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The Orchid Bee Communities in different phytophysiognomies in the Atlantic Forest: from lowland to montane rainforests

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Abstract

To investigate species composition, diversity, richness, and monthly abundance of orchid bees in different types of dense rainforest in different altitudes, the orchid bees were collected for 11 months in three vegetation types: dense montane ombrophilous forest (DMOF), dense submontane ombrophilous forest (DSOF_1 and DSOF_2 in different altitudes), and dense lowland ombrophilous forest (DLOF). Using aromatic traps 1,945 males of 20 species were collected. The DMOF showed the highest species richness (19), and diversity (H' = 2.42), whereas the DSOF_1 showed the highest abundance (775). The DLOF which is the largest continuous forest remnant sampled (15,300 ha), showed the lowest species richness (11) and diversity index (1.63), additionally presented the highest dominance (0,49). *Euglossa cordata* (Linnaeus) was the most abundant species in DLOF, DSOF_1 and DMOF, while *Euglossa ignita* Smith was the most abundant species in the DSOF_2. Abiotic factors (temperature and humidity) were correlated with abundance of species; already the altitude had correlation with dominance and diversity parameters. Our results demonstrate that phytophysiognomy influences the richness and diversity of orchid bees. The results obtained in DLOF did not corroborate what was expected, we attributed these results to the management and extractivism activities of the palm tree *Attalea funifera* Martius. Finally, our study reveals the importance of preserving different phytophysiognomies within the same biome to conserve orchid bees.

Introduction

Euglossini bees, also known as orchid bees, have high biological relevance as pollinators of native and some cultivated plants (Ramírez et al., 2002; Roubik & Hanson, 2004; Singer, 2004; Farias et al., 2007). Several studies as Tonhasca et al. (2002a), Ramalho et al. (2009), Rosa et al. (2015) and Aguiar et al. (2015) have reported the sensitivity of these bees to environmental degradation. For this reason, some species have been suggested as bioindicators of habitat quality, such as *Euglossa marianae* Nemésio, *Euglossa iopoecila* Dressler, and *Euglossa imperialis* Cockerell (Tonhasca et al., 2002; Nemésio 2009; Ramalho et al., 2009)

Orchid bees are diverse in tropical rainforests. The Atlantic Forest is the second richest biome in orchid bee species in Brazil, with over 60 species recorded. Among those species, 27 are endemic (Nemésio & Silveira, 2007; Nemésio, 2009; Moure et al., 2012). The environmental heterogeneity of this forest, together with climatic variations, contribute to the high species diversity and endemism (Silva & Casteleti, 2003; Galindo-Leal & Câmara, 2003). However, this high biodiversity for the orchid bees is susceptible to the effects of habitat fragmentation (Brosi et al., 2007; Brosi, 2009; Aguiar & Gaglianone, 2012; Aguiar et al., 2015). In addition to these factors, the differences in climate and local phytophysionomic features can interfere in the distribution patterns of the orchid bees (Roubik & Hanson, 2004). According to Nemésio and Silveira (2007), differences in the composition species in the Atlantic Forest domain may be associated with floristic differences between Dense and Semi-deciduous

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Ombrophylous Forests. The altitude is another factor that has shown a strong influence on the geographic distribution of these bees (Ramirez et al., 2002).

In the Brazilian Atlantic forest, three regions are considered to be biodiversity corridors: northeastern Brazil (the Pernambuco Corridor), southern Bahia and Espírito Santo State (the Central Corridor), and the Serra do Mar Corridor (Fonseca et al., 2004; Galindo-Leal & Câmara, 2005). One of the last remnants of Atlantic Forest in the Central Corridor lies in southern Bahia (Franke et al., 2005; Saatchi et al., 2001). The region is known as an important endemism area for several taxonomic groups, and has been considered one of the most important in the Atlantic Forest (Franke et al., 2005; Nemésio, 2013a). The orchid bee fauna of the Central Atlantic Forest Corridor was studied in the southern portion of Bahia (Nemésio, 2013a; Nemésio, 2013b; Nemésio, 2013c; Nemésio, 2014) and in a region near to our study area (Rosa et al., 2015). However, large variations have been observed in orchid bee communities, even in neighboring regions, which could be related to habitat heterogeneity (Tonhasca et al., 2002b).

The objective of the present study was to investigate species composition, diversity, richness, and monthly abundance of orchid bees in different types of dense rainforest in different altitudes.

Material and Methods

Study area

The present study was carried out in Atlantic Forest remnants located in the Pratigi Environmental Protection Area, southern Bahia, northeastern Brazil. The reserve has an area of approximately 85,686 ha, covered by three vegetation types: montane, submontane, and lowland rainforests, at different conservation status (Lopes et al., 2011). The annual rainfall is of approximately 2,000 mm; the average relative humidity varies between 80% and 90%; and the average temperature, between 21 ºC and 25 ºC (Fischer, 2007). For the inventory of Euglossini bees, four sampling sites were selected. Each sampling site had two sampling points, 500 m apart from one another. Data sampled in each of these sampling-point pairs were lumped in a single sample. The site covered by dense lowland ombrophilous forest (DLOF, 13º41'07" S; 39º05'18" W) is located at 70 m a.s.l. It is characterized as a transition zone from dense rainforest to sandbank "(restinga)" and mangrove. The first area of dense submontane ombrophilous forest (DSOF_1, 13º50'36" S; 39º17'21" W) is located at 250 m a.s.l. It is an agroforestry system producing cacao, clove, and peach-palm, and it is also a rubber tapping pole. The second area of dense submontane ombrophilous forest (DSOF_2, 13º54'50" S; 39º27'24" W) is located at 470 m a.s.l. In this sampling site, the hills form mountain ranges interconnected to one another by ecological microcorridors. The sampling point in the vegetation type by dense montane ombrophilous forest (DMOF) is located around 2,5 kilometers nearby DSOF_2, but the sampling site was defined (13º53'52" S, 39º27'45" W) at a higher altitude (690 m a.s.l.).

Sampling and taxonomic identification

The males of the orchid bees were sampled monthly, from July 2012 to May 2013, from 09:00 a.m. to 3 p.m. Strong rains hindered sampling in April 2013, when only DSOF_2 and DMOF were sampled. These bees were captured when attracted by seven aromatic baits (eugenol, methyl cinnamate, vanillin, eucalyptol, benzyl acetate, methyl salicylate, and beta-ionone). Attractive baits were imbibed in cotton ball inside traps placed at 1.5 m above the ground with a minimum distance of 2 m between baits (Aguiar et al., 2014). The captured specimens were deposited in the Entomological Collection of the Laboratory of Environmental Studies (Laboratório de Estudos Ambientais - LEA) of the Universidade Estadual de Feira de Santana.

Digital thermo-hygrometer was employed to measure humidity and temperature, and remained in the sampling sites during the entire study, recording daily minimum and maximum values throughout the sampling period. Daily rainfall data were obtained from the Estação Meteorológica do Vale Juliana.

Data analysis

The Shannon species diversity index (H') was calculated, using the formula $H' = -\Sigma (p_i)(\log_2 p_i)$, where, p_i = proportion of individuals of species *i* and $\log_2 p_i$ = logarithm base 2 of p_i (Magurran, 2003).

The Berger-Parker index was calculated to species dominance using the formula $d = N_{max}/N_{total}$, where: N_{max} is the number of individuals of the most abundant species and N_{total} is the total of individuals sampled. Evenness (J') was calculated with the Pielou index (Magurran, 2003): $J' = H'/\log_2 S$, where: J' = Pielou evenness index, H' = Shannon diversity index, and $\log_2 S$ = logarithm base 2 of the species richness. To evaluate the similarity between areas, was applied the Morisita Index, which analyzes abundance data of the species in the community.

To test for relationships between community parameters (abundance, richness, diversity, and dominance) and environmental variables (temperature, humidity, rainfall, and altitude), Spearman linear correlations were performed. The rarefaction curve was estimated with 1,000 randomizations, following Magurran (2003). All analyses were carried out in the program Past 1.91 (Hammer et al., 2001).

Bee identification was based on taxonomical keys and other studies (Nemésio, 2009; Nemésio & Engel, 2012; Hinojosa-Diaz et al., 2012; Faria Jr & Melo, 2012), and later confirmed by Dr. Gabriel A. R. Melo (Universidade Federal do Paraná).

Results

In the four sites we captured 1,945 male orchid bees of 20 species. The site with the highest abundance was a submontane forest area (DSOF_1) with 775 individuals of 17 species. DMOF showed the highest species richness (19 species). Species diversity (H') in the four areas varied from 1.63 (DLOF) to 2.41 (DMOF). Evenness (J') varied from 0.68 to 0.82 (Table 1). The species rarefaction curves built for the four study sites (Fig 1) suggest that sampling was complete in all areas, as the curve tended to stabilization between 200 and 300 individuals (Fig 1).

The composition of species among the sites sampled showed important differences, for example: *Euglossa amazonica* Dressler, *E. clausi* Nemésio and Engel, *E. imperialis* Cockerell, *E. marianae* Nemésio, and *E. iopoecila* Dressler were not registered in the DLOF. On the other hand, *Euglossa pepei* Nemésio and Engel occurred only in DSOF_1 and DMOF, and *E. botocuda* Faria and Melo sampling only in the DSOF_2 and DMOF, whereas *E. ioprosopa* Dressler was exclusive to the DMOF (Table 1). The Morisita index showed two clusters (70% similarity), one corresponding to the DSOF_2 and DMOF with 88% similarity and the other formed by DSOF_1 and DLOF with similarity of 95% (Fig 2).

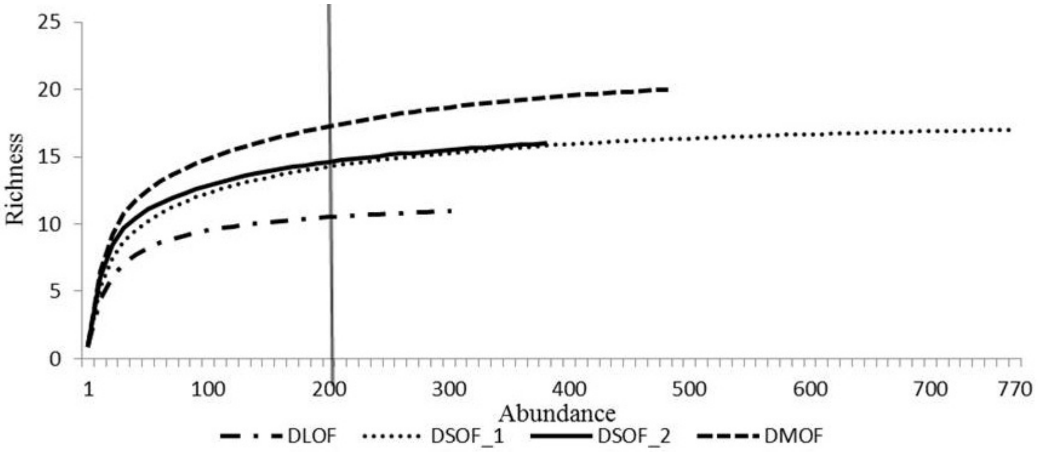

Fig 1. Rarefaction curves for four areas in the Brazilian Atlantic forest. DLOF: dense lowland ombrophilous forest, DSOF_1: dense submontane ombrophilous forest, area 1, DSOF_2: dense submontane ombrophilous forest, area 2, DMOF: dense montane ombrophilous forest.

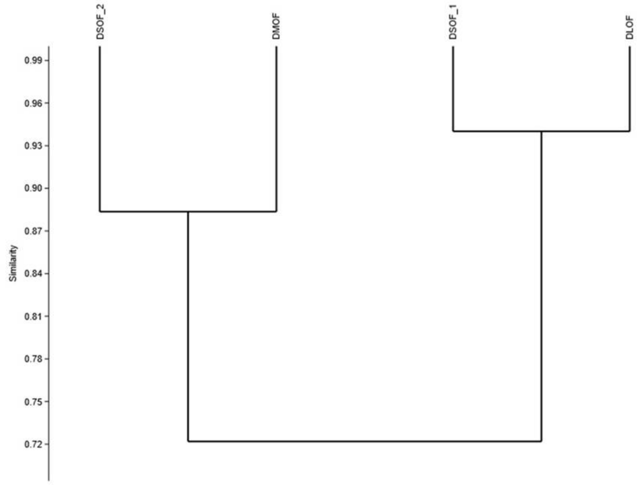

Fig 2. Similarity index (Jaccard) for species composition of four areas in the Brazilian Atlantic forest. DLOF: dense lowland ombrophilous forest), DSOF_1: dense submontane ombrophilous forest, area 1, DSOF_2: dense submontane ombrophilous forest, area 2, DMOF: dense montane ombrophilous forest.

The dominant species showed different abundance distribution patterns in the four areas. *Euglossa cordata* (Linnaeus) was dominant in all areas except for the DSOF_2, where *E. ignita* Smith was the most abundant species. *Euglossa milenae* Bembé was the second most abundant species in the DSOF_2 and DMOF (Table 1, Fig 3).

The highest peaks of abundance occurred in the period of moderate rainfall, from November to January (Fig 4). The monthly variation in Euglossini abundance was mainly influenced by the two most abundant species, *E. cordata* and *E. ignita*.

The abiotic variables (temperature, humidity, rainfall, and altitude) had no influence on species richness ($p > 0.05$). On the other hand, abundance was slightly influenced by temperature ($r = 0.35$; $p = 0.03$), slightly negatively influenced by humidity ($r = -0.40$; $p = 0.02$) and not influenced by rainfall and altitude ($p > 0.05$). Diversity and dominance were only affected by altitude ($r = 0.36$; $p = 0.03$; and $r = -0.37$; $p = 0.03$, respectively).

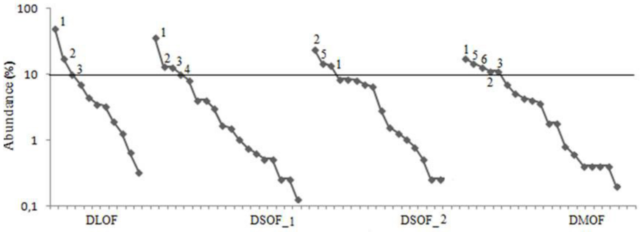

Fig 3. Rank abundance plot for Euglossini species in four areas in the Brazilian Atlantic forest. DLOF: dense lowland ombrophilous forest), DSOF_1: dense submontane ombrophilous forest, area 1, DSOF_2: dense submontane ombrophilous forest, area 2, DMOF: dense montane ombrophilous forest. 1: *Euglossa cordata* 2: *Euglossa ignita* 3: *Eulaema atleticana* 4: *Euglossa imperialis*; 5: *Euglossa milenae*; 6: *Eulaema nigrita.*

Table 1. Species composition, abundance, richness, diversity and evenness in Euglossini bee communities in different phytophysiognomies of the Atlantic Forest in southern Bahia, Brazil. DLOF: dense lowland ombrophilous forest), DSOF_1: dense submontane ombrophilous forest, area 1, DSOF_2: dense submontane ombrophilous forest, area 2, DMOF: dense montane ombrophilous forest.

Species	DLOF	DSOF_1	DSOF_2	DMOF	Total
Euglossa amazonica Dressler	-	5	1	2	8
Euglossa augaspis Dressler	1	-	2	2	5
Euglossa cordata (Linnaeus)	151	287	52	85	575
Euglossa clausi Nemésio & Engel	-	8	6	23	37
Euglossa despecta Moure	11	32	5	18	66
Euglossa ignita Smith	54	102	93	55	304
Euglossa imperialis Cockerell	-	79	27	35	141
Euglossa iopoecila Dressler	-	4	4	20	28
Euglossa ioprosopa Dressler	-	-	-	2	2
Euglossa botocuda Faria & Melo	-	-	1	4	5
Euglossa liopoda Dressler	6	8	3	2	19
Euglossa marianae Nemésio	-	12	11	3	26
Euglossa milenae Bembé	22	63	56	71	212
Euglossa pepei Nemésio & Engel	-	1	-	1	2
Euglossa securigera Dressler	2	2	-	9	13
Eulaema atleticana Nemésio	31	99	32	55	217
Eulaema cingulata (Fabricius)	10	32	31	25	98
Eulaema nigrita Lepeletier	14	24	32	62	132
Eulaema niveofasciata (Friese)	4	13	25	9	51
Exaerete sp.	-	4	-	-	4
Total abundance	306	775	381	484	1.945
Species richness	11	17	16	19	20
Diversity (H')	1.63	2.04	2.25	2.41	2.22
Dominance (D)	0.49	0.37	0.24	0.18	0.30
Evenness (J')	0.68	0.72	0.81	0.82	0.74

Discussion

The orchid bee richness recorded in the Pratigi Environmental Protection Area was similar to that in an area within the Atlantic Forest, in the Serra do Conduru State Park, where 22 species were reported (Nemésio, 2011a); and in the Michelin Ecological Reserve, where 21 species were registered, both localized in the southern Bahia, Brazil (Rosa et al., 2015) However, the species richness found was lower than that reported for the Monte Pascoal National Park, located 360 km away from the Pratigi Environmental Protection Area (36 species) (Nemésio, 2013b). According to Souza et al. (2005), variation in of orchid-bee richness can be influenced by differences factors, including in collection method, types of fragrances used and the sampling effort. Additionality,

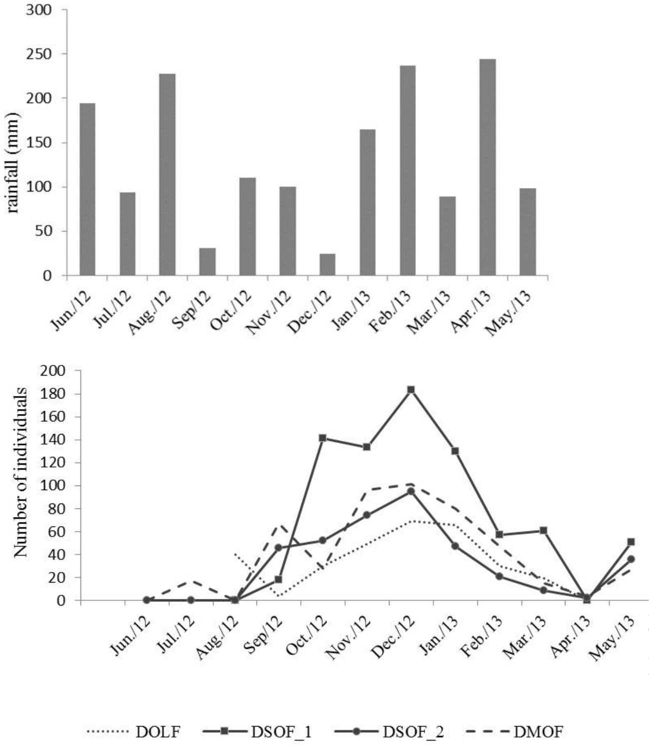

Fig 4. Rainfall (a) and monthly fluctuations in the abundance of Euglossini bees (b) in four areas in the Brazilian Atlantic forest, from June 2012 to May 2013. DLOF: dense lowland ombrophilous forest), DSOF_1: dense submontane ombrophilous forest, area 1, DSOF_2: dense submontane ombrophilous forest, area 2, DMOF: dense montane ombrophilous forest.

studies suggest that differences in local floristic composition, resource availability, and habitat heterogeneity may influence richness, abundance, and species composition of orchid bees (Armbruster, 1993; Souza et al., 2005; Tonhasca et al., 2002b).

Considering our data observed in the four sampling sites located in the Atlantic forest, from lowland to montane rainforests it demonstrated differences in their orchid bee community. Differences related to phyto physiognomy influenced species richness and diversity. Variations in species richness can also reflect differences in floristic composition and resource availability for feeding, nesting and reproduction, since *Euglossa* species, for example, require resin and preexisting cavities for nesting (Roubik & Hanson, 2004). The dense montane ombrophilous forest (DMOF) showed the highest richness and diversity. On the other hand, the dense lowland rainforest (DLOF), which is the largest continuous forest remnant sampled (15,300 ha), showed the lowest species richness and a lower diversity index than the other areas. In this lowland rainforest, there is extractivism of the palm tree *Attalea funifera* Martius (piassava), an endemic plant used in handicraft (Barreto, 2009). The habitat underwent some changes resulting from this extractive activity, as there is the removal of surrounding plants to favor the palm tree growth (R.L.S. Medeiros, personal observation). This practice apparently contributes to the lower plant diversity in the DLOF area, resulting in a less heterogeneous habitat. In this case, our study shows that the low abundance, richness and diversity of species seems to be more related to the conservation status of the DLOF area than to the phytophysiognomic type and altitude, however, new efforts must be applied to prove this perspective. An important aspect to be considered is that some Euglossini species are sensitive to environmental changes (Powell & Powell, 1987), which seems to be the case of *E. marianae* (= *Euglossa analis* Westood; see Nemésio, 2011a), absent in the lowland rainforest (DLOF) and present in the other areas. This species is considered a bioindicator of habitat quality due to its sensitivity to environmental change (Tonhasca et al., 2002; Ramalho et al., 2009)**.** *Euglossa amazonica, E. clausi*, and *E. imperialis* were not recorded in the DLOF. In spite of being recorded in the other three areas, *E. amazonica* was not an abundant species, as other authors have already pointed out (Nemésio, 2011b, 2013a, b; Pires et al., 2013, mentioned as *Euglossa aratingae* Nemésio). According to Nemésio (2009), this species has a broad distribution, but it is rarely sampled. *Euglossa clausi* has been sampled in well preserved areas in the Atlantic Forest (mentioned as *Euglossa sapphirina* Moure) (Tonhasca et al., 2002a; Nemésio & Silveira, 2006; Ramalho et al., 2009; Nemesio, 2013b). Its absence in the DLOF can be related to the conservation status of this area, . Alternatively, its abundance can be locally low, and therefore, this species may not have been sampled. It was recorded in low frequency in other areas, as in the Michelin Ecological Reserve, approximately 25 km away (Ramalho et al., 2013), which is included in the Pratigi Environmental Protection Area. *Euglossa imperialis* showed expressive abundance in three of our four study areas. Rosa et al. (2015) suggested that *E. imperialis* is sensitive to changes in forest cover. Our results corroborate Rosa et al. (2015), as the abundance of this species in the lowland rainforest area seems to be related to the conservation status of the area.

The high abundance of *E. cordata* in the DLOF was responsible for the low local evenness and high dominance (Berger-Parker index). Studies carried out in other Atlantic forest areas also pointed out to the dominance of this species (Aguiar & Gaglianone, 2008, 2012; Ramalho et al., 2009; Ramalho et al., 2013; Nemésio, 2013a, 2013b). *Euglossa cordata* has been frequently considered a bioindicator of environmental disturbances, as this species if favored by dry and/or perturbed environments (Peruquetti et al., 1999; Silva & Rebelo, 2002; Aguiar & Gaglianone, 2008; Rocha-Filho & Garófalo, 2013). However, *E. cordata* has also been recorded in well preserved areas (Tonhasca et al., 2002a; Ramalho et al., 2009, Aguiar & Gaglianone, 2008, 2012; Nemésio, 2013a, 2013b), as observed in our study. Hence, our data, like those of Ramalho et al. (2009) and Aguiar et al. (2014), suggest that *E. cordata* occurs in high abundance in areas at different conservation status.

The two clusters of similarity (70%) for the orchid bee community are apparently related to the altitude and phytophysiognomic type, since it the occurrence of some elements of the Euglossini fauna varied among the areas. *Euglossa ioprosopa* Dressler was exclusive to the montane rainforest (DMOF). This species occurs in the Amazon and Atlantic Forest (Nemésio & Silveira, 2007), and it has been associated with well-preserved environments (Nemésio, 2009; Ramalho et al., 2009; Rocha-Filho & Garófalo, 2013). The highest richness and diversity at DMOF suggests that this area has a better conservation status than the other areas analyzed.

We observed two abundance peaks, one in the period of lowest rainfall, which was influenced mainly by *E. cordata* and *E. ignita*, and another in the beginning of the rainy season, influenced by *E. cordata.* The trends in monthly variation in abundance found in the studied areas were similar to those found by Aguiar & Gaglianone, (2008) and Aguiar et al. (2014) in the semideciduous seasonal forest and dense ombrophilous forest in the Rio de Janeiro state, in the region with altitudes between 40 and 1000 m a.s.l . These authors observed two abundance peaks, one in the dry season and another in the rainy season in Atlantic Forest areas. Rebelo and Cabral (1997) made similar reports for the Brazilian savanna, and Oliveira (1999) for the Amazon forest. Orchid bees abundance peaks in the rainy season have been recorded in several types of vegetation, such as Atlantic forest (Rebêlo & Garófalo, 1997; Ramalho et al., 2009), savanna (Brito & Rego, 2001), sandbank coastal vegetation (“restinga”) (Viana et al., 2002; Silva et al., 2009), and xerophilous vegetation (“caatinga”) (Andrade-Silva et al., 2012).

Abiotic variables did not influence species richness, but temperature influenced abundance. Other studies in the Atlantic Forest (Bezerra & Martins, 2001) and savanna (Carvalho et al., 2006; Mendes et al., 2008) also reported a positive correlation between abundance and temperature. The correlation between Euglossini abundance and relative humidity is controversial. As in this study, Carvalho et al. (2006) observed higher activity of orchid bees in lower air relative humidity in Atlantic Forest areas. However, other studies reported higher abundance of orchid bees in periods of higher air relative humidity in the Atlantic Forest (Aguiar & Gaglianone, 2012; Andrade-Silva et al., 2012) and savanna (Mendes et al., 2008). This suggests that air relative humidity alone does not explain orchid bees abundance. Nemésio and Silveira, (2006) argued that, variations in the abundance of orchid bees may represent responses to small changes in light incidence, temperature and humidity, and other variables difficult to measure, including the dispersal of scents inside the forest. In the case of the studied areas, the humidity is relatively high throughout the year (80-90%), with little variation due to the high rainfall rates in the region (2000 mm) (Fisher, 2007), so other factors such as specific periods of nesting and availability of resources should control the patterns of abundance and occurrence of the species. Apart from phytophysiognomic and climate factors, altitude variation is among the main factors that influence the geographic distribution of orchid bees (Ramirez et al., 2002). We did not find a correlation between species abundance and richness and altitude (70-690 m a.s.l. variation). On the other hand, diversity showed a positive correlation, and dominance showed a negative correlation with altitude, which suggests a more equitable distribution of orchid bee communities at higher altitudes. Aguiar and Gaglianone (2012) found a positive relationship between abundance of orchid bees and altitude in a study carried out in Atlantic Forest fragments with an altitudinal range similar to our study (between 40 and 825 m a.s.l.). Other studies on the influence of altitude on the distribution of those bees observed a decrease in abundance with altitude, but not richness, another hand, Nemésio (2008) observed that bee species richness decreases markedly with altitude, in the range between 850 and 1,350 m a.s.l., however the range analyzed differs from that studied in this work. Dias (unpublished data) observed differences in species composition of orchid bees along a broad altitudinal gradient (125 m – 2,150 m). We investigated whether the altitude influenced richness and abundance of orchid bees along a narrower altitudinal gradient than those analyzed in other studies that found a correlation between those variables

The results obtained in the present study suggest that differences in phytophysiognomy affect the richness and abundance of orchid bees in the Atlantic Forest. Differences in species composition and abundance of these bees between close areas in the same region highlight the importance of conserving different portions of the habitat to assure the conservation of orchid bees in different phytophysiognomies within the same ecosystem.

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