



RESEARCH ARTICLE - BEES

Nest Architecture of *Tetragona clavipes* (Fabricius) (Hymenoptera: Apidae: Meliponini)

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Abstract

The aim of this study was to describe the nest architecture of *Tetragona clavipes* colonies, to gather information to be used on scaling a species-adapted beehive, and to contribute to the necessary knowledge for the preparation of management and conservation plans for this species. The measurements were made on 10 colonies in terms of brood chamber, brood cells, royal cells, food pots, pillars between combs, and population. Brood chamber ($\bar{x} = 3117.9 \pm 1230.6 \text{ cm}^3$) of spiral-shaped combs were all in the center of the nest. The equatorial diameter of brood chambers ($\bar{x} = 14.33 \pm 3.03 \text{ cm}$) can adapt to a 15.0 cm internal beehive width. The height and width of brood cells ($\bar{x} = 6.37 \pm 0.32 \text{ mm}$; $\bar{x} = 3.73 \pm 0.35 \text{ mm}$), and food pots ($\bar{x} = 3.42 \pm 0.39 \text{ cm}$; $\bar{x} = 2.21 \pm 0.23 \text{ cm}$) were statistically different among colonies. The height of food pots was useful to estimate the internal height of a honey super (around 4 cm). The population size found was considerable high ($\bar{x} = 50,920 \pm 20,100$), which might represent an advantage for the commercial raising of the species. The measurements of the internal nest structures of *T. clavipes* were similar to the few other reported studies of this species, and presented only few differences from other stingless bee species. The results of this study seemed to contribute to phylogenetic analyses, allow designing a beehive, consider a potential commercial stingless beekeeping, and add information about the biology and conservation of *T. clavipes* in its natural environment.

Introduction

Meliponini are believed to be the most important pollinators in the tropical American continent. Unfortunately, due to human deforestation and transformation of natural landscapes, these bee species have been suffering large intervention, which can become endangered or disappear. Research related to bee nest architecture contributes to the necessary knowledge for the preparation of management and conservation plans for these species. The measurements of the nest help to gather important information that would be crucial to build adapted boxes for the raising and propagation of social bees.

Tetragona clavipes (F.) (Fig. 1) is a native stingless bee species of South America (Camargo & Pedro, 2015) with little information about its nest architecture in literature. Bertoni (1912) described, in a short paragraph, that wild colonies nest mostly in trees, producing great amount of honey. Along with some species of the genera *Scaptotrigona*, *Melipona* and *Cephalotrigona*, *T. clavipes* has a potential to storage a

large amount of food inside its nest (Cortopassi-Laurino et al., 2006), fact of great interest to commercial honey production. Sakagami and Zucchi (1967) observed only one wild colony, in which they measured the brood chamber volume and the approximately 30 liters of food storage area; however, the authors focused their study on the oviposition process of the queen in a small observation hive. Nogueira-Neto (1970) also described aspects of this species such as behavioral facts; however, very few measurements and quantification of the nest characteristics was performed. Tóth et al. (2004) estimated the colony population based on the reviewed literature data on the oviposition of queens and workers.

The lack of engagement of beekeepers and researchers on keeping this species restricted the discovery of sufficient and more precise information about its biology and nest architecture. The aim of this study was to describe the nest architecture of *T. clavipes* colonies and to compare these features with those already described in literature about *T. clavipes* and other stingless bee species. In addition, help



gather information to be used on scaling a beehive adapted for raising this species.



Fig 1. Worker of *Tetragona clavipes* taking flight. (Photo: Raoni Duarte)

Materials and methods

The nest architecture description of *T. clavipes* was made on 10 colonies located within the campus of the University of São Paulo, Ribeirão Preto, Brazil (21° 10' S, 47° 51' W, and 530m above sea level). All colonies were naturally nested on dead tree trunks. The study was carried out from March 2010 to April 2012, when colonies were transferred to wooden bee boxes. In all data collection, observations, and manipulation process of colonies, researchers wore a body covering protective suit.

Volume and equatorial width of brood chambers, height and width of brood cells, royal cells and food pots, height of pillars between combs were measured. The oldest brood cells were selected to be measured, that were located on the brood comb layer, where adults were emerging from cocoons. A digital caliper rule of 0.01mm accuracy and a common ruler of 0.1cm accuracy were used to measure these features. To estimate colony population (egg, larvae, pupae and adults), the Ihering's (1930) formula ($x + x/2$) was used, where x is equal to the number of brood cells in the nest. The one-way Analysis of Variance (ANOVA) and the Tukey test were applied to find possible significant differences among colonies ($p < 0.01$), within variables height and width of brood cells and food pots.

Results

Colonies were taken from trunks that were already cut down, so, the distance of nests from the ground was not measured. Trunk thickness varied from 11 to 28 cm (Fig 2). Colonies were nested in seven tree species: *Eucalyptus* sp., *Cupressus* sp., *Persea americana* (M.), *Poincianella pluviosa* (DC.), *Pterogyne nitens* (T.), *Tabebuia roseoalba* (R.), and *Tipuana tipu* (B.). The wood density varied according to the specific characteristics of plants.

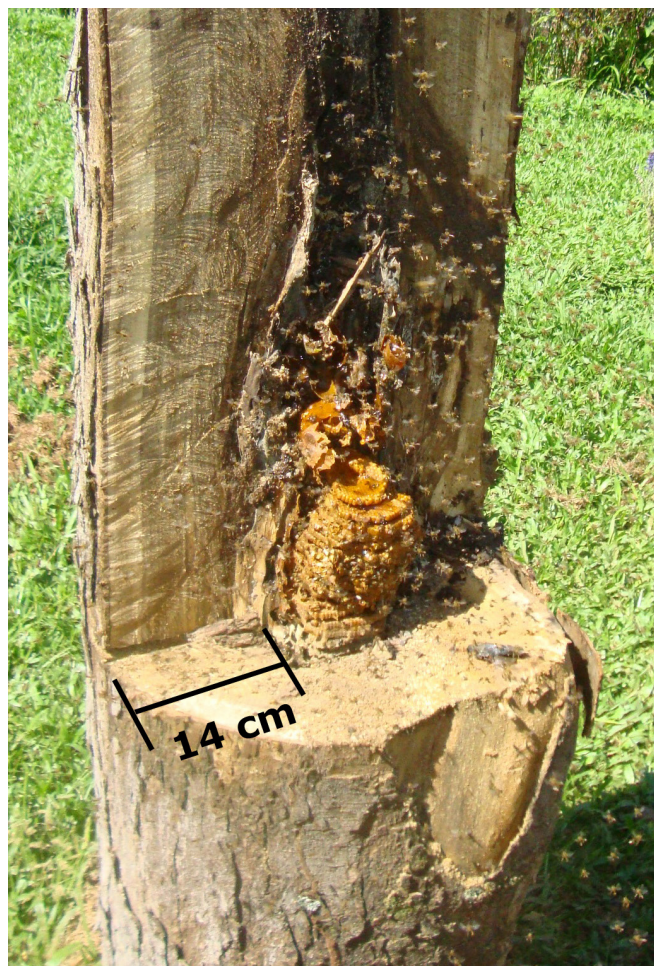


Fig 2. Trunk thickness of a *Tetragona clavipes* natural nesting site. (Photo: Raoni Duarte)

The entrance hole, unique to each colony, was ellipsoidal and constructed with hardened resin and dark propolis (Fig 3). The entrance tunnel ranged from 11 to 70 cm, passing through the timber, not extending inside the cavity, and ending mostly in the upper part of the nest. The cavity surface was dry and coated with white and grey resin.

Wax layers, up to 2 cm thick, covered the chamber of spiral-shaped (Fig 4) brood combs. The majority of brood chamber had the shape of an ellipsoid of revolution and stood in the central area of the nest with food pots around them. There was a statistically significant difference among colonies for height and width of the elongated brood cells ($F_{(10,121)} = 32.91$; $P < 0.01$; $F_{(10,121)} = 21.76$; $P < 0.01$).



Fig 3. Nest entrance of *Tetrasona clavipes*. (Photo: Ivan de Castro)



Fig 4. Superior view of the spiral brood combs of *Tetrasona clavipes*. (Photo: Raoni Duarte)

Regarding size, shape and distribution, there was a mean of 10.89 ± 0.07 brood cells per cm^3 of brood. Therefore, the population of the 10 colonies was on average $50,920 \pm 20,100$ individuals (Table 1). Royal cells were positioned at the edge of combs, and randomly distributed into the brood chamber, which had up to 17 cells per colony.

Table 1. Internal nest features of *Tetrasona clavipes*, Ribeirão Preto-SP.

Nest feature	N	Mean	SD	SE	Range
Brood chamber - width (cm)	20	14.33	3.03	0.96	11.75 – 21.0
Brood chamber - volume (cm^3)	10	3117.90	1230.6	389.15	1301.2 – 5318.1
Brood cells - height (mm)	132	6.37	0.32	0.02	5.39 – 7.04
Brood cells - width (mm)	132	3.73	0.35	0.03	2.75 – 4.56
Pillars between combs - height (mm)	35	3.64	0.39	0.06	2.94 – 4.35
Royal cells - height (mm)	25	8.47	0.41	0.08	7.81 – 9.39
Royal cells - width (mm)	25	6.05	0.17	0.03	5.54 – 6.29
Food pots - height (cm)	100	3.42	0.39	0.39	2.15 - 5.02
Food pots - width (cm)	100	2.21	0.23	0.23	1.74 – 2.93
Estimated population *	10	50,927	20,100	6,356.27	21,253 – 86,864

*Formula used by Ihering (1930). (N = number of observations; SD = standard deviation; SE = standard error; Range = minimum and maximum values)

For height and width of the oval shaped food pots (Fig 5), a statistically significant difference among colonies was found ($F_{(9,90)} = 3.79$; $P < 0.01$; $F_{(9,90)} = 4.21$; $P < 0.01$). There was no clear segregation of honey and pollen pots in separate clusters. However, pollen pots were mainly near the brood, and there was apparently higher amount of honey pots than pollen. The bees built pots in widely dispersed locations, as the shapes of natural cavities were very irregular. The amount of honey was not measured due to food pot damage, and unwanted visitors, such as phorid flies (*Pseudohypocera* sp. (Diptera, Phoridae)) rapidly appeared.



Fig 5. Honey pots of *Tetrasona clavipes* in a honey super. (Photo: Raoni Duarte)

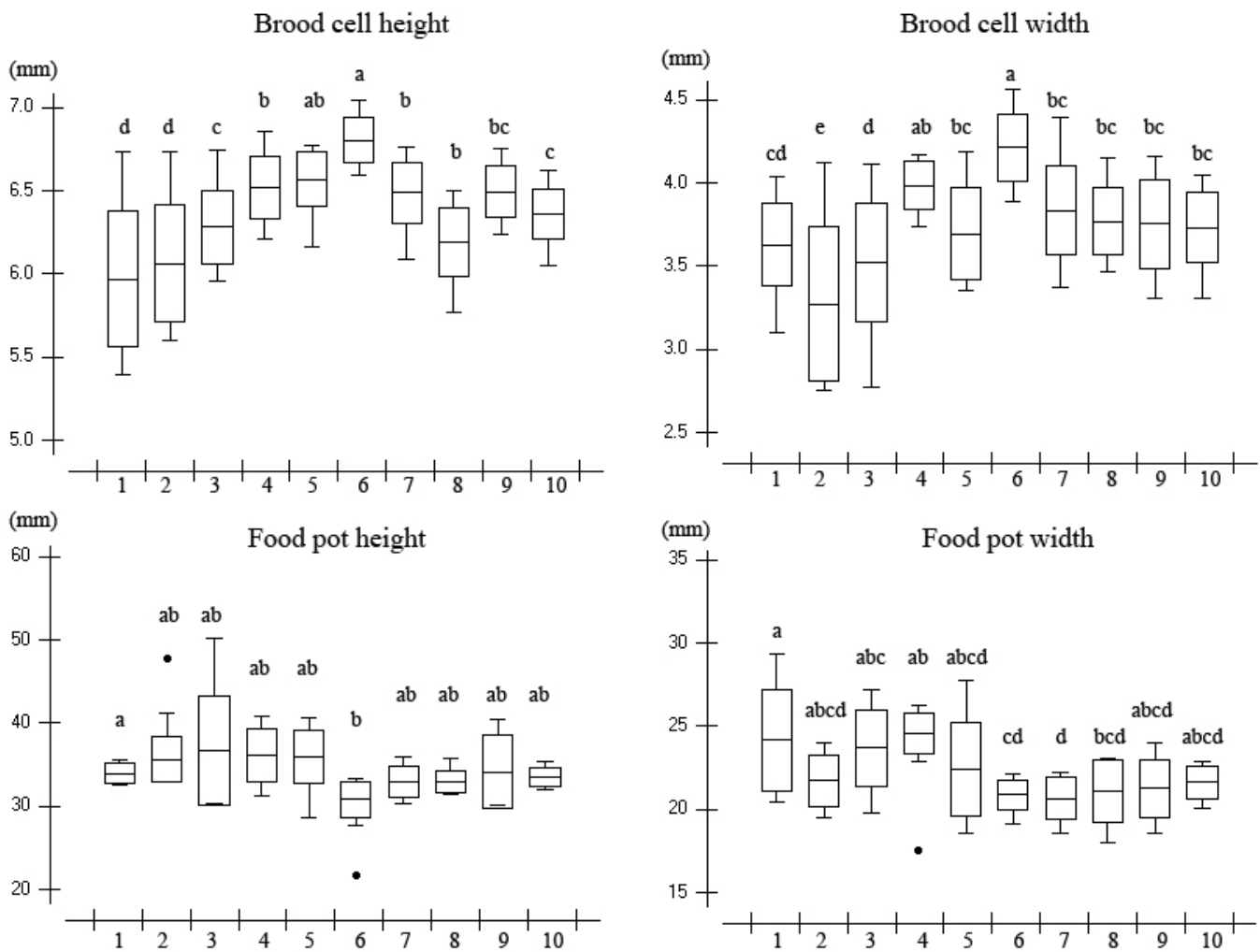


Fig 6. Height and width (mean, stand. dev., max., min.) of brood cells and food pots of 10 *Tetragona clavipes* colonies. Letters (a, b, c, d) indicates grouping differences from ANOVA and Tukey method.

Discussion

As reported by Siqueira et al. (2012) and Lima et al. (2013), all colonies in this study were also nested in trees. Dissimilarly, Wille and Michener (1973), and Freitas (2001) observed this species nested in other locations, such as buildings, brick walls, chimney, and underground plumbing.

Roubik (2006) reported that, within the genus *Tetragona*, some colonies might present the biggest entrance holes within Meliponini along with *Trigona* and *Scaptotrigona* genus. In addition, they build multiple entrance holes, which helps on defending the nest. However, it was not found multiple entrances in these colonies at Ribeirão Preto.

Spiral brood comb was observed in all colonies studied here, corroborating findings reported by Sakagami and Zucchi (1967) and Nogueira-Neto (1970), and can occur in other Meliponini (Laroca, 1971; Wille & Michener, 1973; Nogueira-Neto, 1997; Souza et al., 2007; Brito et al., 2012; Barbosa et al., 2013). The population of colonies in Ribeirão Preto was far above the maximum population of 10,000 bees calculated by Tóth et al. (2004), possibly due to the different estimation methods.

The large number of *T. clavipes* worker bees might represent an advantage for the commercial raising of this species, since a high number of foragers will determine efficient foraging and flower visitation. Consequently, this could favor honey production, pollen collecting, or even the success of pollination services. *T. clavipes* visit flower plants of economic interest such as sunflower (*Helianthus annuus* (L.)), citric fruits (*Citrus* sp.), “butiá” palm (*Butia eriostatha* (M.)), “pequi” (*Caryocar brasiliense* (C.)), “murici” (*Byrsonima crassa* (N.)) and some plants of the Myrtaceae family, such as wild guava (*Psidium incanescens* (M.)). Among other species, also visit Brazilian native plants such as *Poincianella pluviosa* (DC.), *Guazuma ulmifolia* (L.), *Croton urucurana* (B.), *Libidibia ferrea* (M.), and *Smilax fluminensis* (S.) (Sofia, 1992; Pedro, 1992; Mateus, 1998; Almeida-Anacleto, 2007; Duarte, 2012; Pessoa et al., 2013).

The size of brood cells showed higher statistical differences among colonies than food pots (Fig 6), but the reasons are yet unknown. In another Meliponini, *Tetragonisca angustula* L., was observed a size differentiation between worker bees inside each colony. The authors provided evidence

for a physical soldier subcaste, whereas guard bees are 30% heavier than foragers and of different shape. The exoskeleton of holometabolous insects, including stingless bees, does not grow after adult emergence, thus, the authors observed newly emerged adult worker bees with physical sizes classified as guards or foragers. The size of the brood cells from which worker bee emerged was not measured, so the correlation of size of brood cell with size of bee is still not clear (Grüter et al., 2012). This discovery was not expanded to other Meliponini, and so it can not yet explain the statistical differences of the brood cell size in *T. clavipes* measured at Ribeirão Preto, therefore more research is needed.

Parameters of stingless bee nest architecture were used to scale beehives by Portugal-Araujo (1955) in his research on African stingless bees, and by Nogueira-Neto (1970, 1997) on Brazilian stingless bees. Therefore, the measurement values of *T. clavipes* nest features, made in this study, can be useful to design a beehive fitted for this species. The mean 14.33 ± 3.03 cm of brood chamber equatorial diameter can adapt to a 15.0 cm internal width of a brood module. The volume value of the brood chamber may fit to a specific number of brood modules added for each colony. The height of food pots ($\bar{x} = 3.42 \pm 0.39$) can be used to scale the internal height of honey supers, which could be around 4 cm, leaving enough space for building only one layer of food pots by the bees. The overlap of food pot layers can difficult some beekeepers' activities such as inspection of colonies, honey harvest, and colony division. The thickness of natural nesting sites chosen by the colonies observed in this study (up to 28 cm) demonstrated isolation to the external environment, which can be related to temperature control, and is important to determine the wall thickness of a hive.

The mean height of food pots (around 3 cm) was similar to data reported by Sakagami and Zucchi (1967). Statistical differences found among sizes of food pots may be due to the different shape and size of natural nesting sites cavities available for the bees to build pots. The total food volume could not be measured, because pots were found in irregular and difficult accessing locations, not unique in Meliponini (Camargo, 1970). The access to the measurement of individual food pots resulted in pot damaged, honey oozed, fermented pollen exposed, phorid flies appearance and laid their eggs on the colony, compromising the post-transference survival of colonies. However, this species apparently seems to have a better ability to store food compared to other Meliponini, which has not yet been explored. Therefore, this topic deserves attention in future measurement studies.

The internal nest structures of *T. clavipes* were similar to data found on the reported articles about the species. In general, few differences were found between *T. clavipes* and other stingless bee species. The nests measurement data of this study seem to contribute to phylogenetic analyses, allow designing a beehive, consider a potential commercial stingless beekeeping, and add information about the biology and conservation of *T. clavipes* in its natural environment.

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