Morphology of the male genitalia of *Brachymyrmex* and their implications in the Formicinae phylogeny

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Abstract

The male genitalia of *Brachymyrmex* are examined and terminology clarified. We document two conformation groups based on the lateral carina, conformation 1 has a complete lateral carina that reaches the apex of the valviceps and in conformation 2 the lateral carina is broken with the apical end projected dorsally. Previously documented species are evaluated in the context of this new information. We offer support for the movement of *Brachymyrmex* into the resurrected tribe Myrmelachistini based on the morphology of the penisvalvae, and offer definitive methods of assigning unknown specimens to this genus. This study provides histological methodologies for the preparation, differentiation, and permanent storage of minute ant genitalia structures and the associated musculature.

Keywords

*Brachymyrmex*, genitalia, morphology, penisvalvae, phylogeny
Introduction

The ant genus *Brachymyrmex* Mayr, 1868 exists in a state of taxonomic confusion. The small workers are notably devoid of good species delineating characters, and their distributions are not well represented in collections. This “miserable little genus” (Creighton 1950) has been largely ignored in systematic myrmecological works. Currently there are 60 valid species, of which only 21 contain male descriptions, and five of these have genitalia illustrations. The male genitalia offer species level characters that are desperately needed for this taxonomically challenging group (Quirán et al. 2004). In order to utilize the characters to the best of our ability we must first understand their morphology. Boudinot (2013) has provided a comprehensive review of the morphological structures and musculature associated with ant male genitalia, allowing for comparative studies across ants as a group.

Taxonomic history of Brachymyrmex

*Brachymyrmex* was described by Mayr (1868) from the type species *Brachymyrmex patagonicus*. Forel (1878) included *Brachymyrmex* in the subfamily Camponotinae, and in the tribe Plagiolepidini (Forel 1893). Ashmead (1905) included the Plagiolepidini in the subfamily Formicinae. Forel (1912) erected the tribe Myrmelachistini and defined the tribe as having females with an antennomere count of 9 or 10, males being 10 or 11-merous, and included the genera *Aphomyrmex*, *Brachymyrmex*, *Myrmelachista*, and *Rhopalomymrex*. Emery (1925) redefined the Myrmelachistini as having fewer than 12 antennomeres, with a distinct antennal club. This redefinition removed *Brachymyrmex* from the Myrmelachistini and Emery placed it in the tribe Dimorphomyrmicini. Emery included along with *Brachymyrmex*, the genera *Aphomyrmex*, *Cladomyrma*, *Dimorphomyrmex*, and *Gesomyrmex*, based on the 8 to 9 merous antennomeric count lacking an antennal club, short frontal carinae, and large eyes. Emery further considered *Aphomyrmex*, *Brachymyrmex*, and *Cladomyrma* to be closely related and had them comprise the subtribe Brachymyrmicini. Wheeler (1929) treated *Dimorphomyrmex* as a junior synonym of *Gesomyrmex*, and concluded that *Gesomyrmex* was not closely related to the other members of Dimorphomyrmicini. As a result Wheeler raised Emery’s subtribe Brachymyrmicini to tribal status, and resurrected Gesomyrmicini to include *Gesomyrmex*. Wheeler and Wheeler (1976, 1985) concluded *Aphomomyrmex*, *Brachymyrex*, *Cladomyrma*, *Euprenolpis*, *Paratrechina*, *Petelomyrmex*, *Prenolepis*, and *Stigmacros* to be in the Brachymyrmicini, using larval characters. Agosti (1991) placed *Brachymyrmex* within the *Pseudolasius* genus-group defined by widely separated hind coxae, the petiole ventrally u-shaped, a simple helcium which is antero-ventrally often concealed by the anteriorly fused sternite and tergite. Bolton (2003) transferred *Brachymyrmex* to the Plagiolepidin. Recently Ward et al. (2016) transferred both *Brachymyrmex* and *Myrmelachista* to the Myrmelachistini, which they resurrected. Ward et al. define this group as having the following shared worker characters: a reduced antennomere count (9–10-merous), five mandibular teeth, petiolar node inclined anteriorly, with the base of abdominal segment III completely
fused tergosternally on each side of the helcium, and a plesiomorphic 6,4 palp formula that is reduced to 5,4 in some Myrmelachista (Ward et al. 2016).

Santschi (1923) provided the last revision of the group, designating many new species and subspecies. His descriptions, however, are of little value, as they are short and include few comparative characters. In his revision Santschi erected the subgenus Bryscha, which he defined by having dimorphic workers, short and flared sepals of the proventriculus, and erect hairs on the scape and legs. Bryscha was provisionally synonymized under Brachymyrmex by Brown (1973). Although most Brachymyrmex have monomorphic workers; at least two species are dimorphic and Bryscha may need to be resurrected (Ortiz and Fernández 2014).

The genus has a wide diversity of life histories. Most species are generalist nesters that nest in leaf litter, under stones, or under bark; at least two species have a distinct replete caste; while others are arboreal nesting under epiphyte mats or in small plant cavities. By contrast, the closely-related Myrmelachista are obligate arboreal nesting ants (Longino 2006).

As our understanding of ant evolution progresses, the need for additional taxonomic and systematic information is increasing. The primary source for morphological characters in ants is worker morphology. This trend is easy to understand as workers are ubiquitous in the environment, often being the only caste collected and certainly the most prevalent. Queens and males on the other hand are less-frequently encountered, as they are usually within nests or produced only for brief periods. The reproductive casts have thus been largely neglected. Males are largely ignored, and therefore represent an untapped source of morphological characters for taxonomic and phylogenetic use. Moreover, when collected, males may be difficult or even impossible to associate with their workers. However, males may be associated through careful collecting and molecular phylogenies. A recent movement among ant researchers has been to pay more attention to males, both when collecting in the field and unassociated males (Boudinot 2013, Boudinot 2015, Deyrup and Cover 2004, LaPolla et al. 2012, and Yoshimura and Fisher 2007, 2009, 2012).

The most useful character for males are arguably the genitalia, and males, like workers, range in size from the Dorylus “sausage flies” to remarkably small Brachymyrmex. Viewing and manipulating the male genitalia of larger species is often simple enough; most genitalia are even large enough to point mount for permanent storage. Smaller species, however, are neither simple to manipulate nor store. This has led some authors to not fully dissect the genitalia from small males obtaining images in situ either through compound microscopy or scanning electron microscopy (SEM) (LaPolla et al. 2012, and Ortiz and Fernández 2014). With the high cost and low availability of SEM, stereoscopic microscopy is more available, although resolution at 40× is often poor. Due to the low resolution at this magnification, small genitalic structures tend to blend together and become difficult to interpret in situ. While there is value in the structure of an intact genital capsule, there remains a large portion of both the volsella, and penisvalva that cannot be viewed, losing potentially important characters.

Here we present methods for dissection and permanent storage via slide mounting of small ant genitalia, with an additional option for muscle fixture and the ability to
clear structures for SEM. Slide mounting genitalia allows examination at much higher magnifications than under stereoscopic microscopy, without the need for sputter coating or environmental SEM prep. Additionally the remainder of the specimen remains intact, losing, at most, only the last three segments. Notes on the genitalia of Brachymyrmex and their implications on the Formicinae phylogeny are presented using the described dissection methodologies. It is our hope that this paper provides a greater understanding of the male genitalia characters for Brachymyrmex, and encourages renewed interest in this “miserable little genus”.

Materials and methods

Tissue sampling

For this study we sampled 16 species/morphospecies of Brachymyrmex, specimens were taken from the first author’s personal collection (CMWC), the Sam Houston State University Entomology Collection (SHSUE), the John T. Longino collection (JTLC), or accessed via AntWeb.org. Specimens from the collections were either in 95% EtOH or dried. Once dissected, bodies without genitalia were returned to the collections dried and point mounted, with the genitalia deposited in the CMWC on slides. The genitalia where dissected under an Olympus SZX12 dissecting microscope with a maximum 115× magnification mounted with a Olympus DP72 camera. Examined under an Olympus BX53 compound microscope with 10×, 40×, and 60× planapochromatic objectives with differential interference contrast prism mounted with a Olympus DP72 camera, and an Olympus B-Max 41 compound microscope with 10×, 40×, and 60×, with universal and planapochromatic objectives with either bright-field or phase-contrast condenser mounted with a Olympus DP-12 camera. Additional specimens were prepared for SEM using a Vega Scan high vacuum micrograph. Photos taken with the aforementioned cameras were stacked by hand using Adobe Photoshop CS6.

Microtechnique

The procedures for the unstained whole mounts are as follows. The terminal segments of male reproductive organs were removed with a surgical scalpel and size 000 insect pins. Tissues were transferred and presoaked in a 20% sodium hydroxide (NaOH) solution for ca. 30 minutes to dissolve muscle and soft tissues. The remaining sclerites were neutralized with a 20% solution of Acetic acid (C₂H₄O₂) for 5 minutes. Dehydration of tissues was initiated in 70% ethanol and continued at 20 min intervals through a graded series of ethanol washes, ending in two final washes with 100% ethanol. Tissues were cleared to near transparency with an initial introduction to xylene via a 50:50 solution of xylene:ethanol, and two 20 minutes washes in histological grade xylene. Prepared specimens were permanently mounted with dammar balsam mounting me-
dia. Alternatively, specimens can be directly taken form 100% ethanol and mounted in euparal. Slides are placed on a pre-warmed slide warmer and allowed to cure overnight.

The procedures for stained whole mounts are as follows. The terminal segments of male reproductive organs were removed with a surgical scalpel and size 000 insect pins. In order to examine the insertion and attachment sites for all muscles, excised tissues were not dissolved. Hydration of reproductive tissues were initiated in 70% ethanol and regressed to a final wash in deionized water. Prepared tissues were stained for 20 minutes in Harris Hematoxylin (without counterstain), and washed in an acid alcohol de-stain for 5–10 seconds. Stained tissues were then dehydrated with ethanol through an ascending graded series, cleared in xylene and permanently mounted with Damar Balsam mounting media. Examination of prepared tissue mounts follows the description above. The figures presented here are grouped by similar morphology and grouping statements accompany the respective figures.

Results

The terminology herein follows Boudinot (2013). The genital capsule is composed of three paired valves, sternum IX, and the cupula. Working from medial to lateral we find a paired median valve termed the penisvalva, each segment of the penisvalva is composed of an arm-like apophysis termed the valvura, and the distal components or valviceps. The paired penisvalvae are joined dorsomedially by the penisvalva membrane. On either side of the penisvalva are the paired volsellae, which are attached to the basimere via the basivolsella and volsellar membrane. The volsellae themselves are composed of the parossiculus, the cuspis, and the digitus which is basolaterally articulated with the parossiculus. The lateral most paired valves are the parameres, which are composed of a large dish like basimere, and an elongated distal telomere. The cupula is a small thin sclerite attached to abdominal tergum IX and the volsella, and is easily left behind when removing the genital capsule. We have not examined the cupula of Brachymyrmex.

Penisvalvae

The paired penisvalvae of Brachymyrmex show tremendous variation in general shape, ranging from scimitar, to sickle-shaped, and quadrate. All species in which the males have been examined show dentition on the ventral ridge of the penisvalvae, which is conspicuously absent in Myrmelachista (though it should be noted that dentition is present throughout many of the formicids) (Fig. 1).

LaPolla and Longino (2006) suggest that the dorsal placement of the apodemal ridge in Brachymyrmex, Cladomyrma, and Myrmelachista as a potential synapomorphy for these three genera. A clarification of terms is needed. The penisvalva is composed of two parts, the valvura and the valviceps. The valvura bears all of the muscular insertions, and is an internal apophysis while the valviceps is an external blade like
structure. The insertion of muscle $k$ (= dorsal gonostyle/volsella complex penisvalval muscle) delimits the valvura posterodorsally.

As the base of the valvura is produced laterally, it may be referred to as the “lateral apodeme” (Fig. 1). Muscles $i$ (= ventroapical parameral-penisvalvar muscle) and $l$ (= lateral parameral-penisvalvar muscle) attach to the penisvalva via the lateral apodeme. The apodeme and its associated musculature allow the penisvalvae to pivot on the mediosagittal plane. The lateral apodeme in most ants is situated at the anterior end of a lateral carina (referred to by Lapolla and Longino as the “apodemal ridge”). This lateral carina is on the external surface of the valviceps (i.e. is not internal) and therefore cannot be an apodeme. The lateral carina cups the volsella dorsally in situ, and is a structure which displays taxonomically valuable variation. Additionally the “dorsally placed” carina (“apodemal ridge”) interpretation of Lapolla and Longino (2006) is anatomically imprecise and suggest that the lateral carina has migrated dorsally to a position which is dissimilar to other Formicinae. Instead, it is apparent that the lateral carina is unmodified in position and that, rather, the anterodorsal region of the valviceps has been reduced such that the lateral apodeme delimits the valviceps anterodorsally.
Two main conformation patterns can be seen in the penisvalval morphology among the *Brachymyrmex* we sampled. In conformation 1 the lateral carina is complete, running from the lateral apodeme to the apex of the valviceps. In this conforma-
tion group the lateral carina forms a sharp curve dorsally near the carina’s base (Fig. 1C). This group includes *Brachymyrmex cordemoyi* Forel, 1895, *B. patagonicus* and *Brachymyrmex obscurior* Forel, 1893 (Figs 2A, 3C, F). The general shape, most notably the lack of a raised anterior portion along the valviceps blade, can also be seen in the illustrations of *Brachymyrmex breviceps* Emery, 1906 (Quirán 2005), *Brachymyrmex bruchi* Forel, 1912, and *Brachymyrmex oculatus* Santschi, 1919 (Quirán et al. 2004). This conformation is rather distinct among the Formicinae which tend to have a fairly flat, or gently forming a convex lateral carinae (Fig. 1D–I).

The lateral carina of conformation 2 is incomplete being broken apically of the lateral apodeme into an anterior portion, which is continuous with the lateral apodeme, and a posterior portion that is raised dorsally above the blade of the valviceps (Fig. 1B). This conformation group includes all other taxa we examined (Figs 4–6). The broken lateral carina is almost certainly unique and is likely to be a synapomorphy for species with this morphology. Though this is a tentative evaluation that requires further sampling to fully elucidate.

Quirán’s (2007) illustration of *Brachymyrmex australis* Forel, 1901 is difficult to place in either conformation group. The penisvalvae illustration lacks a raised anterior portion, however the lateral carina is not indicated, furthermore the volsella is similar to those in conformation 2, with a medial concavity (see discussion of the volsella below). Ortiz and Fernández’s (2014) description, and accompanying figure for the male of *Brachymyrmex pilipes* Mayr, 1887 is inconclusive. The majority of the valviceps is covered by the telomere, and the volsella is completely hidden. That being said, the dorsum of the apical end of the valviceps does appear to be projected upwards, as we see in conformation 2.
Figure 4. Paramere, volsella, and penisvalva of *Brachymyrmex* CMW-011 (basivolsella fragmented) A–C *Brachymyrmex* CMW-012 (basivolsella fragmented) D–F *Brachymyrmex cavernicola* G–I *Brachymyrmex heeri* J–L Grouping: lateral carinae broken; digitus elongate, apex curving ventrad and not bifurcate apically; valvula oblique to the long axis of the valviceps.

**Volsella**

The volsella of *Brachymyrmex* follows the pattern of some *Myrmelachista* where the cuspis is reduced to a small triangular protrusion affixed to the wall of the digitus (*B. obscurior*, (Fig. 3B) *B. CMW-006, B. CMW-003, B. CMW-008, B. CMW-010, (Fig. 5B, E, H, K) B. JTL-004, B. JTL-005, and *Brachymyrmex depilis* Emery, 1893 (Fig. 6H, K)), or completely absent (*B. CMW-007, B. CMW-009 (Fig. 6B, E)). Unlike *Myrmelachista*, there is little variation in the cuspis, though in some species the cuspis is slightly protruded (*B. CMW-011, B. CMW-012, Brachymyrmex cavernicola* Wheeler, 1938 and *Brachymyrmex heeri* Forel, 1874 (Fig. 4B, E, H, K)). *Brachymyrmex*
Figure 5. Paramere, volsella, and penisalva of *Brachymyrmex* CMW-006 (basivolsella fragmented) A–C *Brachymyrmex* CMW-003 D–F *Brachymyrmex* CMW-008 G–I *Brachymyrmex* CMW-010 J–L Grouping: lateral carinae broken; digitus lobate; valvula perpendicular to the long axis of the valviceps.

cordemoyi is unique amongst the specimens we examined, the cuspis is ovate, and well developed (in regards to *Brachymyrmex*), and a number of flexuous setae are present (Fig. 3E). In addition the digitus also bears setae, which is similarly unique. A row of flexuous setae is present ventromedially on the basivolsella in all taxa we examined and can serve as a method of orienting the dissected volsella. The shape of the digitus ranges from a subtriangular, or subquadrate short broad projection (*B. patagonicus* (Fig. 7C), *B. CMW-006, B. CMW-003, B. CMW-008, B. CMW-010* (Fig. 5B, E, H, K)) to a long narrow structure with the apical portion either curving ventrad (*B. CMW-0011, B. CMW-0012, B. cavernicola, and B. heeri* (Fig. 4B, E, H, K)), or bifurcate (*B. cordemoyi, and B. obscurior* (Fig. 3B, E)). The basivolsella is broadly flattened bending to
Figure 6. Paramere, volsella, and penisvalva of Brachymyrmex CMW-007 (basivolsella fragmented) A–C Brachymyrmex CMW-009 (basivolsella fragmented) D–F Brachymyrmex JTL-004 (basivolsella fragmented) G–I Brachymyrmex JTL-005 (basivolsella fragmented) J–L Brachymyrmex depilis (basivolsella fragmented) M–O Grouping: lateral carinae broken; digitus lobate; valvura perpendicular to the long axis of the valviceps.

curve around the penisvalva (Fig. 2E, F), as a result of disarticulating the volsella from the baimere the basivolsellae are prone to fragmentation this has been noted in the figure legends were it has occurred.
The modification to the lateral carina of the valviceps seen in both conformation groups seem to be related to the method in which the volsella rests against the valviceps in situ. As a result the dorsal surface of the volsella in conformation 1 is unbroken, while the dorsal surface of conformation 2 has a distinct concavity medially (Fig. 7).

Paramere

The telomeres are separated from the basimere by a membrane. Of the structures associated with the genital capsule, the telomeres of *Brachymyrmex* show the least variation in terms of overall shape and size. They are generally short and broad with numerous elongate flexuous setae. Of the taxa we examined, the telomeres of *B. CMW-007*, and *B. CMW-009* (Fig. 6A, D) are as broad as long and hemispherical in shape; those of *B. cavernicola, B. heeri*, (Fig. 4G, J) *B. CMW-006*, *B. CMW-003 B. CMW-008*, *B. CMW-010* (Fig. 5A, D, G, J), *B. CMW-012* (Fig. 4D), *B. JTL-004, B. JTL-005*, and *B. depilis* (Fig. 6G, J, M) are slightly longer than broad and subtriangular; and those of *B. cordemoyi, B. obscurior* (Fig. 3A, D) and *B. CMW-11* (Fig. 4A) are longer than broad, and finger like in shape. The basimere of *Brachymyrmex* are sub-triangular, and broad dorsally. The basimere are also difficult to separate from the volsella completely intact and often fragment.

Discussion

Our findings demonstrate that males of this genus may offer more taxonomic information for understating the evolution within the genus, and for species delineations than their worker counterparts. The morphology of the penisvalva lateral carina displayed in the two conformation groups seen in *Brachymyrmex* likely represent two distinct clades within the genus, and when compared on an intraspecific level there seems to be little variation in genital morphology. Of course additional sampling is needed to confirm this. In regard to the tribal position of *Brachymyrmex* in light of the conclu-
sive phylogeny of Blaimer et al. (2015) the characters that have previously been used
to unite various genera with *Brachymyrmex*, namely that of the reduced antennomere
count, and the reduction of the anterodorsal region of the valviceps must be considered
homoplasious.

Within *Brachymyrmex* Ortiz and Fernández (2014) redescribed two dimorphic
species of *Brachymyrmex*, *B. pilipes*, and *Brachymyrmex micromegas* Emery, in Santschi,
1923. While these ants do fall within the current definition of the genus, the authors
raised questions as to the generic placement of these species. As noted previously their
figure for the male of *B. pilipes* is inconclusive, however it does outwardly resemble the
morphology seen in our conformation 2. Unfortunately the male of *B. micromegas* is
unknown. We hope that our work here stimulates future studies on these dimorphic
species to include dissected male genitalia, as these traits may help to accurately place
dimorphic species.

Future researchers collecting *Brachymyrmex* are encouraged to exhaustively, and
carefully hunt for males along with associated workers. Those running Malaise and
UV light traps should also be alert for male specimens. To that regard *Brachymyrmex*
holds a number of invasive ant species, *B. patagonicus* for example is a widespread
and has reached pest status in the United States (MacGown et al. 2007). The males
of this species can accumulate in UV light traps in the hundreds (Danny McDonald
personal communication). Our results here should be useful in obtaining a definitive
genus diagnosis, and in the United States our native species, *B. depilis*, belongs to con-
formation group 2 which is easily separated from the invasive *B. patagonicus*, and *B.
obscurior* both of which belong to conformation group 1. Outside of the United States
the uniqueness of *Brachymyrmex* genitalia should serve as a valuable tool for invasive
species detection with the implementation of general collecting methods.

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Morphology of the male genitalia of Brachymyrmex and their implications...


Supplementary material 1

**Specimen locality data**

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Data type: Specimen locality data

Explanation note: Locality data for dissected specimens.

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