time. Ten feral colonies were found. These colonies were marked individually, and the nest tree was identified. The marked colonies were carefully removed from the nest tree, placed separately in sacks, and tied up, maintaining their original orientation to prevent bees' damage and death. Nest examination took place at the Central Bicol State University of Agriculture's Crop Protection Laboratory in Pili, Camarines Sur, Bicol, Philippines.

### *Morphological Characterization*

Collected specimens of *T. biroii* (200 workers and 5 queens) were examined in detail under a digital binocular stereomicroscope with top and bottom dual light illumination system and a USB digital camera of 60 times magnification (Amscope 10x- 20x-30x-60x). For detailed examination of some parts, a digital handheld microscope (Dinolite Digital Microscope Pro) of 200 times magnification was used.

All specimens were identified using available published keys (Dollin *et al.* 1997; Schwarz 1939; Michener 1990; Sakagami 1978), descriptions, illustrations and figures. To highlight the specific parts of the stingless bees showcasing its diagnostic characters, specimens were photographed using a digital handheld microscope (Dinolite Digital Microscope Pro) attached to a laptop computer. To obtain the whole images of the specimens, photos were taken using a digital camera attached to a stereomicroscope. The images were then captured and combined using the Auto Montage Software.

### *Nest Characterization*

The characteristics of the inner and outer portion of the colonies were examined and described based on the work of Wille and Michener (1973), as presented by Souza *et al.* (2008). The nests were devoid of dirt and other debris before the examination.

The morphological examination of the nests took note of the following: measurements of the external cavity, nest entrance, and food storage pots; estimates of the space occupied by the brood based on the height and diameter of the combs; the volume of the honey pots taken through complete removal using a 0.50-mL graduated syringe; the weight of pollen using analytical laboratory scale; and estimates of population size based on the equation of Aidar (1996).

The following external and internal nest characters were examined, measured, and compared: (1) nest above the ground; (2) nest length; (3) nest height; (4) nest width; (5) nest volume; (6) diameter of the brood area; (7) height of the brood area; (8) diameter of nest entrance; (9) height of nest entrance; (10) number of brood combs; (11) number of cells/cm<sup>2</sup> in brood combs; (12) height of the brood cells; (13) diameter of the brood cells; (14) volume of honey pot; (15) height of the pollen pot; (16) diameter of pollen pot; (17) volume of pollen pot; (18) number of pollen pot/4 cm<sup>2</sup> in pollen; (19) pillar height; (20) pillar thickness; (21) thickness of inner involucrum; (22) thickness of outer involucrum; and, (23) population estimate. Other data gathered were on the inquilines and bee population.

The internal and external parts of all the nests were photographed. Similarities and differences in the nest morphological structures were observed, recorded, and compared. Based on the colored photographs, line drawings of the nest architecture were made.

### Behavioral Characterization

Other relevant information on *T. biroi*'s behavior, such as nesting ecology and defense mechanisms, was likewise considered in this study to augment the data on morphological and nest characters using a qualitative method. This method helped in describing and understanding the behavior of *T. biroi*. Previously published literature on stingless bee ethology was also used to guide in differentiating this species from its congeners.

## RESULTS AND DISCUSSIONS

### Body Morphology

Below are the results of the morphological examination done on the workers and queens of *T. biroi*. Table 1 shows the various characters measured to determine the structural dimensions of this species.

#### 1. Worker

**Head.** Blackish, as wide as mesosoma, punctate, dull, rough. Vertex, OOD and IOD with sparse, semi-erect, stout, dark bristles (Fig. 1a). Bristles on vertex admixed with short, weakly plumose, pale testaceous hairs. Frontal hairs dark, not plumose, appressed. Frontal suture shallow but defined. Paraocular areas below and above covered with weakly plumose, very short, appressed, dark hairs. Antenna paler below (testaceous), darker above (blackish brown); scape slightly more than 1/2 the eyelength. Clypeus generally dark, bristleless, covered with minute, appressed, not plumose, dark hairs; flat; with a thin, black and thick orange brown transverse bands along apical margin. Epistomal suture slightly curved laterally. Labrum dark. Mandible bidentate; blackish basally and apically, relatively paler at the median portion. Compound eye inner and outer margin defined, wider than gena, glabrous. Gena punctate, dull, rough; covered with minute, appressed, dark hairs; much narrower than compound eyes (Fig. 1b). Malar

Table 1 Structures and characters measured and their abbreviations (*adopted from Nicolas 2013*)

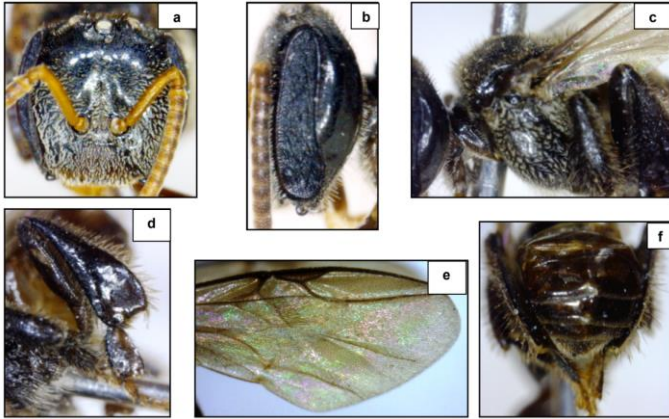
Abbreviation	Meaning
BL	Length of body
WL1	Length of forewing including tegula
HW	Maximum width of head
WL2	Distance between M-Cu bifurcation and basal tip of marginal cell
HTL	Distance between upper basal end to midpoint of apical margin of hindtibia
EL	Length of compound eye
LOD	Lower interorbital distance
MOD	Maximum interorbital distance
IOD	Interocellar distance
OOD	Ocellocular distance
GW	Maximum width of gena
ML	Minimum length of malar area
SC	Length of scape seen laterally
F4L	Length of flagellomere IV seen frontally
F4W	Width of flagellomere IV seen frontally
HTW	Maximum width of hindtibia
HBW	Maximum width of hindbasitarsus
EW	Width of compound eye
HL	Length of head
WL2/HW	Relation of wing length to head width
HTL/HW	Relation of hindtibia length to head width
HTL/WL2	Relation of hindtibia length to wing length
EL/MOD	Relation of eye length to maximum interorbital distance
LOD/MOD	Relation of lower interorbital distance to maximum interorbital distance
IOD/OOD	Relation of interocellar distance to ocellocular distance
GW/EW	Relation of gena width to eye width
ML/FW	Relation of malar length to flagellomere IV width
SC/EL	Relation of scape length to eye length
FL/FW	Relation of flagellomere IV length to flagellomere IV width
HTW/HTL	Relation of hind tibia width to hind tibia length

space entirely covered with minute, appressed, dark hairs; very long, 2x if the flagellum is wide; punctate, dull, rough.

**Mesosoma.** Same coloration and punctuation with head. Mesonotum bordered with short, thick, plumose, pale hairs with few admixtures of erect, stout, dark bristles. Mesoscutum not banded, covered with very short, weakly plumose, pale hairs admixed with sparse, stout, erect, long, dark bristles. Scutellum blackish, fringed with relatively short, plumose, short, pale hairs admixed with sparse, erect, stout, long, dark hairs; scutellar fovea deep; extending to the mesonotum; long. Mesopleuron evenly covered with short, plumose, appressed, dense, pale hairs (Fig. 1c); hypoemiral areacompletely covered. Metapleuron with plumose, not woolly, semi-appressed, dense, relatively pale hairs, quite longer than in mesopleuron. Tegula relatively dark, bristleless. Propodeum less punctate, of subdued sheen, less smooth; glabrous. Legs relatively dark (Fig. 1d). Hindtibia width less than 1/2 its length; expanded apically. Corbicula little excavated, pubescent. Anterior corbicular fringe dark. Hairs on hindtibial fringe dark, plumose. Hind basitarsus basally with dark sericeous area basally, pale bristles apically; posterior margin gently arched. Forewing long, exceeds much the metasoma; venation reduced; with deepening of color below the prestigma; subhyaline; non-iridescent (Fig. 1e). Anterior vein and stigma blackish

brown. Transverse cubital veins strongly outlined. Median cell highly stained. Pterostigma large, not round. Marginal cell almost closed.

*Metasoma.* Abdomen generally dark, pale basally; narrower than mesosoma; less punctate, of subdued sheen, less smooth; devoid of mosslike pubescence. No bands on tergites (Fig. 1f); last abdominal tergite pubescent, surface covered with minute hairs, fringed with setae laterally. Erect hairs on sternites present, pale.



**Fig. 1 (a-f)** Images of the morphological features of *Tetragonula biroi* (Friese) worker (a) frontal view of the head; (b) head in profile; (c) lateral view of the thorax; (d) left hindleg; (e) left forewing; (f) dorsal view of the abdomen. (Photographs a-f were taken from a worker collected from Camarines Sur, CAR-LUZ-BIC-001.)

*Morphometry.* Structural dimensions (ranges) are referred in mm, following Sakagami (1978) and Dollin *et al.* (1997): BL, 3.50-3.65; WL1, 4.10-4.40.; HW, 1.51-1.58; HL, 1.33-1.35; WL2, 1.12-1.18; HTL, 1.53-1.61. WL2/HW, 0.71-0.72; HTL/HW, 0.97-1.03; HTL/WL2, 1.37-1.39; EL/MOD, 0.93-0.95; LOD/MOD, 0.79-0.83; IOD/OOD, 1.81-1.86; GW/EW, 0.59-0.63; ML/FW, 0.5; SC/EL, 0.53; FL/FW, 0.92-1.0; HTW/HTL, 0.34-0.35; HBW/HTW, 0.57-0.6.

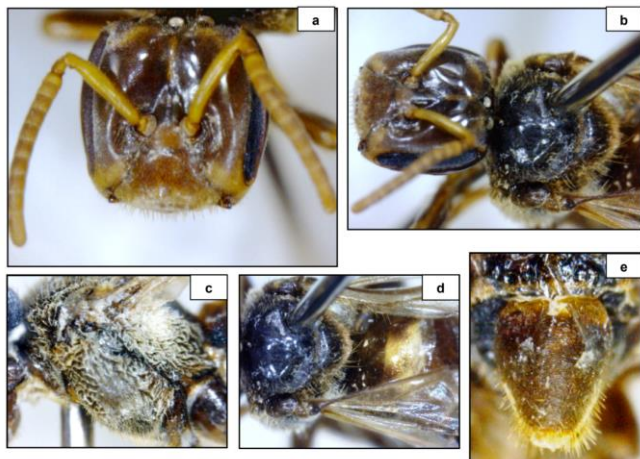
## 2. Queen

*Head.* Head blackish brown, slightly narrower than mesosoma, not extending beyond the outer rims of tegulae as in the case of workers (Fig. 2a). Vertex with some medium-length, semi-erect, ferruginous bristles. Lateral ocelli on vertex, almost parallel with median ocellus. Frontal suture deep. Antenna below ferruginous, darker above; 10-segmented as in workers; last flagellomere longest; 1<sup>st</sup> flagellomere palest; 2nd to 9th flagellomere with thin, pale bands prebasally. Face pilosity above distinctly sparser than in workers; face medially more depressed than in workers. Clypeus dark brown, entirely covered with appressed, microscopic, dense, ferruginous hairs, not plumose; much raised than face on the upper half, lower half distinctly depressed. Malar space fulvous, notably greater than in workers; surface entirely covered with appressed, microscopic, ferruginous hairs. Mandible bidentate, dark brown, not as protuberant as in workers, with black

apical edge; bristlelike, semi-erect hairs on outer margin of mandible present, denser than in workers. Labrum pale yellow, covered with sparse, bristlelike, ferruginous hairs of unequal length. Compound eye much narrower than gena. Gena covered with minute, appressed, ferruginous hairs.

*Mesosoma.* Black. Mesonotum not framed with short, thick hairs. Mesoscutum not banded, covered with short, erect, inconspicuously plumose hairs admixed with relatively few, semi-erect, longer, bristlelike hairs (Fig. 2b); more raised and rounded above than in workers. Mesoscutellum fringed with fulvous hairs admixed with ferruginous, semi-erect bristles, more than 2x as long as the longest bristlelike hairs in mesoscutum; less depressed basally than in workers and much less projecting apically relative to propodeum. Mesopleuron evenly and densely covered with soft, short, fine, matted, semi-appressed hair except for a stretch of glabrous portion below the hypoepimeral area similar to workers (Fig. 2c); hypoepimeral area pubescent. Metapleuron tomentose, completely clothed with appressed, woolly, whitish hairs. Propodeum medially less projecting than in workers; of subdued sheen, dark, glabrous medially. Legs reddish brown to partly blackish, with shorter and fewer hairs on femora than on tibiae. Hairs on fore tibia short, much shorter than those of mid and hind tibiae, unbranched unlike those of workers; distribution of hairs over the outer surface of tibiae fairly dense and uniform. Hind basitarsi devoid of differentiated, oval, sericeous, bristleless area on the inner face; parallel-sided, slightly constricted at the base, almost half the width of tibiae. Hindtibia somewhat club-shaped but gradually expanded toward apex, posterior contour gently convex. Mid tibia and basitarsi are similar to those of workers. Tegula blackish brown, fringed with sparse, ferruginous, bristlelike hairs. Wings actually slightly longer than of workers but relatively much shorter, tip extending only about to apex of T3. Hamuli five. Radial, median, basal and transverse cubital veins with erect, ferruginous bristles, absent in workers. Median cell not stained; transverse cubital vein and marginal cell feebly traced.

*Metasoma.* Blackish with invasions of reddish brown, much distended, longer by about 1/3 than combined length of head and thorax and much wider than either. S1- S3 with erect, dense, long ferruginous bristles medially, S4 with bristles mesally and laterally; S5 devoid of long bristles. T1-T4 virtually hairless (Fig. 2d); T5 surface densely covered with fine, short, appressed, fulvous hairs admixed with sparse, stiff, short hairs, appearing punctate (Fig. 2e); entirely fringed with longish, erect, fulvous bristles, denser apically; T1 and T2 relatively paler.



**Fig. 2 (a-e).** Images of the morphological features of *Tetragonula biroi* (Friese) queen (a) frontal view of the head; (b) dorsal view of the thorax; (c) lateral view of the thorax; (d) dorsal view of the abdomen; (e) dorsal view of the last abdominal tergite. (Photographs a-e were taken from a queen collected from Camarines Sur, CARLUZ-BIC-Q001.)

**Morphometry.** Structural dimensions (ranges) are referred in mm, following Sakagami (1978) and Dollin *et al.* (1997): BL, 6.24; WL1, 4.22-4.32.; HW, 1.58-1.61; HL, 1.48-1.52; WL2, 1.11; HTL, 1.86. WL2/HW, 0.68-0.70; HTL/HW, 1.16-1.18; HTL/WL2, 1.69; EL/MOD, 0.88; LOD/MOD, 0.93-0.97; IOD/OOD, 1.28-1.73; GW/EW, 1.0-1.47; ML/FW, 1.0; SC/EL, 0.66-0.68; FL/FW, 1.0-1.08; HTW/HTL, 0.34-0.35; HBW/HTW, 0.40-0.42.

#### *External Nest Architecture*

**Nest entrance.** Most of the nest entrances of *T. biroi* are at the center, the lowermost part of the colony, single-holed, and made up of hard, sticky, and black propolis as the involucre. On average, it measures 3.44 cm wide and 4.57 long. Pillar-like structures accentuate the opening, probably offering defense against possible predators and intruders (Fig. 3). The hive entrance of stingless bees, to some degree, is taxon-specific (Wille and Michener 1973) but also exhibits plasticity according to situations (Sakagami and Inoue 1985). A smaller hive entrance offers higher defensibility against predators and robbers. But to permit foraging and allow easy passage of traffic, the opening should be more prominent. The aforementioned factors may affect hive entrance size (Couvillon *et al.* 2008).



**Fig. 3** Hive entrance of *T. biroi* with pillar-like structure

**Batumen.** The ten nests samples varied in shape as affected by how they were attached to the nest tree. Black, rigid but pliable batumen that protects the bees from exposure to adverse environments and predators covered the nests entirely. The batumen becomes more robust due to a mixture of fern leaves and small tree branches (Fig. 4).



Fig. 4 A hunted feral colony of *T. biroi* with sturdy and robust batumen

*Internal Nest Architecture*

Fig. 5 presents the actual photo of the lateral view of *T. biroi*'s nest showing the internal structure.



Fig. 5 The exposed part of the feral colony of *T. biroi* showing the brood arrangement laterally, food pots relative to the brood area, involucrum enveloping the brood, and the thick batumen protecting the entire nest

Table 2 Quantitative data on the different external and internal features of *T. biroi* nests collected from Camarines Sur, Bicol, Philippines

Variables	n	Unit	Range	Mean±SD
Nest above the ground	10	cm	744-1121	920.9± 136.02
Nest length	10	cm	18.5-47.5	28.74± 8.16
Nest height	10	cm	14.6-41.0	26.98± 9.08
Nest width	10	cm	15.0-36.7	21.57± 6.39
Nest volume	10	kg	0.85-5.50	2.9324± 1.53
Diameter of brood area	10	cm	8.1-19.0	13.93± 3.55
Height of brood area	10	cm	7.5-19.0	11.81± 3.67
Diameter of nest entrance	10	cm	2.1-5.6	3.44± 0.98
Height of nest entrance	10	cm	0.9-9.5	4.57± 2.27
Number of brood combs	10	un	18-34	24.5± 5.77
Number of cells/1cm <sup>2</sup> in brood combs	10	un	17-24	19.6± 2.42
Height of brood cells	100	mm	3.15-3.56	3.3134± 0.18
Diameter of brood cells	100	mm	2.07-2.37	2.2394± 0.25
Volume of honey pot	100	ml	0.36-0.40	0.3779±0.03
Height of pollen pot	420	gm	1.00-1.19	1.074± 0.16
Diameter of pollen pot	420	cm	0.839-1.01	0.9102± 0.14
Volume of pollen pot	420	g	0.314-0.462	0.3792± 0.12
Number of pollen pot/4 cm <sup>2</sup>	10	un	23-29	24.7± 1.68
Pillar height	100	mm	7.43-10.64	8.6983± 3.3
Pillar thickness	100	mm	1.30-1.70	1.4622± 0.41
Thickness of inner involucrum	10	mm	1.02-2.25	1.497± 0.49
Thickness of outer involucrum	10	mm	1.03-3.18	1.897± 0.9
Population estimate	10	un	3141-10730	6945.1± 2574.71

Legend: n= number of units assessed

*Involucrum.* It as a single or series of sheaths, made of cerumen, surrounding brood (Roubik 2006). All the nests opened had their brood covered with dark involucrum of two to four layers. The outer involucrum is thicker and darker than the inner involucrum. The average thickness of the outer and inner involucrum is 1.90 mm and 1.50 mm, respectively.

*Storage Pots.* Honey and pollen are contained in pots made of cerumen located near or around the brood area.

*Pollen and pollen pots.* These egg-shaped pots, covered with involucrum, surround the brood area and are directly attached to honey pots. Each pot is formed with brown cerumen and measures 1.00 cm to 1.19 cm. The weight of the pollen in pots ranges from 0.314 g to 0.462 g. On average, each pot contains 0.38 g. The largest pots have as much as 0.46 g of pollen. In a 4 cm<sup>2</sup>-area, 23 to 29 pots are present.

*Honey and honey pots.* Honey pots are amorphous and made up of brown cerumen. Pots are either sealed or not sealed. Partially or widely opened pots signify that the honey it contains is still unripe. The wholly sealed pots have ripe honey. The average honey volume in pots is 0.38 ml, with 0.36 ml as the lowest and 0.40 ml as the highest.

*Pillars.* These are cerumen used as anchors of nest elements (Roubik 2006). They hold, support, separate, attach, serve as passageways to the inside of the nest for returning foragers carrying food and other materials. These structures even regulate the temperature inside the nest. Its color varies depending on the area where support is provided. *T. biroi* has an anastomosing pillar system. The height and thickness of the pillars range from 7.43 mm to 10.64 mm and 1.30 mm to 1.70 mm, respectively.

*In the involucrum.* The inner and outer layers of the involucrum are supported and separated by black, slightly stiff but sticky, and relatively short pillars.

*In the brood area.* The brood combs are layered spherically with the support of brown, soft, pliable, and relatively thinner and longer pillars.

*Brood Area.* In all the nests examined, the height of the entire brood area ranges from 7.5 cm to 19.0 cm. The brood area is located at the center of the nest. This area is composed of eggs, larvae, and pupae.

*Brood combs.* These are spirally arranged in layers, ranging from 18 to 34. The size/diameter of the combs varies from layer to layer. The outermost layer is the smallest (1.68 cm), while the innermost layer is the largest (19.0 cm).

*Brood cells.* The height and diameter of brood cells range from 3.15 mm to 3.56 mm and 2.07 mm to 2.37, respectively. They are cylindrical, interconnected, and made up of brown cerumen.

Queen cells are larger than the cells of workers and drones, having a diameter of 4mm and a height of 5mm.

*Population.* Based on estimates, the population of *T. biroi* ranges from 3,141 to 10,730 bees.

*Inquilines.* There were no inquilines found in the nest living in association with *T. biroi*.

### *Ethology*

*Nesting Ecology.* It discusses the nesting habit, nest preference, and nest aggregation behavior of *T. biroi*.

*Nesting habit.* *T. biroi*'s nest is neither exposed nor concealed, hence, considered a semi-exposed nester (Nicolas 2013). It starts building its nest in a living *Drynaria* sp. firmly fixed to a live *Ficus*-enclasp *Cordia* tree. As the bee colony matures, *Drynaria* begins to dry up and eventually die. Preference to utilizing live trees for nesting might illustrate how they generally prevent living in a locality that gets devastated by termites rapidly (Martins *et al.* 2004).

The distance between colonies ranged from 744 cm to 1121 cm above the ground. Each wild nest weighed 0.90 kg to 5.5 kg. Nest weight can be related to the strength and age of a colony. Old and robust colonies were heavier than those young and weak ones. The average length, height, and width were 28.74 cm, 26.98 cm, and 21.57 cm, respectively. Once a colony reaches its supposed "maximum size," the bees start may to prepare for a swarm to build a new colony.

*Nest aggregation.* *T. biroi* exhibits nest aggregation behavior (Rebaya 2015). A total of 10 nests had aggregated in a single nest tree (Fig. 6). This trend was particularly apparent in *Tetragonula collina* and *Heterotrigona pendlebury*. Several authors have previously reported the clustering of nests in trees or artificial structures (Starr and Sakagami 1987; Roubik 1996; Salmah *et al.* 1990), but aggregating is poorly understood up until now. One possible cause of aggregation might be the limited availability of suitable nest sites, especially in degraded areas that lack sufficient numbers of natural tree cavities. On the other hand, the presence of cavities and crevices in the construction material of farmhouses can permit phenomenal concentrations of colonies (Starr and Sakagami 1987). In undisturbed forests, however, nest cavities are less likely to be limited. Here, mechanisms related to how new nest sites are located by bees may favor clustering. Nevertheless, it holds only for cavity-nesting stingless bees.



Fig. 6 Line drawing from an actual photo of *T. biroi* multiple feral colonies nesting in *Cordia* sp. showing nest aggregation

Multiple nest construction per tree might have been due to less pronounced interspecific aggression between *T. biroi* workers or sufficient food sources. It is argued that aggressive competition for food is the ultimate reason for uniform nest dispersion in group-foraging neotropical meliponines (Hubbell and Johnson 1977). Also, aggressive encounters proximately mediate nest spacing between colonies competing for new nest sites. Another possible reason for nest aggregation could be their preference for a tree. Some Meliponinae, which have colonies with prolonged longevity and low swarming frequency, select or choose trees that will give good protection to predators and parasites for a large number of years (Roubik 1989).

**Nesting preference.** Stingless bees seem opportunistic in their selection of nest sites and are likely to colonize any tree that offers a suitable nesting space that meets the requirements for their optimal growth and development. *Cordia* sp. appeared to be the most preferred tree for nesting by *T. biroi* based on this study. While other potential nest trees are present in the study area, the bees only nested in *Cordia* sp. This tree species might have characteristics necessary for the effective defense against predator attacks.

**Defense Mechanisms.** Since stingless bees are incapable of stinging, they rely on a variety of other strategies to defend their nests. Stingless bees, depending on species, employ different defensive strategies (Shanahan and Spivak 2021). They also use resin for protection from intruders, predators and other natural enemies. This is a type of resin-

based defense triggered by visual stimulation (Shanahan and Spivak 2021).

**Biting.** *T. biroi* burrows and clings to the hairs on the head and body, eyelashes, and eyebrows. They nibble on the exposed parts of the body. They attack would-be invaders outside the nest by plastering resin to human hair. They become aggressive when they perceive dark-colored objects, like clothing. They attack and adhere firmly to the dark clothing and never let go even when brushed off until their body parts get torn apart. They seem to exhibit suicidal behavior. It looks like they are willing to give up their own life in defending their colony.

**Storing balls of propolis.** Deposits of ball-like fresh propolis are visible on the internal surface of the hive, both in boxes and coconut shells (Fig. 7). When threatened, *T. biroi* guard bees, just like in *Austroplebeia australis*, defend their colony by harvesting fresh propolis from the deposits, carrying it in their mandibles and corbiculae, and applying to their predators to immobilize or totally mummify them. The color, shininess, and consistency of the propolis indicate its freshness. The bees deposit these substances on the sides of the food pots and brood combs, usually near the hive entrance. It also is an indication of a healthy and strong colony. Most of the weak colonies have less or no propolis deposits in the internal surface of the hive (*pers. obs*).

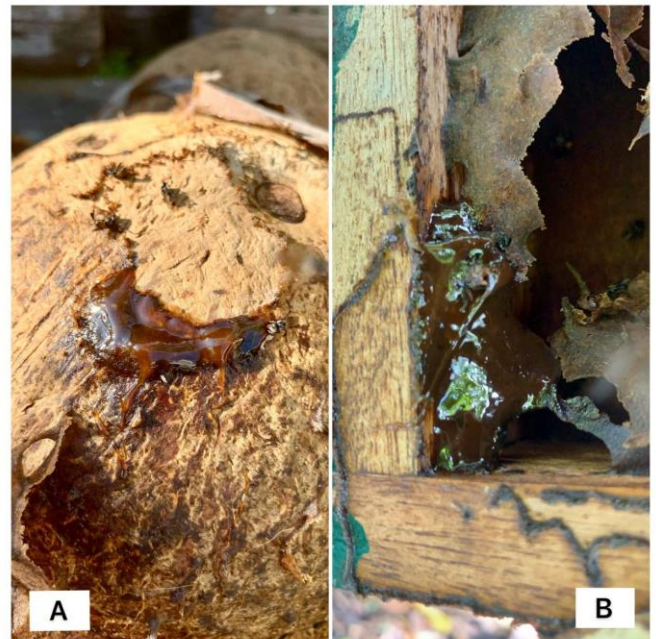


Fig. 7 Balls of resin deposited by *T. biroi* in (a) coconut shell hive and (b) box hive

**Plastering propolis on external hive surface.** *T. biroi* workers apply fresh propolis on the external surface, surrounding the hive entrance and the hive. The bees manifested this behavior consistently when kept either in box or coconut shell hives. They start applying droplets of propolis only a few hours after coconut shells (Fig. 8).



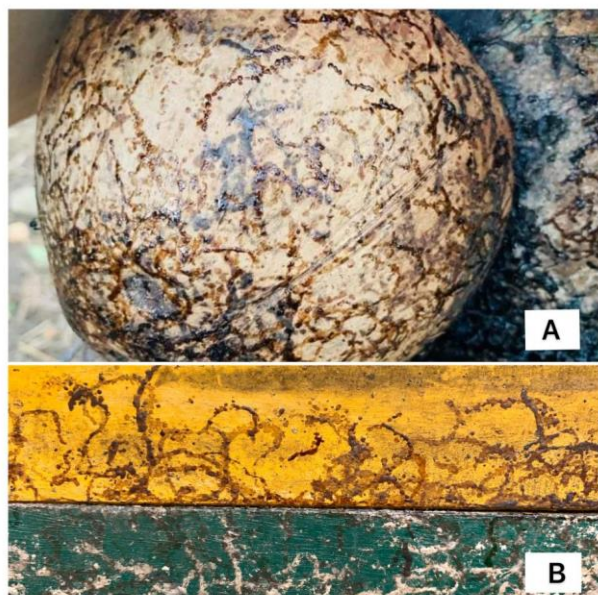


Fig. 8 Streaks of resin applied by *T. biroi* workers on (a) coconut shell hive and (b) hive box

They start applying droplets of propolis only a few hours after colony division and settling in a new hive, forming streak-like patterns. When the entire surface is covered, the bees stop and concentrate more on foraging (*pers. obs.*). Some ants get trapped in the propolis streaks.

#### CONCLUSION

The detailed description of the morphology, nest architecture, and ethology are necessary in the easy identification and separation of *T. biroi* from other members of the genus *Tetragonula*. Results of this study are expected to resolve the taxonomic ambiguities in the identity of this amazing stingless bee species.

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