ZooKeys 586: 1–36 (2016)
doi: 10.3897/zookeys.586.7832
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

New species in the *Sitalcina sura* species group (Opiliones, Laniatores, Phalangodidae), with evidence for a biogeographic link between California desert canyons and Arizona sky islands

Angela DiDomenico[1], Marshal Hedin[1]

1 *Department of Biology, 5500 Campanile Drive, San Diego State University, San Diego, CA 92182-4614, USA*

Corresponding author: *Marshal Hedin* (mhedin@mail.sdsu.edu)

Academic editor: *G. Giribet* | Received 19 January 2016 | Accepted 23 March 2016 | Published 4 May 2016

http://zoobank.org/B1EDEEFC-364A-414A-840A-835E9648557C

Citation: DiDomenico A, Hedin M (2016) New species in the *Sitalcina sura* species group (Opiliones, Laniatores, Phalangodidae), with evidence for a biogeographic link between California desert canyons and Arizona sky islands. ZooKeys 586: 1–36. doi: 10.3897/zookeys.586.7832

Abstract

The western United States is home to numerous narrowly endemic harvestman taxa (Arachnida, Opiliones), including members of the genus *Sitalcina* Banks, 1911. *Sitalcina* is comprised of three species groups, including the monospecific *S. californica* and *S. lobata* groups, and the *S. sura* group with eight described species. All species in the *S. sura* group have very small geographic distributions, with group members distributed like disjunct "beads on a string" from Monterey south to southern California and southeast to the sky-island mountain ranges of southern Arizona. Here, molecular phylogenetic and species delimitation analyses were conducted for all described species in the *S. sura* group, plus several newly discovered populations. Species trees were reconstructed using multispecies coalescent methods implemented in *BEAST, and species delimitation was accomplished using Bayes Factor Delimitation (BFD). Based on quantitative species delimitation results supported by consideration of morphological characters, two new species (*Sitalcina oasiensis* **sp. n.**, *Sitalcina ubicki* **sp. n.**) are described. We also provide a description of the previously unknown male of *S. borregoensis* Briggs, 1968. Molecular phylogenetic evidence strongly supports distinctive desert versus coastal clades, with desert canyon taxa from southern California more closely related to Arizona taxa than to geographically proximate California coastal taxa. We hypothesize that southern ancestry and plate tectonics have played a role in the diversification history of this animal lineage, similar to sclerophyllous plant taxa of the Madro-Tertiary Geoflora. Molecular clock analyses for the *S. sura* group are generally consistent with these hypotheses. We also propose that additional *Sitalcina* species await discovery in the desert canyons of southern California and northern Baja, and the mountains of northwestern mainland Mexico.

Copyright *Angela DiDomenico, Marshal Hedin.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Keywords
Species delimitation, plate tectonics, short-range endemism, historical biogeography, Bayes Factor Delimitation, Madro-Tertiary Geoflora

Introduction

Laniatorean harvestmen comprise the majority of Opiliones diversity, with more than 4100 described species (Kury 2013). North temperate Laniatores are typically small (body length usually less than 3 mm), short-legged animals, most often found in sheltered microhabitats such as under rocks and logs. The western United States is home to a diverse laniatorean fauna, with more genera and species than any other region in the Nearctic, making this area a harvestman "hotspot" (e.g., Briggs 1971, Ubick and Briggs 1989, Ubick and Briggs 2008, Derkarabetian and Hedin 2014). Laniatores of the family Phalangodidae are mostly Holarctic in distribution, but particularly diverse in the Nearctic (Ubick 2007). In California there are about 70 described phalangodid species, and this number has been growing with further sampling and use of SEM studies of morphology. Most of the described species have very small geographic distributions, and deserve more evolutionary, biogeographic, and conservation interest than currently afforded (Ubick and Briggs 2008, Emata and Hedin 2016).

The phalangodid genus *Sitalcina* is comprised of three species groups (Ubick and Briggs 2008), including the monospecific *S. californica* and *S. lobata* groups, and the *S. sura* group which includes eight described species: *S. sura* Briggs, 1968, *S. seca* Ubick & Briggs, 2008, *S. chalona* (Briggs, 1968), *S. flava* Briggs, 1968, *S. borregoensis*, *S. rothi* Ubick & Briggs, 2008, *S. catalina* Ubick & Briggs, 2008 and *S. peacheyi* Ubick & Briggs, 2008. With a known distribution from Monterey south to southern California and southeast to the sky-island mountain ranges of southern Arizona (Figure 1), species of the *S. sura* group occupy a variety of upland habitats. Most of these habitats are dominated by sclerophyllous woody plant taxa (e.g., *Quercus*, Pinyon pine, *Arctostaphylos*, *Ceanothus*, etc.), part of the Madro-Tertiary Geoflora (MTG, Raven and Axelrod 1978, Lancaster and Kay 2013, Baldwin 2014). Exceptions include *S. sura* from redwood forests, and *S. borregoensis* from desert canyons, below the elevational level of desert chaparral. Species from the sky-island mountain ranges of southern Arizona occur in mid-elevation Madro-Tertiary habitats, largely below higher elevation conifer forest, but above low desert habitats. In all habitats *S. sura* group members occupy seemingly similar microhabitats, typically under rocks on shaded north-facing slopes (Ubick and Briggs 2008, Figure 2).

Because of apparent limited dispersal abilities and microhabitat specificity, extreme population genetic structuring and divergence can be expected in *Sitalcina*, as is observed in other low vagility Laniatores (e.g., Thomas and Hedin 2008, Hedin and Thomas 2010, Derkarabetian et al. 2011, Starrett et al. 2016, Emata and Hedin 2016). As such, some currently described species with naturally fragmented distributions (e.g., *S. peacheyi* on isolated mountaintops and in caves) may actually comprise

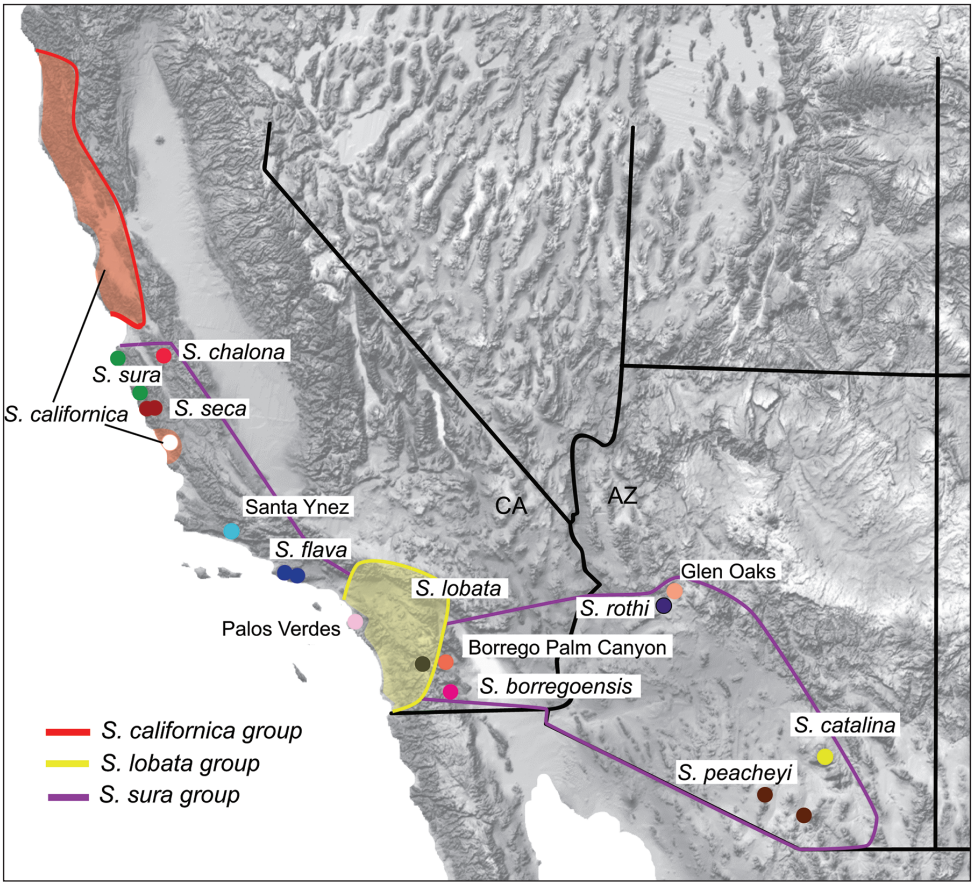


Figure 1. Distribution of *Sitalcina* and geographic sample. Groups include *S. californica* (1 species), *S. lobata* (1 species), and *S. sura* (8 described species, 4 geographically novel populations). Sampled populations indicated by circles. General species distributions follow Ubick and Briggs (2008).

multiple cryptic species. Also, the geographic sampling of previous studies may have missed unique species. Finally, members of the *S. sura* group are very similar in somatic morphology, likely due to niche conservatism (Wiens and Graham 2005, Keith and Hedin 2012). Overall, the *S. sura* group has the potential to include both newly discovered and cryptic species.

A variety of objective species delimitation methods have been developed in recent years (e.g., Yang and Rannala 2010, Rannala and Yang 2013, Grummer et al. 2014). Several of these species delimitation methods are founded on the multispecies coalescent model, utilizing multilocus genetic data (summarized in Fujita et al. 2012, Carstens et al. 2013). The probable existence of morphologically cryptic species in *Sitalcina* makes the use of such genetic methods attractive. Also, because *Sitalcina* populations are allopatric and mostly separated by unsuitable habitat, it can be assumed that interspecific gene flow is minimal. As such, incongruence in gene tree topologies

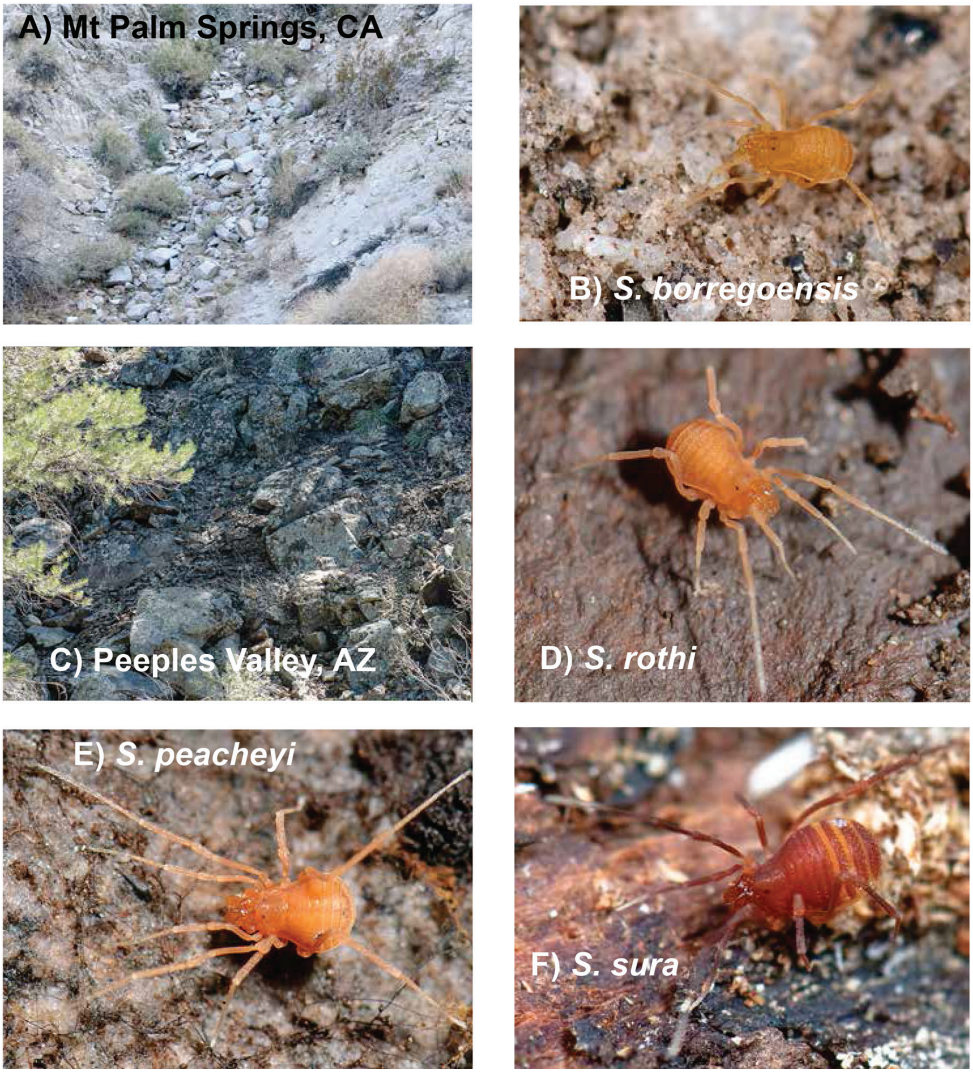


Figure 2. Habitats and live *in situ* specimens. **A** Granite talus at Mt Palm Springs, CA – creosote + ocotillo + bursage habitat **B** live *S. borregoensis* from Mt Palm Springs **C** Volcanic talus at Peeples Valley, AZ – pinyon, juniper habitat **D** live *S. rothi* from Peeples Valley **E** live *S. peacheyi* from Madera Canyon, AZ **F** live *S. sura* from Palo Colorado Road, CA. Specimen images not at same scale.

should largely reflect incomplete lineage sorting, consistent with the assumptions of most multispecies coalescent methods (Fujita et al. 2012). Conversely, extreme population genetic structuring across naturally fragmented habitats may represent a deviation from model assumptions (Niemiller et al. 2012, Hedin et al. 2015).

In this research we first use species discovery approaches to formulate alternative species delimitation hypotheses for the *S. sura* group. Based on genetic species delimitation results, supported by consideration of morphology, two new species and the

previously unknown male of *S. borregoensis* are described. A time-calibrated multilocus species tree is used as a framework to interpret the biogeographic history of the *S. sura* group. We hypothesize that this history is linked to both plate tectonics and southern ancestry, similar to elements of the MTG.

Methods

Taxon sampling

Fieldwork was conducted in the winter and spring months when surface microhabitats were most suitable for successful collections. Voucher specimens used for morphological study and species descriptions were preserved in 80% ETOH, while those used in genetic analyses were preserved in 100% ETOH at -80 °C. All described species of the *S. sura* group were collected from at or near type localities (Ubick and Briggs 2008). This sampling included previously discussed (Ubick and Briggs 2008), but undescribed, specimens from the Santa Ynez Mountains, California. In addition, several new populations from California and Arizona were discovered in appropriate habitats. Two specimens per locality were used for phylogenetic analyses when available, with the final sample including 29 ingroup specimens from 16 localities (Figure 1, Suppl. material 1: Table S1). Locality data for all specimens are also available on the Symbiota Collections of Arthropods Network (http://symbiota4.acis.ufl.edu/scan/portal/index.php). Specimens are housed in the San Diego State Terrestrial Arthropod Collection (with SDSU_TAC or SDSU_OP catalog numbers); type specimens are deposited at the California Academy of Sciences (CAS). *Sitalcina californica* (Banks, 1893) and *S. lobata* Goodnight & Goodnight, 1942 were used as outgroup taxa in all analyses. Here we accept the hypothesis that *Sitalcina* is monophyletic, as argued on morphological grounds by Ubick and Briggs (2008).

DNA isolation, amplification, and sequencing

Genomic DNA was extracted from leg tissue (2–3 legs) using the Qiagen DNeasy Kit (Qiagen, Valencia, CA). DNA fragments for mitochondrial cytochrome oxidase I (COI) and nuclear 28S rRNA, plus five additional protein-coding nuclear genes, were amplified using PCR (Table 1). Primers and cycling conditions are reported in Suppl. material 1: Table S2. Primers for protein-coding nuclear genes targeted terminal exon plus associated 3' untranslated regions (3'-UTRs), and were developed by comparing transcriptomes of *S. lobata* (Hedin et al. 2012) to the phalangodid taxon *Texella bifurcata* (Briggs, 1968) (SRA numbers reported in Emata and Hedin 2016). Both transcriptomes were generated using Illumina short-read technology, and assembled using Trinity software (Grabherr et al. 2011). PCR products were purified using Millipore plates, Sanger sequenced in both directions at Macrogen USA (Rockville, MD),

Table 1. Gene name, matrix completeness, aligned length, parsimony informative sites (ingroup), evolutionary and clock models.

Gene name	*Ixodes* homolog	Matrix % Complete	Aligned length	PI sites	Model of evolution	Clock model
Ecotropic viral integration site protein, putative	ISCW 021220	75%	508 bp	58	HKY+I	Relaxed
Protein phosphatase 2A regulatory subunit A, putative	ISCW 003443	>75%	242 bp	37	HKY+ Γ	Strict
RING finger protein, putative	ISCW 003817	>95%	214 bp	32	GTR+I	Strict
Protein transport protein Sec24A, putative	ISCW 016134	>95%	364 bp	37	GTR+ Γ	Relaxed
Neuromusculin, putative	ISCW 006547	>95%	197 bp	22	HKY+ Γ	Relaxed
28S		>95%	1094 bp	71	GTR+I+ Γ	Relaxed
COI (all)		100%	549 bp	249	GTR+ Γ	Relaxed
COI (pos1)					GTR+ Γ	
COI (pos2)					HKY+I+ Γ	
COI (pos 3)					GTR+Γ	

Note: PI, parsimony informative.

and edited using Geneious Pro 6 (Kearse et al. 2012). Sequences were aligned using MUSCLE (Edgar 2004), with the exception of 28S data, which were aligned using MAFFT (Katoh and Standley 2013) and the G_INS-I alignment algorithm (Wilm 2006). Alleles from heterozygous nuclear sequences were inferred using PHASE 2.1.1 (Stephens et al. 2001, Stephens and Scheet 2005), with each analysis repeated twice to ensure consistent results.

Gene trees and genetic clustering

Models of DNA sequence evolution were chosen using jModeltest2 (Guindon and Gascuel 2003, Darriba et al. 2012), with the Akaike Information Criterion (AIC) used to select models (Table 1). Mitochondrial COI gene tree analyses were conducted using both codon partitioned and un-partitioned models. Gene trees for individual loci were reconstructed using MrBayes 3.2 (Ronquist et al. 2011) run on the Cyber Infrastructure for Phylogenetic Research (CIPRES; Miller et al. 2010). Bayesian MCMC analyses for each gene were run for 50 million generations sampling every 5000 generations. Each analysis was repeated twice to confirm results. ESS values along with –ln*L* scores were evaluated for convergence using Tracer v.1.6 (Rambaut et al. 2014). A 50% majority rule consensus tree from the resulting posterior distribution was constructed for each gene region.

We used genetic clustering in the initial "discovery" phase of species delimitation, a procedure common in the recent literature (reviewed in Carstens et al. 2013). STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to analyze biallelic nuclear genotypic data. Each unique haplotype was treated as a single allele and was called

using SNAP-map (Aylor et al. 2006). STRUCTURE runs were conducted with 20 iterations for multiple K values (K = number of genetic clusters). Separate runs were conducted with coastal clade species (see Results) with a K of 1-9, and desert clade species (see Results) with a K of 1-8. Analyses were run for 100,000 generations, with the first 10,000 generations removed as burnin. Analyses were run using both a no-admixture model (assumes each individual comes from one of the K distinct populations) and an admixture model (allows for population admixture). All other priors were left as default. Structure Harvester (Earl 2012) was used to find the best-fit K utilizing the ΔK method of Evanno et al. (2005). Data were summarized using the *FullSearch* algorithm of CLUMPP (Jakobsson and Rosenberg 2007), and visualized with DISTRUCT (Rosenberg 2004).

Species trees and divergence times

Multilocus species trees were reconstructed using *BEAST 1.8 (Drummond et al. 2012). *A priori* species limits were based on genetic clusters identified by STRUCTURE admixture and no-admixture model results. For each analysis, substitution models, clock models, and trees were unlinked across loci. A best-fit model of molecular evolution was applied to each gene region (Table 1), with mitochondrial COI partitioned by codon and a Yule process applied for the species tree prior. Clock rate parameters were examined using Tracer v1.6 (Rambaut et al. 2014). If the 95% highest posterior density (HPD) of the coefficient of variation for any individual gene included zero (indicating that a strict molecular clock could not be statistically rejected), a strict clock was used for that gene region. Subsequent analyses were run with the appropriate uncorrelated relaxed or strict clock models (Table 1). Analyses were run for 200 million generations logging every 2000 generations. ESS values along with $-\ln L$ scores were evaluated for convergence using Tracer. Each species tree analysis was repeated twice to ensure consistent results. Posterior distributions of repeated runs were combined using LogCombiner, and a maximum clade credibility (MCC) tree was constructed from the resulting combined posterior distributions using TreeAnnotator.

Divergence time analyses were performed in *BEAST (Drummond et al. 2012). Because of general uncertainty in rates of molecular evolution in Laniatorean harvestmen, we conducted three separate analyses to provide bounds on possible dates. First, a well-accepted arthropod COI clock rate of 3.54% per million years (Ma) (arithmetic mean of branch rates (ucld) setting in *BEAST = 0.0178) was specified for the partitioned COI data (Papadopoulou et al. 2010). Second, the unpartitioned COI rate of Papadopoulou et al. (2010) was used (ucld mean = 0.0169). Finally, a slower COI rate calculated for the laniatorean genus *Sclerobunus* (ucld mean = 0.01115) was specified for the COI data partition (Derkarabetian et al. 2010). This latter rate was inferred indirectly based on combined biogeographic and fossil calibrations. For each analysis, a Yule process was specified for the species tree prior and a pairwise linear and constant root was applied for the population model, with a uniform distribution on the upper and

lower bounds of the age. Substitution models, clock models, and trees were unlinked across loci. The appropriate model of molecular evolution and clock-like rate was applied to each gene fragment (see above). Analyses were run for 50 million generations sampling every 500, and repeated to confirm consistent results. Repeated runs were combined using LogCombiner, and a MCC tree was constructed using TreeAnnotator.

Genetic species delimitation

Bayes factor delimitation (BFD, Grummer et al. 2014) allows testing of alternative species delimitation models by assigning individuals to different lineages, with subsequent comparisons of marginal likelihoods of alternative models. We tested the alternative species delimitation models summarized in Table 2 – these alternative models are based on a combination of STRUCTURE results, geographic considerations, and prior taxonomy. Marginal likelihood estimations were run for 100,000 generations, sampled every 1000 with 100 steps using path sampling (Lartillot and Philippe 2006) and stepping stone (Xie et al. 2011) methods. A comparison of marginal likelihoods was conducted using Bayes factors, with values above 10 considered as decisive support (Kass and Raftery 1995).

Niche modeling

With incomplete knowledge of the full distribution of the *S. sura* group, possible regions with additional new populations or species were identified via ecological niche modeling (ENM). DIVA-GIS (Hijmans et al. 2004) and Maxent (Phillips and Dukid 2008) were used to construct niche models, using known localities for the species group as input. Niche models have previously successfully predicted the potential habitats of animal taxa with small and fragmented distributions (e.g., Rissler and Apodaca 2007, Bond and Stockman 2008, Bond 2012). Predicted distributions of the *S. sura* group were reconstructed using altitudinal and climatic layers for nineteen quarterly and annual measurements of temperature and precipitation (Bioclimatic layers 1-19), obtained from the WorldClim dataset (Hijmans et al. 2005). A jackknife analysis was conducted in Maxent to discover the most likely environmental factors impacting species' distributions. Output from Maxent was converted to raster format and reclassified to binary presence/absence in ArcMap 10.3 (ESRI) using the 10 percentile training presence logistic threshold.

Study of morphology

Male penises that were not protruding from the genital operculum were physically extracted using a blunt insect micro pin. Exposed penises were placed in room temperature (or hot) 10% KOH for 1-2 minutes for expansion. Female ovipositors were

Table 2. Alternative species delimitation hypotheses used in BFD analyses.

Hypothesis	Distinct Species (total in parentheses)	Motivation
H1	Glen Oaks, Santa Ynez, *S. borregoensis*, Borrego Palm Canyon, *S. catalina*, *S. chalona*, *S. flava* Topanga, *S. flava* Piuma + Palos Verdes, *S. peacheyi*, *S. peacheyi* MAD, *S. rothi*, *S. sura*, *S. seca* (13 species)	Following STRUCTURE admixture model
H2	Glen Oaks, Santa Ynez, *S. borregoensis*, Borrego Palm Canyon, *S. catalina*, *S. chalona*, *S. flava* Topanga, *S. flava* Piuma + Palos Verdes, *S. peacheyi*, *S. peacheyi* MAD, *S. rothi*, ***S. sura*** + ***S. seca*** (12 species)	Adjacent *S. sura* + *S. seca* considered a single species
H3	Glen Oaks, Santa Ynez, ***S. borregoensis*** + Borrego Palm Canyon, *S. catalina*, *S. chalona*, *S. flava* Topanga, *S. flava* Piuma + Palos Verdes, *S. peacheyi*, *S. peacheyi* MAD, *S. rothi*, *S. sura*, *S. seca* (12 species)	Anza-Borrego specimens considered a single species
H4	Glen Oaks, Santa Ynez, *S. borregoensis*, Borrego Palm Canyon, *S. catalina*, *S. chalona*, *S. flava* Topanga, *S. flava* Piuma + Palos Verdes, *S. peacheyi* + *S. peacheyi* MAD, *S. rothi*, *S. sura*, *S. seca* (12 species)	*S. peacheyi* as single species
H5	Santa Ynez, *S. borregoensis*, Borrego Palm Canyon, *S. catalina*, *S. chalona*, *S. flava* Topanga, *S. flava* Piuma + Palos Verdes, *S. peacheyi*, *S. peacheyi* MAD, Glen Oaks + ***S. rothi***, *S. sura*, *S. seca* (12 species)	Coastal STRUCTURE results + Desert no-admixture model results
H6	Glen Oaks, Santa Ynez, *S. borregoensis*, Borrego Palm Canyon, *S. catalina*, *S. chalona*, Palos Verdes + ***S. flava***, *S. peacheyi*, *S. peacheyi* MAD, *S. rothi*, *S. sura*, *S. seca* (12 species)	*S. flava* and Palos Verdes as single species
H7	Glen Oaks, *S. borregoensis*, Borrego Palm Canyon, *S. catalina*, *S. chalona*, Santa Ynez + *S. flava* + Palos Verdes, *S. peacheyi*, *S. peacheyi* MAD, *S. rothi*, *S. sura*, *S. seca* (11 species)	coastal southern CA a single species
H8	Glen Oaks, Santa Ynez, ***S. borregoensis*** + Borrego Palm Canyon, *S. catalina*, *S. chalona*, *S. flava* Topanga, *S. flava* Piuma + Palos Verdes, *S. peacheyi* + *S. peacheyi* MAD, *S. rothi*, *S. sura*, *S. seca* (11 species)	*S. peacheyi* as single species; Anza-Borrego specimens considered a single species
H9	*S. borregoensis* + Borrego Palm Canyon, *S. catalina*, *S. chalona*, *S. flava* +Santa Ynez + Palos Verdes, *S. peacheyi* + *S. peacheyi* MAD, *S. rothi* + Glen Oaks, *S. sura* + *S. seca* (7 species)	Putative species grouped with geographic neighbors

exposed using the same blunt pin procedure. Specimens were imaged using a Quanta 450 scanning electron microscope (SEM) after being mounted and coated with 20nm platinum. One or two specimens were used for SEM as needed. Whole specimen digital images were captured using a Visionary Digital BK plus system (http://www.visionarydigital.com). Individual images were merged into a composite image using Helicon Focus 6.2.2 software (http://www.heliconsoft.com/heliconfocus.html). Specimen measurements were taken with an SZX12 Olympus dissecting scope equipped with an ocular micrometer, at 50× magnification.

Results

Data characteristics

DNA sequence data were collected for two outgroup taxa (data for *S. lobata* from transcriptomes), eight described species from the *S. sura* group, and four novel geo-

graphic populations with morphological features placing them in the *S. sura* group (see diagnostic features below). Not all specimens were successfully amplified for all gene regions, resulting in some missing data (Table 1). All heterozygous nuclear sequences were PHASED with 100% certainty. GenBank accession numbers for unphased sequences are reported in Suppl. material 1: Table S3, and phased data matrices have been submitted to the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.4gk4f).

Gene trees and genetic clustering

Mitochondrial COI gene trees were reconstructed using both a codon partitioned and un-partitioned model, and are generally topologically similar (Figure 3). A clade that includes southern California desert populations together with Arizona sky-island populations (hereafter named the "desert" clade) is recovered in both analyses. Geographically proximate populations from canyons in the Anza Borrego desert (Borrego Palm Canyon, *S. borregoensis*) are surprisingly genetically divergent, with average Kimura 2-parameter distances exceeding 17% (calculated using MEGA6, Tamura et al. 2013), and do not form a clade on COI trees. An important incongruence between codon partitioned versus un-partitioned results concerns the uncertain placement of the *S. flava* + Palos Verdes clade. These populations are consistently placed with other coastal CA populations (hereafter named the "coastal" clade, see below) in nuclear gene tree analyses, but group with the desert clade with low support (posterior probability (PP) = 0.59) in codon partitioned COI analyses.

Despite expected variance in nuclear gene tree topologies, several general trends are apparent. First, a coastal CA clade is recovered (generally with strong support, PP > 0.95) in all six nuclear gene trees (Figure 4). This clade includes *S. sura*, *S. seca*, *S. chalona*, *S. flava*, Santa Ynez, and Palos Verdes populations. Specimens from Santa Ynez are genetically distinct in all nuclear gene trees. A desert clade is strongly supported in four of six nuclear gene trees; paraphyly of this group in two gene trees may reflect a gene tree rooting issue. Within the desert clade, Borrego Palm Canyon specimens are genetically distinct from neighboring *S. borregoensis* in four gene trees where both populations were sampled (Figure 4).

STRUCTURE analyses for coastal clade specimens favor a K = 6 model (Figure 5A). Both no-admixture and admixture models place *S. sura*, *S. seca*, *S. chalona*, and Santa Ynez individuals into four separate genetic clusters. Specimens from Palos Verdes and *S. flava* from Piuma Road group together into a fifth genetic cluster, while Topanga Canyon *S. flava* specimens represent a sixth cluster. The STRUCTURE admixture model favors all discrete desert populations (either found on isolated mountain ranges or in isolated desert canyons) as unique genetic clusters (K = 7, Figure 5B). A no-admixture model favors K = 6, grouping geographically adjacent Glen Oaks and *S. rothi* specimens (Figure 5C).

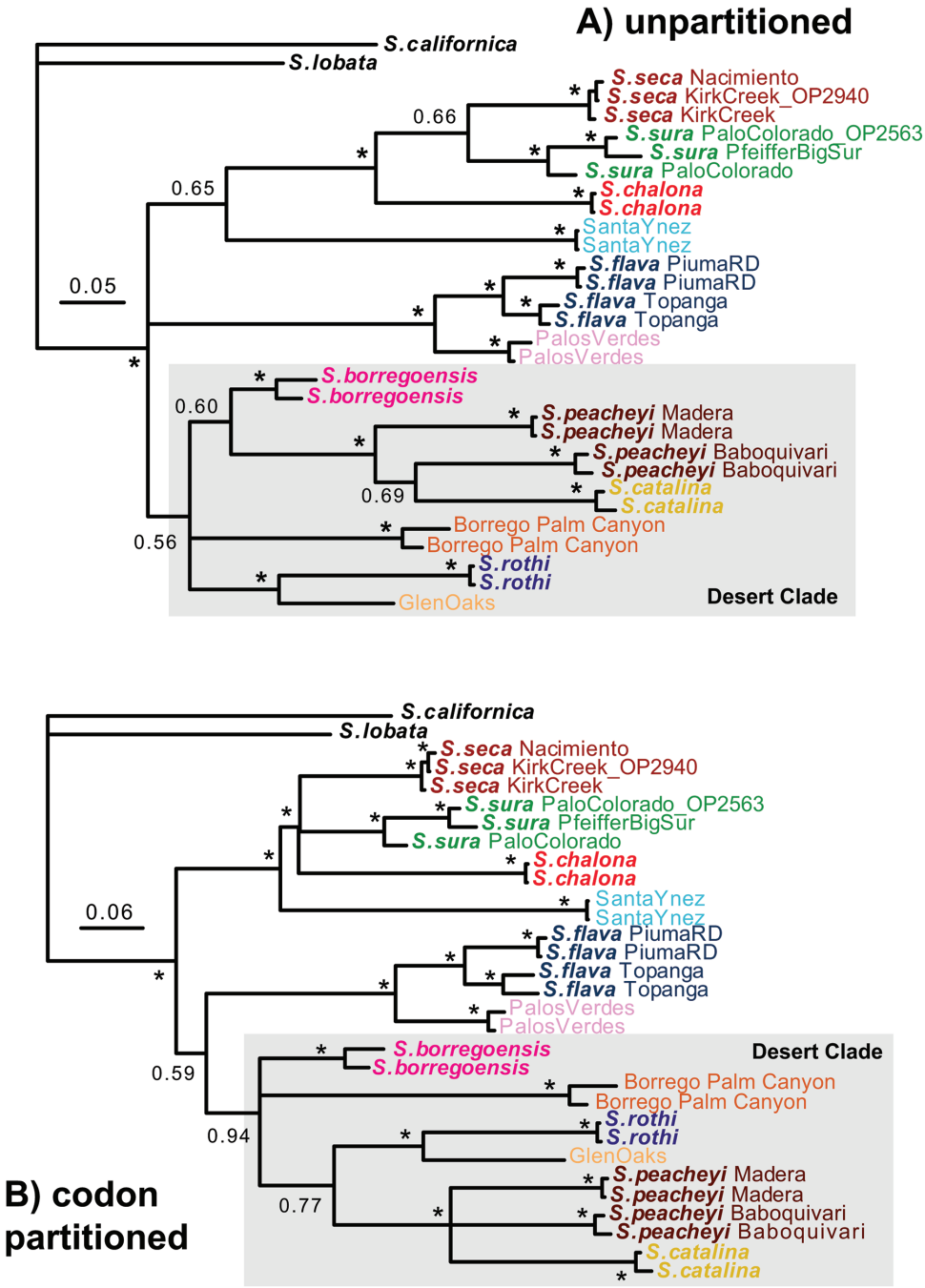


Figure 3. Mitochondrial COI gene trees. Results based on **A** un-partitioned and **B** codon partitioned analyses. Asterisks indicate posterior probabilities above 0.95. Members of desert clade (see text) highlighted.

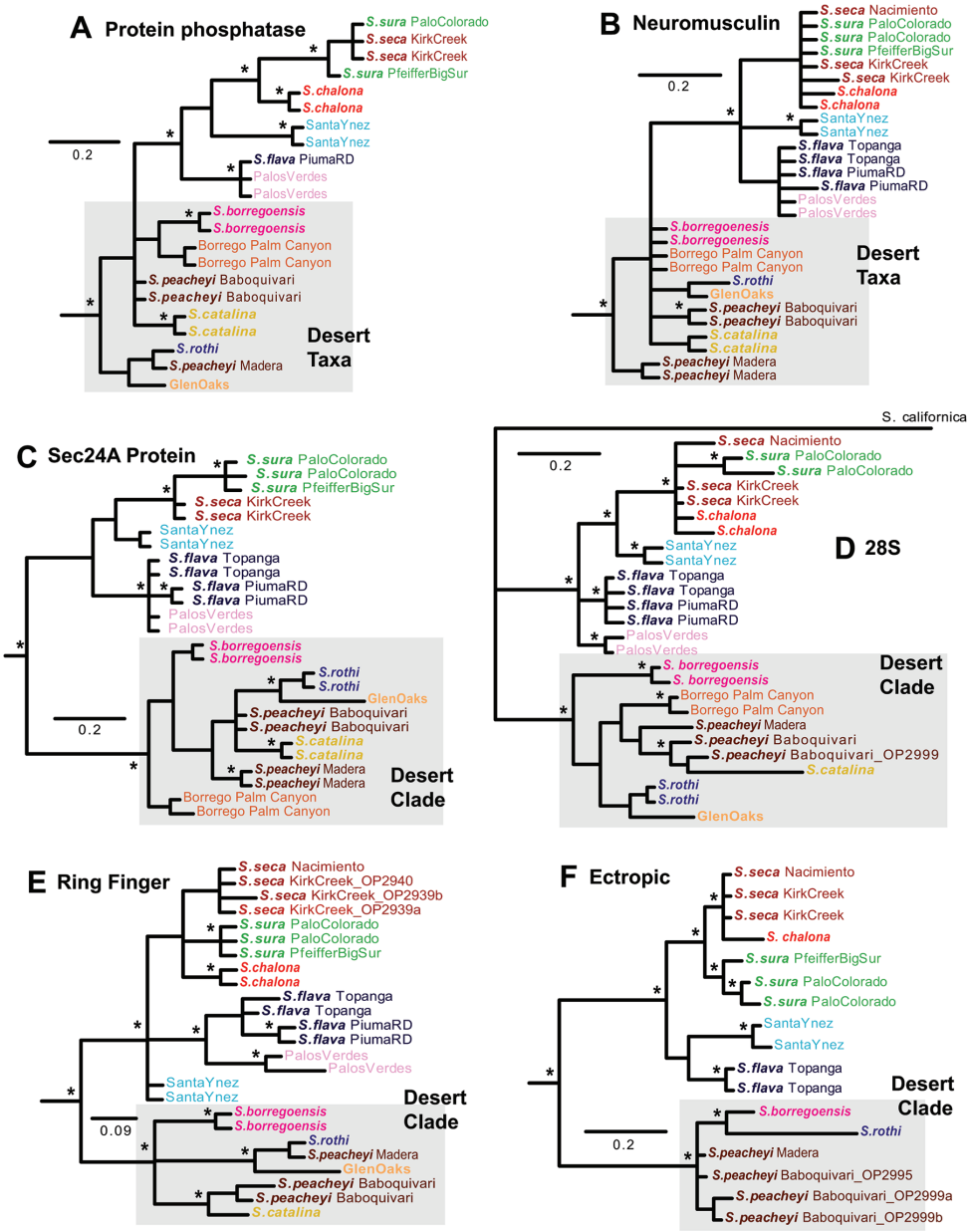


Figure 4. Nuclear gene trees. Individual genes include **A** Protein phosphatase 2A regulatory subunit A **B** Neuromusculin **C** Protein transport protein Sec24A **D** 28S, **E** RING finger protein, and **F** Ecotropic viral integration site protein. Outgroups trimmed from all trees except for 28S. Asterisks indicate posterior probabilities above 0.95. Desert taxa shaded.

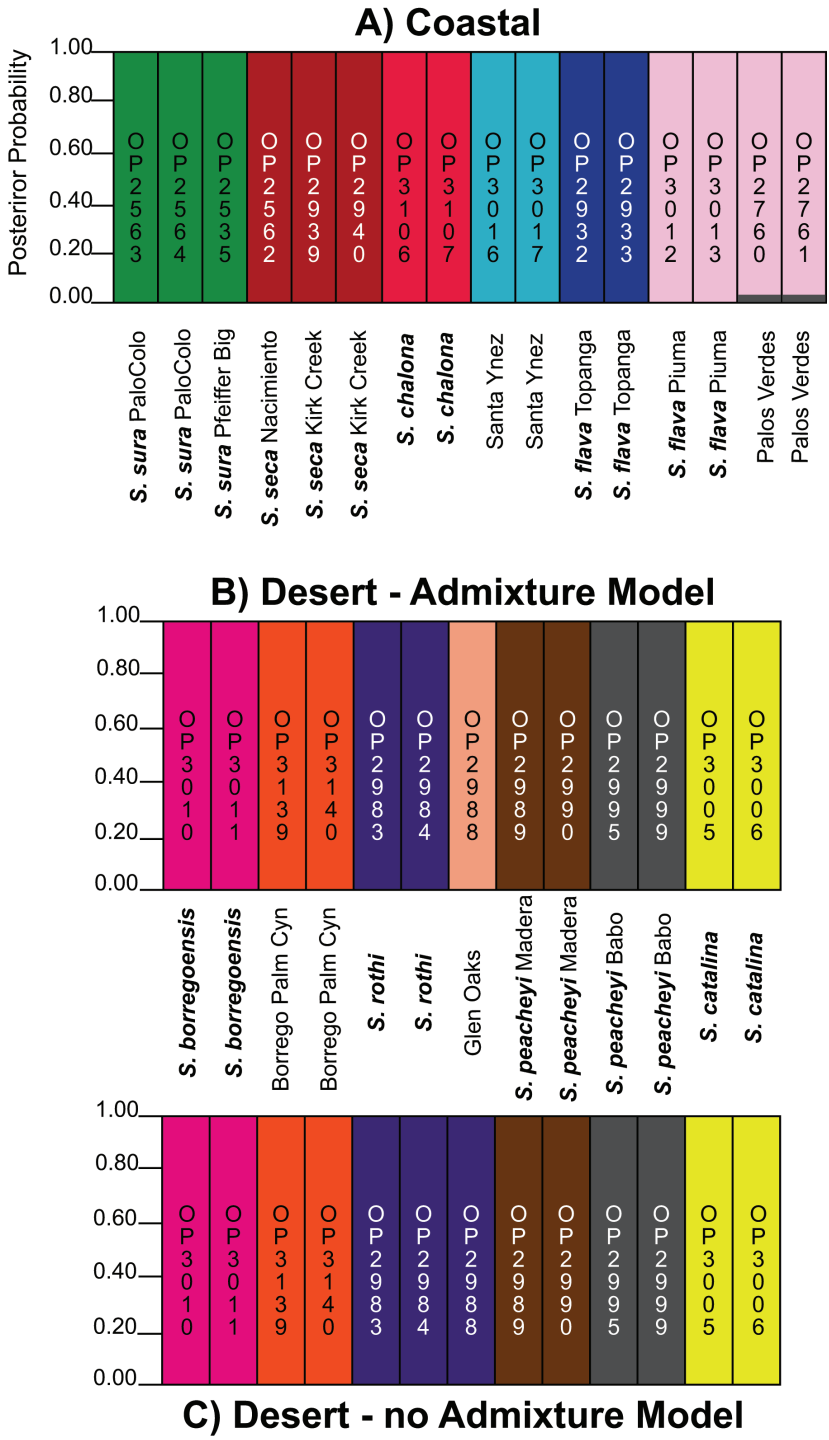


Figure 5. STRUCTURE results. Results for **A** coastal clade using admixture and no admixture models (K=6) **B** desert clade using admixture model (K=7), and **C** desert clade using no admixture model (K=6).

Species trees and divergence times

*BEAST analyses conducted with admixture STRUCTURE clusters as *a priori* species recover desert and coastal clades with strong support (PP > 0.95, Figure 6A). Relationships among coastal taxa are well supported, with an internal topology that mirrors geography (early-diverging southern lineages, Santa Ynez intermediate, derived northern lineages). Relationships between desert taxa are less strongly supported, with CA desert canyon populations forming a clade sister to montane Arizona populations.

Using the Papadopoulou et al. (2010) partitioned COI rate, the estimated divergence time for the most recent common ancestor (tMRCA) of the *S. sura* group is ~18 Ma, the tMRCA for the coastal clade is ~10 Ma, and the tMRCA for the desert clade is ~7 Ma (Figure 6B). The Papadopoulou et al. (2010) unpartitioned COI rate provides very similar time estimates, while the slower *Sclerobunus* rate results in clearly older time estimates for these same nodes (Table 3). In general, we have no *a priori* reason to favor one of these rates, given the general lack of knowledge of rates of molecular evolution in laniatorean harvestmen. However, we favor the younger dates for three reasons. First, the Papadopoulou et al. (2010) rates result in date estimates that coincide with important biogeographic events in the region (see Discussion). Second, various temporal analyses conducted by Emata and Hedin (2016) for the California phalangodid genus *Calicina* also showed that the Papadopoulou et al. (2010) rate provided biologically realistic dates for a California taxon, while slower rates suggested unrealistically old divergence dates. Finally, we note that recent studies of *Sclerobunus* using whole genome SNP data (Derkarabetian et al. 2016) provide younger divergence ages than previously hypothesized in that system, suggesting that the Derkarabetian et al. (2010) COI rate may have been underestimated. All of these arguments are *ad hoc*, with resolution in the *Sitalcina* system ultimately requiring additional data.

Niche modeling

Precipitation in the coldest quarter (BIO19) and precipitation in the driest month (BIO14) were the best predictors of *S. sura* group distributions. ENMs based on these variables provide a visualization of possible sampling gaps within the *S. sura* group (Figure 7). In California and Baja California Norte, areas of high suitability that represent possible gaps include the Santa Ynez Mountains northwest of Santa Barbara, and the southern Santa Lucia Mountains. Some of these habitats are occupied by *S. californica*, which has an apparently exclusive distribution with members of the *S. sura* group (i.e., no sympatry has ever been recorded, Figure 1). Similarly, we expect *S. lobata* to occupy the predicted suitable habitats of coastal southern CA and northern Baja (Figure 1). In these particular cases, we hypothesize that the ENM has over-predicted the distribution of the *S. sura* group. California desert canyon habitats, both north and south of our sampled populations, have predicted suitability. In Arizona and northern

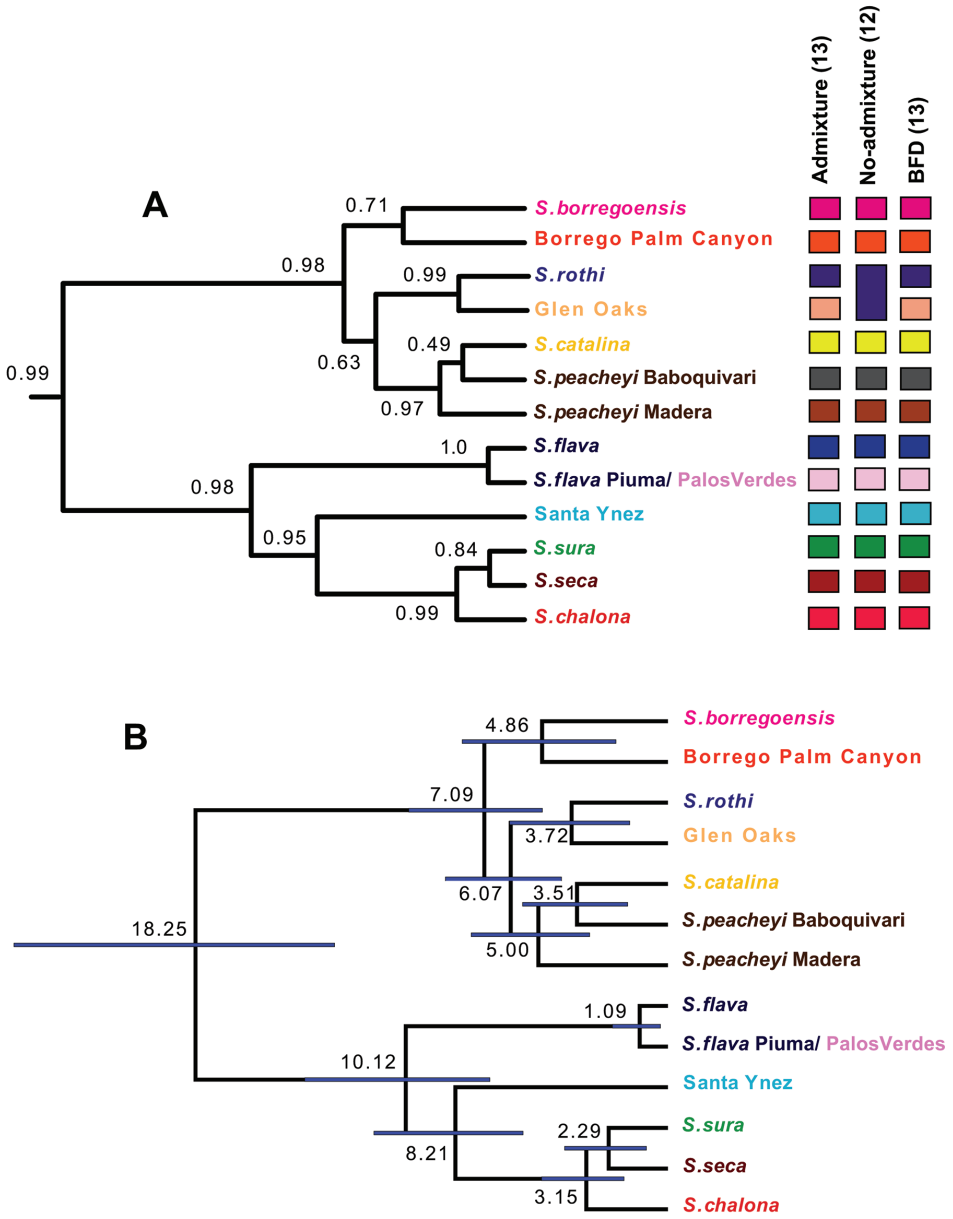


Figure 6. *BEAST species trees. **A** Includes summary of STRUCTURE and BFD species delimitation results. Outgroup taxa not shown **B** Includes divergence time estimates based on Papadopoulou et al. (2010) partitioned COI rate. Ages (in millions of years) and bars indicating 95% highest posterior density (HPD). Outgroup taxa not shown.

mainland Mexico (Sonora), unsampled populations are predicted to occur in montane habitats between known *S. catalina* - *S. rothi* populations, and south of our current sample. We are not aware of *Sitalcina* records from northern mainland Mexico, but

Table 3. Divergence time estimates from alternative COI molecular clock rates.

Model	Papadopoulou partitioned COI rate	Papadopoulou unpartitioned COI rate	Derkarabetian COI rate
tMRCA *S. sura* group	18.25 (12.88–25.26)	19.36 (13.58–26.47)	29.43 (19.56–40.87)
tMRCA coastal clade	10.12 (6.88–14.03)	10.98 (7.56–14.85)	16.25 (10.57–23.07)
tMRCA desert clade	7.09 (4.85–10.01)	7.49 (5.09–10.37)	11.39 (7.64–16.04)
tMRCA *S. borregoensis*, *S. oasiensis*	4.86 (2–7.95)	5.32 (2.09–8.57)	7.87 (3–13.22)

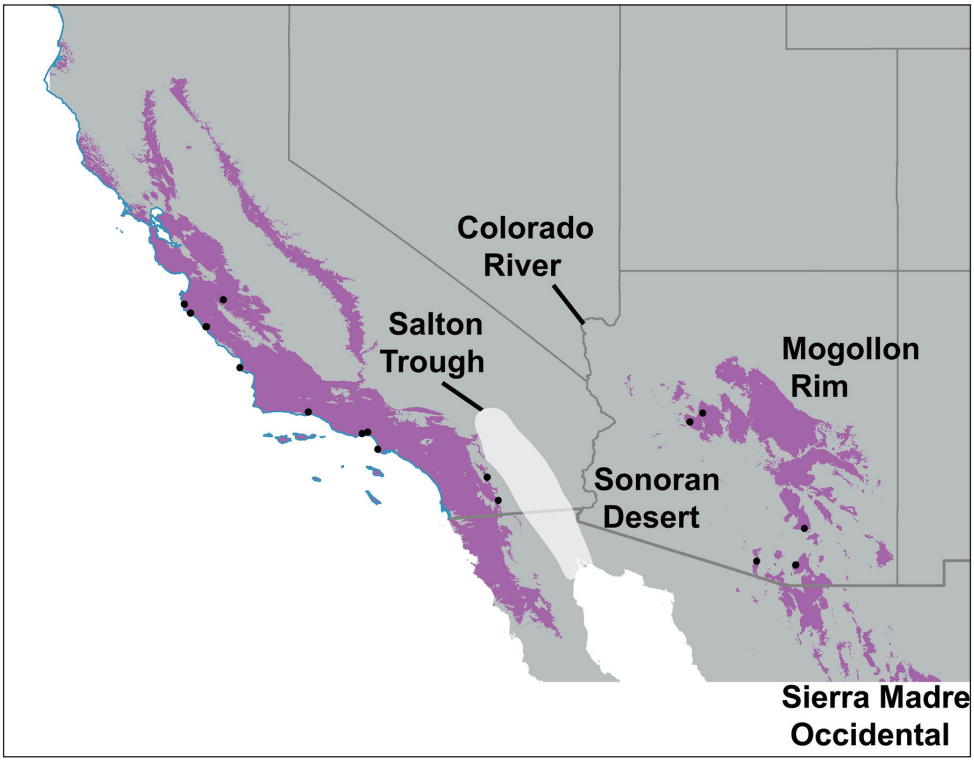


Figure 7. Binary ENM for *Sitalcina sura* species group. Purple regions indicate predicted habitat and dots indicate collection localities used in model estimation. Major geographic features highlighted.

based on habitat and distribution of other regional sky-island animal taxa (e.g., Maddison and McMahon 2000, Bryson et al. 2011, Bryson et al. 2013, Grummer et al. 2014), we expect *Sitalcina* to occur in the mountains of Sonora.

Genetic species delimitation

BFD results support a 13 species model, following the K = 6 STRUCTURE model for coastal taxa and the K = 7 model for desert taxa (Tables 2 & 4, Figure 6). This 13 spe-

Table 4. BFD results.

Model	Path Sampling	Bayes Factor PS	Stepping Stone	Bayes Factor SS
H1	-12137	**NA**	-11953	**NA**
H2	-12387	499	-12366	827
H3	-12308	342	-12252	598
H4	-12258	241	-12137	369
H5	-12303	331	-12235	563
H6	-12235	196	-12129	351
H7	-12264	253	-12140	373
H8	-12352	429	-12300	693
H9	-12287	300	-12131	357

Note: Hypotheses as in Table 2.

cies model supports three new populations as putative species (Borrego Palm Canyon, Santa Ynez, Glen Oaks), and the division of both *S. peacheyi* and *S. flava* into separate species. This model is decisively supported over alternative species delimitation models (Table 4). In translating BFD results into formal taxonomy, we have taken a conservative approach, also considering support from other lines of evidence. Because Glen Oaks is known only from a single adult female specimen, we defer formal species description to a later date, when additional specimens can be collected and studied. Further attention should be also directed at the geographically disjunct Palos Verdes population, which is genetically distinct from *S. flava* for multiple genes (Figures 3, 4). Finally, because we failed to find obvious morphological differences between disjunct populations of *S. peacheyi* and *S. flava*, and because multispecies coalescent models might oversplit genetically structured populations (Niemiller et al. 2012, Hedin et al. 2015), we do not further split *S. peacheyi* and *S. flava* at this time.

Genetically distinct and morphologically diagnosable populations from Borrego Palm Canyon and Santa Ynez are formally described below. Also, previously unknown males of *S. borregoensis* are described. Following Ubick and Briggs (2008), we emphasize both somatic and genitalic characters in our descriptions.

Taxonomy

Morphological abbreviations (following Ubick and Briggs 2008): 1. Somatic morphology: AT = anterior tubercles of scute, BL = body length (viewed laterally), EM = eye mound, GO = genital operculum, Fm = femur, SL = scute length (from front edge of EM to posterior edge of scute, viewed dorsally), SW = scute width at widest point, viewed dorsally), TC = tarsal count, TrIV = trochanter of leg IV. 2. Penis morphology: DL = dorsal lobe of glans, PSL = parastylar lobe(s) of glans, S = stylus, VP = ventral plate of penis, VS = ventral setae of ventral plate, AS = apical spine of ventral plate. 3. Ovipositor morphology: OV = ovipositor, OVM = ovipositor microspines, OVS = ovipositor apical setae. All measurements are in millimeters unless noted otherwise.

Sitalcina Banks, 1911

Diagnosis. As presented in Ubick and Briggs (2008).

Sitalcina sura Group

Diagnosis. Members of the *S. sura* group are distinguished from related *S. californica* and *S. lobata* by the following characters (Ubick and Briggs 2008): Both sexes possess a short row of dorsomesal asetose tubercles on the palpal Fm. Males lack an AS, possess a bilobed PSL, and a curved to straight ectal spur on TrIV. Females with imbricate OVM (absent/weak in the (*S. chalona*, (*S. sura*, *S. seca*)) clade), and curved OVS with brush-like tips.

Sitalcina borregoensis Briggs, 1968

Figures: map Figure 1; habitus Figure 2B; male Figure 8A–D

Sitalcina borregoensis Briggs, 1968: 30. Ubick and Briggs 2008: 22, fig. 25.

Type material examined. Holotype female from California, San Diego County, Anza- Borrego Desert State Park, Mountain Palm Springs, collected by T. Briggs, April 5, 1967 (CAS).

Diagnosis. This small-bodied species is most similar to *S. rothi* and *S. oasiensis*, with a low EM and a flattened body profile in both sexes. Females can be diagnosed by the moderately imbricate OVM. Males possess a TrIV spur that is approximately straight, and longer than in *S. oasiensis*. The distal end of PSL is conspicuously serrate.

Genetic data. GenBank Accession numbers: KX064802, KX064803, KX064830, KX064831, KX064855, KX064856, KX064876, KX064898, KX064899, KX064924, KX064925, KX064954, KX064955.

Description. FEMALE. As in Ubick and Briggs 2008.

MALE. Integument color pale orange, appendages lighter. Body finely rugose with a few large tubercles on posterior tergites, one pair anteriorly on EM; 3 pairs of AT. EM low, flattened, eyes present. Palpal Fm with median dorsobasal row of 4 asetose tubercles and one small mesal tubercle. Palpal megaspines: trochanter one ventral and small; Fm 3 ventrobasal, one mesodistal; patella 2 mesal, one ectal; tibia and tarsus 2 mesal, 2 ectal. TC 3-5-5-5.

Measurements taken from following specimens: SDSU_OP3011 (SDSU_OP3010): BL 1.32 (1.24). SL 0.75 (0.84), SW 0.75 (0.78). EM width 0.19 (0.18), height 0.12 (0.10). GO length 0.17 (0.14), width 0.17 (0.14). Leg II length - missing (2.78), Leg II/SL - missing (3.31). TrIV spur present, nearly straight. Penis VP entire, apically pointed, with 8 pairs of setae, AS absent; glans DL quadrate; PSL serrate distally; S not visible.

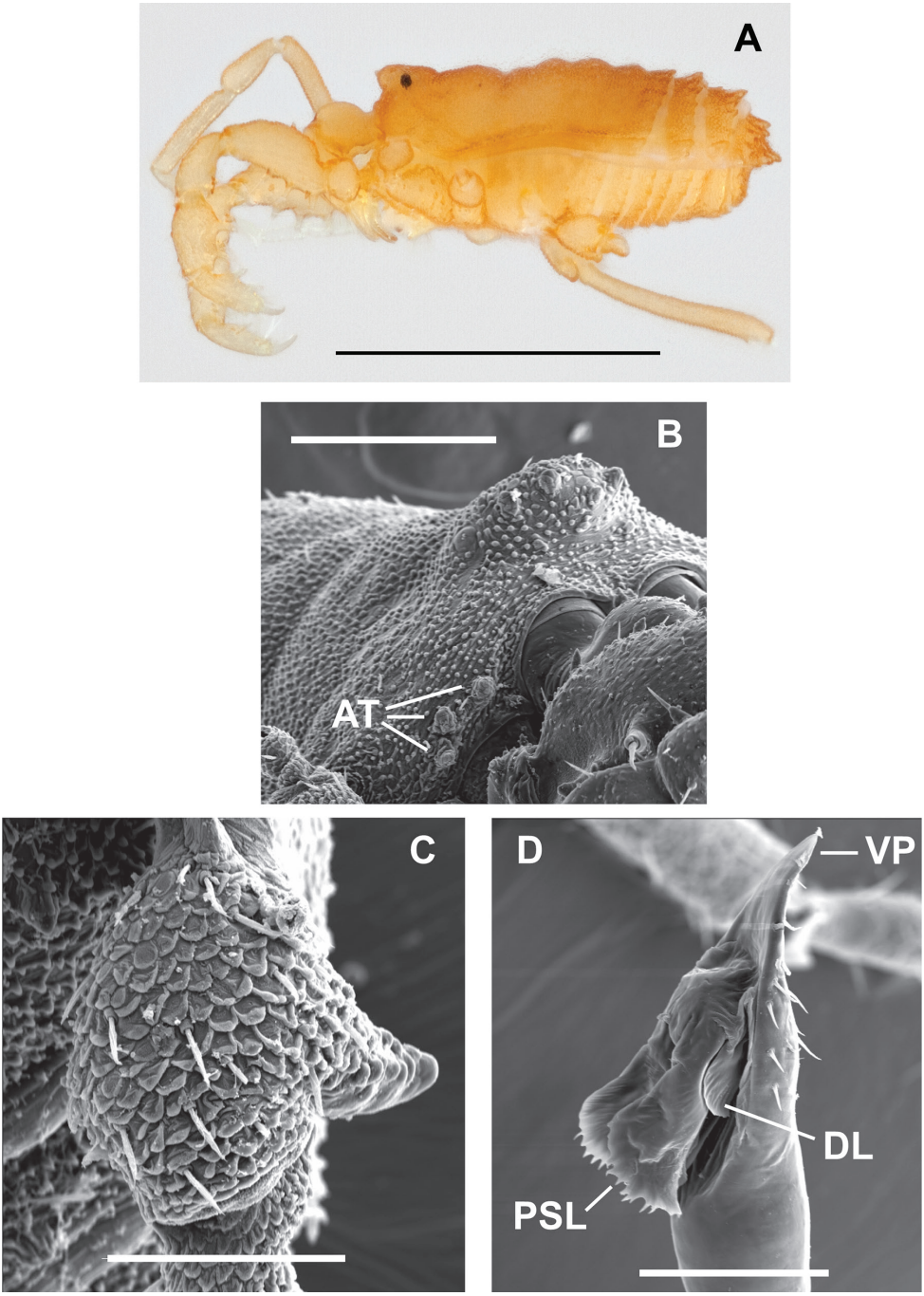


Figure 8. Male *Sitalcina borregoensis* (ETOH - SDSU_OP3011, SEM prep - SDSU_TAC000293) – **A** habitus **B** EM **C** TrIV **D** penis. Scale bar = 1 mm (**A**), 200 µm (**B**), 100 µm (**C, D**).

Other material examined. New males were collected on 19 February, 2012 from the vicinity of the type locality. Suppl. Material 1: Table S1 provides additional locality information for specimens examined.

Distribution and habitat. Known only from the vicinity of Mountain Palm Springs, Anza Borrego Desert State Park. New collections are from a north-facing slope, under the first layer of granite rocks in a small ravine adjacent to a palm grove (Figure 2A).

***Sitalcina oasiensis* DiDomenico & Hedin, sp. n.**
http://zoobank.org/12D14E94-D653-4340-A4AA-4D0ECD9E1400
Figures: map Figure 1; male Figure 9A–E; female Figure 10A–D

Type material. Holotype male (SDSU_TAC000211, CASENT 9029998) from California, San Diego County, Anza-Borrego Desert State Park, Borrego Palm Canyon. N33.28025°, W116.43369° elev. ca. 430 m. Collected by A. DiDomenico, D. Carlson, S. Derkarabetian, S. Bejarano, February 23, 2013.

Etymology. Named for the well-known palm oases of Borrego Palm Canyon.

Diagnosis. Both sexes are small-bodied, with a low EM and a flattened body profile, similar to *S. borregoensis*. Females can be distinguished by the more strongly imbricate OVM. The male TrIV spur is approximately straight, shorter than in *S. borregoensis*.

Genetic data. GenBank Accession numbers: KX064804, KX064805, KX064832, KX064833, KX064857, KX064858, KX064900, KX064901, KX064926, KX064927.

Description. Integument color pale orange with lighter appendages. Body finely rugose with larger tubercles along tergal margins and small tubercles anteriorly on EM; 2-3 pairs of AT. EM low and rounded, eyes present. Palpal Fm with median dorsobasal row of 3 asetose tubercles and one small mesal tubercle. Palpal megaspines: trochanter 2 ventral; Fm 3 ventrobasal, one mesodistal; patella 2 mesal, one ectal; tibia and tarsus 2 mesal, 2 ectal. TC 3-5-5-5.

MALE. Holotype (paratypes SDSU_TAC000297, SDSU_TAC000298): BL 1.3 (1.13–1.28). SL 0.81 (0.75- 0.8), SW 0.78 (0.73–0.88). EM width 0.19 (0.20), height 0.11 (0.10- 0.13). GO length 0.14, width 0.15. Leg II length 2.7 (2.34–2.64), Leg II/SL 3.33 (2.93–3.12). TrIV spur short, straight. Penis VP entire, apically rounded, with 9 pairs of setae, AS absent; glans DL quadrate; PSL simple and bilobed, rounded at apical end; S not visible.

FEMALE paratype SDSU_TAC000299: BL 1.5. SL 0.8, SW 0.9. EM width 0.25, height 0.13. Leg II length 3.0, Leg II/SL 3.75. Strongly imbricate OVM, apical teeth absent, 7 pairs of OVS, curved, multifurcate.

Other material examined. See Suppl. material 1: Table S1 for locality information for specimens examined.

Distribution and habitat. Known only from the type locality. Specimens were collected from sparse desert chaparral habitat, under small rocks amongst larger granite boulders, NE-facing slope above palm oases.

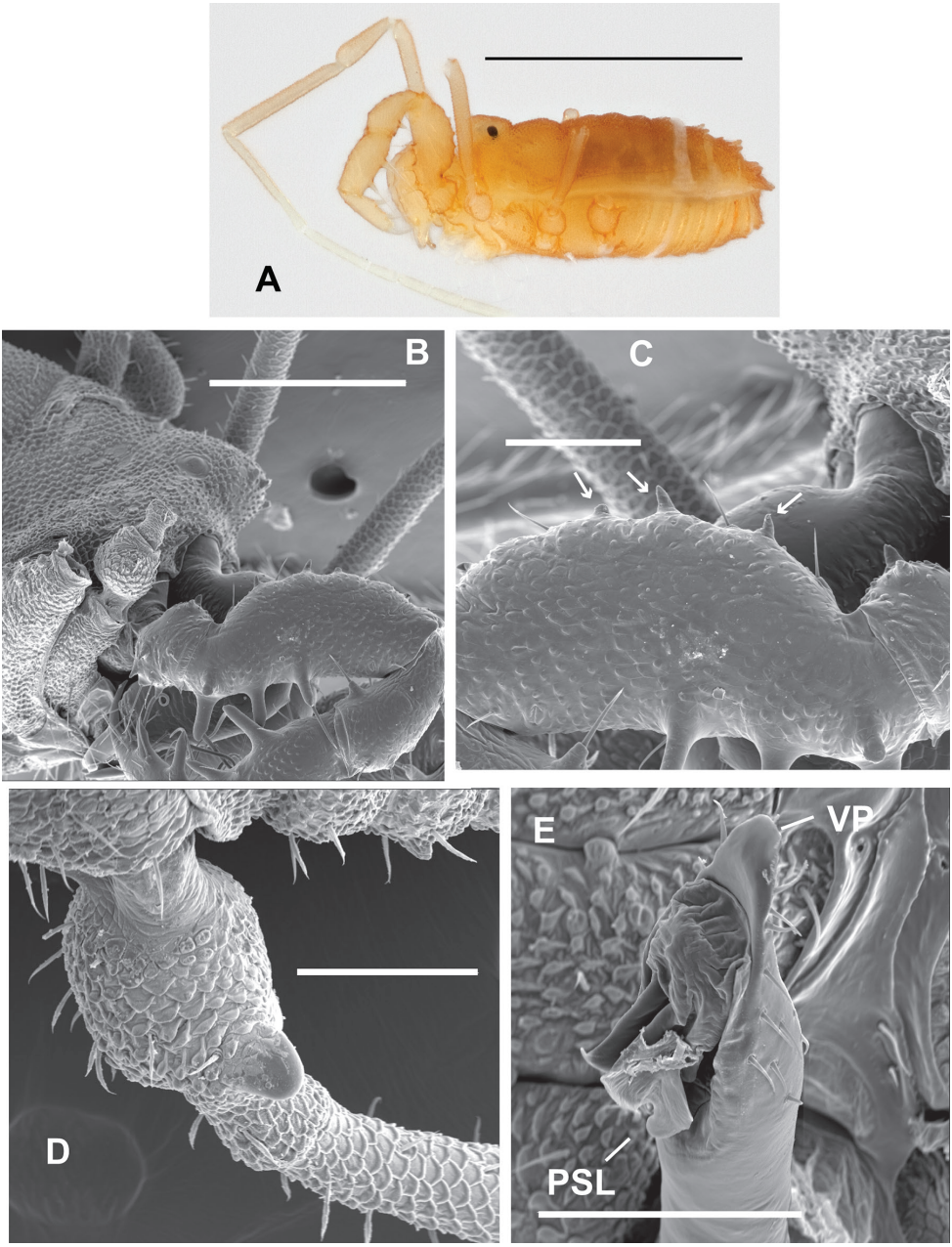


Figure 9. Male *Sitalcina oasiensis* (ETOH - SDSU_ OP3140, SEM prep - SDSU_TAC000289) – **A** habitus **B** EM **C** palpal Fm, asetose tubercles at arrows **D** TrIV **E** penis. Scale bar: 1 mm (**A**), 300 µm (**B**), 100 µm (**C–E**).

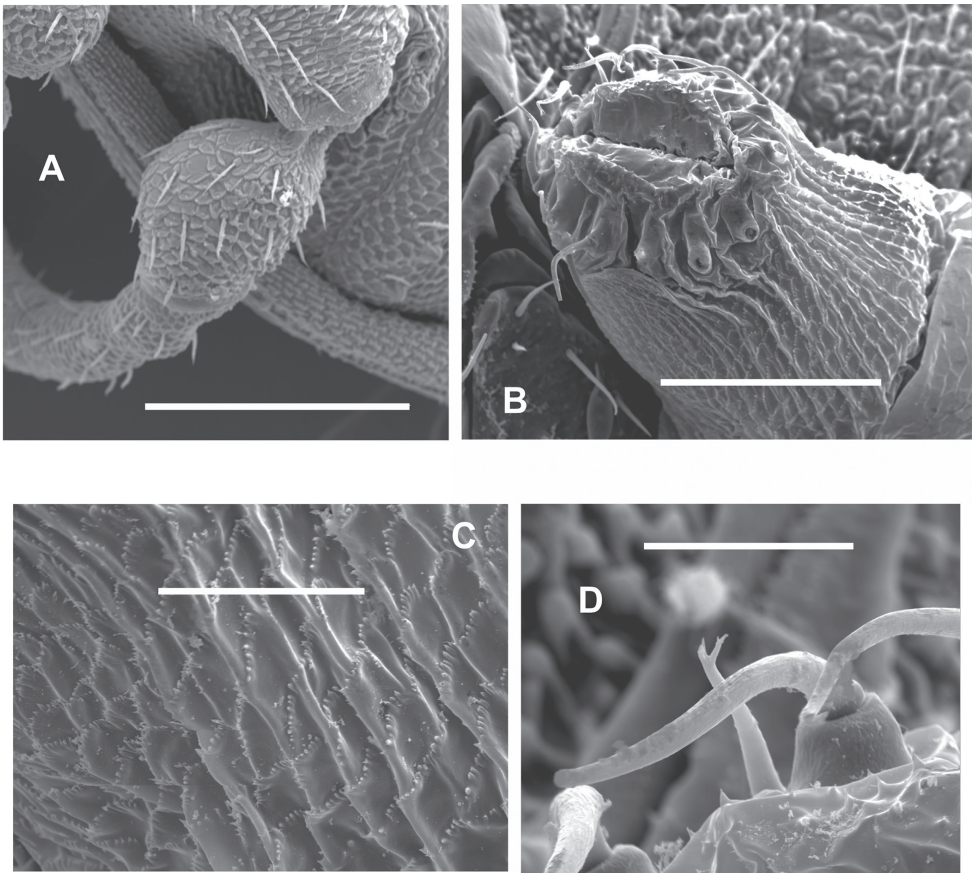


Figure 10. Female *Sitalcina oasiensis* (SDSU_TAC000290) – **A** TrIV **B** OV **C** OVM **D** OVS. Scale bar: 200 µm (**A**), 100 µm (**B**), 30 µm (**C**), 20 µm (**D**).

Sitalcina ubicki **DiDomenico & Hedin, sp. n.**
http://zoobank.org/ECA3E4F6-7182-4974-8875-5FFF30EDB6B6
Figures: map Figure 1; male Figure 11A–D; female Figure 12A–D

Type material. Holotype male (SDSU_TAC000216, CASENT 9029999) from California, Santa Barbara County, Santa Ynez Mtns, Montecito, E Mountain Drive, 0.4 mi NE from jct. with Cold Spring Road N34.45496°, W119.65288°, elev. ca. 230 m. Collected by A. DiDomenico, K. Emata, E. Garcia, A. Schönhofer, February 18, 2012.

Etymology. This species is named in honor of Darrell Ubick (CAS) whose foundational taxonomic research with *Sitalcina* made this project possible.

Diagnosis. Similar to other members of the northern coastal clade (*S. sura*, *S. seca*, *S. chalona*) in body form, relatively large-bodied, with a tall and pointed, tuberculate EM. Male TrIV spur curved, penis PSL similar to but distinguishable from *S. chalona*.

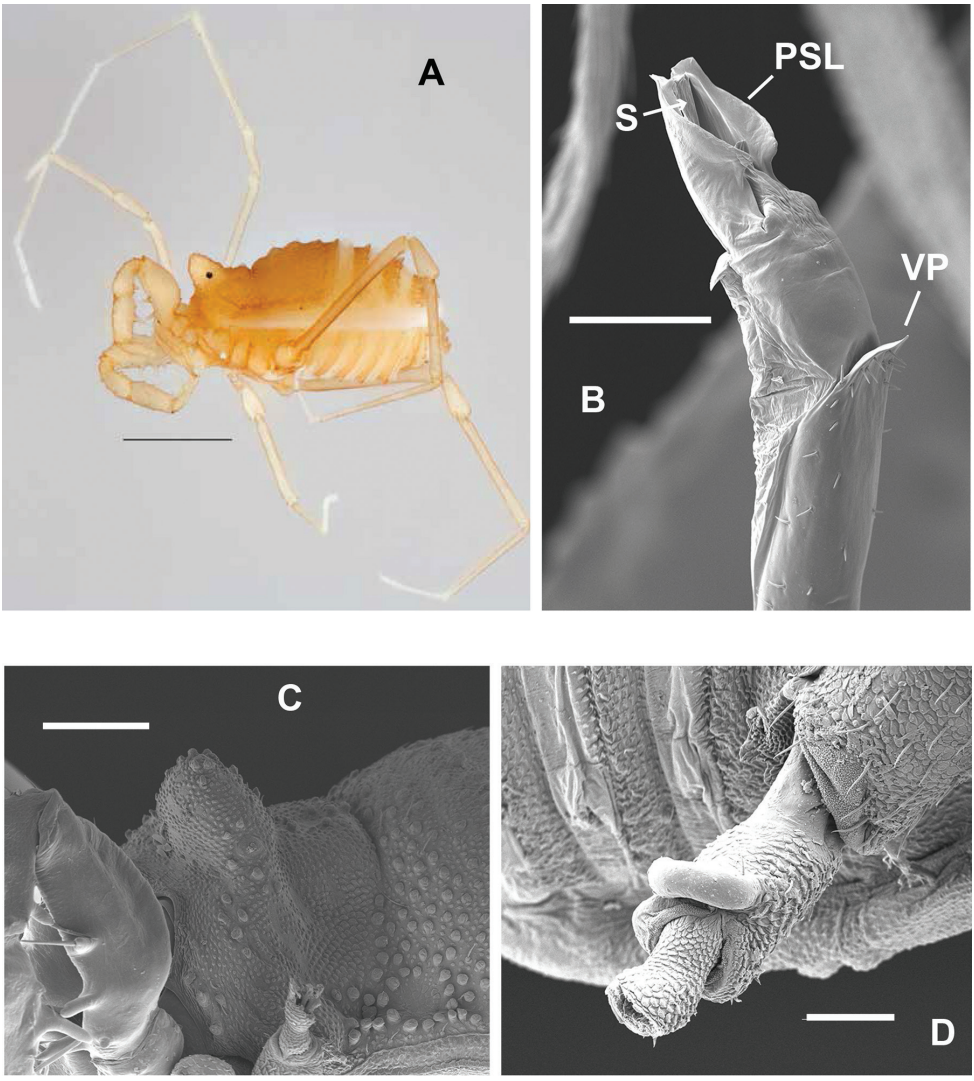


Figure 11. Male *Sitalcina ubicki* (ETOH - SDSU_TAC000216, SEM prep - SDSU_TAC000291) – **A** habitus **B** penis, stylus at arrow **C** EM **D** TrIV. Scale bar: 1 mm (**A**), 200 µm (**C**), 100 µm (**B, D**).

Genetic data. GenBank Accession numbers: KX064794, KX064795, KX064822, KX064823, KX064850, KX064851, KX064873, KX064890, KX064891, KX064916, KX064917, KX064946, KX064947

Description. Integument color light orange with lighter appendages. Body finely rugose with larger tubercles along tergal margins and anteriorly on EM; 3-4 pairs of AT. EM tall, slightly pointed, eyes present. Palpal Fm with median dorsobasal row of 4 asetose tubercles and one small mesal tubercle. Palpal megaspines: trochanter 2 ventral; Fm 3 ventrobasal, one mesodistal; patella 2 mesal, one ectal; tibia and tarsus 2 mesal, 2 ectal. TC 3-5-5-5.

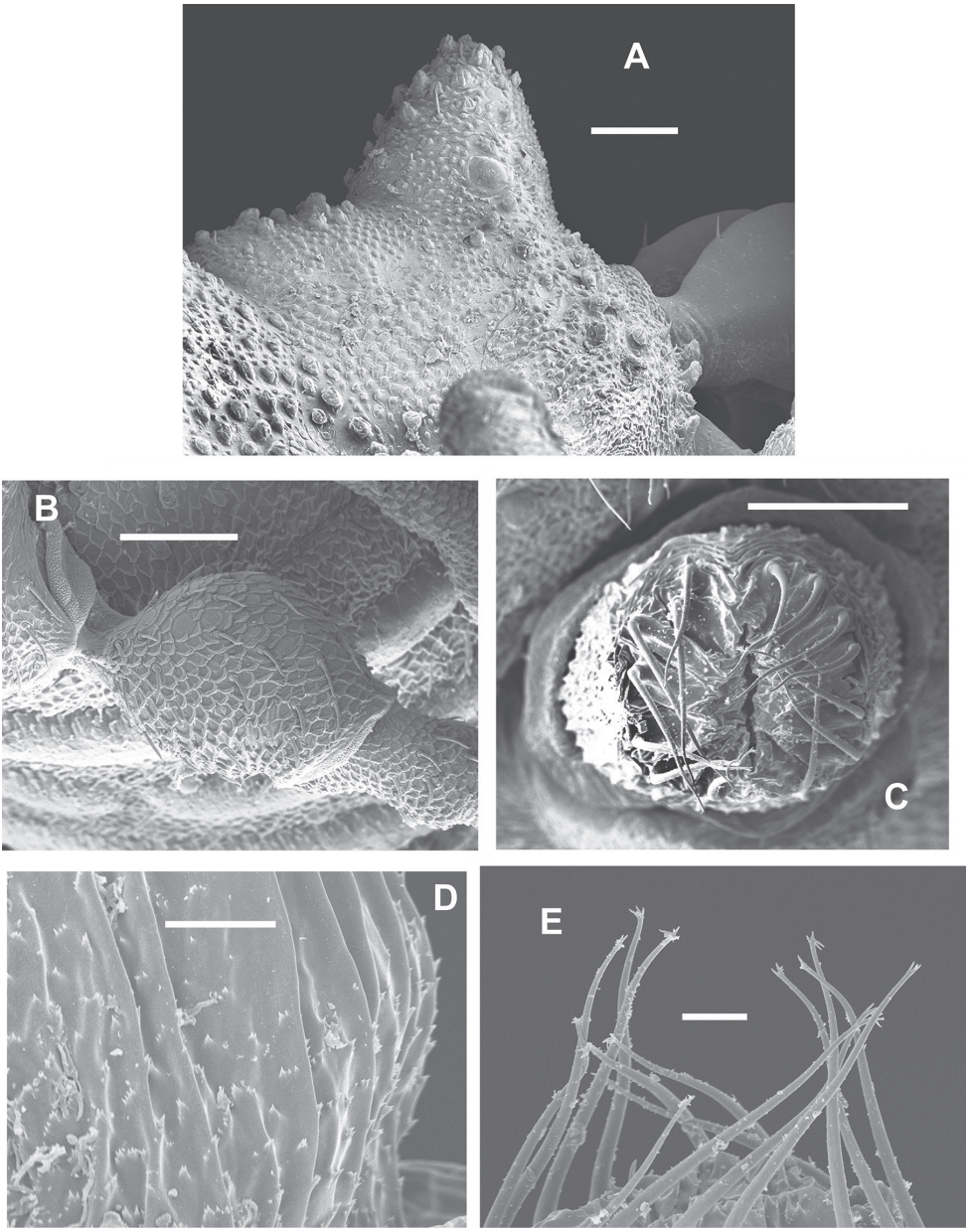


Figure 12. Female *Sitalcina ubicki* (SDSU_TAC000292) – **A** EM **B** TrIV **C** OV **D** OVM **E** OVS. Scale bar: 100 μm (**A, B, C**), 20 μm (**D, E**).

MALE. Holotype (paratypes SDSU_TAC000300, SDSU_TAC000301, SDSU_TAC000302): BL 2.1 (1.68–2.4). SL 1.14 (1.15–1.4), SW 1.3 (1.2–1.4). EM width 0.33 (0.35–0.4), height 0.23 (0.25–0.35). GO length 0.21, width 0.21. Leg II length 5.16 (4.2–5.12), Leg II/Scute Length 4.52 (3.14–3.66). TrIV spur short, curved. Penis

VP entire, apically pointed, with 12 pairs of setae, AS absent; glans DL quadrate; PSL simple, rounded at apical end; S thick.

FEMALE. Paratypes SDSU_TAC000207 (SDSU_TAC000303, SDSU_TAC000304): BL 1.88 (1.7–2.0). SL 1.36 (1.0–1.25), SW 1.4 (1.25–1.35). EM width 0.36 (0.35), height 0.36 (0.2–0.25). Leg II length 4.48 (3.75–4.4), Leg II/Scute Length 3.29 (3.0–3.52). Slightly imbricate OVM, 6 pairs of OVS, straight, multifurcate.

Other material examined. See Suppl. Material 1: Table S1 for locality information of all specimens examined.

Distribution and habitat. Known only from the vicinity of Montecito, Santa Ynez Mountains, Santa Barbara County. Specimens were found under stones, in narrow ravine with stream, in a *Quercus* and *Platanus* forest.

Note. This population was mentioned by Ubick and Briggs (2008), but specimens were damaged and left undescribed. The new material collected for this study is from the same general vicinity as specimens studied by Ubick and Briggs (2008); northern edge of Montecito, off East Mountain Drive.

Discussion

Sitalcina remains poorly known

Species limits in the *S. sura* group were first studied by Ubick and Briggs (2008) based on consideration of somatic and genital morphology. In the current study, these and other species hypotheses (Table 2) were formally tested using multilocus DNA sequence data and a Bayes Factor Delimitation approach. With two newly described species, *Sitalcina* now includes a dozen species, and we expect future research to uncover additional undescribed taxa. Ultimately, we predict that *Sitalcina* diversity might approach that observed in the phalangodid genus *Calicina*, a taxon that includes over 25 short-range endemic species from central and northern California (Ubick and Briggs 1989, Emata and Hedin 2016).

Our results imply that some described species (e.g., *S. peacheyi*, *S. flava*) may actually comprise multiple cryptic species. This pattern is expected in a morphologically conservative group that occupies naturally fragmented habitats. This prediction would also apply to the geographically widespread *S. californica* (see Ubick and Briggs 2008), which has an interesting disjunct distribution in California (Figure 1). We have also discovered new geographic populations (e.g., Glen Oaks, Palos Verdes) that may represent new species. For both new populations and potential cryptic species, future research implementing genomic-scale datasets would be informative. Finally, our results confirm highly localized geographic speciation in these sedentary animals. Examples include geographically adjacent *S. seca*, *S. sura*, and *S. chalona*, as well as *S. borregoensis* plus *S. oasiensis*. If we extrapolate this pattern of localized divergence to regions where *Sitalcina* is likely, but currently unknown, this suggests many additional species. If *Sitalcina* is distributed like "beads on a string", we are missing many of the beads.

Sitalcina specimens are sometimes very challenging to collect. Specimens only occur near the surface (i.e., under accessible rocks or woody debris) at certain times of the year, apparently migrating deep into the soil matrix as surface conditions dry (Ubick and Briggs 2008). This means that collections must take place in "good" years (e.g., non-drought years), in appropriate months, by experienced collectors in suitable microhabitats. An example is the Mountain Palm Springs population of *S. borregoensis*, which was first collected in 1967 (Ubick and Briggs 2008), but not recollected until 2012. Glen Oaks is another example, with only a single specimen collected despite over 3 hours of search time by a team of six experienced collectors. In this context, new populations of *Sitalcina* in poorly collected regions are likely, and we highlight the montane sky islands of northern Mexico and the desert canyons of southern California plus Baja California Norte as potential undiscovered diversification hotspots.

If multiple known species occur on the handful of sky-islands in southeastern Arizona, we expect additional undescribed species on the dozens of montane sky islands in northern Sonora. For many lineages, montane populations in southern Arizona constitute a "northern tip of a southern iceberg", with centers of distribution found in northern Mexico (e.g., montane jumping spiders - Maddison and McMahon 2000, montane rattlesnakes - Bryson et al. 2011). The desert canyons of southern California plus Baja California Norte constitute another distributional area with potentially high unknown diversity. This region is tectonically active. For example, the population at Mountain Palm Springs lays almost directly on the Elsinore Fault, while the Borrego Palm Canyon population inhabits the area between the San Felipe Fault and the more easterly San Andreas Fault (Steely et al. 2009). Furthermore, relictual canyon populations are expected to be strongly isolated by unsuitable xeric habitats, leading to active speciation. As an example, Bond (2012) described multiple species of cryptic trapdoor spider species from a small region in Anza-Borrego Desert State park, with each species known only from one or two locations. Similar desert canyon local endemics likely occur in *Sitalcina*.

Historical biogeography

Niche modeling reveals a conspicuous geographic gap between habitats that are suitable for *Sitalcina*, separating eastern habitats in Arizona from western habitats in California (Figure 7). This gap is not only consistent with biotic features that separate these regions (i.e., low elevation Sonoran desert), but also coincides with major modern day and historical biogeographic barriers. These biogeographic barriers include marine incursions into the Salton Trough associated with the opening of the Gulf of California (ca. 6.3 Ma, Oskin and Stock 2003, Dolby et al. 2015), and the more recent drainage of the Colorado River into the Gulf (ca. 4.1 Ma, Dorsey et al 2007, Dolby et al. 2015). Moreover, populations on either side of this biogeographic gap are currently found on different continental plates – all *S. sura* group populations from California are found on the Pacific plate, while all populations from Arizona are found on the North American plate (we note here that Anza-Borrego canyon populations are close

to plate boundaries, further discussed below). For these reasons, a logical prediction would include a primary phylogenetic division in this region, resulting in California versus Arizona clades. Major phylogenetic or phylogeographic splits coincident with the Colorado River (or Salton Trough) have been found in many animal taxa (e.g., Riddle et al 2000, Riddle and Hafner 2006, Crews and Hedin 2006), although we emphasize that these studies have focused mostly on low elevation desert species.

We failed to recover this predicted pattern, but instead recovered a well-supported phylogenetic pattern in both gene and species trees that includes the separation of *S. sura* group members into coastal versus desert clades (Figures 3, 4, 6). Here, southern Californian populations from the Anza Borrego desert region are phylogenetically allied with Arizonan populations, rather than with geographically proximate Californian populations. Because all lines of evidence indicate that *Sitalcina* is dispersal-limited, we hypothesize that vicariance has dominated the biogeographic history of this lineage. Below we present a biogeographic model for *Sitalcina* that emphasizes the role of plate tectonics and habitat connections to elements of a MTG.

The fundamental spatial premise of our model is that the *S. sura* group has a center of origin in northwestern Mexico, perhaps centered around the region which is today the northern Gulf of California. The fundamental temporal premise is that initial diversification in the group happened before the Pacific plate began to migrate actively northwestwards (along the San Andreas Fault system) against a stationary North American plate (Figure 13). Geological evidence indicates older rifting beginning as early as ca. 30 Ma in northwestern Mexico, with more active northwestern plate movement sometime after ca. 12.3 Ma (Atwater and Stock 1998, Dolby et al. 2015). Furthermore, our model postulates initial diversification along a west to east axis, with westerly coastal clade members (plus *S. lobata* and *S. californica*), and easterly desert clade members. Importantly, this eastern lineage is hypothesized to have straddled both continental plates prior to active plate movements (Figure 13). Our estimates for the overall age of the *S. sura* group, and for the tMRCA of desert versus coastal clades are generally consistent with this predominantly Miocene timeframe. Also, estimated times for the separation of CA canyon taxa from remaining desert clade members are consistent with the evolution of the Gulf of California (tMRCA of 7.09, Figure 6B, versus ca. 6.3 Ma, Oskin and Stock 2003, Dolby et al. 2015).

Our model has interesting connections to the botanical literature. As noted in the Introduction, most members of the *S. sura* group (and *Sitalcina* more generally) are found in habitats dominated by sclerophyllous woody plant taxa (e.g., *Quercus*, Pinyon pine, *Arctostaphylos*, *Ceanothus*, etc.). These plant lineages are part of a MTG, as originally defined by Raven and Axelrod (1978). Although this concept was invoked prior to modern phylogenetic analyses, recent molecular studies have generally supported core aspects of this idea (Lancaster and Kay 2013, Baldwin 2014). Important properties of plant lineages included in this flora include southwestern ancestry, deepest divergences occurring in the Oligocene and/or Miocene (e.g., Lancaster and Kay 2013, figure 2), a proposed role for plate tectonics (e.g., Raven and Axelrod 1978, fig. 4), and biogeographic connections between California and Arizona upland taxa (Raven and Axelrod

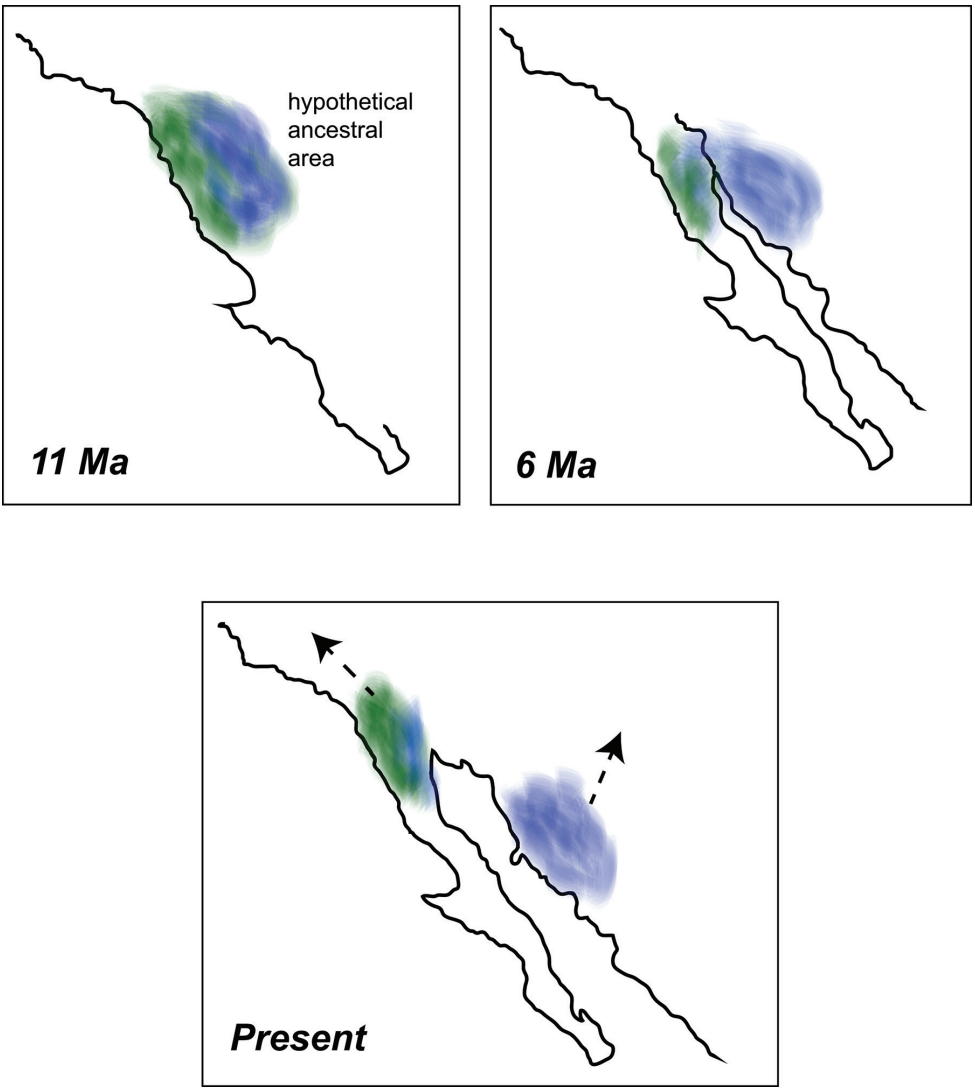


Figure 13. Biogeographic model. General timeframe and plate positions follow Dolby et al. (2015). The labeled "hypothetical ancestral area" is only approximate; arrows indicate present distribution of lineages.

1978, table 7). These aspects of the MTG are consistent with our biogeographic model. Another interesting parallel is that many Madro-Tertiary taxa now found in coastal southern California are hypothesized phylogenetic relicts, found only in suitable habitats directly adjacent to a marine influence (Raven and Axelrod 1978). In this regard, we note that coastal clade *S. sura* group species are all found in moist mountains with direct marine influence (e.g., Palos Verdes peninsula, Santa Monica Mountains, Santa Ynez Mountains, Santa Lucia Range), with the single exception of *S. chalona*.

Several predictions can be derived from our biogeographic model. First, we expect undescribed species in upland habitats of western Sonora, some of which may be related to California desert canyon taxa. Second, we predict undiscovered populations in desert canyons of northern Baja, and again predict possible mixed phylogenetic affinities (some coastal clade, some desert clade). Third, we predict that northern populations of *S. californica* on the North American plate (north of San Francisco) should be phylogenetically derived from southern populations on the Pacific plate (Figure 1). Finally, if our model has generality, we predict that future studies of other regional dispersal-limited animal lineages will show similar biogeographic patterns.

Both California and deserts of the American southwest are active areas for modern biogeographic research in animals. Despite this fact, we have found relatively few animal taxa that show biogeographic patterns similar to those observed in *Sitalcina*. Salamanders in the *Batrachoseps pacificus* group have a distribution much like the coastal clade (from Monterey south to northern Baja, almost all populations restricted to Pacific plate, Jockusch et al. 2014), and the group includes isolated southern California desert populations, but these populations have apparent western ancestry (Martínez-Solano et al. 2012,). *Aptostichus* trapdoor spiders include many California coastal, California desert canyon, and Arizona sky island species (Bond 2012), but phylogenetic details remain uncertain. One compelling parallel is found in night lizards (*Xantusia*). Leavitt et al. (2007) found that taxa from mostly transmontane southern California and northern Baja (including *X. wigginsi*, "San Jacinto" Clade, and "Yucca Valley" Clade) are sister to *X. bezyi* from uplands of central Arizona. Western species are found in pinyon-juniper and desert chaparral, while eastern *X. bezyi* occur in desert chaparral. These lizard taxa are hypothesized to have diverged 6.4 Ma, coincident with the evolution of the Gulf of California (Leavitt et al. 2007). All of these biogeographic patterns are similar to those observed in the *Sitalcina* desert clade.

Conclusions

We have uncovered an apparently novel phylogenetic pattern in a biogeographically well-studied region. Our biogeographic model can be tested with additional research, both in *Sitalcina*, but also in other plant and animal lineages with similar geographic distributions. Ubick and Briggs (2008) suggested that *Sitalcina* likely included additional undescribed species – our data support this contention, and suggest even more undiscovered richness. Basically all members of the *S. sura* group have very small geographic distributions, and many represent apparently old lineages found in often disturbed habitats. Examples include the Palos Verdes population, known only from a single impacted canyon in a matrix of urban development. Similarly, the two known species from Anza-Borrego Desert State Park are each known only from single desert canyons, both located in close proximity to popular hiking trails. These short-range endemic taxa should receive more conservation attention than currently afforded (Harvey 2002, Harvey et al. 2011).

Acknowledgements

We would like to thank Darrell Ubick and Thomas Briggs for their foundational work on *Sitalcina*, and expertise when needed for this project. We would also like to thank Dave Carlson, Alphonse DiDomenico, Kristen Emata, Erika Garcia, Casey Richart, Axel Schönhofer, Daniel Sitzmann, and Erik Stiner for their help with specimen collection. Sophia Bejarano, Alexa Feist, and Jeanne Rahe provided lab assistance, Joshua Coleman and Erik Ciaccio helped with image editing. Steve Barlow provided SEM support at SDSU. Shahan Derkarabetian helped to collect specimens, worked with ENM files, and edited the manuscript. Dean Leavitt clarified biogeographic patterns in *Xantusia*, and provided comments that greatly improved the manuscript. Ronald Clouse, Adriano Kury, Darrell Ubick and Gonzalo Giribet provided additional comments that improved the manuscript. Research was funded by grants to AD from the American Arachnological Society, Community Foundation Desert Research Grant, and the Anza-Borrego Foundation Howie Wier Memorial Conservation Grant, and an NSF grant awarded to MH (DEB 1354558).

References

Atwater T, Stock J (1998) Pacific-North America plate tectonics of the Neogene southwestern United States: an update. International Geology Review 40: 375–402. doi: 10.1080/00206819809465216

Aylor DL, Price EW, Carbone I (2006) SNAP: Combine and map modules for multilocus population genetic analysis. Bioinformatics 22: 1399–1401. doi: 10.1093/bioinformatics/btl136

Baldwin BG (2014) Origins of plant diversity in the California floristic province. Annual Review of Ecology, Evolution, and Systematics 45: 347–69. doi: 10.1146/annurev-ecolsys-110512-135847

Banks N (1893) The Phalangida Mecostethi of the United States. Transactions of the American Entomological Society 20: 149–152.

Banks N (1911) The Phalangida of California. Pomona College Journal of Entomology 3: 412–421.

Bond JE (2012) Phylogenetic treatment and taxonomic revision of the trapdoor spider genus *Aptostichus* Simon (Araneae, Mygalomorphae, Euctenizidae). ZooKeys 252: 1–209. doi: 10.3897/zookeys.252.3588

Bond JE, Stockman AK (2008) An integrative method for delimiting cohesion species: Finding the population-species interface in a group of Californian trapdoor spiders with extreme genetic divergence and geographic structuring. Systematic Biology 57: 628–646. doi: 10.1080/10635150802302443

Briggs TS (1968) Phalangids of the laniatorid genus *Sitalcina* (Phalangodidae: Opiliones). Proceedings of the California Academy of Sciences 36: 1–32.

Briggs TS (1971) The harvestmen of family Triaenonychidae in North America (Opiliones). Occasional Papers of the California Academy of Sciences 90: 1–43.

Bryson RW, Murphy RW, Graham MR, Lathrop A, Lazcano-Villareal D (2011) Ephemeral Pleistocene woodlands connect the dots for highland rattlesnakes of the *Crotalus intermedius* group. Journal of Biogeography 38: 2299–2310. doi: 10.1111/j.1365-2699.2011.02565.x

Bryson Jr RW, Riddle BR, Graham MR, Smith BT, Prendini L (2013) As old as the hills: Montane scorpions in southwestern North America reveal ancient associations between biotic diversification and landscape history. PLoS ONE 8: e52822. doi: 10.1371/journal.pone.0052822

Carstens BC, Pelletier TA, Reid NM, Satler JD (2013) How to fail at species delimitation. Molecular Ecology 22: 4369–4383. doi: 10.1111/mec.12413

Crews SC, Hedin M (2006) Studies of morphological and molecular phylogenetic divergence in spiders (Araneae: *Homalonychus*) from the American southwest, including divergence along the Baja California Peninsula. Molecular Phylogenetics and Evolution 38: 470–487. doi: 10.1016/j.ympev.2005.11.010

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. doi: 10.1038/nmeth.2109

Derkarabetian S, Steinmann DB, Hedin M (2010) Repeated and time-correlated morphological convergence in cave-dwelling harvestmen (Opiliones, Laniatores) from montane western North America. PLoS ONE 5(5): e10388. doi: 10.1371/journal.pone.0010388

Derkarabetian S, Ledford J, Hedin M (2011) Genetic diversification without obvious genitalic morphological divergence in harvestman (Opiliones, Laniatores, *Sclerobunus robustus*) from montane sky islands of western North America. Molecular Phylogenetics and Evolution 61: 844–853. doi: 10.1016/j.ympev.2011.08.004

Derkarabetian S, Hedin M (2014) Integrative taxonomy and species delimitation in harvestmen: a revision of the Western North America genus *Sclerobunus* (Opiliones: Laniatores: Travunioidea). PLoS ONE 9: e104982. doi: 10.1371/journal.pone.0104982

Derkarabetian S, Burns M, Starrett J, Hedin M (2016) Population genomic evidence for multiple refugia in a montane-restricted harvestman (Arachnida, Opiliones, *Sclerobunus robustus*) from the southwestern United States. Molecular Ecology, submitted.

Dolby GA, Bennett SEK, Lira-Noriega A, Wilder BT, Munguia-Vega A (2015) Assessing the geological and climatic forcing of biodiversity and evolution surrounding the Gulf of California. Journal of the Southwest 57: 391–455. doi: 10.1353/jsw.2015.0005

Dorsey RJ, Fluette A, McDougall KA, Housen BA, Janecke SU, Axen GJ, Shirvell CR (2007) Chronology of Miocene-Pliocene deposits at Split Mountain Gorge, southern California: A record of regional tectonics and Colorado River evolution. Geology 35: 57–60. doi: 10.1130/G23139A.1

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973. doi: 10.1093/molbev/mss075

Earl DA (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4: 359–361. doi: 10.1007/s12686-011-9548-7

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. doi: 10.1093/nar/gkh340

Emata KN, Hedin M (2016) From the mountains to the coast and back again: Ancient biogeography in a radiation of short-range endemic harvestmen from California. Molecular Phylogenetics and Evolution 989: 233–243. doi: 10.1016/j.ympev.2016.02.002

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x

Fujita MK, Leaché AD, Burbrink FT, McGuire JA, Moritz C (2012) Coalescent- based species delimitation in an integrative taxonomy. Trends in Ecology and Evolution 27: 480–488. doi: 10.1016/j.tree.2012.04.012

Goodnight CJ, Goodnight ML (1942) New Phalangodidae (Phalangida) from the United States. American Museum Novitates 1188: 1–18.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-seq data without a reference genome. Nature Biotechnology 29: 644–52. doi: 10.1038/nbt.1883

Grummer JA, Bryson Jr. RW, Reeder TW (2014) Species delimitation using Bayes factors: simulations and application to the *Sceloporus scalaris* species group (Squamata: Phrynosomatidae). Systematic Biology 63: 119–133. doi: 10.1093/sysbio/syt069

Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52: 696–704. doi: 10.1080/10635150390235520

Harvey MS (2002) Short-range endemism the Australian fauna: some examples from non-marine environments. Invertebrate Systematics 16: 555–570. doi: 10.1071/IS02009

Harvey MS, Rix MG, Framenau VW, Hamilton ZR, Johnson MS, Teale RJ, Humphreys G, Humphreys WF (2011) Protecting the innocent: studying short-range endemic taxa enhances conservation outcomes. Invertebrate Systematics 25: 1–10. doi: 10.1071/IS11011

Hedin M, Thomas SM (2010) Molecular systematic of eastern North American Phalangodidae (Arachnida: Opiliones: Laniatores), demonstrating convergent morphological evolution in caves. Molecular Phylogenetics and Evolution 54: 107–121. doi: 10.1016/j.ympev.2009.08.020

Hedin M, Starrett J, Akhter S, Schönhofer AL, Shultz JW (2012) Phylogenomic resolution of paleozoic divergences in harvestmen (Arachnida, Opiliones) via analysis of next-generation transcriptome data. PLoS ONE 7: e42888. doi: 10.1371/journal.pone.0042888

Hedin M, Carlson D, Coyle F (2015) Sky island diversification meets the multispecies coalescent – divergence in the spruce-fir moss spider (*Microhexura montivaga*, Araneae, Mygalomorphae) on the highest peaks in southern Appalachia. Molecular Ecology 24: 3467–3484. doi: 10.1111/mec.13248

Hijmans RJ, Guarino L, Bussink C, Mathur P, Cruz M, Barrentes I, Rojas E (2004) DIVA-GIS. Vsn. 5.0. A geographic information system for the analysis of species distribution data. http://www.diva-gis.org

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965–1978. doi: 10.1002/joc.1276

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23: 1801–1806. doi: 10.1093/bioinformatics/btm233

Jockusch EL, Martínez-Solano Í, Timpe EK (2014) The effects of inference method, population sampling, and gene sampling on species tree inferences: An empirical study in slender salamanders (Plethodontidae: *Batrachoseps*). Systematic Biology 64: 66–83. doi: 10.1093/sysbio/syu078

Kass RE, Raftery AE (1995) Bayes factors. Journal of the American Statistical Association 90: 773–795. doi: 10.1080/01621459.1995.10476572

Katoh D, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. doi: 10.1093/molbev/mst010

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. doi: 10.1093/bioinformatics/bts199

Keith R, Hedin M (2012) Extreme mitochondrial population subdivision in southern Appalachian paleoendemic spiders (Araneae: Hypochilidae: *Hypochilus*), with implications for species delimitation. Journal of Arachnology 40: 167–181. doi: 10.1636/A11-49.1

Kury AB (2013) Order Opiliones Sundevall, 1833. In: Zhang Z-Q (Ed.) Animal Biodiversity: An outline of higher–level classification and survey of taxonomic richness. Zootaxa 3703: 27–33. doi: 10.11646/zootaxa.3703.1.7

Lancaster LT, Kay KM (2013) Origin and diversification of the California flora: re-examining classic hypotheses with molecular phylogenies. Evolution 67: 1041–1054. doi: 10.1111/evo.12016

Lartillot N, Philippe H (2006) Computing Bayes factors using thermodynamic integration. Systematic Biology 55: 195–207. doi: 10.1080/10635150500433722

Leavitt DH, Bezy RL, Crandall KA, Sites Jr. JW (2007) Multi-locus DNA sequence data reveal a history of deep cryptic vicariance and habitat-driven convergence in the desert night lizard *Xantusia vigilis* species complex (Squamata: Xantusiidae). Molecular Ecology 16: 4455–4481. doi: 10.1111/j.1365-294X.2007.03496.x

Maddison WP, McMahon MM (2000) Divergence and reticulation among montane populations of the jumping spider *Habronattus pugillis* Griswold. Systematic Biology 49: 400–421. doi: 10.1080/10635159950127312

Martínez-Solano Í, Peralta-García A, Jockusch EL, Wake DB, Vázquez-Domínguez E, Parra-Olea G (2012) Molecular systematics of *Batrachoseps* (Caudata, Plethodontidae) in southern California and Baja California: mitochondrial-nuclear DNA discordance and the evolutionary history of *B. major*. Molecular Phylogenetics and Evolution 63: 131–149. doi: 10.1016/j.ympev.2011.12.026

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 N. 2010, New Orleans, LA, 1–8. doi: 10.1109/GCE.2010.5676129

Niemiller ML, Near TJ, Fitzpatrick BM (2012) Delimiting species using multilocus data: diagnosing cryptic diversity in the southern cavefish, *Typhlichthys subterraneus* (Teleostei: Amblyopsidae). Evolution 66: 846–866. doi: 10.1111/j.1558-5646.2011.01480.x

Oskin M, Stock JM (2003) Marine incursion synchronous with plate-boundary localization in the Gulf of California. Geology 31: 23–26. doi: 10.1130/0091-7613(2003)031<0023:MISWPB>2.0.CO;2

Papadopoulou A, Anastasiou I, Vogler AP (2010) Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. Molecular Biology and Evolution 27: 1659–1672. doi: 10.1093/molbev/msq051

Phillips SJ, Dudík M (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31: 161–175. doi: 10.1111/j.0906-7590.2008.5203.x

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. http://beast.bio.ed.ac.uk/Tracer

Rannala B, Yang Z (2013) Improved reversible jump algorithms for Bayesian species delimitation. Genetics 194: 245–253. doi: 10.1534/genetics.112.149039

Raven PH, Axelrod DI (1978) Origin and relationships of the California flora. University of California Publications in Botany 72: 1–134.

Riddle BR, Hafner DJ, Alexander LF (2000) Evolution of Baileys' pocket mouse (*Chaetodipus baileyi*), comparative biogeography of *C. baileyi* and the *Peromyscus eremicus* species-group, and historical vicariance of peninsular and continental deserts in western North America. Molecular Phylogenetics and Evolution 17: 161–172. doi: 10.1006/mpev.2000.0842

Riddle BR, Hafner DJ (2006) A step-wise approach to integrating phylogeographic and phylogenetic biogeographic perspectives on the history of a core North American warm deserts biota. Journal of Arid Environments 66: 435–461. doi: 10.1016/j.jaridenv.2006.01.014

Rissler LJ, Apodaca JJ (2007) Adding more ecology into species delimitation: Ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). Systematic Biology 56: 924–942. doi: 10.1080/10635150701703063

Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2011) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. doi: 10.1093/sysbio/sys029

Rosenberg NA (2004) *Distruct*: a program for the graphical display of population structure. Molecular Ecology Notes 4: 137–138. doi: 10.1046/j.1471-8286.2003.00566.x

Starrett J, Derkarabetian S, Richart CH, Cabrero A, Hedin M (2016) A new monster from southwest Oregon forests: *Cryptomaster behemoth* sp. n. (Opiliones, Laniatores, Travunioidea). ZooKeys 555: 11–35. doi: 10.3897/zookeys.555.6274

Steely AN, Janecke SU, Dorsey RJ, Axen GJ (2009) Early Pleistocene initiation of the San Felipe fault zone, SW Salton Trough, during reorganization of the San Andreas fault system. GAS Bulletin 121: 663–687. doi: 10.1130/B26239.1

Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. The American Journal of Human Genetics 76: 449–462. doi: 10.1086/428594

Stephens M, Smith N, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. The American Journal of Human Genetics 68: 978–989. doi: 10.1086/319501

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725–2729. doi: 10.1093/molbev/mst197

Thomas SM, Hedin M (2008) Multigenic phylogeographic divergence in the paleoendemic southern Appalachian opilionid *Fumontana deprehendor* Shear (Opiliones, Laniatores, Triaenonychidae). Molecular Phylogenetics and Evolution 46: 645–658. doi: 10.1016/j.ympev.2007.10.013

Ubick D (2007) Phalangodidae. In: Pinto-da-Rocha R, Machado M, Giribet G (Eds) Harvestmen, The Biology of Opiliones, Harvard University Press, Cambridge and London, 217–221.

Ubick D, Briggs TS (1989) The harvestmen family Phalangodidae. 1. The new genus *Calicina*, with notes on *Sitalcina* (Opiliones: Laniatores). Proceedings of the California Academy of Sciences 46: 95–136.

Ubick D, Briggs TS (2008) The harvestman family Phalangodidae 6. Revision of the *Sitalcina* complex (Opiliones, Laniatores). Proceedings of the California Academy of Sciences 59: 1–108.

Wiens JJ, Graham CH (2005) Niche conservatism: integrating evolution, ecology, and conservation biology. Annual Review of Ecology, Evolution, and Systematics 36: 519–539. doi: 10.1146/annurev.ecolsys.36.102803.095431

Wilm A, Mainz I, Steger G (2006) An enhanced RNA alignment benchmark for sequence alignment programs. Algorithms for Molecular Biology 1: 19. doi: 10.1186/1748-7188-1-19

Xie WG, Lewis PO, Fan Y, Kuo L, Chen MH (2011) Improving marginal likelihood estimation for Bayesian phylogenetic model selection. Systematic Biology 60: 150–160. doi: 10.1093/sysbio/syq085

Yang Z, Rannala BR (2010) Bayesian species delimitation using multilocus sequence data. Proceedings of the National Academy of Sciences 107: 9264–9269. doi: 10.1073/pnas.0913022107

Supplementary material 1

Supplementary Tables 1–3
Authors: Angela DiDomenico, Marshal Hedin
Data type: Excel Table
Explanation note:
Table S1. Voucher and locality data
Table S2. PCR primer and reaction information
Table S3. GenBank accession information
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.

ZooKeys 586: 37–93 (2016)
doi: 10.3897/zookeys.586.6774
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

New species of *Austropurcellia*, cryptic short-range endemic mite harvestmen (Arachnida, Opiliones, Cyphophthalmi) from Australia's Wet Tropics biodiversity hotspot

Katya R. Jay[1], Zachary R. Popkin-Hall[2], Michelle J. Coblens[1], Jill T. Oberski[1], Prashant P. Sharma[3], Sarah L. Boyer[1]

1 *Macalester College, Saint Paul, United States of America* **2** *Texas A&M University, College Station, United States of America* **3** *University of Wisconsin, Madison, USA*

Corresponding author: *Sarah L. Boyer* (boyer@macalester.edu)

Academic editor: *A. Kury* | Received 6 October 2015 | Accepted 8 March 2016 | Published 4 May 2016

http://zoobank.org/A8926908-7D99-452D-BFD3-A8970561F317

Citation: Jay KR, Popkin-Hall ZR, Coblens MJ, Oberski JT, Sharma PP, Boyer SL (2016) New species of *Austropurcellia*, cryptic short-range endemic mite harvestmen (Arachnida, Opiliones, Cyphophthalmi) from Australia's Wet Tropics biodiversity hotspot. ZooKeys 586: 37–93. doi: 10.3897/zookeys.586.6774

Abstract

The genus *Austropurcellia* is a lineage of tiny leaf-litter arachnids that inhabit tropical rainforests throughout the eastern coast of Queensland, Australia. The majority of their diversity is found within the Wet Tropics rainforests of northeast Queensland, an area known for its exceptionally high levels of biodiversity and endemism. Studying the biogeographic history of limited-dispersal invertebrates in the Wet Tropics can provide insight into the role of climatic changes such as rainforest contraction in shaping rainforest biodiversity patterns. Here we describe six new species of mite harvestmen from the Wet Tropics rainforests, identified using morphological data, and discuss the biogeography of *Austropurcellia* with distributions of all known species. With this taxonomic contribution, the majority of the known diversity of the genus has been documented.

Keywords

Queensland, rainforest, biogeography, morphology, taxonomy

Copyright *Katya R. Jay et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

The mite harvestmen (order Opiliones, suborder Cyphophthalmi) are a globally distributed suborder of tiny (1.5–5 mm), cryptic arachnids that are extremely dispersal-limited, making them ideal for fine-scale historical biogeographic studies. Nearly all species are known from pristine leaf-litter habitats in tropical, subtropical, and temperate forests, with a few others from caves (Juberthie 1971, Giribet et al. 2012) . The mite harvestmen that are endemic to the Wet Tropics World Heritage Area (WT) of Queensland, in northeast Australia, are members of the genus *Austropurcellia* Juberthie, 1988, with a range spanning the WT in the north to the Queensland-New South Wales border in the south. The highest diversity of species is found in the rainforests of the WT (Figs 1–4). *Austropurcellia* is a member of the family Pettalidae Shear, 1980, a lineage with a classical temperate Gondwanan distribution that includes species from Chile, South Africa, Madagascar, Sri Lanka, Western Australia, and New Zealand (Boyer and Giribet 2007). Phylogenetic analyses of this group have demonstrated monophyly of all Queensland mite harvestmen (Boyer and Giribet 2007, Giribet et al. 2012, Boyer et al. 2015). Therefore, all Queensland species were transferred to *Austropurcellia* by Boyer and Giribet (2007), including species originally described as members of the genera *Neopurcellia* Forster, 1948 and *Rakaia* Hirst, 1925, whose type species occur in New Zealand.

Prior to 2012, only five *Austropurcellia* species were known (four from the WT and one from Central Queensland). Thus, little was known about the evolutionary history of the genus and its diversity in the region. Subsequently, Boyer and Reuter (2012) described four new species of mite harvestmen from the WT, and Popkin-Hall and Boyer (2014) described three new species from southeast Queensland. Boyer et al. (2015) presented six new species in a phylogenetic study of *Austropurcellia*, providing further insight into the historical biogeography of the genus. Together, these new species expanded *Austropurcellia*'s known range to cover most of Queensland's coast. After intensive examination of museum collections and a series of collecting campaigns by the authors and collaborators, there are currently 19 described species within *Austropurcellia*, including 15 species from the WT alone.

Austropurcellia is an ancient lineage, and its evolutionary history has no doubt been shaped by the turbulent geological and climatic history of the Australian continent. Molecular dating suggests that *Austropurcellia* underwent initial diversification in the late Cretaceous (Giribet et al. 2012, Giribet et al. in press). Since then, the genus has persisted despite significant climatic changes in the region. Following the separation of the Australian continent from East Antarctica and the establishment of the Antarctic Circumpolar Current (ACC) in the Oligocene, global cooling occurred and latitudinal temperature gradients steepened (Crisp et al. 2004, Byrne et al. 2008). Australia drifted north into warmer latitudes, partially offsetting the cooling effects of the ACC, leading to a drier and more seasonal climate by the onset of the Miocene (~23 Ma). Rainforest habitats suitable for *Austropurcellia* were widespread throughout the Australian continent during the early Miocene, before they were largely replaced

by sclerophyllous vegetation during a late Tertiary phase of long-term climate change and aridification (Adam 1992, Truswell 1993, Schneider et al. 1998, Crisp et al. 2004, Byrne et al. 2008). Miocene climatic changes have been invoked as a putative driver of speciation processes in other ancient Australian lineages. For example, phylogenetic and biogeographic analyses of Australian Archaeidae (assassin spiders), another limited-dispersal temperate Gondwanan arachnid group found in Queensland, point to evolutionary divergence as a result of Miocene aridification events (Rix and Harvey 2012). *Austropurcellia* provides a relevant point of comparison with this group, and work in preparation by the authors will examine tempo and age of speciation events within the genus.

The WT is considered to be a model system for studying biogeographic processes that shape rainforest diversity because it contains disproportionately large percentages of Australia's fauna (despite comprising only 0.12% of the continent by area), as well as unusually high rates of endemism (Nix 1991, Williams 2006, Rix and Harvey 2012). Palynological records from the WT suggest that significant range contractions and expansions of forest habitats have occurred as a result of more recent climate change. In particular, angiosperm rainforests were replaced by sclerophyllous or drier gymnosperm-dominated forests during Pleistocene glacial and interglacial cycles prior to the establishment of the current climate (Kershaw 1994, Graham et al. 2006, Bell et al. 2007). Rainforests have persisted in some areas of the WT much more consistently than others, leading to identification of potential species refugia by Webb and Tracey (1981), which take the form of small upland rainforest fragments scattered throughout areas of warmer and drier habitats (Schneider et al. 1998, Graham et al. 2006, Graham et al. 2010). Mite harvestmen only need small patches of suitable habitat to persist, and are thus able to survive these severe rainforest contraction events, making them an ideal group to study historical biogeography and speciation in the WT (e.g. Boyer et al. 2005, 2007b, Clouse and Giribet 2007, 2010, Boyer and Giribet 2009, Giribet et al. 2012, Boyer et al. 2015). Boyer et al. (2016) modeled suitable climatic conditions for *Austropurcellia* and projected them onto paleoclimate data layers from time slices going back to the Last Glacial Maximum (LGM). They found that differences in LGM climatic suitability across the WT were a strong predictor of present-day diversity, outperforming current climatic suitability. This suggests that the LGM climatic refugia acted as museums of biodiversity, preserving lineages during a restrictive climatic regime and shaping the distribution of biodiversity across the WT that is seen today.

Mite harvestmen are known to have very low dispersal rates, with species even in well-surveyed areas generally found in only a few localities within a 50-km radius (Boyer and Giribet 2009, Boyer et al. 2015, Clouse et al. 2016) (Figs 1–4). Previous phylogenetic and biogeographic work has indicated that different closely related groups of species within *Austropurcellia* occupy distinct geographic areas of the WT. As examples, species north of the Black Mountain Corridor (BMC), an area that experienced loss of rainforest habitat during the Last Glacial Maximum, such as *A. articosa* and *A. giribeti*, form a distinct clade. *Austropurcellia* from the north-central WT and central WT uplands regions comprise another species group (Boyer et al. 2015)

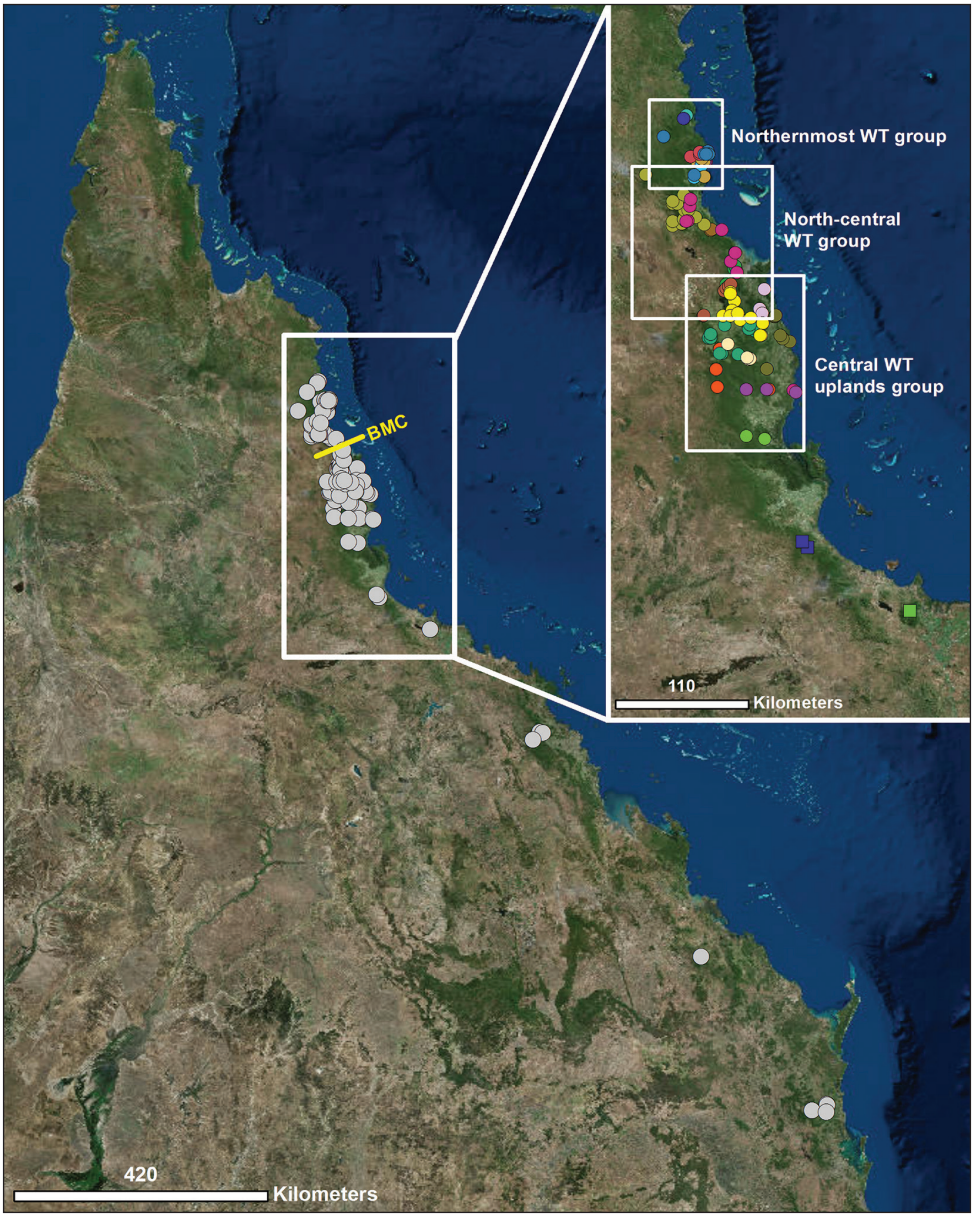


Figure 1. Distribution of all *Austropurcellia* species found throughout Queensland, Australia. Largest white box outlines the Wet Tropics World Heritage Area, shown in closer detail to the right. Smaller boxes within inset map represent groups of closely related species found within the Wet Tropics by Boyer et al. (2015), shown in larger detail in Figs 2, 3, & 4. Each circle denotes a locality and each colored circle denotes a different species found in one of the three relevant groups. Colored squares indicate the two species found in the southern Wet Tropics, which was excluded for the purposes of this study because it does not contain any of the new species presented.

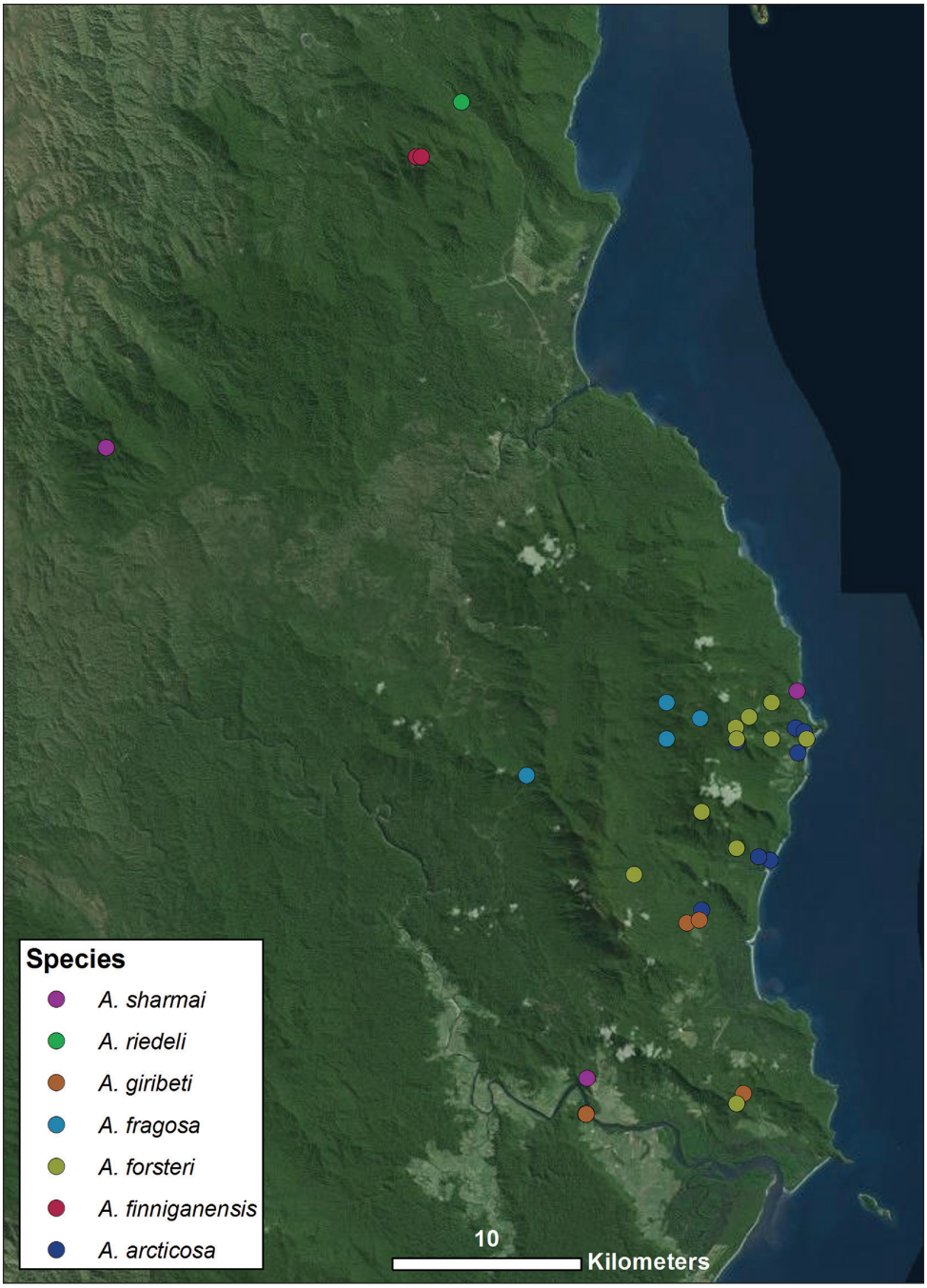


Figure 2. Distribution of all species found within northernmost WT group, corresponding with Fig. 1. Each colored icon denotes a different species, as indicated in the legend.

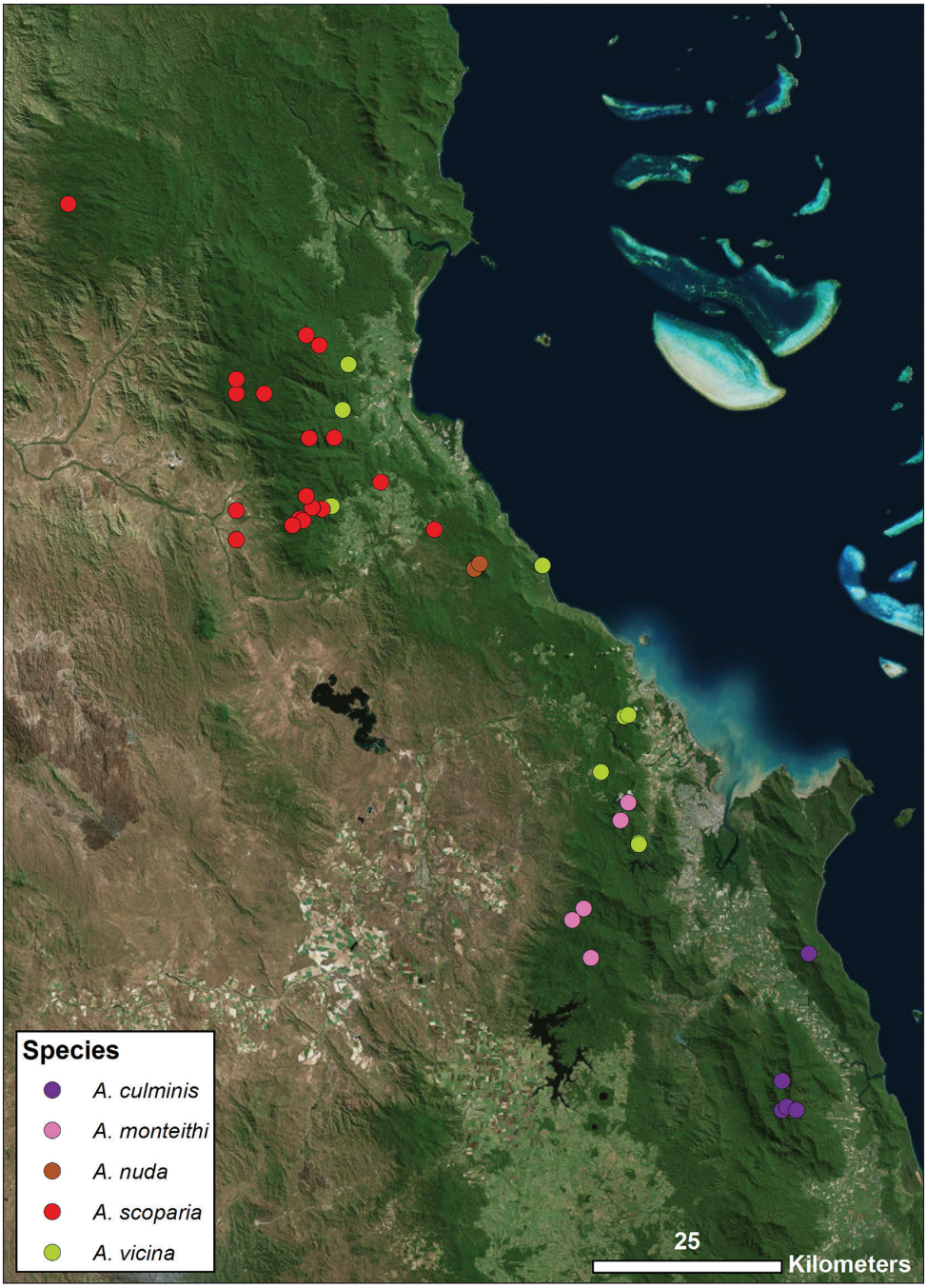


Figure 3. Distribution of all species found within north-central WT group, corresponding with Fig. 1. Each colored icon denotes a different species, as indicated in the legend.

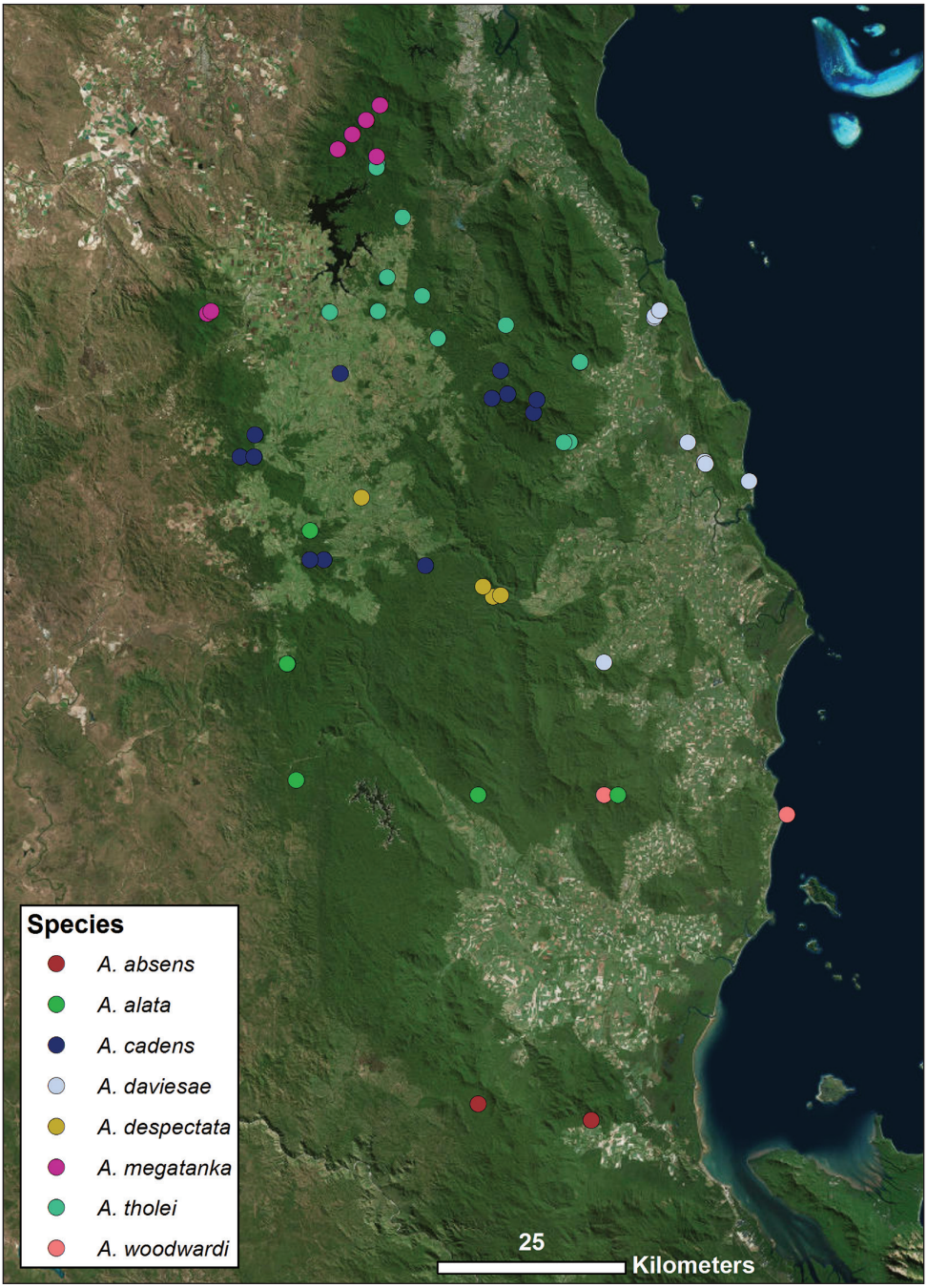


Figure 4. Distribution of all species found within central WT uplands group, corresponding with Fig. 1. Each colored icon denotes a different species, as indicated in the legend.

(Figs 1–4). Unique morphological features also tend to be shared between closely related species. Therefore, combining morphological and geographic data can provide reciprocally corroborative insights into the evolutionary history of the genus.

Here we present six new species of mite harvestmen from the WT that are morphologically distinct from other members of *Austropurcellia*. We identify several diagnostic characters that vary between groups of species whose ranges are geographically proximate, and use this information to form hypotheses about the new species' phylogenetic relationships.

Methods

Specimens were hand-collected by the authors and colleagues in the WT of Queensland, Australia by sifting leaf litter during 2011-2015 and preserved in 95% ethanol. Additional specimens were provided by collections from Harvard's Museum of Comparative Zoology (MCZ), the Queensland Museum (QM), and the Australian National Insect Collection (ANIC). GPS data were recorded at each locality.

Collected specimens were examined for morphological differences using light microscopy and sorted into putative morphospecies. Due to their small size and highly conserved morphology, species-level differences are often only visible using an SEM. Therefore, males from different localities were examined on a scanning electron microscope (SEM). Only males possess characters that are diagnostic at the level of species.

Holotype specimens were photographed using an Olympus SZX10 light microscope driven by Leica Acquire software (Leica Microsystems) at multiple focal planes. Image series were integrated using Helicon Focus (Helicon Soft Limited). Specimens were placed in hand sanitizer for lateral images.

Paratype males chosen for SEM were dissected under the light microscope and mounted on stubs. One of each walking leg (I-III) as well as one palp and one chelicera were removed and mounted on a single stub. Both legs IV were mounted to provide a lateral and medial view of distinguishing features. One female leg IV was also mounted for comparison. Males were mounted ventrally on another stub to allow for close examination of the anal plate and scopula, and remounted for examination of dorsal ornamentation. Stubs were coated with gold-palladium alloy using a Denton Vacuum Desk III sputter coater and imaged using a JEOL JSM-6610LV SEM. Appendage measurements were made using the digital scalar tool included in the JEOL software package. New species were diagnosed based on several key character systems that are demonstrably informative in *Austropurcellia* taxonomy: male anal plate shape, scopula size and shape, tarsus IV segmentation and shape, and adenostyle shape (Fig. 5) (Boyer and Reuter 2012, Boyer et al. 2015).

SEM images for new species were edited to have a uniform black background using Adobe Photoshop CS6 Extended and compiled into plates using Adobe Illustrator CS6. ArcGIS 10.2.1 was used to create distribution maps for species.

Taxonomy

All material examined is from Queensland, Australia.

Order OPILIONES Sundevall, 1833
Suborder CYPHOPHTHALMI Simon, 1879
Infraorder SCOPULOPHTHALMI Giribet, Sharma, Benavides, Boyer, Clouse, de Bivort, Dimitrov, Kawauchi, Murienne & Schwendinger, 2011
Family PETALLIDAE Shear, 1980

Genus *Austropurcellia* Juberthie, 1988

Type species. *Austropurcellia scoparia* Juberthie, 1988

Species included. *Austropurcellia absens* Boyer & Popkin-Hall, 2015, *A. acuta* Popkin-Hall & Boyer, 2014, *A. alata* Boyer & Reuter, 2012, *A. arcticosa* Cantrell, 1980, *A. barbata* Popkin-Hall & Boyer, 2014, *A. cadens* Baker & Boyer, 2015, *A. capricornia* Todd Davies, 1977, *A. clousei* Boyer, Baker & Popkin-Hall, 2015, *A. culminis* Boyer & Reuter, 2012, *A. daviesae* Juberthie, 1989, *A. despectata* Boyer & Reuter, 2012, *A. forsteri* Juberthie, 2000, *A. giribeti* Boyer & Quay, 2015, *A. scoparia* Juberthie, 1988, *A. sharmai* Boyer & Quay, 2015, *A. superbensis* Popkin-Hall & Boyer, 2014, *A. tholei* Baker & Boyer, 2015, *A. vicina* Boyer & Reuter, 2012, *A. woodwardi* Forster 1955.

***Austropurcellia finniganensis* Popkin-Hall, Jay & Boyer, sp. n.**
http://zoobank.org/33A77AC4-9D33-4DAA-A0C7-E6FC0C6FE311
Figs 6–11

Material examined. *Holotype.* Male (QM 102446), Mt. Finnigan (sample 1, AR4), 15.816°S, 145.280°E, coll. Alex Riedel 28.iv.2014.

Paratypes. 3 males, 3 females, same collecting data as holotype, QM 102447, Macalester SEM stubs M30.11, M30.12.

Additional material. 1 female, Mt. Finnigan (sample 2, AR3), 15.816°S, 145.278°E, coll. Alex Riedel 28.iv.2014, MCZ IZ 68947.

4 males, 5 females, 2 juveniles, Mt. Finnigan 37 km S Cooktown, 15.817°S, 145.283°E, coll. G. B. Monteith, D. Yeates, and D. Cook 22.iv.1982, QM berlesate 401A, Macalester SEM stubs M8.1, M8.2.

3 males, 1 female, Mt. Finnigan Summit, 15.817°S, 145.283°E, coll. G. B. Monteith 21.xi.1998, QM berlesate 981, Macalester SEM stubs M6.11, M6.12.

Diagnosis. Distinguished from congeners by very short, round scopula emerging from posterior quarter of fully granulated anal plate. Distinctive lack of granulation on sutures of dorsal scutum, including medial sulcus.

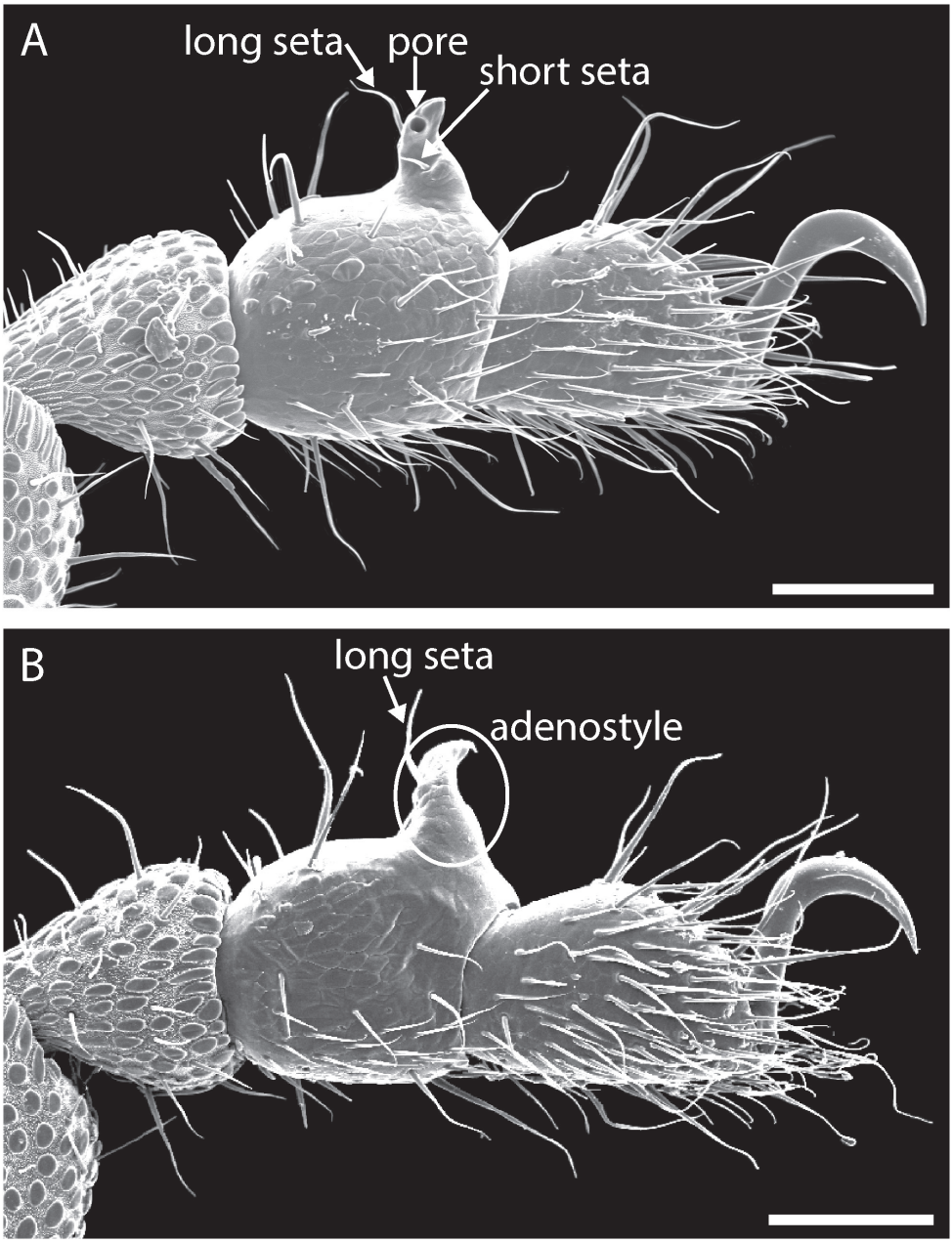


Figure 5. *Austropurcellia megatanka* sp. n., tarsus and metatarsus IV with diagnostic features labeled. **A** male tarsus and metatarsus IV, lateral view, showing adenostyle pore and setae **B** male tarsus and metatarsus IV, medial view, showing adenostyle and long seta. Scale bars: 100 μm.

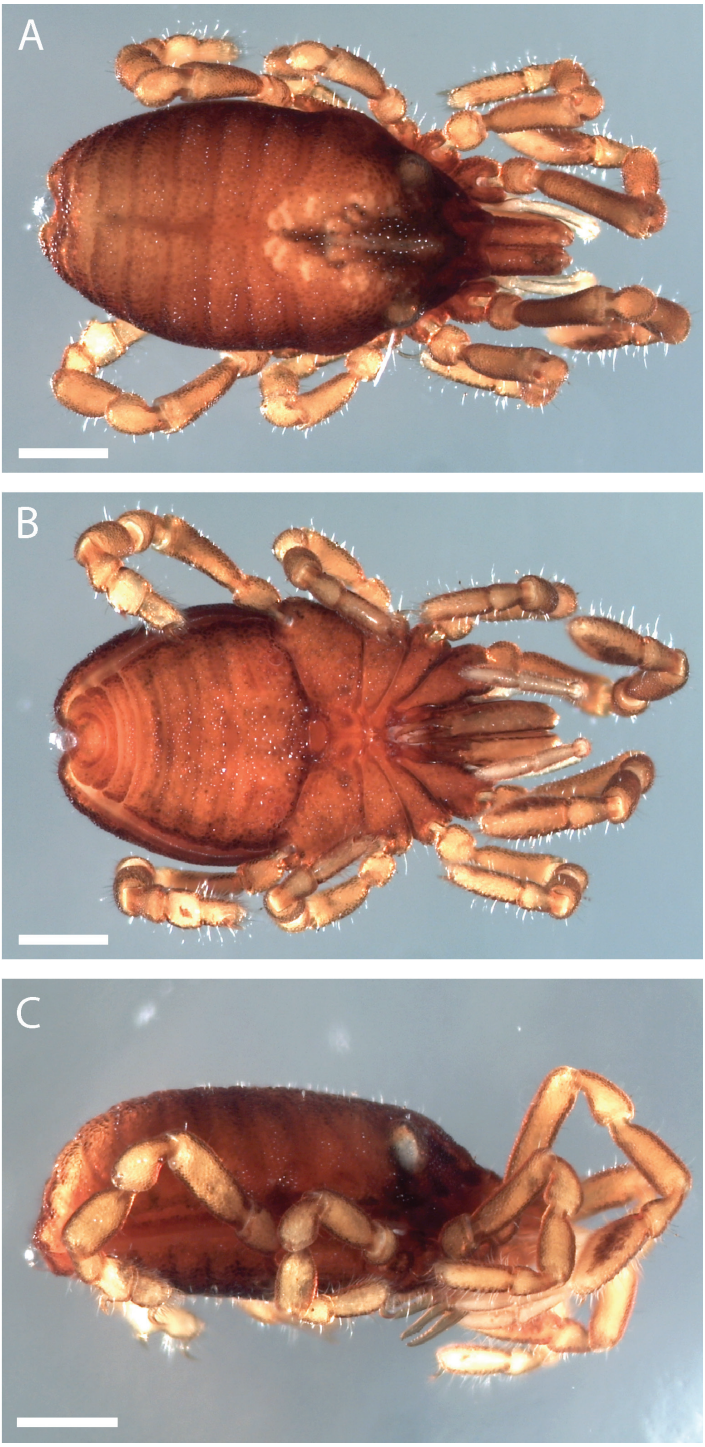


Figure 6. *Austropurcellia finniganensis* sp. n., holotype male, QM 102446. **A** dorsal view **B** ventral view **C** lateral view. Scale bars: 0.5 mm.

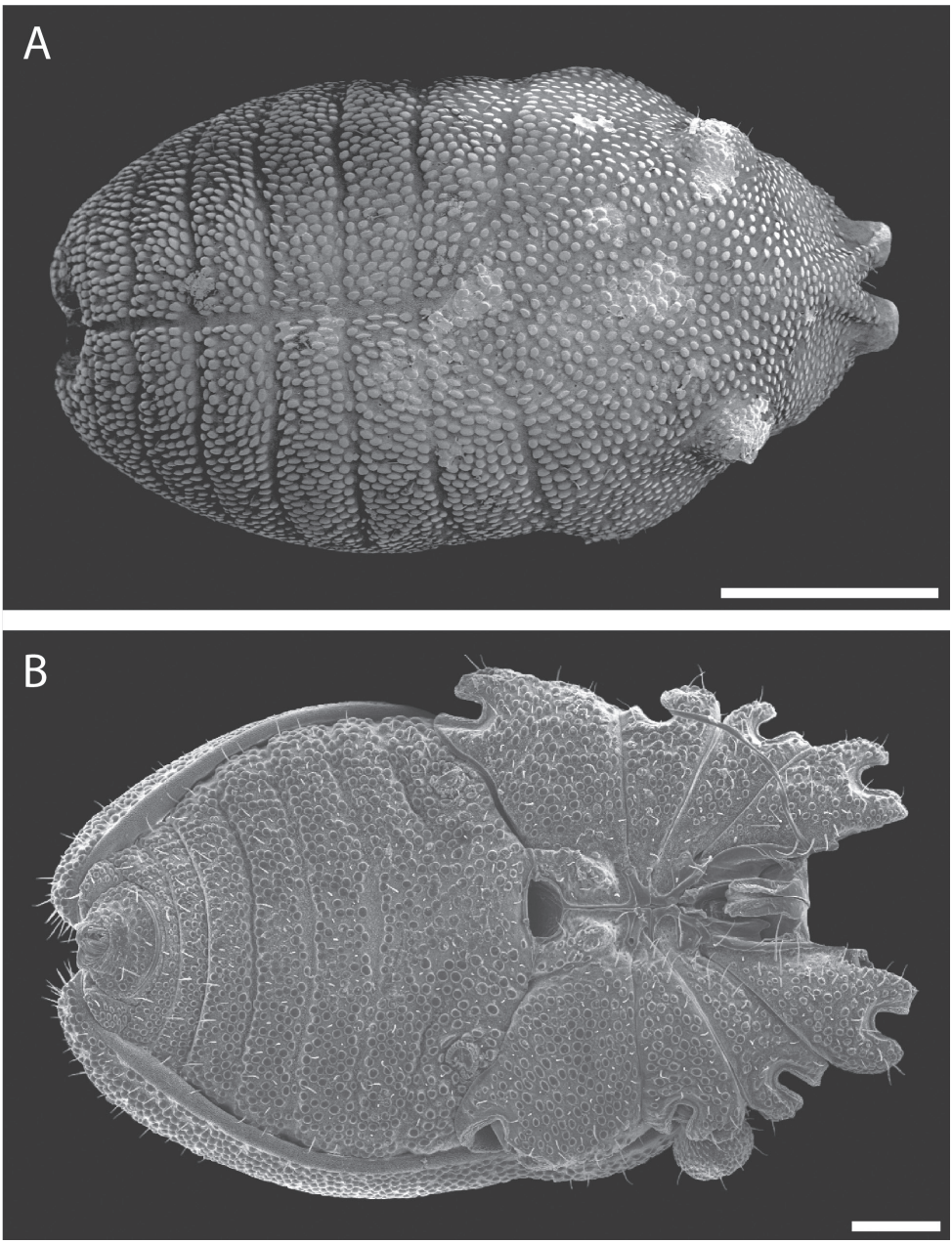


Figure 7. *Austropurcellia finniganensis* sp. n., males. **A** dorsal view, QM berlesate 981 **B** ventral view, QM 102447, paratype. Scale bar: 0.5 μm (**A**); 200 μm (**B**).

Description. Pettalid with tergite VIII bilobed (Figs 6A–B, 7). Posterior margin of dorsal scutum curves ventrally (Fig. 6C). Length of male holotype (Fig. 6) 2.1 mm, width at widest point in posterior third of prosoma 1.2 mm, width at ozophores 0.8

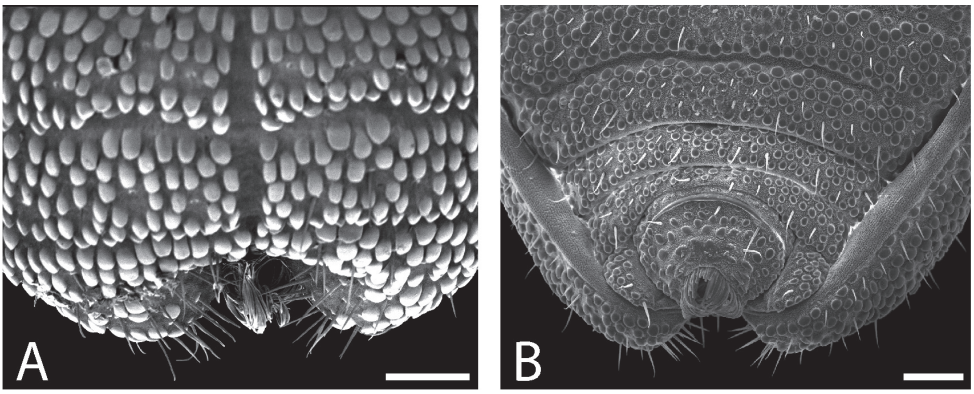


Figure 8. *Austropurcellia finniganensis* sp. n., paratype male, QM 102447. **A** dorsal view of posterior tergites **B** anal plate. Scale bars: 100 µm.

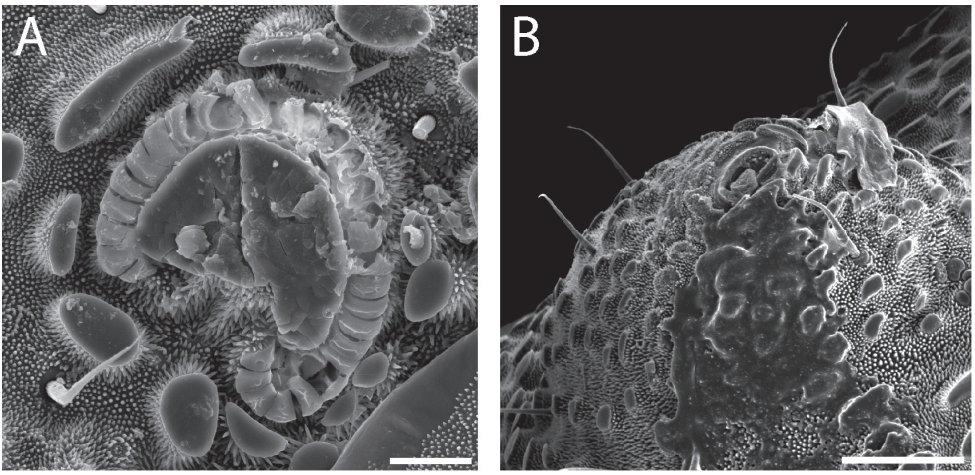


Figure 9. *Austropurcellia finniganensis* sp. n., males. **A** spiracle, QM 102447, paratype **B** ozophore, QM berlesate 981. Scale bar: 20 µm (**A**); 50 µm (**B**).

mm. Most of body surface covered in microstructure of tubercles and granules (Fig. 7). Dorsal transverse sulci present and very prominent by lack of granulation (Fig. 7A). Dorsal longitudinal sulcus lacking granulation but with adjacent band of elongated granules oriented parallel to medial sulcus (Figs 7A, 8A). Granulation medially absent in anterior portions of sternites II-V; area of absent granulation approximately equal to width of gonostome (Fig. 7).

Ozophores relatively conical, of type III *sensu* Juberthie (1970) (Figs 7A, 9A). Coxae of legs I and II mobile, coxae of remaining legs fixed. Male coxae II–IV meeting in the midline (Fig. 7B). Male gonostome small, subtriangular, wider than long (Fig. 7B). Spiracles circular and C-shaped with slightly recurved edges (Fig. 9), as found in "open circle" type of Giribet and Boyer (2002). Anal region of "pettalid type" (Giribet

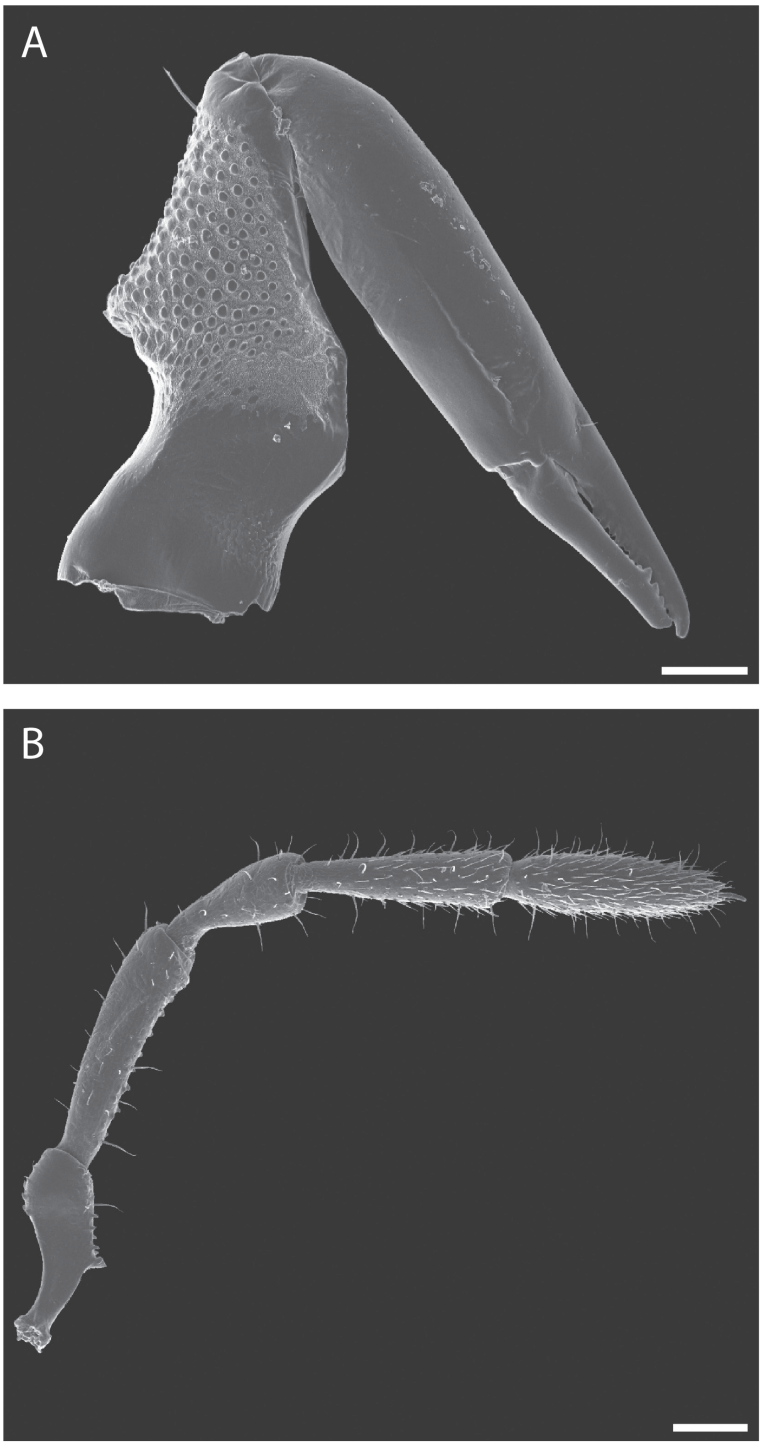


Figure 10. *Austropurcellia finniganensis* sp. n., paratype male and female, QM 102447. **A** female chelicera **B** male palp. Scale bars: 100 μm.

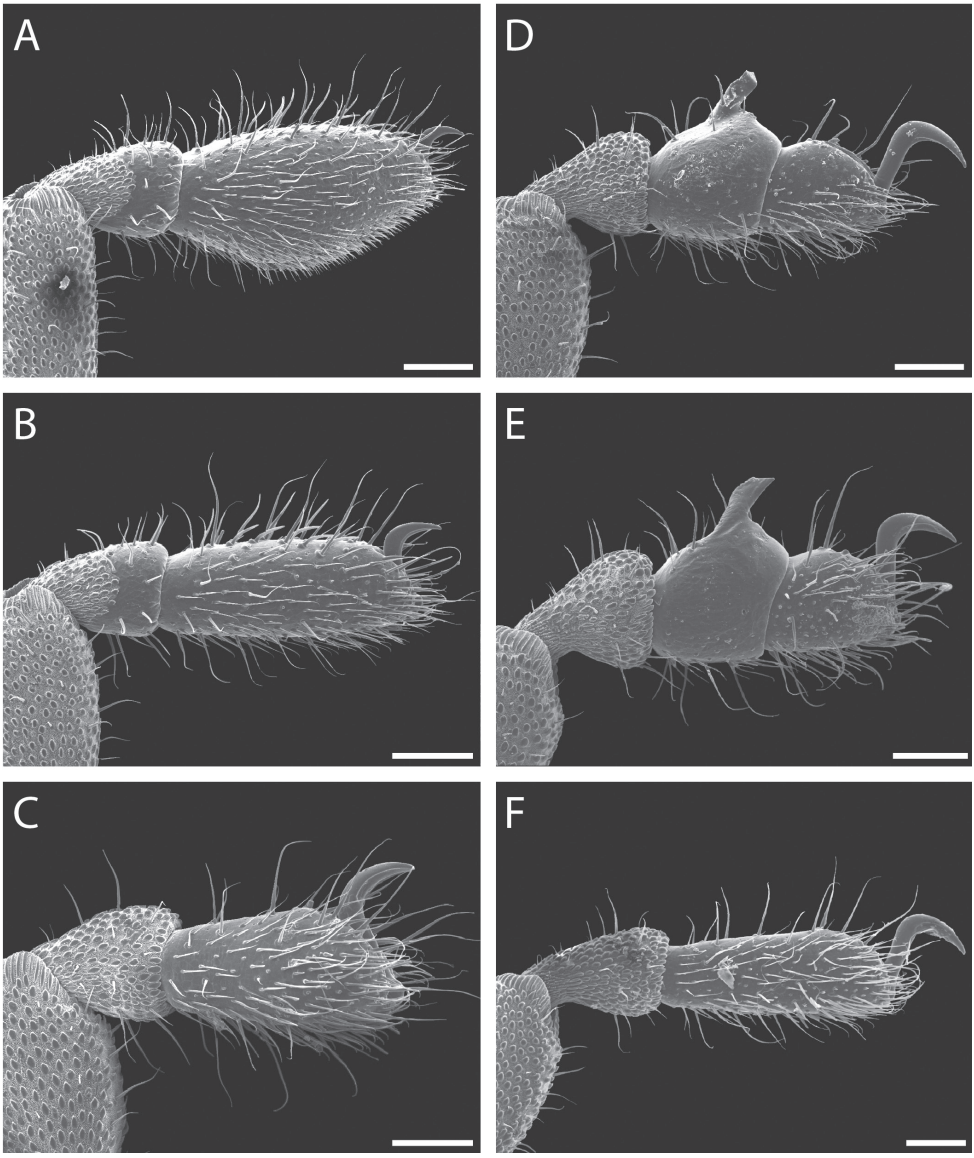


Figure 11. *Austropurcellia finniganensis* sp. n., paratype male and female, QM 102447. **A** male tarsus and metatarsus I **B** male tarsus and metatarsus II **C** female tarsus and metatarsus III **D** male tarsus and metatarsus IV, lateral view; **E** male tarsus and metatarsus IV, medial view **F** female tarsus and metatarsus IV. Scale bars: 100 μm.

and Boyer 2002). Anal plate convex and granulated (Fig. 8B). Short, round scopula extruding from posterior third of anal plate and extending just past posterior margin of anal plate (Fig. 8B). Orientation of scopula obscures anal pores, which are not visible (Fig. 8B).

Chelicerae (Fig. 10A) short and relatively robust. Proximal article of chelicerae with dorsal crest, without ventral process. Median article with prominent apodeme. Chela with two types of dentition typical in pettalids (Fig. 10A). Measurements of cheliceral articles of male paratype, from proximal to distal (in mm): 0.85, 0.94, 0.27. Palp (Fig. 10B) with prominent ventral process on trochanter. Measurements of palpal articles of male paratype from proximal to distal (in mm): 0.27, 0.28, 0.20, 0.31, 0.27.

Legs with all claws smooth, without ventral dentition or lateral pegs (Fig. 11). All tarsi smooth (Fig. 11). Distinct solea present on ventral surface of tarsus I (Fig. 11A). Metatarsi I and II heavily ornamented on proximal half, with distal half smooth (Fig. 11A, B). Remaining metatarsi with full ornamentation (Fig. 11C–F). Male tarsus IV completely divided into two tarsomeres (Fig. 11D, E). Adenostyle with relatively robust, pointed claw, wider base, and small pore at apex on lateral (external) side (Fig. 11D). Seta on lateral surface of adenostyle (Fig. 11D–E) (example with adenostyle features labeled, Fig. 5).

Length measurements from male paratype of leg articles from proximal to distal (in mm): leg I [trochanter damaged], 0.59, 0.26, 0.43, 0.20, 0.40; leg II 0.26, 0.42, 0.25, 0.30, 0.15, 0.34; leg III [trochanter damaged], 0.48, 0.20, 0.33, 0.15, 0.31; leg IV [trochanter damaged], 0.50, 0.24, 0.39, 0.16, 0.35. Width measurements from male paratype of leg articles from proximal to distal (in mm): leg I [trochanter damaged], 0.18, 0.18, 0.17, 0.15, 0.20; leg II 0.17, 0.15, 0.16, 0.18, 0.13, 0.14; leg III [trochanter damaged], 0.18, 0.18, 0.19, 0.12, 0.14; leg IV 0.16, 0.21, 0.20, 0.21, 0.17, 0.18.

Etymology. The specific epithet refers to the type locality, Mt. Finnigan.

Austropurcellia fragosa Popkin-Hall, Jay & Boyer, sp. n.

http://zoobank.org/9DE6E237-E3C7-411C-95D6-3441E83C22BB
Figs 12–17

Material examined. *Holotype.* Male (QM 102445 [ex QM 38121]), Roaring Meg Creek, 16.074°S, 145.416°E, coll. K. Aland and G. B. Monteith 1.v.2015, QM 38121.

Paratypes. 5 males, 2 females, same collecting data as holotype, QM 38121, Macalester SEM stub M30.10.

Additional material. 2 females, 4 juveniles, McDowall Range 17 km N Daintree, 16.100°S, 145.333°E, coll. G. B. Monteith 27.xi.1985, QM berlesate 684.

1 male, 2 females, Roaring Meg Creek 6 km W Cape Tribulation, 16.083°S, 145.4°E, coll. G. B. Monteith, D. Yeates, G. Thompson 5.x.1982, QM berlesate 448, Macalester SEM stubs M22.11, M22.12.

1 female, Roaring Meg Creek 6 km W Cape Tribulation, 16.067°S, 145.400°E, coll. G. B. Monteith, D. Yeates, G. Thompson 5.x.1982, QM berlesate 453, Macalester SEM stubs M22.9, M22.10.

Diagnosis. Distinguished from congeners by convex anal plate with long, narrow scopula emerging from anterior quarter of anal plate and occupying a rectangular indented area for its entire length. Distinctive ungranulated areas cause ventral sutures to appear fused.

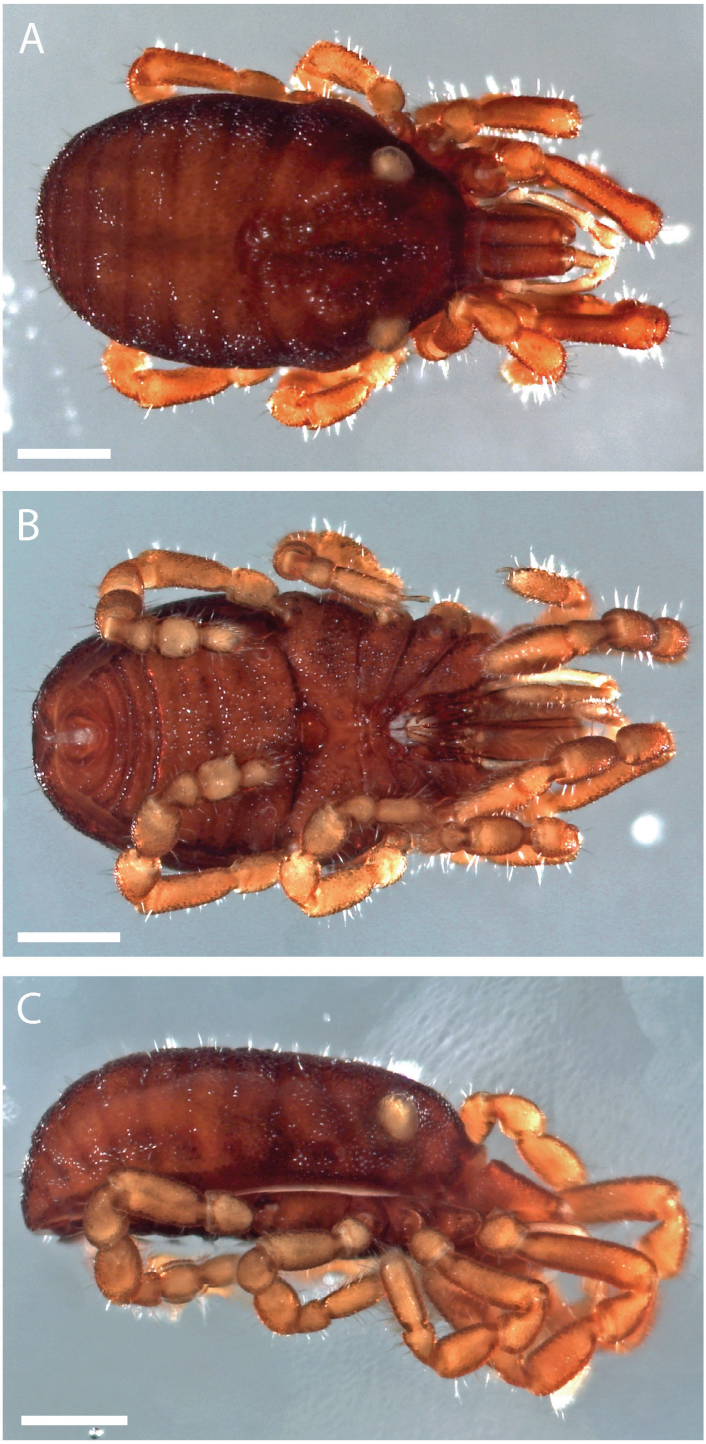


Figure 12. *Austropurcellia fragosa* sp. n., holotype male, QM 102445. **A** dorsal view **B** ventral view **C** lateral view. Scale bars: 0.5 mm.

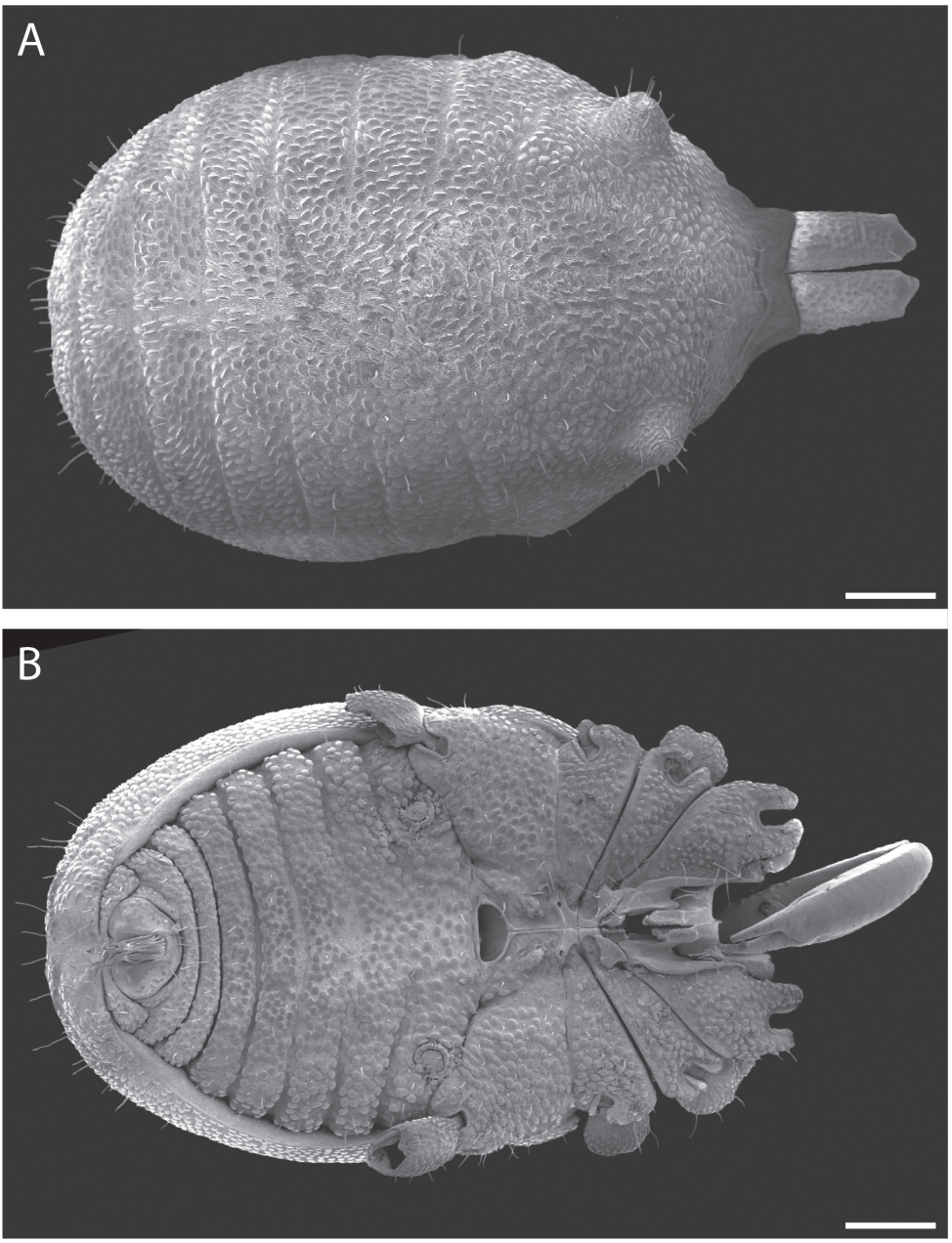


Figure 13. *Austropurcellia fragosa* sp. n., males. **A** dorsal view, QM berlesate 38121, paratype **B** ventral view, QM berlesate 448. Scale bars: 200 μm.

Description. Pettalid with tergite VIII bilobed (Fig. 13). Length of male holotype (Fig. 12) 2.0 mm, width at widest point in posterior third of prosoma 1.2 mm, width at ozophores 0.8 mm. Most of body surface covered in microstructure of tubercles and

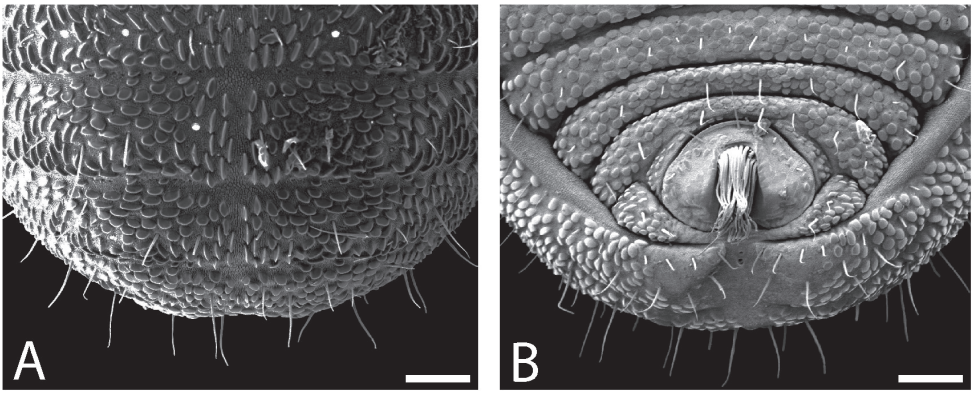


Figure 14. *Austropurcellia fragosa* sp. n., males. **A** dorsal view of posterior tergites, QM berlesate 453 **B** anal plate, QM berlesate 448. Scale bars: 100 μm.

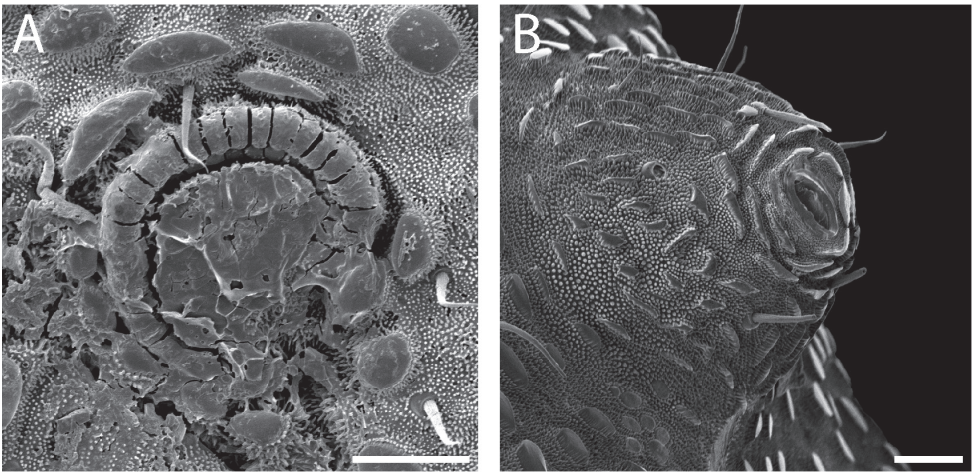


Figure 15. *Austropurcellia fragosa* sp. n., males. **A** spiracle, QM berlesate 448 **B** ozophore, QM berlesate 453. Scale bar: 20 μm (**A**); 50 μm (**B**).

granules (Fig. 13). Posterior ventral body margin flexed anteriorly. Dorsal transverse sulci present and distinct by lack of granulation (Figs 13A, 14A). Dorsal longitudinal sulcus lacking granulation, with adjacent band of elongated granules flanking dorsal longitudinal sulcus (Figs 13A, 14A). Granulation medially absent in anterior portions of sternites II-VI; area of absent granulation approximately equal to width of gonostome (Fig. 13B).

Ozophores relatively tall and conical, of type III *sensu* Juberthie (1970) (Figs 13A, 15B). Coxae of legs I and II mobile, coxae of remaining legs fixed. Male coxae II–IV meeting in the midline (Fig. 13B). Male gonostome small, subtriangular, wider than long (Fig. 13B). Spiracles circular and C-shaped with slightly recurved edges (Fig. 15A), as found in "open circle" type of Giribet and Boyer (2002). Anal region of

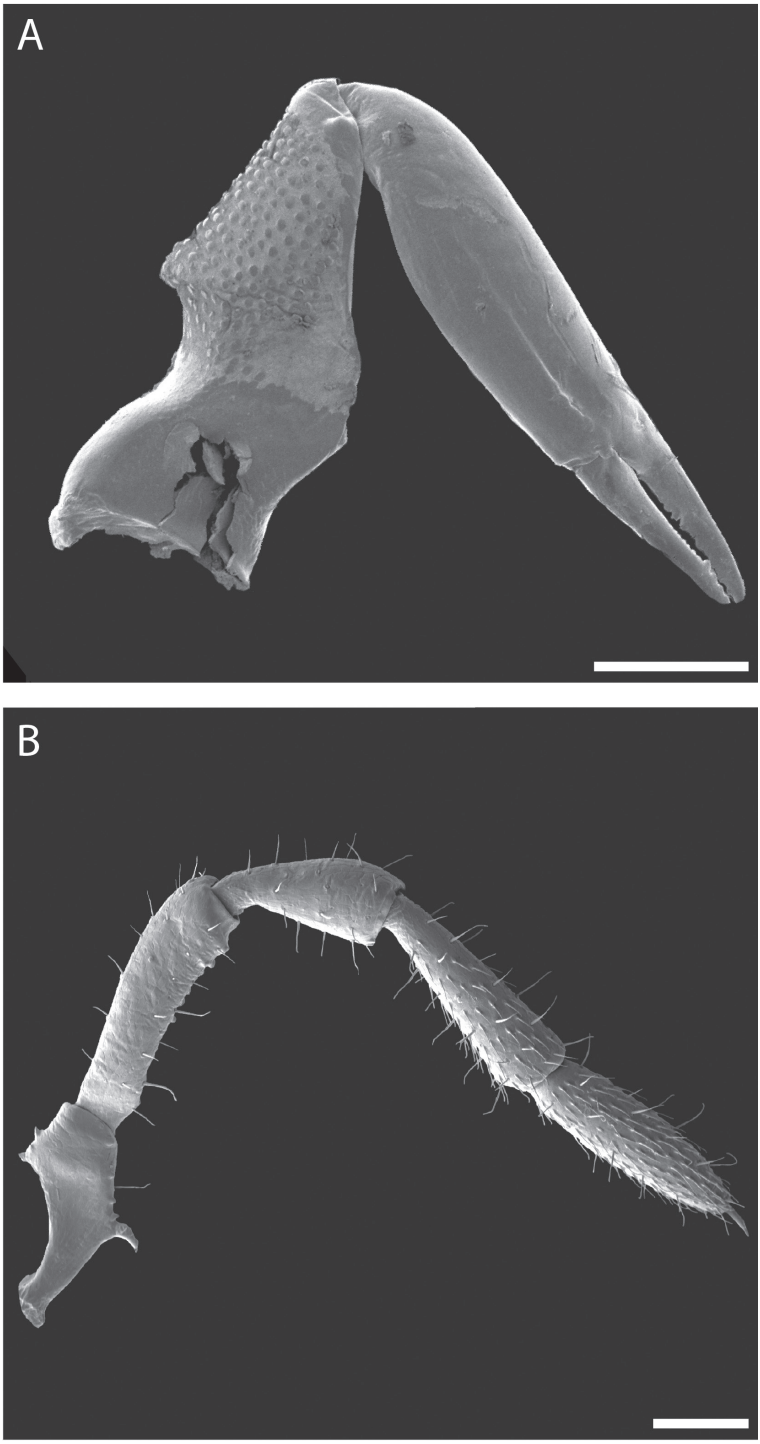


Figure 16. *Austropurcellia fragosa* sp. n., males. **A** chelicera, QM berlesate 448 **B** palp, QM berlesate 453. Scale bar: 200 μm (**A**); 100 μm (**B**).

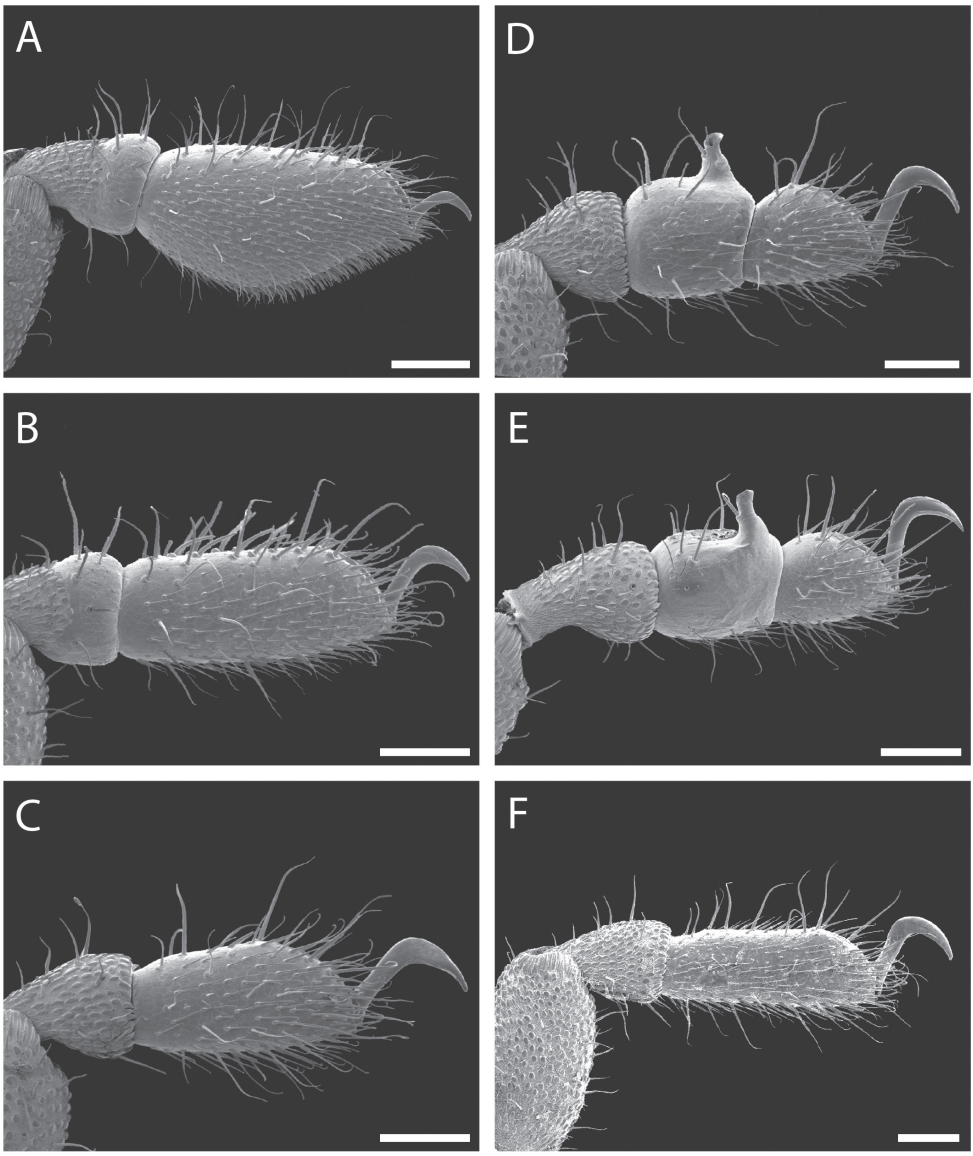


Figure 17. *Austropurcellia fragosa* sp. n., males and female. **A** male tarsus and metatarsus I, QM berlesate 448 **B** male tarsus and metatarsus II, QM berlesate 448; **C** male tarsus and metatarsus III, QM berlesate 448 **D** male tarsus and metatarsus IV, lateral view, QM berlesate 448 **E** male tarsus and metatarsus IV, medial view, QM berlesate 453 **F** female tarsus and metatarsus IV, QM berlesate 684. Scale bars: 100 μm.

"pettalid type" (Giribet and Boyer 2002). Anal plate convex and largely ungranulated, with light granulation along anterior margin (Fig. 14B). Long, narrow scopula emerging at anterior quarter of anal plate and extending past posterior margin of anal plate (Fig. 14B). Scopula inset into rectangular area at center of anal plate (Fig. 14B). Three anal pores visible, located between lobes of tergite VIII (Fig. 14B).

Chelicerae (Fig. 16A) short and relatively robust. Proximal article of chelicerae with dorsal crest, without ventral process. Median article with prominent apodeme. Chela with two types of dentition typical in pettalids (Fig. 16A). Measurements of cheliceral articles of male paratype from proximal to distal (in mm): 0.70, 0.76, 0.24. Palp (Fig. 16B) with prominent ventral process on trochanter. Measurements of palpal articles of male paratype from proximal to distal (in mm): 0.22, 0.27, 0.15, 0.22, 0.23.

Legs with all claws smooth, without ventral dentition or lateral pegs (Fig. 17). All tarsi smooth (Fig. 17). Distinct solea present on ventral surface of tarsus I (Fig. 17A). Metatarsi I and II heavily ornamented on proximal half, with smooth distal half (Fig. 17A, B). Remaining metatarsi with full ornamentation (Fig. 17C–F). Male tarsus IV completely divided into two tarsomeres (Fig. 17D, E). Adenostyle with relatively robust, blunt claw, wide base, and small pore at apex on lateral (external) side (Fig. 17D). Long seta on lateral surface of adenostyle from below pore to above apex (Fig. 17D, E); very short seta rising from adenostyle base below pore (Fig. 17D) (example with adenostyle features labeled, Fig. 5).

Measurements from male paratype of leg articles from proximal to distal (in mm): leg I [trochanter damaged], 0.51, 0.17, 0.33, 0.18, 0.35; leg II [trochanter damaged], 0.38, 0.18, 0.27, 0.14, 0.30; leg III 0.14, 0.29, 0.18, 0.24, 0.12, 0.26; leg IV [trochanter damaged], 0.42, 0.23, 0.29, 0.17, 0.31. Width measurements from male paratype of leg articles from proximal to distal (in mm): leg I [trochanter damaged], 0.16, 0.16, 0.16, 0.14, 0.20; leg II [trochanter damaged], 0.15, 0.15, 0.17, 0.12, 0.12; leg III 0.16, 0.16, 0.15, 0.17, 0.12, 0.13; leg IV [trochanter damaged], 0.18, 0.17, 0.19, 0.14, 0.15.

Etymology. The specific epithet is derived from the first declension form of *fragōsus*, from Latin, meaning "roaring" or "crashing", a reference to the type locality, Roaring Meg Creek.

Austropurcellia megatanka Jay, Coblens & Boyer, sp. n.

http://zoobank.org/16C62C3B-BCE8-4EC0-8CD5-14D81C68ED0F
Figs 18–23

Material examined. *Holotype.* Male (QM 102440 [ex MCZ IZ 68951]), Baldy Mountain Road, Herberton Range National Park, 17.287°S, 145.427°E, coll. S. L. Boyer, M. J. Coblens, K. R. Jay and P. P. Sharma 29.v.2014.

Paratypes. 2 males, 1 female, QM 102441 (ex MCZ IZ 68948), same collecting data as holotype. 2 males, 1 female, 3 juveniles, same collecting data as holotype, MCZ IZ 68949, Macalester SEM stubs M27.7, M27.8, M30.2.

Additional material. 1 male, 3 females, Baldy Mountain Road, Herberton Range National Park, 17.267°S, 145.267°E, coll. D. Yeates and D. Cook 25.xi.1985. QM berlesate 683, S 1755, Macalester SEM stubs M23.3, M23.4.

4 males, 1 female, Baldy Mountain, 17.284°S, 145.432°E, coll. G. B. Monteith 10.x.1980, QM S 2281.

1 male, CSIRO Trail (after hut), 17.108°S, 145.629°E, coll. S. L. Boyer, M. J. Coblens, K. R. Jay and P. P. Sharma 29.v.2014, MCZ IZ 68950.

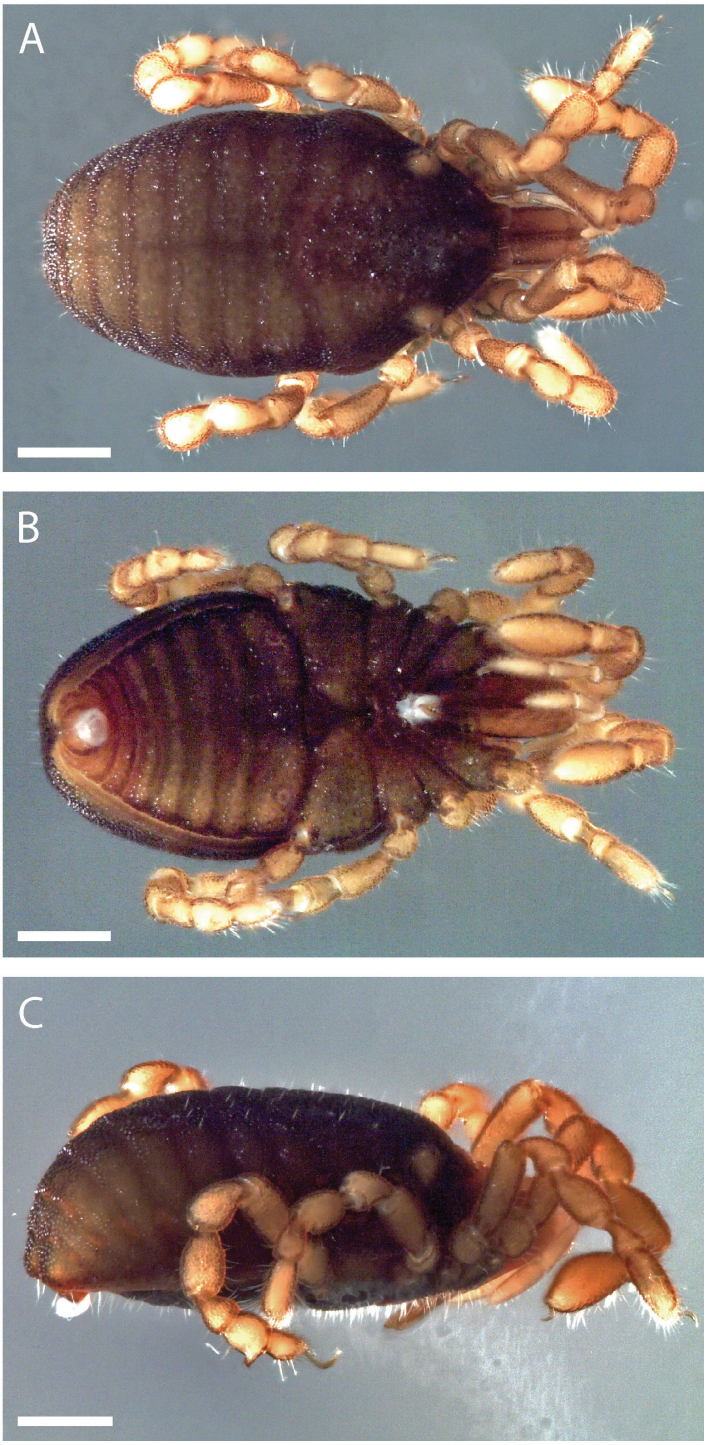


Figure 18. *Austropurcellia megatanka* sp. n., holotype male, QM 102440. **A** dorsal view **B** ventral view **C** lateral view. Scale bars: 0.5 mm.

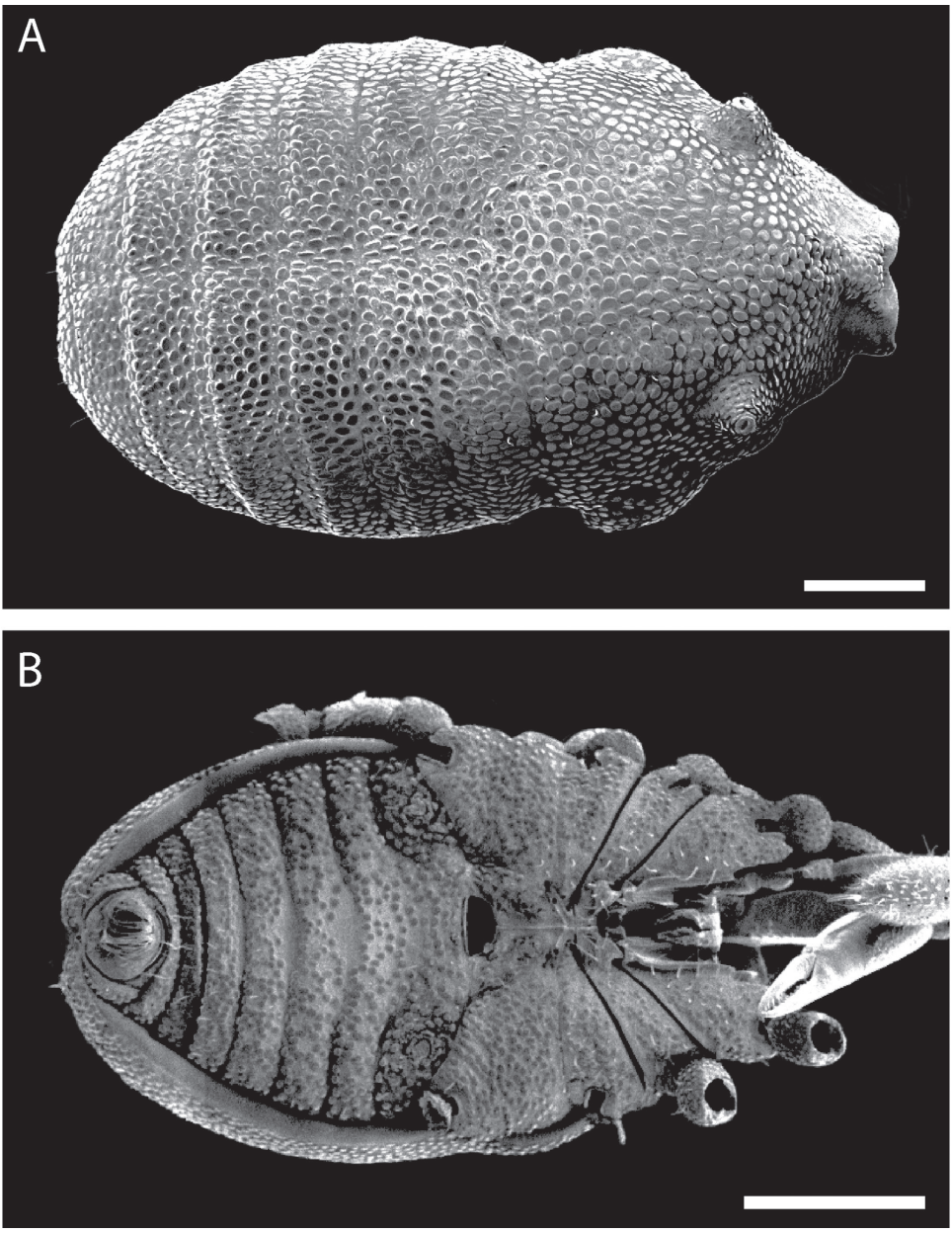


Figure 19. *Austropurcellia megatanka* sp. n., paratype male, QM 102441. **A** dorsal view **B** ventral view. Scale bar: 200 μm (**A**); 500 μm (**B**).

4 juveniles, Mt. Haig, 17.1°S, 145.583°E, coll. Taylor and Feehan 30.vi.1971, ANIC 349.

1 male, 3 females, 5 juveniles, Mt. Haig, Lamb Range, 17.083°S, 145.6°E, coll. G. B. Monteith 25.ii.1997, QM berlesate 918, Macalester SEM stubs M20.11, M20.12.

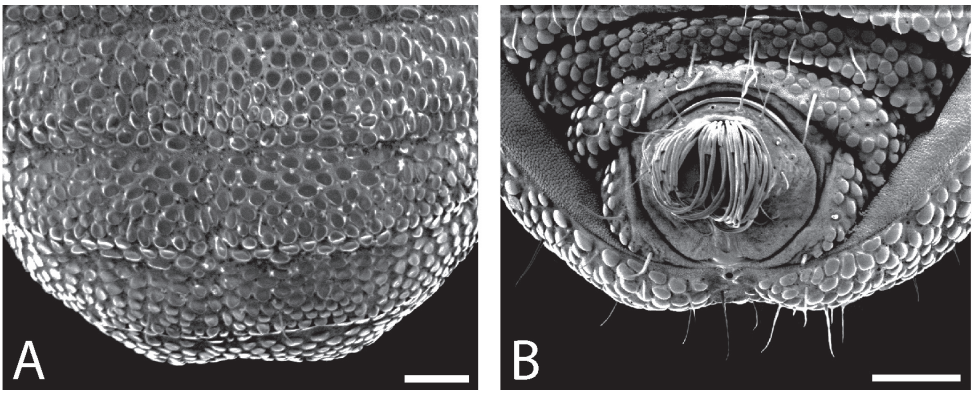


Figure 20. *Austropurcellia megatanka* sp. n., paratype male, QM 102441. **A** dorsal view of posterior tergites **B** anal plate. Scale bars: 100 μm.

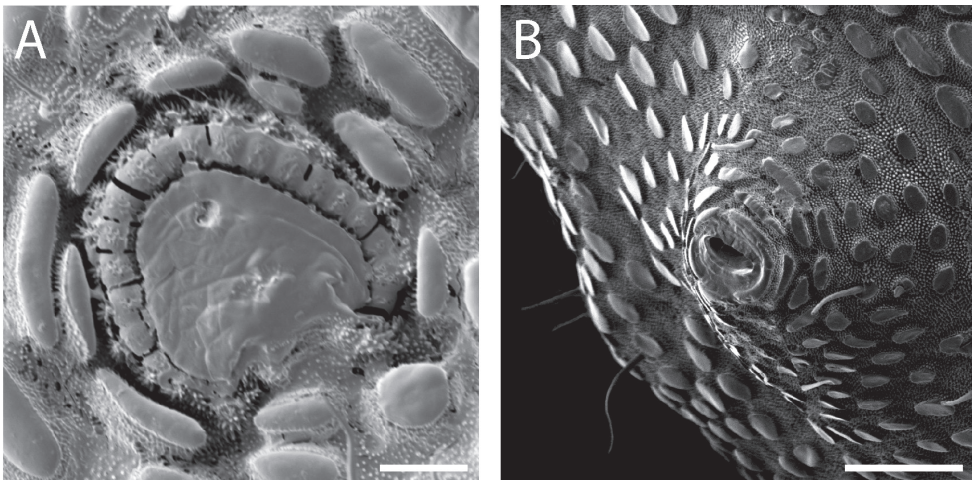


Figure 21. *Austropurcellia megatanka* sp. n., males. **A** spiracle, QM berlesate 918 **B** ozophore, QM 102441, paratype. Scale bar: 20 μm (**A**); 50 μm (**B**).

4 males, 2 females, 22 juveniles, Mt. Tiptree, 17.067°S, 145.617°E, coll. Taylor and Feehan 29.vi.1971, ANIC 345, ANIC 346, ANIC 347, ANIC 348.

Diagnosis. Distinguished from congeners by an usually wide and long scopula emerging from anterior quarter of male anal plate and easily visible in lateral view. Anal plate is very flat compared to the more rounded anal plates of geographically proximate species such as *A. tholei* and *A. despectata*. Distinctive areas lacking granulation cause ventral sutures to appear fused. Male tarsus IV is fully bisegmented rather than partially bisegmented as in *A. tholei* and *A. despectata*.

Description. Pettalid with tergite VIII bilobed (Fig. 19). Length of male holotype (Fig. 18) 2.1 mm, width at widest point in posterior third of prosoma 1.2 mm,

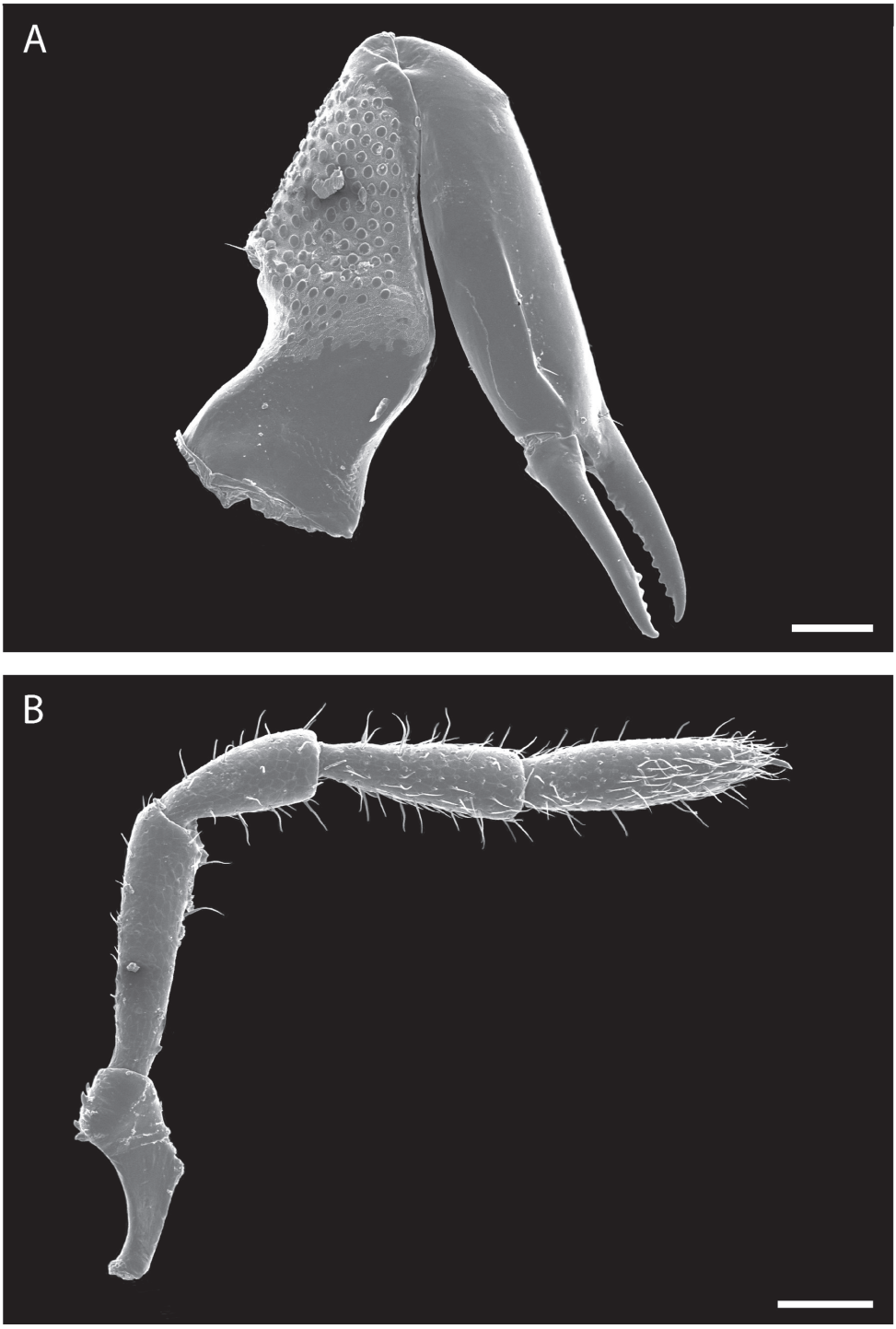


Figure 22. *Austropurcellia megatanka* sp. n., paratype male, QM 102441. **A** chelicera **B** palp. Scale bars: 100 μm.

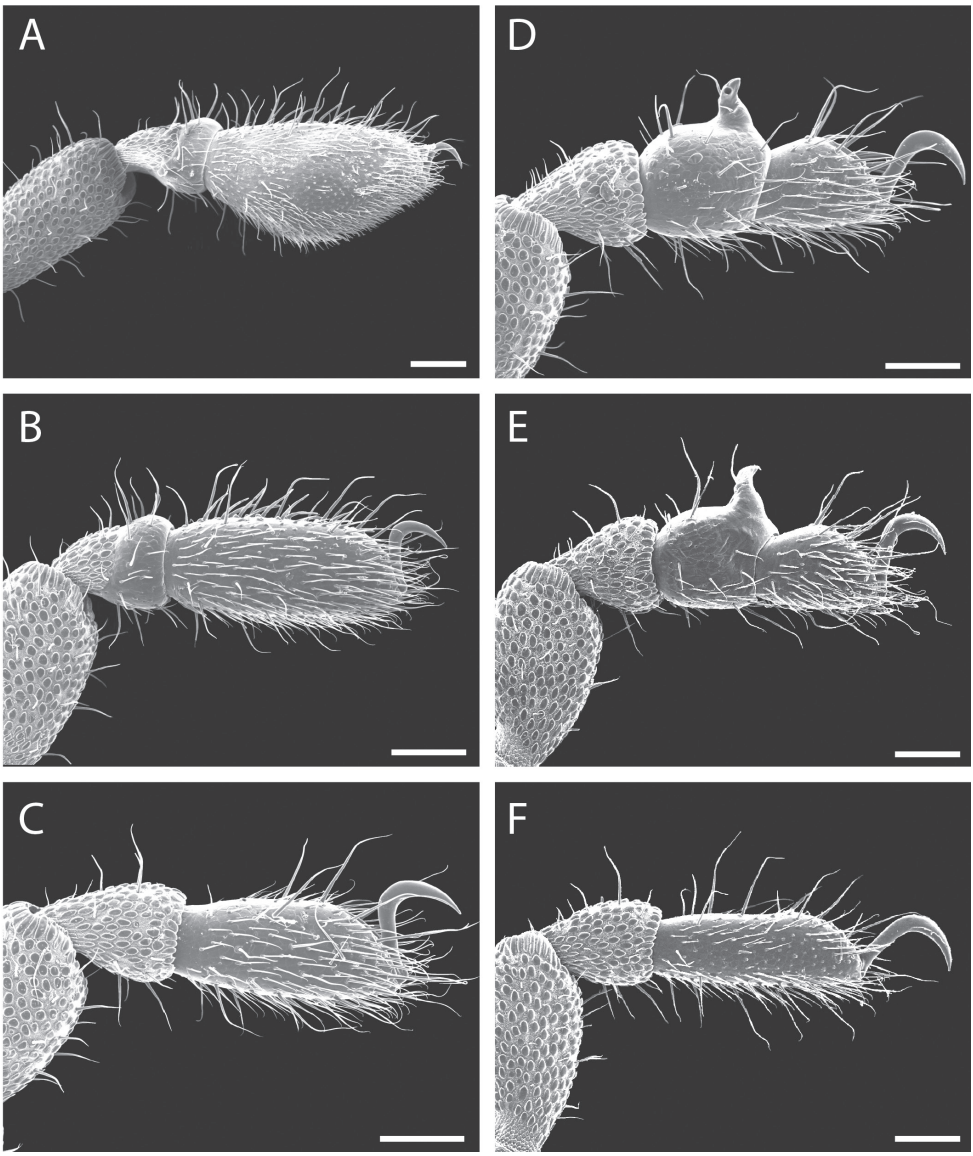


Figure 23. *Austropurcellia megatanka* sp. n., paratype male and female, QM 102441. **A** male tarsus and metatarsus I **B** male tarsus and metatarsus II; **C** male tarsus and metatarsus III **D** male tarsus and metatarsus IV, lateral view **E** male tarsus and metatarsus IV, medial view **F** female tarsus and metatarsus IV. Scale bars: 100 μm.

width at ozophores 0.8 mm. Most of body surface covered in microstructure of tubercles and granules (Fig. 19). Transverse sulci present and granulated (Fig. 19A). Dorsal longitudinal sulcus granulose (Figs 19A, 20A). Granulation medially absent in sternites II-V; area of absent granulation approximately equal to width of gonostome (Fig. 19B).

Ozophores relatively conical, of type III *sensu* Juberthie (1970) (Figs 19A, 21B). Coxae of legs I and II mobile, coxae of remaining legs fixed. Male coxae II–IV meeting in the midline (Fig. 19B). Male gonostome small, subtriangular, and wider than long (Fig. 19). Spiracles circular and C-shaped with slightly recurved edges, as found in "open circle" type of Giribet and Boyer (2002) (Fig. 21). Anal region of "pettalid type" (Giribet and Boyer 2002). Anal plate flat and largely ungranulated (Fig. 20B). Long, full scopula emerging from anterior quarter of anal plate and curling into toward posterior quarter of anal plate (Fig. 20B). Anal pore visible (Fig. 20B).

Chelicerae (Fig. 22A) short and relatively robust. Proximal article of chelicerae with dorsal crest, without ventral process. Median article with prominent apodeme. Chela with two types of dentition typical in pettalids (Fig. 22A). Measurements of cheliceral articles of male paratype from proximal to distal (in mm): 0.51, 0.72, 0.25. Palp (Fig. 22B) with prominent ventral process on trochanter. Measurements of palpal articles of male paratype from proximal to distal (in mm): 0.20, 0.25, 0.17, 0.22, 0.24.

Legs with all claws smooth, without ventral dentition or lateral pegs (Fig. 23). All tarsi smooth (Fig. 23). Distinct solea present on ventral surface of tarsus I (Fig. 23A). Metatarsi I and II heavily ornamented on proximal half, with distal half smooth (Fig. 23A, B). Remaining metatarsi with full ornamentation (Fig. 23C–F). Male tarsus IV fully divided into two tarsomeres (Fig. 23D, E). Adenostyle with relatively robust claw, wide base, and small pore at apex on lateral (external) side (Fig. 23D). Long seta rising from medial (internal) face of adenostyle from below pore to above apex (Fig. 23D, E); very short seta rising from adenostyle base below pore on lateral (external) face (Fig. 23D) (example with adenostyle features labeled, Fig. 5).

Measurements from male paratype of leg articles from proximal to distal (in mm): leg I 0.14, 0.48, 0.20, 0.30, 0.16, 0.39; leg II [trochanter damaged], 0.37, 0.17, 0.26, 0.13, 0.32; leg III [trochanter damaged], 0.30, 0.18, 0.23, 0.15, 0.26; leg IV [trochanter damaged], 0.36, 0.20, 0.28, 0.14, 0.32. Width measurements from male paratype of leg articles from proximal to distal (in mm): leg I [trochanter damaged], 0.15, 0.17, 0.16, 0.14, 0.19; leg II [trochanter damaged], 0.14, 0.16, 0.17, 0.12, 0.13; leg III 0.18, 0.15, 0.15, 0.16, 0.12, 0.11; leg IV 0.16, 0.17, 0.16, 0.18, 0.14, 0.14.

Etymology. The specific epithet, a noun in apposition, honors a Mitsubishi Pajero four-wheel drive vehicle, nicknamed 'Big Tank,' which made it possible to access various remote localities in the WT, including the type locality of *A. megatanka* sp. n.

Austropurcellia monteithi Jay, Popkin-Hall, Coblens & Boyer, sp. n.

http://zoobank.org/BF2F8D6F-27F6-4421-BD92-43C6676602ED
Figs 24–29

Material examined. *Holotype.* Male (QM 102442 [ex MCZ IZ 68951]), Kahlpahlim Rock (Lambs Head) Trail trailhead, Dinden National Park, 17.037°S, 145.613°E, coll. S. L. Boyer, M. J. Coblens, K. R. Jay and P. P. Sharma 30.v.2014.

Paratypes. 1 male, 1 female, same collecting data as holotype, QM 102443 (ex MCZ IZ 69023). 1 male, 1 female, same collecting data as holotype, MCZ IZ 69024, Macalester SEM stubs M28.9, M28.10, M30.3.

Additional material. 1 male, Mt. Edith Summit, 17.093°S, 145.622°E, coll. G. B. Monteith 8.iv.2014, MCZ IZ 69025.

1 male, Davies Creek Road, 17.050°S, 145.600°E, coll. G. B. Monteith and G. Thompson 17.xii.1989, QM berlesate 836, S 25699, Macalester SEM stubs M21.5, M21.6.

1 male, Chujeba Peak Summit, 16.936°S, 145.657°E, coll. G. B. Monteith and G. Thompson 14-16.xii.1989, QM S 41074, Macalester SEM stubs M21.1, M21.2.

4 males, 1 female, 1 juvenile, Mount Williams Summit, 16.917°S, 145.667°E, coll. G. B. Monteith 28.xi.1997, QM berlesate 962, S 35866, Macalester SEM stubs M19.9, M19.10.

1 male, 3 females, 1 juvenile, Mount Williams, 16.917°S, 145.667°E, coll. G. B. Monteith 28.xi.1997, QM berlesate 961, S 35868, Macalester SEM stubs M20.5, M20.6.

1 male, 1 female, 2 juveniles, Mount Williams, 16.917°S, 145.667°E, coll. G. B. Monteith and H. Janetzki 3.xii.1993, QM berlesate 867, S 49641, Macalester SEM stubs M20.7, M20.8.

Diagnosis. Distinguished from congeners by an unusually wide scopula emerging from anterior margin or anterior quarter of male anal plate and covering entire width of anal plate. Closely resembles *A. megatanka* sp. n., due to full scopula covering most of anal plate, but distinguished from *A. megatanka* by differences in scopula shape and ubiquity of ornamentation on opisthosomal sternites.

Description. Pettalid with tergite VIII bilobed (Fig. 25). Length of male holotype (Fig. 24) 2.0 mm, width at widest point in posterior third of prosoma 1.2 mm, width at ozophores 0.8 mm. Most of body surface covered in microstructure of tubercles and granules (Fig. 25). Transverse sulci present and granulated (Figs 25A, 26A). Medial sulcus present, oriented parallel to posterior-anterior axis, containing elongated granules oriented parallel to medial sulcus (Fig. 25A).

Ozophores tall and conical, of type III *sensu* Juberthie (1970) (Figs 25A, 27B). Coxae of legs I and II mobile, coxae of remaining legs fixed. Male coxae II–IV meeting in the midline (Fig. 25B). Male gonostome small, subtriangular, wider than long (Fig. 25B). Spiracles circular and C-shaped with slightly recurved edges (Fig. 27A), as found in "open circle" type of Giribet and Boyer (2002). Anal region of "pettalid type" (Giribet and Boyer 2002). Anal plate convex and sparsely granulated near anterior margin, with granulation density increasing laterally (Fig. 26B). Very wide scopula emerging from anterior quarter of anal plate or from anterior margin and continuing past posterior margin of anal plate (Fig. 26B). Two anal pores visible, one at suture between anal plate and tergite IX and one between lobes of tergite VIII (Fig. 26B).

Chelicerae (Fig. 28A) short and relatively robust. Proximal article of chelicerae with dorsal crest, without ventral process. Median article with apodeme. Chela with two types of dentition typical in pettalids (Fig. 28A). Measurements from male paratype of cheliceral articles from proximal to distal (in mm): 0.61, 0.83. Palp (Fig. 28B)

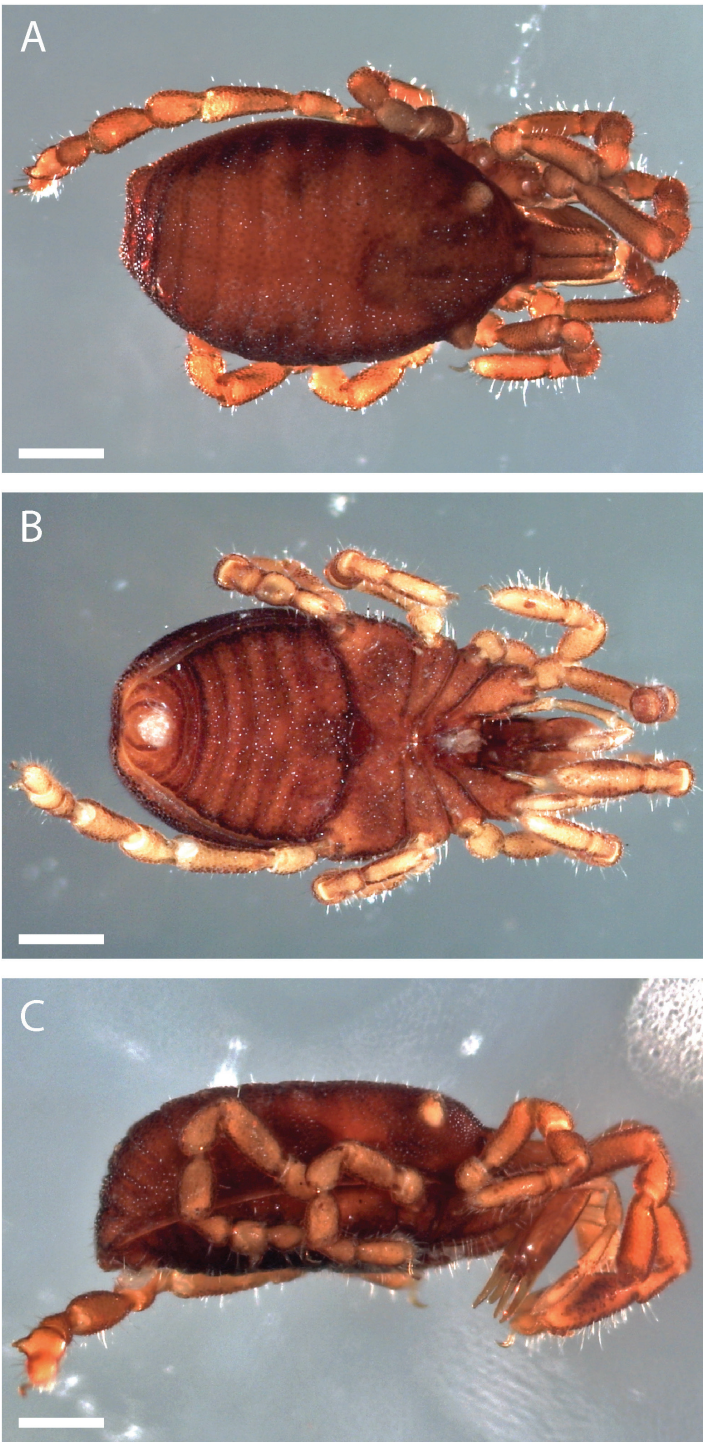


Figure 24. *Austropurcellia monteithi* sp. n., holotype male, QM 102442. **A** dorsal view **B** ventral view **C** lateral view. Scale bars: 0.5 mm.

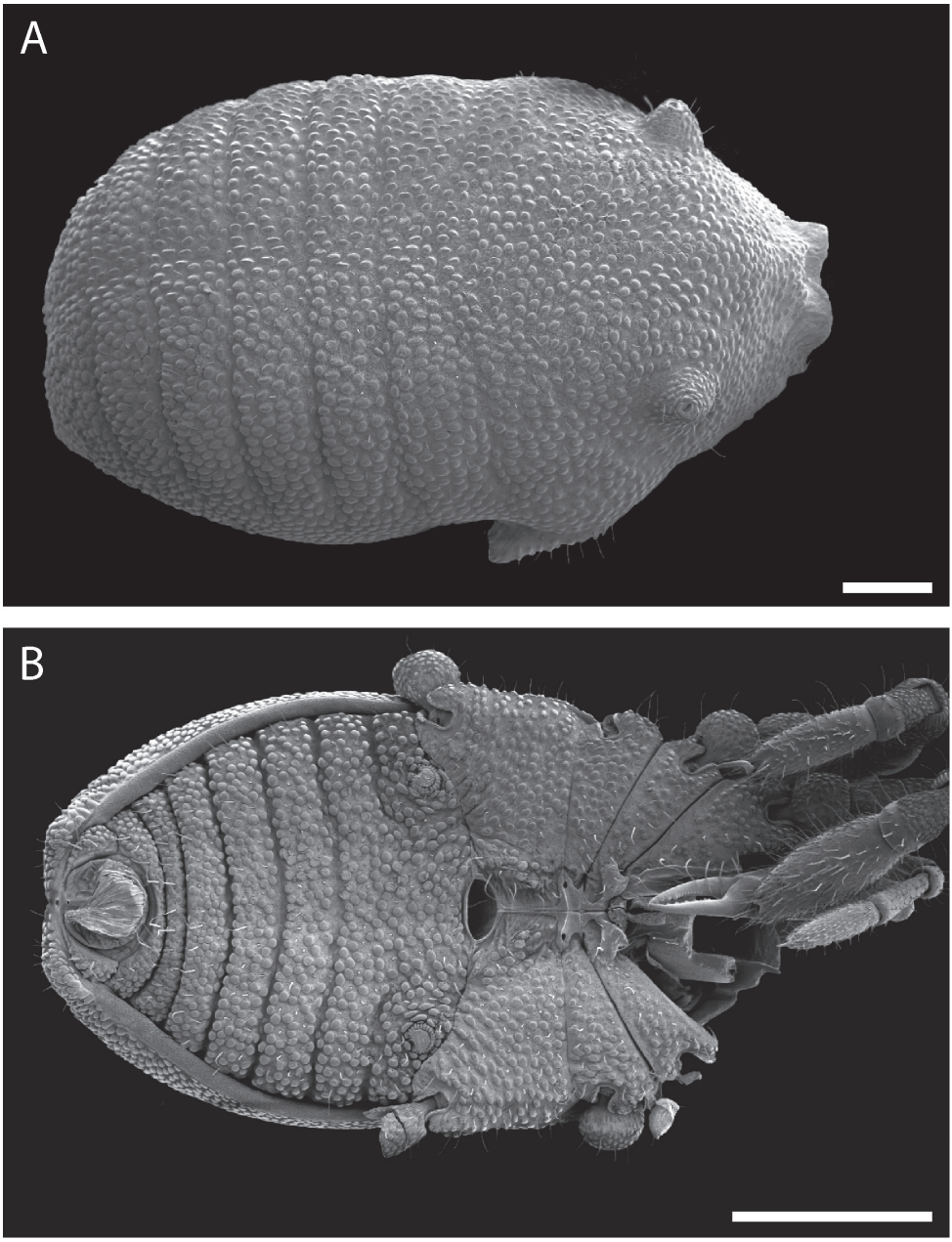


Figure 25. *Austropurcellia monteithi* sp. n., paratype male, QM 102443. **A** dorsal view **B** ventral view. Scale bar: 200 µm (**A**); 500 µm (**B**).

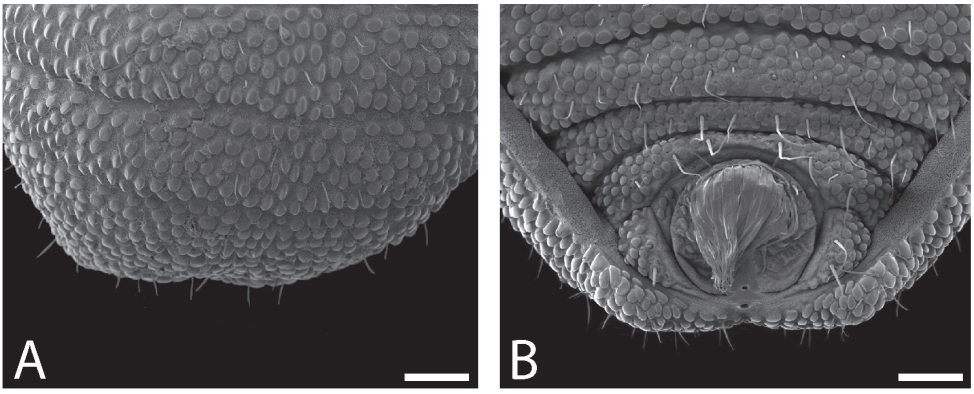


Figure 26. *Austropurcellia monteithi* sp. n., paratype male, QM 102443. **A** dorsal view of posterior tergites **B** anal plate. Scale bars: 100 μm.

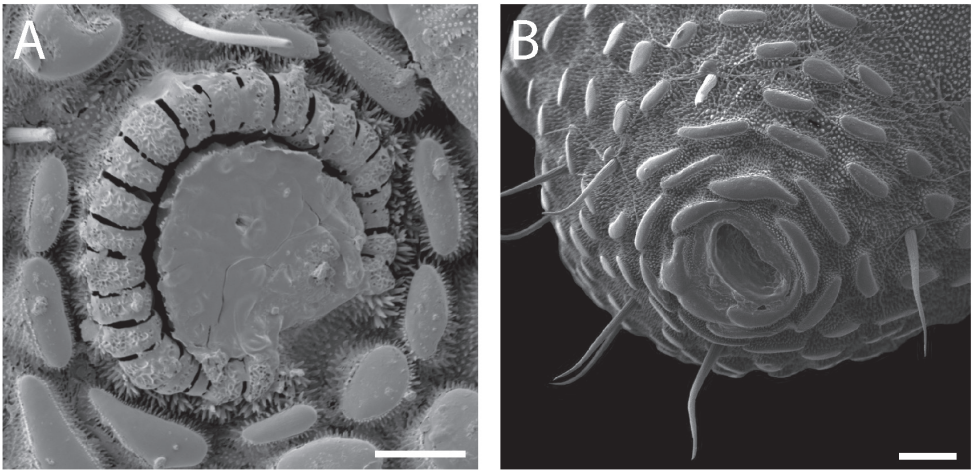


Figure 27. *Austropurcellia monteithi* sp. n., males. **A** spiracle, QM berlesate 836 **B** ozophore, QM 102443, paratype. Scale bars: 20 μm.

with prominent ventral process on trochanter. Measurements from male paratype of palp articles from proximal to distal (in mm): 0.23, 0.28, 0.20, 0.23, 0.27.

Legs with all claws smooth, without ventral dentition or lateral pegs (Fig. 29). All tarsi smooth (Fig. 29). Distinct solea present on ventral surface of tarsus I (Fig. 29A). Metatarsi I and II heavily ornamented on proximal half, with distal half smooth (Fig. 29A, B). Remaining metatarsi with full ornamentation (Fig. 29C–F). Male tarsus IV fully divided into two tarsomeres (Fig. 29D, E). Adenostyle with relatively robust claw, wide base, and small pore at apex on lateral (external) side (Fig. 29D). Long seta rising from medial (internal) face of adenostyle from below pore to above apex (Fig. 29D, E); very short seta rising from adenostyle base below pore on lateral (external) face (Fig. 29D) (example with adenostyle features labeled, Fig. 5).

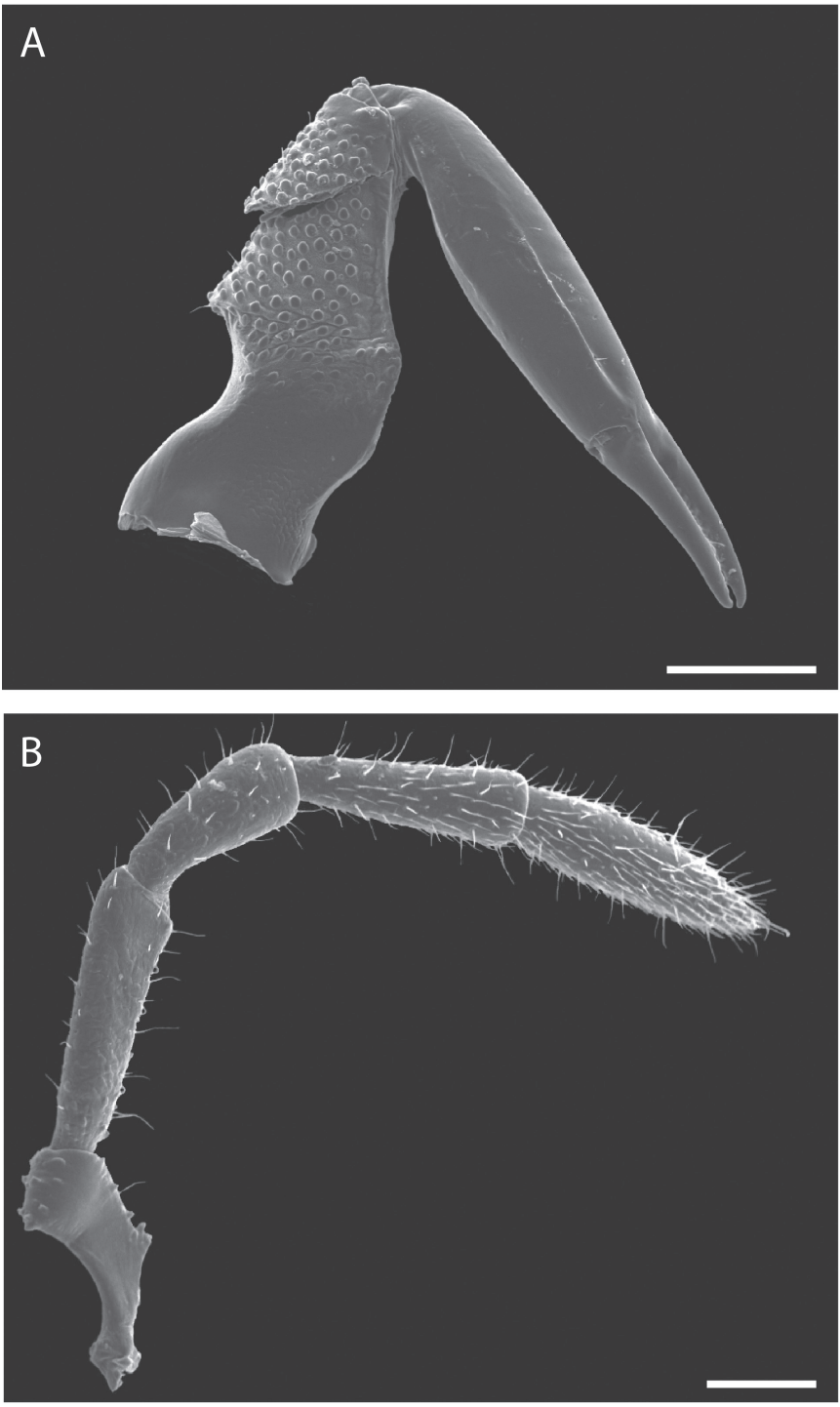


Figure 28. *Austropurcellia monteithi* sp. n., paratype male, QM 102443. **A** chelicera **B** palp. Scale bar: 200 μm (**A**); 200 μm (**B**).

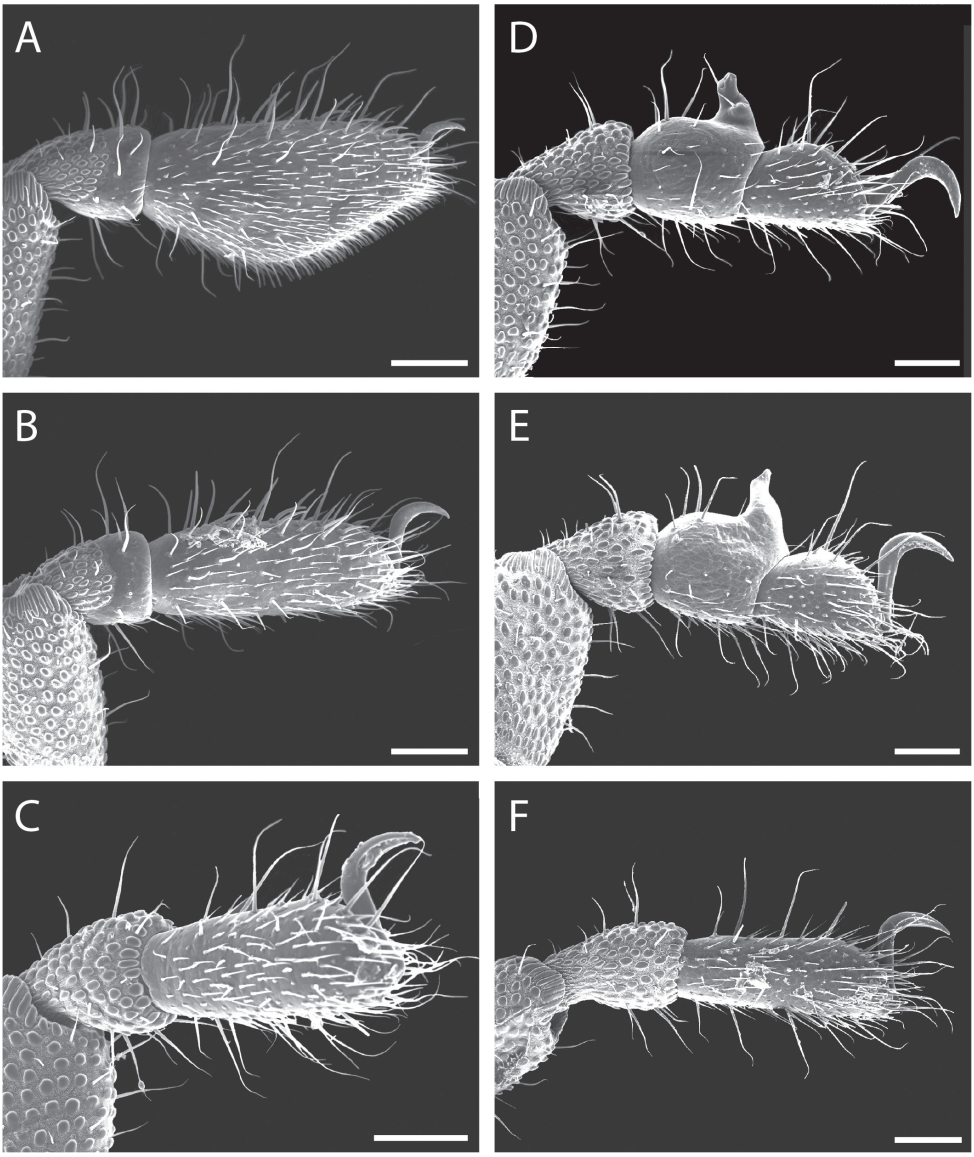


Figure 29. *Austropurcellia monteithi* sp. n., paratype male and female, QM 102443. **A** male tarsus and metatarsus I **B** male tarsus and metatarsus II **C** male tarsus and metatarsus III **D** male tarsus and metatarsus IV, lateral view **E** male tarsus and metatarsus IV, medial view **F** female tarsus and metatarsus IV. Scale bars: 100 μm.

Measurements from male paratype of leg articles from proximal to distal (in mm): leg I 0.15, 0.50, 0.27, 0.38, 0.19, 0.43; leg II 0.17, 0.39, 0.19, 0.31, 0.12, 0.34; leg III [trochanter damaged], [femur damaged] 0.20, 0.25, 0.11, 0.27; leg IV [trochanter damaged], 0.41, 0.23, 0.33, 0.13, 0.37. Width measurements from male paratype of

leg articles from proximal to distal (in mm): leg I [trochanter damaged], 0.18, 0.18, 0.17, 0.15, 0.22; leg II [trochanter damaged], 0.15, 0.16, 0.17, 0.13, 0.14; leg III [trochanter damaged], 0.18, 0.17, 0.18, 0.12, 0.13; leg IV [trochanter damaged], 0.20, 0.17, 0.19, 0.17, 0.16.

Etymology. The specific epithet is a tribute to the legendary Queensland field biologist Geoff Monteith for his invaluable knowledge of Wet Tropics entomology, which guided much of our fieldwork. The authors also wish to recognize his outsize generosity and hospitality to visiting researchers. In addition, he collected many of the specimens used in this study, including the holotype for *A. monteithi* sp. n.

Austropurcellia nuda Popkin-Hall, Jay & Boyer, sp. n.

http://zoobank.org/1E45BE1D-2DA4-47AD-BAC6-063C417E05DC
Figs 30–35

Material examined. *Holotype.* Male (QM 102444 [ex QM 38118]), Black Mountain Summit, 16.644°S, 145.49°E, coll. K. Aland and G. B. Monteith 30.iv.2015, QM 38118.

Paratypes. 10 males, 19 females, 24 juveniles, same collecting data as holotype, QM 38118, Macalester SEM stubs M30.4, M30.8, M30.9.

Additional material. 2 males, 2 females, Black Mountain 17 km ESE Julatten, 16.650°S, 145.483°E, coll. G. B. Monteith, D. Yeates, and D. Cook 29.iv.1982, S 2302, QM berlesate 413, Macalester SEM stubs M20.1, M20.2.

Diagnosis. Distinguished from congeners by lack of scopula on the male anal plate, a trait shared only with *A. absens*. Anal plate is flat and entirely ungranulated; *A. absens* anal plate is convex, bilobed, and mostly granulated.

Description. Pettalid with tergite VIII bilobed (Fig. 31). Length of male holotype (Fig. 30) 2.1 mm, width at widest point in posterior third of prosoma 1.2 mm, width at ozophores 0.8 mm. Most of body surface covered in microstructure of tubercles and granules (Fig. 31). Transverse sulci present and distinct by lack of granulation (Figs 31A, 32B). Dorsal longitudinal sulcus containing elongated granules oriented flanking dorsal longitudinal sulcus (Figs 31A, 32B). Sternites ubiquitously granulated (Fig. 31B).

Ozophores relatively conical, of type III *sensu* Juberthie (1970) (Figs 31A, 33B). Coxae of legs I and II mobile, coxae of remaining legs fixed. Male coxae II–IV meeting in the midline (Fig. 31B). Male gonostome small, subtriangular, and wider than long (Fig. 31B). Spiracles circular and C-shaped with slightly recurved edges (Fig. 33A), as found in "open circle" type of Giribet and Boyer (2002). Anal region of "pettalid type" (Giribet and Boyer 2002). Male tergite VIII bilobed, with lobes ornamented dorsally and ventrally (Fig. 32). Anal plate relatively flat and ungranulated over entire surface with narrow, smooth area running vertically through center from just above the center down to the posterior margin (Fig. 32B). Scopula absent (Fig. 32B). Two anal pores visible (Fig. 32B).

Chelicerae (Fig. 34A) short and relatively robust. Proximal article of chelicerae with dorsal crest, without ventral process. Median article with prominent apodeme.

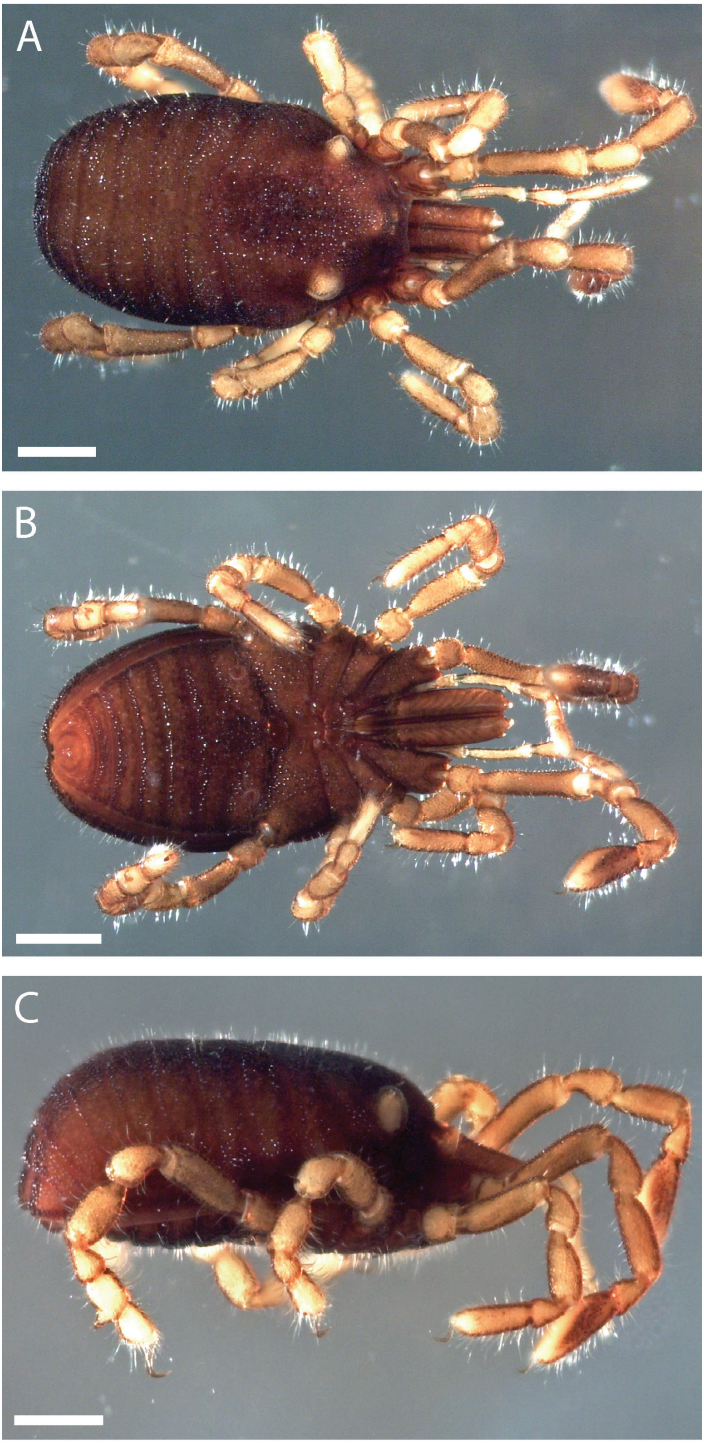


Figure 30. *Austropurcellia nuda* sp. n., holotype male, QM 102444. **A** dorsal view **B** ventral view **C** lateral view. Scale bars: 0.5 mm.

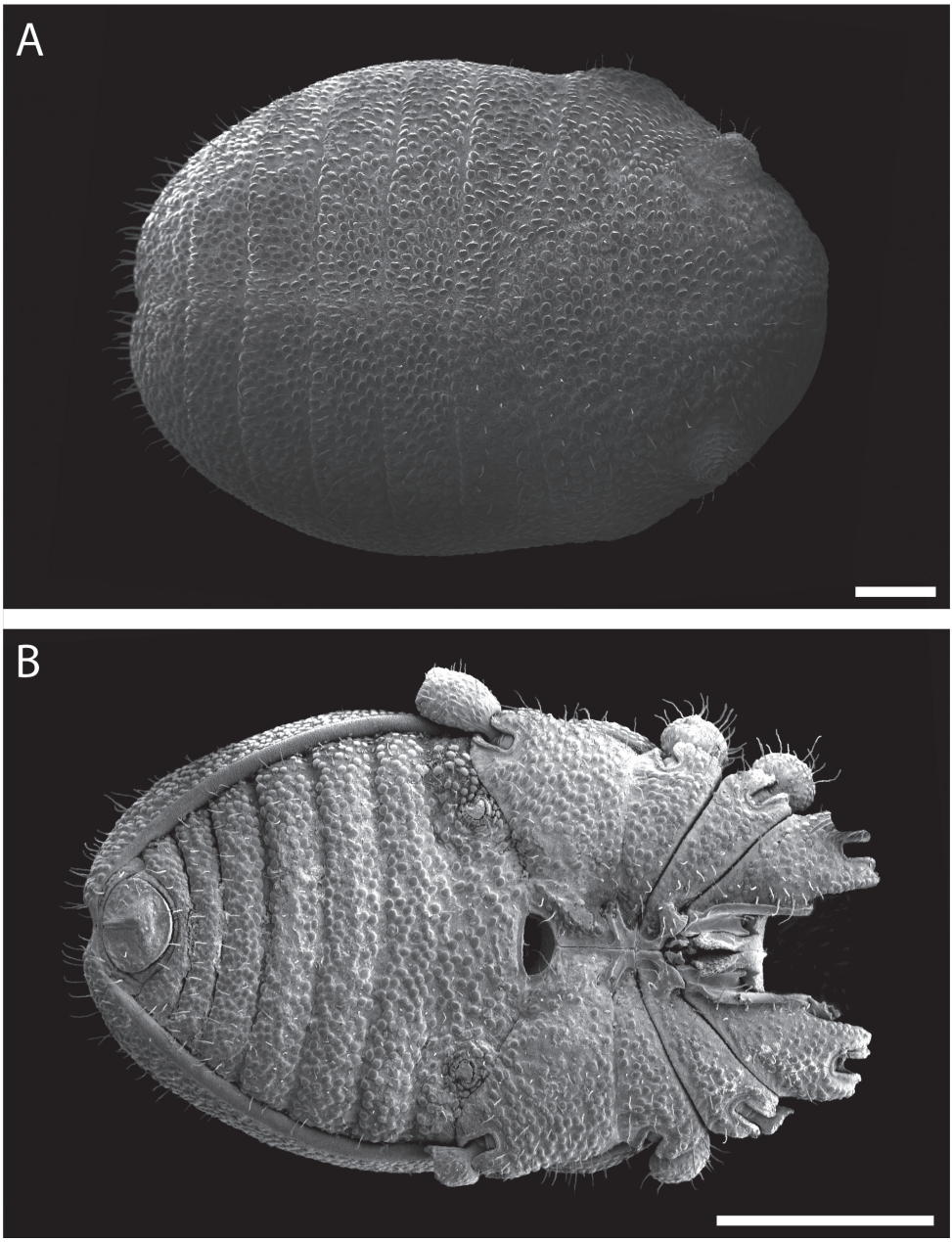


Figure 31. *Austropurcellia nuda* sp. n., males. **A** dorsal view, QM berlesate 413 **B** ventral view, QM berlesate 38118, paratype. Scale bar: 200 μm (**A**); 500 μm (**B**).

Chela with two types of dentition typical in pettalids (Fig. 34A). Measurements of cheliceral articles of male paratype from proximal to distal (in mm): 0.61, 0.84, 0.28. Palp (Fig. 34B) with prominent ventral process on trochanter. Measurements

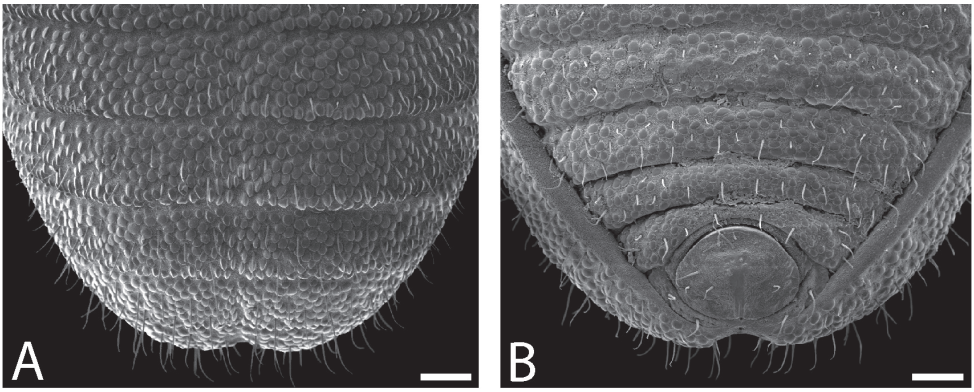


Figure 32. *Austropurcellia nuda* sp. n., males. **A** dorsal view of posterior tergites, QM berlesate 38118, paratype **B** anal plate, QM berlesate 413. Scale bars: 100 μm.

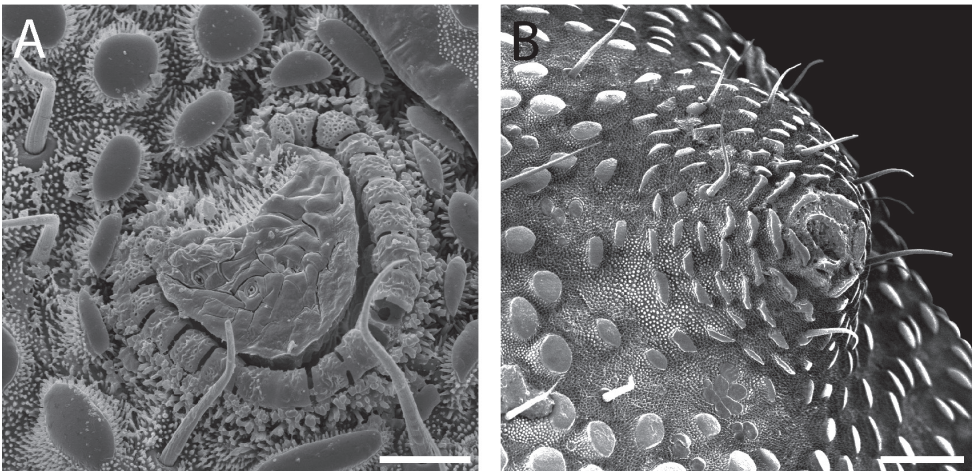


Figure 33. *Austropurcellia nuda* sp. n., males. **A** spiracle, QM berlesate 413 **B** ozophore, QM berlesate 38118, paratype. Scale bar: 20 μm (**A**); 50 μm (**B**).

of palpal articles of male paratype from proximal to distal (in mm): 0.20, 0.29, 0.18, 0.24, 0.27.

Legs with all claws smooth, without ventral dentition or lateral pegs (Fig. 35). All tarsi smooth (Fig. 35). Distinct solea present on ventral surface of tarsus I (Fig. 35A). Metatarsi I and II heavily ornamented on proximal half, with distal half smooth (Fig. 35A, B). Remaining metatarsi with full ornamentation (Fig. 35C-F). Male tarsus IV fully divided into two tarsomeres (Fig. 35D, E). Adenostyle with robust claw, wide base, and small pore at apex on lateral (external) side (Fig. 35D). Long seta rising from medial (internal) face of adenostyle from below pore to above apex (Fig. 35D, E); very short seta rising from adenostyle base below pore on lateral (external) face (Fig. 35D) (example with adenostyle features labeled, Fig. 5).

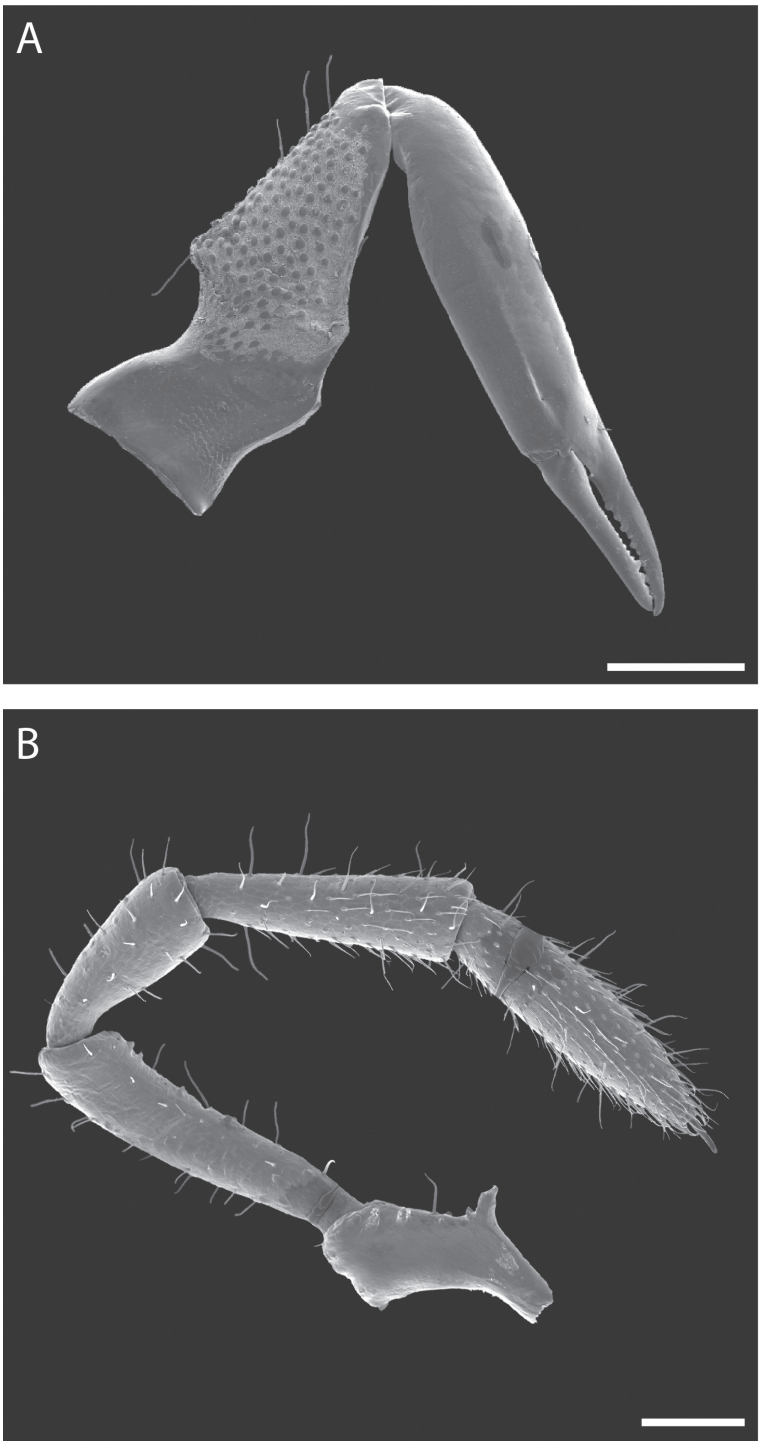


Figure 34. *Austropurcellia nuda* sp. n., males, QM berlesate 413. **A** chelicera **B** palp. Scale bar: 200 µm (**A**); 100 µm (**B**).

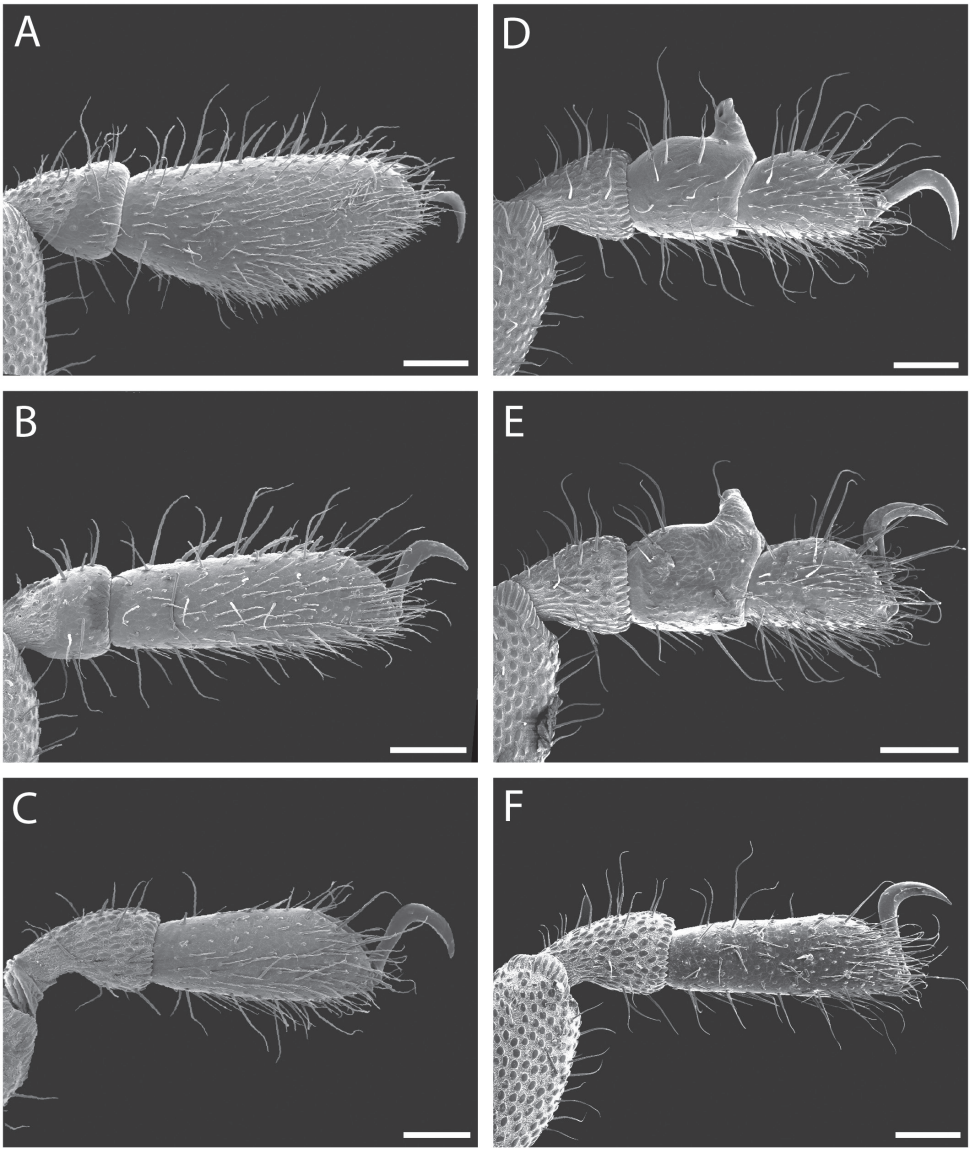


Figure 35. *Austropurcellia nuda* sp. n., males and female. **A** male tarsus and metatarsus I, QM berlesate 413 **B** male tarsus and metatarsus II, QM berlesate 413 **C** male tarsus and metatarsus III, QM berlesate 413 **D** male tarsus and metatarsus IV, lateral view, QM berlesate 38118, paratype **E** male tarsus and metatarsus IV, medial view, QM berlesate 38118, paratype **F** female tarsus and metatarsus IV, QM berlesate 413. Scale bars: 100 μm.

Measurements from male paratype of leg articles from proximal to distal (in mm): leg I 0.18, 0.52, 0.20, 0.39, 0.14, 0.41; leg II 0.15, 0.41, 0.19, 0.33, 0.14, 0.37; leg III [trochanter damaged], 0.37, 0.20, 0.29, 0.23, 0.32; leg IV 0.19, 0.44, 0.19, 0.35, 0.16, 0.39. Width measurements from male paratype of leg articles from proximal to

distal (in mm): leg I 0.20, 0.16, 0.17, 0.16, 0.14, 0.20; leg II 0.18, 0.15, 0.15, 0.15, 0.12, 0.13; leg III [trochanter damaged], 0.17, 0.16, 0.19, 0.13, 0.14; leg IV 0.18, 0.19, 0.18, 0.18, 0.15, 0.17.

Etymology. The specific epithet is derived from the first declension form of *nūdus*, from Latin, meaning "bare" or "naked", a reference to diagnostic absence of the scopula or ornamentation of the anal plate in this species.

***Austropurcellia riedeli* Jay, Oberski & Boyer, sp. n.**
http://zoobank.org/672A8F03-19C4-47CE-BD10-85156747B57F
Figs 36–41

Material examined. *Holotype.* Male (QM 102448), Rossville, Bloomfield (sample 2B, AR2), 15.792°S, 145.302°E, coll. Alex Riedel 1.v.2014.

Paratype. 1 female, Rossville, Bloomfield (sample 2B, AR2), 15.792°S, 145.302°E, coll. Alex Riedel 1.v.2014, QM 102449.

Additional material. 1 male, 2 females, Rossville, Bloomfield (sample 2, AR1), 15.792°S, 145.302°E, coll. Alex Riedel 29.iv.2014, MCZ IZ 69026, Macalester SEM stubs M30.13, M30.14, M30.15.

Diagnosis. Distinguished from congeners by flat anal plate granulated anteriorly, with very short and round scopula emerging from posterior third of plate. Lobes of tergites VIII and IX rounded and prominent in dorsal view; absence of granulation in junction of the anal plate, the lobes of tergite VIII, and the dorsal scutum. Closely resembles *A. finniganensis*, but distinguished by its larger body size (0.4 mm longer, 0.2 mm wider).

Description. Pettalid with tergite VIII bilobed (Fig. 37). Posterior margin of dorsal scutum curves ventrally (Fig. 36C). Length of male holotype (Fig. 36) 2.5 mm, width at widest point in posterior third of prosoma 1.4 mm, width at ozophores 1.0 mm. Most of body surface covered in microstructure of tubercles and granules (Fig. 37). Transverse sulci present and distinct by lack of granulation (Figs 36A, 37A). Dorsal longitudinal sulcus lacking granulation, with adjacent flanking granules oriented parallel to dorsal longitudinal sulcus (Figs 36A, 37A). Anterior edge of sternites IV and V lacking granulation medially (Fig. 37B).

Ozophores relatively conical, of type III *sensu* Juberthie (1970) (Fig. 37A, 39B). Coxae of legs I and II mobile; coxae of remaining legs fixed. Male coxae II-IV meeting in the midline (Fig. 37B). Male gonostome small, subtriangular, wider than long (Fig. 37B). Spiracles circular and C-shaped with slightly recurved edges (Fig. 39A), as found in "open circle" type of Giribet and Boyer (2002). Anal region of "pettalid type" (Giribet and Boyer 2002). Anal plate flat, posteriorly convex, with anterior granulation (Fig. 38B). Short, round scopula extruding from circular area on posterior third of anal plate and extending just past posterior margin of anal plate (Fig. 38B). Orientation of scopula obscures anal pores, which are not visible (Fig. 38B).

Chelicerae (Fig. 40A) short and relatively robust. Proximal article of chelicerae with dorsal crest, without ventral process. Median article with prominent apodeme.

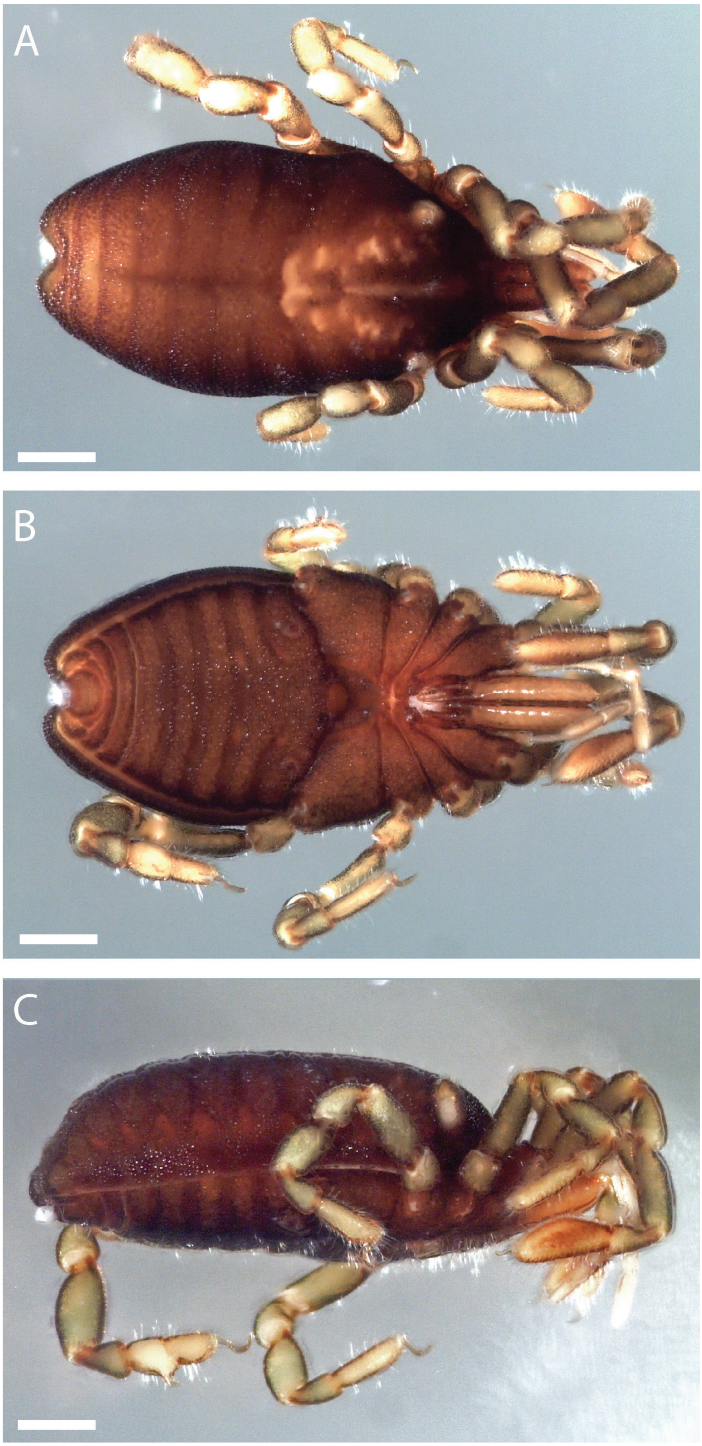


Figure 36. *Austropurcellia riedeli* sp. n., holotype male, QM 102448. **A** dorsal view **B** ventral view **C** lateral view. Scale bars: 0.5 mm.

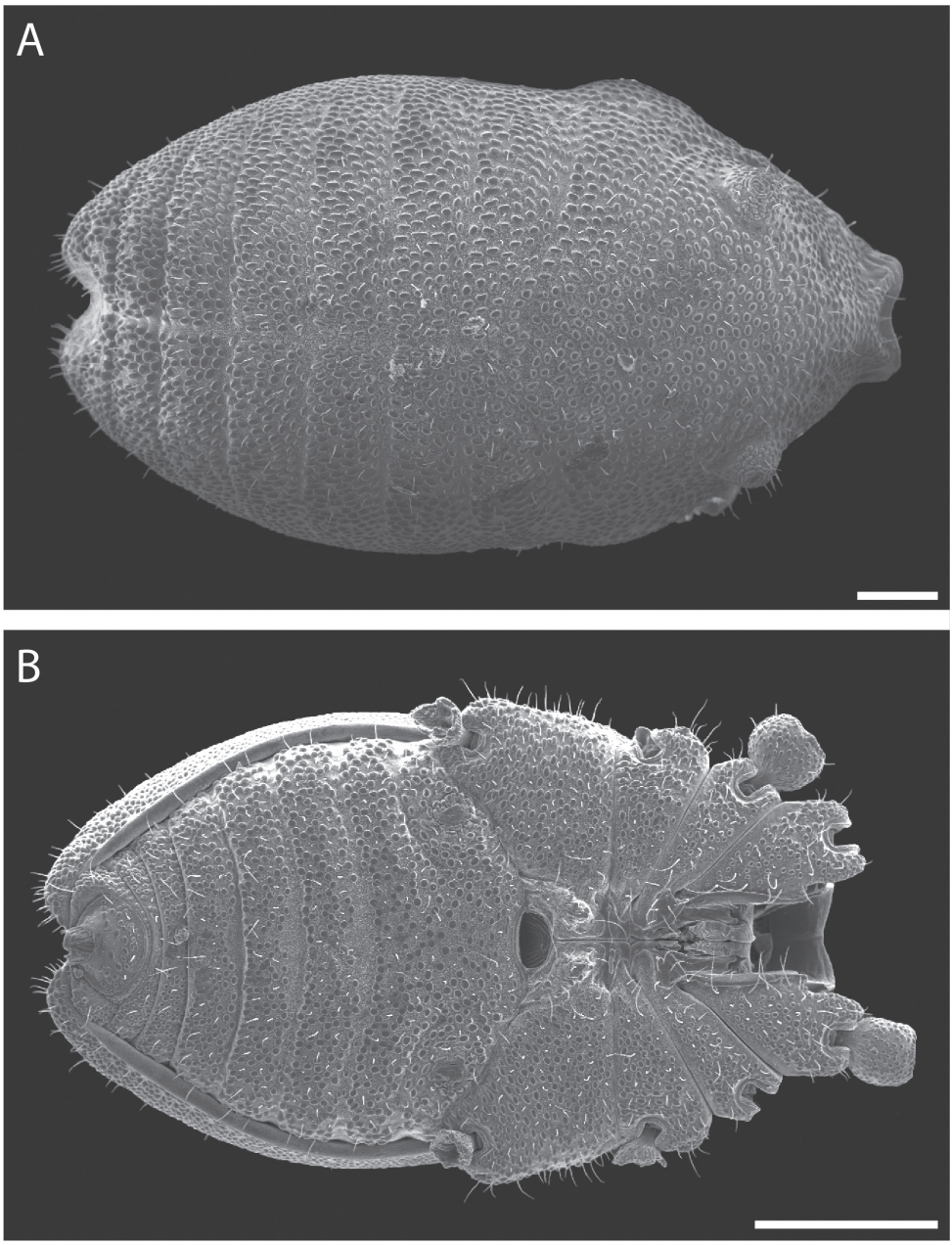


Figure 37. *Austropurcellia riedeli* sp. n., male, MCZ IZ 69026. **A** dorsal view **B** ventral view. Scale bar: 200 µm (**A**); 500 µm (**B**).

Chela with two types of dentition typical in pettalids (Fig. 40A). Measurements of cheliceral articles of male paratype from proximal to distal (in mm): 0.81, 1.02, 0.33. Palp (Fig. 40B) with prominent ventral process on trochanter. Measurements from

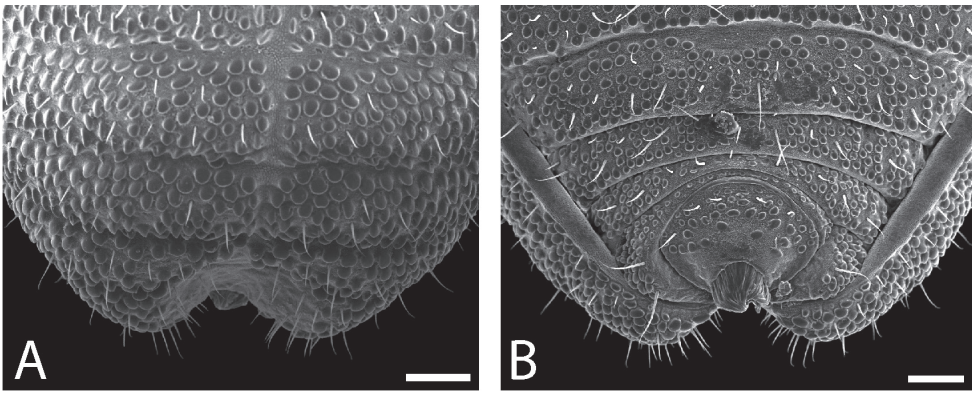


Figure 38. *Austropurcellia riedeli* sp. n., male, MCZ IZ 69026. **A** dorsal view of posterior tergites **B** anal plate. Scale bars: 100 μm.

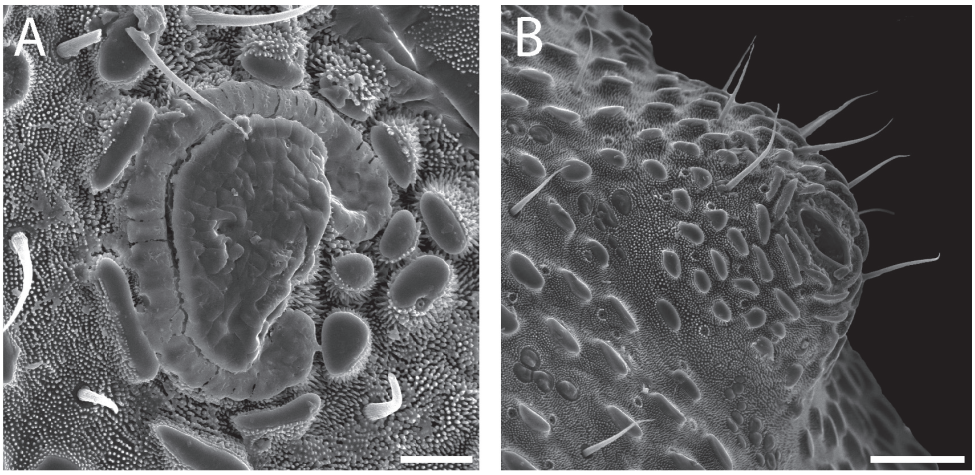


Figure 39. *Austropurcellia riedeli* sp. n., male, MCZ IZ 69026. **A** spiracle **B** ozophore. Scale bar: 20 μm (**A**); 50 μm (**B**).

palpal articles of male paratype from proximal to distal (in mm): 0.22, 0.39, 0.21, 0.35, 0.29.

Legs with all claws smooth, without ventral dentition or lateral pegs (Fig. 41). All tarsi smooth (Fig. 41). Distinct solea present on ventral surface of tarsus I (Fig. 41A). Metatarsi I and II heavily ornamented on proximal half, with distal half smooth (Fig. 40A, B). Remaining metatarsi with full ornamentation (Fig. 41C–F). Male tarsus IV fully divided into two tarsomeres (Fig. 41D, E). Adenostyle with relatively robust, pointed claw curving distally, wider base, and small pore at apex on lateral (external) side (Fig. 41D). Long seta rising from medial (internal) face of adenostyle from below pore to above apex (Fig. 41D, E); very short seta rising from adenostyle base below pore on lateral (external) face (Fig. 41D) (example with adenostyle features labeled, Fig. 5).

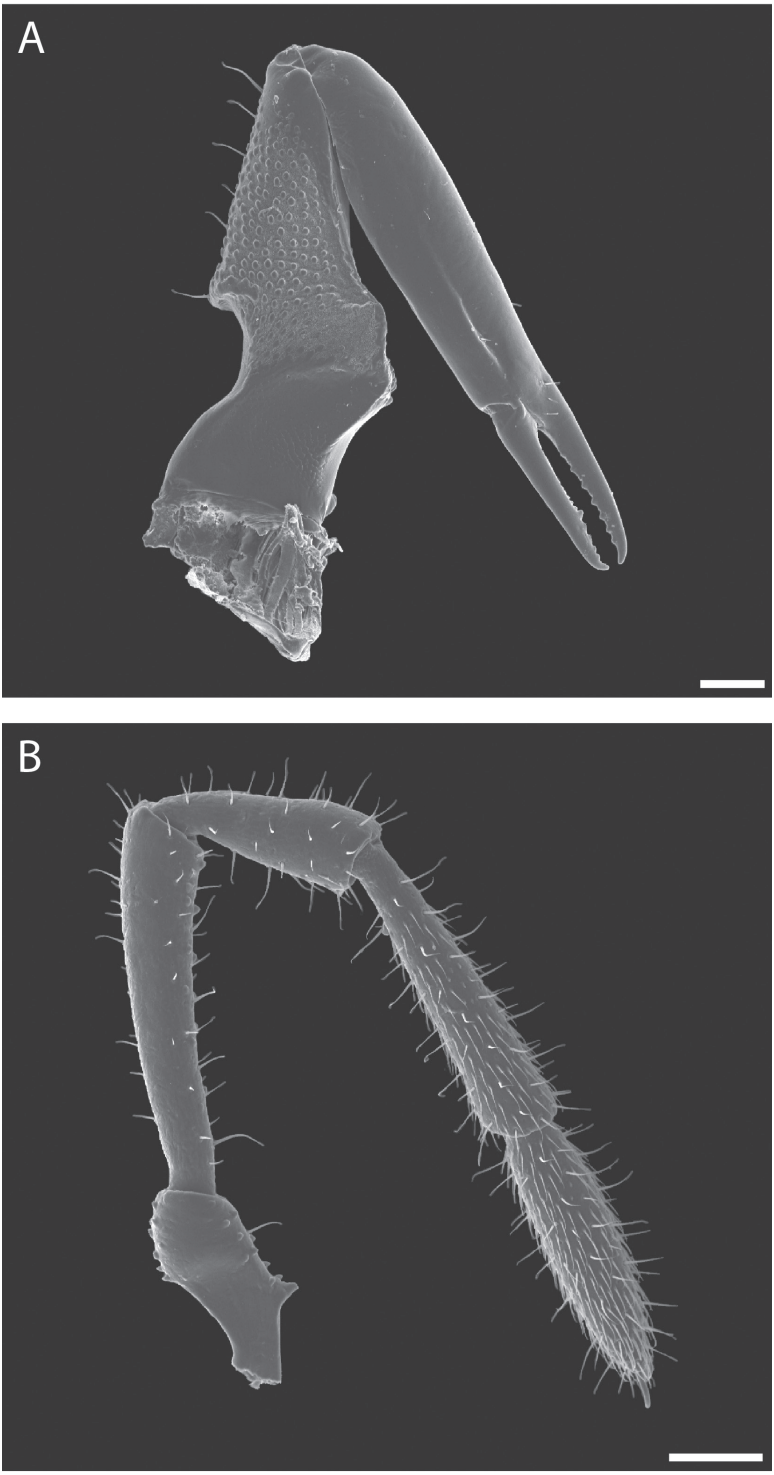


Figure 40. *Austropurcellia riedeli* sp. n., male, MCZ IZ 69026. **A** chelicera **B** palp. Scale bars: 100 μm.

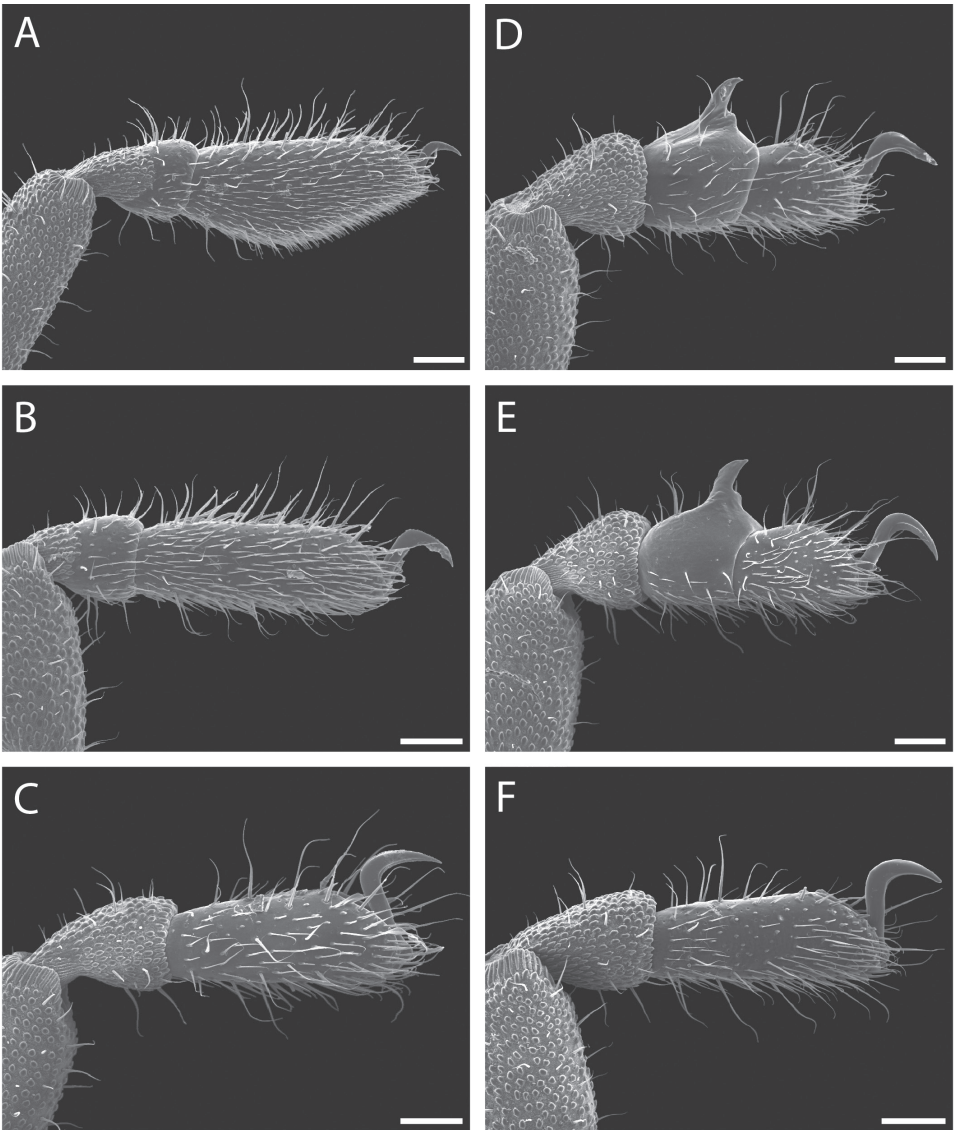


Figure 41. *Austropurcellia riedeli* sp. n., male and female, MCZ IZ 69026. **A** male tarsus and metatarsus I **B** male tarsus and metatarsus II **C** male tarsus and metatarsus III **D** male tarsus and metatarsus IV **E** male tarsus and metatarsus IV **F** female tarsus and metatarsus IV. Scale bars: 100 μm.

Measurements from male paratype of leg articles from proximal to distal (in mm): leg I 0.28, 0.64, 0.25, 0.48, 0.23, 0.46; leg II 0.22, 0.49, 0.25, 0.39, 0.16, 0.42; leg III 0.17, 0.42, 0.25, 0.35, 0.20, 0.39; leg IV 0.22, 0.61, 0.29, 0.44, 0.25, 0.41. Width measurements from male paratype of leg articles from proximal to distal (in mm): leg I 0.23, 0.21, 0.21, 0.20, 0.17, 0.23; leg II 0.21, 0.19, 0.19, 0.20, 0.15, 0.15; leg III 0.22, 0.21, 0.20, 0.20, 0.15, 0.16; leg IV 0.25, 0.23, 0.24, 0.25, 0.21, 0.20.

Etymology. This species is named after Alex Riedel, the German entomologist who provided us with several collections of animals from key localities, including Mount Finnigan.

Discussion

To develop *Austropurcellia* further as a system for studying WT biodiversity and biogeography, it is critical to describe and map the diversity of these cryptic, dispersal-limited arachnids. Significant progress has been made toward this aim; with the six new *Austropurcellia* species described here in addition to other recent work (Boyer and Reuter 2012, Popkin-Hall and Boyer 2014, Boyer et al. 2015), the majority of mite harvestman diversity throughout Queensland is presently thought to be documented. These new species bring the total number of described species within *Austropurcellia* to 25, 21 of which are found within the WT biodiversity hotspot (Fig. 1).

With a greater knowledge of the diversity of *Austropurcellia* comes greater challenge in diagnosing the genus, as previously discussed by Popkin-Hall and Boyer (2014). In their 2007 phylogenetic analysis of the family Pettalidae, Boyer and Giribet re-diagnosed *Austropurcellia* to include the presence of a scopula in the anal plate. However, we now know of two species of *Austropurcellia* that lack a scopula in the anal plate, *A. absens* (Fig. 44E) and *A. nuda* sp. n. That said, the most recent phylogenetic study of the genus indicates that this loss is secondary (i.e. not the ancestral state). The re-diagnosis identified the robustness of the adenostyle, with height no more than twice base length, as an important feature uniting *Austropurcellia*. Although this is present in many species (e.g. *A. daviesae*, Fig. 45C), there are also species within the genus that have as thin, bladelike adenostyles (e.g. *A. acuta*, Fig. 45F), and in this case the diagnostic character is likely ancestral with respect to *Austropurcellia*. Other diagnostic characters of Boyer and Giribet (2007) are still valid, including prominent ventral process on trochanter of the palp, lack of robust ventral process on the chelicera, solea in tarsus I, and male tarsus IV bisegmented dorsally to fully bisegmented (Fig. 45). However, all of these characters are shared with another lineage of pettalids, the New Zealand genus *Rakaia*. Several phylogenetic analyses of the family Pettalidae have indicated that these two genera are reciprocally monophyletic (Boyer and Giribet 2007, Boyer and Giribet 2009, Giribet et al. 2012, Boyer et al. 2015). However, those same analyses have remained equivocal about the relationship of *Austropurcellia* and *Rakaia* to each other. While there is some suggestion that they may be sister taxa (e.g. Boyer et al. 2015), support for that hypothesis is low and studies with extensive taxon sampling across the genus have suggested other possible relationships, also with low support (e.g. Giribet et al. 2012). Regardless of the relationship of *Austropurcellia* to other pettalids, it is clearly reciprocally monophyletic with all other genera. Therefore, we are left with a genus that is valid based on molecular characters and the phylogenetic criterion of monophyly, but currently lacks a robust diagnosis grounded in anatomy. Fortunately, *Austropurcellia* does not co-occur geographically with any other pettalid genus.

Differences in morphological features within the genus can provide insight into evolutionary relationships, especially in the context of geographic distributions of character states. For example, Popkin-Hall and Boyer (2014) described the geographic variation of two morphological features: adenostyle shape and posterior lobe shape in tergite VIII, and found that all species from the WT tend to have a robust, blunt adenostyle morphology (e.g. Fig. 45A, D, E, C), while those south of the WT possess a thin, blade-like adenostyle shape (e.g. Fig. 5F). The six new species described herein are all distributed within the WT, and accord with the pattern of robust, blunt adenostyles being geographically concentrated in the northern end of *Austropurcellia's* range. Previous work also demonstrates that male body shape varies with geography; species within the WT tend to have a rounded posterior, in contrast to the more triangular posterior and pointed lobes of tergite VIII found in species south of the WT. The six new species we describe support the pattern, as they all have rounded posterior lobes like those found in previously described WT species (Figs 7, 13, 19, 25, 31, 37, 42, 43, 44). However, a handful of species arguably defy this trend—*A. giribeti* and *A. articosa* (Fig. 42D, F), both found in the northern WT, share the more triangular posterior shape typically found in species south of the WT, while *A. superbensis* from Southeast Queensland has a more rounded posterior shape.

Further defining characters that vary significantly between species include the size, position, and shape of both the anal plate and the scopula (Figs 42–44). The anal plate is flat in some species and convex to a variable degree in others. Scopula morphology varies in terms of its position on the anal plate and its size; in some species it emerges from the anterior portion of the anal plate while in others it emerges near the posterior margin of the anal plate. Species found in the northernmost WT tend to have a long, narrow scopula that emerges from the posterior end of the anal plate and is oriented parallel to the body (Fig. 42). Although they both possess a somewhat shorter scopula, both *A. finniganensis* sp. n. and *A. riedeli* sp. n. (blue and green points, respectively, on Fig. 2) have the same scopula orientation and scopula placement within the anal plate as other northernmost WT species (Fig. 42A, C). *A. fragosa* sp. n. (white points on Fig. 2) has a unique scopula and anal plate shape that distinguishes it from all other *Austropurcellia* species found in the WT (Fig. 42B). Within species found further south in the rest of the WT, there is much greater variation in scopula morphology (Fig. 43, 44). However, the scopula emerges from the center or near the anterior margin of the anal plate in all of these species, distinguishing them from the northernmost WT species. One exception is a *A. nuda* sp. n., (blue points on Fig. 3) which lacks a scopula on its anal plate (Fig. 32B), a trait that it shares only with *A. absens*.

While this pattern of geographic distributions of shared characters states suggests closer relationships of geographically proximate species, an alternative interpretation is that unrelated species in certain regions of the WT have experienced morphological convergence. However, Boyer et al. (2015, 2016) found a distinct correlation between phylogenetic position within *Austropurcellia* and geographic proximity of species' ranges, as inferred from a four-gene phylogeny of the genus; species that formed monophyletic or paraphyletic groups in the phylogeny were also recovered in close

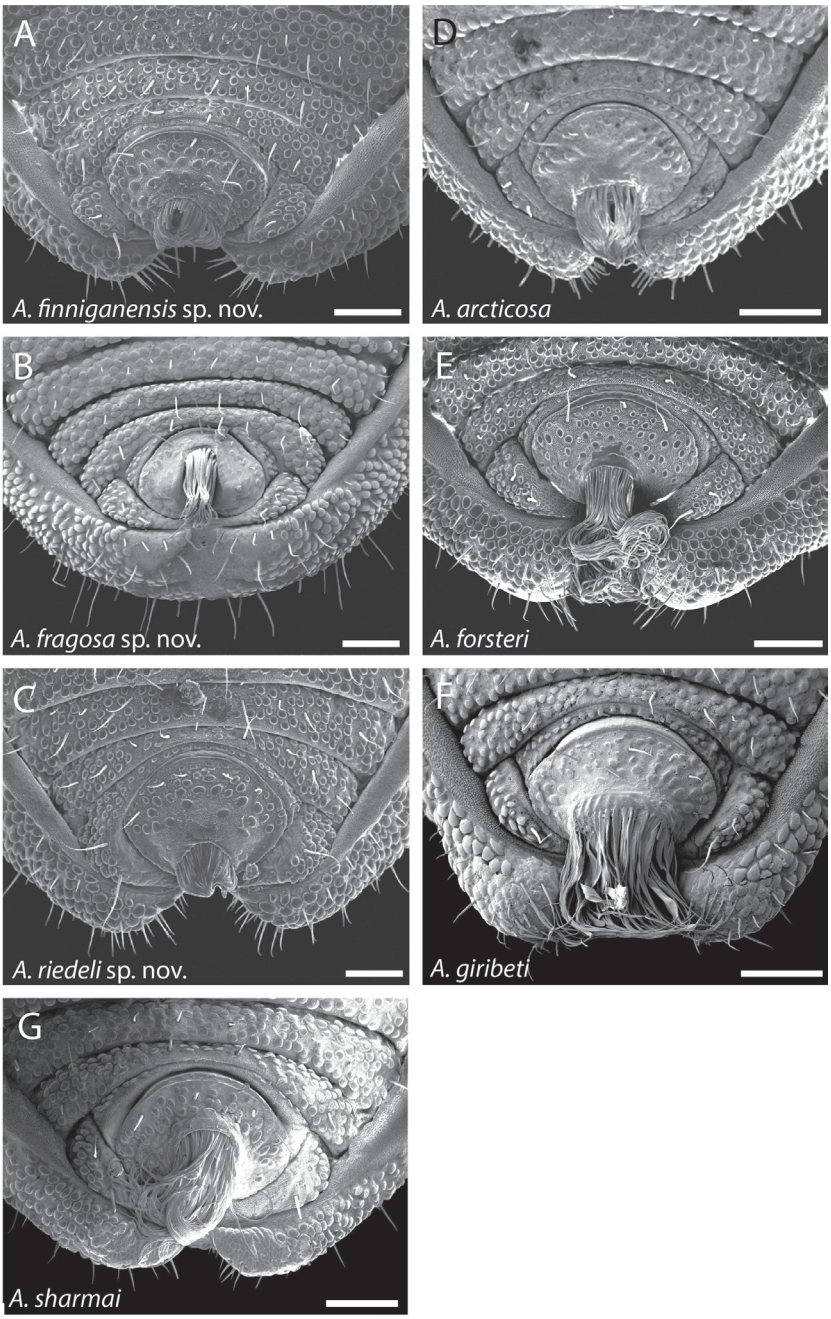


Figure 42. Ventral posterior morphology and anal plate of all seven species from the northernmost WT. Three new species are shown on left, with three previously described species on right. **A** *A. finniganensis* from Mt. Finnigan, QM 102447 (paratype) **B** *A. fragosa* from Roaring Meg Creek, QM berlesate 448 **C** *A. riedeli* from Rossville, Boomfield, MCZ IZ 69026 **D** *A. arcticosa*, from Dubuji Boardwalk, MCZ IZ 132327 **E** *A. forsteri* from Cape Tribulation Top Camp, QM berlesate 486 **F** *A. giribeti* from Alexandra Range, QM berlesate 252 **G** *A. sharmai* from Emmagen Creek, MCZ IZ 132317. Scale bars: 100 μm.

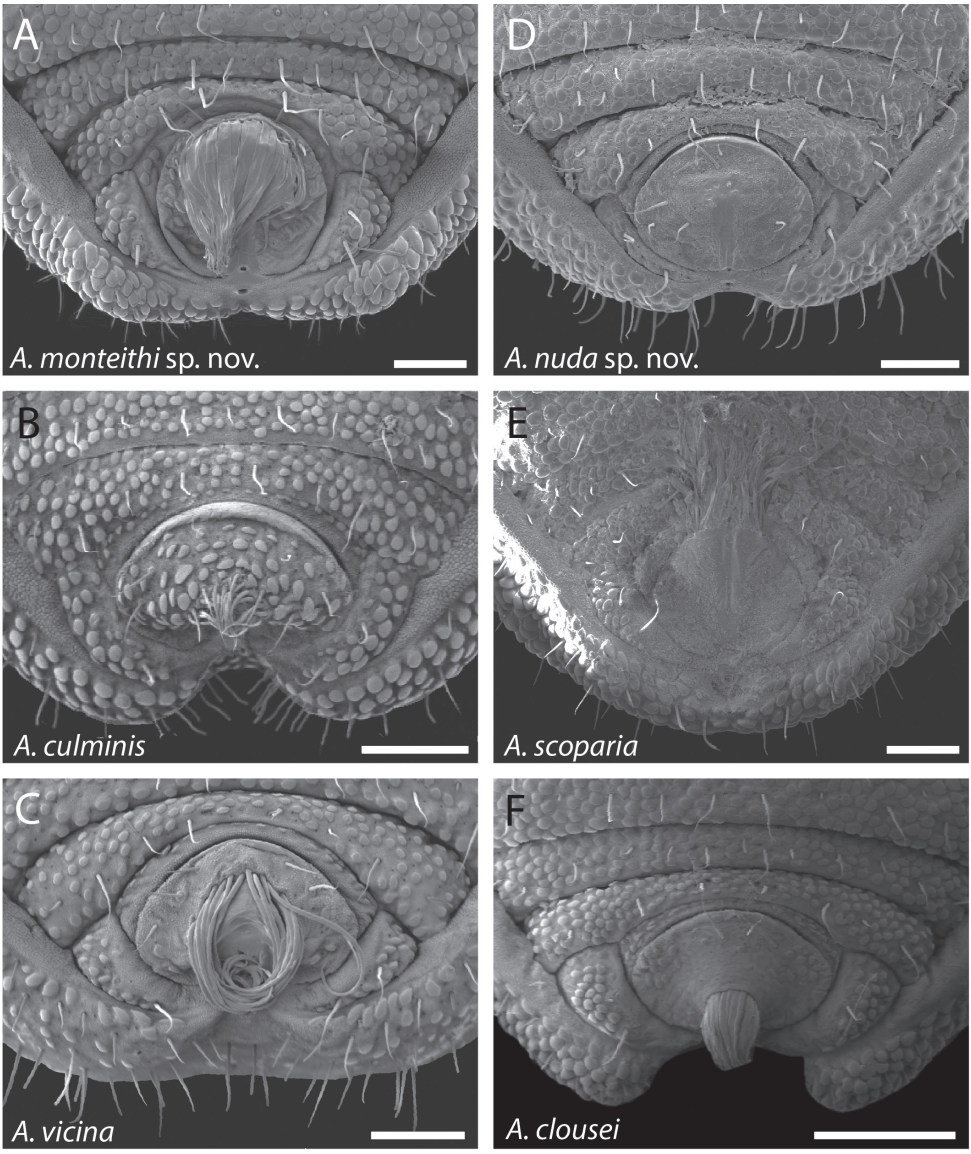


Figure 43. Ventral posterior morphology and anal plate of all five species from the north-central WT and one species from the southern WT (*A. clousei*). Two new species are shown on top row, with four previously described species below. **A** *A. monteithi*, from Kahlpahlim Rock (Lambs Head) Trail trailhead, QM 102443 (paratype) **B** *A. culminis* from Bellenden Ker Summit, MHNG **C** *A. vicina* from Crystal Cascades, ANIC berlesate 679 **D** *A. nuda*, from Black Mountain, QM berlesate 38118 **E** *A. scoparia* from Mt Spurgeon, QM S35834 **F** *A. clousei* from Paluma Range National Park, MCZ IZ 132339 (paratype). Scale bars: 100 μm.

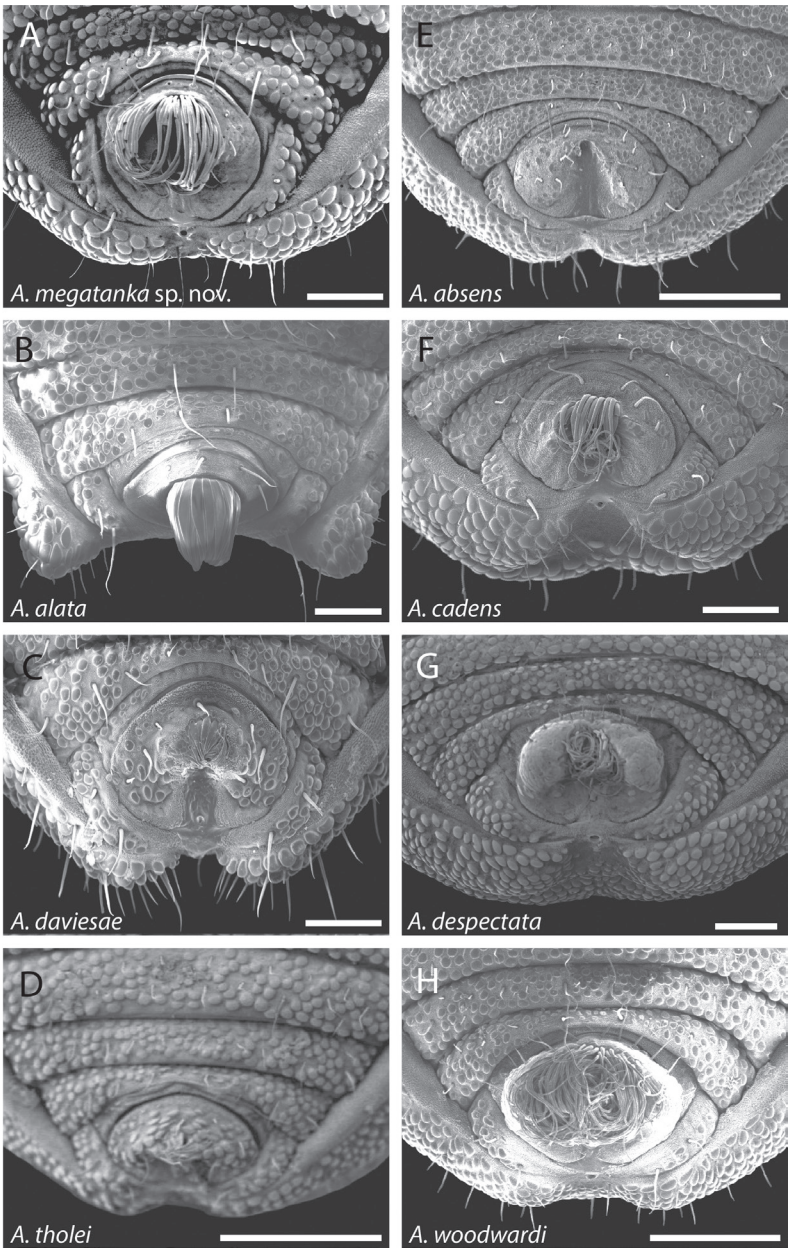


Figure 44. Ventral posterior morphology and anal plate of all eight species from the central WT uplands. One new species is shown alongside seven previously described species. **A** *A. megatanka* from Baldy Mountain Road, QM 102441 (paratype) **B** *A. alata* from Upper Boulder, QM berlesate 829 **C** *A. daviesae* from Ella Bay, MCZ IZ 132343 **D** *A. tholei* from Cathedral Fig Tree, Danville State Forest, MCZ IZ 132330 (paratype) **E** *A. absens* from Range Road, Kirrama Range, MCZ IZ 132316 (paratype) **F** *A. cadens* from Mt. Bartle Frere, CAS HW0038 **G** *A. despectata* from Millaa Millaa, ANIC berlesate 674 **H** *A. woodwardi* from Boulder Creek, QM 1742. Scale bar: 100 µm (**A, B, C, F, G**); 200 µm (**H**); 250 µm (**D**).

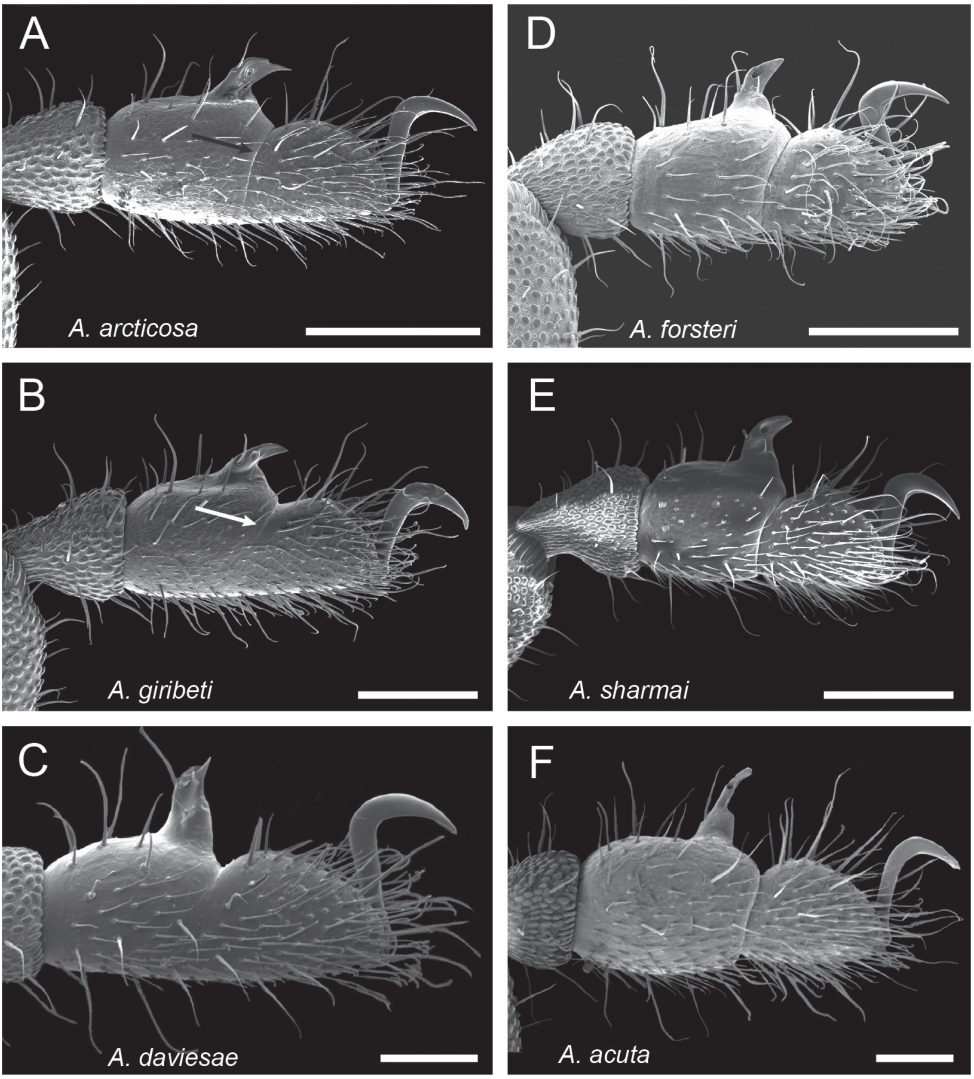


Figure 45. Fourth tarsus of male in lateral (external) view. **A** *A. articosa* from Dubuji Boardwalk, MCZ IZ 132327 **B** *A. giribeti* from Daintree Village, MCZ IZ 132344 **C** *A. daviesae* from Ella Bay, MCZ DNA 106378 **D** *A. forsteri* from Cape Tribulation Top Camp, QM berlese 486 **E** *A. sharmai* from Emmagen Creek, MCZ IZ 132698 **F** *A. acuta* from Bonyee Walk, MCZ IZ 132701. Scale bar: 100 μm (**A, B, C, F**); 250 μm (**D, E**).

proximity to one another on distribution maps. Distinct geographic and phylogenetic groups emerged from the northernmost WT, the north-central WT, the central WT uplands, and the southern WT (Boyer et al. 2015) (Figs 1–4). Based upon these trends reported by Boyer et al. (2015), we postulate that geographic distance serves as a generally reliable proxy for phylogenetic affinity in *Austropurcellia*. Using distribution maps of the new species described here, we predict below the putative clades to which the

new species described herein would belong, and used the distribution of shared morphological character states as a corroborative litmus test for inferred relationships.

Two of the new species described here have already been included in a recent molecular phylogenetic analysis (Boyer et al. 2016). One of them, *A. megatanka* sp. n., is found at the top of Mt. Baldy as well as several localities within the Lamb Range including Mt. Tiptree, Mt. Haig, and a CSIRO trail in Danbulla State Forest (Fig. 4). These sites in the center of the Atherton Tablelands place *A. megatanka* sp. n. in the northern end of the central WT uplands region (Fig. 4), suggesting that its closest relatives should include *A. daviesae*, *A. cadens*, and *A. tholei*. Apropos, *A. megatanka* sp. n. is similar to these species with regard to overall body shape and degree of tarsus IV bisegmentation, with its unusually long and wide scopula being its main distinguishing feature (Figs 19, 20, 44). Boyer et al. (2016) found that *A. megatanka* (identified as "*Austropurcellia* sp. n. Baldy Mt.) is indeed a member of a clade that also includes *A. daviesae*, *A. cadens*, and *A. tholei*.

The other species whose phylogenetic relationships have already been investigated is *A. monteithi* sp. n., which is known from five localities throughout the Lamb Range (Davies Creek, Chujeba Peak summit, Mt. Edith summit, Mt. Williams summit, and the Kahlpahlim Rock trail), geographically placing this species in the north-central WT (Fig. 3). Boyer et al. (2016) found that this species (identified as "*Austropurcellia* sp. n. Lamb Range") is related, as we would expect based on geography and morphology, as a member of a clade that also includes *A. culminis*, *A. scoparia*, and *A. vicina*. *A. nuda* sp. n. is found from only two localities (Black Mtn. and Black Mtn. summit), both located in the center of the distribution of this same north-central WT group (Fig. 3). We expect that it, too, is a close relative of *A. culminis*, *A. scoparia*, and *A. vicina*. Both of these new species share morphological features such as body shape and adenostyle morphology with the north-central WT group, with the main exception again being differences in the scopula and anal plate. *A. monteithi* sp. n. has a very long and wide scopula and *A. nuda* sp. n. lacks a scopula entirely, both in contrast to the relatively narrow, short scopula of *A. vicina* and *A. culminis* and the very unusual scopula of *A. scoparia*, which originates from the anterior margin of the anal plate (Figs 25, 26, 31, 32, 43).

A. finniganensis sp. n., *A. fragosa* sp. n., and *A. riedeli* sp. n. all have distributions in the northernmost WT (Fig. 2). *A. finniganensis* sp. n. is found in two localities on Mt. Finnigan, *A. fragosa* sp. n. is found in three very proximate localities by Roaring Meg Creek as well as a locality in the McDowall Range, and *A. riedeli* sp. n. was collected from a single locality along the Rossville-Bloomfield Road (Fig. 2). This suggests that these three species may be closely related to the other northernmost WT species such as *A. articosa*, *A. giribeti*, *A. forsteri* Juberthie, 2000 and *A. sharmai*, which have been found to form a paraphyletic grade at the base of the WT endemic clade within *Austropurcellia* (Boyer et al. 2015). This prediction is partially supported by morphology; *A. finniganensis* sp. n., *A. riedeli* sp. n. and *A. fragosa* sp. n. all share morphological features such as body shape with *A. sharmai* and *A. forsteri*, but they lack the more pointed, elongated body shape shared by *A. arcticosa* and *A. giribeti*. *A. finniganensis* sp. n., *A. fragosa* sp. n., and *A. riedeli* sp. n. also share another trait that central WT species lack—a very defined, ungranulated dorsal medial sulcus (Figs 7, 8, 13, 14, 37, 38, 42).

The WT is an important system for studying patterns and causes of rainforest endemism in both vertebrates and invertebrates. Yeates et al. (2002) investigated patterns and levels of endemism in 274 flightless insects at the scale of the 23 subregions within the WT (as defined by Winter 1984 and Williams and Pearson 1997). They found that 50% of species were endemic not only to the WT, but also to a single subregion within the WT, compared to only 15% subregional endemism within WT vertebrates (Yeates et al. 2002). Four subregions were found to contain the highest levels of subregional endemism in flightless insects: Finnigan Uplands, Carbine Uplands, Bellenden Ker/Bartle Frere Uplands, and Atherton Uplands (Yeates et al. 2002). When distributional data for the new species described here are combined with unpublished data collected in the lab of author SLB, we find that *Austropurcellia* conforms to the patterns found in other small flightless arthropods, with 50% of species endemic to single subregions. Only two of the 23 subregions contain more than one subregional endemic mite harvestman: Atherton Uplands and Finnigan Uplands. Of the six new species presented here: *A. finniganensis* sp. n. and *A. riedeli* sp. n. are both from the Finnigan Uplands, and indeed all of the species except for *A. megatanka* are subregional endemics. Williams and Pearson (1997) articulated the hypothesis that the distribution of diversity and endemism across WT subregions could be explained by differential extinction during Pleistocene glacial cycling, when rainforest persisted in some subregions but was extirpated from others. Work in the lab of SLB modeling historical distribution of climatic conditions suitable for *Austropurcellia* from the Last Glacial Maximum to the present confirms that this pattern holds for our system (Boyer et al. 2016).

These predictions, and the postulated covariance of geographic distance, morphology, and phylogenetic relatedness in Cyphophthalmi more broadly, should be tested in the future using multilocus molecular phylogenies including the new species of *Austropurcellia* described herein. Such an approach would enable quantification of phylogenetic signal inherent to male morphological characters such as scopula and adenostyle shape, toward integrative taxonomy of mite harvestmen. Two putative new species from the WT remain undescribed due to incomplete collections and a lack of sufficient data, both in terms of morphology and DNA; however, we are confident that the majority of Queensland's mite harvestmen diversity has now been documented. As we continue to approach complete species-level sampling of *Austropurcellia*'s extant diversity, we anticipate this genus will serve as robust model system to test hypotheses of how climatic and geologic changes in the WT have affected the distribution of genetic diversity, and how such processes leave their signature in the evolutionary history of Queensland's paleoendemic fauna.

Acknowledgements

We thank Geoff Monteith, Kieran Aland, and Alex Riedel for their collecting efforts. Jeff Thole provided invaluable technical support for SEM work. Rachel Quay and

Macalester students enrolled in the Spring 2015 iteration of Biology 476: Research in Biodiversity and Evolution (Mito Imagawa, Joanne Johnson, Kaelyn Lemon, Kenji Shoemaker, and Mo Usavage) provided help with SEM imaging and discussion of the project. We also thank curators who lent material: Gonzalo Giribet (Harvard MCZ), Beth Mantle (Australian National Insect Collection), and Robert Raven (Queensland Museum). Sharon Jansa and Mike Anderson provided helpful discussion of the undergraduate honors thesis of ZRPH, which formed the basis of several of the species descriptions. Ralph Holzenthal assisted with imaging of holotype specimens. Joanne Johnson assisted with generation of maps in ArcGIS. Ligia Benavides and Ron Clouse reviewed a previous version of the manuscript and offered helpful feedback that improved our work. This project was supported by NSF grant DEB-1020809 to SLB and a grant from the Macalester College Keck Summer Research Fund to KRJ and SLB.

References

Adam P (1992) Australian Rainforests. Clarendon Press, Oxford, 308 pp.

Bell KL, Moritz C, Moussalli A, Yeates DK (2007) Comparative phylogeography and speciation of dung beetles from the Australian Wet Tropics rainforest. Molecular Ecology 16(23): 4984–4998. doi: 10.1111/j.1365-294X.2007.03533.x

Boyer SL, Baker CM, Popkin-Hall ZR, Laukó DI, Wiesner HA, Quay RH (2015) Phylogeny and biogeography of the mite harvestmen (Arachnida: Opiliones: Cyphophthalmi) of Queensland, Australia, with a description of six new species from the rainforests of the Wet Tropics. Invertebrate Systematics 29(1): 37–70. doi: 10.1071/IS14025

Boyer SL, Clouse RM, Benavides LR, Sharma PP, Schwendinger PJ, Karunarathna I, Giribet G (2007) Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. Journal of Biogeography 34(12): 2070–2085. doi: 10.1111/j.1365-2699.2007.01755.x

Boyer SL, Giribet G (2009) Welcome back New Zealand: regional biogeography and Gondwanan origin of three endemic genera of mite harvestmen (Arachnida, Opiliones, Cyphophthalmi). Journal of Biogeography 36(6): 1084–1099. doi: 10.1111/j.1365-2699.2009.02092.x

Boyer SL, Giribet G (2007) A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. Cladistics 23: 337–361. doi: 10.1111/j.1096-0031.2007.00149.x

Boyer SL, Karaman I, Giribet G (2005) The genus *Cyphophthalmus* (Arachnida, Opiliones, Cyphophthalmi) in Europe: A phylogenetic approach to Balkan Peninsula biogeography. Molecular Phylogenetics and Evolution 36: 554–567. doi: 10.1016/j.ympev.2005.04.004

Boyer SL, Markle TM, Baker CM, Luxbacher AM, Kozak KH (2016) Historical refugia have shaped biogeographical patterns of species richness and phylogenetic diversity in mite harvestmen (Arachnida, Opiliones, Cyphophthalmi) endemic to the Australian Wet Tropics. Journal of Biogeography. doi: 10.1111/jbi.12717

Boyer SL, Reuter CN (2012) New species of mite harvestmen from the Wet Tropics of Queensland, Australia, with commentary on biogeography of the genus *Austropurcellia* (Opiliones: Cyphophthalmi: Pettalidae). Journal of Arachnology 40: 96–112. doi: 10.1636/Ha11-44.1

Byrne M, Yeates DK, Joseph L, Kearney M, Bowler J, Williams MAJ, Cooper S, Donnellan SC, Keogh JS, Leys R, Melville J, Murphy DJ, Porch N, Wyrwoll K-H (2008) Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. Molecular Ecology 17: 4398–4417. doi: 10.1111/j.1365-294X.2008.03899.x

Clouse RM, Giribet G (2007) Across Lydekker's Line - first report of mite harvestmen (Opiliones: Cyphophthalmi: Stylocellidae) from New Guinea. Invertebrate Systematics 21(3): 207–227. doi: 10.1071/IS06046

Clouse RM, Giribet G (2010) When Thailand was an island - the phylogeny and biogeography of mite harvestmen (Opiliones, Cyphophthalmi, Stylocellidae) in Southeast Asia. Journal of Biogeography 37(6): 1114–1130. doi: 10.1111/j.1365-2699.2010.02274.x

Clouse RM, Sharma PP, Stuart JC, Davis LR, Giribet G, Boyer SL, Wheeler WC (2016) Phylogeography of the harvestman *Metasiro* (Arthropoda, Arachnida, Opiliones) reveals a potential solution to the Pangean paradox. Organisms Diversity and Evolution 16(1): 167–184. doi: 10.1007/s13127-015-0233-7

Crisp M, Cook L, Steane D (2004) Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present–day communities? Philosophical Transactions of the Royal Society of London B: Biological Sciences 359(1450): 1551–1571. doi: 1098/rstb.2004.1528

Giribet G, Boyer SL (2002) A cladistic analysis of the Cyphophthalmid genera (Opiliones, Cyphophthalmi). Journal of Arachnology 30: 110–128. doi: 10.1636/0161-8202(2002)030[0110:ACAOTC]2.0.CO;2

Giribet G, Sharma PP, Benavides LR, Boyer SL, Clouse RM, De Bivort BL, Dimitrov D, Kawauchi GY, Murienne J, Schwendinger PJ (2012) Evolutionary and biogeographical history of an ancient and global group of arachnids (Arachnida: Opiliones: Cyphophthalmi) with a new taxonomic arrangement. Biological Journal of the Linnean Society 105: 92–130. doi: 10.1111/j.1095-8312.2011.01774.x

Graham CH, Moritz C, Williams SE (2006) Habitat history improves prediction of biodiversity in rainforest fauna. Proceedings of the National Academy of Sciences of the United States of America 103(3): 632–636. doi: 10.1073/pnas.0505754103

Graham CH, VanDerWal J, Phillips SJ, Moritz C, Williams SE (2010) Dynamic refugia and species persistence: tracking spatial shifts in habitat through time. Ecography 33(6): 1062–1069. doi: 10.1111/j.1600-0587.2010.06430.x

Juberthie C (1970) Les genres d'opilions Sironinae (Cyphophthalmes). Bulletin du Muséum National d'Histoire Naturelle 41: 1371–1390.

Juberthie C (1971) Les opilions cyphophthalmes cavernicoles. Notes sur *Speleosiro argasiformis* Lawrence. Bulletin du Muséum National d'Histoire Naturelle 42: 864–871.

Kershaw AP (1994) Pleistocene vegetation of the humid tropics of northeastern Queensland, Australia. Palaeogeography, Palaeoclimatology, Palaeoecology 109(2): 399–412. doi: 10.1016/0031-0182(94)90188-0

Nix H (1991) Biogeography, pattern and process. In: Nix H, Switzer M (Eds) Rainforest Animals – Atlas of Vertebrates Endemic to Australia's Wet Tropics. Australian National Parks and Wildlife Service, Canberra, 11–41.

Popkin-Hall ZR, Boyer SL (2014) New species of mite harvestmen from southeast Queensland, Australia greatly extend the known distribution of the genus *Austropurcellia* (Arachnida, Opiliones, Cyphophthalmi). Zootaxa 3827(4): 517–541. doi: 10.11646/zootaxa.3827.4.5

Rix MG, Harvey MS (2012) Phylogeny and historical biogeography of ancient assassin spiders (Araneae: Archaeidae) in the Australian mesic zone: Evidence for Miocene speciation within Tertiary refugia. Molecular Phylogenetics and Evolution 62: 375–396. doi: 10.1016/j.ympev.2011.10.009

Schneider CJ, Cunningham M, Moritz C (1998) Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. Molecular Ecology 7: 487–498. doi: 10.1046/j.1365-294x.1998.00334.x

Truswell EM (1993) Vegetation in the Australian Tertiary in response to climatic and phytogeographic forcing factors. Australian Systematic Botany 6(6): 533–557. doi: 10.1071/SB9930533

Webb L, Tracey J (1981) Australian rainforests; pattern and change. In: Keast JA (Ed.) Ecological Biogeography of Australia. W. Junk Publishers, The Hague, 607–694.

Williams SE (2006) Vertebrates of the Wet Tropics Rainforests of Australia: Species Distributions and Biodiversity. Cooperative Research Centre for Tropical Rainforest Ecology and Management, Cairns, Australia, 282 pp.

Williams SE, Pearson RG (1997) Historical rainforest contractions, localized extinctions and patterns of vertebrate endemism in the rainforests of Australia's Wet Tropics. Proceedings: Biological Sciences 264: 709–716.

Winter J (1984) Conservation studies of Australian rainforest possums. In: Smith A, Hume I (Eds) Possums and Gliders. Australian Mammal Society, Sydney.

Yeates DK, Bouchard P, Monteith GB (2002) Patterns and levels of endemism in the Australian Wet Tropics rainforest: evidence from flightless insects. Invertebrate Systematics 16: 605–619. doi: 10.1046/j.1365-294x.1998.00334.x

ZooKeys 586: 95–119 (2016)
doi: 10.3897/zookeys.586.8209
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Position of the *dentifera*-group in the *Coronatella*-branch and its relocation to a new genus: *Magnospina* gen. n. (Crustacea, Chydoridae, Aloninae)

Francisco Diogo R. Sousa[1,2], Lourdes Maria Abdu Elmoor-Loureiro[2], Sandro Santos[1]

1 *Núcleo de Estudos em Biodiversidade Aquática, Programa de Pós-graduação em Biodiversidade Animal, Universidade Federal de Santa Maria – UFSM, Av. Roraima 1000, Camobi, CEP 97105-900, Santa Maria, RS, Brazil* **2** *Laboratório de Biodiversidade Aquática, Universidade Católica de Brasília – UCB, QS7 lote 1, Bloco M, sala 204, CEP 71966-700, Taguatinga, DF, Brazil*

Corresponding author: *Francisco Diogo R. Sousa* (sousa_bio@yahoo.com.br; fdiogo.rs@gmail.com)

Academic editor: *A. Myers* | Received 22 February 2016 | Accepted 2 April 2016 | Published 4 May 2016

http://zoobank.org/D247100A-6343-4E19-BCDA-93B8E3122D10

Citation: Sousa FDR, Elmoor-Loureiro LMA, Santos S (2016) Position of the *dentifera*-group in the *Coronatella*-branch and its relocation to a new genus: *Magnospina* gen. n. (Crustacea, Chydoridae, Aloninae). ZooKeys 586: 95–119. doi: 10.3897/zookeys.586.8209

Abstract

Magnospina **gen. n.** was created to relocate species of the *dentifera*-group from *Alona* sensu lato (Crustacea: Cladocera) and include *Magnospina dentifera* **comb. n.** and *Magnospina siamensis* **comb. n.** The synapomorphies of the *Magnospina* **gen. n.** are (1) basal spines longer than 2/3 of the postabdominal claw, (2) presence of 1–4 large denticles, broad at their bases, protruding downwards, without setules between them. Morphological traits such as habitus, rostrum and postabdomen shape, armature of IDL setae, number of setae on the exopod of limb III are also important in the distinction between *Magnospina* **gen. n.** and other genera from the *Coronatella*-branch. The morphology of *Magnospina dentifera* **comb. n.** male confirms the closer relationship with the clade composed by the *elgans*-group from *Alona* sensu lato, *Ovalona* and *Leberis*, but the external morphology, morphology of the postabdominal claw, basal spine and setae 2–3 of IDL support their separation from any of the group cited. It is concluded that the *Coronatella*-lineage of Aloninae is composed of the genera *Coronatella*, *Anthalona*, *Karualona*, *Bergamina*, *Extremalona*, *Ovalona*, *Celsinotum*, *Leberis* and *Magnospina* **gen. n.** The *elegans*-group from *Alona* sensu lato also belongs to *Coronatella*-lineage, but still need formal allocation.

Keywords

Alona broaensis, *Alona dentifera*, *Alona siamensis*, *Celsinotum*, *Leberis*, male, morphology, *Ovalona*

Copyright *Francisco Diogo R. Sousa et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

The taxonomic status of *Alona dentifera* (Sars, 1901) (Crustacea: Cladocera) was discussed by Sinev et al. (2004). In this study, the authors relocated *Alonella dentifera* to the genus *Alona* Baird, 1843 based on the absence of typical morphological traits of the subfamily Chydorinae Dybowsky & Grochowski, 1894 *emend.* Frey, 1967 and presence of some morphological traits of Aloninae Dybowsky & Grochowski, 1894 *emend.* Frey, 1967. Because of the polyphyletic nature of *Alona* (Sacherová and Hebert 2003, Elmoor-Loureiro 2004, Eliáz-Gutiérrez et al. 2008; Van Damme et al. 2010), allocations of species groups to different genera were made: *Phreatalona*, which corresponds to the *protzi*-complex (Van Damme et al. 2009); *Coronatella*, which corresponds to the *rectangula*-complex (Van Damme and Dumont 2008a); *Brancelia* (Van Damme and Sinev 2011), which corresponds to the *hercegovinae*-complex; and *Anthalona* Van Damme, Sinev & Dumont, 2011, which includes species of the *verrucosa*-complex (Van Damme et al. 2011a). Most recently, Sinev (2015a) included the *pulchella*-group in *Ovalona* Van Damme & Dumont, 2008.

Likewise, the position of *A. dentifera* is doubtful because its morphology is very different from that of the "true *Alona*", which is represented by the *quadrangularis*-group only (Van Damme and Dumont 2008b; Van Damme et al. 2010). Van Damme and Dumont (2008a) suggested that *A. dentifera* belongs to a large lineage of *Alona* sensu lato, named the *Coronatella*-branch, and that it may be close to *Leberis*, as evidenced by molecular tools (Elias-Gutiérrez et al. 2008) or to the *Coronatella* genus. Although Chatterjee et al. (2013) consider *A. dentifera* as a member of the *Coronatella* genus, this species seems to be part of a group with separate evolution, together with *Alona siamensis* Sinev & Sanoamuang, 2007. *Alona dentifera* and *A. siamensis* share synapomorphies, as showed by Sinev and Sanoamuang (2007). Besides, the male morphology of *A. dentifera* is quite different from *Coronatella* and *Leberis* (see description below).

Thus, our aim is to evaluate the morphological traits of *Alona dentifera*, based on original material from Brazil and Argentina, and to describe the adult male, for the first time. Additionally, we relocate *A. dentifera* to a new genus, which also includes *A. siamensis*.

Methods

The description of the new genus was based on material collected in different localities in Brazil and Argentina (see material examined) and data from the literature (Sinev et al. 2004). The selected animals were transferred to drops of glycerol on slides and dissected under a stereomicroscope. The morphology of appendages and other structures was studied using a phase contrast microscope Olympus BX41. To enumerate the setae of limbs, we used the proposal of homology from Kotov (2000a, b), which presented stability when tested in different cladoceran groups (Kotov et al. 2010). Drawings were prepared using a camera lucida attached to a phase contrast microscope Olympus BX41.

The following abbreviations were used in the text, table and figures:

A1	antennule;
A2	antenna;
as	accessory seta;
CBS	copulatory brush seta;
en	endite;
ep	epipod;
ex	exopod;
fc	filter comb;
gfp	gnathobasic filter plate;
gn	gnathobase;
IDL	inner distal lobe;
il	inner lobe; ms: male seta;
ODL	outer distal lobe;
P1-4	limbs I-IV;
PA	postabdomen;
pep	pre-epipod;
s	sensillum.

Depository abbreviations

FDRS	Personal collection of Francisco Diogo R. Sousa;
CLLA	Slides collection of the GEEA, at Universidade Católica de Brasília, Brazil;
ZMOU	Zoological Museum of Oslo University;
ZMMU	Zoological Museum of Moscow State University.

Taxonomy

Class Branchiopoda Latreille, 1817
Order Anomopoda Sars, 1865
Family Chydoridae Dybowsky & Grochowski, 1894 *emend.* Frey, 1967
Subfamily Aloninae Dybowsky & Grochowski, 1894 *emend.* Frey, 1967

***Magnospina* gen. n.**
http://zoobank.org/8BA31D3E-9088-4642-B489-EB9DB45FE9FA

Type species of the genus. *Magnospina dentifera* comb. n. = *Alona dentifera* (Sars, 1901).

Etymology. The name "*Magnospina*" is derived from two Latin words, *magna* = large, long and *spina* = spine. The generic name refers to the long basal spine on the postabdominal claw.

Description. Parthenogenetic female. *Habitus* without dorsal keel, ovoid or with moderate lateral compression, length 0.32–0.48 mm, maximum height before the mid-length of body; body height/length about 1.3-.17. *Head* Eye and ocellus of subequal or different sizes. Rostrum short from a lateral view, wide from a frontal view, not pointed, rounded or truncated; head shield wide, with the distance between the mandibular articulations higher than length of its posterior portion, with or without ornamentation; head pores absent or presents, in last case three connected main head pores, lateral head pores minute. *Labral keel* wide, oval and naked, apex not elongated. *Carapace* ornamentation not evident, slightly punctuated or with narrow longitudinal lines; valves armed with 40–53 setae internally inserted at the ventral margin and differentiated in three groups, setae from the anterior group markedly longer than median and posterior groups; ventral margin with a distinctive rounded angle at 1/3 of the length of the margin. Anteroventral corner of valves rounded; posteroventral corner armed with 1–4 large denticles, broad at their bases, protruding downwards, without setules between them; posterodorsal corner poorly defined. Posterior margin almost straight, armed with inner setules on the carapace which are not arranged in groups. *Antennule* not exceeding the tip of the rostrum, about 2.5 times as long as it is wide; three or four rows of setules on the antennular body. Antennular sensory seta about 1/2 length of antennular body. Nine aesthetascs of different length present in a distal position not exceeding the length of antennular body. *Antenna* with formula of antennal setae 003/113, spines 101/001; first segment of endopod and exopod elongated, about two times longer than the others segments; weak setules or spicules on the segments. First exopod segment with a narrow, naked or plumose seta, with length similar or slightly longer than the branches. Spine on the first endopod segment longer than second endopod segment. Apical spines slightly longer than the apical segments or about two times shorter than the apical segment itself. Three plumose apical setae not differentiated in size among themselves. *Postabdomen* approxamately 1.3–2.5 times as long as wide, narrowing distally. Dorsal margin weakly convex or straight. Preanal angle clearly prominent; preanal, anal and postanal margins of different length; postanal margin about 1.5–1.8 longer than anal margin, armed with 9–13 marginal denticles, of which the most distal (1–4) might be individualized, proximal denticles organized in clusters; 8–10 lateral fascicles with setules relatively weak. *Postabdominal claw* inserted on the projection of postabdomen, 1.3–1.5 times longer than anal margin; spinules on the ventral margin may be present; pecten of spinules on the internal and external face of claw, median pecten with strong spinules; base of the claw armed with 1–5 long and strong spinules. *Basal spine* almost straight, remarkably long, longer than 2/3 of the postabdominal claw length, with or without spinules on the dorsal margin. *First Maxilla* with two setulated setae. *Limb I* with epipod oval, with a finger-like projection. ODL with bisegmented seta, serrated from middle portion towards the distal portion; accessory seta implanted near the base of the ODL. IDL (en 4) with two robust setae (2–3), seta (1) rudimentary or absent; IDL setae 2–3 thick, armed with thick basal denticles. Endite 3 with four setae, anterior seta (1) shorter or similar in length to posterior setae (a-b); setae (a-b) of similar or different length; a sensillum might

be present on the endite. Endite 2 with three posterior long setae (d-f) which differ strongly in length among themselves; seta (d) shorter than the seta (e), setae (e-f) with thick spinules on the lateral face; a sensillum might be present on the endite. Endite 3 armed with three posterior setae (g-i); seta (i) plumose, about 1/2 of the setae (g-h). Ejector hooks relatively short. Ventral face of the limb with six-seven groups of setules organized in clusters. *Limb II* with exopod elongated, short seta present which might be plumose, about two-three times shorter than exopod itself. Inner portion armed with eight scrapers not specialized and decreasing in length towards distal portion, but with some denticles on the scrapers 6–8; anterior soft setae absent; gnathobase armed with four elements, filter comb armed with seven setae, of which two proximal are shorter than the others. *Limb III* with pre-epipod rounded and setulated, epipod oval with a short finger-like projection. Exopod with four distal and two lateral setae; fifth and sixth setae differentiated in length, third and fourth setae long; second seta about 1.4–1.7 times longer than first setae. Setae 3–6 clearly plumose. Distal endite armed with three setae and one sensillum, setae 1–2 scraper-like of different length; third seta curved and armed with many bilaterally implanted setules (3); four plumose posterior setae present. Basal endite with four soft anterior setae increasing in length towards the gnathobase, a sensillum might be present. Gnathobase with three elements, filtercomb with seven setae. *Limb IV* with pre-epipod rounded or rectangular and setulated, epipod oval with a long finger-like projection. Exopod with six marginal setae; first and second setae long, not plumose; third seta plumose, short, about two times shorter than the second seta; fourth seta long and plumose; fifth and sixth setae plumose and with similar lengths; Distal endite with four setae (1–4), one scraper-like (1), three flaming-torch-like (3–4); flaming-torch setae not modified. Basal endite with three slightly setulated soft setae. Gnathobase armed with a setulated seta shorter than the length endite itself, filter comb with five setae. *Limb V* with pre-epipod rounded or rectangular and setulated, epipod oval with a long finger-like projection. Exopod not divided in lobes, armed with four plumose setae. Setae 2–4 of subequal lengths; first seta about two-three times shorter than the other setae. Internal lobe wide, oval and with long setules apically and laterally implanted; two setulated setae on the inner face which are shorter than the length of lobe itself. Filter comb with one or without seta. *Limb VI* absent.

Adult male. As for *Magnospina dentifera* comb. n.

Diagnosis of the genus. Parthenogenetic female. *Habitus* ovoid, without dorsal keel. *Head* with rostrum wide, not pointed; head shield wide with distance between mandibular articulations higher than length of its posterior portion, main head pores absent in adults of *M. dentifera* comb. n. or with three connected main head pores in *M. siamensis* comb. n.; lateral head pores absent (*M. detifera* comb. n.) or present (*M. siamensis* comb. n.). *Labral keel* wide and naked, apex not elongated. *Carapace* ornamentation not evident, punctuated or with narrow longitudinal lines; valves armed with 40–53 setae internally inserted at the ventral margin and differentiated in three groups, setae from the anterior group markedly longer than median and posterior groups; ventral margin with a distinctive rounded angle at 1/3 of the margin length;

posteroventral corner armed with 1–4 large denticles, broad at their bases, protruding downwards, without setules between them. *Antennule* not exceeding the tip of the rostrum, nine aesthetascs of different lengths present distally. *Antenna* with formula of antennal setae 003/113; spines 101/001; basal segments on the exopod and endopod about two times longer than the other segments; weak setules or spicules on the segments. *Postabdomen* narrowing distally, preanal angle prominent; postanal margin armed with 9–13 marginal denticles which the most distal (1–4) might be individualized, proximal denticles organized in clusters; eight-10 lateral fascicles with weak setules. *Postabdominal claw* inserted on the projection of postabdomen, longer than anal margin; spinules on the ventral margin may be present; pecten of spinules on the internal and external face of the claw, base of claw armed with 1–5 long and spinules. *Basal spine* remarkably long, longer than 2/3 of postabdominal claw length, with or without spinules on the dorsal margin (absent in *M. siamensis* comb. n.). *Limb I* with endite 1 armed with three setae (g-i); IDL (en 4) with two robust setae (2–3), seta 1 rudimentary (*M. dentifera* comb. n.) or absent (*M. siamensis* comb. n.); IDL setae 2–3 thick, armed with thick basal denticles. *Limb II* without soft setae; short seta on the exopod; scrapers not specialized, but with some denticles, especially on scrapers 6–8; gnathobase armed with four elements, filter comb armed with seven setae, of which two proximal are shorter than others. *Limb III* with six setae on the exopod, third and fourth setae long; distal endite armed with three setae and one sensillum; gnathobase with three elements, filter comb with seven setae. *Limb IV* relatively short, six setae on the exopod; third seta plumose, short, about two times shorter than the second seta; flaming-torch setae on the distal endite not modified; gnathobase armed with a setulated setae shorter than the length of endite itself, filter comb with five setae. *Limb V* relatively short, setae 3–4 of exopod subequal in length; filter comb reduced, with one short seta in *M. dentifera* comb. n. and none in *M. siamensis* comb. n.. *Limb VI* absent.

Adult male. Habitus smaller than female (Figure 27). *Postabdomen* strongly narrowing distally. *Postabdominal claw* short and ticker than female (Figures 30–31). *Basal spine* about half-length of postabdominal claw, with tip forked (Figure 31). *Limb I* with two setae on the IDL (en4), setae armed with denticles; male seta with tip slightly curved; copulatory hook with one projection on the tip (Figures 32–33).

Differential diagnosis. The synapomorphies of *Magnospina* gen. n. are (1) basal spines longer than 2/3 of postabdominal claw, (2) presence of 1–4 large denticles, broad at their bases, protruding downwards, without setules between them. *Magnospina* gen. n. can also be differentiated from the genus *Coronatella* because it has a distinctive rounded angle at 1/3 of the length of the ventral margin, marginal setae of valves differentiated in three groups, setae from anterior group markedly longer, wide rostrum, postabdomen narrowing distally; the males of *Coronatella* do not bear two lateral aesthetascs on the antennules. *Magnospina* gen. n. differs from *Anthalona* in the presence of a distinctive rounded angle at 1/3 of the length of the ventral margin, sacks underneath lateral head pores (cosmaria) being absent, shape of postabdomen, poorly developed setules of the lateral fascicles, morphology of IDL (which does not have specialized denticles), and armature of limb I; the males of *Anthalona* also do not

bear lateral aesthetascs on the antennules. The new genus differs from *Karualona* in the morphology of IDL setae, shape of the postabdomen, poorly developed setules of the lateral fascicles, seta on the exopod of limb II (present in *Magnospina* gen. n. and absent in *Karualona*) and endite basal of limb IV armed with three flaming-torch; the antennule of *Karualona* males bear just one lateral aesthetasc. *Magnospina* gen. n. is closer to *Leberis* according to Eliáz-Gutiérrez et al. (2008); however, it is distinguished by the presence of long setae on anterior group of ventral margin of the carapace, in the morphology of setae 2–3 of the IDL (Figures 18–19), presence of seta on exopod of limb II, absence of a dorsal keel (Figures 1–5), and presence of a long basal spine on postabdominal claw of postabdomen (Figures 13–16); males of *Leberis* also do not bear denticles on the posteroventral corner of valves. *Magnospina* gen. n. differs from *Celsinotum* Frey, 1991 in the absence of a dorsal keel, absence of spine-like setae on the posterior portion of valves, presence of relatively long apical (endopod and exopod) and basal (endopod) spines on the segment of the antenna, long basal spine on the postadbominal claw, absence of a rudimentary seta (i) on endite 1 of limb I (in *Magnospina* gen. n. setae (i) is developed). The new genus differs clearly from *Bergamina* Elmoor-Loureiro, Santos-Wisniewski & Rocha, 2013 in morphology of postabdomen, presence of denticles on the posteroventral margin of valves and absence of anterior seta between endites 1–2 of limb I (see Elmoor-Loureiro et al. 2013). *Magnospina* gen. n. differs from *Extremalona* Sinev & Shiel, 2012 in general morphology, presence of denticles on the posteroventral margin of valves, postabdomen morphology and armature of setae 2–3 of IDL; male of *Extremalona* also bears six lateral aesthetascs on the antennules. *Ovalona* Van Damme & Dumont, 2008 has a well-developed seta 1 on the IDL, endite 1 of the limb I without seta (i) and exopod of the limb III armed with seven setae. *Magnospina* gen. n. does not present any of aforementioned morphological traits to *Ovalona*. Table 1 shows the main differences and similarities between genera of the *Coronatella*-branch.

Magnospina dentifera **(Sars, 1901), comb. n.**
Figures 1–33

Alona dentifera (Sars, 1901): Sinev et al. 2004: 101, 103–104, figures 1–39; Güntzel et al. 2010: 95, table 1; Sousa and Elmoor-Loureiro 2012: 356, table 2; Debastiani-Júnior et al. 2015: 24, table 2.

Alona broaensis: Matsumura-Tundisi and Smirnov 1984: 327–328, figures 15–21; Güntzel et al. 2010: 95, table 1; Debastiani-Júnior et al. 2015: 24, table 2.

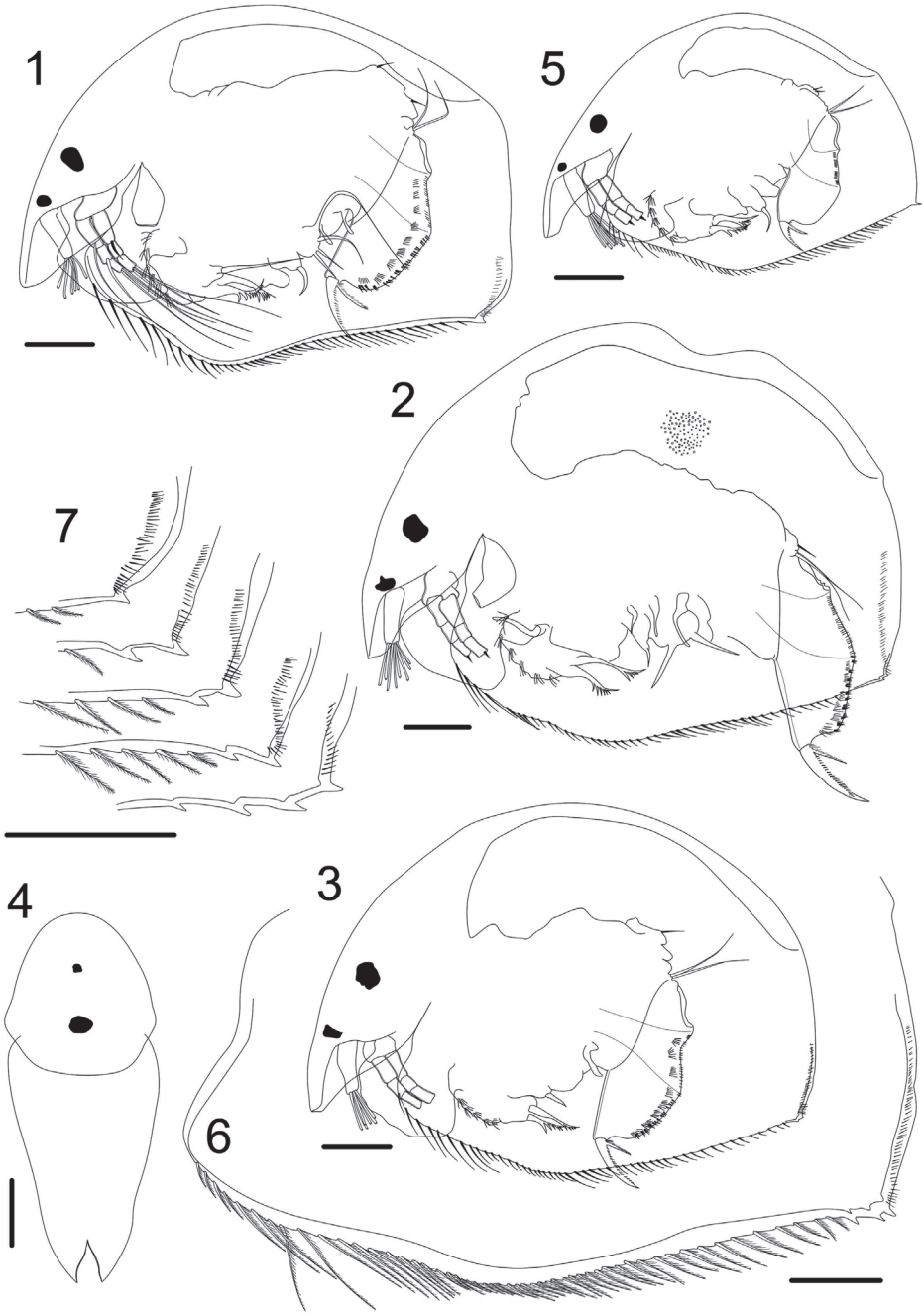
Type locality. "neighborhood of São Paulo", State of São Paulo, Brazil.

Material type. Lectotype: Parthenogenetic ♀, ZMOU F12341a, selected by D. Frey. Paralectotype: 5 parthenogenetic ♀♀, ZMOU F12341b; 1 parthenogenetic ♀ F12341c; 4 parthenogenetic ♀♀, I instar juvenile ♀, ZMOU F12386g; 2 instar II juvenile ♀ ZMOU F12386q; 2 partenogenetic ♀♀, instar II juvenile #, ZMOU, slide

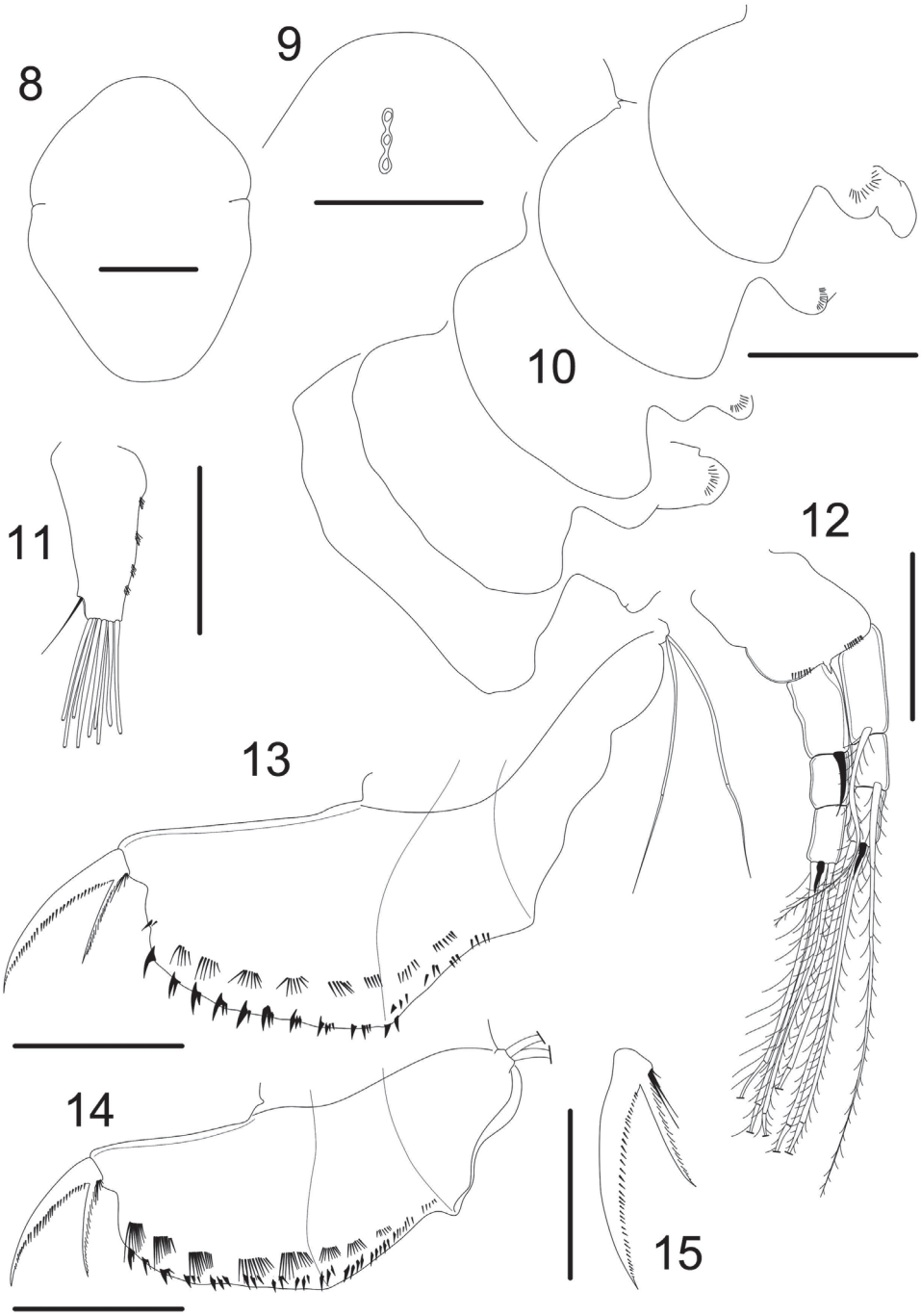
Table 1. Main differences and similarities between genera related to *Coronatella*-branch. To date, there is no description of males of *Bergamina*. From *Karualona*, only the male of *K. iberica* is known (see Alonso and Pretus 1989). From *Magnospina* gen. n., only the male of *M. dentifera* comb. n. is known.

	Extremalona	***Ovalona***	***Leberis***	***Celsinotum***	***Magnospina***	***Coronatella***	***Anthalona***	***Karualona***	***Bergamina***
Female characters									
Maximum length	0.5	0.35–0.7	0.5–1.1	0.53–0.9	0.32–0.48	0.25–0.6	0.3–04	0.4–0.45	0.68
Dorsal keel	absent	absent	present	present	absent	present in one species	absent	absent	absent
Labral denticle	absente	absent	absent	absent	absent	present in one species	absent in three species	absent	absent
Rostrum	rounded	rounded	truncated	truncated	rounded or truncated	rounded	rounded	rounded	rounded
Main head pores	three connected	three connected, connection interrupted or absent	three connected	three connected	absent or three connected	three connected	two connected	two connected	three connected
Lateral head pores	minute	minute, except in one species	minute	minute	absent or minute	minute	specialized, with underneath sacks	minute	minute
A2 – spine on the apical segments	large	large	small	small	large	large	large	large	small
Valves – ventral margin with a distinctive rounded angle	absent	absent	absent or slightly expressed	absent	present	absent	absent	absent	absent
Valves – length of ventral setae from anterior group/ posterior group	not differentiated	anterior longer than posterior	not differentiated	anterior shorter than posterior	anterior very longer than posterior	not differentiated	anterior longer than posterior in three species	anterior longer than posterior	not differentiated
Valves – denticles	absent	1–2 denticles in one species	absent	absent	1–5 large denticles	1–2 short denticles in two species	absent	short denticles in most species	absent
PA – shape of postanal ventral margin	short, narrowing	short and rounded or elongated and narrow	elongated, narrowing	elongated, narrowing	elongated, narrowing	short, rounded	short, rounded	short, rounded	elongated, straight

	Extremalona	***Ovalona***	***Leberis***	***Celsinotum***	***Magnospina***	***Coronatella***	***Anthalona***	***Karualona***	***Bergamina***
PA – basal spine	short	short	short	well-developed, shorter than 2/3 of postabdominal claw	well-developed, longer 2/3 of postabdominal claw	short	short	reduced or short	short
PA – setae on the lateral fascicles	well- developed	well- developed	weakly developed	well- developed	weakly developed	well-developed	well- developed	well- developed	weakly developed
P1 – IDL seta 1	well- developed	well- developed	rudimentary	rudimentary	rudimentary or absent	rudimentary or absent	absent	well- developed	absent
P1 – IDL setae 2–3	with thin setules	with thin setules	with hard setules	with thin or hard setules	with basal denticles	with denticles or hard setules in one species	with denticles or thin setules in three species	with thin setules	with thin setules
P1 – long anterior seta on endite 1	absent	absent	absent	absent	absent	absent	absent	absent	present
P2 – exopod seta	very short	short or moderated length	absent	absent	short	moderated length, rudimentary in two species	present in one species	absent	absent
P3 – number of setae on the exopod	six	seven	six	six	six	six	six	six	six
P4 – Number of flaming-torch	three	three	three	three	three	three	generally three, two in one species	two	three
Male characters									
A1 – lateral aesthetascs	six	two	two	two-six	two	absent	absent	one	unknown
PA – dorsal and ventral margins	almost parallel	parallel or narrowing	narrowing	narrowing	narrowing	almost parallel	almost parallel	almost parallel	unknown
PA – basal spine length	up 0.4 of postabdominal claw	absent or 0.5 of postabdominal claw	0.2–0.5 of postabdominal claw	0.1–0.3 of postabdominal claw	up 0.5 of postabdominal claw	0.2–0.5 of postabdominal claw	0.1–0.3 of postabdominal claw	0.1 of postabdominal claw	unknown
PA – position of gonopores	above of projection for claw insertion	above of projection for claw insertion	at base of postabdominal claw	above of projection for claw insertion or lateral	at base of postabdominal claw	above of projection for clauw insertion	above of projection for claw insertion	above of projection for claw insertion	unknown



Figures 1–7. *Magnospina dentifera* comb. n., parthenogenetic female. **1** *habitus* from Pantanal, Mato Grosso do Sul, Brazil **2** *habitus* from São Paulo, Brazil **3–4** *habitus* from San Pedro, Argentina parthenogenetic female adult from **5** *habitus*, parthenogenetic female juvenile from San Pedro, Argentina **6** ventral margin of carapace from Distrito Federal, Brazil **7** denticles on the posteroventral margin of carapace. Scale bars: 50 μm.



Figures 8–15. *Magnospina dentifera* comb. n., parthenogenetic females. **8** head shield **9** main head pores, female juvenile **10** labral kell **11** antennule **12** antenna **13 –14** postabdomen **15** postabdominal claw. Scale bars: 50 μm.

F9130; 6 parthenogenetic ♀♀ , ephippial ♀, ZMOU, slide F9131; 2 parthenogenetic ♀♀, ephippial ♀, ZMOU, slide F9131.

Material examined. Nine parthenogenetic females and one adult male from Henrique pond, Brasília National Park, Distrito Federal, Brazil (15°41'18"S; 47°56'26.10"W), material collected by Grupo de Estudos de Ecossistemas Aquáticos (GEEA) in ix.2009 (FDRS048). One parthenogenetic female from Henrique pond, Brasília National Park, Distrito Federal, Brazil (15°41'16.5"S; 47°56'22.2"W), material collected by Lourdes M. A. Elmoor-Loureiro on 27.v.2002 (FDRS049). One parthenogenetic female from Cabocla II pond, Campo de Instrução de Formosa, Goiás, Brazil (15°48'21"S; 47°17'09.20"W), material collected by Grupo de Estudos de Ecossistemas Aquáticos (GEEA) on viii.2009 (FDRS050). Six adult parthenogenetic females and one juvenile from Baía da Célia, Fazenda Nhumirim (18°59'27.5"S, 56°39'41.0"W), Pantanal, Mato Grosso do Sul, Brazil, material collected on 07.ix.2000 by Valéria Barros. Four parthenogenetic females from Criminosa Pond (21°40'28.8"S, 57°53'28.5"W) identified as *Alona broaensis*, Porto Murtinho, Pantanal, Mato Grosso do Sul, Brazil, material collected on 19.i.2010, leg Adriana Maria Güntzel (FDRS054). Two parthenogenetic females from Coqueiral Pond, Paranapanema River, Angatuba, São Paulo, Brazil (23°29'22.64"S; 48°37'6.65"W). Material collected by Lourdes M. A. Elmoor-Loureiro on 30.v.2001 (CLLA063, 65-66). Two parthenogenetic females from Esquina, Middle Paraná River, Argentina (30°00.54'59"S; 59°32'51.93"W). Material collected by José Roberto Debastiani Júnior on 12.vi.2010 (FDRS052). Six parthenogenetic females from San Pedro, Lower Paraná River, Argentina (30°40'49"S; 59°18'48.80"W). Material collected by José Roberto Debastiani Júnior on 14.vi.2010 (FDRS053). Three parthenogenetic females from Pimenteira pond, Mata da Pimenteira State Park, Serra Talhada, Pernambuco, Brazil (7°53'48.96"S, 38°18'14.30"W). Material collected by Leidiane Pereira Diniz on 13.iv.2014 (FDRS407).

Differential diagnosis. *Magnospina dentifera* comb. n. differs from *Magnospina siamensis* comb. n. because it has a rounded and wide rostrum and main and lateral head pores are absent in adult. Apical spines of the antenna about two times shorter than the apical segments. On the limbs, the main differences are: *M. dentifera* comb. n. bears a rudimentary seta 1 on the IDL, setae 2-3 of IDL armed with at least seven denticles and the presence of one seta on the filter comb of limb V.

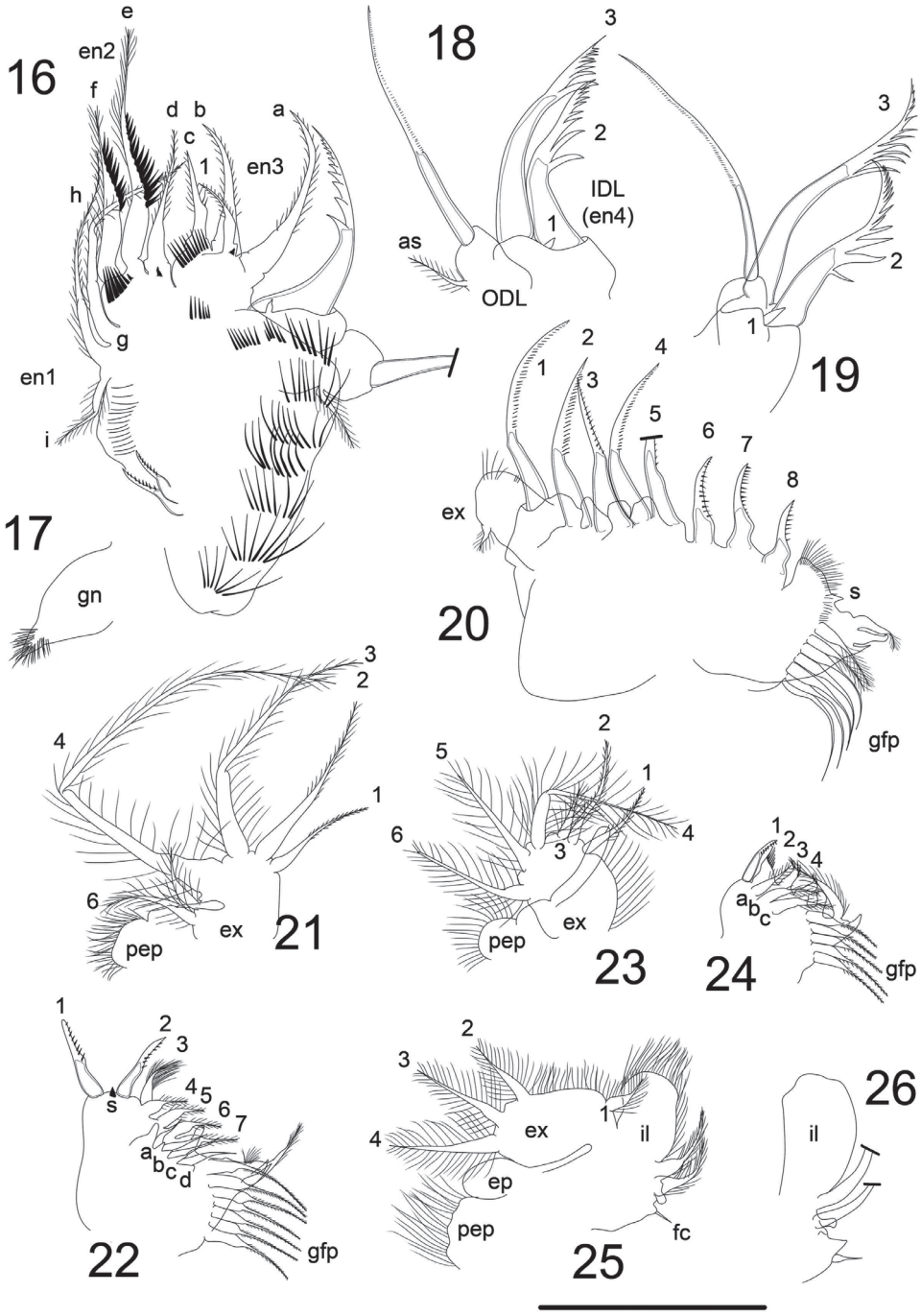
Diagnosis. *Habitus* ovoid, without dorsal keel, not compressed laterally, length 0.32–0.48 mm; eye and ocellus of different sizes. *Head* with rostrum wide, rounded, not pointed; head shield wide, with broadly rounded posterior margin, distance between mandibular articulations higher than length of its posterior portion, main head pores absent in adults, two or three connected main head pores in juveniles (Figures 8–9); lateral head pores absent. *Labral keel* wide, large and naked, apex not elongated (Figure 10). *Carapace* ornamentation slightly punctuated or not evident; ventral margin of carapace with a distinctive rounded angle at 1/3 of length; valves armed with 40–53 setae internally inserted at the valve ventral margin and differentiated in three groups, setae from anterior group markedly longer than median and posterior groups (Figures 1–6); posteroventral corner armed with 1–4 large denticles, broad at their

bases, protruding downwards, without setules between them (Figure 7). *Antennule* do not exceed the tip of the rostrum, nine apical aesthetascs of different lengths which do not exceed the length of the antennular body (Figure 11). *Antenna* with formula of antennal setae 003/113, spines 101/001; first segment of endopod and exopod elongated; weak setules or spicules on the segments; spine on the first segment of the endopod longer than second segment length; apical spines about two times shorter than the apical segments (Figure 12). *Postabdomen* narrowing distally, length about 1.3 times its height; preanal angle prominent; postanal margin about 1.5 times longer than the anal margin armed with 10–13 groups of denticles, 1–3 most distal denticle might be individualized; 8–10 lateral fascicles armed with weak setules (Figures 13–14). *Postabdominal claw* inserted on the projection of postabdomen, longer than anal margin; spinules on the ventral margin may be present; pecten of spinules on the internal and external face of the claw, base of claw armed with 1–5 long and strong spinules (Figures 14–15). *Basal spine* remarkably long, longer than 2/3 of postabdominal claw length, with spinules on the dorsal margin (Figures 14–15). *Limb I* with IDL (en 4) armed with one rudimentary seta (1) and two well-developed setae (2–3) which bears at least seven distinguishable denticles, basal denticles thick; seta (1) on endite 3 shorter than setae (a-c), setae (a-b) of different length, endite 3 with an element; endite 2 armed with three setae (d-f), element present; endite 1 with three setae (g-i) (Figures 16–19). *Limb II* without anterior soft setae; seta on the exopod short, slightly plumose; scrapers not specialized, but with some denticles, especially on scrapers 6–8; gnathobase armed with four elements, filter comb armed with seven setae, of which two are shorter than others (Figure 20). *Limb III* with six setae on the exopod, third and fourth setae long; third seta longer than the second seta; distal endite armed with three setae and one sensillum; gnathobase with three elements, filter comb with seven setae (Figures 21–22). *Limb IV* relatively short, six setae on the exopod; setae 1–2 of different lengths, flaming-torch setae on the distal endite not modified with weak setules; gnathobase armed with a setulated seta shorter than the length of endite itself, filter comb with five setae (Figures 23–26). *Limb V* relatively short, setae 3–4 of exopod similar in length; filter comb reduced with one short seta (Figures 25–26). *Limb VI* absent.

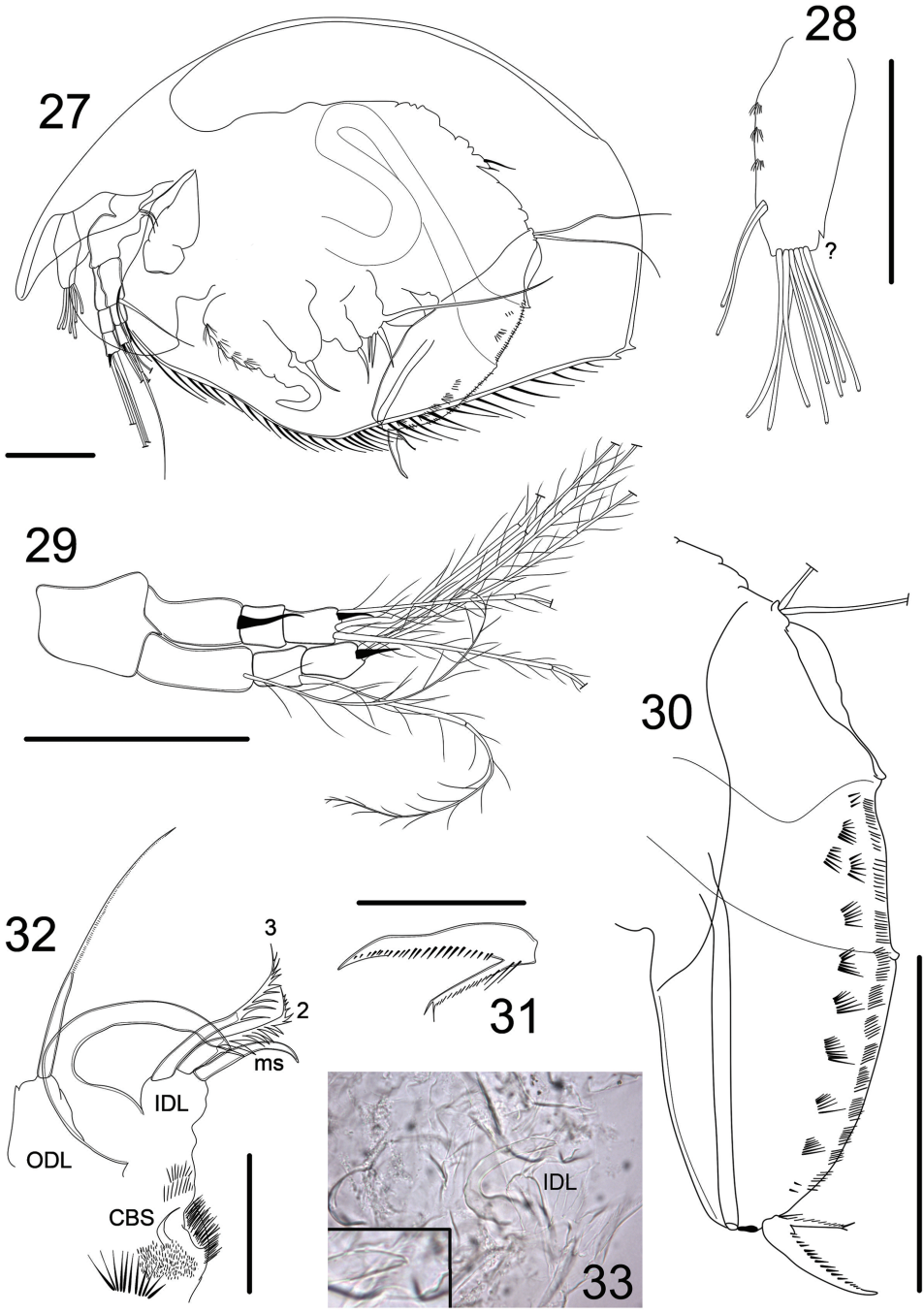
Ephippial female. Not studied.

Adult male. Habitus smaller than female (Figure 27). *Postabdomen* strongly narrowing distally. *Postabdominal claw* short and ticker than female (Figures 30–31). *Basal spine* about half-length of postabdominal claw, with tip forked (Figure 31). *Limb I* with two setae on the IDL (en4), setae armed with denticles; male seta with tip slightly curved; copulatory hook with one projection on the tip (Figures 32–33).

Description of adult male. *Habitus* ovoid, smaller than that in female, length about 0.35 mm, maximum height in the middle of the body (Figure 27). *Head* with rostrum elongated, not blunt, main head pores absent (Figure 27). *Carapace* without ornamentations; ventral margin with a distinctive rounded angle at 1/2 of the margin length, margin armed with about 37 setae, posteroventral corner with two large denticles, broad at their bases, without setules between them (Figure 27). *Antennule* not exceeding the tip of rostrum, about 2.5 times as long as it is wide, with three rows



Figures 16–26. *Magnospina dentifera* comb. n., adult parthenogenetic females. **16** limb I **17** limb I, gnathobase **18–19** limb I, IDL and ODL **20** limb II **21** limb III, exopod **22** limb III, endites **23** limb IV, exopod **24** limb IV, endites **25** limb V **26** limb V, internal lobe. Scale bars: 50 μm.



Figures 27–33. *Magnospina dentifera* comb. n., adult male from Henrique Pond, Brasília National Park, Distrito Federal, Brazil. **27** *habitus* **28** antennule **29** antenna **30** postabdomen **31** postabdominal claw **32** limb I, IDL and ODL **33** limb I, copulatory hook. Scale bars: 50 µm (**27–30)**; 25 µm (**31–33**).

of short setules on body antennular; eleven aesthetascs, two lateral and nine apical ones. Sensory seta and male seta not studied (Figures 27–28). *Antenna* as described for females, however, apical spines relatively longer (Figure 29). *Postabdomen* as long as in female, strongly narrowing distally. Anal margin shorter than postanal margin; 12 rows of thin setules on the anal and postanal margin; eight lateral fascicles with weak setules of which do not exceed postanal margin (Figure 30). *Postabdominal claw* smaller and more robust as comapared with female, base armed with long and strong spinule, pecten armed with strong spinules at the median portion of the claw (Figures 30–31). *Basal spine* long, about half-length of postabdominal claw, with a forked tip, ventral margin armed with spinules (Figure 31). *Limb I* with copulatory hook curved, U-shaped, projection at the tip present, copulatory brush seta shorter than male seta on IDL (en4), the latter armed with three setae; male setae thick with tip slightly curved; setae 2–3 armed with proximal denticles (as observed in female); ODL seta longer than IDL setae (Figures 32–33).

Distribution. Neotropics, from Southern U.S.A to Argentina (Sinev et al. 2004).

Magnospina siamensis comb. n.

Alona siamensis: Sinev and Sanoamuang 2007: 145, 147–148, figures 1–30; Van Damme and Sinev 2013: 226–228; Korovinchinsky 2013: 114, 119, tables 1–2.
Coronatella dentifera (Sars, 1901): Chartejee et al. 2013: 43.

Type locality. Rice field at Ban Bayao Baghe Sub-district, Phannanichom District, Sakhonnakhon Province, Thailand, 01.09.2004.

Material type. Holotype: parthenogenetic female, ZMMU, MI-73. Paratypes: 2 parthenogentic females, ZMMU, MI74.

Differential diagnosis. *Magnospina siamensis* comb. n. differs from *Magnospina dentifera* comb. n. because it has a truncated rostrum, three connected main head pores, minute lateral head pores and a prominent sculpture on the carapace. Apical spines of the antenna are longer than the apical segments. On the limbs, the main differences are: IDL is armed with two setae (2-3), seta 2 with two thick basal denticles, seta 3 with one thick basal denticle, limb V without filter comb.

Diagnosis. Female. According to the literature (Sinev and Sanoamuang 2007).

Habitus without dorsal keel, moderately compressed laterally, length 0.35–0.42 mm; eye and ocellus of subequal sizes. *Head* with rostrum wide, truncated; head shield ornamented with longitudinal lines, wide, posterior margin broadly rounded, distance between mandibular articulations higher than length of its posterior portion, three connected main head pores, lateral head pores minute. *Labral keel* wide, oval and naked, apex not elongated. *Carapace* covered with narrow longitudinal lines; ventral margin of carapace with a distinctive rounded angle at 1/3 of length; valves armed with 45 setae internally inserted at the ventral margin and differentiated in three groups, setae from anterior group markedly longer; posteroventral corner armed with 2–3

large denticles, broad at their bases, protruding downwards, without setules between them. *Antennule* do not exceed the tip of the rostrum, nine apical aesthetascs of different length which do not exceed the length of the antennular body. *Antenna* with formula of antennal setae 003/113, spines 101/001; first segment of endopod and exopod elongated; weak setules or spicules on the segments; spine on the first segment of the endopod longer than the second segment; apical spines longer than the apical segments. *Postabdomen* narrowing distally, length about 2.5 it is height; preanal angle prominent; postanal margin about 1.7–1.8 times longer than the anal margin armed with 3–4 single most distal denticles and 5 cluster of denticles; About 10 lateral fascicles with weak setules. *Postabdominal claw* inserted on the projection of postabdomen, longer than anal margin; base of claw armed with 1–2 long and strong spinules. *Basal spine* remarkably long, longer than 2/3 of postabdominal claw length, without spinules on the dorsal margin. *Limb I* with two thick setae (2–3) on the IDL (en 4), seta (2) with two thick basal denticles, seta (3) with one thick basal denticle; setae (1, a-c) on the endite 3 of subequal length, endite 3 without element; endite 2 armed with three setae (d-f), without element; endite 1 with three setae (g-i). *Limb II* without anterior soft setae; seta on the exopod short, not plumose; scrapers not specialized, but with some denticles, especially on scrapers 6–8; gnathobase armed with four elements, filter comb armed with seven setae, of which two are shorter than the others. *Limb III* with six setae on the exopod, third and fourth setae long; third seta slightly shorter than the second seta; distal endite armed with three setae and one sensillum; gnathobase with three elements, filter comb with seven setae. *Limb IV* relatively short, six setae on the exopod; setae 1–2 of similar lengths; flaming-torch setae on the distal endite not modified, setules on the first flaming-torch relatively longer that one observed in setae 2–3; gnathobase armed with a setulated seta shorter than length of endite itself, filter comb with five setae. *Limb V* with setae 3–4 of exopod similar in lengths; filter comb absent. *Limb VI* absent.

Ephippial female and male. Unknown.

Distribution. Malysia, Thailand (Sinev 2007; Van Damme and Sinev 2013; Korovinchinsky 2013) and probably India (Chartejee et al. 2013).

Discussion

Morphological analyses

In the redescription of *Alona dentifera*, Sinev et al. (2004) suggested that specific morphological traits observed in this species were not enough to create a new genus, however, the description of *A. siamensis* (Sinev and Sanoamuang 2007) showed a new perspective about *dentifera*-group. Thus, separation of *Magnospina* gen. n. is mainly supported by such characters as: (1) basal spines longer than 2/3 of postabdominal claw, (2) presence of 1-4 large denticles, broad at their bases, protruding downwards, without setules between them. Other specific morphological traits also are observed

in *Magnospina* gen. n.: presence of a distinctive rounded angle at 1/3 of the length of ventral margin of carapace, setae on the valves differentiated in three groups with the anterior group markedly longer than median and posterior groups, prominent preanal angle at postabdomen, setae 2–3 of IDL armed with basal denticles, six setae on limb III, and absent limb VI. The presence of six setae on exopod of limb III and absence of limb VI may be considered as simplesiomorphies of the clade *Magnospina dentifera*/ *M. siamensis*, and ancestral state for the *Coronatella*-branch.

The morphology of head shield, main head pores and of some structures of the limbs are different between *M. dentifera* comb. n. and *M. siamensis* comb. n.; however, analogous variation in these structures was already observed in *Euryalona* (Rajapaksa and Fernando 1987). Species-groups of *Alona* sensu lato, such as the *costata*-group (Sinev 1999b, 2001a, 2008, Van Damme and Eggermont 2011, Van Damme et al. 2011b), *verrucosa*-group (Van Damme et al. 2011a, Sinev and Kotov 2012), *rectangula*-group (Van Damme and Dumont 2008b, Sousa et al. 2015a), and *pulchella*-group (Sinev 2001b, c, 2009, Sinev and Silva-Briano 2012, Van Damme et al. 2013, Sousa et al. 2015b) also have differences in structures on head, postabdomen, and limbs.

Recently, Sinev (2014, 2015b) reviewed the morphology of *Camptocercus* Baird, 1843 species and showed significant differences in structures on the limbs among different species of this genus. In the same way, *Celsinotum* also has many differences in the morphology of head shield, postabdomen and limbs (Frey 1991, Sinev and Elmoor-Loureiro 2010, Sinev and Kotov 2012). This endorses our conclusion that the differences between *M. dentifera* comb. n. and *M. siamensis* comb. n. should be considered at a specific level in the *dentifera*-group (also suggested by Sinev and Sanoamuang 2007). For Van Damme and Sinev (2013), this small lineage may represent an ancient vicariant divergence, presenting currently an Amphi-Pacific distribution, i.e. keeping in mind an antiquity of the cladoceran taxa of different ranks (Frey 1987; Kotov and Taylor 2011). Aforementioned differences in the morphology between *M. dentifera* comb. n. and *M. siamensis* comb. n. may be the result of adaptations to different environmental pressure on a micro-scale.

The trend in morphological radiation in the clade *Magnospina* gen. n. concerns the external morphology but not to features of the trunk limbs (such as in the *pulchella*-group). It has been observed that a wide rostrum and the maintenance of primitive ovoid body shape, shared with other species-groups, possibly result from convergence or parallelism (Sinev et al. 2005, Van Damme and Dumont 2008b, Sinev et al. 2009, Van Damme and Sinev 2011). Regarding the limbs, an exception to the aforementioned trend seems be the armature of the IDL setae, which is more specialized in *M. siamensis* comb. n. when compared to *M. dentifera* comb. n. (which has the armature of IDL setae similar to genus *Coronatella*). A similar trend was observed in species of *Anthalona* whose evolution of IDL setae are related to feeding strategies (Van Damme et al. 2011a). Thus, distinct evolutionary pressure on the food handling should be considered to explain differences observed on the IDL setae of *Magnospina* gen. n. species.

The morphology of the postabdomen is the most evident trait of *Magnospina* gen. n. in contrast to *Leberis*, *Coronatella*, *Anthalona*, *Karualona*, *Extremalona*, *Bergamina*,

Celsinotum, *Ovalona* or *Alona* senso stricto; however, this morphological feature does not show a clear relationship with habitat and/or evolutionary history. Generally, specialized species have their morphology linked to habitat conditions (Van Damme et al. 2003, Kotov 2000a, b, Kotov 2006, Van Damme et al. 2009, Kotov et al. 2010, Van Damme and Sinev 2011, Van Damme et al. 2011a, Sinev 2014), but, apparently, this is not case of the two species from the *dentifera*-group, because they may occur in different kinds of habitats (Sinev and Sanoamuang 2007, Güntzel et al. 2010, Sousa et al. 2012, Kotov et al. 2013, Van Damme and Sinev 2013).

Some studies observed that the male's morphology is very important in making any inference about the relationship between closer species or between species groups in Aloninae (Sinev 1999a, 2013) as well as other cladoceran groups (Goulden 1968; Belyaeva and Taylor 2009; Kotov et al. 2009). Indeed, the morphology of the *M. dentifera* comb. n. male indicates more affinities with *Leberis* than with any genus from the *Coronatella*-branch. For instance, the general shape of postabdomen and antennules is similar to that described for adult males of *L. davidi* (Richard, 1895) (Sinev et al. 2005) and *L. colombiensis* Kotov & Fuentes-Reines 2015 (Kotov and Fuentes-Reines 2015). However, there are clear differences between *Leberis* and *Magnospina* gen. n.: the presence of denticles on posteroventral corner of valves, shape of postabdominal claw, length of the basal spine, and armature of IDL setae (for *Magnospina dentifera* comb. n., Figures 30–32).

When evaluating the morphology of species of the *elegans*-group from Palearctic zone, Sinev et al. (2009) highlighted the morphological traits that support that this group in *Coronatella*-branch, as well as its presumed genus-level. Thus, the main difference between females from *Magnospina* gen. n. and species of the *elegans*-group are related to the external morphology (shape of the body, rostrum, postabdomen and presence of denticles on the posteroventral corner of the carapace in *Magnospina* gen. n.); differences on the limbs are observed in the armature of IDL setae and the length of the seta 3 on the exopod of limb III. Males from the *elegans*-group share with *Magnospina* gen. n. the presence of two lateral aesthetascs on the antennules (Figure 28), which is considered the main synapomorphy of clade *Ovalona*/*elegans*-group/*Leberis* (Sinev 2015a; Neretina and Sinev 2016). This confirms the phylogenetic position of *Magnospina* gen. n., which is closely related to *Leberis*.

The male of *Ovalona* genus also has two lateral aesthetascs on the antennules, but differs from *Magnospina* gen. n. because it has straight dorsal and ventral postabdominal margins, gonopores opening above projection to insertion of postabdominal claw, and setae 2–3 of IDL armed with setules. According to Sinev (2015a) and Neretina and Sinev (2016), *Celsinotum* is closer to *Leberis* and *Ovalona*, and thus, its close relationship with *Magnospina* gen. n. could be inferred. *Celsinotum* females differ quantitatively from *Magnospina* gen. n. in external and limb structures (see Frey 1991, Sinev and Elmoor-Loureiro 2010, Sinev and Kotov 2012). The males of *Celsinotum* differ from *Magnospina* gen. n. in the shape of postabdomen, length of the basal spines on the postabdominal claw, presence of two-six lateral aesthetascs on antennules and setae 2–3 of IDL armed with setules (see Frey 1991, Sinev and Kotov 2012).

Differently from *Magnospina* gen. n., males of the *Coronatella* genus have dorsal and ventral margins of the postabomen almost straight and lateral aesthetascs on antennules absent (Van Damme and Dumont 2008a, Sousa et al. 2015a). *Anthalona* males have a short basal spine, well-developed setules of the lateral fascicles on the postabdomen and lateral aesthetascs on antennules absent (Van Damme et al. 2011a, Sinev and Kotov 2012). *Karualona* males has postabdomen very similar to the one observed in *Anthalona*, with well-developed lateral fascicles on the postabdomen and a very short basal spine on the postabdominal claw. However, antennules of *Karualona* males bear one lateral and ten apical aesthetascs (see Alonso and Petrus 1989). Besides short postabdomen, the male of *Extremalona* has six lateral aesthetascs on antennules and well developed seta 1 of IDL and setae 2–3 armed with setules (Sinev and Shiel 2012). Differences between *Magnospina* gen. n. male and *Bergamina* cannot be stated because the male is not known, so far.

Notes on *Alona broaensis* Matsumura-Tundisi & Smirnov, 1984

Alona broaensis species was described from Broa Reservoir, São Paulo, Brazil (Matsumura-Tundisi and Smirnov 1984) and it has not often been found in fauna studies conducted in many regions (including type region). The absence of some information on the morphology, including details from trunk limbs, led Van Damme et al. (2010) to list this species as a junior synonym of *M. dentifera* comb. n.. Indeed, the morphological variation observed between *M. dentifera* comb. n. populations studied here and by Rey and Vasquez (1986) for number of denticles on the posteroventral corner of carapace (Figure 8), morphology of the postabdominal claw, basal spine, and rostrum, include the features signed as diagnostic for *A. broaensis* (see Matsumura-Tundisi and Smirnov 1984). They are like those observed in description of *A. broaensis* (see Matsumura-Tundisi and Smirnov 1984). We analyzed one population identified as *Alona broaensis* from the Pantanal, Brazil, and morphological traits distinct from *M. dentifera* comb. n. were not observed. In the other words, there are not morphological traits that support the validity of *Alona broaensis*. We agree with the suggestion of Van Damme et al. (2010), and *Alona broaensis* is here considered as a junior synonym of *M. dentifera* comb. n..

Conclusions

Magnospina gen. n. is one more genus derived from *Alona* sensu lato and belongs to the *Coronatella*-branch, being close to *Leberis*, as suggested by the phylogenetic analysis based on molecular data. The synapomorphies of the *Magnospina* gen. n. are: (1) basal spines longer than 2/3 of postabdominal claw, (2) presence of 1-4 large denticles, broad at their bases, protruding downwards, without setules between them. *Magnospina* gen. n. also has a wide rostrum, prominent preanal angle at postabdomen, setae 2-3 of IDL armed with basal denticles, six setae on limb III and limb VI absent. In addition to the

female morphology presenting consistent differences when compared to other genera from the *Coronatella*-branch, the male features also support the creation of this new genus that includes *M. dentifera* comb. n. and *M. siamensis* comb. n.

Acknowledgements

We thank Prof. Dra. Adriana M. Güntzel (FUNDECT/MS), Dr. Jose Roberto Debastiani Junior and Biologist Lediane Pereira Diniz for providing the material of *Alona broaensis* from Pantanal and of *Alona dentifera* from Argentine and Pernambuco, respectively. We also thank Dr. Marcelo M. Dalosto for revising the English version of this manuscript. We are grateful to Dr. Eliana A. Panarelli, Dr. Gilmar Perbiche Neves and Dr. Ricardo Lourenço Pinto for the suggestions on the initial draft of this manuscript. We are deeply grateful to Dr. Alexey A. Kotov and Dr. Artem Yu. Sinev for the valuable criticisms, comments and suggestions that greatly improved this manuscript. The first author of this paper was awarded a grant from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Alonso MJ, Pretus L (1989) *Alona iberica*, New species: First Evidence of Noncosmopolitanism within the *A. karua* complex (Cladocera: Chydoridae). Journal of Crustacean Biology 9: 453–476. doi: 10.2307/1548571

Belyaeva M, Taylor DJ (2009) Cryptic species within the *Chydorus sphaericus* species complex (Crustacea: Cladocera) revealed by molecular markers and sexual stage morphology. Molecular Phylogenetics and Evolution 50: 534–546. doi: 10.1016/j.ympev.2008.11.007

Chatterjee T, Kotov AA, Van Damme K, Chandrasekhar SVA, Padhye S (2013) An annotated checklist of the Cladocera (Crustacea: Branchiopoda) from India. Zootaxa 3667(1): 1–89. doi: 10.11646/zootaxa.3667.1.1

Debastiani-Júnior JR, Elmoor-Loureiro LMA, Nogueira MG (2015) High taxonomic resolution as a determinant on finding new species and new record in the Río de La Plata basin: a case on Chydoridae (Crustacea: Branchiopoda: Cladocera). Nauplius 23(1): 21–30. doi: 10.1590/S0104-64972015002301

Elías-Guttiérez M, Martínez-Jerónimo F, Ivanova NV, Valdez-Moreno M, Hebert PDN (2008) DNA barcodes for Cladocera and Copepoda from Mexico and Guatemala, highlights and new discoveries. Zootaxa 1839: 1–42.

Elmoor-Loureiro LMA (2004) Phylogenetic relationships among families of the order Anomopoda (Crustacea, Branchiopoda, Cladocera). Zootaxa 760: 1–26.

Elmoor-Loureiro LMA, Santos-Wisniewski MJ, Rocha O (2013) Redescription of *Alonella lineolata* Sars, 1901 (Crustacea, Cladocera, Chydoridae) and its translocation to the subfamily Aloninae and to the new genus *Bergamina* gen. nov. Zootaxa 3630(3): 571–581. doi: 10.11646/zootaxa.3630.3.11

Frey DG (1987) The taxonomy and biogeography of the Cladocera. Hydrobiologia 145: 5–17. doi: 10.1007/BF02530260

Frey DG (1991) A new genus of alanine chydorid cladocerans from athalassic saline waters of New South Wales, Australia. Hydrobiologia 224: 11–48. doi: 10.1007/BF00006361

Goulden CE (1968) The systematics and evolution of the Moinidae. Transactions of the American Philosophical Society Held at Philadelphia, New Series 58(6): 1–101. doi: 10.2307/1006102

Güntzel AM, Panarelli EA, Silva WM, Rochhe KF (2010) Influence of connectivity on Cladocera diversity in oxbow lakes in the Taquari River floodplain (MS, Brazil). Acta Limnolgica Brasiliensia 22(1): 93–101. doi: 10.4322/actalb.02201012

Korovchinsky NM (2013) Cladocera (Crustacea: Branchiopoda) of South East Asia: history of exploration, taxon richness and notes on the zoogeography. Journal of Limnology 72(s2): 109–124. doi: 10.4081/jlimnol.2013.s2.e7

Kotov AA (2000a) Analysis of *Kozhowia* Vasiljeva & Smirnov, 1969 (Chydoridae, Anomopoda, Branchiopoda), and a description of *Parakozhowia* n. gen. Hydrobiologia 437: 17–56. doi: 10.1023/A:1026507529975

Kotov AA (2000b) Redescription and assignment of the chydorid *Indialona ganapati* Petkovski, 1966 (Branchiopoda: Anomopoda: Aloninae) to Indialonini, new tribus. Hydrobiologia 439: 161–178. doi: 10.1023/A:1004187007890

Kotov AA (2006) Adaptations of Anomopoda Crustaceans (Cladocera) to the benthic mode of Life. Zoologichesky Zhurnal 85: 1043–1059. doi: 10.1134/s0013873806110157

Kotov AA, Fuentes-Reines JM (2015) A new species of *Leberis* Smirnov, 1989 (Cladocera: Chydoridae) from Colombia. Zootaxa 3957(5): 553–566.

Kotov AA, Ishida S, Taylor DJ (2009) Revision of the genus *Bosmina* Baird, 1845 (Cladocera: Bosminidae), based on evidence from male morphological characters and molecular phylogenies. Zoological Journal of the Linnean Society 156: 1–56. doi: 10.1111/j.1096-3642.2008.00475.x

Kotov AA, Sinev AY, Berrios VL (2010) The Cladocera (Crustacea: Branchiopoda) of six high altitude water bodies in the North Chilean Andes, with discussion of Andean endemism. Zootaxa 2430: 1–66.

Kotov AA, Taylor DJ (2011) Mesozoic fossils (>145 Mya) suggest the antiquity of the subgenera of *Daphnia* and their coevolution with chaoborid predators. BMC Evolutionary Biology 11: 129. doi: 10.1186/1471-2148-11-129

Kotov AA, Van Damme K, Bekker EI, Siboualipha S, Silva-Briano M, Ortiz AA, De la Rosa RG, Sanoamuang L (2013) Cladocera (Crustacea: Branchiopoda) of Vientiane province and municipality, Laos. Journal of Limnology 72(s2): 81–108. doi: 10.4081/jlimnol.2013.s2.e6

Matsumura-Tundisi T, Smirnov NN (1984) Description of Alona broaensis sp. n. (Crustacea: Cladocera). Hydrobiologia 113: 327–329. doi: 10.1007/BF00026619

Neretina AN, Sinev AY (2016) A revision of the genus *Leberis* Smirnov, 1989 (Cladocera: Chydoridae) in the Old World and Australia. Zootaxa 4079: 501–533. doi: 10.11646/zootaxa.4079.5.1

Rajapksa R, Fernando CH (1987) A redescription of *Euryalona orientalis* (Daday, 1898), with a consideration of the other species in the genus *Euryalona* (Cladocera: Chydoridae). Hydrobiologia 150: 75–90. doi: 10.1007/BF00006611

Rey J, Vasquez E (1986) Cladocères de quelques corps d'eaux du basin moyen de l'Orénoque (Vénézuéla) Annales de Limnologie – International Journal of Limnology 22(2): 137–168. doi: 10.1051/limn/1986013

Sacherová V, Hebert PDN (2003) The evolutionary history of the Chydoridae (Crustacea: Cladocera). Biological Journal of the Linnean Society 79: 629–643. doi: 10.1046/j.1095-8312.2003.00216.x

Sinev AY (1999a) *Alona werestchangini* sp.n., a new spcies of the genus *Alona* Baird, 1843 related to *A. guttata* Sars, 1862 (Anomopoda: Chydoridae). Arthropoda Selecta 8: 23–30.

Sinev AY (1999b) *Alona costata* Sars, 1862 versus related palaeotropical species: the first example of close relations between species with a different number of main head pores among Chydoridae (Crustacea: Anomopoda). Arthropoda Selecta 8: 131–148.

Sinev AY (2001a) Redescription of *Alona iheringi* Sars, 1901 (Chydoridae, Anomopoda, Branchiopoda), a South American species related to *A. rustica* Scott, 1895. Hydrobiologia 464: 113–119. doi: 10.1023/A:1013935100483

Sinev AY (2001b) Redescription of *Alona glabra* Sars, 1901, a South American species of the *pulchella*-group (Chydoridae, Anomopoda, Branchiopoda). Arthropoda Selecta 10: 273–280.

Sinev AY (2001c) Separation of *Alona camboui* Guerne & Richard, 1893 from *Alona pulchella* King, 1853 (Branchiopoda, Anomopoda, Chydoridae). Arthropoda Selecta 10: 5–18.

Sinev AY (2008) A new species related to *Alona costata* Sars, 1862 (Cladocera: Anomopoda: Chydoridae) from South Africa. Zootaxa 1707: 23–36.

Sinev AY (2009) Notes on morphology and taxonomic status of some North American species of the genus *Alona* Baird, 1843 (Cladocera: Anomopoda: Chydoridae). Fundamental Applied Limnology 175: 59–77. doi: 10.1127/1863-9135/2009/0175-0059

Sinev AY (2013) Cladocerans of *Alona affinis* group (Cladocera: Anomopoda: Chydoridae) from North America. Zootaxa 3693: 329–343. doi: 10.11646/zootaxa.3693.3.3

Sinev AY (2014) A comparative morphological analyses of four species of *Camptocercus* Baird, 1843 (Cladocera: Anomopoda: Chydoridae). Zootaxa 3895: 183–207. doi: 10.11646/zootaxa.3895.2.3

Sinev AY (2015a) Revision of the *puchella*-group of *Alona* s. lato leads to its translocation to *Ovalona* Van Damme et Dumont, 2008 (Branchiopoda: Anomopoda: Chydoridae). Zootaxa 4044: 451–492. doi: 10.11646/zootaxa.4044.4.1

Sinev AY (2015b) Morphology and phylogenetic position of three species of genus Camptocercus Baird, 1843 (Cladocera: Anomopoda: Chydoridae). Zootaxa 4040: 169–186. doi: 10.11646/zootaxa.4040.2.4

Sinev AY, Elmoor-Loureiro LMA (2010) Three new species of chydrorid cladocerans of subfamily Aloninae (Brachiopoda: Anomopoda: Chydoridae) from Brazil. Zootaxa 2390: 1–25.

Sinev AY, Kotov AA (2012) New and rare Aloninae (Cladocera: Anomopoda: Chydoridae) from Indochina. Zootaxa 3334: 1–28.

Sinev AY, Sanoamuang L (2007) *Alona siamensis* sp. n., a new species of Cladocera from South-East Asia, related to *Alona dentifera* (Sars, 1901) (Anomopoda: Chydoridae). Arthropoda Selecta 16: 143–150.

Sinev AY, Shiel RJ (2012) *Extremalona timmsi* gen. nov., sp. nov., a new cladoceran (Cladocera: Anomopoda: Chydoridae) from an acid saline lake in southwest Western Australia. Journal of Natural History 46: 45–46. doi: 10.1080/00222933.2012.727215

Sinev AY, Silva-Briano M (2012) Cladocerans of genus *Alona* Baird, 1843 (Cladocera: Anomopoda: Chydoridae) and related genera from Aguascalientes State, Mexico. Zootaxa 3569: 1–24.

Sinev AY, Alonso M, Sheveleva LG (2009) New species of *Alona* from South-East Russia and Mongolia related to *Alona salina* Alonso, 1996 (Cladocera: Anomopoda: Chydoridae). Zootaxa 2326: 1–23.

Sinev AY, Kotov AA, Van Damme K (2004) Morphology of a Neotropical cladoceran *Alona dentifera* (Sars, 1901), and its position within the Chydoridae Stebbing, 1902 (Branchiopoda: Anomoda). Arthropoda Selecta 13: 99–107.

Sinev AY, Van Damme K, Kotov AA (2005) Redescription of tropical-temperate cladocerans *Alona diaphana* King, 1853 and *Alona davidi* Richard, 1895 and their translocation to Leberis Smirnov, 1989 (Branchiopoda: Anomopoda: Chydoridae). Arthropoda Selecta 14(3): 183–205.

Sousa FDR, Elmoor-Loureiro LMA (2012) How many species of cladocerans (Crustacea, Branchiopoda) are found in Brazilian Federal District? Acta Limnologica Brasiliensia 24(4): 351–362. doi: 10.1590/S2179-975X2013005000008

Sousa FDR, Elmoor-Loureiro LMA, Santos S (2015a) Redescription of *Coronatella poppei* (Richard, 1897) (Crustacea, Branchiopoda, Chydoridae) and a revision of the genus in Brasil, with description of new taxa. Zootaxa 3955(2): 211–244. doi: 10.11646/zootaxa.3955.2.3

Sousa FDR, Elmoor-Loureiro LMA, Santos S (2015b) *Alona kaingang* sp. nov (Crustacea, Cladocera, Chydoridae): a new species of the pulchella-group, with identification key to Neotropical species. Zoological Studies 54(48): 1–12.

Van Damme K, Dumont HJ (2008a) Further division of *Alona* Baird, 1843: separation and position of *Coronatella* Dybowski & Grochowski and *Ovalona* gen.n. (Crustacea: Cladocera). Zootaxa 1960: 1–44.

Van Damme K, Dumont HJ (2008b) The 'true' genus *Alona* Baird, 1843 (Crustacea: Cladocera: Anomopoda): characters of the *A. quadrangularis*-group and description of a new species from Democratic Republic Congo. Zootaxa 1945: 1–25.

Van Damme K, Eggermont H (2011) The Afromontane Cladocera (Crustacea: Branchiopoda) of the Rwenzori (Uganda–D. R. Congo): taxonomy, ecology and biogeography. Hydrobiologia 675: 57–100. doi: 10.1007/s10750-011-0892-0

Van Damme K, Sinev AY (2011) A new genus of cave-dwelling microcrustaceans from the Dinaric Region (south-east Europe): adaptations of true stygobitic Cladocera (Crustacea: Branchiopoda). Zoological Journal of Linnenan Society 161: 31–52. doi: 10.1111/j.1096-3642.2010.00639.x

Van Damme K, Sinev AY (2013) Tropical Amphi-Pacific disjunctions in Cladocera (Crustacea: Branchiopoda). Journal of Limnology 72 (s2): 209–244. doi: 10.4081/jlimnol.2013.s2.e11

Van Damme K, Bekker E, Kotov AA (2013) Endemism in the Cladocera (Crustacea: Branchiopoda) of Southern Africa. Journal of Limnology 72: 440–463. doi: 10.4081/jlimnol.2013.e36

Van Damme K, Brancelj A, Dumont HJ (2009) Adaptations to the hyporheic in Aloninae (Crustacea: Cladocera): allocation of *Alona protzi* Hartwig, 1900 and related species to *Phreatalona* gen. nov. Hydrobiologia 618: 1–34. doi: 10.1007/s10750-008-9607-6

Van Damme K, Elías-Gutiérrez M, Dumont HJ (2011b) Three rare European "*Alona*" taxa (Branchiopoda: Cladocera: Chydoridae), with notes on distribution and taxonomy. Annales de Limnologie – International Journal of Limnology 47: 45–63. doi: 10.1051/limn/2010034

Van Damme K, Kotov AA, Dumont HJ (2010) A checklist of names in *Alona* Baird 1843 (Crustacea: Cladocera: Chydoridae) and their current status: an analysis of the taxonomy of a lump genus. Zootaxa 2330: 1–63.

Van Damme K, Sinev AY, Dumont HJ (2011a) Separation of *Anthalona* gen.n. from *Alona* Baird, 1843 (Branchiopoda: Cladocera: Anomopoda): morphology and evolution of scraping stenothermic Alonine. Zootaxa 2875: 1–64.

Van Damme K, Chiambeng G, Maiphae S, Dumont HJ (2003) New species in the rheophilous genus *Nicsmirnovius* Chiambeng&Dumont, 1999 (Branchiopoda: Anomopoda: Chydoridae) and reassignment of *Alona eximia* Kiser, 1948 and *Alonella fitzpatricki* Chien, 1970. Hydrobiologia 499: 25–49. doi: 10.1023/A:1026391501312

ZooKeys 586: 121–134 (2016)
doi: 10.3897/zookeys.586.7758
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

The larvae of the European species of genus *Apataniana* Mosely, 1936 (Trichoptera, Apataniidae): descriptions, key and ecology

Johann Waringer[1], Hans Malicky[2]

1 *Department of Limnology and Bio-Oceanography, Faculty of Life Sciences, University of Vienna, Austria*
2 *Sonnengasse 13, A-3293 Lunz am See, Austria*

Corresponding author: *Johann Waringer* (johann.waringer@univie.ac.at)

Academic editor: *R. Holzenthal* | Received 11 January 2016 | Accepted 4 April 2016 | Published 4 May 2016

http://zoobank.org/E9FA6422-FCCD-4D85-9141-1F0CEC01A66E

Citation: Waringer J, Malicky H (2016) The larvae of the European species of genus *Apataniana* Mosely, 1936 (Trichoptera, Apataniidae): descriptions, key and ecology. ZooKeys 586: 121–134. doi: 10.3897/zookeys.586.7758

Abstract

This paper describes the previously unknown or insufficiently known larvae of *Apataniana hellenica*, *Apataniana stropones* and *Apataniana vardusia*. Species association was enabled by the fact that the three micro-endemic *Apataniana* larvae are restricted to Greece and the only Apataniidae species recorded in European ecoregion 6 (Hellenic Western Balkan; Graf et al. 2008), and that the endemic status of the three species clearly defined their non-overlapping sampling ranges. Information on the morphology of the larvae is given, and the most important diagnostic features are illustrated.

Keywords

Description, distribution, larvae, identification, ecology

Introduction

In Europe, the genus *Apataniana* was unknown until 1987 when Malicky discovered a large number of unknown Apataniidae larvae in a small stream at 1200 m a.s.l. in the Ossa mountains at the southern side of the Olymp massif; at the same location, a number of adults were caught in light traps and described as *A. hellenica*. The second new species was *A. vardusia* from a small stream above the tree line at 1750 m above

Copyright *Johann Waringer, Hans Malicky.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

sea level in the Vardusia mountains in Central Greece (Malicky 1992). Finally, *A. stropones* was discovered in August 1993 at the eastern slopes of the Dirfis mountains on the island of Euböa (Malicky 1993). Interestingly, the closest relatives of the three Greek *Apataniana* species are reported from Asia Minor (Turkey; *A. borcka* Sipahiler, 1996) and Asia (Kyrghyzstan: *A. rauschorum* Malicky, 1999; Tadzhikistan: *A. pamirensis* Mey and Levanidova, 1989; Kazakhstan, Tadzhikistan, Uzbekistan: *A. cornuta* Ivanov, 1991, *A. elongata* (McLachlan, 1875), *A. propria* Mey, 1986; Mongolia, Tibet: *A. impexa* Schmid, 1968, *A. hutchinsoni* Mosely, 1936, *A. spinosa* Yang & Tao, 2011; Indian Himalaya: *A. charadija* Schmid, 1968; Russia: vicinity of lake Baikal: *A. bulbosa* Martynov, 1918; Chukotski Peninsula: *A. tschuktschorum* Levanidova, 1979) (Mey and Levanidova 1989; Malicky 2005b; Morse 2015).

Mey and Levanidova (1989) proposed three species groups, based on phylogenetic and biogeographical considerations (*impexa*, *bulbosa*, and *elongata* group) and presented morphological information for characterizing genus *Apataniana* in the larval stage which is morphologically very close to *Apatania* Kolenati, 1848. The same authors also provided a first key for larval *Apataniana* including *A. hellenica*, *A. pamirensis*, *A. tschuktschorum*, *A. elongata* and *A. impexa*. With our descriptions, proposed here, the identification of all three European *Apataniana* larvae is now possible even without adults.

Material and methods

The larval material was sampled by Hans Malicky at the following locations and dates: *Apataniana hellenica* Malicky, 1987: Ossa mountains, '*Apataniana* stream' (22°42'E, 39°50'N, 1200 m a.s.l.) on 28 July1991; *Apataniana stropones* Malicky, 1993: Euböa, Dirfis mountains near Stropones (23°53'E, 38°36'N, 700–900 m a.s.l.) on 24 May1974 and 5 August 1993; *Apataniana vardusia* Malicky, 1992: Vardusia mountains above tree line, Central Greece (22°08'E, 38°42'N, 1750 m a.s.l.) on 22 October 1991.

A hand net was used to collect larvae, and light trapping obtained the adult material of the three *Apataniana* species which also included the holo- and paratypes for the species descriptions (details on the latter are given by Malicky 1987, 1992, 1993, 2005b). The material was preserved in 70% ethanol. A Nikon SMZ 1500 binocular microscope with DS-Fi1 camera and NIS-elements D 3.1 image stacking software for combining 7–38 frames in one focused image were used to study and photograph the larvae.

Species association was enabled by the fact that the three European *Apataniana* larvae are the only Apataniidae species recorded in European ecoregion 6 (Hellenic Western Balkan; Graf et al. 2008) and that the endemic status of the three species restricts their distribution range, preventing confusion with other closely related species. The three 5th instar larvae of each species used for the descriptions are deposited in the collection of J. Waringer (Vienna, Austria); further larval, pupal and adult material is stored in the collection of H. Malicky (Lunz am See, Austria). We used the morphological terminology by Wiggins (1998), Wallace et al. (2003) and Waringer and Graf (2011).

Results

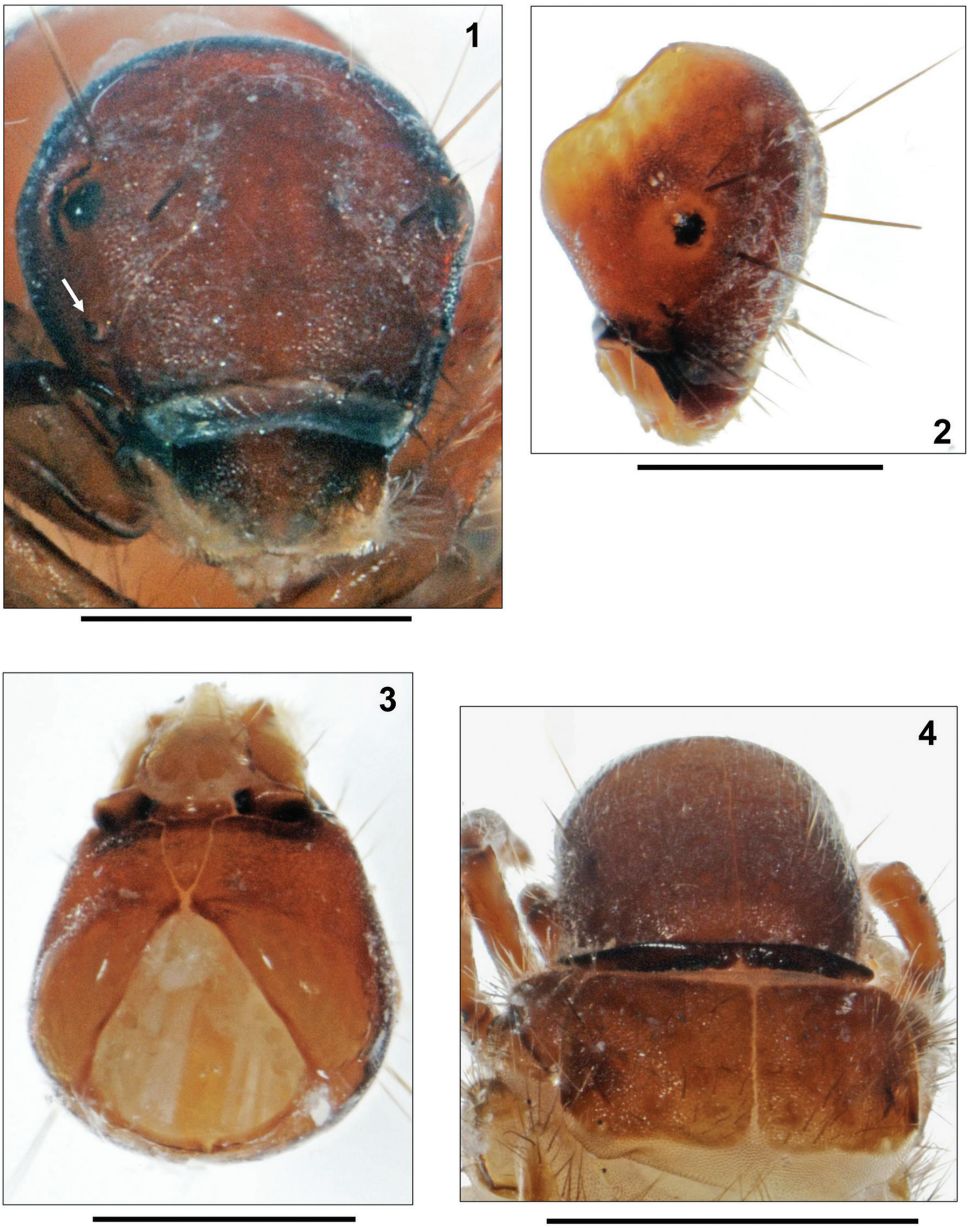
Apataniana stropones Malicky, 1993

Description of the 5th instar larva. Diagnosis. Setae at anterior edge of pronotum long, tapering and with flexuous tips; setal transversal band at 1st abdominal dorsum continuous; 2nd tarsal claw shorter than half tarsal length; central submentum sides converging.

Biometry. Body length of 5th instar larvae ranging from 4.9 to 5.0 mm, head width from 0.73 to 0.74 mm (n= 3).

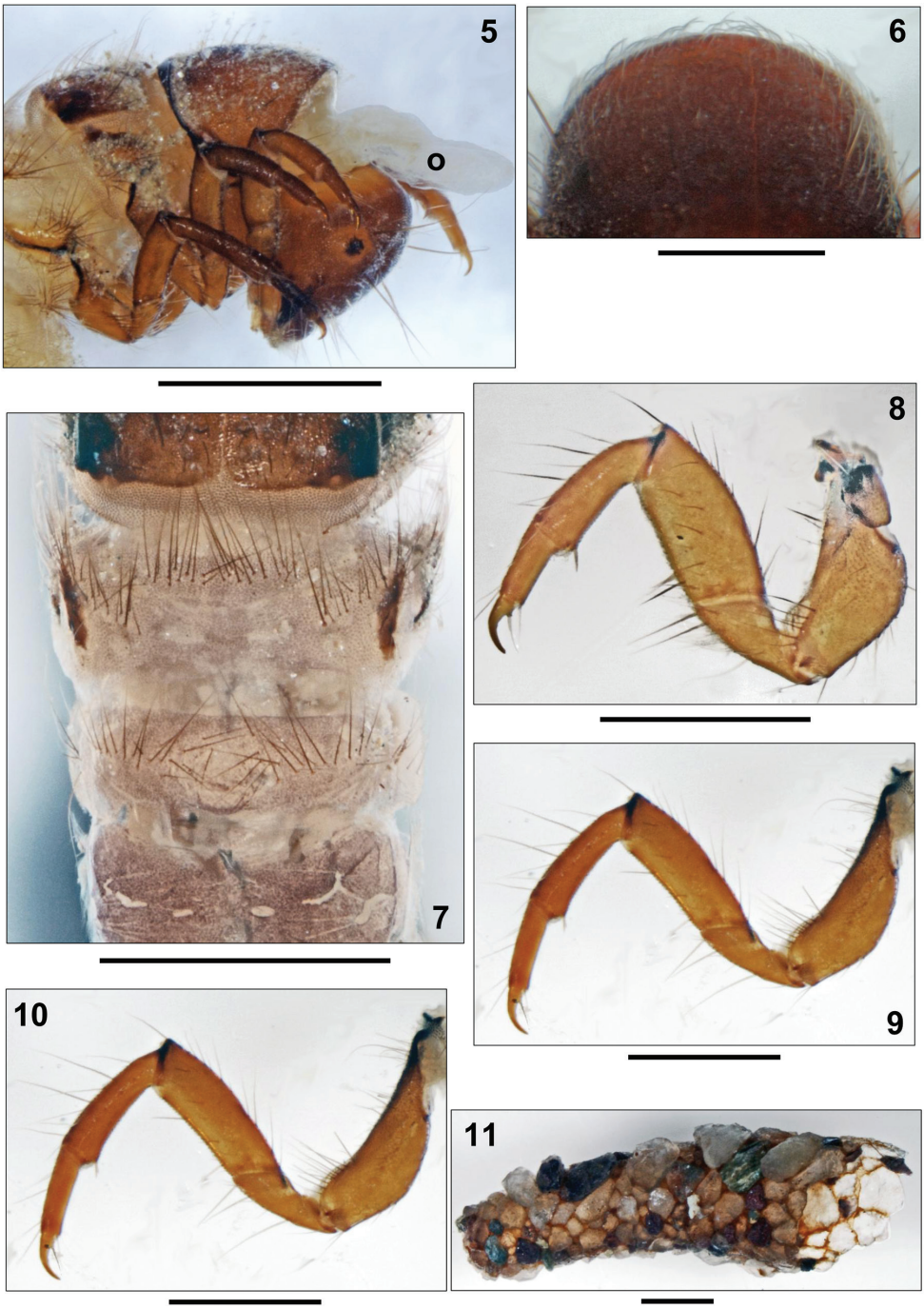
Head. Head capsule with dense cover of microspinules, roundish in shape and hypognathous (Figs 1–3). Coloration medium to dark brown; paler areas around eyes and around foramen occipitale (Figs 1, 2). Muscle attachment spots on frontoclypeus and parietalia small and indistinct (Figs 1, 2). In addition to complete set of primary setae, head capsule with many short, pale and almost translucent secondary setae (Fig. 2). Frontoclypeus bell-shaped, with narrow central constriction (Fig. 1). Antennae situated halfway between eye and anterior head margin (Fig. 1, arrow), short, each consisting of 1 short cylindrical base and 1 prominent lateral seta. Labrum dark brown, with setal brush at anterolateral corners originating from whitish pads of soft cuticle. Submentum wedge-shaped, sclerite almost as wide as long, convex-sided at center; light brown with medium brown pre-apical transverse band. Postgenal suture less than 20% of apotome length. Scraper-type mandibles (as in Fig. 15) black, brownish terminally, elongated quadrangular and without terminal teeth along cutting edge (as in Fig. 15).

Thorax. Pronotum medium brown, surface densely granulated, posterior margins thickened and darkly striped (Figs 4, 5). Pronotal transverse groove lacking, as typical for Apataniidae larvae (Figs 5, 6). In profile, pronotum slightly rounded (Fig. 5). Pronotal surface densely covered by pale, translucent, tapering setae with flexuous tips, especially along the anterior border (Fig. 6); in addition 30–40 longer setae present on each pronotal half (Figs 4, 5). As in other Apataniidae larvae, a defensive gland is situated beneath the pronotal sclerites. In all three European *Apataniana* larvae, an additional Y-shaped appendix is present which can be extended at the cervix between pronotum and head (=osmeterium; Fig. 5o); when threatened, the gland is able to produce a mix of up to 40 fatty acids as a defense agent against predacious invertebrates (Wagner et al. 1990). Pentangular prosternite densely covered by microspinules, very pale and indistinct in its anterior and lateral sections; along posterior border with light brown transverse band; with distinct, light brown and triangular lateral sclerites. Prosternal horn present. Mesonotum completely covered by 2 light brown sclerites; their anterior, lateral and postero-lateral margins darker sclerotized; mesonotal surface with medium brown muscle attachment spots (Fig. 4). Metanotum partially covered by only 1 pair of yellowish lateral sclerites with anterior groups of approximately 20 setae per sclerite and dark brown muscle attachment spots; anterior and posterior metanotal sclerites completely lacking and replaced by groups of setae: 15–20 at each anterior and 15–18 at each posterior metanotal position (Fig. 7). Legs yellowish to light brown with



Figures 1–4. *Apataniana stropones* Malicky 1993, 5th instar larva. **1** Head, frontal view (arrow: antenna) **2** Head, right fronto-lateral view **3** Head, ventral view **4** Pro- and mesonotum, dorsal view. Scale bars: 0.5 mm (except Fig. **4**: 1 mm).

numerous setae on coxae, trochanters and femora; tibiae and tarsi with only a small number of setae (Figs 8–10). Femora each with more than 1 proximodorsal seta. Coxa, femur and tibia of each foreleg wider than those of mid- and hind legs. Additional se-



Figures 5–11. *Apataniana stropones* Malicky 1993, 5th instar larva. **5** Head and thorax, right lateral view (o: osmeterium) **6** Pronotum, dorsal view **7** Metathorax and anterior abdominal segments, dorsal view **8** Left foreleg, posterior face **9** Left midleg, posterior face **10** Left hind leg, posterior face **11** Larval case, right lateral view. Scale bars: 0.5 mm (except Figs **5**, **7**, **11**: 1 mm).

tae present at anterior and posterior faces of all femora. Setae lacking at distal sections of trochanter on all legs. Mid tarsal claw shorter than half tarsal length; in addition, tarsal claw seta long and almost reaching tip of tarsal claw (Fig. 12).

Abdomen. 1st abdominal segment with 1 dorsal and 2 lateral fleshy protuberances densely covered by microspinules. Setal transversal band at 1st abdominal dorsum continuous at center, consisting of 45–60 setae (Fig. 7); dorsal of each lateral protuberance, an additional group of 7–10 setae is present. Ventral section of lateral protuberances and 1st abdominal sternum with continuous field of setae with basal sclerites minute and inconspicuous; total setal number is 130–150.

8th abdominal dorsum with 14–18 posterodorsal setae; several posterolateral setae on each half of 9th abdominal dorsum. All gills single filaments. Dorsal gills present at most from 2nd segment (postsegmental position) to 4th segment (postsegmental position). Ventral gills ranging from 2nd (postsegmental) to 6th segment (postsegmental). Lateral gills lacking. Lateral fringe extending from start of 2nd to mid 8th abdominal segment. Dorsal of lateral fringe a small number of forked lamellae is present per segment (as in Figs 20, 21).

Light brown sclerite on 9th abdominal segment semicircular, with light muscle attachment spots; with 26–33 setae, 6 long and the remainder short to medium in length (Fig. 14). Anal prolegs of the limnephilid type, light brown and with dark brown bar at anterodorsal border of claw base. Anal proleg lateral sclerite with 5 setae along posterior edge (Fig. 13). Anal claws dark brown, with tiny dorsal accessory hook.

Case. Fifth instar larval case 5.4–5.5 mm long (n= 3), curved, tapering posteriorly (width at anterior opening 1.8–2.0 mm and at posterior opening 0.9–1.1 mm), consisting of mineral particles of varying size, sometimes mixed with larger particles attached mostly laterally, thereby creating wing-like structures (Fig. 11).

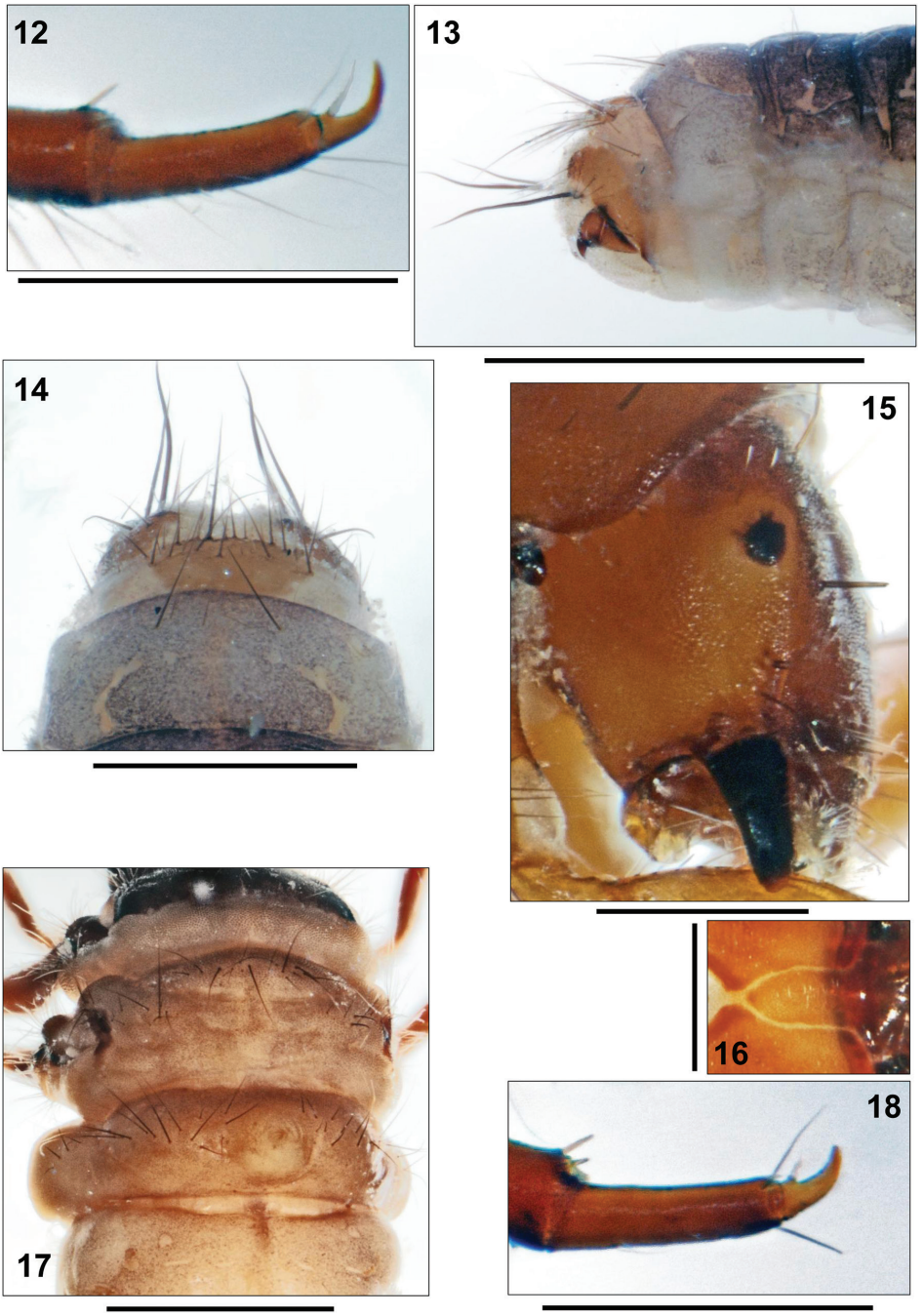
Apataniana hellenica Malicky, 1987

Description of the 5th instar larva (all morphological characters identical to those of *A. stropones* except as noted below). **Diagnosis.** Setal transversal band at 1st abdominal dorsum interrupted at center; central submentum parallel-sided.

Biometry. Body length of 5th instar larvae ranging from 6.4 to 6.9 mm, head width from 0.76 to 0.83 mm (n= 3).

Head. Head reddish brown with paler, wedge-shaped areas from eyes to mandible bases and around anterior border of head capsule (Fig. 15). Labrum medium to dark brown. Submentum narrower than in *A. stropones*, wedge-shaped, parallel-sided at center (Fig. 16); light to yellowish brown, with dark brown anterior, broadened section and medium brown posterior tip.

Thorax. Pronotum dark brown. Pentangular prosternite with medium brown transverse band; lateral sclerites indistinct. Mesonotum medium brown, posterolateral corners black (Fig. 19). Lateral sclerites with anterior groups of 10–20 setae per sclerite; anterior and posterior metanotal sclerites completely lacking and replaced by



Figures 12–18. 12–14 *Apataniana stropones* Malicky 1993, 5th instar larva. **12** Mid tarsus and claw, anterior face **13** Tip of abdomen, right lateral view **14** Ninth abdominal dorsum, dorsal view **15–18** *Apataniana hellenica* Malicky 1987, 5th instar larva. **15** Head, right lateral view **16** Submentum **17** Metathorax and anterior abdominal segments, dorsal view **18** Mid tarsus and claw, anterior face. Scale bars: 0.5 mm (except Figs **12**, **16**, **18**: 0.25 mm and Figs **13**, **17**: 1 mm).

groups of setae: 10–28 at each anterior and 10–15 at each posterior metanotal position (Fig. 17). Mid tarsal claw shorter than half tarsal length (Fig. 18).

Abdomen. Setal transversal band at 1st abdominal dorsum interrupted at center, consisting of 30–40 setae (Fig. 17); setal group dorsal of lateral protuberance with 4–7 setae each. Ventral section of lateral protuberances and 1st abdominal sternum with continuous field of setae with basal sclerites minute and inconspicuous; total setal number is 120–140.

Dorsal gills present at 3rd segment (postsegmental position), ventral gills ranging from 2nd (postsegmental) to 6th segment (postsegmental). Lateral gills lacking.

Case. Fifth instar larval case 8.3-8.7 mm long (n= 3), curved, tapering posteriorly (width at anterior opening 2.5–4.9 mm and at posterior opening 1.5–1.8 mm).

Apataniana vardusia Malicky, 1992

Description of the 5th instar larva (all morphological characters identical to those of *A. stropones* except as noted below). **Diagnosis.** Setal transversal band at 1st abdominal dorsum interrupted at center; 2nd tarsal claw longer than half tarsal length; central submentum parallel-sided.

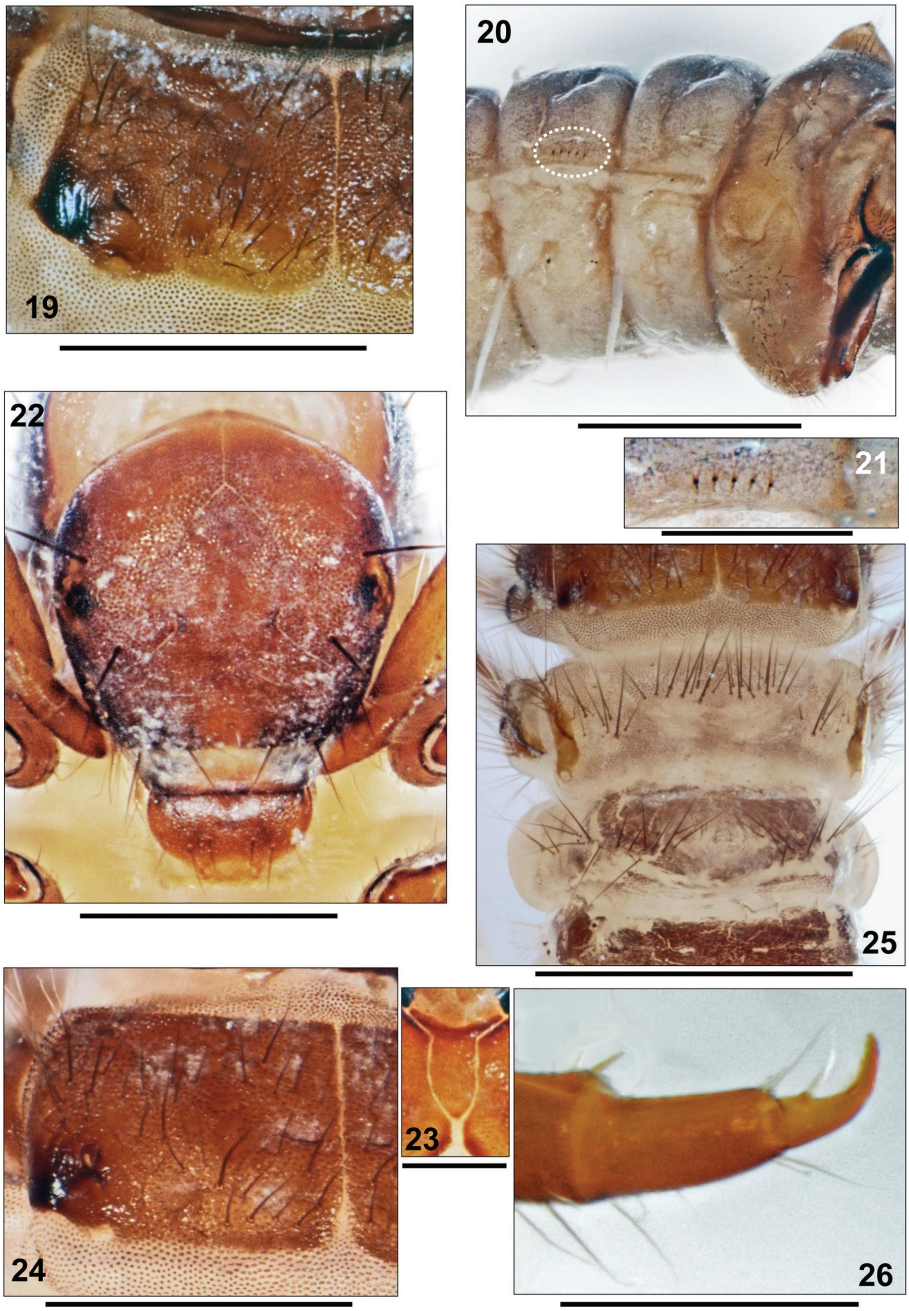
Biometry. Body length of 5th instar larvae ranging from 4.4 to 5.3 mm, head width from 0.70 to 0.73 mm (n= 3).

Head. Head medium brown (Fig. 22). Submentum narrower than in *A. stropones*, wedge-shaped, parallel-sided at center (Fig. 23); light to yellowish brown, with medium brown anterior, broadened section and pale posterior tip. Postgenal suture approximately 30% of apotome length (Fig. 23).

Thorax. Pronotal surface medium brown, with pale, small, roundish muscle attachment spots. In addition to dense cover of pale, translucent, tapering setae with flexuous tips 20–30 longer setae present on each pronotal half. Pentangular prosternite pale, with medium brown posterior transverse band; lateral sclerites indistinct. Mesonotum medium brown, posterolateral corners black; near anteromedian corner of this black spot with ear-like porus (Fig. 24). Lateral sclerites with anterior groups of approximately 10 setae per sclerite; anterior and posterior metanotal sclerites completely lacking and replaced by groups of setae: 5–10 at each anterior and 5–10 at each posterior metanotal position (Fig. 25). Mid tarsal claw longer than half tarsal length (Fig. 26).

Abdomen. Setal transversal band at 1st abdominal dorsum interrupted at center, consisting of 30–40 setae (Fig. 25); setal group dorsal of lateral protuberance with 4–7 setae each. Ventral section of lateral protuberances and 1st abdominal sternum with continuous field of setae with basal sclerites minute and inconspicuous; total setal number is 110–120. 8th abdominal dorsum with 18–20 posterodorsal setae. Light brown sclerite on 9th abdominal segment with 22–34 setae, 6 long and the remainder short to medium in length.

Case. Fifth instar larval case 6.3–6.4 mm long (n= 3), curved, tapering posteriorly (width at anterior opening 2.4–2.5 mm and at posterior opening 1.3–1.4 mm).



Figures 19–26. 19–21 *Apataniana hellenica* Malicky 1987, 5th instar larva. **19** Left mesonotum, dorsal view **20** Anterior abdominal segments, right lateral view (dotted oval: forked lamellae) **21** Detail of forked lamellae situated dorsal of lateral fringe **22–26.** *Apataniana vardusia* Malicky 1992, 5th instar larva. **22** Head, frontal view **23** Submentum **24** Left mesonotum, dorsal view **25** Metathorax and anterior abdominal segments, dorsal view **26** Mid tarsus and claw, anterior face. Scale bars: 0.5 mm (except Figs **20**, **25**: 1 mm and Figs **23**, **26**: 0.25 mm).

Synoptic key for the European *Apataniana* larvae (final instars)

Larval Apataniidae share the following set of morphological characters (Pitsch 1993, Solem 1985, Wallace et al. 2003, Waringer and Graf 2011, Wiggins 1998): transportable case present (Fig. 11); sclerites present on pro-, meso- and metanota (Fig. 5); no transverse rim at the anterior 3rd of the pronotum (Fig. 5); pronotum and mesonotum completely covered by 2 sclerites in close contact, separated by an unbranched longitudinal suture (Fig. 4); median and posterior metanotal sclerites reduced and represented only by setal groups (Fig. 7); prosternal horn present; antennae situated halfway between eye and anterior head margin (Fig. 1, arrow); scraper-type mandibles without terminal teeth along cutting edge (Fig. 15); submentum wedge-shaped (Fig. 3); head with many secondary setae (Fig. 2); fleshy protuberances present laterally and dorsally on the 1st abdominal segment (e.g., Fig. 7); all gills consisting of single filaments (Fig. 20).

In the framework of the larval key to European Apataniidae of Waringer et al. (2015) the three European species of genus *Apataniana* can be easily integrated by using the morphology of setae at the anterior edge of pronotum which are long, tapering and with flexuous tips (Fig. 6) and by the fact that the genus is restricted to the Hellenic Western Balkan (Greece).

Within the trio of *Apataniana* species, the setal transversal band at 1st abdominal dorsum is interrupted at center in *Apataniana vardusia* and A. *hellenica* (Figs 17, 25) but continuous in *A. stropones* (Fig. 7). The former species pair can be separated by the mid tarsal claw/tarsus ratio (Figs 18, 26), and by differences in head capsule width (Table 1). Submentum morphology (Figs 3, 16, 23) provides additional characters for identification of the three species.

Ecology, phenology and distribution

With respect to distribution, *Apatania hellenica* is an endemic species of the Ossa mountains in Eastern Thessalia, *A. stropones* is endemic to the Dirfis moutains on the island of Euböa and *A. vardusia* is an endemic species of the Vardusia mountains in Central Greece. The larvae of the three *Apataniana* species inhabit small brooklets with low water temperatures (Malicky 1987, 1992, 1993, 2005b, 2014a, b). *A. vardusia* was sampled above the tree line at an altitude of 1750 m a.s.l. where water temperature in the source of the brook was, as common for this altitude, 5.7°C in October and 5.7°C at the end of May. In the other two species, typical habitats are situated at lower altitudes: *A. hellenica* lives in the spring area and a short distance downstream of a brook in the Ossa Mountains situated at 1200 m a.s.l. (photograph in Malicky 2014b, p. 190) where water temperature was 5.4°C in May, 5.5°C at the end of July, and 5.5°C on the onset of October. Only 300 m downstream of this spot, the water temperature was 6.1–6.8°C at the end of May, 6.6–6.8°C at the beginning of June, 7.8–10.0°C at the end of July, and 7.6–8.0°C at the onset of October. A few specimens were occasionally found in other nearby springbrooks. *A. stropones* was collected in spring brooklets

Table 1. Synopsis of characters separating the currently known European *Apataniana* larvae (5th instars; Trichoptera: Apataniidae) (Malicky 1987, 1992, 1993, 2004, 2005a, b; Graf et al. 2008).

Species/ character	Setal transversal band at 1st abdominal dorsum interrupted at center (Fig 17)?	2nd tarsal claw shorter than half tarsal length (Fig 12)?	Submentum almost parallel-sided at mid section (Fig 16)?	Head width (mm)	Distribution
Apataniana vardusia	yes	no	yes	0.70–0.73	endemic of Vardusia mountains, Central Greece
Apataniana hellenica	yes	yes	yes	0.76–0.83	endemic of Ossa mountains, Eastern Thessalia, Greece
Apataniana stropones	no	yes	no	0.73–0.74	endemic of Dirfis moutains, Euböa, Greece

at the northern slope of Mt. Dirfis on the island of Euböa, at about 700 to 900 m a.s.l., where the water temperatures were 10.9°C in May, 10.5°C in June, and 10.2°C in August. A similar brook without *Apataniana* only 5 km eastward had water temperatures of 13.6–15.5°C in June, which is the usual temperature to be expected in Greek mountains at these elevations. Obviously, the three species are cold stenothermous. We have no explanation for the low water temperatures of the spring areas inhabited by *A. hellenica* and *A. stropones*. Karst phenomena are unknown in these areas, as the underground consists of various kinds of silicate rocks, and the area is covered by dense natural forest. Both areas are situated at the slopes of the mountains close to the sea. Dense cloud banks may be sometimes seen at mid-slope, often for many hours during fine weather, which may contribute to the low water temperatures of these spring locations.

As in the other known larvae of Apataniidae, the mandibles in the three Greek *Apataniana* species take the shape of scraper blades and lack terminal teeth along their cutting edges; larvae graze autotrophic biofilm and epilithic algae.

Adults of *A. hellenica* were found in June and July, but not in May and October; ultimate and penultimate larval instars were collected in May, June, July and October, mature pupae at the end of July. Therefore, the adults are on the wing between June and September, with one generation per year, but the presence of many ultimate instar larvae in October may be an indication that some individuals may need more than one year for their development. The phenology of *A. stropones* is similar. Only one male was collected at the end of June, but at the onset of August not only high numbers of adults but also mature and immature pupae and many ultimate and penultimate instar larvae were observed. In October no adults were present. In *A. vardusia*, on the other hand, the adult stage is entered later in the year. At the end of May, many larvae but no adults were found. At the end of October, adults, many fresh egg clutches, many empty cases and some ultimate and penultimate larvae were observed.

The three Greek *Apataniana* species are confined to extremely small areas. *A. vardusia* was only found in one spring brook some metres long. *A. hellenica* and *A. stropones*

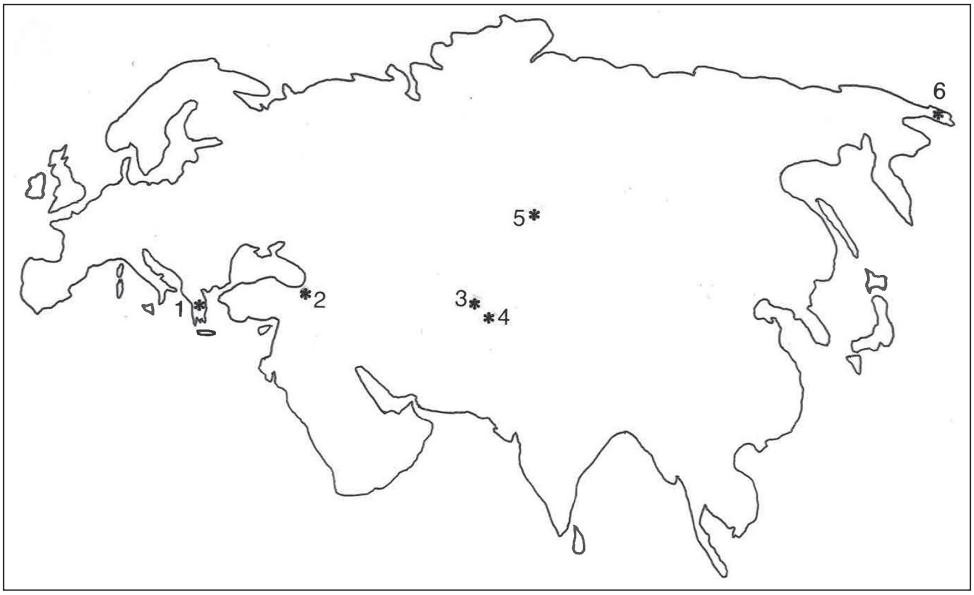


Figure 27. Distribution of *Apataniana* species of the *bulbosa* group: **1** *A. hellenica*, *A. stropones*, *A. vardusia* **2** *A. borcka* **3** *A. rauschorum* **4** *A. pamirensis* **5** *A. bulbosa* **6** *A. tschuktschorum*.

are restricted to a couple of brooklets in distances of several hundred metres. These tiny distribution ranges and the extremely low water temperatures of their habitats earmark the trio of Greek *Apataniana* species as glacial relics. It would be no surprise to detect some more closely related species in other mountain massifs in Greece.

The three species described above belong to the *Apatania bulbosa* group (Mey and Levanidova 1989). The other known species of this group include *A. borcka* from the Caukasus (Turkey, Province of Artvin), *A. rauschorum* from Kyrghyzstan, *A. bulbosa* from the Sajan Mountains, *A. pamirensis* from Tadzhikistan, and *A. tschuktschorum* from the Chukotski Peninsula in the extreme east of Siberia (Fig. 27). All of these species are extremely relictary, and to the best of our knowledge they live in cold streams. They are obviously Pleistocene relics, but the long distances between their areas, averaging thousands of kilometres, suggest that they are relics from one of the earlier (about 20) glaciation periods. Relics from the last (Würm) glaciation period are more often found, but they usually have other patterns of distribution (Malicky 1988). A parallel case in the European fauna is *Apatania volscorum* Moretti, 1988, restricted to a karst area south of Rome at 300 m a.s.l. It is only known from cold springbrooks with water temperatures of 9.6–10.2°C throughout the year. Its next relative, *Apatania ulmeri* Schmid, 1950, is restricted to the Sajan Mountains (near Lake Baikal) in a distance of more than 5000 kilometres away (Bicchierai and Moretti 1988; Corallini and Moretti 1988; Malicky 2005b; Moretti et al. 1988; Spinelli and Moretti 1988).

Acknowledgements

This paper is part of the outcomes of the project "The Drusinae (Insecta: Trichoptera) in a world of global change" (project number P23687-B17, PI: J.Waringer) funded by the Austrian Science Fund (FWF).

References

Bicchierai MC, Moretti GP (1988) Le ali di *Apatania volscorum* sp.n. (Trichoptera, Apataniinae). Atti XV Congresso Nazionale Italiano di Entomologia, L'Aquila 1988: 169–176.

Corallini C, Moretti GP (1988) *Apatania volscorum* sp.n. (Trichoptera, Apataniinae). La larva: morfologia, regime dietetico, parassiti ed epibionti. Atti XV Congresso Nazionale Italiano di Entomologia, L'Aquila 1988: 153–160.

Graf W, Murphy J, Dahl J, Zamora-Muñoz C, López-Rodríguez MJ (2008) Volume 1 – Trichoptera. In: Schmidt-Kloiber A, Hering D (Eds) Distribution and Ecological Preferences of European Freshwater Organisms. Pensoft Publishers, Sofia, Moscow, 388 pp.

Malicky H (1987) The first European *Apataniana* (Trichoptera, Limnephilidae): *Apataniana hellenica* sp. n., from Greece. Aquatic Insects 9: 210. doi: 10.1080/01650428709361298

Malicky H (1992) Vier neue griechische Köcherfliegen (Trichoptera). Entomologische Zeitschrift 102: 40–45.

Malicky H (1988) Spuren der Eiszeit in der Trichopterenfauna Europas (Insecta, Trichoptera). Rivista di Idrobiologia 27: 247–297.

Malicky H (1993) Eine dritte *Apataniana* aus Griechenland. Entomologische Zeitschrift 103: 352–356.

Malicky H (2004) Atlas of European Trichoptera, Second edition. Springer, Dordrecht, 35 pp.

Malicky H (2005a) Ein kommentiertes Verzeichnis der Köcherfliegen (Trichoptera) Europas und des Mediterrangebietes. Linzer biologische Beiträge 37: 533–596.

Malicky H (2005b) Die Köcherfliegen Griechenlands. Denisia 17: 1–240.

Malicky H (2014a) Veränderungen von Köcherfliegenfaunen am Beispiel einiger griechischer Fließgewässer im Verlauf von drei bis vier Jahrzehnten (Trichoptera). Entomologische Zeitschrift 124: 187–197.

Malicky H (2014b) Lebensräume von Köcherfliegen (Trichoptera). Denisia 34: 1–280.

Mey W, Levanidova IM (1989) Revision der Gattung *Apataniana* Mosely, 1936 (Trichoptera, Limnephilidae). Deutsche entomologische Zeitschrift N.F. 36: 65–98.

Moretti GP, Cianficconi F, Papagno F (1988) *Apatania volscorum* sp.n. (Trichoptera, Apataniinae): Morfologia, Tassonomia, ciclo biologico e geonemia. Atti XV Congresso Nazionale Italiano di Entomologia, L'Aquila 1988: 145–152.

Morse JC (Ed.) (2015) Trichoptera World Checklist. http://entweb.clemson.edu/database/trichopt/index.htm [accessed 25 November 2015]

Pitsch T (1993) Zur Larvaltaxonomie, Faunistik und Ökologie mitteleuropäischer Fließwasser-Köcherfliegen (Insecta:Trichoptera). Landschaftsentwicklung und Umweltforschung

- Schriftenreihe des Fachbereichs Landschaftsentwicklung. Sonderheft S8, Technische Universität Berlin, Berlin, 316 pp.

Solem J (1985) Norwegian *Apatania* Kolenati (Trichoptera: Limnephilidae): Identification of larvae and aspects of their biology in a high-altitude zone. Entomologica scandinavica 16: 161–174. doi: 10.1163/187631285X00252

Spinelli G, Moretti GP (1988) *Apatania volscorum* sp.n., (Trichoptera). La pupa, l'esuvia, il fodero. Morfologia e chetotassi. Atti XV Congresso Nazionale Italiano di Entomologia, L'Aquila 1988: 161–168.

Wagner R, Aurich M, Reder E, Veith HJ (1990) Defensive secretion from the larvae of *Apatania fimbriata* (Pictet) (Trichoptera: Limnephilidae). Chemoecology 1: 96–104. doi: 10.1007/BF01241650

Wallace ID, Wallace B, Philipson GN (2003) Keys to the case-bearing caddis larvae of Britain and Ireland. Freshwater Biological Association Scientific Publication 61: 1–259.

Waringer J, Graf W (2011) Atlas of Central European Trichoptera Larvae. Erik Mauch Publishers, 468 pp.

Waringer J, Lubini V, Hoppeler F, Pauls SU (2015) DNA-based association and description of the larval stage of *Apatania helvetica* Schmid 1954 (Trichoptera, Apataniidae) with notes on ecology and zoogeography. Zootaxa 4020: 244–256. doi: 10.11646/zootaxa.4020.2.2

Wiggins GB (1998) Larvae of the North American Caddisfly Genera (Trichoptera), 2nd Edition. University of Toronto Press, Toronto, 457 pp.

ZooKeys 586: 135–144 (2016)
doi: 10.3897/zookeys.586.7423
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Taxonomic notes relating to *Glenea diversenotata* Schwarzer and *G. quadriguttata* Pic (Coleoptera, Cerambycidae, Lamiinae)

Mei-Ying Lin[1], Steven W. Lingafelter[2]

1 *Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beichen West Road, Chaoyang Dist., Beijing, 100101, China* **2** *Systematic Entomology Lab, ARS, USDA, National Museum of Natural History, MRC-168, P.O. Box 37012, Washington, DC 20013-7012, U. S. A.*

Corresponding authors: *Mei-Ying Lin* (linmeiying@ioz.ac.cn); *Steven W. Lingafelter* (elaphidion@gmail.com)

Academic editor: *Ann Ray* | Received 6 December 2015 | Accepted 8 April 2016 | Published 4 May 2016

http://zoobank.org/750A2837-0038-499B-9AB2-0FD7213DBC4B

Citation: Lin M-Y, Lingafelter SW (2016) Taxonomic notes relating to *Glenea diversenotata* Schwarzer and *G. quadriguttata* Pic (Coleoptera, Cerambycidae, Lamiinae). ZooKeys 586: 135–144. doi: 10.3897/zookeys.586.7423

Abstract

Glenea diversenotata Schwarzer, 1925 is reinstated from a subspecies of *G. tonkinea* Aurivillius, 1925 to species level and *G. neohumerosa* Lin & Yang, 2011 is a new junior synonym. Some biological information on *G. diversenotata* is recorded for the first time, including pictures of the larva and pupa. *G. quadriguttata* Pic, 1926 is reinstated from a subspecies of *G. lacteomaculata* Schwarzer, 1925 to species level. Lectotypes for *G. lacteomaculata* and *G. quadriguttata* are designated. A modified key to the related species is presented.

Keywords

Saperdini, new synonym, lectotype, Taiwan, Oriental region

Introduction

Glenea diversenotata Schwarzer, 1925 and *G. quadriguttata* Pic, 1926 were described from Taiwan and N. Vietnam, respectively. Later, the former was downgraded as a subspecies of *G. tonkinea* Aurivillius, 1925 and the latter as a subspecies of *G. lacteomaculata* Schwarzer, 1925 by Breuning (1956). Comparison to the type materials, however, reveals that each of them should be reinstated to species level from subspecies level. We therefore present the new taxonomic decisions, justifications, and full synonymies below.

Copyright *Mei-Ying Lin, Steven W. Lingafelter.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Materials

Types and other material studied are deposited in the following institutions:

IZAS	Institute of Zoology, Chinese Academy of Sciences, Beijing, China
MNHN	Muséum national d'Histoire naturelle, Paris, France
SMF	Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt-am-Main, Germany
SDEI	Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany

Results

Glenea diversenotata Schwarzer, 1925

Glenea diversenotata Schwarzer, 1925: 152. Type locality: China, Taiwan, Kosempo. Type depository: SDEI.
Glenea (*s. str.*) *diversenotata*; Gressitt 1951: 575.
Glenea (*Glenea*) *tonkinea* sbsp. *diversenotata*; Breuning 1956b: 743; Breuning 1966: 689.
Glenea (*Glenea*) *tonkinea diversenotata*; Yu and Nara 1988: 45, 92, pl. 20, fig. 23; Yu, Nara and Chu 2002: 68, 119, pl. 25, fig. 11; Löbl and Smetana 2010: 327.
Glenea (*G.*) *tonkinea* subsp. *diversenotata*; Nakamura, Makihara, Saito 1992: 104; Nakamura et al. 2014: 175.
Glenea diversenotata; Hua 2002: 210; Behne and Gaedike 2013: 177.
Glenea tonkinea diversenotata; Hua 2002: 210; Hua et al. 2009, 219, 360 (the picture pl. LXXXIV, fig. 967 is a *G. coomani* Pic).
Glenea neohumerosa Lin & Yang, 2011: 62, figs 12–23. **New synonym**.
Glenea neohumerosa; Lin 2015a: 204, fig. 205-3; 2015b: 290, figs 1859448, 1859455, 1859450.

Remarks. Breuning (1956) treated *G. diversenotata* Schwarzer as a subspecies of *G. tonkinea* Aurivillius, 1925. The first author examined a photograph of the holotype of *G. diversenotata* Schwarzer, 1925 (taken by Nobuo Ohbayashi, Japan) and a photograph of a live specimen matching it from Taiwan (taken by Yu-Long Lin, Taiwan). Our study of these additional materials necessitates a new synonymy of *G. neohumerosa* Lin & Yang, 2011. We can find no morphological differences to maintain them as separate species. Likewise, our examination of the types of *G. diversenotata* and *G. tonkinea* leads us to review the taxonomic position of them since Breuning (1956) that *G. diversenotata* is a subspecies of *G. tonkinea*. We can find no morphological support for that treatment by Breuning. They are easily separated from each other by the following characters – *Glenea tonkinea*: vertex and occiput of the head with two separate, longitudinal vittae of white pubescence; white elytral maculae more slender and transverse; outer, basal, white, elytral maculae anterolaterally positioned relative to the larger, basal, sutural maculae; outer apical spine of

Figures 1–5. *Glenea diversenotata* Schwarzer, 1925 from Hainan, Diaoluoshan, taken in 2010.IV.23, by Zi-Wei Yin. **1** The broad-leaved forest located in Hainan, Diaoluoshan **2–3** A large decomposing log with *Glenea diversenotata* Schwarzer, 1925 inside **4** Larva **5** Pupa.

elytra weakly produced; — *G. diversenotata*: vertex and occiput with vittae partially fused, not separate; white elytral maculae more rounded; outer basal elytral maculae parallel with the larger, basal, sutural maculae; outer apical spine of elytra pronounced.

Distribution. China (Fujian, Taiwan, Hainan, Guangxi); Vietnam (Tonkin).

Additional specimens examined (Lin and Yang 2011). Holotype, female (Figs 9a, 9b), Formosa, Kosempo,1912.VI.7, leg. H. Sauter (SDEI); 1 male, Taiwan, Pingdong County, Mt. Dahanshan, 2010. VIII.29, leg. Yu-Long Lin & Wenhsin Lin (examined through a live picture, specimen is deposited in private collection of Yu-Long Lin, Taiwan).

Biological notes. A larva (Fig. 4), two pupae (Fig. 5) and two adults (Fig. 6) were extracted from a large, decomposing log (Figs 2–3) found from a creek valley in a broad-leaved evergreen forest (Fig. 1) in 23 April, 2010 (according to personal communication with Zi-Wei Yin, in December, 2013). The stream is located in Hainan, Lingshui County, Diaoluoshan, ca. 935 m, 18°43'36"N, 100°52'14"E. The fresh emerged adults (Fig. 6) have the pubescence white which becomes yellow afterwards (Figs 7–8), and becoming white again after pinned and dried.

Glenea tonkinea Aurivillius, 1925

Glenea tonkinea Aurivillius, 1925: 521, fig. 160. Type locality: Vietnam, Tonkin, Hoa Binh. Type depository: MNHN.
Glenea (*s. str.*) *tonkinea*; Gressitt 1951: 580.
Glenea (*Glenea*) *tonkinea* m. *basirufofemorata* Breuning, 1956a: 698; Breuning 1956b: 743; Breuning 1966: 689.
Glenea (*Glenea*) *tonkinea*; Breuning 1966: 689.
Glenea (*Glenea*) *tonkinea* m. *apicetruncata* Breuning, 1956b: 743; Breuning 1966: 689.
Glenea (*G.*) *tonkinea*; Nakamura, Makihara, Saito, 1992: 104; Nakamura et al. 2014: 175.
Glenea tonkinea tonkinea; Hua, Nara and Yu 1993: 165, 297, pl. XXII, fig. 370b; Hua 2002: 210; Hua et al. 2009, 219, 360, pl. LXXXIV, fig. 967.
Glenea (*Glenea*) *tonkinea tonkinea*; Löbl and Smetana 2010: 327.
Glenea tonkinea; Lin and Yang 2011: 67, figs 30–33.

Remarks. The record from Taiwan (Nakamura, Makihara, Saito, 1992; Hua 2002; Löbl and Smetana 2010; Nakamura et al. 2014) is doubtful. This taxon was not mentioned in the four volumes of Taiwanese fauna book (Yu and Nara 1988; Yu, Nara and Chu 2002; Chou 2004, 2008).

Distribution. China (Guangdong, Hainan, Guangxi, Taiwan?); Vietnam (Tonkin), Myanmar.

Glenea lacteomaculata Schwarzer, 1925, lectotype designation

Glenea lacteomaculata Schwarzer, 1925: 151. Type locality: China, Taiwan, Kosempo, Sokutsu. Type depository: SDEI.

Figures 6–8. *Glenea diversenotata* Schwarzer, 1925, adult, live pictures. **6** A fresh emerged adult from the log in fig. 2, from Hainan, Diaoluoshan, showing the white pubescence, taken in 2010.IV.23, by Zi-Wei Yin **7** An active adult from Hainan, Jianfengling, showing the yellow pubescence, taken in 2011.V.23, by Wen-Xuan Bi **8** An active adult from Taiwan, Dahanshan, showing the yellow pubescence, taken in 2010.VIII.29, by Yu-Long Lin.

Glenea (*s. str.*) *lacteomaculata*; Gressitt 1951: 575.
Glenea (*Glenea*) *lacteomaculata*; Breuning 1956b: 743; Breuning 1966: 689; Yu and Nara 1988: 45, 92, pl. 20, fig. 18; Yu, Nara and Chu 2002: 68, 119, pl. 25, fig. 10.
Glenea (*G.*) *lacteomaculata*; Nakamura, Makihara, Saito, 1992: 105; Nakamura et al. 2014: 174.
Glenea lacteomaculata; Hua 2002: 210; Hua et al. 2009, 216, 358, pl. LXXXII, fig. 939.
Glenea (*Glenea*) *lacteomaculata lacteomaculata*; Löbl and Smetana 2010: 325; Danilevsky 2013: 201.
Glenea lacteomaculata; Lin and Yang 2011: 65, fig. 24; Behne and Gaedike 2013: 183.

Remarks. Lin and Yang (2011) were unable to define *G. lacteomaculata* Schwarzer, 1925. Subsequently, the first author examined a photograph of a syntype of *G. lacteomaculata*

(taken by Nobuo Ohbayashi, Japan). Comparing that photograph with another of *G. quadriguttata* Pic, 1926, it is possible to conclude that the latter must be elevated to species. Hua (2002) indexed Taiwan and Guangxi and this was followed by Löbl and Smetana (2010). However, the specimens from Guangxi should be *G. quadriguttata* Pic, 1926 (Lin and Yang 2011) and only Taiwan was the known locality of *G. lacteomaculata* Schwarzer, 1925.

Distribution. China: Taiwan.

Lectotype designation. According to the original description (Schwarzer 1925), there were multiple type specimens. The syntypes were deposited in SDEI and SMF. In order to fix the species concept and ensure universal and consistent interpretation of this species, we designate the female specimen in SDEI as the lectotype (Fig. 10a, b), the female in SMF (Fig. 11a, b) and another one with same collecting data to the lectotype (Behne and Gaedike 2013) as the paralectotypes of *G. lacteomaculata* Schwarzer, 1925. Though the female in SMF is in better condition than the female with antennae mostly lost in SDEI, we pick up the SDEI one as lectotype according to ICZN Recommendation 74D. The majority of Schwarzer's types collected by Hans Sauter is contained in SDEI (listed by Behne and Gaedike 2013; Stephan Blank and Junsuke Yamasako, personal communication in November, 2015). The lectotype has the following labels: "Sokutsu/ Formosa/ H. Sauter 1912" printed, "7.VI." printed, "Glenea lacteomaculata / Cotype! Schwarzer" handwritten, "Syntypus" printed on red label, "DEI Coleoptera # 200572" printed on white label. And a lectotype label will be added by the managers in SDEI after this paper.

Glenea quadriguttata Pic, 1926, revised status, lectotype designation

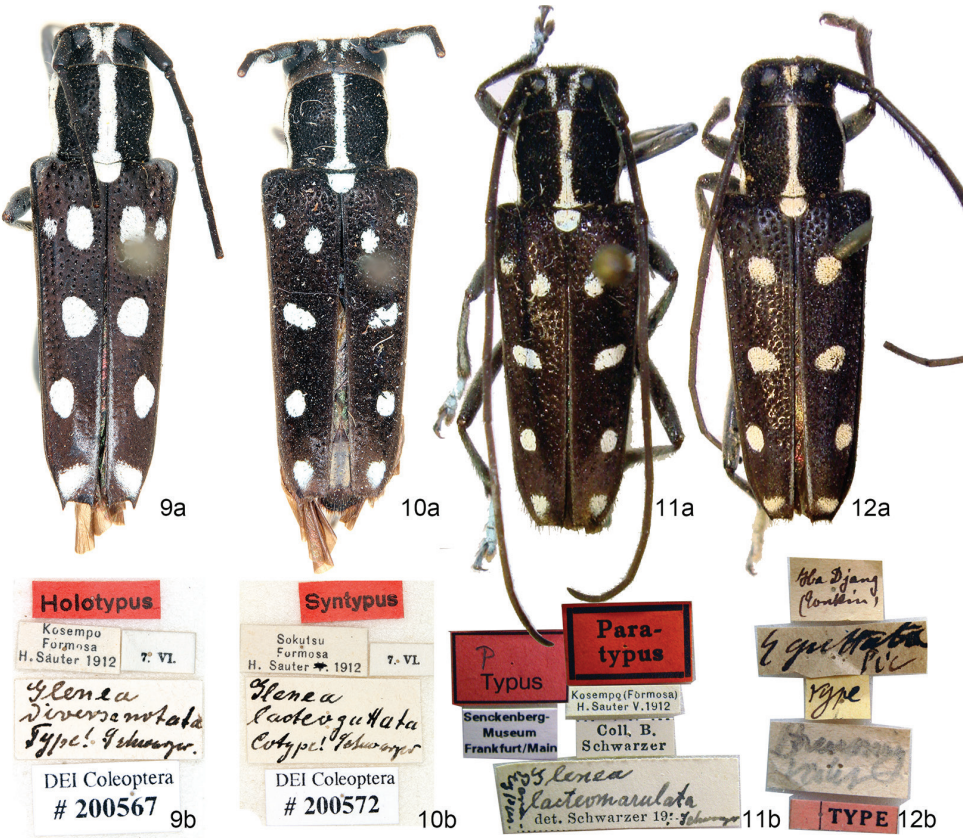
Glenea 4-guttata Pic, 1926: 22. Type locality: Vietnam, Tonkin, Djang. Type depository: MNHN.

Glenea (*Glenea*) *lacteomaculata* sbsp. *quadriguttata*; Breuning 1956b: 743, 744; Breuning 1966: 689.

Glenea lacteomaculata quadriguttata; Lin and Yang 2011: 65, figs 25–26.

Glenea (*Glenea*) *lacteomaculata quadriguttata*; Danilevsky 2013: 201.

Remarks. Breuning (1956) had previously treated *G. quadriguttata* as a subspecies of *G. lacteomaculata*. We have found the following morphological differences between them — *Glenea lacteomaculata*: vertex and occiput of the head with two separate, longitudinal vittae of white pubescence; punctures at the base of the elytra denser and more irregularly spaced than in *G. quadriguttata*; central-most, white elytral maculae more slender, transverse and oblique; elytral apex without acute or toothed angles; — *Glenea quadriguttata*: head with vittae fused, not separate; central-most, white elytral maculae almost rounded; elytral apex with small sutural and outer apical teeth. With these differences, *G. quadriguttata* is reinstated as an independent species.



Figures 9–12. *Glenea* spp., **a** habitus, dorsal view **b** labels **9** *Glenea diversenotata* Schwarzer, 1925, holotype, female, in SDEI (photographed by Nobuo Ohbayashi) **10–11** *Glenea lacteomaculata* Schwarzer, 1925 **10** Lectotype, female, in SDEI (photographed by Nobuo Ohbayashi) **11** paralectotype, female, in SMF (photographed by Mei-Ying Lin) **12** *Glenea quadriguttata* Pic, 1926, lectotype, female, in MNHN (photographed by Mei-Ying Lin). All pictures not to scale.

Distribution. China (Guangxi, Yunnan); Vietnam (Tonkin).

Lectotype designation. The original description does not allow to know the number of specimens used by Pic (1926). In order to fix the species concept and ensure universal and consistent interpretation of this species, we designate the female specimen with Pic's handwriting labels as the lectotype (Fig. 12a, b), and the first author did not find another syntype to be paralectotype when she worked in MNHN in 2007–2008. The lectotype has the following labels: "Ha Djang/ (Tonkin)" handwritten, "G. quadriguttata Pic" handwritten, "type" handwritten on yellow label, "Breuning valiv" handwritten, "TYPE" printed on pink red label. And a lectotype label will be added by the managers in MNHN after this paper.

Key to *Glenea coomani* group

(modified from Lin and Yang 2011)

1 Elytral apex having only a short tooth at the outer angle (subequal to that at the sutural angle, fig. 34 in Lin and Yang 2011) **2**

– Elytral apex having a long and sharp spine at the outer angle (much longer than that at the sutural angle, fig. 35 in Lin and Yang 2011) **5**

2 Elytron having only one big oval macula at basal quarter; the second macula is the smallest (Figs 1–4 in Lin and Yang 2011) ..... ***G. coomani***

– Elytron having two small spots at basal quarter, the first spot is the smallest.... **3**

3 Vertex with one pubescent spot between upper eye lobes; the middle spot on elytron almost rounded (Fig. 12) ..... ***G. quadriguttata***

– Vertex with two pubescent vittae between upper eye lobes; the middle spot on elytron transverse and oblique..... **4**

4 Suture without pubescence stripe; the middle vitta shorter and still far from suture; the apical pubescent vitta small and with distance from suture (Figs 10–11)..... ***G. lacteomaculata***

– Suture with pubescence stripe; the middle vitta longer and almost touching sutural stripe; the apical pubescent vitta larger and fused with sutural stripe (Figs 30–33 in Lin and Yang 2011) .....***G. tonkinea***

5 Elytron having 5 white or yellow maculae (figs 12–15 in Lin and Yang 2011; Figs 6–9)..... ***G. diversenotata***

– Elytron having 6 white or yellowbrown maculae..... **6**

6 Legs testaceous; elytral apical spot smaller, not touching suture; vertex with two yellowish-brown spots between upper eye lobes (fig. 27 in Lin and Yang 2011)..... ***G. laodice***

– Legs black; elytral apical spot larger and touching suture; vertex with one yellowish-brown spot between upper eye lobes (figs 28–29 in Lin and Yang 2011)..... ***G. subalcyone***

Acknowledgements

We are grateful to Gérard Tavakilian and Olivier Montreuil (MNHN), Stephan Blank (SDEI), and Andrea Hastenpflug-Vesmanis (SMF) for providing access to the collections and the allowance to use the pictures. We wish to express our sincere thanks to Nobuo Ohbayashi and Junsuke Yamasako (Japan), Yu-Long Lin (Taiwan, China), Zi-Wei Yin and Wen-Xuan Bi (Shanghai, China) for sharing pictures and information. We thank two reviewers and the editor of Zookeys for critical comments. This research was supported by NSFC programs 31472029 and J1210002, and partly by a grant (No. O529YX5105) from the Key Laboratory of the Zoological Systematics and Evolution of the Chinese Academy of Sciences.

References

Aurivillius C (1925) Neue oder wenig bekannte Coleoptera Longicornia. 21. Arkiv för Zoologi, Uppsala 18A(9): 503–524, 23 figs.

Behne L, Gaedike H (2013) Katalog der in den Sammlungen des Senckenberg Deutschen Entomologischen Instituts aufbewahrten Typen – Coleoptera: Cerambycidae. Contributions to Entomology: Beiträge zur Entomologie 63(1): 169–198.

Breuning S (1956a) Lamiaires nouveaux de la collection Lepesme (2e note). Longicornia III: 665–702, 12 figs.

Breuning S (1956b) Revision der Gattung *Glenea* Newm. (1. Fortsetzung). Entomologischen Arbeiten aus dem Museum G. Frey, Tutzing bei München 7(2): 671–893.

Breuning S (1966) Catalogue des Lamiaires du Monde (Col. Céramb.). Verlag des Museums G. Frey, Tutzing bei München (9): 659–765.

Chou WI (2004) The Atlas of Taiwanese Cerambycidae. Owl Publishing House, Taipei, 408 pp.

Chou WI (2008) The Atlas of Taiwanese Cerambycidae (second edition). Owl Publishing House, Taipei, 408 pp.

Danilevsky ML (2013) Additions and corrections to the new Catalogue of Palaearctic Cerambycidae (Coleoptera) edited by I. Löbl and A. Smetana, 2010. Part. VII. Humanity space - International Almanac 2(1): 170–210.

Gressitt JL (1951) Longicorn beetles of China. In: Lepesme P (Ed.) Longicornia, études et notes sur les longicornes, Volume 2. Paul Lechevalier, Paris, 667 pp., 22 pls.

Hua LZ (2002) List of Chinese Insects. Zhongshan (Sun Yat-sen) University Press, Guangzhou, 2: 1–612.

Hua LZ, Nara H, Yu CK (1993) Longicorn Beetles of Hainan & Guangdong. Muh-Sheng Museum of Entomology, Taiwan, 320 pp, 24 pls.

Hua LZ, Nara H, Samuelson GA, Lingafelter SW (2009) Iconography of Chinese Longicorn Beetles (1406 species) in Color. Sun Yat-sen University Press, Guangzhou, 474 pp, 125 pls.

Lin MY (2015a) A Photographic Guide to Longhorn Beetles of China. Chongqing University Publishing house, Chongqing, 227 pp.

Lin MY (2015b) Album of Type Specimens of Longhorn Beetles Deposited in National Zoological Museum of China. Henan Science and Technology Press, Zhengzhou, 374 pp.

Lin MY, Yang XK (2011) *Glenea coomani* Pic, 1926 and its related species of South China with description of a new species. ZooKeys 153: 57–71. doi: 10.3897/zookeys.153.2106

Löbl I, Smetana A (2010) Catalogue of Palaearctic Coleoptera. Vol. 6. Apollo Books, Stenstrup, 924 pp.

Nakamura S, Makihara H, Kurihara T, Yamasako J (2014) Check-list of Longicorn-beetles of Taiwan. Miscellaneous Reports of the Hiwa Museum for Natural History, Shobara (55): 1–277.

Nakamura S, Makihara H, Saito A (1992) Check-list of Longicorn-Beetles of Taiwan. Miscellaneous Reports of the Hiwa Museum for Natural History, Shobara (33): 1–111.

Pic M (1926) Nouveautés diverses. Mélanges Exotico-Entomologiques 47: 1–32.

Schwarzer B (1925) Sauters Formosa-Ausbeute (Cerambycidae. Col.). (Subfamilie Lamiinae.). Entomologische Blätter 21(4): 145–154.

Tavakilian G, Chevillotte H (2015) Titan: base de données internationales sur les Cerambycidae ou Longicornes. Version 3.0. http://lis-02.snv.jussieu.fr/titan/ [accessed on Novermber 3, 2015]

Yu CK, Nara H (1988) The Longicorn beetles of Taiwan. Muh-Sheng Museum of Entomology, Taiwan, 112 pp.

Yu CK, Nara H, Chu YI (2002) The Longicorn beetles of Taiwan (new edition). Muh-Sheng Museum of Entomology, Taiwan, 151 pp.

ZooKeys 586: 145–162 (2016)
doi: 10.3897/zookeys.586.8375
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

A new genus and species of leaf-mining moth from the French Alps, *Mercantouria neli* gen. n., sp. n. (Lepidoptera, Gracillariidae)

Peter Huemer[1], Carlos Lopez-Vaamonde[2,3], Paolo Triberti[4]

1 *Tiroler Landesmuseen Betriebsgesellschaft m.b.H., Naturwissenschaftliche Sammlungen, Feldstr. 11 a, A-6020 Innsbruck, Austria* **2** *INRA, UR0633 Zoologie Forestière, F-45075 Orléans, France* **3** *Institut de Recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université François-Rabelais de Tours, UFR Sciences et Techniques, 37200 Tours, France* **4** *Museo Civico di Storia Naturale, Lungadige Porta Vittoria 9, I-37129 Verona, Italy*

Corresponding author: *Peter Huemer* (p.huemer@tiroler-landesmuseen.at)

Academic editor: *R. Rougerie* | Received 4 March 2016 | Accepted 13 April 2016 | Published 4 May 2016

http://zoobank.org/FC5F4BB9-FD1F-4B9B-A124-F721DCC265D2

Citation: Huemer P, Lopez-Vaamonde C, Triberti P (2016) A new genus and species of leaf-mining moth from the French Alps, *Mercantouria neli* gen. n., sp. n. (Lepidoptera, Gracillariidae). ZooKeys 586: 145–162. doi: 10.3897/zookeys.586.8375

Abstract

The Alps are a hotspot of biodiversity in Europe with many Lepidoptera species still to be discovered. Here we describe a new gracillariid genus and species, *Mercantouria neli* **gen. n.** and **sp. n.** The morphology of the male genitalia is highly differentiated with unique features. DNA barcodes show that its nearest neighbor is the North American species '*Caloptilia*' *scutellariella* (Braun, 1923). *M. neli* is known from four adults (two males and two females) collected at two localities in the French Alps. Its host plant and life cycle remain unknown.

Keywords

COI, DNA barcoding, histone 3, *Gracillaria* group, new genus, new species, Alps

Copyright *Peter Huemer et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

For more than two centuries the Alpine Lepidoptera fauna has been at the focus of intense taxonomic and faunistic work. As a result, an estimated 5000 Lepidoptera species are known to occur in the Alps, of which about 250 species (ca. 5%) but only a single monotypic genus (*Lunakia* Klimesch, 1941, Plutellidae) are known to be endemic to the alpine region (Huemer 1998, unpubl. data). Several additional genera from various families, e.g. *Kessleria* Nowicki, 1864 (Yponomeutidae), *Sattleria* Povolný, 1965 (Gelechiidae), *Sphaleroptera* Guenée, 1845 (Tortricidae), *Erebia* Dalman, 1816 (Nymhpalidae), *Sciadia* Hübner, 1822 and *Glacies* Milliére, 1874 (Geometridae), show strong diversification and endemism in the alpine region. However, despite the relatively good knowledge of the Alpine Lepidoptera fauna, the recent use of DNA barcoding has helped to reveal an increasing number of new species. Many of these newly discovered taxa are cryptic or morphologically difficult to distinguish (Buchner 2015; Huemer 2011; Huemer and Hausmann 2011; Huemer and Hebert 2011; Huemer et al. 2013; Huemer and Timossi 2014; Huemer et al. 2014a,b; Huemer and Mutanen 2015; Kirichenko et al. 2015; Tabell and Baldizzone 2014; Whitebread 2007; Zeller and Huemer 2015). However, here we report the remarkable discovery of a genetically and morphologically highly divergent micro moth species of the family Gracillariidae from the French Alps.

Gracillariidae are a relatively well known family in Europe with 23 genera and 260 species recorded (De Prins and De Prins 2015). However, new species have been discovered recently (Laštůvka and Laštůvka 2006; 2012; Triberti 2007; Laštůvka et al. 2013; Kirichenko et al. 2015).

The new genus and species described here belongs to the Gracillariinae. This subfamily contains four groups of genera: *Acrocercops*, *Gracillaria*, *Parectopa* and *Parornix* (Kumata et al. 1988a,b). The new taxon belongs to the *Gracillaria* group, which is characterized by the presence of the vein R_{2+3} on the hindwing (Kumata 1982). In the Western Palearctic eight genera are recognized to belong to the *Gracillaria* group: *Gracillaria* Haworth, 1828; *Caloptilia* Hübner, 1825; *Povolnya* Kuznetzov, 1979; *Calybites* Hübner, 1822; *Euspilapteryx* Stephens, 1835; *Aspilapteryx* Spuler, 1910; *Aristaea* Meyrick, 1907; *Cupedia* Klimesch & Kumata, 1973 (Kumata 1982; 1995). In this study also the monotypic Eastern Palearctic genus *Eucalybites* Kumata, 1982, has been included in the comparison for some similarities.

To date, over 40 species of the *Gracillaria* group are known to occur in Europe, about 30 included in the genus *Caloptilia*. In the larval stage most species are leaf miners in early instars and leaf rollers in late instars, while some are leaf miners throughout the whole feeding stage. The majority of species prefer the leaves of bushy and woody plants, included mainly in the families Aceraceae and Betulaceae (especially favored), Fagaceae, Oleaceae and Anacardiaceae. More rarely they also feed on herbaceous plants, particularly in the families Plantaginaceae, Hypericaceae and Asteraceae (De Prins and De Prins 2015).

Here we present genetic (mitochondrial and nuclear) and morphological data that support the hypothesis that individuals of a highly differentiated *Gracillariinae* collected in the French Alps represent a distinct lineage that we formally describe as a new genus and a new species – *Mercantouria neli* Huemer, Lopez-Vaamonde & Triberti, gen. n., sp. n.

Materials and methods

Taxon sampling

Specimens examined in this study were obtained by light trapping integrating UV tubes and mercury lamp. A single specimen was collected flying freely above low vegetation at dusk. Specimens were preserved in tubes, pinned and wings spread in the next morning.

Morphology and nomenclature

We examined the morphology of four dried, pinned specimens belonging to *Mercantouria neli*. The holotype was photographed with an Olympus SZX 10 binocular microscope and an Olympus E 3 digital camera and processed using the software Helicon Focus 4.3 and Adobe Photoshop CS4 and Lightroom 2.3. Genitalia photographs were taken with an Olympus E1 Digital Camera from Olympus BH2 microscope.

Genitalia dissections and slide mounts followed Robinson (1976). Terminology of the genitalia follows Klots (1970) and Kristensen (2003); wing venation Kumata (1982).

Type material is deposited in the collection of TLMF = Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria.

DNA sequencing and analysis

DNA extracts were prepared from a single hind leg removed from three of the four specimens of *C. neli*. DNA extraction, PCR amplification and sequencing of the barcode region were carried out at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) following standard protocols (deWaard et al. 2008). Sequence divergences were quantified using the Kimura 2-parameter model implemented within the analytical tools on BOLD (www.boldsystems.org) (Ratnasingham and Hebert 2007).

In addition, an aliquot of DNA of sample TLMF Lep 08375 was received from CCDB (Guelph). Because DNA concentration was low (0.28 ng/µl), we performed a whole genome amplification using REPLI-g Mini Kit (Qiagen). Then a 350 bp fragment of the nuclear gene histone H3 was sequenced using primers and PCR condi-

tions as described in Kirichenko et al. (2015). This was done at Marko Mutanen's lab (University of Oulu, Finland).

To explore the phylogenetic position of the new species and its generic classification we combined the mitochondrial and nuclear data for *M. neli* with a published dataset of 39 Gracillariidae species and one outgroup (Kirichenko et al 2016, Gutzwiller et al. 2015; Kawahara et al. 2011) (Suppl. material 1). All new specimens and sequence data are available in BOLD in the public dataset dx.doi.org/10.5883/DS-CAYOLLE. Sequences are also deposited in GenBank and accession codes are provided in Suppl. material 1. Sequences were concatenated and aligned using Geneious 9.05 (http://www.geneious.com/).

Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed using PAUP* version 4.0 a 147 (Swofford 2002).

Results

Morphology

***Mercantouria* Huemer, Lopez-Vaamonde & Triberti, gen. n.**
http://zoobank.org/4B34364D-EDD2-4E73-A2A8-903EC332015C
Figs 1–7

Type species. *Mercantouria neli* Huemer, Lopez-Vaamonde & Triberti, sp. n.

Description. Adult (Fig. 1). Forewing length 5.1–5.8 mm. Head. Vertex and face loosely scaled; ocelli absent; proboscis naked, well developed. Antenna about as long as forewing, smooth, each flagellomere with an annulus of slender scales basally and another of shorter scales at apex, about 0.2× length of basal ones, completely covered by the first (Triberti 1998); scape moderate, about 3.0× length of pedicel, pecten missing. Labial palpus long, upturned, pointed apically, segment 2 as long as apical one, slightly thickened with scales towards apex. Maxillary palpus smooth, shorter than apical segment of labial palpus.

Thorax. Smoothly scaled. Forewing narrow, lanceolate; discoidal cell with distal margin nearly vertical, 13-veined; all radial veins separated but vein R4, R5 and M1 very close at their bases; veins M2 and M3 connate and arising from lower angle of cell; Cu1b arising from cell more apical than R2. Hindwing narrowly lanceolate, 8-veined; cell opened between M2 and M3, R4+R5 not parallel to the costal margin and meeting directly with M1+M2 (Fig. 2). Legs with tibial spur pattern 0-2-4; epiphysis present but partly hidden by long scales.

Abdomen. In the male segment 7 and 8 weakly membraneous, with a pair of coremata on each segment; anterior pair of coremata consisting of hairlike scales, longer and thicker than the posterior pair (Fig. 3). Sternum and tergum 7 reduced into a thin sclerites; sternum 8 also reduced but tergum is formed by a small, fan-shaped sclerite, with a narrow median ridge. Female postabdominal segments unmodified.

Male genitalia (Figs 4–5). Tuba analis produced beyond tegumen, membraneous, with a narrowly sclerotized subscaphium, widened basally. Tegumen weakly sclerotized, simple. Valva stout, with sacculus distinctly protruded and rounded apically, setose; cucullus straight, upturned, covered with strong setae on dorso-distal area; costal margin irregular with similar setae medio-distally. Diaphragma with some fine setae at base of anellus. Phallus slightly shorter than valva, apically with long rod-like sclerite branching off at right angle, no cornuti are visible.

Female genitalia (Figs 6–7). Lamella postvaginalis not connected with apophyses anteriores. Ostium bursae located under a lobate sternite 7. Ductus bursae completely membraneous, slender; corpus bursae ellipsoidal with two curved sickle-shaped signa, one of which is slightly longer than the other.

Etymology. The generic name refers to the region of Mercantour National Park (France).

Diagnosis. *Mercantouria* belongs to the *Gracillaria* group for the presence of a very short vein R_{2+3} in the hindwing, running in parallel with apical part of vein $Sc+R_1$. Moreover this new genus shares with most genera of the *Gracillaria* group the following characters: legs more or less smooth-scaled except for mid femur and tibia thickened with raised scales; forewing 13-veined with M_2 and M_3 connate, R_1 arising from cell near base of wing, upper vein of cell weakened on basal part just beyond the point where R_1 branches off; hindwing 8-veined, with radial veins always 2-branched, veins M_1 and Cu_{1a} stalked with veins M_2 and Cu_{1b} respectively, vein M_3 branched from vein Cu_{1a}, cell opened between M_2 and M_3; in male genitalia abdominal segment 7 and 8 weakly membraneous, each of them having a pair of coremata which are in a bundle of long and hairy scales, the latter covered with more or less deformed scales; in female, corpus bursae with two large sickle-shaped signa.

Within *Gracillaria* group the genera are difficult to identify on the basis of apomorphies and comparisons are rather complicated due to the "cross" distribution of characters. *Mercantouria* shows some similarity to the genus *Caloptilia* and allied genera (*Gracillaria*, *Povolnya*, *Euspilapteryx*, *Aspilapteryx* and *Eucalybites*): (1) forewing 13-veined and hindwing 8-veined, albeit with slight differences in the relative positions of some veins; also in *Gracillaria* and *Povolnya* there is a similar venation but in the former there are strong differences in the pregenital segments, the segment 7 being like the preceding and without coremata and the latter with peculiar male genitalia, with the tegumen having a pair of peniculi projected from caudal margin of tegumen; (2) male abdomen with two pairs of coremata more or less similar in length and thickness; a similar condition is found in *Povolnya* and *Euspilapteryx* but the latter differs from the new species in the forewing venation (12-veined) and female genitalia (only one signum); (3) in the female genitalia, the bursa copulatrix has two corniform signa; this character is shared with *Aspilapteryx* and *Eucalybites* however both differ from forewing venation (12-veined) and coremata of different size or only one pair.

Mercantouria differs from these genera in the following morphological characters: 1) the forewings show veins R_4, R_5 and M_1 very close, weakened or obsolescent at their bases; this character is unknown within *Gracillaria* group and it seems closer to

Acrocercops group. 2) The hindwings show veins R_4+R_5 directly connected with radial vein and divergent from costa, this condition is only similar to *Eucalybites* and *Aspilapteryx*; however both clearly differ by having the forewing 12-veined. 3) Lack of pecten which also occurs in a few taxa closely related to *Caloptilia*: subgenera *Timodora* Meyrick, 1886, *Phylloptilia* Kumata, 1982 and the genus *Povolnya*, sometimes considered as another subgenus of *Caloptilia* (Kumata 1982). 4) The male genitalia has a highly modified valva (Fig. 4) unlike any other known in the genus *Caloptilia*; only a somewhat similar shape of the valva is seen in *Aspilapteryx spectabilis* (Fig. 8) (Huemer 1994) and *E. aureola* (Kumata 1982) but easily distinguishable from the cucullus, which is straight and covered with strong setae along its margin in the new genus.

Mercantouria neli **Huemer, Lopez-Vaamonde & Triberti, sp. n.**

http://zoobank.org/FF3D1062-2586-4C50-AE95-440A1AC90230
Figs 1–7

Type material. Holotype ♂ (Fig. 1): "Frankreich Dep. Alpes-Maritimes Col de la Cayolle N 6°44'21"E, 44°16'49"N 2080 m, 19.7.2013 leg. Mayr" "P.Huemer TIN 94 ♂" "DNA Barcode TLMF Lep 16937" (TLMF).

Paratypes: 1 ♀, Frankreich, Alpes-Maritimes, N Col de la Cayolle, Col de la Boucharde N, 6°44'36"E, 44°17'0"N, 1950m, 7.7.2012 leg. Huemer, TLMF 2013-010 (gen.slide P.Huemer TIN 93 ♀; DNA Barcode ID TLMF Lep 08375); 1 ♂, Frankreich, Alpe-Maritimes, PN Mercantour, 2115 m, Col de la Cayolle Nord,

Figure 1. *Mercantouria neli* sp. n., holotype; France, Dep. Alpes-Maritimes, Col de la Cayolle N, 2080 m, 19.7.2013, leg. Mayr.

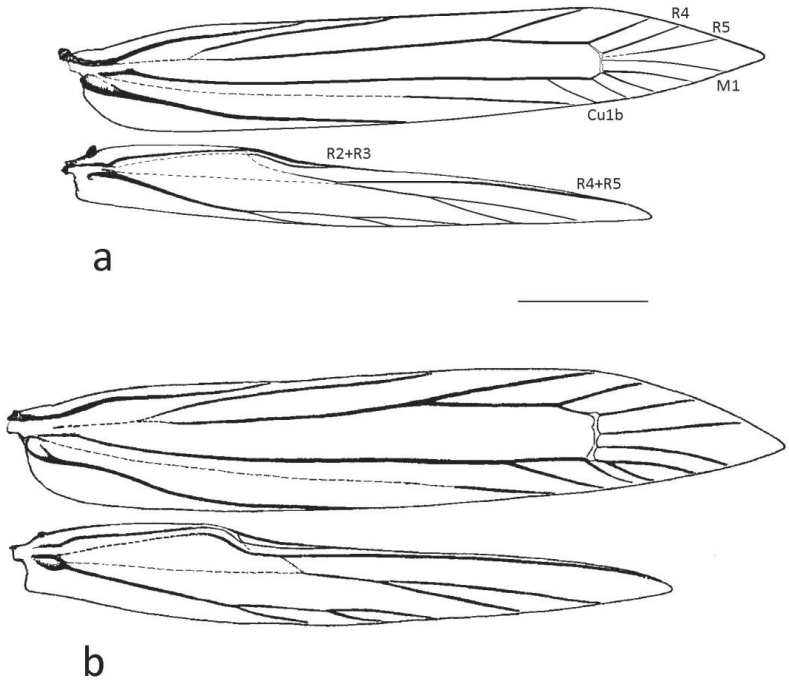
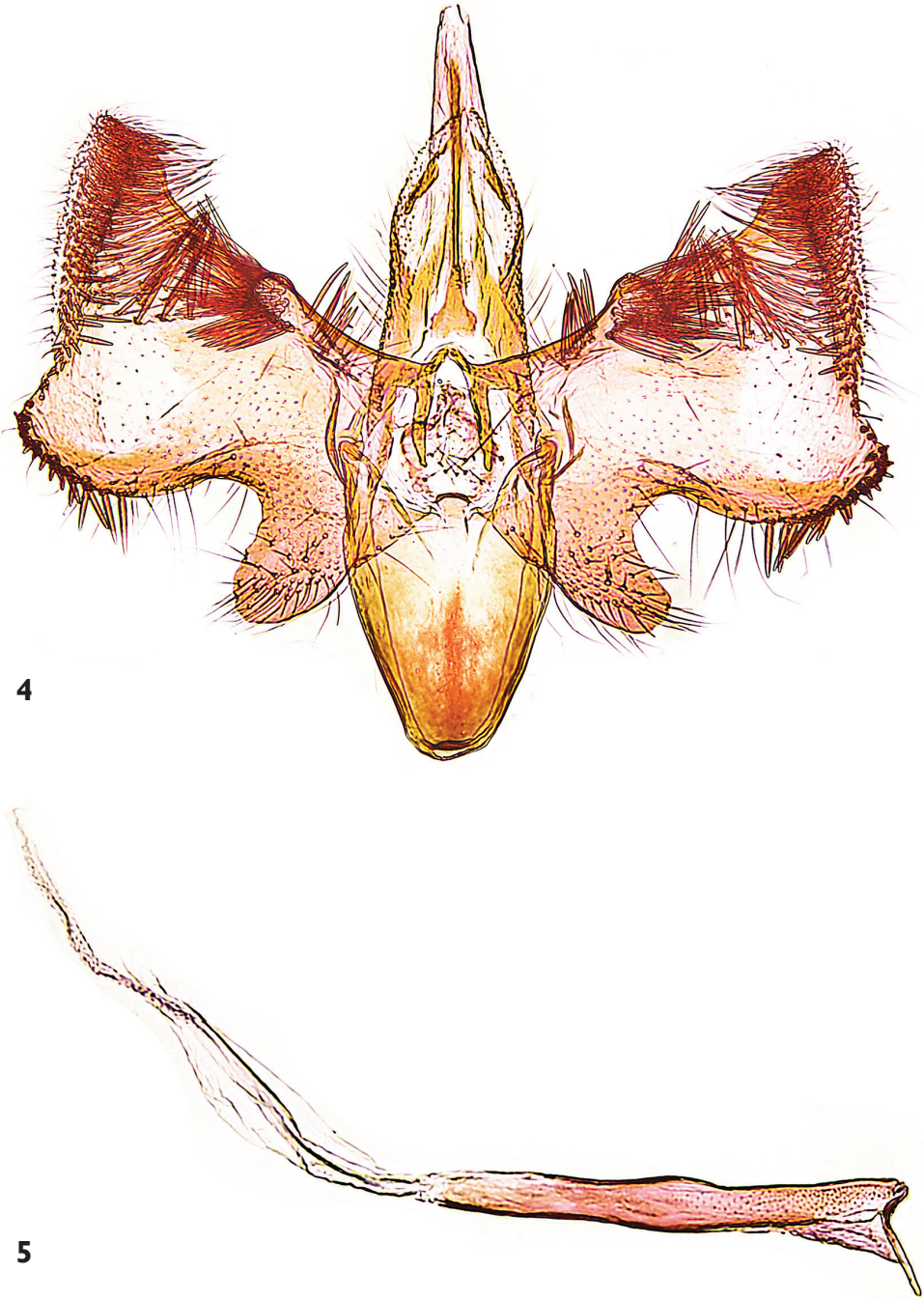


Figure 2. Wing venation: **a** *Mercantouria neli* sp. n.; **b** *Caloptilia stigmatella* (Fabricius, 1781). Scale length = 1 mm.

Figure 3. *Mercantouria neli* sp. n., holotype, abdominal segments 7–8; France, Dep. Alpes-Maritimes, Col de la Cayolle N, 2080 m, 19.7.2013, leg. Mayr; genitalia slide P.Huemer TIN 94 ♂.



Figures 4–5. *Mercantouria neli* sp. n., holotype, male genitalia; France, Dep. Alpes-Maritimes, Col de la Cayolle N, 2080 m, 19.7.2013, leg. Mayr; genitalia slide P.Huemer TIN 94 ♂ **4** tegumen-vinculum-valva complex **5** phallus.

Figures 6–7. *Mercantouria neli* sp. n., paratype, female genitalia; France, Alpes-Maritimes, N Col de la Cayolle, Col de la Boucharde N, 1950m, 7.7.2012, leg. Huemer; genitalia slide P.Huemer TIN 93 ♀ **6** last segments **7** corpus bursae-signa.

N44°16,78', E6°44,32', 21.7.2014, leg. Drouet (gen. slide P.Huemer TIN 95 ♂; DNA Barcode ID TLMF Lep 16938); 1 ♀, Htes-Alpes, Ristolas, La Roche Ecroute, 1750 m, 12.7.2010, leg. Nel, genitalia slide 24139 J. Nel (all coll. TLMF).

Description (Fig. 1). Head. Labial palpus pale ochre-yellowish, apical segment dark brown medio-basally. Legs smooth scaled, dark brown with exception of hind leg that are lighter; all tarsi white.

Thorax. Dorsum and tegulae ochre yellow. Forewing pale ochre yellow with small spots or suffusion of dark brown, mostly along the discoidal cell and sometimes forming, in the apical third of the wing, an irregular fascia. Hindwing light ochre-greyish.

Abdomen, male and female genitalia. See under the genus description.

Etymology. Named in honour of Dr. Jacques Nel (La Ciotat, France) who independently recognized and collected the new species.

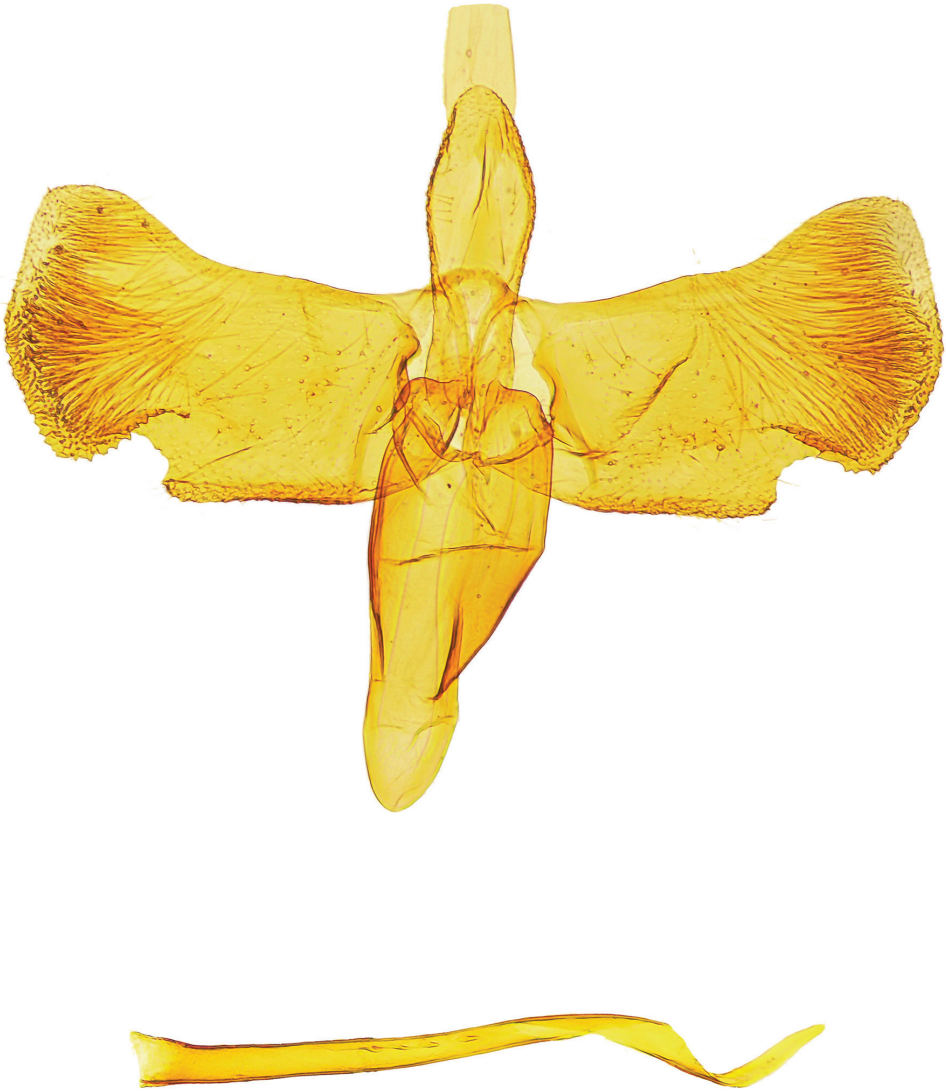


Figure 8. *Aspilapterix spectabilis*, paratype, male genitalia; Austria, Osttirol, Virgental, Venedigergruppe, Sajatmähder, 2150–2350 m, 31.7.1993, leg. Ryrholm; genitalia slide P.Huemer TIN 33 ♂.

Diagnosis. Superficially the adult of *Mercantouria neli* can be confused with some members of the *Gracillaria* group, like light coloured specimens of *Caloptilia roscipennella* (Hübner, 1796) and *Aspilapteryx limosella* (Duponchel, 1843). However, in both species a trace remains of neat rows of darkish small spots, along the costa in the former and in the middle of wing in the latter, while in *M. neli* the dark scales create confused and ill-defined spots. In the male genitalia, the short valva with a protruded sacculus shows some affinity to *Aspilapteryx* and *Eucalybites* species, particularly *A.*

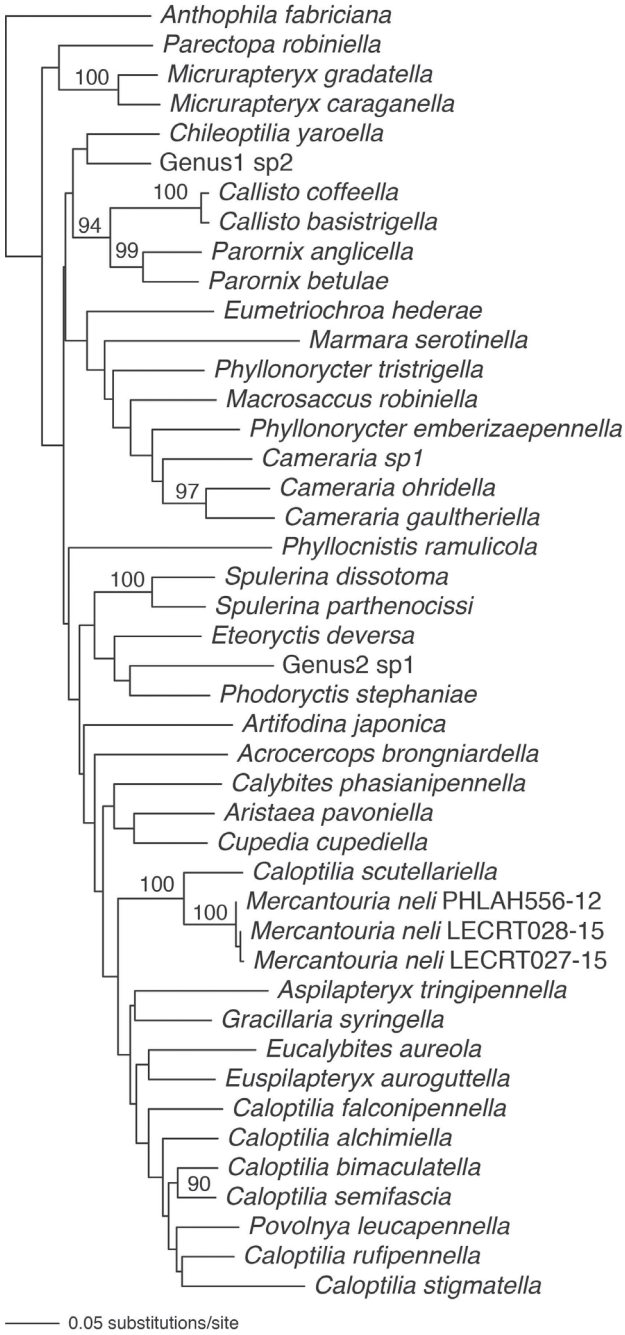


Figure 9. Maximum likelihood tree based on COI and H3 sequences for 43 Gracillariid species. –ln likelihood= 11983.13. Bootstrap values are indicated for nodes more than 50% support (1000 replications). The general time reversible model of sequence evolution was used with the following settings: LSet nst=6 rclass=(abcdef) rmatrix=(2.9833028 10.54651 15.145156 9.0102019 24.833933) basefreq=(0.26825699 0.20834936 0.17265202) rates=gamma shape=1.0542648 pinv=0.53477118).

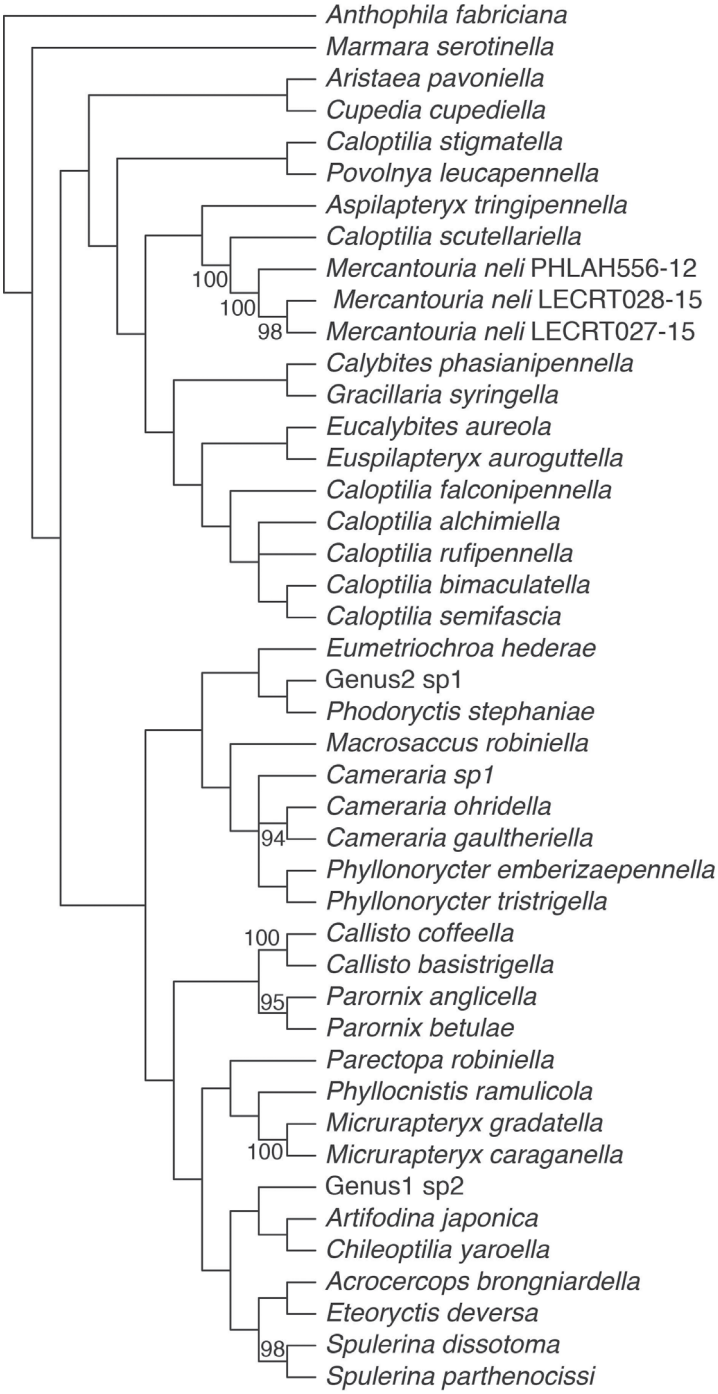


Figure 10. Semistrict consensus tree of four most parsimonious trees (length 2618, consistency index (CI) 0.272, and retention index (RI) 0.385). Bootstrap values are indicated for nodes more than 50% support (1000 replications).

Figure 11. Type locality of *Mercantouria neli* sp. n. near Col de la Cayolle.

spectabilis and *E. aureola*. However, the new species can be easily separated by the straight cucullus and the numerous, thickened setae along its margin and costa. The female genitalia are easily distinguishable from other species of the *Gracillaria* group by the heavily sclerotized sternum 7, which is flap shaped, lobate on caudal margin and about as long as tergum 7. A similar structure is present in *E. aureola* but with the sternum 7 much narrower, about half of tergum, and a heavily sclerotized sterigma with a complicated shape (Kumata 1982).

Molecular data. We obtained DNA barcode data for all 39 individuals and H3 data for 32 out of the 39 samples (Suppl. material 1). The three DNA barcodes obtained for *Mercantouria neli* (maximum intraspecific distance = 0.49%) fall within the same Barcode Index Number (BOLD:ACA9784) allowing the unequivocal identification of the new species. The nearest neighbor is the North American species *Caloptilia scutellariella* (Braun, 1923) (BOLD:AAU2901) and associated or possibly misidentified DNA clusters (BOLD:ABX8283, BOLD:AAP8031) at a genetic distance of 8.41%.That would suggest that the new species could be a representative of the genus *Caloptilia*. However, the generic assignment of *C. scutellariella* seems doubtful from genitalia morphology and needs further revision.

The ML analysis shows that the new species falls within a clade formed by six *Caloptilia* species and *Gracillaria syringella*, although with low bootstrap support (Fig. 9). MP analysis returned four most parsimonious trees. The semistrict consensus is shown in Fig. 10.

Biology. Host-plant and early stages are unknown. *Mercantouria neli* was collected only in singletons so far, either at dusk or during the night at light. The flight period seems to be short, lasting from mid- to late July. The habitat (Fig. 11) is dominated by subalpine scree and grassland on limestone soil. Vertical distribution: from about 1750 to 2100 m.s.l.

Distribution. The new species is so far known from a small area of the French Hautes-Alpes and Alpes-Maritimes.

Discussion

The description of a new genus is an arbitrary decision (Hennig 1966; Humphreys and Barraclough 2014) and a particularly difficult one to make when the genus is monotypic. We based our decision on the fact that neither morphological nor DNA sequence data support the placement of the new taxon within any of the extant Gracillariidae genera. Indeed, the highly differentiated male genitalia with unique structures of the valva, and the forewing venation support the hypothesis of a new genus. Both mitochondrial and nuclear sequence data show that the new taxon might be closely related to the genus *Caloptilia* and in particular to the North American *Caloptilia scutellariella*. However, we think that *C. scutellariella* belongs most likely to a different genus, not *Caloptilia*, but more data is needed to test this hypothesis.

Mercantouria neli could represent a non native species introducted into the Alps. Indeed there are several species of non-native Gracillariidae established in Europe (Lopez-Vaamonde et al. 2010). However, based on the repeated collection of several individuals in different years in such remote alpine habitat we think an anthropogenic introduction is highly unlikely.

M. neli most likely represents a xero-montane relict alpine species like the recently discovered *Callisto basistrigella* (Kirichenko et al. 2015). However, gracillariid species thought to be endemic to the Alps such as *A. spectabilis* have been discovered in other mountain ranges (Huemer 2011) and thus further work is needed to confirm the endemism status of *M. neli* in the Alps.

Like other alpine Lepidoptera such as the recently described *Syrianarpia faunieralis* Gianti, 2005 (Crambidae), a species endemic to the Cottian Alps but with congeneric relatives in Turkey and on the Krim peninsula (Gianti 2005; Goater et al. 2005; Huemer 2009), *M. neli* could also have its closest relatives in Asia. Indeed, there is an undescribed species of a Gracillariinae collected in Turkey (specimens deposited at the Natural History Museum in Copenhagen) whose morphology shows some affinities to *M. neli* (unpublished morphological data). However, genetic data is necessary to support potential congenerity of these two taxa.

Finally, additional biological and molecular data are needed to understand the interrelationships of *M. neli* with the other genera within the *Gracillaria* group.

Acknowledgments

We are particularly grateful to Paul Hebert and his team at the Canadian Centre for DNA Barcoding (Guelph, Canada) whose sequencing work was enabled by funding from the Government of Canada to Genome Canada through the Ontario Genomics Institute. We are also grateful to the Ontario Ministry of Research and Innovation and to NSERC for their support of the BOLD informatics platform. PH thanks the Promotion of Educational Policies, University and Research Department of the Autonomous Province of Bolzano – South Tyrol for helping to fund the project "Genetic biodiversity archive – DNA barcoding of Lepidoptera of the central Alpine region (South, East and North Tyrol)", and the Austrian Federal Ministry of Science, Research and Economics for funds received in the framework of ABOL (Austrian Barcode of Life).

Marie-France Leccia from Parc National Mercantour supplied us with the necessary collecting permits. Eric Drouet (Gap, France), Ole Karsholt (Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark), Natalia Kirichenko (Krasnoyarsk, Russia), Toni Mayr (Feldkirch, Austria) and Jacques Nel (La Ciotat, France) kindly lent or presented us material used in this study and/or supported us during field and lab work. Andreas Eckelt (TLMF), Stefan Heim (TLMF) and Marlies Mayr (Feldkirch, Austria) assisted with photographic work. To both David Plotkin and Akito Kawahara for discussion and help about the combined molecular analysis. To Marko Mutanen, Kyung Min Lee and Laura Törmälä (Oulu, Finland) for sequencing H3 of *M. neli*. To both Jean François Landry (Ottawa) and Jeremy deWaard (Guelph) for allowing us to use unpublished barcode data.

Finally, we are thankful to David Lees (NHM, London) for providing insightful comments on the manuscript.

References

Buchner P (2015) Two new species of *Agonopterix* (Depressariidae, Lepidoptera) from Europe. Zootaxa 3986(1): 101–114. doi: 10.11646/zootaxa.3986.1.5

De Prins J, De Prins W (2015) Global Taxonomic Database of Gracillariidae (Lepidoptera). World Wide Web electronic publication. Available from: http://www.gracillariidae.net [November 2015]

deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA Barcodes: Analytical Protocols. In: Cristofre M (Ed.) Methods in Molecular Biology: Environmental Genetics. Humana Press Inc., Totowa, USA, 275–293. doi: 10.1007/978-1-59745-548-0_15

Gianti M (2005) *Syrianarpia faunieralis* sp. n. from the Cottian Alps of Italy (Crambidae: Scopariinae). Nota lepidopterologica 27: 299–302.

Goater B, Nuss M, Speidel W (2005) Pyraloidea I (Crambidae: Acentropinae, Evergestinae, Heliothelinae, Schoenobiinae, Scopariinae). In: Huemer P, Karsholt O (Eds) Microlepidoptera of Europe 4, Stenstrup, 304 pp.

Gutzwiller F, Dedeine F, Kaiser W, Giron D, Lopez-Vaamonde C (2015) Correlation between the green-island phenotype and *Wolbachia* infections during the evolutionary diversification of Gracillariidae leaf-mining moths. Ecology & Evolution 18: 4049–4062. doi: 10.1002/ece3.1580

Hennig W (1966) Phylogenetic systematics. University of Illinois Press, Urbana, IL, USA.

Huemer P (1994) *Aspilapteryx spectabilis* sp. n., eine neue Schmetterlingsart aus dem Gebiet des Nationalparks Hohe Tauern (Osttirol, Österreich) (Lepidoptera: Gracillariidae). Zeitschrift der Arbeitgemeinschaft Österreichischer Entomologen 46: 1–8.

Huemer P (1998) Endemische Schmetterlinge der Alpen - ein Überblick (Lepidoptera). Stapfia 55: 229–256.

Huemer P (2009) Auf der Suche nach Schmetterlings-Endemiten (Lepidoptera) in den Cottischen Alpen (Prov. Cuneo, Italien). Wissenschaftliches Jahrbuch der Tiroiler Landesmuseen 2: 120–129.

Huemer P (2011) Pseudo-endemism and cryptic diversity in Lepidoptera – case studies from the Alps and the Abruzzi. eco.mont 3(1): 11–18. doi: 10.1553/eco.mont-3-1s11

Huemer P, Hausmann A (2009) A new expanded revision of the European high mountain *Sciadia tenebraria* species group (Lepidoptera, Geometridae). Zootaxa 2117: 1–30.

Huemer P, Hebert PDN (2011) Cryptic diversity and phylogeography of high alpine *Sattleria*—a case study combining DNA barcodes and morphology (Lepidoptera: Gelechiidae). Zootaxa 2981: 1–22.

Huemer P, Karsholt O, Mutanen M (2014a) DNA barcoding as a screening tool for potential unrecognized cryptic diversity exemplified by the genus *Caryocolum*, with description of a new species (Lepidoptera, Gelechiidae). ZooKeys 404: 91–111. doi:10.3897/zookeys.404.7234

Huemer P, Timossi G (2014) *Sattleria* revisited: Unexpected cryptic diversity on the Balkan Peninsula and in the south-eastern Alps (Lepidoptera: Gelechiidae). Zootaxa 3780(2): 282–296. doi:10.11646/zootaxa.3780.2.4

Huemer P, Wieser C, Mutanen M (2014b) *Rhigognostis scharnikensis* sp. n., eine morphologisch und genetisch differenzierte neue Schmetterlingsart aus den Hohen Tauern (Lepidoptera, Plutellidae). Carinthia II 204./124.: 443–454.

Huemer P, Mutanen M (2015) Alpha taxonomy of the genus *Kessleria* Nowicki, 1864, revisited in light of DNA-barcoding (Lepidoptera, Yponomeutidae). ZooKeys 503: 89–133. doi: 10.3897/zookeys.503.9590

Humphreys AM, Barraclough TG (2014) The evolutionary reality of higher taxa in mammals. Proceedings of the Royal Society B: Biological Sciences 281: 20132750. doi: 10.1098/rspb.2013.2750

Kawahara AY, Ohshima I, Kawakita A, Regier JC, Mitter C, Cummings MP, et al. (2011) Increased gene sampling provides stronger support for higher-level groups within gracillariid leaf mining moths and relatives (Lepidoptera: Gracillariidae). BMC Evolutionary Biology 11: 182. doi: 10.1186/1471-2148-11-182

Kirichenko N, Huemer P, Deutsch H, Triberti P, Rougerie R, Lopez-Vaamonde C (2015) Integrative taxonomy reveals a new species of *Callisto* (Lepidoptera, Gracillariidae) in the Alps. ZooKeys 473: 157–176. doi: 10.3897/zookeys.473.8543

Kirichenko N, Triberti P, Mutanen M, Magnoux E, Landry J-F, Lopez-Vaamonde C (2016) Systematics and biology of some species of *Micrurapteryx* Spuler (Lepidoptera, Gracillariidae) from the Holarctic Region, with re-description of *M. caraganella* (Hering) from Siberia. ZooKeys 579: 99–156. doi: 10.3897/zookeys.579.7166

Klots AB (1970) Lepidoptera. In: Tuxen SL (Ed.) Taxonomist's glossary of genitalia in insects. (Second revised and enlarged edition). Munksgaard, Copenhagen, 115–130.

Kristensen NP (2003) Skeleton and muscles: adults. In: Kristensen NP (Ed.) Lepidoptera, moths and butterflies. Vol. 2. Morphology, physiology, and development. Handbook of Zoology IV (36). Walter de Gruyter, Berlin, New York, 39–131. doi: 10.1515/9783110893724.39

Kumata T (1982) A taxonomic revision of the *Gracillaria* group occurring in Japan (Lepidoptera: Gracillariidae). Insecta Matsumurana 26: 1–186.

Kumata T, Kuroko H, Ermolaev VP (1988a) Japanese species of the *Acrocercops*-group (Lepidoptera: Gracillariidae). Part I. Insecta Matsumurana, New Series 38: 1–111.

Kumata T, Kuroko H, Ermolaev VP (1988b) Japanese species of the *Acrocercops*-group (Lepidoptera: Gracillariidae). Part II. Insecta Matsumurana, New Series 40: 1–133.

Kumata T (1995) *Ketapangia*, a new genus for *Macarostola leucochorda* and *Acrocercops reguliferа* (Gracillariidae, Lepidoptera). Insecta Matsumurana 52: 133–148.

Kumata T, Kuroko H, Ermolaev VP (1988) Japanese species of the *Acrocercops*-group (Lepidoptera: Gracillariidae). Part I. Insecta Matsumurana, New Series 38: 1–111.

Laštuvka A, Laštuvka Z (2006) The European *Phyllonorycter* species feeding on the plants of the tribe Genisteae (Fabaceae), with descriptions of twelve new species (Lepidoptera: Gracillariidae). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 54: 65–83. doi: 10.11118/actaun200654050065

Laštuvka Z, Laštuvka A (2012) Additional data on the *Phyllonorycter haasi*-group with description of two new species. SHILAP, Revista de Lepidopterologia 40: 231–239.

Laštůvka Z, Laštůvka A, Lopez-Vaamonde C (2013) A revision of the *Phyllonorycter ulicicolella* species group with description of a new species (Lepidoptera: Gracillariidae). SHILAP Revista de Lepidopterologia 41: 251–265.

Lopez-Vaamonde C, Agassiz DVL, Augustin S, De Prins J, De Prins W, Gomboc S, Ivinskis P, Karsholt O, Koutroumpas A, Koutroumpa F, Laštůvka Z, Karsholt O, Marabuto E, Olivella E, Przybylowicz L, Roques A, Ryrholm N, Šefrová H, Šima P, Sims O, Sinev S, Tomov R, Zilli A, Lees DC (2010a) Lepidoptera. Chapter 11. In: Roques A et al. (Eds) Alien terrestrial arthropods of Europe. BioRisk 4(2): 603–668. doi: 10.3897/biorisk.4.50

Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System. Molecular Ecology Notes 7: 355–364. doi: 10.1111/j.14718286.2007.01678.x Available from: http://www.barcodinglife.org [November 2015]

Robinson GS (1976) The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. Entomologist´s Gazette 27: 127–132.

Swofford DL (2002) Phylogenetic analysis using parsimony (* and other methods). Version 4.0b10. Sinauer Associates, Inc., Sunderland MA, USA.

Tabell J, Baldizzone G (2014) *Coleophora mareki* Tabell & Baldizzone, sp. n., a new coleophorid moth of the *serpylletorum* species-group (Lepidoptera: Coleophoridae). SHILAP Revista de Lepidopterología 42(167): 399–408.

Triberti P (1998) Remarks on the phylogeny of the genera *Parornix* Spuler and *Callisto* Stephens (Lepidoptera Gracillariidae). Museo Civico di Storia Naturale di Verona 22: 175–197.

Triberti P (2007) The *Phyllonorycter* species from Palaearctic Region feeding on Rosaceae (Lepidoptera, Gracillariidae). Museo Civico di Storia Naturale di Verona 31: 147–221.

Whitebread S (2007) *Sphaleroptera alpicolana* (Frölich, 1830) (Lepidoptera, Tortricidae, Cnephasiini): a species complex. Veröffentlichungen des Tiroler Landesmuseums Ferdinandeum 86: 177–204.

Zeller HC, Huemer P (2015) A new species of *Micropterix* Hübner, 1825 from the Orobian Alps (Italy) (Lepidoptera, Micropterigidae). Nota Lepidopterologica 38: 133–146. doi: 10.3897/nl.38.5058

Supplementary material 1

Sample information for specimens used in this study

Authors: Peter Huemer, Carlos Lopez-Vaamonde, Paolo Triberti

Data type: Species data

Explanation note: Details of collecting data, images, sequences, and trace files for the barcoded specimens are available in the public BOLD dataset "DS-CAYOLLE", accessed at http://dx.doi.org/10.5883/DS-CAYOLLE

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.