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## *Eufriesea zhangii* sp. n. (Hymenoptera: Apidae: Euglossina), a new orchid bee from Brazil revealed by molecular and morphological characters

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### Abstract

*Eufriesea zhangii* Nemésio & Santos Júnior sp. n. is described from the ‘Parque Nacional de Ubajara’, state of Ceará, northeastern Brazil. This orchid-bee species is superficially similar to the blackish species belonging to the *Eufriesea mussitans* (Fabricius, 1787) species-group. Molecular and morphological (both external and genital) characters were used to demonstrate that *Eufriesea zhangii* sp. n. is distinct from morphologically similar *Eufriesea nordestina* (Moure, 1999) and *Eufriesea auriceps* (Friese, 1899). Molecular data vaguely suggested that its closest relative is *Eufriesea nigrohirta* (Friese, 1899). This new species, as well as the recently described *Eulaema quadragintanovem* Nemésio & Ferrari, 2012, seems to be geographically restricted to ‘brejos de altitude’—Atlantic Forest physiognomies at the top of mountains in northeastern Brazil—in the state of Ceará. Due to their conspicuous isolation, these areas appear to be a rich source of unknown species that may rapidly vanish due to environmental disturbances.

**Key words:** Atlantic Forest, ‘Brejo de altitude’, conservation, new species, orchid bee, taxonomy

### Introduction

The genus *Eufriesea* Cockerell, 1908 (Hymenoptera: Apidae: Apini: Euglossina) is the second most diverse genus of orchid bees and comprises about 65 species (Nemésio & Rasmussen 2011). Euglossina is an exclusively Neotropical group of insects widely known for their vivid metallic colors and the association between the males and orchids (see Dressler 1982). Despite being species-rich, *Eufriesea* is one of the least known groups of orchid bees, mainly due to the fact that its species are univoltine, highly seasonal, with adults usually only active for two or three months yearly, during the rainy season (see Kimsey 1982). As a consequence, relevant biological data (such as nesting behavior and plants visited) are missing for most species (Kimsey 1982; Ramírez *et al.* 2002); for example, precise geographic distributions of most species are unknown and, for many species, only one of the genders is known. This makes it difficult to study morphological variations in larger series in order to define species limits. Recently, some of these “unknown genders” have been described (*e.g.* Moure 1999; Nemésio 2005, 2011a; Ferrari & Nemésio 2011), and data on geographic distribution of some of the rarest species have become available (*e.g.* Nemésio & Silveira 2004; Nemésio 2011e).

Kimsey (1982) made the first attempt to understand the relationships within *Eufriesea* and to split the genus into subgroups. Nevertheless, the resulting subgroups were not grounded on formal phylogenetic hypotheses, and subsequent phylogenetic studies based on morphological (Faria Jr. 2009) and molecular (Ramírez *et al.* 2010) characters revealed that most of Kimsey’s (1982) subgroups seemed to be paraphyletic. Faria Jr. (2009) used 128 morphological characters of a small subset of 33 species of *Eufriesea* belonging to all 12 groups proposed by Kimsey (1982) in three analyses (equal weights parsimony, implicit weights parsimony, and Bayesian analysis) and found that only four species-groups resulted as monophyletic in all analyses. One of these groups is the *Eufriesea mussitans* (Fabricius, 1787) species group, delimited by Kimsey (1982) as comprising three species: *E. mussitans*, *E. tucumana* (Schrottky, 1902), and *E. violascens* (Mocsáry, 1898).

*Eufriesea tucumana* was interpreted by Nemésio & Rasmussen (2011) as a junior synonym of *E. mariana* (Mocsáry, 1896). The identity of *E. mussitans* is well-established (see Nemésio 2009). On the other hand, much debate still remains concerning the identity of *E. violascens*. Moure's (1976) study of *E. violascens* type specimens revealed that the original syntypic series consisted of two specimens belonging to different species, *E. violascens* proper [designated as lectotype by Moure (1967)] and *E. auriceps* (Friese, 1899). In spite of this, Kimsey (1982) treated both species as synonyms and virtually all records for *E. violascens* in the literature after 1982 [including, most probably, the phylogenetic studies by Faria Jr. (2009) and Ramírez *et al.* (2010)] may, in fact, refer to *E. auriceps* (see Nemésio 2009: 29–39).

We here follow Moure's (1976) interpretation that *E. violascens* and *E. auriceps* are distinct species and, as shown by illustrations presented in Nemésio (2009: 29–39), the structural characters of both species (based on female specimens) suggest they most probably do not even belong to the same species-group. If our interpretation is correct, *E. auriceps* is a widespread bee in Paraguay, northern Argentina, central, southern and southeastern Brazil and eastern Bolivia, and *E. violascens* is an endemic species in the Zongo river valley in Bolivia, following the interpretation by Nemésio (2009) and Nemésio & Rasmussen (2011). Thus, the *E. mussitans* group *sensu* Kimsey (1982) should be constituted of *E. mussitans*, *E. mariana* and *E. auriceps*. A second problem, however, concerns the identity of *E. auriceps* itself.

*Eufriesea auriceps* was described based on a single male from Asunción, Paraguay (Friese, 1899). Nemésio (2009: 29–39) considered that all predominantly black *Eufriesea* occurring in eastern Brazil belong to this species. Thus, according to his interpretation, three species were synonymized under *E. auriceps*: *E. danielis* (Schrottky, 1907), *E. nordestina* (Moure, 1999), and *E. aridicola* (Moure, Neves and Viana, 2001). *Eufriesea danielis* was described based on a single female from Asunción, the same type-locality of the male *E. auriceps*. Although the type-specimen of *E. danielis* is lost, there is a female deposited at 'Museu de Zoologia da Universidade de São Paulo' collected by Schrottky himself that perfectly matches Schrottky's (1907) description of the species – this specimen is illustrated in Nemésio (2009: 34), who considered it as the holotype, following Moure (1999). Later, Nemésio & Rasmussen (2011) considered that it may not indeed be the holotype, but suggested it should be instead designated as a neotype if the holotype is not found. Moure *et al.* (2012) seemed to agree with the above interpretation, stating that “although the type material is missing, and the future interpretation probably pending on designation of a neotype, considering the type locality of *E. danielis*, it is likely the female of *E. auriceps*, as previously interpreted by Nemésio (2009:29) (G. A. R. Melo, unpubl.)”.

Both *E. nordestina* and *E. aridicola* were synonymized under *E. auriceps* by Nemésio (2009) based on the study of photographs of the holotypes. This interpretation, however, was later questioned by Faria & Melo (2011:37) who argued that “some of the species treated by Nemésio (2009) as junior synonyms of *E. auriceps* (Friese, 1899) constitute distinct valid species...”. Since these same authors (Moure *et al.* 2012) consider *E. danielis* a potential junior synonym of *E. auriceps*, and *E. aridicola* a junior synonym of *E. nordestina*, they must refer to *E. nordestina*—although Faria & Melo (2011:37) also consider that there remains some undescribed species in this “complex”.

Lastly, but by no means less important, the type specimen of *E. auriceps* is missing. Moure (1967) suggested it is deposited at the American Museum of Natural History, New York (access number 26.522), but this specimen could not be located there (see Nemésio 2009). A specimen labeled as “type” is deposited at the 'Zoologisches Museum der Humboldt-Universität', Berlin, but it was collected in 1910 and obviously cannot be the type specimen (A. Nemésio, unpub. data). If, as suggested by Faria & Melo (2011), *E. auriceps* is a complex of cryptic species with some of them still undescribed, it is important to establish the actual identity of *E. auriceps*, especially if more than one species occur sympatrically at or close to the type locality of *E. auriceps*. This will require the designation of a neotype unless a true type specimen can be located.

Recent surveys from the westernmost limits of the Atlantic Forest in northeastern Brazil where orchid-bee faunas were not previously known revealed some unexpected results including the presence of new and apparently endemic species (Nemésio & Ferrari 2012). Few remote and isolated areas typically associated to the Atlantic Forest domain still exist in the state of Ceará (Galindo-Leal & Câmara 2003), and these areas are usually located on mountain tops (ranging from 500 m to 1,000 m a.s.l.) and known as 'brejos de altitude' – Atlantic Forest formations situated at the mountain tops, surrounded by more xeric environments, such as 'caatinga', at the lower areas. These areas are among the most threatened in the entire Atlantic Forest domain and may disappear in a few decades if urgent conservation policies are not established (Silva & Tabarelli 2000, 2001) or due to climatic changes (Williams *et al.* 2007).

After a survey of the orchid-bee fauna of 'Parque Nacional de Ubajara', in the municipality of 'Ubajara', in the state of Ceará, in April, 2012, by one of us (JESJ), about 40 specimens of predominantly black *Eufriesea* belonging to the *E. auriceps* complex aforementioned were collected. Among them, one specimen revealed to belong to a new species, which is here described and illustrated. Since this particular group of *Eufriesea* may be under study in the near future (cf. Faria & Melo 2011), recognition of this new species may be of importance for our understanding of its actual diversity.

## Material and methods

The specimens belonging to a previously unknown species were compared to specimens belonging to the *Eufriesea auriceps* complex, namely *E. auriceps* proper and *E. nordestina*. Since this group is potentially the subject of a forthcoming study, as anticipated by Faria & Melo (2011), we refrain here to introduce any nomenclatural acts such as designation of neotypes and/or synonymization or revalidation of species. We do, however, recognize that *E. nordestina* is a species distinct from *E. auriceps* (*contra* Nemésio 2009), and follow Moure *et al.* (2012) in this regard. All specimens were studied and measured under a Leica MZ12 microscope lit. Measurements for the new species were taken from the holotype.

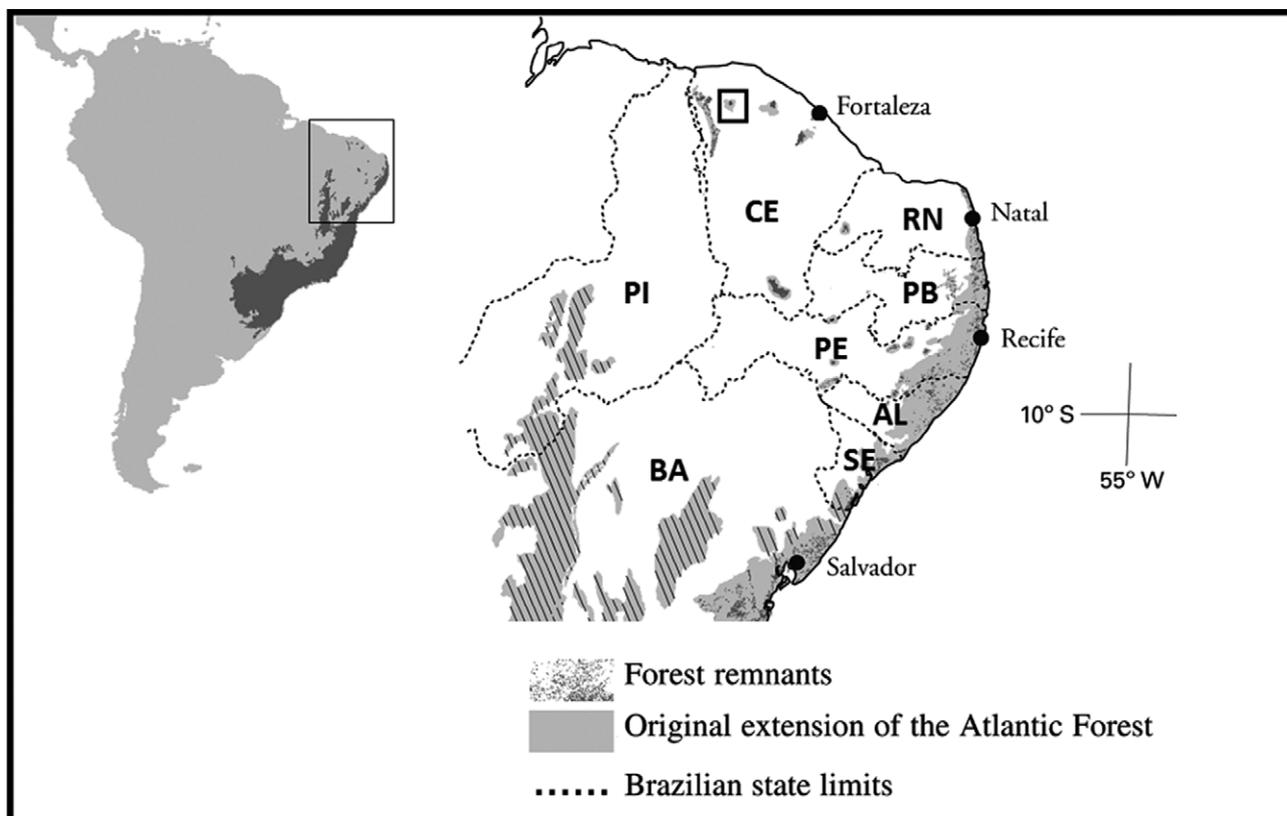
**Morphological terminology.** Terminology follows Michener (2007) and Nemésio (2009: 10). Metasomal terga and sterna are referred to as T1, T2, T3, etc., and S1, S2, S3, etc., respectively. Tongue length was measured as indicated by Kimsey (1982).

**Morphometric analysis.** Four measures were taken to compare sizes of 36 bees (35 *E. nordestina* and one undescribed species from Ceará): scutellum width (SCTW), scutellum length (SCTL), interorbital distance at the base of the scape (IOD), and eye length (EL). These characters were chosen because they are not influenced by the way the specimen is pinned and mounted, contrary to body length, for example, which may vary depending on the position of the metasoma or the extent each tergum is retracted. Means (and standard deviations) of all four measured characters were taken. Furthermore, all the bees were grouped and clustered using statistical software SAS (SAS Institute 1999) according to the measurements of the four characters employed.

**Molecular analyses.** DNA was extracted and purified using a modified phenol-chloroform method (Vilaça *et al.* 2006), and re-suspended in 40 µL of TE buffer. The 5' region of the mtDNA COI gene was amplified using the LepF1 and LepR1 primers (Hebert *et al.* 2004) in 30 µL polymerase chain reaction (PCR) mixes including 0.5 units of Taq DNA polymerase, 2 mM MgCl<sub>2</sub>, in 1x PCR buffer, 0.5 µM of each primer, 2.5 mM dNTPs and about 20 ng of genomic DNA. Amplification was carried out in a thermocycler using a program consisting of 5 min of denaturation at 94°C followed by 35 cycles of 30 s at 94°C, 40 s at 51°C, 1 min at 72°C, and a final extension for 10 min at 72°C. PCR products were visualized in a 0.8% agarose gel. All PCRs that generated a single product were purified using polyethylene glycol 20% (PEG) precipitation (Chaves *et al.* 2008). This purified PCR product was sequenced in both directions using the MegaBACE 1000 sequencer (GE Healthcare) with the same amplification primers following manufacturer's recommendations. The raw sequences were obtained using the Phred v 0.20425 program (Ewing *et al.* 1998). The final sequences were assembled with the programs Phrap v. 0.990319 (<http://www.phrap.org>) and Consed 19.0 (Gordon *et al.* 1998); the latter was used to view and edit the high quality consensus sequences. Alignments of the consensus sequences for all individuals were generated using MUSCLE (Edgar 2004) implemented in the program MEGA 5.01 (Tamura *et al.* 2011). The average genetic distances between sampled species of *Eufriesea* were obtained by the program Mega 5.01, using the model of Kimura 2 parameters. The best fit substitution model for the COI sequences was estimated by the program Modeltest 3.7 (Posada & Krandaall 1998), which indicated the GTR+I model. Phylogenies were generated with Bayesian Inference (BI) in MrBayes 3.1 (Huelsenbeck & Ronquist 2001), with Maximum Parsimony (MP), and Maximum Likelihood (ML) algorithms using the program PAUP\* (Swofford 2002). Inferences of trees by MP and ML methods were performed by heuristic search, with the following parameters: random stepwise addition and branch swapping (TBR - tree bisection and Reconnection). The bootstrap method was used as a measure of branch support of the recovered phylogenies in both programs, using a total of 10,000 replications. Phylogenetic analyses using BI used two sets of Markov chains, each containing three hot chains and one cold, with 10 million of generations to ensure statistical convergence between both sets.

**Type material repositories.** All new type material mentioned in the present study is currently deposited at the Entomological Collection (Centro de Coleções Taxonômicas) of the 'Universidade Federal de Minas Gerais', Belo Horizonte, Brazil (UFMG).

**Taxonomy.** Taxonomy follows Nemésio & Rasmussen (2011), except that we follow Moure *et al.* (2012) and consider *E. nordestina* as a valid species.

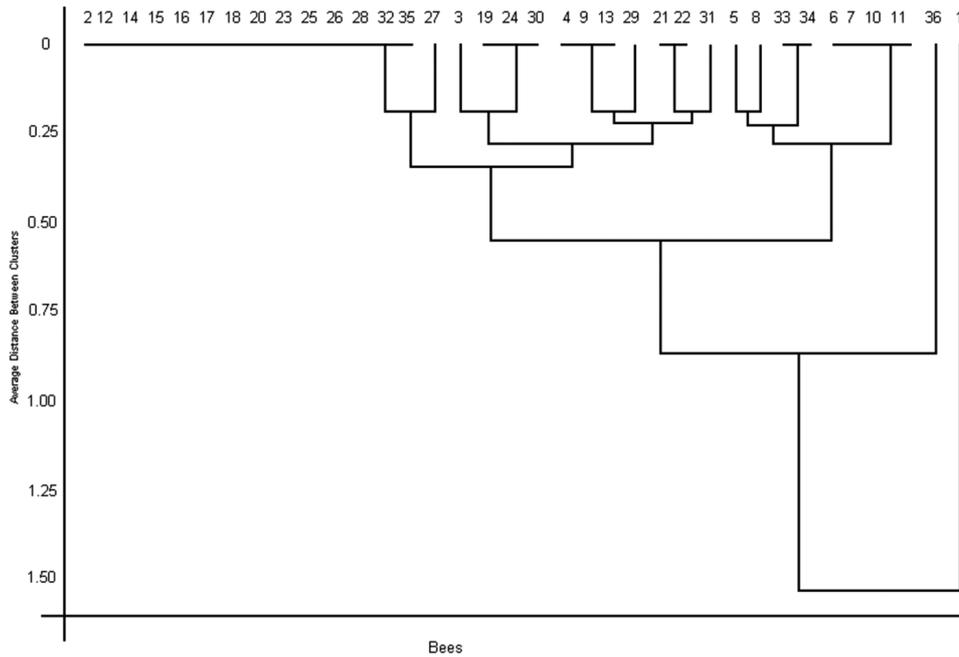


**FIGURE 1.** Map illustrating northeastern Brazil and the type locality (black square) of *Eufriesea zhangii* sp. n. Acronyms represent Brazilian states, as following: AL: Alagoas; BA: Bahia; CE: Ceará; PB: Paraíba; PE: Pernambuco; PI: Piauí; RN: Rio Grande do Norte; SE: Sergipe.

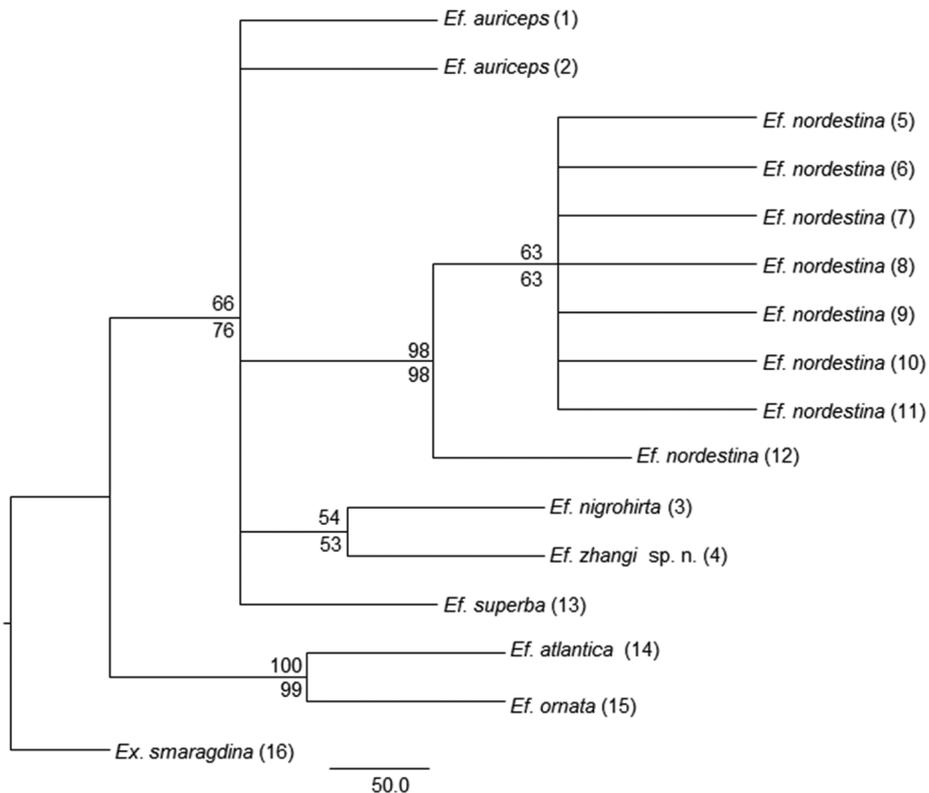
## Results

Specimens identified as *E. nordestina* revealed to belong to a single taxon based on morphological (see Diagnosis) and molecular characters. The clustering of the bees based on the four morphometric characters have shown that *E. nordestina* is consistently smaller than the single specimen here hypothesized to belong to a new species (Figure 2, Appendix 1). The same can be said when molecular data are taken into consideration (Figure 3). The specimen being considered new is nested inside a paraphyletic cluster of specimens belonging to the group *auriceps*. All phylogenetic reconstruction methods placed the putative new species as the sister species of *E. nigrohirta* (Friese, 1899), although we have to take into consideration that not all species belonging to the *E. mussitans* species-group are represented here. The Bayesian analysis groups these two taxa with posterior probability = 0.7, this tree was not presented here because it has a structure very similar to the ML and MP trees. The observed genetic distance between *E. nigrohirta* and the new species was 1.5%, the same interspecific distance observed between *E. nigrohirta* and *E. auriceps* (Table 1). Besides, we present also COI gene distances for other lineages of *E. auriceps* and *E. nordestina* that were suggested to be also distinct species (Faria & Melo 2011), which depicts a large diversity in the *E. auriceps* complex of species, from which the new species is derived (Table 1).

Based on the present morphological and molecular evidences, and given that the single unidentified specimen revealed not to belong to any of the known *Eufriesea* species, including similar looking *E. auriceps*, *E. nordestina*, *E. fuscitra* (Moure, 1999), *E. buchwaldi* (Friese, 1923) and *E. venezolana* (Schrottky, 1913), we here propose that it belongs to a new species, described below.



**FIGURE 2.** Cluster of the 35 specimens (numbers 2 to 36) of *Eufriesea nordestina* (Moure, 1999) and the single specimen of *Eufriesea zanghi* sp. n. (number 1, right side) according to measurements of four morphological characters through SAS (see ‘Material and Methods’). Each specimen is represented by numbers 1 to 36. The correspondent identification and access number at UFMG collection is shown in Appendix 1.



**FIGURE 3.** Tree constructed from the COI gene sequences using Maximum Likelihood (ML). An identical topology was obtained with Maximum Parsimony (MP), and bootstrap values for both reconstruction methods are shown above (ML) and below (MP) branches. The numbers shown in parentheses after the taxon names refer to the lineages and locations presented in Table 2. *Exaerete smaragdina* (Guérin-Méneville, 1844) was used as outgroup.

**TABLE 1.** COI sequence distance variability (%) among taxa of species *Eufriesea auriceps* (Friese, 1899) and *Eufriesea nordestina* (Moure, 1999). The numbers following species names relate to numbers 1, 2 and 12 of the specimens represented in Table 2 (column ‘Number’) and after species names in Figure 3; \*Represents the remaining specimens of *E. nordestina*. (samples 5–11, Figure 3). The model used was the Kimura 2-parameter.

Taxon 1	Taxon 2	Average distance	Standard Deviation
<i>E. auriceps</i> 1	<i>E. auriceps</i> 2	1.2	0.5
<i>E. auriceps</i> 1	<i>E. nordestina</i> *	1.6	0.5
<i>E. auriceps</i> 1	<i>E. nordestina</i> 12	1.5	0.5
<i>E. auriceps</i> 2	<i>E. nordestina</i> *	1.7	0.5
<i>E. auriceps</i> 2	<i>E. nordestina</i> 12	1.7	0.5
<i>E. auriceps</i>	<i>E. zhangii</i> sp. n.	1.6	0.5
<i>E. nigrohirta</i>	<i>E. auriceps</i>	1.5	0.4
<i>E. nigrohirta</i>	<i>E. zhangii</i> sp. n.	1.5	0.5
<i>E. nordestina</i>	<i>E. nigrohirta</i>	2.1	0.6
<i>E. nordestina</i> *	<i>E. nordestina</i> 12	0.4	0.2
<i>E. nordestina</i>	<i>E. zhangii</i> sp. n.	1.9	0.5

### *Eufriesea zhangii* Nemésio & Santos Júnior, sp. n.

**Diagnosis (males only).** *Eufriesea zhangii* sp. n. is easily assigned to the genus *Eufriesea* due to the following characters (Faria Jr. 2009): bilobed gonostylus, tentorial pit situated on a conspicuous depression at the edge of clypeus, and lower limit of clypeus arch-shaped, with edges projected over the labrum. It is also easily assigned to the *E. mussitans* species group due to (Kimsey 1982): head and mesosoma darkly colored, three clypeal ridges, short tongue, not exceeding hindcoxa. *Eufriesea zhangii* sp. n. can be readily distinguished from the very similar *E. nordestina* by the presence of a prominent, elevated medial ridge on the clypeus (only an impunctated stripe in *E. nordestina*), mesobasitarsus black without metallic hues clothed in dense golden hairs medially and black bristles laterally (in *E. nordestina* mesobasitarsus is black with purple hues clothed in sparser golden and black hairs mixed), mesotibial brush vestigial, connected to mesotibial tuft (in *E. nordestina* mesotibial brush is well developed and not connected to mesobital tuft), metatibia with very dense punctation (punctures separated by 0.05 mm to 0.07 mm) and entirely clothed in golden setae (in *E. nordestina* punctation on metatibia is very sparse – punctures separated by 0.07 mm to 0.18 mm—and it is clothed in sparse black setae), S7 with two long lobes (in fact, the longest S7 lobes of all known species of *Eufriesea*) basally glabrous, as well as the region between lobes, and clothed in dense and long black setae distally (Figure 6A) (in *E. nordestina* lobes are shorter and the region between lobes is clothed in dense and long setae; Figure 6B), gonostylar ventral lobe slightly curved and pointed (Figure 6E, G) (in *E. nordestina* it is straight and rounded; Figure 6F, H). *Eufriesea zhangii* sp. n. is consistently larger than *E. nordestina* (see Appendix 1). *Eufriesea zhangii* sp. n. can also be easily distinguished from all other predominantly black species of *Eufriesea*—*E. auriceps*, *E. buchwaldi*, *E. fuscitra*, *E. nordestina*, and *E. venezolana* due to its metatibia entirely clothed in dense golden setae (Figure 4E–F) (sparser blackish setae in the other species). Although molecular data placed *Eufriesea zhangii* sp. n. as closer to *E. nigrohirta* than to any of the blackish species of *Eufriesea*, both species can be easily distinguished due to mesosomal (entirely blackish in *Eufriesea zhangii* sp. n. and with a dense stripe of yellow setae on basal portion of scutum in *E. nigrohirta*) and metasomal coloration (entirely blackish in *Eufriesea zhangii* sp. n. and greenish with reddish hues on T2–T7 in *E. nigrohirta*). A black morph also occurs in *E. nigrohirta*, but even these specimens present strong reddish hues on metasoma (particularly on T2) and also have the yellowish stripe of setae on mesosoma (see Nemésio 2005: 43).

**Type material.** HOLOTYPE: male, with the following label data: “BRASIL, CE, Ubajara, Parque Nacional Ubajara, 03°49’53.4”, 40°54’02.4W, 456m 26.iv.2012 Vanilina, J.E. Santos Jr, UFMG IHY 1207628.

**Description** (Male, Figures 4, 6): *Color and vestiture.* Face metallic green; lower clypeus and malar area metallic purple (Figure 4B); gena and upper frons blackish without metallic hues; mesoscutum metallic dark green basally and black without metallic hues distally entirely clothed in dense black setae (Figure 4A), tegulae bright

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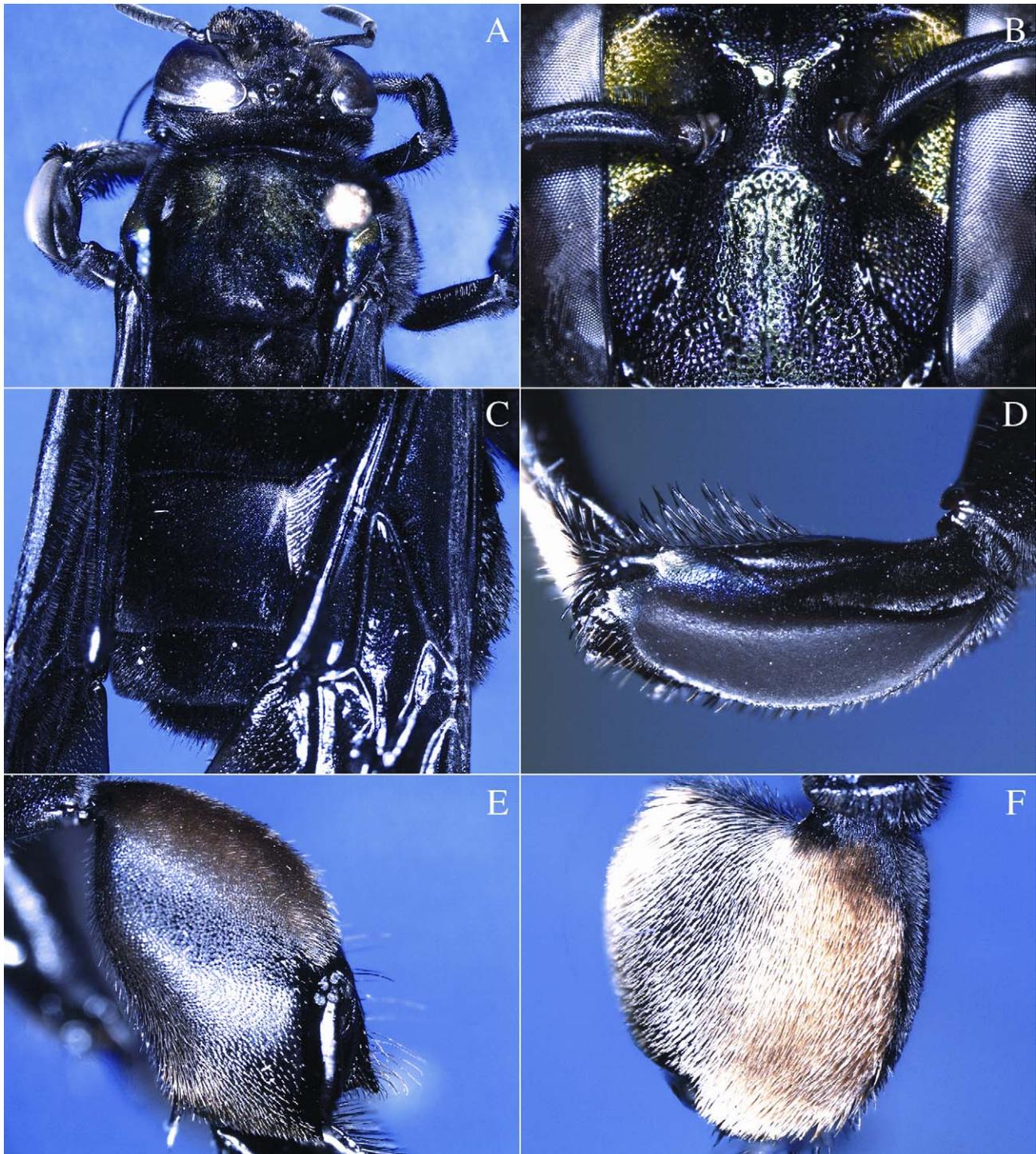
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green anteriorly and black with strong dark blue and purple hues distally; scutellum black without metallic hues clothed in dense black setae (Figure 4A); metasomal terga and sterna black without metallic hues clothed in sparse, short and appressed black setae (Figure 4C).

*Head.* Width 7.13 mm; interorbital distance at base of scape 3.35 mm; maximum interorbital distance 3.72 mm; scape 2.17 mm; eye length 4.46 mm. Clypeus with strong medial ridge (Figure 4B), densely punctate; punctures irregular in shape and size (0.03 mm to 0.1 mm).

*Body.* Body length ca. 18.35 mm; anterior wing ca. 18.23 mm; tongue length 8.06 mm, reaching S1; scutellum 5.33-mm wide and 2.6-mm long; abdominal width 7.9 mm.

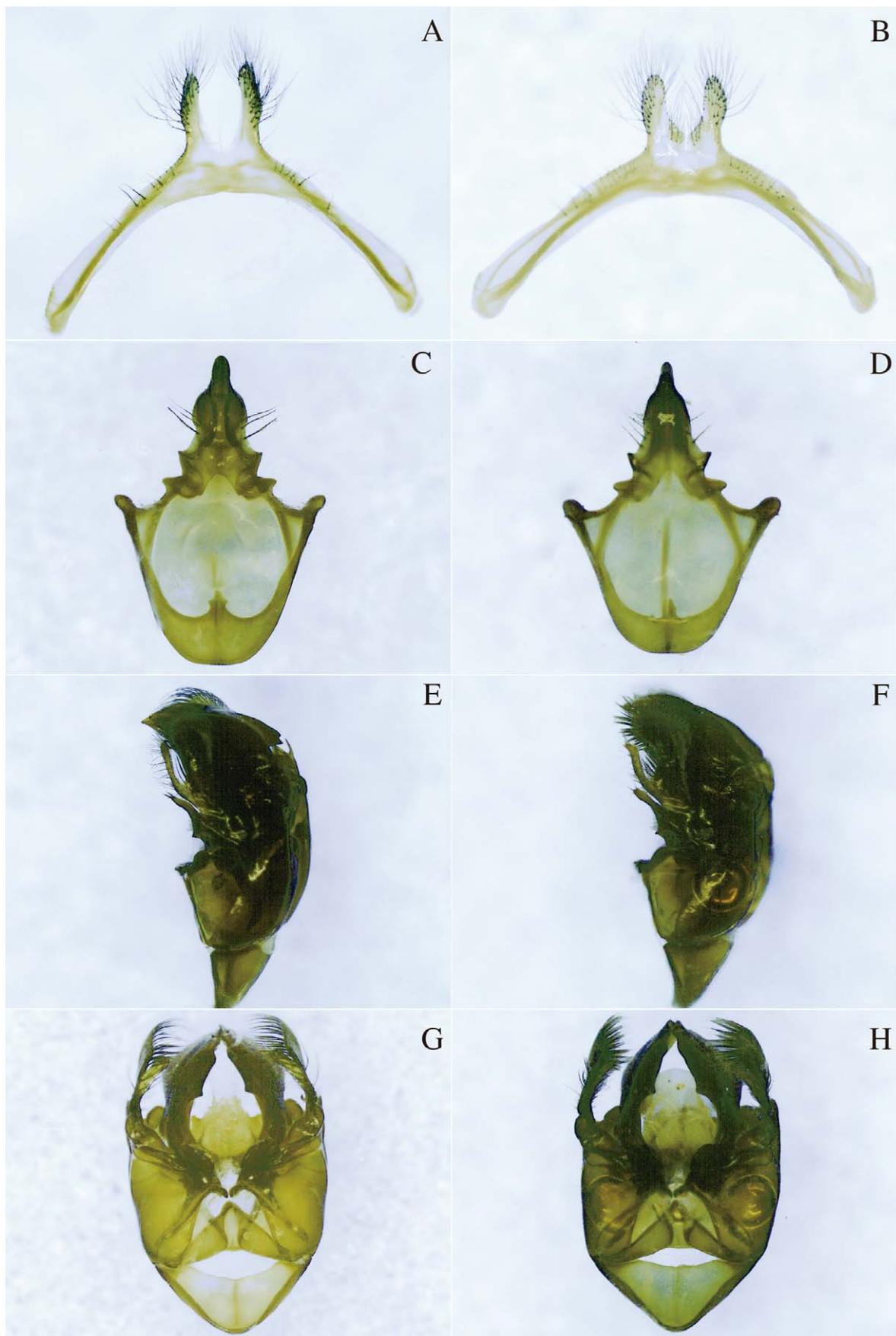


**FIGURE 4.** Holotype *Eufriesea zhangi* sp. n. A: dorsal view of head and mesosoma; B: frontal view of face; C: dorsal view of metasoma; D: ventral view of mesotibia. E: frontal view of metatibia; F: metatibia seen from above.



**FIGURE 5.** *Eufriesea nordestina* (Moure, 1999). A: dorsal view of head and mesosoma; B: frontal view of face; C: dorsal view of metasoma; D: ventral view of mesotibia. E: frontal view of metatibia; F: metatibia seen from above.

*Legs.* Foretibia and forebasitarsus black with purple hues fringed with long and dense, black hairs; mesobasitarsus black without metallic hues, clothed in dense golden setae medially and long black bristles laterally; velvet area occupying the entire outer surface of mesotibia, mesotibial tuft long and triangular, occupying about 1/6 of velvet area, marginally connect to an almost vestigial brush (Figure 4D); metatibia oblong, swollen, black without metallic hues clothed in dense golden hairs, dense punctation comprised of circular and small (0.05 mm in diameter) punctures separated from each other by 0.05 mm to 0.07 mm; post-glandular area reaching apex (Figure 4E–F).



**FIGURE 6.** Comparison of genitalia of *Eufriesea zhangi* sp. n. (left column) and *E. nordestina* Moure, 1999 (right column). A–B: ventral view of S7; C–D: ventral view of S8; E–F: lateral view of genital capsule; G–H: ventral view of genital capsule.

*Terminalia*. S7 arch-shaped and strongly bilobed, both lobes basally glabrous, as well as region between lobes, and medially to apically covered with dense black setae (Figure 6A); S8 pointed apically (Figure 6C); gonostylar dorsal lobe longer than ventral one and clothed in dense setae; gonostylus three to four times longer than gonocoxal dorsal lobe (Figure 6G).

**Female.** Unknown.

**Etymology.** The specific epithet honors Dr. Zhi-Qiang Zhang, editor-in-chief of *Zootaxa*. One decade ago, Krell (2002), in an influential paper in *Nature*, stated that one of the reasons for the low impact factor of taxonomy was the lack of “core journals for general taxonomy. These exist for cladistics, biogeography, chemical systematics, and so on, but not for species descriptions, revisions of genera, identification keys or inventories.” This situation has dramatically changed in the course of only ten years and *Zootaxa* became the most influential core journal on zoological alpha-taxonomy ever, publishing around 20% of all new nomina in zoology yearly. This is thanks to Dr. Zhang’s visionary perspective and we here dedicate this beautiful new orchid bee to him in recognition of his accomplishments in this fantastic enterprise.

**Type locality.** Parque Nacional de Ubajara, municipality of Ubajara, state of Ceará, northeastern Brazil, at 03°49′02.4″S/40°54′02.4″W, 456 m above sea level.

**Attractive baits:** Vanillin.

## Discussion

Although outstandingly conspicuous orchid-bee species have recently been described from time to time (e.g. Moure 1996, Nemésio 2006, Faria & Melo 2011, Oliveira 2011), the recent boom in orchid-bee alpha-taxonomy is mostly due to the recognition of cryptic or sibling species among long established taxa (e.g. Roubik 2004; Rasmussen & Skov 2006; Hinojosa-Díaz & Engel 2007; Bembé 2007, 2008; Ayala & Engel 2008; Nemésio & Bembé 2008; Nemésio 2008, 2010, 2011b, c, d, 2012; Eltz *et al.* 2011; Hinojosa-Díaz *et al.* 2011, 2012; Nemésio & Engel 2012). We here continue this recent tradition by describing a species similar to *E. auriceps* and *E. nordestina*, but as far as we know, geographically restricted to an Atlantic Forest remnant at the top of a mountain in the state of Ceará, northeastern Brazil. Although the most conspicuous character distinguishing this newly described species from other black *Eufriesea* is the presence of dense golden setae on metatibiae, it was here shown that this character is not just an extreme color variation within the sympatric *E. nordestina*. In fact, *E. nordestina* presents a wide range of color variation, especially concerning the metasoma, which can be entirely clothed in black setae (most specimens) or present yellowish setae as well. One extreme of these yellowish color variants was even described by Moure *et al.* (2001) as *E. aridicola*—specimen illustrated by Nemésio (2009: 37). This phenomenon has also been seen among *E. auriceps* specimens (Nemésio 2009: 36) and usually affects most strongly T2 and T3, although yellowish setae may be found on T4-T7 as well in some specimens (A. Nemésio, pers. obs.). The distinctness of *Eufriesea zhangii* sp. n., on the other hand, is supported not only by the diagnostic golden setae of the metatibia (a trait not subject to such a wide variation in both *E. auriceps* and *E. nordestina*), but it is corroborated by its larger size (Figure 2, Appendix 1), a set of distinctive morphological features, including morphology of genitalia (see ‘Diagnosis’ section), and also by molecular data. Since it was anticipated by Faria & Melo (2011) that a full revision of the black species of the *E. mussitans* species group may be in course, recognition of *Eufriesea zhangii* sp. n. as a distinct (and probably rare) species from a remote site in northeastern Brazil may be of importance for a thorough understanding of the richness and limits of speciation within this particular group of bees.

Although *Eufriesea zhangii* sp. n. seems to be morphologically closer to *E. auriceps* and *E. nordestina*, the phylogenetic analyses carried out with COI suggested that *E. nigrohirta* might be closely related to the new species. Interestingly, *E. nigrohirta* is also restricted to mountain tops, but in the states of Minas Gerais and Bahia (Nemésio 2005; Azevedo *et al.* 2010). Nevertheless, *E. nigrohirta* displays several contrasting morphological characteristics that distinguish it from *Eufriesea zhangii* sp. n. (see Nemésio 2005). It should be emphasized, however, that the association between both species in our molecular analyses presented a relatively low support and future studies must be carried out to further investigate their relationships. In fact, detailed phylogenetic studies are still needed to elucidate the relationships among species and groups of *Eufriesea* to overcome limitations revealed in previous studies (e.g. Faria Jr. 2009; Ramírez *et al.* 2010).

Sheffield *et al.* (2009), studying the bee fauna of Nova Scotia, established that individuals of a given species share little intra-specific variation with COI divergences averaging just 0.49%. Our results have shown an average

distance exceeding 1.4% between *Eufriesea zhanghi* **sp. n.** and the other taxa analyzed (Table 1). The relationship among the studied specimens of *E. auriceps* is also noticeable. The average distance among them was 1.2%, whereas the average distance between both groups of *E. nordestina* was 0.4%. These results support Moure's (1999) and Faria & Melo's (2011) hypothesis that *E. auriceps* is a species complex (*contra* Nemésio 2009).

Finally, the position of *Eufriesea zanghi* **sp. n.** as potentially close to *E. nigrohirta* suggests that this latter species may be part of the *E. mussitans* species group. If this is true, the re-arrangement of the *Eufriesea auripes* (Gribodo, 1882) species-group proposed by Nemésio & Bembé (2008) is not correct. In fact, *E. auripes* species-group was supported as monophyletic neither by Faria (2009) nor by Ramírez *et al.* (2010).

**TABLE 2.** Specimens used in genetic analyzes with their respective localities. The column 'Number' refers to the number in parentheses located after the name of the species in Figure 3. CE: Ceará; MG: Minas Gerais; MS: Mato Grosso do Sul; RO: Rondônia.

Species	Number	Specimen	Genbank accession numbers	Municipality	State
<i>Eufriesea auriceps</i> (Friese, 1899)	1	LE290-1	KC313063	Matias Cardoso	MG
<i>E. auriceps</i>	2	75838	KC313064	Serra do Salitre	MG
<i>E. nigrohirta</i>	3	1208243	KC313065	Porteirinha	MG
<i>E. zhanghi</i> <b>sp. n.</b>	4	1207628	KC313066	Ubajara	CE
<i>E. nordestina</i> (Moure, 1999)	5	77107	KC313067	Taiobeiras	MG
<i>E. nordestina</i>	6	LE328-1	KC313068	Matias Cardoso	MG
<i>E. nordestina</i>	7	LE311-1	KC313069	Matias Cardoso	MG
<i>E. nordestina</i>	8	LE286-2	KC313070	Matias Cardoso	MG
<i>E. nordestina</i>	9	LE286-1	KC313071	Matias Cardoso	MG
<i>E. nordestina</i>	10	LE238-1	KC313072	Matias Cardoso	MG
<i>E. nordestina</i>	11	1207230	KC313073	Ubajara	CE
<i>E. nordestina</i>	12	1207570	KC313074	Ubajara	CE
<i>E. superba</i> (Hoffmannsegg, 1817)	13	76385	KC313075	Porto Velho	RO
<i>E. atlantica</i> Nemésio, 2008	14	53072	KC313076	Santa Maria do Salto	MG
<i>E. ornata</i> (Mocsáry, 1896)	15	76195	KC313077	Porto Velho	RO
<i>Exaerete smaragdina</i> (Guérin-Méneville, 1844)	16	72455	KC313078	Corumbá	MS

## Biogeography and conservation

As pointed out by Nemésio (2010) and Nemésio & Ferrari (2012), hypotheses dealing with the possible past connections between the Amazon and the Atlantic forests have been long proposed (*e.g.*, Rizzini 1963, Bigarella *et al.* 1975, Ledru 1993; Coimbra-Filho & Câmara 1996; Vivo 1997; Costa 2003; Vivo & Carmignotto 2004). Isolation of both biomes allowed speciation in many taxa, orchid bees included (reviewed by Nemésio 2010), and studies in intermediate areas, as the state of Ceará, can reveal the distributional limits of Amazonian and Atlantic Forest species (Guimarães 2011; Nemésio & Ferrari 2012). The recent recognition of a new species of *Eulaema* exactly in the same area where the only known specimen of *Eufriesea zhanghi* **sp. n.** comes from, suggests that the Atlantic Forest remnants situated at the top of mountains in northeastern Brazil, the so-called "brejos de altitude", may hold endemic and still unknown species that deserve further investigation. Moreover, although most similar to that of the Atlantic Forest, the orchid-bee fauna of such remote places hold viable populations of species of clear Amazonian origin as well (Guimarães 2011; Nemésio & Ferrari 2012).

Contrary to lowland Atlantic Forest areas, which are currently highly fragmented due to man-made deforestation, the Atlantic Forest vegetation found in 'brejos de altitude' is immersed in a matrix of drier vegetation (especially 'Caatinga'). 'Brejos de altitude' are, thus, naturally "fragmented" and populations of some species may

be consequently isolated for a much longer time, as seems to be the case with *Eulaema quadragintanovem* Nemésio & Ferrari, 2012 and *Eufriesea zhangii* sp. n.

It is impossible at the current stage to estimate the conservation status of *Eufriesea zhangii* sp. n. As stated above, most *Eufriesea* species are only active during a few weeks yearly, and we do not know whether the only specimen collected at 'Parque Nacional de Ubajara' belongs to a rare local species or if collections took place at the very beginning or the end of this species' activity period. It should be stressed that samplings at the type locality were carried out during only eight consecutive days in April 2012. Moreover, it is also not known whether males of this species are strongly attracted to the scents ordinarily used in orchid-bee inventories. Although almost 40 specimens of the sympatric *E. nordestina* were collected during the same period, it is possible that the activity periods of both species weakly overlap, as happens to other species of *Eufriesea* that occur sympatrically in the Atlantic Forest of southern Bahia (A. Nemésio, unpub. data). More collections in the area are needed to estimate the period of activity of this species and its population density before any assumption concerning its status can be confidently made.

Whatever is the situation, however, it should be pointed out that if *Eufriesea zhangii* sp. n. is actually restricted to the 'brejos de altitude' of northeastern Brazil, the habitat where this species occurs is severely threatened (Silva & Tabarelli 2000, 2001) and it may be under the risk of extinction due to the loss of suitable habitat, as are other limited range orchid-bee species recently described from northeastern Brazil (Nemésio 2010; Faria & Melo 2011; Nemésio & Ferrari 2012). It should be also emphasized that predicted climatic changes along the 21<sup>st</sup> century in the region (Williams *et al.* 2007) suggest that even if no deforestation happens in these areas, environmental suitability may be lost as a consequence of a rise in temperature and a decline in humidity by 2100 AD. The 'Parque Nacional de Ubajara', with a total area of only ca. 600 ha, is probably too small to hold viable populations of some species for a long time. More samplings are thus urgently needed both in Ceará and in the neighbor states of Paraíba and Pernambuco, where the last remnants of the 'brejos de altitude' still exist, to search for this species and understand its actual geographic distribution and biology.

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**APPENDIX 1.** Morphometric data, access number (at UFMG) and specimen code (for Figure 2) belonging to specimens of *Eufriesea* collected at 'Parque Nacional de Ubajara' in April, 2012. SCTW = scutelar width; SCTL = scutelar length; IOD = interorbital distance at scape; EL = eye length.

Species	Specimen access number (UFMG collection)	Specimen code (for Figure 2)	SCTW	SCTL	IOD	EL
<i>Eufriesea zhangi</i> sp. n.	1207628	1	5.33	2.6	3.35	4.46
<i>E. nordestina</i> (Moure, 1999)	1207612	2	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207662	3	4.59	1.86	3.1	4.22
<i>E. nordestina</i>	1207570	4	4.59	1.98	3.1	4.34
<i>E. nordestina</i>	988cfc	5	4.34	1.86	3.1	4.34
<i>E. nordestina</i>	992cfc	6	4.46	1.98	3.1	4.34
<i>E. nordestina</i>	992cfc	7	4.46	1.98	3.1	4.34
<i>E. nordestina</i>	992cfc	8	4.34	1.86	3.1	4.22
<i>E. nordestina</i>	992cfc	9	4.59	1.98	3.1	4.34
<i>E. nordestina</i>	992cfc	10	4.46	1.98	3.1	4.34
<i>E. nordestina</i>	1000cfc	11	4.46	1.98	3.1	4.34
<i>E. nordestina</i>	1003cfc	12	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207230	13	4.59	1.98	3.1	4.34
<i>E. nordestina</i>	1207260	14	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207390	15	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207287	16	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207384	17	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207385	18	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207386	19	4.71	1.86	3.1	4.22
<i>E. nordestina</i>	1207387	20	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207390	21	4.71	1.98	3.1	4.34
<i>E. nordestina</i>	1207407	22	4.71	1.98	3.1	4.34
<i>E. nordestina</i>	1207408	23	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207409	24	4.71	1.86	3.1	4.22
<i>E. nordestina</i>	1207410	25	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207411	26	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207412	27	4.84	1.98	3.1	4.22
<i>E. nordestina</i>	1207413	28	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207571	29	4.59	1.86	3.1	4.34
<i>E. nordestina</i>	1207579	30	4.71	1.86	3.1	4.22
<i>E. nordestina</i>	1207580	31	4.71	1.86	3.1	4.34
<i>E. nordestina</i>	1207611	32	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207625	33	4.46	1.86	3.1	4.22
<i>E. nordestina</i>	1207626	34	4.46	1.86	3.1	4.22
<i>E. nordestina</i>	1207627	35	4.84	1.98	3.1	4.34
<i>E. nordestina</i>	1207658	36	4.34	1.74	2.98	3.97