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# *Protanthomyza grimaldii* sp. nov., a further member of the extinct subfamily Protanthomyzinae (Diptera, Anthomyzidae) from Baltic amber

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## Abstract

A new fossil species, *Protanthomyza grimaldii* **sp. nov.** (Diptera, Anthomyzidae), is described from Baltic amber (Eocene, 48–34 Ma) based on two (male and female) inclusions. It is the ninth species of the †genus *Protanthomyza* Hennig, 1965 and †subfamily Protanthomyzinae Roháček, 1998. Adult morphology of *P. grimaldii* **sp. nov.** revealed that the rich chaetotaxy of the thoracic pleuron, two anal veins and presence of the anteroventral process of the epandrium are plausibly shared by all species of *Protanthomyza*. Relationships of the new species, which belongs to a group lacking the ctenidial spine on the fore femur, are discussed.

## Keywords

Anthomyzid flies, adult morphology, Eocene, new extinct species, relationships, taxonomy, Tertiary

## Introduction

Fossil taxa of Anthomyzidae (Diptera) were reviewed by Roháček (2013a), with one species subsequently added (Roháček 2014). A total of 12 ancient (all Tertiary) valid and named species belonging to four genera are currently recognized. The majority of fossil species of Anthomyzidae were described from Baltic amber (48–34 Ma) and only one, viz. *Grimalantha vulnerata* Roháček, 1998, originates from Dominican amber (Miocene, 18–16 Ma). Only four of the ancient species belong to the "modern" subfamily Anthomyzinae Czerny, 1903, viz. the above *Grimalantha vulnerata* from the Miocene (see Roháček, 1998) and *Lacrimyza lacrimosa* Roháček, 2013, *L. christelae* Roháček, 2013 and *Reliquantha eocena* Roháček, 2014 from the Eocene. The latter species belongs to a genus originally described by Roháček (2013b) for an extant species, *R. variipes* Roháček, 2013 from Great Britain. All remaining ancient Anthomyzidae are members of the exclusively fossil subfamily Protanthomyzinae Roháček, 1998 and belong to its only genus *Protanthomyza* Hennig, 1965. Eight named species of this genus are currently recognized, viz. *P. collarti* Hennig, 1965, *P. hennigi* Roháček, 2013, *P. hoffeinsorum* Roháček, 2013, *P. krylovi* Roháček, 2013, *P. loewi* Roháček, 2013, *P. meunieri* Roháček, 2013, *P. presli* Roháček, 2013 and *P. tschirnhausi* Roháček, 2013, all of which were treated in detail by Roháček (2013a). However, Roháček (2013a: 451) also presented the diagnosis of one more new species of *Protanthomyza* which was left unnamed due to insufficient material (a single female with some parts of the body obscured). This unnamed species was considered distinctly different from all other known members of the genus and, therefore, it was also counted among them for the estimation of species diversity of Anthomyzidae in the Eocene Baltic amber forest ecosystem (Roháček 2013a: 470). Recently, Mrs. Christel Hoffeins purchased a nicely preserved *Protanthomyza* inclusion and provided it for study. Detailed examination of this specimen revealed it to be the formerly unknown male conspecific with the unnamed "*Protanthomyza* sp. nov." female dealt with in Roháček (2013a). To supplement the latter monographic treatment, this new species is described in detail below, including the re-examination of the above female specimen. This is only the third fossil species of *Protanthomyza* (and the fourth of all ancient Anthomyzidae) in which both sexes are known.

## Material and methods

### Material

Two amber pieces with 2 anthomyzid inclusions were examined. Abbreviations of amber sources and depositories: **AMNH** – American Museum of Natural History, New York, USA; **CCHH** – collection of C. and H. W. Hoffeins, Hamburg, Germany; **CMTB** – collection of M. von Tschirnhaus, Bielefeld, Germany; **SDEI** – Senckenberg Deutsches Entomologisches Institut (Senckenberg German Entomological Institute) in Müncheberg, Germany.

## Preparation of amber specimens

The methods of preparation of amber stones with fly inclusions were described in detail by von Tschirnhaus and Hoffeins (2009). The amber specimens examined had already been cut out of the original stones, ground and polished as close and as parallel as possible to the frontal, dorsal and lateral sides of the fly and one of them had been subsequently embedded in artificial resin (also ground and polished) (Hoffeins 2001) to facilitate its stereoscopic investigation. This process left a significant cut portion of one stone bearing syninclusions, which remains deposited in CCHH.

## Techniques of investigation

The amber inclusions were examined, drawn and measured using two types of binocular stereoscopic microscopes (Reichert, Olympus). Legs were drawn on squared paper using a Reichert binocular microscope with an ocular screen. The specimens were either photographed by a Canon EOS 60D digital camera with Canon MP-E 65 mm 1–5× macro lens or by a Canon EOS 5D Mark III digital camera with a Nikon CFI Plan 10×/0.25NA 10.5mm WD objective attached to a Canon EF 70–200 mm f/4L USM zoom lens. The specimen photographed by means of the latter equipment was repositioned upwards between each exposure using a Cognisys StackShot Macro Rail and the final photograph was compiled from multiple layers (35) using Helicon Focus Pro 7.0.2. The final images were edited in Adobe Photoshop CS6. Some illustrations were drawn from these and some other macrophotographs and details were inked based on direct observation at higher magnification using a binocular microscope. Measurements: Six characteristics were measured – body length (measured from anterior margin of head to end of cercus, thus excluding the antenna), wing length (from wing base to wing tip), wing width (maximum width), index $Cs_3 : Cs_4$ (= ratio of length of 3rd costal sector : length of 4th costal sector), index *r-m\dm-cu* : *dm-cu* (= ratio of length of section between *r-m* and *dm-cu* on cell *dm* : length of *dm-cu*) and index *r-m\dm-cu* : $CuA_1$ (= ratio of length of section between *r-m* and *dm-cu* on cell *dm*: length of apical portion of $CuA_1$).

**Morphological terminology** follows that used in Roháček (2006, 2009) and Roháček and Barber (2016), including terms of the male hypopygium to be in continuation with Roháček (2013a), except where "orbit" is replaced with "orbital plate". Male terminalia terminology is largely based on the "hinge" hypothesis of the origin of the eremoneuran hypopygium, re-discovered and documented by Zatwarnicki (1996) and, therefore, the following alterations of terms of the male genitalia (against those used by other hypotheses) need to be listed (terms used here first): epandrium = periandrium, gonostylus = surstylus. Morphological terms of the male abdomen and terminalia are depicted in Figs 8, 9, those of the female abdomen in Fig. 13. The synonymous morphological terms of adult structures and their abbreviations as used in the recent manual of Afrotropical Diptera (Cumming and Wood 2017) are given in parentheses in the list of abbreviations below.

## Abbreviations of morphological terms used in text and/or figures

| | |
|---|---|
| **$A_1$** | first anal vein (= anterior + posterior branch of cubital vein, *CuA*+ *CuP*) |
| **$A_2$** | second anal vein (= first branch of anal vein, $A_1$) |
| ***ac*** | acrostichal (seta) (*acr*) |
| ***ar*** | arista |
| ***avp*** | anteroventral process of epandrium; |
| ***C*** | costa |
| ***ce*** | cercus |
| **$CuA_1$** | cubitus (= fourth branch of media, $M_4$) |
| ***dc*** | dorsocentral (seta) |
| ***dm*** | discal medial cell |
| ***dm-cu*** | discal medial-cubital (= discal medial, *dm-m*) cross-vein |
| ***ep*** | epandrium |
| **$f_1$, $f_2$, $f_3$** | fore, mid, hind femur |
| ***ha*** | haltere |
| ***hu*** | humeral (= postpronotal, *pprn*) (seta) |
| ***lbl*** | labellum |
| ***M*** | media (= first branch of media, $M_1$) |
| ***mspl*** | mesopleural (= anepisternal, *anepst*) (seta) |
| ***npl*** | notopleural (seta) |
| ***oc*** | ocellar (seta) |
| ***ors*** | orbital (seta) (*orb*) |
| ***pa*** | postalar (seta) (*pal*) |
| ***pk*** | preapical kink |
| ***plp*** | maxillary palpus |
| ***poc*** | postocular (setulae) |
| ***ppl*** | propleural (= proepisternal + proepimeral, *prepst* + *prepm*) (seta) |
| ***prs*** | presutural (= presutural intraalar, *ial*) (seta) |
| ***prsc*** | prescutellar acrostichal (seta) |
| ***pvt*** | postvertical (= postocellar, *poc*) (seta) |
| **$R_1$, $R_{2+3}$, $R_{4+5}$** | 1st, 2nd, 3rd branches of radius |
| ***r-m*** | radial-medial cross-vein |
| ***S1–S8*** | abdominal sterna |
| ***sa*** | supraalar (seta) (*spal*) |
| ***sc*** | scutellar (seta) (*sctl*) |
| ***Sc*** | subcosta |
| ***stpl*** | sternopleural (= katepisternal, *kepst*) (seta) |
| ***T1–T8*** | abdominal terga |
| **$t_1$, $t_2$, $t_3$** | fore, mid, hind tibia |
| ***vi*** | vibrissa (*vb*) |
| ***vte*** | outer vertical (seta) (*o vt*) |
| ***vti*** | inner vertical (seta) (*i vt*) |

## Systematic palaeontology

**Class Insecta Linnaeus, 1758**
**Order Diptera Linnaeus, 1758**
**Superfamily Opomyzoidea Fallén, 1820**
**Family Anthomyzidae Czerny, 1903**
**Subfamily Protanthomyzinae Roháček, 1998**

### Genus *Protanthomyza* Hennig, 1965

**Type species.** *Protanthomyza collarti* Hennig, 1965; Baltic amber (Eocene).

### *Protanthomyza grimaldii* sp. nov.

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Figures 1–14

*Protanthomyza* sp. nov.: Roháček 2013a: 451–452, fig. 7E, F (diagnosis, female only).

**Etymology.** The species is dedicated to Prof. Dr. David Grimaldi (New York, U. S. A.), the distinguished American dipterist and palaeontologist, in recognition of his valuable contribution to the knowledge of amber fossil flies, including acalyptrates (largely from Dominican amber).

**Type material.** ***Holotype*** ♂ labelled "Faszination Bernstein, Christel Hoffeins, Hans Werner Hoffeins" (framed on obverse), "1040-5a Diptera: Acalyptratae, Anthomyzidae ♂" (handwritten by C. Hoffeins, on reverse), "Baltic amber, Russia: Kaliningrad region, Yantarny", "obtained in May 2010 from Dr. Andrey Krylov, Kaliningrad, Russia", and "Holotypus ♂, *Protanthomyza grimaldii* sp.n., J. Roháček det. 2020" (red label) [amber piece embedded in polyester resin, size 8.6 × 7.9 × 4.8 mm], deposited in SDEI (inventory number Dip-00821). The original amber stone (in form of an icicle = Zapfenschlaube in German), size about 45 × 20 × 11 mm, with multiple layers and aggregation of inclusions, was cut in two pieces; that with the inclusion of *P. grimaldii* (No. 1040-5a) was separated, manually prepared and embedded in polyester resin by H. W. Hoffeins in August 2015. Syninclusions in 1040-5a: 1 stellate hair, pollen grains. Syninclusions in the remaining part (1040-5b, deposited in CCHH): Diptera: Empididae: 1 *Rhamphomyia* sp. female; 2 Mycetophilidae males + fragment; 1 Simuliidae; 2 Chironomidae female and male; Trichoptera, not identifiable; Coleoptera fragment; Araneae fragment; stellate hairs. Paratype ♀ labelled "47b-1, (Baltic A.), 15.1 × 10.7 × 3.3 [mm]" (handwritten), "*Protanthomyza* sp.n. ♀, J. Roháček det. 2011" (yellow label) and "Paratypus ♀, *Protanthomyza grimaldii* sp.n., J. Roháček det. 2020" (yellow label) [shape of stone irregularly pentagonal, thin], temporarily held in CMTB; it will be deposited in AMNH. Syninclusions: only stellate hairs.

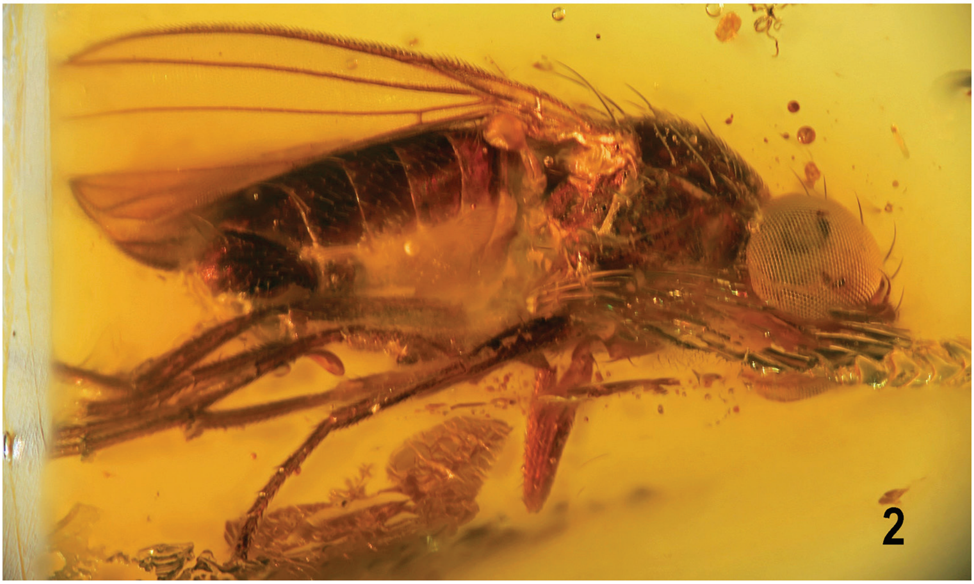
**Type locality and age.** Russia: Kaliningrad region, Yantarny mine. Middle to Late Eocene, 48–34 Ma (cf. Seyfullah et al. 2018).

**Diagnosis.** Ocellar triangle delimited by a groove; frontal triangle not delimited; 1st antennal flagellomere normal, not enlarged; arista bare; 3 or 4 *dc* macrosetae; $f_1$ without a ctenidial spine; male epandrium elongate and posteriorly tapered, with a robust, hook-like, curved anteroventral process; female T7 short; female S6 and S7 broadly transverse.

**Description. Male** (Figs 1, 2). Total body length ca 2.6 mm; general colour brown to blackish brown; only head and some extremities partly ochreous to yellow.

***Head*** (Figs 3–5) higher than long, anteriorly somewhat angularly protruding in front of eye margin (Fig. 5). Occiput very slightly concave, blackish brown. Frons relatively narrow, blackish brown only posteriorly around ocellar triangle, pale brown in anterior half to ochreous yellow at anterior marginal area surrounding frontal lunule. Frontal triangle not developed; ocellar triangle blackish brown, distinctly protruding and delimited by marginal groove. Ocelli large (Figs 3, 4). Orbital plate lighter (ochreous yellow) anteriorly, becoming pale brown posteriorly where contrasting with blackish-brown vicinity of ocellar triangle. Frontal lunule long, ochreous yellow. Face relatively narrow, ochreous, medially lighter and somewhat depressed; parafacialia and anterior half of gena dirty yellow and narrowly brown bordered (gena ventrally); posterior half of gena and postgena brown; mouthparts yellow to ochreous, clypeus pale brown, palpus dirty yellow. Cephalic chaetotaxy (Figs 4, 5): *pvt* (only left one visible) relatively short, convergent but not crossed; *vti* longest of cephalic setae, slightly inclinate; *vte* strongly exclinate and only slightly shorter than *vti*; 3 distinct *ors*, all slightly reclinate, posterior *ors* longest (about as long as *vte*), others becoming slightly shorter anteriorly; *oc* relatively thin (not longer than middle *ors*), proclinate (and unnaturally crossed in holotype), arising inside ocellar triangle; anterior half of frons with about 5 or 6 pairs of microsetae, mostly medially in front of ocellar triangle but a few (1 or 2) also between anterior and middle *ors*; *vi* distinct (Fig. 5), about 3 times as long as foremost peristomal setula; no subvibrissa; 4 or 5 weak proclinate peristomals; postocular setulae in two rows as usual but with only 3 setulae in inner row (Fig. 4); outer row of postoculars long, reaching ventral eye margin; postgena with 2 setae, anterior short, posterior longer. Palpus slender, elongate, with a few (3 visible) minute setulae distally (Fig. 5). Mouthparts relatively short. Eye bare, relatively large and strongly convex, suboval, anteriorly regularly rounded, with only posterior margin somewhat straighter; its longest diameter almost vertical and 1.16 times as long as shortest diameter. Gena low, its shortest height about 0.08 times as long as shortest eye diameter. Antenna medium-sized, generally porrect (Fig. 5) but directed anteroventrally to ventrally, with dark-brown basal segments and pale-brown to ochreous 1st flagellomere. Pedicel with 1 longer seta and several microsetae; 1st flagellomere oval, laterally compressed, with very short, dense and dark pilosity; arista about 1.8 times as long as antenna, entirely bare (Fig. 5), 2 basal segments slightly widened.

***Thorax*** hardly narrower than head, brown to blackish brown, with shining mesonotum and duller pleural part. Mesonotum relatively convex, separated from scutellum by deep suture. Scutellum rounded subtriangular, wider than long, convex dorsally; postscutellum not visible. Thoracic chaetotaxy (see Figs 3, 4, 7) rich as usual in



**Figures 1, 2.** *Protanthomyza grimaldii* sp. nov., holotype male (Baltic amber) **1** entire specimen, left laterodorsal view **2** ditto, right lateroventral view. Body length ca 2.6 mm. Photographs by J. Roháček.

the genus: 1 short *hu* (markedly shorter than anterior *npl*) and only 2 or 3 setulae on humeral callus (= postpronotal lobe); 2 long *npl*, anterior slightly longer; 1 distinct *prs* (about as long as posterior *npl*); 1 long *sa*; 2 *pa*, external very long (longer than *sa*), internal shorter (not visible on left side in Fig. 4); 3 or 4 postsutural *dc* (number different on left and right sides) becoming shorter anteriorly, the hindmost longest (together with apical *sc* longest thoracic setae); *ac* microsetae dense, in 9 or 10 rows on suture,

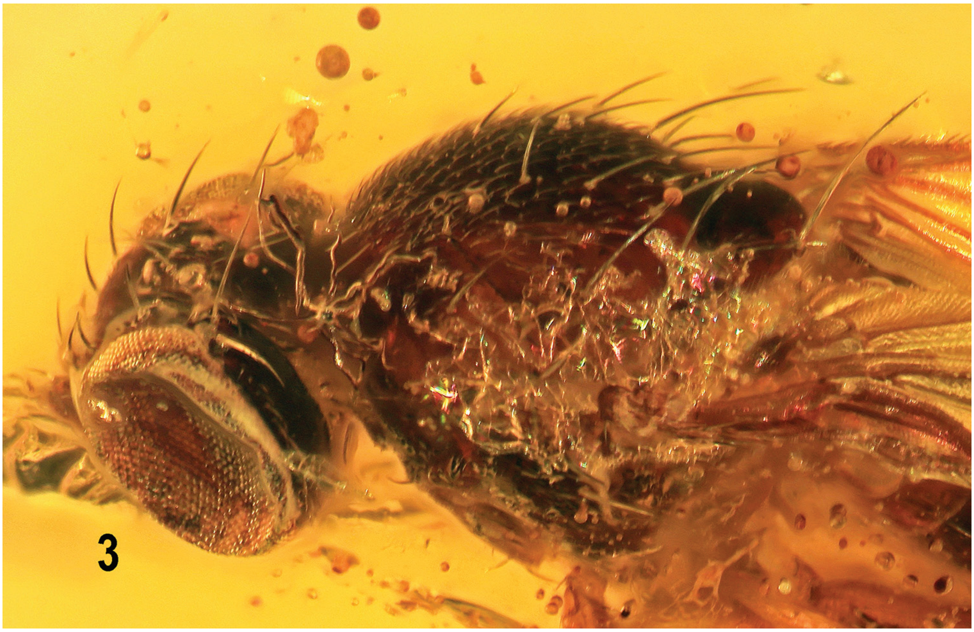


**Figure 3.** *Protanthomyza grimaldii* sp. nov., holotype male (Baltic amber). Head and thorax, left latero-dorsal view. Photograph by J. Roháček.

those in medial rows reaching up to posterior *dc*; prescutellar *ac* macrosetae long and strong, as long as *sa*; 2 *sc*, laterobasal shorter (about as long as *prs*), apical very long; no additional setulae on scutellum; 2 upcurved *ppl*, anterior distinct (as long as *hu*), posterior small. Mesopleuron (anepisternum) and sternopleuron (katepisternum) setose (Figs 4, 7) as in most other species of *Protanthomyza*: 3 long *mspl* (1 dorsal upcurved and shortest, 1 posterodorsal and 1 posterior longest, cf. Fig. 7) and numerous setulae in posterodorsal half of mesopleuron; 1 long posterior *stpl* and about 9 or 10 setulae in posterior half of sternopleuron; other sclerites of pleural part of thorax bare.

***Legs*** brown to ochreous, femora darkest, fore coxa pale ochreous. $f_1$ lacking ctenidial spine; 5 (2 longer) distinct widely-spaced setae in posterodorsal row; setae in posteroventral row more numerous but short and weak. $f_2$ with 2 or 3 anterior setae near middle (cf. Fig. 14), otherwise shortly setulose as is $f_3$. $t_2$ with distinct ventroapical seta (about as long as maximum width of $t_2$) and 2 or 3 small setae adjacent to the latter; $t_1$, $t_3$ and all tarsi simply setulose but basitarsi of all legs with ventrobasal setulae somewhat longer than others.

***Wing*** (Figs 1, 6) moderately long and narrow, widest at distal third; veins brown to pale brown, membrane unicolourous, pale-brown tinged; *C* with more or less distinct subcostal break and somewhat attenuated at humeral cross-vein. *C* extended to apex of *M*, densely uniformly setulose on $Cs_2$ (from subcostal break to apex of $R_{2+3}$), finely short-pilose more distally (on $Cs_3$ and $Cs_4$); *Sc* distinct, separate almost along its entire length, only apically fused with $R_1$ to form preapical kink (see Fig. 6, *pk*); $R_1$ short, dilated distally due to fusion with *Sc*; $R_{2+3}$ long, very slightly sinuate to almost straight

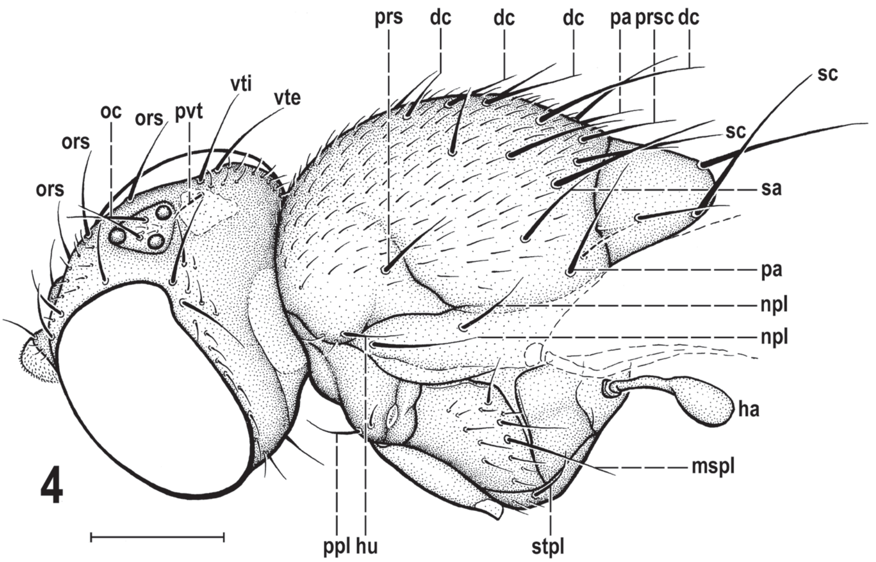
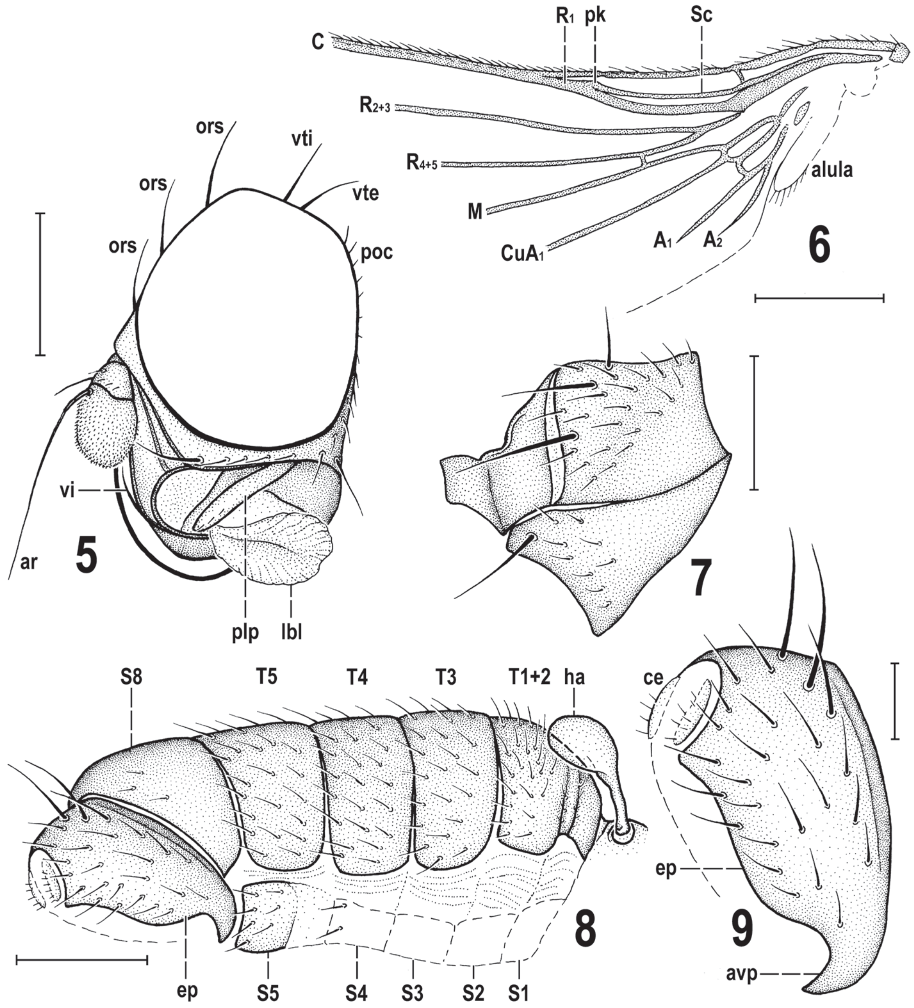


**Figure 4.** *Protanthomyza grimaldii* sp. nov., holotype male (Baltic amber). Head and thorax, left latero-dorsal view. Scale bar: 0.3 mm. For abbreviations see p. 4.

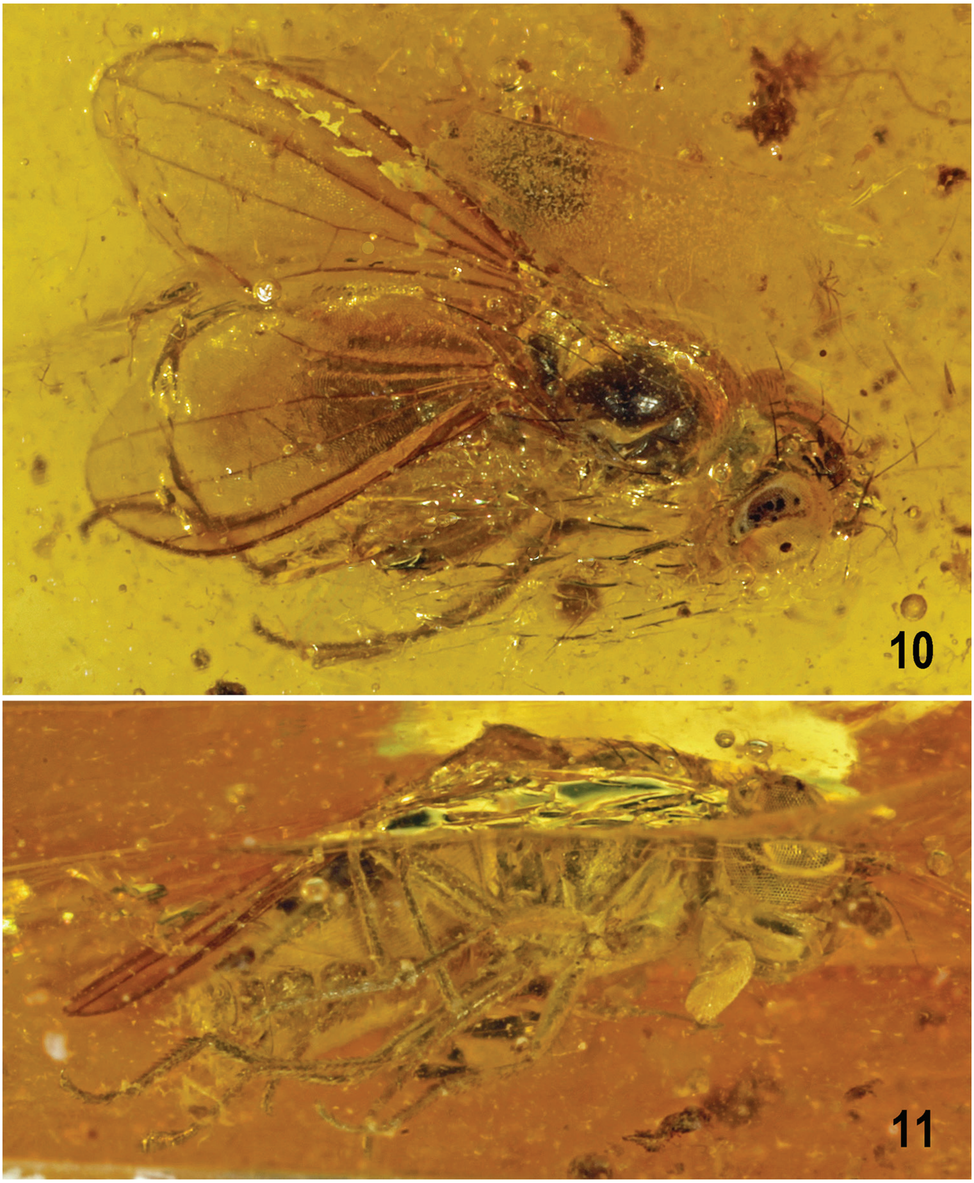
and only its extreme apex slightly upcurved to *C*; $R_{4+5}$ slightly recurved in distal half, divergent from $R_{2+3}$ and apically slightly convergent with *M*; *M* almost straight. Cell *dm* of moderate length, narrow proximally and much widened distally, with angle of anterior outer corner obtuse while that of posterior outer corner distinctly acute (cf. Fig. 12); *r-m* situated in basal third of cell *dm*; *dm-cu* straight; apical portion of $CuA_1$ much shorter than distance between *r-m* and *dm-cu*, distinctly longer than *dm-cu* and almost reaching wing margin; $A_1$ relatively long but ending far from wing margin; $A_2$ well developed, slightly shorter than $A_1$ (Fig. 6); alula distinct but narrow (Fig. 6). Wing measurements: length ca 2.4 mm, width ca 0.8 mm, $Cs_3 : Cs_4 = 1.38$, *r-m\dm-cu* : *dm-cu* = 3.50, *r-m\dm-cu* : $CuA_1 = 2.50$. Haltere (Figs 4, 8) pale ochreous, knob relatively large, darker dorsally.

***Abdomen*** (Figs 1, 2, 8) relatively short, robust but not very broad. Preabdominal terga blackish brown; *T1* fused with *T2* but delimited by a groove (Fig. 8); *T1* finely short-setose, *T2–T5* with relatively long and dense setae (Fig. 8); *T2* somewhat shorter than *T3*, *T3–T5* subequal in length, all relatively short and transverse (Figs 2, 8). Preabdominal sterna mostly invisible (Figs 2, 8) but probably small (narrow) and pale-pigmented; only *S5* discernible (Fig. 8), paler brown and shorter than adjacent *T5*, with sparse setae. Also 2 setae of *S4* visible on right side of abdomen (Fig. 8) but margins of sclerite are not recognizable. Postabdomen: *S6* and *S7* not visible because situated on obscured left side of abdomen (cf. Fig. 1), probably asymmetrical and (partly) fused together (dorsally also with *S8*) as in other *Protanthomyza* species (cf. Roháček 2013a, fig. 4H). *S8* relatively long, blackish brown, situated dorsally and readily visible on right side (Figs 2, 8), almost bare, with only a few setulae.



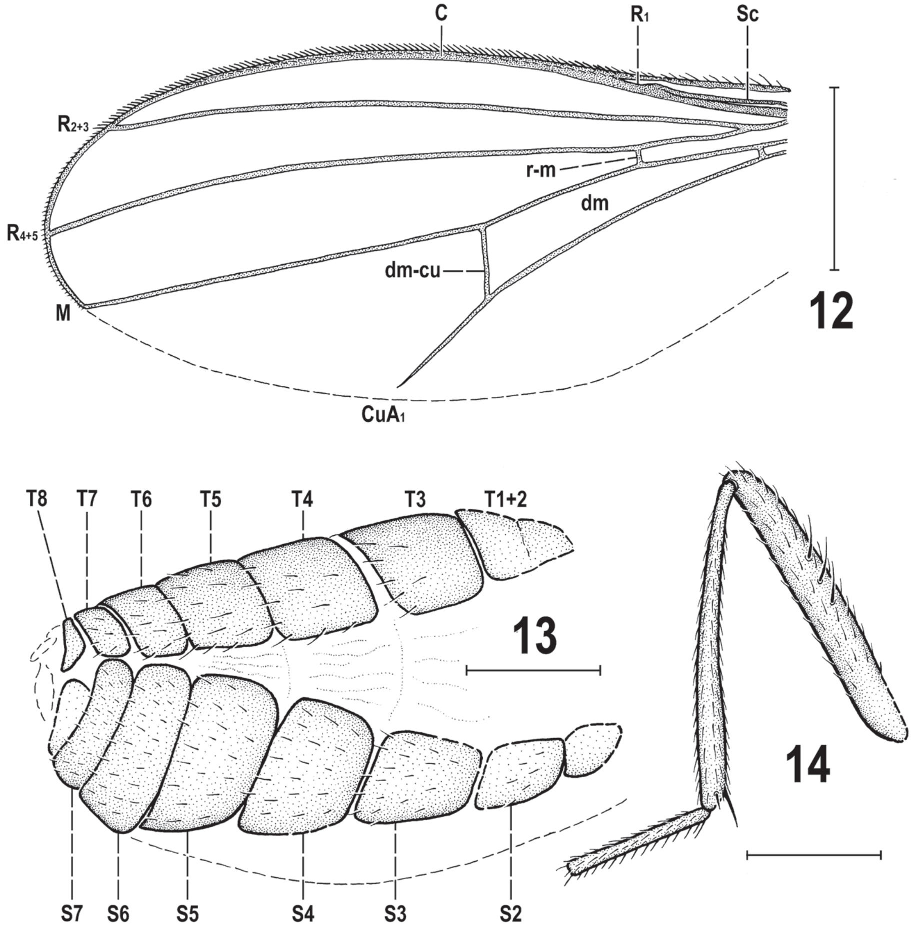
**Figures 5–9.** *Protanthomyza grimaldii* sp. nov., holotype male (Baltic amber) **5** head, left sublateral view **6** base of left wing **7** right mesopleuron, sternopleuron and pteropleuron, lateral view **8** abdomen and haltere, right lateral view **9** terminalia, right lateral view. Scale bars: 0.5 mm (**5**); 0.3 mm (**6–8**); 0.1 mm (**9**). For abbreviations see p. 4.

***Genitalia.*** Epandrium (Figs 8, 9) relatively long but tapered posteriorly, with anteroventral corner modified to distinct, flat and somewhat hook-like projection (see Fig. 9, *avp*) distinctly different from those in other *Protanthomyza* species where known (cf. Roháček 2013a, figs 1F, 2E, 4H, 6B, 12C). Epandrium anterodorsally with 3 pairs of long erect setae (the most lateral markedly shorter), otherwise with scattered short setae. Anal fissure relatively small and cercus reduced (small and narrow) with fine short pubescence (Fig. 9). Gonostylus not discernible (on any side) but probably small and pale. No structures of internal genitalia visible.



**Figures 10, 11.** *Protanthomyza grimaldii* sp. nov., female paratype (Baltic amber) **10** entire specimen, subdorsal view **11** ditto, lateroventral view. Body length ca 2.9 mm. Photographs by J. Roháček (adapted from Roháček 2013a, fig. 7E, F).

**Female** (Figs 10, 11). Similar to male unless mentioned otherwise. Total body length ca 2.9 mm. *pvt* short, strongly convergent, with apices meeting medially; *oc* proclinate and divergent; setae in posteroventral corner of postgena subequal. Palpus with more setulae (5 or 6) visible ventrally, subapically and apically. Mesonotum with 3 strong *dc* in left row and with 4 *dc* (2 posterior strong, 2 anterior short and



**Figures 12–14.** *Protanthomyza grimaldii* sp. nov., female paratype (Baltic amber) **12** left wing (invisible parts omitted) **13** abdomen, right lateroventral view **14** right $f_2$, $t_2$ and mid basitarsus, anterior view. Scale bars: 0.5 mm (**12**), 0.3 mm (**13**, **14**). For abbreviations see p. 4.

weaker) in right row; prescutellar *ac* macrosetae longer than *sa*; about 8 rows of *ac* microsetae on suture. Legs more slender but with same chaetotaxy as in male, except $t_2$ (Fig. 14) with ventroapical seta longer. Wing (Fig. 12) venation resembling that of male but cell *dm* shorter and cross-vein r-m situated more distally. Wing measurements: length 2.58 mm, width 0.95 mm, $Cs_3 : Cs_4 = 1.44$, $rm\backslash dm\text{-}cu : dm\text{-}cu = 2.37$, $rm\backslash dm\text{-}cu : CuA_1 = 1.97$.

***Abdomen*** (Figs 11, 13) only partly visible. Preabdomen with terga (*T1+2, T3–T5*) obviously darker brown than sterna, relatively narrow, hence pleural part of preabdominal segments large; setae on *T3–T5* (and also on *T6* and *T7*) longer than those on adjacent sterna. Preabdominal sterna pale brown to ochreous. *S1–S5* becoming distinctly wider posteriorly, *S5* widest and largest.

***Postabdomen.*** *T6* distinctly shorter than *T5*, transverse; *T7* yet shorter and also narrower than *T6*. *S6* strikingly broad and transverse, wider but shorter than *S5* and apparently larger than adjacent *T6* and laterally almost reaching the latter; *S7* only half length of *S6*, strongly transverse and laterally meeting with sides of *T7* (Fig. 13); both *S6* and *S7* finely setulose. Apex of postabdomen obscured, only *T8* discernible as small bare(?) sclerite (Fig. 13), less than half length of *T7*. Cercus not visible.

## Discussion

*Protanthomyza grimaldii* sp. nov. was previously recognized as a new species by Roháček (2013a: 451) but left unnamed because of insufficient material (a single female having a number of characters not visible). Thanks to the efforts of Christel Hoffeins, a male specimen conspecific with this female was recently obtained for examination and enabled the description and naming of this species. This is the third species of *Protanthomyza* where both sexes are known; formerly, the male and female were described only in *P. krylovi* (1 male and 2 females found in one piece of Baltic amber) and *P. tschirnhausi* (1 male and 1 female in separate pieces of Bitterfeld amber), see Roháček (2013a).

*Protanthomyza grimaldii* belongs to a group of species lacking a ctenidial spine on the fore femur and both sexes are correctly keyed by Roháček (2013a: 442, as *P.* sp. nov.). With its bare arista and generally similar chaetotaxies of the head and thorax, it most closely resembles *P. loewi* (known only from the female), which could be its nearest relative. However, *P. grimaldii* can be easily distinguished from *P. loewi* by the distinctly smaller 1st flagellomere of the antenna (Fig. 5, cf. Roháček 2013a, fig. 8B), the ocellar triangle delimited by a groove (Fig. 4), the less elongate wing with more divergent $R_{2+3}$ and $R_{4+5}$ (Fig. 12, cf. Roháček 2013a, fig. 7D) and the shorter and wider female abdomen with short T7 and broadly transverse S6 and S7 (Fig. 13, cf. Roháček 2013a, fig. 8A), apart from other smaller dissimilarities in head colouration, length of *ppl* setae, $f_1$ and $t_2$ chaetotaxy, etc. In the male, *P. grimaldii* differs from all five other species where the male is known (cf. Roháček 2013a) by the elongate and posteriorly tapered epandrium with a robust, hook-like, curved anteroventral process (Fig. 9, *avp*). Only in *P. tschirnhausi* is the epandrial process similarly robust and flat but it is simply triangular (not hooked) and the epandrium is short, almost globose (not elongate) (cf. Roháček 2013a, fig. 12C). Moreover, *P. tschirnhausi* differs markedly from *P. grimaldii* in a number of other characters including the ciliate arista, very large eyes, only 2 *dc* and a strong ctenidial spine on $f_1$, see Roháček (2013a). Unfortunately, the male of *P. loewi* remains unknown and, consequently, the previously suggested relationship of that species with *P. grimaldii* cannot be confirmed by examination of characters of the male terminalia.

## Conclusions

Based on the data presented above, it can be concluded that:

(1) *P. grimaldii* is the ninth named species of the genus *Protanthomyza*, making this genus the most species-rich fossil genus of Anthomyzidae.

(2) The description of a new *Protanthomyza* species extends the morphological diversity evident in the male and female terminalia of this genus. This adds to the previously documented wide morphological diversity among species seen in the head structures, chaetotaxies, and formation of the sclerites of the abdomen besides the male and female terminalia.

(3) Two anal veins ($A_1$, $A_2$) can continue to be treated as a subfamily/generic character for Protanthomyzinae and *Protanthomyza*, respectively.

(4) The chaetotaxy of the mesopleuron (usually with 3 posterior *mspl* macrosetae + numerous setulae more anteriorly) and sternopleuron (with 1 posterior *stpl* macroseta + setulae) seems to be rather uniform within the genus, although in a few species some setulae on the mesopleuron can be enlarged and/or one (usually the dorsal) *mspl* macroseta is reduced (cf. Roháček 1913a).

(5) The anteroventral process of the epandrium is a synapomorphic character of *Protanthomyza*. It is expected that this process will be found in all other species where the male is unknown, viz. in *P. loewi*, *P. meunieri* and *P. presli*.

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I am very grateful to Mrs Christel Hoffeins (Hamburg, Germany) and Dr Michael von Tschirnhaus (Bielefeld, Germany) for making interesting amber specimens available for examination. Dr Andrey Krylov (Kaliningrad, Russia) is thanked for his continuing support with Baltic amber Diptera, especially acalyptrates, and Mr Peter Chandler (Melksham, England, UK) and both reviewers for their comments, improvements and language corrections of the manuscript. This research on fossil Anthomyzidae was supported by the Ministry of Culture of the Czech Republic, with institutional financing of the long-term conceptual development of the research institution (the Silesian Museum, MK000100595).

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RESEARCH ARTICLE

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# A new species of *Kerkia* Radoman, 1978 (Caenogastropoda, Hydrobiidae) from Bosnia and Herzegovina

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## Abstract

A new species of *Kerkia*, *K. briani* Rysiewska & Osikowski, **sp. nov.** is described from the spring Polički Studenac Vrelo (Crkvina), adjacent to the Trebišnjica River (Bosnia and Herzegovina) collected with Bou-Rouch technique, pumped from an interstitial habitat 50 cm below the bottom of the spring. The shell, female reproductive organs, and the penis are described and illustrated. Mitochondrial cytochrome oxidase subunit I (COI) and nuclear histone H3 partial sequences confirm the distinctness of the new species, and molecularly based phylogenetic relationships of *Kerkia* are briefly presented.

## Keywords

Balkans, cytochrome oxidase, Gastropoda, histone, interstitial, molecular taxonomy, morphology, stygobiont

## Introduction

Mud snails Hydrobiidae are very small or minute snails, whose shells are often approximately 1 mm high. They inhabit surface and subterranean freshwater habitats, although some can also be found in brackish and even marine environments. The family comprises more than 400 extant genera (Kabat and Hershler 1993), many of which are stygobionts. The Balkans, especially their western region, harbours the world's most diverse stygobiont malacofauna (e.g., Culver and Sket 2000; Culver 2012). The minute dimensions of those snails, coupled with low population densities (e.g., Culver and Pipan 2009, 2014), result in very poor knowledge of their biology, speciation, and taxonomy. A few specimens are sometimes flooded out of the substrate to the surface. Otherwise, extensive pumping of the interstitial habitats, applying the Bou and Rouch technique sometimes result in more numerous living specimens.

Radoman (1978) established the genus *Kerkia* Radoman, 1978, with the type species *Hauffenia kusceri* Bole, 1961, known only from the cave Krška jama in Slovenia. He described morphology and anatomy of those minute snails, clinging to the rocks in the underground section of the sinking river Krka (Radoman 1973, 1978, 1983). Later, another species of the genus, *K. brezicensis* Bodon & Cianfanelli, 1996, was described from a karstic spring at the entry to Dvorce village, southeast of Brežice in Slovenia. *Hauffenia jadertina* Kuščer, 1933 from the source of the river Jadro near Split in Croatia, as well as *H. jadertina sinjana* Kuščer, 1933 from a spring Zužino Vrelo in the Cetina valley also in Croatia, based on their anatomy, were synonymised and transferred to the genus *Kerkia* by Beran et al. (2014), who also described a new species *Kerkia kareli* Beran, Bodon & Cianfanelli, 2014, from an old well near Povljana on island Pag in Croatia. They provided descriptions and illustrations of the shells, protoconchs, radulae, and soft part morphology and anatomy as well for all the three Croatian taxa. Rysiewska et al. (2017) demonstrated molecular distinctness of those species of *Kerkia*.

In September of 2019, in the spring Polički Studenac Vrelo (Crkvina), adjacent to the Trebišnjica River, we found *Emmericia expansilabris* Bourguignat, 1880, *Sadleriana* sp., *Anagastina vidrovani* (Radoman, 1973), and *Ancylus recurvus* Martens, 1873. Pumping of the interstitial fauna from sediments below the spring resulted in the collection of a few most probably stygophilic *Radomaniola*, but also the typically stygobiont *Montenegrospeum* Pešić & Glöer, 2013 and *Kerkia*. The representatives of the latter genus did not belong to any species known so far, and in the present paper we describe this new species and discuss its relationships.

## Materials and methods

The snails were collected at the spring Polički Studenac Vrelo (Crkvina), adjacent to the Trebišnjica River (42°42'46.4"N, 18°21'54.5"E), near Trebinje, Bosnia and Herzegovina (Fig. 1). The spring, situated at the right bank of the river (Fig. 2A, B) was in the form of a small shallow pool surrounded by a wall made of stones, with a gravel bottom (Fig. 2C). The Bou–Rouch method (Bou and Rouch 1967) was used to sample interstitial fauna below the spring bottom, at the depth of ca. 50 cm. The tube was inserted in the substrate five times, and 20 litres were pumped each time. Samples were sieved through 500 μm sieve and fixed in 80% analytically pure ethanol, replaced two

**Figure 1.** Localities of *Kerkia* used for phylogeny.

times, and later sorted. Next, the snails were put in fresh 80% analytically pure ethanol and kept in -20 °C temperature in a refrigerator.

The shells were photographed with a Canon EOS 50D digital camera, under a Nikon SMZ18 microscope. The dissections were done under a Nikon SMZ18 microscope with dark field, equipped with Nikon DS-5 digital camera, whose captured images were used to draw anatomical structures with a graphic tablet. Measurements of the shell (Fig. 3) were taken using ImageJ image analysis software (Rueden et al. 2017).

Snails for molecular analysis were fixed in 80% ethanol, changed twice, and later stored in 80% ethanol. DNA was extracted from whole specimens; tissues were hydrated in TE buffer (3 × 10 min), total genomic DNA was extracted with the SHERLOCK extraction kit (A&A Biotechnology), and the final product was dissolved in 20 μl of tris-EDTA (TE) buffer. The extracted DNA was stored at -80 °C at the Department of Malacology, Institute of Zoology and Biomedical Research, Jagiellonian University in Kraków (Poland).

DNA coding for mitochondrial cytochrome oxidase subunit I (COI) and nuclear histone 3 (H3) were sequenced. Details of PCR conditions, primers used, and sequenc-

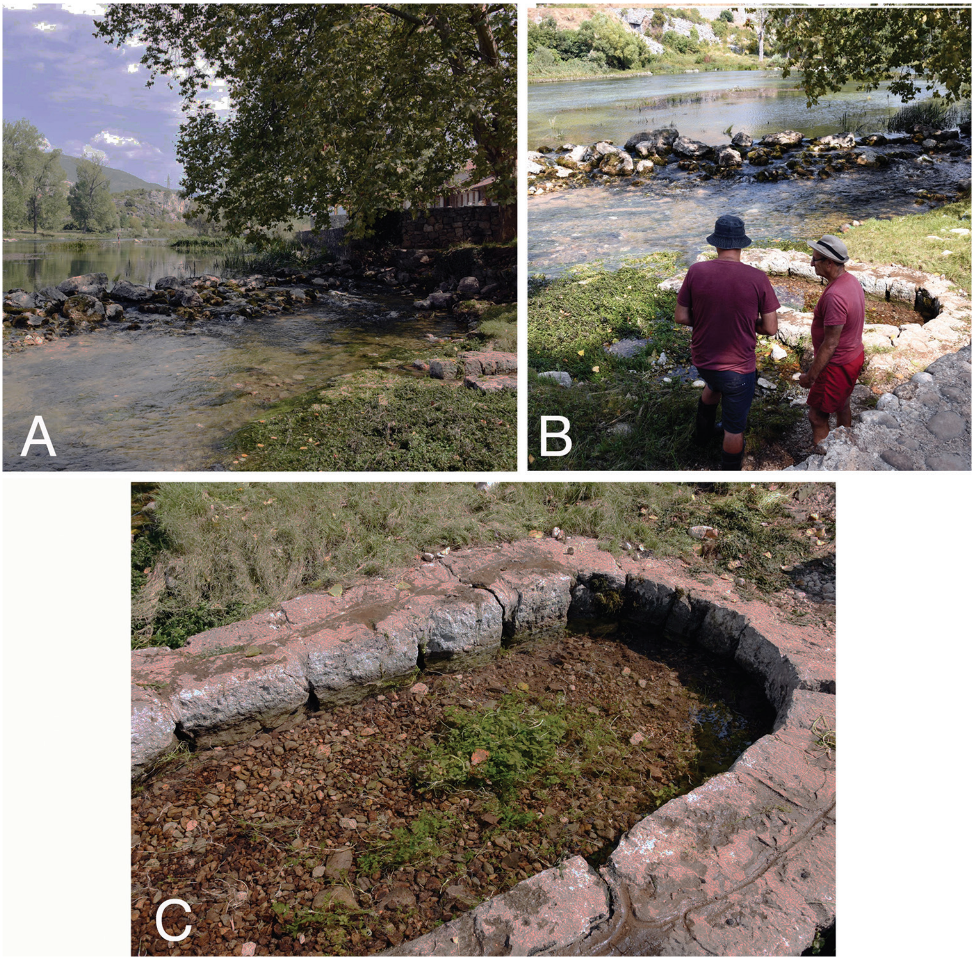

**Figure 2.** Type locality of *Kerkia briani* sp. nov.: **A, B** River Trebišnjica with the spring Polički Studenac Vrelo at its right bank **C** the spring from where interstitial snails were pumped.

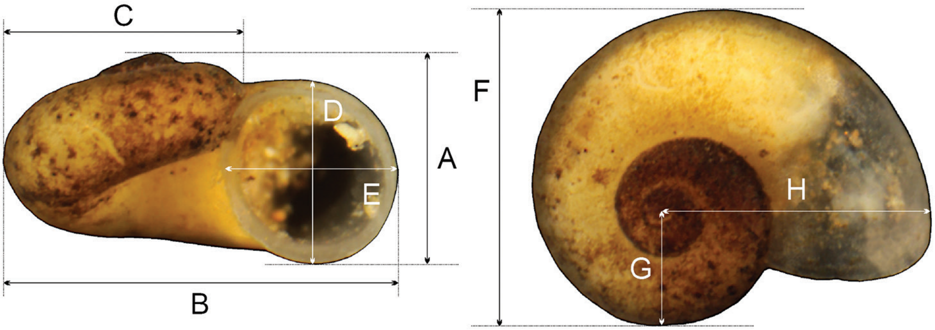

**Figure 3.** Measurements of the shell.

ing are given in Szarowska et al. (2016b). Sequences were initially aligned in the MUSCLE (Edgar 2004) programme in MEGA 6 (Tamura et al. 2013) and then checked in BIOEDIT 7.1.3.0 (Hall 1999). Uncorrected p-distances were calculated in MEGA 6. The estimation of the proportion of invariant sites and the saturation test (Xia 2000; Xia et al. 2003) were performed using DAMBE (Xia 2013). In the phylogenetic analysis additional sequences from GenBank were used as reference (Table 1). The data were analysed using approaches based on Bayesian Inference (BI) and Maximum Likelihood (ML). We applied the GTR model whose parameters were estimated by RaxML (Stamatakis 2014). The Bayesian analyses were run using MrBayes v. 3.2.3 (Ronquist et al. 2012) with defaults of most priors. Two simultaneous analyses were performed, each with 10,000,000 generations, with one cold chain and three heated chains, starting from random trees and sampling the trees every 1,000 generations. The first 25% of the trees were discarded as burn-in. The analyses were summarised as a 50% majority-rule tree. The Maximum Likelihood analysis was conducted in RAxML v. 8.2.12 (Stamatakis 2014) using the 'RAxML-HPC v.8 on XSEDE (8.2.12)' tool via the CIPRES Science Gateway (Miller et al. 2010). Two species delimitation methods were performed: Poisson Tree Processes (PTP) (Zhang et al. 2013) and Automatic Barcode Gap Discovery (ABGD). The PTP approach was run using the web server https://species.h-its.org/ptp/, with 100 000 MCMC generations, 100 thinning and 0.1 burn-in. We used RAxML output phylogenetic tree. The ABGD approach using the web server (http://www.abi.snv.jussieu.fr/public/abgd/abgdweb.html) and the default parameters.

## Results

### Systematic part

**Family Hydrobiidae Stimpson, 1865**
**Subfamily Sadlerianinae Szarowska, 2006**
**Genus *Kerkia* Radoman, 1978**

***Kerkia briani* Rysiewska & Osikowski, sp. nov.**
http://zoobank.org/1F772BD0-3172-42E7-B559-EEA20773BCF1
Figures 4, 5, 6A, B, 7

**Holotype.** Ethanol-fixed specimen (Fig. 4), spring Polički Studenac Vrelo (Crkvina), adjacent to the Trebišnjica River (42°42'46.4"N, 18°21'54.5"E), close to Trebinje (Bosnia and Herzegovina interstitially in the gravel 50 cm below the bottom of the spring. It is deposited in the Museum of Natural History of the University of Wroclaw, Poland, signature: MNHW-1350.

**Paratypes.** Twelve paratypes, ethanol-fixed, in the collection of the Department of Malacology of Jagiellonian University.

**Table 1.** Taxa used for phylogenetic analyses with their GenBank accession numbers and references.

| Species | COI/H3 GB numbers | References |
|---|---|---|
| *Agrafia wiktori* Szarowska & Falniowski, 2011 | JF906762/MG543158 | Szarowska and Falniowski 2011/Grego et al. 2017) |
| *Alzoniella finalina* Giusti & Bodon, 1984 | AF367650 | Wilke et al. 2001 |
| *Amnicola limosus* (Say, 1817) | AF213348 | Wilke et al. 2000b |
| *Anagastina zetavalis* (Radoman, 1973) | EF070616 | Szarowska 2006 |
| *Avenionia brevis berenguieri* (Draparnaud, 1805) | AF367638 | Wilke et al. 2001 |
| *Belgrandia thermalis* (Linnaeus, 1767) | AF367648 | Wilke et al. 2001 |
| *Belgrandiella kuesteri* (Boeters, 1970) | MG551325/MG551366 | Osikowski et al. 2018 |
| *Bithynia tentaculata* (Linnaeus, 1758) | AF367643 | Wilke et al. 2001 |
| *Bythinella cretensis* Schütt, 1980 | KT353689 | Szarowska et al. 2016a |
| *Bythinella hansboetersi* Glöer & Pešić, 2006 | KT381101 | Osikowski et al. 2015 |
| *Bythiospeum acicula* (Hartmann, 1821) | KU341350/ MK609536 | Richling et al. 2016/Falniowski et al. 2019 |
| *Bythiospeum alzense* Boeters, 2001 | KU341355 | Richling et al. 2016 |
| *Ecrobia maritima* (Milaschewitsch, 1916) | KX355835/MG551322 | Osikowski et al. 2016/Grego et al. 2017 |
| *Daphniola louisi* Falniowski & Szarowska, 2000 | KM887915 | Szarowska et al. 2014c |
| *Dalmatinella fluviatilis* Radoman, 1973 | KC344541 | Falniowski and Szarowska 2013 |
| *Emmericia expansilabris* Bourguignat, 1880 | KC810060 | Szarowska and Falniowski 2013a |
| *Erhaia jianouensis* (Y.-Y. Liu & W.-Z. Zhang, 1979) | AF367652 | Wilke et al. 2001 |
| *Fissuria boui* Boeters, 1981 | AF367654 | Wilke et al. 2001 |
| *Graecoarganiella parnassiana* Falniowski & Szarowska, 2011 | JN202352 | Falniowski and Szarowska 2011 |
| *Graziana alpestris* (Frauenfeld, 1863) | AF367641 | Wilke et al. 2001 |
| *Grossuana angeltsekovi* Glöer & Georgiev, 2009 | KU201090 | Falniowski et al. 2016 |
| *Hauffenia michleri* (Kuščer, 1932) | KT236156/KY087878 | Falniowski and Szarowska 2015/ Rysiewska et al. 2017 |
| *Heleobia maltzani* (Westerlund, 1886) | KM213723/ MK609534 | Szarowska et al. 2014b/ Falniowski et al. 2019 |
| *Horatia klecakiana* Bourguignat 1887 | KJ159128 | Szarowska and Falniowski 2014 |
| *Hydrobia acuta* (Draparnaud, 1805) | AF278808 | Wilke et al. 2000a |
| *Iglica* cf. *gracilis* (Clessin, 1882) | MH720985/ MH721003 | Hofman et al. 2018 |
| *Iglica hellenica* Falniowski & Sarbu, 2015 | KT825581/MH721007 | Falniowski and Sarbu 2015/Hofman et al. 2018 |
| *Islamia zermanica* (Radoman, 1973) | KU662362/MG551320 | Beran et al. 2016/Grego et al. 2017 |
| *Kerkia jadertina* (Kuščer, 1933) | KY087868/KY087885 | Rysiewska et al. 2017 |
| *Kerkia jadertina sinjana* (Kuščer, 1933) | KY087873-74/ KY087890-91 | Rysiewska et al. 2017 |
| *Kerkia kareli* Beran, Bodon & Cianfanelli, 2014 | KY087875-77/ KY087892-94 | Rysiewska et al. 2017 |
| *Kerkia kusceri* (Bole, 1961) | KY087867/KY087884 | Rysiewska et al. 2017 |

| Species | COI/H3 GB numbers | References |
|---|---|---|
| *Kerkia* sp. Ljubač | KY087872/KY087889 | Rysiewska et al. 2017 |
| *Littorina littorea* (Linnaeus, 1758) | KF644330/KP113574 | Layton et al. 2014/Neretina 2014, unpublished |
| *Littorina plena* Gould, 1849 | KF643257 | Layton et al. 2014 |
| *Lithoglyphus prasinus* (Küster, 1852) | JX073651 | Falniowski and Szarowska 2012 |
| *Marstoniopsis insubrica* (Küster, 1853) | AF322408 | Falniowski and Wilke 2001 |
| *Moitessieria* cf. *puteana* Coutagne, 1883 | AF367635/MH721012 | Wilke et al. 2001/ Hofman et al. 2018 |
| *Montenegrospeum bogici* (Pešić & Glöer, 2012) | KM875510/MG880218 | Falniowski et al. 2014/Grego et al. 2018 |
| *Paladilhiopsis grobbeni* Kuščer, 1928 | MH720991/MH721014 | Hofman et al. 2018 |
| *Peringia ulvae* (Pennant, 1777) | AF118302 | Wilke and Davis 2000 |
| *Pomatiopsis lapidaria* (Say, 1817) | AF367636 | Wilke et al. 2001 |
| *Pontobelgrandiella* sp. Radoman, 1978 | KU497024/MG551321 | Rysiewska et al. 2016/Grego et al. 2017 |
| *Pseudamnicola chia* (E. von Martens, 1889) | KT710656 | Szarowska et al. 2016b |
| *Pseudorientalia* Radoman, 1973 – Lesvos | KJ920490 | Szarowska et al. 2014a |
| *Radomaniola curta* (Küster, 1853) | KC011814 | Falniowski et al. 2012 |
| *Sadleriana fluminensis* (Küster, 1853) | KF193067 | Szarowska and Falniowski 2013b |
| *Sadleriana robici* (Clessin, 1890) | KF193071 | Szarowska and Falniowski 2013b |
| *Salenthydrobia ferrerii* Wilke, 2003 | AF449213 | Wilke 2003 |
| *Sarajana apfelbecki* (Brancsik, 1888) | MN031432 | Hofman et al. 2019 |
| *Tanousia zrmanjae* (Brusina, 1866) | KU041812 | Beran et al. 2015 |
| *Tricula sp.* Benson, 1843 | AF253071 | Davis et al. 1998 |

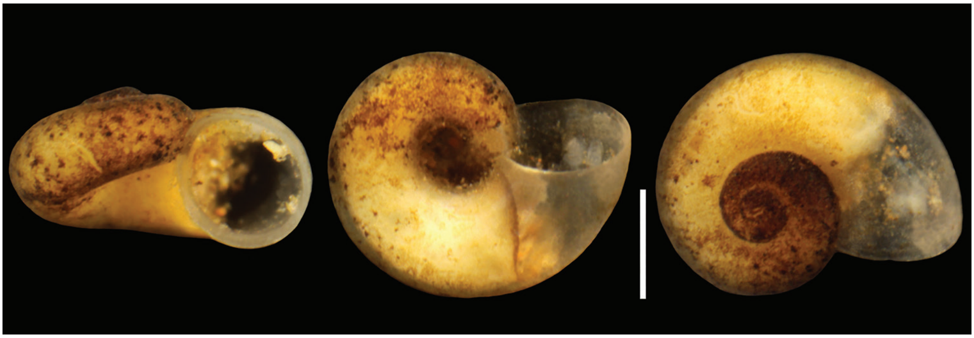

**Figure 4.** Holotype of *Kerkia briani.* Scale bar: 0.5 mm.

**Diagnosis.** Shell minute, nearly planispiral, distinguished from *K. kusceri* by its lower aperture of the shell and smaller non-glandular outgrowth on the left side of the penis, and from *K. jadertina* and *K. kareli* by its higher aperture of the shell and bigger the non-glandular outgrowth on the left side of the penis.

**Description.** ***Shell*** (Fig. 4) up to 0.77 mm high and 1.39 mm broad, nearly planispiral, whitish, translucent, thin-walled, consisted of approximately five whorls, growing rapidly and separated by moderately deep suture. Spire low and flat, body

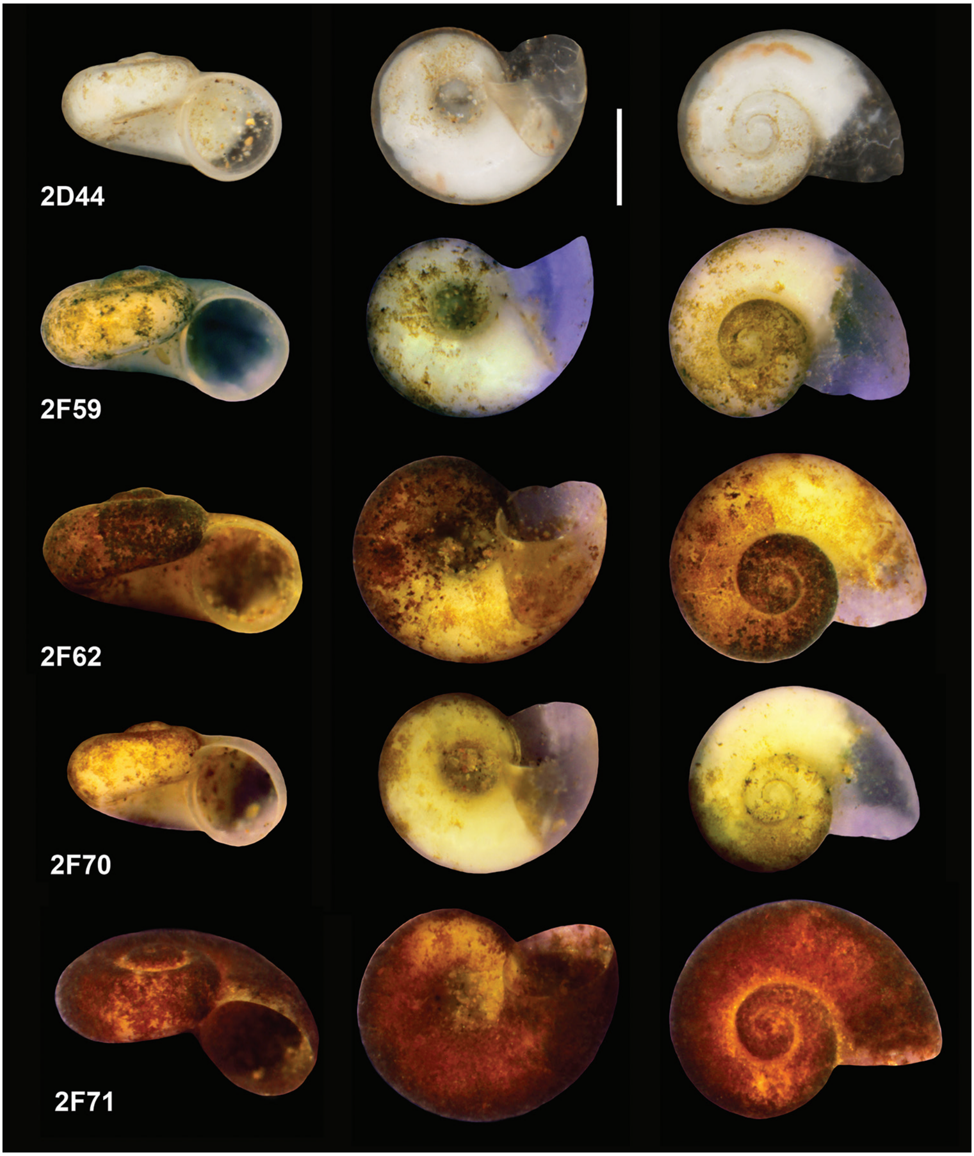

**Figure 5.** Shell variability of *Kerkia briani*, labels the same as in the molecular trees. Scale bar: 0.5 mm.

whorl large. Aperture prosocline, nearly circular in shape, peristome complete and thin, somewhat swollen, in contact with the wall of the body whorl; umbilicus wide, with the earlier whorls visible inside. Shell surface smooth, growth lines hardly visible.

***Measurements*** of holotype, sequenced, and illustrated shells: see Table 2. Shell variability slight (Fig. 5).

***Soft parts morphology and anatomy.*** Body white, without pigment, with no eyes. The ctenidium with twelve short lamellae, osphradium short and broad. Rectum forming characteristic broad loop (Fig. 6A). The female reproductive organs (Fig. 6A, B) with

**Table 2.** Shell measurements (in mm) of *Kerkia briani*. For explanation of the symbols A–H, see Fig. 3.

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| holotype | 0.77 | 1.39 | 0.87 | 0.62 | 0.60 | 1.09 | 0.41 | 0.97 |
| 2D44 | 0.72 | 1.12 | 0.73 | 0.54 | 0.55 | 0.93 | 0.37 | 0.75 |
| 2F59 | 0.73 | 1.26 | 0.82 | 0.54 | 0.55 | 0.95 | 0.41 | 0.80 |
| 2F62 | 0.72 | 1.35 | 0.86 | 0.57 | 0.57 | 1.03 | 0.36 | 0.73 |
| 2F70 | 0.67 | 1.12 | 0.72 | 0.52 | 0.48 | 0.97 | 0.40 | 0.72 |
| 2F71 | 0.75 | 1.37 | 0.85 | 0.46 | 0.60 | 1.02 | 0.41 | 0.84 |
| *M* | 0.73 | 1.27 | 0.81 | 0.54 | 0.56 | 1.00 | 0.39 | 0.80 |
| SD | 0.034 | 0.123 | 0.067 | 0.053 | 0.044 | 0.059 | 0.023 | 0.094 |
| Min | 0.67 | 1.12 | 0.72 | 0.46 | 0.48 | 0.93 | 0.36 | 0.72 |
| Max | 0.77 | 1.39 | 0.87 | 0.62 | 0.60 | 1.09 | 0.41 | 0.97 |

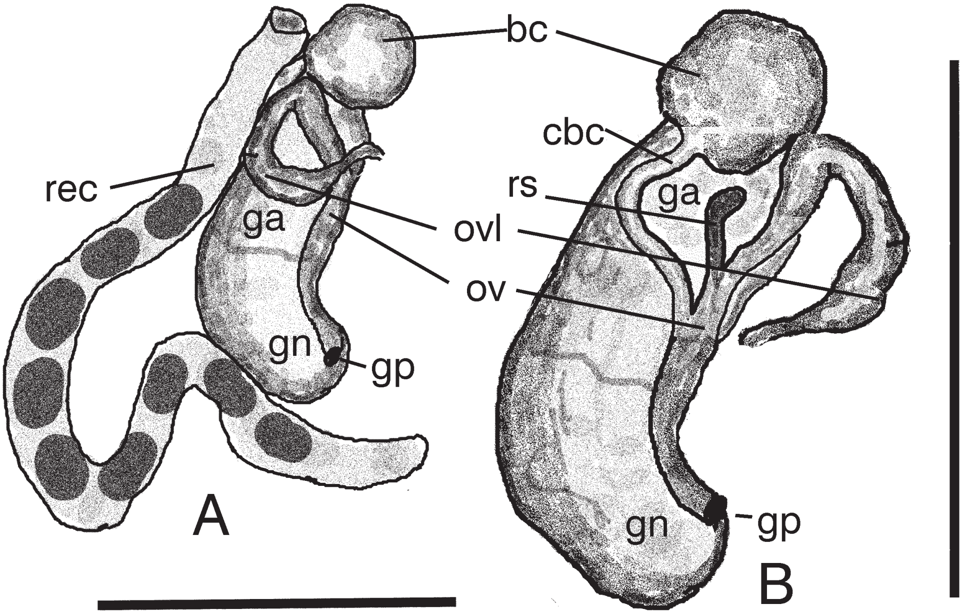

**Figure 6.** Renal and pallial section of the female reproductive organs of *Kerkia briani*: **A** the loop of oviduct in its normal position and the loop of the rectum **B** the loop of oviduct moved to show the receptaculum seminis and duct of bursa. Abbreviations: bc – bursa copulatrix, cbc – duct of bursa, ga – albuminoid gland, gn – nidamental gland, gp – gonoporus, ov – oviduct, ovl – loop of renal oviduct, rec – rectum, rs – receptaculum seminis. Scale bars: 1 mm.

a long, moderately broad loop of renal oviduct and relatively big spherical bursa copulatrix (Fig. 6A) with a long bent duct (Fig. 6B), and one distal receptaculum seminis, long and worm-shaped. The penis (Fig. 7) elongated triangular, with a rather sharp tip and small non-glandular outgrowth on its left side, the vas deferens inside running in zigzags.

**Derivatio nominis.** The specific epithet *briani* refers to our friend Brian Lewarne, Honorary Science Officer of The Devon Karst Research Society, and the Director for "Proteus Project in the Trebišnjica River Basin", deeply devoted to the protection of *Proteus* as well as the study and protection of the subterranean habitats in Bosnia and Herzegovina.

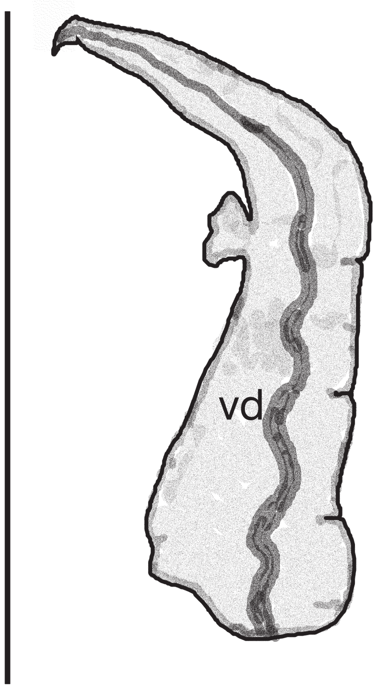

**Figure 7.** Penis of *Kerkia briani*. Abbreviation: vd – vas deferens.Scale bar: 1 mm.

**Distribution and habitat.** Known from the type locality only.

## Molecular distinctness and relationships of *Kerkia briani*

We obtained six new sequences of COI (479 bp, GenBank Accession Numbers MT780191–MT780196), and six new sequences of H3 (309 bp, GenBank Accession Numbers MT786730–MT786735). The tests by Xia et al. (2003) for COI and H3 revealed no saturation. Phylograms were constructed for COI, H3 and for combined COI-H3 dataset. In all analyses, the topologies of the resulting phylograms were identical in both the ML and BI. The ABGD and PTP approaches gave the same results (Fig. 8).

The sequences of the *Kerkia briani* formed distinct clade on COI, H3 as well as combined phylograms (Fig. 8). At the same time all *Kerkia* sequences formed distinct linage with five different species. The p-distance of *Kerkia briani* with other *Kerkia* species varied from 0.123 to 0.146 for COI and from 0.007 to 0.023 for H3 (Table 2). The sister clade of *Kerkia* (bootstrap support 98%) were *Islamia* Radoman, 1973, *Pontobelgrandiella* Radoman, 1978, *Belgrandiella* Wagner, 1927, *Montenegrospeum* Pešić & Glöer, 2013, *Hauffenia* Pollonera, 1898, and *Agrafia* Szarowska & Falniowski, 2011 (Fig. 8, the tree for concatenated COI and H3 sequences).

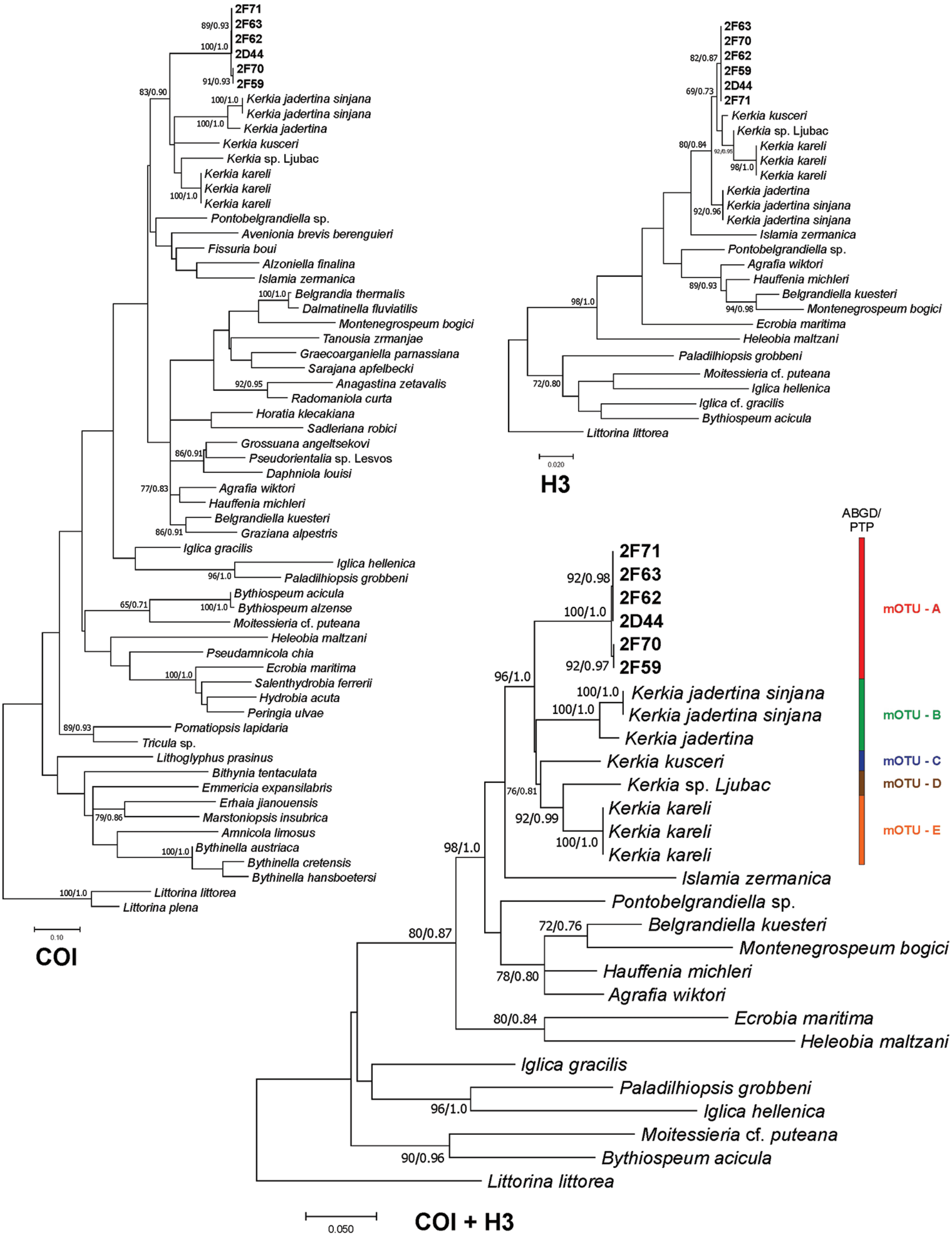

**Figure 8.** Maximum Likelihood tree inferred from mitochondrial COI. Bootstrap supports above 60% with corresponding Bayesian probabilities are given.

**Table 3.** p-distances between *Kerkia* mOTUs for the COI (below diagonal) and H3 genes.

| | mOTU – A | mOTU – B | mOTU – C | mOTU – D | mOTU – E |
|---|---|---|---|---|---|
| mOTU – A | – | 0.010 | 0.007 | 0.010 | 0.023 |
| mOTU – B | 0.135 | – | 0.017 | 0.020 | 0.033 |
| mOTU – C | 0.126 | 0.124 | – | 0.010 | 0.023 |
| mOTU – D | 0.146 | 0.138 | 0.124 | – | 0.013 |
| mOTU – E | 0.123 | 0.110 | 0.095 | 0.093 | – |

## Discussion

Following the terminology of Hershler and Ponder (1998), the habitus of the shell of *Kerkia* is depressed valvatiform (trochiform) or just planispiral. However, the latter term should not be used, since there is no planispiral shell in any recent gastropod (e.g., Falniowski 1993). The ctenidium, osphradium, and loop of oviduct are as in the other species of *Kerkia* (Bodon et al. 2001; Beran et al. 2014). The female reproductive organs are also typical of *Kerkia* (Radoman 1978, 1983; Bodon et al. 2001; Beran et al. 2014). The single receptaculum seminis is situated distally, in the position of $rs_1$ after Radoman (1973, 1983). The penis is similar to that described and drawn by Radoman (1978, 1983), Bodon et al. (2001), and Beran et al. (2014), but the outgrowth of its left side in *K. briani* is smaller than in *K. kusceri*, but larger than that in *K. jadertina* and *K. kareli* (in the latter the outgrowth is nearly vestigial).

Falniowski (1987) demonstrated high variability of the shell, but also of the morphology and anatomy of the soft parts in the Truncatelloidea. In the latter, miniaturisation is one more a source of slight morphological diversity, decreasing the number of possible taxonomically useful characters (Falniowski 2018); in this regard, Szarowska and Falniowski (2008) stressed the narrow limits of morphology-based taxonomy within the Truncatelloidea. On the other hand, Szarowska (2006) demonstrated that such simple structures like the outgrowths on the penis and bursae/receptacula in the female reproductive organs are surprisingly evolutionary stable in position, although not in size and shape, whose variability – physiologically, ontogenetically, and artifactually (as a result of fixation of the snails) based – is striking. Moreover, problems can increase with taxa living in habitats of limited accessibility (such as caves and/or interstitial habitats) for which molecular studies often reveal numerous species but only a few or single living specimens of each species could be found. Thus, the anatomy is basic in distinction of the families and even genera, but the stable and reliable differences between congeneric species are hardly observable. However, the molecular distinctness of *Kerkia briani* is clear.

Finally, it has to be pointed out that *K. briani* inhabits the southernmost locality of *Kerkia*, expanding the range of the genus ca. 190 km ESE.

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# The Pleidae (Hemiptera, Heteroptera) of Thailand, with the descriptions of two new species and a discussion of species from Southeast Asia

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## Abstract

The family Pleidae is represented in Thailand by four species in the genus *Paraplea*. Two of these species, *P. frontalis* and *P. liturata*, are widespread and relatively common in Southeast Asia. Two other species, *P. lateromaculata* Cook, **sp. nov.** and *P. melanodera* Cook, **sp. nov.,** are described and only known from Thailand. Full descriptions are provided for all four species. The distributions of these species are discussed, with an emphasis on Thailand. *Paraplea frontalis*, *P. liturata*, and *P. lateromaculata* Cook, **sp. nov.** are relatively widespread within Thailand and have overlapping distributions whereas *P. melanodera* Cook, **sp. nov.** appears restricted to small brackish ponds near western coastal areas of peninsular Thailand.

## Keywords

distribution, *Paraplea*, taxonomy

## Introduction

The family Pleidae in Southeast Asia is represented only by species in the genus *Paraplea*. *Paraplea areolata* Paiva, 1918 was described from Myanmar (Burma,) and *P. davaoensis* Miyamota, 1981 and *Paraplea sobrina* (Stål, 1860) are known from the Philippines. Two species, *P. frontalis* (Fieber, 1844) and *P. liturata* (Fieber, 1844) are likely widespread in Southeast Asia, and also occur outside this region. In Southeast Asia, *P. frontalis* and *P. liturata* have been recorded from Indonesia, Myanmar (Burma), West Malaysia, Singapore, Taiwan, and Thailand. *Paraplea frontalis* is also known from China, India, Sri Lanka, and Taiwan, and *P. liturata* from Australia, Japan, and New Caledonia. *Paraplea vittifrons* (Horváth, 1919) is known from the type specimen from the Aru Islands of Indonesia, which is in maritime Southeast Asia.

Members of the family Pleidae are rarely collected. These are very small aquatic bugs that are often overlooked in nature and collections, and they occur in habitats that are rarely sampled. The most common habitat where pleids occur is in slow moving or stagnant waters, with rich vegetation (Andersen and Weir 2004), although this may not be true for some regions, such as Thailand. Besides being overlooked and occurring in a habitat that is rarely collected, there has been little recent research emphasis on this family. Chen et al. (2006) recorded aquatic Hemiptera in Thailand, and identified *P. frontalis* and *P. liturata* in northeastern Thailand. Herein we report results of extensive collections throughout Thailand and describe two new species of *Paraplea*, as well as report the distribution of the species of *Paraplea* of Thailand.

The taxonomy of species in Pleidae is based primarily on a few key characters of uncertain taxonomic importance. Traditionally, genera could be identified solely on the index of their tarsal segments (Cook 2011). All known pleid species have three tarsal segments on their metathoracic leg. Species with two tarsal segments on their prothoracic and metathoracic legs are included in *Paraplea*, whereas those with two prothoracic tarsal segments and three mesothoracic tarsal segments are placed in *Neoplea*. Species with three tarsal segments on all legs are in *Plea* or *Heteroplea*, the latter having a callus posteriorly on the head. However, this classification may be artificial as there has been no phylogeny of the family. Other characters that appear to be valid for taxonomic purposes include the tooth pattern of the ovipositor (Sublett and Cook 2015), profile of the sternum, widths of the pronotum and scutellum, state of body sculpturing, male and female opercula, male parameres, and form of the clavus (Drake and Chapman 1953). Cook (2017) added indices of body shape, an ocular index, pronotal index, scutellar index and scutellar length index to the evaluation of species in Pleidae. The descriptions herein use all of these characters to delineate species.

The genus *Paraplea* is the most widespread genus in Pleidae with 19 valid species. Two species are from the New World, known primarily from the Caribbean and the southeastern United States; four species are from Africa; two from India; two from Australia; three from Japan or Taiwan; and six from Southeast Asia. With the addition of the two species from Thailand described in this study, this region is clearly the most diverse for *Paraplea*. However, there are likely many more species that remain unknown and await discovery and description.

## Materials and methods

Most specimens for this study were field collected by co-authors Sites and Vitheepradit and their colleagues. Photographs of these collection sites, identified as L-numbers, are available in a Locality Image Database via a link from the internet site of the Enns Entomology Museum, University of Missouri. Other specimens were from previously collected materials deposited in the Snow Entomology Collection, University of Kansas (**SEMC**) and the United States National Museum (**USNM**). The recently collected specimens for this study are housed primarily in the Enns Entomology Museum, University of Missouri (**UMC**) and Sam Houston State University Natural History Collections (**SHSU**). Maps using data reported in the text were produced with SimpleMapper (Shorthouse 2010). Observations and measurements were made using an Olympus SZX16 microscope with an ocular micrometer and a Keyence VHX-6000 digital microscope. In total, 1279 adult specimens of Thai Pleidae were examined for this study, with 948 used for measurements due to their overall condition and orientation. Indices used for the description are:

| | |
|---|---|
| **BSI** | Body Shape Index (body width/body length) x 100 |
| **OI** | Ocular Index (narrowest width between eyes/width of head across the eyes) x 100; width between eyes is taken anteriorly in dorsal view, width of head is at widest point including eyes |
| **PI** | Pronotal Index (length of pronotum/width of pronotum) x 100 |
| **SI** | Scutellar Index (scutellum width/scutellum length) x 100 |

Description of the ovipositor (gonapophyses) follows the terminology of Sublett and Cook (2015).

## Results and discussion

Four species were found in Thailand, two previously described and two new. Although the two previously described species, *P. frontalis* and *P. liturata*, are relatively well known, they lack a complete modern description. Both were partially redescribed by Lundblad (1933) but not all meaningful taxonomic characters were addressed. All species of *Paraplea* found in Thailand are described below. Two species are new descriptions and two species are supplementary redescriptions.

### ***Paraplea frontalis*** **(Fieber, 1844)**

Figures 1–5, 24A

= *Ploa frontalis* Fieber, 1844: Entomol. Monogr. p. 18.
= *Plea frontalis*: (Kirkaldy, 1898): Wien. Entomol. Zeit. 17: 141.
= *Plea pelopea* Distant, 1911: Fauna Brit. India 5: 336–337 (synonymized by Lundblad 1933: 138).

= *Plea quinquemaculata* Lundblad, 1933: Arch Hydrobiol. 12: 135–138 (synonymized by Nieser 2004: 82).

*Plea* (*Paraplea*) *frontalis*: Esaki and China 1928: Rev. Esp. Entomol. 4: 166 (subgenus description).

*Paraplea frontalis*: Drake and Maldonado-Capriles 1956 (elevation to genus) 51: 53.

**Remarks.** *Paraplea frontalis* was described as *Ploa frontalis* by Fieber (1844) for specimens collected in the East Indies. The original description was not extensive and relied heavily on coloration, which has proven to be a somewhat variable character in pleids. However, this description included a documentation of the distinctive markings of the face and vertex that is found in most specimens of this species. The figures provided with the original description are not very helpful in distinguishing *P. frontalis* from other pleid species. No types were designated by Fieber; however, the distinctive markings of the head made associations with subsequent collections possible with a relatively high degree of certainty. Kirkaldy (1898) reported on a specimen from Rangoon (now Yangon) in present day Myanmar (Burma), and in this and his later publication (Kirkaldy 1904), he followed Leach (1817) in putting all of Pleinae into the genus *Plea*, thus changing the name of this species to *Plea frontalis*. Kirkaldy (1904) also included a range extension of the species into Bengal, which is now in Bangladesh, although it is possible that he did not distinguish between West Bengal and East Bengal, leaving uncertainty to the exact region, which would now be in either India or Bangladesh, respectively. Kirkaldy also reported specimens from Pondicherry (India) and Cochin, China, which is now in Vietnam. Kirkaldy did not provide a description of the specimens he included in *P. frontalis*. Distant (1906) provided an English translation of the original description by Fieber but stated that he was unable to view any specimens of the species. Distant (1910) described a species from Calcutta and Madupur, West Bengal, India as *Plea pelopea* based primarily on coloration, including having a head with four dark spots and commented that he thought this could be an “extreme variety” of *Plea pallescens*. *Plea* (*Paraplea*) *pelopea* was considered by Lundblad (1933) to be the same as *P. frontalis*, although there was no type available for comparison. Lundblad (1933) determined that all previous treatments of *P. frontalis* did not allow for precise identifications. He commented on the most common head marking of having five dark markings and provided drawings of the head, antenna, legs, pronotum, sternal crest and parameres, but he did not provide a complete re-description. Lundblad (1933) also described a new species, *Plea* (*Paraplea*) *quinquemaculata*, which is now considered a synonym of *P. frontalis* (Nieser 2004). Benzie (1989) redescribed the species using specimens from Sri Lanka, including many of the characters used for modern descriptions in this family. Although all of these forms are now included as *P. frontalis*, it is possible that this represents a species complex. Below is a supplemental description of the species, based primarily on specimens from Thailand that fit within *P. frontalis* as it is currently defined.

**Type information.** No holotype is designated. Fieber (1844) reported that the description was made from specimens collected by Dr. Helfer in the East Indies, although the exact location in this region of Southeast Asia is not documented in the literature. A specimen of Pleidae collected by Dr. Helfer is deposited the National Museum in Prague (NMPC) that could give an indication about the area where the original *P. frontalis* was collected (Nico Nieser communication). This specimen could become a neotype if it is determined to be *P. frontalis*, however this specimen has not yet been evaluated.

**Supplementary description.** All measurements are given in millimeters from 456 adult specimens from throughout Thailand as reported in the distribution portion of this paper. Specimens used for this description are deposited at SEMC, SHSU, and UMC. Other specimens from Thailand and areas outside Thailand were surveyed but were not included in measurements due to condition or availability.

***Body size.*** Total length, 1.89–2.32 (average 2.07) in Thailand specimens. Fieber (1844) reported total length of 2, but Lundblad (1933) reported that the species could reach 2.3 to 2.5. Greatest body width in Thailand specimens, 0.92–1.28 (average 1.04); BSI, 50–58.

***Color.*** Color may vary among individuals (Fig. 1A, D). Base color of body ranges from light brown to almost cream-colored, some with light honeycomb pattern, espe-

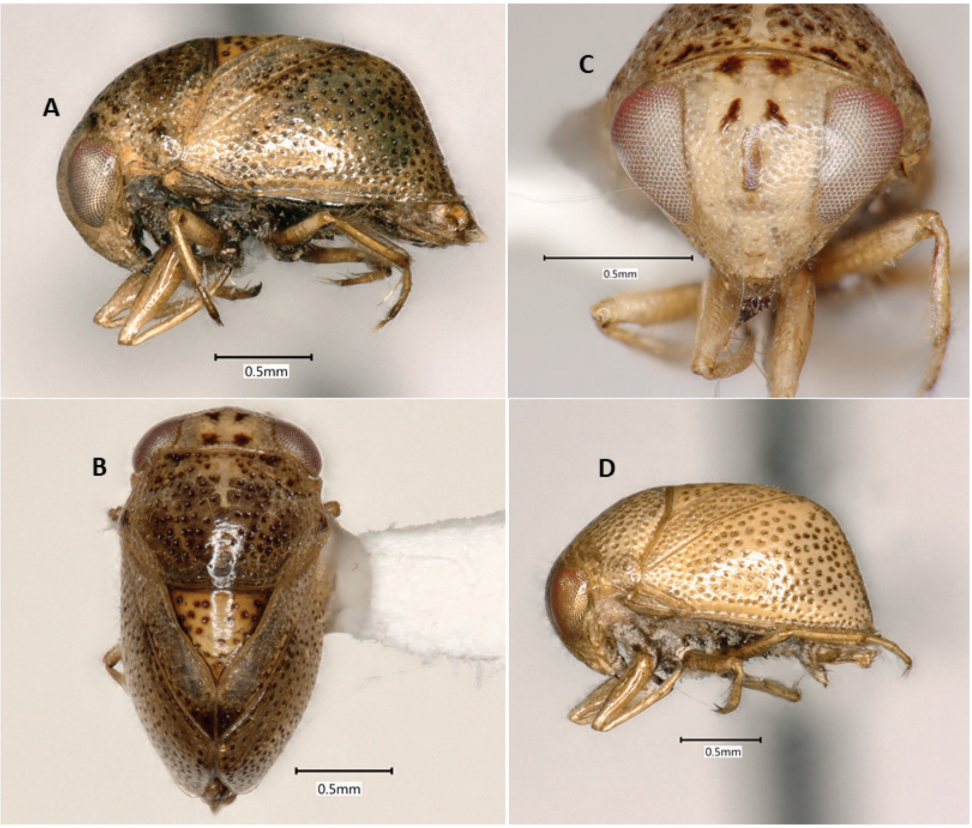


**Figure 1.** *Paraplea frontalis* specimens from Thailand. Male with most typical coloration in (**A**) lateral view, (**B**) dorsal view, and (**C**) frontal view. (**D**) Female showing an alternatively colored form in lateral view.

cially on pronotum; punctures throughout body usually with dark center; scutellum usually golden-tan; legs light brown; sternum and venter darker brown; eyes red to golden to silver in dried specimens, dark blotches seen in various places on some specimens, distinctive dark spots on face and vertex (Fig. 1C).

***Head.*** Head (Fig. 1C) generally light brown to cream colored with dark brown markings, mouthparts dark brown. Face and vertex normally with distinctive markings consisting of pair of spots near vertex, pair of spots between top margin of eyes and vertical line along midline of face, between middle of eyes; markings can differ between individuals; 64% with dark bar and four spots, 25% with light brown bar and four spots, 10% with light brown bar and two spots, 1% with either dark or light brown bar only (see Benzie 1989, Fig. 12 for diagram showing differences). Antenna three-segmented, usually hidden from view below eye. Head size similar among Thailand specimens, head width at widest point including eyes 0.92–1.04, head width at narrowest point between eyes, 0.47–0.56, OI 49–55.

***Pronotum.*** Base color ranging from cream to light brown, honeycombing apparent in some specimens; most have visible central lighter colored vertical band without punctures (Fig. 1B); slight humeral bulge present laterally; posterior margin with thick sclerotized border; punctures relatively large, 0.03–0.05, with dark centers; pronotum 1.01–1.17; length 0.62–0.79; PI 61–75 (Fig. 1B).

***Wings.*** Complete to posterior; punctures generally in irregular rows (0.02 in diameter) (Fig. 1A); underlying honeycomb structure sometimes present; claval suture distinct, complete; scutellum with distinct, dark punctures, more widely spaced than other punctures (Fig. 1B); scutellum base color often golden compared to tan base of hemelytra (Fig. 1B), although sometimes both cream colored (Fig. 1D); yellowish–brown spot at terminal angles of corium often present as reported in original description, but not always readily apparent, posterior half rarely brownish as in original description; lateral view shows distinct dorsal horizontal shape and posterior near vertical aspects with a transition of nearly 90° (Fig. 1A, C); scutellum slightly wider than long (Fig. 1B), scutellum length 0.46–0.57; scutellum width 0.54–0.66; SI 104–120. Hind wings membranous, fully developed, completely concealed by hemelytra.

***Legs.*** Typical leg measurements: prothoracic leg coxa 0.07, trochanter 0.11, femur 0.45, tibia 0.34, first tarsomere 0.02, second tarsomere 0.16,pretarsal claw 0.10; mesothoracic leg coxa 0.04, trochanter 0.18, femur 0.39, tibia 0.25, first tarsomere 0.02, second tarsomere 0.16, pretarsal claw 0.08; metathoracic leg coxa 0.04, trochanter 0.17, femur 0.47, tibia 0.53, first tarsomere 0.04, second tarsomere 0.18, third tarsomere 0.19, pretarsal claw 0.13 (see Benzie 1989, Fig. 9 for leg shapes).

***Median ventral keel.*** Thoracic portions distinct from each other; prosternal keel rounded with posterior blunt tooth; mesothoracic keel almost rectangular; metathoracic keel irregularly shaped, somewhat in lobes, including posterior projecting small tooth, closely associated with abdominal keel, appearing almost fused; thoracic segments relatively similar between specimens. Abdominal keel variable, usually partially fused between segments, usually with four distinct teeth corresponding with first four

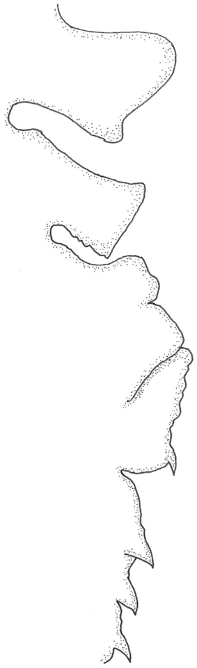


**Figure 2.** Ventral keel of *Paraplea frontalis*, anterior region to the left, ventral to the top.

abdominal segments. Figure of typical Thailand specimen in Fig. 2, but also see drawing by Lundblad (1933, fig. 44H).

***Characters of female.*** Ovipositor roughly triangular in shape, 0.25 in length, with wide side apically (gonapophysis 1) at end of rectangular shaft (gonapophysis 2) (Fig. 3); six distinct teeth along posterior border (apical row) plus two teeth on ventral border (ventral 1 and 2), three rows of small teeth away from apex, two primary, three secondary, and usually three tertiary, although there is variation in number of tertiary teeth; one long hair on ventral side where triangular apex of gonapophysis 1 and basal rectangle of gonapophysis 2 meet. Subgenital plate as in Fig. 4; wider than long; width ~ 0.67, length ~ 0.41; faintly rugose in basal one third; tufts of relatively long hairs on each side near apex; short inconspicuous hairs throughout genital plate darker v-shaped prominence in center, near apex.

***Characters of male.*** Aedeagus bulbous and somewhat asymmetrical in the typical fashion of the family; operculum (subgenital plate) generally triangular, slightly wider than long (Fig. 5), width ~ 0.46, length ~ 0.41; lightly rugose throughout; with short hairs throughout.

**Distribution.** *Paraplea frontalis* is a relatively widespread species in Thailand (Fig. 24A) as well as other parts of Southeast Asia. This study adds the first records from Laos.

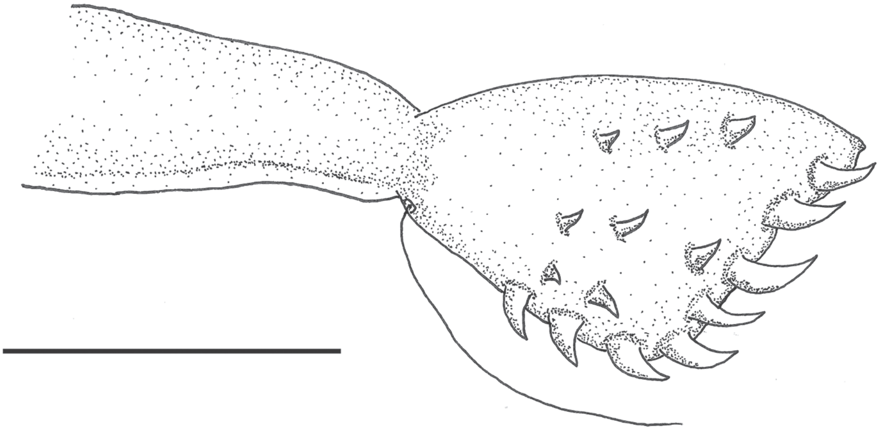
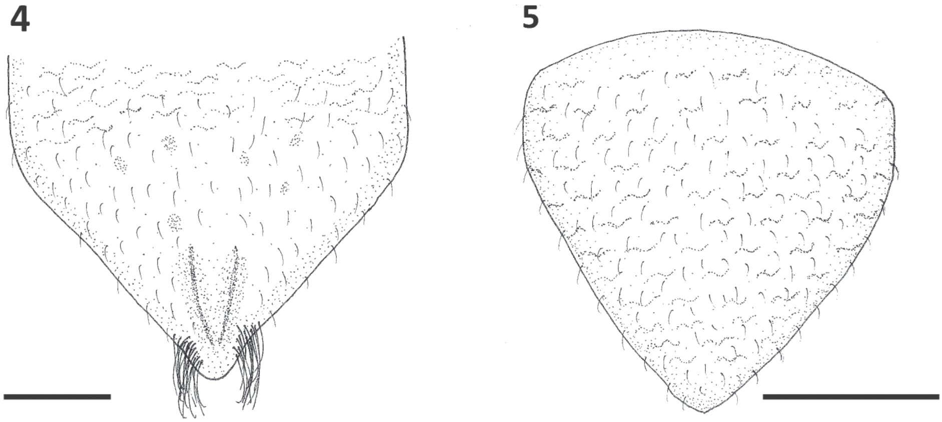


**Figure 3.** Ovipositor of *Paraplea frontalis* with gonapophysis 1 to right and gonapophysis 2 to left. Scale bar: 0.1 mm.



**Figures 4–5.** Genital plates of *Paraplea frontalis*. **4** female, **5** male. Scale bar: 0.2 mm.

**Material examined.** Hong Kong: New Territories, Yuen Long, 23 II 1971, P. & P. Spangler (5 specimens USNM). India: Pondicherry, Maindeon, 1901 (4 specimens USNM); India, Tanquebor (South India) 1951, P. S. Nathan (1 specimen USNM); Karikal, VII 1956, P. S. Nathan (5 specimens USNM). Laos: Vientaine, along Mekong River, 17°58'02.6"N, 102°36'17.6"E, 6 VIII 1997 Wolfgang G. Ulrich (1 specimens USNM). Malaysia: Penang, University of Sains Malay, 29 I 1983, H. C. Chapman (15 specimens USNM). Myanmar: Rangoon Burma (= Yangon, Myanmar) Kemmencline 10 I 1927 (2 specimens USNM); Rangoon Burma (= Yangon, Myanmar) Kemmencline 29 I 1927 (4 specimens USNM). Singapore: 1 IX 1955. Marshall Laird (8 specimens SEMC). Thailand: **Ayutthaya Province:** roadside pond ca. 5 km E of Ayutthaya, 2 VII 1997, L-126, R. W. Sites (2 specimens UMC). **Bangkok Province:** 9–10 V 1959, Manop, col., light (1 specimen USNM); Bangkok, 28 II 1971, P. & P.

Spangler (10 specimens USNM). **Chiang Mai Province:** Amphur Hot, 18°09.930'N, 98°13.496'E, 870 m, pond, 18 V 2004, L-695 (69 specimens UMC); Chiang Mai, 10 III, 1952 m D. C. & E. B. Thurman (2 specimens SEMC). **Chaiyaphum Province:** Tad Tone National Park, Namtok Tad Tone, 15°58.796'N, 102°02.079'E, 210 m, 29 IV 2004, A. Vitheepradit, L-650 (1 specimens UMC); Amphur Chum Pae, 16°34.585'N, 102°01.668'E, 217 m, 3 V 2004, A. Vitheepradit, L-660, (4 specimens UMC). **Chumphon Province:** Amphur Sa Wi, Ban Kron, 10°14.542'N, 99°05.555'E, 6 m, 30 V 2004, L-729, Vitheepradit & Prommi (3 specimens UMC); Amphur Lamae, Ban Suan Som Boon, 09°43.311'N, 99°06.208'E, 13 m, 20 VI 2006, L-964, Vitheepradit & Prommi (1 specimen UMC). **Khon Kaen Province:** Amphur Chum Pae, Tumbon Noan Udom, 16°31.267'N, 102°11.323'E, 217 m, 15 IV 2009, vegetated pond, L-1039 (25 specimens UMC, 1 specimen SHSU); Khon Kaen, Khon Kaen, Bung Kaen Nakhon, 19 II 1994, William D. Shepard (1 specimen USNM); Khon Kaen City, 21 V 1954, R. E. Elbel (1 specimens USNM). **Krabi Province:** Amphur Mueang, Noppharat Thara Beach, 08°02.625'N, 98°48.517'E, 13 m, 8 I 2006, L-902 (8 specimens UMC). **Loei Province:** Amphur Nong Hin, Tumbon Nong Hin, 17°05.658'N, 101°49.193'E, 328 m, 4 V 2004, L-662, A. Vitheepradit (2 specimens UMC); Amphur Nong Hin, Ban Huay Deur, 17°05.804'N, 101°49.823'E, 316 m, 4 V 2004, L-663, A. Vitheepradit (1 specimens UMC). **Mae Hong Son Province:** Tam Pla Resort at Tam Pla River, 19°25'34.2"N, 97°59'16.7"E, 21 X 1997, Wolfgang G. Ulrich (1 specimen USNM). **Nakhon Ratchasima Province:** Nakhon Ratchasima, 60 km S of Sakaerat Experimental Station, 30–600 m, 14°30'N, 101°55'E, 2–4 III 1972, P. & P. Spangler (18 specimens USNM). **Nakhon Sawan Province:** Tumbon Mae Poen, 15°39.054'N, 99°28.727'E, 119 m, 23 V 2004, L-708, Vitheepradit & Prommi (11 specimens UMC); Kamphaeng San, Kasetsart University Campus, 14°00.790'N, 99°59.359'E, 15 I 2012, L-1321, among *Azolla pinnata* in pond, A. Vitheepradit, T. O. Prommi & R. W. Sites (1 specimen SHSU). **Phetchaburi Province:** Amphur Nong Ya Plong, Tumbon Nong Ya Plong, 13°09'N, 99°41'E, 69 m, 15 V 2003, L-533, Vitheepradit & Ferro (10 specimens UMC); Amphur Tha Yang, Mae Nam Phetchaburi, on HWY 3499, 12°55'N, 99°51'E, 39 m, 19 IV 2002, Vitheepradit & Kirawanich, L-354 (1 specimen). **Phuket Province:** mtn. stream 3 III 1968, B. A. Harrison (15 specimens). **Prachuap Khiri Khan Province:** Amphur Hua Hin, Ban Nong Yai Oum, 12°35'N, 99°46'E, 83 m, 16 V 2003, Vitheepradit & Ferro, L-539 (12 specimens UMC); Amphur Thap Sakae, Ban Huay Yang, 11°36'N, 99°38'E, 25 m, 18 V 2003, Vitheepradit & Ferro, L-543 (2 specimens UMC). **Sakon Nakhon Province:** Sakonnakhora (sic) city reservoir, 3 II 1952, M. E. Griffith (16 specimens SEMC). **Sara Buri Province:** Amphur Sao Hal (2.7 km west), 14°33'N, 100°49'E, 11 III 1971, P. & P. Spangler (1 specimen USNM). **Songkhla Province:** Amphur Rataphum, Tumbon Kampangphet, 07°08.030'N, 100°18.437'E, 30 m, 2 VI 2004, Vitheepradit & Prommi, L-732 (38 specimens UMC, 1 specimen SHSU); Amphur Hat Yai, Prince of Songkla University, pond near reservoir, 07°00'N, 100°30'E, 58 m, 4 V 2002, Vitheepradit & Kirawanich, L-391 (1 specimen UMC). **Surat Thani Province:** Amphur Phuphin, Tumbon Khao Kwai, 09°03.773'N, 99°14.521'E, 17 m, 16 VI 2004, Sites, Vitheepradit, & Prommi, L-771 (2 specimens SHSU); Amphur Ban Na, Ban Huay Harng, 08°50.925'N, 99°18.215'E , 24 m, pond, 20 VI 2004, Vitheepradit & Prommi, L-781 (1 specimen, UMC); Amphur Ban Na, Ban Tha Rau Tai, 08°56.567'N, 99°15.130'E, 4 m, 20 VI 2004, L-782, Vitheepradit & Prommi (1 specimen SHSU); Amphur Ban Na San, Ban Poo Pea, 08°40.490'N, 99°20.092'E, 4 m, 19 VI 2006, L-962, Vitheepradit & Prommi (36 specimens UMC, 1 specimen SHSU). **Tak Province:** Amphur Meung, Tumbon Nhong Bua Tai, 16°49'N, 99°07'E, 106 m, 9 V 2003, L-514 (50 specimens UMC, 1 specimen SHSU). **Trang Province:** Amphur Sikao, pond at Chao Mai Beach, 07°26.842'N, 99°20.647'E, 3 m, 9 I 2003, Vitheepradit & Prommi, L-907 (28 specimens UMC); Prince of Songkla University, 7°31'N, 99°35'E, 55 m, 7 VI 2003, CMU and PSU teams, L-597 (13 specimens UMC, 1 specimen SHSU). **Uthai Thani Province:** Amphur Ban Rai, Tumbon Kang Roong, 15°14.121'N, 99°41.002'E, 69 m, 24 V 2004, L-712, Vitheepradit & Prommi, (3 specimens UMC); Amphur Mueang, Tumbon Nam Serm, 15°20.886'N, 100°02.120'E, 20 m, 24 V 2004, L-713, Vitheepradit & Prommi (1 specimen UMC). **Northeast** Thailand, 15 I 1953, M. E. Griffith (90 specimens SEMC, 5 specimens SHSU).

**Discussion.** Because of the wide geographic distribution and variable characters, it is possible that what has previously been considered to be *P. frontalis* may include more than one species. Previously, it has not been possible to differentiate between species variation and species boundaries. However, after viewing hundreds of specimens, it appears that there are some reliable characters as long as there is a series of specimens in the sample to account for the variation. Lundblad (1933) noted that this is a species with variable characters, which is supported by the findings of this study. If a single specimen is used for identification, there may be some uncertainty in obtaining an accurate identification. The most apparent diagnostic character of *P. frontalis* is the facial marking found in most specimens, consisting of five dark marks, one vertical stripe on the center of the face and two pairs of horizontal stripes (Fig. 1C). Nearly 90% of specimens examined had these markings (although with variation in how dark these markings appear); thus, with a series of specimens, the species as now defined is readily identified. The current data did not include the type specimens (because they were never designated); however, they did match this diagnostic characteristic given in the original description. *Paraplea brunni* (Kirkaldy) and *P. halei* (Lundblad) also commonly have facial markings but they are usually restricted to the center vertical stripe and are not known to have the full component in the pattern shown in Fig. 1C. The scutellum in most specimens of *P. frontalis* is a contrasting lighter color and is often golden-orange. The keel of these specimens is also usually diagnostic, having well-defined teeth on the abdominal segments that are relatively longer than in other species, but there is variation in this character. Some specimens have smaller teeth, which could have resulted from wear, and the shape of these teeth can vary from being relatively straight to curved. The drawings of the keel by Lundblad (1933) of specimens from Indonesia had smaller teeth on the abdominal keel than did the majority of those examined in this study, but without examining the Indonesian specimens, it is uncertain if this is typical of specimens from these islands. As with most species of Pleidae, the ovipositor and subgenital plates of both sexes are diagnostic of the species. The ovipositor of *P. frontalis* most commonly has the teeth as shown in Fig. 3, but some specimens appear to be lacking inner teeth. This could be slight intraspecific variation or possibly interspecific differences if *P. frontalis* proves to be a species complex.

### ***Paraplea lateromaculata*** **Cook, sp. nov.**

http://zoobank.org/377ED572-8BF9-4EAA-850B-7852358A97D3

Figures 6–11, 24B

**Description.** All measurements are in millimeters and were taken from 224 adult specimens from throughout Thailand as reported in the distribution portion of this paper. Specimens used for this description are deposited at SEMC, SHSU, and UMC.

***Body size.*** Total length, 1.21–1.58 (average 1.42) (Fig. 6A, B, D); two specimens not included in this range unusually large, 2.35 and 2.52, but consistent in all other morphological characters with this species.

***Color.*** Color may vary slightly among individuals (Fig. 6A, B, D). Base color of body most often golden-tan with some darker brown markings. A few specimens exhibit a weak banding pattern of the hemelytra (Fig. 6A), banding more pronounced in small percentage of individuals that are more lightly colored (Fig. 6B); many with some honeycombing (Fig. 6B, D); small percentage of lightly colored specimens with red patches on vertex of head. Nearly all specimens with three dark spots on pronotum, two near posterolateral region and one at dorsal middle posterior. Distinctive dark spot on hemelytra above metacoxa (Fig. 6A, D).

***Head.*** Head (Fig. 6C) generally light-brown to cream-colored, often with darker markings between eyes. Face and vertex often with a distinctive vertical bar (Fig. 6C), although sometimes missing or not distinctively bar-shaped. Antenna three-segmented, usually hidden from view below eye (extended and visible in Fig. 1A). Head size similar among Thai specimens, head width (excluding two unusually large specimens noted above) at widest point including eyes 0.62–074 (average 0.67), head width at narrowest point between eyes, 0.29–0.39 (average 0.35), OI 45–55 (average 50).

***Pronotum.*** Base color usually light-tan but ranging between nearly white to brown, usually with lighter colored honeycombing apparent; most specimens have three distinct dark spots, one near posterolateral edge and one near the central posterior margin of pronotum (Fig. 6B); most have visible central vertical band without punctures, at least at anterior end (Fig. 6B, C); slight bulge present toward lateral posterior; punctures present, ~ 0.02; if honeycombing present, punctures located between honeycomb bars (Fig. 6B, D); pronotum width 0.58–0.84 (average 0.74); pronotum length 0.29–0.63 (average=0.42); PI 38–69 (average 57).

***Wings.*** Complete to posterior; punctures evenly dispersed with only small distance between punctures, not in rows (0.02 – 0.03 in diameter) (Fig. 6A, D); underlying honeycomb structure sometimes present; claval suture present in most (Fig. 6A) but absent in some (Fig. 6D); scutellum with distinct punctures, usually darkened in

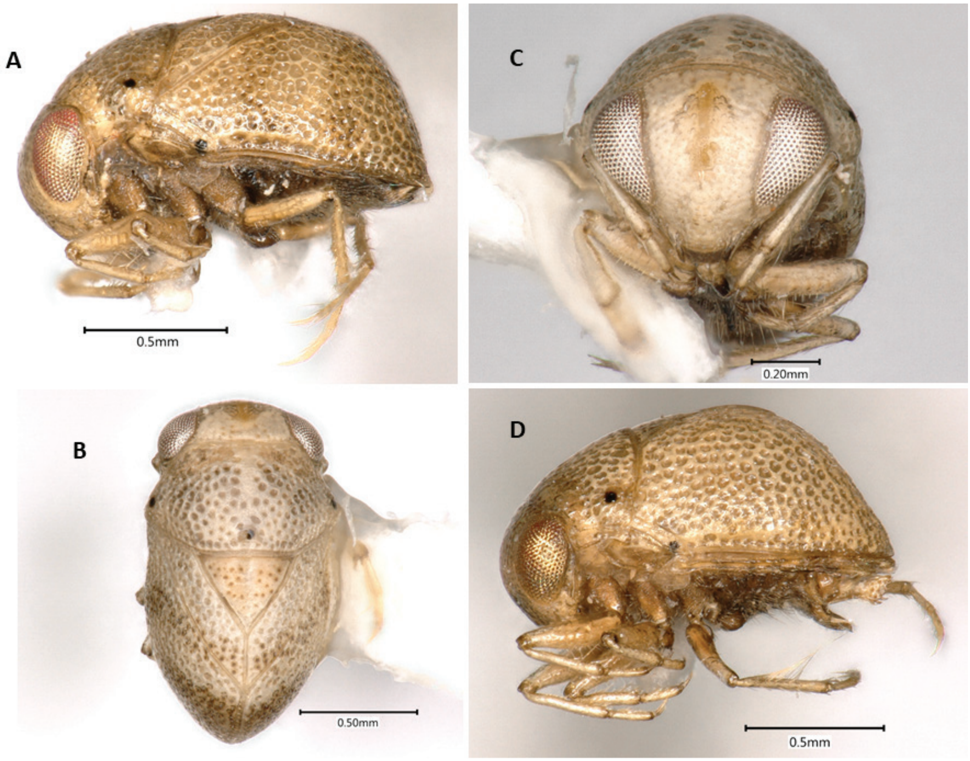
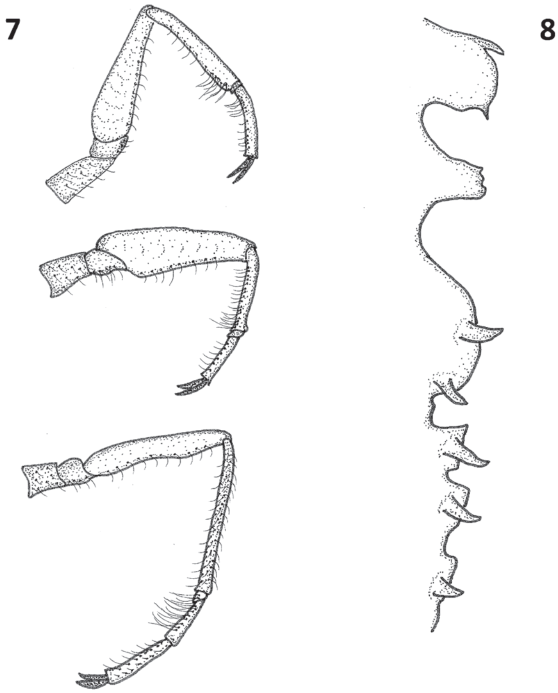


**Figure 6.** *Paraplea lateromaculata* sp. nov. specimens from Thailand. **A** male in lateral view with typical coloration. **B** dorsal view of a light colored morph that shows dark banding and honeycombing. **C** frontal view of specimen in B. **D** female showing an alternatively colored form in lateral view without dark bands.

center (Fig. 6B), more widely spaced than other punctures, punctures not in apparent order; scutellum base color similar to hemelytra, almost white to golden-brown; lateral view shows distinct black spot near margin (Fig. 6A, D), resembling spots on prothorax; darker vertical band on some specimens (Fig. 6B); shape of hemelytra ranging from rectangular (Fig. 6A) to having a dorsal bulge (Fig. 6D); scutellum slightly wider than long but often almost triangular (Fig. 6B), scutellum length 0.21 – 0.43 (average 0.30); scutellum width 0.28–0.48 (average .36); SI 106–148 (average 120). Hind wings membranous, fully-developed, completely concealed by hemelytra.

***Legs.*** Shape of legs as in Fig. 7. Typical leg measurements: prothoracic leg coxa 0.14, trochanter 0.06, femur 0.37, tibia 0.30, first tarsomere 0.02, second tarsomere 0.15,pretarsal claw 0.10; mesothoracic leg coxa 0.14, trochanter 0.06, femur 0.37, tibia 0.21, first tarsomere 0.02, second tarsomere 0.12, pretarsal claw 0.09; metathoracic leg coxa 0.13, trochanter 0.06, femur 0.40, tibia 0.40, first tarsomere 0.05, second tarsomere 0.17, third tarsomere 0.17, pretarsal claw 0.10; several long hairs along ventral side of trochanter, femur, tibia and tarsus, especially at base of hind tarsus where some hairs reach 0.25 (Fig. 7).



**Figures 7–8.** *Paraplea lateromaculata* sp. nov. **7** prothoracic leg above, mesothoracic leg in the middle and metathoracic leg below. **8** profile of the ventral keel with the anterior end (thoracic) to the top and ventral to the left.

***Median ventral keel.*** Thoracic portions distinct from each other, prosternal keel somewhat rectangular with small teeth at anterior and posterior edges; mesosternal keel small but distinctly squared in profile, slightly serrated; metathoracic keel segment somewhat rounded with prominent teeth. Abdominal keel on segments I-IV with distinct teeth, segment I appears fused to metathoracic keel. Figure of typical specimen in Fig. 8.

***Characters of female.*** Ovipositor roughly rectangular but apical side slightly wider (gonapophysis 1) at end of fused rectangular shaft (gonapophysis 2) (Fig. 9); five distinct teeth along posterior border (apical row), although fourth tooth is smaller and somewhat recessed. In some specimens this small tooth appears to be missing; three teeth on ventral border, decreasing in size posteriorly; two rows of three teeth each away from apex, three primary and three secondary, but usually no tertiary teeth; one long hair on ventral side where triangular apex of gonapophysis 1 and basal rectangle of gonapophysis 2 meet, however this hair is sometimes inconspicuous except at high magnification; subgenital plate as in Fig. 10; slightly wider than long; width ~ 0.28, length ~ 0.25; relatively smooth but with hairs emerging from shallow pits; tufts of relatively long hairs on each side near apex; slightly shorter hairs spaced throughout genital plate darker v-shaped prominence in center, near apex and extending 2/3 to posterior border.

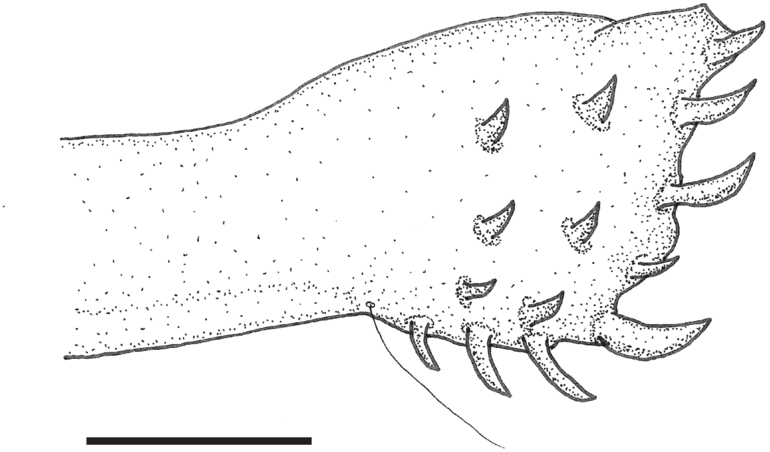
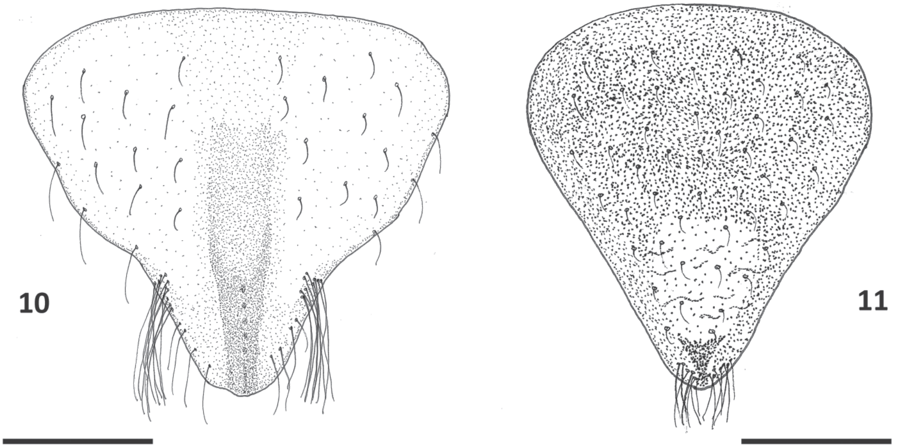


**Figure 9.** Ovipositor of *Paraplea lateromaculata* sp. nov. Scale bar: 0.05 mm.



**Figures 10–11.** Genital plates of *Paraplea lateromaculata* sp. nov. **10** female, **11** male. Scale bar: 0.1 mm.

***Characters of male.*** Aedeagus bulbous and somewhat asymmetrical in typical fashion of family; operculum (subgenital plate) generally triangular, slightly longer than wide (Fig. 11), width ~ 0.23, length ~ 0.25, lightly rugulose in center before apex but otherwise smooth to granular, with short hairs throughout, several longer hairs at apex.

**Distribution.** *Paraplea lateromaculata* is found throughout most of peninsular Thailand, and few specimens have also been collected in eastern Thailand (Fig. 24B). Specimens observed include a single specimen collected from Singapore.

**Type material examined.** ***Holotype*** female, Thailand: **Krabi Province**, Amphur Nuea Khlong, Tumbon Klong Kanarn, Ban Klong Kanarn, pond 8°01.045'N, 99°00.450'E, 37 m, 8 I 2006, Vitheepradit and Prommi, L-903 (UMC). ***Paratypes*** (38 TOTAL): Singapore: Federated Malay States, 31 X 1955, Marshall Laird (1 paratype

SEMC). Thailand: **Chumphon Province:** Amphur Se Wi, Ban Kron, 10°17.390'N, 99°05.464'E, 5 m, 30 V 2004, Vitheepradit and Prommi, L-728 (3 paratypes UMC, 1 paratype SHSU); Amphur Sa Wi, Ban Kron, pond, 10°14.542'N, 99°05.555'E, 6 m, 30 V 2004, Vitheepradit and Prommi, L-729 (5 paratypes UMC); Amphur Lamae, Ban Suan Som Boon, 09°43.311'N, 99°06.208'E, 20 VI 2006, Vitheepradit and Prommi, L-964 (4 paratypes UMC, 1 paratype SHSU). **Kalasin Province:** Phu Pan National Park, Lahm Huay Noi, 8 km S of Ban Kahm Perm, vegetated margins of river, 7 VI 1998, Vitheepradit, Sites and Simpson, L-162 (1 paratype UMC). **Loei Province:** Amphur Phukraduna, pond 1 km W of intersection of Hwy 201 and 2019, 21 VI 1998, Vitheepradit and Sawangsak, L-201 (1 paratype UMC). **Nong Bua Lamphu Province:** Phu Kao-Phu Pan Kham National Park, Namtok Tad Fah Waterfall, 16°55.259'N, 102°27.659'E, 201 m, 10 V 2004, Prommi and Vitheepradit, L-674 (2 paratypes UMC). **Phatthalung Province:** Praiwan Waterfall, 3 km W of Ban Phut, pond with vegetation, 11 VII 1997, Sites and Permkam, L-135 (2 paratypes UMC). **Phetchaburi Province:** Amphur Tha Yang, Ban Yang Chum, stream, 12°47'N, 99°40'E, 46 m, 15 V 2003, Vitheepradit and Ferro, L-536 (3 paratypes UMC, 1 paratype SHSU). **Prachuap Khiri Khan Province:** Amphur Kui Buri Forest, Forest Plantation Station, 12°04'N, 99°37'E, 17 V 2003, 117 m, Ferro and Vitheepradit, L-540 (1 paratype UMC). **Songkhla Province:** Amphur Ratephum, Tumbon Kampangphet, 7°08.030'N, 100° 18.437'E, 30 m, 2 VI 2004, 30 m, Vitheepradit and Prommi, L-732 (1 paratype UMC). **Trang Province:** Amphur Sikao, Tumbon Mai Fard, Ban Klong Maeng, pond, 7°30'N, 99°20'E, 6 m, 10 VIII 2005, Vitheepradit, Simpson and Prommi, L-868 (4 paratypes UMC, 2 paratypes SHSU); Amphur Sikao, Tumbon Mai Fard, Ban Klong Maeng, pond, 7°30.170'N, 99°20.541'E, 6 m, 10 I 2006, Vitheepradit and Prommi, L-908 (1 paratype UMC). **Northeast** Thailand, 15 I 1953 M. E. Griffith (4 paratypes SEMC, 1 paratype SHSU).

**Additional material examined.** Thailand: **Chumphon Province:** Amphur Lamae, Ban Suan Som Boon, 09°43.311'N, 99°06.208'E, 13 m, 20 VI 2006, Vitheepradit and Prommi, L-964 (7 specimens UMC); Amphur Sa Wi, Ban Kron, 10°17.390'N, 99°05.464'E, 5 m, 30 V 2004, Vitheepradit and Prommi L-728 (2 specimens UMC); Amphur Sa Wi, Ban Kron, pond, 10°14.542'N, 99°05.555'E, 6 m, 30 V 2004, Vitheepradit and Prommi, L-729 (5 specimens UMC, 1 specimen SHSU). **Kalasin Province:** Phu Pan National Park, black light at Park Headquarters near pond; 7 VI 1998, Sites, Simpson, and Vitheepradit, L-166 (42 specimens UMC, 1 specimen SHSU); Phu Pan National Park, Lahm Huay Noi, 8 miles S of Ban Kahm Perm, vegetated margins of river, 7 VI 1998, Vitheepradit, Sites and Simpson, L-162 (3 specimens UMC). **Kanchanaburi Province:** Thong Pha Phum, Reforestation Station, black light, 14°39'N, 98°35'E, 211 m, 11 IV 2002, UMC & CMU teams, L-333 (1 specimen UMC). **Krabi Province:** Amphur Nuea Khlong, Tumbon Klong Kanarn, Ban Klong Kanarn, pond 8°01'N, 99°00'E, 37 m, 8 I 2006, Vitheepradit and Prommi, L-903 (2 specimens UMC). **Loei Province:** Amphur Phukradung, pond 2 km W of intersection of Hwy 201 and 2019, 21 VI 1998, Vitheepradit and Sawangsak, L-201 (6 specimens UMC). **Nakhon Si Thammarat Province:** 6 km N of Amphur Chulabhorn on Hwy 41, pond, 8°07.625'N, 99°51.540'E, 31 m, 8 VI 2004, Vitheepradit and

Prommi, L-756 (1 specimen UMC); Amphur Chulabhorn, Tumbon Na Moh Boon, 8°01.664'N, 98°53.763'E, pond, 20 m, 4 VI 2004, Vitheepradit and Prommi, L-741 (1 specimen UMC); Nopphitam Khlong Yod Leung, stream, 8°38'N, 99°44'E, 78 m, 26 V 2005, Vitheepradit and Prommi, L-800 (1 specimen UMC). **Nong Bua Lamphu Province:** Phu Kao-Phu Pan Kham National Park, Namtok Tad Fah, 16°55.259'N, 102°27.659'E, 201 m, 10 V 2004, Prommi and Vitheepradit, L-674 (1 specimen UMC). **Phatthalung Province:** Praiwan Waterfall, 3 km W of Ban Phut, pond with vegetation, 11 VII 1997, Sites and Permkam, L-135 (2 specimens UMC); Amphur Phayom, Ban Pa Phayom, pond, 7°50'N, 99°56'E, 19 m, 31 V 2003, Vitheepradit and Ferro, L-584 (3 specimens UMC). **Phetchaburi Province:** Amphur Tha Yang, Ban Yang Chum, stream, 12°47'N, 99°40'E, 46 m, 15 V 2003, Vitheepradit and Ferro, L-536 (8 specimens UMC). **Songkhla Province:** Amphur Rataphum, Tumbon Kampangphet, 7°08.030'N, 100°18.437'E, 30 m, 2 VI 2004, Vitheepradit and Prommi, L-732 (3 specimens UMC); Hat Yai, Prince of Songkla University campus ponds, 21 VI 2002, Sites and Permkam, L-411 (4 specimens UMC); Prince of Songkla University, pond near reservoir, 7°00'N, 100°30'E, 58 m, 8 VI 2005, Prommi, Sites and Vitheepradit, L-834 (7 specimens UMC). **Surat Thani Province:** Amphur Ban Na, Ban Tha Rau Tai, pond, 8°56.567'N, 99°15.130'E, 4 m, 20 VI 2004, Vitheepradit and Prommi, L-782 (3 specimens UMC). **Trang Province:** Amphur Sikao, Tumbon Mai Fard, Ban Khlong Maeng, pond, 7°30'N, 99°20'E, 6 m, 4 June 2005, Sites, Vitheepradit and Prommi, L-831 (1 specimen UMC); Amphur Sikao, Tumbon Mai Fard, Ban Khlong Maeng, pond, 7°30'N, 99°20'E, 6 m, 10 I 2006, Vitheepradit and Prommi, L-908 (44 specimens UMC); Amphur Sikao, Tumbon Mai Fard, Ban Khlong Maeng, pond, 7°30'N, 99°20'E, 6 m, 14 VI 2006,Vitheepradit, Sites and Prommi, L-955 (25 specimens UMC). **Northeast** Thailand, 15 I 1953, M. E. Griffith (30 specimens SEMC).

**Etymology.** The specific epithet combines two Latin roots, *latero-* meaning the side and *–macula* meaning spot. Thus, the name refers the distinct dark spot on the lateral side of the hemelytra. This spot is similar to the dorsal pronotal spots found in this species and *P. liturata*.

**Discussion.** In general appearance, *P. lateromaculata* sp. nov. could be misidentified as *P. liturata* that is missing two of its dark pronotal spots. However, several consistent characters separate these species. The most obvious of these characters is that *P. lateromaculata* sp. nov. has one dark spot on each side of the hemelytra, anteriorly near the costal margin, which is absent in *P. frontalis*. The ovipositors of these species are quite different (compare Fig. 3 with Fig. 9), and diagnostic characters of the ventral keel and genital plates also differ between these species. *Paraplea lateromaculata* sp. nov. is much smaller, none of which had body lengths reaching a length of 1.60 compared to the smallest measured *P. frontalis* at 1.89.

*Paraplea lateromaculata* sp. nov. can be differentiated from *P. melanodera* sp. nov. by their colored markings. More specifically, *Paraplea lateromaculata* sp. nov. has the distinctive black spots whereas *P. melanodera* sp. nov. has no black spots but has a black band at the posterior margin of the head. Although the size is similar between *P. lateromaculata* sp. nov. and *P. melanodera* sp. nov., recognizable differences exist in other characters as listed in their respective descriptions. *Paraplea lateromaculata* sp. nov. often has three pronotal black spots on the prothorax as is found in less than 10% of *P. liturata*; however, *P. liturata* has never been observed to have the black spots on the hemelytral costal margin as is seen in all specimens of *P. lateromaculata* sp. nov.

### ***Paraplea liturata*** **(Fieber, 1844)**

Figures 12–17, 24C

= *Ploa liturata* Fieber, 1844: Entomol. Monogr. p. 19.
= *Plea liturata*: (Kirkaldy, 1904): Wien. Entomol. Zeit. 23: 129.
= *Plea metiadusa* Distant, 1910: Fauna Brit. India 5: 337.
= *Plea rufonotata* Distant, 1914: Rech. Sci.N.-Calédonie 2: 387.
= *Plea quinquenota* Paiva, 1918: Rec. India. Mus. 14: 29.
= *Plea fasciata* Horváth, 1918: Ann. Mus. Nat. Hung. 16: 144.
*Plea* (*Paraplea*) *liturata*: Esaki and China 1928: Rev. Esp. Entomol. 4: 166 (subgenus description).
*Paraplea liturata*: Drake and Maldonado-Capriles 1956 (elevation to genus) 51: 53.

**Remarks.** *Paraplea liturata* shares some of the same taxonomic history as *P. frontalis* since both were described in the same paper. *Paraplea liturata* was described as *Ploa liturata* by Fieber (1844) for specimens collected in the East Indies. This original description was not only brief but did not capture an important set of markings that is commonly present on specimens of *P. liturata*, even though the description relied almost completely on coloration. Fieber noted the typical two dashes between the eyes and the pair of lateral and single midline spots on the posterior margin of the pronotum. However, two spots on the anterior part of the pronotum were not listed in the description nor on his plate. The figure does show the hemelytral banding that is common in many specimens. Although types were not designated by Fieber, subsequent researchers were able to associate the description with many specimens of this common species, although none of these were those used by Fieber. Placement of this species into the genus *Paraplea* followed the same sequence as reported for *P. frontalis*. Kirkaldy (1904) moved this species into the genus *Plea* but made no comments on the species. Distant (1906) translated Fieber's description but did not further report on this species. Distant (1910) described *Plea metiadusa* from Calcutta, India and reported that it had no maculations, but this species was still determined to be a variant of *P. liturata* by Lundblad (1933). In doing so, Lundblad (1933) commented on the variability of the pronotal spots that are most commonly five in number but can range from none to seven. Distant (1914) also described *Plea rufonotata* but did not associate it with *P. liturata*. The description of *P. rufonotata* from New Caledonia was the first time that a pronotum with five spots was described, which now appears to be the most common state for *P. liturata*. Again, Lundblad (1933) made the association of these species as being synonyms. Horváth (1918) described *Plea fasciata* from specimens from Batavia, Java (Indonesia) but this was considered a strongly-colored example of *P. liturata* (Lundblad 1933). *Plea quinquenota* (Paiva 1918) was described from a single specimen from Inlé Lake, Yawnghe State (now in Myanmar) and was not examined by Lundblad (1933), but was still synonymized with *P. liturata* based on the illustration of the new species perfectly matching the typical form of *P. liturata*. Along with the taxonomic clarifications, Lundblad (1933) also redescribed the species. Below is a supplemental description of the species, incorporating specimens from Thailand.

***Holotype.*** None designated.

**Type locality.** Fieber (1844) reported that the description was made from specimens collected by Dr. Helfer in the East Indies but the exact location in South or Southeast Asia is unknown.

**Supplementary description.** All measurements are in millimeters and were taken from 221 adult specimens from throughout Thailand as reported in the distribution portion of this paper. Specimens used for this description are deposited at UMC and SHSU.

***Body size.*** Total length, 1.27–1.68 (average 1.48) in Thailand specimens. Fieber (1844) reported total length of "approximately 2 mm." Distant (1910) gave the size of his later synonymized *P. metiadusa* from India and his later synonymized *P. rufonotata* from Caledonia as 2. The specimen used to describe the later synonymized *P. quinquenotata* was listed as 1.5 (Paiva 1918). Anderson and Weir (2004) reported a length of 1.8–2.0 for this species in their key and reported it from Northern Territory and Western Australia.

***Color.*** Color may be quite variable among individuals within a population. Base color of body ranges from tan (Fig. 12A, B) to white (Fig. 12D). Darker bands on the sides of the hemelytra are common but not observed in all individuals. When present, bands are darker than the base color, ranging from light orange-brown (Fig. 12D) to tan (Fig. 12A) to brown (Fig. 12B). Punctures are sometimes a darker shade (Fig. 12A). Honeycombing matches the base color. Most specimens have five characteristic dark spots on the pronotum (Fig. 12B); two on the anteromedial portion (Fig. 12 B), two on the posterolateral region (one on each side) (Fig. 12B, D), and one on the posteromedial region (Fig. 12B, D). Conversely, these spots are sometimes faint or absent in some individuals and at some locations.

***Head.*** Head (Fig. 12C) colored with base body coloration, ranging from white to brown. Many specimens with a vertical light-colored bar between eyes. If present, bar can be thin (Fig. 12C) to wider, sometimes occupying nearly a third of width between eyes. Two dark spots common on face, one on each side between central bar and eyes (Fig. 12C). Eyes in dried specimens range from red to gold. Mouthparts usually darker that the rest of head. Antenna three-segmented, usually hidden from view below eye. Head size similar among Thailand specimens, head width at widest point including eyes 0.62–0.79 (average 0.72), head width at narrowest point between eyes, 0.31–0.43 (average 0.40), OI 47–58 (average 53).

***Pronotum*** (Fig. 12B). Base color ranging from white to light brown and honeycombing apparent in most specimens; most specimens with five dark spots on pronotum (91% of Thailand specimens with five spots, ~ 9% with three spots and lacking

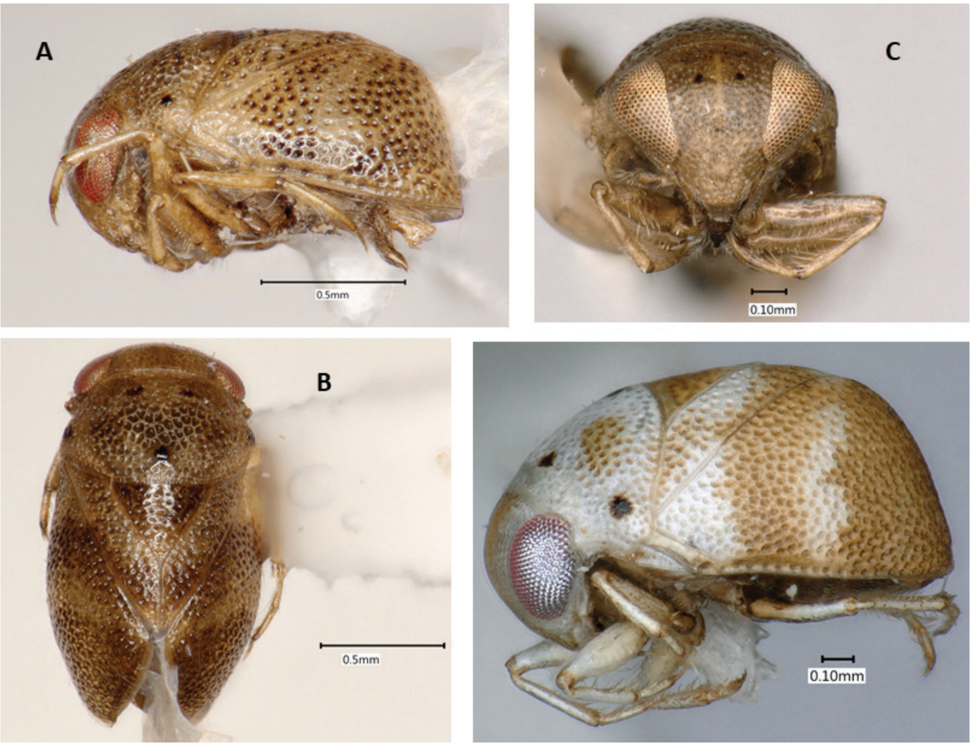
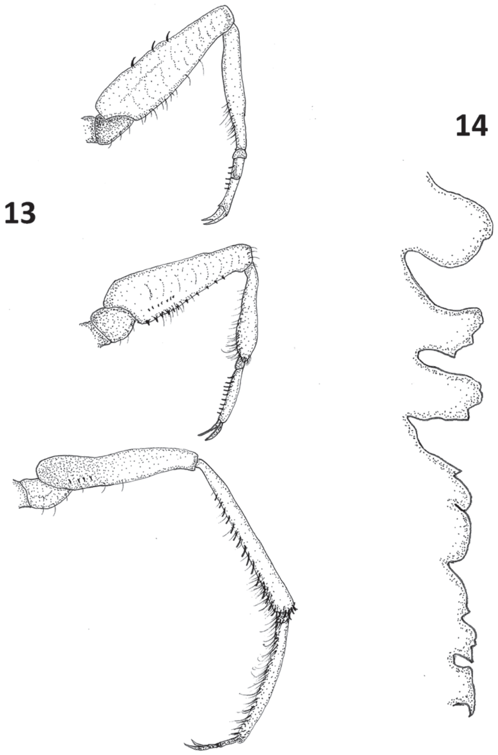


**Figure 12.** *Paraplea liturata* specimens from Thailand. **A** female in lateral view with typical coloration, **B** dorsal view in that shows dark banding and honeycombing. **C** frontal view of specimen in B. **D** male showing an alternatively colored form in lateral view.

anterior pair, less than 1% with no spots); a shallow puncture in center of each cell of honeycomb and under high magnification a minute hair can usually be seen coming from each pore; with slight bulge posteriorly, wider than long, width 0.66–0.92 (average 0.79); pronotum length 0.33–0.57 (average 0.46); PI 39–68 (average 58).

***Wings.*** Complete to posterior; punctures equally spaced but not generally in rows (0.03 in diameter) (Fig. 12A, D); underlying honeycomb structure usually present; claval suture distinct, complete; scutellum with punctures smaller (0.01 in diameter), more widely spaced than other punctures, scutellum base color often golden but sometimes dark brown and often darker than hemelytra; honeycombing absent from scutellum, making it appear somewhat transparent; lateral view shows distinct horizontal dorsal profile and near vertical aspects posterior profile with a transition of nearly 90° (Fig. 12A, C); scutellum slightly wider than long (Fig. 12B), length 0.28–0.43 (average 0.35); width 0.31–0.50 (average 0.40); SI 103–139 (average 116). Hind wings membranous, fully developed, completely concealed by hemelytra.

***Legs.*** Legs with numerous hairs and small spines (Fig. 13), hairs prevalent on apical half of prothoracic and mesothoracic tibiae, without numerous long hairs as found on tibia and tarsus of many *Paraplea*. Typical leg measurements: prothoracic leg coxa



**Figures 13, 14.** *Paraplea liturata.* **13** prothoracic leg above, mesothoracic leg in the middle and metathoracic leg below. **14** profile of the ventral keel with the anterior end (thoracic) to the top and ventral to the left.

0.05, trochanter 0.09, femur 0.42, tibia 0.35, first tarsomere 0.04, second tarsomere 0.09,pretarsal claw 0.08; mesothoracic leg coxa 0.06, trochanter 0.10, femur 0.41, tibia 0.28, first tarsomere 0.03, second tarsomere 0.13, pretarsal claw 0.08; metathoracic leg coxa 0.05, trochanter 0.10, femur 0.39, tibia 0.49, first tarsomere 0.02, second tarsomere 0.13, third tarsomere 0.18, pretarsal claw 0.11.

***Median ventral keel.*** Thoracic portions distinctly separate, prothoracic keel generally rounded, two posterior thoracic segments serrated; abdominal keel with first two segments fused, teeth more pronounced posteriorly (Fig. 15). Several minor differences between Thailand specimens reported here and those reported by Lundblad (1933: fig. 42C–E).

***Characters of female.*** Ovipositor most commonly as in Fig. 15. Ovipositor roughly rectangular in shape but with apical gonapophysis 1 slightly wider; five distinct teeth along posterior border (apical row) plus two teeth on ventral border (ventral 1 and 2); two rows of small teeth away from apex, three primary, three secondary, and occasionally one tertiary (not shown in Fig. 15); bottom secondary tooth larger and extends slightly beyond end of ovipositor, making it sometimes appear as being along posterior margin; three to five long hairs on ventral side of region where gonapophyses 1 and 2

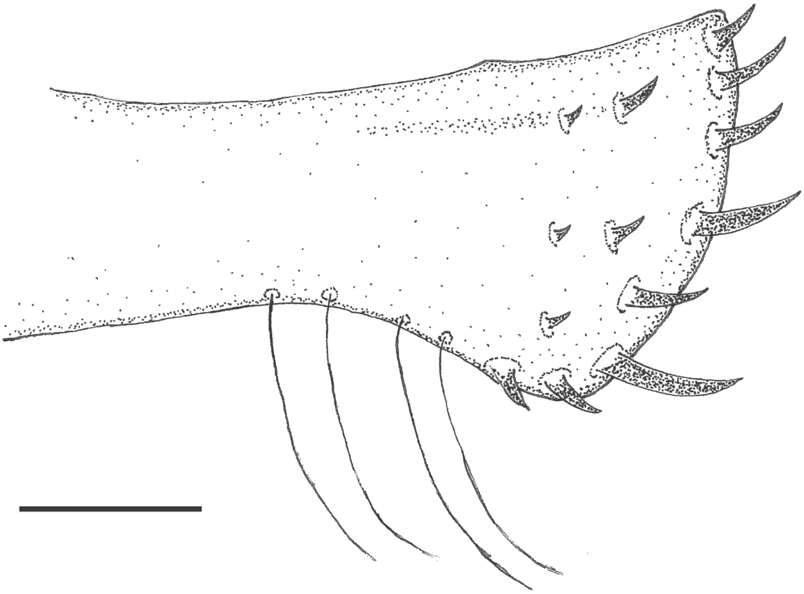


**Figure 15.** Ovipositor of *Paraplea liturata*. Scale bar: 0.05 mm.

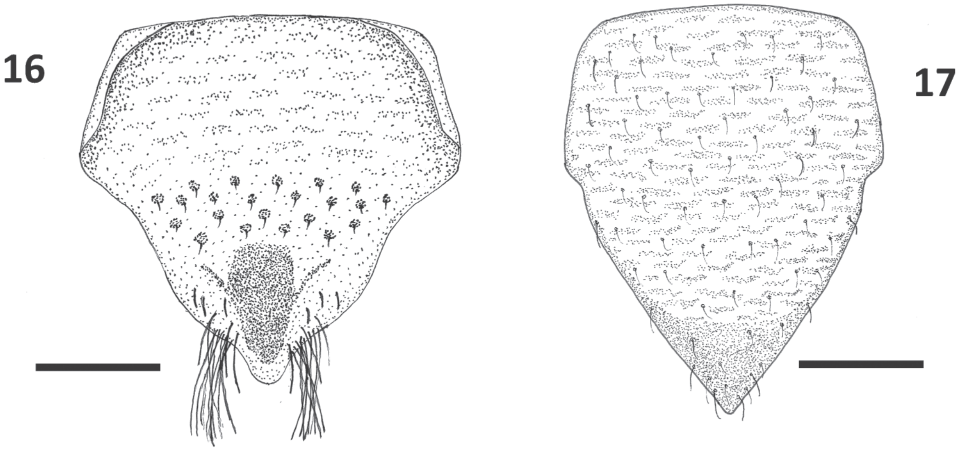


**Figure 16, 17.** Genital plates of *Paraplea liturata*. **16** female **17** male. Scale bar: 0.1 mm.

meet; Subgenital plate slightly longer than wide (Fig. 16), length ~ 0.30, width ~ 0.26, lightly rugose in basal half followed apically by a series of pits, dark region in center near apex, pair of tufted hairs on each side near apex.

***Characters of male.*** Aedeagus bulbous and somewhat asymmetrical in typical fashion of family; operculum (subgenital plate) as in Fig. 17, most of surface slightly rugose, longer than wide, length ~ 0.27, width ~ 0.20.

**Distribution.** In Thailand, *Paraplea liturata* appears to be mostly a peninsular species on the southwest side of the country, although there are two records of it in the eastern region of Thailand; one in Sakon Nakhon Province, which was reported by Chen et al. (2006), and one by RWS and AV in a pond in Ubon Ratchathani Province (Fig. 24C). It is also known from Australia (Anderson and Weir 2004), India (Paiva 1918. Lundblad 1933), Indonesia (Lundblad 1933, Nieser and Chen 1999), Malaysia (Fernando and Cheng 1974), Myanmar (Paiva 1918), New Caledonia (Lundblad 1933), Philippines (Lundblad 1933, Nieser and Chen 1999), and Taiwan (Mitamura et al. 2018).

**Material examined. Chai Nat Province:** Amphur Neon, Kham, Ban Wang Kor Hai, 14°57.934'N, 99°50.668'E, 24 V 2004, Vitheepradit & Prommi, L-710 (1 specimen UMC). **Chumphon Province:** Amphur Sa Wi, Tumbon Kron, 10°17.499'N, 99°05.530'E, 21 VI 2006, Vitheepradit & Prommi, L-967 (4 specimens UMC). **Kalasin Province:** Phu Pan National Park, 7 VI 1998, Sites, Simpson & Vitheepradit, L-165 (3 specimens UMC, 1 specimen SHSU). **Krabi Province:** Amphur Mueang, Klong Muang Beach, pond, 8°02.979'N, 98°45.540'E, 13 m, 8 VIII 2005, Sites, Vitheepradit, Simpson & Prommi L-862 (8 specimens UMC); Amphur Mueang, Nopphorat Thara Beach, pond, 8°02.625'N, 98°48.517'E, 8 V 2005, Sites, Vitheepradit & Prommi, L-805 (1 specimen UMC); Amphur Nuea Khlong, Tumbon, Nuea Khlong, Ban Paga Sai, pond, 8°02.619'N, 99°01.144'E, 27 m, 8 VIII 2005, Sites, Vitheepradit, Simpson & Prommi, L-864 (2 specimens UMC); Amphur Nuea Khong, Pan Paga Sai, pond, 8°02.619'N, 99°01.144'E, 27 m, 13 VI 2006, Vitheepradit & Prommi, L-951 (1 specimen UMC); Tumbon Klong Kanarn, Ban Klong Kanarn, pond, 8°01.045'N, 99°00.450'E, 37 m, 9 VIII 2005, Sites, Vitheepradit, Simpson & Prommi, L-867 (2 specimens UMC). **Phang Nga Province:** Amphur Mueang, Tumbon Na Pring, pond, 8°31.750'N, 98°32.001'E, 5 I 2006, Sites, Vitheepradit & Prommi, L-887 (2 specimens UMC); Amphur Mueang, Tumbon Na Prig, pond, 8°31.750'N, 98°32.001'E, 12 VI 2006, Sites, Vitheepradit & Prommi, L-946 (4 specimens UMC); Amphur Takua Thung, Tumbon Krasom, Ban Bang Mak, pond, 8°24.553'N, 98°27.434'E, 12 VI 2006, Sites, Vitheepradit & Prommi, L-945 (1 specimen UMC); Amphur Takua Pa, Tumbon Bang Nai Si, Ban Bang Yai, pond, 08°25.950'N, 98°23.192'E, 20 m, 8 VI 2006, Sites, Vitheepradit & Prommi, L-927 (1 specimen); Amphur Thai Mueang, Tumbon Na Teoy, Ban Bang Klee, 8°18.655'N, 98°17.552'E, 19 m, 2 VI 2005, Sites, Vitheepradit & Prommi, L-825 (8 specimens UMC); Amphur Thai Mueang, Tumbon Na Teoy, Ban Bang Klee, 8°18.655'N, 98°17.552'E, 19 m, 5 I 2006, Sites, Vitheepradit & Prommi, L-886 (11 specimens UMC); Amphur Thai Mueang, Tumbon Na Teoy, Ban Bang Klee, 8°18.655'N, 98°17.552'E, 9 VI 2006, Sites, Vitheepradit & Prommi, L-935 (11 specimens UMC). **Phatthalung Province:** Amphur Tamot, Tumbon Loh Jak Kra, 7°20.244'N, 100°01.285'E, 44 m, 3 VI 2004, Vitheepradit & Prommi, L-736 (1 specimen UMC). **Phuket Province:** Amphur Thalang, Jud peat swamp, UV pan trap, 8°07.930'N, 98°18.156'E, 24 m, 7 I 2006, Sites, Vitheepradit & Prommi, L-899 (92 specimens UMC, 5 specimens SHSU); Amphur Thalang, Jig peat swamp, 8°08.772'N, 98°17.892'E, 23 m, 7 I 2006, Sites, Vitheepradit & Prommi, L-906 (44 specimens UMC, 3 specimens SHSU); Amphur Thalang, Laem Yot peat swamp, 8°08.325'N, 98°17.927'E, 28 m, 29 V 2005, Sites, Vitheepradit & Prommi, L-807 (1 specimen UMC); Amphur Thalang, Mai Khoa peat swamp, 8°07.461'N, 98°18.193'E, 19 m, 7 I 2006, Sites, Vitheepradit & Prommi, L-900 (42 specimens UMC, 1 specimen SHSU); Amphur Thalang, Tumbon Mai Khao, Moo Ha, 8°10.718'N, 98°17.611'E, 23 m, 31 V 2005, Sites, Vitheepradit & Prommi, L-816 (1 specimen SHSU); Amphur Thalang, Tumbon Mai Khao, Moo Ha, pond, 8°10.718'N, 98°17.611'E, 23 m, 5 I 2006, Sites, Vitheepradit & Prommi, L-889 (17 specimens UMC). **Songkhla Province:** Amphur Rataphum, Tumbon Kampangphet, 07°08.030'N, 100°18.437'E, 30 m, 2 VI 2004, Vitheepradit & Prommi, L-732 (9 specimens UMC). **Surathani Province:** Amphur Ban Na Ban Huay Hamg, 8°08.925'N, 99°18.215'E, 24 m, 20 VI 2004, Vitheepradit & Prommi, L-781 (3 specimens UMC); Amphur Phunphin, Tumbon Boh Rai, 8°53.866'N, 98°08.961'E, 10 m, 7 VI 2004, Vitheepradit & Prommi, L-751 (1 specimen SHSU); Amphur Ban Na, Ban Tha Rau Tai, 8°56.567'N, 99°15.130'E, 4 m, 20 VI 2004, Vitheepradit & Prommi, L-782 (3 specimens UMC). **Trang Province:** Amphur Sikao, Tumbon Mai Fard Ban, Klong Maeng, pond, 8°30.170'N, 99°20.541'E, 6 m, 10 VIII 2005, Sites, Vitheepradit, Simpson & Prommi, L-868 (2 specimens UMC, 1 specimen SHSU). **Uthai Thani Province:** Amphur Mueang, Tumbon Nam Serm, 15°20.886'N, 100°02.120'E, 24 V 2004, Vitheepradit & Prommi, L-713 (3 specimens UMC). **Ubon Ratchathani Province:** Amphur Nam Khoon, Ban Non Yang, pond, 14°28.176'N, 104°53.782'E, 190 m, 10 IV 2004, Sites & Vitheepradit, L-613 (10 specimens UMC).

**Discussion.** There is considerable variation in *P. liturata* if this is a single species. Lundblad (1933) mentioned this variation when synonymizing *P. fasciata*, *P. metiadusa*, *P. quinquenotata,* and *P. rufonotata* with *P. liturata*. The main basis for Lundblad synonymizing these species was the similarity of the abdominal keel. The drawings in his paper (Lundblad 1933) show similarities but there are also subtle variations. The Thailand specimens are also somewhat different from those in the Lundblad drawings. These data preserve the question as to whether this is a variable species or multiple species. Several of the species Lundblad synonymized had been described as having a length of 2 mm, although he stated that this was a small species varying between 1.3 to 1.7 mm. This size range of Lundblad's specimens coincides with that of specimens from Thailand; however, it still does not account for those described from India, New Caledonia, and Australia. The original description of *P. liturata* by Fieber (1844) listed the length imprecisely as "approximately 2 mm" and it is uncertain where in the East Indies these specimens were collected or how accurately that measurement was made. Likewise, some distinct differences in coloration and characters occur in specimens from the various regions.

A character that links all of these specimens into one species is the general state of having five spots on the pronotum. In many populations there can be specimens without these markings although the majority of specimens tend to always have five black pronotal spots. Thus, with a single specimen it may not be possible to rely on this trait but with a series of specimens it is easy to determine the species as *P. liturata*, as it is now defined. There appears to also be some consistency in the characters of the ovipositor. In many of the Thailand specimens, the ovipositor appears remarkably like that figured by Lundblad (1933: fig. 42H) from Java (Indonesia) which is consistent with the ovipositor being a reliable character for species recognition (see Sublett and Cook 2015); however, a small number of specimens lack some or all of the secondary and tertiary teeth. Since the ovipositors of specimens from India, New Caledonia and Australia are unknown, there is still a question as to if specimens from these regions are actually *P. liturata*. Like *P. frontalis*, *P. liturata* could be a species complex. Both these species need additional study to determine their status.

*Ecology*: The habitat of *P. liturata* in Thailand is typical for the majority of pleids. This species was often found in ponds, in shallow water with vegetation.

### *Paraplea melanodera* Cook, sp. nov.

http://zoobank.org/B9B8C42C-2439-4A6D-9FC3-9F038C3CF863
Figures 18–23, 24D

**Description.** All measurements are in millimeters and were taken from 47 adult specimens from Thailand as reported in the distribution portion of this paper. Specimens used for this description are deposited at UMC and SHSU.

***Body size.*** Total length, 1.28–1.66 (average=1.49) (Fig. 18A, B, D).

***Color.*** Color may vary slightly among individuals (Fig. 18A, D) but all specimens with a dark brown to black band at back of vertex of head. Base color of body usually light tan to golden-tan with some darker brown markings (Fig. 18A); although, some specimens have a base color almost white (Fig. 18C, D). A few specimens with weak banding pattern of hemelytra (Fig. 18A, C); honeycombing present but often sparse and difficult to see in some specimens.

***Head.*** Head generally light brown, without markings except distinctive dark band posteriorly. Antenna three-segmented. Head width at widest point including eyes 0.62–074 (average 0.68), head width at narrowest point between eyes, 0.29–0.39 (average 0.34), OI 46–52 (average 49).

***Pronotum.*** Base color brown to light tan (Fig. 18C), usually with lighter colored honeycombing apparent although often not observable throughout; most specimens without dark spots; without distinct humeral or lateral bulges: punctures present, ~ 0.02, spacing between punctures ~ 0.02; if honeycombing present, punctures located between honeycomb bars (Fig. 18C); pronotum width 0.66–0.90 (average 0.78); pronotum length 0.42–0.57 (average 0.50); PI 56–73 (average 64).

***Wings.*** Hemelytra complete to posterior; punctures evenly dispersed with only small distance between punctures, punctures not in rows, ~ 0.02 diameter, evenly spaced (Fig. 18A, C, D); underlying honeycomb structure sometimes present; claval suture present (Fig. 18C, D); scutellum with distinct punctures, usually without darkened center, more widely spaced than other punctures, punctures not in apparent order; scutellum base color more yellow than hemelytra; hemelytra without spots near margin but sometimes with broad vertical bands (Fig. 18A, C); shape of hemelytra flat

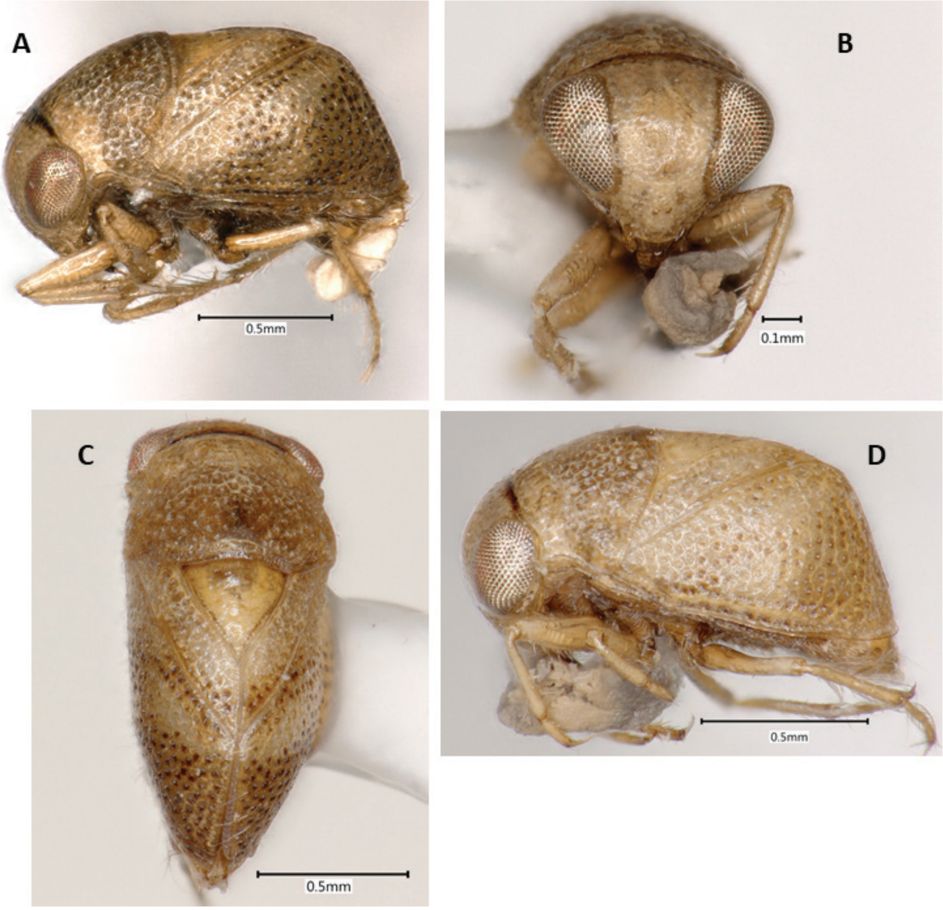
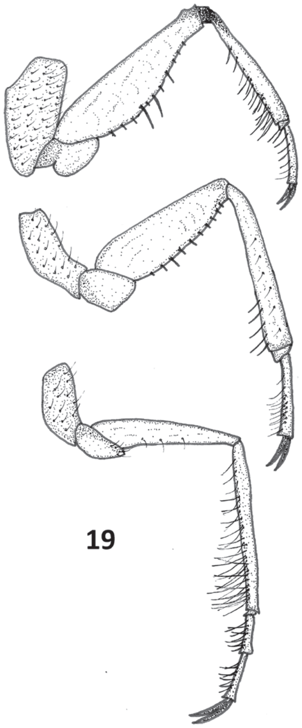


**Figure 18.** *Paraplea melanodera* sp. nov. **A** female in lateral view with typical coloration. **B** dorsal view of a light colored morph that shows dark banding and typical minimum amount of honeycombing. **C** frontal view of specimens in B. **D** female showing a lighter colored lateral view without dark hemelytral bands.

dorsally with posterior face at almost 45° angle (Fig. 18A, D); scutellum often slightly wider than long, sometimes as long as wide (Fig. 18C), length 0.28–0.39 (average 0.34); scutellum width 0.31–0.48 (average 0.39); SI 103–128 (average 115). Hind wings membranous, fully developed, completely concealed by hemelytra.

***Legs.*** Coxae and trochanters relatively very long compared to most pleid species (Fig. 19); hairs numerous on all coxae, relatively long and common on tibiae an tarsi, thickened on prothoracic tibia and tarsus; small spines on prothoracic and mesothoracic femora, small number longer on prothoracic femur; base of prothoracic tibia darker than other parts of leg. Typical leg measurements: prothoracic leg coxa 0.20, trochanter 0.11, femur 0.42, tibia 0.29, first tarsomere 0.03, second tarsomere 0.13, pretarsal claw 0.07; mesothoracic leg coxa 0.25, trochanter 0.08, femur 0.35, tibia 0.29, first tarsomere 0.02, second tarsomere 0.14, pretarsal claw 0.06; metathoracic leg coxa 0.19, trochanter 0.13, femur 0.39, tibia 0.42, first tarsomere 0.03, second tarsomere 0.14, third tar-



**19**

**20**

**Figure 19, 20.** *Paraplea melanodera* sp. nov. **19** prothoracic leg at the top, mesothoracic leg in the middle and metathoracic leg below. **20** profile of the ventral keel with the anterior end (thoracic) to the top and ventral to the left.

somere 0.15, pretarsal claw 0.12; several long hairs along ventral side of trochanter, femur, tibia and tarsus, especially along hind tarsus where some hairs reach 0.18.

***Median ventral keel.*** Thoracic portions distinctively shaped but often hidden by enlarged coxa; prosternal keel broadly rounded, with irregular edges; mesosternal keel narrow, distinctive finger-like projection posteriorly; metathoracic keel segment somewhat rounded with prominent cleft towards center, sometimes appearing to almost overlap first abdominal section; abdominal keel I somewhat rectangular, with distinct tooth, abdominal keel II somewhat square, posterior tooth, abdominal keel III and IV shaped like posteriorly projecting teeth, IV longer than III (Fig. 20).

***Characters of female.*** Ovipositor expanded apically (Fig. 21); five distinct teeth along posterior border (apical row), two large teeth in ventral half, three smaller teeth in dorsal half; three teeth on ventral margin, decreasing in size basally. Two rows of three teeth each away from apex, three primary and three secondary; most specimens with one tertiary tooth; several hairs along basal region of gonapophysis 1 that can be seen under high magnification. Subgenital plate triangular, slightly wider than long (Fig. 22), width ~ 0.42, length ~ 0.37; central anterior area of plate raised above other parts, with v-shaped sub-apical prominence; rugose in basal one third; central region

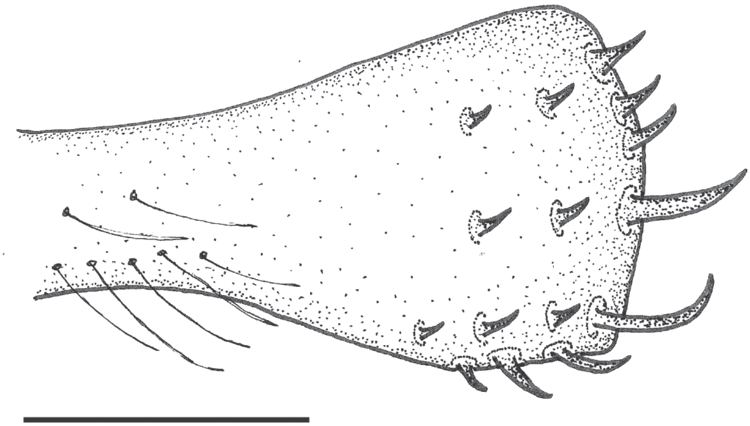
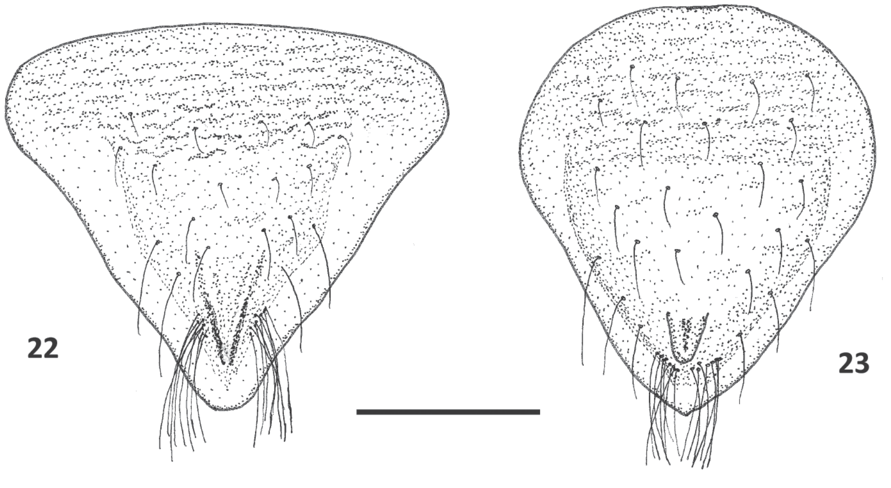


**Figure 21.** Ovipositor of *Paraplea melanodera* sp. nov. Scale bar: 0.05 mm.



**Figures 22, 23.** Genital plates of *Paraplea melanodera* sp. nov.: **22** female, **23** male. Scale bar: 0.1 mm.

towards apex mildly rugose; two distinct tufts of longer hairs on each side of center near apex; other single hairs present, especially toward apex.

*Characters of male*: Aedeagus bulbous and somewhat asymmetrical in the typical fashion of family; operculum (subgenital plate), longer than wide (Fig. 23); width ~ 0.29, length ~ 0.33, rugulose in basal one third but only lightly rugose anterior to that region; central finger-like projection in center towards apex; central region raised above marginal areas; long hairs clumped near apex, other single hairs throughout.

**Distribution.** *Paraplea melanodera* appears to be a species found only in peninsular Thailand along the west coast (Fig. 24D). Most of the known specimens are from ponds near the beach, thus it may be an endemic species to this region of Thailand.

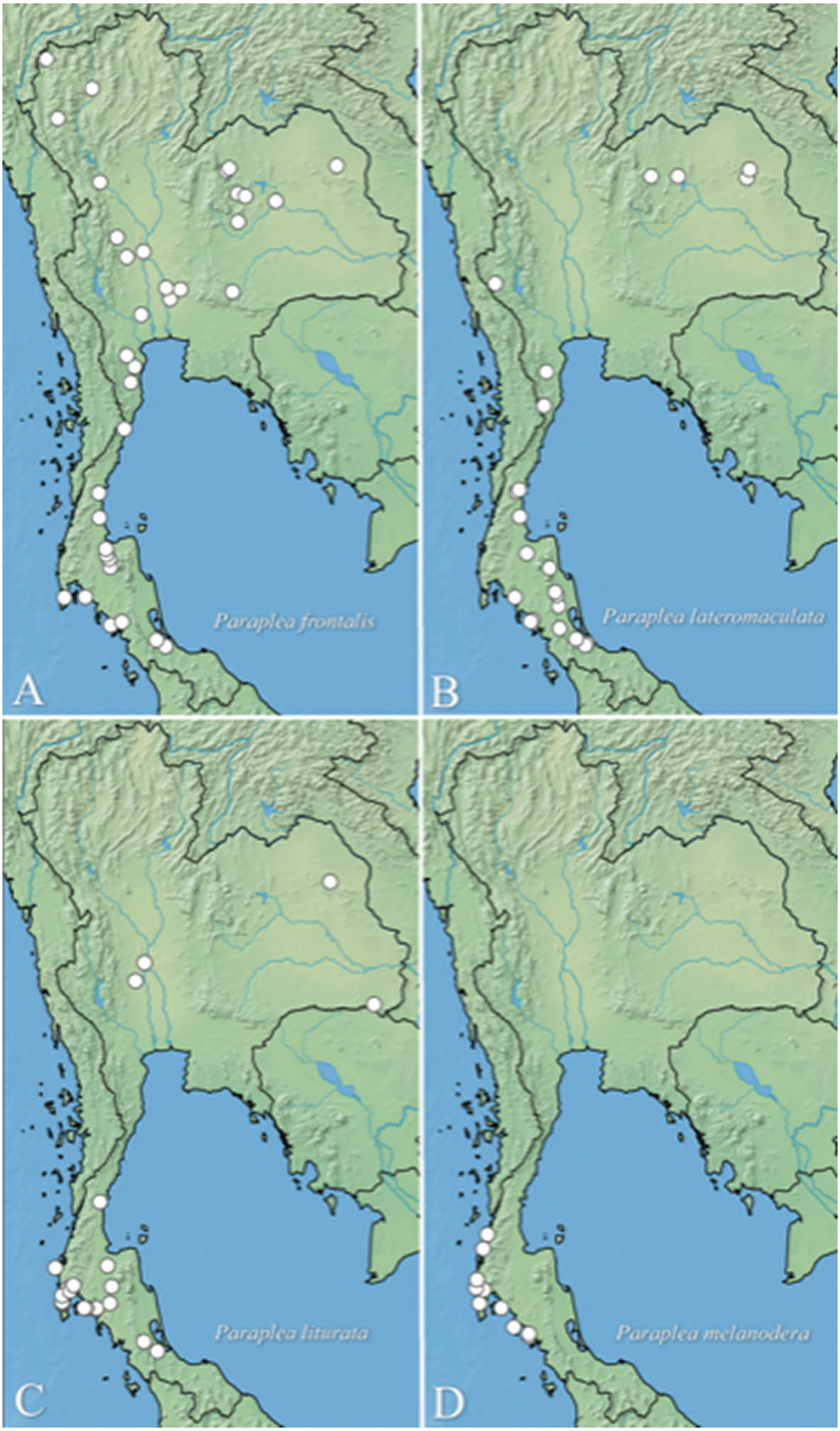


**Figure 24.** Distribution records in Thailand of **A** *Paraplea frontalis* **B** *P. lateromaculata* sp. nov., **C** *P. liturata* **D** *P. melanodera* sp. nov.

**Type material examined.** ***Holotype*:** female, Thailand: **Trang Province**, Amphur Sikao, pond at Chao Mai Beach, 7°26.842'N, 99°20.647'E, 3 m, 9 I 2006, Vitheepradit and Prommi, L-907 (UMC). ***Paratypes*** (10 total): Thailand: **Krabi Province:** Amphur Mueang, Khlong Muang Beach, 8°02.979'N, 98°45.540'E, 13 m, 8 I 2006, Vitheepradit and Prommi, L-901 (3 paratypes UMC, 2 paratypes SHSU). **Phang Nga Province:** Amphur Takua Pa, Nang Tong Beach, pond, 8°38.906'N, 98°14.833'E, 16 m, 4 I 2006, Vitheepradit, Sites and Prommi, L-883 (2 paratypes UMC). **Ranong Province:** Laem Son National Park, pond in front of officers house, 9°36.118'N, 98°28.074'E, 6 m, 2 VIII 2005, Vitheepradit, Prommi and Simpson, L-838 (1 paratype UMC); Laem Son National Park, pond near headquarters, 9°36.247'N, 98°28.005'E, 6 m, 3 I 2006, Vitheepradit, Sites and Prommi, L-875 (1 paratype UMC); Laem Son National Park, pond near headquarters, 9°36.247'N, 98°28.002'E, 6 m, 7 VI 2006, Vitheepradit, Sites and Prommi, L-922 (1 paratype UMC).

**Additional material examined.** Thailand, **Krabi Province:** Amphur Ko Lanta, Khlong Dao Beach pond, 07°38.662'N, 99°01.395'E, 10 m, 9 VIII 2005, Sites, Vitheepradit, Simpson and Prommi, L-865 (5 specimens UMC); Amphur Mueang, Khlong Muang Beach, 8°02.979'N, 98°45.540'E, 13 m, 8 I 2006, Vitheepradit and Prommi, L-901 (12 specimens UMC, 1 specimen SHSU). **Phang Nga Province:** Amphur Khura Buri, Aow Kuey Beach, pond, 9°18.005'N, 98°22.798'E, 5 m, 7 VI 2006, Sites, Vitheepradit and Prommi, L-924 (2 specimens UMC); Amphur Takua Pa, Nang Tong Beach pond, 8°38.906'N, 98°14.833'E, 16 m, 4 I 2006, Vitheepradit, Sites and Prommi, L-883 (2 specimens UMC); Amphur Takua Pa, Tumbon Bang Nai Si, Ban Bang Yai, 8°25.950'N, 98°23.192'E, 20 m, 8 VI 2006, Vitheepradit, Sites and Prommi, L-927 (2 specimens UMC); Khao Lampi-Hat Thai Mueang National Park, pond near beach, 8°28.312'N, 98°13.672'E, 1 m, 2 VI 2005, Vitheepradit and Prommi, L-824 (1 specimen UMC). **Phuket Province:** Amphur Thalang, Jig peat swamp, 8°08.772'N, 98°17.892'E, 23 m, 10 VI 2006, Vitheepradit, Sites and Prommi, L-942 (1 specimen UMC). **Ranong Province:** Laem Son National Park, pond in front of officers house, 9°36.118'N, 98°28.074'E, 6 m, 7 VI 2006, Vitheepradit, Sites and Prommi, L-923 (1 specimen UMC); Laem Son National Park, pond near headquarters, 9°36.247'N, 98°28.005'E, 6 m, 2 VIII 2005, Vitheepradit, Prommi and Simpson, L-837 (2 specimens UMC); Laem Son National Park, pond near headquarters, 9°36.247'N, 98°28.005'E, 6 m, 7 VI 2006, Vitheepradit, Sites and Prommi, L-922 (5 specimens UMC). **Trang Province:** Tumbon Mai Fard, Ban Klong Maeng, pond, 7°30.170'N, 99°20.541'E, 6 m, 10 I 2006, Vitheepradit and Prommi, L-908 (4 specimens UMC).

**Etymology.** The specific epithet combines two Greek roots, *melano-* meaning black and *–dero* meaning the neck. Thus, the name refers the distinct dark line at the back of the head, which is distinctive of this species and a character not found in other members of the genus.

**Discussion.** The distinctive character of *P. melanodera* sp. nov. is the dark band at the posterior margin of the head. This character is seen in all specimens of this species and is not observed in any other species of *Paraplea*. *Paraplea melanodera* sp. nov. also has a raised central portion of the subgenital plate in both sexes and is most pronounced in the male. Other species of *Paraplea* in Southeast Asia do not have this character state. The coxae of *P. melanodera* sp. nov. are quite long. *Paraplea lateromaculata* sp. nov. also has long coxae, but are comparably shorter than those of *P. melanodera* sp. nov. Spines on the metathoracic femur are not common in *Paraplea* but are present in both *P. melanodera* sp. nov. and *P. liturata*. *Paraplea melanodera* also has spines on the prothoracic femur, including a couple that are longer than the others. Characters of the ventral keel, ovipositor, and subgenital plates also have distinct differences compared to other species of *Paraplea*.

In a study to determine the recovery of the lentic insect community following the Indian Ocean tsunami of 2004, Sites & Vitheepradit (2010) sampled ponds along the Thai coastline at four time intervals, including beginning five months after the tsunami, which marked the end of the dry season. *Paraplea melanodera* sp. nov. was collected in 11 of the 12 ponds inundated by the tsunami, including during the first sampling period, and in only two of the ten un-inundated reference ponds. The mean conductivity of all inundated ponds over all dates from which *P. melanodera* sp. nov. was collected was 1,714 µS, including one at 13,040 µS. Conductivity of the reference ponds further inland was < 100 µS and Indian Ocean seawater was over 41,000 µS. Thus, the waterbodies in which *P. melanodera* sp. nov. occurred had distinctly elevated levels of salinity. It is likely that *P. melanodera* sp. nov. also occurs further north and south along the coastlines to Burma and Malaysia.

**Distribution of *Paraplea* in mainland Southeast Asia.** *Paraplea frontalis* is one of the most widespread species of the genus and is prominent in Southeast Asia and beyond. In addition to the records reported here, *P. frontalis* has also been reported in other studies. Nieser (2004) stated that he had seen specimens of *P. frontalis* from Thailand, but these remain unpublished. The first published record of *P. frontalis* in Thailand did not appear until 2006 when it was reported from material collected in 1999 in Sakon Nakhon Province (Chen et al. 2006), which do not include those eluded to by Nieser (2004) (Nico Nieser pers. comm.). Lundblad (1933) described *P. quinquemaculata* (now a synonym of *P. frontalis*) from specimens from northern Sumatra. This region is directly west of mainland Malaysia and is considered maritime Southeast Asia, although with the short geographic distance across the Andaman Sea, it is not unexpected that these regions share species. Lundblad (1933) also reported two specimens of *P. frontalis* from Lake Toba, northern Sumatra and from East Java. Both of these specimens are in maritime Southeast Asia. Fernando (1961) collected *P. frontalis* (reported as *P. quinquemaculata*) at lights from Tanjong Karang, Selangor (Malaysia), which is close to the location of the Chapman specimens reported here. Fernando and Cheng (1974) mentioned that *P. frontalis* (reported as *P. quinquemaculata*) was collected once from a pond in Singapore but also stated that this species had never been collected in Malaya (Malaysia) even though Fernando (1961) had previously reported it from this region. Nieser and Chen (1999) reported on numerous collections of *P. frontalis* from Sulawesi and Sumatra (Indonesia). Esaki (1940) reported four specimens of *P. frontalis* from Chusan, which is an island off the southeastern coast of China. This region is not considered part of the political designation of Southeast Asia but is geographically close and this record would not be unusual with a mainland Southeast

Asia species distribution. Esaki (1940) also stated that *P. frontalis* is widely distributed in India, Indochina, China, Java, Sumatra, Nicobar Islands and Formosa. However, no specimen data or source of that information was provided, nor did he mention any other Pleidae from this region. Esaki's distribution could include data from several species, or it might include undocumented distribution records for *P. frontalis*. Distant (1910) used specimens from Calcutta and Madhupur, India to describe *Plea pelopea*, which is now a synonym of *P. frontalis*. Hafiz and Pradhan (1947) also reported *P. frontalis* from the nearby locality of Patnagarh, India and further north at Gait Sarovar, Bolangir, India. These areas of India share many species with a mainland Southeast Asia distribution; thus, these records might not be considered unusual. Benzie (1989) found *P. frontalis* from waterholes in Yala National Park in southeastern Sri Lanka.

It is uncertain what factors influence the distribution of *P. frontalis*. The limited number of collections that represent our knowledge of this species suggest that it is most common in mainland Southeast Asia, but its distribution extends to the north into eastern Asia in China and Taiwan, west to India and Sri Lanka, and south to maritime Southeast Asia on islands of Indonesia. This paper represents the only data where an area was more thoroughly collected, although even this effort provides fewer than 500 specimens. When these collections are plotted on a distribution map (Fig. 24A), there is no perceptible indication of reasons for this distribution. In fact, with more collections, *P. frontalis* may be found throughout nearly all provinces of Thailand.

Another factor to consider is that what is reported as *P. frontalis* could be a species complex. This would not pertain to the distribution shown in Fig. 24A but could be a factor in reports in other areas of its distribution. There are noted morphological differences among specimens of *P. frontalis* from different parts of its range, including size differences, some differences in markings and other minor morphological variation. More study and many more specimens are needed to sort out this situation, probably including modern molecular comparisons of specimens from these various regions. Until this future study, *P. frontalis* will be considered a variable species with a wide distribution.

*Paraplea liturata* is widely distributed in Thailand and some predict that it could have the largest distribution of any species in the genus (Lundblad 1933, Fernando 1961). Its distribution in Thailand (Fig. 24C) does not appear as extensive of that of *P. frontalis* but it is a common inhabitant within a wide geographic distribution. Fernando and Cheng (1974) collected *P. liturata* at several locations in Malaysia and recorded it as being "fairly common" in this region. Nieser and Chen (1999) likewise found it in several locations in Indonesia as well as documenting the species in the Philippines.

*Paraplea liturata* does not appear to be restricted to any general habitat type or biogeographical region. Chen et al. (2006) stated that it was "common throughout Thailand in vegetation rich stagnant waters." The present study also indicates that the species occurs in ponds and slow-moving streams, both containing aquatic vegetation. Anderson and Weir (2004) reported *P. liturata* from Western Australia and the Australian Northern Territory, areas biogeographically quite different from Thailand and Malaysia, but did not comment on the specifics of the collection sites.

The distribution of *P. lateromaculata* sp. nov. (Fig. 24B) is very similar to that of *P. liturata* (Fig. 24C) in Thailand, including collection sites in common between these species. Both of these species were common in the peninsular region of Thailand.

The documented distribution of *P. melanodera* sp. nov. includes only the central part of peninsular Thailand (Fig. 24D). Even more restrictive is that it was collected only from small ponds near beaches along the coastline. The apparent halophilic nature of *P. melanodera* sp. nov. is well-documented because all specimens were collecting during the tsunami study of Sites & Vitheepradit (2010). The other three species of *Paraplea* also were collected during the study; however, these were mostly from uninundated reference ponds. More specifically, *P. lateromaculata* sp. nov. was collected in two reference ponds, including during all four sampling periods. *Paraplea liturata* was collected in all reference ponds over all sampling periods as well as a single individual from an inundated pond on the first sampling date, which we consider an adventitious occurrence. *Paraplea frontalis* was collected in two inundated ponds with elevated levels of salinity on the last sampling date, which was 17 months after the tsunami. Because eight and 28 specimens were collected in those two ponds, its occurrence was not adventitious; thus, *P. frontalis* appears to have a tolerance for salinity, but is not as adept at dispersing as is *P. melanodera* sp. nov.

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RESEARCH ARTICLE

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# Contribution to the knowledge of the bumblebee fauna of Afghanistan (Hymenoptera, Apidae, *Bombus* Latreille)

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## Abstract

Bumblebees (Hymenoptera: Apidae: genus *Bombus* Latreille, 1802) constitute an important group of pollinators for many wild plants and crops in north temperate regions and South America. Although knowledge of these insects has been increasing over the last decades, some geographic areas remain poorly studied and additions to the knowledge of their faunas are infrequent. Afghanistan is one example of a country that is currently underrepresented in the scientific literature despite its high species diversity. For this study, more than 420 new occurrence records were gathered for 17 bumblebee species belonging to all eight subgenera recorded in the country, including the first record of a species closely related to the *B. longipennis* group. Additionally, the first standardized database for Afghan bees is launched, which we hope will be enriched in the future to allow further assessments of population trends for the bumblebees of Afghanistan. Finally, the previously published species records for the country are discussed considering the most recent taxonomic revisions of the genus and key perspectives are highlighted for further work in this understudied country and neighboring regions.

## Keywords

Asian bees, checklist, Pamir, pollinators, taxonomy

## Introduction

Bumblebees (Hymenoptera: Apidae: genus *Bombus*) constitute a key group of widespread cold-adapted insects, substantially contributing to ecosystem services around the globe through the pollination of numerous wild plants and agricultural crops (Velthuis and van Doorn 2006; Klein et al. 2007). These large colorful bees, represented by ~ 260 described species worldwide (Williams et al. 1998), form an increasingly popular model group for large-scale studies in the fields of biogeography (Williams et al. 2017), population genetics (Ghisbain et al. 2020), evolutionary biology (Tian et al. 2019) and more worryingly, global change biology (Kerr et al. 2015; Rasmont et al. 2015). Bumblebee populations are indeed undergoing serious regressions worldwide, mostly attributable to anthropogenic disturbance such as habitat destruction or climate change (Williams and Osborne 2009; Cameron et al. 2011; Cameron and Sadd 2020; Rollin et al. 2020).

Although long-term and detailed data of bumblebee species are extensively documented in some parts of the world such as Europe (Rasmont et al. 2015) or North America (Williams et al. 2014, 2019) and allow large-scale meta-analyses on their decline (e.g., Kerr et al. 2015; Sirois-Delisle and Kerr 2018), other areas presently remain poorly represented in scientific publications. Afghanistan is a prime example of such a place. Although Afghanistan is particularly diverse in terms of its ecosystems and therefore its flora and fauna, the country remains poorly represented in the scientific literature on insects, with the only exception being the order Lepidoptera (Howarth and Povolný 1973, 1976; Wyatt and Omoto 1966 and more recently Tshikolovets 2017 and Tshikolovets et al. 2018). For bumblebees, it has been recently suggested that Afghan mountains were likely to have acted as a climatically suitable historical bridge allowing the spread of *Mendacibombus* bumblebees from the high elevations of Central Asia toward the Middle East and Europe (Williams et al. 2016, 2017). The Afghan mountains therefore constitute an interesting area for faunal assessments and studies in the field of biogeography.

After the contributions and faunal reviews of Reinig (1930, 1940), Richards (1951), and Tkalců (1968), no subsequent studies have added consequential new data to the bumblebee fauna of Afghanistan. To our knowledge, no occurrence records of bumblebee specimens have been published from Afghanistan in recent decades due to the obvious reason of prolonged human conflict within its territory. Turbyville et al. (2013) and Dunford et al. (2014) discussed and presented a checklist of Afghan Hymenoptera, including a partial list of the bumblebee fauna, but these lists were only meant to include insects potentially harmful to soldiers during their stay in the country, and cannot therefore be regarded as proper additions to the present knowledge of the Afghan bumblebee fauna.

The latest faunal review to date (Tkalců 1968) recorded 21 bumblebee species for Afghanistan. Taking into account more recent taxonomic revisions and current synonymies, Tkalců's checklist would now be reduced to 17 valid species. In this paper, we provide new occurrence records for several of those species, considering the currently accepted synonymies of the genus.

## Materials and methods

We studied the personal collections of the entomologists G. Ebert, H. Huss, C. Naumann, and W.F. Reinig (deposited in the University of Mons, UMons, Belgium) as well as the museum collections of the Natural History Museum (NHMUK, London, England), and the State Museum of Natural History Karlsruhe (Karlsruhe, Germany), gathering *inter alia* specimens from Afghanistan mainly collected in the 1960's and 1970's. Identifications of the specimens were performed using reference specimens from the NHMUK as well as previous taxonomic studies of the bumblebee fauna of Afghanistan, the region of Kashmir and the mountain range of Pamir (mainly Reinig 1930; Tkalců 1968 and Williams 1991). All of the information present on the labels of the examined specimens is listed in the results section, sorted by subgenus and species. However, in order to facilitate easy reuse and updates to the bumblebee Afghan data as part of further studies, we gathered and standardized all the available information into a separate database published with the present study (Suppl. material 1). This appendix gathers all label information from examined specimens (location, date, altitude, collector, identifier) and whenever possible includes GPS coordinates to specimens based on the label data and using the World Geodetic System 1984 (WGS84). This was only done if the coordinates were very likely to correspond to original collecting sites. Because of uncertainty in precisely locating some sites, we assigned an error measurement to coordinates from 1 to 100 km. No coordinates were assigned to specimens whose original collecting locality labelling was highly uncertain or could correspond to multiple sites separated by more than 100 km.

Finally, because a few of our specimens belong to the almost morphologically unidentifiable bumblebees from the subgenus *Bombus s. str.* (see Williams et al. 2012), we used preliminary phylogenetic results from an ongoing study that aims to resolve problematic taxonomic cases within the genus *Bombus*. Briefly, this molecular approach combines the targeted enrichment of ultra-conserved elements (UCEs) with multiplexed next-generation sequencing (NGS) (Branstetter et al. 2017a, b) and allows for efficient recovery of many nuclear and mitochondrial (e.g., COI) loci from museum-preserved specimens. These data were integrated with previously published phylogenies (Williams et al. 2012) to give insights into the phylogenetic relationships between our examined specimens and what is known from the most up-to-date taxonomic literature.

## Results

### New occurrence records for the Afghan bumblebee fauna

The subgenera are classified following the currently accepted phylogenetic relationships of the genus *Bombus* (Cameron et al. 2007; Williams et al. 2008) and species by alphabetical order. The following symbols are used: ♂ = male, ☿ = worker and ♀ = queen.

Sampling locations for which GPS coordinates could be assigned are represented by a number in square brackets (see the corresponding name site and associated latitude, longitude and altitude in the Table 1 and their mapping in the Figure 1). Sampling sites that could not be geographically identified with a high degree of certainty are associated with a question mark in square brackets. All the information on the whole sampling is standardized in the database in Suppl. material 1.

## Subgenus *Mendacibombus* Skorikov, 1914

### *Bombus* (*Mendacibombus*) *makarjini* Skorikov, 1910

**Published data.** Williams et al. (2016).

**Material examined.** Kotal-e-Wazir, 07.viii.71, 4400 m, leg. C. Naumann (1♂) (UMons) [12].

**Global distribution.** Palaearctic region.

### *Bombus* (*Mendacibombus*) *marussinus* Skorikov, 1910

**Published data.** Reinig 1940; Tkalců 1968; Williams et al. (2016).

**Material examined.** Badakshan, Anjuman Pass, 12.viii.52 (1♀, 6☿☿), 13.viii.52 (1☿), 4200 m, leg. J. Klapperich (NHMUK) [18]; Hindukusch, Andarab, 4250 m, leg. H. Kotzsch E. Kotzsch (1☿) (NHMUK) [6]; Hindukusch, Chodja-Mahomed [Kwaja Muhammed], 3900 m, leg. H. Kotzsch E. Kotzsch, (1♂) [9]; Hindukusch, Nuksan Pass [? near Chitral, Konar], 3750 m, leg. H. Kotzsch E. Kotzsch (1☿, 1♂) (NHMUK) [on the border with Pakistan, at approximately 36.33N, 71.58E]; Issik, 3500 m, leg. H. Huss (no date for 1☿, 1♂; 18.viii.75: 1☿; 22.viii.75: 1☿; 23.viii.75: 8☿; 24.viii.75: 1☿, 1♂; 25.viii.75: 1☿) (UMons) [15]; Kotal-e-Wazir, 07.viii.71, 4400 m, leg. C. Naumann (1♀) (UMons) [12]; Pagmangebirge [Paghman mts], 26.viii.53, 2800 m, leg. J Klapperich, (1☿) (NHMUK) [4]; Hindu Kush, Tarest Mts., high valley, 1.ix.67, 3250 m, leg. D.K. Mardon, 36°20'N, 69°50'E (2☿☿) (NHMUK) [7].

**Global distribution.** Palaearctic region.

### *Bombus* (*Mendacibombus*) *turkestanicus* Skorikov, 1910

**Published data.** Williams et al. (2016).

**Material examined.** Hindu Kush, upper Lezdi valley, 21.viii.67, 2240 m, leg. D.K. Mardon, 36°20'N, 69°50'E (1☿) (NHMUK) [7]; Shiva-See, 2–5.viii.71, 3100 m, D. Müting (2☿☿, 1♂) (NHMUK) [10]; Hindu Kush, Tarest Mts., high valley, 1.ix.67, 3250 m, leg. D.K. Mardon, 36°20'N, 69°50'E (1☿) (NHMUK) [7]; Issik, 4200 m, leg. H. Huss (1☿) (NHMUK) [15].

**Global distribution.** Palaearctic region.

**Table 1.** Locations in Afghanistan for which GPS coordinates could be assigned. Latitude (Lat.) and longitude (Long.) are given in decimal degrees and rounded to two decimal places. Site numbers correspond to the locations on the map in Figure 1 (ordered in a clockwise pattern) and to the numbers in square brackets in the Results section. See the associated database (Suppl. material 1) for more details about the samples.

| Labelled site name | Site number | Lat. (DD) and Long. (DD) | Approximate elevation (m a.s.l.) |
|---|---|---|---|
| Kotal-e-Asgharat E-Ste | 1 | 34.38N, 66.65E | 3200 |
| Kotal-e-Narges, D.-e-Godar | 2 | 34.38N, 66.87E | 3150 |
| Ghorghori-e-Panjao, Gaukhana | 3 | 34.38N, 67.02E | 2800 |
| Pagmangebirge | 4 | 34.61N, 68.9E | 2800 |
| Salang-Nord /Salang-Paß | 5 | 35.31N, 69.04E | 2100 |
| Andarab | 6 | 35.67N, 69.32E | 4250 |
| Upper Lezdi valley | 7 | 36.33N, 69.83E | 2240 |
| Lezdi | 8 | 36.36N, 69.91E | 1560 |
| Kwaja Muhammed | 9 | 36.41N, 70.58E | 3900 |
| Shiva-See | 10 | 37.39N, 71.36E | 3100 |
| Bala Kuran | 11 | 36.02N, 70.77E | 3200 |
| Kotal-e-Wazir | 12 | 36.98N, 72.783E | 4400 |
| Darrah-e-Istmotsh | 13 | 37.23N, 72.83E | 4300 |
| Issiktal | 14 | 37.03N, 73.33E | 3500 |
| Ptukh | 15 | 37.01N, 73.37E | 4900 |
| Issik | 16 | 37.00N, 73.33E | 4200 |
| Ahmad Diwana (Baba) | 17 | 35.91N, 71.3E | 2600 |
| Bashgal river | 18 | 35.61N, 71.33E | 2900 |
| Kamdesh | 19 | 35.75N, 71.25E | 3350 |
| Badakshan, Anjuman Pass | 20 | 35.80N, 70.24E | 4200 |
| Dar-e-Pandjshir, Kotal-e-Tal | 21 | 35.27N, 69.47E | 3800 |
| Sarobi | 22 | 34.59N, 69.76E | 1100 |
| Safed Koh, Kotkai | 23 | 34.01N, 69.71E | 2350 |

## Subgenus *Subterraneobombus* Vogt, 1911

### *Bombus* (*Subterraneobombus*) *melanurus* Lepeletier, 1835
Figure 2

**Published data.** Reinig 1940; Richards 1951; Tkalců 1968.

**Material examined.** C-Afghanistan, Prov. Ghor, Kotal-e-Asgharat E-Ste., 9.vii.1976, 3200 m, leg. C. Naumann (6☿☿) (UMons) [1]; C-Afghanistan, Prov. Ghor, Kotal-e-Narges, West-Ste; D.-e-Godar, 09.vii.76, 3100–3200 m, leg. C. Naumann (1☿) (UMons) [2]; E-Afghanistan, Dar-e-Pandjshir, Kotal-e-tal, 30.vii.73, 3800 m, M. N. Khoram (1♀, 3♂♂) (UMons) [19]; Ghilzai, 05.viii.48, 1780 m, N. Haarlov (3♂♂) (NHMUK) [?]; Grosser Pamir, Issiktal, Frostbodenflur, 24.viii.75, 4100–4350 m, leg. H. Huss, (1♂) (UMons) [possibly close to 13]; Grosser Pamir, Issiktal, Quellflur in Artemisia-Chenopodiensteppe, 16.viii.75 (1♀, 4☿☿), 17.viii.75 (1☿), 22.viii.75 (3☿☿, 6♂♂), 23.viii.75 (11☿☿, 2♂♂), 25.viii.75 (1♀, 5☿☿, 3♂♂), 3500 m, leg. H. Huss, 37°02'N, 73°20'E (UMons) [13]; Grosser Pamir, Ptukh, 30.vii.75, leg. H. Huss (1♀) (UMons) [14]; Hazaradjat, Koh-i-Baba, Pandjao-Umg., 26.vii.61, 2500 m, leg. G. Ebert (1☿) (UMons) [reference coordinates of the Koh-i-Baba 34.64N, 67.62E]; Haz-

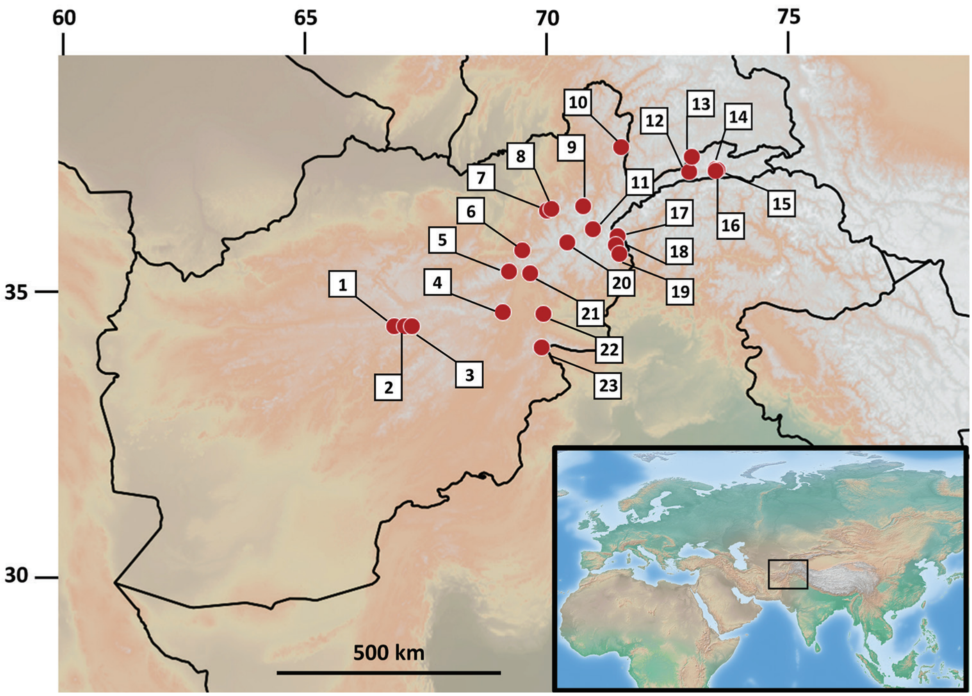

**Figure 1.** Map of Afghanistan and neighboring regions depicting the sampling examined as part of this study. Numbers correspond to the following locations: 1) Kotal-e-Asgharat; 2) Kotal-e-Narges, D-e-Godar; 3) Ghorghori-e-Panjao, Gaukhana; 4) Pagmangebirge; 5) Salang-Nord, vic. Khindjan / Salang-Paß; 6) Andarab; 7) upper Lezdi valley; 8) Lezdi; 9) Chodja-Mahomed [Kwaja Muhammed]; 10) Shiva-See; 11) Bala Kuran; 12) Kotal-e-Wazir; 13) Darrah-e-Istmotsh 14) Issiktal [Quellflur in Artemisia-Chenopodiensteppe]; 15) Ptukh; 16) Issik; 17) Ahmad Diwana (Baba); 18) Bashgal river; 19) Kamdesh, near Suingal/Shkurigal confluence; 20) Badakshan, Anjuman Pass; 21) Dar.-e-Pandjshir, Kotal-e-Tal; 22) Sarobi; 23) Safed Koh, Kotkai. GPS coordinates and altitude of these sites are given in Table 1 and in the associated database (Suppl. material 1).

aradjat, Koh-i-Baba, Shah-tu-Kotal, 20–21.vi.1961, 4000m, leg. G. Ebert (1♀, 2☿☿) (UMons) [reference coordinates of the Koh-i-Baba 34.64N, 67.62E]; Hindu Kush, 06.viii.68, 2290 m, leg. M. Tong (1☿) (NHMUK) [sampling site not possible to locate, given that the Hindu Kush is – 800 km long]; Hindu Kush, nr Kamdesh confluence of R. Suingal and R. Shkurigal, viii.1977, 11000 ft., P.H. Ryley, 35°45'N, 71°15'E (1♀) (NHMUK) [17]; Tarest Mts., 01.ix.67, 3250 m, leg. D.K. Mardon (1☿); Z-Afghanistan, Koh-i-Baba, S-Seite, Shah-tu-Pass, 17–19.vii.1966, 3000 m, leg. G. Ebert (1♀, 2☿☿, 5♂♂) (UMons) [reference coordinates of the Koh-i-Baba 34.64N, 67.62E].

**Global distribution.** Palaearctic and Oriental regions.

### Subgenus *Psithyrus* Lepeletier, 1832

### *Bombus* (*Psithyrus*) *branickii* (Radoszkowski, 1893)

**Published data.** Reinig 1940; Tkalců 1968.

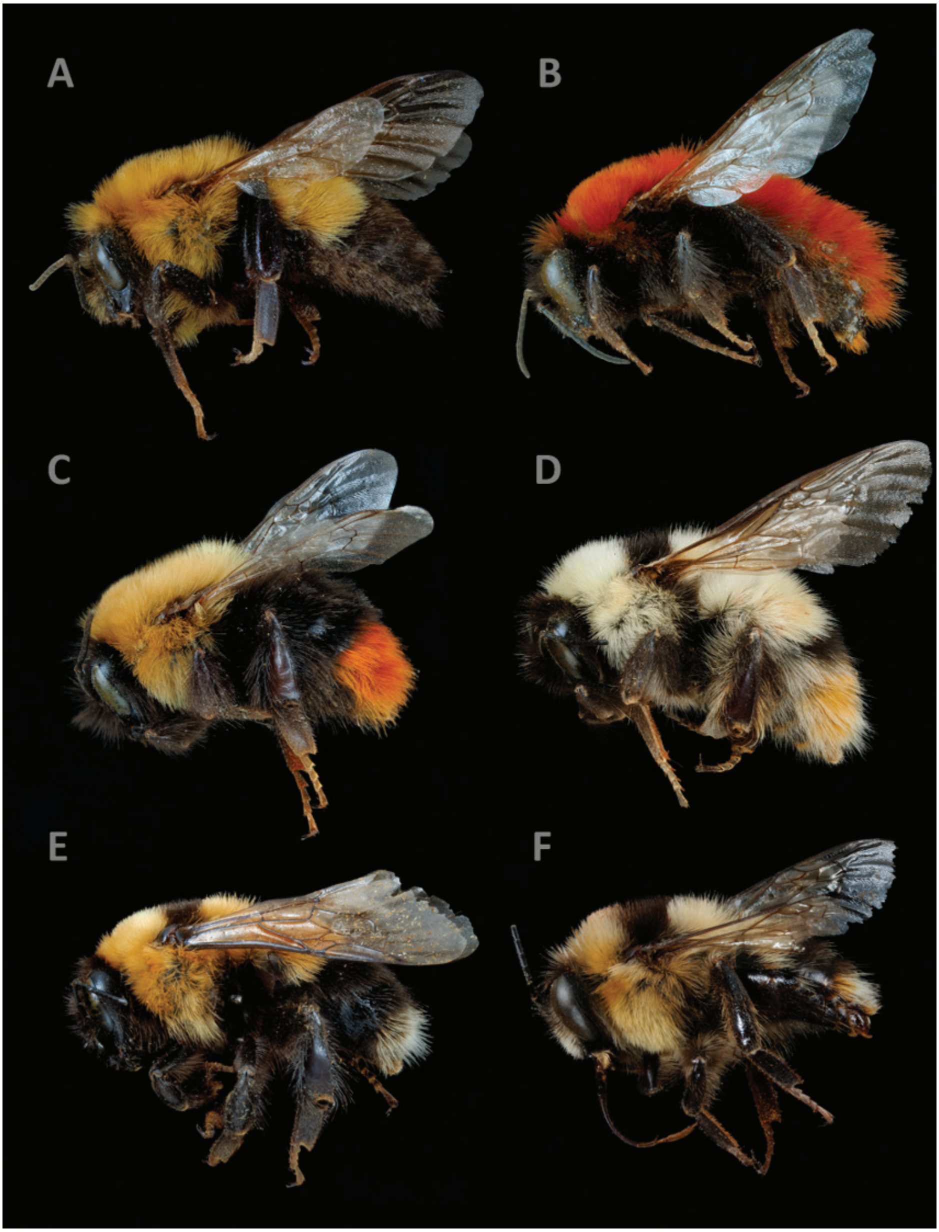

**Figure 2.** Some of the Afghan bumblebee taxa examined as part of this study. **A** *Bombus* (*Subterraneobombus*) *melanurus* **B** *B.* (*Sibiricobombus*) *morawitzi* **C** *B.* (*Melanobombus*) *semenovianus* **D** *B.* (*Melanobombus*) *keriensis s. s.* **E** *B.* (*Sibiricobombus*) *obtusus* **F** *B.* (*Sibiricobombus*) *asiaticus*. Photograph credits P. Rasmont.

**Material examined.** Hindu Kush, Tarest Mts., high valley, 29.viii–3.ix.67, 3250 m, leg. D.K. Mardon, 36°20'N, 69°50'E (2♂♂) (NHMUK) [7]; Shiva-See, 2–5.viii.71, 3100 m, D. Müting (4♂♂) (NHMUK) [10].

**Global distribution.** Palaearctic region and Oriental regions.

### *Bombus* (*Psithyrus*) *ferganicus* (Radoszkowski, 1893)

**Published data.** Tkalců 1968.

**Material examined.** Peniger, R. Bashgal, 4–5.viii.65, 9500 ft., leg. G.W. Johnstone (1♀) [other name: Landay-Sin River, 35.61N, 71.33E].

**Global distribution.** Palaearctic and Oriental regions.

## Subgenus *Pyrobombus* Dalla Torre, 1880

### *Bombus* (*Pyrobombus*) *biroi* Vogt, 1911

**Published data.** Reinig 1940; Tkalců 1968.

**Material examined.** Grosser Pamir, Issiktal, Quellflur in Artemisia-Chenopodiensteppe, 23.viii.75, 3500 m, leg. H. Huss, 37°02'N, 73°20'E (1☿) (UMons) [13]; Shiva-See, 05.viii.71, 3100 m, D. Müting (1☿) (NHMUK) [10].

**Global distribution.** Palaearctic and Oriental regions.

### *Bombus* (*Pyrobombus*) *kotzschi* Reinig, 1940

**Published data.** Reinig 1940; Richards 1951; Tkalců 1968.

**Material examined.** Hindu Kush, Tarest Mts., high valley, 29.viii–3.ix.67, 3250 m, leg. D.K. Mardon, 36°20'N, 69°50'E (1♂, 1☿) (NHMUK) [7].

**Global distribution.** Palaearctic region and Oriental regions.

### *Bombus* (*Pyrobombus*) *subtypicus* (Skorikov, 1914)

**Published data.** Tkalců 1968.

**Material examined.** 3. Danske Exp. Til Centralasien, [manuscript: St.108], ST. [manuscript] Ghilzai, 05.viii.48 (2♂♂), 11.viii.48 in Surfa? (1♂), N. Haarlov (NHMUK) [?]; Ahmad Diwana (Baba), R. Bashgal Valley, 3.viii.1965, 8500 ft., leg. G.W. Johnstone (2♀, 9☿☿) (NHMUK) [16]; E-Afghanistan, Dar-e-Pandjshir, Kotal-e-tal, 30.vii.73, 3800 m, M. N. Khoram (1♂) (UMons) [19]; Grosser Pamir, Darrah-e-Istmotsh (nördl. Zweig), südl. Seitental, 05.viii.71, 4200–4400 m, leg. Ebert and Naumann (1♀) (UMons) [Darya-e Istmotsh: 37.23N, 72.83E]; Hindu Kush, 10.viii.68, 9300 ft., leg. M. Tong (3☿☿); Hindu Kush, nr Kamdesh confluence of R. Suingal and R. Shkurigal, viii.1977, 11000 ft., P.H. Ryley, 35°45'N, 71°15'E (1♂) (NHMUK) [17]; Hindu Kush, Rocky gorge above Lezdi, 17–19.viii.67, 1800–1920 m, leg. D.K. Mardon , 36°20'N, 69°50'E (1♂, 1♀, 7☿☿) (NHMUK) [8]; Hindu Kush, upper Lezdi valley, 21.viii.67, 2240 m, leg. D.K. Mardon, 36°20'N, 69°50'E (1☿) (NHMUK) [7]; NO-Afghanistan, Badachschan, Bala Kuran, 26.vii.1961, 3200 m, leg. G. Ebert (2☿☿) (UMons) [11]; O-Afghanistan, Sarobi, 1.viii.61, 1100 m, leg. G. Ebert (1☿) (UMons) [20]; Peniger, R. Bashgal, 4–5.viii.1965, 9500 ft., leg. G.W. Johnstone (2♀♀, 1☿)

(NHMUK) [other name: Landay-Sin River, 35.61N, 71.33E]; SO-Afghanistan, Safed Koh, S-Seite, Kotkai, 19–23.6.1966, 2350 m (1☿) (UMons) [21].

**Global distribution.** Palaearctic and Oriental regions.

## Subgenus *Bombus sensu stricto* Latreille, 1802

### *Bombus* (*Bombus*) aff. *longipennis* Friese, 1918

**Notes.** New record for Afghanistan.

**Material examined.** Afghanistan, Grosser Pamir, Issiktal, Quellflur in Artemisia-Chenoponiensteppe, 3500 m, 23.viii.1975 (3☿☿); 22.viii.1975 (4☿☿); 17.viii.1975 (1♂) (UMons) [13].

**Global distribution.** Palaearctic and Oriental regions.

### *Bombus* (*Bombus*) *tunicatus* Smith, 1852

**Published data.** Tkalců 1968.

**Material examined.** SO-Afghanistan, Prov. Pastia, Safed Koh, S-Seite, Kotkai, 2350 m, 16–17.vi.1971, rec. Ebert and Naumann, (3♀♀) (UMons) [21]; SO-Afghanistan, Safed-Koh, S-Seite, Kotkai, 2350m, 14–23.vi.1966 (1♀) (UMons) [21].

**Global distribution.** Palaearctic and Oriental regions.

## Subgenus *Melanobombus* Dalla Torre, 1880

### *Bombus* (*Melanobombus*) *keriensis s. s.* Morawitz, 1887

Figure 2

**Published data.** Reinig 1940; Richards 1951; Tkalců 1968.

**Material examined.** Hindu Kush, Tarest Mts., high valley, 29.viii–3.ix.67, 3250 m, leg. D.K. Mardon, 36°20'N, 69°50'E (4☿☿) (NHMUK) [7]; 3. Danske Exp. Til Centralasien, [manuscript: St.108], ST. [manuscript] Ghilzai, 05.viii.48 (3☿☿) (NHMUK) [?]; Grosser Pamir Issiktal, 1975–08 (1♀, 4☿☿), 12.viii.75 (at 4200 m, 1☿), leg. H. Huss (UMons) [possibly close to 13]; Grosser Pamir Issiktal, Salix-bestande, 12.viii.75, 3600 m, leg. H. Huss (3☿☿) (NHMUK) [possibly close to 13]; Grosser Pamir, Darrah-e-Istmotsh (nördl. Zweig), südl. Seitental, 05.viii.71, 4200–4400 m, leg. Ebert and Naumann (2♀♀) (UMons) [Darya-e Istmotsh: 37.23N, 72.83E]; Grosser Pamir, Issiktal, Frostbodenflur, 24.viii.75, 4100–4350 m, leg. H. Huss, (1☿) (UMons) [possibly close to 13]; Grosser Pamir, Issiktal, Quellflur in Artemisia-Chenopodiensteppe, 25.viii.75 (42☿☿), 23.viii.75 (10☿☿), 22.viii.75 (7☿☿); 17.viii.75 (5☿☿), 16.viii.75 (2☿☿), 09.viii.75 (2☿☿), 3500 m, leg. H. Huss, 37°02'N, 73°20'E (UMons) [13]; Hindu Kush, 3.viii.68, 10500 ft., leg. M. Tong (1☿) (NHMUK) [sampling site not possible to locate, given that the Hindu Kush is ~ 800 km long]; Hindu Kush, 6.viii.68, 11500 ft., leg. M. Tong (5☿☿) (NHMUK) [sampling site not possible to locate, given that the Hindu

Kush is ~ 800 km long]; N-Afghanistan, Prov. Badakhshan, Grosser Pamir, Kotal-e-Wazir, 4400m, leg. C. Naumann (4☿☿), 07.viii.71 (2♀♀, 6☿☿, 2♂♂) (UMons) [12]; Salang-Paß, Hindikusch, 13.vii.69, 3500 m, D. Müting (1☿, 2♂♂) (UMons) [5]; Z-Afghanistan, Koh-i-Baba, S-Seite, Shah-tu-Pass, 17–19.vii.1966, 3000m, leg. G. Ebert (2☿☿) (UMons) [reference coordinates of the Koh-i-Baba 34.64N, 67.62E].

**Global distribution.** Palaearctic and Oriental regions.

### ***Bombus* (*Melanobombus*) *semenovianus* (Skorikov, 1914)**

Figure 2

**Published data.** Reinig 1940; Richards 1951; Tkalců 1968.

**Material examined.** Hindu Kush, Tarest Mts., high valley, 29.viii–3.ix.67, 3250m, leg. D.K. Mardon, 36°20'N, 69°50'E (9☿☿) (NHMUK) [7]; Hindu Kush, nr Kamdesh confluence of R. Suingal and R. Shkurigal, viii.1977, 11000 ft., P.H. Ryley, 35°45'N, 71°15'E (2☿☿) (NHMUK) [17]; Hindu Kush, Rocky gorge above Lezdi, 17–19.viii.67, 1800–1920 m, leg. D.K. Mardon, 36°20'N, 69°50'E (18☿☿) (NHMUK) [7]; Hindu Kush, upper Lezdi valley, 21.viii.67, 2240 m, leg. D.K. Mardon, 36°20'N, 69°50'E (6☿☿) (NHMUK) [7]; Hindu Kush, Lezdi, 15–16.viii.67, 1560 m, leg. D.K. Mardon, 36°20'N, 69°50'E (10☿☿) (NHMUK) [8]; 3. Danske Exp. Til Centralasien, [manuscript: St.126], ST. [manuscript] Marak [?]; 16.viii.48, N. Haarlov (2☿☿) (NHMUK) [?]; 3. Danske Exp. Til Centralasien, [manuscript: St.118], ST. [manuscript] Surfa?, 11.viii.48, N. Haarlov (1☿) (NHMUK) [?]; 3. Danske Exp. Til Centralasien, [manuscript: St.124], ST. [manuscript] Marak, 14.viii.48, N. Haarlov (1☿) (NHMUK) [?]; Hindu Kush, 4.viii.68, 8500 ft., leg. M. Tong (1☿) (NHMUK) [sampling site not possible to locate, given that the Hindu Kush is ~ 800 km long]; Hindu Kush, 6.viii.68, 11500 ft., leg. M. Tong (2☿☿) (NHMUK) [sampling site not possible to locate, given that the Hindu Kush is ~ 800 km long]; Hindu Kush, 3.viii.68, 10500 ft. (19☿☿) (NHMUK) [?]; E-Afghanistan, Salang-Nord, 2100 m, vic. Khindjan, 13.vi.70, 2100 m, leg. C. Naumann (1☿) (UMons) [5]; Salang-Paß, Hindikusch, 13.vii.69, 3500 m, D. Müting (4☿☿, 2♂♂) (UMons) [5]; Afghanistan, Salang-Pafs, Nordseite, 17.vi.66, 2650 m, K. Ornoto (1♀) (UMons) [5]; SO-Afghanistan, Safed Koh, S-Seite, Kotkai, 19–23.vi.1966 (1☿) (UMons) [21].

**Global distribution.** Palaearctic region.

### **Subgenus *Sibiricobombus* Vogt, 1911**

### ***Bombus* (*Sibiricobombus*) *asiaticus* Morawitz, 1875 *sensu lato***

Figure 2

**Published data.** Reinig 1940; Richards 1951; Tkalců 1968.

**Material examined.** 3. Danske Exp. Til Centralasien, [manuscript: St.108], ST. [manuscript] Ghilzai, 05.viii.48, N. Harloov (1♀, 2☿☿, 1♂) (NHMUK) [?]; 3. Danske Exp. Til Centralasien, Puistagoli, ST. [manuscript] 106, 02.viii.48, N. Harloov (1♂)

(NHMUK) [?]; Ahmad Diwana (Baba), R. Bashgal Valley, 3.viii.1965, 8500 ft., leg. G.W. Johnstone (1♀, 1☿) (NHMUK) [16]; C-Afghanistan, Prov. Bamian, Ghorghori-e-Panjao, Gaukhana, 11.vii.76, 2800 m, leg. C. Naumann (1☿) (UMons) [3]; C-Afghanistan, Prov. Ghor, Kotal-e-Narges, West-Ste; D.-e-Godar, 09.vii.76, 3100–3200 m, leg. C. Naumann (1♀, 3☿☿) (UMons) [2]; Grosser Pamir, Darrah-e-Istmotsh (nördl. Zweig), südl. Seitental, 05.viii.71, 4200–4400 m, leg. Ebert and Naumann (1♂) (UMons) [Darya-e Istmotsh: 37.23N, 72.83E]; Afghanistan, Grosser Pamir, Issiktal, Quellflur in Artemisia-Chenoponiensteppe, 3500m, 22.viii.1975 (1☿), 25.viii.1975, leg. H. Huss (1♀, 2☿☿, 1♂) (UMons) [13]; Hindu Kush, 8.viii.68, 9500 ft. (1♂), 3.viii.68 (6☿☿; 4♂♂), 6.viii.68 (1♀), leg. M. Tong (NHMUK) [sampling site not possible to locate, given that the Hindu Kush is ~ 800 km long]; Hindu Kush, Rocky gorge above Lezdi, 17–19.viii.67, 1800–1920 m, leg. D.K. Mardon, 36°20'N, 69°50'E (2☿☿, 3♂♂) (NHMUK) [8]; Hindu Kush, Tarest Mts., high valley, 29.viii–3.ix.67, 3250 m, leg. D.K. Mardon, 36°20'N, 69°50'E (1☿) (NHMUK) [7]; Hindu Kush, upper Lezdi valley, 21.viii.67, 2240 m, leg. D.K. Mardon, 36°20'N, 69°50'E (1♂) (NHMUK) [7]; Kl. Pamir, Seitental südl. W-Ende des Kol-e-Tshagmagtin, 20.vii.1971, 4200–4400 m, leg. Ebert and Naumann (1☿) (UMons) [?]; O-Afghanistan, Salang-Paß, N-Seite (Khinjan), 5–11.vii.1966, 2200 m, leg. G. Ebert (1♂) (UMons) [5]; SO-Afghanistan, Prov. Paktia, Safed Koh, S-Seite, Kotkai, 16/17.6.1971, 2350 m, leg. Ebert and Naumann (2☿☿) (UMons) [21]; SO-Afghanistan, Safed Koh, S-Seite, Kotkai, 19–23.6.1966, 2350 m (1☿) (UMons) [21]; Z-Afghanistan, Koh-i-Baba, S-Seite, Shah-tu-Pass, 17–19.vii.1966, 3000 m, leg. G. Ebert (1☿, 1♂) (UMons) [reference coordinates of the Koh-i-Baba 34.64N, 67.62E].

**Global distribution.** Palaearctic and Oriental regions.

### ***Bombus* (*Sibiricobombus*) *morawitzi* Radoszkowski, 1876**

Figure 2

**Published data.** Reinig 1940; Tkalců 1968.

**Material examined.** Grosser Pamir, Issiktal, Frostbodenflur, 24.viii.75, 4100 m, leg. H. Huss, 37°02', 73°20' (2♂♂) (UMons) [13]; Wakhan-Tal, Kotal-e-Dalez, W-Seite, 09.vii.71, 3200–3400 m, leg. Ebert and Naumann (1☿) (UMons) [around the Wakhan corridor in the NE of Afghanistan: 37.09N, 73.63E].

**Global distribution.** Palaearctic region.

### ***Bombus* (*Sibiricobombus*) *obtusus* Richards, 1951**

Figure 2

**Published data.** Richards 1951; Tkalců 1968.

**Material examined.** Hindu Kush, 10.viii.68 at 9300 ft. (1♂, 2☿☿), 8.viii.68 at 9500 ft. (2☿☿), leg. M. Tong (NHMUK) [sampling site not possible to locate, given that the Hindu Kush is ~ 800 km long]; 3. Danske Exp. Til Centralasien, [manuscript:

**Table 2.** Species examined as part of the present study (✓ = taxon examined in the present study; X = taxon reported in Afghanistan but not examined).

| Subgenus(following Williams et al. 2008) | Taxon (according to the latest revisions of Tkalců 1968, Williams et al. 2016, and the present work) | Taxonomic status according to the types examined by PH Williams (https://www.nhm.ac.uk/research-curation/research/projects/bombus/) | Present study |
|---|---|---|---|
| *Mendacibombus* | *afghanus* | Junior synonym of *B. marussinus* | |
| | *makarjini* | Valid | ✓ |
| | *marussinus* | Valid | ✓ |
| | *turkestanicus* | Valid | ✓ |
| *Subterraneobombus* | *melanurus* | Valid | ✓ |
| | *subdistinctus* | Junior synonym of *B. melanurus* | |
| *Psithyrus* | *branickii* | Valid | ✓ |
| | *ferganicus* | Valid | ✓ |
| | *morawitzianus* | Valid | X |
| *Pyrobombus* | *biroi* | Valid | ✓ |
| | *kotzschi* | Valid | ✓ |
| | *subtypicus* | Valid | ✓ |
| *Bombus s. str.* | *lucorum jacobsoni* | *B. jacobsoni* | X |
| | *tunicatus* | Valid | ✓ |
| | aff. *longipennis* | Uncertain taxonomic status | ✓ |
| *Melanobombus* | *keriensis* | Valid | ✓ |
| | *incertoides* | Valid | X |
| | *semenovianus* | Valid | ✓ |
| *Sibiricobombus* | *asiaticus* | Referred to here as the accepted taxon *B. asiaticus s. l.* | ✓ |
| | *miniatocaudatus* | | |
| | *longiceps* | | |
| | *morawitzi* | Valid | ✓ |
| | *obtusus* (sspp. *badakshanensis* and *obtusus*) | Valid | ✓ |
| *Cullumanobombus* | *cullumanus serrisquama* | Valid | ✓ |

St.108], ST. [manuscript] Ghilzai, 05.viii.48, N. Haarlov (1☿) [?]; 3. Danske Exp. Til Centralasien, [manuscript: St.126], ST. [manuscript] Marak ; 16.viii.48, N. Haarlov (3☿☿) (NHMUK) [?]; 3. Danske Exp. Til Centralasien, [manuscript: St.124], ST. [manuscript] Marak, 14.viii.48, N. Haarlov (2♂♂) (NHMUK) [?]; C-Afghanistan, Prov. Ghor, Kotal-e-Narges, West-Ste; D.-e-Godar, 09.vii.76, 3100–3200 m, leg. C. Naumann (4♀♀) (UMons) [2]; Afghanistan Centr., Prov. Bamian, Koh-e-Shorakarak, vic. Samadi, 12.vii.76, 3200 m, leg. C. Naumann (1☿) (UMons) [reference coordinates of Kōh-e Shōrah Kharak: 34.72N, 67.09E].

**Global distribution.** Palaearctic region.

## Subgenus *Cullumanobombus* Vogt, 1911

### *Bombus* (*Cullumanobombus*) *cullumanus* (Kirby, 1802)

**Published data.** Richards 1951; Tkalců 1968.

**Material examined.** C-Afghanistan, Prov. Bamian, Ghorghori-e-Panjao, Gaukhana, 11.vii.76, 2800 m, leg. C. Naumann (1♀) (UMons) [3]; Hazaradjat, Koh-i-Baba, Shah-tu-Kotal, 20–21.vi.1961, 4000 m, leg. G. Ebert (1♀) (UMons) [reference coordinates of the Koh-i-Baba 34.64N, 67.62E]; Z-Afghanistan, Koh-i-Baba, S-Seite, Shah-tu-Pass, 17–19.vii.1966, 3000 m, leg. G. Ebert (1♀, 4☿☿) (UMons) [reference coordinates of the Koh-i-Baba 34.64N, 67.62E].

**Global distribution.** Palaearctic region.

## Discussion

While the bumblebee fauna of Western and Central Asia has received substantial attention within the last several decades, most notably in the Middle-East (Rasmont and Flagothier 1996; Özbek 1997, 1998, 2000; Monfared et al. 2008, 2009; Boustani et al. 2020) and in the Himalaya (Williams 1991; Williams et al. 2010; Streinzer et al. 2019), Afghanistan however has remained under-studied due to the effects of long-standing effect of human conflict on scientific work in the country.

Most identified specimens in the present work were collected at high elevation in the Afghan Pamir (Wakhan Corridor) and Hindu-Kush mountains (Fig. 1). Bumblebees are indeed regarded as species adapted to cool climates and are especially diversified in montane areas (Williams 1991; Williams et al. 2010; Iserbyt and Rasmont 2012; Rasmont et al. 2015). This habitat preference is explained by the species of the genus being able to (i) thermoregulate efficiently in cold environments (Heinrich 1979), (ii) utilize thermally insulted underground nests built by other inhabitants (e.g., small mammals) and (iii) overwinter with very low food requirements. The Pamir mountain range offers a very hospitable habitat for bumblebees, providing suitable habitat and host plants, including long-corolla flowering plants such as Fabaceae, Scrophulariaceae and Boraginaceae, which have been shown to be attractive for the indigenous *Bombus* species (Reinig, 1930). In the same study, the latter author highlights the short summer period suitable for bumblebees in the Pamir region, from July at 4000 m to September in the Kara Kul Lake (Tadjik Pamir). These observations are congruent with ours, with most specimens being recorded in the month of August. Williams (1991) makes parallel comments for Kashmir, on the other side of the northeastern Afghanistan panhandle, where a short annual season above the freezing point constraints the time available for bumblebee colony development.

Moreover, Reinig (1930) underscores the rarity of the specimens from the subgenus *Psithyrus*, recording only three specimens of the 1,350 that he caught in the Russian-German expedition he joined in 1928. Our records therefore provide interesting data to improve understanding of the phenology of these rare bumblebees, whose inquiline-host associations can sometimes be speculative or rely on uncertain, old records (Williams 2008; Lhomme and Hines 2019). One of the cuckoo bumblebee species recorded here, *B. branickii*, is suggested to be a social parasite of *B.* (*Melanobombus*) *keriensis* (Williams et al. 1991, 2009). Due to the geographical proximity of the collecting sites of both *B. banickii* and *B. keriensis*, we provide additional evidence of a probable host-inquiline association of the two species.

Gupta (2004) reports *B. terrestris* (based on one female only) and *B. lucorum* as occurring in Afghanistan. However, the author treated the morphologically similar species *B. jacobsoni* as a synonym of *B. lucorum*, whereas they are now considered to be distinct species (Williams et al. 2012). Separating species in the *B. terrestris* complex based on morphology and color pattern is an arduous task, likely to be unreliable in most cases, especially for workers or discolored males (Rasmont 1984; Carolan et al. 2012; Williams et al. 2012). In the Pamir range, many taxa of this group have been reported: *B. lucorum alaiensis* (described in Reinig 1930), *B. lucorum* (reported in Reinig 1940) and *B. lucorum jacobsoni* (reported by Tkalců 1968). The latter taxon, *jacobsoni* Skorikov (1912), is now strongly supported to represent a separate species and is presently reported as endemic to Kashmir (Williams et al. 2012). The status of the taxon *alaiensis* Reinig (1930) remains unclear but could be a synonym of *lucorum* (Williams et al. 2012). Regarding our specimens that appear very similar to *B. lucorum sensu lato*, preliminary DNA sequencing efforts with the UCE approach (to be presented in a later study), indicate that the present individuals are more closely related to the *B. longipennis* species complex than *B. lucorum*. We therefore have decided to assign the name *B.* (*Bombus*) aff. *longipennis* to these specimens until further work (e.g., in neighboring regions) can clarify the situation.

Although we did not examine all of the bumblebee collections of Tkalců, Reinig, or Richards, the previous records of *B.* (*Melanobombus*) *incertoides* could correspond to specimens of *B. keriensis s. str.*, according to the ongoing global revision of *Melanobombus* (Williams et al. in prep) that suggests that the taxon *B. incertoides* is only present in Mongolia.

Studies addressing the taxonomic relationships of the examined taxa will constitute an essential starting point for further revisions of the Afghan fauna. Highly polymorphic species complexes such as *B. asiaticus* remain enigmatic due to morphological convergence, and many others have not even been collected recently enough to be added in the latest comprehensive phylogeny of world bumblebees (Cameron et al. 2007) or even barcoded (e.g., all the Afghan *Pyrobombus* and *Psithyrus* species). Uncertainties also remain for the specimens of the cryptic yellow-banded *Bombus sensu stricto* complex, despite an extensive revision of the subgenus having been performed at the world scale (Williams et al. 2012). Cephalic labial secretions, now studied from dozens of species (e.g., Brasero et al. 2018a, b, Valterová et al. 2020) are totally unknown for all Afghan taxa. Furthermore, while large-scale meta-analyses on numerous bumblebee species have been performed by gathering old and recent material in the Nearctic and West-Palearctic regions (e.g., Kerr et al. 2015; Rasmont et al. 2015), the very scarce Afghan data coming from museum collections and the total absence of recent surveys makes the establishment of such assessments and conservation measures totally impracticable. Collection of fresh material preserved in adequate conditions and more extensive studies of museum collections are therefore essential to fully describe Afghan species and to protect them from the various factors causing declines that impact the genus throughout the world. Above all, we hope that the present study and database will encourage further work on the rich fauna and flora of Afghanistan.

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## Supplementary material 1

**Table S1. Bumblebee records examined in the present study**

Authors: Guillaume Ghisbain, Paul H. Williams, Denis Michez, Michael G. Branstetter, Pierre Rasmont

Data type: species data

Link: https://doi.org/10.3897/zookeys.973.54796.suppl1

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# A remarkable new species of the rove beetle genus *Anthobium* Leach, 1819 from Eocene Baltic amber (Coleoptera, Staphylinidae, Omaliinae)

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## Abstract

An unusual new omaliine species, *Anthobium alekseevi* **sp. nov.**, is described and illustrated from Eocene Baltic amber, tentatively placed in the megadiverse genus *Anthobium* Leach, 1819. A new monotypic species-group is established. The new species can easily be distinguished from other species of the genus by the larger body, shape of the subrectangular pronotum, and the presence of a median carina on the prosternum and large, subtriangular tooth on the inner side of each mesotibia, likely exhibiting a peculiar sexual dimorphism in the male. Based on the study of the specimen with support of microtomographic images, a brief comparative analysis of a new species with described extant species of *Anthobium* is provided.

## Keywords

*Anthobium*, Anthophagini, fossil, Omaliini, palaeontology, sexual dimorphism, x-ray micro-CT

## Introduction

The rove beetles of the subfamily Omaliinae, with about 1700 species in 118 extant and 14 extinct genera, are distributed in northern temperate areas, with greatest diversity in the Holarctic region. Omaliines are common in various types of biotopes. An overwhelming number of species are hygrophiles, and they can be found near swamps, banks of rivers, mountain streams at high elevations, etc. Most species are predators of small invertebrates or are sapro- and mycophagous, and some species are even pollen-feeders (e.g. *Amphichroum* Kraatz, 1858).

The fossil history of Omaliinae was briefly discussed by Chatzimanolis (2018) and in more detail by Shavrin and Yamamoto (2019). Several extinct taxa were described based on impressions from the Jurassic (Tikhomirova 1968; Ryvkin 1985; Cai and Huang 2013), Cretaceous (Ryvkin 1990), or Cenozoic eras (Scudder 1900). Recently, seven species in three tribes of Omaliinae were reported from Eocene Baltic amber (Zanetti et al. 2016; Shavrin and Yamamoto 2019), but only one of them, *Geodromicus balticus* Shavrin & Yamamoto, 2019, belongs to the tribe Anthophagini.

Due to the poor visibility of several main details of the body, the single specimen of a possible omaliine, as an inclusion within a piece of the Baltic amber from the collection of V. Alekseev (Kaliningrad), was difficult to attribute to any taxon, and therefore it was not included in our last study on fossil omaliines (Shavrin and Yamamoto 2019). However, we recently obtained microtomographic images of this specimen, which allowed us not only to ascertain that it belongs to the subfamily Omaliinae but even to assign to the tribe Anthophagini. Based on the combination of morphological characters such as the general shape of the convex body, shapes of two preapical palpomeres of maxillary palps, the presence of postocular ridge, the shape of gular structures, located close together, the structure of the pronotum with deep mediolateral pits, and explanate lateral portions, this species clearly belongs to the *Anthobium* group of genera (e.g. Campbell 1987; Shavrin and Smetana 2017). This group contains 11 extant genera distributed in the Holarctic region: *Acidota* Stephens, 1829, *Anthobioides* Campbell, 1987, *Anthobiomorphus* Shavrin & Smetana, 2020, *Anthobium* Leach, 1819, *Arpedium* Erichson, 1839, *Camioleum* Lewis, 1893, *Caucanthobium* Assing, 2018, *Deinopteroloma* Jansson, 1946, *Deliphrosoma* Reitter, 1909, *Deliphrum* Erichson, 1839, and *Olophrum* Erichson, 1839. In addition, this group contains two extinct genera, which were described on impressions: Mesozoic *Mesodeliphrum* Ryvkin, 1990 from Turga, Transbaikal Russia (Ryvkin 1990) and *Sinanthobium* Cai & Huang, 2013 from the Middle Jurassic Jiulongshan Formation at Daohugou, Inner Mongolia, China (Cai and Huang 2013). Based on the shape of the body, features of sculpture of the forebody, the presence of long grooves in front of the ocelli (tentorial pits), the proportions of palpomeres of the maxillary palp, and the structure of the setation of the meso- and metatibia, the new species can be tentatively attributed to the megadiverse genus *Anthobium*. The senior author is currently actively exploring this genus and has published already several papers on a few established groups of species from Eastern Palaearctic Region (Shavrin and Smetana

2017, 2018, 2019; Shavrin 2020). To date, 70 species (plus four *nomina dubia*) of the genus are known from the Holarctic Region: 19 species from the western and 38 from the eastern Palaearctic regions, and 13 species from the Nearctic Region. The revision of the entire genus is still in progress and the extant taxonomic diversity has not yet been fully revealed, as several groups and species are still awaiting to be described and its phylogeny thoroughly investigated. Meanwhile, despite some morphological differences from other known taxa, the new species is nevertheless assigned to *Anthobium*, as a separate group of species. The new species represents the second species of Anthophagini known from Eocene Baltic amber. The obtained palaeontological data will undoubtedly be useful in constructing possible phylogenetic relationships in the *Anthobium* group of genera in the future.

## Materials and methods

The studied material is housed in the private collection of Vitalii I. Alekseev (Kaliningrad, Russia) and eventually will be deposited in Borissiak Paleontological Institute of the Russian Academy of Sciences, Moscow, Russia (PIN). This piece of amber was collected by net in the Baltic Sea (Kaliningrad Area, Russia) in the beginning of 2017. It was polished, embedded in a block of GTS-polyester resin, and polished again according the method of Hoffeins (2001).

All measurements are given in millimeters and were made with a stereoscopic microscope equipped with an ocular micrometer. Measurements were made from the dorsal side of the specimen except for ocular length and width of the abdomen which were made from the ventral side. Measurement of the total length of the body was difficult to do because of the specimen's orientation within the amber piece; the resulting approximate values are marked with "~". The type labels are cited in inverted commas and separated from each other by a comma, different lines in labels are separated with '|'; explanations of the type labels are given in square brackets, necessary notes within the label are given in angle brackets.

The specimen was examined using a Nikon SMZ 745T stereomicroscope. A Sony Alpha DSLR-A300digital camera was used for photographs of amber, habitus, and its details. Micro-CT observations of the specimens were conducted at the Daugavpils University (Daugavpils, Latvia) using Zeiss Xradia 510 Versa system. Scans were performed with a polychromatic x-ray beam at an energy of 40 kV and power of 3 W. Sample-detector distance was set to 17.6 mm and source to sample distance 32.6 mm. Tomographic slices were generated from 3001 rotation steps through a 360-degree rotation, using a 4× objective, and exposure time during each projection was set to 3 s. Acquired images were binned (2 × 2 × 2) giving a voxel size of 4.3 µm. Images were imported into Dragonfly PRO (ver. 4.1) software platform for interactive segmentation and 3D visualization. Prior to the full scan a 23-minute warmup scan was conducted with the same scan parameters except rotation steps which had been reduced to 201 and exposure time which was reduced to 1 s.

## Systematic palaeontology

### Order Coleoptera Linnaeus, 1758
### Family Staphylinidae Latreille, 1802
### Subfamily Omaliinae MacLeay, 1825
### Tribe Anthophagini Thomson, 1859

### Genus *Anthobium* Leach, 1819

**Type species.** *Omalium atrocephalum* Gyllenhal (= *Silpha melanocephalum* sensu Marsham), for details see Herman (2001).

### *Anthobium alekseevi* group of species

**Diagnosis.** Body medium-sized; forebody convex, shiny; apical segment of maxillary palp twice as long as preceding segment; anterior angles of subrectangular pronotum slightly protruded anteriad, mediobasal third of pronotum with oval impression; lateral edges of pronotum without crenulation; prosternum with distinct median carina; surface of elytra without elevations; inner side of each mesotibia with large tooth in middle.

**Species included.** †*Anthobium alekseevi* sp. nov.

**Remarks.** *Anthobium alekseevi* sp. nov. differs from the remaining species of the genus by the larger body, the shape of the subrectangular pronotum, the presence of distinct median carina on the prosternum, and the presence of a large median tooth on the inner side of the mesotibia.

### †*Anthobium alekseevi* Shavrin & Yamamoto, sp. nov.

http://zoobank.org/A1797770-BE2C-4B9A-99B1-5D93D54B7943
Figures 1–19

**Type material examined. Holotype:** male, complete specimen as inclusion in a piece of small yellow Baltic amber, 11.0 mm × 0.7 mm × 0.5 mm in size (Figs 1, 2), with glued small paper on side of an amber labeled “AWI148” and additional labels within a plastic envelope: “AWI-148 | Omaliinae | gen. nov. | (mesotibia!)” <handwritten>, “**HOLOTYPE** | *Anthobium* | *alekseevi* sp. nov. | Shavrin A. & Yamamoto S. des. 2020” <red rectangular label, printed> (PIN).

**Preservation.** The specimen is poorly visible because it is partially covered with white microbubbles, and some details of the structure of the body are not visible: head, median portion of pronotum and scutellum, ventral side of the body and abdomen (Figs 1, 2). The basal part of the abdomen ventrally is covered by left hind wing.

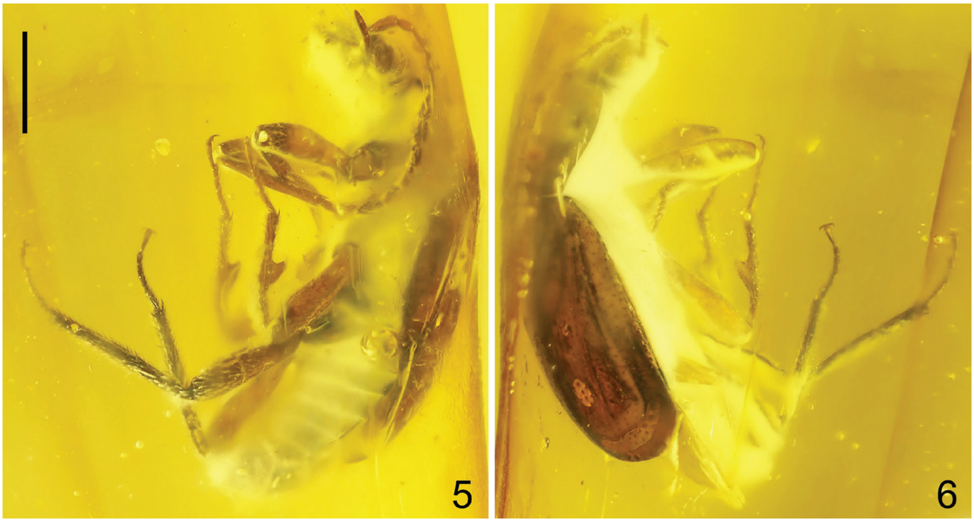
**Locality and horizon.** Baltic amber from Kaliningrad Area, westernmost Russia; mid-Eocene (ca 44 Ma; Wappler 2005).

**Figures 1–4.** *Anthobium alekseevi* sp. nov. **1, 2** amber specimen with inclusion **3** habitus, dorsal view **4** habitus, dorsoventral view. Scale bar: 1.0 mm (**3, 4**).

**Description.** Measurements: maximum width of head including eyes: 1.30; length of head (from base of labrum to neck constriction along head midline in dorsal view): 0.75; ocular length: 0.40; length × width of segments III and IV of maxillary palpi: III: 0.15 × 0.10, IV: 0.30 × 0.10; length of antenna: 2.70; length of pronotum: 1.35; maximum width of pronotum: 1.75; sutural length of elytra from apex of scutellum to posterior margin of sutural angle: 2.60; length of elytron from basal to apical margin: 2.95; maximum width of elytra: 2.30; length of metatibia: 1.60; length of metatarsus: 0.80; maximum width of abdomen (at segment IV): 2.10; total length (from anterior margin of clypeus to apex of abdomen): ~5.40.

Body oblong, moderately wide, shiny (Fig. 3); body laterally as in Figures 5, 6, 9, and 10; body dorsolaterally as in Figure 4; body ventrally as in Figures 11 and 12; forebody as in Figure 14. Body and antennomeres 3–11, brown; legs and mouthparts reddish-brown; antennomeres 1 and 2 yellow-brown.

Head transverse (Figs. 15, 16), 1.7 times as wide as long; anterior portion of frons with obliquely elevated supra-antennal prominences and moderately wide impressions behind them; middle portion slightly elevated, with distinct, deep, narrow dorsal tentorial pits (grooves) in front of ocelli, diagonally stretching apicad to level of middle length of eyes; basal portion with distinct, narrow impression between ocelli; postocular ridge distinct, acute, located relatively close to posterior margin of eye, if see laterally (Figs 5, 9, 18). Eyes large, convex. Ocelli large, situated at about level of postocular ridges; distance between ocelli slightly shorter than distance between ocellus and posterior margin of

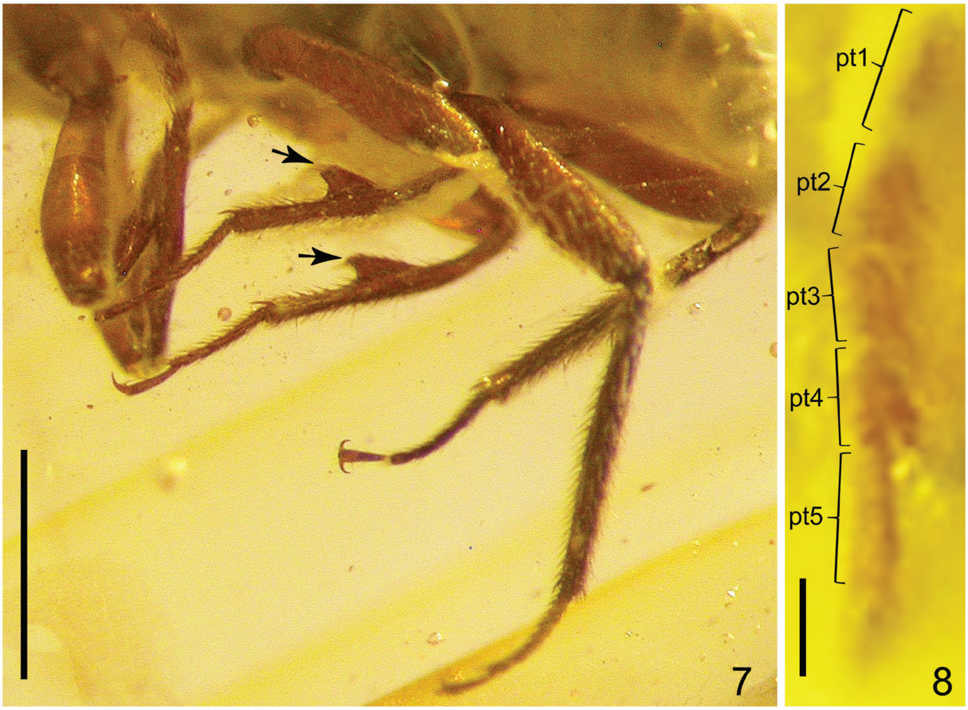


**Figures 5, 6.** *Anthobium alekseevi* sp. nov., habitus (lateral view). Scale bar: 1.0 mm.

eye (Figs 15, 16). Labrum wide, transverse. Mentum and labium narrow, with distinctly elongate apical labial palpomere (Fig. 12). Preapical segment of maxillary palpus moderately long, 1.5 times as long as wide, and as wide as apical segment; apical segment about twice as long as preceding segment, from middle gradually narrowing toward apex (Figs 5, 11, 18). Gular sutures with shortest distance located at level of posterior third of eyes (Fig. 12). Antenna moderately short, exceeding apical third of elytra, with elongate antennomeres 5–10 and long preapical setae, antennomeres 3–11 covered by dense pubescence; basal antennomere moderately wide, 1.5 times as long as 2, antennomere 2 ovoid, about twice as long as wide and slightly shorter than 3, 3 and 4 as wide as 2, 5–7 slightly shorter than 4, 8 and 9 slightly shorter than 7, 10 distinctly shorter than 9, apical antennomere 1.6 times as long as 10, from middle gradually narrowed toward apex (Fig. 3).

Pronotum subrectangular, 1.2 times as wide as long, 1.3 times as wide as head, widest in middle, evenly rounded both anteriad and posteriad (Fig. 14); apical margin rounded, distinctly narrower than posterior margin; anterior angles widely rounded, slightly protruded anteriad; posterior angles obtuse; lateral edges bordered, without visible crenulation; disc with middle portion widely elevated, with indistinct, wide, semioval impression on mediobasal third, and lateral portions moderately wide, slightly explanate, each with deep pit in middle (Fig. 16). Laterobasal and basal portions of pronotum with dense and fine punctation (Fig. 3). Pronotal hypomeron and postcoxal process well developed; intercoxal process elongate and moderately wide; prosternum with distinct median carina (Figs 11, 18). Scutellum large and wide, with rounded apex. Metaventrite wide, convex.

Elytra convex, slightly longer than wide, about twice as long as pronotum, indistinctly widened in middle, reaching basal margin of abdominal tergite VI, with widely rounded apical margins (Fig. 17); shoulders of elytra rounded; lateral portions narrow,

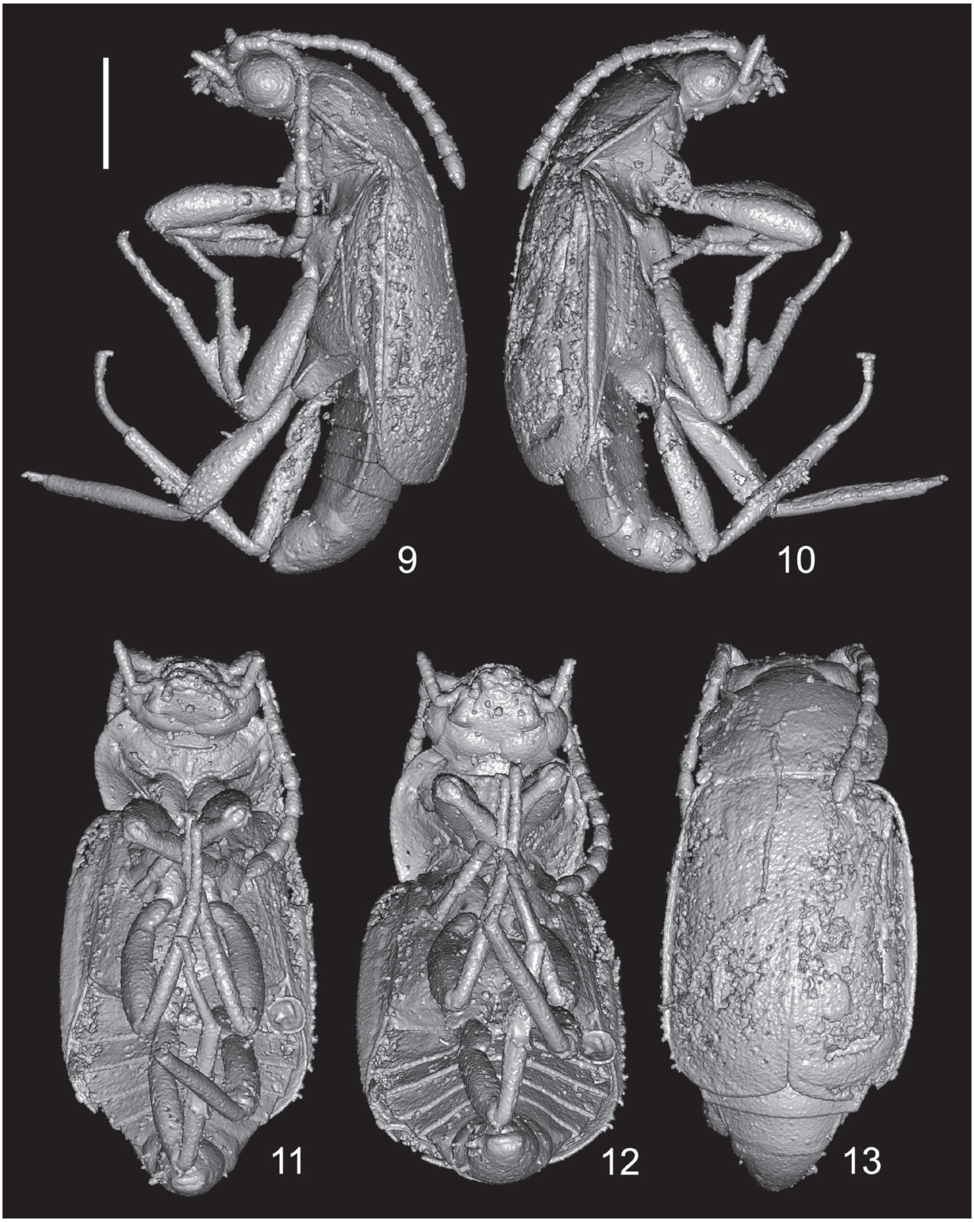


**Figures 7, 8.** *Anthobium alekseevi* sp. nov. **7** legs **8** protarsus. Scale bars: 1.0 mm (**7**); 0.1 mm (**8**). Abbreviations: pt1–pt5 = protarsomeres 1–5.

explanate; surface of elytra without visible elevations. Punctation sparse and moderately small, each elytron with longitudinal rows of vague serial punctures in middle (Fig. 3). Hind wings fully developed.

Legs long (Fig. 7); procoxae wide, protruding ventrad, contiguous; mesocoxae large, convex; metacoxae strongly transverse; pro-, meso-, and metatrochanter relatively narrow, elongate (Figs 11, 12); all femora widest at about middle, profemora slightly wider than meso- and metafemora; pro- and mesotibiae about as long as femora; protibiae covered with dense long setae; protarsomeres 1–5 as in Fig. 8; mesotibiae indistinctly curved in middle, inner side of each mesotibia with large and wide subtriangular, fin-shaped tooth in middle (Fig. 7, *arrows*); meso- and metatibiae covered by very dense, long and strong setae (including apex of tooth on each mesotibia); metatibiae with additional very long setae; metatibiae distinctly longer than metafemora, slightly widened in about middle, covered by dense, strong and long setae, with a few additional spines around apical margin; all tarsi 5-segmented, all tarsi combined shorter than tibia; tarsomeres 1–4 with long and dense lateral setation; apical tarsomere about as long as preceding three tarsomeres together; tarsal claws simple, without modifications (Fig. 7).

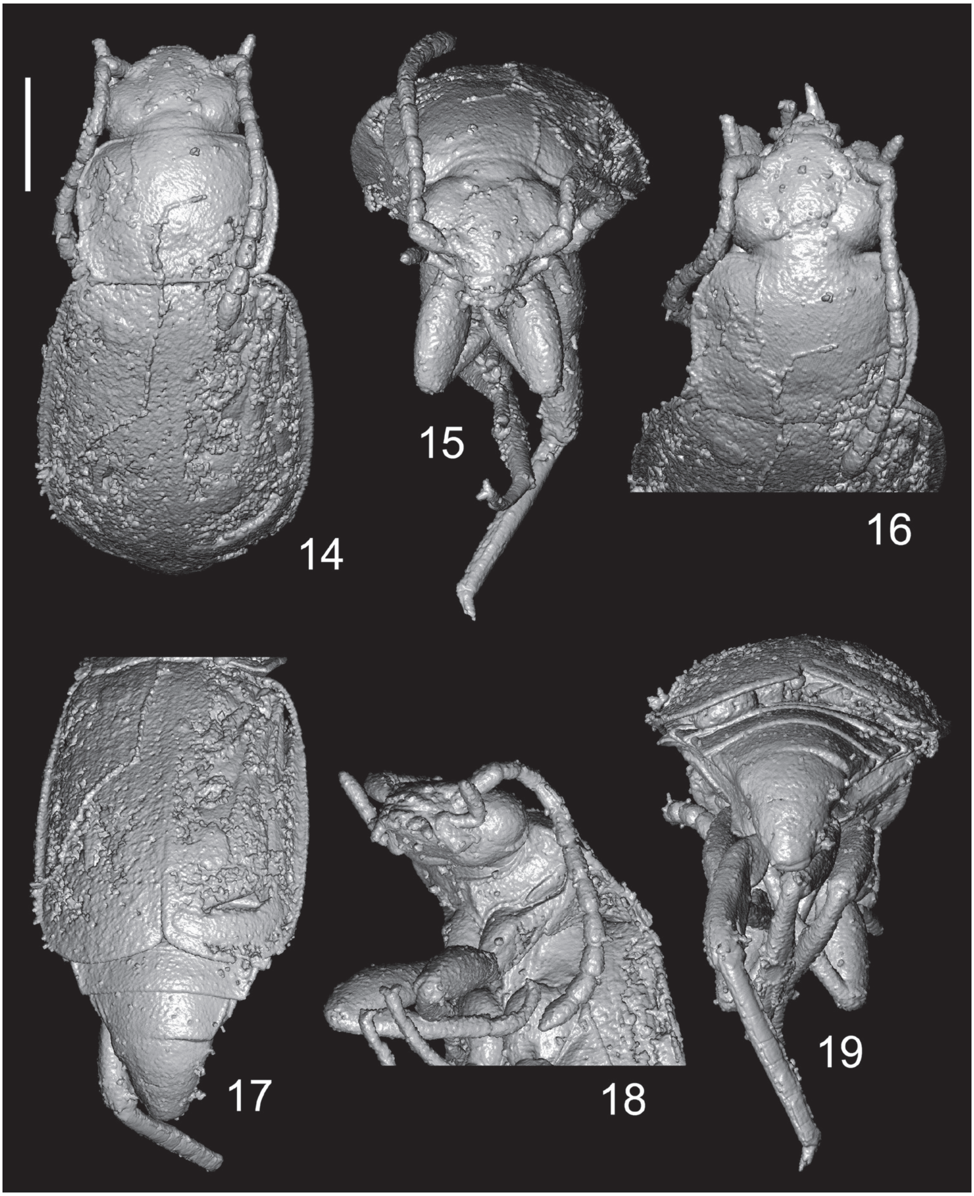
Abdomen distinctly narrower than elytra (Fig. 17), from segment IV significantly narrowing apicad (Fig. 11); abdominal segment IX elongate. Abdomen ventrally as in Figures 11 and 12.



**Figures 9–13.** Habitus of *Anthobium alekseevi* sp. nov. **9, 10** lateral view **11, 12** ventral view **13** dorsal view. Scale bar: 1.0 mm.

Male. Protarsomeres 1–4 distinctly wide, with very long lateral setae (Fig. 8). Apical margin of abdominal tergite VIII rounded (Fig. 17). Apical margin of sternite VIII sinuate (Fig. 12).

Female unknown.



**Figures 14–19.** *Anthobium alekseevi* sp. nov. **14** forebody, dorsal view **15** head, apical portion of pronotum and legs, frontal view **16** head, pronotum and antenna, dorsal view **17** elytra and abdomen, dorsal view **18** head and thorax, lateroventral view **19** apical portion of elytra, abdomen and legs, posterodorsal view. Scale bar: 1.0 mm.

**Etymology.** Patronymic, the species is named to honor our colleague Vitalii I. Alekseev (Kaliningrad), great contributor to the knowledge of the fossil beetle fauna from Baltic amber.

## Discussion

In general, *A. alekseevi* sp. nov. can be characterized by the similar coloration and shape of the convex, shiny, and glabrous body, as in many species of *Anthobium*. The punctation of the forebody is poorly visible except in the lateral and basal portions of the pronotum and elytra; the median portion of the elytra bears longitudinal rows of punctures. The pronotum is shiny, and the microsculpture between punctures is missing. Similar punctation on the shiny forebody is specific for some East Asiatic species groups (e.g. *gracilipalpe* and *nigrum*). Similar to many species of the genus, the head of *A. alekseevi* sp. nov. has distinct median elevation and elongate grooves in front of the ocelli. Unfortunately, each latero-apical portion of the head is hidden by adjoining basal anttennomeres, so the presence and shape of an antocular identation are invisible. Usually, the antocular identation of *Anthobium* is variable in its shape: some species have distinct and semicircular notch as figured by Zanetti (1987: fig. 73a), and many species from Asia have a smooth or very indistinct notch. The shapes of the two preapical palpomeres of the maxillary palpus of *A. alekseevi* sp. nov. are similar to that of many Asiatic congeners. These palpomeres are approximately equal in their width, a characteristic shared in common with almost all the other species of the genus. The shape of the antennomeres of *A. alekseevi* sp. nov. is as usual for *Anthobium*, with the elongate antennomeres 5–10 recalling some species from Asia (e.g. *Anthobium daliense* Shavrin & Smetana, 2017, species of the *morchella* group). The presence of a distinct mediobasal impression on the pronotum is similar to that of some Eastern Palaearctic species. On the contrary, the smooth margins of the elytra without distinct crenulation is suggestive of species from the Western Palaearctic Region. The microtomography of the specimen has not shown the presence of the aedeagus within the abdomen. Usually, species of *Anthobium* have the median lobe variable in width, narrow and long parameres, and a simple internal sac, sometimes with sclerotized additional structures.

*Anthobium alekseevi* sp. nov. has several significant morphological features that distinguish it from other known species of the genus, which led us to propose a new, separate species group for it. The first peculiar feature is the large body of the new species, which is roughly 5.4 mm long. So far, the largest specimens of *Anthobium* have been 4.75 mm long: Himalayan *A. nigrum* (Cameron, 1924) and Chinese *A. puetzi* Shavrin & Smetana, 2017. On average, the body length of known species of the genus varies from 3.0 to 4.0 mm, and the smallest species is the Chinese *A. liliputense* Shavrin & Smetana, 2018, with specimens as small as 1.8 mm in body length. The second peculiar feature is the shape of subrectangular pronotum (Fig. 17). The pronotum of known species of *Anthobium* is distinctly transverse, and the minimum width of it in some species is usually 1.4 times as wide as long. The third peculiar one is the presence of a distinct median carina on the prosternum (Figs 11, 18). Most species of *Anthobium* have no similar structure on the prosternum. A similar structure can be found in some species of *Arpedium*, which have an indistinct carina-like elevation in the middle of the prosternum (see Campbell 1984: figs 14, 15). And finally, one of the interesting features of the new species is the presence of highly modified mesotibia, which possess a very large tooth in the middle (Fig. 7). Various modifications of the male tibia are frequent in some Anthophagini. A small subtriangular tooth or large triangular dilatation on inner margin of the protibia are known in some species of *Anthobium* (e.g. *A. gracilipalpe* (Champion, 1920); *A. unicolor* (Marsham, 1802), and *A. atrocephalum* (Gyllenhal, 1827); see Palm 1948: figs 155f, 156f), *Arpedium* (see Campbell 1984: figs 60, 64), *Camioleum* (see Shin and Ahn 2006: fig. 1) or *Olophrum* (e.g. *Olophrum tadashii* Watanabe, 1990; see Watanabe 1990: fig. 134). Some species have different modifications of the metatibia, such as the very long and acute, median, spine-shaped protrusion on inner side of each metatibia of *Trichodromeus armatus* (Cameron, 1941) (see Coiffait 1983: fig. 2M). Some species of *Amphichroum* are characterized by a deep and wide indentation in the middle of metatibia, sometimes with an indistinct median tooth (see Zanetti 1987: fig. 86). The inner sides of the pro- and mesotibia of *Anthobioides* have a large, convex swelling on their apical portion (see Campbell 1987: figs 1, 34, 35). In light of the examples above, *A. alekseevi* sp. nov. has one of the most unusual sexual modifications of the mesotibia within the tribe.

Species of *Anthobium* are strongly dependent on a temperate climate, living in forest litter and wet moss, and most commonly inhabit wet habitats near swamps and along banks of streams and rivers. Hypothetically, *A. alekseevi* sp. nov. may have lived in wet biotopes near rivers or swamps. A similar temperate-loving, extinct, and potentially rheophilous species, *Geodromicus balticus* Shavrin & Yamamoto, 2019, was also described from Eocene Baltic amber.

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# The Dacini fruit fly fauna of Sulawesi fits Lydekker's line but also supports Wallacea as a biogeographic region (Diptera, Tephritidae)

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## Abstract

Although there is scientific consensus on most of the major biogeographic regions in the world, the demarcation of the area connecting Southeast Asia with Australia and Oceania remains debated. Two candidate boundaries potentially explain faunistic diversity patterns in the regions: Lydekker's and Wallace's lines. The islands in between both 'lines' are jointly termed Wallacea, with Sulawesi as the largest landmass. We surveyed Dacini fruit flies (Tephritidae: Dacinae) in Sulawesi between 2016 and 2019 using traps baited with male lures, resulting in 4,517 collected flies. We identified all specimens to species level, which adds 15 new species records to the island, bringing the total number of Dacini species in Sulawesi to 83. The biogeographic affinity of species in the updated checklist reveals a strong connection with former 'Sunda' (41% of species); validating Lydekker's line, but also a high level of endemism (47% of species), confirming the uniqueness of Wallacea as a biogeographic region. We further describe a new species, *Bactrocera* (*Bactrocera*) *niogreta* Doorenweerd, **sp. nov.** and discuss the taxonomy of several interesting species.

## Keywords

*Bactrocera*, biogeography, *Dacus*, pest, taxonomy, *Zeugodacus*, zoogeographic

## Introduction

Biogeographic boundaries were initially established to indicate stark and sudden differences between faunas of neighboring areas, as noted by early explorers like Alfred Russel Wallace, and proved fundamental to the understanding of tectonic plate movement (Wallace 1876; Lydekker 1896; Mayr 1944; Simpson 1977; Whitmore 1982). Today, they incorporate phylogenetic considerations and define biogeographical regions that can be of broad practical use including for regional identification keys, for understanding dispersal patterns, and designation of biogeographic hotspots for critical conservation considerations (Kreft and Jetz 2010; van Welzen et al. 2011; Holt et al. 2013). The area that connects Southeast Asia with Australia – also termed the Malay Archipelago or Malesia – probably contains the largest number of named biogeographic boundaries anywhere on the planet (Simpson 1977). The two designations that have held up best following extensive studies of both fauna and flora are known as Wallace's line and Lydekker's line, with the area in between often referred to as Wallacea (Fig. 1).

Wallace's line runs south of the Philippines, east of Borneo and continues south between Bali and Lombok (van Welzen et al. 2011). The boundary was hypothesized in works by Wallace (1860), and not long after the term "Wallace's line" was coined by Huxley (1868). Islands and landmasses west of Wallace's line are jointly termed the Sunda Shelf and were intermittently connected by land during the Pleistocene ice ages, up to as recently as 21,000 years ago when the sea level was as much as 120 m below current levels (for a review see van Welzen et al. 2011). Lydekker's line, on the other hand, suggests an alternative separation that would potentially explain the broader faunistic diversity patterns in the regions better. It was proposed by Lydekker (1896), but its significance became more recognized in later studies (Simpson 1977). This boundary runs west of Papua and north of Australia. Papua and Australia are located on the Sahul Shelf and were connected by land during roughly the same periods where Sundaland existed. There has been much debate on which line more accurately indicates the changes in biodiversity composition in the Indomalayan region (Simpson 1977; van Welzen et al. 2011). In some studies, the area between Wallace's and Lydekker's lines has been termed Wallacea and interpreted as a separate biogeographic region altogether, as it generally has high levels of endemism. Sulawesi is the largest island in Wallacea, where it is joined with the Moluccas and the lesser Sunda Islands. The area has known substantial geological turmoil: Sulawesi was separated into three parts until the late Miocene (~ 10 Ma). It was cut into the West Sulawesi ophiolite and North Sulawesi ophiolite that have a closer geographic affinity to Eurasia, and the East Sulawesi ophiolite, which was geographically closer to Australia (Hall 1998; Spakman and Hall 2010). The exact timing of the joining of these fragments is still uncertain, partly because it is unclear which areas were submerged in the past 10 Ma (Hall 2009). In any case, the geographic history of the islands has undoubtedly played a large role in the evolution of the fauna of the Sulawesi.

Dacini fruit flies (Tephritidae: Dacinae) are a tribe of 938 described exclusively Old World species (e.g., Doorenweerd et al. 2018). They are mostly known for their

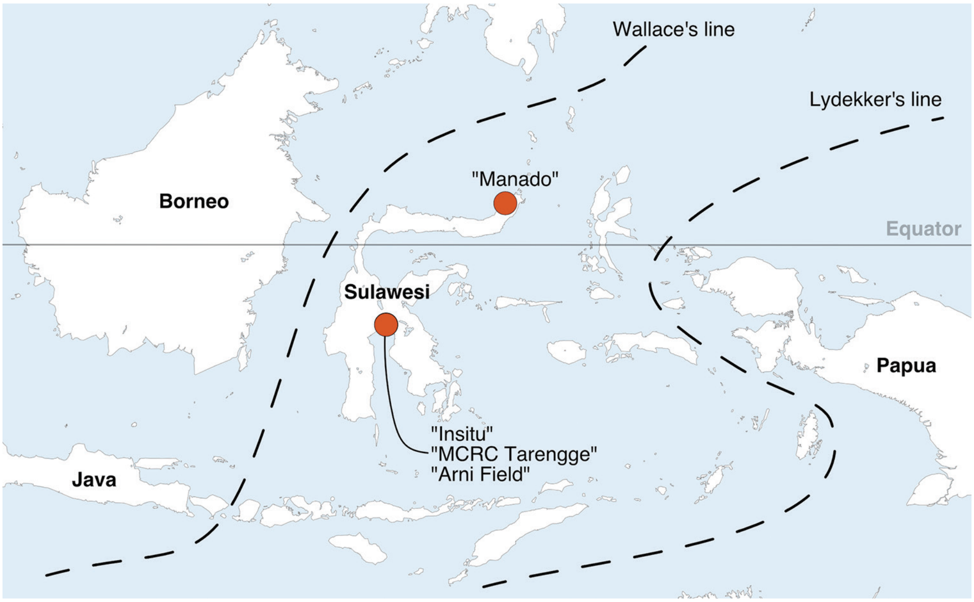


**Figure 1.** Map of Sulawesi and neighboring areas showing the four sampling localities with orange spots; the three localities in South Sulawesi were in close proximity to each other. Two typical biogeographical boundaries are indicated with dotted lines: Wallace's line and Lydekker's line. Land masses west of Wallace's line were connected during ice ages as Sunda, east of Lydekker's line land masses were connected as Sahul. Islands in between the two biogeographical boundaries were never connected by land and are jointly known as Wallacea.

potential to damage fruit crop production, as the majority of species are frugivorous and the larvae will feed on many fleshy fruits also used for human consumption (Vargas et al. 2015; Ekesi et al. 2016). The taxonomic and phylogenetic insights in the group have only recently begun to stabilize (Schutze et al. 2015, 2017; Dupuis et al. 2018; San Jose et al. 2018a), and there are likely many species yet undescribed. The first recorded Dacini fruit flies from Sulawesi were four species collected by Wallace in the mid 19th century (Hardy 1982). After those initial collections, the Dacini fauna of the island went unstudied for over a century until Hardy recorded 34 species during a sabbatical leave in 1975 (Hardy 1982). Many of those species were new to science and are endemic to Sulawesi. More recent studies have added further records (Drew and Romig 2013; Drew and Hancock 1994), and this current study adds another 15, which brings the total of Dacini fruit fly species known from Sulawesi to 83. We here provide a checklist of all species, describe *Bactrocera* (*Bactrocera*) *niogreta* sp. nov. as new to science and discuss new species forms, and assess the faunistic affinity of Sulawesi Dacini with neighboring biogeographic areas. In the last comprehensive overview, Hardy wrote: "The *Dacus* [ed: now Dacini] of Sulawesi fit more closely with the fauna of the Australian Region than with that of the Oriental". The Australian region in this sense included the Moluccas and Papua, thus agreeing with Wallace's line. We re-evaluate this statement based on the updated species list.

# Materials and methods

## Sampling

We collected Dacini flies using handmade bottle traps. A 3 cm-diameter hole was cut 15 cm from the base of a 500 ml plastic water bottle. Male attractant lures: methyl eugenol 10 g cones (Scentry Biologicals Inc., Billings MT, USA), cue lure 2 g cones (Scentry Biologicals Inc., Billings MT, USA), and zingerone (Sigma-Aldrich, St. Louis MO, USA) were individually suspended by a string inside the bottle, 5 cm from the top. A 100 ml water solution of Fisherbrand Sparkleen detergent (Fisher Scientific, Pittsburgh, PA, USA) poured at the bottom of each bottle trap was used as a killing agent. The traps were then hung from a cacao tree (*Theobroma cacao* L.) branch at 1.5 m high. Traps were checked every 2–5 days, and trapped flies were transferred to 95% ethanol. Trapping was mainly conducted in Wotu, Kabupaten Luwu Timur, South Sulawesi at sites named "Insitu" [WGS84 N 2.5587 E 120.7935], "MCRC Tarengge" [WGS84 N 2.5547 E 120.8047] and "Arni field" [WGS84 N 2.5587 E 120.7935] (Fig. 1).

Planting at Insitu was composed primarily of cacao clones PBC123 and BR25 that were planted 3.5 m apart within a row, with 3.5 m spacing between rows, irregularly shaded by a diversity of fruit trees. This site was the most diversified among the trapping sites, including more than 100 banana (*Musa* sp.), four large durian tree (*Durio* sp.), eight rambutan (*Nephelium lappaceum* L.), six coconut trees (*Cocos nucifera* L.), as well as some ginger (*Alpinia* sp.), Luffa (*Luffa acutangula* L.), papaya (*Carica papaya* L.), chili peppers (*Capsicum* sp.) and corns. The cacao trees were not regularly pruned but were treated with an unknown pesticide, and were not artificially irrigated. This farm was surrounded by neighboring cacao farms with a similar diversified composition. In addition, some jackfruit (*Artocarpus heterophyllus* Lam.), mango (*Mangifera indica* L.), guava (*Psidium guavaja* L.), rose apple (*Syzygium* sp.), as well as breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) were present around the neighboring farms. The site at MCRC Tarengge represents a 1 ha of cacao trees of clone M01 with a 1.5 × 3 m density, without any other fruit trees within the block. However, several langsat trees (*Lansium parasiticum* (Osbeck) Sahni & Bennet), banana and a couple of durian trees were present in the neighborhood farms 100 m away from the trapping sites, as well as 20 papaya, 10 rambutan trees (*Nephelium lappaceum* L.) within 200 m, and several mango trees, jackfruit, guava, and rose apple trees within 400 m radius from the trapping site. No pesticide was applied during our field collection, but both surrounding blocks were regularly treated with pesticides. The site 'Arni field' was also mainly composed of cacao trees at lower density (3 × 3 m). Various fruit trees disseminated around the farm, including some banana, rambutans, jackfruit, mangos, guava, and Ambarella (*Spondias dulcis* L.), with rows of corn (*Zea mays* L.) and several durian trees within 50 m, as well as jambu putik (*Syzygium* sp.), rose apple, and breadfruit within 300 m.

In total, the trapping effort at Arni field was approximately four months, five months at MCRC Tarengge, and six and half months at Insitu, spread over different periods during 2016–2019 (Suppl. material 1: Table S1). The site "Manado" [WGS84 1.3973N, 124.6488E], near the city of Manado in North Sulawesi, had three trapping days. At all sites combined, we collected 4,517 Dacini flies and identified all specimens to species level, initially based on external morphology. In cases where the morphology was inconclusive, we used DNA sequences of Cytochrome C Oxidase I and/or Elongation Factor 1-alpha for a total evidence identification approach. In 2016, some additional collecting was done with torula yeast dissolved in water, which attracts females. A full overview of all traps and localities can be found in Suppl. material 1: Table S1. All voucher material is stored at the University of Hawaii Insect Museum (**UHIM**). Photographs of adult specimens were taken using a Zeiss Discovery.V8 stereomicroscope with an attached Sony alpha-6300 camera. Photographs from multiple focal plains were combined into a single stacked image using Affinity Photo 1.7.3 and optimized for publication. Plates with multiple images were assembled in Affinity Designer 1.7.3. Wings of selected specimens were removed and mounted in euparal on glass-slides and photographed in a similar manner as the adults.

### DNA extraction, PCR, sequencing, and analyses

Methods for DNA extraction, PCR primers and conditions, and Sanger sequencing follow those of San Jose et al. (2018a). For the present study, we sequenced a Cytochrome C Oxidase I (COI) 809 base pair 3P' fragment and an Elongation Factor 1-alpha (EF1-alpha) 762 bp gene fragment for *Bactrocera niogreta*. We compared the sequences to our (partially unpublished) sequence database and here release sequences of the most closely related species to establish the diagnostic discrimination of COI and EF1-a sequences. We also sequenced COI and EF1-a for several specimens of *Bactrocera melastomatos* Drew & Hancock, 1994 and *Dacus longicornis* (Wiedemann, 1830), to confirm if the different morphological forms were mirrored in mitochondrial and/or nuclear genetic variation. Finally, we sequenced EF1-alpha for the two specimens of *B. carambolae* Drew & Hancock, 1994, which is diagnostic at five positions, to confirm its identity (see also Leblanc et al. 2019). All specimen collecting details and DNA sequences are available through BOLD dataset DOI: http://dx.doi.org/10.5883/DS-DACSU, and GenBank accessions: MT456325–MT456363 [COI] and MT456286–MT456324 [EF1-alpha]). We performed maximum likelihood analyses for each subset of sequences using IQTree 1.6.10 (Nguyen et al. 2015). We allowed IQTree to determine the substitution model via its integrated modeltest and ran maximum likelihood analyses with 5,000 ultrafast bootstraps and 5,000 Sh-aLRT bootstraps. We consider branches with support values > 95 % for ultrafast bootstraps and > 80 % for Sh-aLRT bootstraps as well supported. Resulting trees were optimized for publication using FigTree 1.4.3 and Affinity Designer 1.7.3.

## Results

We list 83 species of Dacini for Sulawesi (Table 1): 51 species of *Bactrocera*, 7 *Dacus*, and 25 *Zeugodacus*. We collected 29 species during our surveys, of which 15 are new island records. The biogeographic affinity of most species is with the Sunda region; 34

**Table 1.** Checklist of Dacini in Sulawesi.

| Species | Sulawesi record | Male lure | Insitu | MCRC Tarengge | Arni Field | Manado | Biogeographic affinity |
|---|---|---|---|---|---|---|---|
| *B. abbreviata* (Hardy, 1974) | This study | ZN | x |  | x |  | Sunda |
| *B. affinibancroftii* Drew & Romig, 2013 | Drew and Romig 2013 | ME |  |  |  |  | Sulawesi endemic |
| *B. affinidorsalis* (Hardy, 1982) | Hardy 1982 | CL |  |  |  |  | Sunda |
| *B. albistrigata* de Meijere, 1911 | Drew and Romig 2013 | CL | x | x | x | x | Sunda |
| *B. beckerae* (Hardy, 1982) | Hardy 1982 | CL |  |  |  |  | Sulawesi endemic |
| *B. bifasciata* (Hardy, 1982) | Hardy 1982 | CL |  |  |  |  | Wallacea |
| *B. bitungiae* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *B. carambolae* Drew & Hancock, 1994 | This study | ME | x |  |  |  | Sunda |
| *B. careofascia* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *B. commensurata* Drew & Romig, 2013 | This study | ME | x | x | x |  | Sunda |
| *B. curvosterna* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *B. dispar* (Hardy, 1982) | Hardy 1982 | – |  |  |  |  | Sulawesi endemic |
| *B. dorsalis* (Hendel, 1912) | Drew and Romig 2013 | ME | x | x | x | x | Sunda |
| *B. elongata* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *B. flavipennis* (Hardy, 1982) | Hardy 1982 | CL |  |  |  |  | Sulawesi endemic |
| *B. flavosterna* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *B. floresiae* Drew & Hancock, 1994 | Drew and Romig 2013 | ME |  |  |  |  | Sunda |
| *B. fuscitibia* Drew & Hancock, 1994 | Drew and Romig 2013 | CL/ZN* |  |  |  |  | Sunda |
| *B. fuscolobata* Drew & Romig, 2013 | Drew and Romig 2013 | CL | x |  |  |  | Sulawesi endemic |
| *B. fuscoptera* Drew & Romig, 2013 | Drew and Romig 2013 | ME |  |  |  |  | Sulawesi endemic |
| *B. hantanae* Tsuruta & White, 2001 | This study | CL | x |  |  |  | Sunda |
| *B. infulata* Drew & Hancock, 1994 | Drew and Hancock 1994 | ME |  |  |  |  | Sulawesi endemic |
| *B. involuta* (Hardy, 1982) | Hardy 1982 | CL |  |  |  |  | Sulawesi endemic |
| *B. latifrons* (Hendel, 1915) | Drew and Romig 2013 | – |  |  |  |  | Sunda |
| *B. limbifera* (Bezzi, 1919) | Drew and Romig 2013 | CL | x | x |  | x | Sunda |
| *B. linduensis* Drew & Romig, 2013 | Drew and Romig 2013 | CL | x |  |  |  | Wallacea |
| *B. megaspilus* (Hardy, 1982) | Hardy 1982 | CL |  |  | x |  | Sulawesi endemic |
| *B. melastomatos* Drew & Hancock, 1994 | This study | CL | x | x | x |  | Sunda |
| *B. moluccensis* (Perkins, 1939) | Drew and Romig 2013 | CL/ZN |  |  |  |  | Sunda |
| *B. nanoarcuata* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *B. nationigrotibialis* Drew & Romig, 2013 | Drew and Romig 2013 | ME |  |  |  |  | Sulawesi endemic |
| *B. neoritsemai* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *B. niogreta* Doorenweerd sp. nov. | This study | ZN | x |  |  |  | Sulawesi endemic |
| *B. ochroma* Drew & Romig, 2013 | Drew and Romig 2013 | ME |  |  |  |  | Sunda |
| *B. pendleburyi* (Perkins, 1938) | This study | ZN |  | x | x |  | Sunda |
| *B. penebeckerae* Drew & Romig, 2013 | Drew and Romig 2013 | – |  |  |  |  | Wallacea |
| *B. penecostalis* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *B. perkinsi* (Drew & Hancock, 1981) | This study | CL | x |  |  |  | Sahul |
| *B. pernigra* Ito, 1983 | This study | CL |  |  |  |  | Sunda |
| *B. propinqua* (Hardy & Adachi, 1954) | This study | CL | x |  |  |  | Sunda |
| *B. pseudobeckerae* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *B. ritsemai* (Weyenbergh, 1869) | Drew and Romig 2013 | CL |  |  |  |  | Sunda |
| *B. splendida* (Perkins, 1938) | This study | ZN* | x |  |  |  | Sunda |
| *B. sulawesiae* Drew & Hancock, 1994 | Drew and Hancock 1994 | ME |  |  |  |  | Sulawesi endemic |
| *B. suliae* Drew & Romig, 2013 | Drew and Romig 2013 | ME |  |  |  |  | Wallacea |
| *B. syzygii* White & Tsuruta, 2001 | This study | ZN | x | x | x |  | Sunda |
| *B. terminifer* (Walker, 1860) | Drew 1989 | – |  |  |  |  | Sulawesi endemic |
| *B. trifasciata* (Hardy, 1982) | Hardy 1982 | CL |  |  |  |  | Sulawesi endemic |
| *B. umbrosa* (Fabricius, 1805) | Drew and Romig 2013 | ME | x | x | x | x | Sunda; Sahul |
| *B. usitata* Drew & Hancock, 1994 | This study | CL | x |  |  |  | Sunda |
| *B. wuzhishana* Li & Wang, 2006 | Drew and Romig 2013 | ME |  |  |  |  | Sunda |
| *D. donggaliae* Drew & Romig, 2013 | Drew and Romig 2013 | CL |  |  |  |  | Sulawesi endemic |
| *D. longicornis* (Wiedemann, 1830) | Walker 1860; Drew 1989 | CL | x |  |  |  | Sunda |
| *D. melanopectus* Drew & Romig, 2013 | Drew and Romig 2013 | ME |  |  |  |  | Sulawesi endemic |
| *D. nanggalae* Drew & Hancock, 1998 | Drew and Hancock 1998 | CL |  |  |  |  | Sulawesi endemic |
| *D. ortholomatus* Hardy, 1982 | Hardy 1982 | – |  |  |  |  | Sulawesi endemic |
| *D. pedunculatus* (Bezzi, 1919) | This study | ZN* | x | x |  |  | Sunda |

| Species | Sulawesi record | Male lure | Insitu | MCRC Tarengge | Arni Field | Manado | Biogeographic affinity |
|---|---|---|---|---|---|---|---|
| *D. pullus* (Hardy, 1982) | Hardy 1982 | ZN* | x | | | | Sulawesi endemic |
| *Z. abnormis* (Hardy, 1982) | Hardy 1982 | CL | | | | | Sunda |
| *Z. angustifinis* (Hardy, 1982) | Hardy 1982 | CL | x | | | | Sulawesi endemic |
| *Z. apicalis* (de Meijere, 1911) | Hardy 1982 | CL | x | | | | Sunda |
| *Z. bogorensis* (Hardy, 1983) | Hardy 1982 | CL | | | | | Sunda |
| *Z. buruensis* (White, 1999) | Hardy 1982 | CL | | | | | Wallacea |
| *Z. connexus* (Hardy, 1982) | Hardy 1982 | – | | | | | Sulawesi endemic |
| *Z. cucurbitae* (Coquillett, 1899) | Drew and Romig 2013 | CL | x | x | | | Sunda |
| *Z. dubiosus* (Hardy, 1982) | Hardy 1982 | CL | | | | | Sulawesi endemic |
| *Z. emittens* (Walker, 1860) | Walker 1860; Drew 1989 | CL | | | | | Wallacea |
| *Z. eurylomatus* (Hardy, 1982) | Hardy 1982 | – | | | | | Sulawesi endemic |
| *Z. exornatus* (Hering, 1941) | Drew and Romig 2013 | CL | x | | | | Sunda |
| *Z. flavipilosus* (Hardy, 1982) | Drew and Romig 2013 | CL | | | | | Sulawesi endemic |
| *Z. fulvipes* (Perkins, 1938) | Hancock and Drew 2017 | CL | | | | | Sunda |
| *Z. hancocki* (Drew & Romig, 2013) | Drew and Romig 2013 | CL | | | | | Sulawesi endemic |
| *Z. heinrichi* (Hering, 1941) | Hering 1941 | CL/ZN | | | | | Sunda |
| *Z. melanopsis* (Hardy, 1982) | Hardy 1982 | CL | | | | | Sulawesi endemic |
| *Z. neoflavipilosus* (Drew & Romig, 2013) | Drew and Romig 2013 | CL | | | | | Sulawesi endemic |
| *Z. neolipsanus* (Drew & Romig, 2013) | Drew and Romig 2013 | CL | | | | | Wallacea |
| *Z. persignatus* (Hering, 1941) | Drew and Romig 2013 | CL | x | | | | Wallacea |
| *Z. proprescutellatus* (Zhang Che & Gao, 2011) | This study | CL | x | | | | Sunda |
| *Z. synnephes* (Hendel, 1913) | Drew and Romig 2013 | CL | | | | | Sunda |
| *Z. tebeduiae* (Drew & Romig, 2013) | Drew and Romig 2013 | CL | | | | | Sunda |
| *Z. transversus* (Hardy, 1982) | Hardy 1982 | CL | x | | | | Sulawesi endemic |
| *Z. ujungpandangiae* (Drew & Romig, 2013) | Drew and Romig 2013 | CL | | | | | Sulawesi endemic |
| *Z. vargus* (Hardy, 1982) | Hardy 1982 | CL | | | | | Sulawesi endemic |

*: new lure record. Male lure abbreviations: ME = methyl eugenol, CL = cue lure, ZN = zingerone.

of the species in the checklist can also currently be found in areas formerly connected under Sunda. This is in stark contrast with affinities related to Sahul; only one species is currently also found there, and an additional single species is found across Sunda, Wallacea and Sahul (*B. umbrosa* (Fabricius, 1805); see also Krosch et al. 2018). All 47 other species are endemic to Wallacea, and 39 of those are known from Sulawesi only, indicating the high levels of endemicity of the region, even for these volant insects.

We report four new male lure records of species attracted to zingerone: *Bactrocera splendida* (Perkins, 1938), *B. fuscitibia* Drew & Hancock, 1994 (attracted to both cue lure and zingerone), *Dacus pedunculatus* (Bezzi, 1919), and *D. pullus* (Hardy, 1982). Although the three localities "Insitu", "MCRC Tarengge" and "Arni Field" are geographically within two kilometers of each other, Insitu had a distinctly higher diversity with 28 species, whereas we only collected ten species at MCRC Tarengge, and nine at the Arni Field, with similar collecting efforts. We collected only four species at the "Manado" site, but this is likely due to less trapping days, and possibly because this was a less forested site just 50 m from the coastline. The major, widely distributed, pest species *B. albistrigata* (de Meijere, 1911) and *B. dorsalis* (Hendel, 1912) were present at all sites and made up 70.6 % of all specimens collected (Suppl. material 1: Table S1).

Below, we describe two new species, provide more information on the first records of *B. carambolae* for Sulawesi, and discuss the presence of *B. melastomatos*. We also describe the second specimen ever collected of *Dacus pullus*, and provide morphological and molecular evidence for two species forms of *Dacus longicornis*.

## ***Bactrocera* (*Bactrocera*) *niogreta* Doorenweerd, sp. nov.**

http://zoobank.org/AEC5FE4F-A4F4-4C48-AEB6-27A1A74B6F58
Figures 2–7

**Holotype.** Male. Labelled: "Indonesia: Sulawesi: South Sulawesi: Insitu. WGS84 -2.5464 120.7921 16–23.i.2019 Zingerone trap. Leg. Jerome Niogret. DNA sample ms09121". Deposited at the University of Hawaii Insect Museum (UHIM).

**Differential diagnosis.** *Bactrocera* (*Bactrocera*) *niogreta* sp. nov. is most similar to *B.* (*Tetradacus*) *brachycera* (Bezzi, 1916), which is known from India, Bhutan, and China (Drew and Romig 2013). Both species have an incomplete black 'T' marking on the abdomen, and a costal band that follows vein $R_{4+5}$ and expands distally to reach vein M. *Bactrocera niogreta* can be distinguished by the connection of the yellow presutural marking with the notopleuron, which resembles a yellow curly bracket '{' in dorsal view. *Bactrocera niogreta* further has smaller facial spots, not filling the basal ½ of the socket, and in the male genitalia it has a deep emargination of sternum V, which is shallow in *B. brachycera*. *Bactrocera niogreta* may in Sulawesi be most easily be confused with *B. megaspilus*, but the latter has a more angular expansion of the costal band, no medial black markings on the abdomen, no presutural yellow markings and all fulvous legs.

**Molecular diagnostics.** The COI sequence of *Bactrocera niogreta* is, in our database, most similar to *Bactrocera fuscitibia*, which can morphologically easily be distinguished by not having a clearly expanded costal band. The EF1-alpha sequences are most similar to *B. enochra* (Drew, 1972), which is morphologically different in not having a wide costal band, and has a wide red band medially across the scutum and three longitudinal black bands along the abdomen. Both COI and EF1-alpha are diagnostic to identify *B. niogreta* (See BOLD Dataset DOI: http://doi.org/10.5883/DS-DACSU).

**Description of adult.** ***Head*** (Fig. 3). All parts uniformly fulvous to yellow, ocellar triangle slightly darker. Face fulvous with rectangular spot in each antennal furrow. Antennae uniformly fulvous. ***Thorax*** (Figs 2, 4). Scutum and pleural areas black with narrow red-brown areas lateral of the yellow postsutural lateral vittae. Yellow markings: postpronotal lobes; notopleura; postsutular lateral vittae broad and parallel sided, reaching intra-alar seta; presutural marking to the lateral vittae that connects to the notopleura and in dorsal view resembles a curly bracket '{'; broad mesopleural stripe, almost reaching posterior level of postpronotal lobe, continuing onto katepisternum as a broad transverse spot, anterior margin slightly convex; katatergite; anatergite. Medial vitta absent. Scutellum yellow except for narrow black basal band. Setae: two scutellar; one prescutellar; one intraalar; one posterior supraalar; one anterior supraalar; one mesopleural; two notopleural; four scapular; all setae well developed and red-brown. ***Abdomen*** (Figs 2, 5). Oval to diamond shaped; terga free; pecten present on tergum III; posterior lobe of surstylus short (Fig. 7); abdominal sternum V with a deep concavity on posterior margin that reaches the center of the sternum. Tergum I fulvous with apical margin narrowly yellow. Tergum II yellow with anteromedial dark marking. Tergum III mostly dark, with a narrow concave posterior fulvous band. Terga IV and

**Figures 2–7.** *Bactrocera* (*Bactrocera*) *niogreta* sp. nov. Holotype, ms09121 **2** dorsal view **3** frontal view of the face **4** lateral view **5** posterior view of the abdomen showing the ceromatae **6** dissected wing **7** lateral close-up of the genitalia.

V with a medial longitudinal dark marking. Tergum IV with triangular anterolateral dark markings, tergum V with narrow anterolateral dark markings. Ceromatae (shining spots) contrasting red-brown. ***Legs*** (Fig. 4). All leg segments fulvous to yellow; tibiae fulvous with apical black spur on mid tibiae; tarsi fulvous to yellow. ***Wings*** (Fig. 6). Length 6.1 mm, basal costal and costal cells fuscous, increasingly darker distally; microtrichia in outer corner of cell costal only; remainder of wings with a pale fulvous tint except fuscous subcostal (anal) cell; broad fuscous costal band that reaches vein $R_{4+5}$, gradually darker distally until dark brown and expands to reach vein M; a broad fuscous anal streak ending at apex of $A_1$ + $CuA_2$; dense aggregation of microtrichia around $A_1$ + $CuA_2$; supernumerary lobe not pronounced.

**Male lure.** Zingerone.

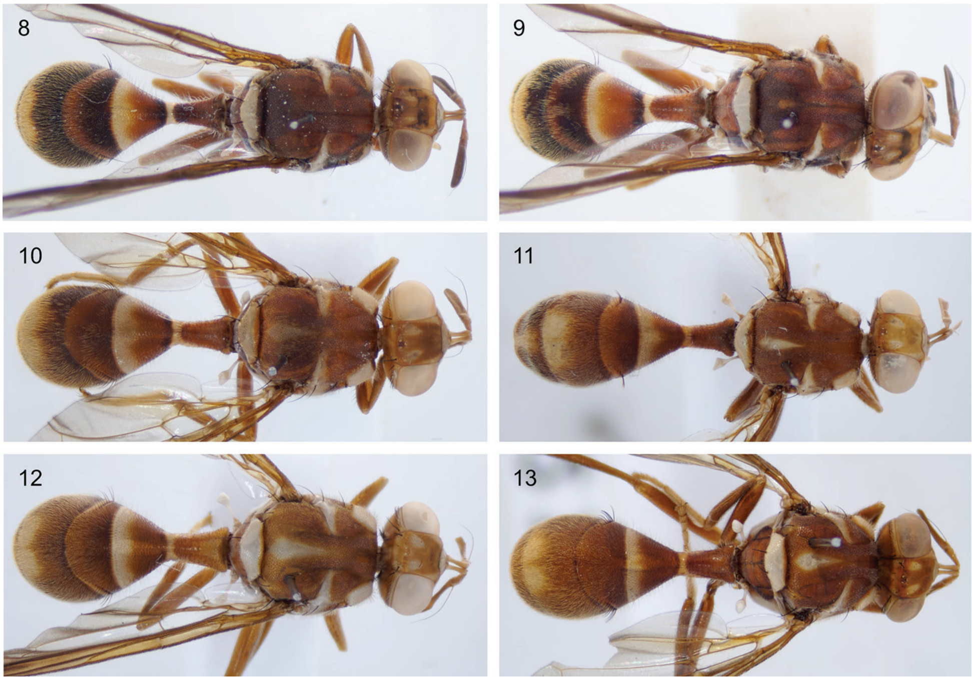
**Host plant.** Unknown.

**Etymology.** The species name is an adjective that refers to instigator of the 2016–2019 Dacini surveys in Sulawesi: Jerome Niogret.

**Comments.** the morphology of *B. niogreta* overall most closely resembles *B.* (*Tetradacus*) *brachycera*, the combination of a short posterior lobe of the surstylus in the male genitalia and a deep concavity on sternum V support placement in subgenus *Bactrocera*. In the Drew and Romig (2016) key to the Southeast Asian fruit flies, *B. niogreta* characters lead to the Indian species *Bactrocera* (*Bactrocera*) *andamanensis*, couplet 90 on page 140. The key can there be adapted to include that *B. niogreta* differs from *B. andamanensis* in having an all-black scutum, broad postsutural yellow lateral vittae, and dark lateral markings on abdominal segment IV, with no dark markings on the legs.

### ***Dacus longicornis* form *icariiformis***

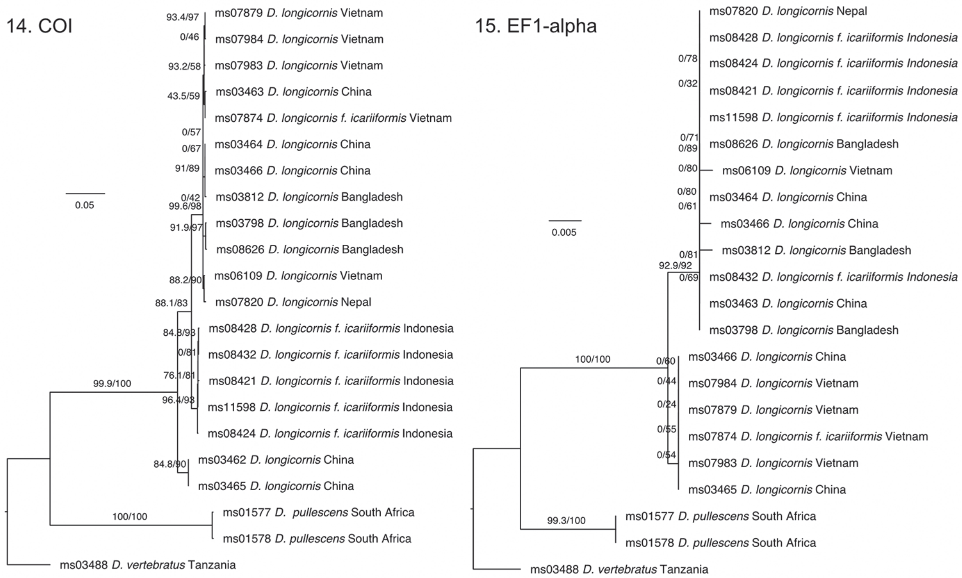
*Dacus longicornis* Wiedemann is a widespread Southeast Asian species that is a minor pest: the larvae feed on *Luffa*, *Trichosanthes* and some other Cucurbitaceae (Allwood et al. 1999; Drew et al. 1998; Hardy and Adachi 1954). The morphology of *D. longicornis* is most extensively treated by Drew et al. (1998), where many synonyms were established and the variability of the species was first documented. In particular, this was the first, and only, publication that noted two forms: "There are two forms of *D. longicornis*, one with and one without a small medial postsutural vitta" (Drew 1998). However, this knowledge was not incorporated in subsequent publications, such as the Drew and Romig (2013) treatment of the Southeast Asian fauna, nor the accompanying Drew and Romig (2016) identification keys. We here provide the first figures of both forms (Figs 8–13). The postsutural medial vitta is absent in Bangladesh specimens (Figs 8, 9), but always present in Sulawesi specimens (Figs 10–13), although sometimes indistinct (Fig. 10). The dark markings on the anterior sides of the abdominal segments are more pronounced in Bangladesh specimens of *D. longicornis*, and Bangladesh specimens have a dark band across the occiput, connecting the compound eyes. The variable presence or absence of a medial vitta is not known for any other Dacinae species, but with all data considered, we see no reason at present to establish this form as a new species. Both COI and EF1-alpha sequence data reveal some genetic substructure in *D. longicornis*, but the structure differs between the two markers and does not match with the morphological forms (Figs 14, 15). Drew and Romig (2013) had studied the type material of *D. icariiformis* Enderlein, from India, and concluded that of the three type specimens –no holotype had been designated– the female was actually *D. longicornis* and only the two males are now regarded as lectotype and paralectotype. This confusion indicates that it is difficult to distinguish *D. longicornis* from *D. icariiformis*, and we here refer to the specimens of *D. longicornis* with a medial vitta that are genetically indistinguishable as *D. longicornis* form *icariiformis*. Because we did not study any type material, we refrain from synonymizing *D. icariiformis* with *D. longicornis*. However, we note that there are no diagnostic characters indicated in the literature to distinguish *D. longicornis* form *icariiformis* from *D. icariiformis*.



**Figures 8–13.** Two forms of *Dacus longicornis* **8** *D. longicornis* collected in Bangladesh, Pabna district, 30-ix-3-x-2013 Leg. M. A. Hossain **9** *D. longicornis* collected in Bangladesh, Maulvi Bazar Rainforest resort, Leg. L. Leblanc & M. A. Hossain **10** specimen ms08424, collected in Sulawesi, with a faint medial postsutural yellow vitta **11** specimen ms08432, collected in Sulawesi **12** specimen ms08428, collected in Sulawesi **13** specimen ms08421, collected in Sulawesi.

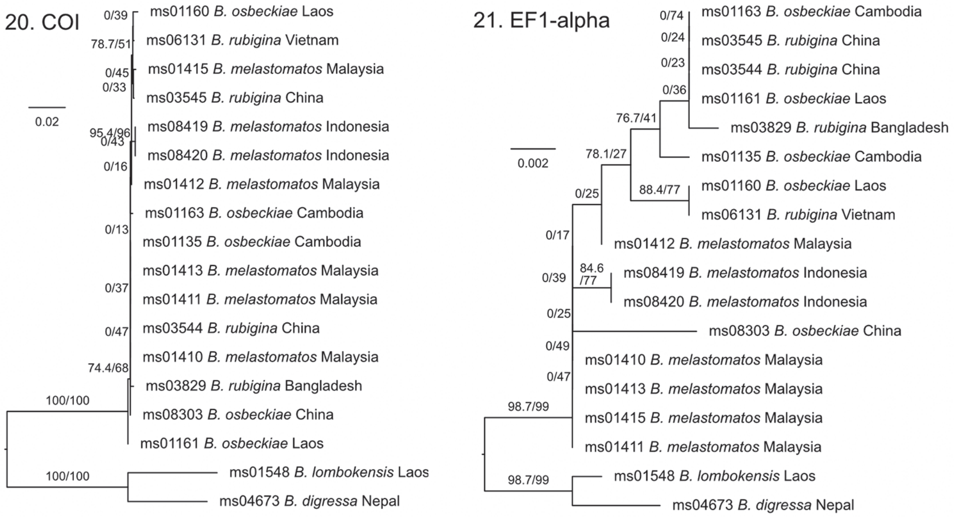
## Sulawesi *Bactrocera melastomatos*

We collected more than 300 specimens with a uniform morphotype that are tentatively included in the checklist as *B. melastomatos* (Table 1, Suppl. material 1: Table S1, Figs 16–19). We sequenced COI and EF1-alpha fragments for multiple specimens: they are genetically indistinguishable from specimens morphologically identified as *B. rubigina* (Wang & Zhao, 1989), *B. melastomatos* and *B. osbeckiae* Drew & Hancock, 1994 in both markers (Figs 20, 21). Morphologically, the specimens from Sulawesi are an imperfect fit for all three genetically suggested candidate species. Instead, they are more similar to the sympatric *B. usitata* Drew & Hancock, 1994 (Figs 16–19), but *B. usitata* has a medial black line across abdominal segments III–V, forming the typical *Bactrocera* black 'T', which is never present in Sulawesi *B. melastomatos*. The costal band of *B. usitata* and *B. melastomatos*, including the Sulawesi specimens, extends to vein $R_{4+5}$, a character also shared with the southern Vietnam form of *B. rubigina* (Drew and Romig 2013). In the Drew & Romig (Drew and Romig 2016) identification keys, the absence of a black 'T' shape on the abdomen will lead to *Batrocera latifrons*, but that species is not attracted to cue lure, and has parallel yellow lateral postsutural vittae,



**Figures 14, 15.** Maximum Likelihood trees based on COI (**14**) and EF1-alpha (**15**) DNA sequence data for *Dacus longicornis*, with *D. pullescens* Munro and *D. vertebratus* Bezzi as outgroups. Branch support values are rapid bootstrap values and approximate-likelihood ratio test values, scale bar indicates substitutions per site. Full details on the samples can be found in BOLD dataset DOI: http://dx.doi.org/10.5883/DS-DACSU.

**Figures 16–19.** Sulawesi *Bactrocera melastomatos* resemble sympatric *Bactrocera usitata* **16** specimen ms09144 *B. usitata*, dorsal view **17** close up of abdomen of ms09144 **18** specimen ms08838 *B. melastomatos*, dorsal view **19** close up of abdomen of ms08838.

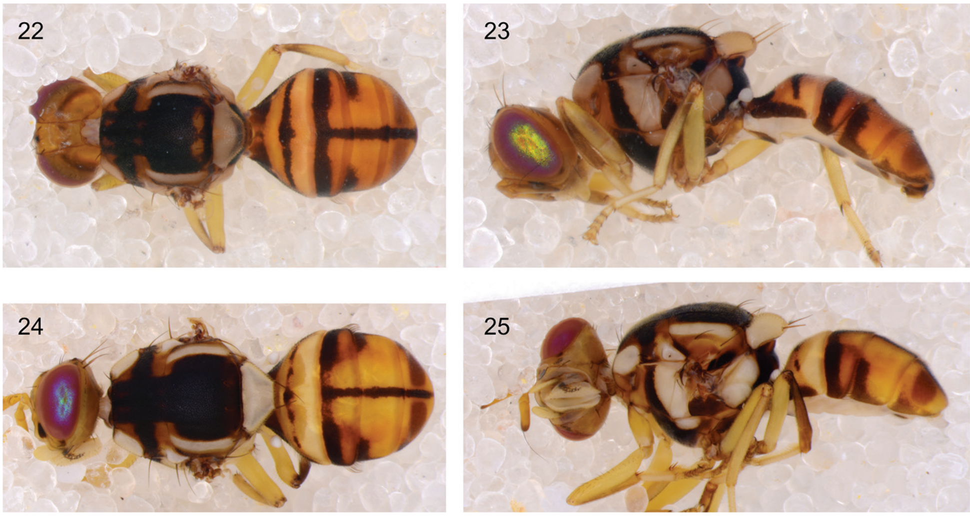


**Figures 20, 21.** Maximum Likelihood trees based on COI (**20**) and EF1-alpha (**21**) DNA sequence data for *Bactrocera melastomatos* and allied species, using *B. lombokensis* Drew & Hancock and *B. digressa* Radhakrishnan as outgroup. Branch support values are rapid bootstrap values and approximate-likelihood ratio test values, scale bar indicates substitutions per site. Full details on the samples can be found in BOLD dataset DOI: http://dx.doi.org/10.5883/DS-DACSU.

which are strongly tapering, almost triangular, in Sulawesi *B. melastomatos*. Because there is no genetic support at present to describe this taxon as a separate species, we interpret the morphology of Sulawesi specimens as regional variation and leave their designation as *B. melastomatos* until more (genomic) data becomes available. Rearing specimens from host fruit would present important ecological data; the currently recorded hosts for this group are all Melastomataceae or Lauraceae (Liang et al. 1993; Allwood et al. 1999).

## *Bactrocera carambolae* Drew & Hancock, 1994

We collected two specimens of *Bactrocera carambolae*, both at the Insitu locality, which represent the first records for Sulawesi (Figs 22–25). The morphology of the collected specimens is consistent with the description (Drew and Hancock 1994). However, because *B. carambolae* is morphologically very similar to *B. dorsalis* and they have intermingled mitochondrial DNA (San Jose et al. 2018b), we confirmed the identification with EF1-alpha DNA sequences. EF1-alpha is diagnostic for this species pair based on five positions (see also Leblanc et al. 2019). *Bactrocera carambolae* was already known from Java and Borneo (Vargas et al. 2015), so its presence in Sulawesi could be through natural dispersal, and it may have been missed during previous surveys. Alternatively, it could have been introduced through fruit transport, as it is a pest species on commercial fruit. Its natural distribution includes Vietnam,

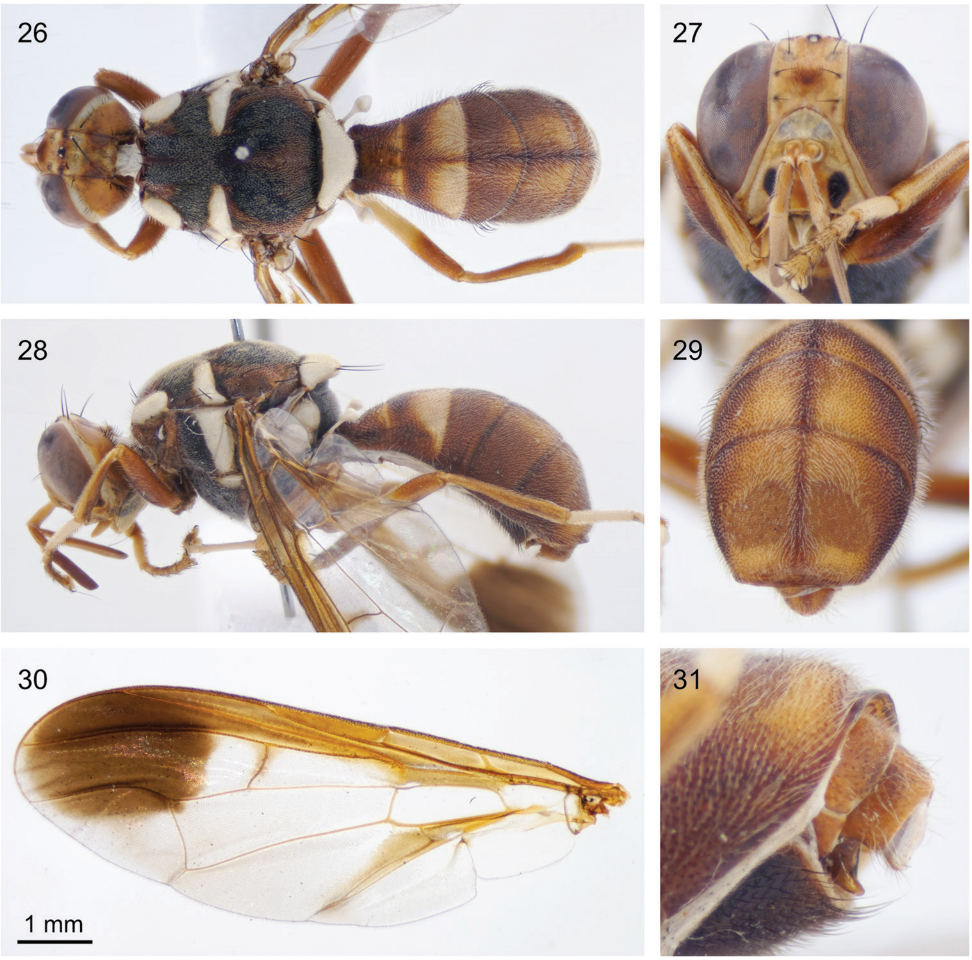


**Figures 22–25.** The two specimens of *Bactrocera carambolae* that represent the first records for Sulawesi, photographed in ethanol (wings were removed) **22** dorsal view of specimen ms08439 **23** lateral view of specimens ms08439 **24** dorsal view of specimen ms10710 **25** lateral view of specimen ms10710. Both specimens have the typical rectangular black mark on the lateral sides of the fourth abdominal segment, but lack the black mark on the fore femur, which can further help to distinguish *B. carambolae* from *B. dorsalis*.

Laos, Cambodia, Thailand, Malaysia, and Indonesia (Java and Kalimantan), and it was recently reported in Bangladesh (Leblanc et al. 2019). In addition, it is highly invasive in agricultural areas in the Guianas in South America, where it is the only representative of Dacini.

### *Dacus pullus* (Hardy 1982)

We record specimen ms09122 as a representative of *Dacus pullus* (Figs 26–31), although the wing markings are somewhat incongruent with the description and illustration of the only known other specimen of this species. The original species description states: "Costal band broad extending through upper half of cell Rs for its entire distance and expanded in apical portion to fill entire wing apex below upper edge of cell 2nd M2" (Hardy 1982). However, the illustration does not depict an expansion in the apical portion. Drew and Romig (2013) also illustrated the holotype, again not showing a significant apical expansion of the costal band. The costal band of specimen ms09122 is mostly confined by vein $R_{4+5}$, although there is infuscation of crossvein r-m, and distally expands to cross vein M. We opt to err on the side of caution and interpret this as intraspecific variation, and do not describe this specimen as a separate species, also considering the limited availability of material and the fact that both specimens were collected in Sulawesi.



**Figures 26–31.** *Dacus pullus* **26** dorsal view **27** frontal view of the face **28** lateral view **29** posterior view of the abdomen showing the ceromae **30** dissected wing **31** lateral close-up of the genitalia.

# Discussion

## Between the lines

Sulawesi is a dispersal crossroads for the biotas of Southeast Asia, Australia, and Oceania. The updated species checklist we present here shows that Sulawesi is unique, with many endemic species, but that there are also strong connections with Southeast Asia, at least for the taxa under study. This finding does not support the earlier working hypotheses that posited a closer connection to the Sahul fauna, including Papua (Hardy 1982). Based on this recent data, it seems likely that Wallacea has been a "stepping-stone" for Dacini to reach Australasia and Oceania; 30 Sulawesi species are also found in (former)

Sunda, while only one is shared with Sahul. This can provide crucial insight into the timing of the diversification of the group in the latter areas. Wallacea, and Sulawesi with it, was separated in discontinuous landmasses with fluctuating sea-levels until the mid-Miocene, 10–15 Ma (Hall 2009). Before this connection, Sunda and Sahul were separated by vast oceanic distances that were unlikely to be crossed by fruit flies. There are currently hundreds of Dacini species known from Australia and Oceania (Drew 1989, Drew and Romig 2001), which may have resulted from rapid radiation after reaching these new areas of ecological opportunity. Similarly, the timing of the formation of Wallacea suggests a relatively recent origin for the 34 Sulawesi endemic Dacini species. However, it should be noted that the Dacini fauna of Papua is understudied (White and Evenhuis 1999) and further surveys in this area may reveal shared geographic ranges with some of the species now presumed to be Wallacean endemics.

It has been advocated by some that the categorization of biogeographic regions should follow more quantitative measures (Kreft and Jetz 2010), as opposed to the qualitative assessments from the early explorers. Using a non-metric multidimensional scaling approach across a wide range of taxa, Kreft and Jetz (2010) suggested that Lydekker's line was most appropriate for separating the Oriental region from Australasia. This agrees with our findings for Dacini, although, depending on the scale of the patterns in question, maintaining Wallacea as a separate biogeographic region can equally well be argued. Other authors have further included a phylogenetic component in the delimitation of biogeographic areas, which resulted in a suggested split of Wallacea: Sulawesi and the Lesser Sunda Islands were grouped with Southeast Asia into the Oriental region, whereas the Moluccas were grouped with Papua in the Oceanian region (Holt et al. 2013). However, none of these broad-scale assessments of biogeographic categorization include or consider invertebrate taxa. Moreover, as phytophagous insects, it might be expected that the biogeographic pattern of Dacini more closely tracks phytogeographic regions. A broad study that included 7,340 plant species across Southeast Asia suggested 'central Wallacea' [defined to encompass the Philippines, Sulawesi, lesser Sunda islands, Moluccas, and Java] as a separate region (van Welzen et al. 2011). This categorization is further corroborated by the climatic conditions; central Wallacea has a yearly dry season and monsoon, whereas both neighboring regions lack a prolonged dry season. For Dacini, we find few connections between the Philippines and Wallacea, but there are some, e.g., *B. commensurata*. Future surveys of the Papuan Dacini fauna, and placement of the Wallacean taxa in a phylogenetic framework, can further inform which biogeographic delimitation fits best with this group of fruit flies, and it is clear that more studies on invertebrate groups will be important to fully understand the biogeographic affinities of the islands that connect Asia with Australia and Oceania.

## Pests

As a tropical island, Sulawesi has a rich diversity of fruiting plants and, consequently, insects that utilize them. Our surveys were performed in cacao plantations; the only Dacini that is known to feed on cacao is the polyphagous *Bactrocera dorsalis* (Allwood et al. 1999). However, we have never observed flies attempting to oviposit on cacao nor have we found maggots inside the pods. Cacao is likely a very rare host for *B. dorsalis*, if at all, and potentially only fallen and dehiscent fruit where the tough skin is cracked is susceptible. We commonly found *B. dorsalis*, but also another pest species; *B. albistrigata* at all sites. Together they made up 70.6 % of all individuals collected. The host records of *B. albistrigata* include jackfruit (*Artocarpus heterophyllus*), jambu putik (*Syzygium* sp.), mango (*Mangifera indica* L.), guava (*Psidium guajava* L.) and rose apple (*Syzygium* sp.) some of which were planted near the cacao. It is interesting to note that although we encountered the cucurbit pest *Zeugodacus cucurbitae*, it was surprisingly rare. Possibly, this is due to the limited availability of melon hosts in the area (none were observed), although it is also known to feed on papaya, which were recurrent in the cacao orchards sampled, and non-commercial cucurbits that commonly occur as weeds in gardens and plantations. We further encountered small numbers of *B. umbrosa*, a pest of breadfruit (*Artocarpus altilis*) that has an extraordinarily wide distribution; it is the only species in the checklist that is known to be naturally dispersed across Southeast Asia, Wallacea, Australia and Oceania (Krosch et al. 2018). We suggest that, if desired, the population densities of pests in our survey areas can likely be decreased significantly with sanitation measures, most importantly the removal of fallen fruit and pruning of damaged fruit unfit for consumption.

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## Supplementary material 1

**Table S1**

Authors: Camiel Doorenweerd, Arni Ekayanti, Daniel Rubinoff

Data type: Spreadsheet with trapping records

Explanation note: Table including the lot numbers for each trapping event, fly identification, fly counts, lure used and exact locality.

Link: https://doi.org/10.3897/zookeys.973.55327.suppl1

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RESEARCH ARTICLE

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# *Nephrotoma* Meigen (Diptera, Tipulidae) from Xizang Autonomous Region, China

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**Abstract**

Eight species of the genus *Nephrotoma* were previously known to occur in Xizang Autonomous Region. Here, three species are added to the fauna of Xizang. Among them two species, *N. beibengensis* **sp. nov.** and *N. hanae* **sp. nov.** are described and illustrated as new to science, and one species, *N. evittata* Alexander, 1935 is recorded from Xizang for the first time. The following four species are redescribed: *N. claviformis* Yang & Yang, 1987, *N. didyma* Yang & Yang, 1987, *N. nigrohalterata* Edwards, 1928, and *N. xizangensis* Yang & Yang, 1987. A key to the species of *Nephrotoma* from Xizang is presented.

**Keywords**

Crane flies, new species, taxonomy, Tibet, Tipulinae

## Introduction

The genus *Nephrotoma* Meigen is one of the largest genera in the family Tipulidae. It is distributed worldwide with 163 known taxa from the Palaearctic Region and 127 taxa from the Oriental Region. Until now, 96 taxa are known in China (Oosterbroek 2020). In recent years, Men et al. (2015, 2016, 2017) and Ren and Yang

(2017) increased our knowledge of the genus *Nephrotoma* in China. This genus is characterized by the following features: body usually yellow with dark stripes at top of prescutum or nearly entirely black; Rs short, cell $m_1$ sessile or shortly petiolate; male tergite 9 separated from sternite 9; posterior margin of tergite 9 varied in shape, usually with small black spines; outer gonostylus usually flattened and fleshy, and more or less acuminate; female cercus longer than hypovalva, blunt at tip, and hypovalva tapered or parallel-sided (Oosterbroek 1978; Tangelder 1983).

Xizang Autonomous Region (hereafter referred to as Xizang) is located in southwestern China and on the Qinghai-Tibet Plateau. This area is in the Palaearctic Region and on the border of the Oriental Region. With the formation and uplift of the Qinghai-Tibet Plateau, it has an average altitude of more than 4,500 m and a wide elevation range (Su et al. 2019). The formation of the Himalayas and the Qinghai-Tibet Plateau created a variety of climates and vicariance (Li and Fang 1999). Therefore, Xizang, is one of the most important biodiversity hotspots in the world. So far, only the following eight species of *Nephrotoma* are known to occur in this area: *N. claviformis* Yang & Yang, 1987, *N. didyma* Yang & Yang, 1987, *N. distans* Edwards, 1928, *N. inorata* Alexander, 1951, *N. kaulbacki* Alexander, 1951, *N. libra* Alexander, 1951, *N. nigrohalterata* Edwards, 1928, and *N. xizangensis* Yang & Yang, 1987. In the present paper, three species, including two new species, are added to the fauna of Xizang, and four species are redescribed. A key to the species of *Nephrotoma* from Xizang is presented.

## Materials and methods

The specimens were studied and illustrated with a ZEISS Stemi 2000-c stereo microscope. Details of coloration were checked in specimens immersed in 75% ethyl alcohol (C2H5OH), except the dried specimens of *N. claviformis* Yang & Yang, 1987. Genitalic preparations of males were made using lactic acid solution (C3H6O3 > 85%) heated in a water-bath to 95 °C for 4–6 minutes. After examination, it was transferred to fresh glycerin (C3H8O3) and stored in a microvial pinned below the specimen. Type specimens are deposited in the Entomological Museum of China Agricultural University (CAU), Beijing.

The morphological terminology mostly follows Alexander and Byers (1981), McAlpine (1981), Tangelder (1985), and de Jong (2017). The terminology applied to the wing veins follows the interpretation of de Jong (2017). Terminology of male hypopygium follows Alexander and Byers (1981) and Tangelder (1985).

## Taxonomy

### Key to species (males) of *Nephrotoma* from Xizang (Tibet), China

1 Vertex with triangular spots near inner margin of eye; prescutum with narrow black margin (Edwards 1928: 700) ..................... ***N. distans* Edwards, 1928**
– Vertex and prescutum not as above ........................................................ **2**

2 Mediotergite with a pair of black spots; tergite 9 with black caudal margin, lateral angle produced into a decurved spine (Yang and Yang 1987: 128)..... .................................................................***N. libra* Alexander, 1951**

– Mediotergite and tergite 9 not as above.................................................. **3**

3 Scutellum mainly black or dark brown..................................................... **4**

– Scutellum mainly yellow.......................................................................... **5**

4 Occipital marking square, extended anteriorly to inner margin of eye (Figs 1, 2); thoracic pleuron without obvious massive black spots; tergite 9 with a deep median notch (Figs 7, 8)..........................................***N. beibengensis* sp. nov.**

– Occipital marking not as above; thoracic pleuron with obvious massive black spots; tergite 9 without deep median notch............................................... **6**

5 Mediotergite and scutellum with brownish longitudinal spot at middle..... **7**

– Mediotergite and scutellum not as above.................................................. **8**

6 Lateral prescutal stripe straight; tergite 9 with a pair of rod-like projections on posterior margin (Figs 10–12).......... ***N. claviformis* Yang & Yang, 1987**

– Anterior end of lateral prescutal stripe bent outward (Figs 43, 44); posterior extension of tergite 9 depressed with two pairs of short projections (Fig. 46).... ..............................................................***N. nigrohalterata* Edwards, 1928**

7 Posterior margin of mediotergite slightly dark; tergite 9 with a pair of large aduncous protuberances on posterior margin (Yang and Yang 1987: 128).... ......................................................................***N. inorata* Alexander, 1951**

– Posterior margin of mediotergite not as above; tergite 9 with a pair of short sharp protuberances on posterior margin (Figs 27, 32) ... ***N. evittata* Alexander, 1935**

8 Occipital marking subtriangular; scutellum with pale brown middle stripe and brown posterior margin (Fig. 53) .......... ***N. xizangensis* Yang & Yang, 1987**

– Occipital marking and scutellum not as above ........................................ **9**

9 Occipital marking dark brown, bell-shaped, extended to top of vertex; tergite 9 strongly protruded at posterior margin (Figs 19, 22)............................... ................................................................. ***N. didyma* Yang & Yang, 1987**

– Occipital marking and tergite 9 not as above ........................................ **10**

10 Posterior margin of tergite 9 produced at middle, with a pair of pointed lateral projections; sternite 8 depressed at posterior margin, medially with a sickle fleshy appendage bearing long apical hairs (Figs 37, 39, 41)............***N. hanae* sp. nov.**

– Posterior margin of tergite 9depressed at middle, without pointed lateral projections; sternite 8 with a very deep and narrow notch at posterior margin (Alexander 1953: 332) ................................ ***N. kaulbacki* Alexander, 1951**

### ***Nephrotoma beibengensis* sp. nov.**

http://zoobank.org/01272B09-7641-4EAD-BA49-A378DA40D942

Figs 1–9, 60

**Diagnosis.** Frontal tubercle raised into discoid protuberance. Occipital marking dark brown, square, anteriorly extending to eye. Mediotergite with an I-shaped, dark-brown, longitudinal stripe. Legs approximately three times as long as body (Figs 1, 2). Posterior extension of tergite 9 slightly depressed with two pairs of short, obtuse projections bearing tiny, black spines, medial pair slightly longer than lateral pair (Figs 7, 8).

**Material examined.** ***Holotype*** male (CAU), China: Xizang, Motuo (Medog, Metok), Beibeng, 2017.VI.11, 859 m, Qicheng Yang (light trap). ***Paratype*** 1 male (CAU), China: Xizang, Motuo, Beibeng, Gelincun, 2018.VI.27, 1400 m, Qicheng Yang (light trap).

**Description.** Male (*n* = 2): body length 10.4–10.5 mm, wing length 10.0 mm, antenna length 2.5 mm.

***Head*** (Figs 1, 2). Mainly yellow. Frontal tubercle raised into discoid. Occipital marking dark brown, square, extended anteriorly to inner margin of eye. Rostrum yellow and nasus brown. Hairs on head black. Antenna brownish yellow, base of each segment darker; first flagellomere 1.3 times longer than second one. Proboscis brownish yellow, with black hairs. Palpus brown, with black hairs.

***Thorax*** (Figs 1, 2). Mainly yellow. Pronotum yellow, lateral side dark brown, with yellow hairs. Prescutum with three black, longitudinal stripes; anterior end of lateral prescutal stripe bent outward, outer part brown and dull; prescutum with posterolateral dark-brown margin. Scutum with two black spots. Scutellum dark brown, with two triangular, yellow spots anteriorly. Mediotergite with an I-shaped, dark-brown marking. Anepisternum and katepisternum yellow. Anepimeron yellow with brown spot at antero-dorsal corner. Parascutellum pale brown. Anatergite brown; katatergite yellow. Meron yellow. Hairs on thorax dark yellow. Legs long, approximately three times as long as body, yellow except apices of tibiae and tarsi dark brown; hairs brown. Wing subhyaline, tinged with light brown; pterostigma pale with macrotrichiae; cell $m_1$ shortly petiolate, virtually invisible; apices of $R_5$ not curve up (Fig. 3). Halter with stem brownish yellow; knob pale yellow.

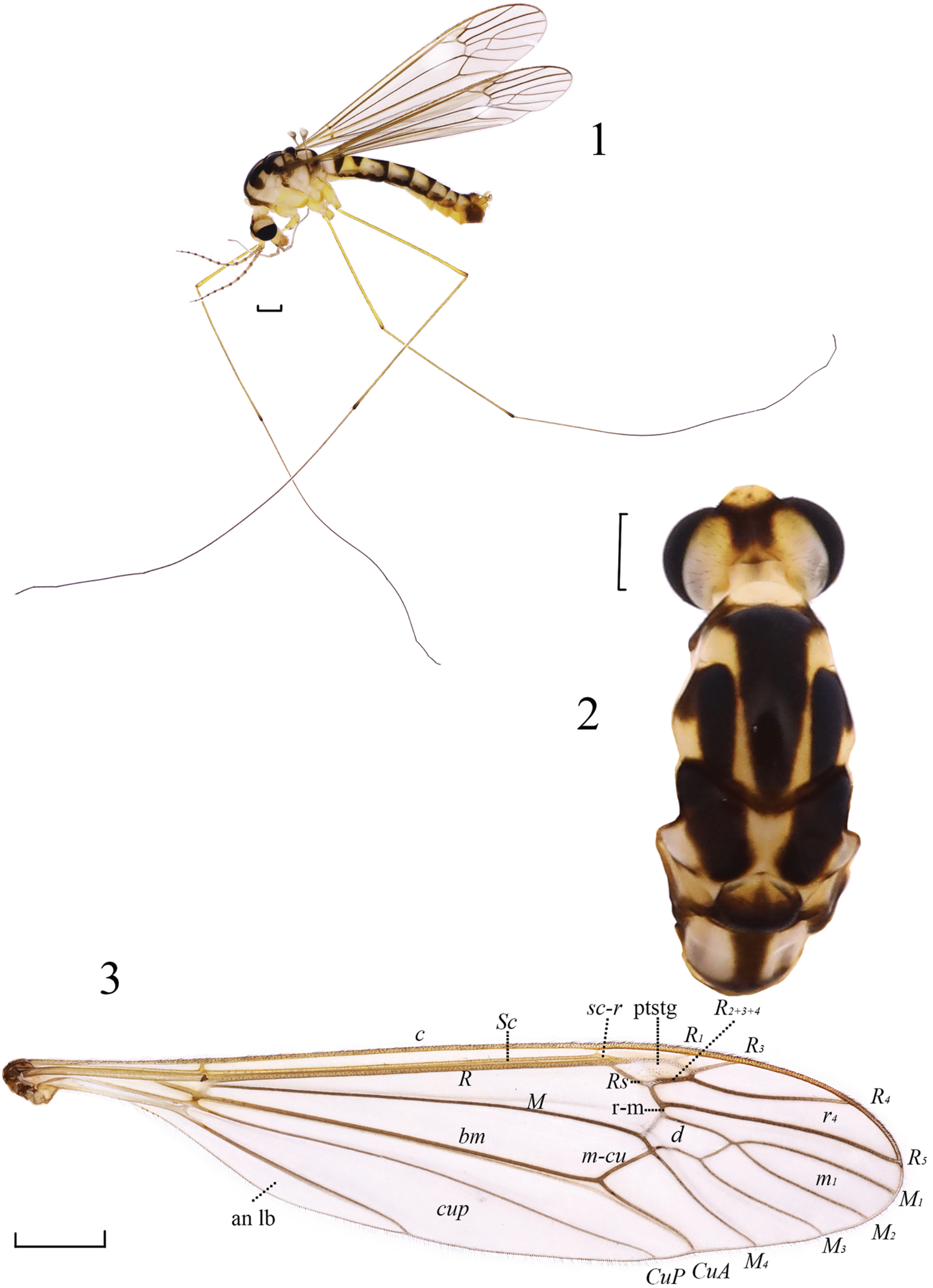
***Abdomen*** (Fig. 1). Mainly yellow. Tergites each with dark-brown, median spot and lateral, longitudinal stripe; median spot nearly square on tergite 1, fan-shaped on tergite 2, and those on tergite 3–7 triangular. Sternite 8 dark brown. Hairs on abdomen blackish.

***Hypopygium*** (Figs 4–9) mainly brown, posterior margin yellow. Posterior margin of tergite 9 medially produced with a deep notch, apices of produced part slightly depressed, with a pair of pointed lateral projections (Figs 7, 8). Mid-posterior margin of sternite 8 depressed, with round process (Fig. 9). Gonapophyses of adminiculum twisted (Fig. 9). Outer gonostylus fleshy, small, with curly tip (Fig. 6). Inner gonostylus with large concavity at base; beak slightly obtuse, slightly produced at top (Fig. 5).

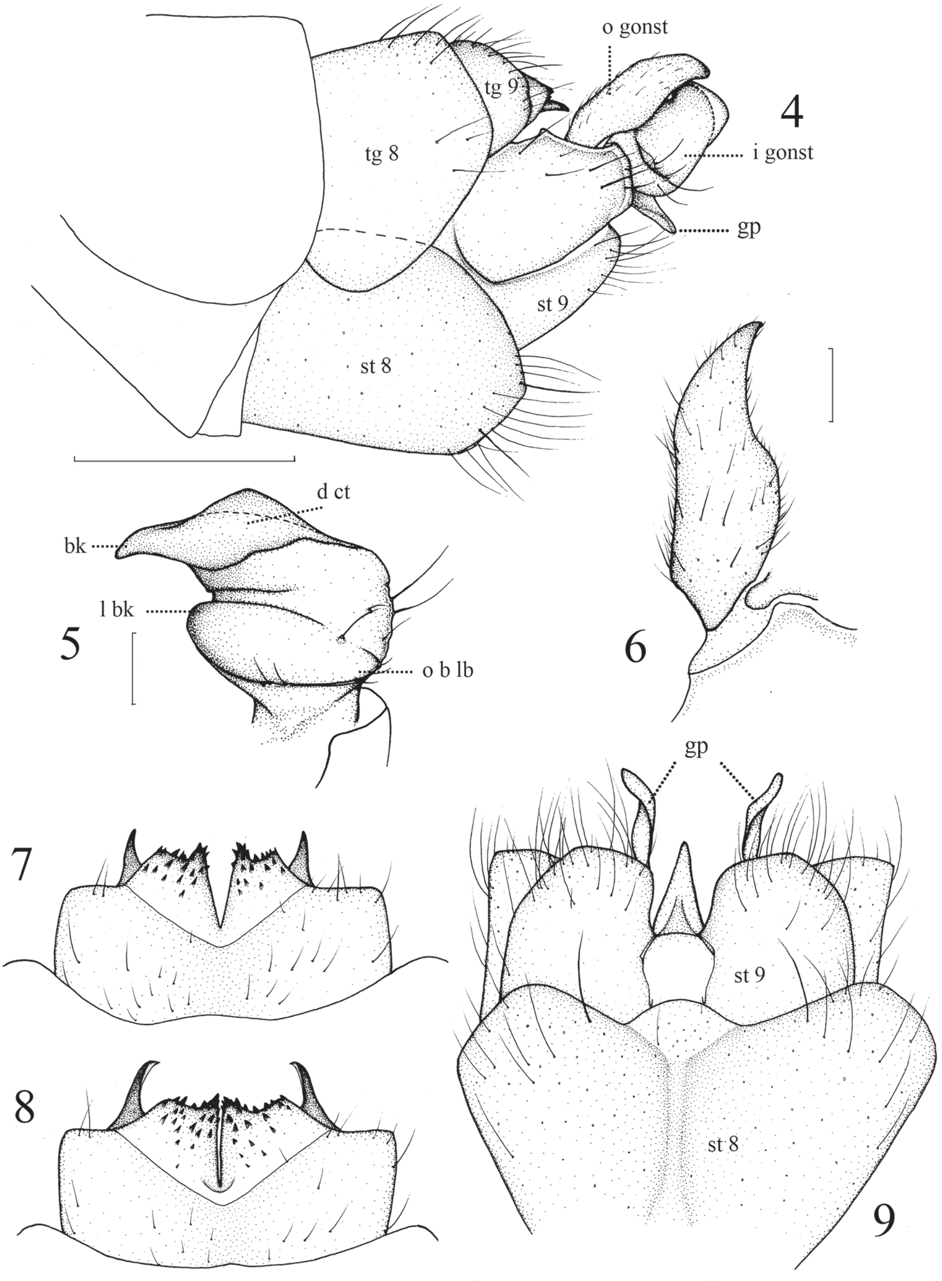
**Distribution.** China (Xizang).

**Remarks.** This new species is similar to *N. globate* Alexander, 1951 from India, but the latter differs in the following characters: tergite 9 with lateral projections tipped with two or three spicules; outer gonostylus long and slender; inner gonostylus long and narrow, lower beak like a slender blackened rod; sternite 8 with two tufts of hair.

**Etymology.** This species is named after the type locality, Beibeng, Xizang, China.



**Figures 1–3.** *Nephrotoma beibengensis* sp. nov. **1** Male habitus, lateral view **2** head and thorax, dorsal view **3** wing. Abbreviations: ptstg = pterostigma. Scale bars: 1.0 mm.



**Figures 4–9.** *Nephrotoma beibengensis* sp. nov. **4** Hypopygium, lateral view **5** inner gonostylus, lateral external view **6** outer gonostylus, lateral external view, before softened **7** tergite 9, dorsal view, softened **8** tergite 9, dorsal view. before softened **9** hypopygium, ventral view. Abbreviations: bk = beak, d ct = dorsal crest, gp = gonapophyses, l bk = lower beak, i gonst = inner gonostylus, o b lb = outer basal lobe, o gonst = outer gonostylus, pct= posterior crest, tg = tergite, st = sternite. Scale bars: 0.5 mm (**4, 7–9**); 0.1 mm (**5, 6**).

## ***Nephrotoma claviformis* Yang & Yang, 1987**

Figs 10–16

*Nephrotoma claviformis* Yang & Yang, 1987: 129. Type locality: China: Xizang, Nyingchi Co.

**Diagnosis.** Spots on head velvety. Vertex with rectangular, dark-brown spots near inner margin of eye. Scutellum dark brown. Mediotergite with a brown longitudinal spot at middle (Fig. 11). Tergite 9 with a pair of short rod-like projections (Fig. 12).

**Material examined.** 2 males (CAU), China: Xizang, Lulang, 1978.VI.10, 3400 m, Fasheng Li.

**Description.** Males (*n* = 2): body length 11.0–11.1 mm, wing length 11.8–12.0 mm, antenna length 3.0 mm.

***Head*** (Figs 10, 11). Mainly yellow. Vertex with velvety, dark-brown, rectangular spots near inner margin of eye. Occipital marking annular, velvety, dark brown. Face with short, linear marking. Dorsal part of rostrum including nasus brownish black. Head with brown hairs. Antenna brownish black, with dark-brown hairs, except scape and pedicel dark brownish yellow; first flagellomere 1.6 times as long as second one. Proboscis brown, with dark-brown hairs. Palpus brown, with brownish-yellow hairs.

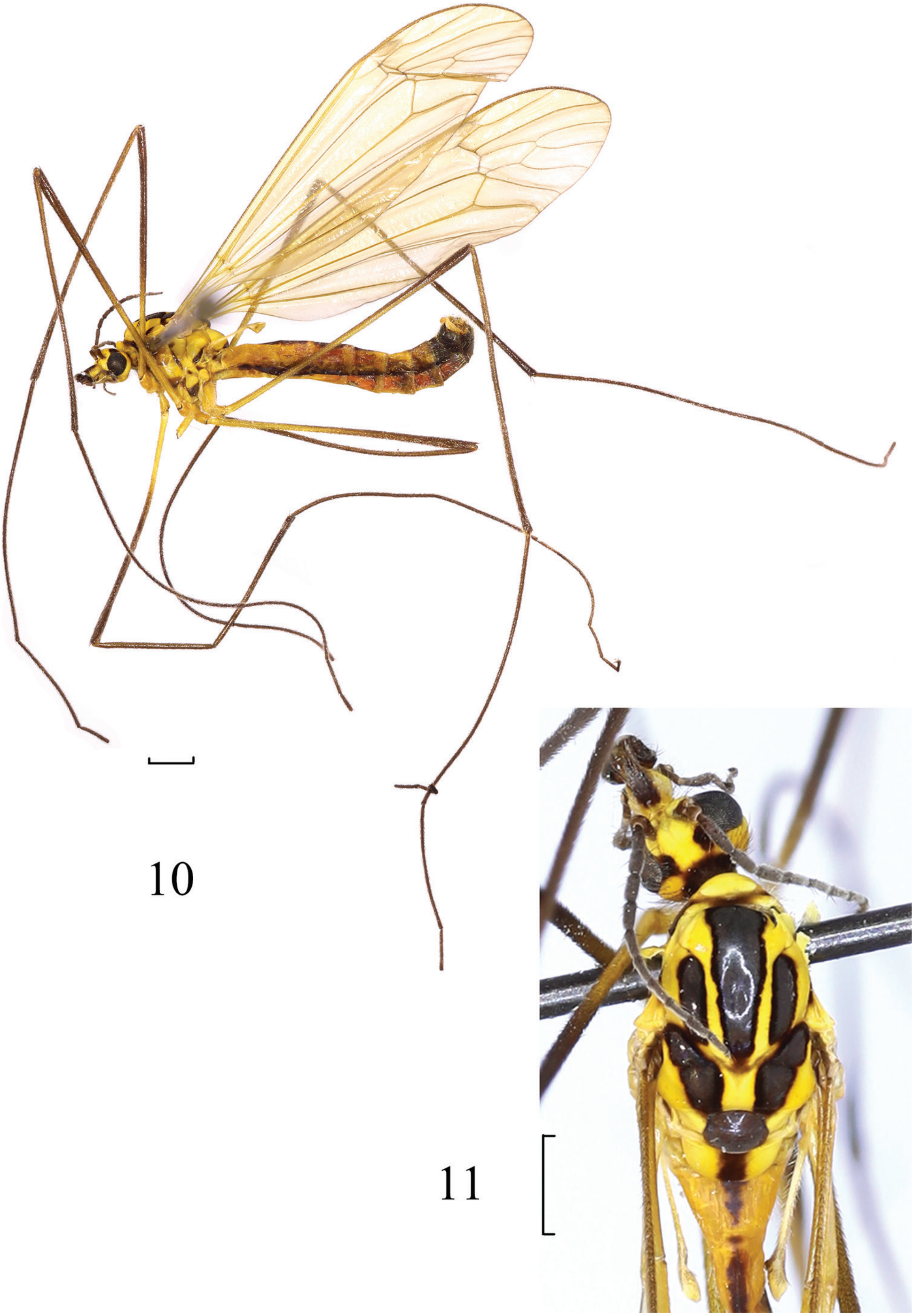
***Thorax*** (Figs 10, 11). Mainly yellow. Pronotum mainly yellow, with dark-brown spots on lateral side. Prescutum with three black, longitudinal stripes bearing velvety margin. Scutum with two black spots bearing velvety margin. Scutellum dark brown. Mediotergite yellow, with a brown, velvety, longitudinal stripe at middle. Anepisternum and katepisternum each with a brown lower spot and black posterior margin. Anepimeron and katepimeron each with a small, pale-brown spot. Meron with black lower part. Parascutellum yellow, anatergite yellow, katatergite with black posterior margin. Legs dark yellow except tips of tibiae, femora, and tarsi dark brown. Hairs on legs brownish, except those on coxae yellow. Wing subhyaline, tinged with light brown; pterostigma brownish; vein of cell $m_1$ sessile (Fig. 10). Halter with yellow stem; knob dark yellow.

***Abdomen*** (Fig. 10). Mainly yellow. Abdominal tergites with brown mid-longitudinal stripe and brownish-black lateral stripe. Hairs on abdomen yellow.

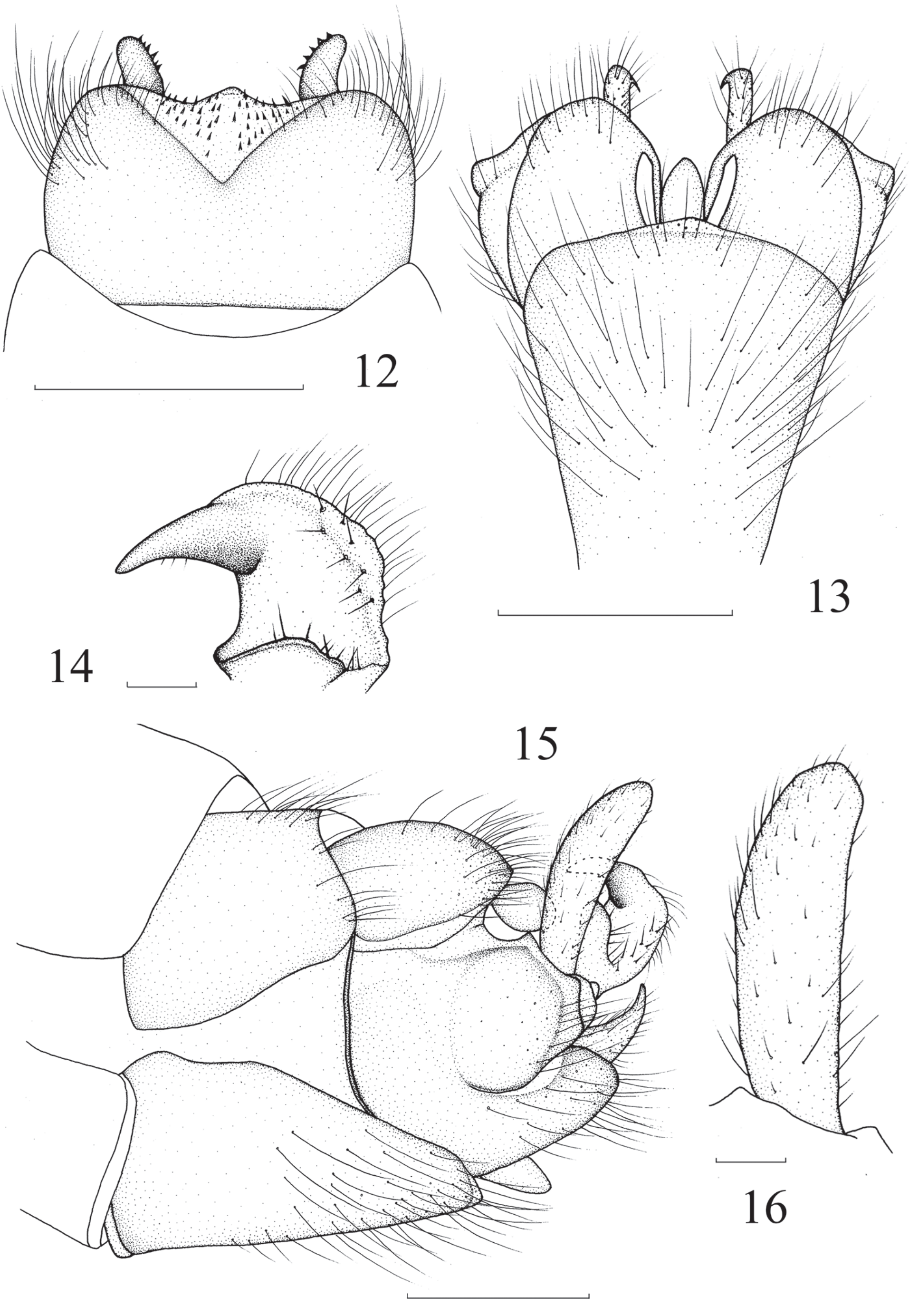
***Hypopygium*** (Figs 12–16) brownish black. Tergite 9 medially slightly produced at posterior margin, tergite 9 with a pair of rod-like projections on posterior margin (Fig. 12). Outer gonostylus short rod-like (Fig. 16). Inner gonostylus anteriorly with a sharp beak at tip, laterally with a lobe at base (Fig. 14).

**Distribution.** China (Xizang).

**Remarks.** This species is similar to *N. distans* Edwards, 1928 from Xizang, the inner gonostylus is similar, but the latter differs in the following characters: occipital marking triangular, scutellum and postnotum pale; tergite 9 without rod-like projections on posterior margin.



**Figures 10, 11.** *Nephrotoma claviformis* Yang & Yang, 1987. **10** Male habitus, lateral view **11** head and thorax, dorsal view. Scale bars: 1.0 mm.



**Figures 12–16.** *Nephrotoma claviformis* Yang & Yang, 1987. **12** Tergite 9, dorsal view **13** hypopygium, ventral view **14** inner gonostylus, lateral external view **15** hypopygium, lateral view **16** outer gonostylus, lateral external view. Scale bars: 0.5 mm (**12, 13, 15**); 0.1 mm (**14, 16**).

## *Nephrotoma didyma* Yang & Yang, 1987
Figs 17–25

*Nephrotoma didyma* Yang & Yang, 1987: 131. Type localty: China: Xizang, Mainling Co.

**Diagnosis.** Pronotum yellow with a brownish spot on lateral side. Mediotergite yellow with dark-brown posterior margin (Figs 17, 19). Posterior margin of tergite 9 with a jaw-shaped protuberance; spade-shaped protuberance with two short horns at posterior margin (Fig. 22). Posterior margin of sternite 8 with meniscus-shaped protrusion (Figs 20, 23).

**Material examined.** 4 males (CAU), China: Xizang, Yigong, 2017.VI.7, 2236 m, Qicheng Yang (light trap). 3 males (CAU), China: Xizang, Yigong, 2017.V 6, 2274 m, Qicheng Yang (light trap). 6 males (CAU), China: Xizang, 106K, 2017.V.17, 2289 m, Qicheng Yang (light trap).

**Description.** Male (*n* = 13): body length 9.5–12.0 mm, wing length 10.0–12.0 mm, antenna length 3.0–4.0 mm.

***Head*** (Figs 17, 19). Mainly yellow. Vertex with brown spot near inner margin of eye. Occipital marking dark brown, bell-shaped, extended to top of vertex. Frontal tubercle relatively high. Dorsal part of rostrum including nasus brownish black. Hairs on head dark brown. Antenna dark brown except scape yellow and pedicel dark yellow; first flagellomere 1.4 times longer than second one. Proboscis yellow, with brown hairs. Palpus brownish grey, with brown hairs.

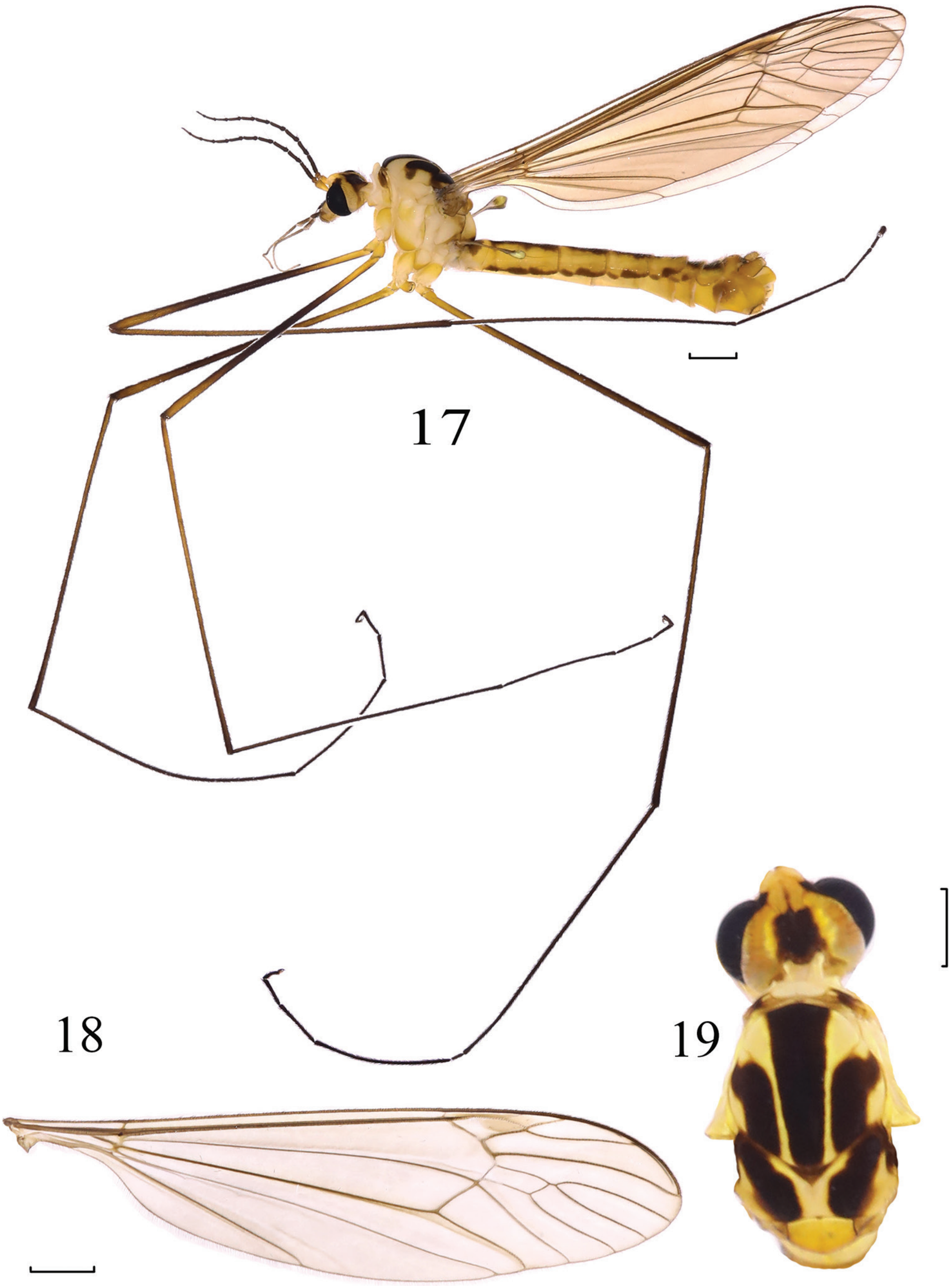
***Thorax*** (Figs 17, 19). Mainly yellow. Pronotum yellow with a brownish spot on lateral side. Prescutum with three black longitudinal stripes; anterior end of lateral prescutal stripe bent outward, outer part brown. Scutum with two large, dark-brown spots. Scutellum dark yellow. Mediotergite yellow with dark-brown posterior margin. Anepisternum and katepisternum pale yellow with yellow lower part. Anepimeron pale yellow. Parascutellum pale brown; anatergite and katatergite pale yellow. Legs yellow except middle portions of femora, tips of tibiae, and tarsi dark brown; hairs dark brown, except those on coxae and trochanters yellow. Wing subhyaline, tinged with light brown; pterostigma pale brown; cell $m_1$ nearly sessile (Fig. 18). Halter with stem brownish grey; knob yellowish brown.

***Abdomen*** (Fig. 17). Mainly yellow. Abdominal tergites with three brown longitudinal stripes. Hypopygium mainly dark yellow. Abdomen with yellow hairs.

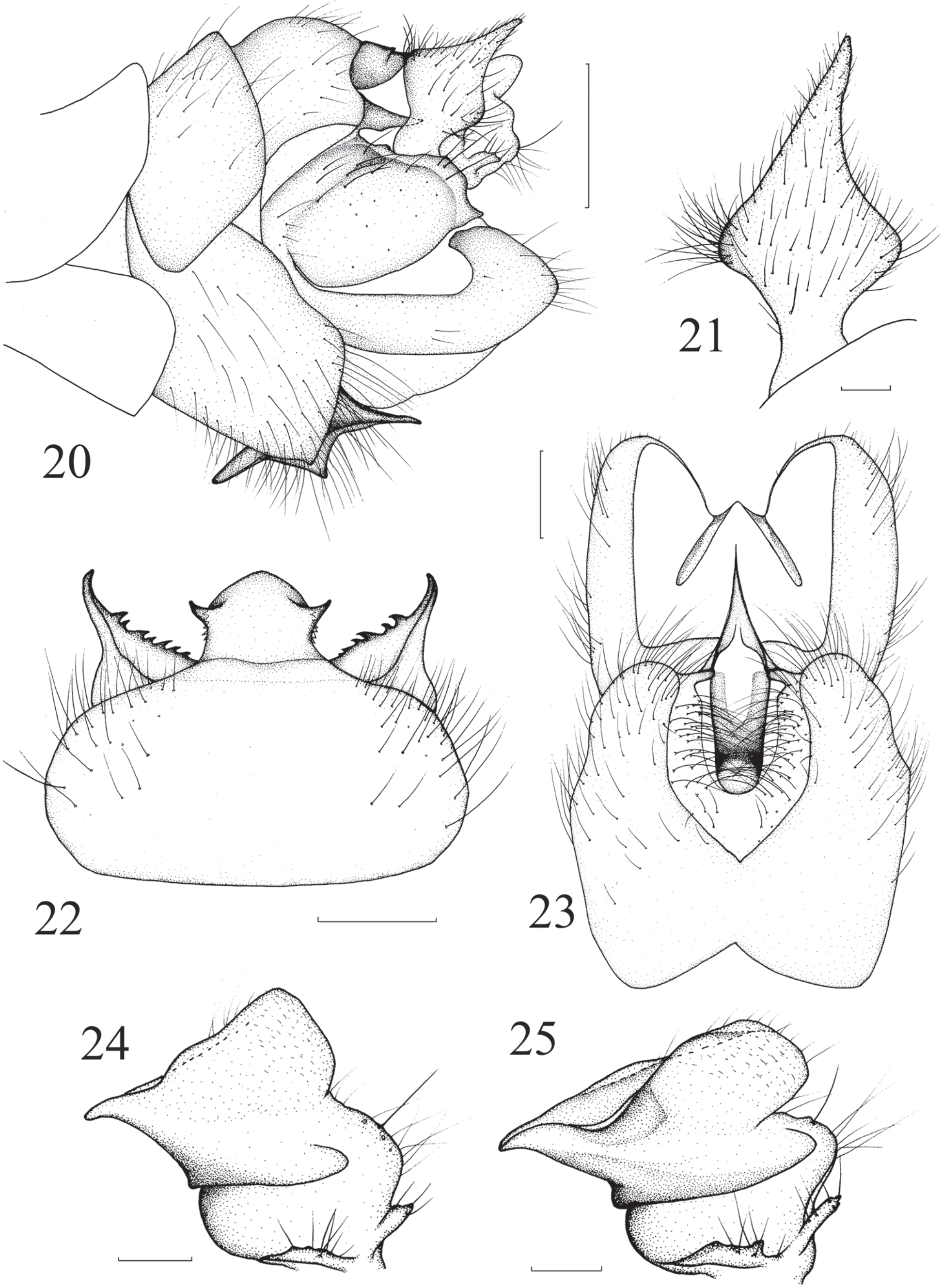
***Hypopygium*** (Figs 20–25) brownish yellow. Tergite 9 ellipse with spade-shaped protuberance bearing two short horns at posterior margin; posterolateral margin of tergite 9 with a large jaw-shaped protuberance (Fig. 22). Posterior margin of sternite 8 with obvious appendages, meniscus-shaped in lateral view, covered with dense yellow hairs (Fig. 20). Outer gonostylus fleshy, spade-shaped, wide at middle, narrowed toward tip (Fig. 21). Inner gonostylus with large concavity, basally with a posterior projection (Figs 24, 25).

**Distribution.** China (Xizang).

**Remarks.** Hypopygium of this species is very specific; no similar species.



**Figures 17–19.** *Nephrotoma didyma* Yang & Yang, 1987. **17** Male habitus, lateral view **18** wing **19** head and thorax, dorsal view. Scale bars: 1.0 mm.



**Figures 20–25.** *Nephrotoma didyma* Yang & Yang, 1987. **20** Hypopygium, lateral view **21** outer gonostylus, lateral external view **22** tergite 9, dorsal view, softened **23** hypopygium, ventral view **24** inner gonostylus, lateral external view, softened **25** inner gonostylus, lateral external view, before softened. Scale bars: 0.5 mm (**20, 22, 23**); 0.1 mm (**21, 24, 25**).

### ***Nephrotoma distans*** **Edwards, 1928**

*Nephrotoma distans* Edwards, 1928: 700. Type locality: China: Tibet, Rongshar Valley.

**Diagnosis.** Vertex with triangular black spot near inner margin of eye. Prescutum with narrow black margin. Scutellum dark. Wing completely brownish. Gonapophyses of adminiculum hooked (Edwards 1928).

**Distribution.** China (Sichuan and Yunnan).

### ***Nephrotoma evittata*** **Alexander, 1935**

Figs 26–33

*Nephrotoma evittata* Alexander, 1935: 200. Type locality: China: Szechwan, Shin-Kai-Si, Mount Omei.

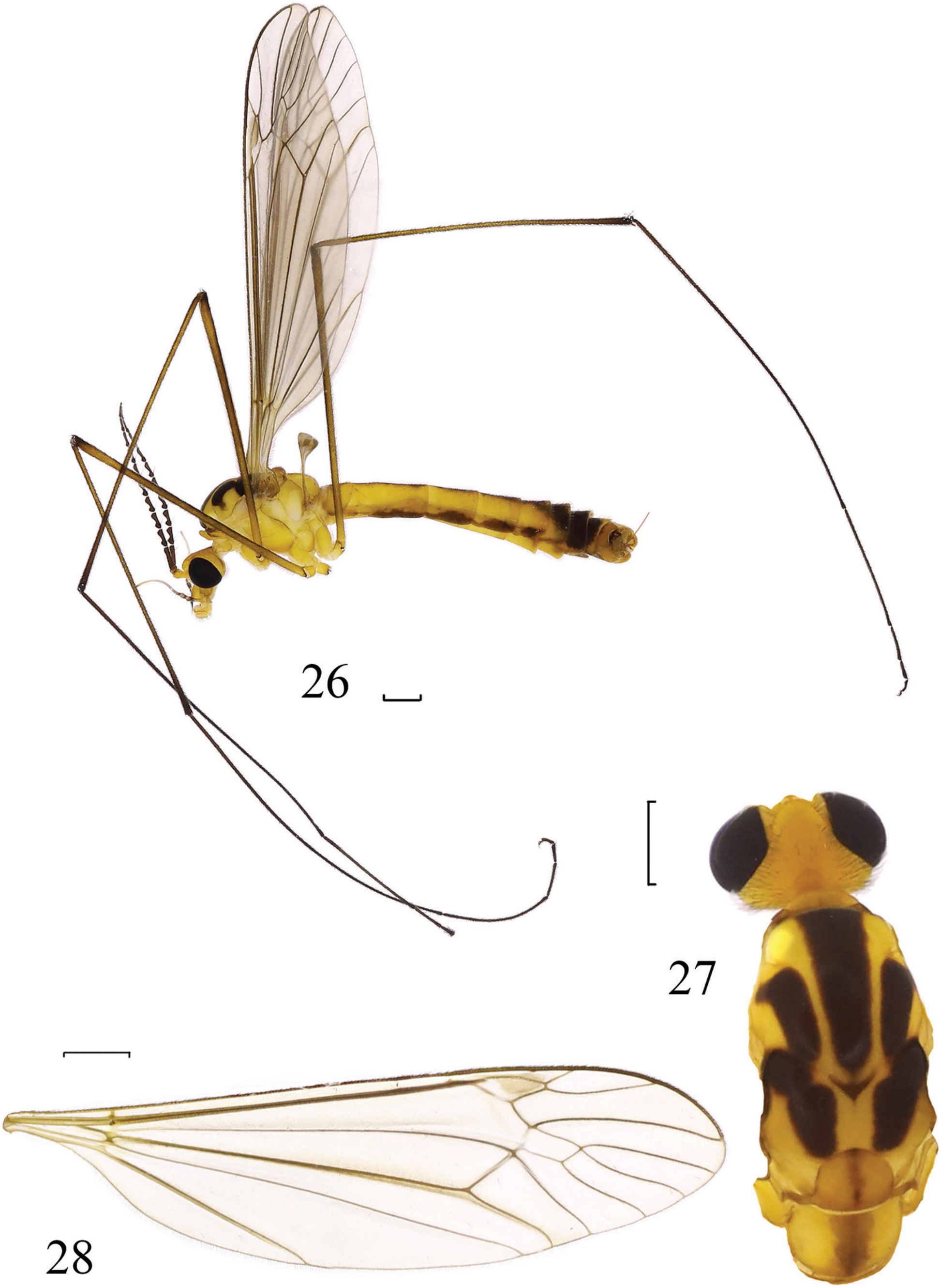
**Diagnosis.** Antenna mainly dark brown except scape yellow, pedicel and first flagellomere brown; both ends of flagellomere with obvious expansion (Fig. 26). Scutellum dark yellow, with brown middle stripe. Mediotergite yellow with brown to dark-yellow middle stripe (Fig. 27). Tergite 9 semicircular, depressed at middle, with a pair of sharp short protuberances at posterior margin (Fig. 32). Inner gonostylus with flat lower beak bearing bristles (Fig. 31).

**Material examined.** 2 males (CAU), China: Xizang, Yigong, 2017.VI.7, 2236 m, Qicheng Yang (light trap). 2 males (CAU), China: Xizang, Yigong, 2017.VIII.5, 2183 m, Qicheng Yang (light trap). 1 male (CAU), China: Xizang, 80K, 2017.VI.13, 2023 m, Qicheng Yang (light trap). 1 male (CAU), China: Xizang, 106K, 2017.V.17, 2289 m, Qicheng Yang (light trap).

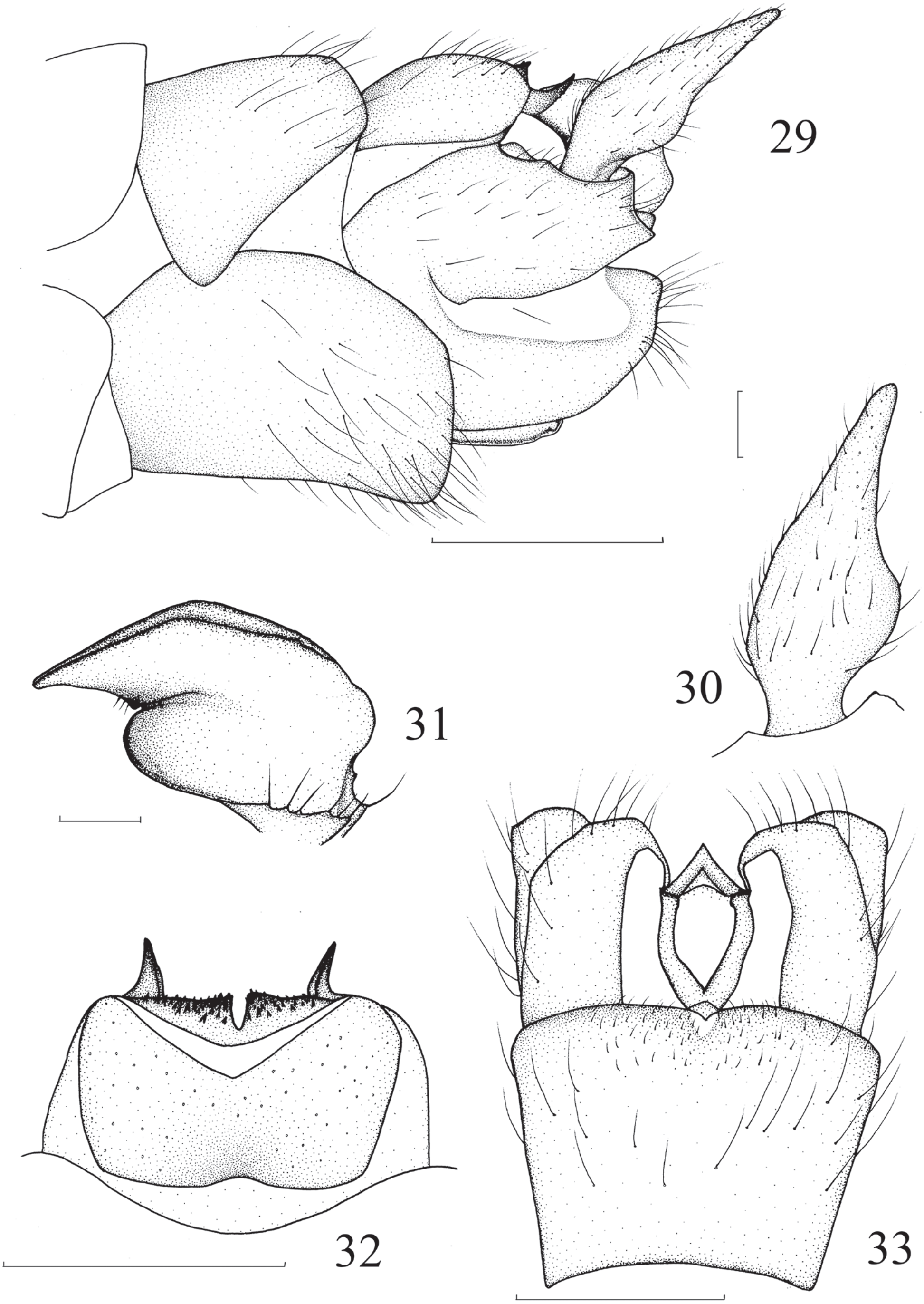
**Description.** Male ($n$ = 6): body length 11.0–12.5 mm, wing length 11.0–12.0 mm, antenna length 2.5–3.5 mm.

***Head*** (Figs 26, 27). Mainly yellow. Occipital marking brown, faint. Frontal tubercle relatively high. Dorsal part of rostrum, including nasus, dark brown. Head with black hairs. Antenna dark brown except scape yellow, pedicel and first flagellomere brown; first flagellomere 1.2 times as long as second one; both ends of flagellomere obvious expansion. Proboscis mainly yellow, with black hairs. First palpal segment yellow, second one brown, with black hairs.

***Thorax*** (Figs 26, 27). Mainly yellow. Pronotum yellow, pale brown on lateral side. Prescutum with three black longitudinal stripes; anterior end of lateral prescutal stripe bent outward, outer part brown. Scutum with four black spots. Scutellum dark yellow, with brown middle stripe. Mediotergite yellow, with brown to dark-yellow middle stripe. Anepisternum and katepisternum yellow; katepisternum with yellow lower part. Anepimeron pale yellow, lower part dark yellow. Anatergite and katatergite yellow. Parascutellum yellow. Legs yellow except tips of tibiae brown and tarsi dark brown; hairs dark brown except those



**Figures 26–28.** *Nephrotoma evittata* Alexander, 1935. **26** Male habitus, lateral view **27** head and thorax, dorsal view **28** wing. Scale bars: 1.0 mm.



**Figures 29–33.** *Nephrotoma evittata* Alexander, 1935. **29** Hypopygium, lateral view **30** outer gonostylus, lateral external view **31** inner gonostylus, lateral external view **32** tergite 9, dorsal view **33** hypopygium, ventral view. Scale bars: 0.5 mm (**29, 32, 33**); 0.1 mm (**30, 31**).

on coxae dark yellow. Wing subhyaline, tinged with light brown; pterostigma slightly deepened; cell $m_1$ shortly petiolate (Fig. 28). Halter with stem pale brown; knob pale yellow.

***Abdomen*** (Fig. 26). Mainly yellow. Abdominal tergites with three brown longitudinal stripes. Abdominal segments 6–8 entirely dark brown to black; hypopygium mainly yellow; tergite 9 brown or yellow. Hairs on abdomen blackish or yellow.

***Hypopygium*** (Figs 29–33) brownish yellow. Tergite 9 semicircular, depressed at middle, with a pair of sharp short protuberances on posterior margin (Fig. 32). Posterior margin of sternite 8 with dense bristles at middle (Fig. 33). Outer gonostylus fleshy, protruded at middle, narrowed toward tip (Fig. 30). Inner gonostylus flat, with large concavity at base; lower beak with bristles (Fig. 31).

**Distribution.** China (Sichuan, Yunnan and Xizang).

**Remarks.** First record for Xizang. This species is similar to *N. impigra impigra* Alexander, 1935 from China (Hubei, Sichuan, Zhejiang, Fujian, Guizhou, Jiangxi), but the latter differs in the following characters: occipital marking distinct; scutellum dark brown. The hypopygium of this species is consistent with holotype, and we found that its occipital marking is variable. We think the difference between the veins is caused by the origin, which is also variable.

### ***Nephrotoma hanae*** **sp. nov.**

http://zoobank.org/25F93152-725A-41E5-913D-A266A4FFF631
Figs 34–42

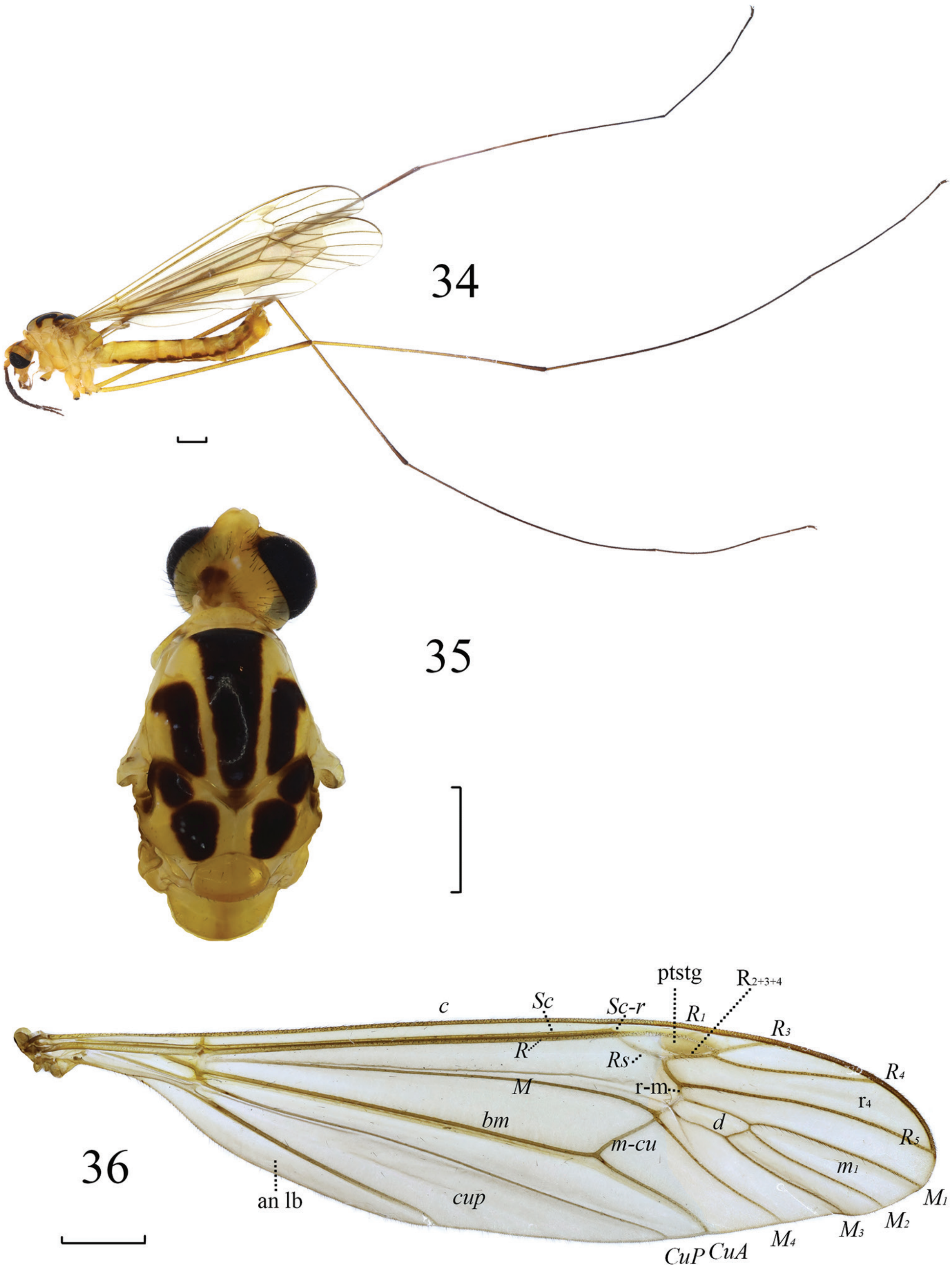
**Diagnosis.** Occipital marking variable, small, brown. Antennal scape yellow, pedicel brown, flagellum brownish black (Figs 34, 35). Tergite 9 medially produced with a pair of projections and a subequal notch at posterior margin; laterally with a pair of pointed projections with spines (Fig. 42). Sternite 8 depressed at posterior margin, medially with a sickle-shaped, fleshy appendage bearing long apical hairs (Figs 37, 39). Outer gonostylus slightly expanded anteriorly (Fig. 38). Inner gonostylus with a weak protuberance at posterior margin (Figs 40, 41).

**Material examined.** ***Holotype*** male (CAU), China: Xizang, Bomi (Pome), 2016. VII.12–26, 2700 m, Shaolin Han (light trap). ***Paratype***: male (CAU), China: Xizang, Bomi, Bagai, 2018.VII.1–2, 2823 m, Qicheng Yang (light trap). 1 male, 1 female (CAU), China: Xizang, Yadong, 2018.VII.12, 4000 m, Yajun Zhu. 1 male, 2 female (CAU), China: Xizang, Bomi, Bagai, 2018.VII.1–2, 2823 m, Qicheng Yang (light trap).

**Description.** Male ($n$ = 4): body length 10.3–10.6 mm, wing length 11.0–11.6 mm, antenna length 2.8–3.0 mm.

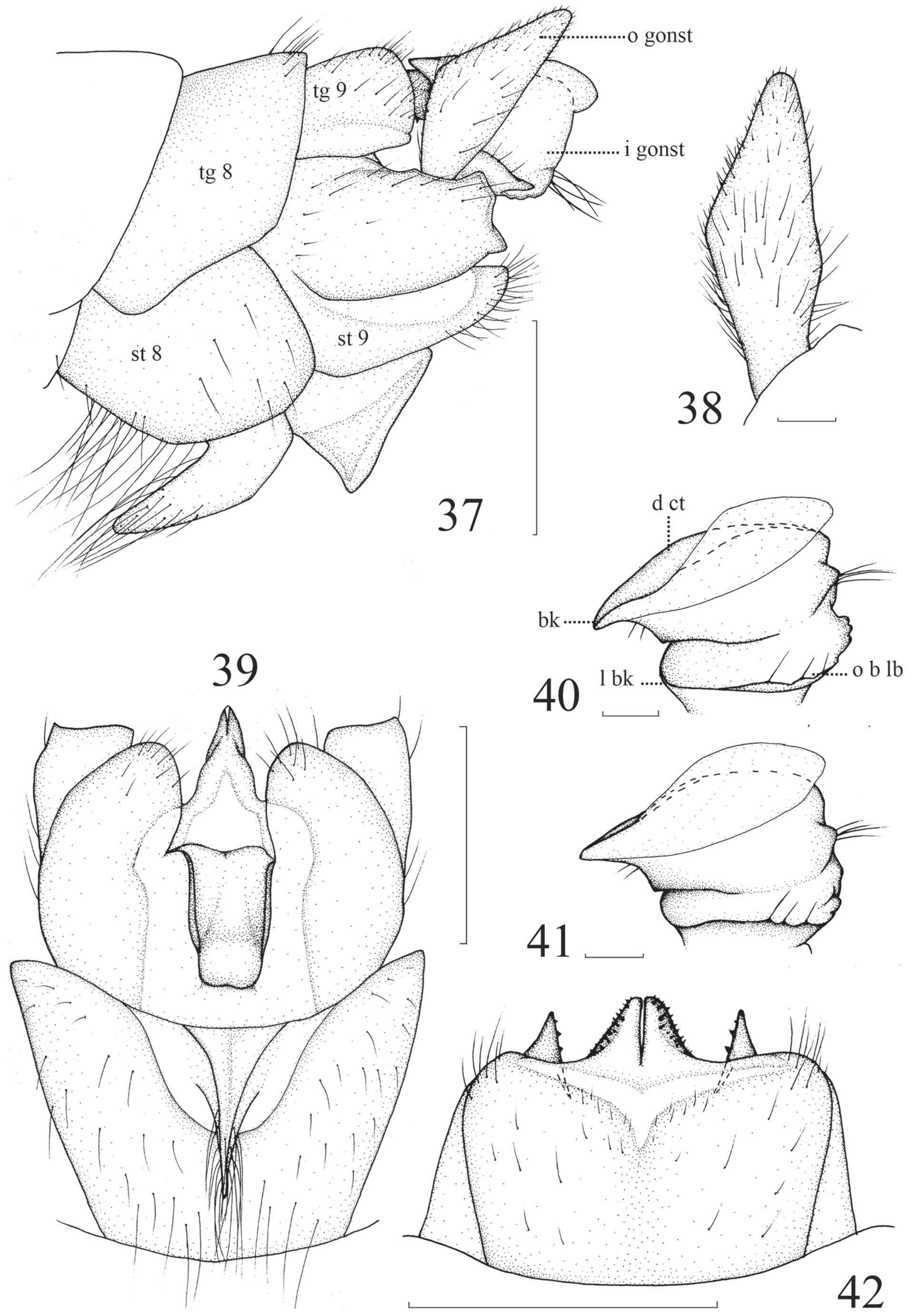
***Head*** (Figs 34, 35). Mostly yellow. Occipital marking variable, small, brown. Frontal tubercle relatively high. Rostrum and nasus brown. Hairs on head dark brown. Antennal scape yellow, pedicel brown, flagellum brownish black; first flagellomere 1.3 times longer than second segment. Proboscis brown, with dark-brown hairs. Palpus brownish yellow, with dark-brown hairs.

***Thorax*** (Figs 34, 35). Mostly yellow. Pronotum yellow, with a small, pale-brown spot on lateral side. Prescutum with three black longitudinal stripes; anterior end of



**Figures 34–36.** *Nephrotoma hanae* sp. nov. **34** Male habitus, lateral view **35** head and thorax, dorsal view **36** wing. Abbreviations: ptstg = pterostigma. Scale bars: 1.0 mm.

lateral prescutal stripe bent outward, outer part brown. Scutum with four brownish-black spots, anterior margin of lateral spot brown. Scutellum and mediotergite yellow. Pleuron pale yellow, but lower part of katepisternum and katatergite yellow.



**Figures 37–42.** *Nephrotoma hanae* sp. nov. **37** Hypopygium, lateral view **38** outer gonostylus, lateral external view **39** hypopygium, ventral view **40, 41** inner gonostylus, lateral external view **42** tergite 9, dorsal view. Abbreviations: bk = beak, d ct = dorsal crest, l bk = lower beak, i gonst = inner gonostylus, o b lb = outer basal lobe, o gonst = outer gonostylus, tg = tergite, st = sternite. Scale bars: 0.5 mm (**37, 39, 42**); 0.1 mm (**38, 40, 41**).

Parascutellum yellow. Legs yellow, except tips of femora dark yellow, tips of tibiae and tarsi dark brown; hairs brown, except those on coxae and trochanters yellow. Wing subhyaline, tinged with light brown; pterostigma brown; cell $m_1$ sessile, cell $m_1$ and cell d narrow; apices of $R_5$ not curving up (Fig. 36). Halter pale yellowish brown.

***Abdomen*** (Fig. 34). Mainly yellow. Abdominal tergites each with three brown spots, lateral spots rather narrow and fused with each other; tergite 1 without spot, median spot on tergites 2 and 3 roughly rectangular, on tergites 4–8 subtriangular. Abdomen with yellow and brown hairs.

***Hypopygium*** (Figs 37–42) mainly dark yellow. Tergite 9 medially produced, with notch, lateral side with a pointed projection with spines (Fig. 42). Sternite 8 depressed at posterior margin, medially with a sickle-shaped, fleshy appendage bearing long apical hairs (Figs 37, 39). Sternite 9 with a subtriangular appendage (Figs 37, 39). Outer gonostylus fleshy, small, anteriorly slightly expanded, apically pointed (Fig. 38). Inner gonostylus with concavity at base; beak sharp, posterior margin with a small protuberance with five bristles (Figs 40, 41).

**Distribution.** China (Xizang).

**Remarks.** This new species is similar to *N. korpa* Alexander, 1967 from India (Sikkim), but the latter differs in the following characters: posterior vertex with three brown spots at narrowest point; projections of tergite 9 smaller, but lateral projections without spines; outer gonostylus unusually long, basally expanded.

**Etymology.** This species is named after the collector, Shaolin Han.

## *Nephrotoma inorata* Alexander, 1951

*Nephrotoma inorata* Alexander, 1951: 1096. Type locality: China: south-eastern Tibet, Eong To Valley.

**Diagnosis.** Frontal tubercle slightly forked, clover-shaped. Scutum and scutellum with brownish longitudinal spot at middle. Posterior margin of mediotergite slightly darker. Sternite 8 with long hairs, but without process at posterior margin (Alexander 1951, 1967).

**Distribution.** China (Xizang), India (W Bengal).

## *Nephrotoma kaulbacki* Alexander, 1951

*Nephrotoma kaulbacki* Alexander, 1951: 1094. Type locality: China: eastern Tibet, Poshö, Kyari Dzong.

**Diagnosis.** Antennal scape and pedicel pale yellow, flagellum black. Scutellum and mediotergite without spot. Tergite 9 with a deep median notch. Sternite 8 with a very deep and narrow notch at posterior margin. Gonapophyses of adminiculum hooked (Alexander 1951, 1953).

**Distribution.** China (Xizang).

### *Nephrotoma libra* Alexander, 1951

*Nephrotoma libra* Alexander, 1951: 1092. Type locality: China: Tibet, Gyantse.

**Diagnosis.** Antennal scape reddish brown, pedicel blackish brown, flagellum black. Mediotergite with a pair of black spots. Tergite 9 with thick, black caudal margin, lateral angle produced into a decurved spine. Sternite 8 without process. Sternite 9 with a finger-like process. Gonapophyses of adminiculum hooked (Alexander 1951, 1953).

**Distribution.** China (Xizang).

### *Nephrotoma nigrohalterata* Edwards, 1928

Figs 43–51, 62

*Nephrotoma nigrohalterata* Edwards, 1928: 700. Type locality: China: Szechwan-Tibet border, Yien-Long-Shien.

*Nephrotoma attenuata* Alexander, 1935: 135. Type locality: China: Szechwan-Tibet border, Yien-Long-Shien.

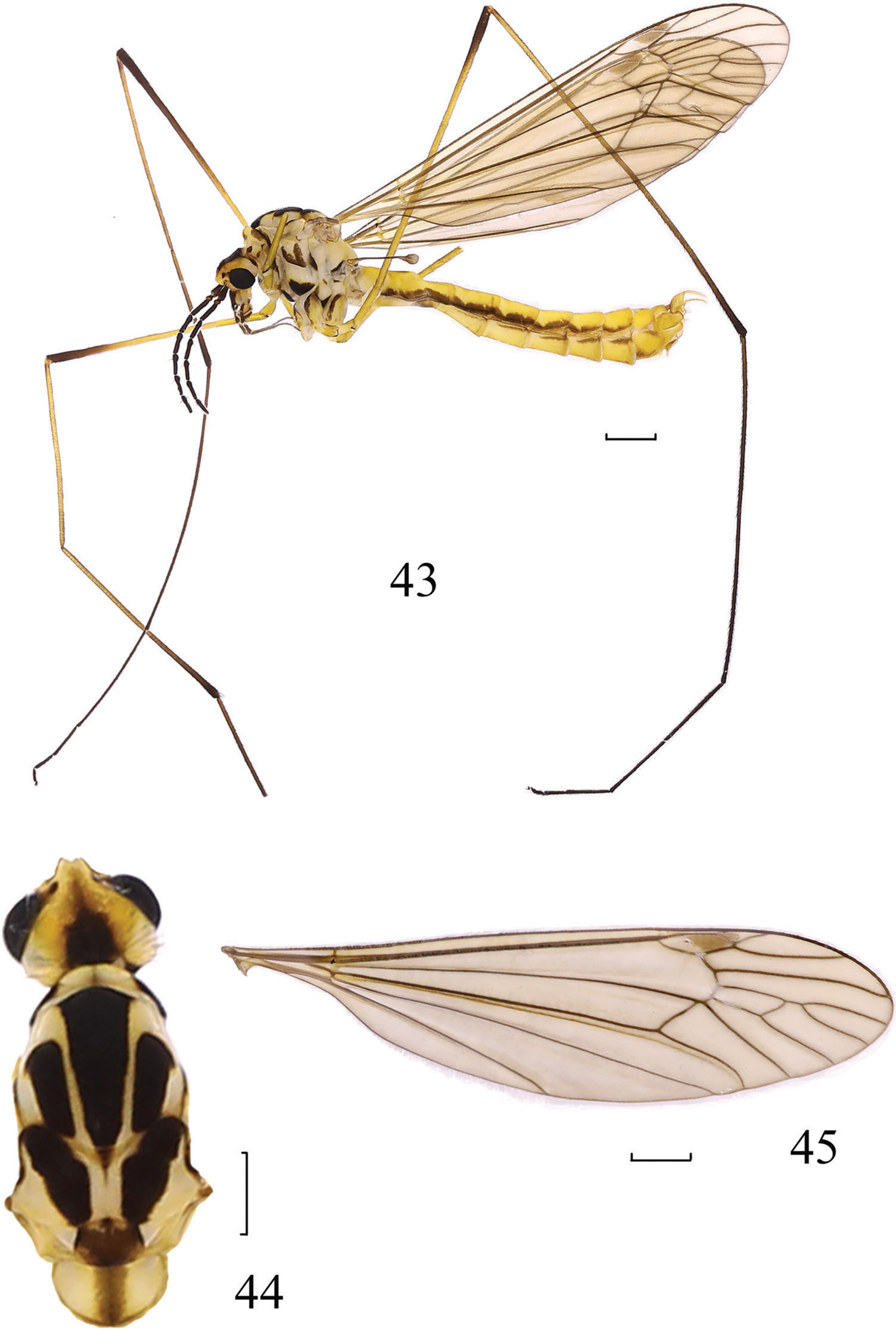
**Diagnosis.** Vertex with round, dark-brown spot near inner margin of eye; dorsal part of rostrum including nasus brownish black. Antenna black (Figs 43, 44). Tergite 9 distinctly depressed (Fig. 46). Sternite 9 with a brown, horn-like projection. Outer gonostylus greatly produced and slender (Figs 47, 50).

**Material examined.** 12 male (CAU), China: Xizang, Bayi, 2017.VI.2, 2950 m, Qicheng Yang (light trap).

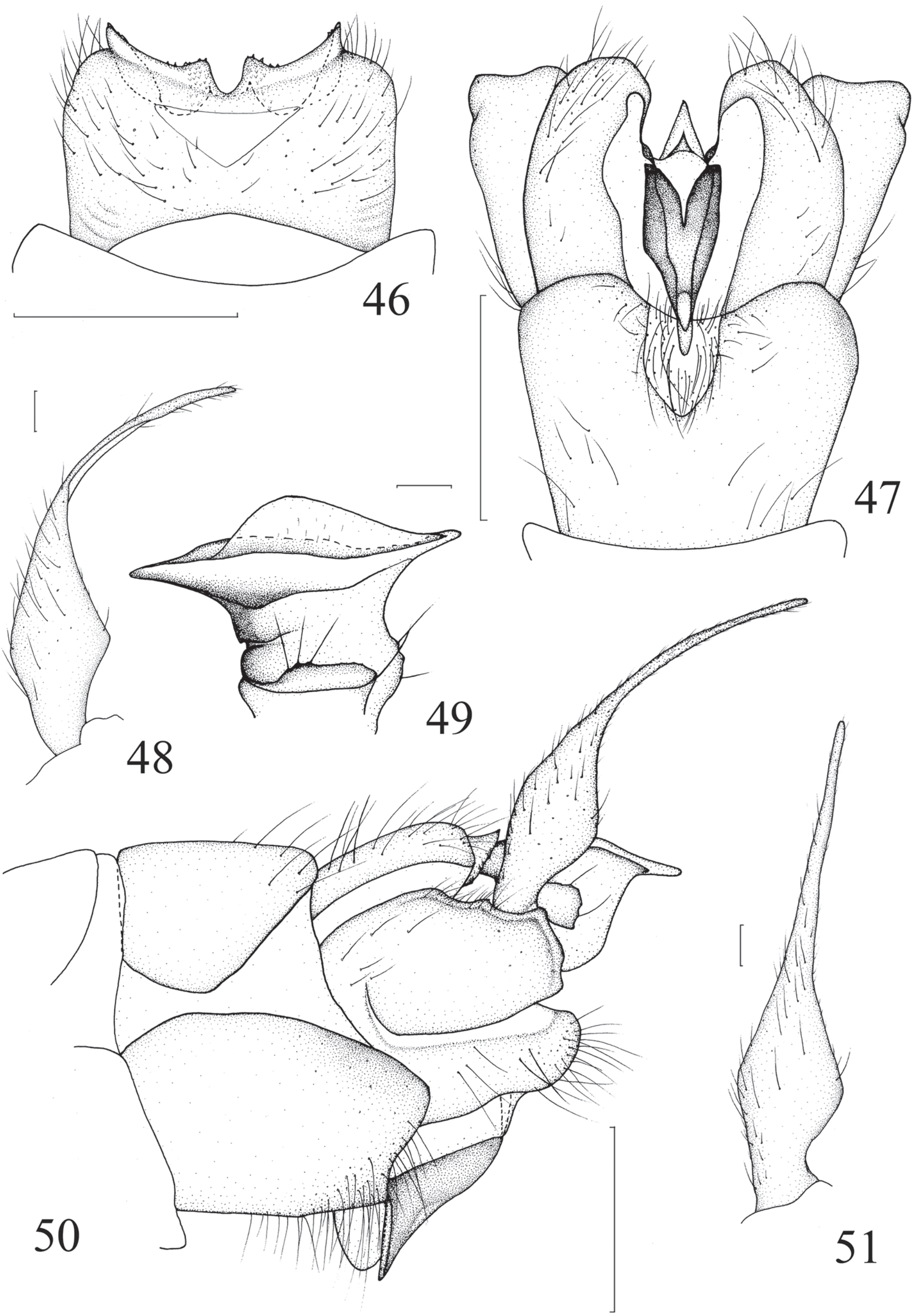
**Description.** Male (*n* = 12): body length 10.5–12.5 mm, wing length 10.5–12.5 mm, antenna length 3.5–5.0 mm.

***Head*** (Figs 43, 44). Mainly yellow. Vertex with round, dark-brown spot near inner margin of eye. Occipital marking dark brown, subtriangular. Face with obvious linear marking. Posterior margin of postgena brown. Dorsal part of rostrum including nasus brownish black. Head with black hairs. Antenna black with dense villi; first flagellomere 1.2 times longer than second one. Proboscis mainly yellow, with brown hairs. Palpus greyish brown, with brown hairs.

***Thorax*** (Figs 43, 44). Mainly yellow. Pronotum mainly yellow, with large, brown spots on lateral side. Prescutum with three black longitudinal stripes, middle stripe extended to scutum, anterior end of lateral prescutal stripe slightly bent outward. Scutum with two black spots. Scutellum dark brown. Mediotergite with a brown longitudinal stripe at middle. Anepisternum and katepisternum each with large black spot, posterior margin of anepisternum and katepisternum black; spot of anepisternum tilted V-shaped. Anepimeron and katepimeron each with small pale brown spot. Meron with black lower portion. Anatergite yellow, katatergite with black lower margin. Parascutellum yellow. Legs yellow, except anterior margin of coxae brown black, tips of tibiae and femora brown; hairs brownish except those on coxae yellow. Wing subhyaline, tinged



**Figures 43–45.** *Nephrotoma nigrohalterata* Edwards, 1928. **43** Male habitus, lateral view **44** head and thorax, dorsal view **45** wing. Scale bars: 1.0 mm.



**Figures 46–51.** *Nephrotoma nigrohalterata* Edwards, 1928. **46** Tergite 9, dorsal view, softened **47** hypopygium, ventral view **48** outer gonostylus, lateral external view, before softened **49** inner gonostylus, lateral external view **50** hypopygium, lateral view **51** outer gonostylus, lateral external view, softened. Scale bars: 0.5 mm (**46, 47, 50**); 0.1 mm (**48, 49, 51**).

with light brown; pterostigma greyish brown; vein of cell $m_1$ shortly petiolate, cell d almost as long as cell $m_1$ (Fig. 45). Halter with stem brown; knob yellowish brown.

***Abdomen*** (Fig. 43). Mainly yellow. Abdominal tergites with three dark-brown longitudinal stripes. Abdomen with yellow hairs.

***Hypopygium*** (Figs 46–51) yellow. Posterior extension of tergite 9 depressed, with two pairs of short projections. Posterior margin of sternite 8 depressed, medially with long hairs, produced ventrad into a small, pale, fleshy lobe (Figs 46, 47). Sternite 9 with a brown, horn-like projection (Figs 47, 50). Outer gonostylus greatly produced and slender (Figs 48, 51). Inner gonostylus with sharp beak, posterior crest produced backward; outer basal lobe with three strong, black setae (Fig. 49).

**Distribution.** China (Sichuan, Xizang)

**Remarks.** This species is similar to *N. geniculata* Yang & Yang, 1987 from China (Hubei, Inner Mongolia, Ningxia, Sichuan), but the latter differs in the following characters: without occipital marking; dorsal part of rostrum including nasus yellow. Posterior margin of sternite 8 undepressed, without fleshy lobe ventrally.

## ***Nephrotoma xizangensis*** **Yang & Yang, 1987**

Figs 52–59, 61

*Nephrotoma xizangensis* Yang & Yang, 1987: 129. Type locality: China: Xizang, Nyingchi Co.

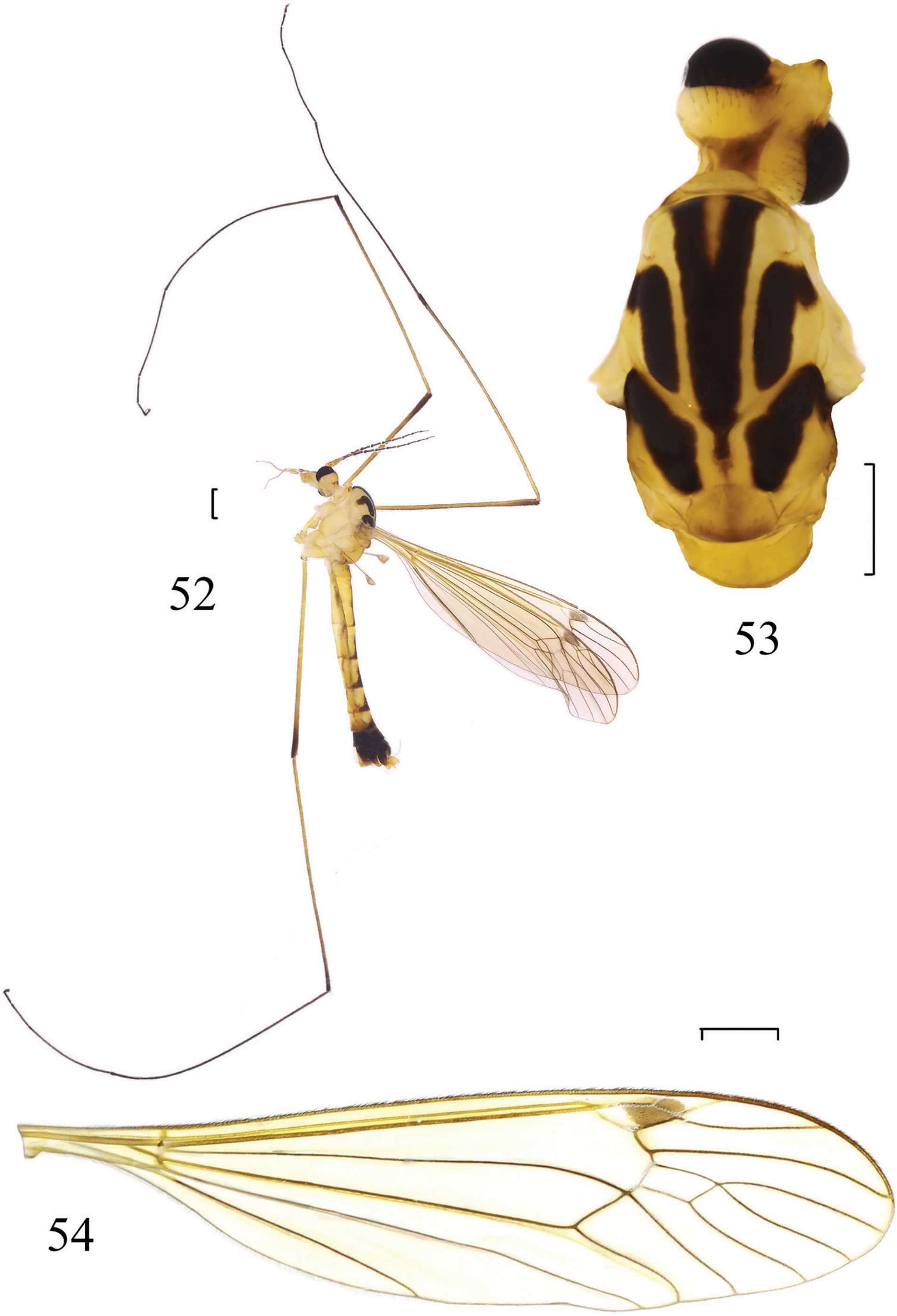
**Diagnosis.** Antennal scape yellow, pedicel pale brown, flagellum mainly dark brown, except first flagellomere pale brown (Fig. 52). Mid-longitudinal stripe of prescutum V-shaped. Scutellum with pale brown middle stripe and brown posterior margin. Mediotergite yellow (Fig. 53). Posterior extension of tergite 9 slightly depressed, with two pairs of short obtuse projections (Fig. 58).

**Material examined.** 2 males (CAU), China: Xizang, Yigong, 2017.VI.7, 2236 m, Qicheng Yang (light trap). 1 male (CAU), China: Xizang, Bayi, 2017.VI.4, 2955 m, Qicheng Yang (light trap).

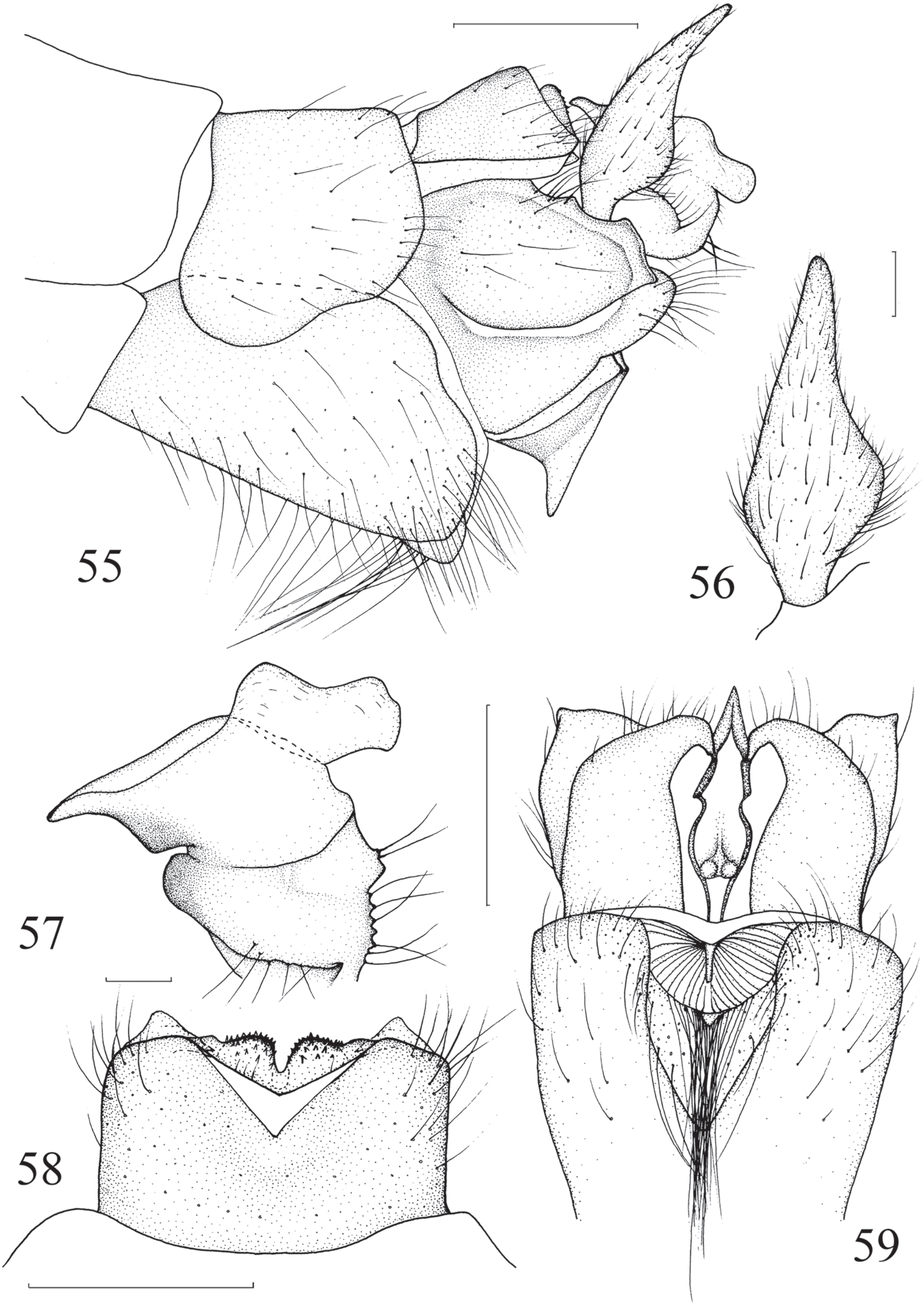
**Description.** Male ($n$ = 3): body length 11.5–12.5 mm, wing length 11.5–12.5 mm, antenna length 3.5–4.0 mm.

***Head*** (Figs 52, 53). Mainly yellow. Vertex with brown spot near inner margin of eye. Occipital marking brown, subtriangular. Dorsal part of rostrum including nasus brown. Head with black hairs. Antennal scape yellow, pedicel pale brown, flagellum mainly dark brown except first flagellomere pale brown; first flagellomere 1.5 times longer than second one. Proboscis yellow, with brown hairs. First palpal segment greyish brown, second one yellow, with brown hairs.

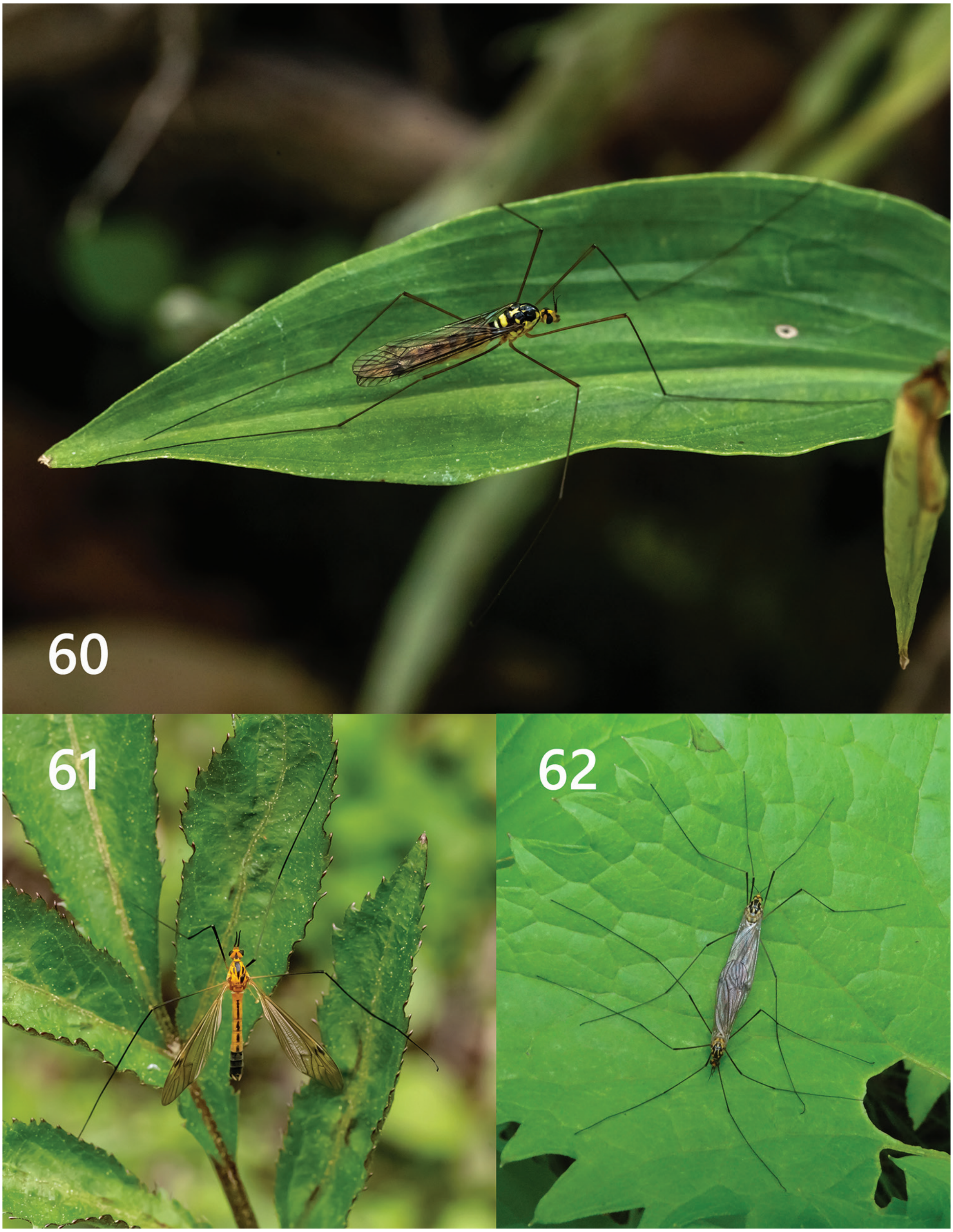
***Thorax*** (Figs 52, 53). Mainly yellow. Pronotum yellow. Prescutum with three black longitudinal stripes; middle stripe V-shaped, anterior end of lateral prescutal stripe bent outward, outer curved part brown. Scutum yellow, with two black subtriangular spots. Scutellum with pale brown middle stripe and brown posterior margin. Mediotergite yellow.



**Figures 52–54.** *Nephrotoma xizangensis* Yang & Yang, 1987. **52** Male habitus, lateral view **53** head and thorax, dorsal view **54** wing. Scale bars: 1.0 mm.



**Figures 55–59.** *Nephrotoma xizangensis* Yang & Yang, 1987. **55** Hypopygium, lateral view **56** outer gonostylus, lateral external view **57** inner gonostylus, lateral external view **58** tergite 9, dorsal view **59** hypopygium, ventral view. Scale bars: 0.5 mm (**55, 58, 59**); 0.1 mm (**56, 57**).



**Figures 60–62.** Ecograph. **60** *Nephrotoma beibengensis* sp. nov. **61** *Nephrotoma xizangensis* Yang & Yang, 1987 (variation) **62** *Nephrotoma nigrohalterata* Edwards, 1928 (mating) (**60, 61** by Zhenqi Song **62** by Qicheng Yang).

Pleuron pale yellow. Parascutellum yellow. Legs yellow, except tips of femora and tibiae brown, tarsi dark brown; hairs dark brown. Wing subhyaline, tinged with light brown; pterostigma brown; cell $m_1$ sessile (Fig. 54). Halter brownish grey.

***Abdomen*** (Fig. 52). Mainly yellow. Abdominal tergites with three longitudinal stripes, middle stripe brown, triangular; stripes on lateral side brownish grey. Abdominal segments 7 and 8 entirely dark brown to black; hypopygium mainly dark brown, tergite 9 black. Hairs on abdomen dark brown or golden.

***Hypopygium*** (Figs 55–59) mainly black. Posterior extension of tergite 9 slightly depressed with two pairs of short obtuse projections; posterolateral margin with a pair of ventrad curved appendages each with two cusps (Fig. 58). Posterior margin of sternite 8 slightly depressed, middle part pleated, sclerotized, anterior part with long hairs (Fig. 55). Sternite 9 with a horn-like projection (Figs 55, 59). Outer gonostylus fleshy, widened at middle, narrowed toward tip (Fig. 56). Inner gonostylus with large concavity at base; beak sharp; outer basal lobe with hairy protuberance; posterior crest with a large membranous area (Fig. 57).

**Distribution.** China (Xizang).

**Remarks.** This species is similar to *N. flavonota* Alexander, 1914 from Japan (Honshu, Shikoku, Kyushu), China (Zhejiang, Fujian, Hainan), but the latter differs in the following characters: Sternite 8 sheathing very slightly narrowed outwardly, terminating in two broad lobes separated by a V-shaped emargination filled with pale membrane; lobes bearing abundant long yellow setae.

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# Cuba in Mexico: first record of *Phyllops falcatus* (Gray, 1839) (Chiroptera, Phyllostomidae) for Mexico and other new records of bats from Cozumel, Quintana Roo

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## Abstract

The first record of *Phyllops falcatus* (Gray, 1839) in Mexico is documented from the island of Cozumel, Quintana Roo. This species is present in the Antilles, distributed in all the Cuban archipelago, Cayman Islands, and Hispaniola. It is likely that a hurricane moved these bats from Cuba to Cozumel. The Cozumel record extends the distribution more than 200 km west. Two new records from Cozumel of the bats *Lasiurus ega* and *Molossus alvarezi* are also provided.

## Keywords

Caribbean islands, Cuban archipelago, dispersal, hurricanes, range extension, Yucatan Peninsula

## Introduction

Cozumel is an island in the Mexican Caribbean with an area of about 647 km$^2$ and about 20 km off the coast of the state of Quintana Roo (Orellana et al. 2007). It is the Mexican island with not only the greatest species richness but also the highest endemism of any island of the country (ECOSUR 2018). However, there are few works addressing the bat diversity of Cozumel. Koopman (1959), based on the then known 19 species of mammals of Cozumel, outlined the limits of the West Indies and discarded Cozumel as an island that does not belong to the Antilles subregion, as all the mammals known in Cozumel at the time had affinities with the Yucatan Peninsula or were undetermined. Jones and Lawlor (1965) recorded eight species of bats and their conclusions were similar, that Cozumel is a continental island with affinities to Yucatan, and not to the Antilles, because of the absence of endemic Antillean species. CONABIO (2008) presented a list of 22 species of bats for the island, although several of them are undoubtedly erroneous, like *Glossophaga leachii* Gray, 1844 and *Rhogeessa parvula* H. Allen, 1866, which are only found on the Pacific tropical dry forest (Medellin et al. 2008) and *Artibeus intermedius* J. A. Allen, 1897 that has been included as a synonym of *A. lituratus* (Olfers, 1818) (Simmons 2005). Similarly, Rincón-Sandoval (2013) reported a list of 22 species from Cozumel, including three new records that we consider dubious for the island. *Molossus sinaloae* J. A. Allen, 1906 was recorded from echolocation recordings, but it is well known that the family Molossidae features great diversity in the design of their echolocation calls, and that their calls are similar within the genus (Jung et al. 2014). However, this report of *M. sinaloae* may have instead been *M. alvarezi* González-Ruiz et al. (2011). Likewise, the echolocation call of *Pteronotus davyi* Gray, 1838 can be confused with that of *P. gymnonotus* (J. A. Wagner, 1843) and also possibly with *P. personatus* (J. A. Wagner, 1843) (Ibañez et al. 2000). Orozco-Lugo (pers. comm. to N. Rivas 2000, 2006) by means of mist nets and ultrasound detectors reported the possible presence of *Lasiurus ega* (Gervais, 1856), *Diphylla ecaudata* Spix, 1823, and *Mormoops megalophylla* (Peters, 1864) on Cozumel. However, the calls of *Lasiurus intermedius* H. Allen, 1862 can also be confused with those of other congeneric species. Given that there are no specimens that backup the correct identification of these species, we consider their presence unconfirmed in Cozumel.

In previous lists, *Corynorhinus mexicanus* G. M. Allen, 1916 has been reported from Cozumel, but Koopman (1959), Jones and Lawlor (1965), and other authors have clarified that *Corynorhinus* sp. is not present in Cozumel or even the Yucatan Peninsula. Likewise, *Lasiurus borealis* (Müller, 1776) only exists in northeastern Mexico (Medellin et al. 2008).

Previous to this study, the only species of the genus *Molossus* known from Cozumel was *M. rufus* É. Geoffroy 1805. González-Ruiz et al. (2011) described a new species of the genus *Molossus* for the populations from the Yucatan Peninsula formerly ascribed to *M. sinaloae*. They described *M. alvarezi* on the basis of morphometric characters that clearly distinguished it as a species, endemic to Yucatan.

The genus *Phyllops* (Chiroptera, Phyllostomidae) is endemic to the Antilles (Da Cunha and Mancina 2008). This genus has one living species, *Phyllops falcatus* (Gray, 1839), with two subspecies, *P. falcatus falcatus* that inhabits Cuba, Grand Cayman, and Cayman Brac, and *P. falcatus haitiensis* in Hispaniola. The genus also has an extinct species, *P. vetus*, from Cuba and Isla de la Juventud (Silva 1979; Suárez and Díaz-Franco 2003). This species is assessed as Least Concern by the IUCN (Solari et al. 2019).

*Phyllops* can be distinguished from other Caribbean species by the presence of three upper molars, well-developed post-orbital processes, a small gap between the incisor foramina and the incisor root, and a broad noseleaf that is broader in its midsection than at its base. The skull is tall and round, without a developed sagittal crest, and with a well-developed metaconid in m1 (Da Cunha and Mancina 2008). With the objective to contribute to the understanding and knowledge of the bat fauna of the island, we provide an updated list of bats of Cozumel and report for the first time the presence of three species, including one that is new for Mexico (Table 1).

## Material and methods

### Area of study

Cozumel is the largest island of Mexican Caribbean and is comprised of limestone. The soil is very permeable, which causes the water to rapidly filter (Téllez-Valdez et al. 1989), and due to this, the flow of water is primarily subterranean (Alcocer and Escobar 1996). The coastal ecosystems of the Yucatan Peninsula have three well-defined seasons: the dry season from March to May, the hot rainy season from June to October, and cold rainy season from November to February, which is driven by cold fronts (Herrera-Silveira et al. 2010). The annual total precipitation varies

**Table 1.** Updated list of the 19 bat species from Cozumel, Mexico including the first reports of each species in the island. * indicates species newly recorded from the island as per our work.

| Species | First record for Cozumel | NOM-059 Status |
|---|---|---|
| **Family Phyllostomidae** | | |
| *Artibeus jamaicensis* | Koopman 1959 | |
| *Artibeus lituratus* | Jones and Lawlor 1965 | |
| *Artibeus phaeotis* | Jones and Lawlor 1965 | |
| *Centurio senex* | Koopman 1959 | |
| *Glossophaga soricina* | Koopman 1959 | |
| *Micronycteris microtis* | Jones and Lawlor 1965 | |
| *Mimon cozumelae* | Goldman 1914 | THREATENED |
| *Phyllops falcatus** | This study | |
| **Family Mormoopidae** | | |
| *Pteronotus mesoamericanus* | Engstrom et al. 1989 | |
| **Family Natalidae** | | |
| *Natalus mexicanus* | Jones and Lawlor 1965 | |
| FAMILY MOLOSSIDAE | | |
| *Eumops bonariensis* | Engstrom et al. 1989 | |
| *Molossus alvarezi* * | This study | |
| *Molossus rufus* | Koopman 1959 | |
| *Nyctinomops laticaudatus* | Koopman 1959 | |
| **Family Vespertilionidae** | | |
| *Eptesicus furinalis* | Rincón-Sandoval 2013 | |
| *Lasiurus frantzii* | Koopman 1959 | |
| *Lasiurus ega* * | This study | |
| *Myotis pilosatibialis* | LaVal 1973 | |
| *Rhogeessa aeneus* | Jones et al. 1973 | |

between 800 and 1500 mm. The driest months are March and April; September is the month with the most of rain due to the passage of hurricanes (Orellana et al. 2007). Cozumel has the Am(f) climate subtype, which is characterized by a warm, humid summer with rain. The annual average temperature is between 26 and 27 °C (Orellana et al. 2007).

The flora of the island is only 40% of that reported from the state of Quintana Roo (Téllez-Valdez et al. 1989). Dominant vegetation in Cozumel is middle subdeciduous forest. The island also has low subdeciduous forest, magrove forest, and wetlands with reeds, palms, etc., and halophyte vegetation or coastal dune (Téllez-Valdez et al. 1989). The island also has several endemic vertebrates such as pygmy Cozumel raccoon (*Procyon pygmaeus* Merriam, 1901), Cozumel harvest mouse (*Reithrodontomys spectabilis* Jones & Lawlor, 1965), Cozumel emerald (*Chlorostilbon forficatus* Ridgway, 1885), Cozumel vireo (*Vireo bairdi* Ridgway, 1885) among other species (Jones and Lawlor 1965; McFadden et al. 2006; Aves de Cozumel 2020).

## Field surveys

As part of an ongoing biological inventorying of mammals and birds, field surveys have been conducted for the past 3.5 years. We started in February 2017 using 12-m long mist nets. Sampling sites were Parque Ecológico Estatal Laguna Colombia, El Cedral, and the San Gervasio Archeological Site. Parque Ecológico Estatal Laguna Colombia is located in southwestern Cozumel. It is covered by coastal dune vegetation, subdeciduous low forest, and mangroves with a brackish lagoon. El Cedral is a small human community that is also located to the southwest, activities of agriculture and livestock as well as human habitation occur in the settlement. The San Gervasio Archeological Site is 7 km east of the city of San Miguel de Cozumel. Both El Cedral and San Gervasio sites are covered by middle subdeciduous forest.

On April 5, 10, and 25, 2019, we captured bats in the San Gervasio Archaeological Site. Dominant arboreal species included: *Manilkara zapota*, *Bursera simaruba*, *Cedrela odorata* L. (1759), *Metopium brownei*, *Ceiba aesculifolia*, *Lysiloma latisiliquum*, and *Sideroxylon foetidissimum* (Téllez-Valdez et al. 1989).

We set two or three 12 m long mist nets, fixed with poles and cords, in the vegetation on April 25, 2019 and on March 2, 2020. On each capturing night, we opened the nets at 19:00 hr and closed them at 00:00 hr. The species were identified using the guide by Medellin et al. (2008). An additional bat record came from a local inhabitant who had found a dead bat on October 6, 2017. We visited the location which was at km 4.5 of the road to San Gervasio and collected the specimen. All bats were handled according to the guidelines for the use of wild mammals in research (Sikes et al. 2016).

To compile the list of bat species present in Cozumel, we examined all the literature containing reports of bats from the island. In addition, we added our own information, the results of the ongoing inventory, and talked to other researchers working on the island. Bat nomenclature and taxonomy follows Ceballos et al. (in press).

## Results

During our first field survey on April 5, 2019, we captured a bat that did not match any species known from México (Ceballos and Oliva 2005; Medellin et al. 2008). This bat was a lactating female. It had two white spots on the shoulders, the central part of the noseleaf was wider than its base, the tragus was yellowish, and the forearm length was 49 mm (Fig. 1). The second metacarpal of the index finger was curved and the first dactylopatagium was wide and translucent. This bat had three upper molars. The specimen was photographed, weighed, morphometric measurements were obtained using manual calipers and then it was released.

We identified this bat using a field key including the four short-faced stenodermatine genera found on the Antilles, and it was identified as *Phyllops falcatus*. On April 10, we caught three adult specimens of *P. falcatus*. Their forearm lengths were 45, 42, and 44 mm and body masses were 17, 17.8, 20.3 g, for one male with scrotal testes, one non-reproductive male, and one non-reproductive female, respectively. The length of the thumb was 10 mm for all three specimens. We collected one of these males, which was deposited in Mexico's Colección Nacional de Mamíferos at the Institute of Biology (UNAM). Unfortunately the skull was lost. On April 25 an additional non-reproductive female of *Phyllops falcatus* was caught; it had a forearm of 47 mm and body mass of 14.5 g. All five individuals of *P. falcatus* were caught between 20:38 and 21:51 hrs.

On February 22, 2019, we captured an adult male *Lasiurus ega*, with a forearm of 43.5 mm and body mass of 19 g. This is the first specimen of this species to be captured on the island. Finally, on March 2, 2020, we captured an adult male *Molossus alvarezi*, which is clearly distinguishable from the other *Molossus* known from the island by its smaller size. This species was considered endemic to the north and east parts of the Yucatan Peninsula, and was not known from Cozumel. A subsequent study by Loureiro et al. (2019) extended its distribution to Central America and French Guiana in northern South America. The specimen reported on Oct 6, 2017 was determined to be a male *Lasiurus frantzii* Peters, 1870, the first individual of this species to be found on Cozumel in 100 years since Gaumer collected one individual on the island (Koopman 1959).

The following species were recorded from February 2017 to August 2019 from the Punta Sur area: *Rhogeessa aeneus* Goodwin, 1958, *Myotis pilosatibialis* LaVal, 1973, *Artibeus jamaicensis* Leach, 1821, and *Natalus mexicanus* Miller, 1902. From March 2019 to March 2020, the species captured at the San Gervasio site were: *Artibeus jamaicensis*, *Artibeus phaeotis* (Miller, 1902), *Artibeus lituratus*, *Centurio senex* Gray, 1842, *Glossophaga soricina* (Pallas, 1766), *Phyllops falcatus*, *Pteronotus mesoamericanus* Smith, 1972, *Molossus alvarezi*, *Eptesicus furinalis* (d'Orbigny, 1847), *Myotis pilosatibialis*, *Rhogeessa aeneus*, and *Lasiurus ega*. Lastly, in the zone of El Cedral, from March 2019 to December 2019, we recorded these species: *Artibeus jamaicensis*, *Centurio senex*, *Micronycteris microtis* Miller, 1898, *Rhogeessa aeneus*, *Myotis pilosatibialis*, *Eptesicus furinalis*, and *Pteronotus mesoamericanus*. This brings the number of bats known from Cozumel to 19 (Table 1).

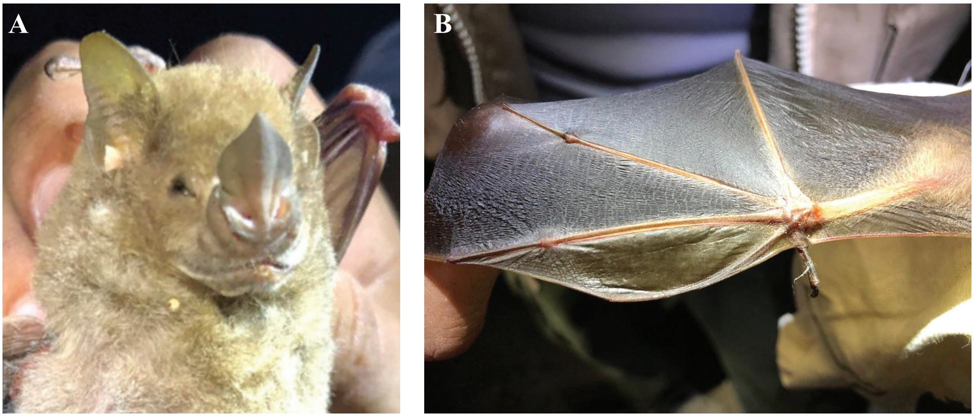

**Figure 1.** *Phyllops falcatus* from an area adjacent to the San Gervasio Archeological Site, Cozumel island, Mexico **A** lactating female showing the noseleaf that is broader in its midsection than its base **B** recurved second finger and translucent dactylopatagium minus. Photograph by P. Sabido (**A**) and N. Rivas (**B**).

## Discussion

The distance between the northeastern point of Cozumel and the western limit of the distribution of *P. falcatus* in Villa Cabo San Antonio in western Cuba is 235 km, separated by the Caribbean Sea. Furthermore, the distance between Villa Cabo San Antonio and Cancun is less than 210 km and without and islands or islets of any kind. This distance is too far for *P. falcatus* to disperse by normal flight, given its assumed flight capability. For example, a related stenodermatine, *Sturnira lilium* (E. Geoffroy, 1810), is known to move a maximum of 760 m from roost to foraging area (Mello et al. 2008). Another phyllostomid, the insectivorous trawler *Macrophyllum macrophyllum* (Schinz, 1821), can move 7 km from the roost, although in foraging bouts it may reach up to 47 km, a distance is considered extreme (Meyer et al. 2005), but less than a quarter of the distance from Cuba to Cozumel. We speculate that *P. falcatus* could have been carried by a hurricane that passed by Cuba and continued its path to the Yucatan Peninsula and Cozumel. Both Cuba and Cozumel are located in the Caribbean Hurricane Alley. From 2000 to 2016, more than 20 hurricanes passed over Cuba (Hypothetical hurricanes 2020). From 1879 to 2016, 34 hurricanes passed over the Yucatan Peninsula, including Cozumel Island (Aves de Cozumel 2020).

Three invasion routes for overwater dispersal of bats in the Caribbean have been proposed and identified as the northern, western, and southern routes (Rodriguez-Duran and Kunz 2001). However, these routes were proposed for mainland species invading Caribbean islands, and *P. falcatus* has clearly dispersed in the reverse direction, coming from the Caribbean island of Cuba and invading the mainland. Hurricanes seem to have a severe negative effect on bat and bird populations (Pedersen et al. 1996; Rodríguez-Durán and Kunz 2001), but clearly for *P. falcatus*, hurricanes have allowed this species to reach and colonize a new area. It is likely that *P. falcatus* may continue to disperse and easily reach the mainland of the Yucatan Peninsula. Based on the available data, we conclude that passive dispersal by tropical storms, not purposeful bat dispersal behavior, are responsible for insular dispersal and gene flow of bat populations living in the Lesser Antilles (Pedersen et al. 2013).

The presence of *P. falcatus* in Cozumel is not incidental, given that we have captured a total of five specimens, and that the very first specimen was a lactating female. This indicates that the species is present in the island with a breeding population. Given the geography of the Caribbean, and the presence of *P. falcatus* in Cozumel, it is possible that other species of bats, notably *Ardops*, *Stenoderma*, and *Ariteus* may also be recorded in the future. Likewise, we predict that with more surveys, *P. falcatus* is likely to be recorded in the vicinity of Cancun.

Given its recent detection in Mexico and that this may be the only population in the country, this species may warrant conservation status of at least Threatened under Mexican legislation. We are in the process of conducting genetic studies to determine the relationship of animals from Cozumel with those from Cuba. The presence of this species increases the number of known bat species in Mexico to 140.

Very little is known still about bats of Cozumel over time because there are fewer than five publications documenting them and many errors in labeling and identification of specimens. Information on the species richness of this group in Cozumel is muddled. The bad reputation that these flying mammals have with the general public makes educating the people of Cozumel about bats, the value of the island's fauna, and the importance of bat conservation necessary.

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