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ORIGINAL RESEARCH ARTICLE

Taxonomic revision of the leaf-cutting ant *Acromyrmex subterraneus* (Forel, 1893)

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Abstract
Leaf-cutting ants (genera *Acromyrmex*, *Amoimyrmex* and *Atta*) are the most important generalist herbivores in the Neotropical Region. The subspecies of *Acromyrmex subterraneus* are morphologically similar but differing from the other species of this genus. Acromyrmex *subterraneus* complex may be three non-described leaf-cutting ant species. The taxonomic revision of *Acromyrmex subterraneus* (Hymenoptera: Formicidae) and of three of its subspecies, *A. s. brunneus* Forel 1911, *A. s. molestans* Santschi (1925) and *A. s. subterraneus* Forel 1893 was made based on its workers and male morphology and genome sequencing with mt-COI and mt-COII. *Acromyrmex molestans* stat. n., *Acromymex brunneus* stat. n. and *Acromyrmex subterraneus* stat. n. are here raised to species. The results of this study contribute to the taxonomic knowledge and reaffirm the complex evolutionary history of leaf-cutting ants.

Keywords A. s. brunneus · A. s. molestans · A. s. subterraneus

Introduction

Thirty-tree species and 29 subspecies are worldwide recognized for the ant genus *Acromyrmex* (AntWeb 2021). Three of the five *Acromyrmex subterraneus* (Hymenoptera: Formicidae) subspecies occur in Brazil. The polymorphism and variation between individuals make the taxonomy of the *Acromyrmex* species among the most difficult in the Formicidae family. The head and thorax spines are important diagnostic characters, but their proportions vary between individuals of different and, even, of the same colony (Gonçalves 1961, 1967a, 1982; Fowler et al. 1986; Fowler 1988; Della-Lucia et al. 1993; Diehl-Fleig 1995; Rabeling et al. 2015). A new genus of leaf-cutting ant, *Amoimyrmex* Cristiano et al. (2020) has been proposed based on molecular phylogenetic and morphological data, to include the species *Amoimyrmex striatus* (Roger 1863) and *Amoimyrmex silvestrii* (Emery 1905) and elevating *Ac. silvestrii bruchi* to the species level as *Amoimyrmex bruchi* (Forel 1912) (Cristiano et al. 2020).

Two identification keys have been made to determining *Acromyrmex* species in Brazil (Gonçalves 1961, 1967a, b, 1982; Fowler et al. 1986; Fowler 1988; Della-Lucia et al. 1993; Diehl-Fleig 1995). The first is based in species from Argentine (Santschi 1925) and the second in those of the Amazonian biome. A revision of this genus was based on species occurring in the São Paulo State, Brazil with illustrations (Andrade 1991). A pair of inferior pronotal spines strongly bent forward and the head with dark forehead are the main characteristics of *A. s. molestans* subspecies and the inferior pronotal spine of the *A. s. subterraneus* and *A. s. brunneus* subspecies is faced forward (Gonçalves 1961).

Difficulties to separate *A. subterraneus* subspecies by morphological characters and the observation of more than one subspecies in the same area refute the characteristics of allopatric populations (Fowler and Ketelhut 1993). A survey of *Acromyrmex subterraneus* complex in the São Paulo State registered three of its subspecies in the same area (Andrade 1991) with the need of further research on *A. subterraneus* subspecies taxonomy. The objective was

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to develop a taxonomic study on *A. subterraneus* based on its worker morphology and the male genitalia. A genomic analysis employing the 2mtDNA genes was also used to support the morphological study.

Materials and methods

Collection of the subspecies studied

Colonies of *Acromyrmex subterraneus* subspecies were collected in the municipalities of Eldorado, Jacareí and Piracicaba, São Paulo State and in Viçosa, Minas Gerais, all in Brazil. These colonies were maintained in the Laboratory of Social Insect-Pests of the Universidade Estadual Paulista (UNESP)] in Botucatu, São Paulo State, Brazil.

Subspecies identification through worker morphology

The subspecies were identified based on studies of general morphology of their workers and male genitalia. Specimens from the largest workers of the nests were collected and identified under stereomicroscope with an identification key (Gonçalves 1961) and comparisons with reference keys (Andrade 1991; Mayhé-Nunes 1991). The male genitalia morphology was analyzed on permanent slides with photos of this structure of ants collected in the nests of Eldorado, Jacareí and Piracicaba (*A. s. brunneus*), São Paulo State and Viçosa, Minas Gerais State (*A. s. molestans* and *A. s. subterraneus*).

Study of male genitalia

The slides were prepared using a generic preparation method (Galati 1995). The ant specimens were placed in Dietrich Solution (96% alcohol 30 mL; formalin 10 mL; glacial acetic acid 10 mL; distilled water 60 mL) for 24 h and washed in 70% alcohol. After placement and washing, they were processed according to the following sequence: (a) clearing with 10% KOH at 40 °C in an oven for 10 h; (b) quick washing in distilled water; (c) immersion for one to two minutes in 2% acetic acid; (d) dehydration in phenol for 12 h; (f) immersion in xylol for 5 min; (g) mounting in Canada balsam. The specimens were dissected during mounting and their genitalia removed. A small holder was placed to avoid crushing the material. The slides were put in an oven at 40 °C for five days.

Scientific drawings of the male genitalia were made using a camera lucida and this structure was compared between the three subspecies and also with their descriptions (Zolessi and Abenante 1973, 1975; Zolessi and González 1974, 1978).

Genome sequencing

This study was conducted in the Center for Studies on Social Insects of the São Paulo State University in Rio Claro, São Paulo, Brazil. Genes cytochrome oxidase I (CO-I) and cytochrome oxidase II (COII) were sequenced for the subspecies. The PCR from the total DNA extracted was used with primers designed in the Center for Studies on Social Insects (Centro de Estudos de Insetos Sociais-CEIS), São Paulo State University (Universidade Estadual Paulista) (UNESP). The amplified material served as a template for the sequencing reaction.

Genomic DNA isolation

Samples of the mandible or prothorax muscles of the three *A. subterraneus* subspecies workers were subjected to three DNA extraction procedures. The material in the first extraction was transferred to 1.5 mL Eppendorf tubes, immediately frozen in liquid nitrogen and ground using a plastic pestle. The ground material was dissolved in TE buffer (Tris–acetate-EDTA), pH = 8.0 and sequential extractions made with phenol (2×), phenol: chloroform: isoamyl alcohol 25: 24: 1 (3×) and finally with chloroform: isoamyl alcohol 24: 1 (2×). The DNA was precipitated in a solution of 3 M sodium acetate at -20 °C for 1 h, centrifuged, washed with 70% ethanol, and suspended in TE. The other 2 DNA extractions were based on the GenomicPrep (27–5237-01) or Nucleon Hard Tissue (8509) commercial kits (both by Amerham, Phamarcia Biotech) according to the manufacturer's instructions. The samples were ground in 50 µl of frozen cell lysis solution and the total DNA extracted (Amersham 27–5237-01 Extraction Kit's protocol). Subsequently, they were incubated at 65 °C for 15 to 60 min. A 5µL of Proteinase K solution (20 mg per mL) was added to the sample, with incubation at 55 °C for 3 to 12 h. The protein precipitation was made using a sample cooled and with 180 µL of protein precipitation solution added to the lysed cell for maximum yield. Centrifugation was done at 15,000 × g for 4 min, and the supernatant containing the DNA placed carefully inside a clean 1.5 mL tube containing 600µL of 100% isopropanol. The sample was gently inverted 50 × and centrifuged again at 15,000 × g for 4 min. The pooled DNA was washed with 600µL of 70% ethanol and left drying at room temperature for 1 h. A total of 100µL of DNA hybridization solution was added to the DNA pellet with incubation for 12 h in the refrigerator, periodically inverting the tube to help in the dispersion of the DNA. The sample was stored at -20 °C.

The DNA was quantified in a spectrophotometer (Pharmacia) at 260 nm in 1% agarose gel at 5 V per cm in TBE buffer (0.1 M Tris–HCl, 0.1 M boric acid, and 0.02 mM

EDTA pH 8.3) after stained with ethidium bromide (10 mg per mL) (Sambrook et al. 1989).

PCR and sequencing of genes Cytochrome Oxidase I (COI) and Cytochrome Oxidase II (COII)

The region of the mitochondrial genome of ants comprising part of gene COI, a spacer region, the gene for Leucine tRNA (Trna-Leu), and part of the gene COII (Simon et al. 1994), was expanded with AntF primers (TATATTCAT TTGAAATTCTTTCTTTCAA) and Ant Rprimers (TGT GTGCAT GATAATCACATGTTT), designed at the Center for Studies on Social Insects (Centro de Estudos de Insetos Sociais), São Paulo State University (Universidade Estadual Paulista), Rio Claro, São Paulo. Each reaction contained 0.1 µg of genomic DNA, 1 pmol of each primer, 1 portion of Ready to Go PCR reagent (Pharmacia 27–9555-01) and included denaturation (94 °C, 3 min) followed by 30 cycles of PCR (94 °C 10 s, 37 °C 1 min, 72 °C 3 min).

Sequencing of genes Cytochrome Oxidase I (CO-I) and Cytochrome Oxidase II (COII)

The PCR products were purified using the Wizard PCR Preps Kit (Promega A7170) and sequenced. The sequencing reaction contained 100 ng of purified DNA, 1 pmol of primers (the same used in the amplification), 4 µL of Big Dye (PE Applied Biosystems 4,303,153) and conducted with initial denaturation (90 s at 96 °C), followed by 25 PCR cycles (12 s at 96 °C, 6 s at 50 °C, 4 min at 60 °C). After reaction, the sequenced products were purified as recommended by the manufacturer (PE Applied Biosystems), separated in polyacrylamide gel and sequenced on an ABI 377 device (PE Applied Biosystems).

Phylogenetic relationship between the subspecies

Generated sequences were compared to those deposited in GenBank (ncbi.nlm.nih.gov) through the BLASTN application and their counterparts aligned by Claustal W applications (Thompson et al. 1994) refined manually. Ambiguous regions as to the alignment were excluded from the phylogenetic analyses made by maximum parsimony using the PAUP* 4.0b4a application (Sambrook et al. 1989).

Results

Subspecies identification through worker morphology

The ant colonies from Eldorado, Jacareí and Piracicaba, São Paulo State, were identified as belonging to the *A. s. brunneus* subspecies. Thirty-six nests were collected in Viçosa, Minas Gerais State, being 17 of *A. s. subterraneus* subspecies and 19 of *A. s. molestans* subspecies.

The largest workers of the *A. s. brunneus* subspecies differed only from those of *A. s. subterraneus* by their black or dark brown color, light brown or yellowish (Fig. 1). However, the larger workers with a peculiar characteristic; usually a darker frons, and the inferior pronotal spines are strongly bent forward facilitates the differentiation of the *A. subterraneus molestans* subspecies (Fig. 1).

The *A. s. molestans* may be a different species and a taxonomic status problem also exists between the *A. s. brunneus* and *A. s. subterraneus* subspecies.

Male genitalia morphology

Male genitalia are composed of a basal ring as a single plate in the proximal region of the structure. The gonocoxite (paramellar blades) below open up in the proximal region and gonostyles are found in the distal region. Internally, there are penis valves or blades. Volsellas, on each side of the penis valves are gonocoxite expansions (Fig. 2) with different composition between the subspecies.

Variations in some genitalia structures of *A. s. brunneus* males collected in Eldorado, São Paulo includes a wider gonocoxite and a narrower and more curved gonostyle compared to individuals of other locations (Fig. 2). However, the volsellas characteristics were the same even with these differences. The morphology and color of the *A. s. brunneus* workers from the three locations were identical.

The gonocoxite and gonostyle of the subspecies *A. s. subterraneus* and *A. s. brunneus* did not vary but their volsellas differed in most cases (Figs. 2 and 3).

Genome sequencing

Sequencing was performed in a region of the mitochondrial genome containing part of the gene COI, a spacer region, gene Leucine tRNA, and part of gene COII, for the three *A. subterraneus* subspecies. Fourteen other representatives of the Attini Tribe were included for comparison in the parsimony analysis (Fig. 4).

Six other sequences obtained from the National Center for Biotechnology Information (NCBI) were included in the analyses in addition to the genes nucleotide sequences and the mitochondrial spacer regions for the 17 species of Attini. The alignment generated an array with 600 characters with 227 of them corresponding to the spacer region (IGS) with ambiguous alignment and therefore they were excluded from the analysis. A total of 174 characters from the remaining 373 being was considered informative for the parsimony analysis and their results are presented in a dendrogram (Fig. 4).

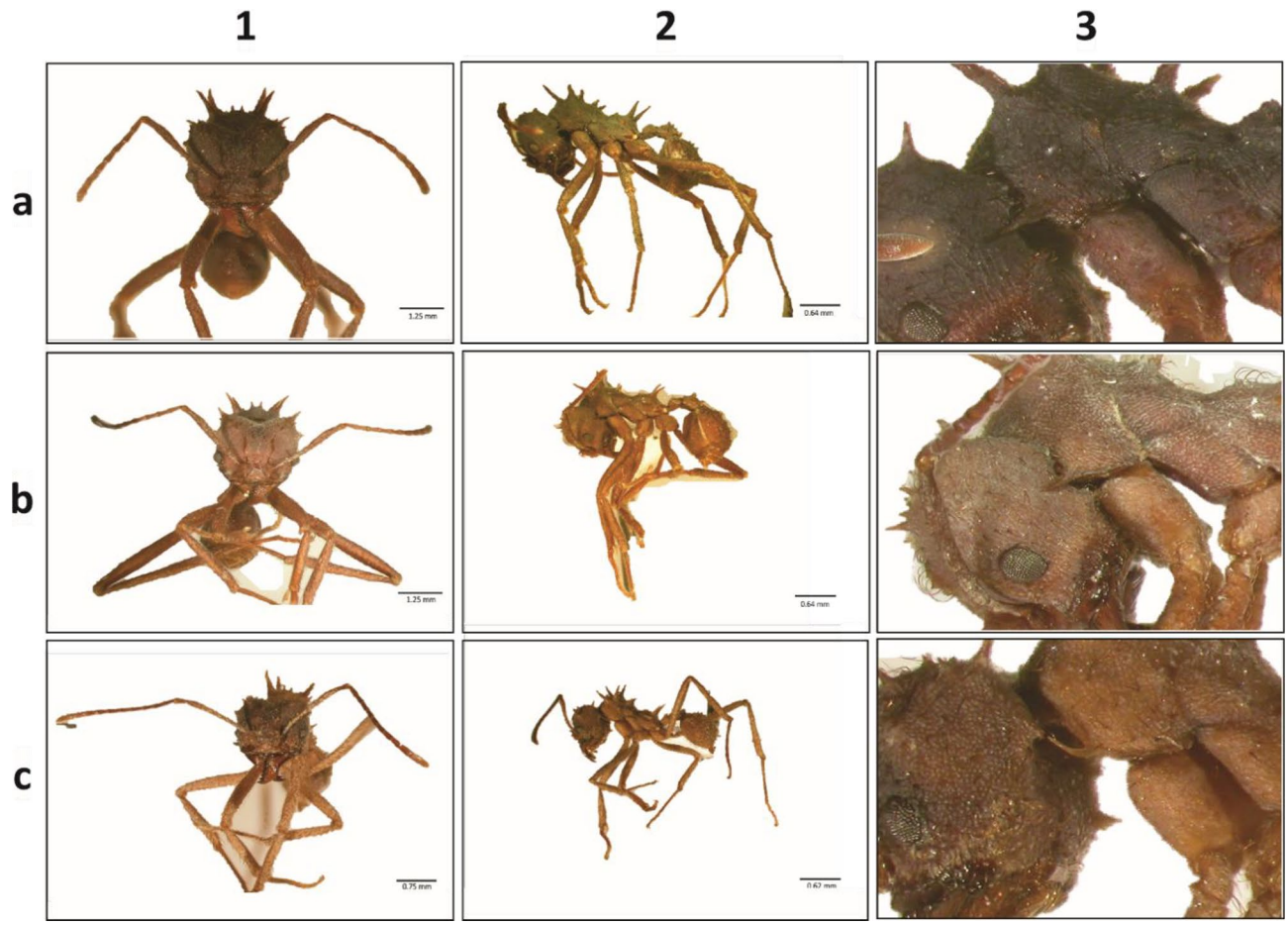


Fig. 1 (**a**) *Acromyrmex subterraneus brunneus*: **a1**. Head; **a2**. Side; **a3**. Side 2x; (**b**) *Acromyrmex subterraneus subterraneus*: **b1**. Head; **b2**. Side; **b3**. Side 2x; (**c**) *Acromyrmex subterraneus molestans*: **c1**. Head; **c2**. Side**; c3**. Side 2xs

Discussion

Subspecies identification through worker morphology

The identification keys for the two subspecies *A. s. subterraneus* and *A. s. brunneus* of the genus *Acromyrmex* (Gonçalves 1961; Andrade 1991; Mayhé-Nunes 1991) were based in a very questionable character, the color of the largest worker. Difficulties in distinguishing the three subspecies by morphological characters have been published (Gonçalves 1961; Pacheco and Berti Filho 1987; Fowler and Ketelhut 1993).

The *A. s. brunneus* characters match those used in a taxonomic key (Gonçalves 1961) developed in Piracicaba, São Paulo and to the subspecies reported in another work in the same region (Andrade 1991). The individuals of the five nests were identified as the subspecies *A. s. brunneus* and other subspecies was found in this region and in Atlantic Forest area in Eldorado, São Paulo, Brazil (Andrade 1991). The largest ant workers from nests collected in Viçosa, Minas Gerais were predominantly light brown or brown, but individuals from some colonies were black or dark brown. Specimens with very different color tegument were observed from nests in this region (Gonçalves 1961) what difficults distinguishing *A. s. brunneus* from *A. s. subterraneus* subspecies.

The spine arrangement, similar between the subspecies, especially the inferior pronotal spine, is an important character in keys (Gonçalves 1961; Andrade 1991; Mayhé-Nunes 1991). This was straight and faced forward in all *A. s. subterraneus* and *A. s. brunneus* subspecies workers. Thus, the color character of the subspecies from the municipality of Viçosa identified it as *A. s. subterraneus* and some other individuals collected as of *A. s. molestans* (Gonçalves 1961) based on the pair of the inferior pronotal spines strongly bent forward and the head with "a dark frons". Thus, two coexisting subspecies were found in the same location, which refutes the characteristics of allopatric populations (Andrade 1991; Fowler and Ketelhut 1993).

Male genitalia morphology

Terminologies describing the structures of the male genitalia (Table 1) came from different studies (Zolessi and

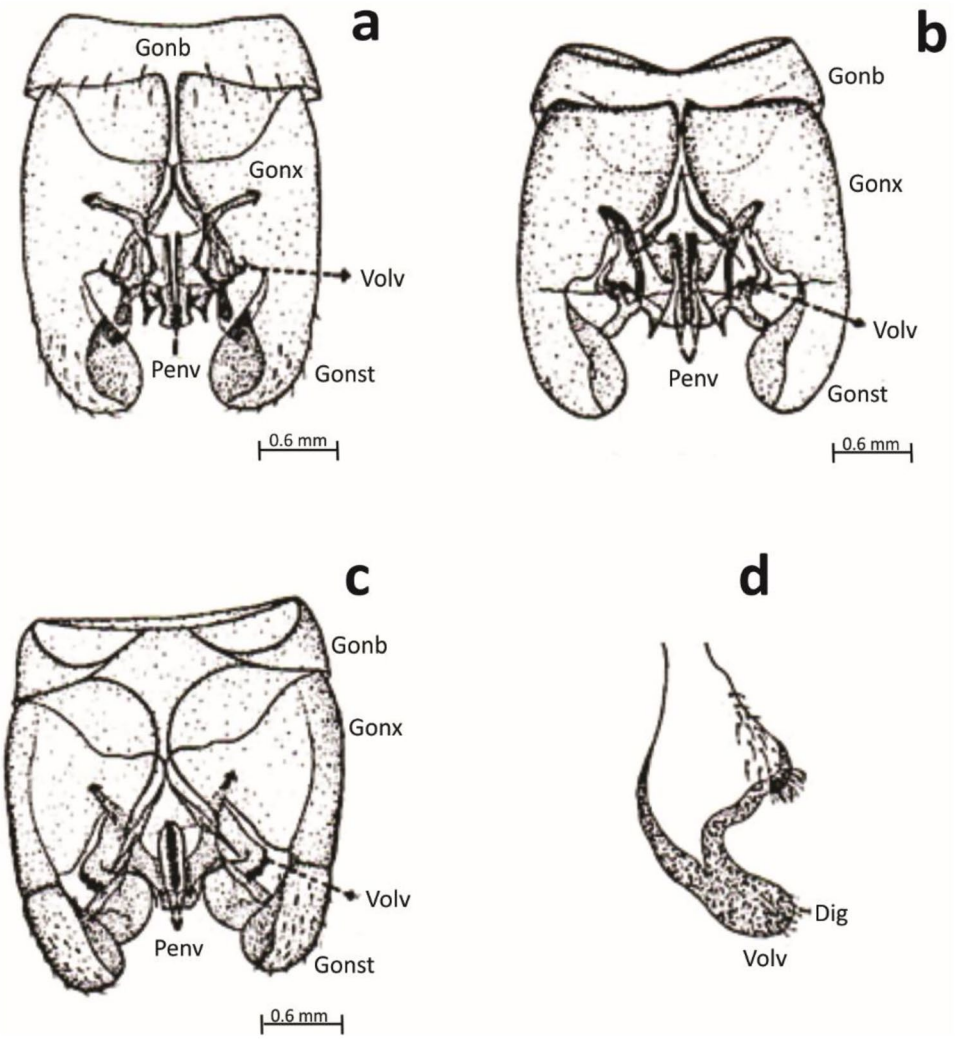


Fig. 2 Ventral view of the male genitalia of *Acromyrmex subterraneus brunneus*. Genitalia of a male from nest J1 (Jacareí, São Paulo) (**a**); nest P3 (Piracicaba, São Paulo) (**b**); nest E5 (Eldorado, São Paulo) (**c**); Volsella (100×) highlighting the bristles in the digitus (**d**). Abbreviations: Basal ring (BR), gonocoxite (Gnc), gonostyle (Gnt), penis valves (PV), volsella (Vo) and digitus (Dig)

Abenante 1973, 1975; Zolessi and González 1974, 1978) and morphological differences between the genitalia of *A. s. subterraneus* and *A. s. brunneus* males were not found (Fowler and Ketelhut 1993).

Differences in the morphology of the *A. s. molestans* male genitalia from that of the other two subspecies is important to separate them. The the volsella is an important structure to differentiate ant species because it is the coupling structure between the male and the female genitalia during copulation and it is used to distinguish *A. subterraneus* subspecies (Zolessi and González 1974, 1978). The volsella and the sub genital plate are also important male morphological characters to differentiate *Acromyrmex* species (Zolessi and Abenante 1973; Zolessi and González 1974, 1978). The sagittal morphology differentiated *Atta* species (Gonçalves 1942, 1944; Borgmeier 1950, 1959) and the gonostyle shape the *Acromyrmex landolti* subspecies (Fowler 1988).

Genome sequencing

Each gene of *Atta*, *Acromyrmex* and *Trachymyrmex* was monophyletic and those of *A. s. brunneus* and *A. s. subterraneus* species derivative as to genus. The sequences obtained for these subspecies indicated 95% identity in 457 analyzed nucleotides and most of the 19 substitutions observed in the IGS region. These results are very similar to those of the same gene region in *Atta sexdens rubropilosa* and *Atta sexdens sexdens* with identity values from 97 to 100%. This fact suggests that *A. s. brunneus* and *A. s. subterraneus* are indeed subspecies or even distinct populations within the same specific unit. The identity of *A. s. molestans* subspecies was 72% similar to the other two *A. subterraneus* subspecies, what indicates that the first is a different species. The parsimony analysis also indicates this species as the oldest radiation of the genus replacing the *Acromyrmex coronatus* (Wetterer 1999) (Fig. 4). This author made this proposal

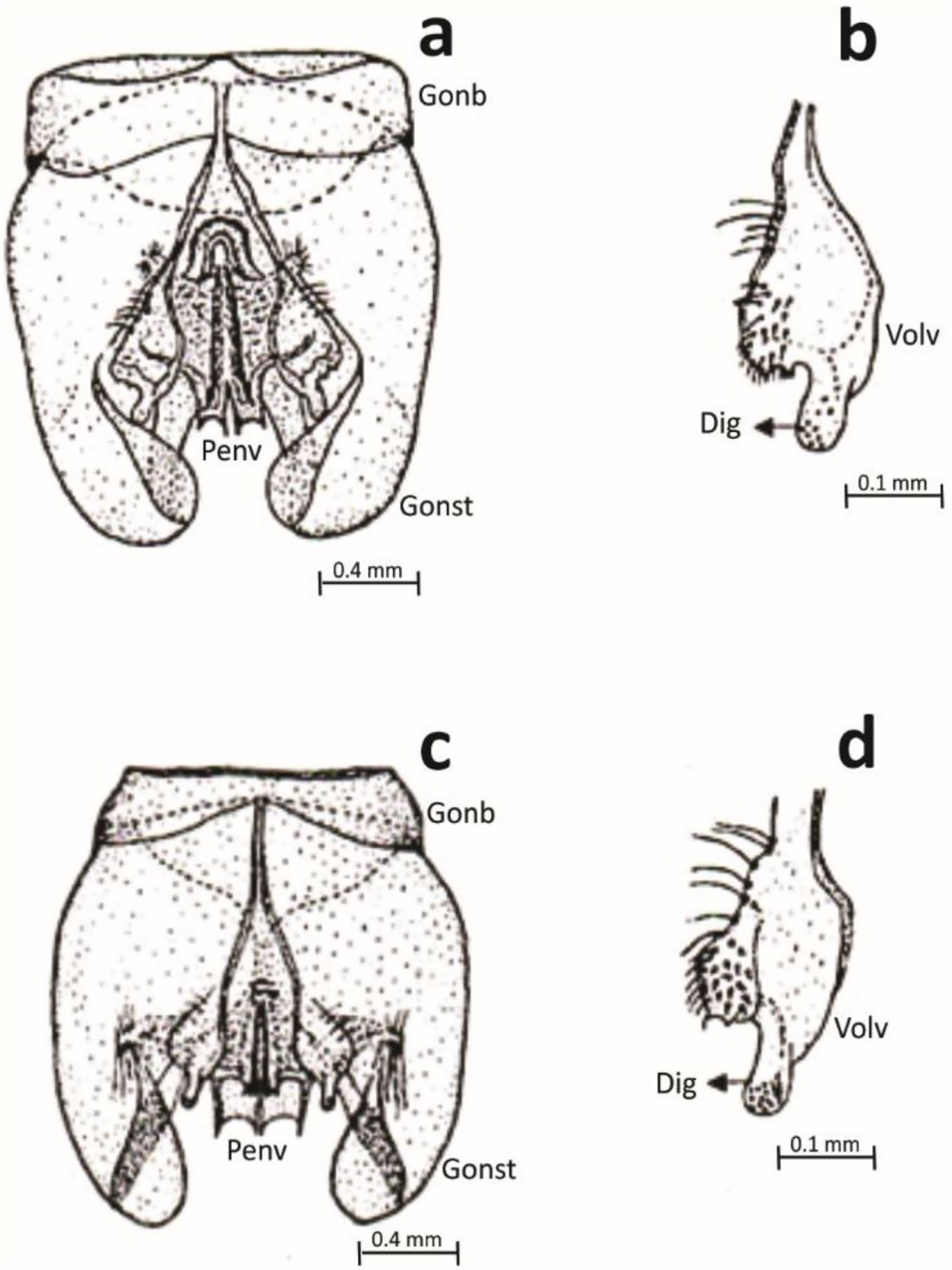


Fig. 3 (**a**) Ventral view of the genitalia of *Acromyrmex subterraneus subterraneus* males (nest V12, Viçosa, Minas Gerais); (**b**) Volsella (10×) highlighting the absence of bristles in the digitus; (**c**) Ventral view of the genitalia of *A. subterraneus molestans* males; (**d**) Volsella (10×) highlighting the absence of bristles in the digitus. Abbreviations: Basal ring (BR), gonocoxite (Gnx), gonostyle (Gnt), penis valves (PV), volsella (Vo), digitus (Dig)

through a parsimony analysis of morphological data based on the size distribution of workers of various species of the Attini tribe.

Comparisons of male genitalia and parsimony analysis provided sufficient evidence to differentiate and to raise *A. s. molestans* to the species category as *Acromyrmex molestans*. Despite doubts on the external morphology of its workers, the parsimony analysis reinforced the differences found in the genitalia morphology between *A. s. brunneus* and *A. s. subterraneus* males showing that these two taxa can be separated species.

The use of mitochondrial genome sequencing containing regions of the gene cytochrome oxidase is important to elucidate phylogenetic problems of insect groups, including Coleoptera (Dobler and Müller 2000), Diptera (Smith-Caldas et al. 2001) and Dermaptera (Wirth et al. 1999). A review in the molecular systematic of insects reported studies related to the use of gene cytochrome oxidase I (COI) and cytochrome oxidase II (COII) (Caterino et al. 2000). The phylogeny of fungi-cultivating ants of the Attini tribe was based on their mtDNA sequences and morphological characters of their larvae (Williams et al. 1990; Wetterer

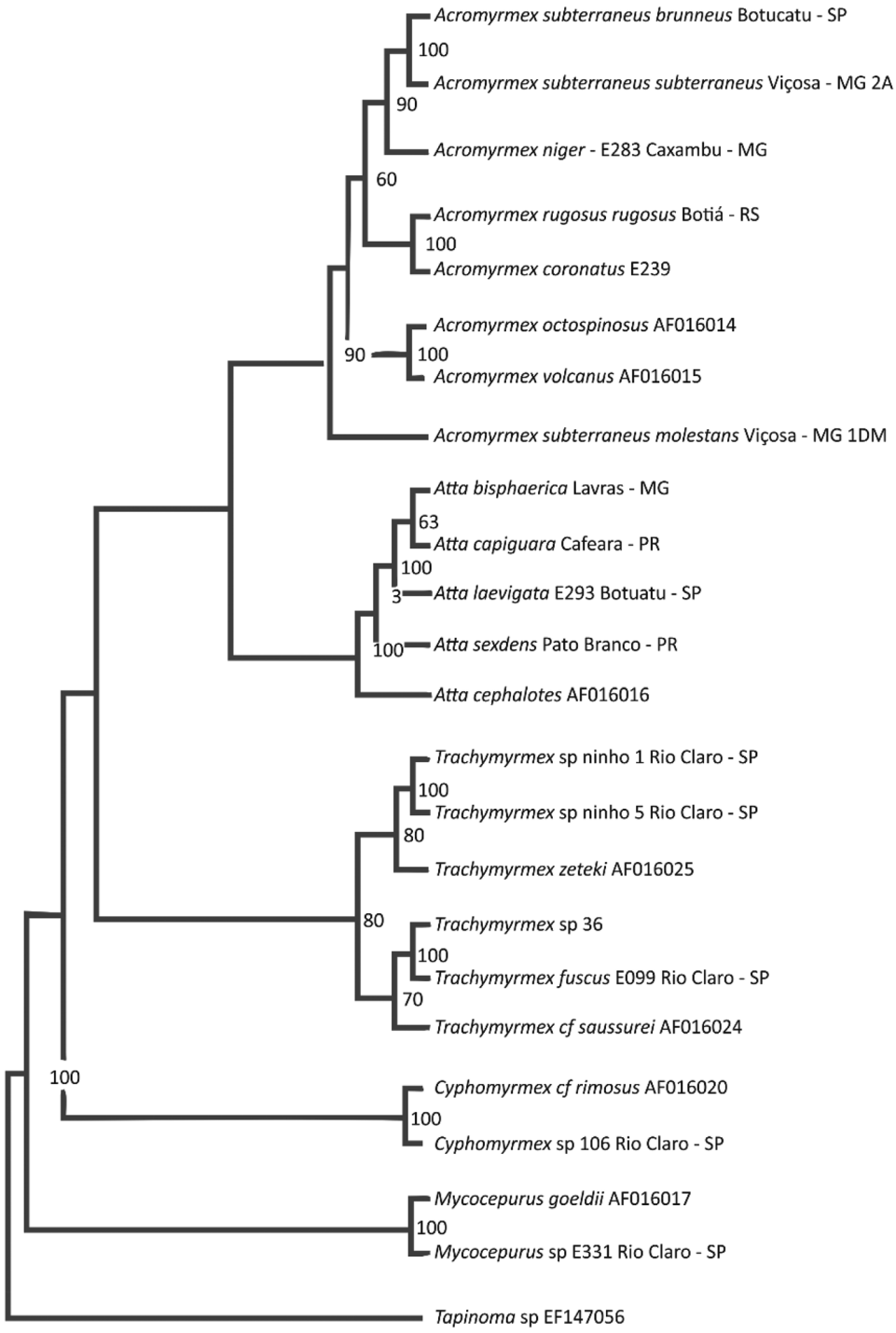


Fig. 4 Phylogenetic relationship resulting from the parsimony analysis of 174 informative characters of COI, IGS, Leu-tRNA and COII of 23 representatives of the Attini tribe with *Tapinoma* sp. as an outgroup. Steps = 765, CI = 0.4247, RI = 0.6076. The numbers ahead of the clades are bootstrap values, which were omitted when lower than 50%. The numbers ahead of the taxa indicate the sequenced species laboratory code or the NCBI access number

et al. 1998; Chiotis et al. 2000; Schultz 2000). However, this technique has not been used to elucidate taxonomic problems at species and subspecies levels for the *Atta* and *Acromyrmex* genus.

The differentiation of *Atta* species was also based on the PCR-RAPD (Polymerase Chain Reaction—Random Amplification of Polymorphic DNA) (Williams et al. 1990). This technique allows amplifying numerous DNA fragments delimited by a 10-base primer of arbitrary sequence that hybridize into complementary sequences randomly distributed in the DNA. The variations produced by mutations at hybridization sites or by insertion or deletion of bases between sites detected have been used to identify species and subspecies of bacteria, fungi, insects, mites, nematodes, and plants (Cenis and Beitia 1994). In addition, it is possible to calculate the genetic distance (similarity) between two or

Table 1 The structures basal ring (BR), gonocoxite (Gnc), gonostyle (Gnt), penis valves (PV) and volsella (Vo) composing the male genitalia (MG) of the *Acromyrmex subterraneus* (Hymenoptera: Formicidae) subspecies

MG	*Acromyrmex subterraneus subterraneus*	*Acromyrmex subterraneus brunneus*	*Acromyrmex subterraneus molestans*
BR	Wide	Wide	Wide
Gnc	Wide in the proximal region	Wide in the proximal region	Wide and rounded in the proximal region
Gnt	In the distal region, they are curved and internally concave	In the distal region, they are curved and internally concave	In the distal region, they are curved and more internally dug than the others
PV	Phallus is laminar, rather sclerotized and internally with two series of small and thick bristles	Phallus is laminar, rather sclerotized and internally with two series of small and thick bristles	Phallus is laminar, rather sclerotized and internally with two series of small and thick bristles
Vo	Internal portion covered by bristles, and, in its curvature, two small protuberances covered by bristles too. The digitus is very prominent and without bristles	The digitus is wide and rather prominent, with bristles. Its curvature is smooth with a tuft of bristles on the largest end and the internal portion	Narrower than the others. Curvature is rather prominent, with a small protuberance, but without bristles on it. The digitus is longer and narrower than the others and without bristles

more individuals allowing the construction of a dendrogram by means of cluster analysis techniques without using molecular data representing the genotype of individuals (Nei 1987).

Molecular techniques to identify markers capable of characterizing *Atta* species and subspecies have been developed (Carvalho 2000). The analysis of the genetic diversity of *Atta* species allowed to group them almost entirely within a subgenera (Borgmeier 1950) with the separation of *Atta sexdens* in three distinct nuclei—*Atta sexdens sexdens*, *Atta sexdens piriventris*, and *Atta sexdens rupropilosa* (Carvalho 2000)-supporting the classification of another study (Gonçalves 1963) but contrary the one, which synonymies these three subspecies (Bolton 1995). The *Acromyrmex molestans* stat. n., *Acromymex brunneus* stat. n., and *Acromyrmex subterraneus* stat. n. are here raised to species.

Illustrated key for the identification of major *Acromyrmex* workers in São Paulo State, Brazil.

1. Supraocular spine (Supra Sp.) present and prolonged mandible (Md.) (Fig. 5a) ... 2

   1' Supra Sp. absent and short mandible; no prominent eyes, both lateral pronotal spines (L.Pn.Sp.) and medium lateral pronotal spines (M.L.Pn.Sp.) are tuberciform (Fig 5b) ... *Acromyrmex balzani*

2. Integument with dense pubescence (Fig. 5c) ... *Acromyrmex disciger*

   2' Integument with microscopic reticulation and shiny (Fig. 5d) *Acromyrmex ambiguous*

   2" Opaque integument ... 3

3. Anterior mesonotal spines (A.Mn.sp.) much longer and robust than the lateral pronotal spines and slightly frontward directed (Fig. 5e) ... *Acromyrmex asperses*

   3' A.Mn.sp. pointy, erect and upward directed, slightly sideward directed and much longer than the others (Fig. 5f) ... *Acromyrmex diasi*

   3" A.Mn.sp. less robust than the others ... 4

4. L.Pn.sp. much longer than the A.Mn.Sp. and frontward and sideward directed (Fig. 5g) ... *Acromyrmex coronatus*

   4' L.Pn.sp. not remarkably prolonged ... 5

5. Tubercles over the garter dorsum (T.G.) randomly distributed; inferior pronotal spines (I.Pn.sp.) backwards directed (Fig. 5h) ... 6

   5' T.G. distributed in 4 longitudinal series and sometimes inconspicuous (Fig. 5i) ... 7

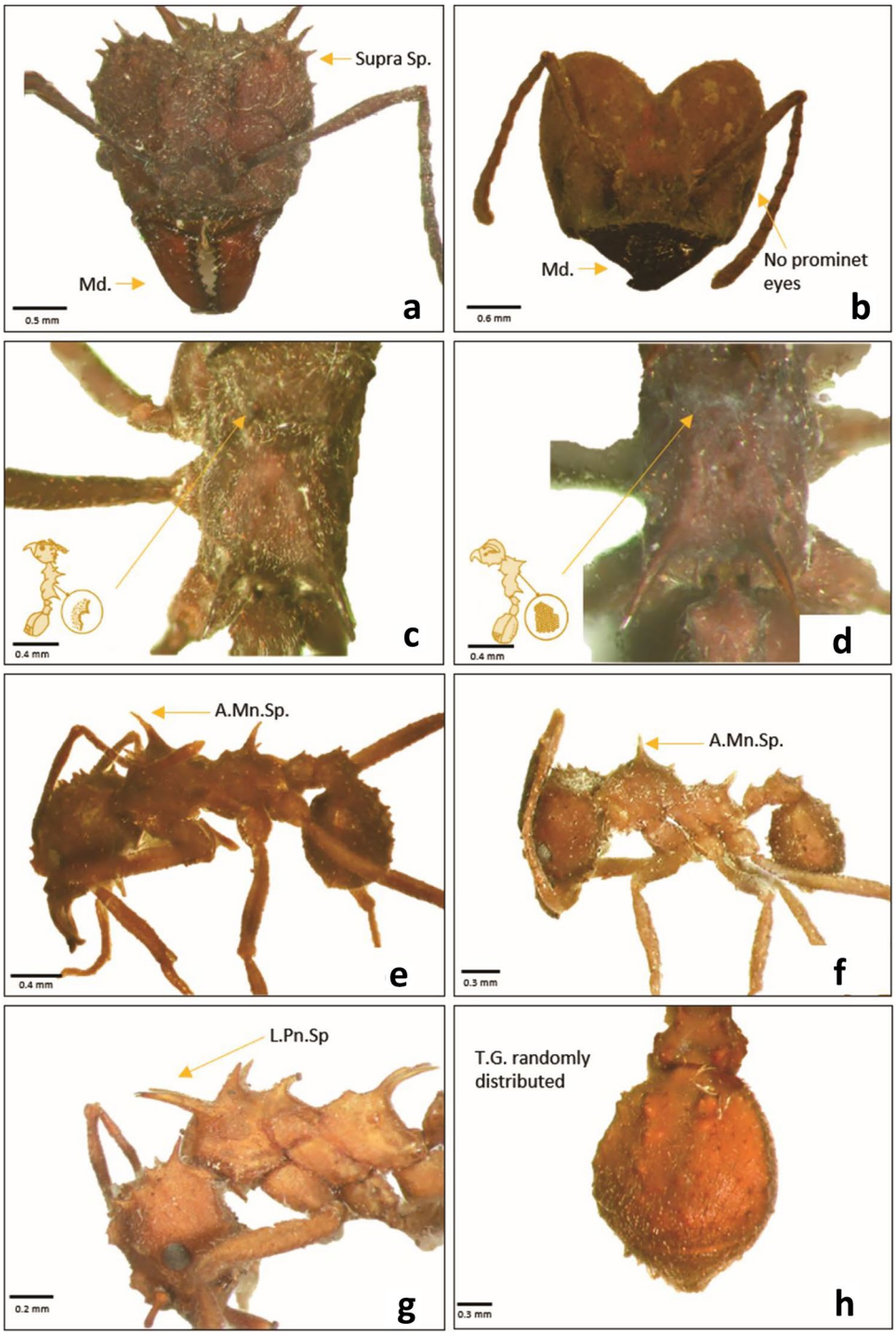
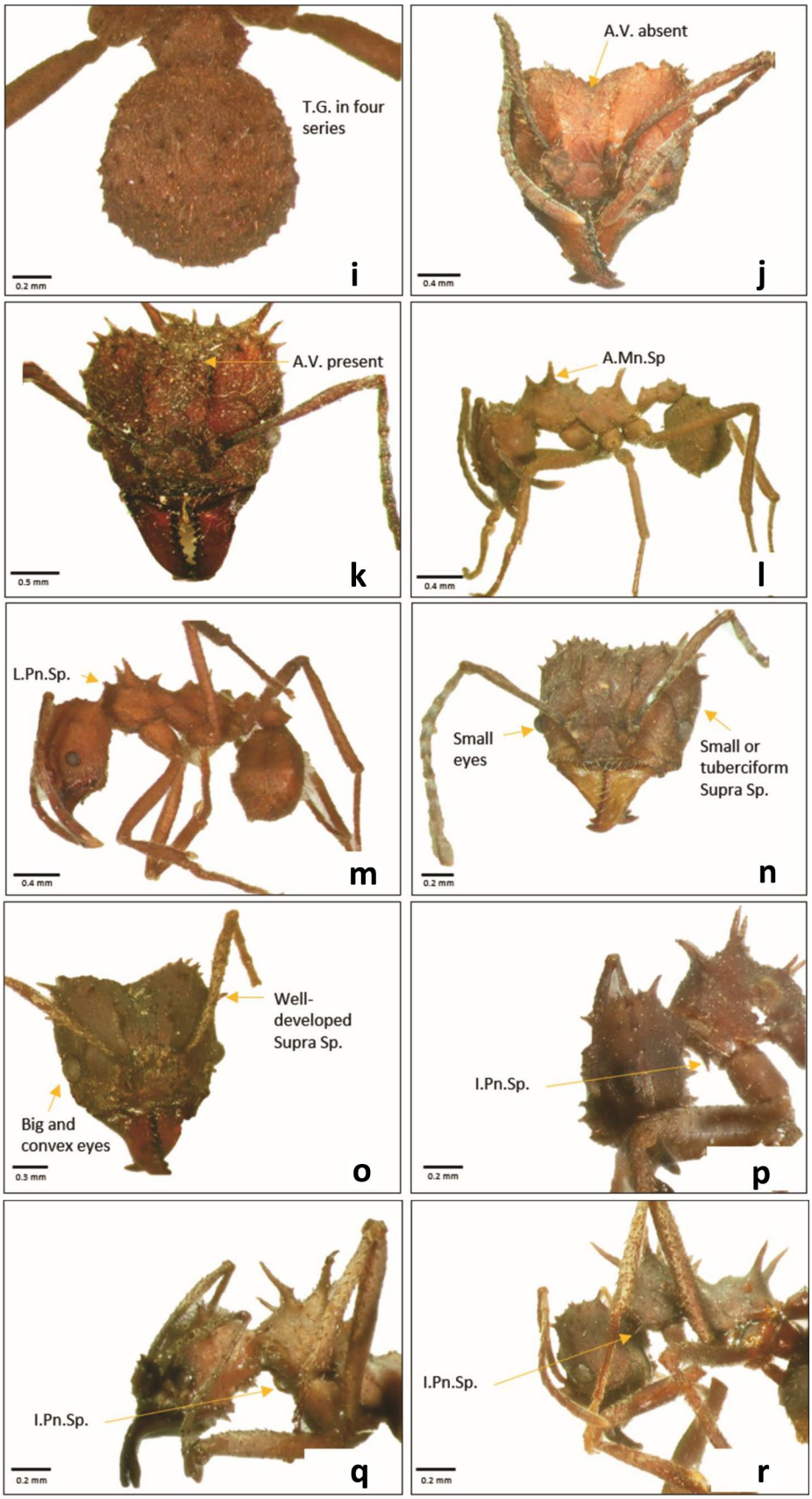


Fig. 5 Illustrated key for the identification of major *Acromyrmex* workers of São Paulo State, Brazil

Fig. 5 (continued)



6. Big enlarged head, with very rounded occipital lobes (Occ.L.), edge of vertex (A.V.) absent or vestigial (Fig. 5j) ... *Acromyrmex laticeps nigrosetosus*

   6' Less enlarged head, less rounded Occ.L., well-defined A.V.; A.Mn.Sp. thicker and longer than the L.Pn.sp., those sometimes backwards directed (Fig. 5k) ... *Acromyrmex crassispinus*

7. Base of A.Mn.p. much more robust than L.Pn.sp.1, both pairs of spine are the same size, convex eyes (Fig. 5l) ... *Acromyrmex rugosus rugosus*

   7' Base of A.Mn.sp. much more robust than L.Pn.sp., which are shorter or reduced in tubercles, convex eyes (Fig. 5m) ... *Acromyrmex rugosus rochai*

      7" A.Mn.sp. with the same thickness as the L.Pn. sp., which are shorter or with the same size as the A.Mn.p. ... 8

8. Small or tuberciform Supra sp.; small eyes (Fig. 5n) ... *Acromyrmex niger*

   8' Well-developed Supra sp.; big and convex eyes (Fig. 5o) ... 9

9. I.Pn.sp. straight and frontward directed; head with the same color as the body; light-brown workers (Fig. 5p) ... *Acromyrmex subterraneus* stat. n.

   9' I.Pn.sp. frontward bent; head usually with darkened front; light-brown workers (Fig. 5q) ... *Acromyrmex molestans* stat. n.

      9″ I.Pn.sp. straight and frontward directed; black or blackened workers (Fig. 5r) … *Acromyrmex brunneus* stat. n

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Declarations

Conflict of interest The authors declare no conficts of interest.

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