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Original article

An integrative approach identifies a new species of bumblebee (Hymenoptera: Apidae: Bombini) from northeastern Brazil

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Abstract – Here, we describe a new species of genus *Bombus* Latreille, 1802 from northeastern Brazil, *Bombus* (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. Molecular analysis was initially performed to confirm the new species placement within the genus *Bombus*. Afterward, we performed an integrative approach combining molecular data (DNA barcoding and two nuclear regions) and morphology to confirm its taxonomic status. The genetic and morphological data were very consistent and congruent supporting this group as a new species. *B*. (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. has the body totally covered by black pilosity and can be distinguished from closer and/or sympatric species by clypeus shape, which has a distinct flattened dorsal platform, by length of malar area and the length and shape of the hairs, shorter and aligned as if it was trimmed. In addition, brief taxonomic notes on *Bombus* (*Thoracobombus*) *brevivillus* Franklin (1913) and *Bombus* (*Thoracobombus*) *morio* Swederus (1787), the sympatric species, are provided. A key for identification of Brazilian *Bombus* species, including the new species, was elaborated.

Bumblebee / *Bombus* sp. nov. / DNA barcoding / nuclear markers / traditional taxonomy

1. INTRODUCTION

Bumblebees belong to the genus *Bombus* (Hymenoptera: Apidae, Bombini) that encompasses about 250 species. These bees provide important ecosystem services by their extraordinary ability to pollinate (Corbet et al. 1991; Kevan 1991; Memmott et al. 2004; Pywell et al. 2006; Goulson et al. 2008). They occur primarily in cold climate zones in Eurasia and North America (Michener 2007). In Brazil, six species are currently recognized: *Bombus bellicosus* Smith (1879), *Bombus brasiliensis* Lepeletier (1836), *Bombus brevivillus* Franklin (1913), *Bombus morio* Swederus (1787), *Bombus pauloensis* Friese (1913) (also known as *Bombus atratus* Franklin; 1913), and *Bombus transversalis* Olivier (1789), all belonging to the subgenus *Thoracobombus* (Williams et al. 2008). The low number of species in Brazil, despite their wide distribution, is very contrasting to the high number found in other world regions as the Palaearctic (120 species), Orient (108 species), and Japan (23 species) (Williams 1996, 1998).

In general, species of *Bombus* present homogeneity in morphology, an unusual feature for bees. This uniformity is an obstacle to their identification, and consequently, cryptic species may be still unrecognized (Moure and Sakagami 1962;

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Williams 1998; Michener 2007; Murray et al. 2008). Conversely, Brazilian species present high intra-specific variability, and parallelism in color patterns is observed in sympatric species. As stated by Moure and Sakagami (1962), *Bombus* is a real "puzzles" for taxonomists and the "most difficult group to classify".

The DNA barcode has been proposed to supplement current and traditional methods based on morphological traits (Hebert et al. 2004). For animals in general, this method is based on the sequencing of the mitochondrial gene *cytochrome C oxidase I* (*COI*) region. Recent works have employed this molecular method with success in the identification of a great variety of taxa, including the genus *Bombus* (Murray et al. 2008; Williams et al. 2011, 2012). Nevertheless, subsequent to an initial DNA barcoding, an integrative approach using multilocus analysis and traditional taxonomy is still necessary for species delimitation accuracy (Sites and Marshall 2004; Collins and Cruickshank 2012).

To develop a comprehensive phylogeography study based on molecular data, we have been collecting *Bombus* specimens in Brazil during these past years. Also, we had access to samples kept in collections for DNA extraction (Table I). The preliminary analysis revealed that some specimens from northeastern Brazil were genetically "different" from all other samples. In addition of being more robust, these distinctive specimens were slightly different morphologically from the sympatric species (*B. brevivillus* and *B. morio*), according to the morphological key proposed by Moure and Sakagami (1962).

Considering these molecular and morphological evidences, the goals of this research were as follows: (a) verify the placement of these specimens within the genus *Bombus* (whether they are closely related to the Brazilian species) and ensure that these specimens do not belong to a species already described; (b) test if these specimens constitute a new species by gathering molecular data from the DNA barcode and nuclear regions for all Brazilian *Bombus* species and performing phylogenetic analysis; (c) characterize these specimens morphologically; (d) describe the new species; and (e) provide a key for identification of Brazilian *Bombus* species, including the new species.

2. MATERIALS AND METHODS

2.1. Samples and molecular markers

Table I summarizes the Brazilian species studied and the general information for each specimen. We were able to obtain 17 specimens of the suspected species either from nature or from collections. The specimens were identified according to the morphological key proposed by Moure and Sakagami (1962). One middle leg of frozen specimens was used for DNA extraction following the Chelex® 100 (Bio-Rad, UK) method (Walsh et al. 1991). For pinned specimens, we also used one middle leg for DNA extraction using the DNeasy Tissue Kit (Qiagen, Germany) following the supplier's recommendations.

Primers (Online resource 1) and PCR conditions for amplification of the *COI* barcode region were as described by Françoso and Arias (2013). The *COI* sequences for *B. bellicosus*, *B. brevivillus*, and *B. transversalis* were obtained previously in our laboratory, using primers described by Simon et al. (1994; online resource 1). Also, the large ribosomal RNA subunit (*16S*) region was amplified according to the same authors. We amplified exons and introns from two nuclear genes, *arginine kinase* (ArgK) (Kawakita et al. 2003) and *elongation factor-1* α (*EF-1* α) (Hines et al. 2006), to test for mitochondrial introgression and to improve our phylogenetic analyses. All PCR amplifications were according to the articles cited above and performed in a Mastercycler Pro (Eppendorf, Germany). For amplifications using the primers described by Simon et al. (1994), we used the same PCR cycling conditions as for the DNA barcode region. PCR products were separated on a 0.8 % agarose gel, stained with Gel Red 10.000X (Biotium, USA), and visualized under UV light. All PCR products were purified with 0.5 μl of ExoSAP-IT® (USB, USA) following the manufacturer's recommendations and were sequenced from the forward direction by the Macrogen Company (South Korea). The program Muscle (Edgar 2004) included in Geneious Pro 7.0.3 software (http://www.geneious.com) was used to align the sequences. The *COI* sequences were edited and translated into amino acid sequences. These DNA sequences were

Table I. Collection information for specimens analyzed in this study.

Species name	Index	Collection locality	Year	Collector	Voucher number/ identification	Institutional acronym
B. applanatus	A	Abel Figueiredo, PA	2002	EAB Almeida	7855-23796 (EF129)	LBEA
B. applanatus	B	Brasília, DF	1999	FA Silveira	4900-13443 (EF116)	LBEA
B. applanatus	C	Igrapiúna, BA	2011	E Françoso	EF202	LGEA
B. applanatus	D	Igrapiúna, BA	2011	E Françoso	EF203	LGEA
B. applanatus	E	Igrapiúna, BA	2011	E Françoso	EF204	MHNBA-MZUFBA
B. applanatus	F	Igrapiúna, BA	2011	E Françoso	EF205	MHNBA-MZUFBA
B. applanatus	G	Igrapiúna, BA	2011	E Françoso	EF206	MHNBA-MZUFBA
B. applanatus	H	Igrapiúna, BA	2011	E Françoso	EF207	LGEA
B. applanatus	I	Igrapiúna, BA	2011	E Françoso	EF209	LGEA
B. applanatus	J	Igrapiúna, BA	2011	E Françoso	EF210	LGEA
B. applanatus	K	Igrapiúna, BA	2011	E Françoso	EF212	LGEA
B. applanatus	L	Igrapiúna, BA	2011	E Françoso	EF213	LGEA
B. applanatus	M	Igrapiúna, BA	2011	E Françoso	EF214	LGEA
B. applanatus	N	Igrapiúna, BA	2011	E Françoso	EF215	LGEA
B. applanatus	O	Igrapiúna, BA	2011	E Françoso	EF216	MHNBA-MZUFBA
B. applanatus	P	Itacajá, TO	1993	JMF Camargo, JA Tavares, SRM Pedro	930450 (EF148)	RPSP
B. applanatus	Q	João Pessoa, PB	2009	SS Neto	6464 (EF20)	Esalq
B. bellicosus	–	São Joaquim, SC	2006	A Aguiar, A Matins, LRR Faria Jr	29219	DZUP
B. brasiliensis	A	Biguaçu, SC	2011	FO Francisco	FOFBigu4	LGEA
B. brasiliensis	B	Ilha do Cardoso, SP	2011	FO Francisco	FOFIcard13	LGEA
B. brasiliensis	C	Ribeirão Preto, SP	2006	A Assis	–	LGEA
B. brasiliensis	D	Teresópolis, RJ	2011	FO Francisco	FOFTere30	LGEA
B. brevivillus	–	Oriximiná, PA	1968	Exp. Perm. Amaz.	–	MZUSP
B. morio	A	Brasília, DF	2008	SC Cappellari	240708-16 (EF188)	UNB
B. morio	B	Igrapiúna, BA	2011	E Françoso, AR Zuntini	EF211	LGEA
B. morio	C	Jaboticatubas, MG	2011	AR Zuntini	EF177	LGEA
B. morio	D	Teresópolis, RJ	2009	FO Francisco	FOF255 (EF30)	LGEA
B. pauloensis	A	Brasília, DF	2009	SC Cappellar	2401_9_1 (EF181)	UNB
B. pauloensis	B	Caçador, SC	2011	FO Francisco	FOFCaça2 (EF176)	LGEA
B. pauloensis	C	Guaratuba, PR	2011	FO Francisco	Guarat1 (EF170)	LGEA
B. pauloensis	D	Jaboticatubas, MG	2011	AR Zuntini	EF178	LGEA

Table I (continued)

Species name	Index	Collection locality	Year	Collector	Voucher number/ identification	Institutional acronym
B. pauloensis	E	Londrina, PR	2011	AN Alves	EF219	LGEA
B. transversalis	–	Porto Velho, RO	1983	CEA Coimbra	–	MZUSP

DZUP Coleção entomológica Pe. Jesus Santiago Moure (Hymenoptera), UFPR; *LGEA* Laboratório de Genética e Evolução de Abelhas, USP; *MZUSP* Museu de Zoologia da USP; *UNB* Coleção de insetos da Universidade de Brasília; *LBEA* Laboratório de Sistemática e Ecologia de Abelhas, UFMG; *MHNBA-MZUFBA* Coleção Entomológica do Museu de História Natural da Universidade Federal da Bahia; *RPSP* Coleção Camargo, USP; *Esalq* Museu de Entomologia da Esalq

compared with GenBank using BLAST tool (Altschul et al. 1990) and Bold databases (Ratnasingham and Hebert 2007). These two procedures were employed to verify inadvertent amplification of homologous *COI* from *Wolbachia* or numts. The DNA sequences obtained were deposited in GenBank (Accession numbers: KT187861–KT187933).

2.2. Phylogenetic analysis

To confirm the placement within the genus and ensure that these specimens could not be a species already known from another region, we selected one *Bombus* sp. nov. (EF204) *COI* sequence to compare with the *COI* sequences deposited at BOLD and GenBank databases. Also, we performed phylogenetic analysis using the sequence alignment of 218 taxa (Hines 2008) already published for the entire genus (Cameron et al. 2007) including the suspect group sequences. Three out of five molecular markers used by Cameron et al. (2007) were used in the new species: *16S*, *ArgK*, and *EF-1α*. We added the suspect specimen sequences to the data matrix (Study ID: S1927) available in TreeBASE (http://www.treebase.org) and applied the same models of nucleotide substitution and the same phylogenetic reconstruction parameters used in Hines (2008) for Bayesian analysis.

Phylogenetic reconstruction for the Brazilian group of bumblebees was performed by Bayesian analysis through MrBayes 3.2.2 (Huelsenbeck and Ronquist 2001). The program JModelTest 2.1.4 (Darriba et al. 2012) selected the models GTR+I+R for the mitochondrial *COI* and TIM1+G for the nuclear *ArgK* and *EF-1 α* genes. The analysis was run with 10 million generations with a burn-in of 25 %. *Bombus* (*Bombus*) *ignitus* Smith (1869), *Bombus* (*Bombus*) *lucorum* Linnaeus (1761), and *Bombus* (*Cullumanobombus*) *volucelloides* Gribodo (1891) (GenBank accession numbers: 010967.1, AY267133, and AY739522, respectively) were used as outgroups.

The genetic distance between the new species clade and the other Brazilian species of bumblebee was taken through the sum of the length of branches of a UPGMA tree obtained in Geneious Pro 5.6.3 software.

2.3. Traditional morphology

Four female (f) specimens (queens) were morphologically analyzed and deposited at Coleção Entomológica do Museu de História Natural da Universidade Federal da Bahia (MHNBA-MZUFBA), Salvador, Bahia, Brazil. The other 13 specimens were analyzed only molecularly and were included as paratypes (Table I). General morphological terminology is according to Michener (2007), and the standard abbreviations are as follows: antennal flagellomeres (F1, F2…), metasomal terga (T1 to T6), and puncture diameter (PD). The upper and lower interocular distances were measured using the shortest distance between the compound eyes in frontal view. All measurements are given in millimeters (mm). Label information from separate labels is separated by double slashes, "//." Photomicrographs were prepared using a Leica M165C stereomicroscope coupled with a Leica DFC295 and a Leica Application Suite V4.1 Interactive Measurements, Montage. All the observation and measurements for all species cited in here were made based in female queen specimens. A key for identification of Brazilian *Bombus* species was prepared, adapted from Moure and Sakagami (1962), including the new species (see below).

3. RESULTS

3.1. Molecular markers and phylogeny

The following sequence sizes were generated: 376 base pairs (bp) for *16S*, 430 bp for *COI* (with primers MtD7 and MtD9), 631 bp for *COI* (with primers BarbeeF and MtD9), 820 bp for *ArgK*, and 733 bp for *EF-1* α.

The *Bombus* sp. nov. (EF204) *COI* sequence was used for comparisons to our *Bombus* COI sequence, to *Bombus COI* sequences homologous from GenBank (1607 sequences) and Bold (1269 sequences), totaling 2893 sequences (about 130 species). The sequence sizes were not identical, which could compromise the accuracy of the genetic distance between them. However, the 20 highest hits (sequence similarities) obtained for *Bombus* sp. nov. (EF204) encompass two Brazilian species, *B. brasiliensis* and *B. pauloensis*, and one North American, *Bombus* (*Thoracobombus*) *fervidus* Fabricius, 1798 (Online resource 2). These species are all united in *Thoracobombus* subgenus. No *Wolbachia* or other contaminants were verified.

According to the phylogeny of the entire genus (Figure 1a), *Bombus* sp. nov. is related to the following Brazilian bumblebee species: *B. pauloensis*, *B. brasiliensis*, and *B. transversalis*, with high values of posterior probabilities. For DNA barcode and nuclear regions, in all topologies, samples of *Bombus* sp. nov. constituted a clade strongly supported by posterior probabilities and are well separated from the other Brazilian bumblebee species (Figure 1). The DNA barcode data revealed a 6.11 % genetic distance between the new lineage and its sister group, comprised of *B. bellicosus*, *B. brasiliensis*, *B. brevivillus*, and *B. transversalis*.

The clades obtained from *COI* sequences were well resolved, for all species (Figure 1b, c). The nuclear genetic markers gave support for the *Bombus* sp. nov. as monophyletic group but did not provide enough signal to solve all of the other Brazilian species as distinct from each other, except for *B. morio*, the most distant in the Brazilian bumblebee group (Cameron et al. 2007; Figure 1d, e). The DNA barcode data also revealed that *B. brasiliensis* is polyphyletic and apparently represents a species complex.

3.2. Morphology

Morphological characters allowed the recognition of these specimens as a new species, as the flattened structure of clypeus on the middle portion (Figure 2 (1)), its general aligned body pubescence (uniform in length) with straight hair (as if they had been trimmed) (Figure 3 (11)), and also the relatively shorter pubescence on head (Figure 2 (2)). *Bombus* sp. nov. is quite similar to *B. brevivillus* and *B. morio*, mainly by similar integument color (black) and a very dense pubescence and punctures in general. Nevertheless, these three species can be distinguished. The malar area is shorter and wider in the new species in comparison to *B. morio*. *B. brevivillus* has the shortest malar area among the three species, and it is narrower compared to the new species. The discal glabrous area of mesoscutum is smooth and shiny in *B. brevivillus* and *Bombus* sp. nov., but narrower in *B. brevivillus*, and microreticulated and dull in *B. morio* (Figures 2 and 3), as described below in the taxonomic treatment.

4. DISCUSSION

Specimens of the genus *Bombus* are not easily identified by morphology (Murray et al. 2008). This is an impediment to understanding the taxonomy and systematics of bumblebees, such as revealing new species. For example, *Bombus* (*Bombus*) *cryptarum* Fabricius, 1775, a common and widespread species, remained undetected until 2005 due to the high morphological similarity to *B. lucorum* (Bertsch et al. 2005; Murray et al. 2008). According to Williams et al. (2012), *Bombus* s. str. is likely to require additional characters beyond morphology in order to add substantial progress toward resolving cryptic species.

The low number of *Bombus* species found in Brazil may be a consequence of ecological and environmental features, among other factors. The tropical climate may facilitate dispersion allowing gene flow even in large areas; thus, the species integrity can be maintained. However, the low number of *Bombus* species presenting large geographic distribution in Brazil may be unreal. The lack of morphological characters seems to be the major constraint to species discrimination in this

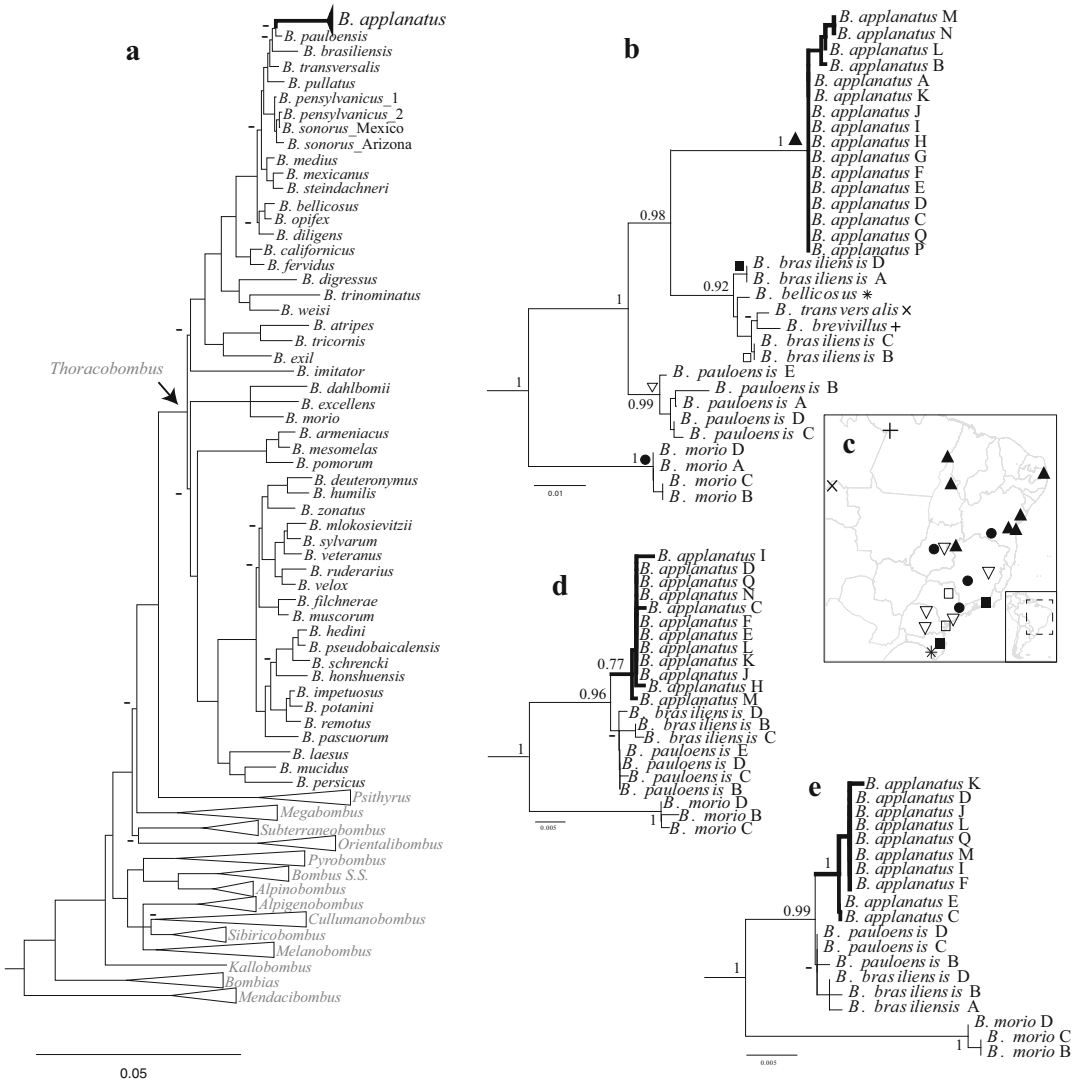

Figure 1. Bayesian phylogenetic trees obtained through molecular markers analysis (**a** , **b** , **d** , **e**). Values in node represent posterior probabilities, and *dashes* represent posterior probabilities lower than 90 %. *Letters beside the species name* match to the index in Table I. **a** Phylogeny of the genus *Bombus* (Cameron et al. 2007; Hines 2008) obtained by analyzing 218 taxa (*16S*, *ArgK*, *EF-1α*, *opsin*, and *PEPCK*) with the placement of *Bombus* (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. **b** Phylogeny of the Brazilian species obtained by *cytochrome C oxidase I* sequences. **c** Geographic distribution of samples in **b. d** Phylogeny of the Brazilian species obtained by *elongation factor-1 α* sequences. **e** Phylogeny of the Brazilian species obtained by *arginine kinase* sequences. Only posterior probabilities lower than 90 % are represented.

bee group. Even for well-known taxa, the existence of morphologically unrecognized (or cryptic) species indicates that there are likely to be many more species than has been currently estimated (Frankham et al. 2002; Blaxter 2004; Bickford et al. 2007; Murray et al. 2008). Williams et al. (2011), studying the subgenus *Subterraneobombus* Vogt (1911), suggested that *COI* barcodes are a cost-effective source of additional characters where morphological information has been insufficient. Here, after confirming the placement in the subgenus, the DNA

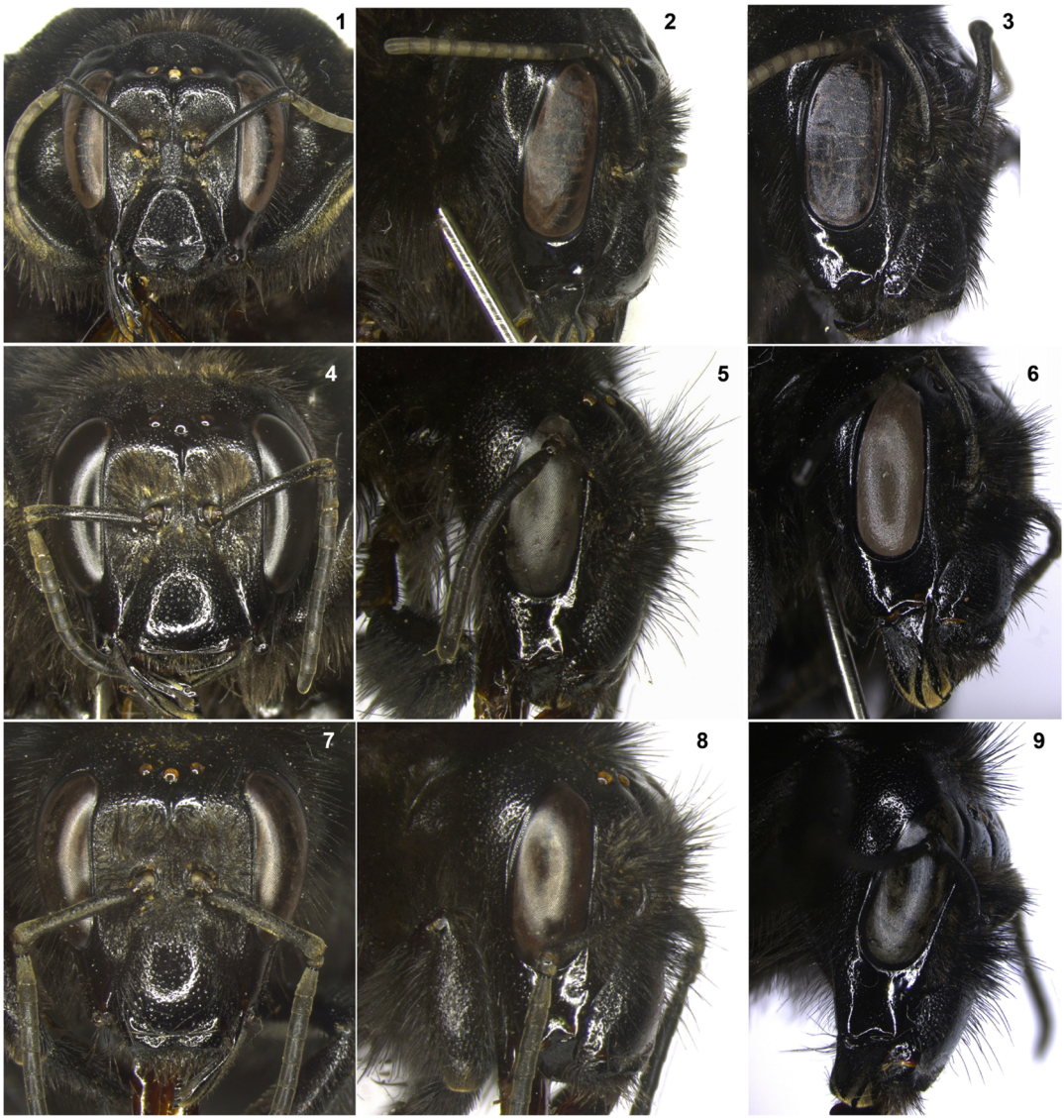

Figure 2. Frontal view of the head (*1* , *4* , *7*), head in profile (*2* , *5* , *8*), and malar area (*3* , *6* , *9*) of species of *Bombus* Latreille, 1802 (Hymenoptera: Apidae: Bombini). *1–3* : *Bombus* (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. (Holotype); *4–6* : *Bombus* (*Thoracobombus*) *brevivillus* Franklin, 1913; *7–9* : *Bombus* (*Thoracobombus*) *morio* (Swederus, 1787).

barcoding approach enabled the unveiling of a new species of *Bombus* endemic to Brazil and also suggests that *B. brasiliensis* is a non-monophyletic species, likely a cryptic species complex. Subsequently, nuclear markers corroborate the mtDNA findings for the new species. The clade comprising the new species lineage was distinct and well supported. It is worth mentioning that although the nuclear markers supported the new species as a distinctive clade, those markers could not discriminate several other Brazilians species, probably due to recent divergence time among *B. bellicosus* , *B. brasiliensis* , *B. brevivillus* , and *B. transversalis* , estimated in less than 3 Mya (Hines 2008).

The *COI* divergence between the new species and its nearest neighbor was relatively high (6.11 %) when comparing to other *Bombus*

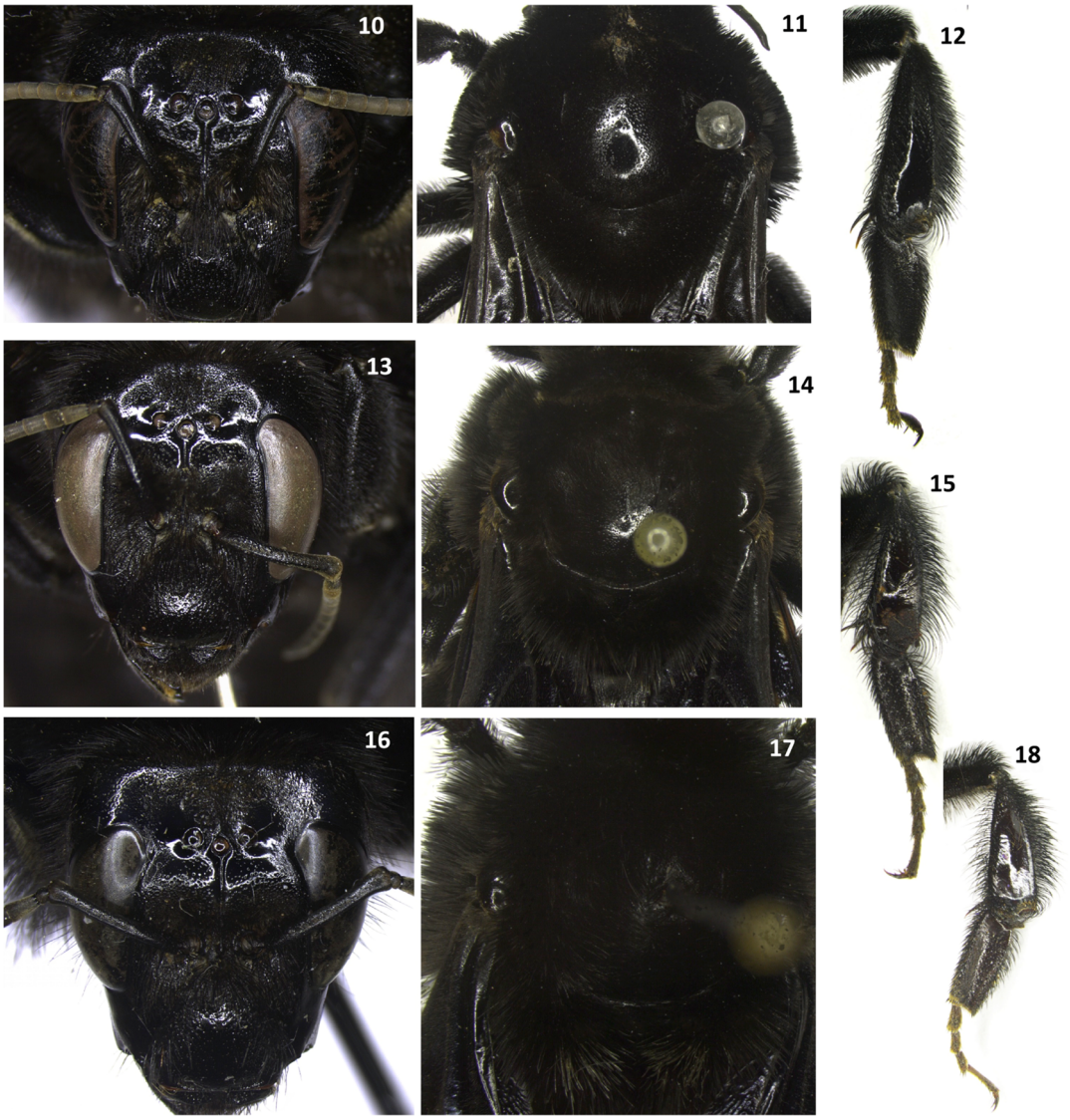

Figure 3. Dorsal view of the head (vertex) (*10*, *13*, *16*), mesoscutum (*11*, *14*, *17*), and hind tibia (*12*, *15*, *18*) of species of *Bombus* Latreille, 1802 (Hymenoptera: Apidae: Bombini). *10–12*: *Bombus* (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. (Holotype); *13–15*: *Bombus* (*Thoracobombus*) *brevivillus* Franklin, 1913; *16–18*: *Bombus* (*Thoracobombus*) *morio* Swederus (1787).

species. Among the Brazilian species that share a common ancestor (Cameron et al. 2007), the lowest value of the minimum interspecific sequence divergence was 0.8 % (between *B. transversalis* and *B. brevivillus*), and the highest value was 4.7 % (between *B. pauloensis* and *B. transversalis*). In the subgenus *Bombus*, the average was 3.2 % (minimum of 1.57 % and maximum of 6.07 %; Williams et al. 2012). Species boundaries signaled by deep *COI* divergence are generally congruent with those established through classical taxonomic work (Sheffield et al. 2009). In fact, herein, the new species can be distinguished morphologically from their sympatric species on the basis of shape of the clypeus, having a distinct dorsal platform and lateral area, length of malar area, and the length and shape of the hairs (shorter and aligned).

In this study, the DNA barcode methodology revealed a new bumblebee species from northeastern Brazil, with agreement between mitochondrial, nuclear, and morphological data. The large ranges of bumblebee species in Brazil, in addition to the lack of basic biological studies, constitute an auspicious scenario for further unveiling new species currently overlooked in species complexes. The recent reports about the decline in range or abundance of some of these valuable pollinators mean that there is a pressing need to monitor all bumblebee species (Williams and Osborne 2009). Hence, there is a need for revision of the taxonomy and nomenclature of the Brazilian species to give reliable support to further research (Moure and Sakagami 1962) and to guide conservation efforts.

Taxonomic treatment

Genus *Bombus* Latreille, 1802

B. (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. (Figures 2, 3, and 4)

Holotype. f (Queen): Holótipo // Favizia 005263 // Brasil, Bahia, Igrapiúna, BR 101-BA001, 12.X.2011, n. 216, E. Françoso leg. // *B*. (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. The specimen is in excellent condition, only with the right middle leg removed from the body for the molecular analysis, and is deposited in MZUFBA.

Paratypes. (3f, Queen): Parátipo // Favízia 005264 // Brasil, Bahia, Igrapiúna, Reserva Ecológica da Michelin, 11.X.2011, n. 204, E. Françoso leg. // *B*. (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. (1f, MZUFBA); Parátipo // Favízia 005265 // Brasil, Bahia, Igrapiúna, Reserva Ecológica da Michelin, 11.X.2011, n. 205, E. Françoso leg. // *B*. (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. (1f, MZUFBA); Parátipo // Favízia 005266 // Brasil, Bahia, Igrapiúna, Reserva Ecológica da Michelin, 11.X.2011, n. 206, E. Françoso leg. // *B*. (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. (1f, MZUFBA). Other specimens (13), studied only for molecular biology analysis, where also included here as Paratypes: Paratype: Parátipo // Brasil, Pará, Abel Figueiredo, 1.VII.2002, E.A.B.. Almeida // 7855–23796 (EF129) (1f, UFMG, LBEA); Parátipo // Brasil, Distrito Federal, Brasília, 27.VIII.1999, F. A. Silveira // 4900–13443 (EF116) (1f, UFMG, LBEA); Parátipo // Brasil, Tocantins, Itacajá, 17.I.1993, J.M.F. Camargo, J.A. Tavares, S.R.M. Pedro // 930450 (EF148) (1f, RPSP); Parátipo // Brasil, Paraíba, João Pessoa, IV.2009, S.S. Neto 6464 (EF20) (1f, ESALQ); Parátipo // Brasil, Bahia, Igrapiúna, 10.XI.2011, E. Françoso leg. // EF202 (1f, LGEA); Parátipo // Brasil, Bahia, Igrapiúna, 10.XI.2011, E. Françoso leg. // EF203 (1f, LGEA); Parátipo // Brasil, Bahia, Igrapiúna, 10.XI.2011, E. Françoso leg. // EF207 (1f, LGEA); Parátipo // Brasil, Bahia, Igrapiúna, 10.XI.2011, E. Françoso leg. // EF209 (1f, LGEA); Parátipo // Brasil, Bahia, Igrapiúna, 10.XI.2011, E. Françoso leg. // EF210 (1f, LGEA); Parátipo // Brasil, Bahia, Igrapiúna, 10.XI.2011, E. Françoso leg. // EF212 (1f, LGEA); Parátipo // Brasil, Bahia, Igrapiúna, 10.XI.2011, E. Françoso leg. // EF213 (1 f. LGEA); Parátipo // Brasil, Bahia, Igrapiúna, 10.XI.2011, E. Françoso leg. // EF214 (1 f. LGEA); Parátipo // Brasil, Bahia, Igrapiúna, 10.XI.2011, E. Françoso leg. // EF215 (1 f. LGEA).

Diagnosis: Integument predominantly black, except by a very small spot on mandibular condyle and the light brown strigilis, plumose setae across the body brownish and simple setae black; pubescence on clypeus and labrum more sparse, on clypeus leaving a large area without pubescence; general pubescence of body shorter and aligned, especially on the head, with the tip of hair straight as if trimmed (Figures 2 (2) and 3 (11)). Clypeus flattened on the middle portion longitudinally, with a large subtriangular dorsal area contrasting with an almost vertical lateral area (Figure 2 (1)); discal glabrous area of mesoscutum wide, polished, and shiny (Figure 3 (11)).

Description: f: *Structure*: Total body length 27.6; forewing length 20.0; head length 6.8, width 6.1; clypeus length 2.2, width 2.9 (clypeus dorsal area length 1.8, larger width 1.9, smaller width 0.66; lateral area width 0.7); malar area length 1.2; malar area width: greatest width 1.9, mandible basis 1.4; scape length 2.7, width 0.3 (apex 0.4);

Figure 4. Head in profile (*19*), frontal view of the head (vertex) (*20*), mesosoma in dorsal view (*21*, *22*, *26*, *30*, *34*, *39*), lateral view of the body (*23*, *27*, *31*, *35*, *36*), dorsal view of the head (vertex) (*24*, *28*, *32*, *37*), and mesoscutum (discal glabrous area) (*25*, *29*, *33*, *38*) of species of *Bombus* Latreille (1802) (Hymenoptera: Apidae, Bombini). *19–21*, *24–27*: *Bombus* (*Thoracobombus*) *pauloensis* Friese (1913); *22–23*: *Bombus* (*Thoracobombus*) *brevivillus* Franklin (1913); *28–31*: *Bombus* (*Thoracobombus*) *bellicosus* Smith (1879); *32–35*: *Bombus* (*Thoracobombus*) *transversalis* Olivier (1789); *36–39*: *Bombus* (*Thoracobombus*) *brasiliensis* Lepeletier (1836).

length of compound eye 4.1, width 1.9; ocellus diameter: median 0.43, lateral 0.44; ocellocular distance 0.8 (1.82× lateral ocellar diameter); interocellar distance: lateral to median 0.2,

between lateral 0.74; alveolocular distance 1.0; interalveolar distance 0.88; interocular distance: upper 3.4, median 3.6, lower 4.3; metatibia length 7.8, width 2.4; metabasitarsus length 5.2, width 2.0. Clypeus flattened on the middle portion longitudinally, what provide it a large flat dorsal area contrasting with an almost vertical lateral area (Figure 2 (1)).

Coloration: Integument predominantly black (Figures 2 (1–3) and 3 (10–12)), except by a very small spot on mandibular condyle and the anterior tibial spur (strigilis), light brown; antennal flagellum grayish from F3 to apex on ventral surface; tegula black; wing membrane dark amber brownish, a little darker in the anterior border and apex of the marginal and on basis of first submarginal cell, the veins blackish.

Pubescence: General setae on body long, but comparatively shorter than other similar species, and extremely dense, except on head, relatively uniform in length on the regions of body, especially on the head and mesoscutum and posterior edge of the posterior tibia, aligned with the tip of hair straight like they had been trimmed (Figures 2 (2, 3) and 3 (11, 12)). Body entirely covered by long black thick branched setae (the branches are extremely short and compact, almost imperceptible), interspersed by shorter plumose brown hairs, easily visible on the parocular area near the alveoli, on pronotal lobes, on sides of propodeum, apical border of the sternum, and on the last segment of the metasoma (dorsal and ventral sides); the longer bristles on sides of clypeus (0.61), vertex (0.8), mesoscutum (1.5), scutellum (2.0), and mesepisternum (1.8); setae of legs long, thicker, black, longer, and denser in the femurs than in the tibias, on posterior border of metatibia a little shorter (1.5; 1.0 on the anterior border), uniform in length and directed downward (0.8 on the posterior border of the metabasitarsus) (Figure 3 (12)); pilosity even longer on terga (3.2), except T1, where is extremely short and thin on disk and longer on the sides of the apical borer (0.8); microtrichia of forewing relatively long and dense in the basal two thirds of wing (0.2). A pale-yellowish-golden decumbent velvety microvilli, resembling that which covers the flagellum but longer and denser, is also observed on the external surface of the mandibles (denser in apical half), the apex of the coxae and first sternum, and ventral face of femora (denser in apical half), covering the apical third of basitarsus and entirely the other tarsomeres, especially the latter which is denser.

Sculpturing: Punctures extremely dense and marked, interspaces polished and mostly smaller than 0.3PD, the space between the points mostly represented by only the edge of the points, on vertex, mesepisternum, mesoscutum, and scutellum; some punctures a little larger on clypeus and labrum, but remarkable less marked; punctures very sparse on head, especially on clypeus (reach 3PD) (Figure 2 (1–3)), fore tibiae and corbicula and malar area where there is a large smooth area. Clypeus flattened on the middle portion longitudinally, with a large subtriangular dorsal area contrasting with an almost vertical lateral area (Figures 2 (1) and 3 (10)); discal glabrous area of mesoscutum relatively wide, polished and shiny (Figure 3 (11)).

Male: unknown.

Geographical distribution: Pará (Cabo Branco Beach, Abel Figueiredo), Distrito Federal (Brasília), Tocantins (Itacajá), Paraíba (João Pessoa), and Bahia (Igrapiúna). Basically in areas of sub-evergreen Equatorial forest, Brazilian savannah and Atlantic forest.

Comments. Among the Brazilian species of *Bombus*, *B. brevivillus* and *B. morio* are totally black, making them difficult to identify, especially in the field. *B. brasiliensis* has yellow bands on tergal hairiness, and *B. pauloensis* can have both morphotypes (totally black or with yellow stripes).

Although *B.* (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. is quite similar to *B. brevivillus* and *B. morio* in the color of integument, very dense pubescence and punctures, in general, it can be easily differentiated from both species by the flattened structure of clypeus on the middle portion and by its general pubescence of body aligned (uniform in length), with the tip of hair straight like they had been trimmed, and also by the relatively shorter

pubescence on head (Figures 2 and 3). Although the malar area is a little shorter in *B.* (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. than in *B. morio*, it is wider in the new species; *B. brevivillus* has the shorter malar area among the three species, and it is narrower than the new species. Comparing the discal glabrous area of mesoscutum in the three species, it is smooth and shiny in *B.* (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. and in *B. brevivillus* (narrower in the new species) and microreticulated and dull in *B. morio*.

Etymology: The specific epithet, from Latin (applanatis=flattened), is a reference to flatten clypeus, main character distinguishing *B.* (*Thoracobombus*) *applanatus* Oliveira, Françoso & Arias, sp. nov. from the other similar black species.

Diagnosis: *B.* (*Thoracobombus*) *brevivillus* f (Figures 2 (4–6) and 3 (13–15)): *Structure*: Total body length 26.5; forewing length 18.2; head length 7.0, width 5.8; clypeus length 2.1, width 2.8; malar area length 1.0; malar area width: greatest width 1.6, mandible basis 1.2; scape length 2.8, width 0.32 (apex 0.56); length of compound eye 3.9, width 1.6; ocellus diameter: median 0.34, lateral 0.33; ocellocular distance 0.8 (2.42× lateral ocellar diameter); interocellar distance: median to lateral 0.24, between lateral 0.72; alveolocular distance 0.9; interalveolar distance 0.8; interocular distance: upper 3.2, median 3.4, lower 3.9; metatibia length 7.4, width 2.3; metabasitarsus length 4.8, width 1.8. Clypeus slightly convex on the middle portion longitudinally, without flat dorsal area (Figures 2 (4) and 3 (13)); discal glabrous area of the mesoscutum relatively narrow, but polished and shiny (Figure 3 (14)). *Pubescence*: General setae on body long and extremely dense, except on head, not uniform in length on the regions of body, and with the apical extremity of the bristle very thinned in relation to other portions along its length (Figures 2 (5, 6) and 3 (14, 15)); some setae of body 2× longer than others, especially on the head and mesoscutum; the longer bristles on sides of clypeus (0.87), supraclypeal area (0.5–1.7), vertex (1.1), and mesepisternum (1.4); setae of legs long, thicker, black, longer, and denser in the femurs than in the tibias, on posterior border of metatibia a little shorter (1.6; 0.83 on the anterior border), not uniform in length (0.85 on the posterior border of the metabasitarsus and 0.7 on anterior border) (Figure 3 (15)); even longer terga (2.5), except T1, where is extremely short and thin on disk and longer on the sides of the apical borer (0.4).

Diagnosis: *Bombus morio* f (Figures 2 (7–9) and 3 (16–18)): *Structure*: Total body length 28.0; forewing length 20.8; head length 7.7, width 6.1; clypeus length 2.6, width 2.8; malar area length 1.45; malar area width: greatest width 1.4, mandible basis 1.3; scape length 2.9, width 0.3 (apex 0.5); length of compound eye 4.1, width 1.8; ocellus diameter: median 0.37, lateral 0.42; ocellocular distance 0.8 (1.9× lateral ocellar diameter); interocellar distance: lateral to median 0.2, between lateral 0.7; alveolocular distance 0.9; interalveolar distance 0.8; interocular distance: upper 3.3, median 3.5, lower 4.2; metatibia length 7.4, width 2.3; metabasitarsus length 3.5, width 1.3. Clypeus convex on the middle portion longitudinally, without flat dorsal area (Figures 2 (7) and 3 (16)); discal glabrous area of the mesoscutum microreticulated and dull, well-defined (Figure 3 (17)). General setae on body long and extremely dense, except on head, not uniform in length on the regions of body, and with the apical extremity of the bristle very thinned in relation to other portions along its length (Figures 2 (8, 9) and 3 (17, 18)); some setae of body 2× longer than others, especially on the head and mesoscutum; some extremely long on lateral of clypeus (1.4); the longer bristles on sides of clypeus (1.51), supraclypeal area (1.53), vertex (1.2), and mesepisternum (1.4); setae of legs long, thicker, black, longer, and denser in the femurs than in the tibias, on posterior border of metatibia a little shorter (1.7; 1.13 on the anterior border), not uniform in length (1.64 on the posterior border of the

metabasitarsus and 0.66 on anterior border) (Figure 3 (18)); even longer terga (2.2), except T1, where is extremely short and thin on disk and longer on the sides of the apical borer (1.2).

Morphological identification key for Brazilian species of *Bombus* Latreille (1802), modified from Moure and Sakagami (1962)

Females

1. Pubescence entirely black, except for some brownish hairs or slightly whitish at the apical end, observed in the terga and ventral half of the body (Figures 2 (1–9), 3 (10–18) and 4 (21-23))..... 2

1′. Yellow or reddish pubescence on the thorax (mesosoma) and/or abdomen (metasoma), forming stripes for both, or fully covering the dorsal side of the thorax (Figure 4 (19, 20), (24–39))..... 5

2. Clypeus flattened medially, so that features a large flat subtriangular in the central area with a lateral area almost vertical (Figures 2 (1–9) and 3 (10)); pubescence of the head, thorax, and posterior edge of the posterior tibial relatively long and dense, uniform in length, with the tips of hair straight, as if they had been trimmed (Figures 2 (2, 3) and 3 (11, 12)); discal glabrous area of mesoscutum well developed, smooth, and glossy (Figure 3 (11))..... *B*. (*T*.) *applanatus* Oliveira, Françoso & Arias, sp. nov.

2′. Clypeus moderately convex or slightly convex, without central flat area (Figures 2 (4, 7) and 4 (19, 20)); pubescence of the head and thorax relatively long, but not uniform in length around the head and thorax, bristles with the apical end quite tapered (Figure 2 (5, 6, 8, 9)); discal glabrous area of the mesoscutum variable..... 3

3. Malar area longer than its width of the jaw base (Figure 2 (9)); discal glabrous area of mesoscutum microreticulated and dull, well defined (Figure 3 (17))..... *B*. (*T*.) *morio*

3′. Malar area shorter than its width of the jaw base (Figure 2 (5, 6)); discal glabrous area of mesoscutum somewhat or very developed, however shiny (Figure 3 (14))..... 4

4. Superior micropunctuated justaorbital area, at least as wide as the diameter of medium ocellus (Figure 3 (13)); shorter hairiness (1.1 in T3), thick and relatively dense, velvety (Figure 4 (22))..... *B*. (*T*.) *brevivillus*

4′. Superior micropunctuated justaorbital area very narrow, generally around one third of the ocelorbital distance; hairiness longer in general (1.4 in T3), thin and looser, especially in T4 and T5, easily allowing easy viewing of the integument through the bristles (Figure 4 (21)). melanocytic form..... *B*. (*T*.) *pauloensis*

5. Pronotum, mesoscutum, and scutellum covered with yellowish or rusty pubescence, without a black hairy interalar stripe; sometimes, some yellowish hairs on T1 and T2; the last three terga rusty-hairy (Figure 4 (28–31)) ..... *B*. (*T*.) *bellicosus*

5′. Pronotum, mesoscutum, and scutellum covered with yellowish or rusty pubescence more or less developed, but always with a black hairy interalar stripe (Figure 4 (25, 33, 38)); usually with a yellow stripe on the third tergum and the last segments black-hairy and black (Figure 4 (26, 34, 39))..... 6

6. Posterior glabrous discal area of mesoscutum undefined, with quite strong punctuation and somewhat more sparsely than in other regions of mesoscutum (Figure 4 (33)); short pubescence (around 0.9 in T3), stripe of pronotum, mesoscutum, scutellum, and third tergum with an intense ocher yellow (Figure 4 (33–35))..... *B*. (*T*.) *transversalis*

6′. Posterior glabrous discal area of mesoscutum well defined, shiny, more or less infundibuliform, without point in the middle (Figure 4 (25, 38)); the hairiness longer and looser (minimum 1.2 in T3) (Figure 4 (26, 39))..... 7

7. The yellow pubescence fully covering the mesepisternum and propodeum to bottom, beyond the pronotum strips, scutellum, first and third tergum (Figure 4 (36–39)) ..... *B*. (*T*.) *brasiliensis*

7′. The yellow pubescence slightly paler, restricted to the pronotum, dorsal portion of mesosoma, and T3 (flavinic form; Figure 4 (24–27)) ..... *B*. (*T*.) *pauloensis*

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Une approche intégrative identifie une nouvelle espèce de bourdon (Hymenoptera : Apidae : Bombini) du nord-est du Brésil

***Bombus* sp. nov. / barcoding moléculaire / marqueurs nucléaires / taxonomie traditionnelle**

Eine integrative Methode identifiziert eine neue Hummelart (Hymenoptera : Apidae : Bombini) in Nordost-Brasilien

Hummel / *Bombus* sp. nov. / DNA barcoding / nukleare Marker / traditionelle Taxonomie

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