



Delimitation of tribes in the subfamily Leptanillinae (Hymenoptera: Formicidae), with a description of the male of *Protanilla lini* TERAYAMA, 2009

Zachary GRIEBENOW

Abstract

The subfamily Leptanillinae EMERY, 1910 (Hymenoptera: Formicidae) is a clade of cryptic subterranean ants, which is restricted to the tropics and warm temperate regions of the Old World. Due to acquisition bias against the minute and hypogaecic workers, most known leptanilline specimens are male, with four genera described solely from males. The sexes have been associated in only two out of 69 described species, meaning that redundant naming of taxa is likely. Herein, the male of *Protanilla lini* TERAYAMA, 2009 is associated with corresponding workers collected on Okinawa-jima, Japan, by means of genome-scale data, allowing the first published description of male ants belonging to the Anomalomyrmini TAYLOR, 1990, one of the two established tribes within the Leptanillinae. The first male-based diagnoses of these tribes are provided, based on a phylogeny of the Leptanillinae inferred from ultra-conserved elements using maximum-likelihood and Bayesian inference, along with a dichotomous key to all described male-based species within the Leptanillinae and to undescribed male morphospecies sequenced in this study. With molecular data enabling the association of separately collected sexes and phylogenomic inference contextualizing morphological observations, the parallel taxonomy that afflicts this enigmatic group of ants can begin to be resolved.

Key words: Leptanillinae, Anomalomyrmini, male morphology, phylogenomics.

Received 24 June 2020; revision received 29 September 2020; accepted 2 October 2020

Subject Editor: John S. LaPolla

Zachary Griebenow, Department of Entomology & Nematology, University of California, Davis 95616, USA.

E-mail: zgriebenow@ucdavis.edu

Introduction

The ant subfamily Leptanillinae (Hymenoptera: Formicidae) EMERY, 1910 is a group of small, hypogaecic ants largely restricted to the Old World tropics and subtropics. Little is known of the biology of these ants; the few species for which detailed behavioral observations exist appear to be specialist predators of geophilomorph centipedes (MASUKO 1990, HSU & al. 2017). Known gynes of *Leptanilla* EMERY, 1870 are dichthadiiform (e.g., EMERY 1870, MASUKO 1990, LÓPEZ & al. 1994), whereas those of other genera are alate or ergatoid (BOLTON 1990a, BARONI URBANI & DE ANDRADE 2006, BOROWIEC & al. 2011, BILLEN & al. 2013, CHEN & al. 2017, HSU & al. 2017). The position of the leptanillines within ant phylogeny has been extensively debated (MOREAU & al. 2006, RABELING & al. 2008, KÜCK & al. 2011, MOREAU & BELL 2013, BOROWIEC & al. 2019), but molecular evidence indicates that the Leptanillinae are sister to *Martialis heureka* RABELING & VERHAAGH, 2008, with these two taxa constituting a clade that is sister to all other crown-group ants (BOROWIEC & al. 2019).

The subfamily Leptanillinae is formally divided into the tribes Leptanillini and Anomalomyrmini (BOLTON

1990a), with the monotypic *Opamyрма* YAMANE & al., 2008 unplaced to tribe and sister to the remainder of the Leptanillinae (WARD & FISHER 2016, BOROWIEC & al. 2019). The Leptanillini EMERY, 1910 consist of the genus *Leptanilla* EMERY, 1870 (46 spp.; BOLTON 2020), which is known from both sexes, and four genera known only from males: *Scyphodon* BRUES, 1925; *Phaulomyrma* WHEELER & WHEELER, 1930; *Noonilla* PETERSEN, 1968; and *Yavnella* KUGLER, 1987 (BOLTON 1990a). Of these male-based genera only *Yavnella* is not monotypic (2 spp.; BOLTON 2020). The Anomalomyrmini BOLTON, 1990 include the genera *Anomalomyrma* TAYLOR, 1990 (3 spp.; BOLTON 2020) and *Protanilla* TAYLOR, 1990 (13 spp.; BOLTON 2020) (BOLTON 1990a, HSU & al. 2017). BOROWIEC & al. (2019) extensively sampled molecular data from across the Leptanillinae, attempting to resolve basal divergences within the Formicidae with model-based inference from 11 nuclear loci. Otherwise, only WARD & FISHER (2016) have made explicitly phylogenetic contributions to our understanding of this clade – an understanding hampered by dissociation of male and worker specimens.

Due to collection bias most known leptanilline material is male, including most of the terminals sampled by BOROWIEC & al. (2019). Males attributable to the Anomalomyrmini by molecular data have been discovered and sequenced but remain undescribed and unassociated with female counterparts (BOUDINOT 2015, BOROWIEC & al. 2019). Within the Leptanillini, the only species for which both sexes have been identified is *Leptanilla japonica* BARONI URBANI, 1977 (OGATA & al. 1995); 28% of all described *Leptanilla* spp. are known only from males (BOLTON 2020). The male of *Opamyрма* has also been described (YAMADA & al. 2020).

The description of ant taxa based solely upon males results in parallel taxonomy, culminating in taxonomic “confusion rather than enlightenment” (BOLTON 1990a). Therefore, any phylogenetic reclassification of the Leptanillinae that clarifies the interrelationships of the male-based and worker-based taxa must necessarily integrate morphological and molecular data for both sexes, preferably with association of sexes for each species. To this end, the aim of the current paper is to 1) describe the male of *Protanilla lini* TERAYAMA, 2009 – a species heretofore known from workers and queens – from material collected on Okinawa-jima, Japan, associated with the conspecific worker using genome-scale data; and 2) provide the first formal morphological male-based definitions of the Anomalomyrmini and Leptanillini, with these definitions informed by inference from phylogenomic data.

Materials and methods

Material sampled: Morphology. The following material was physically examined: a series of four male *Protanilla* (M. Yoshimura det.), inferred to be *Protanilla lini* (see below), collected on Okinawa-jima; a series of four males belonging to an undescribed species of *Protanilla*, resembling the inferred *P. lini*, collected in northern Vietnam; and 37 additional male morphospecies attributed to the Leptanillinae according to the definition provided by BOUDINOT (2015). When necessary, images of male Leptanillinae already available on AntWeb (www.AntWeb.org) were used. A worker representative of *P. lini* (S. Iniyama & T. Yoshida det.) collected with a Sea, Air & Malaise (SLAM) trap in 2016 on Okinawa-jima was also examined and used for DNA sequencing, along with one of the males.

All specimens were examined with a Leica MZ75 compound microscope, except for the male of *Martialis heureka*, for which observations were derived from BOUDINOT (2015). Specimens were imaged using a JVC KY-F75 digital camera and color photographs were compiled from these with the Syncrosopy AutoMontage Program (v. 5.02.0096). Scanning electron microscopy was undertaken using a Hitachi TM4000 tabletop microscope. Material is deposited in the following repositories: the Okinawa Institute of Science and Technology, Onna, Okinawa, Japan (OIST); 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).

Phylogenomics. Phylogenomic data were acquired from 39 specimens of Leptanillinae, along with *Martialis heureka* as an outgroup (Tabs. 1 - 2), using ultra-conserved elements (UCEs) (see below, “Genomic Data Generation & Processing”). This approach was used as an alternative to barcoding methods, the reliance of which upon a single locus (usually cytochrome oxidase c, subunit 1, COI) can produce spurious phylogenetic inferences at a macro-evolutionary scale (TALAVERA & VILA 2011, CARAVAS & FRIEDRICH 2012, SIMON & HADRYIS 2013, CHEN & al. 2014) unless topologies are constrained by prior inference from other datasets (ZHOU & al. 2016). Mitogenomes can be retrieved from raw data generated in UCE sequencing; if needed for this purpose, the raw reads used for this study are publicly available (Tab. 2).

The phylogenomic sampling of the Leptanillinae in this study is the first to encompass both described species of the male-based genus *Yavnella* (or morphospecies with close affinity to them) and morphospecies attributable to the monotypic male-based genus *Noonilla*. *Noonilla zhg-my04* closely resembles *Noonilla copiosa* PETERSEN, 1968 but cannot be identified as such without better intraspecific sampling. The remaining four sampled *Noonilla* morphospecies diverge from the habitus of *N. copiosa* in that the mesoscutum is flattened relative to that species, resembling *Scyphodon anomalum* in this respect; however, their highly distinctive genital morphology conforms to that of *Noonilla* as described by PETERSEN (1968), and will be described in future publications (Z. Griebenow, G. Fischer & E. Economo, unpubl.).

Genomic data generation and processing: DNA was extracted non-destructively using a DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA) according to manufacturer instructions. Genomic concentrations were quantified for each sample with a Qubit 2.0 fluorometer (Life Technologies Inc., Carlsbad, CA). Phylogenomic data were generated using the ant-specific version of the UCE probe set *hym-v2* (BRANSTETER & al. 2017), with libraries being prepared and target loci enriched using the protocol of BRANSTETER & 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 an equimolar final pool. Depending on the lane in question, the contents of this final pool were sequenced on an Illumina HiSeq 2500 at the High Throughput Genomics Facility, University of Utah, Salt Lake City, UT; or an Illumina HiSeq 4000 at Novogene, Sacramento, CA. Sequence Read Archive (SRA) accession numbers for all raw reads are presented in Table 2.

The FASTQ output was demultiplexed and cleansed of adapter contamination and low-quality reads using *illumiprocessor* (FAIRCLOTH 2013) in the PHYLUCE package. Raw reads were assembled with *trinity* v. 2013-02-25 (GRABHERR & al. 2011) or with SPAdes v. 3.12.0

Tab. 1: Collection data for all terminals sequenced in this study. Taxon names are followed with Ward Laboratory extraction codes.

Terminal name	CASENT #	Form	BOROWIEC & al. (2019) designation	Collection origin	Voucher type
<i>Anomalomyrma boltoni</i> D0953	CASENT0217032	♀	<i>Anomalomyrma boltoni</i>	Malaysia: Perak	Same specimen
<i>Leptanilla</i> GR03 D0965	CASENT0106058	♂	N/A	Greece: Rhodes	Same specimen
<i>Leptanilla</i> GR01 D0969	CASENT0106236	♂	<i>Leptanilla</i> GR01	Greece: Rhodes	Same series
<i>Leptanilla</i> GR02 D0967	CASENT0106060	♂	<i>Leptanilla</i> GR02	Greece: Rhodes	Same specimen
<i>Leptanilla revelierii</i> D1866	CASENT0842627	♀	N/A	France: Corsica	Same specimen
<i>Leptanilla</i> TH01 D0679	CASENT0119792	♂	<i>Leptanilla</i> TH01	Thailand: Chiang Mai	Same specimen
<i>Leptanilla</i> zhg-au01 D1544	CASENT0758873	♂	N/A	Australia: Queensland	Same specimen
<i>Leptanilla</i> zhg-au02 D1545	CASENT0758864	♂	N/A	Australia: New South Wales	Same specimen
<i>Leptanilla</i> zhg-bt01 D1682	CASENT0842617	♂	N/A	Bhutan: Sarpang	Same specimen
<i>Leptanilla</i> zhg-id01 D1754	CASENT0842626	♂	N/A	Indonesia: Kalimantan Barat	Same specimen
<i>Leptanilla</i> zhg-my02 D1683	CASENT0106451	♂	N/A	Malaysia: Sabah	Same specimen
<i>Leptanilla</i> zhg-my03 D1686	CASENT0842553	♂	N/A	Malaysia: Sabah	Same specimen
<i>Leptanilla</i> zhg-my04 D1684	CASENT0842568	♂	N/A	Malaysia: Sabah	Same specimen
<i>Leptanilla</i> zhg-my05 D1685	CASENT0842618	♂	N/A	Malaysia: Sabah	Same specimen
<i>Leptanilla</i> zhg-th01 D1679	CASENT0842614	♂	N/A	Thailand: Chiang Mai	Same specimen
<i>Martialis heureka</i> D0751	CASENT0106181	♀	<i>Martialis heureka</i>	Brazil: Amazonas	Same specimen
<i>Noonilla</i> zhg-my01 D1690	CASENT0842585	♀	N/A	Malaysia: Sabah	Same specimen
<i>Noonilla</i> zhg-my02 D1691	CASENT0842599	♂	N/A	Malaysia: Sabah	Same specimen
<i>Noonilla</i> zhg-my04 D1694	CASENT0842610	♂	N/A	Malaysia: Sabah	Same specimen
<i>Noonilla</i> zhg-my06 D1688	CASENT0106373	♂	N/A	Malaysia: Sabah	Same specimen
<i>Opamyрма hungvuong</i> D0734	CASENT0178347	♂	<i>Opamyрма hungvuong</i>	Vietnam: Ha Tinh	Same specimen
<i>Protanilla</i> JP01 D0381	CASENT0007002	♀	<i>Protanilla</i> JP01	Japan: Kagoshima	Same specimen
<i>Protanilla</i> TH02 D0806	CASENT0128922	♀	<i>Protanilla</i> TH02	Thailand: Chaiyaphum	Same specimen
<i>Protanilla</i> TH03 D0807	CASENT0119791	♂	<i>Protanilla</i> TH03	Thailand: Chiang Mai	Same specimen
<i>Protanilla</i> VN01 D0895	CASENT0179564	♀	<i>Protanilla</i> VN01	Vietnam: Bac Giang	Same specimen
<i>Protanilla</i> VN03 D0896	CASENT0179565	♀	<i>Protanilla</i> VN03	Vietnam: Dong Nai	Same specimen
<i>Protanilla lini</i> D1755	OKENT0035688	♀	N/A	Japan: Okinawa	Same specimen
<i>Protanilla lini</i> D1756	OKENT0018456	♂	N/A	Japan: Okinawa	Same specimen
<i>Protanilla</i> TH01 D0678	CASENT0119776	♀	<i>Protanilla</i> TH01	Thailand: Khon Kaen	Same specimen
<i>Protanilla</i> zhg-vn01 D1678	CASENT0842613	♂	N/A	Vietnam: Vinh Phuc	Same specimen
<i>Yavnella</i> MM01 D0876	CASENT0179537	♂	<i>Phaulomyrma</i> MM01	Myanmar: Rakhine	Same specimen
<i>Yavnella</i> TH03 D0800	CASENT0129721	♂	<i>Leptanilla</i> TH03	Thailand: Chiang Mai	Same specimen
<i>Yavnella</i> TH04 D0801	CASENT0129695	♂	<i>Leptanilla</i> TH04	Thailand: Chiang Mai	Same specimen
<i>Yavnella</i> TH06 D0803	CASENT0129609	♂	<i>Leptanilla</i> TH06	Thailand: Chiang Mai	Same specimen
<i>Yavnella argamani</i> D1081	CASENT0235253	♂	N/A	Israel: Mehoz HaTzafon	Same specimen
<i>Yavnella</i> cf. <i>indica</i> D1689	CASENT0106375	♂	N/A	Sri Lanka: Kandy	Same specimen
<i>Yavnella</i> TH02 D0677	CASENT0119531	♂	<i>Leptanilla</i> TH02	Thailand: Khon Kaen	Same specimen
<i>Yavnella</i> TH08 D1052	CASENT0227775	♂	<i>Leptanilla</i> TH08	Thailand: Surat Thani	Same specimen
<i>Yavnella</i> zhg-bt01 D1681	CASENT0842616	♂	N/A	Bhutan: Sarpang	Same specimen
<i>Yavnella</i> zhg-th01 D1680	CASENT0842615	♂	N/A	Thailand: Phetchabun	Same specimen

Tab. 2: Summary statistics for final 368,656-bp UCE alignment, along with iTru primer sequences and Sequence Read Archive (SRA) accession numbers. Taxon names are followed with Ward Laboratory extraction codes.

Terminal name	i7 primer	i5 primer	Missing %	AT content	Accession # for SRA
<i>Anomalomyrma boltoni</i> D0953	ATCCGTTG	ACCGCTAT	6.401	0.599	SRR11742957
<i>Leptanilla</i> GR03 D0965	CGACCATT	AGATCGTC	16.033	0.606	SRR11793843
<i>Leptanilla</i> GR01 D0969	GAGATGTC	CGTGACT	8.224	0.612	SRR11881502
<i>Leptanilla</i> GR02 D0967	TAAGTGGC	ACAGACCT	61.795	0.587	SRR11881501
<i>Leptanilla revelierii</i> D1866	CGACGTTA	TCCTACCT	4.673	0.610	SRR11881510
<i>Leptanilla</i> TH01 D0679	GCTTCGAA	TTGTGTGC	2.181	0.599	SRR11881509
<i>Leptanilla</i> zhg-au01 D1544	AACCTACG	TCGACAAG	38.315	0.597	SRR11793860
<i>Leptanilla</i> zhg-au02 D1545	AGAACCAG	TATGACCG	29.168	0.606	SRR11793848
<i>Leptanilla</i> zhg-bt01 D1682	GTCCACAT	ACAGCAAG	23.968	0.603	SRR11793849
<i>Leptanilla</i> zhg-id01 D1754	GTGGTGTG	CGGTTGTT	49.660	0.580	SRR11881505
<i>Leptanilla</i> zhg-my02 D1683	CTGACACA	GTTATGGC	40.244	0.592	SRR11793840
<i>Leptanilla</i> zhg-my03 D1686	GTGAAGTG	ACGAATCC	7.929	0.602	SRR11793851
<i>Leptanilla</i> zhg-my04 D1684	GACATTCC	ACGAACGA	19.731	0.594	SRR11793838
<i>Leptanilla</i> zhg-my05 D1685	TGCACCAA	GGATGTAG	31.422	0.590	SRR11793837
<i>Leptanilla</i> zhg-th01 D1679	CCTCAGTT	TGGTTCGA	19.428	0.593	SRR11793854
<i>Martialis heureka</i> D0751	TCTTACGG	CACAGGAA	21.045	0.556	SRR11881511
<i>Noonilla</i> zhg-my01 D1690	TTACGGCT	GGCAAGTT	64.911	0.582	SRR1793857
<i>Noonilla</i> zhg-my02 D1691	CGCTAGTA	ACGTATGG	36.678	0.590	SRR11793856
<i>Noonilla</i> zhg-my04 D1694	AGTTCGTC	ACGTCCAA	58.794	0.577	SRR11793855
<i>Noonilla</i> zhg-my06 D1688	ACTGCTAG	GGAGTCTT	16.252	0.594	SRR11793842
<i>Opamyрма hungvuong</i> D0734	ACAGTGAC	TGAGCTGT	24.566	0.557	SRR11742960
<i>Protanilla</i> JP01 D0381	TGCACTTG	ACATGCCA	5.685	0.591	SRR11742961
<i>Protanilla</i> TH02 D0806	TTGGACTG	GCATAACG	5.818	0.599	SRR11742959
<i>Protanilla</i> TH03 D0807	GATGTCGA	CAGTGCTT	6.571	0.580	SRR11742954
<i>Protanilla</i> VN01 D0895	CACGCAAT	CGTCAAGA	9.189	0.580	SRR11742952
<i>Protanilla</i> VN03 D0896	AGTTCGCA	CCATGAAC	6.215	0.580	SRR11742951
<i>Protanilla lini</i> D1755	AAGTCCGT	TACCAACC	2.759	0.591	SRR11881503
<i>Protanilla lini</i> D1756	ATAGCGGT	TCGCTGTT	3.493	0.591	SRR11881504
<i>Protanilla</i> TH01 D0678	CTGAAGCT	CAACACCT	6.120	0.601	SRR12006305
<i>Protanilla</i> zhg-vn01 D1678	GAGCTTGT	ACACCGAT	36.354	0.575	SRR11793859
<i>Yavnella</i> MM01 D0876	AGAGACTC	CGTATCTC	14.154	0.581	SRR11742953
<i>Yavnella</i> TH03 D0800	TGCAAGAC	TGACAACC	5.389	0.589	SRR11742956
<i>Yavnella</i> TH04 D0801	ACCTCAGT	TGTTCCGT	5.127	0.584	SRR11742958
<i>Yavnella</i> TH06 D0803	TGTCAGTG	CCTAGAGA	6.232	0.585	SRR11742955
<i>Yavnella argamani</i> D1081	TGCTTGCT	GGTACGAA	5.723	0.592	SRR11793861
<i>Yavnella cf. indica</i> D1689	ACCACGAT	ACGGTACA	32.766	0.570	SRR11793841
<i>Yavnella</i> TH02 D0677	TGGTACAG	TTGCTGGA	2.710	0.585	SRR11881508
<i>Yavnella</i> TH08 D1052	TCGTGGAT	TAAGTGGC	8.005	0.584	SRR11881506
<i>Yavnella</i> zhg-bt01 D1681	ACGACAGA	TGGTATCC	11.895	0.583	SRR1793850
<i>Yavnella</i> zhg-th01 D1680	CGTACGAA	ACACTCTG	5.460	0.585	SRR11793853

(BANKEVICH & al. 2012). All PHYLUCE commands hereinafter are cited from FAIRCLOTH (2016). Species-specific contig assemblies were obtained with the ant-specific *hym-v2* probe set (BRANSTETTER & al. 2017) using *phyluce_assembly_match_contigs_to_probes.py* (min_coverage=80), with min_identity = 90 to minimize the influence of possible contamination; and a list of UCE loci shared across all taxa was generated using *phyluce_assembly_get_match_counts.py*, and separate FASTA files for each locus were created using these outputs. Sequences were aligned separately by locus using MAFFT L-INS-i (KATO & TOH 2008), rather than the default version of MAFFT implemented in *phyluce*, implemented with the command *phyluce_assembly_seqcap_align.py*. These sequences 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 dataset that was 90% complete with respect to taxon coverage per locus was generated using the script *phyluce_align_get_only_loci_with_min_taxa.py*, consisting of 580 loci. The final alignment was then concatenated and converted to PHYLIP format with *phyluce_align_format_nexus_files_for_raxml* and was 368,656 bp in length, with 19.07% missing data, 135,832 parsimony-informative sites, and mean locus length being 634 bp. This alignment, along with the partition scheme, is available on Dryad (doi: 10.25338/B8490T). Summary statistics for this alignment were computed with the *summary* command in AMAS (BOROWIEC 2016) (Tab. 2).

Phylogenetic inference: Evolutionary processes operating on ultra-conserved elements and their flanking regions vary due to differing constraints, both between loci and among sites within a single locus (TAGLIACOLLO & LANFEAR 2018). Failing to accommodate this variation (i.e., model misspecification) can result in erroneous inferences (LANFEAR & al. 2014; KAINER & LANFEAR 2015). Using the corrected Akaike Information Criterion (AICc) TAGLIACOLLO & LANFEAR (2018) found that for six empirical phylogenomic datasets, partitioning drastically improved model fit. Therefore the final alignment in this study was partitioned according to, and within each, UCE locus using the *analysis.py* script of PartitionUCE (available at <https://github.com/Tagliacollo/PartitionUCE/tree/master/scripts>) using the SWSC partitioning algorithm, with site entropy as the nucleotide property by which data blocks were derived (SWSC-EN; TAGLIACOLLO & LANFEAR 2018). Substitution models were then selected for these partitions using ModelFinder (KALYAANAMOORTHY & al. 2017) in IQ-Tree v. 1.4.2-beta (NGUYEN & al. 2015), with the AICc as test statistic, and only the top 20% of partition schemes considered using the relaxed hierarchical clustering algorithm (LANFEAR & al. 2014); all substitution models were considered, with the exception of those with I + G extensions (YANG 1996). The resulting scheme consisted of 1221 partitions.

The phylogeny of the Leptanillinae was inferred using IQ-Tree v. 1.6.10 (NGUYEN & al. 2015) on the CIPRES Science Gateway (v. 3.3) (MILLER & al. 2010) with 1,000 ultrafast bootstrap replicates (HOANG & al. 2018), both unpartitioned and using the partition scheme (CHERNOMOR & al. 2016) inferred using the SWSC-EN algorithm and ModelFinder. Bayesian inference was performed in ExaBayes (ABERER & al. 2014) using two cloned analyses on the CIPRES Science Gateway with the same partitioning scheme as above, but with GTR + G imposed as the substitution model across all partitions. Each analysis involved two runs, each proceeding for 240,000 generations and consisting of four Metropolis-coupled continuous-time Markov chains (three of them “heated”, with an increment of 0.5). Topology was considered converged once the average standard deviation of split frequencies (ASDSF) equaled 0.05. Apparent convergence of the Markov-chain Monte Carlo (MCMC) with respect to continuous parameters was assessed with Tracer v. 1.7.1 (RAMBAUT & al. 2018). The output of these Bayesian analyses is available on Dryad (doi: 10.25338/B8490T).

Measurements and indices: Out of all terminals sampled in this study for which males are known, *Protanilla zhg-vn01* is the most closely related to *P. lini* (Figs. 1, 2). Therefore, morphometric comparisons were made between males of these two lineages (Tabs. 3 - 4). Unlike the examined males of *P. lini* all examined *Protanilla zhg-vn01* males were syntopic, and so less morphometric variation among them is expected than in examined *P. lini*.

Protanilla zhg-vn01 material examined. CASENT0106382, CASENT0842613, CASENT0842655-6. Vietnam: Vĩnh Phúc, Tam Đảo National Park (21.46667° N, 105.65° E), 1200 m elevation, 19 - 22.VI.2014, leg. M. Hauser and N. von Ellenreider, 4 males (CSCA).

Measurements.

DPW = Dorsal Petiole Width, maximum width of the petiole measured in dorsal view

EL = Eye Length, maximum measurable length of compound eye parallel to anteroposterior axis of head

EW = Eye Width, maximum measurable length of eye parallel to dorsoventral axis of head

FrW = Frons Width, the shortest distance between the medial margins of the compound eyes measured in full-face view

HL = Head Length, maximum length of head in full-face view from anterior clypeal margin to posterior head margin between lateral ocelli, ignoring distance which ocelli project

LF1 = First Funicular Segment Length, maximum length of 1st funicular segment (pedicel) in dorsal view (WARD 1985)

LF2 = Second Funicular Segment Length, maximum length of 2nd funicular segment (most basal flagellomere) in medial view

LOD = Lateral Ocellus Length, maximum diameter of lateral ocellus with head oriented such that anterior and posterior lateral ocellus margins are in same plane of focus

MFL = Metafemur Length, maximum length of metafemur in profile view

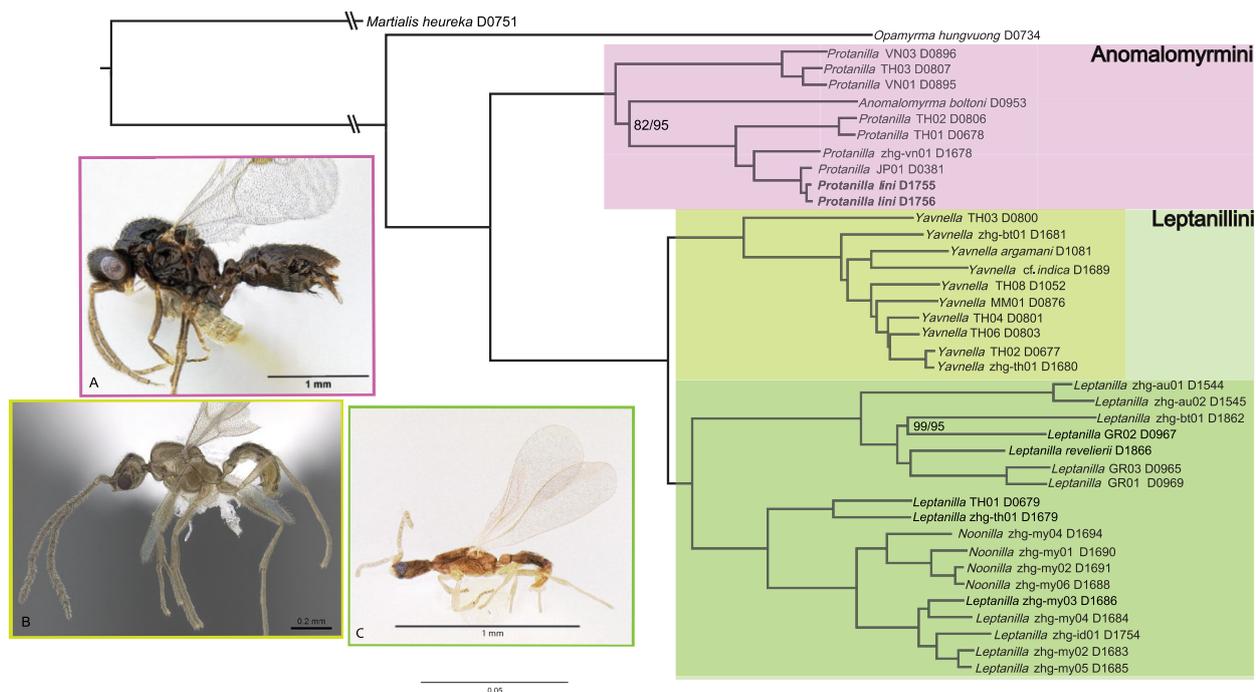


Fig. 1: Maximum-likelihood phylogeny of the Leptanillinae based upon ultra-conserved elements. Non-parametric bootstrap values are given for both unpartitioned and partitioned analyses, respectively, where non-parametric bootstrap values < 100. (A) *Protanilla lini* (CASENT0011097); (B) *Yavnella* TH08 (CASENT022755; Shannon Hartman, image courtesy of AntWeb, www.AntWeb.org); (C) *Leptanilla* zhg-bt01 (CASENT084612). Bootstrap support = 100 unless otherwise noted.

Tab. 3: Metrics useful in distinguishing male *P. lini* and *Protanilla* zhg-vn01, whether directly or via indices derived from them. *P. lini* = pale gray; *Protanilla* zhg-vn01 = dark gray. All measurements given in millimeters.

Specimen identifier	HL	FrW	SL	LF1	LF2	EL	EW	LOD
OKENT0018456	0.520	0.409	0.162	0.091	0.140	0.234	0.301	0.089
OKENT0028803	0.468	0.349	0.150	0.083	0.131	0.208	0.273	0.076
OKENT0011097	0.495	0.402	0.173	0.087	0.131	0.206	0.279	0.090
OKENT0027514	0.468	0.373	0.155	0.075	0.137	0.212	0.274	0.074
CASENT0842655	0.443	0.349	0.121	0.072	0.095	0.206	0.260	0.065
CASENT0842656	0.446	0.329	0.115	0.060	0.089	0.204	0.264	0.072
CASENT0106382	0.444	0.340	0.109	0.065	0.092	0.198	0.249	0.065
CASENT0842613	0.430	0.337	0.113	0.062	0.098	0.196	0.250	0.070
	MOD	PFL	MFL	DPW	PTH	PTL	TW3	TW4
OKENT0018456	0.085	0.521	0.696	0.199	0.200	0.239	0.321	0.579
OKENT0028803	0.077	0.451	0.499	0.164	0.232	0.201	0.294	0.543
OKENT0011097	0.077	0.508	0.519	0.183	0.289	0.242	0.247	0.487
OKENT0027514	0.072	0.471	0.490	0.168	0.246	0.206	0.284	0.523
CASENT0842655	0.064	0.385	0.410	0.202	0.270	0.199	0.269	0.349
CASENT0842656	0.065	0.389	0.399	0.174	0.269	0.200	0.233	0.335
CASENT0106382	0.060	0.381	0.398	0.195	0.268	0.195	0.247	0.343
CASENT0842613	0.053	0.292	0.398	0.174	N/A	0.201	0.262	0.347

Tab. 4: Indices useful in distinguishing male *P. lini* and *Protanilla zhg-vn01*. *P. lini* = pale gray; *Protanilla zhg-vn01* = dark gray.

Specimen identifier	CS	SEI	SI	FI	PTI	TI1
OKENT0018456	0.465	144.444	39.609	349.749	83.682	55.440
OKENT0028803	0.409	138.667	42.980	304.268	115.423	54.144
OKENT0011097	0.448	119.080	43.035	283.607	119.421	50.719
OKENT0027514	0.421	136.774	41.555	291.667	119.418	54.302
CASENT0842655	0.396	170.248	34.670	202.970	135.678	77.077
CASENT0842656	0.388	177.391	34.954	229.310	134.500	69.552
CASENT0106382	0.392	181.651	32.059	204.102	137.436	72.011
CASENT0842617	0.384	173.451	33.531	228.735	N/A	75.504

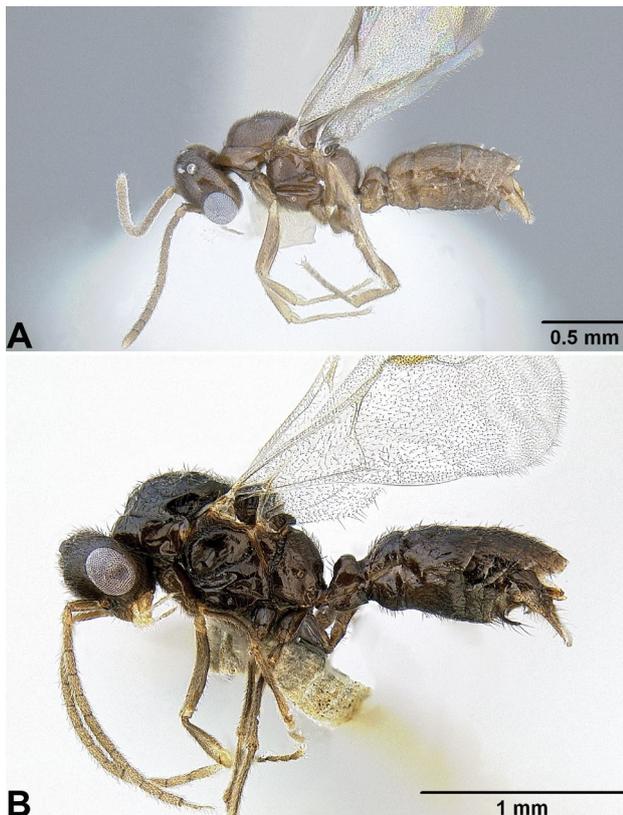


Fig. 2: Profile view of (A) *Protanilla zhg-vn01* (CASENT0106382) and (B) *Protanilla lini* (OKENT0011097).

MOD = Median Ocellus Width, maximum diameter of median ocellus in full-face view

PFL = Profemur Length, maximum length of profemur in profile view

PTH = Petiole Height, maximum width of the petiole in profile view along dorsoventral axis.

PTL = Petiole Length, length of petiole in profile view along anteroposterior axis from inflection point of petiolar presclerites (the articular surfaces) to most posterior point of posterior margin

SL = Scape Length, maximum length of scape in medial view, excluding condylar neck

TW3 = Width of Tergite III, maximum width of abdominal tergite III measured in dorsal view orthogonal to dorsal vertex of abdominal tergite III

TW4 = Width of Tergite IV, maximum width of abdominal tergite IV measured in dorsal view orthogonal to dorsal vertex of abdominal tergite IV

Indices. CS = Cephalic Size (FrW + HL) / 2

FI = Femora Index PFL / MFL × 100

PTI = Petiole Index PTH / PTL × 100

SEI = Scape-Eye Index EL / SL × 100

SI = Scape Index SL / FrW × 100

TI1 = Tergal Index 1 TW3 / TW4 × 100

All continuous morphometric data for *P. lini* and *Protanilla zhg-vn01* that are of discriminatory use are summarized in Tables 3 - 4.

Terminology. Morphological terminology follows that promulgated by the Hymenoptera Anatomy Ontology project (YODER & al. 2010), except for terminology for the male genitalia, which follows BOUDINOT (2018), and terms pertaining to metasomal sclerites, which are derived from BOLTON (1990b) and KELLER (2011). Hamuli are termed according to the nomenclature of BASIBUYUK & QUICKE (1997). Terminology for sculpturation and setation follows HARRIS (1979) and WILSON (1955: p. 23, Fig. 3), respectively.

Results

Phylogenomic inference: The maximum-likelihood (ML) phylogeny of the Leptanillinae presented here (Fig. 1) recovers the same topology whether based upon partitioned or unpartitioned genome-scale UCE alignments, and corroborates the previous molecular phylogenetic studies of the subfamily (WARD & FISHER 2016, BOROWIEC & al. 2019). Non-parametric bootstrap values are maximal throughout, except for two internal nodes (Fig. 1): 1) *Anomalomyrma boltoni* BOROWIEC & al., 2011 as sister to the anomalomyrmine subclade containing *P. lini*; and 2) the sister-group relationship of *Leptanilla* GR02 and *Leptanilla zhg-bt01*. *Opamyrra* is recovered as sister to all remaining Leptanillinae, which bifurcate into two well-supported nodes corresponding to the tribes Leptanillini and Anomalomyrmini. Bayesian inference with



Fig. 3: CASENT0178838 (*Leptanilla* indet.), image courtesy of AntWeb (www.AntWeb.org), photographer April Nobile.

ExaBayes under the same partitioning scheme as the ML analyses corroborates the ML phylogeny exactly, but with Bayesian Posterior Probabilities (BPP) maximal across all nodes.

These phylogenies also indicate that the *Protanilla lini* worker and putative male are conspecific, given the lack of other putative anomalomyrmine male morphospecies on Okinawa-jima (E. Economo, pers. comm.). Such a conclusion is consistent with preliminary maximum-likelihood inference from smaller molecular datasets (E. Economo, pers. comm.). *Protanilla* JP01, from the northern Ryukyu Islands, is robustly recovered sister to the Okinawan *P. lini*, and, given its geographical proximity to Okinawa and the brevity of its subtending branch, can be provisionally judged conspecific with *P. lini* as well. The node corresponding to the Anomalomyrmini itself consists of two major clades, both of which include worker and male material putatively assigned to *Protanilla*. *Anomalomyrma boltoni* is sister to the clade including *P. lini*, but with comparatively weak support, contrasting with the relationship inferred by BOROWIEC & al. (2019), who recovered *A. boltoni* sister to the other major anomalomyrmine clade. If the assignment of these undescribed worker and male morphospecies to *Protanilla* is accurate, then *Anomalomyrma* renders *Protanilla* paraphyletic (cf. BOROWIEC & al. 2019), and should be synonymized with the latter genus. Alternatively, *Protanilla* in the current sense could be divided into at least two genera (see Discussion).

While sampling of the tribe Leptanillini in this study is more extensive than that of BOROWIEC & al. (2019), and the loci and inferential framework different, the results of these two studies are largely congruent, with the most conspicuous difference being the placement of *Anomalomyrma* within the Anomalomyrmini (see Discussion). The two described species of *Yavnella* are nested within a clade consisting of morphospecies provisionally attributed to *Leptanilla*, and a single male specimen incorrectly assigned to the male-based genus *Phaulomyrma* (Z. Griebenow, in review); this clade is distinguishable based

upon male morphology (e.g., posterodorsal propodeal outline concave in profile view, noted by KUGLER (1986) as contrasting *Yavnella* with *Noonilla*), and is hereafter referred to as *Yavnella* s.l. The sister clade to *Yavnella* s.l. contains all the morphospecies attributed to *Noonilla*, itself monophyletic, nested within an assemblage of specimens provisionally identified as *Leptanilla*; among these is *Leptanilla revelierii* EMERY, 1870, the type species of the genus. This clade is termed *Leptanilla* s.l. Examination of male morphological diversity across this node, informed by phylogenomic data, is in progress and will clarify the boundaries and status of the four male-based genera currently placed within the Leptanillini.

Diagnosis of leptanilline tribes based upon the male sex: BOUDINOT (2015) acknowledged the existence of male Leptanillinae that were inferred to be anomalomyrmines based upon molecular data, as later published in BOROWIEC & al. (2019). Due to an error on AntWeb that linked specimens with the wrong images, the male leptanilline in Figs. 6F and 10A of BOUDINOT (2015), said to be that of *Protanilla* TH01 (CASENT0119776), is actually that of CASENT0119531 (named *Leptanilla* TH02 by BOROWIEC & al. 2019), which closely resembles the male-based genus *Yavnella* (Leptanillini; KUGLER 1986, BOROWIEC & al. 2019). As noted by BOUDINOT (2015: p. 14), this morphospecies is notable among the Formicidae for the complete loss of metasomal petiolation. CASENT0119531 is called *Yavnella* TH02 in this paper (Tabs. 1 - 2). Examination of CASENT0119776 demonstrated that this specimen in fact closely resembles other male Anomalomyrmini.

Moreover, the specimen in Figure 12D of BOUDINOT (2015), identified as *Protanilla* indet. (CASENT0178838) (Fig. 3), is qualitatively like five male morphospecies sequenced in this study (*Leptanilla* zhg-my02-05, -id01) and robustly recovered within a clade corresponding to the Leptanillini. The rationale for labeling CASENT0178838 as *Protanilla* is not given (BOUDINOT 2015), nor was it noted anywhere outside of peer review (B. Boudinot, pers. comm.). Thus, there is no justification for regarding CASENT0178838 as *Protanilla*. CASENT0178838 and those five male-based morphospecies provisionally assigned to *Leptanilla* exhibit such peculiar attributes as protibial combs of robust setae and ventrolateral setose metasomal processes. The latter are unique among the whole of the Hymenoptera (L. Kimsey & B. Boudinot, pers. comm.), and were previously hypothesized (BOUDINOT 2015) to be 1) filiform extensions of the gonocoxae sensu BOUDINOT (2018) (i.e., basimeres sensu SCHULMEISTER 2001) or 2) pygostyles.

Therefore, the known phenotypic diversity of male anomalomyrmines is circumscribed relative to what is implied by Fig. 12 of BOUDINOT (2015). All 5 anomalomyrmine morphotaxa represented by male material that were sampled in this study, including *P. lini*, consistently contrast with males of the Leptanillini in several respects. The two previously established tribes and *Opamyрма hungvuong* YAMANE & al., 2008 are diagnosed below

according to these character states, in addition to a male-based definition of the Leptanillinae relative to other Formicidae; character states that are apomorphic within the Leptanillinae are underlined. *Opamyрма* is left unplaced to tribe because there is no comparative basis for defining such a tribe morphologically, given that said tribe would be monobasic. For taxonomic synopses of *Opamyрма*, Leptanillini, and Anomalomyrmini refer to BOLTON (2020).

Subfamily Leptanillinae EMERY, 1910

Leptanillinae EMERY, 1910: 32 (as tribe of subfamily Dorylinae). Type-genus: *Leptanilla* EMERY, 1870: 196.

Male Diagnosis (modified from BOUDINOT 2015).

1. Mandibles edentate; minute and nub-like, or hypertrophied and spatulate
2. Frontal carinae and lobes absent
3. Anterior clypeal margin without pegs
4. Antenna 13-merous; funiculus filiform to submoniliform
5. Mesopleural sulcus present or absent
6. Metapleural spiracular plate absent
7. Propodeal lobes inconspicuous or absent
8. Metacoxal cavities closed
9. Tibial spur formula 2s,2s; 1s,2(1s,1p); 1s,2s; or 0,1p
10. Metatarsus lacking posterolateral line of dense differentiated setae
11. Pretarsal claws edentate
12. Wing venation Ogata Type IVb
13. Hindwing venation reduced, at most R+Rs and 1A tubular
14. Jugal lobe absent
15. Petiolar tergum not forming anteroventral collar around sternum
16. Helcium axial or infra-axial
17. Abdominal segment IV not vaulted, as long as, or distinctly longer than, more posterior abdominal segments
18. Abdominal spiracles IV-VIII obscured by preceding tergites
19. Posterior margin of abdominal sternite IX with posteromedian process, entire, or with posterolateral processes
20. Pygostyles (i.e., cerci) absent
21. Cupula present or absent
22. Parossiculus and lateropenite (BOUDINOT 2018) (i.e., cuspis and digitus) distinct or indistinct

***Opamyрма* YAMANE, BUI & EGUCHI, 2008**

Opamyрма YAMANE, BUI & EGUCHI 2008: 56. Type-species: *Opamyрма hungvuong*, by monotypy.

Male Diagnosis.

1. Ocelli not set on tubercle
2. Four maxillary palpomeres
3. Pronotum not prolonged posteriorly
4. Mesoscutum not prolonged posteriorly
5. Notauli present
6. Rs+M and 1m-cu present in forewing
7. 1A present in hindwing

8. Pterostigma present
9. Petiole distinct, without tergo-sternal fusion
10. Postpetiole absent
11. Parossiculus and lateropenite distinct; lateropenite club-shaped

Tribe Leptanillini EMERY, 1910

Leptanillini EMERY, 1910: 32 (as tribe of subfamily Dorylinae). Type genus: *Leptanilla* EMERY, 1870: 196.

Constituent genera: *Leptanilla*; *Scyphodon*; *Phaulomyрма*; *Noonilla*; *Yavnella*

Male Diagnosis.

1. Ocelli, when present, set on tubercle (with exception of *Leptanilla zhg-my05*)
2. One maxillary palpomere
3. Pronotum prolonged posteriorly, or not prolonged posteriorly
4. Mesoscutum prolonged posteriorly, or not prolonged posteriorly
5. Notauli absent
6. Rs + M and 1m-cu absent in forewing
7. 1A absent in hindwing
8. Pterostigma absent
9. Petiole distinct to absent and with tergo-sternal fusion
10. Postpetiole absent
11. Parossiculus and lateropenite not distinct

Tribe Anomalomyrmini TAYLOR, 1990

Anomalomyrmini TAYLOR, 1990 in BOLTON 1990a: 278. Type-genus: *Anomalomyрма* TAYLOR, 1990 in BOLTON 1990a: 278.

Constituent genera: *Anomalomyрма*; *Protanilla*

Male diagnosis.

1. Ocelli not set on tubercle
2. Four maxillary palpomeres
3. Pronotum not prolonged posteriorly
4. Mesoscutum not prolonged posteriorly
5. Notauli present or absent
6. Rs + M and 1m-cu absent in forewing
7. 1A present or absent in hindwing
8. Pterostigma present
9. Petiole distinct, with tergo-sternal fusion
10. Postpetiole present or absent
11. Parossiculus and lateropenite distinct

Discussion

Delimitation of major lineages within the Leptanillinae: According to the admittedly “tentative and unsatisfactory” diagnosis of BOLTON (1990a), the males of Leptanillinae are identified by 7 co-occurring character states, most of them vague (e.g., “genitalia large to .. hypertrophied; not retractile”) and so of limited utility. None of these character states are unique to the subfamily. Later male-based revision of the Formicidae (BOUDINOT 2015) recognized the reduction of the propodeal lobe (BOUDINOT 2015: Fig. 4G) as diagnostic of leptanilline males in combination with other character states.

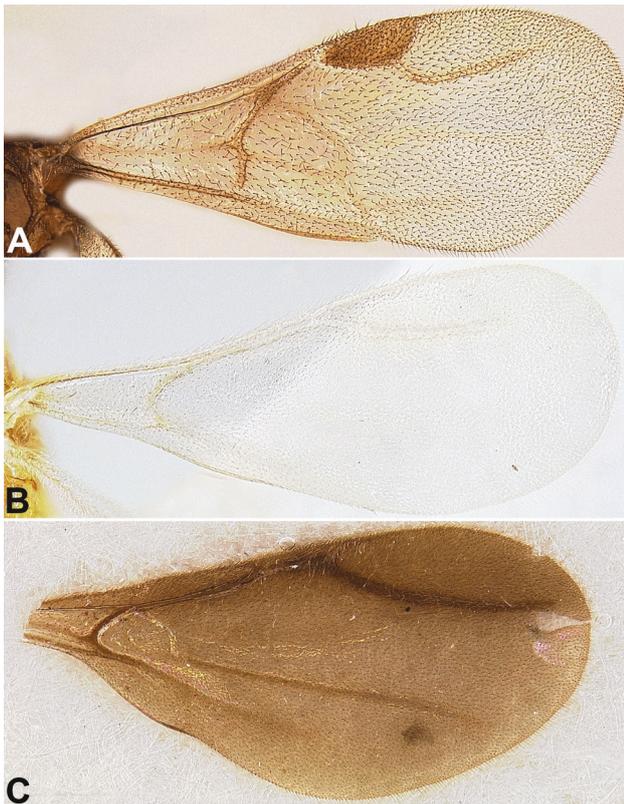


Fig. 4: Forewings of (A) *Protanilla* zhg-vn01 (CASENT0842613); (B) *Yavnella* cf. *indica* (CASENT0106378); (C) *Leptanilla* zhg-my05 (CASENT0842571).

Male Leptanillini are far better represented in this study than males of their sister group, and correspondingly exhibit morphologies that are not only more diverse, but qualitatively more disparate, than those of anomalomyrmine males. Even so, there are two morphological characters that reliably separate males of Anomalomyrmini and Leptanillini: the presence / absence of the pterostigma (Figs. 4A - B) and the presence/absence of an ocellar tubercle or swelling (Figs. 5A - B). While better sampling may bring further male leptanilline morphological diversity to light and so necessitate revision of this morphological diagnosis, it is satisfactory given the material available.

Males of the clade to which CASENT0178838 putatively belongs (consisting of *Leptanilla* zhg-my02 through -05 and zhg-id01) display what resembles a pterostigma with the confluence of Rf and 2s - rs + Rs + 4 - 6 (Fig. 4C), but this condition is likely homoplasious with that seen in the Anomalomyrmini. Pterostigmal condition remains the most useful character for discriminating males of the two tribes: although there are lineages within the Leptanillini with males that have deciduous forewings (pers. obs.) and therefore for which the condition of the pterostigma is unknown, in no known male Anomalomyrmini are the forewings deciduous. Exceptions to the diagnosis of male Leptanillini as possessing an ocellar tubercle do occur, but these either occupy a unique character state in that they lack ocelli entirely (Fig. 6B; *Yavnella* TH03 and zhg-bt01) or are distinct from the Anomalomyrmini in that the anteromedian ocellus is not orthogonally dorsal to the compound eyes (e.g., *Leptanilla* zhg-my05; Fig. 7).

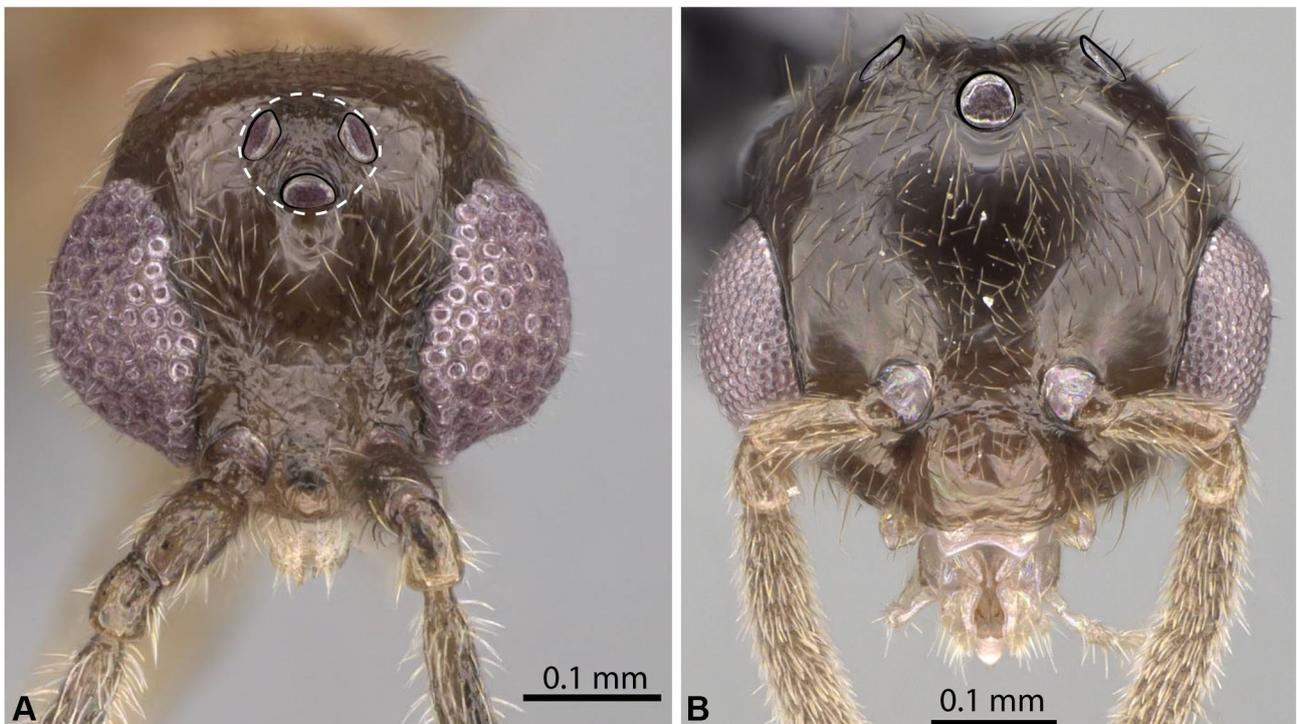


Fig. 5: Presence (A) (*Yavnella* TH02; CASENT0119531) vs. absence (B) (*Protanilla* TH01; CASENT0119776) of an ocellar tubercle. Photographer Michele Esposito, images courtesy of AntWeb (www.AntWeb.org).

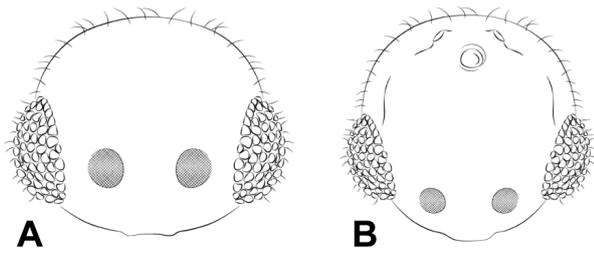


Fig. 6: Presence (B) (“*Yavnella*” TH10; CASENT0227557) vs. absence (A) (*Yavnella* TH03; CASENT0129721) of the ocelli in the Leptanillini.



Fig. 7: Head of *Leptanilla zhg-my05* (CASENT0842573), profile view.

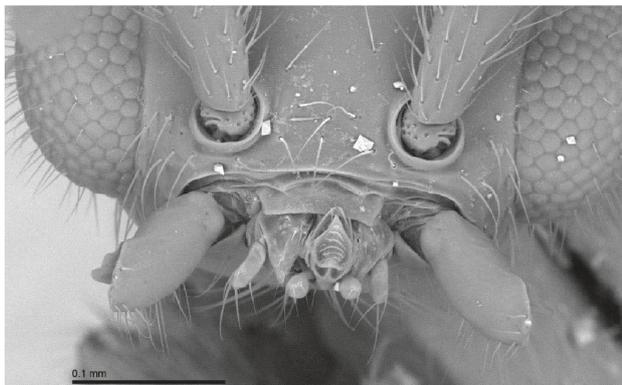


Fig. 8: Mouthparts of *Noonilla zhg-my02* (CASENT0842591), full-face view.

This survey of novel male material attributable to the Leptanillinae refines and extends the previous male-based diagnoses of the subfamily. It is apparent that 1) there are 4 maxillary palpomeres in all known anomalomyrmine males (and *Opamyрма hungvuong*, in which the palpal formula is 4,2; YAMADA & al. 2020), in contrast with the single maxillary palpomere of both males (Fig. 8) and workers (BOLTON 1990a, OGATA & al. 1995, BOUDINOT 2015) of the Leptanillini (KUGLER 1986 reported a vestigial constriction of the single worker maxillary palpomere in *Leptanilla escheri* KUTTER, 1948 and *Leptanilla judaica* KUGLER, 1987); 2) the mesoscutum is not always flattened

and posteriorly extended in the males of Leptanillinae, with *O. hungvuong*, the Anomalomyrmini and *Yavnella* s.l. being the exceptions; and 3) the propodeal lobe is inconspicuous to absent in all leptanilline male morphospecies examined in this study that were not cited by BOUDINOT (2015: p. 31) and in *O. hungvuong* (YAMADA & al. 2020), corroborating the male diagnosis of the subfamily by BOUDINOT (2015: p. 29).

In the context of male Formicidae, I infer the anomalomyrmine habitus to be plesiomorphic overall relative to that of previously described male Leptanillinae (PETERSEN 1968, KUGLER 1986, BOLTON 1990a, OGATA & al. 1995, BOUDINOT 2015, YAMADA & al. 2020), e.g., in the presence of the pterostigma. Micro-CT scans of *Protanilla zhg-vn01* genitalia (Z. Griebenow, G. Fischer & E. Economo, unpubl.) indicate that a reduced ventral cupula (SCHULMEISTER 2001) is present in that morphospecies, contrasting with all males attributable to Leptanillini in which this character can be observed; while the genitalia of other anomalomyrmine male material were not examined by dissection (virtual or otherwise), I predict that the cupula is present in *Protanilla lini* and the Anomalomyrmini as a whole. YAMADA & al. (2020: Fig. 15D) report the presence of a similarly reduced cupula in the male of *Opamyрма hungvuong*, the sister group of the remaining Leptanillinae (YAMADA & al. 2020: Fig. 14A), and like the Anomalomyrmini, male *O. hungvuong* exhibit a pterostigma and lack an ocellar tubercle; moreover, YAMADA & al. (2020) report lack of tergosternal fusion in the petiole of male *O. hungvuong*, the converse of which is here described in male *P. lini*. Tergosternal fusion is present in all males so far included in phylogenomic analysis, in addition to *Scyphodon anomalum* BRUES, 1925 and *Noonilla copiosa* PETERSEN, 1968. The lack of tergosternal fusion in male *O. hungvuong* is likely plesiomorphic within the Leptanillinae. Conversely, the male genitalia of *O. hungvuong* exhibit derived character states not seen in *P. lini*, including medial gonocoxal fusion (YAMADA & al. 2020: Figs. 13A - B), which is otherwise known only in *Yavnella* TH03 and some subclades of *Leptanilla* s.l. The pedunculate lateropenites (YAMADA & al. 2020: Figs. 13E - F) and penial “spinescent lobes” (YAMADA & al. 2020: p. 45, Figs. 13A, G) are unparalleled among male Leptanillinae surveyed in this study.

Delimitation of anomalomyrmine genera with male morphology: It would be premature to define *Protanilla* based upon the male sex, since molecular evidence indicates that the boundaries of the anomalomyrmine genera require revision. *Protanilla* was described based upon worker material, whereas *Anomalomyрма* was described from a dealate gyne (BOLTON 1990a): the initially unknown female castes of both were described subsequently (BARONI URBANI & DE ANDRADE 2006, BOROWIEC & al. 2011). Mandibular characters were initially used to distinguish these genera (BOLTON 1990a, 1994, IMAI & al. 2003), but with the description of the worker caste of *Anomalomyрма* it became arguable that abdominal morphology provided more consistent diagnoses (BOROWIEC & al. 2011).

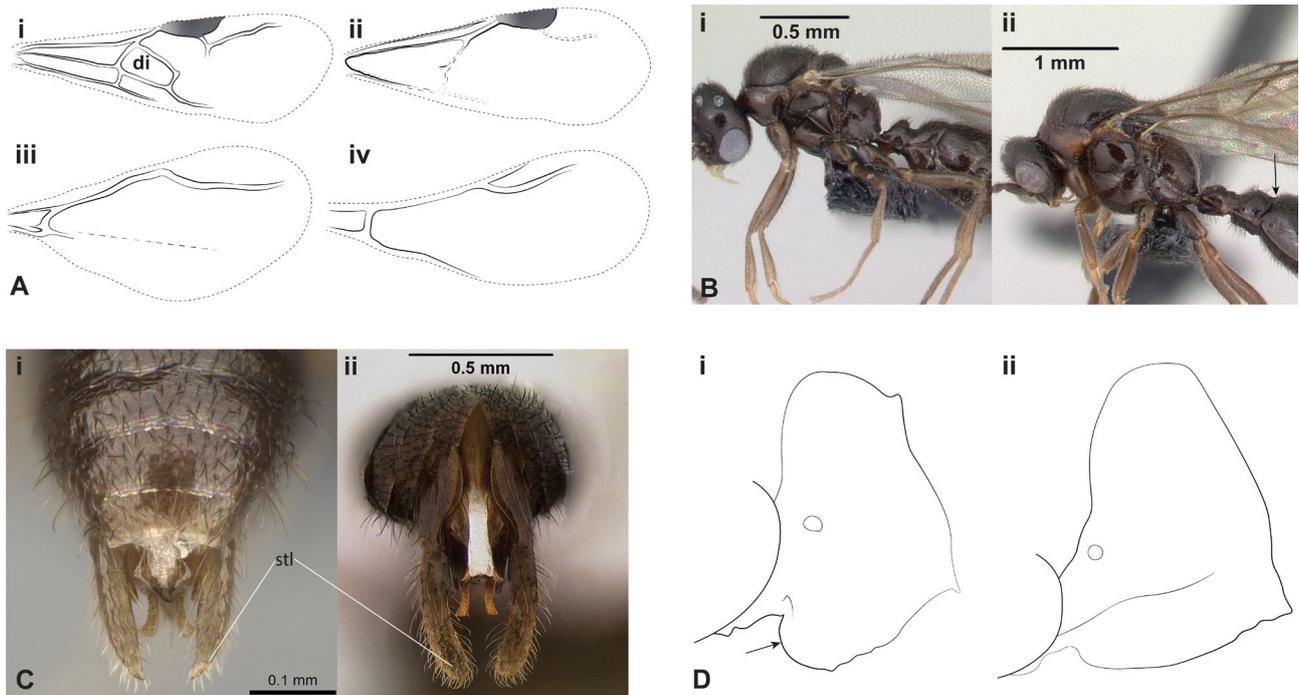


Fig. 9: (A) Male forewing venation across the Leptanillinae in (i) *Opamyрма hungvuong* (after YAMADA & al. 2020: Fig. 14), (ii) *Protanilla lini*, (iii) *Leptanilla zhg-my05*, and (iv) *Phaulomyrma javana*; (B) condition of abdominal segment III in (i) *Protanilla* TH02 (CASENT0128922; Erin Prado) and (ii) *Protanilla* TH03 (CASENT0119791; Erin Prado), with post-petiole in *Protanilla* TH03 marked; (C) posterior view of male genitalia in (i) *Protanilla* TH01 and (ii) *P. lini*; (D) profile of male petiole in (i) *Protanilla* zhg-vn01 and (ii) *Protanilla lini*, with ventral bulge of abdominal sternite II in *Protanilla* zhg-vn01 marked. 9B-Ci courtesy of AntWeb (www.AntWeb.org).

The first molecular study to include exemplars of both anomalomyrmine genera (BOROWIEC & al. 2019) recovered the sole sampled *Anomalomyrma* on a long branch nested deep within two well-resolved clades, both identified as *Protanilla*; and irrespective of dataset or statistical framework weakly recovered *Anomalomyrma boltoni* sister to the clade consisting of *Protanilla* TH03, *Protanilla* VN01, and *Protanilla* VN03. However, the ML and Bayesian UCE-based phylogenies presented herein recover *A. boltoni* as

sister to the major sampled clade of Anomalomyrmini, which is here found to include *P. lini*, with strong but sub-maximal support under ML inference (Fig. 1) and maximal support under Bayesian inference. Either result implies that the two anomalomyrmine genera could be synonymized, or *Protanilla* divided into at least two genera. Future phylogenomic work will explore the influence of different methods on inference of basal divergences within the Anomalomyrmini – in particular, the effects of including more UCE loci, and filtering those loci in order to compensate for systematic biases in the data.

Protanilla TH03 is conspicuously different from the remaining known anomalomyrmine males in the presence of a postpetiole (Fig. 9B) and apical maxillary palpomeres that are subequal in length (Fig. 10B); this is consistent with its position in a clade sister to that containing all other sequenced anomalomyrmine male material and subtended by a long branch. *Protanilla* TH03 is certainly not a male of *Anomalomyrma* under the present definition of that genus, as this undescribed male is embedded within a clade consisting of morphospecies known only from workers that clearly have little morphological affinity to *Anomalomyrma*, and among described anomalomyrmine species appear closely akin to *Protanilla bicolor* Xu, 2002 and *Protanilla gengma* Xu, 2011. The future status of *Anomalomyrma* will depend on scrutiny of morphological differences between the two major anomalomyrmine clades, with reference to both sexes. Sequencing of and

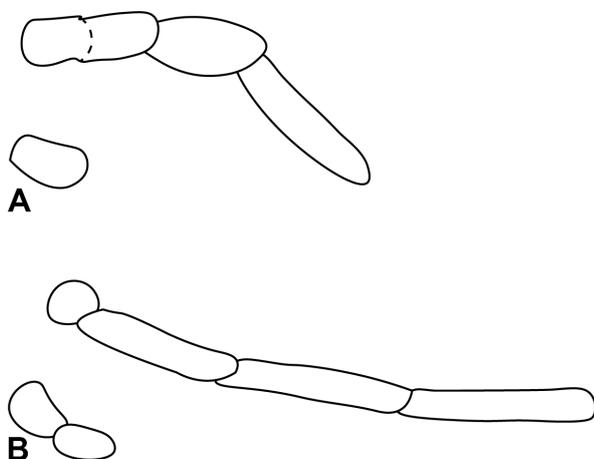


Fig. 10: Palpi of (A) *Protanilla* TH01 and (B) *Protanilla* TH03, after sketches by P. S. Ward.

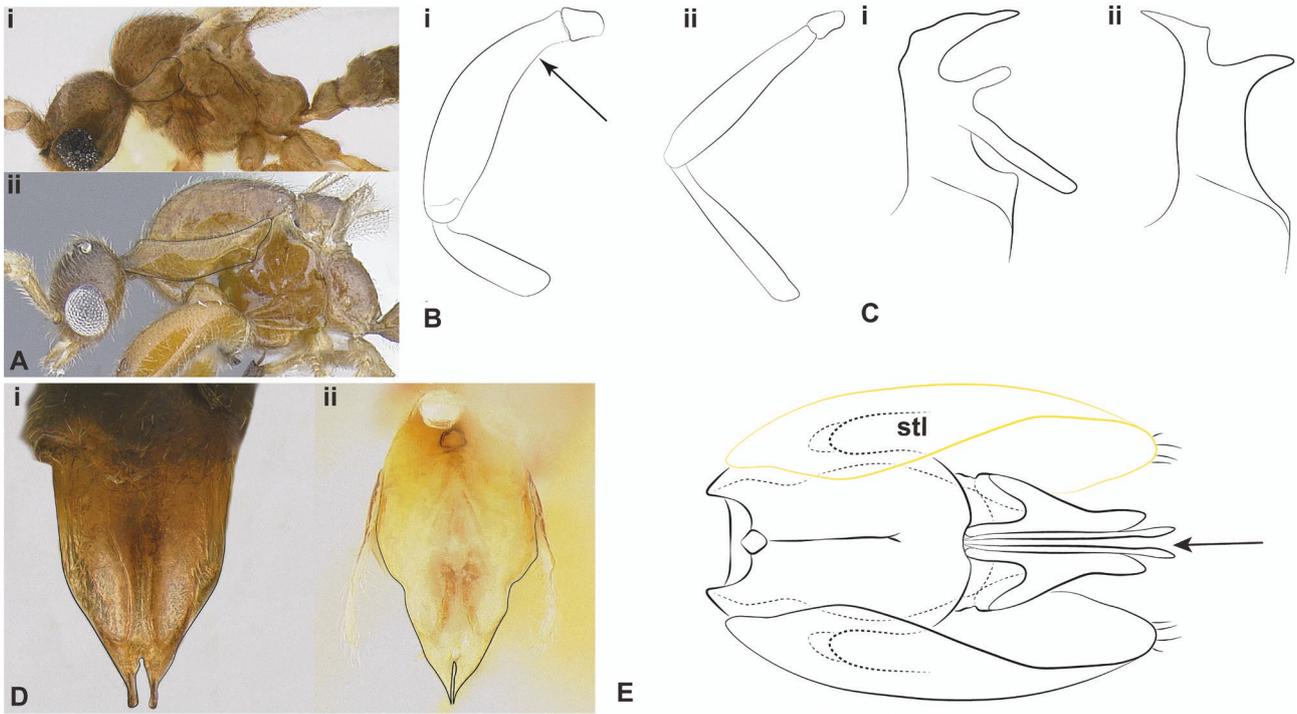


Fig. 11: (A) profile view of mesosoma in (i) *Yavnella zhg-bt01* and (ii) *Noonilla zhg-my04*, with pronotum marked in blue and mesoscutum marked in black; (B) male foreleg of (i) *Yavnella zhg-th01* and (ii) *Yavnella argamani*, with basal constriction of the profemur in *Yavnella zhg-th01* marked; (C) mesal view of volsella in (i) *Yavnella zhg-th01* and (ii) *Yavnella TH02* (after sketch by P.S. Ward); (D) posterodorsal view of penial sclerites in (i) *Yavnella argamani* (CASENT0235253) and (ii) *Yavnella cf. indica* (CASENT0106378); (E) ventral view of the male genitalia of *Leptanilla astylina* (after PETERSEN 1968: Fig. 3), with stylus in yellow and penial apex marked. Abbreviation: stl = stylus.

description of further anomalomyrmine males and worker material will clarify the best course for taxonomic revision.

Note on male morphospecies delimitation in *Protanilla*: While formally describing males of single species without description of males belonging to similar species is limiting, I conclude that it is warranted in this case. Based on phylogenomic data, the males described herein are indubitably conspecific with worker material collected in sympatry and identified as *P. lini*, justifying the description of the males of those species; doing so expands our holomorphological knowledge of *P. lini* (cf. TERAYAMA 2009, HSU & al. 2017).

We lack confident associations of the sexes in leptanilline species aside from *O. hungvuong*, *P. lini*, and *L. japonica*, but these species can be discriminated from other male morphospecies included in this study by means of the key that follows. Those undescribed morphospecies that were included in BOROWIEC & al. (2019) are renamed as needed according to provisional conclusions from this and other phylogenetic studies (see Tables 1 - 2). Some *Leptanilla* spp. have been described only from males (SANTSCHI 1907, 1908, WHEELER & WHEELER 1930, SMITH 1953, PETERSEN 1968, DLUSSKY 1969, BARONI URBANI 1977, KUGLER 1986), and these are included in the key below based upon published descriptions. Undescribed leptanilline material figured in previous publications but not yet sequenced is excluded from this key (PETERSEN 1968, BARONI URBANI 1977, OGATA & al. 1995, SCUPOLA & BALLARIN 2009).

Key to leptanilline male morphotaxa for which phylogenomic data are available and described species for which male morphology is known:

1. Discal cell present (Fig. 9Ai); parossiculus and lateropenite distinct.
- ...*Opamyрма hungvuong* YAMANE & al., 2008
- Discal cell absent (Fig. 9Aii-iv); if volsella discernible, parossiculus and lateropenite distinct or indistinct. 2
2. Pterostigma present (Fig. 9Ai-ii), wings never deciduous; ocellar tubercle absent..... 3
- Pterostigma absent (Fig. 9Aiii-iv), wings seldom deciduous; ocellar tubercle usually present. 7
3. Notauli present. 4
- Notauli absent. 5
4. Notauli scrobiculate; postpetiole present (Fig. 9Bii)..... ***Protanilla TH03***
- Notauli not scrobiculate; postpetiole absent (Fig. 9Bi). ***Protanilla TH02***
5. Styler (i.e., telomerale sensu SCHULMEISTER 2001) apex pointed (Fig. 9Ci). ***Protanilla TH01***
- Styler apex rounded (Fig. 9Cii). 6
6. Anterior face of abdominal sternite II nearly perpendicular to craniocaudal axis in profile view (Fig. 9Di); abdominal tergum III slightly narrower than IV in dorsal view (TII 70-77).
- ***Protanilla zhg-vn01***

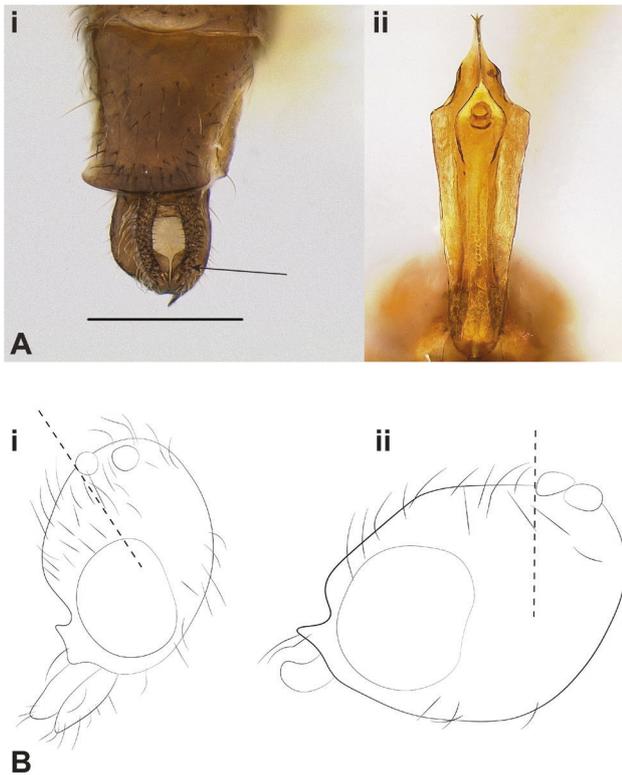


Fig. 12: (A) posterodorsal view of penial sclerites in (i) *Noonilla zhg-my02* and (ii) *Leptanilla zhg-my05* (CASENT0106432), with phallotrematic setae marked; (B) profile view of male head in (i) *Noonilla zhg-my04* and (ii) *Noonilla zhg-my01*. Abbreviations: di = discal cell; stl = stylus.

- Anterior face of abdominal sternite II gently sloping relative to craniocaudal axis (Fig. 9Dii); abdominal tergum III much narrower than IV in dorsal view (TII 50-55). ***Protanilla lini* TERAYAMA, 2009**
- 7. Propodeum concave in profile view; pronotum and mesoscutum not posteriorly prolonged (Fig. 11Ai). ... 8
- Propodeum not concave in profile view; mesoscutum and pronotum posteriorly prolonged (Fig. 11Aii). 17
- 8. Gonocoxae entirely fused medially; posterior margin of abdominal sternite IX with median extension. ***Yavnella* TH03**
- Gonocoxae partly to fully separate medially; posterior margin of abdominal sternite IX entire. 9
- 9. Ocelli absent (Fig. 6A). ***Yavnella zhg-bt01***
- Ocelli present (Fig. 6B). 10
- 10. Gonopodite (i.e., paramere sensu SCHULMEISTER 2001) longer than penial sclerites (i.e., penisvalvae). 11
- Gonopodite shorter than, or equal in length to, penial sclerites. 16
- 11. Profemur curved (Fig. 11Bi), constricted basally. .. 12
- Profemur not curved nor constricted basally (Fig. 10Bii). 13
- 12. Volsella bifid (Fig. 11Ci), ventral process bifurcated. ***Yavnella zhg-th01***

- Volsella bifid (Fig. 11Cii), ventral process entire. .
..... ***Yavnella* TH02**
- 13. Styler apex subtriangular, entire.
..... ***Yavnella* MM01**
- Styler apex tapering, entire or bifid. 14
- 14. Posterodorsal margin of gonopodite with vestiture of sparse setae; styler apex bifurcated.
..... ***Yavnella* TH08**
- Posterodorsal margin of gonopodite with vestiture of dense setae; styler apex entire. 15
- 15. Volsella bifid; mandible articulated to gena.
..... ***Yavnella* TH04**
- Volsella entire; mandible fused to gena.
..... ***Yavnella* TH06**
- 16. Internal margins of apical penial cleft distinctly separated (Fig. 11Di); posteroventral gonocoxal margin entire. ***Yavnella argamani* KUGLER, 1987**
- Internal margins of apical cleft of penial sclerites subparallel (Fig. 11Dii); posteroventral gonocoxal margin sinuate. 17
- 17. Color castaneous; posterior margin of compound eye linear in profile view (India: Kerala).
..... ***Yavnella indica* KUGLER, 1987**
- Color flavous to pallid; posterior margin of compound eye convex in profile view (Sri Lanka).
..... ***Yavnella cf. indica***
- 18. Dorsolateral carina present on propodeum.
..... ***Leptanilla palauensis* (M.R. SMITH, 1953)**
- Dorsolateral carina absent from propodeum. 19
- 19. Stylus lenticular in outline; penial sclerites with medial conjunctiva (Fig. 11E).
..... ***Leptanilla astyline* PETERSEN, 1968**
- Stylus not lenticular in outline; penial sclerites usually without medial conjunctiva. 20
- 20. Phallotreme surrounded with dense setae (Fig. 12Ai). 21
- Phallotreme bare (Fig. 12Aii). 24
- 21. Mandalus not extending to mandibular apex; anteromedian ocellus orthogonally dorsal to compound eye in profile view (Fig. 12Bi).
..... ***Noonilla copiosa* PETERSEN, 1968;**
Noonilla zhg-my04
- Mandalus extending to mandibular apex; anteromedian ocellus positioned posterodorsal to compound eye in profile view (Fig. 12Bii). 22
- 22. Stylus longer than gonocoxa (Fig. 13Ai).
..... ***Noonilla zhg-my01***
- Stylus shorter than, or subequal in length to, gonocoxa (Fig. 13Aii). 23
- 23. Penial apex entire. ***Noonilla zhg-my06***
- Penial apex cleft. ***Noonilla zhg-my02***
- 24. Dorsal propodeal face long, parallel to craniocaudal axis; protibial comb present. 25
- Dorsal propodeal face short, with propodeal outline in profile view convex; protibial comb absent. 29
- 25. Phallotreme at penial apex (Fig. 13Bi). 26
- Phallotreme basal to penial apex, anatomically ventral (Fig. 13Bii). 27

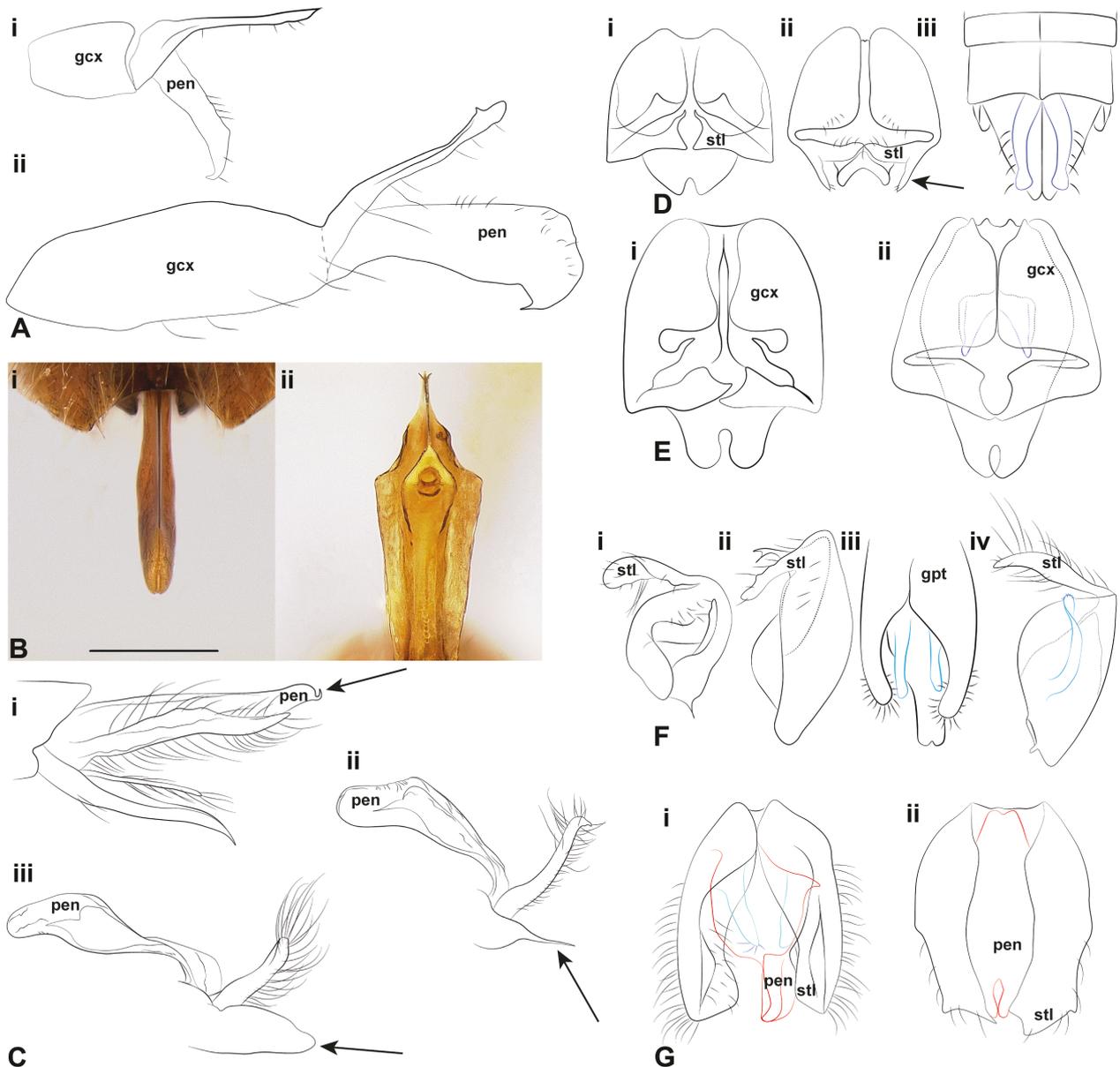


Fig. 13: (A) Profile view of male genitalia of (i) *Noonilla zhg-my01* and (ii) *Noonilla zhg-my02*, to scale; (B) posterior view of penial sclerites in (i) *Leptanilla zhg-my04* (CASENT0842553) and (ii) *Leptanilla zhg-my05* (CASENT0106432); (C) profile view of male genitalia (not to scale) in (i) *Leptanilla zhg-id01* (hook at penial apex marked), (ii) *Leptanilla zhg-my02*, and (iii) *Leptanilla zhg-my05*, basolateral gonocoxal laminae marked for Cii-iii; (D) ventral view of male genitalia in (i) *Leptanilla tenuis* (after SANTSCHI 1907: Fig. 1C), (ii) *Leptanilla bifurcata* (after KUGLER 1986: Fig. 8), and (iii) *Leptanilla santschii* (after WHEELER & WHEELER 1930: Fig. 2D); (E) ventral view of genitalia of (i) *Leptanilla tanit* (after SANTSCHI 1907: Fig. 2B) and (ii) *Leptanilla israelis* (after KUGLER 1986: Fig. 14); (F) ventral view of gonopodite in (i) *Leptanilla islamica* (after BARONI URBANI 1977: Fig. 39), (ii) *Leptanilla australis* (after BARONI URBANI 1977: Fig. 38), (iv) *Leptanilla africana* (after BARONI URBANI 1977: Fig. 37), and of the entire genitalia (iii) in *Leptanilla exigua* (after SANTSCHI 1908: Fig. 1A); (G) dorsal view of the male genitalia in (i) *Leptanilla alexandri* (after DLUSSKY 1969: Fig. 3), and (ii) *Leptanilla japonica* (after OGATA & al. 1995: Fig. 12), with penial sclerites marked in red. Volsellae marked in blue. Abbreviations: pen = penial sclerites; gcx = gonocoxa; stl = stylus.

- | | |
|---|-----------------------------------|
| 26. Penial sclerites dorsoventrally compressed at apex. | <i>Leptanilla zhg-my03</i> |
| – Penial sclerites lateromedially compressed at apex. | <i>Leptanilla zhg-my04</i> |
| 27. Stylus present, penial sclerites with recurved apical hook (Fig. 13Ci). | <i>Leptanilla zhg-id01</i> |
| – Stylus absent, penial sclerites without recurved apical hook (Fig. 13Cii-iii). | 28 |
| 28. Basolateral gonocoxal lamina subulate (Fig. 13Cii). | <i>Leptanilla zhg-my02</i> |
| – Basolateral gonocoxal lamina lanceolate (Fig. 13Ciii). | <i>Leptanilla zhg-my05</i> |
| 29. Mesoscutellum produced into recurved posterior process. | <i>Leptanilla zhg-th01</i> |
| – Mesoscutellum not produced into recurved posterior process. | 30 |

30. Stylus bifurcated. 31
 – Stylus entire. 38
31. Petiole without distinct dorsal node.
 ***Leptanilla minuscula* SANTSCHI, 1907**
 – Petiole with distinct dorsal node. 32
32. Ventromesal gonocoxal margin with sinuate process
 (Fig. 13Ei). ***Leptanilla tanit* SANTSCHI, 1907**
 – Ventromesal gonocoxal margin entire (Fig. 13Eii). 33
33. Styler apex with obtuse tooth subtending dorsal
 process. ***Leptanilla* GR02**
 – Styler apex lacking obtuse tooth subtending dorsal
 process. 34
34. Ventromedian margin of stylus excavated basal to
 apical furca. ***Leptanilla zhg-au02***
 – Ventromedian margin of stylus entire basal to
 apical furca. 35
35. Dorsal process of stylar apex acuminate.
 ***Leptanilla tenuis* SANTSCHI, 1907**
 – Dorsal process of stylar apex rounded. 36
36. Penial apex entire. ***Leptanilla* GR01**
 – Penial apex emarginate. 37
37. Internal margins of apical penial cleft distinctly
 separated, ventral stylar process narrower than
 dorsal process (Fig. 13Dii).
 ***Leptanilla bifurcata* KUGLER, 1987**
 – Internal margins of apical penial cleft adjacent,
 stylar processes subequal in breadth (Fig. 13Eii).
 ***Leptanilla israelis* KUGLER, 1987**
38. Stylus not tapered. 39
 – Stylus tapered. 42
39. Volsella with expanded apex (Fig. 13Diii).
 ***Leptanilla santschii* WHEELER & WHEELER, 1930**
 – Volsella (when visible) without expanded apex
 (Figs. 13Eii, Fiii-iv, Gi). 40
40. Stylus with expanded, rounded apex (Fig. 13Fi). ..
 ***Leptanilla islamica* BARONI URBANI, 1977**
 – Stylus with apex not expanded (Figs. 13Fii-iv,
 Gi-ii). 41
41. Penial outline attenuate in posterodorsal view
 (13Gi). ***Leptanilla alexandri* DLUSSKY, 1969**
 – Penial outline elliptic in posterodorsal view
 (Fig. 13Gii).
 ***Leptanilla japonica* BARONI URBANI, 1977**
42. Stylus ligulate in outline (Fig. 13Fiv).
 ***Leptanilla africana* BARONI URBANI, 1977**
 – Stylus not ligulate in outline. 43
43. Styler apex acuminate (Fig. 13Fii). 44
 – Styler apex digitate (Fig. 13Fiii). 45
44. Mesopleural sulcus traversing most of mesopleu-
 ron; abdominal sternite II without ventral projec-
 tion. ***Phaulomyrma javana***
WHEELER & WHEELER, 1930
 – Mesopleural sulcus traversing posterior 1/3 of
 mesopleuron; abdominal sternite II with ventral
 projection. ***Leptanilla zhg-bt01***
45. Penial sclerites broader than long.
 ***Leptanilla* GR03**
 – Penial sclerites longer than broad. 46

46. Stylus not articulated to gonocoxa.
 ***Leptanilla exigua* SANTSCHI, 1908**
 – Stylus articulated to gonocoxa. 47
47. Mesopleural sulcus present; Sc+R+Rs tubular.
 ***Leptanilla zhg-au01***
 – Mesopleural sulcus absent; Sc+R+Rs absent.
 ***Leptanilla australis* BARONI URBANI, 1977**

Description of *Protanilla lini* male

***Protanilla* TAYLOR, 1990**

Protanilla TAYLOR, 1990 in BOLTON 1990a: 279, Figs. 1-6. SINGAPORE. Type-species: *Protanilla rafflesi*, by original designation.

***Protanilla lini* TERAYAMA, 2009**

P. lini TERAYAMA, 2009: 126, Figs. 113 - 118 (worker). TAIWAN, Fusan: Taipei Prefecture, 18.III.2006.

Material examined (4 males): OKENT0027514. Japan, Okinawa Is.: Ogimi, Hentona High School (26.70134° N, 128.13156° E), 13 - 27.V.2016, 21 m elevation, leg. OKEON, SLAM trap (S0015), OK01355 (OIST).

OKENT0028803. Japan, Okinawa Is.: Nago, Nago Central Pk. (26.590068° N, 127.99402° E), 26.V - 9.VI.2016, 86 m elevation, leg. OKEON, SLAM trap (S0068), OK01516 (OIST).

OKENT0018456. Japan, Okinawa Is.: Naha, Sueyoshi Pk. (26.22831° N, 127.71600° E) 1 - 15.VII.2016, 65 m elevation, leg. OKEON, SLAM trap (S0057), OK01851 (OIST).

OKENT0011097. Japan, Okinawa Is.: Onna, OIST Campus Forest Site (26.48509° N, 127.84190° E), 17.VI. - 1.VII.2015, 107 m elevation, leg. OKEON, SLAM trap (S0008), OK00017 (OIST).

Male description: Head. In full-face view head slightly broader than long (CS 0.409 - 0.465), excluding compound eyes (Fig. 15A). Labrum reduced, lateromedially compressed, bare of apparent vestiture. Mandibles reduced, nub-like, edentate, articulated to cranium (“mdb” in Fig. 14); mandalus (“mdl” in Fig. 15C) large, covering entire anterodorsal mandibular surface in full-face view. Palpal formula assessed to be 4,1 *in situ*; maxillary palp

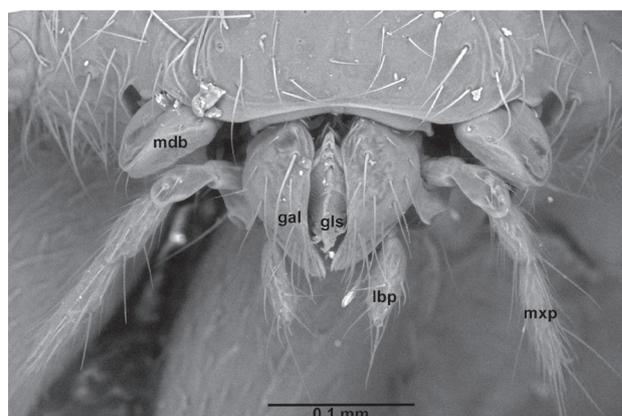


Fig. 14: Mouthparts of male *Protanilla lini*, full-face view. Abbreviations: mdb = mandible; gal = galea; gls = glossa; lbp = labial palp; mxp = maxillary palp.

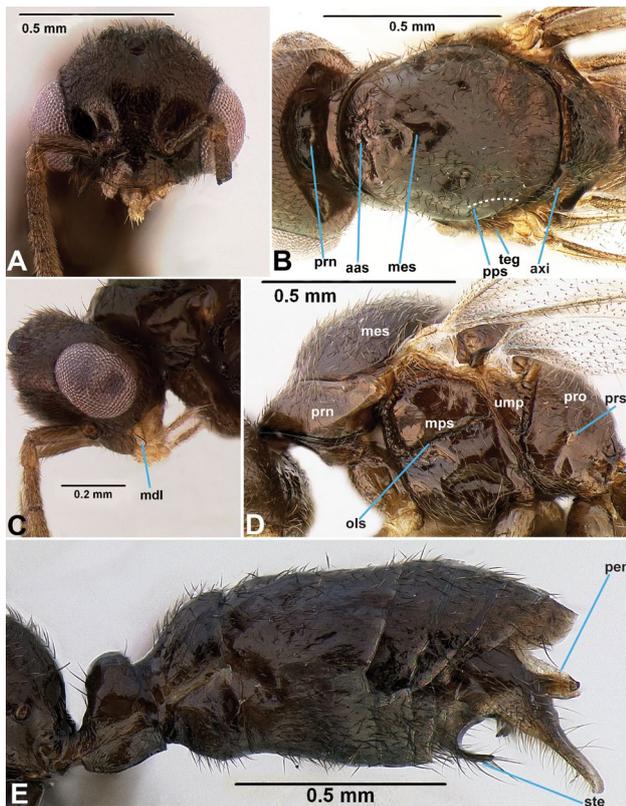


Fig. 15: *Protanilla lini* male. (A) full-face view of head (OKENT0018456); (B) dorsal view of mesosoma (OKENT0011097); (C) profile of head (OKENT0011097); (D) profile of mesosoma (OKENT0028803); (E) profile of metasoma (OKENT0011097). Abbreviations: prn = pronotum; aas = antero-admedian line; mes = mesonotum; pps = parapsidal line; teg = tegula; axi = axilla; mdl = mandalus; mps = mesopectus; ols = mesopleural sulcus; ump = upper metapleuron; pro = propodeum; prs = propodeal spiracle; ste = abdominal sternite IX; pen = penial sclerites.

(“mxp” in Fig. 14) extending past hypostomal margin, covered with dense setae, articulation between palpomeres 1 - 2 indistinct; labial palp (“lbp” in Fig. 14) short and robust, sparsely setose. Premental shield broadly truncate at apex. Galea (“gal” in Fig. 14) simple, sparsely setose, twice the length of mandible in full-face view. Clypeus with medial anteroposterior length about twice the diameter of the torulus, anterior margin entire, posterior margin not produced between toruli (Fig. 15A). Anterior tentorial pits situated directly anterior to antennal toruli, with no part of torular lobe extending anterad of anterior tentorial pit. Ocellar region bulging moderately, but ocelli not set on distinct tubercle; posterior ocellar line longer than lateral ocellar line. Occipital carina present dorsally, not enclosing occiput. Hypostomal carina present, not laminate. Compound eyes wider than long in profile view, slightly convex in full-face view, medial margin slightly convex, all margins entire (Fig. 15A, C). Antennae 13-merous; scape cylindrical, shorter (SL 0.150 - 0.173 mm) than width (EW 0.273 - 0.301 mm) or length (EL 0.206 - 0.234 mm) of compound eye (Fig. 15C); pedicel short, subcylindrical, dilated apically, 2 / 3 length of scape (LF1 0.075 - 0.091 mm); an-

tennomere 3 long, cylindrical, twice the length of pedicel (LF2 0.131 - 0.140 mm); antenna filiform, slightly longer than mesosoma.

Mesosoma. In profile view anterodorsal pronotal face linear, diagonal to craniocaudal axis at ~60° angle; anterior and posterior pronotal margins subparallel in profile view (Fig. 15D). Mesoscutum expanded dorsally, strongly convex (“mes” in Fig. 15B, D); mesoscutal width measured in dorsal view between pronotal lobes subequal to mesoscutal length measured in same view from anterior mesoscutal margin to transscutal line. Notauli absent. Antero-admedian line present on mesoscutum (“aas” in Fig. 15B). Parapsidal signa (“pps”, Fig. 15B) present, divergent, slightly impressed. Parascutal carina present. Axillae (“axi” in Fig. 15B) small and well-separated in dorsal view, anteroposteriorly expanded laterally, lateral faces concave. Preaxilla present, impressed. Axillula (“axu” in Fig. 16) impressed into shallow trough; axillular line indistinct. Mesoscutellum longer than tall, dorsum lower than that of mesoscutum, posterodorsal face of mesoscutellum (“msd” in Fig. 16) convex and without posterodorsal process(es); mesoscutellar arm (“msa” in Fig. 16) strongly elevated. Metascutellum small and anteroposteriorly narrow, extending posterior to mesoscutellum in dorsal view (“met” in Fig. 16). Metanotal trough deeply excavated, with coarse longitudinal sulci; metascutellar arm (“mta” in Fig. 15) moderately elevated. Mesopleural sulcus (“ols”) bisecting mesopectus (“mps”) (Fig. 15D). Longitudinal metapleurale sulcus absent. Upper metapleuron (“ump” in Figs. 13 - 14) distinct from propodeum, constricted ventrally in profile view. Lower metapleuron fused insensibly to propodeum. Metapleurale gland absent. Propodeum (“pro” in Figs. 15D - 16) parabolic in profile view, with narrow distinct dorsal face; propodeal spiracle (“prs” in Figs. 15D - 16) circular, facing posteriorly, slightly more adjacent to propodeal foramen than to metapleuron; propodeal lobe absent. All pairs of legs with similar proportions; procoxa without anteroventral transverse carina; protrochanters twice as long as wide; profemur not markedly constricted at base, not incrassate, lacking ectoventral flange at apex, carina absent from mesal face; femora moderately anteroposteriorly compressed; dorsoventral protibial width greatest at apex, apex not dorsoventrally flattened; ventral protibial face without distinct margins, convex in cross-section. Mesotibial spur not apparent; pro- and metatibial spurs conspicuous.

Forewing. Tegula small (“teg” in Fig. 15B). Membrane hyaline. Venation of Ogata Type IVb (Fig. 17A). C, Sc + R + Rs, Rf, Mf1, and 1A tubular; M + Cu nebulous at juncture with *cu-a*, spectral and disappearing basally. *Cu-a* with weakening adjacent to 1A. Pterostigma present, heavily infuscated, with all enclosing abscissae tubular, although Rf with weakening basal to pterostigma. 2s-rs + Rs + 4 - 6 tubular and spectral apically, not reaching costal margin (Fig. 17A).

Hindwing. Membrane hyaline. Four distal hamuli present (“ham” in Fig. 17B). Venation reduced: R+Rs tubular, extending < 1 / 5 of distance along costal margin;

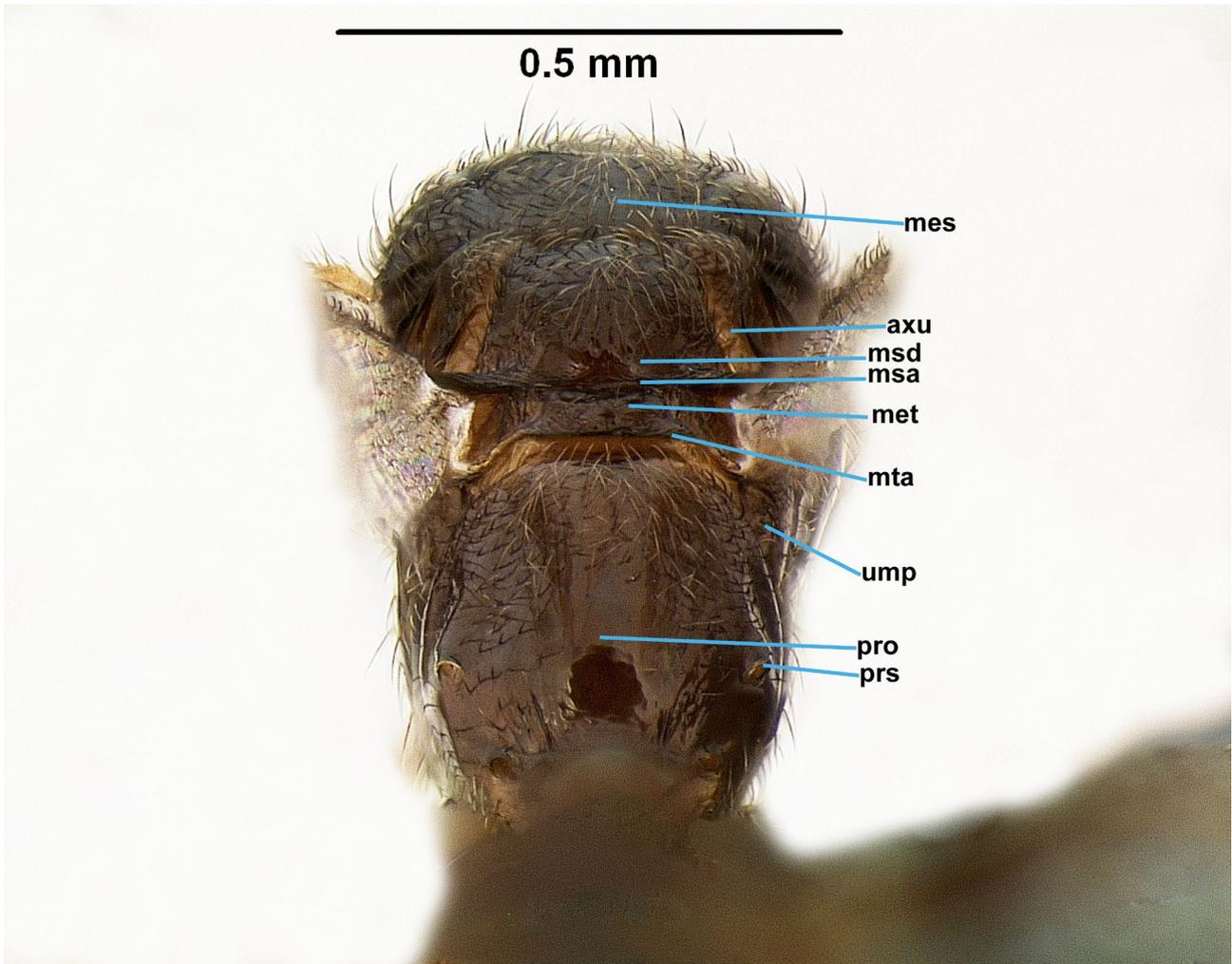


Fig. 16: Male mesosoma of *Protanilla lini* (OKENT0028803), posterior view. Abbreviations: mes = mesonotum; axu = axillula; msd = mesoscutellar disc; msa = mesoscutellar arm; ump = upper metapleuron; pro = propodeum; prs = propodeal spiracle.

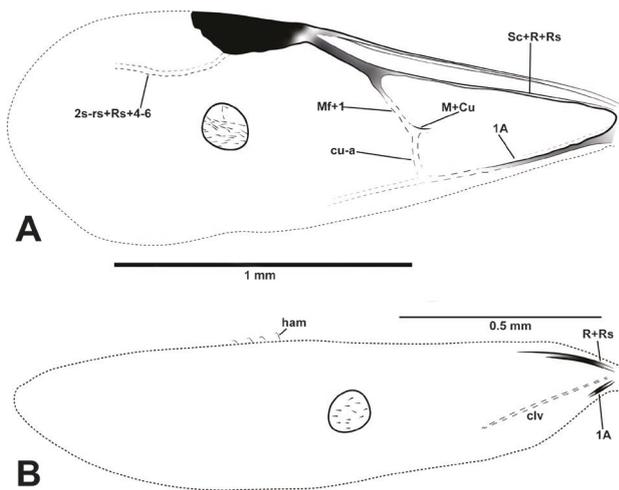


Fig. 17: (A) Forewing and (B) hindwing of *Protanilla lini*. Abbreviations: ham = hamuli; clv = clavus.

1A spectral, not extending to anal margin, one quarter of R+Rs length. Jugum absent. Clavus ("clv" in Fig. 17B) weakly developed.

Metasoma. Petiole (Fig. 18) anteroposteriorly compressed (DPW 0.164 - 0.199 mm, PTL 0.201 - 0.242 mm), without peduncle, lateral faces subparallel in dorsal view; posterior petiolar foramen elevated dorsad anterior petiolar foramen; tergal spiracle present, situated anteriorly on petiole; longitudinal carinulae of tergum II absent from petiole; petiolar tergite and sternite fused, delimited by faint longitudinal suture ("ls" in Fig. 18) visible in ventral profile view; petiolar tergite with pronounced dorsal node, with dorsal face of node slightly convex; petiolar sternite lateromedially compressed into process occupying the anterior 2 / 3 of petiole length. Pretergite III with lateral margins diverging dorsally; presternite III with lateral margins converging ventrally; medial anteroposterior length of pretergite III greater than that of presternite III. Dorsomedial length of helcium 1 / 3 × that of petiole; helcium axial (Fig. 15E). Abdominal segment III distinctly narrower than IV in dorsal view (TW3 0.247 - 0.321 mm, TW4 0.487 - 0.579 mm), with margins of tergite III subparallel in dorsal view; sternite III convex, without prora. Presclerites of abdominal segment IV visible with girdling constriction present but indistinct; presclerites of abdominal

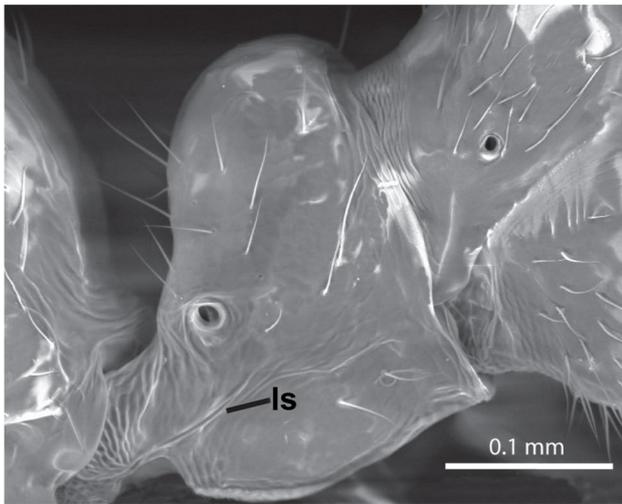


Fig. 18: Male petiole of *Protanilla lini*, profile view. Abbreviation: ls = longitudinal suture.

segments V - VIII inconspicuous. Abdominal segments III-VIII without tergosternal fusion. Abdominal tergite VIII broader than long, posterior margin somewhat laminate. Abdominal sternite IV moderately convex medially; abdominal sternites IV-VIII unmodified, not convex; all sternites visible *in situ*. Posterior margin of sternite VIII entire and unmodified. Abdominal sternite IX with margin extended into posteroventral process ("ste" in Figs. 15E, 19B, D), its anterodorsal surface concave, tip rounded, three times longer than visible lateral length of sternite IX; posterior margin of sternite IX lateral to process with rounded laminae.

Genitalia. Pygostyles absent. Gonopodites partially articulated, with faint ventral articulation discernible in profile view. Gonocoxae ("gcx" in Fig. 19A, D) glabrous, somewhat dorsoventrally compressed; distinct from one another along entire ventromedial length; posteroventral marginal laminae absent. Styli ("stl" in Fig. 19A, D) not compressed at base, dorsoventrally compressed towards apex with distinct ectal and dorsal faces, all stylar surfaces with posteriorly directed setae, dorsal face slightly convex, medial margin of stylus with dorsal carina along basal 2 / 5 of stylar length; stylar apex blunt. Parossiculus ("prs" in Fig. 19A - B, D) lateromedially compressed towards apex, margins rounded; lateropenite ("ltp" in Fig. 19A - B, D) extending posterad parossiculus, ectal surfaces convex, lateropenital apex ectally recurved. Penial sclerites ("pen" in Fig. 19A - B, D) lateromedially compressed, ectal surfaces convex; medial conjunctiva extending from gonocoxae to penial apex; dorsal margins of valviceps ("vlv" in Fig. 19A) extended into triangular processes, tips curving laterally; ventral margins of valviceps ventrally extended into triangular processes.

Sculpturation. Sculpturation weak to lacking on most sclerites. Fine piligerous punctae present on head, femora, and tibiae; mesosomal and metasomal piligerous punctae, where present, coarser; punctae on mesosoma and petiole sparse by comparison to those on head, ab-

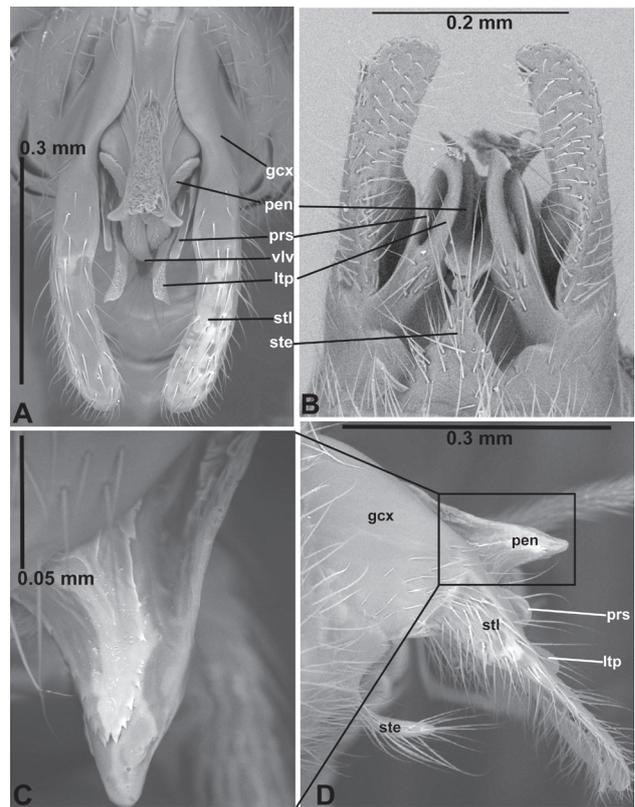


Fig. 19: Male genitalia of *Protanilla lini* (OKENT0011097). (A) posterodorsal aspect; (B) ventral aspect; (C) ectal aspect of valviceps; (D) profile view of genitalia and abdominal sternite IX. Abbreviations: gcx = gonocoxa; pen = penial sclerites; prs = parossiculus; vlv = valviceps; ltp = lateropenites; stl = stylus; ste = abdominal sternite IX.

dominal segments III - IX, and limbs; piligerous punctae absent from propleuron, pronotum, and mesopectus. Subalar areas of upper mesopectus and upper metapleuron intricately, confusedly striolate; metanotal trough porcate; sclerites otherwise glabrous. Dorso-anterior mesopectal margin, mesopleural suture, and anterior margin of metapectal-propodeal complex scrobiculate. Irregular denticle-like microsculpture on dorso-ectal (Fig. 19C) and posteroventral faces of valviceps.

Coloration. Coloration dark brownish-gray with pallid highlights throughout. Axillulae and sulci of metanotal trough pale. Lateropenites moderately to strongly castaneous.

Setation. Vestiture coarse overall, with most somal and appendicular setae short (~30 μ m) to moderate (~80 μ m) in length, suberect to decumbent, variable among setae on given sclerites. Pedicel and flagellum densely covered with short decumbent or appressed setae, with longer subdecumbent setae scattered over surface of antennomeres. Ectal pro- and metacoxal surfaces bare; posterior metacoxal surfaces bare; tarsi covered with vestiture like that present on pedicel and flagellum. Head, scape, and mesosoma with moderately dense setae; prosternite, antero-alar region of pronotum, and upper metapleuron with setae sparse, surfaces of these sclerites being almost bare.

Wings covered with short subdecumbent setae. Petiolar setae dense only on anterior face of petiolar node; abdominal segment II otherwise largely bare. Remainder of metasoma covered with dense setae; setal length gradually increasing posteriorly, with those on abdominal sternites VII - IX especially long (0.10 - 0.13 mm; ranges given representing inter-specimen variation in maximum length) and recurved, this tendency being most pronounced on posteroventral median process of sternite IX. Genital setae restricted to stylus and basivolsella, except for some basodorsad the gonopodital articulation. Some dorsal stylar setae unusually long (~100 - 115 μ m) and recurved.

Conclusions

Ant systematics generally relies upon the worker caste to the exclusion of males: short-lived, male ants are less likely to be collected than their female counterparts, and male morphology has only occasionally (e.g., WILD 2007, LA POLLA & al. 2012, BARDEN & al. 2017) been found to contain phylogenetic signal not already provided by workers. The Leptanillinae are unusual in that in their case this acquisition bias is reversed, necessitating focus upon male specimens. By describing a male that is confirmed by phylogenomic inference to belong to the Anomalomyrmini and to be conspecific with a *P. lini* worker, our knowledge of male leptanilline morphology is expanded without propagating the parallel taxonomy that has dogged this group since the description of the first putative males; moreover, by providing male-based definitions of the three main leptanilline lineages, male morphology is now grounded in phylogeny. Future work will use phylogenetic inference from molecular data and male morphology for the purpose of delimiting subclades of the tribe Leptanillini, and so resolve the status of the many taxa therein that are known only from males.

Acknowledgments

I first must thank Masashi Yoshimura and Evan Economo for their generosity in loaning the male specimens of *Protanilla lini* that are here described, along with conspecific worker material. The generosity of José María Gómez Durán, Jadranka Rota (MZLU), Debbie Jennings (ANIC), Kevin Williams (CSCA), and Brian Fisher (CASC) in loaning material for this paper is also greatly appreciated; thanks are also extended to Bui Tuan Viet, Katsuyuki Eguchi, Michael Ohl, Christian Rabeling, Michael Sharkey, and Masashi Yoshimura, who provided material for BOROWIEC & al. (2019) that is also used here. The help and advice of Michael Branstetter was indispensable in upload of raw reads to the Sequence Read Archive. Jill Oberski, Matt Prebus, Antoine Abrieux, Chris Pagan, and Lam-Tung Nguyen are all to be thanked for their help in acquiring and interpreting the sequence data obtained in this study. Matt Prebus' advice in interpreting ExaBayes output is particularly appreciated. I am grateful to Mia Lippey for creating line drawings, both from images and from existing figures; I also thank Jason Bond for allowing me the use of a Hitachi TM4000. Lastly, I must thank my lab-mate Brendon Boud-

inot and adviser Phil Ward, along with two anonymous reviewers, for their feedback on the descriptive content and overall layout of this paper. This research was supported by the University of California, Davis and by National Science Foundation grant DEB-1932405 to P.S. Ward.

References

- ABERER, A.J., KOBERT, K. & STAMATAKIS, A. 2014: ExaBayes: massively parallel Bayesian inference for the whole-genome era. – *Molecular Biology and Evolution* 31: 2553-2556.
- BANKEVICH, A., NURK, S., ANTIPOV, D., GUREVICH, A.A., DVORKIN, M., KULIKOV, A.S., LESIN, V.M., NIKOLENKO, S.I., PHAM, S., PRJIBELSKI, A.D., PYSHKIN, A.V., SIROTKIN A.V., VYAHHI, N., TESLER, G., ALEKSEYEV, M.A. & PEVZNER, P.A. 2012: SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. – *Journal of Computational Biology* 19: 455-477.
- BARDEN, P., BOUDINOT, B. & LUCKY, A. 2017: Where fossils dare and males matter: Combined morphological and molecular analysis untangles the evolutionary history of the ant genus *Leptomyrma* MAYR (Hymenoptera: Dolichoderinae). – *Invertebrate Systematics* 31: 765-780.
- BARONI URBANI, C. 1977: Materiali per una revisione della sottofamiglia Leptanillinae EMERY (Hymenoptera: Formicidae). – *Entomologica Basiliensia* 2: 427-488.
- BARONI URBANI, C. & DE ANDRADE, M.L. 2006: A new *Protanilla* TAYLOR, 1990 (Hymenoptera: Formicidae: Leptanillinae) from Sri Lanka. – *Myrmecologische Nachrichten* 8: 45-47.
- BASIBUYUK, H.H. & QUICKE, D.L.J. 1997: Hamuli in the Hymenoptera (Insecta) and their phylogenetic implications. – *Journal of Natural History* 31: 1563-1585.
- BILLEN, J., BAUWELEERS, E., HASHIM, R. & ITO, F. 2013: Survey of the exocrine system in *Protanilla wallacei* (Hymenoptera: Formicidae). – *Arthropod Structure and Development* 42: 173-183.
- BOLTON, B. 1990a: The higher classification of the ant subfamily Leptanillinae (Hymenoptera: Formicidae). – *Systematic Entomology* 15: 267-282.
- BOLTON, B. 1990b: Abdominal characters and status of the cerapachyine ants (Hymenoptera: Formicidae). – *Journal of Natural History* 24: 53-68.
- BOLTON, B. 1994: Identification guide to the ant genera of the world. – Harvard University Press, Cambridge, MA, 222 pp.
- BOLTON, B. 2020: An online catalog of the ants of the world. – <<https://antcat.org>>, retrieved on 25 September 2020.
- BOROWIEC, M.L. 2016: AMAS: a fast tool for alignment manipulation and computing of summary statistics. – *PeerJ* 4: art. e1660.
- BOROWIEC, M.L., RABELING, C., BRADY, S.G., FISHER, B.L., SCHULTZ, T.R. & WARD, P.S. 2019: Compositional heterogeneity and outgroup choice influence the internal phylogeny of the ants. – *Molecular Phylogenetics & Evolution* 134: 111-121.
- BOROWIEC, M.L., SCHULZ, A., ALPERT, G.D. & BAÑAR, 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.
- 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 & Development* 47: 563-613.

- BRANSTETTER, M.G., LONGINO, J.T., WARD, P.S. & 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 and Evolution* 8: 768-776.
- CARAVAS, J. & FRIEDRICH, M. 2012: Shaking the Diptera tree of life: performance analysis of nuclear and mitochondrial sequence data partitions. – *Systematic Entomology* 38: 93-103.
- CASTRESANA, J. 2000: Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. – *Molecular Biology and Evolution* 17: 540-552.
- CHEN, W.-J., KOCH, M., MALLATT, J.M. & LUAN, Y.-X. 2014: Comparative analysis of mitochondrial genomes in Diplura (Hexapoda, Arthropoda): taxon sampling is crucial for phylogenetic inferences. – *Genome Biology and Evolution* 6: 105-120.
- CHEN, Z.-L., SHI, F.-M. & ZHOU, S.-Y. 2017: First record of the monotypic genus *Opamyрма* (Hymenoptera: Formicidae) from China. – *Far Eastern Entomologist* 335: 7-11.
- CHEMNOMOR, O., VAN HAESELER, A. & MINH, B.Q. 2016: Terrace aware data structure for phylogenomic inference from supermatrices. – *Systematic Biology* 65: 997-1008.
- DLUSSKY, D. 1969: First finding of an ant from the subfamily Leptanillinae (Hymenoptera: Formicidae) in the USSR. – *Zoologicheskii Zhurnal* 48: 1666-1671. (in Russian)
- EMERY, C. 1870: Studi mirmecologici. – *Bullettino della Società Entomologica Italiana* 2: 193-201.
- EMERY, C. 1910: Hymenoptera. Fam. Formicidae. Subfam. Dorylinae. – *Genera Insectorum* 102: 1-34.
- FAIRCLOTH, B.C. 2013: Illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming. – <<http://dx.doi.org/10.6079/J9ILL>>, retrieved on 2 November 2020.
- FAIRCLOTH, B.C. 2016: PHYLUCE is a software package for the analysis of conserved genomic loci. – *Bioinformatics* 32: 786-788.
- GRABHERR, M. G., HAAS, B. J., YASSOUR, M., LEVIN, J. Z., THOMPSON, D. A., AMIT, I., ADICONIS, X., FAN, L., RAYCHOWDHURY, R., ZENG, Q., CHEN, Z., MAUCALI, E., HACOEN, N., GNIRKE, A., RHIND, N., DI PALMA, F., BIRREN, B.W., NUSBAUM, C., LINDBLAD-TOH, K., FRIEDMAN, N. & REGEV, A. 2011: Full-length transcriptome assembly from RNA-seq data without a reference genome. – *Nature Biotechnology* 15: 644-652.
- HARRIS, R.A. 1979: A glossary of surface sculpturing. – *Occasional Papers in Entomology* 28: 1-31.
- HOANG, D.P., CHEMNOMOR, O., VON HAESELER, A., MINH, B.Q. & VINH, L.S. 2018: UFBoot2: improving the ultrafast bootstrap approximation. – *Molecular Biology and Evolution* 35: 518-522.
- HSU, P.-W., HSU, F.-C., HSIAO, Y. & LIN, C.-C. 2017: Taxonomic notes on the genus *Protanilla* (Hymenoptera: Formicidae: Leptanillinae) from Taiwan. – *Zootaxa* 4268: 117-130.
- IMAI, H.T., KIHARA, A., KONDOH, M., KUBOTA, M., KURIBAYASHI, S., OGATA, K., ONOYAMA, K., TAYLOR, R.W., TERAYAMA, M., TSUKI, Y., YOSHIMURA, M. & UGAWA, Y. 2003: *Ants of Japan*. – Gakken, Tokyo, 224 pp.
- KAINER, D. & LANFEAR, R. 2015: The effects of partitioning on phylogenetic inference. – *Molecular Biology and Evolution* 32: 1611-1627.
- KALYAANAMORTHY, S., MINH, B.Q., WONG, T.K.F., VON HAESELER, A. & JERMIIN L.S. 2017: ModelFinder: fast model selection for accurate phylogenetic estimates. – *Nature Methods* 14: 587-589.
- KATO, K. & TOH, H. 2008: Recent developments in the MAFFT multiple sequence alignment program. – *Briefings in Bioinformatics* 9: 286-298.
- KELLER, R.A. 2011: A phylogenetic analysis of ant morphology (Hymenoptera: Formicidae) with special reference to the poneromorph subfamilies. – *Bulletin of the American Museum of Natural History* 355: 1-90.
- KÜCK, P., HITA GARCIA, F., MISOF, B. & MEUSEMANN, K. 2011: Improved phylogenetic analyses corroborate a plausible position of *Martialis heureka* in the ant tree of life. – *Public Library of Science One* 6: art. 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.
- LANFEAR, R., CALCOTT, B., KAINER, D., MAYER, C. & STAMATAKIS, A. 2014: Selecting optimal partitioning schemes for phylogenomic datasets. – *BioMed Central Evolutionary Biology* 14: art. 82.
- LAPOLLA, J.L., KALLAL, R.J. & BRADY, S.G. 2012: A new ant genus from the Greater Antilles and Central America, *Zatania* (Hymenoptera: Formicidae), exemplifies the utility of male and molecular character systems. – *Systematic Entomology* 37: 200-214.
- LÓPEZ, F., MARTÍNEZ, M.D. & BARANDICA, J.M. 1994: Four new species of the genus *Leptanilla* (Formicidae: Leptanillinae) from Spain: relationships to other species and ecological issues. – *Sociobiology* 24: 179-212.
- MASUKO, K. 1990: Behavior and ecology of the enigmatic ant *Leptanilla japonica* BARONI URBANI (Hymenoptera: Formicidae: Leptanillinae). – *Insectes Sociaux* 37: 31-57.
- MILLER, M.A., PFEIFFER, W. & SCHWARTZ, T. 2010: Creating the CIPRES Science Gateway. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*. – Institute of Electrical and Electronics Engineers, Piscataway, pp. 1-8.
- MOREAU, C.S. & BELL, C.D. 2013: Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. – *Evolution* 67: 2240-2257.
- MOREAU, C.S., BELL, C.D., VILA, R., ARCHIBALD, S.B. & PIERCE, N.E. 2006: Phylogeny of the ants: diversification in the age of angiosperms. – *Science* 312: 101-104.
- NGUYEN, L.-T., SCHMIDT, H.A., VON HAESELER, A. & MINH, B.Q. 2015: IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. – *Molecular Biology and Evolution* 32: 268-274.
- OGATA, K., TERAYAMA, M. & 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.
- PETERSEN, B. 1968: Some novelties in presumed males of Leptanillinae (Hym.: Formicidae). – *Entomologiske Meddelelser* 36: 577-598.
- RABELING, C., BROWN, J.M. & VERHAAGH, M. 2008: Newly discovered sister lineage sheds light on early ant evolution. – *Proceedings of the National Academy of Sciences of the United States of America* 105: 14913-14917.
- RAMBAUT, A., DRUMMOND, A. J., XIE, D., BAELE, G. & SUCHARD, M.A. 2018: Posterior summarization in Bayesian phylogenetics using Tracer 1.7. – *Systematic Biology* 67: 901-904.
- SCHULMEISTER, S. 2001: Functional morphology of the male genitalia and copulation in lower Hymenoptera, with special emphasis on the Tenthredinoidea s. str. (Insecta, Hymenoptera, 'Symphyta'). – *Acta Zoologica* 82: 331-349.
- SCUPOLA, A. & BALLARIN, R. 2009: The genus *Leptanilla* EMERY, 1870 in Sicily (Hymenoptera: Formicidae). – *Myrmecological News* 12: 129-132.

- SANTSCHI, F. 1907: Fourmis de Tunisie capturées en 1906. – *Revue Suisse de Zoologie* 15: 305-334.
- SANTSCHI, F. 1908: Nouvelles fourmis de l'Afrique du Nord (Égypte, Canaries, Tunisie). – *Annales de la Société Entomologique de France* 77: 517-534.
- SIMON, S. & HADRY, H. 2013: A comparative analysis of complete mitochondrial genomes among Hexapoda. – *Molecular Phylogenetics and Evolution* 69: 393-403.
- SMITH, M.R. 1953: A new species of *Probolomyrmex*, and the first description of a *Probolomyrmex* male (Hymenoptera: Formicidae). – *Journal of the New York Entomological Society* 61: 127-129.
- TAGLIACOLLO, V.A. & LANFEAR, R. 2018: Estimating improved partitioning schemes for ultraconserved elements. – *Molecular Biology and Evolution* 35: 1798-1811.
- TALAVERA, G. & VILA, R. 2011: What is the phylogenetic signal limit from mitogenomes? The reconciliation between mitochondrial and nuclear data in the Insecta class phylogeny. – *BioMed Central Evolutionary Biology* 11: art. 315.
- TERAYAMA, M. 2009. A synopsis of the family Formicidae of Taiwan (Insecta, Hymenoptera). – *Bulletin of Kanto Gakuen University, Liberal Arts* 17: 81-266.
- WARD, P.S. 1985: The Nearctic species of the genus *Pseudomyrmex* (Hymenoptera: Formicidae). – *Quaestiones Entomologicae* 21: 209-246.
- WARD, P.S. & FISHER, B.L. 2016: Tales of dracula ants: the evolutionary history of the ant subfamily Amblyoponinae (Hymenoptera: Formicidae). – *Systematic Entomology* 41: 683-693.
- WHEELER, G.C. & WHEELER, E.W. 1930: Two new ants from Java. – *Psyche* 37: 193-201.
- WILD, A.L. 2007: Taxonomic revision of the ant genus *Linepithema* (Hymenoptera: Formicidae). – *University of California Publications in Entomology* 126: 1-151.
- WILSON, E.O. 1955: A monographic revision of the ant genus *Lasius*. – *Bulletin of the Museum of Comparative Zoology* 113: 1-201.
- YAMADA, A., NGUYEN, D.D. & EGUCHI, K. 2020: Unveiling the morphology of the Oriental rare monotypic ant genus *Opamyрма* 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.
- YAMANE, S., BUI, T.V. & EGUCHI, K. 2008: *Opamyрма hungvuong*, a new genus and species of ant related to *Apomyрма* (Hymenoptera: Formicidae: Amblyoponinae). – *Zootaxa* 1767: 55-63.
- YANG, Z. 1996: Among-site rate variation and its impact on phylogenetic analyses. – *Trends in Ecology & Evolution* 11: 367-372.
- YODER, M.J., MIKO, I., SELTMANN, K.C., BERTONE, M.A. & DEANS, A.R. 2010: A gross anatomy ontology for Hymenoptera. – *Public Library of Science One* 5: art. e15991.
- ZHOU, X., FRANDSEN, P.B., HOLZENTHAL, R.W., BEET, C.R., BENNETT, K.R., BLAHNIK, R.J., BONADA, N., CARTWRIGHT, D., CHULUUNBAT, S., COCKS, G.V., COLLINS, G.E., DEWAARD, J., DEAN, J., FLINT, O.S., HAUSMANN, A., HENDRICH, L., HESS, M., HOGG, I.D., KONDRATIEFF, B.C., MALICKY, H., MILTON, M.A., MORINIÈRE, J., MORSE, J.C., MWANGI, F.N., PAULS, S.U., GONZALEZ, M.R., RINNE, A., ROBINSON, J.L., SALOKANNEL, J., SHACKLETON, M., SMITH, B., STAMATAKIS, A., STCLAIR, R., THOMAS, J.A., ZAMORA-MUÑOZ, C., ZIESMANN, T. & KJER, K.M. 2016: The Trichoptera barcode initiative: a strategy for generating a species-level Tree of Life. – *Philosophical Transactions of the Royal Society B-Biological Sciences* 371: art. 20160025.