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Trap-nesting bees and wasps (Hymenoptera, Aculeata) in a Semidecidual Seasonal Forest fragment, southern Brazil

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ABSTRACT

Trap-nesting bee and wasp inventories are common in Brazil but many phytophysiognomies are still poorly studied. The main objective of this study is to survey trap-nesting bees and wasps in a Semidecidual Seasonal Forest fragment. Also, we test the differences on nesting between interior and edge transects. A sum of 1,500 trap nests was made with bamboo cane internodes and two consecutive years were monitored. In the first year 46 nests were occupied by Pachodynerus grandis *(19 nests),* Pachodynerus guadulpensis *(19),* Centris analis *(two), and* Centris tarsata, Megachile fiebrigi, Megachile guaranitica, Megachile susurrans, Trypoxylon *sp and* Zethus smithii *with one nest each. No statistical differences were found between interior and edge transects for richness and occupation rate, but the species composition was different. In the second year 39 nests were occupied by four species, three previously recorded,* C. analis *(seven nests),* P. guadulpensis *and* P. grandis *(six nests each), plus* Monobia angulosa *with 15 nests. Parasitoids from four families and one cleptoparasite were recorded and the mortality rate was higher in bees than in wasps. These findings reinforce the notion that trap nests assemblages from different studies are not directly comparable for richness and composition.*

Key-Words: Apidae; Crabronidae; Diversity; Eumeninae; Nesting.

INTRODUCTION

Hymenoptera (Aculeata) includes key organisms, as parasitoids, pollinators and predators, which can respond to habitat fragmentation acting as ecological indicators (Calvillo *et al.,* 2010) since its local diversity are frequently correlated to variables of forest fragments as size, connectivity and edge effect (Gonçalves *et al.,* 2014). Bees and wasps have distinct needs of food sources and some groups of species are specialized in certain nesting substrates. Although most nests are excavated in the soil, some females excavate their nests in natural or even human made cavities (Camillo *et al.,* 1995). Bees and wasps that nest in wood cavities usually are solitary and make one to several brood cells divided by transverse partitions, and, in addition to the place, the females also require other resources to nest construction such as mud, wax, oil, plant leaves and petals (Camillo, 2000).

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Artificial tubes and bamboo internodes as nesting substrate have been used as sampling method in inventories (Krug & Alves-dos-Santos, 2008) or as ecological indicators of habitat disturbances, such as habitat loss or edge effect (Stangler *et al.,* 2015). A recent study shows that trap nests have higher occupancy compared to natural potential nesting cavities, possibly because they offer an appropriate shelter for offspring and food storage (Westerfelt *et al.,* 2015). An important advantage about trap-nesting methodology is the possibility of replication along the study site, allowing a sufficient sampling effort for statistical analyses and avoiding the sampling of transitory species (Camillo *et al.,* 1995; Tscharntke *et al.,* 1998). Still, trap nests studies can focus on aspects of natural history like nest and cell architecture, materials and resources, sex ratio, mortality, and association with parasites (Camillo, 2000). There are three types of trap nests commonly used in literature: (1) black cardboard paper; (2) drilled wooden blocks; and (3) plant internodes, like common reed and bamboo cane. The trap nests are grouped in larger blocks or tubes, the sampling stations, and settled in the field (Garófalo *et al.,* 1993; Camillo *et al.,* 1995).

In Brazil there is a vast scientific literature on trap nest based inventories, but published studies in the state of Paraná are scarce and cover the Araucaria Forest (Buschini, 2006; Buschini & Woiski, 2008; Woiski, 2009) and Dense Ombrophylous Forest (Marchi, 2005), lacking initiatives under Semidecidual Seasonal Forest in the State. This study aimed to provide survey data on bees and wasps nesting in bamboo trap nests in a Semidecidual Seasonal Forest fragment. Specific goals are: to compare sampling rate on edge and interior transects; and to investigate general aspects of natural history as nests architecture, sex ratio, mortality rates, phenology and associations with parasitoids and cleptoparasites.

MATERIAL AND METHODS

The study was conducted at Parque Estadual São Camilo (PESC), located in the Palotina municipality, western region of Paraná state in Brazil (geographic UTM coordinates -24.312998, -53.917491). PESC is a 385 ha conservation unit, with a humid subtropical climate, with hot summers and is located under a Submontane Semideciduous Seasonal forest, which belongs to Atlantic Forest biome (IAP, 2006). The area is surrounded by alternate soybean and corn crops, being one of the few forest fragments under conservation on western Paraná.

This study was divided in two phases, corresponding to two consecutive and uninterrupted years of sampling. In the first phase of the study (from May 2013 to April 2014) the traps were placed in two habitats, the first transect (T1) was a trail inside the forest and the second transect (T2) was a trail between the border of PESC fragment and the surrounding crops. Each sampling transect was two kilometers long and two meters wide and the trap sets were arbitrarily installed along them. In a second phase (from May 2014 to April 2015) the traps were placed only in T2, also the sets were arbitrarily installed. There was no minimum distance between each set of trap, because their installation sites were randomly selected based on the total trail distance.

The trap nests were handmade with bamboo cane internodes, approximately 20 cm long and with internal diameters varying randomly from 0.5 to 3 cm. We made trap nests sets using 2 L pet bottles as protective cases, and each set had 25 nest traps of different diameters (Fig. 1). These sets were installed on 1.5 m height tree branches. On the first phase, 15 sets were installed on each transect, totaling 375 trap nests by transect, and on the second phase, 30 sets were installed on T2, totaling 750 trap nests. A total of 1,500 trap nests were installed along the two years of sampling.

After the nest trap sets installation on the beginning of each phase, the traps were individually inspected twice a month. The occupied nests, defined here by the closed entrances, were collected and immediately replaced by an empty nest in the field. In the first phase, the nests were isolated with plastic tubes until the emergence of the adults, and posteriorly the nests were dissected for cell examination. In the second phase, the collected nests were opened for visual inspection of the cells and closed and isolated with plastic tubes until the emergence of the adults. Data retrieved from the nests of each species were the mean diameter of the nest (mdn), the mean number

FIGURE 1: Trap nests installed on Parque Estadual São Camilo.

TABLE 1: Number of nests and emerged adults of trap-nesting bees and wasps in Parque Estadual São Camilo (Palotina, Paraná). Phase 1 (from May 2013 to April 2014), Phase 2 (from May 2014 to April 2015).

Species	T1, Phase 1		T2, Phase 1		T2, Phase 2	
	Nests	Emerged adults	Nests	Emerged adults	Nests	Emerged adults
Apidae						
Centris (Hemisiella) tarsata Smith, 1874	1	1	—	—	—	—
Centris (Heterocentris) analis Fabricius, 1804	—	—	2	3	7	14
Megachile (Austromegachile) fiebrigi Schrottky, 1908	—	—	1	11	—	—
Megachile (Austromegachile) susurrans Haliday, 1836	1	11	—	—	—	—
Megachile (Chrysosarus) guaranitica Schrottky, 1908	1	2	—	—	—	—
Cabronidae					—	—
Trypoxylon sp	1	5	—	—	—	—
Vespidae						
Monobia angulosa Saussure, 1852	—	—	—	—	15	66
Pachodynerus guadulpensis Saussure, 1853	6	14	13	73	6	43
Pachodynerus grandis Willink e Roig-Alsina, 1998	17	75	2	6	6	19
Zethus smithii Saussure, 1856	1	3	—	—	—	—
Total	**28**	**111**	**18**	**93**	**34**	**142**

of provisioned cells (mnc), the mean length of the cells (mlc), and the sexual rate (sr) of adults (female/male). The mortality rate is defined as the mean of the ratio of number of provisioned cells by the number of emerged adult. All insects were pinned, databased, identified and were deposited at Universidade Federal do Paraná (DZUP).

We used the Mann-Whitney test, with bilateral p, to compare transects, using as ecological descriptors the number of occupied nests and the mortality rate. The choice of parametric statistics was made after considering the normality, using the Shapiro-Wilk test. All variables were log transformed and $\alpha = 0.05$ for all tests. All analyses were performed using package Stats in the plataform R 3.1.3 (R Core Team, 2015).

RESULTS

Occupied nests and emerged adults

In the first phase of the study 46 nests were occupied (Table 1), corresponding to 0.06% of the installed trap nests. From these nests, 204 individuals emerged, belonging to nine bees and wasps species. The nest builders were distributed in three families (Apidae, Crabronidae and Vespidae). Apidae was the richer family with five species: *Centris (Heterocentris) analis* Fabricius, 1804, *Centris (Hemisiella) tarsata* Smith, 1874, *Megachile (Austromegachile) fiebrigi* Schrottky, 1908, *Megachile (Chrysosarus) guaranitica* Schrottky, 1908, and *Megachile (Austromegachile) susurrans* Haliday, 1836. Crabronidae was represented only by *Trypoxylon* sp. Vespidae was the most abundant group, represented by three species of Eumeninae, *Pachodynerus guadulpensis* Saussure, 1853, *Pachodynerus grandis* Willink & Roig-Alsina, 1998, and *Zethus smithii* Saussure, 1856. The species of *Pachodynerus* were the most abundant, either in number of nests and emerged adults; the other species were only represented by one or two nests. From four nests, different hymenopteran parasitoids emerged, belonging to Chrysididae, Ichneumonidae, and Leucospidae (two species, Table 2).

In the second phase, 34 nests were occupied, corresponding to 0.04% of the installed traps. From these nests 142 individuals emerged, belonging to Apidae (*Centris analis*) and three Vespidae species, the previously recorded *P. guadulpensis* and *P. grandis* and *Monobia angulosa* Saussure, 1852. Differently from the first phase when it was not even sampled, *M. angulosa* was the most abundant species in phase 2. In eleven nests (32% of the total) parasitoids of Chrysididae, Leucospidae, Tachinidae (Diptera) and one Apidae cleptoparasite (*Coelioxys*) were found (Table 2).

The mean mortality rate (mmr) varied among the species from 0 to 0.8, higher values were found in bees, *C. analis, C. tarsata* and *M. guaranitica.* The wasps showed lower mortality rates, including the abundant species of *Monobia* and *Pachodynerus.* Still, *Trypoxylon* sp and *Zethus smithii* did not presented mortality rates, both species were not parasitized.

Transect comparison

Mann Whitney test did not detect statistical differences between occupied nests ($p = 0.6781$) and mortality rates ($p>0.05$) with sampled transects. In spite of the similar number of nests between the two

TABLE 2: Natural history data samplesd on trap-nesting bees and wasps in Parque Estadual São Camilo (Palotina, Paraná). Columns indicate (cm): mean diameter of the nest (mdn), mean number of provisioned cells (mnc), mean length of the cells (mlc), sexual rate (sr) of adults (male/female) and mean mortality rate (mmr).

Specie	mdn	mnc	mlc	sr	mmr	parasite
Apidae						
C. (Hemisiella) tarsata	0.7	8	1.65	1 male	0.8	*Leucospis cayannensis* (Leucospidae)
C. (Heterocentris) analis	0.9	4.5	1.17	1.17	0.6	*Chrysis* (Chrysididae), *Coelioxys* (Apidae), *Leucospis cayannensis*
M. (Austromegachile) fiebrigi	1	13	1.47	0.5	0.8	*Neotheronia* sp. (Ichneumonidae)
M. (Austromegachile) susurrans	0.8	8	1.29	0.6	0.2	*Chrysis*
M. (Chrysosarus) guaranitica	0.7	5	0.88	1	0.6	—
Crabronidae						
Trypoxylon sp	0.6	5	1.78	4	0	—
Vespidae						
Monobia angulosa	1.31	7	1.32	1.53	0.3	Tachinidae (Exoneurinae), *Chrysis*
Pachodynerus guadulpensis	0.7	8.5	1.3	0.85	0.4	*Chrysis, Leucospis propingua*
Pachodynerus grandis	0.9	12	1,1	0.67	0.3	*Chrysis*
Zethus smithii	0.6	10	1.16	0.5	0	—

transects, a different species composition (Table 1) was evidenced, from the nine sampled species, five were exclusive to T1 and two were exclusive of T2. Most species nested in transect in the forest (T1). Also, in the first phase, *Pachodynerus* species showed different preferences on transects. In the forest transect (T1) *P. grandis* occupied 17 nests with 75 emerged wasps while *P. guadulpensis* occupied six nests with 14 wasps; in border transect (T2) *P. grandis* occupied two nests with six individuals and *P. guadulpensis* occupied 13 nests with 73 individuals.

Nests architecture and phenology

The description of the nests, mean diameter of the nest entrance, mean number of provisioned cells, mean length of the cells and the sexual rate can be seen in Table 2 and Fig. 1. The mean diameters range from 0.6 to 1.3 among the species corresponding to the lower diameters provided here (0.5 to 3 cm). *Megachile* species showed a great variation in cell number, with *M. guaranitica* with five cells and *M. fiebrigi* with 13 cells, the lowest and the highest values obtained here. For the sexual ratio, five species presented more females than males, *M. guaranitica* showed the same ratio and *C. analis, C. tarsata, M. angulosa* and *Trypoxylon* sp had more males than females.

The nests of all species were already known and are described in the literature (Fig. 2A-F), except for *Z. smithii,* which was not previously recorded in trap nests. *Zethus smithii* built the nest uniquely with mud, forming a thick layer covering the walls among the cells, the last one being thicker. The closure plug was also made of a thick layer of mud. The diameter of the entrance was 0.6 cm and the mean length of the cells was 1.16 cm. The nest presented ten cells, and only two females and one male emerged from it.

Centris analis nested from January to February 2014 and *Centris tarsata* nested in March 2014, both during the summer season. The *Megachile* species nested in winter season, *M. susurrans* and *M. guaranitica* nested in July 2013 and *M. fiebrigi* in August 2013.The crabronid *Trypoxylon* sp. nested in December 2013 and February 2014, this later being a shared nest with *P. grandis. Zethus smithii* nested in November 2013. On the first phase *Pachodynerus guadulpensis* occurred from September 2013 to March 2014 (Fig. 3A), except for October 2013, while *Pachodynerus grandis* nested from October 2013 to March 2014.

Centris analis nested on two seasons, spring (November) and summer (February and March 2015). The *Pachodynerus* species showed a different pattern (Fig. 3B), ranging from the spring to early summer, with *P. guadulpensis* nesting in November 2014 and January 2015 and *P. grandis* nesting in October, November 2014, and January 2015. *Monobia angulosa,* absent in the first sampling phase, nested in November 2014 and January 2015, when showed the highest nesting rate in this study.

DISCUSSION

Trap nesting studies have provided important information about species occurrence and nesting biology in Brazil (Camillo *et al.,* 1995; Assis & Camilo, 1997; Morato & Campos, 2000; Gazola, 2003; Zanette *et al.,* 2004; Buschini *et al.,* 2006; Buschini & Woiski, 2008). However, the diversity estimation in trap nests assemblages varies with sampling design, collecting period, trap nesting type, biomes and habitats (Aguiar *et al.,* 1995). Thus, authors emphasize the difficulty in comparing data from different localities in spite of the observed assemblage's general patterns (Buschini & Woiski, 2008; Pires *et al.,* 2011; Nascimento & Garófalo, 2014). Here, ten species colonized the trap nests and except for the abundant *Monobia* and *Pachodynerus,* the remaining species occupied only one or two nests. The assemblage composed by few abundant species should reflect a characteristic of most biological communities (Krebs, 1994; Magurran, 2004) being similar to previous trap-nesting studies (Camillo *et al.,* 1995; Assis & Camillo, 1997; Buschini & Woiski, 2008). Another general pattern evidenced here is the highest number of nest foundation in the warm and rainy season (summer), already evidenced by different authors (Loyola & Martins 2006; Pires *et al.,* 2011; Nascimento & Garófalo, 2014).

The aculeate nest occupation rate obtained in the present study, less than 1%, is considered very low when compared with other studies. The occupation rate is variable, a 7% was found by Buschini &

FIGURE 2: Trap nests in Parque Estadual São Camilo (Palotina, Paraná), **(A)** *Centris analis,* **(B)** *Megachile susurrans,* **(C)** *Monobia angulosa,* **(D)** *Pachodynerus grandis,* **(E)** *Pachodynerus guadulpensis,* **(F)** *Zethus smithii.* Scale bars: 1 cm.

Buss (2010) for *Pachodynerus,* a number considered low by the author, but higher than ours. As examples of considered low values we cite 8.5% of Krug & Alves-dos-Santos (2008), who used bamboo nests and also wooden tubes and 10% of Loyola & Martins (2006), which uses wooden tubes. It is possibly that the additional use of wooden tubes could raise the number of species and nests (Teixeira, 2011). Our results indicate a great similarity in nesting abundance in forest border and inside forest, but a difference in species composition being the transect more diverse. The same transects were previously compared using bowl traps and differences were also restricted to species composition, and T2 showed greater abundance and richness (Gonçalves & Oliveira, 2013). Studies comparing transects in the same physiognomy are scarce and due to their methodology not comparable to our data, but Woiski (2009) also showed that the variation of nesting in relation to the edge could be an individual response of the species.Eumeninae was the most abundant group in trap nest assemblage and both species of *Pachodynerus* are common in trap-nesting inventories. *Pachodynerus guadulpensis* was the most common species in grassland and Araucaria forest as found by Buschini & Buss (2010) and *P. grandis* was the most abundant in Atlantic forest fragments in the inventories of Carvalho (2011) and Teixeira (2011). These species showed different preferences for transects in the first phase and could be investigated as habitat quality indicators, Fye (1972) indicates that these species are more frequently found

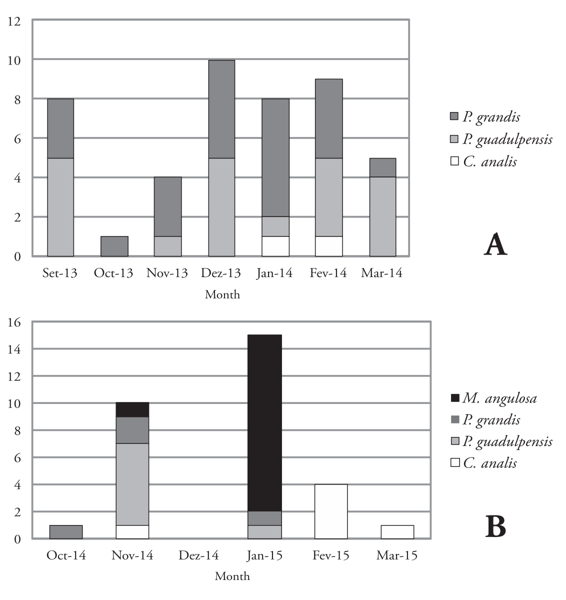

FIGURE 3: Phenology of most common trap-nesting Aculeata in Parque Estadual São Camilo (Palotina, Paraná), **(A)** from September 2014 to March 2014, **(B)** from October 2014 to March 2015.

in more impacted or open areas. *Pachodynerus* species occupied nests from September to March in the first phase, a pattern discussed by Buschini & Buss (2010) for *P. guadulpensis,* but in the second phase the nesting activity was concentrated in October to January.

Monobia angulosa was the most common species in the second phase of the study, being absent in the first phase. This species has a widespread distribution and is commonly sampled in trap nests (Melo & Zanella, 2012), and the absence in the first phase can be considered as a sampling artifact. Silva (2008) observed the highest nesting activity in hot and rainy seasons, similar with our data, and Camillo *et al.* (1997) found the activity peak of this species being on spring.

Zethus was reported in trap nest studies (Buschini & Woiski, 2008; Woiski, 2009; Teixeira, 2011), but in the literature we found no record of *Zethus smithii* in trap nests, so we considered this as the first published record of this species nesting in cavities. *Zethus smithii* nest agree with the pattern described for *Zethus* by Buschini & Woiski (2008), but differs from the notion that *Zethus* does not use clay in nest building (Wenzel, 1998; Carpenter & Marques, 2001). However, it should be noted that Eumeninae nests are highly variable, even within genera (Camillo *et al.* 1997).

Crabonidae wasps also are common elements in trap-nesting studies, but only one nest of *Trypoxylon* was recorded in the present study contrary to the high richness and abundance reported previously (Woiski, 2009; Santos, 2011; Melo & Zanella 2012). A possible explanation for this low incidence is that the genus prefers open areas, with high insolation rate (Morato & Campos, 2000)

According to Gonçalves *et al.* (2014) there are at least 18 bee species in the study region that use cavities for nesting, but only five species were sampled here, two *Centris* and three species of *Megachile.* Nine *Centris* species were recorded in trap nests (Garófalo *et al.,* 2004). *Centris* species are probably the most abundant species sampled in trap nest, for example *Centris tarsata* (Buschini, 2006). However *Centris tarsata* occupied only one nest in this study, and *Centris analis* occupied nine nests, seven on the second phase. *Megachile* is a large and diverse group of bees with about 200 species in Brazil (Moure *et al.,* 2012). The three species of *Megachile* sampled here present only one occupied nest and were already recorded in previous studies. Buschini (2006) recorded two nests of *M. fiebrigi* in wood blocks in two habitats of Araucaria forest and Woiski (2009) recorded one nest of *M.* aff. *susurrans* also in Araucaria forest. *Megachile* *guaranitica* was previously recorded for seasonal semideciduous forest by Rocha-Filho & Garófalo (2015), with a large number of occupied nests (26). The use of mud together with leaves was previously recorded for some *Megachile* (Rocha-Filho & Garófalo, 2015), and it was also recorded here for *M. guaranitica.* Differently from the other bees and wasps, *Megachile* nesting activity was in the winter, but *M. guaranitica* activity was in the end of summer in the study of Rocha-Filho & Garófalo (2015), unfortunately there is no trap nesting information about the other two *Megachile* species.

The recorded natural enemies were distributed in six species and five different families. The parasitoids and cleptoparasites were found in the nests of most species, except for *M. guaranitica, Trypoxylon* and *Z. smithii.* There was no clear preference of natural enemies of bees or wasps and this could not explain the higher mortality rates in bees (60%), higher than the mortality rate reported by most authors (Nascimento & Garófalo, 2014). Buschini & Buss (2010) found variable mortality rates for *Pachodynerus* species and it was low for *P. guadulpensis,* which is similar to our data (20%).

CONCLUSION

We found ten species in trap nests in a seasonal Semidecidual Forest Fragment in southern Brazil, with Eumeninae wasps as the most abundant group and bees and crabronids with few occupied nests. The assemblage diversity is similar with previous studies but the species composition can be considered different, our findings reinforce the notion that trap nests assemblages are not directly comparable (Aguiar *et al.,* 1995) because the highly variable sampling design and frequent small richness to be representative of the Aculeate community.

RESUMO

Inventários com ninho armadilha são comuns no Brasil, mas muitas fitofisionomias ainda são pouco estudadas. O principal objetivo deste trabalho foi realizar um levantamento de abelhas e vespas que nidificam em armadilhas em um fragmento de Floresta Estacional Semidecidual. Além disso, testamos as diferenças de nidificação entre transectos, interior e borda. Um total de 1.500 ninhos-armadilha feitos de bambu foi monitorado em dois anos consecutivos. No primeiro ano 46 ninhos foram ocupados por Pachodynerus grandis *(19 ninhos),* Pachodynerus guadulpensis *(19),* Centris analis *(dois), e* Centris tarsata, Megachile fiebrigi, Megachile guaranitica, Megachile susurrans, Trypoxylon *sp e* Zethus smithii *com um ninho cada. Este é o primeiro registro de Z. smithii em ninhos-armadilha, as demais espécies são comumente amostradas em ninhos-armadilha. Não foram encontradas diferenças estatísticas entre os transectos de interior e de borda para riqueza e para taxa de ocupação, mas para composição de espécies houve diferença. No segundo ano 39 ninhos foram ocupados por quatro espécies, três registradas anteriormente,* C. analis *(sete ninhos),* P. guadulpensis *e* P. grandis *(seis ninhos cada), além de* Monobia angulosa *com 15 ninhos. Parasitóides de quatro famílias e um cleptoparasita foram registrados e a taxa de mortalidade foi maior nas abelhas do que em vespas. Estes registros reforçam a noção de que assembleias de ninhos-armadilha de diferentes estudos não são diretamente comparáveis quanto à riqueza e composição.*

Palavras-Chave: Apidae; Crabronidae; Diversidade; Eumeninae; Nidificação.

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