Species delimitation and sex associations in the bee genus *Thygater*, with the aid of molecular data, and the description of a new species

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Abstract – *Thygater* Holmberg 1884, a Neotropical bee genus included in the tribe Eucerini, is distributed from Argentina to Mexico, was last revised almost 50 years ago, and included 30 species. Significant problems in *Thygater* taxonomy are like those found in other bee taxa: sexual dimorphism (sometimes accentuated); large intraspecific variation in some taxa, especially in color patterns; great similarity among putative recently-diverging species; and scarcity of specimens for study of several apparently rare species. These problems hinder the correct delimitation of species boundaries and could result in an underestimated number of species and incorrect association of sexes. In this taxonomic and phylogenetic study of the genus, morphological and molecular evidence are considered together to elucidate the taxonomy of several Brazilian species of *Thygater*. The analyses allowed the description of two new species (one already described and other described here), additional support for the synonyms proposed elsewhere and sex associations for several species.

Apoidea / sexual dimorphism / Eucerini / Neotropics / new synonym / reciprocal monophyly / species delimitation

1. INTRODUCTION

The Neotropical bee genus *Thygater* Holmberg (1884), a member of the tribe Eucerini, includes 30 species distributed from Argentina to Mexico, but absent in Chile and the Antilles (Michener 2007; Urban et al. 2012). Some of its

species, such as *Thygater analis* (Lepeletier 1841), are relatively common throughout their geographic ranges (Urban 1967). Others, however, are relatively rare, and some are narrowly geographically and environmentally restricted (Urban 1967, 1999; Freitas and Silveira 2017).

The main problems found when working on *Thygater* taxonomy are the sexual dimorphism (sometimes very accentuated); the large intraspecific variation in some species, especially in color patterns; the great similarity among putative recently-diverging species; and the scarcity of specimens for study of several rare species. These problems, which hinder the correct delimitation of species boundaries (including association of

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sexes), are not exclusive of *Thygater*. In fact, molecular studies are showing that the number of recognized species is underestimated due to such problems, especially among invertebrates, including bees (e.g., Duda Jr. et al. 2008; Fontaneto et al. 2009; Gibbs 2009a; Koch 2010; Soldati et al. 2014; Wolinska et al. 2009). In dealing with such problems, the association of molecular and morphological characters has proven to be of help in Hymenoptera, including several groups of bees (e.g., Gibbs 2009a, b; Hurtado-Burillo et al. 2017; Nemésio et al. 2013; Pilgrim et al. 2008; Rehan and Sheffield 2011; Santos Júnior et al. 2015; Sheffield et al. 2009). This approach, however, has never been applied to *Thygater*.

As part of a comprehensive study on the phylogeny and taxonomy of *Thygater* and related genera, the principles of integrative taxonomy, with the combined use of molecular and morphological data (Dayrat 2005; Padial et al. 2010; Schlick-Steiner et al. 2010; Will et al. 2005), were employed here to test previous sex associations, to search for unknown sexes of species described previously on the basis of a single sex, and to test the boundaries of species of *Thygater* previously described on the basis of morphological characters alone, especially of those occurring in Brazil, always under a phylogenetic perspective.

2. MATERIAL AND METHOD

A total of 356 specimens of *Thygater* were examined for this study. Specimens employed in molecular analyses are listed in Table 1; type specimens of *Thygater carijo* sp. n. are listed in the species description, in Section 3.3; other specimens studied are listed in Freitas and Silveira (2017). The studied material belongs to the following collections: *Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais* (UFMG); *Museu Regional de Entomologia da Universidade Federal de Viçosa* (UFV); *Coleção Entomológica Prof. J. M. F. Camargo* of the *Universidade de São Paulo*, *Ribeirão Preto* (RPS); *Coleção Entomológica Padre Jesus Santiago Moure* of the *Universidade Federal do Paraná*, *Curitiba* (DZUP); and the *Laurence Packer Collection* of the *York University*, *Toronto* (PCYU). Those bees were examined under a *Leica M125[®]* dissection microscope in search for

distinctive taxonomic characters. Male genitalia of most species were dissected for study as described in Freitas and Silveira (2017).

The following species were employed as the ingroup in the analyses described below: *Thygater anae* Urban 1999, *Thygater armandoi* Urban 1999, *Thygater chaetaspis* Moure 1941, *Thygater latitarsis* Urban 1967, *Thygater luederwaldti* (Schrottky 1910), *Thygater minarum* Urban 1999, *Thygater palliventris* (Friese 1908), and *Thygater paranaensis* Urban 1967. Additionally, a few distinct morphospecies, all identifiable as *T. analis* (Lepelletier 1841), were also included in the analyses. Of the 15 species of *Thygater* known to occur in Brazil, these are all for which material could be obtained for molecular analysis.

Trichocerapis mirabilis (Smith 1865) was chosen as the outgroup, considering that the genus is part of a putative clade in Eucerini, also including *Thygater*, the so-called *Thygater-Trichocerapis* group (Urban 1967; Michener 2007).

An effort was made to extract DNA from the largest possible number of specimens of each species. For abundant taxa, newer specimens were preferred for maximum extraction efficiency. DNA samples were extracted from legs taken from dry specimens, using the phenol–chloroform method (Sambrook & Russel 2001). The cytochrome oxidase I (COI) was then amplified, using the primers LepF1 and LepR1 (Hebert et al. 2004) and mtd_6 and mtd_9 (Simon et al. 1994). Amplification was done in 15 µL polymerase chain reaction (PCR) mix, including 5 units of Taq polymerase, 0.6 µL of MgCl₂ (50 mM), 1.5 µL of PCR buffer (10×), 0.5 µL of each primer (25 µM), 1.2 µL of dNTPs (2.5 mM), and about 1 µL of genomic DNA (20 ng). Amplification was carried out using a thermocycler program consisting of 5 min of denaturation at 94 °C, followed by 37 cycles of 30 s at 94 °C, 40 s at 48–50 °C, 1.30 min at 72 °C, and a final extension for 10 min at 72 °C. PCR products were visualized in a 2% agarose gel. All PCRs that generated a single product were purified using 20% polyethylene glycol (PEG) precipitation (Santos Júnior et al. 2015). Sequencing reactions were carried out using BigDye[®] version 3.1 in both directions using an ABI 3130xl Genetic Analyzer[®] sequencer, with the same amplification primers, and following the manufacturer's recommendations.

Table 1. Specimens employed in the phylogenetic analyses performed here

Species	ID	Sex	Depository collection		Locality (country—state/ province)	GenBank access number
			Acronym	Access no.		
<i>T. anae</i>	1	Female	UFMG	IHY 38332	Brazil—Minas Gerais	MF544041
	2	Female	UFMG	IHY 65124	Brazil—Rio de Janeiro	MF544042
	3	Male	UFMG	IHY 1200097	Brazil—Rio de Janeiro	MF544043
<i>T. analis</i>	4	Male	UFMG	IHY 1217311	Brazil—Paraíba	MF544044
	5	Male	UFMG	IHY 1217358	Brazil—Paraíba	MF544045
	6	Female	UFMG	IHY 1300785	Brazil—Minas Gerais	MF544046
	7	Male	UFMG	IHY 1316999	Brazil—Rio de Janeiro	MF544047
	8	Male	UFMG	IHY 1317001	Brazil—Rio de Janeiro	MF544048
	9	Male	UFMG	IHY 1402951	Brazil—Minas Gerais	MF544049
	10	Male	UFMG	IHY 1530265	Brazil—Santa Catarina	MF544050
	11	Male	UFMG	IHY 1600325	Brazil—Minas Gerais	MF544051
	12	Male	UFMG	IHY 1208352	Brazil—Minas Gerais	MF544052
	13	Female	PCYU	NSV 4971	Argentina—Misiones	MF544053
	14	Female	PCYU	NSV 3243	Argentina—Formosa	MF544054
<i>T. carijo</i> sp. n.	15	Male	UFMG	IHY 1200664	Brazil—Santa Catarina	MF544055
	16	Male	UFMG	IHY 1200675	Brazil—Santa Catarina	MF544056
	17	Female	UFMG	IHY 1200670	Brazil—Santa Catarina	MF544057
	18	Female	UFMG	IHY 1200704	Brazil—Santa Catarina	MF544058
	19	Female	UFMG	IHY 1200686	Brazil—Santa Catarina	MF544059
	20	Male	UFMG	IHY 1200689	Brazil—Santa Catarina	MF544060
<i>T. chaetaspis</i>	21	Female	UFMG	IHY 37872	Brazil—Rio de Janeiro	MF544061
	22	Female	UFMG	IHY 38303	Brazil—Espírito Santo	MF544062
	23	Macho	UFMG	IHY 38306	Brazil—Espírito Santo	MF544063
	24	Female	UFMG	IHY 38338	Brazil—Minas Gerais	MF544064
<i>T. minarum</i>	25	Female	UFMG	IHY 37965	Brazil—Minas Gerais	MF544065
	26	Male	UFMG	IHY 53640	Brazil—Minas Gerais	MF544066
	27	Male	UFMG	IHY 53641	Brazil—Minas Gerais	MF544067
	28	Male	UFMG	IHY 53642	Brazil—Minas Gerais	MF544068
<i>T. palliventris</i>	29	Male	UFMG	IHY 53700	Brazil—Minas Gerais	MF544069
	30	Female	UFMG	IHY 1205207	Brazil—Minas Gerais	MF544070
	31	Male	UFMG	IHY 1500415	Brazil—Minas Gerais	MF544071
	32	Male	UFMG	IHY 1500418	Brazil—Minas Gerais	MF544072
	33	Male	UFMG	IHY 1500419	Brazil—Minas Gerais	MF544073
	34	Male	UFMG	IHY 1500422	Brazil—Minas Gerais	MF544074
	35	Female	UFMG	IHY 1501392	Brazil—Minas Gerais	MF544075
	36*	Female	UFMG	IHY 1530285	Brazil—Santa Catarina	MF544076
	37	Female	UFMG	IHY 1530286	Brazil—Santa Catarina	MF544077
	38	Male	UFMG	IHY 1600305	Brazil—Minas Gerais	MF544078
	39	Male	PCYU	NSV 3502	Argentina—Misiones	MF544079
<i>T. paranaensis</i>	40	Female	UFMG	IHY 1200448	Brazil—Santa Catarina	MF544080
	41	Female	UFMG	IHY 1200449	Brazil—Santa Catarina	MF544081

Table 1 (continued)

Species	ID	Sex	Depository collection		Locality (country—state/ province)	GenBank access number
			Acronym	Access no.		
<i>T. danunciae</i>	42	Female	UFMG	IHY 65133	Brazil—Rio de Janeiro	MF544082
	43	Female	UFMG	IHY 92245	Brazil—Minas Gerais	MF544083
	44	Female	UFMG	IHY 1200451	Brazil—Santa Catarina	MF544084
	45	Male	UFMG	IHY 76767	Brazil—Minas Gerais	MF544085
	46	Female	UFMG	IHY 39828	Brazil—Minas Gerais	MF544086
<i>T. luederwaldti</i>	47	Female	PCYU	06728H10_BRA	Brazil—Minas Gerais	MF544087
<i>T. latitarsis</i>	48*	Male	UFMG	IHY 37922	Brazil—Rio de Janeiro	MF544088
	49	Female	UFMG	IHY 37880	Brazil—Rio de Janeiro	MF544089
	50	Female	UFMG	IHY 37923	Brazil—Rio de Janeiro	MF544090
<i>Trichocerapis mirabilis</i>	51	Male	UFMG	IHY 65105	Brazil—Minas Gerais	MF544091

The specimens marked with asterisk were previously identified as *T. nigrilabris* (27), which is synonymized to *T. latitarsis*, and *T. armandoi* (36), synonymized to *T. palliventris*

The consensus sequences were obtained using the software SeqScape® v2.6. At least two sequencing reactions were done in each direction to help in the elucidation of doubts concerning chromatogram interpretation. A complete list of the specimens employed in the phylogenetic analyses from which DNA was extracted and sequenced is given in Table 1.

2.1. Matrix construction

COI sequences were aligned using the algorithm MUSCLE (Edgar 2004) with the default settings in MEGA 7 (Kumar et al. 2016), resulting in an alignment of 726 bp. After that, the matrix was submitted to the algorithm Gblocks (Castresana 2000), in the online server http://molevol.cmima.csic.es/castresana/Gblocks_server.html, with all options to a less stringent selection marked (“Allow smaller final blocks,” “Allow gap positions within the final blocks,” and “Allow less strict flanking positions”) to reduce the missing data in the extremities of the alignment, resulting in a new matrix, now with 652 bp, that was used in the phylogenetic analyses.

The nucleotide substitution models and partitioning schemes were chosen with the aid of the software PartitionFinder2 (Lanfear et al. 2016), with each COI codon position included as a partition, the branch lengths unlinked, BIC as

the criterion for model selection, and “greedy” as the option for best scheme searching.

Phylogenetic analyses were conducted using the online server CIPRES (<https://www.phylo.org/portal2/home.action>; Miller et al. 2010). Maximum likelihood (ML) analysis was conducted using RAXML 8.2.9 (Stamatakis 2014) with 100 starting trees and the bootstrap configured to halt automatically with the auto RME criterium. The substitution model used for each partition (COI codon positions) was the GTR + GAMMA (the only one implemented). Default parameters were used in all other instances. Bayesian inference (BI) was performed with MrBayes 3.2.6 (Ronquist et al. 2012) using two independent runs with 10 million generations, with four Markov chains each, and a 25% burn-in. HKY + GAMMA, F81 + I, and HKY + GAMMA were the models indicated by PartitionFinder2 and used for each one of the partitions in sequence. Other parameters were the default ones. Convergence for BI analysis was checked using Tracer 1.6 (Rambaut, et al. 2014); ESS values were confirmed to be all above 200.

Average intra- and interspecific genetic distances were obtained for all species analyzed. For this, DNA originally extracted for this paper and COI data available in GenBank and BOLD online databases were employed (sequence access numbers are available in the [supplementary](#)

material). Since sequencing primers used to generate these sequences were not always the same, only the 379 bp present in all sequences were employed here. Genetic distances were calculated with MEGA 7 (Kumar et al. 2016), using the Kimura two-parameter (K2P) model with 1000 bootstrap replications (Nei and Kumar 2005). Intraspecific distances were estimated only for species represented by three or more specimens and with at least two different haplotypes.

In the diagnoses and description below, the morphological nomenclature employed was generally that of Michener (2007), except that the divisions of the antennal flagellum were called “flagellomeres” instead of “flagellar segments” and were usually abbreviated to F1, F2, etc. Similarly, divisions of the palpi were called palpomeres. Metasomal terga and sterna were called T1, T2, etc., and S1, S2, etc., respectively. Additionally, terminology referring to male genitalia follows Urban (1967). Distance between punctures is expressed as number of diameters of a puncture (dp).

In the taxonomic sections of this paper, information on the holotype is reproduced as presented on its labels, with information in different lines of a label separated by semicolons, and information from each label separated by double quotes. Information on the other examined specimens is given in the following format: country—state: municipality—specific locality (when available), collection date (as dd/mm/yyyy), collector name, number of females and males examined [acronym of the depository collection]. Information on specimens collected in each municipality is separated by a semicolon from those referring to specimens collected in the same municipality but on different dates, and/or by different collectors.

The diagnostic characters found for each of the species or sexes recognized here are in boldface in the diagnoses presented in the taxonomic results below.

3. RESULTS

3.1. Phylogenetic analyses

The results of both analyses [ML (Fig. S1 supplementary material) and BI (Figure 1)] were congruent in three different ways: (1) both recovered all the species previously recognized

morphologically, as well as the candidate new species (*T. carijo* sp. n., see Section 3.3), as reciprocally monophyletic; (2) both suggested the same sex associations for all the species for which male and female were included in the analyses; and (3) both grouped the species in two major, strongly supported clades with the same species composition.

The analyses also indicated that males of *T. analis* and those of *T. palliventris* were being confused (males of the latter were unknown until now). Moreover, they allowed for the identification of male *Thygater danunciae* [described in Freitas and Silveira (2017) and identified with the support of these results] and *T. carijo* sp. n. Males of both species were previously identified as *T. analis* with the use of Urban's (1967) key to the species of *Thygater*. Genetic separation of the males of these four species led to their careful examination and to the discovery of subtle morphological characters allowing for their distinction. These characters were employed in the description of *T. carijo* sp. n. (see Section 3.3) and in another taxonomic study of *Thygater* (Freitas and Silveira 2017). The genetic analyses also lead to the recognition of males of *T. minarum*, a species known only by its females until now (Urban 1999; Urban et al. 2012). Moreover, the results also confirmed the preliminary suspicion by Freitas and Silveira (2017), based on morphological similarity, that *T. armandoi* and *Thygater nigrilabris* were junior synonyms of *T. palliventris* and *T. latitarsis*, respectively.

The only difference between ML and BI results was the position of the specimens of *T. anae* and *T. minarum*, which were sister species, sister to the remaining species in the clade (*T. danunciae*, *T. analis*, *T. carijo* sp. n., and *T. palliventris*) in the BI result (see Figure 1), while in the ML tree, *T. minarum* was sister to *T. anae* + the remaining species in the clade.

3.2. Genetic distances

Thygater paranaensis presented the largest average intraspecific distance ($0.50\% \pm 0.25$, Table S1 Supplementary Material); however, this is still smaller than the smallest average interspecific distances, those between *T. analis* and

Figure 1. Consensus tree resulting from Bayesian inference. All posterior probabilities above 95% and those inside the clades corresponding to the species were omitted. The branch between the outgroup and the ingroup was shortened so that the tree would fit in the page. Males attributed to *T. minarum* in the tree were previously undescribed; males attributed to *T. palliventris* and *T. danunciae* and males and females attributed to *T. carijo* sp. n. were previously identified as *T. analis*. Specimen 36-F included among *T. palliventris* was formerly identified as *T. armandoi* (now a junior synonym of *T. palliventris*), and specimen 48-M was previously identified as *T. nigrilabris* (synonymized to *T. latitarsis*), both of them are marked with an asterisk. The specimen ID numbers are those in Table I. The letters M and F in the specimen codes identify males and females, respectively.

T. danunciae ($0.80\% \pm 0.55$), between *T. palliventris* and *T. danunciae* ($0.83\% \pm 0.45$), between *T. carijo* sp. n. and *T. palliventris* ($0.88\% \pm 0.45$), and between *T. carijo* sp. n. and *T. danunciae* ($1.05\% \pm 0.50$) (Table S1 Supplementary Material).

3.3. Taxonomic results

Thygater carijo sp. n. is described; males of *T. palliventris* and *T. minarum* are diagnosed (their complete descriptions, as well as the description of *T. danunciae*, were presented elsewhere (Freitas and Silveira 2017)).

3.3.1. *Thygater palliventris* (Fries 1908) (Figures 2a–c and 4b, e)

Tetralonia palliventris Fries 1908, p. 47

Thygater (*Thygater*) *palliventris* (Moure & Michener 1955; Urban 1967; Moure et al. 2012)

Thygater (*Thygater*) *arandoi* Urban 1999, p. 219

Diagnosis (male) Labrum yellowish (Figure 2a); basal depression of clypeus short (one fifth of clypeal length; Figure 2a); pilosity of mesosoma always predominantly whitish

(Figure 2a–c); hind basitarsus parallel-sided; pilosity on marginal zones of T5 and T6 always white; apical emargination of T7 deep, separating two triangular lobes; marginal fringe of S4 incomplete; fringe of thick setae present only along posterior margin of proximal lobe of median plate of S7, absent on the anterior margin of distal lobe (Figure 4b); proximal lobe of S7 wide, its anterior margin bulging, anterior and lateral margins meeting on a wide angle (Figure 4b). *Thygater palliventris* can be confused with those males of *T. analis* with predominantly white pilosity on T4–T7 but can be distinguished from them by the characters in S7 highlighted above. These two species are sympatric throughout the known range of *T. palliventris*.

Examined material Argentina—Misiones: Puerto Iguazu–Parque Nacional Iguazu (17 July 2009), N. Veiga, 1♂ [PCYU]. Brazil—Minas Gerais: Alto Caparaó (12 March 2015), J. E. Santos-Junior and J. C. Oliveira, 1♂ [UFMG]; Caldas–Pedreira Juparana (10 November 2014), B. C. Figueiredo, 10♂♂ [UFMG]. Itamonte (20 October 2006), M.F. Goulart, 1♂ [UFMG]. Nova Lima (12 May 2015), A. R. Lima, 1♂ [UFMG]; 26

Figure 2. a–c Male of *T. palliventris* (UFMG IHY 1602166). a Frontal view. b Lateral view. c Dorsal view. d–f Male of *T. minarum* (UFMG IHY 53639). d Frontal view. e Lateral view. f Dorsal view. Scale bars = 1 mm.

April 2015, A. R. Lima, 1♂ [UFMG]. Sabará (24 November 1996), F. A. Silveira, 1♂ [UFMG]. Santa Maria do Salto (13 February 2009), A. Nemésio (13 February 2009), 1♂ [UFMG]; 14 February 2009, A. Nemésio, 1♂ [UFMG]. Santa Catarina: São Francisco do Sul—Bairro Vila da Glória (20 December 2011), F. A. Silveira, 2♂♂ [UFMG]. São Paulo: Bauru—UNESP (1 July 1994), F. Knoll, 1♂ [UFMG].

the posterior margin of the proximal lobe of median plate of S7 (Figure 4c), those setae thicker than in any other Brazilian species of the genus.

Examined material/Brazil—Bahia: 8 km from Camacã—Serra Bonita (21 January 2011), G. Melo, 6♂ [DZUP]. Prado (5 November 1971), C. Elias, 7♂ [DZUP]. Minas Gerais: Santa Maria do Salto (13 February 2009), A. Nemésio, 7♂♂; 14 February 2009, A. Nemésio, 1♂ [UFMG].

3.3.2. *Thygater minarum* Urban 1999 (Figures 2d–f and 4c, f)

Thygater (*Thygater*) *minarum* Urban 1999, p. 217; Moure et al. 2012

Diagnosis (male) Males of *T. minarum* can be confused with those of *T. analis* and *T. palliventris*, from which they can be distinguished by the projections of the apical margin of T7, which are rounded in *T. minarum* and triangular in those other species; by the presence of a fringe of very thick setae present only along

3.3.3. *Thygater carijo* Freitas & Silveira sp. n. (Figures 3 and 4a, d)

Holotype (♀): “Brasil, SC, São Francisco; do Sul, B. Vila da Glória; 26° 13' 17.4" S; 48° 41' 11.3" W; 20.xii.2011. Em flor; F. A. Silveira. UFMG IHY 1200666”; “Holotypus; *Thygater* (*Thygater*) *carijo* ♀; F.V. Freitas & F. A. Silveira 2018”. At UFMG.

Paratypes (3♀♀ and 4♂♂): “Brasil, SC, São Francisco do Sul, B. Vila da Glória, 26° 13' 17.4" S; 48° 41' 11.3" W. 20.xii.2011. Em flor. F. A.

Figure 3. *T. carijo* sp. n. Female (holotype, UFMG IHY 1200666). **a** Frontal view. **b** Lateral view. **c** Ventral view. Male (paratype, UFMG IHY 1200664). **d** Frontal view. **e** Lateral view. **f** Ventral view. Scale bars = 1 mm.

Figure 4. Seventh sterna (a–c) and genitalia (d–f) of a, d *Thygater carijo* sp. n. (UFMG IHY 1200664); b, e *T. palliventris* (UFMG IHY 1602166); and c, f *T. minarum* (UFMG IHY 53639). Scale bars = 500 μ m.

Silveira. UFMG-IHY-1200664, 1200670, 1200675, 1200686, 1200689, 1200690, 1200704”.

Etymology: Homage to the Carij Indians, original inhabitants of the coastal lands that encompass the type locality.

Flower records: unknown.

Diagnosis Females of *T. carijo* sp. n. can be distinguished mainly for their completely black integument and almost completely black pilosity (Figure 3a–c). They have a smooth mid-longitudinal stripe on clypeal integument, which never reaches the apex of clypeus (Figure 3a) similar to that in *T. palliventris*, but in this latter species, the integument is yellowish or brownish, never black as in *T. carijo* sp. n. Males may be confused with *T. analis* and *T. palliventris*, but

the pilosity of F1 is golden in *T. carijo* sp. n. and black or fuscous in the two other species. Besides that, *T. carijo* sp. n. has the median plate of S7 with thick setae along the posterior margin of its proximal lobe and the anterior margin of its distal lobe, these setae almost reaching the base of both lobes, as shown in Figure 4a; in *T. analis* and *T. palliventris*, those setae never reach the base of those lobes (Figure 4b).

Description ♀ (holotype) Measurements (mm): total body length, 13.0; maximum head width, 4.9; largest distance between the external margins of tegulae, 5.2; length and width of clypeus, 1.5 and 2.2, respectively; inter antennal distance, 1.1; malar area, 0.3 (one fourth width of mandibular base); maximum labrum width, 1.8. **Integument** black, except translucent-amber on apex of

marginal zones of terga and sterna; on clypeus densely punctate, reticulate on basal two thirds, except for smooth mid-longitudinal stripe; mesosoma reticulate, densely punctate (< 1 dp); metasoma lineolate, finely and densely punctate (< 1 dp); marginal depression of terga smooth. *Pilosity* black, except whitish on labrum, laterally on clypeus, lower paraocular area, gena near the posterior eye margin, and on lateral tufts of S3 and S4; fuscous on venter of mesosoma and metasoma; on mesosoma dense; on metasoma sparse, with predominantly simple setae on terga; T5 with thick, long setae mixed with plumose ones; sterna with complete apical fringe, especially dense on S5. *Structure*—clypeus gently convex, with light longitudinal, preapical elevation, its basal depression reaching one third of clypeal length; marginal zone of terga short (shorter than F2), except that of T1, almost as long as pedicel; translucent-amber areas on apex of marginal zone of sterna short, shorter than pedicel; S6 with mid-longitudinal carina.

♂ (UFMG IHY 1200664, Brazil, Santa Catarina, São Francisco do Sul, B. Vila da Glória, 20.xii.2011. Em flor. F. A. Silveira). *Integument* black, except whitish-yellow on labrum; head reticulate, except smooth on mid-longitudinal stripe (almost as wide as the length of F1) of clypeus, and around ocelli; mesosoma reticulate and densely punctate (≈ 1 dp), mesoscutum somewhat shining, with lighter reticulation; metasoma strongly reticulate, especially on terga, somewhat shining with lighter reticulation on sterna; terga finely and sparsely punctate (≈ 1.5 dp), sterna still finer and sparser. *Pilosity* fuscous, except golden on F1, white on head, pronotum, axillae, posterior margin of mesoscutum, metanotum, propodeum, all venter and including forelegs, coxa, trochanter, and femur of mid and hind legs, and T1 basally; black apically on T1 and on T2–T4 throughout; a mixture of black and white hairs on T5–T7, the white ones more abundant on disc; on T2–T4 forming medially-interrupted fringes; on T5 reduced to lateral tufts; on T6 forming an hourglass-shaped patch of simple and short hairs. *Structure*—malar area two thirds as long as basal mandibular width; clypeus slightly convex on

disc, its basal depression reaching one third of clypeal length; translucent-amber areas on apex of marginal zones short, S5 without marginal projections; S6 without lateral and mid-longitudinal carinae; T7 deeply emarginate forming two flat triangular projections.

4. DISCUSSION

The genetic divergences between some of the species of *Thygater* were relatively small, even if compared to species in other genera of Eucerini (Table S2 Supplementary Material). However, there are other examples of bee taxa with small genetic distances between closely related species, for example *Bombus* (Santos Júnior et al. 2015 and references therein), *Dialictus* (Gibbs 2009a), and Euglossina (Dick et al. 2004; Nemésio et al. 2013). As in other bee groups, a “barcode gap” can be identified, with interspecific distances being always larger than the largest intraspecific distances. Of course, this gap by itself is not irrefutable evidence for species distinction (e.g., Čandek and Kuntner 2014; Magnacca and Brown 2010; Meyer and Paulay 2005; Wiemers and Fiedler 2007) but gives additional support to the recognition of sibling species, when considered with other sources of evidence, such as the reciprocal monophyly of species, and the recognition of morphological autapomorphies or consistent diagnostic characters (see also Freitas and Silveira (2017)).

In the opposite direction, analyses of molecular data gave additional support to the recognition of synonyms when species were originally distinguished by subtle characters (Urban 1999), which were found to be variable (see a discussion of these characters in Freitas and Silveira (2017)).

The analyses above also corroborated the sex associations suggested by previous authors for the species of *Thygater* and allowed new associations, reinforcing the utility of the genetic barcode as an auxiliary tool in systematics, especially in sex association, a hard task in the taxonomy of

highly sexual dimorphic insects, as already shown for other invertebrates (e.g., Barrett and Hebert 2005; Carolan et al. 2012; Koch 2010; Packer et al. 2009; Ståhls et al. 2009) and especially bees (e.g., Carolan et al. 2012; Gibbs 2009a, b; Williams et al. 2012).

Regarding the phylogenetic relations of the species employed here, the two major, strongly supported clades could coincide with the subgenera currently accepted in the classifications of *Thygater* (Urban et al. 2012; Michener 2007; Ascher and Pickering 2017). However, our results do not corroborate the distinction made by Urban (1967) between the subgenera *Thygater* s. str. and *Thygater* (*Nectarodiaeta*), based upon the number of maxillary palpomeres [*Thygater* s. str. having three and *Thygater* (*Nectarodiaeta*) four]. Thus, *T. paranaensis* and *T. chaetaspis*, species with three maxillary palpomeres, grouped with *T. latitarsis* and *T. luederwaldti*, the only species with four palpomeres included in the analyzes. Michener (2007) already questioned the classification proposed by Urban (1967), suggesting that, thus defined, the subgenera would not be monophyletic. Here, no taxonomic decision on the subgeneric classification of *Thygater* is made, because more taxa and characters (both molecular and morphologic) would be required to produce a robust phylogenetic hypothesis and that was not the aim of this study. Such analysis is being undertaken by the authors.

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AUTHOR CONTRIBUTIONS

FVF and FAS conceived this research. FVF and JESJ performed the experiments and analyzed the data. FRS and FAS contributed with the reagents, materials, and analysis tools. FVF and FAS wrote the paper. All authors read and approved the final manuscript.

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Délimitation d'espèces et associations sexuelles dans le genre *Thygater* à l'aide de données moléculaires, et description d'une nouvelle espèce

Apoidea / Dimorphisme sexuel / Eucerini / Néotropiques / Nouveau synonyme / Monophylie réciproque / Délimitation des espèces

Bestimmung von Artgrenzen und Geschlechtsmerkmalen in der Bienengattung *Thygater* mithilfe molekularer Daten, und die Beschreibung einer neuen Art

Apoidea / Geschlechtsdimorphismus / Eucerini / Neotropen / neues Synonym / reziproke Monophylie / Artgrenzen

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