

Taxonomic status of the Columbia dusksnail (Truncatelloidea, Amnicolidae, Colligyrus)

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Abstract

Undescribed freshwater snails (Amnicolidae: *Colligyrus*) from the Mount Hood region (northwestern United States) identified as a new species (commonly known as the Columbia dusksnail) in grey literature have been provided federal protection under the “survey and manage” provisions of the Northwest Forest Plan and have been placed on conservation watch lists. However, there are no published studies of the identity of these snails aside from a molecular phylogenetic analysis which delineated a close relationship between the single sampled population and *C. greggi*, which is distributed more than 750 km to the east of the Mount Hood area. Here we examine the taxonomic status of the Columbia dusksnail based on additional molecular sampling of mitochondrial DNA sequences (COI) and morphological evidence. We found that the Columbia dusksnail is not a monophyletic group and forms a strongly supported clade with *C. greggi*. The COI divergence between these broadly disjunct groups (2.1%) was somewhat larger than that within *C. greggi* (1.0%) but considerably less than that among the three currently recognized species of *Colligyrus* (8.7–12.1%). Additionally we found that the Columbia dusksnail and *C. greggi* cannot be consistently differentiated by previously reported diagnostic characters (size and shape of shell spire, pigmentation of body and penis) and are closely similar in other aspects of morphology. Based on these results we conclude that the Columbia dusksnail is conspecific with *C. greggi*.

Keywords

Gastropoda, aquatic, western United States, systematics, phylogeny, conservation

Introduction

The freshwater gastropod genus *Colligyrus* contains three currently recognized species (commonly known as dusksnails) that live in cold water seeps and springs in the northwestern United States (Hershler 1999, Hershler et al. 2003). *Colligyrus greggi* is distributed in the upper Snake River drainage and a small portion of the northeastern Great Basin while the other two congeners are narrowly ranging in the northwest Great Basin (*C. depressus*) and Pit River drainage (*C. convexus*) (Fig. 1). There are also numerous undescribed populations in other portions of the northwestern United States (e.g., Klamath River basin) that may belong to this little studied genus.

The cluster of undescribed dusksnail populations in the vicinity of Mount Hood (Columbia River basin) was identified in grey literature as a new species, commonly known as the Columbia dusksnail (Frest and Johannes 1993), and differentiated from morphologically similar *C. depressus* by its smaller size; and from *C. greggi* by its smaller, less attenuated (shell) spire, and lighter pigmentation of the body and penis (Frest and Johannes 1995). The description of this putative novelty did not include supporting data, illustrations, or voucher details. There have been no subsequently published studies of the Columbia dusksnail aside from a molecular phylogenetic analysis of *Colligyrus* (Hershler et al. 2003, fig. 6) which delineated a close relationship between the population in Oak Grove Fork (Willamette River basin) and *C. greggi*, which is distributed more than 750 km to the east of the Mount Hood area.

The Columbia dusksnail has received considerable attention from the conservation community owing to its supposedly narrow distribution, and threats that include road construction, logging, and water diversions (USFWS 2011). It was listed as a Record of Decision (ROD) Survey and Manage species under the Northwest Forest Plan (USDA and USDI 1994) and has been placed on several conservation watch lists (e.g., NatureServe 2015). However, in response to a recent listing petition, the USFWS (2012) found that addition of the Columbia dusksnail to the federal list of threatened or endangered species was not warranted at this time owing to the absence of published evidence that it is a “listable entity” (i.e., a distinct species).

Clearly there is a need to clarify the taxonomic status of the Columbia dusksnail as a prerequisite for protection under the Endangered Species Act and other possible conservation measures. Here we address this research gap by further analysis of mtCOI sequences (for which six populations of these animals and two populations of *C. greggi* were newly sampled) and assessment of reported diagnostic morphologic characters.

Methods

For the molecular component of this study we newly sampled two populations of *C. greggi*, six populations of the Columbia dusksnail from the Lower Deschutes River and Middle Columbia-Hood River basins, and a population of another putatively undescribed species of dusksnail (from the Puget Sound region) recognized in grey

literature (Johannes 2010). Specimens were preserved in 90% ethanol in the field. Genomic DNA was extracted from entire snails (1–4 specimens per sample) using a CTAB protocol (Bucklin 1992); each specimen was analyzed for mtDNA individually. LCO1490 (Folmer et al. 1994) and COH743 (5'GGT AAA ATT AAA ATA TAT ACT T3') were used to amplify a 720 base pair (bp) fragment of COI. Amplification conditions and sequencing of amplified polymerase chain reaction product followed Liu et al. (2003). Sequences were determined for both strands and then edited and aligned using SEQUENCHER® version 5.0.1. The 29 newly sequenced specimens were analyzed together with our previously published *Colligyrus* dataset (Hershler et al. 2003); *Amnicola limosa* (AF213348) was used as the root in each analysis. One example of each haplotype detected in a given sample was used in the analyses. The new haplotypes from each sampling locality were deposited in GenBank (accession numbers KT248021–KT248031). Sample information and GenBank accession numbers are given in Table 1; the locations of the *Colligyrus* populations from which sequences were obtained are shown in Figure 1.

MRMODELTEST 2.3 (Nylander 2004) was used to obtain an appropriate substitution model (using the Akaike Information Criterion) and parameter values for the molecular phylogenetic analyses. This program selected HKY + I model parameters as the best fit model for the COI dataset. Phylogenetic analyses were performed using four different methodologies—distance, maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference. The distance, MP, and ML analyses were performed using PAUP*4.ob10 (Swofford 2002), and the Bayesian analyses were conducted using MRBAYES 3.2.3 (Ronquist and Huelsenbeck 2003). For the distance analyses, HKY distance was used to generate a neighbor-joining (NJ) tree (Saitou and Nei 1987). The MP analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping and 100 random additions. The ML analyses were performed using the HKY + I model; a HKY distance based NJ tree was used as the initial topology for branch-swapping. Node support was evaluated by 10,000 bootstrap pseudo-replicates except for the ML analysis, for which support values were based on 1000 replications. For the Bayesian analyses Metropolis-coupled Markov chain Monte Carlo simulations were run with four chains (using the model selected through MRMODELTEST) for 2,000,000 generations. Markov chains were sampled at intervals of 10 generations to obtain 200,000 sample points. We used the default settings for the priors on topologies and the HKY + I model parameters selected by MRMODELTEST as the best fit model for both analyses. At the end of the analyses, the average standard deviation of split frequencies was 0.0018 and the Potential Scale Reduction Factor (PSRF) was 1, indicating that the runs had reached convergence. The sampled trees with branch lengths were used to generate a 50% majority rule consensus tree, with the first 25% of the samples removed to ensure that the chain sampled a stationary portion.

Genetic distances within and between samples were calculated using MEGA6 (Tamura et al. 2013), with standard errors estimated by 1,000 bootstrap replications with pairwise deletion of missing data. Since MEGA does not contain the HKY model that

Table 1. Samples used for molecular analysis, with codes (used in Figs. 1-2), locality details, and GenBank accession numbers for COI.

Taxon	Code	Locality (voucher catalog number)	GenBank accession number
Columbia duskysnail	COL1 (N=4)	Small spring, Brooks Meadow, middle Columbia River basin, Hood River Co., OR	KT248021
	COL2 (N=4)	Spring tributary, Tony Creek, middle Columbia River basin, Hood River Co., OR	KT248022
	COL3 (N=4)	Bottle Prairie, middle Columbia River basin, Hood River Co., OR	KT248023
	COL4 (N=4)	Spring tributary, Ramsey Creek, middle Columbia River basin, Wasco Co., OR	KT248024
	COL5 (N=4)	Spring tributary, Clear Creek, Deschutes River basin, Wasco Co., OR	KT248025 (COL5A, N=3) KT248026 (COL5C, N=1)
	COL6 (N=4)	Bear Creek, Hood River Co., OR	KT248027 (COL6A, N=3) KT248028 (COL6C, N=1)
	CL	Oak Grove Fork, Willamette River basin, Clackamas Co., OR	AY196174
<i>Colligyrus convexus</i>	BL	Baum Lake, Pit River basin, Shasta Co., CA	AY196166
	TS	Fall River (spring source), Pit River basin, Shasta Co., CA	AY196167
	MBa	Burney Creek, Pit River basin, Shasta Co., CA	AY196168
	MBb	Burney Creek, Pit River basin, Shasta Co., CA	AY196169
<i>Colligyrus depressus</i>	SRa	Second spring south of Turner Ranch, Silvies River basin, Harney Co., OR	AY196170
	SRb	Third spring south of Turner Ranch, Silvies River basin, Harney Co., OR	AY196171
<i>Colligyrus greggi</i>	SN	Springs along Cliff Creek, upper Snake River basin, Sublette Co., WY	AY196172
	BR	Spring at Saint Charles campground, Bear Lake basin, Bear Lake Co., ID	AY196173
	AM17 (N=2)	Spring at Porcupine campground, Bear Lake basin, Bear Lake Co., ID	KT248030
	AM20 (N=2)	Springs along Trail Creek, upper Snake River basin, Caribou Co., ID	KT248031
<i>Colligyrus</i> sp.	KL	Link River, Klamath River basin, Klamath Co., OR	AY196175
<i>Colligyrus?</i> sp.	COL7 (N=1)	Allison Springs, Puget Sound drainage, Thurston Co., WA	KT248029
<i>Amnicola limosa</i>	-	Blind Lake, Lake Michigan basin, Washtenow Co., MI	AF213348

was selected by MRMODELTEST, we used the Tajima-Nei distance, which is the nearest model.

The morphologic component of the study was focused in large part on evaluating the purported diagnostic differences between the Columbia duskysnail and *C. greggi*. Shell parameters were compiled for two samples of the former and five samples of the latter to assess variation in spire size and shape, and other aspects of shell form. Ten to 20 adult specimens (having fully formed apertural lips) were selected from amongst the largest specimens of each sample. The height of the entire shell (SH), width of the body whorl (WBW), and height of the aperture (AH) were measured from camera lucida outline

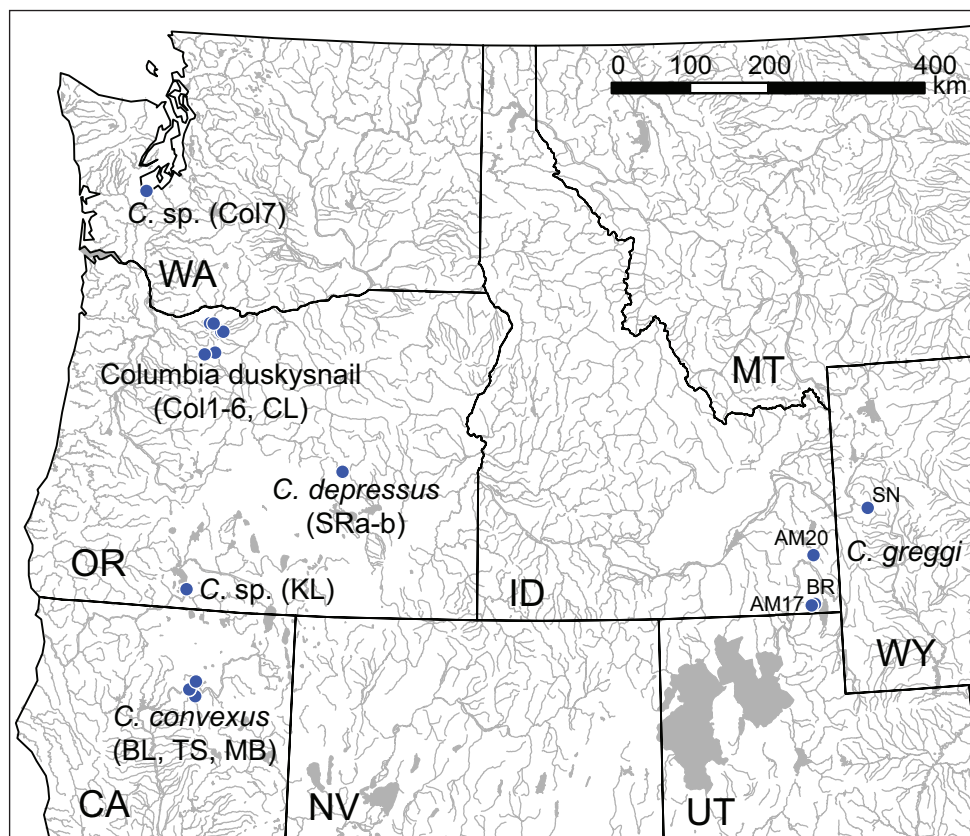


Figure 1. Map of the northwestern United States showing the collecting localities for *Colligyrus* samples used in the molecular analysis. Specimen codes are from Table 1.

drawings using a digitizing pad linked to a personal computer (see Hershler 1989). Ratios were generated from the raw data to estimate the size of the spire relative to aperture height (SH-AH/AH) and shape of the spire (SH-AH/WBW). Sample heterogeneity of these parameters was examined through analysis of variance (ANOVA), with post-hoc testing of differences among means using the Bonferroni correction for multiple comparisons. We also performed a discriminant analysis of seven standard shell parameters (total number of whorls, height and width of entire shell, body whorl, and aperture) obtained from this same set of specimens (measurement methods as above). A classification matrix based on the resulting canonical scores was generated to assess accuracy of assignment of individual specimens to the Columbia dusksnail and *C. greggi*. Analyses were performed using Systat for Windows 11.00.00 (SSI 2004). Several recently collected ethanol-preserved samples of the Columbia dusksnail were examined to assess purportedly diagnostic (soft part) pigmentation patterns, and to further evaluate the distinctiveness of these animals relative to *C. greggi*. Variation in the number of cusps on the radular teeth ($N=5$) was assessed using the method of Hershler et al. (2007).

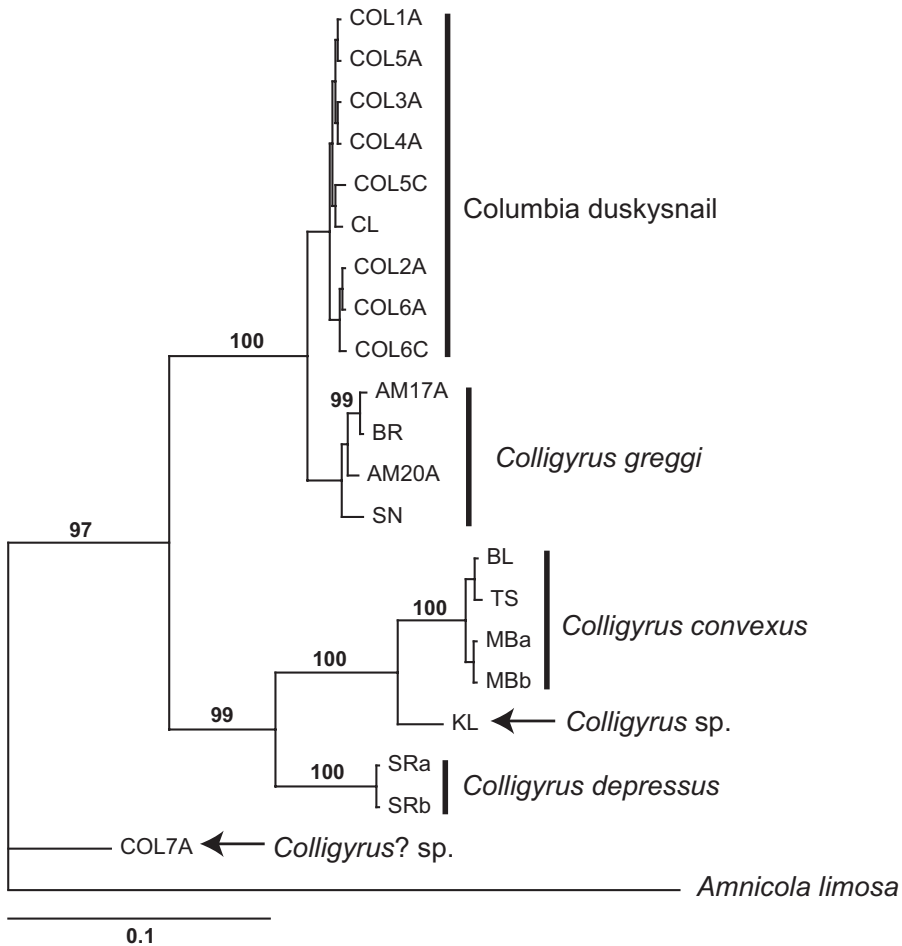


Figure 2. Bayesian tree based on the COI dataset. Nodes having posterior probabilities >95% are shown. Specimen codes are from Table 1.

Results

The Columbia duskysnail COI sequences formed a strongly supported clade with *C. greggi* in all but the ML tree; the Bayesian topology is shown in Figure 2. This clade differed genetically from *C. convexus* and *C. depressus* by >10% (Table 2). The Columbia duskysnail and *C. greggi* differed from each other by $2.1 \pm 0.5\%$ (ranging from 1.7–2.7%) and formed mutually exclusive sub-clades (albeit without strong support) in all but the ML tree in which the latter formed a clade while the former was paraphyletic. There was little variation among Columbia duskysnail specimens ($0.3 \pm 0.1\%$, ranging from 0.0–0.8%) and somewhat greater variation within *C. greggi* ($1.0 \pm 0.3\%$, ranging from 0.2–1.5%). Note that the sequenced specimen from the Puget Sound area (Co-17A) was positioned basally outside of the *Colligyrus* clade in all of the resulting trees.

Table 2. Mean mtCOI sequence divergence (Tajima-Nei distance) among amnicolid lineages. Values are percentage $\pm$ standard deviation.

Lineage	<i>C. greggi</i> + <i>Columbia duskysnail</i>	<i>C. convexus</i>	<i>C. depressus</i>	<i>C. sp.</i> (KL)	<i>C. sp.</i> (COL7)
<i>C. greggi</i> + <i>Columbia duskysnail</i>	1.2 $\pm$ 0.3				
<i>C. convexus</i>	11.6 $\pm$ 1.5	0.0. $\pm$ 0.2			
<i>C. depressus</i>	10.5 $\pm$ 1.4	8.7 $\pm$ 1.3	0.0 $\pm$ 0.0		
<i>C. sp.</i> (KL)	10.7 $\pm$ 1.4	4.0 $\pm$ 0.9	8.5 $\pm$ 1.3		
<i>C. sp.</i> (COL7)	12.1 $\pm$ 1.5	12.8 $\pm$ 1.7	13.2 $\pm$ 1.6	11.9 $\pm$ 1.5	
<i>A. limosa</i>	19.0 $\pm$ 1.9	19.2 $\pm$ 2.1	19.6 $\pm$ 2.1	19.4 $\pm$ 2.1	16.4 $\pm$ 1.8

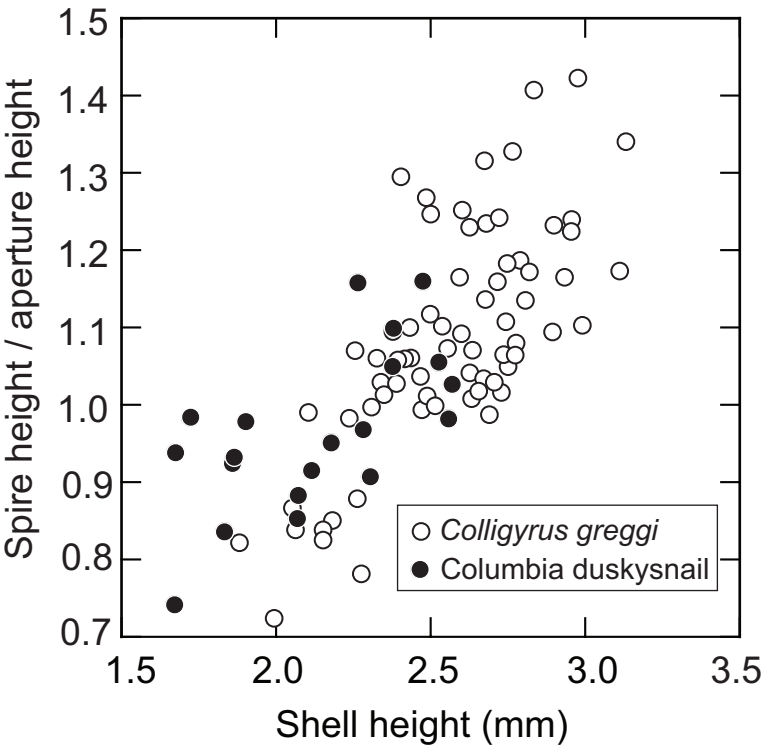


Figure 3. Scatterplot of shell size (SH + SW) and spire size (SH-AH/WBW) for specimens from five samples of *C. greggi* and two samples of the *Columbia duskysnail* (Table 3).

Shell parameters (shell height, spire size and shape) and ANOVA results are reported in Table 3. Spire size overlapped considerably among specimens of the *Columbia duskysnail* and *C. greggi* (Figs 3–4) and was significantly associated with shell height (Pearson correlation, $r^2 = 0.73$, $P < 0.01$). The same patterns were observed for spire shape. Sample heterogeneity was highly significant for shell size, spire size, and spire shape (Table 3). *Colligyrus greggi* had significantly larger and more elongate spires

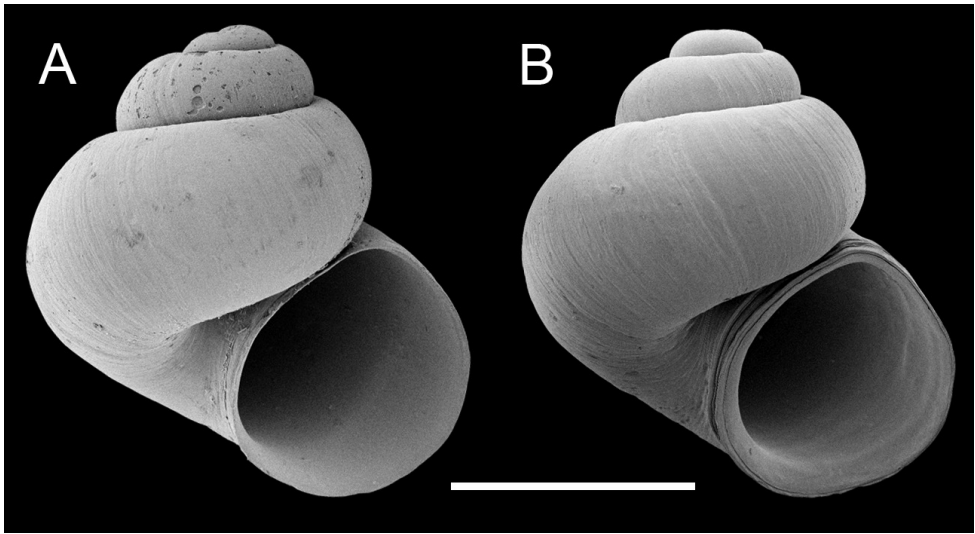


Figure 4. Scanning electron micrographs of shells of the Columbia dusksnail (**A** USNM 1256484) and *C. greggi* (**B** USNM 905375) having similarly sized spires. Scale bar: 1.0 mm.

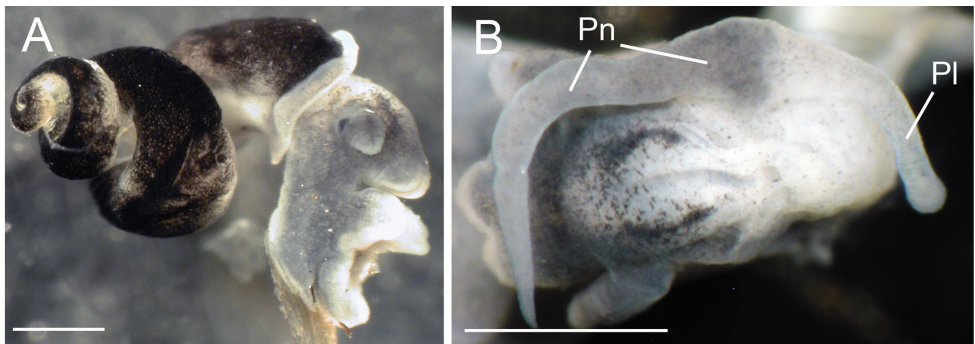


Figure 5. Photographs of ethanol preserved specimens of the Columbia dusksnail (USNM 1256484) showing pigmentation of body (**A**) and penis (**B**). Scale bars: 500 μ m. **Pl** penial lobe, **Pn** penis.

($P < 0.05$) than the Columbia dusksnail in five of 10, and seven of 10 pairwise comparisons (among samples), respectively. However, the differences in these parameters were not significant in seven of eight comparisons between samples of the Columbia dusksnail and similar sized *C. greggi* (USNM 905275, USNM 1003672). The discriminant function analysis of the standard shell parameters delineated significant differences between the Columbia dusksnail and *C. greggi* (Wilk's lambda = 0.5678, $F = 9.0257$, $df = 7$, $P < 0.0001$). However, the classification matrix correctly distinguished only 14/20 (70%) of the Columbia dusksnails while 67/71 (94%) of the *C. greggi* specimens were correctly distinguished. (This dataset is available from the first author on request.)

Table 3. Variation in shell parameters among samples of *C. greggi* and the *Columbia duskysnail*. Values are mean $\pm$ standard deviation, and range.

Sample	SH (mm)	Spire size (SH-AH/AH)	Spire shape (SH-AH/WBW)
<i>C. greggi</i>			
USNM 883531 (<i>N</i> =12)	2.65 $\pm$ 0.17 2.40–2.98	1.27 $\pm$ 0.09 1.11–1.42	0.88 $\pm$ 0.06 0.77–0.96
USNM 905375 (<i>N</i> =11)	2.14 $\pm$ 0.15 1.88–2.44	0.87 $\pm$ 0.11 0.72–1.10	0.65 $\pm$ 0.06 0.58–0.76
USNM 905382 (<i>N</i> =20)	2.64 $\pm$ 0.13 2.40–2.95	1.08 $\pm$ 0.07 1.00–1.23	0.78 $\pm$ 0.04 0.73–0.88
USNM 1003672 (<i>N</i> =11)	2.35 $\pm$ 0.07 2.25–2.47	1.03 $\pm$ 0.06 0.88–1.10	0.72 $\pm$ 0.04 0.64–0.77
USNM 1075739 (<i>N</i> =17)	2.82 $\pm$ 0.19 2.43–3.13	1.14 $\pm$ 0.10 0.99–1.34	0.83 $\pm$ 0.05 0.75–0.90
<i>Columbia duskysnail</i>			
USNM 1256484 (<i>N</i> =10)	2.34 $\pm$ 0.18 2.08–2.57	0.98 $\pm$ 0.08 0.64–0.80	0.72 $\pm$ 0.05 0.64–0.80
USNM 1256489 (<i>N</i> =10)	1.94 $\pm$ 0.26 1.67–2.47	0.95 $\pm$ 0.13 0.75–1.16	0.67 $\pm$ 0.06 0.57–0.78
*ANOVA	** <i>F</i> =43.717	** <i>F</i> =24.601	** <i>F</i> =35.301

* *DF* for all parameters was 6, 84

** Highly significant (*P*<0.01)

Table 4. Radular cusp counts for *C. greggi* (from Hershler 1999) and the *Columbia duskysnail* (USNM 1256484).

	<i>C. greggi</i>	<i>Columbia duskysnail</i>
Central teeth, lateral cusps	4–7	4–7
Central teeth, basal cusps	1–2	1–2
Lateral teeth, cusps on inner side	2–4	3–5
Lateral teeth, cusps on outer side	3–4	4–5
Inner marginal teeth	24–27	24–30
Outer marginal teeth	25–33	23–29

We were unable to confirm the purported differences in soft part pigmentation between the *Columbia duskysnail* and *C. greggi*. The pallial roof and visceral coil of the *Columbia duskysnail* is darkly pigmented and often black (Fig. 5A) as was described for *C. greggi* (Hershler 1999). The penis also conformed to that of *C. greggi* in having a basally concentrated internal core of dark pigment (Fig. 5B). The *Columbia duskysnail* closely resembled *C. greggi* in most other details (i.e., the number of cusps on the radular teeth, Table 4), although it appears to have a relatively longer penial lobe based on examination of a half dozen males (Fig. 6A–B).

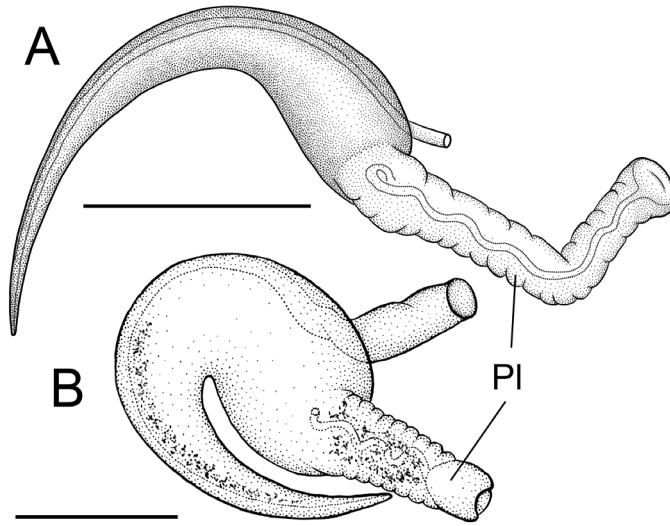


Figure 6. Dorsal views of penes of the Columbia dusksnail (**A** USNM 1256484) and *C. greggi* (**B** USNM 883531, reproduced from Hershler 1999, Fig. 2C). Scale bars: 250 μ m. **PI** penial lobe.

Discussion

Our molecular analysis further confirms a close relationship between the Columbia dusksnail and *C. greggi* and also indicates that populations of the former do not form an evolutionarily distinct, monophyletic unit. The COI sequence divergence between the Columbia dusksnail and *C. greggi* ($2.1 \pm 0.5\%$) is somewhat larger than differentiation within the latter ($1.0 \pm 0.3\%$) but considerably less than that among the three currently recognized congeners ($8.7\text{--}12.1\%$). We also found that the Columbia dusksnail closely resembles *C. greggi* morphologically and cannot be consistently distinguished from it based on the diagnostic characters reported in grey literature (or other shell parameters). Consequently we conclude that the Columbia dusksnail is conspecific with *C. greggi*.

Colligyrus greggi can be added to a long list of plant and animal species that have broadly disjunct, coastal-inland distributions in the Pacific Northwest (Brunsfield et al. 2001, Bjork 2010, Shafer et al. 2010). The full extent of the geographic range *C. greggi* is uncertain pending resolution of the taxonomic status of dusksnail populations in western Montana and northern Idaho. The *C. greggi* populations in the Mount Hood area are geographically isolated and somewhat differentiated genetically relative to other members of this species. There is also evidence of minor morphological differentiation of these animals—i.e., they tend to be smaller and have a longer penial lobe than other *C. greggi*. Collectively this evidence suggests that the populations in the Mount Hood area should be recognized as a distinct conservation unit (within *C. greggi*) that may merit monitoring and other protective measures.

As mentioned in the introduction to this paper, there are numerous taxonomically unstudied populations in the northwestern United States that may be assignable to *Colligyrus*; it is likely that some of these are new species. Although our findings have shown that the *Columbia duskysnail* cannot be considered a distinct congener, the undescribed populations in the Klamath Lake basin (KL) and Puget Sound area (COL7) merit further study as candidate species given that they differ from other *Colligyrus* lineages by 4.0–13.2% COI sequence divergence. The positioning of the latter outside of the *Colligyrus* clade, together with the unusual (near planispiral) shells of these snails suggests that they may belong to a previously unrecognized component of the North American amnicolid radiation.

Material examined (* voucher material for new sequences presented herein)

Colligyrus greggi.—IDAHO. Bear Lake County. USNM 905382, spring at Saint Charles campground, Bear Lake basin, 42.11°N, 111.4662°W. USNM 905375, spring at Porcupine campground, Bear Lake basin, 42.0951°N, 111.5179°W. Fremont County. USNM 1003672, Otter Springs, upper Snake River basin, 44.1545°N, 111.2132°W. OREGON. Clackamas County. USNM 1019124, Oak Grove Fork, 0.24 km upstream from Timothy Lake, Willamette River basin, 45.11076°N, 121.76156°W. Hood River County. *USNM 1256484, small spring, Brooks Meadow, middle Columbia River basin, 45.41389°N, 121.52659°W. *USNM 1256483, spring tributary, Tony Creek, middle Columbia River basin, 45.49263°N, 121.70352°W. *USNM 1256485, Bottle Prairie, west of Eightmile Creek, middle Columbia River basin, 45.39471°N, 121.49992°W. *USNM 1256488, Bear Creek, side channel, Hood River Co., OR, 45.49386°N, 121.64251°W. Wasco County. *USNM 1256486, spring tributary, Ramsey Creek, middle Columbia River basin, 45.40065°N, 121.46345°W. *USNM 1256487, spring tributary, Clear Creek, Deschutes River basin, 45.14148°N, 121.58495°W. WYOMING. Sublette County. USNM 883531, Cliff Creek, tributary springs, upper Snake River basin, 43.2454°N, 110.5002°W.

Colligyrus sp.—WASHINGTON. Thurston County. *USNM 1258917, Allison Springs, Puget Sound drainage, 47.04432°N, 122.98454°W.

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A new species of *Zachaeus* C.L. Koch from Turkey (Opiliones, Phalangiidae)

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Abstract

A new species of harvestmen, *Zachaeus seyyari* **sp. n.** (Opiliones, Phalangiidae), is described and illustrated on the basis of both sexes from Şirnak Province in Turkey. Differences between the new species and related species are indicated. Photographs of its characteristic structures are also provided.

Keywords

Opiliones, Phalangiidae, *Zachaeus*, new species, Turkey

Introduction

Zachaeus C.L. Koch, 1839 is a genus belonging to the subfamily Phalangiinae of the Phalangiidae and it is distributed in the eastern part of the Mediterranean Region, south-eastern Europe, and western Asia (Snegovaya and Staręga 2008). The genus includes 12 species: *Z. anatolicus* (Kulczynski, 1903), *Z. birulai* (Redikorzev, 1936), *Z. crista* (Brullé, 1832), *Z. hebraicus* (Simon, 1884), *Z. hyrcanus* (Redikorzev, 1936), *Z. kervillei* (Sørensen, 1912) species inquirenda, *Z. lupatus* (Eichwald, 1830), *Z. mirabilis* (Caporiacco, 1949), *Z. orchimonti* (Giltay, 1933), *Z. redikorzevi* (Staręga & Chevrizov, 1978), *Z. shachdag* (Snegovaya & Staręga, 2008), and *Z. simferopolensis* (Chemeris &

Kovblyuk, 2005) [Giltay 1933; Redikorzev 1936; Caporiacco 1949; Snegovaya and Staręga 2008], of which five are known in Turkey: *Z. anatolicus*, *Z. crista*, *Z. hebraicus*, *Z. orchimonti*, and *Z. redikorzevi* (Kurt, 2014).

The genus is characterized by the following morphological characteristics: body large, heavily denticulated dorsally; chelicerae usually strong, second segment enlarged; pedipalps normally structured, strong, robust; legs short and first pair much thicker than the others; truncus penis basally widened, parallel-sided on the distal half, distally shallow spoon-shaped, glans usually banana-shaped (shorter in *Z. anatolicus* and *Z. seyyari*), stylus long (Snegovaya and Staręga 2008, 2009). Here, we describe a new species of the genus *Zachaeus* from Şırnak Province in Turkey, and compare it to the most similar species.

Material and methods

Samples were collected by hand from the meadows and grassland in Şırnak Province, Turkey. Species identification was conducted using a Leica EZ4 stereomicroscope. Specimens are preserved in 70% ethanol and deposited in the collection of the Arachnological Laboratory of Şiran Vocational School, Gümüşhane University (GUSAL), Turkey. All measurements are given in millimeters.

Results

Taxonomy

Family Phalangiidae Latreille, 1802

Genus *Zachaeus* C.L. Koch, 1839

Zachaeus seyyari sp. n.

<http://zoobank.org/89BE1BA3-8AEE-4CB1-A1F2-17B789F8BB6C>

Figs 1–4

Type material. Holotype: 1♂ (GUSAL), Turkey: Şırnak Province, İdil District, Yörük Village (37°16'47.54"N, 42°1'17.18"E), 655 m, 12 May 2007, leg. E.A. Yağmur and H. Koç.

Paratypes. 2♂, 4♀ (GUSAL), 1♂, 1♀ (AZMM=Alaşehir Zoological Museum, Manisa) same data as holotype.

Distribution. Up to now only known from type locality in the Şırnak Province, Turkey.

Diagnosis. The new species is similar to *Z. anatolicus* (Kulczyński 1903: 660; Šilhavý 1956: 34, figs 1–5; -1965: 382–384, figs 1–13, Staręga 1976: 376, figs 75–77; Chevrizov 1979: 22, figs 119–121) and *Z. redikorzevi* (Staręga and Chevrizov 1978: 419–422, figs 1–2; Staręga 1978: 219; Chevrizov 1979: 22, figs 122–124; Kurt et al. 2011: 146–147, figs 1–8). The differences between these species are given in Table 1.

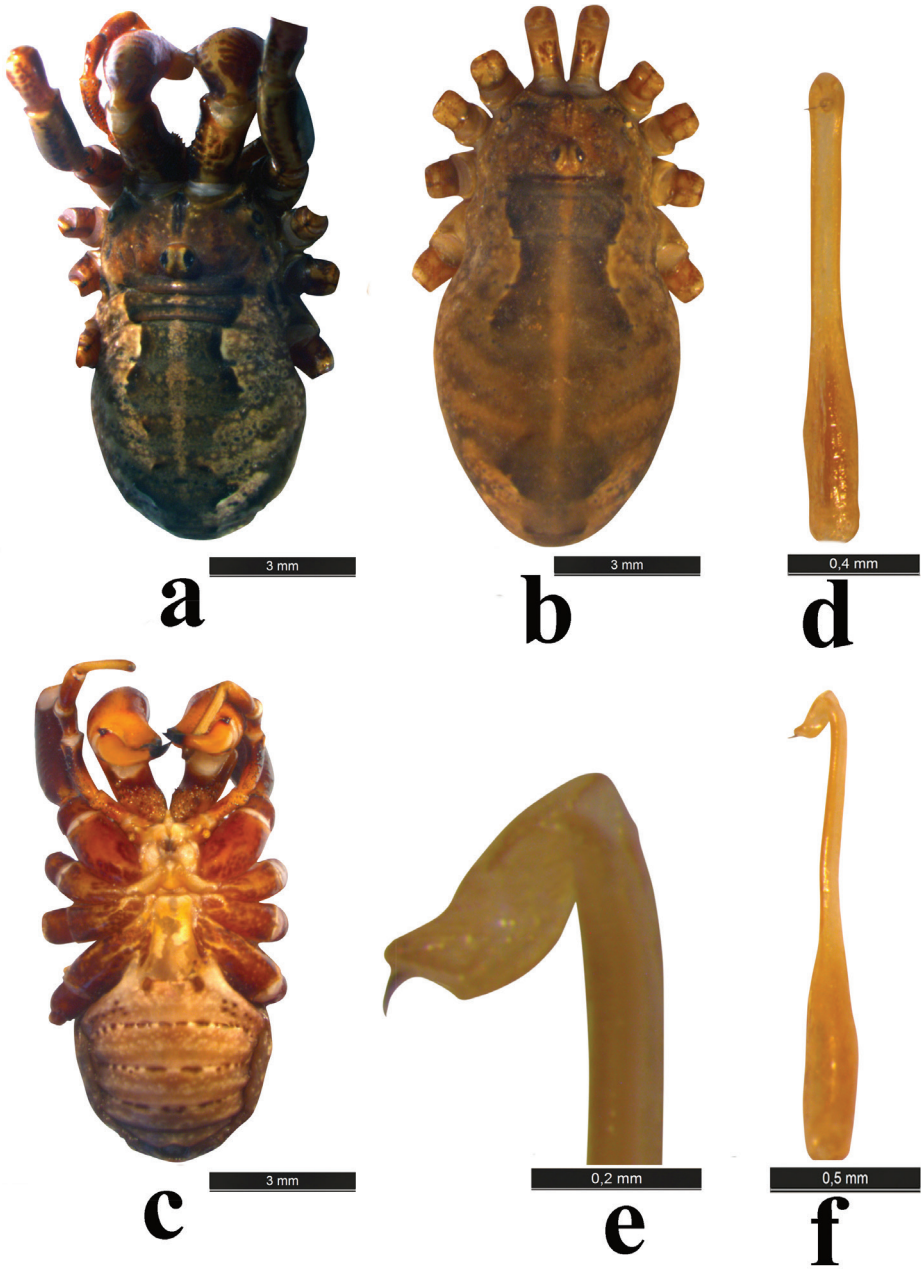


Figure 1. *Z. seyyari* sp. n.: **a** body, male, dorsal view **b** body, female, dorsal view **c** body, male, ventral view **d** penis, dorsal view **e** glans, lateral view **f** penis, lateral view.

Derivatio nominis. The specific epithet is in honor of Dr. Osman SEYYAR (Niğde University, Niğde, Turkey), who has made important contributions to Turkish arachnology.

Table 1. Main diagnostic characters of most closely related species in the genus *Zachaeus*.

Characters	<i>Z. seyyari</i> sp. n.	<i>Z. anatolicus</i>	<i>Z. redikorzevi</i>
Body	cephalothorax dorsally with small denticles; abdomen dorsally not denticulated	cephalothorax and abdomen dorsally with numerous denticles (Starega 1976).	cephalothorax dorsally only granulated on surface, abdomen dorsally not denticulated (Starega and Chevrizov 1978)
Tuber oculorum	relatively low and 1–2 setae in two rows	low and 4–8 tubercles in two rows (Starega 1976).	low and 5–6 tubercles in two rows (Starega and Chevrizov 1978)
Chelicerae of male	second segment swollen (more cylindrical), covered with setae.	second segment not swollen, covered with setae and microdenticles (Starega 1976).	second segment extraordinarily swollen, covered with setae and microdenticles (Starega and Chevrizov 1978)
Palp of male	patella dorsally with setae; tibia with setae.	patella dorsally with microdenticles, tibia with setae and microdenticles (Starega 1976).	patella dorsally with setae; tibia only with setae (Starega and Chevrizov 1978)
Leg	femur I–III with setae; femur IV ventrally with denticles, dorsally setae.	femur I–IV with denticles (Starega 1976).	femur I–III with setae; femur IV ventrally with denticles (Starega and Chevrizov 1978)
Penis	truncus wide at the base, basal to center with straight sides; then slightly narrowed at the center; then not widened, straight-sided at the subapex; glans stocky, not elongated, parallel sided, ventrally slightly oval-sided, apical outline rectangular.	truncus wide at the base, base to center narrowed; then widened at the subapex; glans, stocky, not elongated, ventrally oval (Starega 1976).	truncus slightly enlarged at the proximal half; then straight-sided in distal half; glans elongated and narrow, ventrally oval-sided, apical outline triangular (Starega and Chevrizov 1978)

Table 2. Measurements (in mm) of male holotype (female paratype).

	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
Palp	1.7 (1.5)	0.9 (0.85)	1.2 (0.95)	- (-)	1.9 (1.7)	5.7 (5.0)
Leg I	3.2 (2.8)	1.4 (1.2)	2.94 (2.2)	2.7 (2.1)	4.2 (4.0)	14.44 (12.3)
Leg II	3.8 (3.4)	1.5 (1.4)	3.5 (3.1)	3.7 (3.1)	7.9 (7.8)	20.4 (18.8)
Leg III	2.3 (2.1)	1.3 (1.1)	2.5 (2.1)	3.0 (2.9)	4.8 (4.7)	13.9 (12.9)
Leg IV	3.5 (3.5)	1.4 (1.3)	3.3 (3.2)	3.5 (3.5)	7.2 (7.0)	18.9 (18.5)

Description. Male: body length 7.2 mm, width 4.5 mm; chelicera basal segment 2.8 mm, second segment 3.7 mm.

Body (Fig. 1a): approximately oval-shaped in dorsal view. Opening of odoriferous gland prominent with 1–2 black denticles. Cephalothorax covered with small black denticles. Carapace ochre-brown. Abdomen dorsally with distinct brownish-gray saddle. Saddle with longitudinal whitish-yellow stripe in the center. Abdominal tergites with transverse rows of dark brown spots, not denticulated.

Tuber oculorum (Fig. 4f): nearly hemispherical, median furrow present, relatively low and with 1–2 setae on each side.

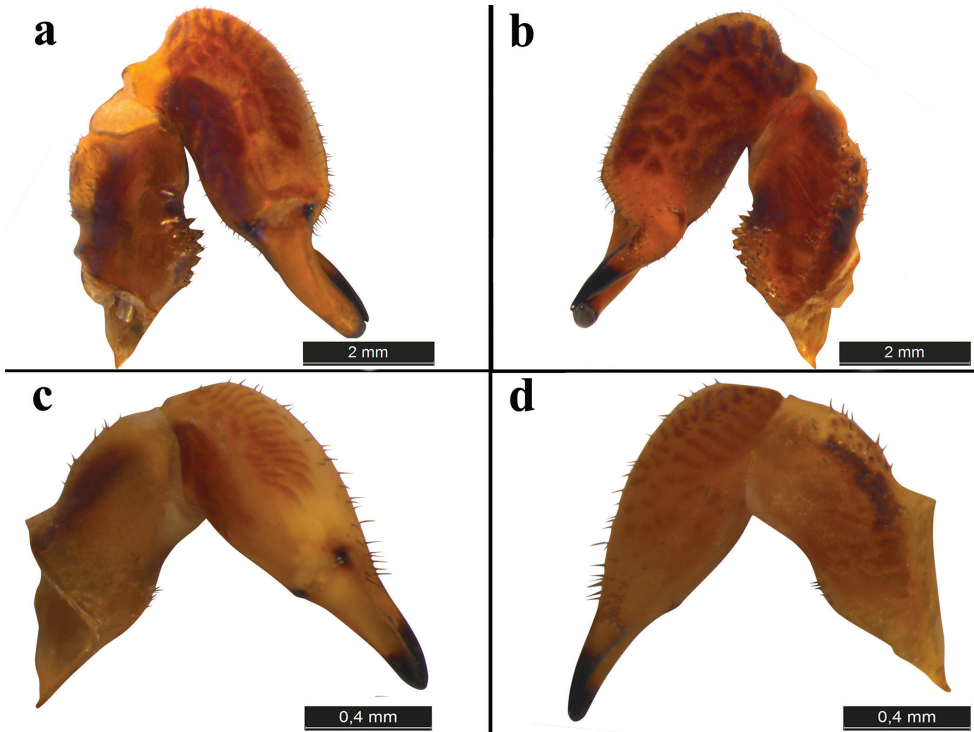


Figure 2. Chelicerae of *Z. seyyari* sp. n.: **a, b** chelicerae, male, lateral view **c, d** chelicera, female, lateral view.

Ventral side (Fig. 1c): coxae and genital operculum covered with sparse hairs. Abdomen ventrally with transverse rows of brown spots, and with sparse hairs.

Chelicerae (Fig. 2, 4e): strong, robust and dark ochre brown. Basal segment apically not widened and slightly bent, dorsally with small black-tipped tubercles and setae, ventrally with long black-tipped tubercles. Second segment apically widened, zebra-like stripe pattern of pigmentation, and covered with setae.

Pedipalp (Fig. 3): normally structured, strong; ochre-brown and with dark brown spots. Coxae with finger-shaped apophysis and covered with long setae; trochanter relatively long, ventrally and dorsally with black tubercles and setae; femur of male slightly curved, dorsally and ventrally covered with black-tipped tubercles and setae; patella distally with usual bulge densely hairy in female, less developed in male: similar in tibia; tibia and tarsus only with setae, but male tarsus ventrally bearing black microdenticles, tarsal claw smooth.

Legs (Fig. 4a–d): short and strong, light ochre-brown and with dark brown spots. Femur to tarsus I relatively thicker than in legs II to IV. Femur and patella I with setae, tibia ventrally with microdenticles and dorsally with setae, metatarsus ventrally covered with densely spaced microdenticles, tarsus bearing only setae. Leg pairs II and III with sparse setae. Femur and tibia IV ventrally covered with black denticles, and dorsally setae; metatarsus IV ventrally with bristle, dorsally with microdenticles; tarsus ventrally bristle, dorsally with setae.

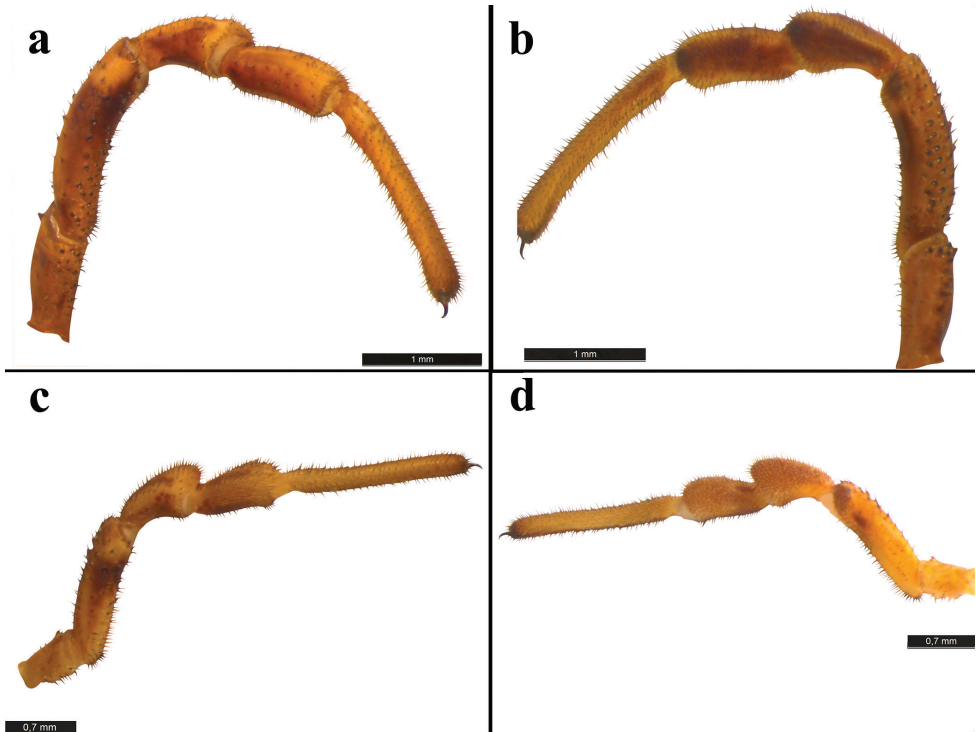


Figure 3. Pedipalp of *Z. seyyari* sp. n.: **a, b** pedipalp, male, lateral view **c, d** pedipalp, female, lateral view.

Male genital morphology (Fig. 1d–f): truncus wide at base, proximal fourth of shaft straight-sided; then slightly narrowed at the center; straight-sided from center to distal end of shaft, forming a spoon-shape, wings not very wide; glans stocky, widened, not elongated, not banana-shaped; stylus long.

Female: body length 9.0 mm, width 4.7 mm; chelicera basal segment 1.5 mm, second segment 2.1 mm. General appearance similar to that of male, but body larger and wider (Fig. 1b). Second segment of chelicerae normally structured, not enlarged, basal segment ventrally without tubercles.

Discussion. This paper describes a new species belonging to the genus *Zachaeus*. This genus has five species (*Z. anatolicus*, *Z. crista*, *Z. hebraicus*, *Z. orchimonti*, and *Z. redikorzevi*) in Turkey. *Z. anatolicus* is distributed in Bulgaria, Caucasus, Crimea, Greece, former Yugoslavia, and Turkey (Adana, Ankara, Bayburt, Gümüşhane, Kayseri and Manisa Provinces) and *Z. redikorzevi* is recorded from Russia and Turkey (Bayburt, Gümüşhane, and Osmaniye Provinces) (Kurt 2014). *Z. crista* is distributed throughout South and Eastern Europe, Caucasus. It is widespread in Turkey (Ankara, Antalya, Bayburt, Bilecik, Bolu, Denizli, Gümüşhane, İzmir, Kırıkkale, Niğde and Osmaniye Provinces). *Z. hebraicus* is known from Jordan, Israel, Lebanon, Libya, Syria and Turkey (Adana, Manisa Provinces). *Z. orchimonti* is only known from Turkey (Aydın, Denizli, İzmir and Manisa Provinces) (Giltay, 1932) and *Z. kervillei* is known

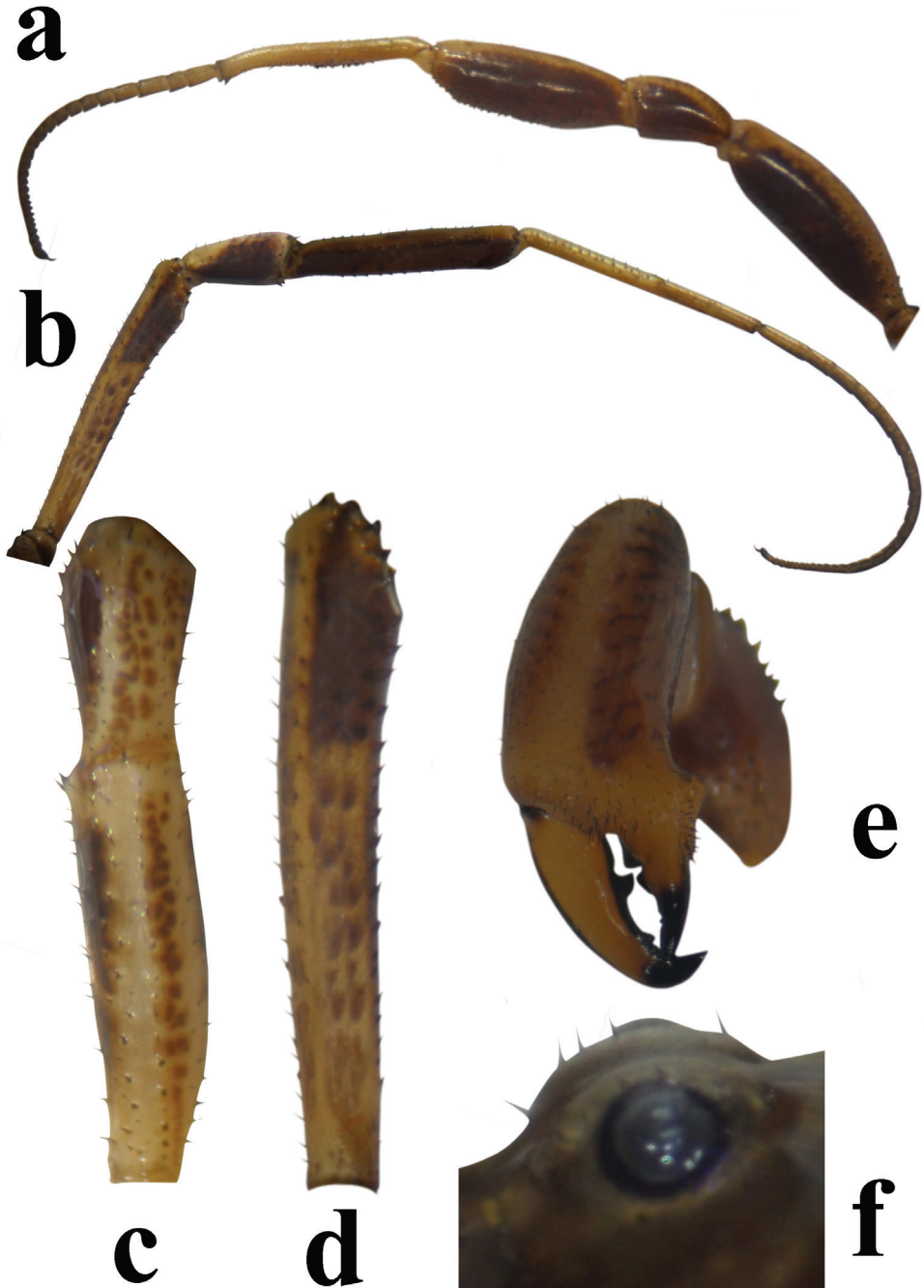


Figure 4. **a, b** Leg of *Z. seyyari* sp. n.: **a** pair I, male, lateral view **b** pair IV, male, lateral view **c** pair I femur and patella, female, dorsal view **d** pair IV femur, lateral view **e** chelicerae, male, frontal view **f** tuber oculorum, male, lateral view.

from Syria (Roewer 1923). The new species differs from *Z. hebraicus*, *Z. crista*, and *Z. kervillei* by the presence of setae only on the ocularium, abdomen not denticulated dorsally, and legs I–III femora covered with setae only (*Z. hebraicus*, *Z. crista*, and *Z. kervillei* are characterized by ocularium with denticles, abdomen dorsally denticulated, and leg I–III femora covered with denticles). *Zachaeus seyyari* sp. nov. differs from *Z. orchimonti* by a setose ocularium, femur of pedipalp dorsally and ventrally covered with black-tipped tubercles and setae (in *Z. orchimonti*, ocularium with 5–7 small denticles and femur of pedipalp with setae).

With *Zachaeus seyyari* sp. n., the number of *Zachaeus* species known from Turkey is now increased to 6. Considering the geographical features of Turkey and the habitat preferences of the genus, the number of species will surely increase with ongoing studies in the future.

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Five new species of *Phintella* Strand, 1906 (Araneae, Salticidae) from the Wuling Mountains, China

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Abstract

Five new species of *Phintella* are described from the Wuling Mountains, China: *Phintella arcuata* **sp. n.** (male and female), *Phintella levii* **sp. n.** (female), *Phintella panda* **sp. n.** (female), *Phintella pulcherrima* **sp. n.** (male and female), and *Phintella wulingensis* **sp. n.** (female). Distribution data, detailed morphological characteristics, and illustrations of body and genital organs are presented.

Keywords

Jumping spider, southern Central China

Introduction

Phintella was established by Strand in 1906 with the type species *P. bifurcilinea*. A total of 54 species has been reported mainly from the Oriental and Palearctic regions (World Spider Catalog 2015), including 25 species transferred mainly from *Chrysilla* Thorell, 1887, *Telamonia* Thorell, 1887, *Icius* Simon, 1876, *Jotus* L. Koch, 1881 and 29 species described as new species. To date, there are 20 species known from China: *P. abnormis* (Bösenberg & Strand, 1906), *P. accentifera* (Simon, 1901), *P. aequipeiformis* (Zabka, 1985), *P. arenicolor* (Grube, 1861), *P. bifurcilinea* (Bösenberg & Strand, 1906), *P. cavaleriei* (Schenkel, 1963), *P. debilis* (Thorell, 1891), *P. hainani* Song et al. (1988), *P. linea* (Karsch, 1879), *P. longapophysis* (Lei & Peng, 2013), *P. longlinensis* (Lei &

Peng, 2013), *P. parva* (Wesolowska, 1981), *P. popovi* (Proszyn'ski, 1979), *P. pygmaea* (Wesolowska, 1981), *P. suavis* (Simon, 1885), *P. suavisoides* (Lei & Peng, 2013), *P. tengchongensis* (Lei & Peng, 2013), *P. versicolor* (Koch, 1846), *P. vittata* (Koch, 1846) and *P. yinae* (Lei & Peng, 2013). The genus can be identified by: palpal tegulum with lobe and bump, embolus sets apically, usually short, pointed or furcated, tibia with one or more apophyses, female internal genitalia simple, couplulatory ducts of different length, usually not twisted, spermathecae round in most species (Zabka 2012).

The Wuling Mountains are located in southern Central China. All the areas are covered with folded mountains, the elevation generally above 1000 meters, the average temperature about 13.4 °C and the average precipitation reach to 1100–1600 millimeters. Vegetation are mainly composed of trees, forest coverage rate reached 80%. East-west of the mountains are range with karst geomorphology and stretch across Chongqing, Hunan, Hubei and Guizhou Provinces (Chen and Li 2003). Salticidae species richness in Wuling Mountains, up to now, more than 100 species including 6 known and several new species of *Phintella* have been collected. The present paper reports five new species of *Phintella* identified from the collections from Wuling Mountains.

Material and methods

Descriptions were made based on specimens fixed in 75% ethanol. The specimens were examined and measured using an Olympus SZX16 stereomicroscope. The details were studied with an Olympus BX53 compound microscope. Male palp and female genitalia were drawn after they were dissected from the spiders. Photos were taken with a Canon PowerShot G12 digital camera mounted on an Olympus SZX16. Compound focus images were generated using Helicon Focus software.

All measurements are given in millimeters. Leg measurements are giving as total length (femur, patella + tibia, metatarsus, tarsus). Abbreviations used are as follows: **AER** anterior eye row; **AERW** anterior eye row width; **ALE** anterior lateral eyes; **AME** anterior median eyes; **EL** eye field length; **PER** posterior eye row, **PERW** posterior eye row; **PLE** posterior lateral eyes. Specimens are deposited in the College of Life Sciences, Hunan Normal University in Changsha, China.

Taxonomy

Phintella Strand, 1906

Phintella arcuata sp. n.

<http://zoobank.org/0C74E8A6-EC9A-4601-BFFE-97833B79A042>

Figs 1–3

Type material. Holotype: ♂, **China, Hunan:** Shimen County, Huping mountain Township, Jinban Mountain Village, (29°26.288'N, 110°46.681'E, 554 m), 12 June

2014, C. Wang, B. Zhou, JH. Gan and YH. Gong leg. **Paratypes:** 1♀, Daling Village, (30°02'20.22N, 110°37'30.25E, 436 m), 18 October 2014, the collectors same as holotype; 1♀, Daling Village, (30°01'37.69N, 110°37'32.56E, 341 m), 19 October 2014, the collector same as holotype; 1♀, Daling Village, (30°01.681'N, 110°37.681'E, 677 m), 18 June 2014, the collectors same as holotype.

Etymology. The specific name comes from the Latin *arcuata* (curved), referring to the form of yellow area at the middle part of male carapace.

Diagnosis. The male of this new species is very similar to *P. aequipeiformis* Zabka, 1985, especially in retrolateral view of male palp, but can be distinguished from the latter by: 1) the terminal sperm duct angle (TSDA) almost 60° (Fig. 3A) versus about 15° in *P. aequipeiformis*; 2) the distal end of retrolateral tibial apophysis curved in ventral view (Figs 1C, 3A) versus straight in *P. aequipeiformis*; 3) Lamellar process almost semicircular (Figs 1C, 3A) versus almost triangular in *P. aequipeiformis*; 4) dorsum of opisthosoma with 3 lines of markings, the first and second lines composed of 4 and 3 white stripes respectively (Fig. 1A) versus only with 2 lines in *P. aequipeiformis*. The female of this new species is similar to *P. linea* (Karsch, 1879), but can be distinguished from the latter by: 1) atrium margin distinct, located at the terminal portion of epigyne (Figs 2B, 3D) versus indistinct in *P. linea*; 2) spermathecae pyriform (Figs 2C, 3E) versus scutiform in *P. linea*; 3) spermathecae separated by less than one-seventh of their width in dorsal view (Figs 2C, 3E) versus about one-third of their width in *P. linea*; 4) base of fertilization ducts extend beyond the base of copulatory ducts in dorsal view (Figs 2C, 3E) versus almost at same level in *P. linea*.

Description. Male: Total length 4.20. Prosoma 2.15 long, 1.75 wide. Opisthosoma 2.05 long, 1.30 wide. Clypeus 0.14 high. Carapace (Fig. 1A) blackish-brown, inflated, covered with white and brown long hair. Bilateral of eye field and posterior sides of carapace with white curved stripes covered by white hair, anterior of thorax with a curved yellowish area behind eye field. Eye bases and margins of carapace black. Fovea reddish-brown, longitudinal, cervical and radial grooves indistinct. Eye sizes and inter-distances: AME 0.50, ALE 0.31, PLE 0.28, AERW 1.35, PERW 1.40, EL 1.03. Chelicerae (Figs 1B, 3C) dark brown, with 2 promarginal teeth and 1 retro-marginal. Endites broader at base, anterior margin with bristles. Labium dark brown, with brown thin hair. Sternum colored as labium, anteriorly straight and posteriorly subacute, with thin hair. Leg trochanters, coxae and tarsi yellowish-brown, others dark brown. Leg spination: tibiae I and II with three pairs, metatarsi I and II with two pairs of long spines. Measurements of legs: I 7.16 (2.05, 3.01, 1.55, 0.55), II 5.55 (1.70, 2.10, 1.20, 0.55), III 5.70 (1.75, 1.95, 1.45, 0.55), IV 5.80 (1.80, 2.00, 1.45, 0.55). Leg formula: 1432. Dorsum of opisthosoma (Fig. 1A) long oval, anterior area with two pairs of white stripes, median area with two pairs of muscle impressions and three transverse white stripes, posterior end with one cambered white stripes, covered with light dots. Venter pale brown, with four longitudinal lines formed by light dots at middle part.

Palp (Figs 1C–D, 3A–B): tibia slightly longer than wide, retrolateral apophysis thin, with a swollen base and slightly curved tip. Posterior lobe large, curved at terminal end and slightly sharp at the tip. Tegulum bump situated posteriorly, almost



Figure 1. *Phintella arcuata* sp. n., **A** male body, dorsal view **B** left chelicerae of male, posterior view **C** male palp, ventral view **D** male palp, retrolateral view. Scale bars: 1.0 mm (**A**); 0.1 mm (**B**); 0.2 mm (**C–D**).

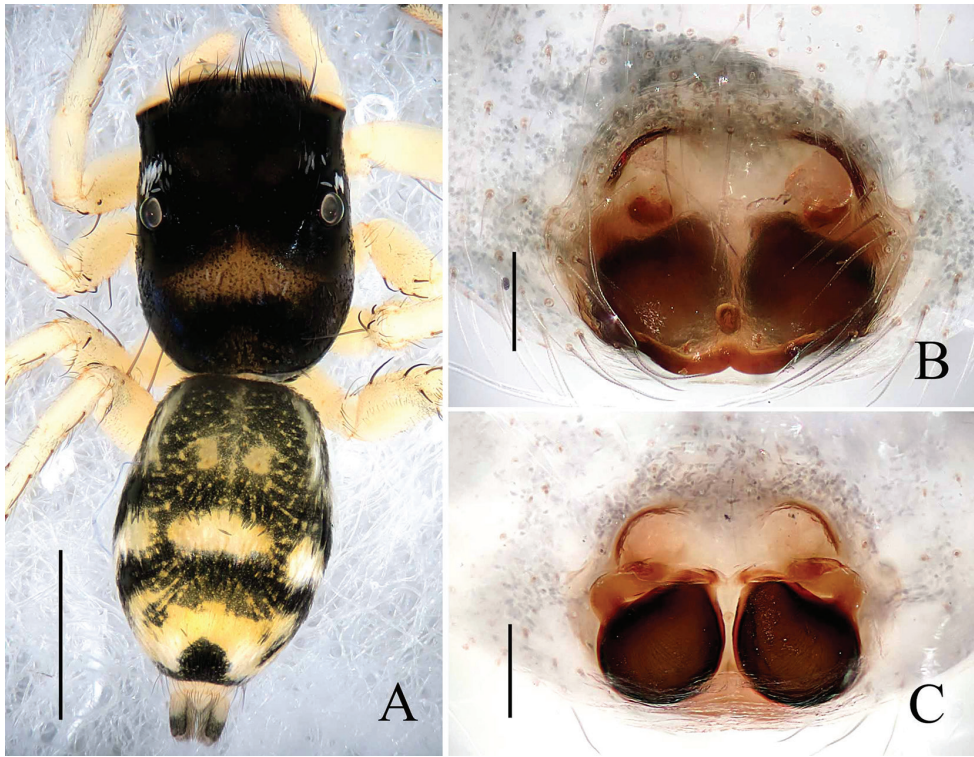


Figure 2. *Phintella arcuata* sp. n., **A** female body, dorsal view **B** epigyne, ventral view **C** vulva, dorsal view. Scale bars: 1.0 mm (**A**); 0.1 mm (**B–C**).

triangular in retrolateral view. Embolus thin, originated at the top on tegulum, the tip almost extended to the position of 1:00 o'clock. Lamellar process big, almost semicircular. Sperm duct visible and the terminal sperm duct angle almost 60°.

Female: Total length 4.10. Prosoma 1.97 long, 1.41 wide. Opisthosoma 2.06 long, 1.43 wide. Clypeus 0.14 high. Carapace (Fig. 2A) dark brown, anterior margin covered with dark brown hair. Bilateral of eye field with white stripes formed by white hair, anterior median of thorax with a triangular yellowish area behind eye field. Margins of carapace and eye bases black. Eye sizes and inter-distances: AME 0.48, ALE 0.27, PLE 0.26, AERW 1.41, PERW 1.33, EL 1.02. Fovea reddish-brown, longitudinal, cervical and radial grooves indistinct. Chelicerae, endites, labium, sternum similar to male except for the lighter color. Legs yellow. Leg spination: as same as male. Measurements of legs: I 3.05 (0.95, 1.20, 0.50, 0.40), II 2.90 (0.90, 1.10, 0.50, 0.40), III 3.45 (1.00, 1.30, 0.75, 0.40), V 4.00 (1.25, 1.45, 0.90, 0.40). Leg formula: 4312. Dorsum of opisthosoma (Fig. 2A) pale brown, the markings similar to male, covered with light dots. Venter grey brown, bilateral of posterior portion with two longitudinal white stripes, and two longitudinal lines formed by light dots behind epigastric furrow.

Epigyne (Figs 2B–C, 3D–E) with arc band-shaped atrium margins anteriorly. Copulatory openings small, situated at the median area, the distance between them

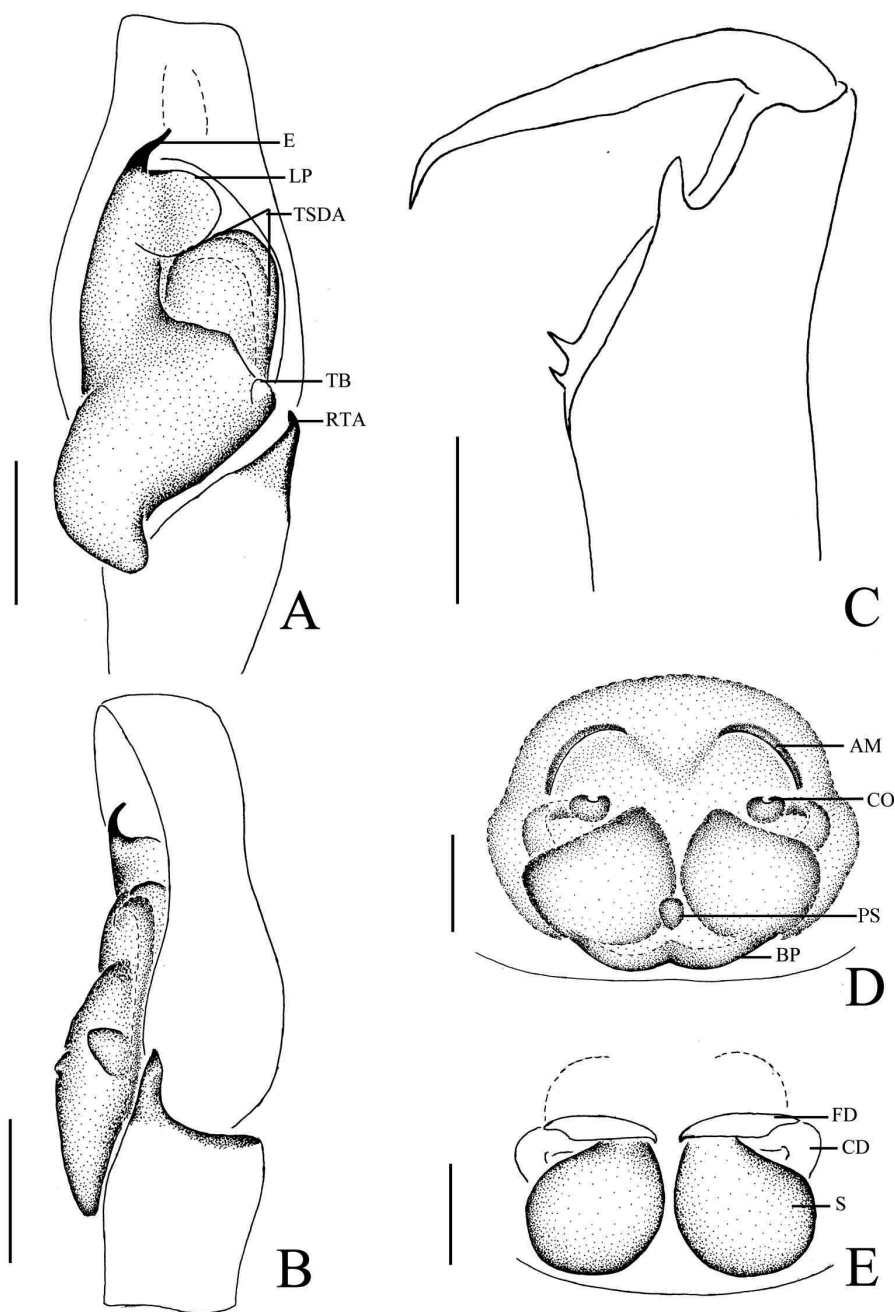


Figure 3. *Phintella arcuata* sp. n., **A** male palp, ventral view **B** male palp, retrolateral view **C** left chelicerae of male, posterior view **D** epigyne, ventral view **E** vulva, dorsal view. **AM** atrium margin **BP** basal plate **CD** copulatory duct **E** embolus **FD** fertilization duct **LP** lamellar process **PL** posterior lobe **PS** poriform structure **RTA** retrolateral tibial apophysis **TB** tegulum bump **TSDA** terminal sperm duct angle **S** spermathecae. Scale bars: 0.1 mm (**A–E**).

about equal to spermathecal width. Basal plate arched, with wave-like protruding parts. Copulatory ducts slightly thick, curved at middle part. Spermathecae pyriform, close to each other, separated by less than one-tenth of their width.

Distribution. China (Hunan).

***Phintella levii* sp. n.**

<http://zoobank.org/5C5C50FD-C08D-40FA-884C-ED5129EC25CB>

Figs 4–5

Type material. Holotype: ♀, **China, Hunan:** Shimen County, Hupingshan Township, Quanping Village, (30°00.786'N, 110°35.822'E, 611 m), 15 June 2014, C. Wang, B. Zhou, JH. Gan and YH. Gong leg. **Paratypes:** 1 ♀, same data as Holotype.

Etymology. The specific name is in honor of Dr. H. Levi, a famous American arachnoid scholar.

Diagnosis. This new species is similar to *P. nigirica* Proszyn'ski, 1992 in having copulatory ducts originated from spermathecal base and the terminal part inflated, but can be distinguished from the latter by: 1) epigyne almost round (Figs 4B, 5A) versus triangular in *P. nigirica*; 2) the distance between atrium margins wider than half of spermathecal width in ventral view (Figs 4B, 5A) versus distinctly narrower than half of spermathecal width in *P. nigirica*; 3) copulatory ducts about half length of epigyne in ventral view (Figs 4B, 5A) versus distinctly longer than half length of epigyne in *P. nigirica*; 4) epigyne with poriform structure situated at the area between bases of two spermathecae (Figs 4B, 5A) versus absent in *P. nigirica*; 5) spermathecae pyriform (Figs 4C, 5B) versus scutiform in *P. nigirica*; 6) dorsum of opisthosoma with yellow stripes and brown stripes alternately arranged (Fig. 4A) versus with two dark submarginal streaks with brown scales along edges in *P. nigirica*.

Description. Female: Total length 4.04. Prosoma 1.61 long, 1.11 wide. Opisthosoma 2.35 long, 1.71 wide. Clypeus 0.15 high. Carapace (Fig. 4A) blackish-brown, covered with long brown and white hair. Bilateral of eye field and posterior sides of carapace with white curved stripes covered by white hair, middle part of carapace with one a W-shaped yellowish brown area. Margin of carapace and eye bases black. Fovea reddish-brown, longitudinal, cervical and radial grooves indistinct. Eye sizes and inter-distances: AME 0.46, ALE 0.22, PLE 0.24, AERW 1.11, PERW 1.05, EL 0.88. Chelicerae yellowish-brown, with 2 promarginal teeth and 1 retromarginal. Endites dark brown, with narrower base, anterior margin with bristles. Labium colored as endites, broader at base, covered with black hair at terminal. Sternum dark brown, anteriorly straight and posteriorly subacute, covered with thin hair. Terminal part of femur, anterior and terminal parts of patella with dark annuli, others yellow. Leg spination: tibiae I and II with two pairs, metatarsi I and II also with two pairs of long spines. Measurements of legs: I 3.00 (0.95, 1.10, 0.55, 0.40), II 2.80 (0.90, 1.00, 0.50, 0.40), III 3.45 (1.10, 1.15, 0.80, 0.40), IV 3.90 (1.20, 1.40, 0.90, 0.40). Leg formula: 4312. Dorsum of opisthosoma (Fig. 4A) oval, yellow stripes and brown stripes alternately ar-

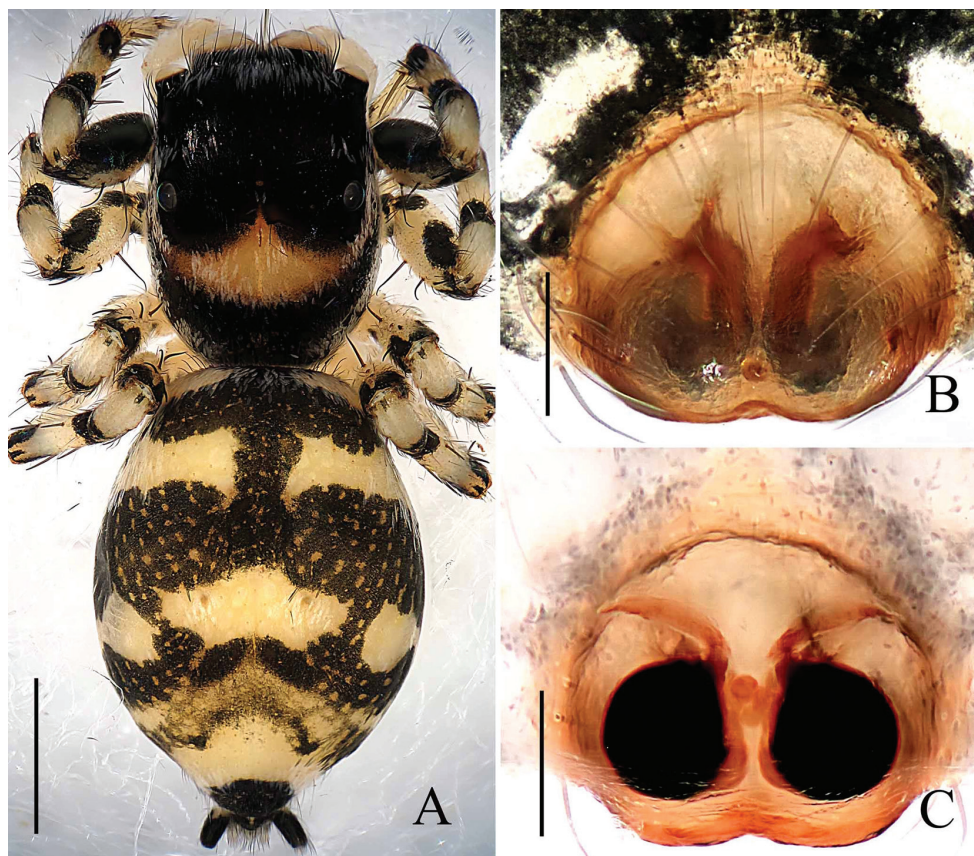


Figure 4. *Phintella levii* sp. n., **A** female body, dorsal view **B** epigyne, ventral view **C** vulva, dorsal view. Scale bars: 1.0 mm (**A**); 0.1 mm (**B–C**).

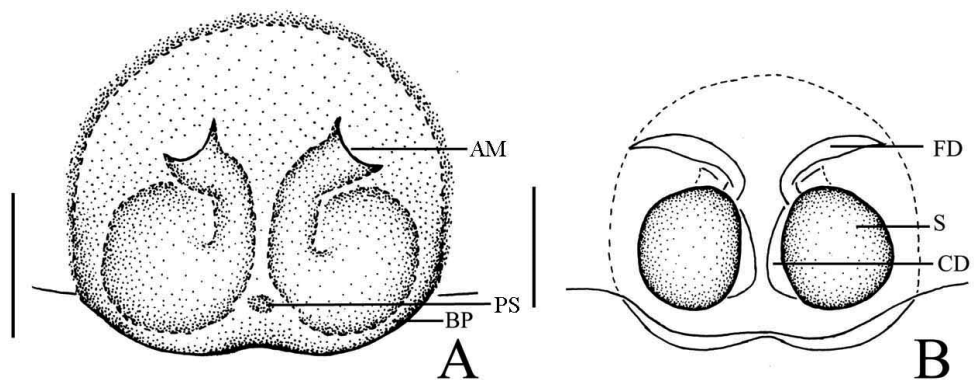


Figure 5. *Phintella levii* sp. n., **A** epigynum, ventral view **B** vulva, dorsal view. **AM** atrium margin **BP** basal plate **CD** copulatory duct **FD** fertilization duct **PS** poriform structure **S** spermathecae. Scale bars: 0.1 mm (**A–B**).

ranged, median area with two pairs of muscle impressions. Venter brown, the middle part with one broad longitudinal pale brown stripe covered with two longitudinal lines formed by light dots. Spinnerets black.

Epigyne (Figs 4B–C, 5A–B) almost round. Atrium margins situated at anterior-median area. Basal plate big, below epigastric furrow. Copulatory ducts long, about half length of epigyne, and the terminal part slightly inflated. Spermathecae pyriform, separated by one-third of their width.

Male: unknown.

Distribution. China (Hunan).

***Phintella panda* sp. n.**

<http://zoobank.org/02F5BDC9-EE3A-453F-92CF-5E813C96872D>

Figs 6–7

Type material. Holotype: ♀, **China, Hunan:** Shimen County, Hupingshan Township, Daling Village, (30°02.359'N, 110°37.301'E, 892 m), 19 June 2014, C. Wang, B. Zhou, JH. Gan and YH. Gong leg.

Etymology. The specific name comes from the Latin *panda* (panda), referring to the form of markings between the posterior lateral eyes, which is similar to the markings of the pandas' eyes..

Diagnosis. This new species is somewhat similar to *P. arcuata* sp. n. in having pyriform spermathecae and a similar basal plate, but can be distinguished from the latter by: 1) atrium margins slit-like, longitudinal (Figs 6B, 7A) versus arc band-shaped, diagonal in *P. arcuata*; 2) base of spermathecae far from basal plate in dorsal view (Figs 6C, 7B) versus almost at same level in *P. arcuata*; 3) distance between the two protruding parts of basal plate wider distinctly, and the protruding parts hornlike (Figs 6B, 7A) versus wave-like in *P. arcuata*; 4) spermathecae touching each other in middle part (Figs 6C, 7B) versus separated distinctly in *P. arcuata*; 5) dorsum of opisthosoma with only one black spot (Fig. 6A) versus with complicated markings in *P. arcuata*.

Description. Female: Total length 4.68. Prosoma 1.96 long, 1.36 wide. Opisthosoma 2.54 long, 1.61 wide. Clypeus 0.15 high. Carapace (Fig. 6A) yellowish-brown, color of cephalic region darker, with one pair of black markings between PER bases. Eye bases black, eye field covered with sparse yellowish-brown bristles, denser in vicinity of eyes. Fovea short and thin, reddish-brown, longitudinal, cervical and radial grooves indistinct. Eye sizes and inter-distances: AME 0.45, ALE 0.23, PLE 0.25. AERW 1.38, PERW 1.30, EL 1.14. Chelicerae yellow, with 2 promarginal teeth and 1 retromarginal. Endites narrower at base, anterior margin with bristles. Labium broader at base, covered with brown thin hair, denser in anterior part. Sternum pale yellow, anteriorly straight and posteriorly subacute, covered with brown thin hair. Legs pale yellow to yellow. Leg spination: tibiae I and II with three pairs, metatarsi I and II with two pairs of long spines. Measurements of legs: I 3.01 (0.93, 1.15, 0.50, 0.43), II

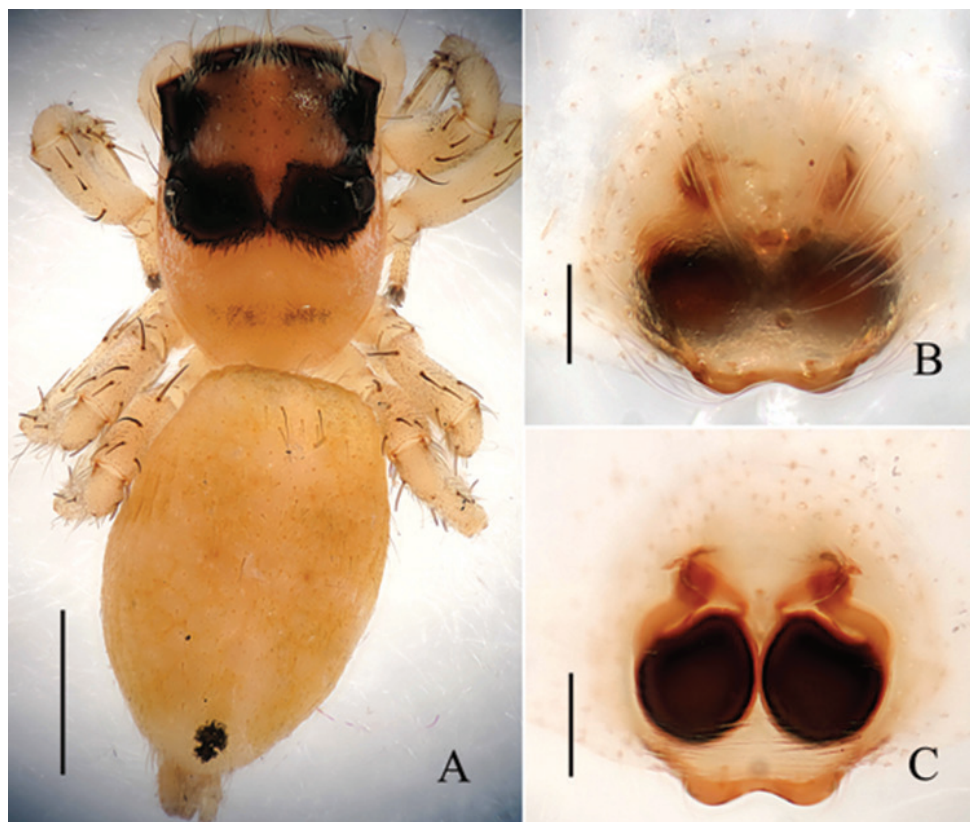


Figure 6. *Phintella panda* sp. n., **A** female body, dorsal view **B** epigyne, ventral view **C** vulva, dorsal view. Scale bars: 1.0 mm (**A**); 0.1 mm (**B–C**).

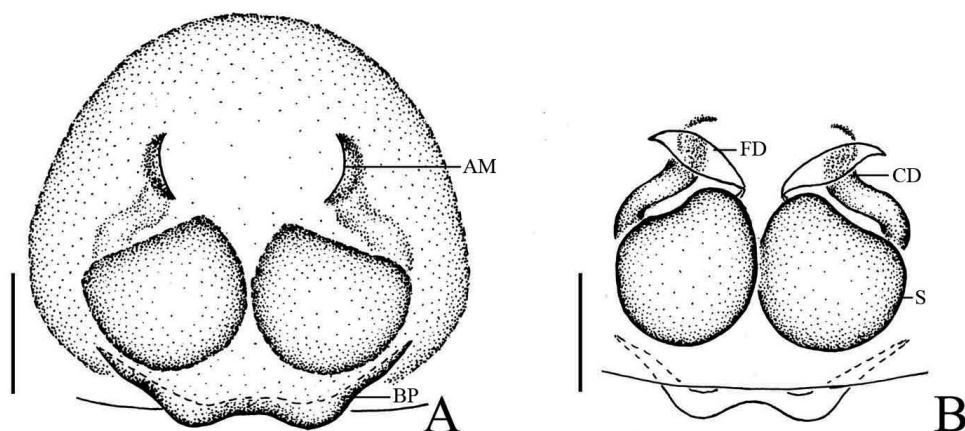


Figure 7. *Phintella panda* sp. n., **A** epigyne, ventral view **B** vulva, dorsal view. AM atrium margin BP basal plate CD copulatory ducts FD fertilization duct S spermathecae. Scale bars: 0.1 mm (**A–B**).

2.88 (0.90, 1.05, 0.50, 0.43), III 3.58 (1.05, 1.20, 0.90, 0.43), IV 3.78 (1.15, 1.30, 0.90, 0.43). Leg formula: 4312. Dorsum of opisthosoma (Fig. 6A) long oval, yellow, with lighter area, covered with sparse thin hair, median area with two pairs of muscle impressions, posterior area with one black spot. Venter pale yellow, without distinct marking.

Epigyne (Figs 6B–C, 7A–B) slightly longer than wide. Atrium margins slit-like, longitudinal, situated anteriorly. Basal plate arched, with two protruding parts below epigastric furrow. Copulatory ducts long and thick, originated from the middle part of outer margin of spermathecae, slightly snaky. Spermathecae pyriform, touching each other in the middle section.

Male: unknown.

Distribution. China (Hunan).

***Phintella pulcherrima* sp. n.**

<http://zoobank.org/B66C79A1-15E6-4F25-86F4-6D936B105624>

Figs 8–10

Type material. Holotype: ♂, **China, Guizhou:** Tongren City, Wenbi Mountains, (27°43.168'N, 109°10.077'E, 475 m), 26 July 2014, XQ. Mi, Y. Huang, C. Wang, B. Zhou and MY. Liao leg. **Paratypes:** 3♀7♂, same data as Holotype.

Etymology. The specific name comes from the Latin *pulcherrima* (very beautiful), referring to the beautiful appearance of the specimens of this new species in alcohol.

Diagnosis. This new species is very similar to *P. linea* (Karsch, 1879) in having similar palps and epigynes, but the males can be distinguished from the latter by: 1) tibia slender relatively, longer than wide (Figs 8B, 10A) versus dumpy, wider than long in *P. linea*; 2) the posterior lobe only extending to tibial terminal in ventral view (Figs 8B, 10A) versus extending to tibial base in *P. linea*; 3) the distal end of retrolateral tibial apophysis curved in ventral view (Figs 8B, 10A) versus straight in *P. linea*; 4) dorsum of opisthosoma with several white round markings and covered with light dots (Fig. 8A) versus with dark brown pattern composed of longitudinal and diagonal stripes in *P. linea*. The females can be distinguished from the latter by: 1) spermathecae almost spherical (Figs 9C, 10D) versus pyriform in *P. linea*; 2) the distance between copulatory openings narrower than spermathecal width in ventral view (Figs 9B, 10C) versus almost equal to spermathecal width in *P. linea*; 3) epigyne with a broad, band-shaped basal plate (Figs 9B, 10C) versus the basal plate divided into three parts in *P. linea*; 4) markings on dorsum of opisthosoma (Fig. 9A) also different.

Description. Male: Total length 4.63. Prosoma 2.37 long, 1.78 wide. Opisthosoma 2.26 long, 1.42 wide. Clypeus 0.15 high. Carapace (Fig. 8A) reddish-brown, widest at coxae II and III. Posterior margins of carapace with yellow curved stripes covered with whiter hair, anterior median of thorax with a quadrangular yellowish area covered by white hair. Eye field with black patches medially, white hair posterior-bilaterally

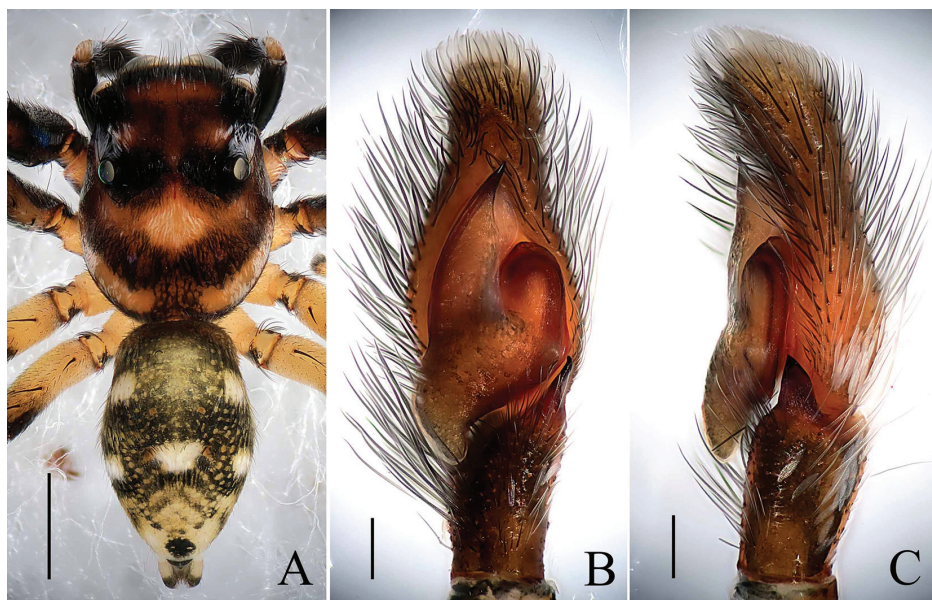


Figure 8. *Phintella pulcherrima* sp. n., **A** male body, dorsal view **B** male palp, ventral view **C** male palp, retrolateral view. Scale bars: 1.0 mm (**A**); 0.1 mm (**B–C**).

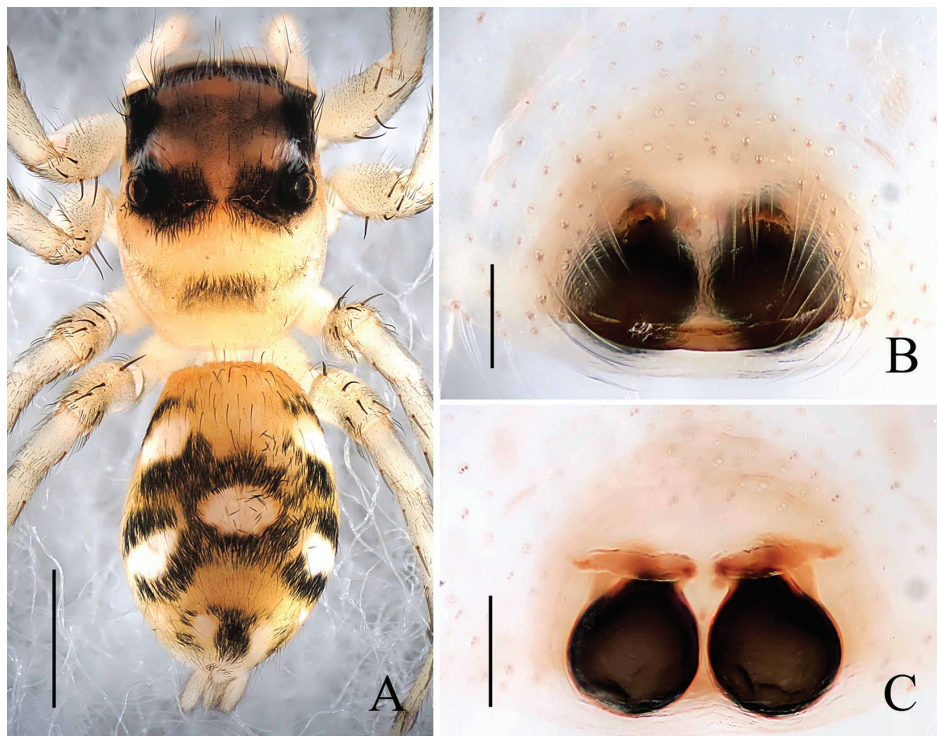


Figure 9. *Phintella pulcherrima* sp. n., **A** female body, dorsal view **B** epigyne, ventral view **C** vulva, dorsal view. Scale bars: 1.0 mm (**A**); 0.1 mm (**B–C**).

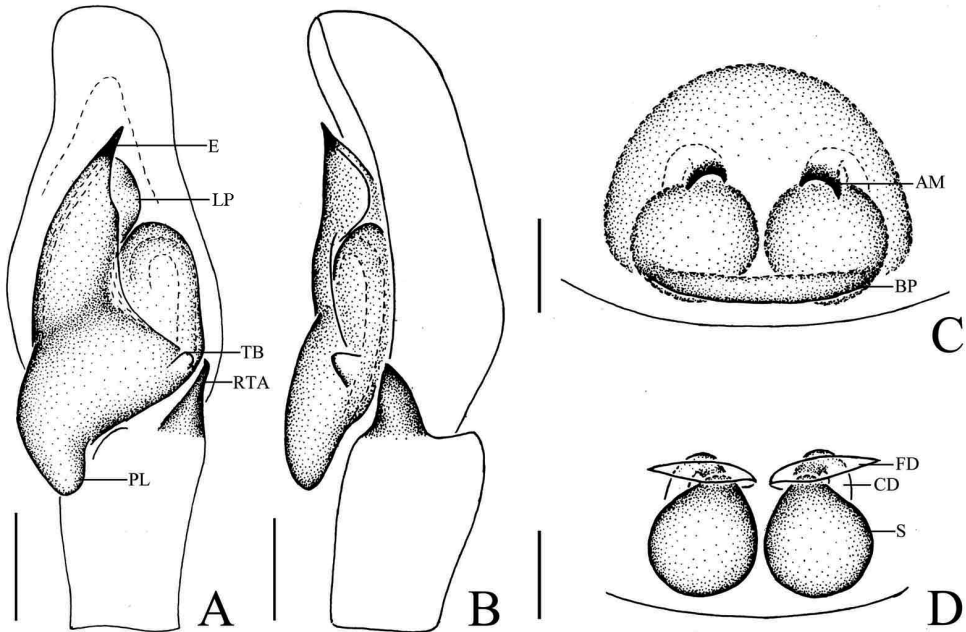


Figure 10. *Phintella pulcherrima* sp. n., **A** male palp, ventral view **B** male palp, retrolateral view **C** epigyne, ventral view **D** vulva, dorsal view. Scale bars: 0.1 mm (**A–D**). **AM** atrium margin **BP** basal plate **CD** copulatory ducts **E** embolus **FD** fertilization ducts **LP** lamellar process **PL** posterior lobe **RTA** retrolateral tibial apophysis **TB** tegulum bump; **S** spermathecae.

situated, covered with sparse brown hair, denser in eye bases. Fovea reddish-brown, longitudinal, cervical and radial grooves indistinct. Eye sizes and inter-distances: AME 0.51, ALE 0.29, PLE 0.29, AERW 1.55, PERW 1.43, EL 1.21. Chelicerae reddish-brown, with 2 promarginal teeth and 1 retromarginal. Endites yellowish-brown, with broader bases, anterior margin with bristles. Labium dark brown, covered with brown thin hair, denser in anterior part. Sternum yellow, anteriorly straight and posteriorly curved. Legs I and II dark brown except middle of patella, metatarsi and tarsus yellow; Legs III and IV yellow except terminal of femur, middle of tibia and terminal of metatarsi dark brown. Leg spination: tibiae I and II with three pairs, metatarsi I and II with two pairs of long spines. Measurements of legs: I 7.35 (2.15, 3.05, 1.55, 0.60), II 5.85 (1.80, 2.25, 1.25, 0.55), III 6.05 (1.85, 2.10, 1.55, 0.55), IV 6.45 (1.95, 2.15, 1.75, 0.60). Leg formula: 1432. Dorsum of opisthosoma (Fig. 8A) long oval, anterior-bilateral area with one pair of round white markings, median area with two pairs of muscle impressions and three white markings, posterior end with two white markings separated by a black spot, covered with light dots. Venter pale brown, with four longitudinal lines formed by light dots at middle part.

Palp (Figs 8B–C, 10A–B): tibia longer than wide distinctly, retrolateral apophysis thin, with a swollen base and slightly curved tip in ventral view, broad base and sharp tip in retrolateral view. Poster lobe big, terminal curved and the tip blunt. Tegulum

bump situated posteriorly, almost at same level with the tip of retrolateral tibial apophysis in ventral view, almost triangular in retrolateral view. Embolus thin, short, originated from top of bulb, the tip about pointed to the position of 1:00 o'clock. Lamellar process small relatively, almost crescent. Sperm ducts visible, running submarginally along retrolateral margin of tegulum in ventral view.

Female: Total length 4.45. Prosoma 2.04 long, 1.48 wide. Opisthosoma 2.31 long, 1.59 wide. Clypeus 0.15 high. Carapace (Fig. 9A) yellowish-brown, darker in cephalic region. Sparse brown bristles on eye field, denser in vicinity of eyes. Posterior bilateral of eye field with white hair and big brown spots between PLE bases. Fovea, cervical and radial grooves indistinct. Eye sizes and inter-distances: AME 0.49, ALE 0.28, PLE 0.28, AERW 1.45, PERW 1.36, EL 1.09. Chelicerae, endites, labium, sternum similar to male except the lighter color. Legs yellow. Leg spination: as same as male. Measurements of legs: I 4.15 (1.30, 1.70, 0.70, 0.45), II 3.90 (1.25, 1.50, 0.70, 0.45), III 4.45 (1.45, 1.55, 1.00, 0.45), IV 5.00 (1.55, 1.75, 1.25, 0.45). Leg formula: 4312. Dorsum of opisthosoma (Fig. 9A) yellow, the markings similar to male except the white markings around with black area. Venter pale yellow.

Epigyne (Figs 9B–C, 10C–D) slightly wider than long. Atrium margins curved, the distance between them less than spermathecal width. Basal plate band-shaped, slightly curved. Copulatory ducts short, curved at middle part and most parts covered by spermathecae and fertilization ducts. Spermathecae almost spherical, close to each other, separated by less than one-twelfth of their width.

Distribution. China (Guizhou).

***Phintella wulingensis* sp. n.**

<http://zoobank.org/D0D2987D-2D40-4BD0-9601-88E953C9C338>

Figs 11–12

Type material. Holotype: ♀, **China, Guizhou:** Songtao County, Fanjing Mountains national native reserve, Wuluo Township, Taoyuan Village, (28°00'0113N, 108°46'4784E, 880 m), 31 July 2014, XJ. Peng, Y. Huang, P. Liu, C. Wang, B. Zhou and MY. Liao leg. **Paratypes:** 1♀, **Hunan:** Shimen County, Hupingshan Township, Daling Village, (30°01.681'N, 110°37.681'E, 677 m), 18 June 2014, C. Wang, B. Zhou, JH. Gan and YH. Gong leg; 1♀, Daling Village, (30°02.175'N, 110°37.455'E, 710 m), 19 June 2014, C. Wang, B. Zhou, JH. Gan and YH. Gong leg.

Etymology. The specific name refers to the type locality; the Wuling Mountains.

Diagnosis. This new species is somewhat similar to *P. panda* sp. n. in having a similar appearance and epigyne with an arched basal plate, but can be distinguished from the latter by: 1) atrium margins diagonal (Figs 11B, 12A) versus longitudinal in *P. panda*; 2) copulatory openings round (Figs 11B, 12A) versus invisible in *P. panda*; 3) situation of atrium margins close to the top of spermathecae (Figs 11B, 12A) versus far from the top of spermathecae in *P. panda*; 4) copulatory ducts thinner, narrower

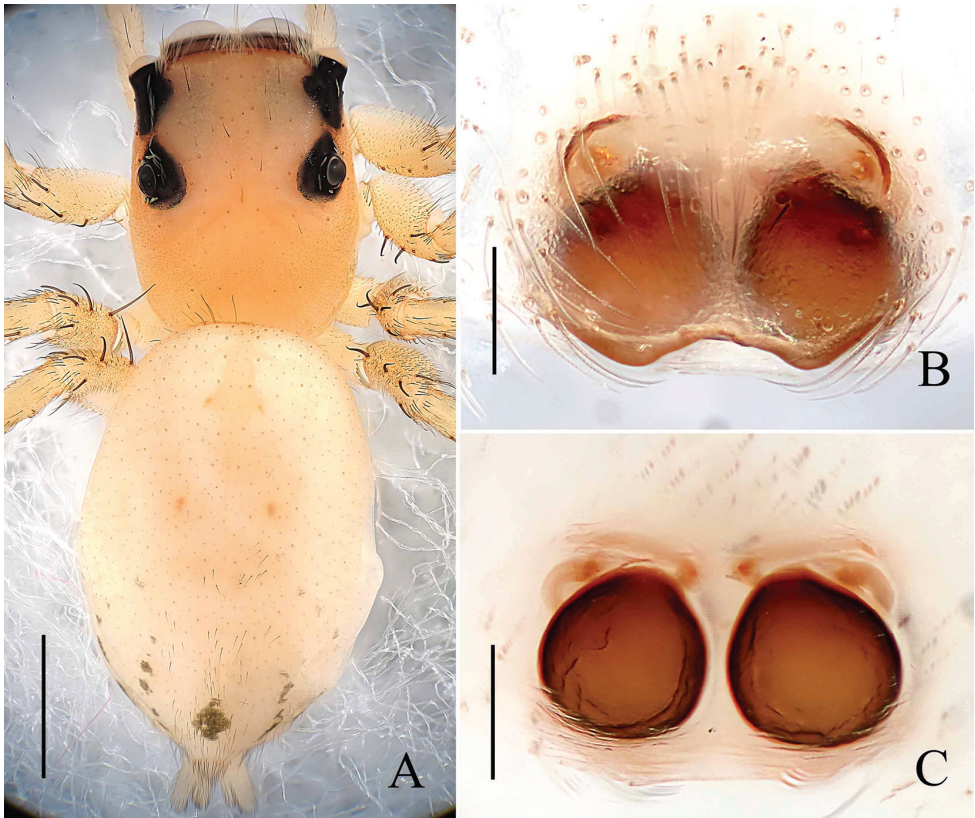


Figure 11. *Phintella wulingensis* sp. n., **A** female body, dorsal view **B** epigyne, ventral view **C** vulva, dorsal view. Scale bars: 1.0 mm (**A**); 0.1 mm (**B–C**).

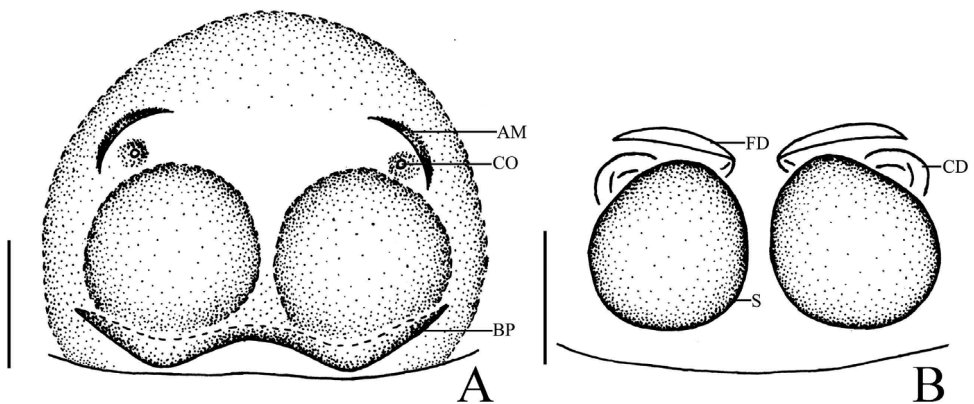


Figure 12. *Phintella wulingensis* sp. n., **A** epigynum, ventral view **B** vulva, dorsal view. **AM** atrium margin **BP** basal plate **CD** copulatory duct **CO** copulatory opening **FD** fertilization duct **S** spermathecae. Scale bars: 0.1 mm (**A–B**).

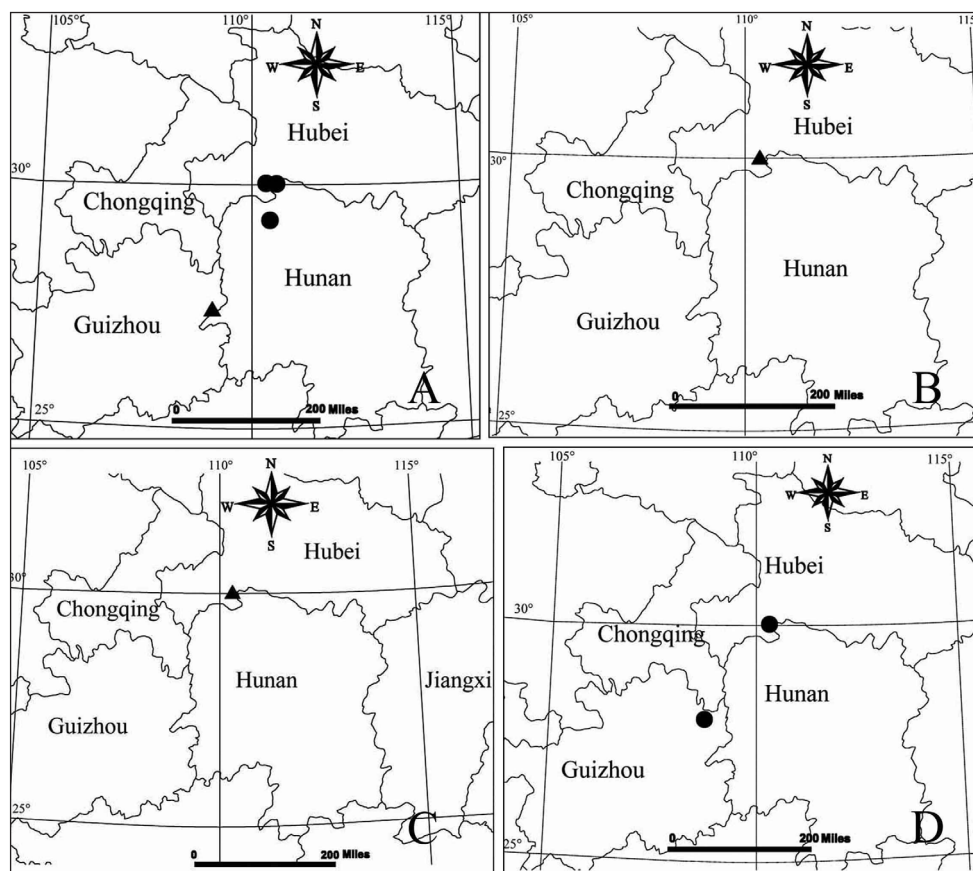


Figure 13. Distribution records of all new species. **A** • *P. arcuata*; ▲ *P. pulcherrima* **B** *P. levii* **C** *P. panda* **D** *P. wulingensis*.

than one-tenth of spermathecal width (Figs 11C, 12B) versus about two-seventh of spermathecal width in *P. panda*; 5) spermathecae almost spherical (Figs 11C, 12B) versus pyriform in *P. panda*; 6) carapace without markings (Fig. 11A) versus with one pair of black markings between PER bases in *P. panda*.

Description. Female: Total length 5.07. Prosoma 1.96 long, 1.55 wide. Opisthosoma 3.07 long, 2.07 wide. Clypeus 0.16 high. Carapace (Fig. 11A) yellow, cephalic region square and thoracic region acutely declining. Eye bases black except PME bases brown, eye field covered with sparse brown hairs. Fovea thin and short, longitudinal, cervical and radial grooves indistinct. Eye sizes and inter-distances: AME 0.52, ALE 0.29, PLE 0.28, AERW 1.52, PERW 1.41, EL 1.04. Chelicerae yellow, with 2 promarginal teeth and 1 retromarginal. Endites narrower at base, anterior margin with bristles, almost parallel. Labium yellow, hair dark and thin, denser in anterior area. Sternum anteriorly straight and posteriorly subacute, covered with brown thin hair. Legs pale yellow to yellow. Leg spination: tibiae I and II with three pairs, metatarsi I and II with two pairs of long spines. Measurements of legs: I 3.38 (1.05, 1.30, 0.60, 0.43), II 3.23

(1.00, 1.25, 0.55, 0.43), III 3.73 (1.15, 1.35, 0.80, 0.43), IV 4.23 (1.45, 1.45, 0.90, 0.43). Leg formula: 4312. Dorsum of opisthosoma (Fig. 11A) long oval, pale yellow, median area with two pairs of muscle impressions, posterior area with small brown spots, covered with recumbent hair. Venter pale yellow, without distinct markings.

Epigyne (Figs 11B–C, 12A–B) slightly wider than long, atrium margins curved, diagonal, situated anteriorly. Copulatory openings small, situated anteriorly, separated from each other distinctly. Basal plate arched, with two protruding parts close to epigastric furrow. Copulatory ducts thin, narrower than one-tenth of spermathecal width, curved at middle part. Spermathecae almost spherical, close to each other, separated by less than two-seventh of their width.

Male: unknown.

Distribution. China (Guizhou, Hunan).

Acknowledgements

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Pushing the limits – two new species of *Pteromalus* (Hymenoptera, Chalcidoidea, Pteromalidae) from Central Europe with remarkable morphology

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Abstract

Two new species, *Pteromalus briani* **sp. n.** and *P. janstai* **sp. n.**, with unusual characters are described from the Central Plateau and the Alps in Switzerland, respectively. *P. briani* **sp. n.** is remarkable in that it has the metatibia quite abruptly expanded before the middle. This type of modification of the hind tibia is unique within the Pteromalidae and probably also the entire Chalcidoidea. It is also very rare in other parasitic wasps, where it is suspected to be associated with pheromone glands. The species is a gregarious endoparasitoid of pupae of *Vanessa atalanta* (Linnaeus) and *Aglais urticae* (Linnaeus), two common butterflies (Lepidoptera: Nymphalidae) in Europe. It is furthermore a koinobiont parasitoid ovipositing in an early larval stage of the host. The other species, *P. janstai* **sp. n.**, shows a flattened mesosoma. A dorsoventrally depressed body is a unique feature within the genus *Pteromalus*, but known from a number species in unrelated genera and subfamilies. The two records demonstrate that it is possible to discover entirely new species with extraordinary characters even in one of the taxonomically most thoroughly explored parts of the world.

Keywords

Systematics, taxonomy, thorax, morphometry, distance measurements, *P. apum*, *P. bifoveolatus*, *P. cassotis*, *P. puparum*, *P. squamifer*, *P. vanessae*, Pteromalinae, Pireninae, Papilionidae, Pieridae

Introduction

In Western Europe the vast majority of newly discovered insect species usually belong to complexes of cryptic species. Morphologically, such new species are therefore quite similar to known species and in many cases even rather difficult to separate from those (e.g., Huber et al. 2013, Alkhatib et al. 2014, Baur et al. 2014, Schmidt et al. 2015). Today the discovery of species with an entirely distinct morphology happens quite rarely and they are then usually found in remote places such as the recently described, spectacular *Cyranobracon depardieui* Quicke and Butcher (Hymenoptera: Braconidae) from tropical Papua New Guinea (Butcher and Quicke 2015) or *Norbanus draco* Mitroiu (Hymenoptera: Pteromalidae) from Central and Southern Africa (Mitroiu 2015). Here I describe two new species of Pteromalidae (Chalcidoidea) with outstanding morphological characters from Central Europe. Although both species are clearly referable to the genus *Pteromalus*, some of their characters stretch the limits of the genus, and in one case the character state may not even be known in Chalcidoidea.

The genus *Pteromalus* contains 485 species world wide, with the majority (371 species) having been described from Europe (Noyes 2015). It is thus the most species-rich genus of Pteromalidae. All species are parasitoids of larvae and pupae of various holometabolous insects, for instance Lepidoptera, Coleoptera, gall forming Hymenoptera (Cynipidae, Tenthredinidae) and Diptera (Tephritidae). No recent study is available that delineates *Pteromalus* based on phylogenetic principals. However, the genus can easily be recognized by a combination of characters (Graham 1969, Bouček and Rasplus 1991, Bouček and Heydon 1997): clypeus striate, its anterior margin truncate or weakly to strongly emarginate, always without a median tooth; flagellum with 2 anelli and 6 funicular segments; clava in females symmetrical; prepectus with relatively small upper triangular area; paraspiracular sulci rather deep and usually with some transverse costulae. *Pteromalus puparum* (Linnaeus, 1758) and *P. cerealellae* (Ashmead, 1902) are among the best-known species of the genus, while for the majority of species little is known except for an occasional distributional or host record (Noyes 2015). However, some *Pteromalus* species attacking fruit flies (Diptera: Tephritidae) have received attention as potential biological control agents (Kapaun et al. 2010) and in community ecology (e.g., Hoffmeister 1992).

Material and methods

Specimens are deposited in the following collections (acronyms mostly according to Noyes 2015): The Natural History Museum, London, UK (BMNH); Canadian National Collection, Ottawa, Canada (CNC); Swiss Federal Institute of Technology, Entomology Collection, Zurich, Switzerland (ETHZ); Jacqueline Grosjean, Niederwangen, Switzerland (JGC, private collection); Biological Museum (Entomology), Lund University, Lund, Sweden (LUZM); Muséum d'histoire naturelle, Geneva, Switzerland (MHNG); Natural History Museum, Vienna, Austria (NHMV); Natural History Museum Bern, Bern, Switzerland (NMBE); Staatliches Museum für Naturkunde, Stutt-

gart, Germany (SMNS); University of Riverside, Riverside, California, USA (UCR); United States National Museum, Washington DC, USA (USNM); Veli Vikberg, Turenki, Finland (VVC, private collection); Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK). All specimens were killed with ethyl acetate, mounted on card rectangles following the method described by Noyes (1982), and finally air dried. Some specimens were later dissected for taking photographs.

Geographical coordinates on data labels of type specimens are indicated as WGS 84 latitude and longitude.

Nomenclature and classification of Chalcidoidea follow Noyes (2015). Terminology of body parts follows Gibson (1997), for terms concerning sculpture of the integument and for some particular expressions used in the description I refer to Graham (1969). The separation of the plica of the propodeum into an anterior and a posterior plica is according to Baur (2000). The Appendix gives an overview of the basic descriptive statistics for each body measurement (in μm) and species as well as the sample sizes. The selected measurements correspond to those used in the taxonomy of Pteromalidae for calculating standard ratios (e.g., Graham 1969; see Table 1), except for body length. Body length of Pteromalidae is usually measured in dorsal view from anterior margin of head to tip of ovipositor sheaths (Graham 1969, Bouček and Rasplus 1991). It is thus often quite variable due to the varying position and angle of head and gaster relative to mesosoma. I therefore have preferred to indicate body length as the sum of lengths (in mm) of head, mesosoma and gaster, each of which could be measured rather more precisely. I also give mesoscutum breadth (in μm), which is considered by Ohl and Thiele (2007) as the most universal measure of size in some Apoidea (Hymenoptera). Note that such a measure is the best way to compare the size of females and males in Chalcidoidea, since body length is usually strongly affected by sex related differences of the gaster (see Bouček 1988, Gibson et al. 1997).

Most characters were measured on photographs taken by Lisa Wilmsmeier with a Leica DFC425 camera mounted on a Leica M16 stereomicroscope. Photographs were taken at different magnifications depending on the size of the character, in order to reduce measurement error for the smaller ones. For all measurements, it was ensured that the points of reference were in perfect focus and that the diaphragm of the lens was fully open. The distances were finally measured using ImageJ, version 1.46v (Schneider et al. 2012). Body parts on the images were zoomed-in on screen up to four times before measuring. To avoid variation due to fluctuating asymmetry (e.g., Palmer and Strobeck 1986, Bechshøft et al. 2008), measurements of paired characters were taken on the left hand side.

I measured three characters, eye length, head length, and temple length, on a single stack photograph taken with a Keyence VHX 2000 digital photomicroscope and a VH-Z20R/W zoom lens at a magnification of $200\times$ (i.e., $1000\text{ }\mu\text{m}$ corresponded to 888 pixels, see Table 1), also using ImageJ, version 1.48v. Stack photos were used because the reference points do not lie in the same focal plane. Accuracy of measurement is thus critically dependent on an exact positioning of the head in dorsal view. Naturally, measurement error should be higher for such characters.

Table 1. Abbreviation, name, definition, and magnification of the 41 measurements used in the description (see Material and methods for further information).

Abbreviation	Character name	Definition	Magnification in pixel/mm*
ant.l	Pedicle plus flagellum length	Combined length of pedicle plus flagellum, outer aspect (Graham 1969)	1742
clv.b	Clava breadth	Greatest breadth of clava, outer aspect	3910
clv.l	Clava length	Greatest length of clava, outer aspect	3910
eye.b	Eye breadth	Greatest breadth of eye, lateral view	3910
eye.d	Eye distance	Shortest distance between eyes, dorsal view	1742
eye.h	Eye height	Greatest length of eye height, lateral view	2549
eye.l	Eye length	Length of eye, dorsal view (Graham 1969)	888
f13.b	First funicular segment breadth	Greatest breadth of first funicular segment (= third flagellar segment), outer aspect	3910
f13.l	First funicular segment length	Greatest length of first funicular segment (= third flagellar segment), outer aspect	3910
f18.b	Sixth funicular segment breadth	Greatest breadth of sixth funicular segment (= eighth flagellar segment), outer aspect	3910
f18.l	Sixth funicular segment length	Greatest length of sixth funicular segment (= eighth flagellar segment), outer aspect	3910
fm3.b	Metafemur breadth	Greatest breadth of metafemur, outer aspect	3910
fm3.l	Metafemur length	Length of metafemur, from distal end of trochanter to tip of metafemur, measured along midline, outer aspect	1742
fwi.b	Fore wing breadth	Greatest breadth of fore wing, measured at about right angle to marginal and postmarginal veins	1742/1089
fwi.l	Fore wing length	Greatest length of fore wing, measured from end of humeral plate to tip of wing	1089
gst.b	Gaster breadth	Greatest breadth of gaster, distance between the outermost lateral edges of the gaster, dorsal view	1742/2549
gst.l	Gaster length	Length of gaster along median line from posterior edge of nucha to tip of ovipositor sheath, dorsal view	1089
hea.b	Head breadth	Greatest breadth of head, dorsal view	1742
hea.h	Head height	Distance between anterior margin of clypeus and anterior edge of anterior ocellus, frontal view	1742
hea.l	Head length	Length of head, dorsal view (Graham 1969)	888
lof.h	Lower face height	Distance between anterior margin of clypeus and lower margin of torulus	2549
mav.l	Marginal vein length	Length of marginal vein, distance between the point at which the submarginal vein touches the leading edge of the wing and the point at which stigmal vein and postmarginal vein unite (Graham 1969)	2549

Abbreviation	Character name	Definition	Magnification in pixel/mm*
msc.b	Mesoscutum breadth	Greatest breadth of mesoscutum just in front of level of tegula, dorsal view	1742
msc.l	Mesoscutum length	Length of mesoscutum along median line from posterior edge of pronotum to posterior edge of mesoscutum, dorsal view	2549
mcp.l	Malar space	Distance between the point where malar sulcus enters mouth margin and malar sulcus enters lower edge of eye, lateral view (Graham 1969)	3910
mss.l	Mesosoma length	Length of mesosoma along median line from anterior edge of pronotum collar to posterior edge of nucha, dorsal view	1089
ool.l	OOL	Shortest distance between posterior ocellus and eye margin, dorsal view (Graham 1969)	3910
pdl.b	Pedicel breadth	Greatest breadth of pedicel, outer aspect	3910
pdl.l	Pedicel length	Length of pedicel, outer aspect	3910
plc.d	Plica distance	Greatest distance between upper edge of anterior plica	2549
pmv.l	Postmarginal vein length	Length of postmarginal vein (Graham 1969), distance between the point at which the stigmal vein and postmarginal vein unite, apically to where the vein appears to end	2549
pol.l	POL	Shortest distance between posterior ocelli, dorsal view (Graham 1969)	3910
ppd.l	Propodeum length	Length of propodeum measured along median line from anterior edge to posterior edge of nucha, dorsal view	2549
scp.b	Scape breadth	Greatest breadth of scape, outer aspect	3910
scp.l	Scape length	Length of scape exclusive of radicle, outer aspect (Graham 1969)	2549
sct.l	Scutellum length	Length of scutellum along median line from posterior edge of mesoscutum to posterior edge of scutellum, dorsal view	2549
stv.l	Stigmal vein length	Length of stigmal vein, distance between the point at which stigmal vein and postmarginal vein unite apically, and the distal end of the stigma (Graham 1969)	2549
ta3.l	Metatarsus length	Length of metatarsus, including pretarsus	2549
tb3.b	Metatibia breadth	Apical breadth of metatibia, outer aspect	3910
tb3.l	Metatibia length	Length of metatibia, measured along midline, outer aspect	1742
tmp.l	Temple length	Length of temple, dorsal view (Graham 1969)	888

* Where more than one number is present, the first refers to the magnification used for *P. briani* sp. n., the second to the one used for *P. janstai* sp. n.

I also used the Keyence microscope for making stack-images of qualitative character states. A 4-digit individual code including the notion “Baur” (e.g., “Baur 2410”) was provided for specimens that have been measured or photographed, or used as reference specimens for comparison with newly described species.

Data resources

I compiled all morphological data in a FileMaker Pro 12®, version 12.0v5, database, of which natural language descriptions as well as ranges of body ratios were generated using the FileMaker script language. Because this is commercial software, a qualitative and a quantitative data matrix (raw values in μm) were exported as comma separated values (CSV) files made available at the BMNH data portal at DOI: <http://dx.doi.org/10.5519/0056966>. The repository furthermore contains all photographs used for measurements, photographs of reference specimens (sometimes provided by other institutions, see acknowledgments) and of labels of the holotypes of the newly described species.

Results

Pteromalus briani sp. n.

<http://zoobank.org/58D10F28-31F6-4E6C-AC8C-90FFBDA10ADC>

Figs 1, 2A, B

Type material. Holotype ♀ Switzerland, Canton Bern, Köniz, Niederwangen, 570 m, 46.92361°N, 7.37266°E, leg. Jacqueline Grosjean, 28-ii-2004, ex pupa 16-iii-2004, host *Vanessa atalanta* (Linnaeus, 1758) (Lepidoptera: Nymphalidae), deposited in NMBE (Baur 2129). The host pupa was collected in sheltered cavity of a pedestrian underpass beneath the highway and railway line in Niederwangen. Paratypes 46 ♀ 2 ♂, emerging from the same host pupa as the holotype, deposited in: 2 ♀ BMNH, 2 ♀ CNC, 2 ♀ ETHZ, 5 ♀ JGC, 2 ♀ LUZN, 2 ♀ MHNG, 2 ♀ NHMV, 19 ♀ (Baur 2408, 2414, 2416, 2418–2421, 2423–2426) 2 ♂ (Baur 2139, 2415) NMBE, 2 ♀ SMNS, 2 ♀ UCR, 2 ♀ USNM, 2 ♀ VVC, 2 ♀ ZFMK. Paratypes 6 ♀ Switzerland, Canton Bern, Reichenbach, Kien, 560 m, 46.6132°N, 7.6854°E, v-2008, leg. Rahel Schnidrig, reared from pupa of *Aglais urticae* (Linnaeus, 1758) (Lepidoptera: Nymphalidae), deposited in: 1 ♀ CNC, 5 ♀ NMBE. According to Schnidrig (pers. comm.), the host was collected as a larva (size about 2.5 mm) and afterwards reared under protected conditions. A total of 40–50 specimens emerged from the pupa but only the paratypes were preserved.

Description, female. *Color:* Head and mesosoma: green to blue-green with metallic luster; setae on head and mesosoma: whitish, inconspicuous; tegula: testaceous; setae on callus of propodeum: whitish.

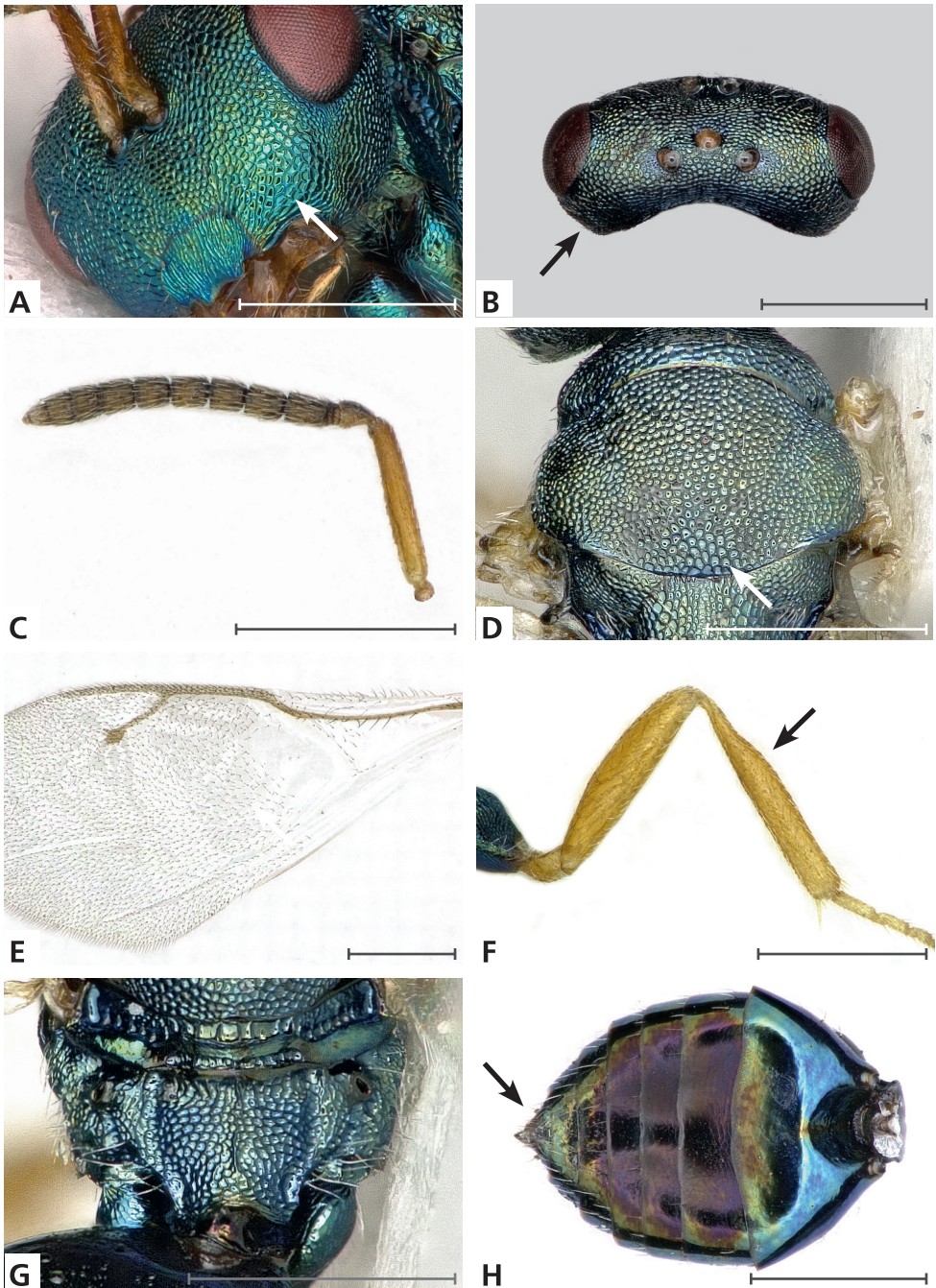


Figure 1. **A, D, G** *Pteromalus briani* sp. n. holotype ♀, **B, C, E, F, H** paratype ♀. **A** gena, anterolateral view **B** head, dorsal view **C** left antenna, outer aspect **D** mesoscutum, dorsal view **E** fore wing venation **F** left metatibia, outer aspect **G** propodeum, dorsal view **H** gaster, dorsal view. Arrows mark important character states; scale bars 0.5 mm.

Scape: testaceous; pedicel: testaceous, slightly infusate dorsally; flagellum: brown.

Fore wing: hyaline; fore wing venation: testaceous; setae on fore wing: fuscous; hind wing: hyaline.

Coxae: green; trochanters: testaceous; femora: testaceous; tibiae: testaceous; tarsi: testaceous with fifth segment slightly infusate; pretarsi: slightly infusate.

Petiole: green with purplish tinge; gaster: green; gastral terga: one to five with strong purplish tinge.

Sculpture: Head in frontal view: finely reticulate with relatively high dividing septa; clypeus: finely striate (Fig. 1A); area between clypeus and malar sulcus: meshes of reticulation conspicuously enlarged (Fig. 1A).

Mesoscutum: finely reticulate, meshes rather high, areoles small and only moderately enlarged in posterior part of sclerite (Fig. 1D); scutellum: reticulate, meshes about as strong and coarse as on posterior part of mesoscutum, but with a narrow band of smaller areoles in anterior half of median longitudinal line; frenum: reticulate, meshes of similar size to those on scutellum; axilla: reticulate, about as strong as on central part of scutellum; prepectus upper triangular area: reticulate; upper mesepimeron: anteriorly smooth, posterior corner distinctly alutaceous; upper mesepisternum: reticulate, about as strong as on mesoscutum; metapleuron: reticulate, about as strong as on mesepisternum.

Pro- and mesocoxa: finely alutaceous, metacoxa: finely reticulate.

Median area of propodeum: evenly reticulate, as strong as on mesoscutum (Fig. 1G); inner corner of anterior plica: with a depression, weakly reticulate; nucha: reticulate, as strong as on median area of propodeum; callus of propodeum: reticulate; paraspiracular sulcus: reticulate with few transverse costulae.

Petiole in dorsal view: smooth; gastral terga: smooth and shining, sixth tergum and syntergum alutaceous (Fig. 1H).

Shape and structure: Head in frontal view: subtrapezoid; gena in frontal view: rounded; temple in dorsal view: obtuse (Fig. 1B); forming an angle with occiput of: 120 degrees; occipital carina: absent; torulus position with respect to lower ocular line: distinctly above; lower face in lateral view: flat, receding with respect to upper face: weakly, forming an angle of: 35 degrees; scrobe: narrow, rather shallow; malar sulcus: superficial, but traceable; clypeus, anterior margin: widely and shallowly emarginate, without a slight depression above emarginate edge; gena near mouth: terete; tentorial pit: distinctly visible (Fig. 1A); mouth extension: not conspicuously enlarged; mandibular formula: 4-4.

Antenna (Fig. 1C). Antennal formula: 11263; scape reaching: distinctly above level of vertex; flagellum: filiform; first anellus: strongly transverse; second anellus: strongly transverse; first funicular segment: cylindrical; setae on flagellum: moderately thickly clothed with setae standing out at an angle of 30 degrees, length of setae less than half the breadth of flagellar segments; number of rows of longitudinal sensilla on first funicular segment: 2, on sixth: 1–2.

Mesosoma in lateral view: moderately strongly bent; propodeum in lateral view sloping with respect to dorsal plane of mesoscutum and scutellum at an angle of: 45 degrees;

pronotum breadth with respect to mesoscutum breadth: distinctly narrower; pronotum collar: horizontal, well defined, its length with respect to mesoscutum length: one sixth, its anterior margin: rounded edge; pronotum posterior margin: thin, shiny strip; notaulus: extremely superficial, hardly traceable, reaching: about half along mesoscutum (Fig. 1D); scutellum in lateral view: moderately convex; scutellum in posterior view: moderately convex; scutellum posterior margin projection: level of anterior margin of dorsellum; scutellum posterior margin in posterior view: narrowly emarginate in the middle; frenal line: finely indicated, especially on sides; prepectus upper triangular area: not separated by oblique carina; upper mesepimeron: strongly narrowing below, not reaching base of mesopleuron; propodeum (Fig. 1G): anterior plica: bent inwards in anterior two fifths and strong; posterior plica: present, joining or almost joining anterior plica; orientation of posterior plicae: almost parallel; median carina of propodeum: weakly indicated, irregular; nucha: elevated but not clearly differentiated from median area of propodeum; spiracle: oval, size: small, separated from anterior margin of propodeum by: shortest diameter; callus pilosity: numerous long setae; paraspiracular sulcus: narrow and deep.

Fore wing (Fig. 1E). Fore wing apex with respect to apex of gaster when folded back: distinctly exceeding; basal cell number of setae: 7; basal setal line: complete, with: 6 setae; cubital setal line: incomplete, with: 4 setae; costal cell pilosity on dorsal side: bare; costal cell pilosity on lower side: with numerous setae in distal half and a complete setal line extending to base; speculum on upper side: bare, widely open below; fore wing disc: rather thickly pilose; marginal setae: present, short; stigma: sub-circular, small; uncus: short.

Femora: moderately slender; metatibia: quite abruptly expanded before the middle (Fig. 1F); metacoxa pilosity, dorsally: bare.

Petiole in dorsal view: conical, in ventral view: open; gaster in dorsal view: ovate, obtusely pointed (Fig. 1G); gastral terga: weakly sunken; posterior margin of first gastral tergum: slightly curved backwards medially; first gastral tergum reaching: two fifths of gaster; tip of hypopygium reaching: slightly beyond middle of gaster; ovipositor sheath: slightly protruding.

Length and body ratios: Body length: 2.3–2.9 mm; mesoscutum breadth: 591–806 μm .

Head breadth to height: 1.2–1.41; head breadth to length: 2.02–2.08; head breadth to mesoscutum breadth: 1.26–1.34; lower face height to head height: 0.5–0.58; POL to OOL: 0.76–0.87; eye height to breadth: 1.3–1.36; eye distance to height: 1.74–1.88; temple length to eye length: 0.35–0.44; malar space to eye height: 0.68–0.76.

Pedicle plus flagellum length to head breadth: 0.72–0.87; scape length to eye height: 0.99–1.04; scape length to breadth: 5.24–5.82; pedicle length to breadth: 1.22–1.54; pedicle length to first funicular segment length: 0.84–1.13; first funicular segment length to breadth: 0.91–1.33; sixth funicular segment length to breadth: 0.85–1.04; first funicular segment breadth to clava breadth: 0.85–1.06; clava length to breadth: 2.01–2.57.

Mesosoma length to mesoscutum breadth: 1.5–1.6; mesoscutum breadth to length: 1.57–1.76; mesoscutum length to scutellum length: 1.03–1.1; propodeum length to scutellum length: 0.57–0.62; plica distance to propodeum length: 1.21–1.31.

Fore wing length to breadth: 2–2.18; marginal vein length to stigmal vein length: 1.51–1.68; postmarginal vein length to stigmal vein length: 0.78–1.01.

Metafemur length to breadth: 3.27–4.47; metatibia length to breadth: 5.61–7.82; metatarsus length to metatibia length: 0.65–0.89.

Gaster length to breadth: 1.17–1.62; gaster length to mesosoma length: 0.82–1.11.

Description, male. *Color:* Head and mesosoma: bright green to blue-green with metallic luster; setae on head and mesosoma: whitish, inconspicuous; tegula: testaceous; setae on callus of propodeum: whitish.

Scape: testaceous; pedicel: testaceous, slightly infusate dorsally; flagellum: testaceous, slightly infusate dorsally.

Fore wing: hyaline; fore wing venation: testaceous; setae on fore wing: fuscous; hind wing: hyaline.

Coxae: green; trochanters: testaceous; femora: testaceous; tibiae: testaceous; tarsi: testaceous with fifth segment slightly infusate; pretarsi: slightly infusate.

Petiole: green with purplish tinge; gaster: green; gastral terga: one to three with an indistinct yellowish spot.

Sculpture: Head in frontal view: finely reticulate with relatively high septae; clypeus: finely striate; area between clypeus and malar sulcus: meshes conspicuously enlarged (Fig. 2A).

Mesoscutum: finely reticulate, meshes rather high, areoles small and only moderately enlarged in posterior part of sclerite; scutellum: reticulate, meshes about as strong and coarse as on posterior part of mesoscutum, but with a narrow band of smaller areoles in anterior half of median longitudinal line; frenum: reticulate, meshes of similar size to those on scutellum; axilla: reticulate, about as strong as on central part of scutellum; prepectus upper triangular area: reticulate; upper mesepimeron: anteriorly smooth, posterior corner distinctly alutaceous; upper mesepisternum: reticulate, about as strong as on mesoscutum; metapleuron: reticulate, about as strong as on mesepisternum.

Pro- and mesocoxa: finely alutaceous, metacoxa: finely reticulate.

Median area of propodeum: evenly reticulate, as strong as on mesoscutum; inner corner of anterior plica: with a depression, weakly reticulate; nucha: reticulate, as strong as on median area of propodeum; callus of propodeum: reticulate; paraspiracular sulcus: reticulate with few transverse costulae.

Petiole in dorsal view: smooth; gastral terga: smooth and shining, sixth tergum and syntergum alutaceous.

Shape and structure: Head in frontal view: subtrapezoid; gena in frontal view: rounded; temple in dorsal view: obtuse; forming an angle with occiput of: 120 degrees; occipital carina: absent; torulus position with respect to lower ocular line: distinctly above; lower face in lateral view: flat, receding with respect to upper face: weakly, forming an angle of: 35 degrees; scrobe: narrow, rather shallow; malar sulcus: superficial, but traceable; clypeus, anterior margin: widely and shallowly emarginate, without a median depression above emarginate edge; gena near mouth: terete; tentorial pit: distinctly visible; mouth extension: not conspicuously enlarged (Fig. 2A); mandibular formula: 4-4.

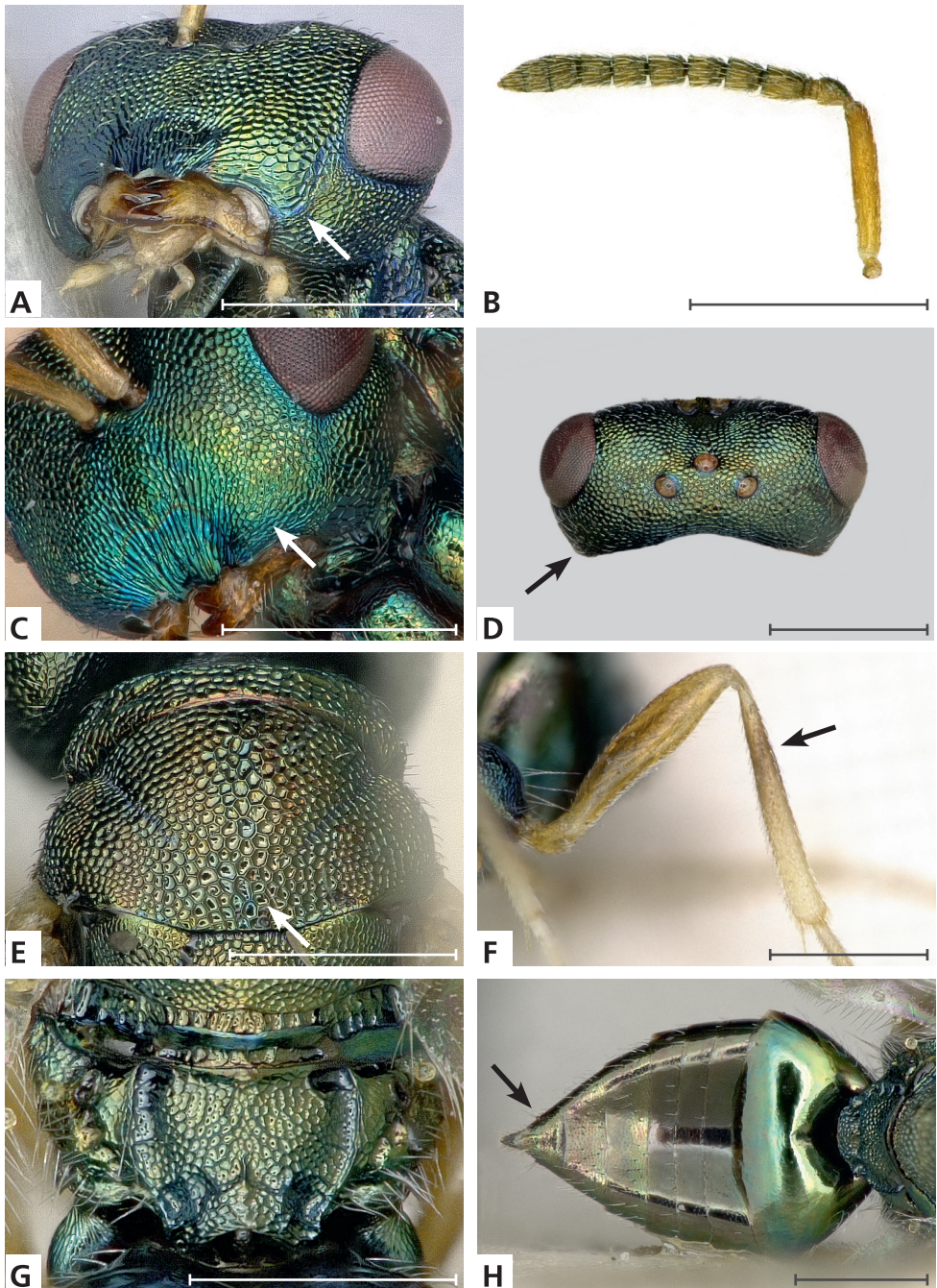


Figure 2. **A, B** *Pteromalus briani* sp. n. paratype ♂, **C–H** *Pteromalus squamifer* Thomson ♀, from Sweden. **A** head, ventral view **B** left antenna, outer aspect **C** gena, anterolateral view **D** head, dorsal view **E** mesoscutum, dorsal view **F** left metatibia, outer aspect **G** propodeum, dorsal view **H** gaster, dorsal view. Arrows mark important character states; scale bars 0.5 mm.

Antenna (Fig. 2B). Antennal formula: 11263; scape reaching: distinctly above level of vertex; flagellum: filiform; first anellus: strongly transverse; second anellus: strongly transverse; setae on flagellum: thickly clothed with setae standing out at an angle of 40 degrees, length of setae less than half the breadth of flagellar segments; number of rows of longitudinal sensilla on first funicular segment: 1, on sixth: 1.

Mesosoma in lateral view: moderately strongly bent; propodeum in lateral view sloping with respect to dorsal plane of mesoscutum and scutellum at an angle of: 50 degrees; pronotum breadth with respect to mesoscutum breadth: distinctly narrower; pronotum collar: horizontal, well defined, its length with respect to mesoscutum length: one sixth, its anterior margin: rounded edge; pronotum posterior margin: thin, shiny strip; notaulus: extremely superficial, hardly traceable, reaching: about half along mesoscutum; scutellum in lateral view: moderately convex; scutellum in posterior view: moderately convex; scutellum posterior margin projection: level of anterior margin of dorsellum; scutellum posterior margin in posterior view: narrowly emarginate in the middle; frenal line: finely indicated, especially on sides; prepectus upper triangular area: separated by a fine oblique carina; upper mesepimeron: strongly narrowing below, not reaching base of mesopleuron; anterior plica: bent inwards in anterior two fifths and strong; posterior plica: present, joining anterior plica; orientation of posterior plicae: almost parallel; median carina of propodeum: weakly indicated, irregular; nucha: elevated but not clearly differentiated from median area of propodeum; spiracle: oval, size: small, separated from anterior margin of propodeum by: shortest diameter; callus pilosity: numerous long setae; paraspircular sulcus: narrow and deep.

Fore wing apex with respect to apex of gaster when folded back: distinctly exceeding; basal cell number of setae: 6; basal setal line: complete, with: 8 setae; cubital setal line: incomplete, with: 4 setae; costal cell pilosity on dorsal side: bare; costal cell pilosity on lower side: numerous setae in distal half and a complete setal line extending to base; speculum on upper side: bare, widely open below; fore wing disc: rather thickly pilose; marginal setae: present, short; stigma: subcircular, small; uncus: short.

Femora: moderately slender; metatibia: quite abruptly expanded before the middle; metacoxa pilosity, dorsally: bare.

Petiole in dorsal view: conical, in ventral view: open; gaster in dorsal view: ovate; gastral terga: weakly sunken.

Length and body ratios: Body length: 2.7 mm; mesoscutum breadth: 682–684 μm .

Head breadth to height: 1.46–1.47; head breadth to length: 2.02–2.03; head breadth to mesoscutum breadth: 1.3; lower face height to head height: 0.59–0.6; POL to OOL: 0.89–0.96; eye height to breadth: 1.29–1.3; eye distance to height: 1.78; temple length to eye length: 0.39–0.43; malar space to eye height: 0.58–0.61.

Pedical plus flagellum length to head breadth: 0.84; scape length to eye height: 0.97–0.98; scape length to breadth: 4.89–5.15; pedicel length to breadth: 1.37; pedicel length to first funicular segment length: 0.85–0.96; first funicular segment length to breadth: 1.27–1.57; sixth funicular segment length to breadth: 1.02–1.05; first funicular segment breadth to clava breadth: 0.91–0.98; clava length to breadth: 2.44–3.13.

Mesosoma length to mesoscutum breadth: 1.63–1.64; mesoscutum breadth to length: 1.48–1.5; mesoscutum length to scutellum length: 1.08–1.12; propodeum length to scutellum length: 0.55–0.59; plica distance to propodeum length: 1.21–1.39.

Fore wing length to breadth: 2–2.02; marginal vein length to stigmal vein length: 1.39–1.56; postmarginal vein length to stigmal vein length: 0.84–0.93.

Metafemur length to breadth: 4.23–4.62; metatibia length to breadth: 7.16–7.35; metatarsus length to metatibia length: 0.72–0.74.

Gaster length to breadth: 1.68–1.71; gaster length to mesosoma length: 1–1.01.

Comment. Close examination of the expanded metatibia under a stereomicroscope did not reveal any distinctive characteristics compared to the “normal”, i.e. unexpanded, metatibia of the other *Pteromalus* species. It should be noted that for some of the specimens reared from *Aglaia urticae* the expansion is slightly less abrupt than shown in Fig. 1F.

Diagnosis. The female of *P. briani* sp. n. keys out in Graham (1969) via couplets 1, 2, 7–9, 11, 12, 14, 49, 52–57, 88–90 (alternatively couplets 49, 70, 72, 74, 78, 79, 84, 88–90) to *P. smaragdus* Graham. The male keys out via couplets 1–3, 5, 7, 10, 11, 14–19, 40, 44, 45, 54–56, 65 to *P. semotus* and *P. varians* [sub *P. grandis*]. The species belongs to a group of species with 4 teeth in both mandibles and a large reticulate nucha (i.e., to *Pteromalus* sensu stricto of Graham 1969). In this group it is most similar to *P. puparum* and *P. squamifer*, especially in the structure of the propodeum (compare Figs 1G and 2G). It is distinguished from those and all other species by the following combination of characters: female legs except coxae bright testaceous (Fig. 1F); reticulation between clypeus and malar sulcus with enlarged meshes (Fig. 1A, 2A); POL distinctly less than OOL (Fig. 1B); tentorial pit distinctly visible (Fig. 1A); antenna inserted high on face, lower edge of torulus above the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite (Fig. 1D); scutellum in lateral view moderately convex; metatibia quite abruptly expanded before the middle (Fig. 1F); female gaster obtusely pointed (Fig. 1H), usually less than 1.6 times as long as broad.

Below the most important differences are given for those species with which *P. briani* sp. n. might be most easily confounded. Because of the difficulty to identify some of them, a rather large number of taxa either related to *P. puparum* or with similar hosts (Lepidoptera: Papilionidae, Nymphalidae or Pieridae) has been considered.

P. apum (Retzius, 1783): female femora infusate; reticulation between clypeus and malar sulcus without enlarged meshes; POL greater than OOL; tentorial pit indistinct; antenna inserted less high on face, lower edge of torulus below the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view flattened; metatibia gradually widening towards apex; female gaster acuminate, often more than 1.6 times as long as broad. Source of information: 2 ♀ 2 ♂ from Switzerland in NMBE (Baur 2517–2520), also compared with the key by Askew and Shaw (1997).

P. bifoveolatus (Förster, 1861): female femora infusate; reticulation between clypeus and malar sulcus without enlarged meshes; POL slightly greater than OOL; tentorial pit indistinct; antenna high on face, lower edge of torulus at about the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster acuminate, often more than 1.6 times as long as broad. In addition, the male of *P. bifoveolatus* is special in that the mouth is very wide, so that the malar space is much less than 0.1 times as long as eye height (0.58–0.61 in *P. briani* sp. n.). Source of information: syntype ♂ in NHMV, 2 ♀ 2 ♂ (Baur 2521–2524) from Switzerland in NMBE.

P. cassotis Walker, 1847 (syn. *P. archippi* Howard, 1889: 1891): female legs except coxae testaceous; reticulation between clypeus and malar sulcus without enlarged meshes; POL about as great as OOL; tentorial pit indistinct; antenna high on face, lower edge of torulus at about the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster acuminate, about 1.25 times as long as broad. Source of information: photographs of lectotype ♀ in BMNH, provided by N. Dale-Skey Papilloud; lectotype ♀ of *P. archippi* in USNM.

P. fuscipes (Provancher, 1881): The lectotype is deposited in the Laval University, Quebec, Canada (Noyes 2015; Huber, pers. comm.), but was not available for examination. The original description (see Provancher 1881: 295) suggests a species with dark legs (“Pattes brunes” = legs brown), which naturally excludes an identity with *P. briani* sp. n. Burks (1963: 1262) suggested that *P. fuscipes* might be the same as *P. p. vanessae* (see also below).

P. luzonensis Gahan, 1925: female femora infusate; reticulation between clypeus and malar sulcus without enlarged meshes; POL about as great as OOL; tentorial pit indistinct; antenna high on face, lower edge of torulus at about the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster obtusely pointed, 1.4–1.6 times as long as broad. Source of information: photographs of a syntype ♀ from Luzon, Mount Makiling, provided by the USNM Chalcidoidea type catalog. 5 ♀ 5 ♂ from Assam and Nepal, in BMNH, compared with the original description by Gahan (1925: 99–100).

P. melitaeae Dzhanokmen, 1998: female femora infusate; reticulation between clypeus and malar sulcus without enlarged meshes; POL greater than OOL; tentorial pit indistinct; antenna less high on face, lower edge of torulus slightly below the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster acuminate, about 2.3 times as long as broad. Source of information: 2 ♀ from

Switzerland in NMBE (Baur 2525, 2526), compared with a paratype 1 ♀ in BMNH and the English version of the original description by Dzhankov (1998).

P. platensis Brèthes in Massini, 1913 (syn. *P. caridei* Brèthes, 1913: 93, synonymized by De Santis 1967: 197): The name-bearing types are not available for examination (Noyes 2015). The descriptions of *P. platensis* and *P. caridei* (see Massini 1913: 517, Brèthes 1913: 93, and Massini and Brèthes 1918, 2. plate), suggest a species with dark femora close to *P. puparum*, which thus excludes it from being the same as *P. briani* sp. n.

P. platyphilus Walker, 1874: female femora infuscate; reticulation between clypeus and malar sulcus without enlarged meshes; POL greater than OOL; tentorial pit indistinct; antenna less high on face, lower edge of torulus distinctly below the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster obtusely pointed, about 1.3 times as long as broad. Source of information: 1 ♀ from Morocco in NMBE (Baur 2527), det. Z. Bouček 1996.

P. puparum (Linnaeus, 1758): female femora infuscate; reticulation between clypeus and malar sulcus without enlarged meshes; POL slightly greater than OOL; tentorial pit indistinct; antenna high on face, lower edge of torulus at about the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster obtusely pointed, rarely more than 1.6 times as long as broad. Source of information: 3 ♀ 2 ♂ from Switzerland in NMBE (Baur 2528–2531, 2549).

P. puparum vanessae Howard, 1889: Harris (1841: 220–221) originally proposed the specific name “*Pteromalus vanessae*” but without accompanying description. Hence it has to be considered as a nomen nudum (Noyes 2015). Howard (1889: 1891–1892) who gave a brief description based on material reared from *Nymphalis antiopa* (Linnaeus, 1758) (sub *Eu Vanessa antiopa*) and *Polygonia interrogationis* (Fabricius, 1798) (both Lepidoptera: Nymphalidae), eventually made the name available. The whereabouts of the syntypes is unknown (Noyes 2015) and they thus could not be checked. However, Howard (1889) evidently considered *P. p. vanessae* to be only a larger and darker variety of *P. puparum*, of which he gave a redescription (p. 1890). The latter is said to have dark legs, which differentiates the species from *P. archippi* (= *P. cassotis*, see above) with pale legs described by Howard in the same paper (p. 1891). Therefore, *P. p. vanessae* also must have dark legs, which clearly separates it from *P. briani* sp. n.

P. semotus (Walker, 1834): female femora infuscate; reticulation between clypeus and malar sulcus without enlarged meshes; POL distinctly greater than OOL; tentorial pits indistinct; antenna less high on face, lower edge of torulus slightly below the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster

acuminate, distinctly more than twice as long as broad. Source of information: 1 ♀ from Switzerland in NMBE (Baur 2532), compared with the lectotype ♀ in BMNH.

P. smaragdus Graham, 1969: female legs except coxae bright testaceous [this is in contrast to the original description, where it is stated on p. 494 that the legs have the same color as *P. procerus* (Graham, 1969) which is said to have the femora infusate (p. 493)]; reticulation between clypeus and malar sulcus without enlarged meshes; POL slightly greater than OOL; tentorial pit indistinct; antenna high on face, lower edge of torulus at about the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster acuminate, about 1.3 times as long as broad. Source of information: photographs of holotype ♀ in BMNH, provided by N. Dale-Skey Papilloud.

P. squamifer (Thomson, 1878): female legs except coxae testaceous (Fig. 2F); reticulation between clypeus and malar sulcus without enlarged meshes (Fig. 2C); POL slightly less than OOL (Fig. 2D); tentorial pit indistinct (Fig. 2C); antenna high on face, lower edge of torulus at about the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles large and rather strongly enlarged in posterior part of sclerite (Fig. 2E); scutellum in lateral view moderately convex; metatibia gradually widening towards apex (Fig. 2F); female gaster acuminate (Fig. 2H), 1.55–1.6 times as long as broad. As in *P. bifoveolatus*, the male has the mouth very large (see Graham 1969: 399, figure 338) and malar space much less than 0.1 times as long as eye height (0.58–0.61 in male *P. briani* sp. n., Fig. 2A). Source of information: photographs of lectotype ♀ in LUZM, provided by C. Hansson; 1 ♀ from Italy in NMBE (Baur 2533) and 4 ♀ from Sweden in BMNH (Baur 2545–2548). It should be noted that in the key of Graham (1969: 513–514) couplet 91 to *P. squamifer* might be misleading, in that he stated “temples about two thirds as long as eyes”. In fact, my measurements on a photograph as well as on the other specimens showed that the temple is at most 0.6 times as long as the eye (Fig. 1C). This value is also strongly depending on how the head is positioned. In another photograph after re-positioning of the same specimen, the ratio was only 0.5!

P. varians (Spinola, 1808): female femora varying from infusate to testaceous; reticulation between clypeus and malar sulcus without enlarged meshes; POL distinctly greater than OOL; tentorial pits indistinct; antenna high on face, lower edge of torulus at about the middle between anterior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster acuminate, distinctly more than twice as long as broad. Source of information: 4 ♀ 1 ♂ from France, Moldavia, and Switzerland in NMBE (Baur 2534–2539), compared with lectotypes of synonyms of *P. varians*, that is, ♀ *P. grandis* Walker, 1835 and ♀ *P. latipennis* Walker, 1835 in BMNH.

P. vopiscus Walker, 1839: female femora infusate; reticulation between clypeus and malar sulcus without enlarged meshes; POL slightly greater than OOL; tentorial pit indistinct; antenna high on face, lower edge of torulus at about the middle between an-

terior margin of clypeus and anterior edge of anterior ocellus; mesoscutum with areoles small and only moderately enlarged in posterior part of sclerite; scutellum in lateral view moderately convex; metatibia gradually widening towards apex; female gaster acuminate, often more than 1.6 times as long as broad. Source of information: 2 ♀ from Switzerland, in NMBE (Baur 2540, 2541). Identification originally based on Graham's (1995) redescription of the species, however, the specimens were later also compared with specimens from Southern France in BMNH identified by Graham himself.

Etymology. Following the suggestion of the collector of the new species, Jacqueline Grosjean, *Pteromalus briani* sp. n. is named after Brian Jones, since the *V. atalanta* pupa was collected on his birthday. The name "briani" is a noun in the genitive case and need not agree in gender with the generic name.

Biology. *Pteromalus briani* sp. n. is a gregarious, primary endoparasitoid of pupae of Nymphalidae (Lepidoptera). Currently, *Vanessa atalanta* and *Aglais urticae* are known as hosts but the species is likely to attack pupae of other nymphalids or possibly of related families. About 58–60 specimens emerged from the overwintering pupa of *V. atalanta* (only 51 ♀, 2 ♂ preserved). According to Rahel Schnidrig (pers. com.) about 40–50 specimens emerged from the pupa of *Aglais urticae* but only 6 ♀ were preserved. The investigation of Schnidrig suggests a koinobiont life history strategy, because the host was collected in an early larval stage (body length 2.5 mm), which was afterwards protected from further parasitization during captive rearing.

***Pteromalus janstai* sp. n.**

<http://zoobank.org/856D795F-691C-41EE-9E54-89FA67700253>

Fig. 3

Type material. Holotype ♀ Switzerland, Canton Wallis, Kippel, Zend, 2100 m, 46.4069°N, 7.7494°E, 15.07.2005, leg. P. Jansta & H. Baur, 15-vii-2005, on Larch (*Larix decidua* Mill.), NMBE (Baur 2410). Paratype 1 ♀, same data as holotype, BMNH (Baur 2411). Paratype 1 ♂ Switzerland, Canton Grisons, Samedan, Blais Granda, 2100 m, 46.4412°N, 9.86456°E, 10-viii-1998, leg. H. Baur, NMBE (Baur 2412).

Description, female. *Color:* Head and mesosoma: green to blue-green with metallic luster; setae on head and mesosoma: fuscous, inconspicuous; tegula: green; setae on callus of propodeum: whitish.

Scape: fuscous with basal third testaceous; pedicel: fuscous; flagellum: fuscous.

Fore wing: hyaline; fore wing venation: brownish; setae on fore wing: fuscous; hind wing: hyaline.

Coxae: green; trochanters: slightly greenish, testaceous at tips; pro- and mesofemur: green, testaceous in apical quarter, metafemur: green, testaceous in apical sixth; protibia: testaceous, meso- and metatibia: testaceous, medially slightly infuscate; tarsi: testaceous, apical segments slightly infuscate; pretarsi: slightly infuscate.

Petiole: dark purplish; gaster: green to blue-green with metallic luster; gastral terga: one to five with strong purplish tinge.

Sculpture: Head in frontal view: finely reticulate with moderately high septae; clypeus: striate; area between clypeus and malar sulcus: finely reticulate.

Mesoscutum: finely reticulate, meshes moderately high, areoles small and not enlarged in posterior part of sclerite; scutellum: reticulate, meshes about as strong and coarse as on posterior part of mesoscutum; frenum: reticulate, meshes larger than those on scutellum; axilla: reticulate, about as strong as on central part of scutellum; prepectus upper triangular area: weakly reticulate; upper mesepimeron: anteriorly smooth, posterior corner distinctly alutaceous; upper mesepisternum: reticulate, about as strong as on mesoscutum; metapleuron: weakly reticulate, less strong as on mesepisternum.

Coxae: weakly reticulate.

Median area of propodeum: uniformly reticulate, as strong as on mesoscutum; inner corner of anterior plica: with a smooth depression and transverse carinae; nucha: reticulate, as strong as on median area of propodeum; callus of propodeum: weakly reticulate; paraspircular sulcus: smooth with few transverse costulae.

Petiole in dorsal view: smooth; gastral terga: smooth and shining, third to fifth tergum anteriorly, sixth tergum and syntergum wholly alutaceous.

Shape and structure: Head in frontal view: subtrapezoid (Fig. 3A); gena in frontal view: buccate; temple in dorsal view: obtuse (Fig. 3B); forming an angle with occiput of: 110 degrees; occipital carina: absent; torulus position with respect to lower ocular line: above; lower face in lateral view: weakly curved, receding with respect to upper face: weakly, forming an angle of: 35 degrees; scrobe: narrow, moderately deep; malar sulcus: superficial, but traceable; clypeus, anterior margin: widely and shallowly emarginate, medially slightly inclined above anterior edge (Fig. 3A); gena near mouth: terete; tentorial pit: indistinct (Fig. 3A); mouth extension: not conspicuously enlarged (Fig. 3A); mandibular formula: 3-4.

Antenna (Fig. 3C). Antennal formula: 11263; scape reaching: middle of anterior ocellus; flagellum: almost filiform; first anellus: strongly transverse; second anellus: strongly transverse; first funicular segment: very slightly constricted at base; setae on flagellum: moderately thickly clothed with setae standing out at an angle of 10–20 degrees, length of setae less than half the breadth of flagellar segments; number of rows of longitudinal sensilla on first funicular segment: 2, on sixth: 1–2.

Mesosoma in lateral view: rather flattened; propodeum in lateral view sloping with respect to dorsal plane of mesoscutum and scutellum at an angle of: 20 degrees (Fig. 3D); pronotum breadth with respect to mesoscutum breadth: distinctly narrower; pronotum collar: horizontal, well defined, its length with respect to mesoscutum length: one sixth, its anterior margin: finely carinate; pronotum posterior margin: thin, shiny strip; notaulus: superficial, reaching: two thirds along mesoscutum; scutellum in lateral view: almost flat; scutellum in posterior view: almost flat medially; scutellum posterior margin projection: level of anterior margin of dorsellum; scutellum posterior margin in posterior view: straight; frenal line: finely indicated, especially on sides; prepectus upper triangular area: separated by a strong carina; upper mesepimeron: strongly narrowing below, not reaching base of mesopleuron; propodeum (Fig. 3F): anterior plica:

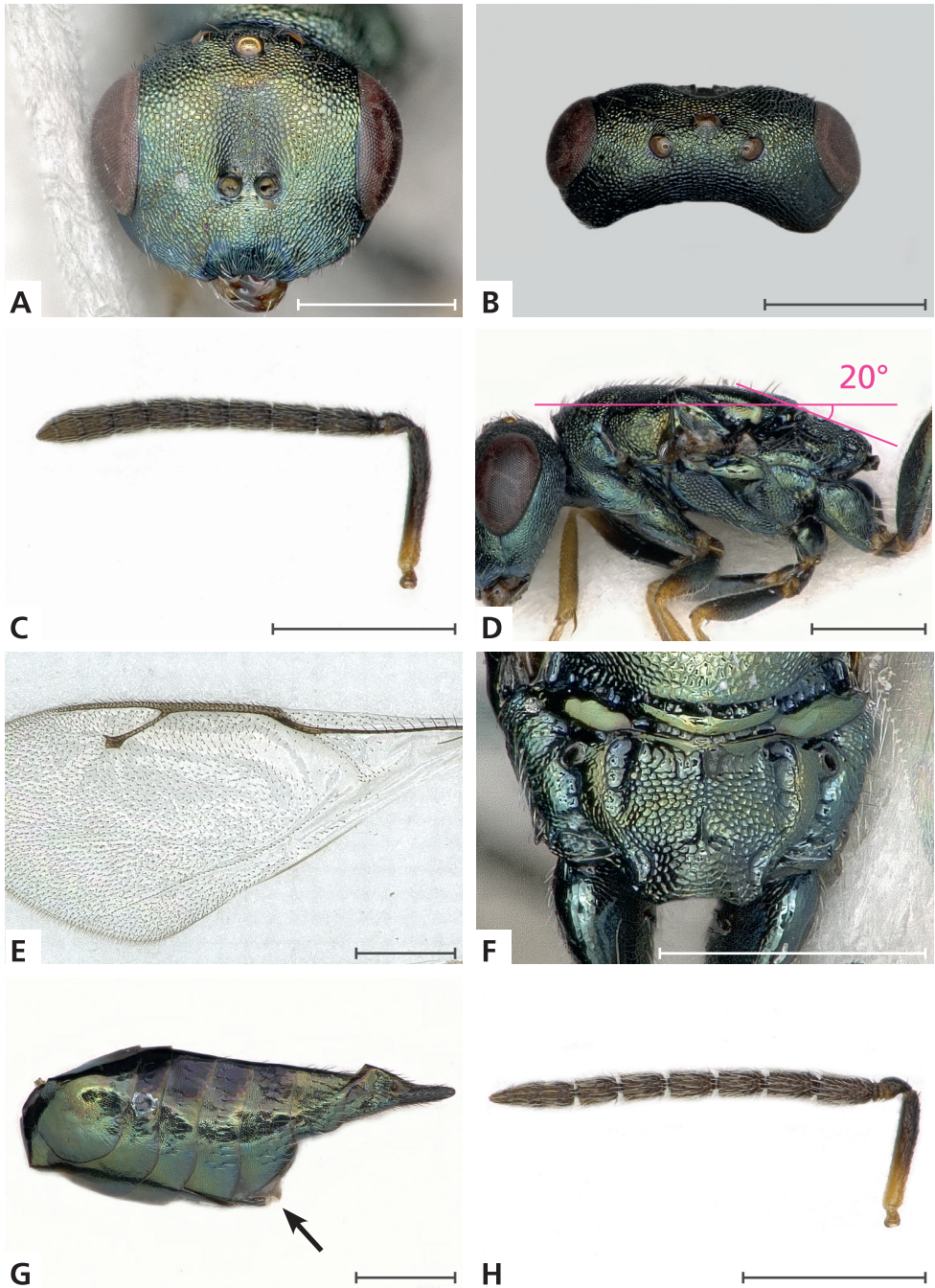


Figure 3. **A–G** *Pteromalus janstai* sp. n. paratype ♀, **H** paratype ♂. **A** head, frontal view **B** head, dorsal view **C** left antenna, outer aspect ♀ **D** mesosoma, lateral view **E** fore wing venation **F** propodeum, dorsal view **G** gaster, lateral view **H** left antenna, outer aspect ♂. Arrows mark important character states; scale bars 0.5 mm.

present, almost straight in anterior part; posterior plica: present, joining or almost joining anterior plica; orientation of posterior plicae: almost parallel; median carina of propodeum: mostly effaced; nucha: elevated but not clearly differentiated from median area of propodeum; spiracle: oval, size: small, separated from anterior margin of propodeum by: shortest diameter; callus pilosity: relatively sparsely setose; paraspicular sulcus: narrow and deep.

Fore wing (Fig. 3E). Fore wing apex with respect to apex of gaster when folded back: just reaching; basal cell number of setae: 9–12 setae in distal part; basal setal line: complete, with: 11–12 setae; cubital setal line: incomplete, with: 4–8 setae; costal cell pilosity on dorsal side: bare; costal cell pilosity on lower side: with numerous setae in distal half and one setal line extending to base; speculum on upper side: bare, widely open below; fore wing disc: moderately thickly pilose; marginal setae: present, short; stigma: subrectangular, small; uncus: short.

Femora: slender; metatibia: gradually widening towards apex; metacoxa pilosity, dorsally: bare.

Petiole in dorsal view: conical, in ventral view: open; gaster in dorsal view: very elongate and acuminate; gastral terga: strongly convex; posterior margin of first gastral tergum: entire; first gastral tergum reaching: one fourth of gaster; tip of hypopygium reaching: almost three fifths of gaster (Fig. 3G); ovipositor sheath: distinctly protruding.

Length and body ratios: Body length: 3.9–4 mm; mesoscutum breadth: 815–829 μm .

Head breadth to height: 1.35–1.39; head breadth to length: 2.08–2.1; head breadth to mesoscutum breadth: 1.18; lower face height to head height: 0.42–0.44; POL to OOL: 1.19–1.2; eye height to breadth: 1.54–1.65; eye distance to height: 1.4–1.46; temple length to eye length: 0.36–0.45; malar space to eye height: 0.45–0.48.

Pedicle plus flagellum length to head breadth: 1.05–1.07; scape length to eye height: 0.81–0.86; scape length to breadth: 6.05–6.39; pedicle length to breadth: 1.51; pedicle length to first funicular segment length: 0.64–0.68; first funicular segment length to breadth: 1.77–1.83; sixth funicular segment length to breadth: 1.06–1.1; first funicular segment breadth to clava breadth: 0.8–0.84; clava length to breadth: 2.09–2.24.

Mesosoma length to mesoscutum breadth: 1.7–1.71; mesoscutum breadth to length: 1.44–1.52; mesoscutum length to scutellum length: 1.13–1.22; propodeum length to scutellum length: 0.59–0.61; plica distance to propodeum length: 1.2–1.36.

Fore wing length to breadth: 2.24–2.3; marginal vein length to stigmal vein length: 1.7–1.78; postmarginal vein length to stigmal vein length: 0.93–0.99.

Metafemur length to breadth: 3.88–4.47; metatibia length to breadth: 7.19–7.29; metatarsus length to metatibia length: 0.8.

Gaster length to breadth: 5.04–5.35; gaster length to mesosoma length: 1.51–1.52.

Description, male. *Color:* Head and mesosoma: bright green to blue-green with metallic luster; setae on head: whitish, inconspicuous, on mesosoma: whitish, inconspicuous; tegula: green; setae on callus of propodeum: whitish.

Scape: fuscous with basal two fifths testaceous; pedicle: fuscous; flagellum: brown.

Fore wing: hyaline; fore wing venation: brownish testaceous; setae on fore wing: fuscous; hind wing: hyaline.

Coxae: green; pro- and mesotrochanter: slightly infusate, metatrochanter: fuscous; pro- and mesofemur: infusate, testaceous in apical third, metafemur: green, testaceous on tips; tibiae: testaceous; protarsus: slightly infusate, meso- and metatarsus: testaceous, apical segments slightly infusate; pretarsi: slightly infusate.

Petiole: dark purplish; gaster: green; gastral terga: basal terga with large dark yellow spot.

Sculpture: Head in frontal view: finely reticulate with moderately high septae; clypeus: striate; area between clypeus and malar sulcus: finely reticulate.

Mesoscutum: finely reticulate, meshes moderately high, areoles small and not enlarged in posterior part of sclerite; scutellum: weakly reticulate, meshes less strong and coarse than on posterior part of mesoscutum; frenum: weakly reticulate, meshes larger than those on scutellum; axilla: reticulate, about as strong as on lateral part of scutellum; prepectus upper triangular area: weakly reticulate; upper mesepimeron: anteriorly smooth, posterior corner distinctly alutaceous; upper mesepisternum: reticulate, about as strong as on mesoscutum; metapleuron: weakly reticulate, less strong than on mesepisternum.

Pro- and mesocoxa: finely alutaceous, metacoxa: finely reticulate.

Median area of propodeum: uniformly reticulate, as strong as on mesoscutum but with smaller meshes; inner corner of anterior plica: with a smooth depression and transverse carinae; nucha: reticulate, as strong as on median area of propodeum; callus of propodeum: weakly reticulate; paraspiracular sulcus: smooth with few transverse costulae.

Petiole in dorsal view: smooth; gastral terga: smooth and shining, second to sixth tergum and syntergum alutaceous.

Shape and structure: Head in frontal view: subtrapezoid; gena in frontal view: buccate; temple in dorsal view: obtuse; occipital carina: absent; torulus position with respect to lower ocular line: distinctly above; lower face in lateral view: rather flat, receding with respect to upper face: weakly, forming an angle of: 35 degrees; scrobe: narrow, moderately deep; malar sulcus: superficial, but traceable; clypeus, anterior margin: widely and shallowly emarginate, medially slightly inclined above anterior edge; gena near mouth: terete; tentorial pit: indistinct; mouth extension: not conspicuously enlarged; mandibular formula: ?3-4 (the mandibles are in the single male concealed, but the mandibular formula is most likely the same as in females).

Antenna (Fig. 3H). Antennal formula: 11263; scape reaching: posterior edge of anterior ocellus; flagellum: filiform; first anellus: strongly transverse; second anellus: strongly transverse; first funicular segment: slightly conical; setae on flagellum: thickly clothed with setae standing out at an angle of 50–60 degrees, length of setae slightly shorter than half the breadth of flagellar segments; number of rows of longitudinal sensilla on first funicular segment: 1, on sixth: 1.

Mesosoma in lateral view: rather flattened; propodeum in lateral view sloping with respect to dorsal plane of mesoscutum and scutellum at an angle of: about 25 degrees; pronotum breadth with respect to mesoscutum breadth: distinctly narrower; pronotum collar: horizontal, well defined, its length with respect to mesoscutum length: one sixth, its anterior margin: slightly elevated edge, medially carinate; pronotum posterior

margin: thin, shiny strip; notaulus: superficial, reaching: two thirds along mesoscutum; scutellum in lateral view: almost flat; scutellum in posterior view: almost flat medially; scutellum posterior margin projection: level of anterior margin of dorsellum; scutellum posterior margin in posterior view: narrowly emarginate in the middle; frenal line: finely indicated, especially on sides; prepectus upper triangular area: ? (the lower part of the prepectus is concealed in the single male, but the character state is likely to be the same as for the females); upper mesepimeron: strongly narrowing below, not reaching base of mesopleuron; anterior plica: present, almost straight in anterior part; posterior plica: present, joining anterior plica; orientation of posterior plicae: almost parallel; median carina of propodeum: anteriorly indicated, effaced posteriorly; nucha: elevated but not clearly differentiated from median area of propodeum; spiracle: oval, size: small, separated from anterior margin of propodeum by: shortest diameter; callus pilosity: relatively sparsely setose; paraspiracular sulcus: narrow and deep.

Fore wing apex with respect to apex of gaster when folded back: not exceeding; basal cell number of setae: with up to 10 setae in distal part; basal setal line: complete, with: 11 setae; cubital setal line: incomplete, with: 4 setae; costal cell pilosity on dorsal side: bare; costal cell pilosity on lower side: with numerous setae in distal half and a complete setal line extending to base; speculum on upper side: bare, widely open below; fore wing disc: moderately thickly pilose; marginal setae: present, short; stigma: subrectangular, small; uncus: short.

Femora: slender; metatibia: gradually widening towards apex; metacoxa pilosity, dorsally: bare.

Petiole in dorsal view: conical, in ventral view: open; gaster in dorsal view: elongate, obtuse; gastral terga: weakly sunken; posterior margin of first gastral tergum: entire; first gastral tergum reaching: slightly less than one third of gaster.

Length and body ratios: Body length: 3.1 mm; mesoscutum breadth: 732 μm .

Head breadth to height: 1.44; head breadth to length: 2.06; head breadth to mesoscutum breadth: 1.17; lower face height to head height: 0.51; POL to OOL: 1.33; eye height to breadth: 1.39; eye distance to height: 1.46; temple length to eye length: 0.38; malar space to eye height: 0.44.

Pedicle plus flagellum length to head breadth: 1.3; scape length to eye height: 0.84; scape length to breadth: 5.42; pedicle length to breadth: 1.28; pedicle length to first funicular segment length: 0.54; first funicular segment length to breadth: 2.08; sixth funicular segment length to breadth: 1.41; first funicular segment breadth to clava breadth: 0.97; clava length to breadth: 3.25.

Mesosoma length to mesoscutum breadth: 1.65; mesoscutum breadth to length: 1.44; mesoscutum length to scutellum length: 1.23; propodeum length to scutellum length: 0.64; plica distance to propodeum length: 1.1.

Fore wing length to breadth: 2.11; marginal vein length to stigmal vein length: 1.75; postmarginal vein length to stigmal vein length: 0.91.

Metafemur length to breadth: 4.3; metatibia length to breadth: 7.04; metatarsus length to metatibia length: 0.79.

Gaster length to breadth: 3.38; gaster length to mesosoma length: 1.19.

Comment. The dorsoventrally compressed mesosoma and the shape of the propodeum allowed an easy association of the females with the male even though they were collected in separate localities (about 160 km as the crow flies).

Diagnosis. *P. janstai* sp. n. is distinguished from all known species of *Pteromalus* species by the following combination of characters: mesosoma strongly flattened; female gaster elongate, laterally strongly compressed, more than 5 times as long as broad.

The female keys out in Graham (1969) via couplets 1, 2, 7–9, 11, 12, 14, 49, 52–56, 58, 59, 60, 62 to couplet 63, where it fits neither of the two species, *P. dispar* (see below) and “H. sp. indet. C”. The male keys out via couplets 1–3, 5, 7, 10, 11, 14–19, 40, 44, 50, 52, 53, 54, 55, 56, 57, and 59 where both options don’t match well.

Most similar are the following species but they differ – among many other characters mentioned in the description – by a rather more strongly bent mesosoma and a much less elongate female gaster:

P. cyniphidis (Linnaeus, 1758) (syn. *P. capreae* (Linnaeus, 1761)), *P. dispar* (Curtis, 1827), *P. dolichurus* (Thomson, 1878), *P. fasciatus* (Thomson, 1878), *P. pontaniae* (Askew, 1985) and *P. tereus* Walker, 1839. Source of information, beside the keys of Graham (1969) and Askew (1995): *P. dispar* 2 ♀ 1 ♂ from Denmark and Switzerland in NMBE (Baur 2542–2544); specimens compared with material identified by Graham and Bouček as well as lectotypes of synonyms of *P. dispar*, that is, ♀ *P. mesochlorus* Walker, 1835, ♀ *P. saravus* Walker, 1845, ♀ *P. basalis* Walker, 1835, and ♂ *P. cabarnos* Walker, 1839 in BMNH. Lectotype ♀ of *P. dolichurus* and of *P. fasciatus* in LUZM, and lectotype ♂ of *P. tereus* in BMNH. Furthermore, Veli Vikberg kindly compared photographs of *P. janstai* sp. n. with specimens of *P. cyniphidis* in VVC. Some of these specimens belong to the same reared series from which the neotype ♂ of *P. cyniphidis* was selected by Vikberg and Askew (2006). Vikberg (pers. comm.) confirmed that the two species are clearly separated by the mentioned characters.

Etymology. *Pteromalus janstai* sp. n. is named after Petr Jansta, who collected the female specimens. The name “janstai” is a noun in the genitive case and need not agree in gender with the generic name.

Biology. Host unknown. The females of *Pteromalus janstai* sp. n. were swept on some isolated Larch trees (*Larix decidua*) in an Alpine meadow. The male was swept in a similar habitat, but it cannot be determined whether it was swept from trees.

Discussion

Although the two new species are clearly placed within the genus *Pteromalus*, their morphology and some life history traits are remarkable and merit discussion. The most notable morphological feature concerns the metatibia of *P. briani* sp. n. Its abrupt expansion in proximal half is unique, not only within the genus but also within the family Pteromalidae and – as far as I can judge – the entire Chalcidoidea. Expansions of tibiae are known from some Pteromalidae, but here they look quite different. For instance, in *Spathopus* (Pireninae) the metatibia is conspicuously but very uniformly

swollen only in males. Furthermore, the mesotibia of males of some *Mesopolobus*, *Pegopus*, and *Spaniopus* (Pteromalinae) differs in that the expansion is accompanied by a flattening or at least lateral compression of the tibia (Graham 1969, Bouček 1972). In the case of *Mesopolobus* the mesotibia also shows some special processes and coloration (Graham 1969, Mitroiu 2010). While such an ornamentation may play a role in courtship or during mating (Assem 1974; reviewed by Wehling 1986), a possible behavioral function of the expanded metatibia in *P. briani* sp. n. remains unknown.

The expansion of the metatibia is a very rare phenomenon in parasitoid wasps. Quicke and Falco (1998) have reported for *Vipio moneilemae* Gahan, 1930 a putative pheromone gland associated modification, which they assumed is unique within the Braconidae. Here, the swelling is present only in males and the dorsal side of the tibia has a longitudinal groove bordered by lateral ridges. In *P. briani* sp. n., the expansion is the same in both sexes and is not accompanied by a structural modification of the integument. However, only the use of scanning electron microscopy and histological serial sections of fresh material could possibly reveal the structure and function of this particular character. Special attention should be paid to the presence of metatibial glands, such as those found in some aculeate Hymenoptera (Hölldobler et al. 1996).

The rearing of the host larva under protected condition suggests that *P. briani* sp. n. develops as a gregarious, koinobiont endoparasitoid, since the host was allowed to continue its development after oviposition in an early larval stage and was only killed in the pupal stage. This is in contrast to some related gregarious endoparasitoids of Lepidoptera pupa. For instance, *P. puparum*, a widespread parasitoid of Papilionidae and Pieridae, immobilizes the pupal stage of its host on which the development also takes place (Takagi 1985, 1986, 1987). This species thus shows an idiobiont life history strategy (Fortuna et al. 2012).

The other species, *P. janstai* sp. n., is unique within *Pteromalus* because of its flattened mesosoma. This trait is reported from species of a number of other genera of Pteromalidae, for instance *Macroglenes* (Pireninae), *Anogmus*, *Guancheria*, *Monoksa*, *Pachyneuron*, *Platyptermalus*, *Psilonotus*, *Rakosina*, *Syntomopus*, and *Zdenekiana* (Pteromalinae) (Graham 1969, Bouček and Rasplus 1991), but also from various other families, e.g., some species of *Baryscapus* and *Pronotalia* (Eulophidae) (Graham 1987, 1991). In certain families of Chalcidoidea, like the Encyrtidae and Aphelinidae, a flattened body characterizes most species. The function of the flattening remains unclear in a particular case. In Pteromalinae, species of genera, which are more closely related to *Pteromalus* (e.g., *Anogmus*, *Psilonotus*), are often parasitoids of gall midges (Diptera: Cecidomyiidae) (Noyes 2015), and perhaps the trait could be indicative for the unknown host of *P. janstai* sp. n.

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Appendix

Overview of 41 measurements (in μm) of *Pteromalus brianii* sp. n. and *P. janstai* sp. n., showing minimum, maximum, mean, and standard deviation (except for *P. janstai* male with n = 1). For character name and definition, see Table 1.

Character	<i>P. brianii</i> , females, n = 11					<i>P. brianii</i> , males, n = 2					<i>P. janstai</i> , females, n = 2					<i>P. janstai</i> , male, n = 1	
	MIN	MAX	MEAN	SD		MIN	MAX	MEAN	SD		MIN	MAX	MEAN	SD		VALUE	
ant.l	577	815	749.2	73.72		743	743	743.4	0.01		1033	1035	1033.9	0.93		1116	
clv.b	65	90	77.2	6.88		64	75	69.3	8.23		94	97	95.4	1.64		72	
clv.l	168	197	184	8.31		183	199	190.9	10.9		201	211	206.1	6.58		235	
eye.b	237	304	280.4	28.08		272	274	273.4	1.47		291	299	295.2	5.42		282	
eye.d	559	739	673.6	79.6		631	631	631.2	0.34		668	674	671.2	4.3		572	
eye.h	315	408	371.3	38.59		355	355	355	0.01		459	481	470	15.25		393	
eye.l	231	301	276	28.51		262	267	264.9	3.57		291	297	293.9	4.8		280	
fl3.b	68	84	74	4.86		62	69	65.4	4.82		77	79	78.4	1.5		70	
fl3.l	63	110	88.4	15.64		87	97	92.3	6.99		140	141	140.7	0.55		146	
fl8.b	65	83	75.5	5.85		64	65	64.7	1.03		81	85	83	3.15		71	
fl8.l	63	82	73.3	6.22		65	69	67	2.61		85	94	89.8	6.18		101	
fm3.b	115	155	138.6	17		128	137	132.6	5.85		151	175	163.1	17.35		134	
fm3.l	496	659	581.7	70.88		578	594	586.2	11.28		675	681	677.7	3.99		577	
fwi.b	794	1054	961	110.3		903	905	903.8	1.32		1118	1142	1130.1	17.32		928	
fwi.l	1721	2214	2002.6	208.5		1813	1825	1818.8	8.59		2564	2568	2565.9	2.79		1963	
gst.b	622	875	765.9	91.68		650	673	661.4	16.26		403	415	409.1	8.2		424	
gst.l	932	1111	1045.7	46.68		1113	1133	1123.1	14.28		2090	2157	2123.4	47.45		1435	
hea.b	789	1036	946.4	102.7		886	889	887.8	1.96		965	981	972.8	11.33		859	
hea.h	575	740	688.1	60.65		604	610	607.1	3.76		694	727	710.4	23.5		598	
hea.l	380	507	461.5	53.02		436	440	437.8	2.81		459	472	465.5	9.54		416	
lofh	333	423	388.8	36.03		355	367	361.1	8.45		303	303	303	0.5		304	
mav.l	349	475	419.8	48.08		362	381	371.4	12.89		533	557	545	16.8		433	
msc.b	591	806	731	87.75		682	684	683.3	1.43		815	829	822	9.83		732	

Character	<i>P. briani</i> , females, n = 11					<i>P. briani</i> , males, n = 2					<i>P. janstai</i> , females, n = 2				<i>P. janstai</i> , male, n = 1	
	MIN	MAX	MEAN	SD		MIN	MAX	MEAN	SD		MIN	MAX	MEAN	SD	VALUE	VALUE
msc.l	363	511	451.8	61.49		456	462	458.9	4.39		536	577	556.7	29.11	509	509
msh.l	222	299	262.4	27.02		205	217	211.1	8.98		214	219	216.4	3.43	171	171
mss.l	940	1279	1140.1	136.82		1118	1118	1118.3	0.32		1384	1419	1401.7	24.4	1208	1208
ool.l	170	236	211.3	25.8		187	189	187.9	1.93		185	188	186.3	2.03	153	153
pdl.b	53	67	62.5	5.33		61	61	60.9	0.52		59	63	61.1	2.45	61	61
pdl.l	69	97	86.5	10.33		83	84	83.4	0.99		90	95	92.2	3.68	79	79
plc.d	256	358	314.9	40.58		299	318	308.5	13.17		345	380	362.6	25.06	294	294
pmv.l	301	414	364.9	38.59		320	339	329.3	13.34		518	530	524.2	8.42	392	392
pol.l	142	186	167.2	17.67		168	179	173.5	7.83		222	224	222.9	0.97	202	202
ppd.l	202	286	251.1	30.68		228	248	238.3	14.05		279	288	283.4	6.24	267	267
sqp.b	59	78	69.2	6.93		67	71	69	3.07		61	65	62.8	3.12	61	61
scp.l	320	412	375.9	37.81		344	348	346	2.7		387	393	390.2	4.27	329	329
scr.l	346	463	421.5	51.33		413	422	417.5	6.71		474	475	474.3	0.55	414	414
str.l	215	293	263.8	32.12		244	260	251.9	11.59		314	314	313.9	0.41	248	248
ta3.l	400	529	485.5	48.7		468	478	473.1	7.13		672	675	673.4	2.51	572	572
tb3.b	78	110	98.5	10.45		88	91	89.6	2.31		115	117	116.1	0.94	102	102
tb3.l	586	778	696.5	80.08		646	653	649.8	4.77		840	842	840.9	0.83	721	721
tmp.l	84	130	108.8	18.49		103	113	107.8	6.77		107	130	118.6	15.93	107	107

A new species of nectar-feeding bat, genus *Lonchophylla*, from the Caatinga of Brazil (Chiroptera, Phyllostomidae)

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Abstract

We describe *Lonchophylla inexpectata* **sp. n.** from the Caatinga of Brazil. This new species can be distinguished from all known species of *Lonchophylla* that occur in Brazil by dental traits, cranial size, and fur colour. Specimens of *L. inexpectata* have been misidentified as *L. mordax*; but *L. inexpectata* is a pale-venter species, similar in external appearance to *L. dekeyseri*. We have found *L. inexpectata* in the Caatinga of North-eastern Brazil; *L. mordax* along the eastern border of the Caatinga and in the Atlantic Forest–Caatinga ecotone in North-eastern Brazil; and *L. dekeyseri* in the Cerrado of Mid-western Brazil, in the Brazilian Cerrado–Caatinga ecotone, and as far west as the Cerrado of Bolivia.

Keywords

Atlantic Forest, Caatinga, Cerrado, *Lonchophylla inexpectata*, *Lonchophylla dekeyseri*, *Lonchophylla mordax*, North-eastern Brazil

Introduction

Lonchophylla Thomas, 1903 (Phyllostomidae) comprises 12 species of nectar-feeding bats restricted to the Neotropics (Griffiths and Gardner 2008, Parlos et al. 2014). Parlos et al. (2014) revised the Lonchophyllinae and established *Hsunycteris* as a new genus to include the smaller species formerly known as the *Lonchophylla thomasi* complex.

However, their revision did not include the Brazilian species *L. mordax* Thomas, 1903, *L. bokermanni* Sazima et al., 1978, *L. dekeyseri* Taddei et al., 1983, and *L. peracchii* Dias et al., 2013. During our assessment of these Brazilian species we found evidence of another new taxon based on specimens from the Brazilian Caatinga we found in museum collections. Some specimens of this previously undescribed species have been misidentified as *L. mordax* for more than a century.

Lonchophylla mordax was described from Lamarão, Bahia (Thomas 1903), with subsequent records ascribed to specimens from other localities in Northern ([N] Handley 1967, Piccinini 1974, Koopman 1981), North-eastern ([NE] Vieira 1955, Sazima et al. 1978, 1983, Mares et al. 1981, Willig 1983, Astúa and Guerra 2008), Mid-western ([MW] Peracchi et al. 2011), and South-eastern Brazil ([SE] Pereira-Barreto et al. 1968, Taddei et al. 1978, Pedro and Passos 1995, Esbérard et al. 2006, Dias et al. 2002, Esbérard 2003). Handley (1966) synonymized *L. concava* Goldman, 1914 under *L. mordax*, thus enlarging its geographic distribution westward into western Colombia and Ecuador, and northwestward into Costa Rica. This arrangement was rejected by Albuja and Gardner (2005), who recognized *L. concava* as a distinct species. Based on the records available, bat biologists have assumed that *L. mordax* was restricted to eastern South America, with records from the Amazon Forest of N Brazil, eastward to xeric habitats in NE Brazil, and southward to the Atlantic Forest of SE Brazil, including transitional areas between these last two biomes (see Griffiths and Gardner 2008, Peracchi et al. 2011).

Thomas's (1903) description of *L. mordax* is based on eight specimens from Lamarão, Bahia collected by Alphonse Robert in 1903. Lamarão is in the *agreste* sub region of NE Brazil, which is a narrow transition zone between the coastal Atlantic Forest to the east and the semiarid Caatinga on the west (Prado 2003). According to local residents, the vegetation in Lamarão and adjacent areas during the first half of the 20th century was dominated by tall forests, which is characteristic of the transitional vegetation between the Atlantic Forest and Caatinga. Throughout the last century, land-use practices have converted the region into a semi-arid environment that resembles caatinga habitats. The type material of *L. mordax*, originally deposited in the Natural History Museum, London (BM), includes the holotype (BM 1903.9.5.34) and seven paratypes. One of the paratypes was sent to the Smithsonian's National Museum of Natural History, Washington, DC (USNM 123392). A few years after Thomas described *L. mordax*, a series of *Lonchophylla* were collected in Barra, Bahia by Ernest Garbe and Robert H. Becker in 1908 and 1914, respectively. Barra, Bahia is in the *sertão* sub region (450 to 500 km west of Lamarão), a semi-arid environment that is characteristic of the Caatinga (Prado 2003). According to their labels, Garbe's and Becker's specimens from Barra were identified as *L. mordax* and either originally deposited or subsequently sent to museums in Brazil and United States of America. This material has been the basis for several subsequent published accounts on *L. mordax* (e.g., Lima 1926: 36, Vieira 1942: 321). As with the paratype of *L. mordax* (USNM 123392), one of those specimens collected by Garbe is housed in the Smithsonian's National Museum of Natural History (USNM 238008). After comparing skins and skulls of Garbe's and Thomas's USNM specimens from Barra (Caatinga, USNM 238008) and Lamarão (Atlantic Forest/Caatinga, USNM 123392),

we determined that the pale-venter *Lonchophylla* from Barra could be distinguished from *L. mordax*, and represented an undescribed species. Among distinctive traits distinguishing the Barra specimen from *L. mordax* are the paler colour of the ventral fur and the smaller skull that has a narrower and more delicate rostrum.

To test this hypothesis and further understand the geographic distribution of Brazilian species, we examined series of *Lonchophylla* from localities in the Caatinga, Cerrado, and Atlantic Forest, as well as from transitional zones between these habitats. The material used in our comparisons represents all *Lonchophylla* species known to occur in Brazil. During this process we found additional features that support our hypothesis that the pale-venter *Lonchophylla* from the Caatinga represents a new species, which we describe below.

Methods

The material we used in the comparisons includes series of *Lonchophylla* from the Caatinga of NE Brazil (Bahia [municipalities of Andaraí, Barra, Buíque], Ceará, Pernambuco, Piauí, Sergipe [Grota do Angico]); Cerrado of Bolivia (Santa Cruz) and Mid-western Brazil (Distrito Federal, Goiás, Mato Grosso do Sul); Atlantic Forest of SE Brazil (Espírito Santo, Rio de Janeiro); and the Atlantic Forest–Caatinga ecotone in NE Brazil (Bahia [Lamarão], Sergipe [Itabaiana]). This material includes representatives of all currently recognized Brazilian species of *Lonchophylla*, and includes primary and secondary types of *L. bokermanni* (6 specimens from the type series), *L. dekeyseri* (holotype and one paratype), *L. mordax* (holotype and one paratype), and *L. peracchii* (holotype and two paratypes). Vouchers are preserved in the American Museum of Natural History (AMNH, New York, USA); Carnegie Museum of Natural History (CM, Pittsburgh, USA); Museu Nacional (MN, Rio de Janeiro, Brazil); Muséum d'histoire naturelle (MHNG, Geneva, Switzerland); Natural History Museum (BM, London, England); Smithsonian's National Museum of Natural History (USNM, Washington DC, USA); Universidade Estadual Paulista Júlio de Mesquita Filho (DZSJRP, São José do Rio Preto, Brazil); Universidade Federal do Espírito Santo (UFES, Espírito Santo, Brazil); Universidade Federal Rural do Rio de Janeiro (ALP, LMD, Seropédica, Brazil). A complete list of specimens examined is in the Appendix. Most geographical coordinates follow Gardner's (2008) gazetteer of marginal localities.

Measurements in this report are from adults, and are either in millimetres (mm) or grams ([g] body mass). The body mass was recorded from skin labels. Other dimensions include: the forearm length (FA), from the elbow to the distal end of the forearm including carpals, measured with the wing partially folded; greatest length of skull (GLS), from the posteriormost point of the occiput to the tips of the upper inner incisors; condylo-incisive length (CIL), from the line connecting the occipital condyles to the tips of the upper inner incisors; basal length (BAL), from the anterior margin of the foramen magnum to the tips of the upper inner incisors; maxillary toothrow length (MTL), from the anterior surface of the upper canine, including the cingulum, to the posterior surface of M3; molariform toothrow length (M1M3), from the crown of M1 to

the crown of M3; breadth across canines (BAC), greatest breadth across outer surface of the crowns of upper canines, including cingulae; breadth across molars (BAM), greatest breadth across outer edges of the crowns of upper molars; postorbital breadth (POB), least breadth across frontals posterior to the postorbital bulges; braincase breadth (BCB), greatest breadth of the globular part of the braincase; mastoid breadth (MAB), greatest breadth across the mastoid region; mandibular length (MAL), from the mandibular symphysis to the condyloid process; and the mandibular tooththrow length (MAN), from the anterior crown of the lower canine, including cingulum, to the posterior crown of m3. Craniodental measurements were taken under binocular dissection microscopes with low magnification (usually 6×). Dimensions were taken by only one of us, using digital callipers accurate to 0.02 mm. Measurements were recorded and analysed to the nearest 0.01 mm, but values were rounded off to 0.1 mm throughout the text because this is the smallest unit that allows accurate repeatability with callipers (Voss et al. 2013). Descriptive statistics (mean and range) were calculated for all dimensions. The statistical significance of differences among samples was assessed by single analyses of variance (one-way ANOVA). This statistics was performed in PAST (Hamer et al. 2001).

Discriminant Function Analysis (DFA) was used to compare taxa. For the analysis, we selected a subset of the cranial dimensions (GLS, CIL, MAB, BCB, POB, BAC, BAM, M1M3, MTL, MAL) to represent different axes of length and width of the skull. As multivariate procedures require complete datasets, missing values (< 3% of the total dataset) were substituted by means. Measurements were transformed to natural logarithms and the covariance matrices were computed considering all variables. DFA was performed in SPSS.

Nomenclature of tooth morphology follows Phillips (1971). Capitalized colour nomenclature follows Ridgway (1912).

Taxonomy

Lonchophylla inexpectata sp. n.

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Figures 1, 2, 4, 5; Table 1

Lonchophylla mordax: Lima 1926: 76; not *Lonchophylla mordax* Thomas, 1903.

Lonchophylla mordax: Vieira 1942: 321; not *Lonchophylla mordax* Thomas, 1903.

Lonchophylla mordax: Taddei, Vizotto and Sazima 1983; not *Lonchophylla mordax* Thomas, 1903.

Lonchophylla dekeyseri: Woodman and Timm 2006: 450; part, not *Lonchophylla dekeyseri* Taddei, Vizotto & Sazima, 1983.

Lonchophylla mordax: Woodman and Timm 2006: 475; part, not *Lonchophylla mordax* Thomas, 1903.

Lonchophylla dekeyseri: Woodman 2007. Part, not *Lonchophylla dekeyseri* Taddei, Vizotto & Sazima, 1983.



Figure 1. Dorsal **A** and ventral **B** pelage of the holotype of *L. inexpectata* (USNM 238008). Scale bar: 10 mm.

Holotype. An adult male, USNM 238008, with skin and skull (Figures 1, 2), including mandible, collected by E. Garbe at Barra (12°42'S, 41°33'W), Bahia, Brazil, on January 1908. Skull and mandible are in good condition except for the minimally damaged anteriormost portion of the foramen magnum. The body is prepared as dry skin. Woodman and Timm (2006: 450) described USNM 238008 as a faded skin, but after comparison of its pelage colour with those from other specimens, only mem-

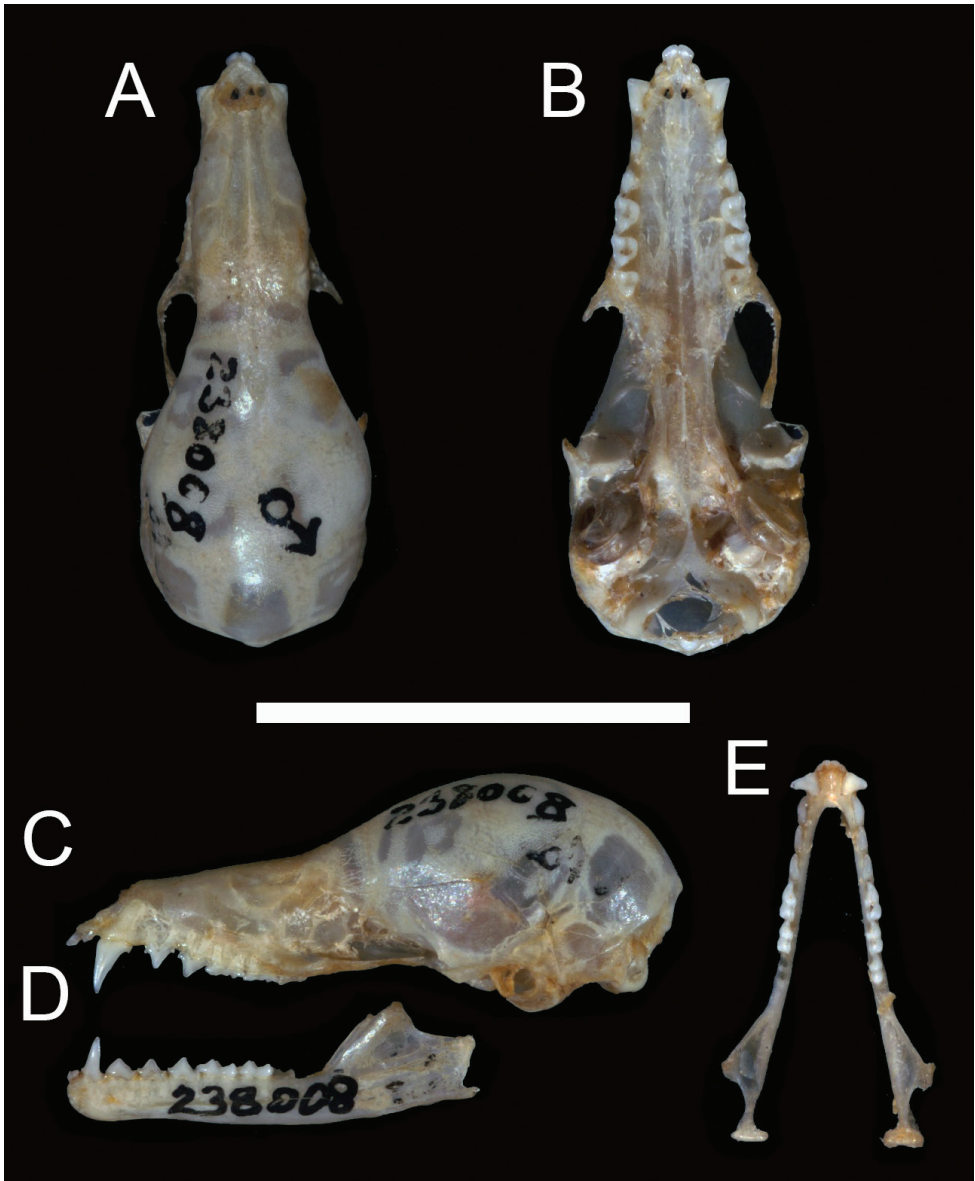


Figure 2. Dorsal **A**, ventral **B**, and lateral **C** views of the cranium, and lateral **D** and dorsal **E** views of the mandible of the holotype of *L. inexpectata* (USNM 238008). Scale bar: 15 mm.

branes seem to be faded. External and craniodental measurements for the holotype and paratypes are in Table 1.

Paratypes. The paratype series comprises 46 vouchers. Three paratypes are from the type locality in Barra, Bahia (AMNH 235608, FMNH 21077, 21078), and were collected by R. H. Becker in 1914. One is from Serra do Catimbau, Buíque, Pernambuco (FMNH 137414; 08°37'S, 37°09'W [coordinates for Catimbau National Park]),

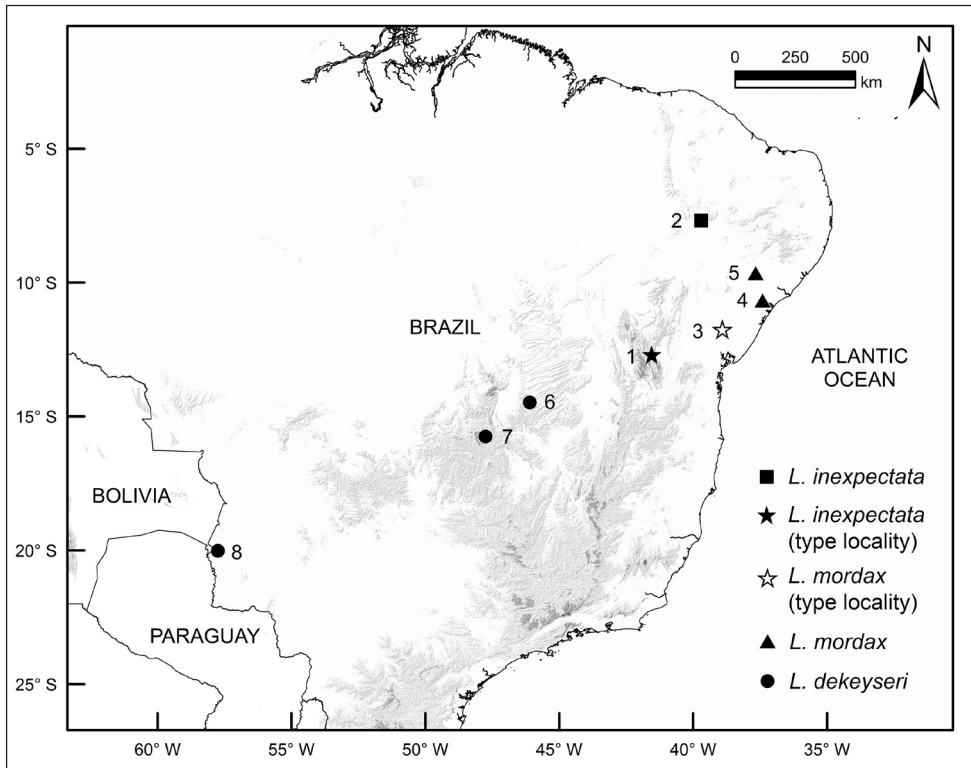


Figure 3. Map of part of South America showing the geographic distribution of samples we confirmed as *L. inexpectata* (black star [type locality] and square), *L. dekeyseri* (circles), and *L. mordax* (white star [type locality] and triangles). Localities 1, 2, 5 are in the Caatinga; localities 3, 4 are in the Caatinga–Atlantic Forest ecotone; and localities 6–8 are in the Cerrado.

and was collected by D. Guerra in 1970. Thirty-eight vouchers are from 17 km south of Exu, Pernambuco (CM 99413–99450; 07°41'S, 39°32'W), elevation ca. 480 m, and were collected by M. R. Willig in 1976. Paratypes from Barra (AMNH 235608, FMNH 21077, 21078), and Buíque (FMNH 137414) are in spirits, others are prepared as dry skin.

Other specimen. One additional specimen (ALP 3686) from the Caatinga of Andaraí, Bahia may represent *L. inexpectata*. The specimen is preserved in spirit, and the dentition is partially worn, preventing its unambiguous identification.

Distribution. *Lonchophylla inexpectata* occurs in the Caatinga of North-eastern (NE) Brazil, with confirmed records from Pernambuco (NE), and Bahia (NE) (Figure 3).

Diagnosis. *Lonchophylla inexpectata* can be distinguished from all South American species that occur east of the Andes by the following set of traits: presence of a lingual cusp in the P4, absence of a lingual cusp in the P3, absence of a deep longitudinal groove in the posterior face of the upper canine, proximal portion of the dorsal surface of the forearm not furred, and ventral fur pale.

Table 1. Body mass (g) and external and skull measurements (mm) of the holotype (USNM 238008) of *L. inexpectata*, and descriptive statistics for *L. inexpectata* (from Caatinga [type series]), *L. dekeyseri* (from Cerrado), and *L. mordax* (from Caatinga and Caatinga–Atlantic Forest ecotone).

	<i>L. inexpectata</i>	<i>L. inexpectata</i>	<i>L. dekeyseri</i>	<i>L. mordax</i>
	Holotype	Mean	Mean	Mean
	USNM 238008	(Min.–Max.) <i>N</i>	(Min.–Max.) <i>N</i>	(Min.–Max.) <i>N</i>
Body mass	–	8.2	–	–
		(7.0–9.5) 15		
FA	33.7	34.6	36.9	35.8
		(32.3–36.4) 62	(35.5–38.0) 15	(34.5–37.4) 32
GLS	22.3	23.1	22.4***	23.6**
		(22.0–23.9) 38	(22.0–22.7) 16	(22.6–24.5) 24
CIL	20.8	21.7	21.0***	22.2***
		(20.5–22.6) 37	(20.4–21.4) 16	(21.3–23.2) 24
BAL	19.1	19.8	19.1***	20.2**
		(18.7–20.7) 36	(18.5–19.6) 16	(19.6–20.8) 20
MTL	7.6	7.8	7.6**	8.0***
		(7.4–8.2) 45	(7.3–7.9) 16	(7.6–8.4) 26
M1M3	–	3.3	3.4*	3.5***
		(3.1–3.6) 40	(3.3–3.6) 14	(3.3–3.7) 30
BAC	3.4	3.6	3.7**	3.7*
		(3.3–3.8) 44	(3.4–3.9) 16	(3.5–4.1) 27
BAM	4.8	5.1	5.1	5.3*
		(4.8–5.5) 43	(4.9–5.3) 16	(4.7–5.7) 26
POB	4.1	4.3	4.5***	4.3
		(4.1–4.7) 46	(4.2–4.6) 16	(4.0–4.6) 27
BCB	7.9	8.3	8.4*	8.5
		(7.9–8.6) 46	(8.0–8.7) 16	(8.1–8.9) 27
MAB	8.5	9.0	9.1***	9.3*
		(8.5–9.6) 44	(8.8–9.4) 16	(8.9–9.7) 27
MAL	14.9	15.6	15.1***	16.1***
		(14.1–16.3) 44	(14.8–15.4) 16	(15.5–17.0) 25
MAN	8.0	8.2	8.1*	8.4***
		(7.8–8.5) 43	(7.7–8.4) 16	(7.9–8.9) 25

N = sample size (adults only, males and females combined). See "Methods" for variable abbreviations and Appendix for localities of specimens used in comparisons. One-way ANOVA for skull measurements is comparing *L. inexpectata* with *L. dekeyseri* and *L. mordax*: * *p* ≤ 0.05, ** *p* ≤ 0.01, *** *p* ≤ 0.001.

Description and comparisons. Like other *Lonchophylla*, the dental formula of *L. inexpectata* is 2/2, 1/1, 2/3, 3/3 = 34. *Lonchophylla inexpectata*, *L. dekeyseri* and *L. bokermanni* are the three pale-venter Brazilian species of the genus, whereas *L. mordax* and *L. peracchii* have pale-brown ventral pelage. We did not find evidence of *L. bokermanni* and *L. peracchii* in sympatry with *L. inexpectata*—*L. bokermanni* is restricted to a small area in the Serra do Espinhaço, Cerrado of Minas Gerais; and

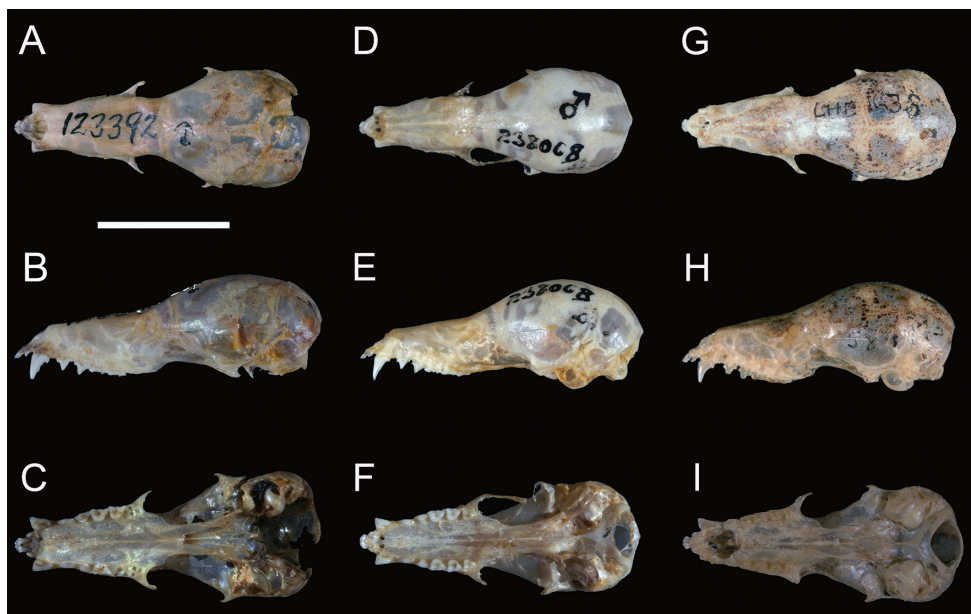


Figure 4. Dorsal (above), lateral (middle), and ventral (below) views of the skull of *L. mordax* (A–C [USNM 123392, paratype]), *L. inexpectata* (D–F [USNM 238008, holotype]), and *L. dekeyseri* (G–I [USNM 584472]). Scale bar: 10 mm.

L. peracchii occurs in the Atlantic Forest, from Espírito Santo southward to São Paulo. *Lonchophylla inexpectata* can be distinguished from these two species by the presence of a well-developed lingual cusp in the P4, with lingual root in the median portion of the tooth; absence of a groove along the anterior surface of the upper canines; and proximal portion of the dorsal surface of the forearm not covered with fur.

Based on the samples we have available, *L. inexpectata* resembles *L. dekeyseri* in the pale ventral fur, and *L. mordax* in the dental morphology. These three species overlap partially in external and cranial size, but in general, cranial measurements for *L. inexpectata* average significantly larger than those for *L. dekeyseri* and smaller than those for *L. mordax* (Table 1).

Lonchophylla mordax has been reported in the literature as a pale-venter species (e.g., Lima 1926, Vieira 1942, Taddei et al. 1983, Nogueira et al. 2007), and subsequent to the description of *L. dekeyseri*, these taxa have been considered the two pale-venter species from NE Brazil (see Taddei et al. 1983, Nogueira et al. 2007, Dias et al. 2013). However, after examining part of the type series of *L. mordax* (BM 1903.9.5.34 [holotype], USNM 123392 [paratype]), along with one other specimen from the same locality of the type series (MHNG 667.13 [identified as *L. mordax* by Thomas]), and samples from a nearby locality having similar habitat (Itabaiana, Sergipe)—whose external and skull morphology fit with those of the type series of *L. mordax* (ALP 8768–8770, 8812–8819)—we concluded that *L. mordax* has a light-brown ventral pelage, which is consistently darker than the paler ventral pelage of the type material of *L.*

dekeyseri and other samples of this species. The ventral pelage of specimens from Barra, Bahia (*L. inexpectata*) is similar to that of *L. dekeyseri*. Under “historical remarks” we discuss the reasons for previous assignments of pale-venter samples from the Caatinga of NE Brazil (= *L. inexpectata*) to *L. mordax*.

Lonchophylla inexpectata averages significantly smaller than *L. mordax* in all cranial dimensions except in POB and BCB (Table 1, Figure 4). This is particularly notable in the length of the mandible (MAL $\bar{x}$ = 15.6 mm, range [R] = 14.1–16.3 mm [*inexpectata*] versus $\bar{x}$ = 16.1 mm, R = 15.5–17.0 mm [*mordax*]). *L. inexpectata* can also be distinguished by the ventral pelage, which varies from whitish (e.g., USNM 238008, CM 99415) to pale greyish (near Avelaneous [e.g., CM 99432, 99437]), but near Buffy Brown in *L. mordax* (e.g., BM 1903.9.5.34, USNM 123392). The throat and the posterior region of the belly are consistently paler, tending to whitish, in *L. inexpectata* (Figure 5).

Lonchophylla inexpectata resembles *L. dekeyseri* in the pelage colour, but these species can be distinguished by qualitative and quantitative cranial characteristics. *Lonchophylla inexpectata* is significantly larger than *L. dekeyseri* in all length measurements of skull and rostrum (GLS, CIL, BAL, MTL, M1M3, MAL, MAN), but *L. dekeyseri* averages slightly larger in those measurements of the width of skull and rostrum (BAC, POB, BCB, MAB), indicating a longer but narrower skull in *L. inexpectata* (Table 1). *L. inexpectata* can be distinguished from *L. dekeyseri* by the narrower first upper premolar (P3) in occlusal view, with lingual lobe absent or obsolete (in contrast with the usually more robust P3, which has a small or moderately developed inner lobe in *dekeyseri* [Figure 6]); absence of a deep longitudinal groove in the posterior surface of the canine; narrower and uninflated rostrum, with more widely projecting lacrimals (wider and more inflated rostrum, and lacrimal region almost indistinguishable in *dekeyseri*); upper molars (M1 and M2) with low crowns in lateral view (molars with higher crowns in *dekeyseri*); parastyle of M1 projecting labially over the posterior labial margin of the last upper premolar (P4); mesostyle of M1 shorter; metastyle of M1 well developed (reduced or absent in *dekeyseri* [Figure 6]); parastyle of M2 well developed but slender (well developed and more rounded in *dekeyseri*); mesostyle of M2 shorter; metastyle of M2 distinct, moderate or well developed (reduced or absent in *dekeyseri*).

Multivariate analysis. To test the results obtained from the morphological analyses, we performed a discriminant function analysis including samples we confidently assigned to *L. dekeyseri* (three groups from the Cerrado of Mid-western Brazil), *L. inexpectata* (two groups from the Caatinga of NE Brazil), and *L. mordax* (one group from the Caatinga of NE Brazil, and one group from the Atlantic Forest–Caatinga ecotone in NE Brazil). The first two discriminant functions (DF1, DF2) summarized 47% and 40% of the total variation, respectively (Table 2). All samples grouped as expected, confirming the cohesive pattern retrieved from the morphological analysis. Centroids for samples assigned to *L. inexpectata* were distinct from those of *L. dekeyseri* and *L. mordax* across the first two axes, and only a few scores of *L. inexpectata* are within the dispersal cloud of *L. mordax* (Figure 7). The three species overlap partially across the first axis, but *L. inexpectata* distinguishes from *L. dekeyseri* and *L. mordax*

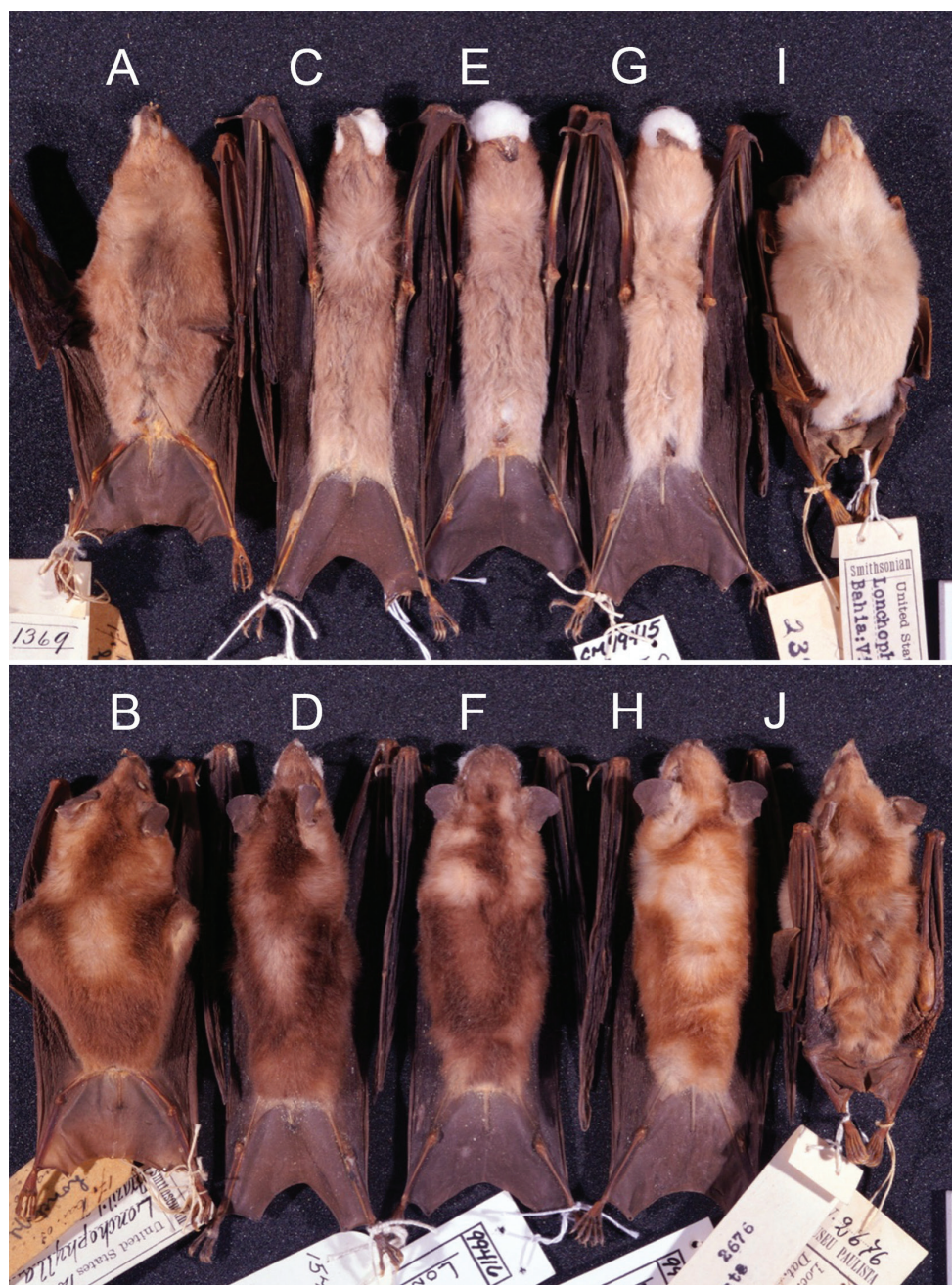


Figure 5. Ventral (above) and dorsal (below) pelage colours of *L. mordax* **A, B** (USNM 123392, paratype), and *L. inexpectata* **C, D** (CM 99432) **E, F** (CM 99416) **G, H** (CM 99415) **I, J** (USNM 238008, holotype).

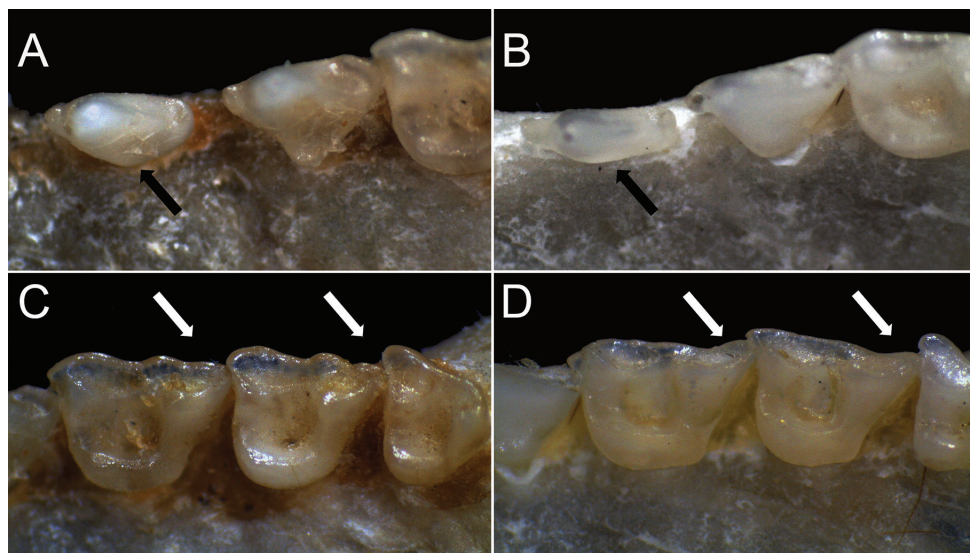


Figure 6. Upper dentition of *L. dekeyseri* **A, C** (LDM 3185) and *L. mordax* **B, D** (ALP 6149). **A, B** Moderate inner lobe in the first upper premolar (P3) of *L. dekeyseri* **A** contrasting with the lingual lobe of P3 absent or very reduced in *L. mordax* **B** (similar condition observed in *L. inexpectata*) **C, D** metastyles of M1 and M2 reduced or absent in *dekeyseri* **C** contrasting with the metastyles well developed and distinct in *L. inexpectata* and *L. mordax* **D**.

Table 2. Vector correlation coefficients (loadings) between original variables and discriminant functions (DF1, DF2) for samples of *L. dekeyseri*, *L. inexpectata* and *L. mordax*.

	DF1	DF2
Characters	46.5%	40.4%
GLS	0.724	0.021
CIL	0.706	-0.130
MAB	0.240	0.388
BCB	0.268	0.413
POB	-0.149	0.261
BAC	0.117	0.336
BAM	0.413	0.193
M1M3	0.226	0.477
MTL	0.523	0.151
MAL	0.645	0.100

along the second axis. Scores for *L. inexpectata* had very low positive to high negative values along the DF2, whereas those for *L. dekeyseri* and *L. mordax* have low negative to high positive values along this axis.

Etymology. The name “*inexpectata*” is Latin for “unexpected”, in allusion to the unexpected (at least for the authors) new taxonomic status of pale-venter populations of *Lonchophylla* from the Caatinga of North-eastern Brazil.

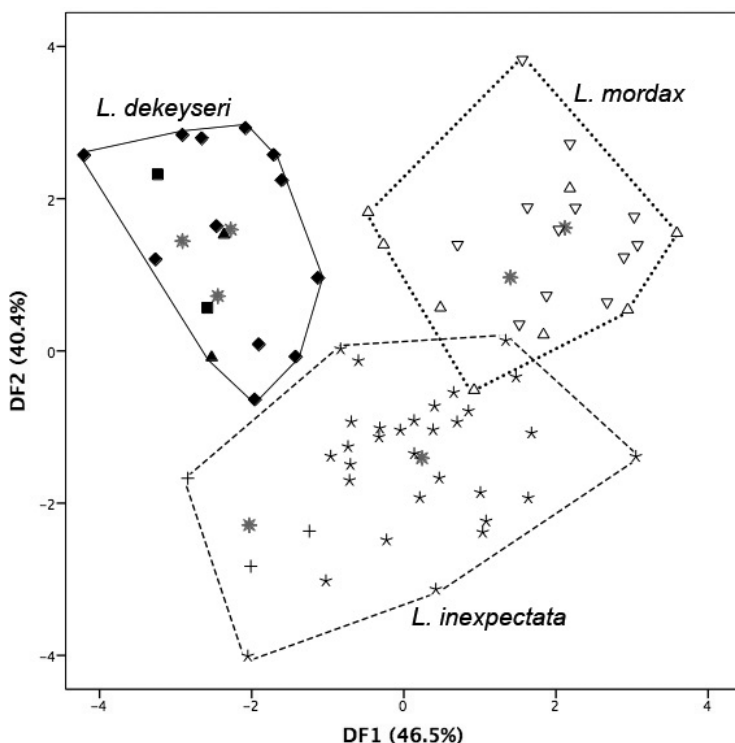


Figure 7. Plots of multivariate individual scores in the first two discriminant functions (DF1, DF2). Samples: *Lonchophylla dekeyseri* (Goiás [black diamonds, $N = 12$]; Mato Grosso do Sul [black squares, $N = 2$]; Distrito Federal [black triangles, $N = 2$]), *L. inexpectata* (Barra, Bahia [crosses, $N = 3$]; Exu, Pernambuco [stars, $N = 31$]), and *L. mordax* (Itabaiana, Sergipe [white triangles, $N = 8$]; Grota do Angico, Sergipe [white inverted triangles, $N = 12$]). Centroid groups are marked with grey asterisks.

Key to the Brazil's species of *Lonchophylla*

- 1 Proximal portion of the dorsal surface of the forearm covered with fur; upper canines distinctly grooved along the anterior surface; P4 narrow in occlusal view, with inner lobe reduced and lingual root displaced posteriorly.....**2**
- Proximal portion of the dorsal surface of the forearm not conspicuously furred; upper canines lacking a groove along the anterior surface; P4 robust, with inner lobe well developed and lingual root in the median portion of the tooth.....**3**
- 2 Smaller size; forearm length 37 mm or less; pale-brownish ventral fur; tip of the tragus rounded; parastyles, mesostyles and metastyles of M1 and M2 absent or poorly developed *Lonchophylla peracchii*
- Larger size; forearm length 39 mm or more; pale-greyish ventral fur; tip of the tragus pointed; parastyles, mesostyles and metastyles of M1 and M2 well developed..... *Lonchophylla bokermanni*

- 3 P3 robust in occlusal view, with lingual lobe varying from small to moderately developed projection; presence of a conspicuous longitudinal groove along the posterior surface of the canine; metastyle of M1 and M2 absent or reduced ...
 *Lonchophylla dekeyseri*
- P3 narrow in occlusal view, usually without inner lobe or with a reduced lobe; absence of a conspicuous longitudinal groove along the posterior surface of the canine; metastyle of M1 and M2 distinct and developed.....4
- 4 Ventral fur pale-brownish; mandibular length 15.5–17.0 mm
 *Lonchophylla mordax*
- Ventral fur whitish or pale-greyish on the throat and abdomen (particularly on the posterior region of the belly); mandibular length 14.1–16.3 mm
 *Lonchophylla inexpectata*

Discussion

Historical remarks. Previous assignments of *L. inexpectata* to *L. mordax* seem to have originated with Lima (1926: 36) who based his account of *L. mordax* on the series from Barra, which was collected by Garbe and deposited in the Museu de Zoologia da Universidade de São Paulo. Barra is in the *sertão* of Bahia (Caatinga), ca. 450–500 km west of Lamarão, which is in the *agreste* of Bahia (transition between Atlantic Forest and Caatinga; type locality of *L. mordax*). Thomas (1903: 459) described *L. mordax* as follows:

General external appearance, so far as can be judged by skins, exactly as in Glossophaga soricina, except that the colour averages paler. The type is near “cinnamon-brown” above, the bases of the hairs whitish, and “wood-brown” below, but there is some variation in tone, and the darker specimens are quite as dark as the paler examples of Glossophaga obtained at the same place.

Lima (1926) seems to have misinterpreted Thomas (1903) where he reported that “darker specimens [of *L. mordax*] are quite as dark as the paler examples of *Glossophaga* obtained at the same place.” Lima’s conclusion might be biased by the series he had at hand, primarily composed by pale-venter specimens from Barra, Bahia. However, at that time, *L. mordax* was unquestionably the closest species—geographically and morphologically. Although Lima had identified this series from Barra as *L. mordax*, the label of the USNM 238008 bears the notation “*Subsp. n. ?*”

Vieira (1942: 321) followed Lima (1926) and based his account of *L. mordax* on the same specimens collected by Garbe. Both recognized *L. mordax* as a pale-venter species. This was followed by Taddei et al. (1983) who compared the species they were describing (*L. dekeyseri*) with “*L. mordax*”—the other pale-venter species from NE Brazil, according to those authors. However, according to Thomas (1903), the ventral pelage of *L. mordax* is “wood-brown”, but with some variation, with darker specimens almost as dark as paler specimens of *Glossophaga* from the same area. *Glossophaga*

soricina (Phyllostomidae)—the only species of the genus that occur in the region—has ventral pelage varying from “buffy to fuscous” (Alvarez et al. 1991).

Taxonomic remarks. Molecular and morphological analyses have recovered *Lonchophylla* (sensu Griffiths and Gardner 2008) as a paraphyletic assemblage (Dávalos and Jansa 2004, Woodman and Timm 2006, Woodman 2007). Combining evidence from nuclear and mitochondrial genes, karyotypes and skull morphology, Parlos et al. (2014) also retrieved *Lonchophylla* as paraphyletic. Based on their findings, Parlos et al. (2014) described *Hsunycteris* and moved three species into this new genus—*thomasi* J. A. Allen, 1904; *cadenai* Woodman & Timm, 2006; and *pattoni* Woodman & Timm, 2006. As a result, *Lonchophylla* comprised 12 South and Central American species (Parlos et al. 2014). However, several species were not assessed, including *L. mordax*—the type species of *Lonchophylla*. According to Parlos et al. (2014), the two genera can be distinguished by size (with species in *Lonchophylla* larger than those in *Hsunycteris*), qualitative cranial features, and karyotypes (*Lonchophylla* spp.: diploid number $[2n] = 48$, fundamental autosomal number $[NF] = 50$; *Hsunycteris* spp.: $2n = 30\text{--}36$, $NF = 34\text{--}50$).

The samples we have available show that *L. dekeyseri* and *L. mordax* are in parapatry with *L. inexpectata*: *L. dekeyseri* occurs in the Cerrado of Brazil and possibly in the Bolivian savannah (USNM 584472, 584473) and the Cerrado–Caatinga ecotone in NE Brazil (DZSJRP 11459); and *L. mordax* occurs in the Atlantic Forest–Caatinga ecotone (*agreste*), and along the eastern border of the Caatinga (*sertão*). We are not convinced that *L. dekeyseri* occurs in the Bolivian savannah and in the Cerrado–Caatinga ecotone in NE Brazil. One of the specimens supporting these records was examined a long time ago (DZSJRP 11459), and the other two (USNM 584472, 584473) are distinct from other samples of *L. dekeyseri* as determined in a previous discriminant function analysis. These specimens are not included in this analysis because we were not able to compare them with samples from other localities. Records previously assigned to *L. mordax* from N Brazil are based primarily on Handley (1967) and Piccinini (1974), and those identifications were not confirmed in subsequent surveys. We speculate that they are misidentifications of *L. thomasi*, now *Hsunycteris thomasi*. Similarly, previous unvouchered records of *L. mordax* from the Atlantic Forest of SE Brazil apparently represent *L. peracchii* based on the identity of material we have examined from nearby localities.

After Parlos et al.’s (2014) assignment of *L. thomasi* J. A. Allen, 1904 to *Hsunycteris*, *L. inexpectata* is the fifth *Lonchophylla* reported from Brazil—all pending phylogenetic positioning. There are several specimens pending verification of identity, particularly those from the Caatinga. Additional material, particularly from NE and Mid-western Brazil, will be important to a clearer understanding of the taxonomic diversity, and the geographic distribution of Brazilian species of *Lonchophylla*.

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Appendix

Specimens examined. Abbreviations for collections are in “Methods”.

- Lonchophylla bokermanni* (08): Brazil, Minas Gerais: Diamantina (18°23'S, 43°61'W: MN 79996, MN 79997); Serra do Cipó (19°16'S, 43°36'W: DZSJRP 10342 [paratype], 10347 [holotype], 10408 [paratype], 11410 [paratype], 11411 [paratype], 10412 [paratype; referred in the original description as ZUEC 585]).
- Lonchophylla dekeyseri* (16): Brazil, Distrito Federal: Parque Nacional de Brasília (15°41'S, 47°59'W: DZSJRP 10099 [holotype]); unknown locality (ALP 6706, 6707). Brazil, Goiás: Mambai (14°29'S, 46°06'W: LDM 283, 3008, 3065, 3066, 3104, 3169, 3170, 3184, 3185, 3201, 3215, 3270). Brazil, Mato Grosso do Sul: Corumbá (19°61'S, 57°45'W: LDM 2642).
- Lonchophylla* cf. *dekeyseri* (3): Bolivia, Santa Cruz: Velasco (13°54'27"S, 60°48'52.92"W: USNM 584472, 584473). Brazil, Piauí: Sete Cidades, Piracuruca (03°56'S, 41°44'W: DZSJRP 11459 [paratype of *dekeyseri*]).
- Lonchophylla inexpectata*: Brazil, Bahia (43): Barra (12°42'S, 41°33'W: USNM 238008 [holotype], AMNH 235608, FMNH 21077, 21078 [paratypes]). Brazil, Pernambuco: Buíque, Serra do Catimbau (08°37'S, 37°09'W: FMNH 137414 [paratype]); 17 km south of Exu (07°41'S, 39°32'W: CM 99413–99450).

Lonchophylla cf. *inexpectata* (1): Brazil, Bahia: Andaraí, unknown locality (ALP 3686).

Lonchophylla mordax (35): Brazil, Bahia: Lamarão (11°45'S, 38°55'W: BM 1903.9.5.34 [holotype], USNM 123392 [paratype]). Brazil, Sergipe: Itabaiana (10°68'S, 37°42'W: ALP 6149, 8769, 8770, 8812–8819); Parque Nacional Grota do Angico (09°65'S, 37°67'W: ALP 9747, 9752, 9755, 9757, 9759, 9761, 9762, 9768, 9769, 10075–10082, 10084–10088).

Lonchophylla peracchii (75): Brazil, Espírito Santo: Sooretama, BR-101, Km 105, Reserva Biológica de Sooretama (19°1'48.97"S, 40°1'8.976" W: UFES 2046, 2047, 2117) Brazil, Rio de Janeiro: Angra dos Reis, Ilha da Gipóia (23°02'S, 44°21'W: LDM 4200, 4423); Angra dos Reis, Ilha Grande (23°10'S, 44°12'W: DZSJRP 15159 [paratype], 15160, 15161, 15162 [holotype], 15163 [paratype], LDM 246, 2090, 3450, 3896, 3897, 4052, 4233, 4533); Cambuci (21°34'S, 41°54'W: LDM 4250, 4253, 4477); Casimiro de Abreu, Morro de São João (22°29'S, 41°58'W: LDM 2219, 2245, 4113, 4222, 4226, 4227); Itaguaí, Ilha de Itacuruçá (23°56'S, 43°53'W: LDM 5085); Mangaratiba, Vale do Rio Sahy (23°55'S, 43°59'W: LDM 5128); Nova Iguaçu, Reserva Biológica do Tinguá (22°39'S, 43°34'W: ALP 6265, 6560, 6561, 6283, 6284, 6556, 6656–6559); Parati (23°19'S, 44°38'W: LDM 996, 997); Rio de Janeiro, Estrada Rio-Santos (23°55'S, 43°16'W: LDM 5008, 5010); Rio de Janeiro, Floresta da Tijuca (22°57'S, 43°24'W: LDM 1064, 1460); Rio de Janeiro, Jardim Botânico (22°58'S, 43°13'W: LDM 875); Rio de Janeiro, Parque Estadual da Pedra Branca (22°52'S, 43°23'W: ALP 5664, 5820, 5860); Rio de Janeiro, Reserva do Grajaú (22°55'S, 43°16'W: ALP 1783–1785, LDM 237, 238, 246–248, 250, 270, 280, 281, 345, 395, 531–533, 1359, 1495–1497, 1499); Rio de Janeiro, Reserva Rio das Pedras (22°59'S, 44°06'W: LDM 1781, 3700); Teresópolis, Parque Nacional da Serra dos Órgãos (22°26'S, 42°59'W: ALP 6482). Brazil, São Paulo: Ubatuba, Picinguaba (23°18'S, 44°53'W: ALP 10242).

Supplementary material I

Occurrence localities for Bolivian and Brazilian species of *Lonchophylla*

Authors: Ricardo Moratelli, Daniela Dias

Data type: Occurrence localities

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Cytotaxonomy of unionid freshwater mussels (Unionoida, Unionidae) from northeastern Thailand with description of a new species

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Abstract

Morphological and chromosomal characteristics of a number of unionid freshwater mussels were studied from northeastern Thailand. Karyotypes of eight species from seven genera (*Chamberlainia*, *Ensidens*, *Hyriopsis*, *Physunio*, *Pseudodon*, *Scabies* and *Trapezoideus*) were examined. Six species possess $2n = 38$ karyotypes, whereas *Scabies crispata* and an unidentified *Scabies* sp. lack three small chromosome pairs, giving a diploid number of 32. Moreover, the karyotypes of the unidentified *Scabies* differ from *S. crispata* as it exhibits a telocentric chromosome pair ($6m + 7sm + 2st + 1t$). Most of the conchological characters also differ between the two species – adult size, colour pattern, muscle scars, pseudocardinal and lateral teeth. The name *Scabies songkramensis* **sp. n.** is proposed for the unidentified species, and its description is included in this paper. Interestingly, seven species contain mostly bi-armed chromosomes, but only the mud-dweller in stagnant water, *Ensidens ingallsianus*, contains predominantly five telocentric pairs. In addition, the marker chromosome characteristics of an unbalanced long arm, twisted centromere, a wider angle 180° arrangement, a twisted arm and telomeric end union reported in this study are described for the first time for unionid mussels.

Keywords

Chromosome, mussel, karyotype, systematics, Southeast Asia, cryptic species

Introduction

The Unionidae is numerically the largest family of both extant and extinct freshwater mussels and includes over 670 species worldwide with about 220 species occurring in Indotropa (Graf and Cummings 2007). Such high species diversity and wide distribution make the unionid mussels very attractive for systematic and bio-geographical studies. However, environmental problems, including water pollution, threatens the survival of many species today, and many populations in many parts of the world have been reported as declining (Williams et al. 1993; Vaughn and Tayler 1999; Sethi et al. 2004; Haag and Williams 2014). As a response, taxonomic and systematic studies of unionids that integrate conchological and anatomical analyses with molecular phylogenies have increased over the last two decades.

Most studies have dealt with American, European and Australasian taxa (Rosenberg et al. 1994, 1997; Graf and Ó Foighil 2000; Hoeh et al. 2001; Graf and Cummings 2011; Graf 2013; Lopes-Lima et al. 2014; Prié and Puillandre 2014; Graf et al. 2015), whereas Asian taxa have largely been neglected. The monographs by Haas (1969) and Brandt (1974) have reported taxonomic surveys of Thai species. Nevertheless, recent reassessments by other malacologists have revealed some new and unknown species (Graf 2002; Deen et al. 2003) and there are still many localities that have never been surveyed. Owing to their conservative morphological diversity, it has not been easy to establish a reliable phylogeny for unionids. Identification of species is often difficult due to morphological variation among individuals and within regional populations, termed ecophenotypic variability (Roe et al. 2001; Plouviez et al. 2009; Vannarattanarat et al. 2014). The plasticity of shell characters is well-known amongst the Unionoida (e.g. Graf 2000; Baker et al. 2004; Marshall et al. 2014). Tests of phylogenetic hypotheses on the basis of other data sources, such as those derived from molecules and chromosomes, are therefore likely to be informative. However, such approaches have as yet been attempted only on a limited number of taxa and there are still very few studies in Asian and African regions (Lopes-Lima et al. 2014; Marshall et al. 2014; Graf et al. 2015).

Several sympatric species have been recorded in numerous Thai localities (Brandt 1974; Panha 1990), raising many interesting taxonomic and ecological questions. Some of these questions have been addressed in a few publications on some biological aspects such as the relationships of mussels and their fish hosts or 'glochidiosis' (Panha 1992; 1993a,b). Whilst chromosomal data of some Thai unionids have been described (Meesukko 1996; Deen et al. 2003; see also Table 1), the number of karyotyped species comprise fewer than 30% of the total species diversity in the family.

Here we examine the karyotypes of eight species of unionids from northeastern Thai that represent seven genera (and four subfamilies): *Chamberlainia*, *Hyriopsis* (Hyriopsinae); *Scabies* (Parreysiinae); *Pseudodon* (Pseudodontinae); *Ensidents*, *Physunio*, *Trapezoides* (Rectidentinae). All these genera are considered to be completely different from each other on a morphological basis (Brandt 1974; Panha 1990).

Materials and methods

The localities and shell characteristics of each species are given in Figs 1, 2 and Table 1. Species identifications were made using Brant (1974) and Sutcharit et al. (2013). Comparisons with type specimens in the Senckenberg Museum, Frankfurt (SMF) were also conducted. Chromosome preparations were made from gill tissue by the air-drying method, modified from Patterson and Burch (1978), Deen et al. (2003) and Kongim et al. (2006, 2009, 2010). Living animals recently collected from the wild were treated with colchicine solution for 4 h at a final concentration of 0.01 M. Gill filaments were removed, cut into small pieces, and soaked in 0.075 M KCl for 45 min. The cells were then harvested by centrifugation at 1500 rpm. After fixation and rinsing in 3:1 (v/v) methanol: acetic acid, the cell suspension was pipetted onto microscope slides on warm plates (60 °C) and allowed to dry under controlled conditions for optimum spread. Chromosomes were stained with 4% (w/v) Giemsa solution for 10 min. For the karyotype analysis, metaphase plates in which the chromosomes were clearly differentiated within the cells were selected for study. Photomicrographs of 25 well-spread metaphase cells were measured for relative chromosome length and centromeric index. Mitotic karyotypes were arranged and numbered for chromosome pairs in order of decreasing mean relative length. The nomenclature for morphological chromosome types was derived from Levan et al. (1964).

Abbreviations for figures and measurements: aa, anterior adductor; muscle scar; lt, lateral teeth ; pa, posterior adductor muscle scar; pl, pallial line; pt, pseudocardinal tooth; H, height of valves; L, length of valves; W, width of valves.

Institutional abbreviations

CUMZ Chulalongkorn University, Museum of Zoology, Bangkok, Thailand
SMF Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt, Germany
ZMMSU Zoological Museum of Mahasarakham University, Thailand.

Results

Karyotype

The karyotype of six species consists of $2n = 38$ chromosomes, but two species (*Scabies crispata* and an unidentified *Scabies* sp.) showed $2n = 32$. In all samples examined, no sex chromosome heteromorphism or secondary constrictions were evident. The fundamental numbers (FN) varied among species, ranging from 46 to 76 (Figs 3, 4 and Table 1). Seven species contain metacentric dominant chromosomes (12–13 pairs), but only *Ensidents ingallsianus* contains 12 pairs of the telocentric dominant category.

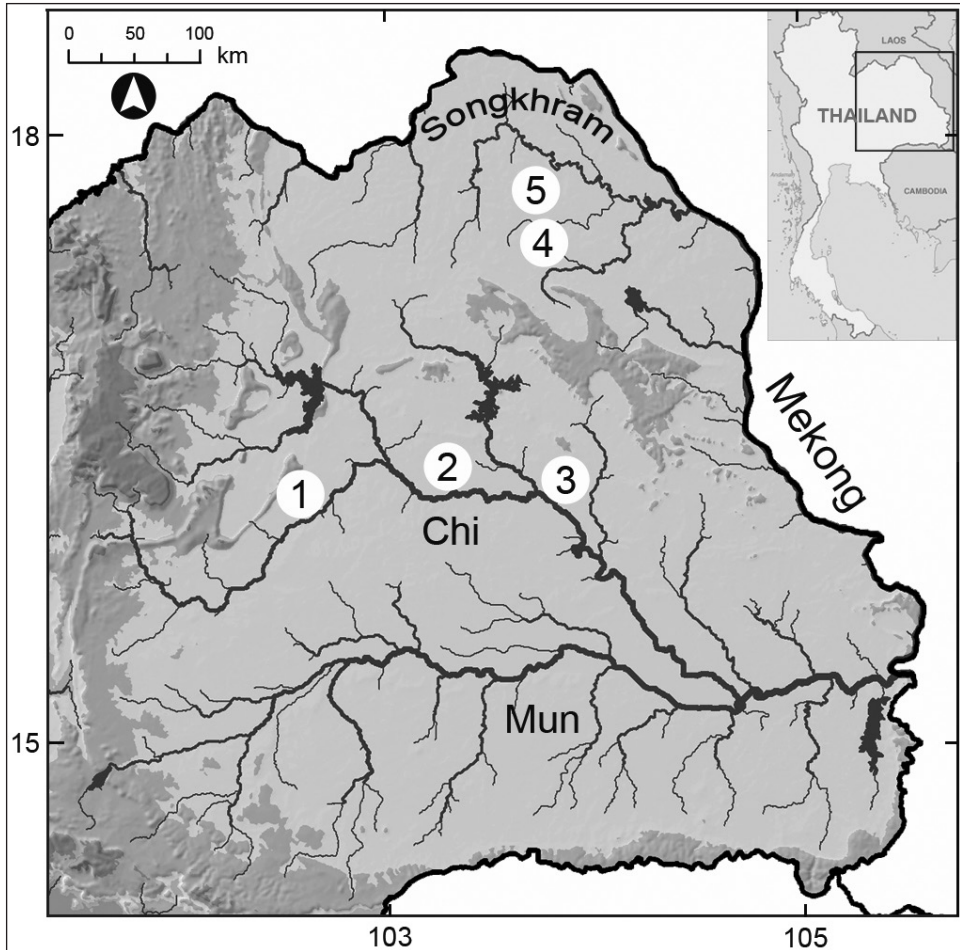


Figure 1. Sampling locations for unionids in northeastern Thailand: **1** Ban Tha Nanglian, Chonnabot, Khon Kaen (16°1'21"N; 102°33'34"E) **2** Ban Tha Khonyang, Kantharawichai, Maha Sarakham (16°14'1"N; 103°16'1"E) **3** Ban Tha Krai, Selaphum, Roi Et (16°2'0"N; 103°56'2"E) **4** Ban Klang Charern, Pangkon, Sakon Nakorn (17°24'22"N; 103°50'1"E) **5** Kamtakla, Sakon Nakorn (17°49'32"N; 103°47'10"E).

The two large pearl mussels (*Chamberlainia hainesiana* and *Hyriopsis bialatus*) plus one medium-sized species (*Trapezoides exolecens*) have the same numbers of metacentric and telocentric chromosomes consisting of 13 + 6 pairs with slightly different arrangements (Table 1). *Chamberlainia hainesiana* possesses the largest chromosome pair 1, and has unbalanced arms on chromosome pairs 5 and 13. *Hyriopsis bialatus* possesses distinct chromosome markers in having a short arm pair 6 with a telomere end union.

The karyotype of *Scabies crispata* is almost identical to that of *Scabies songkramensis* sp. n., but the latter differs in having a telocentric pair 7. The FN values were dissimilar at 64 and 62, respectively (Figs 3, 4 and Table 1). Both species show chromosome markers of a twisted arm on chromosome pair 10 and 15, respectively.

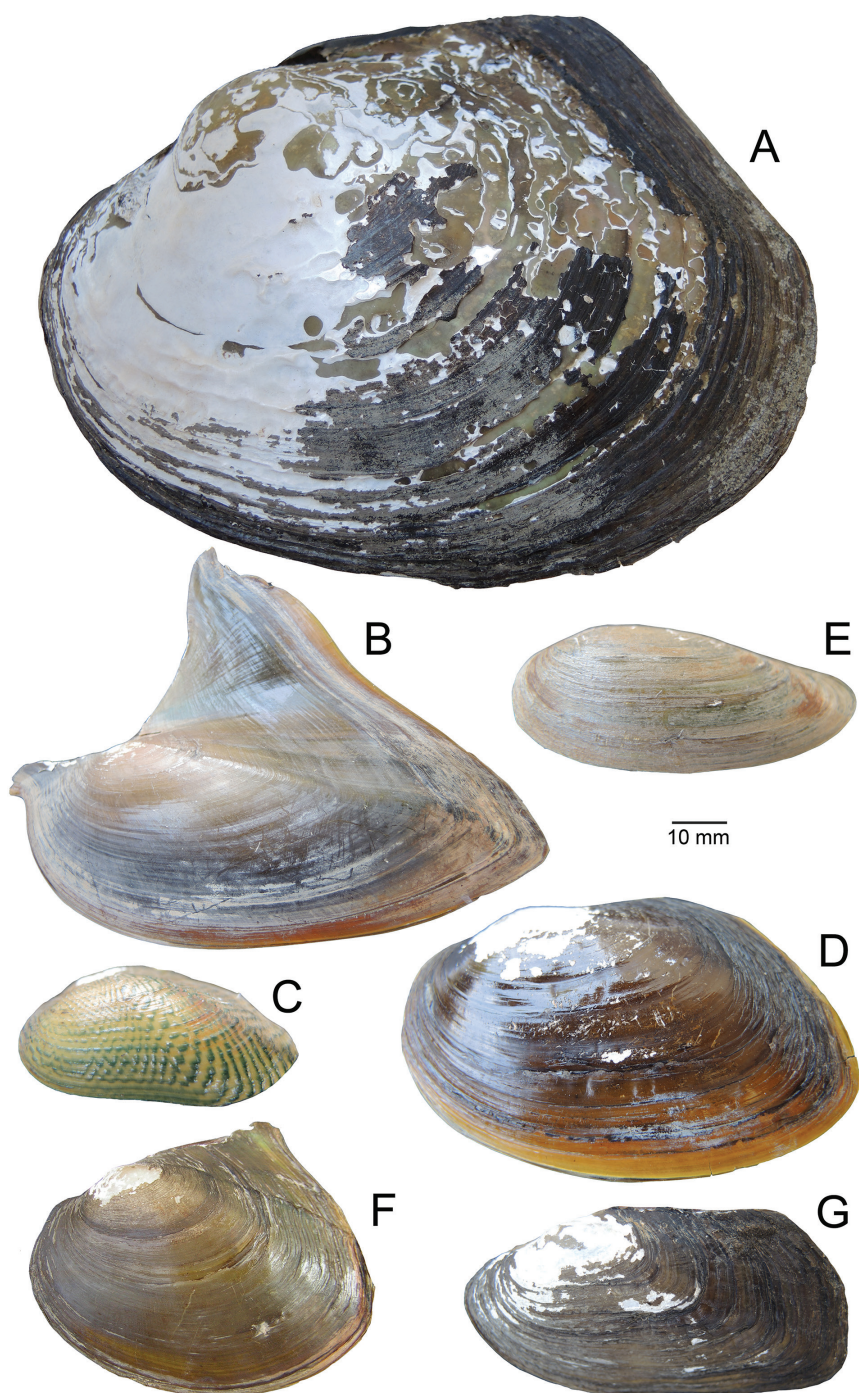


Figure 2. Comparative external views of shell valves of unionids studied: **A** *Chamberlainia hainesiana* **B** *Hyriopsis bialatus* **C** *Scabies crispata* **D** *Pseudodon mouhoti* **E** *Ensidents ingallsianus* **F** *Physunio inornatus* **G** *Trapezoideus exolescens*.

Table 1. Data summary. This table shows the number of specimens examined (No.), locality, diploid number (2*n*), fundamental number (FN), karyotype pattern and chromosome markers of the Unionidae species included the present study. The numbered localities are presented in Figure 1. Abbreviations: m, metacentric; sm, submetacentric; st, subtelocentric; t, telocentric.

Species	No.	Locality	2 <i>n</i>	FN	Karyotype formula	Marker chromosome (pair number)
UNIONIDAE						
Subfamily Hyriopsinae						
<i>Chamberlainia hainesiana</i>	2	2,3	38	76	4m + 9sm + 6st	5 and 13 unbalance of long arm
<i>Hyriopsis bialatus</i>	10	1, 2, 3	38	72	6m + 7sm + 4st + 2t	6 telomeric end union
Subfamily Parreysiinae						
<i>Scabies crispata</i>	10	1, 2, 3	32	64	6m + 7sm + 3st	10 twisted arm
<i>Scabies songkeramensis</i> sp. n.	10	4, 5	32	62	6m + 7sm + 2st + 1t	15 small and twisted arm
Subfamily Pseudodontinae						
<i>Pseudodon mouhoti</i>	6	1, 2, 3	38	74	6m + 6sm + 6st + 1t	7 twisted centromere
Subfamily Rectidentinae						
<i>Ensidents ingallsianus</i>	6	1, 2, 3	38	46	3m + 4sm + 7st + 5t	1 unbalance of long arm 6 and 13 wider angle 180° arrangement and twisted centromere
<i>Physunio inornatus</i>	10	1, 2, 3	38	74	3m + 9sm + 6st + 1t	4 wider angle 180° arrangement 8 twisted centromere
<i>Trapezoideus exolescens</i>	10	1, 2, 3	38	74	3m + 10sm + 5st + 1t	3 unbalance of long arm

The karyotypes of *Pseudodon mouhoti* consists of 6m + 6sm + 6st + 1t with twisted centromere pair 7. The three members of the subfamily Rectidentinae (i.e. *Ensidents ingallsianus*, *Physunio inornatus* and *Trapezoideus exolescens*) are different from each other in FN value, size arrangement and morphology of chromosomes (Table 1), but all three exhibit the largest chromosome pair 1. *Ensidents ingallsianus* distinct chromosome markers of having long arm characters of the first pairs, with the non-identical left and right long arms, as well as exhibiting a remarkably wide angle (about 180°) arrangement of chromosome pairs 6 and 13. *Physunio inornatus* also exhibits a slightly smaller angle at 100° in pair 4, and pair 8 shows a twisted centromere. The distinct chromosome markers in *Trapezoideus exolescens* are the non-identical left and right long arms in pair 3 (Table 1).

Systematics

Family Unionidae Rafinesque, 1820

Genus *Scabies* Haas, 1911

Type species (by subsequent designation of Haas 1969: 63) *Unio scobinatus* Lea, 1856. Recent, Southeast Asia. Gender masculine.

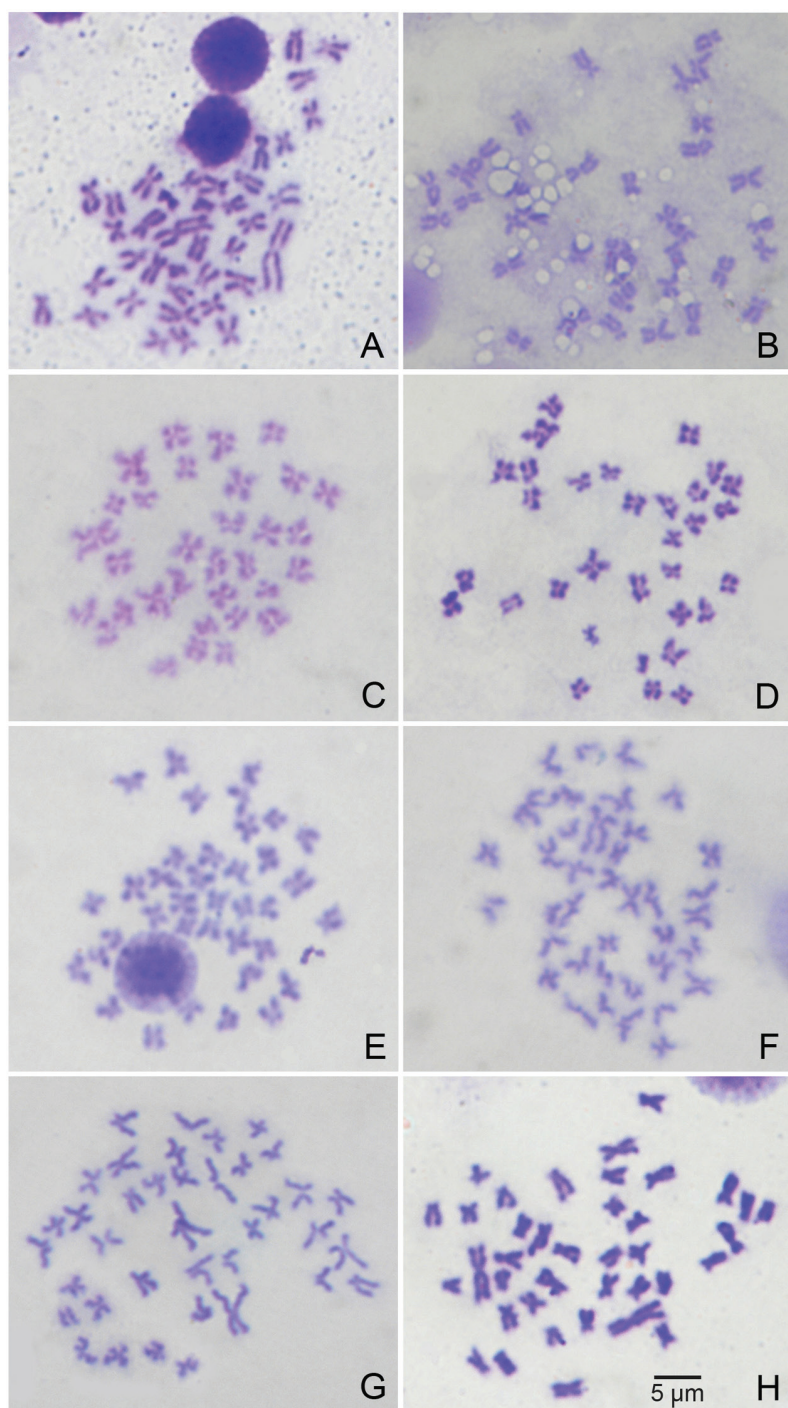


Figure 3. Mitotic chromosomes of unionids studied: **A** *Chamberlainia hainesiana* **B** *Hyriopsis bialatus* **C** *Scabies crispata* **D** *Scabies songkramensis* sp. n. **E** *Pseudodon mouboti* **F** *Ensidens ingallsianus* **G** *Physunio inornatus* **H** *Trapezoideus exolescens*.

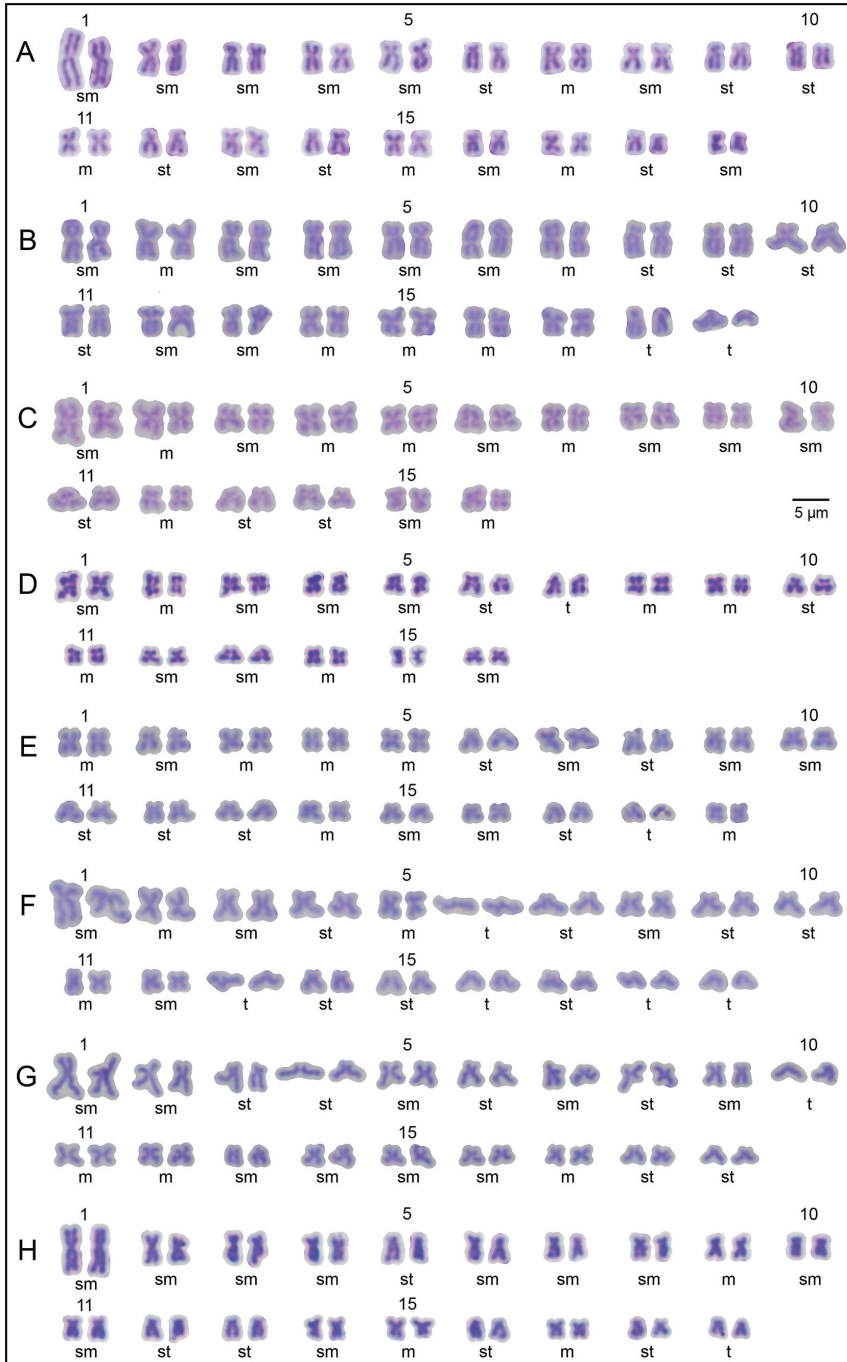


Figure 4. Karyotypes of unionids studied: **A** *Chamberlainia hainesiana* **B** *Hyriopsis bialatus* **C** *Scabies crispata* **D** *Scabies songkramensis* sp. n. **E** *Pseudodon mouhoti* **F** *Ensidents ingallsianus* **G** *Physunio inornatus* **H** *Trapezoides exolecens*. Abbreviations: m, metacentric; sm, submetacentric; st, subtelocentric; t, telocentric; numbers 1, 5, 10, 11, 15 represent the pair numbers.

***Scabies songkramensis* Kongim & Panha, sp. n.**

<http://zoobank.org/C55BB4DA-BACA-40A6-AF97-8496C3B2FC14>

Fig. 5A, B, F; Table 3

Type material. Holotype ZMMSU 00500 (length 30 mm, height 18 mm, width 7.5 mm) Paratypes: ZMMSU 00501 (20 shells; length 29–33 mm, height 17–19 mm, width 7–8 mm); CUMZ (five shells).

Type locality. Houy Plahang stream in Songkram River Basin, Ban Klang Char-ern, Pangkon, Sakon Nakorn, Thailand – 17°24'22"N, 103°50'1"E. Type locality indicated in Fig. 1, locality 4).

Etymology. The specific name *songkramensis* refers to the Songkram River, type locality of this new species. Authorship of this new species is to be credited to Kongim and Panha in Kongim, Sutcharit and Panha.

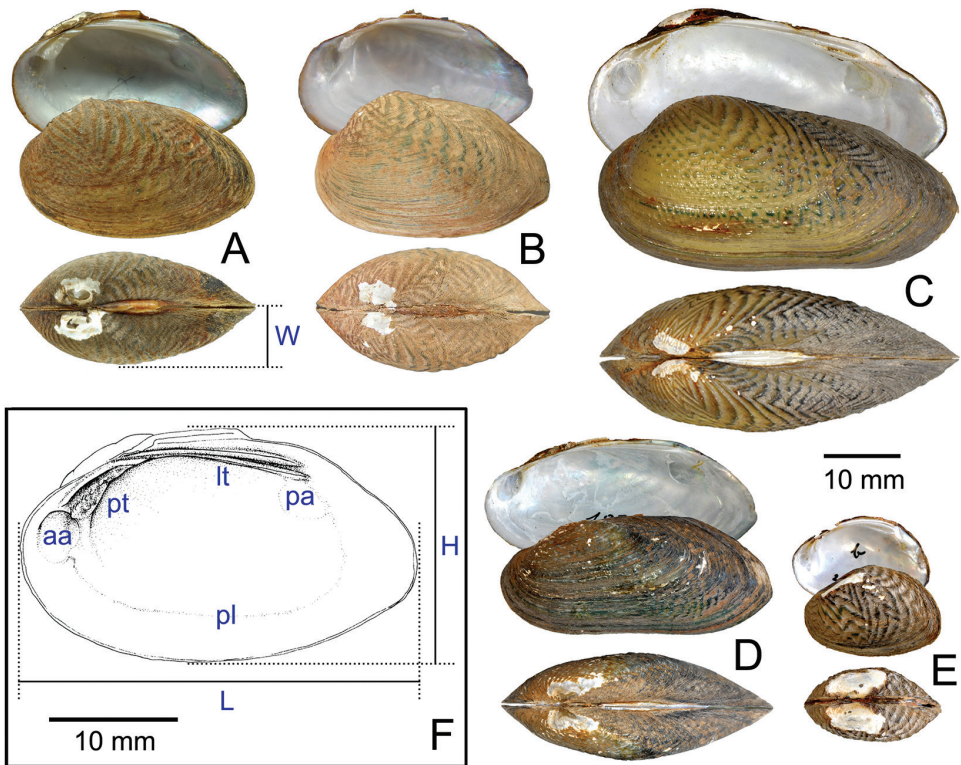


Figure 5. Shell valves of **A, B** *Scabies songkramensis* sp. n., **A** holotype ZMMSU 00500 and **B** paratype ZMMSU 00501. **C** *Scabies crispata*, Brandt collection SMF 188682 from Bangkok, Thailand **D** *Scabies nucleus* Brandt collection SMF 198394 from Mekong River, Pakse, Laos **E** *Scabies phaselus* Brandt collection SMF 188695 from Takrong River, Nakon Ratchsrima, and **F** hinge plates of *Scabies songkramensis* sp. n., holotype, with illustrating and measurements terminology. Abbreviations: aa, anterior adductor muscle scar; lt, lateral teeth; pa, posterior adductor muscle scar; pl, pallial line; pt, pseudocardinal tooth; H, height of valves; L, length of valves; and W, width of valves.

Description. Shell of medium size (length 29–33 mm), ovate in outline, H/L ratio = 0.59, anterior portion rounded, umbonal area elevated and sloping downwards posteriorly. Underlying shell colour brown. Shell sculptured with a series of coarse, v-shaped ribs radiating outwards from umbo; v-line arrangement loose, with 4-fold number on 10 mm; posterior slope with coarse and distinct ridges. Sculpture reduced to nearly obsolete near ventral and posterior shell margin. Periostracum brown, tending towards dark green where ribs are worn. Hinge plate well-developed; pseudocardinal tooth (pt) forming a thickened plate and raised lamelliform on right valve, but thinner and also raised lamelliform on left valve. Two well-developed posterior lateral teeth (lt) present in each valve, long and sharp. Anterior adductor muscle scar (aa) prominent and deeply impressed; posterior adductor muscle scar (pa) shallow; pallial line (pl) faintly impressed. Nacre bluish-white with little iridescence.

Remarks. The new species differs from the closely related *Scabies crispata* (Gould) and *S. phaselus* (Lea) by having a smaller, harder, thicker, ovate shell that is brown in colour, with dark brown v-line sculpture. The two other species have larger, more elongate shells that are yellowish brown in colour, combined with greenish v-line sculpturing in *S. crispata* and a nearly smooth shell surface in *S. phaselus*. *Scabies songkramensis* sp. n. differs from *S. nucleus* (Lea) in having a larger shell and v-line sculpture, compared with w-line sculpture in *S. nucleus*.

Habitat. *Scabies songkramensis* sp. n. occurs in a small tributary of the Songkhram River. It lives in shallow water in a sandy-gravel substrate, or less frequently in sandy-mud. This new species is currently known only from the type locality, approximately 100 km from the main stem of the Songkhram River (Fig. 1, locality 4), in slow moving water at depths that ranged from 0.5 to 2 m in the wet season (i.e. from June to October).

Discussion

The diploid numbers of six species in the three subfamilies, Hyriopsinae, Pseudodontinae and Rectidentinae, showed the same chromosome number ($2n = 38$), which is similar to unionid taxa in other regions (Vitturi et al. 1982; Meesukko 1996; Jenkinson 2014; see also Table 2). An investigation of two species of *Alasmidonta* and four species of *Anodonta* also showed a similar chromosome number ($2n = 38$) and fundamental arm number, FN = 76 (see Table 2). In other regions, the Parreysiinae is traditionally considered as more primitive than other subfamilies (Bieler et al. 2010; Carter et al. 2011; Whelan et al. 2011; Graf 2013). However, our data showed that *S. crispata* and *S. songkramensis* sp. n. (Parreysiinae) had the lowest diploid number among the Unionidae ($2n = 32$), which is the same as *Elliptio complanata* (Table 2, Lillie 1901), although Park and Burch (1995) reported the chromosome number of *E. complanata* from Ocqueoc River, Michigan, USA, as being $2n = 38$. This case should be re-evaluated carefully, especially in terms of the species identification. Unfortunately, we cannot

Table 2. The diploid ($2n$), haploid (n) and fundamental number (FN) for the Unionoida. Data for the Unionidae plus three additional families (Hyriidae, Mutelidae and Margaritiferidae) are included in the table. References as follows: (1) Lillie (1901); (2) McMichael and Hiscock (1958); (3) Griethuysen et al. (1969); (4) Nadamitsu and Kanai (1975); (5) Jenkinson (1976); (6) Vitturi et al. (1982); (7) Park and Burch (1995); (8) Ebied (1998); (9) Jara-Seguel et al. (2000); (10) Wang et al. (2000); (11) Shan et al. (2001) (12) Deen et al. (2003); (13) Woznicki (2004); (14) Woznicki and Jankun (2004) and (15) Carrilho et al. (2008). Abbreviations: m, metacentric; sm, submetacentric; st, subtelocentric; t, telocentric; a, acrocentric.

Species	$2n$	n	FN	Karyotype	Locality	References
Family Hyriidae						
<i>Diplodon chilensis</i>	34	—	—	9m + 8sm	Chile	9
Family Mutelidae						
<i>Alathyria pertexta</i>	34	—	—	—	Australia	2
<i>Mutela rostrata</i>	20	—	—	2m + 2sm + 6a	Egypt	8
<i>Vesunio ambiguus</i>	34	—	—	—	Australia	2
<i>Vesunio legrandi</i>	34	—	—	—	Tazmania	2
Family Margaritiferidae						
<i>Margaritifera margaritifera</i>	38	—	—	—	USA	5
<i>Margaritifera laevis</i>	38	19	76	19sm	Japan	4
Family Unionidae						
<i>Alasmidonta arcula</i>	38	—	—	—	USA	5
<i>Alasmidonta marginata</i>	38	—	—	10m + 7sm + 2sm	USA	5
<i>Anodonta anatina</i>	38	—	76	10m + 3s/m + 6sm	Netherlands	3
<i>Anodonta anatina</i>	38	—	76	6m + 12sm + 1st	Poland	14
<i>Anodonta cygnea</i>	38	—	76	6m + 12sm + 1st	Portugal	15
<i>Anodonta grandis</i>	38	—	—	6m + 12sm + 1st	USA	5
<i>Anodonta woodiana</i>	38	—	76	—	Poland	13
<i>Anodonta woodiana woodiana</i>	38	—	76	—	China	11
<i>Anodontoides ferussacianus</i>	38	—	—	9m + 10sm	USA	5
<i>Elliptio complanata</i>	—	16	—	—	USA	1
<i>Elliptio complanata</i>	38	—	—	—	USA	7
<i>Gonidea angulata</i>	38	—	—	—	USA	5
<i>Hyriopsis cumingii</i>	38	—	—	—	China	10
<i>Inversidens japonensis</i>	38	—	76	6m + 13sm	Japan	4
<i>Lampsillis radiata luteola</i>	38	—	—	—	USA	5
<i>Lasmigona costata</i>	38	—	—	9m + 7sm + 3st	USA	5
<i>Potamilus alatus</i>	38	—	—	—	USA	5
<i>Pseudodon obovalis omiensis</i>	38	—	76	9m + 10sm	Japan	4
<i>Ptychobranchus fasciolaris</i>	38	—	—	8m + 10sm + 1st	USA	5
<i>Quadrula quadrula</i>	38	—	—	—	USA	5
<i>Soleniaia khwaenoiensis</i>	37	19	—	3m + 15sm + 1st	Thailand	12
<i>Toxolasma lividus grans</i>	38	—	—	—	USA	5
<i>Tritigonia verrucosa</i>	38	—	—	—	USA	5
<i>Unio elongatulus</i>	28	—	—	10m + 4sm	Egypt	8
<i>Unio elongatulus</i>	—	19	—	—	Italy	6
<i>Unio pictorum</i>	38	—	76	8m + 1m/sm + 10sm	Netherlands	3
<i>Villosa iris</i>	38	—	—	11m + 6sm + 2st	USA	5
<i>Villosa lienosa</i>	38	—	—	—	USA	5

Table 3. Comparisons of shell characteristics of the new species compared with those of the three other Thai species of *Scabies*.

Characteristics	<i>S. songkramensis</i> sp. n.	<i>S. crispata</i>	<i>S. nucleus</i>	<i>S. phaselus</i>
Length of valves; L (mm)	29–33 29.60 ± 0.57	30–39 35.40 ± 2.33	16–19 18.00 ± 0.40	30–35 32.60 ± 1.85
Height of valves; H (mm)	17–19 17.60 ± 0.57	14–17 15.88 ± 0.68	11–13 12.20 ± 0.67	13–17 15.26 ± 0.55
Width of valves; W (mm)	7–8 7.51 ± 0.35	5.5–7.5 6.57 ± 0.42	3.5–4.5 4.23 ± 0.87	5.5–7 5.95 ± 0.39
H/L ratio	0.59 ± 0.01	0.46 ± 0.31	0.71 ± 0.01	0.48 ± 0.32
Shell shape	Ovate	Elongate cuneiform	Subquadrate	Elongate with ventral margin concave
Shell colour	Greenish brown	Dark greenish	Greenish	Dark greenish
Shell sculpture	Coarse, obtuse	Fine, glossy	Coarse, obtuse	Fine, glossy
Line of shell sculpture	Loose, distinct v-line	v or w-line	v-line	Dense, wavy line
Fold number on shell sculpture on 10 mm	4	6	6	9
Shell thickness	Thick	Thin	Thick	Thin
Nacre colour	Bluish-white	Milky-white	Bluish-white	Milky-white
Pseudocardinals tooth	Thick plate	Large, deep fracture	Thick, stumpy, short, deep fracture	Large, short, triangular, pointed crest
Muscle scars	Deep and narrow in anterior, shallow in posterior	Deep in anterior, very shallow in posterior	Distinct, deep in anterior	Deep in anterior

clarify the taxonomic status of the previous *E. complanata* to determine this variation in the chromosome number.

McMichael and Hiscock (1958) identified $2n = 34$ as the chromosome number for three species of Mutelidae and 1 species of Hyriidae, the latter a more primitive family than the Unionidae. However, chromosome number has been, so far, of little used for the taxonomy of unionid mussels. The other recent reports of different diploid number are from *Solenia khwaenoiensis* from Thailand with the unusual 37 ($2n$) chromosomes (Deein et al. 2003) and from *Unio elongatulus* from Egypt with 28 ($2n$) (Ebied 1998). However, *U. elongatulus* was previously karyotyped from Italy and this *Unio* species has only been described from the upper Nile in Ethiopia, whereas these were caught in the lower part of this river in Egypt. This misidentification was made probably with one of the common genus *Coelatura* in the lower Nile River. Interestingly *Coelatura* also belongs within Parreysiinae. The karyotype of most species has not been studied in detail and additional characters might be useful for identification to species level. This study revealed that the Parreysiinae genus *Scabies*, which possesses a lower chromosome number than others of its subfamily, is significant because it has not been reported previously.

The karyotypes of all eight species of unionids studied here differ in the degree of asymmetry (sub-telocentric and telocentric). Primitive karyotypes typically exhibit low asymmetry and derived karyotypes show higher asymmetry (Diupotex-Chong et al. 2004; Kongim et al. 2010). Thus, the karyotype of *Scabies crispata* is assumed to exhibit a primitive character among Southeast Asian unionids, whereas the karyotype with the highest asymmetry was exhibited by *Ensidents ingallsianus* (Rectidentinae), which is assumed to be a derived form.

Marker chromosomes such as telomeric end union, wider angle arrangement and others, are useful in taxonomy and systematics (Gomes et al. 2011). Our data show that marker chromosome arrangement varies among species and so may have diagnostic significance. The unbalance of the long arm and the twisted centromere are found in most cases in four chromosome pairs in three species. The latter wider angle 180° arrangement, and twisted arm are found in two chromosome pairs in two species. The last telomeric end union is found in only one pair of a single species (*Hyriopsis bialatus*) and that could be a diagnostic feature for this species. All of the marker chromosomes are different in their chromosome structure, especially the telomeric end union, whereby the sticky end in the telomere of the two chromatids cause the fusing together that is the telomeric arrangement. Overall, the data indicated that several chromosomal re-arrangements seem to have taken place during the karyo-evolutionary history of unionid species, mainly driven by reciprocal translocation (Halnan 1989; Rooney and Czepulkowski 1992; Clark and Wall 1996; Rickart et al. 1999). This karyological differentiation is not only related to geographical isolation, but it also indicates reproductive incompatibility and the occurrence of different evolutionary mechanisms of translocation. This karyological evidence was supported by the differences in their morphology and geographic separation. The Parreysiinae has been reported to be an early branch from the common ancestor leading to the other subfamilies with the other subfamilies being proposed as sister groups (Whelan et al. 2011; Graft 2013). Differences in chromosome number may be an isolation mechanism in each subfamily, as supported by the molecular phylogenetic tree of freshwater mussels (Bieler et al. 2010; Carter et al. 2011; Whelan et al. 2011; Graf and Cummings 2011; Graf 2013).

The karyotype is generally a species-specific character, and as such is useful in species discrimination (White 1978; Halnan 1989; King 1993; Clark and Wall 1996; Kolnicki 2000). Karyological data have been used for species-level classification in several molluscan groups, including *Atlanta*, *Bellamya*, *Goniobasis* and *Viviparus* (Zhou et al. 1988; Dillon 1991; Thiriot-Quièreux and Seapy 1997; Baršienė et al. 2000). Chromosome variations, in terms of both the number, karyotype pattern, and the marker chromosome, have been implicated as a primary isolating mechanism for speciation in the polymorphic *Sphaerium corneum* (see Petkevičiūtė et al. 2006). Therefore, cytogenetic study is an efficient tool for systematic approaches (cytotaxonomy) in several molluscan groups, where it is helpful in discriminating between morphologically similar species (cryptic species), since the karyotype itself probably represents a character that is resistant to environmental, behavioural or physiological influences (White 1978; Baršienė 1994; Aldridge 2000; Bauer 2001; Sumner 2003).

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Palaeartic species of *Rhamphomyia* (*Pararhamphomyia*) *anfractuosa* group (Diptera, Empididae)

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Abstract

Palaeartic species of the *Rhamphomyia* (*Pararhamphomyia*) *anfractuosa* group are revised. *Rhamphomyia* (*Pararhamphomyia*) *biflexata* sp. n., *R. (Pararhamphomyia) lineodorsata* sp. n., *R. (Pararhamphomyia) nudiscutellata* sp. n., and *R. (Pararhamphomyia) shatalkini* sp. n. (all from Russian Far East) are described and illustrated. A key to Palaeartic species of the *Rhamphomyia* (*Pararhamphomyia*) *anfractuosa* group is provided.

Keywords

Empidoidea, *Rhamphomyia*, taxonomy, key, new species, Palaeartic

Introduction

Rhamphomyia Meigen is one of the three megadiverse groups of Empididae, alongside *Empis* Linnaeus and *Hilara* Meigen. Almost 600 species, distributed mostly in the Northern Hemisphere have been described worldwide (Yang et al. 2007; Barták 2007; Barták et al. 2007; Barták and Kubík 2008a, b, c, 2009, 2010, 2012; Saigusa 2012; Barták et al. 2014), but many more await description.

Rhamphomyia (*Pararhamphomyia*) *anfractuosa* group is delimited here as probably a natural group of *Pararhamphomyia* (as fixed by Barták and Sinclair 2003 and diagnosed by Barták 1982). The most important characters shared by members of this group are as follows (* for characters considered synapomorphic):

- acrostichals present, biserial
- male head holoptic
- legs brown to black
- proepisternal depression without setae
- phallus forms several tightly coiled loops*
- cercus simple (“subcercal process” or “posterior cercus” absent)*
- hypandrium membranose posteriorly, anterior part desclerotized medially*
- tip of epandrial lamellae with expanding membranose pouch (see Fig. 1 and 9)*
- tergite and sternite 8 fused*
- axillary angle acute to right angled
- anal vein complete or depigmented in middle
- halter yellow
- female leg parts broadly pennate

As usual in *Rhamphomyia*, it is not always easy to arrange single females into groups or even subgenera (compare species group approach in Barták 2002, 2003; Barták and Kubík 2009), so, separate key to females of this group would be meaningless.

The most allied species to this group are species of *Pararhamphomyia* sharing most characters with this group mentioned above, except the tightly coiled phallus and/or yellow halter (e.g., *R. slovakii* Barták, *R. plumifera* Zetterstedt, *R. deformata* Frey, *R. deformatella* Barták). The subgenus *Calorhamphomyia* Saigusa with similarly modified last abdominal segments and phallus may be allied to this group of species, however its members may be easily separated by at least partly yellow legs.

Material and methods

The material studied is deposited in the following collections: CULSP (Czech University of Life Sciences Prague), ZMMU (Zoological Museum of Moscow State University). Acronyms are used further in the text.

Terminal abdominal segments (Figs 1–8) and hind legs (Figs 13–16) were photographed by means of Nikon Digital sight DS Fi-1. Each image resulted usually from combining 7–10 layers by means of Nish element. Legs were illustrated from these images; details were added by direct observation. Genitalia (Figs 9–12) together with 2–3 pregenital segments were removed and macerated in potassium hydroxide solution (approx. 10 %) in small vials submerged in hot water for 1–2 hours. After neutralizing with 8 % acetic acid, the genitalia were dissected in glycerine and their parts photographed by means of an Olympus E-41 digital camera mounted on an Olympus BX51

compound microscope. Images were edited with the computer software Quick Foto micro 2.3 provided with Deep focus 3.1. Each image resulted usually from combining 7–15 layers. Images were improved by means of Adobe Photoshop.

The morphological terms used here follow Merz and Haenni (2000), Sinclair (2000) and Sinclair and Cumming (2006). All body measurements (including body and setae length) were taken from dry specimens (therefore the actual length may differ) by means of an ocular micrometer on a Nikon SMZ 1500 binocular microscope.

Length of antennal segments = length of scape: pedicel: postpedicel: style (in 0.01 mm). Male body length was measured from antennal base to the tip of last abdominal segment (without the genitalia) and female body length from the base of antennae to the tip of the cerci. Wing measurements: M_2/d = length of vein M_2 : greatest length of discal medial cell (discal cell); CuA_1 ratio = length of apical: preapical sections of vein CuA_1 ; lw/ww = greatest length of wing (from basicosta to apex): greatest width of wing. Length of frons is measured from front margin of front ocellus to antennal base.

Taxonomic account

Rhamphomyia (Pararhamphomyia) biflexata sp. n.

<http://zoobank.org/2715DE4F-1E0A-4636-825F-751DC030B876>

Figs 2, 9, 14

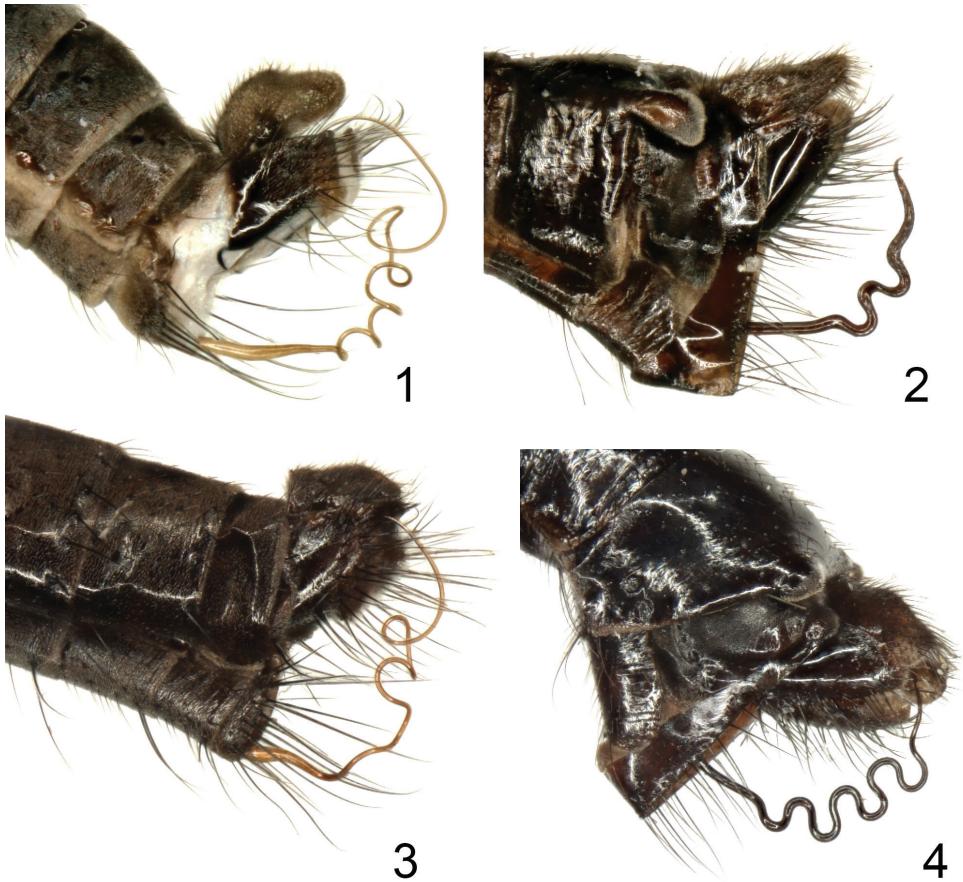
Type material. HOLOTYPE male, Russia, Amurskaja oblast, g. (= city) Zeja, 8.vii.1981, A. Shatalkin, deposited in ZMMU; PARATYPE: 1 male, same data as holotype (CULSP).

Diagnosis. Species of the subgenus *Pararhamphomyia* with pair of long thumb-like dorsomarginal processes on tergite 6, lustrous abdomen, uniserial dorsocentrals and two outgoing loops of the phallus.

Etymology. The species epithet stresses two outgoing loops on the phallus.

Description. Male. Head holoptic, facets in dorsal third of eye enlarged. Frons blackish brown, light grey microtrichose, without setae. Ocellar setae less than half as long as frons, black, ocellar triangle without additional setae. Face blackish brown, light grey microtrichose dorsally and broadly lustrous in ventral third, 0.28 mm broad ventrally and 0.32 mm long, bare. Occiput blackish brown, microtrichose, sparsely black setose, setae rather thick and short, ventrally longer and finer. Antenna black, basal segment brown, length of antennal segments = 11: 12: 40: 19, setae on basal two segments up to 0.10 mm long. Labrum brown, lustrous, slightly longer than head is high. Palpus brown, short, covered with several short setae and one strong preapical seta (0.25 mm long). Gena narrow and lustrous, clypeus lustrous.

Thorax brownish-black, light grey microtrichose (without brownish-yellow tinge), darker stripes below rows of acrostichals and dorsocentrals scarcely visible. All setae black. Chaetotaxy: proepisternum with about 10 setae, both propleural depression and prosternum bare; acrostichals damaged but probably irregularly biserial and short; 8



Figures 1–4. Male terminalia, lateral view: **1** *Rhamphomyia* (*Pararhamphomyia*) *anfractuosa* Bezzi **2** *Rhamphomyia* (*Pararhamphomyia*) *biflexata* sp. n. **3** *Rhamphomyia* (*Pararhamphomyia*) *lineodorsata* sp. n. **4** *Rhamphomyia* (*Pararhamphomyia*) *multisinuosa* Frey.

uniserial dorsocentrals, 0.20 mm long in middle of rows, ending in 2 prescutellars, 1 short intrahumeral, 1 strong posthumeral; postpronotum with 1 strong seta and about 8–10 smaller setae; 3–4 notopleurals (1–2 setae on anterior part of notopleuron); 1 supraalar and 1 prealar; 1 long and 1 shorter postalar; 3 pairs of scutellars (outer pair short); laterotergite with black setae.

Legs brown, lustrous, black setose. Coxae blackish-brown, microtrichose (only hind coxa with lustrous spot anteriorly near base), black setose. One long seta in posteroapical comb of hind tibia. Fore femur with fine anteroventral setae $1/3$ as long as depth of femur, dorsal setae shorter, posteroventrals almost absent. Fore tibia with 4 posterodorsal setae slightly longer than width of tibia, ventral and anterodorsal setae short. Mid femur with two rows of spiny setae ventrally, anteroventrals half as long as depth of femur, posteroventrals longer than depth of femur, other setae short. Mid tibia with two rows of setae dorsally nearly $2\times$ longer than width of tibia (each row consists

of 3–4 setae including preapicals), two rows of ventral setae about as long as width of tibia. Hind femur (Fig. 14) with row of fine anteroventrals shorter than depth of femur, posteroventrals equally fine and irregularly arranged. Hind tibia with 5–6 pairs of antero- and posterodorsal setae 1.5× longer than width of tibia, ventral setae short. Basal tarsomeres of fore and mid legs thin and short setose, with several short ventral spines. Basal tarsomere of hind leg thin, with several setae dorsally 2× longer than diameter of tarsomere and with several ventral spines slightly longer than diameter of tarsomere.

Wing clear to light brownish, stigma slightly darker, veins brown, anal vein almost complete. Costal seta strong and long (several other setae between costal seta and wing base relatively strong and long), axillary angle acute. Measurements: $M_2/d = 1.3$, CuA_1 ratio = 1.6–1.7, $lw/ww = 2.5–3.1$. Halter yellow, calypter yellow with dark fringes.

Abdomen brown, lustrous (only segment 1 and part of segment 7 microtrichose as well as tips of thumb-like processes). All setae dark. Hind marginal setae on sides of tergites 2–4 nearly as long as their corresponding segments (discal setae shorter), on segments 5–6 shorter (but still slightly longer than discal setae), segment 7 very short setose. Dorsum of abdomen with short setae. Tergite 6 (Fig. 2) with two thumb-like processes on dorsal hind side; tergite 8 fused with sternite. Phallus (Fig. 9) with two outgoing loops; hypandrium membranose on posterior part (this part covers only part of ventral “ciliation” of phallus); phallus with hair-like “ciliation” ventrobasally and not much produced basal bulge.

Length: Body 5.5 mm, wing 5.5 mm.

Female. Unknown.

Remarks. *Rhamphomyia (Pararhamphomyia) biflexata* sp. n. may be easily identified according to the key. It is the only species with long dorsomarginal processes on tergite 6 (similarly as *R. shatalkini*) and simultaneously with two outgoing loops on phallus. Female is unknown with certainty, see also Remarks under *R. shatalkini* sp. n.

Distribution. Russia (Far East).

Dates of occurrence. July.

***Rhamphomyia (Pararhamphomyia) lineodorsata* sp. n.**

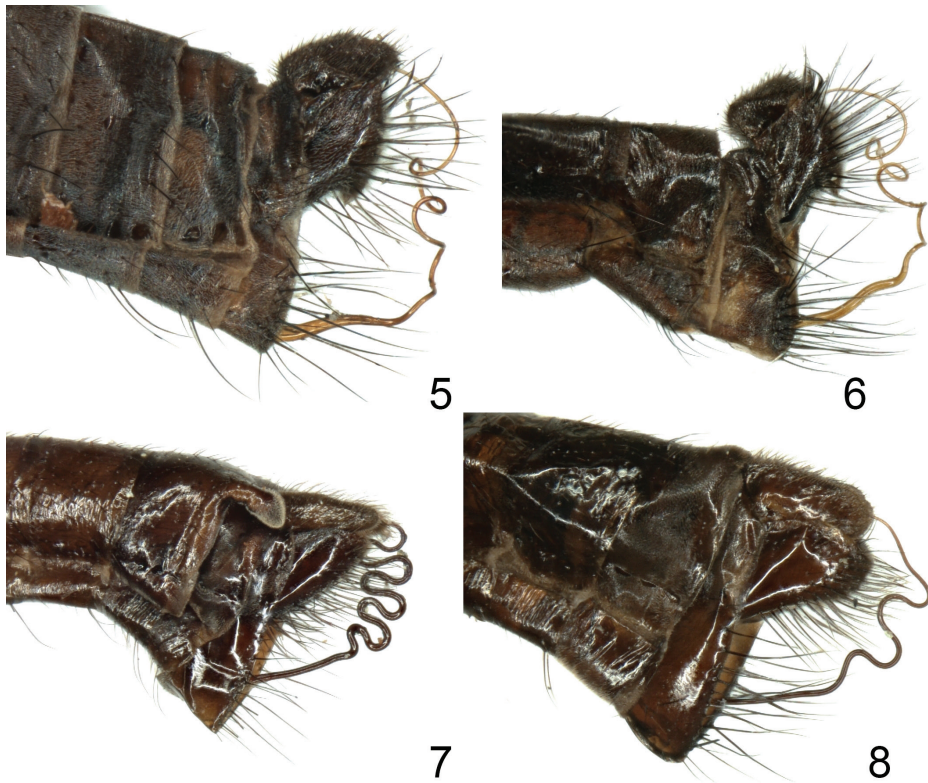
<http://zoobank.org/945770C7-B15A-40B5-B524-5B6548EEC453>

Figs 3, 10, 13

Type material. HOLOTYPE male: Russia, Amurskaja oblast, g. (= city) Zeja, 31.viii.1982, A. Shatalkin, deposited in ZMMU; PARATYPES: 2 males, same data as holotype; 1 male, same locality as holotype, 29.viii.1981, A. Shatalkin; paratypes depositories: ZMMU, CULSP.

Additional material examined (excluded from type series): 2 females, same locality as holotype, 4.ix.1981; 1 female, same locality as holotype, 13.ix.1981 – all A. Shatalkin (ZMMU and CULSP).

Diagnosis. Species of the subgenus *Pararhamphomyia* with phallus forming loops in space, dark brownish black mesoscutum with darker stripes below rows of setae and



Figures 5–8. Male terminalia, lateral view: **5** *Rhamphomyia* (*Pararhamphomyia*) *nudiscutellata* sp. n. **6** *Rhamphomyia* (*Pararhamphomyia*) *robustior* Frey **7** *Rhamphomyia* (*Pararhamphomyia*) *shatalkini* sp. n. **8** *Rhamphomyia* (*Pararhamphomyia*) sp. 2.

uniserial dorsocentrals. Female tergites 6 and 7 lustrous and hind tibia without pen-nate ciliation dorsally.

Etymology. The name of the species is derived from dark stripes on the mesoscutum, differing from closely allied species, *R. robustior* Frey.

Description. Male. Head holoptic, facets in dorsal half of eye enlarged. Frons blackish brown, light grey microtrichose, without setae. Ocellar setae one third as long as frons, black, ocellar triangle with 1–2 pairs of additional setae. Face blackish brown, light grey microtrichose, 0.30 mm broad ventrally and equally long, without setae. Occiput blackish brown, microtrichose, sparsely black setose, setae rather thick and short, longer and finer ventrally. Antenna black, scape and pedicel brown, length of antennal segments = 16–18: 12: 48–50: 16, setae on basal two segments up to 0.12 mm long. Labrum brown, lustrous, slightly longer than height of head. Palpus brown, short, covered with several setae and one strong preapical seta (0.20 mm long). Gena narrow and lustrous, clypeus microtrichose.

Thorax brownish-black, rather dark brownish grey microtrichose, scutum with distinct darker stripes below rows of acrostichals and dorsocentrals (best visible in

posterior view). All setae black. Chaetotaxy: proepisternum with about 10 setae, both propleural depression and prosternum bare; 10–16 narrowly biserial (anteriorly almost uniserial), fine acrostichals about 0.20 mm long; 8–11 almost regularly uniserial dorsocentrals slightly longer (about 0.30 mm in middle of rows), ending in 2–3 long prescutellars, 1–2 strong intrahumerals, 1 strong posthumeral; postpronotum with 2–3 long and 10–15 gradually shorter setae; 4 notopleurals (2–3 long setae on anterior part of notopleuron); 1 long supraalar and 2–3 smaller prealar; 1 long and 1 shorter postalar; 3 pairs of scutellars; laterotergite with black setae.

Legs brown, microtrichose, black setose. Coxae blackish-brown, microtrichose (only hind coxa with lustrous spot anteriorly near base), black setose. One long seta present in comb at tip of hind tibia. Fore femur with fine anteroventral setae up to half as long as depth of femur, dorsal setae shorter, posteroventrals up to half as long as depth of femur, present mostly only on proximal half. Fore tibia with 4–6 posterodorsal setae about as long as width of tibia, ventral and anterodorsal setae short. Mid femur with two rows of spiny setae ventrally, anteroventrals one third as long as depth of femur, posteroventrals slightly longer than depth of femur, other setation short. Mid tibia with only two anterodorsal setae (one subbasal and one preapical – but holotype on one leg atypically with three such setae), and 4–6 posterodorsals slightly longer than depth of tibia, two rows of ventral setae somewhat shorter than width of tibia (several posteroventrals may be longer than remaining ones). Hind femur (Fig. 13) with ventral microtrichosity up to 0.04 mm long, with rather fine anteroventral setae about as long as depth of femur in basal half and sometimes apically, very short to absent on third quarter of femur, posteroventrals present only on extreme base of femur. Hind tibia with 3–5 antero- and 6–8 posterodorsal setae slightly longer than width of tibia, ventral setae short. Basal tarsomeres of fore and mid legs thin and short setose, mid one with several short ventral spines, basal tarsomere of hind leg slightly swollen, with several setae dorsally and spine like setae ventrally up to 2× longer than diameter of tarsomere.

Wing light brown, stigma slightly darker, veins brown (yellowish in basal part of wing), anal vein almost complete or indistinct about middle. Costal seta present, axillary angle acute. Measurements: $M_2/d = 1.4\text{--}1.6$, CuA_1 ratio = $1.4\text{--}2.1$, $lw/ww = 2.7\text{--}3.1$. Halter yellow, calypter brownish-yellow with dark fringes.

Abdomen brown, dark brown microtrichose (dark brown in both lateral and dorsal views), setae all dark. Hind marginal setae almost as long as corresponding segments, discal setae shorter. Dorsum of abdomen with short setae. Phallus (Figs 3, 10) with three twists in space.

Length: Body 4.5–5.3 mm, wing 5.1–5.8 mm.

Female. Head dichoptic, frons approximately 0.35 mm long and 0.25 mm wide, subparallel, with two rows of 5–7 relatively long setae on sides. Face approximately 0.25 mm long and subequally wide in middle (slightly broadening ventrally), without setae. Palpus lighter than in male, yellowish red. Thorax as in male, only setae shorter. Fore femur with anterodorsal row of almost pennate setae slightly shorter than depth of femur, with anteroventral row of thin setae as long as depth of femur and with posteroventral



Figures 9–12. Male genitalia (macrated), lateral view: **9** *Rhamphomyia* (*Pararhamphomyia*) *biflexata* sp. n. **10** *Rhamphomyia* (*Pararhamphomyia*) *lineodorsata* sp. n. **11** *Rhamphomyia* (*Pararhamphomyia*) *nudiscutellata* sp. n. **12** *Rhamphomyia* (*Pararhamphomyia*) *shatalkini* sp. n.

row of pennate setae slightly longer than depth of femur. Fore tibia as in male, only posterodorsal setae less differentiated. Mid femur with both (antero)dorsal and (postero)ventral pennation about as long as depth of femur. Mid tibia with short subpennate ciliation both dorsally and ventrally in addition to several slightly longer setae on both sides. Hind femur with long pennate ciliation ventrally in addition to several setae and shorter dorsal subpennate ciliation. Hind tibia with two rows of dorsal setae slightly longer than width of tibia, ventral setae short and slightly subpennate. Wing light brown as in male, measurements: $M_2/d = 1.4\text{--}1.6$, CuA_1 ratio = $1.5\text{--}1.9$, $lw/ww = 2.8\text{--}3.0$. Abdomen microtrichose, with tergites 6 to 8 and sternite 8 lustrous and sternite 7 sublustrous. Hind marginal setae on segments 2–6 $2/3$ as long as corresponding segments.

Length: Body 5.3–5.8 mm, wing 5.5–6.0 mm.

Remarks. *Rhamphomyia* (*Pararhamphomyia*) *lineodorsata* sp. n. is closely allied to remaining three Palaearctic species of *R. anfractuosa* group of species with phallus twisted in space, viz *R. anfractuosa* Bezzi, *R. nudiscutellata* sp. n. and *R. robustior*. Most

specimens of this complex examined differ from specimens of *R. multisinuosa* complex (with phallus twisted in a single plain) by absence of submedian anterodorsal setae on mid tibia in addition to characters given in the key. However, as mentioned above, holotype of the above described species has atypically one such seta present on one leg. We excluded females from type series because of problems with exact identification of females in this group of species and because we had no pairs taken in copula. But we believe we identified them properly. See under *R. nudiscutellata* for discussions of the differences between females of this complex.

Distribution. Russia (Far East).

Dates of occurrence. August–September.

***Rhamphomyia (Pararhamphomyia) nudiscutellata* sp. n.**

<http://zoobank.org/BB33F445-E025-4126-8C47-007857B89D56>

Figs 5, 11, 15

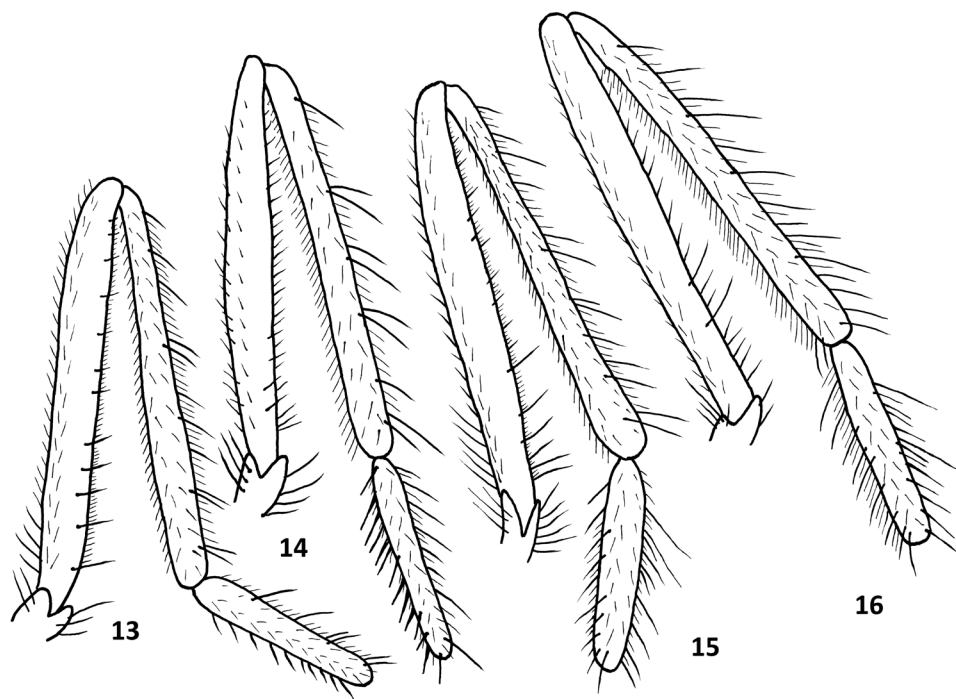
Type material. HOLOTYPE male: Russia, Amurskaja oblast, g. (gorod = city) Zeja, 14.ix.1981, A. Shatalkin, deposited in ZMMU; PARATYPES: 2 males, same data as holotype; 4 males, Tuva, okr. (=region) Baj-Chaaka, Berezovka, 5.ix.1973, V. Kovalev; 2 males, same locality, 3.ix.1973, V. Kovalev; 2 males, same locality, 7.ix.1973, V. Kovalev; 2 males, Tuva, okr. Saryg Sep, listvennicznik na granice lesa (= larch on forest boundary), 28.viii.1973, V. Kovalev; 1 male Chitin, r. (=river) Kuenga, vyche (= above) Chernyshevskaja, 24.viii.1977, V. Kovalev. Paratypes depositories: ZMMU, CULSP.

Additional material examined (excluded from type series): 2 females, Chitin, r. (reka = river) Kuenga, vyche Chernyshevskaja, 26.viii.1977, V. Kovalev; 1 female, Tuva, okr. (= region) Baj-Chaaka, Berezovka, 5.ix.1973, V. Kovalev; 1 female, same locality, 2.ix.1973, V. Kovalev; 2 females, Tuva, okr. (= region) Shagonar, Ishtii-Khem, 21.viii.1973, V. Kovalev; 1 female, same locality, 24.viii.1973, V. Kovalev; (ZMMU and CULSP).

Diagnosis. Species of the subgenus *Pararhamphomyia* with phallus forming loops in space, light grey mesoscutum and uniserial dorsocentrals. Female front femur with two rows of ventral setae longer than depth of femur, hind tibia not pennate, wing light brownish and tergites 6 and 7 lustrous.

Etymology. The name of the species is derived from the relatively naked scutellum bearing only four setae (nudus, Latin = naked).

Description. Male. Head holoptic, facets in dorsal half of eye enlarged. Frons blackish brown, light grey microtrichose, without setae. Ocellar setae one third as long as frons, black, ocellar triangle with 1–2 pairs of additional setae. Face blackish brown, light grey microtrichose, 0.20 mm broad ventrally and 0.25 mm long, without setae. Occiput brownish black, rather light grey microtrichose, black setose. Antenna black, scape and pedicel brown, length of antennal segments = 15–16: 11: 45–50: 12–14, setae on basal two segments about 0.10 mm long. Labrum brown, lustrous, slightly shorter than head is high. Palpus brown, short, covered with several rather long setae



Figures 13–16. Male hind legs (femur, tibia and basitarsus): **13** *Rhamphomyia* (*Pararhamphomyia*) *lineodorsata* sp. n. **14** *Rhamphomyia* (*Pararhamphomyia*) *biflexata* sp. n. **15** *Rhamphomyia* (*Pararhamphomyia*) *nudiscutellata* sp. n. **16** *Rhamphomyia* (*Pararhamphomyia*) *shataalkini* sp. n.

(0.20 mm long), preapical seta poorly differentiated. Gena narrow and lustrous, clypeus microtrichose.

Thorax brownish-black, rather light grey microtrichose, scutum without stripes, only in immature specimens with poorly visible darker stripes below rows of acrostichals and dorsocentrals. All setae black. Chaetotaxy: proepisternum with 5–8 setae, both propleural depression and prosternum bare; 6–10 biserial, short and fine acrostichals (about 0.15 mm long); 7–10 regularly uniserial dorsocentrals (about 0.25 mm in middle of rows), ending in 2 long prescutellars, 1–2 fine and long intrahumerals, 1 strong posthumeral; postpronotum with 2–3 long and 6–10 gradually shorter setae; 3 notopleurals (0–2 long setae on anterior part of notopleuron); 1 long supraalar, prealar absent; 1 long and 1 shorter postalar; 2 pairs of scutellars; laterotergite with black setae.

Legs brown, microtrichose, black setose. Coxae blackish-brown, microtrichose (only hind coxa with lustrous spot anteriorly near base), black setose. One long seta present in comb at tip of hind tibia. Fore femur with complete rows of fine antero- and posteroventral setae up to 2/3 as long as depth of femur, dorsal setae shorter. Fore tibia short setose, without differentiated setae except preapical. Mid femur with two rows of spiny setae ventrally, anteroventrals up to one-third as long as depth of femur, posteroventrals slightly longer than depth of femur, other setation short. Mid

tibia with only two anterodorsal setae (one short subbasal and one long preapical), and 3–4 posterodorsals slightly longer than depth of tibia, two rows of ventral setae somewhat shorter than width of tibia (several posteroventrals may be longer than remaining ones). Hind femur (Fig. 15) with ventral microtrichosity up to 0.05 mm long, with rather fine anteroventral setae about as long as depth of femur in basal half and in some specimens apically, very short to absent on third quarter of femur, posteroventrals present only on extreme base of femur. Hind tibia with 3–4 antero- and 5–6 posterodorsal setae slightly longer than width of tibia, ventral setae short. Basal tarsomeres of fore and mid legs thin and short setose, mid basal tarsomere with several short ventral spines; basal tarsomere of hind leg slightly swollen, with several setae dorsally and spine like setae ventrally up to 2× longer than diameter of tarsomere.

Wing clear, stigma scarcely darker, veins brown and yellowish in basal part of wing, anal vein indistinct about middle. Costal seta present, axillary angle right. Measurements: $M_2/d = 1.2\text{--}1.7$, CuA_1 ratio = 1.6–1.9, $lw/ww = 2.7\text{--}3.3$. Halter yellow, calypter yellow with dark fringes.

Abdomen brown, entirely light grey microtrichose (light grey from both lateral and dorsal views), setae all dark. Hind marginal setae on tergites 2–3 somewhat longer and on tergites 4–6 as long as corresponding segments, discal setae shorter. Dorsum of abdomen with short setae. Phallus (Figs 5, 11) with three twists in space.

Length: Body 3.8–4.0 mm, wing 4.5–5.8 mm.

Female. Head dichoptic, frons approximately 0.25 mm long and 0.20 mm wide, subparallel, with two rows of 4–6 relatively long setae on sides. Face approximately 0.20 mm long and subequally wide in middle (strongly divergent ventrally), without setae. Palpus brown as in male. Thorax as in male. Fore femur with both antero- and posteroventral rows of setae as long as depth of femur, posteroventrals mostly thin but in some specimens on one or both legs thickened – almost pennate, dorsal ciliation short and thin. Fore tibia as in male, only posteroventral setae slightly differentiated. Mid femur with both (antero)dorsal and (postero)ventral pennation about as long as depth of femur. Mid tibia short setose, most specimens with several antero- and posteroventral setae and/or several setae dorsally shorter than depth of tibia. Hind femur with long pennate ciliation ventrally in addition to several setae and shorter dorsal subpennate ciliation. Hind tibia slightly flattened, with two rows of dorsal setae slightly longer than width of tibia, ventral setae short, short ciliation slightly subpennate. Wing clear with only indistinct brownish tinge, measurements: $M_2/d = 1.3\text{--}1.6$, CuA_1 ratio = 1.6–1.8, $lw/ww = 2.8\text{--}3.0$. Abdomen microtrichose, with tergites 6 to 8 and sternites 7 and 8 lustrous. Hind marginal setae on segments 2–4 as long as corresponding segments, on segments 5–7 2/3 as long as corresponding segments.

Length: Body 4.0–4.4 mm, wing 4.6–5.2 mm.

Remarks. *Rhamphomyia* (*Pararhamphomyia*) *nudiscutellata* sp. n. is closely allied to the remaining three Palearctic species of the *R. anfractuosa* group of species with phallus twisted in space, viz *R. anfractuosa*, *R. lineodorsata* sp. n. and *R. robustior*. All four species may be identified according to the key. We excluded females from the type series because of problems with exact identification of females in this group of species

and because we had no pairs taken in copula. But we believe we identified them properly. Female differs from *R. robustior* and *R. anfractuosa* in lustrous abdominal tergites 6 and 7 (microtrichose in both *R. anfractuosa* and *R. robustior*), from *R. lineodorsata* by brown palpus (yellowish red in *R. lineodorsata*) and from *R. anfractuosa* also by front femur with two rows of ventral setae longer than depth of femur, hind tibia without broad pennate ciliation and light brownish wing (in *R. anfractuosa*, front femur has almost no ventral setae, hind tibia is broadly pennate both dorsally and ventrally and wing is deep brown).

Distribution. Russia (Far East).

Dates of occurrence. August–September.

***Rhamphomyia (Pararhamphomyia) shatalkini* sp. n.**

<http://zoobank.org/11ADE0FE-A4E1-48FB-BAF6-1923EBE2BC2A>

Figs 7, 12, 16

Type material. HOLOTYPE male: Russia, Amurskaja oblast, g. (= city) Zeja, 22.vi.1978, leg. A. Shatalkin, deposited in ZMMU; PARATYPES: 2 males, same data as holotype; 2 males, same locality, 23.vi.1978; 2 males, same locality, 24.vi.1978; 1 male, same locality, 21.vi.1979 – all A. Shatalkin; 1 male, same locality, 25.vi.1982, A. Ozerov; 3 males, Russia, Juzhnoje Primorije, Kamenushka, 9.vi.1984, A. Shatalkin; 1 male, Russia, Irkutskaja o. (= oblast, = region), Listvjanka, 21.vi.1965, O.P. Negrobov; paratypes depositories: ZMMU, CULSP.

Diagnosis. Species of the subgenus *Pararhamphomyia* with uniserial dorsocentrals, lustrous abdomen, tergite 6 with two thumb-like dorsomarginal processes, mesoscutum without lustrous stripes and phallus with four outgoing loops.

Etymology. The species is named after Anatole Shatalkin, dipterist from Moscow Museum and collector of part of type series.

Description. Male. Head holoptic, facets in dorsal third of eye enlarged. Frons blackish brown, light grey microtrichose, without setae. Ocellar setae fine, half as long as frons, black, ocellar triangle without additional setae. Face blackish brown, light grey microtrichose dorsally and broadly lustrous along ventral margin, 0.25 mm broad ventrally and 0.30 mm long, without setae. Occiput blackish brown, grey microtrichose, sparsely black setose, setae rather thick and short, ventrally longer and finer. Antenna black, scape brown, pedicel and extreme base of postpedicel brownish-yellow, length of antennal segments = 13: 10: 40: 17, setae on basal two segments nearly 0.12 mm long. Labrum brown, lustrous, about as long as or slightly longer than height of head. Palpus brown, short, with several setae almost 0.30 mm long. Gena narrow and lustrous, clypeus lustrous.

Thorax brownish-black, light grey microtrichose (with slight brownish-yellow tinge), scutum with somewhat darker stripes below rows of acrostichals and dorsocentrals. All setae black. Chaetotaxy: proepisternum with 10–15 setae, both propleural depression and prosternum bare; 14–20 irregularly biserial, fine acrostichals about 0.20

mm long; almost regularly uniserial slightly longer dorsocentrals (about 0.25 mm in middle of rows), ending in 2–3 strong and long prescutellars, 1 small intrahumeral, 1 strong posthumeral; postpronotum with 1 strong seta and about 15 additional finer setae; 4 notopleurals (1–2 long setae on anterior part of notopleuron); 1 supraalar and 1 equally strong prealar; 1 long and 1 shorter postalar; 3 pairs of scutellars (rarely two pairs); laterotergite with black setae.

Legs brown, lustrous, black setose. Coxae blackish-brown, microtrichose (only hind coxa with two lustrous spots anteriorly near base and at apex), black setose. One long seta in posteroapical comb of hind tibia. Fore femur with fine anteroventral setae $1/3$ as long as depth of femur, posteroventral and dorsal setae shorter. Fore tibia with 4–5 strong posterodorsal setae $2\times$ longer than width of tibia, ventral and anterodorsal setae short. Mid femur with two rows of spiny setae ventrally, anteroventrals half as long as depth of femur and densely arranged, posteroventrals sparse and longer than depth of femur, other setation short. Mid tibia with two rows of setae dorsally nearly $2\times$ longer than width of tibia (each row consists of 4–5 setae), row of short posteroventral setae, anteroventrals more irregularly arranged and somewhat longer than posteroventrals. Hind femur (Fig. 16) with anteroventral row of rather fine setae nearly as long as depth of femur (1 or 2 of them may be stronger than remaining), other setae including posteroventrals short and fine. Hind tibia about as thick as hind femur, with 6–8 pairs of antero- and posterodorsal setae $1.5\times$ longer than width of tibia, ventral setae short. Basal tarsomeres of fore and mid legs thin and short setose, with several short ventral spines. Basal tarsomere of hind leg thin, with several setae dorsally $2\times$ longer than width of tarsomere and with several ventral spines slightly longer than width of tarsomere.

Wing light brownish, stigma slightly darker, veins brown, anal vein almost complete. Costal seta strong and long (several other setae between costal seta and wing base relatively strong and long), axillary angle acute. Measurements $M_2/d = 1.3\text{--}1.4$, CuA_1 ratio = $2.2\text{--}2.5$, $lw/ww = 2.6\text{--}3.0$. Halter yellow, calypter brownish-yellow with dark fringes. Abdomen brown, lustrous (only segment 1 and small spots on 3 pregenital segments microtrichose). All setae dark. Hind marginal setae on sides of tergite 2 nearly as long as segment, on segments 3–5 gradually shorter (discal setae shorter), marginals on tergite 6 short, tergite 7 bare. Dorsum of abdomen with short setae. Abdominal tergite 6 with two thumb-like processes dorsally (Fig. 7). Phallus (Fig. 12) with four outgoing loops in a single plain; phallus with hair-like “ciliation” ventrobasally and produced basal bulge; hypandrium membranose on posterior part (this part covers whole ventrobasal “ciliation” of phallus).

Length. Body 5.3–6.4 mm, wing 5.7–6.4 mm.

Female. Unknown.

Remarks. *Rhamphomyia* (*Pararhamphomyia*) *shatalkini* sp. n. may be easily distinguished from all other Palaearctic species of *Rhamphomyia* (except unnamed species *R. sp. 1*) by peculiar shape of phallus forming four outgoing loops in a single plain and simultaneously tergite 6 bearing two long thumb-like dorsomarginal processes. However, the mesoscutum in the new species is entirely microtrichose but with three lustrous stripes below lines of setae in *R. sp. 1*. Other species with similarly formed

phallus are *R. multisinuosa* Frey and *R. spectabilis* Frey, both without long thumb-like processes on tergite 6. Female of *R. shatalkini* remains unknown with certainty. We have at our disposal several females which may belong to either *R. shatalkini*, *R. spectabilis* or *R. biflexata*, but we are unable to associate them with particular males. Males of all three species have very similar microtrichosity pattern of mesoscutum which otherwise helps to associate males with females even if not taken in copula. These females differ from all other Palearctic *Pararhamphomyia* by the following combination of characters: body entirely dark setose, dorsocentrals almost regularly uniserial, halter yellow, clypeus lustrous, both mid and hind femora broadly pennate, tibiae without pennation and abdomen lustrous except the first segment.

Distribution. Russia (Far East).

Dates of occurrence. June.

Unnamed species

Rhamphomyia (*Pararhamphomyia*) sp. 1

Material examined. 1 male, Russia, Amurskaja oblast, g. (= city) Zeja, 26.vi.1982, M. Krivosheina, deposited in ZMMU.

Remarks. Species very similar to *R. shatalkini* sp. n. with the exception of characters given in the key. We hesitate to describe new species from only a single specimen.

Rhamphomyia (*Pararhamphomyia*) sp. 2

Material examined. 1 male, Russia, Amurskaja oblast, g. (= city) Zeja, 26.vii.1978, A. Shatalkin, deposited in ZMMU.

Remarks. Species very similar to *R. spectabilis* Frey with the exception of characters given in the key. We hesitate to describe new species from only a single specimen; moreover, we do not have *R. spectabilis* at our disposal.

Key to males of Palearctic species of the *Rhamphomyia anfractuosa* group

- 1 Phallus forms loops in space. Abdomen microtrichose. Hind femur with ventral microtrichosity. No modifications of tergites 6 and 7. No lustrous stripes below rows of acrostichals and dorsocentrals.....2
- Phallus forms loops in flat plain. Abdomen lustrous. Hind femur without ventral microtrichosity. Tergites 6 and/or 7 modified. Lustrous stripes below rows of acrostichals and dorsocentrals present or absent.....5
- 2 (1) Mesoscutum dark brownish black. Abdomen dark brown. Usually three (rarely four) pairs of scutellar setae. Axillary angle acute.....3

- Mesoscutum light grey microtrichose. Abdomen light grey. Usually two (rarely one or three) pairs of scutellar setae. Axillary right angled **4**
- 3 (2) Mesoscutum with distinct darker stripes below acrostichals and dorsocentrals. Dorsocentrals regularly uniserial (Additional characters opposite of *R. nudiscutellata*: fore tibia with differentiated posterodorsal setae, wing light brown). (Additional characters: female tergites 6 and 7 lustrous and hind tibia without pennate ciliation dorsally, palpus yellowish red). Phallus as in Fig. 3 ***R. lineodorsata* sp. n.**
- Mesoscutum subpolished, without distinct stripes. Dorsocentrals irregularly biserial. (Additional characters: female tergites 6 and 7 microtrichose and hind tibia with broad pennate ciliation dorsally). Phallus as in Fig. 6 ***R. robustior* Frey**
- 4 (2) Hind marginal setae on tergites 5–6 nearly absent. Wings brown. Antennal stylus slightly shorter than postpedicel. Phallus with five twists (Fig. 1). (Additional characters: female front femur nearly without setae, hind tibia broadly pennate both dorsally and ventrally, wing deep brown and tergites 6 and 7 microtrichose) ***R. anfractuosa* Bezzi**
- Hind marginal setae on tergites 5–6 about as long as these segments. Wings clear. Antennal stylus 1/4 as long as postpedicel. Phallus with three twists (Fig. 5). (Additional characters opposite of *R. lineodorsata*: fore tibia without differentiated posterodorsal setae, wing clear) (Additional characters: female fore femur with two rows of ventral setae longer than depth of femur, hind tibia without pinnate setae, wing light brownish and tergites 6 and 7 lustrous) ***R. nudiscutellata* sp. n.**
- 5 (1) Phallus with two outgoing loops (Fig. 2) ***R. biflexata* sp. n.**
- Phallus with 3 or 4 outgoing loops **6**
- 6 (5) Tergite 6 with two long thumb-like dorsomarginal processes **7**
- Tergite 6 without processes or with only small triangular shaped dorsomarginal projections **8**
- 7 (6) Mesoscutum microtrichose, without lustrous stripes. Dorsocentrals uniserial. Syntergosternite 8 without dorsomarginal projections. Phallus as in Fig. 7 ... ***R. shatalkini* sp. n.**
- Mesoscutum with lustrous stripes below rows of acrostichals and dorsocentrals. Dorsocentrals irregularly biserial. Syntergosternite 8 with two dorsomarginal triangular projections ***R. sp. 1***
- 8 (6) Tergite 6 with two small triangular shaped dorsomarginal projections. Mesoscutum with lustrous stripes below rows of acrostichals and dorsocentrals. Phallus as in Fig. 4 ***R. multisinuosa* Frey**
- Tergite 6 without projections. Mesoscutum microtrichose, without lustrous stripes **9**
- 9 (8) Dorsocentrals irregularly biserial. Phallus with four outgoing loops ***R. spectabilis* Frey**
- Dorsocentrals uniserial. Phallus with three outgoing loops (Fig. 8) ... ***R. sp. 2***

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An Asiatic Chironomid in Brazil: morphology, DNA barcode and bionomics

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Abstract

In most freshwater ecosystems, aquatic insects are dominant in terms of diversity; however, there is a disproportionately low number of records of alien species when compared to other freshwater organisms. The Chironomidae is one aquatic insect family that includes some examples of alien species around the world. During a study on aquatic insects in Amazonas state (Brazil), we collected specimens of Chironomidae that are similar, at the morphological level, to *Chironomus kiiensis* Tokunaga and *Chironomus striatipennis* Kieffer, both with distributions restricted to Asia. The objectives of this study were to provide morphological information on this *Chironomus* population, to investigate its identity using DNA barcoding and, to provide bionomic information about this species. *Chironomus* DNA barcode data were obtained from GenBank and Barcode of Life Data Systems (BOLD) and, together with our data, were analyzed using the neighbor-joining method with 1000 bootstrap replicates and the genetic distances were estimated using the Kimura-2-parameter. At the morphological level, the Brazilian population cannot be distinguished either from *C. striatipennis* or *C. kiiensis*, configuring a species complex but, at the molecular level our studied population is placed in a clade together with *C. striatipennis*, from South Korea. Bionomic characteristics of the Brazilian *Chironomus* population differ from the ones of *C. kiiensis* from Japan, the only species in this species complex with bionomic information available. The Brazilian *Chironomus* population has a smaller size, the double of the number of eggs and inhabits oligotrophic water, in artificial container. In the molecular analysis, populations of *C. striatipennis* and *C. kiiensis* are placed in a clade, formed by two groups: Group A (which includes populations from both named species, from different Asiatic regions and our Brazilian population) and Group B (with populations of *C. kiiensis* from Japan and South Korea). Genetic distance between the Brazilian population and specimens in Group A suggests that it was recently introduced in Brazil, and that its country of origin is probably South Korea.

Keywords

Aquatic insects, non-native species, Amazonas, *Chironomus kiiensis*, *Chironomus striatipennis*, sibling species

Introduction

Alien species represent one of the most serious threats to biodiversity at different taxonomic levels (Mack et al. 2000) including freshwater ecosystems (Gherardi 2007). Human activities have been contributing to the increase and to the strengthening of this process (Lodge 1993) as many species can be transported around the world on human transportation systems such as ships, airplanes and automobiles. Examples include the green crab (*Carcinus maenas* Linnaeus, 1758), mud crab (*Rhithropanopeus harrisi* Gould, 1841) and blue mussel (*Mytilus galloprovincialis* Lamark, 1819), which were recorded being transported in ballast tanks (Briski et al. 2012). The Asian tiger mosquito (*Aedes albopictus* (Skuse, 1894)), which is a vector of the dengue viruses, was introduced in several countries through the importation of tires from Asia (Fontenille and Toto 2001).

Despite their dominance in terms of diversity in most freshwater ecosystems, aquatic insects have a disproportionately low number of alien species when compared to other freshwater macroinvertebrates (Karatayev et al. 2009). Exceptions include several examples of recognized alien species of Ephemeroptera (Zimmermann 1957), including one in Brazil, a Baetidae species from Africa reported in Brazil's Espírito Santo state (Salles et al. 2014).

Among the necessary characteristics for a species to become a successful invasive alien are: phenotypic plasticity, ability for uniparental reproduction and fast growth in disturbed habitats (Kleunen et al. 2010). Additional important characteristics for alien aquatic insects are: generalist feeding (e.g., detritivores), year-round breeding capacity, ability to colonize peri-urban environments and artificial water bodies, and the climatic similarity of invaded and source environments (De Moor 1992). Chironomidae species often have characteristics mentioned above, and cases of successfully introduced *Chironomus* species have been reported around the world (Jacobsen and Perry 2007, Hribar et al. 2008; Gray et al. 2012).

During a study on aquatic insects in Amazonas state (Brazil), we collected specimens of Chironomidae that were similar, at the morphological level, to *Chironomus kiiensis* Tokunaga and, we have named it as such (Lacerda et al. 2014). However, Martin (2014) reported that *Chironomus striatipennis* Kieffer is morphologically similar to *C. kiiensis* and, that the latter is treated as a junior synonym of *C. striatipennis*; cytogenetic studies also indicated that both species are included in the pseudothummi-cyctocomplex species. Both species have geographic distribution restricted to Asia. *Chironomus striatipennis* is widely distributed in South and Southeast Asia and it is a common species in rice fields and other wetlands in India (Chaudhuri and Chattopadhyay 1990), while *C. kiiensis* is reported as the most prevalent species in South Korea and Japan (Ree 1993). The problem is that identification of these species is based, mainly, on their geographical distribution since it is not possible to distinguish them at the mor-

phological level (Martin 2014). This fact results in specimens collected in South and Southeast Asia being identified as *C. striatipennis* and those collected in South Korea and Japan being identified as *C. kiiensis* (e.g., Nath and Lakhota 1989; Yong et al. 1999; Jeong et al. 2004; Nandi et al. 2011).

In view of this complex situation, our objectives were to register a *Chironomus* population of this Asiatic species complex in Brazil, to provide morphological information on this population, to investigate its identity using DNA barcoding and, to provide bionomic information about this species.

Methods

Study area and field collection

Egg masses of the *Chironomus* Brazilian population were collected in tap water accumulated in a 10 L plastic container, for several days (5th, 6th, 8th, 10th and 14th), in January 2011 in the urban area of Manaus municipality Amazonas, Brazil (03°06'50.17"S, 59°58'30.99"W). The egg masses were placed individually in 80 mL plastic vials, which were labeled with collection information, covered with a screen and observed daily until the larvae hatched and abandoned the gelatinous mass. Larvae from each egg batch were transferred to a plastic tray (19.5×31×6.5 cm) containing burned sand as substrate and 1.5 L of water (pH = 5.9; electrical conductivity of 20.7 $\mu\text{S cm}^{-1}$). The trays were covered with wooden structures (40×21×32 cm) serving as frames for screens (2 mm mesh), following a model modified from Fonseca and Rocha (2004) for retention of adults and behavioral observations.

Larvae were fed fish food (TETRAMIM®) every 48 hours. The colony established using this collected material was kept in the insect-raising facility at the Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia (INPA), at environmental conditions similar to those of the climate in Manaus during the months from May to September 2011: temperature of 26 ± 0.3 °C; air humidity of $75 \pm 7.7\%$ and photoperiod of 12/12 hours (data obtained from <http://www.inmet.gov.br>). The specimens analyzed in the present study were obtained from this colony.

Species identification based on morphological and molecular analysis

Emerged adults with pupal and larval exuviae from the colony were dissected and mounted on slides with Euparal® following the procedures outlined by Epler (1988). The morphological characteristics analyzed were male genitalia, anal spur and distribution of shagreen in pupae and, in structures present on the larval head capsule (antenna, pecten epipharyngis, premandible, mandible, mentum, labral setae). Comparisons between the Brazilian *Chironomus* population (n = 40; 10 adult male, 10 adult female, 10 pupae and 10 larvae) and other *Chironomus* species were made using available species descriptions

and related literature (Kieffer 1910; Tokunaga 1936; Chaudhuri et al. 1992; Martin 2014). We also examined specimens of *C. striatipennis* (n = 4; 1 male adult, 1 pupa and 2 larvae) from India (lent by Dr. Jon Martin, University of Melbourne, Australia) and specimens of *C. kiiensis* (n = 30; 10 male adult, 5 adult female, 5 pupae and 10 larvae) from Japan (lent by Dr. Masaru Yamamoto, from Yamaguchi Prefecture). The measurements and images presented in this study were obtained using an Olympus compound microscope with a mounted digital photographic camera, model Olympus DP72, using Cell D (Olympus) software and a stereomicroscope (Leica M165C), with Leica software (auto montage, Application Suite V3).

Molecular analyses were done using the DNeasy Blood & Tissue (Qiagen) kit following the manufacturer's recommendations. Amplifications of the extracted DNA from three specimens (2 larvae and 1 adult male) of the Brazilian *Chironomus* population were made using primers developed by Folmer et al. (1994) that are specific for the Cytochrome Oxidase I (COI) gene in the mitochondrial DNA. The amplified fragments were purified by the EXO-SAP (Exonuclease I-Shrimp Alkaline Phosphatase) method and sent to the Centro de Estudo do Genoma Humano (Universidade de São Paulo), where they were sequenced using an ABI 3730 DNA Analyzer.

Sequences for 14 *Chironomus* species and *Lipiniella fujiprimus* (Sasa, 1985) available at the GenBank and Barcode of Life Data Systems (BOLD) were used in the analysis (accession numbers in Table 1). The alignment and editing of the sequences were done in BioEdit software v.5.0.6 (Hall 1999). A tree was constructed using the neighbor-joining method with 1000 bootstrap replicates, and genetic distances were estimated using the Kimura-2-parameter (K_2P) model in the Mega5 program (Tamura et al. 2011).

Voucher specimens of the Brazilian population are deposited in the Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia. Haplotype sequences are deposited in GenBank under the accession numbers KJ424334–KJ424336.

Biological information

The egg masses were characterized by their shape, length, width and number of eggs; the maximum length and width of eggs were measured. In order to determine the development time of the egg stage, five egg masses were isolated and observed every hour until first instar hatched.

Ten egg masses were isolated to determine the development time of each of the four larval instars; starting from the moment at which the first instar hatched, three larvae were fixed (in 80% ethanol) daily, until the last larva of the egg mass pupated. To identify the instar of each of the fixed larvae; they were mounted between slide and coverslip (using Hoyer as the mounting medium) to measure the ventral length of the head capsule, following the methodology of Strixino (1973). To classify larvae into one of the four larval instars, the measurements obtained were subjected to a frequency-distribution analysis; each peak in the graph indicates a larval instar (Strixino 1973). The development time of each instar was determined based on the size limits of each

Table 1. GenBank and BOLD accession numbers of the sequences from species of *Chironomus* and *Lipiniella fujiprimus* included in the analysis.

Species	Accession numbers	Reference
<i>C. kiiensis</i>	JF412086–JF412089*;	Kim et al. 2012
	KC407765*; AB838642–AB838646*; JQ350720*; AB740240–AB740241*	-
<i>C. striatipennis</i>	COTW008-08, COTW011-08, COTW012-08, COTW027-10**	-
<i>C. balatonicus</i>	JN016827*	-
<i>C. calligraphus</i>	KF278357*;	Proulx et al. 2013
	COTW041-11–COTW042-11**	-
<i>C. columbiensis</i>	COTW001-08–COTW002-08**	-
<i>C. curabilis</i>	JN016822*	-
<i>C. flaviplumus</i>	JF412077*	Kim et al. 2012
<i>C. javanus</i>	JF412085*	Kim et al. 2012
<i>C. nipponensis</i>	JN887053*	-
<i>C. plumosus</i>	JF412198*	Kim et al. 2012
<i>C. salinarius</i>	KC250756*	Kim et al. 2012
<i>C. usenicus</i>	JN016819*	-
<i>C. xanthus</i>	DQ648209*	-
<i>L. fujiprimus</i>	JF412078*	Kim et al. 2012

*GenBank; **BOLD; - data unpublished.

larval instar obtained in the frequency-distribution graph and the day when they were preserved. The development time of each larval instar was determined by combining information gathered from the frequency distribution graph with the measurements of the head capsule of the larvae collected daily.

To estimate pupal development time, 50 pupae were observed every 12 hours from the moment of pupation until adult emergence. Longevity of adults was estimated using 50 adults that emerged in the laboratory on the same day; these were isolated in pairs (male and female) in cages made of PET bottles and observed until there were no more survivors.

Results

Morphological analysis

The morphology of the Brazilian *Chironomus* population (adult, pupal and larval) is identical to that presented in the original descriptions of *C. striatipennis* and of *C. kiiensis* (Kieffer 1910; Tokunaga 1936), and in other taxonomic papers on these two species (Chaudhuri et al. 1992; Martin 2014), it being impossible to distinguish them from each other morphologically. The examined specimens of *C. striatipennis* (Fig. 1A, D, G) from India and of *C. kiiensis* (Fig. 1B, E, H) from Japan were also morphologically indistinguishable from the Brazilian *Chironomus* population (Fig. 1C, F, I).

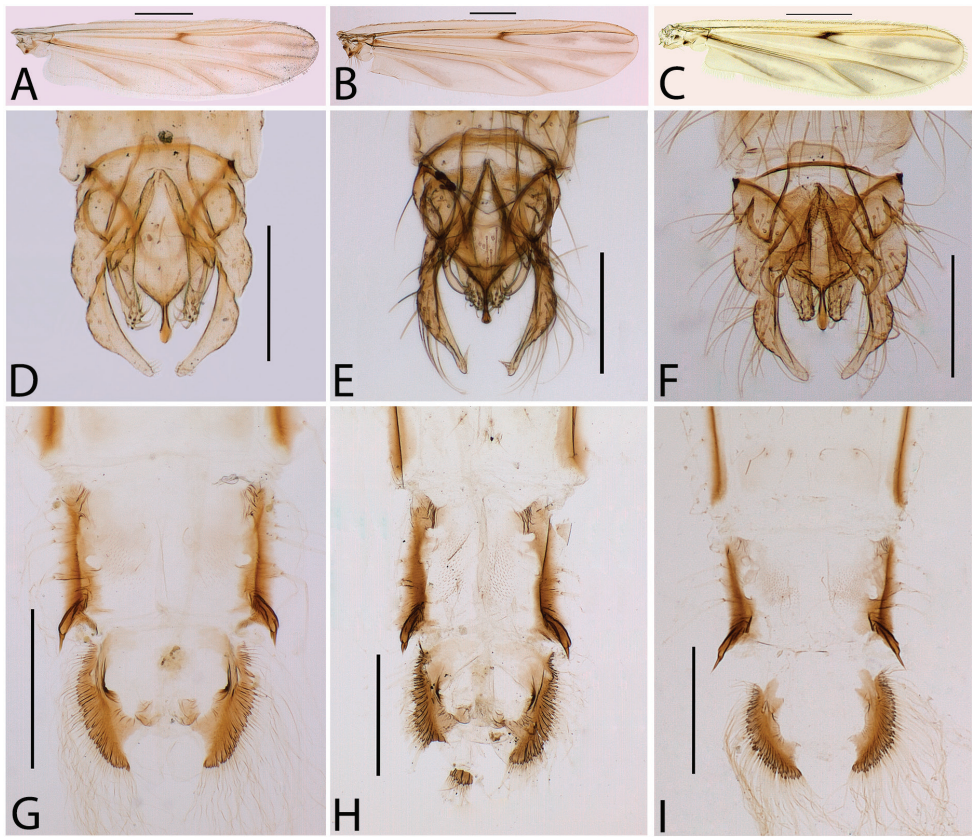


Figure 1. Adult male and pupae. *Chironomus striatipennis*, Indian population. **A** Wing **D** Hypopygium, dorsal view **G** Anal spur, dorsal view. *Chironomus kiiensis*, Japanese population **B** Wing **E** Hypopygium, dorsal view **H** Anal spur, dorsal view. *Chironomus striatipennis*, Brazilian population **C** Wing **F** Hypopygium, dorsal view **I** Anal spur, dorsal view. Scale bar: 500 μm (**A**, **B**, **C**, **G**, **H**, **I**); 200 μm (**D**, **E**, **F**).

Molecular analysis

In the neighbor-joining tree (Fig. 2), we observed that the Brazilian *Chironomus* population (*C. sp1BRA*, *C. sp2BRA* and *C. sp3BRA*) grouped, with 94% bootstrap support, with others specimens of *C. striatipennis* and of *C. kiiensis* from different regions of East Asia. The analysis resulted in two groups, each with 100% bootstrap support, which we named “Group A” and “Group B”. Group A is composed of the Brazilian *Chironomus* population and specimens identified as *C. kiiensis* from South Korea and Japan and *C. striatipennis* from Malaysia and India. Group B included specimens identified as *C. kiiensis*, also from South Korea and Japan.

Chironomus species from other regions, including the three specimens from Brazil, were included in a distinct clade (Fig. 2). Considering that Groups A and B represent monophyletic groups, based on the intraspecific genetic distance between their members, we observe that in Group A the genetic distance varied from 0.0 to 3.3%, while in

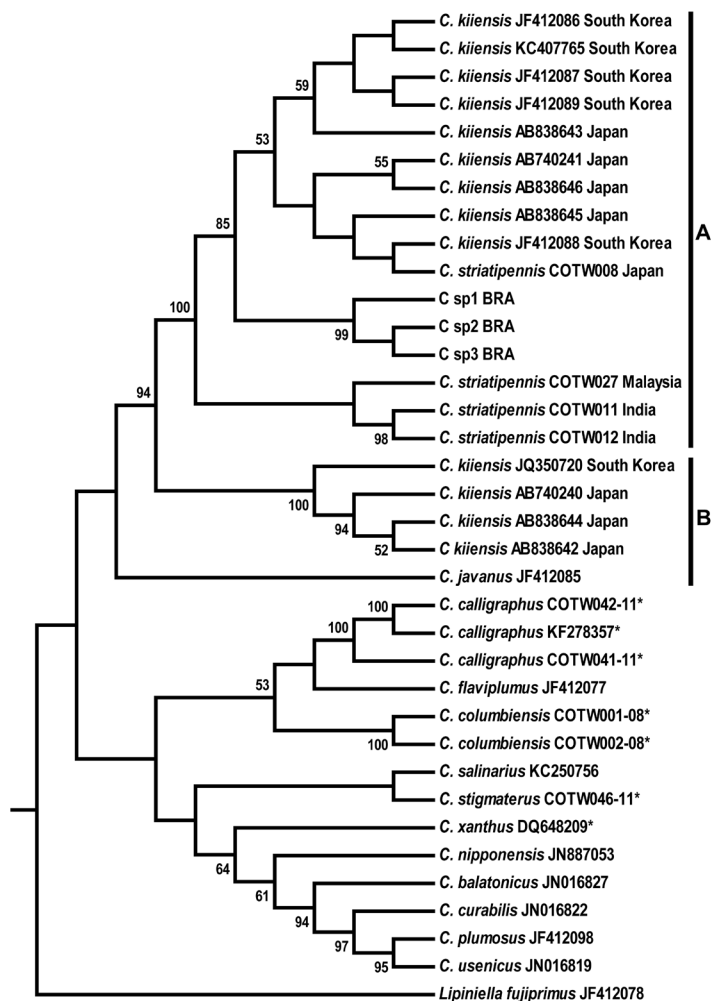


Figure 2. NJ tree based on the COI sequences of the mtDNA of *Chironomus* (Diptera: Chironomidae) species. The sequence of *Lipiniella fujiiprimus* was used as the outgroup. Bootstrap values > 50% are shown on branches. Accession numbers and countries are provided beside the species names. Species flagged with an asterisk (*) are neotropical species. Brazilian *Chironomus* population: *C. sp1BRA*; *C. sp2BRA*; *C. sp3BRA*

Group B the distance varied from 0.6 to 2.0% (Table 2). The average genetic distance between sequences from Groups A and B was 9.6% (Table 3), and within each group the average genetic distance was 1.3%.

Within Group A there are three specimens of *C. striatipennis* from Malaysia and India with a mean genetic divergence of 2.6%, a higher value considering that the mean divergence between the remaining group members was 0.6% (Table 3). The mean genetic distance between Groups A, B and other *Chironomus* species, including Neotropical species, was 15.6%, ranging from 10.5 to 19.5% (Table 3).

Table 2. Genetic distance between Groups A and B and other *Chironomus* species based on the COI gene in the mtDNA. Values in bold in the first two lines indicate genetic distance within each group, and in the remaining lines bold values indicate intraspecific genetic distances. Analyses were conducted using Kimura-2-parameter model.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1.	Group A	1.3												
2.	Group B	9.6	1.3											
3.	<i>C. nipponensis</i>	17.0	15.6											
4.	<i>C. plumosus</i>	17.2	19.5	12.0										
5.	<i>C. flaviplumus</i>	14.4	13.3	13.5	17.9									
6.	<i>C. javanus</i>	13.3	13.6	15.4	16.7	15.0								
7.	<i>C. curabilis</i>	17.2	17.0	11.0	5.5	16.3	17.4							
8.	<i>C. usenicus</i>	16.5	17.5	10.5	3.1	16.8	17.2	4.0						
9.	<i>C. balatonicus</i>	18.2	17.2	12.0	8.8	16.1	16.5	8.4	8.2					
10.	<i>C. salinarius</i>	17.9	15.7	15.4	18.7	15.1	18.3	16.4	17.0	18.4				
11.	<i>C. calligraphus</i>	14.8	16.1	16.1	17.2	12.1	15.5	17.2	16.2	16.3	17.0	1.8		
12.	<i>C. columbiensis</i>	13.7	12.2	14.3	15.7	11.7	13.8	15.6	15.0	16.6	16.6	12.3	1.2	
13.	<i>C. stigmatheus</i>	16.9	15.6	16.1	18.6	16.2	15.3	17.0	17.6	19.6	15.7	17.5	15.7	
14.	<i>C. xanthus</i>	10.5	10.6	10.1	5.9	13.8	9.4	7.4	6.7	6.5	12.3	9.7	10.5	13.8

Biological information

Egg masses of the Brazilian *Chironomus* population were found attached by a stem in the wall of a plastic tray with water. The egg masses measured 16.6 mm (SD = 3.1; n = 5) in length and 1.59 mm (SD = 0.04; n = 5) in width (median region). Each mass contained an average of 600 eggs (SD = 104; n = 5). The eggs were elliptical in shape and measured, on average, 0.24 mm (SD = 0.02; n = 50) in length and 0.10 mm (SD = 0.01; n = 50) in width. The mean incubation time of the eggs was two days (SD = 0.5; n = 50) at 26 °C, with a hatching rate of 89.9%. The eggs were distributed in a pseudo-spiral pattern, in alternate parallel rows along the primary axis of the gelatinous mass (Fig. 3A) and eggs were elliptic format (Fig. 3B).

The four instars of the Brazilian *Chironomus* population were well defined using the ventral length of the head capsule. Mean length of the head capsule of the 1st-instar is 54.7 µm (SD = 4.2; n = 81); the 2nd 93.7 µm (SD = 4.5; n = 53); the 3rd 157.1 µm (SD = 7.2; n = 67) and the 4th 257.6 µm (SD = 16.7; n = 316) (Fig. 4).

Development times of the 1st (SD = 0.6; n = 81), 2nd (SD = 1.0; n = 53) and 3rd instars (SD = 1.1; n = 67) were similar, each averaging three days; the 4th instar was the longest, with mean development time of 10 days (SD = 2.5; n = 316). Mean time for complete larval stage development was 19 days (SD = 5.2; n = 517). Mean development time for the pupal stage, at 26 °C, was two days (SD = 0.24, n = 50). The life span of adults, on average, at 26 °C, was three days for both males (SD = 0.70, n = 50) and females (SD = 0.65, n = 50). Development time of the Brazilian *Chironomus* population from the time larvae hatch to the adult stage was 27 days at 26°C. The emergence percentage was 42.9% for females and 57.1% for males.

Table 3. Pairwise per cent nucleotide differences (p-distance) between all specimens based on the COI gene in the mtDNA. Analyses were conducted using the Kimura-2-parameter model. Values in bold are genetic distances between Groups A and B.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. <i>C. sp1BRA</i>																			
2. <i>C. sp2BRA</i>	0.0																		
3. <i>C. sp3BRA</i>	0.0	0.0																	
4. <i>C. ktiensis_JF412086_South_Korea</i>	0.8	0.8	0.8																
5. <i>C. ktiensis_JF412087_South_Korea</i>	0.8	0.8	0.8	0.0															
6. <i>C. ktiensis_JF412089_South_Korea</i>	0.8	0.8	0.8	0.0	0.0														
7. <i>C. ktiensis_KC407765_South_Korea</i>	0.8	0.8	0.8	0.0	0.0	0.0													
8. <i>C. ktiensis_JF412088_South_Korea</i>	1.1	1.1	1.1	0.3	0.3	0.3	0.3												
9. <i>C. ktiensis_AB740241_Japan</i>	1.2	1.2	1.2	0.6	0.6	0.6	0.6	0.6											
10. <i>C. ktiensis_AB838643_Japan</i>	1.7	1.7	1.7	0.9	0.9	0.9	0.9	1.2	1.5										
11. <i>C. ktiensis_AB838645_Japan</i>	0.9	0.9	0.9	0.2	0.2	0.2	0.2	0.2	0.5	1.1									
12. <i>C. ktiensis_AB838646_Japan</i>	0.9	0.9	0.9	0.3	0.3	0.3	0.3	0.3	0.3	1.2	0.2								
13. <i>C. striatipennis_COTW008_Japan</i>	0.9	0.9	0.9	0.2	0.2	0.2	0.2	0.2	0.5	1.1	0.0	0.2							
14. <i>C. striatipennis_COTW011_India</i>	3.0	3.0	3.0	2.5	2.5	2.5	2.5	2.8	3.1	3.3	2.6	2.8	2.6						
15. <i>C. striatipennis_COTW012_India</i>	2.8	2.8	2.8	2.6	2.6	2.6	2.6	2.6	2.9	3.3	2.4	2.6	2.4	1.3					
16. <i>C. striatipennis_COTW027_Malaysia</i>	2.8	2.8	2.8	2.2	2.2	2.2	2.2	2.2	1.9	3.1	2.0	1.9	2.0	3.3	2.8				
17. <i>C. ktiensis_AB740240_Japan</i>	9.2	9.2	9.2	9.1	9.1	9.1	9.1	9.1	9.2	9.7	9.1	9.2	9.1	10.4	10.0	9.6			
18. <i>C. ktiensis_AB838642_Japan</i>	9.1	9.1	9.1	9.2	9.2	9.2	9.2	9.4	9.4	9.9	9.2	9.4	9.2	10.3	9.8	9.9	0.6		
19. <i>C. ktiensis_AB838644_Japan</i>	9.2	9.2	9.2	9.4	9.4	9.4	9.4	9.6	9.6	10.3	9.4	9.6	9.4	10.1	9.6	9.9	0.6	0.6	
20. <i>C. ktiensis_JQ350720_South_Korea</i>	10.1	10.1	10.1	10.1	10.1	10.1	10.1	10.3	9.9	11.2	10.1	9.9	10.1	11.1	10.7	10.3	1.9	2.2	2.0

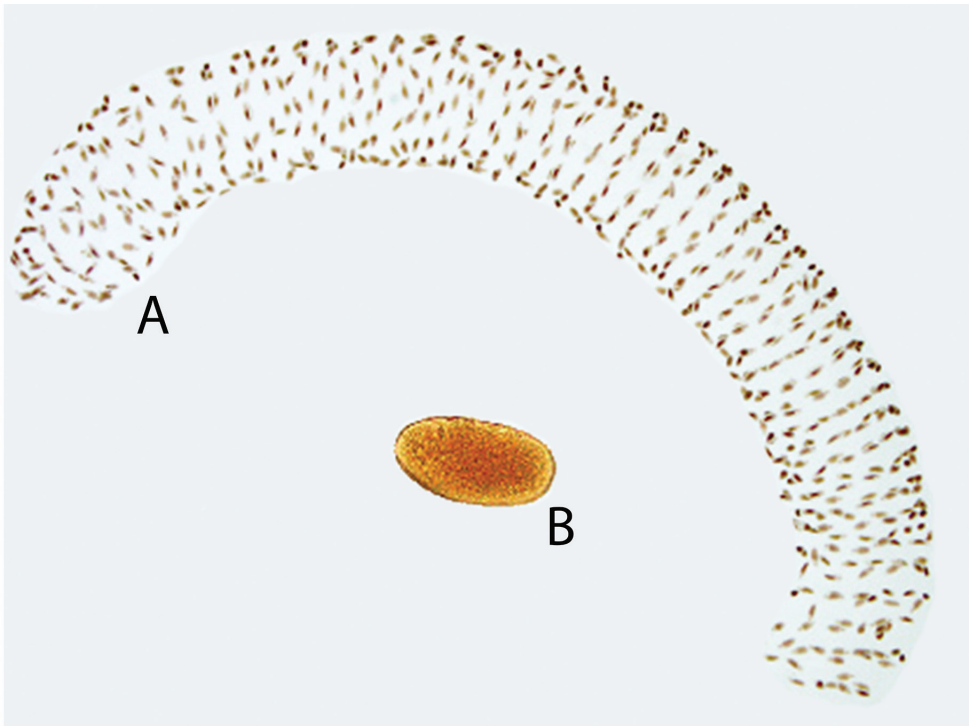


Figure 3. *Chironomus striatipennis*, Brazilian population. **A** Egg mass **B** Egg.

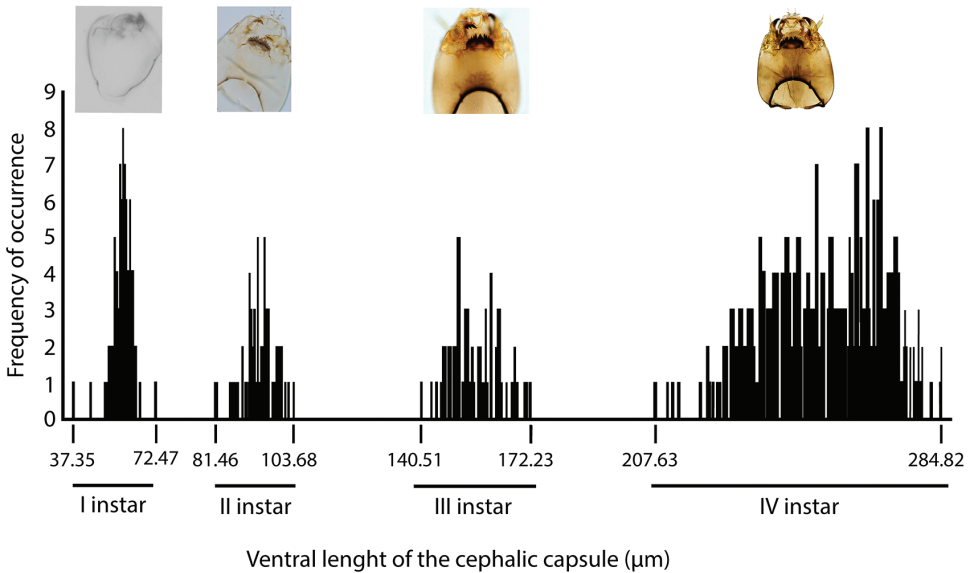


Figure 4. Frequency of occurrence of the ventral length of the cephalic capsule of a Brazilian *Chironomus* population (Diptera: Chironomidae) showing the four larval instars.

Discussion

Species identification

High morphological similarities observed between the Brazilian *Chironomus* population and both *C. striatipennis* and *C. kiiensis* (Kieffer 1910; Tokunaga 1936) corroborate the studies that reported the difficulty in distinguishing the latter two species at the morphological level, including the one that proposed *C. kiiensis* as a synonym of *C. striatipennis* (Martin 2014). However, cytotaxonomic studies have indicated that they are sibling species that belong to the same cytocomplex (Martin 2014). Our molecular analysis showed that both species names have been applied to specimens with high genetic divergence, and that the identification based on geographical distribution, which is currently in common usage to circumscribe both species (e.g., Nath and Lakhota 1989; Yong et al. 1999; Jeong et al. 2004; Nandi et al. 2011), is not a good taxonomic practice.

Studies on the genetic intraspecific divergence in some Chironomidae genera have reported values between 0.5 and 2.3% (Sinclair and Gresens 2008; Silva et al. 2013; Proulx et al. 2013; Trivinho-Strixino et al. 2012). Similar values were observed in studies on other insects, including some Diptera families (e.g., Beckenbach and Borkent 2003; Hamada et al. 2010; Hernández-Triana et al. 2012). Although Silva et al. (2013) attributed low intraspecific divergence (mean 0.91%) to the specimens having been collected in the same place, our results and other studies, for example, with Lepidoptera and Simuliidae, do not corroborate this hypothesis (e.g., Hebert et al. 2004; Hernández-Triana et al. 2012).

The interspecific genetic divergence observed in our data is in accordance with results for other groups of Chironomidae and other aquatic Diptera families, with values around 15% (Beckenbach and Borkent 2003; Sinclair and Gresens 2008; Proulx et al. 2013; Silva et al. 2013), corroborating the hypothesis that Groups A and B represents at least two distinct species. Group A is composed of specimens identified as *C. striatipennis* and *C. kiiensis* from different regions of Asia. Within this group, there are three specimens identified as *C. striatipennis* from Malaysia and India (the country where this species was described) with high genetic divergence (mean 2.62%) when compared to other members in Group A (Table 3.). This fact might be an indication of the presence of two sibling species in Group A. But, since we have no detailed information on the morphology of all life stages of the populations included in Group A, we suggest that this group should be treated as a species complex. Based on ICNZ rules (Principle of Priority – Article 23.3), and as encouraged by Martin (2014), we named Group A as the *C. striatipennis* species complex.

The large genetic distance (mean 9%) between Group B specimens (composed of specimens identified as *C. kiiensis* from Japan and South Korea) and Group A is a clear indication that each clade represents a distinct species. This is also corroborated by the genetic distance observed between specimens in each species group (maximum = 3.3%), and by the interspecific genetic distance values reported by other studies. For example, a study on three *Podonomus* populations observed that the genetic distances

between them were greater than 7%, indicating the presence of three species (Trivinho-Strixino et al. 2012). Since *C. kiiensis* was described from Japan, we hypothesized that the specimens in Group B may represent this species. On the other hand, because of the lack of information on the morphology of all life stages of these populations, and based on genetic information obtained from GenBank for specimens collected near the type locality of *C. kiiensis*, we choose do not propose any name for Group B specimens.

The placement of the Brazilian *Chironomus* population in Group A and its small genetic distance from South Korean specimens (0.8%) indicates that this might represents a recently introduced species in Brazil, probably, from a population from South Korea. This could have occurred due to the fact that Manaus is located in a port zone, receiving many cargo ships from different continents, including Asia, due to the presence of industries in the Manaus Tax-Free Zone (SUFRAMA). We therefore assume that specimens of the Brazilian *Chironomus* species arrived in Brazil by ship. Other exotic species have been entering the country by this way, perhaps using ballast water or some other source of standing water (e.g. Santos and Lamonica 2008; Brodin and Anderson 2009; Raunio et al. 2009; Jensen 2010).

Biological information

Environmental conditions where the Brazilian *Chironomus* population and the Japanese *C. kiiensis* population were collected were different: the Brazilian population was observed inhabiting oligotrophic water and the Japanese population eutrophic water (Inoue et al. 2008; Al-Shami et al. 2010). The Brazilian *Chironomus* population has double the number of eggs (~ 600 eggs) compared to *C. kiiensis* from Japan, although they were reared at similar water temperatures (25–26 °C), feeding methodology and food type were not the same (Maeda and Yano 1988). Larval development time of the Brazilian *Chironomus* population was shorter (10 days) than that of the Japanese *C. kiiensis* population (14–20 days) (Nandi et al. 2011). Development times of the 1st, 2nd and the 3rd instars of the Brazilian *Chironomus* population was shorter than that of the 4th instar, as has also been observed in other *Chironomus* species (Fonseca and Rocha 2004; Zilli et al. 2008). The 4th instar's long larval development time is probably due to the greatest body growth occurring in this period (Tokeshi 1995), as well the production and maturation of the oocytes (Strixino and Trivinho-Strixino 1985).

The size of last-instar larvae, represented by the ventral length of the head capsule, of the Brazilian *Chironomus* population was smaller than that of the Japanese *C. kiiensis* population (Maeda and Yano 1998). Male survival time of the Brazilian *Chironomus* population under laboratory conditions was shorter (three days) than in the Japanese *C. kiiensis* population at similar air temperature (25 °C) (Maeda and Yano 1988; Nandi et al. 2011). Environmental conditions can have an effect on species biology and information in this area is important for characterizing the habitat of each species in a species complex and can help to delimit each species in the complex.

Concluding remarks

In this study we addressed the taxonomic problem involving the species named *C. striatipennis* and *C. kiiensis* from Asia, since these names have been used for specimens morphologically similar and closely related. Regional variation among Asian populations of the two above mentioned species was observed, but only at molecular level, based on the genetic distance estimated using a partial COI sequence. We hypothesize that these two names encompass at least two, and perhaps three, species, based on sequences deposited in GenBank and BOLD. We indicated that the usual way to identify these two species, which are practically indistinguishable at the morphological level, based on geographical distribution is not a feasible approach, since specimens identified with the same name (*C. kiiensis*) from Japan and South Korea have highly genetic divergent (genetic distance of 9%). A detailed study, including the morphology of all life stages and a multilocus molecular analysis of populations from the entire distribution needs to be done to solve this taxonomic problem. The presence of an Asiatic *Chironomus* in Brazil might be the consequence of human globalization, where fast and easy global transportation systems are available to any organisms with the minimum characteristics of alien species. This example also demonstrates that the geographic limits of species cannot be considered in isolation, but rather need to be examined from a broad perspective to avoid mistakes.

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