

# Is the “Centro de Endemismo Pernambuco” a biodiversity hotspot for orchid bees?

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Received: December 12, 2012 – Accepted: June 11, 2013 – Distributed: November 30, 2014  
(With 2 figures)

## Abstract

The orchid-bee faunas (Hymenoptera: Apidae: Euglossina) of the three largest forest remnants in the “Centro de Endemismo Pernambuco”, northeastern Brazil, namely Estação Ecológica de Murici (ESEC Murici), RPPN Frei Caneca, and a forest preserve belonging to Usina Serra Grande, in the states of Alagoas and Pernambuco, were surveyed using seventeen different scents as baits to attract orchid-bee males. Eight sites were established in the three preserves, where samplings were carried out using two protocols: insect netting and bait trapping. We collected 3,479 orchid-bee males belonging to 29 species during 160 hours in early October, 2012. Seven species were collected in the “Centro de Endemismo Pernambuco” for the first time. Richness proved to be one of the highest of the entire Atlantic Forest domain, and diversity in some sites, especially at ESEC Murici, revealed to be one of the highest in the Neotropics. *Eulaema felipei* Nemésio, 2010, a species previously recorded only at ESEC Murici, was found in no other preserve in the region and its conservation status is discussed.

**Keywords:** Atlantic Forest, Euglossina, euglossine bees, Hexapoda.

## O Centro de Endemismo Pernambuco é um ‘hotspot’ de biodiversidade para abelhas-das-orquídeas?

### Resumo

As faunas de abelhas-das-orquídeas (Hymenoptera: Apidae: Euglossina) dos três maiores remanescentes florestais do Centro de Endemismo Pernambuco, nordeste do Brasil, sendo elas a Estação Ecológica de Murici (ESEC Murici), a RPPN Frei Caneca e um remanescente florestal pertencente à Usina Serra Grande, foram amostradas com o uso de dezessete diferentes iscas aromáticas para atrair machos dessas abelhas. Oito sítios amostrais foram selecionados nas três áreas, onde amostragens foram realizadas sob duas metodologias: coleta ativa com rede entomológica e coleta com armadilhas. Foram coletados 3.479 machos de abelhas euglossinas durante 160 horas no início de outubro de 2012. Sete espécies foram registradas no Centro de Endemismo Pernambuco pela primeira vez. A riqueza da região se mostrou uma das mais altas de toda a Mata Atlântica, e a diversidade em alguns locais, especialmente na ESEC Murici, revelou-se uma das mais altas de toda a região Neotropical. *Eulaema felipei* Nemésio, 2010, uma espécie que só havia sido registrada na ESEC Murici, não foi encontrada em nenhuma outra área e seu estado de conservação é discutido.

**Palavras-chave:** Mata Atlântica, Euglossina, abelhas euglossinas, Hexapoda.

### 1. Introduction

Orchid bees (Hymenoptera: Apidae: Euglossina), important Neotropical pollinators (see Dressler, 1982a; Roubik and Hanson, 2004), are the focus of dozens of ecological studies (e.g. Ackerman, 1983, 1989; Janzen et al., 1982; Pearson and Dressler, 1985; Roubik and Ackerman, 1987; Powell and Powell, 1987; Nemésio and Silveira, 2006a, b, 2007a, 2010; Rasmussen, 2009; Abrahamczyk et al., 2011; Nemésio and Vasconcelos, 2013). The ease of collecting

their males, which are strongly and readily attracted to synthetic aromatic scents that mimic natural floral fragrances (Vogel, 1966; Dodson et al., 1969), greatly contributes to the popularity of field studies involving these bees.

Although taxonomic studies on orchid bees have recently revealed new species throughout the Neotropical region (e.g. Roubik, 2004b; Oliveira, 2006; Rasmussen and Skov, 2006; Nemésio, 2006, 2007, 2008, 2009, 2010a,

2011b, c, d, 2012a; Ayala and Engel, 2008; Bembé, 2008; Hinojosa-Díaz and Engel, 2011a, b; Hinojosa-Díaz et al., 2011, 2012; Nemésio and Engel, 2012), faunistic and ecological studies in the last decade have been mainly focused on the Atlantic Forest of eastern Brazil (e.g. Bezerra and Martins, 2001; Tonhasca Júnior et al., 2002; Santos and Sofia, 2002; Martins and Souza, 2005; Milet-Pinheiro and Schlindwein 2005; Darrault et al., 2006; Nemésio and Silveira, 2006b, 2007a, 2010; Farias et al., 2007; Farias et al. 2008; Aguiar and Gaglianone, 2008, 2011; Moura and Schlindwein, 2009; Nemésio, 2010b, 2011a, b, e, 2012c; Mattozo et al., 2011; Nemésio et al., 2012; Cordeiro et al., 2013; Nemésio and Vasconcelos, 2013), although a few studies have also been conducted in the Amazon (Nemésio and Morato, 2004, 2006; Storck-Tonon et al., 2009; Rasmussen, 2009; Abrahamczyk et al., 2011; Nemésio et al., 2014).

Despite this recent “boom” of studies in the Atlantic Forest domain, there still are many areas that could be considered “data deficient” concerning our knowledge on their orchid-bee fauna. Areas in northeastern Brazil, particularly at the northern margin of the São Francisco river, are a good example. Only a few areas have been effectively sampled in Paraíba (Bezerra and Martins, 2001; Farias et al., 2007; Farias et al., 2008), Pernambuco (Milet-Pinheiro and Schlindwein, 2005; Darrault et al., 2006), and Alagoas (Darrault et al., 2006; Moura and Schlindwein, 2009; Nemésio 2010b). Almost nothing is known from the states of Ceará, Sergipe and Rio Grande do Norte, although a new orchid bee has recently been described from Ceará (Nemésio and Ferrari, 2012).

The Atlantic Forest situated to the north of the São Francisco river is often called the “Centro de Endemismo Pernambuco” (hereafter CEPE). Compared to other areas in the Atlantic Forest domain, CEPE is the most devastated, the least known and the least protected (e.g. Hayer, 1988; Coimbra-Filho and Câmara, 1996; da Silva and Tabarelli, 2001). Originally the forested area ranged from the state of Alagoas to the state of Rio Grande do Norte covering a total area of almost 60,000 km<sup>2</sup> (Brown, 1982; Prance, 1982). Only ca. 2,000 km<sup>2</sup> (less than 5% of its original cover) remains, scattered in small fragments (Cardoso da Silva and Tabarelli, 2000; Ribeiro et al., 2009). Deforestation of CEPE began early in Brazilian history (Coimbra-Filho and Câmara, 1996), but reached catastrophic levels during the 1970's, when forests in Alagoas and Pernambuco gave way to sugar cane plantations, largely due to fiscal incentives provided by the Brazilian government under a development plan known as “Pró-Álcool”; this program consisted of sugar cane cultivation to produce ethanol fuel for motor vehicles (Goerck and Wege, 2005). Nevertheless, many plant and animal species considered endemic in this region (e.g. Teixeira and Gonzaga, 1983a, b, 1985; Prance, 1987; Teixeira, 1987; Pennington, 1990; Siqueira Filho, 1998; Olmos, 2005; Nemésio, 2010a) have been resilient enough to survive in the remaining forest patches, although some species have most probably vanished from these areas (Asfora and Pontes, 2009). Besides the endemic

species, CEPE holds ca. 50% of all bird species of the entire Atlantic Forest (Roda and Pereira, 2006) and at least 8% of all tree species (Uchoa Neto and Tabarelli, 2002).

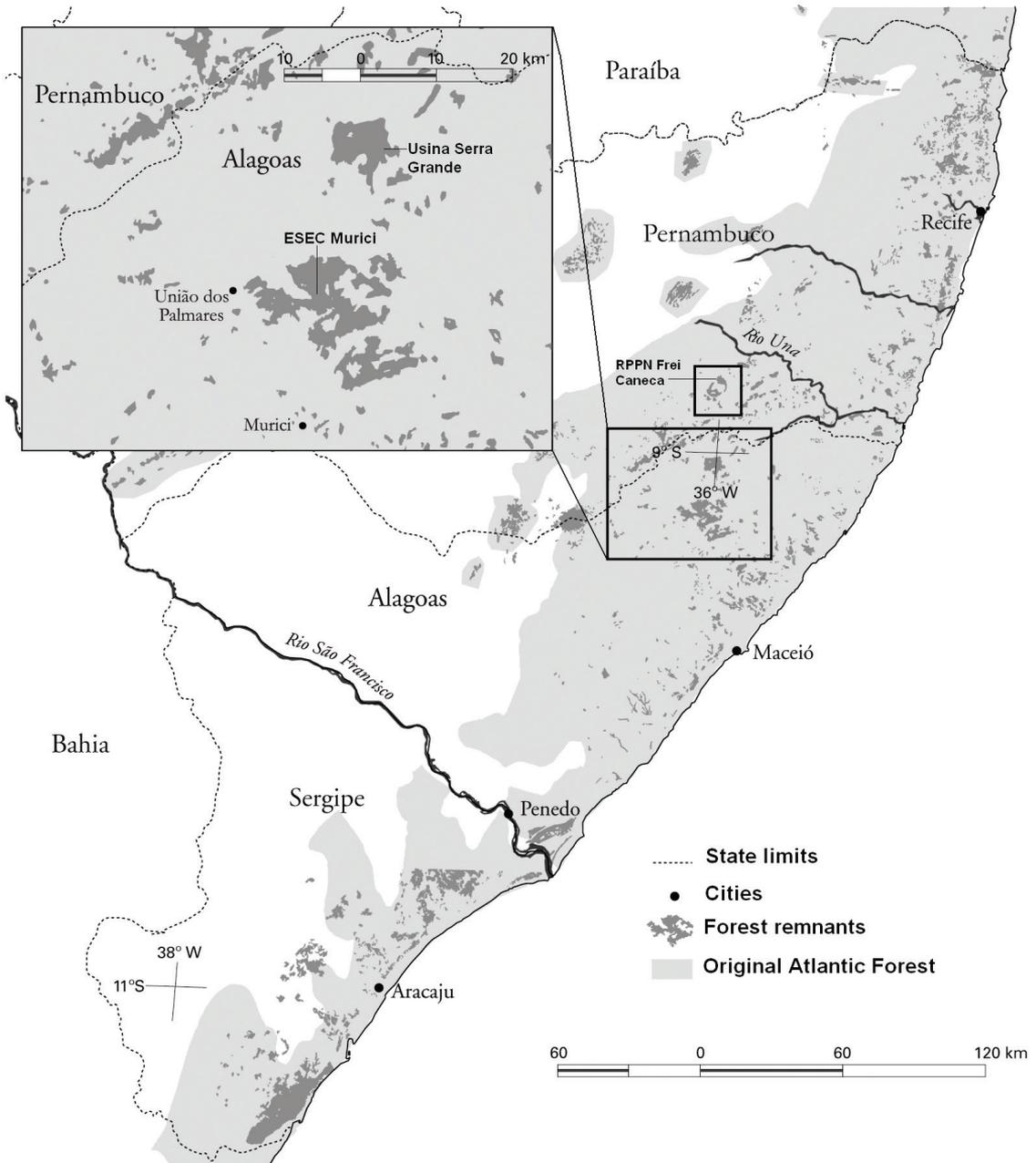
Currently, the largest forest preserves in CEPE are located in the states of Alagoas and Pernambuco, namely Estação Ecológica de Murici, in the municipality of Murici, and Mata do Coimbra, in the municipality of São José da Laje, both in the state of Alagoas; and Reserva Particular do Patrimônio Natural (RPPN) Frei Caneca, in the municipality of Jaqueira, southern Pernambuco (Figure 1). The orchid-bee fauna of the former area was sampled in 2009 (see Nemésio, 2010b) and the two latter areas were sampled in 2003 by Darrault et al. (2006). Nonetheless, sampling protocols used in both studies were different and Darrault et al. (2006) did not present data on abundance of each species. More importantly, many species were not identified at species level by Darrault et al. (2006). Nemésio (2010b) recognised 25 species for the region, but the absence of species-level identification for some taxa in Darrault et al. (2006) suggests that richness in the region may be higher.

The goals of the present study were, thus, to investigate the actual richness and diversity of orchid bees in CEPE using the same sampling protocol simultaneously in the three largest forest remnants in the region. If we assume that most orchid-bee species are forest dependent (Dressler, 1982a; Roubik and Hanson, 2004; Nemésio, 2009), then the best chances of recording the highest richness and diversity of these insects would be in the largest forest patches, theoretically the best preserved ones. Besides, we also used simultaneously different protocols (handnet collecting *versus* bait trapping) in order to investigate whether differences in sampling protocols would influence the results, a current topic in orchid-bee studies (Nemésio and Morato, 2004, 2006; Mattozo et al., 2011; Nemésio, 2012b).

## 2. Material and Methods

### 2.1. Study sites

This study was conducted in three forest remnants in CEPE, two in the state of Alagoas and one in the state of Pernambuco (Figure 1). In the state of Pernambuco, samplings were performed at RPPN Frei Caneca (RFC), a private protected forest remnant with a total area of about 1,000 ha, split into several forest fragments. Three sampling sites were selected at RFC: the largest (RFC-1; 500 ha) fragment, locally known as “Serra do Quengo”; a 50-ha fragment, known as “Mata do Ageró” (RFC-2); and another 50-ha fragment, known as “Mata do Espelho” (RFC-3). At the two former sites orchid-bee males were actively collected with insect nets, whereas in the latter site bees were bait trapped (see below). All three fragments are connected by corridors, although the small ones present more disturbed vegetation. In the state of Alagoas, two areas were sampled: Mata do Coimbra, a private area belonging to Usina Serra Grande (USG), with a continuous block of forest of about 3,500 ha, and (ii) Estação Ecológica de



**Figure 1.** Map of northeastern Brazil showing the location of RPPN Frei Caneca, Usina Serra Grande and ESEC Murici. See Material and Methods for exact coordinates.

Murici, a protected federal area of about 6,000 ha split into two larger fragments (about 2,500 ha each) and several smaller ones (see Nemésio, 2010a, b). Four sites were sampled at USG, one at the forest edge and three in the interior of the forest, one of the latter ones with bait traps, all the other ones with insect nets. Only one sampling site was selected at ESEC Murici, and sampled with insect nets since three sites had been sampled in this area three years before under the same protocol employed here. All these areas are immersed in a matrix of sugar cane plantations

and pastures. Samplings were carried out from the 2<sup>nd</sup> to the 11<sup>th</sup> of October, 2012.

### 2.2. Sampling

Twenty hours of sampling were performed at each of the eight selected sites in the areas (using either insect nets or bait traps), totalling 160 hours of field work, following the protocol established and discussed by Nemésio (2010b, 2011a, b, 2012c). The eight sampling sites were: RFC-1 (08°42'48"S, 35°50'30"W, ca. 737 m a.s.l., ca. 500 ha, interior of forest), RFC-2 (08°44'20"S, 35°50'31"W, ca. 483

m a.s.l., ca. 50 ha, interior of forest), RFC-3 (08°43'13"S, 35°50'37"W, ca. 633 m a.s.l., ca. 50 ha, interior of forest), USG-1 (08°59'44"S, 35°50'26"W, ca. 393 m a.s.l., ca. 3,500 ha, forest edge), USG-2 (09°00'05"S, 35°50'24"W, ca. 391 m a.s.l., ca. 3,500 ha, interior of forest), USG-3 (09°00'09"S, 35°50'42"W, ca. 442 m a.s.l., ca. 3,500 ha, interior of forest), USG-4 (09°00'01"S, 35°50'30"W, ca. 510 m a.s.l., ca. 3,500 ha, interior of forest), and MUR (09°13'06"S, 35°52'40"W, ca. 570 m a.s.l., ca. 2,700 ha, interior of forest). At sites RFC-1, RFC-2, USG-1, USG-2, USG-3 and MUR, 17 different scent baits were placed ca. 2.0 metres apart from each other at about 1.5 m above the ground. These baits were made of cotton waddings soaked with one of the following substances, known or believed to be attractive to orchid bees: benzyl acetate, benzyl alcohol, *r*-carvone, 1,8-cineole, *p*-cresol acetate, dimethoxybenzene, eugenol,  $\beta$ -ionone, methyl benzoate, methyl *trans*-cinnamate, heneicosane, linalool, methyl salicylate, skatole, tricosane, *p*-tolyl acetate, vanillin. Baits with cineole, the most volatile compound, were recharged every hour. Bees arriving on the baits during the sampling period (usually from 08:00h to 16:00h during three days, consecutive or not, until 20 hours were completed) were collected with insect nets, killed with ethyl acetate and pinned for posterior identification. At sites RFC-3 and USG-4, twenty-four bait traps modified according Campos et al. (1989) were placed ca. 1.5 metres apart from each other at about 1.5 m above the ground. These 24 bait traps consisted of six sets of four traps, each set containing one different scent. Thus, six scents were used, each scent offered in four traps. We selected the seven most attractive scents, but eliminated benzyl acetate and skatole because *Eulaema marcii* Nemésio, 2009, one of the dominant species in the region, is heavily attracted by these scents and we avoided overcollecting it, since many specimens had already been captured with insect nets. *Trans*-methyl cinnamate was, then, used to replace both scents. The six scents used were: 1,8-cineole, eugenol,  $\beta$ -ionone, methyl *trans*-cinnamate, methyl salicylate, and vanillin. Traps were exposed around 08:00h in the morning and left unattended until 16:00h, when all trapped specimens were killed with ethyl acetate and pinned for posterior identification. In order to allow comparison of both sampling protocols, two datasets were generated: the first one containing only data on bees collected with insect nets, with 17 scents; the second one containing data on bees collected with both methods, but only bees attracted to the six scents used in bait trapping were counted (see Tables 1 and 2). For analysing bait preference, only data on bees collected with insect nets with all 17 scents were used (Tables 3 and 4).

### 2.3. Data analysis

Diversity was estimated through Shannon-Wiener diversity index ( $H'$ ), as  $H' = -\sum p_i \ln(p_i)$ , where  $p_i$  is the proportion of total number of species made up of the  $i$ th species (Pielou, 1975). Evenness ( $E$ ) was estimated through the formula  $E = H'/\ln(S)$ , where  $S$  is the species richness. The

similarity in faunistic composition among sites was estimated by the Renkonen similarity coefficient, recommended by Wolda (1981) for small samples. Similarity indexes that take into account not only presence of species, but also their relative abundances, are strongly recommended by Balmer (2002) since theoretically they better reflect natural processes. For the similarity analysis we only included the six sites where bees were collected with insect nets, since the sampling protocol was identical and, thus, comparable. Based on those similarities, the areas were grouped using UPGMA (Sneath and Sokal, 1973).

### 2.4. Taxonomy

Taxonomy follows Nemésio and Rasmussen (2011) with the additions provided by Nemésio (2012a), Hinojosa-Díaz et al. (2012), and Nemésio and Engel (2012).

## 3. Results

Three thousand, four hundred and seventy-nine orchid-bee males belonging to 29 species were collected in all eight sites (Tables 1 and 2). Of these, 3,189 bees were collected with insect nets and 290 bees in bait traps. *Euglossa carolina* Nemésio, 2009, *Eulaema marcii* Nemésio, 2009, *Eulaema nigrita* Lepeletier, 1841, and *Euglossa imperialis* Cockerell, 1922 were the most abundant species throughout the region (Tables 1 and 2). *Euglossa marianae* Nemésio, 2011b was also a common species, but restricted to Usina Serra Grande and ESEC Murici, the largest forest remnants. Richness, diversity and evenness were the lowest at the sites situated at the forest edge (USG-1) at Usina Serra Grande and at the small fragment (RFC-2) at RPPN Frei Caneca ( $H' = 1.86$  and  $1.8$ ,  $E = 0.67$  and  $0.61$ , respectively). ESEC Murici, on the other hand, presented the highest richness (23 species), diversity ( $H' = 2.55$ ) and evenness ( $E = 0.81$ ) of all eight sampled sites. *Euglossa carinilabris* Dressler, 1982c and *Euglossa viridis* (Perty, 1833) were only collected at RPPN Frei Caneca, but both species were represented by singletons (Table 1); *Euglossa aratingae* Nemésio, 2009 and *eg. perpulchra* Moure and Schindwein, 2002 were only collected at RPPN Frei Caneca and Usina Serra Grande; *Euglossa adiastrata* Hinojosa-Díaz, Nemésio and Engel, 2012, *eg. hemichlora* Cockerell, 1917, *eg. monnei* Nemésio, 2012a, *eg. marianae* and *eg. pleosticta* Dressler, 1982d were only collected at Usina Serra Grande and ESEC Murici; *Eulaema felipei* Nemésio, 2010a and *Euglossa pepeii* Nemésio and Engel, 2012, were only collected at ESEC Murici (Tables 1 and 2). *Eulaema atleticana* Nemésio, 2009 and *Euglossa nanomelanotricha* Nemésio, 2009 were not recorded at ESEC Murici during the present study, but both species were collected there three years before (Nemésio, 2010b). All species collected by Darrault et al. (2006) and Nemésio (2010b) in the region were recorded in the present study, except for bees belonging to *Eufriesea* Cockerell, 1908. All 29 species were collected with insect nets, whereas only 19 species were collected with bait traps. Abundance ranged from 15.6 bees collected per hour at USG-1 to 36.6 bees per hour at USG-2, but it must be

**Table 1.** Diversity, evenness, species richness and number of specimens of each orchid-bee species collected with insect nets with 17 different scents (see Material and Methods) in six sampling sites in “Centro de Endemismo Pernambuco”, northeastern Brazil, after 20 hours of sampling in each site. See text for location of each site.

Species	Usina Serra Grande				RPPN Frei Caneca			ESEC Murici	Total
	USG-1	USG-2	USG-3	Subtotal	RFC-1	RFC-2	Subtotal		
<i>Euglossa (Euglossa) amazonica</i> Dressler, 1982d	40	86	59	185	17	20	37	47	269
<i>Eg. aratingae</i> Nemésio, 2009	0	2	1	3	2	0	2	0	5
<i>Eg. bembei</i> Nemésio, 2011d	0	0	0	0	9	0	9	5	14
<i>Eg. calycina</i> Faria and Melo, 2012	1	6	6	13	10	3	13	21	47
<i>Eg. carolina</i> Nemésio, 2009	143	191	109	443	37	174	211	62	716
<i>Eg. despecta</i> Moure, 1968	16	35	14	65	4	2	6	61	132
<i>Eg. hemichlora</i> Cockerell, 1917	0	1	1	2	0	0	0	1	3
<i>Eg. marianae</i> Nemésio, 2011b	10	66	56	132	0	0	0	50	182
<i>Eg. milenae</i> Bembé, 2007	16	26	5	47	0	3	3	54	104
<i>Eg. monnei</i> Nemésio, 2012a	0	1	2	3	0	0	0	3	6
<i>Eg. nanomelanotricha</i> Nemésio, 2009	0	0	0	0	1	2	3	0	3
<i>Eg. pleosticta</i> Dressler, 1982d	1	2	0	3	0	0	0	1	4
<i>Eg. securigera</i> Dressler, 1982d	7	11	5	23	7	4	11	8	42
<i>Eg. (Euglossella) perpulchra</i> Moure and Schindwein, 2002	0	0	1	1	6	13	19	0	20
<i>Eg. viridis</i> (Perty, 1833)	0	0	0	0	1	0	1	0	1
<i>Eg. (Glossura) ignita</i> Smith, 1874	3	37	10	50	5	6	11	14	75
<i>Eg. imperialis</i> Cockerell, 1822	4	31	34	69	64	9	73	84	226
<i>Eg. roubiki</i> Nemésio, 2009	0	0	7	7	17	3	20	46	73
<i>Eg. (Glossurella) adiaetola</i> Hinojosa-Díaz, Nemésio and Engel, 2012	0	9	2	11	0	0	0	1	12
<i>Eg. carinilabris</i> Dressler, 1982c	0	0	0	0	0	1	1	0	1
<i>Eg. clausi</i> Nemésio and Engel, 2012	10	46	27	83	2	2	4	51	138
<i>Eg. pepeii</i> Nemésio and Engel, 2012	0	0	0	0	0	0	0	2	2
<i>Eulaema (Apeulaema) felipei</i> Nemésio, 2010a	0	0	0	0	0	0	0	15	15
<i>El. marcii</i> Nemésio, 2009	38	119	162	319	127	99	226	132	677
<i>El. nigrita</i> Lepeletier, 1841	19	50	45	114	113	71	184	65	363
<i>El. (Eulaema) atleticana</i> Nemésio, 2009	1	6	1	8	13	11	24	0	32
<i>El. niveofasciata</i> (Friese, 1899)	1	1	3	5	1	3	4	1	10
<i>Exaerete frontalis</i> (Guérin-Méneville, 1844)	2	5	2	9	0	1	1	1	11
<i>Ex. smaragdina</i> (Guérin-Méneville, 1844)	0	2	0	2	0	3	3	1	6
Total (N)	312	733	552	1597	436	430	866	726	3189
Richness	16	21	21	23	18	19	22	23	29
Diversity (H')	1.86	2.32	2.17	2.24	2.04	1.8	2.04	2.55	2.4
Evenness (E)	0.67	0.76	0.71	0.72	0.7	0.61	0.66	0.81	0.71

emphasised that it rained substantially at ESEC Murici for ca. six hours on October 11<sup>th</sup>, and if we consider only the 14 hours of effective sampling, abundance at this site would be around 52 bees collected per hour.

The ordination of the sites according to the similarity of their faunas showed a medium to high overall (55% to 80%) similarity among the three forest areas (see Figure 2). The sites situated at the interior of the forest at

Usina Serra Grande showed the highest similarity (80%) and grouped with the site at ESEC Murici (69.9%), also in the interior of the forest. The site situated at the edge of the forest at Usina Serra Grande grouped first with the small site at RPPN Frei Caneca (69.4%) and, then, to the first group (67.8%). The site situated at the interior of the forest in the largest fragment at RPPN Frei Caneca was the most dissimilar of all six sites, sharing only 55.2% of

**Table 2.** Diversity, evenness, species richness and number of specimens of each orchid-bee species collected with insect nets and bait traps with six different scents (see Material and Methods) in eight sampling sites in “Centro de Endemismo Pernambuco”, northeastern Brazil, after 20 hours of sampling in each site. See text for location of each site.

Species	Usina Serra Grande				RPPN Frei Caneca				Total	
	USG-1	USG-2	USG-3	USG-4 (traps)	Subtotal	RFC-1	RFC-2	RFC-3 (traps)		Subtotal
<i>Euglossa (Euglossa) amazonica</i>	31	68	45	2	146	13	19	2	34	180
<i>Eg. aratingae</i>	0	0	1	0	1	2	0	0	2	3
<i>Eg. bembei</i>	0	0	0	0	0	3	0	0	3	3
<i>Eg. calycina</i>	1	4	1	0	6	5	0	2	7	13
<i>Eg. carolina</i>	131	169	79	62	441	22	166	27	215	656
<i>Eg. despecta</i>	7	6	5	1	19	0	0	0	0	19
<i>Eg. hemichlora</i>	0	1	0	0	1	0	0	0	0	1
<i>Eg. marianae</i>	8	38	30	4	80	0	0	0	0	80
<i>Eg. milenae</i>	14	24	5	20	63	0	3	0	3	66
<i>Eg. monnei</i>	0	0	2	0	2	0	0	0	0	2
<i>Eg. nanomelanotricha</i>	0	0	0	1	1	0	2	0	2	3
<i>Eg. pleosticta</i>	1	2	0	0	3	0	0	0	0	3
<i>Eg. securigera</i>	7	11	5	2	25	7	4	2	13	38
<i>Eg. (Euglossella) perpulchra</i>	0	0	1	1	2	6	13	0	19	21
<i>Eg. viridis</i>	0	0	0	0	0	1	0	0	1	1
<i>Eg. (Glossura) ignita</i>	2	9	3	1	15	2	2	3	7	22
<i>Eg. imperialis</i>	3	29	28	29	89	55	3	12	70	159
<i>Eg. roubiki</i>	0	0	7	0	7	17	3	0	20	27
<i>Eg. (Glossurella) adiaetola</i>	0	9	2	0	11	0	0	0	0	11
<i>Eg. carinilabris</i>	0	0	0	0	0	0	1	0	1	1
<i>Eg. clausi</i>	7	46	27	7	87	2	1	0	3	90
<i>Eg. pepeii</i>	0	0	0	0	0	0	0	0	0	0
<i>Eulaema (Apeulaema) felipei</i>	0	0	0	0	0	0	0	0	0	0
<i>El. marcii</i>	11	53	56	23	143	82	49	31	162	305
<i>El. nigrita</i>	0	7	10	2	19	44	8	38	90	109
<i>El. (Eulaema) atleticana</i>	1	3	1	2	7	11	6	9	26	33
<i>El. niveofasciata</i>	1	1	2	3	7	0	3	1	4	11
<i>Exaerete frontalis</i>	1	5	0	1	7	0	1	0	1	8
<i>Ex. smaragdina</i>	0	2	0	1	3	0	2	1	3	6
Total (N)	226	487	310	162	1185	272	286	128	686	1871
Richness	15	19	19	17	24	15	17	11	21	27
Diversity (H')	1.57	2.16	2.21	1.92	2.14	2.06	1.53	1.8	2.04	2.22
Evenness (E)	0.58	0.73	0.75	0.68	0.67	0.76	0.54	0.75	0.67	0.67

similarity with the other five ones (Figure 2). This latter site was the only one where specimens belonging to *Eulaema* Lepeletier, 1841 together represented more than 50% of the orchid-bee community (Table 1).

Samplings carried out with bait traps captured substantially less bees than samplings performed with insect nets (Table 2), even considering that four traps were used for each scent. Besides the lowest abundance, richness, diversity and evenness were also among the

lowest when compared to sites sampled with insect nets (Table 2). Larger bees belonging to *Eulaema* were more commonly captured with bait traps than insect nets. At Usina Serra Grande, 18.5% of the trapped bees belonged to the genus *Eulaema*, whereas 14.3% of the bees captured with insect nets were *Eulaema* spp. at the other three sites. At RPPN Frei Caneca, 61.7% of the trapped bees were *Eulaema* spp., whereas 36.4% of the bees collected with insect nets were *Eulaema* at the other two sites (Table 2).

**Table 3.** Diversity, evenness, species richness and number of specimens of each orchid-bee species collected with insect nets attracted to the seven most efficient scents in six sampling sites in “Centro de Endemismo Pernambuco”, northeastern Brazil, after 20 hours of sampling in each site. See text for location of each site.

Species	BA	BI	CI	SK	EU	MS	VA	TOTAL
<i>Euglossa (Euglossa) amazonica</i>	0	3	118	51	92	0	1	265
<i>Eg. aratingae</i>	0	0	0	0	0	0	3	3
<i>Eg. bembéi</i>	0	0	0	10	0	0	4	14
<i>Eg. calycina</i>	0	0	1	21	0	21	3	46
<i>Eg. carolina</i>	0	379	220	40	5	0	6	650
<i>Eg. despecta</i>	0	0	12	102	16	0	0	130
<i>Eg. hemichlora</i>	0	0	0	0	0	2	0	2
<i>Eg. marianae</i>	0	0	96	85	0	0	0	181
<i>Eg. milenae</i>	3	92	3	0	0	0	0	98
<i>Eg. monnei</i>	0	1	0	1	0	0	4	6
<i>Eg. nanomelanotricha</i>	0	0	1	0	1	0	0	2
<i>Eg. pleosticta</i>	0	0	4	0	0	0	0	4
<i>Eg. securigera</i>	0	1	14	0	26	0	1	42
<i>Eg. (Euglossella) perpulchra</i>	0	12	0	0	0	0	8	20
<i>Eg. viridis</i>	0	0	0	0	0	1	0	1
<i>Eg. (Glossura) ignita</i>	29	0	3	1	0	25	0	58
<i>Eg. imperialis</i>	0	0	62	3	1	132	0	198
<i>Eg. roubiki</i>	0	0	29	0	28	1	15	73
<i>Eg. (Glossurella) adiastrata</i>	0	0	10	0	1	1	0	12
<i>Eg. carinilabris</i>	0	0	0	0	1	0	0	1
<i>Eg. clausi</i>	0	1	52	2	3	0	76	134
<i>Eg. pepei</i>	0	0	0	0	0	0	2	2
<i>Eulaema (Apeulaema) felipei</i>	0	0	0	0	0	15	0	15
<i>El. marcii</i>	128	85	1	189	42	0	198	643
<i>El. nigrita</i>	0	0	22	286	0	0	52	360
<i>El. (Eulaema) athleticana</i>	8	0	12	0	0	3	7	30
<i>El. niveofasciata</i>	0	0	0	0	0	7	0	7
<i>Exaerete frontalis</i>	1	0	5	1	2	0	0	9
<i>Ex. smaragdina</i>	0	0	3	0	1	0	0	4
Total (N)	169	574	668	792	219	208	380	3010
Richness	5	8	19	13	13	10	14	29
Diversity (H')	0.76	0.99	2.08	1.75	1.7	1.27	1.51	2.39
Evenness (E)	0.47	0.48	0.71	0.68	0.66	0.55	0.57	0.71

BA: benzyl acetate; BI:  $\beta$ -ionone; CI: 1,8 cineole; SK: skatole; EU: eugenol; MS: methyl salicylate; VA: vanillin.

Sixteen of the 17 scents used in the present study were attractive to orchid-bee males (Tables 3 and 4). No specimen was collected on linalool. More than 94% of the specimens and all species were collected on seven scents: skatole (25%), cineole (21%),  $\beta$ -ionone (18%), vanillin (12%), eugenol (7%), methyl-salicylate (6.5%) and benzyl acetate (5.3%) (Table 3). Cineole attracted the highest number of species (19). None of the ten lesser scents attracted exclusive species (Table 4).

## 4. Discussion

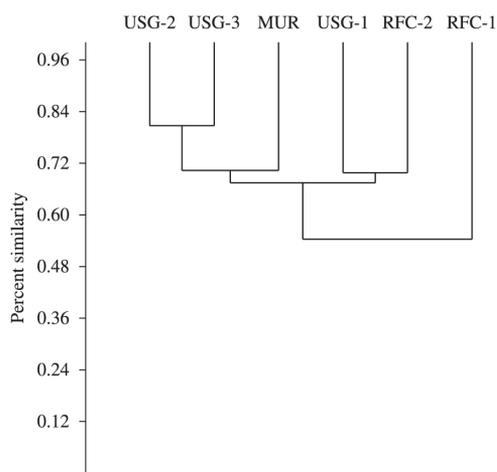
### 4.1. Sampling protocol

The lack of standardisation in orchid-bee inventories has been pointed out as a main concern for comparative studies for over a decade (Morato, 1998; Nemésio and Silveira, 2007b; Nemésio, 2012b). Recently, Nemésio (2010b) proposed a 20-hour sampling methodology that consists in collecting all bees in a given site over 20 hours

**Table 4.** Diversity, evenness, species richness and number of specimens of each orchid-bee species collected with insect nets attracted to the nine less efficient scents in six sampling sites in “Centro de Endemismo Pernambuco”, northeastern Brazil, after 20 hours of sampling in each site. See text for location of each site.

Species	BA	MB	MC	CR	DB	FL	HE	RC	TO	TR	TOTAL
<i>Euglossa (Euglossa) amazonica</i>	3	0	1	0	0	0	0	0	0	0	4
<i>Eg. aratingae</i>	0	0	0	0	0	2	0	0	0	0	2
<i>Eg. calycina</i>	0	0	0	0	0	0	1	0	0	0	1
<i>Eg. carolina</i>	0	1	14	0	1	14	1	33	1	0	65
<i>Eg. despecta</i>	0	0	1	0	0	1	0	0	0	0	2
<i>Eg. marianae</i>	0	0	0	0	0	1	0	0	0	0	1
<i>Eg. milenae</i>	0	0	1	0	0	2	0	3	0	0	6
<i>Eg. nanomelanotricha</i>	0	0	0	0	0	1	0	0	0	0	1
<i>Eg. (Glossura) ignita</i>	9	0	0	0	0	4	0	1	3	0	17
<i>Eg. imperialis</i>	0	0	0	2	1	1	0	0	24	0	28
<i>Eg. clausi</i>	0	0	0	0	0	4	0	0	0	0	4
<i>El. marcii</i>	16	2	0	1	1	12	1	0	0	1	34
<i>El. nigrita</i>	0	0	0	0	0	2	0	0	1	0	3
<i>El. (Eulaema) atleticana</i>	0	0	0	0	0	0	0	0	1	0	1
<i>El. niveofasciata</i>	0	0	1	0	0	0	0	0	2	0	3
<i>Exaerete frontalis</i>	0	0	1	0	0	0	0	0	1	0	2
<i>Ex. smaragdina</i>	0	0	1	0	0	1	0	0	0	0	2
Total (N)	28	3	20	3	3	45	3	37	33	1	176
Richness	3	2	7	2	3	12	3	3	7	1	17
Diversity (H')	0.92	0.64	1.15	0.64	1.1	1.98	1.1	0.4	1.04	0.0	1.95
Evenness (E)	0.84	0.92	0.59	0.92	1.0	0.8	1.0	0.37	0.54	-	0.69

BA: benzyl alcohol; MB: methyl benzoate; MC: *trans*-methyl cinnamate; CR: *p*-cresol acetate; DB: dimethoxybenzene; FL: in flight (attracted to the sampling area but not to any specific scent); HE: heneicosane; RC: *r*-carvone; TO: *p*-tolyl acetate; TR: tricosane.



**Figure 2.** Clustering of the six sites where sampling was conducted with insect nets and 17 scent baits according to their similarity. MUR: ESEC Murici; RFC: RPPN Frei Caneca; USG: Usina Serra Grande. See Material and Methods for exact coordinates of each sampled site.

(in consecutive days or not) in any period from 07:00h to 17:00h, using as many scents as possible, during the season when orchid bees are most active. Roubik (2004a) had already suggested that surveys of orchid-bee males with scent baits during a single day have great utility, and may reveal almost as much about local community structure as studies lasting a full year. The protocol proposed by Nemésio (2010b) has already been used in more than twenty areas in northeastern Brazil (Nemésio, 2010b; this study), southern Bahia (Nemésio, 2011a, 2013a, c, d), northern Espírito Santo (Nemésio, 2011b, 2013b), Minas Gerais (Nemésio, 2012c; Nemésio and Paula, 2013), and the Peruvian Amazon (Nemésio et al., 2014), always with a high number of collected specimens during a relatively short period of time. With more than 3,000 bees collected in only ten days, the present study revealed one of the highest abundances ever recorded with this 20-hour sampling strategy, only rivalled by the regions of Monte Pascoal, in southern Bahia (Nemésio, 2013d), and Linhares, in northern Espírito Santo (Nemésio, 2013b). On the other hand, it must be emphasised that this protocol potentially fails to record the actual richness of species

belonging to *Eufriesea* Cockerell, 1908, since most species in this genus are univoltine, highly seasonal (Kimsey, 1982), and it becomes a matter of chance reaching the sampling areas when one or more species of this genus are active. It is particularly true in northeastern Brazil, since the rainy season, when most bees are active (see Bezerra and Martins, 2001), occurs during winter, with no active species of *Eufriesea*.

#### 4.2. Insect nets versus bait traps

Although some authors (Aguiar and Gaglianone, 2008, 2011) insist that bait traps may be an efficient sampling method, there is now a growing body of evidence to contradict it in different parts of the Neotropics (see Nemésio and Morato, 2004, 2006; Mattozo et al., 2011; this study). When both methodologies are used simultaneously, the commonly used bait traps are always less efficient than active collecting with insect nets (Nemésio and Morato, 2004, 2006; Mattozo et al., 2011; this study). Specimens belonging to *Eulaema* are more commonly found trapped than specimens belonging to *Euglossa* Latreille, 1802, suggesting that small bees belonging to *Euglossa* escape more easily than the larger *Eulaema* spp. Results obtained with the sole use of bait traps should, thus, be considered with great care. If, as pointed out by Nemésio (2012b), our main objective is to understand the actual relative abundance of each species in a given orchid-bee community, then the most efficient sampling methodology should always be preferred. The sole use of bait traps should be avoided whenever possible, since it is not only the lower number of captured bees that counts here (which, theoretically, could be counterbalanced through a larger number of traps), but the serious bias introduced by this methodology, given that it favours trapping the largest bees.

#### 4.3. Scent preferences

Although sixteen scents were attractive to orchid-bee males, our data strongly suggest that almost the same results could be reached with the use of only the seven most efficient scents, since more than 94% of the specimens and all 29 species were attracted to them (see Tables 3 and 4). The most efficient scents may vary from region to region, depending on the species composition of each community, since some species present particular scent preferences (e.g. Ackerman, 1989). For example,  $\beta$ -ionone was an important scent in the present study, but only three specimens were attracted to it during 120 hours of active sampling in Peruvian Amazon only two months before this study (Nemésio et al., 2014). Cineole, methyl salicylate and vanillin, among the most powerful attractants in the present study, are widely recognised as strong attractants. On the other hand, skatole has been neglected by most researchers, who argue that it stinks. Although it is true, it is a powerful attractant. In the present study it attracted the largest number of specimens and it was an important scent for collecting *Euglossa marianae*, a species only known to visit skatole and cineole baits. In a recent survey in Peru, skatole attracted the highest number

of species (Nemésio et al., 2014). Other scents that were not particularly strong attractants in the present study, such as *p*-tolyl acetate and *trans*-methyl cinnamate, have shown to be important baits in southern Bahia (A. Nemésio, unpublished data) and in the Amazon (Nemésio et al., 2014). Based on the results here presented and those presented by Nemésio et al. (2014) for the Amazon, we encourage researchers to use a wider array (more than the usual five or six) of scents in future orchid-bee studies to better sample those odd little-responsive species that usually escape our attention, especially in highly species-rich areas (see also Nemésio, 2012b).

#### 4.4. Faunistics, richness and diversity

Twenty-nine species were recorded in the present study, the highest number ever recorded for this part of the Atlantic Forest. Nemésio (2010b), retrieving data from all published inventories in the “Centro de Endemismo Pernambuco”, assumed that 25 orchid-bee species were known to occur in the region. That study, however, recorded three species of *Eufriesea* not collected in the present study since no species of *Eufriesea* was apparently active during field samplings. On the other hand, seven species of *Euglossa* are here recorded for the first time in the “Centro de Endemismo Pernambuco” and are dealt with below.

##### 4.4.1 *Euglossa adiastrata* Hinojosa-Díaz, Nemésio and Engel, 2012

This species was considered in previous studies in the Atlantic Forest as *Euglossa augaspi* Dressler, 1982c. It is a common species in “Hileia Baiana” (the Atlantic Forest of southern Bahia and northern Espírito Santo), occurring in most forest remnants (Nemésio, 2011a, b, 2013a-d), and reaching the northeastern portion of the state of Minas Gerais (Nemésio, 2012c). The records of this species in ESEC Murici and Usina Serra Grande extend its known geographic distribution over 650 km northwards.

##### 4.4.2. *Euglossa aratingae* Nemésio, 2009

This species is apparently widespread in southeastern Brazil (Nemésio, 2009) and its previous northernmost record in the Atlantic Forest was in southern Bahia. The records presented here extend its geographic distribution over 700 km northwards. There is also a male recently collected in the state of Paraíba currently deposited in the Entomological Collection of the Universidade Federal de Minas Gerais (UFMG), which would represent the northernmost record of this species in the Atlantic Forest.

##### 4.4.3. *Euglossa bembéi* Nemésio, 2011d

This species had already been recorded for the state of Pernambuco (at RPPN Frei Caneca) by Darrault et al. (2006) (as *Eg. ioprosopa* Dressler, 1982b). It is here recorded for Alagoas for the first time, at ESEC Murici. Its discontinuous distribution (it was not collected in the intermediately located Usina Serra Grande) may be partially explained since this species apparently prefers higher elevations (see Nemésio, 2011d, 2012c; Nemésio

and Vasconcelos, 2013) and sites at Usina Serra Grande were those situated at the lowest elevations.

#### 4.4.4. *Euglossa carinilabris* Dressler, 1982c

This is another species considered to be found only in “Hileia Baiana” by Nemésio (2009). Nevertheless, it seems to be rarely attracted to scent baits. During a recent inventory throughout the “Hileia Baiana”, over 15,000 orchid-bee males were collected (see locations in Nemésio et al., 2012) and only one specimen of *Eg. carinilabris* was collected. The record of this species at RPPN Frei Caneca extended its known geographic distribution over 700 km northwards.

#### 4.4.5. *Euglossa hemichlora* Cockerell, 1917

This species is not restricted to the Atlantic Forest, being also present in the Amazon Basin (in most studies it is treated as *Eg. galianii* Dressler, 1982d, a junior synonym). In the Atlantic Forest it had been recorded in Rio de Janeiro (Tonhasca Junior et al., 2002), Minas Gerais (Nemésio and Silveira, 2006), Espírito Santo (Nemésio, 2011b) and Bahia (Nemésio, 2013d). The present record represents a range extension of over 700 km northwards, but it must be pointed out that specimens belonging to this species have been also collected in the states of Ceará and Piauí, and currently deposited at the Entomological Collection of Universidade Federal de Uberlândia.

#### 4.4.6. *Euglossa monnei* Nemésio, 2012a

This species was recently described from “Hileia Baiana”, where it was recorded in low abundance (only 14 males collected among over 15,000 orchid bees) from Linhares, in Espírito Santo, to Igrapiúna, in Bahia (see Nemésio, 2012a). The present record extends its known geographic distribution over 650 km northwards from Igrapiúna.

#### 4.4.7. *Euglossa pepeii* Nemésio and Engel, 2012

This small *Euglossa* was described from Parque Nacional do Pau Brasil (Porto Seguro, Bahia) based on only four specimens. It has been subsequently found in Linhares (Nemésio, 2013b) and, now, in ESEC Murici, greatly extending its geographic distribution.

#### 4.4.8. *Euglossa viridis* (Perty, 1833)

This species seems to present a wide distributional range in South America, occurring in the Amazon Basin and the Atlantic Forest. In the latter biome, it was recorded from the state of Paraná, in the south (Giangarelli and Sofia, 2011) to Bahia, in the north (Nemésio, 2011a). Since this species is weakly attracted to common scents in orchid-bee inventories, its rareness seems to be an artifact. The record of this species at RPPN Frei Caneca is the northernmost record for this species in the Atlantic Forest.

Another species that deserves further consideration, especially concerning nomenclature, is *Euglossa calycina* Faria and Melo, 2012 (treated as *Euglossa mixta* Friese, 1899 by Nemésio, 2009 and as *Eg. iopyrrha* Dressler, 1982b by Darrault et al., 2006). Additionally, although *Euglossa marianae* Nemésio, 2011b had already been recorded

for Alagoas (at ESEC Murici, as *Eg. analis* Westwood, 1840 – see Nemésio, 2010b) – it is here recorded for Usina Serra Grande for the first time (it was not listed by Darrault et al., 2006), which represents the northernmost record for this species.

At least four species should be added to the 29 species collected in the present study: *Eufriesea atlantica* Nemésio, 2008, *Ef. mussitans* Fabricius, 1787, *Ef. nordestina* Moure, 1999 and *Ef. pyrrhopyga* Faria and Melo, 2011 were already recorded for the “Centro de Endemismo Pernambuco”. Thus, orchid-bee richness in the region reaches at least 33 species, more than 50% of all Atlantic Forest species. Some species weakly attracted to synthetic scents, as *Exaerete dentata* (Linnaeus, 1758), are expected to occur in the region. This latter species, for example, occurs, in eastern Brazil, from Piauí (Perty, 1833, as *Chrysanthea nitida*), in the north, to São Paulo, in the south. According to GAR Melo (personal communication), a specimen of *Ex. salsai* Nemésio, 2011c from Pernambuco is also deposited in the Entomological Collection of the Universidade Federal do Paraná. Given the number of species formerly believed to be endemic in “Hileia Baiana” now found to also occur in the “Centro de Endemismo Pernambuco”, it would be no surprise if *Eulaema seabrai* Moure, 1960 and *Euglossa cyanochlora* Moure, 1996, two species weakly attracted to synthetic scents (the latter one considered to be endemic in “Hileia Baiana”), were collected in the region in the future.

The above considerations show that the “Centro de Endemismo Pernambuco” holds one of the richest orchid-bee faunas of the entire Atlantic Forest, only surpassed by that recorded in “Hileia Baiana”, with more than 40 species ever recorded (reviewed by Nemésio, 2013d). Diversity is also extremely high in the region, with the diversity recorded for ESEC Murici ( $H' = 2.55$ ) being one of the highest ever recorded for the entire Neotropical region (see Storck-Tonon et al., 2009, p. 700-701 for a review). On the other hand, the number of endemic species in the “Centro de Endemismo Pernambuco” is particularly low: only three species (*Eufriesea pyrrhopyga*, *Euglossa perpulchra*, and *Eulaema felipei*) were exclusively collected in this region to date. The new records presented in this study, however, show that the number of endemic species formerly believed to occur in “Hileia Baiana” was overestimated, since many endemics to the latter region were now found to occur in the “Centro de Endemismo Pernambuco”, with the sole exception of *Euglossa cyanochlora*. This finding strongly suggests that both subunits of the Atlantic Forest, well characterised by vegetation and other animal groups as strongly distinct, are apparently a continuous and indistinct biome for orchid bees, the rarest species of *Euglossa* and *Eulaema* in “Hileia Baiana” are also the rarest species in the “Centro de Endemismo Pernambuco”. The absence of some species currently found in “Hileia Baiana” from the “Centro de Endemismo Pernambuco”, such as *Euglossa cognata* Moure, 1970, may be just a consequence of the more dramatic devastation that took place in the Atlantic Forest of Alagoas, Pernambuco and Paraíba.

#### 4.5. Conservation

Only a few forest fragments remain in the “Centro de Endemismo Pernambuco” and none exceeds 10,000 ha (Ribeiro et al., 2009). The few forest areas left are, thus, extremely threatened, although still holding a huge diversity of life forms (Cardoso da Silva and Tabarelli, 2000; da Silva and Tabarelli, 2001; Uchoa Neto and Tabarelli, 2002; Goerck and Wege, 2005; Olmos, 2005; Asfora and Pontes, 2006; Nemésio, 2010b). The situation of these few areas is dramatic because strong anthropogenic pressures still exist in the region (reviewed by Nemésio, 2010a, b) and predicted climatic changes can heavily impact the forest remnants (Williams et al., 2007), changing their suitability for many species. Concerning orchid bees, two species deserve particular attention: *Euglossa marianae* and *Eulaema felipei*.

*Euglossa marianae* is perhaps the most sensitive forest-dependent of all orchid-bee species in the Atlantic Forest. Nemésio (2011b) realized that this species was only recorded in 13 forest remnants along the Atlantic Forest, the smallest of them with ca. 3,000 ha (ESEC Murici, see Nemésio, 2010b). The record of this species in Alagoas by Nemésio (2010b) was mysteriously ignored by Faria and Melo (2012), although these latter authors followed Nemésio’s (2010b) data on the close ally *Euglossa calycina* for the region. Nine specimens were collected previously in ESEC Murici (Nemésio, 2010b) and in the present study we demonstrate that this species also occur at Usina Serra Grande, where it is one of the most abundant species (Table 1), especially at sites in the interior of the forest (as previously suggested by Tonhasca Junior et al., 2002 and Nemésio and Silveira, 2006, as *Eg. analis*). The presence of this species at Usina Serra Grande is outstanding, because it apparently remained unnoticed in a previous sampling in that area (Darrault et al., 2006). This species was not found at RPPN Frei Caneca, a smaller forest patch, supporting Nemésio’s (2011b) point of view that ESEC Murici, and now Usina Serra Grande, present the smallest area possible to maintain viable populations of this sensitive species. Since many small fragments persist between both preserves, which are separated by ca. 40 km, there is a remarkable opportunity to test if they function as corridors for this species or if both populations are isolated. Anyway, any further deforestation in both areas may add a potential risk of extirpating this species from the only two areas where this species is known to occur in the “Centro de Endemismo Pernambuco”.

*Eulaema felipei* presents a still more dramatic situation if compared to *Euglossa marianae*. Although apparently as sensitive as the latter species, *El. felipei* is only known from ESEC Murici. This species would be expected to occur at both Usina Serra Grande and RPPN Frei Caneca, close to ESEC Murici. Nevertheless, our field work carried out simultaneously in all three areas revealed that it is absent from the latter two. We can only speculate about the reasons why it is restricted to ESEC Murici. Pristine vegetation can be found more abundantly in ESEC Murici than in the other two areas. Moreover, ESEC Murici is

closer to the coast, presenting the highest humidity levels. Whatever the reasons, the fact is that *El. felipei* was never recorded outside ESEC Murici in any previous field study in the “Centro de Endemismo Pernambuco” (Bezerra and Martins, 2001; Martins and Souza, 2005; Milet-Pinheiro and Schlindwein, 2005; Darrault et al., 2006; Farias et al., 2007, 2008; Moura and Schlindwein, 2009; this study). If it is true, we hypothesise that a high level of endogamy may be present in this species, one additional factor, besides (and most probably a consequence of) its restricted geographic distribution, to threaten this species. Further samplings in nearby forest remnants are needed to search for other populations of this species and urgent measures are needed to protect other forest patches in the area. Except for ESEC Murici, almost all other forest remnants in the region are situated in private areas and most of them are completely unprotected.

#### Acknowledgements

The Brazilian government, through the environmental departments IBAMA and ICMBio, provided the collecting permits (#19100-1, 29472-1). Mr. Gustavo Jardim Pedrosa da Silveira Barros, owner of RPPN Frei Caneca, and Mr. Paulo, from Usina Serra Grande, kindly allowed and supported our research in the areas under their responsibilities during our field work. We are most grateful to Prof. Angelo Machado, who partially supported this study.

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