

# The orchid-bee fauna (Hymenoptera: Apidae) of Acre state (northwestern Brazil) and a re-evaluation of euglossine bait-trapping

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## Abstract

Male orchid bees were sampled with chemical baits from May 1996 to March 1998 in two forested areas in the state of Acre, Brazilian Amazonia. The two most used sampling methods in euglossine studies were used simultaneously: insect nets and bait traps. We collected 1,744 euglossine specimens belonging to at least 33 species. Of these, 1,221 were collected with insect nets and 523 in bait traps. *Eulaema cingulata* (Fabricius) and *Eulaema meriana* (Olivier) were the commonest species in both the Parque Zoobotânico and the Catuaba Reserve, followed by *Euglossa amazonica* Dressler, *Euglossa ignita* Smith, and *Euglossa mixta* Friese. Due to a combination of 'dominant species' and the specific composition of the orchid-bee fauna of the studied areas, the Acrean orchid-bee fauna has shown to be quite different from other areas sampled in Amazonia. As the sampling effort with bait traps was twice as higher as that with insect nets, the efficiency of the insect net method was 4.7 times greater, in average, than that with bait traps. Thirty-two of the 33 species were collected with insect nets, whereas only 24 species were collected with bait traps. The resulting community of orchid bees was also different. The large bees of the genus *Eulaema* Lepeletier were much more abundant in bait-trap (74% - 79%) than in insect net samples (37% - 39%). The considerable differences in efficiency between insect nets and bait trap collections suggest that the sole use of bait traps should be avoided in orchid bee studies or restricted to situations in which the use of insect nets is impossible.

**Key words:** Amazonia, chemical baits, euglossine bees, Hymenoptera, insect nets, Insecta.

## Introduction

The orchid bees belong to the Euglossina (Hymenoptera: Apidae: Apini), a strictly Neotropical group of bees occurring from northern Argentina to northern Mexico (Pearson & Dressler, 1985). Their males are remarkable for collecting aromatic compounds at flowers and storing them in a special organ in their posterior tibiae (Dodson et al., 1969). The biological function of those compounds is not known, although they are believed to act in the recognition of males by females during mating (Eltz et al., 1999).

Since aromatic compounds attractive to orchid bees were discovered in the 1960's (Dodson et al., 1969), several inventories have been carried out in an attempt to characterize

the local faunas of these neotropical bees (e. g. Bennett, 1972; Pearson & Dressler, 1985; Becker et al., 1991; Rebêlo & Garófalo, 1991, 1997; Morato et al., 1992; Oliveira & Campos, 1995; Neves & Viana, 1997, 1999; Bonilla-Gomez, 1999; Bezerra & Martins, 2001; Tonhasca Jr. et al., 2002; Nemésio, 2003, Nemésio & Faria Jr., 2004). Although some parts of the Amazon Basin were already sampled for orchid bees (e. g. Pearson & Dressler, 1985; Bonilla-Gómez & Nates-Parra, 1991; Morato et al., 1992; Oliveira & Campos, 1995), the euglossine bee fauna of the state of Acre remains virtually unknown.

Orchid bees have also become a favorite subject in ecological studies (e.g. Powell & Powell, 1987; Becker et al., 1991; Morato, 1994; Tonhasca Jr. et al., 2002; Nemésio & Silveira, 2006), due to the ease of attracting them to the baits. Methodologies for sampling such faunas, however, have been far from uniform. Comparisons among studies and general approaches used for studying the distribution of these bees are two major problems, as noted by Morato (1998). The number of chemical baits used to attract bees varies greatly among studies, as do the

Received: 08.VI.05

Accepted: 15.VIII.06

Distributed: 30.XII.06

durations of the seasonal and daily sampling periods. Differences in sampling methods are an important issue, which makes direct comparisons among studies difficult. In some studies, bees were collected with insect nets (e. g. Rebêlo & Garófalo, 1991, 1997; Morato et al., 1992; Neves & Viana, 1997, 1999; Bezerra & Martins, 2001; Tonhasca Jr. et al., 2002; Nemésio, 2003, Nemésio & Faria Jr., 2004), while in others bees were collected with baited traps (e. g. Bennett, 1972; Becker et al., 1991; Oliveira & Campos, 1995). In certain instances, bees were mostly identified in the field, but only seldom collected (e.g. Bonilla-Gomez, 1999).

The lists of species presented in most papers are believed to represent the orchid bee fauna of particular areas, although Armbruster (1993) considered that the sampled fauna may be only representative of the particular site where sampling was carried out, and not of the total surrounding area. Regardless of this debate, no work to date has focused attention on the possible differences in efficiency between the two most used methods for sampling orchid bees — hand-netting and bait-trapping. Becker et al. (1991) had already noticed that bait trapping might not be much efficient. However, since their own study was only based on this methodology, no comparison could be made. Besides, the total number of captured bees in their study was very low (less than 300 specimens), which makes any inferences quite difficult. Only recently, Nemésio & Morato (2004) compared both methods and noticed that some bias may happen, but as the number of sampled bees was also very low, the issue remained open. In studies based on hand netting, pieces of paper or cotton are imbued in the chemical baits and exposed to the bees, generally suspended or attached to the vegetation. In these cases, the collector remains during all sampling period near the baits and tries to collect all bees attracted to them. On the other hand, in baited-trap studies, traps are left unattended in the field, generally during early morning, and the collected bees are removed at the end of the day or in the beginning of the following day.

The main aims of this paper were to sample, for the first time, the orchid-bee fauna of eastern state of Acre, Brazilian Amazonia, and to compare the efficiency of the two most commonly used methods of studying orchid bee faunas — hand-netting and bait-trapping.

## Material and Methods

### Study Area

This study was carried out at the Parque Zoobotânico (PZ) and in the Catuaba Reserve (CR), both belonging to the Universidade Federal do Acre and located in the municipality of Rio Branco, in the Brazilian state of Acre (09°15' S - 10°30' S; 67°00' W - 69°30' W; ~ 200 m above sea level). The regional climate is tropical, hot and humid (transition from Am to Aw, according to Köppen's classification), with annual average temperature between 22°C and 24°C and the total annual precipitation between 1,900 mm and 2,100 mm. The Catuaba Reserve (10°04' S; 67°37' W) is an 820 ha area covered, basically, by well-preserved primary Tropical Humid Forest. The *Terra Firme* forest occupies ca. 77% of the CR, although it presents a conspicuously open canopy and high concentration of

palms (Arecaceae) and bamboos (Poaceae) (EFM, pers. obs.). The CR is surrounded by disturbed areas, such as secondary forests and pastures. The Parque Zoobotânico is a 100 ha area inside the campus of the Universidade Federal do Acre (09°56'43" S - 67°52'28" W), with a total area of 252 ha (Guilherme, 2001). The vegetation at PZ is essentially secondary forest, although some typical primary-forest species are found, such as *Hevea brasiliensis* (Euphorbiaceae) and *Bertolletia excelsa* (Lecythidaceae). Some patches of bamboo (*Guadua weberbaueri*) are also found (Guilherme, 2001).

### Sampling

Male orchid bees were attracted with seven aromatic compounds (1,8-cineole, benzyl acetate, benzyl benzoate, eugenol, methyl salicylate, skatole, and vanillin), during fourteen months between May 1996 and March 1998 (in the Parque Zoobotânico, from August to November, 1996; February, March, and June to December, 1997; and March 1998. In Catuaba Reserve, in May and from August to November 1996; and in February to April and June to November 1997). As Armbruster (1993) suggested that a single site may not be enough to sample the whole fauna of a given area, each month a different site was chosen for collecting bees. Once sampled in a month, that specific site was never sampled again. In the chosen sites, three points were established: in the first point, bees were collected with hand nets when arriving at cotton packs soaked with the chemical baits. These lures were hanged by a string at about 2 m above the soil surface and distant ca. 2 m from each other. The other two sites were established ca. 500 m north and ca. 500 m south of the first point. In each of these two points, seven baited traps (see below) each one containing one of the seven different aromatic compounds, were also hanged by strings at about 2 m above the soil surface and distant at least 2 m from each other. Both sets of bait traps were installed exactly at the same day and time in which the hand net sampling was carried out. Sampling was always carried out between 07:00-15:00. Although 1,8-cineole is highly volatile, since the traps set with this lure could not be replenished, the cineole lure in the "hand netting area" was also not replenished, so that comparable data was generated. All specimens captured with hand nets or traps were killed with ethyl acetate and pinned. They are currently deposited at the Entomological Collection of the Taxonomic Collections of the Universidade Federal de Minas Gerais. Bees were identified with the aid of taxonomic keys (e.g. Dressler, 1982 a,b,c; Nates-Parra & Bonilla-Gómez, 1991; Roubik & Hanson, 2004) and by comparison with specimens previously identified by specialists.

### Bait traps

The bait traps used were based on those described by Campos et al. (1989). Plastic bottles (21 cm high) were used as traps. Three holes (1.5 cm in diameter each) were made at the heights of 8 cm, 10 cm, and 12 cm from the base, in opposing sides of the bottle. A plastic cylinder ca. 2.5 cm long was placed in each hole, so that bees could walk inside it to enter the trap. This structure is absent from the traps described by Campos et al. (1989), in which the bees entered the trap directly through the holes. These plastic cylinders were placed to avoid or diminish the possibility of escapes. Inside each bottle, a piece of cotton in

which the scent baits were imbibed hanged from a 12 cm long string attached to the bottle lid.

#### Data analysis

To assess the efficiency of each sampling method (insect net  $\times$  bait trap) in terms of size of the samples generated, the number of individuals collected was divided by the number of samples (14), for the hand-net sampling, and by twice the number of samples (28) for bait traps (since two sets of traps were used simultaneously each month). To compare the efficiency in terms of the number of species recorded, two sets of data were used: (i) all the individuals collected with each method and (ii) the same data obtained in (i) excluding the singletons. Singletons are defined here as those species represented by a single specimen for one method and area.

#### Results

The two sampling methods, in both areas together, yielded 1,744 bees belonging to 33 species. Of these, 1,024 were captured in the Catuaba Reserve and 720 in the Parque Zoobotânico. The hand netting procedure yielded 1,221 bees, whereas 523 were captured by the baited traps (Table I). In the Parque Zoobotânico, collecting rates were of 36.1 specimens by sampling date for hand netting and of 7.6 for trapping. In the Catuaba Reserve, those values were 51.1 and 11, respectively. In the Parque Zoobotânico the collecting rate for hand-netting was 4.75 times greater than with bait traps, a very similar result to that obtained in the Catuaba Reserve (4.65). The large specimens of *Eulaema* Lepeletier were much more abundant in the bait-trap (between 74 % and 79%) than in the hand-net samples (between 37% and 39%). When all individuals of all species are considered, 32 of the 33 species were collected with insect nets, whereas only 24 species were collected with the bait traps. Only one species collected with bait traps was not collected with insect nets (Table I). When singletons (as defined in the material and methods) were excluded, 27 species remained. All of them were represented in the hand-net samples (25 in CR and 19 in PZ), whereas only 16 were captured with the bait traps (15 in CR and nine in PZ). Thirty-eight individuals of *Euglossa* Latreille could not be identified to species because they were lost during transportation. They were treated as *Euglossa* spp. and included in Table I so that an accurate result was obtained when the percentages of individuals belonging to *Eulaema* and *Euglossa* were calculated.

*Eulaema cingulata* (Fabricius) and *Eulaema meriana* (Olivier) were the commonest species in both the Parque Zoobotânico and the Catuaba Reserve, followed by *Euglossa amazonica* Dressler, *Euglossa ignita* Smith, and *Euglossa mixta* Friese.

#### Discussion

##### The Acrean orchid-bee fauna

As already observed by Nemésio & Morato (2004) for a previous sample of the Acrean orchid-bee fauna, the high frequencies of *Eulaema* species are outstanding. That study,

however, was based on a small sample of bees ( $n = 254$ ). The present results, however, based on almost 2,000 bees (a number similar to other long term studies in the Amazon Basin – Morato et al., 1992; Oliveira & Campos, 1995) contradicts the general belief that the abundance of species of *Eulaema* in the Atlantic Forest domain (e. g. Rebêlo & Garófalo, 1991, 1997; Bezerra & Martins, 2001; Tonhasca Jr. et al., 2002; Nemésio, 2003), a highly fragmented landscape, would be an indication of disturbed areas. The contrary would be true for most surveys in the Amazon (Pearson & Dressler, 1985; Powell & Powell, 1987; Morato et al., 1992; Oliveira & Campos, 1995) where, in spite of being much more diverse, *Eulaema* species generally represent a small fraction of the total number of orchid bees. Even *Eulaema nigríta* Lepeletier, rarely collected in Amazonia, is consistently present in Acrean samples (ca. 3.0% in Parque Zoobotânico). The absence of *Euglossa stilbonota* Dressler, the commonest species in Central Amazonia (Powell & Powell, 1987; Becker et al., 1991; Morato et al., 1992; Oliveira & Campos, 1995), is also remarkable, and was also noticed in the Humaitá Reserve, state of Acre (Nemésio & Morato, 2004). This species is also absent from the Tambopata Reserve, in the Peruvian Amazonia (Pearson & Dressler, 1985), and from the Parque Nacional da Serra do Divisor (Nemésio & Morato, 2006), western state of Acre, seeming not to be present in westernmost Amazonia. Moreover, a recent study (Nemésio, 2005) also did not record *Eg. stilbonota* in northern Amazonia, what may be an indication that this species is restricted to Central Amazonia.

The dominant species at the Parque Zoobotânico and the Catuaba Reserve were completely different from those found in studies carried out in Central Amazonia, in the Brazilian state of Amazonas, by Powell & Powell (1987), Becker et al. (1991), Morato et al. (1992), and Oliveira & Campos (1995). However, regardless the differences in the community structure and among the lists of species of the orchid-bee fauna of these two Acrean areas and those in other parts of Amazonia, the 33 species found in southeastern Acre compose a highly diverse assemblage. For example, in the Tambopata Reserve, Peruvian Amazonia, ca. 40 species were recorded in a long term study (Pearson & Dressler, 1985), approximately the same figure obtained by Oliveira & Campos (1995) for Central Amazonia.

##### Insect nets $\times$ bait traps

The results presented here clearly show that the dataset obtained may vary greatly, depending on the sampling method used. If the data presented here had come exclusively from trap samples, the relative bee-abundance reported would be about 20% of the one actually observed and the observed species richness would represent ca. 75% of that observed with insect nets. Since in hand netting almost all individuals attracted to the baits are collected (although the odd specimen always escapes), the results obtained through this method should be regarded as those most closely reflecting reality. Thus the use of bait traps underestimates both the abundance and the species richness of local euglossine faunas, distorting, in addition, our view of the community structure. In the sites studied here, the four commonest species were quite different, depending on the sampling method employed (Table I), with the exception of the most common of all, *El. cingulata*, which was the most abundant species in the samples, regardless of the method. However, its

**Table I** - Number of individuals of each species collected at Parque Zoobotânico (PZ) and Catuaba Reserve (CR) with bait traps and insect nets.

	Baited traps		Insect nets	
	PZ	CR	PZ	CR
<i>Eufriesea eburneocincta</i> (Kimsey)	-	-	-	1
<i>Ef. flaviventris</i> (Friese)	-	-	1	-
<i>Ef. fragrocara</i> Kimsey	-	-	-	10
<i>Ef. ornata</i> (Mocsáry)	-	3	-	7
<i>Ef. pulchra</i> (Smith)	1	2	-	3
<i>Ef. superba</i> (Hoffmannsegg)	-	1	1	-
<i>Ef. vidua</i> (Moure)	-	1	-	1
<i>Euglossa allosticta</i> Moure	-	1	12	1
<i>Eg. amazonica</i> Dressler	1	6	67	83
<i>Eg. analis</i> Westwood	-	-	1	-
<i>Eg. augaspis</i> Dressler	7	4	24	29
<i>Eg. bursigera</i> Moure	1	-	4	-
<i>Eg. chalybeata</i> Friese	-	1	6	52
<i>Eg. cognata</i> Moure	1	-	2	11
<i>Eg. decorata</i> Smith	1	-	-	4
<i>Eg. despecta</i> Moure	-	-	5	2
<i>Eg. ignita</i> Smith	12	7	87	32
<i>Eg. imperialis</i> Cockerell	-	8	2	31
<i>Eg. intersecta</i> Latreille	-	-	-	4
<i>Eg. mixta</i> Friese	2	14	46	69
<i>Eg. modestior</i> Dressler	1	-	24	12
<i>Eg. pleosticta</i> Dressler	-	-	-	2
<i>Eg. townsendi</i> Cockerell	-	-	5	11
<i>Euglossa</i> spp.	-	10	1	27
<i>Eulaema bombiformis</i> (Packard)	-	4	-	1
<i>El. cingulata</i> (Fabricius)	73	111	96	196
<i>El. meriana</i> (Olivier)	58	108	51	64
<i>El. mocsaryi</i> (Friese)	3	4	14	15
<i>El. nigrata</i> Lepeletier	22	2	6	3
<i>El. polyzona</i> (Mocsáry)	-	-	1	-
<i>El. pseudocingulata</i> Oliveira	12	-	19	3
<i>Exaerete frontalis</i> (Guérin-Méneville)	1	5	2	13
<i>Ex. smaragdina</i> (Guérin-Méneville)	17	17	29	28
<i>Ex. lepeletieri</i> Oliveira & Nemésio	1	-	-	-
<b>Total number of species</b>	<b>17</b>	<b>18</b>	<b>23</b>	<b>27</b>
<b>Grand total for species</b>	<b>24</b>		<b>32</b>	
<b>Total number of individuals</b>	<b>214</b>	<b>309</b>	<b>506</b>	<b>715</b>
<b>Total number of individuals of <i>Eulaema</i></b>	<b>168</b>	<b>229</b>	<b>187</b>	<b>282</b>
<b>% <i>Eulaema</i></b>	<b>79</b>	<b>74</b>	<b>37</b>	<b>39</b>

frequency varied from 19% (PZ) and 27% (CR) for hand netting to 34% and 36%, respectively, when traps were employed. No *Euglossa* species appears among the four commonest species in PZ, when only bait trapping is considered, and only one such species appears, as the fourth commonest, in CR. On the other hand, when hand-net samples are considered, the second and third commonest species in both areas belong to *Euglossa*. *Eg. amazonica*, one of the commonest species in hand-net samples, both in PZ and CR, is a singleton in the PZ trap samples. Moreover, several more *Euglossa* species are singletons in bait-trap samples than in hand-net samples.

The interpretation of most data obtained in euglossine studies should be, then, reconsidered in the light of the results presented here. For example, Peruquetti et al. (1999), sampling orchid bee fauna with bait traps at Parque Estadual do Rio Doce, a large Atlantic Forest remnant in southeastern Brazil, found that *El. cingulata* was the commonest species (53% of all euglossine specimens) and *Euglossa analis* Westwood represented only 2% of the collected individuals. Nemésio & Silveira (2006) studied the same area one year later using insect nets, and found that *Eg. analis* represented ca. 47% of the euglossine community, whereas *El. cingulata* responded for 20% of the specimens collected. The high numbers of bees obtained using baited traps by Oliveira & Campos (1995) in Central Amazon (2,422 bees collected), must be compared with caution to this and other studies in which bait traps were used. This is so, firstly, because that study was carried out during a whole year, with four sets of traps. More important, contrary to most studies with baited traps, in which the traps are left unattended for many hours, the study by Oliveira & Campos (1995) involved researchers checking the traps every hour (see Oliveira, 1999) and this could prevent many bees from escaping the traps.

The results presented here suggest, thus, that the sole use of baited traps should be avoided or used only as a complimentary method when sampling orchid bees. It may be hypothesized that where the large individuals of *Eulaema* are abundant, as in the areas sampled for the present work, individuals of *Euglossa* are often disturbed by *Eulaema* while trying to enter the trap. Aggressive behavior of this nature has been observed in the field (AN, pers. obs.). Moreover, males of at least some species of *Euglossa* are extremely aggressive while trying to approach the pieces of cotton containing aromatic compounds. One of us (AN) has seen several such conflicts involving individuals of *Eg. analis* in the Parque Estadual do Rio Doce (state of Minas Gerais – unpubl. data). It is possible that conflicts between male *Eulaema* and *Euglossa* and also between different males of *Euglossa* prevent or delay the entrance of many male *Euglossa* into the traps, increasing the relative frequency of individuals of *Eulaema* in traps in relation to those observed in hand-net samples.

The lower abundance of individuals in traps, however, may involve other factors. *Eulaema* bees generally spend more time trying to collect the compounds than do *Euglossa* (AN, pers. obs.). That means that a single individual may “close” an entrance to the trap for several minutes before entering, whereas if it appeared in the presence of the researcher, the bee would be removed quickly by means of a hand net. Moreover, individuals of *Euglossa* escape more frequently from the traps than do *Eulaema* (AN and EFM, pers. obs.), and this may be due to two reasons: (i) the smaller size of *Euglossa*, which allows them to

escape through a small hole (the entrance of the trap), and (ii) the fact that *Eulaema*, spending more time trying to enter the trap, spend also more energy and thus, tire before finding their way out of the traps. These hypotheses, however, should be tested in the field in order to establish the reasons why the number of bees caught in the traps is so low compared to that obtained with insect nets.

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