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Estimating colonies of *Plebeia droryana* (Friese, 1900) (Hymenoptera: Apidae: Meliponini): adults, brood and nest structure

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Abstract

Estimate of stingless bee colonies including nest structures and quantitative brood and adult individuals are scarce. Here, we describe a new approach to estimate colonial parameters from nest structure, adults and brood. We used five colonies of *Plebeia droryana* (Friese, 1900) to evaluate colony size and weight of adult and brood. Nest architecture in *P. droryana* is similar to the species of the same genus but differ to the other stingless bees. In this species, we counted a total of 9 to 12 brood combs and a total of 19 to 25 food pots in the nests. The number of individuals in the colonies is considered small and our estimate was based on individual and group weight. Our study approach may contribute to further detailed studies of the species nest and considering the stingless bees to the pollination of agricultural crops and native flora of tropical regions, it is important to add information about the biology of *P. droryana*.

Introduction

Stingless bees are found in tropical and subtropical regions all around the world (Sakagami, 1982) and they have populated tropical regions for over 65 million years – longer than *Apis*, the honeybees (Camargo & Pedro, 1992; Michener, 2000). However, stingless bees have 50 times more species and, as emphasized here, differ from *Apis* in many biologically significant ways (Roubik, 2006). The colonies have individuals active every day that perform their activities and, therefore, have supported climate impact of the environment (Roubik, 1989; Hansell, 1993). The colonies of stingless bees present structural, behavioral and physiological adaptations, which protect bees from the external environment (Roubik & Peralta, 1983).

Their nests are perennial, and they are active throughout the year (Michener, 1974; Sakagami, 1982). Stingless bees usually build their nests in holes in trees, cavity in the soil, and even in cavities in human constructions, but some also construct aerial nests (Nogueira-Neto, 1997), and some species that regularly establish their nests within nests of Nasutitermes or ants (Michener, 2000). In contrast to the Apini, new stingless bee nests are started by workers going back and forward from an existing old colony. They transfer building materials and food to the new site. Ultimately, a young queen flies to the new site, workers stay there, and independence from the old colony is gradually attained over a period of weeks or months (Michener, 1946; Inoue et al., 1984; Van-Veen & Sommeijer, 2000).

Nests are made of wax secreted from the metasomal terga mixed with resins and gums collected by the foragers. Some species add mud, feces, or other materials in several parts of the nest. In all species, the composition and texture differ in different parts of the nest (Michener, 2000). In some species,

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the brood combs are covered by layers of wax involucrum and the storage pots are made with cerumen. Several bee species build a strong structure with cerumen, mud, and resin called scutellum or batumen (Zucchi & Sakagami, 1972). Some of the supports and batumen plates are of tough material, and the external sheet of batumen around some exposed nests is harder and brittle (Michener, 2000).

Nest characteristics such as the nesting site, architectural complexity, and building materials may be taxon-specific and provide an excellent opportunity to assess information about underlying behavioral evolution of the respective taxa and higher groups (Rasmussen & Camargo, 2008). The nest architecture of stingless bees consists of an entrance, access tunnel, food pots (nectar and pollen storage) and brood area, with horizontal combs or in cluster (Zucchi & Sakagami, 1972; Wille & Michener, 1973; Michener, 1974). Michener (1974) described the colony size of various social bees, especially the genus *Trigona*. In addition, Lindauer and Kerr (1960) presented an estimate of adult workers in colonies of stingless bee colonies. They evaluated 10 species and among them *Plebeia droryana* (formerly known as *Trigona droryana*) had 2,000 to 3,000 adults. Wille and Michener (1973) showed another estimate in which measured nest structures as food pots and brood cells diameter and length. Using Lindauer and Kerr (1960) estimative references to *P. droryana* and other bees, Duarte et al. (2016) studied nest architecture of *Tetragona clavipes* and presented structures and individuals estimative.

The genus *Plebeia* Schwarz, 1938 consists a very diverse group and is distributed in the Neotropical region. *Plebeia* is morphologically the most primitive taxa within stingless bees and is mainly found in the southeast region in Brazil (Wittmann, 1989; Michener, 1990; Witter et al., 2007). The stingless bee *P. droryana* has a wide geographic distribution, which includes Argentina, Bolivia, Paraguay and some Brazilian states as Bahia, Espírito Santo, Minas Gerais, Paraná, Pernambuco, Rio Grande do Sul, Rio de Janeiro, Santa Catarina and São Paulo (Moure et al., 2007; Camargo & Pedro, 2013). These bees are small (4 mm) and not aggressive, build royal cells for queen production (Nogueira-Neto, 1970).

Although some estimative recordings for *P. droryana* nests and other stingless bee species have been made, studies on basic biology aspects are still scarce. The stingless bees are important for pollination of agricultural crops and native flora of tropical regions and income source as the meliponiculture. Therefore, new studies that provide information about the biology of Neotropical stingless bees and can collaborate with the conservation of the species by measuring the nest structures and to allow better handling conditions are necessary (Cortopassi-Laurino et al., 2016; Jaffé et al., 2015). Here, we present a description of the nest structure and an evaluation of adults and brood of *P. droryana*. Moreover, we provide a new method to estimate stingless bee colonies.

Material and methods

Study site

The study was carried out in the Campus of the University of São Paulo at Ribeirão Preto, Brazil (21°10’39’’S, 47°48’24’’W). Five colonies of the stingless bee *P. droryana* were kept in hives made with wood (14.0 ´ 14.0 ´ 12 cm; walls: 2 cm; volume of 2.352 cm^3) in the Laboratório de Comportamento e Ecologia de Insetos Sociais. The hives were connected to the outside via plastic tubes through which the bees could freely exit and enter in the nest. The bees collected pollen and nectar freely because they no received artificial feeding.

Colony size

We evaluated colony size by weighting *P. droryana* colonies (hereafter PD1, PD2, PD3, PD4 and PD5) between 17th September and 27th September 2013. We used carbon dioxide to anesthetize individuals and to preserve the structure of the nest. We weighted the combs, the involucrum wax and the adult individuals. We estimated the weight of food pots before removing the combs. Workers and males were weighted together, but males were found only in one nest.

We counted the number of the food pots (honey and pollen), brood combs with egg, larvae (small and medium) and pupae, resin piles and garbage dump. We also estimated the population size of adult and immature individuals. To estimate adult population, we weighted separately 42 workers in order to know the individual weight, and divided by the total weight of nest individuals, representing the total estimated number of the nest. We used a formula to calculate the total weighted of adult bee population and nest material:

$$\text{Total}_{\text{col size}} = (\text{In}_{w} + \text{C} + \text{Box}_{s} + \text{Q} + \text{W}) + \text{Total}_{ap}$$

where, the total colony size ($\text{Total}_{\text{col size}}$) was equal to the sum of the involucrum wax (In_w), combs (C), box with structures (Box_s), queen (Q), waste (W) and the total weight of the adult individuals (Total_{ap} - estimative). The waste was the variation found in the colonies weighted because the manipulation of the colonies and lost of some materials (wax, honey and others).

To estimate immature individuals, we weighted one comb and counted the number of cells. We measured the comb (length x width) and related the number of cells with the number of combs for each nest (Fig 2). The data were analyzed with Microsoft Excel.

Results and discussion

Nest and colony estimative

Nests had from 9 to 12 brood combs and 19 to 25 food pots. Also, there were one to four resin piles and one to three garbage dumps (Table 1). In the total combs in every nest, we found a proportion of three to four combs with eggs and larvae stage, and six to eight combs with pupae stage (Table 1). We counted one pollen pot only in the PD4 nest. In the PD1 nest, we observed one royal cell and one giant male individual (Fig 1; Table 1).

Fig 1. Nest architecture of the stingless bee *Plebeia droryana*. (A) Food pots (honey and pollen). (B) Resin pile. (C) The nest with all structures. (D) The amount of the combs (brood area). Scale bar 3 mm.

The characteristics of nest architecture in *P. droryana* were similar to those described by Zucchi and Sakagami (1972) and Wille and Michener (1973) in other species. We found the occurrence of an involucrum and the resin pile, typical of this species. In the brood area, the involucrum layers keep the temperature by retaining the heat production from brood metabolism (Wille, 1983; Engels et al., 1995; Barbosa et al., 2013). The presence of garbage dump deposit was observed in this study such as previously reported by Nogueira-Neto (1997) in other stingless bee species.

Weight Estimative

Although some authors described previously the nest structures, the weight of them was not reported as in our study. The weight of the five colonies ranged from 987.7 to 1109.9g (Table 2). The brood combs weighted 31g in the PD1 colony, 44.1g in the PD2 colony, 38.3g in the PD3 colony,

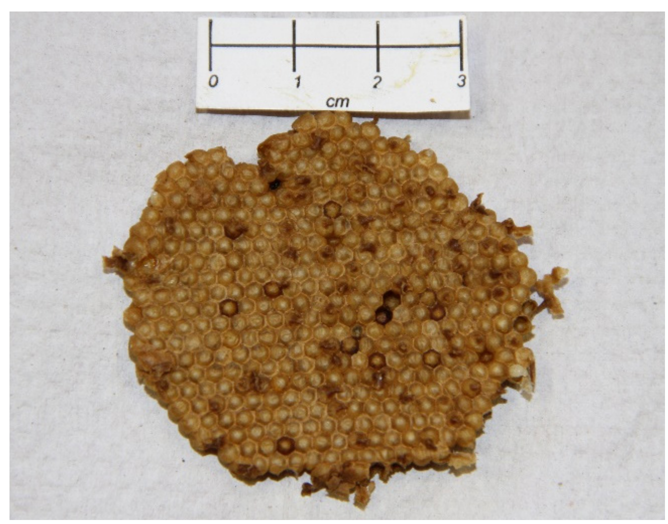

Fig 2. Brood comb of the *Plebeia droryana* used to estimate immatures. Scale bar = 3 cm.

Table 1. Number of the nest structures, brood combs, adult individuals estimative and immature estimative and the mean ±SD, respectively.

	PD1	PD2	PD3	PD4	PD5	Mean ± SD
Total brood combs	11	10	10	9	12	10.40 ± 1.14
Combs (eggs and small larvae)	3	4	3	3	4	3.40 ± 0.55
Combs (larvae medium and pupae)	8	6	7	6	8	7.00 ± 1.00
Total adult individuals estimative	2507	1548	2414	1917	1069	1891.00 ± 601.45
Total immature estimative	4158	5915	5137	4212	4727	4829.80 ± 727.25
Food pots	25	22	19	19	22	21.40 ± 2.51
Resin piles	1	4	2	2	1	2.00 ± 1.22
Garbage dump	1	3	3	3	2	2.40 ± 0.89

Table 2. Weight of the structures and individuals in the five different nests (in grams) and the mean ± SD to each item. The weight of the empty wooden boxes was the average between all colonies studied. The "box with structures" refers to the weight of the wooden box with food pots, garbage dump and resin piles; and "structures without the box" refers to the weight without the mean of the empty wooden box, to calculate approximately the weight of the structures that remained inside the box.

	PD1	PD2	PD3	PD4	PD5	Mean ± SD
Total nest - hive	1075.3	987.7	1094.7	1042.6	1109.9	1062.04 ± 48.57
Involucrum wax	3.6	8.9	6.1	6.7	7.8	6.62 ± 2.00
Combs	31	44.1	38.3	31.4	31.4	35.24 ± 5.82
Empty wooden boxes	701.5	701.5	701.5	701.5	701.5	-
Box with structures	878.1	916	702.3	985.1	1096.6	915.62 ± 145.34
Structures without the box	176.6	214.5	0.8	283.6	395.1	214.12 ± 145.32
Queen	0.045	0.0582	0.0536	0.0484	0.0499	0.051 ± 0.005
Total adult individuals	13.6	8.4	13.1	10.4	5.8	10.26 ± 3.26

31.4g in the PD4 and PD5 colonies (mean of 35.2 ± 5.82g). The weight of the involucrum wax varied between 3.6 to 8.9g (mean±SD = 6.62 ± 2.0g) (Table 2). Queens weighted 0.045g (PD1 colony), 0.058g (PD2 colony), 0.053g (PD3 colony), 0.048g (PD4 colony) and 0.049g (PD5 colony). The average of the weight to the total adult individuals was of 13.6g to PD1 colony, 8.4g to PD2 colony, 13.1g to PD3 colony, 10.4 g to PD4 colony and 5.8g to PD5 colony (mean: 10.26 ± 3.26) (Table 2).

Finally, the weight of foragers and comb workers were obtained (Fig 1) and we used the total weight (42 individuals) for the total individual nest estimative (Fig 3). The number estimated of adult individuals in the colonies was 2,507 (PD1), 1,548 (PD2), 2,414 (PD3), 1,917 (PD4) and 1,069 (PD5), mean of the 1,891 ± 601.45 (Table 1). The estimated number of immature individuals was 4,158 (PD1), 5,915 (PD2), 5,137 (PD3), 4,212 (PD4) and 4,727 (PD5), mean of the 4,829.8 ± 727.25 (Table 1).

The weight of individuals has an important role for the estimative of the population. Duarte et al. (2016) have estimated nest architecture by measuring (in centimeters), and they found an approximate population of 50,927 individuals in *T. clavipes* by using a formula (Ihering, 1930). Our study

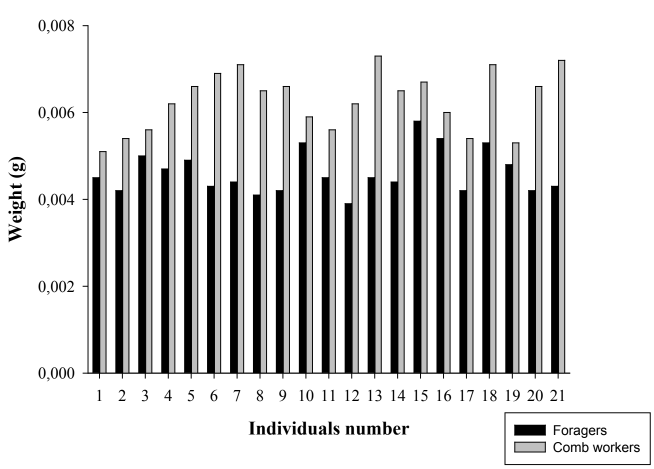

Fig 3. Weight of individuals separately (21 foragers and 21 comb workers). The foragers were lighter because they have less adipose tissue for the flight (Michener, 1974).

showed an effective estimative by using the weight. Nests of *P. droryana* presented 1,069 to 2,507 individuals, observed also by Lindauer and Kerr (1960) that estimated to *P. droryana* between 2,000 and 3,000 individuals. Cortopassi-Laurino (1979) estimated the adults' population of the *P. droryana* using another parameter. The colonies were considered "strong" when they had approximately 1,500 to 2,000 individuals and this result agreed with our estimated adult population (1,069 to 2,507). The immature estimate was of 4,727 individuals.

Conclusion

We provided a new method to estimate *Plebeia droryana*. The present study contributes to provide more details about the nests of this species and the estimate can be useful to further studies as well to the management and conservation of stingless bees.

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