



RESEARCH ARTICLE - BEES

Male Orchid Bees (Hymenoptera: Apidae: Euglossini) in Canopy and Understory of Amazon *Várzea* Floodplain Forest. I. Microclimatic, Seasonal and Faunal Aspects

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Article History

Edited by

Og de Souza, UFV, Brazil

Received 01 November 2016

Initial acceptance 07 March 2017

Final acceptance 01 May 2017

Publication date 21 September 2017

Keywords

Amapá, vertical stratification, diversity, euglossine bees.

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Abstract

Floodplain forests are important ecosystems of the Amazon Basin. The aim of the present study was to investigate whether male orchid bees have preference for arboreal strata in a forest with a dynamic understory. Traps with aromatic scents to attract bees were placed at heights of 25 m and 1.5 m. Data loggers were used to register temperature and relative humidity at both heights. A total of 835 individuals belonging to 28 species were sampled, 645 males of 23 species in the understory and 190 males of 24 species in the canopy. The temperature was 1.4 °C higher in the canopy and the air relative humidity was 11.58% higher in the understory. Temperature, relative humidity and winds were the most important abiotic variables influencing the assemblage in the canopy. The abundance increased in the understory and decreased in the canopy in the less rainy season. *Euglossa cognata* Moure, *Eulaema meriana* Olivier and *Euglossa ignita* Smith were species closely associated with strata and seasons. The present study revealed higher richness and abundance in the understory all months of the year, reinforcing the likelihood that orchid bees forage preferably in the lower layer of rainforests.

Introduction

Studies have shown significant differences in the diversity and community composition of insects across distinct arboreal strata, such as canopy and understory, regardless of the taxon and type of forest analyzed (Smith, 1973; Dulmen, 2001; Giovanni et al., 2015). These differences may be related to abiotic conditions and biotic resources, such as light availability, temperature, wind exposure, forest structure and composition, food and nesting sites, which differ in intensity and availability between strata (Ulyshen, 2011). For some groups of insects these differences are fairly important so that there are species restricted to specific strata (Erwin, 1983).

As emphasized by Ulyshen et al. (2010), few studies have been conducted to determine the occurrence of the vertical distribution of bees. Among such studies, Bawa et al. (1985)

found that tree species pollinated by small bees were especially common in the understory, whereas species pollinated by medium-sized to large bees were predominant in the canopy of a tropical forest. Roubik (1993) performed systematic collections of bees from the canopy and understory in two forests in Panama and found that two species of nocturnal bees were conspicuously abundant in the canopy, despite of that most of the species did not have a preference for a particular stratum. Sobek et al. (2009) reported spatial stratification in the abundance of cavity-nesting bees and wasps and their parasitoids as well as higher parasitism rates in the canopy than the understory in temperate deciduous forests in Germany. In a study on the vertical distribution of trap-nesting bees along a fragmentation gradient of secondary forest remnants in Costa Rica, Stangler et al. (2016) found that bees were more abundant in the canopy and understory in comparison to an intermediate height but the diversity was higher in the canopy.



Some authors have investigated the occurrence of vertical stratification in populations of orchid bees. Roubik (1993) found that large euglossine bees demonstrated a tendency to forage high, which was directly related to their capacity for heat loss during flight, compared to smaller euglossine bees. Oliveira and Campos (1996), in two areas of *Terra Firme* forest in the Amazon, found similar values of richness and abundance between canopy and understory, but higher diversity in the canopy; the authors also reported that some species were more associated with the canopy and others were more common in the understory. In areas of the Atlantic Rainforest (coastal Brazil), Martins and Souza (2005) found that the abundance and richness of euglossine bees were higher in absolute numbers in the understory, but the diversity was higher in the canopy. Studying population fluctuations of euglossine bees in a remnant of *Cerrado* scrubland in the state of Mato Grosso do Sul, Brazil, Ferreira et al. (2011) found no differences in species richness or diversity between strata, but the abundance of males was higher in the canopy than the understory. All these studies carried out with orchid bees had focused on differences and similarities in the richness, abundance and diversity between canopy and understory, but none of them assessed the influence of abiotic conditions on the community and distribution of species across the different strata.

Therefore, the aim of the present study was to investigate how the assemblage of male orchid bees is structured in canopy and understory of a *várzea* floodplain forest in the Brazilian Amazon, taking into consideration that floodplains have a more dynamic understory than non-flooded forests, which could favor faunal differences in both strata. Microclimatic differences regarding temperature and air relative humidity were analyzed in each stratum and the influence of such abiotic variables, together with others as rainfall, wind speed and tidal height, was investigated on the assemblage.

Material and Methods

Study area

This study was conducted in the Protected Area of Fazendinha, category V of the IUCN list (Dudley, 2008), which is located in the southeastern portion of the state of Amapá, Eastern Amazon, Brazil (00°03'02"S 051°07'51" W). The area encompasses 137 ha comprised mostly of dense tidal *várzea* forest (Prance, 1979), a kind of floodplain subject to floods by white-water rivers, which is different of *igapó*, another kind of Amazonian floodplain subject to floods by black or clean-water rivers (Prance, 1979). These forests represent about 27% of the Amazon Basin and differ one from each other regarding many aspects, as forest structure and composition, number of arboreal strata, height and architecture of the canopies, density coverage, soil composition, and so on (Pires & Prance, 1985; Piedade et al., 2001; Carim, 2017). Large species of trees adapted to flood regimes are present in the area, such as rubber tree (*Hevea*

brasiliensis Muell. Arg., Euphorbiaceae), *andiroba* nut (*Carapa guianensis* Aubl., Meliaceae), *pracuíba* (*Mora paraensis* Ducke, Leguminosae-Caesalpinioideae), palm species, such as *açaí* (*Euterpe oleracea* Mart., Arecaceae) and others (Prance, 1979; Carim et al., 2017). This vegetation gives to the forest an over-20-m canopy in height and a clear understory that is flooded up to 2 m by tides mainly from the Amazon River and some creeks (*igarapés*), such as Paxicú and Fortaleza, that flow throughout the forest (Drummond et al., 2008).

The climate is typical of Tropical Forest, Af according to Köppen's classification (Peel et al., 2007), with high rainfall throughout most of the year. Mean annual precipitation is approximately 2100 mm. A rainier season extends from January to July and a less rainy season extends from August to December. Mean annual temperature is about 27 °C and mean annual humidity is higher than 78% (Drummond et al., 2008).

Sampling

The fieldwork was carried out once a month, from January 2011 to March 2012, in an open natural area 500 m from the edge of the forest. Twenty-six traps were used to capture the bees. Each trap consisted of a 2-L plastic bottle with four holes measuring 3 cm in diameter spaced 5 cm apart and 17 cm up from the bottom of the bottle. Transparent hoses measuring 4 cm in length were cut in half longitudinally and attached to the holes to facilitate the entrance of the males. Three cotton swabs with extra cotton (ball diameter: 2 cm) were contained in each trap and impregnated with an aromatic scent. Thirteen scents were used: 1.8-cineole, eugenol, vanillin, benzyl acetate, methyl salicylate, methyl cinnamate, β -ionone, benzyl benzoate, β -myrcene, ethyl butyrate, linalool, methyl benzoate and 2-phenyletanol. One pair of traps with the same scent was securely fixed, to avoid wind shaking, on the same tree at the height of 25 m (canopy) and 1.5 m above the ground (understory). The trees were approximately 10 m one from each other, encompassing an area of approximately 150 m². The traps were monitored from 8:00 a.m. to 6:00 p.m., being replenished every four hours, when the attracted males were removed.

Abiotic variables

General abiotic data of the area: rainfall (mm), temperature (°C), air relative humidity (%) and wind speed (m/s) were obtained from the Núcleo de Hidrometeorologia e Energias Renováveis/ Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá (NHMET/ IEPA). Microclimatic data of each arboreal stratum [temperature (°C) and air relative humidity (%)] were obtained from two climatic data loggers (HOBO® Pro Series) placed at 25 m and 1.5 m in height to record data on an hourly basis. These loggers were used from April 2011 to March 2012 only on sampling days. Data on tidal flows [height (m)] were obtained from the Capitania dos Portos do Amapá/ Marinha do Brasil.

Data analysis

The Kolmogorov-Smirnov test was used to determine the normality of the data of richness and abundance. Depending on the results, either the Wilcoxon test (W) or paired t -test (t) were used to determine the significance of such data comparing between strata and seasons (Zar, 2014). Principal Component Analysis (PCA) was performed to determine how species were associated with each stratum and season. Pearson's correlation coefficients (r) were calculated to determine the strength of relationships between faunal data (richness and abundance) and abiotic variables in each stratum. Similarity coefficients were used to compare the strata with regard to diversity (Whittaker - β_w), number of shared species (Sørensen - S_s) and exclusive species (Jaccard - S_j). Pielou evenness index (J'), Berger-Parker dominance (d) and Simpson index (used its reciprocal form $1-D$, in which the assemblage becomes more even when the value rises (Magurran, 2004) were calculated for each stratum to determine how individuals were distributed per species. Shannon-Wiener index (H') was calculated to determine diversity in each stratum regarding the presence of rare species. Hutcheson's t -test was used to determine the significance of differences in the Shannon-Wiener diversity index (Magurran, 2004).

Results

A total of 835 individuals belonging to 28 species was sampled (Table 1). The abundance was significantly higher in the understory considering the accumulated number in each stratum ($N_{und.} = 645$ (77.25%), $N_{can.} = 190$ (22.75%); $W = 168.0$, $Z = 2.265$, $p = 0.024$) and the relative abundance in each stratum per month ($t = -5.587$, $df = 14$, $p = <0.001$). The richness was similar in both strata considering the accumulated number ($S_{und.} = 23$, $S_{can.} = 24$; $W = -5.00$, $Z = -0.333$, $p = 0.820$), but it was significantly higher in the understory considering the relative richness in each stratum per month ($t = -5.565$, $df = 14$, $p = <0.001$) (Fig 1).

Microclimatic differences between strata

The temperature was 1.4 °C higher in the canopy (range: 25.60 to 30.83; mean: 28.62 ± 1.79) in comparison to the understory (range: 24.89 to 29.27; mean: 27.22 ± 1.56), representing a statistically significant difference between the strata ($t = 13.083$, $df = 11$, $p = <0.001$). The air relative humidity was higher 11.58% in the understory (range: 80.73 to 104.10; mean: 93.53 ± 9.14) than the canopy (range: 68.32 to 99.20; mean: 81.95 ± 11.68) being a statistically significant difference ($t = -9.790$, $df = 11$, $p = <0.001$). From April to June/2011 and February to March/2012 the air relative humidity of the understory was higher than 100%, revealing a supersaturated environment, which coincided with the highest levels of tides and rainfall and lowest levels of winds and temperatures (Fig 1, Supple. Mat. 1).

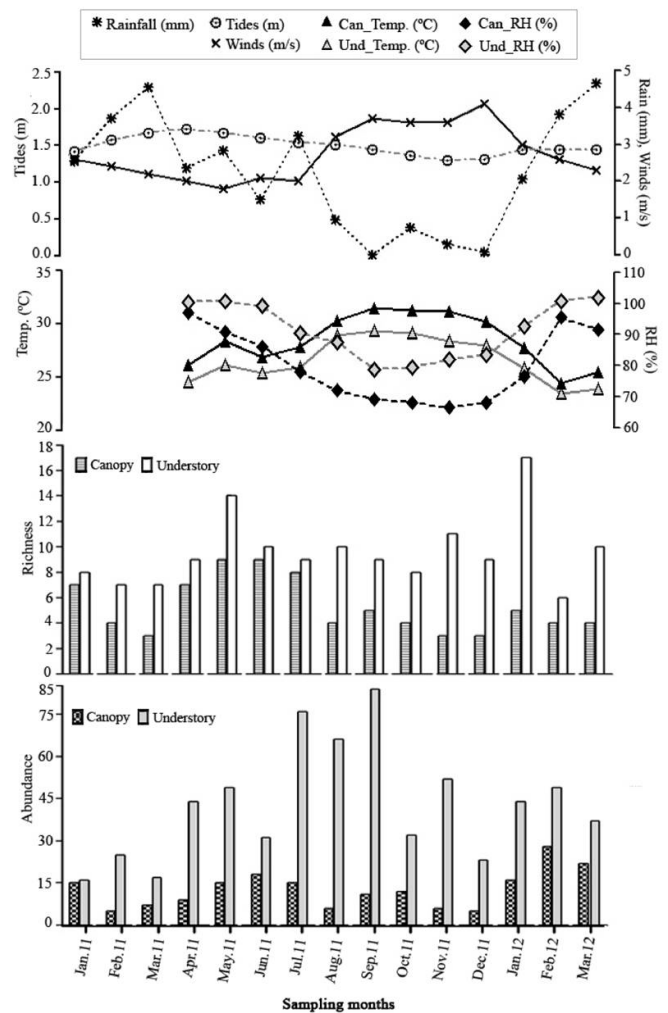


Fig 1. Monthly fluctuation of abiotic variables, abundance and richness of male euglossine bees sampled in canopy (25 m) and understory (1.5 m) of Amazon várzea floodplain forest. **Can_Temp.** (°C) = mean temperature (°C) in canopy; **Und_Temp.** (°C) = mean temperature (°C) in understory; **Can_RH** (%) = relative humidity (%) in canopy; **Und_RH** (%) = relative humidity (%) in understory. Obs.: **Rainfall** (mm) values divided by 100 to fit of image.

Correlation with abiotic variables

In the canopy, the number of males was negatively correlated with the temperature ($r_{can.temp.} = -0.78$, $p = <0.01$) and positively correlated with the air relative humidity ($r_{both} = 0.66$, $p = 0.02$) (Supple. Mat. 1). The richness in the canopy was negatively correlated with the wind speed ($r = -0.64$, $p = 0.001$) and positively correlated with the tidal height ($r = 0.53$, $p = 0.044$). In the understory, neither richness nor abundance were significantly correlated with the abiotic variables analyzed (Supple. Mat. 1).

Seasonal aspects

Considering accumulated numbers per season, richness ($W = -55.0$, $Z = -3.162$, $p = 0.002$) and abundance ($W = -281.0$, $Z = -3.792$, $p = <0.001$) were higher in the rainier season (Table 1), when tides, rainfall and air relative humidity were higher (Fig 1). Accordingly, fewer individuals and species occurred

in the less rainy season (Table 1), when temperatures were higher and winds were stronger (Fig 1).

The analysis of each stratum per season revealed a significant difference between canopy and understory with regard to the abundance of males (Table 1). In the less rainy season, the abundance was six-fold higher in the understory ($W = 110$, $Z = 2.847$, $p = 0.003$) while in the rainier season it was twofold higher comparing to the canopy ($W = 158.0$, $Z = 2.263$, $p = 0.025$). The richness did not differ significantly between the strata neither in the rainier ($W = 0.00$, $Z = 0.00$, $p = 1.000$) nor in the less rainy season ($W = 9.000$, $Z = 0.707$, $p = 0.547$) (Table 1).

Faunal composition

More species and individuals of the genus *Eufriesea* Cockerell were recorded in the canopy (Table 2). *Euglossa* Latreille, *Eulaema* Lepelletier and *Exaerete* Hoffmannsegg were more abundant in the understory but were quite similar in both strata with regard to richness (Table 2). The genera occurred with higher richness and abundance in the rainier season, except *Eulaema*, which had the same species present in both seasons (Table 2).

Eufriesea auripes, *E. superba*, *E. surinamensis*, *Euglossa augaspis* and *Exaerete dentata* were sampled

Table 1. Species of male euglossine bees sampled in canopy (Can. [25 m]) and understory (Und. [1.5 m]) in rainier (↑Rain) and less rainy (↓Rain) seasons. Amazon várzea floodplain forest, state of Amapá, Brazil, Jan. 2011 to Mar. 2012

Species of euglossine bees	Rainier (↑) season		Less rainy (↓) season		Total per season		Total per strata		Total
	Can.	Und.	Can.	Und.	Total ↑Rain	Total ↓Rain	Total Can.	Total Und.	
<i>Eufriesea auripes</i> (Gribodo, 1882)	4	0	1	0	4	1	5	0	5
<i>Eufriesea concava</i> (Friese, 1899)	5	1	0	0	6	0	5	1	6
<i>Eufriesea flaviventris</i> (Friese, 1899)	1	1	0	0	2	0	1	1	2
<i>Eufriesea superba</i> (Hoffmannsegg, 1817)	1	0	0	0	1	0	1	0	1
<i>Eufriesea surinamensis</i> (Linnaeus, 1758)	1	0	0	0	1	0	1	0	1
<i>Eufriesea vidua</i> (Moure, 1976)	0	1	0	0	1	0	0	1	1
<i>Euglossa (Euglossa) amazonica</i> Dressler, 1982	4	4	1	1	8	2	5	5	10
<i>Euglossa (Euglossa) avicula</i> Dressler, 1982	0	4	0	1	4	1	0	5	5
<i>Euglossa (Euglossa) bidentata</i> Dressler, 1982	0	1	0	0	1	0	0	1	1
<i>Euglossa (Euglossa) cordata</i> (Linnaeus, 1758)	10	35	13	29	45	42	23	64	87
<i>Euglossa (Euglossa) cognata</i> Moure, 1970	47	24	0	0	71	0	47	24	71
<i>Euglossa (Euglossa) galianii</i> Dressler, 1982	2	1	2	0	3	2	4	1	5
<i>Euglossa (Euglossa) modestior</i> Dressler, 1982	3	10	1	12	13	13	4	22	26
<i>Euglossa (Euglossa) mourei</i> Dressler, 1982	1	1	0	0	2	0	1	1	2
<i>Euglossa (Euglossa) sp</i>	3	5	3	0	8	3	6	5	11
<i>Euglossa (Glossura) allosticta</i> Moure, 1969	2	2	0	4	4	4	2	6	8
<i>Euglossa (Glossura) ignita</i> Smith, 1874	5	59	5	98	64	103	10	157	167
<i>Euglossa (Glossura) viridifrons</i> Dressler, 1982	4	6	1	2	10	3	5	8	13
<i>Euglossa (Glossurella) augaspis</i> Dressler, 1982	5	0	0	0	5	0	5	0	5
<i>Euglossa (Glossuropoda) intersecta</i> Latreille, 1817	1	23	0	6	24	6	1	29	30
<i>Eulaema (Apeulaema) nigrita</i> Lepelletier, 1841	7	14	4	11	21	15	11	25	36
<i>Eulaema (Apeulaema) pseudocingulata</i> Oliveira, 2006	24	70	4	15	94	19	28	85	113
<i>Eulaema (Eulaema) bombiformis</i> (Packard, 1869)	0	4	1	1	4	2	1	5	6
<i>Eulaema (Eulaema) meriana</i> (Olivier, 1789)	13	101	3	59	114	62	16	160	176
<i>Eulaema (Eulaema) polyzona</i> (Mocsáry, 1897)	0	3	0	2	3	2	0	5	5
<i>Exaerete dentata</i> (Linnaeus, 1758)	1	0	0	0	1	0	1	0	1
<i>Exaerete lepeletieri</i> Oliveira & Nemésio, 2003	4	10	1	13	14	14	5	23	28
<i>Exaerete smaragdina</i> (Guérin, 1844)	2	8	0	3	10	3	2	11	13
Number of individuals	150	388	40	257	538	297	190	645	835
Number of species	23	23	13	15	28	18	24	23	28

Table 2. Abundance and richness of males per genus of euglossine bees in canopy (Can.) and understory (Und.) in rainier (↑Rain) and less rainy (↓Rain) seasons. Amazon várzea floodplain forest, state of Amapá, Brazil, Jan. 2011 to Mar. 2012

Genus	Number of individuals				Number of species			
	Can.	Und.	↑Rain	↓Rain	Can.	Und.	↑Rain	↓Rain
<i>Eufriesea</i>	13	3	15	1	5	3	6	1
<i>Euglossa</i>	113	328	262	179	12	13	14	10
<i>Eulaema</i>	56	280	236	100	4	5	5	5
<i>Exaerete</i>	8	34	25	17	3	2	3	2
Total	190	645	538	297	24	23	28	18

exclusively in the canopy, whereas *E. vidua*, *E. avicula*, *E. bidentata* and *E. polyzona* were only sampled in the understory (Table 1). However, these exclusive species were represented per few individuals, up to 5 males. Nineteen species (67.86% of the total) were present in both strata. The most abundant species in the canopy were *E. cognata*, *E. pseudocingulata* and *E. cordata*, whereas *E. meriana*, *E. ignita*, *E. pseudocingulata* and *E. cordata* were the most abundant in the understory (Table 1).

Diagnostic species for strata. *Euglossa cognata* was closely related to the canopy, whereas *E. meriana*, *E. ignita*, *E. intersecta*, *E. modestior* and *E. lepeletieri* were closely related to the understory, as demonstrated by the PCA (Fig 2). *Euglossa cordata*, *E. nigrita* and *E. pseudocingulata* were related to both strata (Fig 2).

Diagnostic species for seasons. *Eulaema meriana*, *E. pseudocingulata*, *E. cognata* and *E. intersecta* were strongly associated with the rainier season, whereas *E. ignita* was the only associated with the less rainy season, according to the PCA (Fig 3). Moreover, we add the species of *Eufriesea* as

related to the rainier season (Table 1). *Euglossa cordata*, *E. modestior*, *E. nigrita* and *E. lepeletieri* were related to both seasons (Table 1 and Fig 3).

The ecological analysis demonstrated higher diversity in the canopy based on the presence of rare species, according to the Shannon-Wiener index, and greater evenness in the distribution of individuals per species, according to the Pielou index (Table 3). These results were supported by the Simpson index, which demonstrated higher diversity in the canopy also with regard to abundant species and evenness (Table 3). According to the Berger-Parker index, the dominance of species was similar in both strata (Table 3).

There was high similarity between canopy and understory with regard to the number of species shared between the strata, according to the Sørensen coefficient. High similarity was found considering the composition and diversity in each stratum, according to the Whittaker coefficient. Moderate similarity was found regard to the number of exclusive species in each stratum, according to the Jaccard coefficient (Table 3).

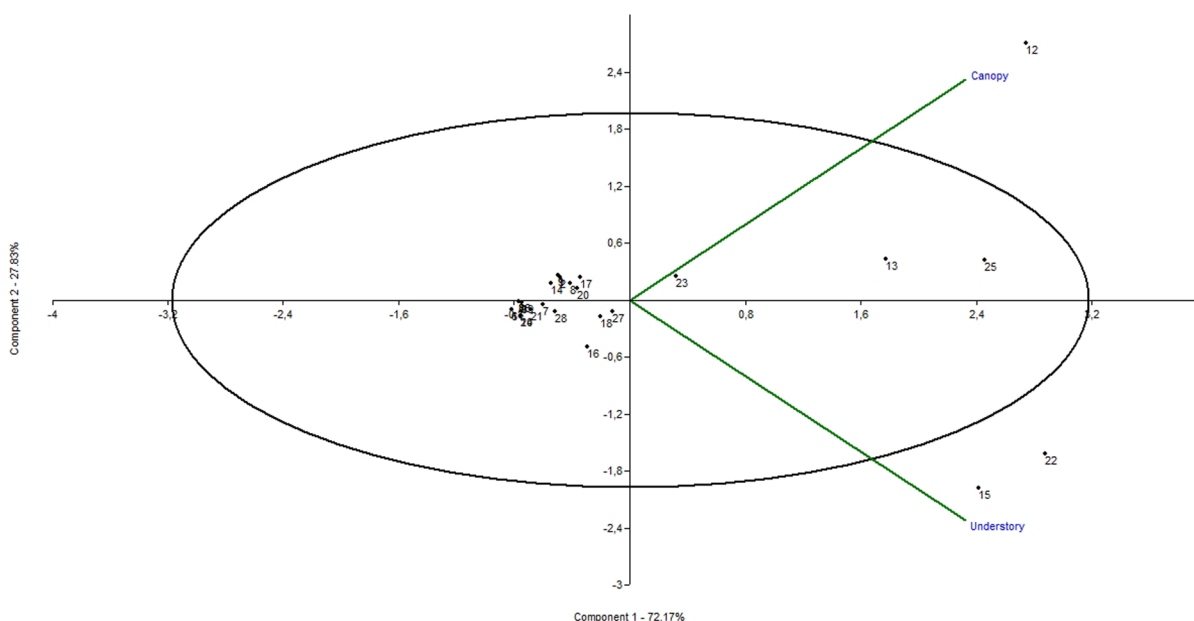


Fig 2. Principal Component Analysis (PCA) between species and strata. Ellipse indicates 95% confidence interval. Species farther from origin of axis are more representative of each stratum. Species names: 1. *E. auripes*; 2. *E. concava*; 3. *E. flaviventris*; 4. *E. superba*; 5. *E. surinamensis*; 6. *E. vidua*; 7. *E. allosticta*; 8. *E. amazonica*; 9. *E. augaspis*; 10. *E. avicula*; 11. *E. bidentata*; 12. *E. cognata*; 13. *E. cordata*; 14. *E. gaianii*; 15. *E. ignita*; 16. *E. intersecta*; 17. *E. sp.*; 18. *E. modestior*; 19. *E. mourei*; 20. *E. viridifrons*; 21. *E. bombiformis*; 22. *E. meriana*; 23. *E. nigrita*; 24. *E. polyzona*; 25. *E. pseudocingulata*; 26. *E. dentata*; 27. *E. lepeletieri*; 28. *E. smaragdina*.

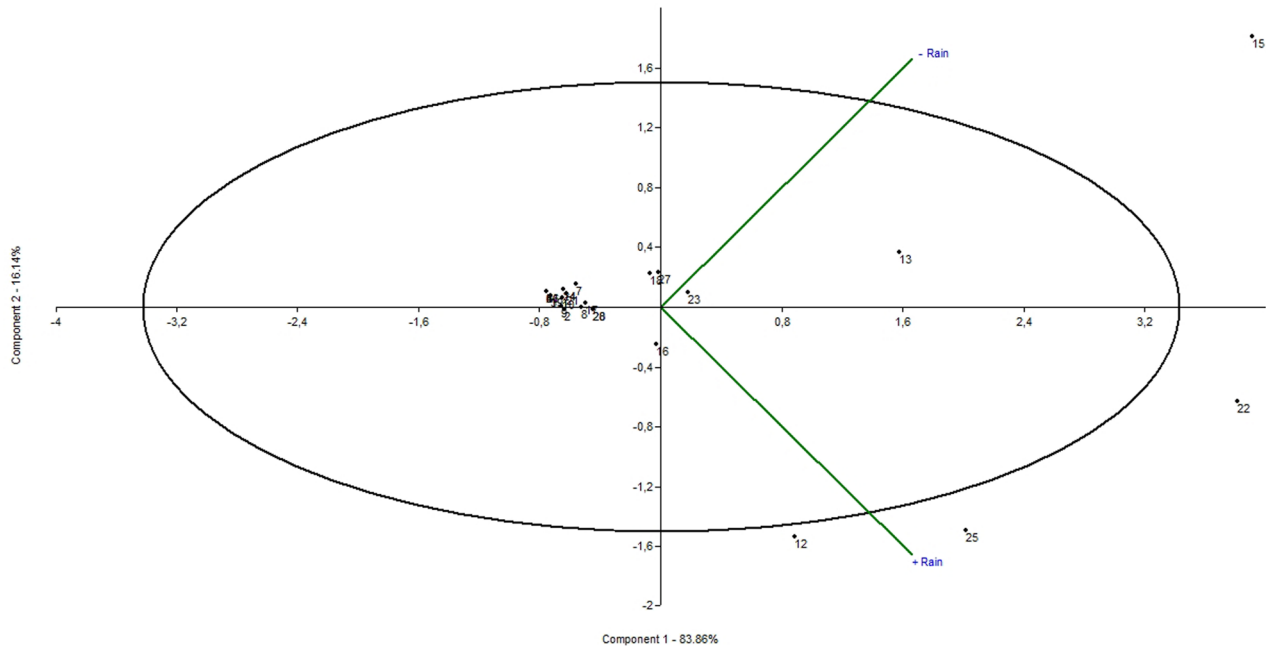


Fig 3. Principal Components Analysis (PCA) between species and seasons. Ellipse indicates 95% confidence interval. Species farther from origin of axis are more representative of each season. Species names: **1.** *E. auripes*; **2.** *E. concava*; **3.** *E. flaviventris*; **4.** *E. superba*; **5.** *E. surinamensis*; **6.** *E. vidua*; **7.** *E. allosticta*; **8.** *E. amazonica*; **9.** *E. augaspis*; **10.** *E. avicula*; **11.** *E. bidentata*; **12.** *E. cognata*; **13.** *E. cordata*; **14.** *E. galianii*; **15.** *E. ignita*; **16.** *E. intersecta*; **17.** *E. sp.*; **18.** *E. modestior*; **19.** *E. mourei*; **20.** *E. viridifrons*; **21.** *E. bombiformis*; **22.** *E. meriana*; **23.** *E. nigrita*; **24.** *E. polyzona*; **25.** *E. pseudocingulata*; **26.** *E. dentata*; **27.** *E. lepeletieri*; **28.** *E. smaragdina*.

Table 3. Ecological indices for assemblage of male euglossine bees in each arboreal stratum. H' = Shannon-Wiener; t = Hutcheson t -Test; J' = Pielou; $1-D$ = Simpson; d = Berger-Parker; S_s = Sørensen; S_j = Jaccard; β_w = Whittaker; Range of similarity indices: 0 to 1 (0 = similar, 1 = different)

Index	Canopy (25 m)	Understory (1.5 m)
Diversity	$H' = 1.1061$ $t = 3.7569, df. 321, p=0.01$	$H' = 0.9659$
Evenness	$J' = 0.8014$	$J' = 0.7093$
Dominance	$1-D = 0.8828$ $d = 0.2474$	$1-D = 0.8438$ $d = 0.2481$
Similarity	$S_s = 0.81; S_j = 0.68; \beta_w = 0.1915$	

Discussion

Analyze the differences between strata considering only accumulated numbers of richness and abundance in each of them, as reported by Oliveira and Campos (1996) and Ferreira et al. (2011), may lead to erroneous interpretations regarding the real distribution or preference of species for strata. This assumption is supported by the present results regarding richness, which revealed to be significantly different between the strata only when the data were analyzed per sampling month. Martins and Souza (2005), studying the vertical stratification of orchid bees in area of Atlantic Rainforest, reported that although there have been higher total abundance

in the understory, no difference was found between the strata when the monthly averages were analyzed. These results reinforce the supposed above and therefore we consider that if species or individuals are much more present in one stratum throughout all or most months of the year, this should be interpreted as the preferred stratum or that more favorable to foraging. Thus, although in our study the accumulated richness have been similar in both strata, we thought the understory as the stratum used by most of the species of orchid bees because along all months of the year more species and individuals were foraging therein than in the canopy.

Roubik (1993) and Otero and Sallenave (2003) found the highest richness and abundance of orchid bees in the understory, reinforcing the likelihood that males forage preferably in the lower layer of rainforests. This preference is supported by some features regarding the biology of these bees, for instance males collect scents from sources usually found in the understory, as the surface of rotting wood, fruits and seeds, leaf litter and terrestrial mushrooms (Whitten et al., 1993; Cappellari & Harter-Marques, 2010). Moreover, their courtship behavior is carried out few meters from the ground (Stern, 1991; Eltz et al., 2003), where they are less exposed to predators, such as birds (Roubik, 1993). In the understory there are more substrates for females build nests, such as large cavities in trunks which can be used by species with large body sizes, as *Eulaema* spp. (Bennet, 1965; Cameron & Ramírez, 2001), and those with communal or primitively social life habit (Roberts & Dodson, 1967; Santos & Garófalo, 1994). The cavities can also be found in the canopy, but they decline in size with height (Ulyshen, 2011).

Microclimatic differences between strata

Studies carried out in Tropical Forests of the Panama (Read, 1977) and Costa Rica (Fetcher et al., 1985) reported wider ranges of temperature in canopy and understory, with at least 14 °C of difference between the minimum and maximum temperatures of each stratum. This was different of the observed in the present study, in which the ranges were approximately of 5 °C, due the absence of low temperatures in the Brazilian Amazon. Regardless of this, in all these studies the higher values of temperature were found in the canopy and the difference between the strata was from 1 to 2 °C, which must be the difference expected between canopy and understory of undisturbed rainforests.

In two areas of *Terra Firme* forest in the Amazon, the males were active in the field when the temperature ranged between 24.5 and 27 °C, with minimal or nonexistent records of males outside these limits (Oliveira, 1999). In the present study, average temperatures within that range occurred only during three months in the canopy and during seven months in the understory. In the remaining months, the average temperatures were higher than that range in both strata, suggesting that males demonstrate greater tolerance to this climatic variable in *várzea* floodplain forest.

The high humidity of understories was reported by Read (1977), when analyzing the potential evaporation ratio in canopy and understory it was accounted a rate of 60% to the canopy and of 45% to the understory, suggesting “an excess of the water needs of the forest”. Fetcher et al. (1985) reported higher values of vapor pressure deficit in the canopy and lower in the understory, suggesting that low values implies in saturated air, which also represents a lower potential of evaporation and, thus, a moister air. These results lay support to the present study and, besides that, we reported that in some months of the year the understory becomes a supersaturated environment and it could represent less capacity of evapotranspiration to plants, warming of air and animals (Read, 1977; Andrews, 2010).

The tides have an important role in this process of supersaturation because they increase the amount of water vapor and gases in the understory (Bartlett et al., 1990). Further, the intense rainfall and the already high humidity of the region also contribute to more vapors. The low temperatures, low wind flows and the canopy coverage difficult the evaporation and the air remains supersaturated in the understory in some days or months of the year. This combination allows an air relative humidity higher than 100% (Read, 1977; Andrews, 2010) and it could be particularly adverse to small bees, such as *Euglossa*, which have lower thermoregulation capacity (May & Casey, 1983).

Abiotic and seasonal aspects

The fact that only the canopy assemblage was significantly affected by abiotic variables suggests that

the understory is a more stable environment for the males, offering protection against adverse weather conditions related mainly to rainfall, temperature and winds. This hypothesis is supported by Read (1977) who reported that canopies intercept approximately 48% of the rains, which does not reach the understory under weak rains, further they have solar radiation 57% higher than the understories. Such conditions significantly increase the energetic costs for living in the canopy, since the bees are required to perform greater thermoregulation activity and exert greater power output of flight muscles to tolerate high temperatures and strong winds many months of the year (Inouye, 1975; May & Casey, 1983). This was corroborated in the less rainy season of the present study, since the peaks of wind and temperature did coincide with the lowest richness and abundance in the canopy and with the highest abundance of males in the understory.

Roubik (1993) reported a general tendency of orchid bees to favor lower traps in the dry season in Panama, except for one species (*E. nigrata*). This author also reported greater difference between canopy and understory in the dry season in comparison to the wet season, supporting our results that in the less rainy season the difference between the strata is more accentuate than in the rainier season.

The period with higher tidal levels did coincide with the rainier season when some species exclusive to this period occurred, such as *E. cognata*, all species of *Eufriesea* and others less abundant species. We consider that the correlation between tidal height and canopy richness was related with the arising of these seasonal species, which were strictly abundant in the canopy. Cameron (1976), comparing insect populations in salt marshes environments during samplings with and without floods, observed that seasonal species remained in the vegetation even when the tidal level was high. This permanence occurred because it was better to stay in that situation than go out whenever the tides rose, therefore they would maximize their lifetime and reproductive success, being regulated primarily by biological rather than physical factors. These results support our assumption that tides would not exert direct effects on the euglossine assemblage in *várzea* forest. Nonetheless, our sampling was not conducted to compare days with and without floods, some of them randomly occurred during few sampling months. Thus, it is necessary studies comparing both situations of the tidal regime to better understand whether this variable have some influence on the orchid bees in floodplains.

Faunal composition

The major difference between the fauna observed in the studied area and that reported in studies carried out in non-flooded Amazonian forests (Oliveira & Campos, 1995; Storck-Tonon et al., 2009) is related to the high abundance of large-sized species in *várzea* forest, such as *Eulaema*, *Exaerete* and large *Euglossa* species, as *E. intersecta*. Such observations are corroborated by Pearson and Dressler (1985),

who reported the occurrence of large orchid bees as *E. meriana*, *E. bombiformis*, *E. smaragdina*, as more abundant in floodplain forest while *E. augaspis*, *E. bidentata* and others small species were significantly more abundant in *Terra Firme* forest. Moreover, Dulmen (2001), in the Colombian Amazon, reported that large bees were the primary pollinators in flooded forest while small bees were in upland forests.

Roubik (1993) reported that species of *Eulaema* and *Exaerete* had greater tendency to forage in the canopy while *Euglossa* species were more common in the understory, suggesting that large bees are better fit to forage in the upper layers due to their efficient thermoregulation capacity (May & Casey, 1983). We assume this hypothesis to support the prevalence of large bees in the understory of the *várzea* forests, because the high air relative humidity present in this stratum increases the sensation of heat and requires species with more thermoregulation capacity, especially in the supersaturated months.

The occurrence of most of the species and individuals of *Eufriesea* in the canopy was also reported by Oliveira and Campos (1996) and Ferreira et al. (2011), laying support about the preference of this genus to canopies. Furthermore, the seasonal habits of these species have been known for a long time, which is attributed to their prepupal diapause (Dressler 1982; Garófalo et al., 1993; Kamke et al., 2008). Because of these aspects, in the present study, *Eufriesea* species were considered diagnostic for stratum and season, despite they have not been highlighted in the PCA due to low abundance values.

If foraging in the canopy requires higher energy costs and greater exposure to predators, there should be a biological trade-off (Kneitel & Chase, 2004) for seasonal and univoltine species, such as *E. cognata* and *Eufriesea* species, since the costs experienced in the canopy is trade-off by a habitat with less competition and with higher likelihood to get quick success in a short lifetime, for instance finding a mate, copulating and finding resources to build and provisioning the nest.

This assumption may also be suggested for *E. nigrita* in environments in which it has a seasonal behavior, mainly related to cool periods (Rebêlo & Garófalo, 1997, Rocha-Filho & Garófalo, 2014). This seasonality would explain the abundance of this species in the forest canopy found in the studies of Roubik (1993) and Ferreira et al. (2011). Similar to reported by Ackerman (1983), in the present study *E. nigrita* did not exhibit seasonality. In this case, behaving like a multivoltine species, it is better to live in the understory and avoid additional costs unless other constraints, such as high population density, conspecific competition, natural enemies, and others, lead them to seek another stratum, as observed in *E. cordata*, *E. nigrita* and *E. pseudocingulata*, species sharing both strata.

Oliveira and Campos (1996), Martins and Souza (2005) and the present study showed that the canopy is the arboreal stratum with greater diversity and evenness. Furthermore, the studies on the vertical stratification of orchid bees (Roubik, 1993; Oliveira & Campos 1996; Martins & Souza, 2005;

Ferreira et al., 2011) agree that the faunal composition of both strata are similar, since the forests share many species between strata, which could be called as permanent species, according to Magurran (2004). Further, there is a low number of exclusive species in each stratum, most of them being *singletons* and *doubletons*, or called as occasional species (Magurran, 2004). The permanent species are important pollinators to both strata, especially to the canopy when the seasonal species disappear.

Although *várzea* forest has a dynamic understory, this was the arboreal stratum used by most of the species and individuals of the orchid bees. In the canopy prevailed seasonal species and others without preference for strata. The fauna was constituted especially by large bees, most of them was found in both strata while few were exclusive in each one. Despite of that, it was possible to suggest diagnostic species to strata and seasons. In the less rainy season there was greater difference between the strata, and the abundance increased in the understory and decreased in the canopy. The microclimatic differences between the strata were significant, being temperature, air relative humidity and winds the most important abiotic variables influencing the canopy assemblage. The understory was not significantly affected by the abiotic variables analyzed, proving that it is a more stable environment to the establishment of the orchid bees, having milder microclimatic conditions most of the months of the year, albeit in some months it becomes a supersaturated environment.

Acknowledgments

The authors are grateful to Márcio Luiz de Oliveira (INPA) for helping with the taxonomic identification of the euglossine species, to the anonymous referees for their contributions to the work, and to the Brazilian fostering agency CNPq for awarding a master's degree scholarship to the first author.

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