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Temporal variation in the Abundance of Orchid Bees (Hymenoptera: Apidae) in a Neotropical Hygrophilous Forest

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Abstract
Although bees are important pollinators in several ecosystems around the world, studies on bee diversity in hygrophilous forests are scarce. This type of vegetation is restricted to permanently wet soils and, therefore, has particular floristic, structure and physiognomy. The goal of the present study was to inventory and analyze the temporal variation of the euglossine bees that occur in a neotropical hygrophilous forest. In order to sample male bees we used four chemical baits, eucalyptol, eugenol, vanillin, and methyl salicylate. The captures were made once a month, from March 2010 to February 2011, from 9:00 am to 12:00 pm. We captured 113 individuals of three genera and five species (in decreasing order of abundance): *Eulaema nigrita* Lepeletier, 1841 (n=52), *Euglossa pleosticta* Dressler, 1982 (34), *Exaerete smaragdina* (Guérin-Méneville, 1844) (12), *Euglossa carolina* Nemésio, 2009 (11), and *Euglossa fimbriata* Rebêlo & Moure, 1968 (4). The most attractive bait was eucalyptol (n=98), followed by vanillin (11), and eugenol (4). Both temperature and rainfall affected significantly the distribution of the number of males throughout the year. The highest number of Euglossini species and individuals was sampled in the warm and rainy season, with activity peaks varying among species.

Introduction

Bees of the tribe Euglossini, also known as orchid bees, are characterized by a relatively long tongue, highly modified hind tibias in males where they can store fragrances and, in most cases, a bright metallic tegument (Cruz Landim et al., 1965). Euglossines occur in the Neotropics and are found from southern North America (Minckley & Reyes, 1996) and northern Mexico to northern Argentina (Dressler, 1982), at altitudes that vary from sea level to 1,500 and 1,600 m a.s.l.; euglossines are rare above 2,000 m a.s.l. (Dressler, 1982).

The fast flight, characteristic of this bee group and the rarity of finding them at flowers (Nemésio & Silveira, 2007), probably explains why these bees have been underestimated in studies on Apoidea communities in the past. However, the accidental discovery of male attraction (Lopez, 1963) to synthetic terpenoids and aromatic hydrocarbons, mimetic of chemical products found in floral fragrances (Rebêlo & Cabral, 1997), allowed to quickly advance the knowledge about the ecology of these bees. In addition to floral fragrances, Euglossini males are also attracted to a variety of non-floral fragrances, which are produced in wood, fungi, tree wounds, fruits (Ackerman, 1983; Whitten et al., 1993), and feces (Eltz et al., 2007). The studies carried out by Roubik and Hanson (2004) and Zimmermann et al. (2006) confirmed that the fragrances collected by males attract conspecific males, supporting the idea that those fragrances are analogous to pheromones and probably involved in mate recognition and choice (Zimmermann et al., 2009).

Although the development of fragrances in the past 40 years allowed a large increase in the sampling of Euglossini communities in several Brazilian ecosystems, hygrophilous forests have been poorly studied. Also known as seasonal semi-deciduous riparian forests with permanent fluvial influence (Rodrigues, 2004), broadleaf hygrophilous forests, swamp forests (Leitão Filho, 1982), or just hygrophilous forests (Toniato et al., 1998), this vegetation is characterized by

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a permanently flooded soil, contrary to periodically flooded riparian forests (Toniato et al., 1998), and has the important function of protecting water sources (Joly, 1992; Marques, 1994; Teixeira et al., 2008). Currently, hygrophilous forests are extremely vulnerable to human impacts (Teixeira et al., 2008). This kind of vegetation has been largely devastated in the State of São Paulo (Torres et al., 1994), and so has progressively disappeared before being studied (Gomes et al., 2006). Until the present study no information on Euglossini communities of hygrophilous forests was available. These bees are important pollinators in several Brazilian biomes and have close relationships with several plants (Rocha-Filho et al., 2012; Silva et al., 2012) of different families (Apocynaceae, Bignoniaceae, Clusiaceae, Commelinaceae, Convolvulaceae, Cucurbitaceae, Leguminosae, Melastomataceae, Myrtaceae, Rubiaceae, Solanaceae and Verbenaceae) that also occur in hygrophilous forests (Castro, personal observation).

Hence, the objective of the present study was to describe the richness, abundance and the diversity of Euglossini bees that occur in a fragment of hygrophilous forest, as well as to assess the efficiency of odor baits in the attraction of males in this environment and to test for an effect of climate on the distribution and temporal fluctuations in the abundance of Euglossini bees throughout the year.

Material and Methods

Study area

The study was carried out in Reserva Toca da Paca (hereafter RTP), classified as a private reserve of natural heritage by the Brazilian environmental law. This private reserve is within the boundaries of Boa Vista Farm, on the margins of the Mogi-Guaçu River (21º27’30” S - 48º05’12” W) at 510m a.s.l., in the city of Guatapará, São Paulo State, southeastern Brazil (Fig 1). The 187ha of RTP’s area has a predominant vegetation of hygrophilous forest which, despite being situated in a transition area of the cerrado and semi-deciduous forest vegetation types, is currently surrounded by agricultural crops, especially sugarcane.

The regional climate type is Aw (Köppen’s classification), with two well-defined seasons: a dry and cold season, from April to September (autumn-winter), with the average temperature varying between 19.3 and 22.9 ºC and 201.5mm of total rainfall; and a rainy and warm season, from October to March (spring-summer), with the average temperature varying between 23.8 and 25.2ºC and 982.1mm of total rainfall (data from Centro de Pesquisas Meteorológicas e Climáticas Aplicadas a Agricultura - UNICAMP).

Sampling of male bees

We sampled bees monthly from March 2010 to February 2011 on a 50m long transect, inside the fragment and parallel to its edge. The distance between transect and the edge of the fragment was 80m and the nearest crop sugarcane was 200m (Fig 1). We placed on this transect four odor baits with eucalyptol, eugenol, vanillin, and methyl salicylate, which proved to be efficient for attracting euglossine male bees (Rebêlo & Garófalo, 1991; Sofia & Suzuki, 2004). We prepared the baits using a wad of paper soaked with only one fragrance, which was tied with a string and attached to the vegetation on

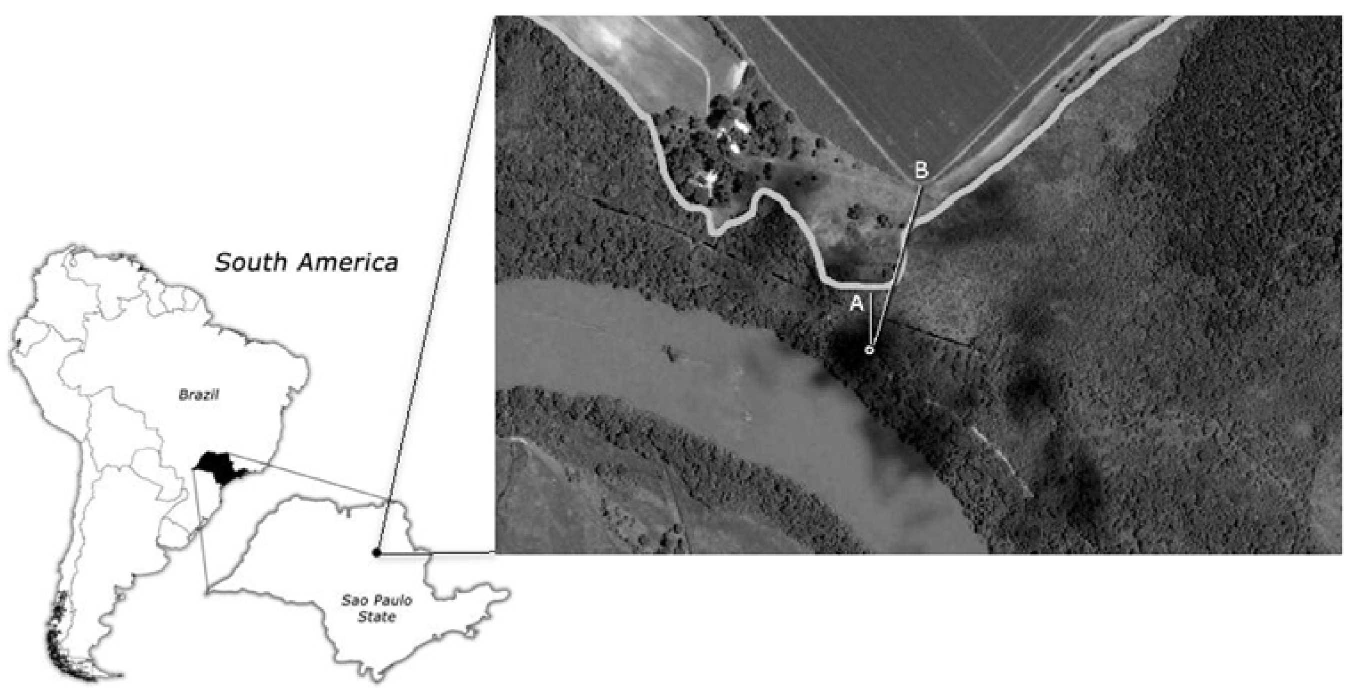

Fig 1. Location of the area where the Euglossini bees were sampled in Guatapará, São Paulo State, Brazil. The thicker gray line marks the edge of Reserva Toca da Paca natural vegetation. A: Distance of 80m from the sample point to the edge of Reserva Toca da Paca’s natural vegetation. B: Distance of 200m from the sample point to the edge the sugarcane plantation.

a shaded place 1.5m above the ground and 10m away from other odor baits.

Sampling was always carried out at the same sites on a transect and each sampling session lasted three hours, from 9:00am to 12:00pm. This interval corresponds to the period of highest activity of males in the field in northeastern São Paulo State (Rebêlo & Garófalo, 1991). At each half an hour we replaced the essences to compensate the loss of volatile compounds, as suggested by Sofia and Suzuki (2004).

We captured the bees attracted to the baits with an insect net and stored them individually in 10ml falcon tubes, where they were killed with ethyl acetate vapor released by a paper soaked with the substance and attached to the cap. The specimens were deposited in the Collection of Bees and Solitary Wasps at the Department of Biology, FFCLRP-USP.

Data analysis

Correlations between species richness, the number of individuals collected each month and average temperature and rainfall were made by Pearson coefficient (r), following Zar (1999). The software used for these calculations was program R version 2.15.2 (R Development Core Team, 2012).

To test the monthly distribution of the males activities in the range of one year, we used the Rayleigh test of uniformity (Z) and ran the calculations in the software Oriana 4.0 (Kovach Computing Services, 2012), in which months were converted to angles, beginning at 30º, which corresponds to January, and ending at 330º, which corresponds to December, in intervals of 30º. After the conversion, we calculated the mean date (a) of the capture frequency of males and the concentration (r) of this event around the mean angle. In the histogram, the vector indicates the mean angle, which corresponds to the mean date of occurrence of the event. The hypotheses were: H0= the males of each species are evenly distributed throughout the year and, hence, there is no temporal variation; H1= the males of each species are unevenly distributed throughout the year and, hence, there is temporal variation. In case H1 is true, i.e., ($P<0.05$), the concentration of male captures around the mean angle, denoted by (r), may be considered a measurement of temporal variation. According to Morellato et al. (2000), r=0 when the distribution is even throughout the year, and r=1 when the distribution is concentrated around a single month.

In discussion, to compare the Euglossini richness of RTP with other areas of the same region in northeastern São Paulo State, a rarefaction curve was made to equalize the differences in sampling effort. The diversity of bees were calculated by Shannon-Wiener index, and the values of the indices were compared using Hutcheson's t-test (Hutcheson, 1970). Uniformity and dominance were quantified following Pielou Index and Berger-Parker Index (Magurran, 2004) respectively. We also applied Sørensen similarity coefficient (Sørensen, 1948) to compare species composition among areas. Since this coefficient does not consider abundance, it reduces the chances of errors caused by studies with different sampling effort. All of the aforementioned statistical analyses were done using the software PAST 2.17c (Hammer et al., 2001).

Results

We collected 113 males of five species and three genera in the studied hygrophilous forest. Among the species studied, the most abundant was *Eulaema nigrita* Lepeletier, 1941, with 46% of all collected individuals, followed by *Euglossa pleosticta* Dressler, 1982 (30.1%), *Exaerete smaragdina* (Guérin-Méneville, 1844) (10.6%), *Euglossa carolina* Nemésio, 2009 (9.7%), and *Euglossa fimbriata* Rebêlo & Moure, 1968 (3.5%) (Table 1).

Among the four odor baits used, eucalyptol attracted males of all species and also the highest number of males (n=98). Except for *Eg. carolina*, and *Eg. fimbriata*, whose males were attracted exclusively to eucalyptol, the other species were attracted to at least two different kinds of odor baits (Table 1). Methyl salicylate did not attract any males.

During the study, the average monthly temperature varied from 18 to 26 ºC and monthly rainfall varied from 0 to 296 mm (Fig 2). We observed the highest rainfall and temperature values between November and March, when the highest number of specimens was sampled (Fig 2). Species richness showed a significant correlation with temperature (r=0.85; $p<0.01$) and rainfall (r=0.83; $p<0.01$); male abundance also showed a significant correlation with temperature (r=0.92; $p<0.01$) and rainfall (r=0.81; $p<0.01$).

Table 1. Number of Euglossini bees collected with Eucalyptol (EC), Vanillin (VA), Eugenol (EG) and Methyl salicylate (MS) in Reserva Toca da Paca, Guatapará, Brazil, from March 2010 to February 2011.

Species	N	%	Aromatic baits			
			EC	VA	EG	MS
Eulaema nigrita	52	46	47	5	0	0
Euglossa pleosticta	34	30.1	29	2	3	0
Exaerete smaragdina	12	10.6	7	4	1	0
Euglossa carolina	11	9.7	11	0	0	0
Euglossa fimbriata	4	3.5	4	0	0	0
Abundance	113	100	98	11	4	0
Richness	5	-	5	3	2	0

The Rayleigh test indicated a temporal variation in the occurrence of bees in RTP (r=0.50; Z=28.35; $P<0.01$) (Fig 3A), with concentration of individuals around January. We also observed temporal variation in different species separately, except for *Eg. fimbriata* (r=0.69; Z=1.91; P=1.15) (Fig 3C), which was not sampled in December; this species, though, has a marked occurrence period between October and February. *Euglossa carolina* (Fig 3B) and *Eg. pleosticta* (Fig 3D) showed activity peaks in January, and *El. nigrita* (Fig 3E) was the only species sampled throughout the year, with a peak in December (Table 2 and Fig 3E). *El. nigrita*, as well as the other species, had no males attracted to the baits in August.

Discussion

The euglossine bees sampled in the studied fragment of hygrophilous forest have also been observed in other environments of northeastern São Paulo State, Brazil. (Table 3). This similarity in bees species occurrence may be explained by the fact that the hygrophilous forest is composed of plant species that are also found in other vegetation types that occur in this region, such as the semi-deciduous forest and the cerrado. In addition, the geographic closeness and the relative similar altitudes of the areas in the northeastern São Paulo State region may influence the share of the species composition of this tribe of bees, as has been observed in other studies (Brosi, 2009; Cordeiro et al., 2013).

In contrast, species richness, expressed by the rarefaction curves (Fig 4), indicates a lower number of species than observed in other areas of northeastern São Paulo State. This reflects in the Sørensen similarity coefficient presented by cluster analysis in which the RTP is the least similar to other areas of same region (Fig 5).

The Euglossini richness in areas of semi-deciduous forest varies from eight to 14 species (Rebêlo & Garófalo, 1997; Jesus & Garófalo, 2000). Also in a semi-deciduous forest, but very close to the ecotone with cerrado, Silveira et al. (2011) sampled 13 species, whereas Hirotsu et al. (2010) in a cerrado and Rebêlo and Garófalo (1991) in a second-growth area (capoeira) sampled eight species each. The difference in Euglossini richness found between RTP and other areas studied by Rebêlo and Garófalo (1991, 1997) and Jesus and Garófalo (2000) may be due to the size of the areas studied, since the fragments studied by these authors were significantly larger than RTP. Another reason for the smaller number of species observed in the present study, compared to the one made by Silveira et al. (2011), could be a difference in sampling effort, which in this latter were analyzed two simultaneous sampling points against one of the RTP. Storck-Tonon et al. (2013) reported that those differences is also caused by differences in the index of edge in the fragment, the connectivity and the landscape structure, or as observed by Milet-Pinheiro and Schlindwein (2005) affected by the surrounding matrix of the studied area.

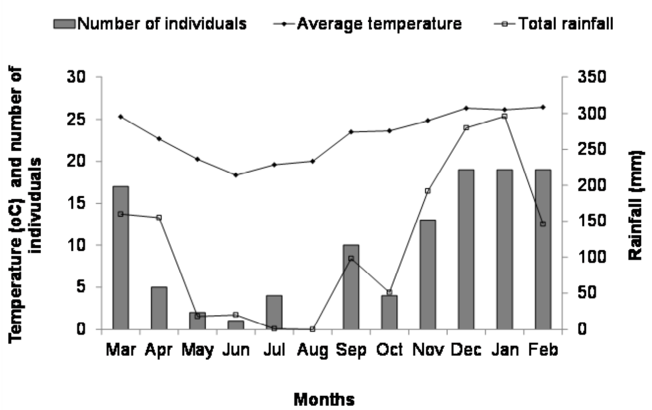

Fig 2. Monthly rainfall (mm), average temperature (°C), and number of Euglossini males collected each month in Reserva Toca da Paca, Guatapará, Brazil. From March 2010 to February 2011.

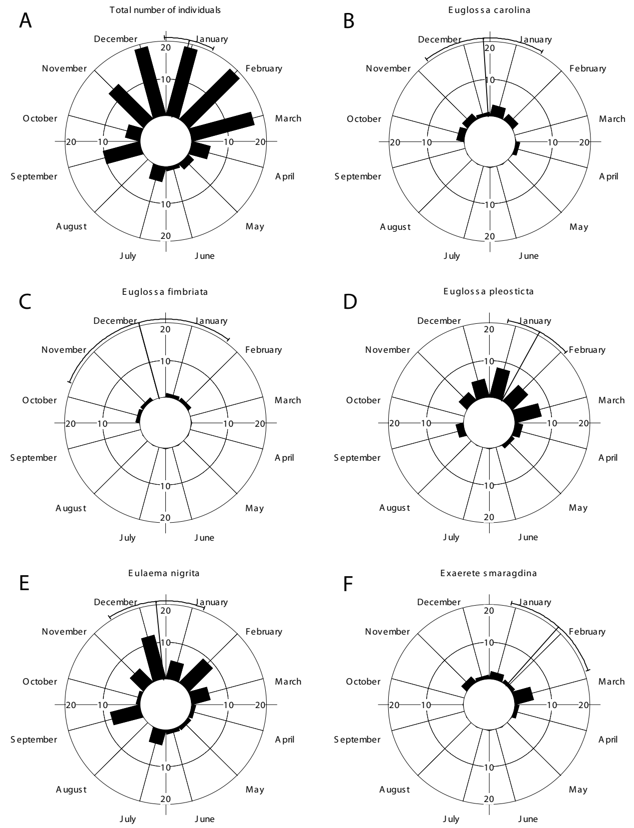

Fig 3. Temporal variation of the total abundance and for each Euglossini species sampled in Reserva Toca da Paca, Guatapará, Brazil, from March 2010 to February 2011. The line at the top of the vector stands for standard deviation. Except for *Eg. fimbriata* with P=0.151, the remaining species presented P<0.01. The test values corresponding to these distributions are shown in Table 2.

The abundance of Euglossini bees in the RTP was also lower than other studied areas in the region, except the area studied by Hirotsu et al. (2010) (Table 3). *Eulaema nigrita* and *Eg. pleostica* were by far the most frequent species through-

Table 2. Results of circular statistic analyses testing for the occurrence of temporal variation in the abundance of Euglossini species sampled in Reserva Toca da Paca, Guatapará, Brazil, from March 2010 to February 2011. Rayleigh test was performed for significance of the mean angle.

	Eulaema nigrita	*Euglossa pleoscticta*	*Exaerete smaragdina*	*Euglossa carolina*	*Euglossa fimbriata*	Total number of individuals
Number of males (n)	52	34	12	11	4	113
Mean angle	354.43°	28.62°	41.45°	356.31°	345°	12.47°
Mean date (*a*)	December	January	February	December	December	January
Concentration (*r*)	0.39	0.64	0.68	0.64	0.69	0.50
Rayleigh test (Z)	7.95	14.08	5.56	4.51	1.90	28.35
Rayleigh test (*P*)	<0.01	<0.01	<0.01	<0.01	0.151	< 0.01

Table 3. Number of Euglossini bees sampled in areas of the northeastern São Paulo State.

Species	Areas in northeastern São Paulo State					
	Gua[1]	Sta	Ser	Mat	Pat	Caj
Eufriesea auriceps (Friese)	-	-	-	-	1	-
Eufriesea violacea (Blanchard)	-	-	24	310	75	19
Euglossa annectans Dressler	-	8	4	27	-	12
Euglossa cordata (Linnaeus)	11	3	5	3	6	2
Euglossa despecta Moure	-	-	-	-	-	-
Euglossa fimbriata Rebêlo & Moure	4	2	51	30	55	232
Euglossa imperialis Cockerell	-	4	-	-	37	2
Euglossa leucotricha Rebêlo & Moure	-	-	-	-	1	1
Euglossa melanotricha Moure	-	-	1	-	15	18
Euglossa pleosticta Dressler	34	27	533	363	392	245
Euglossa securigera Dressler	-	5	-	1	-	1
Euglossa stellfeldi Moure	-	-	-	-	65	-
Euglossa townsendi Cockerell	-	-	1	-	-	2
Euglossa truncata Rebêlo & Moure	-	3	18	12	29	81
Euglossa violaceifrons Rebêlo & Moure	-	-	-	1	-	1
Eulaema nigrita Lepeletier	52	12	83	66	181	251
Exaerete dentata (Linnaeus)	-	-	-	-	1	-
Exaerete smaragdina (Guérin)	12	-	16	1	1	39
Abundance	113	64	736	814	859	906
Richness	5	8	10	10	13	14
Diversity (H')	1.30	1.71	1.03	1.27	1.66	1.69
Uniformity (J')	0.81	0.82	0.45	0.55	0.65	0.64
Dominance	0.46	0.42	0.72	0.45	0.46	0.28

Gua=Guatapará (present study), Sta=Santa Rita do Passa Quatro (Hirotsu et al., 2010), Ser=Sertãzinho (Rebêlo & Garófalo, 1997), Mat=Matão (Jesus & Garófalo, 2000), Pat=Patrocínio Paulista (Silveira et al., 2011), Caj=Cajuru (Rebêlo & Garófalo, 1991, 1997).
[1]*Eg. cordata* is treated as *Eg. carolina* by Nemésio, 2009.

out the year and also the most abundant species, representing together 76% of all individuals collected in the present study. This was expected, since these species were also the most common in all other studies carried out in the same region of São Paulo (Rebêlo & Garófalo, 1991, 1997; Jesus & Garófalo, 2000; Hirotsu et al., 2010; Silveira et al., 2011). However, while in RTP and the area studied by Rebêlo and Garófalo (1997) *El. nigrita* was the most abundant bee, being responsible for a dominance of 0.46 and 0.27 respectively, in the other studied areas *Eg. pleosticta* was the most numerous species, reaching a 0.72 dominance in Rebêlo and Garófalo (1997) studied area (Table 3). The 1.302 diversity index of RTP was very similar to that obtained by Jesus and Garófalo (2000), not been significantly different ($t=0.216$; $p>0.05$) and only higher than the one presented by Rebêlo and Garófalo (1997), which was influenced by its high dominance value (Table 3).

Eucalyptol, or 1,8-cineol, was the most attractive bait for Euglossini males in RTP, which corroborates the efficiency of that compound as attractive scent bait for a large number of species and individuals, as emphasized by Dressler (1982) and observed in other studies carried out in several neotropical regions (e.g., Janzen et al., 1982; Ackerman, 1983; Pearson & Dressler, 1985; Rebêlo & Garófalo, 1991, 1997; Cordeiro et al. 2013; Rocha-Filho & Garófalo, 2013). As reported by Alvarenga et al. (2007) and Neves and Viana (1999) in their study areas, methyl salicylate was also not attractive for males in the RTP.

Despite the significant efficiency of odor baits for the study of Euglossini communities, two species that nested in the study area, *Euglossa townsendi* Cockerell, 1904 and *Euglossa truncata* Rebêlo & Moure, 1996, were not sampled with the baits used in the present study (Castro, personal observation). Similar observations were reported by Rebêlo and Garófalo (1991), who found nests of *Eufriesea auriceps* Friese, 1899 and *Eg. townsendi* in semi-deciduous forests, but did not collect males of these species with odor baits. Recently, Knoll and Penatti (2012) reported that six fragrances used as odor baits, for 77 months, did not attract *Eg. townsendi* males, which were captured only on flowers. These observations reveal a lack of attraction of males of those species by the baits used in those studies or, in the case of *Eg. townsendi*, a weak association, because even when they were attracted by the baits they were always in small numbers (Janzen et al., 1982; Ackerman, 1989; Rebêlo & Garófalo, 1997; Nemésio & Silveira, 2007; Abrahamczyk et al., 2011; Silva, 2012). Such occurrences show the importance of using alternative methods to sample euglossine bees, such as trap nests (Garófalo et al., 1993) and direct collection on flowers

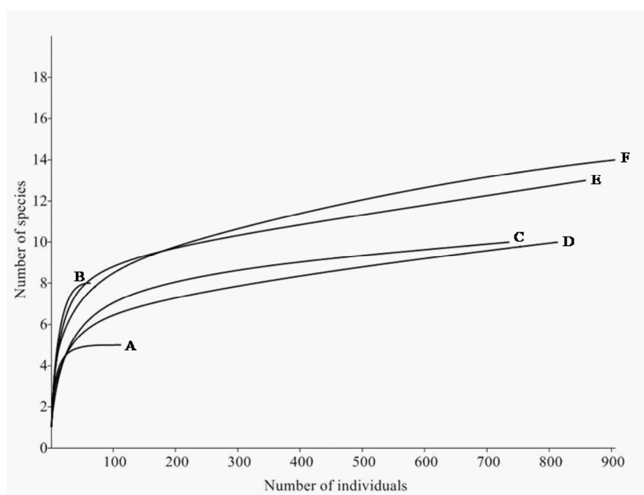

Fig 4. Rarefaction curve for Euglossini species richness in areas of northeastern São Paulo State, Brazil. A- Guatapará (present study), B- Santa Rita do Passa Quatro (Hirotsu et al. 2010), C- Sertãozinho (Rebêlo & Garófalo, 1997), D- Matão (Jesus & Garófalo 2000), E- Patrocínio Paulista (Silveira et al. 2011), F- Cajuru (Rebêlo & Garófalo, 1991, 1997).

with insect nets (Rocha-Filho et al., 2012), in order to obtain representative samples of the species composition. Rebêlo (2001) pointed out that males have different preferences for aromatic fragrances depending on locality and season.

Regarding temporal variation in bee abundance, in the present study, temperature and rainfall affected significantly the distribution of males throughout the year. Both the number of species and number of individuals were higher in the warm and rainy months with activity peaks varying among species. These results are consistent with other studies carried out in different ecosystems (e.g., Rebêlo & Garófalo, 1991, 1997; Rebêlo & Cabral, 1997; Ramalho et al., 2009; Cordeiro et al., 2013; Silva, 2012) suggesting that the peaks in number of individuals and species are probably related to a higher availability of floral resources (Rebêlo & Garófalo, 1997), since the flowering in tropical environments is cued by the rains and many of the orchid bees may also use these rains as a cue

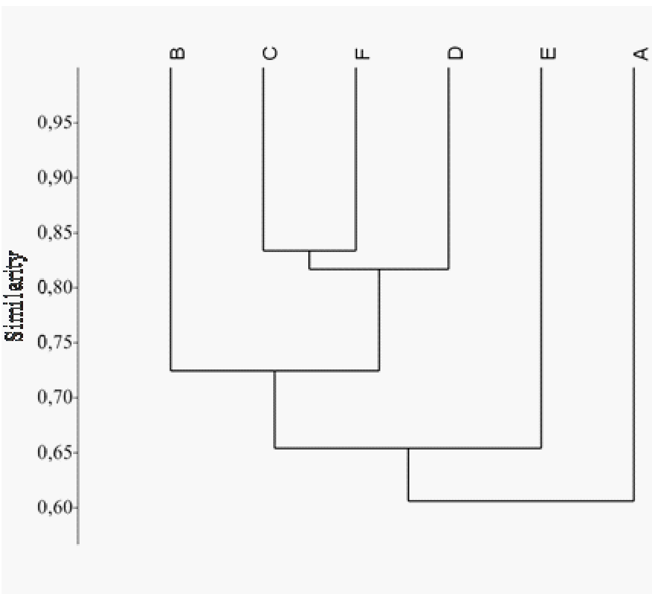

Fig 5. Similarity dendogram based in Sørensen coefficient between areas of northeastern São Paulo State, Brazil. A- Guatapará (present study), B- Santa Rita do Passa Quatro (Hirotsu et al. 2010), C- Sertãozinho (Rebêlo & Garófalo, 1997), D- Matão (Jesus & Garófalo 2000), E- Patrocínio Paulista (Silveira et al. 2011), F- Cajuru (Rebêlo & Garófalo, 1991, 1997).

for emergence (Pearson & Dressler, 1985). However, individual analyzes of the most abundant species in a community may show temporal variations different from that observed in RTP. In Peru, Pearson and Dressler (1985) observed the occurrence of two peaks in the activities of males, a major peak at the end of the dry season and beginning of the wet season, and another minor peak at the end of the wet season and into the dry season. According to those authors, smaller orchid bee species predominate in the hot/dry season. Large and very large species were virtually aseasonal in abundance, while medium species were relatively more common during the cool/dry season. Two peaks of abundance were also reported by Aguiar and Gaglianone (2008) working in remnants of lowland forest on tertiary tabuleiro in the north Rio de Janeiro State, Brazil. The abundance peak in the hot/rainy season was strongly influenced by *El. nigrita* and that in the cool/dry season was influenced by *Eg. cordata*.

The observed low abundance and richness of Euglossini in the hydrophilious forest in relation to those found in other areas of the same geographic region cannot be interpreted only as a feature of this type of vegetation. The similarity in species composition with other regional vegetation types contrasts with the very low number of individuals sampled and suggests a strong effect of area and spatial isolation by sugarcane culture and deforestation. Other studies should be carried out in other hygrophilous forests areas in order to increase the species list and also to advance the knowledge about community composition of these bees in this kind of vegetation, which is still poorly known.

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