



## RESEARCH ARTICLE - BEES

## The Cavity-Nesting Bee Guild (Apoidea) in a Neotropical Sandy Coastal Plain

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### Abstract

Some solitary bees establish their nests in preexisting cavities. Such nesting behavior facilitates the investigation of their life history, as well as the monitoring of their populations in natural, semi-natural and cropped habitats. This study aimed to evaluate the acceptance of artificial substrates by cavity-nesting bees in a heterogeneous landscape. We investigated the percentage of occupation of the different trap-nests, the monthly fluctuations in the nesting activity, offspring sex ratio, mortality and parasitism, in two phytophysiognomies: herbaceous-shrub restinga (site 1) and arboreal restinga (site 2). We used as trap-nests, bamboo canes, large and small straws of cardboard inserted into solid wooden blocks. Five bee species established 193 nests, from which 386 adults emerged. *Centris tarsata* Smith was the most abundant species. Large straws were significantly more occupied than small straws ( $\chi^2 = 19.951$ ;  $df = 1$ ;  $p < 0.0001$ ). Offspring mortality rate for unknown reasons was significant different between sites, 11% (site 1) and 20% (site 2) ( $\chi^2 = 4.203$ ;  $df = 1$ ;  $p = 0.04$ ). The cavity-nesting bee guild had similar composition in both phytophysiognomies, there was a similar rate of occupation of trap-nests in both sites, as well as dominance of *C. tarsata* nests. Offspring mortality and parasites attack rates seem to be the more distinctive aspects between the herbaceous-shrub and arboreal restinga sampled. Our study indicated that remnant fragments of coastal native habitats may be important nesting sites for the maintenance of bee populations, some of which have been indicated as candidates for management as pollinators of cultivated plants in Brazil.

### Introduction

Bee populations are dependent of key resources related to their feeding, mating and nesting (Westrich, 1996). The Brazilian “restingas” (herbaceous-shrub and arboreal vegetation covering the sandy coastal plain) are under intense degradation, which has produced accentuated changes in the landscape and habitat loss (Rocha et al., 2007). The loss of local biodiversity, primarily by fragmentation and habitat loss can reduce the availability of nesting sites and food resources, and threaten bee communities and pollination services (Viana et al., 2012).

Several bee species nest in natural and artificial preexisting cavities (Roubik, 1989). The trap-nests sampling technique was designed by Krombein (1967) in order to obtain nests of these bees. Since then, it has been used to answer questions related to breeding and nesting biology (Garofalo et al., 2012). It also has allowed a management of bee pollinators in agricultural and natural landscapes, resulting in the increasing of the agricultural production (Bosch & Kemp, 2000; Magalhães & Freitas, 2013; Yamamoto et al., 2014).

Some studies have investigated the nesting biology of solitary bee species inhabiting the Atlantic coastal plain (Viana et al., 2001; Aguiar & Martins, 2002; Camarotti-de-Lima



& Martins, 2005; Bernardino & Gaglianone, 2008; Martins et al., 2014). Some of these cavity-nesting bee species, such as *Centris* and *Xylocopa* spp. are involved in pollination of crops and wild plants (Gottsberger et al., 1988; Buchmann, 2004; Gaglianone et al., 2010; Yamamoto et al., 2012; Oliveira et al., 2013). This study aimed to evaluate the acceptance of artificial substrates (trap-nests) for nest by cavity-nesting bees in a heterogeneous landscape covered by different phytophysiognomies of "restingas". We investigated the percentage of occupation of the different trap-nests, the monthly fluctuations in the nesting activity of these species, as well as the sex ratio of the offspring and the incidence of mortality and parasitism of the brood cells.

## Material and methods

### Study area

This study was carried out in a private reserve (RPPN-DSA, Reserva Particular do Patrimônio Natural Dunas de Santo Antônio; 12°27'S; 37°56'W), in Mata de São João, state of Bahia, Northeast of Brazil. According to the classification of Köppen, the local climate is Tropical (Af), hot and humid without dry seasons. The average temperature is between 21°C and 31 °C, and annual rainfall varies from 1,600 to 2,000 mm. The rainiest period occurs from April to June (SEI, 1999).

The phytophysiognomies of this restinga are similar to those described by Cogliatti-Carvalho et al. (2001), the herbaceous-shrub vegetation of the restingas are characterized by bare sand corridors, allowing a higher incidence of sunlight, while in the arboreal restinga, there is a greater density of woody vegetation, less sunlight incidence, increased availability of litter on the ground and high humidity. The study site is covered by an herbaceous-shrub vegetation in which *Humiria balsamifera*, *Waltheria cinerescens*, *Chamaecrista ramosa*, *Chrysobalanus icaco*, and *Cuphea brachiata* were plants often found, and by arboreal vegetation, a kind of restinga forest, in which *Bowdichia virgilioides*, *Stryphnodendron pulcherrimum*, *Andira nitida* and *Anacardium occidentale* are common plant species and can exceed 12 m in height (Queiroz, 2007). These plant species are the most important food resources for wild bees in sandy coastal plain of Bahia, Brazil (Viana & Kleinert, 2006).

### Sampling

The sampling was carried out in two sites 2.5 km distant from each other: one in the herbaceous-shrub vegetation (site 1), and other in arboreal vegetation (site 2). Although the sampling points are located in different phytophysiognomies, they are not far from each other enough to suggest that these bee populations are distinct.

The trap-nests were kept in steel shelves covered with a plastic tarpaulin. We used as trap-nests straws made of black cardboard inserted into solid wooden blocks, as well as bamboo canes following Camillo et al. (1995), and

Aguiar and Garofalo (2004). Six blocks of wood perforated by holes were placed at each site. Three blocks contained 60 holes each, in which black cardboard straws measuring 58 x 6 mm (SS = small cardboard straws) were inserted. Three other blocks contained 56 holes each, in which trap-nests of 105 x 8 mm (LS = large cardboard straws) were placed. In addition, 18 hollow bamboo canes were arranged in groups containing six bamboo canes of variable lengths (90 to 220 mm) and diameters (8 to 16 mm).

The trap-nests were inspected once a month, from November 2006 to November 2007. The trap-nests used by bees were collected and replaced by new ones. Each nest was maintained in a glass tube at room temperature, and they were opened after the emergence of the imagos. The number of brood cells, the presence of dead individuals and their stage of development, as well as the presence of other insects in the brood cells were recorded.

### Data analysis

The  $\chi^2$  test was used to compare trap-nest occupation in each site and the occupied trap-nests of each size (Zar, 2011). The  $\chi^2$  Test goodness of fit tests was used to compare the following aspects between the two sites: length of trap-nest and the frequency of occupation of the trap-nest (is the percentage the ratio between the total number of nests founded by the total number of nests available large and small card nests plus cane bamboo in each site), in order to check the extent to which the observed sex ratio deviated from the expected frequency (1M:1F), and to analyze differences in progeny sex ratio among nests of different size and nests from different sites. Heterogeneity  $\chi^2$  test was used to analyze the rates of immature mortality and parasitism (Zar, 2011). The Mann-Whitney test was used to monthly assess whether there were preferably in the two types of trap-nest offered, and also to check the difference on the development time between males and females emerged in the nest (Zar, 2011).

Spearman correlation ( $r_s$ ) analyses were performed to assess monthly temperature and precipitation effects on the monthly frequency of new nests, being considered significant  $p < 0.05$  by the Student's t-test (Zar, 2011). Sørensen index ( $C_s$ ) was used to evaluate the degree of similarity in the species composition of the cavity-nesting guild in both sites (Magurran, 2011). The analyzes were performed using R software (R Development Core Team, 2016).

## Results

### Trap-nests occupation, species richness and abundance

Five bee species nested in the trap-nests (Table 1). Although the phytophysiognomies are different in the two sampling points, there was no significant difference in the total number of nests between these sites ( $\chi^2 = 0.375$ ;  $df = 1$ ;  $p = 0.54$ ). A similar species composition of the cavity-nesting bee guild was found in the two sites ( $C_s = 0.88$ ). Only *Tetrapedia diversipes* Klug occurred exclusively in the site 2.

The occupation rates of the trap-nests were also similar, 25% (site 1) and 28% (site 2). *Centris tarsata* Smith established the highest number of nests in both sites, 18% of the all nests in the site 1 and 20% in the site 2 (Table 1).

Regarding to the occupation of the straws of different dimensions, the total occupation rate (the two sites together) was 36% to the large straws (122 nests established), and 11% to the small straws (39 nests). There was a statistically significant difference in the occupation rates of these straws of different dimensions ( $\chi^2 = 19.951$ ;  $df = 1$ ;  $p < 0.001$ ). *Centris analis* Fabricius, *C. tarsata*, and *T. diversipes* used both types

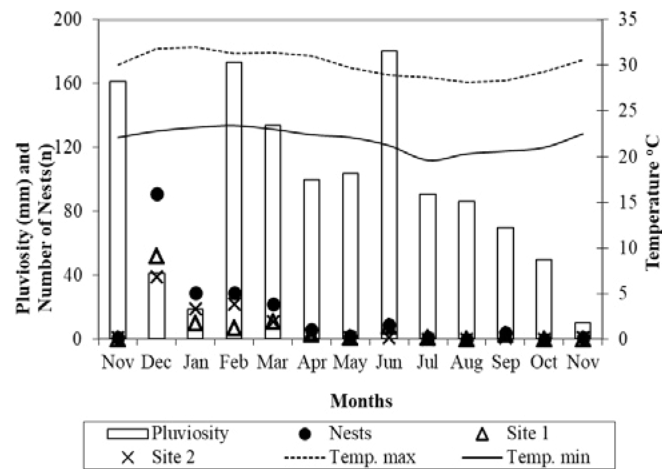
of cardboard straws, while *Centris trigonoides* Lepeletier used only the large straws. *C. tarsata* established significantly more nests in large straws than in small ones ( $\chi^2 = 82.6$ ;  $df = 1$ ;  $p < 0.001$ ), while *C. analis* established a higher number of nests in small straws ( $\chi^2 = 12.772$ ;  $df = 1$ ;  $p < 0.001$ ). There was no significant difference in the occupation of large and small straws by *T. diversipes* ( $\chi^2 = 3.469$ ;  $df = 1$ ;  $p > 0.05$ ) (Table 1). Regarding to the occupation of bamboo canes, 89% of those that were made available to the bees were occupied. *C. tarsata* used mainly those from 10 to 13 mm diameter, while *Euglossa cordata* Linnaeus, occupied only those with 18 mm in diameter.

**Table 1.** Number of nests provisioned (N) and number of imagos emerged (I) in restinga vegetation, Bahia, Brazil. Site 1: herbaceous-shrub vegetation, site 2: arboreal vegetation. LS = large cardboard straws, SS = small cardboard straws, BC = bamboo canes.

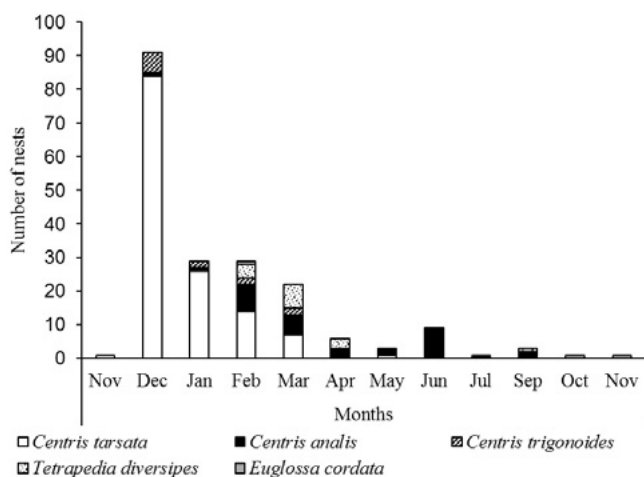
Bee species	Site 1								Site 2							
	Total		LS		SS		BC		Total		LS		SS		BC	
	N	I	N	I	N	I	N	I	N	I	N	I	N	I	N	I
<i>Centris analis</i>	15	26	5	5	10	21	-	-	15	15	-	-	15	15	-	-
<i>Centris tarsata</i>	64	178	51	106	-	-	13	72	73	113	53	60	4	-	16	53
<i>Centris trigonoides</i>	10	23	10	23	-	-	-	-	1	2	1	2	-	-	-	-
<i>Tetrapedia diversipes</i>	-	-	-	-	-	-	-	-	12	19	2	4	10	15	-	-
<i>Euglossa cordata</i>	2	1	-	-	-	-	2	1	1	9	-	-	-	-	1	9
Total	91	228	66	134	10	21	15	73	102	158	56	66	29	30	17	62

*Temporal distribution of nesting activity*

Nesting activity in this restinga occurred mainly from December to June, with an abundance peak of nests in December (Fig 1). The total number of nests established per month, was influenced by the nesting activity of *C. tarsata*, which established the highest number of nests. The temporal distribution of its nesting activity was more restricted



**Fig 1.** Number of nests established by bees in trap-nests, maximum and minimum temperature, and monthly pluviosity, in Mata de S o Jo o, state of Bahia, Brazil, from November 2006 to November 2007.



**Fig 2.** Number of nests established by bee species in restinga vegetation.

(six months) than that of other species, such as *C. analis*, which had the longest nesting period (nine months) (Fig 2).

There was a significant positive correlation between minimum monthly temperature and the total number of nests built ( $r_s = 0.71$ ;  $p < 0.05$ ), for the nests built by *C. tarsata* ( $r_s = 0.62$ ;  $p < 0.05$ ) and *C. trigonoides* ( $r_s = 0.64$ ;  $p < 0.05$ ), but not for the other species. There was no correlation between monthly rainfall and the total number of nests ( $r_s = 0.03$ ;  $p < 0.05$ ).



**Table 4.** Mortality percentage in bee brood cells due to unknown causes or parasitism. Site 1: herbaceous-shrub vegetation, site 2: arboreal vegetation. NBC: total number of brood cells attacked by natural enemies (parasites).

Bee species	Unknown causes (%)			Parasitism (%)			Natural enemies	NBC (N)
	site 1	site 2	Total	site 1	site 2	Total		
<i>C. tarsata</i> (bamboo canes)	1.4	7.0	3.9	2.7	12.3	6.9	<i>Anthrax</i> sp.	1
							<i>Mesocheira bicolor</i>	8
							<i>Anthrax</i> sp.	7
<i>C. tarsata</i> (cardboard straws)	10.1	29.6	19.4	4.3	8.8	6.5	<i>Mesocheira bicolor</i>	7
							<i>Leucospis</i> sp.	3
							<i>Leucospis</i> sp.	7
<i>C. analis</i>	20.5	37.8	28.4	6.8	10.8	8.6	<i>Leucospis</i> sp.	7
<i>C. trigonoides</i>	29.4	-	27.8	5.9	-	5.6	<i>Leucospis</i> sp.	2
<i>T. diversipes</i>	-	18.2	18.2	-	21.2	21.2	<i>Leucospis</i> sp.	6
							<i>Anthrax</i> sp.	1
<i>E. cordata</i>	50.0	-	9.1	-	33.3	33.3	<i>Anthrax</i> sp.	3

## Discussion

The cavity-nest bee guild in the RPPN-DSA had few species, as well as in other coastal habitats in the Northeast of Brazil, as sand dunes (Viana et al., 2001), coastal savanna (coastal “*tabuleiros*”), and coastal rainforest (Aguiar & Martins, 2002). Differences in species richness in cavity-nesting guilds may be related to differences in the species composition of each bee assemblage, as they can also be influenced by sampling, since there are different probabilities of nesting females finding the trap-nests and accepting them as substrates of nesting. Flower-visiting data from RPPN-DSA have indicated that there are at least ten other cavity-nesting bee species in this area. The abundance of several of these cavity-nesting bees in flowers was low, suggesting that their populations are small (P. Oliveira-Rebouças, unpublished data). The absence of *Xylocopa* species using the trap-nests was surprising, since they are usual components of the cavity-nesting bees guild in the Brazilian coastal plain (Viana et al., 2001; Aguiar & Martins, 2002; Viana & Alves-dos-Santos, 2002; Silva et al., 2015). Five *Xylocopa* species were collected visiting flowers in the RPPN-DSA, and two of them (*Xylocopa cearensis* Ducke and *Xylocopa subcyanea* Pérez) were very abundant in this bee assemblage (P. Oliveira-Rebouças, unpublished data). However, they did not use the trap-nests available, although there were adequate for their nesting (bamboo canes up to 220 mm in diameter).

The percentage of trap-nests occupied was moderate, both in site 1 (25%) and site 2 (28%). These trap-nests occupation rates were higher than those recorded in other coastal habitats in Northeast Brazil. Viana et al. (2001) reported that solitary bees used only 14% of the trap-nests available in a fragment of tropical sand dunes, and Aguiar and Martins (2002) recorded that bees occupied 7% of the trap-nests in a coastal savanna (*tabuleiros* vegetation). On the other hand, these authors recorded occupancy of 25% of trap-nests in a coastal rainforest. The trap-nests occupation rates by nesting bees can be influenced by several factors, such as the local availability of natural substrates for nesting (Frankie et al., 1988; Viana et al., 2001; Silva & Viana, 2002),

the diversity of the trap-nests available (Vandenberg, 1995), the degree of exposure to the sun (Frankie et al., 1988), and the local availability of trophic resources (Gathmann et al., 1994). These factors appear to have not interfered in the trap-nest occupation rates between sites in the RPPN-DSA, since there was no difference in the occupation of the trap-nests in herbaceous-shrub and arboreal phytophysiognomies.

The abundance of bee nests established in each site (91 and 102 nests) in the restinga RPPN-DSA was higher than those found in sand dunes (n = 62) (Viana et al., 2001), in coastal rainforest (n = 19), in coastal savanna vegetation (n = 47), and in a mosaic of these two latter habitats (n = 69) (Aguiar & Martins, 2002). Differences in the number of bee nests may be related to the number of sampled sites in each area, to the sampling effort, as well as to the local dominance of some species that can establish many nests. In the RPPN-DSA, the high number of nests in both phytophysiognomies was due to the high nesting success of *C. tarsata* in these habitats. This high dominance of *C. tarsata* on artificial nesting substrates has been recorded in other habitats in Northeast Brazil, as coastal sand dunes (Viana et al., 2001), coastal savanna (Aguiar & Martins, 2002), dry forest (“*Caatinga*”) and semideciduous forest (Aguiar & Garófalo, 2004). These findings support the hypothesis raised by Aguiar and Garófalo (2004) about the high ability of *C. tarsata* populations to occupy open environments, with high temperatures and high insolation. Probably, this factor contributed to the large number of nests founded by *C. tarsata* females in RPPN-DSA, because much of the this area is covered by an herbaceous-shrub vegetation, which allows high incidence of sunlight.

These solitary bees used different types and sizes of trap-nests in unequal proportions. In large straws there were higher number of nests, and they attracted more bee species than small straws, as was also observed in other habitats (Aguiar & Garófalo, 2004; Pina & Aguiar, 2011). *C. tarsata* showed more affinity for large cardboard straws, while *C. analis* used mainly small cardboard straws, similar to that observed in coastal savanna, where 54% of the *C. tarsata* nests were built in large straws and 88% of *C. analis* nests were in small straws (Aguiar & Martins, 2002).

We registered high amount of trap-nests occupied by solitary bees (especially *C. tarsata*) in the hottest months (summer) in the RPPN-DSA, with a peak of abundance in December, when rainfall was low. In other studies, variations were observed between seasons and years. In coastal savanna, the highest number of nests was registered in summer (November), a rainy and hot season (Aguiar & Martins 2002). According to Viana et al. (2001), in coastal sand dunes of Northeastern Brazil, the dominant species, *C. tarsata*, had abundance peak of nests in December (in summer, the dry season) in the first year, while a higher number of nests was found in the rainy months (autumn) in the second year. The fluctuations in the frequency of nesting can be related to intrinsic factors of the cavity-nesting species or to environmental factors, such as weather patterns, or the dynamic of floral resources availability (Frankie et al., 1988).

The number of brood cells built per nest was variable in all species, as reported in other habitats, for *C. tarsata* (Aguiar & Garófalo, 2004; Buschini & Wolff, 2006) and *C. analis* (Jesus & Garófalo, 2000). Large variations in the number of brood cells were also found in different types of trap-nests (cardboard straws and bamboo canes) in the studied area. The number of brood cells built per nest, as well as the brood cells arrangement in nests, depend upon the size of the cavity. On the other hand, behavioral decisions of the female bees regarding time spent in the same cavity to produce offspring affect the number of brood cells produced by nest, since the more time invested in the same nest the more brood cells are expected to be produced. Alternatively, distributing the reproductive effort in several nests should also have some effect on variability in the number of cells per nest (Jesus & Garófalo, 2000).

The total sex ratio of *C. tarsata* offspring was biased to males, mainly in cardboard straws (4M:1F), but not in bamboo canes (1M:1F). Bias to males have been recorded in some populations of *C. tarsata* (Aguiar & Martins 2002; Aguiar & Garófalo, 2004; Buschini & Wolff, 2006). However, in other populations, the sex ratio of the offspring was 1:1 (Silva et al., 2001; Aguiar & Garófalo, 2004). Several factors can affect the offspring sex ratio of cavity-nesting bees, as the length (Alonso et al., 2012; Gruber et al., 2011; Stephen & Osgood, 1965) and diameter of the cavities (Rust 1998), the abundance of food resources in the environment, the foraging efficiency of nesting females (Torchio & Tepedino, 1980), and conditions of the mother (Seidelmann et al., 2010).

The mortality rate by unknown reasons varied among the species. *C. tarsata* offspring had low mortality in bamboo canes (< 4%), and higher in cardboard straws (19%). These values were lower than those in other habitats, as reported by Aguiar and Garófalo (2004), who recorded 41% in dry forest and 42% in a semideciduous forest in Brazil. Buschini and Wolff (2006) found high rates of offspring mortality (58% to 70%) by unknown reasons in swamp habitat and grasslands in southern Brazil. The mortality rate in *C. analis* offspring (35%) was moderate similar to that observed in agricultural

areas in Brazil (24-25%) (Aguiar & Pina, 2012). Higher mortality rates were found in an urban area in southeastern Brazil, 63% (Jesus & Garófalo, 2000) and 42% (Couto & Camillo, 2007). Offspring mortality may be related to failures in development, environmental factors, such as temperature (Jesus & Garófalo, 2000; Gazola & Garófalo, 2009), changes in air humidity conditions (Buschini & Wolff, 2006), or even to the handling of the nests (Aguiar & Pina, 2012).

Although the phytophysiognomies sampled in the RPPN-DSA were different, the cavity-nesting bee guild had a similar composition in the studied sites, there was a similar rate of occupation of trap-nests in both sites, as well as dominance of *C. tarsata* nests. Mortality rates due to unknown causes and by parasite attack on the offspring of some cavity-nesting bee species seem to be the most distinctive aspect between herbaceous-shrub restinga and arboreal restinga phytophysiognomies sampled. Another relevant aspect was the lack of records on both sites of some cavity-nesting species, collected in flowers, but did not use the available artificial nesting substrates, indicating that the trap-nest sampling method alone is not enough to adequately sample the species richness of the cavity-nesting bee guild. Finally, our study indicated that remnant fragments of coastal native habitats may be refuges for the maintenance of bee populations, some of which, such as *C. tarsata* and *C. analis*, have been indicated as candidates for management as pollinators of cultivated plants in Brazil.

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