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An integrative taxonomic and phylogenetic approach reveals a new Neotropical swarm-founding social wasp, *Pseudopolybia cryptica* sp. n. (Vespidae: Polistinae: Epiponini)

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Abstract

Phenotypic characters are traditionally the main information for species discrimination in taxonomic studies of invertebrates. However, the presence of inter- and intraspecific polymorphism makes it difficult to identify species in many groups such as Neotropical social wasps. Herein, we examined different sources of biological information such as adult morphology, male genitalia, nest architecture, and genetic data applying an integrative taxonomic approach to study pinned museum specimens belonging to the social wasp genus *Pseudopolybia* de Saussure, 1863. Based on multiple independent lines of evidence, we described a new Neotropical swarm-founding social wasp, *Pseudopolybia cryptica* sp. n. Moreover, we proposed a phylogenetic hypothesis for *Pseudopolybia* including this new species. Our taxonomic findings applying an integrative approach reinforce that the social wasp diversity in the Neotropics may be underestimated due to morphological similarity.

Keywords

Epiponini, integrative taxonomy, molecular systematics; mtDNA, paper wasps, *Pseudopolybia*, social wasps, species delimitation.

1. Introduction

Accurate species delimitation is essential for multiple disciplines such as ecology, evolution, conservation biology, among others (Adams et al. 2014). However, species delimitation between closely related species is often difficult since traditional taxonomy depends on the evolution of informative and consistent phenotypic characters (Bickford et al. 2007; Gokhman 2018; Matos-Maraví et al. 2019). Social wasps (Hymenoptera: Vespidae) exemplify this issue since they show different sibling species morphologically similar (Menezes et al. 2011; Buck et al. 2012; Lopes and Menezes 2017; Schmid-Egger et al. 2017). Hence, as in other taxonomic groups, the combination of different sources of biological information such as morphology and genetics is necessary to better understand the diversity of social wasps.

Pseudopolybia de Saussure, 1863 is a Neotropical genus of swarm-founding social wasps (Vespidae: Polistinae: Epiponini) comprising four species: *Pseudopolybia compressa* (de Saussure, 1854), *Pseudopolybia difficilis* (Ducke, 1905), *Pseudopolybia langi* Bequaert, 1944, and *Pseudopolybia vespiceps* (de Saussure, 1863). They are distinguished by the third segment of the labial palpi bearing a short, stout, curved bristle near its apex, and the number of palpal segments being six maxillary and four labial (Richards 1978; Andena et al. 2007). The genus distribution is from Nicaragua to southern Brazil, and they are found mainly in rainforests (Richards and Richards 1951; Richards 1978; West-Eberhard et al. 1995). Nests of *P. compressa*, *P. difficilis*, and *P. vespiceps* have been described (Wenzel 1998). They are usually arboreal with spherical or tapering envelope and the entrance hole is a simple hole at the lowest point of the envelope (Richards 1978).

Although there are only four described species within *Pseudopolybia* several taxonomic problems persist (e.g. Bequaert 1938; Richards 1978; Andena et al. 2007). For instance, *P. compressa* and *P. vespiceps* show striking color variation that was treated as different "morphs" (see Richards 1978). Thus, *P. compressa* included the morphs *compressa* (de Saussure), *luctuosa* (Smith), and *laticincta* (Ducke), while *P. vespiceps* comprised the "morphs" *vespiceps* (de Saussure) and *testacea* (Ducke). These names were first described as distinct species or varieties, but later reduced to varieties of their respective species (Bequaert 1938). Interestingly, the forms of *P. compressa* (e.g. *laticincta* and *luctuosa*) were found on the same nest from Ecuador (Ducke 1910). Hence, Andena et al. (2007) conclude that these "morphs" suggested by Richards (1978) "do not merit any treatment other than synonyms".

In the present paper, we use an integrative taxonomic approach, i.e. analyzing the variation of mitochondrial genetic markers with molecular species delimitation methods, adult morphology, nest architecture, and male genitalia, to assess the taxonomic status of pinned museum specimens previously identified as *P. compressa* from Central-West and Northeast of Brazil. Based on multiple independent lines of evidence, we described a new Neotropical swarm-founding social wasp, *Pseudopolybia cryptica* sp. n.

2. Methods

2.1. Taxon sampling, morphological analysis, and distribution map for *Pseudopolybia* species

Three colonies from the new species were collected during a survey in Central-West and Northeast of Brazil in the years 1997, 2000, and 2008 by SM in the Brazilian states of Mato Grosso and Bahia. We analyzed a total of 83 *Pseudopolybia* specimens deposited in the Invertebrate Collection of Instituto Nacional de Pesquisas da Amazônia (INPA, Manaus, Brazil) and Museum of Zoology of the Universidade de São Paulo (MZUSP, São Paulo, Brazil) (Supplementary material S1). Additionally, we also examined and compared the type material of three *Pseudopolybia* species: the holotypes of *Pseudopolybia compressa* morph. *compressa* and *Pseudopolybia vespiceps* (= *Xanthocaba nigrolineata*) deposited in the Natural History Museum (London, England), and the lectotype of *Pseudopolybia difficilis* as well as a specimen of *P. compressa* morph. *laticincta* deposited in the Muséum National d'Histoire Naturelle (Paris, France) (Supplementary material S1). We examined *Pseudopolybia langi* and *Pseudopolybia compressa* morph. *luctuosa* using only the original description (for details see Bequaert (1944) and Smith (1857), respectively).

For adult morphological structures and male genitalia we used the terminology following the original descriptions, Richards (1978), Andena et al. (2007), and Somavilla et al. (2018). The procedure for extraction and clearing of male genitalia followed the protocol proposed by Somavilla et al. (2018). We separated for study and description the following male genitalia structures: paramere, aedeagus, digitus, and cuspis. All observations and photographs of adult morphological structures and male genitalia were performed through a digital camera Leica DMC4500 coupled to a stereomicroscope Leica M205A and also using the Leica Application Suite software v4.10.0. A nest collected in Nova Mutum (Mato Grosso, Brazil) was photographed by SM, in situ, with a film camera and we edited the photographs using Adobe Photoshop CS6 Extended v13.

Additionally, we produced a distribution map for *Pseudopolybia* species based on a compilation of literature and collection records from museums (see Supplementary material S1). We georeferenced the localities from each specimen using the on-line Google Maps Platform. We excluded missing or inaccurate locality data, but the exception was a *P. vespiceps* specimen from Santa Catarina. The geographical coordinates were converted to decimal degrees and imported into a free and open-source geographic information system, QUANTUM-GIS

v2.18.18 (Open Source Geospatial Foundation Project, Beaverton, OR, USA), to produce a distribution map.

2.2. Molecular dataset, phylogenetic inference, and species delimitation analyses

We followed standard laboratory protocols (Menezes et al. 2015, 2017) to sequence two gene fragments from six specimens collected between 1999 and 2016: the mitochondrial loci Cytochrome oxidase subunit I (COI) and 16S ribosomal DNA (16S). For PCR amplification we used the following primers: for COI, 5'-GGAGGATTTGGAAATTGATTAGTTCC-3' (CI-J-1718) and 5′-GGTAAAATTAAAATATAAACTTC-3′ (CI-N-2191) (Simon et al., 1994), and for 16S, 5'-CACCTGTTTATCAAAAACAT-3' (LR13943F) and 5`-CGTCGATTTGAACTCAAATC-3` (LR13392R) (Costa et al. 2003). Sanger sequencing was conducted by Centro de Recursos Biológicos e Biologia Genômica (CREBIO), Universidade Estadual Paulista 'Júlio de Mesquita Filho' (Jaboticabal, São Paulo, Brazil). We carried out sequence quality control and assembly using the program GENEIOUS R7. We retrieved from GenBank nine DNA sequences (see Supplementary Table S1). All new DNA sequences were deposited in GenBank (accession numbers MT740709–MT740713 and MT738236–MT738241). Sequences were aligned using the default parameters of ClustalW (Thompson et al. 1994) implemented in MEGA v7.0.26 (Kumar et al. 2016). All alignments were visually inspected and corrected. The most appropriate model of nucleotide evolution and the best-fitting partitioning scheme were selected using PartitionFinder2 (Lanfear et al. 2017) under the Bayesian information criterion (see Supplementary Table S2). We also computed a Kimura Two-Parameter (K2P) pairwise distances using MEGA v7.0.26 (Supplementary Table S3).

Phylogenetic inference was conducted by maximum likelihood using IQ-TREE (ver. 1.6.12, see http://www.cibiv.at/software/iqtree; Nguyen et al. 2015) with a combined morphological and molecular dataset (concatenated COI and 16S sequences). We used the morphological matrix provided by Andena et al. (2007) with few modifications: we excluded the characters 2, 3, 13, 15, and 23, because they were not informative for our analysis, and adapted the character states of the characters 11, 17, and 21 (Supplementary Table S4). We used *Agelaia pallipes* (Olivier, 1792), *Angiopolybia pallens* (Lepeletier, 1836), and *Apoica pallens* (Fabricius, 1804) as outgroups based on previous phylogenetic studies (Andena et al. 2007; Menezes et al. 2020). Finally, we used the Jukes-Cantor type model for morphological data with ascertainment bias correction (MK+ASC) and calculated branch supports with 1000 replicates of ultrafast bootstrap approximation (UFboot) (Minh et al. 2013) and SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010) as implemented in the IQ-tree software. The consensus tree was visualized and edited using the program FigTree v1.4.0.

We carried out single-locus molecular species delimitation analyses with three methods based on different approaches: a sequence similarity clustering method, the automatic barcode gap discovery (ABGD) (Puillandre et al. 2012); and two tree-based coalescence methods, the Bayesian Poisson Tree Processes (bPTP) (Zhang et al. 2013) and the multi-rate Poisson Tree Processes (mPTP) (Kapli et al. 2017). The ABGD analyses were performed on the web-based interface http://wwwabi.snv.jussieu.fr/public/abgd/ (Puillandre et al. 2012), with default values for both the proxy of the minimum relative gap width (X = 1.5) and the scanned range of prior intraspecific divergence (Pmin-Pmax = 0.001-0.1). Moreover, we checked the robustness of the ABGD results by changing the parameter values one at a time, particularly by increasing Pmax to 0.2 and by decreasing/increasing X (to 1 and 2). The bPTP and mPTP analyses were performed through the web interfaces http://species.h-its.org/ptp/ and https://mptp.h-its.org/#/tree, respectively. We used as input maximum likelihood COI and 16S trees and concatenated maximum likelihood tree (generated by MEGA v7.0.26), removing the outgroups and using 300000 Markov chain Monte Carlo (MCMC) generations.

3. Results

Based on our integrative taxonomic approach, we can hypothesize that *Pseudopolybia* specimens collected in the Brazilian states of Mato Grosso and Bahia belong to a new species that we describe below. We consider several morphological characters including male genitalia (Fig. 1 and Table 1), nest architecture (Fig. 2), and two mitochondrial markers, COI and 16S, under a phylogenetic and molecular species delimitation approach (Fig. 3).

3.1. Taxonomy

Pseudopolybia cryptica Somavilla & Menezes, sp. nov.

http://zoobank.org/6EF0EE9C-A6B5-43AD-9CB2-4C5AEC299480

Fig. 1A–I

Type locality. Nova Mutum, Mato Grosso, Brazil.

Description. Female (Fig. 1A, B, C). ***Size***: 13.0 mm. Forewing in length 9.5 mm. ***Head***: (1) clypeus about 1.3 times wider than long, evenly convex in medial portion with lateral lobe flat; in contact with the eyes by less than the width of the antennal socket; lateral margins of the clypeus and upper margin sinuous; upper margin separated by less than the width of the antennal socket; apex of clypeus acute; entire surface of clypeus silver pubescent with yellowish and long bristles in apical portion;

Table 1. Morphological differences between *P. compressa* (de Saussure, 1854) and *Pseudopolybia cryptica* sp. n.

Morphological characters	***P. compressa* (de Saussure)**	***P. cryptica* sp. n.**
General color	black, extensive yellow marks	black, few yellow marks mainly on the face
Frontal furrow	narrow	deep
Distance between the lateral ocelli	short, less than the diameter of the ocelli	larger, equal to the diameter of the ocelli
Pronotal carina, in the middle region	lower	high
Tegula shape	elongated	globose
Posterior margin of the propodeum and orifice of the propodeal muscle	elevated	not elevated
Propodeal valve	translucid	not translucid
Tergum I–VI	black, with or without yellow apical band, sparse bristles	entire dark brownish, row of bristles
Aedeagus, apical portion	fine denticulation	intermediate denticulation
Aedeagus, lateral apodema	with a projection in the central curvature	without a projection in the central curvature
Paramere, spine base	with long and dense bristles	with long and sparse bristles
Digitus	with dense bristles	with sparse bristles

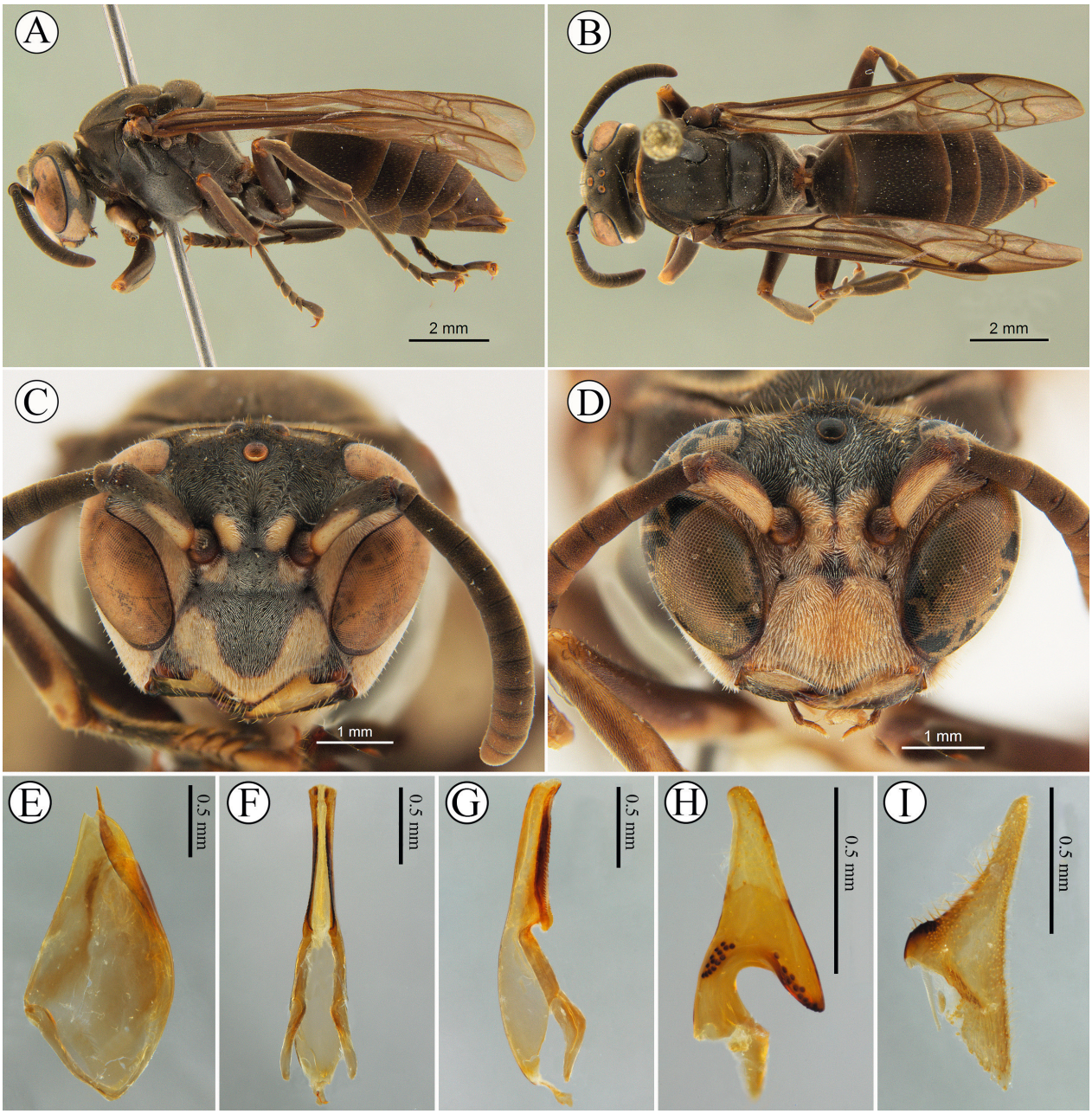

Figure 1. *Pseudopolybia cryptica* Somavilla & Menezes, new species. **A**: Female, lateral view. **B**: Female, dorsal view. **C**: Female face, frontal view. **D**: Male face, frontal view. **E-I**: Male genitalia. **E**: Paramere inner view. **F**: Aedeagus ventral view. **G**: Aedeagus lateral view. **H**: Digitus lateral view. **I**: Cuspis lateral view.

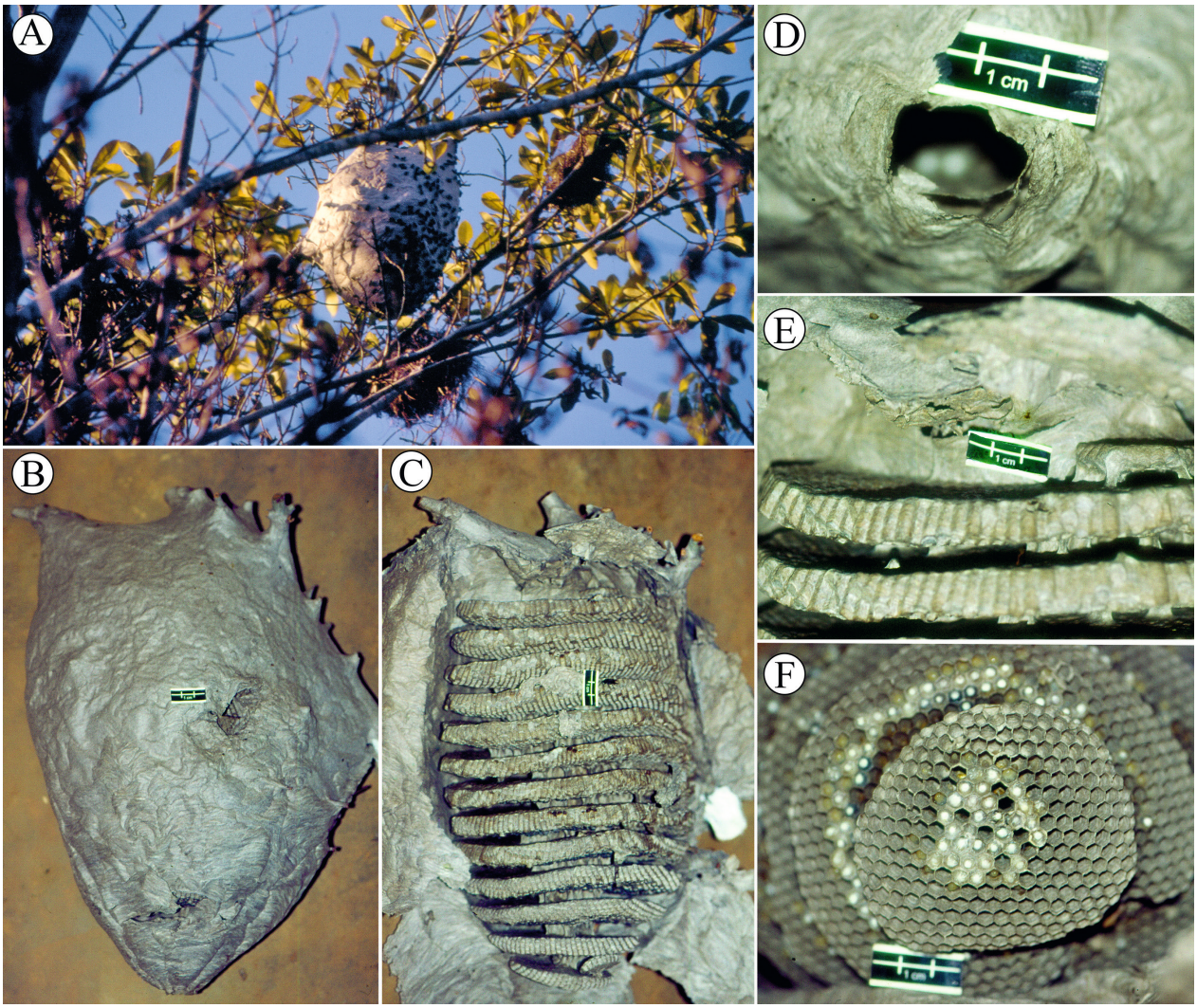

Figure 2. Nest of *Pseudopolybia cryptica* Somavilla & Menezes, new species. **A**: *In situ*. **B**: Frontal view. **C**: Internal comb view. **D**: Entrance. **E**: Comb lateral view. **F**: Comb ventral view.

punctures very shallow and spaced; (2) frons and vertex with long and spaced bristles and yellowish pubescence; punctures shallow, medium sized, separated by approximately one diameter; (3) eyes with middle bristles and spaced; (4) malar space with half of the antennal socket; (5) mandible with approximately 2.3 times longer than wide; with a long bristle band in the lower region; (6) middle region of the gena with width smaller than the eyes in profile; silver pubescence evident; middle bristles spaced; punctures very shallow and spaced; (7) diameter of the medial ocellus, 0,24 mm; (8) interocelar distance, 0,22 mm; (9) posterior region of head without occipital carena. ***Mesosoma***: (1) pronotum with short and dense pubescence, concentrated on lateral part; pronotal carina produced high, lamellated with long bristles in front; punctures shallow, separated by less than one diameter; (2) Mesepisternum with dorsal groove present, at least as anterior trace with the same pattern of punctuation but becoming sparser laterally; short and dense pubescence; scrobal furrow wide, shallow; (3) tegula globose; (4) scutum with dense pubescence, central area more shinning; punctuation small, shallow, separated by one diameter or more, becoming sparser centrally; with a thin line in the upper central region; (5) scutellum with the same pattern of punctuation in the scutum, with a thin line in the entire central region; (6) propodeum with dense silver pubescent; long yellowish bristles laterally; posterior margin of the propodeum not elevated, muscle propodeal valve not translucid; (7) propodeal muscle large. ***Metasoma***: (1) Tergum I compressed, cap-shape; punctures very weak, spaced; short and dense goldenish pubescence; (2) tergum II wider than long; punctures very weak, spaced; short and dense goldenish pubescence, row of short bristles; (3) tergum III–VI punctures pubescence like tergum II; (4) sternum II–V with punctures very weak; short and dense goldenish pubescence. ***Color***: Black, mandibles, and malar space is blackish and yellowish; the lateral and apical part of clypeus, inner orbits, two spots on base of antennal scape, and part of antennal pedicel yellowish; flagellomere brownish; gena with a small yellow band, sometimes reaching the gena basis. Scutum, scutellum, metanotum, and mesepisternum blackish; margin of pronotum in dorsal view, yellow; tegula black; legs black to dark-brown in the portion of tibiae and tarsi; wings hyaline and venation dark-brown; tergum I–VI and sternum dark brownish and without apical yellow bands.

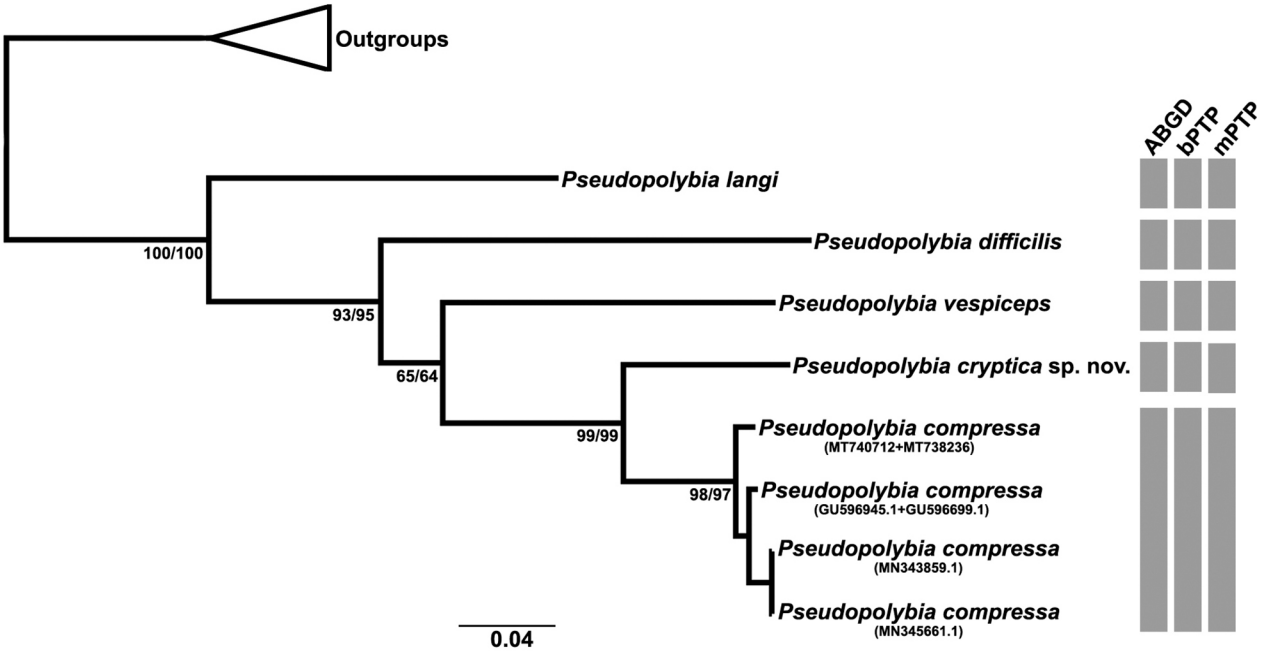

Figure 3. Phylogeny of *Pseudopolybia* inferred from the combined Maximum likelihood analysis of molecules (mitochondrial markers COI and 16S) and morphology, with molecular species delimitation analyses ABGD, bPTP, and mPTP (vertical bars). Values below to the nodes represent ultrafast bootstrap (left) and SH-aLRT (right) supports as reported by IQ-Tree.

Male (Fig. 1D). ***Size***: 11.0 mm. Forewing in length 8.5 mm. Like female except that the gena and clypeus are narrower and entirely yellow; the mandible is blackish and yellowish; the clypeus is covered with a long and dense golden pubescence; the frons is also yellowish in the interantennal area and antennal pedicel; small yellowish marks are present in the pronotal carina, end of tegula, anterior mesepisternum region, beneath of the coxae and femur; legs brownish. ***Male genitalia*** (Fig. 1E): (1) Paramere more long than wide, approximately two times longer than wide at the middle, basal angle obtuse, apical angle broad, long and curved spine very pointed apically, with long and dense bristles, mainly on spine base; (2) aedeagus apical portion with intermediate denticulation, extended on the apical portion to the end of the median expansion, an area near serration darker, lateral margin straight, penis valve widely dilated and with a slight central entrance, ventral process rounded and dilated in the sides, forming a "U", lateral apodema without a projection in the central curvature; (3) digitus pointed apically, with long and spaced hairs, punctation strong and forming a central band around the base of the digitus; (4) cuspis very pointed and tapering abruptly apically, with small and spaced hairs.

Nest (Fig. 2)**.** The single arboreal nest of the new species used for nest's architecture description was collected by SM in the Buriti Farm, Nova Mutum, Mato Grosso, Brazil. It was in a rubber plantation close to a small lake. The spherical nest form flattened laterally was built around five meters from the ground in a rubber tree with thin branches incorporated in the upper part of the nest, and it measured approximately 42 cm in length and 27 cm wide (Fig. 2A and B). The nest's envelope is predominantly gray (Fig. 2B) made with long fiber, typical of supple paper (Wenzel 1998). The external envelope is composed of many layers of sheets, and in the upper part of the nest is very hard and the lateral downward to the entrance is soft. The fourteen spherical multipedicellate combs stacked downward, growing gradually at margins sometimes in contact with envelope, cocoon with simple caps (Fig. 2C, E and F). The simple entrance hole at the bottom usually has than more one layer (Fig. 2D).

Diagnosis. Color black, with few yellow marks mainly on the face and mandibles, clypeus, inner orbits, base of antennal scape, antennal pedicel and gena; clypeus wider than long, in contact with the eyes by less than the width of the antennal socket; frontal furrow deep; tegula globose; tergum I–VI dark brownish; sternum dark brownish; tergum and sternum without apical yellow bands.

Etymology. The specific epithet "cryptica" refers to the Greek word kryptikos that means hidden because the species was previously classified as *Pseudopolybia compressa*.

Distribution. Approximate range of the new species is shown in Fig. 4. To date, *Pseudopolybia cryptica* sp. n. has been recorded in Bahia and Mato Grosso Brazilian states.

Material examined. ***Type material***: Holotype ♀, 'Brasil, Mato Grosso | Nova Mutum, Buriti Farm | 13º51′90.3″S, 056º11′61.9″W | 23.viii.2000, Nest, S. Mateus leg.' (INPA). – Paratypes 1 ♂, 5 ♀, 'Brasil, Mato Grosso | Nova Mutum, Buriti Farm | 13º51′90.3″S, 056º11′61.9″W | 23.viii.2000, Nest, S. Mateus leg.' (INPA); 1 ♂, 3 ♀, 'Brasil: Bahia, Lençóis | 12º35′17.0″S, 041º22′96.3″W | 23.x.2008, S. Mateus leg.'

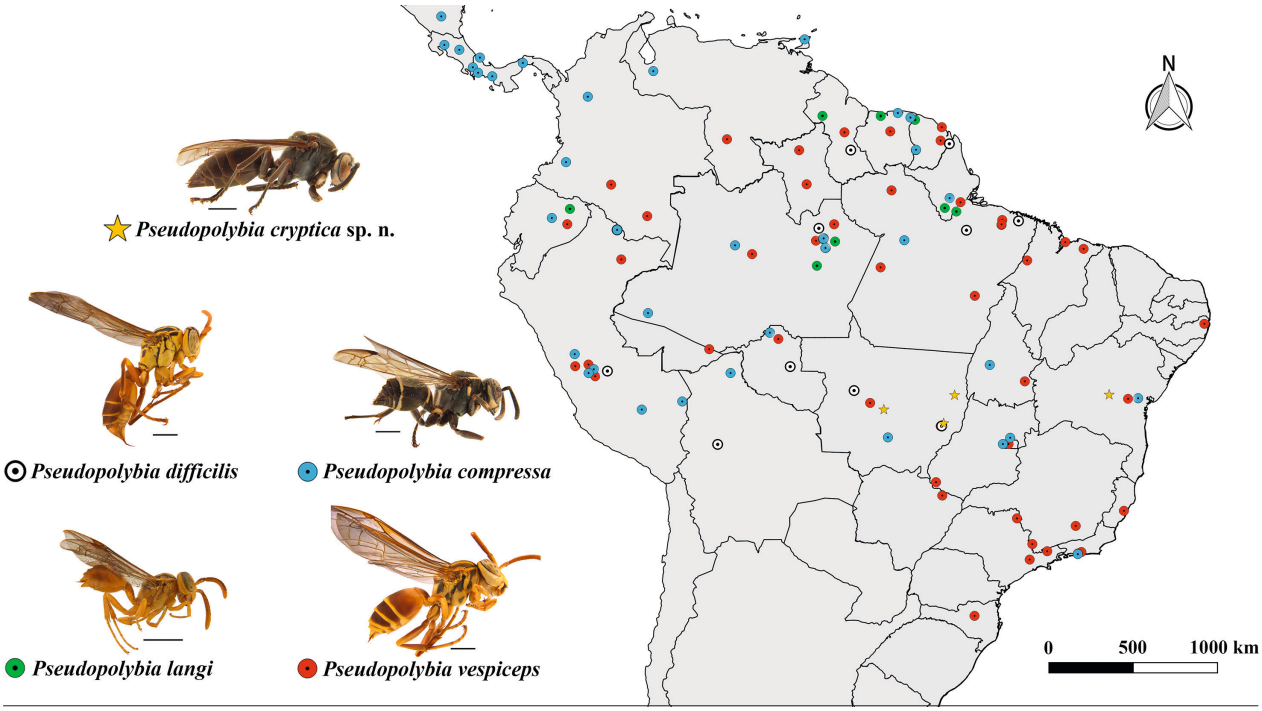

Figure 4. Map of South and Central America showing the current geographic distribution of *Pseudopolybia* species. For locality information see Supplementary material S1.

(INPA); 1 ♂, 12 ♀, 'Brasil, Mato Grosso | Serra Dourada, 04.xi.1997 | S. Mateus & Noll leg.' (INPA); 1 ♀, 'Brasil, Mato Grosso | Serra Dourada, 04.xi.1997 | S. Mateus & Noll leg.' (Coleção Entomológica "Prof. J.M. F.Camargo"); 1 ♀, 'Brasil, Mato Grosso | Serra Dourada, 04.xi.1997 | S. Mateus & Noll leg.' (MZUSP); 1 ♀, 'Brasil, Mato Grosso | Serra Dourada, 04.xi.1997 | S. Mateus & Noll leg.' (AMNH).

Phylogenetic position. The new species is reconstructed as sister to *P. compressa* based on the analysis of partial sequences of COI and 16S and morphological characters (Fig. 3). The detected level of genetic divergence between the new species and other congeneric range from 8.29–18.44% for COI and 3.34–11.08% for 16S (Supplementary Table S3).

3.2. Key to *Pseudopolybia* species

The following key is a revised and adapted version with few modifications of the key provided by Andena et al. (2007). Thus, *P. cryptica* would run down to *P. compressa* and *P. vespiceps* (couplet 3) and the following changes could be made to include the new species.

1 Dorsal pronotal carina is very weak; first metasomal segment much longer than wide; eyes densely haired; small species (approximately 6–8 mm); yellow with three brown stripes on the scutum; metasoma brownish ..... ***P. langi*** Bequaert (Fig. 5A)

1' Dorsal pronotal carina lamellate laterally; first metasomal tergum nearly as broad as or much broader than long; eyes with or without hairs; larger species (approximately 13–15 mm); yellow to black **2**

2 The first and second metasomal segment a little longer than wide; metanotum not compressed; yellow with black marks on the head and mesosoma; three black stripes on the scutum; metasoma brownish ..... ***P. difficilis*** (Ducke) (Fig. 5B)

2' The first and second metasomal segment approximately twice as wide as long; metanotum slightly to strongly compressed; dark yellow with three black stripes on the scutum or blackish species with pale marks **3**

3 Dark yellow species with brown to blackish marks; prestigma much longer than wide; clypeus narrowly separated from eyes; eyes bare; the tip of the clypeus weakly pointed; metanotum slightly compressed; the malar space longer than fourth antennal article ..... ***P. vespiceps*** (de Saussure) (Fig. 5C)

3' Blackish species with pale marks; prestigma approximately as long as wide; clypeus touching the eyes; eyes with hairs; the tip of the clypeus sharply pointed; metanotum strongly compressed; the malar space about as long as fourth antennal article **4**

4 Blackish species with wide pale marks in the face; pronotal carina lower in the middle region; tegula elongated with an inferior yellow spot; tergum I–VI black, with or without a yellow apical band, sparse bristles ..... ***P. compressa*** (de Saussure) (Fig. 5D)

4' Blackish species with few pale marks in face; pronotal carina high in the middle region; tegula more globose entirely black; tergum I-VI entire dark brownish, row of bristles ..... ***P. cryptica* sp. n** (Fig. 1).

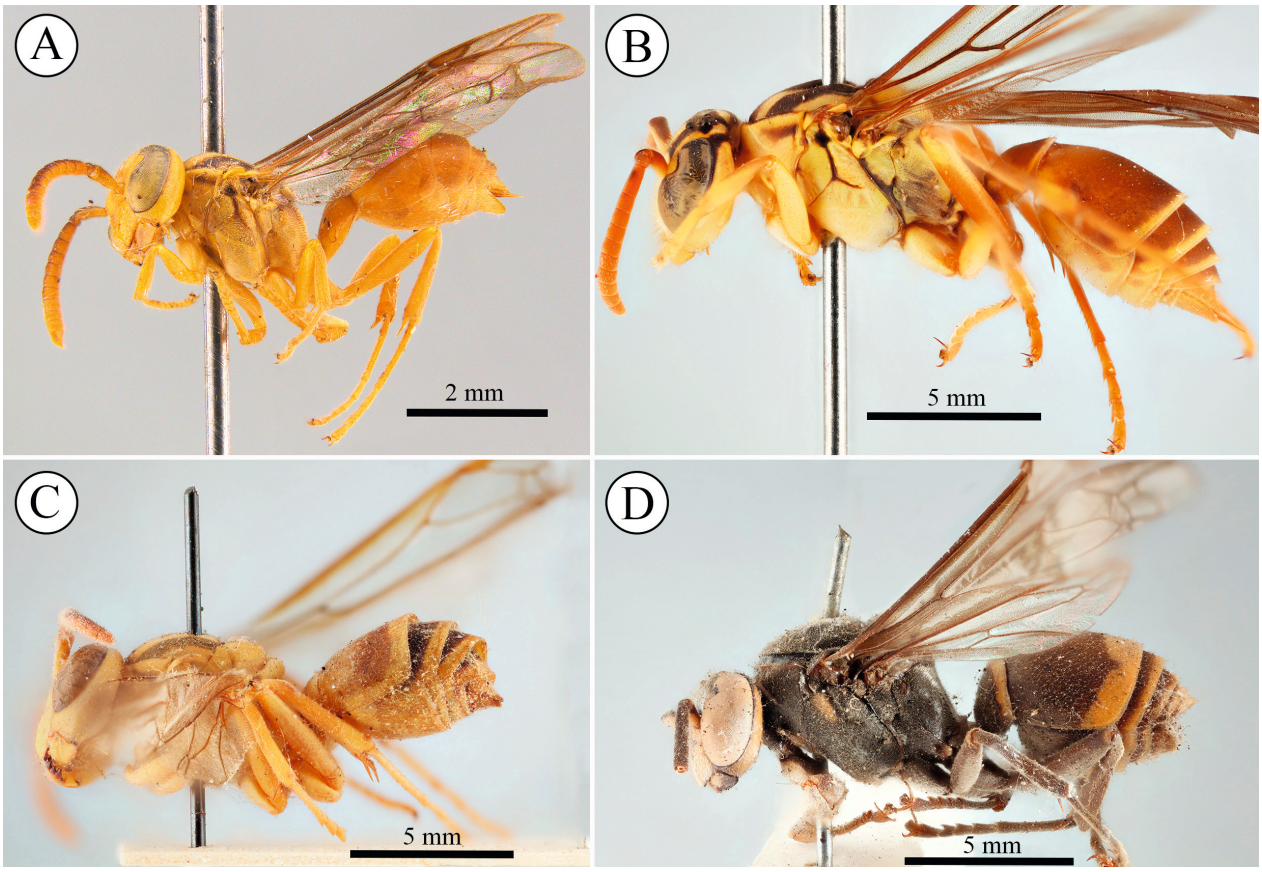

Figure 5. *Pseudopolybia* species**,** lateral view. **A**: *Pseudopolybia langi* Bequaert, 1944. **B**: *Pseudopolybia difficilis* (Ducke, 1905) lectotype. **C**: *Pseudopolybia vespiceps* (de Saussure, 1863) type. **D**: *Pseudopolybia compressa* (de Saussure, 1854) holotype.

3.4. Phylogenetic reconstruction and molecular species delimitation analyses

The phylogenetic inference using a concatenated matrix (morphology and mitochondrial DNA) is summarized in Fig. 3. *Pseudopolybia* is supported as a monophyletic group. *Pseudopolybia langi* is sister to the clade with all other species (100% UFboot and SH-aLRT) and *P. difficilis* is sister to a clade composed of *P. vespiceps*, *P. cryptica*, and *P. compressa* (93% UFboot and 95% SH-aLRT). Our phylogenetic tree is very similar to previously proposed phylogeny for the genus (see Andena et al. 2007), but here we included the new species that was recovered with high support (99% UFboot and SH-aLRT) as sister of *P. compressa*.

The evolutionary divergences of COI and 16S using K2P are given in Supplementary Table S3. The average genetic distance within *Pseudopolybia* for COI was 12.09% and 16S was 7.8%. *Pseudopolybia cryptica* differs from *P. compressa* by a genetic distance of more than 8% for COI and 3.3% for 16S. The intraspecific genetic distance for COI and 16S within *P. compressa* range from 0.0%–3.36% and 0.47%, respectively (Supplementary Table S3). The ABGD, bPTP, and mPTP methods consistently identify *P. cryptica* as a distinct hypothetical species and also the other *Pseudopolybia* species already described (see bars in Fig. 3).

4. Discussion

Our integrated evaluation of adult morphology, male genitalia, nest architecture, and results from molecular species delimitation support the new species *P. cryptica* and indicate a sister species relationship with *P. compressa*. From the morphological point of view, *P. cryptica* cannot be confused with any *Pseudopolybia* species, except for *P. compressa* (Fig. 5D). The blackish general color of *P. cryptica* is very similar to *P. compressa*, but *P. cryptica* does not present extensive yellow marks, mainly in the tergum I and II. Moreover, the metasoma of *P. cryptica* is entire brownish and not black like *P. compressa*. Despite *P. compressa* has color variants described as three "morphotypes" [*P. compressa* morph. *compressa* (de Saussure, 1854), *P. compressa* morph. *luctuosa* (Smith, 1857), and *P. compressa* morph. *laticincta* (Ducke, 1904)], we noticed that they are morphological different of *P. cryptica*. According to Smith (1857) and Richards (1978), the *P.* morph. *luctuosa* is smaller and black, with a few yellow marks, and it is very similar to the *P. compressa* morph. *compressa* and *P. compressa* morph. *laticincta*. *Pseudopolybia cryptica* is different, since it is darker than all other *P. compressa* morphs, and it is larger than the *P. compressa* morph. *luctuosa*. Additionally, the male genitalia was another important character in the diagnosis of this new species, since it has aedeagus with intermediate den-

ticulation in apical portion and paramere with long and sparse bristles in the spine base as differences when compared with the male genitalia of *P. compressa* (Table 1).

Concerning our molecular data, the species delimitation analyses have been carried out with a clustering (ABGD) and tree-based (bPTP and mPTP) methods on two mitochondrial markers, COI and 16S. Empirical studies have shown that species delimitation methods based on single-locus data tend to under- and oversplit species (da Silva et al. 2017; Pentinsaari et al. 2017; Renner et al. 2017). Additionally, despite *P. cryptica* showed high genetic distance when compared with other congeners (e.g. more than 8% for COI), a species-delimitation decision considering genetic distance using only mitochondrial markers has to be taken with extreme caution (e.g. Mueller 2006; Waichert et al. 2019). First, in the Epiponini wasps queen number may vary from many to few during the colonial cycle (cyclic oligogyny) and such colony strategy can lead to differences between nuclear and mitochondrial genetic structure at microgeographical scales (Ross and Shoemaker 1997). Second, mitochondrial markers are supposed to be linked, non-recombining locus with maternal inheritance, and likely evolved under similar constraints. We are aware of the limitations caused by stochasticity of the coalescent process and gene flow for single-locus species delimitation methods as well as the use of only mitochondrial markers, but for our study, we take advantage of using these methods within an integrative taxonomic framework. For instance, our morphology-based analysis and the three molecular species delimitation methods with two mitochondrial markers consistently recognize *P. cryptica* as a distinct taxon and confirm that it cannot be assigned to any known described *Pseudopolybia* species including the three "morphotypes".

Despite several studies performed molecular species delimitation approaches to identify Hymenoptera species (Fernández-Flores et al. 2013; Schwarzfeld and Sperling 2015; Williams et al. 2015; Hurtado-Burillo et al. 2017; Waichert et al. 2019; Brasero et al. 2020; Sabadini et al. 2020), molecular methods have not previously been applied to alpha-taxonomic problems in Epiponini wasps. Notably, based on morphological and molecular results for *Synoeca* species, Menezes et al. (2015) and Lopes and Menezes (2017) argued that the social wasp diversity in the Neotropical region may be underestimated due to morphological similarity of these insects, and hence they recommended the combination of morphology, population-level sampling, and genetics to systematic studies for the group. Our findings here reinforce the need of combining independent sources of biological data for the diversity study of social wasps.

5. Authors' contributions

A.S. and R.S.T.M. designed the study, funding acquisition, project administration, and led the writing of the manuscript. P.C.S.B. carried out the molecular laboratory work under supervision of R.S.T.M. S.M. collected samples. A.S., R.S.T.M., P.C.S.B., and M.A. performed the analyses. All authors gave final approval for publication.

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Supplementary material 1

File 1

Authors: Somavilla A, Barroso PCS, Aragão M, Mateus S, Menezes RST (2021)

Data type: .pdf

Explanation note: File 1: Supplemental material.pdf — **Supplementary material S1.** *Pseudopolybia* specimens examined (type and additional material) and the current geographic distribution of each species based on data from literature and museum collections. — **Supplementary Table S1.** Geographical information, collection year, preservation type, and GenBank accession reference for all samples used in this study. *Represent new sequences generated in this study. — **Supplementary Table S2.** Models of molecular evolution by genes and codon positions implemented in the Maximum likelihood analysis to infer the phylogenetic relationship of *Pseudopolybia* species. — **Supplementary Table S3.** Pairwise genetic divergence between sequences of COI and 16S of *Pseudopolybia* species. Analyses were conducted using the Kimura 2-paramenter model. — **Supplementary Table S4.** Character matrix for *Pseudopolybia* based on Andena et al. (2017) with few modifications. The following symbols are used: (–) inapplicable and (P) polymorphic.

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