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Nests of *Eufriesea* aff. *auriceps* (Hymenoptera, Apidae, Euglossini) in remnants of Atlantic Forest and reforested areas

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Abstract

In the last decades, the use of the trap-nest technique has helped to increase knowledge on the nest architecture of many orchid bee species. This study describes the nest architecture of *Eufriesea* aff. *auriceps* constructed in trap-nests made of dried bamboo internodes (canes). The nests were placed in remnants of Atlantic Forest and in reforested areas next to forest remnants and monitored monthly from August 2015 to August 2016 and from August 2018 to August 2019 in southern Brazil. The bamboo internodes occupied by bees varied in internal diameter from 1.0 cm to 2.0 cm ($\overline{X}$ = 1.7; SD = 0.3; N = 12) and in length from 11.0 cm to 28.0 cm ($\overline{X}$ = 19.5; SD = 4.8; N = 12). The total size of the nests inside the bamboo internodes ranged from 9.0 cm to 19.9 cm ($\overline{X}$ = 14.3; SD = 3.9; N = 12). The number of brood cells constructed per nest varied from 1 to 10 ($\overline{X}$ = 4.0; SD = 2.3; N = 15). The cells were built with small pieces of bark cemented with resin, linearly arranged along the bamboo tube. Internally, the cell wall was lined with resin. The cells measured 1.5-3.0 cm ($\overline{X}$ = 2.3 ± 0.5; N = 48) in length and 1.4-1.7 cm ($\overline{X}$ = 1.5 ± 0.1; N = 17 cm) in width. The internal contour of the cells was elliptical. Females of *Eufriesea* aff. *auriceps* occupied trap-nests in forest remnants and in areas undergoing restoration.

Introduction

Euglossine bees or orchid bees are a diverse group of neotropical pollinators, assembling approximately 250 species, distributed in five genera (*Euglossa, Eufriesea, Eulaema, Exaerete* and *Aglae*), belonging to the corbiculate bee clade (Moure et al., 2012; Saleh & Ramírez, 2019; Ascher & Pickering, 2020). The group is best known for their metallic and shiny integument and the very long tongues of its representants, as well as by the males' behavior of collecting fragrances from many orchid species to use during courtship (Dressler, 1982; Roubik & Hanson, 2004; Eltz et al., 2015).

The group has become better known over the last five decades after the use of scent baits to attract males in several surveys carried out in different regions and ecosystems in Central and South America (Dressler, 1982; Ramírez et al., 2002; Roubik & Hanson, 2004; Nemésio, 2009). The scent baits are very efficient to attract males, who visit them searching for the synthetic chemicals which mimic the aromatic compounds produced by orchids (Dodson et al., 1969; Dressler, 1982). However, the use of this method in orchid bee surveys has some limitations, since only males visit the baits and males of some species are not or are only rarely attracted to baits (Nemésio, 2012). As a consequence, as almost the totality of the ecological studies involving orchid bees are based on sampling male specimens attracted to scent baits (Nemésio, 2012), the literature still needs more surveys and information concerning euglossine females.

In the last three decades, information on euglossine females has been obtained mostly though the trap-nest technique (Garófalo et al., 1993, 1998; Augusto & Garófalo, 2004), which is useful for collecting biological data on solitary bees nesting in preexisting cavities (Garófalo et al., 2004). Since female orchid bees rarely excavate their nests (Solano-Brenes et al., 2018) and several species build their nests in preexisting cavities in nature or even in human buildings (Ramírez et al., 2002), the use of trap-nests for euglossine studies has provided, for instance, information on nesting biology of different species (Garófalo et al., 1993, 1998; Viana et al., 2001; Augusto & Garófalo, 2004). In fact, the use of trap-nests can be more effective than searching for euglossine nests in the field (Garófalo et al., 1993). Moreover, surveys with trap-nests have contributed by providing geographical records of those species for which males are only rarely or are not attracted to scent baits during inventories on orchid bees (see Nemésio, 2012). Despite this, the use of the trap-nest method in surveys involving orchid bees is still scarce in the literature.

Recently, a study using chemical bait to attract males compared the euglossine assemblages from Atlantic Forest remnants and from reforested areas (Ferronato et al., 2018). Even though the occurrence of this species has been reported in the studied region (Sofia et al., 2004), males of *Eufriesea auriceps* (Friese, 1889) were not attracted to baits in any of the studied areas surveyed by Ferronato et al. (2018). The absence of males of this species is not surprising, since, with rare exceptions (e.g. Andrade et al., 2012), only a few males have been sampled at scent baits in different surveys conducted in areas for which the occurrence of this species is known (e.g. Sofia et al., 2004; Rocha-Filho & Garófalo, 2013; Viotti et al., 2013; Martins et al., 2018; Dec & Alves-dos-Santos, 2019).

Eufriesea auriceps (Friese, 1899) is a solitary species of euglossine bees, widely distributed in Brazilian territory (Moure et al., 2012), with occurrences recorded for different ecosystems, including the *Cerrado* biome (Martins et al., 2018), *Campos Rupestres* (high-altitude rocky fields; Viotti et al., 2013), and Atlantic Forest (Sofia et al., 2004; Nemésio, 2009; Rocha-Filho & Garófalo, 2013; Dec & Alves-dos-Santos, 2019), from the northeastern to southern regions (Moure et al., 2012; Martins et al., 2018; Dec & Alves-dos-Santos, 2019). Occurrences of *E. auriceps* have also been reported in Paraguay and Argentina.

In this study, we describe the nest architecture of nests of *Eufriesea* aff. *auriceps* sampled in trap-nests placed in forest remnants of the Atlantic Forest (AF) and in reforested areas of this biome. Although the structure of nests of this species has already been described elsewhere (Garófalo et al., 1993), the current study aims both to add more information on the theme and to compare our findings concerning the preferences of *E. auriceps* in the use of trap-nests, with those reported by Garófalo et al. (1993). Moreover, we discuss our findings on the perspective of the reestablishment of orchid bee fauna in forest habitats under restoration processes, since studies on this subject are still very scarce in the literature (Rasmussen, 2009; Ferronato et al., 2018).

Material and Methods

Study species

Considering that many species of the 'auriceps' group, to which *E. auriceps* belongs, are currently the focus of taxonomic discussion (Nemésio 2009; Moure et al., 2012), as well as which, the entire group needs a thorough revision (Moure et al., 2012), the specimens of our study were identified as *Eufriesea* aff. *auriceps*, by Dr. Gabriel A. R. Melo (Universidade Federal do Paraná, Brazil).

Study areas and samplings

The study was conducted in the north of the state of Paraná, in southern Brazil, in an area covered by seasonal semi-deciduous forest (SSF), a type of vegetation formation of AF, often called matas de planalto (plateau forests), due to their distribution on the hinterland highlands (Oliveira & Fontes, 2000). The deforestation in this region occurred over a period of approximately a hundred years, with only 2-4% of native forests remaining, distributed in a fragmented landscape and embedded in agricultural areas (Torezan, 2002).

We conducted our surveys in three remnants of SSF and nine reforested areas, in both cases with variations in sizes (Table 1). Other relevant information regarding the study sites, such as the age of reforestation of each area, are also given in Table 1.

The samplings were conducted from August 2015 to Augusto 2016 and from August 2018 to August 2019 (Table 1).

The methodology of the study was based on Garófalo et al. (1993), with some modifications. In our study, the trap-nests used to attract euglossine females, consisted only of bamboo internodes (canes), with diameters from 0.5 to 3.0 cm and variable lengths (mostly between 14 and 30 cm), which are cut so that the nodal septum closes one end of the internode (Garófalo et al., 2004). Twenty-five units of the bamboo canes were arranged in bundles inside a PET bottle cut open at one end. The bamboo bundles were placed horizontally on wooden platforms. Each platform contained four bamboo bundles, totaling a hundred canes per platform. The platforms were fixed 1.5 m above the ground, attached to a wooden rod, sunk 30 cm deep into the soil. In the first year, the number of platforms per study area was two (1st year of study) and five (2nd year). A higher number of platforms was implemented in the second year of the study (August 2018 to August 2019) in an attempt to improve the efficiency of the surveys. In the first year of sampling, in each forest remnant and in restored areas, two platforms were placed, one 20 m from the edge and one 500 m from the edge, totaling 200 nests per study area. In the second year, in each forest remnant (A, B, and C) and

in nine restored areas, five platforms were placed 20 m from the edge and 100 m apart from each other. In this latter case, distributed across a transect of 500 m towards the center of the fragment, totaling 500 trap-nests in each area.

The inspections of the trap-nests in each study site were conducted once a month, at intervals of approximately 30 days. As in Garófalo et al. (1993), the trap-nests occupied by females were marked and left in the location until the next inspection, when the entrance was closed with a stopper and they were moved to the laboratory. Each inspection was performed with the aid of an otoscope. In the laboratory, nests were maintained in a cabinet and inspected regularly for adult emergences. When emergences did not occur, the trap-nests were opened for final inspections and measurements.

For measurement procedures of brood cells and internal observation of the nests, the bamboo canes were opened along their lengths, resulting in two halves. All measures were taken with a digital caliper.

We compared the similarity of both the diameter and length of traps used by *E. auriceps* between the current study and those obtained by Garófalo et al. (1993, using the Sorensen index). For this purpose, we clustered the different sizes of bamboo, as follows: into five classes of diameters (1.0-1.4; 1.5-1.9; 2.0-2.4; 2.5-2.9; 3.0-3.5 cm) and eight classes of lengths (11.0-13.9; 14.0-16.9; 17.0-19.9; 20.0-22.9; 23.0-25.9; 26.0-28.9; 29.0-31.9; 32.0-34.9 cm), which included all sizes of both measures of trap-nests occupied by females in our study and in the study of Garófalo et al. (1993).

Table 1. Municipality location, geographic coordinates, fragment size (ha), and forest stage (i.e., mature x secondary forests) for all study areas surveyed at northern region of the State of Paraná (PR), southern Brazil. In this table was also included the age of each reforested area. The mean altitude (m) for all areas is provided in the latter column. The vegetation type of all study areas is seasonal semi-deciduous forest. FR = forest remnant; R = restored areas.

Area code	Type of area	Municipality	Geographic coordinates	Size (ha)	Forest stage	Mean altitude (m)	Implantation date
A	FR	Alvorada do Sul	22°49'1.89"S 51°11'25.77"W	137.3	Secondary	350	
B	FR	Rancho Alegre	23° 0'5.15"S 50°56'38.00"W	107.3	Secondary	361.5	
C	FR	Sertanópolis	22°56'29.33"S 50°57'4.68"W	28.7	Secondary	345.5	
D	R	Alvorada do Sul	22°48'45.31"S 51°11'52.05"W	115.6	Secondary	350	May 2004
E	R	Rancho Alegre	22°59'50.09"S 50°56'38.05"W	197.8	Secondary	361.5	June 2003
F	R	Sertanópolis	22°56'10.68"S 50°56'59.81"W	263.1	Secondary	345.5	Sept 2003
G	R	Primeiro de Maio	22°46'50.10"S 51°10'5.85"W	142.6	Secondary	350	May 2003
H	R	Alvorada do Sul	22°45'8.58"S 51°13'29.93"W	115.30	Secondary	350	Nov 2003
I	R	Primeiro de Maio	22°54'33.57"S 50°57'5.70"W	125.1	Secondary	337	August 2005
J	R	Primeiro de Maio	22°48'24.55"S 51° 5'35.04"W	150.4	Secondary	335	Nov 2004
K	R	Alvorada do Sul	22°49'16.07"S 51°18'28.31"W	151.9	Secondary	340	October 2002
L	R	Rancho Alegre	23° 3'1.33"S 50°57'46.18"W	191	Secondary	338	April 2004

Results

During the surveys, a total of 15 nests of *E.* aff. *auriceps* were obtained in the bamboo trap-nests. From these, four (26.6% of the total) were in restored areas (F and G), being three from F and one from G. Out of the 11 nests of *E.* aff. *auriceps* constructed in bamboo canes in the three forest remnants, one was in fragment A, while 4 and 6 were in remnants B and C, respectively.

Table 2 contains information on the bamboo dimensions, nests, and brood cells of 12 nests. For three out of 15 nests obtained in the surveys, measures of nest dimensions and cells were not included in the analysis due to mistakes made during the measurement procedures.

The bamboo internodes occupied by females varied in internal diameter from 1.2 cm to 2.0 cm ($\overline{X}$ = 1.7; SD = 0.3; N = 12) and lengths ranged from 11.0 cm to 28.0 cm ($\overline{X}$ = 19.5; SD = 4.8; N = 12). The total sizes of the nests inside the

bamboo internode ranged from 9.0 cm to 19.9 cm ($\overline{X}$ = 14.3; SD = 3.9; N = 12). The number of brood cells constructed per nest varied from 1 to 10 ($\overline{X}$ = 4.1; SD = 2.6; N = 12), (Table 2).

Brood cells were built with pieces of vegetal bark and resin, linearly arranged along the bamboo tube (Fig 1A-H). Externally, the wall of the brood cells was covered by bark, arranged in variable positions, firmly fixed to each other with resin, which seemed to act as cement to join the pieces of bark (Fig 1A-L). Resin was also present between cells and the inner wall of the bamboo and in small deposits across the bamboo length (Fig 1 E), probably to be used in the construction. Internally, the cell wall was lined with resin and quite smooth (Fig 1L).

Fig 1. A-E and I: nests of *Eufriesea* aff. *auriceps* established in trap-nests of bamboo. C-D: nests of *E.* aff. *auriceps* and nests of the wasp *Auplopus* sp (cells constructed with mud) in the same cavity. E: nests showing four brood cells and two males. F: detail of seven brood cells (closed). H: the same sevens cells (open) and larvae. I: nest with two cells and resin deposit in the trap-nest wall. J: detail of a pupa (male) and a cell. K: detail of an adult bee inside the cell; L: detail of a brood cell (open), showing its interior.

The cells measured 1.5-3.0 cm ($\overline{X}$ = 2.3 ± 0.5; N = 48) in length and 1.4-1.7 ($\overline{X}$ = 1.5 ± 0.1; N = 17 cm) in width. The thickness of the cell ranged from 1.5 to 2.7 mm ($\overline{X}$ = 1.9 ± 0.3; N = 17). The cells were ovoid (externally) and elliptical in their internal form, clearly showing a higher axis (diagonal), demonstrated by the position of the bee inside the cell (Fig 1K). Although resin could be detected between cells, no conspicuous demarcation was detected between them. Cells were closed with a plug made exclusively of resin. In some nests it was detected the presence of pieces of barks mixed with resin deposited between the last cell constructed by females and the nest entrance (Figs 1D and 1G). Two nests of *E.* aff. *auriceps* were also occupied by wasps of the genus *Auplopus* sp (Fig 1C-D).

We recorded only few emergences of adults in December 2019, from nests collected in February and March 2019 (N = 8; Table 2). In these cases, we observed that the cocoon was clear (beige) in most cases, although cocoons with a light brown shade were also found. On emergence from the cell, the bees opened a circular hole, smaller than the diameter of the cell plug.

Activity of *E.* aff. *auriceps* females occurred mainly during the warm-wet season, since most nests were surveyed from January to March. Only three nests were collected in June and July, however they were probably out of activity (see Table 2).

The percentages of similarity obtained in the comparisons between the data from the current study and those reported by Garófalo et al. (1993), considering the five classes of diameters and eight classes of length of trap-nests of bamboo occupied by females of *E. auriceps*, the estimates of similarity, given by Sorensen index, were 50.0% and 80%, respectively.

Discussion

Information on nesting biology and nest structure of euglossine bees remains restricted to only about 20% of the species (Ramírez et al., 2002; Cameron, 2004; Solano-Brenes et al., 2018). In our study, we describe the nest architecture of the orchid bee *E.* aff *auriceps*, constructed in bamboo trap-nests. The nest of this species has previously been described, in cavities of bamboo trap-nests, by Garófalo et al. (1993).

Table 2. Identification of the nests and study areas, measures of bamboo trap-nest occupied by females of *Eufriesea* aff. *auriceps*, number of brood cells per nest and measures of the main nest structures. A, B, C, D, F and G = study areas. FR = forest remnants of Atlantic Forest; R = reforested areas; M = Male; Fe = Female.

Nest Number*	Site	Fragment type	Data collection	Bamboo size (cm)	Bamboo ∅ (cm)	Total size of the nest (cm)	No. of cells (no. of individuals in development)	Cell length (cm)		Additional information
								Variation	$\overline{X}$ ± SD	
1	A	FR	15/02/15	20.3	1.4	13.7	5 (5)	2.0-2.5	2.2 ± 0.2	No emergence
2	B	FR	03/03/16	16.0	1.4	16.0	4 (4)	2.7-3.5	2.9 ± 0.5	No emergence
3	F	R	03/03/16	22.0	1.9	19.1	5 (5)	2.2-2.7	2.4 ± 0.2	No emergence
4	B	FR	17/03/16	15.5	1.5	15.5	1** (1)	-	-	No emergence
8	C	FR	25/02/19	28.0	1.7	9.00	4 (4)	1.9-2.3	2.1 ± 0.2	1 M and 3 Fe (only 2 emerged in 12/12/19); with nest of *Auplopus* sp
9	G	R	10/07/19	11.0	1.5	9.00	1** (0)	-	-	Nest probably abandoned (only resin and bark)
10	C	FR	26/03/19	26.9	2.0	19.9	10 (6)	1.5-2.8	2.1 ± 0.4	4 M and 2 Fe emerged (in 18/12/19)
11	C	FR	05/06/19	17.1	2.0	10.5	4 (2)	2.2-3.0	2.7 ± 0.4	2 Fe (no emerged)
12	C	FR	05/06/19	20.0	2.0	10.5	4 (0)	2.2-3.0	2.7 ± 0.4	No emergence
13	C	FR	28/01/19	22.0	2.0	13.0	2 (0)	2.3-2.7	2.5 ± 0.3	Larvae didn't develop
14	C	FR	26/03/19	18.1	1.4	18.1	3 (0)	2.0-3.0	2.5 ± 0.5	No emergence (attacked by ants); with nest of *Auplopus* sp
15	F	R	26/03/19	17.0	1.5	17.0	7 (7)	1.5-2.3	1.7 ± 0.3	No emergence

While our results reveal remarkable similarities in most aspects of nesting habit of this species between both studies, some differences are pinpointed here. One of them is the fact that in all the nests we surveyed brood cells were in a conspicuous linear arrangement, while Garófalo et al. (1993) reported that in 20% of the sampled nests of *E. auriceps*, cells were in an irregular cluster arrangement inside the bamboo cavity (Garófalo et al., 1993). On the other hand, those authors speculated that the irregular cluster arrangement of the cells, in the two nests where this arrangement was found, was probably due to the wider diameters of the bamboo canes (≥2.2 cm). Since in our study females of *E.* aff. *auriceps* did not use bamboo canes with diameters above 2.0 cm, the absence of brood cells in a cluster arrangement among the 15 nests could be because the females did not occupy wider cavities. Furthermore, we can conclude that females of this species preferred bamboo canes with a diameter ≤ 2.0 cm, since although bamboo canes with a broader diameter were available, they were never used by the females.

The *Eufriesea* species are medium sized to quite large bees (14-26 mm long; Dressler, 1982). *Eufriesea auriceps* shows a total length *ca.* 18 mm (Nemésio, 2009) and can be considered a large sized bee, a condition that could explain the preferential use of bamboo canes with widths between 1.5 and 2.0 cm and lengths, mostly, above 13.0 cm, both in our study and in that of Garófalo et al. (1993). In fact, in the few studies involving *Eufriesea* in which measures of brood cells were taken, the internal widths and lengths of the cells usually remained above 1.0 cm and 1.5 cm, respectively (Garófalo et al., 1993; Viana et al., 2001). Only for *Eufriesea violacea* (Blanchard), the internal contour of cells did not reach 1.0 cm (Sakagami & Michener, 1965). Moreover, the wall thickness of the cells, which are made from tree bark and resin in all species of *Eufriesea* so far studied, contribute to increase the cell width (Garófalo et al., 1993; Viana et al., 2001; Rocha-Filho et al., 2016; Carvalho-Filho & Oliveira, 2017). All these conditions certainly explain the preference of *E. auriceps* for bamboo trap-nests herein described.

Many species of *Eufriesea* are highly seasonal (Dressler, 1982; Garófalo et al., 1993; Peruquetti & Campos, 1997). Our results, based on adult emergences and nesting activity of females, indicate that *E.* aff. *auriceps* has a univoltive life cycle, being active during the warm-wet season, which in the studied region ranges from October to March (Sofia et al., 2004). Our results also revealed that females of this species were active until the end of this season.

With 67 species catalogued (Moure et al., 2012) of the *Eufriesea* genus, at most, the nests of a dozen species have been studied (Sakagami & Michener, 1965; Kimsey, 1982; Viana et al., 2001; Ramírez et al., 2002; Carvalho-Silva & Oliveira, 2017). Our results on the nest architecture of *E.* aff. *auriceps* are in line with the few studies describing the nesting habit of *Eufriesea*, which showed the use of tree bark associated with resin in cell construction (e.g. Sakagami & Michener, 1965; Viana et al., 2001; Carvalho-Silva & Oliveira, 2017; Rocha-Filho et al., 2017) and both the size and elliptical form of the brood cells (Sakagami & Michener, 1965; Garófalo et al., 1993; Viana et al., 2001). In this context, our results contribute to supporting the idea that no obvious taxonomic pattern exists among species of *Eufriesea* for nesting behavior, at least not based on current recognition of species groups and available biological data (Cameron, 2004).

Lastly, our findings add light on the strategy of using trap-nests for managing orchid bee fauna in forest fragments under anthropogenic interference. More than that, we showed here that the use of bamboo trap-nests can also help the colonization of forest areas undergoing restoration by orchid bee females, as observed for *E.* aff. *auriceps*. In a scenario where several studies have suggested that orchid bees or, at least some species of the group, are sensitive to environmental disturbances, such as habitat loss and forest fragmentation (Tonhasca et al., 2002; Brosi, 2009; Giangarelli et al., 2009, Nemésio, 2011, 2013), strategies to manage impacted areas become essential for the conservation of euglossine bees and their habitats. Therefore, despite the colonization of nests in the restored areas studied herein, these accounted for only approximately a quarter of the nests sampled and were restricted to only two of the nine restored areas. Thus, we recommend the continued use of trap-nests as a potential measure for managing the establishment of euglossine fauna in these areas.

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Disclosure statement

The authors declares that they have no conflict of interest.

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