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UCE Phylogenomics Resolves Major Relationships Among Ectaheteromorph Ants (Hymenoptera: Formicidae: Ectatomminae, Heteroponerinae): A New Classification For the Subfamilies and the Description of a New Genus

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Abstract

Uncovering the evolutionary history of the subfamilies Ectatomminae and Heteroponerinae, or ectaheteromorphs, is key to understanding a major branch of the ant tree of life. Despite their diversity and ecological importance, phylogenetic relationships in the group have not been well explored. One particularly suitable tool for resolving phylogeny is the use of ultraconserved elements (UCEs), which have been shown to be ideal markers at a variety of evolutionary time scales. In the present study, we enriched and sequenced 2,127 UCEs from 135 specimens of ectaheteromorph ants and investigated phylogeny using a variety of model-based phylogenomic methods. Trees recovered from partitioned maximum-likelihood and species-tree analyses were well resolved and largely congruent. The results are consistent with an expanded concept of Ectatomminae that now includes the subfamily Heteroponerinae **new synonym** and its single tribe Heteroponerini **new combination**. Eleven monophyletic groups are recognized as genera: *Acanthoponera, Alfaria* **status revived**, *Boltonia* Camacho and Feitosa **new genus**, *Ectatomma*, *Gnamptogenys*, *Heteroponera*, *Holcoponera* **status revived**, *Poneracantha* **status revived**, *Rhytidoponera*, *Stictoponera* **status revived**, and *Typhlomyrmex*. The new phylogenetic framework and classification proposed here will shed light on the study of Ectatomminae taxonomy and systematics, as well as on the morphological evolution of the groups that it comprises.

Key words: phylogenomics, ultraconserved elements, Formicidae, Ectatomminae, Heteroponerinae

Ants are a globally diverse lineage of eusocial aculeate wasps and represent one of the great success stories of evolution, being the richest and most ecologically dominant group among all social insects (Hölldobler and Wilson 2008). The taxonomy and internal phylogeny of Formicidae have been significantly stabilized in recent decades due to extensive study of ant systematics on a global scale (Baroni Urbani et al. 1992, Bolton 1995, Brady et al. 2006, Moreau et al. 2006, Ward 2014). Many of the findings from these studies have shown a great congruence between existing morphological and molecular hypotheses, such as the monophyly of the subfamily Proceratiinae and the recognition of the subfamily Paraponerinae as a distinct lineage among poneroid ants (Ouellette et al. 2006). Other findings, however, highlight the need for a better understanding of the ancestral morphology and biology of ants. The subfamilies

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Ectatomminae and Heteroponerinae (commonly referred to as ectaheteromorphs) are important in this regard. Although possessing morphological and behavioral traits thought to be plesiomorphic for ants as a whole (Baroni Urbani 1989, Hölldobler and Wilson 1990, Keller 2000, Bolton 2003, Ward and Brady 2003), the two subfamilies are part of the large formicoid clade, in which they are sister to the highly derived Myrmicinae (Brady et al. 2006, Moreau et al. 2006, Ouellette et al. 2006, Branstetter et al. 2017).

The ectaheteromorphs contain 302 described ant species (Bolton 2021) distributed across most tropical and subtropical regions of the world, with a substantial number of species also occurring in hot temperate environments (Camacho and Feitosa 2015, Feitosa 2015). Species live and forage in the soil and vegetation and are known to nest underground, in rotting logs, in leaf litter, or in trees, with colony sizes ranging from a few dozen to a few hundred workers. Ectaheteromorph workers vary morphologically, from large ants with robust bodies and well-developed compound eyes to tiny and totally blind (Fig. 1). They also range from possessing very short to very long appendages. The cuticle varies from coarsely sculptured to polished and shiny. Coloration can be drab or highly conspicuous (Camacho and Feitosa 2015, Feitosa 2015).

The clade has a disjunct distribution, occurring in the Neotropical region (with a minor extension into the southern Nearctic) and in the Australian and Indomalayan regions (Janicki et al. 2016). Currently, the subfamily Ectatomminae is divided into two tribes: (1) Ectatommini, composed of the genera *Ectatomma* Fr. Smith (Hymenoptera: Formicidae), exclusive to the Neotropical region, *Rhytidoponera* Mayr (Hymenoptera: Formicidae), occurring only in the Australian region, and *Gnamptogenys* Roger (Hymenoptera: Formicidae), present in the Neotropical, Nearctic, Indomalayan, and Australasian regions; and (2) Typhlomyrmecini, composed of the single genus *Typhlomyrmex* Mayr (Hymenoptera: Formicidae), which is strictly Neotropical in distribution. Heteroponerinae contains a single tribe, Heteroponerini, which includes the genus *Acanthoponera* Mayr (Hymenoptera: Formicidae), strictly Neotropical, and *Heteroponera* Mayr (Hymenoptera: Formicidae), which has a disjunct distribution between the Neotropical and Australian regions. The enigmatic genus *Aulacopone* Arnoldi (Hymenoptera: Formicidae), known only from two collection events in Azerbaijan, including the type locality, is currently *incertae sedis* in the subfamily. The taxonomic limits of the ectaheteromorph genera have been relatively stable since they were originally proposed and there have been numerous species-level treatments within individual genera (e.g., Ward 1980, Ward 1984, Lattke 1995, Lattke 2004, Nettel-Hernanz et al. 2015, Camacho et al. 2020). There have been multiple attempts to understand relationships among the genera using morphology alone (Emery 1911, Brown 1965, Lattke 1994, Keller 2011, Feitosa 2015) but the results have been contradictory or poorly supported. The monophyly of genera has also never been formally tested using molecular data.

The incorporation of molecular biology into phylogenetic inference has greatly advanced understanding of ant evolution and ecological success. Several studies investigated the early evolution and diversification of ants (Brady et al. 2006, Ouellette et al. 2006, Moreau and Bell 2013), resolving most of the relationships among subfamilies (Branstetter et al. 2017, Borowiec et al. 2019). Among the 17 subfamilies of Formicidae, internal phylogenetic relationships have been extensively studied using molecular data in only ten, accounting for 94% of the described species diversity within the family (Ward and Brady 2003 (Myrmeciinae); Ward et al. 2010 (Aneuretinae and Dolichoderinae); Schmidt 2013 (Ponerinae); Brady et al. 2014, Borowiec 2019 (Dorylinae); Ward et al. 2015 (Agroecomyrmecinae and Myrmicinae); Chomicki et al. 2015 (Pseudomyrmecinae); Blaimer et al. 2015, Ward et al. 2016 (Formicinae); Ward and Fisher 2016 (Amblyoponinae)). However, most of these studies were limited to analyzing only a relatively low number of mitochondrial and nuclear genes, sequenced using traditional Sanger sequencing methods (except for Blaimer et al. 2015, Branstetter et al. 2017, and Borowiec 2019).

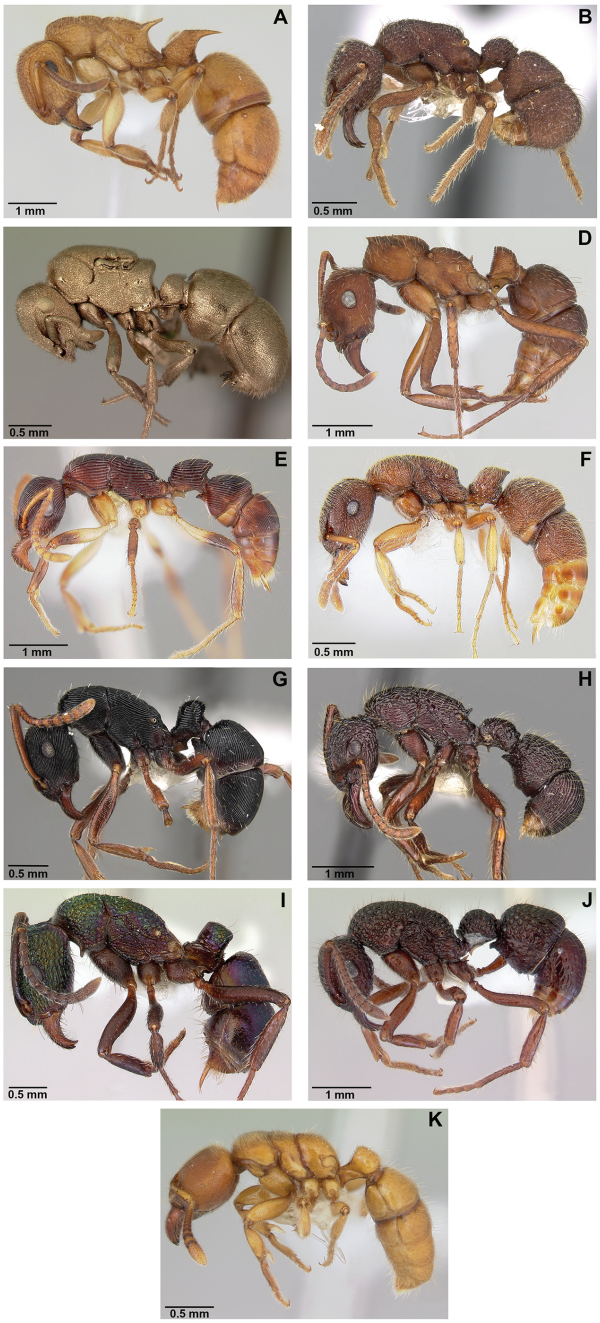

Fig. 1. In lateral view, workers of the Ectatomminae genera, showing the morphological diversity within the clade. (A) *Acanthoponera mucronata* (CASENT0173540), (B) *Alfaria minuta* (CASENT0281213), (C) *Ectatomma planidens* (CASENT0173379), (D) *Gnamptogenys acuminata* (USNMENT00441095), (E) *Heteroponera panamensis* (CASENT0106021), (F) *Holcoponera ammophila* (CASENT0281512), (G) *Poneracantha mecotyle* (CASENT0281530), (H) *Rhytidoponera metallica* (CASENT0172345), (I) *Stictoponera biroi* (CASENT0172380), (J) *Typhlomyrmex rogenhoferi* (CASENT0173390). See Fig. 3 for images of *Boltonia microps*. Images by April Nobile, Estella Ortega, Michael Branstetter, Zach Lieberman, and Jeffrey Sosa-Calvo; available from www.antweb.org (Antweb 2021).

Phylogenomic methods, in contrast, can efficiently generate hundreds to thousands of loci for phylogenetic inference, allowing for the resolution of previously intractable phylogenetic problems and providing increased confidence (Johnson et al. 2013, Blaimer et al. 2015, Branstetter et al. 2017). Phylogenomic approaches can increase the number of characters available hundreds to thousands of times, which can reduce stochastic error for phylogenetic inference (Delsuc et al. 2005) and help to overcome phylogenetic conflict among gene trees (Camacho et al. 2019). Among alternative phylogenomic markers, ultraconserved elements (UCEs) are ideal for the study of evolutionary relationships at different time scales (Faircloth et al. 2015). Enrichment of UCE loci has been used to investigate issues involving older phylogenetic divergences for various vertebrates (Crawford et al. 2012, Faircloth et al. 2013a; McCormack et al. 2013), several insect groups (Faircloth et al. 2015, Blaimer et al. 2016a), and ants (Blaimer et al. 2015, Branstetter et al. 2017). The technique is also useful for understanding relationships at the species and population level (Smith et al. 2013, Ješovnik et al. 2017; Ströher et al. 2019, Branstetter and Longino 2019, Longino and Branstetter 2020, Prebus 2021). UCE enrichment is effective even for poorly preserved specimens with degraded DNA (Blaimer et al. 2016b), and the cost is relatively low compared to other DNA-sequencing methods (Branstetter et al. 2017).

Previous studies have supported the monophyly of the ectaheteromorphs and their placement near the Myrmicinae. They have long been thought to be closely related to the subfamily Myrmicinae, based on morphology (Brown 1958, Bolton 2003). Feitosa (2015) discovered ten diagnostic characters for the ectaheteromorph group, providing morphological support for monophyly. Early molecular datasets supported the monophyly of the ectaheteromorphs, but estimates of their placement relative to other subfamilies were uncertain (Brady et al. 2006, Moreau et al. 2006). Using UCEs, Branstetter et al. (2017) found the first strong evidence that ectaheteromorphs were a sister clade to Myrmicinae, the most diverse subfamily of ants. The study, however, focused on relationships among subfamilies and included UCE data for only four ectaheteromorph species. Thus, this and all previous molecular studies have been based on very limited taxon sampling within the ectaheteromorphs.

Here, we use UCE data to reconstruct the phylogeny of ectaheteromorph ants and improve ectaheteromorph systematics. To do so, we assembled a data set of 2,127 UCE loci by means of target enrichment and multiplexed sequencing of 135 ectaheteromorph taxa, greatly expanding the taxon sampling of Branstetter et al. (2017). We selected taxa to contain a broad representation of species across genera and were able to include six of the seven currently valid ectaheteromorph genera and many of the species groups within genera. Our detailed objectives were to (1) use phylogenomic information and dense taxon sampling to test the monophyly of subfamilies, tribes, and genera within the ectaheteromorphs; (2) resolve phylogenetic relationships among lineages; and (3) use the results to improve the ectaheteromorph classification at all taxonomic levels. Based on the phylogenetic results and morphology, and in order to establish an evolutionary classification in which higher taxa are monophyletic, we: (i) synonymize Heteroponerinae under Ectatomminae; (ii) describe one new genus, *Boltonia* Camacho and Feitosa **gen.n.** (Hymenoptera: Formicidae); (iii) revive the genera *Alfaria* Emery (Hymenoptera: Formicidae), *Holcoponera* Mayr (Hymenoptera: Formicidae), *Poneracantha* Emery (Hymenoptera: Formicidae), and *Stictoponera* Mayr (Hymenoptera: Formicidae) from synonymy; and (iv) provide an illustrated identification key for the Ectatomminae genera.

Methods

Taxon Sampling

Our dataset comprised 135 individuals belonging to 130 species of ectaheteromorph ants (Supp Table S1 [online only]). The only genus we could not sample was *Aulacopone*, which is a monotypic genus known only from its holotype (collected in 1929 and currently lost) and by another specimen that was collected in 1936, which was coated with gold-palladium for scanning electron microscopy long ago. We maximized the sampling breadth by including at least one representative from each biogeographic region in which a genus occurs and by sampling across morphologically disparate groups within genera. In addition, we included 15 taxa to serve as closely related outgroups from six ant subfamilies (Myrmicinae, Dorylinae, Pseudomyrmecinae, Formicinae, Myrmeciinae, and Dolichoderinae) (Supp Table S1 [online only]) belonging to the formicoid clade of ants (*sensu* Brady et al. 2006). Trees were rooted using Dorylinae. The total sample comprised 73 species of *Gnamptogenys* (77 terminals), 13 species of *Heteroponera* (14 terminals), four species of *Acanthoponera* (four terminals), three species of *Typhlomyrmex* (three terminals), 26 species of *Rhytidoponera* (26 terminals), and 11 species of *Ectatomma* (11 terminals). All specimens included in this study were collected in accordance with local regulations and all necessary permits were obtained. Voucher specimens have been deposited at the Entomological Collection *Padre Jesus Santiago Moure* of the Federal University of Paraná (DZUP), Brazil; at the John T. Longino personal collection (JTLC), University of Utah, Salt Lake City, UT, USA; and at the Smithsonian Institution National Museum of Natural History (NMNH/USNM), Washington, DC, USA.

Morphological Data

We examined the external morphology of adult forms to produce diagnostic information for the formal and informal groupings proposed in this study (Supp Table S2 [online only]), following the terminology traditionally used for myrmecological revisions (Keller 2011). For the surface sculpturing, we followed the terminology proposed by Harris (1979). The type material was examined in person or by photographs, when available at www.antweb.org (Antweb 2021). Taxonomic history for the species follows Bolton (2021).

Molecular Data Collection

DNA was extracted destructively or non-destructively from adult workers using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). We quantified DNA for each sample using a Qubit fluorometer (High sensitivity kit, Life Technologies, Inc.) and sheared 5.7– 271 ng ($\bar{x}$ = 47 ng) of DNA to a target size of approximately 600 bp by sonication (Qsonica). The sheared DNA was used as input for a modified genomic DNA library preparation protocol (Kapa Hyper Prep Library Kit, Kapa Biosystems) that incorporated 'with-bead' cleanup steps (Fisher et al. 2011) and a generic SPRI substitute (Rohland and Reich 2012), "speedbeads" hereafter, as described by (Faircloth et al. 2015). We used TruSeq-style dual indexing adapters during adapter ligation (Glenn et al. 2019), and PCR-amplified 50% of the resulting library volume. After rehydrating and purifying reactions, we combined groups of ten libraries at equimolar ratios into enrichment pools having final concentrations of 153–178 ng/μL.

We enriched each pool using a set of 9,898 custom-designed probes (MYcroarray, Inc., now Arbor Biosciences) targeting 2,524 UCE loci specific for ants (Branstetter et al. 2017, 'hym-v2-ant-specific'). We followed library enrichment procedures for the MYcroarray MYBaits kit (Blumenstiel et al. 2010), except we used

a 0.1X concentration of the standard MYBaits concentration and added 0.7 µL of 500 µM custom blocking oligos designed against our custom sequence tags. We ran the hybridization reaction for 24 h at 65 °C, subsequently bound all pools to streptavidin beads (MyOne C1; Life Technologies), and washed bound libraries according to a standard target enrichment protocol (Faircloth et al. 2012). We used the with-bead approach for PCR recovery of enriched libraries as described in Faircloth et al. (2012). We combined 15 µL of streptavidin bead-bound, enriched library with 25 µL HiFi Ready Mix (Kapa Biosystems), 5 µL of Illumina TruSeq primer mix (2.5 µM each), and 5 µL of ddH2O. We purified resulting reactions using 1.0X speedbeads, and we rehydrated the enriched pools in 22 µL EB. We quantified 2 µL of each enriched pool using a Qubit fluorometer (broad range kit). Enriched DNA samples were sequenced on four Illumina HiSeq 2500 lanes (2x125bp v4 chemistry) at the High Throughput Genomics Lab at the University of Utah. All of the UCE laboratory work was conducted at the University of Utah.

Processing and Alignment of UCE Data

The sequencing facility demultiplexed and converted raw data from BCl to FASTQ format using BCL2FASTQ (available at http://support.illumina.com/downloads/bcl2fastq_conversion_software_184.html). We trimmed the demultiplexed FASTQ data output for adapter contamination and low-quality bases using Illumiprocessor (Faircloth 2013b), which is a wrapper program around TRIMMOMATIC (Bolger et al. 2014). All further data processing described in the following relied on scripts within the PHYLUCE v1.5. package. We computed summary statistics on the data using the get_fastq_stats.py script, and assembled the cleaned reads using the assemblo_trinity.py wrapper around the program Trinity (v2013-02-25) (Grabherr et al. 2011). Average sequencing coverage across assembled contigs was calculated using get_trinity_coverage.py. To identify assembled contigs representing enriched UCE loci from each species, species-specific contig assemblies were aligned to the ant-specific hym-v2 bait file (Branstetter et al. 2017) using match_contigs_to_probes.py (min_coverage = 50, min_identity = 80), and sequence coverage statistics (avg, min, max) for contigs containing UCE loci were calculated using get_trinity_coverage_for_uce_loci.py. Subsequently, we used get_match_counts.py to query the relational database containing matched probes created in the previous step, in order to generate a list of UCE loci shared across all taxa. This list of UCE loci was then used in the get_fastas_from_match_counts.py script to create FASTA files for each UCE locus, which contain sequence data for taxa present at that particular locus (Supp Table S3 [online only]). We aligned all data in all these FASTA files using MAFFT (Katoh et al. 2009) through seqcap_align_2.py (min-length = 20, no-trim). Following alignment, we further trimmed our alignment using a wrapper script (get_gblocks_trimmed_alignment_from_untrimmed.py) for Gblocks (Castresana 2000) using the following settings: b1=0.5, b2=0.5, b3=12, b4=7.We then used get_only_loci_with_min_taxa.py to filter the initial set of alignments to include only loci with data for more than 75% of taxa (>112 of 150) or 90% of taxa (>135 of 150). These are referred to as the 75p-matrix and 90p-matrix, respectively (Supp Table S4 [online only]).

Phylogenetic Inference

We performed a set of sensitivity analyses of our dataset, by employing both concatenated and species-tree analyses on the different data matrices, and also by recoding the nucleotide data to RY-characters. This set of sensitivity analysis was performed to allow for assumptions that differ from those used in the primary analysis and to check the robustness of the results.

For the concatenated analyses, we used the Sliding-Window Site Characteristics based on Entropy method (SWSC-EN; Tagliacollo and Lanfear 2018) to partition the UCE data for phylogenetic analysis. This method breaks UCE loci into three regions, corresponding to the right flank, core, and left flank. The theoretical underpinning of the approach comes from the observation that UCE core regions are conserved, whereas the flanking regions become increasingly more variable (Faircloth et al. 2012). After running the SWSC-EN algorithm, the resulting data subsets were analyzed using PARTITIONFINDER2 (Lanfear et al. 2012). For this analysis we used the rclusterf algorithm, AICc model-selection criterion, and the GTR+G model of sequence evolution. Using the SWSC-EN partitioning scheme and concatenated matrices, we inferred phylogenetic relationships of ectaheteromorphs with the likelihood-based program IQ-TREE v1.5.5 (Nguyen et al. 2015). For the analysis we selected the '-spp' option for partitioning and the "-m MFP" option for ModelFinder (Kalyaanamoorthy et al. 2017) to select the best model of sequence evolution. To assess branch support, we performed 1,000 replicates of the ultrafast bootstrap approximation (UFB) (Minh et al. 2013, Hoang et al. 2018). Additionally, we performed matched-pair tests of symmetry to test the assumptions of stationarity and homogeneity for the partition scheme. We used the '—symtest-remove-bad' option on IQ-TREE v.2.1.3 to remove all 'bad' partitions (pvalue cutoff = 0.050) and continued the analysis with the remaining 'good' partitions, as described by Naser-Khdour et al. (2019). The resulting best-fit partitioning scheme included 1,427 data subsets (245 'bad' partitions removed) for the 75p-matrix and 902 data subsets (147 'bad' partitions removed) for the 90p-matrix and had a significantly better log likelihood than alternative partitioning schemes (75p-matrix: SWSC-EN-symtest: -13272290.208; SWSC-EN: -16,476,333.842; By Locus: -16,773,830.932; Unpartitioned: -16,912,745.749; 90p-matrix: SWSC-EN-symtest: -8903417.276; SWSC-EN: -10,811,172.113; By Locus: -11,010,444.832; Unpartitioned: -11,093,438.532). We also recoded the nucleotides to RY-characters for both matrices in an attempt to reduce possible negative effects caused by base composition heterogeneity or saturation (Phillips and Penny 2003). For these support measures, values ≥ 95% signal were regarded as well supported in this study.

For species-tree analyses, we used the SWSC-EN partitioning scheme to estimate gene trees for the 2,180 UCE loci in the 75p-matrix and the 1,351 UCE loci in the 90p-matrix, since partitioning the UCE loci can improve gene-tree resolution (Freitas et al. 2021). Each partitioned gene tree reconstruction was done with IQ-TREE using the "-m MFP" option for ModelFinder for the best model fit with 1000 UFB replicates. We also contracted very low support branches (e.g., below 10% bootstrap support) from gene trees, since Zhang et al. (2018) showed that this can improve accuracy in species tree estimation. Species-tree analyses with local posterior probability support values were performed in ASTRAL-III (Zhang et al. 2018) using the manipulated gene trees as input.

Finally, given that there was relatively low support for some of the inferred nodes (see results), we explicitly explored the level of support of each locus for competing topologies. First, we obtained gene trees for all 2,520 loci in our dataset without partitioning, as well as the mean ultrafast-bootstrap support and GC content for the corresponding locus. We then counted how many gene trees supported each competing topology using the testMono function in 'ape' (Paradis and Schliep 2019) in R v.3.6.3. (R Core Team 2020). This also allowed us to test if a given topology was supported by loci with biased base composition and/or low signal (i.e., low mean average bootstrap support across all nodes). All the above phylogenetic analyses were performed on the Smithsonian Institution's High-Performance Computing Cluster (SI/HPC).

Data Availability

All phylogenetic datasets are available in the Dryad data repository under https://doi.org/10.5061/dryad.sxksn034j. Raw sequence data files have further been submitted to NCBI's Sequencing Read Archive (BioProject PRJNA668430) (Supp Table S6 [online only]).

Nomenclature

This paper and the nomenclatural act(s) it contains have been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:55B5ECCD-6C6E-4721-B094-ADDC2275CEE6

Results

UCE Capture Statistics

An average of 45,784 contigs with a mean length of 420.2 bp were assembled by Trinity after adapter- and quality-trimming of raw reads, with an average contig coverage of 7.3X (Supp Table S3 [online only]). From the bulk set of contigs, we extracted an average of 2,126 UCE loci per sample and these had a mean contig length of 722.3 bp and average coverage of 39.2X. The 75p-matrix retained 2,180 loci, which provided 1,205,560 bp of sequence data, 558,013 informative sites, and only 10.05% missing data. The 90p-matrix retained 1,351 loci, generating 792,650 bp of sequence data, of which 368,562 were informative, with 7.1% missing data. For additional sequencing and assembly information see Supplementary Material (Supp Tables S3 and S4 [online only]).

Phylogenetic Results

Our concatenated, RY-recoded, and species-tree analyses recovered highly congruent topologies for Ectatomminae, with only a few incongruences at the genus and species levels. Analysis of the concatenated 90p-matrix recovered a highly resolved phylogeny for the ectaheteromorphs with most nodes displaying maximum UFB support (Fig. 2). Only a few nodes were recovered with a UFB score lower than 95%, mainly involving interspecific relationships among closely related species within a genus (Fig. 2). For the 90p-RY concatenated analysis, we also recovered a highly resolved phylogeny with high support, but with some differences in generic relationships from the 90p-matrix, most notably the paraphyly of *Heteroponera* in relation to *Acanthoponera* (Supp Fig. S1 [online only]). The 75p-matrix analysis recovered results very similar results to those of the 90p-RY analysis. Relationships among species were congruent, except for the position of *Heteroponera* sp._GPC22 (Supp Fig. S2 [online only]). The 75p-RY concatenated analysis was also mostly congruent with the 90p-RY dataset, but recovered some conflicting relationships between *Poneracantha*, *Alfaria,* and *Holcoponera* (Supp Fig. S3 [online only]). The species trees estimated by ASTRAL-III closely matched the topology estimated by the 90p-matrix concatenated analysis of nucleotide data, with most nodes showing maximum local posterior probability (LPP) support values (Supp Figs. S4 and S5 [online only]). All of the results discussed below refer to the 90p-matrix concatenated tree, except where noted, since this was the topology with the highest likelihood value and because the completeness of the matrix minimizes the effect of missing data.

The ectaheteromorphs, as currently defined, encompass two different subfamilies. We found strong support for the monophyly of both subfamilies (heteroponerines: UFB = 100, LPP = 1; ectatommines: UFB = 100, LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]) and for the sister-group relationship between them (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). We also recovered the ectaheteromorphs (heteroponerines + ectatommines) as the sister clade of the Myrmicinae.

The heteroponerines include the genera *Heteroponera* and *Acanthoponera*. *Acanthoponera* was recovered as monophyletic in all analyses (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). *Heteroponera*, in contrast, was recovered as paraphyletic with respect to *Acanthoponera,* with a single species, *Boltonia microps* (Borgmeier) **new combination** (formerly classified as *Heteroponera*), clearly separated from the other species of *Heteroponera* and sister to all other Heteroponerinae with maximum support (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). The remaining *Heteroponera* species were recovered as a monophyletic group in the concatenated analyses of the 90p-matrix (UFB = 76) (Fig. 2, Supp Fig. S2 [online only]) and in the species-tree analyses, although with low support (90p-matrix: LPP = 0.49; 75p-matrix: LPP = 0.86) (Supp Figs. S4 and S5 [online only])). Analsyes of the 75p-matrix, as well as of both RY-coded matrices, recovered *Heteroponera monticola* Kempf and Brown, a South American species, as sister to a clade comprising *Acanthoponera* and *Heteroponera* (UFB = 100) (Supp Figs. S1–S3 [online only]).

Ectatommini, as classified here, comprises eight extant genera, all of them included in our analyses, and together they formed a clade with maximum support in all analyses (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). Within the tribe the reciprocally monophyletic genera *Rhytidoponera* (UFB = 100; LPP = 1) and *Ectatomma* (UFB = 100; LPP = 1) formed a clade in the concatenated and species-tree analyses (UFB =100; LPP = 1) (Fig. 2, Supp Figs. S2, S4, and S5 [online only]), as well as for the 75p-matrix converted to RY-coding (UFB = 98) (Supp Fig. S3 [online only]), and the clade was recovered as sister to all other ectatommines. For the 90p-matrix converted to RY-coding, *Ectatomma* was recovered as sister to all remaining ectatommines with full support, and *Rhytidoponera* was sister to the remaining genera (UFB = 97) (Supp Fig. S1 [online only]).

The genus *Gnamptogenys* was found to be paraphyletic with respect to *Typhlomyrmex*, with full support (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]) and, consequently, a series of independent clades within *Gnamptogenys* are here redefined as different genera (Fig. 2). The Indomalayan genus *Stictoponera* **status revived** (formerly the *coxalis*, *laevior,* and *taivanensis* groups of *Gnamptogenys* sensu Lattke 2004) was recovered as a single clade with maximum support (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). The genus *Poneracantha* **status revived,** a lineage comprised mainly of species specialized in preying on myriapods and diplopods (formed mostly by species representing the *rastrata* group of *Gnamptogenys* sensu Lattke 1995), was recovered with maximum support (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). The very distinctive *Alfaria* **status revived** (formerly the *minuta* group of *Gnamptogenys* sensu Brandão and Lattke, 1990) formed a clade also recovered with maximum support (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). Our analyses also recovered a clade formed by *Holcoponera* **status revived** (UFB = 100; LPP = 1) comprising Australasian, Indomalayan, and Neotropical species (most of the species of the *striatula* group of *Gnamptogenys* sensu Lattke (1995) and the *albiclava* and *epinotalis* groups of *Gnamptogenys* sensu Lattke (2004)) (Fig. 2, Supp Figs. S1–S5 [online only]). We recovered, with maximum support, a monophyletic *Typhlomyrmex* including two small-sized species formerly assigned to *Gnamptogenys* (*T. reichenspergeri* (Santschi) and *T. lavra* (Lattke)) (BS = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). The discovery of this clade is a very surprising result of

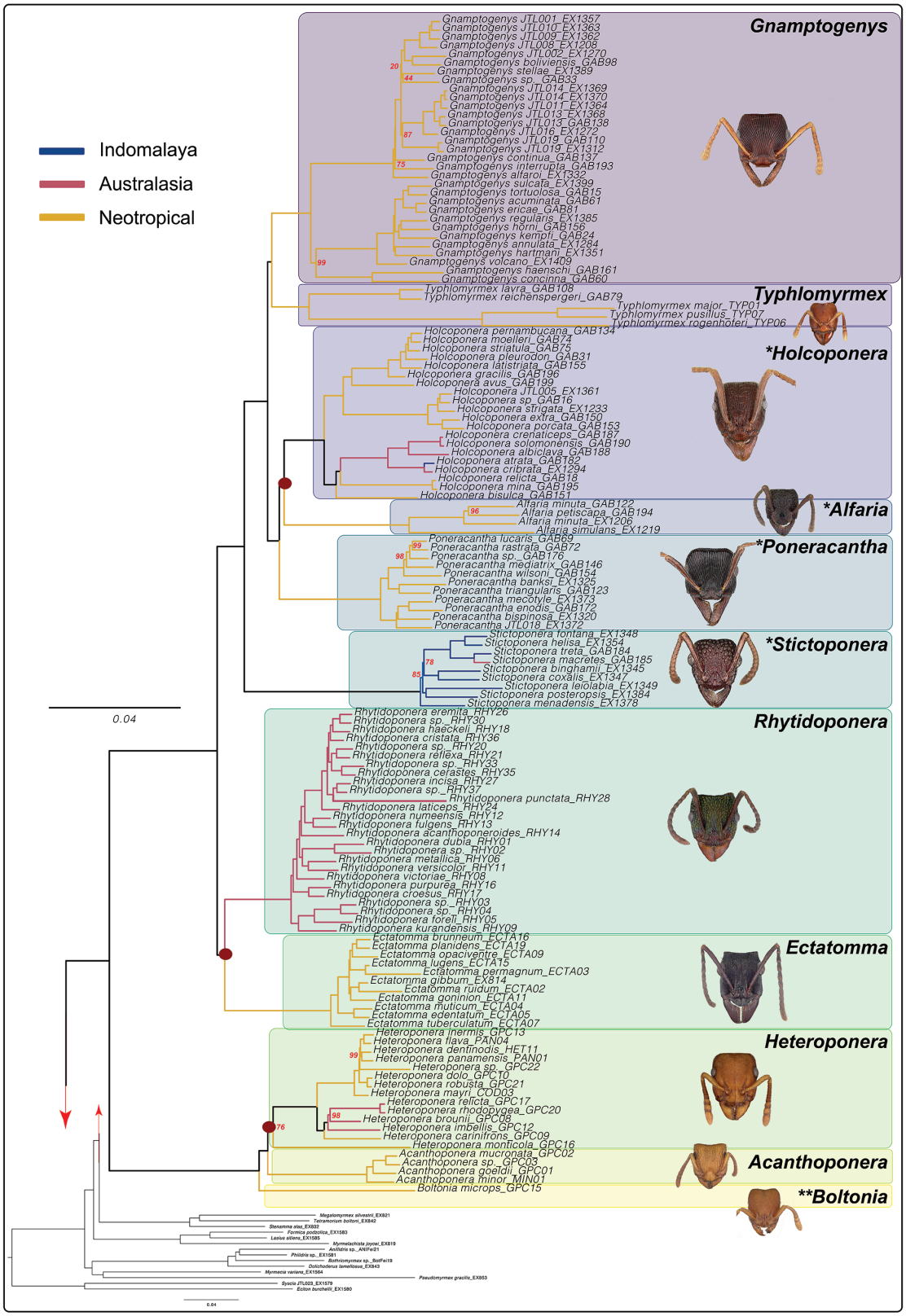

Fig. 2. Phylogeny of the subfamily Ectatomminae based on phylogenomic analyses of the UCE 90% complete data set (150 taxa). Figure is based on IQ-Tree best-tree searches with ultrafast bootstrap (UFB) frequencies of less than 100% mapped onto the respective nodes. UFB searches consisted of 1000 replicates. The eleven larger ectatommine lineages are indicated. Branch color indicates the biogeographical range of the species. Taxa marked with asterisk (*) were classified in *Gnamptogenys* prior to this revision and those with double asterisk (**) were included in *Heteroponera* prior to this revision. See Supplementary material for the 75% complete matrix (Supp Fig. S1 [online only]). Ant photos show heads in frontal view of, from top to bottom: *Gnamptogenys acuminata* (USNMENT00441095), *Typhlomyrmex rogenhoferi* (CASENT0004700), *Holcoponera striatula* (CASENT0106042), *Alfaria simulans* (CASENT0603729), *Poneracantha rastrata*

our study, since these former *Gnamptogenys* species were thought to be closely related to *Holcoponera* (former *striatula* group sensu Lattke (1995)) due to their remarkable morphological similarities shared with other small-sized *Holcoponera* species (i.e., *H. mina* (Brown), *H. haytiana* (Wheeler and Mann), and *H. relicta* (Mann)).

The genus *Gnamptogenys* was recovered as a clade consisting of species from the *sulcata*, *concinna*, and *mordax* groups (sensu Lattke 1995) with full support (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). The *concinna* group (UFB = 100; LPP = 1), also recognized by Lattke (1995), contains two of the largest species in the genus, *G. concinna* (Smith) and *G. haenschi* (Emery). Although morphologically quite different, the differences are probably due to microhabitat differences (*G. concinna* has large eyes and bright color and is a canopy ant; *G. haenschi* has small eyes and drab color and occurs on the ground and in litter samples, and occasionally under rotten wood). The *sulcata* and *mordax* groups (UFB = 100; LPP = 1) recognized here each contain multiple species and only partially correspond to Lattke's (1995) concepts for these groups (Fig. 2, Supp Figs. S1–S5 [online only]).

Regarding relationships among genera within what was previously *Gnamptogenys*, *Stictoponera* was recovered as sister to all other lineages in all analyses (UFB = 100, LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). *Gnamptogenys* and *Typhlomyrmex* were recovered as sister groups in all analyses with full support (UFB = 100, LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). *Holcoponera* as sister to *Alfaria* was recovered by the concatenated (90p-matrix and 75p-matrix) and the 75p-matrix species-tree analyses with full support (UFB = 100; LPP = 1) (Fig. 2, Supp Figs. S2 and S5 [online only]), and with lower support by the 90p-matrix converted to RY-coding (UFB = 78; Supp Fig. S1 [online only]) and the 90p-matrix species tree (LPP = 0.85; Supp Fig. S4 [online only]), which also recovered *Poneracantha* as sister to both genera with full support (UFB = 100, LPP = 1) (Fig. 2, Supp Figs. S1–S5 [online only]). The 75p-matrix converted to RY-coding, in contrast, recovered *Poneracantha* as sister to *Holcoponera* (UFB = 93), and *Alfaria* as sister to both genera with full support (UFB = 100) (Supp Fig. S3 [online only]).

Gene Support for Alternative Topologies

Despite the large dataset used in the present study, some nodes showed relatively low support or were incongruent between different datasets (see red dots in Fig. 2), which could indicate either low or conflicting phylogenetic signals. To explore these possibilities, we looked at the support of gene trees for competing topologies. We found that, of all 2,520 gene trees, 620 recovered *Heteroponera* as monophyletic (including *H. monticola*) (Supp Fig. S6 [online only], N1), and 526 recovered *H. monticola* as sister to a clade formed by the remaining *Heteroponera* + *Acanthoponera* (Supp Fig. S6 [online only], N2). Regarding the sister group relationship between *Rhytidoponera* and *Ectatomma*, 16 gene trees recovered both genera as sister groups (Supp Fig. S6 [online only], N3), while 31 recovered *Ectatomma* as sister to all other Ectatommini genera (Supp Fig. S6 [online only], N4). Lastly, 454 gene trees recovered *Holcoponera* as sister to *Alfaria* (Supp Fig. S6 [online only], N5), while 412 gene trees recovered *Poneracantha* as sister to *Holcoponera* (Supp Fig. S6 [online only], N6). These results indeed support the existence of considerable incongruence among loci for those particular nodes. For each of those problematic nodes, gene trees mostly supported two alternative topological hypotheses, with significantly less support for other topologies. The favored topologies have significantly higher ($p < 0.05$) mean bootstrap values, and the loci supporting the favored topologies have significantly lower GC content than loci supporting alternative topologies. But relatively low numbers of loci support the favored topologies, and in each case the majority of loci do not support the favored topologies. Among the favored topologies there is nearly equal evidence supporting the alternatives, in terms of both bootstrap support and GC content.

Discussion

Phylogenomics Resolves Relationships Among Ectatomminae Genera

Our concatenated and species-tree analyses recovered a well resolved and highly congruent phylogeny for Ectatomminae, while identifying possible incongruences that need to be further investigated (Figs. 2, Supp Figs. S1–S5 [online only]). These results, based on our 2,520 UCE loci dataset, are congruent with prior research that suggests that having a greater number of loci is beneficial (Borowiec et al. 2015, Branstetter et al. 2017), although it remains unclear how many loci are necessary to resolve phylogenetic relationships. However, it has long been recognized that simply increasing the amount of data can exacerbate systematic bias in phylogenetic estimation (Phillips et al. 2004, Philippe et al. 2011, Borowiec et al. 2015) and that to improve phylogenetic inference data quality is key (Borowiec et al. 2015). We showed that, despite the incongruencies found among different datasets for some nodes (see red dots in Fig. 2), all alternative topologies are supported by good-quality data with strong phylogenetic signal. However, recoding nucleotides to RY characters suggests that composition bias may be contributing to support for nodes where gene-tree incongruence is pervasive (Supp Fig. S6 [online only]). RY-coding reduces such biases and increases the signal on internal branches relative to external, increasing phylogenetic signal in mitochondrial genome data (Phillips and Penny 2003). Nevertheless, using RY-coding reduces the dataset size and, as shown in Supp Fig. S6 (online only), nodes that are incongruent between the nucleotide and RY-character data are supported by relatively few loci, which may suggest that dataset size may be important for resolving phylogenetic relationships in Ectatomminae. If loci are discordant, it is expected that numerous additional markers are necessary to generate a robust tree, allowing for an amplification of phylogenetic signal with the increase of the amount of data (Camacho et al 2019).

Previous research has shown that taxonomic balance within a data set has a large impact on phylogenetic results (Branstetter et al. 2017), emphasizing the importance of both broad taxonomic sampling (i.e., covering taxonomic disparity and geographic coverage) and taxonomic evenness across samples (i.e., having comparable samples sizes among the groups, according to their diversity). The fact that we recover alternative hypotheses for some nodes may suggest that a larger sampling of those groups might shed light on their relationships in the future. Despite the fact that our phylogeny includes a broad representation of *Heteroponera*, the addition of *H. inca* to the phylogeny could help elucidate the position of *H. monticola*, since both species seem to be morphologically similar and possibly closely related. Regarding the relationship among *Rhytidoponera* and *Ectatomma*, even though the 26 species of *Rhytidoponera* included

(CASENT0281223), *Stictoponera biroi* (CASENT0281519), *Rythidoponera metallica* (CASENT0172345), *Ectatomma lugens* (USNMENT00445341), *Heteroponera brounii* (CASENT0172105), *Acanthoponera mucronata* (CASENT0173540), and *Boltonia microps* (CASENT0173544). Images by April Nobile, Jeffrey Sosa-Calvo, Zach Lieberman, Will Ericson, Michael Branstetter, and Estella Ortega; available from www.antweb.org (Antweb 2021).

in our phylogeny represents a broad sampling for the genus, there are 104 species currently described and a more complete phylogeny for the genus could provide increased support. Similarly, a larger sampling of *Holcoponera*, *Alfaria,* and *Poneracantha* species could elucidate the relationships among those genera. Nevertheless, our results recover a robust and fully resolved topology that we discuss below through an in-depth discussion of the morphological hypotheses available for the group.

Taxonomy of Ectatomminae Revisited

We propose taxonomic changes for the subfamily that improve ant systematics, i.e., by ensuring that formally named taxa are monophyletic, while simultaneously keeping names fairly stable. At the subfamily level, our decision to synonymize Heteroponerinae under Ectatomminae is not based on the monophyly of these groups, since both are reciprocally monophyletic as currently circumscribed, and their sister-group relationship has been broadly discussed. Historically, the close relationship between both groups has been supported by morphological (Brown 1958, Bolton 2003, Ward 2007, Keller 2011) and molecular data (Brady et al. 2006, Moreau et al. 2006, Moreau and Bell 2013, Branstetter et al. 2017). However, morphology can be misleading, especially when defining the diagnostic characters for the groups separately. When describing Heteroponerinae, Bolton (2003) stated that there is no unequivocal apomorphy for the subfamily, suggesting a number of characters that could have this status. Feitosa (2015) investigated the phylogeny of Heteroponerinae using morphological data, testing the characters suggested by Bolton (2003), as well as by several others, and also could not identify any apomorphy for the group. However, in his work, Feitosa included species of Ectatomminae as outgroups and his analysis suggested at least ten diagnostic characters for the clade comprising both Ectatomminae and Heteroponerinae. For this reason, we reclassify all ectaheteromorph ants as members of a single subfamily, ensuring the monophyly criterion that already applies to all other ant subfamilies but, most importantly, providing a clear diagnosis for the subfamily based on morphological synapomorphies.

Regarding taxonomic changes at the tribal level, our aim is to keep the classification stable. In this sense, the new combination of the tribe Heteroponerini and the synonymy of Typhlomyrmecini are made to ensure the correct placement of the former, and the monophyly of Ectatommini in the case of the latter. At a generic level within the tribe Heteroponerini, the paraphyly of *Heteroponera* is a striking result, unpredicted by morphology, with *B. microps* appearing as a separately diverging lineage. This result is congruent with the previous hypotheses of Borgmeier (1957) and Feitosa (2015), which suggested that the diagnostic characters for this species are highly divergent from the morphological patterns for *Heteroponera*, but its placement as a separate genus is supported here for the first time. Similarly, the position of *H. monticola,* recovered as sister to all the other *Heteroponera* species, as well as the recovery of two separate clades, the first comprising *H. carinifrons* (from Chile) as sister to the Australasian species and the second comprising the remaining Neotropical species, are also entirely new evolutionary hypotheses for the genus, with strong implications for its biogeographical history.

This phylogenetic scenario suggests that the common ancestor of Heteroponerini morphologically resembled a modern member of *Acanthoponera*, with a relatively large body, prominent spines, well developed eyes, and long palps. An early lineage probably split off and evolved to occupy the epigaeic and hypogaeic strata of the environment, maybe displaced by an emerging dominant lineage of ants (e.g., Myrmicinae). This now cryptic early lineage of heteroponerines has undergone a drastic reduction of body size, appendages, and eyes, as we can see in the extant *Boltonia*. Later, a second divergence event separated two lineages of *Heteroponera* and adaptation for living in the ground was repeated. In this second process, *H. monticola* and *H. inca* retained several plesiomorphic traits, also related to *Acanthoponera*, but the remaining *Heteroponera* gradually lost these characters as they made their way to the soil and morphologically converged on *Boltonia* in the reduction of appendages and body size. This scenario is supported by the presence of tarsal teeth and lobes in *Acanthoponera*, traits strictly related to arboreal habits that were lost in the remaining lineages of heteroponerine adapted to nesting and foraging in the ground (Feitosa 2015). Our results regarding relationships among species in Heteroponerini shed new light on the study of their morphological evolution. We believe that, in order to ensure the stability of the classification, to best understand the evolution of this group, and to make the most significant contribution to ant systematics, the assessment of relationships among the species should combine both molecular and morphological approaches. Unfortunately, the genus *Aulacopone* was not included in our analysis due to the unavailability of specimens and difficulties of collecting in its type locality. The genus is monotypic and was collected only twice in the 1920/30s, with the only known specimen currently metal-coated, making recovery of DNA information from the pinned specimen a risk to the only specimen available. The distribution of this genus is singular within the Ectatomminae, being the only group to occur in the Palearctic region. *Aulacopone* is said to share several morphological similarities with the other heteroponerines (Brown 1958, Taylor 1980, Lattke 1994, Bolton 2003, Feitosa 2015), but its position among the Ectatomminae is still not well defined due to the impossibility of examining important characters in the previous phylogenetic study (Feitosa 2015).

The eight genera that comprise Ectatommini are shown to form a well-supported clade, a result that is congruent with previous morphological hypotheses for the group (Bolton 2003, Ward 2007, Keller 2011), although these works considered the four genera as previously defined. In the molecular phylogenies published so far, only one or a few specimens of each genus were included, limiting their conclusions regarding the relationships among them (Brady et al. 2006, Moreau et al. 2006, Moreau and Bell 2013, Branstetter et al. 2017). Given these limitations, this is the first molecular study that aimed to investigate the genus-level relationships in Ectatomminae. A fairly novel result, the sister-group relationship between *Ectatomma* and *Rhytidoponera,* is congruent with previous morphological hypotheses by Keller (2000, 2011) and was suggested by other broad-scale molecular phylogenies of Formicidae that did not focus specifically on these groups (Brady et al. 2006, Moreau et al. 2006, Moreau and Bell 2013). Brown (1958) noticed some similarities between the two genera, noting similarities in wing venation and male genitalia and absence of a metacoxal spine (present in *Holcoponera*, *Gnamptogenys,* and *Stictoponera*). Also, Brown (1958) called attention to similarities between *Ectatomma* workers and those of the largest species of *Rhytidoponera*. Our results are the first to include broad species-level representatives of those genera and our results shed light on the evolution of these groups.

Perhaps the most strikingly novel result in our study is the strong support for the paraphyly of the former *Gnamptogenys* in relation to *Typhlomyrmex*. This result was never previously predicted by any morphological or molecular study. Historically, the position of *Typhlomyrmex* relative to the Ectatommini was first addressed by Emery (1911), but Brown (1965) later placed the genus in its own tribe, Typhlomyrmecini, considering it to be closely related to

the Amblyoponini. Lattke (1994) suggested that the similarities of Typhlomyrmecini with Ectatommini required further exploration and Bolton (2003) most recently considered the Typhlomyrmecini to be a member of Ectatomminae. However, rather than forming a separate tribe in Ectatomminae, it now appears that this group of ants is a highly derived lineage among the former species of *Gnamptogenys* with a distinctive cryptic morphology.

The paraphyly of *Gnamptogenys* in relation to *Typhlomyrmex* provided two different alternatives for the taxonomic treatment of the genera in Ectatomminae, the first being the synonymization of *Gnamptogenys* under *Typhlomyrmex*, since the latter is the oldest available name. However, we recognize the importance of the name *Gnamptogenys* within the myrmecological literature and, with a nomenclatural gender change from feminine (*Gnamptogenys*) to masculine (*Typhlomyrmex*) for most species, this would not be the most parsimonious treatment. The second possibility, chosen here, involved reviving available names for the different clades recovered in our phylogeny, considering the similar phylogenetic distances between those clades and between other Ectatomminae genera, and the strong diagnostic morphological characters recovered for each of the lineages. The availability of generic names for each of those clades shows that hypotheses for those groups were once presented, but morphological data were not sufficient to define them at the time, and they were later synonymized under *Gnamptogenys* (Brown 1958). With our molecular dataset we recovered each clade with strong support and, by reciprocal illumination, defined the morphological characters that separate each genus from any other genus in Ectatomminae.

The generic status of *Holcoponera*, *Stictoponera,* and *Alfaria* were subjects of long and arduous inquiry into the myrmecological literature since they were first proposed as subgenera of *Ectatomma* in the case of the first two, or as a genus, in the case of *Alfaria*. Brown (1958) found no basis for maintaining the generic status of those names, but divided *Gnamptogenys* into four groups, namely the *Gnamptogenys* group, the *Stictoponera* group, the *Holcoponera* group, and the *Alfaria* group. Brown considered *Holcoponera* to be a well-defined genus based on its more compact, dorsally convex mesosoma with a marked promesonotal suture interrupting the sculpture and on the form of the petiolar node, as well as by characters of wing venation and larval hairs. However, when analyzing the similarities between the species *Typhlomyrmex reichenspergeri, Holcoponera relicta,* and *Holcoponera mina*, he considered the lack of gastric sculpture in *T. reichenspergeri* as evidence against its placement in a separate genus. In our study, we recovered *T. reichenspergeri* as sister to *Typhlomyrmex* and relatively distant from *Holcoponera* and we found that *Holcoponera* is not a strictly Neotropical genus because it also includes Indomalayan and Australian species formerly described as *Rhopalopone* and *Wheeleripone*. Brown considered it impossible to define the genus *Stictoponera* because of dissimilarities among the Old World species. We resolve the problem by showing that Old World species fall into two independent clades, one within *Holcoponera*. Brown considered the genus *Alfaria* to be the most distinct of the ectatommine genera but felt that *A. striolata* cast doubts on its generic status due to the less inflated second gastric segment and to its sculpture, which is similar to that of *Stictoponera*. Our genomic data, however, show that *Alfaria* forms a distinct clade among the Ectatomminae and, even though *A. striolata* was not included in the phylogeny, the presence of an expanded frontal carina suggests that this species placement is correct.

The current definition of the genus *Poneracantha* is a novel result, as this was proposed as a monotypic subgenus to contain the highly divergent *P. bispinosa*. However, Lattke (1995) proposed that the specialized millipede predators that belong to this genus formed the *Gnamptogenys rastrata* group and considered them to be closer to *Holcoponera* than to the present definition of *Gnamptogenys* based on the presence of triangular mandibles, long and typically sculptured scapes, the convex clypeal lamella, and the well-developed metacoxal tooth, a result that is also recovered by our molecular data. Lattke (1995) also recovered the *sulcata* and *mordax* groups as sister groups, with the *concinna* group as closely related to them, but not monophyletic. We obtained similar results, except for the monophyly of the *concinna* group, and redefine the *sulcata, concinna,* and *mordax* groups as a smaller, strictly Neotropical *Gnamptogenys*. Finally, the sister-group relationship between the species *T. reichenspergeri*, *T. lenis,* and *T. lavra* and the remaining *Typhlomyrmex* is a result never predicted by morphology and, in fact, the phylogenetic distance among those species is similar to the distance among other genera. Those species have in common absent or reduced eyes, with less than 15 ommatidia; promesonotal suture well marked, totally interrupting dorsal mesosomal sculpture; propodeal spiracle separated from declivity margin by a distance longer than its diameter; metacoxal dorsum unarmed; and petiole pedunculate. *T. reichenspergeri*, *T. lenis,* and *T. lavra* lack a well-defined antennal club and a prominent anteroventral process on the petiole. We chose to combine those species into *Typhlomyrmex* based on these shared diagnostic characteristics, in the interest of a more stable classification.

Additional work is necessary because we strongly believe that the molecular phylogenetic data should be combined with the study of morphological characters that are diagnostic for the newly defined genera and for the new generic combinations, so that the final classification can be functional and useful to any researcher studying specimens in the laboratory or in the field. In this study, we demonstrate that UCE data provide a robust source of phylogenomic data for the Ectatomminae ants. Morphological evolution, interpreted with reference to our resulting phylogeny, has produced diagnostic characters for defining taxonomic groups. We believe that the phylogenetic framework and the new classification proposed here provides a solid foundation for the further study of Ectatomminae taxonomy and systematics, as well as for reconstructing the morphological evolution of the genera, species groups, and species that it comprises.

Taxonomic Account

In order to erect a phylogenetic classification for the subfamily, with monophyletic tribes and subfamilies (Ward 2011), we propose a number of higher-level taxonomic changes. New and revived combinations include the junior synonyms of the species names listed below. Author and year of publication for all genus and species names can be found in AntCat (http://antcat.org/). The tribal and generic classifications of ectaheteromorphs are here modified to achieve consistency with our molecular phylogenetic results. We maintain the existing classification as far as possible, while striving to ensure that all recognized tribes are monophyletic. Genera known only from fossils are indicated with a dagger; most of these are unplaced to tribe and are treated as *incertae sedis* within the subfamily.

Ectatomminae Emery

= Heteroponerinae Bolton **new synonym**

Diagnosis: Presenting the characters of 'poneromorph' subfamilies described by Bolton (2003: p.40). Clypeus broadly inserted between frontal lobes (Bolton 2003); anterior clypeal margin with a narrow lamellar apron (Bolton 2003). Torulus not completely fused to frontal lobe (Bolton 2003). Antenna with 12 segments (Bolton

2003). Pronotum with the humeral corners angled, forming a distinct delimitation between the anterior and lateral margins (Lattke 2004). Antero-ventral angle of pronotum triangular (Feitosa 2015). Pretarsus without arolium (Lattke 2004). Petiole pedunculate (Keller 2011). Petiolar node as wide or wider than long (Feitosa 2015). Subpetiolar process very well developed, occupying more than one-third of the ventral portion of the petiolar sternite (Feitosa 2015). Helcium projecting from about midheight of the anterior face of abdominal segment III. Abdominal segment IV presclerites separated from the rest of segment by a constriction or slight thickening (Lattke 1994). Fourth abdominal tergite arched and larger than the sternite, giving the segment a curved appearance (Keller 2011).

Tribes: Ectatommini and Heteroponerini

Incertae sedis: †*Canapone*, †*Electroponera*, †*Pseudectatomma*.

Notes: In spite of the reciprocal monophyly of the subfamilies Ectatomminae and Heteroponerinae, the morphological evidence strongly suggests that all ectaheteromorph genera could be combined into a single subfamily Ectatomminae, which is the oldest available name. Ectatomminae, as defined here, presents a combination of 10 diagnostic characters that can be used to differentiate those ants from any other ant subfamily, making the identification of those groups more accessible.

Tribe Ectatommini Emery
= Stictoponerini Arnol'di
= Typhlomyrmecini Emery **new synonym**

Diagnosis (Females): Ectatommine ants of small to large size (head width 0.44-2.84mm, head length 0.56-3.8mm). Antennal scrobe usually absent. Eye absent to well-developed (Bolton 2003). Acetabulum of antennal socket apparatus spherical (Keller 2011); accessory chamber of antennal socket present (Keller 2011). Labial palp with two palpomeres (Keller 2011). Promesonotal suture fused and immobile to complete and flexible (Bolton 2003). Ventral flap on metapleural gland opening present (Keller 2011). Metacoxal cavity open (Bolton 2003). Petiolar sternite fused with tergite over its entire length (except in *Rhytidoponera)* (Bolton 2003, Keller 2011); laterotergites of petiole indistinct to absent.

Genera: Alfaria **status revived**, *Ectatomma, Gnamptogenys, Holcoponera* **status revived**, *Poneracantha* **status revived**, *Rhytidoponera, Stictoponera* **status revived**, and *Typhlomyrmex*.

***Alfaria* Emery status revived**
= *Opisthoscyphus* Mann **new combination**

Type Species: Alfaria simulans Emery

Diagnosis (Females): Head subquadrate; occipital lobe usually present; frontal carina broadly expanded laterad; row of stout setae on base of foretarsus opposite to strigil present; promesonotal suture absent to lightly impressed, never interrupting dorsal mesosomal sculpture; petiolar spiracle facing directly ventrad and sunken within a pit; second gastral (IV abdominal) sternite usually strongly reduced, so that the gaster is directed ventrally and anterad.

Species: caelata **new combination**, *falcifera* **new combination**, *fieldi* **new combination**, *minuta* **revived combination**, *petiscapa* **new combination**, *piei* **new combination**, *simulans* **revived combination**, *striolata* **revived combination**, and *vriesi* **new combination** (and the junior synonyms *soror* **new combination**, *carinata* **revived combination**, *emeryi* **revived combination**, *mus* **revived combination**, *panamensis* **revived combination**, *pneodonax* **new combination**, *scabrosus* **new combination**, and *bufonis* **revived combination**).

Distribution: Exclusively Neotropical, from southern Mexico to northern Argentina.

Notes: Alfaria is a very morphologically distinct lineage among the Ectatommini, given the extreme anterior curvature of the gaster in profile. In fact, these ants are usually mistakenly identified as *Proceratium* Roger, 1863, due to the impressive convergence in this character. We here resurrect the name *Alfaria*, firstly proposed by Emery (1896) and synonymized under *Gnamptogenys* by Brown (1958), to comprise the species previously included in the *minuta* group of *Gnamptogenys* sensu Brandão and Lattke (1990). All *Alfaria* species can be identified using the work of Camacho et. al. (2020) under the previous combination in *Gnamptogenys*.

***Ectatomma* Smith**

Type Species: Ectatomma tuberculatum (Olivier)

Diagnosis (Females): Occipital lobe absent. Antennal club absent. Palp formula 2,2. Pronotum usually with two or three tubercles. Mesonotum prominent and clearly differentiated from propodeum, separated by a deep transverse suture. Promesonotal suture well marked, interrupting or not the dorsal mesosomal sculpture. Propodeal spiracle elliptical or slit-shaped and separated from the declivous face of propodeum by a distance longer than its diameter. Apex of protibia with a stout seta close to the strigil base; dorsum of posterior coxa without projections.

Species: brunneum, confine, edentatum, gibbum, goninion, †gracile, lugens, muticum, opaciventre, parasiticum, permagnum, planidens, ruidum, suzanae, tuberculatum, and *vizottoi*.

Distribution: Exclusively found in the New World, from USA (Texas) to Argentina (Buenos Aires).

Notes: Ectatomma are among the most conspicuous elements of the ant fauna in Neotropical ecosystems. Currently, the most comprehensive work including an identification key for the species in the genus is the revision by Kugler and Brown (1982). However, this work does not include the species *Ectatomma parasiticum* Feitosa and Fresneau, in Feitosa et al. (2008), *E. suzannae* Almeida (1986) and *E. vizottoi* Almeida (1987).

***Gnamptogenys* Roger**
= *Commateta* Santschi
= *Emeryella* Forel
= *Tammoteca* Santschi

Type Species: Gnamptogenys sulcata (Smith)

Diagnosis (Females): Head subquadrate to elongate. Mandible subtriangular to subfalcate. Occipital lobe absent. Antennal club absent. Palp formula 2,2 to 3,2. Pronotum unarmed and without tubercles. Promesonotal suture feebly impressed to absent, never

interrupting dorsal mesosomal sculpture, sometimes with a small pit frequently situated medially on a weakly impressed promesonotal suture. Mesonotum not prominent, forming a continuous line with the propodeum, separated by a transverse suture. Propodeal spiracle oval or rounded, separated from the declivous face of propodeum by a distance longer or shorter than its diameter. Apex of protibia without a stout seta close to the strigil base; dorsum of posterior coxae frequently with a lobe or spine.

Species: acuminata, alfaroi, andersoni, annulata, biquetra, boliviensis, bruchi, †casca, concinna, continua, curvoclypeata, ericae, †europaea, falcaria, fernandezi, flava, haenschi, hartmani, horni, interrupta, kempfi, †levinates, lucaris, mordax, nana, †pristina, regularis, rimulosa, † rohdendorfi, rugimala, rumba, schmitti, siapensis, stellae, sulcata, tortuolosa, transversa, and *volcano*.

Distribution: Exclusively found in the New World, from USA (Texas) to Argentina (Buenos Aires), with one species occurring in Cuba.

Notes: In the new concept proposed here, *Gnamptogenys* is now restricted to the species from the previous *sulcata, concinna,* and *mordax* groups (sensu Lattke 1995), considering that *G. sulcata* is the type-species of the genus. All except one of the species of *Gnamptogenys* can be identified using the work of Camacho et. al. (2020). *Gnamptogenys rugimala*, a newly described species, can be identified using the paper by Marcineiro and Lattke (2020).

***Holcoponera* Mayr status revived**
= *Mictoponera* Forel
= *Rhopalopone* Emery
= *Spaniopone* Wheeler and Mann
= *Wheeleripone* Mann

Type Species: Holcoponera striatula (Mayr)

Diagnosis (Females): Head wider posterad than anterad; mandible triangular with striae or rugulae on frontal surface; anterior clypeal margin convex; scape usually surpassing vertexal margin; eye slightly behind cephalic midlength; promesonotal suture frequently well marked, totally interrupting dorsal mesosomal sculpture; propodeal spiracle close to the declivous face of propodeum; propodeum unarmed; anterior prosternal process broadly concave medially; metacoxal dorsum always with a denticle or lobe; petiolar node high; anteroventral postpetiolar process relatively wide; second gastric segment only slightly arched ventrally.

Species: acuta **revived combination**, *albiclava* **new combination**, *ammophila* **new combination**, *andina* **new combination**, *aspera* **new combination**, *aterrima* **new combination**, *atrata* **new combination**, *auricula* **new combination**, *avus* **new combination**, *bisulca* **new combination**, *brunnea* **new combination**, *crenaticeps* **new combination**, *cribrata* **new combination**, *dichotoma* **new combination**, *ejuncida* **new combination**, *epinotalis* **new combination**, *extra* **new combination**, *gentryi* **new combination**, *gracilis* **revived combination**, *haytiana* **new combination**, *ilimani* **new combination**, *latistriata* **new combination**, *lucida* **new combination**, *luzonensis* **new combination**, *major* **new combination**, *malaensis* **new combination**, *mina* **revived combination**, *moelleri* **revived combination**, *nigrivitrea* **new combination**, *pernambucana* **revived combination**, *pilosa* **new combination**, *pittieri* **new combination**, *pleurodon* **revived combination**, *porcata* **revived combination**, *preciosa* **new combination**, *relicta* **new combination**, *sila* **new combination**, *solomonensis* **new combination**, *striatula* **new combination**, and *strigata* **new combination** (and the junior synonyms *dammermani* **new combination**, *diehlii* **new combination**, *moelleri splendens* **revived combination**, *teffensis* **revived combination**, *teffensis concinna* **revived combination**, *emeryi* **revived combination**, *vidua* **revived combination**, *magnifica* **revived combination**, *striatula angustipleura* **new combination**, *arcuata* **revived combination**, *brasiliensis* **revived combination**, *brasiliensis calcarata* **revived combination**, *brasiliensis mayri* **revived combination**, *curtula* **revived combination**, *curutlum paulina* **new combination**, *curtulum stolli* **new combination**, *curtulum vollenweideri* **revived combination**, *emeryi recta* **revived combination**, *rustica* **revived combination**, *simplicoides* **revived combination**, *striatula angustiloba* **new combination**, *striatula antillana* **revived combination**, *striatula obscura* **new combination**, *wasmanni* **revived combination**, *wasmanni isthmica* **revived combination**, *wheeleri* **revived combination**, *concentrica* **new combination**, *satzgeri* **new combination**, *simplex* **revived combination**, *simplex spurium* **new combination**).

Distribution: Neotropical, Indomalayan, and Australasian.

Notes: In the new classification proposed here the available name *Holcoponera* is resurrected from synonymy under *Gnamptogenys* to include most of the species of the *striatula* group sensu Lattke (1995), and the *albiclava* and *epinotalis* groups sensu Lattke (2004). The only species from the former *striatula* group not included in *Holcoponera* are *lavra, lenis,* and *reichenspergeri*, which were transferred to *Typhlomyrmex* in this study. The Neotropical species of *Holcoponera* can be identified using the work of Camacho et. al. (2020). Oriental species can be identified using the key in Lattke (2004).

***Poneracantha* Emery status revived**
= *Barbourella* Wheeler
= *Parectatomma* Emery

Type Species: Poneracantha bispinosa (Emery)

Diagnosis (Females): Head subquadrate or wider anterad than posterad in frontal view; anterior clypeal margin usually straight; frontal surface of mandible usually striate or rugulose; scape usually surpassing vertex; promesonotal suture feebly impressed to absent, never interrupting dorsal mesosomal sculpture; metanotal suture well impressed; propodeum usually armed with denticles or spines; petiolar node low; subpetiolar process shape variable, usually projecting anterad but sometimes subquadrate; metacoxal teeth generally present, usually acicular; second gastric segment slightly arched ventrally.

Species: *banksi* **new combination**, *bispinosa* **revived combination**, *†brunoi*, *cuneiforma* **new combination**, *enodis* **new combination**, *ingeborgae* **new combination**, *insularis* **new combination**, *lanei* **new combination**, *laticephala* **new combination**, *lineolata* **new combination**, *lucaris* **new combination**, *mecotyle* **new combination**, *mediatrix* **new combination**, *menozzii* **revived combination**, *perspicax* **new combination**, *rastrata* **new combination**, *semiferox* **new combination**, *triangularis* **new combination**, and *wilsoni* **new combination** (and the junior synonyms *schubarti* **new combination**, *trigona* **new combination**, *aculeaticoxae* **new combination**, and *triangularis richteri* **new combination**).

Distribution: Exclusively Neotropical, occurring from Guatemala to Uruguay, and in the Caribbean islands of Hispaniola and Lesser Antilles.

Notes: Here we revive the name *Poneracantha* from synonymy under *Gnamptogenys* to include all the species representing the previous *rastrata* group sensu Lattke (1995). All *Poneracantha* species can be identified using the work of Camacho et. al. (2020) under the previous combination in *Gnamptogenys*.

Rhytidoponera **Mayr**
= *Chalcoponera* Emery

Type Species: Rhytidoponera araneoides (Le Guillou)

Diagnosis (Females): Occipital lobe frequently present. Antennal club absent. Palp formula 2,2 to 3,2. Pronotum unarmed. Mesonotum not prominent, forming a continuous line with the propodeum, separated by a transverse suture. Promesonotal suture well marked, totally interrupting dorsal mesosomal sculpture. Propodeal spiracle oval or rounded, separated from the declivous face of propodeum by a distance longer than its diameter. Apex of protibia with a stout seta close to the strigil base; dorsum of posterior coxa without projections.

Species: abdominalis, acanthoponeroides, aciculata, aenescens, anceps, aquila, araneoides, arborea, aspera, atropurpurea, aurata, barnardi, barretti, borealis, carinata, celtinodis, cerastes, chalybaea, chnoopyx, clarki, confusa, convexa, cornuta, crassinodis, cristata, croesus, depilis, dubia, enigmatica, eremita, ferruginea, flavicornis, flavipes, flindersi, foreli, foveolata, fulgens, fuliginosa, †gibsoni, greavesi, gregoryi, haeckeli, hanieli, hilli, impressa, incisa, inops, inornata, insularis, †kirghizorum, koumensis, kurandensis, laciniosa, lamellinodis, laticeps, levior, litoralis, luteipes, maledicta, maniae, mayri, metallica, micans, mimica, mirabilis, nexa, nitida, nitidiventris, nodifera, nudata, numeensis, opaciventris, peninsularis, pilosula, pulchella, punctata, punctigera, punctiventris, purpurea, reflexa, reticulata, rotundiceps, rufescens, rufithorax, rufiventris, rufonigra, scaberrima, scabra, scabrior, socrus, spoliata, strigosa, subcyanea, tasmaniensis, taurus, tenuis, terrestris, trachypyx, turneri, tyloxys, versicolor, victoriae, violacea, viridis, †waipiata, wilsoni, and yorkensis.

Distribution: Exclusively Australasian.

Notes: This speciose ectatommine genus could be considered an ecological equivalent of *Ectatomma* in the Australian region. The most recent taxonomic tools for the identification of *Rhytidoponera* species include the papers by Ward (1980, 1984) and Heterick (2009).

Stictoponera **Mayr status revived**

Type Species: Stictoponera coxalis (Roger)

Diagnosis (Females): Occipital lobe present. Antennal club absent. Palp formula 3,2. Pronotum usually unarmed, occasionally with humeral projections. Mesonotum not prominent, forming a continuous line with the propodeum, separated by a transverse suture. Promesonotal suture absent to feebly impressed, never interrupting the dorsal mesosomal sculpture. Propodeal spiracle oval to rounded and separated from the declivous face of propodeum by a distance longer than its diameter. Apex of protibia without a stout seta close to the strigil base; apex of meso- and metatibia with two spurs; dorsum of posterior coxae frequently with a lobe or spine.

Species. bicolor **revived combination**, *biloba* **new combination**, *binghamii* **revived combination**, *biroi* **revived combination**, *bulbopila* **new combination**, *chapmani* **new combination**, *coccina* **new combination**, *coxalis* **revived combination**, *crassicornis* **revived combination**, *delta* **new combination**, *dentihumera* **new combination**, *fistulosa* **new combination**, *fontana* **new combination**, *gabata* **new combination**, *gastrodeia* **new combination**, *grammodes* **new combination**, *helisa* **new combination**, *hyalina* **new combination**, *lacunosa* **new combination**, *laevior* **revived combination**, *leiolabia* **new combination**, *macretes* **new combination**, *meghalaya* **new combination**, *menadensis* **revived combination**, *nanlingensis* **new combination**, *niuguinensis* **new combination**, *ortostoma* **new combination**, *palamala* **new combination**, *panda* **revived combination**, *paso* **new combination**, *pertusa* **new combination**, *polytreta* **new combination**, *posteropsis* **revived combination**, *quadrutinodules* **new combination**, *rugodens* **new combination**, *scalpta* **new combination**, *sichuanensis* **new combination**, *sinensis* **new combination**, *sinhala* **new combination**, *taivanensis* **revived combination**, *toronates* **new combination**, and *treta* **new combination** (and the junior synonyms *banana* **new combination**, *bicolor minor* **new combination**, *borneensis* **revived combination**, *costata* **revived combination**, *costata pinealis* **revived combination**, *costata simalurensis* **revived combination**, *costata unicolor* **revived combination**, *laevior avia* **revived combination**, *kalabit* **new combination**, *menadensis obscura* **revived combination**, *parva* **revived combination**, *rugosa wallacei* **revived combination**, *spiralis* **revived combination**, and *stylata* **revived combination**)

Distribution: Oriental region, into South-East Asia, including southern China, covering the Sundas and Melanesia all the way to Fiji, including the Philippines.

Notes: Our phylogenomic results suggest that the Indomalayan *Stictoponera* species represent a separate evolutionary lineage, not strictly related to the other Australasian lineages in the subfamily. We here resurrect the name *Stictoponera* from synonymy under *Gnamptogenys* in order to accommodate the species previously included in the *coxalis*, *laevior*, and *taivanensis* groups of *Gnamptogenys* (Lattke 2004, Chen et al. 2017). These species comprise a well-supported clade forming the sister group of *Gnamptogenys* in the new sense. Its placement as sister to the former *Gnamptogenys* is congruent with Lattke (2004), who predicted it based on morphological features and morphological phylogenetic analysis.

Typhlomyrmex **Mayr**

Type Species: Typhlomyrmex rogenhoferi Mayr

Diagnosis (Females): Head subquadrate; antennal club sometimes well-defined and formed by 3 or 4 segments; cephalic vertex mostly smooth and shining, sometimes presenting faded striae or rugulae; eye absent or reduced, with less than 15 ommatidia; promesonotal suture well marked, totally interrupting dorsal mesosomal sculpture; propodeal spiracle separated from declivity margin by a distance longer than its diameter; metacoxal dorsum unarmed or at most with a small lobe or denticle; petiole pedunculate, sometimes with a prominent anteroventral process.

Species: clavicornis, foreli, lavra **new combination**, *lenis* **new combination**, *major, meire, prolatus, pusillus, reichenspergeri* **new combination**, and *rogenhoferi.*

Distribution: Exclusively Neotropical, occurring from Mexico to Argentina (Buenos Aires).

Notes: Most species of *Typhlomyrmex* can be identified using the key of Lacau et al. (2008), while *T. lavra*, *T. lenis*, and *T. reichenspergeri* (formerly included in the *striatula* group of *Gnamptogenys*) can be identified using Camacho et al. (2020).

Tribe Heteroponerini Bolton new combination

Diagnosis: Ectatommine ants of small to medium size (head width 0.42–1.61 mm, head length 0.53–1.75 mm); cephalic dorsum with a longitudinal carina extending from anterior margin of clypeus to posterior margin of head (Bolton 2003); antennal scrobe present (Bolton 2003); eye present; acetabulum of antennal socket apparatus hemispherical (Keller 2011); accessory chamber of antennal socket absent (Keller 2011); labial palp with three or four palpomeres (Bolton 2003, Keller 2011); promesonotal suture complete and flexible (Bolton 2003); ventral flap on metapleural gland opening absent (Keller 2011); metacoxal cavity closed (Bolton 2003); petiolar sternite articulated with tergite over its entire length (Bolton 2003, Keller 2011); laterotergites of petiole present.

Genera: Acanthoponera, *Aulacopone*, *Boltonia* **new genus**, *Heteroponera*.

***Acanthoponera* Mayr**

Type Species Acanthoponera mucronata (Roger)

Diagnosis (Females): Ants of comparatively medium size (head width 0.90–1.61, head length 1.00–1.75). Mandible triangular. Palp formula 6,4. Frontal lobe reduced, only partially covering antennal insertions. Antennal club with four antennomeres. Antennal scrobe deeply impressed. Eye well-developed, with clear limits between ommatidia. Propodeum with a pair of well-developed spines. Tarsal claw with conspicuous preapical teeth and a basal lobe. Petiole with a long posterodorsal projection. Anterior face of abdominal segment III with an arched carina above the helcium.

Species: goeldii, minor, mucronata, and *peruviana.*

Distribution: Exclusively Neotropical, from southern Mexico to northern Argentina.

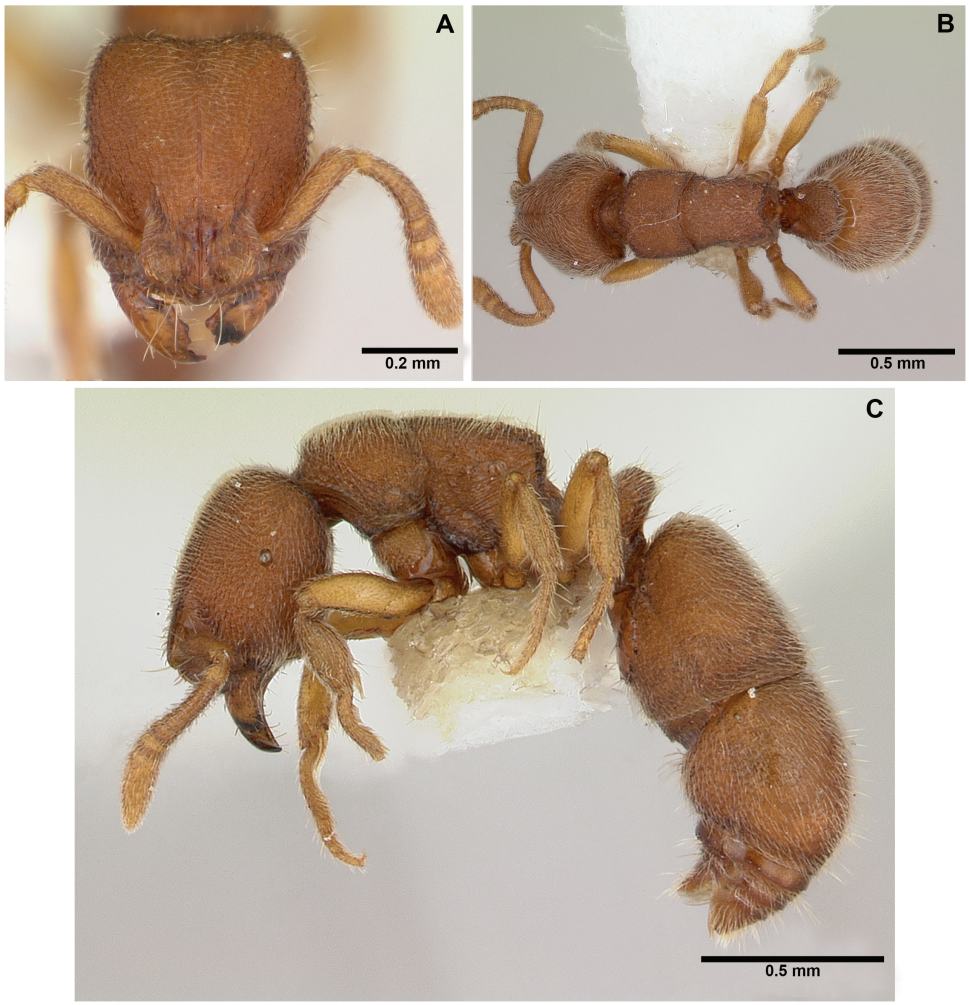

Fig. 3. Worker of *Boltonia microps* in A) frontal view; B) dorsal view; and C) lateral view. Images by April Nobile (CASENT0173544); available from www.antweb.org (Antweb 2021).

Notes: Acanthoponera represents a lineage of arboreal and nocturnal Neotropical ants. Most species can be identified using the work of Feitosa and Prada-Achiardi (2019) for the Colombian fauna.

***Aulacopone* Arnol'di**

Type Species: Aulacopone relicta Arnol'di

Diagnosis (Queens): Ants of comparatively medium size. Mandibles subfalcate. Median portion of clypeus modified, raised as a short, blunt triangular point projecting from the antennal insertions to the mandible. Frontal lobe expanded, extending from the clypeal posterior margin to the vertex. Antennal scrobe wide and deep.

Species: relicta.

Distribution: The only known specimens were collected in Azerbaijan in mountainous forests.

Notes: The genus is only known from two queens collected in Azerbaijan. The first specimen was collected in 1929 in Alazapin on the border with Iran, and later designated as the holotype by Arnol'di and deposited at the Zoological Institute of the Russian Academy of Sciences. The second specimen was collected in 1936, also by Arnol'di in the same country, in the region of Khachmaz, and later deposited in his personal collection at the Institute of Evolutionary Animal Morphology in Moscow. However, the holotype has been missing since 1979 and has not been examined for any study other than the original description. The second specimen was coated in gold-palladium for the study of its external morphology using scanning electron microscopy by Taylor (1980).

***Boltonia* Camacho and Feitosa new genus**

Type Species: Boltonia microps (Borgmeier) **new combinationtion** (Fig. 3)

Diagnosis (Females): Ants of comparatively small size (head width 0.42-0.53, head length 0.53-0.67). Mandible subfalcate. Palp formula 3,2. Frontal lobe expanded, completely covering antennal insertions. Antennal club with three antennomeres. Antennal scrobe absent. Eye drastically reduced, without conspicuous limits between ommatidia. Propodeum unarmed. Tarsal claw simple, without conspicuous preapical teeth nor a basal lobe. Petiole unarmed. Anterior face of abdominal segment III without an arched carina above the helcium.

Species: microps.

Distribution: Exclusively Neotropical, from Costa Rica to northern Argentina and southern Brazil.

Notes: We here propose the new genus *Boltonia* to accommodate a single species, *B. microps* (Borgmeier 1957), formerly a member of *Heteroponera*. This species represents a divergent lineage at the base of Heteroponerini and is the sister-group of all the remaining species in the tribe. The genus name is an homage to Barry Bolton, legendary ant taxonomist and author of Bolton's Catalogue of Ants of the World, which is the very foundation of all taxonomic papers published in myrmecology since 1994.
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***Heteroponera* Mayr**
= *Anacanthoponera* Wheeler
= *Paranomopone* Wheeler

Type Species: Heteroponera carinifrons Mayr

Diagnosis (Females): Ants of comparatively small to medium size (head width 0.63-1.37; head length 0.72-1.54). Mandible triangular. Palp formula 3,2 to 4,3. Frontal lobe expanded, completely covering antennal insertions. Antennal club with three antennomeres. Antennal scrobe shallowly to deeply impressed. Eye well-developed to reduced, with clear limits between ommatidia. Propodeal spine absent to well-developed. Tarsal claw simple, without conspicuous preapical teeth (except in *H. dolo* and *H. robusta*) nor a basal lobe. Petiole with or without posterodorsal projections. Anterior face of abdominal segment III with an arched carina above the helcium.

Species: angulata, brounii, carinifrons, crozieri, darlingtonorum, dentinodis, dolo, ecarinata, flava, georgesi, imbellis, inca, inermis, leae, lioprocta, majeri, mayri, monteithi, monticola, panamensis, pendergrasti, relicta, rhodopygea, robusta, trachypyx, viviennae, and *wilsoni*.

Fig. 4. Dorsal view of head, showing: A) Cephalic median longitudinal carina present, extending from the anterior clypeal margin to the vertex (*Acanthoponera minor*—CASENT0178699); B) Cephalic median longitudinal carina not extending from the anterior clypeal margin to the vertex (*Ectatomma tuberculatum*—CASENT0173380); C) Cephalic median longitudinal carina absent (*Holcoponera striatula*—CASENT0173386). Photos by April Nobile; available from www.antweb.org (Antweb 2021).

Distribution: Neotropical, from Nicaragua to southern Chile; and Australian, including New Zealand.

Notes: Identification tools for the species of *Heteroponera* include the works of Feitosa and Prada-Achiardi (2019) for the Colombian fauna and Taylor (2011; 2015) for Australian groups.

Key to the Ectatomminae Genera

1. Cephalic median longitudinal carina present, extending from the anterior clypeal margin to the vertex (Fig. 4A). Metapleural gland orifice simple, directed posteriorly or laterally (tribe Heteroponerini) 2

- Cephalic median longitudinal carina absent or not extending from the anterior clypeal margin to the vertex (Fig. 4B, C). Metapleural gland orifice forming an oblique curved slit bounded below by a convex rim of cuticule that directs the orifice dorsally to posterodorsally (tribe Ectatommini) 5

2(1). Median portion of clypeus modified, raised as a short, blunt triangular point projecting from the antennal insertions to the mandible. Antennal scrobe wide and very deep (exclusively Paleartic) (known only by queens) ..... *Aulacopone*

- Median portion of clypeus not raised, not or only to a small extent covering the mandible. Antennal scrobe deep to absent 3

3(2). Tarsal claws with a prominent basal lobe and a long preapical tooth. Propodeum armed with prominent spines (exclusively Neotropical)..... *Acanthoponera*

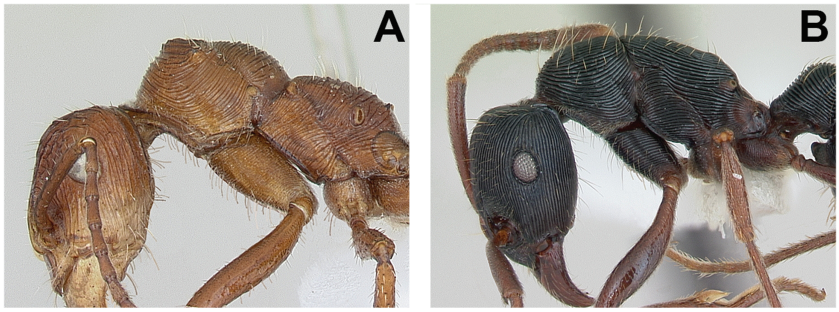

Fig. 5. Lateral view of pronotum, showing: A) Pronotal tubercles present; mesonotum prominent, separated from propodeum by a deep transversal suture (*Ectatomma tuberculatum*—CASENT0173380); B) Pronotal tubercles or projections absent; mesonotum not prominent, forming a continuous profile with propodeum (*Holcoponera striatula*—CASENT0173386). Photos by April Nobile; available from www.antweb.org (Antweb 2021).

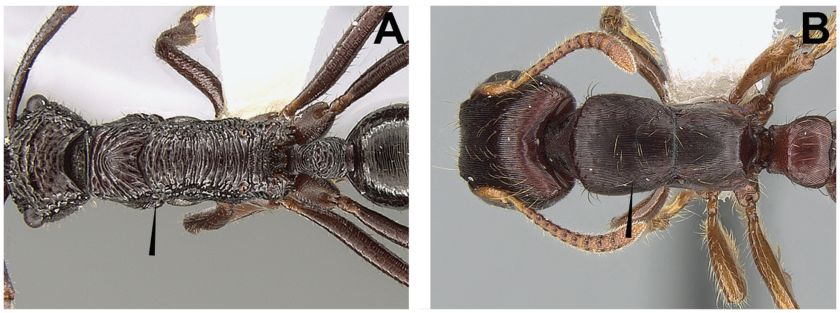

Fig. 6. Dorsal view of pronotum, showing: A) Pronotum and mesonotum separated by a distinct suture (*Rhytidoponera abdominalis*—CASENT0281333); B) Pronotum and mesonotum continuous with a discrete groove (*Gnamptogenys stellae*—CASENT0281227). Photos by Cerise Chen (A) and Estella Ortega (B) available from www.antweb.org (Antweb 2021).

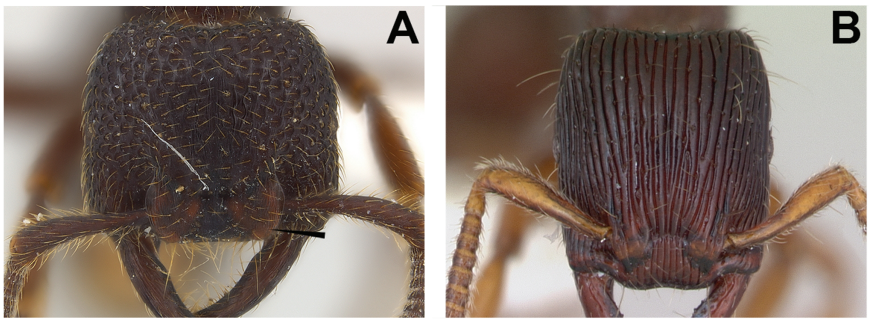

Fig. 7. Frontal view of head, showing: A) Expanded frontal lobes (*Alfaria falcifera*—CASENT0179971); B) Occipital lobes absent (*Gnamptogenys continua*—CASENT0173383). Photos by Erin Prado (A) and April Nobile (B); available from www.antweb.org (Antweb 2021).

- Tarsal claws simple, without a prominent basal lobe or preapical tooth. Propodeum generally angled or with small rhomboidal teeth at most 4

4(3). In frontal view, mandible subfalcate, with around four teeth on the masticatory margin. Antennal scrobe absent. Eye drastically reduced, without conspicuous limits between ommatidia (exclusively Neotropical) ..... *Boltonia*

- In frontal view, mandible subtriangular, with six to eight teeth on the masticatory margin. Antennal scrobe shallowly to deeply impressed. Eye well-developed, with clear limits between ommatidia (Neotropical and Australian) .... *Heteroponera*

5(1). Pronotum usually with 2 or 3 tubercles. Mesonotum prominent and clearly differentiated from propodeum, separated by a deep transverse suture (Fig. 5A). Apex of anterior tibia in outer lateral view with a seta close to the spur base (exclusively Neotropical) ..... *Ectatomma*

- Pronotum unarmed and without tubercles. Mesonotum not prominent, forming a continuous profile with the propodeum (Fig. 5B). Apex of anterior tibia in outer lateral view without a seta close to the spur base; if seta present, then species distribution is exclusively Australasian 6

6(5). In dorsal view, pronotum and mesonotum always separated by a distinct suture, so that each tergite forms a separate plate

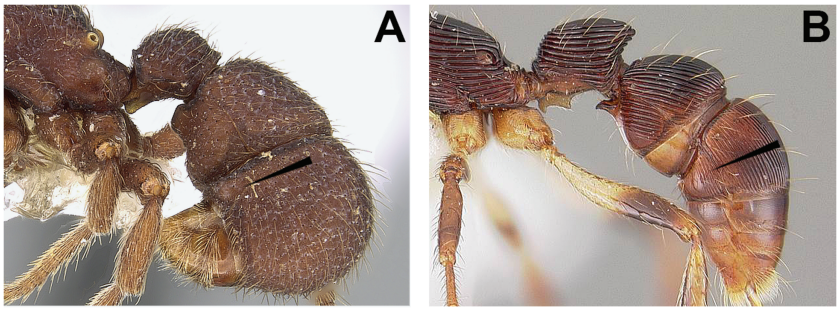

Fig. 8. Lateral view of gaster, showing: A) Second gastral (IV abdominal) sternite not strongly reduced in relation to the tergite; dorsal profile of gaster gently convex, so that the apex of gaster is only discretely directed ventrally (*Gnamptogenys acuminata*—USNMENT00441095); B) Second gastral (IV abdominal) sternite strongly reduced in relation to the tergite; dorsal profile of gaster extremely convex, so that the gaster is strongly directed ventrally and anterad (*Alfaria minuta*—CASENT0281213). Photos by Jeffrey Sosa-Calvo (A) and Estella Ortega (B); available from www.antweb.org (Antweb 2021).

Fig. 9. Dorsal view of mesosoma, showing: A) Promesonotal suture absent (*Gnamptogenys acuminata*—USNMENT00441095); B) Promesonotal suture feeble, never interrupting dorsal mesosomal sculpture (*Poneracantha banksi*—INBIOCRI001281007); C) Promesonotal suture well marked, totally interrupting dorsal mesosomal sculpture (*Holcoponera moelleri*—CASENT0173384). Photos by Jeffrey Sosa-Calvo (A), Estella Ortega (B), and April Nobile (C); available from www.antweb.org (Antweb 2021).

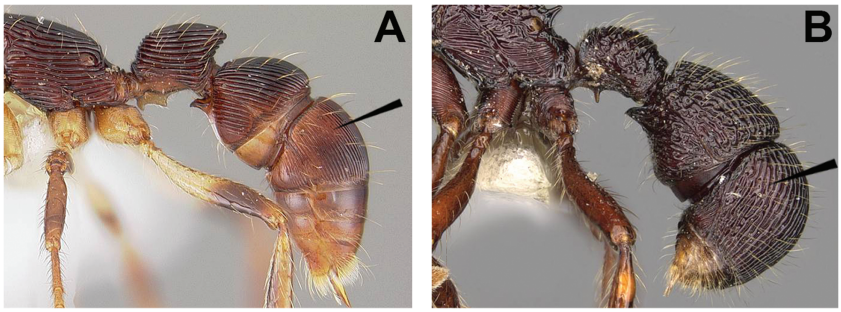

Fig. 10. Lateral view of gaster, showing: A) Second gastric segment (IV abdominal) relatively straight (*Gnamptogenys acuminata*—USNMENT00441095); B) Second gastric segment (IV abdominal) slightly arched ventrally (*Poneracantha mecotyle*—CASENT0281530). Photos by Jeffrey Sosa-Calvo (A) and Zach Lieberman (B); available from www.antweb.org (Antweb 2021).

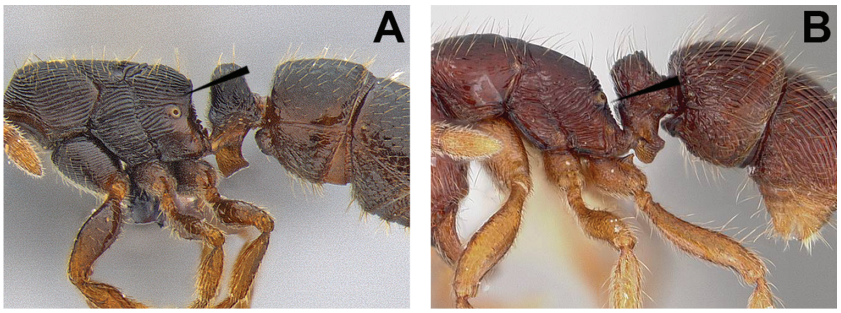

Fig. 11. Lateral view of propodeum, showing: A) Propodeal spiracle separated from declivity margin by a distance longer than its diameter (*Typhlomyrmex lavra*); **B**) Propodeal spiracle close to the declivous face of propodeum (*Holcoponera relicta*—USNMENT00412058). Photos by Gabriela Camacho (A) and Jeffrey Sosa-Calvo; available from www.antweb.org (Antweb 2021).

(Fig. 6A). Dorsum of posterior coxa never with a lobe or spine. Petiolar sternite tightly attached but never fused to the tergite (exclusively Australasian) ..... *Rhytidoponera*

- In dorsal view, pronotum and mesonotum usually continuous or separated by a discrete groove (Fig. 6B); if a well-impressed promesonotal suture is present (as in *Holcoponera* and *Typhlomyrmex*), then the dorsum of posterior coxa frequently with a lobe or spine. Petiolar sternite fused with tergite over its entire length 7

7(6). Frontal lobes strongly expanded, entirely covering the antennal insertions (Fig. 7A). Second gastral (IV abdominal) sternite strongly reduced in relation to the tergite; dorsal profile of gaster extremely convex, so that the gaster is usually strongly directed ventrally and anterad (Fig. 8A). Exclusively Neotropical. ..... *Alfaria*

- Frontal lobes less developed, only partially covering the antennal insertions (Fig. 7B). Second gastral (IV abdominal) sternite not strongly reduced in relation to the tergite; dorsal profile of gaster gently convex, so that the apex of gaster is directed ventrally or posteriorly (Fig. 8B). 8

8(7). Promesonotal suture feebly impressed to absent, never interrupting dorsal mesosomal sculpture (Fig. 9A, B) 9

- Promesonotal suture well marked, totally interrupting dorsal mesosomal sculpture (Fig. 9C) 11

9(8). Strictly Indomalayan species ..... *Stictoponera*

- Strictly Neotropical species 10

10(9). Propodeum rarely armed with denticles or spines. Metacoxal teeth present or absent. Second gastric segment (IV abdominal) relatively less curved (Fig. 10A) ..... *Gnamptogenys*

- Propodeum usually armed with denticles or spines. Metacoxal teeth generally present. Second gastric segment (IV abdominal) relatively more curved (Fig. 10B) ..... *Poneracantha*

11(8). Eye absent or reduced. Propodeal spiracle separated from posterior face of propodeum by a distance longer than its diameter (Fig. 11A). Metacoxal dorsum unarmed or at most with a small lobe or denticle ..... *Typhlomyrmex*

- Eye well developed to reduced. Propodeal spiracle close to posterior face of propodeum (Fig. 11B). Metacoxal dorsum always with a denticle or lobe ..... *Holcoponera*

Supplementary Data

Supplementary data are available at *Insect Systematics and Diversity* online.

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Author Contributions

GPC: Conceptualization; Data curation; Formal Analysis; Funding acquisition; Investigation; Methodology; Project administration; Software; Validation; Visualization; Writing – original draft; Writing – review & editing. WF: Data curation; Formal analysis; Investigation; Writing - review & editing. MGB: Data curation; Formal analysis; Investigation; Software; Writing – review & editing. MRP: Conceptualization; Resources; Supervision; Writing – review & editing. JTL: Resources; Formal analysis; Funding acquisition; Writing – review & editing. TRS: Funding acquisition; Resources; Supervision; Writing – review & editing. RMF: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Supervision; Writing – review & editing.

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