



## RESEARCH ARTICLE - BEES

## Continuous Micro-Environments Associated Orchid Bees Benefit from an Atlantic Forest Remnant, Paraná State, Brazil

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### Abstract

The fragmentation and habitat loss are the main causes of pollinators decline worldwide, however very little is known about the composition and distribution of neotropical pollinators along continuous micro-environments. To fill this gap, we carried out samplings of Euglossini bees in a continuous area of forest with micro-environments of primary (remnant) and secondary (regeneration) forest of Atlantic Forest. We evaluated the differences in the composition and uniformity of orchid bees in different micro-environments, in order to characterize the responses of the local environmental changes in the attraction of bees to chemical traps. Our results indicated that the composition and uniformity were similar between the two forest fragments studied here, although there are greater abundance of some species by micro-environments. We conclude that the characteristics of the sites in a continuous environment with primary and secondary forest do not seem to have an effect on the composition of the Euglossini fauna, and that the chemical substances are complementary in the attractiveness of the orchid bee males. Thus, our findings suggest that micro-environments in a continuous matrix near forest remnants can help to promote the reintegration of the orchid bee communities and contribute to the conservation of areas in process of forest regeneration.

### Introduction

The tribe Euglossini comprises more than 200 described species distributed in five genera with two of them being composed of cleptoparasitic species (Dressler, 1982; Cameron, 2004; Nemésio, 2009). These bees are essential pollinators in the Neotropical region and are responsible for pollination of several plants of economic and ecological importance (Dressler, 1982; Ramirez et al., 2002; Milet-Pinheiro & Schindwein, 2005; Santos & Absy, 2012; Gianinni et al., 2015). Moreover some orchid bee species are bioindicators of conserved areas and others are widely distributed in disturbed and fragmented environments (Morato, 1994; Peruquetti et al., 1999; Aguiar & Ganglianone, 2008). Studies exploring

diversity of orchid bees at multiple scales (ecological and molecular level) are common in the Neotropical region (Raw, 1989; Silveira et al., 2011; Boff et al., 2014; Gonçalves et al., 2014; Eltz et al., 2015; Giangarelli et al., 2015; Penha et al., 2015; McCravy et al., 2016) because orchid bee males are easily attracted to chemical compounds (Dodson et al., 1969).

Although a population expansion of few species has been noticed to south of North America (Pemberton & Wheeler, 2006; Eltz et al., 2015; Griswold et al., 2015), orchid bees are extremely diversified in Central and South America (Ramalho et al., 2009; Cordeiro et al., 2012; Aguiar & Ganglianone, 2012; McCravy et al., 2016; Botsch et al., 2017). In South America, the Atlantic Forest (AF) is known to wide host bee diversity. Approximately 50 species of Euglossini have been



already recorded to this ecosystem (Sofia et al., 2004; Milet-Pinheiro & Schlindwein, 2005; Nemésio, 2009; Cordeiro et al., 2012; Gonçalves et al., 2014; Oliveira et al., 2015; Ferronato et al., 2017). Although orchid bee fauna is considered to be diverse in the AF, their communities seem to depend on the traits of local environment (Moreira et al., 2015).

Historical process of land use and occupation in the AF has deeply altered the habitats. From its original cover only 11.73% of its domain remains natural (Ribeiro et al., 2009). Several fragments are mostly surrounded by monoculture, pasture and cities (BRASIL, 2009). Thus, AF fragments are usually both small (< 50 ha) and isolated (Ribeiro et al., 2009). Although orchid bees are considered to be strong flyers, fragmentation may alter their dispersion range and thus affect local diversity, as a consequence, small forest fragments in urban areas may play the role as refuges for these bees (Rosa et al., 2015; Neame et al., 2012; Storck-Tonon et al., 2013; Oliveira et al., 2015; Fischer et al., 2016).

Studies show that Euglossini populations are influenced by landscapes that surround the forest matrix. According to Ferreira et al. (2013), antique urban fragments seem to offer the main conditions for the maintenance of orchid bee community: nest and food. In addition, small forest fragments, mainly in backyards, may serve as stepping stones between forest matrices. In another study, the authors evaluated the permeability of three matrices of arboreal crops (piassava palm, oil palm and rubber tree) and they found that the arboreal matrices are contributing to the landscape mosaic and the Brazilian Atlantic Forest corridor (Rosa et al., 2015). On the other hand, sugarcane crop that surround the forest matrix interferes with species dynamic, negatively affecting the richness and abundance, indicating that the forest edge functions as a barrier in the individuals flow (Milet-Pinheiro & Schlindwein, 2005). Although the studies were carried out in different crops and diversity scale ( $\beta$  and  $\gamma$ ), they show that functional connectivity is an important factor for the reestablishment of the orchid bee populations. Thus, the questions that still remain are: Does forest regeneration surrounding to primary forests favor the connectivity between fragments? Are anthropic landscapes (cities and monocultures) less impacting to the Euglossini community in a forest matrix surrounded by forests in regeneration?

To our knowledge, studies comparing pattern of orchid bee communities in stages of regeneration and environmental preservation in a continuous are scarce (Moreira et al., 2015; Boscolo et al., 2017; Ferronato et al., 2017). In order to provide insights in this topic, we compared the attraction potential of orchid bees to chemical baits in multiple micro-environments distributed in continuous areas with primary and secondary forest.

Here we tested if different continuous micro-environments in primary or secondary forests affect: (i) the diversity indexes of orchid bees and, (ii) at temporal scale patterns of species distribution. We defined primary and

secondary as vegetation heterogeneity at each sampling site, classified according to Brazilian environmental standards (IBAMA, 1991; BRASIL, 1994). Therefore, the present work investigated the differences in the composition and uniformity of orchid bees in different micro-environments, in order to characterize the response of local environment change in the bees attraction to chemical traps.

## Material and methods

### *Study area*

The study was conducted in six different sampling sites selected in forest fragments of a continuous matrix with two distinct habitats: primary and secondary forests. All sampling sites ( $n = 6$ ) were located at Foz do Iguaçu municipality in the Refúgio Biológico Bela Vista (RBV), western Paraná State, Brazil. The RBV reserve covers 1920 ha conservation unit which is part of the Itaipu Binacional complex, under a Seasonal Semi-deciduous Forest. The area is surrounded by Itaipu reservoir, alternate soybean and corn crops and it is 1300 m from city boundaries. This area includes fragments in advanced stages of regeneration and a part with native forest remnant. Of these fragments we selected three sites placed at primary forest and other three sites placed at secondary forest (Fig 1).

### *Sites characteristics and habitat complexity*

We used data from satellite Google Earth Pro and Global Forest Change dataset (Hansen et al., 2013) to distribute sampling sites in different continuous micro-environments. The characteristics of the sites (Table 1) were realized before starting the sampling. The size of each site was calculated using metric applications of the satellite programs (Google Earth Pro and Global Forest Change dataset). The distance from one site to another at each forest was established in an interval between 500-560 m (Fig 1). Georeferencing informations were digitized using software QGIS version 2.18 (2016). The classification of vegetation according to Brasil (1994) is provided in Table 1.

Primary forest is all vegetal community with great biological diversity and minimal anthropic effects, not affecting original characteristics of forest structure (BRASIL, 1994). This forest structure favors the regeneration of late species and the formation of seed banks (BRASIL, 1994; Clements, 1916), also known as “climax forest” or mature forest. In the study region, the forests are classified as Seasonal Semi-deciduous Forest (IBAMA, 1991), one of the main formations of Atlantic Forest (BRASIL, 2010a). The national forest system determines primary formation such as natural forest (BRASIL, 2010b) or forest remnants. In this study, the primary forest is composed by forest remnant (native) and reforestation (which was performed with > 40 years) (ITAIPU, 1978; Ziober & Zanirato, 2014) (Fig 1).

**Table 1.** Environmental gradient: geographic coordinates, elevation (m), age of vegetation (years), size of sites (ha), stage of forest (Brasil, 1994) and continuous sampling sites of primary and secondary forest, western region of Paraná, southern Brazil.

Environmental gradient	Primary forest			Secondary forest		
	Native forest (NA <sub>p</sub> )	Transition between Native and Reforested (NR <sub>p</sub> )	Reforested (RE <sub>p</sub> )	Initial stage of regeneration (IN <sub>s</sub> )	InterMediate stage of regeneration (IM <sub>s</sub> )	ADvanced stage of regeneration (AD <sub>s</sub> )
Geographic coordinates	25°26'2069"S 54°31'2932"O	25°26'3809"S 54°31'2721"O	25°26'5487"S 54°31'2466"O	25°27'3502"S 54°31'3147"O	25°27'2341"S 54°31'2849"O	25°27'2861"S 54°31'1164"O
Elevation (m)	251	246	247	224	224	238
Age (years)	> 40 <sup>a</sup>	> 40 <sup>a</sup>	> 40 <sup>a</sup>	≤ 5 <sup>b</sup>	≤ 10 <sup>b</sup>	≤ 15 <sup>b</sup>
Size (ha)	315.70	---	96.30	19.98	27.32	23.70
Forest stage	Primary (remaining)	Primary (transition)	Primary (reforested)	Secondary (initial)	Secondary (intermediate)	Secondary (Advanced)
Environmental aspects of sites	Native forest with difficult access, pristine	native/ reforested transition	Plantations of seedlings native to the Atlantic Forest	An open field covered by dense vines	Forest with few trees that were distant from each other, thereby forming clearings as woodland	Greenwood, forest and flooded environment

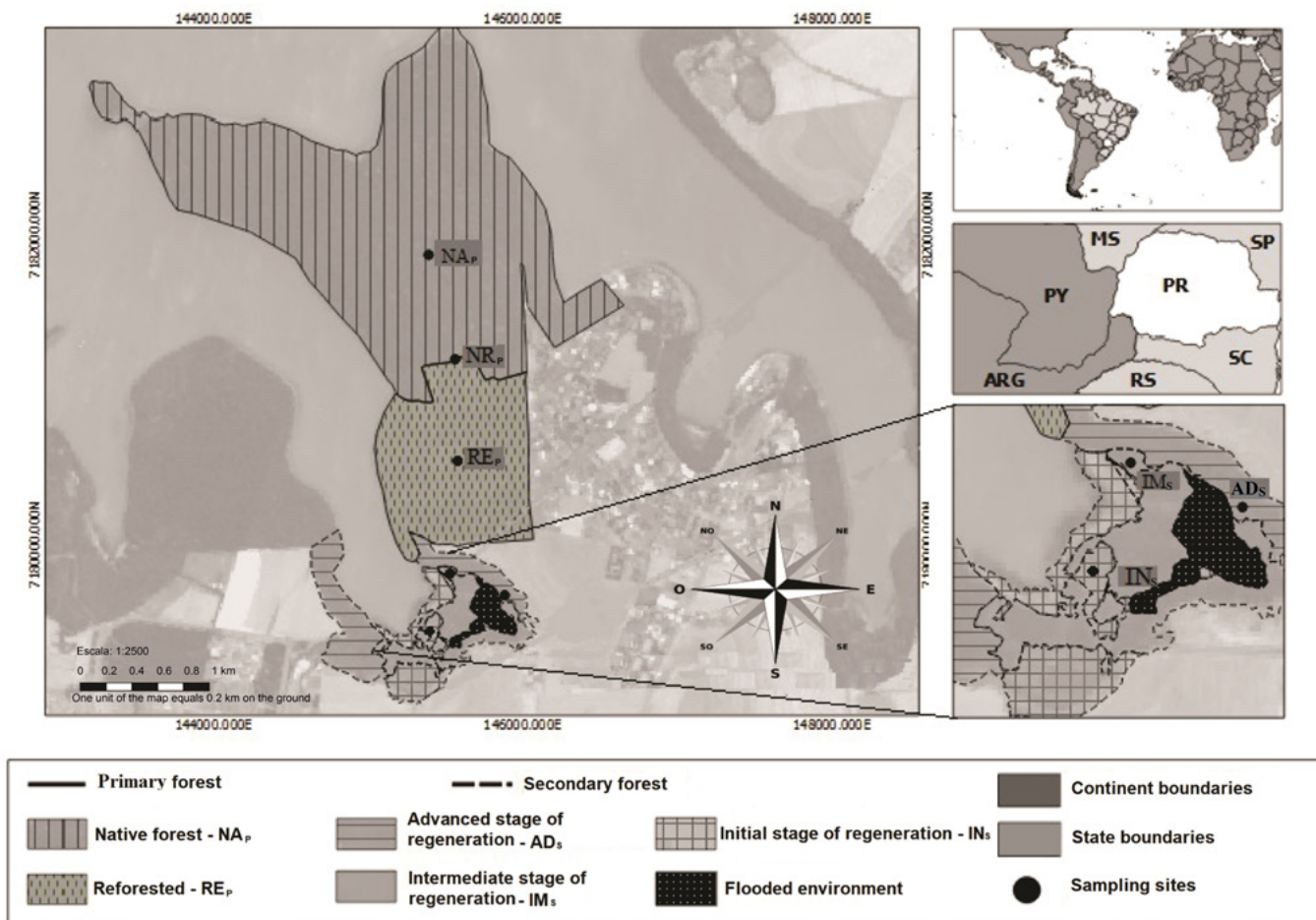
<sup>a</sup> Reference used to characterize the primary forest sites (ITAIPU, 1978).

<sup>b</sup> Reference used to characterize the secondary forest sites (Ziobar & Zanirato, 2014).

--- Division between NA<sub>p</sub> and RE<sub>p</sub> sites, cannot measure size (ha).

Secondary forest is a habitat that has recovered after a great disturbance, resulting from natural processes of succession (BRASIL, 1994). Usually young vegetation (< 15 years), consisting of open fields, high sunlight incidence and pioneering plant species with evident stages of regeneration (initial,

intermediate and advanced) (Clements, 1916; BRASIL, 1994). In this study, the secondary forest is composed by three stages of regeneration (initial, intermediate, and advanced), being a forest fragment that lost the original vegetation and it is naturally regenerating (Fig 1).



**Fig 1.** Location of the two forest fragments and each sampling site used in the study for the Euglossini bee communities in Paraná State, Brazil. Primary forest: sampling sites (NA<sub>p</sub>, NR<sub>p</sub> and RE<sub>p</sub>). Secondary forest: sampling sites (IN<sub>s</sub>, IM<sub>s</sub> and AD<sub>s</sub>). NR<sub>p</sub>= transition between native and reforested.

## Survey

Male Euglossini bees were sampled during 12 months from October 2013 to September 2014 at all sites (Fig 1). At each site four bait traps were used, each one with a different fragrance (cineole, eugenol, methyl salicylate, and vanillin). The traps remained at each site for the entire months, covering after 12 months, 8640 hours (12 months x 30 days x 24 hours) of trap exposition. Traps were fixed by a wooden support at a mean height of 1.5 m from the ground. The traps were visited monthly for the bee collections, cleaning the traps, and fragrances were refilled.

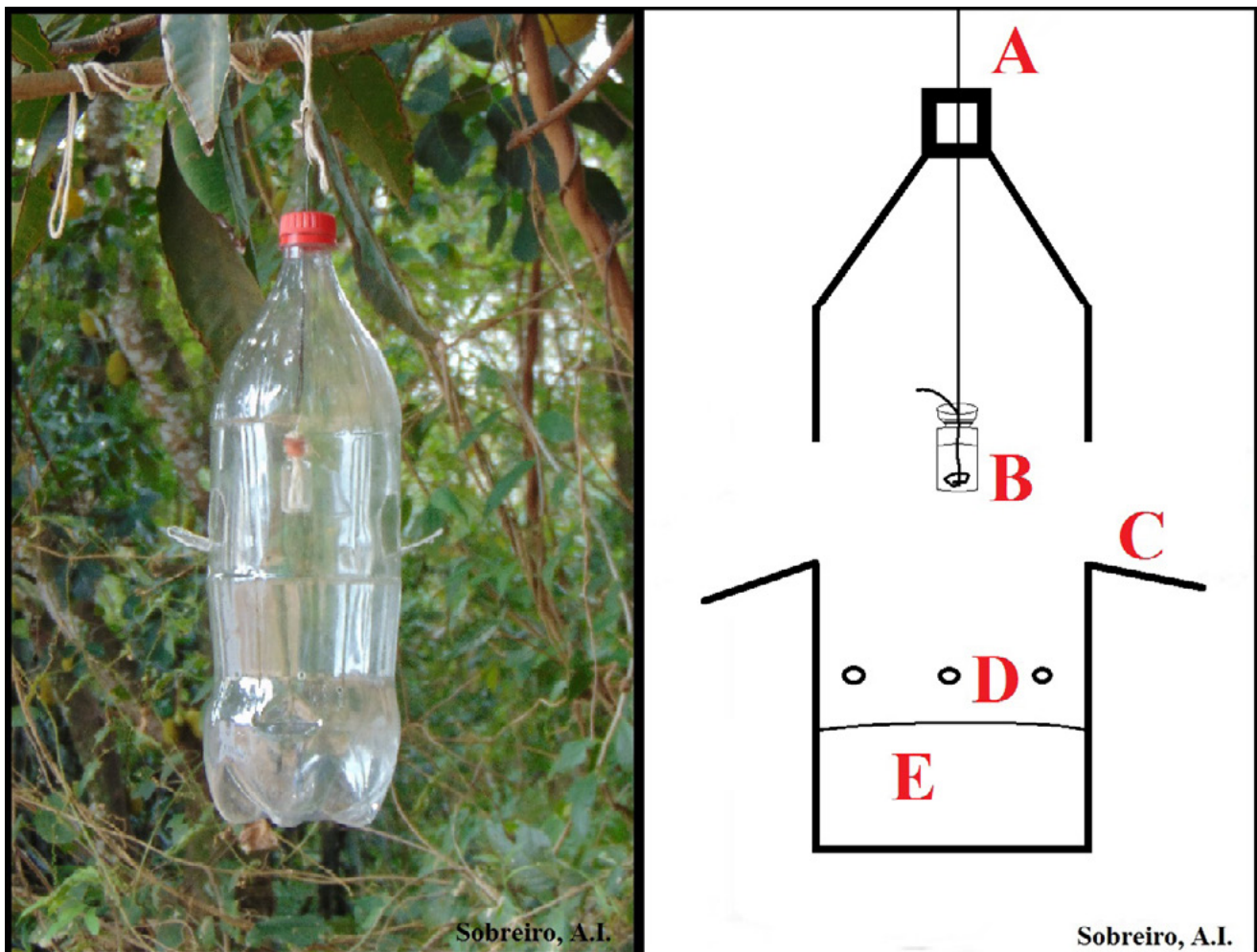
The traps were made from plastic bottles (with modifications from the model of Campos et al., 1989) (Fig 2). At the bottom of each trap (bottle), it was add a solution containing the following proportions: water (200 ml), alcohol 92.6% (100 ml), two spoons of salt (5 g) and one spoon of neutral detergent (15 ml) (colorless and without flavor). The solution was refilled once a month. The essences were stored in small glass bottles and a small opening was made to pass a string where the fragrance would volatilize by capillarity. In the laboratory, the bees were cleaned, mounted

in entomological pins and identified with the aid of specialist (R. B. Gonçalves, Federal University of Paraná).

## Data analysis

To compare the diversity of the six sites we used the Shannon-Wiener diversity ( $H'$ ). The dominance of the species at each site was calculated using Berger-Parker ( $d$ ) and Simpson ( $D-1$ ) Index (Melo, 2008). Lastly, Evenness ( $J'$ ) was used to define uniformity or homogeneity of species distribution at the sites (Magurran, 2004).

The rarefaction curve for the species richness of each studied site was obtained using 1000 randomizations, ~~and it is commonly used to evaluate the sampling effort.~~ In this study, we used the rarefaction curve to compare sampling effort among micro-environments (6 sites) and between two forests. The total sampling of study was represented by 72 samples, with 12 samples (one sample of bees per month during 12 months) at each site (total of 6 sites), i.e., 12 samples x 6 sites = 72 total samples. The Detrended Correspondence Analysis (DCA) was used to evaluate the relationship between the bee communities in the continuous micro-environments.



**Fig 2.** Bait trap made with commercial 2000 ml plastic bottle. (A) Nozzle with wire rod for support the suspended trap; (B) Small glass bottle filled with chemical essence and a piece of string that served as wick for dispersion of essence; (C) Two lateral openings of diameter equal with flaps for “landing area” of the bees; (D) Six circular openings for draining excess water; (E) Solution. Modifications from the model of Campos et al. (1989).



Sørensen-Dice index was used to compare similarities between sites and species richness. To perform the DCA and rarefaction curve, we employed the vegan package (Oksanen et al., 2018) using the software R version 3.4.1 (R Development Core Team, 2017).

This software was also used to realized the Analysis of Variance (ANOVA) and to determine whether there were significant differences for: (i) species abundance per month; (ii) total abundance of species between forest types; (iii) species abundance among sites; (iv) species abundance by chemical essence. The specnumber function locates the number of species using the *MARGIN* argument. If the experiment has more than one fragment or sampling site, it uses if the parameter *groups* and the function finds the total number of species for each parameter (Hurlbert, 1971). We used this function to determine if the richness data were significant per month, forest types, among sites and chemical essence. In addition, this function was also used to calculate the diversity index, so we applied a test of ANOVA to determine if there was significant difference.

Finally, aiming to compare the temporal distribution of the Euglossini species in the two forests, we performed a histogram with all the species frequencies per sampled month. To make the histogram, we used the poncho.R function, and this analysis was performed with the aid of software R (R Development Core Team, 2013). The poncho function is used to create histograms and multiple graphs of species distribution in a community in relation to environmental gradients or experimental variables (Dambros, 2014). In the current study, the confidence interval was of 99%.

## Results

Throughout the study, we collected 586 orchid bee males belonging to four genera and eight species. Of these, 285 were collected in the primary forest and 301 specimens

were collected in the secondary forest. The abundance was statistically different per month ( $df = 11$ ,  $F = 3.987$ ,  $p = 0.0002$ ), although it did not vary significantly between forest types ( $df = 1$ ,  $F = 0.0451$ ,  $p = 0.832$ ). The same orchid bee species ( $n = 8$ ) were sampled in both primary and secondary forest. The highest abundance of orchid bees in the secondary forest was found at  $AD_s$  site ( $n = 162$  individuals) and in the primary forest at  $RE_p$  site ( $n = 131$  individuals). The site with the lowest abundance and diversity was at  $NR_p$  with 62 individuals (6 spp.) and at  $IN_s$  with 66 individuals (7 spp.). The abundance did not vary significantly among the sites ( $df = 5$ ,  $F = 0.9439$ ,  $p = 0.4559$ ).

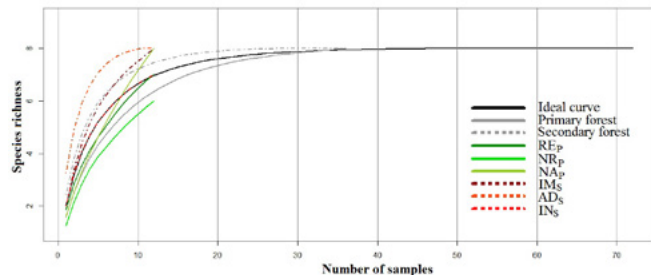
*Euglossa annectans* Dressler was the most abundant species in both primary and secondary fragment, with relative abundance of 70.52% ( $n = 201$ ) and 42.19% ( $n = 127$ ), respectively. *Eufriesea aff. auriceps* (Friese) was the less abundant species with 0.35% ( $n = 1$ ) in the primary forest and 2.32% ( $n = 7$ ) in the secondary forest.

Shannon-Wiener Index ( $H'$ ) indicated the secondary forest with higher diversity ( $H' = 1.66$ ) than the primary forest ( $H' = 1.08$ ). The diversity was the highest at  $AD_s$  ( $H' = 1.69$ ) and the lowest at  $NR_p$  ( $H' = 0.80$ ). Regarding the Berger-Parker Index ( $d$ ), the dominance was higher in the primary forest fragment ( $d = 0.70$ ) than in the secondary forest fragment ( $d = 0.41$ ). Among sites, the highest dominance was found at  $NR_p$  ( $d = 0.79$ ), probably due to the higher abundance of *Eg. annectans*, whereas the lowest dominance was recorded at the  $AD_s$  site ( $d = 0.38$ ). The Simpson Index was lower in the secondary forest ( $D = 0.75$ ) than in the primary forest ( $D = 0.48$ ). For the sampled sites, the Simpson Index at  $AD_s$  showed the lowest dominance ( $D = 0.77$ ), while at  $NR_p$  presented the highest dominance ( $D = 0.36$ ). The evenness was higher in the secondary forest ( $J' = 0.80$ ) than in the primary forest ( $J' = 0.52$ ). Among sites, the  $IN_s$  site obtained the maximum evenness ( $J' = 0.83$ ), followed by  $AD_s$  ( $J' = 0.81$ ). The sites with the lowest evenness were  $NA_p$  ( $J' = 0.41$ ) and  $NR_p$  ( $J' = 0.44$ ) (Table 2).

**Table 2.** Orchid bee species sampled with chemical compounds in continuous micro-environments of two forest fragments (primary and secondary) of Atlantic Forest, Brazil. Total abundance, divergence index ( $H'$ ), dominance ( $D-1$  and  $d$ ), and Evenness ( $J'$ ).

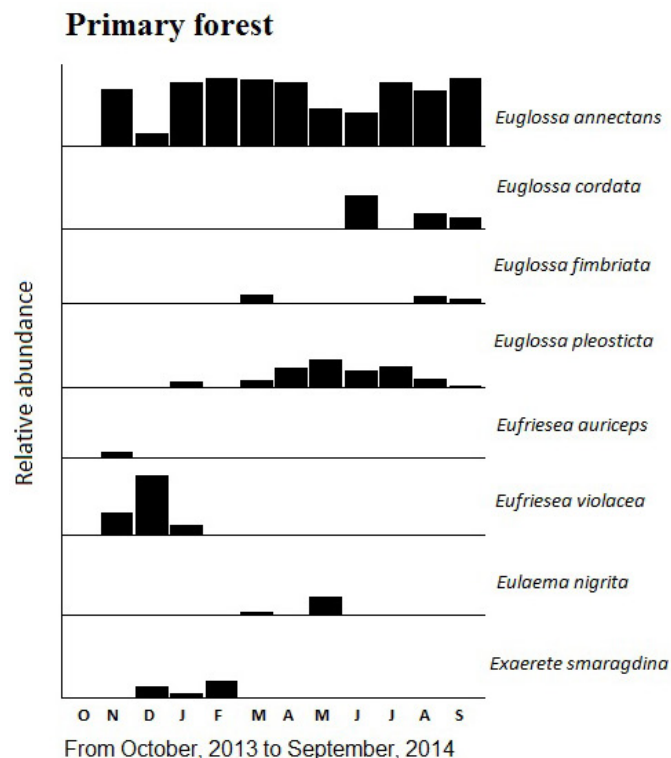
Species	Primary forest			Secondary forest		
	$NA_p$	$NR_p$	$RE_p$	$IN_s$	$IM_s$	$AD_s$
<i>Euglossa annectans</i> Dressler	72	49	80	24	41	62
<i>Euglossa cordata</i> (Linnaeus)	2	3	23	6	4	26
<i>Euglossa fimbriata</i> Moure	6	0	5	7	1	5
<i>Euglossa pleosticta</i> Dressler	1	6	14	12	6	25
<i>Eufriesea aff. auriceps</i> (Friese)	1	0	0	0	3	4
<i>Eufriesea violacea</i> (Blanchard)	8	1	7	14	11	27
<i>Eulaema nigrita</i> Lepeletier	1	1	1	2	6	9
<i>Exaerete smaragdina</i> (Guérin)	1	2	1	1	1	4
Total of individuals	92	62	131	66	73	162
Total of species	8	6	7	7	8	8
Shannon-Wiener ( $H'$ )	0.86	0.80	1.20	1.63	1.42	1.69
Simpson ( $D-1$ )	0.37	0.36	0.58	0.76	0.64	0.77
Berger-Parker ( $d$ )	0.78	0.79	0.61	0.36	0.56	0.38
Evenness ( $J'$ )	0.41	0.44	0.61	0.83	0.66	0.81

The rarefaction curve used to compare species richness suggests that the richness of the primary and secondary forest stabilized reaching the asymptote. Being that in the primary forest stabilized at the 34th sample, while in the secondary forest stabilized at the 36th sample. On the other hand, comparing the rarefaction curve for the six sampling sites, the only site that approached of stabilizing was AD<sub>s</sub>, while the other sites did not reach the total number of species richness for the micro-environment, sample adequacy was not sufficient at the NA<sub>p</sub>, NR<sub>p</sub>, RE<sub>p</sub>, IM<sub>s</sub>, and IN<sub>s</sub> sites. The sampling efficiency was 99.78 and the average estimated richness was 8.01 (Fig 3).



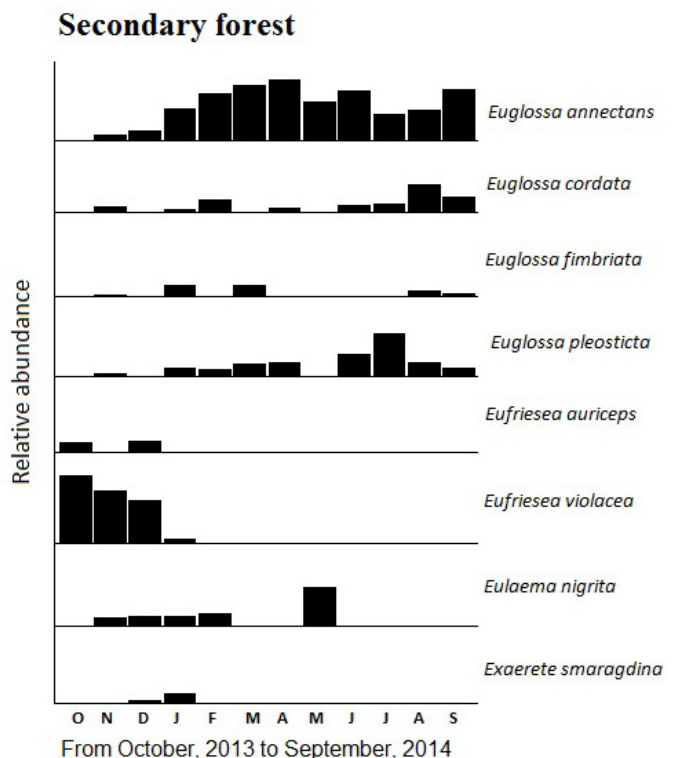
**Fig 3.** Rarefaction curve (1000 randomizations) for the species richness and individuals of orchid bees as a function of their abundance in the six sites of primary and secondary fragment of Atlantic Forest in Paraná State, Brazil.

We verified that the distribution pattern of species per month in the secondary forest showed a higher frequency and abundance for all Euglossini species, except *Eg. annectans* which was most frequent and abundant in the primary forest.

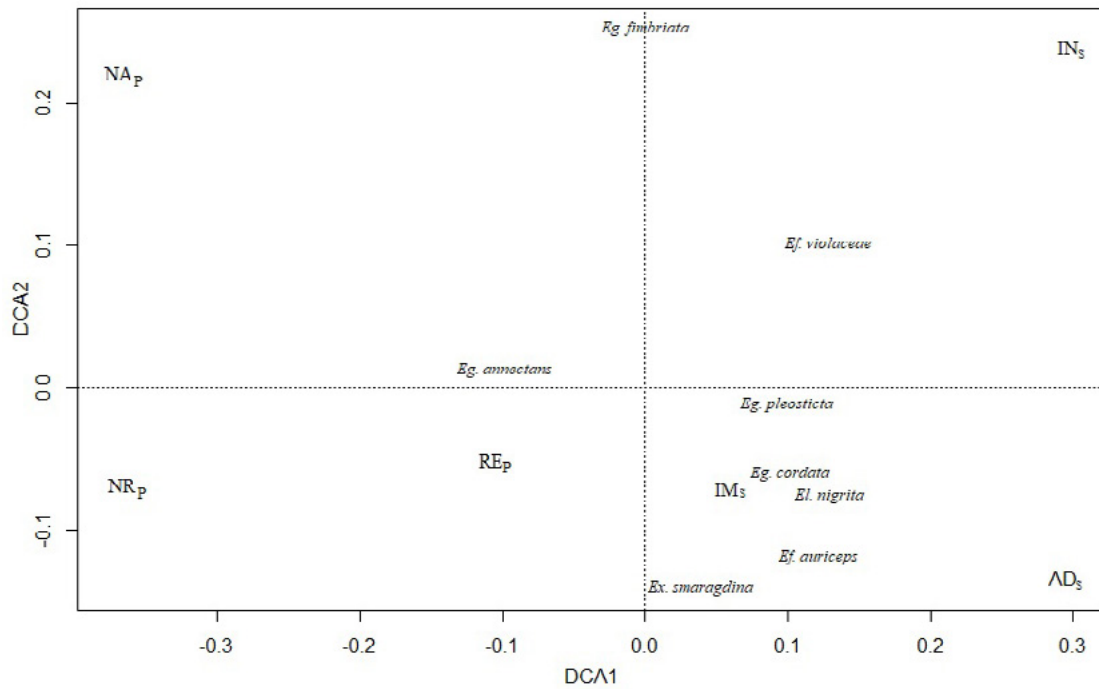


This one was the only species that occurred during 11 months and the most abundant during 8 months in both forests, representing 55.97% (328 individuals) of the total number of sampled bees. The month with the highest abundance in the primary forest was September 2014 with 36.84% (105 individuals), while in the secondary forest August 2014 with 17.60% (53 individuals) presented the highest abundance. In October 2013, only *Ef. aff. auriceps* and *Ef. violacea* were recorded in the secondary forest fragment. In addition, ANOVA indicated that the species richness did not change per month ( $df = 11$ ,  $F = 1.713$ ,  $p = 0.0703$ ) and between forests types ( $df = 1$ ,  $F = 3.16$ ,  $p = 0.0765$ ), although it varied significantly among sites ( $df = 5$ ,  $F = 15.54$ ,  $p = 0.00732$  (Fig 4).

The Detrended Correspondence Analysis (DCA) revealed differences in the Euglossini fauna ordination among the sampling sites (Fig 5). The RE<sub>p</sub> and IM<sub>s</sub> sites are grouped by the occurrence pattern of species with diversity, dominance and evenness similar among the micro-environments. We observed that the primary forest sites (NA<sub>p</sub>, NR<sub>p</sub> and RE<sub>p</sub>) are grouped on the left side of the ordination and secondary forest sites (IN<sub>s</sub>, IM<sub>s</sub> and AD<sub>s</sub>) are grouped on the right side of the ordination, connected by similarity between RE<sub>p</sub> and IM<sub>s</sub>. Besides, it seems that *Eg. annectans* is the species positioned closer to the primary forest grouped on the left side of the ordination (primary forest sites), as well as it is noticeable that *Ef. violacea* is closer to the secondary forest sites on the right side of the ordination. *Euglossa cordata* and *Eg. pleosticta* were grouped among the geographically closest sites (RE<sub>p</sub>, IN<sub>s</sub>, and AD<sub>s</sub> sites) with different conservation characteristics among the micro-environments (see Fig 1) (Fig 5).



**Fig 4.** Temporal distribution histogram of Euglossini bees in the primary and secondary Atlantic Forest fragment in Paraná State, Brazil from October 2013 to September 2014.

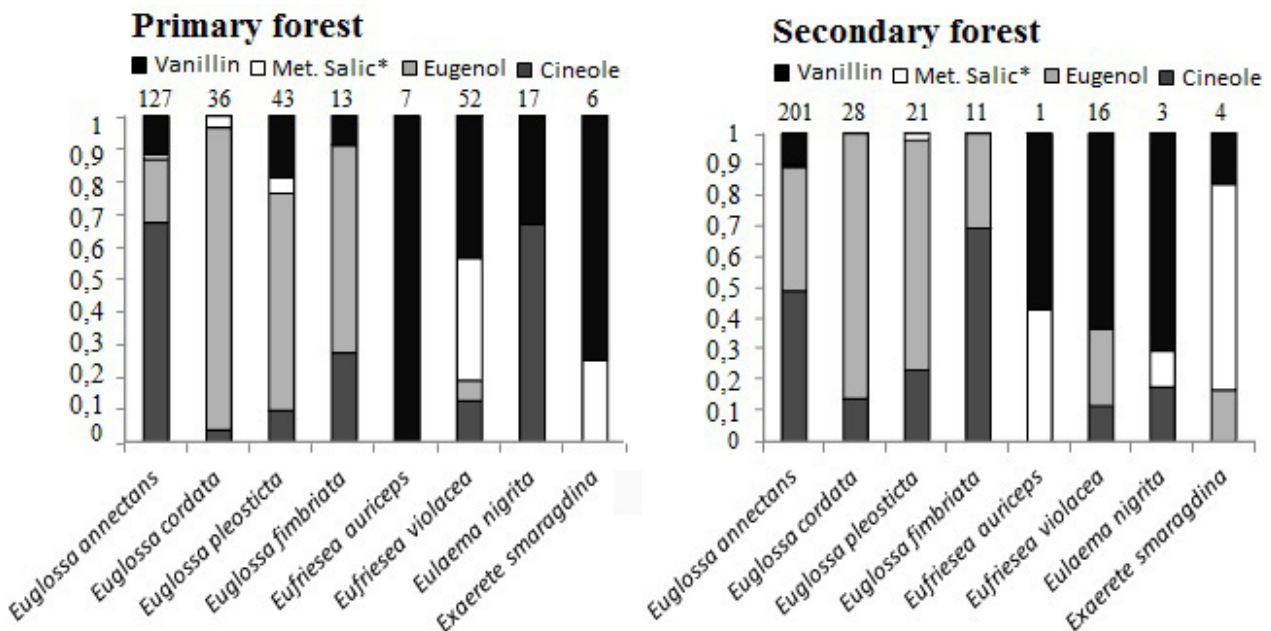


**Fig 5.** Detrended Correspondence Analysis (DCA) of the Euglossini community at the sites (n = 6) in primary (NA<sub>p</sub>, NR<sub>p</sub> and RE<sub>p</sub>) and secondary forest fragment (IN<sub>s</sub>, IM<sub>s</sub> and AD<sub>s</sub>) of Atlantic Forest in Paraná State, Brazil. According to Sørensen-Dice coefficients.

*The role of scents*

Cineole was the most efficient scent in the species abundance attraction, accounting for 40.95% (240 individuals), followed by eugenol, which attracted 37.37% (219 individuals) of all sampled bees. In both forests, the vanillin and methyl salicylate traps attracted the lowest abundances with 17.74% (104 individuals) and 3.92% (23 individuals), respectively. In primary forest, the most attractant fragrance was eugenol with 47.01% (134 individuals) and in the secondary forest the

cineole was more attractant with 43.85% (132 individuals). For micro-environments (sites), the eugenol was the most attractant essence with 10.75% (n = 63 individuals) at NA<sub>p</sub> and 9.55% (n = 59 individuals) at RE<sub>p</sub>. Cineole attracted the highest abundance at AD<sub>s</sub> (11.77%, n = 69 individuals), followed by NR<sub>p</sub> (6.82%, n = 40 individuals), IM<sub>s</sub> (5.46%, n = 32 individuals) and IN<sub>s</sub> (5.29%, n = 31 individuals). Methyl salicylate was the less attractant scent with 0.17% (n = 1 individual) at IN<sub>s</sub> and neither individual at NA<sub>p</sub> (Fig 6).



**Fig 6.** Frequency of male Euglossini bees captured using different chemical baits (cineole, eugenol, methyl salicylate and vanillin) within of primary and secondary Atlantic Forest fragment in Paraná State, Brazil. \*Methyl salicylate.

The attractiveness of the essences was significantly different for the species richness ( $df = 3$ ,  $F = 11.51$ ,  $p = 3.84e-07$ ) and diversity ( $df = 3$ ,  $F = 6.043$ ,  $p = 0.000531$ ), but it was not different for the abundance ( $df = 3$ ,  $F = 0.9479$ ,  $p = 0.4203$ ). We observed here that the essences present an attraction pattern for the Euglossini genera, being that 89.79% of all the individuals of *Euglossa* were attracted by eugenol and cineole, whereas 73.58% of all the individuals of *Eufriesea*, *Eulaema*, and *Exaerete* were attracted by methyl salicylate and vanillin. In addition, the fragrances that attracted more species were methyl salicylate and vanillin with 7 species each, followed by cineole and eugenol that attracted six species each.

## Discussion

Our analyzes showed that the species composition, abundance and indexes of diversity did not differ between the primary and secondary forest fragments. Interestingly, the micro-environments of the primary and secondary forest seem to influence the preference of the species for conserved or altered sites. Recently, Ferronato et al. (2017) investigated the Euglossini community in native and reforested forests and they observed that some species of orchid bees seem to indicate a better quality of habitat, besides the possible reintegration of the bee community from the reforested forests being favored by native forest. Although studies comparing the richness and abundance of Euglossini among several forest fragments are not scarce (Sofia et al., 2004; Aguiar et al., 2014; Gonçalves et al., 2014; Giangarelli et al., 2015; Ferronato et al., 2017), very little is known about the distribution of these species in micro-environments, mainly comparing the micro-environments mosaic within the primary and secondary forest and if the distribution of the species affect the attractiveness of the essences in these different micro-environments.

Some studies suggest negative effects on the Euglossini community in fragmented habitats (Sofia et al., 2004; Aguiar et al., 2014; Gonçalves et al., 2014; Giangarelli et al., 2015), however our results seem to indicate that fragmented habitats in process of regeneration can support the reintegration of the orchid bee community, as suggested by Ferronato et al. (2017) for the restored forest areas. A possible evidence for this fact is the presence of *Ef. violacea* in micro-environments of the primary and secondary forest, being more predominant in the forest in process of regeneration (secondary). This species was suggested as sensitive to fragment size reduction in studies involving small open areas or greenwoods and large forests (Sofia & Suzuki, 2004; Giangarelli et al., 2009). Our results seem to indicate that the Euglossini species were little sensitive to fragmentation. This finding could be explained by the proximity of the secondary fragment to primary forest, and that the continuous micro-environments between primary and secondary forest cannot adversely affect the foraging dynamic of these bee species. It is worth emphasizing that the results presented here should be interpreted with caution,

mainly due to the small geographic distance between the sampled fragments and that Euglossini males could fly long distances in search of the display territory, not being restricted to a specific location until establishment of the territory (Janzen, 1971; Pokorný et al., 2014).

The frequency and abundance for all Euglossini species were higher in the secondary forest, except *Eg. annectans* which predominated in the primary forest. An effect which may play an important role in orchid bees attraction is the scent volatilization of each compound (Silva & Rebêlo, 2002). Bees in general can use scent as main driver to find resource. Thus, if air flow is stronger in open areas than within the forests, the scent can be spread easier in the secondary forest (in process of regeneration) than within primary forest. Although similar findings in different environments and previous studies have providing insights in this topic (Raw, 1989; Milet-Pinheiro & Schlindwein, 2005; Nemésio & Silveira, 2006), a more controlled experiment to understand the air flow effect in orchid bees attraction in these forest types is still missing. And too, factors such as wind speed, air temperatures and wind direction may cause different concentrations of odours dispersion (Murlis et al., 1992), because the density between greenwoods and forests is different (Raw, 1989; Nemésio & Silveira, 2006). Concerning the predominance of *Eg. annectans* in the primary forest, our findings corroborated with the studies of Knoll and Penatti (2012) and Ferronato et al. (2017) suggesting that the species' distribution may be related to the more humid and preserved regions of the Atlantic Forest.

On the other hand, *Eg. cordata*, *Eg. pleosticta*, *Ef. violacea* and *El. nigrita* were more abundant and more frequently occurred in the secondary forest, which may be due to responses to the micro-factors that do not play or are rare in primary forests (as temperature variation, sunlight incidence and relative humidity) (Nemésio & Silveira, 2006). *Eufriesea violacea* was clearly predominant in the secondary forest, although this fragment had been altered and it is in process of natural regeneration, the preference of the species for this fragment may suggest environmental quality in forest recovery, as suggested by Giangarelli et al. (2009). The number of *Ef. aff. auriceps* recorded in this study was higher to those found in other studies in Paraná State (Santos & Sofia, 2002; Sofia et al., 2004; Gonçalves et al., 2014), assuming that the more preserved conditions of the primary forest may favor the regeneration of the secondary forest in this study, influencing the ecological rebalancing of the Euglossini community, and this condition could explain the record of *Ef. aff. auriceps*, besides highlighting the importance of preserving small fragments for the Euglossini community.

It has been suggested that some species are indicators of disturbed environments, such as *Eg. cordata* and *El. nigrita* (Rebêlo & Cabral, 1997; Peruquetti et al., 1999; Silva & Rebêlo, 2002). However, our data confirm that forests in regeneration are important for the Euglossini communities and



can have an effect on the micro-distribution (and maybe in the foraging behaviour). The current data are not congruent that *Eg. cordata* and *El. nigrita* are indicators of disturbed forest in this study, as stressed by Ramalho et al. (2009) and Aguiar and Ganglianone (2012), despite their tolerance to disturbed forest be evident (Santos & Sofia, 2002; Aguiar & Ganglianone, 2012; Nemésio, 2013; Giangarelli et al., 2015; Mateus et al., 2015; Ferronato et al., 2017; Medeiros et al., 2017). This leads us to the question: Do the different degrees of regeneration in continuous micro-environments not change the community of orchid bees or change to the point that negatively affect the community in the conserved sites of the primary forest? We highlight here that *Eg. annectans* was more abundant in the preserved sites and it seems to indicate the positive effect of continuous micro-environments in regeneration on the community of orchid bees, considering the potential of the species to act as bioindicator of conserved habitats or with more success in recovery or reforested forests (Aguiar & Ganglianone, 2012; Knoll & Penatti, 2012; Ferronato et al., 2017). In addition, the less preserved micro-environments had a lower abundance of this species, suggesting some restriction of the species in these sites, or that the Euglossini species respond differently to local characteristics of micro-environments (Moreira et al., 2015). As well as, the male Euglossini bees perform their mating flights in sunlight open spaces (Stern, 1991), which is another factor that could have contributed to the higher uniformity of species assemblages (except *Eg. annectans*) at sites of less conserved continuous micro-environments of primary and secondary forest (RE<sub>p</sub>, AD<sub>s</sub>, IM<sub>s</sub> and IN<sub>s</sub>).

When we compared the micro-environments sampled within the two forest types we found that species composition and uniformity indexes were different among micro-environments, this may occur because some Euglossini species require different habitats to meet their specific foraging and nesting requirements (Roubik, 1989; Roubik & Hanson, 2004). Further, although the investigations involving the flight distance of Euglossini are not conclusive (Dressler, 1982; Pokorný et al., 2014), the great flight capacity of some orchid bee species is evident (Janzen, 1971; Wikelski et al., 2010; Nemésio, 2012), this potential of long-range flight can have contributed to the presence of almost all species in the micro-environments, which may be promoting pollen flow across the undisturbed and disturbed areas and contributing to the forest regeneration (Gathmann & Tschamtkke, 2002; Jha & Dick, 2010).

The occurrence of Euglossini in continuous micro-environments can be interpreted as a possibility of gene flow among plants of the different micro-environments, considering the pollination potential of these bees, responsible for pollination of a wide variety of Orchidaceae species and many plant species of other botanical families (Dressler, 1982; Cameron, 2004; Roubik & Hanson, 2004), and probably they are involved in success of regeneration favoring the forest recovery and maintenance of the ecological system (Ferronato et al., 2017).

### *The role of scents*

It is common the use of aromatic baits in studies carried out to attract the male Euglossini bees (Sofia & Suzuki, 2004; Silveira et al., 2011; Gonçalves et al., 2014; Giangarelli et al., 2015). Scent traps offered for short periods is the most used method (Aguiar & Ganglianone, 2008; Silveira et al., 2011; Gonçalves et al., 2014; Giangarelli et al., 2015; Oliveira-Junior et al., 2015), however the method that we used was of traps offered for long period (8640 hours/12 months), in order to maximize the efficiency of field samplings. According to Storck-Tonon et al. (2013), research on the Euglossini fauna requires an efficient sampling effort for better estimates of species richness, including rare species. In this perspective, we highlight that even the volatility being an important factor in the attractiveness of individuals, the method that we used here (long period) presented as a positive point the efficiency in the sampling effort (99.78) and the estimation of richness in the primary (8 spp.) and secondary (8 spp.) forest near the ideal (8.01 spp.), which means **thatmost** of the sensitive species the four essences used in this study were sampled, maybe occurring a very rare species or some species that are passing by the forest environments, thus attending to an efficient sampling method that it is not commonly employed.

Concerning the attractiveness of the essences, we found that there is a complementarity of the scents used and the Euglossini genera attracted. The cineole and eugenol attracted approximately 90% of the total of *Euglossa* species, and methyl salicylate and vanillin attracted approximately 75% of *Eufriesea*, *Eulaema* and *Exaerete* individuals. This result showed that the variety of essences chosen may affect the diversity of Euglossini species sampled. Therefore, we emphasize the importance of cautiously evaluating the preference of the chemical compounds for Euglossini, since multiple factors, besides the essences, can affect the attractiveness of the bees (Stern, 1991; Murlis et al., 1992; Nemésio & Silveira, 2006; Oliveira-Junior et al., 2015).

Here, we showed that continuous micro-environments seem to favor the reintegration of the Euglossini community in forests in regeneration (secondary) close to primary forests. Moreover, despite of temporal effect and Euglossini fauna composition in different micro-environments, the primary and secondary forest do not seem to have an effect on the temporal variation and species richness. Another important point is the complementarity of the essences in the attractiveness of Euglossini genera, aiming that the choice of the compound can influence the composition and abundance of the species. Although previous studies have focused on the responses of orchid bees to habitats and regions diversity (Aguiar & Ganglianone, 2012; Aguiar et al., 2014; Cordeiro et al., 2012; Gonçalves et al., 2014; Giangarelli et al., 2015; Botsch et al., 2017; Ferronato et al., 2017), few studies have investigated until now how micro-environments affect the structure and responses of these bees to local characteristics (Abrahamczyk

et al., 2011), especially between nearby fragments or in micro-environments that form a forest matrix. Forest fragments are generally small and isolated (Ribeiro et al., 2009) and in urban centers are restricted to green areas of different extensions which may affect the Euglossini responses to forest structure of these green environments (Ferreira et al., 2013), but our study showed that continuous micro-environments can favor the uniformity and reintegration of orchid bees, so we suggest that these facts should be considered in future actions of conservation and recovery of areas.

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### Authors' contribution

AI Sobreiro, LLS Peres and VV Alves-Junior: experimental design. AI Sobreiro and LLS Peres: fieldwork and data collection. AI Sobreiro: data analysis. AI Sobreiro, LLS Peres, S Boff, JR Amaral and VV Alves-Junior: writing of the manuscript. ~~S Boff: revision of the manuscript with important changes.~~

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