ZooKeys 313: 1–8 (2013)
doi: 10.3897/zookeys.313.5106
www.zookeys.org

RESEARCH ARTICLE

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A review of the Oriental genus *Eudeferunda* Chen, Yang & Wilson (Hemiptera, Fulgoromorpha, Achilidae) with description of one new species from Hainan, China

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Academic editor: *Mike Wilson* | Received 11 March 2013 | Accepted 27 June 2013 | Published 27 June 2013

http://zoobank.org/?lsid=urn:lsid:zoobank.org:pub:870A72A2-6238-4765-AE29-8D6DCA80EF9D

Citation: Long J-K, Yang L, Chen X-S (2013) A review of the Oriental genus *Eudeferunda* Chen, Yang & Wilson (Hemiptera, Fulgoromorpha, Achilidae) with description of one new species from Hainan, China. ZooKeys 313: 1–8. doi: 10.3897/zookeys.313.5106

Abstract

A new species of *Eudeferunda* Chen, Yang & Wilson, 1989 (Hemiptera: Fulgoromorpha: Achilidae: Plectoderini), *E. alatea* Long & Chen **sp. n.** from Hainan, China, is described and illustrated. The generic characters are modified, including the addition of male genitalia characters. A key to the species of the genus is provided.

Keywords

Achilid, Fulgoroidea, Oriental region, planthopper, taxonomy

Introduction

The achilid genus *Eudeferunda* (Hemiptera: Fulgoromorpha: Achilidae: Plectoderini) was established by Chen et al. (1989), and with *E. lenita* Chen, Yang & Wilson, 1989 as its type species. To date, this genus contains only the type species. Here, we described and illustrated a new species: *Eudeferunda alatea* Long & Chen sp. n. from South China. A key to separate these two species of *Eudeferunda* is provided.

Materials and methods

Specimens were collected by sweeping. Dry specimens were used for the description and illustration. External morphology was observed under a stereoscopic microscope and characters were measured with an ocular micrometer. The genital segments of the examined specimens were macerated in 10% KOH and drawn from preparations in glycerin jelly using a Leica MZ 12.5 stereomicroscope. Illustrations were scanned with Canon CanoScan LiDE 200 and imported into Adobe Photoshop CS3 for labeling and plate composition. Terminology for morphology following by Szwedo and Żyła (2009) and Bourgoin and Huang (1990). The type material of specimens are deposited in the Institute of Entomology, Guizhou University, Guiyang, Guizhou Province, China (IEGU).

Taxonomy

Eudeferunda Chen, Yang & Wilson, 1989

http://species-id.net/wiki/Eudeferunda
Figs 1–28

Eudeferunda Chen et al. 1989: 16.

Type species. *Eudeferunda lenita* Chen, Yang & Wilson, 1989, by original designation.

Diagnosis. Small size. Head with eyes narrower than pronotum (0.7–0.8:1) (Figs 7, 19). Vertex produced before eyes about 0.4 times of its length, longer in middle line than wide at base (1.4:1), disk distinctly depressed, median carina basal half prominent, anterior half obsolete, anterior margin acutely rounded convex, lateral margins carinate, straight, diverging posteriorly, posterior margin in middle angulately convex (Figs 7, 19). Frons longer in middle line than widest part (1.25–1.4:1), basal margin subtruncate or truncate, about quarter length of widest part, lateral margins strongly foliate basally, slightly convex laterally, thence incurved into suture (Figs 8, 20). Postclypeus shorter than frons in middle line (0.4–0.6:1) (Figs 8, 20). Eyes incised beneath, not or slightly overlapping pronotum (Figs 7, 9, 19, 21). Ocelli detached from eyes. Antennae with pedicel subglobose. Pronotum moderately short, as long behind eyes as in middle line, disc with median carina shorter than lateral carinae (0.5:1) (Figs 7, 19). Mesonotum tricarinate, longer than vertex and pronotum together, lateral carinae slightly diverging posteriorly, middle carina apically obsolete (Figs 7, 19). Forewing 2.5–2.9 times as long as broad, CuA_1 not convex strongly, Sc+R forking level of CuA_1 fork, both slightly distally union of PCu with A_1, clavus terminating at midway of forewing (Figs 10, 22). Post-tibia with a single lateral spine at basal 2/5, metatibio-tarsal formula of hind leg 8–7–6 (Figs 5–6). Anal segment relatively short, in dorsal view apical margin concave in middle (Figs 12, 23). Pygofer in lateral view distinctly longer ventrally than dorsally, anterior margin concave, posterior margin convex and produced a curved process directing downward (Figs 13, 24); in ventral view medioventral processes paired, median cleft deep (Figs 14, 25). Dorsal margin of gonostyle with a large triangular process laterad, thence laterad convex near base (Figs 15–16, 26). Male genitalia with phallobase submembraneous, tube-like, phallobase with dorsal and ventral lobes; ventral lobe of phallobase in ventral view cleft at apical margin in middle, each lateral side with a pointed processes, bilateral areas with thorns; in dorsal view, dorsal lobe of phallobase with apical margin broadly incised in middle; phallic appendages longer than phallobase about 3:1, gradually narrowing apically (Figs 17–18, 27–28).

Distribution. Oriental region.

Key to species of *Eudeferunda* Chen, Yang & Wilson (male)

1 Medioventral processes of pygofer digitate, gradually narrowing apically (Fig. 25); anal segment in dorsal view with base margin truncate and blunt laterally (Fig. 23); ventral lobe of phallobase with a couple of lateral processes near apex (Figs 27–28) .... ***E. lenita***

– Medioventral processes of pygofer with apex distinctly attenuate and bent towards outboard (Fig. 14); anal segment in dorsal view with basal margin slightly sinuate and angular laterally (Fig. 12); ventral lobe of phallobase with a couple of lateral processes at base 1/3 (Fig. 17).................. ***E. alatea* sp. n.**

Eudeferunda alatea Long & Chen sp. n.

http://zoobank.org/?lsid=urn:lsid:zoobank.org:act:7BB7C6C5-3FAA-4B0B-BCC2-AF560530F217
http://species-id.net/wiki/Eudeferunda_alatea
Figs 1–18

Type material. Holotype: 1♂, **China:** Hainan, Ledong, Jianfengling National Natural Reserve (18°41'N, 108°36'E), 16 Jan. 2011, J.-K Long. Paratypes: 1♂, 3♀♀, Hainan, Ledong, Jianfengling National Natural Reserve (18°41'N, 108°36'E), 13–16

Figures 1–6. *Eudeferunda alatea* Long & Chen sp. n. **1** Male habitus, dorsal view **2** Male habitus, lateral view **3** Female habitus, dorsal view **4** Female habitus, lateral view **5** Hind tibia and tarsus **6** Apex of hind leg.

Jan. 2011, J.-K Long; 1♀, Hainan, Ledong, Jianfengling National Natural Reserve (18°41'N, 108°36'E), 14 Jan. 2011, W.-B. Zheng (IEGU).

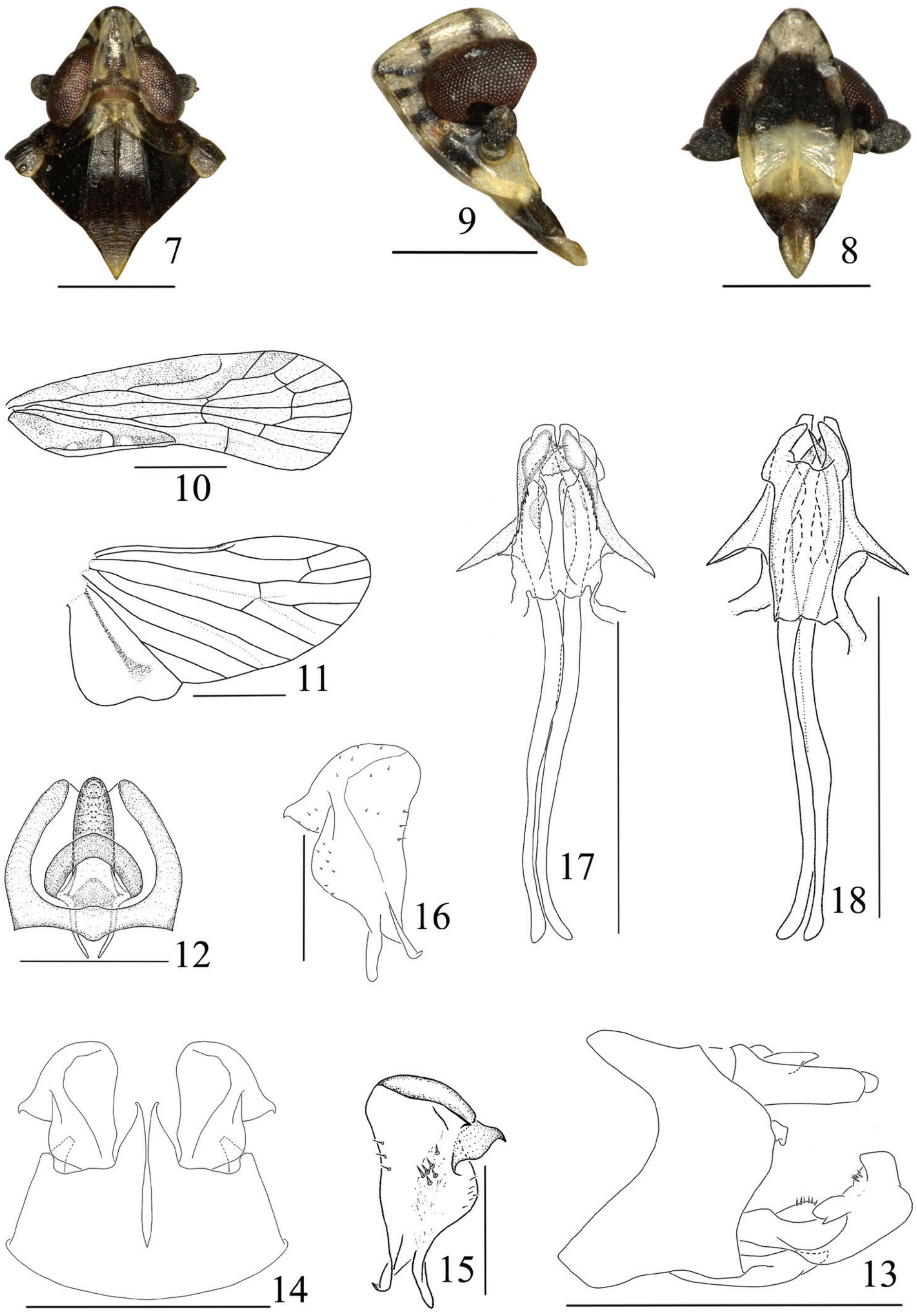
Etymology. The species name is derived from the Latin word "*alate*", indicating the phallobase with a pointedly alate process at base 1/3 of each lateral side.

Description. Body length (from apex of vertex to tip of forewings): male 3.75–3.80 mm (N=2), female 4.50–4.75 mm (N=4); forewing length: male 2.95–3.05 mm (N=2), female 3.55–3.75 mm (N=4).

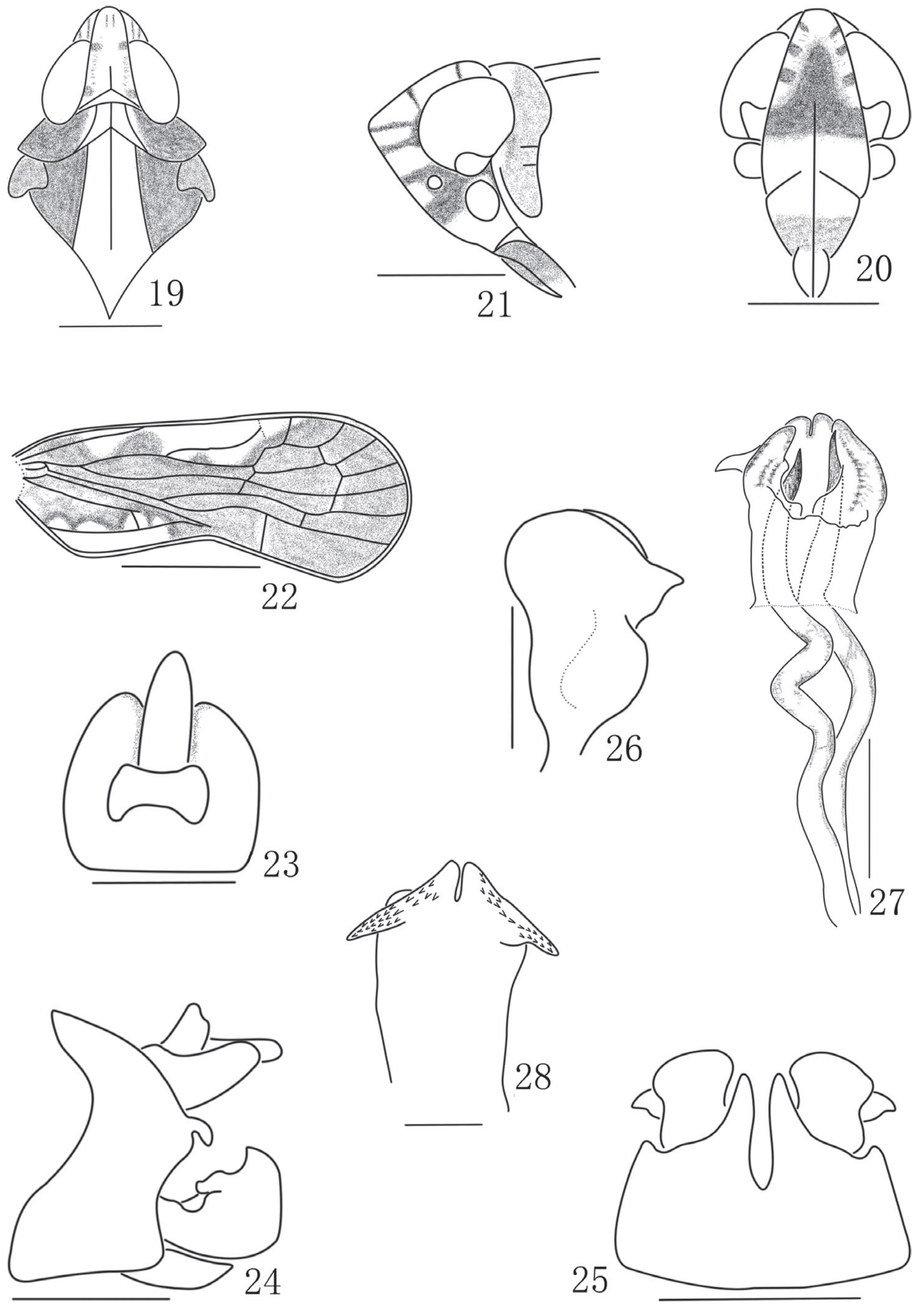
Coloration. Ivory white to black brown (Figs 1–9). Vertex ivory white, with black brown at base, longitudinal black brown stripe, gradually narrowing forward (except middle carina light brown) in middle, anterior and lateral margins brown, lateral margin with two transverse black brown stripes respectively at the level of anterior margin of eyes and base (Fig. 7). Frons with disk in middle black brown, basal and apical fourth ivory white, lateral margins with three big and one small fuscous spots (Fig. 8). Postclypeus with basal half ivory-white and apical half fuscous; preclypeus light brown except the base fuscous (Fig. 8). Genae ivory white, with three fuscous transverse stripes before eyes, two dorsad, area between ocellus and antenna fuscous, genae coloration at level of clypeus same the face (Fig. 9). Eyes reddish brown, ocelli pale reddish-brown (Fig. 9). Antennae fuscous (Figs 7–9). Pronotum dark brown, area between out side of two lateral carinae ivory white; mesonotum dark brown, with yellowish brown at apical angle (Fig. 7). Tegula dark brown, with dorsally lateral margin yellowish brown (Fig. 7). Forewing yellowish brown to dark yellowish brown, costal margin with three ivory white spots, clavus with three ivory white spots inside A_2 and one longitudinal ivory white stripe between A_2 and posterior margin (Fig. 10). Hindwing pale brown, veins pale brown. Legs pale brown (Figs 2, 4, 5–6). Abdomen dark brown.

Head and thorax. Head with eyes narrower than pronotum (0.81:1) (Fig. 7). Vertex long, broad across base than anterior (5.00:1), produced before eyes about 0.36 times of its length, width of vertex measured at base of middle line 0.66 times length along middle, longer in middle line than wide at base (1.41:1). Frons longer in middle line than widest part (1.25:1), basal margin subtruncate, middle carina gradually weakening basally (Fig. 8). Postclypeus shorter than frons in middle line (0.42:1) (Fig. 8). Rostum with apical segment longer than subapical one (1.47:1). Pronotum shorter than vertex in middle line (0.31:1). Mesonotum longer than pronotum in middle line (6.8:1), than vertex and pronotum together (1.61:1) (Fig. 7). Disc of tegula with several small longitudinal ridges (Fig. 7). Forewing 2.93 times as long as broad (Fig. 10). Hindwing 1.78 times as long as broad (Fig. 11).

Male genitalia. Anal segment in dorsal view slightly shorter than broad (0.90: 1), basal margin slightly sinuate and angular laterally, apical margin concave (Fig. 12). Medioventral processes of pygofer paired, with apex distinctly attenuate and bent towards outboard, median cleft deep (Figs 13–14). Dorsal margin of gonostyle with a small dentiform process dorsad and a large triangular process laterad, thence laterad convex near base, apical margin broadly convex (Figs 15–16). Male genitalia with phallobase in ventral view (Fig. 17), ventral lobe cleft at apical margin in middle, bilateral areas hump-shaped and protuberated subapically, a pointedly alate process at base 1/3 of each lateral side, with its base of inside margin denticulated and apically extended; in dorsal view (Fig. 18), dorsal lobe with apical margin broadly incised in middle, bilateral margin ox-horn like produced. Phallic appendages longer than phallobase (3:1), gradually narrowing apically (Figs 17–18).



Figures 7–18. *Eudeferunda alatea* Long & Chen sp. n. **7** Head and thorax, dorsal view **8** Frons and clypeus **9** Head, lateral view **10** Forewing **11** Hindwing **12** Anal segment, dorsal view **13** Male genitalia, lateral view **14** Male genitalia, ventral view **15** Left gonostyle, dorsal view **16** Left gonostyle, ventral view **17** Aedeagus, ventral view **18** Aedeagus, dorsal view. Scale bars: = 1 mm (Figs **10–11**); 0.5 mm (Figs **7–9, 13–14, 17–18**); 0.2mm (Figs **12, 15–16**).



Figures 19–28. *Eudeferunda lenita* Chen, Yang & Wilson, 1989. **19** Head and thorax, dorsal view **20** Frons and clypeus **21** Head, lateral view **22** Forewing **23** Anal segment, dorsal view **24** Male genitalia, lateral view **25** Male genitalia, ventral view **26** Left gonostyle **27** Aedeagus, dorsal view **28** Phallobase, ventral view. Scale bars: = 1 mm (Figs **10–11**); 0.5 mm (Figs **7–9, 13–14, 17–18**); 0.2mm (Figs **12, 15–16**) (all after Chen et al. 1989).

Distribution. South China (Hainan).

Remarks. This new species differs from *Eudeferunda lenita* Chen, Yang & Wilson, 1989 by: mesonotum dark brown, only with yellowish brown at apical angle (between lateral carinae white in *lenita*); forewing with three ivory white spots along costal margin (ivory white area among costal cell, Sc+R, and stigma in *lenita*); disk of tegula with several small longitudinal ridges (without in *lenita*); male pygofer of medioventral processes with apex distinctly attenuate and bent towards outboard (digitate and gradually narrowing apically in *lenita*); anal segment in dorsal view with basal margin slightly sinuate and angular laterally (base margin truncate and blunt laterally in *lenita*); ventral lobe of phallobase with a couple of lateral processes at base 1/3 (near apex in *lenita*).

Eudeferunda lenita Chen, Yang & Wilson, 1989

http://species-id.net/wiki/Eudeferunda_lenita
Figs 19–28

Eudeferunda lenita Chen et al. 1989: 16.

Distribution. South China: Henchun (22°00'N, 120°44'E), Pingtung City, Taiwan.

Material examined. No specimen has been collected by the authors.

Acknowledgements

We are grateful to Wei-Bing Zheng (IEGU) for collecting valuable specimen. This work was supported by the National Natural Science Foundation of China (No. 31060290, 31093430, 31160163) and the International Science and Technology Cooperation Program of Guizhou (20107005).

References

Bourgoin T, Huang J (1990) Morphologie comparée des genitalia mâles des Trypetimorphini et remarques phylogénétiques (Hemiptera: Fulgoromorpha: Tropiduchidae). Ann. Soc. Entomol. Fr. 26: 555–564.

Chen CL, Yang CT, Wilson MR (1989) Achilidae of Taiwan (Homoptera: Fulgoroidea). Taiwan Museum Special Publication Series, 8: 1–64.

Fennah RG (1950) A generic revision of the Achilidae (Homoptera: Fulgoroidea) with descriptions of new species. Bulletin of the British Museum (Natural History) Entomology, 1(1): 1–170.

Szwedo J, Żyła D (2009) New Fulgoridiidae genus from the Upper Jurassic Karatau deposits, Kazakhstan (Hemiptera: Fulgoromorpha: Fulgoroidea). Zootaxa 2281: 40–52

Yang CT, Chang TY (2000) The external male genitalia of Hemiptera (Homoptera – Heteroptera). Taichung, Shih Way Publication, 746 pp.

ZooKeys 313: 9–24 (2013)
doi: 10.3897/zookeys.313.5317
www.zookeys.org

RESEARCH ARTICLE

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A new species of the gamasid mite genus *Arctoseius* Thor, 1930 (Parasitiformes, Mesostigmata, Ascidae) from Russia with a key to the *multidentatus* species-group

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Academic editor: *Farid Faraji* | Received 13 April 2013 | Accepted 18 June 2013 | Published 28 June 2013

urn:lsid:zoobank.org:pub:D8278A74-2879-4B7F-8A69-EB09E70FD0FA

Citation: Makarova OL, Lindquist EE (2013) A new species of the gamasid mite genus *Arctoseius* Thor, 1930 (Parasitiformes, Mesostigmata, Ascidae) from Russia with a key to the *multidentatus* species-group. ZooKeys 313: 9–24. doi: 10.3897/zookeys.313.5317

Abstract

A new gamasid mite species belonging to the genus *Arctoseius* Thor, 1930 is described from Russia. *Arctoseius koltschaki* **sp. n.** is distributed in the plain and mountain tundras from Khibiny Mountains to Chukotka on the north and to West Sayan Mountains on the south. A diagnosis and a key for identification of species comprising the *multidentatus* species-group (*A. multidentatus* Evans, 1955; *Arctoseius wisniewskii* Gwiazdowicz & Kamczyc, 2009; *A. sexsetus* Lindquist & Makarova, 2011; *A. haarlovi* Lindquist & Makarova, 2011; and *A. koltschaki* **sp. n.**) are given.

Keywords

Gamasid mites, Arctic, *Arctoseius koltschaki* sp. n., *multidentatus* species-group, identification key

Introduction

Arctic landscapes are populated by at least 26 mite species belonging to the genus *Arctoseius* Thor, 1930, which constitutes about half of the total diversity of this genus (Makarova in press). Such species richness appears to be unique among terrestrial mite genera within the Arctic. In spite of intensive investigations in the two last decades (Petrova and Makarova 1991; Makarova and Petrova 1992; Makarova 2000a, b; Lindquist and Makarova 2011), several arctic species still remain undescribed, one of which is treated here. It belongs to the *multidentatus* species-group, for which a diagnosis and a key to species are presented.

Material and methods

In total 59 females, 23 males and 19 nymphs were found in 25 localities. Setal notation for the idiosoma follows Lindquist and Evans 1965, with some modification (Lindquist 1994). The leg and palpal chaetotaxy corresponds to Evans 1963, 1964. The poroidotaxy and adenotaxy are given according to Johnston and Moraza 1991, with small modification (Makarova 2003). The length of all shields was measured on the mid-line, the width at the broadest part, except for the sternal/sternitigenital shield, whose length was measured from the level of setae *st*1 to the posterior margin, and the width at the broadest part between levels of setae *st*2 and *st*3 (Makarova 2000a). The length of the epigynal shield was measured to include the anterior flap, the width – next to the posterior margin. The length of legs and tarsi are given excluding the ambulacrum, and also the pedicel on leg I. The subcapitulum length was measured from its anterior margin without appendages (internal malae, corniculi). The length of the chelicera excludes the basal segment, and the cheliceral digit length is based on the movable digit. Measurements were carried out in 20 females and 10 males and their limits are stated in micrometers (μm). The following ratios were used in the description (Makarova 2000a):

lD/wD	length-to-width ratio of the dorsal shield;
_J_(1–4)_min/jmax_	ratio of the length of dorsocentral setae inserted on the opisthonotal and podonotal regions, notably the shortest among *J*1–4 and the longest in the series *j*;
(_J_4–_J_4)/(_J_3–_J_3)	ratio of transverse distances between bases of opisthonotal setae *J*4 and *J*3 in pairs;
_Z_5/(_Z_5–_Z_5)	ratio of setae *Z*5 length to the distance between them;
lSt/wSt, lAn/wAn, lVA/wVA	length-to-width ratios of the sternal, anal, and ventrianal (in males) shields, respectively;
lCo/lD	ratio of the length of the corniculus and of the dorsal shield, %;
lCh/lD	ratio of the length of the chela and of the dorsal shield, %;
lCh/lCo	ratio of the length of the chela and of the corniculus;
_lExI/lD, lT_IV/_lD_	ratio of the length of leg I or tarsus IV and of the dorsal shield;
_lT_III/_wT_III	length-to-width ratio of tarsus III, width taken at basistarsus.

In the male description, the features common with the female are omitted.

Holotype and most of paratypes are deposited in Zoological Institute, Russian Academy of Sciences, Saint Petersburg (ZIRAS). Part of paratypes, 5 females, 1 male, 2 N2, from Suntar-Khayata Range, 1500 m a.s.l. (above sea level), spotty tundra (on one slide) – in Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa (CNC).

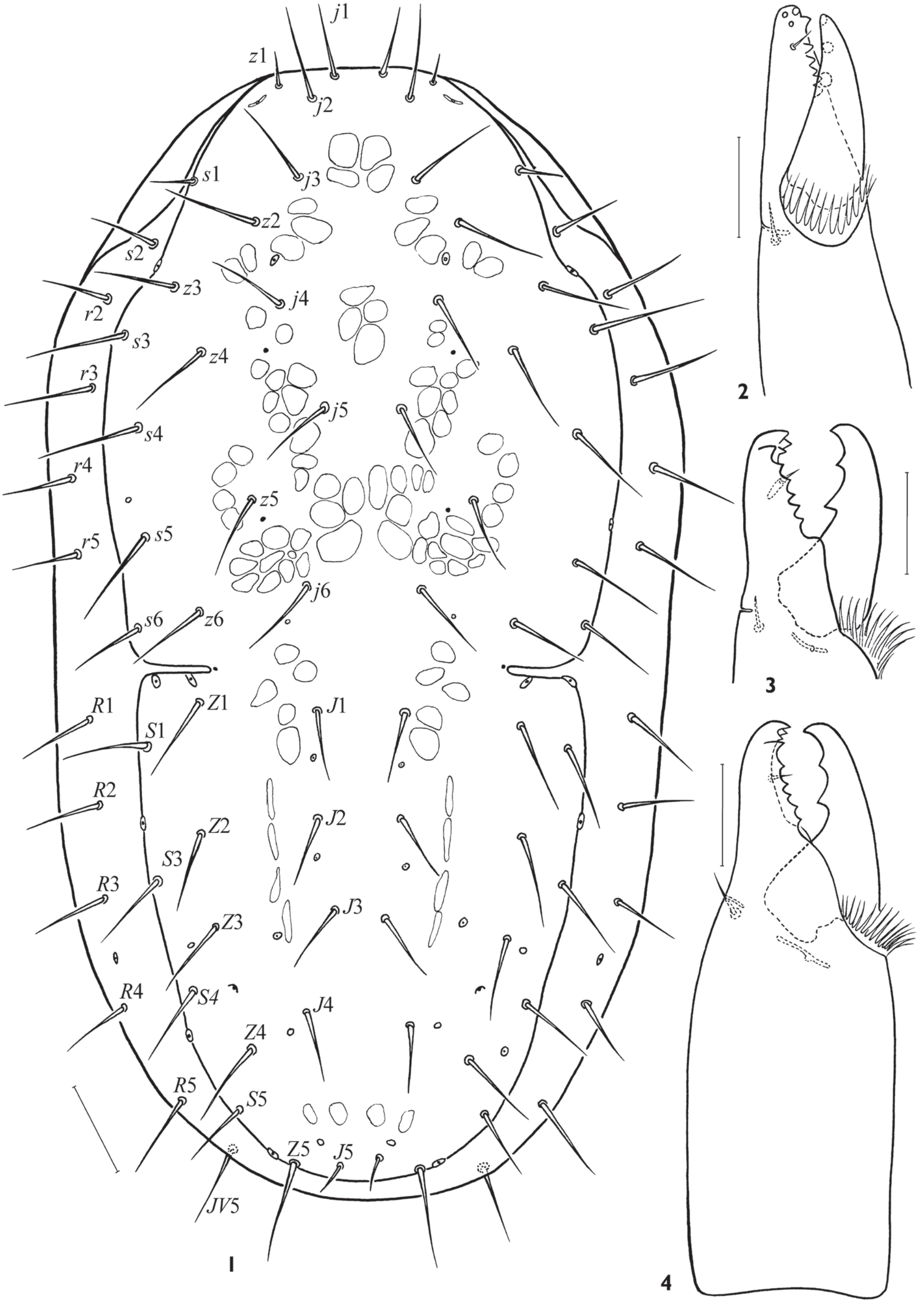
Species description

***Arctoseius koltschaki* Makarova & Lindquist sp. n.**
urn:lsid:zoobank.org:act:25C72458-8447-4610-BCA1-EC2EBC33D2A6
http://species-id.net/wiki/Arctoseius_koltschaki
Figs 1–25

Material. Holotype, female: EAST SIBERIA, YAKUTIYA, Khalerchinskaya Tundra, Chukochiya River, mosses in shrub tundra, IX 1975, leg. E.V. Gordeyeva (ZIRAS).

Paratypes: 5 females, 1 male, 2 N2, EAST SIBERIA, Suntar-Khayata Range, upper reaches of Kyubyume River, 63°13'N, 139°36'E, 1500 m a.s.l., spotty tundra, 29.VII 2002, leg. O.L. Makarova (CNC); 1 male, same district and collector, 1960 m a.s.l., snow bed near small stream, sedge turf, 1.VIII 2002; 1 female, delta of Indigirka River, Russko-Ustinskaya Protoka, hummocky *Dryas*-forb tundra, 14–16.VII 1994, leg. A.B. Babenko; NOVAYA ZEMLYA ARCHIPELAGO, 1 male, Southern Island, Pan'kova Zemlya, lower part of slope, sedge-willow-moss tundra, VIII 1995, leg. S.V. Goryachkin; 1 female, 1 male, Northern Island, Krestovaya Bay, tundra, VIII 1993, leg. V.I. Bulavintsev; 1 female, BOL'SHEZEMEL'SKAYA TUNDRA, Vorkuta Region, Sivaya Maska vicinity, tundra, 20.VII 1978, leg. N.A. Kuznetsova; 3 females, 1 male, KOLA PENINSULA, Khibiny Mountains, Eastern Petrelius Pass, *Dryas*-sedge-moss tundra, 28.VI 2006, leg. A.B. Babenko (ZIRAS).

Other material: NORTH-EAST ASIA, 5 females, 1 male, Chukotka, Daurkin Peninsula, lower reaches of Chegitun River, forb-sedge-moss tundra, 30.VIII 1996, leg. M.V. Berezin; 5 females, 3 males, Magadan Region, upper reaches of Kolyma River, Peak Aborigen vicinity, *Pinus pumila* litter, 24.VIII 2006, leg. A.V. Alfimov; 1 female, Olskoye Plateau, upper reaches of Ola River, 60°39'N, 151°16'E, 1220 m a.s.l., forb meadow, 11.VIII 2011, O.L. Makarova; EAST SIBERIA, delta of Kolyma River, Pokhodskaya Edoma, 1 female, litter in lower part of slope; 1 female, wet grassy meadow, 18.VII 1994, leg. A.B. Babenko; delta of Yana River, Shirokostan Peninsula, vicinity of Ledyanoye Lake, 72°25'N, 141°00'E, 1 female, south slope of valley, forb-grassy meadow; 1 male, high river terrace, *Dryas*-*Carex* tundra, 4–6.VIII 1994, leg. A.B. Babenko; 3 females, 1 N1, Yana Bay, Makar Island, 6.VIII 1985, leg. V.I. Bulavintsev; Suntar-Khayata Range, upper reaches of Kyubyume River, 3 males, 1 N2, 1300 m a.s.l., lichen *Larix*-forest, litter, 25.VII 2002, 2 females, 1 male, 1 N1, 1800 m a.s.l., snow bed, under *Rhododendron aureum,* 2 males, 5 N2, 2 N1, 1800 m a.s.l., hum-

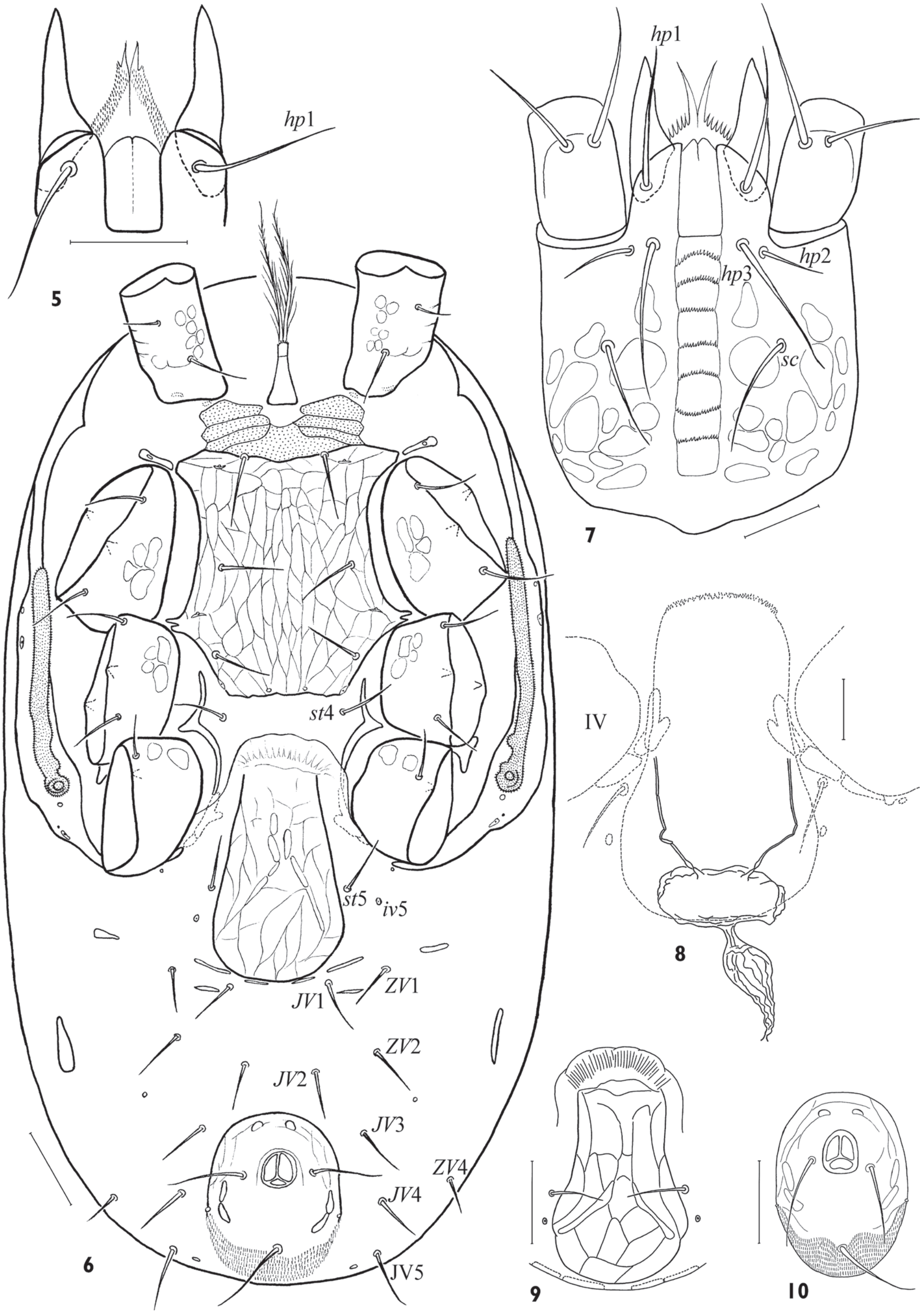


Figures 1–4. *Arctoseius koltschaki* sp. n., female **1** idiosomal dorsum **2–4** cheliceral digits. Scales: **1** – 50 µm, **2–4** – 25 µm.

mocky *Eriophorum*-community, 28.VII 2002, 1 female, 1 N2, 1800 m a.s.l., lichen-willow tundra, 9.VII 2002, 1 female, 1 N2, 1 N1, 2000 m a.s.l., mossy tundra, 15.VII 2002, 4 females, 3 males, 2 N2, 1 N1, *Dryas-Carex-Eriophorum* bog, 19.VII 2002, leg. O.L. Makarova; 1 female, Cherskogo Range, Ust-Nera vicinity, 500 m a.s.l., grassy meadow near stream, 22.VII 1992, leg. N.A. Kuznetsova; TAYMYR PENINSULA, 1 male, N Taymyr, Khariton Laptev Coast, Opalovaya River, seaside sand-rubble terrace with *Salix polaris*, 15–17.VIII 1994, leg. A.B. Babenko; Taymyr Lake, Cape Blizhnyi, 2 females, *Tetraplodon* moss cushion in tundra, 28.VII 1994, 1 female, lemming hill within stony tundra on elevation, grassy turf, 10.VIII 1994, 2 females, lemming burrow, 17.VIII 1994, 5 females, 1 N1, lemming hill within spotty tundra, grassy turf, 17.VIII 1994, leg. O.L. Makarova; 1 female, NW Taymyr, upper reaches of Kolomejtseva River, lemming hill in spotty tundra, grassy turf, 21.VIII 1997, leg. O.L. Makarova; 2 females, 1 male, NW Taymyr, Ragozinka River, spotty tundra, 9.VII 1986, leg. A.B. Babenko; 1 female, NW Taymyr, mouth of the Tareya River, 73°15'N, 90°35'E, dry *Dryas*-community, 22.VII 2010, O.L. Makarova; 1 female, SW Taymyr, vicinity of Pyasino Lake, Nyapan' Upland, zoogenic meadow on a hill, 16.VII 1999, O.L. Makarova; VAIGATCH ISLAND, 1 male, no other data, VII 1984, leg. V.I. Bulavintsev; NOVAYA ZEMLYA ARCHIPELAGO, 1 male, no other data, leg. V.I. Bulavintsev; BOL'SHEZEMEL'SKAYA TUNDRA, 3 females, Yugor Peninsula, Cape Belyi Nos, 25.VI 1983, leg. V.I. Bulavintsev; 1 female, Vorkuta city vicinity, IX 2009, leg. E.M. Perminova; 1 female, Pechora Bay, Kuznetskoye Lake, polar fox hill, litter under *Cornus suecica*, 25.VIII 1994, leg. A.B. Babenko; KOLGUEV ISLAND, 3 females, 1 male, 2 N2, 1 N1, tundra litter, VII 2011, leg. S.B. Rosenfeld; SOUTH SIBERIA, WEST SAYAN MOUNTAINS, 1 female, 50°23'N, 90°26'E, Tsagan-Shibetu Range, Mugur-Aksy vicinity, 2800 m a.s.l., tundra, litter, 22.VII 1993, leg. S.K. Stebaeva.

Description. Adults of middle size, yellowish or brownish, with idiosoma rather narrow and appendages of normal proportions. Idiosomal shields moderately sclerotized, very finely punctate (punctation rarely visible), with clearly reticulate ornamentation only on sternal, genital or sternitigenital shields; dorsal shield smooth with distinct sigillae. Most body setae of moderate size, needle-shaped. Some setae of distal leg segments, especially of leg IV, elongated and finely tipped, subapical setae *av*-1 and *pv*-1 on tarsi II-IV strongly formed and blunt.

Female. *Idiosomal dorsum*. Dorsal shield 528-616 × 248-304, narrowing posteriad, *lD*/*wD* ca 1.96–2.24, its maximal width at level of setae *s*4 (Fig. 1); lateral incisions of moderate length (30–44). Podonotal region normally with 17 pairs of simple setae (*z*3 present); *z*1 and *s*1 seldom asymmetrically absent; *s*2 usually on soft cuticle, sometimes asymmetrically on shield margin, sometimes symmetrically absent; *s*6 rarely off shield. Opisthonotal region with 13–14 pairs of setae (*S*2 always absent, *S*3 or *S*5 rarely asymmetrically off shield margin). Among podonotal setae, *z*1 (13–22) and *s*1 (20–35) distinguished by clearly shorter lengths, seta *j*1 30–36, *j*2 32–42, length of other setae 30–50. On opisthonotal region, setae *J*1-4 (25–35) slightly shorter than others (30–44), except *J*5 clearly shortest (9–17) and *Z*5 clearly longest (48–63); *J*(1–4)*min*/*jmax* 0.65–0.80,

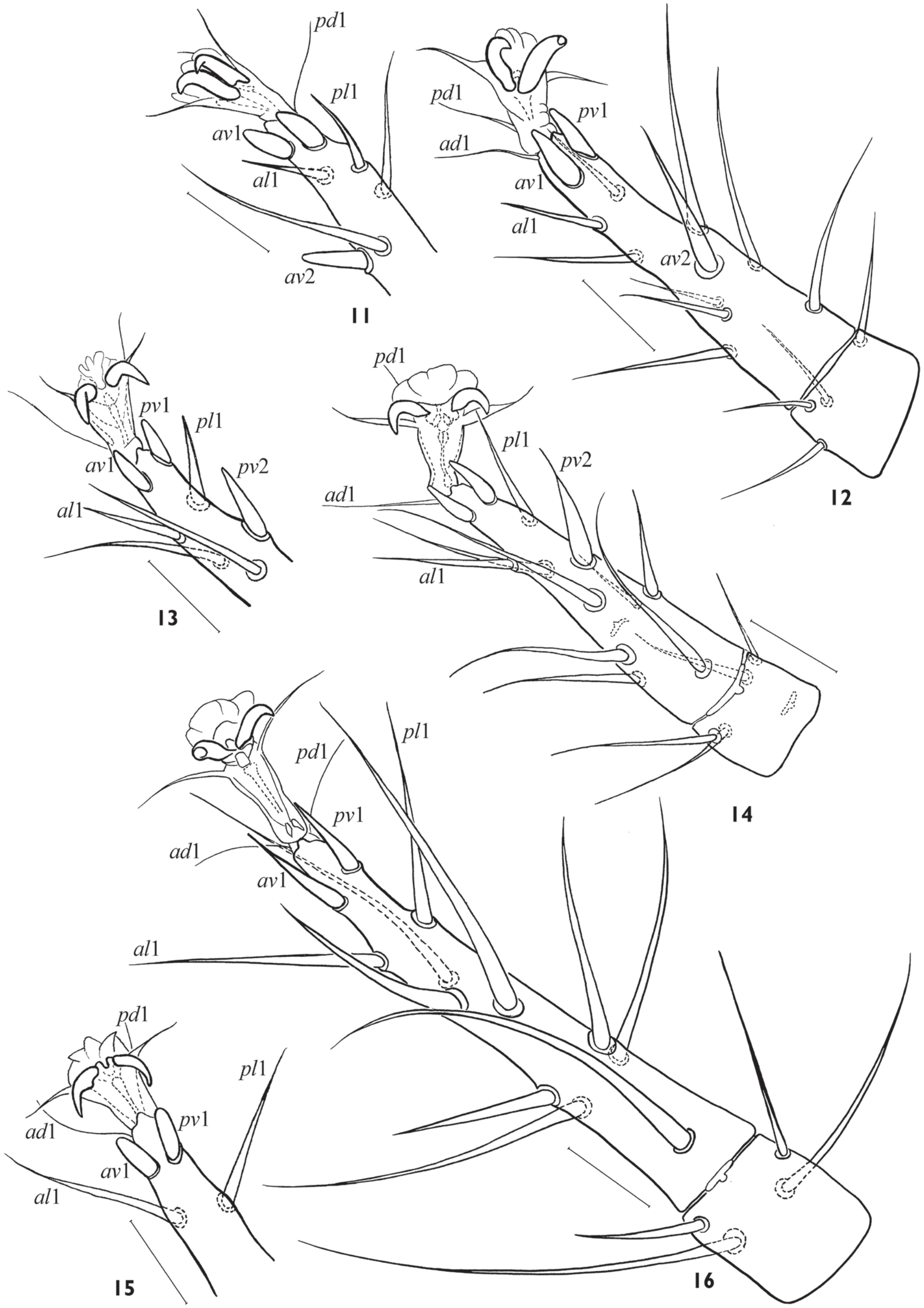


Figures 5–10. *Arctoseius koltschaki* sp. n., male (**5**) and female **(6–10) 5** hypostome **6** idiosomal venter **7** subcapitulum **8** inner part of spermathecal apparatus **9** variant of genital shield form **10** variant of anal shield form. Scales: **5, 7, 8** – 25 µm, **6, 9, 10** – 50 µm.

*Z*5/(*Z*5-*Z*5) 0.76–1.26; (*J*4-*J*4)/(*J*3-*J*3)1.31–2.00. Dorsal shield with 5 pairs of gland pores: *gdj*4, *gdj*6, *gdz*5, *gdz*6, all poorly visible and *gdZ*3 distinct. All marginal setae on soft cuticle (their length 28–44, only *r*3 39–48); 4 (rarely 3) setae in series *r*, 5 setae in series *R*; marginal poroid *Rp* in usual position between setae *R*3 and *R*4.

Idiosomal venter. Base of tritosternum narrow (20–35 × 16–22); laciniae with sparse large barbs, free for ca 0.8–0.9 of lengths, their fused basal area fimbriated anteriorly (Fig. 20); rarely barbs are hardly separated (Fig. 21); length of laciniae free part 60–80. Presternal platelets large, consolidated with each other and sternal shield, lineate and clearly punctate (Fig. 6). Sternal shield commonly longer (146–164) than wide (124–160), *lSt*/*wSt* 1.00–1.23, merged with endopodal platelets between coxae II-III. Endopodal projections between coxae I-II small, nearly bacilliform, commonly separated, their lateral part encompassing opening of gland *gvb*. Sternal shield entirely reticulated; anterior margin straight or slightly concave, posterior margin straight. Sternal shield with typical setae *st*1-3 (32–44), lyrifissures *iv*1-3, and with vestiges of gland *gv*1 on posterior margin. Setae *st*4 (24–34) on soft cuticle. Endopodal strips between coxae III and IV free, rather narrow, partly hidden under epigynal flap. Epigynal shield (140–164 × 72–82) distinctly reticulated (Fig. 9), broadly axe- or flask-shaped, with evenly convex hyaline flap well distant from sternal shield, and posterior margin broadly convex; lateral margins widening behind level of setae *st*5, but *st*5 (26–36) and paragenital poroids *iv*5 remain on soft cuticle. Opening of gland *gv*2 on soft cuticle close beside end of peritrematal-exopodal strip behind coxa IV. Two pairs of postgenital platelets in fold of soft cuticle adjacent to posterior margin of epigynal shield, another pair between setae *JV*1 and *ZV*1. Anal shield rather small, ovate, weakly reticulated (Fig. 10), longer (94–112) than wide (76–93), *lAn*/*wAn* 1.08–1.30; paranal setae (36–46) variably inserted, usually between mid-level of anus and its posterior margin, and often nearly as long as postanal seta (42–64); opening of gland *gv*3 inconspicuous; cribrum ordinary. Opisthogastral setae (8 pairs, *JV*1–5, *ZV*1, 2, 4) of moderate length (most setae 30–40), *JV*5 longest (35–52), *ZV*1 shortest (20–34). Anterior metapodal platelets small, of variable form; posterior metapodal platelets elongate, often bacilliform (24–39 × 4–7). Exopodal platelets between coxae II and III, as well as between coxae III and IV, small, triangular, rarely visible in ventral aspect. Peritrematal shield reduced, of uneven width, with angular extension at level of coxa I; its anterior end abutting dorsal shield; its posterior edge connecting with exopodal strip enveloping coxa IV posteriorly; lyrifissures *ip*1–3 and glands *gp*1, 2 present. Peritreme shortened (128–156 × 8–11), extending to mid-level of coxa II anteriorly. Spermathecal apparatus seldom visible, with very narrow tubuli, thin-walled sperm receptacle and sperm duct strongly expanded medially (Fig. 8).

Gnathosoma. Gnathotectum (28–39 × 40–56) triramous, with projections of almost equal length, sometimes divided apically (Figs 23, 24); middle process sometimes narrower basally than lateral ones. Subcapitulum always slightly longer (116–128) than wide (104–120) (Fig. 7). Deutosternum with 7 (rarely 8) rows of denticles (15–22 denticles in each row); groove delineated laterally, its width 15–19 (Fig. 7). Hypostomatic pair *hp*3 (46–58) longer than other subcapitular setae (*hp*1 31–40, *sc*



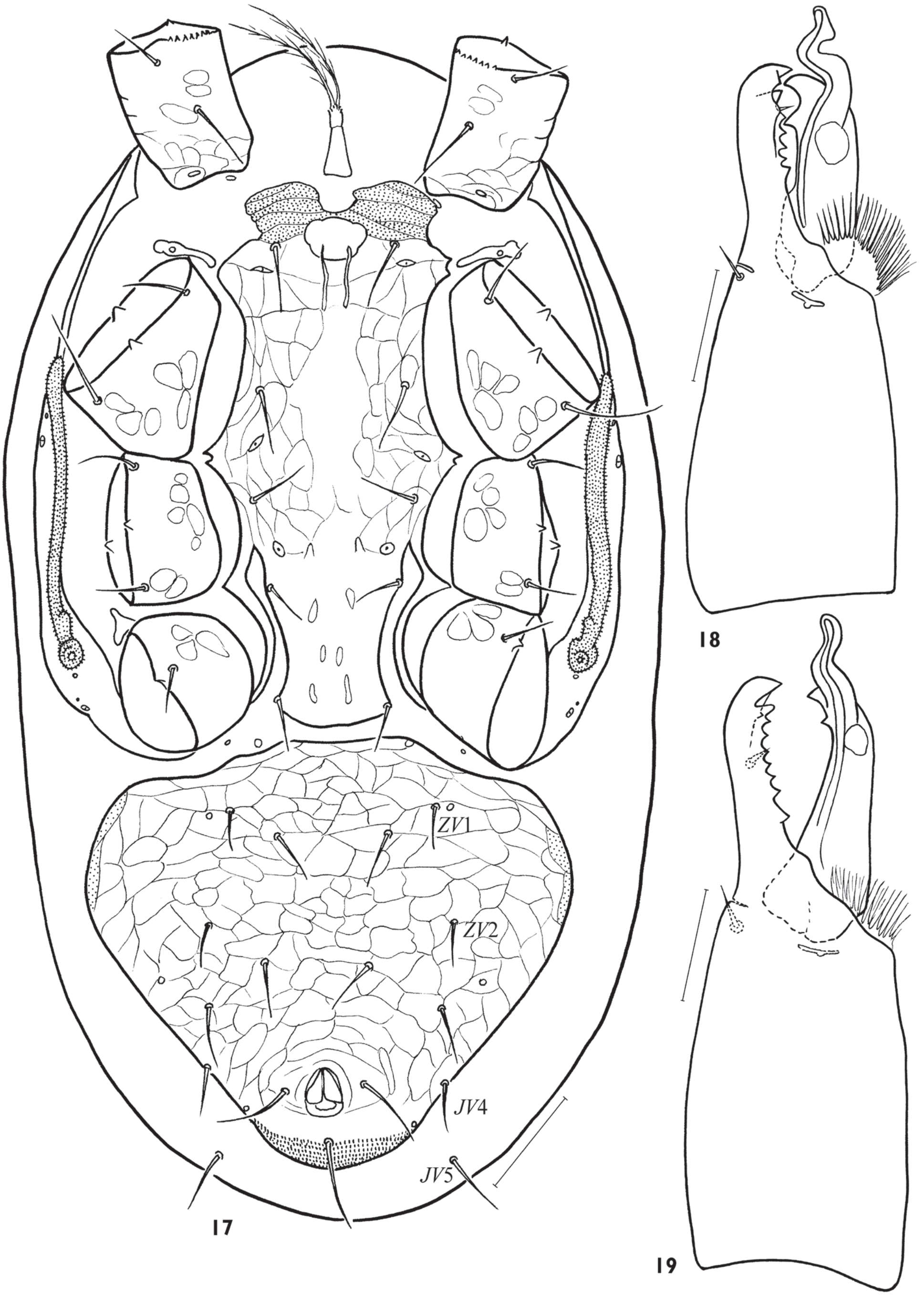
Figures 11–16. *Arctoseius koltschaki* sp. n., female **11, 12** variants of subapical setation of tarsus II **13, 14** variants of subapical setation of tarsus III **15, 16** variants of subapical setation of tarsus IV. Scales 25 µm.

26–42, *hp*2 18–26); all setae simple, attenuate. Corniculi somewhat elongated, 46–53 × 15–19; *lCo*/*lD* 7.66–9.56. Internal malae slightly shorter than corniculi, gradually tapering to tip, with lateral margins roughly fimbriated basally. Chelicera medium-sized, length of middle article 132–156; movable digit moderate sized (50–56, *lCh*/*lD* 8.65–9.71), slightly longer than corniculus (*lCh*/*lCo* 1.03–1.17). Fixed digit of chela ending in apical trident, masticatory surface with a row of 5–6 teeth in paraxial position and pilus dentilis in antaxial position (Figs 2–4). Movable digit bidentate. Palp length 180–204; internal seta of trochanter (39–43) slightly longer than external seta (34–38); palpi with typically specialized setae on femur (*al*) and genu (*al*1, *al*2) large, thick, with oblique tip.

Legs. Legs of moderate length (I 512–624, II 432–480, III 352–424, IV 512–592); leg I similar in length to dorsal shield, *lExI*/*lD* 0.91–1.09. Length of tarsi I 152–180, II 108–134, III 96–132, IV 160–192; tarsi of normal proportions: *lT*IV/*lD* 0.28–0.34, *lT*III/*wT*III 3.25–4.10. Leg II significantly thicker than others (width of genu II 56–68, genu I 36–40, genua III, IV 44–48). Leg chaetome in general as described for genus (Lindquist and Evans 1965); tibia IV with 7 setae, *pl* present. Most leg setae simple, of moderate lengths; length of *pd*2 on genu II (42–48) by a third shorter than width of segment (55–64). Tarsi II–IV each with some rather long setae (up to 100 on tarsus IV), many of them with fine tips (Figs 12, 14, 16); with lateral subapical setae *al*1 and *pl*1 needle-shaped, strong, rather long (24–32 on leg II, 21–39 on leg III, 36–56 on leg IV), two times more distant from tarsus apex and much thinner than robust blunt ventral subapical setae *av*1 and *pv*1 (14–19, 12–20, 16–27 respectively) (Figs 11–16); *av*2 on tarsus II and *pv*2 on tarsus III also thick, sometimes shortened, with blunt tips (Figs 11, 13). Ambulacrum I on pedicellate base, claws I (12–13) smaller than claws II-IV (16–21). Tarsus I distally with 6 rod-like solenidia, 4 of them inserted apically; length of sensillum with lanceolate apex 36–42. Ambulacra of legs II-IV (length 30–40) with rather long paradactyli (14–20 on legs II and III; 18–26 on leg IV) extending clearly beyond apices of claws. Tarsi II-IV with apical setae *ad*1 and *pd*1 (24–39 on legs II, III; 30–40 on leg IV) subequally as long as ambulacrum.

Male. *Idiosomal dorsum*. Dorsal shield 446–552 × 228–260, more narrowed posteriad than in female (*lD*/*wD* ca 2.05–2.17); lateral incisions on average shorter than in female (20-40); chaetome in general as in female, but setae commonly slightly shorter (by 5–15 %); 9 pairs of marginal setae (*r*2-*r*5, *R*1-*R*5) on soft cuticle; *s*2 usually on soft cuticle, rarely on shield margin or absent.

Idiosomal venter. Tritosternum base (16–20 × 12–16) slightly smaller than in female (Fig. 17). Presternal platelets connected with sternitigenital shield. Sternitigenital shield united with endopodal platelets developed between coxae II-III, partly separated from ones situated between coxae III-IV and only abut ones developed between coxae I-II; its posterior margin slightly convex or truncate; length of shield 212–248, width 124–140, its narrowest part (44–48) between coxae IV. Sternitigenital shield without reticulation in the central and posterior areas, with setae *st*1–3 (30–38) longer than setae *st*4, 5 (20–28) and distinct lyrifissures *iv*1–3; vestiges of glands *gv*1 not discernible. Ventrianal shield well separated from sternitigenital and peritrematal shields,



Figures 17–19. Males, *Arctoseius koltschaki* sp. n. **(17, 18)** and *Arctoseius multidentatus* Evans, 1955 **(19)** **17** idiosomal venter **18, 19** chelicera. Scales: **17** – 50 µm, **18, 19** – 25 µm.

subtriangular, often with small anterior ledge, fully reticulated, its length 188–224, width 184–240, *lVa*/*wVa* 0.83–1.09, normally consolidated with metapodal platelet sigillae laterally; its anterolateral margins evenly convex, cribrum formed as in female. Ventrianal shield with 6 pairs of opisthogastral setae (*JV*1–4, *ZV*1, 2), 2–3 pairs of poroids and gland opening *gv*3, sometimes *ZV*1 asymmetrically absent, *JV*4 rarely asymmetrically off shield margin; *ZV*1, 2 (20–28) somewhat shorter than others (26–35), paranals 32–42, postanal seta 40–52; seta *JV*5 (34–40) on soft cuticle.

Gnathosoma. Gnathotectum as in female. Corniculi slightly thinner and more parallel than in female (Fig. 5). Internal malae unlike female finely and densely fimbriate. Cheliceral digits 46–52 (*lCh*/*lD* 8.58–10.94), longer than corniculus (42–47 × 12–15, *lCh*/*lCo* 1.02–1.30, *lCo*/*lD* 7.52–9.40). Fixed digit with dentition similar to female, but apical trident not developed and one denticle present subapically in antaxial position, apart from paraxial row of 5–6 denticles. Movable digit with one large denticle and tightly (at a sharp angle) sinuate spermatodactyl (length of free part 26–29), half protruding beyond tip of digit (Fig. 18).

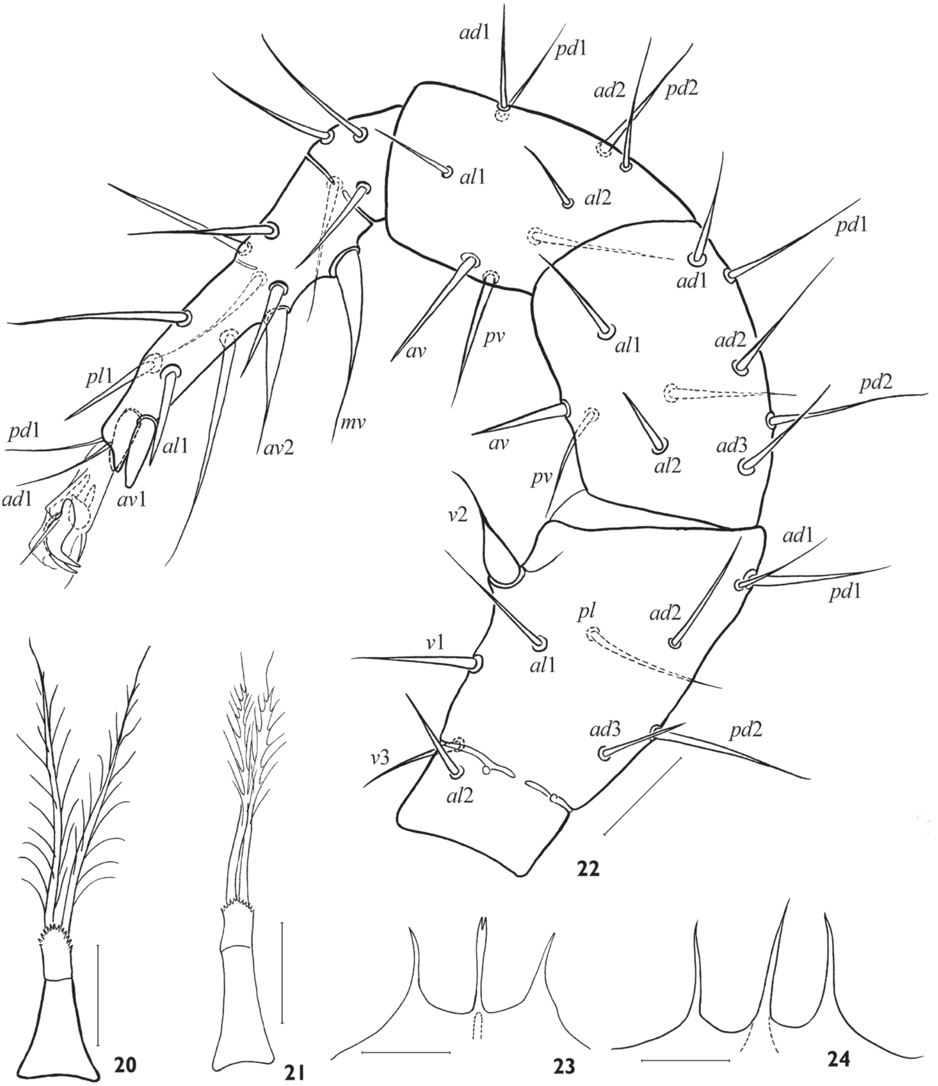
Legs. Length of legs I to IV 492–596 (*lExI*/*lD* 0.96–1.13), 364–432, 336–416, and 472–568 respectively; length of tarsi I to IV 140–168, 96–124, 104–122, and 140–170 respectively. Leg II stouter than other legs, with dimorphically modified setae. Three setae on leg II, namely *v*2 on femur, *av*2 and *mv* on telotarsus, remarkably stout basally and attenuate apically, on elevated bases; opposable ventral setae *v*-1 on femur II and *av* on genu II and tibia II also usually slightly spinelike (Fig. 22). Other leg structure and setation generally as in female.

Differential diagnosis. Adults of *Arctoseius koltschaki* sp. n. are similar to those of *Arctoseius multidentatus* Evans, 1955, but the dorsal shield in *A. koltschaki* sp. n. is often narrower (*lD*/*wD* in female 1.96–2.24, in male 2.05–2.17 instead of 1.91–2.05 and 1.85–2.03 respectively), and tarsi II-IV have subapical setae *av*1 and *pv*1 more stout and blunt (Figs 11–15). In male of *A. koltschaki* sp. n., the ventrianal shield is relatively wide (Fig. 17), subtriangular (narrowly oval in *A. multidentatus*), incorporating the metapodal platelets (free in *A. multidentatus*); the spermatodactyl is more tightly or sharply sinuate, and more angled apically (not as gently sinuate, and digitiform apically as in *A. multidentatus*) (cf. Figs 18 and 19). In female of *A. koltschaki* sp. n., the sternal shield is uniformly reticulated (whereas in *A. multidentatus*, the reticulation is developed laterally, and separately within an elongate posteromedial “window”). Female of *A. koltschaki* sp. n. is often smaller (dorsal shield length 528–616 instead of 566–688 in *A. multidentatus*).

Etymology. The species named in honor of Admiral Alexander Koltschak, the prominent Arctic hydrologist and outstanding Citizen of Russia.

Distribution. At present known from the Russian Arctic and Siberian mountain regions (Fig. 25).

Ecology. Recorded from dry and humid tundra sites, various meadows including zoogenic ones (e.g. polar fox and lemming hills), snow-beds, bogs, larch forests and shrub (*Salix* spp., *Rhododendron aureum, Pinus pumila*) thickets.



Figures 20–24. *Arctoseius koltschaki* sp. n., female **(20, 21, 23, 24)** and male **(22) 20, 21** tritosternum **22** leg II **23, 24** gnathotectum. Scales 25 µm.

Variability. The species is rather stable morphologically. The form of female epigynal and anal shields varies a little (Figs 6, 9, 10). The variability concerns mainly the form of tectum (Figs 23, 24), the position of setae *s*2, *s*6, *S*3, *S*5 (on or off shield margin), and the form and size of leg subapical setae (Figs 11–16). The robust subapical setae *av*1 and *pv*1 sometimes may be acuminate, more often on leg IV (Fig. 16). The deutosternum rarely has 8 (instead normal 7) rows of denticles. The lacinia of tritosternum seldom has adnate barbs (Fig. 21). One male (from 23 ones) originating from Chukotka has free baculiform metapodal platelets (26 × 4).

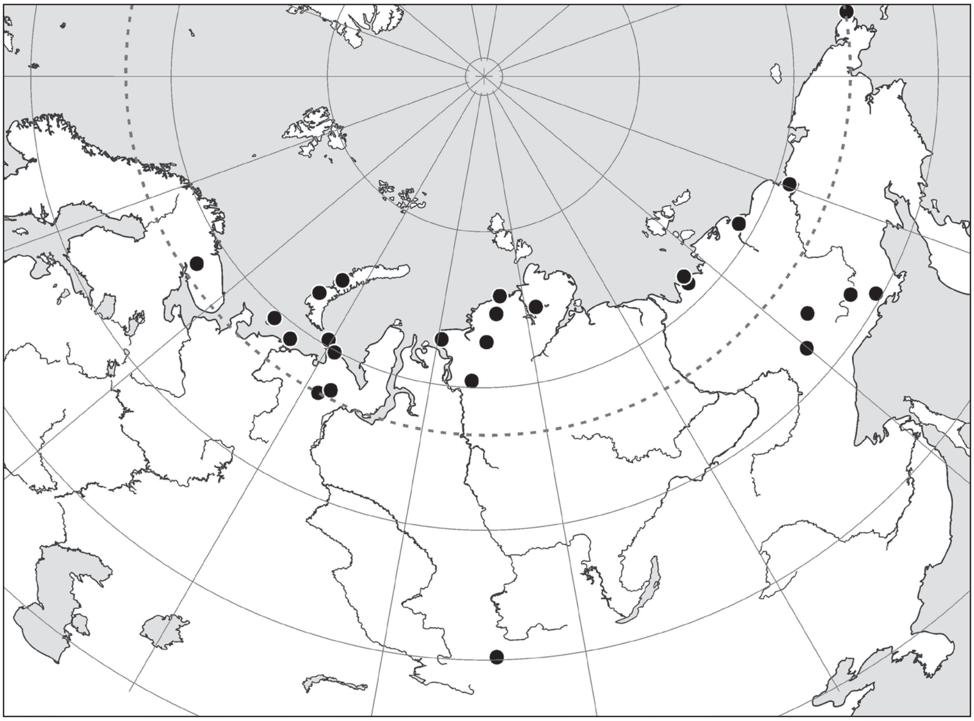


Figure 25. Records of *Arctoseius koltschaki* sp. n.

Discussion. The *multidentatus*-group

History

Previously and provisionally, there were some indications about the presence of new species close to *Arctoseius multidentatus* Evans, 1955, namely *Arctoseius* sp. aff. *multidentatus* [*A. koltschaki* sp. n.], «*A. haarlovi* Lindquist, 1963 ms.» (Lindquist 1964) [*A. haarlovi* Lindquist & Makarova, 2011], together forming a natural group. Their distinguishing features were given in a key to high-arctic *Arctoseius* species (Makarova 2000b). Recently Lindquist and Makarova (2011) augmented this group by the inclusion of *Arctoseius wisniewskii* Gwiazdowicz & Kamczyc, 2009 and *Arctoseius sexsetus* Lindquist & Makarova, 2011. Moreover, they pointed out the similarity between all mentioned species and *Arctoseius laterincisus* Thor, 1930, the type-species of the genus *Arctoseius*. In spite of its doubtful validity, this species can be formally considered as a member of the *multidentatus*-group, in view of its having the following features: shortened peritremes, elongated corniculi, and elongated posterior idiosomatic setae (Lindquist and Makarova 2011). However, subsequent to its original description, this species has not been found either on Svalbard (Ávila-Jiménez et al. 2011) or anywhere in the European Arctic (Makarova 2013). Very probably, its type series included specimens belonging to more than one species of *Arctoseius* (Lindquist and Makarova 2011). Because of these aspects, *A. laterincisus* is not included in the key below.

Diagnosis

Idiosoma of moderate size (388–688 in females, 348–552 in males); dorsal shield rather narrow (*lD*/*wD* ca 1.9–2.6), not covering entire idiosoma, smooth, with distinct sigillae. Presternal platelets weakly sclerotized, lineate and punctate, fused with sternal shield. Sternal/sternitigenital shield often free from endopodal strips between coxae I–II (excluding *A. sexsetus, A. wisniewskii*), but united with them between coxae II–III. In females, anal shields small, usually not shorter than wide (*lAn*/*wAn* 0.85–1.40). In males, ventrianal shields with convex anterior margin, oval or roundish, leaving the metapodal platelets free on soft cuticle (exception – *A. koltschaki* sp. n. with wider, subtriangular ventrianal shield incorporating metapodal platelets). In both sexes, circumanal setae of similar lengths, with paranal setae relatively long, at least 0.7 as long as postanal seta. In both sexes, peritrematal shield reduced, of uneven width, with angulate extension between coxae I-II; peritreme shortened, extending anteriorly at most to mid-level of coxa II. Gnathotectum with three projections, these sometimes bifurcate apically. Corniculi rather long (*lCo*/*lD* ca 7–13), shorter or longer than cheliceral digits (*lCh*/*lCo* 0.74–1.44). In male, spermatodactyl straight or sinuate; leg II sometimes with dimorphically modified setae. Legs moderately long, leg I similar in length with dorsal shield.

The relatively long paranal setae are considered to be apomorphic for this species-group within the genus *Arctoseius*. The reduced peritremes and peritrematal shields, somewhat elongated corniculi, and complete lack of dorsal shield ornamentation (retention of deutonymphal condition) are also apomorphic, but are derived independently among some other *Arctoseius* species.

Key to species of *Arctoseius* of *multidentatus*-group. Adults[1]

1 Podonotal seta *z*3 and opisthonotal seta *S*1 absent; corniculi large (*lCo*/*lD* 11.52–13.41), longer than cheliceral digits (*lCh*/*lCo* 0.74–0.93); fixed cheliceral digit with at most 4 denticles besides terminal hook; tibia IV with 6 setae, *pl* absent; in male, dimorphically modified setae on leg II absent, spermatodactyl straight **2**

– Podonotal seta *z*3 and opisthonotal seta *S*1 present (Fig. 1); corniculi smaller (*lCo*/*lD* 6.90–10.98), shorter than cheliceral digits (*lCh*/*lCo* 1.02–1.44); fixed cheliceral digit polydont, with 6–14 denticles besides terminal hook (Figs 4, 18); tibia IV with 7 setae, *pl* present; in male, dimorphically modified setae on leg II well developed (Fig. 22), spermatodactyl clearly sinuate (Figs 18, 19) ... **3**

2 Fixed cheliceral digit terminating with trident, behind which one denticle present; in male, single denticle of movable digit situated in subapical position. Female 444–552 µm, male 404–464 µm. Alaska, Eurasian Arctic, Siberian mountains .... ***Arctoseius sexsetus* Lindquist & Makarova, 2011**

– Tip of fixed cheliceral digit of usual form, with three denticles forming a row behind apical hook; in male, single denticle of movable digit situated

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[1] Measuring attributes to the dorsal shield length.

at a greater distance from apex, almost opposite pilus dentilis of fixed digit. Female 550 µm, male 500 µm, Stolovye Mountains, Poland .... ***A. wisniewskii* Gwiazdowicz & Kamczyc, 2009**

3 Smaller mites, dorsal shield length at most in female 465 µm, in male 420 µm; opisthonotal region with 13 pairs of setae (only one pair of setae present in area usually occupied by *J*4 and *Z*4); peritreme much reduced, usually not extending beyond mid-level of coxa III anteriorly. Female 388–465 µm, male 348–420 µm. Circumpolar........***A. haarlovi* Lindquist & Makarova, 2011**

– Larger mites, dorsal shield length at least in female 528 µm, in male 446 µm; opisthonotal region with 14 pairs of setae (*J*1–5, *Z*1–5, *S*1, 3–5); peritreme terminating about mid-level of coxa II anteriorly (Figs 6, 17) **4**

4 Tarsi II-IV with subapical setae *av*-1 and *pv*-1 stout and blunt (Figs 11–15); in male, ventrianal shield relatively wide, subtriangular, incorporating metapodal platelets (Fig. 17); spermatodactyl tightly sinuated, and angled apically (Fig. 18). Female 528–616 µm, male 446–552 µm. Eurasian Arctic and Metaarctic, South Siberia (West Sayan Mountains) .... ***Arctoseius koltschaki* sp. n.**

– Tarsi II-IV with subapical setae *av*-1 and *pv*-1 needle-shaped, but thicker than *pl*-1, *al*-1; in male, ventrianal shield usually narrow, often oval, leaving metapodal platelets free on soft cuticle; spermatodactyl more gently sinuated, and either simple or bluntly angled apically (Fig. 19). Female 566–688 µm, male 488–550 µm. Circumpolar......***Arctoseius multidentatus* Evans, 1955**

Acknowledgements

Many friends and colleagues supplied material from which additional specimens of this new species were found – A.V. Alfimov, A.B. Babenko, M.V. Berezin, D.I. Berman, V.I. Bulavintsev, E.V. Gordeyeva, S.V. Goryachkin, N.A. Kuznetsova, E.M. Perminova, M.B. Potapov, S.B. Rosenfeld, S.K. Stebaeva. The Programs "Problems of Life Origin and Biosphere Formation" and "The Wildlife" of the Russian Academy of Sciences as well as the Russian Fond of Basic Researches supported the first author during certain aspects of this investigation, including field work. Arrangement of figures and the map were prepared for publication with thankful participation of K.V. Makarov. We are very grateful to all.

References

Ávila-Jiménez ML, Gwiazdowicz DJ, Coulson SJ (2011) The mesostigmatid mite (Acari; Parasitiformes) fauna of Svalbard: a revised inventory of a High Arctic archipelago. Zootaxa (3091): 33–41.

Evans GO (1955) A collection of mesostigmatid mites from Alaska. Bulletin of the British Museum (Natural History). Zoology 2(9): 287–307.

Evans GO (1963) Observations on the chaetotaxy of the legs in the free-living Gamasina. Bulletin of the British Museum (Natural History). Zoology 10: 275–303.

Evans GO (1964) Some observations on the chaetotaxy of the pedipalps in the Mesostigmata (Acari). Annals and Magazine of Natural History (Series 13) 6: 513–527.

Gwiazdowicz DJ, Kamczyc J (2009) *Arctoseius wisniewskii* sp. nov. (Acari, Ascidae) from Poland. Annales Zoologici, Warszawa 59(1): 119–123. doi: 10.3161/000345409X432628

Johnston DE, Moraza ML (1991) The idiosomal adenotaxy and poroidotaxy of Zerconidae (Mesostigmata: Zerconina). In: Dusbabek F, Bukva V (Eds) Modern Acarology. V. 2. Academia, Prague, 349–356.

Lindquist EE (1964) A revision of mites of the subfamily Blattisocinae (Acarina: Blattisocidae) in America north of Mexico. PhD thesis, University of California, Berkeley.

Lindquist EE (1994) Some observations on the chaetotaxy of the caudal body region of gamasine mites (Acari: Mesostigmata), with a modified notation for some ventrolateral body setae. Acarologia 35(4): 323–326.

Lindquist EE, Evans GO (1965) Taxonomic concepts in the Ascidae, with a modified setal nomenclature for the idiosoma of the Gamasina (Acarina: Mesostigmata). Memoirs of the Entomological Society of Canada 47: 1–64. doi: 10.4039/entm9747fv

Lindquist EE, Makarova OL (2011) Two new circumpolar mite species of the genus *Arctoseius* (Parasitiformes, Mesostigmata, Ascidae). Entomological Review 91(8): 1054–1072. doi: 10.1134/S0013873811080100

Makarova OL (2000a) To a study of mites of the genus *Arctoseius* (Parasitiformes, Ascidae) from the Far North: 1. Morphometric analysis of the taxonomic features of Arctic species and description of *Arctoseius tschernovi* sp. n. and *A. miranalis* sp. n. Entomological Review 80(Suppl. 1): S112–S130.

Makarova OL (2000b) To a study of mites of the genus *Arctoseius* (Parasitiformes, Ascidae) from the Far North: 2. Description of *A. productus* sp. n. and *A. babenkoi* sp. n. and key to High Arctic species. Entomological Review 80(Suppl. 1): S131–S142.

Makarova OL (2003) A new genus and three new species of the mite family Arctacaridae (Parasitiformes, Mesostigmata) from North America. Entomological Review 83(7): 868–886.

Makarova OL (2013) Gamasid mites (Parasitiformes, Mesostigmata) of the European Arctic and their distribution patterns. Entomological Review 93(1): 113–133. doi: 10.1134/S0013873813010156

Makarova OL (in press) Gamasid mites (Parasitiformes, Mesostigmata) of the Arctic: taxonomical structure of the fauna. Zoologicheskyi Zhurnal, Moscow.

Makarova OL, Petrova AD (1992) Gamasid mites of the genus *Arctoseius* Thor, 1930 (Mesostigmata: Aceosejidae) from the East Palaearctic. 1. Species of the cetratus-group. Biologicheskiye Nauki, Moscow 2: 28–40.

Petrova AD, Makarova OL (1991) *Arctoseius tajmyricus,* a new species of gamasid mites (Mesostigmata, Aceosejidae) phoretic on trichocerid flies (Diptera). Entomological Review 70(5): 139–142.

Thor S (1930) Beiträge zur Kenntnis der invertebraten Fauna von Svalbard. Skrifter om Svalbard og Ishavet 27: 1–156.

ZooKeys 313: 25–43 (2013)
doi: 10.3897/zookeys.313.5363
www.zookeys.org

RESEARCH ARTICLE

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Integrative taxonomy of a new species of planarian from the Lake Ohrid basin, including an analysis of biogeographical patterns in freshwater triclads from the Ohrid region (Platyhelminthes, Tricladida, Dugesiidae)

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Academic editor: *Lyubomir Penev* | Received 19 April 2013 | Accepted 20 June 2013 | Published 28 June 2013

urn:lsid:zoobank.org:pub:B61969DE-DB12-4C74-8299-859F5E05568E

Citation: Stocchino GA, Sluys R, Deri P, Manconi R (2013) Integrative taxonomy of a new species of planarian from the Lake Ohrid basin, including an analysis of biogeographical patterns in freshwater triclads from the Ohrid region (Platyhelminthes, Tricladida, Dugesiidae). ZooKeys 313: 25–43. doi: 10.3897/zookeys.313.5363

Abstract

A new species of the genus *Dugesia* is described from the Lake Ohrid region in the western part of the Balkan Peninsula, forming the first fully documented species description for this genus in the Ohrid area. The morphological species delimitation is supported by complementary molecular, karyological, and cytogenetic data available from the literature. Therefore, species delineation is based on a truly integrative approach. Further, a short account on the degree of freshwater planarian endemicity in the Ohrid region is provided.

Keywords

Platyhelminthes, Tricladida, *Dugesia*, integrative taxonomy, ancient lake, Ohrid, new species, endemicity

Introduction

The oligotrophic karstic Lake Ohrid is located in the western part of the Balkan Peninsula on the Macedonian-Albanian frontier. With a limnological age of 2-5 million years it is considered to be one of the oldest lakes in Europe (Albrecht and Wilke 2008). The lake is characterized by a high degree of biodiversity and endemicity in several groups of organisms (Stanković 1960, Albrecht and Wilke 2008). With more than 210 known endemic species it is probably the most biodiverse lake in the world, at least when one takes surface area into account (Albrecht and Wilke 2008). Due to its peculiarities, Lake Ohrid is considered to be a key site for biodiversity and speciation research (Albrecht and Wilke 2008).

The first studies on the triclad fauna of the Ohrid area date back to the 1920's with the first description of several new species of *Phagocata* Leidy, 1847 and *Dendrocoelum* Örsted, 1844 (cf. Stanković and Komárek 1927). Further important researches carried out during the 20th century, mainly by Stanković (1938, 1960, 1969) and Kenk (1978), contributed to a better knowledge of this very interesting planarian fauna. In his valuable monograph, Stanković (1960) in particular pointed out the extraordinary biogeographical situation of the endemic triclads in the Ohrid region.

In this paper we report on a new species of freshwater planarian of the genus *Dugesia*, forming the first fully documented species description for this genus in the Ohrid area. Our morphological species delimitation was supported by complementary molecular, karyological, and cytogenetic data available from the literature. Therefore, our species delineation is based on a truly integrative approach. Further, we provide a short account on the biogeographical patterns in freshwater planarians and their degree of endemicity in the Ohrid region.

Materials and methods

Planarians were collected in 1995 from the southern section of Lake Ohrid basin, near the town of Çërravë, along the Pogradec-Korçë road, ca. 10 km south-east of Pogradec, at an altitude of ca. 800 m asl (Fig. 1). The animals were found under pebbles and among vegetation in a rivulet, flowing along a steep meadow, joining a tributary stream of the lake. All individuals (n = 20) were asexual at collection. The collected specimens were transferred to the laboratory, reared in glass bowls under semi-dark conditions at 18 +/- 2 °C and fed with fresh beef liver.

After having been kept in the laboratory for about one year, during which the strain notably increased in numbers due to asexual reproduction by fission, approximately 30% of the specimens shifted from the fissiparous reproductive mode towards a tendency to sexualize, i.e. to develop reproductive organs. These sexualized animals displayed the characteristic features of ex-fissiparous individuals: large body size, development of the copulatory apparatus, hyperplasic ovaries.

For morphological study sexualized specimens were fixed for 24 hours in Bouin's fluid, dehydrated in a graded ethanol series, cleared in toluene, and embedded in synthetic paraffin. Serial sections were made at intervals of 5–7 µm and were stained with Harris' haematoxylin-eosin, Mallory's trichrome, or Pasini's reagent.

The material is deposited in the Naturalis Biodiversity Center, Leiden, The Netherlands (collection code: ZMA), and in the Giacinta A. Stocchino collection (CGAS), University of Sassari.

Abbreviations used in the figures

bc: bursal canal; bg: bulb glands; ca: common atrium; cb: copulatory bursa; cg: cement glands; cm: circular muscles; d: diaphragm; e: epithelium; ed: ejaculatory duct; g: gonopore; gd: gonoduct; ie: infranucleate epithelium; l: lumen; lm: longitudinal muscles; lod: left oviduct; ma: male atrium; pb: penis bulb; pf: penial fold; pg: penis papilla glands; ph: pharynx; pp: penis papilla; rod: right oviduct; s: spermatophore; sg: shell glands; sv: seminal vesicle; vd: vas deferens.

Results

Systematic Account

Order Tricladida Lang, 1884
Suborder Continenticola Carranza, Littlewood, Clough, Ruiz-Trillo, Baguñà & Riutort, 1998
Family Dugesiidae Ball, 1974
Genus *Dugesia* Girard, 1850

***Dugesia superioris* Stocchino & Sluys, sp. n.**
urn:lsid:zoobank.org:act:E1A595E2-6466-4F59-99CF-3E846A545332
http://species-id.net/wiki/Dugesia_superioris
Figs 1–4; Table 1

Material examined. Holotype: ZMA V.Pl. 7153.1, Çërravë, Pogradec District (40°50'56"N, 20°42'60"E), Lake Ohrid basin, Albania, August 1995, coll. P. Deri and N. Mazniku, one set of sagittal sections on 50 slides (stained in Harris' haematoxylin-eosin).

Paratypes: CGAS Pla 6. 1, ibid., sagittal sections on 43 slides (stained in Harris' haematoxylin-eosin); CGAS Pla 6. 2, ibid., sagittal sections on 12 slides (stained in Mallory's trichrome); CGAS Pla 6. 3 ibid., transverse sections on 135 slides (stained in Pasini's reagent); ZMA V.Pl. 7153.2, ibid., transverse sections on 131 slides (stained in Harris' haematoxylin-eosin); CGAS Pla 6. 4, ibid., transverse sections on 60 slides

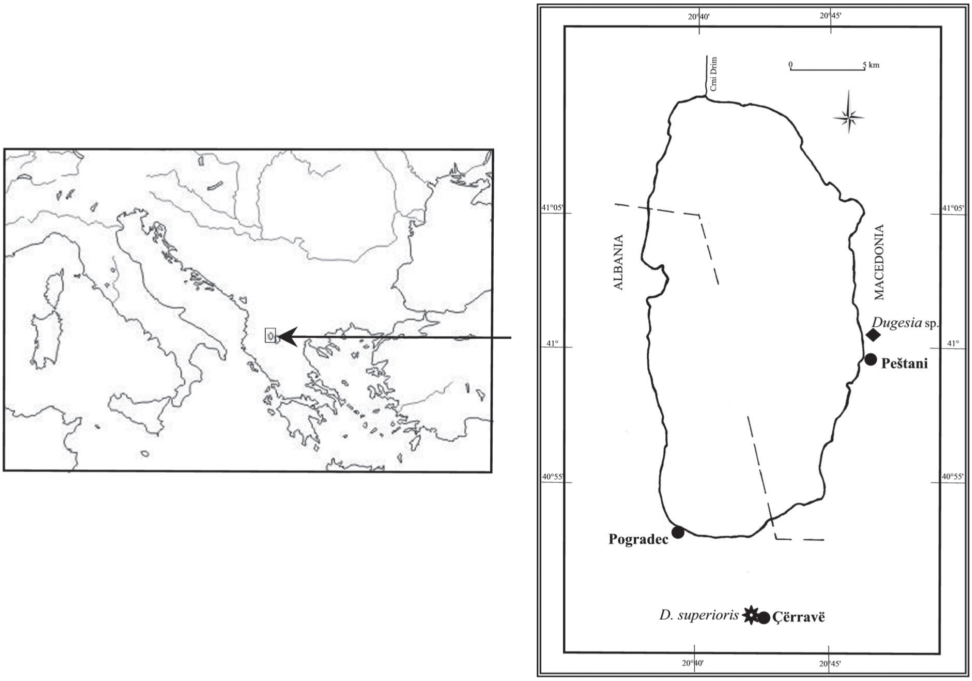

Figure 1. Geographic distribution of *Dugesia superioris* (indicated by an asterisk) and *Dugesia* sp. NMNH 55294 (indicated by black diamond) in the Lake Ohrid region.

(stained in Harris' haematoxylin-eosin); ZMA V.Pl. 7153.3, ibid., horizontal sections on 21 slides (stained in Harris' haematoxylin-eosin).

Diagnosis. *Dugesia superioris* is characterized by the presence of the following features: dorsal course of the ejaculatory duct; subterminal opening of the ejaculatory duct; asymmetrical openings of the oviducts into the bursal canal; openings of vasa deferentia at halfway along the seminal vesicle; plump penis papilla; small diaphragm; triploid chromosome complement of 24 + 1B-chromosomes.

Description. Body size of living fissiparous specimens ranged from 7–10 mm in length and 1.5–2 mm in width (Fig. 2). Sexualized specimens were about 13–16 mm in length and about 3 mm in width. Two eyes are present in the middle of the head, and unpigmented auricular grooves are marginally placed just posteriorly to the eyes. The colour is uniformly brown dorsally, and pale ventrally.

Inner and outer pharyngeal musculature is bilayered, i.e. without an extra, third, outer longitudinal muscle layer. The ovaries are hyperplasic, with several scattered masses at a short distance behind the brain, filling up the entire dorso-ventral space. A degenerative condition is clearly evident in the ovaries, in that maturation of the oocytes is regular up to the beginning of the diplotene stage, whereas diplotenic oocytes show progressive cytoplasm vacuolation, followed by collapse of the entire cell content and by cell necrosis.

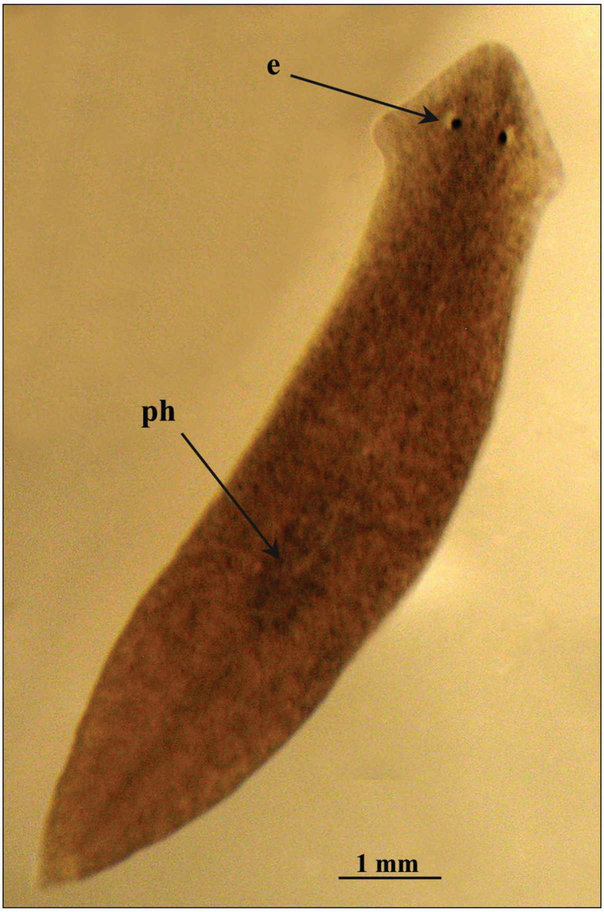

Figure 2. *Dugesia superioris*. Habitus of a living fissiparous specimen.

The anterior portion of the infranucleated oviducts is expanded to form a seminal receptacle that arises in the middle of the ovarian masses at a poorly defined position, dependent upon the hyperplasic condition of the ovaries. The oviducts run ventrally in a caudal direction up to the vaginal area and open asymmetrically into the distal section of the bursal canal. The right oviduct opens dorsally to the left one. The latter opens very close to the point where the canal communicates with the common atrium (Fig. 3). The very abundant shell glands open at the level of the left oviducal opening.

The testes are situated dorsally and extend from just anterior to the ovaries to the posterior end of the body. The testes generally are under-developed in that the majority of germ cells are represented only by spermatogonia (ca. 90%). In only some specimens, and then in only a few follicles, mature sperms are present. However, in all cases anomalies were observed, such as irregularly shaped spermatids and spermatozoa. Vitellaria are located between the testes and the intestinal branches.

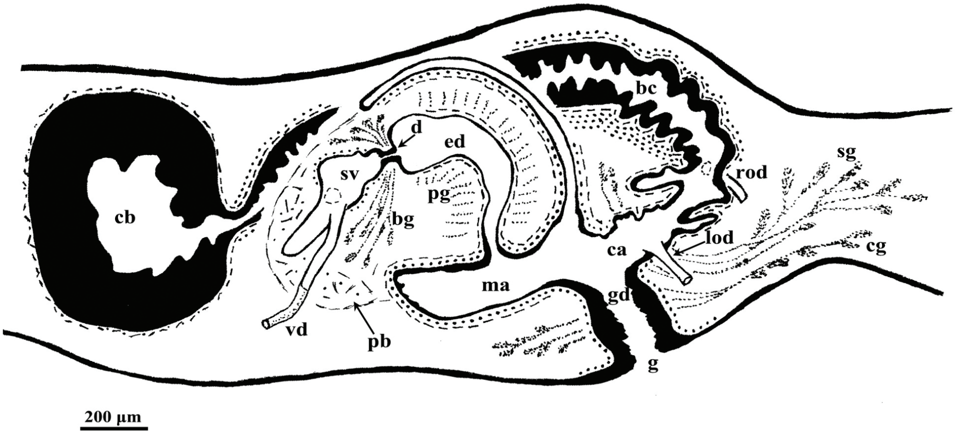

Figure 3. *Dugesia superioris.* Holotype ZMA V.Pl. 7153.1, sagittal reconstruction of the copulatory apparatus (anterior to the left).

The large sac-shaped copulatory bursa is lined by a columnar, glandular epithelium bearing basal nuclei and it is surrounded by a thin layer of muscles. From the mid-posterior wall of the bursa the bursal canal runs in a caudal direction, to the left of the copulatory apparatus. Posteriorly to the gonopore the bursal canal recurves antero-ventrally and, subsequently, opens into the posterior section of the atrium. The bursal canal is lined by a pleated epithelium with cylindrical, infranucleated, and ciliated cells and is surrounded by a thin, subepithelial layer of longitudinal muscles, followed by a thicker layer of circular muscle. Ectal reinforcement is absent (Figs 3, 4C). At its distal section, near the atrium, the bursal canal shows several deep folds.

The moderately developed penis bulb, rich in glands, consists of intermingled longitudinal and circular muscle fibres. It houses an elongated seminal vesicle, which extends through the entire length of the penis bulb. The anterior half of the seminal vesicle is tubular in shape, while its distal, posterior section is considerably expanded.

The vasa deferentia penetrate the antero-lateral wall of the penis bulb and open separately and symmetrically into the seminal vesicle at a position about halfway along the vesicle. No spermiducal vesicles were observed in any of the specimens examined. The seminal vesicle, lined with a flat epithelium and surrounded in its distal, posterior section by layers of circular muscle fibres, opens into the ejaculatory duct via a small diaphragm. The latter, located at the base of the penis papilla, receives the openings of very abundant bulb glands. The blunt penis papilla is lined with an infranucleated epithelium that is underlain with a thin subepithelial layer of circular muscles fibres, followed by a layer of longitudinal muscle fibres.

The ejaculatory duct follows a dorsally displaced course through the penis papilla and has a sub-terminal opening. The spacious lumen of the ejaculatory duct is lined by a cuboidal, infranucleated epithelium that is surrounded by a layer of longitudinal muscles and receives the abundant secretion of penis papilla glands; in the majority

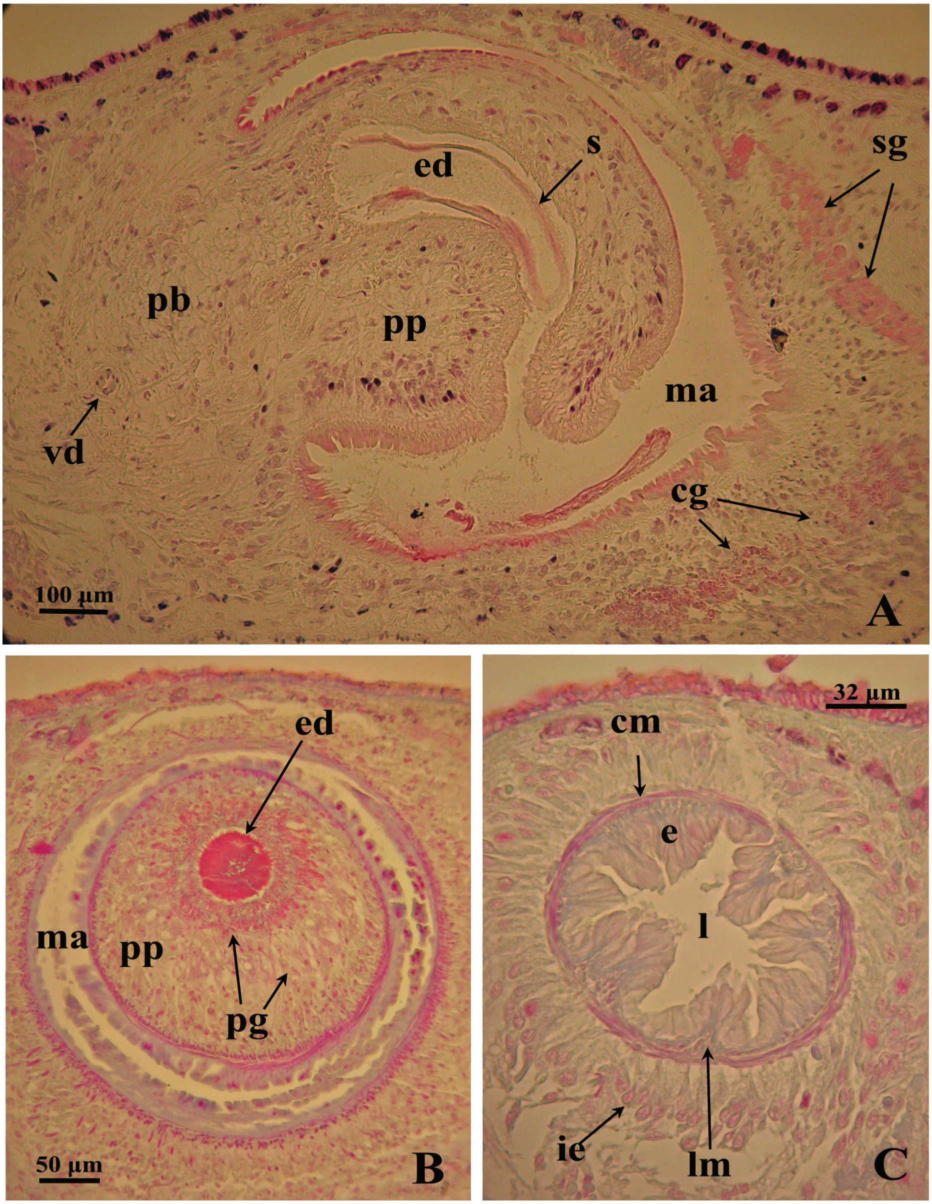

Figure 4. *Dugesia superioris*. Photomicrographs of the copulatory apparatus. **A** Holotype ZMA V.Pl. 7153.1, sagittal section showing the penis bulb and the penis papilla with the ejaculatory duct **B** Paratype CGAS Pla 6. 3, transverse section of the penis papilla and the ejaculatory duct surrounded by numerous glands **C** Paratype CGAS Pla 6. 3, transverse section of the bursal canal.

of examined specimens the ejaculatory duct contained an empty spermatophore (Fig. 4A,B). Both the bulb glands and the penis papilla glands secrete globules that stain purple in Pasini's reagent (Fig. 4B). The acentral, dorsally displaced ejaculatory duct makes the penis papilla asymmetrical, with the ventral part being thicker than the dorsal one (Figs 3, 4A,B).

The genital atrium is lined by an infranucleated epithelium that is underlain by a subepithelial layer of circular muscle, followed by a layer of longitudinal muscle fibres. The common atrium communicates with a gonoduct that is lined by a columnar epithelium, which receives the openings of very abundant cement glands; the gonoduct communicates with the ventral gonopore (Fig. 3).

Etymology. The specific epithet is derived from the Latin *superius*, located at a higher position, and alludes to the dorsally displaced course of the ejaculatory duct in the penis papilla.

Geographical distribution. Known from the type locality and, most likely, also from a second Albanian locality, viz. Voskopojë (see below).

Additional data supporting the status of the new species

A karyological study by Deri et al. (1999) identified for the Pogradec population a complement of 24 standard chromosomes with one B-chromosome, suggesting a triploid condition with a haploid number of n = 8. Moreover, their karyometric analysis indicated a probably aneutriploid condition, due to a constant excess of small, medium-sized chromosomes. A haploid number with n = 8 represents the most common chromosome number among *Dugesia* species. *Dugesia superioris* shares the triploid condition with a haploid number of n = 8 with only a few other species from the Western Palaearctic Region, viz. *D. benazzii* Lepori, 1951, *D. etrusca* Benazzi, 1946, *D. liguriensis* De Vries, 1988a, and *D. subtentaculata* (cf. Benazzi and Benazzi-Lentati 1976, Ribas 1990, Pala 1993, cf. Lázaro et al. 2009).

A molecular cytogenetic comparison of several species and populations of the genus *Dugesia* revealed that these planarians from Pogradec besides two telomeric NOR *loci*, also have a ribosomal site located in an intercalated position on the long arm of one of the largest chromosomes (Batistoni et al. 1999). This peculiar condition differs from other planarian taxa, in which 18S + 28S rRNA genes appeared preferentially located on telomeric regions of medium-sized chromosomes, and was interpreted by the authors as a structural chromosomal rearrangement, such as a paracentric inversion, suggesting a case of speciation.

More recently, a phylogeographic analysis of two Albanian populations, one from Pogradec and the other from Voskopojë (populations 30 and 31, respectively in Lázaro et al. 2009), revealed that they belong to the same clade, which is well-separated from other species and populations of *Dugesia* in the Western Mediterranean region, thus pointing to a new species (Lázaro et al. 2009). In a second study, which included other and more eastern Mediterranean species of *Dugesia*, the population from Pogradec (population 15 in Solà et al. 2013) also sat on its own branch, separate from all other populations of *Dugesia* examined.

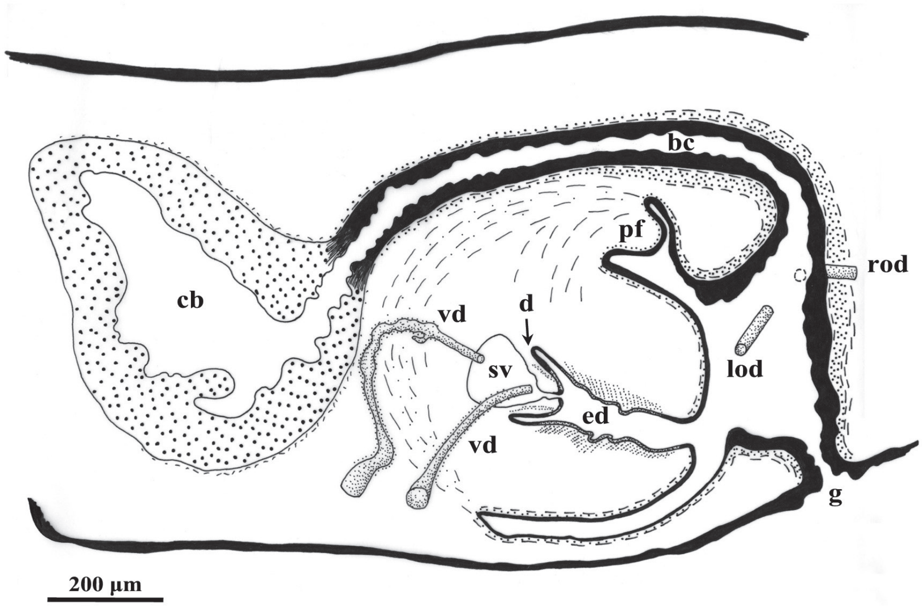

Figure 5. Sagittal reconstruction of the copulatory apparatus of *Dugesia* specimen NMNH 55294.

Previous records of *Dugesia* from the Lake Ohrid region

From Lake Ohrid only one species of *Dugesia* has been reported until now, viz. *D. gonocephala.* Stanković (1960) reported the species from running waters and springs in the Ohrid region, but he did not mention exact localities. However, in view of the fact that in that period all continental European planarians with a triangular head were assigned to this species, the taxonomic status of these Ohrid planarians remains uncertain. Kenk (1978) reported the presence of *D. gonocephala* in the Spring Elešec, about 2 km north of Peštani (Fig. 1). Re-examination of his material (NMNH 55294) revealed that the male copulatory apparatus of the animal consists of a stubby penis papilla, provided with a small, dorsal penial fold (Fig. 5). The diaphragm is rather large and pointed and projects into a broad, central ejaculatory duct, which opens at the tip of the penis papilla. The vasa deferentia open into an intrapenial seminal vesicle, albeit that the precise location of the openings is different. One vas deferens opens close to the diaphragm, whereas the other duct opens through the anterior lining epithelium of the seminal vesicle. A large copulatory bursa is situated immediately behind the pharyngeal pocket. The bursal canal is lined with a tall, nucleated epithelium and is surrounded by a subepithelial layer of longitudinal muscles, followed by a layer of circular muscle. In the vaginal area and on the posterior, curved section of the bursal canal the circular muscle layer is well-developed but becomes gradually thinner on the remaining part of the canal. Ectal reinforcement in the form of an extra, outer layer of longitudinal muscle is present in the vaginal region of the bursal canal. The oviducts open into the most proximal section of the bursal canal, one oviducal opening at the point where the canal communicates with the atrium, while the opening of the other oviduct is located somewhat more dorsally. Shell glands could not be discerned.

Biogeographical patterns of freshwater triclads in the Ohrid region

Out of a total of 27 nominal species of triclads reported from the Lake Ohrid region, 19 are endemic to this area (70% of endemicity) (Table 1). Most of these endemics (15) are restricted to the lake proper: *Dendrocoelum albidum* Kenk, 1978, *D. cruciferum* (Stanković, 1969)*, D. decoratum* Kenk, 1978*, D. dorsivittatum* Kenk, 1978*, D. komareki* (Stanković, 1969)*, D. lacustre* (Stanković, 1938)*, D. lychnidicum* (Stanković, 1969)*, D. magnum* (Stanković, 1969)*, D. minimum* Kenk, 1978*, D. ochridense* (Stanković & Komárek, 1927)*, D. sinisai* Kenk, 1978*, D. translucidum* Kenk, 1978*, Phagocata maculata* (Stanković, 1938)*, P. stankovici* (Reisinger, 1960)*, P. undulata* (Stanković, 1960). The species that live in the lake may inhabit only one of the three major bathymetrical zones of the lacustrine bottom (littoral, sublittoral and profundal) or can be found in two or more zones (Table 1). Only three species are endemic both to the lake and adjacent water systems: *Dendrocoelum maculatum* (Stanković & Komárek, 1927)*, D. sanctinaumi* (Stanković & Komárek, 1927), *Phagocata ochridana* (Stanković & Komárek, 1927).

Dendrocoelum lacteum (Müller, 1774) is a species with a very large distributional range across the Palaearctic Region that occurs both in the lake and in surrounding waters. Sywula et al. (2006) showed that *D. lacteum* from Lake Ohrid is genetically distant from the Central European populations and suggested that the Ohrid population should be considered as a distinct species. However, this study was based only on allozyme data, while its results do not fully support the conclusion of the authors. For example, the genetic distance between the Ohrid population of *D. lacteum* and *D. adenodactylosum* (Stanković & Komárek, 1927) is of the same order of magnitude as the distance to the Central European populations, whereas in the phylogenetic trees the Ohrid population strongly clusters with *D. adenodactylosum*.

Dendrocoelum adenodactylosum is very common in the lake, in its tributary streams and springs and also in Lake Prespa, a nearby lake southeast of Lake Ohrid that is a major water supplier for the latter. Six species are found in surrounding streams and springs and do not occur in the lake proper, viz. *Dugesia superioris*, *Dendrocoelum jablanicense* (Stanković & Komárek, 1927), *Schmidtea lugubris* (Schmidt, 1861), *Crenobia alpina montenigrina* (Mrázek, 1904), *Planaria torva* (Müller, 1774)*,* and *Polycelis tenuis* Ijima, 1884. *Dendrocoelum jablanicense* is endemic of the Lake Ohrid region, while the others concern widespread species.

Table 1. Checklist of Tricladida from the Lake Ohrid hydrographic basin.

Taxa	Lacustrine habitat	Adjacent waters of lake Ohrid	Endemic species	References
Dugesiidae Ball, 1974				
***Dugesia* Girard, 1850**				
Dugesia superioris	–	tributary rivulet	–	Present paper
Dugesia sp.	–	Spring Elešec	?	Kenk 1978
Dugesia gonocephala (Dugès, 1830) (?)	–	tributary streams and the effluent Crni Drim River	–	Stanković 1960
***Schmidtea* Ball, 1974**				
Schmidtea lugubris (Schmidt, 1861)	–	stagnant waters of the Ohrid region; drainage ditch at Teferić	–	Stanković 1960; Kenk 1978
Dendrocoelidae Hallez, 1892				
***Dendrocoelum* Örsted, 1844**				
Dendrocoelum adenodactylosum (Stanković & Komárek, 1927)	littoral, sublittoral, profundal zones	littoral cold springs; tributary streams; a tributary of the effluent Crni Drim River	–	Stanković and Komárek 1927; Kenk 1978
Dendrocoelum albidum Kenk, 1978	sublittoral zone	–	+	Kenk 1978
Dendrocoelum cruciferum (Stanković, 1969)	sublittoral zone	–	+	Stanković 1969; Kenk 1978
Dendrocoelum decoratum Kenk, 1978	sublittoral and profundal zones	–	+	Kenk 1978
Dendrocoelum dorsivittatum Kenk, 1978	profundal zone	–	+	Kenk 1978
Dendrocoelum jablanicense (Stanković & Komárek, 1927)	–	Šum Spring; tributary streams	+	Stanković and Komárek 1927; Stanković 1960; Kenk 1978
Dendrocoelum komareki (Stanković, 1969)	sublittoral zone	–	+	Stanković 1969; Kenk 1978
Dendrocoelum lacteum (Müller, 1774)	sublittoral and profundal zones	stagnant waters	–	Stanković and Komárek 1927; Arndt 1938; Stanković 1960; Kenk 1978
Dendrocoelum lacustre (Stanković, 1938)	sublittoral zone	–	+	Stanković 1938; Stanković 1969; Kenk 1978
Dendrocoelum lychnidicum (Stanković, 1969)	sublittoral zone	–	+	Stanković 1969; Kenk 1978

Taxa	Lacustrine habitat	Adjacent waters of lake Ohrid	Endemic species	References
Dendrocoelum maculatum (Stanković & Komárek, 1927)	littoral zone	tributary streams; littoral springs	+	Stanković 1960; Kenk 1978
Dendrocoelum magnum (Stanković, 1969)	sublittoral zone	–	+	Stanković 1969; Kenk 1978
Dendrocoelum minimum Kenk, 1978	profundal zone	–	+	Kenk 1978
Dendrocoelum ochridense (Stanković & Komárek, 1927)	littoral, sublittoral, profundal zones	–	+	Stanković 1960; Kenk 1978
Dendrocoelum sanctinaumi (Stanković & Komárek, 1927)	littoral and sublittoral zones	tributary streams; littoral springs	+	Stanković 1960; Kenk 1978
Dendrocoelum sinisai Kenk, 1978	profundal zone	–	+	Kenk 1978
Dendrocoelum translucidum Kenk, 1978	profundal zone	–	+	Kenk 1978
Planariidae Stimpson, 1857				
***Crenobia* Kenk, 1930**				
Crenobia alpina montenegrina (Mrázek, 1904)	–	springs; tributary streams and the effluent Crni Drim River	–	Stanković and Komárek 1927; Stanković 1960; Kenk 1978
***Phagocata* Leidy, 1847**				
Phagocata maculata (Stanković, 1938)	sublittoral zone	–	+	Stanković 1938; Stanković 1960; Kenk 1978
Phagocata ochridana (Stanković & Komárek, 1927)	littoral, sublittoral, profundal zones	springs and pools	+	Stanković and Komárek 1927; Stanković 1960; Kenk 1978
Phagocata stankovici (Reisinger, 1960)	sublittoral and profundal zones	–	+	Reisinger 1960; Kenk 1978
Phagocata undulata (Stanković, 1960)	sublittoral zone	–	+	Stanković 1960; Kenk 1978
***Planaria* Müller, 1776**				
Planaria torva (Müller, 1774)	–	only one specimen at the mouth of the Studenčišta brook	–	Stanković 1960; Kenk 1978
***Polycelis* Ehrenberg, 1831**				
Polycelis tenuis Ijima, 1884	–	tributary streams	–	Stanković 1960; Kenk 1978

Discussion

Dugesia superioris differs from its congeners in particular in (a) the dorsal course of the ejaculatory duct, with its sub-terminal opening, (b) the asymmetrical openings of the oviducts into the bursal canal, and (c) the openings of vasa deferentia at about halfway along the seminal vesicle.

For the genus *Dugesia* a dorsal course of the ejaculatory duct was reported for the first time by Stocchino et al. (2005) for the endemic Sardinian species *D. hepta* Pala, Casu & Vacca 1981. However, in this species the opening of the duct is located laterally on the right side, near the tip of the penis papilla. Moreover, this species is characteri-zed by a ventro-lateral penial fold, which is absent in the new species. *Dugesia superioris* therefore represents the second species of the genus showing a dorsal course of the ejaculatory duct. Further, another important difference between *D. hepta* and *D. superioris* is the haploid chromosome number, which counts n = 7 in the former (Pala et al. 1981) and n = 8 in the latter (Deri et al. 1999, see below).

A subterminal opening of the ejaculatory duct, as found in *D. superioris*, occurs in no less than 26 species of *Dugesia*: *D. bakurianica* Porfirjeva, 1958, *D. biblica* Benazzi & Banchetti, 1972, *D. leporii* Pala et al., 2000, and *D. sicula* Lepori, 1948, from the Western Palaearctic; *D. aethiopica* Stocchino et al., 2002, *D. arabica* Harrath & Sluys, 2013, *D. astrocheta* Marcus, 1953, *D. lanzai* Banchetti & Del Papa, 1971, *D. lamottei* De Beauchamp, 1952, *D. neumanni* (Neppi, 1904) and *D. myopa* De Vries, 1988b from the Afrotropical Region; the other 15 species are distributed in the Oriental Region, Eastern Palaearctic and Australasian Region, viz. *D. andamanensis* (Kaburaki, 1925), *D. austroasiatica* Kawakatsu, 1985, *D. batuensis* Ball, 1970, *D. bengalensis* Kawakatsu, 1983, *D. burmanensis* (Kaburaki, 1918), *D. deharvengi* Kawakatsu & Mitchell, 1989, *D. indica* Kawakatsu, 1969, *D. indonesiana* Kawakatsu, 1973, *D. japonica* Ichikawa & Kawakatsu, 1964, *D. leclerci* Kawakatsu & Mitchell, 1995, *D. lindbergi* De Beauchamp, 1959, *D. nannophallus* Ball, 1970, *D. novaguineana* Kawakatsu, 1976, *D. tamilensis* Kawakatsu, 1980, and *D. uenorum* Kawakatsu & Mitchell, 1995. However, in all of these species the ejaculatory duct is ventrally displaced, except for *D. bakurianica* in which the ejaculatory duct is central. Therefore, a dorsal course of the ejaculatory duct and a subterminal opening of the duct represents a new diagnostic combination in the genus *Dugesia*.

The Pogradec population had already been subjected to karyological, cytogenetic, and phylogeographic studies before anything was known about the anatomy of the specimens (see above). All of these analyses pointed to a situation that this *Dugesia* population differs considerably from congeneric populations. Therefore, it was unsurprising that the anatomy of the Pogradec animals suggested also that they represent a new species. As a result of the cumulation of the evidences from these independent datasets, the present delineation of the new species is based on a truly integrative approach to taxonomy.

Studies on the phylogeny of *Dugesia* (Sluys et al. 1998 and references therein) considered the asymmetrical penial papilla to constitute an important taxonomic feature.

However, this asymmetry related to the apomorphic presence of a ventral ejaculatory duct. Our present study shows that in future analyses this asymmetry needs to be specified by adding a third character state to character (1) (Sluys et al. 1998, p. 277 and Table II), i.e. ejaculatory duct located dorsally.

An asexual population of *Dugesia* sp. was collected in 2006 by R. Manconi from Voskopojë, an Albanian locality situated south-west of Lake Ohrid. Unfortunately, we have been unable to ascertain the taxonomic status of this population due to the lack of sexual specimens (Stocchino and Manconi, pers. obs.). However, according to the phylogeographic analysis of Lázaro et al. (2009) this population is molecularly identical to the Pogradec population and therefore should be assigned also to *D. superioris.* It is noteworthy that the Voskopojë locality is outside of the Ohrid basin and therefore signals a wider distribution of *D. superioris.*

That Kenk (1978) identified his *Dugesia* material from Ohrid (NMNH 55294) as *D. gonocephala* is hardly surprising in view of the fact that at that time many European populations were assigned to *D. gonocephala sensu lato*. The precise anatomy of *D. gonocephala sensu stricto* was only resolved by De Vries and Ball (1980) and De Vries (1984a, 1986). A comparison with Kenk's specimen quickly learns that this animal does not conform to *D. gonocephala* because it does not exhibit the muscular ridges, the elongated penis papilla, or the two penial folds (cf. De Vries and Ball 1980, De Vries 1984a). In the presence of a small dorsal penial fold and a central ejaculatory duct the animal resembles *Dugesia benazzii* Lepori, 1951, *D. elegans* De Vries, 1984, *D. taurocaucasica* (Livanov, 1951) and *D. effusa*, the latter recently described from the Greek island Chios (Sluys et al. in prep.). *Dugesia benazzii* from Corsica and Sardinia is characterized by a pointed diaphragm and a penial fold, the position of which is variable but which is usually located dorsally; the size of the penial fold is also variable (Lepori 1951, De Vries 1984b). In *D. benazzii* ectal reinforcement is restricted to the region of the oviducal openings, the latter being symmetrically arranged. In contrast, in the NMNH 55294 specimen the oviducts open asymmetricaly into the bursal canal, while the ectal reinforcement extends further on the bursal canal.

Dugesia elegans from Rhodes differs from NMNH 55294 in the presence of a much larger seminal vesicle, a stubbier diaphragm, and the situation that its bursal canal epithelium is infranucleated (De Vries 1984a).

The penial fold of *D. taurocaucasica* is considerably larger than the one in NMNH 55294, while the fold is also traversed by the abundant secretion of cyanophilic glands, which discharge through the lining epithelium of the penial fold. Furthermore, in *D. taurocaucasica* the ectal reinforcement layer on the bursal canal extends for a considerable distance towards the copulatory bursa (Porfirjeva and Dyganova 1987).

The species *Dugesia effusa* differs from NMNH 55294 in the presence of a short, valve-like diaphragm, a large intrabulbar seminal vesicle, a highly glandular penis papilla, and symmetrical oviducal openings into the bursal canal.

Therefore, the *Dugesia* specimen NMNH 55294 may well represent a new species. However, on the basis of only the presently available material we refrain from describing it as new. Furthermore, the asymmetrical openings of the vasa deferentia into the seminal vesicle of this animal represents a highly unusual condition for a species of *Dugesia* and needs to be checked on additional material.

Present data support Stanković' (1960) suggestion that two faunistic complexes may be distinguished in the Lake Ohrid region, viz. (1) lacustrine endemic forms and (2) inhabitants of other waters outside of the lake with a wider distributional range. In the Ohrid region it is evident that there is a very low degree of exchange between the lacustrine endemic fauna and the non-endemic fauna. Further, the distribution of triclads in the Lake Ohrid area supports the situation for the lake's fauna in general, namely that endemism occurs at different spatial scales, ranging from species endemic to some parts of the lake to species endemic to the whole Ohrid basin (Albrecht and Wilke 2008).

Acknowledgements

This research was funded in part by the Regione Autonoma Sardegna (CRP-60215 "Conservazione e valorizzazione delle grotte sarde: biodiversità e ruolo socio-economico-culturale). G.A.S. acknowledges financial support by the "Fondazione Banco di Sardegna" and SYNTHESYS, a programme of the European Commission under the 7th Research and Technological Development Framework Programme (grant number: NL-TAF 2772). Completion of the manuscript was made possible by a grant from the Naturalis Biodiversity Center to R. Sluys. Dr. Jon Norenburg (National Museum of Natural History (NMNH), Washington, USA) kindly sent on loan the *Dugesia* specimen collected by R. Kenk. We thank Prof. G. Corso for her kind support. G.A.S. is grateful to Prof. M. Pala for her financial support in memory of her husband Prof. N.G. Lepori. R.S. thanks Prof. Dr. M. Kawakatsu for providing a copy of a cited publication.

References

Albrecht C, Wilke T (2008) Ancient Lake Ohrid: biodiversity and evolution. Hydrobiologia 615: 103–140. doi: 10.1007/s10750-008-9558-y

Arndt W (1938) Spongiologische Unterschungen am Ochridasee. Archiv für Hydrobiologie 34: 48–80.

Ball IR (1970) Freshwater Triclads (Turbellaria, Tricladida) from the Oriental Region. Zoological Journal of the Linnean Society 49: 271–294. doi: 10.1111/j.1096-3642.1970.tb00742.x

Banchetti R, Del Papa R (1971) Descrizione della planaria *Dugesia lanzai*, n. sp. del Kenya (Africa). Atti della Società Toscana di Scienze Naturali 78: 20–35.

Benazzi M (1946) Sopra una nuova planaria d'acqua dolce. Archivio Zoologico Italiano 31: 93–102.

Batistoni R, Rossi L, Salvetti A, Deri P (1999) A molecular cytogenetic comparison of planarians from the '*Dugesia gonocephala* group' (Platyhelminthes, Tricladida). Italian Journal of Zoology 66: 239–244. doi: 10.1080/11250009909356261

Benazzi M, Banchetti R (1972) Descrizione di *Dugesia biblica*, nuova microspecie del "gruppo *Dugesia gonocephala*" trovata nel fiume Giordano (Israele). Atti della Società Toscana di Scienze Naturali 79: 83–91.

Benazzi M, Benazzi Lentati G (1976) Platyhelminthes. In: John B (Ed) Animal cytogenetics. Gebrüder Borntraeger, Berlin-Stuttgart, Vol I, 1–182.

De Beauchamp P (1952) Sur les *Dugesia* (Turbellariés Triclades) d'Afrique tropicale et de Madagascar. Bulletin de la Société Zoologique de France 77: 362–370.

De Beauchamp P (1959) Triclades Paludicoles d'Afganistan (Contribution à l'Ètude de la Faune d'Afganistan, I). Kungliga Fysiografiska Sällskapets i Lund Förhandlingar 29: 27–43.

Deri P, Colognato R, Rossi L, Salvetti A, Batistoni R (1999) A karyological study on populations of *Dugesia gonocephala* s.l. (Turbellaria, Tricladida). Italian Journal of Zoology 66: 245–253. doi: 10.1080/11250009909356262

De Vries EJ, Ball IR (1980) On *Dugesia gonocephala* from Western Europe. Bijdragen tot de Dierkunde 50: 342–350.

De Vries EJ (1984a) On the species of the *Dugesia gonocephala* group (Platyheminthes, Turbellaria, Tricladida) from Greece. Bijdragen tot de Dierkunde 54: 101–126.

De Vries EJ (1984b) On the taxonomic status of *Planaria torva* from Corsica (Turbellaria, Tricladida, Paludicola). Mitteilungen aus dem Zoologischen Museum in Berlin 60: 17–21.

De Vries EJ (1986) On the taxonomic status of *Dugesia gonocephala* and *Dugesia subtentaculata* (Turbellaria, Tricladida, Paludicola). Journal of Zoology, London 209: 43–59. doi: 10.1111/j.1469-7998.1986.tb03565.x

De Vries EJ (1988a) Further contributions to the taxonomy and biogeography of the subgenus *Dugesia* (Platyhelminthes: Tricladida: Paludicola) in the Mediterranean region and the Middle East. Israel Journal of Zoology 35: 109–136.

De Vries EJ (1988b) A synopsis of the nominal species of the subgenus *Dugesia* (Platyhelminthes, Tricladida, Paludicola) from Africa and Madagascar. Zoological Journal of the Linnean Society 92: 345–382. doi: 10.1111/j.1096-3642.1988.tb01729.x

Harrath AH, Sluys R, Aldahmash W, Al-Razaki A, Alwasel S (2013) Reproductive strategies, karyology, parasites, and taxonomic status of *Dugesia* populations from Yemen (Platyhelminthes, Tricladida, Dugesiidae). Zoological Science 30: 502–508. doi: 10.2108/zsj.30.502

Ichikawa A, Kawakatsu M (1964) A new freshwater planarian, *Dugesia japonica*, commonly but erroneously known as *Dugesia gonocephala* (Dugès). Annotationes Zoologicae Japonenses 36: 102–107.

Ijima I (1884) Untersuchungen über den Bau und die Entwicklungsgeschichte der Süsawasser-Dendrocoelen (Tricladen). Zeitschrift für wissenschaftliche Zoologie 40: 359–464.

Kaburaki T (1918) Freshwater Triclads from the Basin of the Inlé Lake. Records of the Indian Museum 14: 187–194.

Kaburaki T (1925) Planarians from the Andamans. Records of the Indian Museum 27: 29–32.

Kawakatsu M (1969) Report on Freshwater Planaria from India. Annotationes Zoologicae Japonenses 42: 210–215.

Kawakatsu M (1973) Report on Freshwater Planaria from Indonesia (Sumatra and Java). Contributions from the Biological Laboratory, Kyoto University 24: 87–103.

Kawakatsu M (1976) The Freshwater Planarians from New Guinea and Malaya. Bulletin of the National Museum, Tokyo 2: 143–152.

Kawakatsu M, Mitchell RW (1989) *Dugesia deharvengi* sp. n., a new troglobitic freshwater planarian from Tham Kubio Cave, Thailand (Turbellaria; Tricladida; Paludicola). Bulletin of the Biogeographical Society of Japan 44: 175–182.

Kawakatsu M, Mitchell RW (1995) Two new freshwater cavernicolous planarians (Turbellaria, Tricladida, Paludicola) from Sulawesi (Celebes), Indonesia. Special Bulletin of the Japanese Society of Coleopterology 4: 81–103.

Kawakatsu M, Oki I, Tamura S, Yamayoshi T (1985) Reexamination of freshwater planarians found in tanks of tropical fishes in Japan, with a description of a new species, *Dugesia austroasiatica* sp. n. (Turbellaria; Tricladida; Paludicola). Bulletin of the Biogeographical Society of Japan 40: 1–19.

Kawakatsu M, Tamura S, Yamayoshi T, Oki I (1980) The Freshwater Planaria from Thailand and South India. Annotationes Zoologicae Japonenses 42: 210–215.

Kawakatsu M, Oki I, Tamura S, Yamayoshi T, Aditya AK (1983) A new freshwater planarian from West Bengal. Bulletin of the Biogeographical Society of Japan 38: 1–10.

Kenk R (1978) The planarians (Turbellaria: Tricladida Paludicola) of the Lake Ohrid in Macedonia. Smithsonian Contributions to Zoology 280: 1–56. doi: 10.5479/si.00810282.280

Lázaro EM, Sluys R, Pala M, Stocchino GA, Baguñà J, Riutort M (2009) Molecular barcoding and phylogeography of sexual and asexual freshwater planarians of the genus *Dugesia* in the Western Mediterranean (Platyhelminthes, Tricladida, Dugesiidae). Molecular Phylogenetics and Evolution 52: 835–845. doi: 10.1016/j.ympev.2009.04.022

Lepori NG (1948) Descrizione di *Dugesia sicula*, nuova specie di Triclade d'acqua dolce dei dintorni di Catania. Archivio Zoologico Italiano 33: 461–472.

Lepori NG (1951) Sulle caratteristiche morfologiche e sulla posizione sistematica della planaria di Sardegna e Corsica già ascritta a *Dugesia gonocephala* (Dugès). Atti Società Toscana di Scienze Naturali 58 (B): 1–22.

Livanov NA (1951) Planarii Kopet-Daga i blizkie vidy Kryma, Kavkaza i Zakavkaz'ia. Trudy Murgabskoĭ Gidrobiologicheskoĭ Stantsii 1: 103–114.

Marcus E (1953) Turbellaria Tricladida. In: Institut des Parcs Nationaux du Congo Belge, Exploration du Parc National de l'Upemba, Mission G.F. de Witte, 1946–1949, 21: 1–62.

Mrázek A (1904) Ergebnisse einer von Dr. Al. Mrázek im J. 1902 nach Montenegro unternommenen Sammelreise. Věstnik královské České Společnosti Náuk, Třída mathematic-ko-přirodovědecká, for 1903 (15).

Müller OF (1774) Vermium terrestrium et fluviatilium, seu Animalium infusorium, helminthicorum, et testaceorum, non marinorum, succincta historia. Hauniae & Lipsiae. 1(2).

Neppi V (1904) Über einige exotische Turbellarien. Zoologische Jahrbücher, Abteilung für Systematik Geographie und Biologie der Tiere 21: 303–326.

Pala M (1993) Correlation between chromosome complement and reproduction in a population of a freshwater triclad. In: Animal Biology. 39th Annual Meeting of the Italian Embryology Group, Alghero (Italy), June 1993. 2: 131.

Pala M, Casu S, Vacca RA (1981) *Dugesia hepta*, nuova specie di planaria d'acqua dolce di Sardegna appartenente alla superspecie *Dugesia gonocephala* (Dugès) (Turbellaria, Tricladida). Bollettino della Società Sarda di Scienze Naturali 20: 97–107.

Pala M, Stocchino GA, Corso G, Casu S (2000) A new species of freshwater planarian belonging to the genus *Dugesia* (Platyhelminthes, Tricladida) from Sardinia. Italian Journal of Zoology 67: 379–383. doi: 10.1080/11250000009356343

Porfirjeva NA (1958) Planarii Kavkaza [Planarians of the Caucasus]. Trudy Obshchestva Estestvoispytateleĭ pri Kazanskom gosudarstvennom Universitete 62: 39–89. [in Russian]

Porfirjeva NA, Dyganova RY (1987) Planarii europeiskoi ciasti SSSR [Planarians of the European part of the USSR – Morphology, Systematics, Distribution]. University of Kazan. 188 pp. [in Russian]

Reisinger E (1960) Vitale Nervenfärbungen bei Plathelminthen und ihre Abhängigkeit vom physiologischen Zustand des Organismus. Zeitschrift für wissenschaftliche Zoologie 164: 271–293.

Ribas M (1990) Cariologia, sistematica i biogeografia de les Planaries d'aigues dolces al Paisos Catalans. Barcelona: PhD thesis, Barcelona, Spain: University of Barcelona.

Schmidt O (1861) Ueber *Planaria torva* Autorum. Zeitschrift für wissenschaftliche Zoologie 11: 89–94.

Sluys R, Kawakatsu M, Winsor L (1998) The genus *Dugesia* in Australia, with its phylogenetic analysis and historical biogeography (Platyhelminthes, Tricladida, Dugesiidae). Zoologica Scripta 27: 273–289. doi: 10.1111/j.1463-6409.1998.tb00461.x

Sluys R, Solà E, Gritzalis K, Vila-Farré M, Mateos E, Riutort M (in prep) Integrative delineation of species of Mediterranean freshwater planarians (Platyhelminthes, Tricladida, Dugesiidae).

Solà E, Sluys R, Gritzalis K, Riutort M (2013) Fluvial basin history in the northeastern Mediterranean underlies dispersal and speciation patterns in the genus *Dugesia* (Platyhelminthes, Tricladida, Dugesiidae). Molecular Phylogenetics and Evolution 66: 877–888. doi: 10.1016/j.ympev.2012.11.010

Stanković S (1938) Novi prilozi poznavanju endemičnih triklada Ohridskog jezera (Nouvelle contribution à la connaissance des Triclades endémiques du lac d'Ohrid). Glasnik Skopskog Naučnog Drušva 18: 1–12 [in Serbo-Croatian]

Stanković S (1960) The Balkan lake Ohrid and its living world. Uitgeverij Dr Junk W, Den Haag, 357 pp.

Stanković S (1969) Turbellariés Triclades endémiques nouveaux du lac d'Ohrid. Archiv für Hydrobiologie 65: 413–435.

Stanković S, Komárek J (1927) Die Süsswasser-Tricladen des Westbalkans und die zoogeographischen Probleme dieser Gegend. Zoologische Jahrbücher, Abteilung für Systematik 53: 591–674.

Stocchino GA, Corso G, Manconi R, Pala M (2002) African planarians: *Dugesia aethiopica* sp. n. (Platyhelminthes, Tricladida) from Lake Tana (NW Ethiopia). Italian Journal of Zoology 69: 45–51. doi: 10.1080/11250000209356437

Stocchino GA, Corso G, Manconi R, Casu S, Pala M (2005) Endemic freshwater planarians of Sardinia: Redescription of *Dugesia hepta* (Platyhelminthes, Tricladida) with a comparison of the Mediterranean species of the genus. Journal of Natural History 39: 1947–1960. doi: 10.1080/00222930500060025

Sywula T, Krstanovski Z, Biala A, Wysocka A, Kilikowska A, Sell J (2006) Phylogenetic position of *Dendrocoelum lacteum* (Tricladida) from the Balkan Lake Ohrid evidenced by allozyme data. Biochemical Systematics and Ecology 34: 212–218. doi: 10.1016/j.bse.2005.11.003

ZooKeys 313: 45–59 (2013)
doi: 10.3897/zookeys.313.4904
www.zookeys.org

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A new species of *Elpidium* (Crustacea, Ostracoda) from bromeliads in Cusuco National Park, Honduras

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Academic editor: *Niel Bruce* | Received 22 February 2013 | Accepted 19 June 2013 | Published 1 July 2013

urn:lsid:zoobank.org:pub:5334A1D9-46F9-4D2F-934B-BCE6CEC07166

Citation: Pinto RL, Jocqué M (2013) A new species of *Elpidium* (Crustacea, Ostracoda) from bromeliads in Cusuco National Park, Honduras. ZooKeys 313: 45–59. doi: 10.3897/zookeys.313.4904

Abstract

Passively dispersing aquatic invertebrates such as Ostracoda in restricted aquatic habitats such as bromeliads remain an intriguing observation considering the highly specialised dispersal vectors needed for efficient colonisation. Here we describe a new species of *Elpidium, E. merendonense* **sp. n.**, collected from bromeliads in the cloud forest from Cusuco National Park, Honduras. *Elpidium merendonense* **sp. n.** is a small to medium-sized species that can be easily distinguished from its congeners by its unique outgrowth at the posterior end of the left valve, visible especially in females. The species was common all through the park occurring at a wide range of altitudes and in different species of bromeliads. This finding is the first freshwater ostracod species described from Honduras and is in agreement with the prediction that the genus *Elpidium* contains a large number of species with small geographic distributions. We update the list of described species of *Elpidium* and present a key to species.

Keywords

Timiriaseviinae, phytotelmata, dispersal, endemism, neotropics, cloud forest

Introduction

Phytotelmata are plant structures that hold water, such as tree holes, flowers, husks or leaf brackets. These water bodies are completely rain dependent and often contain communities of highly specialised aquatic invertebrates (Kitching 2000). Well known examples of phytotelmata are several species of Bromeliaceaea, a group of terrestrial and epiphytic perennial plants distributed from Argentina to Florida, and with one representative in Africa. The bromeliads that contain enough water to house aquatic invertebrate communities are called tank bromeliads. As a consequence of their architecture, bromeliads provide high quality living space in the form of humus and moisture for diverse biota (Benzing 2000).

The first discovery of an ostracod species in bromeliad tanks was published by Fritz Müller (1880), based on material collected in the state of Santa Catarina, Southern Brazil. A new genus and species were then described, *Elpidium bromeliarum* Müller, 1880, and one year later an extension of the species description was published (Müller 1881). A putative record of this species was later reported from Costa Rica (Picado 1913). Since then, other species in the genus have been described from the Caribbean, all of them from bromeliad phytotelmata. Tressler (1941) described *E. maricaoensis* (Tressler, 1941) from Puerto Rico. After studying material from Jamaica, Tressler (1956) described *E. laesslei* (Tressler, 1956) and recorded *E. bromeliarum* from that country. Pinto and Purper (1970) published a revision of the genus, wherein a redescription of the type species was provided with the designation of a neotype, since the original type series had been lost. Three new species were described from Cuba (Danielopol 1975) but were left in open nomenclature (*Elpidium* sp. A, *Elpidium* sp. B and *Elpidium* sp. C). These three Cuban species were later named *Elpidium inaequivalve* Danielopol, 1980, *Elpidium pintoi* Danielopol, 1980 and *Elpidium purperae* Danielopol, 1980 respectively (Colin and Danielopol 1980).

Studies on *Elpidium* remained predominantly taxonomic until relatively recently, when Little and Hebert (1996) investigated the evolutionary ecology of *Elpidium* ostracods in Jamaica: nine species could be distinguished based on morphological and genetic evidence, but none of these were formally described. Additionally, most species showed restricted distribution (high endemism) and only rarely species co-occurred, which led the authors to conclude that bromeliads seem to be a source of high biodiversity, working as 'ecological islands' that lead to frequent allopatric speciation events (Little and Hebert 1996). These results suggest that the diversity of the genus, with its current six valid species, is strongly underestimated and that the whole area of distribution of the genus has been thus far poorly investigated.

In the present work, we report on a new species of *Elpidium* from Cusuco National Park, which consists of the first ostracod species collected from bromeliad phytotelmata in Honduras.

Material and methods

Material used for the species description was collected during the survey of aquatic invertebrate communities in bromeliads from 12th July to 22nd August 2006 in Cusuco National Park (CNP), Honduras. This survey is part of the yearly biodiversity survey by the ecovolunteer driven conservation organisation Operation Wallacea (UK) in CNP. CNP is situated in north-western Honduras, within the Merendón Mountain range. The core zone of the park consists of lower montane tropical rain forest (a mix of primary and secondary), with patches of primary cloud forest and upper montane rain forest characterised by high densities of bromeliads. Bromeliads sampled for Ostracoda were *Tillandsia guatemalensis* Smith and *Catopsis* sp. We sampled a total of 110 bromeliads in 12 different sites in the park, for a more detailed description of the study of aquatic invertebrates in bromeliads we refer to the protocol described in Jocque et al. (2010). The material in this study comes from a single bromeliad (*Tillandsia guatemalensis*) in the core zone of the park. The bromeliad was attached at about 1.5 meter above the ground. The collected plants were dismantled leaf by leaf and ostracods were collected from the water in the bromeliads and preserved in 70% ethanol.

For description, specimens were dissected under a stereomicroscope; valves were stored dry in micropaleontological slides and soft parts were mounted in permanent slides with CMC-9AF mounting medium (Masters Company Inc., Bensenville, Illinois, USA). Micrographs of valves and carapaces were obtained with a scanning electron microscope; line drawings of appendages and body parts were made under an optical microscope with the aid of a camera lucida.

All analysed material is deposited in the crustacean collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Higher taxonomy of the Ostracoda follows the synopsis by Horne et al. (2002).

Taxonomy

Class Ostracoda Latreille, 1802
Subclass Podocopa G.W. Müller, 1894
Order Podocopida G.O. Sars, 1866
Suborder Cytherocopina, Baird, 1850
Superfamily Cytheroidea Baird, 1850
Family Limnocytheridae Klie, 1938
Subfamily Timiriaseviinae Mandelstam, 1960

Genus *Elpidium* F. Müller, 1880
http://species-id.net/wiki/Elpidium

Type species (by original designation): *Elpidium bromeliarum* F. Müller, 1880.

Other species allocated: *Elpidium inaequivalve* Danielopol, 1980; *Elpidium laesslei* (Tressler, 1956) Danielopol, 1980; *Elpidium maricaoensis* (Tressler, 1941) Danielopol, 1980; *Elpidium pintoi* Danielopol, 1980; *Elpidium purperae* Danielopol, 1980.

Diagnosis. Medium sized to relatively large ostracods; carapace broad, generally bigger in width than in height; ventral margin flat; pale to dark brown smooth surface; in dorsal and ventral view males with greatest width at midlength, females with posterior part expanded into a brood pouch carrying eggs and greatest width displaced posteriorly; strongly interlocking selvages along ventral margin leaving an anteroventral gap between left and right valve margins; hinge a crenulated cardinal bar on the smaller valve forming rudimentary anterior and well developed posterior teeth; A1 with 5 functional articles, the first one bearing a sub-apical expansion with a tuft of tiny setules on the dorsal margin; in males, A2 with a serrated apical claw on the terminal segment (no such serration in females); terminal segment of A2 with a small lobe (hyaline formation) in both males and females; second and third endites of the maxillule bearing two spatulate claws each; copulatory process of hemipenis a hook-like structure placed ventrally on the muscular body, near the base of distal lobe.

Comparison between *Elpidium* and *Intrepidocythere*. *Elpidium* is closely related (Pinto et al. 2008) to the terrestrial genus *Intrepidocythere* Pinto et al., 2008. Nonetheless several differences can be recognized. The carapace of *Intrepidocythere* is smaller and considerably less broad in dorsal view compared to *Elpidium* species. Furthermore, the marginal zone and hinge structures are different, while *Elpidium* has a long bar on the smaller valve ending in a small anterior tooth and a crenulated posterior tooth, *Intrepidocythere* has a smooth medial ridge and a posterior socket in left valve with the complementary smooth medial groove and posterior tooth in right valve. The antennule has 2 medio-dorsal setae on the fourth segment in *Elpidium*, but only 1 in *Intrepidocythere*; the male antenna has 1 serrated claw on the terminal segment in *Elpidium*, but 2 in *Intrepidocythere*; and the female caudal ramus has three setae in *Elpidium*, but only two in *Intrepidocythere*.

Elpidium merendonense sp. n.

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http://species-id.net/wiki/Elpidium_merendonense
Figs 1–4

Type locality. Cusuco National Park, Honduras at an altitude of 1840 masl. Geographical coordinates: 15.5133; -88.2417. Water accumulated in bromeliad (*Tillandsia guatemalensis*) leaf axils. Material collected on 15 July 2006 by M. Jocqué.

Type material. Holotype: a dissected male, with valves dried and coated for scanning electron microscopy stored in a micropaleontological slide and soft parts mounted in a permanent slide with CMC-9AF mounting medium (MZUSP 29072). Allotype: a dissected ovigerous female, with valves stored dry in a micropaleontological slide and soft parts mounted in a permanent slide with CMC-9AF mount-

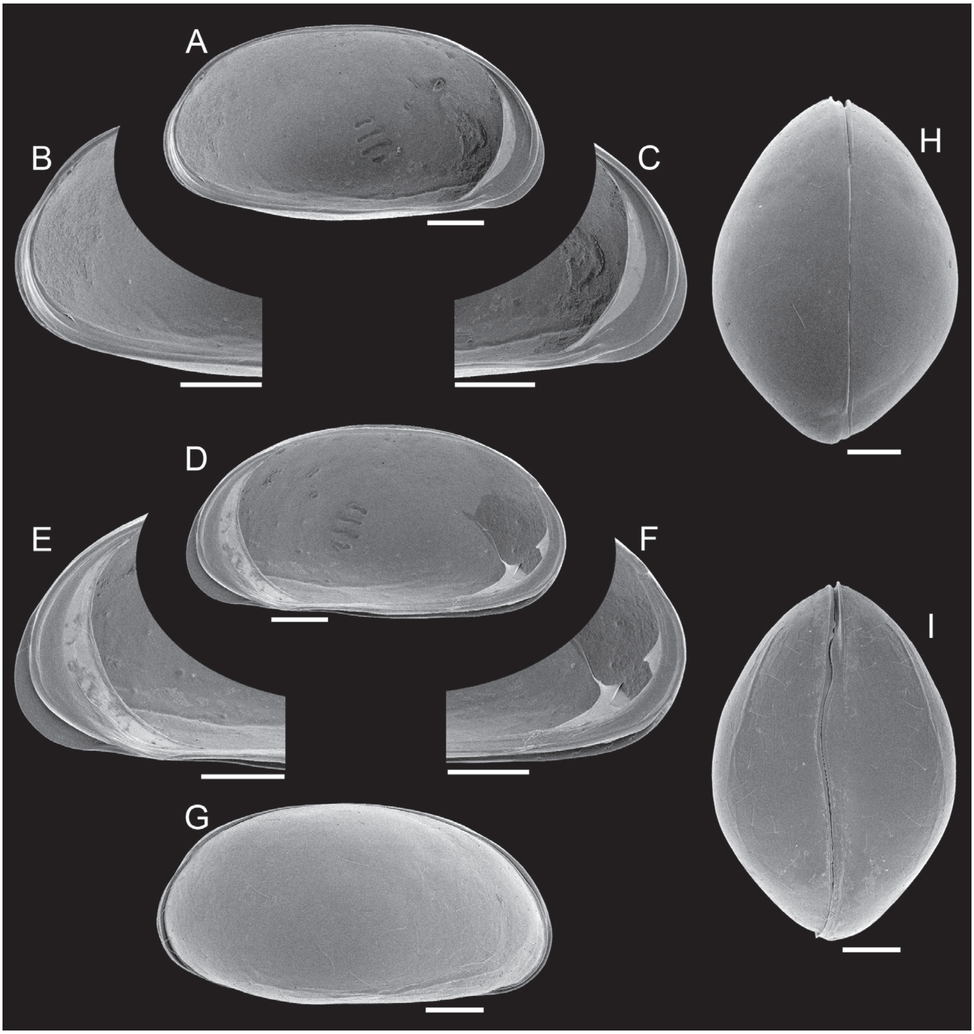

Figure 1. *Elpidium merendonense* sp. n., male. **A** Left valve, internal view **B** left valve, internal view, detail of postero-ventral margin **C** left valve, internal view, detail of antero-ventral margin **D** right valve, internal view **E** right valve, internal view, detail of antero-ventral margin **F** right valve, internal view, detail of postero-ventral margin **G** right lateral view **H** dorsal view **I** ventral view. **A–F** holotype, MZUSP 29072; **G** paratype, MZUSP 29077; **H** paratype, MZUSP 29078; **I** paratype, MZUSP 29079. Scale bars: 100 µm.

ing medium (MZUSP 29073). Paratypes: two males (MZUSP 29074 and MZUSP 29075), dissected and stored like the allotype; an ovigerous female (MZUSP 29076) dissected and stored like the holotype; three males (MZUSP 29077, MZUSP 29078 and MZUSP 29079) and three females (MZUSP 29080, MZUSP 29081 (carapace broken) and MZUSP 29082), dried and coated for scanning electron microscopy stored in micropaleontological slides; 25 males and 8 females kept whole in a vial with 70% ethanol (MZUSP 29083).

Derivation of name. The species is named after the Merendón mountains in Honduras, where the specimens described in the present work were collected.

Diagnosis. Small sized *Elpidium* (c. 700 µm). In dorsal and ventral views carapace relatively elongated for the genus (length/width c. 1,4). In right lateral view carapace elongated (length/height c. 2), with left valve overlapping right valve on all margins, but very strongly at the posterior end of the carapace, especially in females, where this overlap produces a conspicuous outgrowth of the outer lamella, apparently without substantial change to the inner marginal structures. Ventral margin flat, with a subtle ventro-lateral ridge on each valve, at the edge of the flat area. Distal lobe on hemipenis triangular, with a pointed tip and with a finger-like projection at the base of the internal margin next to the dorsal seta; copulatory process a stiff hook-like structure, thick at the first half of its length and then quickly narrowing to the orifice at the tip.

Description of male. Carapace (Fig. 1G–I). Small sized (length = 657–685 µm), with brown surface and sparse setae; surface smooth except for a subtle ventro-lateral ridge on each valve; elliptically elongated in lateral view (length/height=1.96), with greatest height just behind the mid-length; left valve overlapping right valve on all margins, strongly interlocking in antero-ventral, ventral and postero-ventral margins; ventral area flat; dorsal margin arched; posterior and anterior margins rounded, both produced towards the ventral side; oval shaped in ventral and dorsal views, with maximum width behind mid-length; dorsal margin straight in dorsal view; ventral margin sinuous in ventral view with well-marked ridges.

Left valve (Fig. 1A–C). In internal view, anterior and posterior margins rounded, produced towards the ventral side; ventral margin nearly straight; dorsal margin arched; calcified inner lamella well developed anteriorly, with a short line of concrescence near the valve margin leaving a vestibule; vestibule less developed posteriorly, but present; prominent selvage running on all margins except in the middle of dorsal margin; a short flange is formed at the antero-ventral margin; central muscle scars consisting of 4 spots arranged in a vertical row relatively separated from each other and a round small frontal scar.

Right valve (Fig. 1D–F). In internal view, anterior and posterior margins rounded, produced towards the ventral side; ventral margin nearly straight; dorsal margin arched; calcified inner lamella well developed anteriorly, with a short line of concrescence near the valve margin leaving a vestibule; vestibule less developed posteriorly but present; prominent selvage running all around, forming the hinge structures on the dorsal margin; a continuous flange is present, being wide on the antero-ventral margin, very narrow at the mouth region and narrow on the ventral and postero-ventral margins; central muscle scars consisting of 4 spots relatively separated from each other arranged in a vertical row and a round small frontal scar.

Hinge (Fig. 1 A–F). A long (c. 3/4 of the valve length) cardinal ridge is present on the right valve, forming at each end, respectively, a small anterior tooth and a large posterior tooth; the ridge is slightly crenulated, especially at the posterior end; RV with complimentary groove and sockets.

Figure 2. *Elpidium merendonense* sp. n. **A** Antennula **B** Antenna **C** terminal segment of antenna **D** Mandibula **E** Mandibular palp **F** Maxillula **A–B, F** male specimen, holotype, MZUSP 29072 **C** female specimen, allotype, MZUSP 29073 **D–E** male specimen, paratype, MZUSP 29075. Scale bars: **A–B, D–F**: 50 µm; **C**: 20 µm. Ya = aesthetasc on terminal segment of A1. Y = aesthetasc on penultimate segment of antennae. hy = hyaline organ on second antennae.

Pigmented naupliar eye present; carapace less pigmented at the eye region.

Antennula (Fig. 2A). 5 functional articles; first article relatively large, bearing on the dorsal margin a sub-apical expansion with a tuft of tiny setules; second article the longest with a ventro-proximal long and thick seta; third article small with a short dorso-apical seta; fourth article partially subdivided in two, medially (where the segment is subdivided) with two dorsal and one ventral setae, and apically with a long ventro-apical, two short and one long dorso-apical setae; fifth (terminal) article with two long setae, one short seta and a short aesthetasc (Ya).

Antenna (Fig. 2B). Protopodite 2-segmented, the first one very short and the second one long, wide and curved; endopodite 3-articulated; first segment relatively short, bearing a long ventro-apical seta; second segment very long and narrow, dorsally with two subapical setae, one three thirds as long as the other, ventro-medially with a short seta and an aesthetasc (Y), and apically with two setae, one large and one minute; last segment small, with three claws, the ventral one strongly serrated and the other two slender, a minute seta and a tiny lobe (hyaline formation); exopodite with a very small seta and a spinneret seta.

Mandible (Fig. 2D–E). Coxa with 7 strong teeth and 6 setae on inner edge and a seta on outer edge (near the articulation with the palp); palp 4-segmented (basis + 3-segmented endopodite); basis externally with respiratory plate (exopodite) consisting of 3 rays and one reflexed seta, and internally with two setae, one two thirds as long as the other; first endopodal segment with two apical internal setae, one less than half as long as the other; second endopodal segment with an internal apical seta and 4 external apical setae, one short, two long and one intermediate; terminal endopodal segment with 2 setae and one slender claw, all equally long.

Maxillula (Fig. 2F). Internally with three endites, first one with 2 setae, second and third ones each with 3 setae and two claws, the latter with a conspicuous spoon-shaped apex; palp not segmented, tapering, with 2 apical setae; respiratory plate well developed, carrying a reflexed seta (i.e. reversed towards the front) and 16 long rays.

First thoracopod (Fig. 3A). 4-segmented; first segment with a long medio-proximal dorsal seta, a medium-sized medio-ventral seta and two stout short ventro-apical setae; second segment quite long, with a strong ventro-apical seta; third segment devoid of setae; terminal segment with an apical claw that bears a minute seta at its base.

Second thoracopod (Fig. 3B). 4-segmented; first segment with a long medio-proximal dorsal seta, a medium-sized medio-ventral seta and a relatively short ventro-apical seta; second segment long, with a strong ventro-apical seta reaching tip of following segment; third segment devoid of setae; terminal segment with an apical claw bearing a minute seta at its base.

Third thoracopod (Fig. 3C). 4-segmented, quite slender; first segment with a medio-proximal dorsal seta, a medio-ventral seta and a ventro-apical seta; second segment quite long, with a slender ventro-apical seta; third segment devoid of setae; terminal segment with a very long and slim apical claw carrying a minute seta at its base.

Hemipenis (Fig. 3D). Consisting of a large rounded muscular body, an articulating distal lobe and a dorsal seta; distal lobe triangular, with a pointed tip and with a

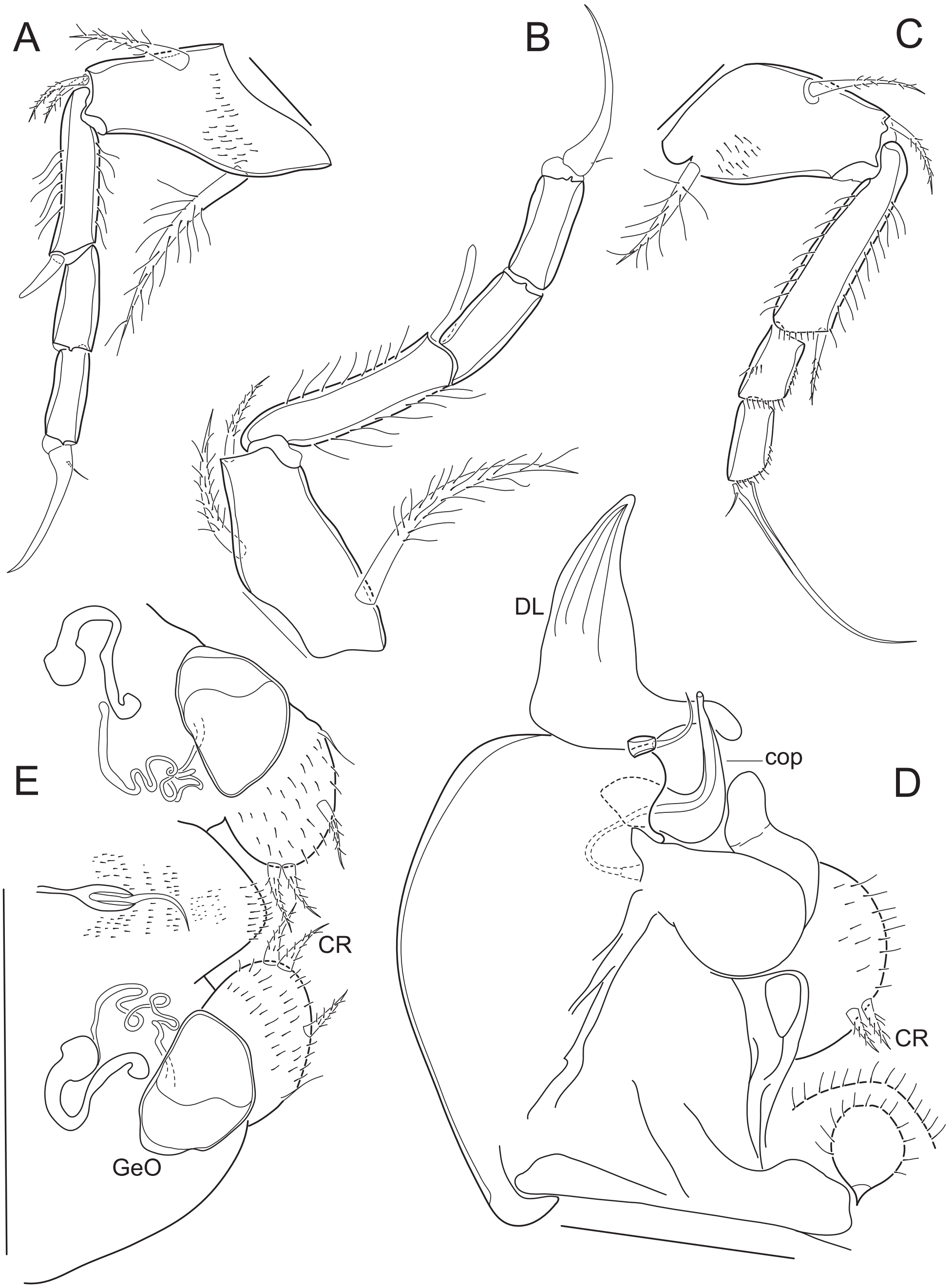

Figure 3. *Elpidium merendonense* sp. n. **A** First thoracic leg **B** second thoracic leg **C** third thoracic leg **D** Hemipenis **E** Abdomen. **A–D** male specimen, holotype, MZUSP 29072 **E** female specimen, allotype, MZUSP 29073. Scale bars: **A–C**: 50 µm; **D**: 100 µm; **E**: 200 µm. DL = distal lobe on hemipenis. cop = copulatory process on hemipenis. CR = caudal ramus. GeO = genital operculum.

finger-like projection at the base of the internal margin, next to the dorsal seta; copulatory process a stiff hook-like structure, thick at the first half of its length and then quickly narrowing to the orifice at the tip; lower ramus ("crochet accessoire") sinuous and with a rounded tip; caudal ramus a hirsute rounded lobe bearing a pair of setae.

Additional description of female. Carapace (Fig. 4G–I). Small sized (length = 697–722 µm), with brown surface and sparse setae; surface smooth except for a subtle ventro-lateral ridge on each valve; elliptically elongated in lateral view (length/height=1.96), with greatest height just behind mid-length; left valve overlapping right valve on all margins, strongly interlocking in antero-ventral, ventral and postero-ventral margins; valve overlap very strong at the posterior end of the carapace, producing a conspicuous outgrowth of the outer lamella, apparently without substantial change to the inner marginal structures; ventral area flat; dorsal margin arched; posterior and anterior margins rounded, both margins produced towards the ventral side; valves oval shaped in ventral and dorsal views, with maximum width displaced towards the posterior end in comparison to the male, producing a brooding cavity; up to 10 eggs were observed in a brood (mean egg size = 4.87 ± 4.0 µm, N = 8); dorsal margin straight in dorsal view; ventral margin sinuous in ventral view with well-marked ridges.

Left valve (Fig.4 A–C). In internal view anterior margin rounded, produced towards the ventral side; posterior margin narrowly rounded, produced towards the ventral side, forming a bulge at the postero-ventral area; ventral margin nearly straight; dorsal margin arched; calcified inner lamella well developed anteriorly, with a short line of concrescence near the valve margin leaving a vestibule; vestibule less developed posteriorly, but present; prominent selvage running on all margins except in the middle of dorsal margin; a short flange is formed at the antero-ventral margin; at the postero-ventral region, with outer lamella expanded towards the posterior end; central muscle scars consisting of 4 spots arranged in a vertical row relatively separated from each other and a round small frontal scar.

Antenna (Fig. 2C). As in the male, except for the small terminal segment with three equally slender claws, a minute seta and a tiny lobe (hyaline formation).

Abdomen (Fig. 3E). Genital operculum rounded, internally connected by tubes to a trabecula; caudal rami two hirsute rounded lobes, each with two apical setae in juxtaposition, a medio-external seta and an inconspicuous external seta nearer the base of the caudal ramus; end of body rounded, with a dorsal seta inserted in a strongly chitinized structure.

Measurements. Male. Holotype: length = 674 µm, height = 347 µm; Paratype MZUSP 29077: length = 685 µm, height = 349 µm; Paratype MZUSP 29078: length = 657 µm, width = 463 µm; Paratype MZUSP 29079: length = 677 µm, width = 461 µm. Female. Paratype MZUSP 29076, LV: length = 721 µm, height = 344 µm; Paratype MZUSP 29080: length = 715 µm, height = 355 µm; Paratype MZUSP 29081: length = 697 µm, width = 513 µm; Paratype MZUSP 29082: length = 722 µm, width = 518 µm.

Comparisons. The carapace of *Elpidium merendonense* sp. n. resembles that of *E. inaequivalve* in being relatively elongated in dorsal view and having a wide valve overlap at the posterior end of the carapace. However, the valve asymmetry is more pronounced

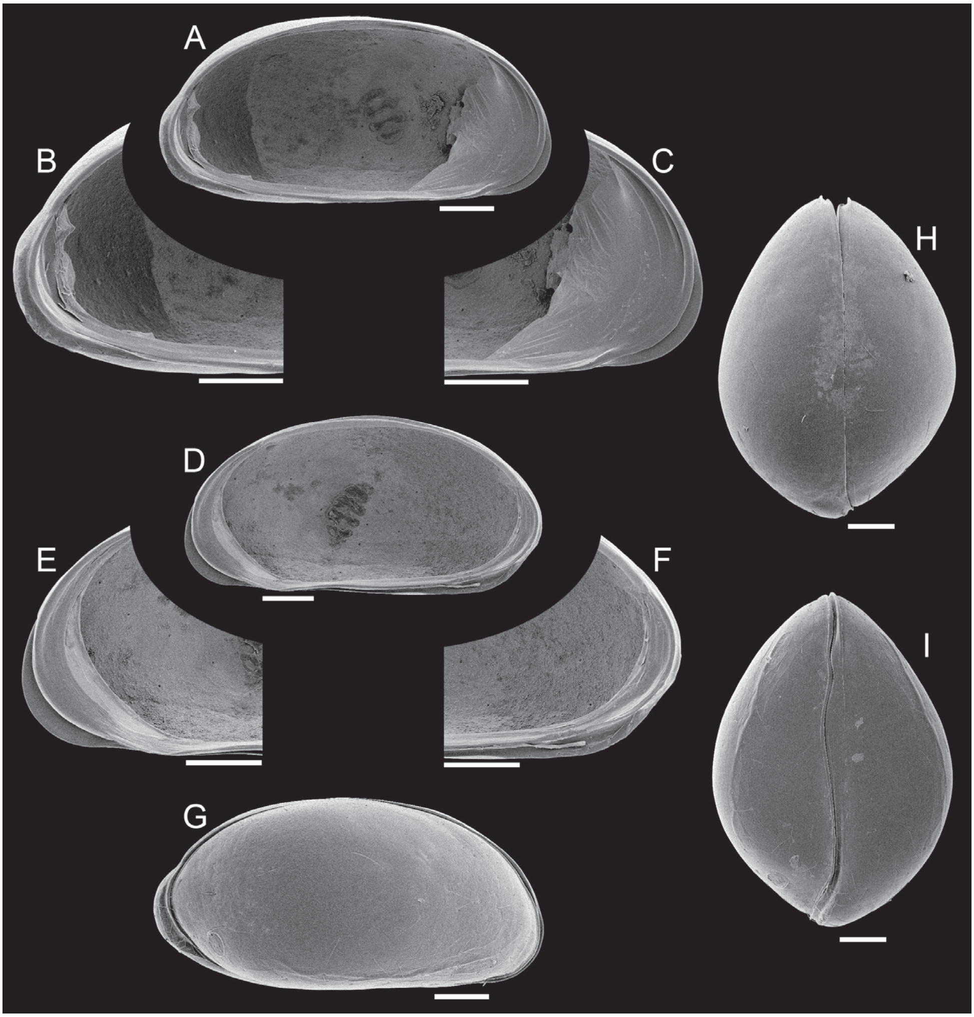

Figure 4. *Elpidium merendonense* sp. n., female. **A** Left valve internal view, general **B** left valve internal view, detail of postero-ventral margin **C** left valve internal view, detail of antero-ventral margin **D** right valve internal view, general **E** right valve internal view, detail of antero-ventral margin **F** right valve internal view, detail of postero-ventral margin **G** right lateral view **H** dorsal view **I** ventral view. **A–F** paratype, MZUSP 29076; **G** paratype, MZUSP 29080; **H** paratype, MZUSP 29081; **I** paratype, MZUSP 29082. Scale bars: 100 µm.

in *E. inaequivalve,* while females of *E. merendonense* sp. n. show a unique outgrowth at the posterior end of the left valve where it overlaps the right valve. *Elpidium merendonense* sp. n. and *E. laesslei* both present a distal lobe on the hemipenis with similar shape, but the copulatory process and the lower ramus are smaller in the latter species. These two species can furthermore be distinguished by the shape of the carapace in dorsal view, which is broad and rounded in *E. laesslei* and oval in *E. merendonense* sp. n.

Ecology and accompanying fauna. *Elpidium merendonense* was found only in water accumulated in leaf axils of bromeliads from 1,400 to 2,242m elevation in CNP. Only *Tillandsia* sp. were examined. In this altitudinal range, bromeliads occur all through the area in varying densities depending on microclimatic conditions. Specimens of this species were common in collections throughout the park, sometimes co-occupying bromeliads with a species of candonid ostracod and/or a species of Anomopoda (*Ceriodaphnia laticaudata* Müller, 1867). Ostracoda were more common in larger bromeliads.

Discussion

Honduras has a complex topography with numerous cloud forested mountain tops characterised by a high endemicity, documented for several taxa such as plants (Bubb et al. 2004), reptiles and amphibians (Wilson and McCranie 2003), and invertebrates (Anderson and Ashe 2000). *Elpidium* species are bromeliad specialists and, considering the high diversity and endemism of *Elpidium* in the molecular study of Jamaican species by Little and Hebert (1996), it is not surprising to find a new species in Honduras. More surprising is that the species described here is the first non-marine ostracod described from inland Honduras (Martens and Behen 1994). Currently, 275 valid species of nonmarine ostracods are recorded from the Neotropical region (Martens et al. 2008), but this figure is highly incomplete. Few localities in this region have been investigated in detail, and large unexplored gaps remain, as is the case with large parts of Honduras. We therefore expect that new sampling campaigns at CNP and elsewhere in Honduras would likely yield more unknown ostracod species, not only from phytotelmata but also from other freshwater habitats.

With the present contribution, seven valid species are currently known in the genus *Elpidium*. Two of these, namely *E. maricaoensis* and *E. laesslei*, are known only from incomplete original descriptions and without records of males, whose reproductive structures bear the most important diagnostic features for identification. Nonetheless, it is still possible to confidently identify all seven species, and an identification key is presented here.

Identification key to species of the genus *Elpidium*

1	Carapace surface smooth	**2**
–	Carapace surface pitted	***E. laesslei***
2	Left valve overlapping right valve	**3**
–	Right valve overlapping left valve	***E. purperae***
3	Female carapace small to medium size, not longer than 0.8 mm	**4**
–	Female carapace large, reaching more than 0.9 mm in length	***E. bromeliarum***

4 in dorsal and ventral views, left valve with posterior end markedly longer than right valve, especially in females **5**

– Valves symmetric **6**

5 Female carapace in dorsal view with length per width ratio smaller than 1.3. .... ***E. inaequivalve***

– Female carapace in dorsal view with length per width ratio bigger than 1.35 .... ***E. merendonense* sp. n.**

6 female carapace with greatest width at mid-length .... ***E. pintoi***

– female carapace with greatest width displaced towards the posterior end....... .... ***E. maricaoensis***

All seven known *Elpidium* species were recovered from water impounded inside bromeliads, and distribution of the genus seems to follow almost the whole geographic range of their host plants. *Elpidium* species have been recorded from a variety of altitudes from southernmost Brazil to Cuba: from near sea level (*E. bromeliarum*; see Pinto and Purper 1970), to 1,000 to 1,200 meters above sea level (*E. maricaoensis* and *E. laesslei*, respectively; see Tressler 1956) and up to 2,200 meters above sea level (present study).

The presence of passively dispersing Ostracoda in tank bromeliads requires specialised dispersal vectors. Already in his earliest description of the genus, Müller (1881) discussed possible dispersal mechanisms of bromeliad inhabiting ostracods. Müller (1881) argued that while it may be easy to understand the presence of aquatic insect larvae inside bromeliads, considering that the adults fly away and lay eggs in other plants, the same does not hold for the strictly aquatic ostracods. Müller (1881) hinted on probable phoretic behaviour (i.e. mechanical transportation by other animals) of this species: “they (*E. bromeliarum*) cannot make the necessary travels (from one plant to another) unless they attach to the body of a bromeliad visitor”. More recent field observations (Lopez et al. 1999) and experimental results (Lopez et al. 2005) have confirmed that *Elpidum* can efficiently use frogs and snakes as phoretic dispersal agents to colonise new plants. This ability of *Elpidium* species to hop from plant to plant on phoretic vectors together with the resistance to environmentally harsh conditions such as dehydration (Lopez et al. 2009) are probably the two main traits causing the generally observed dominance of *Elpidium* ostracods inside bromeliads.

Acknowledgements

We would like to thank the people from Buenos Aires, Cortecito and Tierra Santa around National Park Cusuco for collaboration and guidance on the numerous excursions in the forest. Also the staff and volunteers of Operation Wallacea for supporting the project, in particular Jonathan Kolby for constructive mental and physical support.

References

Anderson RS, Ashe JS (2000) Leaf litter inhabiting beetles as surrogates for establishing priorities for conservation of selected tropical montane cloud forest in Honduras, Central America (Coleoptera; Staphylinidae, Curculionidae). Biodiversity and Conservation 9: 617–653. doi: 10.1023/A:1008937017058

Benzing DH (2000) Bromeliaceae: profile of an adaptive radiation. Cambridge University Press, Cambridge, UK, 710 pp. doi: 10.1017/CBO9780511565175

Bubb P, May I, Miles L, Sayer J (2004) Cloud forest agenda. United Nations Environment Program World Conservation Monitoring Centre, Cambridge, 32 pp.

Colin JP, Danielopol DL (1980) Sur la morphologie, la systématique, la biogéographie et l'évolution des ostracodes Timiriaseviinae (Limnocytheridae). Paleobiologie Continentale 11: 1–51.

Danielopol DL (1975) Remarques sur la diversification morphologique de trois nouvelles espéces d'*Elpidium* (Ostracoda) à Cuba. Bulletin of American Paleontology 65: 47–60.

Jocque M, Kernahan A, Nobes A, Willians C, Field R (2010) How effective are non-destructive sampling methods to assess aquatic invertebrate richness in bromeliads? Hydrobiologia 649: 293–300. doi: 10.1007/s10750-010-0272-1

Kitching RL (2000) Food webs and container habitats: the natural history and ecology of phytotelmata. Cambridge University Press, Cambridge, UK, 448 pp. doi: 10.1017/CBO9780511542107

Little TJ, Hebert PDN (1996) Endemism and ecological islands: the ostracods from Jamaican bromeliads. Freshwater Biology 36: 327–338. doi: 10.1046/j.1365-2427.1996.00094.x

Lopez LCS, Alves RRDN, Rios RI (2009) Micro-environmental factors and the endemism of bromeliad aquatic fauna. Hydrobiologia 625: 151–156. doi: 10.1007/s10750-009-9704-1

Lopez LCS, Rodrigues PP, Rios RI (1999) Frogs and snakes as phoretic dispersal agents of bromeliad ostracods (Limnocytheridae: *Elpidium*) and Annelids (Naididae: *Dero*). Biotropica 31: 705–708. doi: 10.1111/j.1744-7429.1999.tb00421.x

Lopez LCS, Filizola B, Deiss I, Rios RI (2005) Phoretic behaviour of bromeliad annelids (*Dero*) and ostracods (*Elpidium*) using frogs and lizards as dispersal vectors. Hydrobiologia 549: 15–22. doi: 10.1007/s10750-005-1701-4

Martens K, Schön I, Meisch C, Horne DJ (2008) Global biodiversity of non-marine Ostracoda (Crustacea). In: Balian E et al. (Eds) Freshwater animal diversity assessment. Hydrobiologia 595: 185–193.

Martens K, Behen F (1994) A checklist of the non-marine ostracods (Crustacea, Ostracoda) from South-American inland waters and adjacent islands. Travaux scientifiques du Museé d'Histoire naturelle de Luxembourg 22: 81 pp.

Müller F (1880) Wasserthiere in Baumwipfeln *Elpidium bromeliarum*. Kosmos 6: 386–388.

Müller F (1881) Descrição do *Elpidium bromeliarum* crustáceo da família dos Cytherideos. Arquivos do Museu Nacional, Rio de janeiro 4: 27–34.

Picado C (1913) Les broméliaces épiphytes considerées comme milieu biologique. Bulletin Scientifique France Belgique 47: 215–360.

Pinto ID, Purper I (1970) A neotype for *Elpidium bromeliarum* Müller, 1880 (type species for the genus) and a revision of the genus *Elpidium* (Ostracoda). Escola de Geologia, Universidade Federal do Rio Grande do Sul, Publicação Especial 19: 1–23.

Pinto RL, Rocha CEF, Martens K (2008) On the first terrestrial ostracod of the superfamily Cytheroidea (Crustacea, Ostracoda): description of *Intrepidocythere ibipora* n. gen. n. sp. from forest leaf litter in São Paulo state, Brazil. Zootaxa 1828: 29–42.

Tressler WL (1941) Ostracoda from Puerto Rican bromeliads. Journal of the Washington Academy of Sciences 31: 263–269.

Tressler WL (1956) Ostracoda from bromeliads in Jamaica and Florida. Journal of the Washington Academy of Sciences 46: 333–336.

Wilson LD, McCranie JR (2003) The herpetofauna of the cloud forests of Honduras. Amphibian & Reptile Conservation 3: 34–48.

ZooKeys 313: 61–79 (2013)
doi: 10.3897/zookeys.313.4823
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RESEARCH ARTICLE

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Bones and genes: resolution problems in three Vietnamese species of *Crocidura* (Mammalia, Soricomorpha, Soricidae) and the description of an additional new species

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Academic editor: *K. M. Helgen* | Received 4 February 2013 | Accepted 20 June 2013 | Published 2 July 2013

urn:lsid:zoobank.org:pub:313A5DC5-B59C-476C-89C3-AED22353FA59

Citation: Jenkins PD, Abramov AV, Bannikova AA, Rozhnov VV (2013) Bones and genes: resolution problems in three Vietnamese species of *Crocidura* (Mammalia, Soricomorpha, Soricidae) and the description of an additional new species. ZooKeys 313: 61–79. doi: 10.3897/zookeys.313.4823

Abstract

Recent investigations of Southeast Asian white toothed shrews belonging to the genus *Crocidura* have revealed discrepancies between the results of morphological and molecular studies. The following study concerns three species of *Crocidura* occurring in Vietnam, namely *C. attenuata, C. tanakae* and *C. wuchihensis*, and an undescribed fourth species revealed by molecular analysis. For many years *Crocidura attenuata* has been known to occur in Vietnam but, until very recently, the morphologically similar and comparably sized *C. tanakae* was believed to be restricted to Taiwan. Following several molecular studies over the last few years, this species is now believed to be considerably more widespread and recognised as occuring also in Vietnam. The results of one of these recent molecular studies also revealed the presence of an undescribed species of *Crocidura*, similar in size and morphology to *Crocidura wuchihensis*, which is herein described. Data are provided on geographical variation in Vietnam and the problems of defining morphologically similar yet molecularly disparate species are discussed.

Keywords

Crocidura, new species, morphology, molecular analysis, geographical variation

Introduction

From the late 1990s there have been several intensive surveys of the small mammal fauna in various localities in Vietnam, resulting in the discovery of a number of species new to science. Before that time only three species of *Crocidura* had been recorded from Vietnam: *C. attenuata* Milne Edwards, 1872*, C. fuliginosa* (Blyth, 1855) and *C. indochinensis* Robinson & Kloss, 1922 (Van Peenen et al. 1969, Heaney and Timm 1983). Lunde et al. (2003) recorded the occurrence of a fourth species, *C. wuchihensis* Shaw, Wang, Lu & Chang 1966 in northern Vietnam. This was followed by a spate of descriptions of new species of *Crocidura* based entirely on morphology: *C. kegoensis* Lunde, Musser & Ziegler, 2004; *C. sokolovi* Jenkins, Abramov, Rozhnov & Makarova, 2007; *C. zaitsevi* Jenkins, Abramov, Rozhnov & Makarova, 2007; *C. annamitensis* Jenkins, Lunde & Moncrieff, 2009; *C. guy* Jenkins, Lunde & Moncrieff, 2009; *C. phuquocensis* Abramov, Jenkins, Rozhnov & Kalinin, 2008a; *C. phanluongi* Jenkins, Abramov, Rozhnov & Olsson, 2010.

Molecular studies were also being carried out during this period. Significant studies included those of Ohdachi et al. (2006) investigating the mitochondrial cytochrome *b* gene sequences of Soricidae; Esselstyn et al. (2009), Esselstyn and Brown (2009) and Esselstyn and Oliveros (2010) studying mitochondrial and nuclear genes of *Crocidura*. These were broad based studies covering wide geographical regions of Southeast Asia, Indonesia and the Philippines but some samples of Vietnamese *Crocidura* were included in their analyses. Bannikova et al. (2011) studied two mitochondrial genes, cytochrome *b* (cyt*b*) and cytochrome *c* oxidase subunit I gene (COI), of Vietnamese *Crocidura* collected at various localities ranging from the north to the south of the country.

While the molecular studies of Vietnamese material confirmed some of the results of the contemporaneous morphological studies, a number of anomalies were equally revealed, indicating the presence of several morphologically similar but molecularly distinct taxa. Investigation of these incongruent results is the subject of this current study.

Background to identification of species based on DNA analysis

Crocidura attenuata and *C. tanakae*

Crocidura attenuata Milne Edwards, 1872 described originally from Szechuan, China, was regarded as a widespread and common species known throughout much of Asia, including many localities from northern to southern Vietnam.

Crocidura tanakae Kuroda, 1938 from Taiwan was originally described as a full species but was subsequently considered to be either a synonym or subspecies of *C. attenuata* (Ellerman and Morrison-Scott 1951, Jameson and Jones 1977, Fang et al. 1997, Jiang and Hoffmann 2001, Han et al. 2002). Motokawa et al. (2001) demonstrated that the karyotype of Taiwanese specimens differed from that of *C. attenuata* from mainland southern China and suggested that it might represent a distinct species. Although Ohdachi et al. (2006) observed phylogenetic differentiation between the two samples they used from Taiwan and Vietnam, these authors continued to consider the Taiwanese samples as a subspecies of *C. attenuata*.

Esselstyn and Brown (2009) and Esselstyn et al. (2009) studying Southeast Asian shrews, recognised the relationship between samples of *C. tanakae* from Taiwan and a sample from northeastern Vietnam, which they identified in these studies as *Crocidura* cf. *tanakae*. The following year, Esselstyn and Oliveros (2010) demonstrated the presence of two similar sized species of *Crocidura* in Vietnam, namely *C. attenuata* based on samples from northern Vietnam and *C.* cf. *tanakae* based on samples from four separate localities in northern and central Vietnam. Bannikova et al. (2011) included their own recently collected samples from northern, central and southern Vietnam plus information derived from GenBank. They were similarly able to demonstrate the presence of two separate species, *C. attenuata* confined to a single locality in northeastern Vietnam and *C. tanakae* which was widespread in northern, central and southern localities. With a minimum distance of 9.91% between the haplotypes, their cyt*b* tree showed good support for the distinction of *C. tanakae* from a multi-species group comprising *C. attenuata, C. dsinezumi, C. indochinensis, C. lasiura, C. tadae kuroda, C. wuchihensis, C.* sp. AB1 and *C. zaitsevi*.

***Crocidura wuchihensis*, *C. indochinensis* and *C.* sp. AB1**

Although known from few specimens at any one location *Crocidura indochinensis* Robinson & Kloss, 1922 was considered to have a wide, disjunct distribution, occuring in a few widely separated locations in Vietnam and extralimitally in Myanmar and China (Osgood 1932, Anthony 1941, Heaney and Timm 1983, Jiang and Hoffmann 2001, Hutterer 2005, Jenkins et al. 2009). Additional specimens from southern Vietnam were recorded recently (Abramov et al. 2009; Jenkins et al. 2010) and tissue samples from these specimens were included in the molecular analysis by Bannikova et al. (2011).

Crocidura wuchihensis Shaw, Wang, Lu and Chang, 1966 was originally described on the basis of two specimens from Hainan Island, China. Specimens collected from two localities in Vietnam (Lunde et al. 2003; 2004) were referred to this species and specimens from several other locations in Vietnam were also considered to represent *C. wuchihensis* (Jenkins et al. 2009). Samples of *C. wuchihensis* from Ha Giang Province, Mt. Tay Con Linh II, northern Vietnam were included in molecular analyses of Ohdachi et al. (2006) and Esselstyn and Oliveros (2010). A separate analysis of a sample from Vinh Phu Province, Tam Dao, northern Vietnam (Meegaskumbura et al. 2007) from a specimen misidentified as *C. fuliginosa*, also proved to be a representative of *C. wuchihensis* (see Bannikova et al. 2011). Bannikova et al. (2011) added GenBank data from samples from these two localities to their cyt*b* analysis of samples from northern, central and southern Vietnam. Their analysis of cyt*b* revealed the presence of three taxa forming a group with 100% support: *C. wuchihensis* in the two localities (Ha Giang Province and Vinh Phu Province) in northern Vietnam; *C. indochinensis* in a single locality (Bi Doup - Nui Ba Nature Reserve) in southern Vietnam; and an unnamed species, designated as *C.* sp. AB1, from Sa Pa in northern Vietnam. The average distance on the cyt*b* tree between *C. wuchihensis* and the combined *C. indochinensis* / *C.* sp. AB1 branch was 7.75%. The *p*-distance from the cyt*b* tree separating *C. wuchihensis* and *C. indochinensis* is 7.6%, and separating *C. wuchihensis* and *C.* sp. AB1 was 8.0%. There was 100% bootstrap support on the cyt*b* and COI trees for the *C. indochinensis* and *C.* sp. AB1 group but these two taxa were respectively separated at *p*-distances of 4.1% (for cyt*b*) and 4.0% (in the COI analysis).

Methods

This morphological study draws on specimens from a wide range of geographical locations in Vietnam (see Fig. 1) and includes those specimens from which tissue samples were analysed in the papers by Ohdachi et al. (2006), Meegaskumbura et al. (2007), Esselstyn et al. (2009), Esselstyn and Brown (2009), Esselstyn and Oliveros (2010) and Bannikova et al. (2011). The specimens included in this study (see supplementary file) are stored in the collections of the American Museum of Natural History, New York (AMNH); Natural History Museum, London (BMNH); Field Museum of Natural History, Chicago (FMNH); Museum of Vertebrate Zoology, University of California, Berkely (MVZ); National Museum of Natural History, Smithsonian Institution, Washington D.C. (USNM); Zoological Institute, Russian Academy of Sciences, Saint-Petersburg, Russia (ZIN).

Measurements in millimetres were taken with digital callipers. Cranial and dental nomenclature follows that of Meester (1963), Mills (1966), Butler and Greenwood (1979) and Dannelid (1998). Definitions of skull measurements follow Jenkins et al. (2009).

Results

Crocidura sapaensis sp. n.

urn:lsid:zoobank.org:act:6016D7F3-D50E-4DBC-B571-EB54E5062D1E
http://species-id.net/wiki/Crocidura_sapaensis

Holotype. ZIN 96433, genetic analysis code CVN108, BOLD Accession no. ABMIV11408, field no. 132, male, body in ethanol, skull extracted, collected 25 May 2006 by A.V. Abramov.

Figure 1. Geographical distribution of sampling localities in Vietnam: **1** Lao Cai Province, Ngai Tio **2** Lao Cai Province, Sa Pa District **3** Lao Cai Province, Van Ban District **4** Lao Cai Province, Thai Nien **5** Lao Cai Province, Pa Kha **6** Ha Giang Province, Mt. Tay Con Linh II **7** Tuyen Quang Province **8** Vinh Phu Province, Tam Dao **9** Hai Phong Province, Cat Ba Island **10** Ha Tinh Province, Huong Son District **11** Quang Binh Province, Phong Nha - Ke Bang National Park **12** Quang Tri Province, Huong Hoa Nature Reserve **13** Quang Nam – Da Nang Provinces, Ba Na Nature Reserve **14** Kon Tum Province, Ngoc Linh Mt. **15** Kon Tum Province, Dak To **16** Lam Dong Province, Da Lat **17** Lam Dong Province, Bi Doup - Nui Ba Nature Reserve **18** Khanh Hoa Province, Hon Ba Mt.

Type locality. Vicinity of Tram Ton Station of Hoang Lien National Park, north slope of Phansipan Mt. area, 6 km west of Sa Pa Town, Sa Pa District, Lao Cai Province, Vietnam, 22°21'N, 103°46'E, altitude 2200m above sea level.

Paratypes. ZIN 96262, genetic analysis code CVN93, GenBank no. HM587005, BOLD no. ABMIV100 08, field no. 13, male, collected 8 December 2005; ZIN 96264, genetic analysis code CVN94, GenBank no. HM587006, BOLD no. ABMIV101 08, field no. 15, female, collected 8 December 2005; ZIN 96269, genetic analysis code CVN99, BOLD no. ABMIV106 08, field no. 32, male, collected 15 December 2005; ZIN 96271, genetic analysis code CVN101, BOLD no. ABMIV108 08, female, collected 16 December 2005; ZIN 96274, genetic analysis code CVN102, BOLD no. ABMIV109 08, field no. 45, male, collected 17 December 2005; ZIN 96275, genetic analysis code CVN103, BOLD no. ABMIV110 08, field no. 46, male, collected 17 December 2005; ZIN 96276, genetic analysis code CVN104, BOLD no. ABMIV111 08, field no. 66, female, collected 22 December 2005; ZIN 96432, genetic analysis code CVN107, BOLD no. ABIOW074 08, field no. 131, male, collected 25 May 2006; ZIN 96434, genetic analysis code CVN109, BOLD no. ABIOW075 08, field no. 133, male, collected 25 May 2006; ZIN 96436, genetic analysis code CVN111, BOLD no. ABMIV116 08, field no. 136, male, collected 28 May 2006; ZIN 96438, genetic analysis code CVN113, BOLD no. ABMIV117 08, field no. 138, female, collected 28 May 2006; ZIN 96439, genetic analysis code CVN114, BOLD no. ABMIV118 08, field no. 139, female, collected 28 May 2006; ZIN 96442, genetic analysis code CVN117, BOLD no. ABIOW069 08, field no. 144, male, collected 31 May 2006; ZIN 99779, field no. 24, male, collected 10 May 2010. All bodies in ethanol, skulls extracted, collected by A.V. Abramov and A.V. Shchinov from the same locality as the holotype, altitude 1930–2200m above sea level.

Other material. FMNH 39029 Chapa [Sa Pa], Lao Cai Province; BMNH 1925.1.1.24; BMNH 1925.1.1.27 Ngai Tio, Lao Cai Province, 22°36'N, 103°40'E.

Diagnosis. A small shrew distinguished by the mitochondrial genes cytochrome *b* (cyt*b*) and cytochrome oxidase *c* subunit I (COI) and by the shape of the talonid of the third lower molar (m3).

Description. Size small (see Table 1) with a moderately long tail relative to head and body length (62–84%). Dorsal pelage dark greyish brown; tail dark grey dorsally, slightly paler below (see Fig. 2). Skull with a rounded, short rostrum: moderately broad interorbital region; rounded, relatively deep braincase with subangular superior articular facets and lambdoid crests just evident laterally near the junction with the mastoid (see Fig. 3). The first upper incisor is slender with a relatively small posterior cusp, less than half the height of the first upper unicuspid; posterolingual border of upper premolar (P4) deep and rounded, in close contact with the anterolingual margin of M1 in occlusal view; last upper molar (M3) relatively narrow. Lower incisor with two distinct cusps on the occlusal surface in unworn dentition; posterolingual cuspid present on lower premolar (p4); talonid basin of m3 broad and deep with an entoconid ridge and low entoconid (see Fig. 4).

Comparison with other species. *Crocidura sapaensis* averages larger than the very small species of *Crocidura* recorded from Vietnam. The condyloincisive length is

Figure 2. Photograph of adult male *Crocidura sapaensis* (ZIN 99779).

greater than that of *C. guy, C. annamitensis* and *C. kegoensis*, within the upper part of the range of *C. zaitsevi* and the braincase is deeper than that of all four small species. *Crocidura wuchihensis* and *C. sapaensis* are in the same size range. *Crocidura sapaensis* is smaller than or at the lower end of the size range of *C. indochinensis* with a relatively shorter tail (see Table 1 and Fig. 5).

Crocidura sapaensis and *C. wuchihensis* are distinguished by differences in cyt*b* sequences. *Crocidura sapaensis* differs from *C. zaitsevi* and *C. indochinensis* in the cyt*b* and COI gene sequences.

Differences in the shape of the talonid of m3 in northern Vietnamese populations serve to distinguish *C. sapaensis* and *C. wuchihensis* (see Fig. 4). In specimens of *C. sapaensis* from northern Vietnam the talonid basin is broad and deep with an entoconid ridge and low entoconid, whereas in *C. wuchihensis* the talonid basin is narrow. In *C. indochinensis* the talonid basin is broad and deep with a hypoconid, entoconid and marked entoconid ridge (see Fig. 4).

Etymology. The new species is named after Sa Pa, the capital of Sa Pa District in Lao Cai Province of northern Vietnam, with the Latin suffix - *ensis* (belonging to).

Natural history. The series of type specimens was collected from a variety of habitats in the vicinity of Tram Ton Station of Hoang Lien National Park: mixed evergreen forest; forested banks of small streams; open grassy glades (Fig. 6); primary forest with large trees at an elevation 1930–2200m (Abramov et al. 2008b). During 2005–2010 a total of 190 shrews was captured in this area, including 4 species (*Crocidura sapaensis*, *Blarinella griselda* Thomas, 1912, *Anourosorex squamipes* Milne Edwards, 1872, and *Episoriculus leucops* (Horsfield 1855)). *Crocidura sapaensis* was the most numerous species (90% of the total captures), followed by *A. squamipes* and *B. griselda* (5.3% and 4.2% respectively), while only one *E. leucops* was captured (Abramov et al. 2010). *Crocidura sapaensis* was more abundant in

Figure 3. Comparison of crania of *Crocidura wuchihensis* (AMNH 274153), *Crocidura sapaensis* (ZIN 96433) and *Crocidura indochinensis* (ZIN 97668). Top row from left to right: dorsal views of the skulls of *C. wuchihensis, C. sapaensis* and *C. indochinensis*, ventral views of the skulls in the same order. Lower row: left lateral view of skulls and mandibles from left to right of *C. wuchihensis, C. sapaensis* and *C. indochinensis*.

slightly disturbed mixed forest (2.2–3.0 specimens per 100 trap/nights), the occurrence in open glades, amongst shrubs on stream banks and in undisturbed primary forest was 0.3–2.6 specimens per 100 trap/nights. The proportion of males to females in *C. sapaensis* was greater in all seasons; on average the male to female ratio is 2.3. Pregnant females were recorded from May to mid-July. Mean litter size in *C. sapaensis* was 3.0 (2-4, n=15).

Distribution. Confirmed specimens of *C. sapaensis* are recorded from Lao Cai Province, Sa Pa District on the basis of cyt*b* analysis and morphology of m3. On the basis of morphology, specimens from the northern part of Lao Cai Province, Ngai Tio (elevation 1450m) and from the vicinity of Cat Cat Village near Sa Pa Town (elevation 1400–1450m) in relatively close geographical proximity also probably belong to the same species.

Populations of *C. wuchihensis* identified on the basis of cyt*b* and those probably representing this species on the basis of morphology (from Pa Kha and Thai Nien,

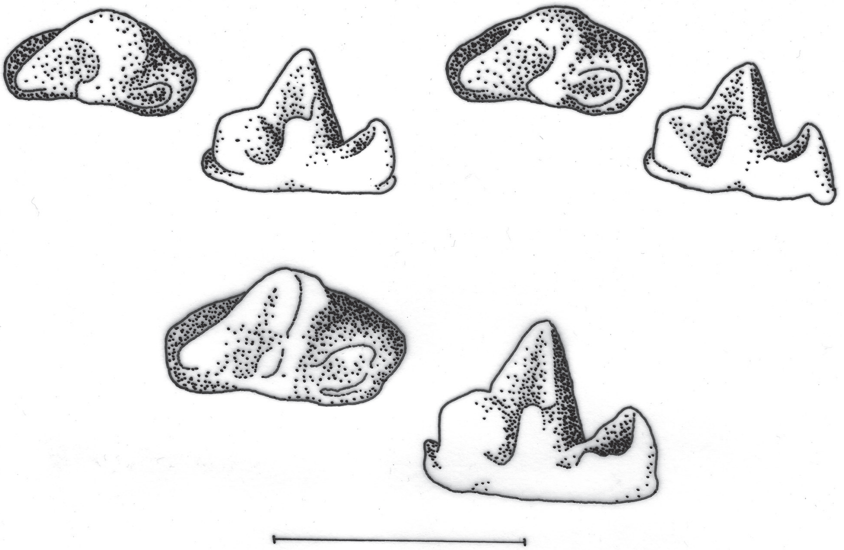

Figure 4. Occlusal (left) and lingual (right) views of right lower third molar to show differences in development of the talonid. Upper row left *Crocidura wuchihensis* AMNH 274168; upper row right *Crocidura sapaensis* ZIN 96439; lower row *Crocidura indochinensis* ZIN 97671. Scale equals 1 mm.

Table 1. Comparison of *Crocidura indochinensis, C. wuchihensis* and *C. sapaensis*. *Crocidura guy* and *C. zaitsevi* B are included as representatives of the very small species in Vietnam. Measurements in millimetres are presented as the mean, standard deviation and range, followed by sample size in parentheses.

Character	***Crocidura zaitsevi*** **B Bi Doup & Hon Ba**	***Crocidura guy*** **Na Hang**	***Crocidura wuchihensis*** **Mt Tay Con Linh II**	***Crocidura sapaensis*** **Sa Pa**	***Crocidura indochinensis*** **Bi Doup**
Condyloincisive length	15.4 ± 0.28 14.9-15.8 (15)	15.4 ± 0.05 15.3-15.4 (4)	16.4 ± 0.5 15.7-17.1 (6)	16.6 ± 0.41 15.6-17.2 (20)	18.1 ± 0.36 17.5-18.7 (10)
Condylobasal length	14.8 ± 0.31 14.2-15.3 (15)	14.9 ± 0.06 14.8-14.9 (4)	15.7 ± 0.47 15.0-16.4 (6)	15.9 ± 0.42 15.0-16.5 (20)	17.4 ± 0.37 16.8-17.8 (10)
Upper toothrow length	6.5 ± 0.14 6.3-6.8 (15)	6.5 ± 0.14 6.4-6.7 (4)	7.0 ± 0.25 6.6-7.2 (6)	7.0 ± 0.17 6.5-7.2 (21)	7.7 ± 0.23 7.3-8.0 (10)
Maxillary breadth at M2	4.5 ± 0.19 4.2-4.9 (15)	4.5 ± 0.14 4.4-4.7 (4)	4.9 ± 0.06 4.8-5 (6)	4.8 ± 0.18 4.4-5.1 (21)	5.2 ± 0.07 5.1-5.3 (10)
Braincase breadth	7.2 ± 0.15 6.9-7.5 (15)	7.2 ± 0.17 7.0-7.4 (4)	7.5 ± 0.16 7.3-7.8 (6)	7.7 ± 0.19 7.4-8.1 (19)	8.2 ± 0.15 7.9-8.4 (10)
Braincase height	3.7 ± 0.13 3.5-3.9 (14)	3.6 ± 0.14 3.5-3.8 (4)	4.0 ± 0.15 3.7-4.1 (6)	4.1 ± 0.17 3.9-4.4 (19)	4.4 ± 0.14 4.1-4.5 (10)
Head and body length	52.7 ± 2.79 49-59 (15)	49.5 ± 2.27 47-53 (4)	60.6 ± 2.7 58-65 (5)	57.4 ± 3.91 50-65 (20)	63.5 ± 3.81 56-68 (10)
Tail length	31.8 ± 1.2 30-34 (15)	35.9 ± 1.4 34-37 (4)	39.8 ± 2.28 37-42 (5)	41.6 ± 2.48 37-47 (20)	55 ± 2.79 50-58 (10)
Ratio of tail length to head and body length	0.61 ± 0.02 0.55-0.65 (15)	0.73 ± 0.03 0.69-0.77 (4)	0.66 ± 0.04 0.63-0.72 (5)	0.73 ± 0.06 0.62-0.84 (20)	0.87 ± 0.05 0.81-0.98 (10)
Ratio of tail length to condyloincisive length	2.1 ± 0.06 2.0-2.2 (15)	2.3 ± 0.09 2.2-2.4 (4)	2.4 ± 0.14 2.2-2.6 (5)	2.5 ± 0.14 2.2-2.7 (20)	3.1 ± 0.12 2.9-3.2 (10)

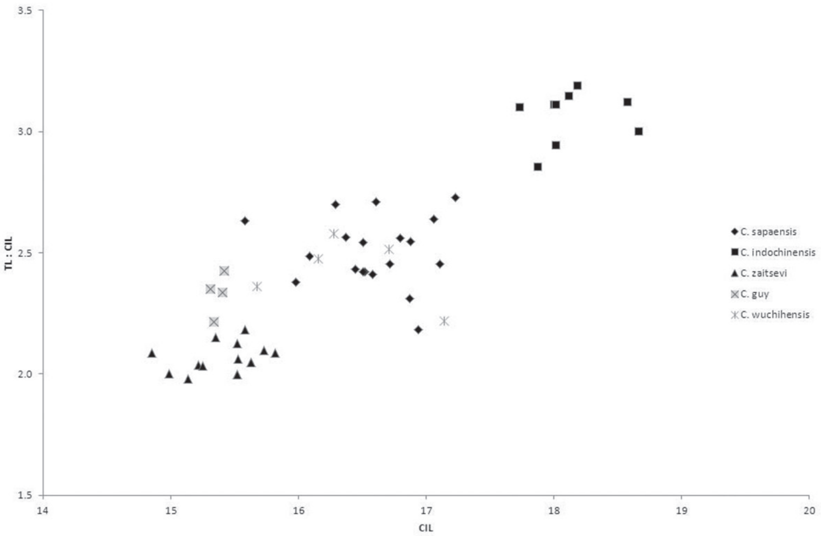

Figure 5. Bivariate plot to show differences in skull size and relative tail length. Horizontal axis: condyloincisive length; vertical axis: ratio of tail length to condyloincisive length.

Figure 6. Habitat typical of the area where *Crocidura sapaensis* was found.

both in Lao Cai Province) all occur in northeastern Vietnam in localities to the east of the Song Hong (Red River). The observation that this river marks the border between the two species, with *C. wuchihensis* to the east and *C. sapaensis* to the west, was made by Bannikova et al. (2011), however this apparent biogeographical separation is based on few locality records. These authors also observed that, in the cyt*b* analysis, the two northern Vietnamese populations of *C. wuchihensis* (from Mt Tay Con Linh II [22°46'N, 104°49'E] and Tam Dao [21°27'N, 105°38'E]) were separated by a *p*-distance of 2.1% suggesting that they probably represent distinct geographic populations.

The population of *C. wuchihensis* recorded from Huong Son, Ha Tinh Province in the southern Annamites by Lunde et al. (2004) and Jenkins et al. (2009), does of course, occur west of the Song Hong and samples have not been included in any of the previous molecular studies. Specimens from Mt Tay Con Linh II are larger on average (CIL 15.7–17.1, mean 16.4) than those from Huong Son (CIL 15.8-16.4, mean 16.0). The Canonical Variate Analysis reported in Jenkins et al. (2009: Fig. 10) shows that these two groups respectively from northern Vietnam and the southern Annamites are moderately well separated from each other. In view of the problems outlined in this paper, lacking further evidence from molecular studies, it is impossible to predict if the population from Huong Son is correctly assigned to *C. wuchihensis*, could belong to *C. sapaensis*, or might indeed represent a further undescribed species.

Crocidura attenuata* and *C. tanakae

Characters separating *C. attenuata* and *C. tanakae*

The population from Mt Tay Con Linh II, Ha Giang Province in northern Vietnam recognised by molecular analysis of cyt*b* as *C. attenuata*, falls within the size range of *C. tanakae* and both species are morphologically very similar in appearance. The two species may be separated by the following characters. The basioccipital region in *C. attenuata* is narrow and ridged particularly anterior to the position of the basioccipital suture, whereas in *C. tanakae* the basioccipital region is broad and flat to concave (see Fig. 7). The palatal suture in *C. attenuata* is a rounded to flat-topped ‘n’ shape, whereas in *C. tanakae* the suture is a shallow to more marked ‘m’ shape (see Fig. 8). The two species also differ in the shape of the talon of the upper premolar (P4). In occlusal view, the talon of *C. attenuata* is broader and more angular than that of *C. tanakae*, the lingual border is straight to concave, with the posterior border shallowly indented so that the whole tooth looks larger in occlusal view. In *C. tanakae* the lingual border of P4 is rounded and the posterior border of the tooth is deeply indented (see Fig. 8).

Figure 7. Comparison of crania of *Crocidura tanakae* (ZIN 91190) and *Crocidura attenuata* (AMNH 274152). Top row from left to right: dorsal views of the skulls of *C. tanakae* and *C. attenuata*, ventral views of the skulls in the same order. Lower row: left lateral view of skulls and mandibles from left to right of *C. tanakae* and *C. attenuata*.

Geographical variation in *C. tanakae*

Esselstyn and Oliveros (2010) did not provide detailed results about Vietnamese samples in the text of their analysis of Asian *C. tanakae*, nevertheless they demonstrated apparent geographical variation in Vietnam. Their illustration of a statistical parsimony network of mitochondrial haplotypes (Esselstyn and Oliveros 2010: Fig. 4) shows northeastern Vietnam samples from Tam Dao (21°27'N, 105°38'E) and Tuyen Quang (22°20'N, 105°25'E) grouped relatively closely, separated from each other by

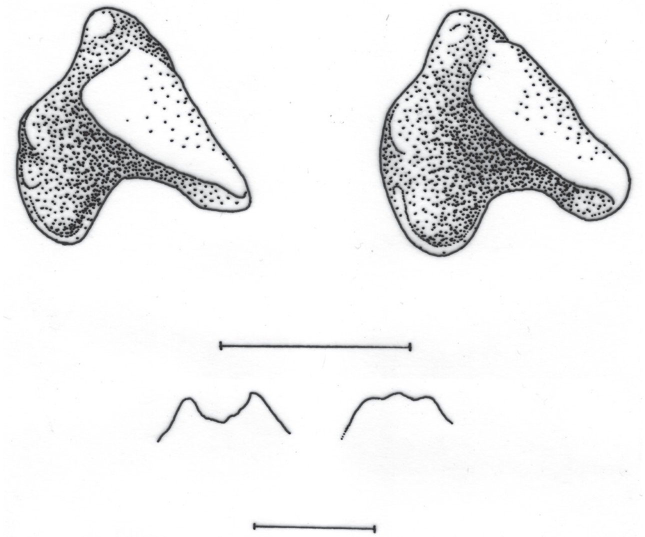

Figure 8. Above: occlusal view of left upper premolar of *Crocidura tanakae* (ZIN 91205) left and *Crocidura attenuata* (AMNH 274232) right. Below: palatal sutures of the same specimens in the same order. Scales equal 1 mm.

relatively few steps but separated by multiple steps from samples from the other two localities, Ha Tinh (18°21'N, 105°13'E) and Quang Nam (15°12'N, 108°02'E), which form a looser group. In their analysis of the COI gene, Bannikova et al. (2011) demonstrated the presence of two clearly defined haplogroups within Vietnamese *C. tanakae*: *C. tanakae* B restricted to the northern part of the country (Hoang Lien Mountains, Van Ban District) and *C. tanakae* A which was more widespread in Central and South Vietnam (Huong Hoa, Phong Nha-Ke Bang, Ngoc Linh, Hon Ba and Bi Doup). The uncorrected *p*-distance between these two groups using the COI gene was about 2.5%.

Populations of *C. tanakae* in Vietnam show a distinct clinal variation in skull size, populations at higher latitudes averaging smaller in size than those at lower latitudes (see Table 2). Although sample sizes are small, this observation is a possible example of the converse Bergmann's rule where body size decreases with latitude.

Geographical variation in *C. attenuata*

Abramov et al. (2012) demonstrated apparent geographical variation of *C. attenuata* in southern China and northern Vietnam. Genetic differentiation of *C. attenuata* is notable and reveals a phylogeographic structure with four haplogroups. The specimens from Cat Ba Island (Hai Phong Province, northeastern Vietnam) formed a single cluster closely related to the group of specimens from northern Vietnam (Ha Giang Province) and southeastern China (Guangxi Province). The genetic distance (*p*-distance)

Table 2. Latitudinal size variation in *Crocidura tanakae*. Measurements in millimetres are presented as the mean, standard deviation and range, followed by sample size in parentheses.

Character	Lao Cai Prov., Van Ban Dist. 21°58'N	Ha Tinh Prov., Huong Son 18°21'N	Quang Tri Prov., Huong Hoa 16°56'N	Kon Tum Prov., Ngoc Linh 15°05'N	Lam Dong Prov,. Bi Doup 12°11'N
Condyloincisive length	19.3 ± 0.47 18.5–20.0 (14)	19.7 ± 0.47 18.4–20.4 (24)	20.1 ± 0.38 19.6–20.6 (5)	20.6 ± 0.36 20.1–20.9 (4)	20.4 ± 0.52 19.6–21.3 (11)
Condylobasal length	18.8 ± 0.48 17.9–19.4 (14)	18.9 ± 0.48 17.7–19.6 (24)	19.2 ± 0.36 18.8–19.6 (5)	19.8 ± 0.29 19.4–20.0 (4)	19.5 ± 0.52 18.8–20.6 (11)
Upper toothrow length	8.3 ± 0.18 7.9–8.6 (14)	8.5 ± 0.22 8.2–9.0 (24)	9.0 ± 0.24 8.6–9.3 (5)	9.1 ± 0.14 8.9–9.2 (4)	9.0 ± 0.23 8.5–9.4 (11)
Maxillary breadth at M2	5.9 ± 0.17 5.6–6.3 (14)	6.0 ± 0.19 5.6–6.4 (24)	6.2 ± 0.17 6.0–6.4 (5)	6.3 ± 0.18 6.1–6.5 (4)	6.2 ± 0.21 5.9–6.6 (11)
Braincase breadth	8.7 ± 0.31 8.3–9.2 (14)	8.9 ± 0.19 8.3–9.1 (24)	9.0 ± 0.14 8.7–9.1 (5)	9.2 ± 0.36 8.9–9.6 (4)	9.3 ± 0.26 8.9–9.6 (11)

between specimens from Cat Ba / Ha Giang as well as Cat Ba / Guangxi is about 2.1%. The specimen of *C. attenuata* from the more north-eastern region of China (Hunan Province) appears basal among all samples of *C. attenuata* from China and Vietnam. Thus, the genetic distance between two specimens from China (Hunan / Guangxi) is 4.3%, which is nearly the same as the distance between *C. indochinensis* and *C. sapaensis* (see Bannikova et al. 2011).

Distribution

While *C. attenuata* in Vietnam appears to occur only to the east of the Song Hong (Red River), in northeastern Vietnam (Bannikova et al. 2011, Abramov et al. 2012), *C. tanakae* does not appear to be so constrained and has been recorded on both sides of the river in northern Vietnam and also in central and southern Vietnam (Esselstyn and Oliveros 2010, Bannikova et al. 2011, this study).

Discussion

For sister species that are recognised on the basis of morphology, cyt*b* distance values typically exceed 5% (Baker and Bradley 2006). The *p*-distance values of at least 9.91% between haplotypes belonging to the *C. tanakae* and *C. attenuata* groups, of 7.6% separating *C. wuchihensis* and *C. indochinensis*, and of 8.0% separating *C. wuchihensis* and *C. sapaensis* provide compelling evidence in support of their taxonomic distinction (Bannikova et al. 2011). The lower *p*-distance values of 4.1% separating *C. in-*

dochinensis from *C. sapaensis* are somewhat less convincing, were it not for the ready morphological distinction of the two species. The conundrum is the morphological distinction yet relatively low molecular separation between *C. indochinensis* and *C. sapaensis*, in contrast to *C. wuchihensis* and *C. sapaensis* which are not easily defined by morphological features but are distinguished by high molecular values. Similar situations are found in the literature, for example amongst different species of *Eumops* Miller, 1906 (bonneted bats) (McDonough et al. 2008). It is possible to speculate that this implies a relatively closer relationship between *C. indochinensis* and *C. sapaensis* but morphological convergence of *C. wuchihensis* and *C. sapaensis* to meet similar ecological requirements.

Crocidura tanakae is currently recognised as being widely distributed in Southeast Asia including southern China, Vietnam, Taiwan and the Philippines (Esselstyn and Oliveros 2010, Bannikova et al. 2011). *Crocidura attenuata* has a more northerly distribution (Esselstyn and Oliveros 2010) and furthermore evidence suggests that, at least in Vietnam, the distribution may be more restricted than formerly understood (Bannikova et al. 2011, this study). It is conceivable that these two morphologically convergent species have secondarily come into contact with each other as there is evidence that the two species are sympatric in at least one Chinese locality (Esselstyn and Oliveros 2010, Judith Eger pers. comm.).

Acknowledgements

The field studies in Vietnam were possible due to the support of the Joint Vietnam-Russian Tropical Research and Technological Centre. We are grateful to the administration of the Hoang Lien National Park for providing us with an opportunity to carry out field surveys. AA thanks A.V. Shchinov, A.E. Anichkin, Vu Van Lien, A.L. Monastyrskii, S.V. Kruskop for their help and scientific expertise during the field work. This study was supported in part by the Russian Foundation for Basic Research (projects 11-04-00020, 12-04-93005).

PJ particularly thanks Darrin Lunde, National Museum of Natural History, Smithsonian Institution, Washington D.C. for generously allowing access to specimens collected at pivotal localities in Vietnam and for many discussions over the problems of identifying morphologically conservative shrews. For providing access to the collections in their care, we are especially grateful to Eileen Westwig and Neil Duncan, American Museum of Natural History, New York; to James Patton and Chris Conroy, Museum of Vertebrate Zoology, University of California, Berkeley and to William Stanley and John Phelps, Field Museum of Natural History, Chicago. As always, grateful thanks to Phil Hurst, Photographic Unit, Natural History Museum, London, for specimen photography and to Roberto Portela Miguez, Mammal Group, Natural History Museum, London for scanning documents. We thank two anonymous reviewers for their helpful and constructive comments and criticisms.

References

Abramov AV, Jenkins PD, Rozhnov VV, Kalinin AA (2008a) Description of a new species of *Crocidura* (Soricomorpha: Soricidae) from the island of Phu Quoc, Vietnam. Mammalia 72: 269–272. doi: 10.1515/MAMM.2008.033

Abramov AV, Shchinov AV, Rozhnov VV (2008b) Study of insectivorous mammals in North Vietnam. Contemporary Problems of Ecology 1: 593–595. doi: 10.1134/S1995425508050135

Abramov AV, Kruskop SV, Shchinov AV (2009) Small mammals of the Dalat Plateau, southern Vietnam. Russian Journal of Theriology 8: 61–73.

Abramov AV, Shchinov AV, Rozhnov VV (2010) Soricidae of Sa Pa Area (Northern Vietnam). In: Advances in the biology of shrews III. Materials of the International Conference: 5. Moscow: KMK Scientific Press.

Abramov AV, Bannikova AA, Rozhnov VV (2012) White-toothed shrews (Mammalia, Soricomorpha, *Crocidura*) of coastal islands of Vietnam. Zookeys 207: 37–47. doi: 10.3897/zookeys.207.3237

Anthony HE (1941) Mammals collected by the Vernay-Cutting Burma expedition. Field Museum of Natural History Publications Zoological Series 27: 37–123.

Baker RJ, Bradley RD (2006) Speciation in mammals and the genetic species concept. Journal of Mammalogy 87: 643–662. doi: 10.1644/06-MAMM-F-038R2.1

Bannikova AA, Abramov AV, Borisenko AV, Lebedev VS, Rozhnov VV (2011) Mitochondrial diversity of the white-toothed shrews (Mammalia, Eulipotyphla, *Crocidura*) in Vietnam. Zootaxa 2812: 1–20. http://www.mapress.com/zootaxa/2011/f/z02812p020f.pdf

Blyth E (1855) Proceedings of the Asiatic Society of Bengal for May 1855. Journal of the Asiatic Society of Bengal 24: 354–364.

Butler PM, Greenwood M (1979) Soricidae (Mammalia) from the early Pleistocene of Olduvai Gorge, Tanzania. Zoological Journal of the Linnaean Society 67: 329–379. doi: 10.1111/j.1096-3642.1979.tb01119.x

Dannelid E (1998) Dental adaptations in shrews. In: Wójcik JM, Wolsan M (Eds) Evolution of shrews. Mammal Research Institute Polish Academy of Sciences, Białowieża, 157–174.

Ellerman JR, Morrison-Scott TCS (1951) Checklist of Palaeartic and Indian mammals 1758 to 1946. Trustees of the British Museum (Natural History), London, 810pp.

Esselstyn JA, Brown RM (2009) The role of repeated sea-level fluctuations in the generation of shrew (Soricidae: *Crocidura*) diversity in the Philippine Archipelago. Molecular Phylogenetics and Evolution 53: 171–181. doi: 10.1016/j.ympev.2009.05.034

Esselstyn JA, Timm RM, Brown RM (2009) Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in Southeast Asian shrews. Evolution 63: 2595–2610. doi: 10.1111/j.1558-5646.2009.00743.x

Esselstyn JA, Oliveros CH (2010) Colonization of the Philippines from Taiwan: a multi-locus test of the biogeographic and phylogenetic relationships of isolated populations of shrews. Journal of Biogeography 37: 1504–1514.

Fang Y-P, Lee L-L, Yew F-H, Yu H-T (1997) Systematics of white-toothed shrews (*Crocidura*) (Mammalia: Insectivora: Soricidae) of Taiwan: karyological and morphological studies. Journal of Zoology (London) 242: 151–166. doi: 10.1111/j.1469-7998.1997.tb02936.x

Han S-H, Iwasa MA, Ohdachi SD, Oh H-S, Suzuki H, Tsuchiya K, Abe H (2002) Molecular phylogeny of *Crocidura* shrews in northeastern Asia: a special reference to specimens on Cheju Island, South Korea. Acta Theriologica 47: 369–379.

Heaney LR, Timm RM (1983) Systematics and distribution of shrews of the genus *Crocidura* (Mammalia: Insectivora) in Vietnam. Proceedings of the Biological Society of Washington 96: 115–120.

Horsfield T (1855) Brief notices of several new or little-known species of Mammalia, lately discovered and collected in Nepal, by Brian Houghton Hodgson. Annals and Magazine of Natural History (2) 16: 101–114. doi: 10.1080/037454809495489

Hutterer RH (2005) Order Soricomorpha. In: Wilson DE, Reeder DM (Eds) Mammal species of the world. A taxonomic and geographic reference. 3rd ed. Johns Hopkins Press, Baltimore, 220–311.

Jameson EW, Jones GS (1977) The Soricidae of Taiwan. Proceedings of the Biological Society of Washington 90: 459–482.

Jenkins PD, Abramov AV, Rozhnov VV, Makarova OV (2007) Description of two new species of white-toothed shrews belonging to the genus *Crocidura* (Soricomorpha: Soricidae) from Ngoc Linh Mountain, Vietnam. Zootaxa 1589: 57–68. http://www.mapress.com/zootaxa/2007f/z01589p068f.pdf

Jenkins PD, Lunde DP, Moncrieff CB (2009) Descriptions of new species of *Crocidura* (Soricomorpha: Soricidae) from mainland Southeast Asia, with synopses of previously described species and remarks on biogeography. Bulletin of the American Museum of Natural History 331: 356–405. doi: 10.1206/582-10.1

Jenkins PD, Abramov AV, Rozhnov VV, Olsson A (2010) A new species of *Crocidura* (Soricomorpha: Soricidae) from southern Vietnam and north-eastern Cambodia. Zootaxa 2345: 60–68. http://www.mapress.com/zootaxa/2010/f/z02345p068f.pdf

Jiang X-L, Hoffmann RS (2001) A revision of the white-toothed shrews (*Crocidura*) of southern China. Journal of Mammalogy 82: 1059–1079. doi: 10.1644/1545-1542(2001)082<1059:AROTWT>2.0.CO;2

Kuroda N (1938) A list of the Japanese mammals. N. Kuroda, Tokyo.

Lunde DP, Musser GG, Son NT (2003) A survey of the small mammals from Mt. Tay Con Linh II, Vietnam, with the description of a new species of *Chodsigoa* (Insectivora: Soricidae). Mammal Study 28: 31–46. doi: 10.3106/mammalstudy.28.31

Lunde DP, Musser GG, Ziegler T (2004) Description of a new species of *Crocidura* (Soricomorpha: Soricidae, Crocidurinae) from Ke Go Nature Reserve, Vietnam. Mammal Study 29: 27–36. doi: 10.3106/mammalstudy.29.27

McDonough MM, Ammerman LK, Timm RM, Genoways HH, Larson PA, Baker RJ (2008) Speciation within bonneted bats (genus *Eumops*): the complexity of morphological, mitochondrial, and nuclear data sets in systematics. Journal of Mammalogy 89: 1306–1315. doi: 10.1644/07-MAMM-A-349.1

Meegaskumbura SM, Meegaskumbura R, Pethiyagoda K, Manamendra-Arachchi, Schneider CJ (2007) *Crocidura hikmiya,* a new shrew (Mammalia: Soricomorpha: Soricidae) from Sri Lanka. Zootaxa 1665: 19–30. http://www.mapress.com/zootaxa/2007f/z01665p041f.pdf

Meester J (1963) A systematic revision of the shrew genus *Crocidura* in southern Africa. Transvaal Museum Memoir 13: 1–127.

Miller GS (1906) Twelve new genera of bats. Proceedings of the Biological Society of Washington 19: 83–86.

Mills JRE (1966) The functional occlusion of the teeth of Insectivora. Journal of the Linnaean Society Zoology 46: 1–25. doi: 10.1111/j.1096-3642.1966.tb00081.x

Milne Edwards A (1872) Mémoire sur la faune mammalogique du Tibet oriental et principalement de la Principauté de Moupin. In: Milne Edwards H, Recherches pour servir à l'histoire naturelle des mammifères. Libraire de l'Académie de Médecine, Paris, 231–304.

Motokawa M, Harada M, Wu Y, Lin L-K, Suzuki H (2001) Chromosomal polymorphism in the gray shrew *Crocidura attenuata* (Mammalia: Insectivora). Zoological Science 18: 1153–1160. doi: 10.2108/zsj.18.1153

Ohdachi SD, Hasegawa M, Iwasa MA, Vogel P, Oshida T, Lin L-K, Abe H (2006) Molecular phylogenetics of soricid shrews (Mammalia) based on mitochondrial cytochrome *b* gene sequences: with special reference to the Soricinae. Journal of Zoology (London) 270: 177–191.

Osgood WH (1932) Mammals of the Kelley-Roosevelts and Delacour Asiatic expeditions. Field Museum of Natural History Publication 312. Zoological Series 18: 193–339.

Robinson HC, Kloss CB (1922) New mammals from French Indo-China and Siam. Annals and Magazine of Natural History (9) 9: 87–99. doi: 10.1080/00222932208632642

Shaw T-H, Wang S, Lu C-K, Chang L-K (1966) A survey of the mammals of Hainan Island, China. Acta Zootaxonomica Sinica 3: 260–275.

Thomas MRO (1912) On a collection of small mammals from the Tsin-ling Mountains, Central China, presented by Mr G. Fenwick Owen to the National Museum. Annals and Magazine of Natural History (8) 10: 395–403. doi: 10.1080/00222931208693252

Van Peenen PFD, Ryan PF, Light RH (1969) Preliminary identification manual for mammals of South Vietnam. United States National Museum, Smithsonian Institution, Washington, DC, 310pp.

Appendix

Specimens included in the morphological study. (doi: 10.3897/zookeys.313.4823.app). File format: Microsoft Word document (doc).

Explanation note: The supplementary file contains a list of all specimens included in this study.

Citation: Jenkins PD, Abramov AV, Bannikova AA, Rozhnov VV (2013) Bones and genes: resolution problems in three Vietnamese species of *Crocidura* (Mammalia, Soricomorpha, Soricidae) and the description of an additional new species. ZooKeys 313: 61–79. doi: 10.3897/zookeys.313.4823 Specimens included in the morphological study. doi: 10.3897/zookeys.313.4823.app

ZooKeys 313: 81–90 (2013)
doi: 10.3897/zookeys.313.5460
www.zookeys.org

RESEARCH ARTICLE

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Two new species in the subfamily Perlinae (Plecoptera, Perlidae) from China

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Academic editor: *Sam Heads* | Received 4 May 2013 | Accepted 27 June 2013 | Published 2 July 2013

urn:lsid:zoobank.org:pub:20982C05-0FC3-45FD-9BF6-E30958669103

Citation: Wang H–L, Wang G–Q, Li W–H (2013) Two new species in the subfamily Perlinae (Plecoptera, Perlidae) from China. ZooKeys 313: 81–90. doi: 10.3897/zookeys.313.5460

Abstract

Two species in the genera *Neoperla* and *Kamimuria* (Plecoptera: Perlidae) from China are described as new: *Kamimuria guangxia* **sp. n.**, and *Neoperla mesostyla* **sp. n.** The new species are compared to similar taxa.

Keywords

Plecoptera, Perlidae, *Neoperla*, *Kamimuria*, new species, China

Introduction

Kamimuria and *Neoperla* (Plecoptera: Perlidae) are the most speciose genera within the subfamily Perlinae within China (Du et al. 1999, DeWalt et al. 2013). *Neoperla* is represented by more than 70 known species in China, comprising about 30% of species in the genus (DeWalt et al. 2013). These were described by Chu (1929), Du

(1999, 2000 a, b), Du and Sivec (2004, 2005), Du and Wang (2005, 2007), Du et al. (2001), Sivec and Zwick (1987), Wu (1935, 1938, 1948, 1962, 1973), Wu and Claassen (1934), Yang and Yang (1990, 1991), Yang and Yang (1992, 1993, 1995 a, b, 1996, 1998), Li et al. (2011), Li and Wang (2011), Li et al. (2012), Li et al. (2012), Li and Li (2013) and Li et al. (2013). *Kamimuria* is represented by nearly 50 species in China, comprising about 70% of the described species within the genus (DeWalt et al. 2013; Sun and Du 2012; Li et al. 2012). In the present paper, we describe two additional Perlinae species as new to science: *Kamimuria guangxia* sp. n., and *Neoperla mesostyla* sp. n. from the Guangxi autonomous region. These species seem most closely related to congeners known from other southeastern Asian areas, as noted in the text. All types, including paratypes, are deposited in the Entomological Museum of China Agricultural University (CAU). Aedeagi were everted using the cold maceration technique of Zwick (1983) or Sivec et al. (1988).

Taxonomy

Kamimuria guangxia Li & Wang, sp. n.

urn:lsid:zoobank.org:act:5BF4961D-1520-418F-B086-F6F168097A51
http://species-id.net/wiki/Kamimuria_guangxia
Figs 1–3

Type material. Holotype: male, originally labeled as China: Guangxi autonomous region, Tian'e County, Buliuhe River, light trap, 25.0005 N, 107.1738 E, 16 Aug. 2002, Ding Yang. Paratypes: 2 males, same data as holotype.

Male. Forewing length 14.8–15.0 mm. General body color dark brown. Head slightly wider than pronotum, generally brown with darker, quadrate interocellar region, the anterior corners of which extend laterally in teneral specimen, M-line pale (Figs 1A-B); compound eyes dark; antennae dark brown. Pronotum dark brown with rugose surface (Fig. 1A); wing membrane brown, veins darker; femora pale basally, otherwise dark (Fig. 1E).

Terminalia. Hemiterga slender, finger-like, and slightly swollen apically, without hidden groove (Figs 2A–B). Tergum 9 with posteromedial patch of sensilla basiconical on a somewhater darker sclerite. Tergum 8 without sensilla patch. (Figs 1C, 2A). Setal brushes present on sterna 4–6. Aedeagus before eversion oval, apex tapering, hidden sac with apex darker and palm-like, with two lateral sharp claws and a median pad (Fig. 2C). Aedeagal sac membranous, medially constricted, apex expanded, heart-shaped and mostly covered with fine spinules, the apex and basal half of the sac bare (Fig. 3).

Female. Unknown.

Etymology. The specific epithet refers to Guangxi autonomous region where the type specimen was collected.

Distribution. China (Guangxi).

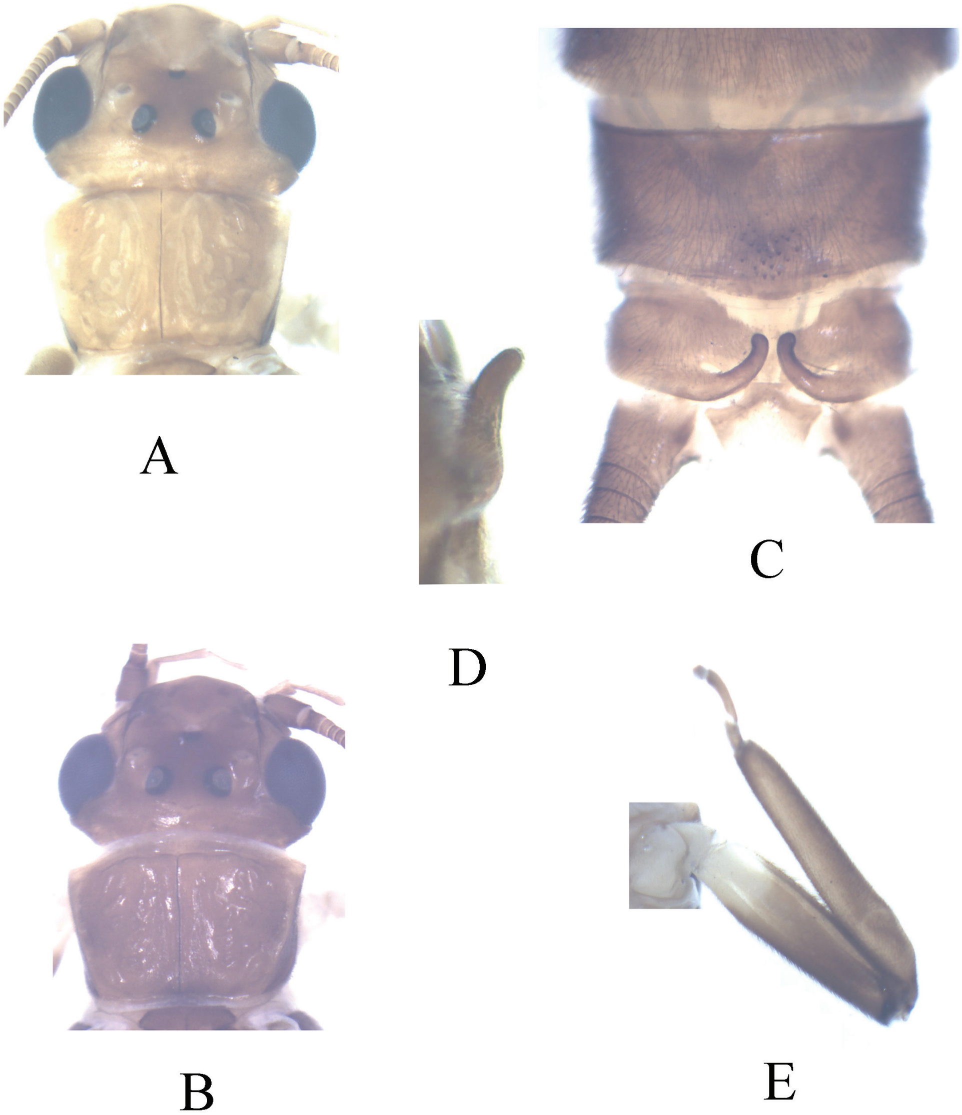

Figure 1. *Kamimuria guangxia* Li & Wang, sp. n. (male). **A** Head and pronotum, dorsal view (teneral specimen) **B** Head and pronotum, dorsal view (older specimen) **C** Terminalia, dorsal view **D** Hemitergal process, lateral view **E** Foreleg, lateral view.

Diagnosis. The male of *K. guangxia* is characterized by the hemiterga being slightly swollen apically. The aedeagal sac is membranous, medially constricted, apex expanded, heart-shaped and mostly covered with fine to tiny spines (Figs 1C, 2B). The new species is similar to *K. atra* Sivec & Stark, 2008, a species known both from Vietnam and Thailand, in general body color and features of terminalia, but tergum 8 of the new species has no sensilla basiconica patch.

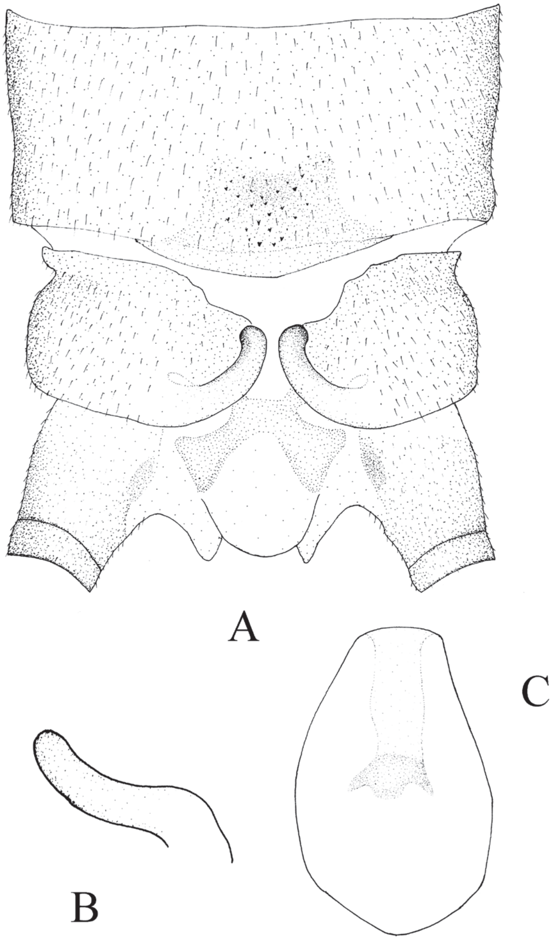

Figure 2. *Kamimuria guangxia* Li & Wang, sp. n. (male). **A** Terminalia, dorsal view **B** Hemitergal process, lateral view **C** Aedeagus before eversion, ventral view.

Neoperla mesostyla Li & Wang, sp. n.

urn:lsid:zoobank.org:act:151A4084-8B37-424B-BD9A-8F8CB4A5857A
http://species-id.net/wiki/Neoperla_mesostyla
Figs 4–5

Type material. Holotype: male, China: Guangxi autonomous region, Mt. Jiuwanshan, Jiuren Station next to Rongjiang River, 950–1150 m, 25.0673 N, 109.2563 E, light trap, 3 Aug. 2003, Zhang Li-Li. Paratype: 1 male, same data as holotype.

Male. Forewing length ca. 14.8 mm. General body color brown. Distance between ocelli nearly as wide as diameter of ocellus. Head slightly wider than pronotum, with a small triangular interocellar patch and another black triangular patch on frons (Fig. 4A); compound eyes dark; antennae dark brown. Pronotum pale brown with darker

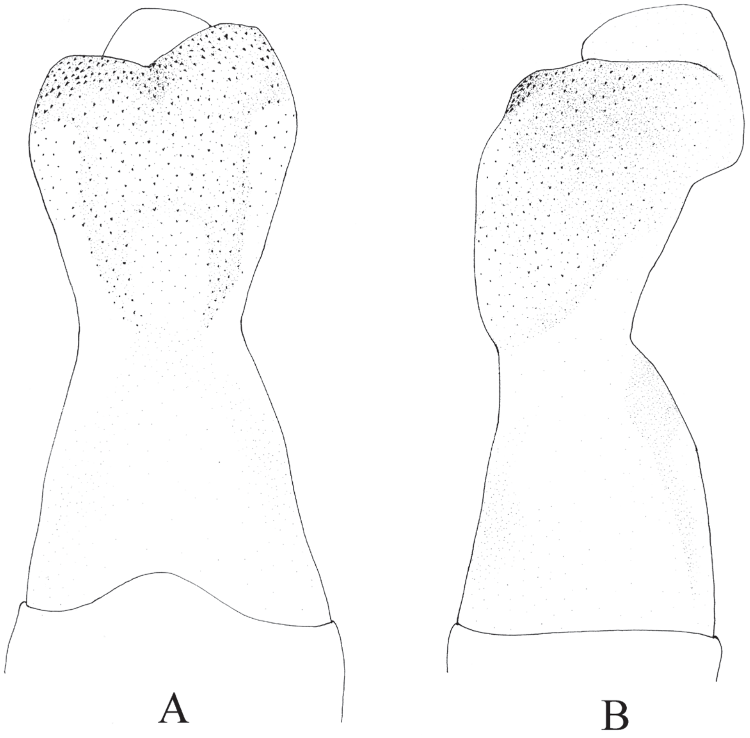

Figure 3. *Kamimuria guangxia* Li & Wang, sp. n. (male). **A** Aedeagus, dorsal view **B** Aedeagus, lateral view.

anterior and median stripes (Fig. 4A); wing membrane pale brown, veins dark; legs yellowish brown, distal fourth of femora, basal fifth of tibiae and tarsi darker (Fig. 4C).

Terminalia. Process of tergum 7 forming a large subquadrate plateau, mostly covered with dense sensilla basiconica patches but with few sensilla basiconica at margins (Fig. 4B). Tergum 8 with an upcurved tongue-shaped process, with sparse ventral tiny spines. Tergum 9 without sensilla basiconica patches. Hemitergal lobes slender and curved laterally near midlength (Fig. 4B). Aedeagal tube plump (length 3× width at basal bulb), ventrally with a mesal bifurcate lobe bearing a basal common stem, dorsal surface heavily sclerotized, the pigmentation slightly expanded mesolaterally, membranous sac 1.5× as long as tube and gradually curved ventrad to form a loop; sharp to stout small spines occur along dorsal surface toward apex (Figs 4D, E, 5).

Zwick & Sivec defined the bulb side of the tube as being ventral, however, the sac of the new species forms a loop so that the spines of apical half of the dorsal surface are actually ventrally directed; we herein discuss dorsal or ventral surfaces of the sac as if it were straight, as such in *Neoperla flagellata* Li & Murányi (Li et al. 2012) and *N. monacha* Stark & Sivec (Stark and Sivec 2008).

Female. Unknown.

Etymology. The specific epithet refers to the mesal position of the bifurcate lobe on the ventral surface of the aedeagal tube.

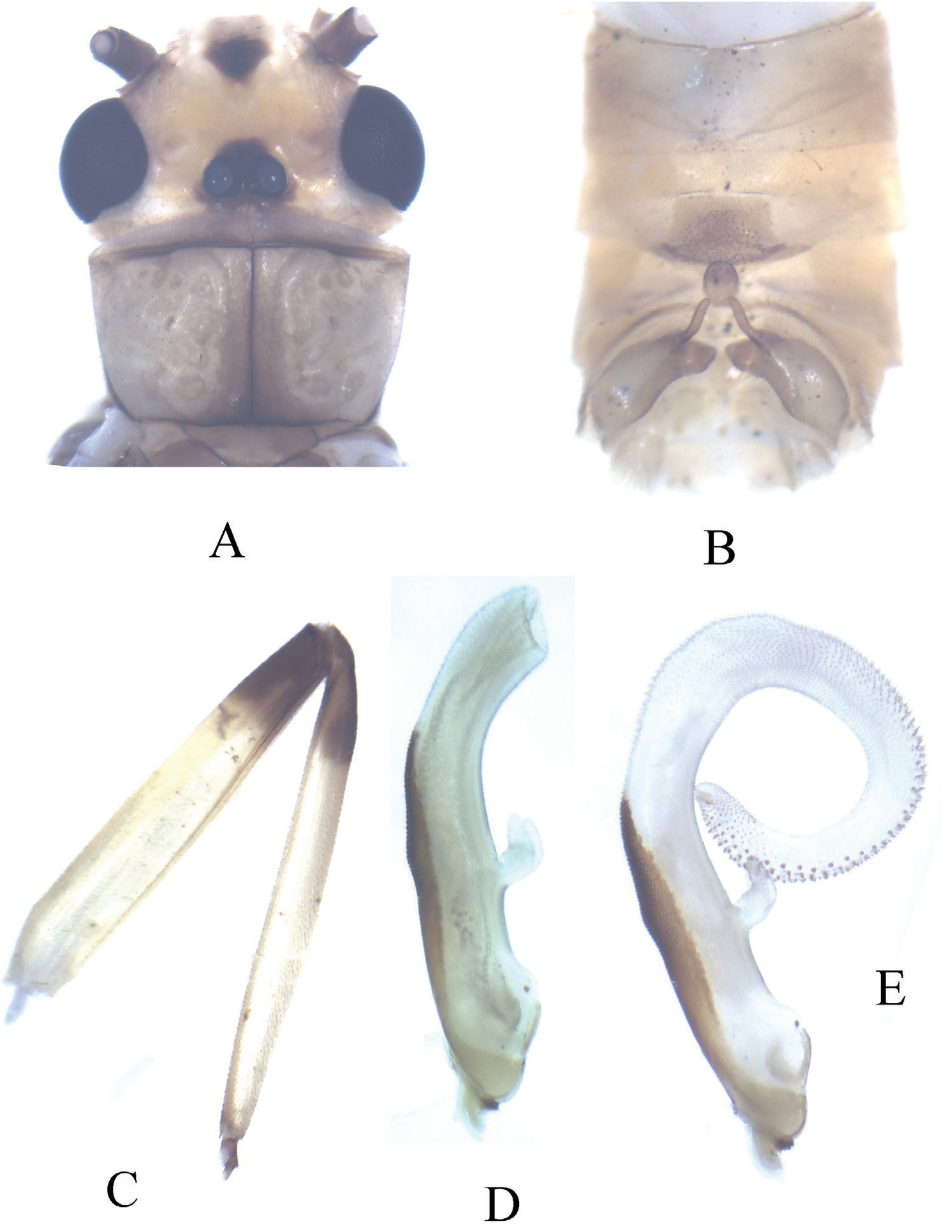

Figure 4. *Neoperla mesostyla* Li & Wang, sp. n. (male). **A** Head and pronotum, dorsal view **B** Terminalia, dorsal view **C** Hindleg (part of tarsi in this leg missing), lateral view **D** Aedeagus before eversion, lateral view **E** Aedeagus, lateral view.

Distribution. China (Guangxi).

Diagnosis. The new species appears to belong to a well differentiated subgroup of the montivaga species group (Zwick 1983) that has as tube characteristics a dark,

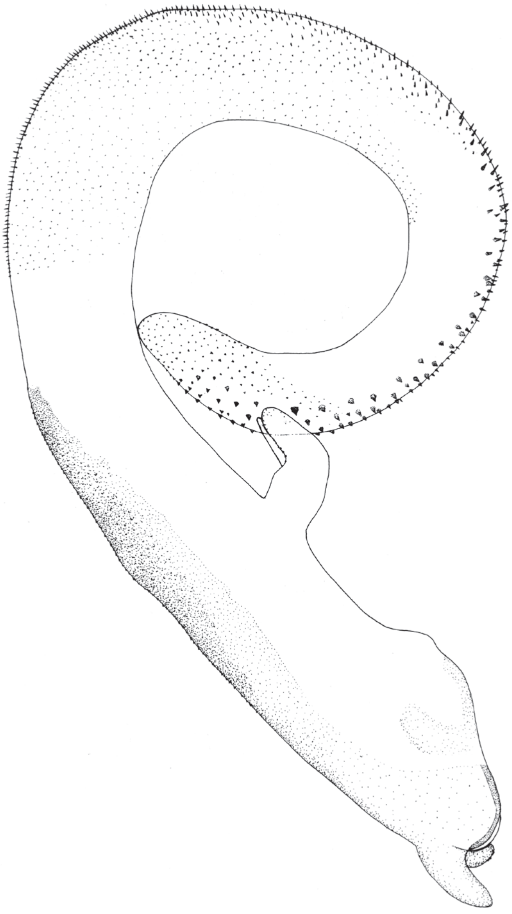

Figure 5. *Neoperla mesostyla* Li & Wang, sp. n. Male aedeagus, lateral view.

elongate sclerite dorsally and a bilobed, mostly membranous process ventrally (Zwick and Sivec 1985). Based on these features, several other species could also be assigned to this *diehli* subgroup: *Neoperla han* Stark, *N. mnong* Stark, *N. furcostyla* Li & Qin, *N. forcipata* Yang and Yang & *N. yao* Stark.

The new species is characterized by the aedeagal tube being short (≤3× width of bulb) and the ventral, bifurcate process being midlength on the tube. Additionally, the apical half of the sac is clothed with heavy spinules on the dorsal surface. *Neoperla diehli* has an elongate slender aedeagal tube (length ≥4× tube width), the ventral processes originate at the apex of the tube, and the heavy spinules are restricted to the tip of the sac (see Fig. 21 in Zwick and Sivec 1985). *Neoperla mesostyla* is easily distinguishable from other members of the group by its very long sac and relatively short Y-lobe. *Neoperla han* Stark and *N. yao* Stark have elongate Y-lobes (Figs 6, 10 in Stark 1987); *N. mnong* Stark, *N. furcostyla* Li & Qin, and *N. forcipata* Yang & Yang have a short or very short aedeagal sac (Fig. 7 in Stark 1987; Figs 2 B, C in Li et al. 2013).

Acknowledgments

We acknowledge the partial support of our research by the National Natural Science Foundation of China (No. 31000977).

References

Chu Y-T (1929) Descriptions of four new species and one new genus of stone-flies in the family Perlidae from Hangchow. The China Journal 10: 88–92.

DeWalt RE, Neu-Becker U, Stueber G (2013) *Plecoptera Species File Online*. Version 5.0/5.0. 6/12/2013. http://Plecoptera.SpeciesFile.org

Du Y-Z (1999) Plecoptera. In: Huang BK (Ed) Fauna of Insects in Fujian Province of China. Vol. 3. Fujian Science and Technology Publishing house, Fuzhou, Fujian, China. 301–335.

Du Y-Z (2000a) *Neoperla magisterchoui*, a new species of the genus *Neoperla* Needham (Plecoptera: Perlidae) from China. In: Zhang YL (Ed) Systematic and faunistic research on Chinese insects. Proceedings of the 5th National Congress of Insect Taxonomy. China Agriculture Press. Beijing, 1–3.

Du Y-Z (2000b) Two new species of the genus *Neoperla* Needham (Plecoptera: Perlidae: Perlinae) from Guizhou, China. Entomotaxonomia 22: 1–5.

Du Y-Z, Sivec I (2004) Plecoptera: Perlidae, Nemouridae, Leuctridae. In: Yang XK (Ed) Insects from Mt. Shiwandashan area of Guangxi. China forestry Publishing House, Beijing, 39–45.

Du Y-Z, Sivec I (2005) Plecoptera. In: Yang XK (Ed) Insect Fauna of Middle-west Qinling Range and South Mountains of Gansu Province. Science Press, Beijing, 38–54.

Du Y-Z, Wang Z-J (2005) Plecoptera: Leuctridae, Nemouridae, Perlidae and Peltoperlidae. In: Yang MF, Jin DC (Eds) Insects from Dashahe Nature Reserve of Guizhou. Guizhou People Press, Guiyang, Guizhou, 51–57.

Du Y-Z, Wang Z-J (2007) Nemouridae and Perlidae. In: Li ZZ, Yang MF, Jin DC (Eds) Insects from Mountain Leigongshan Landscape of Guizhou. Guizhou Science and Technology Publishing house, Guiyang, Guizhou, 84–90.

Du YZ, Sivec I, He J-H (1999) A checklist of the Chinese species of the family Perlidae (Plecoptera: Perloidea). Acta Entomologica Slovenica 7: 59–67.

Du Y-Z, Sivec I, Zhao M-S (2001) Plecoptera. In: Wu H, Pan CW (Eds) Insects of Tianmushan National Nature Reserve. Science Press, Beijing, 69–80.

Li W-H, Li X-P (2013) A new species of the genus *Neoperla* (Plecoptera: Perlidae) from China. Acta Zootaxonomica Sinica 38: 75–77.

Li W-H, Wang R-F (2011) A new species of *Neoperla* (Plecoptera: Perlidae) from China. Entomological News 122: 261–264. doi: 10.3157/021.122.0308

Li W-H, Wang H-L, Lu W-Y (2011) Species of the genus *Neoperla* (Plecoptera: Perlidae) from Henan, China. Zootaxa 2735: 57–63.

Li W-H, Wang G-Q, Lu W-Y (2012) Species of *Neoperla* (Plecoptera: Perlidae) from Hubei, China. Zootaxa 3478: 32–37.

Li W-H, Wang G-Q, Li W-L, Murányi D (2012) Review of *Neoperla* (Plecoptera: Perlidae) from Guangdong Province of China. Zootaxa 3597: 15–24.

Li W-H, Wang G-Q, Qin X-F (2013) Two new species of *Neoperla* (Plecoptera: Perlidae) from China. ZooKeys 290: 21–30. doi: 10.3897/zookeys.290.4568

Li W-H, Wang L-H, Yu H (2012) First record of *Kamimuria* (Plecoptera: Perlidae) from Hainan Island of China, with description of *K. hainana* n. sp. Zootaxa 3500: 84–88.

Li W-H, Wu L-M, Zhang H-R (2011) A new species of the genus *Neoperla* (Plecoptera: Perlidae) from Henan, China. Acta Zootaxonomica Sinica 36: 33–35.

Sivec I, Zwick P (1987) Some *Neoperla* (Plecoptera) from Taiwan. Beiträge zur Entomologie 37: 391–405.

Sivec I, Stark BP (2008) New species of *Kamimuria* Klapálek (Plecoptera: Perlidae) from Thailand and Vietnam, with notes on Chinese species. Illiesia 4: 110–138.

Sivec I, Stark BP, Uchida S (1988) Synopsis of the world genera of Perlinae (Plecoptera: Perlidae). Scopolia 16: 1–66.

Stark BP (1987) Records and descriptions of Oriental Neoperlini (Plecoptera: Perlidae). Aquatic Insects 9: 45–50. doi: 10.1080/01650428709361270

Stark BP, Sivec I (2008) New species and records of *Neoperla* (Plecoptera: Perlidae) from Vietnam. Illiesia 4: 19–54.

Sun H-T, Du Y-Z (2012) Two new species of the genus *Kamimuria* (Plecoptera: Perlidae) from China, with redescription of *Kamimuria cheni* Wu and *Kamimuria chungnanshana* Wu. Zootaxa 3455: 61–68.

Wu C-F (1935) Aquatic insects of China. Article XXI. New species of stoneflies from East and South China. (Order Plecoptera). Peking Natural History Bulletin 9: 227–243.

Wu C-F (1938) Plecopterorum sinensium: A monograph of stoneflies of China (Order Plecoptera). Yenching University, 225 pp.

Wu C-F (1948) Fourth supplement to the stoneflies of China (Order Plecoptera). Peking Natural History Bulletin 17: 75–82.

Wu C-F (1962) Results of the Zoologico-Botanical expedition to Southwest China, 1955-1957 (Plecoptera). Acta Entomologica Sinica 11 (Supplement): 139–153.

Wu C-F (1973) New species of Chinese stoneflies (Order Plecoptera). Acta Entomologica Sinica 16: 97–118.

Wu C-F, Claassen PW (1934) Aquatic insects of China. Article XXI. New species of Chinese stoneflies. (Order Plecoptera). Peking Natural History Bulletin 9: 111–129.

Yang C-K, Yang D (1990) New and little-known species of Plecoptera from Guizhou Province (I).Guizhou Science 8: 1–4.

Yang C-K, Yang D (1991) New and little-known species of Plecoptera from Guizhou Province (II). Guizhou Science 9: 48–50.

Yang D, Yang C-K (1992) Plecoptera: Perlidae. In: Huang FS (Ed) Insects of Wuling Mountains area, Southwestern China. Science Press, Beijing, 62–64.

Yang D, Yang C-K (1993) New and little-known species of Plecoptera from Guizhou Province (III). Entomotaxonomia 15: 235–238.

Yang D, Yang C-K (1995a) Three new species of Plecoptera from Hainan Province. Acta Agriculture Universitatis Pekinensis 21: 223–225.

Yang D, Yang C-K (1995b) Plecoptera: Perlidae. In: Wu H (Ed) Insects of Baishanzu Mountain, Eastern China. China Forestry Publishing House, Beijing, 59–60.

Yang D, Yang C-K (1996) Four new species of Plecoptera from Nei Mongol. Journal of China Agricultural University 1: 115–118.

Yang D, Yang C-K (1998) Plecoptera: Styloperlidae, Perlidae and Leuctridae. In: Wu H (Ed) *Insects of Longwangshan*. China Forestry Publishing House, Beijing, 40–46.

Zwick P (1983) The *Neoperla* of Sumatra and Java (Indonesia) (Plecoptera: Perlidae). Spixiana 6: 167–204.

Zwick P, Sivec I (1985) Supplements to the Perlidae (Plecoptera) of Sumatra. Spixiana 8: 123–133.