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Research Article

Fine Morphological Study of the *Myrmecina graminicola* (Latreille, 1802) Male, With First Description of External Genitalia in the Genus (Hymenoptera: Formicidae: Myrmecinae)

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The genus *Myrmecina* was described basing on males of *M. graminicola*. Though representing the caste type, males were sufficiently described in only two out of 105 species known in the genus. However, the morphology of the male external genitalia remained undescribed. Re-examining by SEM the male of *M. graminicola*, we describe and illustrate in detail for the first time the external genitalia, redefining and updating the morphological male diagnosis of the genus. We also analyze the overall morphology, illustrating additional peculiar characters of this species as follows: (i) a very distinctive stipital groove in the dorsolateral stipes; (ii) a developed uncinate-shaped mesoscutellar arm; (iii) the antennal cleaning; and (iv) the absence of meso- and metatibial spurs. This morphological study will be useful as a base for further morphological descriptions of the males in other species of the same genus to support correct taxonomic identifications.

1. Introduction

In the genus *Myrmecina* Curtis, 1829, one hundred and five species are known [1], distributed in Nearctic, Palearctic, Oriental, Australasian, Indomalesian, and Oceania regions; in the Neotropical region is present only in Mexico [2]. Species of *Myrmecina* live in soil and forest litter [3, 4]. The taxonomic history of the genus dates back two centuries, representing one of the first taxonomic descriptions of the family Formicidae. It was erected basing on the male of *Myrmecina latreillii*, junior synonym of *M. graminicola* (Latreille, 1802) [5], which, therefore, represent the caste type of the genus [6]. Subsequently, other brief morphological descriptions of the *M. graminicola* male were published [7–14]. In very few other species of the genus, the following short morphological descriptions of males are available, unusable for the morphological identification: (i) *M. pilicornis* Smith, 1858 [15]; (ii) *M. sulcata* Emery, 1887 [16]; (iii) *M. sicula* André, 1882 [17]; (iv) *M. modesta* Mann, 1919 [18]; and (v) *M. americana* Emery, 1895 [19]. Recently, more exhaustive morphological diagnoses of the *M. nipponica* Wheeler, 1906 male [20] and a new morphological diagnosis of the males at the genus level have been published [21, 22]. The presence of undescribed males associated with worker types are reported in the species *M. boltoni*, *M. gopa*, *M. itoi*, *M. maryatiae*, and *M. sundanica* [4].

Summing up, males of the genus *Myrmecina*, though representing the caste type [6], were described in only six out of 105 species, only two of which, *M. nipponica* and *M. graminicola*, with appropriate descriptions. Most important, in none of the available morphological descriptions male external genitalia were described.

In this work, we describe for the first time the male external genitalia of *M. graminicola*, type species of the genus, by using optical and scanning electron microscopy

(SEM). This represents the first description of the external genitalia of the genus. In addition, we redescribe the male of *M. graminicola*, redefining the diagnosis of the genus.

2. Materials and Methods

This study is based on 16 male specimens of *Myrmecina graminicola* captured on 12 August 2023 in the Vatican Gardens (Vatican State, 41°54′15.39N; 12°27′61.81E; 80 m.a.s.l.), by using Malaise traps. All the individuals were preserved in 90% alcohol. The taxonomic identification of *M. graminicola* was made based on morphological descriptions [6, 11, 12, 14] and confirmed by molecular analyses.

2.1. Morphological Analysis. The identifications and dissections were performed by using Leica MZ12 (Leica Microsystems, Wetzlar, Germany) and Olympus SZX16 (Olympus, Tokyo, Japan) stereomicroscopes equipped, respectively, with Olympus Highlight 2100 and Olympus KL1500 LCD strong fiber optics. Pictures were acquired with a Zeiss Axio Zoom V16 (Carl Zeiss AG; Oberkochen, Germany) and an Axiocam 503 (Carl Zeiss Microimaging Gmbh, Jena, Germany) equipped with Led dual spot lights Photonic Optische (Vienna, Austria).

The scanning electron microscopy (SEM) analysis was performed at L.I.M.E. lab. (University of Roma Tre, Rome, Italy). Samples were dehydrated in a graded ethanol series (70%, 85%, and 95% for 30 min each and 100% for 2 h), critical point-dried (Balzer Union CPD 030 unit), mounted on aluminum stubs with a conductive adhesive carbon disk, sputtered with a thin layer (30 nm) of gold in a Emithech K550 sputter coater (Emithech, Kent, UK), and analyzed with a Zeiss Gemini 300 field emission SEM microscope at a voltage of 5 kV (Carl Zeiss AG, Jena, Germany); measurements taken with proprietary image analysis software Smart SEM (Carl Zeiss AG; Oberkochen, Germany).

2.2. Molecular Analyses. For a molecular validation of the identification of the male specimens based on morphology, we extracted DNA from four individuals using the © Qiagen DNeasy Blood and Tissue kit following the manufacturer's protocol. The Barcoding region of the Cytochrome C Oxidase I (COI) was amplified using the primer pairs LCO1490/HCO2198 [23]. PCR were carried out in a final volume of 25 μl, containing 3 μl of 10x reaction buffer, 1–3 μl of MgCl2 (50 mM), 0.5–1 μl dNTPs (10 mM), 0.2 μl of TaqDNA polymerase (5 U/μl BIOTAQ DNA Polymerase, Meridian BIOSCIENCE), 0.5 μl of each primer (25 mM), and 0.5–1 μl of DNA template. Thermal cycle conditions consisted of an initial denaturation of 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 50°C, and 1 min at 72°C, with a final elongation step of 10 min at 72 C. Products were visualized on a 1% agarose gel stained using the GelRed nucleic acid gel stain (Biotium). Sequences were manually checked and edited using Geneious Prime 2021.1.1 [24]. To support morphological identification, first we used the identification tool in BOLD [25] and then we aligned our sequences with some of those referred to the genus *Myrmecina* available in BOLD and NCBI repositories. We retained only the sequences from specimens identified at the species level and those for which the collection country was indicated. For each species, we kept maximum 10 sequences per country. The final dataset included the species *M. americana* (from Canada and USA), *M. nipponica* (from Japan and South Korea), and *M. graminicola* (from Germany, Bulgaria, Switzerland, and United Kingdom). *Acanthomyrmex careoscrobis* Moffett, 1986, was used as the outgroup [26]. The distance-based neighbor joining [27] method was used to infer the relationship between our query sequences and those downloaded from online repositories using the Kimura 2-parameter model [28]and 1000 bootstrap replicates. Analyses were performed with MEGA v7 [29].

2.3. Terminology. The terminology used in this study is based on the following references: general morphology [30], head and mesosoma [31], cuticle microsculpture [32], antennal cleaning [33], wings [34–37], and external genitalia [38–40].

2.4. Measurements

HL: head length, in full face view. The midline distance from the level of the maximum posterior projection of the margin of the head (not including the ocelli) to the level of the most anterior projection of the anterior clypeal margin.

HW: head width, in full face view, the maximum width of the head posterior to the compound eyes.

SL: antennal scape length, measured from the apex of the first antennal segment to the base, exclusive of the radicle.

EL: eye length, in lateral view, the length of the compound eye along the longitudinal axis.

EW: eye width, in lateral view, the maximum transverse width of the compound eye.

MML: maximum mesosomal length in lateral view from the anteromedial part of the pronotum to the posteromedial part of the propodeum.

WL: forewing length, the maximum distance between the insertion of the sclerotized wing veins and the distal margin of the wing.

WHL: hindwing length, the maximum distance between the insertion of the sclerotized wing veins and the distal margin of the wing.

Indices: CI: cephalic index 100x HW/HL, SI: scape index 100x SL/HL, OI: ocular index 100x EL/HL, ES: eye size 100x ELxEW, WI: wing index 10x WL/MML.

3. Results

3.1. Male Morphological Diagnosis of the Genus Myrmecina.

3.1.1. Genus Myrmecina Curtis 1829. Head subglobose, eyes convex occupying most of the anterolateral side of the head, ocelli prominent. Antennae filiform with 13

articles, scape short, longer than the second article of the funiculus, last article the longest. Clypeus convex medially. Mandibles very reduced in lobe, with setae. Palp formula 4 : 3 (*M. graminicola*) or 3 : 2 (*M. nipponica*). Mesonotum with distinct notauli. Meso and Metatibiae without spur (*M. graminicola*) or with spur (*M. nipponica*). Forewings with pterostigma, submarginal 1 cell, no discoidal cell, and marginal cell closed and appendiculate. Hindwing without M2 vein. Propodeum with pair of dentiform projections posterodorsally. Petiole sessile and subcilindric in shape and postpetiole subglobose in shape. Pygostyles present. External genitalia: paramere with basimere developed and telomere short and lobiform; volsella with digitus falciform and parassiculus with basivolsella and lobate cuspis; penisvalves with valviceps lamina dentate.

3.2. Redescription of *M. graminicola* Male

3.2.1. Myrmecina graminicola (Latreille, 1802). Measurement (in mm, n: 7): HL: 0,541–0,543, HW: 0,639–0,641, SL:0,168–0,169, EL:0,262–0,263, EW: 0,145–0,146, WL:0,145–0,146, MML: 1,167–1,169, WL: 2,86–2,87, and WHL: 2,30–2,31.

Indices: CI: 118, SI: 31, OI: 48,5, ES: 3,80, and WI: 24,5.

Habitus (Figure 1): color black with tarsi and antennae brown. Head, mesosoma, and metasoma with long decumbent setae. Wings fuscate.

Head (Figures 2 and 3): head smooth with erect and decumbent long setae dorsally and ventrally; hypostoma entire, without anteromedial stipital notch. Malar area is very short. Anterolateral frontal area with microsculpture striate transversally. Clypeus is very convex medially and with straight anterior margin. Antennae is with scape slightly longer than the second article of the funiculus. Labrum bilobate, separate by a deep notch, with anterior margin of each lobe rounded. Maxillae elongate, galea with long setae anteromedially. Stipes very developed dorsolaterally with stipital groove very characteristics dorsally and laterally. Maxillary palps with four articles: first short and thick, second longer than third, and fourth the longest; labial palps with three articles: first thick and as long as the second and third the longest.

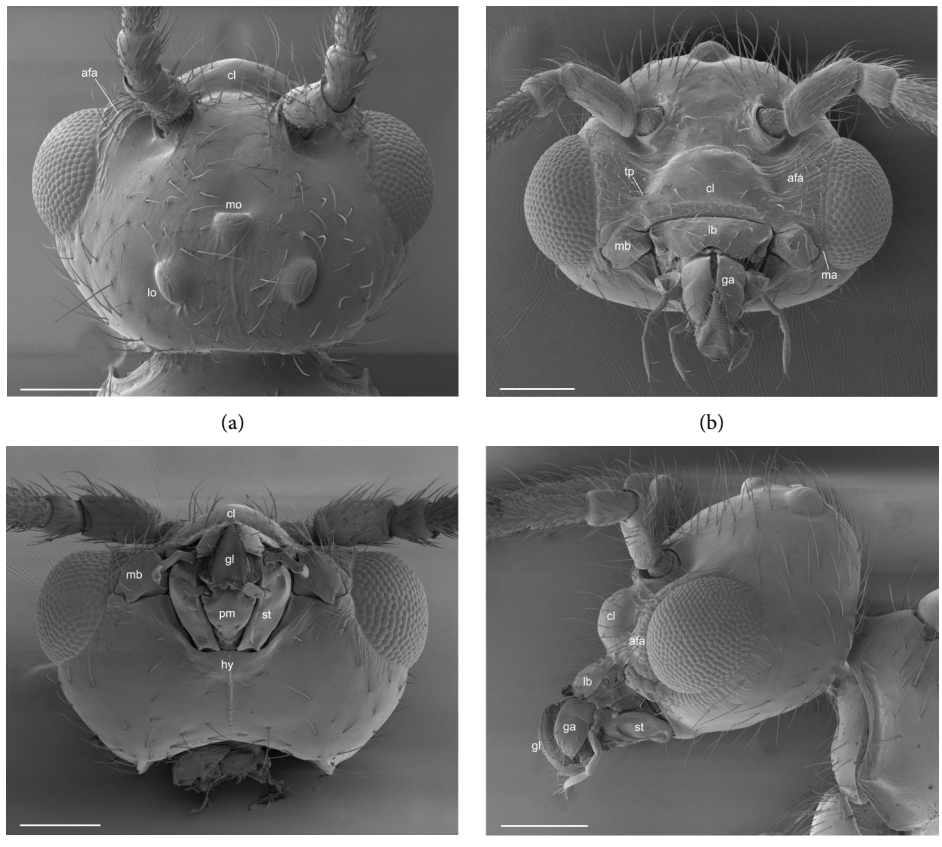
Mesosoma (Figures 4–6): pronotum short and smooth entirely with long setae anteriorly. Mesonotum not overhanging the pronotum, smooth dorsally with long setae and notauli distinctly impressed; anepisternum with longitudinal striate microsculptures anterodorsally and smooth posteroventrally, katepisternum smooth. Mesoscutellum slightly lower than mesoscutum with long decumbent setae, convex dorsally with longitudinal strong striate microsculpture anterodorsally and smooth posterodorsally, longitudinal striate microsculpture dorsolaterally; scutoscutellar sulcus present; mesoscutellar arm developed into a hook-like shape. Metascutellum lower than mesoscutellum; low and upper metapleuron fused with the propodeum and with striate microsculpture longitudinally; low metapleuron without metapleural gland orifice. Propodeum dorsal face short, with striate macrosculpture longitudinally, with 6-7 strong striae on each side and long setae; propodeal spiracle elliptical; pair of short teeth posterodorsally; declivous face concave. Forewing venation as in Figure 5 with marginal cell appendiculate; hindwing venation as Figure 5 with Cu, Rs and 1R veins nebulous, 2M vein absent, and median anterior margin with 4-5 hamuli. Antennal cleaning presents a calcar with comb and external short cuticular fringes, and basitarsus with comb; meso and metatibiae without spurs; pretarsus with developed arolium and claw simple.

Metasoma (Figure 7): petiole dorsally slightly convex medially, with long decumbent setae; ventrally with small anteroventral process. Postpetiole convex dorsally with long decumbent setae dorsally and long setae ventrally, located on the small ventral lobe; petiole and postpetiole longitudinally striate laterally and ventrally, smooth anterodorsally. Gaster smooth, with abdominal segment IV very developed with long erect or decumbent setae ventrally and dorsally. Other segments of gaster with long decumbent setae located only posteriorly on the tergites and sternites. Pygostyles present.

External genitalia (Figures 8–10): abdominal sternite IX tapered posteriorly, with long setae only posteriorly and with median spiniform process anteriorly. Paramere with basimere and telomere with parameral suture only lateroventrally; telomere with setae apically; telomere medially produced into and hook-like flattened fold, similar in shape to the apicodorsal process of digitus, with which is medially in contact. Volsella with median falciform digitus, with posterolateral sensorial conical sensilla in the apicodorsal process of digitus, representing the volsella sensorium [40]; parassiculus with median conical process and cuspis developed in large flattened lobe, which reaches laterodorsally the apicodorsal process of the digitus, and ventral basivolsella flat with short and long setae. Penisvalve with valviceps lamina slightly convex and dentate ventrally and edentate and straight posteriorly.

(a) (b)

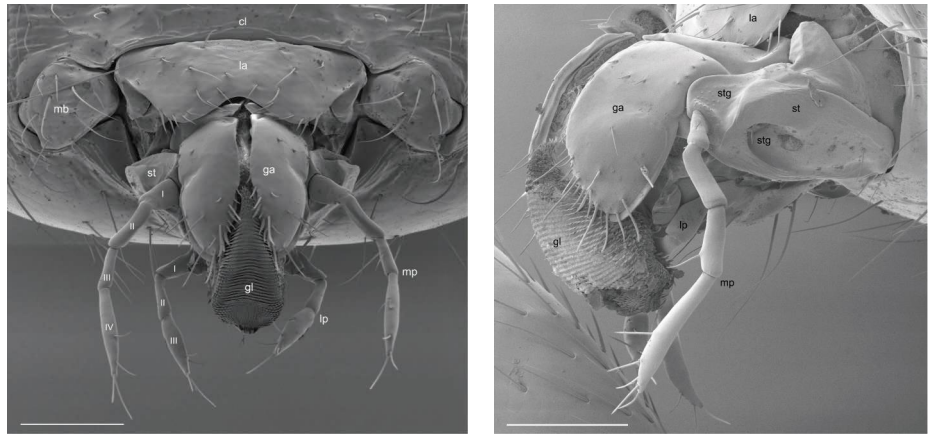
Figure 1: *Myrmecina graminicola* male. (a) Habitus with wings in flight and (b) Habitus with wings at rest.



(a) (b)

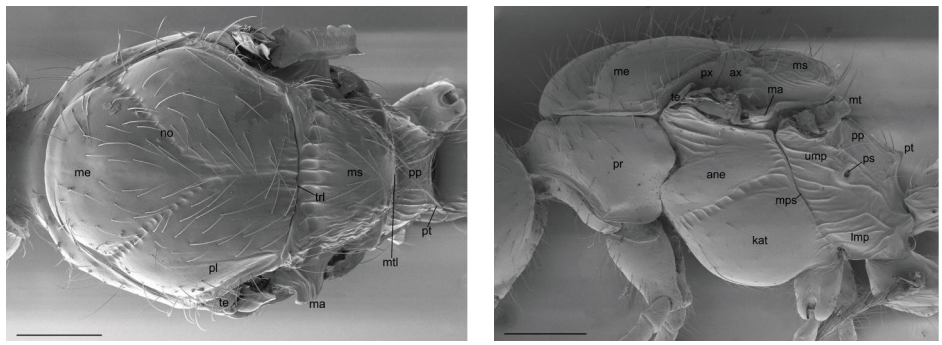
(c) (d)

Figure 2: *Myrmecina graminicola* male. (a) Head in dorsal view; (b) head in frontal view; (c) head in ventral view; and (d) head in left lateral view. Abbreviations: afa: anterolateral frontal area; cl: clypeus; ga: galae; gl: glossa; hy: hypostoma; lb: labrum; lo: lateral ocellus; ma: malar area; mb: mandible; mo: median ocellus; pm: prementum; st: stipes; tp: tentorial pit. Scale-bars: a–c = 150 μm, d = 200 μm.



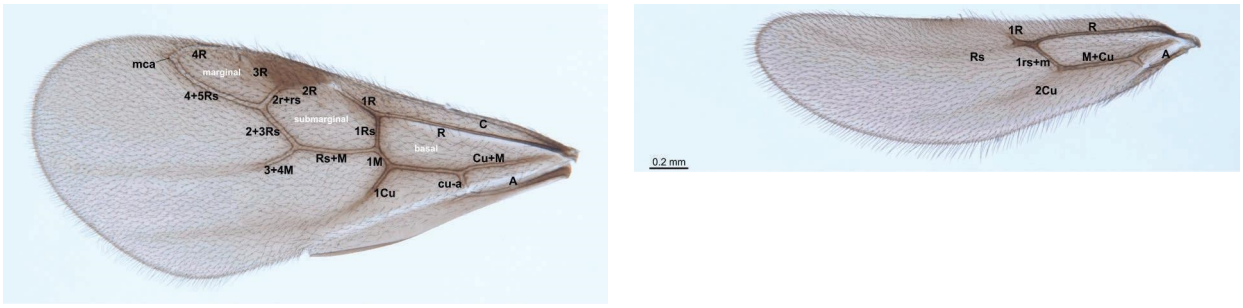
(a) (b)

FIGURE 3: *Myrmecina graminicola* male. (a) Buccal structure in frontal view and (b) buccal structure in lateral view. Abbreviations: cl: clypeus; ga: galea; gl: glossa; lb: labrum; lp: labial palp; mb: mandible; mp: maxillar palp; st: stipes; stg: stipital groove. Scale-bar: a-b = 100 μm.



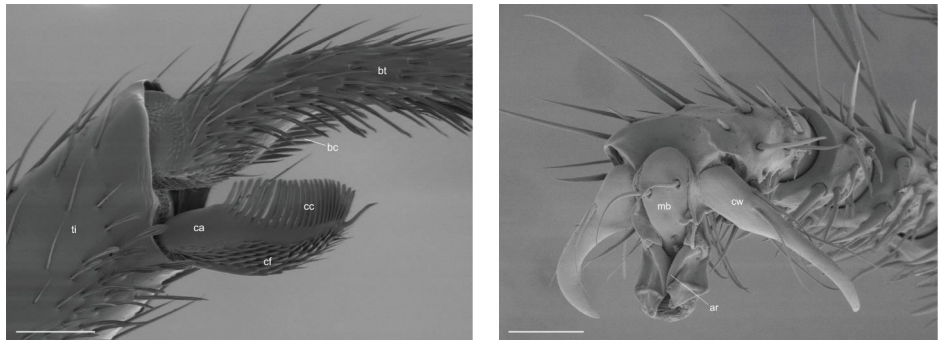
(a) (b)

FIGURE 4: *Myrmecina graminicola* male. (a) Mesosoma in dorsal view and (b) mesosoma in lateral view. Abbreviations: ane: anepisternum; ax: axilla; kat: katepisternum; lmp: low metapleuron; ma: mesoscutellar arm; me: mesonotum; mps: mesopleural suture; ms: mesoscutellum; mt: metanotum; mtl: mesoscutellar line; no: notauli; px: preaxilla; te: tegula; pl: parapsidial line; pp: propodeum; pr: pronotum; ps: propodeal spiracle; pt: propodeal tooth; trl: transcutellar line ump: upper metapleuron. Scale-bars: a–b = 250 μm.



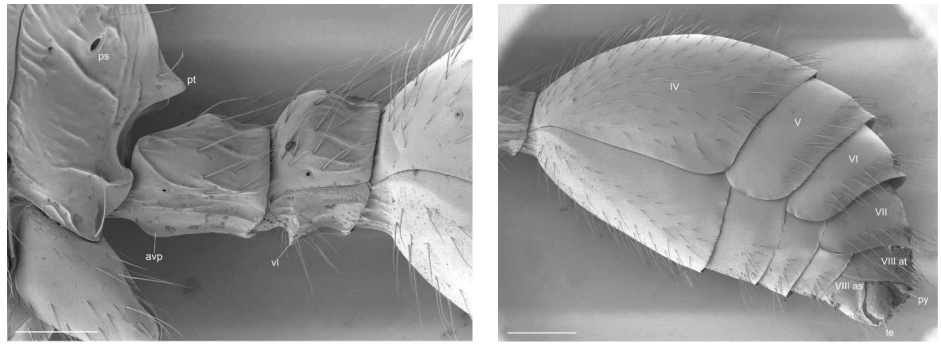
(a) (b)

FIGURE 5: *Myrmecina graminicola* male. (a) Forewing and (b) hindwing. Abbreviations: A: anal vein; C: costa vein; Cu: cubitus vein; Cu + M: cubitus vein + Media vein; cu-a: cubitus-anal crossvein; M: Media vein; mca: marginal cell appendiculate; R: radial vein; Rs: radial sector vein; r + rs: radial + radial sector crossvein.



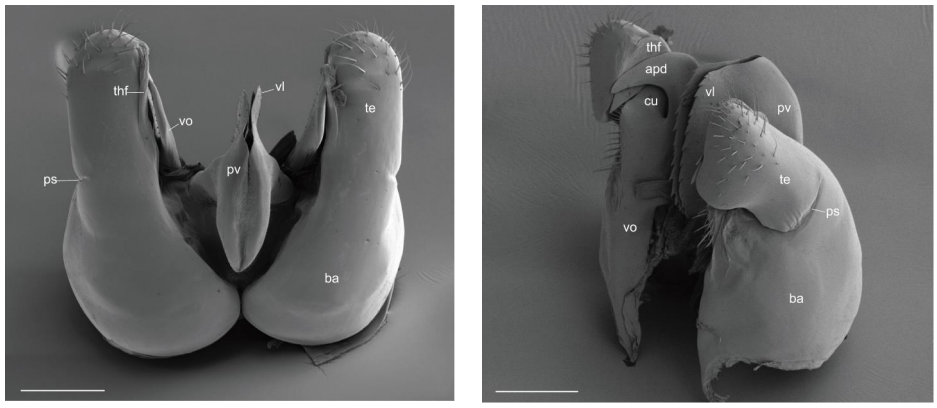
(a) (b)

Figure 6: *Myrmecina graminicola* male. (a) Antennal cleaning and (b) pretarsal claw. Abbreviations: ar: arolium; bc: basitarsal comb; bt: probasitarsus; ca: calcar; cc: calcar comb; cf: external cuticolar fringes; cw: claw; mb: manubrium; ti: protibia. Scale-bars: a = 50, b = 25 μm.



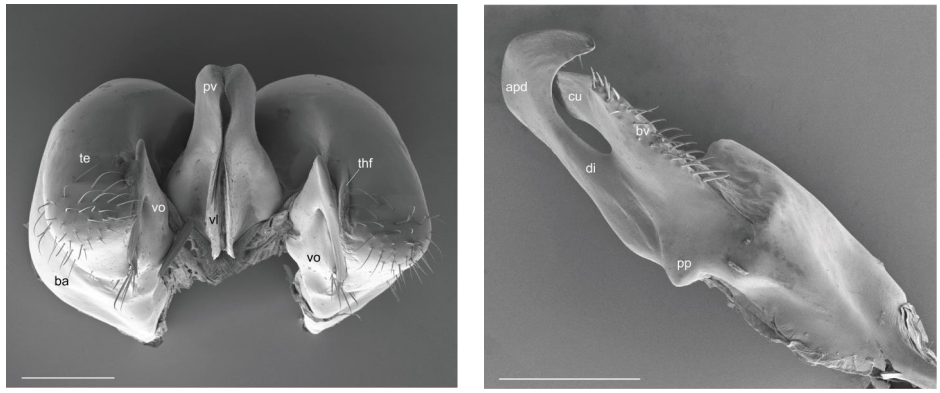
(a) (b)

Figure 7: *Myrmecina graminicola* male. (a) Petiole in lateral view and (b) gaster. Abbreviations: as: abdominal sternite; at: abdominal tergite; avp: anteroventral process; ps: propodeal spiracle; pt: propodeal tooth; py: pygostyle; te: telomere; vl: ventral lobe. Scale-bars: a = 150 μm and b = 250 μm.



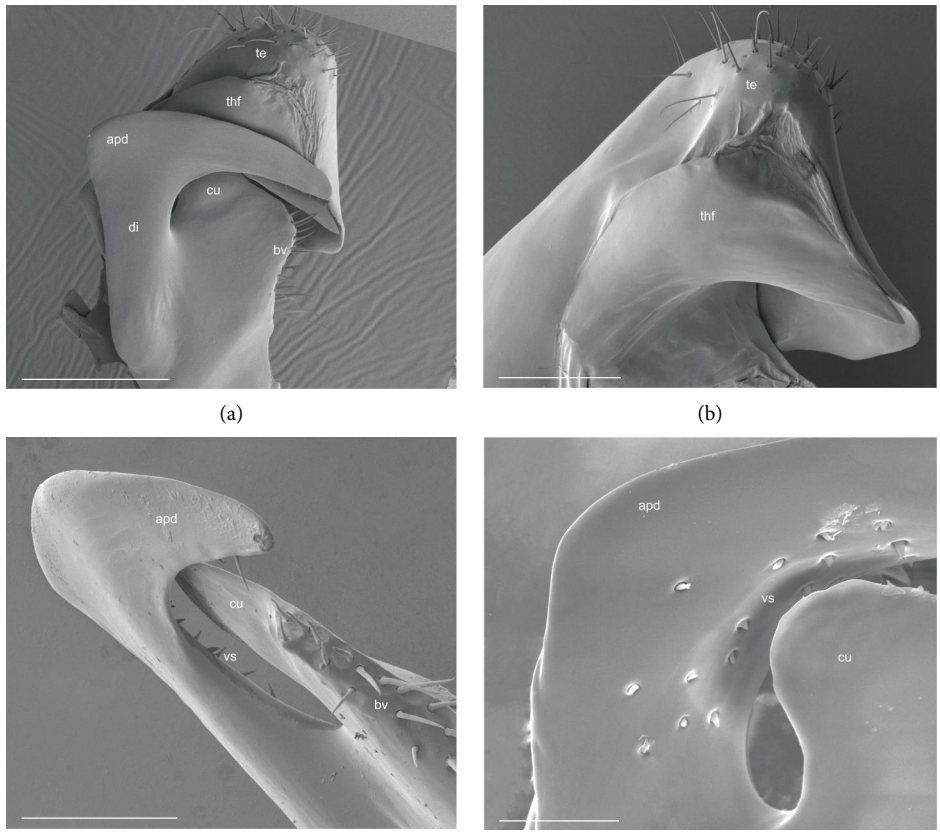
(a) (b)

Figure 8: Continued.



(c) (d)

Figure 8: *Myrmecina graminicola* male. (a) External genitalia in posterodorsal view; (b) external genitalia in ventrolateral view; (c) external genitalia in posterior view; and (d) volsella in medioventral view; Abbreviations: apd: apicodorsal process of digitus; ba: basimere; bv: basivolsella; cu: cuspis; di: digitus; pp: parassiculus process; ps: parameral suture; pv: penisvalve; te: telomere; thf: telomere hook-like fold; vo: volsella; vl: valviceps lamina; vs: volsella sensorium. Scale-bars: a–d = 100 μm.



(a) (b)

(c) (d)

Figure 9: *Myrmecina gram*inicola male (a) Telomere and volsella in median view; (b) telomere in medial view; (c) volsella in medianventral view; and (d) volsella with volsella sensorium in lateral view. Abbreviations: apd: apicodorsal process of digitus; bv: basivolsella; cu: cuspis; di: digitus; te: telomere; thf: telomere hook-like fold; vs: volsella sensorium. Scale-bars: a: 100 μm; b: 50 μm; c: 40 μm; and d = 20 μm.

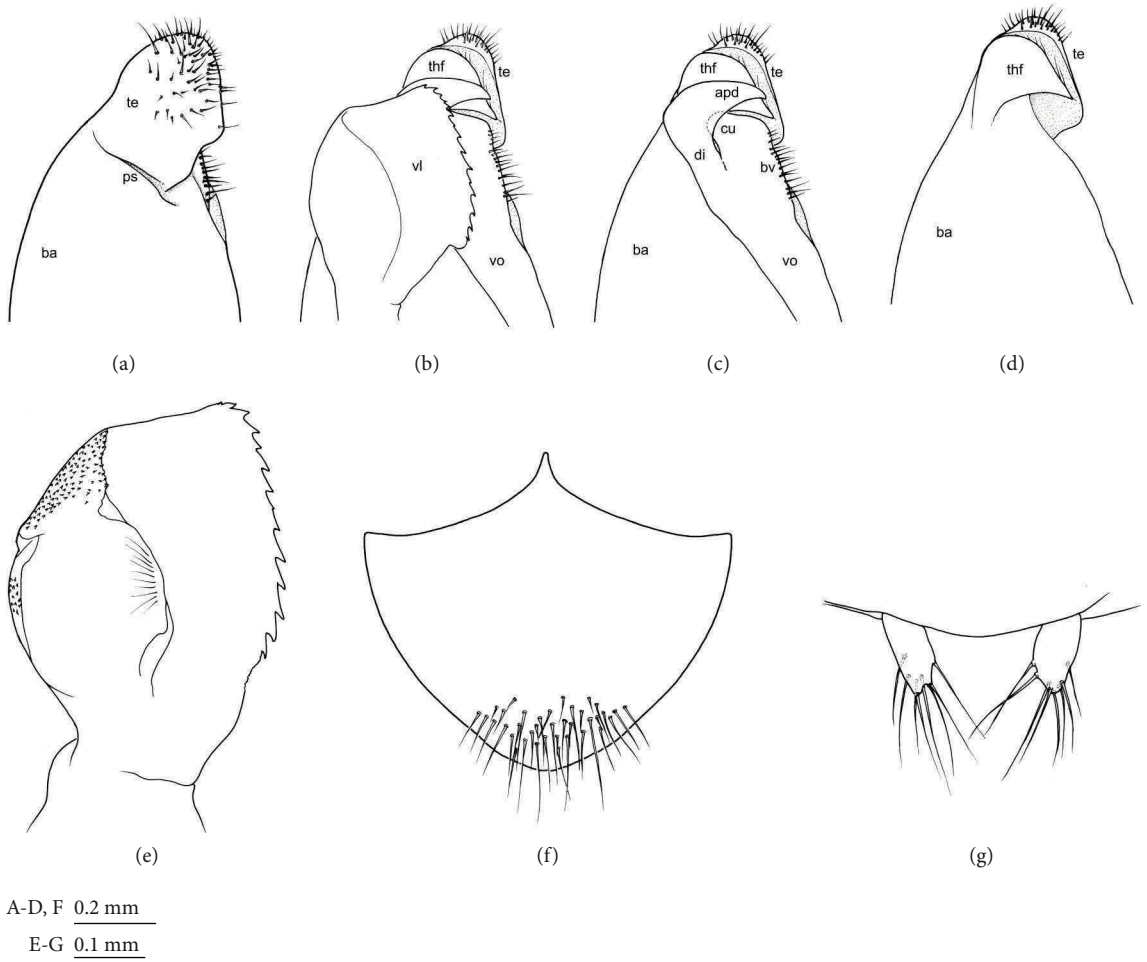


Figure 10: *Myrmecina graminicola* male (a) paramere lateral view; B-C-D: external genitalia in median view, showing the relative position and morphology of valviceps lamina (b), volsella (c) and telomere hook-like fold (d); (e) valviceps lamina; (f) IX ambdominal sternite; (g) pygostyles. Abbreviations: apd: apicodorsal process of digitus; ba: basimere; bv: basivolsella; cu: cuspis; di: digitus; ps: parameral suture; te: telomere; thf: telomere hook-like fold; vo: volsella; vl: valviceps lamina.

3.3. Molecular Analysis of M. graminicola. The four COI fragments analyzed had a final length of 626 bp and were all attributed to *M. graminicola* with BOLD % similarity >99 and resulted included in the clade with other *M. graminicola* in the NJ tree (bootstrap value = 100, Figure 11), supporting morphological identification. GenBank accession numbers are PP774379, PP774380, PP774381, and PP774382.

4. Discussion

In the updated genus male diagnosis supplied in the present study, we outline a variability in the mesotibial and metatibial spurs; in fact, spurs are absent in *M. graminicola* and, according to Ogata [20], present in *M. nipponica*. This variability is quite atypical since the presence or absence of the spurs in metatibiae is an invariant diagnostic character at the genus level in most ant genera [41]. The articles of the maxillary and labial palpi are also different between the two species, being the palp formula of *M. nipponica* 3 : 2 [20] and of *M. graminicola* 4 : 3.

Due to the poor available descriptions, it is not possible to make comparisons or to identify males of the few other species with the described males. A very brief comparative note between males of *M. graminicola* and *M. americana* var. *brevispinosa* was published by Emery [19], highlighting that males of *M. americana* var. *brevispinosa* differ in having light yellow antennae, slimmer legs, and sharper sculpture of the mesoscutellum. De Stefani [17] also described the wings of the male of *M. sicula* as hyaline, differentiating them from those fuscatae of *M. graminicola*.

In *M. graminicola*, we here analyze the morphological characteristics in detail with photos at SEM, and we describe for the first time (i) a very distinctive stipital groove in the dorsolateral stipes (Figure 3); (ii) a developed uncinate shaped

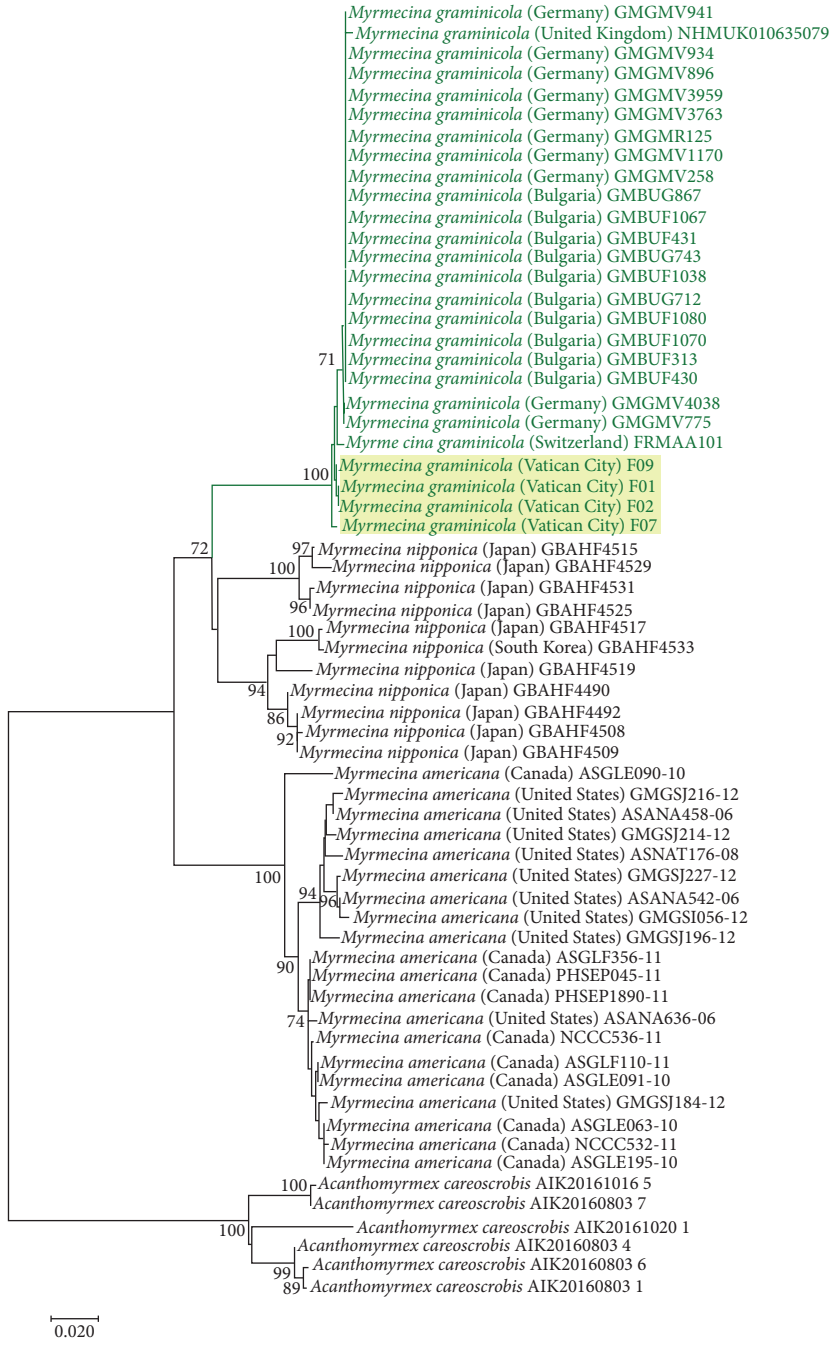


Figure 11: Neighbor joining tree based on COI (K2P-corrected distances). Only bootstrap values > 70 are shown. The clade corresponding to *M. graminicola* is highlighted in green, while the samples newly sequenced for this work are in the light green square.

mesoscutellar arm (Figure 4); (iii) the antennal cleaning (Figure 6); (iv) the absence of meso- and metatibial spurs (Figure 1); and (v) the external genitalia (Figures 8–10). In addition, we here supply the first barcoding sequences of this species from the Italian region, supporting the morphological identification of males, and make a neighbor joining tree based on COI of the available species of the genus.

Here, we provide a detailed description of the external genitalia of *M. graminicola*, which is the species type of the genus *Myrmecina*, adding these characters to the genus male diagnosis. This morphological description will be useful as the base for correctly identifying the species of this genus. In fact, the male external genitalia show species-specific morphologies, used in taxonomic identifications in all insects. For this reason, though the specimens here analyzed were collected from a single site (Vatican State), we do not expect significant intraspecific morphological differences in these characters among populations.

Unfortunately, the species of genus *Myrmecina* are based only on the morphologies of the workers, caste sterile with phenotype which may vary between populations, ignoring the caste type defined on males [6] and molecular analyses. The use of male morphology, and in particular of the male external genitalia, would guarantee a correct taxonomical diagnosis of new species or resolve doubts in taxonomical identification in cases where only workers have been described.

Data Availability

The images used to support the findings of this study are included within the article. The male specimens used to support the findings of this study are available in the Formicidae collection of the authors upon request. The genetic data will be deposited in GenBank repository and freely accessible.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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