Sapienza Università Editrice

E-ISSN 2284-4880
ISSN 0429-288X

Fragmenta entomologica, 54 (1): 179–184 (2022)

Research article
Submitted: February 4th, 2022 – *Accepted:* April 20th, 2022 – *Published:* May 15th, 2022
Doi: 10.13133/2284-4880/714

Nest architectures of myrmecophilous stingless bees, *Trigona* sp. cfr. *cilipes* and *Paratrigona* sp., from Peruvian Amazon (Hymenoptera: Apidae, Apinae, Meliponini)

Marilena MARCONI[1,*], Alessandro MODESTI[2], Carlos Daniel VECCO GIOVE[3], Emiliano MANCINI[2], Andrea DI GIULIO[1]

[1] *Department of Science, Roma Tre University, Viale Guglielmo Marconi, 446, 00146 Rome, Italy* – andrea.digiulio@uniroma3.it; marilena.marconi@uniroma3.it
[2] *Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Viale dell'Università, 32, 00185 Rome, Italy* – emiliano.mancini@uniroma1.it
[3] *National University of San Martín, Jr. Maynas, 177, 22200 Tarapoto, Peru* – cdvecco@unsm.edu.pe
* Corresponding Author

Abstract
Stingless bees (Hymenoptera: Apidae, Meliponini) are corbiculate and eusocial bees, including over 500 species distributed in the tropical and sub-tropical regions of the world. They establish perennial colonies using a large variety of nesting sites, as well as colonies of ants, wasps and termites. Compared to termitophily, myrmecophily is an uncommon feature and little studied aspect among Meliponini. In Peru there are few records relating to these types of associations, and in general little is known about the architecture of the nests of bees and ants that live together. For the first time, the nest architecture of *Trigona* sp. cfr. *cilipes* in association with *Dolichoderus quadridenticulatus* (Formicidae) is described from the Peruvian Amazon. The record of another association, *Paratrigona* sp. - *Camponotus* sp. (Formicidae) is also reported. As the nests are arranged, the types of building materials used and some behavioral defense observations of ants and bees are also provided.

Keywords: Meliponini, myrmecophily, *Dolichoderus quadridenticulatus, Camponotus* sp., nest biology, Peru.

Introduction

Stingless bees (Hymenoptera: Meliponini) are the most diverse group among the corbiculate bees, with over 500 species living in tropical and sub-tropical regions of the world (Michener 2000).

Meliponini established perennial colonies using a great diversity of substrates, ranging from tree hollows, cracks in the rocks, underground cavities to human constructions (Roubik 2006); moreover the ability of many stingless bee species to found colonies in nests of other social insects such as ants, termites and wasps, is also well known (Wille & Michener 1973). Termite nests are more preferred by stingless bees, whereas myrmecophily is less common, probably due to the aggressive behavior of ants (Sakagami et al. 1989). Species of the genera *Trigona* Jurine, 1807, *Paratrigona* Schwarz, 1938 and *Partamona* Schwarz, 1938 have been found associated with *Acromyrmex* Mayr, 1865*, Atta* Fabricius, 1805, *Azteca* Forel, 1878, *Camponotus* Mayr, 1861, *Crematogaster* Lund, 1831 and *Dolichoderus* Lund, 1831 ants (Kerr et al. 1967; Wille & Michener 1973; Rasmussen & Camargo 2008).

These relationships are often species specific and can be obligate, facultative or, in other cases, accidental (Wille & Michener 1973). Stingless bees benefit from these symbioses, as hosts provide protective spaces against enemies, and likely assist in temperature regulation and homeostasis of the colony (Grüter 2020).

Studying this type of association is generally complex. From the external observation of the nests, it is not easy to understand the interactions that exist between the associated colonies. On the other hand, when the nests are opened, the hosts can invade the colony of bees and thus invalidate the observational work. In this regard, the description of the internal architectures of the nests and the building materials used by bees, provides useful hints on the kind of interactions that may take place in these associations. The rapid construction of sticky resin walls for example, is a critical step in establishing a new colony for *Sundatrigona moorei* Schwarz, 1937 in a *Crematogaster* ant hill (Sakagami et al. 1989), as well as the resin structures that form the "false nests" in the entrances of *Partamona* nests, which probably have the function of distracting ants or other enemies that try to enter (Camargo & Pedro 2003).

Fig. 1 – Nest of *Trigona* **sp. cfr**. *cilipes – Dolichoderus quadridenticulatus*. The long tube is the entrance to the hive.

Fig. 2 – Nest of *Paratrigona* **sp.** – *Camponotus* **sp**. The white arrow indicates the tube of entrance of the hive.

In Peruvian Amazon there is a great diversity of Meliponini (Rasmussen & Gonzalez, 2009), but many aspects of their biology, ecology and behaviors still remain to be investigated.

To date, *Partamona testacea* Klug, 1807 has been observed to form associations with *Atta* or *Paraponera clavata* Fabricius, 1775 ants in a secondary forest of Peru (Bordoni et al. 2019), but the internal structure of the nests and how they are arranged is not known. Here, we add two more records of myrmecophilous stingless bees, with the associations *Trigona* sp. cfr. *cilipes* Fabricius, 1804 - *Dolichoderus quadridenticulatus* Roger in 1862 and *Paratrigona* sp. - *Camponotus* sp*.,* both from the Peruvian Amazon.

Furthermore, for the first time, the internal architectures of the nests *Trigona* sp. cfr. *cilipes- Dolichoderus quadridenticulatus* are described and observations on the defense behavior of the bees and ants are also reported.

Material and methods

Opening of the nests

The nest of *Trigona* sp. cfr. *cilipes* - *Dolichoderus quadridenticulatus* was kept on 2 May 2020 from an apiary located near the city of Tarapoto, Department of San Martin, in a secondary forest of Peru (-6.27.57N, -76.21.2E, 350 m). At the time of observation, few ants remained from the colony of origin (Fig. 1). The nest of *Paratrigona* sp.-*Camponotus* sp. was found on 4 June 2020 close to a mandarin tree, near the bank of the Shilcayo River, also near the city of Tarapoto (-6.27.37N, -76.21.4E). This nest probably fell recently, as the leaves woven into the outer shell were still green, but the ants had already left the nest (Fig. 2).

The two nests were dissected using a surgical scalpel. Measurements were taken with a ruler and a Vernier caliper. The weights were measured with a digital scale.

Identification of stingless bees and ants

For each colony of bees and ants, two specimens were extracted for taxonomic identification. *Paratrigona* sp. and *Trigona* sp. cfr. *cilipes* were recognised based on the available morphological key (Silveira et al. 2002; Camargo & Pedro 2007; de Oliveira et al. 2013).

In respect to ant species, *Dolichoderus quadridenticulatus* was identified by Dr S. Cantone at the Department of Science of Roma Tre University. *Camponotus* sp. was recognized only based on the characteristics of the nest and the building materials (Santos & Del-Claro 2009), also referring to the description and photographs shown in Laroca and Almeida (1989) for *Camponotus senex* Smith, 1858.

Fig. 3 – Sticky resin deposits between the outer layers of the *Dolichoderus quadridenticulatus* nest.

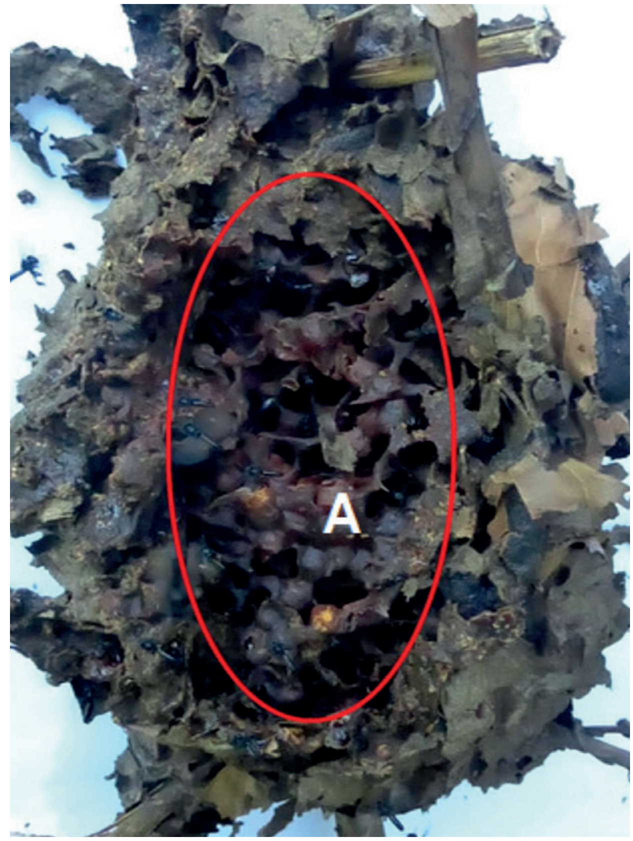

Fig. 4 – Inside the nest of *Paratrigona* **sp.** – *Camponotus* **sp**. The red circle indicates the cavity occupied by the bee colony. **A**, Storage pots.

Results

***Trigona* sp. cfr. *cilipes* – *Dolichoderus quadridenticulatus* association**

Ant nest architecture

The nest was covered with a dark brown paper-like envelope. The material used was most likely organic matter obtained from the environment. It was oval in shape (major axis= 47.4 cm, minor axis= 25.5 cm, weight= 1075 g). Between the layers of the outer envelope, there were accumulations of sticky black resin deposited by bees (Fig. 3). Dry leaves formed the outer covering of the nest, while two large vertical and six horizontal branches formed the internal skeleton, maintaining the integrity of the structure. The nest was in a state of semi-abandonment as no broods were found inside and few ants came out when the nest was opened.

Bee nest architecture

The entrance extended in the shape of a tube at the bottom of the ant nest with a length of 57 cm and a diameter of 1 cm. It was constructed of hard black cerumen in the basal part, followed by a 22 cm long portion of stacked rings and a final part of 3 cm consisting of pale brown cerumen. Near the entrance, several semicircular folds of lamellar wax were noted which probably indicate a reworking of the tube during its lengthening. Sticky resin grains were observed on the outer surface of the tube in the distal part up to some rings. The portion that adhered to the nest was covered with some paper strips built by the ants.

Internally, the cavity of the nest was oval in shape (major axis= 22.6 cm, minor axis=10.4 cm), extending for almost the entire nest of the ants (Fig. 4). It was coated with a thin layer (1 mm) of black sticky batumen, which adhered to the paper layers of the nest of the ants. Internally, there was a thin (1 cm), non-sticky envelope. Between the first and second layers, empty spaces and communicating tunnels made of batumen were observed (Fig. 5).

Brood area was made up of 10 cells arranged in a horizontal comb. Cells were cylindrical (l= 0.6 cm; Ø= 0.4 cm), built with pale brown cerumen and located in the lower part of the cavity, surrounded by food pots. All cells appeared open, containing larval food. There were no queen cells present, but the queen was observed in the entrance tube, indicating that the colony was stable.

Storage pots occupied the entire cavity of the nest, forming a continuous mass. The vessels were brown in color and ovals (h= 1.1 cm; Ø= 0.7 cm), being those containing pollen of a reddish color.

***Paratrigona* sp. - *Camponotus* sp. association**

Ant nest architecture

Fig. 5 – Batumen tunnels between the first and second outer layers of the stingless bee's (*Trigona* **sp. cfr**. *cilipes*) nest.

Fig. 6 – Inside the nest. Horizontal honeycombs of the *Paratrigona* **sp**. are visible. The red circle indicates a real cell.

The nest had an oval shape (major axis= 57 cm, minor axis= 32 cm, weight= 450 g). The outward appearance showed a woven silk material with tree leaves and thin adherent branches. Several holes were spread over the external surface, which were probably used by ants to get out of the nest. One side of the nest was torn showing the internal structure consisting of several membranous layers communicating with each other. Two branches formed a central skeleton inside the nest. No ants, broods or larvae were found.

Bee nest architecture

The entrance was located on the top of the ant nest. Externally, it looked like a 5.7 cm long right tube with an elliptical section (major axis= 1.1 cm, minor axis= 0.7 cm). It was constructed of hard black cerumen in the basal part, pale brown, malleable and sticky in the distal portion (l= 3 cm). A dilated part of batumen-like material was observed where the tube adhered to the outer envelope of the nest of the ants and sticky resin deposits were observed.

Nest cavity was oval in shape (major axis= 16.5 cm, minor axis= 7.8 cm), located 8.3 cm away from the entrance (Fig. 6). It was coated with a thin layer (1 mm) of dark sticky batumen, which adhered to the membranous layers of the nest of the ants. Internally, the wrapper showed up thin (less than 1 mm), made up of pale brown cerumen, less sticky. The number of lamellae varied, being more numerous in the apical and basal part of the nest cavity.

Brood area was made up of 18 horizontal honeycombs separated from each other by a distance of 0.2 - 0.3 mm. The combs were elliptical in shape with pale brown cells. Worker bee cells were cylindrical (l= 0.5 mm; Ø= 0.3 mm) with an apex and base round. Four queen bee cells have been counted (l= 0.8 mm; Ø= 0.5 mm). They were located outside the honeycombs, all built with pale brown cerumen.

Storage pots were located at the bottom of the nest cavity forming a single mass. The vessels were ovals (h= 1.2 mm; Ø= 1 cm), without differences between those containing honey and those with pollen. Pollen pots were grouped in the upper part of the storage mass, which was separated from the brood area from a cerumen lamella of the wrapper.

Behavioral observations

Before the opening of the nests, the defensive behavior of stingless bees and ants was evaluated by placing a stick on the entrances of the hives. The guardians of *Trigona* sp. cfr. *cilipes* responded by flying in a disorderly manner around the operator, while the guardians of *Paratrigona* sp. withdrawn into the hive. *Dolichoderus quadridenticulatus* ants do not approach the entrance of the hive of *T.* cfr.

cilipes, but they bite the operator also showing the typical ventral flexion of the gaster. The relation between *T.* sp. cfr. *cilipes* and *D. quadridenticulatus* was peaceful as no ants were observed approaching the entrance to the nest of the bees.

Discussion

The ability to establish colonies in nests of other social insects is well known in stingless bees, but myrmecophily is reported infrequently in literature (Wille and Michener 1973; Sakagami et al. 1989; Roubik 2006).

Trigona cilipes is usually found nesting with *Azteca* or *Crematogaster* ants (Kempf 1962; Camargo 1988). Also, nests of *T. cilipes* have been reported in association with termites (Rasmussen and Camargo 2008), *Brachygastra* sp. and *Epipona tatua* Cuvier, 1797 wasps (Silva-Matos et al. 2000; Rasmussen 2004). Nesting plasticity of *T. cilipes* offers a great advantage, exploiting the nests of different social insects and also the nests of different ant species. This allows it to colonize different environments depending on the species of ants present.

Based on our knowledge, until now the internal architecture of the nest of *Trigona cilipes* in association with *Dolichoderus quadridenticulatus* has not yet been described. The nest architecture described here has the same characteristics reported in other associations with ants. For example, the long tube of entrance, which is characteristic of *T. cilipes*, can reach a length of 1.2 m (Camargo and Pedro 2003). It could have a function of distancing the hive entrance from the external surface of the nest of the ants, probably because the entry and exit activities of the foraging bees take place here.

The presence of a hard and continuous envelope of batumen around the stingless bee colony, already reported in the nests of *T. cilipes* and *Azteca* ants (Kerr et al. 1967), would suggest its isolation function with respect to the ant colony. Instead, the presence of communication tunnels between the first and second batumen layers could play some role in facilitating the movement of bees out of their colony without coming into direct contact with the ants.

The sticky resin that has been observed between the outer layers of the nest of the ants has instead an evident role in excluding the ants that would otherwise remain entangled. Finally, the aggressive behavior of the *Dolichoderus* ants could benefit the docile *T.* cfr. *cilipes* in defending the colony.

The structure of *Paratrigona* sp.-*Camponotus* sp. nest described here, is similar to that described of *Paratrigona myrmecophila* Moure, 1989 - *Camponotus senex* (Laroca & Almeida 1989) and *Paratrigona peltata peltata* Spinola, 1853 - *Camponotus senex* associations (Wille & Michener 1973), even if in the latter the entrance tube of the hive is located in the lower part of the nest of the ants, and the size of the nests generally show some variations. *Paratrigona* sp. probably colonized the *Camponotus* nest a long time ago as the honeycombs occupied almost all the space of the nest of the ants. The presence of the small resin deposits on the entrance tube of the hive could have a defensive function towards the host, as well as the presence of a sticky batumen to isolate the internal honeycombs. Also in this association, the aggressive behavior of the *Camponotus* ants could benefit the meek *Paratrigona* bees.

The few records of myrmecophilus stingless bees do not always allow understanding how these relations were initiated and maintained. In most cases, stingless bees living in association with ants are considered nesting parasites (Sakagami et al. 1989; Roubik 2006), but the interactions may be different and more complex than expected, ranging from the sole exploitation of ant nest cavities to parasitism and parabiotic interactions (Bordoni et al. 2019). Describing nest architectures and building materials is a useful approach to studying associations between stingless bees and other social insects, because it provides hints for understanding these interactions.

Much remains to be understood about the complex relationships between stingless bees and other social insects, particularly those with ants. Our records of the two myrmecophilous bees from the Peruvian Amazon contribute with the knowledge about the Meliponini of Peru and provide useful elements for interpreting the interactions between stingless bees and ants living associated.

Acknowledgments – The research was made possible thanks to the collaboration between the Science Department of the Roma Tre University, the Department of Biology and Biotechnology, Sapienza University of Rome (Italy) and Urku Estudios Amazonicos Association (Peru). We thank the beekeeper Mr. Francisco Bartra for indicating the nest of *T.* cfr. *cilipes*-*D. quadridenticulatus*.

References

Bordoni A., Mocilnik G., Forni G., Bercigli M., Giove C.D.V., Luchetti A., Turillazzi S., Dapporto L., Marconi M. 2020. Two aggressive neighbours living peacefully: the nesting association between a stingless bee and the bullet ant. Insectes Sociaux, 67: 103–112, Doi: 10.1007/2Fs00040-019-00733-9

Camargo J.M.F. 1988. Meliponinae (Hymenoptera, Apidae) da coleção do ‘‘Instituto di entomologia agraria’’, Portici, Itália. Revista Brasileira de Entomologia, 32: 351–374.

Camargo J.M.F., Pedro S.R. 2003. Neotropical Meliponini: the genus *Partamona* Schwarz, 1939 (Hymenoptera, Apidae, Apinae)-bionomy and biogeography. Revista Brasileira de Entomologia, 47(3): 311–372, Doi: 10.1590/S0085-56262003000300001

de Oliveira F.F., Trautman Richers B.T., da Silva J.R., Farias R.C., de Lima Matos T.A. 2013. Guia Ilustrado das Abelhas “Sem-Ferrão” das Reservas Amanã e Mamirauá, Brasil (Hymenoptera, Apidae, Meliponini). Instituto de Desenvolvimento Sustentável Mamirauá, Brazil, 267 pp.

Grüter C. 2020. Stingless bees. Springer, Switzerland, 385 pp., Doi: 10.1007/978-3-030-60090-7

Kerr W.E., Sakagami S.F., Zucchi R., Portugal-Araújo V.D., Camargo J.M.F. 1967. Observações sobre a arquitetura dos ninhos e comportamento de algumas espécies de abelhas sem ferrão das vizinhanças de Manaus, Amazonas (Hymenoptera, Apoidea). Proceedings of the Simpósio sobre a biota Amazônica, Conselho Nacional de Pesquisas Rio de Janeiro, Brazil, pp. 255–309.

Kempf N. 1962. Mutualism between Trigona compressa Latr. and Crematogaster stolli Forel (Hymenoptera: Apidae). New York Entomological Society, 70: 215–217.

Laroca S., Almeida M.C. 1989. Coexistência entre abelhas sem ferrão e formigas: ninho de *Paratrigona myrmecophila* (Apidae) construído em ninho de *Camponotus senex* (Formicidae). Revista Brasileira de Zoologia, 6: 671–680, Doi: 10.1590/S0101-81751989000400012

Michener C.D. 2000. Bees of the world. Johns Hopkins Press, Baltimore, 992 pp.

Rasmussen C. 2004. A Stingless Bee Nesting with a Paper Wasp (Hymenoptera: Apidae, Vespidae). Journal of the Kansas Entomological Society, 77(4): 593–601, Doi: 10.2317/E29.1

Rasmussen C., Camargo J.M. 2008. A molecular phylogeny and the evolution of nest architecture and behavior in *Trigona* s.s. (Hymenoptera: Apidae: Meliponini). Apidologie, 39: 102-118. DOI: 10.1051/apido:2007051

Rasmussen C., González V. 2009. Abejas sin aguijón (Hymenoptera: Apidae: Meliponini) del Cerro Escalera, San Martín, Perú. Sistemas Agroecológicos y Modelos Biomatemáticos, 2(2): 26–32.

Roubik D.W. 2006. Stingless bee nesting biology. Apidologie, 37: 124–143, DOI: 10.1051/apido:2006026

Sakagami S.F., Inoue T., Yamane S., Salmah S. 1989. Nests of the myrmecophilous stingless bee, *Trigona moorei*: how do bees initiate their nest within an arboreal ant nest? Biotropica, 21: 265–274. Doi: 10.2307/2388654

Santos J.C., Del-Claro K. 2009. Ecology and behaviour of the weaver ant *Camponotus* (*Myrmobrachys*) *senex*. Journal of Natural History, 43 (23–24): 1423–1435. Doi: 10.1080/00222930902903236

Silva-Matos E.V., Noll F.B., Zucchi R. 2000. Sistemas de regulação social encontrados em abelhas altamente eussociais (Hymenoptera: Apidae: Meliponinae). Proceedings of the IV Encontro sobre Abelhas, Ribeirão Preto-São Paulo, pp. 95–101.

Silveira F.A., Melo G.A., Almeida E.A. 2002. Abelhas Brasileiras sistemática e identificação. Belo Horizonte, Minas Gerais, 253 pp.

Wille A., Michener C.D. 1973. The nest architecture of stingless bees with special reference to those of Costa Rica (Hymenoptera, Apidae). Revista de Biología Tropical, 21: 1–278.