RESEARCH ARTICLE



# Two new species of Fluminicola (Caenogastropoda, Lithoglyphidae) from southwest Oregon, USA, and a range extension for F. multifarius

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# Abstract

We describe two new species of pebblesnails (Lithoglyphidae: *Fluminicola*) from southwest Oregon based on morphologic and mitochondrial DNA (COI, cytB) evidence. *Fluminicola umpquaensis* **sp. n.**, which had been traditionally identified as *E virens* prior to the recent restriction of the latter to the lower Columbia River drainage, lives in lotic habitats in the Umpqua River basin. This species is readily distinguished from closely related *F. gustafsoni* and *F. virens* by shell and anatomical characters, and by its mtDNA sequences (divergence  $\geq$ 3.6% for both genes). *Fluminicola fresti* **sp. n.** ranges among lotic habitats in the North Umpqua River basin, and in the upper Rogue River drainage north of Little Butte Creek. This species differs from other congeners by >9.1% for both genes and is distinguished from closely similar and geographically proximal *F. multifarius* by several anatomical characters. Additionally, new records are provided for *F. multifarius* from the upper Rogue River basin south of Little Butte Creek, which extend the geographic range of this species about 80 km northward from the Sacramento River headwater region. This continues a recent series of taxonomic papers on the poorly known and little studied pebblesnail fauna of the vast Pacific watershed from northern California to southern British Columbia.

#### **Keywords**

Caenogastropoda, Truncatelloidea, freshwater, North America, systematics, morphology, mitochondrial DNA

#### Introduction

*Fluminicola* (Truncatelloidea, Lithoglyphidae) is a western North American genus of small freshwater gastropods with globose to conical shells, commonly known as pebblesnails, which is distributed in diverse habitats ranging from small seeps to spring-influenced lacustrine reaches to large rivers. Both morphological (Hershler and Frest 1996) and molecular (Hershler and Liu 2012) evidence suggest that the 25 currently recognized *Fluminicola* species belong to two evolutionarily distinct lineages. One of the lineages contains two species in the Columbia River basin – *F. gustafsoni* Hershler & Liu, 2012; *F. virens* (Lea, 1838) – while the other (containing the remaining congeners) is distributed in this drainage and also the Great Basin, upper Colorado River basin, and Sacramento River basin. *Fluminicola* continues to be recognized as a non-monophyletic genus pending clarification of the phylogenetic relationships of its poorly known and possibly extinct type species, *F. nuttallianus* (Lea, 1838).

The broad geographic range of *Fluminicola* includes most of the Pacific Coastal watershed from northern California to southern British Columbia. Pebblesnail populations are scattered throughout much of this huge area, yet are currently undescribed aside from six species in the Columbia River basin (Hershler and Frest 1996, Hershler and Liu 2012, Liu et al. 2013) and 14 species in the Sacramento River basin (Hershler et al. 2007). This taxonomic knowledge gap is hampering efforts by the conservation community to obtain legal protection for pebblesnails (e.g., USFWS 2012), which are groundwater-dependent and threatened by various anthropogenic activities.

The *Fluminicola* fauna of southwestern Oregon includes a relatively large pebblesnail in the Umpqua River basin that was traditionally identified as *F. virens* prior to the restriction of the latter to the lower Columbia River drainage (Hershler and Frest 1996), and numerous unstudied populations of smaller pebblesnails in the upper reaches of both the Rogue and Umpqua River basins that were recently reported in grey literature (Frest and Johannes 1999, 2000, 2004, 2005). Herein we utilize DNA sequences from two mitochondrial genes in delineating the *Fluminicola* species in the Rogue-Umpqua basins. This continues our recent series of integrative taxonomic studies of the pebblesnails of the Pacific Coastal drainages (Hershler et al. 2007, Hershler and Liu 2012, Liu et al. 2013).

#### **Methods**

We sequenced specimens from 35 sites in the Rogue-Umpqua basins, including eight localities containing the large pebblesnail resembling *F. virens* and 27 localities containing smaller pebblesnails. Thirty-two of these localities were sampled by RH and HPL during September 2015 specifically for this project. Specimens were collected by hand or with a small sieve and preserved in 90% (non-denatured) ethanol in the field. Vouchers were deposited in the Smithsonian Institution's National Museum of Natural History (USNM) collection. Other relevant material from the USNM

and the Bell Museum of Natural History (BellMNH) was also examined during the course of this study.

A total of 155 and 146 sequences of cytochrome c oxidase subunit I (COI) and cytochrome B (cytB), respectively were obtained from 161 analyzed Rogue-Umpqua pebblesnails. Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin 1992); each specimen was analyzed for mtDNA individually. LCO1490 and HCO2198 (Folmer et al. 1994) were used to amplify a 708 base pair (bp) fragment of COI; cytB427F (5'TGA GGK GCN ACT GTT ATT ACT AA3') and cytB1049R (5'GTG AAA ACT TGS CCR ATT TGC TC3') were used to amplify a 644 bp fragment of the cytB gene. The cytB427F and cytB1049R primers were designed based on conserved regions of cytB in an alignment using previously published sequences from Oncomelania hupensis (Gredler) (NC13073) and Potamopyrgus antipodarum (Gray) (GQ996433). Amplification conditions and sequencing of amplified polymerase chain reaction product methods were those of Liu et al. (2013). Sequences were determined for both strands and then edited and aligned using SEQUENCHER<sup>™</sup> version 5.4.1 (Gene Codes Corporation, Ann Arbor, MI). Our analysis of the COI dataset also included 35 previously published sequences from 23 congeners, two taxonomically indeterminate Fluminicola lineages from the Sacramento River basin (F. sp. A, F. sp. B, Hershler et al. 2007), and representatives of two other North American lithogyphid genera (Somatogyrus, Taylorconcha). Trees were rooted with Pristinicola hemphilli (Pilsbry) (Hydrobiidae). The cytB dataset also included 34 previously published sequences from 22 Fluminicola species (a cytB sequence is not available for F. gustafsoni) and the two taxonomically indeterminate Fluminicola lineages. Given that cytB sequences are not available for other North American lithoglyphid genera, we used basally positioned F. virens to root the trees (Hershler et al. 2007, Hershler and Liu 2012). In order to generate easily readable topologies, one example of each detected haplotype was used in the phylogenetic analyses, which were performed separately for the COI and cytB datasets. Sample codes, locality and voucher details, and GenBank accession numbers for the sequences used in the molecular phylogenetic analyses are in Suppl. material 1.

Genetic distances were calculated using MEGA7 (Kumar et al. 2016), with standard errors estimated by 1,000 bootstrap replications with pairwise deletion of missing data. MRMODELTEST v. 2.3 (Nylander 2004) selected the GTR + I + G model parameters as the best fit for both the COI and cytB datasets (using the Akaike Information Criterion). Phylogenetic analyses were performed using three different methodologies – maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. The MP and ML analyses were performed using PAUP\* v. 4.0b10 (Swofford 2003) and the Bayesian analyses were conducted using MRBAYES v. 3.2.6 (Ronquist and Huelsenbeck 2003). The MP analysis was conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping and 100 random additions. Nodal support was evaluated by 10,000 bootstrap replicates. The ML analysis was performed using the GTR + I + G model. The optimized parameter values for COI were base frequencies of A = 0.3097, T = 0.3952, C = 0.1612, G = 0.1339; shape of gamma distribution = 1.3430; proportion of invariant sites = 0.5912. The optimized parameter values for cytB were base frequencies of A = 0.3274, T = 0.3671, C = 0.1919, G = 0.1145; shape of gamma distribution = 0.7958; proportion of invariant sites = 0.54237. A GTR distance based neighbor-joining (NJ) tree was used as the initial topology for branch-swapping. Nodal support was evaluated by 1,000 bootstrap pseudoreplicates. For the Bayesian analyses Metropolis-coupled Markov chain Monte Carlo simulations were run with four chains (using the model selected by MRMODELTEST) for 5,000,000 generations. Markov chains were sampled at intervals of 100 generations to obtain 50,000 sample points. We used the default settings for the priors on topologies and the GTR + I + G model parameters. At the end of the analyses, the average standard deviations of split frequencies were 0.005692 (COI dataset) and 0.004193 (cytB dataset) 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 50% majority rule consensus trees, with the first 25% of the samples removed to ensure that the chain sampled a stationary portion.

Large adult females were used for shell measurements. The total number of shell whorls (WH) was counted for each specimen; and the height and width of the entire shell (SH, SW), body whorl (HBW, WBW), and aperture (AH, AW) were measured from camera lucida outline drawings (Hershler 1989). Descriptive statistics were generated using SYSTAT FOR WINDOWS 11.01 (SSI 2004). Other methods of morphological study were routine (Hershler et al. 2007).

#### Results

Forty-one COI and 55 cytB haplotypes were detected in the analyzed specimens from the Rogue-Umpqua basins (Suppl. material 2–3, respectively). The molecular phylogenetic analyses of both the COI and cytB datasets consistently resolved the Rogue-Umpqua haplotypes into three distinct clades. The trees generated by the three methods of phylogenetic analysis were closely similar; the Bayesian topology based on the COI dataset is shown in Figure 1.

Two of the clades (clades A, B, Fig. 1) contained the smaller Rogue-Umpqua pebblesnails. Clade A, which was weakly supported generally, but well supported in the cytB Bayesian topology (100% posterior probability), was composed of the haplotypes from populations in the upper Rogue River basin to the south of Little Butte Creek (Fig. 2) and the two representative sequences of *F. multifarius* Hershler, Liu, Frest & Johannes, 2012, a morphologically variable species that is distributed in the upper Sacramento River basin in northern California (Hershler et al. 2007). The divergence between the Rogue pebblesnails in clade A and all published sequences of *F. multifarius* (2.6% for COI and 3.6% for cytB, Hershler et al. 2007) was slightly greater than the variation within these two groups (2.2 and 1.4% for COI; and 3.5 and 2.4% for cytB, respectively) and falls into the range of pairwise differences among currently recognized *Fluminicola* species: 1.4–18.7% for COI and 1.6–25.7% for



**Figure 1.** Bayesian tree based on the COI dataset. The three clades (**A–C**) containing Rogue-Umpqua haplotypes are color coded as in Figure 2. Posterior probabilities for nodes are shown when  $\geq$ 95%. Specimen codes are from Suppl. material 1; Rogue-Umpqua haplotype codes (in parentheses) are from Suppl. material 2.



**Figure 2.** Map of southwest Oregon and northwest California showing the distribution of mtDNA clades **A–C** with color codes matching those in Fig. 1. Specimen codes are from Suppl. material 1.

cytB (Hershler et al. 2007, Hershler and Liu 2012). However, we could not differentiate these geographically disjunct yet phylogenetically intermixed groups of populations based on morphologic criteria and thus are treating them as conspecific. Clade B, which was well supported in all but the cytB Bayesian analysis, is composed of the small pebblesnails in the North Umpqua basin, and the upper Rogue River basin north of Little Butte Creek (Fig. 2). The members of this clade differ from all currently recognized *Fluminicola* species by >9.1% for both genes (the sequence divergence within the clade is *c*. 2% for both genes) and although having the generalized morphology shared by most of the smaller *Fluminicola* species, can also be distinguished from closely similar and geographically proximal *F. multifarius* by several anatomical characters. Based on the sum of this evidence we recognize clade B as a somewhat variable new species which is described below.

The third clade (C), which was moderately supported in most of the analyses and well supported in the COI ML tree (100%), contained the haplotypes detected in the large pebblesnails from the Umpqua River basin (Fig. 2). The sequence divergence within this clade was slight – 0.3% for COI and 0.6% for cytB, respectively. Clade C, in turn, formed a well-supported monophyletic group with *F. gustafsoni* and *F. virens*, and is most similar genetically to the latter, from which it differs by 3.6% for COI and 3.9% for cytB. The large pebblesnail in the Umpqua River basin is also readily differentiated morphologically from both *F. gustafsoni* and *F. virens*, and thus we recognize it as a new species which is described below.

#### Systematic descriptions

Family Lithoglyphidae Troschel, 1857 Genus *Fluminicola* Carpenter, 1864

# Fluminicola multifarius Hershler, Liu, Frest & Johannes, 2007

Figs 3, 4A–D

*Fluminicola multifarius.* – Hershler et al. 2007: 415, 417, 419, figs. 7M, 24, 25 (Big Springs (source) at Big Springs City Park northwest of the city of Mount Shasta, south of Spring Hill, Siskiyou County, California ([UTM zone 10] 556400 E, 4575265 N, 1092 m).

**Distribution.** Sacramento River headwater region (as far downflow as Conant), and a few sites in the upper reaches of the McCloud River drainage (Hershler et al. 2007).

**Referred material.** OREGON. *Jackson County.* USNM 1144635, USNM 1145050, USNM 1145051, USNM 1297159, spring run on east side of BLM 37-3E-31.0, 0.24 rd. km north of BLM 38-2E-11 junction (42.2996°N, 122.5198°W), USNM 1144556, USNM 1145001, USNM 1144557, USNM 1145002, USNM 1297160, spring influenced creek at BLM 37-3E-31.0 crossing, 0.32 rd. km north of BLM 38-2E-11.0 junction (42.3001°N, 122.5198°W), USNM 1144554, USNM 1144555, USNM 1144999, USNM 1145000, USNM 1297161, spring influenced creek at crossing of BLM 38-2E-11, 1.26 rd. km east of BLM 37-3E-31.0



**Figure 3.** Shells, opercula and radula, *F. multifarius*. **A** Shell, USNM 1144567 **B** Shell, USNM 1144997 **C** Shell, USNM 1145051 **D**, **E** Opercula (outer, inner sides), USNM 1144567 **F** Portion of radular ribbon, USNM 1144567 **G** Central teeth, USNM 1144567 **H** Lateral teeth, USNM 1144567. Scale bars: **A–B** 1.0 mm; **D–E** 250 μm; **F**, 100 μm; **G–H** 10 μm.

(42.2890°N, 122.5262°W), USNM 1144582, USNM 1144583, USNM 1144584, USNM 1144682, USNM 1145010, USNM 1145011, USNM 1145012, USNM 1297162, spring on north side of Burnt Creek Road (BLM 39-3E-21.0), 0.64 rd. km



**Figure 4.** Reproductive anatomy. **A–D** *F. multifarius* **A**, **B**, **D** USNM 1144567, **C** USNM 1070753 **E–G** *F. fresti*, sp.n., USNM 1422223 **H–J** *F. umpquaensis*, sp. n., USNM 1144736. **A**, **E**, **G** Female glandular oviduct and associated structures (viewed from left side) **B**, **C**, **F**, **I** Bursa copulatrix and seminal receptacle, **D**, **G**, **J** Penis, dorsal surface. The bursa copulatrix and seminal receptacle of type material of *F. multifarius* (**C**), which was not illustrated by Hershler et al. (2007), is shown for comparative purposes. Scale bars, 500 μm. **Ag** albumen gland **Bu** bursa copulatrix **Cg** capsule gland **Cov** coiled oviduct **Dsr** seminal receptacle duct **Ga** genital aperture **Pd** penial duct **Pw** posterior wall of pallial cavity **Sr** seminal receptacle **Vc** ventral channel of capsule gland.

south of BLM 39-3E-32.1 junction, 1.29 km northwest of Cottonwood Glades, 3.22 rd. km south of Dead Indian Memorial Road (Jackson County 722) (42.2212°N, 122.5008°W), USNM 1144580, USNM 1144581, USNM 1144928, USNM 1297163, Cold Spring on south side of Burnt Creek Road (BLM 39-3E-21.0), 0.8 rd. km east of junction with BLM 39-3E-17.0 (42.1944°N, 122.5120°W), USNM 1144547, USNM 1144548, USNM 1144681, USNM 1144927, USNM 1144996, USNM 1144997, USNM 1297164, spring run above (north of) and below BLM 39-3E-17.0, ca. 0.56 rd. km north of BLM 39-3E-18.1 junction, northeast of Round Mountain (42.1814°N, 122.5076°W), USNM 1144566, USNM 1144567, USNM 1144909, USNM 1297165, spring on east side of (above) BLM 40-2E-35.0 at MP 0.35 off BLM 40-2E-33, east of Emigrant Creek, north of Pilot Rock, west of Porcupine Mountain (42.0438°N, 122.5612°W), USNM 1144607, USNM 1144916, spring on south side of BLM road, 0.16 km off BLM 40-2E-33, east of Emigrant Creek, north of Pilot Rock, southwest of Porcupine Mountain (42.0387°N, 122.5614°W), USNM 1144609, USNM 1144930, Sampson Creek on both sides of BLM 38-3E-18.1 (42.1869°N, 122.5167°W), USNM 1144614, USNM 1144686, USNM 1145027, USNM 1145028, spring south of Hobart Lake, west of Hobart Bluff, 0.21 km east of BLM 39-3E-32.3, 0.64 km north of Hobart Peak (42.0933°N, 122.4799°W).

**Comparative material.** CALIFORNIA. *Siskiyou County*. USNM 1070753, Big Springs (source) at Big Springs City Park northwest of the city of Mount Shasta, south of Spring Hill, USNM 1020771, Bundoora Spring, west of access road off FS40N44.

**Remarks.** The newly reported populations closely conform to the original description of *F. multifarius* in all respects aside from a slightly larger maximum shell height (5.02 vs. 4.64 mm). Representative Rogue basin specimens are illustrated in Figures 3A–C (shells), 3D–E (opercula), 3F–H (radula) and 4A, B, D (reproductive anatomy). Ten COI and cytB haplotypes were detected in the Rogue basin populations (Suppl. material 2–3, respectively).

The new records detailed herein extend the range of *F. multifarius* about 80 km northward from the Sacramento River headwaters (Fig. 2). It is not known whether *F. multifarius* is also distributed in the intervening Klamath River basin; the pebblesnail fauna of this large watershed is currently undescribed. Populations of *F. multifarius* in the Rogue basin were referred to as the Chinquapin pebblesnail, Emigrant pebblesnail, Keene Creek pebblesnail, Little Butte pebblesnail, and Pilot Rock pebblesnail by Frest and Johannes (2000, 2004, 2005).

# *Fluminicola fresti* Hershler, Liu & Hubbart, sp. n. http://zoobank.org/75362D82-9786-4423-9435-CD99A795C050 Figs 4E–G, 5

**Types.** Holotype, USNM 1144376, diversion from Big Butte Springs through Butte Falls Hatchery, just south of Butte Falls-Fish Lake Road (Jackson County 321) and 0.16 km west of Butte Falls-Prospect Road (Jackson County 922), Jackson County, Oregon, 42.5389°N, 122.5551°W, 10/22/1994, Terrence J. Frest and Edward J. Jo-

hannes. Paratypes, USNM 1422223 (a large series of dry shells and alcohol-preserved specimens), from same lot.

Referred material. OREGON. Douglas County: USNM 1297126, Trap Creek at crossing of FS4788, 0.48 km south of its confluence with the Clearwater River (43.2431°N, 122.2886°W). Jackson County. USNM 1144448, USNM 1297142, Rogue River on north side at Rogue Elk County Park, east of Rogue Elk, south of OR62 (42.6618°N 122.7524°W), USNM 1144532, USNM 1297143, Vine Creek on east side of McNeil Road (42.6270°N, 122.6722°W), USNM 1144525, USNM 1297144, Rogue River, upriver from Casey State Park boat ramp, west of McLeod (42.6591°N, 122.6993°W), USNM 1297145, Mill Creek at Mill Creek Campground off OR62 on FS030, ca. 5.6 km northeast of Prospect (42.7937°N, 122.4679°W), USNM 1144901, USNM 1145101, USNM 1145102, USNM 1297146, Evergreen Spring above Lost Creek Lake (42.7026°N, 122.6090°W), USNM 1297147, spring outflow, at foot bridge crossing, Joseph Stewart State Park (42.6810°N, 122.6216°W), USNM 1297149, spring outflow, first crossing above foot bridge, Joseph Stewart State Park (42.6811°N, 122.6204°W), USNM 1297150, Lost Creek at BLM 34-2E-8 (Medco A Road) crossing (north side), south of Joseph Stewart State Park (42.6570°N, 122.6152°W), USNM 1144528, USNM 1297152, Middle Fork Lost Creek at BLM 34-2E-8 (Medco A Road) crossing (east side), south of Joseph Stewart State Park (42.6571°N, 122.6150°W), USNM 1297153, Clark Creek collected east of BLM 34-2E-9.02 crossing off Clark Creek Road (BLM 34-2E-7) (42.6305°N, 122.5865°W), USNM 1144526, USNM 1297154, spring below (northeast of) BLM 33-2E-13.02, 0.32 rd.km off of BLM 33-2E-13.01, west of Smith Creek (42.7015°N, 122.5347°W), USNM 1144531, USNM 1297155, southern-most spring of three on west side of BLM 33-3E-28.01, 0.8 rd. km north of BLM 33-3E-34 junction, east of Reinecke Burn (42.6733°N, 122.4749°W), USNM 1297156, diversion from Big Butte Springs through Butte Falls Hatchery, USNM 1144375, USNM 1297157, Whiskey Spring at Whiskey Spring Campground on FS100 off FS37 (42.4949°N, 122.4151°W), USNM 1144533, USNM 1144534, USNM 1297158, spring in Wasson Canyon on south side of Wasson Canyon Creek above (east of) BLM 36-2E-19.2 off of BLM 36-2E-26 (42.4240°N, 122.5206°W).

**Diagnosis.** A small to medium-sized *Fluminicola* (2.3–5.5 mm shell height) having a trochoidal to ovate-conic shell and small, gently tapered penis. Differs from closely similar and geographically proximal *F. multifarius* in the hooked shape of the anterior end of the osphradium, larger number of ctenidial (gill) filaments, smaller seminal receptacle, and in its mtDNA sequences.

**Description.** Shell (Fig. 5A–D) trochoidal to narrow-conic, whorls 3.5–4.0. Teleoconch whorls medium convex, sometimes weakly shouldered. Aperture ovate, slightly angled above; inner lip complete, variably thickened and reflected, sometimes forming a rather wide parietal-columellar shelf that sometimes covers the umbilical region. Outer lip thin, prosocline. Umbilicus very small or absent, umbilical region sometimes excavated. Shell white, periostracum brown, sometimes covered with thick black deposits. Shell measurements and whorl count data are summarized in Table 1.



Figure 5. Shells, opercula and radula, *F. fresti*, sp. n. A Holotype, USNM 1144376 B, C Sympatric ovate-conic and trochiform shell morphs, respectively, USNM 1297149 D Shell, USNM 1297155
E, F Opercula (outer, inner sides), USNM 1422223 G Portion of radular ribbon, USNM 1422223
H Central teeth, USNM 1422223 I Lateral teeth, USNM 1422223. Scale bars: A–D 1.0 mm; E–F 250 µm; G 100 µm; H–I 20 µm.

Operculum (Fig. 5E–F) as for genus; muscle attachment margin thickened on inner side. Radula (Fig. 5G–I) as for genus; dorsal edge of central teeth concave, lateral cusps two–five, basal cusp one–two. Lateral teeth having two–three cusps on inner side and three–four cusps on outer side; length of outer wing 175–185% length of cutting

	WH	SH	SW	HBW	WBW	AH	AW		
Holotype, USNM 1144376									
	4.00	4.36	3.12	3.55	2.76	2.20	2.03		
Paratypes, USNM 1422223 (N = 17)									
Mean	4.03	4.37	3.42	3.61	2.82	2.28	2.09		
S.D.	0.17	0.41	0.30	0.31	0.23	0.22	0.19		
Range	3.75-4.25	3.93-5.46	3.07-4.18	3.18-4.31	2.58-3.37	1.92-2.77	1.77-2.48		

Table 1. Shell parameters for *F. fresti*. Measurements are in mm.

edge. Inner marginal teeth with 23–31 cusps, outer marginal teeth with 27–40 cusps. Radula data are from USNM 1422223, USNM 1144426.

Snout, cephalic tentacles, pallial roof, visceral coil usually medium pigmented (brown); foot varying from near pale to medium pigmented along anterior edges. Distal section of penis having dense core of internal black pigment. Ctenidial filaments 21-24 (N = 5), lateral surfaces smooth. Anterior end of osphradium distinctly hooked (not illustrated). Glandular oviduct and associated structures shown in Figure 4E–F. Coiled oviduct circular, proximal arm kinked, posterior arm sometimes having small pouch containing sperm. Bursa copulatrix large, reniform, partly overlapped by albumen gland. Bursal duct slightly shorter than bursa copulatrix, narrow. Seminal receptacle small, sac-like, almost completely overlapped by albumen gland. Albumen gland having short pallial component. Capsule gland longer than albumen gland, composed of two glandular zones. Genital aperture a small, sub-terminal pore. Penis (Fig. 4G) small, slightly curved, gently tapering, distal section abruptly narrowing to small pointed tip. Medial section having a few weak folds along inner edge. Penial duct near centrally positioned, straight, narrow.

**Etymology.** This species name is a patronym (in the genitive singular) honoring recently deceased malacologist Terrence Frest for his many contributions to the documentation of molluscan biodiversity in the northwestern United States.

**Distribution.** *Fluminicola fresti* is distributed in spring-fed habitats in the North Umpqua River drainage and in the Rogue River basin north of Little Butte Creek.

**Remarks.** As noted above, the shells of *F. fresti* vary in overall shape and in the width of the inner apertural lip. Although this variation is generally continuous in the material that we examined, two rather distinct forms – ovate-conic with a narrow inner apertural lip (Fig. 5B) and trochoidal with a wide parietal-columellar shelf (Fig. 5C) – can be identified in one of the springs in Joseph Stewart State Park. We sequenced specimens from this locality and found that the two shell forms (samples RU18 and RU17, respectively) differed by 0.7% for COI and 1.3% for cytB, which was less than the variation within these two groups (0.2 and 1.2% for COI; and 0.2 and 1.5% for cytB, respectively). The scant genetic differentiation of the two shell forms when in sympatry provides additional evidence that they are conspecific. Additional studies incorporating rapidly evolving nuclear markers (e.g., microsatellites) may help sift through the possible explanations for the interesting variation in the shells of this species (e.g., ecophenotypic plasticity, incipient speciation). Twenty-three COI hap-

lotypes and 34 cytB haplotypes were detected in the sequenced specimens of *F. fresti* (Suppl. material 2–3, respectively).

Populations of *F. fresti* were referred to as the Beaverdam pebblesnail, Camp Creek pebblesnail, Clark pebblesnail, Evergreen pebblesnail, Rogue pebblesnail, Stewart pebblesnail, and Umpqua pebblesnail by Frest and Johannes (1999, 2000, 2004, 2005). In order to avoid confusion, we suggest that "Frest's pebblesnail" be used as the common name for *F. fresti*.

#### Fluminicola umpquaensis Hershler, Liu & Hubbart, sp. n.

http://zoobank.org/7EEF6D2F-5CAB-4CD2-A41C-E1EB8D556EBD Figs 4H–I, 6

*Fluminicola virens.*–Pilsbry 1899: 122-123 (in part). Henderson 1929: 169 (in part). Burch and Tottenham 1980: 102 (in part).

**Types.** Holotype, USNM 1144535, Umpqua River at Bunch Bar Access (County), west of OR38 (Umpqua Highway), Douglas County, Oregon, 43.6462°N, 123.6670°W, 9/27/1998, Terrence J. Frest and Edward J. Johannes. Paratypes, USNM 1422224 (a large series of dry shells and alcohol-preserved specimens), from same lot.

Referred material. OREGON. Jackson County. USNM 1296909, Umpqua River at Bunch Bar Access, USNM 1296910, Umpqua River at Umpqua County Landing upriver of Sutherlin-Umpqua Road Bridge (Douglas County 9) on east side, north of the mouth of Calapooya Creek, opposite of the town of Umpqua, RM 102.7 (43.3661°N, 123.4677°W), USNM 1296911, east side of Umpqua River at Cleveland Rapids, Cleveland Rapids Park, opposite and northeast of Cleveland (43.2966°N, 123.4705°W), USNM 1144361, USNM 126912, east side of North Umpqua River near boat ramp on west side of Whistlers Bend County Park (43.3101°N, 123.2168°W), USNM 1297138, bedrock shelf and bar on west side of North Umpgua River, ca. 0.48 km southwest of mouth of Swamp Creek (43.3015°N, 122.8692°W), USNM 1297139, Myrtle Creek just upstream of OR99 (Myrtle Creek Highway) bridge (43.0237°N, 123.2890°W), USNM 1297140, Elk Creek just below confluence of Drew Creek (42.8907°N, 122.9227°W), USNM 1144544, USNM 1297141, Elk Creek just south of bridge of Callahan Creek Road (FS3230), west and below Tiller Trail Highway (Douglas Co. 1, OR227) (42.8973°N, 122.9308°W), USNM 1144751, Pass Creek at site of Comstock in Pass Creek County Park, west side of I-5 off exit 164 (43.7186°N, 123.2076°W), USNM 1144360, USNM 1144656, south side of North Umpqua River at John P. Amacher Park, under and down river of railroad bridge west of I-5 and Winchester (43.2796°N, 123.3557°W), USNM 1144726, west side of North Umpqua River at and 0.32 km upstream of Chris Hestness Landing (43.2843°N, 123.1674°W), USNM 1144730, Umpqua River at Umpqua County Landing upriver of Sutherlin-Umpqua Road Bridge (Douglas County 9) on east side, north of the mouth of Calapooya Creek, opposite of Umpqua (43.3643°N, 123.4665°W), USNM



**Figure 6.** Shells, opercula and radula, *F. umpquaensis*, sp. n. **A** Holotype, USNM 1144535 **B** Shell, USNM 1144736 **C** Shell, USNM 1144371 **D**, **E** Opercula (outer, inner sides), USNM 114535 **F** Portion of radular ribbon, USNM 1422224 **G** Central teeth, USNM 1422224 **H** Lateral teeth, USNM 1422224. Scale bars: **A–E**, 1.0 mm; **F**, 100 μm; **G–H**, 10 μm.

1144736, east side of Umpqua River at Cleveland Rapids, Cleveland Rapids Park, opposite and northeast of Cleveland (43.2972°N, 123.4686°W), USNM 1144748, Myrtle Creek just upstream of OR99 (Myrtle Creek Highway) bridge (43.0220°N,

123.2871°W), USNM 1144371, USNM 1144948, west side of South Umpqua River, east of I-5 and north of OR99 (Myrtle Creek Highway) Bridge, west of town of Myrtle Creek (43.0238°N, 123.2954°W), USNM 1144372, South Myrtle Creek, ca. 2.82 km from junction with North Myrtle Creek, below Days Creek Road (43.0176°N, 123.2666°W), USNM 1144473, north side of South Umpgua River just upstream (east) of mouth of Dumont Creek at Dumont Creek Campground (43.0337°N, 122.8010°W), USNM 1144545, Umpqua River on south side, Scottsburg County Park boat ramp (upstream side), southwest of Scottsburg (43.6480°N, 123.8377°W), USNM 1144546, USNM 1144995, Cow Creek at mouth of Salt Creek, west of Cow Creek Road (Douglas Co. 39), east of Byers (42.9240°N, 123.4897°W), BellMNH uncat., North Umpqua River at fish traps above Idevld (43.3220°N, 123.0248°W), BellMNH uncat., North Umpqua River, Glide (43.302°N, 123.101°W), BellMNH uncat., forks of Umpqua River below Roseburg, BellMNH uncat., North Umpqua River at Winchester (43.282°N, 123.355°W), BellMNH uncat., North Umpqua River near Glide, BellMNH uncat., South Umpqua River near Canyonsville, BellM-NH uncat., Umpqua River, Basket Point, BellMNH uncat., Umpqua River, Elkton (43.635°N, 123.570°W).

**Diagnosis.** A large *Fluminicola* (maximum shell height, 9.5 mm) having a conical shell with eroded spire and a broad, little tapered penis. Differs from closely related *F. gustafsoni* and *F. virens* in its reniform-shaped bursa copulatrix and in its mtDNA sequences. Further differentiated from *F. gustafsoni* by its more elongate shell, longer outer wing of the lateral radular teeth, and smaller seminal receptacle; and from *F. virens* by its more convex shell whorls and longer bursal duct.

**Description.** Shell (Fig. 6A–C) conical, spire usually eroded, whorls >3.5. Teleoconch whorls medium convex, narrowly shouldered. Aperture ovate, slightly angled above; inner lip complete, thickened. Outer lip thin, prosocline. Umbilicus absent. Shell white, periostracum tan or olive. Shell measurements and whorl count data are summarized in Table 2.

Operculum (Fig. 6D–E) as for genus; inner side smooth. Radula (Fig. 6F–H) as for genus; dorsal edge of central teeth concave, lateral cusps four–five, basal cusp two–four. Lateral teeth having three–six cusps on inner side and four–six cusps on outer side; length of outer wing 180–200% length of cutting edge. Inner marginal teeth with 13–18 cusps, outer marginal teeth with 11–17 cusps. Radula data are from USNM 1422224.

Head-foot rather lightly pigmented, cephalic tentacles with central brown longitudinal stripe along length. Pallial roof, visceral coil dark brown, almost black. Penis having dense core of internal black pigment along penial duct. Ctenidial filaments 34-36(N = 5), lateral surfaces ridged. Glandular oviduct and associated structures shown in Figure 4H–I. Coiled oviduct vertical or posterior-oblique, proximal arm kinked. Bursa copulatrix medium-sized, reniform, largely overlapped by albumen gland. Bursal duct about twice as long as bursa copulatrix, very narrow. Seminal receptacle small, sac-like, completely overlapped by albumen gland. Albumen gland having short pallial component. Capsule gland slightly shorter than albumen gland, composed of two glandular

	SH	SW	HBW	WBW	AH	AW		
Holotype, USNM 1144535								
	7.77	5.97	6.64	4.93	4.39	3.88		
Paratypes, USNM 1422224 (N = 10)								
Mean	7.37	5.71	6.57	4.91	4.24	3.80		
S.D.	0.36	0.35	0.28	0.33	0.22	0.22		
Range	6.89–7.96	5.37-6.48	6.21–7.25	4.59-5.73	3.89-4.59	3.56-4.29		

Table 2. Shell parameters for *F. umpquaensis*. Measurements are in mm.

zones. Genital aperture a small, sub-terminal pore. Penis (Fig. 4J) large, straight, broad, little tapered, distally rounded, deeply folded along most of length. Penial duct near centrally positioned, rather wide, undulating along entire length, opening through small terminal papilla.

**Etymology.** The species name is an adjectival geographic epithet referring to the distribution of this pebblesnail in the Umpqua River basin.

**Distribution.** *Fluminicola umpquaensis* is widely ranging in the Umpqua River basin, and is distributed in riverine habitats as well as springs and streams.

**Remarks.** As mentioned above, the smaller of the two divergent *Fluminicola* clades (containing *F. gustafsoni* and *F. virens*) was previously confined to the Columbia River basin (Hershler and Liu 2012). *Fluminicola umpquaensis* extends the geographic range of this lineage >200 km southward from the lower Columbia River. Eight COI haplotypes and 11 cytB haplotypes were detected in the sequenced specimens of *F. umpquaensis* (Suppl. material 2–3, respectively).

Populations identified herein as *F. umpquaensis* were referred to as the Jade pebblesnail by Frest and Johannes (2000:182). We propose that this common name continue to be applied to this species.

#### Acknowledgements

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# Supplementary material I

# Sample codes, locality details (voucher numbers for newly sequenced specimens in parentheses), and GenBank accession numbers for COI and cytB sequences Authors: Robert Hershler, Hsiu-Ping Liu, Niko Hubbart

Data type: Specimen data

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Link: https://doi.org/10.3897/zookeys.679.13472.suppl1

# Supplementary material 2

#### Distribution of COI haplotypes

Authors: Robert Hershler, Hsiu-Ping Liu, Niko Hubbart

Data type: Genetic data

Explanation note: Exemplars used in the phylogenetic analyses are in parentheses.

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Link: https://doi.org/10.3897/zookeys.679.13472.suppl2

# Supplementary material 3

# Distribution of cytB haplotypes

Authors: Robert Hershler, Hsiu-Ping Liu, Niko Hubbart

Data type: Genetic data

Explanation note: Exemplars used in the phylogenetic analyses are in parentheses.

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RESEARCH ARTICLE



# *Erhaia* Davis & Kuo (Gastropoda, Rissooidea, Amnicolidae) also in Bhutan

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# Abstract

The occurrence of at least one species of *Erhaia* in Bhutan, viz. *Erhaia wangchuki* **sp. n.**, is confirmed by DNA sequencing. A second unnamed species from Bhutan, that might be congeneric, is known from only a single shell. According to the molecular analysis, *E. wangchuki* is most closely related to a still undescribed *Erhaia* species from China. These two species together with *E. jianouensis* and *Akiyoshia kobayashii*, both also from China, form a well supported clade. Awaiting additional molecular data, the apparent inconsistency regarding *Erhaia* versus *Akiyoshia* is not dealt with here. The extant true sister species of *E. wangchuki* could be among the four SE Himalayan species from Bhutan and Nepal that are classified with *Erhaia* on the basis of conchological data only.

#### Keywords

Erhaia, Akiyoshia, 16S, taxonomy, distribution, Nepal, Bhutan

# Introduction

The extremely speciose superfamily Rissooidea, with over 400 recent genera 'one of the largest gastropod families' (Wilke et al. 2001: 1), encompasses very many species that cannot be identified on the basis of only shell characters, distribution and ecology. Anatomical characters may additionally be used, when the equipment for dissection is available, but even that technically demanding approach does not always bring conclusive results. Therefore, molecular analyses are advisable in cases like this. This has resulted in a more reliable classification allowing these snails to be used as ecological indicator species on the one hand and model organisms in historical biogeography on the other hand.

After its description by Davis and Kuo in Davis et al. (1985), the genus *Erhaia* turned out to be widespread in Asia. From Nepal, two species of *Erhaia* are reported by Nesemann et al. (2007) on the basis of shell shape and ecology. Here, we report the occurrence of a species of *Erhaia* in Bhutan, confirmed by a molecular analysis. The shell of a species that might be congeneric is additionally described but without naming it.

#### Material and methods

The first author coincidentally collected a minute snail in a marshy source area in Bhutan, in the district Thimphu, W of Geneykha at 2825 m altitude. The locality could not yet be visited again. The specimen got lost after being photographed with a Ricoh WG-4 digital camera, using the extreme macro facility. Some equally small snails were found by Jigme Wangchuk of the Ugyen Wangchuck Institute for Conservation and Environment, Bumthang, in a source in the district Wangdue Phodrang at 2883 m altitude, and preserved in alcohol 70%. One specimen (Fig. 1) of the latter sample, a paratype, was photographed with a digital microscope system (KEYENCE VHX-2000; KEYENCE Corp., Itasca, IL, USA) and then used for a molecular analysis. Another shell, the holotype (Fig. 2), was photographed with a Canon EOS 7D, using a Canon extension tube EF25 and a Sigma DC 18-50 mm lens.

DNA was isolated using standard protocols for molluscs (see Stelbrink et al. 2016 for details). A mitochondrial DNA fragment (16S rRNA) was amplified and sequenced (GenBank accession number: KY798003). In addition, a maximum likelihood tree (Fig. 4) was obtained using RAxML BlackBox (substitution model: GTR+ $\Gamma$ , 100 bootstrap replicates; Stamatakis et al. 2008) by using the 16S rRNA dataset of Liu et al. (2014).

# Results

Our molecular analysis resulted in a tree (Fig. 4) with *Erhaia wangchuki* sp. n. as the sister taxon of an undescribed *Erhaia* sp. from China, Guangxi, Xiangjiang river

at Quanzhou (after Liu et al., 2014: 22). The sister taxon of these two species is unclear, but a clade formed by four species, viz. *E. wangchuki*, *E.* spec., *Akiyoshia kobayashii* Kuroda & Habe, 1958 and *E. jianouensis* (Liu & Zhang, 1979), is highly supported.

#### **Systematics**

Superfamilia Rissooidea Gray, 1847 Family Amnicolidae Tryon, 1863

#### Genus Erhaia Davis & Kuo, 1985

**Type species.** *Erhaia daliensis* Davis & Kuo, in Davis, Kuo, Hoagland, Chen, Yang and Chen, 1985.

#### Erhaia wangchuki sp. n.

http://zoobank.org/304DE8F4-959A-4C7E-A497-00DA959CB99D Figs 1, 2

**Material.** District Wangdue Phodrang, Gangchhu (Figs 5, 6), 2883 m alt.; 27°26'N 90°11'E; Jigme Wangchuk leg. 21.iii.2015. National Biodiversity Centre, Serbithang, Thimphu [holotype NBCB1013, paratypes NBCB1014/2].

**Shell.** Conical, broader than high, with a flat apex because the initial <sup>3</sup>/<sub>4</sub>-1 whorl is planispiral; <sup>3</sup>/<sub>4</sub> whorls in total. Body whorl large, the height of the aperture exceeds that of the spire. Aperture with a broadly rounded outer lip and a nearly straight parietal side, so that a columellar border is hardly recognizable. Growthlines moderately strong, with a more prominent periostracal ridge at more or less regular distances. Teleoconch whorls broadly shouldered and separated by a deeply incised suture. Aperture oblique ovoid, smooth inside; apertural edge not touching the penultimate whorl. Umbilicus widely open. The holotype is the largest shell and measures 2.15×1.77 mm.

The shell differs from the shells of the three '*Erhaia*' species reported from Nepal by Nesemann et al. (2007) by the large body whorl, the relative height of the aperture, and by being broader than high. The other species that are referred to as *Erhaia* in the literature, from areas that are further apart than Bhutan and Nepal, also have different combinations of character states.

**Notes.** This species is known from the source of the Gangzetem brooklet, emerging from an underground spring aquifer surrounded by blue pine (*Pinus wallichina*) and a small open meadow (Figs 5, 6). The stream bed substrate, viz. pebbles, small rocks and parts of plants, is covered with dark-green algae, housing an abundant diversity of aquatic invertebrates. Alongside the brooklet are rhododendrons (*Rhododendron thomsonii, R. arboretum, R. kesangae*), berries (*Berberies asiatica, Rosa sericea*), betula



**Figure 1.** *Erhaia wangchuki* sp. n., sequenced paratype; scale bar 0.5 mm (photographs by B.S.); Bhutan, district Wangdue Phodrang, Gangchhu, 2883 m alt.; 27°26'N, 90°11'E; Jigme Wangchuk leg. 21.iii.2015.



**Figure 2.** *Erhaia wangchuki* sp. n., holotype NBCB1013, measurements 2.15×1.77 mm (photographs by E.G.); Bhutan, district Wangdue Phodrang, Gangchhu, 2883 m alt.; 27°26'N 90°11'E; Jigme Wang-chuk leg. 21.iii.2015.

(*Betula utilis*), larch (*Larix griffithii*), daphne (*Daphne bholua*) and remnants of dead dwarf bamboo (*Yushania microphyllus*).

A farm road to the villages of Gangphel and Zizi crosses over the stream. The source is very close (~50m) to that road. The stream also spins a *chhukhor*, i.e. a water powered

prayer wheel. At the very outlet of the stream is a water tank, which supplies drinking water to Damchu Lhakhang. The brooklet meanders into the Phobji main stream, and measures about 1100 meters. During the pre-monsoon (21.03.2015) and post-monsoon (29.11.2015), physiochemical properties of the stream were measured. The water is almost neutral (pH 7.06, 7.58) and has a nearly stable temperature (6.76, 6.20° C).

Etymology. wangchuki, after Jigme Wangchuk, who discovered these minute snails.

#### Erhaia sp.

Fig. 3

**Material.** District Thimphu, 4.5 km E of Chuzom, W of Genekha, 2750 m alt.; 27°19'N 89°36'E; E. Gittenberger leg. 21.vi.2012.

**Shell.** Elongated ovoid, higher than broad, with a last whorl measuring more than <sup>3</sup>/<sub>4</sub> of the total shell height; aperture attached to the penultimate whorl for less than <sup>1</sup>/<sub>3</sub> of the parietal-columellar side. Umbilicus very narrow. Shell height ca. 2 mm.

**Notes.** The shell is most similar in size and shape to '*Erhaia*' chandeshwariensis Nesemann & Sharma, 2007, and '*Erhaia*' banepaensis Nesemann & Sharma, 2007, as figured by Nesemann et al. (2007: 78, figs 4–5). *Erhaia wangchuki* sp. n. differs clearly by the broader shell with a lower spire.



**Figure 3.** *Erhaia* spec., measurements c. 2.0×1.35 mm (photograph by E.G.). Bhutan, district Thimphu, 4.5 km E of Chuzom, W of Genekha, 2750 m alt.; 27°19'N 89°36'E; E. Gittenberger leg. 21.vi.2012.



**Figure 4.** Maximum likelihood tree based on the 16S rRNA dataset of Liu et al. (2014). Numbers on branches denote bootstrap values >50.



**Figures 5–7.** The Gangzetem brooklet (**5**), with the watertank at the source (**6**), and the site where the brooklet crosses the road (**7**). Photographs by Damber Bdr Chhetri.

# Discussion

After its introduction by Davis et al. (1985), the classification of the genus Erhaia remained uncertain for some time. Wilke et al. (2000) eventually published maximum likelihood phylogenies based on sequences of both nuclear and mitochondrial markers, viz. 18S, and 16S and COI, and a combination of all three markers. COI supported a clade formed by *Erhaia* and the combined *Amnicola* Gould & Haldeman, 1840 and Bythinella Moquin-Tandon, 1856. Both 18S and 16S placed Amnicola next to the combined Erhaia and Moria Kuroda & Habe, 1958. The combined data showed the combined Erhaia and Moria as the sister group of Amnicola, and these three genera together in a sister group relation with *Bythinella*. Later on, maximum likelihood phylogenies were published using data from 18S and COI and a combination of both (Wilke et al. 2001). According to this study, Erhaia, Amnicola and Marstoniopsis Van Regteren Altena, 1936 together form a clade in the COI and in the combined tree as the sister group of Bithynella; the same three genera cluster together in the 18S-based tree, where the position of Bythinella remains unresolved. Liu et al. (2014), while including other genera in their molecular study, once again confirmed the systematic position of Erhaia together with Akiyoshia Kuroda & Habe, 1954 and Bythinella within the Amnicolidae.

*Erhaia* is known by several species in China, from the Yangtze River drainage and, by one species, from the Mekong River drainage (Davis and Kang 1995, Davis and Rao 1997). The probability that *Erhaia* is represented in Nepal indeed (Nesemann et al. 2007) is considerably enhanced by the anatomically confirmed occurrence of *E. nainitalensis* Davis & Rao, 1997, in Nainital in northern India west of Nepal, and *E. wangchuki* sp. n. in Bhutan east of Nepal. It may be hypothesized that there is a radiation in *Erhaia* along the southern border of the Himalaya, far west and northwest of its large range in China. With hardly any spring area in Bhutan searched for micro-snails, additional species may wait for discovery there at least.

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RESEARCH ARTICLE



# A new species of the genus *Parachemmis* Chickering, 1937 from Colombia (Araneae, Corinnidae, Corinninae)

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# Abstract

The spider genus *Parachemmis* Chickering, 1937 (Araneae: Corinnidae: Corinninae) is reported from Colombia for the first time. *Parachemmis julioblancoi* **sp. n.** Martinez-G & Villarreal is described and illustrated from the Sierra Nevada de Santa Marta, Magdalena department. The exclusive morphology of the short and apically truncated retrolateral tibial apophysis and club-like tegular laminar process of the male palp indicates that the specimens described herein belong to a new species of *Parachemmis*. A map of the distribution of species in the genus is included.

#### **Keywords**

Dionycha, distribution, Neotropical Region, Sierra Nevada de Santa Marta, taxonomy

# Introduction

The family Corinnidae Karsch, 1880 currently includes 754 species in 67 genera (World Spider Catalog 2017) in two subfamilies (Ramírez 2014). The subfamily Corinninae includes 17 genera, characterized by two synapomorphies: the male palpal reservoir primarily coiled and with a sclerotized distal sector, this last is share too with some Castianeiriniae (Platnick and Baptista 1995, Bonaldo 2000, Ramírez 2014). The genus *Para*-

*chemmis* was proposed by Chickering (1937) with *Parachemmis fuscus* Chickering, 1937 as type species, and placed in the subfamily Micariinae (Clubionidae). Posteriorly Reiskind (1969) considered *Parachemmis* as a Liocraninae by the presence of four to six pairs of long ventral spines on the tibiae of the first pair of legs and setae simply on abdomen, however, Bonaldo and Brescovit (1994) examined this characters and transferred *Parachemmis* to the subfamily Corinninae, because several pairs of ventral spines on anterior tibiae are common among Corinninae and the photograph of the *Parachemmis hassleri* (Gertsch 1942) reveal feathery setae on abdomen and legs (Bonaldo and Brescovit 1994). Currently this genus includes three valid species, distributed in Brazil, Guyana, and Panamá. Nevertheless, these numbers are highly conservative, because in countries considered megadiverse as Colombia, only has reported 12 genera and 25 species for the family Corinnidae (World Spider Catalog 2017; William Galvis pers. comm.). Therefore, it is expected that with greater effort and review of material deposited in Natural History Museums our understanding of this diversity will increase considerably.

The species of the genus *Parachemmis* can be recognized by having serrula in the lateral edge of the endites, subovate carapace, abdominal traqueal tubercule, male palp with retrolateral tibial apophysis (RTA) entire and by the presence of an articulated ventral tibial apophysis (VTA), as well as by the presence of a prolateral laminar process (PLP) arising from the tegulum. In females, the epigyne have two copulatory openings, the copulatory ducts are large and the secondary spermathecae are poorly-developed. On the other hand, specimens of the genus also have anterior eye row strongly procurved, posterior eye row lesser procurved, and anterior median eyes clearly larger than the others, in addition to having the sternum with two external anterolateral excavations; the latter is also characteristic of the genera *Stethorrhagus* Simon, 1896 and *Tupirinna* Bonaldo, 2000 (Bonaldo 2000).

In this paper, both sexes of a new species of *Parachemmis* Chickering, 1937 from Colombia is described and illustrated: *P. julioblancoi* sp. n. from the Sierra Nevada de Santa Marta (SNSM), Magdalena, Colombia. Finally, a map of the distribution of the genus is included.

#### Material and methods

The specimens examined are deposited in the Arachnological Collection of the Instituto de Ciencias Naturales of the Universidad Nacional de Colombia (ICN-Ar, Eduardo Flórez), Bogotá. The multifocal photographs of the copulatory structures and the measurements of the specimens were taken with a Leica MC–120 HD digital camera attached to a Leica S8AP0A stereomicroscope, the photographs were united by the image stacking software Leica Application Suite version 4.1.0. The illustrations of the palp and epigyne were made with a light camera attached to a Leica M125 stereomicroscope and the software Inkscape version 0.91. Platnick and Shadab (1975) is used as model for describing leg spination (with minor changes). For visualization of female genitalia, the epigynal plate was dissected and cleared in KOH solution 10% concentration, following the guideline proposed by Platnick et al. (1999).

Abbreviations used in the text and figures are:

AER	anterior eye row	PLP	prolateral laminar process
ALE	anterior lateral eye	PME	posterior median eye
AME	anterior median eye	ро	epigynal pocket
с	conductor	pr	proximal
cd	copulatory duct	r	retrolateral
со	copulatory opening	RTA	retrolateral tibial apophysis
d	dorsal	sp	spermathecal
e	embolus	Spe	spermophore
fd	fertilization duct	ST I	primary spermatheca
m	meters above mean sea level	ST II	secondary spermatheca
р	prolateral	v	ventral
PER	posterior eye row	VTA	ventral tibial apophysis
PLE	posterior lateral eye		

The map was prepared in the Geographic Information System QGIS "Las Palmas" (version 2.18.0, http://www.qgis.org/es/site/). The measurements are given in millimeters.

# Taxonomy

# Parachemmis Chickering, 1937

Parachemmis Chickering, 1937:38 (type species: Parachemmis fuscus Chickering, 1937)

Diagnosis and description. See Bonaldo 2000: 126.

*Parachemmis julioblancoi* Martinez-G & Villarreal, sp. n. http://zoobank.org/EE03D67B-E20A-4DCB-98F3-4911195437DE Figs 1, 2, 3

**Holotype.** Male in ICN-Ar, from Colombia, Magdalena, Sierra Nevada de Santa Marta, San Pedro de la Sierra, 2104 m, 10.895138°N, 73.999611°W, 28 Mar.2017, L. Martínez (ICN-Ar 8420). **Paratypes.** 1 $\bigcirc$ , same data (ICN-Ar 8421); 2 $\bigcirc$  (ICN-Ar 8422- 8423) and 4 $\bigcirc$  (ICN-Ar 8424) from same locality; 1 $\bigcirc$  and 1 $\bigcirc$  (ICN-Ar 8327-8328) same locality. 6 May 2016.

**Etymology.** The specific epithet is a patronym in honor of Dr. Julio Enrique Blanco (Founder of the Universidad del Atlántico), for his many contributions to art and education in Colombia.



Figure 1. *P. julioblancoi* sp. n., holotype (ICN–Ar 8420), **A** habitus **C** sternum, paratype female (ICN-Ar 8421) **B** habitus **D** sternum. Scale bars 2 mm (**A**), 5 mm (**B**), 0.3 mm (**C**), 0.5 mm (**D**).

**Diagnosis.** Males of *P. julioblancoi* sp. n. can be distinguished from all remaining species of the genus by the large, club-like PLP, which extends towards the middle and distal part of the tegulum, wide conductor that ends next to embolus, a short, apically truncated RTA and a wide VTA (Fig. 2A–C), Females of *P. julioblancoi* sp. n. resembles those of *P. fuscus* by having the copulatory openings placed medially and by the non-coiled copulatory ducts, but can be diagnosed by the short, wide and rounded copulatory ducts and very short ST II (Fig. 2D–E).

**Description. Male (holotype, ICN–Ar 8327).** Total length: 7.77. Carapace brown, 3.20 long, 2.65 wide, 1.15 high. Eyes AER 1.18 wide, PER 1.32 wide. AME 0.25, PME 0.17, ALE 0.20, PLE 0.21. (Fig. 1A). Sternum brown 1.69 long, 1.65 wide (Fig. 1C). Legs 4123, I-femora 3.10/ patella 0.94/ tibiae 3.40/ Meta-tarsus 3.01/ tarsus 1.65/total = 12.10; II 3.29/0.99/2.75/2.85/1.63/11.51; III 2.33/0.70/2.32/2.05/1.21/8.61; IV 3.2/1.25/2.67/3.65/1.47/12.24. Chelicerae dark brown, with three promarginal and four retromarginal teeth. Labium brown. Legs yellow (Fig. 1A). Leg macrosetae: femora, I d 1-1-0, p 1 di; II d=I, p 1-1-1; III d 1-1-1, p 1-0-1; IV d=III, p 1 di, r 1 di. Tibiae, I v 1–2–2; II v 0–2–2; III v 2–2–2, p 1 pr, r 1-1-0; IV v=III, p 1-1-0, r=III. Abdomen Dark gray, with an anterior yellow patch (Fig. 1A); ventrally gray. Spinnerets gray.



**Figure 2.** *P. julioblancoi* sp. n., holotype male (ICN–Ar 8420) **A** left male palp, prolateral view **B** same, ventral view **C** same, retrolateral view; paratype female (ICN–Ar 8421) **D** epigyne, dorsal view **E** same, ventral view. Abbreviations: c = conductor; cd = copulatory duct; co = copulatory opening; e = embolus; fd = fertilization duct; PLP = prolateral laminar process; po = epigynal; RTA = retrolateral tibial apophysis; sp = spermathecae; Spe = spermophore; VTA = ventral tibial apophysis. Scale bars 1 mm (**A–C**), 0.5 mm (**D–E**).

**Female. (paratype, ICN–Ar 8328).** Total length: 11.00. Carapace brown with yellowish posterior borders, 4.72 long, 3.84 wide, 1.97 high. Eyes AER 1.35 wide, PER 1.56 wide. AME 0.29, PME 0.24, ALE 0.27, PLE 0.19 (Fig. 1B). Sternum brown 2.42 long, 2.04 wide (Fig. 1D). Legs 4123. I-femora 4.26/ patella 1.46/ tibiae 4.41/ Metatarsus 3.77/ tarsus 1.01/total= 14.91; II 4.32/1.15/4.16/3.41/0.97/14.01; III 4.02/1.16/3.52/3.65/1.36/13.71; IV 5.17/1.49/4.45/5.32/1.61/18.04. Chelicerae dark brown with two promarginal and four retromarginal teeth. Labium brown. Legs yellow (Fig. 1B). Leg macrosetae: femora, I d 1-1-0, p 0-1-1; II d 1-1-1, p 0-2-1; III d 1 di, p 2-1-1, 1, r 0-1-1; IV d 1-1-1, p 1 di, r 1 di. Tibiae, I-II v 2–2-2; III v 2-2-2-2, p



Figure 3. Distribution of the species of the genus Parachemmis in South America.

0-1-1, 0-1-1; IV v 2-2-2, p 1 di, 1-0-1. Metatarsus, I v 2-0-2; II v 2-1-2; III v 2-1-2, p 1-1-1, r 1-1-1; IV v 1-1-2-2, p 1-1-0, r 1-0-1. Abdomen dorsal gray, with several anterior yellow patches. (Fig. 1B); ventrally gray. Spinnerets gray.

**Natural history.** The type material was collected manually, on leaf litter, in a conserved high mountain wet forest ecosystem.

Distribution. Only known from the type locality (Fig. 3).

Authors' contributions. LM and EV collected, and identified the material. LM was responsible for species description. LM, NM and EV reviewed the literature, drafted the manuscript, and contributed to the critical discussion. EV and LM prepared the images. All authors read and approved the final manuscript.

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SHORT COMMUNICATION



# New and interesting Orthoptera from the Arabian Peninsula and Socotra

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## Abstract

This paper reports on some interesting taxa recently found in the Arabian Peninsula and the island of Socotra. Among them is a new species of brachypterous grasshopper *Sphodromerus carapezzanus* **sp. n.** (Acrididae: Calliptaminae), described from an isolated area in Dhofar (Oman). A female *Heteracris hemiptera* (Uvarov, 1935) (Acrididae: Eyprepocnemidinae) is reported, with morphological characters which do not fully comply with those of any known subspecies. Two species, hitherto rarely documented, are also reported, *Phaneroptila insularis* Uvarov, 1957 (Tettigoniidae: Phaneropterinae) from Socotra and *Cataloipus thomasi* Uvarov, 1933 (Acrididae: Eyprepocnemidinae) from Oman. *Pycnodictya dentata* Krauss, 1902 (Acrididae: Oedipodinae) is reported from Saudi Arabia, constituting a new record for the country.

## **Keywords**

new records, new species, Oman, Sphodromerus carapezzanus sp. n., taxonomy

# Introduction

The Arabian Peninsula is located between the two wide continents of Africa and Asia, its fauna containing species of Asian and African origin. The Orthoptera fauna of the Arabian Peninsula is quite well known; the list of Ensifera and Caelifera is very long and no less than 190 taxa have been reported (Cigliano et al. 2016). Many contributions have been published in recent years (see Popov 1980, 1981a, 1981b, 1984, 1985,

1997, Ingrisch 1999, Massa et al. 2010, and Buzzetti et al. 2014). In spite of this, there are some isolated regions (like Dhofar in Oman), which are poorly explored. A number of entomological expeditions were carried out by the Museum of Cardiff, and Orthoptera material was also collected by Attilio Carapezza (as part of the Cardiff expeditions), who kindly made it available for study. Among the material identified, a few, but interesting, taxa were found. In this paper these taxa are presented along with some other interesting records from other Arabian localities and the island of Socotra.

## Material and methods

Taxonomical arrangement follows that of Cigliano et al. (2017). Specimens were photographed with a Nikon Coolpix 4500 digital camera, mounted on a Wild M5 Stereomicroscope, and photographs were integrated using the freeware CombineZP (Hadley 2008). Mounted specimens were measured with a digital calliper (precision 0.01 mm). The following measurements were taken (all in mm); body length: dorsal length from the head to the apex of the abdomen (ovipositor excluded in females), pronotum length: length of the pronotum along dorsal median line, pronotum height: maximum height of the pronotum, hind femur: length of hind femur, tegmina: length of tegmina.

## Abbreviations

BMPC Collection Bruno Massa, University of Palermo (Italy);MSNG Museo Civico di Storia Naturale 'G. Doria', Genoa (Italy).

## **Results and discussion**

Tettigoniidae Phaneropterinae

*Phaneroptila insularis* Uvarov, 1957 Figs 1–3

**Material examined.** Yemen, Socotra, Wadi Ayhaft 23.I.2014, 12°23'35"N, 53°59'18"E, A. Carapezza (2♂) (BMPC).

**Remarks.** Only one species, endemic to Socotra, is known from this genus. A previous record from this taxon is that of a male holotype, collected on 15 March 1953 along the northern slopes of the Hagghier, at Hijama (Hadiboh Plain) (Uvarov and Popov 1957, Popov 1981a), ca. 15 km away from Wadi Ayhaft. It appears to be a very rare species, where the female still remains unknown. *Phaneroptila insularis* is



Figures 1–3. *Phaneroptila insularis* Uvarov, 1957 l habitus of male 2 stridulatory file below the left tegmen 3 subgenital plate and cerci of male.

characterized by the 2<sup>nd</sup> pair of wings as long as the tegmina (Fig. 1). The stridulatory file, previously undescribed, is 0.6 mm long and consists of approximately 80 evenly spaced teeth (Fig. 2); the subgenital plate of the male is narrow and long, and apically concave. Cerci are robust and incurved (Fig. 3).

# Acrididae Eyprepocnemidinae

# *Cataloipus thomasi* Uvarov, 1933 Fig. 4

**Material examined.** Oman, Dhofar, Wadi Ayun (680 m) 18.XI.2016, A. Carapezza (13); Oman, Dhofar, Jebel Qamar (650 m) 14.XI.2016, A. Carapezza (13) (BMPC).

**Remarks.** This species is considered to be an endemic taxon to the southern Arabian Peninsula, known only from Dhofar region. According to Popov (1980) it is related to *C. oberthuri* (Bolívar, 1890) from central-east Africa.

## Eyprepocnemidinae

# Heteracris hemiptera (Uvarov, 1935)

Figs 5-6

**Material examined.** Oman, Dhofar, Wadi Mugshail (light trap) 18.XI.2016, A. Carapezza (1♀) (BMPC).

Remarks. Popov (1981b) proposed considering the genus Cyclopternacris Ramme, 1928 as a subgenus of Heteracris Walker, 1870, but Grunshaw (1991), on the basis of male genitalia, decided to synonymize the former. Heteracris hemiptera aja was described by Popov (1981b) from a male collected from north-central Saudi Arabia (Shammar). It is different from the typical subspecies, described from Yemen, due to its smaller size, shorter antennae (scarcely longer than head and pronotum together), more rounded hind margin of pronotum, more slender hind femur, shorter and broader supra-anal plate and a more attenuate cercus. In addition, the dark transverse fasciae on the hind femora are well expressed on the upper and inner median areas, while the coloration of the lower sulcus is slate-blue instead of red (Figs 5–6). The female of this taxon was previously unknown. Measurements of the specimen collected from Dhofar lie within the range of females reported by Popov (1981b) for the subspecies *hemiptera*, the antennae are longer than the head and pronotum combined, the hind femur is not as slender as recorded by Popov (1981b) for the subspecies *aja*, and fasciae on hind femora are inappreciable. However, the typical subspecies has the base of the hind femur red, while in the specimen from Dhofar here discussed the color is blue, tibiae are red and hind tarsus is brown (not purple as in *aja* or red as in *hemiptera*). Taking into consideration the geographical position of Yemen, Shammar region, and Dhofar, it seems possible that the female from Dhofar belongs to an unknown taxon, but due to the lack of a male specimens, it is not being described as yet.

Measurements (in mm). Female. Body length: 30.3; length of pronotum: 7.0; height of pronotum: 6.0; length of tegmina: 16.2; length of hind femur: 18.9; height of hind femur: 4.6.

## Oedipodinae

#### Pycnodictya dentata Krauss, 1902

**Material examined.** Oman, Dhofar, Jebel Qara, Jabal Darabab (1100 m) 16.XI.2016, A. Carapezza (1♀); Oman, Dhofar, Wadi Ayun (680 m) 18.XI.2016, A. Carapezza (1♀); Saudi Arabia, Wadi Jizan 11.VIII.1978, Filipponi (1♂) (BMPC).

**Remarks.** *Pycnodictya dentata* can be separated from *P. galinieri* (Reiche & Fairmaire, 1849) by its blue hind tibiae (purplish in *P. galinieri*) and sinuated posterior lower angle of the pronotal lobes (not sinuated in *P. galinieri*); the color of the hind wings may vary and is not diagnostic (Popov 1980, Ingrisch 1999, Haggag 2016). Specimens here listed as having reddish hind wings. The presence of this species in central-west Saudi Arabia is being recorded for the first time; it was previously reported from Oman and Yemen.



**Figures 4–6.** *Cataloipus thomasi* Uvarov, 1933: **4** habitus of male **5** *Heteracris hemiptera* (Uvarov, 1935) ssp.: habitus of female **6** detail of the hind femur of the same.

# Calliptaminae

## Sphodromerus carapezzanus sp. n.

http://zoobank.org/05EA1E6D-7E89-475A-BEFF-B8FA0AD9DF28 Figs 7–17

**Material examined.** Oman, Dhofar, Wadi Ayun (680 m)  $17^{\circ}14'53.37"N$ ,  $53^{\circ}53'16.29"E$ , 18.XI.2016, A. Carapezza (1 holotype, 1 paratype) (BMPC); (1 paratype) (MSNG).

**Diagnosis.** *Sphodromerus carapezzanus* is very peculiar for its color, brown with tegmina venation being dark and cream spotted. Hind femora with a white base and red outer carinulae, lower genicular lobe white, with the upper part brown, inner face of femora black-reddish, inner tibiae red, outer face of tibiae whitish, spines are black tipped. Carinae of pronotum are distinct in prozona, well visible in metazona.

Description. Male (Figs 7, 9, 11, 13, 14, 16, 17). Integument finely rugose. Head hypognathous, frontal ridge flat, punctate with margins diverging gradually. Fastigium of vertex depressed longitudinally, narrow, concave, without median carina, margins evident. Frons vertical, slightly convex. Frontal ridge narrow, flat, with a small depression under ocellus. Eyes oval, longer than subocular groove. Antennae filiform, 24 segmented, barely longer than the head and pronotum together (Fig. 7). Pronotum robust, slightly tectiform, lateral carinae distinct, less in metazona, median carina distinct along its entire length, slightly raised, intersected by anterior and posterior sulci (Figs 7, 9, 11). The posterior margin of pronotum obtuse, anterior rounded (Fig. 11), lateral lobes of pronotum with large dots. Prosternal process subconical, with obtuse apex. Hind femora 2.8 times longer than wide, its maximum width behind the middle (Figs 7, 13). Tegmina abbreviated, as long as 2/3 of the abdomen, but shorter than the hind knee (Figs 7, 13). Mesosternal space is 2 times longer than high. Epiproct elongate, with converging lateral margins, margins basally enlarged (Fig. 16), dorsal surface with three longitudinal furrows. Cerci incurved and stout, which is typical of the genus, they are flat with parallel margins, slightly curved and apically divided into two lobes (Figs 13, 14). Subgenital plate conical. Epiphallus broad, ventro-lateral angles projecting, anchorae short, but evident, lophi absent (Fig. 17), ectophallus with a large sclerite, aedeagus with a slender tip (Fig. 17). Hairs sparse in the body, mainly on the legs.

Female (Figs 8, 10, 12, 15). Characters are similar to those of the male, but it is of larger size (see Measurements below), cerci conical, valves of ovipositor short, robust, black-tipped with curved apices (Figs 8, 15).

Affinities. The species is assigned to the Calliptaminae genus *Sphodromerus* Stål, 1873, based on modified male cerci with a single apical tooth and robust femora. The majority of species are described on the basis of their coloration, which seems to be unreliable in similar genera (e.g. *Calliptamus* Serville, 1831) (Uvarov 1922). Three *Sphodromerus* species from the Arabian Peninsula are known to date: (1) *S. pantherinus* Krauss, 1902 from Saudi Arabia, (2) *S. serapis* (Serville, 1838) = *S. scriptipennis* (Walker, 1870) from Arabian Peninsula, and (3) *S. rathjensi* Uvarov, 1936 from Yemen, represented by two subspecies, the nominotypical and *S. r. montanus* Uvarov, 1943. *S. pantherinus* is similar to *S. serapis* from Sinai (Egypt), with tegmina reaching abdominal apex, head and pronotum with blackish spots, inner face of hind femora red, inner hind tibiae red, with inner spines red, outer whitish. *S. scriptipennis* (= *S. serapis*) has tegmina as long as abdomen, hind tibiae yellowish, inner face of hind femora blackish-purple, while *S. r. montanus* has these parts pale yellow (see also Ingrisch 1999).

**Measurements** (in mm). Male. Body length: 23.6; length of pronotum: 4.8; height of pronotum: 5.3; length of tegmina: 11.4; length of hind femur: 12.8; height of hind femur: 4.5. Female. Body length: 28.7–31.9; length of pronotum: 8.3–8.6; height of pronotum: 7.7–8.3; length of tegmina: 16.8–17.9; length of hind femur: 18.6–18.8; height of hind femur: 6.4–6.7.

**Etymology.** Named for Attilio Carapezza, distinguished Italian heteropterologist, who collected most of the Orthoptera here reported in Oman.



**Figures 7–12.** *Sphodromerus carapezzanus* sp. n.: **7** habitus of male **8** habitus of female **9** lateral view of head and pronotum of male **10** lateral view of head and pronotum of female **11** dorsal view of head and pronotum of male **12** dorsal view of head and pronotum of female.



Figures 13–17. *Sphodromerus carapezzanus* sp. n.: 13 particular of hind femur and last abdominal segments of male 14 left cercus 15 detail of hind femur and last abdominal segments of female 16 dorsal view of last tergites and epiproct of male 17 dorsal view of phallic complex.



**Figure 18.** The habitat at Wadi Ayun (Dhofar, Oman) where *Sphodromerus carapezzanus* sp. n. was collected (photo by A. Carapezza).

**Habitat.** Wadi Ayun (Arab = Valley of sources) is a very isolated narrow strip of green in an otherwise parched region; at the bottom there are deep pools of flowing blue-green water around sedges and grasses. A rocky desert surrounds it for dozens of kilometers (Fig. 18). Specimens of *Sphodromerus carapezzanus* sp. n. were collected on the ground ca. 200–300 m away from the water.

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RESEARCH ARTICLE



# A new species of psallopinous plant bug from the Malay Peninsula (Heteroptera, Miridae, Psallopinae)

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## Abstract

The paper presents description and illustrations of a new peculiar species from the genus *Psallops*, *P. coloratus* **sp. n.** from Southeast Asia (Singapore). Photographs, line drawings of the general habitus and a short comparison with a species from Thailand are provided.

## Keywords

Heteroptera, Miridae, new species, Psallopinae, Psallops, Southern Asia

# Introduction

Schuh (1976) established a new subfamily Psallopinae (Miridae) for the single member *Psallops ocullatus* Usinger, 1946. Among the main characters of *Psallops* the author listed anterior margin of pronotum slightly bent inwards, 2–segmented tarsi, subapical tooth on the tarsal claw, bristle–like parempodia, phallotheca fused with the phallobase, a simple form of vesical. Additionally, many authors consider the presence of nine metafemoral trichobothria, the head rounded in frontal view, and one or two closed cells on the membrane to be characteristic. In our estimation, one of the basic characters of the Psallopinae is the presence of enlarged eyes that are exceptionally well-developed dorso-ventrally and almost touch each other reaching the pharynx (Herczek and Popov 2014). However, it should be noted that these features are not unique for *Psallopinae*. They appear in some other subfamilies among the Miridae (e.g., Isometopinae, Cylapine or Phylinae) although some of these features are not fixed within *Psallopinae*. This is the main difficulty in providing a clear, morphological definition of this subfamily. Therefore, most often we rely on a combination of morphological features. Konstantinov (2003) analysed in detail the male genitalia in *Psallopinae* based on two species, one of which also came from Singapore (Psallops sp. no. 1). According to this author, it is premature to draw any taxonomic conclusion because too little material has been examined so far. The morphology of genitalia shows many plesiomorphic features and cannot be used to delineate the systematic position of the group. Having agreed with this statement, we should not underestimate the usefulness of such characters in the determination of species (despite the fact that species variations for these structures are still poorly examined ). After comparing the specimens described so far, we have noticed clear differences in the construction of left paramere (despite the constancy with the overall schema) and aedeagus (especially the endosoma).

There are nine species representing the subfamily Psallopinae that are known from the Southeast Asia. The first reports came from Japan where three species were described by Yasunaga (1999), *Psallops myiocephalus* (Nagasaki), *P.nakatanii* (Fanaura) and *P. yaeyamanus* (Yaeyema). The next was Lin's paper (2004), describing *P. chinensis* (Tijain, China), *P. formosanus* (Nautan, Taiwan), and *P. leeae* (Pintung, Taiwan). *Psallops luteus* (Lin, 2006) also comes from Taiwan. Recent reports (Yasunaga et. al. 2010) introduced the Thai species *P. fulvoides* and *P. sakaerat*, which had been caught in the Sakaerat National Environmental Research Station (northeastern Thailand). Among the species listed, four were captured using a light trap, two were found on the trunks of deciduous trees (*Quercus* and fabaceous broadleaf), one was captured in a sweep net, and one in a malaise trap.

## Material and methods

While studying the material in the collection of the Zoologisk Museum Copenhagen, the authors found a specimen of Psallopinae that has not been described to date. This specimen was recorded from Singapore, collected by O. Martin in the Seletar Reservoir and deposited in the collection of the museum. The abdomen was dissected and placed in a separate vial. The parameres and aedeagus were sectioned, immersed in Berlese liquid, and placed on a celluloid board. The board was placed under the specimen. Coloured photographs and drawings were obtained using Nikon Eclipse E 600 microscope and the computer program NIS Elements, Ver. 4.10. Measurements were taken with a micrometre. Classification terminology of the male genitalia follows Konstantinov (2003).

## Taxonomy

Family Miridae Subfamily Psallopinae

Genus Psallops Usinger, 1946

Type species. Psallops oculatus Usinger, 1946; 86.

#### Psallops coloratus sp. n.

http://zoobank.org/7BD9DB14-59E4-419C-B640-E6D0ACE1505C Figs 1–7

**Diagnosis.** Pronotum covered with long, strong, protruding black setae; corium with two types of setae: pale, long, semi erect hairs and shorter, dark and adpressed. Second and third antennal segments with long, pale setae, more than twice as segment diameter. Ratio of eye width to vertex width 2.67; ratio of head width to vertex width 6.51; ratio of corium length to cuneus length 4.55.

Description. Male. Body elongated, 2.67 long as wide. Head 2.33 as wide as long. Eyes large and discoid, occupying nearly entire sides of head, contiguous with anterior margin of pronotum (Figs 1, 2). Vertex not very broad, at the narrowest point 0.37 times as wide as one eye. Clypeus smoothly flush with convex frons; base of clypeus located slightly lower than half eye height; mandibular plate relatively broad, nearly reaching apex of clypeus (Fig. 3). Antennal fossa situated at base of maxillary plate. Antennae four-segmented, segment I shortest, nearly 1/5 length of second; segment III 0.8 times as long as II; segments IV missing. Maxillary plate relatively broad, buccula narrow. Thorax: Pronotum without calli with collar-like, flattened anterior margin (Figs 2, 3). Pronotum 1.54 as long as head and 2.22 times wider at basal margin as long. Exposed part of mesoscutum convex, 0.52 times as long as scutellum. Length of mesoscutum and scutellum slightly longer than the length of claval commissure. Corium 4.55 as long as cuneus. Hemelytral membrane with well large developed cell, 2.16 times as long as wide. Legs: hind femur approximately 3.6 times longer than maximum width, hind tibia 3.63 times longer than tarsus length. Tarsi two-segmented, second tarsal segment 1.3 times as long as the first; Inner surface of tibia with two rows of bright spines, which length is slightly greater than diameter of tibia. Male genitalia: Aedeagus membranous with strong sclerotized dorsal part (Figs 4, 5). Endosoma with complicated, strong sclerotized structure; paramere structure Lygus-type (Konstantinov 2003), left paramere scythe-shaped, apical process with seven small teeth. Body of paramere with several short setae. Sensory lobe convex, knee-shaped (Figs 6, 7). Right paramere missing. Head and antennae smoky-yellow with dark back vertex and fuscous clypeus. Eyes silver, labium brown. Pronotum wrinkled, dark brown. Mesos-



**Figures 1–3. 1,2** *Psallus coloratus* sp. n. **I** Photograph of dorsal view **2** Drawing of dorsal view **3** Lateral drawing of head. Scale bars: **1,2** = 0.5 mm; **3** = 0.1 mm.



**Figures 4–7. 4, 5** Photograph and drawing of aedeagus and endosoma **6, 7** Photograph and drawing of left paramere. Scale bars 0.1 mm **4, 5**; 0.05 mm **6, 7**.

cutum rust, shiny. Apical half part of scutellum reddish, basal part yellowish with a few reddish spots. Basal part of clavus and corium, apex of clavus, external margins of corium and apical 1/2 of cuneus reddish. The remainder of corium brown (just like basal part of cuneus), slightly brighter in middle. Membrane grey dark, with clearly marked cells. Ostriolar peritreme dark-reddish with ivory edge. Median vein distinctly marked. Proepisternum, propleuron and mesoepisternum black-brown. Mesoepisternum reddish brown with ivory back. Metapleurum brown. Fore coxae brown, middle and hind pale yellow; femora brown, weakly thickened, tibia and tarsi yellowish.

## Female. Uunknown.

Material examined. Holotype: male. Singapore, Seletar Reservoir, 1°24'N, 103°48'E; 7.XI.1991. O. Martin leg., Zoologisk Museum Copenhagen.

**Measurements (in mm).** Male: body length - 2.48; width - 0.93; length of head - 0.24; width - 0.56; height - 0.44; dorsal width of eye - 0.23; width of vertex - 0.09; antennal segments: I - 0.14; II - 0.64; III - 0.52, IV - missing; rostral segments: I - 0.32; II - 0.37; III - 0.34; IV - 0.11 (?); length of pronotum - 0.37; anterior width - 0.42; length of mesoscutum - 0.16; length of scutellum - 0.31; length of claval commissura - 0.42; length of fore femur - 0.57, width - 0.13; tibia length - 0.62, width - 0.08; tarsus length - 0.23; (I - 0.10, II - 0.16); middle femur length - 0.65, width - 0.14 (tibia and tarsus missing); hind femur length - 0.80, width - 0.22; tibia length - 0.98, width - 0.10; tarsus length - 0.27 (I - 0.11, II - 0.14); length of corium - 1.50; length of cuneus - 0.33; cell length - 0.41; width - 0.19

**Etymology.** From the Latin *coloratus* (variegated), referring to the different colours of the dorsal surface.

**Remarks.** The new species is distinguished from the one known from southwestern Asia by a combination of colour features and the construction of a copulatory apparatus. It is distinct in the colour of head (yellow, back of the vertex dark), the third part of the antennae (yellow), the scutellum (1/2 apical part reddish, the basal part yellowish with the reddish spots) and the colour of the legs (forecoxa brown, the middle and the hind pale yellow; femora brown, tibia and tarsus pale yellow). *P. coloratus* sp. n. is by colour related to *Psallops formosanus* Lin, but scutellum, the middle part of corium with clavus and the basal part of cuneus are different. On the other hand, the colour of pronotum is similar to that in *P. nakatani*, *P. ponapensis* and *P. yaeyamanus*. In turn, the colour of head shows affinities with that found in *P. sakaerat*, and antennal segments I , II are coloured like those in *P. yaeyamanus*. The colour of mesoscutum is similar to the one described in *P. leeae*, *P. formosanus* and *P. yapensis*.

Some metric features decisively distinguish *P. coloratus* from the species known from the Southeast Asia regions. These are: the ratio of the eye width to the vertex width (2.67), the head width to the vertex width (6.51), antennal segment II length to the pronotum width (1.73), and others. The construction of left paramere is also different. Although the shape of the lob sensor is reminiscent of the one observed in *Psallops sakaerat* Yasunaga, the paramere body and the apical process are developed differently. Additionally, a vast difference is observed in the construction of the aedeagus, which is characterised by a complex of highly sclerotized structures in the endosoma (Figs 4, 5).

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RESEARCH ARTICLE



# One new species and one new record for the genus Ninodes Warren from China (Lepidoptera, Geometridae, Ennominae)

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## Abstract

A new species of the genus *Ninodes* Warren, *N. quadratus* **sp. n.**, is described from China and compared with related species, based on numerous museum specimens. *N. albarius* Beljaev & Park, 1998, described from Korea, is newly recorded for China. Illustrations of external features and genitalia for each species of *Ninodes* are presented.

## Keywords

Cassymini, diagnosis, genitalia, morphology, N. albarius, N. quadratus, taxonomy

# Introduction

The genus *Ninodes* was described by Warren (1894) on the basis of *Ephyra splendens* Butler, 1878 from Japan, and is currently placed in the tribe Cassymini within the subfamily Ennominae. *Ninodes* Warren is a small genus with four recognized species, *N. splendens* (Butler, 1878), *N. albarius* Beljaev and Park, 1998, *N. flavimedia* Warren, 1907 and *N. watanabei* Inoue, 1976 (Beljaev and Park 1998; Scoble 1999). The species of *Ninodes* are mainly distributed in East Asia, but only *N. splendens* has been recorded from China (Prout 1915; Wehrli 1939; Heppner and Inoue 1992; Xue 1992, 2001).

The purpose of this paper is to describe one new species, *N. quadratus* sp. n., and to provide a differential diagnosis of the new species and its relatives, to report *N. albarius* as newly recorded for China, and to provide illustrations of external features and genitalia for all species of *Ninodes* to facilitate identification of the species belonging to this genus.

#### Materials and methods

Specimens of *Ninodes* were mainly taken from collections of the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (**IZCAS**), the Museum National d'Histoire Naturelle, Paris, France (**MNHN**), the Natural History Museum, London, UK (**NHM**), the Senckenberg Museum für Tierkunde, Dresden, Germany (**SMTD**) and the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (**ZFMK**). A further institution cited as type depositary is the Laboratory of Insect Taxonomy, National Institute of Agricultural Science and Technology, Suwon, Korea (**NIAST**).

Terminology for wing venation follows the Comstock-Needham System (Comstock 1918), and that for the genitalia is based on Pierce (1914, reprint 1976), Klots (1970) and Nichols (1989). Photographs of the moths were taken with digital cameras. Composite images were generated using Auto-Montage software version 5.03.0061 (Synoptics Ltd). The illustrations were compiled using Adobe Photoshop software.

## Taxonomy

## Genus Ninodes Warren, 1894

Ninodes Warren, 1894, Novit. zool., 1: 407. Type species: *Ephyra splendens* Butler, 1878, by original designation.

**Generic characters.** Antenna filiform, ciliate in both sexes. Frons narrow, not protruding. Labial palpi narrow and short. Apex of forewing rounded; outer margin of both wings smooth, or slightly rounded. Forewing length: 7–13 mm. Wings usually pale yellow to yellow, with black or greyish brown markings. Forewing with Sc free,  $R_1$  coincident with  $R_2$ ,  $R_{3+4}$  stalked with  $R_5$ ,  $R_{3-5}$  arising before anterior angle of cell, CuA<sub>1</sub> arising from or before posterior angle of cell; hindwing with Sc+R<sub>1</sub> close to cell less than one half of length of cell; Rs arising before anterior angle of cell; CuA<sub>1</sub> arising from or before posterior angle of cell. An elongate, unscaled, translucent fovea present in male. Male genitalia with uncus almost triangular; median process of gnathos small; valva with a narrow and strongly curved dorsal arm at valva base, with a tiny spine apically; juxta short; saccus broad and rounded; aedeagus short and narrow; vesica often with a sclerotized lobe and a patch of tiny spines. Female genitalia with papillae anales not elongate, slightly narrower terminally; antrum well sclerotized; ductus bursae short; ductus seminalis often with a sclerotized base, and separate from posterior part of corpus bursae; corpus bursae sclerotized and with longitudinal ribbing posteriorly; signum rounded, with long marginal and smaller central spines.

Distribution. China, Japan, Korean Peninsula, (Papua New Guinea, see below).

**Remarks.** Only one female specimen (the holotype) of *N. flavimedia* was located in NHM. Close examination of the holotype (Fig. 9) revealed that *N. flavimedia* is quite different from its present congeners in both wing patterns and female genitalia. Further morphological study of *Ninodes* and its related genera is needed to determine the correct taxonomic position of *N. flavimedia*. Characters of *N. flavimedia* are not included in the generic description above.

One male and one female probably belonging to *N. watanabei* are kept in the collection of ZFMK (collected at "Tapaishan", Shaanxi). Before recording this species as new to the Chinese fauna, the specimens must be checked closely and the identity proved by dissection (Dieter Stüning, pers. comm.).

Ninodes miegi Sterneck, 1931 has been treated as a junior synonym of N. splendens since Inoue (1956). However, we found that the wing pattern of the holotype of N. miegi (Fig. 6) is quite similar to N. albarius (Figs 4, 5). The above two species were both originally described from Korea. We suspect that N. miegi Sterneck, 1931 may be a senior synonym of N. albarius and probably has to be restored from synonymy of N. splendens. The female holotype of N. miegi has to be checked carefully. We will discuss this taxonomic problem in the future revision of Ninodes.

Ninodes scintillans Thierry-Mieg, 1915 was described as a distinct species, but listed as a junior synonym of *N. splendens* by Scoble (1999). After examining the type of *N. splendens* at BMNH, a photo of a syntype of *N. scintillans* (coll. MNHN) and specimens of both taxa in IZCAS, we found that the male and female genitalia of them are in fact identical (Figs 16–18, 22, 23), although the wing patterns of *N. splendens* (Figs 7, 8) and *N. scintillans* (Figs 10–12) are quite different, as described by Prout (1915) and Wehrli (1939). The identical genitalia are probably the reason why Scoble (1999) listed *N. scintillans* as a junior synonym of *N. splendens*. However, the genitalia of the syntypes of *N. scintillans* have not been studied so far. Examination of the collecting localities and data of the Chinese specimens in IZCAS and ZFMK revealed that *N. splendens* and *N. scintillans* are probably just seasonal forms of the same species, with typical *N. splendens* being the first generation and *N. scintillans* the second. However, further morphological, biological, and molecular studies are needed to test this hypothesis. We will also discuss this problem in the future revision of *Ninodes*.

#### Ninodes quadratus sp. n.

http://zoobank.org/09828123-6308-415C-9E52-801B86921A1B Figs 1, 2, 13, 19

**Description.** *Head.* Antenna filiform, ciliate in both sexes (ciliae of males longer), with mixed pale yellow mixed and black scales dorsally. Frons narrow, black, not protruding. Labial palpus narrow, scaled black. Vertex with large and black scales.



Figures 1–12. Adults of *Ninodes*. 1–2 *N. quadratus* sp. n. 1 male (holotype) 2 ditto, underside 3 *N. watanabei*, male (holotype, coll. NHM) 4–5 *N. albarius* 4 male 5 ditto, underside 6 *N. miegi*, female (holotype, coll. SMTD) 7–8 *N. splendens* 7 male collected from Shanghai, May 8 ditto, underside 9 *N. fla-vimedia*, female (holotype, coll. NHM) 10–12 *N. splendens* ("scintillans") 10 male, collected from Gansu in July 11 ditto, underside 12 male, collected from shanghai (syntype of *N. scintillans*, coll. MNHN). Scale bar 1 cm.

Thorax. Patagia, tegulae and dorsal side of thorax black. Hind tibia with two pairs of spurs in both sexes, without hair-pencil in male. Forewing length: 9–10 mm. Apex of forewing rounded; outer margin of forewing only slightly oblique, evenly curved outwards. Ground colour of wings pale yellow. Forewing black from base to antemedial line; discal spot pale grey, indistinct; medial and postmedial line yellow, narrow and wavy; a black quadrate patch present near tornus; terminal line present as a series of short black streaks between veins; fringe pale yellow. Hindwing pale yellow at base, discal spot hardly visible; a broad black band present between medial and postmedial line; submarginal line yellow and wavy, more distinct than that of forewing; terminal line and fringe similar to those of forewing. Underside with discal spot of forewing more distinct than that on upperside; black postmedial bands present on both wings, that of forewing elongate, not quadrate as on upperside, that of hindwing narrower than on upperside, other pattern elements less distinct than those on upperside. Wing venation and fovea of male as mentioned in generic characters.

*Abdomen.* Abdomen dorsally suffused with black scales. Setal comb absent on male sternite III.

*Male genitalia*. Uncus almost triangular, strongly sclerotized and shallowly bifurcate at apex. Gnathos with a small median process, rounded terminally. Valva short, elongate-triangular, narrow and rounded terminally, with a sclerotized ridge, evenly curved outwards, costal margin curved outwards, with a row of long and spine-like setae medially. A well-developed dorsal arm present at valva base, strongly curved to Sshape, with a tiny spine apically and a long, curved, rod-like basal process. Juxta short and broad, tapering terminally. Saccus broad and rounded. Aedeagus short and narrow; vesica with a large spatulate lobe and a large dense patch of of tiny spines, laterally extended into a row of short, stout spines.

*Female genitalia*. Papillae anales not elongate, slightly narrower terminally. Apophyses anteriores about 3/5 length of apophyses posteriores. Antrum well sclerotized, about one half length of corpus bursae. Ductus bursae very short. Ductus seminalis with a small sclerotized base, separated from posterior part of corpus bursae. Corpus bursae sclerotized and with longitudinal ribbing at posterior half; signum on ventral side, rounded, margins and internal surface covered with spines.

Diagnosis. Concerning wing patterns, the new species can be distinguished from the other congeners by the broad black quadrate patch at medial to postmedial area of the forewing, becoming indistinct above CuA<sub>3</sub>; the discal spot of the forewing is more distinct on the underside than that on the upperside. In the male genitalia, the new species has a distinctive shape of valva and arrangement of cornuti: the costal margin of the valva is curved outwards medially, while it is almost straight in N. albarius (Fig. 15), slightly curved inwards in N. splendens (Figs 16-18), and forming a semicircular cleft in N. watanabei (Fig. 14); the spatulate lobe of the vesica is similar to that of N. splendens, but larger and the large dense patch of small spines present in *N. quadratus* is rather arranged in a longitudinal line of much larger spines in *N*. splendens and a very short row of small spines in N. albarius. The lateral row of short stout spines is absent in N. splendens and N. albarius. The female genitalia of the new species are similar to N. albarius, but the base of the ductus seminalis is almost conic and separated from the corpus bursae in N. albarius (Fig. 21), while this character is absent in the new species. The antrum is similar in length to N. albarius, but much shorter than that of N. splendens (Figs 22, 23), and is much narrower compared to N. watanabei (Fig. 20). The signum of N. quadratus is larger and has more but smaller marginal spines than those of N. splendens and N. albarius.

Material examined. Holotype, *A*, CHINA: Henan (IZCAS): Baotianman, 623 m, 12.VIII.2008, coll. Xue Dayong. Paratypes: 1*A*, same data as holotype. Henan (IZCAS): 1*A*, Haoxian, Baiyunshan, 115 m, 7–9.VIII.2013, coll. Jiang Nan. Shaanxi



Figures 13–18. Male genitalia of *Ninodes*. 13 *N. quadratus* sp. n. (paratype, IZCAS slide no. Geom-04014) 14 *N. watanabei* (NHM Inoue slide no. 05182). 15 *N. albarius* (IZCAS slide no. Geom-04015) 16–18 *N. splendens* 16 collected from Japan, (syntype, NHM Geometridae slide no. 07899) 17 collected from Shanghai in May (IZCAS slide no. Geom-04311) 18 collected from Gansu in July ("scintillans") (IZCAS slide no. Geom-02339). Scale bars 1 mm.

(IZCAS):  $5\overset{\circ}{\partial}2\overset{\circ}{\Box}$ , Shangnan, Jinsixia, 777 m, 23–25.VII.2013, coll. Cui Le & Jiang Nan;  $3\overset{\circ}{\partial}$ , Ningshan, Guanghuojie, Baohuzhan, 1081–1189 m, 26–28.VII.2014, coll. Liu Shuxian & Ban Xiaoshuang. **Gansu** (IZCAS):  $1\overset{\circ}{\partial}$ , Wenxian, Qiujiaba, 2200–2300 m, 16–19.VII.2003, coll. Wang Hongjian;  $4\overset{\circ}{\partial}5\overset{\circ}{\Box}$ , Wenxian, VI–IX.2002, coll. Wang Hongjian;  $1\overset{\circ}{\partial}$ , Bikou, Baohuzhan, 645 m, 6–7.VIII.2016, coll. Cheng Rui & Jiang Shan;  $5\overset{\circ}{\partial}1\overset{\circ}{\Box}$ , Bikou, Bifenggou, 720 m, 8–10.VIII.2016, coll. Cheng Rui & Jiang Shan. **Zhejiang** (ZFMK):  $1\overset{\circ}{\partial}$ , West-Tien-Mu-Shan, 1600 m, 14.IV.1932, coll. H. Höne.

Distribution. China (Henan, Shaanxi, Gansu, Zhejiang).

**Etymology.** The specific name refers to the quadrate (Latin: *quadratus*) black patch near the tornus of the forewing.



Figures 19–24. Female genitalia of *Ninodes*. 19 *N. quadratus* sp. n. (paratype, IZCAS slide no. Geom-04005) 20 *N. watanabei* (NHM Inoue slide no. 05183) 21 *N. albarius* (IZCAS slide no. Geom-04046); 22–23 *N. splendens* 22 collected from Shanghai in June (IZCAS slide no. Geom-04052) 23 collected from Hubei in July ("*scintillans*") (IZCAS slide no. Geom-02966) 24 *N. flavimedia* (holotype, NHM Geometridae slide no. 7901, without scale). Scale bars 1 mm.

## Ninodes albarius Beljaev & Park, 1998

Figs 4, 5, 15, 21

Ninodes albarius Beljaev & Park, 1998, Tinea, 15 (3): 243, figs 1, 4, 13. Holotype &, Gwangleung, Korea (NIAST).

**Diagnosis.** *N. albarius* is similar to *N. splendens* externally, but can be distinguished by the following features: a broad, black band, reaching from costa to anal margin, is present at the base of the forewing, while in *N. splendens*, it is absent or only present

near the anal margin; in the male genitalia, the valvae are longer and narrower, more strongly pointed apically and with a group of strong spines near apex, the terminal part of the juxta is broader and incised, the cornuti are smaller and arranged in a short band. In the female genitalia, the antrum is shorter, the posterior part of the corpus bursae is narrower and more strongly sclerotized, and the signum is larger in *N. albarius*. By the wing patterns, *N. albarius* also resembles *N. quadratus* and *N. watanabei* in the basal black or greyish brown band, but it is different from *N. quadratus* by the absence of the quadrate patch near the tornus and the distinct distal spot on the upperside of the forewing, and differs from *N. watanabei* by the smaller size and the absence of the postmedial band between veins M, and M, of the forewing.

**Material examined. CHINA: Henan** (IZCAS): 1Å, Tongbai, Shuiliandong, 300 m, coll. Shen Xiaocheng and Ren Yingdang; 2Å, Xinyang, Jigongshan, 250 m, 20–21.VII.2002, coll. Han Hongxiang. **Shaanxi** (IZCAS): 1Å, Zhouzhi, Houzhenzi, 1350 m, 24.VI.1999, coll. Zhu Chaodong; 2Å1 $\updownarrow$ , Shangnan, Jinsixia, 777 m, 23–25. VII.2013, coll. Cui Le & Jiang Nan; 1Å, Xunyang, Jinxinyuan, Shanzhuang, 386 m, 1–3.VIII.2014, coll. Ban Xiaoshuang. **Gansu** (IZCAS): 3Å, Bikou, Baohuzhan, 645 m, 6–11.VIII.2016, coll. Cheng Rui & Jiang Shan; 2Å1 $\updownarrow$ , Bikou, Bifenggou, 720 m, 8–10.VIII.2016, coll. Cheng Rui & Jiang Shan. **Hubei** (IZCAS): 11Å1 $\updownarrow$ , Yingshan, Wujiashan, 860 m, 28–30.VI.2014, coll. Cui Le, Jiang Nan & Xue Dayong; 3Å1 $\clubsuit$ , Yingshan, Wujiashan, Jingqu, 500 m, 30.VI.2015, coll. Xue Dayong; 1Å, Yunxi, Guanyinzhen, 289–305 m, coll. Ban Xiaoshuang.

**Distribution.** Korean peninsula and China (Henan, Shaanxi, Gansu, Hubei); this is a new record for China.

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RESEARCH ARTICLE



# A new cryptic species of *Nagiella* Munroe from China revealed by DNA barcodes and morphological evidence (Lepidoptera, Crambidae, Spilomelinae)

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## Abstract

*Nagiella occultalis* Misbah & Yang, **sp. n**. from China is described and illustrated. This new species is very similar to *N. quadrimaculalis* (Kollar, 1844) in general morphological characters of forewing and male genitalia. Molecular evidence shows that these two species diverge in COI barcode region by more than 3.2%. Sequence divergence among the two species is congruent with subtle morphological differences. Wing venation and male genitalia of the two species are compared and illustrated.

## Keywords

COI gene, genitalia, Scopula quadrimaculalis, taxonomy

# Introduction

The subfamily Spilomelinae (Crambidae) is the largest subfamily of pyraloid moths including about 3300 species in more than 300 genera having worldwide distribution (Munroe and Solis 1999). The genus *Nagiella* Munroe, 1976 is one of the less speciose genera of Spilomelinae (Munroe 1976). Compared to other genera of this subfamily and despite its small size, *Nagiella* has been little studied and no comprehensive stud-

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ies have been made on the taxonomy of its constituent species. The only taxonomic efforts were made by Munroe in 1976. This genus was originally described as *Nagia* by Walker in 1866 based on the type species *Nagia desmialis* Walker, 1866. Munroe (1976) recognized that *Nagia* Walker, 1866 is a junior homonym of *Nagia* Walker, 1858 (Lepidoptera: Noctuidae) and replaced it with the new name *Nagiella* Munroe, 1976. This genus is widely distributed in Malaysia (Borneo and Sarawak), Burma, China, and Japan (Munroe 1976; Inoue 1982; Wang 1980). The genus comprises three described species: *Nagiella inferior* (Hampson, 1898), *Nagiella quadrimacualis* (Kollar, 1844) with two junior subjective synonyms, *desmialis* Walker, 1866 and *incomitata* Swinhoe, 1894, and *Nagiella hortulatoides* Munroe, 1976) are: uncus truncate, short and wide; gnathos ribbon-like; subscaphium elongate; valva broader with stout setae subapically, sella digitiform, elongate and sharp; cornutus absent. This provides the baseline description of the genus on which the present study is based.

Recently the integration of DNA barcoding and morphological approaches opened the field for researchers in accelerating species identification and assisted in detecting previously undetected cryptic species (Sutrisno 2005; Mutanen et al. 2012; Haines et al. 2012; Yang et al. 2012; Rajaeish et al. 2013; Yang et al. 2016; Mally et al. 2016). The taxonomic placement of *N. occultalis* sp. n. has been unclear; therefore, an integrative approach was designed to study the generic differences (Munroe 1976). In the present integrative taxonomic study, *N. occultalis* sp. n. collected from Shaanxi and Hubei Province, China, is described.

## Materials and methods

## **Taxon sampling**

Three specimens of *N. occultalis* sp. n. were collected from Taibai Mountain, Shaanxi and Wufeng, Hubei in China and 15 specimens of *N. quadrimaculalis* were collected from various localities (Table 1). Genitalia preparation mainly follows Landry (2007) and Yang et al. (2012) and terminology follows Kristensen (2003). The images of adults and genitalia were captured with a Canon Power Shot SX60 digital camera and (ZEISS Discovery V20) stereomicroscope equipped with an AxioCam ICc5 camera, respectively and measurement was taken in mm by scale bar equipped in stereomicroscope. Type material of the new species is deposited in the Entomological Museum, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China (NWAFU).

#### DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from insect legs by following the method of Ivanova et al. (2006). PCR amplifications were conducted to amplify a full-length (658 bp)

Identification	BIN	Process ID	Sample ID	Length of sequence (bp)	GenBank Accession	Province	Genitalia slide number
N. occultalis sp. n.	BOLD:AAD8179	CNPYB439-16	NAFU Pyr002290	658	KY080696	Shaanxi	
N. occultalis sp. n.	BOLD:AAD8179	CNPYB407-16	NAFU Pyr002397	658	KY080703	Shaanxi	NAFU Pyr002065
N. occultalis sp. n.	BOLD:AAD8179	CNPYD499-10	$P_{yr}000499$	658	HM908668	Hubei	
N. quadrimaculalis		CNPYA401-10	NAFU Pyr000401	0		Yunnan	
N. quadrimaculalis		CNPYA402-10	NAFU Pyr000402	0		Sichuan	
N. quadrimaculalis		CNPYA403-10	NAFU Pyr000403	0		Yunnan	
N. quadrimaculalis		CNPYA404-10	NAFU Pyr000404	0		Yunnan	
N. quadrimaculalis		CNPYB409-16	NAFU Pyr002070	0		Shaanxi	NAFU Pyr002070
N. quadrimaculalis		CNPYB410-16	NAFU Pyr002261	0		Shaanxi	NAFU Pyr002261
N. quadrimaculalis		CNPYB411-16	NAFU Pyr002262	0		Shaanxi	
N. quadrimaculalis	BOLD:AAD8178	CNPYB412-16	NAFU Pyr002263	658	KY080700	Shaanxi	
N. quadrimaculalis	BOLD:AAD8178	CNPYB413-16	NAFU Pyr002264	658	KY080702	Shaanxi	
N. quadrimaculalis	BOLD:AAD8178	CNPYB414-16	NAFU Pyr002265	658	KY080704	Shaanxi	
N. quadrimaculalis	BOLD:AAD8178	CNPYB415-16	NAFU Pyr002266	658	KY080698	Shaanxi	
N. quadrimaculalis	BOLD:AAD8178	CNPYB416-16	NAFU Pyr002267	658	KY080694	Shaanxi	
N. quadrimaculalis	BOLD:AAD8178	CNPYB417-16	NAFU Pyr002268	658	KY080705	Shaanxi	
N. quadrimaculalis	BOLD:AAD8178	CNPYB418-16	NAFU Pyr002269	658	KY080697	Shaanxi	
N. quadrimaculalis		CNPYB419-16	NAFU Pyr002270	0		Shaanxi	
N. quadrimaculalis		CNPYB420-16	NAFU Pyr002271	0		Shaanxi	
N. quadrimaculalis		CNPYB421-16	NAFU Pyr002272	0		Henan	NAFU Pyr002272
N. quadrimaculalis		CNPYB422-16	NAFU Pyr002273	0		Henan	NAFU Pyr002273
N. quadrimaculalis		CNPYB423-16	NAFU Pyr002274	0			
N. quadrimaculalis		CNPYB424-16	NAFU Pyr002275	0		Hunan	
N. quadrimaculalis		CNPYB425-16	NAFU Pyr002276	0		Hunan	
N. quadrimaculalis		CNPYB426-16	NAFU Pyr002277	0			
N. quadrimaculalis		CNPYB427-16	NAFU Pyr002278	0		Fujian	
N. quadrimaculalis		CNPYB428-16	NAFU Pyr002279	0		Hainan	
N. quadrimaculalis		CNPYB429-16	NAFU Pyr002280	0		Hainan	

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Table

Identification	BIN	Process ID	Sample ID	Length of sequence (bp)	GenBank Accession	Province	Genitalia slide number
N. quadrimaculalis		CNPYB430-16	NAFU Pyr002281	0			
N. quadrimaculalis		CNPYB431-16	NAFU Pyr002282	0		Zhejiang	
N. quadrimaculalis		CNPYB432-16	NAFU Pyr002283	0		Yunnan	
N. quadrimaculalis		CNPYB433-16	NAFU Pyr002284	0			NAFU Pyr002284
N. quadrimaculalis		CNPYB434-16	NAFU Pyr002285	0			
N. quadrimaculalis		CNPYB435-16	NAFU Pyr002286	0			
N. quadrimaculalis		CNPYB436-16	NAFU Pyr002287	0			NAFU Pyr002287
N. quadrimaculalis		CNPYB437-16	NAFU Pyr002288	0			
N. quadrimaculalis	BOLD:AAD8178	CNPYB438-16	NAFU Pyr002289	658	KY080695	Shaanxi	
N. quadrimaculalis	BOLD:AAD8178	CNPYB440-16	NAFU Pyr002291	658	KY080701	Shaanxi	NAFU Pyr002291
N. quadrimaculalis	BOLD:AAD8178	CNPYB441-16	NAFU Pyr002292	658	KY080699	Shaanxi	
N. quadrimaculalis		CNPYB408-16	NAFU Pyr002398	0		Shaanxi	NAFU Pyr002067
N. quadrimaculalis	BOLD:AAD8178	CNPYD497-10	Pyr000497	622	HM908666	Hubei	
N. quadrimaculalis	BOLD:AAD8178	CNPYD498-10	Pyr000498	658	HM908667	Hubei	
N. quadrimaculalis		CNPYD500-10	Pyr000500	0		Hubei	
N. quadrimaculalis		CNPYD501-10	Pyr000501	0		Hubei	
N. quadrimaculalis		CNPYD502-10	Pyr000502	0		Hubei	
N. quadrimaculalis	BOLD:AAD8178	CNPYD503-10	Pyr000503	658	HM908669	Hubei	
N. quadrimaculalis	BOLD:AAD8178	CNPYD504-10	Pyr000504	658	HM908670	Sichuan	
N. quadrimaculalis	BOLD:AAD8178	CNPYD505-10	Pyr000505	658	HM908671	Sichuan	

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barcode region of the mitochondrial COI gene by the primers pairs, *LepF1* and *LepR1* (Hajibabaei et al. (2006). After the PCR products were checked with 1% agarose gel, sequencing was performed at Sangon Biotechnology Co., Ltd. (Shanghai, China) using the same primers as in PCR.

# Data analysis

Sequence alignment was carried out by using MUSCLE algorithm implemented in MEGA 6.0 (Tamura et al. 2013). MEGA 6.0 was also used to perform genetic distances under the Kimura 2-parameter model of base substitution, to produce the Neighbor-Joining (NJ) tree, and to perform bootstrap analysis (1000 replicates) (Kimura 1980). In the present study, we included four sequences of *Nagiella inferior* and selected *Patania ruralis* (Scopoli, 1763) as the primary out-group to build the tree which is most closely related genus. Sequences obtained from the current study were deposited in GenBank, in addition to being available in the BOLD dataset DS-PLEQUA.

# Results

# DNA sequence analysis

A total of 18 COI gene sequences of *N. occultalis* sp. n. and *N. quadrimaculalis* were obtained. The lengths were from 622–658 bp (mean 656 bp). The genetic distances within and between these two species of *Nagiella* are given in Table 2. Intraspecific genetic divergences ranged from 0.00–0.16 % (mean 0.078 %), whereas interspecific genetic divergence ranged from 3.12–3.28 % (mean 3.21 %). The neighbor-joining (NJ) tree (Fig. 1) showed two distinct barcode clusters that correspond to morphological differences between these two species.

**Table 2.** Kimura 2-parameter genetic distances calculated within (in italic) and between three species ofNagiella.

	Nagiella occultalis	Nagiella	Nagiella	Patania ruralis
	sp. n.	quadrimaculalis	inferior	(outgroup)
Nagiella occultalis sp. n.	0.0000000	0.0072358	0.0086344	
Nagiella quadrimaculalis	0.0320975	0.000787822	0.0101216	
Nagiella inferior	0.0475427	0.0598071	0.000761036	
Patania ruralis (Outgroup)	0.1156349	0.1165689	0.1134248	0.009202714

The diagonal row of values (in bold) indicates intra specific distances, the values below the diagonal indicates mean interspecific distances and values above the diagonal indicates SE estimates obtained by bootstrap procedure (1000 replicates) as implemented in MEGA 6.0. The three species were defined using the 2.0% divergence.



0.01

**Figure 1.** Neighbor-joining tree (K2P) based on the 22 COI sequences of the three *Nagiella* species from China, rooted with *Patania ruralis* as outgroup. Bootstrap values <75 are not shown.

#### Taxonomy

*Nagiella occultalis* Misbah & Yang, sp. n. http://zoobank.org/C252DFC4-FA47-4A75-85CE-3D7E99E25177

**Etymology.** The specific epithet refers to "cryptic", as this previously undetected species stood within the *N. quadrimaculalis* complex.

**Diagnosis.** This species can be distinguished from *N. quadrimaculalis* by the width and length of the uncus, the proportions of the valva and transtilla, and size of the forewing, as described in Table 3.

**Description (Figs 2A, 3).** Body yellowish brown to black with white patches on wings. Length of forewing 15–16 mm. Head with frons shiny white, labial palpus bent over top of head. Patagium shiny black. Forewing dark brown, with small bean-shaped white spot of varying size near middle of reniform stigma in the base of discal cell; rectangular subdiscal white spot proportionally narrower or elongate.  $R_1$  arising from cell at about apical third and almost parallel to Sc,  $R_2$  parallel to  $R_1$  but close to  $R_{3+4}$ ,  $R_3$  and  $R_4$  long stalked and reached apical margin.  $M_2$  and  $M_3$  closer to each other at base than  $M_1$  (almost of the same length) but all median veins on equal distance on outer margin. Vein Cu<sub>2</sub> originating from 2/3 of the cell. Anal vein  $A_{1+2}$  prominent and complete while  $A_3$  diminished before mid-length of wing. Hind wing with bean-shaped white spots near outer margin of medial line at terminal part of discal cell; Sc, radial and  $M_1$  on same stalk, anal vein  $A_3$  incomplete.



Figure 2. Adults, dorsal aspect A N. occultalis sp. n. B N. quadrimaculalis.



Figure 3. Wing venation of *N. occultalis* sp. n.

Characteristics	<i>N. occultalis</i> sp. n.	N. quadrimaculalis
Forewing length	15–16 mm (Fig. 2A)	18-20 mm (Fig. 2B)
Small subdiscal spot on forewing	Proportionally narrower or elongate	Sub-quadrate
Uncus width and length	0.4 × 0.6 mm (Fig. 4A)	0.3 × 0.68 mm (Fig. 4C)
Posterior margin of uncus	Slightly notched medially	Evenly rounded
Valva	Broader, W/L 0.91 × 3.09 mm	Slender, W/L 0.7 mm × 2.08 mm
Sella with ventral edge	Straight	Slightly incurved
Subscaphium	Elongate, conical sclerotized	Unsclerotized
Size of transtilla	Narrower, 0.28 × 0.8 mm	Broadly triangular 0.3 × 0.9 mm
Phallus	Phallus L/valva L ratio 1.19 (Fig. 4B)	Phallus L/valva L ratio 1.7 (Fig. 4D)

Table 3. Morphological differences between Nagiella occultalis sp. n. and N. qudrimaculalis.



Figure 4. Male genitalia A, B *N. occultalis* sp. n., genitalia slide NAFU PYR 002397 C, D *N. quadrimaculalis*, genitalia slide NAFU PYR 002069.

Male genitalia (Fig. 4A, B). Uncus subtrapezoid in outline, posterolateral angles rounded, distal margin slightly notched medially. Gnathos with proximal arms extended transversely from teguminal margin and joined mesially into subclavate distal projection extended almost to level of apex of uncus. Subscaphium very elongate, apex extended beyond apex of valvae. Transtilla triangular, broad basally and apically narrower. Valva relatively short and broad with several thickened setae on posterior margin. Sella elongate, digitiform, straight laterally, apex rounded. Saccus roundly conical. Phallus cylindrical, terminal end somewhat tapered, cornutus absent.
Female. Unknown

Distribution. China (Taibai Mountain, Shaanxi; Wufeng, Hubei).

**Type material. Holotype.** ♂: China: Shaanxi, Taibai Mountain, 1051 m, 25 July 2014, Zhou Lin (NWAFU), Specimen ID: NAFU PYR002397. Genitalia slide number: NAFU PYR002397. **Paratypes.** 1 ♂, same data as the holotype except 24 July 2014; 1 ♂, China, Hubei, Wufeng, Changleping town, 14 July 2008, Zhao Lu.

**Remarks.** The genus *Nagiella*, formerly comprised of three recognized species widespread in Burma, China, Japan and Malaysia (Borneo and Sarawak), is now increased to four with *N. occultalis* sp. n.

#### Discussion

Munroe (1976) indicated that *Nagiella* differs from *Pleuroptya* Meyrick, 1890 in several genital characters, i.e. short, wide uncus, gnathos developed, cornutus absent, valva broader with stout setae subapically, as well as in type of wing maculation. This taxonomic treatment was followed by Kirti and Sodhi (2001) and Rose (2002). However, members of the genus *Nagiella* have been placed in various genera, namely *Pleuroptya* Meyrick, 1890, *Syllepte* Hübner, 1823, *Patania* Moore, 1888 (Inoue, 1982; Wang, 1980; Li et al. 2009; Xu 2015; Irungbam et al. 2016; Kirti et al. 2016). Leraut (1997) also listed *Nagiella* as a junior synonym of *Pleuroptya*. Kirti and Gill (2007) synonymized *Pleuroptya* Meyrick, 1890 under *Patania* on the basis of shared characters such as the lack of gnathos, the valvae leaf-like and without setae, and the presence of distinct cornuti present in the phallus. In *Nagiella* the gnathos is present, the valvae are broader and bear stout subapical setae, and the cornuti are absent. Based on this morphological evidence and online Lepindex (Beccaloni et al. 2003), we consider that *Nagiella* warrants distinct generic status and we re-instate it as valid.

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RESEARCH ARTICLE



# The genus Apodrosus Marshall, 1922 in Cuba (Coleoptera, Curculionidae, Entiminae, Polydrusini)

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#### Abstract

The genus *Apodrosus* Marshall is newly recorded for, and revised for Cuba. Nine new species are recognized as follows: *Apodrosus alberti* (type locality, Granma, Parque Nacional Pico Turquino), *A. alternatus* (type locality, Guantánamo, El Yunque), *A. franklyni* (type locality, Cienfuegos, Parque Nacional Pico San Juan), *A. griseus* (type locality, Santiago de Cuba, Siboney-Jutici Ecological Reserve), *A. mensurensis* (type locality, Holguin, Parque Nacional La Mensura-Piloto), *A. pseudoalternatus* (type locality, Matanzas, Varahicacos), *A. beckeli* (type locality, Guantánamo, 8 km W. Imias), *A. sandersoni* (type locality, Guantánamo, Loma Lafarola), and *A. zayasi* (type locality, Cienfuegos, Parque Nacional Pico San Juan). A key for their identification, descriptions, summaries of natural history information and data on distributions are presented. A molecular phylogeny based on 11 species of *Apodrosus* from Cuba, Hispaniola and Puerto Rico is reconstructed. A sister group relationship between *Polydrusus* and *Apodrosus* is recovered with a limited sampling of the former genus. The monophyly of *Apodrosus* is recovered with strong support. Cuban *Apodrosus* are not monophyletic. Five of the six sampled Cuban species form a clade, sister to an undescribed *Apodrosus* species from the Dominican Republic; and, *Apodrosus alternatus* is sister to *A. quisqueyanus* Girón & Franz, 2010, a species from the Dominican Republic. Biogeographic implications for Cuban species are discussed in light of the phylogeny.

#### **Keywords**

New species, weevils, West Indies, Caribbean, biodiversity

# Introduction

The genus *Apodrosus* Marshall, 1922 was recently comprehensively revised by Girón and Franz (2010). They documented the presence of 13 species throughout the Caribbean, eleven of which they described as new. No species of the genus were known from Cuba. Following three recent (2012, 2013, 2014) expeditions to Cuba we have now recorded nine additional endemic species of the genus from throughout the country. These species are described herein.

Girón and Franz (2010) also presented a morphological phylogeny and biogeographic analysis of the genus with two main clades represented; the Hispaniolan/ Puerto Rican *A. artus-A. wolcotti* clade from elevations over 1000 m and the more speciose and widespread western Caribbean *A. eximius-A. empherefasciatus* clade from arid, lower-elevation, coastal habitats. Here, we reinterpret their results based on a limited molecular phylogeny incorporating data from 11 species of the genus from Cuba, Hispaniola and Puerto Rico. Host plant associations continue to remain uncertain.

The phylogenetic study by Girón and Franz (2010) questioned the placement of *Apodrosus* in Polydrusini. At present, we do not address this issue as any changes in tribal placement would require definition of the pertinent tribes of Entiminae and this is well beyond the scope of this study.

#### Materials and methods

The approach used in this study follows that of Girón and Franz (2010) for ease of comparison of species from Cuba with the rest of the genus. A differential diagnosis is presented allowing for separation from other Cuban species only. Cuban species are considered distinct based on comparison of male genitalia with figures in Girón and Franz (2010) and on geography. Specimens are deposited in the Hasbrouck Insect Collection of Arizona State University, Tempe, AZ (ASUHIC); Canadian Museum of Nature, Ottawa, Canada (CMNC); Charles W. O'Brien Collection, Green Valley, AZ (CWOB); Illinois Natural History Survey, Champaign, IL (INHS); and United States National Museum, Washington D.C. (USNM). Species descriptions were prepared by RSA and phylogenetic analysis by GYZ. Species are listed in alphabetical order. Latitude and longitude data are given in decimal degrees. A map of Cuban *Apodrosus* is included.

Phylogenies of *Apodrosus* species and their allies were reconstructed using molecular data. Two successive analyses were conducted, first with a broad outgroup sample, and the second focusing on just Apodrosus species (details of samples including geography, vouchering and Genbank numbers are provided in Suppl. material 1). Eleven species of *Apodrosus* were sampled for molecular data, with six from Cuba and five from Puerto Rico or Hispaniola (represented by the Dominican Republic). Other species of Apodrosus were not sampled due to a lack of DNA-quality specimens or failed molecular experiments. In the first analysis, sequences of eight species of *Poly*drusus and one species of Pachyrhinus, all Palearctic, were downloaded from Genbank. These were included to test the relationship between Apodrosus and Polydrusus, both currently placed in Polydrusini (Alonso-Zarazaga & Lyal, 1999). Representatives of New World Polydrusus were not sampled due to absence of published data or fresh specimens. Two genes (Cytochrome c Oxidase subunit I [COI], and Cytochrome c Oxidase subunit II [COII]), are available for *Polydrusus* and *Pachyrhinus*. Eleven other species of various other Neotropical entimines were also included to position Polydrusini as sampled in the current study in a broader phylogenetic context, an exercise hitherto not performed. This selection represents a broad coverage of Caribbean entimine diversity at the tribal level, but is also limited by sample availability. Girón and Franz (2010) placed Anypotactus bicaudatus as the sister of Apodrosus sampled in that study. Due to lack of DNA-quality material, this genus is not sampled in the current study. Anthonomus grandis was used to root the 33-taxon phylogeny. This phylogeny recovered a sister relationship between *Polydrusus (Pachyrhinus)* and *Apodrosus* (Suppl. material 2). In a second analysis, only species of Apodrosus and a Polydrusus (as root) were retained and this phylogeny is presented in the main text of the article. DNA extractions were performed using the Qiagen DNeasy<sup>®</sup> Blood and Tissue Kit (Qiagen). The right hind leg was excised from a specimen and extracted. Six mitochondrial, nuclear, protein-coding or ribosomal gene fragments - COI, COII, 12s, Arginine Kinase (AK), Elongation Factor 1-alpha (Ef  $1-\alpha$ ), and 28s (D2-D4 regions) – were amplified with polymerase chain reaction using EmeraldAmp MAX PCR Master Mix in an Eppendorf vapo protect thermal cycler. Primer and PCR programming information is the same as in Zhang et al. (2017). DNA sequencing was performed at the Arizona State University DNA Laboratory on an Applied Biosystems 3730 capillary sequencer. DNA sequences were edited with the software Geneious R7 and uploaded to Genbank. The software program MAFFT (Katoh and Standley 2013) was used to align DNA sequences, applying the auto alignment strategy for protein coding genes (COI, COII, AK and Ef-1a) and L-INS-i for ribosomal genes (12s and 28s). The aligned, individual gene data sets were concatenated using SequenceMatrix (Vaidya et al. 2011). A matrix of 33 terminals and 4539 aligned nucleotide sites was generated for the first analysis and another with 13 terminals and 4494 sites for the second analysis. The maximum likelihood method was used to reconstruct phylogenies using the program RAxML (Stamatakis 2014) via the CIPRES (www.phylo.org) supercomputer cluster (Miller et al. 2010). The analysis was partitioned with each gene fragment considered a different partition.

#### Taxonomy

#### Apodrosus Marshall, 1922

*Apodrosus* Marshall, 1922: 59. *Apodrusus* Marshall (in Wolcott 1924: 130, error).

Gender masculine. Type species *Apodrosus wolcotti* Marshall 1922: 59, by original designation.

**Diagnosis and description.** Girón and Franz (2010) present a detailed diagnosis and description of the genus. Their diagnosis (slightly modified) follows here: *Apodrosus* is a genus of relatively small sized (2-7 mm), often metallic colored (but not among the Cuban species), exclusively Caribbean entimine weevils without a pronotal postocular lobe and postocular vibrissae, and with the humeri and wings well developed. Species of *Apodrosus* may resemble members of the Anypotactini and Polydrusini; however, *Apodrosus* can be distinguished from *Polydrusus* and other polydrusine genera by a particular combination of characters including a median furrow on the head, a large, bare, and smooth triangular area formed by the epistome on the rostrum; the presence of premucro; the presence of a median fovea on sternum VII; and an either J- or Y-shaped female spermatheca. *Apodrosus* is furthermore distinguished from an undescribed though apparently closely related genus that also occurs at higher elevations in the Hispaniolan Cordillera by having a well defined epistome, well developed elytral humeri, and fully developed wings. Finally, *Apodrosus* differs from *Anypotactus* Schoenherr in having connate (as opposed to free) claws.

**Distribution.** Species of *Apodrosus* are known from the Bahamas, Cuba, Dominican Republic, Haiti, Mona Island, Puerto Rico, and the Turks and Caicos Islands. The records of the genus from Cuba are reported herein for the first time.

#### **Description of species**

#### Apodrosus alberti Anderson, sp. n.

http://zoobank.org/4704330D-0DD7-4513-8F72-1D4DE2CDF21C Figures 1–3

**Specimens examined.** 5 males, 3 females. Holotype male (CMNC), labelled CUBA: Province Granma, Parque Nacional Pico Turquino, 1103 m, 20.0107, -76.8843, IV.2012, CarBio Team, montane forest, CU-03. Paratypes. Alto de Merino, near biological station, 958 m, 5–10.III.2013, 19.9858333, -077.0158333, pluviselva litter, F. Cala Riquelme, A. Deler Hernandez (1 female; CMNC). Parque Nacional Pico Turquino, on the trail up to ca. 0.5 km (by air) from La Platica, 20.0083333, -76.8883333, 920 m, 23–27.VI.2012, A. Deler Hernández & M. Fikacek, sweeping exposed vegetation, secondary evergreen forest, MF-20 (1 female; CMNC). Province

Santiago de Cuba, Parque Nacional Gran Piedra, Gran Piedra and surrounding trails, general collecting, 20.01177, -75.62595, 1200 m, leg. N. Franz, 29.I.2012 (1 male; ASUHIC). Gran Piedra, along trail Museo Isabelica to Gran Sofía, on plants, 20.0010, -75.5940, 800 m, leg N. Franz, 28.I.2012 (1 male, 1 female; ASUHIC). Gran Piedra, nr. Santiago, May 30–31, 1957, M.W. Sanderson (2 males; CWOB, INHS).

**Diagnosis.** This species is easily distinguished from other Cuban species by larger eyes, elytra with all intervals of equal elevation, elytra with stria 10 continuous throughout length, body with most scales brown or copper in color, and distinctive male genitalia.

Description. Male. Body length 3.6-4.2 mm; in dorsal view about 2.3 times longer than greatest width which is at about second third of elytra; dorsal outline in lateral view quite flat. Vestiture composed of pale to dark brown scales, with very small recurved, fine brown setae and a few scattered broader white scales on elytra.. Eyes 1.4 times longer than wide, projected, separated from anterior margin of prothorax by 0.3 times greatest diameter of eye; line of anterior margin of eyes very slightly impressed; shortest distance between eyes (dorsal view) 0.35 times greatest width of pronotum; median furrow linear, narrow and deep, extending from anterior to posterior margin of eyes. Rostrum slightly longer than wide; epistoma apically with three setae situated on each side; nasal plate well defined, v-shaped, hind margin tumid, not declivious, continuing onto rostrum as an elevated median carina. Antennal insertion apicad of midpoint of rostrum; scrobe curved downwards by 45°, directed posteriorly at end, barely reaching anterior margin of eye, separated from it by 2.5 times width of scrobe. Mandibles with 2 lateral setae. Antennae reddish brown; antennal scape extending to just slightly before posterior margin of eye; desmomere I about same length as II. Pronotum cylindrical, slightly wider than long, greatest width from midlength to near base; dorsal surface shallowly punctate, scales sparse, each puncture with a curved, fine brown seta; posterior margin slightly bisinuate, slightly wider than anterior margin; scutellum subcircular, glabrous. Mesocoxal cavities about 5 times width of intercoxal process. Metasternum with lateral portions tumid, not posteriorly produced. Elytra in dorsal view 1.75 times their greatest width; anterior margin sinuate; humeral region of elytra 1.5 times width of posterior margin of pronotum; lateral margins slightly divergent until second third, thereafter convergent; apex acutely rounded; in lateral view with dorsal outline quite flat; posterior declivity gradually descending; striae 9 and 10 complete throughout length although punctures of 10 faintly defined beyond metacoxa; intervals largely covered with scales, with dark and light areas forming an irregular pattern; all intervals equally flat, humerus angled; interval 9 very slightly tumid just anterior to metacoxa; all intervals with recurved, fine brown setae, a few scattered broader white scales on elytral declivity. Venter with scales sparse, small on ventrites, middle of ventrites 1 and 2 with moderately dense, long, fine, erect hairs, ventrites 3 and 4 subequal in length, their combined length slightly longer than ventrite 5; posterior margin of ventrite 5 widely rounded, apex at middle narrowly impressed. Tergum VII of male emarginate. Tegmen with tegminal apodeme 0.5 times length of aedeagus; tegminal plate simple. Aedeagus in dorsal view about 5 times longer than its greatest width; apex rounded. Endophallus



Figures 1-3. *Apodrosus alberti.* I Lateral habitus, female 2 Dorsal habitus, female 3 Male aedeagus, dorsal.

extended to about midlength of aedeagal apodemes, with only a pair of inverted cshaped sclerites at about one-third length of aedeagus. Aedeagus in lateral view slightly evenly convex. Aedeagal apodemes about 0.8 times length of aedeagus.

*Female.* Body length 3.9–4.0 mm. Differing from male as follows: elytra in lateral view with posterior declivity angulate, sutural interval very slightly inflated at about midheight declivity.

**Etymology.** This species is named after Albert Deler Hernández, coleopterist, of Santiago de Cuba, Cuba.

Natural history. Adults were collected on vegetation along trails in montane forest. Comments. Dissected males from Parque Nacional Pico Turquino and Parque Nacional Gran Piedra have indistinguishable genitalia and are here considered conspecific.

#### Apodrosus alternatus Anderson, sp. n.

http://zoobank.org/11001A82-F052-46CB-8835-8EB117681F96 Figures 4–6

**Specimens examined.** 5 males, 8 females. Holotype male (CMNC), labelled CUBA: Province Guantanamo, El Yunque, 360 m, 20.34501, -74.56642, IV.2014, CarBio Team, CU-15. Paratypes. Same data as holotype (1 male, 2 females; CMNC). El Yunque, 20–150 m, 20.317, -74.571, 31.I.2012, R. Anderson, wet rainforest (1 male; CMNC). El Yunque, along trail to peak, 20.35060, -74.57497, 550 m, beating shrubs and trees, leg. N. Franz, 1.II.2012 (4 females; ASUHIC). El Yunque, Finca La Delicia, 4 km SW Flora y Fauna Station, 20.32085, -74.57014, 150 m, F. Cala, 1.II.2012 (1 female; ASUHIC). El Yunque, along road near Flora y Fauna Station, 20.32775, -74.56941, 100 m, N. Franz, 31.I.2012 (2 males; ASUHIC). Nibujón, Parque Nacional Humboldt, Sendero Mirador, 3 km N. Nibujón, 20.52036, -74.69018, 100 m, on plants, N. Franz, 2.II.2012 (1 female; ASUHIC).

**Diagnosis.** This species is distinguished from other Cuban species by larger eyes, elytra with intervals 3 (subbasally and discally), 5 (subbasally) and 7 (at humerus) elevated above adjacent intervals (moreso in female than in male), and distinctive male genitalia with the aedeagus length about 4.5 times maximum width.

Description. Male. Body length 3.0-3.4 mm; in dorsal view 2.1-2.3 times longer than greatest width which is between midlength and second third of elytra; dorsal outline in lateral view slightly convex. Vestiture composed of pink, pinkish-white to brown scales, with very small recurved, fine brown setae. Eyes 1.6 times longer than wide, projected, separated from anterior margin of prothorax by 0.25 times greatest diameter of eye; line of anterior margin of eyes slightly impressed; shortest distance between eyes (dorsal view) 0.3-0.4 times greatest width of pronotum; median furrow linear, narrow and deep, extending from anterior margin of eye but not reaching anterior margin of pronotum. Rostrum slightly longer than wide; epistoma apically with a single seta situated on each side; nasal plate well defined, tumid, steeply declivious. Antennal insertion apicad of midpoint of rostrum; scrobe curved downwards by 45°, directed posteriorly at end, barely reaching anterior margin of eye, separated from it by 1.5 times width of scrobe. Mandibles with 2 lateral setae. Antennae reddish brown; antennal scape extending to just slightly before posterior margin of eyes; desmomere I about same length as II. Pronotum cylindrical, about as long as wide, greatest width near base; dorsal surface shallowly punctate, each puncture with a curved, fine brown seta; posterior margin slightly bisinuate, slightly wider than anterior margin; scutellum subcircular, rugose and glabrous. Mesocoxal cavities about 3 times width of intercoxal process. Metasternum with lateral portions posteriorly produced. Elytra in dorsal



Figures 4-6. *Apodrosus alternatus.* 4 Lateral habitus, male 5 Dorsal habitus, male 6 Male aedeagus, dorsal.

view 1.5 times their greatest width; anterior margin slightly sinuate; humeral region of elytra 1.5 times width of posterior margin of pronotum; lateral margins slightly divergent until second third, thereafter convergent; apex acutely rounded; in lateral view with dorsal outline slightly convex; posterior declivity gradually descending; striae 9 and 10 separate along entire length; intervals completely covered with scales, with dark and light areas forming an irregular pattern; interval 3 slightly produced at base and again at middle on disc, interval 4 tumid at base, interval 5 produced at base, then less so, then pronounced again throughout most of length, humerus distinctly angled; interval 9 slightly tumid above metacoxa; all intervals with recurved, fine brown setae. Venter with scales denser, larger on ventrites 1 and 2, small and fine, some seta-like, on ventrites 3–5; ventrite 2 about as long as ventrite 1 (medially), ventrites 3 and 4 subequal in length, their combined length slightly shorter than ventrite 5; posterior margin of ventrite 5 widely rounded, finely narrowly emarginate at middle, apex at middle narrowly impressed. Tegmen with tegminal apodeme 0.6 times length of ae-deagus; tegminal plate simple. Aedeagus in dorsal view about 4.5 times longer than its greatest width; apex rounded. Endophallus with a pair of large asymmetrical hook-like sclerites positioned near midlength, two elongate fields of microtrichiae extended between aedeagal apodemes, and a faint apical sclerite complex. Aedeagus in lateral view slightly evenly convex. Aedeagal apodemes about 0.5 times length of aedeagus.

*Female*. Body length 3.3–3.8 mm. Differing from male as follows: elytra with interval 3 produced at base and again more strongly so at middle on disc, interval 4 tumid at base, interval 5 strongly produced at base, then less so, then moderately pronounced again throughout most of length, humerus distinctly angled, humeral interval somewhat produced a short distance beyond humerus.

**Etymology.** This species is named after the Latin adjective "alternatus", referring to the alternating elevation of the elytral intervals.

**Natural history.** Adults were collected beating vegetation along trails in tropical wet forest.

**Comments.** During the initial phase of this study, this species and *A. pseudoalternatus* were considered as conspecific. Males can be separated by the form of the aedeagus but females at present are not separable using external features.

## Apodrosus beckeli Anderson, sp. n.

http://zoobank.org/CF91E267-40E2-4BFC-BA5F-BB6E503D1269 Figures 7–9

**Specimens examined.** 4 males, 10 females. Holotype male (CMNC), labelled CUBA: Province Guantánamo, 8 km W. Imías, 27 m, 20.05421, -74.71520, 4.x.2014, R. Anderson, F. Cala Riquelme, A. Deler Hernandez, 2014-034, beating, coastal scrub. Paratypes. Data as holotype (2 males, 9 females; ASUHIC, CMNC, CWOB). Baracoa, Aug. [18]90, Busch collector (1 male, 1 female; USNM).

**Diagnosis.** This species is difficult to distinguish from other Cuban species especially *A. franklyni* and *A. griseus*. This group of three species can be separated from other Cuban species by larger eyes, elytra with all intervals of equal elevation, and elytra with stria 10 interrupted above metacoxa. Males of the three species can be separated on the basis of distinctive male genitalia. Females of this species can be separated from *A. franklyni* by the form of the elytral declivity in lateral view but are not separable from *A. griseus* using external features.

**Description.** *Male.* Body length 2.2–2.3 mm; in dorsal view about 2.2 times longer than greatest width which is at about second third of elytra; dorsal outline in lateral



Figures 7-9. Apodrosus beckeli. 7 Lateral habitus, male 8 Dorsal habitus, male 9 Male aedeagus, dorsal.

view quite flat. Vestiture composed of grey, greyish-white to brown scales, with very small recurved, fine brown setae. Eyes 1.3 times longer than wide, projected, separated from anterior margin of prothorax by 0.6 times greatest diameter of eye; line of anterior margin of eyes very slightly impressed; shortest distance between eyes (dorsal view) 0.5 times greatest width of pronotum; median furrow linear, narrow and shallow, extending from anterior margin of eyes but not reaching anterior margin of pronotum. Rostrum slightly longer than wide; epistoma apically with three setae situated on each side; nasal plate well defined, v-shaped, slightly tumid, not declivious. Antennal insertion apicad of midpoint of rostrum; scrobe curved downwards by 60°, directed posteriorly at end, barely reaching anterior margin of eye, separated from it by 1.5 times width of scrobe. Mandibles with 2 lateral setae. Antennae reddish brown; antennal scape extending to

just slightly before posterior margin of eye; desmomere I about same length as II. Pronotum cylindrical, slightly wider than long, greatest width from midlength to near base; dorsal surface shallowly punctate but largely obscured by scales, each puncture with a curved, fine brown seta; posterior margin slightly bisinuate, slightly wider than anterior margin; scutellum subcircular, glabrous. Mesocoxal cavities about 3 times width of intercoxal process. Metasternum with lateral portions slightly tumid, not posteriorly produced. Elytra in dorsal view 1.7-1.8 times their greatest width; anterior margin sinuate; humeral region of elytra 1.5 times width of posterior margin of pronotum; lateral margins subparallel until second third, thereafter convergent; apex acutely rounded; in lateral view with dorsal outline quite flat; posterior declivity gradually descending; stria 9 complete, stria 10 interrupted above metacoxa, resuming at suture between ventrites 1 and 2; intervals completely covered with scales, with dark and light areas forming an irregular pattern; all intervals equally flat, humerus angled; interval 9 very slightly tumid above metacoxa; all intervals with recurved, fine brown setae. Venter with scales dense, large on ventrites; ventrites 3 and 4 subequal in length, their combined length slightly shorter than ventrite 5; posterior margin of ventrite 5 widely rounded, apex at middle narrowly impressed. Tegmen with tegminal apodeme 0.5 times length of aedeagus; tegminal plate simple. Aedeagus in dorsal view about 4.5 times longer than its greatest width; apex rounded. Endophallus extended to about midlength of aedeagal apodemes, with only an apical hooked sclerite complex. Aedeagus in lateral view slightly evenly convex. Aedeagal apodemes about same length as aedeagus.

Female. Body length 2.5–3.0 mm.

**Etymology.** This species is named after William Edwin Beckel, PhD., Entomology, father of Margaret Beckel, President and CEO of the Canadian Museum of Nature, for his generous support of insect taxonomy.

Natural history. Adults were collected beating vegetation in dry coastal scrub.

**Comments.** Externally, this species is very similar to *A. griseus* and although males can be separated by details in the structure of the endophallus (see key), females cannot be separated using external features.

#### Apodrosus franklyni Anderson, sp. n.

http://zoobank.org/326B2551-FD08-4A05-A684-AEE7A05091B8 Figures 10–12

**Specimens examined.** 9 males, 4 females. Holotype male (CMNC), labelled CUBA: Province Cienfuegos, Parque Nacional Pico San Juan, road, 21.98812, -80.14632, 1086 m, 19.v.2013, R. Anderson, 2013-022X, hand collections. Paratypes. Data as holotype (5 males, 3 females; CMNC, CWOB). Pico San Juan, near peak, 21.9886833, -80.1465833, 1105 m, 19.V.2013, G. Zhang, CB-13, L.22 (3 males, 1 female; AS-UHIC).

**Diagnosis.** This species is difficult to distinguish from other Cuban species especially *A. beckeli* and *A. griseus*. This group of three species can be separated from other



Figures 10–12. *Apodrosus franklyni*. 10 Lateral habitus, male 11 Dorsal habitus, male 12 Male aedeagus, dorsal.

Cuban species by larger eyes, elytra with all intervals of equal elevation, and elytra with stria 10 interrupted above metacoxa. Males of the three species can be separated on the basis of distinctive male genitalia. Females of this species can be separated from *A. beckeli* and *A. griseus* by the elytral profile at apical declivity distinctly angulate.

**Description.** *Male.* Body length 2.8–3.2 mm; in dorsal view about 2.2 times longer than greatest width which is at about second third of elytra; dorsal outline in lateral view quite flat. Vestiture composed of grey, greyish-white to brown scales, with very small recurved, fine brown setae. Eyes 1.3 times longer than wide, projected, separated from anterior margin of prothorax by 0.5 times greatest diameter of eye;

line of anterior margin of eyes very slightly impressed; shortest distance between eyes (dorsal view) 0.6 times greatest width of pronotum; median furrow linear, narrow and shallow, extending from anterior margin of eyes but not reaching anterior margin of pronotum. Rostrum slightly longer than wide; epistoma apically with three setae situated on each side; nasal plate well defined, v-shaped, slightly tumid, not declivious. Antennal insertion apicad of midpoint of rostrum; scrobe curved downwards by 60°, directed posteriorly at end, not reaching anterior margin of eye, separated from it by 2.0 times width of scrobe. Mandibles with 2 lateral setae. Antennae reddish brown; antennal scape extending to slightly beyond midlength of eye; desmomere I slightly longer than II. Pronotum cylindrical, slightly longer than wide, greatest width from midlength to near base; dorsal surface shallowly punctate, each puncture with a curved, fine brown seta; posterior margin slightly bisinuate, slightly wider than anterior margin; scutellum subcircular, glabrous. Mesocoxal cavities about 5 times width of intercoxal process. Metasternum with lateral portions slightly tumid, not posteriorly produced. Elytra in dorsal view 1.4-1.5 times their greatest width; anterior margin sinuate; humeral region of elytra 1.7 times width of posterior margin of pronotum; lateral margins slightly divergent until second third, thereafter convergent; apex acutely rounded; in lateral view with dorsal outline quite flat; posterior declivity gradually descending; stria 9 complete, stria 10 interrupted above metacoxa, resuming at suture between ventrites 1 and 2; intervals completely covered with scales, with dark and light areas forming an irregular pattern although many specimens with a more or less distinct transverse dark macula at about posterior one-third of elytral length; all intervals equally flat, humerus angled; interval 9 very slightly tumid above metacoxa; all intervals with recurved, fine brown setae. Venter with scales dense, large on ventrites, ventrites 3 and 4 subequal in length, their combined length slightly about same length as ventrite 5; posterior margin of ventrite 5 widely rounded, apex at middle narrowly impressed. Tegmen with tegminal apodeme 0.55 times length of aedeagus; tegminal plate simple. Aedeagus in dorsal view about 4.7 times longer than its greatest width; apex rounded. Endophallus extended almost to apex of aedeagal apodemes, with a narrow u-shaped sclerite at midlength aedeagus and apical hooked sclerite complex. Aedeagus in lateral view slightly evenly convex. Aedeagal apodemes slightly shorter than length aedeagus.

*Female*. Body length 3.4–3.6 mm. Differing from male as follows: elytra in lateral view slightly tumid dorsally and with posterior declivity angulate, sutural interval very slightly inflated at about midheight of declivity.

**Etymology.** This species is named after Franklyn Cala Riquelme, arachnologist, of Santiago de Cuba, Cuba.

**Natural history.** Adults were collected beating montane vegetation along the upper part of the road to Pico San Juan.

**Comments.** This species and *A. griseus* and *A. beckeli* are superficially very similar. Males can be separated by the form of genitalia but females are very similar with females of *A. franklyni*, differing in the form of the elytral declivity in lateral view.

#### Apodrosus griseus Anderson, sp. n.

http://zoobank.org/E4B8DAB9-E57A-4536-9313-363A008ECAF4 Figures 13–15

**Specimens examined.** 6 males, 4 females. Holotype male (CMNC), labelled CUBA: Province Santiago de Cuba, Siboney-Jutici Ecological Reserve, 60 m, near Biological Station, 19.96158, -75.71534, 1.IV.2012, CarBio Team, forest semi-dry, broadleaf, CU-07. Paratypes. Siboney-Jutici Ecological Reserve, Estación Ecologica Siboney, 50 m, 19.961, -75.715, 6.II.2012, R. Anderson, dry thorn scrub (2 males, 2 females; CMNC). Siboney-Jutici Ecological Reserve, 19.96227, -75.71684, 100 m, beating shrubs at night, leg. F. Cala, 26.I.2012 (2 males, 1 female; ASUHIC). Siboney-Jutici Ecological Reserve, 19.96227, -75.71684, 100 m, beating shrubs at night, leg. N. Franz, 6.II.2012 (1 male, 1 female; ASUHIC).

**Diagnosis.** This species is difficult to distinguish from other Cuban species especially *A. franklyni* and *A. beckeli*. This group of three species can be separated from other Cuban species by larger eyes, elytra with all intervals of equal elevation, and elytra with stria 10 interrupted above metacoxa. Males of the three species can be separated on the basis of distinctive male genitalia. Females of this species can be separated from *A. franklyni* by the form of the elytral declivity in lateral view but are not separable from *A. beckeli* using external features.

Description. Male. Body length 2.6-2.9 mm; in dorsal view 2.2-2.4 times longer than greatest width which is between first and second third of elytra; dorsal outline in lateral view quite flat. Vestiture composed of grey, greyish-white to brown scales, with very small recurved, fine brown setae. Eyes 1.3 times longer than wide, projected, separated from anterior margin of prothorax by 0.7 times greatest diameter of eye; line of anterior margin of eyes very slightly impressed; shortest distance between eyes (dorsal view) 0.3–0.4 times greatest width of pronotum; median furrow linear, narrow and shallow, extending from anterior margin of eyes but not reaching anterior margin of pronotum. Rostrum slightly longer than wide; epistoma apically with three setae situated on each side; nasal plate well defined, v-shaped, slightly tumid, not declivious. Antennal insertion apicad of midpoint of rostrum; scrobe curved downwards by 60°, directed posteriorly at end, barely reaching anterior margin of eye, separated from it by 1.5 times width of scrobe. Mandibles with 2 lateral setae. Antennae reddish brown; antennal scape extending to just slightly before posterior margin of eye; desmomere I very slightly longer than II. Pronotum cylindrical, slightly wider than long, greatest width near base; dorsal surface shallowly punctate but largely obscured by scales, each puncture with a curved, fine brown seta; posterior margin slightly bisinuate, slightly wider than anterior margin; scutellum subcircular, glabrous. Mesocoxal cavities about 3 times width of intercoxal process. Metasternum with lateral portions slightly tumid, not posteriorly produced. Elytra in dorsal view 1.7-1.8 times their greatest width; anterior margin sinuate; humeral region of elytra 1.5 times width of posterior margin of pronotum; lateral margins subparallel until second third, thereafter convergent; apex acutely rounded; in lateral view with dorsal outline quite flat; posterior declivity



Figures 13–15. *Apodrosus griseus.* 13 Lateral habitus, male 14 Dorsal habitus, male 15 Male aedeagus, dorsal.

gradually descending; stria 9 complete, stria 10 interrupted above metacoxa, resuming at suture between ventrites 1 and 2; intervals completely covered with scales, with dark and light areas forming an irregular pattern; all intervals equally flat, humerus angled; interval 9 very slightly tumid above metacoxa; all intervals with recurved, fine brown setae. Venter with scales dense, large laterally on ventrites, smaller and less dense medially, ventrites 3 and 4 subequal in length, their combined length slightly shorter than ventrite 5; posterior margin of ventrite 5 widely rounded, apex at middle narrowly impressed. Tegmen with tegminal apodeme 0.8 times length of aedeagus; tegminal plate simple. Aedeagus in dorsal view about 6.5 times longer than its greatest width; apex rounded. Endophallus extended to apical two-thirds of aedeagal apodemes, with a narrow, scythe-like sclerite positioned near basal two-thirds of length, an elongate field of microtrichia positioned at base of aedeagus, and an apical hooked sclerite complex. Aedeagus in lateral view slightly evenly convex. Aedeagal apodemes about one-half length aedeagus.

Female. Body length 3.3–3.8 mm.

**Etymology.** This species is named after the Latin adjective "griseus" meaning grey, after the predominantly grey scales of the body vestiture.

Natural history. Adults were collected beating vegetation in dry thorn scrub.

**Comments.** Externally, this species is very similar to *A. beckeli* and although males can be separated by details in the structure of the endophallus (see key), females cannot be separated using external features.

#### Apodrosus mensurensis Anderson, sp. n.

http://zoobank.org/417AA45B-F711-4A1C-92BE-9259930BB9EB Figures 16–18

**Specimens examined.** 2 males. Holotype male (ASUHIC), labelled CUBA: Province Holguin, Mayari, Parque Nacional La Mensura-Piloto, 20.5288333, -75.7683000, 750 m, 09 May 2013, G. Zhang, (CB13\_L2). Paratype. Data as holotype (1 male; CMNC).

**Diagnosis.** This species is distinguished from other Cuban species by larger eyes, elytra with all intervals of equal elevation, elytra with stria 10 continuous throughout length, body with most scales brown or copper in color, and distinctive male genitalia.

Description. Male. Body length 2.7-3.0 mm; in dorsal view about 2.2 times longer than greatest width which is at about second third of elytra; dorsal outline in lateral view quite flat. Vestiture composed of predominantly light to dark brown scales, with very small recurved, fine dark brown setae. Eyes 1.3 times longer than wide, projected, separated from anterior margin of prothorax by 0.6 times greatest diameter of eye; line of anterior margin of eyes very slightly impressed; shortest distance between eyes (dorsal view) 0.4 times greatest width of pronotum; median furrow linear, narrow and shallow, extending from just behind anterior margin of eyes, not reaching anterior margin of pronotum. Rostrum slightly longer than wide; epistoma apically with four setae situated on each side; nasal plate well defined, v-shaped, slightly tumid, slightly declivious. Antennal insertion apicad of midpoint of rostrum; scrobe curved downwards by about 45°, directed posteriorly towards apex, not reaching anterior margin of eye, separated from it by about width of scrobe. Mandibles with 2 lateral setae. Antennae reddish brown; antennal scape extending to just slightly before posterior margin of eye; desmomere I slightly longer than II. Pronotum cylindrical, very slightly wider than long, greatest width at about midlength; dorsal surface shallowly punctate but largely obscured by scales, each puncture with a curved, fine brown seta; posterior margin slightly bisinuate, about same width as anterior margin; scutellum subcircular,



Figures 16–18. *Apodrosus mensurensis.* 16 Lateral habitus, male 17 Dorsal habitus, male 18 Male aedeagus, dorsal.

glabrous. Mesocoxal cavities about 6 times width of intercoxal process. Metasternum with lateral portions slightly tumid, not posteriorly produced. Elytra in dorsal view 1.5–1.6 times their greatest width; anterior margin sinuate; humeral region of elytra 1.5 times width of posterior margin of pronotum; lateral margins subparallel until second third, thereafter convergent; apex acutely rounded; in lateral view with dorsal outline quite flat; posterior declivity gradually descending; striae 9 and 10 complete; intervals completely covered with scales, with dark and light areas forming an irregular pattern but with declivity mainly of paler scales; all intervals equally flat, humerus angled; interval 9 very slightly tumid above metacoxa; all intervals with recurved, fine brown setae. Venter with scales dense, large on ventrites, ventrites 3 and 4 subequal in length, their combined length slightly shorter than ventrite 5; posterior margin

of ventrite 5 widely rounded, finely narrowly emarginate at middle, apex at middle deeply narrowly impressed. Tegmen with tegminal apodeme 0.5 times length of aedeagus; tegminal plate simple. Aedeagus in dorsal view about 4.5 times longer than its greatest width; tapered in apical 2/5 to narrowly rounded apex. Endophallus extended to about midlength of aedeagal apodemes, with only pair of small curved sclerites just before midlength, and a pair of narrow, faint fields of microtrichiae near base aedeagus. Aedeagus in lateral view slightly evenly convex. Aedeagal apodemes about same length as aedeagus.

Female. Unknown.

**Etymology.** This species is named after the type locality Parque Nacional La Mensura-Piloto. The specific name is an adjective.

Natural history. Adults were collected beating vegetation in a mixed pine scrub.

#### Apodrosus pseudoalternatus Anderson, sp. n.

http://zoobank.org/621091D2-ED04-4D2A-97DA-647D353DF260 Figures 19–21

**Specimens examined.** 1 male, 1 female. Holotype male (CMNC), labelled CUBA: Province Matanzas, Varadero, Varahicacos, 8 m, 23.194, -81.154, III.2014, F. Cala Riquelme. Paratype. Same data as holotype (1 female; CMNC).

**Diagnosis.** This species is distinguished from other Cuban species by larger eyes, elytra with intervals 3 (subbasally and discally), 5 (subbasally) and 7 (at humerus) elevated above adjacent intervals (more so in female than in male), and distinctive male genitalia with the aedeagus very long and slender, length about 10 times maximum width.

Description. Male. Body length 3.0 mm; in dorsal view 2.3 times longer than greatest width which is between midlength and second third of elytra; dorsal outline in lateral view moderately convex. Vestiture composed of pink, pinkish-white to brown scales, with very small recurved, fine brown setae. Eyes 1.6 times longer than wide, projected, separated from anterior margin of prothorax by 0.25 times greatest diameter of eye; line of anterior margin of eyes slightly impressed; shortest distance between eyes (dorsal view) 0.4 times greatest width of pronotum; median furrow linear, narrow and deep, extending from anterior margin of eye but not reaching anterior margin of pronotum. Rostrum slightly longer than wide; epistoma apically with single setasituated on each side; nasal plate well defined, tumid, steeply declivious. Antennal insertion apicad of midpoint of rostrum; scrobe curved downwards by 45°, directed posteriorly at end, barely reaching anterior margin of eye, separated from it by 1.5 times width of scrobe. Mandibles with 2 lateral setae. Antennae reddish brown; antennal scape extending to just slightly before posterior margin of eyes; desmomere I about same length as II. Pronotum cylindrical, about as long as wide, greatest width near base; dorsal surface shallowly punctate, each puncture with a curved, fine brown seta; posterior margin slightly bisinuate, slightly wider than anterior margin; scutellum subcircular, rugose and glabrous. Mesocoxal cavities about 3 times width of intercoxal



Figures 19–21. *Apodrosus pseudoalternatus.* 19 Lateral habitus, female 20 Dorsal habitus, female 21 Male aedeagus, dorsal.

process. Metasternum with lateral portions posteriorly produced. Elytra in dorsal view 1.5 times their greatest width; anterior margin sinuate; humeral region of elytra 1.5 times width of posterior margin of pronotum; lateral margins slightly divergent until second third, thereafter convergent; apex acutely rounded; in lateral view with dorsal outline slightly convex; posterior declivity gradually descending; striae 9 and 10 separate along entire length; intervals completely covered with scales, with dark and light areas forming an irregular pattern; interval 3 slightly produced at base and again at middle on disc, interval 4 tumid at base, interval 5 produced at base, then less so, then pronounced again throughout most of length, humerus distinctly angled; interval 9 slightly tumid above metacoxa; all intervals with recurved, fine brown setae. Venter with scales denser, larger on ventrites 1 and 2, small and fine, some seta-like, on ven-

trites 3-5; ventrite 2 about as long as ventrite 1 (medially), ventrites 3 and 4 subequal in length, their combined length slightly shorter than ventrite 5; posterior margin of ventrite 5 widely rounded, finely narrowly emarginate at middle, apex at middle narrowly impressed. Tegmen with tegminal apodeme 0.3 times length of aedeagus; tegminal plate simple. Aedeagus in dorsal view about 10 times longer than its greatest width; apex rounded. Endophallus with a pair of asymmetrical hook-like sclerites positioned near midlength otherwise no distinct internal sclerotization. Aedeagus in lateral view slightly evenly convex. Aedeagal apodemes about 0.4 times length of aedeagus.

*Female*. Body length 3.5 mm. Differing from male as follows: elytra with interval 3 produced at base and again more strongly so at middle on disc, interval 4 tumid at base, interval 5 strongly produced at base, then less so, then moderately pronounced again throughout most of length, humerus distinctly angled, humeral interval somewhat produced a short distance beyond humerus.

**Etymology.** This species is named because of the initial confusion of this species with *A. alternatus*. The specific name is an adjective.

# Natural history. No information.

**Comments.** During the initial phase of this study this species and *A. alternatus* were placed as conspecific. Males can be separated by the form of the aedeagus but females at present are not separable using external features.

#### Apodrosus sandersoni Anderson, sp. n.

http://zoobank.org/38EB6069-43D2-4BB5-A6EF-A3FB2252E63A Figures 22–23

**Specimens examined.** 2 females. Holotype female (CWOB), labelled CUBA: Province Oriente, Loma Lafarola, along Ríoo Cajobado, 2.VI.1959, M.W. Sanderson, C59-12. Paratype. Data as holotype (1 female; CMNC).

**Diagnosis.** This species is distinguished from other Cuban species by larger eyes, elytra with all intervals of equal elevation, elytra with stria 10 continuous throughout length, body with most scales scales grey, greyish white or pearlescent in color. Only females are known.

**Description.** *Female.* Body length 3.2–3.3 mm; in dorsal view 2.3 times longer than greatest width which is at about second third of elytra; dorsal outline in lateral view slightly convex. Vestiture composed of pale grey to pearlescent scales, with very small recurved, fine greyish setae. Eyes 1.3 times longer than wide, quite flat, separated from anterior margin of prothorax by 0.5 times greatest diameter of eye; line of anterior margin of eyes not at all impressed, frons continuous with base of rostrum; shortest distance between eyes (dorsal view) 0.45 times greatest width of pronotum; median furrow linear, narrow and deep, confined to area between eyes. Rostrum slightly longer than wide; epistoma apically with three setae situated on each side; nasal plate well defined, glabrous, not declivious, continuing onto rostrum as a number of indistinct, small striae, not carinate posteriorly. Antennal insertion apicad of midpoint of rost



Figures 22-23. Apodrosus sandersoni. 22 Lateral habitus, female 23 Dorsal habitus, female.

trum; scrobe curved downwards by 60°, directed posteriorly at end, barely reaching anterior margin of eye, separated from it by about width of scrobe. Mandibles with 2 lateral setae. Antennae reddish brown; antennal scape extending to just slightly before posterior margin of eye; desmomere I very slightly longer than II. Pronotum cylindrical, slightly wider than long, greatest width from midlength to base; dorsal surface shallowly punctate, scales moderately dense, each puncture with a suberect, fine grey seta; posterior margin slightly bisinuate, slightly wider than anterior margin; scutellum subcircular, with a few scattered scales. Mesocoxal cavities about 5 times width of intercoxal process. Metasternum with lateral portions tumid, not posteriorly produced. Elytra in dorsal view 1.6 times their greatest width; anterior margin sinuate; humeral region of elytra 1.5 times width of posterior margin of pronotum; lateral margins slightly divergent until second third, thereafter convergent; apex acutely rounded; in lateral view with dorsal outline slightly convex; posterior declivity gradually descending; striae 9 and 10 complete throughout their length although punctures of 10 faintly defined beyond metacoxa; intervals largely covered with uniformly grey to pearlescent scales; all intervals equally flat, humerus angled; interval 9 very slightly tumid just anterior to metacoxa; all intervals with minuet fine grey setae. Venter with scales scattered, small on ventrites; ventrites 3 and 4 subequal in length, their combined length slightly shorter than ventrite 5; ventrite 5 setose, posterior margin widely rounded, impressed and very narrowly emarginated medially. Female not dissected.

Male. Unknown.

**Etymology.** This species is named after Milton W. Sanderson (1910-2012), entomologist and botanist, Illinois Natural History Survey, Champaign, Illinois.

Natural history. No information.

**Comments.** This species bears a superficial resemblance to *A. argentatus* from the Dominican Republic and Puerto Rico and can be separated by geographic distribution and the differently sculptured dorsal surface of the rostrum. Males are not available of *A. sandersoni* for comparison of genitalia. We have found a latitude and longitude for Loma La Farola in Guantánamo Province as 20.1592 -74.4686 and we believe these to be the approximate coordinates of specimen capture.

#### Apodrosus zayasi Anderson, sp. n.

http://zoobank.org/3C5872C4-1EE5-4262-B986-FD5D1F7A4785 Figures 24–26

**Specimens examined.** 1 male, 2 females. Holotype male (CMNC), labelled CUBA: Province Cienfuegos, Parque Nacional Pico San Juan, road, 21.98812, -80.14632, 1086 m, 19.V.2013, R. Anderson, 2013-022X, hand collections. Paratype. Data as holotype (1 female; CMNC). Pico San Juan, near peak, 21.9886833, -80.1465833, 1105 m, 19.V.2013, G. Zhang, CB-13, L.22 (1 female; ASUHIC).

**Diagnosis.** This species is distinguished from other Cuban species by the eyes small, rounded, the distance from posterior margin of eye to posterior margin of head about the same as greatest diameter of an eye, and by distinctive male genitalia. It is the only Cuban species with such small, rounded eyes.

**Description.** *Male.* Body length 3.6 mm; in dorsal view about 2.3 times longer than greatest width which is at about second third of elytra; dorsal outline in lateral view quite tumid. Vestiture composed of pale to dark brown scales, with very small recurved, fine brown setae. Eyes 1.1 times longer than wide, projected, separated from anterior margin of prothorax by about greatest diameter of eye; line of anterior margin of eyes very slightly impressed; shortest distance between eyes (dorsal view) 0.5 times greatest width of pronotum; median furrow linear, narrow and shallow, extending from anterior margin of eyes but not reaching anterior margin of pronotum, partially obscured by scales. Rostrum slightly longer than wide; epistoma apically with two setae



Figures 24–26. *Apodrosus zayasi*. 24 Lateral habitus, female 25 Dorsal habitus, female 26 Male aedeagus, dorsal.

situated on each side; nasal plate well defined, v-shaped, slightly tumid, not declivious. Antennal insertion apicad of midpoint of rostrum; scrobe curved downwards by 45°, directed posteriorly at end, barely reaching anterior margin of eye, separated from it by 2.0 times width of scrobe. Mandibles with 2 lateral setae. Antennae reddish brown; antennal scape extending to just slightly beyond posterior margin of eye; desmomere I about same length as II. Pronotum cylindrical, slightly wider than long, greatest width at midlength; dorsal surface shallowly punctate but largely obscured by scales, each puncture with a curved, fine brown seta; posterior margin slightly bisinuate, slightly wider than anterior margin; scutellum subcircular, glabrous. Mesocoxal cavities about

5 times width of intercoxal process. Metasternum with lateral portions slightly tumid, not posteriorly produced. Elytra in dorsal view 1.8 times their greatest width; anterior margin sinuate; humeral region of elytra 1.5 times width of posterior margin of pronotum; lateral margins slightly divergent until second third, thereafter convergent; apex acutely rounded; in lateral view with dorsal outline tumid; posterior declivity gradually descending; stria 9 complete, stria 10 interrupted above metacoxa (appearing to merge with stria 9), resuming at suture between ventrites 1 and 2; intervals completely covered with scales, with dark and light areas forming an irregular pattern; all intervals equally flat, humerus angled; interval 9 very slightly tumid above metacoxa; all intervals with recurved, fine brown setae. Venter with scales very sparse, linear and hair-like on all ventrites; ventrites 3 and 4 subequal in length, their combined length shorter than ventrite 5; posterior margin of ventrite 5 widely rounded, finely narrowly emarginate at middle, apex at middle narrowly impressed. Tegmen with tegminal apodeme 0.8 times length of aedeagus; tegminal plate simple. Aedeagus short and robust, in dorsal view about 3.0 times longer than its greatest width; apex rounded, deflexed ventrally. Endophallus extended to just beyond base of aedeagus, with no visible internal sclerotization. Aedeagus in lateral view slightly evenly convex. Aedeagal apodemes about same length as aedeagus.

*Female*. Body length 3.4–3.6 mm.

**Etymology.** This species is named after Fernando de Zayas (1912–1983), entomologist, Cuban Academy of Sciences.

**Natural history.** Adults were collected beating vegetation along the upper part of the road to Pico San Juan.

## Key to Cuban species of Apodrosus

Eyes small, rounded, the distance from posterior margin of eye to posterior
margin of head about the same as greatest diameter of an eye (Fig. 24); male
with aedeagus very short and stout, length about 3 times maximum width
(Fig. 26)
Eyes larger, more elongate oval, the distance from posterior margin of eye
to posterior margin of head about 0.3-0.7 times greatest diameter of an eye;
male with aedeagus with length more than 4 times maximum width
Elytra with intervals 3 (subbasally and discally), 5 (subbasally) and 7 (at hu-
merus) elevated above adjacent intervals (more so in female than in male)
(Figs 5, 20) <b>3</b>
Elytra with all intervals of equal elevation4
Male with aedeagus very long and slender, length about 10 times maximum
width (Fig. 21); endophallus lacking any fields of microtrichiae (Fig. 21);
females not separable
Male with aedeagus moderately long and less slender, length about 4.5 times
maximum width (Fig. 6); endophallus with two elongate fields of microtri-

chiae at base extended between apodemes (Fig. 6); females not separable..... 4 Elytra with stria 10 continuous throughout length (Figs 1, 16, 22)......5 Elytra with stria 10 interrupted above metacoxa (Figs 7, 13).....7 5 Body with most scales grey, greyish white or pearlescent in color (Figs 22, 23); in lateral view with frons and base of rostrum continuous, in same plane (Fig. 22); ventrites 1 and 2 with only scales, not setose; males not known..... Body with most scales brown or copper in color (Figs 1, 2, 16, 17); frons and base of rostrum separated by a slight transverse impression in lateral view 6 Male with aedeagus in dorsal view broadly rounded at apex, subtruncate (Fig. 3); male tergite VII with apex emarginate; male with apex of ventrite 5 im-Male with aedeagus in dorsal view tapered in apical 2/5 to narrowly rounded apex (Fig. 18); male tergite VII with apex rounded; male with apex of ventrite 5 deeply, narrowly impressed medially..... A. mensurensis Anderson, sp. n. 7 Male with aedeagus with endophallus extended almost to apex of aedeagal apodemes, with a narrow u-shaped sclerite at midlength of aedeagus and an apical hooked sclerite complex (Fig. 12); female with elytral profile at apical Male with aedeagus with endophallus extended from about midlength of aedeagal apodemes to apical two-thirds of aedeagal apodemes, with either a narrow, scythe-like sclerite positioned near basal two-thirds of length, an elongate field of microtrichia positioned at base of aedeagus, and an apical hooked sclerite complex (Fig. 15), or with only an apical hooked sclerite complex (Fig. 9); female with elytral profile at apical declivity evenly rounded .......8 8 Male with aedeagus with endophallus extended from about midlength of aedeagal apodemes to apical two-thirds of aedeagal apodemes, with either a narrow, scythe-like sclerite positioned near basal two-thirds of length, an elongate field of microtrichia positioned at base of aedeagus, and an apical hooked sclerite complex (Fig. 15); females not separable..... Male with aedeagus with endophallus extended to about midlength of aedeagal apodemes, with only an apical hooked sclerite complex (Fig. 9); females 

# Phylogeny and biogeographic implications

A distribution map of Cuban Apodrosus is shown in Fig. 27. The 33-taxon molecular phylogeny (Suppl. material 2), reconstructed using a maximum likelihood method, recovers the monophyly of *Apodrosus* (bootstrap value 100%) represented by 11 species



Figure 27. Distribution map of Cuban species of Apodrosus, made using simplemappr.net.

in the current study. Apodrosus and Polydrusus are recovered as sister groups, although with poor nodal support. In other words, the monophyly of Polydrusini as sampled in the current study is recovered. It is worth pointing out that numerous genera of this tribe are not sampled here and the phylogenetic coherence or monophyly of Polydrusini remain to be fully tested. Most critically, Anypotactus, sister group of Apodrosus recovered by Girón and Franz (2010) was not sampled here. It would be premature to draw a conclusion on the placement of Apodrosus. Pachyrhinus lethierryi is nested within Polydrusus, necessitating the synonymy of the two genera, but this question is left for future investigations. The 13-taxon Apodrosus focal phylogeny is congruent with the 33-taxon phylogeny in the "deeper" relationships within Apodrosus, but the two differ in relationships among five Cuban species (A. zayasi-A. mensurensis clade in Fig. 28). We will base our phylogenetic and biogeographic discussions on the 13-taxon phylogeny, as our biogeographic discussions primarily concern inter-island patterns and the phylogenetic resolution within Cuban species is inconsequential in that regard. We recognize that bootstrap nodal support values are low for several clades, reflecting potential inconsistency in the data. We opted for presenting a fully dichotomous phylogeny, as is the case in many publications. Biogeographic scenarios are discussed in light of this dichotomous phylogeny (which is the best phylogeny available), but it is understood that they also are subject to future testing and revisions when additional data is acquired. Apodrosus argentatus Wolcott, 1924, a widespread species distributed in both Puerto Rico and the Dominican Republic, is recovered as sister to the remaining species of Apodrosus. The six Cuban species sampled here are not monophyletic. Five of the Cuban species form a clade (A. zayasi-A. mensurensis), which is sister to an unidentified species from Dominican Republic (which might represent a new species as it could not be keyed out using Girón and Franz 2010). A single species, Apodrosus alternatus, forms a sister relationship with A. quisqueyanus Girón & Franz, 2010, a spe-



**Figure 28.** Maximum likelihood phylogeny of *Apodrosus*, based on six gene fragments. Colored boxes indicate species distributions. Bootstrap support values (1000 replications) are drawn at nodes. Hispaniola is represented by the Dominican Republic for all instances. Scale bar indicates the average number of nucleotide substitutions per site.

cies from the Dominican Republic. Together, they are part of a larger clade that also includes two Puerto Rican species, *A. wolcotti* and *A. epipolevatus* Girón & Franz, 2010.

The current molecular phylogeny (Fig. 28) and the morphological phylogeny by Girón and Franz (2010) differ in taxon sampling, but congruence and conflict between these phylogenies can be observed and are presented as follows. *Apodrosus wolcotti* and *A. epipolevatus*, both from Puerto Rico, are sister species in both analyses. In Girón and Franz (2010), *A. quisqueyanus* is more closely related to *A. argentatus* than either is to the two Puerto Rican species. In contrast, the current phylogeny posits a closer relationship between *A. quisqueyanus* and the Puerto Rican species, which are in the same clade, whereas *A. argentatus* is outside that clade.

According to the current phylogenetic hypothesis (Fig. 28), species of *Apodrosus* from the same island are distributed in multiple clades and some sister groups are formed by species from different islands. This pattern of the geographic distributions of species of *Apodrosus* with regards to the phylogeny is reminiscent of other Caribbean entimines (Zhang et al. 2017), and is indicative of multiple successive dispersal events between islands. A "stepping-stone" scenario of an origin in Puerto Rico and subsequent dispersal to Hispaniola and Cuba is plausible. An alternative explanation of the biogeographic pattern is island to island vicariance. According to the geological evolution model proposed by Iturralde-Vinent and MacPhee (1999), the three Greater Antillean islands, Cuba, Hispaniola and Puerto Rico, were connected to form a land-span in the Late Eocene and Early Oligocene (35–33 million-years ago [Ma]). Eastern Cuba and northern Hispaniola were physically connected during the Early Oligocene, which split apart later in that epoch. On the other hand, central Hispaniola and Puerto

Rico remained connected probably until late in the Miocene (14 Ma). A sister relationship between Cuba and the Dominican Republic appears twice in our phylogeny, which is in accordance with the vicariance of the two islands. However, in the clade "*A.epipolevatus–A. alternatus*", Puerto Rico is sister to Cuba and the Dominican Republic, implying an early split of Puerto Rico from the two larger islands, which is at odds with the vicariance model proposed by Iturralde-Vinent and MacPhee (1999) (i.e., a late split between Puerto Rico and Hispaniola).

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# Supplementary material I

# Information of specimens of *Apodrosus* used in molecular analyses, including Genbank accession numbers.

Authors: Robert S. Anderson, Guanyang Zhang

Data type: Occurrence and genbank accession numbers (table).

- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: https://doi.org/10.3897/zookeys.679.12805.suppl1

# Supplementary material 2

33-taxon maximum likelihood phylogeny of *Apodrosus*, *Polydrusus* and representatives of other Neotropical entimines. Colored boxes indicate species distributions. Bootstrap support values (500 replications) are drawn at nodes. Hispaniola is represented by the Dominican Republic for all instances. Scale bar indicates the average number of nucleotide substitutions per site.

Authors: Robert S. Anderson, Guanyang Zhang

Data type: Figure.

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RESEARCH ARTICLE



# Description of the immature stages of Larinus vulpes and notes on its biology (Coleoptera, Curculionidae, Lixinae)

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# Abstract

Mature larva and pupa of *Larinus vulpes* (Olivier, 1807) (Curculionidae: Lixinae: Lixini) are morphologically described for the first time and compared with known larvae and pupae of other *Larinus* species. Very high counts of larval body setae (pronotum with more than 25 setae and postdorsum on meso- and metathorax and also on abdominal segments I–VII with more than 12 setae) are characteristic features of the nominotypical subgenus *Larinus*. The biology of the species was studied in Ukraine. *Echinops ruthenicus* and *E. sphaerocephalus* were identified as host plants of both larvae and adults of this weevil based on the present research in Ukraine, which shows probably oligophagous. Overwintering beetles emerged at the end of May or earlier, then feeding and mating on the host plants. The highest level of adult activity was observed at the end of June. Larvae were endophagous within the flower heads. In July and August, the larvae pupated within inflorescences in a pupation cell. Adults exited the cells at the end of August and did not hibernate on the host plants. Sometimes, larvae and imagines of a new generation were found outside the flower heads in chambers constructed on the stems.

#### **Keywords**

Asteraceae, *Echinops*, eastern Europe, host plant, larva, larval development, life history, morphology, Palaearctic Region, pupa, weevil

### Introduction

The weevil genus *Larinus* Dejean, 1821 belongs into the tribe Lixini Schoenherr, 1823 and is represented by ca. 180 species (Csiki 1934; Ter-Minassian 1967; Gültekin 2006) of which more than 110 are known in the Palaearctic (Gültekin and Fremuth 2013; Gültekin and Shahreyary-Nejad 2015). A further 40 species are recorded from the Ethiopian region, only three species from the Oriental region, four (introduced species) from the Nearctic region (McClay 1988; Gültekin 2006), and one in New Zealand (Woodburn and Briese 1996; Gültekin 2006). The valid systematic position of this genus has been assigned for Palaearctic species in the Catalogue of Palaearctic Coleoptera (Gültekin and Fremuth 2013). The genus is divided into four subgenera: *Cryphopus* Petri, 1907; *Larinus* Dejean, 1821; *Larinomesius* Reitter, 1924; and *Phyllonomeus* Gistel, 1856 (Gültekin and Fremuth 2013). Knowledge of the morphology of immature stages in *Larinus* is incomplete in comparison to the total number of species in this genus and to the importance of several species as potential biological control agents against weeds (Nikulina et al. 2004; Seastedt et al. 2007).

The species *Larinus vulpes* (Olivier, 1807) belongs to the nominotypical subgenus *Larinus*, which includes 35 species in the Palaearctic region (Gültekin and Fremuth 2013; Gültekin and Shahreyary-Nejad 2015), and is distributed in the western Palaearctic, east Siberia and central Asia (Gültekin and Podlussány 2012; Gültekin and Fremuth 2013). The most northern area of its range is Kungur, Russia (56–57°N) (Dedyukhin 2011). The life cycle of *L. vulpes* is associated only with globe thistles, the genus *Echinops* L. (Asteraceae). Similar to other *Larinus* species, adults feed on the leaves and stems. Eggs, larvae, and pupae develop in the inflorescences. Imagines of a new generation hibernate outside the host plants. Circumstantial observations of reproduction and preimaginal development of *L. vulpes* were reported by Fabre (1922; as *Larinus maculosus* Schoenherr, 1832). Next, Volovnik (1996, 2016) provided sufficient details on the biology of this species. The immature stages of this species have never been morphologically described.

Some species from the genus *Echinops* are very important invasive weeds (Czarapata 2005; Reddy et al. 2008) and also have medicinal uses (Murch et al. 2003; Eram et al. 2013; Parhat et al. 2014). The globe thistles are nectar (Wroblewska et al. 1993; Jabłoński and Kołtowski 2005) and ornamental plants (Wiersema and León 2016), a potential source of natural insecticide (Gemechu et al. 2013; Liu et al. 2013), molluscicid (Hymete et al. 2005), and energetic oil (Horn et al. 2008). The root extract of *E. giganteus* A. Rich. is used as a mosquito repellent (Karunamoorthi and Hailu 2014) and also as perfume (The Green Vision 2017), and these roots are used as dietary spice (Stève et al. 2016). Because of their deep roots, the globe thistles are widely used for mechanical stabilisation of banks, ravines, and slopes (Chopik et al. 1983). Therefore, there are strong arguments for a detailed investigation of the weevil *L. vulpes* and then promote the use of larvae of *L. vulpes* as potential biological control agents against this plant. The knowledge of bionomy of immature stages of *Larinus* species is also
important for further taxonomic studies at different levels and for effective protection of endangered species. In this paper, we describe the immature stages of *L. vulpes* and provide details of its life history based on field observations in Ukraine.

# Materials and methods

# Insect collection and laboratory breeding

The material used to describe the immature stages was collected and field observations were conducted in Ukraine in the following localities:

- The eastern shore of Molochnyi Estuary between the two villages of Altagir (= Bogatyr) and Radyvonivka (46°38'29"N, 35°16'59"E). Altitude: up to 20 m a. s. l. Bedrock: loess loam with herbaceous covering. Dominant plant species: *Agropyron pectinatum* (M. Bieb.) P. Beauv., *Festuca valesiaca* Schleich. ex Gaudin, *Koeleria cristata* (Ledeb.) Schult., *Artemisia marschalliana* Spreng., *Ephedra distachya* L., and *Helichrysum arenarium* (L.) Moench, 1794 (Kolomiychuk and Vynokurov 2016). Globe thistle *Echinops ruthenicus* M. Bieb. usually grows along the top of the slope.
- 2) Man-made forest near the Kamyana Mohyla Reserve (46°57'01"N, 35°28'12"E). Altitude: up to 10 m a. s. l. Bedrock: sandy chernozem. Dominant plant species: *Robinia pseudoacacia* L. and *Morus nigra* L., with herbaceous plants (i.e., *Echinops ruthenicus, Centaurea adpressa* Ledeb. ex Steud., *Melilotus albus* Medik.) in the clearings.
- 3) NE Cyrilivska Spit located between the Sea of Azov and Molochnyi Estuary (46°25'12"N, 35°25'09"E). Altitude: 5 m a. s. l. Bedrock: flats of quartz sand, shells and mud. Habitats: salty grasslands with dominant herbaceous species *Elytrigia elongata* (Host) Nevski, *Puccinellia distans* (Jacq.) Parl., *Aeluropus littoralis* (Gouan) Parl., 1850 and *Juncus gerardii* Loisel.; coastal communities with *Phragmites australis* (Cav.) Trin. ex Steud., *Bolboschoenus maritimus* (L.) Palla and *Juncus maritimus* Lam. (Kolomiychuk and Vynokurov 2016). Globe thistle *Echinops ruthenicus* is locally distributed between the central road and the coast of the estuary (Fig. 21).

In the above-mentioned localities, life cycle, including feeding of adults, oviposition, and early development of larvae were observed directly during the vegetation growing seasons of *Echinops ruthenicus* and *E. sphaerocephalus* L. in the time period 2012–2016.

The compound flower heads of globe thistles consist of simple capitula, each of which has only one floret. These primary capitula are aggregated in globose secondary capitula (Kadereit and Jeffrey 2007). For convenience, below, we shall name as capitula only secondary ones. The second author collected all larvae and pupae of *L. vulpes* within inflorescence. Some inflorescences (n = 42) were dissected to investigate preimaginal development, and a further 250 were dissected to determine the quantity of preimaginal specimens of *L. vulpes* within an inflorescence. All photographs in the field were taken with digital cameras, a Nikon Coolpix 4600 and a Canon PowerShot SX500 IS.

Laboratory observations were conducted in Melitopol, Ukraine (46°50'N,  $35^{\circ}22$ 'E). The dry inflorescences (n = 7) with developing mature larvae or pupae were placed into cardboard boxes. A small hole was opened in every inflorescence for possible observations of insect development. Measurements of flower head were performed with a slide caliper and ocular micrometre. The size of flower heads was determined at the greatest diameter.

Geographical distribution and phenology were studied from several entomological collections, specifically: Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine (Kyiv), TG Shevchenko Kyiv National University Zoological Museum, Zoological Institute of Russian Academy of Sciences (St. Petersburg), VN Karazin Kharkiv National University Museum of Natural History, and Igor Maltzevs' private collection (Odessa). In total, more than 130 pinned specimens were studied.

### Morphological descriptions

Part of the larval and pupal material was preserved in Pampel fixation liquid (see Skuhrovec et al. 2015) and used for the morphological descriptions. These specimens are deposited in the collection of Group Function of Invertebrate and Plant Biodiversity in Agro-Ecosystems of the Crop Research Institute (Prague, Czech Republic). The collectors identified the plants. To prepare slides, we followed May (1994).

The observations and measurements were conducted using a light microscope with calibrated oculars (Olympus BX 40, SZ 11, and Nikon Eclipse 80i). The following characteristics were measured for each larva: head width, length of the body (larvae fixed in a C-shape were measured in the middle of the segments in lateral view), and width of the body in the widest place (i.e., meso- and metathorax). For the pupae, the length and the width at the widest place were measured.

Drawings were created with a drawing tube on a light microscope and edited by the programs Adobe Photoshop 10, Corel Photo-Paint X7, and GIMP 2.

We used the terms and abbreviations for the setae of the mature larva and pupa found in Scherf (1964), May (1977, 1994) and Marvaldi (1998, 1999). The numbers of setae of the bilateral structures are given for one side.

The counts of some of the setae on the epipharynx (particularly *ams* and *mes*) have not been completely resolved. According to Marvaldi (1998, 1999), the standard status of the epipharynx in weevils is 2 *ams* and 3 *mes*; however, when the position of the distal *mes* is very close to the anterior margin, it appears as *ams*. The final decision was to add this problematic seta to the latter group (*ams*), and the position of this seta

is similar to that in other genera, e.g., in *Coniocleonus* Motschulsky, 1860 or *Tychius* Germar, 1817. We did not follow Stejskal et al. (2014) and Skuhrovec et al. (2014) who accepted the standard status in weevils and counted the seta as *mes*, but we followed Trnka et al. (2015) and Skuhrovec et al. (2015), e.g., in *Adosomus* Faust, 1904 or *Sibinia* Germar, 1817. The thoracic spiracle was located on the prothorax near the boundary of the prothorax and mesothorax, as shown in the drawing (see Fig. 10), but this spiracle is of mesothoracic origin (Marvaldi et al. 2002; Marvaldi 2003).

#### **Results and discussion**

## Morphology of immature stages

#### Larinus (Larinus) vulpes (Olivier, 1807)

**Material examined. UKRAINE:** Cyrilivska Spit (locality 3 in Materials and methods), 12–29.viii.2015, 15 larvae, 16 pupae  $(5 \Im \Im, 11 \Im \Im)$ , leg. S Volovnik.

**Description of mature larva.** *Measurements* (in mm, n = 15). Body length: 13.4–21.2 (mean 18.1). Body width: (meso- and metathorax) 4.03–5.01 (mean 4.46). Head width: 1.99–2.29 (mean 2.14).

*General.* Body stocky, slightly curved, rounded in cross section (Fig. 7). Cuticle densely spiculate (Figs 8–9).

*Colouration.* Head light brown or brown with a distinct pale pattern around the frontal and epicranial sutures (Fig. 7). All thoracic and abdominal segments are dark yellow with a light brown, elongate stripe on the dorsum of the pronotum (Fig. 7).

Vestiture. Setae on body thin, short, light yellow or orange (Figs 7-9).

*Head capsule* (Fig. 1). Head suboval, slightly longer than wide, endocarinal line weak, but long as a half-length of frons. Frontal sutures distinct, wide, and extended to the antennae. Single anterior stemma (st) distinct, in the form of a slightly pigmented spot.  $Des_1$  and  $des_2$  located in the upper part of the central part of the epicranium,  $des_1$  near the middle part of the epicranium and  $des_2$  near the side of the epicranium,  $des_3$  located anteriorly near the frontal suture,  $des_4$  located in the central part of the epicranium,  $des_5$  located anterolaterally; all des long, nearly subequal in length, except  $des_4$  distinctly shorter (Fig. 1).  $Fs_1$ ,  $fs_2$  and  $fs_3$  placed medially,  $fs_4$  located anteromedially,  $fs_{4b}$  located laterally close to  $fs_4$ ; and  $fs_5$  near the source than very long  $fs_{1.4}$  (Fig. 1).  $Les_{1.2}$  and  $ves_{1.2}$  very long, as long as  $des_5$ . Epicranial area with two sensilla, one upper  $des_1$  and the second in upper part of posterior; and also with 3 *pes* in line with upper  $des_2$ .

Antennae located at the end of the frontal suture on each side, membranous and slightly convex basal article bearing one conical sensorium, relatively long; basal membranous article with 5 sensilla, different in both shape and length (Fig. 4).

*Clypeus* (Fig. 2) trapezoid-shaped, approximately twice as wide as long, with two relatively long *cls*, *cls*<sub>2</sub> slightly shorter than *cls*<sub>1</sub>, localized posterolaterally and 1 sensil-lum located close to *cls*; anterior margin concave.

Mouth parts. Labrum (Fig. 2) approximately twice as wide as long, with 3 pairs of piliform *lms*, of different lengths; *lms*<sub>3</sub> distinctly shorter than very long *lms*<sub>1</sub> and long lms2; lms1 located close to the margin with clypeus, lms2 located anteromedially, and *lms*, located anterolaterally; anterior margin bisinuate. Epipharynx (Fig. 3) with 4 blunt, finger-like *als*, unequal in length,  $als_{1-2}$  shorter than  $als_{3-4}$ ; 3 ams: ams, and ams, distinctly thinner than ams, and ams, piliform, ams, blunt, finger-like; 2 mes and one sensillum close to mes; mes, distinctly smaller than mes,, both located close to lr; labral rods (lr) elongated, slightly diverging on distal half. Mandibles (Fig. 5) relatively broad, bifid, teeth of unequal height; slightly truncate; both mds relatively long, piliform. Maxilla (Fig. 6) stipes with 2 stps, 2 pfs, and 1 mbs; both stps and pfs, very long and 1.5 times longer than long *pfs*<sub>1</sub>, *mbs* very short; mala with 9 bacilliform *dms*<sub>1-9</sub>; 5 short *vms*<sub>1,5</sub> in two sizes: 2 *vms* short, and 3 very short; all *vms* distinctly shorter than dms. Maxillary palpi with two palpomeres; basal palpomere with 1 short mxps and two sensilla; length ratio of basal and distal palpomeres: 1:0.9; distal palpomere with one sensillum and a group of conical, apical sensorial papillae. Praelabium (Fig. 6) heartshaped, with 1 relatively long *prms*; ligula with sinuate margin and 2 piliform very short *ligs*, unequal in length; premental sclerite V-shaped clearly visible. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres: 1:0.9; distal palpomere with one sensillum and short, apical sensorial papillae; basal palpomere with 1 ventral sensillum. Postlabium (Fig. 6) with 4 pms, 1 pms located anteriorly, and 3 pms laterally; very long, almost of equal length; surface of postlabium densely covered by distinct asperities.

*Thorax.* Prothorax larger than meso- and metathorax. Meso- and metathorax distinctly wider than abdominal segments I–IV. Spiracle unicameral. Cuticle densely spiculate and with distinct thorn-like cuticular processes, primarily on dorsal parts but also on pleural parts (Fig. 7). Prothorax (Fig. 10) with ca. 30–35 relatively long to short *prns* unequal in length, 25 on pigmented pronotal sclerite, which is subdivided medially into two triangular plates, next 5–10 *prns* placed below; 20 relatively long *ps* also on pigmented sclerite, and 12 relatively long *eus*. Mesothorax (Fig. 10) with 3 short *prs*; 13 relatively long to short *pds*; 6–7 relatively long to short *as*; 6 relatively long to short *ss* on pigmented sclerite; 6–9 relatively long to short *eps* on pigmented sclerite; 14 relatively long to short *ps* on pigmented sclerite and 12 relatively long to long *eus*. Chaetotaxy of meso- and metathorax (Fig. 10) almost identical, but some specimens partly variable in the exact count of setae. Each pedal area of the thoracic segments well separated and pigmented, with 10 long *pda* on pigmented sclerite, unequal in length.

*Abdomen.* Abdominal segments I–IV of almost equal length, subsequent abdominal segments decreasing gradually to the terminal parts of the body. Abdominal segment X reduced to four anal lobes of unequal size, the dorsal being distinctly the largest, the lateral pair equal in size, and the ventral lobe very small. Anus located



**Figure I.** *Larinus vulpes* mature larva head, frontal view. Abbreviations: *des* – dorsal epicranial s., *fs* – frontal epicranial s., *les* – lateral epicranial s., *ves* – ventral epicranial s., at – antenna, st – stemma. Scale bar 1 mm.



**Figures 2–3.** *Larinus vulpes* mature larva, mouth parts. **2** Labrum and clypeus **3** Epipharynx. Abbreviations: *ams* – anteromedial s., *als* – anteriolateral s., *cls* – clypeal s., *mes* – median s., *lms* – labral s., lr – labral rods. Scale bar 0.2 mm.



**Figures 4–5.** *Larinus vulpes* mature larva, antenna, and mouth parts. **4** Antenna **5** Right mandible (*mds* – mandible dorsal s.). Scale bars 0.05 mm (**4**) and 0.5 mm (**5**).



**Figure 6.** *Larinus vulpes* larval mouthparts, maxillolabial complex, ventral view: right maxilla. Abbreviations: *dms* – dorsal malar s., *vms* – ventral malar s., *mpxs* – maxillary palps s., *mbs* – basioventral s., *pfs* – palpiferal s., *stps* – stipital s.), prementum and postmentum, ventral view (*prms* – premental s., *pms* – postmental s., *ligs* – ligular s.). Scale bar 1 mm.

terminally. Spiracles unicameral, the eight abdominal spiracles located laterally, close to the anterior margin of abdominal segments I–VIII. Cuticle also densely spiculate and with distinct thorn-like cuticular processes, primarily on dorsal parts but also on pleural parts (Figs 8–9). Abdominal segments I–VII (Figs 11–12) with 2 relatively



Figure 7–9. *Larinus vulpes* mature larva. 7 Habitus, lateral view. Scale bar: 2 mm. 8 Detail lateral view on dorsum of abdominal segment I with densely spiculate cuticle 9 Detail lateral view on dorsum of abdominal segment VIII–IX.

long to short *prs*; 13 relatively long to short *pds*, 10 *pds* in line, and 3 *pds* in the below part partly anteriorly; 7 relatively long to short *ss*, 5 *ss* under *pds* (abdominal segment VII only with 3 setae), and 2 *ss* in below part of dorsal lobe; 13 (10–14) relatively long



**Figures 10–12.** *Larinus vulpes* mature larva, habitus. **10** Lateral view of thoracic segments **11** Lateral view of abdominal segment I **12** Lateral view of abdominal segments VII-X. Abbreviations: *prns* – pronotal s., *prs* – prodorsal s., *pds* – postdorsal s., *as* – alar s., *ss* – spiracular s., *eps* – epipleural s., *ps* – pleural s., *pda* – pedal s., *lsts* – laterosternal s., *eus* – eusternal s., *ds* – dorsal s., *sts* – sternal s., Th1-3 – number of thoracic segments, Ab1-10 – number of abdominal seg. Scale bar 2 mm.

to short *eps* on pigmented sclerite (only on abdominal segments I–II); 9 relatively long *ps* of unequal length; 2 short *kts* and 2 short *eus*. Abdominal segment VIII (Fig. 12) with 2 relatively long *prs*; 10 relatively long to long *pds* in line; 2 relatively long *ss* in below part of dorsal lobe; 13 relatively long to short *eps*; 9 relatively long *ps* of unequal length; 2 short *kts* and 2 short *eus*. Abdominal segment IX (Fig. 12) with 7 *ds* (6 long *ds* near posterior margin, and 1 short *ds* medially); 13 relatively long to long *ps*; and 2 relatively long and 2 short *sts*. Abdominal segment X (Fig. 12) with 2 very short setae (*ts*) on each lateral anal lobe, and 1 very short seta (*ts*) on dorsal anal lobe.

**Description of pupa.** *Measurements* (in mm; 5  $\Diamond \Diamond$ , 11  $\bigcirc \bigcirc$ ). Body length:  $\Diamond$  11.8–13.2 (mean 12.5),  $\bigcirc$  12.6–15.2 (mean 14.0). Body width:  $\Diamond$  6.5–7.8 (mean 6.8),  $\bigcirc$  7.0–8.20 (mean 7.5). Thorax width:  $\Diamond$  4.6–4.9 (mean 4.7),  $\bigcirc$  4.8–5.1 (mean 4.9). Head width:  $\Diamond$  1.8–2.0,  $\bigcirc$  1.9–2.2.

*Colouration.* All thoracic and abdominal segments light yellow or greenish-white. Cuticle smooth, except thorn-like processes on abdominal segments III–VIII.

*Morphology* (Figs 13–20). Body moderately slender and elongated. Rostrum long, approximately 2.5 times as long as wide, extended to mesocoxae. Antennae relatively long and slender. Pronotum 2.5 times as wide as long. Meso- and metanotum of equal length. Abdominal segments I–V of equal length, abdominal segments V–VII diminish gradually, abdominal segment VIII almost semi-circular, and abdominal segment IX distinctly smaller than other segments. Urogomphi very short, almond-shaped with acute sclerotised apexes. Spiracles placed dorso-laterally; 5 pairs functional on abdominal segments I–V and one atrophied on abdominal segment VI, on next abdominal segment segment IX: gonotheca of Q divided (Fig. 19),  $\delta$  undivided (Fig. 20).

Chaetotaxy (Figs 13-20). Setae distinct, of different length, light brown. Head capsule includes 1 vertical seta (vs); 3 super-orbital setae (sos,  $_{3}$ ) (equal in length); 1 orbital seta (os) and 4 post-antennal setae  $(pas_{1-4})$  (equal in length). Rostrum with 8 rostral setae  $(r_{s_{1-8}})$  (different in length);  $r_{s_{1-4}}$  located apically,  $r_{s_{5-7}}$  latero-apically, and  $rs_8$  latero-medially. Mandibular theca with 1 epistomal seta (es). Setae on head capsule and rostrum straight or slightly curved, shorter than setae on pronotum. Pronotum with many setae, which created a characteristic pattern that consisted of: 1 super apical seta (sas), 4 super lateral setae ( $sl_{1,a}$ ) (equal in length), distributed along a horizontal line, 3 discal setae  $(ds_{1-3})$  equal in length, forming a group medially, and 21 posterolateral setae  $(pk_{1-2l})$  (different in length) of which:  $pk_{1-ll}$  located along posterior margin of pronotum and next 10 setae  $(pk_{12-21})$  form groups ventrally. Lateral setae in two groups;  $k_{1-5}$  located more or less along margin of pronotum, next five  $(k_{6-10})$  form a group (sometimes covered by antennae). Setae on pronotum different in length:  $pl_{1-1}$ the longest,  $ds_{1-3}$  the shortest (Fig. 16). Prolegs with 5 trochanter setae ( $trs_{1-5}$ ). Mesonotum with 14 setae  $(d_{1-14})$ :  $d_{1-3}$  located anteromedially,  $d_4$  posteromedially and  $d_{5-14}$  mediolaterally. Metanotum with 14 setae  $(d_{1-14})$ :  $d_{1-3}$  located anteromedially,  $d_{4-14}$  form a line medially (Fig. 16). Apex of femora with 3 or 4 long femoral setae (fes) (Fig. 18). Setae on abdominal segments various in size, sometimes replaced by thorn-like cuticu-



**Figures 13–15.** *Larinus vulpes* pupa habitus. **13** Dorsal view **14** Lateral view **15** Ventral view. Abbreviations: Ab1-9 – number of abdominal segments, Th1-3 – number of thoracic segments, ur – urogomphi. Scale bar 5 mm.



**Figures 16–20.** *Larinus vulpes* pupa chaetotaxy. **16** Dorsal view **17** Head and rostrum **18** Lateral view of abdominal segments 3-7 **19** Ventral view of lasts abdominal segments of female **20** Ventral view of lasts abdominal segments of male. Abbreviations: Ab1-9 – number of abdominal segments, Th1-3 – number of thoracic segments, ur – urogomphies. Setae: *sas* – super apical, *l*, *ls* – lateral, *sls* – super lateral, *d* – dorsal, *ds* – discal, *pls* – posterolateral, *v* – ventral, *vs* – vertical, *sos* – super orbital, *os* – orbital, *pas* – postantennal, *rs* – rostral, *es* – epistomal, *fes* – femoral. Scale bars 5 mm (**16–18**), and 2 mm (**19–20**).

lar processes; a reason the exact number of setae remained difficult to precisely determine. Approximately 40 setae are on dorsal parts of each of abdominal segments I and II, and approximately 30 on each of abdominal segments III–VIII. Generally: 4–6 very short setae placed along anterior margin of each segment, next 20 long and short setae (or ca. 10 long setae and ca. 10 thorn-like cuticular processes) form a regular, horizontal line on median parts; next approximately 15 thin setae form groups dorsolaterally on each segment. Some small setae are replaced by thorn-like cuticular processes, which increase gradually from abdominal segment III to VII. Cuticular processes on abdominal segment VIII distinctly smaller than those on abdominal segments VI and VII. Abdominal segment VIII with 5 small setae along anterior margin, 6 medium size setae and some small cuticular processes medially. Abdominal segments I–VIII with approximately 20 thin setae ( $l_{1-20}$ ) laterally (Fig. 18); ventral parts of abdominal segment IX with 5 short setae, located ventrally along anterior margin of segment, and next single seta on gonothecae. External parts of urogomphi are densely covered by thin setae.

Comparison with larvae of other Larinus species. To date, larvae of 16 Larinus species have been described (Gardner 1934; Scherf 1964; Lee and Morimoto 1988; Nikulina et al. 2004; Zotov 2009a, 2010; Gosik and Skuhrovec 2011; Nikulina and Gültekin 2014), while detailed descriptions of the pupae are known for only 8 Larinus species (Zotov 2009a, 2010; Gosik and Skuhrovec 2011; Nikulina and Gültekin 2014). The comparison with previously described immatures of some other species, primarily of L. (Phyllonomeus) saussureae Marshall, 1924 (Gardner 1934), L. (Phyllonomeus) carlinae (Olivier, 1807) (as L. planus F.) and L. (Phyllonomeus) iaceae (Fabricius, 1775) (both in Scherf 1964), was somewhat problematic because of missing details of chaetotaxy and/or absence of quality drawings; therefore, a comparison of these three species with other known Larinus species was not possible to the level of detail required to incorporate them in the key (see Key to the immature stages of the Larinus). Lee and Morimoto (1988) provide a general larval description of the genus Larinus based on two species: L. (Phyllonomeus) latissimus Roelofs, 1873 and L. (Phyllonomeus) meleagris Petri, 1907. However, they did not present any differences between these two species (see aggregation of both species in the key at dichotomy 12).

According to May (1993), the increased number of *pds* on the meso- and metathorax and abdominal segments I–VII and of setae on the epipharyngeal lining (*als*) (i.e., more than the most frequent number of setae in weevils) are diagnostic of the mature larva of the subfamily Lixinae. The following descriptions of mature larvae from the tribe Lixini confirmed this diagnosis: genus *Larinus* (Scherf 1964; Lee and Morimoto 1988; Nikulina et al. 2004; Zotov 2009a, 2010; Gosik and Skuhrovec 2011; Nikulina and Gültekin 2014); genus *Lixus* (Scherf 1964; Lee and Morimoto 1988; May 1994; Nikulina 2001, 2007; Zotov 2009a, b; Nikulina and Gültekin 2011; Gosik and Wanat 2014; Skuhrovec and Volovnik 2015; Trnka et al. 2016); and *Rhinocyllus conicus* (May 1994), in addition to descriptions of all known species from the tribe Cleonini (Zotov 2011; Stejskal et al. 2014; Trnka et al. 2015). For a proper comparison of both tribes, including a key and detailed generic studies, further descriptions of immature stages of several Cleonini would be required.

Gosik and Wanat (2014), in a precise general description of the larvae of the tribe Lixini, summarized the tribe by 16 character sets (for details, see Gosik and Wanat 2014), but some of these characters (primarily chaetotaxy on the body) do not correspond exactly with most Larinus species from the nominotypical subgenus, including the recently described L. vulpes. The species from the subgenus Larinus (except L. idoneus Gyllenhal, 1835 and L. latus (Herbst, 1783)) had very high counts of larval body setae; e.g., pronotum with more than 25 setae and postdorsum on meso- and metathorax and also on abdominal segments I-VII with more than 12 setae (see details in Key to the immature stages of the Larinus and Table 1). The pupal number of setae was identical to that of all known pupae of species from the subgenus Larinus (except L. idoneus) with a pronotum with 25 or even more setae (see details in Key to the immature stages of the Larinus and Table 2). Morphological characters of larvae and pupae distinctly separated the subgenus Larinus from the other subgenera Phyllonomeus Gistel, 1856 and Larinomesius Reitter, 1924. Only two species (L. idoneus and L. latus) from the nominotypical subgenus did not correspond with the described chaetotaxy, which could be explained considering three hypotheses: (1) the nominotypical subgenus can be divided into two distinct groups, (2) these two species do not belong in this subgenus, or (3) these species show a peculiar autapomorphy; a change in a setal number can be a mere convergence (or coincidence). To solve this problem further morphological and molecular studies would be necessary.

The immature stages of L. vulpes had the closest affinity to the larvae of L. (L.) inaequalicollis Capiomont, 1874 and L. (L.) capsulatus Gültekin, 2008 based on five larval morphological characters: (1) frons with 6 or 7 fs; (2) postlabium with 4 or 5 setae; (3) stipes with 2 long sts; (4) prodorsum on meso- and metathorax with 3 prs; and (5) dorsal part of body distinctly spiculate; and two pupal morphological characters: (6) cuticle around setae dark-pigmented, visible spots formed; and (7) rostrum with 3 pas and only 1 rs. The primary differences between L. vulpes and L. inaequalicollis were as follow (see key to the immature stages of the Larinus): postepicranial setae pes, -pes, distinct (versus L. inaequalicollis very small, indistinct); frons with 6 fs (versus with 7 fs); endocarina not distinct, its length is as half-length of frons or less (versus distinct, massive, approximately 2/3 the length of frons), and ligula with 2 very thin ligs (versus with 1 micro *ligs* and two sensillae). The primary differences between *L. vulpes* and *L.* capsulatus were are as follows (see key to the immature stages of the Larinus): postlabium with 4 setae (versus L. capsulatus with 5 setae); meso- and metathorax with 6-7 as, 6 ss and 6-9 eps (versus with 4 as, 4 ss and 5 eps); abdominal segments I-VII with fewer than 14 pds and more than 10 eps (versus more than 15 pds and 8 or fewer eps); and lateral lobe of abdominal segment X with 2 setae (versus 3 setae).

Moreover, detailed descriptions of immature stages of *Larinus* species are also important for further studies on generic and evidently also subgeneric taxonomic relationships within Lixini and to effectively protect endangered species and promote the use of larvae of *Larinus* species as potential biological control agents against weeds

Part of body		a	b	с	d	e	f	g	h
	Pronotal	~24	~26	12	26–29	12	Σ	32	~30-35
- 1	Pleural	18	2	2	11	2	5	2	20
Prothorax	Eusternal	5	?	1	10	2	5	3	12
	Pedal area	12	?	7	11	8	12	7	10
	Prodorsal	3-4	5–9	2	3	1	9	4	3
	Postdorsal	14	18	5	14	4	0	11-12	13
	Alar area	4	1	3	7	1	11	3–4	6–7
Mass Matathanan	Spiracular area	4–5	?	?	?	?		?	6
Meso- Metathorax	Epipleural	4–5	5	1	8–9	4?	1-2	1	6–9
	Pleural	12	2	2	8	2	5	2	14
	Eusternal	5	6	1	4–5	2	5	2–3	13
	Pedal area	12	7	7	11-12	8	12	8	10
	Prodorsal I-VII	3	6	2	3	1	9	5	2
	VIII	?	?	1	?	?	3	?	2
	Postdorsal I-VII	19	24	8	17	8	~40	17	13
	VIII	?	?	5	?	?	8	?	10
	Spiracular I-VII	3	3	2	6	1	9	3	7
	VIII	?	?	?	?	?	0	?	2
Abd segment I VIII	Epipleural I-VII	8	3	2	12	2	6	3	10-14
Abd. segment 1-v III	VIII	?	?	?	?	?	5	?	13
	Pleural I-VII	7	1	2	6	2	3	1	9
	VIII	?	?	?	?	?	3	?	9
	Laterosternal I-VII	1	1	?	1	0	2	0	2
	VIII	?	?	?	?	0	2	?	2
	Eusternal I-VII	4	2	3	2	0	3	2	2
	VIII	?	?	?	?	0	1	?	2
	Dorsal	5	?	6	12	5	6	7	7
Abd. segment IX	Lateral	9	?	2	9	2	1	2	13
	Sternal	11	?	2	3	2	1	1	4
Abd. segment X	Anal area	4	4	1	2	4	3	2	3
	Dorsal	5	5	5	4	5	5	5	5
	Posterior	2	2	0	1	0	0	1	3
	Lateral	3	3	2	2	2	1	3	2
Head cansule	Ventral	?	?	?	?	?	1	?	2
ileud cupsule	Frontal	6	5	2	7	5	5	4	6
	Clypeal	2	2	2	2	2	2	2	2
	Labral	3	3	3	3	3	3	3	3
	Mandibular	2	2	2	0	2	2	0	2
	Anterolateral	2	3	4	4	4	5	3	4
Epipharynx	Anteromedial	?	2	2	3	4	2	2	3
	Medial	?	0	2	2	0	2	2	2

**Table 1.** Larval setal index of species from the subgenus *Larinus*: (a) *L. capsulatus*, (b) *L. fucatus*, (c) *L. idoneus*, (d) *L. inaequalicollis*, (e) *L. latus*, (f) *L. pollinis*, (g) *L. sibiricus* and (h) *L. vulpes* (italics – microsetae;  $\Sigma$  – uncountable, defined as more than 50; ? – no data).

Part of body			b	с	d	e	f	g	h
	Lacinia (dorsal)	7	9	8	9	8	10	8	9
	Lacinia (ventral)	3	5	6	3	4	4	4	5
Maxilla	Palpal	1	1	1	1	1	1	1	1
	Stipital	2	1	1	2	1	1	1	2
	Palpiferal	2	2	2	2	2	2	2	2
	Postlabial	5	?	3	5	3	3	3	4
Labium	Prelabial	1	1	1	1	1	1	1	1
	Ligular	2	2	2	2	2	2	3	2

**Table 2.** Pupal setal index of selected *Larinus* species: (a) *L. idoneus*, (b) *L. inaequalicolllis*, (c) *L. obtusus*, (d) *L. pollinis*, (e) *L. sibiricus*, (f) *L. sturnus*, (g) *L. turbinatus* and (h) *L. vulpes* ( $\Sigma$  – uncountable, defined as more than 50; ? – no data).

Part of body	a	Ь	с	d	e	f	g	h	
	vertical s.	-	1	1	1	2	1	-	1
TT II.	super orbital s.	1	2	2	3	2	2	1	2
Head capsule	orbital s.	2	1	1	1	1	1	2	1
	post antennal s.	5	4	4	4	4	4	3	3
D	rostral s.	3	2	1	8	5	4	1	1
Rostrum	epistomal s.	2	1	0	1	1	1	1	2
	superapical s.	1	1	Σ	1	1	1	1	-
	apical s.	0	0	Σ	0	0	0	2	2
Duration	lateral s.	~14	5	Σ	10	5	6	5	5
Prothorax	superlateral s.	5	1	Σ	4	1	2	-	2
	discal s.	2	2	Σ	3	2	2	1	3
	posterolateral s.	19	4	Σ	21	4	4	3	6
Mesothorax		6	12	6	Σ	16	6	6	~14
Metathorax		6	12	6	Σ	16	6	6	~14
	dorsal	~20	9	Σ	~40	9	9	8	~25
Abd. segment I-VII	ventral	3	3	3	3	3	3	4	3
	lateral	~20	4	7	~20	4	4	2	3
	dorsal	8	9	Σ	~10	8	8	8	~10
Abd. segment VIII	ventral	3	3	Σ	3	3	3	4	4
	lateral	~10	4	7	~20	4	4	2	3
41.1 . 197	ventral	?	2	3	5	4	4	?	?
Abd. segment IX	dorsal	?	1	2	Σ	1	3	?	?
Legs-femoral	*	3	3	3	6	3	3	3	3(4)

(e.g., *Carduus*, *Cirsium*, *Echinops*). Species identification of larvae with morphological evidence is relatively easy, and it is generally much cheaper than identification by molecular methods (Hirsch et al. 2010). The largest problem in the identification of the immature stages is the relatively low number of available larval descriptions in comparison to the many species only known at the adult stage. However, the problem is not exclusive to Curculionidae, being common to many other beetle groups.

# Key to the immature stages of Larinus

# Larvae (last instar)

The following key is based on the larvae of *Larinus vulpes* described in this paper and on 13 selected descriptions of larvae in the genus *Larinus* published previously (Lee and Morimoto 1988; Nikulina et al. 2004; Zotov 2009a, 2010; Gosik and Skuhrovec 2011; Nikulina and Gültekin 2014). Unfortunately, Lee and Morimoto (1988) only provide a general description of the genus for two species, and it was not possible to divide these two species (see aggregation of both species at point 12). Further comments are given in the previous section under Discussion.

1	Body with high count of setae; pronotum with more than 25 setae, postdor- sum on meso- and metathorax and also on abdominal segments I–VII with more than 12 setae
_	Body with usual count of setae; pronotum with less than 15 setae, postdor- sum on meso- and metathorax and also on abdominal segments I–VII with fewer than 10(12) setae
2	Frons with 6 or 7 <i>fs</i> . Postlabium with 4 or 5 setae. Stipes with 2 long <i>sts</i> . Pre- dorsum on meso- and metathorax with 3 <i>prs</i> . Dorsal part of body distinctly spiculate
_	Frons with 4 or 5 <i>fs</i> . Postlabium with 3 setae. Stipes with 1 long <i>sts</i> . Predorsum on meso- and metathorax up to 4 <i>prs</i> . Dorsal part of body not distinctly spiculate <b>5</b>
3	Postepicranial setae $pes_1 - pes_2$ distinct. Frons with 6 <i>fs</i> . Endocarina not distinct, its length is as half-length of frons or less. Ligula with 2 very thin <i>ligs</i> <b>4</b>
_	Postepicranial seta $pes_1$ very small, indistinct. Frons with 7 <i>fs</i> . Endocarina distinct, massive, approximately 2/3 the length of frons. Ligula with 1 micro <i>lies</i> and two sensilla <i>L</i> . ( <i>L</i> .) <i>inaequalicollis</i>
4	Postlabium with 5 setae. Meso- and metathorax with 4 <i>as</i> , 4 <i>ss</i> and 5 <i>eps</i> . Abdominal segments I–VII with more than 15 <i>pds</i> and 8 or fewer <i>eps</i> . Lateral lobe of abdominal segment X with 3 setae <i>L</i> . ( <i>L</i> .) <i>catsulatus</i>
_	Postlabium with 4 setae. Meso- and metathorax with 6–7 <i>as</i> , 6 <i>ss</i> and 6–9 <i>eps</i> . Abdominal segments I–VII with fewer than 14 <i>pds</i> and more than 10 <i>eps</i> . Lateral lobe of abdominal segment X with 2 setae
5	Frons with 5 setae. Endocarina either very thin and short (less than $1/3$ of the length of frons) or absent. Prodorsum on metathorax with more than 5 <i>prs.</i> .
_	Frons with 4 setae. Endocarina distinct, length of approximately $1/3$ of the length of from a Prodorsum on matchborar with 4 transmitted $I$ ( $I$ ) <i>eibining</i>
6	Endocarina very thin, length of less than 1/3 of the length of frons. Epiphar- ynx with 3 <i>als</i> . Pronotum with 26 <i>prns</i> . Meso- and metathorax with 18 <i>pds</i> ,

	and abdominal segments I–VII with approximately 24 <i>pds</i>
_	Endocarina absent. Epipharynx with 5 als. Pronotum with more than 50
	prns. Meso- and metathorax with more than 20 pds, and abdominal segments
	I-VII with approximately 40 <i>pdsL.</i> ( <i>L.</i> ) <i>pollinis</i>
7	Abdominal segments I-VII with more than 1 prs, 7 or 8 pds, and 1 or 2 ss .8
_	Abdominal segments I–VII without prs, only 5 pds, and 1 ss
8	Frons with 5 fs. Meso- and metathorax and also abdominal segments I–VII
	with 1 prs. Meso- and metathorax with 1 as and 2-3 ss9
_	Frons with 2 fs. Meso- and metathorax and also abdominal segments I-VII
	with 2 prs. Meso- and metathorax with 3 as and 1 ss L. (L.) idoneus
9	Meso- and metathorax with 5 pds, 1 as and 3 ss. Abdominal segments I-VII
	with 8 <i>pds</i> <b>10</b>
-	Meso- and metathorax with 4 pds, 1 as and 2-3 ss. Abdominal segments I-
	VII with 7 <i>pds</i> <b>11</b>
10	Head with wide, bright stripes on sides. Abdominal segments I-VII with 1
	eps. Lateral lobes of abdominal segment X with 1 tiny seta
	L. (Phyllonomeus) sp. aff. leuzeae
-	Head with barely noticeable bright stripes on sides. Abdominal segments I–VII
	with 2 eps. Lateral lobes of abdominal segment X with 4 setaeL. (L.) latus
11	Abdominal segments I–VII with 2 eps and 2 ps12
-	Abdominal segments I–VII with 1 eps and 1 ps L. (Phyllonomeus) turbinatus
12	Stipes with 1 long sts. Epipharynx with 4 als. Pronotum with 9 prns. Meso-
	and metathorax with 2 ss
	L. (Phyllonomeus) meleagris, L. (Phyllonomeus) latissimus
-	Stipes with 2 long sts. Epipharynx with 5 als. Pronotum with 11 prns. Meso-
	and metathorax with 3 ss L. (Phyllonomeus) sturnus

# Pupae

The following key is based on the pupa of *L. vulpes* described in this paper and on seven descriptions of pupae of the genus *Larinus* published previously (Zotov 2009a, 2010; Gosik and Skuhrovec 2011).

1	Pronotum with 25 or even more setae. Sas without special protuberances2
_	Pronotum with fewer than 20 setae. Sas placed on thorn-like protuberances 5
2	Setae on pronotum form a regular pattern, which consists of lines and groups
	of setae (counting of setae possible)
_	Pronotum densely covered by very long setae (counting of setae impossible)

3	Cuticle around setae not more pigmented than rest of pronotum. Rostrum
	with 3 or 4 <i>pas</i> and 3 or more <i>rs</i>
_	Cuticle around setae dark-pigmented, visible spots formed. Rostrum with 3
	pas and only 1 rs
4	Head with 1 sos; vs absent. Rostrum with 5 pas and 3 rs
	L. (L.) inaequalicolllis
_	Head with 3 sos; vs present. Rostrum with 4 pas and 8 rs
5	Body rather elongated. Urogomphi distinct
_	Body rather stout. Urogomphi very short, almost not visible7
6	Body length over 10 mm. Sas on head as horns. Urogomphi with dark, scle-
	rotised apex L. (Phyllonomeus) sturnus
_	Body length under 8 mm. Sas on head, no protuberance shaped as horns.
	Urogomphi without sclerotised apex
7	Head with 2 os. Rostrum with 3 pas. Pronotum with 3 pls L. (L.) idoneus
_	Head with 1 os. Rostrum with 4 pas. Pronotum with 4 pls
	L. (Larinomesius) obtusus

## Biology and ecology of Larinus vulpes

**Habitats.** *Larinus vulpes* occurred in the primary and degraded steppe lands, slopes, limestone and chalk cliffs of low mountains, forest edges, man-made treelines, roadsides and other ruderal plots. This weevil preferred open, sunny areas. In Iran, the weevil was recorded as high as 2580 m a. s. l. in the mountains (Gültekin and Podlussány 2012).

Adult behaviour. Adults feed on the upper surface of the leaf. As feeding was initiated, an adult raised and strongly lowered its head onto the leaf surface, which was followed by some motions of the mandibles and repeated "peck-like" motions by its rostrum. Apparently, the motion created additional pressure and helped to break through cuticle and epidermis covered with woolly hairs. Following this behaviour, an imago gnawed on mesophyll tissue, moving the head away from itself, and at one feeding, a weevil could gnaw out an irregularly shaped piece of leaf (approximately  $2 \times 8$  mm). The translucent cuticle of the leaf downside covered with dense woolly hairs remained intact (Fig. 22). During feeding, some short pauses by the weevil were observed. After eating, the weevil cleaned the apex of its rostrum using the apexes of both tibias. Weevils moved from one flower head to another by walking; they flew very reluctantly.

**Host plant.** Both adults and larvae were recorded feeding exclusively on *Echinops ruthenicus* and *E. sphaerocephalus*. We never observed *L. vulpes* on other plant species. According to Zwölfer (1985) and Nicolas (1895), weevils feed on *Echinops microcephalus* Sibth. and Sm. and *E. ruthenicus* (as *E. ritro* L.). In this case, *L. vulpes* is oligophagous (or monophagous *sensu* Jolivet 1992). Imagines fed on the leaves and on the apexes of the stems, and larvae gnawed tissues in the flower head. Hoffmann (1954) noted that *L. vulpes* were often recorded on *Cirsium ferox*, but whether this plant was a host for the weevil was not resolved.



Figures 21-28. Habitat, life cycle, and immature stages of *Larinus vulpes*. 21 Habitat with flowering *Echinops ruthenicus* (Cyrilivska Spit) 22 Adult and the damaged leaf of *Echinops ruthenicus* by weevil 23 When larva begins its development, upper parts of the flower head die and are visible as a light brown spot 24 Pupa cell results in deforming of flower head 25 Larva and pupa in the same inflorescence 26 Two larvae in the same flower head, right larva is younger and dead 27 Pupa in the inflorescence 28 Mature larva in a chamber outside a flower head. All photos SV Volovnik.

Life cycle. In Ukraine, adults were recorded from the end of April (usually from the end of May) onwards. The primary peak in the population of adults was reached at the end of June and then decreased. Active imagines from the new generation were observed from the beginning of August to early September. The phenology of the weevil is closely synchronized with the phenology of its host plant (Volovnik 1996). In spring, the weevils began to feed first on the rosettes of host plants and then gnawed the young stems and leaves (Fig. 22). *Larinus vulpes* is a univoltine species. Mating and oviposition occurred from the second part of June to the middle of July. The common scenario of oviposition and subsequent development from egg to adult were the typical for the genus *Larinus* (Volovnik 2016).

Female *L. vulpes* preferred to lay eggs in the larger flower heads on the side stems (Volovnik 2016). In this case, the female makes a hole in the flower head. Freshly laid, eggs were oval, milky white, glossy, 1.3–1.6 mm long and 0.7–1.2 mm wide. The eggs were laid solitarily in the deepening in the receptacle, gnawed by the female, or very close to the receptacle (maybe, the rostrum of some weevils was too short for access). The process of oviposition has been described in detail previously (Volovnik 1996). Eggs were found in inflorescences from the end of June to the end of July. Very occasionally, dry fragments of the egg and also a small larva were found in the same flower head. In other instances, the fragments of eggs were likely destroyed by parasites or abiotic factors (i.e., desiccation).

Later, after hatching of larva, the oviposition site became a visible, brownish tiny spot (Fig. 23). Apparently, this spot was a result of damage to the flower head by the larva. Larvae nibbled the receptacle of the flower head and the bases of primary capitula, with the damaged capitula fixed with a sticky liquid that flowed out of receptacle, resulting in a deformed flower head (Fig. 24). The mature larva colouration was peach-orange (Figs 26, 28) and that of the young pupa yellow-orange (Figs 25, 27). Fabre (1922) wrote that he found "six and more" larvae in the same inflorescence and that three larvae in the same flower head "frequently happens". Our data differed greatly from the observations of Fabre (see Table 3). Apparently, two larvae could finish full development in a medium-sized flower head (Fig. 26). Larvae were recorded from the end of June to the end of August. Larval development has been described and discussed in detail by Fabre (1922). According to direct observations by Fabre, larvae feed primarily on the sap of the receptacle. Mature larvae build a pupation cell ( $4-5 \times 7-10 \text{ mm}$ ) (Figs 29–31), with the walls significantly stronger than those of the larval cell (Figs 32–33).

After emergence, adults remained in the dry inflorescence for 5–7 days until fully sclerotised (Fig. 37), with an upper surface that was usually densely covered with a rust, pollen-like flush (Figs 34–36). Adults hibernated outside host plants, most likely in the top layer of soil or among dry plant debris.

**Preimaginal development outside of flower heads.** In the second part of the summer in 2015, the second author found seven chambers on the side of the stem of *Echinops ruthenicus*. All were located 1–2 cm below the inflorescence. Two of the



**Figures 29–37.** Hatching, pupal cells, and adults of *Larinus vulpes.* **29** Dead inflorescence with pupal cell inside **30** Pupation cell and dry fragments removed from outside of the cell **31** Pupation cell with a fresh, not fully coloured adult **32, 33** Pupation cell at the beginning (left) and after finishing construction. The inner layer of the finished wall is hard and glanced **34** Adult in pupation cell **35** Adult leaving the pupa cell **36** Exit hole of adult of new generation **37** Fresh adult in pupa cell. All photos: SV Volovnik.

seven capsules were located on the same stem and touched one another. The largest chamber was approximately  $0.7 \times 1.25$  cm. The walls of all chambers were solid but rather brittle and easily crushed by the fingers. The material of the walls had no taste.

**Table 3.** Abundance of *Larinus vulpes* larvae in flower heads of *Echinops sphaerocephalus* (Khomutovskyi Step Reserve, 30–31.07.1982; n=77).

Count of larvae in one flower head	0	1	2	3	4	5
Count of the inflorescences with specific count of larvae	13	58	1	4	-	1

The external surface of the capsule wall was brown to black, rough, earthy coloured, lumpy and mat; with incrustations of elements characteristic for *Echinops*, i.e., wool-like coating of the stems, small leaves with spines and tips of spines (Figs 28, 38–39).

The inner surface of the wall was denser than that of the external item and was bright red-brown with yellowish spots and veins, glossy, and 0.8–1.0 mm thick. The inner surface was also smooth and appeared varnished and polished, similar to inner surface of the wall of the usual pupa chamber of *L. vulpes*, but without any incrustations. The contents of the chambers included the following: 2 chambers with dead imagines, 1 chamber with a living imago, one with only the head capsule of a larva, one chamber with a mature larva alive (Fig. 34), one with only pieces of a pupa and one with only parts of exuviae or cuticle remains. No excrement was observed but clearly the larvae incorporated their faeces into the wall during construction.

**Biotic interactions.** A parasitic wasp, *Bracon urinator* (Fabricius, 1898) (Hymenoptera: Braconidae), was reared from the pupae of *L. vulpes*. Sometimes the capitulum dried out, cracking the walls of larval or pupal cells, and ants, *Formica imitans* Ruzsky, 1902, destroyed the larvae and pupae of the weevil. Feeding on the inflorescences of *Echinops*, the rose beetle, *Protaetia metallica* (Herbst, 1782) (Coleoptera: Scarabaeidae), harmed larvae of *L. vulpes* developing in the same inflorescence. We found cells with dead weevil larvae together and simultaneously with living larvae or adults of carnivorous bugs, *Orius* sp. (Heteroptera: Anthocoridae) (Volovnik 1994).

Larinus vulpes and its host-plants. Larvae of L. vulpes living in flower heads of globe thistle consumed the ovaries and unripe seeds. The prevalence of weevils in globe thistles sometimes reached 33% of inflorescences (SV, unpublished data). Some flower heads lost all their seeds, although in an overall view, the loss of some seeds may be expected. Globe thistles have a special morphological structure that separates the compound flower head into small, single primary capitula with one seed each (Mulkijanian 1951). The durable walls of the pupal cell were very strong, which might prevent the possible separation of each capitulum. Furthermore, Mulkijanian (1951) also recorded stems breaking in the wind, primarily when larvae damaged the receptacle. Most likely, the larvae of Larinus vulpes or some similar species of Larinus damaged the receptacles. Clearly, the specialisation of globe thistles to a specific environment, such as rocky slopes, also constrained the weevils, being adapted to that same environment. The chemical composition of *Echinops* is also somewhat specific, and some species in this genus have insecticidal, nematicidal, antifungal, bactericidal and also antiviral properities (i.e. Fokialakis et al. 2006; Zhanget al. 2009; Abdelnabby and Abdelrahman 2012; Tekwu et al. 2012; Gemechu et al. 2013; Liu et al. 2013). Therefore, the inhabitants of Echinops flower heads might also be protected from some parasites and



**Figures 38–40.** Pupal chamber outside inflorescence and habitat in 2006. **38, 39** Pupal chamber in the axil of a leaf **40** Habitat in dry, hot season in 2016; flower heads were small and died early. All photos SV Volovnik.

predators because of the chemical compounds in the plant. This aspect requires further experimental investigations.

The absence of preimaginal development outside of flower heads is a characteristic of not only L. vulpes but also for the genus Larinus in general. Only six Larinus species construct analogous capsules (pupal chambers) with a sweet taste (known as "trehala") on plant stems, namely: L. capsulatus Gültekin, 2008; L. hefenborgi Boheman, 1845; L. nidificans Guibourt, 1858; L. ruficollis Petri, 1907 (Gültekin 2008); L. trehalanus Gültekin and Shahreyary-Nejad, 2015 (Gültekin and Shahreyary-Nejad 2015); and L. onopordi (Fabricius, 1787) (Khnzoryan 1951). According to Zwölfer et al. (1971), larvae of L. vulpes sometimes construct capsules on the stems, but the authors did not provide further details for this observation. Fabre (1922) once recorded the larval chamber built in the axil of the leaf of Echinops sp. Of note, L. vulpes and the trehala building species develop on plants of the same genus, *Echinops*, and perhaps the construction activity outside of flower heads might be to avoid properties of the thick adhesive sap of these plants. Of the seven Larinus species that develop on Echinops spp., all construct outer pupal chambers (L. vulpes included). According to Iljin (1953), E. ruthenicus and other globe thistles contain the type of rubber that turns into a dense substance when exposed to air. Closely related to Larinus weevils, Afrolarinus moestus (Chevrolat, 1882) also develops on *Echinops*, but only in the flower heads (Gültekin 2013).

Two important moments in the life cycle of *Larinus vulpes* remain unclear: (1) why do eggs appear outside flower heads? (2) can imagines open their chambers on the stems and emerge at the proper time? According to the assumptions of Fabre (1922), an egg could fall from the inflorescence after being laid, or the female could lay an egg in an unusual place "either by inadvertence or by intention". This event is unlikely because females preferred to lay eggs in the top of flower heads. Occasionally, females laid an egg on a lateral part but never on the bottom of flower head. Thus, falling out into the axil of a leaf is doubtful. Unlikely also was "inadvertence", because this

phenomenon is not that rare. On a relatively small patch of grassland (approximately 150 m<sup>2</sup>), seven chambers were found. Therefore, females laid eggs in an unusual place, for enigmatic reasons. In previous years, Volovnik conducted direct observations of *L. vulpes* in the field but never saw its capsules on plant stems. In the region of investigation, the growing season for vegetation during 2016 was abnormally dry and hot. Most of the globe thistles were short with small inflorescences and died earlier than usual (Fig. 40). It is possible that such an extreme situation resulted in an acute shortage of suitable places for normal oviposition; thus, a significant portion of eggs were laid in unconventional places.

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COMMENTARY



# The importance of validated alpha taxonomy for phylogenetic and DNA barcoding studies: a comment on species identification of pygmy grasshoppers (Orthoptera, Tetrigidae)

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### Abstract

In a recently published paper on colour polymorphism in a Pygmy grasshopper from China (Zhao et al 2016) an unidentified *Paratettix* sp. was misidentified as *Tetrix bolivari*. This case highlights the need for correct species identification and provides an opportunity to recommend some aspects of Good Taxonomic Practice (GTP) in Tetrigidae to reduce the number of erroneous identifications.

### **Keywords**

Orthoptera, Tetrigidae, Barcoding, taxonomy, misidentification

## Introduction

For a number of reasons, nobody can avoid misidentifications of insect species. However, verified species identification is a backbone for many biological disciplines. Among Orthoptera, the members of the family Tetrigidae have a quite complicated taxonomic history, including long list of synonyms and large collections of unidentified specimens. In Europe, it took almost 200 years, from Linnean time to the publication of the European handbook by Harz (1975), to provide reasonable taxonomic stability. With increasing speed of taxonomic research in other regions of the world, we observe similar problems of taxonomic inflation. With respect to Tetrigidae taxonomy, researchers from China are currently particularly active, having described 795 species (compare Cigliano et al. 2017). While we applaud the effort of our Chinese colleagues, we observe some problems with interpretation of well-known Palaearctic species.

In a recent publication in ZooKeys, Zhao et al. (2016) have shown that genetic haplotype diversity of the Barcoding locus COI is incongruent with colour polymorphism found in a Chinese pygmy grasshopper (Tetrigidae). While the molecular data by Zhao et al. (2016) of 57 individuals supports the hypothesis that the colour morphs studied by them all belong to a single species, we have evidence against the identification of the species. A great benefit of the publication by Zhao et al. (2016) is that it not only includes COI barcodes, but also presents photos of a number of specimens. This allows us to reflect on the identity of the species under study. We first provide some evidence that the species depicted in the publication of *Zhao* et al. (2016) is misidentified. For clarification, we outline the known distribution of *Tetrix bolivari*, which is unlikely to occur in China and recommend some aspects of Good Taxonomic Practice (GTP) in Tetrigidae.

The photos of several individuals presented by Zhao et al. (2016) in their figure 3 show individuals belonging to the genus Paratettix Bolívar, 1887. This is evident from the traits visible (see for example Devriese (1999) and Tumbrinck (2014) for detailed descriptions of useful morphological characters in Tetrigidae). (1) The Median Carina (CM) of the pronotum terminates before reaching the distal pronotal end, (2) the interocular distance is as broad as the eye-span, (3) the fastigium does not protrude in front of the eyes and (4) the long antennae segments slightly broaden towards their terminal end. The first character is very distinctive and the single-most important character aligning the specimens to the genus *Paratettix*, as outlined in the detailed keys for Europe (Harz 1975) and sub-Saharan Africa (Günther 1979). Interestingly, these characters are also referred to in a key presented by three Chinese authors (Deng et al. 2007) in which the senior author of Zhao et al. (2016) is involved. Using their key (Deng et al. 2007, p. 422) to identify the specimens shown in their figure 3 we end up with the genus Paratettix. This genus has a global distribution and occurs mainly in the tropics, including many extremely common species in open tropical lowland habitats (e.g. Günther 1979). The quality of the photos does not allow a proper identification to the species level. Without material at hand, our best guess is a species identical with or close to Paratettix obesus Bolívar, 1887 (compare Tumbrinck 2015).

This is a Neighbour-joining tree without distance corrections.



**Figure 1.** Neighbour-joining tree based upon the COI data, illustrating the clustering of the Chinese samples by Zhao et al. (2016) together with *Paratettix* and not *Tetrix* species, data extracted from the Barcoding of life project.

A second line of evidence comes from genetic data. The authors sequenced the gene COI (which is commonly used as Barcoding gene) to examine a proposed correlation between colour morphs and haplotypes. As sequences of COI are available for a wide range of taxa, we deployed a quick analysis of the mitochondrial COI data from the Barcoding of life database (BOLD version 3; Ratnasingham and Hebert 2007) in conjunction with some of the Chinese data by Zhao et al. (2016). We chose gene sequences from a selected subset of species belonging either to the genus Tetrix or Paratettix, together with three sequences from the study of Zhao et al. (2016). Detailed information on the determination and sequencing history can be found in the BOLD system (www.barcodinglife.org). A neighbour-joining tree was produced running the software MUSCLE (Edgar 2004) online on the EBI Server (www.ebi.ac.uk/Tools/ msa/muscle/). The resulting tree (Figure 1) is in line with the morphological evidence, as the Chinese individuals build a monophyletic cluster with Paratettix meridionalis (Rambur, [1838]) and Paratettix pullus Bolívar, 1887, rather than to group with selected members of the genus Tetrix Latreille, [1802]. It should be noted that the gene COI contains a reasonable phylogenetic signal for a smaller number of species of moderate separation time, and was shown to be quite useful for Barcoding purposes in Orthoptera as well (Hawlitschek et al. 2016).

We can add a biogeographic argument, why we question the occurrence of *Te-trix bolivari* in China in general. This species has been studied in detail for ecological (Kočárek et al. 2011) and faunistic reasons. *Tetrix bolivari* is distributed from southern Europe (Devriese 1996), the Middle East (Lehmann and Monnerat 2014) to Central Asia, where it is found in Turkmenistan (Podgornaya 1983; Storozhenko et al. 1994) and Tadzhikistan (Harz 1979). The tropical and subtropical regions of China have a very distinct fauna, making the occurrence of a Mediterranean-Central-Asian species unlikely. We therefore encourage our Chinese colleagues to re-examine their material. Interestingly, when adding 12S rDNA sequence data published independently under the name *Tetrix bolivari* from China (Chen and Jiang 2004) to our own alignment of Palaearctic Tetrigidae (Hochkirch et al., in prep.), they cluster with the widespread

East-Asian species *Tetrix japonica* (Bolívar, 1887). In summary, both testable records of *Tetrix bolivari* from China appear to belong to other species.

We summarize some recommendations for a Good Taxonomic Practice (GTP) to reduce the taxonomic instability in Tetrigidae:

A fundamental aspect of morphological work on Tetrigidae should be a common terminology of the body parts. A large set of morphological traits are depicted and named by Devriese (1996, 1999) and recently by Tumbrinck (2014). We strongly recommend using this terminology for an easier comparison of characters.

An astonishing but common mistake in Tetrigidae identification is the misinterpretation of nymphs for adults. Tetrigidae can show interesting phenologies, including cohort splitting between years. This phenomenon is quite well studied in European species (e.g. Schulte 2003, Pushkar 2009) such phenomena can also be expected in the tropics, less so in lowlands but especially on mountains. Especially in species with unknown adult phenology the collection of individuals from the same locality over the season might reduce this problem.

Along with studies on the morphology we need a better understanding of phenotypic variation between individuals. Such phenotypic data of adults have been shown to be helpful to reduce the instability in hard to distinguish *Paratettix* species from Africa (Günther 1979).

Tetrigidae have a tendency to exhibit extensive morphological polymorphisms, such as different wing morphs. Wing dimorphism is best studied and understood in *Tetrix subulata* (e.g. Steenman et al. 2013, 2015), and a frequent phenomenon in European (summarized in Devriese 1996) and African species (Günther 1979). The easiest way to test for wing dimorphism in Tetrigidae is obviously to study larger sample sizes and obtain data on body and hind wing dimensions. No such data seem to exist to date for Asian species, but we would not be surprised if some of the newly described species from China would turn out to represent wing morphs of previously described species.

A further striking polymorphism in Tetrigidae is found in body colouration. Colour polymorphism is widespread within Tetrigidae species, even within the same populations (e.g. Nabours 1930; Hochkirch et al. 2007, 2008), see also the current study by Zhao et al. (2016). From the beginning of Tetrigidae taxonomy, colour variation has caused taxonomic uncertainties until it was recognized as an intraspecific phenomenon, see e.g. the list of synonyms for *Tetrix subulata* in Harz (1975). We have to assume that a number of newly described Tetrigidae species from China merely represent colour morphs rather than new species.

Until improved determination keys (using multiple characters being complemented by drawings or photographs) along with verified molecular data are available, showing photographs of the studied specimens is a helpful practice. It was of great benefit that the article by Zhao et al. (2016) provided photos of specimens in combination with gene sequences, which allowed us to reflect on the species identity.

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