Synonymization of the male-based ant genus *Phaulomyrma* (Hymenoptera, Formicidae) with *Leptanilla* based upon Bayesian total-evidence phylogenetic inference

Zachary H. Griebenow

Abstract

Although molecular data have proven indispensable in confidently resolving the phylogeny of many clades across the tree of life, these data may be inaccessible for certain taxa. The resolution of taxonomy in the ant subfamily Leptanillinae is made problematic by the absence of DNA sequence data for leptanilline taxa that are known only from male specimens, including the monotypic genus *Phaulomyrma* Wheeler & Wheeler. Focusing upon the considerable diversity of undescribed male leptanilline morphospecies, the phylogeny of 38 putative morphospecies sampled from across the Leptanillinae, plus an outgroup, is inferred from 11 nuclear loci and 41 discrete male morphological characters using a Bayesian total-evidence framework, with *Phaulomyrma* represented by morphological data only. Based upon the results of this analysis *Phaulomyrma* is synonymized with *Leptanilla* Emery, and male-based diagnoses for *Leptanilla* that are grounded in phylogeny are provided, under both broad and narrow circumscriptions of that genus. This demonstrates the potential utility of a total-evidence approach in inferring the phylogeny of rare extant taxa for which molecular data are unavailable and begins a long-overdue systematic revision of the Leptanillinae that is focused on male material.

Introduction

Over the past three decades, DNA sequences have provided great insight into phylogenetic relationships across the Metazoa, including the insects (Kjer *et al.* 2018). The application of maximum-likelihood and Bayesian statistical methods to analysis of genetic data has robustly resolved many problems that were intractable when using morphological data alone (e.g. Niehuis *et al.* 2012; Wipfler *et al.* 2019). However, DNA sequences may be unavailable for some taxa, necessitating the integration of morphological and molecular data under the same inferential framework. Fossils are the most obvious example of this: these are valuable for calibration of phylogenies in absolute time under a Bayesian approach, preferably with their topological position being inferred from the data (Ronquist *et al.* 2012; O'Reilly *et al.* 2015; Bapst et al. 2016; Matzke & Wright 2016). Although the inclusion of fossils for the purposes of "tip-dating" has received the bulk of attention in Bayesian total-evidence phylogenetic inference, the lack of molecular data may afflict rare extant taxa as well (Sánchez *et al.* 2016; Robertson & Moore 2016). This is problematic if the affinities of these taxa are not immediately clear from morphology alone.

The ant subfamily Leptanillinae (Hymenoptera: Formicidae) is an apt test case for methods to resolve this problem. A group of small, hypogaeic ants largely restricted to the Old World tropics and subtropics, the Leptanillinae are understood to be one of the earliest-diverging lineages in the ant crown-group (Rabeling *et al.* 2008; Kück *et al.* 2011; Borowiec *et al.* 2019; Boudinot *et al.* submitted). Four leptanilline genera—*Scyphodon* Brues, 1925; *Phaulomyrma* Wheeler & Wheeler, 1930; *Noonilla* Petersen, 1968; and *Yavnella* Kugler, 1986—have been described solely from males, as have many species of *Leptanilla* Emery, 1870 (cf. Bolton 1990). Recent molecular data indicate that the type species of *Yavnella* and a specimen provisionally assigned

to *Phaulomyrma* are nested within a clade of putative *Leptanilla* morphospecies (Borowiec *et al.* 2019). Moreover, although *Scyphodon anomalum* Brues, 1925 and *Noonilla copiosa* Petersen, 1968 exhibit bizarre autapomorphies such as hypertrophied mandibles (Brues 1925) and a ventromedian genital "trigger" (Petersen 1968), respectively, these ants are otherwise similar to males attributed to *Leptanilla* (Boudinot 2015).

This indicates a need for a systematic revision of the Leptanilline, but almost all published taxonomic study of the group has been descriptive without recourse to molecular phylogeny, with the exceptions being revisions to our concept of the subfamily. Multi-locus DNA datasets demonstrated that the enigmatic Afrotropical genus *Apomyrma* Gotwald, Brown & Lévieux, 1971 is closely related to the Amblyoponinae rather than the Leptanillinae (Brady *et al.* 2006; Moreau *et al.* 2006), and that the superficially similar Asian genus *Opamyrma* Yamane, Bui & Eguchi, 2008 is in fact sister to the remaining Leptanillinae (Ward & Fisher 2016). None of these studies focused upon the Leptanillinae or the internal phylogeny of this clade. Such a study must confront two challenges: first, the lack of DNA sequences for certain critical taxa across the Leptanillinae (e.g., *Scyphodon*), which hampers any attempt to confidently resolve relationships among these; second, the definition of genera based only upon males, which prevents an integrated phylogenetic classification of the Leptanillinae, since phenotypes of only one sex are considered.

The dissociation of leptanilline castes results from collecting bias. Subterranean workers have been largely collected with *lavage de terre* methodology (López *et al.* 1994; Wong and Guénard 2016), Winkler trapping (Belshaw & Bolton 1994; Leong *et al.* 2018), and subterranean pitfall traps (Wong & Guénard 2016; Man *et al.* 2017); whereas male leptanillines are typically collected by sweeping foliage or by deploying Malaise or pan traps (Robertson 2000). None of

these methods are likely to collect males in association with workers, nor is the queen caste often collected in association with conspecifics. Contrasting with the alate condition observed in most ants, queens described from the tribe Leptanillini are completely wingless and blind (Emery 1870; Kutter 1948; Masuko 1990; López *et al.* 1994; Ogata *et al.* 1995), meaning that these are no more likely to be collected than corresponding workers. Queens belonging to other leptanilline lineages (*Opamyrma* and Anomalomyrmini) are alate so far as is known (Bolton 1990; Baroni Urbani & de Andrade 2008; Borowiec *et al.* 2011; Chen *et al.* 2017; Hsu *et al.* 2017; Man *et al.* 2017), save for an apparent record of queen polyphenism in an undescribed *Protanilla* Taylor, 1990 (Billen *et al.* 2013), but are infrequently collected.

Therefore, the bulk of known leptanilline diversity, most of it undescribed, is represented by exclusively male material. In some cases, molecular data are inaccessible for male morphotaxa due to paucity of suitably recent specimens, obliging a total-evidence approach to infer the phylogeny of these lineages. This study uses such an approach to resolve the position of the male-based species *Phaulomyrma javana* Wheeler & Wheeler, 1930, the sole species included in this genus. Here, the phylogeny of the Leptanillinae is inferred jointly from 10 protein-coding genes, 28S rRNA, and 41 discrete male morphological characters under a Bayesian statistical framework. This is the first combined-evidence Bayesian analysis to include the Leptanillinae and is novel among studies of ant phylogeny in its inclusion of exclusively male morphological characters (Barden *et al.* [2017] used both worker and male morphology in their Bayesian total-evidence inference). Despite the absence of nucleotide sequences for *P. javana* a total-evidence approach facilitates the inclusion of this terminal and its confident phylogenetic placement. Based upon the results of this joint molecular and morphological phylogenetic analysis, a revised

male-based definition of *Leptanilla* is provided, and *Phaulomyrma* is synonymized with that genus.

Materials and Methods

Taxon Sampling

Thirty-eight terminals were included in total (Table 1). Discrete morphological data were scored for those 35 terminals for which male material was available. These observations were completed in November 2019, meaning that these did not include the recently described male of *Opamyrma hungvuong* Yamane, Bui & Eguchi, 2008 (Yamada *et al.* 2020). In addition to *O. hungvuong*, *Anomalomyrma boltoni* Borowiec, Schulz, Alpert & Baňar, 2011 and *Leptanilla revelierii* Emery, 1870 were represented in this study by workers alone. The latter was included on account of its status as the type species of that genus: regardless of future systematic revision to the Leptanillinae, the concept of the genus *Leptanilla* will not exclude this species. DNA sequences for the outgroup *M. heureka* were obtained from a worker ant, as published in Borowiec *et al.* (2019).

Representatives of all male-based genera were included in total-evidence analyses, except for *Scyphodon*. These include both *Yavnella argamani* Kugler, 1986 and *Yavnella* cf. *indica*, along with two undescribed *Yavnella* morphospecies from Bhutan and Thailand, respectively; *P. javana* and *Phaulomyrma* MM01, the latter morphospecies provisionally assigned to *Phaulomyrma* by Boudinot (2015) and Borowiec *et al.* (2019); and a morphospecies of *Noonilla* close to the type species *Noonilla copiosa* Petersen, 1968, along with three additional *Noonilla* morphospecies identified as such according to the definition given by Petersen (1968).

Material is deposited in the following repositories: the Bohart Museum of Entomology, University of California, Davis, CA, USA (UCDC); the California Academy of Sciences, San Francisco, CA, USA (CASC); the California State Collection of Arthropods, Sacramento, CA, USA (CSCA); the Lund Museum of Zoology, Lund, Sweden (MZLU); and the Australian National Insect Collection, Canberra, Australia (ANIC).

Molecular Dataset

Phylogenetic inference was based upon 11 nuclear loci: *28S ribosomal DNA* (*28S*)*, abdominal-A* (*abdA*)*, arginine kinase* (*argK*)*, antennapedia* (*Antp*)*, elongation factor 1-alpha F2 copy* (*EF1αF2*)*, long wavelength rhodopsin* (*LW Rh*)*, NaK ATPase* (*NaK*)*, DNA pol-delta* (*POLD1*)*, topoisomerase I* (*Top1*)*, ultrabithorax* (*Ubx*)*,* and *wingless* (*Wg*). I derived these "legacy loci" for 19 terminals from the alignment of Borowiec *et al.* (2019) (doi: 10.5281/zenodo.2549806) but expanded to include autapomorphic indels and introns, and constituting 11,090 bp. For further detail on the protocols for the extraction and amplification of these genetic data, refer to Ward *et al.* (2010) and Ward & Fisher (2016). I added 18 terminals to this "legacy-locus" intron-inclusive dataset by retrieving orthologous loci from phylogenomic data acquired with the ultra-conserved element (UCE) probe set *hym-v2* (Branstetter *et al.* 2017), as follows.

In the case of these 18 terminals, DNA was extracted non-destructively using a DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA) according to manufacturer instructions. DNA was quantified for each sample with a Qubit 2.0 fluorometer (Life Technologies Inc., Carlsbad, CA). Phylogenomic data were obtained from these taxa using the *hym-v2* probe set, with libraries being prepared and target loci enriched using the protocol of Branstetter *et al.* (2017). Enrichment success and size-adjusted DNA concentrations of pools were assessed using the SYBR FAST qPCR kit (Kapa Biosystems, Wilmington, MA) and all pools were combined into

132 an equimolar final pool. The contents of this final pool were sequenced by an Illumina HiSeq
133 2500 at the University of Utah's High Throughput Genomics Facility. The FASTQ output was
134 demultiplexed and cleansed of adapter contamination and low-quality reads using
135 *illumiprocessor* (Faircloth 2013) in the PHYLUCE package. Raw reads were then assembled
136 with *trinity* v. 2013-02-25 (Grabherr *et al.* 2011).

137 The possibility of genetic contamination across UCE samples was examined by inferring the
138 phylogeny from a concatenated UCE alignment, unpartitioned, using IQ-Tree v. 1.6.10 (Nguyen
139 *et al.* 2015) on the CIPRES Science Gateway (v. 3.3) (Miller *et al.* 2010) with the GTR+G model
140 of nucleotide substitution for 1,000 ultrafast bootstrap replicates (Hoang *et al.* 2018): topologies
141 and branch lengths were plausible given preliminary morphological hypotheses, indicating that
142 contamination had not occurred.

143 Legacy loci orthologous with those used by Borowiec *et al.* (2019) were then recovered from
144 genome-scale data as follows with PHYLUCE (Faircloth 2016). I derived single exon sequences
145 representing each locus for *Leptanilla* GR02 from the alignment ANT-exon-sequences-40-taxa-
146 reduced.fasta published by Branstetter *et al.* (2017), given the comparative completeness of the
147 matrix for that species, and its phylogenetic position nested well within the Leptanillinae. These
148 sequences were then used analogously to probes. Species-specific contig assemblies were
149 aligned using *phyluce_assembly_match_contigs_to_probes.py* (min_coverage = 50, min_identity
150 = 85), a list of legacy loci shared across all taxa was generated using
151 *phyluce_assembly_get_match_counts.py*, and separate FASTA files for each locus were created
152 using these outputs. Sequences were aligned separately by locus using MAFFT (Katoh *et al.*
153 2009) implemented with the command *phyluce_assembly_seqcap_align.py*, and these sequences
154 were then trimmed with Gblocks (Castresana 2000) as implemented by the wrapper script

phyluce_assembly_get_gblocks_trimmed_alignment_from_untrimmed.py (settings: b1 = 0.5, b2 = 0.5, b3 = 12, b4 = 7). Alignment statistics for the output FASTA files were calculated with *phyluce_align_get_align_summary_data.py*. Finally, a matrix that was 80% complete with respect to locus coverage was generated using the script *phyluce_align_get_only_loci_with_min_taxa.py*. This contained only 6 out of the 10 protein-coding loci that I attempted to recover using the exon-based bioinformatic protocol of Branstetter *et al.* (2017). Legacy loci recovered from UCE assemblies often included non-coding sequences adjacent to the regions included in Borowiec *et al.* (2019), which were trimmed manually in AliView. In whichever cases those loci had been recovered, sequences for the taxa represented only in the dataset of Borowiec *et al.* (2019) were then aligned with the recovered legacy loci using the online MAFFT interface (Katoh *et al.* 2019) with default settings. In cases where legacy loci were not successfully recovered or were incomplete relative to preexisting Sanger-derived sequences, these loci were derived from the dataset of Borowiec *et al.* (2019) or, in the case of *Leptanilla* GR03, Ward & Sumnicht (2012). These data were concatenated with UCE-derived sequences across all FASTA files, inasmuch all sequences for each morphospecies were derived from the same specimen; and all loci were concatenated to produce a final alignment, which was 9,011 bp in length. Further summary statistics for this final alignment are provided in Table 1. *P. javana* was the only terminal for which molecular data were not obtained: this species is known only from two slide-mounted syntypes collected in 1907.

Alignment was unambiguous once all loci were brought into their respective reading frames. GenBank accession numbers for all loci used in this study, and NCBI accession numbers for the raw UCE reads from which some loci were derived, are provided in Table 2.

Morphological Dataset

Forty-one discrete binary morphological characters were coded for all 35 morphospecies known from males. All these specimens were examined with a Leica MZ75 compound microscope or by reference to images on AntWeb, except for the male of *M. heureka*, in the case of which observations were derived from Boudinot (2015: Figs. 11-12). I imaged specimens when necessary using a JVC KY-F75 digital camera and compiled color photographs from these with the Syncroscopy AutoMontage Program. Scanning electron microscopy was undertaken using a Hitachi TM4000 tabletop microscope. Morphological terminology follows the Hymenoptera Anatomy Ontology (Yoder *et al.* 2010), with some exceptions being derived from Bolton (2003) and Boudinot (2018). The character coding scheme was binary and non-additive (Pleijel 1995). Missing data were scored as '?'. Autapomorphic characters were included. Numerical scores for all morphological characters are presented in Supplemental Table 1.

Non-additive binary coding has been criticized for its susceptibility to redundancy (Strong & Lipscomb 1999), stipulation of compound characters, and the inadvertent conflation of morphological absences that are not hierarchically equivalent (Brazeau 2011). These problems largely result from careless character delimitation. I compensated for these potential flaws by defining and using only characters that do not logically depend upon other characters. Definitions of morphological character states are provided in Supplemental Material 1.

Phylogenetic Analyses

Best-fitting partitions of the molecular data, and substitution models for these partitions, were inferred synchronously with PartitionFinder2 v. 2.1.1 (Guindon *et al.* 2010; Lanfear *et al.* 2012, 2017) on the CIPRES Science Gateway (Miller *et al.* 2010), with subsets being asserted *a priori* according to locus and codon position. Introns were included. I+G general time-reversible mixture models were excluded from consideration due to undesirable behavior under a

probabilistic framework (Yang 1996). As an alternative *ad hoc* partitioning scheme for molecular data, I respectively partitioned all exonic loci so that 1st-2nd codon positions were placed in their own partition separate from the 3rd, and modeled nucleotide substitution in all partitions under GTR+G. Using AMAS (Borowiec 2016), the full 9,011-bp alignment was split according to both partition schemes.

The Mkv model (Lewis 2001) was used to model substitution of morphological character states, albeit with stationary frequencies of character states treated as free parameters (Felsenstein 1981) in order to accommodate asymmetry in character state frequencies. Variation in evolutionary rate among characters was accommodated by drawing rates from a gamma-distributed prior probability distribution (+G), approximated with 8 discrete categories *k*.

Phylogenetic analyses were performed in a Bayesian statistical framework using RevBayes v. 1.0.11 (Höhna *et al.* 2017) compiled on Ubuntu Linux v. 13.04. Each analysis consisted of four independent Markov chain Monte Carlo (MCMC) chains, each run for 50,000 generations. Trees were sampled every 10 generations, with the first 25% of the run being discarded as burn-in. MCMCs with respect to all continuous parameters were considered converged if the effective sample sizes as given in Tracer v. 1.7.1 (Rambaut *et al.* 2018) were >200, with sufficiency of MCMC mixing across posterior probability landscapes being qualitatively assessed using traces of the respective log-likelihoods of each parameter across the course of the analysis. Maximum *a posteriori* trees were compiled from this sample of each run, with node support expressed as Bayesian posterior probability (BPP).

Data Availability and Nomenclature

222 All nucleotide and morphological data along with PF2 configuration files, RevBayes scripts, and
223 output of both phylogenetic analyses, are available at the Dryad Digital Repository
224 (doi:10.25338/B8GP7C). Sequence Raw Archives (SRAs) of raw UCE reads, and UCE
225 assemblies, are publicly available on NCBI (Table 2).

226 This article has been registered in Zoobank (www.zoobank.org). The LSID number is
227 5F3BECF6-3715-47B3-8D0F-DE7D66E1DA0A.

228 **Results**

229 Bayesian total-evidence inference of leptanilline phylogeny under the two partitioning schemes
230 resulted in similar topologies, with none of the differences affecting the composition or
231 interrelationship of major clades, and therefore not influencing the generic status of
232 *Phaulomyrma*. The phylogeny inferred under the *ad hoc* partitioning scheme is provided on
233 Dryad (doi: 10.25338/B8GP7C). All discussion from here on will refer to the phylogeny inferred
234 under the scheme derived with PartitionFinder2 (Fig. 1). Most nodes in this phylogeny were
235 supported with BPP≥0.95 (Fig. 1). Those that were not were scattered and shallow, meaning that
236 the interrelationships among all major leptanilline clades are well-resolved. Although the
237 sampling of the Leptanillinae was more extensive than that of Borowiec *et al.* (2019), our
238 inferences were largely congruent.

239 The clade corresponding to the tribe Anomalomyrmini (labeled as *Protanilla sensu lato* in Fig.
240 1) is recovered with maximal support (BPP = 1), with *A. boltoni* sister to all sampled *Protanilla*
241 save *Protanilla* TH03—thus rendering *Protanilla* paraphyletic—moderately supported (BPP =
242 0.9009). Borowiec *et al.* (2019) recovered *A. boltoni* as sister to *Protanilla* TH03 with weak
243 support irrespective of statistical framework, albeit with more extensive sampling within the

Anomalomyrmini. However, the internal topology of the Anomalomyrmini does not have any bearing upon the status of *Phaulomyrma* relative to other male-based leptanilline genera, nor its status relative to *Leptanilla*.

Noonilla, *Yavnella argamani* and *Yavnella* cf. *indica*, *Leptanilla revelierii*, and *Phaulomyrma javana* were firmly recovered within a clade corresponding to the Leptanillini (BPP = 1). As in Borowiec *et al.* (2019) the Leptanillini bifurcate robustly, with *Y. argamani* (and *Yavnella* cf. *indica*, which was not included in Borowiec *et al.* [2019]) recovered in a clade otherwise without described representatives, which is hereinafter designated *Yavnella sensu lato* (BPP = 1). Although morphologically diverse (Fig. 2), the male morphospecies that comprise the sister-group to *Yavnella s. l.* are distinguished from *Yavnella s. l.* by 1) clypeus with a medial axis no longer than the diameter of the torulus, where the epistomal sulcus is distinct; and 2) pronotum and mesoscutum that are not extended posteriorly in profile view. Since *L. revelierii* is recovered within this clade, it is hereinafter referred to as *Leptanilla sensu lato* (BPP = 0.9999).

Leptanilla s. l. bifurcates into two well-supported clades: one is broadly Eurasian and Australian in its representation (with a single Afrotropical representative included in this study), including *L. revelierii* and *P. javana* (BPP = 0.9971); the other is Indo-Malayan, and includes *Noonilla* (BPP = 0.9998). Of the 11 terminals recovered in this latter subclade, only *Leptanilla* TH01 was included in Borowiec *et al.* (2019). *Noonilla* (BPP = 0.9769) is sister to a clade of highly distinctive male morphospecies, recovered with maximal support (BPP = 1), that are immediately recognizable by bizarre metasomal processes (heretofore hypothesized to be extensions of the gonocoxae *sensu* Boudinot [2018] [Boudinot 2015: Fig. 10D]) and a comb-like row of robust bristles on the protibia (Fig. 3). These morphospecies remain undescribed, but I provisionally refer to the clade as the “Bornean morphospecies-group”: while present sampling is

too sparse to judge whether this clade is precinctive to Borneo, available material exclusively originates on that island. The rather disparate morphospecies *Leptanilla* TH01 and *Leptanilla* zhg-th01 are recovered as a clade with maximum support (BPP = 1), and this clade is in turn sister to *Noonilla* + Bornean morphospecies-group. *Leptanilla* zhg-th01 is unique among the Leptanillinae in possessing a recurved mesoscutellar horn (Fig. 4B). Since the type species of *Leptanilla* (*L. revelierii*) is included within the Eurasian-Australian clade, this clade is hereinafter referred to as *Leptanilla sensu stricto.* These two circumscriptions of the name *Leptanilla* are supported by male morphology (see Discussion).

The support values of internal nodes within *Leptanilla s. str.* are generally poor, with the placement of *Leptanilla* ZA01 and *Leptanilla* zhg-bt01 differing according to the partitioning scheme. In either case the topology of *Leptanilla s. str.* is likely subject to strong stochastic error due to the inclusion of *P. javana*, for which molecular data are entirely absent. While the phylogeny of *Leptanilla s. str.* cannot be resolved with the inferential approach and dataset used herein, the monophyly of this clade is robustly supported (BPP = 0.9971). The recovery of *P. javana* within *Leptanilla s. str.* is therefore probable based upon Bayesian total-evidence inference (see Discussion for description of morphological characters supporting this conclusion).

The syntype of *P. javana* and the taxon dubbed "*Phaulomyrma*" MM01 by Boudinot (2015) and Borowiec *et al.* (2019) were recovered distant from one another in the leptanilline phylogeny (Fig. 1). Total-evidence phylogenetic inference recovered the latter terminal within *Yavnella s. l.*, indicating that it was incorrectly assigned to *Phaulomyrma* by these authorities; another undescribed male morphospecies referred to as *Phaulomyrma* by Boudinot (2015: Fig. 4F) was not sequenced in this study, but conforms morphologically to *Yavnella s. l.*, and so likewise was

incorrectly identified as *Phaulomyrma*. Conversely, *P. javana* is here robustly recovered within *Leptanilla s. l.*, and moreover within *Leptanilla s. str.* While the internal phylogeny of *Leptanilla s. str.* remains uncertain due to *P. javana* lacking molecular data, the monophyly of this clade inclusive of *Phaulomyrma* is strongly supported by current findings.

Discussion

Delimitation of Subclades in Leptanillinae using Male Morphology

Male morphological characters corroborate phylogeny at nodes of variable depth. The four male representatives of the Anomalomyrmini included in the present study can easily be distinguished from male Leptanillini by the presence of a pterostigma (although wing venation may be inaccessible due to deciduous wings in some male Leptanillini [pers. obs.]) and the absence of an ocellar tubercle. Griebenow (in review) will provide a formal description of female-associated male *Protanilla* and a male-based definition of the leptanilline tribes. *Yavnella s. l.* is likewise well-supported by Bayesian total-evidence inference, as is *Leptanilla s. l.*, with the former clade diagnosed almost entirely by symplesiomorphies: the only putative autapomorphy of *Yavnella s. l.* is concavity of the propodeum in profile view (Fig. 5A), which was previously noted by Kugler (1986) as being distinctive to *Yavnella*.

Leptanilla s. str. is identifiable relative to other subclades of *Leptanilla s. l.* based upon the following combination of male morphological characters: absence of posterior mesoscutellar prolongation (observed in *Leptanilla* zhg-th01 and *Leptanilla* TH01); propodeum convex and without distinct dorsal face (Fig. 5C); gonopodites articulated (otherwise among the Leptanillini articulated only in *Leptanilla* zhg-th01 and some *Noonilla*); gonocoxae fully separated ventrally (this character state [Fig. 6A] elsewhere observed among sampled Leptanillini in all *Yavnella s. l.* except for "*Leptanilla*" TH03; and *Leptanilla* zhg-th01); and penial sclerites dorsoventrally

compressed along their entire length, entire, and lacking sculpture (Fig. 7A), this character state elsewhere observed in Leptanillini only among *Yavnella s. l.* (excluding "*Leptanilla*" TH03) and *Leptanilla* zhg-th01.

Unlike *Scyphodon*, *Noonilla*, and even the male-based species *Leptanilla palauensis* (Petersen, 1968: p. 593), the status of *Phaulomyrma* as a leptanilline—and as an ant—has never been debated. Wheeler & Wheeler (1930) established the genus based upon the presence of wing veins and "unusually large genitalia" (Wheeler & Wheeler 1930: p. 193). Their argument regarding wing venation has no merit, given that the forewing venation of *P. javana* falls within the range of variation observed in putative *Leptanilla* morphospecies (Petersen, 1968: pp. 594-595), with all leptanilline males examined by Boudinot (2015) exhibiting at least one compound abscissa on the forewing. Petersen (1968: p. 597) even referred to *Leptanilla* and *Phaulomyrma* as "nearly identical" (when comparing these taxa to *L. palauensis*), but refrained from synonymizing *Leptanilla* and *Phaulomyrma* on account of the apparent uniqueness of the genitalia of *P. javana* as illustrated by Wheeler & Wheeler (1930: Figs. 2A, C). In passing, Taylor (1965: p. 365) also mentioned *Phaulomyrma* as being "possibly synonymous" with *Leptanilla*.

Examination of a syntype of *P. javana* (below designated as lectotype) demonstrates that its genitalia are consistent with other sampled male *Leptanilla s. str.* to the exclusion of males within the Indo-Malayan sister-group of *Leptanilla s. str.* (Fig. 8). Although the preservation of this *P. javana* syntype on a slide prevents us from directly confirming stylar articulation, the sharply recurved styli are consistent with the syndrome seen in dried male leptanillines with articulated gonopodites (Kugler 1986; Ward and Sumnicht 2012), indicating that the gonopodites are articulated in *P. javana*. *Contra* Fig. 2C of Wheeler & Wheeler (1930), the volsellae of *P.*

javana are not discernible *in situ* (Fig. 8D). If their condition is truly "plate-like" as described by Wheeler & Wheeler (1930: p. 196), the volsellae of *P. javana* resemble those observed in undescribed Sicilian male morphospecies attributed to *Leptanilla* (Scupola & Ballarin 2009). Dissection of Anatolian *Leptanilla* GR03, and Spanish material that closely resembles sequenced males of *Leptanilla s. str.*, demonstrates that the volsellae are likewise lamellate in these morphospecies, having much the same condition as in *Leptanilla africana* (Baroni Urbani 1977: Fig. 37) (not included in this study). Therefore, given the phylogeny of *P. javana* and its morphological conformity to *Leptanilla s. str.* there is no justification for maintaining the genus *Phaulomyrma*. It is perhaps a nomenclatural irony that Wheeler & Wheeler (1930: p. 193) note that the derivation of the genus name is from the Greek *phaulus*, which they translate as "trifling or paltry": the justification for establishing *Phaulomyrma* as a genus was trifling indeed.

Leptanilla javana (Wheeler & Wheeler, 1930) comb. nov.

Fig. 8A-D.

Phaulomyrma javana Wheeler & Wheeler 1930: Figs. 1, 2C.

Phaulomyrma javana – Petersen 1968: 293. Figs. 16A-C.

Lectotype

INDONESIA • ♂; Jawa Barat, "Buitenzorg" [Bogor]; Mar. 1907; F.A.G. Muir leg.; MCZ 31142.

Paralectotype

Same data as for lectotype.

A complete male-based diagnosis of *Leptanilla* relative to other Leptanillinae under both broad and strict circumscriptions of *Leptanilla* is provided below, with putative synapomorphies represented in italics for the two circumscriptions. Only genital characters could be scored for *Leptanilla* ZA01.

Genus *Leptanilla* Emery, 1870

Type species: *Leptanilla revelierii* Emery, 1870: 196.

= *Leptomesites* Kutter, 1948 (286). Synonymy by Baroni Urbani, 1977 (433). Holotype deposited at MHNG (Muséum d'Histoire Naturelle, Geneva).

= *Phaulomyrma* Wheeler & Wheeler, 1930 (193); **syn. nov.** Lecto- and paralectotype deposited at MCZC (Museum of Comparative Zoology, Cambridge, Massachusetts).

Male Diagnosis of Leptanilla s. l. *relative to other Leptanillinae*

1. Mandibles articulated to gena (Fig. 9B).
2. *Medial axis of clypeus no longer than diameter of torulus, when epistomal sulcus is distinct*.
3. Antennomere 3 shorter than scape.
4. Ocelli present and set on tubercle (Fig. 10) (with exception of *Leptanilla* [Bornean morphospecies-group] zhg-my05).
5. *Pronotum and mesoscutum posteriorly extended (Fig. 11B-C).*
6. Notauli absent.
7. Pterostigma absent.
8. Propodeum not concave in profile view.

Male Diagnosis of Leptanilla s. str. *relative to other* Leptanilla s. l.

9. *Anteromedian ocellus and compound eye not intersecting line parallel to dorsoventral axis of cranium.*
10. Profemoral ventral cuticular hooks absent.
11. Ventromedian protibial comb-like row of setae absent.

12. Infuscation at juncture of Rf and 2s-rs+Rs+4-6 absent.
13. Antero-admedian line absent (HAO: 0000128).
14. Mesoscutellum not posteriorly prolonged.
15. Propodeum convex in profile view, without distinct dorsal face.
16. Abdominal sternite IX without posterolateral filiform processes.
17. Abdominal tergite VIII broader than long.
18. Gonocoxae medially separated*.
19. Gonopodites articulated.
20. *Volsella lamellate, entire distally, without denticles**.
21. Penial sclerites dorsoventrally compressed, dorsomedian carina absent, ventromedian carina sometimes present.
22. Phallotreme situated at penial apex, without vestiture.

*These character states observed so far as is possible with available specimens.

Notes.

1. The mandibles are fused to the gena (Fig. 9B) in sampled *Yavnella s. l.* except for "*Leptanilla*" TH04: this character state is seen elsewhere in male ants only among some *Simopelta* spp. (Ponerinae) (Brendon Boudinot, pers. comm.).
2. The epistomal sulcus is often difficult to distinguish in *Leptanilla s. l.*, but the anteroposterior reduction of the clypeus can be inferred by the situation of the toruli at the anterior-most margin of the head (cf. Boudinot 2015: p. 30).
3. Antennomere 3 is longer than the scape in all sampled *Yavnella s. l.* except for "*Leptanilla*" TH05.

4. Ocelli are entirely absent in "*Leptanilla*" TH03 and *Yavnella* zhg-bt01. The ocellar tubercle is absent in the Anomalomyrmini and *O. hungvuong*; within *Leptanilla s. l.* it is absent in *Leptanilla* zhg-my05, which is here inferred to be a secondary loss.
5. As noted by Petersen (1968: p. 87), *N. copiosa* contrasts with other described male Leptanillinae by the lack of an "elongated, laterally compressed" mesosoma. *Yavnella* was described by Kugler (1986) as sharing this condition, which Petersen (1968) adduced as plesiomorphic for the Leptanillinae. While the relative modification of the mesosoma—here approximated by the proportions of the pronotum and mesoscutum—forms a morphocline across the male Leptanillinae, this morphocline is discontinuous, with a gap between the morphospace occupied by *Leptanilla s. l.* (Fig. 11B-C) and that occupied by *O. hungvuong*, the Anomalomyrmini, and *Yavnella s. l.* (Fig. 11A). Future sampling of male Leptanillinae may close this gap in morphospace, which would limit the diagnostic utility of pronotal and mesonotal length.
6. The absence of notauli is a synapomorphy of the tribe Leptanillini. The notauli in *Protanilla* TH01 and *Protanilla* zhg-vn01, in the tribe Anomalomyrmini, are homoplastically absent.
7. The absence of the pterostigma (Figs. 12A, C) is a synapomorphy of the Leptanillini.
8. The convexity of the propodeum in profile view is plesiomorphic for the Leptanillinae. Its concave condition in *Yavnella* (Kugler 1986) is apomorphic for that genus.
9. The anteromedian ocellus is not situated orthogonally to the compound eye in profile view in *Leptanilla s. str. Leptanilla* TH01 and zhg-th01, the Bornean morphospecies-group, and all examined *Noonilla* except for *Noonilla* zhg-my04. The concomitant prognathy of the male cranium is unique among male Leptanillinae to *Leptanilla s. l.*, and

as adduced by Petersen (1968), this condition appears apomorphic among the Leptanillinae.

10. A profemoral ventral cuticular hook (Fig. 13B) is unique among the morphospecies sampled herein to *Leptanilla* ("Bornean morphospecies-group") zhg-my02 and -05.
11. The ventromedian comb-like row of setae on the protibia is an autapomorphy of the Bornean morphospecies-group.
12. The infuscation observed in the Bornean morphospecies-group at the juncture of Rf and 2s-rs+Rs+4-6 (Fig. 12C) is not enclosed anteriorly by an abscissa and appears to be homoplasious with the pterostigma observed in male Anomalomyrmini. Infuscation of the forewing is otherwise absent in the Leptanillini.
13. The antero-admedian line is present among the Leptanillini only among some *Yavnella s. l.* and in *Noonilla* zhg-my04.
14. The mesoscutellum is posteriorly prolonged in *Leptanilla* TH01 and *Leptanilla* zhg-th01 (Fig. 4B). The differences in mesoscutellar shape between these morphospecies (see Supplemental Material 1) are such that the homology of posterior mesoscutellar prolongation is uncertain.
15. The propodeum has a distinct planar to depressed dorsal face in the Bornean morphospecies-group (Fig. 5B). This condition is an autapomorphy of that clade.
16. The posterior margin of abdominal sternite IX is variously emarginate to entire in male Leptanillinae or with a posteromedian process (e.g. *Protanilla* zhg-vn01, "*Leptanilla*" TH03), but posterolateral filiform processes of abdominal sternite IX are an autapomorphy of the Bornean morphospecies-group.

17. Abdominal tergite VIII is longer than broad only in *Noonilla* (Fig. 14A), *Scyphodon* and a bizarre male morphospecies from Côte d'Ivoire (CASENT0102373) for which molecular data are unavailable.
18. The gonocoxae exhibit partial (Fig. 6B) to full (Fig. 6C) medial fusion at least in ventral view in "*Leptanilla*" TH03, *Noonilla*, and all sampled members of the Bornean morphospecies-group. Within *Leptanilla s. l.*, complete lack of medial gonocoxal fusion (Fig. 6A) is a symplesiomorphy of *Leptanilla s. str.*, *Leptanilla* TH01, and *Leptanilla* zhg-th01.
19. Articulation of the gonopodites encompasses both cases in which conjunctival membrane is visible between the gonocoxa and stylus, and those in which the stylus is recurved relative to the gonocoxa without apparent conjunctival membrane. This character state is a symplesiomorphy of *Leptanilla s. str.*, and among *Leptanilla s. l.* included in this study is observed in *Noonilla* zhg-my01-2,6 and *Leptanilla* zhg-th01.
20. The volsellae cannot be observed without dissection in many male Leptanillinae (e.g., *Noonilla*), limiting my ability to assess their condition. However, *Leptanilla s. str.* contrast with the Anomalomyrmini, *Yavnella s. l.*, and the Bornean morphospecies-group in that the volsellae (where visible) are dorsoventrally flattened, entire, and lacking sculpture (Fig. 15). This is one of only two synapomorphies of *Leptanilla s. str.* relative to other *Leptanilla s. l.*
21. Dorsoventral compression at the penial apex is also observed in *Yavnella s. l.* (except for "*Leptanilla*" TH03). In the Indo-Malayan sister clade of *Leptanilla s. str.* the penial sclerites are lateromedially compressed to subcircular, at least basally. *Leptanilla* zhg-

th01 exhibits an intermediate condition, with the penial apex being lateromedially compressed and this condition less pronounced towards the base.

22. Position of the phallotreme with distal margin adjoining the penial apex appears to be ancestral for the Leptanillini. The phallotreme is shifted basally in *Leptanilla* zhg-my02 and –5 (Fig. 16B), *Noonilla*, and *Scyphodon*. The outline of the phallotreme is subcircular in these morphotaxa. Setae surrounding the phallotreme are observed in *Noonilla* and *Scyphodon*; this character state is likely a synapomorphy of these genera.

Goals of Future Research

Two described male-based species of *Leptanilla* are worth noting here as requiring further study and acquisition of fresh material: *L. palauensis*, which was transferred with some reservation to *Leptanilla* from *Probolomyrmex* (Formicidae: Proceratiinae) by Taylor (1965); and *Leptanilla astylina.* Examination of the holotype of *L. palauensis* demonstrates that according to the morphological hypotheses made herein this species can be confidently referred to *Leptanilla s. l.*, but beyond that its affinities are unclear. Based upon available illustrations (Petersen 1968: Fig. 1) *L. astylina* likewise can be placed in *Leptanilla s. l.*, and closely resembles *Leptanilla s. str.*, excluding its genitalia, which to judge from Petersen (1968) are unlike those of any specimen that was examined in this study, and exclude it from the definition of *Leptanilla s. str.* given herein.

The case of *Scyphodon* must also be briefly addressed here. Examination of a specimen attributable to this monotypic male-based genus shows that it can be placed in *Leptanilla s. l.* As reported by Petersen (1968), the genitalia of *Scyphodon* conspicuously resemble those of *Noonilla*: there is no reason to conclude that *Scyphodon* belongs within *Leptanilla s. str.,* and I

predict that *Scyphodon* is either sister to, or nested within, *Noonilla*. Future total-evidence Bayesian phylogenetic inference will resolve the relation of *Scyphodon* to other *Leptanilla s. l.*

Future acquisition and examination of novel material may necessitate revision of the male diagnosis of *Leptanilla* provided here, but this diagnosis is robust to all morphological observations made with sequenced material. As *Yavnella s. l.*, *Noonilla* and the Bornean morphospecies-group are known only from males, and *L. revelierii* is known only from female castes, no argument can yet be made regarding the ranking of the former clades relative to *Leptanilla*. *Yavnella* is here ranked as a genus, but the description of *Yavnella* workers may reveal a morphological basis for subjective arguments for the subsumption of *Yavnella* within *Leptanilla*. The delimitation of genera within the Leptanillini therefore depends not only upon phylogenetic resolution of the many lineages known only from male material, but upon the morphology of corresponding workers. Future molecular sequencing will be needed to associate workers and/or gynes to leptanilline lineages that are known only from males: such an effort has successfully linked *Protanilla lini* (Anomalomyrmini) with previously unassociated males (Griebenow, in review).

Conclusions

I have here demonstrated the utility of discrete morphological data within a total-evidence framework that includes molecular data in inferring the phylogeny of an ant taxon known only from male morphology. Using probabilistic models, the phylogenetic position of *P. javana* is robustly inferred in conjunction with taxa for which only molecular data, or both these and male morphological data, are available. In that phylogeny, *P. javana* and *L. revelierii* are confidently recovered within a subclade easily diagnosed by male morphological characters; disregarding future retrieval of worker material and/or novel male specimens, *Phaulomyrma* can be

synonymized with *Leptanilla* despite continued uncertainty in the bounds of the latter genus. Future work will employ this Bayesian total-evidence approach to infer the affinity of other, more peculiar leptanilline taxa for which molecular data are unavailable. With a robust phylogeny inferred for the Leptanillinae that is congruent with male morphology, the parallel taxonomy that bedevils this little-understood group of ants can begin to be resolved.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgments

First and foremost, I must thank Ziad Khouri for his generosity in providing indispensable assistance and conceptual advice on the writing of scripts for the Bayesian phylogenetic analyses upon which this project relied. I thank Jadranka Rota (MZLU), Debbie Jennings (ANIC), Kevin Williams (CSCA), and Brian Fisher (CASC) for loans of material for examination and non-destructive DNA extraction; Stefan Cover was of great help in facilitating access to a syntype of *P. javana*. I also thank my present and past lab-mates Jill Oberski and Matt Prebus for their diligent and painstaking work enriching UCEs from the specimens that were used in this study (sometimes with my help, sometimes not). In the realm of data collection, I am grateful to Eli Sarnat, Steve Heydon and Lynn Kimsey for allowing me the usage of equipment for this study; the aid of Michael Branstetter in providing advice on the retrieval of legacy loci from UCE datasets; and my labmate Brendon Boudinot, who was an enduring source of informative feedback on the coding of morphological observation into discrete character states. Lastly, I must thank my adviser Phil Ward for his invaluable feedback on the construction and finer details of this manuscript, along with past work in acquiring the Sanger-sequenced data that I utilized in this study, and for tutoring me in the delimitation of protein-coding loci from flanking introns.

This research was supported by the University of California, Davis and by NSF grant DEB-1932405 to P. S. Ward.

References.

Agosti, D. (1991). Revision of the oriental ant genus *Cladomyrma*, with an outline of the higher classification of the Formicinae (Hymenoptera: Formicidae). *Systematic Entomology* **16**, 293-310.

Bapst, D. W., Wright, A. M., Matzke, N. J., and Lloyd, G. T. (2016). Topology, divergence dates, and macroevolutionary inferences vary between different tip-dating approaches applied to fossil theropods (Dinosauria). *Biology Letters* **12**, 20160237.

Barden, P., Boudinot, B. E., and Lucky, A. (2017). Where fossils dare and males matter: combined morphological and molecular analysis untangles the evolutionary history of the spider ant genus *Leptomyrmex* Mayr (Hymenoptera: Dolichoderinae). *Invertebrate Systematics* **31**, 765-780.

Baroni Urbani, C. (1977). Materiali per una revision della sottofamiglia Leptanillinae Emery (Hymenoptera: Formicidae). *Entomologica Basiliensia* **2**, 427–488.

Baroni Urbani C. and de Andrade, M.L. (2006). A new *Protanilla* Taylor, 1990 (Hymenoptera: Formicidae: Leptanillinae) from Sri Lanka. *Myrmecologische Nachrichten* **8**, 45–47.

Belshaw, R. and Bolton, B. (1994). A survey of the leaf litter ant fauna in Ghana, West Africa (Hymenoptera: Formicidae). *Journal of Hymenoptera Research* **3**, 5–16.

Billen, J., Bauweleers, E., Hashim, R., and Ito, F. (2013). Survey of the exocrine system in *Protanilla wallacei* (Hymenoptera, Formicidae). *Arthropod Structure and Development* **42**(3), 173–183.

Bolton, B. (1990). The higher classification of the ant subfamily Leptanillinae (Hymenoptera: Formicidae). *Systematic Entomology* **15**, 267–282.

Bolton, B. (2003). Synopsis and classification of Formicidae. *Memoirs of the American Entomological Institute* **71**, 1–370.

Borowiec M. L., Schulz, A., Alpert, G.D., and Baňar, P. (2011). Discovery of the worker caste and description of two new species of *Anomalomyrma* (Hymenoptera: Formicidae: Leptanillinae) with unique abdominal morphology. *Zootaxa* **2810**, 1–14.

Borowiec, M. L., C. Rabeling, S. G. Brady, B. L Fisher, T. R. Schultz, and P. S. Ward. (2019). Compositional heterogeneity and outgroup choice influence the internal phylogeny of the ants. *Molecular Phylogenetics & Evolution* **134**, 111–121.

Boudinot, B. E. (2015). Contributions to the knowledge of Formicidae (Hymenoptera, Aculeata): a new diagnosis of the family, the first global male-based key to subfamilies, and a treatment of early-branching lineages. *European Journal of Taxonomy* **120***,* 1–62.

Boudinot, B. E. (2018). A general theory of genital homologies for the Hexapoda (Pancrustacea) derived from skeletomuscular correspondences, with emphasis on the Endopterygota. *Arthropod Structure and Development* **47**(6), 563–613.

Brady, S. G., Schultz, T. R., Fisher, B. L., and Ward, P. S. (2006). Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 18172-18177

Branstetter, M. G., Longino, J. T., Ward, P. S., and Faircloth, B.C. (2017). Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. *Methods in Ecology & Evolution* **8**(1), 768–776.

Brazeau, M. D. (2011). Problematic character coding methods in morphology and their effects. *Biological Journal of the Linnaean Society* **104**, 489–498.

Brues, C. T. (1925). *Scyphodon*, an anomalous genus of Hymenoptera of doubtful affinities. *Treubia* **6**, 93–96.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology & Evolution* **17**, 540–552.

Chen Z.-L., Shi, F.-M., and Zhou, S.-Y. (2017). First record of the monotypic genus *Opamyrma* (Hymenoptera: Formicidae) from China. *Far Eastern Entomologist* **335**, 7–11.

Emery, C. (1870). Studi mirmecologici. *Bullettino della Società Entomologica Italiana* **2**, 193-201.

Faircloth, B. C. (2013). 'Illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming.' Available at http://dx.doi.org/10.6079/J6079ILL

Faircloth, B. C. (2016). PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics* **32**(5), 786–788. https://doi.org/10.1093/bioinformatics/btv646

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* **17**(6), 368–376.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., and Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**(3), 307-321.

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., … Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* **29**(7), 644-652. Published 2011 May 15. doi:10.1038/nbt.1883

Hsu, P.-W., Hsu, F.-C., Hsiao, Y., and Lin, C.-C. (2017). Taxonomic notes on the genus *Protanilla* (Hymenoptera: Formicidae: Leptanillinae) from Taiwan. *Zootaxa* **4268**(1), 117–130. http://dx.doi.org/10.11646/zootaxa.4268.1.7

Höhna, S., Landis, M. J., and Heath, T. A. (2017). Phylogenetic inference using RevBayes. *Current Protocols in Bioinformatics,* https://doi.org/10.1002/cpbi.22

Hoang, D. P., Chernomor, O., von Haeseler, A., Minh, B. Q., and Vinh, L. S. (2018). UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* **35**(2), 518-522.

Katoh, K., Asimenos, G., and Toh, H. (2009). Multiple alignment of DNA sequences with MAFFT. In 'Bioinformatics for DNA Sequence Analysis'. (Ed. D. Posada.) pp. 39–64. (Springer: New York City, NY.)

Katoh, K., Rozewicki, J., and Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**(4), 1160-1166.

Kjer, K., Borowiec, M. L., Frandsen, P. B., Ware, J. and Wiegmann, B. (2018). Advances using molecular data in insect systematics. *Current Opinions in Insect Science* **18**, 40-47.

Kück, P., Hita Garcia, F., Misof, B., and Meusemann, K. (2011). Improved phylogenetic analyses corroborate a plausible position of *Martialis heureka* in the ant tree of life. *PLOS One* **6**, e21031.

Kugler, J. (1986). The Leptanillinae (Hymenoptera: Formicidae) of Israel and a description of a new species from India. *Israel Journal of Entomology* **20**, 45–57.

Kutter, H. (1948). Beitrag zur Kenntnis der Leptanillinae (Hym. Formicidae): eine neue Ameisengattung aus süd-Indien. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* **11**, 286–295.

Lanfear, R., Calcott, B., Ho, S. Y., and Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**(6), 1695-1701.

Lanfear, R., P. B. Frandsen, A. M. Wright, T. Senfeld, and B. Calcott. (2016). PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Molecular Biology and Evolution* **34**(3), 772–773.

Leong, C.-M., Yamane, S., and Guénard, B. (2018). Lost in the city: discovery of the rare ant genus *Leptanilla* (Hymenoptera: Formicidae) in Macau with description of *Leptanilla macauensis* sp. nov. *Asian Myrmecology* **10**, e010001. DOI: 10.20362/am.010001

Lewis, P. O. (2001). A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* **50**(6), 913–925.

López, F., Martinez, M. D., and Barandica, J. M. (1994). Four new species of the genus *Leptanilla* (Hymenoptera: Formicidae) from Spain - relationships to other species and ecological issues. *Sociobiology* **24**, 179–212.

Man, P.; Ran, H., Chen, Z., and Xu, Z. (2017). The northernmost record of Leptanillinae in China with description of *Protanilla beijingensis* sp. nov. (Hymenoptera: Formicidae). *Asian Myrmecology* **9**, e009008. DOI: 0.20362/am.009008

Masuko, K. (1990). Behavior and ecology of the enigmatic ant *Leptanilla japonica* Baroni Urbani (Hymenoptera: Formicidae: Leptanillinae). *Insectes Sociaux* **37**(1), 31–57.

Matzke, N. J. and Wright, A. (2016). Inferring node dates from tip dates in fossil Canidae: the importance of tree priors. *Biology Letters,* https://doi.org/10.1098/rsbl.2016.0328

Miller, M. A., Pfeiffer, W., and Schwartz, T. (2010). Creating the CIPRES Science Gateway. In 'Proceedings, the Gateway Computing Environments Workshop (GCE)', 14 November 2010, New Orleans, LA. pp. 1–8. (Institute of Electrical and Electronics Engineers: Piscataway, NJ.)

Moreau, C. S., Bell, C. D., Vila, R., Archibald, S. B., and Pierce, N. E. (2006). Phylogeny of the ants: diversification in the age of angiosperms. *Science* (Washington, D. C.) **312**, 101-104.

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., and Minh, B. Q. (2015). IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution* **32**(1), 268–274.

Niehuis, O., Hartig, G., Grath, S., Pohl, H., Lehmann, J., Tafer, H., … Misof, B. (2012). Genomic and morphological evidence converge to resolve the enigma of Strepsiptera. *Current Biology* **22**(14), 1309-1313.

Ogata, K., Terayama, M., and Masuko, K. (1995). The ant genus *Leptanilla*: discovery of the worker-associated male of *Leptanilla japonica*, and a description of a new species from Taiwan (Hymenoptera: Formicidae: Leptanillinae). *Systematic Entomology* **20**, 27–34.

O'Reilly, J. E., dos Reis, M., and Donoghue, P. C. J. (2015). Dating tips for divergence-time estimation. *Trends in Genetics* **31**(11), 637-650.

Petersen, B. (1968). Some novelties in presumed males of Leptanillinae (Hym., Formicidae). *Entomlogiske Meddelelser* **36**, 577–598.

de Pinna, M. C. C. (1991). Concepts and tests of homology in the cladistic paradigm. *Cladistics* **7**, 367–394.

Pleijel, P. (1995). On character coding for phylogeny reconstruction. *Cladistics* **11**, 309–315.

Rabeling, C., Brown, J., and Verhaagh, M. (2008). Newly discovered sister lineage sheds light on early ant evolution. *Proceedings of the National Academy of Sciences of the United States of America* **105**(39), 14913–14917.

Rambaut, A., Drummond, A. J., Xie, D., Baele, D., and Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **1;67**(5), 901–904.

Robertson, H. G. (2000). Formicidae (Hymenoptera: Vespoidea). In 'Dâures Biodiversity of the Brandberg Massif, Namibia'. (Eds. A. H. Kirk-Spriggs and E. Marais.) pp. 371–382. Cimbabesia Memoir 9. (National Museum of Namibia: Windhoek.)

Robertson, J. A. and Moore, W. (2016). Phylogeny of *Paussus* L. (Carabidae: Paussinae): unravelling morphological convergence associated with myrmecophilous life histories. *Systematic Entomology* **42**(1), https://doi.org/10.1111/syen.12205

Ronquist, F., Klopfstein, S., Vilhelmsen, L., Schulmeister, S., Murray, D. L., and Rasnitsyn, A. P. (2012). A total-evidence approach to dating with fossils, applied to the early radiation of the Hymenoptera. *Systematic Biology* **61**(6), 973-999.

Scupola, A. and Ballarin, R. (2009). The genus *Leptanilla* Emery, 1870 in Sicily (Hymenoptera: Formicidae). *Myrmecological News* **12**, 129–132.

Sánchez, N., Yamasaki, H., Pardos, F., Sørensen, M. V., Martínez, A. (2016). Morphology disentangles the systematics of a ubiquitous but elusive meiofaunal group (Kinorhyncha: Pyncophyidae). *Cladistics* **32**(5), https://doi.org/10.1111/cla.12143

Strong, E. E. and Lipscomb, D. (1999). Character coding and inapplicable data. *Cladistics* **15**, 363–371.

Taylor, R. W. (1965). A monographic revision of the rare tropicopolitan ant genus *Probolomyrmex* Mayr (Hymenoptera: Formicidae). *Transactions of the Royal Entomological Society of London* **117**, 345–365.

Taylor, R. W. (1980). Notes on the Russian endemic ant genus *Aulacopone* Arnoldi (Hymenoptera: Formicidae). *Psyche* **86**(4), 353-361.

Ward, P. S. and Sumnicht, T. P. (2012). Molecular and morphological evidence for three sympatric species of *Leptanilla* (Hymenoptera: Formicidae). *Myrmecological News* **17**, 5–11.

Ward, P. S., Brady, S. G., Fisher, B. L., and Schultz, T. R. (2010). Phylogeny and biogeography of dolichoderine ants: effects of data partitioning and relict taxa on historical inference. *Systematic Biology* **59**, 342–362.

Wheeler, G. C. and Wheeler, E. W. (1930). Two new ants from Java. *Psyche* **37**(3), 193–201.

Wipfler, B., Letsch, H., Frandsen, P. B., Kapli, P., Mayer, C., Buckley, T. R., Donath, A. … Simon, S. (2019). Evolutionary history of Polyneoptera and its implications for our understanding of early winged insects. *Proceedings of the National Academy of Sciences of the United States of America* **116**(8), 3024-3029.

Wong, M. K. L. and Guénard, B. (2016). *Leptanilla hypodracos* sp. n., a new species of the cryptic ant genus *Leptanilla* (Hymenoptera, Formicidae) from Singapore, with new distribution data and an updated key to Oriental *Leptanilla* species. *ZooKeys* **551**, 129–144.

Yamada, A., Nguyen, D. D., and Eguchi, K. (2020). Unveiling the morphology of the Oriental rare monotypic ant genus *Opamyrma* Yamane, Bui & Eguchi, 2008 (Hymenoptera: Formicidae: Leptanillinae) and its evolutionary implications, with first descriptions of the male, larva, tentorium, and sting apparatus. *Myrmecological News* **30**, 27-52.

Yang, Z. (1996). Among-site rate variation and its impact on phylogenetic analyses. *Trends in Ecology and Evolution* **11**, 367–372.

Yoder, M. J., I. Miko, K. C. Seltmann, M. A. Bertone, and A. R. Deans. (2010). A gross anatomy ontology for Hymenoptera. *PLOS One* **5**, e15991.

Fig. 1. Bayesian total-evidence phylogeny of the Leptanillinae under partitioning scheme inferred with PartitionFinder2. Phylogeny was rooted *a posteriori* on *Martialis heureka*. Nodes with BPP≥0.95 marked in red. (A) *Protanilla* zhg-vn01 (CASENT0842613); (B) "*Leptanilla*" TH08 (CASENT022755; Shannon Hartman); (C) *Leptanilla* zhg-my02 (CASENT0106416).

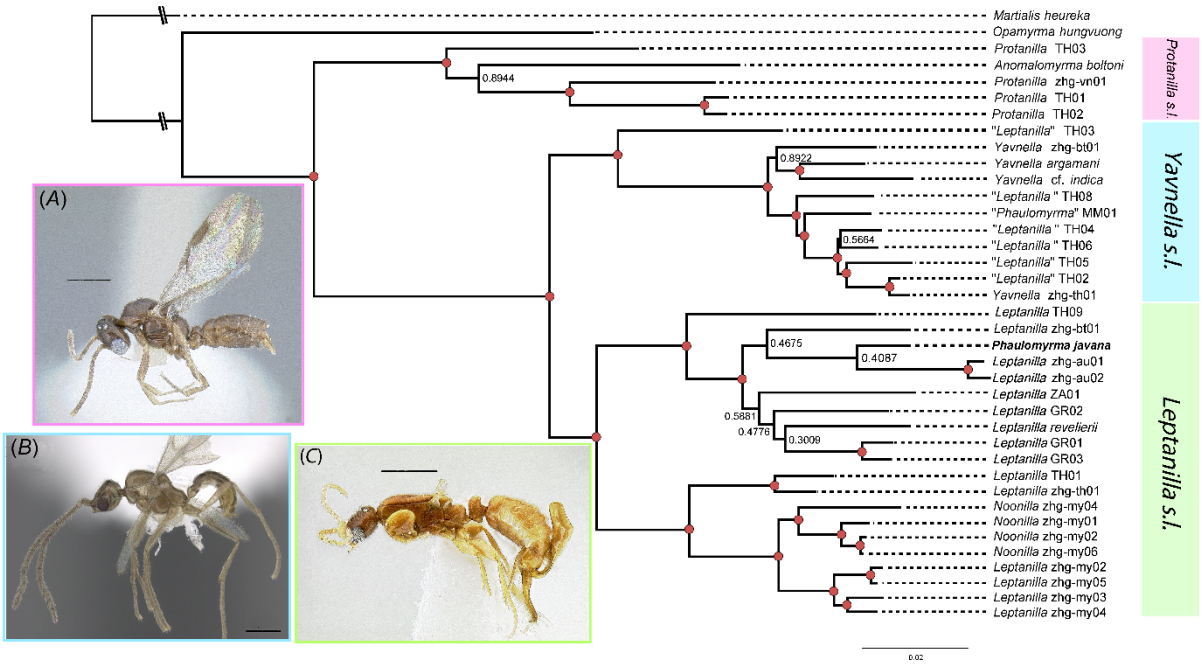


Fig. 2. Selected diversity of male *Leptanilla s. l.* (A) *Leptanilla* TH01 (CASENT0119792; April Nobile); (B) *Leptanilla* zhg-bt02 (CASENT084612; not sequenced in this study); (C) *Noonilla* zhg-my04 (CASENT0842610); (D) *Leptanilla* (Bornean morphospecies-group) zhg-my05 (CASENT0842571). Scale bar A = 0.2 mm.; B = 1 mm.; C-D = 0.5 mm.

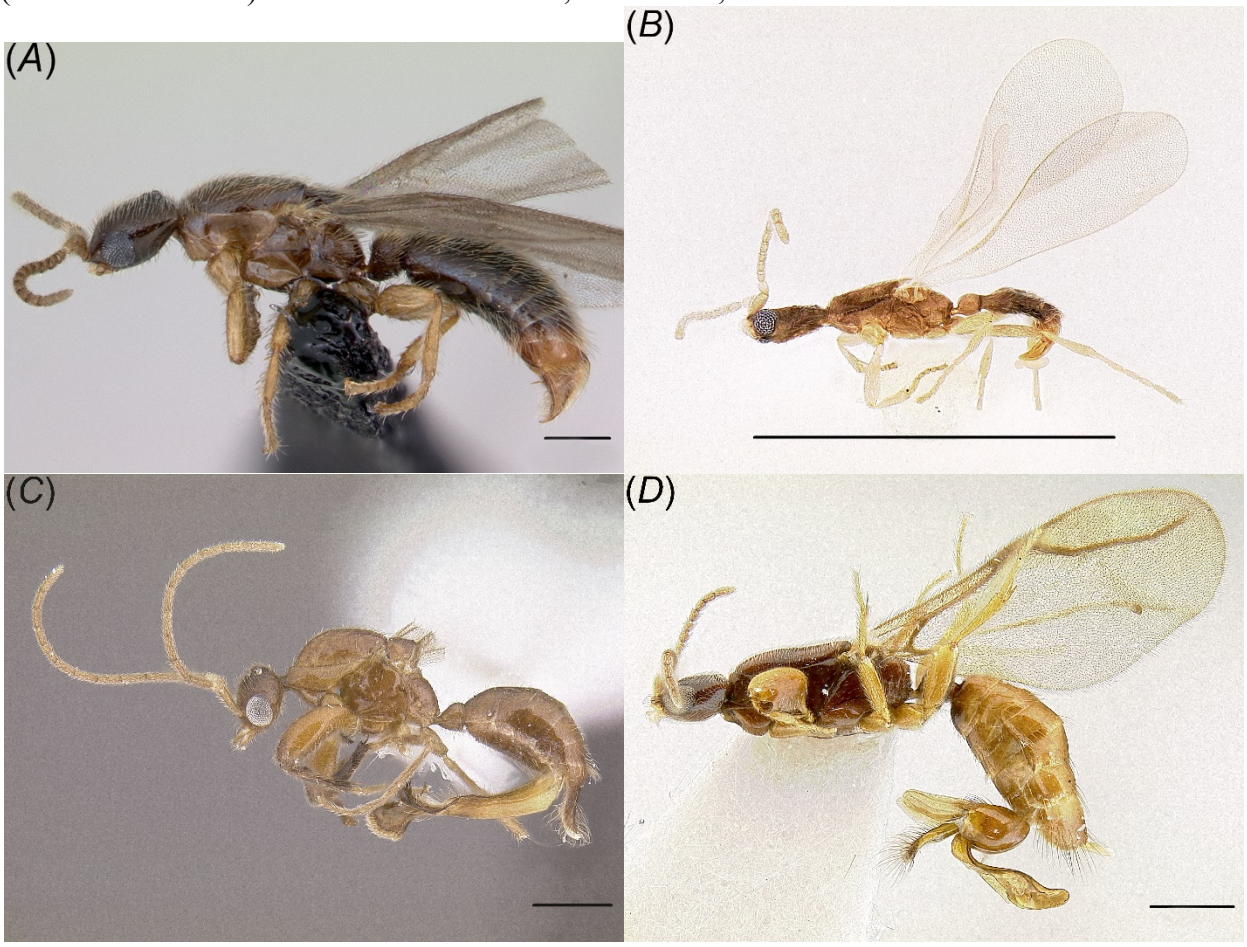


Fig. 3. Protibia of *Leptanilla* zhg-my04 (CASENT0842555). Scale bar = 0.2 mm.

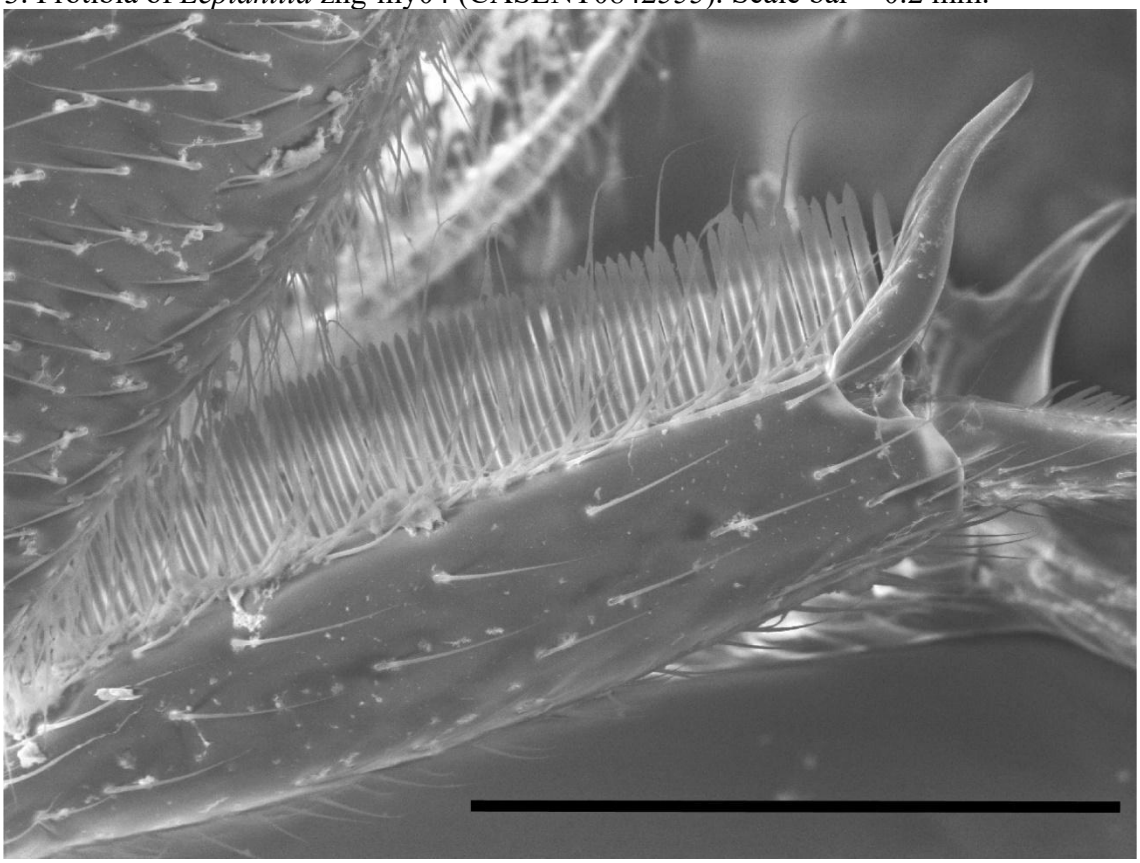


Fig. 4. Presence (B: *Leptanilla* zhg-th01; CASENT0842619) versus absence (A: *Yavnella* zhg-th01; CASENT0842621) of the posterior prolongation of the mesoscutellum in male Leptanillini. Scale bar = 0.3 mm.

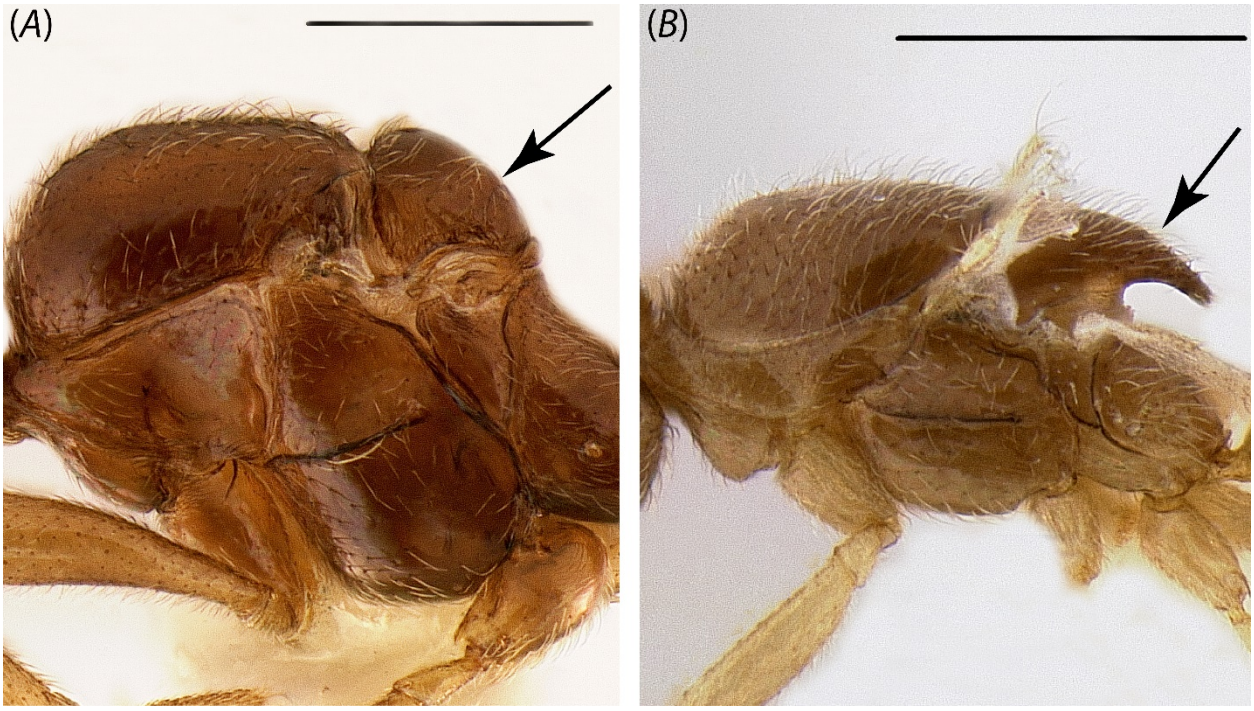


Fig. 5. Conditions of the propodeum in the Leptanilllinae. (A) Concave (*Yavnella* zhg-bt01; CASENT0106384); (B) convex with distinct dorsal face (*Leptanilla* zhg-my02; CASENT0106456); (C) convex without distinct dorsal face (*Protanilla lini* [OKENT0011097]; male described by Griebenow, in review).

Fig. 6. Ventral view of male genitalia across the Leptanillini. (A) *Leptanilla* ZA01 (CASENT0106354), (B) *Noonilla* zhg-my02 (CASENT0842595); (C) *Leptanilla* zhg-my04 (CASENT0842553). Scale bar A = 0.1 mm.; B = 0.3 mm.; and C = 0.5 mm.

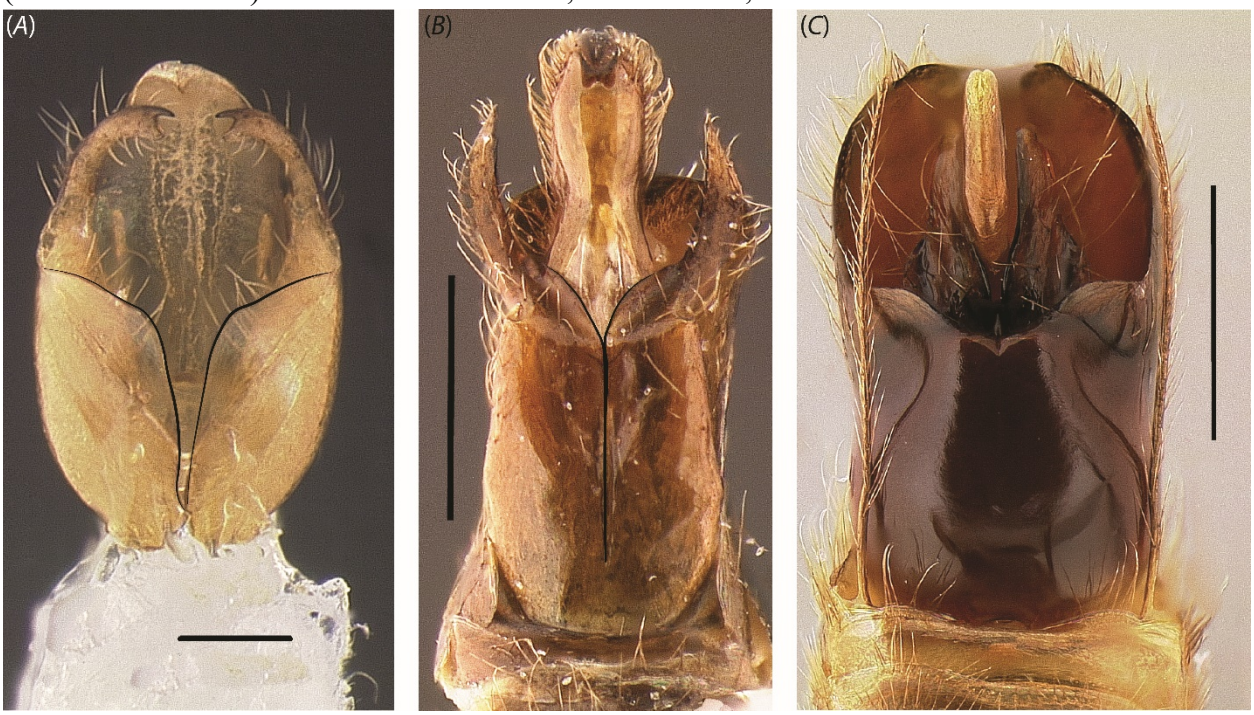


Fig. 7. Dorsoventral (A) (*Yavnella* zhg-th01; CASENT0842620) vs. lateromedial (B) (*Leptanilla* zhg-my04; CASENT0842553) compression of the penial sclerites in posterodorsal view. Dorsomedian carina marked with arrow. Scale bar = 0.3 mm.

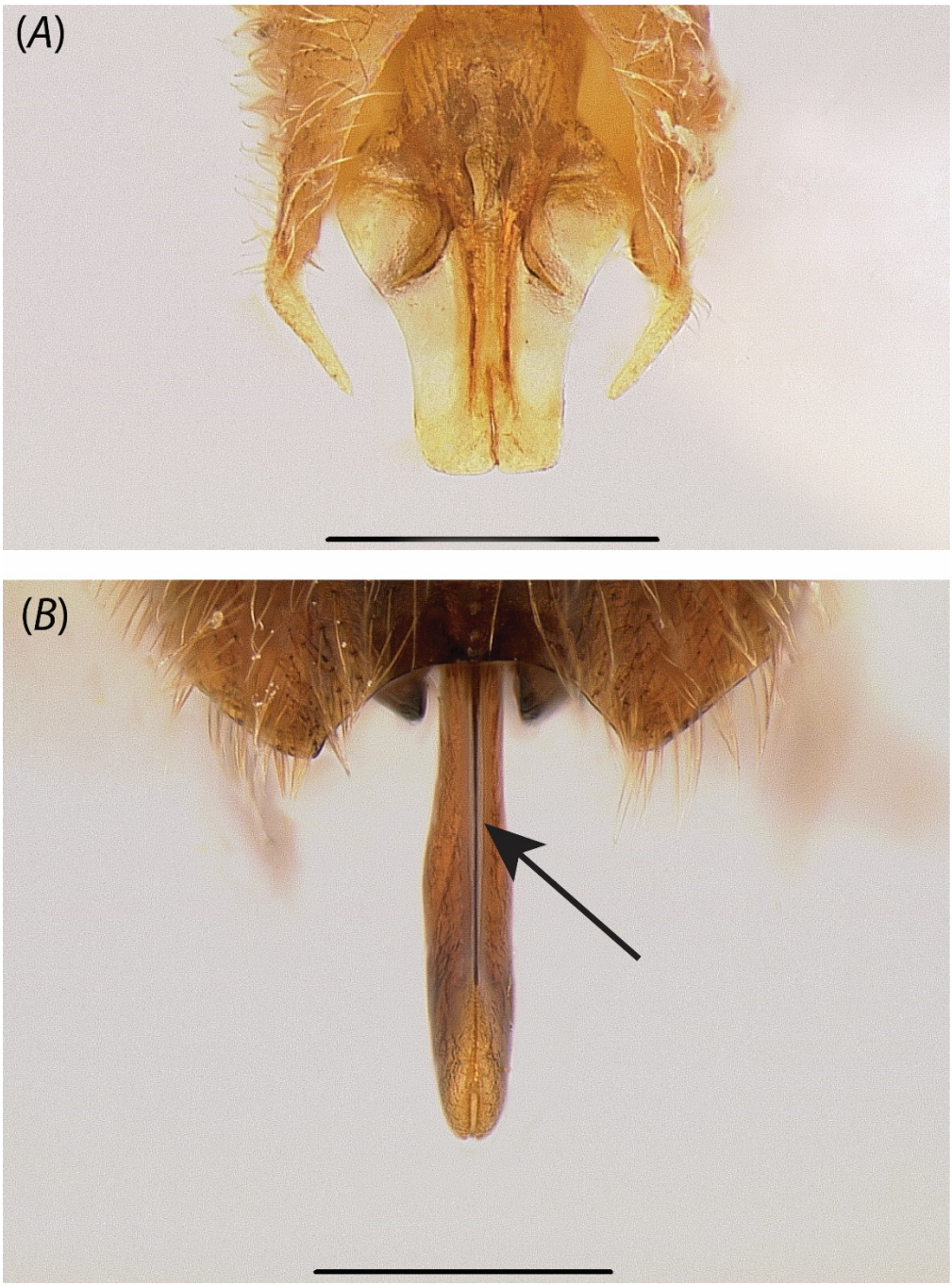


Fig. 8. Syntype of *Phaulomyrma javana* examined in this study (MCZ:Ent:31142). (A) Full-face view; (B) profile view of mesosoma; (C) forewing; (D) metasoma and genitalia. Scale bar = 0.2 mm.

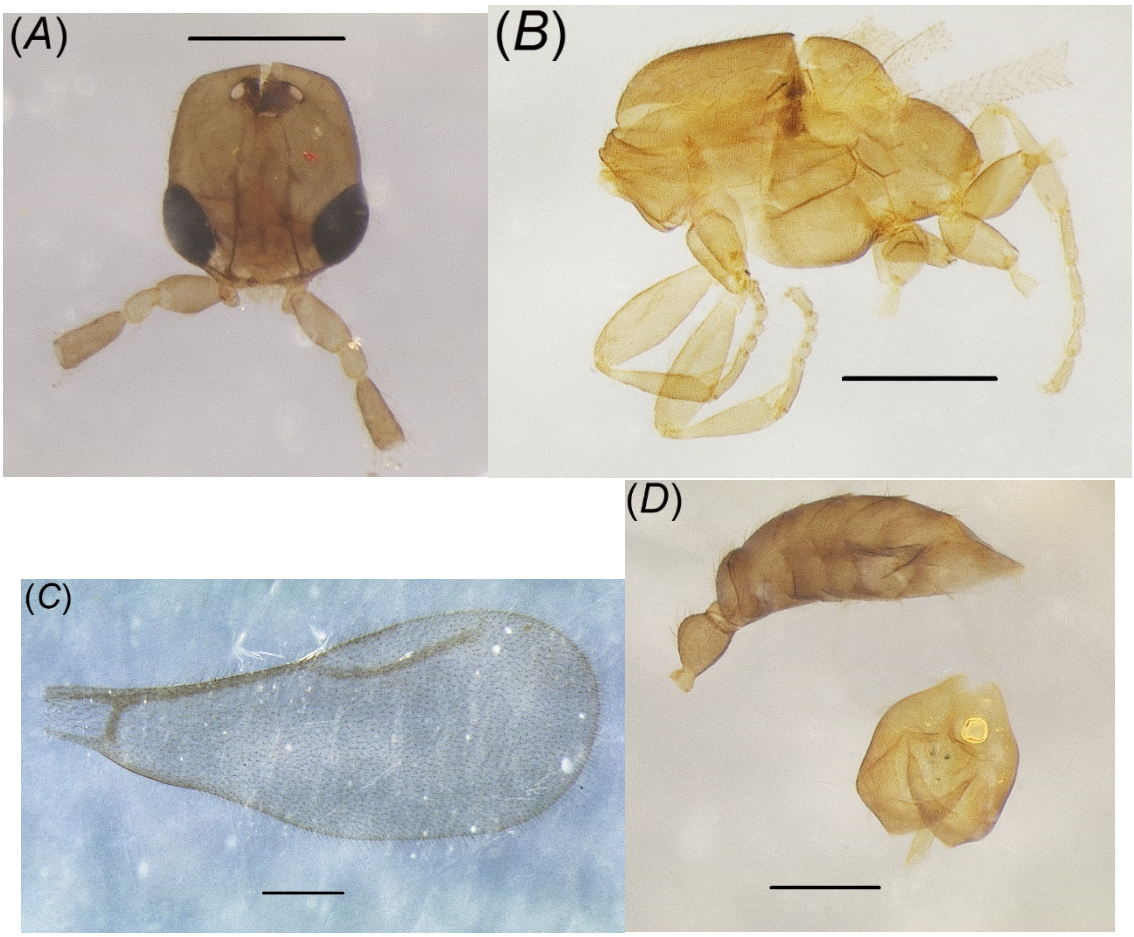


Fig. 9. Mandible of (A) *Yavnella* cf. *indica* (CASENT0106377) and (B) *Leptanilla* zhg-my03 (CASENT0842618). Scale bar A=0.03 mm.; B=0.05 mm.

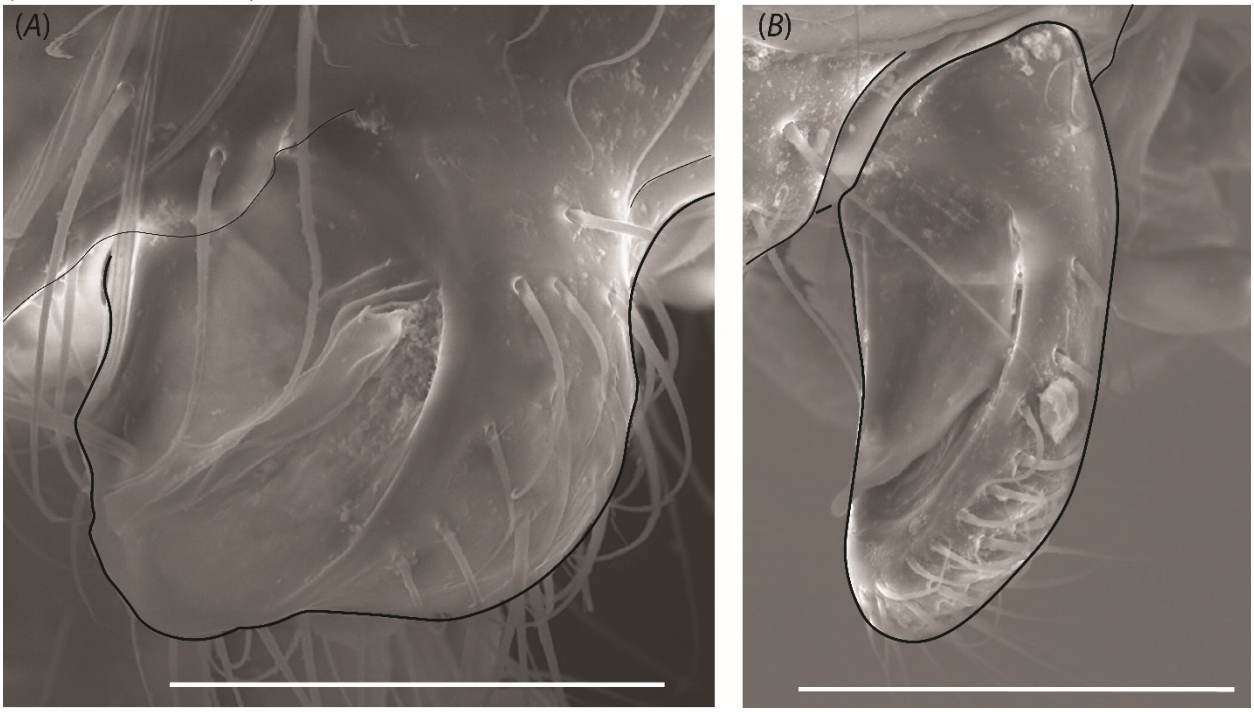


Fig. 10. Full-face view of "*Leptanilla*" TH02 (CASENT0119531; Michele Esposito), with ocellar tubercle marked. Scale bar = 0.1 mm.

Fig. 11. Profile of pronotum (black) and mesoscutum (blue) in male Leptanillini. (A) *Yavnella* zhg-bt01 (CASENT0106384); (B) *Noonilla* zhg-my04 (CASENT0842610); (C) *Leptanilla* (Bornean morphospecies-group) zhg-my03 (CASENT0106416).

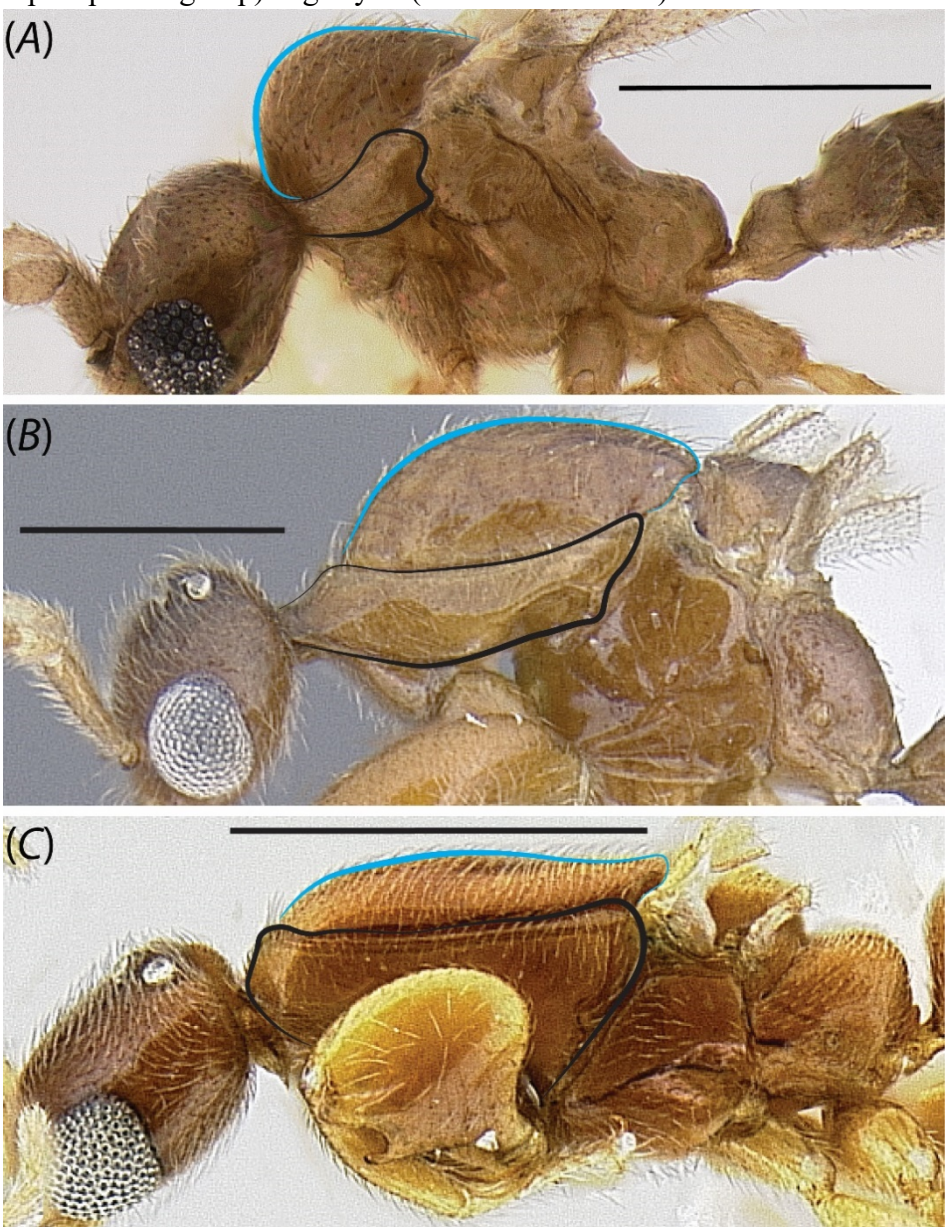


Fig. 12. Examples of male forewing venation across the Leptanillinae. (A) *Yavnella* zhg-bt01 (CASENT0106384); (B) *Protanilla* zhg-vn01 (CASENT0842613); (C) *Leptanilla* (Bornean morphospecies-group) zhg-my05 (CASENT0842571).

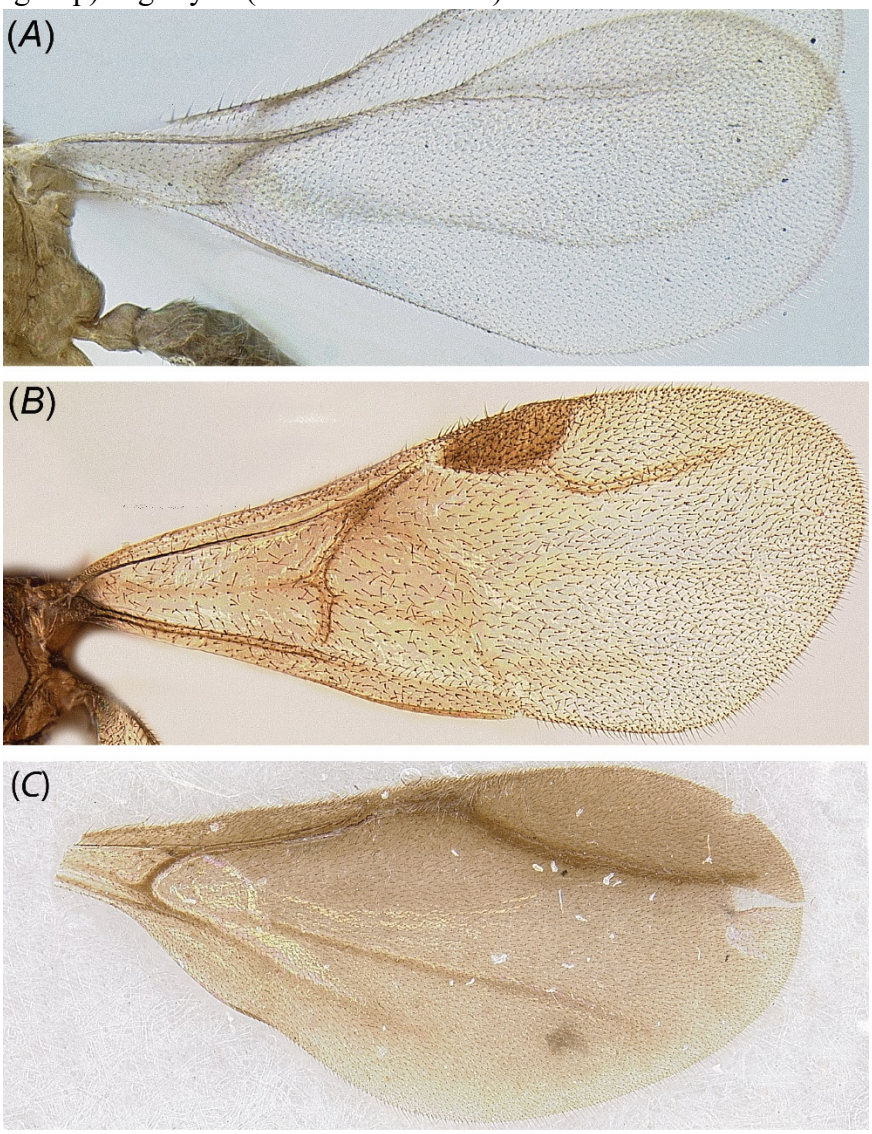


Fig. 13. Foreleg of (A) *Yavnella argamani* (CASENT0235253) and (B) *Leptanilla* zhg-id01 (CASENT0842626; not sequenced in this study). Scale bar=0.3 mm.

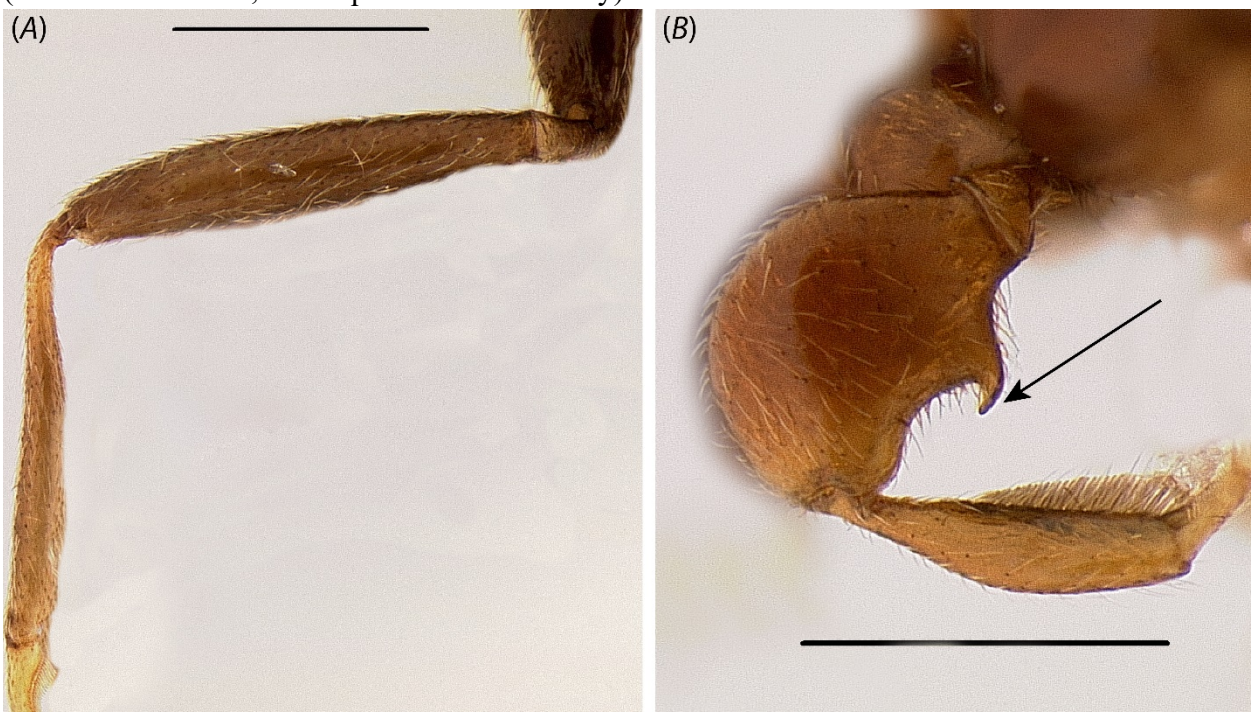


Fig. 14. Posterior view of abdominal tergite VIII in male Leptanillini. (A) *Yavnella* zhg-th01 (CASENT0842620) and (B) *Noonilla* zhg-my02 (CASENT0842592). Scale bar = 0.3 mm.

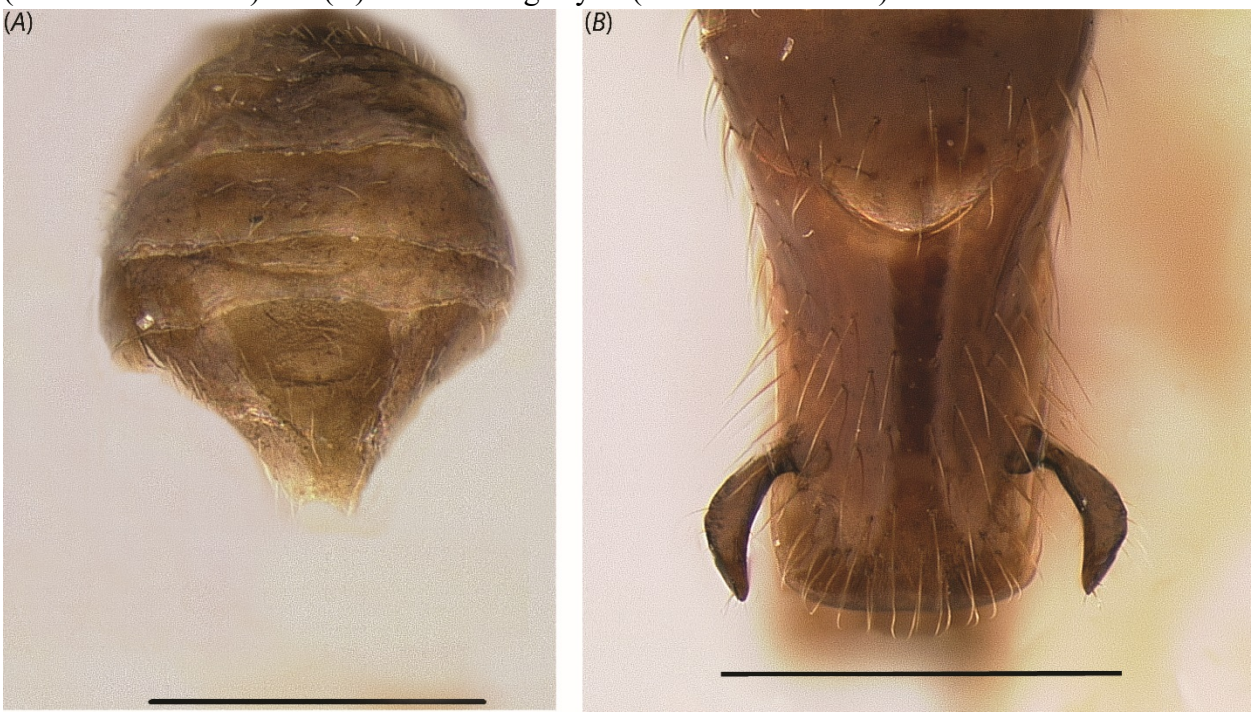


Fig. 15. Gonopodite and volsella (*vol*) of *Leptanilla africana*, sketched after Baroni Urbani (1977: Fig. 37) by M. K. Lippey. Top of image is distal to body.

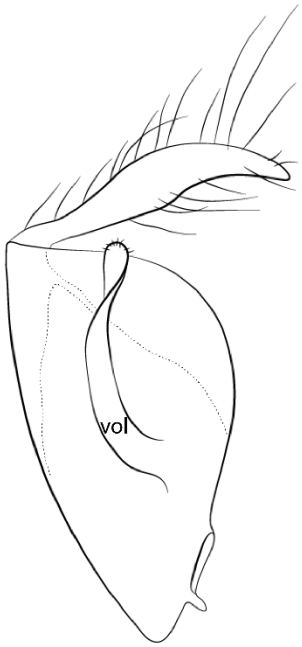
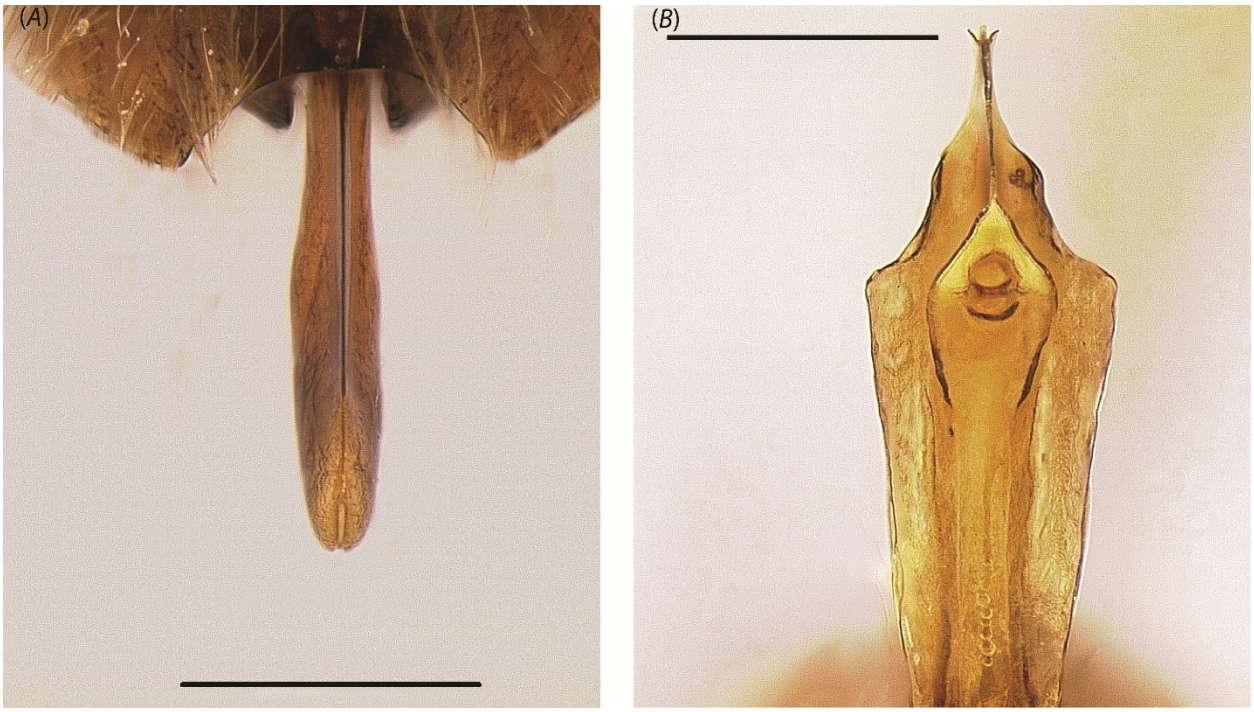


Fig. 16. Dorsoposterior view of phallotreme in (A) *Leptanilla* zhg-my04 (CASENT0842553); ventroposterior view of phallotreme in (B) *Leptanilla* zhg-my05 (CASENT0106432). Scale bar A = 0.3 mm.; B = 0.4 mm.



785 Table 1. Summary statistics for full 9,011-bp DNA sequence alignment. Generic assignments in
786 quotation marks were inherited from Borowiec *et al.* (2019). " – " = absent base; " ? " =
787 unknown base.

Taxon	**Caste/Sex**	**Identifier**	**Percent missing**	**AT content**	**-**	**?**
Anomalomyrma boltoni	Worker	CASENT0217032	16.613	0.525	1497	0
Leptanilla GR01	Male	CASENT0106060	17.434	0.536	1571	0
Leptanilla GR02	Male	CASENT0106236	16.879	0.539	1521	0
Leptanilla GR03	Male	CASENT0106058	7.424	0.529	668	1
Leptanilla TH01	Male	CASENT0119792	17.701	0.523	1595	0
"*Leptanilla*" TH02	Male	CASENT0119531	16.558	0.513	1492	0
"*Leptanilla*" TH03	Male	CASENT0129721	16.68	0.521	1503	0
"*Leptanilla*" TH04	Male	CASENT0129695	16.702	0.512	1505	0
"*Leptanilla*" TH05	Male	CASENT0134656	16.746	0.517	1509	0
"*Leptanilla*" TH06	Male	CASENT0179537	16.857	0.511	1519	0
"*Leptanilla*" TH08	Male	CASENT0227775	16.902	0.515	1523	0
Leptanilla TH09	Male	CASENT0227556	16.757	0.543	1510	0
Leptanilla ZA01	Male	CASENT0106085	16.824	0.545	1516	0
Leptanilla zhg-au01	Male	CASENT0758873	89.568	0.531	1435	6636
Leptanilla zhg-au02	Male	CASENT0758864	62.701	0.536	685	4965
Leptanilla zhg-th01	Male	CASENT0842614	60.448	0.545	801	4646
Leptanilla revelierii	Worker	CASENT0006788	51.038	0.552	536	4063
Leptanilla zhg-bt01	Male	CASENT0842617	64.421	0.517	862	4943
Leptanilla zhg-my02	Male	CASENT0106451	51.027	0.538	535	4063
Leptanilla zhg-my03	Male	CASENT0842618	52.558	0.544	673	4063
Leptanilla zhg-my04	Male	CASENT0842553	52.869	0.537	701	4063
Leptanilla zhg-my05	Male	CASENT0842568	55.499	0.529	619	4382
Martialis heureka	Worker	CASENT0106181	23.915	0.465	2155	0
Noonilla zhg-my01	Male	CASENT0842585	89.258	0.506	1396	6647
Noonilla zhg-my02	Male	CASENT0842599	63.489	0.532	470	5251
Noonilla zhg-my04	Male	CASENT0842610	81.212	0.542	1202	6116
Noonilla zhg-my06	Male	CASENT0106373	51.171	0.543	548	4063
Opamyrma hungvuong	Worker	CASENT0178347	16.347	0.477	1473	0
"*Phaulomyrma*" MM01	Male	CASENT0179537	16.957	0.514	1528	0
Phaulomyrma javana	Male	MCZ:Ent:31142	100	0	9011	0
Protanilla TH01	Male	CASENT0119776	16.258	0.529	1465	0
Protanilla TH02	Male	CASENT0128922	16.28	0.529	1467	0
Protanilla TH03	Male	CASENT0119791	16.89	0.497	1522	0
Protanilla zhg-vn01	Male	CASENT0842613	60.803	0.521	228	5251
Yavnella argamani	Male	CASENT0235253	16.724	0.52	1507	0
Yavnella cf. *indica*	Male	CASENT0106375	61.98	0.516	1203	4382
Yavnella zhg-bt01	Male	CASENT0842616	50.838	0.524	518	4063

Taxon	**Caste/Sex**	**Identifier**	**Percent missing**	**AT content**	**-**	**?**
Yavnella zhg-th01	Male	CASENT0842615	50.882	0.523	522	4063

Table 2. NCBI and SRA accession numbers for DNA sequences used in this study.

Taxon	CASENT #	28S	AbdA	EF2	LwRh	Wg	AP	ArgK	NaK	POLD1	Top1	Ubx
Anomalomyrma boltoni	CASENT0217032	KU671445	KU672069	KU671496	KU671547	KU671598	KU671848	KU671656	KU672002	KU671925	KU671719	KU671782
Leptanilla GR01	CASENT0106236	EF012999	JN967847	JN967830	JN967890	JN967854	MF625736	JN967880	MF626276	MF625821	JN967820	JN967809
Leptanilla GR02	CASENT0106060	JN967864	JN967848	JN967831	JN967891	JN967856	MF625737*	JN967883	MF626277*	MF625822*	JN967823	JN967812
Leptanilla GR03	CASENT0106058	JN967868	JN967851	JN967834	JN967894	JN967859	N/A	JN967885	MT603718	MT526730	JN967826	JN967815
Leptanilla TH01	CASENT0119792	KU671447	JN967845	JN967836	KU671549	JN967853	KU671856	KU671660	KU672010	KU671933	KU671723	KU671786
"*Leptanilla*" TH02	CASENT0119531	MF626108	MF625677	MF625890	MF626217	MF625999	MF625738	MF626161	MF626278	MF625823	MF626052	MF625943
"*Leptanilla*" TH03	CASENT0129721	MF626109	MF625678	MF625891	MF626218	MF626000	MF625739	MF626162	MF626279	MF625824	MF626053	MF625944
"*Leptanilla*" TH04	CASENT0129695	MF626110	MF625679	MF625892	MF626219	MF626001	MF625740	MF626163	MF626280	MF625825	MF626054	MF625945
"*Leptanilla*" TH05	CASENT0134656	MF626111	MF625680	MF625893	MF626220	MF626002	MF625741	MF626164	MF626281	MF625826	MF626055	MF625946
"*Leptanilla*" TH06	CASENT0179537	MF626112	MF625681	MF625894	MF626221	MF626003	MF625742	MF626165	MF626282	MF625827	MF626056	MF625947
"*Leptanilla*" TH08	CASENT0227775	MF626113	MF625682	MF625895	MF626222	MF626004	MF625743	MF626166	MF626283	MF625828	MF626057	MF625948
Leptanilla TH09	CASENT0227556	MF626114	MF625683	MF625896	MF626223	MF626005	MF625744	MF626167	MF626284	MF625829	MF626058	MF625949
Leptanilla ZA01	CASENT0106085	AY867452	AY867468	EF013432	AY867483	AY867421	MF625745	JN967878	MF626285	MF625830	JN967818	JN967807
Leptanilla zhg-au01	CASENT0758873	N/A	N/A	N/A	N/A	N/A	N/A	MT526685	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Leptanilla zhg-au02	CASENT0758864	N/A	N/A	N/A	N/A	N/A	N/A	MT526686	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Leptanilla zhg-th01	CASENT0842614	N/A	N/A	N/A	N/A	N/A	N/A	MT526687	XXXXXXX	MT526731	XXXXXXX	XXXXXXX
Leptanilla revelierii	CASENT0006788	N/A	N/A	N/A	N/A	N/A	N/A	N/A	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Leptanilla zhg-bt01	CASENT0842617	N/A	N/A	N/A	N/A	N/A	N/A	MT526684	XXXXXXX	MT526729	XXXXXXX	XXXXXXX
Leptanilla zhg-my02	CASENT0106451	N/A	N/A	N/A	N/A	N/A	N/A	MT526688	XXXXXXX	MT526732	XXXXXXX	XXXXXXX
Leptanilla zhg-my03	CASENT0842618	N/A	N/A	N/A	N/A	N/A	N/A	XXXXXXX	XXXXXXX	MT526734	XXXXXXX	XXXXXXX
Leptanilla zhg-my04	CASENT0842553	N/A	N/A	N/A	N/A	N/A	N/A	XXXXXXX	XXXXXXX	MT526735	XXXXXXX	XXXXXXX
Leptanilla zhg-my05	CASENT0842568	N/A	N/A	N/A	N/A	N/A	N/A	MT526687	XXXXXXX	MT526733	XXXXXXX	XXXXXXX
Martialis heureka	CASENT0106181	KU671448	KU672072	KU671499	KU671550	KU671601	KU671858	KU671661	KU672012	KU671935	KU671724	KU671787
Noonilla zhg-my01	CASENT0842585	N/A	N/A	N/A	N/A	N/A	N/A	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Noonilla zhg-my02	CASENT0842599	N/A	N/A	N/A	N/A	N/A	N/A	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX
Noonilla zhg-my04	CASENT0842610	N/A	N/A	N/A	N/A	N/A	N/A	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX

*These accession numbers are erroneously attributed to CASENT0106067 *(Leptanilla* GR02) on GenBank.