RESEARCH ARTICLE



# A stable phylogenomic classification of Travunioidea (Arachnida, Opiliones, Laniatores) based on sequence capture of ultraconserved elements

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# Abstract

Molecular phylogenetics has transitioned into the phylogenomic era, with data derived from next-generation sequencing technologies allowing unprecedented phylogenetic resolution in all animal groups, including understudied invertebrate taxa. Within the most diverse harvestmen suborder, Laniatores, most relationships at all taxonomic levels have yet to be explored from a phylogenomics perspective. Travunioidea is an early-diverging lineage of laniatorean harvestmen with a Laurasian distribution, with species distributed in eastern Asia, eastern and western North America, and south-central Europe. This clade has had a challenging taxonomic history, but the current classification consists of ~77 species in three families, the Travuniidae, Paranonychidae, and Nippononychidae. Travunioidea classification has traditionally been based on structure of the tarsal claws of the hind legs. However, it is now clear that tarsal claw structure is a poor taxonomic character due to homoplasy at all taxonomic levels. Here, we utilize DNA sequences derived from capture of ultraconserved elements (UCEs) to reconstruct travunioid relationships. Data matrices consisting of 317–677 loci were used in maximum likelihood, Bayesian, and species tree analyses. Resulting phylogenies recover four consistent and highly supported clades; the phylogenetic position and taxonomic status of the enigmatic genus *Yuria* is less certain. Based on the resulting phylogenies, a revision of Travunioidea is proposed, now consisting of the Travuniidae, Cladonychiidae, Paranonychidae (Nippononychidae is synonymized), and the new family Cryptomastridae Derkarabetian & Hedin, **fam. n.**, diagnosed here. The phylogenetic utility and diagnostic features of the intestinal complex and male genitalia are discussed in light of phylogenomic results, and the inappropriateness of the tarsal claw in diagnosing higher-level taxa is further corroborated.

#### **Keywords**

cave evolution, harvestmen, historical biogeography, Holarctic, target enrichment, taxonomy

# Introduction

The arachnid order Opiliones is taxonomically rich, comprising 46 families, over 1,640 genera, and more than 6,600 described species (summarized in Kury 2000, Machado et al. 2007, Kury 2013). Within Opiliones, considerable phylogenetic progress has been made over the past ~10 years, summarized by Pinto-da-Rocha et al. (2007) and reviewed/updated in Giribet et al. (2010) and Giribet and Sharma (2015). This progress includes transcriptome-based phylogenomic approaches used in Hedin et al. (2012a), Sharma and Giribet (2014) and Fernández et al. (2017). Opiliones diversity falls into four primary clades, including the "mite harvestmen" (Cyphophthalmi), typical "dad-dy longlegs" (Eupnoi and Dyspnoi), and the "short-legged" or "armored" harvestmen (Laniatores). Laniatores is strongly supported as monophyletic, is the most species-rich group of harvestmen (with more than 4,100 described species) and can be found on all continents except for Antarctica. Many laniatoreans are tropical, where these animals are conspicuous and occupy a wide variety of habitats. Temperate laniatoreans are less noticeable, and in the Holarctic, are mostly small-bodied (~1.5–4 mm) predators restricted to cryophilic habitats (e.g., under decaying logs or rocks, in leaf litter, in caves, etc.).

The molecular phylogenetic research of Giribet et al. (2010), which focused on relationships within Laniatores, formed the framework for further systematic research in these arachnids. Following this study, Sharma and Giribet (2011) conducted a phylogenetic analysis representing the most inclusive study of Laniatores to date. These authors recovered four primary laniatorean lineages (Fig. 1A), including the Synthetonychiidae Forster, 1954 (New Zealand), Triaenonychidae Sørensen, 1886 (mostly south temperate), Travunioidea Absolon & Kratochvíl, 1932 (north temperate), and Grassatores Kury, 2002 (broadly distributed, most diversity in the tropics). Although the sampling of travunioid taxa in these studies was incomplete (6-7 of 24 travunioid genera sampled), these molecular phylogenetic results and morphology (reviewed in Giribet and Kury 2007) support the monophyly of a north temperate lineage that constitutes the focal group of this study, the Travunioidea (Figs 1, 2).

Classification and generic level diagnoses within the Travunioidea have traditionally been based on structure of the tarsal claws of hind legs III and IV, particularly the



Figure I. Photographs of live travunioid harvestmen. A Theromaster brunneus B Erebomaster sp. C Cryptomaster leviathan D Holoscotolemon lessiniense E Peltonychia leprieurii F Trojanella serbica G Briggsus sp.
H Isolachus spinosus I Speleonychia sengeri J Yuria pulcra K Paranonychus brunneus L Sclerobunus nondimorphicus M Metanippononychus sp. N Zuma acuta O Kainonychus akamai. All photos by MH, except D, E (courtesy of and copyright A. Schönhofer), and F (courtesy of and copyright I. Karaman).

number of side branches on the median prong. It is now widely-recognized that tarsal claw structure is a poor taxonomic character in this clade, as claw structure is highly homoplastic and variable at all taxonomic levels (e.g., Shear 1977, Maury 1988, Hunt and Hickman 1993, Karaman 2005, Shear and Derkarabetian 2008). For example,



**Figure 2.** Geographic distribution of travunioid genera. Colors correspond to classification proposed in this study. Abbreviations: wNA = western North America, eNA = eastern North America, EUR = central and southern Europe, JPN/KOR = Japan and South Korea.

many travunioids were formerly grouped with the Triaenonychidae, sharing tridentshaped tarsal claw morphology. The transfer of all "north temperate triaenonychids" to the Travunioidea was first hinted at by Staręga (1971), proposed based on intestinal morphology by Dumitrescu (1975, 1976), and has since been supported with additional morphological (Giribet and Kury 2007, Mendes 2009) and molecular phylogenetic data (e.g., Derkarabetian et al. 2010, Giribet et al. 2010, Sharma and Giribet 2011). Other somatic morphological characters have been used to diagnose travunioid taxa (e.g., free lateral sclerites), but these characters may be retained plesiomorphic states (Kury 2007a) and/or potentially neotenic (Rambla 1980, Hunt and Hickman 1993). To illustrate the difficulty in the using these characters specifically in travunioids, Karaman (2005) described the monotypic genus *Trojanella* Karaman, 2005 yet left it unplaced within Travunioidea due to the uncertainty and homoplasy surrounding the diagnostic characters typically used to assign travunioid taxa.

Travunioidea has had a long and complicated taxonomic history dating back to 1861 with the description of the first species. Many European species were described by multiple authors throughout the late 1800s and early 1900s, resulting in many nomenclatural errors, comprehensively discussed in Kury and Mendes (2007). The vast majority of travunioid diversity was described during the mid-1960s to mid-1970s by Briggs (1969, 1971a, 1971b, 1974) in North America and Suzuki (1964, 1972, 1975a, 1975b, 1976) in Japan. Following this burst of taxonomic research very few studies focused on travunioids with the exception of two describing new European species (Tedeschi and Sciaky 1994, Karaman 2005), until sustained research began in the mid-2010s. During the mid-late 2010s, continued morphological work and the incorporation of genetic data confirmed the unsuitability of tarsal-claw taxonomy and resulted in several nomenclatural changes including new familial names (Giribet et al. 2010) and synonymies at the subfamilial, generic, and species levels (Shear and Derkarabet-

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ian 2008, Derkarabetian and Hedin 2014). Recent studies incorporating genetic and morphological analyses have led to the discovery, delimitation, and description of new travunioid species from western North America (Derkarabetian and Hedin 2014, Starrett et al. 2016).

Kury et al. (2014) recently provided a checklist of Travunioidea, made some taxonomic revisions, and provided a new taxonomy which serves as the starting point for this study. In this classification, Travunioidea includes 77 species/subspecies in 24 genera classified into three families, the Travuniidae Absolon & Kratochvíl, 1932 (including the historical Travuniidae, Cladonychiidae Hadži, 1935, and Briggsidae Özdikmen & Demir, 2008 as subfamilies), Paranonychidae Briggs, 1971, and Nippononychidae Suzuki, 1975. A summary of the historical classifications is presented in Table 1. While the monophyly of Travunioidea is almost certain, internal phylogenetic relationships remain largely unresolved. The most comprehensive phylogenetic analyses were conducted by Derkarabetian et al. (2010) and included samples from ten genera. Although this study was not focused on relationships among Travunioidea and only included samples from North America, the resulting phylogeny indicated a need for a taxonomic revision as multiple families and subfamilies were recovered as as para- or polyphyletic.

A robust genus-level phylogeny of Travunioidea and a stable classification would provide an important anchor for future taxonomic and evolutionary studies in this group. The stability of any phylogenetics-based classification relies upon high confidence and support for internal relationships. In other animal groups, genomic- or subgenomic-scale approaches have produced phylogenies with generally higher nodal support and have resolved difficult relationships (e.g., Blaimer et al. 2015, Garrison et al. 2016, Hamilton et al. 2016, Baca et al. 2017, Branstetter et al. 2017, Breinholt et al. 2017, Hedin et al. 2018). In this paper we utilize DNA sequences derived from capture of ultraconserved elements (UCEs; Faircloth 2017) to reconstruct phylogenomic relationships within Travunioidea. The phylogenetic utility of UCE data at multiple evolutionary scales has been demonstrated in other arachnid lineages (Starrett et al. 2017, Hedin et al. 2018). Our taxon sample includes 21 of 24 described genera and all currently and historically recognized travunioid families and subfamilies, plus outgroups. Although previous studies have examined relationships among Laniatores using a wider range of taxa (Giribet et al. 2009, Sharma and Giribet 2011), this study includes the most complete taxon set to date for Travunioidea.

# Materials and methods

#### Taxon sampling

Fifty-seven specimens were included in this study (Suppl. material 1), including 40 Travunioidea, 14 non-travunioid Laniatores (eight Triaenonychidae, two Synthetonychiidae, and four Grassatores), and single representatives from each of the other three harvestmen suborders. Forty-nine samples were newly sequenced for this study; raw

Traditional	Kury et al. (2014)	
Travunioidea	Travunioidea	
Travuniidae	Travuniidae	
Abasola	Travuniinae	
Arbasus	Arbasus	
Buemarinoa	Buemarinoa	
Dinaria	Dinaria	
Kratochviliola	Peltonychia	
Peltonychia	Speleonychia	
Travunia	Travunia	
Speleonychia	Trojanella	
Yuria	Cladonychiinae	
Cladonychiidae	Cryptomaster	
Cryptomaster	Erebomaster	
Erebomaster	Holoscotolemon	
Holoscotolemon	Speleomaster	
Speleomaster	Theromaster	
Theromaster	Briggsinae	
Pentanychidae	Briggsus	
Pentanychus	Isolachus	
Isolachus		
Triaenonychoidea (in part)	Paranonychidae	
"northern" Triaenonychidae	Sclerobuninae	
Sclerobuninae	Sclerobunus	
Sclerobunus	Zuma	
Cyptobunus	Paranonychinae	
Zuma	Paranonychus	
Paranonychinae	Metanonychus	
Paranonychus	Kaolinonychus	
Metanonychus	Kainonychus	
Kainonychus	Nippononychidae	
Kaolinonychinae	Nippononychus	
Kaolinonychus	Metanippononychus	
Mutsunonychus	Izunonychus	
Nippononychinae	Yuria	
Nippononychus		
Metanippononychus		
Izunonychus		

**Table 1.** Historical classification of the Travunioidea. Traditional classification refers to the taxonomy in place after the mid-1970s.

reads for other taxa are from Starrett et al. (2017). We included two samples for most travunioid genera (in most cases from two different species), except for *Trojanella* and *Travunia* Absolon, 1920, both of which were represented by a single specimen. We

were unable to include three European travuniid genera (Arbasus Roewer, 1935, Bue-

*marinoa* Roewer, 1956, and *Dinaria* Roewer, 1935) as these are extremely rare and difficult to obtain cave-dwelling taxa.

#### Molecular data collection

Genomic DNA was extracted from whole bodies using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). For several larger specimens (body size greater than 3–4 mm) only legs, pedipalps, and chelicerae were used in extractions. Extractions were quantified using a Qubit Fluorometer (Life Technologies, Inc.) Broad Range kit, and quality was assessed via gel electrophoresis on a 0.8% agarose gel. Up to 500 ng of genomic DNA was used in sonication procedures, using a Bioruptor for 7 cycles at 30 seconds on and 90 seconds off, or a Covaris M220 Ultrasonicator for 60 seconds with a Peak Incidence Power of 50, Duty Factor of 10%, and 200 cycles per burst. Samples were run out on a gel to verify sonication success.

Library preparation followed the general protocol of Starrett et al. (2017) and the UCE website (ultraconserved.org), with some modifications. Briefly, libraries were prepared using the KAPA Hyper Prep Kit (Kapa Biosystems), using up to 250 ng DNA (i.e., half reaction of manufacturer's protocol) as starting material. Ampure XP beads (Beckman Coulter) were used for all cleanup steps. For samples containing <250 ng total, all DNA was used in library preparation. After end-repair and A-tailing, 5  $\mu$ M universal adapter stubs (University of Georgia, EHS DNA Lab) were ligated onto libraries. Libraries were then amplified in a 50  $\mu$ I reaction, which consisted of 15  $\mu$ I adapter-ligated DNA, 1X HiFi HotStart ReadyMix, and 0.5  $\mu$ M of each Illumina TruSeq dualindexed primer (i5 and i7) with modified 8-bp indexes (Glenn et al. 2016). Amplification conditions were 98 °C for 45 s, then 16 cycles of 98 °C for 15 s, 60 °C for 30 s, and 72 °C for 60 s, followed by a final extension of 72 °C for 60 s. Samples were quantified to ensure amplification success. Equimolar amounts of libraries were combined into 1000 ng pools consisting of eight samples each (125 ng per sample).

Target enrichment was performed on pooled libraries using the MYbaits Arachnida 1.1K version 1 kit (Arbor Biosciences) following the Target Enrichment of Illumina Libraries v. 1.5 protocol (http://ultraconserved.org/#protocols). Hybridization was conducted at 65 °C for 24 hours, then libraries were bound to streptavidin beads (Dynabeads MyOne C1, Invitrogen) and washed. Following hybridization, pools were amplified in a 50 µl reaction consisting of 15 µl of hybridized pools, 1X Kapa HiFi HotStart ReadyMix, 0.25 µM of each of TruSeq forward and reverse primers, and 5 µl dH20. Amplification conditions consisted of 98 °C for 45 s, then 16 cycles of 98 °C for 15 s, 60 °C for 30 s, and 72 °C for 60 s, followed by a final extension of 72 °C for 5 minutes. Following an additional cleanup, libraries were quantified using a Qubit fluorometer. Molarity was determined with an Agilent 2100 Bioanalyzer and equimolar mixes were prepared for sequencing on an Illumina NextSeq (University of California, Riverside Institute for Integrative Genome Biology) with 150 bp PE reads.

# Bioinformatic and phylogenomic analyses

Raw demultiplexed reads were processed entirely in the PHYLUCE pipeline (Faircloth 2015). Quality control and adapter removal were conducted with the ILLUMIPROCESSOR wrapper (Faircloth 2013). Assemblies were created with TRINITY r2013-02-25 (Grabherr et al. 2011) and VELVET 1.21 at default settings. For each sample, the fasta files from both assembly methods were combined into a single file. The combined assembly contigs were matched to probes using minimum coverage and minimum identity values of 65 with a modified version of the "phyluce\_assembly\_match\_contigs\_to\_probes" script to allow multiple hits to a single probe. UCE loci were aligned with MAFFT (Katoh and Standley 2013) and trimmed with GBLOCKS (Castresana 2000, Talavera and Castresana 2007) with custom blocks settings (b1 = 0.5, b2 = 0.5, b3 = 6, b4 = 6) implemented in the PHYLUCE pipeline. Individual UCE alignments were imported into Geneious 11.0.4 (http://www. geneious.com, Kearse et al. 2012) and manually inspected for obvious alignment errors and to remove any potential non-homologous sequences. In this case, ingroup sequences more divergent than outgroup taxa based on pairwise genetic distance calculated in Geneious were flagged and removed as potential non-homologs. Two datasets were produced including loci at two different taxon coverage thresholds (50% and 70%). All bioinformatic analyses were performed on a late 2015 iMac, except for contig assembly, which was run on the University of California, Riverside Institute for Integrative Genome Biology Linux cluster.

Analyses of both 50% and 70% datasets included concatenated maximum likelihood, concatenated Bayesian, and coalescent-based analyses, while partitioned maximum likelihood analyses were run only on the 70% dataset with partitions and models determined by PARTITIONFINDER v1.1.1 (Lanfear et al. 2012). Maximum likelihood trees were estimated with RAXML v8.2 (Stamatakis 2014) using the rapid bootstrap algorithm, 500 bootstrap replicates, and the GTRGAMMA model. Bayesian analyses were conducted using the BEAST v2.4 package (Bouckaert et al. 2014), run for 100 million generations, logging every 1000 generations, with 10% burnin. To assess convergence, Tracer (Rambaut et al. 2007) was used to check for ESS values >200 and examine stationarity of parameters. Two separate analyses were run to check for convergence between runs. All RAXML and BEAST analyses were run through the CIP-RES PORTAL (Miller et al. 2010). To incorporate coalescent approaches, ASTRAL-II (Mirarab et al. 2014, Mirarab and Warnow 2015) was used with individual gene trees estimated in RAXML, and SVDQUARTETS (Chifman and Kubatko 2014, 2015) was run through PAUP\* 4.0a159 (Swofford 2003), with 100 bootstrap replicates.

# Results

#### Sequencing results

Sequencing results and data matrix statistics are presented in Suppl. material 1. Raw sequence reads are available in the NCBI Short Read Archive Accession no. SRP142540

(BioProject ID PRJNA451420). Untrimmed contigs, all trimmed individual locus alignments (pre- and post-manual editing), and trimmed concatenated matrices are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.tj48997. Combining assemblies resulted in higher numbers of resulting UCE loci relative to using only a single assembly method, suggesting assembly-specific contigs as found in Hedin et al. (2018). The average number of UCE loci sequenced was 748 across all travunioid samples and 675 across all samples included in this study. The 70% taxon coverage matrix included 317 loci (272 average loci per sample, total length of 83,990 bp, 253.86 bp average locus length) and the 50% taxon coverage matrix included 677 loci (488 average loci per sample, total length of 165,096 bp, 264.95 average locus length).

#### Phylogenomic analyses

All analyses, with the exception of the 70% concatenated BEAST analysis, recover Travunioidea as sister group to all other Laniatores lineages (Figure 3 and Suppl. material 2: Figure 1), a relationship not recovered in previous molecular phylogenetic studies. However, based on morphological data, this hypothesis has been put forth by Kury (2015) who created the name Tricospilata Kury, 2015 for Triaenonychoidea + Grassatores (the sister group to Travunioidea). The 70% concatenated BEAST analysis recovers Travunioidea + Triaenonychoidea Sørensen, 1886 (= Synthetonychiidae + Triaenonychidae), previously called Insidiatores Loman, 1900, sister group to the Grassatores. The only other phylogenetic analysis resulting in Insidiatores as sister group to Grassatores is the transcriptome-based study of Fernández et al. (2016). Synthetonychiidae is recovered as sister group to Triaenonychidae with full support in all RAXML and SVDQUARTETS analyses and the concatenated BEAST analysis, but is sister group to all non-travunioid Laniatores (Triaenonychidae + Grassatores) in the ASTRAL analyses. It is apparent that denser taxonomic sampling and further phylogenomic datasets will be required to resolve the base of Laniatores.

Travunioidea is monophyletic and fully supported across all analyses (Figure 3). Within Travunioidea, no families (and all but one subfamily) as currently defined in Kury et al. (2014) are monophyletic in any analyses (Figs 4, 5). A highly supported sister relationship between *Travunia* and *Trojanella* is recovered, and this group is sister to all remaining travunioids in all analyses. The western North American genera *Cryptomaster* Briggs, 1969 and *Speleomaster* Briggs, 1969 are recovered as sister taxa, and although the placement of *Cryptomaster* + *Speleomaster* is inconsistent across analyses, they are never sister group to or included within the Travuniidae or Cladonychiinae *sensu* Kury et al. (2014). In all analyses, the traditional Briggsinae (*Briggsus* Özdikmen & Demir, 2008 + *Isolachus* Briggs, 1971) are recovered within a largely travuniid clade, and always the sister group to *Speleonychia* Briggs, 1974. Eastern North American *Erebomaster* Briggs, 1969 (traditional Cladonychiinae) is the sister group to the Briggsinae + *Peltonychia* Clavigera (Simon, 1879) in all analyses. The two samples of the European genus *Peltonychia* Roewer, 1935 included in this study are never sister, *P. leprieurii* (Lucas, 1861) is found as the sister group to *Holoscotolemon* Roewer, 1915 while *P. clavig-*



**Figure 3.** Phylogenetic relationships among major laniatorean lineages. Lower phylogenies correspond to results presented in this study. Nodes are fully supported (100% bootstrap or 1.0 posterior probability), unless indicated otherwise. Numbers in lower left phylogeny correspond to support values from 50% RAxML concatenated (top), and from 70% RAxML partitioned (bottom) analyses. Numbers in ASTRAL phylogeny based on 50% (top) and 70% (bottom) matrices.



**Figure 4.** Phylogenomic relationships among travunioid genera. Left: RAxML and 50% BEAST concatenated topologies, with bootstrap support from the partitioned analysis. All nodes in the BEAST topology have posterior probability of 1.0. Abbreviations indicate placement in classification at the time of Kury et al. (2014): Tt = Travuniidae, Travuniinae; Tc = Travuniidae, Cladonychiinae; Tb = Travuniidae, Briggsinae; Pp = Paranonychiae, Paranonychinae; Ps = Paranonychidae, Sclerobuninae; N = Nippononychidae. Right: 70% BEAST concatenated. Nodes are fully supported (100% bootstrap or 1.0 posterior probability), unless indicated.



**Figure 5.** Phylogenomic relationships among travunioid genera. Left: 70% SVDQUARTETS. Right: 70% ASTRAL. Nodes are fully supported (100% bootstrap), unless indicated.

*era* is the sister group to Briggsinae + *Speleonychia*. The relationships among these lineages are generally weakly supported at multiple nodes. All analyses recover a clade comprised of the former "northern triaenonychids", currently in the families Paranonychidae and Nippononychidae, although neither family as currently defined is monophyletic. The Californian endemic genus *Zuma* Goodnight & Goodnight, 1942 is recovered within a clade comprised of Japanese taxa except *Yuria* Suzuki, 1964 and *Paranonychus fuscus* (Suzuki, 1976). The relationships within this lineage are all highly supported and identical across all analyses. The placement of the Japanese genus *Yuria* differs considerably across analyses and is recovered as the sister group to either the traditional travuniids or to a clade comprising *Cryptomaster* + *Speleomaster* and the Paranonychidae + Nippononychidae. Most importantly, *Yuria* is never recovered with the other Japanese nippononychids.

#### Phylogenomic revision

Our approach to establish a stable classification involved identifying the largest group of terminal taxa that are always monophyletic and always highly supported across all analyses. We discovered four multi-genus clades consistent across all analyses (Figs 4, 5), treated here as families:

- 1) A clade containing *Travunia* + *Trojanella*. Because of the inclusion of *Travunia*, this clade retains the name Travuniidae.
- A clade containing the majority of travuniid genera sensu Kury et al. (2014): Peltonychia, Holoscotolemon, Erebomaster, Theromaster, Speleonychia, Briggsus, and Isolachus. This clade will use the re-elevated and re-circumscribed familial name Cladonychiidae (see below).
- A clade containing all genera currently included in the Paranonychidae and Nippononychidae of Kury et al. (2014). This clade retains the familial name Paranonychidae.
- A clade consisting of the two former Cladonychiinae genera *Cryptomaster* and *Speleomaster*, endemic to the Pacific Northwest of North America, described below as the new family Cryptomastridae fam. n. (Figure 6).

The genus *Yuria* is considered *incertae sedis* given its uncertain phylogenetic placement (see Discussion). The new phylogenomics-based classification, used hereafter, is summarized in Table 2.

# Taxonomy

Below we redefine and diagnose all families of Travunioidea, including the newly described Cryptomastridae. The unsampled European genera are placed into two of these families based on previous morphological studies: *Dinaria* is placed in the Travuniidae with *Travunia* and *Trojanella*, while *Arbasus* and *Buemarinoa* are placed in the Cladonychiidae with *Peltonychia* and *Holoscotolemon*. It is premature to discuss definitive morphological

**Table 2.** Proposed revised classification. The number of described species/subspecies at each taxonomic level is in parentheses. Genera are grouped by phylogenetic affinity, not alphabetically.

Travunioidea (69/+11)		
Travuniidae (6)	Cryptomastridae (4)	
Dinaria (1)	Cryptomaster (2)	
Travunia (4)	Speleomaster (2)	
Trojanella (1)		
Cladonychiidae (30/+1)	Paranonychidae (28/+9)	
Arbasus (1)	Paranonychus (3)	
Buemarinoa (1)	Metanonychus (3/+5)	
Peltonychia (8)	Sclerobunus (12)	
Holoscotolemon (8)	Kaolinonychus (1/+1)	
Erebomaster (3/+1)	Metanippononychus (4/+2)	
Theromaster (2)	Nippononychus (1)	
Briggsus (5)	Zuma (2)	
Isolachus (1)	Kainonychus (1/+1)	
Speleonychia (1)	Izunonychus (1)	
	incertae sedis (1/+1)	
	<i>Yuria</i> (1/+1)	

synapomorphies for all travunioids, as all hypothesized members have never been surveyed for all relevant morphological characters. However, likely morphological synapomorphies include the presence of a four-lobed ovipositor and a bipartite intestinal *diverticulum ter-tium* (OD3 below; Dumitrescu 1975, 1976, reviewed in Giribet and Kury 2007).

# Abbreviations used for intestinal diverticula

D1	diverticulum 1;
OD2	opisthosomal diverticula 2;

**OD3** opisthosomal diverticula 2,

Terminology and homology for penis/glans structure follows Martens (1986).

#### Suborder LANIATORES Thorell, 1876

# Family CRYPTOMASTRIDAE Derkarabetian & Hedin, fam. n. http://zoobank.org/A32A845F-36A7-426B-B3C1-F4E65085F356

#### Type genus. Cryptomaster Briggs, 1969

**Type species.** Cryptomaster leviathan Briggs, 1969

**Diagnosis.** The Cryptomastridae can be diagnosed from all other travunioids by the presence of a distal swelling on tibia II that bears enlarged setae (Figure 6A, C), a sexually dimorphic structure found only in males. Both genera are fairly distinctive. Cryptomaster is easily identified as the largest (>2.5 mm body length) laniatorean in the Pacific Northwest of North America (Figure 6D) and largest member of Travunioidea, although two size forms exist (Starrett et al. 2016). Speleomaster species are restricted to lava tubes showing extreme levels of troglomorphy with complete absence of eyes, extremely reduced pigmentation, and leg elongation (Figure 6B). Although unrelated, Speleomaster and Speleonychia are both highly troglomorphic lava tube dwellers in the Pacific Northwest, found in Idaho and Washington, respectively. Aside from their disjunct geographic distribution, Speleomaster can be differentiated from Speleonychia by the absence of a free ninth tergite and lateral sclerites, and by the presence of bifurcating tarsal claws of the hind legs (Speleonychia with a peltonychium). The cryptomastrid genera can be distinguished from the eastern North American Cladonychiidae (Erebomaster + Theromaster) by the spination of the pedipal tarsus, previously noted by Briggs (1969, 1974). Cryptomastrids possess five prominent spines on the lateral margins of the pedipalpal tarsus, three on the prolateral margin and two on the retrolateral margin. Erebomaster and Theromaster possess three pairs of prominent lateral spines (in some *Theromaster*, the two apical retrolateral spines are fused at the base). The Cryptomastridae are unique in intestinal morphology, possessing a combination of an elongate and triangular DI (similar to Briggsus and Isolachus), and shorter OD2 and OD3 (similar to the Paranonychidae) (Suppl. material 2: Figure 2).



Figure 6. Morphology of Cryptomastridae. A) male *Cryptomaster*, arrow denotes sexually dimorphic swelling diagnostic of Cryptomastridae; B) *Speleomaster*, habitus; C) Scanning electron micrograph of tibial swelling, from Starrett et al. (2016); D) Cryptomastrid distribution.

**Included genera and species.** *Cryptomaster.* Described by Briggs (1969) and originally included only *Cryptomaster leviathan* Briggs, 1969 from the Coastal Range of southwestern Oregon. A second species, *Cryptomaster behemoth* Starrett & Derkarabetian, 2016, was described from the west-central Cascade Range of Oregon (Starrett et al. 2016).

**Speleomaster.** Briggs (1974) described the genus and both known species, *Speleomaster lexi* Briggs, 1974 and *Speleomaster pecki* Briggs, 1974, from lava tubes of the Snake River Plain in southern Idaho.

#### Family TRAVUNIIDAE Absolon & Kratochvíl, 1932

Type genus. Travunia Absolon, 1920.

**Type species.** *Travunia troglodytes* (Roewer, 1915).

**Diagnosis.** It is difficult to diagnose the Travuniidae as all taxa have yet to be examined for all relevant characters. For all species in which male genitalia have been examined, the glans is widened and flattened with lateral extensions, tooth-like in *Trojanella* and wing-like in *Travunia* and *Dinaria*. The Travuniidae as defined here are restricted to the European Dinaric Karst and are highly troglomorphic, completely blind with a highly reduced ocularium (Figure 1F). The penis of *Travunia* and *Dinaria*.

is undifferentiated, while that of cladonychiids shows a clear division between glans and shaft. *Trojanella* shows some similarities in glans structure to *Holoscotolemon* and *Peltonychia* (e.g., divided glans and shaft), but the penis musculature is restricted to the apical portion of the shaft and glans in *Trojanella*, while the musculature of the European cladonychiids are restricted to the basal portion of the shaft.

**Included genera and species.** *Travunia.* The genus *Travunia* includes four described species that are all highly troglomorphic and restricted to caves in the southern Dinaric Karst region of Europe: *T. borisi* (Hadži, 1973) from Bosnia and Herzegovina, *T. hofferi* (Šilhavý, 1937) from Montenegro, *T. jandai* Kratochvíl, 1937 from Croatia, and *T. troglodytes* (Roewer, 1915) from Croatia and Bosnia and Herzegovina.

*Trojanella* (Figure 1F). This monotypic genus is represented by *T. serbica* Karaman, 2005, a highly troglomorphic species restricted to a single cave on Stara Planina Mountain in Serbia.

**Dinaria.** A monotypic genus represented by the highly troglomorphic species *D. vjetrenicae* (Hadži, 1932) known only from Vjetrenica Cave in southern Bosnia and Herzegovina.

**Remarks.** It is not surprising that *Trojanella* is included in the most early-diverging travunioid lineage given Karaman's (2005) statement that this species is a "unique and isolated phylogenetic line in the superfamily". Karaman's decision to leave *Trojanella* unplaced in Travunioidea was made to highlight, and is a consequence of, the commonly used morphological characters that have hindered a reliable taxonomy within this group. It is unclear how many species of *Travunia* actually exist. Novak (2004) questioned the validity of *Travunia, Dinaria*, and *Abasola* at the generic level, and *Abasola* was later synonymized with *Travunia* (Kury and Mendes 2007). Novak (2004, 2005) argues that *Travunia* may be oversplit and includes only 2–3 species of questionable status. The status of *Travunia* and *Dinaria* as distinct genera has been questioned based on similarity in male genitalic morphology (Novak 2004); together these might represent a single lineage.

#### Family CLADONYCHIIDAE Hadži, 1935

#### **Type genus.** *Erebomaster* Briggs, 1969.

Type species. Erebomaster flavescens Cope, 1872.

**Diagnosis.** Some taxa have not been examined for the relevant characters, but tentative diagnostic characters may be found in the intestinal complex (Suppl. material 2: Figure 2). All Cladonychiidae that have been examined show a 2–3 branched, elongate, and triangular D1, and elongate OD3. In the Pacific Northwest of North America, cladonychiids are broadly sympatric with the Cryptomastridae and Paranon-ychidae. The above intestinal characteristics differentiate them from Cryptomastridae, which possess a relatively short and stout OD3, and the Paranonychidae, which possess a simple unbranched D1 (Suppl. material 2: Figure 2). The European taxa can be diagnosed from Travuniidae based on male genital morphology (Figure 7): travuniids have a widened and flattened glans with lateral wing-like extensions; the glans and shaft are



Figure 7. Representative penis morphology of Travunioidea. Clockwise from left: *Trojanella serbica* redrawn from Karaman (2005), *Travunia hofferi* redrawn from Karaman (2005), *Cryptomaster behemoth* adapted from Starrett et al. (2016), *Holoscotolemon jaqueti* redrawn from Martens (1978), *Briggsus hamatus, Yuria pulcra, Paranonychus brunneus, Metanonychus setulus navarrus, Nippononychus japonicus* redrawn from Suzuki (1975), *Kaolinonychus coreanus coreanus* redrawn from Suzuki (1975), *Izunonychus ohruii, Zuma acuta*. All Travuniidae and Cladonychiidae are drawn in ventral view; Cryptomastridae, *Yuria*, and Paranonychidae drawn in lateral. For simplicity, not all travunioid genera are included.

undivided in *Travunia* and *Dinaria*. The penis musculature is restricted to the base in *Holoscotolemon* and *Peltonychia*, while the musculature of *Trojanella* is restricted to the apical portion of the shaft and glans.

**Included genera and species.** *Erebomaster* (Figure 1B). *Erebomaster* is found in the eastern United States, and currently includes three species: *E. flavescens* Cope, 1872 with two subspecies *E. f. flavescens* from Wyandotte Cave in Indiana and *E. f. coecus* (Packard, 1888) from Carter Cave in Kentucky; *E. weyerensis* (Packard, 1888) from caves in West Virginia; and the relatively widespread *E. acanthinus* (Crosby & Bishop, 1924) with a distribution along and west of the Appalachian Mountains. A revision of *Erebomaster* taxa is needed.

**Theromaster** (Figure 1A). Consisting of two described species found in the eastern United States: *T. brunneus* (Banks, 1902) is relatively widespread in the southern Appalachian Mountains; *T. archeri* (Goodnight & Goodnight, 1942) from caves in Alabama. *Speleonychia* (Figure 1I). A monotypic genus, *Speleonychia sengeri* Briggs, 1974 is a highly troglomorphic species restricted to lava tubes near Mt. Adams, in south-central Washington.

**Briggsus** (Figure 1G). The genus and all species were originally described by Briggs (1971b) as *Pentanychus*; Özdikmen and Demir (2008) provided the replacement name. This genus consists of five described species all restricted to the moist coastal forests (>50 inches yearly rainfall) of Oregon and Washington in the Pacific Northwest: *B. bilobatus* (Briggs, 1971), *B. clavatus* (Briggs, 1971), *B. flavescens* (Briggs, 1971), *B. hamatus* (Briggs, 1971), and *B. pacificus* (Briggs, 1971).

*Isolachus* (Figure 1H). A monotypic genus, *Isolachus spinosus* Briggs, 1971 is restricted to northwest Oregon and southwest Washington.

Holoscotolemon (Figure 1D). A European genus with eight species. Six species are restricted to the Alps, primarily from Italy and Austria: *H. unicolor* Roewer, 1915; *H. lessiniensis* Martens, 1978; *H. oreophilus* Martens, 1978; *H. franzinii* Tedeschi & Sciaky, 1994; *H. monzinii* Tedeschi & Sciaky, 1994; and *H. naturae* Tedeschi & Sciaky, 1994. *H. querilhaci* (Lucas, 1864) is found in the Pyrenees of southern France and *H. jaqueti* (Corti, 1905) is recorded from eastern Europe in Romania, Ukraine, and former Yugoslavia.

**Peltonychia** (Figure 1E). A genus with a long history, *Peltonychia* includes the first described travunioid species, *P. leprieurii*. This genus of eight species is almost entirely known only from caves in central Europe throughout the Pyrenees and Alps (Suppl. material 2: Figure 3). *Peltonychia leprieurii* is found in the Alps of northern Italy. *Peltonychia clavigera*, *P. navarica* (Simon, 1879), *P. piochardi* (Simon, 1872), and *P. sarea* (Roewer, 1935) are all found in the Pyrenees of northern Spain and southern France. *P. gabria* Roewer, 1935 is recorded from Trieste, Italy; *P. postumicola* (Roewer, 1935) is recorded from Trieste, Italy and Slovenia are conclusively shown to be in error, and *P. postumicola* and *P. tenuis* are morphologically similar to *P. clavigera* (Novak and Gruber 2000). As such, it is unclear how many actual species are included.

*Arbasus.* A monotypic genus, the highly troglomorphic *Arbasus caecus* (Simon, 1911) is only known from Grotte de Pène Blanque in the Pyrenees of southern France.

**Buemarinoa.** A monotypic genus, the highly troglomorphic *Buemarinoa patrizii* Roewer, 1956 is only known from the Grotte del Bue Marino in Sardinia, Italy.

**Proholoscotolemon** Ubick & Dunlop, 2005. A monotypic genus, *P. nemasto-moides* (Koch & Berendt, 1854) is known from specimens preserved in Baltic amber. The specimens were redescribed by Ubick and Dunlop (2005) and based on morphological similarity and geography it is interpreted as the ancestor of, or sister group to, *Holoscotolemon*.

**Remarks.** *Peltonychia* is polyphyletic, in some cases with strong support (Figure 5). The sampled species are from two separate geographic regions: *P. clavigera* from the Pyrenees of northern Spain and southern France, and *P. leprieurii* from the Alps of northern Italy. Accounting for the locality errors in Italy and Slovenia mentioned above, *Peltonychia* is geographically split into two regions: *P. leprieurii* in northern

Italy, and the remaining species in the Pyrenees. The male genitalia of four species of *Peltonychia* have been examined: *P. leprieurii*, *P. clavigera*, *P. gabria*, and *P. postumicola*. Based on these genitalic drawings (Chemini 1985, Martens 1978, Thaler 1996), it is obvious that *P. leprieurii* is very divergent from the other three *Peltonychia*, which are very similar (Suppl. material 2: Figure 3). This concordance between geography, genital morphology, and our phylogenomic analyses support the separation of *Peltonychia* into two genera. However, we refrain from formally making this taxonomic change until all relevant species can be studied.

The sister relationship of *Speleonychia* to the traditional Briggsinae (*Briggsus* + *Isola-chus*) is not surprising given the close geographic proximity of these genera and shared presence of a free ninth tergite and lateral sclerites. The distinct generic status of *Ar-basus* and *Buemarinoa* has been doubted (Kury and Mendes 2007). The morphological distinction between *Arbasus* and *Buemarinoa* is minimal and entirely based on tarsal segmentation (Kury and Mendes 2007), which is typical of the "Roewerian classification" system that resulted in taxa being over split based on irrelevant characters (e.g., Kury et al. 2014, Kury and Pérez-González 2015). Aside from the original descriptions with basic drawings (Roewer 1935, 1956), virtually no taxonomic work has been conducted on *Arbasus* and *Buemarinoa*. However, Kury and Mendes (2007) note that they "both look superficially like *Hadziani* [=*Peltonychia*], but with clear troglomorphic traits…", and their inclusion in Cladonychiidae here seems justified.

# Family PARANONYCHIDAE Briggs, 1971

#### Type genus. Paranonychus Briggs, 1971

Type species. Paranonychus brunneus (Banks, 1893).

**Diagnosis.** The Paranonychidae can be diagnosed by their relatively complex glans (except *Paranonychus*) (Figure 7 and Suppl. material 2: Figure 4), and by their intestinal complex (Suppl. material 2: Figure 2). For all taxa that have been examined, the paranonychids possess a small D1 that is circular to subtriangular, and a simple and shorter OD3. The paranonychids are restricted to western North America and East Asia. In southern Japan the paranonychids are sympatric with *Yuria* and can be diagnosed by several characteristics: *Yuria* possesses a free ninth tergite, and the penis has a dorsal plate with fused stylus; the paranonychids do not have a free ninth tergite and the penis glans lacks a dorsal plate. In western North America, the paranonychids are sympatric and syntopic in surface habitats with the Cladonychiidae (*Briggsus, Isola-chus*) and Cryptomastridae (*Cryptomaster*). The paranonychids can be differentiated from these families by the structure of D1: paranonychids possess a small circular to subtriangular unbranched D1, while Cladonychiidae and Cryptomastridae possess an elongate, triangular, and branched D1.

Included genera and species. *Paranonychus* (Figure 1K). This trans-Beringian genus includes three known species: *P. brunneus* (Banks, 1893) distributed in the Coast

and Cascade Ranges of Oregon and Washington with records extending north to Alaska; *P. concolor* Briggs, 1971, recorded from a single location in the southern Cascade Range of Oregon; and *P. fuscus* found throughout northern Honshu in Japan.

*Metanonychus* Briggs, 1971. This genus and all species were described by Briggs (1971) and are restricted to the moist forests of the Pacific Northwest of North America. *Metanonychus* includes three species: *M. nigricans* Briggs, 1971 with two subspecies, *M. n. nigricans* and *M. n. oregonus*, found in Oregon; *M. setulus* Briggs, 1971 with five subspecies, *M. s. setulus*, *M. s. cascadus*, *M. s. mazamus*, *M. s. navarrus*, and *M. s. obrieni*, found in Oregon, Washington, and northern California; and *M. idahoensis* Briggs, 1971 found in northern Idaho.

Sclerobunus Banks, 1893 (Figure 1L). Recently revised by Derkarabetian and Hedin (2014), Sclerobunus is distributed throughout western North America and currently includes 12 species divided into three species groups. The nondimorphicus group includes S. nondimorphicus Briggs, 1971 from Oregon, Washington, and British Columbia, and S. idahoensis Briggs, 1971 from northern Idaho. The caveobligate cavicolens group includes: Sclerobunus cavicolens (Banks, 1905) restricted to Lewis and Clark Caverns, Montana; Sclerobunus ungulatus (Briggs, 1971) from caves in Great Basin National Park, Nevada; Sclerobunus madhousensis (Briggs, 1971) from caves near Provo, Utah. The robustus group includes the widespread S. robustus (Packard, 1877), S. glorietus Briggs, 1971, and S. skywalkeri Derkarabetian & Hedin, 2014, all distributed throughout the high elevation forests of the southwestern United States, and S. jemez Derkarabetian & Hedin, 2014, S. klomax Derkarabetian & Hedin, 2014, S. speoventus Derkarabetian & Hedin, 2014, and S. steinmanni Derkarabetian & Hedin, 2014, which are all troglomorphic species restricted to cave and talus habitats along the eastern edge of the southern Rocky Mountains in New Mexico and Colorado.

*Kaolinonychus* Suzuki, 1975. This monotypic genus endemic to South Korea is recorded mostly from caves. *Kaolinonychus coreanus* (Suzuki, 1966) includes two subspecies *K. c. coreanus* and *K. c. longipes*.

*Metanippononychus* Suzuki, 1975. (Figure 1M). Endemic to Japan, *Metanippononychus* is restricted to southern Honshu, Shikoku, and Kyushu and includes four species: *M. daisenensis* Suzuki, 1975; *M. iriei* Suzuki, 1975, with two subspecies *M. i. iriei* and *M. i. yakuensis*; *M. iyanus* Suzuki, 1975; *M. tomishimai* Suzuki, 1975, with two subspecies *M. t. tomishimai* and *M. t. awanus*.

*Nippononychus* Suzuki, 1975. A monotypic genus endemic to Japan, *Nipponon-ychus japonicus* (Miyosi, 1957) is restricted to southern Honshu and Shikoku.

Zuma (Figure 1N). Zuma includes two species restricted to forests of central and northern California: Zuma acuta Goodnight & Goodnight, 1942 restricted to the coastal forests south of San Francisco; Zuma tioga Briggs, 1971 found in the central and northern Sierra Nevada range.

*Izunonychus* Suzuki, 1975. A monotypic genus endemic to Japan, *Izunonychus ohruii* Suzuki, 1975 is restricted to the Izu peninsula and Hakone area in central Honshu.

*Kainonychus* Suzuki, 1975 (Figure 1O). A monotypic genus endemic to Japan, *Kainonychus akamai* (Suzuki, 1972) includes two subspecies, *K. a. akamai* distributed throughout northern Honshu and *K. a. esoensis* restricted to Hokkaido.

**Remarks.** In this study all genera in the Paranonychidae have been sampled and the generic relationships are consistent and highly supported across all analyses (Figs 4, 5). Although the study of Derkarabetian et al. (2010) only included North American taxa, the relationships of paranonychids recovered here are the same, notably *Paranonychus* as the earliest diverging genus, and a sister relationship between *Sclerobunus* and *Metanonychus*. The familial name Sclerobunidae has been used previously (Giribet et al. 2010) for the "northern triaenonychids". However, Paranonychidae and the subfamily Paranonychinae Briggs, 1971 have priority over the names Sclerobunidae and Sclerobuninae Dumitrescu 1976.

The Japanese genera *Metanippononychus* and *Nippononychus* show levels of UCE divergence consistent with congeners (Figs 4, 5). Intermediate morphological forms between *Nippononychus japonicus* and *Metanippononychus daisenensis* can be found where the two species come into contact (Tsurusaki pers. obs.). These genera are differentiated only by tarsal claw structure: *Metanippononychus* possessing a ventral tooth on the median prong of the hind claws. The original drawings of male genitalia show that *M. daisenensis* and *N. japonicus* differ in the width of the stylus (Suzuki 1975b). However, the penis of *N. japonicus* is highly similar to that of the geographically proximate *M. tomishimai tomishimai*.

Kury et al. (2014) includes *Paranonychus fuscus* (formerly *Mutsunonychus fuscus*) as a synonym of *Paranonychus brunneus* (Banks, 1893) based on Shear's (1986) statement "*Paranonychus brunneus* (=*Mutsunonychus fuscus* Suzuki; Paranonychidae)". Later in Shear and Derkarabetian (2008), the genus *Mutsunonychus* was formally synonymized under *Paranonychus*, and although a potential species level synonymy was noted, it was not formally established. The levels of UCE divergence between *P. brunneus* and *P. fuscus* are consistent with species level divergences compared to other pairs of congeneric taxa included (Figs 4 and 5), and as such, *P. fuscus* is again treated as a distinct species here.

# Incertae sedis

**Included genera and species.** *Yuria* (Figure 1J). A monotypic genus endemic to Japan, *Yuria pulcra* Suzuki, 1964 includes two subspecies distributed throughout southern Honshu, Shikoku, and Kyushu: *Y. p. pulcra* and *Y. p. briggsi* Suzuki, 1975.

**Remarks.** When *Yuria pulcra* was first described it was placed in Travuniidae because the tarsal claw is a peltonychium (Suzuki 1964, 1975a). Phylogenetic analysis of morphological data placed *Yuria* as the sister group to *Nippononychus* (Paranonychidae) with two synapomorphies (Mendes 2009). Kury et al. (2014) later transferred this genus to the Nippononychidae, which contained most of the other Japanese travunioids. Placement and support for *Yuria* varies depending on analysis (Figs 4, 5). Morphology complicates matters further, as *Yuria* possesses a free ninth tergite and lateral sclerites, plesiomorphic characters that are potentially neotenic and shared with the Briggsinae. The penis morphology of *Yuria* is also relatively unique within Travunioidea, possessing a dorsal plate with fused stylus (Figure 7 and Suppl. material 2: Figure 4).

# Discussion

# Travunioidea classification and the trouble with travuniids

Travunioidea includes 80 nominal taxa (species/subspecies), four families, and one unplaced genus. Traditionally within Travunioidea the subfamilial rank has been used to further subdivide taxa, and the composition of subfamilies has changed across classification schemes (Table 1). Here, we refrain from using the subfamilial rank for three reasons: 1) the confusing taxonomic history of these lineages, specifically with regards to relative rank and composition; 2) the poorly supported nodes in Cladonychiidae and non-monophyly of *Peltonychia*; 3) and the relatively sparse composition each subfamily would have (i.e., 4/6 subfamilies would contain only 1–2 genera). Based on phylogenomic analyses, the composition of all subfamilies would have changed again (Table 2). Relationships among several traditional Travuniidae genera are still uncertain, and the absolute stability of the familial rank will be dependent upon future incorporation of the unsampled European genera *Arbasus* and *Buemarinoa*.

The traditional Travuniidae have had an incredibly long and complex taxonomic history beginning with the description of the first travunioid in 1861. Kury and Mendes (2007) focus entirely on nomenclatural issues, resolving them at the familial and generic level, and in doing so note that "the [traditional] family Travuniidae constitutes one of the worst problems of the laniatorid taxonomy of the 20th century". Several others have discussed the diverse array of problems plaguing travunioid taxonomy (Novak 2005, Novak and Gruber 2000, Karaman 2005). These issues include, but are not limited to, description of two species in two different genera based on the same material, autosynonymy of a genus name, proposal of unavailable family and genus names, genus description without designation of type species after 1930, species descriptions based on juveniles, mistranslation of foreign languages, disregard for correct taxonomic changes, mismatched type localities, type localities accidentally and intentionally incorrectly named, inability to find further specimens from type localities, and destruction of type localities. Even though the European taxa have received significant attention from a taxonomic standpoint, a great deal of focused and devoted research including fieldwork, and modern morphological and phylogenetic analyses will be needed to fully resolve the taxonomic issues of this notoriously difficult clade.

The goal of this research was to provide a stable classification of Travunioidea at the familial level. This stability relies on incorporating potential future changes if unsam-

pled taxa are included. We believe our familial level classification, disuse of subfamily rank, and leaving *Yuria* unplaced, minimizes future taxonomic changes. All familial name-holding genera are included. Only *Arbasus* and *Buemarinoa* are missing but given morphological similarities and the geographic distribution of these taxa, their inclusion in Cladonychiidae given future sampling seems likely. The stability of the familial level provided by this phylogenomics-based reclassification and the recovered distinction between Travuniidae and European Cladonychiidae can guide future efforts. The morphologically enigmatic *Yuria* remains phylogenetically elusive. A potential solution to the unreliable placement of *Yuria* is to create a monotypic family. However, we refrain from this until all genera can be included in phylogenomic analyses.

#### Morphological reevaluation

The tarsal claw - A type of modified tarsal claw termed a peltonychium united the "traditional Travuniidae", a structure now known to be convergent in several unrelated cave-dwelling taxa (e.g., Peltonychia, Speleonychia, Trojanella). The morphological distinction between the typical trident-shaped tarsal claw (with variable number of sidebranches) and a peltonychium is not entirely clear in some travunioids (e.g., Izunonychus, Metanippononychus), and the transition between forms is best documented in the triaenonychid genus Lomanella Pocock, 1902 (Hunt and Hickman 1993). However, all of the 18 travunioid species (not including subspecies) with a clear peltonychium are found in caves, 10 of which are either described to be troglobitic (cave-obligate) and/or show high levels of troglomorphy. An additional six species are described to have reduced pigmentation (relative to surface-only species), five of which are only reported from caves. Two remaining species are inconsistent with this pattern. First, Peltonychia leprieurii, from the Alps of northern Italy and Switzerland is recorded from both cave and surface habitats (considered a troglophile) and retains black pigment. Second, Y. pulcra pulcra and Y. p. briggsi from southern Japan show some reduced pigmentation, though they differ in their habitat: Y. p. pulcra is recorded from both cave and surface habitats, while Y. p. briggsi is known only from surface habitats under woody debris. It has been suggested that the peltonychium, and other plesiomorphic characters, are convergent through neoteny (Rambla 1980, Hunt and Hickman 1993, Karaman 2005). The hind tarsal claws of juvenile triaenonychids and travunioids have more side branches than adults (e.g., Hunt and Hickman 1993, Suzuki 1975b), and some juveniles additionally possess a pseudonychium (median tarsal claw) (Shultz and Pinto-da-Rocha 2007, Gnaspini 2007). As such, the peltonychium may be a retained adult form of this juvenile structure (Hunt and Hickman 1993).

**The ninth tergite and lateral sclerites** – The traditional Briggsinae (*Briggsus* + *Isola-chus*) were hypothesized to be a relatively early diverging lineage within Travunioidea (Briggs 1971, Giribet and Kury 2007). This was due to the presence of a free ninth tergite, and lateral sclerites, a plesiomorphic condition found in the other suborders of harvestmen. Conversely, penis morphology suggested that this group is derived given

the relatively simple penis structure (Martens 1986). Speleonychia also possess a distinct ninth tergite and lateral sclerites, now shown to be a shared condition with Briggsus + Isolachus. Rambla (1980) argued that these characters are neotenic, retained in adults from nymphal stages, and as such are derived. All phylogenomic analyses here support the derived nature of these characters, as they are recovered well within the Cladonychiidae in all analyses. However, not all travunioid taxa with a free ninth tergite and free lateral sclerites are restricted to this clade, as Yuria also possess both characters (Suzuki 1964, 1975a). While the placement of Yuria is uncertain, it is never recovered with or as the sister group to the traditional Briggsinae + Speleonychia. Outside of Travunioidea, the only other laniatorean taxa known to possess free lateral sclerites are in the genus Hickmanoxyomma Hunt, 1990, a largely cave-dwelling triaenonychid genus endemic to Tasmania (Hunt 1990). In this genus the presence of five pairs of free lateral sclerites (2-3) in travunioids) are a diagnostic character for the *H. cavaticum* species group containing four species, all recorded only from caves and some showing troglomorphy. Hunt (1990) suggested it might be correlated with the overall reduced sclerotization associated with troglomorphy, but also reiterated Rambla's (1980) view of neoteny. No clear phylogenetic, evolutionary, or ecological pattern exists for these characters and their presence in multiple unrelated lineages suggests their plesiomorphic nature.

**The midgut** – Studies focusing on the digestive tract began in the 1920s, but the work of Dumitrescu (e.g., 1974, 1975, 1976) contributed most significantly to the phylogenetic utility of midgut morphology in Opiliones. Through examination of intestinal morphology Dumitrescu (1975, 1976) noticed the "northern triaenonychids" are more similar to the Travuniidae as they share a bipartate OD3, instead of the southern hemisphere triaenonychids (3-branched). As such, he placed the "northern triaenonychid" taxa into the family Paranonychidae Briggs, 1971. The name Paranonychidae was used by a few subsequent authors (e.g., Shear 1982, 1986, Ubick and Dunlop 2005), but from a classification standpoint, was not incorporated into the taxonomy as the taxa were left in the Triaenonychidae (e.g., Kury 2003a, Pinto-da-Rocha and Giribet 2007; Kury 2007b). Later, molecular phylogenetic studies also showed the "northern triaenonychids" being grouped with the travunioid families (Giribet et al. 2010, Hedin and Thomas 2010, Derkarabetian et al. 2010, Sharma and Giribet 2011), and the familial name Paranonychidae was finally included in Opiliones taxonomy by Kury (2013).

Our phylogenomic analyses allow for a reexamination of the intestinal morphology research of Dumitrescu (1975, 1976) (Suppl. material 2: Figure 2). First, the recovery of Travunioidea as the earliest diverging Laniatores lineage is reflected in the branching pattern of OD3. All Travunioidea possess an OD3 with two branches, a characteristic shared with the other harvestmen suborders, while the Synthetonychiidae, Triaenon-ychidae, and Grassatores possess three branches. This phylogenetic utility of OD3 was also noted in the morphological analyses of Mendes (2009), who recovered Travunioidea as the earliest diverging lineage of Laniatores. The intestinal complex also shows some phylogenetic value within Travunioidea; three of four families can be differentiated entirely based on intestinal morphology. Of the taxa examined by Dumitrescu

(1975, 1976), the paranonychids possess a simple and circular D1 with no branches, while all cladonychiid genera possess a relatively complex D1 with 2–3 branches or is distinctly triangular in shape. Karaman (2005) also reports a D1 with two branches for *Trojanella*. Cryptomastridae possess a triangular D1, but the shorter OD2 and OD3 are more similar to those of the paranonychids. Samples of *Briggsus* and *Speleonychia* were included and look very similar to each other, particularly the D1, which is recorded to be 3-branched in *Briggsus* and triangular in *Speleonychia* (Dumitrescu 1976). Dumitrescu did not note the similarity, instead stating *Speleonychia* was most similar to *Nippononychus japonicus* (then *Peltonychia japonica*, placed in Travuniidae), perhaps subjectively limited by the classification system of the time. It is clear that there is phylogenetic utility in midgut morphology at higher taxonomic levels, and further descriptions are needed to confirm the consistency of diagnostic characteristics noted here.

*The penis* – Based on descriptions and drawings, the musculature and glans complexity can be used to diagnose and differentiate travunioid lineages recovered here (Figure 7) (Briggs 1971b, Suzuki 1975a, 1975b, Martens 1986, Karaman 2005, Novak 2005, Pinto-da-Rocha and Giribet 2007, Derkarabetian and Hedin 2014, Starrett et al. 2016). In Travunioidea, the glans is relatively simple and plate-like without dorsal, dorsolateral, or ventral plates in *Trojanella, Travunia*, Cryptomastridae, and Cladon-ychiidae, while in *Yuria* and Paranonychidae (except *Paranonychus*) the glans is relatively complex, and some possess a dorsolateral plate. The dorsal plate is absent from all travunioids, except *Yuria*, which possesses a dorsal plate that is fused to the stylus.

Given the consistent and highly supported relationships within Paranonychidae, diagnostic differences of paranonychid lineages can be seen in penis morphology (Figure 7; Suppl. material 2: Figure 4). Within Paranonychidae, the earliest-diverging genus *Paranonychus* possesses the simplest glans. Dorsolateral plates are present but reduced in two pairs of sister genera *Sclerobunus* + *Metanonychus* and *Metanippononychus* + *Nippononychus* (Briggs 1971b, Suzuki 1975b, Derkarabetian and Hedin 2014). Additionally, *Sclerobunus* + *Metanonychus* show a distinct setae-bearing process similar to a ventral plate ("sensillenträger" of Martens) that is fused to the base of the stylus in the *Zuma* + Japanese taxa. The *Zuma* + *Izunonychus* + *Kainonychus* (late possesses a modified stylus that is expanded distally (Briggs 1971b, Suzuki 1975b). As opposed to other Japanese paranonychus + *Kainonychus* is separated from the ventral plate (Suzuki 1975b).

The overall trend across Opiliones suborders is one of apparently increasing genitalic complexity. The earliest-diverging suborder Cyphophthalmi has a spermatopositor, the Dyspnoi and Eupnoi have a simple penis with relatively little modifications, and the derived Laniatores possess the most complex penes (Macías-Ordóñez et al. 2010). Within Laniatores the trend of increasing complexity is maintained, as Travunioidea with relatively simple glans morphology is possibly the most early-diverging Laniatores. Travunioidea and Triaenonychoidea use muscles for glans expansion (Shultz and Pinto-da-Rocha 2007, Pérez-González and Werneck 2018), a condition shared with Eupnoi and Dyspnoi. Relative to travunioids, the synthetonychiids and triaenonychids have slightly more complex glans structures with dorsolateral plates. Finally, in the most diverse laniatorean lineage Grassatores, muscles are absent, and the glans requires hydraulic pressure to expand (Shultz and Pinto-da-Rocha 2007). In Travunioidea, Martens (1986) noted a tendency towards simplification of the glans, and Karaman (2005) additionally noted a correlation where simplification of the glans structure is associated with reduction of penis musculature to basal portion of the truncus. Both Martens and Karaman argued that the simple glans of travunioids (i.e., traditional travuniids) is a derived condition, as all Triaenonychoidea have relatively complex glans with dorsolateral plates and a distinct process bearing setae, two characters lost in many travunioids. Most phylogenomic analyses conducted here recover Travunioidea as the most early-diverging Laniatores lineage suggesting a trend of simple to complex penis structure. It is interesting to note that the simplest glans and some taxa with relatively reduced musculature are found in cave-inhabiting taxa (e.g., Trojanella, Dinaria, Travunia), which may be a potential confounding factor in establishing a clear evolutionary trend. More detailed morphological examinations of the male and female genitalia using modern approaches (e.g., Pérez-González and Werneck 2018) will help elucidate evolutionary trends and provide more diagnostic characters for the families established here.

# Context for future research

This phylogenomic study provides a more stable taxonomy for Travunioidea, which serves as a starting point for species-level phylogenomics and provides the phylogenetic context to explore evolutionary questions relating to character evolution, alpha taxonomy, and biogeography.

*Morphological and chemical evolution* – Many Travunioidea are cave-obligate taxa with species from 14 genera showing some degree of troglomorphy, possessing homoplastic morphological features that evolve as a response to cave life. Travunioidea can be an excellent system to study the repeated evolution of troglomorphy, as it has evolved at multiple taxonomic levels (e.g., within families, genera, species) with multiple independently derived taxa showing varying degrees of troglomorphy. For example, within the genus *Sclerobunus*, troglomorphy has evolved at least five times independently across multiple species and within single species and is time-correlated (Derkarabetian et al. 2010, Derkarabetian and Hedin 2016). A species-level phylogeny for all travunioids would allow for an accurate estimate of the number of independent evolutions of troglomorphy and allow for in-depth morphological analyses exploring the rate and timing of this potentially adaptive morphology, as well as providing the phylogenetic framework for comparative studies (e.g., gene expression).

Similarly, chemical evolution can be explored in this phylogenomic context. Harvestmen possess repugnatorial glands, which are used to store chemical cocktails that are secreted in defensive behavior. Chemical composition across lineages has been shown to have some phylogenetic value, particularly in Laniatores (Raspotnig 2012, Raspotnig et al. 2015). Similarly, a study focusing on Travunioidea and Triaenonychoidea has shown high levels of divergence in chemical composition between taxa formerly united under the traditional taxonomy (i.e., Cladonychiidae) (Shear et al. 2014). Given the phylogenetic context, detailed chemical analyses can be used to discover novel chemicals, compare pathways of identical chemicals found in independent lineages, and explore the evolution of biochemical pathways across taxa (e.g., Rodriguez et al. 2018).

Biogeography and alpha taxonomy – Cryophilic harvestmen are useful in biogeographic analyses because of restricted ecological constraints and extremely low vagility. Previous molecular phylogenetic studies on harvestmen with these biological characteristics have shown compelling biogeographic patterns (e.g., Boyer et al. 2007, Thomas and Hedin 2008, Giribet et al. 2011, Schönhofer et al. 2015, Boyer et al. 2015). The Travunioidea have a temperate Laurasian distribution with species found in eastern Asia, eastern and western North America, and central Europe, with notable absences from central Asia (Figure 2). Recent divergence dating analyses show an ancient origin of Travunioidea dating to >200 million years (Giribet et al. 2010, Sharma and Giribet 2011), although these time estimates should be revisited. Several travunioid lineages show trans-continental distributions. For example, members of the Cladonychiidae are distributed in Europe and eastern and western North America, while the Paranonychidae show a Beringian distribution with genera in western North America, Japan and South Korea. Many other harvestmen show similar broad distributions within the Holarctic including the Sironidae, Caddo, Leiobunum, and many Dyspnoi (Suzuki 1972b, Suzuki et al. 1977, Shultz and Regier 2009, Hedin et al. 2012a, Hedin et al. 2012b, Schönhofer et al. 2013).

The biological characteristics of essentially all travunioids are quite similar (e.g., dispersal-limited, restricted to cryophilic microhabitats in north temperate latitudes). Regional clades are relatively ancient, allowing ample time for the accumulation of species diversity. In addition, the presence of rare, completely blind, troglobitic species in several different geographic areas speaks to the ancient origin of the Travunioidea. It is likely that troglobitic species, especially those in central Europe, have an unknown diversity concealed by troglomorphy. Similarly, many ancient lineages can be found in the moist, coastal forests of the Pacific Northwest of North America (Briggsus, Metanonychus) that have likely persisted in refugia through climatic cycles. Additionally, all of these taxa consist of species and subspecies that are short-range endemics (Harvey 2002). Congeneric species syntopy is rare, probably because of ecological niche conservatism that prevents resource partitioning. This ecological niche conservatism likely plays an important role in speciation (see model of Wiens 2004). As such, there is a high potential for species discovery, and recent species-level studies focusing on the travunioid genera Sclerobunus and Cryptomaster have resulted in the description of new species (Derkarabetian and Hedin 2014, Starrett et al. 2016). Our ongoing studies of Travunioidea are continuing this trend: every genus currently being revised (Briggsus, Erebomaster, Theromaster, Metanonychus) shows evidence for new species.

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# Supplementary material I

# Data Table. Taxon sample and UCE sequencing results

Authors: Shahan Derkarabetian, James Starrett, Nobuo Tsurusaki, Darrell Ubick, Stephanie Castillo Marshal Hedin

Data type: species data

Explanation note: Samples highlighted in red were sequenced for Starrett et al. (2017). Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.760.24397.suppl1

# Supplementary material 2

# Figures

Authors: Shahan Derkarabetian, James Starrett, Nobuo Tsurusaki, Darrell Ubick, Stephanie Castillo Marshal Hedin

Data type: molecular data

Explanation note:

- **Figure 1.** Outgroup relationships for the 70% dataset. Nodes are fully supported (e.g., 100 bootstrap, 1.0 posterior probability, etc.) unless otherwise indicated. RAxML tree manually rooted according to BEAST topology. Asterisk in ASTRAL tree indicates node not given support values by default.
- Figure 2. Representative midgut morphology. Only the right half of the midgut is illustrated. Drawings for Triaenonychoidea (*Synthetonychia*), Grassatores (*Discocyrtus*), Cladonychiidae, and Paranonychidae redrawn from Dumitrescu (1975, 1976). Abbreviations: D1 = diverticulum 1; OD2 = opisthosomal diverticula 2; OD3 = opisthosomal diverticula 3.
- Figure 3. Representative genital morphology and distribution of *Peltonychia* in Europe. Records from Slovenia and Trieste, Italy (red) are in error. Genitalia from left to right: *P. clavigera*, redrawn from Thaler (1996); *P. leprieurii*, redrawn from Chemini (1985); *P. gabria*, redrawn from Martens (1978).
- Figure 4. SEM penis morphology of *Yuria* and Paranonychidae. Scale bars 500 mm.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

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RESEARCH ARTICLE



# A new Sky Island species of Vaejovis C. L. Koch, 1836 from Sonora, Mexico (Scorpiones, Vaejovidae)

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#### Abstract

*Vaejovis islaserrano* **sp. n.** is described from the Sierras Elenita and La Mariquita, Municipio de Cananea, Sonora, Mexico. This species belongs to the "*vorhiesi*" group of the genus *Vaejovis* and inhabits pine-oak forests in northern Mexico. This species is compared to its most similar species. This new species presents an interesting morphological difference from the rest of the species in the species-group: the absence of a subaculear tubercle or spine.

#### Resumen

Se describe *Vaejovis islaserrano* **sp. n.** de las Sierras Elenita y La Mariquita, en el Municipio de Cananea, Sonora, México. Esta especie pertenece al grupo "*vorhiesi*" dentro del género *Vaejovis* y que habita en los bosques de pino y encino del norte de México. Se le compara con las especies más similares morfológicamente. Esta nueva especie presenta una característica morfológica interesante para las especies del grupo: la ausencia de un tubérculo o espina subaculear.

#### Keywords

Diversity, pine-oak forests, scorpions, Speciation

## Introduction

Approximately 2300 species of scorpions have been described worldwide (Santibáñez-López et al. 2016). The family Vaejovidae is the most diverse scorpion family on North America, with at least 211 described species. The most recent works on Vaejovidae focused mainly on the subfamily Syntropinae (González-Santillán and Prendini 2013, 2014, 2015). In addition, seven new species in the genus *Vaejovis* C. L. Koch, 1836 within the "*vorhiesi*" complex have been described from the Sky Island mountain ranges in the southwestern United States and northwestern Mexico (Graham et al. 2012; Sissom et al. 2012; Ayrey 2012, 2013, Ayrey and Webber 2013, Ayrey and Soleglad 2014, 2015); six of the new species are from the United States and only one from Sonora, Mexico.

The Madrean Archipelago (Bezy and Cole 2014, Bezy et al. 2017) located between the northern Sierra Madre Occidental in Sonora, Mexico and the Mogollon Rim in central Arizona contains 55 Sky Islands and Sky Island complexes (mountain ranges connected by oak woodland passages), 32 of them in Sonora (Van Devender et al. 2013). Sky Islands are isolated mountain ranges with crowns of oak woodland and pine-oak forest surrounded by lowland 'seas' of thorn scrub, desert scrub, or desert grassland. The Sky Islands are well known for their high biodiversity (Van Devender et al. 2013); the fauna and flora of the upper ranges are isolated from each other, and there are endemic species in some groups, but the rates of endemism in the Sky Islands are low compared to oceanic islands (Bowers and McLaughlin 1996, Reina-Guerrero and Van Devender 2005), or the Sierra Madre Occidental near Yécora, Sonora (Van Devender and Reina-Guerrero 2016). The Vaejovis species distributed in the area are the exception, reflecting an important evolutionary radiation on Sky Island mountain tops. Bryson et al. (2013) suggested that each isolated mountain range could harbour an endemic scorpion of the "vorhiesi" species group. If this is true, several species still await discovery and description from the Madrean Archipelago.

The Sierra La Mariquita and La Elenita are two interconnected Sky Islands in northern Sonora located northwest of the copper mining town of Cananea, only 26 km south of the Arizona border. The Sierra La Mariquita and Sierra Elenita are connected by oak woodland in Puerto Cananea. They are geographical sister ranges to the Huachuca Mountains of Arizona.

On the southwestern side, the Sierras La Mariquita rise from 1230 to 2498 m and La Elenita from 1230 to 2305 m at the summits. The vegetation varies from desert grassland and oak woodland on the lower slopes to pine-oak forest above. The montane forest is dominated by Apache pine (*Pinus engelmannii*) and southwestern white pine (piñón, *Pinus strobiformis*). The Sierra Elenita has similar vegetation but the pine-oak forest is better developed in a large area. Both ranges are in the San Pedro River drainage, which flows northward into Arizona.

The Sierra La Mariquita was visited in June 2009, September 2010, and August 2013 as part of the Madrean Archipelago Biodiversity Assessment (MABA) program of the Sky Island Alliance. The Sierra Elenita was visited in September 2016 as part of the Madrean Discovery Expedition program of GreaterGood.org. Animal and plant

observations and collections from these and other Sonoran Sky Islands are available in the Madrean Discovery Expedition (MDE) database (madreandiscovery.org; linked to the MABA database). Here we describe *Vaejovis islaserrano* sp. n. in the "*vorhiesi*" complex collected during these expeditions, as an addition to the scorpion biodiversity of the Sky Island Region.

## Materials and methods

Nomenclature and mensuration follows Stahnke (1970), except for trichobothrial terminology after Vachon (1974), cheliceral dentition after Vachon (1963); metasomal, pedipalpal carination, as well as the hemispermatophore terminology after González-Santillán and Prendini (2013); metasomal setae counts, modified from Santibáñez-López and Sissom (2010); telotarsal spination and setal counts following Contreras-Félix et al. (2015); terminology for the lateral eyes follows Loria and Prendini (2014); laterobasal aculear serrations (= LAS) terminology follows Fet et al. (2006); hemispermatophores were dissected following Vachon (1952), and cleared using the technique of Álvarez-Padilla and Hormiga (2008). Higher level taxonomy of scorpions follows Sharma et al. (2015) and Prendini and Wheeler (2005). Finally, photographs of each metasomal segment using UV light were taken following the recommendations of Prendini (2003) and Volschenk (2005). Images were taken using a digital camera Leica DFC490 (8 mp) attached to a Leica Z16 APO A microscope and prepared using "Leica Application Suite-version 4.3.0 (Build: 600)". Pictures were edited with software Adobe Photoshop CS6. The map was generated with ESRI ArcGIS online suite. Finally, the measurements were taken with an ocular micrometre calibrated at 10X, and are given in millimetres.

## **Systematics**

Family Vaejovidae Thorell, 1879 Genus *Vaejovis* C. L. Koch, 1836

Vaejovis islaserrano sp. n. http://zoobank.org/998D60A1-C6C2-4D2F-81BF-361B33CD3221 Figs 1–9

**Type material.** Holotype Male, MEXICO: Sonora, Municipio Cananea, vicinity of Observatorio Astrofísico Guillermo Haro, Sierra La Mariquita (31.05444°N, 110.38244°W, 2422 m elev) 03-VIII-2013. Cols: T. R. Van Devender, J. D. Palting, and G. Molina. 1 ♂ (CNAN-T01207).

Paratypes: Same data as the holotype 4 males and 5 females (CNAN-T-01208); 2 males and 2 females (AMNH). MEXICO: Sonora, Cananea, Sierra La Elenita. Near

"El 15" (31.00252°N, 110.38944°W, 1911 m) 30-IV-2016. Cols: D. Barrales, J. Cirett, I. Ochoa. Pine-Oak forest.

**Etymology.** The specific epithet is regarding the distribution of the species in the highlands of the Sonoran desert and it is composed by the words in Spanish "isla" in reference of island and "sierra" as in mountain range, being the adjective "serrano" and together they compose the name islaserrano, which is used as a noun in apposition.

**Diagnosis.** *Vaejovis islaserrano* sp. n. belongs to the "*vorhiesi*" group due to the presence of the following characters: the presence of a sclerotized mating plug in the spermatophore; trichobothria ib - it on the base of the fixed finger of the pedipalp chela; the absence of setae on the prolateral and retrolateral sides on the first pair of legs. This is a relatively small scorpion, with adult total length ranging from 18 mm to 24 mm (Table 1). Sternite V with a noticeable whitish oval spot on the posterior fifth, also present on sternite VII. Vesicle of the telson, elongated more than twice longer than wide (L/W: 2.44), and thin, almost as wide as deep (W/D: 1.12). LAS present on both sides of the aculeus. Pedipalp chela fingers dentate margins straight, without scalloping.

*Vaejovis islaserrano* sp. n. is most similar to *Vaejovis bandido* Graham, Ayrey & Bryson, 2012, from Sierra Los Ajos, Sonora, but it is easily differentiated by the following characters: the presence of a subaculear spine in *V. bandido*, whereas in *V. islaserrano* sp. n. does present a vestigial subaculear spine: the presence of a caudal gland of the telson evident on adult males of *V. islaserrano* sp. n., whereas in *V. bandido* it is not evident; the hemispermathophore presents an apical crest on the lamella in *V. bandido*, whereas *V. islaserrano* sp. n., presents a lamella without crest. Another species closely related to *V. islaserrano* sp. n. is *V. vorhiesi* Stahnke, 1940, from the nearby Huachuca Mountains, Arizona, that can be differentiated as follows: *V. vorhiesi* presents a subaculear spine, whereas *V. islaserrano* sp. n. does not present a subaculear spine. Finally, *Vaejovis cashi* Graham, 2007, from the Chiricahua Mountain in Arizona, differs from *V. islaserrano* sp. n. in the following characters: smaller size (19 to 22 mm); a small aculear spine present in *V. cashi*, absent in *V. islaserrano* sp. n.; the hind laminar hook on the hemispermathophore present a deep depression between them in *V. cashi*.

**Description of the holotype male** (Fig. 1a, b). *Coloration:* Chelicerae, pale yellow coloration, with a black pattern on distal margin of chelae. Carapace, pale yellow coloration, with a diffuse fusco-piceus pattern. Mesososma, tergites pale yellow, with a diffuse fusco-piceus pattern. Sternites III-VII pale yellow, with a diffuse dark pattern on the sides; sternite V with a noticeable pale oval spot on posterior fifth, and sternite VII presents also a noticeable triangular spot on the posterior fourth. Metasoma, segments I-V pale yellow, with a very diffuse black pattern, more evident dorsally on each segment. Telson, pale yellow coloration with a diffuse fusco-piceus pattern present on the ventral face and additionally on the dorsal face, but faint. Pedipalp, Femur, patella and chela pale yellow with a diffuse dark coloration intense at the margins of each segment and on the carinae. Legs, pale yellow, with a diffuse dark coloration present, denser on prolateral face of femur and patella and on prolateral and retrolateral margins of basitarsus.

	<b>TT 1</b>	<b>D</b> 1	<b>D</b> 1	<b>D</b> 1	D 1
	Holotype 🔿	Paratype 🔿	Paratype 🔿	Paratype 🔿	Paratype 🔿
Total L	19.5	20.3	18.3	18.3	20
Carapace L	2.5	2.7	2.4	2.1	2.7
Carapace W	1.3	1.4	1.3	1.3	1.4
Mesosoma L	5.4	5.9	5.5	5.4	6.1
SMI L/W/D	1.2/1.5/1.2	1.3/1.5/1.2	1.1/1.4/1.1	1.2/1.5/1.2	1.21.5/1.3
SM II L	1.5	1.5	1.3	1.4	1.4
SM III L	1.6	1.6	1.5	1.5	1.5
SM IV L	2.1	2.1	1.9	1.9	2
SM V L/W/D	3/1.3/1.1	31.2/1.1	2.6/1.3/1	2.71.2/1	2.9/1.3/1.2
Metasoma L	9.4	9.5	8.4	8.7	9
Vesicle L/W/D	2.2/0.9/0.8	2.2/0.9/0.8	2/0.9/0.7	2.1/0.8/0.6	2.2/1/0.8
Femur L/W/D	2.1/0.6/0.5	2.3/0.7/0.5	2.1/0.7/0.4	2.2/0.6/0.4	2.4/0.7/0.4
Patella L/W/D	2.4/0.8/0.7	2.5/0.8/0.6	2.3/0.8/0.5	2.4/0.7/0.6	2.7/0.8/0.6
Chela L/W/D	2/0.8/0.8	2/0.8/0.8	1.7/0.7/0.8	1.8/0.8/0.8	2/0.8/0.8
Movable finger L	2.3	2.3	2	2.2	2.5
Fixed finger L	1.8	1.8	1.7	1.8	2.1
Pectinal tooth counts	13–14	14-14	13-13	14–15	14–15
	<b>Paratype</b> $\bigcirc$	Paratype $\bigcirc$	Paratype $Q$	Paratype $\mathcal{Q}$	Paratype ♀
Total L	20.3	23.8	24.1	23.1	23.9
Carapace L	3.2	3.3	3.4	3.3	3.4
Carapace W	1.7	1.8	1.9	1.8	1.9
Mesosoma L	7.4	7.8	7.9	7.4	7.6
SMI L/W/D	1/1.4/1.1	1.4/1.8/1.5	1.4/1.9/1.5	1.3/1.6/1.4	1.4/2/1.5
SM II L	1.3	1.6	1.5	1.6	1.6
SM III L	1.4	1.7	1.9	1.7	1.7
SM IV L	1.6	2.1	2.3	2.2	2.3
SM V L/W/D	2.4/1.2/1	3.31.6/1.3	2.91.6/1.4	3.21.6/1.3	3.3/1.6/1.4
Metasoma L	7.7	10.1	10	10	10.3
Vesicle L/W/D	2/0.9/0.7	2.6/1/1.1	2.8/1.2/1	2.4/1.1/0.9	2.6/1.2/0.9
Femur L/W/D	2.1/0.7/0.4	2.80.9/0.5	3/1/0.6	2.8/0.9/0.6	3/1/0.7
Patella L/W/D	2.4/0.7/0.6	3.1/1/0.8	3.2/1/0.8	3.2/1/0.8	3.4/1.1/0.9
Chela L/W/D	1.8/0.8/0.8	2.3/1/1	2.5/1.1/1.1	2.3/1.1/1.1	2.5/1.1/1.1
Movable finger L	2.1	3	3.1	2.9	3.2
Fixed finger L	1.9	2.5	2.5	2.3	2.6
Pectinal tooth counts	12–13	13–14	12-12	12–12	13–13

Table 1. Measurements on selected specimens of Vaejovis islaserrano sp. n. The measurements are given in mm.

Prosoma. *Chelicerae*: Serrula present, well-developed. Dorsal margin of movable finger with the basal denticle smaller than the median followed by two small subdistal denticles and a larger distal denticle; ventral edge of movable finger smooth. Fixed finger with basal denticle bicuspid, subdistal denticle small and distal denticle larger compared to each other. *Carapace* (Fig. 2a): Anterior margin slightly concave, almost straight; anteromedian longitudinal sulcus shallow; surface of carapace minutely granular on area surrounding the median ocelli, rest of surface granular. Ocular tubercle with superciliary carinae lower than medial ocelli; lateral ocelli type 3A (Loria and Prendini 2014).



Figure 1. Habitus of *Vaejovis islaserrano* sp. n. **a, b** Habitus of the Holotype male **c, d** Paratype female **a, c** dorsal view **b, d** ventral view. Scale bars: 5 mm.



Figure 2. a Carapace of the holotype male of *Vaejovis islaserrano* sp. n. dorsal view b Holotype mesosoma, ventral view, showing the pectinal teeth and sternite. Scale bar: 1 mm.

*Mesosoma:* Tergites I-II, shagreened, with a granular pattern confined to posterior margin; tergites III-VI with anterior half shagreened and posterior half noticeably granular, with median carina present on posterior half of each segment (Fig. 2b). Tergite VII with strongly developed submedian and lateral carinae, paramedian carinae reaching posterior margin; intercarinal surface noticeably granular. Sternites III-VI smooth, slightly granulated on posterolateral margins; sternite VII intercarinal surface shagreened, slightly granular on the sides and with 11 setae; lateral carinae strong, composed by a row of aggregated granules. Pectinal tooth count: 13–14 (Fig. 2b).

Metasoma (Fig. 3a-c): Dorsal lateral and lateral median carinae on segments I-IV strong, composed by a single line of granules and the distalmost slightly larger than the preceding (Fig. 3a); lateral inframedian carinae on segments I-III strong, composed by a single row of granules and present along the entire segment, on segment IV vestigial, composed by small scattered granules on distal half (Fig. 3b); ventral lateral carinae on segments I-IV strong, composed by a single row of granules; ventral submedian carinae on segment I weak, composed by a row of low granules just above the surface, on segments II-IV, strong, composed by a single row of raised granules. Dorsal and lateral intercarinal surfaces minutely granular, and on ventral face shagreened (Fig. 3c). Segment V: Dorsal lateral carinae strong, composed by a single row of granules on anterior half, wider with scattered granules on posterior half; lateral median carinae strong, composed by an irregular row of granules and present on basal two thirds; ventral lateral carinae strong, composed by a single row of granules; ventral median carina strong, composed by a single row of granules and not reaching posterior margin. Setae count on metasomal segments I-IV as follows: DL: 0/0/1/2; LM: 1/1/0/3; LI: 1/1/0/3; VL: 2/2/0/3; VS: 2/2/0/3. On segment V: DL: 3; LM: 2-3; VL: 3; VM: 3 (Full variation of setal counts in the metasoma, is given in Table 2).



**Figure 3.** Detail of the metasoma, from the Holotype male. **a** Dorsal view **b** Lateral view **c** Ventral view. Scale bar: 2 mm.

*Telson* (Fig. 4): Vesicle elongated, more than twice longer than wide (L/W: 2.44), and thin, almost as wide as deep (W/D: 1.12). Subaculear tubercle vestigial to absent (Fig; 4a) Glandular area on the dorsal face present on distal third, and longer than wide (Fig. 4b). Surface of vesicle smooth on ventral and dorsal faces. LAS present on both sides of the aculeus.

*Pedipalp* (Fig. 5): Orthobothriotaxic type "C". *femur* (Fig. 5a) more than three times longer than wide (L/W: 3.5) and slightly wider than deep (W/D: 1.2); dorsal retrolateral and dorsal prolateral carinae strong, composed by an irregular line of granules; prolateral ventrosubmedian carina strong, composed by a line of large granules along the segment; prolateral ventral carina, vestigial, only present by two larger, separate granules; ventral prolateral carina strong, composed by several rows of aggregated granules; ventral median and retrolateral ventral carinae strong, composed by a line of granules; ventral median carina strong, composed by several rows of aggregated granules; ventral median and retrolateral ventral carinae strong, composed by a line of granules; ventral median carina undistinguishable from other



**Figure 4. a** Male telson lateral view, showing the vestigial subaculear tubercle or spine **b** telson dorsal view, showing the faint and elongated shape of the caudal gland, which in this picture is highlighted with a white oval. Scale bars: 1 mm **a**, 5 mm **b**.

granules of the ventral surface; ventral retrolateral carina weak and smooth; retrolateral dorsosubmedian carina strong, composed by an irregular row of larger granules; intercarinal spaces all surfaces are granular. *Patella* (Fig. 5b–e): Three times longer than wide (L/W: 3) and wider than deep (W/D: 1.2). Dorsal prolateral and dorsal retrolateral carinae strong, composed by several rows of granules; prolateral subdorsal carina absent; prolateral median carina strong, composed by a line of scattered large granules; ventral prolateral carina strong, composed by a line of granules; ventral median carina strong, composed by a line of granules; ventral median carina strong, composed by a line of granules; ventral median carina strong, composed by a line of granules; ventral median carina strong, composed by a line of granules; ventral median carina strong, composed by a line of granules; retrolateral median and retrolateral carina strong, composed by a line of granules; retrolateral median and retrolateral dorsosubmedian carinae weak, almost absent, composed by scattered small granules and a slight costa. Intercarinal spaces shagreened with some scattered granules on ventral face. *Chela* (Fig. 6a–d): Manus more than twice longer than wide (L/W: 2.5) and as wide as deep (W/D: 1). Dorsal retrolateral carina weak, with a costa and some small granules; retrosubmedian accessory carina weak, composed by several rows of aggregated small granules; dorsal median carina weak, composed by a costa



**Figure 5.** Detail of the segments in the pedipalp of the Holotype male of *V. islaserrano.* **a** Femur, dorsal view **b** Patella, ventral view **c** Patella, retrolateral view **d** Patella, dorsal view **e** Patella, prolateral view. Scale bars: 1 mm.



**Figure 6.** Detail of the chela in *V. islaserrano*. **a** Chela holotype male, retrolateral view **b** Chela holotype, prolateral view **c** Chela holotype, ventral view **d** Chela holotype, dorsal view **e** Chela of the a paratype female of *V. islaserrano* sp. n. retrolateral view. Scale bars: 1 mm.



**Figure 7. a** Hemispermatophore of a paratype male of *Vaejovis islaserrano* sp. n. ectal view **b** Hemispermatophore of a paratype male of *V. islaserrano* sp. n. ental view. Scale bars: 0.5 mm **a, c**; 0.2 mm **b**.

and some small granules; dorsal prosubmedian and dorsal prolateral carinae strong, composed by several rows of aggregated granules; prolateral dorsal, ventral median, ventral prosubmedian, retrolateral subventral accessory and retrolateral dorsal carinae absent; prolateral median and prolateral ventrosubmedian carinae strong, composed by a row of aggregated large granules; ventral prolateral and prolateral ventral carinae vestigial, almost absent, composed by a slight costa and some scattered granules; ventral retrolateral carina faint, almost absent; retrolateral subventral carina weak, only present as costa; retrolateral median carina faint, only differentiated by a small line of granules and a slight costa. Intercarinal surfaces shagreened. Dentate margins of the pedipalp chela fingers straight; fixed finger with five inner accessory denticles, movable finger with six inner accessory denticles.

					Metasomal setae o	counts				
DL	0/1/1/2/3	0/1/1/2/3	0/1/1/2/3	0/1/1/2/3	0/1/1/2/3	1-0/1/1/2/3	1-0/1-0/1/2/3	1-0/1/1/2/3	0/1/1/2/3	0/1/1/2/3
LM	0/1/1/2/3-2	0/1/1/2/2	0/1/1/2/2	0/1/1/2/2	0/1/1/2/2	1/1/2-1/2/3	0/1/1/2/2	0/1/1/2/3	0/1/1/2/2-3	0/1/1/2/2
LI	1/0/0/0	1/0/0/0	1/0/0/0	1/0/0/0	1/0/0/0/	1/0/0/0/	1/0/0/0/	1/0/0/0/	1/0/0/0/	1/0/0/0/
٨L	2/3/3/3/3	2/3/3/3/3	2/3/3/3/3	2/2/3/3/3	2/3/3/3/3	2/2-3/3/3/3	2/3/3/3/3	2/3/3/3/3	2/3/3/3/3	2/3/3/3/3
VS/M	3/3/3/3/3	3/3/3/3/3	3/3/3/3/3	3/3/3/3/3	3/3/3/3/3	3-1/3/3/3/3	3/3/3/3/3	3/3/3/3/3	3/3/3/3/3	3/3/3/3/3

Table 2. Metasomal setal counts on selected segments of the type series of Vaejovis islaserrano sp. n. Abbreviations: DL: Dorsal lateral; LM: Lateral median; LI: Lateral inframedian; VL: Ventral lateral and VS/M; ventral submedian/median carinae.

Table 3. Telotarsi setal counts on selected specimens of the type series of Vaejovis islaserrano. Abbreviations: DTS: Distal terminal setae; Pi/Ri: Prolateral internal/ Rotrolateral internal.

	2 2-2-2-2 2-2-2-2 2-2-2-2	2 2-2-3-x-x 2-2-x-x 2-2-2-2		1:1/1 0/0:1/1:1/11 0/0:1/1:x/x:1/1 0/0:1/1:1/1	1:1/2 0/0:1/1:1/1:x/x 0/0:1/1:x/x:x/x 0/0:1/1:1/1	
Legs Distal Terminal Setae counts	-2-2-2 x-2-2-	-2-2-2 2-4-2-	ae counts	/1:1/1:1/1 x/x:1/1:1/	/1:1/1:1/1 0/0:1/1:1/	
	2-2-2-2	2-2-2-2	al and retrolateral setae co	0/0:1/0:1/1:1/1 0/0:1/	0/0:1/1:1/1:1/1   1/0:1/	
	2-3-2-2	2-2-2-2	Prolater	0/0:1/0:1/1:1/1 0	0/0:1/1:1/1:1/1 0	
	2-2-2-2	2-2-2-2		0/0:1/1:1/1:1/1	0/0:1/1:1/1:1/1	
	2-2-2	2-2-x-2			0/0:1/1:1/1:1/1	0/0:1/1:X/X:1/1
	4-4-4-4	4-4-4		0/0:1/1:1/1:1/1	0/0:1/1:1/1:1/1	
	5 TC	017		- /:U	глп	



Figure 8. Map showing the type locality where *Vaejovis islaserrano* sp. n. was collected, and the distribution of the other three geographical and morphological closer species. Key: red star: *Vaejovis islaserrano* sp. n.; blue square: *V. bandido*; green rhombus: *V. cashi*; orange circle *V. vorhiesi*.

*Legs:* Telotarsi on legs I-IV with a single line of spinules ventrally and with two distal spinules on each leg (Table 3). Prolateral and retrolateral setae on the telotarsi as follows: 0/0:1/1:1/1:1/1.

*Hemispermatophore* (Fig. 7): Lamelliform (total length: 1.7; Lamella length: 1; width: 0.6mm). Lamella with a weak basal constriction at level of laminar hooks; dorsal trough long; mating plug present, with the distal barb margin smooth.

*Variation*: The sexual dimorphism in the species is little, but the total length of adult males and females differ by 18.3 to 20.3 mm on males and 20.3 to 24.1 mm on females; the presence of a white patch on mesosomal sternite V and the dorsal face of vesicle present on males and absent in females. The inner denticles, on the pedipalp chela movable finger, vary from five (on three specimens) to six (eight specimens). Carapace longer than pedipalp femur in males (CL/FL: 1.18) than in females (CL/FL: 1.5), but shorter than metasomal segment V (CL/MS V: 0.8) in males, whereas in females it is longer than metasomal segment V (CL/MS V: 1.33). Mesosomal sternite VII, setal counts ranges between eleven and twelve setae. Full variation of measurements is given in Table 1.

**Distribution.** This species is known from a few localities in the higher elevations of the Sierra La Mariquita and Sierra La Elenita in Sonora, Mexico at 1911–2422 m. This currently represents the southwestern-most record for the "*vorhiesi*" group of the genus *Vaejovis* (Fig. 8).



**Figure 9. a** Life female dorsal habitus in sight **b** type locality of *V. islaserrano* sp. n., showing the mixed pine-oak vegetation where it lives.

**Natural history** (Fig. 9). The specimens of *V. islaserrano* sp. n., were collected in August 2013 and September 2016. This species inhabits rocky slopes in pine-oak forest. (Fig. 9b). It was observed active on a cold rainy night, foraging in pine needle litter and living sympatric with *Paravaejovis spinigerus* (Wood, 1863), which inhabits open, rocky outcrops in the same areas.

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RESEARCH ARTICLE



# Two new species of the millipede family Cambalopsidae from Myanmar (Diplopoda, Spirostreptida)

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#### Abstract

Two new species of cave-dwelling millipedes are described from Myanmar, one each in the genera *Plusio-glyphiulus* Silvestri, 1923 and *Trachyjulus* Peters, 1864. *Plusioglyphiulus digitiformis* **sp. n.** joins the small peculiar group of congeners from Thailand which is characterized by such plesiomorphies as the tergal crests on the collum and following metaterga being transversely divided into two, not three parts, as in species of the genus *Glyphiulus* Gervais, 1847. However, this new species differs by the 3-segmented telopodites of  $\delta$  legs 1, the anterior gonopodal coxosternum showing higher and nearly straight apicomesal processes and very evident apicolateral teeth, as well as the higher and acuminate paramedian coxal processes of the posterior gonopods, the latter's telopodites demonstrating an apical fovea bearing a group of microsetae at the bottom. *Trachyjulus bifidus* **sp. n.** is primarily distinguished by the telopodites of their anterior gonopods being strikingly and deeply bifid. A key to the five species of Cambalopsidae currently known to occur in Myanmar is presented, and a map showing their distributions given.

#### Keywords

diplopod, key, map, Myanmar, new species, Plusioglyphiulus, Trachyjulus

# Introduction

Myanmar is globally recognized as a highly important hotspot of biodiversity, supporting a great number of species and abundant forest resources (Myers et al. 2000). Unfortunately, by 2010 the deforested areas in Myanmar totalled 21,178.8 km<sup>2</sup>, with an annual deforestation rate of 0.81% between 1990 and 2010 (Wang and Myint 2016). Yet some regions, especially montane ones, remain rich in woodlands, including primary tropical forest.

Cambalopsidae is the largest family in the suborder Cambalidea, order Spirostreptida, and it currently contains > 100 species in 7–8 genera, all in Southeast Asia and Indo-Australia, up to central China in the north and Borneo in the east (Hoffman 1980, Mauriès 1983). Only a couple of anthropochore species have attained particularly vast pantropical distributions.

Pocock (1893) was the first to describe cambalopsids from Myanmar, three new species currently referred to as *Trachyjulus calvus* (Pocock, 1893), *Podoglyphiulus doriae* (Pocock, 1893) and *P. feae* (Pocock, 1893). According to the latest catalogue of the Diplopoda of Myanmar (Likhitrakarn et al. 2017), the fauna of that country currently amounts to 92 species, including those first three cambalopsids of Pocock.

After more than 120 years of complete inactivity in this respect, the present paper puts on record another two new species of Cambalopsidae from Myanmar. It also provides a key to all five species of this family in that country, as well as a map showing their distributions. The two new species described below are also the first to come from caves in Myanmar.

#### Material and methods

The material was collected in Myanmar in 2015–2016 by Somsak Panha and members of the Animal Systematics Research Unit, Chulalongkorn University, as well as by a French collecting team headed by Louis Deharveng, of the Muséum National d'Histoire Naturelle, Paris, France. Photographs of live animals were taken in the laboratory using a Nikon 700D digital camera with a Nikon AF-S VR 105 mm macro lens. Specimens were preserved in 75% ethanol, and morphological observations made under an Olympus SZX7 stereo microscope.

Scanning electron micrographs (SEM) were taken applying a JEOL, JSM-5410 LV microscope, and the material returned to alcohol upon examination. Pictures of the gonopods of the holotypes were taken in the laboratory and assembled using "Cell<sup>D</sup>" automontage software of the Olympus Soft Imaging Solution package. The key below is primarily based on the descriptions by Golovatch et al. (2007a, 2007b, 2009, 2011). One of the holotypes, as well as most of the paratypes are housed in the Museum of Zoology, Chulalongkorn University (CUMZ), Bangkok, Thailand. The other holotype and several paratypes are stored in the Muséum national d'Histoire naturelle (MNHN), Paris, France, while a few paratypes are deposited in the collection of the

Zoological Museum, State University of Moscow (ZMUM), Russia, as indicated in the text.

The collecting sites were located by GPS using the WGS84 datum.

The carinotaxic formulae in the descriptions follow those in Golovatch et al. (2007a, b, 2009, 2011), while body segment counts are after Enghoff (1993).

#### Taxonomic part

# Family Cambalopsidae Cook, 1895 Genus *Plusioglyphiulus* Silvestri, 1923

Plusioglyphiulus digitiformis sp. n.

http://zoobank.org/0F9B6EEB-4144-48BE-B71E-F4422E7AFA13 Figs 1–4

**Holotype** ♂ (CUMZ), Myanmar, Shan State, Taunggyi, Hopong, Parpant area, cave, 20°43'30"N, 97°08'04"E, 23.09.2015, leg. C. Sutcharit and R. Srisonchai.

**Paratypes.** 7  $\Diamond$ , 18  $\bigcirc$  (CUMZ), same data as holotype. 2  $\Diamond$ , 1  $\bigcirc$  (MNHN, MY15-16/09), Shan State, Jatwet Gu (Linwe Depression Cave #2), limestone, 21°13'40"N, 96°33'24"E, 29.11.2015; 1  $\bigcirc$  (MNHN, MY15-17/10 (SS06)), same State, Kyauk Khaung Cave (Stone Cave), limestone, 21°11'28"N, 96°33'09"E, 29.11.2015; 9  $\Diamond$ , 17  $\bigcirc$ , 1 juv. (MNHN, MY15-18/06), same State, Mondawa Gu Cave, limestone, 20°45'17"N, 97°01'03"E, 01.12.2015; 7  $\bigcirc$  (MNHN, SS11), same locality, 21.09.2015; 13  $\Diamond$ , 15  $\bigcirc$  (MNHN, MY15-20/04), same State, Parpent Cave n°1, Guano, limestone, 20°51'03"N, 97°14'23"E, 02.12.2015; 2  $\Diamond$ , 3  $\bigcirc$ , 4 juv. (MNHN, SS15), same locality, 23.09.2015; 6  $\Diamond$ , 17  $\bigcirc$  (MNHN, MY15-21/07), same State, Parpent Cave n°2, Guano, limestone, 20°51'04"N, 97°14'28"E, 02.12.2015, all leg. F. Bréhier.

**Other material.**  $4 \circlearrowleft, 14 \heartsuit$  (MNHN, MY15-14/09),  $2 \circlearrowright, 3 \heartsuit$  (ZMUM), Mon State, Saddan Sin Gu Cave, limestone, tower karst,  $16^{\circ}31'43"N$ ,  $97^{\circ}43'02"E$ , 26.11.2015; 1 juv. (MNHN, MY15-15/07), same State, Nathack Gu Cave (Two Level Cave), limestone, tower karst,  $16^{\circ}31'33.5"N$ ,  $97^{\circ}42'48.8"E$ , 26.11.2015, all leg. F. Bréhier.

**Etymology.** To emphasize the finger-shaped apicomesal coxoternal processes (**acp**) of the anterior gonopodal coxosternum; adjective.

**Diagnosis.** This new species is apparently most similar to *P. antiquior* Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011, from a cave in Kanchanaburi Province, Thailand (Golovatch et al. 2011), in sharing the special the carinotaxic formulae of the collum and postcollum rings (Fig. 2A–C, H, I, N, O),  $\mathcal{J}$  legs 1 with a short central hook (Figs 3A, B, 4C),  $\mathcal{J}$  legs 2 with modestly enlarged telopodites (Figs 3D, 4D), coupled with the simple plate-like anterior gonopods (Figs 3G, H, 4G), the complex posterior gonopods in which the coxites are densely setose paramedially and each supplied with an evident fovea, and the telopodites are evident and digitiform (Figs



**Figure 1.** *Plusioglyphiulus digitiformis* sp. n., **A**, **B**,  $\bigcirc$  paratype from Parpant area, live animal. Pictures by R. Srisonchai, not to scale.

3K, L, 4H, I). However, the new species differs from *P. antiquior* in the more clearly divided crests on metaterga, the lateral ones being somewhat higher, coupled with the 3-segmented telopodites of  $\Im$  legs 1, the anterior gonopodal coxosternum showing higher and nearly straight apicomesal coxoternal processes (acp) and very evident basolateral coxosternal processes (bcp), as well as the higher and acuminate anterior coxal processes (ap) of the posterior gonopods, the latter's telopodite (te) demonstrating an apical fovea that bears a group of microsetae at the bottom (Figs 3K, L, 4H, I).

**Description.** Length of holotype ca. 18 mm; adult paratypes 12-27 ( $\stackrel{\circ}{\circ}$ ) or 13-29 mm ( $\stackrel{\circ}{\ominus}$ ); midbody segments circular in cross-section (Fig. 2K), width in holotype 0.9 mm; paratypes 0.8-1.0 ( $\stackrel{\circ}{\circ}$ ,  $\stackrel{\circ}{\ominus}$ ).

Colouration of live animals light red-brown (Fig. 1) with lighter anterior and posterior parts of body; antennae, venter and legs light yellowish; coloration in alcohol, after two years of preservation, uniformly light red brownish to dark castaneous brown,



**Figure 2.** *Plusioglyphiulus digitiformis* sp. n., **A–C, F, H–J, L, M**  $\bigcirc$  paratype from Parpant area **D, E, G, K, N–P**  $\bigcirc$  paratype from Parpant area. **A, B** anterior part of body, lateral and dorsal views, respectively **C** collum and body ring 2, dorsal view **D** head, ventral view **E** anterior part of antenna, lateral view **F** second body crest, dorsal view **G** bacilliform sensilla on antennomere 5, lateral view **H, I** midbody rings, lateral and dorsal views, respectively **J** porostele, lateral view **K** cross-section of a midbody segment **L** midbody crests, dorsal view **M** midbody prozona, dorsal view **N–P** posterior part of body, lateral, dorsal and ventral views, respectively.



**Figure 3.** *Plusioglyphiulus digitiformis* sp. n.,  $\eth$  paratype from Parpant area. **A, B**  $\eth$  legs 1, caudal and anterior views, respectively **C** claw of  $\eth$  leg 1, anterior view **D**  $\circlearrowright$  legs 2, caudal views **E, F**  $\circlearrowright$  legs 3, anterior and caudal views, respectively **G, H** anterior gonopods, anterior and caudal views, respectively **I** microsetae on top of coxal processes of anterior gonopods, caudal view **K, L** posterior gonopods, caudal and anterior views, respectively **J** tip of telopodite of posterior gonopod, caudal view **M** setose lobe on telopodite of posterior gonopod, anterior view.



Figure 4. *Plusioglyphiulus digitiformis* sp. n., **A**, **B** ♂ paratype from Mondawa Gu Cave **C**-**H** ♂ paratype from Parpent Cave n°2. **A** antenna, lateral view **B** gnathochilarium, ventral view **C** legs 1, anterior view **D** legs 2, caudal view **E** legs 3, caudal view **F** midbody leg, anterior view **G** anterior gonopods, caudal view **H**, **I** posterior gonopods, caudal and anterior views, respectively. Abbreviations: **acp** apiconmesal coxoternal processes **bcp** basolateral coxosternal process **te** telopodites **ap** anterior coxal processes **cp** caual coxal processes **pp** paramedian coxal processes. Scale bars: 0.2 mm.

dorsal crests and porosteles usually dark brownish. Antennae and venter yellow brownish to brownish. Ommatidia brown to blackish.

Adult body with 46p+3a+T (holotype); paratypes with 37–60p+1–4a+T ( $\mathcal{O}$ ) or 36–66p+1–4a+T ( $\mathcal{Q}$ ). Eye patches transversely ovoid, with 3+(1–2) blackish, rather flat ommatidia in 1–2 longitudinal rows. Antennae short and clavate (Figs 1, 2A, 2B, 2D, 2E, 4A), extending behind ring 4 laterally, antennomeres 5 and 6 each with a small apicodorsal field or corolla of bacilliform sensilla (Figs 2E, 4A). Gnathochilarium oligotrichous, each lamella lingualis with 3–4 setae; mentum undivided (Fig. 4B).

In width, collum = midbody rings (close to  $13^{th}$  to  $15^{th}$ ) > head = ring 4 > 10 > 9 > 8 > 7 > 6 > 4 = 5 > 2 > 3; body abruptly tapering towards telson on a few posteriormost rings (Fig. 2N–P). Postcollar constriction evident due to only a moderately enlarged collum (Fig. 2B, C).

Collum with 6+6 longitudinal crests starting from anterior edge, carinotaxic formula of collum, 1+2p+3+4p/t+5p/t+pp+/ma (Fig. 2A–C).

Following metaterga similarly strongly crested (Figs 1, 2A–C, H, I, L, N, O), especially so from ring 5 onwards, whence porosteles commence, these tubercles clearly reduced on legless segments where ozopores are missing (Fig. 2N). Porosteles large, high, conical, round, directed caudolaterad, wider than high (Fig. 2J); ozoporiferous crests distinctly divided into two about midway, their anterior halves being higher (Fig. 2A, B, C, H, I, L, N, O). Carinotaxic formulae of metaterga 2–4, 2+2/2+M+2/2+2 (Fig. 2A, B); usual formula of following metaterga, 2/2+I/i+3/3+I/i+2/2 (Fig. 2A, B, H, I, L, N, O); all crests and tubercles low.

Tegument delicately alveolate-areolate (Fig. 2B, H, I, L, M, N, O), dull throughout. Fine longitudinal striations in front of stricture between pro- and metazonae, remaining surface of prozonae very delicately shagreened (Fig. 2F, L). Metatergal setae absent. Segments 2 and 3 each with long pleural flaps.

Limbus extremely finely and more or less regularly denticulate.

Epiproct (Fig. 2N–P) broadly rounded apically, with 1+1 paramedian tubercles at midway. Paraprocts rather clearly flattened, each with a faint premarginal sulcus medially (Fig. 2P). Hypoproct emarginated at caudal margin (Fig. 2P)

Ventral flaps behind gonopod aperture on  $3^\circ$  segment 7 barely distinguishable as low swellings, forming no marked transverse ridge.

Legs nearly as long as body diameter (Fig. 2K), claw at base with a strong accessory claw almost half as long as claw itself (Fig. 4F).

Å legs 1 with an unusually short, central hook and relatively strongly reduced, 3-segmented telopodites (Figs 3A, B, 4C), each with a small and sharp claw (Fig. 3C).

 $\Diamond$  legs 2 clearly enlarged, with high and large coxae; telopodites hirsute on anterior face; penes broad, oblong-subtrapeziform, fused at base, each with 3–4 strong setae distolaterally (Figs 3D, 4D).

å legs 3 modified in having coxae especially slender and elongate, but with somewhat shortened telopodites (Figs 3E, F, 4E).

Anterior gonopods (Figs 3G–I, 4G) with a typical shield-like coxosternum, the latter modestly setose on caudal face and provided with a concave notch separating a pair of high, nearly straight, terminally rounded, apicomesal, coxosternal processes

(acp) and a much lower basolateral coxosternal processes (bcp), these being rounded at tip; telopodite (te) typical, rather stout, movable, 1-segmented, lateral in position, with several strong apical setae and a field of small microsetae at base, slightly longer than adjacent bcp.

Posterior gonopods (Figs 3J–M, 4H, I) highly compact, contiguous basally until about midheight; each with a densely setose paramedian coxal process (pp) (Fig. 3M) and with two higher central pieces: anterior coxal process (ap) elongate, distally represented by an acuminate lamina; caudal coxal process (cp) subtriangular, membranous, rounded at tip; each telopodite (te) vase-shaped, with a compact group of coniform microsetae placed at bottom of an apical fovea (Fig. 3J), with another, parabasal field of microsetae on anterior face (Figs 3L, 4H, I).

**Remarks.** The genus *Plusioglyphiulus* Silvestri, 1923 has recently been reviewed (Golovatch et al. 2009, 2011) and shown to comprise 27 species ranging from northern Thailand and Laos in the west to Borneo in the east and southeast. This new species is the first *Plusioglyphiulus* to be recorded from Myanmar. Based on the pigmented body and eye patches, and like most if not all other cave-dwelling congeners known to date, *P. digitiformis* sp. n. seems to be hardly more than a troglophile.

Most species of this genus show particularly enlarged colla with the tergal crests both on the collum and following segments being clearly divided transversely into three parts. Only two species, *P. antiquior* and *P. panhai* Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011, both from caves in Thailand and both found quite close to the frontier to Myanmar, are remarkable in still showing the pattern of carinotaxy observed in the genus *Glyphiulus* Gervais, 1847 (Golovatch et al. 2011).

In particular, while their gonopods are relatively complex and unequivocally the same as in typical *Plusioglyphiulus*, the carinotaxic pattern is simple and typical of *Glyphiulus*, i.e., the crests on their colla and following metaterga are divided transversely into two, not three, parts. In this respect, *P. digitiformis* sp. n. clearly joins the above duet, showing the closest similarities, both morphologically and geographically, to *P. antiquior*.

Non-type material shows all characters of the type series, but their localities lie very far from the others (ca. 470 km) (Fig. 8). We hope that future molecular studies will answer the question of the conspecificity (or not) of all above populations.

Interestingly, the famous Burmese amber, 99–100 Mya, appears to contain a typical *Plusioglyphiulus* yet to be described (Wesener in litt.). This is evidence both of the very old age of this genus and its long presence *in situ*.

## Genus Trachyjulus Peter, 1864

*Trachyjulus bifidus* sp. n. http://zoobank.org/73DE8D2F-8205-4CC9-9B72-8F802F569454 Figs 5–7

**Holotype**  $\delta$  (CUMZ), Myanmar, Tanintharyi Region, San Gu Cave (Elephant Cave), limestone, tower karst, 11°13'55"N, 99°10'32"E, 17.11.2015, leg. F. Bréhier.

**Paratypes.** 3  $\Diamond$ , 1  $\bigcirc$ , 3 juv. (CUMZ), 1  $\Diamond$ , 3 juv. (MNHN, MY15-01/01), same data as holotype. 6  $\Diamond$ , 7  $\bigcirc$ , 4 juv. (MNHN, MY15-02/27), 2  $\Diamond$ , 2  $\bigcirc$  (ZMUM), same Region, Yae Gu Cave (River Cave), limestone, tower karst, 11°13'05"N, 99°10'32"E, 21.11.2015; 12  $\Diamond$ , 10  $\bigcirc$ , 5 juv. (MNHN, MY15-07/13), same Region, Linno Gu n°1 Cave, guano, limestone, tower karst, 76 m a.s.l., 11°13'35"N, 99°10'32"E, 19.11.2015, all leg. F. Bréhier. 3  $\Diamond$ , 2  $\bigcirc$  (MNHN, MY15-09), same Region, Thin Bow Gu Cave (Linno Gu #2), limestone, tower karst, 11°11'23"N, 99°10'18"E, 03.06.2015, leg. C. Rahmadi.

**Etymology.** To emphasize the strongly bifid telopodites of the anterior gonopods; adjective.

**Diagnosis.** Differs from other *Trachyjulus* species based primarily on the following combination characters: the strongly elongated and bifid telopodites (te) of the anterior gonopods, coupled with the absence of flagella and the presence of deeply bipartite posterior gonopods, in which the telopodites (te) are much shorter than the massive, paramedian, coxal processes (cp).

**Description.** Length of holotype ca. 19 mm; adult paratypes 13–30 ( $\eth$ ) or 12–25 mm ( $\updownarrow$ ); midbody segments circular in cross-section (Fig. 5N), width of holotype 1.0 mm, of paratypes 0.8–1.0 ( $\eth$ ) or 0.8–1.1 mm ( $\clubsuit$ ).

Coloration of adults in alcohol light grey-brown to dark castaneous brown, without a clear-cut pattern. Head, antennae and venter light yellowish to brownish. Ommatidia brown to blackish.

Adult body with 45p+4a+T (holotype); paratypes with 39–70p+2–4a +T ( $\mathcal{C}$ ) or 40–60p+2–6a+T ( $\mathcal{Q}$ ). Eye patches transversely ovoid, with 3(4)+3(1) blackish, rather flat ommatidia in 1–2 longitudinal rows. Antennae short and clavate (Figs 5A, B, D, E, 7A), extending behind segment 5 laterally (Fig. 5A), antennomeres 5–7 each with a small apicodorsal group or corolla of bacilliform sensilla (Figs 5A, F, G, H, 7A), surface at base of antennomere 5 very finely scaly (Fig. 5F, I). Gnathochilarium (Figs 5E, 7B) oligotrichous, each lamella lingualis with 3–4 setae; mentum single.

In width, collum = midbody rings (close to  $6^{th}$  to  $8^{th}$ ) > head = ring 2 > 8–10 > 7 > 6 > 5 > 3 = 4; body abruptly tapering towards telson on a few posteriormost rings (Fig. 5R, S).

Collum (Fig. 5A–C) smooth, only near lateral edge with 1–3 light, short, superficial striae (Fig. 5A). Postcollar metaterga clearly, but not particularly strongly carinate (Figs 5A, B, J, K, L, R, S), especially so from segment 5 on, whence porosteles commence, these becoming completely absent from legless segments where ozopores are missing (Fig. 5R). Porosteles large, but low, conical, round, directed caudolaterad, wider than high (Fig. 5Q). Carinotaxic formula of metaterga 2–4, 7/7+m/m+7/7 (Fig. 5A–C). Carinotaxic formulae of following segments typically 10–7/10–7+I/i+2/2+m/m (Fig. 5A, B, C, J, K, L, R, S); all crests and tubercles, including porosteles, low.

Tegument smooth (Fig. 5A, B, J, K, L, R, S), dull throughout. Fine longitudinal striations in front of stricture between pro- and metazonae, remaining surface of prozonae very delicately shagreened (Fig. 5J, K, L, R). Metatergal setae absent. Segments 2 and 3 each with long pleural flaps.



**Figure 5.** *Trachyjulus bifidus* sp. n., **A–C**  $\bigcirc$  paratype from Linno Gu n°1 Cave **D–U**  $\bigcirc$  paratype from Linno Gu n°1 Cave. **A, B** anterior part of body, lateral and dorsal views, respectively **C** collum and body ring 2, dorsal view **D, E** head, anterior and ventral views **F** anterior part of antenna, ventral view **G** bacilliform sensilla on antennomere 5, lateral view **H** tip of antenna **I** base of antennomere 5, lateral view **J, K, O** midbody rings, lateral, dorsal and ventral views, respectively **L** midbody ring, dorsal view **M** midbody prozona, dorsal view **N** cross-section of a midbody ring **P** claws of midbody legs **Q** midbody porostele, dorsal view **R–U**, posterior part of body, lateral, dorsal and ventral views, respectively.



**Figure 6.** *Trachyjulus bifidus* sp. n.,  $\bigcirc$  paratype from Linno Gu n°1 Cave. **A, B** legs 1, anterior and caudal views, respectively **C** leg 2, caudal view **D** penes, caudal view **E** legs 3, caudal view **F, G** anterior gonopods, anterior and caudal views, respectively **H** tip of telopodite of anterior gonopod, caudal view **I, J** posterior gonopods, anterior and caudal views, respectively **K** right posterior gonopod, caudal view.



**Figure 7.** *Trachyjulus bifidus* sp. n.,  $\Diamond$  holotype from San Gu Cave. **A** antenna, lateral view **B** gnathochilarium, ventral view **C** legs 1, anterior view **D** legs 2, caudal view **E** legs 3, caudal view **F** posterior gonopods, caudal view **G H** anterior gonopods, anterior and caudal views, respectively. Abbreviations: **cp** coxal processes **te** telopodites **acp** anterior coxosternal process **pcp** posterior coxoternal process. Scale bars: 0.2 mm.



**Figure 8.** Distribution of two new cambalopsid species. Key:  $\Box$  *Plusioglyphiulus digitiformis* sp. n., Jatwet Gu and Kyauk Khaung Cave  $\blacksquare$  *Plusioglyphiulus digitiformis* sp. n., Mondawa Gu Cave  $\triangle$  *Plusioglyphiulus digitiformis* sp. n., Cave in Parpant area  $\bullet$  *Plusioglyphiulus digitiformis* sp. n., Parpent Cave n°1 and Parpent Cave n°2  $\diamond$  *Plusioglyphiulus digitiformis* sp. n., Sandu Cave, Yae Gu Cave, Linno Gu n°1 Cave and Thin Bow Gu Cave.

Epiproct (Fig. 5R–U) simple, bare, smooth, regularly rounded caudally. Paraprocts smooth, rather regularly convex and densely setose (Fig. 5U). Hypoproct transversely bean-shaped, slightly concave caudally (Fig. 5U).

Ventral flaps behind gonopod aperture on  ${\mathcal 3}$  segment 7 evident swellings, forming a marked transverse ridge.

Legs nearly as long as body diameter (Fig. 5N), claw with an evident and long accessory claw near base (Fig. 5P), the latter up to ca. 2/3<sup>rd</sup>s the length of claw itself (Fig. 5P).

& legs 1 highly characteristic (Figs 6A, B, 7C) in being very strongly reduced, with large 1-segmented telopodites and a pair of large, hook-shaped, medially contiguous, sternal processes with groups of long and strong setae at base on caudal face.

 $\Diamond$  legs 2 slightly reduced, but coxa and femur hypertrophied (Figs 6C, 7D); penes rather small, oblong-subtrapeziform, each with 1–2 strong setae distolaterally (Figs 6C, D, 7D).

 $\circlearrowleft$  legs 3 slightly reduced, modified in having coxae especially slender and elongate (Figs 6E, 7E).

Anterior gonopods (Figs 6F–H, 7G, H)) peculiar in stout telopodites (te) being two curved, widely separated fingers with a setose central field on anterior face (Figs 6F, H, 7H). Anterior coxosternal process (acp) lobe-shaped, caudally about as high as a stout posterior coxosternal process (pcp).

Posterior gonopods (Figs 6I–K, 7F) elongate and finger-shaped, membranous, evidently bipartite, round, with both coxal processes (cp) and telopodites (te) sparsely microspiculate at margin (Fig. 6K); te membranous, slightly curved mesad, clearly shorter than cp, with a parabasal field of coniform microsetae caudally (Figs 6J, 7H).

**Remarks.** The genus *Trachyjulus* Peters, 1864 is currently known to comprise 31 species ranging from Nepal, India, and Sri Lanka in the west, through Bangladesh and Myanmar to Vietnam, Thailand, Malay Peninsula, Singapore, and Indonesia (Sumatra and Java) in the east (Golovatch et al. 2012). Only one species, the pantropical anthropochore *T. calvus*, has hitherto been documented from Myanmar (Likhitrakarn et al. 2017). This species (cf. Golovatch et al. 2012) is similar to *T. bifidus* sp. n., but the latter is clearly distinguished by the bifid telopodites of the anterior and posterior gonopods.

Based on the pigmented body and eye patches, and like most if not all other cavedwelling congeners known to date, *T. bifidus* sp. n. seems to be hardly more than a troglophile.

No special key to relevant genera involved seems to be needed, as the one given below to Myanmar species contains the necessary information.

# Key to Cambalopsidae species currently known to occur in Myanmar, chiefly based on male characters:

3	$\eth$ legs 1 with 3-segmented telopodites (Figs 3A, B, 4C)	
		n.
_	👌 legs 1 with 5-segmented telopodites	.4
4	Carinotaxy of collum: anterior transverse and posterior transverse rows consis	st-
	ing of 10 and 9 crests, respectively Podoglyphiulus doriae (Pocock, 1893	3)
_	Carinotaxy of collum: anterior transverse and posterior transverse rows con	n-
	sisting of 6 and 5 crests, respectively Podoglyphiulus feae (Pocock, 1893	3)

# Conclusions

There are 94 millipede species currently known to occur in Myanmar, including both new ones described above. The new material comes from some of the 27 caves located within a radius of ca. 70 km around the town of Kalaw, Shan State, northeastern Myanmar (Piccini et al. 2009). Studies on the cave fauna of that country have just begun and there can hardly be any doubt that many more interesting discoveries are ahead. These will certainly concern Diplopoda as well. Because Cambalopsidae are especially diverse and common in karsts of the adjacent parts of China, Laos, Thailand and Malaysia, where they are usually associated with bat guano in caves (Golovatch 2015), the same presumption can easily be extended to the karsts of Myanmar, too.

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RESEARCH ARTICLE



# A new species of *Melita* from Japan (Crustacea, Amphipoda, Melitidae)

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#### Abstract

A new brackish-water species of melitid amphipod, *Melita choshigawaensis*, from the Choshigawa River, Mie Prefecture, Japan, is named and described. *Melita choshigawaensis* **sp. n.** is distinguished from the most similar *M. shimizui* (Uéno, 1940) by having an elongate and weakly arched male uropod 3, and a deep and strongly hooked anterior lobe of the coxa on the female's pereopod 6. Nucleotide sequences of the mitochondrial cytochrome *c* oxidase subunit I (COI) of *M. choshigawaensis* and *M. shimizui* support divergence at the species level. A key to the Japanese species of *Melita* is provided.

## Keywords

Brackish water, Choshi River, COI, Mie Prefecture, taxonomy

## Introduction

The amphipod genus *Melita* Leach, 1814 comprises approximately 80 species worldwide (Krapp-Schickel and Sket 2015), most of which occur in marine intertidal and shallow waters, though some inhabit brackish and freshwaters (Jarrett and Bousfield 1996; Krapp-Schickel and Sket 2015). Eleven species of *Melita* have been recorded from Japan: *M. bingoensis* Yamato, 1987; *M. hoshinoi* Yamato, 1990; *M. koreana* Stephensen, 1944; *M. longidactyla* Hirayama, 1987; *M. nagatai* Yamato, 1987; *M. pilopropoda* Hirayama, 1987; *M. quadridentata* Yamato, 1990; *M. rylovae* Bulycheva, 1955; *M. setiflagella* Yamato, 1988; *M. shimizui* (Uéno, 1940); *M. tuberculata* Nagata, 1965. Among them, two species, *M. setiflagella* and *M. shimizui*, are known from brackish lakes and river mouths (Nagata 1965; Hirayama 1987; Yamato 1987, 1988, 1990; Ishimaru 1994). However, it is apparent that the diversity of species of *Melita* in Japanese waters, particularly in brackish environments, is not fully appreciated.

During field surveys of aquatic fish and amphipod faunas in the Choshi River, Mie Prefecture, Japan, a new amphipod species was found. Though DNA nucleotide sequence data have been recently successfully used to differentiate morphologically similar amphipod species (Matsukami et al. 2017; Tomikawa et al. 2016, 2017), previous taxonomic studies on *Melita* in Japan have focused on morphological characteristics only. Here, both molecular and morphological data are used to differentiate this species from others, which is described and illustrated. A key to species of *Melita* in Japanese waters using conventional morphological criteria is provided.

## Materials and methods

#### Specimens

Specimens were collected using a hand net (mouth 25 cm wide, 17 cm high, mesh size 0.1–0.5 mm) from under stones at the mouth of Choshi River, Kihoku, Mie Prefecture (Fig. 1), before being fixed in 99% ethanol. The specimens have been deposited in the National Museum of Nature and Science, Tsukuba (NSMT)

#### Morphological observation

All appendages were dissected in 80% ethanol and mounted in gum-chloral medium on glass slides using a stereomicroscope (Olympus SZX7). Slides were examined using a light microscope (Nikon Eclipse Ni), with appendages illustrated using a camera lucida. Body length (BL, to the nearest 0.1 mm) was measured from the rostrum tip to the telson base, along the dorsal curvature. Type specimens are deposited at the National Museum of Nature and Science, Tsukuba (NSMT).

#### PCR and DNA sequencing

Genomic DNA extraction from body or appendage muscle followed Tomikawa et al. (2014). The cytochrome *c* oxidase subunit I (COI) gene [LCO1490 and HCO2198 (Folmer et al. 1994)] primer set was used for PCR and cycle sequencing (CS) reactions. PCR reactions and DNA sequencing were performed following Tomikawa et



**Figure 1.** Collection locations: *M. choshigawaensis* sp. n. ( $\star$ ) and *M. shimizui* (•). Names of localities are shown in Table 1.

al. (2017). PCR reactions were performed using a PC-320 thermal cycler (ASTEC) with an Ex *Taq* Polymerase Kit (Takara Bio Inc.). PCR mixtures were heated to 94 °C for 7 min, followed by 35 cycles at 94 °C (45 s), 42 °C (1 min), and 72 °C

(1 min), and a final extension at 72 °C for 7 min. Amplification products were purified using the silica method (Boom et al. 1990). All sequencing reactions were performed according to the manufacturer's instructions using the BigDye Terminator v3.1 Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA). Cycle sequencing conditions were 25 cycles of 10 s at 96 °C, 5 s at 50 °C, and 4 min at 60 °C. Sequencing reaction products were purified by ethanol precipitation. Labeled fragments were analyzed using an ABI 3130x Genetic Analyzer (Applied Biosystem). Sequences obtained from both strands of gene segments (for verification using the same primers) were edited using MEGA7 (Kumar et al. 2016). DNA sequences have been deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (DDBJ).

### Taxonomy

Melitidae Bousfield, 1973 *Melita* Leach, 1814

#### Melita choshigawaensis sp. n.

http://zoobank.org/C10A0F95-5419-4534-8923-07D8C2E77F17 Figures 2–7 New Japanese name: Choshigawamerita-yokoebi

**Material examined.** Holotype: male (BL 5.3 mm, NSMT-Cr 25826), Choshi River, Kihoku, Mie Prefecture, Japan (34.108242°N, 136.221998°E), col. Ko Tomikawa, Kentaro Hirashima, Atsushi Hirai, and Ryu Uchiyama, 2 March 2017. Paratypes: male (BL 4.1 mm, NSMT-Cr. 25827), data as for holotype; male (BL 6.8 mm, NSMT-Cr. 25828), female (BL 5.8 mm, NSMT-Cr. 25829), 27 December 2017, locality and collectors as for holotype.

**Diagnosis.** Male gnathopod 2 propodus with oblique palmar margin; anterior lobe of female pereopod 6 coxa deep and strongly hooked; male uropod 3 outer ramus uni-articulate, weakly arched, its length 7–8 times its width, lacking long setae.

**Description male (holotype, NSMT-Cr 25826).** Head (Fig. 2) slightly shorter than pereonites 1 and 2 combined; rostrum short; eyes ovate; lateral cephalic lobe rounded; antennal sinus quadrate, not incised. Pereonites 1–7 (Fig. 2) dorsally smooth with fine setae. Dorsal margins of pleonites 1–3 (Fig. 3A–C) with 2, 2, and 7 setae, respectively; epimeral plate 1 (Fig. 3E) ventral submargin with three robust and one slender setae, posterior margin with two setae, posterodistal corner weakly pointed with seta; epimeral plate 2 (Fig. 3F) ventral margin with three robust setae, posterior margin with robust seta, posterior margin bare, posterodistal corner weakly pointed with seta; epimeral plate 3 (Fig. 3G) ventral margin with robust seta, posterior margin bare, posterodistal corner weakly pointed with seta. Dorsal margin of urosomite 2 (Fig. 3D) with four robust setae.

Antenna 1 (Fig. 3H): length 1.1 times that of body; length ratio of peduncular articles 1–3 as 1.0:1.3:0.7; ventral margin of peduncular article 1 with three robust



**Figure 2.** *Melita choshigawaensis* sp. n., holotype, male, BL 5.3 mm, NSMT-Cr 25826, Choshi River, Kihoku, Mie Prefecture, Japan. Habitus, lateral view.

setae, posterodistal corner with robust seta; primary flagellum 28-articulate with a few setae; accessory flagellum (Fig. 3I) 2-articulare, with short terminal article. Antenna 2 (Fig. 3J) half of antenna 1 length; peduncular article 5 length 0.9 times that of article 4; flagellum 7-articulate, article 1 length 1.5 times that of article 2; calceoli absent.

Upper lip (Fig. 3K) ventral margin convex, rounded, with minute setae. Left and right mandibular incisors (Fig. 3L–N) 4- and 5-dentate, respectively, with left lacinia mobilis quadri-dentate (Fig. 3M) and right (Fig. 3N) multidentate; left and right accessory setal rows (Fig. 3M, N) with five and three bladed setae, respectively; molar process triturative with plumose seta; palp tri-articulate, length ratio of articles 1–3 1.0:2.3:2.0, article 1 bare, article 2 with two setae, article 3 with seven setae. Lower lip (Fig. 3O) outer lobes broad, setulose, mandibular lobes narrow; inner lobes distinct. Maxilla 1 (Fig. 3P, Q) inner plate narrow with six plumose setae; outer plate rectangular with nine serrate robust setae; palp 2-articulate; article 1 rectangular, lacking setae; article 2 expanded, outer margin without setae, apical margin with robust and slender setae. Maxilla 2 (Fig. 3R) inner plate with oblique inner row of seven setae; outer plate slightly longer than inner plate. Maxilliped (Fig. 3S) distal part of inner plate not reaching half of palp article 2; outer plate ovate, exceeding half of palp article 2, apical margin with plumose setae, inner submargin with robust setae; palp quadri-articulate, article 4 with nail.

Gnathopod 1 (Fig. 4A, B) smaller than gnathopod 2; ventral margin and posterior submargin of coxa with setae; basis, anterior and posterior margins with long setae, posterodistal submargin with tiny palmate setae; ischium with tiny palmate



**Figure 3.** *Melita choshigawaensis* sp. n., holotype, male, BL 5.3 mm, NSMT-Cr 25826, Choshi River, Kihoku, Mie Prefecture, Japan. **A–C** dorsal margins of pleonites 1–3, dorsal views **D** dorsal margin of urosomite 2, dorsal view **E–G** epimeral plates 1–3, lateral views **H** right antenna 1, medial view, some articles of main flagellum omitted **I** accessory flagellum of right antenna 1, medial view **J** right antenna 2, medial view, some articles of flagellum omitted **K** upper lip, anterior view **L** left mandible, medial view **M** incisor, lacinia mobilis, and accessory setal row of left mandible, medial view **P** right maxilla 1, anterior view **Q** palp article 2 of left maxilla 1, posterior view **R** left maxilla 2, anterior view **S** left maxilliped, anterior view.



**Figure 4.** *Melita choshigawaensis* sp. n., holotype, male, BL 5.3 mm, NSMT-Cr 25826, Choshi River, Kihoku, Mie Prefecture, Japan. **A** right gnathopod 1, medial view **B** palmar margin of propodus and dactylus of right gnathopod 1, medial view **C** right gnathopod 2, medial view **D** left pereopod 3, lateral view **E** left pereopod 4, lateral view.

setae; merus with small ventral setae; carpus not lobate, length 1.5 times that of propodus, anterior submargin with small setae, posterior margin with clusters of setae; propodus without anterodistal hood, palmar margin convex with two rows of robust setae, proximal part of palmar margin with distinct protuberance; dactylus short, not exceeding palmar margin. Gnathopod 2 (Fig. 4C) coxa subrectangular, ventral margin and submargin with setae; basis anterior margin bare, posterior margin with long setae, antero- and posterodistal corners with small setae, posterodistal submargin with small palmate setae; carpus not lobate, length 0.5 times that of propodus; propodus large, half as wide as long, palmar margin oblique with nine medial and ten lateral robust setae; dactylus with small posterodistal notch, of similar length to palmar margin.

Pereopod 3 (Fig. 4D) coxa subrectangular, ventral margin and submargin with setae; basis arched, anterior and posterior margins with long and short setae; length ratio of merus, carpus, propodus and dactylus 1.0:0.9:0.8:0.3. Pereopod 4 (Fig. 4E): coxa expanded with posterior concavity, bearing ventral and surface setae; basis anterior and posterior margins with long and short setae; length ratio of merus, carpus, propodus and dactylus 1.0:0.9:0.8:0.3. Pereopod 5 (Fig. 5A) coxa bilobate, anterior lobe large with small seta on distal margin, posterior lobe with small setae on ventral margin and posterodistal corner; basis with posterodistal lobe; length ratio of merus, carpus, propodus and dactylus 1.0:0.8:0.9:0.2; merus weakly expanded, half as wide as long. Pereopod 6 (Fig. 5B) coxa bilobate, shallower than that of percopod 5, posterior lobe with small seta on posterodistal corner; basis posterior margin weakly serrate, posterodistal corner lobate; length ratio of merus, carpus, propodus and dactylus 1.0:0.9:1.3:0.3; merus weakly expanded, half as wide as long. Pereopod 7 (Fig. 5C) coxa semicircular, with seta on posterior margin; basis subovate, posterior margin weakly serrate, bearing posterodistal lobe; length ratio of merus, carpus, propodus and dactylus 1.0: 0.8:1.2:0.3; merus 0.4 times as wide as long.

Coxal gills (Fig. 2) present on gnathopod 2, and pereopods 3-6.

Pleopod 1–3 (Fig. 5D) peduncles with paired retinacula (Fig. 5E) on inner distal margin, and bifid plumose setae (clothes-pin setae) on inner ramus inner basal margin.

Uropod 1 (Fig. 5F) extending beyond uropod 2; peduncle with basofacial seta; inner ramus length 0.6 times that of peduncle, with two inner marginal and four distal robust setae, proximal part with slender seta; outer ramus 1.1 times longer than inner ramus, bearing two outer marginal and four distal robust setae. Uropod 2 (Fig. 5G) not extending beyond peduncle of uropod 3; inner ramus 0.9 times as long as peduncle, with two inner robust setae, distal part with five robust setae; outer ramus 0.9 times as long as inner ramus, with one inner and two outer robust setae, distal part with four robust setae. Uropod 3 (Fig. 5H, I) peduncle extending beyond telson; inner ramus length 0.13 times that of outer ramus, with distal robust seta; outer ramus with single article, weakly arched, length 2.9 times that of peduncle and 7.0 times that of outer ramus width, long setae absent. Telson (Fig. 5J) length 1.1 times longer than wide, completely cleft, each lobe with two lateral and three distal robust setae.

**Description female (paratype, NSMT-Cr 25829).** Antenna 1 (Fig. 6A) 0.6 times body length; length ratio of peduncle articles 1–3 1.0:1.2:0.7; ventral margin of peduncular article 1 without robust setae; primary flagellum 17-articulate. Antenna 2 (Fig. 6B) length half that of antenna 1.



**Figure 5.** *Melita choshigawaensis* sp. n., holotype, male, BL 5.3 mm, NSMT-Cr 25826, Choshi River, Kihoku, Mie Prefecture, Japan. **A** right pereopod 5, medial view; **B** left pereopod 6, lateral view **C** left pereopod 7, lateral view **D** pleopod 1, medial view, some setae on rami omitted **E** retinacula on peduncle of pleopod 1 and associated seta, medial view **F** left uropod 1, dorsal view **G** right uropod 2, dorsal view **H** left uropod 3, dorsal view **J** telson, dorsal view.



**Figure 6.** *Melita choshigawaensis* sp. n., paratype, female, BL 5.8 mm, NSMT-Cr 25829, Choshi River, Kihoku, Mie Prefecture, Japan. **A** right antenna 1, medial view, some articles of main flagellum omitted **B** right antenna 2, medial view; **C** right gnathopod 1, medial view **D** palmar margin of propodus and dactylus of right gnathopod 1, medial view **E** right gnathopod 2, medial view **F** palmar margin of propodus and dactylus of right gnathopod 2 **G** left pereopod 5, lateral view, carpus–dactylus omitted **H** left pereopod 6, lateral view, carpus–dactylus omitted I distal part of coxa anterior lobe of left pereopod 6, lateral view **J** left pereopod 7, lateral view, carpus–dactylus omitted; **K** right uropod 3, dorsal views.



**Figure 7.** *Melita choshigawaensis* sp. n. **A, B** live males, BL *ca* 6 mm, lateral views. Photographed by Ryu Uchiyama.

Gnathopod 1 (Fig. 6C, D) coxa elongate, anterior margin weakly concave; carpus length 1.6 times that of propodus; proximal part of palmar margin without protuberance. Gnathopod 2 (Fig. 6E, F): coxa elongate; anterior margin of basis with seta; carpus length 0.8 times that of propodus; propodus 0.6 times as wide as long, palmar margin with six medial and six lateral robust setae.

Pereopods 5–7 (Fig. 6G, H, J). Depth of pereopod 6 (Fig. 6I) anterior lobe equal to coxal width, strongly hooked; merus width 0.4 times that of length.

	Species	Locality	1	2	3	4
1	<i>M. choshigawaensis</i> sp. n.	Choshi River, Mie (1)	0.0-0.2			
2		Lake Hinuma, Ibaraki (2)	14.9	_		
3	M. shimizui (Uéno, 1940)	Seno River, Hiroshima (3)	14.9	4.4	-	
4		Ota River, Hiroshima (4)	14.9	4.4	0.0	_

**Table 1.** Uncorrected *p*-distances (%) of COI sequences (658 bp) among *M. choshigawaensis* sp. n. and three populations of *M. shimizui* (Uéno, 1940). Numbers after localities correspond to locations in Figure 1.

Uropod 3 (Fig. 6K) inner ramus length 0.15 times that of outer ramus; outer ramus sublinear, length 2.5 times that of peduncle and 6.6 times outer ramus width.

#### 16 eggs.

**Variation.** Uropod 3 outer ramus length 2.8 times that of peduncle and 8.2 times outer ramus width (male 6.8 mm, NSMT-Cr 25828).

**Sequences and COI genetic distances.** In total, 658 bp of six nucleotide sequences were determined: paratypes of *M. choshigawaensis* sp. n. (NSMT-Cr 25827–25829), three sequences (LC371923–371925); and *M. shimizui* from three localities, one from Lake Hinuma (LC371926), one from Seno River (LC371927), and one from Ota River (LC371928). Uncorrected *p*-distances between *M. choshigawaensis* and *M. shimizui* were 14.9% (Table 1). Intraspecific distances of *M. choshigawaensis* and *M. shimizui* were up to 0.2% and 4.4%, respectively (Table 1).

Distribution. Known only from the type locality.

**Etymology.** Derived from the name of the type locality.

**Remarks.** Melita choshigawaensis is closely related to M. shimizui (Uéno, 1940), originally described from a freshwater pond on Liaodong Peninsula, China (Uéno 1940), but subsequently recorded from several brackish sites in the Japanese archipelago, such as Honshu, Shikoku, Kyushu, and the main island of Okinawa (Yamato 1988). Recently, Labay (2016) described a new subspecies, M. shimizui sakhalinensis from Sakhalin. The pleonites of both species lack dorsal teeth, urosomite 2 has robust setae on the dorsal margin, the accessory flagellum of antenna 1 is bi-articulate, and the outer ramus of uropod 3 is uni-articulate and lacks long setae. However, M. choshigawaensis can be distinguished from M. shimizui by (features of M. shimizui in parentheses): the outer ramus of male uropod 3 being weakly arched (compared with sublinear) and more than seven times longer than wide (ca. 5), and the anterior lobe of the female percopod 6 coxa is deep, equal in length to coxal width (shorter than width), and strongly (as opposed to weakly) hooked. These two species also differ genetically in COI (14.9%) greater than distances (3.5-4%) proposed as thresholds for amphipod species discrimination (Witt et al. 2006; Rock et al. 2007; Hou et al. 2009). Thus, we determined *M. choshigawaensis* represented a novel species.

*Melita choshigawaensis* is similar to *M. laevidorsum* Stephensen, 1944 from Korea, and *M. myersi* Karaman, 1987 from Australia, Fiji, and New Caledonia in that all three have dorsally smooth pleonites, a urosomite 2 with robust setae on their

dorsal margins, and an elongate outer ramus of uropod 3 (Stephensen 1944; Karaman 1987). However, *M. choshigawaensis* differs from *M. laevidorsum* in having an accessory flagellum of antenna 1 with two articles (compared with four), in lacking an anterodistal hood on the propodus of male gnathopod 1 (compared with having one), and in that the medial surface of the propodus of male gnathopod 2 is sparsely (as opposed to densely) setose. From *M. myersi*, *M. choshigawaensis* differs in having a deep antennal sinus (compared with shallow), in lacking an anterodistal hood on the propodus of the male's gnathopod 1 (compared with having one), and in having the meri of pereopods 5 and 6 weakly expanded (as opposed to their not being expanded).

# Key to species of Melita in Japan

Since records of three species, *M. coroninii* Heller, 1867, *M. dentata* (Krøyer, 1842), and *M. palmata* (Montagu, 1804) from Japanese waters are dubious (Ishimaru 1994), these species are excluded from the key.

1	Uropod 3, outer ramus 1-articulate2
-	Uropod 3, outer ramus 2-articulate
2	Pleonites 1–3 each with dorsal tooth
-	Pleonites 1–3 dorsally smooth
3	Dactylus of pereopods 3 and 4 long, feeble M. longidactyla Hirayama, 1987
-	Dactylus of percopods 3 and 4 short, stout
4	Urosomite 2 with teeth
-	Urosomite 2 without teeth
5	Female pereopod 6, anterior lobe of coxa shallow, weakly hooked
-	Female pereopod 6, anterior lobe of coxa deep, strongly hooked
6	Antenna 2, flagellum strongly setose
-	Antenna 2, flagellum weakly setose7
7	Antenna 1, accessory flagellum 4-articulate; male gnathopod 2, palm quad-
	rate
-	Antenna 1, accessory flagellum 2-articulate; male gnathopod 2, palm
	oblique
8	Male uropod 3 outer ramus weakly arched, more than 7.0 times longer than
	wide; anterior lobe of coxa of female pereopod 6 as deep as coxa is, strongly
	hooked
-	Male uropod 3 outer ramus sublinear, about 5 times as long as wide; ante-
	rior lobe of coxa of female pereopod 6 depth less than coxal width, weakly
	hooked M. shimizui (Uéno, 1940)

9	Antenna 2 flagellum strongly setose; uropod 3 outer ramus with long setae
	<i>M. quadridentata</i> Yamato, 1990
-	Antenna 2 flagellum weakly setose; uropod 3 outer ramus without long
	setae10
10	Inferior antennal sinus absent M. pilopropoda Hirayama, 1987
-	Inferior antennal sinus present11
11	Maxilla 1 palp article 1 with setae; male gnathopod 2 propodus palm
	oblique
-	Maxilla 1 palp article 1 without setae; male gnathopod 2 propodus palm
	quadrateM. rylovae Bulycheva, 1955

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RESEARCH ARTICLE



# The freshwater crabs of Danum Valley Conservation Area in Sabah, East Malaysia, with a description of a new species of *Thelphusula* Bott, 1969 (Crustacea, Brachyura, Gecarcinucidae, Potamidae, Sesarmidae)

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#### Abstract

Seven species of freshwater crabs from three families are recorded from and around the Danum Valley Conservation Area in Sabah, Malaysian Borneo: *Thelphusula capillodigitus* **sp. n.**, *Thelphusula dicerophilus* Ng & Stuebing, 1990, *Arachnothelphusa terrapes* Ng, 1991, *Terrathelphusa secula* Ng & Tan, 2015, *Para-thelphusa valida* Ng & Goh, 1987 (new record) (Gecarcinucidae); *Isolapotamon ingeri* Ng & Tan, 1998 (Potamidae); and *Geosesarma danumense* Ng, 2002 (Sesarmidae). The new species of *Thelphusula* Bott, 1979, can be distinguished from all congeners by a unique combination of morphological features, most notably the presence of dense patches of short setae on the fingers of the adult male chelipeds, as well as the structure of the male first gonopod. *Arachnothelphusa terrapes* is confirmed to be a phytotelm species. A key to all species in the conservation area is provided.

#### Keywords

Taxonomy, Borneo, Thelphusula capillodigitus, Arachnothelphusa, Terrathelphusa, Parathelphusa, Isolapotamon, Geosesarma, phytotelm

## Introduction

Danum Valley Conservation Area, northeastern Borneo, in the Malaysian state of Sabah contains over 400 square kilometres of pristine rainforest and is a key conservation area on the island (Hazebroek et al. 2011). The first author has been involved in studies of the freshwater decapod crustacean fauna in the area since the late 1980s, and five species of freshwater and semiterrestrial crabs have been reported thus far: *Thelphusula dicerophilus* Ng & Stuebing, 1990, *Arachnothelphusa terrapes* Ng, 1991, *Terrathelphusa secula* Ng & Tan, 2015 (Gecarcinucidae Rathbun, 1904); *Isolapotamon ingeri* Ng & Tan, 1998 (Potamidae Ortmann, 1896); and *Geosesarma danumense* Ng, 2002 (Sesarmidae Dana, 1851) (Ng 1991, 2002; Ng and Stuebing 1990; Ng and Tan 1998, 2015).

Here we review and add to the freshwater crab fauna of the Danum Valley Conservation Area. Specimens of a recently collected *Thelphusula* Bott, 1969, from Danum Valley proved to be a new species. While superficially resembling *T. hulu* Tan & Ng, 1997, from the Maliau Basin in Sabah in morphology and habits, it can easily be distinguished from this and all congeners by its setose male cheliped dactyli, as well as a number of other carapace features. It is here described as *T. capillodigitus* sp. n. In addition, *Parathelphusa valida* Ng & Goh, 1987, is added to the fauna for the area. Observations on the ecology of *Arachnothelphusa terrapes* Ng, 1991, originally described from Danum Valley, are also provided, showing that it is only the second confirmed tree-hole crab in South East Asia. A key to the seven species of Gecarcinucidae, Potamidae, and Sesarmidae in the Danum Valley Conservation Area is provided.

## Materials and methods

The terminology used follows that in Ng (1988) and Davie et al. (2015). Measurements provided, in millimetres, are of the maximum carapace width and length, respectively. The abbreviations G1 and G2 are used for the first and second male gonopods, respectively. Specimens examined are deposited in the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum, National University of Singapore.

#### Systematic account

Family Gecarcinucidae Rathbun, 1904

Genus Thelphusula Bott, 1969

Type species. Potamon (Geothelphusa) buergeri De Man, 1899, by original designation. Remarks. Thelphusula Bott, 1969, was established for Potamon (Geothelphusa) buergeri De Man, 1899, and currently contains 11 species (Ng and Tan 1998; Ng et al. 2008; Grinang and Ng 2014), all from Borneo. Of these, five species are found in Sabah: *T. dicerophilus* Ng & Stuebing, 1990, *T. hulu* Tan & Ng, 1997, *T. luidana* (Chace, 1938), *T. sabana* Tan & Ng, 1998, and *T. tawauensis* Tan & Ng, 1998. The genus is characterized by its quadrate carapace shape, the epibranchial tooth being poorly developed or absent, the posterolateral margins which are subparallel, the ambulatory legs being not prominently elongate, the G1 being slender with a relatively long and cylindrical terminal segment, and a relatively short G2 which has a short flagellum (Tan and Ng 1998).

## Thelphusula capillodigitus sp. n.

http://zoobank.org/EBC4CF72-6C54-408A-ADD1-60190EF3FDAF Figures 1–4

**Material examined.** Holotype: male  $(23.9 \times 18.4 \text{ mm})$  (ZRC 2017.1294), coll. Danum Valley, Lahad Datu, Sabah, Borneo, Malaysia, 22 July 2017. Paratypes: 1 male  $(18.8 \times 15.5 \text{ mm})$  (ZRC 2017.1295), same data as holotype; 1 male  $(19.9 \times 16.4 \text{ mm})$  (ZRC 2009.0080), in pitfall trap, Danum Valley Research Centre, Sabah, coll. C. Colón, October 1996. Others: 3 juveniles  $(3.7 \times 3.0 \text{ mm}, 6.5 \times 5.4 \text{ mm}, 6.7 \times 5.5 \text{ mm})$ , 1 young female  $(10.7 \times 9.1 \text{ mm})$  (ZRC 1990.0548–0551), Danum Valley Research Centre, Lahad Datu, Sabah, coll. R. Stuebing, 23 July 1989.

Diagnosis. Carapace broader than long, not raised; dorsal surface with regions clearly demarcated; frontal median triangle absent (Figs 2A–C, 3E); epibranchial tooth low, distinct, separated from external orbital tooth by shallow cleft; epigastric regions raised, rugose, not cristate; postorbital cristae low, distinct, rugose, not confluent with epigastric cristae, not reaching anterolateral margin; cervical grooves and H-shaped gastric depression deep; gastric regions with prominent transverse striae; antero- and posterolateral regions with strong oblique striae (Fig. 2A, B); median lobe on posterior margin of epistome triangular, tip rounded (Figs 2C, 3E). Third maxilliped with subrectangular ischium, distinctly longer than broad (Fig. 2D). Chelipeds with outer surface of palm almost smooth, dorsal and lateral surfaces of adult male dactylus covered with dense short setae (Fig. 3A–D). Ambulatory legs not prominently elongate, dorsal margin of merus gently serrated (Figs 2A, 3F–I). Thoracic sternum with surface evenly pitted to smooth, sternopleonal cavity reaching imaginary line joining anterior edges cheliped coxae (Fig. 2F, G); pleon distinctly T-shaped, somite 6 rectangular, slightly more than twice as long as broad, telson triangular, longer than broad (Fig. 2E, F). G1 relatively slender, almost straight; terminal segment approx. a quarter length of subterminal segment (Fig. 4A-C). G2 approx. two-thirds length of G1, distal article short (Fig. 4D).

**Description of male holotype.** Carapace broader than long, not raised; dorsal surface gently convex, regions clearly demarcated, covered with very short setae which does not obscure surface; frontal margin almost straight, without distinct median concavity, not deflexed, approx. a third carapace width; frontal median triangle absent



**Figure 1.** *Thelphusula capillodigitus* sp. n., colour in life, holotype male (23.9 × 18.4 mm) (ZRC 2017.1294), Sabah. **A** dorsal view **B** frontal view (photographs Dennis Sim).

(Figs 2A–C, 3E); anterolateral margin not clearly separated from posterolateral margin; external orbital tooth low, broadly triangular; epibranchial tooth low but distinct, separated from external orbital tooth by shallow cleft; postfrontal surface slightly rugose; postorbital region surface; epigastric regions raised, rugose, not cristate, divided into 2 parts by narrow, deep median groove; postorbital cristae low but distinct, sharp, not confluent with epigastric cristae, not reaching anterolateral margin; cervical grooves and H-shaped gastric depression deep; gastric regions with prominent transverse striae; antero- and posterolateral regions with strong oblique striae; posterolateral margins concave, gently converging towards posterior carapace margin; posterior carapace margin straight (Fig. 2A, B); pterygostomial, suborbital, sub-branchial, and subhepatic



**Figure 2.** *Thelphusula capillodigitus* sp. n., holotype male (23.9 × 18.4 mm) (ZRC 2017.1294), Sabah. **A** overall view **B** dorsal view of carapace **C** frontal view of cephalothorax **D** left third maxilliped **E** pleon **F** anterior thoracic sternum and pleon **G** sternopleonal cavity.

regions covered with striae (Fig. 2C); orbits large, eyes occupying entire width, supra and suborbital margins entire, cristate; eyes pigmented, well developed, peduncle with low, sinuous median ridge (Fig. 2B, C); median lobe on posterior margin of epistome triangular, tip rounded, lateral margins sinuous (Figs 2C, 3C).



**Figure 3.** *Thelphusula capillodigitus* sp. n., holotype male (23.9 × 18.4 mm) (ZRC 2017.1294), Sabah. A outer views of chelae **B** outer view of right chela **C** dorsal view of dactylus of right chela **D** surface of dactylus showing dense short setae **E** epistome **F–I** first to fourth ambulatory legs, respectively (all to same scale).

Mandibular palp 2-segmented, terminal one distinctly bilobed. Third maxilliped covering majority of buccal cavity when closed; ischium subrectangular, distinctly longer than broad, with shallow submedian groove; merus quadrate, slightly broader than long; exopod long, slender, reaching median part of merus, flagellum long, exceeding width of merus (Fig. 2D).

Chelipeds asymmetrical, right larger; surface of merus slightly rugose, relatively long, trigonal in cross section, margins without teeth or spines; carpus surface distinctly rugose, subovate, inner distal angle with sharp spine with basal tubercle; palm relatively stout, longer than broad, outer surface slightly rugose to almost smooth; fingers subequal in length to palm, dactylus marginally longer than pollex, curving inwards, cutting margin of fingers lined with numerous denticles, fingers pitted (Figs 2A, 3A–D); dorsal and lateral surfaces of most of dactylus covered with dense short setae; lateral surface of pollex with mat of short, relatively less dense setae; tips of fingers strongly curved, corneous, glabrous (Fig. 3B–D).

Ambulatory legs not prominently elongate, third pair longest, fourth leg shortest; segments laterally flattened laterally, surfaces mildly rugose; dorsal margin of merus gently serrated, no visible subdistal tooth; carpus of first to third legs with low median ridge, absent on carpus of fourth leg; margins of propodus and dactylus lined with numerous short spines (Figs 2A, 3F–I).

Thoracic sternum surface evenly pitted to smooth; sternites 1 and 2 completely fused forming triangular structure; suture separating sternites 2 and 3 relatively shallow, sinuous, medially convex with lateral parts concave (towards buccal cavity); sternites 3 and 4 completely fused; sternopleonal cavity almost reaching imaginary line joining anterior edges cheliped coxae, near suture between sternites 2 and 3; part of sternite 8 exposed when pleon closed; tubercle of male pleonal locking mechanism prominent, peg-like, on anterior third of sternite 5 (Fig. 2F, G).

Pleon distinctly T-shaped; somite 1 short, broad, reaching coxae of fourth ambulatory legs; somite 2 slightly longer than somite 1, as broad as somite 1; somite 3 short, broadest, with prominently convex lateral margins; somites 4 and 5 trapezoidal; somite 5 notably narrower than 4, trapezoidal with concave lateral margins; somite 6 rectangular, slightly more than twice as long as broad, lateral margins concave; telson triangular, longer than broad, tip rounded (Fig. 2E, F).

G1 relatively slender, entire structure almost straight; terminal and subterminal segments clearly separated; terminal segment relatively short, approx. a quarter length of subterminal segment, cylindrical with tip tapering to subtruncate tip, margins with short stiff setae, surface just before tip with numerous squamiform setae; lower half of subterminal segment with numerous short setae (Fig. 4A–C). G2 approx. two-thirds length of G1; basal segment long; distal segment short (Fig. 4D).

**Variation.** Unlike the male holotype (the largest specimen), the degree and extent of the setation on the fingers of the chelae of the two smaller paratype males are the same in both chelipeds. Male specimens less than 15 mm in carapace width do not have the setae on the fingers of the chelae. The outer surface of the chela in smaller specimens is also relatively more rugose compared to larger ones.

**Etymology.** The name is derived from the Latin *capillus* for hair and *digitus* for finger. The name is used as a noun in apposition.

**Colour.** In life, the carapace is mostly dark reddish brown; the sub-branchial regions, third maxillipeds, pleon and thoracic sternum is pale yellow; the ambulatory



**Figure 4.** *Thelphusula capillodigitus* sp. n., holotype male (23.9 × 18.4 mm) (ZRC 2017.1294), Sabah. **A** left G1 (ventral view) **B** distal part of left G1 (ventral view) **C** distal part of left G1 (doral view) **D** left G2 (ventral view). Scale bars: 1.0 mm (**A**, **D**); 0.5 mm (**B**, **C**).

legs dark brown, faintly marmorated, with exception of pale yellow, faintly spotted merus; and the chelipeds are yellowish orange, with the inner surfaces paler and the setose patches on the surface of the male fingers light brown (Fig. 1).

Remarks. Thelphusula capillodigitus sp. n. can easily be distinguished from all congeners by the adult male possessing dense setae on the dorsal surfaces of the fingers of the chelipeds (Fig. 3B–D), a character also absent in genera allied to Thelphusula: Adeleana Bott, 1969, Balssiathelphusa Bott, 1969, Stygothelphusa Ng, 1989, Arachnothelphusa Ng, 1991, and Coccusa Tan & Ng, 1998 (cf. Bott 1969, 1970; Ng 1989b, 1991; Tan and Ng 1998; Ng and Guinot 2014). The absence of a clearly discernible frontal median triangle is a character T. capillodigitus shares with T. pueh, T. cristicervix and T. styx, but it can be distinguished from them by the presence of setose patches on the fingers of the adult male chelipeds (Fig. 3B-D) as well as a G1 which is only slightly curved with a relatively shorter terminal segment that is approx. a third the length of the subterminal segment (Fig. 4A-C). In contrast, the G1s in T. pueh and T. cristicervix possess a prominently curved terminal segment which is proportionately longer, being approx. half the length of the subterminal segment (cf. Ng and Grinang 2014: fig 3). In T. styx, the G1 has a relatively broader subterminal segment with the terminal segment distinctly upturned (cf. Ng 1989a: figs 2E, F). Thelphusula capil*lodigitus* can further be distinguished from *T. styx* by its relatively more shallow cervical grooves which end at the H-shaped median depression with a level and straight frontal margin (Fig. 2B) (versus with deeper and distinctly longer cervical grooves that extend

to the posterolateral region of the carapace, and the frontal margin deflexed in *T. styx*; cf. Ng 1989a: fig.1). The carapace of *T. capillodigitus* is gently convex (Fig. 2B, C) whereas in both *T. pueh* and *T. cristicervix*, the carapaces are distinctly inflated (cf. Ng and Grinang 2014: figs 1C, 2C). In addition, *T. pueh*, *T. cristicervix*, and *T. styx* are only known from Sarawak.

In the general form of the carapace (not raised and relatively low) and relatively shorter ambulatory legs, T. capillodigitus most closely resembles T. sabana from Lahad Datu and T. hulu from the Maliau Basin, both in Sabah. Other than in the setose adult male cheliped fingers, T. capillodigitus can also be distinguished by the gastric regions prominently lined with transverse striae (Fig. 2B) (versus gastric regions rugose to smooth in T. hulu; cf. Tan and Ng 1997: fig. 5); the absence of a frontal median triangle (Figs 2C, 3E) (versus frontal median triangle distinct in *T. hulu*; Tan and Ng 1997: fig. 3B); the ischium of the third maxilliped being relatively longer (Fig. 2D) (versus ischium relatively shorter in T. hulu; cf. Tan and Ng 1997: fig. 3D); the outer surface of chela being almost smooth (Fig. 3A, B) (versus covered with prominent striae and scattered granules in T. hulu; cf. Tan and Ng 1997: fig. 3C); the male pleonal somite 6 being proportionately longer (Fig. 2E) (versus male pleonal somite 6 proportionately shorter in *T. hulu*; cf. Tan and Ng 1997: fig. 4B); and the G1 being almost straight (Fig. 4A-C) (versus G1 terminal segment strongly curved outwards in T. hulu; cf. Tan and Ng 1997: fig. 4C, D). Thelphusula capillodigitus resembles T. sabana from Lahad Datu in possessing strong striae and granules on the carapace surface, but can be separated by lacking a frontal median triangle (Figs 2C, 3E) (versus frontal median triangle discernible but incomplete in T. sabana; cf. Tan and Ng 1998: fig. 3C); the male pleonal somite 6 is proportionately longer (Fig. 2E) (versus male pleonal somite 6 proportionately shorter in T. sabana; cf. Tan and Ng 1998: fig. 3B); and the G1 is almost straight with a short terminal segment (Fig. 4A-C) (versus G1 prominently curved outwards with the terminal segment very long in *T. sabana*; cf. Tan and Ng 1998: fig. 3D–G).

Two other species of *Thelphusula* are present in Sabah, *T. dicerophilus* (which occurs in the same area as *T. capillodigitus*) and *T. tawauensis* which occurs to the east. *Thelphusula capillodigitus* can be separated from *T. dicerophilus* easily by its relatively flatter carapace (Fig. 2B, C) (versus carapace very high and raised in *T. dicerophilus*; Fig. 6B; cf. Ng and Stuebing 1990: pl. 1B); and from *T. tawauensis* by the gastric regions covered with prominent striae and the frontal median triangle being absent (Figs 2C, 3E) (versus gastric regions smooth with the frontal median triangle prominent in *T. tawauensis*; cf. Tan and Ng 1998: fig. 4A, C).

Thelphusula capillodigitus was collected in a clear flowing shaded jungle stream with an average temperature range of 26–28 degrees Celsius and near neutral pH. All specimens were collected during the day, under rocks, and appear to be mostly aquatic in habits, although one specimen was collected from a pitfall trap (ZRC 2009.0080). *Parathelphusa valida* was also present in the same stream in larger numbers. The presence of a second species of *Thelphusula* in Danum Valley is not surprising, considering that *T. capillodigitus* has more aquatic habits than *T. dicerophilus* (see next species).

#### Thelphusula dicerophilus Ng & Stuebing, 1990

Figure 6A, B

*Thelphusula dicerophilus* Ng & Stuebing, 1990: 46, fig. 1, pl. 1; Tan and Ng 1998: 813, fig. 6C; Ng et al. 2008: 73.

**Material examined.** Holotype: male  $(14.0 \times 12.0 \text{ mm})$  (ZRC 1989.3588), in pitfall trap, adjacent to pool of rhinoceros mud wallow, Danum Valley, Lahad Datu, Sabah, ca. 4°55'N 117°46', coll. R. Stuebing, 4 March 1988. Paratypes: 1 female (18.3 × 15.0 mm), 2 juveniles (ZRC 1989.3592–3594), in pitfall trap, adjacent to pool of rhinoceros mud wallow, Danum Valley, Lahad Datu, Sabah, ca. 4°55'N 117°46', coll. R. Stuebing, 1 March 1988; 1 male (18.5 × 15.4 mm) (ZRC 1989.3591), in mist net in rhinoceros mud wallow pool, Danum Valley, Lahad Datu, Sabah, ca. 4°55'N 117°46', coll. R. Stuebing, 2 March 1988; 1 male, 1 female (ZRC 1989.3589–3590), in rhinoceros mud wallow pool, Danum Valley, Lahad Datu, Sabah, ca. 4°55'N 117°46', coll. R. Stuebing, 2 March 1988. Others: 1 female (11.0 × 9.5 mm) (ZRC 1997.0138), in pitfall trap, Danum Valley Field Centre, Lahad Datu, Sabah, coll. C. Colón, 14 October 1996; 1 male (16.4 × 13.5 mm) (ZRC 1997.0140), in pitfall trap, Danum Valley Field Centre, Lahad Datu, Sabah, coll. C. Colón, 10 October 1996; 1 male (20.0 × 15.8 mm) (ZRC 1997.0141), in pitfall trap, Danum Valley Field Centre, Lahad Datu, Sabah, coll. C. Colón, 17 October 1996; 1 male (22.0 × 17.6 mm) (ZRC 1997.0142), in pitfall trap, Danum Valley Field Centre, Lahad Datu, Sabah, coll. C. Colón, 19 October 1996; 1 male (10.5 × 9.0 mm), 1 female (17.8 × 14.9 mm) (ZRC 2017.1272), in mud and leaves under wooden walk-way, Orchid Trail, Danum Valley Field Centre, Lahad Datu, Sabah, at night, coll. local rangers, 20 July 2017; 1 female (26.1 × 21.3 mm) (ZRC 2017.1047), in pool along wooden walkway at night, Orchid Trail, Danum Valley Field Centre, Lahad Datu, Sabah, at night, coll. local rangers, 20 July 2017; 1 female (24.0 × 17.5 mm) (ZRC 1997.0139), Kunak, Baturong, Binuang River, Lahad Datu, Sabah, coll. R. Stuebing, 20 March 1989.

**Colour.** In life, the carapace is reddish brown with the ambulatory legs lighter in colour; the chelipeds are orangish red with the fingers pale-yellow (Fig. 6A, B).

**Remarks.** The present series of specimens do not change the original description of this species in any way. The species does grow substantially larger than the type series, with the largest specimen here, a female measuring 26.1 × 21.3 mm (ZRC 2017.1047).

The available collection data indicates *T. dicerophilus* is a semiterrestrial nocturnal species and forages on the forest floor, usually in wet, swampy areas, digging burrows in the soft substrate; they were often caught in pitfall traps set near these areas (see also Ng and Stuebing 1990). This contrasts with the more aquatic habits of its congener in Danum Valley, *T. capillodigitus* sp. n.

#### Genus Arachnothelphusa Ng, 1991

Type species. Potamon (Potamon) melanippe De Man, 1899, by original designation.

#### Arachnothelphusa terrapes Ng, 1991

Figure 5

Arachnothelphusa terrapes Ng, 1991: 8, figs 3-6; Ng et al. 2008: 69.

**Material examined.** Holotype: male (17.6 × 13.3 mm) (ZRC 1992.7918), Danum Valley Field Centre, station 507, in dry stump on ridge, Lahad Datu, Sabah, Borneo, leg. H.K. Voris, 23 October 1990. Paratype: female (25.7 × 18.6 mm) (ZRC 1992.7919), Danum Valley, Lahad Datu, Sabah, Borneo, leg. S.C. Choy, 21 July 1989. Others: 1 male (30.8 × 20.5 mm), 1 female (30.1 × 20.5 mm, with 26 juvenile crabs) (ZRC 2017.1205), from water-filled tree buttress, ca. 35 cm above ground Danum Valley, Lahad Datu, Sabah, Borneo, Malaysia, 20 July 2017.

**Comparative material.** *Arachnothelphusa kadamaiana* (Borradaile, 1900): 1 female (23.2 × 17.1 mm) (ZRC 2009.0094), Poring, Basin 1A, Sabah, Malaysia, Borneo, coll. R.F. Inger et al., 12 August 1992; 3 males (21.1 × 15.8 mm, 22.8 × 16.5 mm, 25.3 × 18.5 mm) (ZRC 2002.0097), Crocker Range, Sabah, 5°27'N 116°03'E, coll. I. Das, 24 April 2001. *Arachnothelphusa* aff. *kadamaiana*: 1 female (19.0 × 14.2 mm) (ZRC 2002.0098), Bako National Park, Sarawak, coll. I. Das and L. Grismer, 27 March 2001. *Arachnothelphusa merarapensis* Grinang, Pui & Ng, 2015: Holotype male (22.5 × 16.8 mm) (ZRC 2016.0297), water-filled tree-hole, ca. 100 cm above ground, steep dipterocarp forest, Merarap Hot Spring Resort, Lawas, northern Sarawak, Malaysia, Borneo, 4°22'25.4"N, 115°26'10.1"E, 485 m asl, coll. J. Grinang and Y.M. Pui, 31 October 2014.

**Colour.** The live coloration of this species observed in the recent pair of specimens is a uniform dark purple colour on the dorsal surface of the carapace, ambulatory legs and chelipeds, with a pale purple to dull white on the thoracic sternum, pleon and distal portions of the ambulatory legs and cheliped fingers (Fig. 5B–F). The dark purple colouration appears considerably darker when the animal is dry, explaining the original paler colour observation by Ng (1991).

**Remarks.** Ng (1991) established *Arachnothelphsua* for several Bornean species previously classified as *Thelphusula* Bott, 1969, with *Potamon* (*Potamon*) *melanippe* De Man, 1899, as the type species. Currently, four other species are recognised: *A. kadamaiana* (Borradaile, 1900), *A. rhadamanthysi* (Ng & Goh, 1987), *A. terrapes* Ng, 1991, and *A. merarapensis* Grinang, Pui & Ng, 2015, all from northern Borneo. One species originally included by Ng (1991) in *Arachnothelphusa*, *Parathelphusa* (*Liotelphusa*) *nobilii* Colosi, 1920, was transferred to *Stygothelphusa* Ng, 1989, by Ng and Álvarez (2000) (see also Ng 2013; Ng and Grinang 2014).

*Arachnothelphusa terrapes* is easily distinguished from congeners by the deep U-shaped sinus separating the truncate external orbital tooth from the epibranchial tooth (Ng 1991: fig. 3). *Arachnothelphusa merarapensis* has a superficially similar anterolateral margin except that the two teeth are separated by an obtusely triangular broad cleft instead (Grinang et al. 2015: fig. 1A, B). Other congeners have the epibranchial tooth separated by a V-shaped notch or the margin is almost entire (De Man 1899: pl. 9; Ng and Goh 1987: pl. 3A; Ng 1991: fig. 1A; Grinang et al. 2015: fig. 6A).



**Figure 5.** Habitat and life colour of *Arachnothelphusa terrapes*. **A** water-filled tree hole at base of tree in Danum Valley where crab was found **B** water filled tree hole where crabs were hiding **C**, **D** male (30.8  $\times$  20.5 mm) (ZRC 2017.1205) **E**, **F** female (30.1  $\times$  20.5 mm, with juvenile crabs) (ZRC 2017.1205).

The biology of species of *Arachnothelphsua* is not well known. All known species are represented by only very few specimens (Ng 1991) and there is often no accompanying ecological data. *Arachnothelphsua melanippe* and *A. kadaimana* were both described without any indication of their biology (De Man 1899; Borradaile 1900). Grinang et al. (2015) reported on a female specimen from Poring in Sabah but there was no information on where it was found. In the ZRC there are two lots of *A. kadaimana* (ZRC 2009.0094, ZRC 2002.0097) from Sabah, also without specific habitat data. A female



**Figure 6.A, B** *Thelphusula dicerophilus*, female (26.1 × 21.3 mm) (ZRC 2017.1047) (in situ) **C, D** *Para-thelphusa valida*, male (27.8 × 22.7 mm) (ZRC 2017.1269) **E** *Terrathelphusa secula*, holotype male (29.2 × 20.4 mm) (ZRC 2018.0297) (preserved colour) **F** *Isolapotamon ingeri*, male (57.4 × 43.5 mm) (ZRC 1997.0799) (preserved colour).

specimen of *Arachnothelphusa*, close to but not conspecific with *A. kadaimana* (ZRC 2002.0098) from Bako National Park in Sarawak was collected from a tree trunk (I. Das, per. comm.). *Arachnothelphsua terrapes* was found low on shrubs (Ng 1991) while *A. rhadamanthysi* was collected on a stalagmite wall inside a cave (Ng and Goh 1987). The most detailed account so far was that by Grinang et al. (2015) for *A. merarapensis* from Merarap Hot Springs in Sarawak, who obtained the species from low tree holes approx. 150 cm from the ground. It is not known if the crabs live in phytotelms higher up on the forest trees. *Arachnothelphsua rhadamanthysi* has since been photographed by naturalists in the forested area outside Gomantong caves where it was first found, suggesting it is only a facultative cave dweller (Christensen 2015).

Arachnothelphusa terrapes was described from a pair of specimens, the first, a female collected in 1989 which moulted shortly after capture and died, leaving both the animal and exuvium in poor condition. The male holotype was collected a year later from a dry tree stump, with the live coloration being a deep reddish brown on dorsal surfaces, chelipeds and legs (Ng 1991: 11). In view of the present observations of this species as a tree hole specialist, it is likely the holotype male was only taking temporary refuge in the tree stump when it was found.

Two individuals of *A. terrapes* were observed at 0030 hours in Danum Valley, less than 50 m apart. The first, a large adult male was observed at the edge of a water-filled hole on a tree buttress, roughly 35 cm above the ground (Fig. 5A). A second, a female carrying newly hatched young under its pleon, was found inside a water filled tree hole approx. 150 cm above ground (Fig. 5F). This species is nocturnal and highly sensitive to light, swiftly retreating into their holes when disturbed. Additional observations by other naturalists who have photographed this species in Danum Valley suggest it is always found on trees and never on the forest floor itself (unpublished data). It is clear that *A. terrapes* is a true phytotelm species and predominantly arboreal in nature, living exclusively in tree-holes; although they will move in search of other tree holes if the one they are residing begins to dry up or when searching for a mate. All specimens have been observed on the lower parts of trees and it is not known if they climb much higher up. All specimens recorded so far have been solitary. The present observation of an adult female carrying young (Fig. 5F) is notable and confirms the species breeds in the phytotelm.

The biology of obligate arboreal crabs has been discussed at length by Sivasothi et al. (1993), Sivasothi (2000), Cumberlidge et al. (2005), Fratini et al. (2005), Grinang et al. (2015), Ng et al. (2015), Wehrtmann et al. (2016) and Kumar et al. (2017). While most are primary freshwater crabs (sensu Yeo et al. 2014) of the families Potamidae and Gecarcinucidae, members of two South East and East Asian sesarmid genera, Geosesarma De Man, 1892, and Scandarma Schubart, Liu & Cuesta, 2003, are primarily arboreal in habits (see Schubart et al. 2003; Naruse and Ng 2007; Ng 2017). There are of course some species of freshwater crabs that occasionally climb trees and use phytotelms but can also be found on the forest floor or nearby streams, and thus are not obligate arboreal species. In Asia, Sundathelphusa celer (Ng, 1991) (Gecarcinucidae), from the Philippines was collected in a tree hollow above ground, but it is not certain if it is a wholly arboreal species (Ng 2010). Perbrinckia scansor (Ng, 1995) from Sri Lanka has also been noted to have arboreal tendencies but is clearly a terrestrial species that occasionally climbs trees (Ng 1995: 183; Ng and Tay 2001: 148-149). In Hainan, China, some species of *Neotiwaripotamon* Dai & Naiyanetr, 1994, are known to be primarily arboreal (unpublished data; Shih 2008). In India, the only known true arboreal phytotelm species is the recently described Kani maranjandu Kumar, Raj & Ng, 2017.

#### Genus Terrathelphusa Ng, 1989

Type species. Geothelphusa kuhli De Man, 1883, by original designation.

## Terrathelphusa secula Ng & Tan, 2015

Figure 6E

Terrathelphusa secula Ng & Tan, 2015: 447, figs 1-3.

**Material examined.** Holotype male (29.2 × 20.4 mm) (ZRC 2018.0297), found dead in pool adjacent to Borneo Rainforest Lodge, next to Danum Valley Conservation Area, Lahad Datu, Sabah, 4°58.2'N 117°41.4'E, ca. 600 m asl, East Malaysia, Borneo, coll. local ranger, 28 May 2015.

**Colour.** The freshly dead type specimen was described as dark brown overall (Ng and Tan 2015: 448).

**Remarks.** The species was described from just outside the Danum Valley Conservation Area by Ng and Tan (2015) from a recently dead specimen. *Terrathelphusa* species are difficult to collect due to their secretive terrestrial habits and tendency to dig deep burrows, coming out only at night and during the wet season (Grinang and Ng 2015).

*Terrathelphusa* was established by Ng (1989c) for a group of terrestrial species from Java and Borneo (type species *Geothelphusa kuhli* De Man, 1883, from Java) and now contains 11 species (Ng et al. 2008; Ng and Tan 2015); although the genus is probably not monophyletic (unpublished data). *Terrathelphusa secula* is unusual among congeners in possessing a very ovate carapace and a G1 that is elongate with a long and strongly curved terminal segment (Ng and Tan 2015).

#### Genus Parathelphusa H. Milne Edwards, 1853

**Type species.** *Parathelphusa tridentata* H. Milne Edwards, 1853, by subsequent designation (Rathbun 1905).

# Parathelphusa valida Ng & Goh, 1987

Figure 6C, D

*Parathelphusa valida* Ng & Goh, 1987: 317, pls 1, 2; fig. 1; Ng 1995: 79; Tan and Ng 1997: 555; Ng et al. 2008: 71; Klaus et al. 2013 68.

**Material examined.** Holotype: male (40.0 × 30.0 mm) (ZRC 1989.2024), stream outside Simud Hitam Cave, Gomantong, Sabah, Borneo, ca 5°33'N 118°06'E, coll. P. Chapman, 27 March 1986. Paratypes: 1 male (ZRC 1989.2192), Simud Puteh Cave, Gomantong, Sabah, Borneo coll. P. Chapman, 27 March 1986; 2 males (ZRC 1990.0445–0446), 1 male (ZRC 1989.3402), Sungei Madai, in stream just outside Madai Caves, Kunak, Lahad Datu district, Sabah, Borneo, ca. 04°44'N 118°12'E, coll. January 1985; 1 male (73.0 × 73.0 mm) (ZRC 1990.0444), Sungei Binuang, stream

adjacent to Baturung Caves, Kunak, Lahad Datu district, Sabah, Borneo, 4°43'N 117°59'E, coll. R. Goh, 20 March 1985. Others: 1 female (ZRC 1989.3403), Sungei Madai, in stream just outside Madai Caves, Kunak, Lahad Datu district, Sabah, Borneo, ca. 04°44'N 118°12'E, coll. R. Goh, January 1985; 1 male (ZRC 1996.1897), Gomantong Caves Sabah, coll. C.L. Chan, January 1995; 1 male, 1 female (ZRC 2009.0091), Gomantong Caves, coll. D. Chia, 21 December 1999; 1 female (ZRC 1996.1999), Sungei Binuang, Banturung Caves, Lahad Datu, coll. R. Goh, 20 March 1989; 1 male (ZRC 1990.0571), Danum Valley, Sabah, coll. R. Stuebing, 1980s; 1 male (ZRC 1990.0568), Danum Valley, Lahad Datu, Sabah, coll. R. Stuebing, 23 July 1989; 1 male, 2 females (ZRC 1008.1346), Sungei Palun Tambun, tributary of Sungei Segama, upstream of Danum Valley Field Centre, Lahad Datu, Sabah, coll. H.H. Tan et al., 1 October 1996; 2 males, 5 females (ZRC 1996.1998), Kallang Sebaru stream, 4°58'4.8"N, 107°48'56.5"E, Danum Valley, Lahad Datu, Sabah, coll. H.H. Tan et al., 1 October 1996; 1 male, 1 female (ZRC 1996.1997), Sepat Kalisun, Danum Valley, Lahad Datu, Sabah, coll. H.H. Tan et al., 1 October 1996; 2 males (ZRC 1996.1995) Ca Gin Stream Right, 4°59'8.5"N, 107°54'5.1"E, Danum Valley, Lahad Datu, Sabah, coll. H.H. Tan et al., 2 October 1996; 2 females (ZRC 1996.2000), Sungei Bole Ketabil tributary, 4°57'33.5"N, 117°51'34.1"E, Danum Valley Field Centre, Lahad Datu, Sabah, coll. H.H. Tan et al., 2 October 1996; 1 male, 2 females (ZRC 1996.1994), Sungei Bole Ketabil tributary, 4°57'33.5"N, 117°51'34.1"E, Danum Valley Field Centre, Lahad Datu, Sabah, coll. H.H. Tan et al., 2 October 1996; 2 males, 2 females (ZRC 2010.0045), West Six stream, tributary of Sungei Segama, 600 m inside conservation area, coll. H.H. Tan et al., 4 October 1996; 1 male, 1 female (ZRC 1996.2004), West Eight, forest stream, 800 m into conservation area, tributary of Sungei Segama, Danum Valley Field Centre, Lahad Datu, Sabah, coll. H.H. Tan et al., 4 October 1996; 2 males (ZRC 2009.0309), Sungei Palum Tambum, near Danum Valley Field Centre, Lahad Datu, Sabah, coll. K. Martin-Smith, 9 October 1996; 1 female (34.7 × 28.4 mm) (ZRC 2017.1268), Danum Valley Field Centre, in forest streams, Lahad Datu, Sabah, Borneo, at night, coll. locals, 20 July 2017; 1 male (27.8 × 22.7 mm), 1 young female (17.4 × 5.3 mm) (ZRC 2017.1269), Danum Valley Field Centre, just outside dorms in streams, at night, Lahad Datu, Sabah, Borneo, coll. 22 July 2017; 1 male, 1 female (ZRC 2008.483), Maliau Basin, stream draining into Sungei Maliau, coll. S.H. Tan and T.H.T. Tan, 15–17 May 1996; 2 males, 1 female (ZRC 2008.0607), Maliau Basin, tributary of Sungei Maliau, adjacent Camp 88, coll. S.H. Tan and T.H.T. Tan 15-17 May 1996; 1 male, 1 female (ZRC 1997.0104), Maliau Basin, coll. S.H. Tan and T.H.T. Tan, 13-17 May 1996; 1 female (ZRC 1989.2194), Maliau Basin, Sabah, coll. Sabah Foundation Expedition 1988; 1 male, 2 females (ZRC 1996.2005), Tawau Plateau, Telupid Sandakan stream, coll. R. Goh, 1990; 5 males (ZRC 2008.1345), stream by Air Panas, near base of Tawau Hills Park, coll. H.H. Tan et al., 5 October 1996; 1 male 4 females (ZRC 1996.2008), Tawau, Sabah, 4°18'03"N, 117°54'20.7"E, coll. H.H. Tan et al., 5 October 1996; 2 males, 4 females (ZRC 1996.2001), Tawau, Sungei Matarid, Gua Madai, Jalan Madai, 4°43'8.7"N, 118°9'14.7"E, Tawau, Sabah, H.H. Tan et al., 6 October

1996; 1 male (ZRC 1994.4201), Danau Biandum, Kinabatangan River, Sabah, coll. S.H. Tan et al., 8 April 1994.

**Colour.** Fresh specimens have an olive-brown carapace with the grooves and striae reddish brown; the ambulatory legs are brown with specks of reddish brown; and the chelipeds are orange, with the fingers black except for the orange tip (Fig. 6C, D).

**Remarks.** The recently collected specimens agree well with the published descriptions and figures of the species, originally described from Gomantong, Bettontan and Lahad Datu in Sabah (Ng and Goh 1987). The species has a wide range in eastern Sabah (see also Ng 1995; Tan and Ng 1997).

*Parathelphusa valida* occurred syntopically with *T. capillodigitus* sp. n. and was present in a variety of habitats including jungle streams, swampy areas and on the forest floor. It has not been previously formally recorded from Danum Valley, which is surprising, considering it is by far the most common species there and there are many specimens in the museum dating back to the 1980s.

#### Family Potamidae Ortmann, 1896

#### Genus Isolapotamon Bott, 1968

Type species. Potamon anomalus Chace, 1938, by original designation.

## Isolapotamon ingeri Ng & Tan, 1998

Figure 6F

*Isolapotamon* sp. – Ng and Goh 1987: 328, pl. 3, D. *Isolapotamon ingeri* Ng & Tan, 1998: 66, figs 6E–H, 7; Ng et al. 2008: 163.

**Material examined.** Holotype: male (44.3 × 33.3 mm) (ZRC 1997.0796), Sungei Tawau, Tawau Hills Park, Tawau, Sabah, coll. P. Yam, 14 December 1991. Paratypes: 1 female (41.4 × 31.0 mm) (ZRC 1997.0797), same data as holotype. Others: 1 male, 2 females, 1 juvenile (ZRC 2000.2217), Lower Segama River, Danum Valley Field Centre, Lahad Datu, Sabah, coll. K. Martin-Smith, June 1996; 1 male (57.4 × 44.8 mm) (ZRC 1997.0798), Sungei Palum Tambum, near Danum Valley Field Centre, Lahad Datu, Sabah, coll. K. Martin-Smith, August 1996; 5 males (largest 57.4 × 43.5 mm), 1 female (ZRC 1997.0799), Sungei Palum Tambun, near Danum Valley Field Centre, Lahad Datu, Sabah, coll. K.M. Martin-Smith, 9 October 1996; 1 female (ZRC 2000.2218), Sepat Kalisun, stream 200 m from 4°58'04.8"N, 117°48'56.5"E, Danum Valley Field Centre, Lahad Datu, Sabah, coll. H.H. Tan et al., 1 October 1996; 1 male, 2 females (ZRC 2000.2210), Sungei Bole Ketabil tributary, 4°57'33.5"N, 117°51'34.1"E, Danum Valley Field Centre, Lahad Datu, Sabah, coll. H.H. Tan et al., 2 October 1996; 1 male, 2 females (ZRC 2000.2220), Cabin Stream, 50 km on road

to Danum Valley Conservation Area, drains from Bukit Rafflesia, Lahad Datu, Sabah, coll. H.H. Tan et al., 2 October 1996; 1 male (ZRC 2000.2221), West Eight, forest stream, 800 m into conservation area, tributary of Sungei Segama, Danum Valley Field Centre, Lahad Datu, Sabah, coll. H.H. Tan et al., 4 October 1996; 2 females (ZRC 2008.0431), Danum Valley Rainforest Lodge, 5°03'2.9"N, 117°34'34.8"E, Lahad Datu, Sabah, 3 October 1996; 1 female (56.0 × 42.0 mm) (ZRC 1989.3419), Sungei Madai, Madai Caves, Sabah, coll. R. Goh, 27 January 1985; 1 male (ZRC 1997.0802), Tawau, Sungei Matarid, Gua Madai, Jalan Madai, 4°43'8.7"N, 118°9'14.7"E, Tawau, Sabah, H.H. Tan et al., 6 October 1996.

Colour. The colour in life is dark green overall (H.H. Tan, pers. comm.).

**Remarks.** *Isolapotamon ingeri* belongs to the same group of species as *I. kinabauense* (Rathbun, 1904) and *I. anomalum* (Chace, 1938) (both from the Mount Kinabalu area in northern Sabah), with the distal part of the terminal segment of the G1 expanded and flap-like (Ng and Tan 1998). Like these species, *I. ingeri* usually occurs in large streams and rivers with large rocks and fast flowing water.

#### Family Sesarmidae Dana, 1851

#### Genus Geosesarma De Man, 1892

**Type species.** *Sesarma* (*Geosesarma*) *nodulifera* De Man, 1892, by subsequent designation (Serène and Soh 1970).

## Geosesarma danumense Ng, 2002

Figure 7A–C

*Geosesarma danumense* Ng, 2002: 303, figs 1–3; Ng et al. 2008: 220.

**Material examined.** Holotype: male  $(14.8 \times 14.6 \text{ mm})$  (ZRC 2017.1298), in pitfall trap, primary forest, Danum Valley Field Centre, Sabah, Malaysia, coll. C. Colón, 22 November 1996. Others: 1 ovigerous female  $(14.9 \times 15. \text{ mm})$  (ZRC 2017.1273), in water filled rotting log, Nature Trail, Danum Valley Field Centre, Sabah, Borneo, coll. local rangers, 21 July 2017.

**Comparative material.** *Geosesarma sabanum* Ng, 1992: holotype male (13.1 × 13.6 mm) (ZRC 2018.0296), on leaf of herb in forest, ca. 50 m from nearest stream, Tawau Hills Park, eastern Sabah, Malaysia, Borneo, coll. R.F. Inger, 3 November 1991.

**Colour.** *Geosesarma danumense* has a dark yellow to orange carapace, purple ambulatory legs with scattered white specks, orange merus and carpus of the chelipeds, with the palm and fingers white (Fig. 7A–C). The eggs of the recently collected ovigerous female were observed to be a bright reddish orange in colour and large in size, indicating that the development is probably direct (see Soh 1969; Ng and Tan 1995). The live coloration is very similar to the related *G. sabanum* Ng, 1992 from Tawau (Fig. 7D).



**Figure 7.A** *Geosesarma danumense*, ovigerous female  $(14.9 \times 15.1 \text{ mm})$  (ZRC 2017.1273) **B, C** *G. danumense*, male (carapace width ca. 1.5–2.0 cm), on log approx. 30 cm from ground, ca. 30 m from river flowing through Danum Valley Field Centre, 10 pm, 25 July 2013, specimen not collected (photographs: Marcus Ng) **D** *G. sabanum*, male, on leaf above ground, Tawau Hills National Park, specimen not collected (photograph Ying Seawei).

**Remarks.** Geosesarma danumense and G. sabanum are morphologically close, although the external orbital tooth of the latter species is proportionately more slender, the frontal margin less truncate, the ambulatory meri proportionately shorter, and most significantly the corneous distal part of the G1 is proportionately longer (see Ng 2002).

The holotype of *G. danumense* was obtained from a pitfall trap while the recent large ovigerous female (ZRC 2017.1273) was collected from under a rotting log. Specimens have also been observed climbing small shrubs. In this respect, it probably has similar habits to *G. sabanum* from Tawau which has been observed by the second author to hide between the leaves of *Pandanus* sp. during the day, emerging only at night to forage on low lying vegetation and occasionally amongst leaf litter (unpublished data). The terrestrial habits of *G. danumense* and *G. sabanum* probably parallel those known for species in Peninsular Malaysia and Indonesian Kalimantan (Ng 2015, 2017).

Manuel-Santos et al. (2016: 336) commented that the three species of *Geosesarma* on Palawan Island in the Philippines, *G. lawrencei* Manuel-Santos & Yeo, 2007, *G. batak* Manuel-Santos, Ng & Freitag, 2016, and *G. tagbanuana* Manuel-Santos, Ng & Freitag, 2016, are morphologically very similar to the Sabahan *G. danumense* and *G. sabanum*, notably in their relative large adult size and long slender ambulatory legs. Their G1 structures, however, are very different, with those of the latter two species proportionately much shorter and stouter (cf. Ng 1992, 2002; Manuel-Santos and Yeo 2007; Manuel-Santos et al. 2016).

# Key to freshwater crabs in the Danum Valley Conservation Area

1	Third maxillipeds forming median rhomboidal gap when fully closed; carapace frontal margin with 4 distinct truncate lobes; cornea large, appearing bulbous in life; frontal and lateral surfaces of carapace with net-like pattern of short setae; exopod of third maxilliped without flagellum; terrestrial species
_	<i>Geosesarma danumense</i> Third maxillipeds closing without any median rhomboidal gap; carapace frontal margin entire with 2 weakly separated rounded lobes; eyes not swol- len in life; frontal and lateral surfaces of carapace may be granular but never with net-like pattern of short setae; exopod of third maxilliped with distinct
2	flagellum; terrestrial and aquatic species
_	Anterolateral margin of carapace rounded or straight, entire or with at most one tooth (external orbital tooth)
3	Frontal margin and orbits of carapace appears sunken in from dorsal view; ambulatory legs very elongate, longest leg 4–5 times longer than carapace length: lives in tree holes
_	Frontal margin and orbits of carapace level with sides from dorsal view; am- bulatory legs proportionately much shorter; free-living
4	Anterolateral carapace margin with a distinct epibranchial tooth clearly sepa- rated from external orbital tooth by V-shaped notch; G1 with neck-like me- dian section and rectangular flap distally; lives under rocks in fast flowing
	waterIsolapotamon ingeri
_	Anterolateral carapace margin appears entire or with a low epibranchial tooth barely separated from external orbital tooth; G1 gradually tapering towards tip, straight or curved; terrestrial, semiterrestrial, in swampy areas or slow
5	Carapace relatively flat, not prominently raised; gastric regions with distinct transverse striae; fingers of adult male chelipeds with dense mat of short setae; mostly aquatic species
_	Carapace prominently raised, appears swollen; gastric regions appears smooth, without prominent transverse striae; fingers of adult male chelipeds granulated without setae: terrestrial to semiterrestrial species
6	Carapace almost squarish to slightly rectangular: G1 terminal segment elon-
0	earapace annost squarisi to signify rectangular, GT terminal segment cloir
_	Carapace transversely ovate, egg-shaped; G1 terminal segment strongly curved, hook-like
#### Acknowledgements

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RESEARCH ARTICLE



# Translating Niphargus barcodes from Switzerland into taxonomy with a description of two new species (Amphipoda, Niphargidae)

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#### Abstract

The amphipod genus *Niphargus* (Amphipoda: Niphargidae Bousfield, 1977) is the most species-rich genus of freshwater amphipods in the World. Species of this genus, which live almost exclusively in subterranean water, offer an interesting model system for basic and applied biodiversity science. Their use, however, is often limited due to the hitherto unresolved taxonomy within the whole genus. As a comprehensive taxonomic revision of the currently >425 *Niphargus* species is too demanding, it has been suggested that the taxonomy of the genus could be advanced in smaller steps, by reviewing regional faunas, that would eventually integrate into a global revision. In this study, we provide such a revision of *Niphargus* in Switzerland. First, we molecularly delimited, morphologically diagnosed, and formally described two new species, namely *Niphargus luchoffmanni* **sp. n.** and *Niphargus tonywhitteni* **sp. n.** Second, we updated and revised a checklist of *Niphargus* in Switzerland with new findings, and prepared a list of reference sequences for routine molecular identification, available at BOLD and GenBank. All available specimens of 22 known species from the area were morphologically examined, and their morphological variation was compiled in a data file of DEscription Language for TAxonomy, which can be used for automated genera-

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tion of dichotomous or interactive keys. The data file is freely available at the World Amphipoda Database. Together, the checklist, the library of reference sequences, the DELTA file, but also a list of hitherto unresolved aspects are an important step towards a complete revision of the genus within a well-defined and biogeographically interesting area in Central Europe.

#### **Keywords**

Amphipoda, barcodes, DELTA, groundwater, integrative taxonomy, Niphargidae, web-taxonomy

#### Introduction

*Niphargus* Schiødte, 1849 (Amphipoda: Niphargidae Bousfield, 1977) is an amphipod genus living almost exclusively in groundwater ecosystems of the West Palearctic (Copilaş-Ciocianu et al. 2018). With >425 described species (Horton et al. 2018) it is among the most species-rich freshwater amphipod genera (Väinölä et al. 2008, Horton et al. 2018), and an important representative of European groundwater macroinvertebrate fauna (Zagmajster et al. 2014). The genus is an interesting model system for biogeography (McInerney et al. 2014), evolutionary ecology (Trontelj et al. 2012, Copilaş-Ciocianu et al. 2018), and applied ecology (Marmonier et al. 2013).

The use of *Niphargus* species in applied ecology is often limited due to the partly unresolved and still incomplete taxonomy within the genus. The taxonomic incompleteness in the first place mirrors the biology and ecology of Niphargus: many species have narrow ranges, sometimes spanning only a few kilometres around their type localities (Meleg et al. 2013) and ranges extending beyond a few hundred kilometres are the exception (Trontelj et al. 2009, Copilaș-Ciocianu et al. 2017, Copilaș-Ciocianu et al. 2018). Consequently, any newly investigated cave can potentially harbour new, undescribed species. Moreover, many species are elusive and can only be found after repeated sampling (Pipan and Culver 2007, Fišer and Zagmajster 2009). This often involves intense fieldwork on a fine scale, with difficulty to access habitats, and often requires advanced caving techniques and the help of local cavers. Second, the genus is characterised by inherent challenges with respect to species delimitations. Morphological differences between species are often subtle, while intraspecific variation can be high (Fišer et al. 2008, Fišer and Zagmajster 2009, Delić et al. 2016). A low number of specimens per sample often hampers further insights into intra- and interspecific variation, and limits taxonomic decisions based on morphology only. Consequently, taxonomic evaluations need to be complemented with molecular data, and in some speciescomplexes diagnoses entirely depend on diagnostic sequences (Delić et al. 2017a, b). With cryptic species (i.e., morphologically indistinguishable species) being repeatedly found in Niphargus, the revision of the genus is feasible only upon critical assessment of molecular, morphological, geographical and ecological data, that is, within an integrative taxonomy framework (Padial et al. 2010, Yeates et al. 2011).

Overall, a comprehensive taxonomic revision of *Niphargus*, encompassing all hitherto described species between Ireland and Iran, is technically challenging and unlikely to be completed in the near future. To make *Niphargus* accessible for various end-users of taxonomy, such as naturalists, ecotoxicological laboratories, or nature conservation agencies, local revisions rather than a single global revision represent a more realistic way forward. Such geographically restricted revisions could link local species checklists, diagnostic morphological traits and barcoding sequences, and thereby make the group accessible to these users. Local revisions can be completed within a realistic time, and also contain fewer morphologically similar species than an eventual global revision. In the long term, carefully composed local revisions, based on the inclusion of appropriate outgroups and representative species from the whole genus, can be integrated into a global revision.

The idea of geographically restricted revisions was already applied to *Niphargus* in the Middle East (Esmaeili-Rineh et al. 2015a). An initial overview of the fauna (Fišer et al. 2009a) was followed by field work. Samples from the Middle East were first delimited using molecular phylogenetic tools (Esmaeili-Rineh et al. 2015b), and then complemented by morphological analyses, morphological diagnoses and a construction of a morphological database using DEscription Language for TAxonomy (DELTA ; Esmaeili-Rineh et al. 2017b). The latter presented the basis for all subsequent morphological comparisons but also for automated constructions of interactive or dichotomous identification keys. Until now, this database has been continuously updated with descriptions of new species (Mamaghani-Shishvan et al. 2017, Esmaeili-Rineh et al. 2017b).

A similar approach was applied to the geographically restricted diversity of *Niphargus* species in Switzerland. An initial checklist and molecular exploration (Altermatt et al. 2014) was complemented by further sampling and descriptions of new species (Fišer et al. 2017). The latter studies identified several species awaiting taxonomic evaluation. Here, we further advance this revision of *Niphargus* from Switzerland. We first described two more species. Second, in order to accelerate further research of groundwater communities in Switzerland, we overviewed current knowledge of the taxonomy of the genus and constructed a DELTA database of morphological characters. Finally, we prepared a reference library of COI sequences, and linked them to species names.

# Materials and methods

# Sampling and origin of specimens

The studied specimens derive from various sampling campaigns (2010–2017). Most of the specimens from the newly described species were sampled for a larger study on springs in the Swiss mountains (Verena Lubini and Aquabug AG, Neuchâtel, Switzerland). The sample from the Töss River interstitial was collected using a hand pump. The sample from Achensee in Austria was sampled using a rectangular kicknet ( $25 \times 25$  cm) with mesh size of 500 µm and disturbing the littoral zone manually. Specimens were conserved in ethanol. Most of the samples (67) were already analysed

in previous studies (Altermatt et al. 2014, Fišer et al. 2017); in this study we molecularly analysed samples from 15 additional locations (Suppl. material 3). Specimens were morphologically examined, and at least one individual per sample was sequenced, as described in the subsequent sections.

#### Morphometric analyses

The specimens were partly dissected in glycerol, and mounted on slides in glycerol gelatine. The animals were observed under a stereomicroscope Olympus SZX9 and a light microscope Zeiss Primo Star. For measurements, photographs and measurements were made using the program cellSense (Olympus); details on landmarks and overview of taxonomic characters are presented in Fišer et al. (2009c). Illustrations were prepared following digital inking (Coleman 2003, 2009) in Adobe Illustrator CS6, using photos as background pictures (taken on a Leica M205C with a mounted Canon EOS 5D Mark III).

# Molecular and phylogenetic analysis

Genomic DNA was isolated from one of the pereiopods or the whole animal (depending on specimen size) using the GenElute Mammalian Genomic DNA (Sigma-Aldrich, United States). We amplified the mitochondrial cytochrome oxidase I (COI) gene and three nuclear DNA gene fragments: part of 28S rRNA gene (28S), histone H3 (H3) and internal transcribed spacer I and II (ITS). A 660 bp long fragment of COI was amplified using primers LCO 1490 and HCO 2198 (Folmer et al. 1994); the part of 28S using primers from Verovnik et al. (2005) and Zakšek et al. (2007) and the H3 gene using primers from Colgan et al. (1998). PCR cycling conditions were the same as described in Fiser et al. (2013). For one of the focal species in this study, a subset of samples was selected for which about 2100 bp long fragments of the complete ITS region, including the flanking proportions of the 18S and 28S genes, were amplified using primers and procedures described by Flot et al. (2010). PCR products were purified using Exonuclease I and FastAP (Thermo Fisher Scientific Inc., United States) according to the manufacturer's instructions. Each fragment was sequenced in both directions using PCR amplifications primers by Macrogen Europe (Amsterdam, Netherlands). An exception was the ITS fragment, which was sequenced using two additional primer pairs: i) ITS sf1– ITS sr1 and ii) ITS sf2– ITS sr2 (Flot et al. 2010). Chromatograms were assembled and edited using Geneious 11.0.3 (Biomatters, New Zealand).

#### **Phylogenetic analyses**

The data for three gene fragments (COI, 28S and H3) were complemented with available sequences from previous studies, with the aim of including different phylogenetic lineages and potentially closely related taxa (Altermatt et al. 2014, Fišer et al. 2017). The dataset for phylogenetic analysis included 126 specimens and 98 taxa (Suppl. material 1). *Microniphargus leruthi* Schellenberg, 1934, the nearest taxon to the genus *Niphargus*, was used as outgroup. Sequences of all sequenced loci were aligned using MAFFT v.7 (Katoh and Standley 2013). The total length of the concatenated dataset was 1953 bp. We searched for the best-fitting substitution models and partitioning scheme using PartitionFinder 2.1.1 (Lanfear et al. 2012). Phylogenetic relationships were reconstructed using Bayesian inference with partition-specific settings in Mr-Bayes 3.2.6 (Ronquist and Huelsenbeck 2003). A Bayesian MCMC tree search with two independent runs with four chains for each was run for 20 million generations, trees were sampled every 1000 generations. After reaching the stationary phase, the first 25% of trees were discarded, and from the remaining trees a 50% majority rule consensus tree was calculated. Phylogenetic analyses were run on the CIPRES Science Gateway (Miller et al. 2010, accessible at www.phylo.org).

#### Species delimitation procedures

The selection of species delimitation methods critically depends on the species concept used. We applied the general lineage species concept, which states that species emerge as independently evolving segments of metapopulations (de Queiroz 2007). Within this concept, a species-taxon is delimited on evidence for a lack of gene flow among segments of the metapopulation. As such, it provides a broad testable framework for different spatial and ecological contexts of speciation, using different lines of evidence (e.g., characters, for more details see discussion in Fišer et al. 2018). The concept has been successfully and broadly used in *Niphargus* taxonomy (Fišer et al. 2009b).

Two different molecular-based species delimitation methods were applied: a distance-based Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012) and a tree-based Poisson Tree Processes (PTP) and Bayesian PTP (bPTP) model (Zhang et al. 2013). ABGD is an automated procedure that clusters sequences into candidate species based on pairwise distances by detecting differences between intra- and interspecific variation (i.e., barcoding gap) without *a priori* species hypothesis. ABGD analyses were performed at the ABGD web-server (http://wwwabi.snv.jussieu.fr/public/abgd/ abgdweb.html ) and analysed for COI sequences using the two available models Jukes-Cantor (JC69) and the Kimura K80, and three different values of relative gap width (X = 1.5, 1, 0.5). For the PTP and bPTP analyses we generated a new dataset from COI sequences used in this study combined with the largest available COI dataset for Niphargus (Eme et al. 2018). The maximum likelihood phylogenetic tree was generated using 855 haplotypes in PhyML 3.0 available at http://www.atgc-montpellier.fr/phyml/ (Guindon et al. 2010) using the GTR+I+G evolutionary model. We used the resulting phylogenetic tree as input for PTP and bPTP analyses. Calculations were conducted on the PTP webserver (http://species.h-its.org/ptp/), with 500,000 MCMC generations, thinning set to 100 and burn-in at 25% and performing a Bayesian search.

In one focal species clade, an additional nuclear marker (ITS) was applied which provides a higher level of genetic variation and combined with mitochondrial COI (Suppl. material 2). The presence of species level lineages in sequence variation within the *N. tonywhitteni* sp. n. – *N. thienemanni* clade was also assessed by means of statistical parsimony (Templeton et al. 1992). Haplotype networks were built for both COI and ITS sequences using PopART software at http://popart.otago.ac.nz (Leigh and Bryant 2015).

The molecular delimitations were revised within the respective spatial context and with analyses of morphological variation. We searched for ecological differences in localities, and diagnostic morphological traits. Considering the paucity of the data, we could not apply statistical tests on the latter.

#### **Identification tools**

In order to ease future molecular species identification, we revised the molecular data for *Niphargus* species reported from Switzerland. For species with unambiguous taxonomy we submitted their COI sequences to the Barcode of Life Data System (BOLD). Some species complexes await taxonomic evaluations. For these, a list of 28S sequences available at GenBank was compiled such that potential taxonomy-users can at least approximately identify the respective lineages.

In order to ease future analyses of morphological variation, we constructed a database in DELTA (Dallwitz et al. 1999). DELTA allows morphological characterization of species with both quantitative and qualitative morphological characters, and this information can be easily converted into species descriptions and dichotomous or interactive identification keys (Coleman et al. 2010).

#### **Results and discussion**

#### Faunistics

New samples yielded mostly species reported from previous studies: *N. styx* Fišer, Konec, Alther, Švara & Altermatt, 2017, *N. puteanus* (Koch, 1836), *N. thienemanni* Schellenberg, 1934, *N. rhenorhodanensis* Schellenberg, 1937, a species labelled as *N.* cf. *thienemanni* in Fišer et al. (2017), and *N. luchoffmanni* sp. n. (see section "Species descriptions" below) labelled as *N.* cf. *stygius* 1 in Fišer et al. (2017) (see Suppl. material 3). In addition, the new samples unveiled a species that morphologically corresponds to the description of *N. aquilex* Schiødte, 1855. We could not verify its molecular identity, however, this is the first finding and confirmation of this species in Switzerland after decades (Strinati 1966). Interestingly, the new finding is not far from the first finding place (Fig. 1). The number of *Niphargus* species from Switzerland has risen to 22. Still unresolved is the status of two taxa, likely distinct and undescribed species, so far only



**Figure 1.** Finding sites of the new species *N. tonywhitteni* sp. n. (purple) and *N. luchoffmanni* sp. n. (green), and the distribution of *N. aquilex* (yellow) in Southern Germany and Northern Switzerland. Data source: Esri, 2013: Data & Maps for ArcGIS for use with Esri software; Elevation map of Europe EEA, Copenhagen, 2004.

represented by two and one individual, and provisionally labelled as *N*. cf. *thienemanni* and *N*. cf. *stygius*, respectively. Another problem is the paraphyletic complex *N*. *rhenorhodanensis*, with at least five distinct lineages, many of which may comprise more than one species, and their occurrence in Switzerland is not yet resolved (Table 1).

# Molecular and phylogenetic analysis, species delimitation, and barcodes

Phylogenetic analyses included new samples of *Niphargus* from Switzerland (12 additional individuals; sequences from three samples could not be obtained). The additional samples did not affect phylogenetic structure (Fig. 2) and the newly obtained phylogeny showed no substantial differences from the previously published one (Fišer

List of <i>Nipbargus</i> species of Switzerland	Reference sequence (28S) for lineage identifcation GenBank Access. No. <sup>1</sup>	Reference sequence (COI) for species identifcation GenBank Access. No. <sup>1</sup>	Morphological information available in DELTA <sup>2</sup>
Niphargus aquilex Schiødte, 1855	/	/	yes
Niphargus auerbachi Schellenberg, 1934	EU693292	KX379130	yes
Niphargus brixianus Ruffo, 1937	KX379011	KX379109	yes
Niphargus caspary Pratz, 1866	KX379003	KX379123	yes
<sup>3</sup> Niphargus cf. stygius	KX379016	KX379103	yes
<sup>3</sup> N. cf. <i>thienemanni</i> Schellenberg, 1934	KX379031	KX379074	yes
Niphargus forelii Humbert, 1877	/	/	yes
Niphargus inopinatus Schellenberg, 1932	/	KY707004	yes
Niphargus luchoffmanni sp. n.	KX379014	KX379105	yes
<i>Niphargus muotae</i> Fišer, Konec, Alther, Švara & Altermatt, 2017	KX379024	KX379095	yes
<i>Niphargus murimali</i> Fišer, Konec, Alther, Švara & Altermatt, 2017	KX379022	KX379097	yes
Niphargus puteanus Koch, 1836	MH172402	MH172434	yes
<sup>3</sup> Niphargus rhenorhodanensis complex Schellenberg, 1937, lineage ABC	KJ566681	KX379117	On a level of complex
<sup>3</sup> Niphargus rhenorhodanensis complex Schellenberg, 1937, lineage FG	KX379042	KX379084	On a level of complex
* <i>Niphargus rhenorhodanensis</i> complex Schellenberg, 1937, lineage H	KJ566685	KX379116	On a level of complex
<sup>3</sup> Niphargus rhenorhodanensis complex Schellenberg, 1937, lineage JK	MH172416	MH172436	On a level of complex
Niphargus setiferus Schellenberg, 1937	/	/	yes
<i>Niphargus styx</i> Fišer, Konec, Alther, Švara & Altermatt, 2017	KX379023	KX379096	yes
Niphargus thienemanni Schellenberg, 1934	KJ566688	KX379114	yes
Niphargus thuringius Schellenberg, 1934	/	KY706717	yes
Niphargus tonywhitteni sp. n.	KX379045	KX379081	yes
Niphargus virei B. Chevreux, 1896	KJ566680	KX379098	yes

Table 1. A check list of Niphargus species from Switzerland, with an overview of the diagnostic traits.

<sup>1</sup>The diagnostic traits of two genes; accessible via GenBank and BOLD.

<sup>2</sup>Morphological diagnostic traits are available in DELTA database.

<sup>3</sup>The taxonomy of the species is not resolved yet; their identity of the species can be assessed only to lineage level.

et al. 2017). The phylogenetic position of the two herein described species, however, deserves more discussion.

The first species, *Niphargus tonywhitteni* sp. n. (see section "Species description" below), is closely related to *N. thienemanni* (Fig. 2, blue and purple shading). Genetic differences between the two sister species are relatively small, but distinct. ABGD analysis using default value of relative gap width (1.5) suggested that the lineage comprised a single species. By contrast, lowering the threshold to 0.5 (also used in Fišer et al. 2017) suggested that the lineage was comprised of two species. This result was



**Figure 2.** Phylogenetic relationships of *Niphargus* species focusing on new taxa from Switzerland. Highlighted are two new species and a sister species (green: *N. luchoffmanni* sp. n., blue: *N. thienemanni*, purple: *N. tonywhitteni* sp. n.) The tree was constructed using Bayesian inference on COI, 28S rRNA and histone gene sequences. The tree was rooted using *Microniphargus leruthi* (not presented). On the right side, two haplotype networks (based on COI and ITS) calculated within *N. thienemanni* and *N. tonywhitteni* sp. n. species pairs are shown. These networks are based on a higher number of samples (N=10, see Suppl. material 2) compared to the phylogenetic tree. Colours in the networks correspond to colours in the tree.

concordant with results from the PTP and bPTP analyses. The results of PTP and bPTP did not differ from each other and both analyses identified two species within this lineage. A more detailed network analysis reinforced the hypothesis that the lineage is comprised of two species. The patterns in differentiation of mitochondrial COI and molecular ITS markers were congruent, and both networks suggest there is no indication of gene flow between the two species (Fig. 2). In addition, the analysis of field notes implied that the two species might differ ecologically (Fig. 1). Niphargus thienemanni was found exclusively in springs, above 1395 m a. s. l. By contrast, the hitherto undescribed putative species lived in interstitial habitats, linked to alluvial plains of the Rhine and the Danube. The two species also differed morphologically (details in the following section). We therefore concluded that N. thienemanni and N. tonywhitteni sp. n. needed to be treated as two distinct, albeit only recently evolved species. The subtle morphological differences, and small genetic distances imply that the two species split relatively recently, perhaps when post-Pleistocene warming and glacier melting made previously non-occupied habitats on higher elevations accessible for colonization.

The second species, *N. luchoffmanni* sp. n. (see section "Species description" below), belongs to a lineage endemic to Switzerland that comprises two sister species, namely *N. luchoffmanni* sp. n. and an as yet undescribed species provisionally named *N. cf. stygius* (an insufficient number of specimens for the latter taxon does not allow a proper description yet). The results of species delimitation analyses (ABGD and PTP) of COI approved their separate species status. We could not assess morphological differences between the two, nor analyse their detailed genetic differentiation, as we had only one damaged male of *N. cf. stygius*. Yet, genetic distinctness (0.045 K2P) suggested that the two species differed to such an extent that interbreeding between them is unlikely (Lagrue et al. 2014). A substantial within-species genetic variation was found, but this was still significantly lower compared to distance to *N. cf. stygius*.

All available COI sequences of *Niphargus* species from Switzerland were submitted to GenBank and can be accessed also through BOLD. Their accession numbers are MH172382-MH 172398 and MH172401-MH172436 and can be viewed in Table 1.

#### Species descriptions

#### Niphargus tonywhitteni sp. n.

http://zoobank.org/E5CE0D3A-2BE9-4794-851F-D1D537EEE767 Figs 3–8

**Holotype.** Male, 9.1 mm. The specimen is mounted on two slides and deposited in the collection of the Musée de Zoologie, Lausanne, Switzerland under voucher number GBIFCH00585714 and GBIFCH00585715. Sampled on 17 October 2014 by Tom Gonser. Paratypes represent one male of length 7.5 mm with voucher numbers GBIFCH00587517.



**Figure 3.** Two new *Niphargus* species from Switzerland. The drawings are scaled to the same size. Top: *N. luchoffmanni* sp. n. (holotype, male 6.7 mm), bottom: *N. tonywhitteni* sp. n. (holotype, male 9.1 mm). Both specimens were rearranged digitally after drawing. Missing parts were taken from the right hand side of the specimen and are depicted in grey.

**Material examined.** Three males of lengths 9.1, 7.5 and 9.1 mm; specimens are partially dissected and mounted on slides with voucher numbers GBIFCH00585714, GBIFCH00585715, and GBIFCH00587517; three other specimens were sequenced.

**Type locality.** Gravel bed of Töss River near Winterthur, Switzerland (CH1903: 697,715/257,410)

**Diagnosis.** Small *Niphargus*, of mid-slender appearance closely resembling *N. fontanus*. Telson narrow, with long apical and lateral spines; dorsal spines lacking. Propodus of gnathopod I of rectangular shape, propodus of gnathopod II almond (hoof) shape. Uropods I with equal rami; uropod III rod shaped, likely sexually dimorphic, with elongated distal article.

**Description (based on dissected specimens).** *Head and trunk* (Figs 3, 8). Body length up to 9.1 mm. Head length approximately 10% of body length; rostrum absent. Pereonites I–VI without setae, pereonite VII with one seta ventro-posteriorly.

Pleonites I–III with up to four setae along the entire respective dorso-posterior margins. Epimeral plate II only slightly inclined, posterior and ventral margins slightly sinusoid and convex, respectively; ventro-postero-distal corner distinct; two spines along ventral margin; four setae along posterior margin. Epimeral plate III inclined, posterior and ventral margin sinusoid and convex, respectively; ventro-postero-distal corner distinct but not produced; two spiniform setae along ventral margin; four thin setae along posterior margin.

Urosomite I postero-dorso-laterally with one strong spiniform seta sometimes accompanied with one slender and flexible seta; urosomite II postero-dorso-laterally with two to three strong spiniform setae; urosomite III without setae. At the base of uropod I a single strong spiniform seta.

Telson length : width ratio is 1 : 0.85–0.90; cleft is 0.6–0.65 telson length; telson margins straight and narrowing apically. Telson spiniform setae (per lobe, left-right lobe asymmetry commonly observed): three to five apical, and none to two lateral spiniform setae; dorsal and mesial setae were not observed. Apical spiniform setae up to 0.5 telson length. Pairs of plumose setae laterally.

*Antennae* (Fig. 4). Antenna I 0.45–0.55 of body length. Flagellum with 21 articles; each article with one aesthetasc. Peduncle articles in ratio 1 : 0.85–0.90 : 0.41–0.45. Proximal article of peduncle dorso-distally slightly produced. Accessory flagellum biarticulated; distal article shorter than one quarter of proximal article length.

Length ratio antenna I : antenna II as 1 : 0.46-0.47. Flagellum of antenna II with seven to eight articles; each article with setae and elongated sensillae of unknown function. Peduncle articles lengths 4 : 5 is 1 : 0.93-0.98; flagellum 0.55-0.58 times length of peduncle articles 4+5.

Mouthparts (Fig. 5). Labrum typical; inner lobes of labium hardly visible.

Left mandible: incisor with five teeth, lacinia mobilis with four teeth; between lacinia and molar a row of serrated setae, few spatulate setae and one long seta at the base of molar. Right mandible: incisor processus with four teeth, lacinia mobilis with several small teeth, between lacinia and molar a row of thick serrated setae. Ratio of mandibular palp article 2 : article 3 (distal) is 1 : 1.12–1.22. Proximal palp article without setae; second article with seven to nine setae; distal article with a group of four A setae; three groups of B setae; 18–19 D setae and five E setae.

Maxilla I distal palp article with seven to eight apical setae. Outer lobe of maxilla I with a row of seven stout setae, inner with many subapical denticles, the remaining setae with one denticle; inner lobe with two apical setae.

Maxilla II inner lobe slightly smaller than outer lobe; both lobes setose apically.



**Figure 4.** Antenna I (left) and II (right) of *N. tonywhitteni* sp. n. (top, holotype, male 9.1 mm) and *N. luchoffmanni* sp. n. (bottom, holotype, male 6.7 mm). Drawings are not scaled to the same size.

Maxilliped palp article 2 with five to eight rows of setae along inner margin; distal article with a dorsal seta, and setae at the base of nail. Maxilliped outer lobe with seven to eight stout setae mesially to subapically, and three setae apically; inner lobe apically with two stout setae and six serrated setae.

*Coxal plates, and gills* (Figs 3, 6, 7). Coxal plate I of parallelogram shape, with rounded antero-ventral corner and armed with three to four setae. Coxal plates II–IV width : depth ratios are 1.09–1.16 : 1, 0.87–0.89 : 1 and 0.85–0.92 : 1 respectively; anterior and ventral margins with five to six, four and four to five setae respectively. Coxal plate IV posteriorly distinctly concave. Coxal plates V–VI anteriorly with large lobe; posterior margins with one seta. Coxal plate VII half-rounded shaped with one posterior seta. Gills II–VI ovoid.

Gnathopod I (Fig. 6). Ischium with one group of two to six postero-distal setae. Carpus 0.58–0.61 of basis length and 0.77–0.80 of propodus length; broadened distally. Carpus with single distal group of setae anteriorly; transverse rows of setae along posterior margin and a row of setae postero-laterally. Propodus rectangular. Along posterior margin five to six rows of setae. Anterior margin with two to three groups of total 11–12 setae in addition to antero-distal group of seven to eight setae. Several groups of short setae on the inner surface present. Palmar corner armed with a long spiniform palmar seta, three serrated spiniform setae, a single supporting spiniform seta on inner surface and three to four long setae below palmar spine. Palm setose. Nail length 0.31–0.32 of total dactylus length; four to six setae along anterior margin; a row of short setae along inner margin.

*Gnathopod II* (Fig. 6). Basis width : length is 0.31–0.32 : 1. Ischium with four postero-distal setae. Carpus 0.56–0.58 of basis length and 0.75–0.85 of propodus length, distally broadened. Carpus with distal group of setae anteriorly; few transverse rows of setae along posterior margin and a row of setae postero-laterally. Propodus of hoof or almond shape, large (circumference measures up to 0.19–0.20 of body length), larger than propodus of gnathopod I (I : II as 0.79–0.81 : 1). Posterior margin with eight to nine rows of setae. Anterior margin with a pair of individual setae in addition to eight to nine antero-distal setae. Individual surface setae present. Palmar corner with one strong palmar spiniform seta, single supporting spiniform seta on inner surface and one to two denticulated thick spiniform setae on outer side. Palm setose, below spiniform palmar seta a group of three long setae. Nail length 0.29–0.36 of total dactylus length; four setae along anterior margin; few short setae along inner margin.

*Pereopods III-IV* (Fig. 7). Lengths of pereopods III and IV subequal. Dactylus IV 0.34–0.43 of propodus IV; nail length 0.47–0.50 of total dactylus length. Dactyli III–IV with dorsal plumose seta; two tiny setae at the base of nail.

*Pereopods V–VII* (Fig. 7). Lengths of pereopods V : VI : VII is 1 : 1.30-1.33 : 1.30-1.41; pereopod VII measures 0.44-0.48 of body length.

Bases V–VII broad, respective length : width ratios as 1 : 0.60-0.65, 1 : 0.55-0.62and 1 : 0.57-0.62; posterior margins straight to convex; bases V–VII with moderate large posterior lobes; posteriorly eight to nine, eight to ten and seven to nine setae, respectively; anteriorly seven to eight, eight and seven to eight groups of spines, respectively. Dactyli V–VII with dorsal plumose seta, with two tiny setae at the base of the nail.

*Pleopods and uropods* (Fig. 8). Pleopods I–III with two hooked retinacles. Pleopod II rami with seven to eight and nine to ten articles.

Uropod I protopodite with six dorso-lateral spiniform setae and three dorso-medial spiniform setae. Exopodite : endopodite lengths is 1 : 1.0–1.03; rami straight. Endopodite with three individual spiniform setae laterally and five spiniform setae apically. Exopodite with five groups of totally nine setae; mesially with individual spiniform setae and laterally with spiniform and flexible setae; five spiniform setae apically.

Uropod II exopodite : endopodite lengths is 1 : 1.09.

Uropod III rod-shaped, 0.25–0.30 of body length. Protopodite with none to one lateral setae and seven to nine apical spiniform setae. Endopodite 0.54–0.61 of protopodite length, laterally without setae, apically with two setae, at least one spiniform. Exopodite of uropod III distal article 0.35–0.41 of the proximal article length. Proximal article with four to six groups of thin-flexible, spiniform and plumose setae along inner margin and four to five groups of thin-flexible and spiniform setae along outer margin. Distal article with two to three groups of thin-flexible setae along each margin, and a pair of setae apically.

**Etymology.** The species is named in honour of Tony Whitten (1953–2017), who devoted his life to nature conservation including conserving life in caves. He was a cochair of the Cave Invertebrate Specialist Group at IUCN.

**Habitat and distribution.** The species is known only from interstitial or related habitats. The species was found along the northern margin of the Alpine arch, between Achensee in Austria, Southern Germany and the type locality in Switzerland.

**Variability.** Only a small sample was available, not all individuals were adult, and many specimens were damaged. The extent of sexual dimorphism in uropod III is unknown; the terminal article of exopodite indicates elongation, as in *N. fontanus* 



**Figure 5.** Mouth parts (mandible & mandibular palp, maxilla I, maxilla II, maxilliped; from left to right) of *N. tonywhitteni* sp. n. (top, holotype, male 9.1 mm) and *N. luchoffmanni* sp. n. (bottom, holotype, male 6.7 mm). Drawings are not scaled to the same size.

from Great Britain, and our observations suggest that this article is longer in males and shorter in females. Most variation noticed can be likely attributed to different sizes of the specimens.

Remarks and affiliation. The species is closely related to N. thienemanni, from which it clearly differs by the almond-hoof shape of propodus of gnathopod II (rectangular in *N. thienemanni*). However, the newly described species is strikingly similar to N. fontanus Bate, 1859 from Great Britain, Belgium and France. The latter comprises a complex of cryptic species, distributed between Great Britain and Alps (McInerney et al. 2014), whereas the newly described N. tonywhitteni sp. n. belongs to a completely different phylogenetic lineage (Fig. 2), ruling out a possible conspecificity. The morphological differences between the two complexes are difficult to evaluate, mainly because we have only limited insights into variation of N. tonywhitteni sp. n. as well as the species complex containing the nominal species. We compared the newly described species with the lectotype and information available in various descriptions (Ginet 1996, Hartke et al. 2011). The only observed difference is in the shape of propodus of gnathopod I, which tends to be more rectangular in the newly described species in contrast to more almond shaped propodus of the nominal lineage. Additional identification traits depend on non-morphological information, i.e., geographic origin of the species, and especially on diagnostic COI sequences. While the description of N. tonywhitteni sp. n. substantially improved the knowledge of Niphargus in Switzerland, it is clear that the polyphyletic complex N. fontanus -N. tonywhitteni sp. n. is awaiting revision, which is beyond the scope of the present paper.

#### Niphargus luchoffmanni sp. n.

http://zoobank.org/E1C7C812-1494-40A8-8844-C6DC45C7AF07 Figs 3–8

**Holotype.** Male, 6.7 mm. The specimen is mounted on two slides and deposited in the collection of the the Musée de Zoologie, Lausanne, Switzerland under voucher numbers GBIFCH00585716 and GBIFCH00585717. Sampled on May 29, 2014 by Verena Lubini. Additional paratypes include 9.15 mm long and partially dissected female deposited under voucher number GBIFCH00587519, a male 6.8 mm long deposited under voucher number GBIFCH00587518 and several un-dissected specimens deposited in vials under GBIFCH00329353 and GBIFCH00329354.

**Material examined.** Two males of lengths 6.7 and 6.8 mm and a female 9.15 mm long; specimens are partially dissected and mounted on slides with voucher numbers GBIFCH00585716, GBIFCH00585717, GBIFCH00587518 and GBIF-CH00587519; seven other specimens were sequenced.

**Type locality.** Marchbachquelle, Wolfenschiessen, Switzerland (CH1903: 672,490/190,300).

**Diagnosis.** Mid-sized species, in general appearance similar to *N. forelii*. Epimeral plates angular. Telson with three long apical spines, one lateral, and one dorsal spine



**Figure 6.** Gnathopod I (left) and II (right) of *N. tonywhitteni* sp. n. (top, holotype, male 9.1 mm) and *N. luchoffmanni* sp. n. (bottom, holotype, male 6.7 mm). Gills are dashed, and drawn only when intact (missing in *N. luchoffmanni* sp. n.). Drawings are not scaled to the same size.

per lobe. Propods of gnathopods I and II of rectangular shape, propodus of gnathopod II large when compared to body length and propodus I. Maxilla outer lobe with seven spiniform setae, the inner four comb-like with long subapical denticles, the remaining three spines with one such denticle. Uropod I inner ramus slightly shorter than outer ramus; uropod II inner ramus slightly longer than outer ramus. Uropod III distal article elongated in males, as long as 0.5 times proximal article.

**Description (based on dissected specimens).** *Head and trunk* (Fig. 3). Body length up to 9.2 mm. Head length approximately 10% of body length; rostrum absent. Pereonites I–VI without setae, pereonite VII with one seta ventro-posteriorly.

Pleonites I–III with up to four setae along the entire respective dorso-posterior margins. Epimeral plate II only slightly inclined, posterior and ventral margins slightly convex; ventro-postero-distal corner distinct; two spines along ventral margin; three to six setae along posterior margin. Epimeral plate III inclined, posterior and ventral margin concave and convex, respectively; ventro-postero-distal corner distinct but not produced; two to three spiniform setae along ventral margin; four to five thin setae along posterior margin. Urosomite I postero-dorso-laterally with one slender and flexible seta; urosomite II postero-dorso-laterally with one strong spiniform setae accompanied with one slender and flexible seta; urosomite III without setae. At the base of uropod I, a single strong spiniform seta.

Telson length : width ratio is 1 : 0.81–0.91; cleft is 0.69–0.72 telson length; telson margins straight and narrowing apically. Telson spiniform setae (per lobe, left-right lobe asymmetry commonly observed): three apical, one dorsal and one lateral spiniform; mesial setae were not observed. Apical spiniform setae 0.44–0.5 telson length. Pairs of plumose setae laterally.

*Antennae* (Fig. 4). Antenna I 0.41–0.56 times body length. Flagellum with 17–20 articles; each article with one aesthetasc. Peduncle articles in ratio 1 : 0.79–0.87 : 0.37–0.47. Proximal article of peduncle dorso-distally slightly produced. Accessory flagellum biarticulated; distal article shorter than one quarter of proximal article length.

Length ratio antenna I : antenna II is 1 : 0.48-0.57. Flagellum of antenna II with nine to ten articles; each article with setae and elongated sensillae of unknown function. Peduncle articles lengths 4 : 5 is 1 : 0.93-0.95; flagellum 0.69-0.77 of length of peduncle articles 4 and 5.

Mouthparts (Fig. 5). Labrum typical; inner lobes of labium hardly visible.

Left mandible: incisor with five teeth, lacinia mobilis with four teeth; between lacinia and molar a row of serrated setae, few spatulate setae and a long seta at the base of molar. Right mandible: incisor processus with four teeth, lacinia mobilis with several small teeth, between lacinia and molar a row of thick serrated setae. Ratio of mandibular palp article 2 : article 3 (distal) is 1 : 1.01–1.11. Proximal palp article without setae; the second article with seven to eleven setae; distal article with a group of two to four A setae; two to three groups of B setae; 15–20 D setae and three E setae.

Maxilla I distal palp article with five to six apical setae. Outer lobe of maxilla I with a row of seven stout setae, inner four comb-like, with many long subapical denticles, the remaining three setae with one denticle; inner lobe with two to three apical setae.

Maxilla II inner lobe slightly smaller than outer lobe; both lobes setose apically.

Maxilliped palp article 2 with seven to eight rows of setae along inner margin; distal article with a dorsal seta, and setae at the base of nail. Maxilliped outer lobe with nine to eleven stout setae mesially to subapically, and three to five setae apically; inner lobe apically with three to four stout setae and seven serrated setae.

*Coxal plates, and gills* (Figs 3, 6, 7). Coxal plate I of parallelogram shape, with rounded antero-ventral corner and armed with four to six setae. Coxal plates II-IV width : depth ratios as 0.87–1.07 : 1, 1.03–1.12 : 1 and 0.96–1.13 : 1; anterior and ventral margins with four to seven, five to six and five to seven setae. Coxal plate IV posteriorly distinctly concave. Coxal plates V–VI anteriorly with large lobes; posterior margins with one seta. Coxal plate VII half-rounded shaped with one posterior seta. Gills II–VI ovoid.

*Gnathopod I* (Fig. 6). Ischium with one group of four to six postero-distal setae. Carpus 0.58–0.61 of basis length and 0.84–0.97 of propodus length; broadened distally. Carpus with single distal group of setae anteriorly, rarely accompanied by an additional



**Figure 7.** Pereopods III-VII (from left to right) of *N. tonywhitteni* sp. n. (top, holotype, male 9.1 mm) and *N. luchoffmanni* sp. n. (bottom, holotype, male 6.7 mm). Pereopods VII on this side were broken; see Fig. 3 for illustration of the entire pereopod. Gills were only drawn when intact; however, they are present on pereopods III-VI. Drawings are not scaled to the same size.

seta in the mid of article; transverse rows of setae along posterior margin and a row of setae postero-laterally. Propodus rectangular. Along posterior margin, three to five rows of setae. Anterior margin with two to three groups of total four to eleven setae in addition to antero-distal group of eight setae. Several groups of short setae on the inner surface present. Palmar corner armed with a long spiniform palmar seta, two to three serrated spiniform seta, a single supporting spiniform seta on inner surface and three to five long setae below palmar spine. Palm setose. Nail length 0.31–0.32 of total dactylus length; four setae along anterior margin; a row of short setae along inner margin.

*Gnathopod II* (Figs 6). Basis width : length is 0.28–0.30 : 1. Ischium with three to four postero-distal setae. Carpus 0.52–0.57 of basis length and 0.83–0.91 of pro-

podus length, distally broadened. Carpus with distal group of setae anteriorly, rarely accompanied by an additional seta in the middle of the article; few transverse rows of setae along posterior margin and a row of setae postero-laterally. Propodus rectangular, large (circumference measures up to 0.20–0.23 of body length), much larger than propodus of gnathopod I (I : II as 0.75–0.76 : 1). Posterior margin with six to eight rows of setae. Anterior margin with two to three groups of total four to six setae in addition to seven to ten antero-distal setae. Individual surface setae present. Palmar corner with one strong palmar spiniform seta, a single supporting spiniform seta on inner surface and two denticulated thick-spiniform setae on outer side. Palm setose, below spiniform palmar seta, a group of three to four long setae. Nail length 0.30–0.32 of total dactylus length; three to six setae along anterior margin; a few short setae along inner margin.

*Pereopods III–IV* (Fig. 7): Lengths of pereopods III and IV subequal. Dactylus IV 0.46–0.52 of propodus IV; nail length 0.52–0.59 of total dactylus length. Dactyli III-IV with a dorsal plumose seta; one spiniform seta at the base of nail, sometimes accompanied by a tiny seta.

*Pereopods V–VII* (Fig. 7): Lengths of pereopods V : VI : VII is 1 : 1.33-1.34 : 1.39-1.42; pereopod VII measures 0.55–0.59 of body length.

Bases V-VII broad, respective length : width ratios as 1 : 0.64-0.67, 1 : 0.60-0.65and 1 : 0.60-0.63; posterior margins straight to convex; bases V-VII with moderate posterior lobes; posteriorly eight to eleven, nine to twelve and seven to ten setae, respectively; anteriorly six to seven, six and five to seven groups of spines, respectively. Dactyli V–VII with dorsal plumose seta; spiniform seta at the base of nail, in most cases accompanied by one tiny seta.

*Pleopods and uropods* (Fig. 8): Pleopods I–III with two hooked retinacles. Pleopod II rami with seven to nine and nine to ten articles.

Uropod I protopodite with three to six dorso-lateral spiniform setae and three to four dorso-medial spiniform setae. Exopodite : endopodite lengths is 1 : 0.82–0.99; rami straight. Endopodite with two individual spiniform setae laterally and five spiniform setae apically. Exopodite with two to four groups totalling three to eight setae; mesial groups comprise individual spiniform setae, whereas lateral groups comprise spiniform and flexible setae groups; apically five spiniform setae.

Uropod II exopodite : endopodite lengths is 1 : 1.02–1.12.

Uropod III rod-shaped, 0.22–0.41 of body length. Protopodite with one to two lateral setae and six to seven apical spiniform setae. Endopodite 0.45–0.50 of protopodite length, laterally with 0–1 seta, apically with two setae, at least one spiniform. Exopodite of uropod III distal article 0.28–0.48 of the proximal article length. Proximal article with four to six groups of thin-flexible, spiniform and plumose setae along inner margin and four groups of thin-flexible and spiniform setae along outer margin. Distal article with one to four groups of thin-flexible setae along each margin, and five to six of setae apically.

**Etymology.** The species is named in honour of Hans Lukas "Luc" Hoffmann (1923–2016), naturalist and ecologists, who importantly influenced nature conserva-



**Figure 8.** Uropod I (left) and III (middle), and Telson (right) of *N. tonywhitteni* sp. n. (top, holotype, male 9.1 mm) and *N. luchoffmanni* sp. n. (bottom, holotype, male 6.7 mm). Drawings are not scaled to the same size.

tion worldwide. Among others, he was the founder of the MAVA foundation and cofounder of the World Wide Fund for Nature (WWF).

Habitat and distribution. The species has been hitherto reported from springs, and seems to be endemic to central Switzerland (Fig. 1).

**Variability.** The variability of the species is poorly understood, as we could analyse relatively little material, with numerous sub-adult and damaged specimens. Males and females differ in length of distal article of uropod III, which is remarkably longer in males. Larger specimens tend to have narrower bases of pereopods V–VII. The pattern of denticulation on spines on outer lobe of maxilla I is, however, stable and the most important diagnostic trait.

**Remarks and affiliation.** In a morphological sense, *N. luchoffmanni* sp. n. shows some similarities to *N. forelii* Humbert, 1876. We compared *N. luchoffmanni* sp. n. with neotypes from Bodensee from Berlin Museum and species descriptions (Karaman and Ruffo 1993, Ginet 1996). Both species have long dactyls, long telson spines, multiple setae along gnathopod dactyls, an elongated distal article of uropod III in males, and the endopodite of uropod I shorter than the exopodite. Yet, there are few distinct traits separating both species. The differences in sizes of propods of gnathopods I and II is more pronounced in *N. luchoffmanni* sp. n. than in *N. forelii*. In addition, endopodite of the uropod II is longer and shorter than the exopodite in *N. luchoffmanni* sp. n. and *N. forelii*, respectively. *Niphargus luchoffmanni* sp. n. has one dorsal spine on telson, while *N. forelii* is lacking dorsal telson spines. Finally, the spines on the outer lobe of maxilla I are different: while *N. luchoffmanni* sp. n. has at least four spines multidenticulate, *N. forelii* has, at most, one such spine.

## **DELTA database**

The present database counts 19 out of 22 species and 19 characters treated for each species. In the database we included two as yet undescribed species (*N. cf. stygius, N. cf. thienemanni*; see also Fišer et al. 2017). The species complex of *N. rhenorhodanensis*, however, containing at least four species is not further resolved and is treated at the complex level.

Easily visible and unambiguous characters were preferentially selected, such as the number and type of setae on maxillae, telson, gnathopod and pereopod dactyls, as well as the urosoma. In addition, characters describing shapes, such as epimeral plates, shapes and size ratios of carpus and propodus of gnathopods, and shapes of coxal plate IV were used. Two characters describe sexual dimorphism, namely the different elongation of rami of uropods. The file is freely available on the website of World Amphipoda Database, and can be used for generating species descriptions, dichotomous identification keys and interactive identification keys (Coleman et al. 2010). The virtue of this file is that anyone can assess their own samples for all traits, add additional taxa and further characters.

# Conclusions

An early attempt of web-initiated collaborative taxonomic research that would foster local taxonomy of *Niphargus* within a unified framework (Fišer et al. 2009b), was only moderately successful (available via http://niphargus.info/morpho-database/). The reasons are unclear, but likely a combination of an insufficient coverage of local faunas and the size of the matrix (high number of characters and many taxa) were not appealing to potential collaborators. By contrast, smaller initiatives, such as presented in this study, seem to be more manageable and in the long run more fruitful. The two DELTA morpho-databases from Middle East and Switzerland are not complete but provide the best overview of the state of *Niphargus* taxonomy in two geographically well-defined regions, and hold promise to stimulate further research in the genus. Both databases are backed with diagnostic sequences. They both enclose a small fraction of morphological variation (19 and 30 characters respectively), making them relatively simple to use. Although each of the two databases contains only a smaller number of species, they jointly present 11 % of all known Niphargus species. The virtue of such small, independent studies limited to specific geographic regions is their immediate availability for non-taxonomists. As the number of such revisions increases over time, they could sum into a comprehensive revision of the entire genus.

Our recent work (Altermatt et al. 2014, Fišer et al. 2017) significantly advanced the knowledge on *Niphargus* in Switzerland, and it seems that new findings, such as of *N. aquilex*, are getting rarer. However, the revision of the genus for that area is still incomplete. There are still several open taxonomic questions, including the complex

*N. rhenorhodanensis* (Lefébure et al. 2007), as yet formally unnamed lineages that have been provisionally named *N. cf. stygius* and *N. cf. thienemanni* (Fišer et al. 2017), and new findings of *N. aquilex* (Trontelj et al. 2009, McInerney et al. 2014). For these species we could not yet draw final taxonomic conclusions with the data at hand, and they require further detailed analyses. Nevertheless, end-users can already combine morphological data and COI sequences and at least identify the main species complexes in Switzerland. We are optimistic this initiative will foster further taxonomic research and, in the near future, close the Swiss chapter of *Niphargus*.

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# Supplementary material I

# Table S1. List of samples used in phylogenetic analyses along with GenBank Accessions

Authors: Cene Fišer, Roman Alther, Valerija Zakšek, Špela Borko, Andreas Fuchs, Florian Altermatt

Data type: species data

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Link: https://doi.org/10.3897/zookeys.760.24978.suppl1

# Supplementary material 2

# Table S2. List of samples used in a detailed network analyses along with Gen-Bank Accessions

Authors: Cene Fišer, Roman Alther, Valerija Zakšek, Špela Borko, Andreas Fuchs, Florian Altermatt

Data type: species data

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Link: https://doi.org/10.3897/zookeys.760.24978.suppl2

# Supplementary material 3

# Table S3. List of new records of Niphargus from Switzerland

Authors: Cene Fišer, Roman Alther, Valerija Zakšek, Špela Borko, Andreas Fuchs, Florian Altermatt

Data type: occurence

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Link: https://doi.org/10.3897/zookeys.760.24978.suppl3

# Morphological database in DELTA

Authors: Cene Fišer, Roman Alther, Valerija Zakšek, Špela Borko, Andreas Fuchs, Florian Altermatt

Data type: molecular data

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Link: https://doi.org/10.3897/zookeys.760.24978.suppl4

RESEARCH ARTICLE



# The genus *Phyllomyza* Fallén from China, with descriptions of three new species (Diptera, Milichiidae)

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#### Abstract

The following three species of the genus *Phyllomyza* Fallén from China are described as new to science: *P. guangxiensis* **sp. n.**, *P. luteigenis* **sp. n.**, and *P. quadratpalpus* **sp. n.** A key to the known species of *Phyllomyza* from China is presented.

# Keywords

China, Milichiidae, Diptera, new species, Phyllomyza

# Introduction

The genus *Phyllomyza* was established by Fallén in 1810. It belongs to the subfamily Phyllomyzinae of the family Milichiidae and most species are recognized by the following characteristics: three lateroclinate orbital setae; occiput not strongly concave when viewed from above; palpus and first flagellomere in male usually greatly enlarged, longer than broad; lunule usually with a pair of setae (Brake 2000). There are 49 described species distributed widely in the world except the Neotropical region (Malloch 1914a; Hennig 1967; Yang 1998; Brake 2000; Iwasa 2003; Xi and Yang 2013, 2015a, b; Xi et al. 2016).

15 species are known from the Palaearctic region (Hennig 1937; Papp 1976, 1984; Papp and Wheeler 1998; Yang 1998) and 29 species from the Oriental region (Brake 2000; Iwasa 2003; Xi and Yang 2013, 2015a, b; Xi et al.2016). There are 20 species occurring in China, of which four species are distributed in Taiwan (Hendel 1914; Malloch 1914b; Yang 1998; Xi and Yang 2013, 2015a, b; Xi et al. 2016). In the present paper, three species of the genus from China are described as new to science. A key to the described species of *Phyllomyza* from China is presented. Larvae of some *Phyllomyza* are generally saprophagous and live in decaying plants, or rear from nests of ants (Donisthorpe 1927). Adults of some species can be collected in open landscapes, such as steppes or meadow, in wadis, at the edges of forests, inside forests, in the forest canopy, in stables or houses, or even in caves, but they don't seem to be found in coastal habitats or to other places near water (Brake 2000), this habit is different from Canacidae and Tethinidae, each of which have members with similar physical characters to the genus *Phyllomyza*.

# Materials and methods

Genitalia preparations were made by removing and macerating the apical portion of the abdomen in glacial acetic acid, then rinsed in distilled water brfore being stored in glycerine filled microvials. After examination, they were transferred to fresh glycerine and stored in a microvial on the pin below the specimen or moved to an ethanol tube together with the wet specimens. Specimens examined were deposited in the Entomological Museum of China Agricultural University (CAU), Beijing; the Entomological Museum of Henan Agricultural University (HAU). The general terminology follows McAlpine (1981) and Brake (2000). The following abbreviations are used:

asc	apical scutellar seta(e),	pa	postalar seta(e),
bsc	basal scutellar seta(e),	pos	postsutural seta(e),
dc	dorsocentral seta(e),	prs	presutural seta(e),
h	humeral seta(e),	prsc	prescutellar seta(e),
ia	intraalar seta(e),	sa	supraalar seta(e),
kepsts	katepisternal seta(e),	S	sternite,
npl	notopleural seta(e),	Т	tergite.

# Taxonomy

# Key to species (males) of Phyllomyza from China

1	Palpus almost bare, without setulae	.2
_	Palpus with short setulae at tip and on ventral side	.7
2	Frons with 3 interfrontal setae P. fuscusa Xi, Yin & Yan	ng
_	Frons with 4 interfrontal setae	.3
3	Arista 2.5 times as long as first flagellomere; cercus with or lacking ventral	
---------	--	
	Arite 2 times a large a fact flag line and a second large of the second large of the first second large of the first second large of the second la	
	Arista 5 times as long as first nagenomere; cercus lacking ventral appendix	
4	Gena approximately one-fourth eye height (Fig. 5); cercus lacking ventral ap-	
	pendix (Fig. /)	
-	Gena approximately one-eighth eye height (Fig. 25); cercus with ventral ap-	
	pendix (Fig. 26) <i>P. nudipalpis</i> Malloch	
5	Gena very narrow, approximately one-fourteenth eye height (Fig. 1); first flagel-	
	lomere irregularly oblong; knob of halter brownish P. guangxiensis sp. n.	
_	Gena at least one-tenth eye height; first flagellomere irregularly quadrate;	
	knob of halter yellowish6	
6	Gena approximately one-sixth eye height; first flagellomere 2 times wider	
	than long	
_	Gena approximately one-tenth eye height; the length of first flagellomere is	
	the same as width	
7	Palpus very long, at least 6 times as long as wide	
_	Palpus relatively short, less than 5 times as long as wide <b>9</b>	
8	First flagellomere long 1.4 times as long as wide: knob of balter vellowish	
0	white D havilature Vi Vin & Vang	
	Einst flagsligman wide 1.1 times as lang as wide lunch of helter darkich	
_	First nagenomere wide, 1.1 times as long as wide; knob of naiter darkisn	
0	brown $P$ . sinensis $AI \otimes Iang$	
9	Cercus with ventral appendix (Fig. 22); first flagellomere broadly elliptoid	
	<i>P. epitacta</i> Hendel	
-	Cercus lacking ventral appendix; first flagellomere very broadly oblong 10	
10	Palpus pointed apically; first flagellomere shallowly panduriform	
	(Figs 15, 16) P. claviconis Yang	
-	Palpus inflated, blunted apically; first flagellomere broadly elliptoid or shal-	
	lowly oblong11	
11	First flagellomere shallowly oblong, 1.3 times as long as wide	
	P. quadratpalpus sp. n.	
_	First flagellomere broadly elliptoid, almost the same length and width12	
12	M, between r-m and dm-cu as long as dm-cu	
_	M, between r-m and dm-cu longer than dm-cu14	
13	Gena narrow, approximately one-eleventh eve height; palpus 5 times as long	
-	as wide (Figs 13, 14) P. angustigenis Xi & Yang	
_	Gena relatively broad, approximately one-seventh eve height: palpus 7 times	
	as long as wide (Figs 23, 24) <b><i>P. euthyinalpis</i> Xi &amp; Yang</b>	
14	M between r-m and dm-cu at least 1.5 times longer than dm-cu 15	
11	M between r m and dm cu less than 1 2 times longer than dm cu 19	
15	Vibrigge leasted at level of lever margin of eve	
1)	Vibrigga logated below level of lower margin of even	
- 16	vibilissa localed below level of lower inargin of eye	
10	raipus yenow; nind tidia yenowisn <i>P. tuteipaipis</i> Malloch	
-	Palpus dark brown; hind tibia yellow to brown	

17	Halter white; palpus curved P. drepanipalpis Xi & Yang
_	Halter dark brown or yellowish; palpus straight18
18	Gena approximately one-seventh eye height; knob of halter yellowish
	P. emeishanensis Xi & Yang
_	Gena approximately one-fifth eye height; knob of halter with upper half
	white and lower half dark brown
19	Gena broad, approximately one-sixth eye height (Figs 19, 20)
	<i>P. dicrana</i> Xi & Yang
_	Gena narrow, less than one-sixth eye height
20	Vibrissal angle relatively acute, the tip less than 60° angle (Figs 17, 18)
	P. cuspigera Xi & Yang
_	Vibrissal angle blunt, the tip almost 90° angle
21	Cercus with thin ventral appendix (Fig. 21); hind tibia brownish
_	Cercus lacking short ventral appendix; hind tibia dark brown22
22	Arista approximately 4.5 times as long as first flagellomere; knob of halter
	with upper half brownish and lower half dark brown (Figs 27, 28)
_	Arista approximately 3.5 times as long as first flagellomere; knob of halter
	with upper half yellow and lower half yellowish (Figs 29, 30)
	<i>P. tibetensis</i> Xi & Yang

## Phyllomyza guangxiensis sp. n.

http://zoobank.org/FCE8BDB1-7760-434F-BCC8-168358778852 Figs 1–4

**Diagnosis.** Gena approximately one-fourteenth of eye height. Upper blade of bifurcated tip of surstylus swollen and apical margin rounded, lower blade thin and longer than upper one; cercus arched with short sparse setae.

Description. Male. Body length 1.6-1.7 mm; wing length 1.6-1.7 mm.

Head (Fig. 1) black with greyish microtomentum; orbital plate satiny blackish brown, with microtomentum, ocellar triangle blackish brown without microtomentum; lunule very depressed falciform, darkish brown with black margin. Posterior eye margin ventrally diverging from head margin; eye 1.4 times as high as long, gena approximately one-fourteenth of eye height. Setae and setulae on head black; ocellar triangle with two ocellar setae and three short setae; frons with three orbital and two frontal setae on black-ish brown orbital stripe, orbital setae lateroclinate and frontal setae medioclinate, four interfrontal setae; postocellar setae cruciate. Lunule with two setae. Vibrissal angle flat, the tip a little more than a 90° angle; vibrissa strong, located below level of lower margin of eye. Antenna darkish brown pedicel with short black setulae at middle and margin, setulae at margin longer than others, longest one approximately five times longer than others; first flagellomere with pubescence, irregularly oblong; arista three times as long as



**Figures 1–4.** *Phyllomyza guangxiensis* sp. n. (male). **I** Head, lateral view **2** wing **3** epandrium, cerci, and surstyli, posterior view **4** epandrium, cerci, and surstyli, lateral view. Scale bars: 0.1 mm.

first flagellomere, black, distinctly pubescent. Proboscis short and folded, darkish brown, with sparse black setulae. Palpus wide, 0.5 mm, with blunt apex in lateral view; darkish brown with short dense black pubescence, margin without sparse setulae.

Thorax brown with grey microtomentum, except scutum shiny brownish with sparse black microtomentum; scutellum brownish with gray microtomentum. Setae and setulae on thorax black; one h, two dc, one prsc, two npl, one prs, one pos, one sa, one pa, one kepsts (setulae at forward position); scutellum 1.5 times wider than long, with pair of asc and bsc, asc three times longer than bsc. Legs slender, coxae and femora darkish brown, tibiae yellow except hind tibia brown with yellow apex, tarsi yellowish. Setae and setulae on legs black, mid tibia with single black preapical dorsal seta. Wing (Fig. 2) hyaline, unspotted; veins brown; Sc strong; M<sub>1</sub> between r-m and dm-cu longer than dm-cu. Calypter yellowish, with dense brownish microtrichae, margin with thin and long setulae. Knob of halter brownish, stalk yellow.

Abdomen blackish brown with grey microtomentum. Setae and setulae on abdomen black; TII-TV with setae, marginal setae longer than others; sternites with sparse black setulae at posterior 3/4. Posteromedial triangular projection of TI into TII present; SII generally luniform, the apex blunt and round, SIII irregularly oblong, SIV generally quadrate, SV depressed panduriform, apical margin arched. Male genitalia (Figs 3–4): epandrium with eight strong black setae; upper blade of bifurcated tip of surstylus swollen and apical margin rounded, lower blade thin and longer than upper one; cercus arched with short sparse setae.

Female. Unknown.

**Material examined.** *Holotype*, ♂, China, Guangxi, Fangchenggang, Shangsi County (21°53'47.61"N, 107°49'20.32"E; 450 m), 18. V. 2012, Guo-Quan Wang (CAU). *Paratypes*, 2 ♂♂, same data as holotype.

Distribution. China (Guangxi).

Etymology. The specific name guangxiensis is derived from the type locality.

**Remarks.** This species is similar to *P. nudipalpis* Malloch, but can be separated by the gena being approximately one-fourteenth of the eye height and the knob of the halter brownish; in *P. nudipalpis*, the gena is approximately one-eighth of the eye height and the knob of the halter is yellowish white (Malloch 1914b).

#### Phyllomyza luteigenis sp. n.

http://zoobank.org/A5CA19E7-9E6F-4EB3-8CB2-E8E0D57B559F Figs 5–8

**Diagnosis.** Gena approximately one-quarter of eye height. Surstylus with upper blade of bifurcated tip extremely swollen, lower blade short and thinner than upper blade.

Description. Male. Body length 1.7-1.9 mm; wing length 1.6-1.8 mm.

Head (Fig. 5) darkish yellow with greyish microtomentum; orbital plate satiny yellow, with microtomentum; ocellar triangle brownish without microtomentum; lunule very depressed luniform, brownish with black margin. Posterior eye margin ventrally diverging from head margin; eye 1.1 times as high as long, gena approximately onefourth of eye height. Setae and setulae on head black; ocellar triangle with two ocellar setae and three short setae; frons with three orbital and two frontal setae, orbital setae lateroclinate and frontal setae medioclinate; four interfrontal setae; postocellar setae cruciate. Lunule with two short setae. Vibrissal angle blunt, the tip almost a 90° angle; vibrissa strong, located at the level of lower margin of eye. Antenna blackish yellow; pedicel with black setulae at middle and margin, setulae at margin longer than others, longest one approximately 3 times longer than others; first flagellomere with pubescence, irregularly quadrate and margin blunt; arista 2.5 times as long as first flagellomere. Proboscis short and folded, brownish, with short sparse black setulae. Palpus slightly flat, 0.4 mm, apex blunt in lateral view, 3 times longer than wide; darkish yellow with short dense brownish pubescence, margin without short sparse setulae.

Thorax yellow with grey microtomentum, except scutum shiny darkish yellow with sparse brownish microtomentum; scutellum brownish yellow with gray microtomentum. Setae and setulae on thorax black; one h, two dc, one prsc, two npl, one prs, one



**Figures 5–8.** *Phyllomyza luteigenis* sp. n. (male). **5** Head, lateral view **6** wing **7** epandrium, cerci, and surstyli, posterior view **8** epandrium, cerci, and surstyli, lateral view. Scale bars: 0.1 mm.

pos, one sa, one ia, two pa, one kepsts (a row of setulae at forward position); scutellum 1.3 times wider than long, with pair of asc and bsc, asc two times longer than bsc. Legs slender, coxae and femora brownish, tibiae brownish except fore tibia darkish yellow, tarsi yellowish. Setae and setulae on legs black. Mid tibia with a single black preapical dorsal seta. Wing (Fig. 6) hyaline, unspotted; veins brown; Sc strong; M<sub>1</sub> between r-m and dm-cu a little longer than dm-cu. Calypter yellowish, with yellowish microtrichae, margin with brownish setulae. Knob of halter white, stalk yellowish.

Abdomen brownish with gray microtomentum. Setae and setulae on abdomen black; TII-TV with setae at posterior 3/4, marginal setae longer than others; sternites with sparse setulae. Posteromedial triangular projection of TI into TII present; SII irregularly luniform, apical margin blunt, SIII irregularly oblong, SIV very broadly panduriform, basal margin a little wider than apical margin, SV very shallowly falciform. Male genitalia (Figs 7–8): epandrium with nine strong black setae; surstylus with upper blade of bifurcated tip extremely swollen, lower one short and slightly thin; cercus arched with short sparse setae.

Female. Unknown.

Material examined. *Holotype*, ♂, China, Gansu, Tianshui City, Maiji Mountain (34°23'30.31"N, 106°06'35.61"E; 150 m), 15. VII. 2012, Li-Hua Wang (CAU). *Paratypes*, 3 ♂♂, China, Gansu, Tianshui City, Maiji Mountain (34°23'30.31"N, 106°06'35.61"E; 150 m), 15. VII. 2012, Ze-Hui Kang (CAU).

Distribution. China (Gansu).

Etymology. The specific name refers to the yellow gena.

**Remarks.** This new species is distinctly different from other species of the genus: the gena is approximately one-fourth of the eye height, eye 1.1 times as high as long, and SV is generally very shallowly falciform.

### Phyllomyza quadratpalpus sp. n.

http://zoobank.org/75DB8CAA-1489-4E94-875A-6B8349603DA7 Figs 9–12

**Diagnosis.** Gena narrowed, approximately one-twelfth of eye height. Surstylus with upper blade of bifurcated tip extremely swollen, lower one slightly swollen and shorter than upper one.

Description. Male. Body length 1.6–1.8 mm; wing length 1.6–1.8 mm.

Head (Fig. 9) black with gravish microtomentum; orbital plate satiny black, with microtomentum, ocellar triangle brownish without microtomentum; lunule transverse luniform, darkish brown with black margin. Posterior eye margin ventrally diverging from head margin; eye 1.4 times as high as long, gena approximately one-twelfth of eye height. Setae and setulae on head black; ocellar triangle with two ocellar setae and three short setae; frons with three orbital and two frontal setae on brownish orbital stripe, orbital setae lateroclinate and frontal setae medioclinate, four interfrontal setae; postocellar setae converging. Lunule with two short setae. Vibrissal angle flat, the tip almost a 90° angle; vibrissa strong, located at level of lower margin of eye. Antenna brownish brown; pedicel with black setulae at middle and margin, setulae at margin longer than others, longest one 4.5 times longer than others; first flagellomere with pubescence, shallowly oblong and margin blunt; arista 2.5 times as long as first flagellomere, black, distinctly pubescent. Proboscis thick and geniculate, 0.4 mm, brownish, with short sparse black setulae. Palpus flat, irregularly quadrate in lateral view, 2.5 times longer than wide; darkish brown with short dense black pubescence, margin with short sparse setulae.

Thorax darkish brown with grey microtomentum, except scutum shiny blackish brown with sparse black microtomentum; scutellum dark brown with grey microtomentum. Setae and setulae on thorax black; one h, two dc, one prsc, two npl, one prs, one pos, one sa, one ia, one pa, one kepsts (a row of setulae at forward position); scutellum 1.3 times wider than long, with pair of asc and bsc, asc three times longer than bsc. Legs slender, coxae and femora dark brown, tibiae brownish yellow except hind tibia darkish brown, tarsi yellowish. Setae and setulae on legs black. Mid tibia with a black preapical dorsal seta. Wing (Fig. 10) hyaline, unspotted; veins brown;



**Figures 9–12.** *Phyllomyza quadratpalpus* sp. n. (male). **9** Head, lateral view **10** wing **11** epandrium, cerci, and surstyli, posterior view **12** epandrium, cerci, and surstyli, lateral view. Scale bars: 0.1 mm.

Sc strong;  $M_1$  between r-m and dm-cu a little longer than dm-cu. Calypter yellowish, with brownish microtrichae, margin with brownish setulae. Knob of halter yellowish white, stalk yellowish.

Abdomen brown with grey microtomentum. Setae and setulae on abdomen black; TII-TV with setae at posterior 3/4, marginal setae longer than others; sternites with sparse setulae. Posteromedial triangular projection of TI into TII present; SII generally luniform, SIII oblong, apical margin blunt and round, SIV very broadly obpanduriform, SV shallowly oblong. Male genitalia (Figs 11–12): epandrium with five strong black setae; surstylus with upper blade of bifurcated tip extremely swollen, lower one slightly swollen and shorter than upper one; cercus irregularly arched with short sparse setae.

Female. Body length 1.8-2.0 mm; wing length 1.8-2.0 mm.

Similar to male, but palpus shorter, approximately four-fifths of males'. Female terminalia: TVIII brown, shallowly oblong, margin with setulae. Supra-anal plate broadly trullate; SVIII very shallowly luniform, subanal plate wide, brownish, very depressed trullate. Cercus with long setulae.

**Material examined.** *Holotype*,  $\Diamond$ , China, Guizhou, Libo County, Maolan National Nature Reserve (25°15'36.67"N, 108°03'21.65"E; 620m), 13. X. 2013, Ding Yang (CAU). *Paratypes*, 5  $\Diamond$ , same data as holotype; 2  $\bigcirc$   $\bigcirc$ , China, Guizhou, Libo County,



Figures 13–18. 13 Phyllomyza angustigenis Xi et Yang (male); head, lateral view 14 Phyllomyza angustigenis Xi et Yang (male); epandrium, cerci, and surstyli, posterior view 15 Phyllomyza claviconis Yang (male); head, lateral view 16 Phyllomyza claviconis Yang (male); epandrium, cerci, and surstyli, posterior view 17 Phyllomyza cuspigera Xi et Yang (male); head, lateral view 18 Phyllomyza cuspigera Xi et Yang (male); epandrium, cerci, and surstyli, posterior view 17 Phyllomyza cuspigera Xi et Yang (male); head, lateral view 18 Phyllomyza cuspigera Xi et Yang (male); epandrium, cerci, and surstyli, posterior view. Scale bars: 0.1 mm.



Figures 19–24. 19 *Phyllomyza dicrana* Xi et Yang (male); head, lateral view 20 *Phyllomyza dicrana* Xi et Yang (male); epandrium, cerci, and surstyli, posterior view 21 *Phyllomyza dilatata* Malloch (male); epandrium, cerci, and surstyli, posterior view 22 *Phyllomyza epitacta* Hendel (male); epandrium, cerci, and surstyli, posterior view 23 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis* Xi et Yang (male); head, lateral view 24 *Phyllomyza euthyipalpis*



Figures 25–30. 25 *Phyllomyza nudipalpis* Malloch (male); head, lateral view 26 *Phyllomyza nudipalpis* Malloch (male); epandrium, cerci, and surstyli, posterior view 27 *Phyllomyza planipalpis* Xi et Yang (male); head, lateral view 28 *Phyllomyza planipalpis* Xi et Yang (male); head, lateral view 29 *Phyllomyza tibetensis* Xi et Yang (male); head, lateral view 30 *Phyllomyza tibetensis* Xi et Yang (male); epandrium, cerci, and surstyli, posterior view. Scale bars: 0.1 mm.



Figure 31. The distribution of *Phyllomyza* species in China.

Maolan National Nature Reserve (25°12'32.50"N, 108°21'20.08"E; 830m), 18. VIII. 2017, Xiaohui Hou (HAU).

**Distribution.** China (Guizhou).

Etymology. The specific name refers to the shaped of palpus.

**Remarks.** This new species is somewhat similar to *P. planipalpis* Xi & Yang, but differs in the palpus irregularly quadrate in lateral view, surstylus with upper blade of bifurcated tip extremely swollen and apical margin smooth; in *P. planipalpis*, the palpus depressed semiluniform in lateral view, surstylus with upper blade of bifurcated tip swollen and apical margin blunt (Xi and Yang 2015a).

# Discussion

*Phyllomyza guangxiensis* sp. n. and *P. quadratpalpus* sp. n. are separately distributed in Guangxi and Guizhou Provience, this is the first reported species of *Phyllomyza* species in the two places. The palpus of *P. guangxiensis* sp. n. is wide and the basally curved, the shaped of the palpus is different with other species and cercus almost as wide as epandrium in lateral view. *P. quadratpalpus* sp. n. has a distinctive palpus, as the shaped of pal-

pus is very shallowly rhombiod in lateral view. In Milichiidae, as far as we know, there is no similar species to *P. luteigenis* sp. n., when you consider the body colour, and palpus and eye shapes. There are 49 species of *Phyllomyza* distributed in the world, until now, 23 species are known to occur in China. Only three species are distributed in the Palaearctic Region, *P. claviconis*, *P. latustigenis*, and *P. luteigenis* sp. n., which have one similar character, which is the wide gena: *P. luteigenis* sp. n. is wider than others, and the species of Oriental Region do not have this obvious character. Twenty species are distributed in the Oriental Region, of which nine are distributed in Yunnan Province (*P. angustigenis*, *P. aureolusa*, *P. basilatusa*, *P. clavellata*, *P. cuspigera*, *P. dicrana*, *P. euthyipalpis*, *P. fuscusa*, *P. leioipalpus*) and four species in Taiwan Province (*P. dilatata*, *P. epitacta*, *P. luteipalpis*, *P. nudipalpis*) (Hendel 1914; Malloch 1914b; Xi and Yang 2013, 2015a; Xi et al. 2016) (Fig. 31). The Chinese fauna of Milichiidae is extraordinarily rich, with the continued discovery and description of further species providing great potential.

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