A new species of *Discothyrea* Roger (Hymenoptera: Formicidae) from the Brazilian Atlantic Rainforest

Uma nova espécie de *Discothyrea* Roger (Hymenoptera: Formicidae) da Mata Atlântica brasileira

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Abstract: A new species of the cosmopolitan genus *Discothyrea* Roger, a member of the Proceratiinae subfamily, is described for the Brazilian Atlantic Forest. The species, *Discothyrea bobi* sp. nov., is compared to the other New World species described for the genus so far. Two putatively important morphological features of the genus, the palpal formula and the antennal pits, are discussed.

Keywords: Biodiversity. Taxonomy. Myrmecology. Morphology.

Resumo: Uma nova espécie do gênero cosmopolita *Discothyrea* Roger, pertencente à subfamília Proceratiinae, é descrita para a Mata Atlântica brasileira. A espécie, *Discothyrea bobi* sp. nov., é comparada com as demais espécies do Novo Mundo descritas para o gênero até o momento. Duas características morfológicas de possível importância para o gênero, a fórmula palpal e os furos antenais, são discutidas.

Palavras-chave: Biodiversidade. Taxonomia. Mirmecologia. Morfologia.

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INTRODUCTION

Discothyrea is a charismatic genus of ants composed of tiny species living in the litter, rotten logs and soil. Observations and studies on a few species indicate the genus is a spider egg predator specialist (Brown Jr., 1979; Dejean & Dejean, 1998; Katayama, 2013). There are currently 48 extant and 2 fossil species (Hita-Garcia *et al*., 2019a) and they are present in all biogeographic regions, although their diversity in the Nearctic and Palearctic regions is very low (Guénard *et al*., 2017; Janicki *et al*., 2016). The first species described for the genus is the only occurring in the Nearctic, *D. testacea* Roger, 1863. New World species described subsequently were all Neotropical and are the following: *D. neotropica* Bruch 1919; *D. horni* Menozzi, 1927; *D. denticulata* Weber, 1939; *D. humilis* Weber, 1939; *D. icta* Weber, 1939; *D. isthmica* Weber, 1940; *D. sexarticulata* Borgmeier, 1954; †*D. gigas* De Andrade, 1998 and †*D. maya* De Andrade, 1998 (from Dominican and Mexican amber inclusions, respectively); and *D. soesilae* Makhan, 2007. Therefore, New World has currently 11 described *Discothyrea*, extant or extinct.

The Afrotropical fauna has recently received a comprehensive treatment (Hita-Garcia *et al*., 2019a) which will potentially serve as a guide for new revisions of other biogeographic regions, not only for representing an in-depth morphological exploration of the genus, but also for its effort towards a standardized terminology. A small survey made by the author in Brazilian institutions examining *Discothyrea* specimens indicates the actual number of Neotropical species might be considerably higher. In fact, *D. sexarticulata* alone might represent a complex of four or more species (personal observation).

Despite the need of a comprehensive revision of the New World *Discothyrea*, this contribution does not intend to do such thorough task. Instead, it brings the description of one species from Minas Gerais state, southeast Brazil. A discussion about palpal formula and on the antennal pits, recently reported for the related genus *Probolomyrmex* (Oliveira & Feitosa, 2019) and here shown to be also present in *Discothyrea*, is also made, as these are potentially important characters at the genus or the subfamily rank. Finally, the importance of antennal count in *Discothyrea* taxonomy is pondered.

MATERIAL AND METHODS

Studied specimens are deposited at the following myrmecological collections:

- Entomological Collection of the Laboratory of Systematics and Biology of Coleoptera at the *Universidade Federal de Viçosa* (CELC) in Viçosa, Minas Gerais, Brazil;
- Padre Moure Entomological Collection at the *Universidade Federal do Paraná* (DZUP), Paraná, Brazil;
- *Museu Paraense Emílio Goeldi* (MPEG), Pará, Brazil;
- *Instituto Nacional de Pesquisas da Amazônia* (INPA), Amazonas, Brazil;
- *Museu de Zoologia da Universidade de São Paulo* (MZSP), São Paulo, Brazil;
- John Longino personal collection at the University of Utah (JTLC), Utah, USA.
- Apart from the physical specimens, specimens of various species of Proceratiinae, especially *Discothyrea*, were examined based on images available online (Antweb, 2020).

Specimens of the type series were under the morphospecies name "*Discothyrea* ufv-03" since 2015 at Antweb until now and images of one specimen was already available during this period.

Various images of specimens of other *Discothyrea* species deposited on Antweb were examined during this study. Collection data of the specimens which were physically examined were uploaded to Antweb and are the following: *D. clavicornis*: UFV-LABECOL-011157, UFV-LABECOL-011171, UFV-LABECOL-011186, UFV-LABECOL-011183, UFV-LABECOL-011021; *D. horni*: UFV-LABECOL-011185, UFV-LABECOL-009622; *D. neotropica*: UFV-LABECOL-000067, UFV-LABECOL-000068, UFV-LABECOL-008587; *D. sexarticulata*: UFV-LABECOL-000011, UFV-LABECOL-000008, UFV-

LABECOL-000052, UFV-LABECOL-000051, UFV-LABECOL-000113, UFV-LABECOL-007679, UFV-LABECOL-008543, ANTWEB1032519, ANTWEB1032520, UFV-LABECOL-000031, UFV-LABECOL-008580, UFV-LABECOL-010769, UFV-LABECOL-010770, UFV-LABECOL-010771, UFV-LABECOL-008966, ANTWEB1032527, UFV-LABECOL-008199, UFV-LABECOL-008170, UFV-LABECOL-000339, UFV-LABECOL-001876, UFV-LABECOL-011175, UFV-LABECOL-011180, UFV-LABECOL-011159, UFV-LABECOL-011179, UFV-LABECOL-011181, UFV-LABECOL-007465, UFV-LABECOL-011161, UFV-LABECOL-011169, UFV-LABECOL-011192, UFV-LABECOL-011184, UFV-LABECOL-010504, UFV-LABECOL-010841, UFV-LABECOL-010842; *Discothyrea* ufv-04: UFV-LABECOL-011174.

Disarticulated specimens have had their sclerites mounted either dry on card points or on mounted slides, embedded in polyvinyl alcohol medium (a variation of that presented in Downs, 1943), between a pair of round, 8 mm cover slides; all sclerites maintained in the same pin, included the mounted ones. Specimens which were already dry were soften prior dissection in a 50/50 hot water ethanol solution. If the specimens were in ethanol, then they were simply dissected straight away. Dissections were made with entomological pins on a flat piece of white EVA foam with a constantly maintained thin layer of ethanol. As sclerites were dissected, they were placed on the round cover glass with a droplet of the gel. After the removal of the wanted sclerites and their positioning on the cover glass, the second cover glass was placed on top of it, heated on a slide, and then a paper card was glued on the edge of the sandwiched cover glasses (see last images of UFV-LABECOL-000032 on Antweb as an example).

Most measurements were based on Hita-Garcia *et al*. (2019a) and are listed in Table 1.

Table 1. Abbreviations of measurements. A few measurements which are not based on Hita-Garcia *et al*. (2019a) were taken and contain a brief explanation of how they were acquired.

Measurements	Descriptions
AantL	Apical antennomere length: with the scape in its dorsal view, the length of the apical flagellomere, the club
AantW	Apical antennomere width: with the scape in its dorsal view, the width of the apical flagellomere, the club
EL	Eye length (queen only)
HFL	Hind femur length
HL	Head length
HW	Head width
LT3	Length of abdominal tergite 3
LT4	Length of abdominal tergite 4
MssctmW	Mesoscutum width (queen only): maximum width of the mesoscutum in dorsal view
MssctmL	Mesoscutum length (queen only): maximum length of mesoscutum in dorsal view
MstxL	Mesothorax length (queen only): the length of entire mesothorax in dorsal view, including mesoscutum, mesoscutellum and scutoscutelar suture
PeL	Petiole length
PeW	Petiole width
PW	Pronotum width
SL	Scape length
TL	Total length, the sum of HL, WL, PeL, LT3 and LT4
WL	Weber's length

I followed Keller (2011) for terminology. I also followed all terminology propositions of Hita-Garcia *et al*. (2019a). For the dealate mesosoma description, I followed Boudinot (2015).

Stereomicroscope images were made in a Zeiss Discovery V20 Stereo. Light microscope images were made in an Olympus CX40 with a Canon 1100D camera attached. Images acquired in both the stereomicroscope and the light microscope were stacked in Zerene software and edited in Gimp 2.10.12 (Kimball & Mattis, 1996).

RESULTS AND DISCUSSION

TAXONOMY

Discothyrea bobi CHAUL, SP. NOV.
(FIGURES 1, 2, 3 AND 5)

http://zoobank.org/urn:lsid:zoobank.org:pub:C1CED939-6D3F-409D-86CA-1F9D6E6743E7

Type Material

HOLOTYPE. Pinned worker. Brazil, MG, P. E. Serra do Brigadeiro, 1,600 m, winkler, jan. 2007 (Solar, R.) [CELC: UFV-LABECOL-000035]. PARATYPES. Five pinned workers and one pinned dealate queen with same data as holotype, except for altitude of sampling point [CELC: UFV-LABECOL-000036, queen, altitude 1,800 m; MZSP: UFV-LABECOL-000033, worker, altitude "1300-1800 m"; DZUP: UFV-LABECOL-000050, worker, altitude "1,300-1,800 m"; MPEG: UFV-LABECOL-010838, worker, altitude 1,600 m; INPA: UFV-LABECOL-000034, worker, altitude 1,500 m; JTLC: UFV-LABECOL-000114, worker].

Non-type material: one disarticulated worker, same data as holotype, except for altitude which is 1500 m [CELC: UFV-LABECOL-000032]

Note: the are no coordinates on the labels of the type series, however all were sampled in the area around the point 20.65 S, 42.40W, with a 1 km error (collector personal communication).

Measurements

Holotype (UFV-LABECOL-000035, worker): HL 0.525, HW 0.43, SL 0.27, AantL 0.23, AantW 0.145, PW 0.31, WL 0.55, HFL 0.32, PeW 0.17, PeL 0.12, LT3 0.4, LT4 0.29, TL 1.885.

Paratype (UFV-LABECOL-000050, worker): HL 0.54, HW 0.54, SL 0.3, AantL 0.24, AantW 0.16 PW 0.31, WL 0.585, HFL 0.35, PeW 0.17, PeL 0.12, LT3 0.4, LT4 0.32, TL 1.965.

Paratype (UFV-LABECOL-000034, worker): HL 0.53, HW 0.44, SL 0.29, AantL 0.23, AantW 0.15, PW 0.32, WL 0.57, HFL 0.33, PeW 0.17, PeL 0.12, LT3 0.42, LT4 0.31, TL 1.95.

Paratype (UFV-LABECOL-000033, worker): HL 0.54, HW 0.44, SL 0.3, AantL 0.24, AantW 0.155, PW 0.32, WL 0.58, HFL 0.35, PeW 0.175, PeL 0.12, LT3 0.4, LT4 0.33, TL 1.97.

Paratype (UFV-LABECOL-010838, worker): HL 0.54, HW 0.44, SL 0.3, AantL 0.24, AantW 0.155, PW 0.32, WL 0.58, HFL 0.35, PeW 0.17, PeL 0.12, LT3 0.42, LT4 0.31, TL 1.97.

Paratype (UFV-LABECOL-000036, queen): HL 0.52, HW 0.46, SL 0.3, EL, 0.125, AantL 0.24, AantW 0.15, WL 0.66, PW 0.35, MssctmW 0.38, MssctmL 0.39, MstxL 0.54, HFL 0.36, PeW 0.19, PeL 0.13, LT3 0.48, LT4 0.33, TL 2.12.

Geographic range

Minas Gerais state, Brazil.

Diagnosis

Antennal with seven antennomeres. Head widest point posterior to its midlength. Frontal lamella in full-face view thickened. Poorly formed corners between lateral and vertexal margins of the head in full-face view. Integument only mildly sculptured, moderately shiny in some parts. Propodeal spiracle very close to metapleural gland bulla,

not protruded in dorsal view. Angle of lateral to declivous propodeal faces round. Subpetiolar process low, triangular, with a sharp anterior angle. Relatively large species.

Description

Matching the diagnosis of the genus given in Hita-Garcia *et al*. (2019a). Worker. Head. In full-face view, head posterior margin without clear distinction of the lateral and vertexal margins, widest at two thirds of its length. Mandible masticatory margin edentate, except for the apical tooth and a subbasal denticle. Inner surface of mandible, close to the masticatory edge, with a row of thick specialized setae. Palpal formula 3,2. Basal maxillary palpomeres 1 and 2 fused, appearing as one, encrusted on apicomedial margin of stipe. Apical maxillary palpomere clavate and small. Labial basal palpomere tube-like, bent at base; labial apical palpomere inflated and covered on various fine setae (while other palpomeres are entirely or mostly glabrous). Labrum roughly rectangular, with a concave middle section of its distal margin, covered with small setae. In profile view of the head, frontal lamella a large and blunt lobe with a translucent anterior patch; in full-face view, the lamella is thickened, roughly oval. Eye small but distinct; in full face view situated anterior to midlength of head. Ommatidia difficult to count, appearing as partially fused to each other. Frontoclypeal structure strongly projected, having somewhat distinct lateral and anterior margins in full-face view; the lateral margin being oblique, and the anterior margin slightly convex. Antenna heptamerous, antennomeres 3 and 6 (flagellomere 1 and 4, respectively) having one tiny pit each, the former dorsolaterally on flagellomere and the latter ventromedially. Apical antennomere 1.3x longer than other flagellomeres and the pedicel combined. Cephalic capsule with weak sculpturing all through, except for hypostomal bridge, which is smooth. Mesosoma. In profile view, dorsum of mesosoma evenly arched, meeting the declivous margin in a round angle. In profile, declivous margin apical half flat, its basal half, the propodeal lobe, protruded, convex. Declivous propodeal surface flat, with poorly defined corners in relation to lateral and dorsal propodeal faces. Katepisternum delimited, somewhat raised from the level of other pleural sclerites around it. Mesosoma lateral margins in dorsal view gradually tapering posteriorly. Propodeal spiracle not protruding in dorsal view; in profile view considerably large and lowered, almost touching the metapleural gland bulla. Mesonotal spiracle about as large as propodeal, entirely covered by the pronotum but clearly distinguishable for being a raised area; metanotal spiracle a minute spot on lateral mesosoma surface, closer to propodeal spiracle than to mesonotal. Anterior tibia having the calcar, mesotibia without spur, and metatibia having a single, large pectinate spur. Mesosomal sculpturing poorly developed, with dorsum of mesosoma being less sculptured than the lateral surfaces; metapleuron and particularly the katepisternum having stronger sculpturing than surrounding sclerites. Metasoma. Subpetiolar process low, developed as a translucent carina which gradually widens anteriorly where it forms well-defined angle. In profile, anterior margin of petiole sinuous, and posterior margin oblique, both meeting to form a peak which is posteriorly displaced along the length of the petiole. The posterior margin ends in a short, flat portion which is the apex of a posterior petiole ring. Petiole spiracle facing ventrally and much smaller than propodeal spiracle. Posterior petiole tube or collar very wide, having about three quarters of petiole height, discounting subpetiolar process. Anterior portion of abdominal segment III forming a concave area where presclerites sit in the middle, the area is well-delimited ventrally by an V-shaped carina (the prora) and dorsally by a non-carinated folding of the tergite. In profile view, length of poststernite III slightly greater than a third of length of posttergite III; length of poststernite IV less than a tenth of length of posttergite IV. Area of abdominal segment III (A3) in profile appearing slightly greater than that of abdominal segment IV (A4). In dorsal view, A3 width approximately 2.4x greater than petiole width. Posttergite III posteromedially having a low tumulus. In the dorsal view

of A3 and A4, A3 only slightly wider than A4 and with a mild constriction between them. Sting small, thick at base and sharp apically. Petiole more sculptured than other parts of the body. A3 smooth laterally, punctate posteromedially. A4 the shiniest sclerite on body, smooth all through. Pilosity and coloration. Body covered with small, filiform, whitish pubescence, only last abdominal segments having suberect small setae, starting on the edge of A4 but denser only on A7, especially on sternite (hypopygium). Body having light brown, ferruginous coloration, with antennae and legs being slightly lighter.

Queen (dealate). Agreeing with the description of the worker except in the following. Head with three small ocelli and large compound eyes. Minute setae in between ommatidia of compound eye. Mesosoma drastically different from workers, much larger and typical of winged queens. Dorsally, mesoscutum very large; scutoscutellar suture poorly marked, therefore weak delimitation of mesoscutellum anteriorly and of axillae posteriorly. Propodeum in dorsal view forming two round projections posterad. In profile view, propodeum dorsal margin short and meeting the declivous margin in a round angle. Propodeum posterior surface concave in dorsal view. Oblique mesopleural suture and mesometapleural suture well-marked. Metapleuropropodeal suture absent. Metasoma larger than in workers. Subpetiolar process having a denticle anteriorly rather than merely an angle as in workers.

Male

Unknown.

Etymology

The species is named after ecologist, myrmecologist, and friend Ricardo Solar, also known as Bob, who has sampled the entire type series.

Comments

With the description of *D. bobi*, extant New World species of *Discothyrea* are now ten. The variation observed in some of these species, which has been reported in Sosa-Calvo & Longino (2008), but also seen by the author (either by examining Antweb images or physical specimens), indicates that, in fact, the genus has more species to be described for the Neotropics, some of which are currently under the name of a given species (*e.g*. *D. sexarticulata*) and others are entirely new (*Discothyrea* ufv-04, UFV-LABECOL-011174). Therefore, identification will often be confusing and only a thorough revision can solve this problem. Considering the small amount of material available to me and the apparently unreported diversity of the genus in the New World, the building of an effective identification key for this fauna would be impossible. Moreover, the lack of images of some species (especially their types) and species described based on winged females further hamper the task of making a key. An effective key to the New World species would be the result of a much more in-depth investigation of the genus than this work represents. However, to ease identification for the time being, I highlight important traits separating *D. bobi* from each New World species as they are currently understood and presented a key to the three species occurring in the Atlantic Rainforest.

Key to the *Discothyrea* workers and queens of the Atlantic Rainforest.

1 In full-face view, frontal lamella thick. Propodeal spiracle distant from metapleural gland bulla by less than its own width. 2

1' In full-face view, frontal lamella thin. Propodeal spiracle distant from metapleural gland bulla by more than its own width. ..... *D. sexarticulata* complex

2 Opaque integument. Propodeal corners well-defined .....*D. neotropica*

2' Smooth integument with shiny portions, especially A4. Propodeal corners poorly marked.....*D. bobi*

Figure 1. *Discothyrea bobi*, holotype worker (UFV-LABECOL-000035, images A-E) and worker (UFV-LABECOL-000032, image F): A) head in full-face view; B) profile view of the body; C) dorsal view of the body; D) detail of antenna; E) mesosoma and petiole profile; F) anterior head profile view. Abbreviations: flm = frontal lamella; fl1-5 = flagellomeres 1 to 5; mgb = metapleural gland bulla; mssp = mesonotal spiracle; mtsp = metanotal spiracle; pe = pedicel; petsp = petiole spiracle; propsp = propodeal spiracle; sc = scape (only apical portion appearing). Scale bars are 0.1 in A, D, E and F and 0.2 in B and C.

Figure 2. *Discothyrea bobi* paratype queen (UFV-LABECOL-000036): A) head in full-face view; B) profile view of body; C) profile view of mesosoma; D) dorsal view of mesosoma. Scale bars are 0.1 mm in A, C and D and 0.2 mm in B.

Note: *D. horni* appears to be erroneously registered for the Atlantic Rainforest, state of Rio de Janeiro, in Antmaps.org (Janicki *et al*., 2016), as it is absent in the list of sampled ants of the work shown as the source of the register in the website (Nascimento Santos *et al*., 2019).

D. bobi can be differentiated from other New World species by the following traits:

- *D. testacea* Roger, 1863 (USA): nine antennomeres; integument entirely opaque; well-defined lateral and vertexal margins of the head in full-face view;

propodeal spiracle separated from the metapleural gland bulla by more than its diameter; well-defined propodeal corners; subpetiolar process long; petiole node posterior margin in profile high (Roger, 1863);

- *D. neotropica* Bruch, 1919 (Argentina, Paraguay, and Brazil): integument entirely opaque; lateral and vertexal margins of the head in full-face view relatively well-defined, head less round (worker) (see additional information on the putative close relationship between *D. neotropica* and *D. bobi* and comments on biogeography below) (Bruch, 1919);
- *D. horni* Menozzi, 1927 (Central America and northern South America): nine or eight antennal segments; integument entirely opaque; well-defined propodeal corners (Menozzi, 1927);
- *D. denticulata* Weber, 1939 (Central America and South America): eight or seven antennal segments; frontal lamella with a distinct tooth; propodeal spiracle separated from the metapleural gland bulla by more than its diameter; propodeal spiracle in dorsal view protruded; subpetiolar process long (Weber, 1939);
- *D. soesilae* Makhan, 2007 (Suriname): appears to have a pointy triangular frontal lamella (see comments below on identification of this species) ;
- *D. humilis* Weber, 1939 (Central America, northern South America, Lesser Antilles): well-defined propodeal corners; posterior margin of petiole node in profile high (Weber, 1939);
- *D. icta* Weber, 1939 (Trinidad, only queen known): projecting propodeal corners; petiole sternite process long; overall smaller (Weber, 1939);
- *D. isthmica* Weber, 1940 (Central America and northern South America): nine antennal segments; overall smaller (Weber, 1940);
- *D. sexarticulata* Borgmeier, 1954 (across most South America and Central America, probably a complex of various species): six antennal segments; integument entirely opaque, densely sculptured; propodeal spiracle separated from the metapleural gland bulla by more than its diameter; subpetiolar process long (Borgmeier, 1954).

D. bobi is most similar to *D. neotropica*, a species described based on a dealate queen from Argentina. Important traits they share are: a 3, 2 palpal formula; the subrectangular frontoclypeal structure (as opposed to semicircular); a thickened frontal lamella in full-face view; the propodeal spiracle very close to the metapleural gland bulla; the overall shape of the petiole, without a long subpetiolar process. There are imaged records of workers of *D. neotropica* from southern Paraguay (Itapúa, CASENT0281861) and southern and southeastern Brazil (Santa Catarina and Espírito Santo: FMNHINS0000050586, UFV-LABECOL-000067, UFV-LABECOL-008587) and these likely represent *D. neotropica* itself. Records from northern Atlantic Forest and the Amazon should be re-evaluated as these registers could either be of *D. neotropica*, or *D. bobi* which were previously identified as *D. neotropica.* All records from Argentina, including the type locality, are from queens (Bruch, 1919; Sosa-Calvo & Longino, 2008; Arcusa & Cicchino, 2017). The queen caste of *D. neotropica* appears to be larger than *D. bobi* and having an opaque integument, as opposed to a weak sculptured and partially polished, as seen in *D. bobi*. There are no images of the type of *D. neotropica* except for Bruch's original drawings. Arcusa & Cicchino (2017) photographed the queen they reported for Buenos Aires province, but the images are unsatisfactory to be compared to *D. bobi* queen and do not reveal further differences other than size and sculpturing. *D. neotropica* differs from *D. bobi* by having the integument entirely opaque; the lateral and vertexal margins of the head in full-face view being relatively well-defined (head appearing less round than in *D. bobi*); angle between propodeal dorsal and declivous margins in profile more defined; denser pubescence on body (Figure 4). Besides of those differences, the following minor differences (Figure 5) must be of importance to separate both species, although needs further confirmation in specimens of *D. neotropica* to verify for intraspecific variation: in dorsal view, posterior end of frontal lamella at about the same level of posteriormost point of the toruli as opposed to

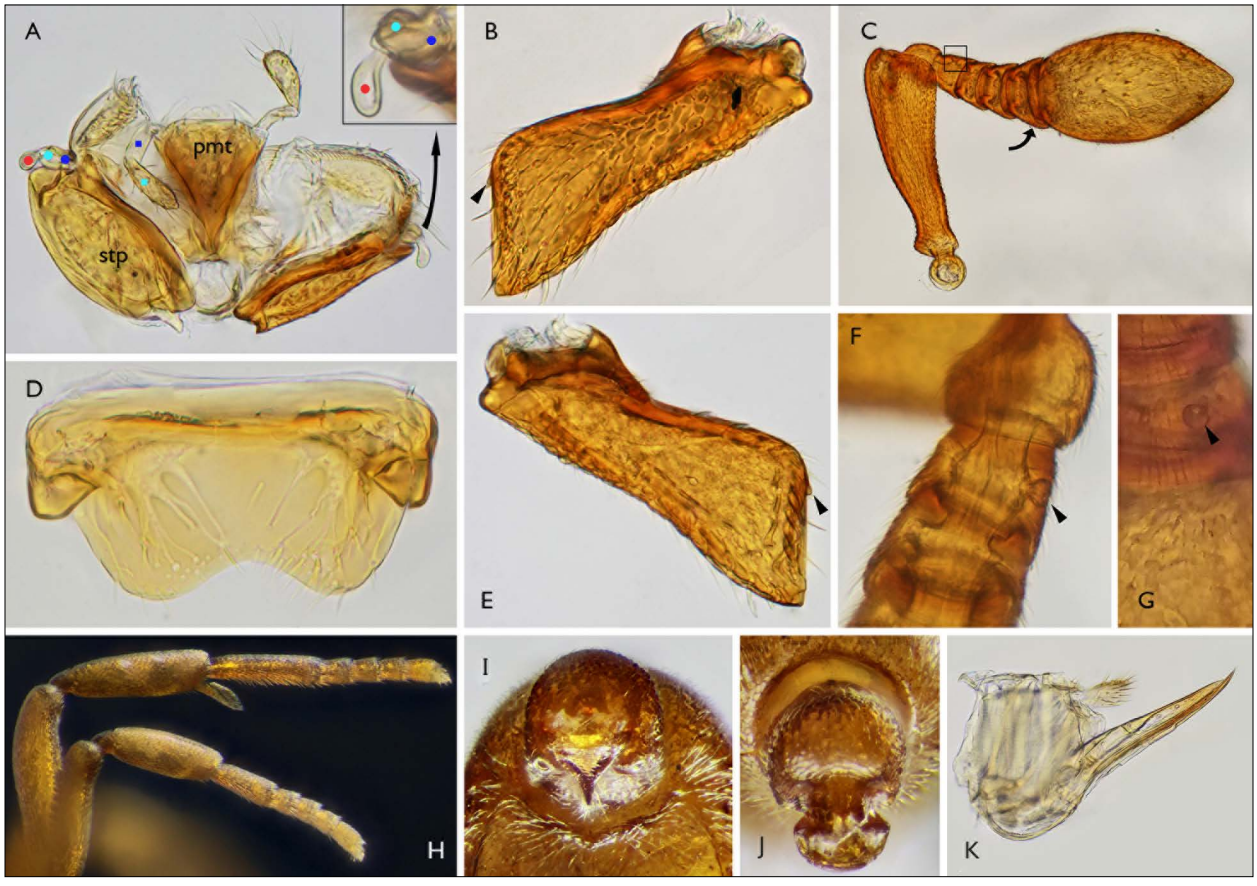

Figure 3. Body parts of dissected specimens of *D. bobi*: A) labiomaxillary complex, right corner having a zoomed, ventral view of left maxillary palps; B and E) dorsal and ventral views of left mandible, respectively, arrowheads evidencing subbasal denticle; C, F, and G) C is the dorsal view of the antenna with regions of the first and penultimate flagellomeres indicated, the square in C is the region zoomed in F, the curved seta in C indicates the ventral region seen in detail in G, arrowheads indicate the pits in F and G; D) labrum; H) mid and hind legs, showing spur present on the latter and absent on the former; I and J) anteroventral and anterodorsal views, respectively, of anterior abdominal III region (petiole still attached), evidencing the shape of the prora in I and the dorsal strong folding of the tergite in J; K) sting apparatus. Abbreviations: pmt = prementum; stp = stipe. Colored circles and squares in A represent palpomeres, see explanation in Figure 6.

surpassing this level as in *D. bobi*; smaller eyes (Figures 5A and 5B); frontal lamella in profile view having different shape than in *D. bobi,* the latter having a deeper notch anteriorly in the lamella (Figures 5E and 5F); propodeal spiracle less developed than in *D. bobi* (Figures 5C and 5D); petiole spiracle laterally rather than ventrolaterally oriented; shape of subpetiolar process differing, in *D. neotropica* being round anteriorly (Figures 5G and 5H); abdominal segment IV in profile appearing relatively more developed in *D. neotropica* (Figures 4B and 1B), except for *D. neotropica* specimen UFV-LABECOL-008587. Moreover, total length of the body usually smaller than in *D. bobi* (although the reverse appears to be the case between the queen caste). Careful should be taken as many measurements were acquired from images or, in the case of the Argentinian queens, from specimens not correctly positioned.

Sosa-Calvo & Longino (2008) reported a *D*. nr. *neotropica* from Colombia and Venezuela with nine antennal segments. Specimen CASENT0178698, which has an apparent antennomere count of eight and is currently identified as *D. horni*, also appears to be a related species of *D. neotropica* and *D. bobi*.

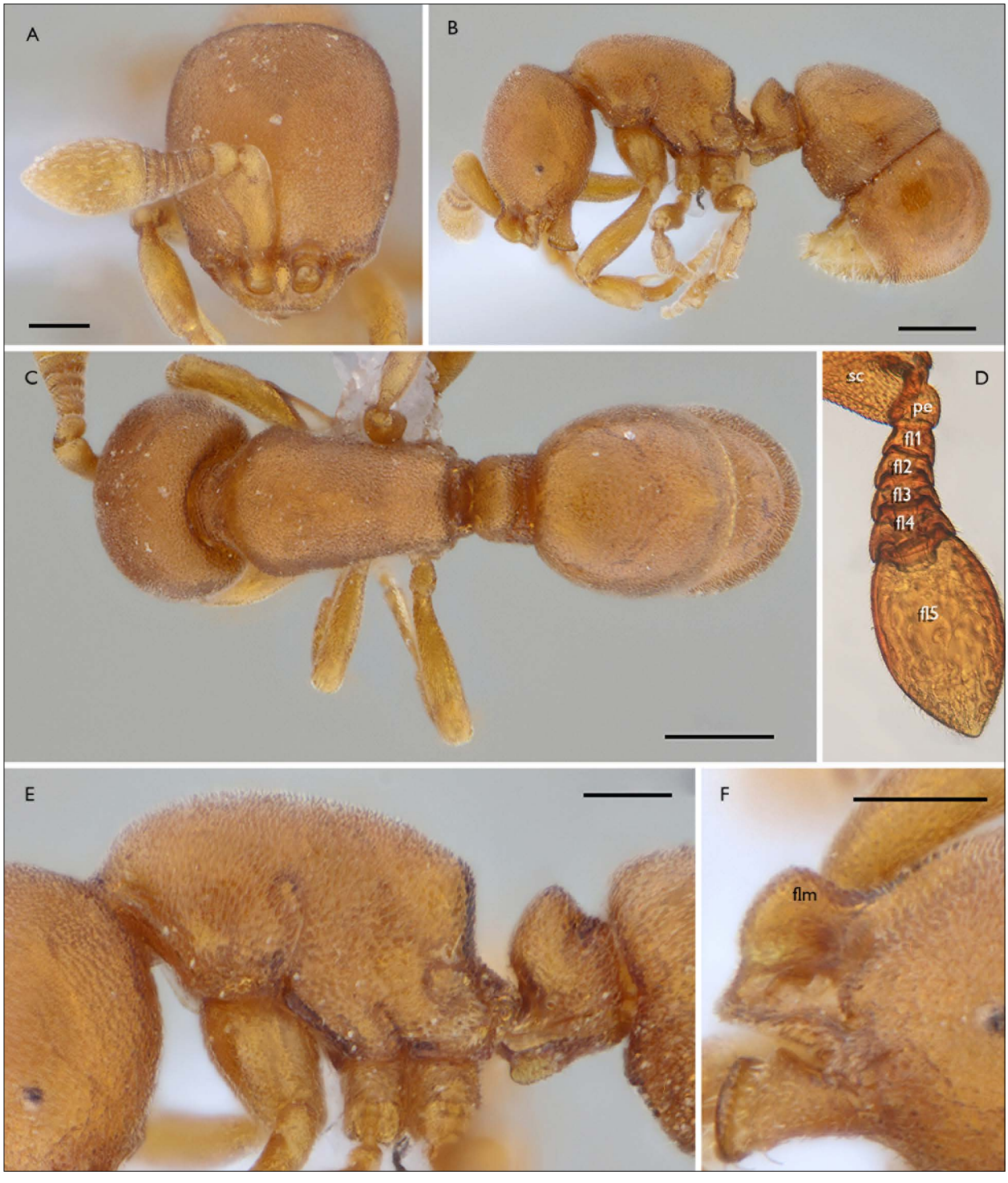

Figure 4. *Discothyrea neotropica*, worker (UFV-LABECOL-000067): A) head in full-face view; B) profile view of the body; C) dorsal view of the body; D) detail of antenna; E) mesosoma and petiole profile; F) anterior head profile view. Abbreviations as in Figure 1. Scale bars are 0.1 mm in A, E and F and 0.2 mm in B and C.

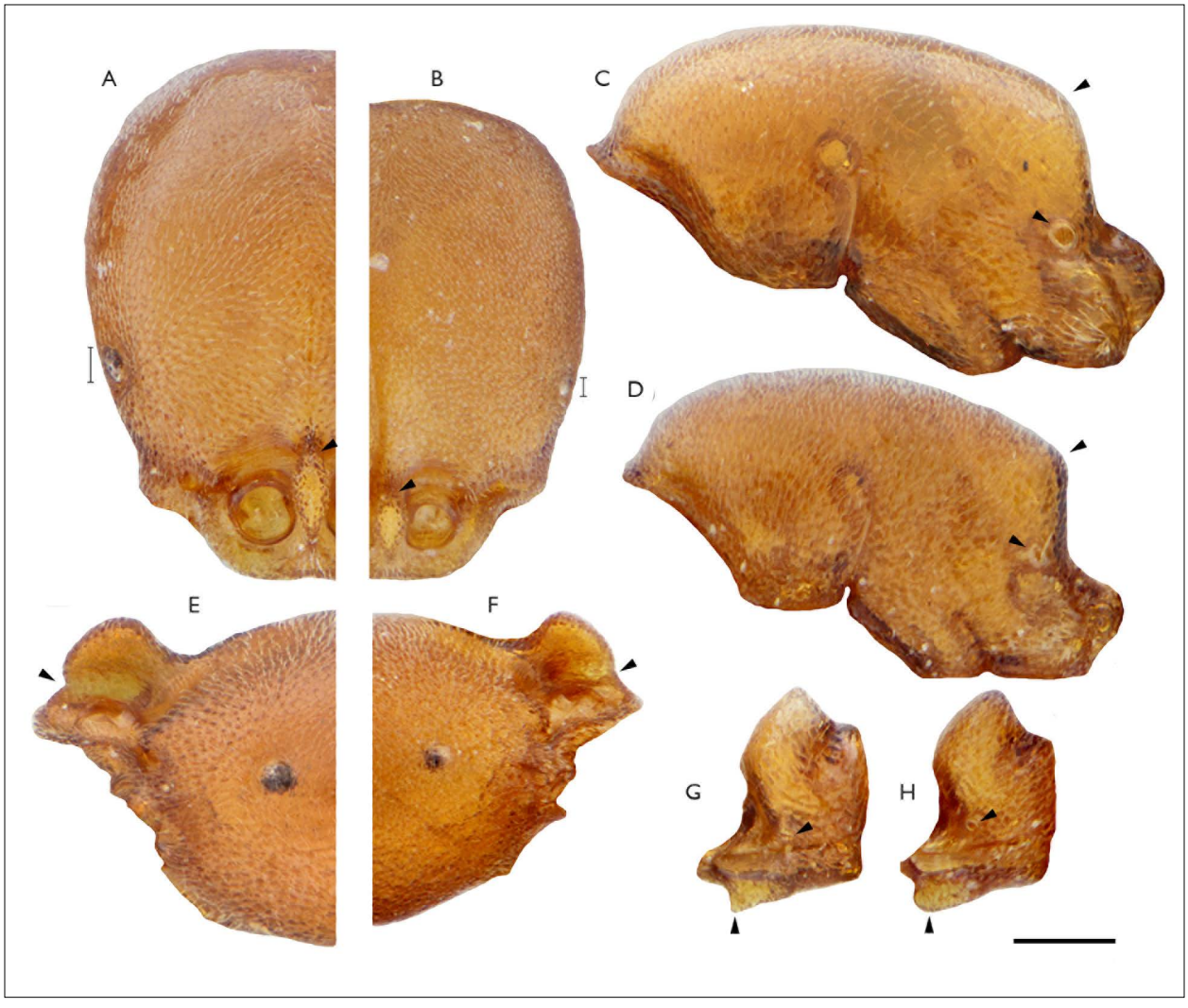

Figure 5. Comparison between some sclerites of the workers of *D. bobi* (A, C, E, G) and *D. neotropica* (B, D, F, H): A and B) half of the head capsule in full face view; C and D) mesosoma in profile; E and F) anterior head in profile evidencing frontal lamella; G and H) petiole in lateral view. Scale bar 0.1 mm, for all images. Arrowheads indicate important features (see in text).

The description of *D. soesilae* Makhan is unsatisfactory and proper images and a redescription are necessary. The species appears to have a seven segmented antennae (Figure 3, right antennae, Makhan, 2007) as some Neotropical species including *D. bobi*. The frontal lamella though, appears to be thin and with at least some kind of sharp projection (Figures 2 and 3, Makhan, 2007), which would differentiate it from *D. bobi*. Additionally, its type locality, Suriname, also favors the interpretation it must not be conspecific to *D. bobi*, as species occurring in both the Atlantic Forest and Amazonian (*e.g*. *D. sexarticulata*, *D. neotropica*) may well be different species within a species complex, as already mentioned.

The fossil *†D. gigas* De Andrade, 1998 and *†D. maya* De Andrade, 1998 are unlikely closely related to *D. bobi* considering the geographical and temporal gap between them (De Andrade, 1998). *†D. maya* cannot be compared to any Neotropical species at the moment since it was

described based on a male from Mexican amber and the males of Neotropical extant *Discothyrea* are poorly known and have not been associated to the females. It will take time until male specimens of extant species accumulate and are properly matched to their females in a way to allow phylogenetic proximity between living lineages and *†D. maya* to be deduced. *†D. gigas*, considered the largest Neotropical species, is not, in fact, much larger than than *D. bobi*. *†D. gigas* can be separated from *D. bobi* by its antennal count of nine.

Finally, an ecological/geographic fact reinforces the argument for the isolation of *D. bobi* as a species. The type locality is a mountain range in southeastern Brazil, the Serra do Brigadeiro, located 40 km from the Viçosa municipality. The latter has a well-known ant fauna due to continuous sampling efforts in the past 25 years, while the former has been only occasionally explored. Nevertheless, the forests of Serra do Brigadeiro have yielded curious species and morphospecies never found in the region of Viçosa. Some of these represent new species awaiting formal description (*e.g*. morphospecies in the *louisianae* group of *Strumigenys* or in the group of *Brachymyrmex* with tumuliform metathoracic spiracles). *D. bobi* type series was sampled in between 1300 and 1800 meters. It has never been sampled in Viçosa (average height 700 m). It is a possibility that the mountains of Serra do Brigadeiro, still poorly explored myrmecologically, might be driving speciation of various lineages in the Atlantic Forest. Indeed, a pattern of geographically restricted species on montane regions in the Afrotropical fauna was recently discovered (Hita-Garcia *et al*., 2019a).

MORPHOLOGICAL CONSIDERATIONS

The examination of various specimens, images, texts and even the 3D models of specimens, which luckily are plenty for *Discothyrea* (Hita-Garcia *et al*., 2019a), made apparent a couple of morphological features which are worth mentioning and might be of importance for future studies on the genus and the subfamily. They are the palpal formula variation and the presence of antennal pits and are discussed below. I also briefly comment on the use of antennomere count in *Discothyrea*.

ANTENNOMERE COUNT

Various authors have questioned the validity of the antennal count as a useful feature in *Discothyrea* taxonomy (Brown Jr., 1958; Sosa-Calvo & Longino, 2008; Hita-Garcia *et al*., 2019a). Hita-Garcia *et al*. (2019a) demonstrated that apparent antennal count, the count made by external examination, may differ from the true antennal count, obtained through dissection (virtual or physical). Due to the difficulties in establishing the true antennal count, the authors recommend it to be avoided in the identification of species (its use in keys or diagnosis). In this work, I used a high-resolution stereomicroscope (160 x magnification) to count antennomeres in dry, pinned specimens, but also chose a small subset of specimens and made dissections of the antennae and mouthparts to be examined on slides under the light microscope. Most of the times, the count I made on the slide matched that previously made on the stereomicroscope. Among the specimens available to me, the sole exception was a specimen which had an apparent antennal count of six, but a true antennomere count of eight. This specimen (UFV-LABECOL-010504) is identified as *D. sexarticulata,* but it might well belong to *D. denticulata* or other species. The differences in the count and/or in the shape of the antennal segments within a species should be seen with skepticism, since species in the New World appear to be poorly defined, and what is called intraspecific variation might well represent differences between closely related species, which are not currently recognized as such. The 3D models generated from micro-CT scans (Hita-Garcia *et al*., 2019a) are probably the most accurate we can currently get in order to ascertain the true antennal count, however the equipment to generate those models is expensive, not available to most laboratories working on ant identification or ant taxonomy. On the other hand, light microscopes

are often available and the preparation of slides, as those made in this study (*e.g*. UFV-LABECOL-010504, UFV-LABECOL-010841, UFV-LABECOL-010842), are a simple, relatively fast, and cheap technique.

I agree with Hita-Garcia *et al*. (2019a) in that the dissections are necessary to establish the true antennal count. Contrary to those authors, however, I encourage the preparation of slides in an attempt to further verify whether the count is indeed misleading in the identification process. If the true count, based on dissected specimens, is available for all species (including many populations within a species) then, we will be able to better understand the importance of the character. Such a state of knowledge will be much easily achieved if not only taxonomists working on *Discothyrea* are extracting this data, but also other myrmecologists (*e.g*. ecologists, ethologists) which by any means are studying species or populations of the genus and need identifications. In the same manner, I strongly encourage that palpal formula, discussed below, deserves more attention, as it varies a lot within the genus, and to score it for each species might help in better understand species limits or in the delimitation of species groups within the genus. In fact, the dissection of one antenna, the labiomaxillary complex, and one mandible, showed to be ideal to get a complete sense of the morphology of any given *Discothyrea* specimen (*e.g*. UFV-LABECOL-010842).

PALPAL FORMULA

Determining the palpal count of *Discothyrea* is challenging. The very small size of these ants make it almost impossible to be count *in situ*. Fortunately, dissected mouthparts prepared on slides can be clearly seen under light microscopes in 200x or 400x magnification. The fused zigzag maxillary palpomeres 1 and 2, a synapomorphy of *Discothyrea* (Keller, 2011), can be interpreted in SEM images, however are hardly revealed by light microscopy. They are seen as one palpomere encrusted on the apicomedial margin of the stipe. Despite that, this "first" maxillary palpomere, as seen in light microscopy, do look strange, sometimes square-shaped, sometimes bent. I followed Keller and considered this palpomere as number 1 and 2 in the counts here presented, therefore *D. bobi* palpal formula is 3,2 and not 2,2 as the observer is inclined to interpret (Figure 3A). Hita-Garcia *et al*. (2019a) called attention to the fact that the variation in the palpal formula showed in Sosa-Calvo & Longino (2008) could be due to a counting error and not true variation within the genus. The latter authors mention the Neotropical species can have 5,4; 4,4; 4,3; 3,4; or 1,3 palpal formula, with *D. sexarticulata* having the formula 1,3 (Borgmeier, 1954). At least in the case of *D. sexarticulata*, a species I was able to dissect specimens from three very different localities, I agree in that the palpal formula was wrongly interpreted.

Processes of reduction and loss of palpomeres appear to have happened in *Discothyrea* lineages, with some having a palpal count of 6,4, as in *D. oculata* and in *D. clavicorni*s (Figures 6A-6C, with *D. clavicornis* showing strong reduction in the apical maxillary and labial palpomeres), while other lineages presenting various counts (Figures 3A and 6D-6F and Table 2). In the former type, the first and second maxillary palpomeres are fused in a zigzag arrangement and the ones apical to them are the "free" palpomeres, not tightly attached to apicomedial margin of stipe as the first two, and completely exposed when the labrum is closed. The labial palpomeres are composed of a tube-like basal palpomere which is bent at base, glabrous and covered by the labrum when the latter is closed. The bent allows it to lay tightly on the prementum. The palpomeres apicad this first one, which might be up to three, are covered on fine setae and are not concealed when the labrum is closed; various reductions were observed in these (Table 2). In the Neotropical species examined, the palpal formula was always very low, either 3,2 in *D. bobi* and *D. neotropica* or 2,2 in *D. horni* and *D. sexarticulata*.

Accordingly, the palpal formula seen in Borgmeier's drawings should be 2,2 and not 1, 3 as reported. The maxillary palpomere interpreted by Borgmeier as one

Table 2. Palpal formula of various species of *Discothyrea*. Abbreviations: PMX = maxillary palpomere; PLB = labial palpomere; BR = biogeographical region (AUS = Australasian; NEO = Neotropical; NEA = Nearctic; AFR = Afrotropical; ORI = Oriental-Indomalayan; PAL = Palearctic; PAC = Pacific). Asterisks mean guessed values, prone to error (see text for explanation). Numbers over species' names: 1 = actual specimen was examined; 2 = based on images from Antweb; 3 = based on SEM images from Antweb; 4 = based on 3D models from the Arilab available at Sketchfab.

Species	PMX	PLB	BR	Species	PMX	PLB	BR
D. bobi[1]	3	2	NEO	*D. patrizii*[4]	3*	3 or 4*	AFR
D. neotropica[1]	3	2	NEO	*D. aisnetu*[4]	3*	3*	AFR
D. sexarticulata[1]	2	2	NEO	*D. athene*[4]	3*	3*	AFR
D. horni [1]	2	2	NEO	*D. gaia*[4]		3 or 4*	AFR
D. testacea[3]	2*	2*	NEA	*D. gryphon*[4]	2*	2*	AFR
D. clavicornis[1]	6	4	AUS	*D. michelae*[4]	3*	3*	AFR
D. kamiteta[4]	6*	4*	PAL	*D. wakanda*[4]	3*	2*	AFR
D. banna[4]	6*	4*	ORI	*D. kalypso*[4]	3 or 4*	3*	AFR
D. diana[4]	6*	4*	ORI	*D. dryad*[4]	3*	2*	AFR
D. mixta[4]	6*	4*	AFR	*D. penthos*[4]	3*	3*	AFR
D. oculata[3]	6	4	AFR	*D. poweri*[4]	5 or 6*	3 or 4*	AFR
D. damato[4]	3*	2*	AFR	*D.* SM01[4]	3*	3*	PAC
D. patrizii[4]	3*	3 or 4*	AFR				

(his figure 1.9), should be interpreted as the fused, zigzag 1+2 palpomeres, therefore making the maxillary count to be two. As for the labial palpomeres, Borgmeier considered them to be three. I suspect Borgmeier wrongly scored the posterolateral prementum projection as a tiny palpomere. In his interpretation, the second labial palpomere is the one which is tube-like. In all specimens I examined the tube-like palpomere was the basal and in Keller's image of *D. oculata* (ANTWEB1008518), this is clearly the case. So, Borgmeier's figure 1.8 can be interpreted as depicting two and not three labial palpomeres, the basal being the cylindrical palpomere, typical of all *Discothyrea*, the apical being the swollen exposed (when labrum is closed) palpomere (Figure 6F).

Dissected specimens, SEM images on Antweb, and 3D models of microCT scans allowed palpal counts of many *Discothyrea* to be ascertained or at least guessed. In all 3D models, most of Afrotropical species, the palps could be seen, however, as most specimens have a closed mouthparts and the technique does not precisely reveal the palpomeres (constrictions between the palpomeres are not always clear), the palpal formula in these were never surely scored.

ANTENNAL PITS IN *DISCOTHYREA* AND OTHER PROCERATIINAE

Antennal pits have been reported for *Probolomyrmex* (Oliveira & Feitosa, 2019). During this study, as I examined various specimens and images to compare them against *D. bobi*, the same structure appeared to be present in *Discothyrea*, although apparently slightly larger and less numerous than in *Probolomyrmex*. At first, only a pit on the dorsolateral surface of the first flagellomere (third antennomere, Figure 3F) was noticed. The pit is not easily seen under the stereomicroscope, but can be confidently confirmed in mounted antennae under the light microscope (or in SEM, as in Oliveira & Feitosa, 2019, or, for example, in the *D. testacea* specimen ANTWEB1008519). It appears to be the opening of a channel, since a tube-like structure can sometimes be seen by transparency connecting it to the main internal

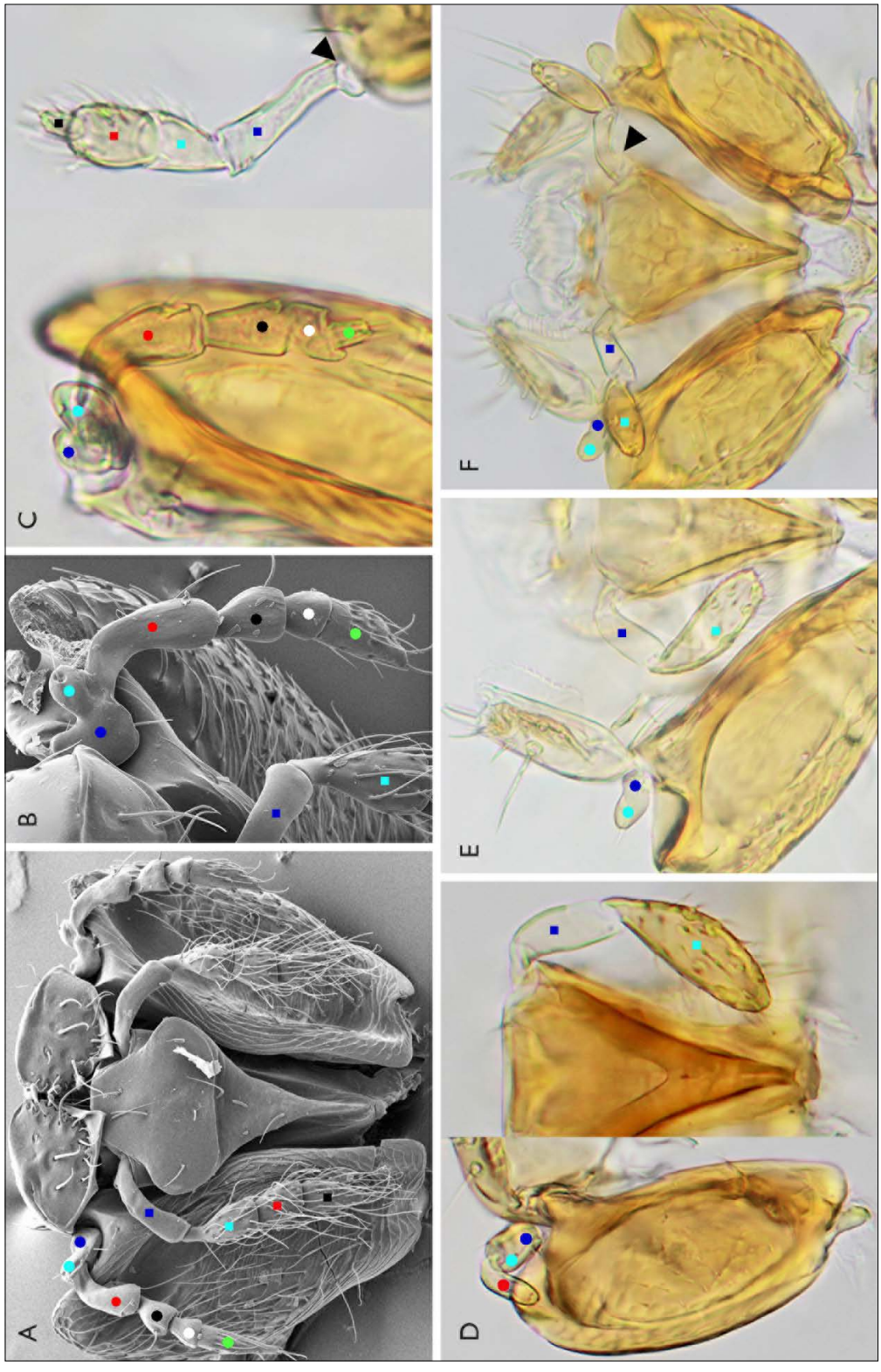

Figure 6. Labiomaxillary complexes of some *Discothyrea*: A-B) SEM images of *D. oculata* labiomaxillary complex (ANTWEB1008518, available at Antweb, images by Roberto Keller); C) *D. clavicornis* (UFV-LABECOL-011021), also showing a 6,4 palpal formula; D) *D. neotropica* (UFV-LABECOL-000067) with a 3,2 palpal formula; E) *D. horni* (UFV-LABECOL-009622), 2,2 palpal formula; F) *D. sexarticulata* (UFV-LABECOL-010770), 2,2 palpal formula. Circles and squares represent the maxillary and labial palpomeres, respectively. Dark blue is the first palpomere; light blue is the second; red is the third; black is the fourth; white is the fifth; and green is the sixth. Arrowheads in C and F indicate basal bent of first palpomere (not a division between two palpomeres).

chamber of the flagellomere. The structure can be seen in Sosa-Calvo & Longino (2008, figures 4a and 4c), although there is no mention about it in the text. Interestingly, the 3D surface models of *Discothyrea*, recently published for Afrotropical species (Hita-Garcia *et al*., 2019a, 2019b, also available at OIST-Economolab, s. d.a), reveal the pits very clearly in all species, either on the same flagellomere and same position or on different flagellomeres and in different positions. Indeed, after finding additional pits in the 3D models, I reexamined the Neotropical specimens paying special attention to the ventral and medial surfaces of the antenna. The pit on the penultimate flagellomere was also confirmed in all Neotropical species (Figure 3G). One common pattern among some Afrotropical species is the combination of one pit on the lateral penultimate flagellomere and one pit on the lateral antepenultimate flagellomere (Figures 7E and 7F). Another pattern is the already mentioned combination of one dorsolateral pit on the first flagellomere and one ventromedial pit on the penultimate flagellomere, this one being the most common pattern, present in all Neotropical and in most Afrotropical species examined (Figures 7A-7D). Other combinations were seen, but were uncommon (Table 3). One 3D model of a *Proceratium* head capsule (available at OIST-Economolab, s. d.b) also show pits laterally on the first to fourth flagellomeres, one on each. I examined in the stereomicroscope a few specimens of two Neotropical species, *Proceratium* sp. (*micrommatum* group) and *P. brasiliense*, as well as various images on Antweb and could not find pits. Apart from the pits, some specimens of *Discothyrea* possessed a peculiar hardened substance, probably a secretion, which was recurrently seen ventrally in between the last and penultimate flagellomeres. By judging from a few specimens which have the substance in less quantity so that it does not block a lot of the ventral view of the flagellomeres, it seems more probable it leaks from the division between the apical and the penultimate flagellomeres, rather than from the antennal pits. The presence of this substance was also scored (Table 3, Figure 7D, blue arrowhead, can be also seen in specimens CASENT0281862 and CASENT0374613, on Antweb, for example). The presence of the pits in various *Discothyrea* species from different biogeographic region, in one *Proceratium*, as well as in various *Probolomyrmex* species, as firstly reported, indicates the antennal pit is, at least, a Proceratiinae trait and it remains to be determined if the structure is absent outside that subfamily. The antennal pits occurrence and their pattern on the flagellomeres might be of phylogenetic importance.

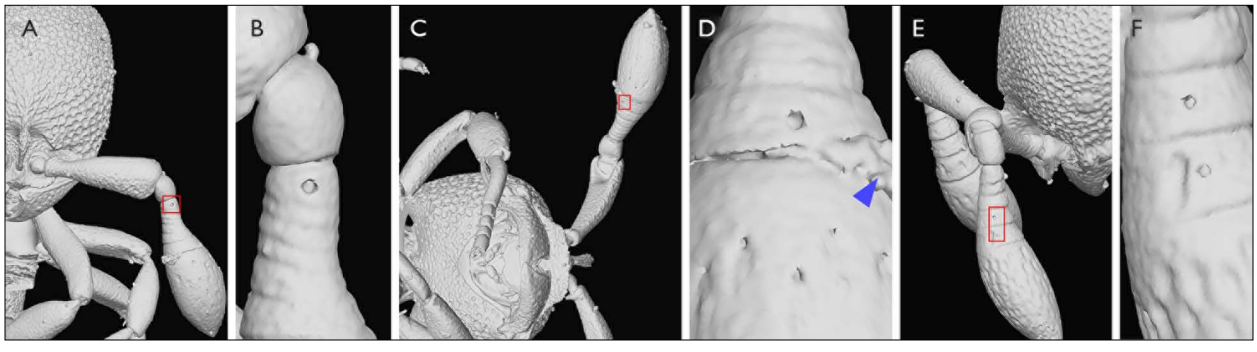

Figure 7. Antennal pits revealed in 3D models of some Afrotropical *Discothyrea*: A and B) *D. michelae*, dorsal view of the antenna and zoom in first flagellomere; C and D) *D. michelae*, ventral view of the antenna and zoom in penultimate flagellomere; E and F) *D. gaia*, lateral view of the antenna and zoom in penultimate and antepenultimate flagellomeres. Zoomed areas of B, D and F highlighted in A, C and E by red squares. The 3D models of the species are available at the Dryad Digital Repository (Hita-Garcia *et al*., 2019a, 2019b), and are also available at the Sketchfab page of the the Economo Lab, from the Okinawa Institute of Science and Technology (OIST-Economolab, s. d.a).

Table 3. Antennal pits in *Discothyrea* species. Abbreviations as follows: A = pit laterally or dorsolaterally on first flagellomere; B = pit ventromedially on penultimate flagellomere; C = pit laterally on antepenultimate flagellomere; D = pit laterally on penultimate flagellomere; E = other pattern of pits; F = secretion (normally ventrally between last and penultimate flagellomeres); BR = biogeographical region (AUS = Australasian; NEO = Neotropical; NEA = Nearctic; AFR = Afrotropical; ORI = Oriental-Indomalayan; PAL = Palearctic; PAC = Pacific). Number over species' names as explained in Table 2. Unchecked boxes must be seen with caution, especially for the specimens not physically examined, as they do not necessarily mean absence.

Species	A	B	C	D	E	F	BR	Species	A	B	C	D	E	F	BR
D. bobi[1]	x	x					NEO	*D. aisnetu*[4]			x	x			AFR
D. neotropica[1]	x	x					NEO	*D. athene*[4]			x	x			AFR
D. sexarticulata[1]	x	x				x	NEO	*D. maia*[4]	x	x					AFR
D. pe01[2]	x					x	NEO	*D. gryphon*[4]	x	x					AFR
D. testacea[3]	x						NEA	*D. schulzei*[4]	x	x					AFR
D. clavicornis[1]	x	x					AUS	*D. michelae*[4]	x	x				x	AFR
D. kamiteta[4]	x				x		PAL	*D. wakanda*[4]	x	x					AFR
D. banna[4]	x	x				x	ORI	*D. kalypso*[4]	x	x					AFR
D. diana[4]	x	x			x		ORI	*D. hawkesi*[4]	x	x					AFR
D. mixta[4]	x	x					AFR	*D. dryad*[4]	x						AFR
D. oculata[3]	x						AFR	*D. penthos*[4]	x	x					AFR
D. damato[4]	x	x					AFR	*D. venus*[4]	x	x					AFR
D. traegaordhi[4]			x	x	x		AFR	*D. poweri*[4]					x		AFR
D. patrizii[4]			x	x			AFR	*D.* SM01[4]	x	x				x	PAC
D. gaia[4]			x	x			AFR								

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