

The mesosomal anatomy of *Myrmecia nigrocincta* workers and evolutionary transformations in Formicidae (Hymenoptera)

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Abstract. The mesosomal skeletomuscular system of workers of *Myrmecia nigrocincta* was examined. A broad spectrum of methods was used, including micro-computed tomography combined with computer-based 3D reconstruction. An optimized combination of advanced techniques not only accelerates the acquisition of high quality anatomical data, but also facilitates a very detailed documentation and visualization. This includes fine surface details, complex configurations of sclerites, and also internal soft parts, for instance muscles with their precise insertion sites. Myrmeciinae have arguably retained a number of plesiomorphic mesosomal features, even though recent molecular phylogenies do not place them close to the root of ants. Our mapping analyses based on previous morphological studies and recent phylogenies revealed few mesosomal apomorphies linking formicid subgroups. Only five apomorphies were retrieved for the family, and interestingly three of them are missing in Myrmeciinae. Nevertheless, it is apparent that profound mesosomal transformations took place in the early evolution of ants, especially in the flightless workers. The modified mesosoma is characterized by four character complexes: **a)** an enlarged prothorax with elongate procoxae, a large plate-like pronotum, strongly developed muscles of the forelegs and especially of the neck region; **b)** highly differentiated legs with complex cleaning and attachment devices; **c)** a reduced flight apparatus with greatly simplified pterothoracic musculature and mechanically reinforced exoskeleton and **d)** strongly developed specialized muscles inserted on the base of the metasoma. Structural modifications of the prothorax and neck region allow ant workers to transport items efficiently with a highly movable head with strongly developed cervical muscles. Their differentiated legs enable them to move efficiently on various surfaces and to maintain their complex apparatus of sensilla. The mechanically reinforced mesosoma provides protection against predators and likely against detrimental environmental agents. The enhanced movability of the metasoma increases the defensive capacity with a sting or other mechanisms.

Key words. *Myrmecia*, Formicidae, ants, mesosoma, phylogeny, evolution.

1. Introduction

Even though most individuals of Formicidae are small and inconspicuous, the group is exceptionally popular and also generally known outside the community of entomologists. The diversity of the family is relatively modest with ca. 12,800 described species (BOUDINOT 2015). However, the enormous biomass, the obviously high impact in many ecosystems, elaborate forms of eusociality and complex behavior patterns have attracted intensive attention of researchers since the early days of scientific entomology (see e.g. HÖLLDOBLER & WILSON 1990).

Morphological research on Formicidae goes back to the late 19th century (e.g. NASSONOFF 1889). Nevertheless, considering the enormous popularity and importance of the group, the available anatomical data are surprisingly scarce. Most morphological investigations were restricted to external skeletal features, whereas detailed and well-documented anatomical studies are still scattered and limited in their taxonomic scope.

A very early study providing anatomical data on “ants, bees and wasps” was presented by LUBBOCK (1881). The

morphology of external mesosomal structures of ants was discussed by NASSONOFF (1889) and JANET (1898). EMERY (1900) homologized structures in different ant castes, and his nomenclature was used by later researchers. SNODGRASS (1910a) briefly described the mesosoma of an ant worker in a comprehensive study on Hymenoptera. TULLOCH (1935) compared external mesosomal structures of alates and workers. The anatomy and life history of workers of *Camponotus herculeanus pennsylvanicus* De Geer, 1773 were described by FORBES (1938) and the anatomy of *Rhytidopenera metallica* (Smith, 1858) by WHELDEN (1960). Workers from seven subfamilies were described by REID (1941) for a general comparison of wingless and short-winged types in Hymenoptera. MARKL (1966) and SAINI et al. (1982) described the mesosomal skeletomuscular system of single species, the former also covering the mesosomal nervous system. The tracheal system was investigated by KEISTER (1962). DE GUSMÃO et al. (2001), BILLEN et al. (2011) and BILLEN (2017) examined the metapleural gland under morphological and functional aspects.

Many studies on external structures were used in a taxonomic context (e.g. WILSON et al. 1967; GOTWALD & KUPIEC 1975; GOTWALD & SCHAEFER 1982; BOLTON 2003; BOUDINOT 2015), partly also covering fossil taxa (e.g. GRIMALDI et al. 1997; ENGEL & GRIMALDI 2005; BARDEN & GRIMALDI 2012). EMERY (1877) proposed the first evolutionary hypothesis of relationships among ant subfamilies. There was a gap until BROWN (1954) presented a revised comprehensive morphology-based phylogeny, based on results of previous studies. He did not explicitly use a Hennigian (or cladistic) approach in this contribution. Nevertheless, due to his profound knowledge of the group and his extensive taxonomic work based on a series of previous investigations (e.g. EMERY 1877; WHEELER 1928), his tentative evolutionary tree is highly consistent with results of some recent analyses of molecular data (e.g. BRADY et al. 2006; MOREAU et al. 2006; KELLER 2011; WARD 2014). Using scanning electronic microscopy, BARONI URBANI et al. (1992) and KELLER (2011) characterized more detail of external morphology for explicit phylogenetic study of Formicidae. Combining anatomical data, phylogenetic systematics and locomotor function, KELLER et al. (2014) analyzed the mesosomal evolution in ant castes and trade-off between different behavioral patterns.

Recently, computer-based 3D reconstruction was used to increase the efficiency and accuracy of the documentation of external and internal characters, and also to facilitate sharing complex morphological data (FRIEDRICH et al. 2013; WIPFLER et al. 2016). HITA GARCIA et al. (2017) applied these methods in descriptions of two new species from Madagascar. However, fine details like muscle insertions were not documented in that study, and generally the use of this approach (and other innovative methods) is still limited in studies on Formicidae and related groups. Consequently, our primary aim was to provide detailed documentation of the mesosoma of an ant worker using a broad spectrum of techniques,

including microphotography, scanning electron microscopy (SEM), micro-computed tomography (μ CT) and 3D reconstruction. For our investigation, we chose a species from the subfamily Myrmeciinae. Even though this group is likely not close to the root of the family (MOREAU & BELL 2013; WARD 2014; BLANCHARD & MOREAU 2017; BOROWIEC et al. 2017; BRANSTETTER et al. 2017a), it shows a high number of discernable mesosomal sclerites, and is therefore possibly close to the plesiomorphic mesosomal status of Formicidae (WARD & BRADY 2003). We homologize the mesosomal muscles observed in *Myrmecia nigrocincta* with those previously described for species of other groups, notably the honeybee (SNODGRASS 1942), but also other representative of Aculeata and taxa close to the root of the hymenopteran tree such as Xyelidae and Tenthredinidae (VILHELMOSEN 2000a,b; MAKI 1938). We compare our observations with conditions found in formicid alates and members of other groups of Aculeata. Finally, we present some interpretations on the functional and evolutionary background of modifications occurring in different castes of ants.

2. Materials and methods

2.1. Specimens examined

Myrmecia nigrocincta Smith, 1858, worker, preserved in 70% ethanol, collected by R. Jordan in Australia, Queensland, Mount Hypipamee on September 2, 2002 in an open forest habitat. The species identification follows the key from OGATA & TAYLOR (1991).

2.2. Hand drawings

One specimen was manually dissected in 70% ethanol under a Zeiss Stemi SV11 with an additional Euromex Illuminator EK-1 lighting system. The mesosomal sclerites were drawn with full lines, margins below other sclerites with dotted lines. The legs were omitted, except the coxal elements. The figures were drawn with pencil under the microscope, scanned into the computer and finished with Adobe Illustrator CC.

2.3. Computer-based 3D reconstruction

One specimen was dehydrated in an ethanol series (from 70% to 100%) transferred into Acetone and dried at the critical point (EmiTech K850 Critical Point Dryer). It was scanned in a SkyScan2211 micro-CT (FSU Jena) with beam settings of 40 kV and 320 μ A. In a 360° scan pictures were taken every 0.2° with an exposure time of 150 ms. A pixel size of 0.9 μ m was achieved. The mesosomal segments of the specimen were reconstructed three-dimensionally based on the μ CT-image stack using

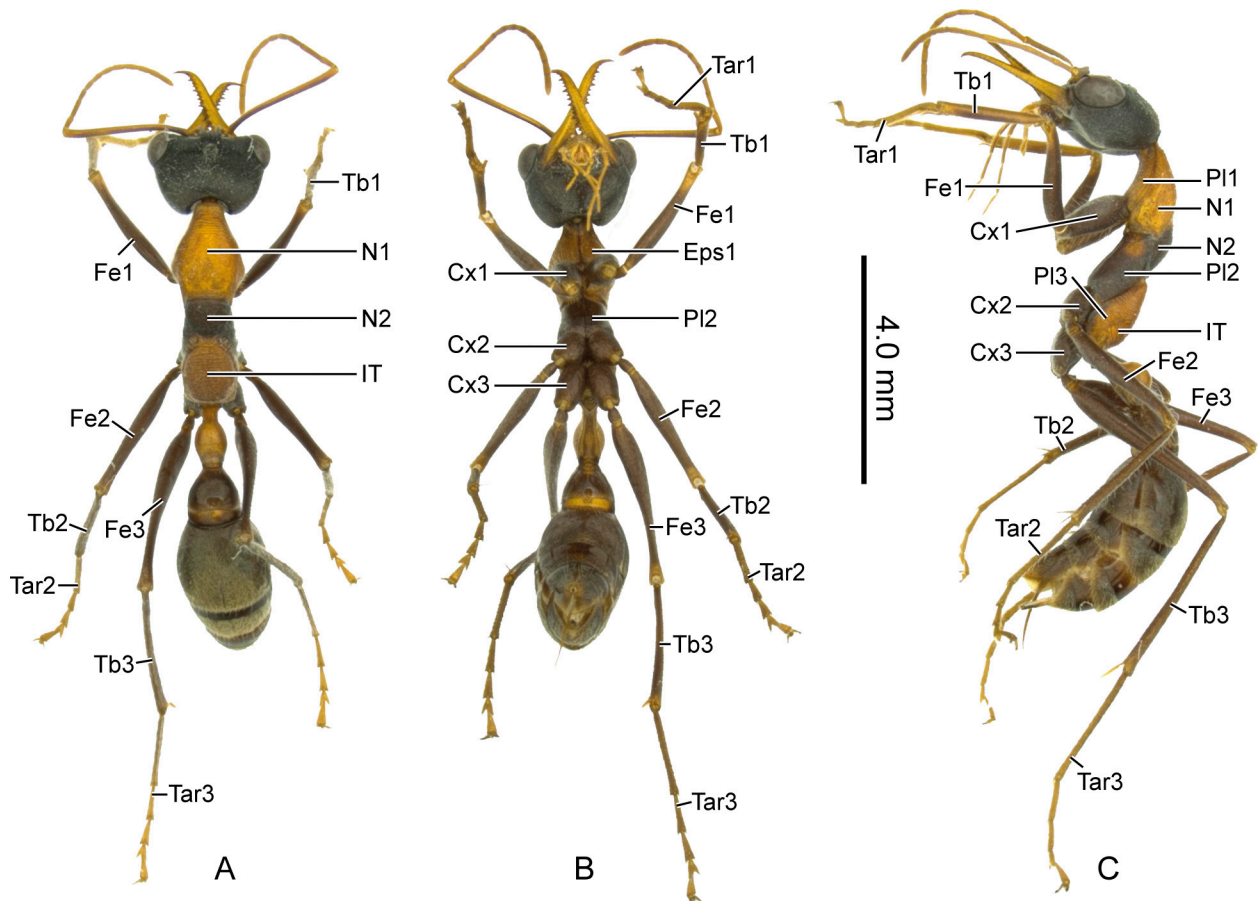


Fig. 1. *Myrmecia nigrocincta*, habitus, digital photographs. **A:** dorsal view, **B:** ventral view, **C:** lateral view.

FEI Amira 6.0 for segmentation and VG-Studio Max 2.0 (Volume Graphics GmbH, Heidelberg, Germany) for volume-/ surface renders.

2.4. Microscopic photography and scanning electronic microscopy

One specimen was air dried after fixing it in position over night in 100% ethanol. It was photographed with a Keyence VH-Z20R to record the general body shape and coloration. Another specimen was coated with gold (EmiTech K500 sputter coater) after critical point drying. Micrographs were taken with Philips XL 30 ESEM (FEI) and ResAlta Scandium software.

2.5. Reconstruction of character evolution

External characters of the mesosoma for the mapping analysis were adopted from previous morphology-based phylogenetic studies including No. 10–15 from BARONI URBANI et al. (1992) and No. 49–94 from KELLER (2011). These two references provide comprehensive data on the external skeletal morphology. We excluded characters No. 10–12 from BARONI URBANI et al. (1992) that are duplicated as characters No. 49, 60 and 62 in KELLER

(2011). Based on parsimony inference we assigned the most plausible character state to the groundplan of each subfamily if it was represented by more than one terminal with variation in this feature (NINOMIYA & YOSHIKAWA 2009). In cases of ambiguity we scored multiple states for the terminal. We also used the same method to combine invalid subfamilies Ecitoninae, Leptanilloidinae, Cera-pachyinae, Aenictinae and Dorylinae into one branch as Dorylinae. We then checked uninformative characters in WinClada with the function “Mop uninformative chars” and deleted them. Finally, 26 characters (Table S1) for 15 formicid subfamilies and 3 outgroup taxa Scoliididae, Bradynobaenidae and Vespidae were mapped on a manually reconstructed tree in WinClada with the function “Move branch mode”, using the phylogenomic topology from BRANSTETTER et al. (2017a). It contains a large number of terminal taxa, extensive gene regions and relatively unambiguous alignments.

We also homologized the mesosomal muscles of 12 species of Hymenoptera with those observed in our studied species *M. nigrocincta* (Tables 1, S2). The muscular characters come from LUBBOCK (1881), MAKI (1938), DUNCAN (1939), SAINI et al. (1982), VIHELMSEN (2000a,b), MIKÓ et al. (2007), SNODGRASS (1942) and ALAM (1951). The information from the last two references was extracted from “TABLE XXII” of MATSUDA (1970). In total, these references cover seven families including

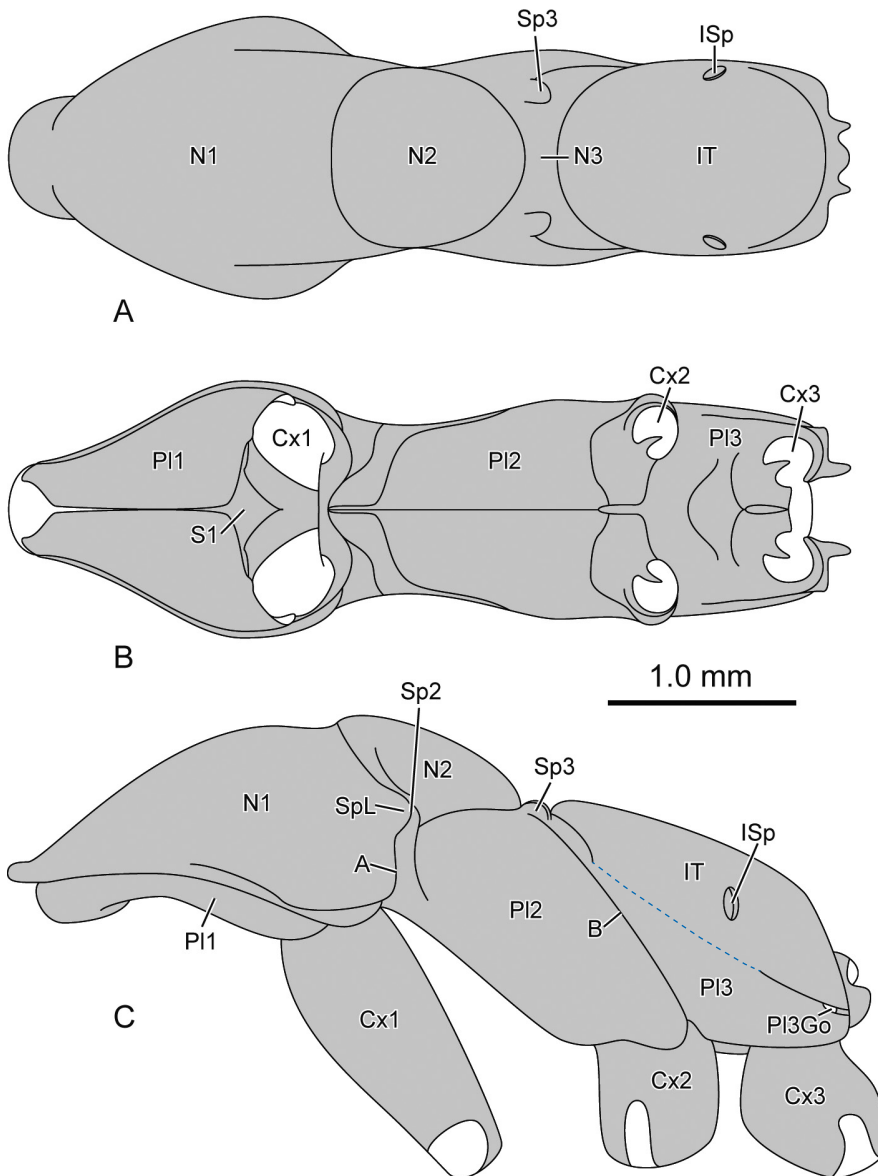


Fig. 2. *Myrmecia nigrocincta*, thoracic exoskeleton, line drawings. **A:** dorsal view, **B:** ventral view, **C:** lateral view, the dotted blue line is the border between propodeum and metapleuron.

Xyelidae, Tenthredinidae, Ichneumonidae, Braconidae, Scelionidae, Vespidae, Apidae and Formicidae. Among them, Vespidae is represented by three species of *Vespa*. Formicidae is represented by three species of Formicinae: two of the ant studies contain information from both alate gynes and flightless workers (LUBBOCK 1881; SAINI et al. 1982), while one contains only information from workers (MARKL 1966).

2.6. Terminology

The terminology for the mesosomal exoskeleton is based on REID (1941) and KELLER (2011), and on FRIEDRICH & BEUTEL (2008a) for internal skeletal structures and muscles.

Abbreviations: A – posterolateral margins of pronotum; Ar1/2/3 – arolium of fore-/mid-/hindleg; B – meso-metapleural suture; Btar1/2/3 – pro-/meso-/metabasitarsus; BtarS – basitarsus setae; Ca – calcar; CaBa – brush on an-

terior side of calcar; CaLa – lamella of calcar; CaPe – pectinate-shaped structure of calcar; C11/2/3 – claw of fore-/mid-/hindleg; Cx1/2/3 – pro-/meso-/metacoxa; Dc2/3 – meso-/metadiscrimen; Fe1/2/3 – pro-/meso-/metafemur; Fu1/2/3 – pro-/meso-/metafurca; Hm – hairy membrane, ISp – propodeal spiracle; IT – propodeum; Lcv – lateral cervical sclerites; Ma2 – manubrium of midleg; N1/2/3 – pro-/meso-/metanotum; P11/2/3 – pro-/meso-/metapleuron; P13G – metapleural gland; P13Go – metapleural gland orifice; P1 – planta; Ses – stout setae; Sp2/3 – meso-/meta-thoracic spiracle; SpL – spiracle lobe; StrC – strigil comb, StrN – strigil notch; S1 – prosternum; Tar1/2/3 – pro-/meso-/metatarsus; Tb1/2/3 – pro-/meso-/metatibia; Tr1/2/3 – pro-/meso-/metatrochanter; TspA2/3 – meso-/metatibia antero-dorsal apical setae; TspP2/3 – meso-/metatibia posterior apical setae. — Abbreviations for muscles are based on the terminology of FRIEDRICH & BEUTEL (2008a). Two newly introduced abbreviations are used, IA1 for the first propodeo-abdominotergal muscle, IA2 for the second propodeo-abdominotergal muscle (LUBBOCK 1881).

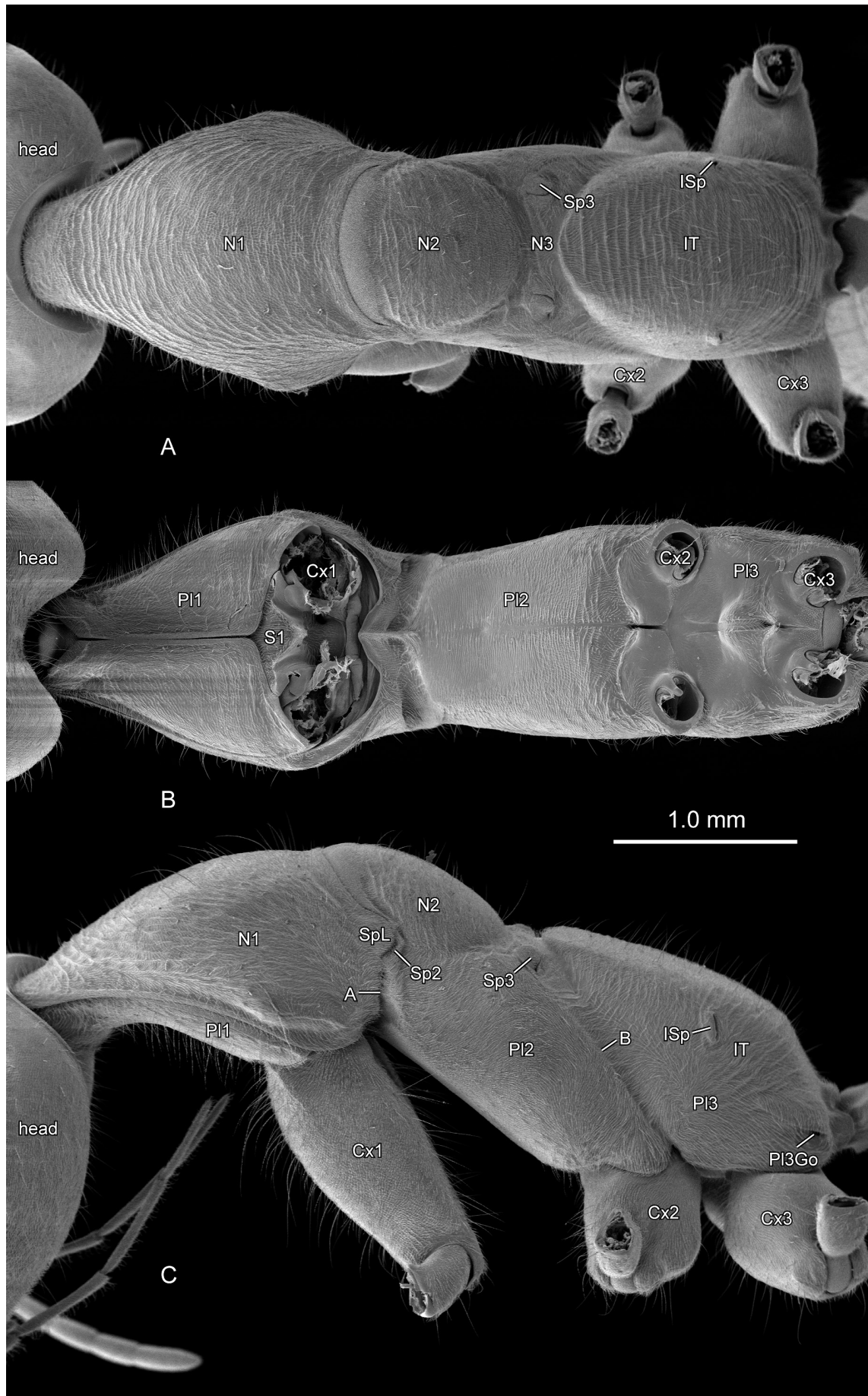


Fig. 3. *Myrmecia nigrocincta*, thoracic exoskeleton, SEM micrographs. A: dorsal view, B: ventral view, C: lateral view.

3. Results

3.1. Skeletal structures

3.1.1. General appearance

The entire tagma appears very slender in dorsal view, and greatly simplified in its entire skeletal configuration compared to more generalized insects (e.g. Zoraptera; FRIEDRICH & BEUTEL 2008a). It is densely covered by pubescence (Fig. 3). In lateral view the pro- and mesothorax form an arch from the strongly narrowed cervical region to the mesocoxal insertion area. The propodeum appears parallelogram-shaped in lateral view, resting like a saddle on the posterior mesothorax. The strongly slanting and very distinct meso-metapleural suture (REID 1941) (Figs. 2C, 3C, 4A: B) extends from the metathoracic spiracle to the lateral mesocoxal articulation.

3.1.2. Cervical region and prothoracic skeleton

The segment appears elongated and pear-shaped in dorsal view, and slightly curved in lateral view, with an evenly curved, ascending pronotum and a long concave anteroventral margin. The cuticle is slightly rugose, without any conspicuous surface modifications.

The cervical membrane connects the very narrow foramen occipitale with the narrow anterior prothoracic margin. The wide and concave surface of the occipital region of the head appears very large in comparison to the anterior prothorax. The neck region lacks exposed sclerotized cervicalia, but an invaginated internal process represents a slightly bulged lateral cervical sclerite (Fig. 4D: Lcv).

The elongate pronotum (Figs. 1A,B, 2A,C, 3A,C, 4A: N1) is evenly rounded along its lateral margins; it reaches its greatest width at its posterior 1/4 and very distinctly narrows before it connects with the mesonotum, with posterolateral edges, which appear evenly rounded in dorsal view (Figs. 2C, 3C, 4A: A); the anterior margin is slightly bent upwards; it is distinctly narrowed, about 1/3 as wide as the maximum width of the segment; the lateral edge of the pronotum is rounded; the posterior edge appears more or less concave depending on the angle of view; the propleura at the hind margin of the pronotum is distinctly reduced. The large propleuron (Figs. 1B,C, 2B,C, 3B,C, 4A: P11) is inflexed medially and curving posteroventrally, thus covering the lateral part of the segment and also the entire ventral area, where both parts meet along a ventromedian line; a distinct, evenly curved articulation separates it from the pronotum, running almost exactly parallel to the lateral segmental border; antero-laterally it forms paired roughly triangular projections, enclosing a V-shaped incision, which forms the propleural antero-ventral edge of the segment.

The strongly elongated procoxae (Figs. 1B,C, 2B,C, 3B,C, 4A: Cx1) extend postero-ventrad from the posterior propleural margin; they are inserted in transversely oval procoxal cavities. Antero-medially the profurca

(Fig. 4B: Fu1) arises from a rhombic prosternum (Figs. 2B, 3B: S1) between the procoxae, the only exposed sternal element; a pair of extensive processes for muscle attachment arise from the anterior side of the apical part of the profurca.

3.1.3. Mesothoracic skeleton

The mesonotum (Figs. 1A,C, 2A,C, 3A,C, 4A: N2) is a completely undivided, moderately large, oval sclerite; it is about $1.5 \times$ as long as wide and evenly rounded on all sides; antero-laterally it bears a pair of mesothoracic spiracles (Figs. 2C, 3C, 4A: Sp2), which are covered by semicircular spiracle lobes (Figs. 2C, 3C, 4A: SpL). The large mesopleuron (= mesoplectus) (Figs. 1B,C, 2B,C, 3B,C, 4A: P12) is dorsally continuous with the lateral mesonotal margin; it expands over the entire lateral and ventral areas; ventro-medially it is invaginated into the mesosomal lumen to form an elongate-triangular meso-discriminal lamella (Fig. 4B: Dc2), which is posteriorly connected to the mesofurcal base. The nearly spherical mesocoxae are distinctly shorter than the procoxae and laterally directed in their resting position (Figs. 1B,C, 2B,C, 3A–C, 4A: Cx2); they are inserted at the posterior margin of the mesothoracic venter; they are almost adjacent medially, even though the internal openings of the mesocoxal cavities are widely separated and distinctly smaller than their prothoracic counterparts. The narrow mesofurca (Fig. 4B: Fu2) is curved in lateral view; it originates from the middle area between the paired mesocoxae; from there it extends antero-dorsad and connects to the dorsal part of the mesopleuron; a slender antero-median mesofurcal process serves as attachment area of a ventral longitudinal muscle (Fig. 4C: Iv1m7).

3.1.4. Skeleton of metathorax and propodeum

The metanotum (Figs. 2A, 3A: N3) is a very narrow and undivided element between the annular metathoracic spiracles (Figs. 2A,C, 3A,C, 4A: Sp3), which are located close to the postero-lateral mesonotal margin. The large propodeum (Figs. 1A, C, 2A,C, 3A,C, 4A: IT) anteriorly connects with the metanotum, and ventrally with the large triangular metapleura (Figs. 2A,C, 3B, 4A: P13). The paired metapleura expand over the entire lateral and ventral areas, and a sclerotized metadiscrimin (Fig. 4C: Dc3) extends from the ventral midline between the paired metapleural halves upward into the mesosomal lumen. Dorso-laterally, paired slit-shaped propodeal spiracles are present (Figs. 2A,C, 3A,C, 4A: ISp), and the paired orifices (Figs. 2C, 3C, 4A: P13Go) of the flocculent metapleural glands (Fig. 4E: P13G) are located postero-laterally. Externally, a low carina is present antero-dorsally to the gland opening. The conical metacoxae (Figs. 1B,C, 2B,C, 3A–C, 4A: Cx3) are longer than the mesocoxae; they are inserted at the posterior edge of the metathoracic venter; the internal openings of the metacoxal cavities are medially connected, thus forming a broad, transverse postero-ventral aperture. The narrow metafurca (Fig. 4B:

Fu3) originates from the median area between the metacoxae and extends antero-dorsad along the anterior metadiscrimen; anteriorly they connect to the postero-dorsal part of the mesofurca; this part forms a semi-cylindrical structure that ensheathes the very long and slim ventral longitudinal muscles (Fig. 4C: IIIvlm7).

3.1.5. Legs

All three pairs of legs are long and slender and covered by short pubescence. Additionally, fine long setae are dispersed over the entire surface, with their length decreasing distally. The foreleg is the shortest and the hindleg the longest. However, the procoxa (Figs. 1B,C, 2C, 3C, 4A, 5A: Cx1) is distinctly elongate, almost twice as long as its meso- (Figs. 1B,C, 2C, 3C, 4A, 5B: Cx2) and metathoracic counterparts (Figs. 1B,C, 2C, 3C, 4A, 5C: Cx3). All trochanters (Fig. 5A–C: Tr1–3) are short, barrel-shaped sclerites. The protrochanter connects laterodistally with the procoxa, probably allowing rotatory movements; the meso- and metatrochanters connect with the distal ends of their respective coxae with a hinge-like contact zone, likely resulting in restricted movability. The femur (Figs. 1A–C, 5A–C: Fe1–3) is the longest element of all three legs. It is slightly bulbous in its proximal half, especially in the fore- and hindlegs, and it narrows distally. The femuro-tibial articulations allow movements in one plane (Fig. 5A). The tibiae (Figs. 1A–C, 5A: Tb1) are rather straight and narrower than the femur. All tarsi (Figs. 1A–C, 5A: Tar1) are pentamerous; the basitarsus is about as long as the remaining tarsomeres combined; tarsomere 4 is the shortest and is bilobed; tarsomere 5 bears paired claws and an arolium (Fig. 5A).

A complex armature is present on the distal parts of all three legs, but most elaborate on the foreleg. The protibia bears a row of four stout setae distally on its posteroventral surface; the posterior side bears three stout setae apically near the calcar (Fig. 5E,F: Ca) of the strigil (FRANCOEUR & LOISELLE 1988: antenna cleaner), which articulates on the ventral side of the protibia. The calcar carries a lamella (Fig. 5F: CaLa) proximally and a pectinate structure (Fig. 5F: CaPe) on its distal region; on its anterior surface, it bears a brush of clubbed microtrichia, running parallel to the lamellate and pectinate edge and covering most of its surface on this side (Fig. 5E: CaBa); densely packed, short, spine-like microtrichia are present on the ventral surface of the calcar. The probasitarsus (Fig. 5E,F: Btar1) forms the corresponding part of the strigil; its anterior surface bears paddle-shaped setae similar to the clubbed microtrichia of the calcar; very similar setae are also inserted distally on the anteroventral protibial surface. The probasitarsus bears shorter, apically rounded setae on its posterior and ventral side; the comb of the strigil (Fig. 5F: StrC) is inserted in a proximal notch of the probasitarsus (Fig. 5F: StrN), and a row of stout setae (Fig. 5F: Ses) extends parallel to it on the posterior surface of this tarsomere; more widely spaced stout setae are continuous with this row and reach the distal end of the probasitarsus.

The meso- and metatibia also bear stout apical setae, antero-dorsally and posteriorly on the midleg and posteriorly on the hindleg; additionally, both carry two spurs distally on the ventral side (Fig. 5G,H). The mesotibial spurs (Fig. 5G: TspA2, TspP2) are very similar to each other in length and configuration, with short, spine-like microtrichia on the ventral side and a row of cuticular teeth on the dorsal edge. The posterior metatibial spur (Fig. 5H: TspP3) is longer and stronger than the anterior one (Fig. 5H: TspA3), and is somewhat similar to the strigil; in contrast, the smaller anterior spur is similar to its mesotibial counterpart. The larger posterior spur carries a comb of microtrichia on its dorsal side, quite similar to that of the calcar; additionally, this spur bears a small brush of club-like microtrichia on its posterior side, smaller than the one on the anterior side of the calcar but otherwise similar; paddle-shaped setae are inserted on the postero-ventral metabasitarsus, similar to those of the anterior probasitarsal surface. A group of blunt setae is inserted distally on the postero-ventral metatibial surface. The meso- and metabasitarsi bear lateral rows of stout setae like the probasitarsus; additionally a sulcus is present on their anterodorsal surface (Fig. 5D: BtarS).

The terminal parts of all legs are similar and well-developed (Fig. 6). The claws articulate with the distal rim of tarsomere five and bear an apically rounded preapical tooth that is broader than the apical part. A vestiture of short setae is present on the proximal two thirds of the claws (Fig. 6A–C: Cl1–3), three long setae are inserted ventro-laterally, and a field of minute hairs is present on the ventral base. The arolium (Fig. 6A–D: Ar1–3) and its supporting sclerites originate between the claws; the humerus-shaped manubrium (Fig. 6B,D: Ma2) is dorsally articulated with tarsomere 5; it bears several small setae proximally and two larger setae proximad its middle region; membranous areas with minute spine-like protuberances are present at the lateral base of the manubrium. The surface structure at the lateral membranous bases of the arolium is spinose, whereas its ventral and lateral regions are smooth. The deeply concave dorsal surface resembles a wicker basket, with scale-like microtrichia along most of its dorsal surface; some of these minute scale-like structures, especially close to the margin, bear a single minute microtrichium on the tip. Ventrally, the planta (Fig. 6A: Pla1) is densely covered with setae. The unguitactor plate could not be observed with the techniques we applied and the arcus is also concealed (FEDERLE et al. 2001).

3.2. Musculature (Fig. 4)

3.2.1. Prothoracic muscles

Dorsal longitudinal muscles. **Idlm1**, *M. prophragma-occipitalis*, long and slender, slightly wider in its middle region; O (= origin): median part of anterior mesonotal margin, I (= insertion): dorsally on posterior edge of occipital region, close to midline. — **Idlm5**, *M. pronotophragmalis anterior*, fan-shaped, strongly narrowing

anteriorly, narrower posteriorly; O: postero-lateral area of pronotum; I: latero-median part of anterior mesonotal margin.

Dorsoventral muscles. **Idvm5**, *M. pronoto-cervicalis anterior*, broad muscle, narrowing towards insertion site on lateral cervical sclerite; O: antero-lateral area of pronotum; I: lateral cervical sclerite. — **Idvm9**, *M. profurca-occipitalis*, long slender bundle; O: anterior area of profurca; I: dorsal occipital region. — **Idvm18**, *M. pronoto-coxalis lateralis*; O: postero-lateral area of pronotum; I: lateral procoxal rim.

Tergo-pleural muscles. **Itpm1**, *M. pleurocrista-occipitalis*, very large muscle, narrowing towards insertion on occipital region; O: postero-lateral area of propleuron; I: dorso-median area of occipital region. — **Itpm2**, *M. propleuro-occipitalis*, very large muscle, larger on propleuron, narrowing towards insertion on occipital region; O: postero-dorsal area of propleuron; I: dorso-median area of occipital region. — **Itpm4**, *M. pronoto-apodemalis anterior*; O: ventro-lateral area of pronotum; I: dorsal area of profurca. — **Itpm5**, *M. pronoto-apodemalis posterior*; O: postero-lateral area of pronotum; I: profurcal anterior process. — **Itpm6**, *M. pronoto-intersegmentalis*; O: postero-ventral area of pronotum; I: basal area of profurca.

Pleuro-coxal muscles. **Ipcm4**, *M. propleuro-coxalis superior*, triangular, narrowing towards insertion on procoxal rim; O: postero-dorsal area of proepimeron; I: lateral procoxal rim. — **Ipcm8**, *M. propleuro-trochanteralis*, long muscle; O: postero-dorsal area of proepimeron; I: protrochanteral tendon.

Ventral longitudinal muscles. **Iv1m1**, *M. profurca-cervicalis*, slender muscle; O: profurcal anterior process; I: lateral cervical sclerite. — **Iv1m3**, *M. profurca-tentorialis*, long slender bundle; O: anterior area of profurca; I: ventral occipital region. — **Iv1m7**, *M. profurca-mesofurcalis*, two subcomponents, the median bundle slender and shorter, the lateral one widening towards mesofurcal insertion site; O: posterior area of profurca; I: median bundle on anterior mesofurcal process, lateral subunit on antero-dorsal part of mesofurca.

Sterno-coxal muscles. **Iscm1**, *M. profurca-coxalis anterior*; O: postero-ventral area of propleuron close to the basal part of profurca; I: anterior procoxal rim. — **Iscm3**, *M. profurca-coxalis medialis*, wider on profurca, narrowing towards insertion on procoxal rim; O: dorsal part of profurca; I: mesally on procoxal rim. — **Iscm4**, *M. profurca-coxalis lateralis*, triangular muscle, wider on profurca, narrowing towards insertion on procoxal rim; O: dorsal part of profurca; I: laterally on procoxal rim. — **Iscm6**, *M. profurca-trochanteralis*, long muscle; O: dorsal part of profurca; I: protrochanteral tendon.

3.2.2. Mesothoracic muscles

Sterno-coxal muscles. **Iiscm1**, *M. mesofurca-coxalis anterior*, very large triangular muscle, narrowing towards insertion on mesocoxal rim; O: ventral area of mesopleuron; I: anteriorly on mesocoxal rim. — **Iiscm3**, *M. mesofurca-coxalis medialis*, triangular muscle, narrow-

ing towards mesocoxal rim; O: basal part of mesofurca; I: mesocoxal rim. — **Iiscm4**, *M. mesofurca-coxalis lateralis*, very large triangular muscle, narrowing towards on mesocoxal rim; O: ventral area of mesopleuron; I: laterally on mesocoxal rim. — **Iiscm6**, *M. mesofurca-trochanteralis*, very large triangular muscle, narrowing towards insertion; O: posterior margin of mesonotum and with a few fibers on the dorsal mesofurca; I: mesotrochanteral tendon.

3.2.3. Muscles of metathorax and propodeum

Dorsoventral muscles. **IIIidvm5**, *M. metanoto-coxalis posterior*, very large muscle, narrowing towards metacoxal rim; O: antero-lateral area of propodeum; I: postero-lateral metacoxal rim.

Ventral longitudinal muscle. **IIIv1m2**, *M. metafurca-abdominosternalis*, slender muscle; O: postero-ventral part of metafurca; I: antero-lateral margin of petiole. — **IIIv1m7**, *M. metafurca-abdominosternalis*, very long and slender; O: postero-dorsal part of metafurca; I: antero-ventral margin of petiole.

Sterno-coxal muscles. **IIIscm1**, *M. metafurca-coxalis anterior*, large triangular muscle, narrowing towards metacoxal rim; O: ventral area of metafurca; I: laterally on metacoxal rim. — **IIIscm4**, *M. metafurca-coxalis posterior*; O: postero-ventral area of metafurca; I: laterally on metacoxal rim. — **IIIscm6**, *M. metafurca-trochanteralis*, very large muscle, narrowing towards insertion; O: anterolateral area of propodeum and a small part on the ventral metacoxa; I: metatrochanteral tendon.

Muscles of propodeum. **IA1**, *M. propodeo-abdominotergalis dorsalis*, long feather-shaped muscle; O: dorso-medial area of propodeum; I: antero-dorsal margin of abdominal segment II. — **IA2**, *M. propodeo-abdomitergalis lateralis*; O: dorso-lateral area of propodeum; I: anterolaterally on dorsal margin of abdominal segment II.

4. Discussion

4.1. Morphological techniques

The anatomical investigations in this study were carried out with only five specimens. Nevertheless, the workflow and combination of different techniques allowed a very detailed documentation of skeletal structures and also internal soft parts. This demonstrates that a combination of traditional and modern techniques can greatly facilitate the acquisition of detailed morphological data. Traditional anatomical studies can be of great value and some of them are highly accurate and detailed (e.g. DUNCAN 1939; MARKL 1966; SAINI et al. 1982; MIKÓ et al. 2007). However, information on coloration and 3-dimensional effects are usually limited or lacking (e.g. MAKI 1938). Complex anatomical illustration usually requires great

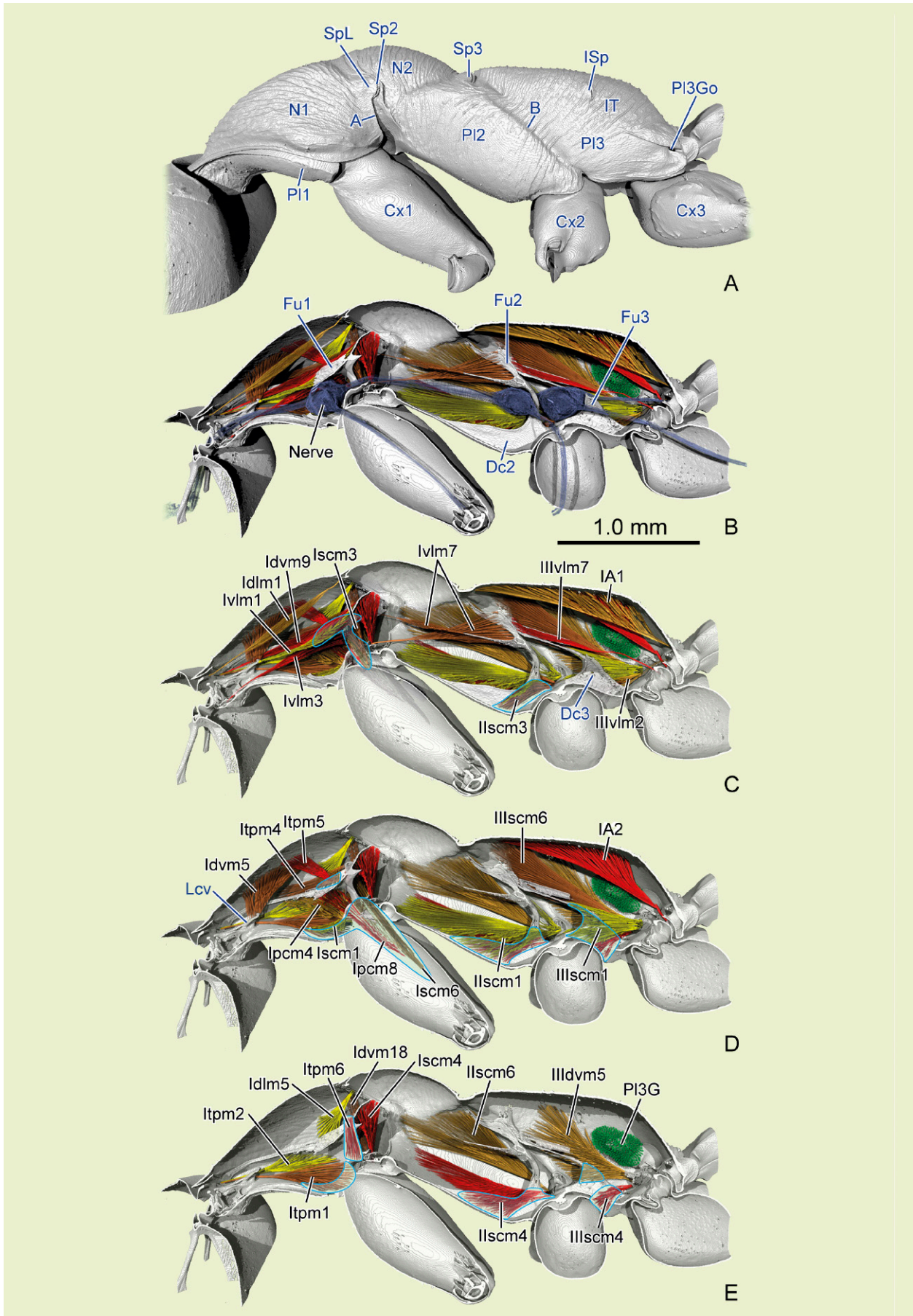


Fig. 4. *Myrmecia nigrocincta*, thorax, 3D reconstruction. **A:** lateral view of exoskeleton; **B–E:** endoskeleton and muscles, muscles removed layer by layer from median plane to lateral body wall.

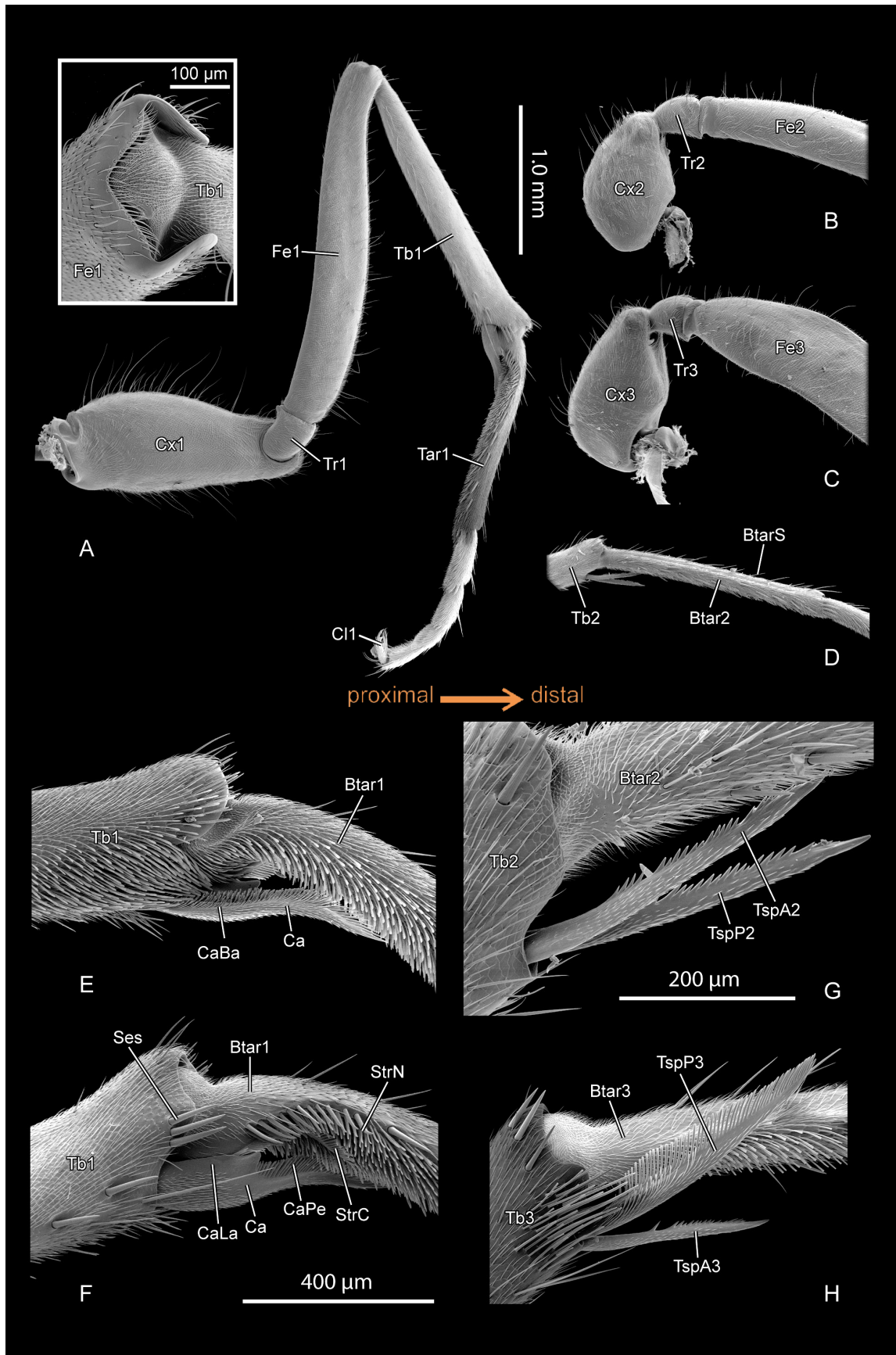


Fig. 5. *Myrmecia nigrocincta*, legs, SEM micrographs. **A:** foreleg, posterior view, insert shows femorotibial articulation in dorsal view; **B:** midleg, proximal part, posterior view; **C:** hindleg, proximal part, posterior view; **D:** tip of mesotibia and mesobasitarsus with basitarsal sulcus, front view; **E:** strigil of foreleg, front view; **F:** strigil of foreleg, posterior view; **G:** mesotibial spurs of midleg, front view; **H:** metatibial spurs of hindleg, posterior view. Scale bars: 1.0 mm for A–D; 100 µm for the insert of A; 400 µm for E, F, H; 200 µm for G. Orange arrow indicates direction from proximal to distal.

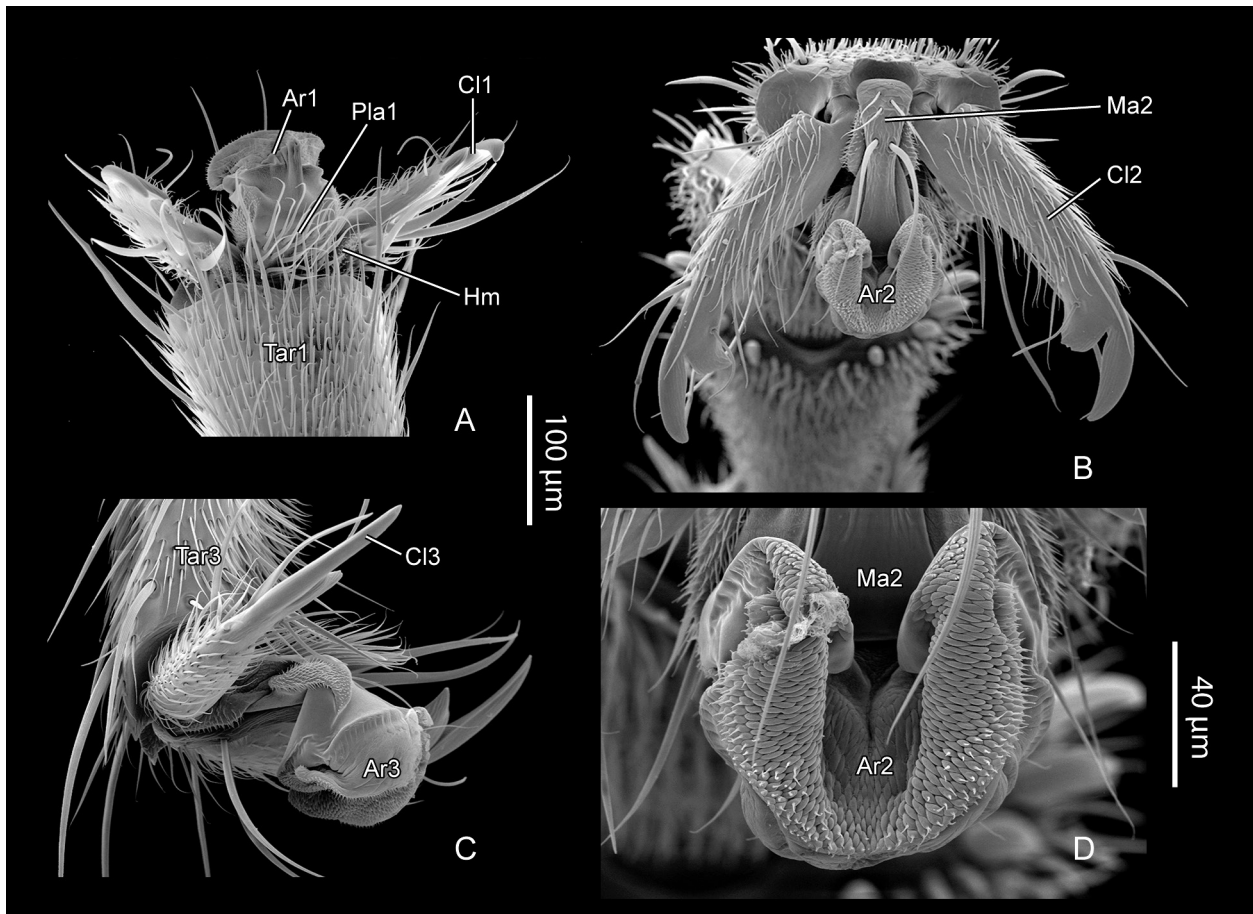


Fig. 6. *Myrmecia nigrocincta*, pretarsal structures, SEM micrographs. **A:** foreleg, dorsal view; **B:** midleg, front view; **C:** hindleg, lateral view; **D:** arolium, midleg. Scale bars: 100 µm for A–C; 40 µm for D.

experience and outstanding drawing skills (e.g. LUBBOCK 1881; WEBER 1969; see ALLGAIER 2008). Besides this, especially in the case of small or very small objects, structural details may be easily overlooked without high quality microtome sections or μ -CT data. In the case of the ant thorax, this may concern minute muscles of the wing base, which are generally difficult to observe based only on dissections.

Microphotography accurately records important characters including coloration (also a tentative indicator of sclerotization) and transparency of cuticle (WIPFLER et al. 2016). Accompanying the written description, microphotographs provide a more intuitive impression of habitus and configuration of an insect specimen (Fig. 1). They are very well suited for gradual changes of color and texture of the cuticle, which are very difficult to reproduce with traditional drawing techniques.

Scanning electron microscopy (SEM) provides precise information on fine surface structures (FRIEDRICH et al. 2014; WIPFLER et al. 2016). Compared with hand drawn figures (Fig. 2), SEM micrographs very clearly depict minute microtrichia or scales with stereo effect as well as fine wrinkles or other patterns of microsculpture (Fig. 3). High magnification and resolution allows the documentation of fine details not accessible with other methods (Figs. 5E–H, 6: e.g. microtrichia and tiny hairs on the

legs and claws), allowing their interpretation in a functional and evolutionary context. Handling of specimens and working with limited material is facilitated by a recently developed rotatable specimen holder (POHL 2010); additional advantages of this device are reduced charging of surfaces and a homogenous black background.

Microcomputed tomography (μ -CT) can greatly accelerate the acquisition of anatomical data and is less cost intensive than histological sectioning (WIPFLER et al. 2016). High quality μ -CT data are an ideal basis for 3D reconstruction, which has arguably triggered a renaissance of insect anatomy in the last 15 years (FRIEDRICH & BEUTEL 2008b; FRIEDRICH et al. 2014; WIPFLER et al. 2016). With μ -CT and suitable reconstruction (e.g. Amira, VGStudio) in an optimized workflow, external and internal structures such as sclerites, muscles, nerves and gland can be very clearly visualized (Fig. 4; WIPFLER et al. 2016). The description of the new species *Terataner balrog* Hita Garcia, 2017 from Madagascar was based on this method (HITA GARCIA et al. 2017: fig. 11). The entire study is an exemplary contribution in taxonomy, even though fine details like muscle insertions or nerves were not identified separately.

A systematic morphological database was established by YODER et al. (2010: Hymenoptera Anatomy Ontology) for the megadiverse Hymenoptera (WIPFLER et al. 2016).

Websites such as antweb.org provide numerous high quality images of Formicidae with standardized terminology. The enormous potential of these anatomical data collections in a taxonomic and phylogenetic context was emphasized by WIPFLER et al. (2016), and has been apparent in the works of ant systematists (e.g., YOSHIMURA & FISHER 2007; HITA GARCIA & FISHER 2011 [taxonomy]; BOUDINOT 2013 [morphology]). The technical procedures and the workflow presented in this study may contribute to the optimization of these ventures in the future.

4.2. Phylogenetic patterns and the “ancestral status” of Myrmeciinae

The first phylogenetic study of Formicidae was presented by EMERY (1877), who considered Dorylinae as closest to the ancestral pattern (“groundplan”) of ants. This hypothesis remained unchallenged until BROWN (1954) suggested a concept with this subfamily as a possible derivative from a “poneroid” ancestor, and a phylogenetic affinity with Myrmeciinae, with this taxon nested within a unit also including Pseudomyrmecinae, dolichoderomorphs and Formicinae. This phylogenetic topology remained a more-or-less preferred hypothesis (WILSON et al. 1967a,b; WILSON 1971), with some aberration (HÖLLDOBLER & WILSON 1990), until BARONI URBANI et al. (1992) conducted a formal parsimony analysis. In a study on the rediscovered Australian genus *Nothomyrmecia*, TAYLOR (1978) characterized Myrmeciinae as “the most structurally generalized ants” and their behavior as “significantly primitive” (WARD & BRADY 2003). In fact, the subfamily has arguably retained an entire series of plesiomorphic features. This includes six maxillary and four labial palpomeres in all castes, 12 antennal annuli in females and 13 in males, paired spurs on the middle and hind tibiae, tarsal claws with a strongly developed median tooth, a furcula and two-segmented gonostyli associated with the stinging apparatus, and an active closing mechanism of the proventriculus of workers (TAYLOR 1978). In an unpublished phylogenetic hypothesis from BROWN’s laboratory (around 1986), BROWN intuited that Myrmeciinae were placed close to the root of the phylogenetic tree of ants, conform with an apparent proximity to the groundplan of Formicidae (KELLER 2011: fig. 4). Subsequently, taxonomic concepts have changed with the application of phylogenetic models to molecular data (e.g. BRADY et al. 2006; RABELING et al. 2008; KÜCK et al. 2011; WARD 2014; BOROWIEC et al. 2017; BRANSTETTER et al. 2017b). Moreover, new ant fossils were discovered, likely belonging to the stem-group of the family, potentially shedding new light on the ancestral morphology of extant Formicidae (e.g. GRIMALDI et al. 1997; ENGEL & GRIMALDI 2005; PERRICHOT et al. 2008; BARDEN & GRIMALDI 2012, 2016; LAPOLLA et al. 2013; BARDEN 2017). These paleontological studies provide new morphological data, which are relevant in the context of ancestral features and the early evolution of the group. Therefore, the “ancestral status” of Myrmeciinae and the groundplan of Formicidae deserve further discussion.

The monophyly of Formicidae is consistently supported in analyses based on morphological (BROTHERS 1975) or molecular data (MOREAU et al. 2006; BRADY et al. 2006; RABELING et al. 2008; MOREAU & BELL 2013; BOROWIEC et al. 2017; BRANSTETTER et al. 2017a,b). In contrast, the branching pattern within the group is affected by changing phylogenetic approaches, with different phylogenies suggested by morphological characters and molecular data sets. BARONI URBANI (1989) and BARONI URBANI et al. (1992) placed Formicinae as an early branch in studies based on morphology (BARONI URBANI 1989: fig. 2, 1992: fig. 5), suggesting that some generalized formicid features were conserved in this subfamily. The analyses of extensive to extremely large molecular data sets with broad taxon sampling (RABELING et al. 2008; KÜCK et al. 2011; MOREAU & BELL 2013; BOROWIEC et al. 2017; BRANSTETTER et al. 2017a) have placed the small and specialized subfamilies Martialinae and Leptanillinae as the first two branches in a tree of extant ant taxa, followed by formicoids with 9 subfamilies (e.g. Formicinae, Myrmicinae), and the poneroids in a large clade with 6 other subfamilies, among them Amblyoponinae and Proceratiinae. In re-analyses of morphological data with fossil taxa included, a basal position of the extinct Sphecomyrmine in Formicidae s.l. (including stemgroup taxa) was supported (GRIMALDI et al. 1997). Preserved plesiomorphies of this group are the presence of two mandibular teeth and an antenna with a short scape and long, flexible funiculus (DLUSSKY 1983; GRIMALDI et al. 1997). Recently, another parsimony analysis placed the Cretaceous counterpart of modern trap-jawed ants haidomyrmecines as the basal branch (BARDEN & GRIMALDI 2016).

Recent phylogenetic results (BRADY et al. 2006; MOREAU et al. 2006; WARD 2014; BOROWIEC et al. 2017; BRANSTETTER et al. 2017a) clearly show that Myrmeciinae are not a “basal” group of ants, but in fact separated by six to seven nodes from the root of the tree. However, this does not exclude that the subfamily has preserved more ancestral features than most other formicid subgroups, as pointed out by TAYLOR (1978). In contrast to Myrmeciinae, the “basally” placed Martialinae are highly specialized, with an array of apomorphies linked with hypogaecic, and predacious habits, aside from some retained plesiomorphic ant features (RABELING et al. 2008; BOUDINOT 2015).

Our mapping analyses (Fig. 7) using the phylogeny of BRANSTETTER et al. (2017a) yields only five apomorphies of Formicidae. The exposed metathoracic spiracle with a round or oval shape [6.1] might be a derived groundplan feature, while concealment of the spiracle by a spiracular lobe has apparently evolved as an apomorphy of “core formicoids”. The presence of metapleural glands [8.1] is very likely an autapomorphy of Formicidae (WHEELER 1928; WILSON et al. 1967a; BARONI URBANI 1989; BOLTON, 2003; BOUDINOT 2015). It protects adults from fungi and bacterial infections and possibly even more immature stages (i.e., eggs, larvae, pupae) (BARONI URBANI 1989; BILLEN et al. 2011; YEK & MUELLER 2011; BILLEN 2017). Its absence in males of many species is

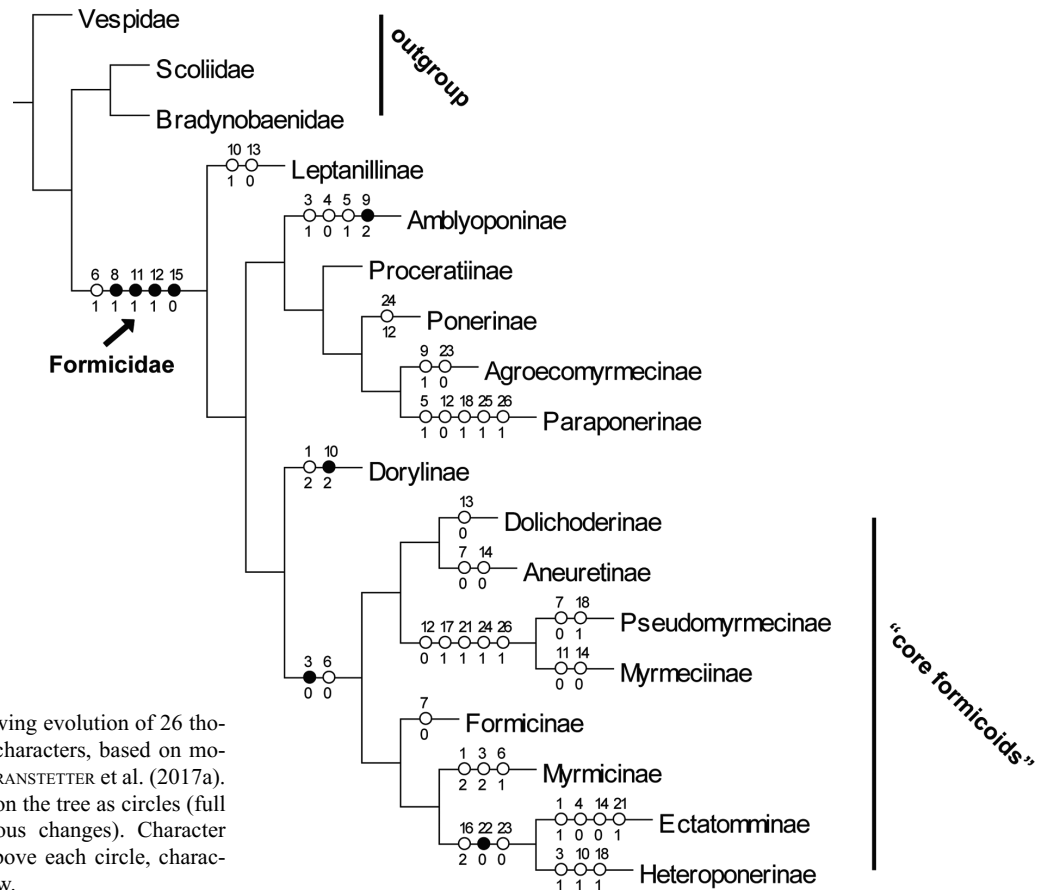


Fig. 7. Cladogram showing evolution of 26 thoracic skeletomuscular characters, based on molecular phylogeny of BRANSTETTER et al. (2017a). Apomorphies mapped on the tree as circles (full circles non-homoplasious changes). Character numbers are labeled above each circle, characters state numbers below.

apparently a secondary feature and probably due to their limited role in cooperative activities (HÖLLDOBLER & ENGEL-SIEGEL 1984; BARONI URBANI 1989). The propodeal spiracle without bulla [11.1], a round to oval atrial opening [12.1] and the absence of stout setae on the posterior protibial apex [15.0] were also retrieved as autapomorphies. The absence of all three features in Myrmeciinae is inferred as a result of reversals in our mapping analysis. A clade Myrmeciinae + Pseudomyrmecinae, the myrmeciomorphs of BOLTON (2003), is supported by three possible mesosomal synapomorphies, a calcar of the strigil with a basal lamella [17.1], propretarsal claws with a preapical tooth [21.1], and the presence of a metabasitarsal sulcus [26.1]. However, apparent homoplasy in these characters makes the polarity assessment ambiguous, except in the case of the last one (BOLTON 2003). This also applies to the posteriorly open metacoxal cavity [14.0], which was retrieved as an autapomorphy of Myrmeciinae. However, this trait optimized as reversal in our analysis, is very likely a retained plesiomorphy in Myrmeciinae (BOLTON 2003), with parallel losses in several formicid subgroups. Similar selective pressure may have resulted in a pattern which is less parsimonious than reversal in Myrmeciinae.

In addition to the derived features presented here, BOUDINOT (2015) also suggested a series of apomorphies of Formicidae: disticoxal foramen directed laterally and completely enclosing protochanteral base (char. 6); all meso- and metacoxal cavities small, circular, monocon-

dylic, ventrally-directed disticoxae strongly produced laterally (all adult castes) (char. 7); propodeal spiracle located on lateral propodeal face distant from anterodorsal propodeal corner, often near midlength of propodeum (all adult castes) (char. 9).

4.3. Mesosomal groundplan of Formicidae

The differentiation into flightless workers and sexual morphs with preserved flight apparatus is a crucial groundplan apomorphy and key innovation of Formicidae (HÖLLDOBLER & WILSON 1990).

Compared with more generalized members of Aculeata (SNODGRASS 1910b, 1925: Apidae; DUNCAN 1939: Vespidae), alate sexual morphs of ants show only limited modifications of the mesosoma, such as for instance wings with a reduced venation, or obsolete mesopleural ridges. The pronotum is larger than the short and clasp-shaped structure of Vespidae and Apidae (SNODGRASS 1910b, 1925; DUNCAN 1939), but distinctly shorter than in workers. The propleuron, a triangular plate-like structure, articulates with its lower posterior angle with the coxa, and with the occipital region on its anterior margin. Cervicalia are fused with the propleura as in the Hymenoptera groundplan postulated by VILHELMESEN (2000a). The small but separate sclerites of the neck region identified in the honeybee by SNODGRASS (1925) are likely free due to reversal. As in Vespidae and Apidae, a functional wing

Table 1. Overview of the musculatures of Formicidae (SAINI et al. 1982; LUBBOCK 1881; MARKL 1966), Vespidae (DUNCAN 1939) and Apidae (SNODGRASS 1942). Present with “+” or muscle name in green, absent with “-” in orange, uncertain with “?” or “/” in yellow. In Formicidae, muscles present only in workers labeled in dark green, those only occurring in the alate castes in dark blue.

Family	Vespidae	Apidae	Formicidae			
Genus	<i>Vespula</i>	<i>Apis</i>	<i>Myrmecia</i>	<i>Camponotus</i>	<i>Lasius</i>	<i>Formica</i>
ldlm1	-	40 + 41	+	1	a	40 & 41
ldlm5	lis1[50] & lis2[51]	45	+	-	-	45
ldvm5	lpm1[37] & lpm2[38]	47	+	6	c	46 & 47
ldvm7	-	46	-	-	-	-
ldvm9	Ois2[33]	43	+	2	a1	43
ldvm18	llm6[48]	55	+	-	-	55
ltpm1	-	42b? & 42c?	+	-	b1	42/1
ltpm2	Ois1[32]	42a?	+	-	c1 & c2	42/2
ltpm3	lpm3[39] & lpm4[40]	48	-	-	-	48
ltpm4	lpm5[41]	49	+	10	e	49
ltpm5	lpm6[42]	50	+	-	f	50
ltpm6	-	-	+	-	g	-
lpcm1	llm7[49]	mcr	-	-	d1	mcr
lpcm3	llm5[47]	57	-	-	-	-
lpcm4	-	-	+	-	h	53
lpcm5	llm2[44]	53	-	-	-	-
lpcm8	llm3[45]	61	+	-	k	-
lvlm1	lfp[36]	51	+	-	d	51
lvlm3	Ois3[34] & Ois4[35]	44	+	3	b	44
lvlm7	lis4[53] & lis5[54]?	52	+	18 & 19	m & n	52
lscm1	llm1[43]	54	+	-	i?	54
lscm2	llm4[46]	56	-	-	i	56
lscm3	-	-	+	-	-	-
lscm4	-	-	+	-	-	-
lscm5	lis3[52]	58	-	-	-	-
lscm6	llm3[45]?	61?	+	-	l	61
lldlm1	lld1[56]	71	-	20	β	-
lldlm3	llis1[72]	70	-	-	-	-
lldvm1	lldv1[57]	72	-	21	θ	-
lldvm6	-	82	-	23	-	-
lldvm7	-	-	-	-	o	-
lldvm8	lldv2[58]	78	-	-	-	-
lltpm2	mut[69]	74	-	-	-	-
lltpm5	llpm4[62]	75	-	-	-	-
lltpm6	-	-	-	25	-	-
lltpm7	m3Ax[63]	76a	-	24a	-	-
lltpm9	llpm2[60] & llpm3[61]	76b & 76c	-	24b	-	-
lltpm11	llpm5[64]	-	-	-	-	-
llppm2	-	-	-	-	-	73
llspm1	llpm1[59]	77	-	-	-	-
llspm2	llfp1[70] & llfp2[71]	79	-	-	-	-
llpcm3	llm1[65]	80	-	-	-	-
llvlm3	llis2[73]	-	-	-	-	79?
llscm1	llm2[66]	81	+	28	p & s	81 & 82
llscm2	llm4[68]	83	-	-	q	83
llscm3	llm4[68]?	83?	+	29 & 31	r	83
llscm4	-	-	+	30	t	80
llscm6	llm3a&b[67]	86	+	-	o	86
llldlm1	llld[75]	96	-	-	-	-
llldvm1	-	-	-	-	θ	-
llldvm4	-	-	-	40	y?	103
llldvm5	-	-	+	-	y?	105
llldvm6	lllpm5[80]	105	-	37	-	-
llldvm7	-	-	-	-	x	-
llltpm5	lllpm4[79]	97 & 98 & 99	-	-	-	-
llltpm6	-	-	-	39	-	-
llltpm7	lllpm2[77]	100	-	38a	-	-
llltpm9	lllpm2[77]?	-	-	38b	-	-
llltpm11	lllpm3[78]	102	-	-	-	-
lllspm1	lllpm1[76]	101	-	-	-	-
lllpcm2	-	-	-	36	-	-
lllpcm3	lllpm4[84]	103	-	-	-	-
lllvlm2	lllis2[86]?	118	+	33	u1	119
lllvlm7	-	-	+	35	v	118
lllscm1	llllm1[81]	104	+	41	z	104 & 105
lllscm2	llllm2[82]	106	-	-	x1	106
lllscm3	llllm2[82]?	106?	-	43	z1	106
lllscm4	-	-	+	42	y1	103
lllscm6	llllm3[83]	109	+	-	x	109
IA1	lad1[88]	120	+	34	u	120
IA2	lad2[89]	121	+	32	w	121

articulation is preserved in the pterothoracic segments of alate ants, with axillary sclerites (SAINI et al. 1982), notal wing processes and mesothoracic basalar and subalar sclerites as attachment sites of direct flight muscles.

The highly modified mesosoma of workers, documented in detail for *M. nigrocincta* in the present study, is part of a complex of groundplan apomorphies. The pronotum is a solid and extensive plate-like structure. The propleura cover the entire lateral and ventral prothoracic regions and meet ventromedially, with only a small exposed prosternal sclerite between the procoxae. A strongly pronounced notopleural edge forms an articulation with the posterolateral head capsule. The cervical sclerite is completely fused with the internal skeleton of the propleuron. The procoxae are distinctly enlarged relative to the meso- and metacoxae in workers of *M. nigrocincta* and other groups, including stemgroup fossils (WILSON et al. 1967a,b). This is arguably an additional groundplan apomorphy of Formicidae in all adult castes, with parallel evolution in other aculeate groups such as Dryinidae (Chrysoidea or Dryinoidea: BRANSTETTER et al. 2017b), which catch prey or hosts for their larvae with the forelegs (WALOFF 1974), and Pompilidae (Vespoidea) (L. Vilhelmsen, personal communication).

A crucial character complex is the complete loss of wings and flight related structures (Figs. 2, 3). The mesonotum (Figs. 1A,C, 2A,C, 3A,C, 4A: N2) forms a single sclerite without separate elements like prescutum, scutum or scutellum, and also without mesophragma (Fig. 4B). The mesopleuron (Figs. 1B,C, 2B,C, 3B,C, 4A: P12) is also undivided, and like in the prothorax its paired halves are fused ventromedially. Axillary sclerites, notal wing processes, basalare and subalare are missing in both pterothoracic segments of workers (Figs. 2A,C, 3A,C, 4A). The metanotum (Figs. 2A, 3A: N3) is very narrow and undivided. The metapleuron (P13: 2A, C, 3B, 4A) is strongly reduced. The elongated metafurca (Fig. 4B: Fu3) fuses anteriorly with the slender mesofurca (Fu2: Fig. 4B) and encloses the very slender ventral longitudinal muscle (Fig. 4C: IIIv1m7).

Compared with members of other families of Hymenoptera (Tables 1, S2), the prothoracic musculature appears largely unmodified in alate and flightless ant castes. The dorsal longitudinal muscles Id1m1 and 5, the dorsoventral muscles Idvm5, 9 and 18, the tergo-pleural muscles Itpm1–5, the pleuro-coxal muscles Ipcm1, 4 and 8, the ventral longitudinal muscle Iv1m1, 3 and 7 and sterno-coxal muscles Iscm1, 2, 4 and 6 are present in workers of *M. nigrocincta* and very likely also in the groundplan of Formicidae. As plesiomorphic features alate queens retain a relatively complete muscle set in the mesothorax. This includes well-developed dorsal longitudinal bundles and also dorsoventral, tergo-pleural and pleuro-coxal muscles (LUBBOCK 1881; SAINI et al. 1982). Among them, the presence of muscles Iid1m1, Iidvm1 and 6, and Iitpm7 and 9 is likely ancestral for Formicidae. In contrast, workers, including those of *M. nigrocincta*, have lost most mesothoracic muscles, except those inserted on the mesocoxal rim and mesotrochanter.

The comparison of queens and workers with representatives of other families of Hymenoptera suggests that the sterno-coxal muscles Iiscm1–3 and 6 belong to the groundplan of Formicidae. The number of metathoracic muscles of queens is only slightly less than in other families of Hymenoptera (Tables 1, S2). In contrast, the muscle set is greatly simplified in workers.

Our results confirm that mesosomal groundplan features differ profoundly between ant castes, except for the procoxal-trochanteral articulations, and the meso- and metathoracic articulations with their respective coxae (BOUDINOT 2015), with moderate modifications in alate forms and far-reaching transformations in workers. Anatomical investigations of stemgroup ant fossils (e.g. GRIMALDI et al. 1997; ENGEL & GRIMALDI 2005; PERRICHOT et al. 2008; BARDEN & GRIMALDI 2012), particularly using micro-computed tomography, should have high priority. Combined with detailed morphological data on related groups, this will not only allow for a more reliable assessment of the groundplan, but also a reconstruction of early evolutionary transformations in the group.

4.4. Mesosomal modifications in workers and their functional background

The mesosomal morphology of ant workers was apparently shaped by different but interrelated phenomena. This includes complete winglessness combined with a mechanically reinforced pterothorax, a very movable head with a strongly developed neck musculature, unusually differentiated legs as efficient cleaning tools and locomotory organs, and a highly movable gaster with a stinging apparatus preserved in *Myrmecia* and in the groundplan of the family.

The loss of wings in ant workers has consequences beyond the loss of the ability to fly. To reduce structures required in the context of flight opens perspectives to specialize in other directions (e.g. BURD 2000; SCHILMAN & ROCES 2005; BOHN et al. 2012). Although workers are “cheaply manufactured” with small and light bodies, loss of wing pairs and flight musculature, short life cycle, and lacking ovaries to support the colonial economy (PEETERS & ITO 2015), considering them as a wingless version of the alate queens would be an oversimplification (KELLER et al. 2014).

Compared with alate forms, the pronotum of workers is greatly enlarged, apparently in correlation with an enhanced muscular apparatus (KELLER et al. 2014). This increases the movability in the neck region (SNODGRASS 1935; HARTENSTEIN 2006; KELLER et al. 2014) and also enables the ants to lift and carry objects with their head, such as for instance prey, pieces of plants or seed (e.g. GORB & GORB 1999; MOLL et al. 2010; KELLER et al. 2014; NGUYEN et al. 2014).

An important modification of workers is the far-reaching fusion of sclerites. The dorsal pterothoracic sclerites are reduced to undivided and undifferentiated notal elements. Notal wing processes and axillary sclerites, im-

portant elements controlling flight movements (BRODSKY 1994), are dispensable in flightless morphs and therefore reduced, resulting in an increased mechanical rigidity of the segments. The pterothoracic sclerites are largely fused in ant workers (e.g. RICHARDS 1956; KELLER 2011). This leads to a mechanically very compact mesosoma with a minimum of exposed membranous surfaces, a condition also occurring in beetles, even though achieved in a different way and in most cases with a retained functional flight apparatus (e.g. BEUTEL & HAAS 2000). The reinforced thorax enhances mechanical protection against predators on the ground and possibly also increases the barrier against harmful environmental agents as well as against the loss of water. Ant workers are the caste that forages outside the nest and are strongly exposed to these factors throughout their adult live span (HÖLDOBLER & WILSON 1990; JEMIELITY et al. 2005). This results in selective pressure favoring the formation of rigid exoskeletal structures, made possible mainly by the reduction of the flight apparatus.

In a study on the mesothorax of workers, KELLER et al. (2014) noted the simplification of the mesonotum, the reduction of the mesophragma and the loss of the dorso-longitudinal muscle *IIdm1*. This structural complex generally initiates the first wing depression in the flight stroke cycle in pterygote insects with retained flight ability (BRODSKY 1994), but is usually modified or distinctly reduced in secondarily flightless insects (WIPFLER et al. 2014; LIU et al. 2017). The absence of dorso-ventral muscles *II/IIIIdvm1* is another common feature related to the loss of the flight capacity (KOZLOV 1986; WIPFLER et al. 2014; LIU et al. 2017). As depressors of the notum during wing elevation (BRODSKY 1994), they are dispensable in flightless forms, and in fact completely absent in all examined ant workers (Tables 1, S2).

Some flight-related muscles are retained in worker ants, such as the dorsoventral muscles *II/IIIIdvm4*, 5 and 7, which are even exceptionally well developed. These elements of the muscular system can fulfill more than one function, as wing levators in forms capable of flight, but also in the context of leg movements (KOZLOV 1986; BRODSKY 1994; LIU et al. 2017).

Linked with the loss of the flight capacity, locomotion on different substrates plays an essential role in ant workers. Consequently, the forelegs differ strongly in their structure and armature from the mid- and hindlegs (Figs. 1, 5), even though similarly well-developed muscles operating leg movements are present in all three thoracic segments. The enlarged, elongated procoxae likely allow more efficient movements of the forelegs, together with a modified coxo-trochanteral articulation described in BOUDINOT (2015). The sterno-procoxal muscles *IIsclm1* and 4 distinctly are greatly enlarged in workers. This supports efficient movements on the ground including a broad range of specific activities (HÖLDOBLER & WILSON 1990), such as jumping (CLARK 1951: 7.5–10 cm), digging (WALLIS 1962; SUDD 1969), trophallaxis, antenna cleaning (WALLIS 1962), prey handling (MASUKO 2009), grooming of queens in the case of leaf cutter ants and be-

havior related to maintaining hygiene in fungus gardens (FERNANDEZ-MARIN et al. 2003).

The differentiated arolia and claws further support efficient movement on various surfaces (FEDERLE et al. 2001), even though well-developed pretarsal attachment devices are a common feature in aculeate hymenopterans (FRANSTEICH & GORB 2004). The unusually complex armature of the distal leg parts, especially in the case of the fore- and hindlegs, also plays a role in different functional contexts, for instance efficient cleaning of the antennae. Perfect functioning of cuticular sensilla on the antennae and other body parts likely plays an essential role for ant workers.

A last essential character complex is the highly movable metasoma, with an aculeate stinging device retained in the groundplan of the family (HÖLDOBLER & WILSON 1990). The increased movability of the gastral segments is supported by a strongly developed and specialized petiole musculature (Fig. 4C,D: *IA1*, *IA2*), with marked effects in the context of defense and prey capture (HASHIMOTO 1996). Protection against predators apparently plays an important role for the flightless workers. Aside from the sting, additional defensive adaptations of workers have evolved, including for instance gland secretions or sharp exoskeletal spines (e.g. BUSCHINGER & MASCHWITZ 1984; BLANCHARD & MOREAU 2017; SARNAT et al. 2017).

Structural modifications of ant workers can be seen as optimization for different tasks important for the colony. At the same time, the simplified structure of the worker thorax means “less investment” compared with winged eusocial insects with a complex flight apparatus. Highly efficient workers produced at “low cost” are likely one of the main factors contributing to the ecological dominance and success of ants (PEETERS & ITO 2015). In summary, the reduced flight apparatus of ant workers is part of an evolutionary trade-off: on one hand obvious advantages of flight like for instance dispersal, and on the other various options of flightlessness to optimize other functions in the context of a particularly successful life strategy (ROFF 1990; WAGNER & LIEBHERR 1992).

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6. References

- AGOSTI D. 2005. Productive ants run ahead. – *Systematic Entomology* **30**: 175–176.
- ALAM S.M. 1951. The skeleton-muscular mechanism of *Stenobraccon deesae* Cameron (Braconidae, Hymenoptera). – Aligarh Muslim University. Publications, (Zoological Series) on Indian Insect Types **3**: 1–76.
- ALLGAIER C. 2008. Die wissenschaftlichen Zeichnungen von Hermann Weber. – *Entomologia Generalis* **31**(2): 119–127.
- BARDEN P. 2017. Fossil ants (Hymenoptera: Formicidae): ancient diversity and the rise of modern lineages. – *Myrmecological News* **24**: 1–30.
- BARDEN P., GRIMALDI D. 2012. Rediscovery of the bizarre Cretaceous ant *Haidomyrmex* Dlussky (Hymenoptera: Formicidae), with two new species. – *American Museum Novitates* **3755**: 1–16.
- BARDEN P., GRIMALDI D. 2016. Adaptive radiation in socially advanced stem-group ants from the Cretaceous. – *Current Biology* **26**(4): 1–7.
- BARONI URBANI C. 1989. Phylogeny and behavioural evolution in ants, with a discussion of the role of behavior in evolutionary process. – *Ethology Ecology & Evolution* **1**: 137–168.
- BARONI URBANI C., BOLTON B., WARD P.S. 1992. The internal phylogeny of ants (Hymenoptera: Formicidae). – *Systematic Entomology* **17**: 301–329.
- BEUTEL R.G., HAAS F. 2000. Phylogenetic relationships of the suborders of Coleoptera (Insecta). – *Cladistics* **16**(1): 103–141.
- BILLEN J. 2017. The metapleural gland of *Aneuretus simoni* (Formicidae, Aneuretinae). – *Asian Myrmecology* **9**: 1–5.
- BILLEN J., HASHIM R., ITO F. 2011. Functional morphology of the metapleural gland in workers of the ant *Crematogaster inflata* (Hymenoptera, Formicidae). – *Invertebrate Biology* **130**(3): 277–281.
- BLANCHARD B.D., MOREAU C.S. 2017. Defensive traits exhibit an evolutionary trade-off and drive diversification in ants. – *Evolution* **71**(2): 315–328.
- BOHN H.F., THORNHAM D.G., FEDERLE W. 2012. Ants swimming in pitcher plants: kinematics of aquatic and terrestrial locomotion in *Camponotus schmitzi*. – *Journal of Comparative Physiology A* **198**: 465–476.
- BOLTON B. 2003. Synopsis and classification of the Formicidae. – *Memoirs of the American Entomological Institute* **71**: 1–370.
- BOROWIEC M.L., RABELING C., BRADY S.G., FISHER B.L., SCHULTZ T.R., WARD P.S. 2017. Compositional heterogeneity and outgroup choice influence the internal phylogeny of ants. – *BioRxiv* (doi.org/10.1101/173393).
- BOUDINOT B.E. 2013. The male genitalia of ants: musculature, homology, and functional morphology (Hymenoptera, Aculeata, Formicidae). – *Journal of Hymenoptera Research* **30**: 29–49.
- BOUDINOT B.E. 2015. Contributions to the knowledge of Formicidae (Hymenoptera, Aculeata): a new diagnosis of the family, the first global male-based key to subfamilies, and a treatment of early branching lineages. – *European Journal of Taxonomy* **120**: 1–62.
- BRADY S.G., SCHULTZ T.R., FISHER B.L., WARD P.S. 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. – *Proceedings of the National Academy of Science of the United States and America* **103**(48): 18172–18177.
- BRANSTETTER M.G., LONGINO J.T., WARD P.S., FAIRCLOTH B.C. 2017a. Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. – *Methods in Ecology & Evolution* **8**(6): 1–9.
- BRANSTETTER M.G., DANFORTH B.N., PITTS J.P., FAIRCLOTH B.C., WARD P.S., BUFFINGTON M.L., GATES M.W., KULA R.R., BRADY S.G. 2017b. Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. – *Current Biology* **27**(7): 1019–1025.
- BRODSKY A.K. 1994. *The Evolution of Insect Flight* (2nd ed.). – Oxford University Press, Oxford.
- BROTHERS D.J. 1975. Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. – *The University of Kansas Science Bulletin* **50**: 483–648.
- BROWN W.L. 1954. Remarks on the internal phylogeny and subfamily classification of the family Formicidae. – *Insectes Sociaux* **1**: 21–31.
- BURD M. 2000. Body size effects on locomotion and load carriage in the highly polymorphic leaf-cutting ants *Atta colombica* and *Atta cephalotes*. – *Behavioral Ecology* **11**(2): 125–131.
- BUSCHINGER A., MSCHWITZ U. 1984. Defensive behavior and defensive mechanisms in ants. Pp. 95–150 in: HERMANN H.R. (ed.), *Defensive Mechanisms in Social Insects*. – Praeger Publishers, Westport.
- CLARK J. 1951. The Formicidae of Australia. Volume I. Subfamily Myrmeciinae. Pp. 172 in: CHAPIN E.A. (ed.), *The Quarterly Review of Biology* **29**(2). – CSIRO, Melbourne.
- DE GEER C. 1773. *Mémoires pour servir à l'histoire des insectes*. Tome troisième. – Pierre Hesselberg, Stockholm. 696 pp.
- DE GUSMÃO L.G., CAETANO F.H. & NAKANO O. 2001. Ultramorphology of the metapleural gland in three species of *Atta* (Hymenoptera, Formicidae). – *Iheringia, Série Zoologia* **91**: 33–36.
- DLUSSKY G.M. 1983. A new family of Upper Cretaceous Hymenoptera – an “intermediate link” between the ants and the scolioids. – *Paleontologicheskii Zhurnal* **3**: 65–78.
- DUNCAN C.D. 1939. A contribution to the biology of North American vespine wasps. – *Annals of the Entomological Society of America* **32**(1): 1–272.
- EMERY C. 1877. Saggio di un ordinamento naturale dei mirmicidae e considerazioni sulla filogenesi delle formiche. – *Bollettino della Società Entomologica Italiana* **9**: 67–83.
- EMERY C. 1900. Intorno al Torace delle Formiche. – *Bollettino della Società Entomologica Italiana* **32**: 103–119.
- ENGEL M.S., GRIMALDI D.A. 2005. Primitive new ants in Cretaceous amber from Myanmar, New Jersey, and Canada (Hymenoptera: Formicidae). – *American Museum Novitates* **3485**: 1–24.
- FEDERLE W., BRAINARD E.L., McMAHON T.A., HÖLDOBLER B. 2001. Biomechanics of the movable pretarsal adhesive organ in ants and bees. – *Proceedings of the National Academy of Sciences* **98**(11): 6215–6220.
- FERNANDEZ-MARIN H., ZIMMERMANN J.K., WCISLO W.T. 2003. Nest-founding in *Acromyrmex octospinosus* (Hymenoptera, Formicidae, Attini): demography and putative prophylactic behaviors. – *Insectes Sociaux* **50**(4): 304–308.
- FORBES J. 1938. Anatomy and history of the worker of *Camponotus herculeanus pennsylvanicus* De Geer (Formicidae, Hymenoptera). – *Annals of the Entomological Society of America* **31**: 181–195.
- FRANCOEUR A., LOISELLE R. 1988. Évolution du strigile chez les fourmis. – *Naturaliste Canadien* **115**: 333–353.
- FRANSTEICH L., GORB S. 2004. Structure and mechanics of the tarsal chain in the hornet, *Vespa crabro* (Hymenoptera: Vespidae): implications on the attachment mechanism. – *Arthropod Structure & Development* **33**(1): 77–89.
- FRIEDRICH F., BEUTEL R.G. 2008a. The thorax of *Zorotypus* (Hexapoda, Zoraptera) and a new nomenclature for the musculature of Neoptera. – *Arthropod Structure & Development* **37**: 29–54.
- FRIEDRICH F., BEUTEL R.G. 2008b. Micro-computer tomography and a renaissance of insect morphology. – *SPIE* **7078**: doi: 10.1117/12.794057.
- FRIEDRICH F., MATSUMURA Y., POHL H., BAI M., HÖRNSCHEMEYER T., BEUTEL R.G. 2014. Insect morphology in the age of phylogenomics: innovative techniques and its future role in systematics. – *Entomological Science* **17**(1): 1–24.
- GORB S.N., GORB E.V. 1999. Effects of ant species composition on seed removal in deciduous forest in Eastern Europe. – *Oikos* **84**(1): 110–118.
- GOTWALD W.H., KUPIEC B.M. 1975. Taxonomic implications of Doryline worker ant morphology: *Cheliomyrmex morosus* (Hymenoptera: Formicidae). – *Annals of the Entomological Society of America* **68**(6): 961–971.

- GOTWALD W.H., SCHAEFER R.F. 1982. Taxonomic implications of Doryline worker ant morphology: *Dorylus* subgenus *Anomma* (Hymenoptera: Formicidae). – *Sociobiology* 7(2): 187–204.
- GRIMALDI D., AGOSTI D., CARPENTER J.M. 1997. New and rediscovered primitive ants (Hymenoptera: Formicidae) in Cretaceous amber from New Jersey, and their phylogenetic relationship. – *American Museum Novitates* 3208.
- HARTENSTEIN V. 2006. The muscle pattern of *Drosophila*. Pp. 8–27 in SINK H. (ed.), *Muscle Development in Drosophila*. – Springer Science+Business Media, New York.
- HASHIMOTO Y. 1996. Skeletomuscular modifications associated with the formation of an additional petiole on the anterior abdominal segments in aculeate Hymenoptera. – *Japanese Journal of Applied Entomology and Zoology* 64(2): 340–356.
- HITA GARCIA F., FISCHER G. 2011. The ant genus *Tetramorium* Mayr (Hymenoptera: Formicidae) in the Malagasy region – introduction, definition of species groups, and revision of the *T. bicarinatum*, *T. obesum*, *T. sericeiventre* and *T. tosii* species groups. – *Zootaxa* 3365: 1–123.
- HITA GARCIA F., FISCHER G., LIU C., AUDISIO T.L., ALPERT G.D., FISHER B.L., ECONOMO E.P. 2017. X-ray microtomography for ant taxonomy: An exploration and case study with two new *Terataner* (Hymenoptera, Formicidae, Myrmicinae) species from Madagascar. – *PLoS One* 12(3): e0172641.
- HÖLDOBLER B., ENGEL-SIEGEL H. 1984. On the metapleural gland of ants. – *Psyche* 91: 201–224.
- HÖLDOBLER B., WILSON E.O. 1990. *The Ants*. 1st edn. – Belknap, Cambridge. 746 pp.
- JANET C. 1897. Limites morphologiques des anneaux post-cephaliques et musculature des anneaux post-thoraciques chez la *Myrmica rubra*. – *Société Zoologique de France*, Paris, 36 pp.
- JEMIELITY S., CHAPUISAT M., PARKER J.D., KELLER L. 2005. Long live the queen: studying aging in social insects. – *Age* 27(3): 241–248.
- KEISTER M. 1962. The anatomy of the tracheal system of *Camponotus pennsylvanius* (Hymenoptera: Formicidae). – *Annals of the Entomological Society of America* 56: 336–340.
- KELLER R.A. 2011. A phylogenetic analysis of ant morphology (Hymenoptera: Formicidae) with special reference to the Poneromorph subfamilies. – *Bulletin of the American Museum of Natural History* 355: 1–90.
- KELLER R.A., PEETERS C., BELDADE P. 2014. Evolution of thorax architecture in ant castes highlights trade-off between flight and ground behaviors. – *eLife* 3: e01539.
- KOZLOV M.V. 1986. Functional morphology of pterothoracic muscles in *Operophtera brumata* L. (Lepidoptera, Geometridae). – *Zhurnal Evolutsionnoi Biokhimii i Fiziologii* 22: 492–496.
- KÜCK P., HITA GARCIA F., MISOF B., MEUSEMANN K. 2011. Improved phylogenetic analyses corroborate a plausible position of *Martialis heureka* in the ant tree of life. – *PLoS ONE* 6: e21031.
- LAPOLLA J.S., DLUSSKY G.M., PERRICHOT V. 2013. Ants and the fossil record. – *Annual Review of Entomology* 58: 609–630.
- LIU S.-P., WIPFLER B., NIITSU S., BEUTEL R.G. 2017. The thoracic anatomy of the male and female winter moth *Nyssiodes lefuarius* (Lepidoptera: Geometridae) and evolutionary changes in the thorax of moths and butterflies. – *Organisms Diversity & Evolution* 17(3): 565–594.
- LUBBOCK J. 1881. III. On the anatomy of ants. – *Transaction of the Linnean Society of London*. 2nd Series. *Zoology* 2(2): 141–154.
- MAKI T. 1938. Studies on the thoracic musculature in insects. – *Memoirs of the Faculty of Science and Agriculture, Taihoku Imperial University* 24(1): 1–343.
- MARKL H. 1966. Peripheres Nervensystem und Muskulatur im Thorax der Arbeiterin von *Apis mellifica* L., *Formica polyctena* Foerster und *Vespa vulgaris* L. und der Grundplan der Innervierung des Insekten thorax. – *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenie der Tiere* 83: 107–184.
- MASUKO K. 2009. Studies on the predatory biology of Oriental dacetine ants (Hymenoptera: Formicidae) II. Novel prey specialization in *Pyramica benten*. – *Journal of Natural History* 43(13–14): 825–841.
- MATSUDA R. 1970. Morphology and evolution of the insect thorax. – *Memoirs of the Entomological Society of Canada* 76: 1–431.
- MIKÓ I., VILHELMSSEN L., JOHNSON N.F., MASNER L., PÉNZES Z. 2007. Skeletomusculature of Scelionidae (Hymenoptera: Platygastroidea): head and mesosoma. – *Zootaxa* 1571: 1–78.
- MOLL K., ROCES F., FEDERLE W. 2010. Foraging grass-cutting ants (*Atta vollenweideri*) maintain stability by balancing their loads with controlled head movement. – *Journal of Comparative Physiology* 196(7): 417–480.
- MOREAU C.S., BELL C.D. 2013. Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. – *Evolution* 67(8): 2240–2257.
- MOREAU C.S., BELL C.D., VILA R., ARCHIBALD S.B., PIERCE N.E. 2006. Phylogeny of the ants: diversification in the age of angiosperms. – *Science* 312(5770): 101–104.
- NASSANOFF N.W. 1889. Materials for the anatomy of the ant (*Lasius flavus* Fabr.). – *Proceedings of the Zoological Museum of the Moscow State University* 4: pt 1.
- NGUYEN V., BLAINE L., CASTRO C. 2014. The exoskeletal structure and tensile loading behavior of an ant neck joint. – *Journal of Biomechanics* 47(2): 497–504.
- NINOMIYA T., YOSHIZAWA K. 2009. A revised interpretation of the wing base structure in Odonata. – *Systematic Entomology* 34: 334–345.
- OGATA K., TAYLOR R.W. 1991. Ants of the genus *Myrmecia* Fabricius: a preliminary review and key to the named species (Hymenoptera: Formicidae: Myrmeciinae). – *Journal of Natural History* 25: 1623–1673.
- PEETERS C., ITO F. 2015. Wingless and dwarf workers underlie the ecological success of ants (Hymenoptera: Formicidae). – *Myrmecological News* 21: 117–130.
- PERRICHOT V., NÉL A., NÉRAUDEAU D., LACAU S., GUYOT T. 2008. New fossil ants in French Cretaceous amber (Hymenoptera: Formicidae). – *Naturwissenschaften* 95(2): 91–97.
- POHL H. 2010. A scanning electron microscopy specimen holder for viewing different angles of a single specimen. – *Microscopy Research and Technique* 73(13): 1073–1076.
- RABELING C., BROWN J.M., VERHAAGH M. 2008. Newly discovered sister lineage sheds light on early ant evolution. – *Proceedings of the National Academy of Sciences* 105(39): 14913–14917.
- REID J.A. 1941. The thorax of the wingless and short-winged Hymenoptera. – *Transactions of the Royal Entomological Society of London* 91(8): 367–446.
- ROFF D. 1990. The evolution of flightlessness in insects. – *Ecological Monographs* 60(4): 389–421.
- SAINI M.S., DHILLON S.S., AGGARWAL R. 1982. Skeletomuscular differences in the thorax of winged and non-winged forms of *Camponotus camelinus* (Smith) (Hym., Formicidae). – *Deutsche Entomologische Zeitschrift* 29(4–5): 447–458.
- SARNAT E.M., FRIEDMAN N.R., FISCHER G., LECROQ-BENNET B., ECONOMO E.P. 2017. Rise of the spiny ants: diversification, ecology and function of extreme traits in the hyperdiverse genus *Phenacolepis* (Hymenoptera: Formicidae). – *Biological Journal of the Linnean Society* 122(3): 514–538.
- SCHILMAN P.E., ROCES F. 2006. Foraging energetics of a nectar-feeding ant: metabolic expenditure as a function of food-source profitability. – *Journal of Experimental Biology* 209: 4091–4101.
- SMITH F. 1858. *Catalogue of Hymenopterous Insects in the Collection of the British Museum*. Part VI. Formicidae. – *British Museum*, London. 216 pp.
- SNODGRASS R.E. 1910a. The thorax of the Hymenoptera. – *Proceedings of the United States National Museum* 39(1774): 37–91.
- SNODGRASS R.E. 1910b. *The Anatomy of the Honey Bee*. – *Government Printing Office*, Washington. 236 pp.
- SNODGRASS R.E. 1925. *Anatomy and Physiology of the Honeybee*. – *McGraw-Hill Book Company*, New York & London. 327 pp.
- SNODGRASS R.E. 1935. *Principles of Insect Morphology*. – *McGraw-Hill Book Company*, New York & London. 667 pp.

- SNODGRASS R.E. 1942. The skeletomuscular mechanism of the honey bee. – *Smithsonian Miscellaneous Collections* **103**(2): 1–120.
- SUDD J.H. 1969. The excavation of soil by ants. – *Ethology* **26**(3): 257–276.
- TAYLOR R.W. 1978. *Nothomyrmecia macrops*: A living-fossil ant rediscovered. – *Science* **201**: 979–985.
- TULLOCH G.S. 1935. Morphological studies of the thorax of the ant. – *Entomologica Americana* **15**(3): 93–131.
- VILHELMESEN L. 2000a. Cervical and prothoracic skeleton-musculature in the basal Hymenoptera (Insecta): Comparative anatomy and phylogenetic implications. – *Zoologischer Anzeiger* **239**: 105–138.
- VILHELMESEN L. 2000b. Before the wasp-waist: Comparative anatomy and phylogenetic implications of the skeleton-musculature of the thoraco-abdominal boundary region in basal Hymenoptera (Insecta). – *Zoomorphology* **119**: 185–221.
- WAGNER D.L., LIEBHERR J.K. 1992. Flightlessness in insects. – *Trend in Ecology and Evolution* **7**(7): 216–220.
- WALLIS D.I. 1962. Aggressive behavior in the ant, *Formica fusca*. – *Animal Behaviour* **10**(3–4): 267–274.
- WALOFF N. 1974. Biology and behaviour of some species of Dryinidae (Hymenoptera). – *Journal of Entomology Series A, General Entomology* **49**(1): 97–109.
- WARD P.S. 2014. The phylogeny and evolution of ants. – *Annual Review of Ecology, Evolution, and Systematics* **45**: 23–43.
- WARD P.S., BRADY S.G. 2003. Phylogeny and biogeography of the ant subfamily Myrmeciinae (Hymenoptera: Formicidae). – *Invertebrate Systematics* **17**: 361–386.
- WEBER H. 1969. Die Elefantenlaus *Haematomyzus elefantis* Piaget 1896. – *Anzeiger für Schädlingkunde* **42**(6): 92–93.
- WILSON E.O., CARPENTER F.M., BROWN JR.W.L. 1967a. The first Mesozoic ants, with the description of a new subfamily. – *Psyche* **74**(1): 1–19.
- WILSON E.O., CARPENTER F.M., BROWN JR.W.L. 1967b. The first Mesozoic ants. – *Science* **157**: 1038–1040.
- WILSON E.O. 1971. *The Insect Societies*. – Harvard University Press, Cambridge. 548 pp.
- WIPFLER B., POHL H., YAVORSKAYA M.I., BEUTEL R.G. 2016. A review of methods for analysing insect structures – the role of morphology in the age of phylogenomics. – *Current Opinion in Insect Science* **18**: 60–68.
- WIPFLER B., KLUG R., GE S.-Q., BAI M., GÖBBELS J., YANG X.-K., HÖRNSCHEMEYER T. 2015. The thorax of Mantophasmatodea, the morphology of flightlessness, and the evolution of the neopteran insects. – *Cladistics* **31**(1): 50–70.
- WHEELER W.M. 1928. *The Social Insects: Their Origin and Evolution*. – Kegan Paul, Trech, Trubner & Co., Ltd., London. 378 pp.
- WHELDEN R.M. 1960. The anatomy of *Rhytidoponera metallica* F. Smith (Hymenoptera: Formicidae). – *Annals of the Entomological Society of America* **53**(6): 793–808.
- YEK S.H., MUELLER M.G. 2011. The metapleural gland of ants. – *Biological Reviews* **86**: 774–791.
- YODER M.J., MIKÓ I., SELTMANN K.C., BERTONE M.A., DEANS A.R. 2010. A gross anatomy ontology for Hymenoptera. – *Plos One* **5**(12): e15991.
- YOSHIMURA M., FISHER B.L. 2007. A revision of male ants of the Malagasy region: key to subfamilies and treatment of the genera of Ponerinae. – *Zootaxa* **1654**: 21–40.

Electronic Supplement Files

at <http://www.senckenberg.de/arthropod-systematics>

File 1: liu&al-myrmeciathorax-asp2019-electronicssupplement-1.xls — **Table S1.** Character list and matrix. — DOI: 10.26049/ASP77-1-2019-01/1

File 2: liu&al-myrmeciathorax-asp2019-electronicssupplement-2.xls — **Table S2.** Muscular homology chart of Hymenoptera (present with “+” or muscular name in green, absent with “-” in pink, uncertain with “?” or “/” in yellow. In Formicidae, the muscles only present in workers are labeled in dark green; muscles only occurring in alate castes labeled in dark blue.) — DOI: 10.26049/ASP77-1-2019-01/2