

Leucosolenia qingdaoensis sp. nov. (Porifera, Calcarea, Calcaronea, Leucosolenida, Leucosoleniidae), a new species from China

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Academic editor: P. Stoev | Received 9 October 2019 | Accepted 4 December 2019 | Published 22 January 2020

<http://zoobank.org/DE8D65F1-1739-4377-8BC4-E42F84E77B08>

Citation: Chu Y-L, Gong L, Li X-Z (2020) *Leucosolenia qingdaoensis* sp. nov. (Porifera, Calcarea, Calcaronea, Leucosolenida, Leucosoleniidae), a new species from China. ZooKeys 906: 1–11. <https://doi.org/10.3897/zookeys.906.47164>

Abstract

A new species of Leucosoleniidae, *Leucosolenia qingdaoensis* sp. nov., is described. This new species was collected in a scallop-breeding pond from the Yellow Sea and preserved in 75% ethanol. This sponge consists of a dense reticulation of ascon tubes, with the surface minutely hispid and the consistency soft and fragile.

Spiculation of the new species consists of diactines, which are smooth, straight or sometimes slightly curved, triactines of two types, and tetractines with short and curved apical actines; spiculation also slightly overlaps and is somewhat irregularly assembled. Together these form a thin layer of skeleton, with a small number of cells, which results in a transparent, white sponge. As a typical asconoid feature, all internal cavities of the sponge are lined with choanocytes, and there is no fully developed inhalant system. Comparisons with other *Leucosolenia* reported from the Pacific Ocean are also made.

Keywords

Sponge, taxonomy, Yellow Sea

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Introduction

The family Leucosoleniidae is characterised by a branched and rarely anastomosed cormus and asconoid aquiferous system; there is neither a common cortex nor a delimited inhalant or exhalant aquiferous system (Minchin 1900). The family includes three genera (Borojevic et al. 2002): *Ascyssa* Haeckel, 1872, *Ascute* Dendy & Row, 1913, and *Leucosolenia* Bowerbank, 1864. They can be easily distinguished by their skeletons: the skeleton of *Ascyssa* contains only diactines; the skeleton of *Ascute* exhibits giant longitudinal diactines forming a continuous layer on the external surface, and includes triactines and tetractines; and the skeleton of *Leucosolenia* lacks any of these obvious characteristics in the above two genera. Instead, the skeleton of *Leucosolenia* is characterised by being composed of diactines, triactines and/or tetractines, without a reinforced external layer on the tubes.

The genus *Leucosolenia* comprises 40 living species worldwide (Van Soest et al. 2019), of which only three species, *L. microspinata* Longo, 2009, *L. salpinx* Van Soest, 2017, and *L. parthenopea* Sarà, 1953, were named after 1950; 11 species were described by Haeckel between 1870 and 1872. The literature of this genus is relatively old, and the descriptions contained therein of the species of *Leucosolenia* were simple, almost without details and illustrations of the body shapes and spicules. Thus, a taxonomic revision of this genus is very difficult, and to date, no worldwide revision of the genus has been made.

The localities of the 15 known species of *Leucosolenia* recorded from the Pacific Ocean are shown in Figure 1. Seven species (*L. eleanor* Urban, 1906, *L. minuta* Tanita, 1943, *L. mollis* Tanita, 1941, *L. pyriformis* Tanita, 1943, *L. serica* Tanita, 1942, *L. tenera* Tanita, 1940, and *L. ventosa* Hôzawa, 1940) were reported from the Japanese waters (Sagimi Sea, Wakayama Prefecture, Onagawa Bay, Mie Prefecture, Matsushima Bay, Izushima, Wagu Miye Prefecture, respectively). *Leucosolenia macquariensis* Dendy, 1918 was reported from the west coast of Macquarie Island; *L. australis* Brøndsted, 1931 was reported from Comau Fjord; *L. albatrossi* Hôzawa, 1918 was reported from Copper Island and the Komandorski Islands; *L. echinata* Kirk, 1893 and *L. rosea* Kirk, 1896 were reported from New Zealand; *L. lucasi* Dendy, 1891 was reported from Port Phillip Heads, Australia; *L. nautilia* Laubenfels, 1930 was reported from California, USA; and *L. feuerlandica* Tanita, 1942 was reported from Tierra del Fuego, South America. The *Leucosolenia* species reported from the coasts of Japan account for most species. The type specimens of new species were found in the Yellow Sea, very close to Japan.

Materials and methods

The specimens were collected in a scallop-breeding pond from the Yellow Sea and were preserved in 75% ethanol. Two specimens were deposited in the Marine Biological Museum of the Institute of Oceanology in the Chinese Academy of Sciences (IOCAS), Qingdao, China.

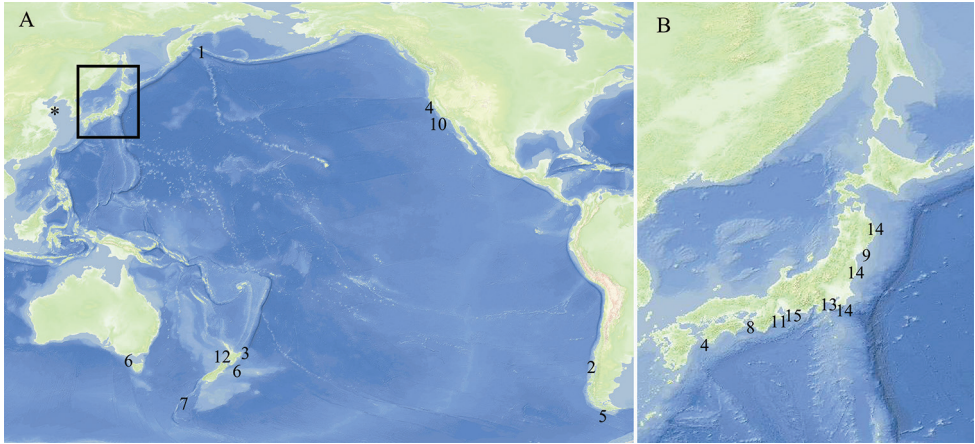


Figure 1. Distribution of *Leucosolenia* **A** location in the Pacific Ocean **B** detail of the type locality in the Japanese coast: (1) Komandorski Islands (*L. albatrossi* Hôzawa, 1918); (2) Comau Fjord (*L. australis* Brøndsted, 1931); (3) Cook Strait, Poverty Bay, Kawakawa (*L. echinata* Kirk, 1893); (4) Francisco Bay, California; Sukumo ôsima, Kôti Prefecture, Sagimi Sea (*L. eleanor* Urban, 1906); (5) Tierra del Fuego (*L. feuerlandica* Tanita, 1942); (6) Port Phillip Heads, Australia, and New Zealand (*L. lucasi* Dendy, 1891); (7) Macquarie Island (*L. macquariensis* Dendy, 1918); (8) Wakayama Prefecture (*L. minuta* Tanita, 1943); (9) Onagawa Bay (*L. mollis* Tanita, 1941); (10) Monterey Bay, California (*L. nautilia* Laubenfels, 1930); (11) Mie Prefecture (*L. pyriformis* Tanita, 1943); (12) New Zealand (*L. rosea* Kirk, 1896); (13) Yodomi, Sagami Sea (*L. serica* Tanita, 1942); (14) Matsushima Bay, Onagawa Bay, Izushima (*L. tenera* Tanita, 1940); (15) Wagu Miye Prefecture (*L. ventosa* Hôzawa, 1940); (*) Qingdao (*L. qingdaoensis* sp. nov.).

For examination of the spicules, a small piece of specimen was cut and placed in a 1.5 mL microcentrifuge tube to which 1000 µL of sodium hypochlorite solution was added (Kersken et al. 2016). The mixture was then vortexed, placed at environmental temperature, and vortexed occasionally during incubation until it was completely lysed. Next, the sample was centrifuged at 8000 rpm for 2 min, the supernatant was poured off, 1000 µL of distilled water was added, and the sample was again centrifuged at 8000 rpm for 2 min. This procedure was repeated four times, then the spicules were washed three times with 96% ethanol and then the spicules were preserved in the third ethanol solution.

Scanning Electron Microscopy (SEM) was performed with a Hitachi S3400N. Preserved spicules for SEM were adhered to stubs with double-sided carbon conductive tape and coverslip. After dehydration, the spicules were coated with gold in a Hitachi MC1000 (LOPES 2018).

Measurements of at least 20 spicules of each type were performed using an optical microscope (Nikon Eclipse Ni) with a micrometric eyepiece. The length from the tip to the base and the thickness at the base of each actine were measured. The reported numbers refer to the range of measurements for each spicule type. Photographs were taken with a stereomicroscope (Zeiss Stemi 2000-c) and an optical microscope (Nikon Eclipse Ni-U) equipped with a digital camera to evaluate difference between the length of the unpaired and paired actines of each type of triactine. For comparison with the new species, we only selected those species of *Leucosolenia* reported from the Pacific Ocean.

Results

Systematics

Class Calcarea Bowerbank, 1862

Subclass Calcaronea Bidder, 1898

Order Leucosolenida Hartman, 1958

Family Leucosoleniidae Minchin, 1900

Genus *Leucosolenia* Bowerbank, 1864

Leucosolenia qingdaoensis sp. nov.

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Figs 1–4; Tables 1, 2

Type material. *Holotype*: MBM181606, scallop-breeding pond on southeastern Shandong Peninsula, China, June 1988, 0–0.3 m depth, collected by Shue Li, 35°58'N, 120°11'E. *Paratype*: MBM181476, Zhonggang, Qingdao, China, 7 June 1984, 0–0.6 m depth, 36°06'N, 120°21'E.

Type locality. Qingdao, Yellow Sea.

Etymology. The name is derived from the type locality, Qingdao, China.

Description. The sponge is arborescent, consisting of many thin-walled tubes, which are copiously ramified but never anastomosed. The sponge occurs as growth form. The oscula are terminal on erect tubes. The color of the sponge is white after being preserved in alcohol and in vivo. The external walls of the tubes are hairy, with diactines protruding at right or oblique angles from the body; the surface is minutely hispid, and the consistency is soft and fragile. The holotype measures 21.32 × 3.38 mm (height × width). The wall of the sponge body is very thin, and there is no fully developed inhalant system, the gap between the skeleton and the cell on the wall arrange evenly (Fig. 2F); only a small amount of cells is distributed on the thin sponge skeleton (Fig. 2C–F), which is a typical asconoid feature. All internal cavities of the sponge are lined by choanocytes.

Skeletal arrangement. The skeleton consists of multifarious diactines, sagittal triactines of two types, sagittal tetractines with bent apical actines and triactine-like basal actines; together these form the wall of the ascon-type sponge body.

In the apical osculum (Fig. 2C, E), there are paired actines of triactines and tetractines, some additional tangential diactines, together forming a clear line dividing the apical oscula, and some radial diactines projecting beyond the apical osculum with different length.

In the sponge body (Fig. 2C, E), the triactines and tetractines are regularly arranged, their paired actines are parallel to the apical oscula, and the unpaired actines point downward, with slight folding allowed, but never overlapping; in contrast to the triactines and tetractines, the diactines are arranged more irregularly but generally point downward.

In the root-like structures (Fig. 2D, F), the arrangement of triactines and tetractines is the same as that in the body, but the arrangement of diactines is different; most of them tangentially project beyond the surface, which results in the surface having a slightly hispid appearance.

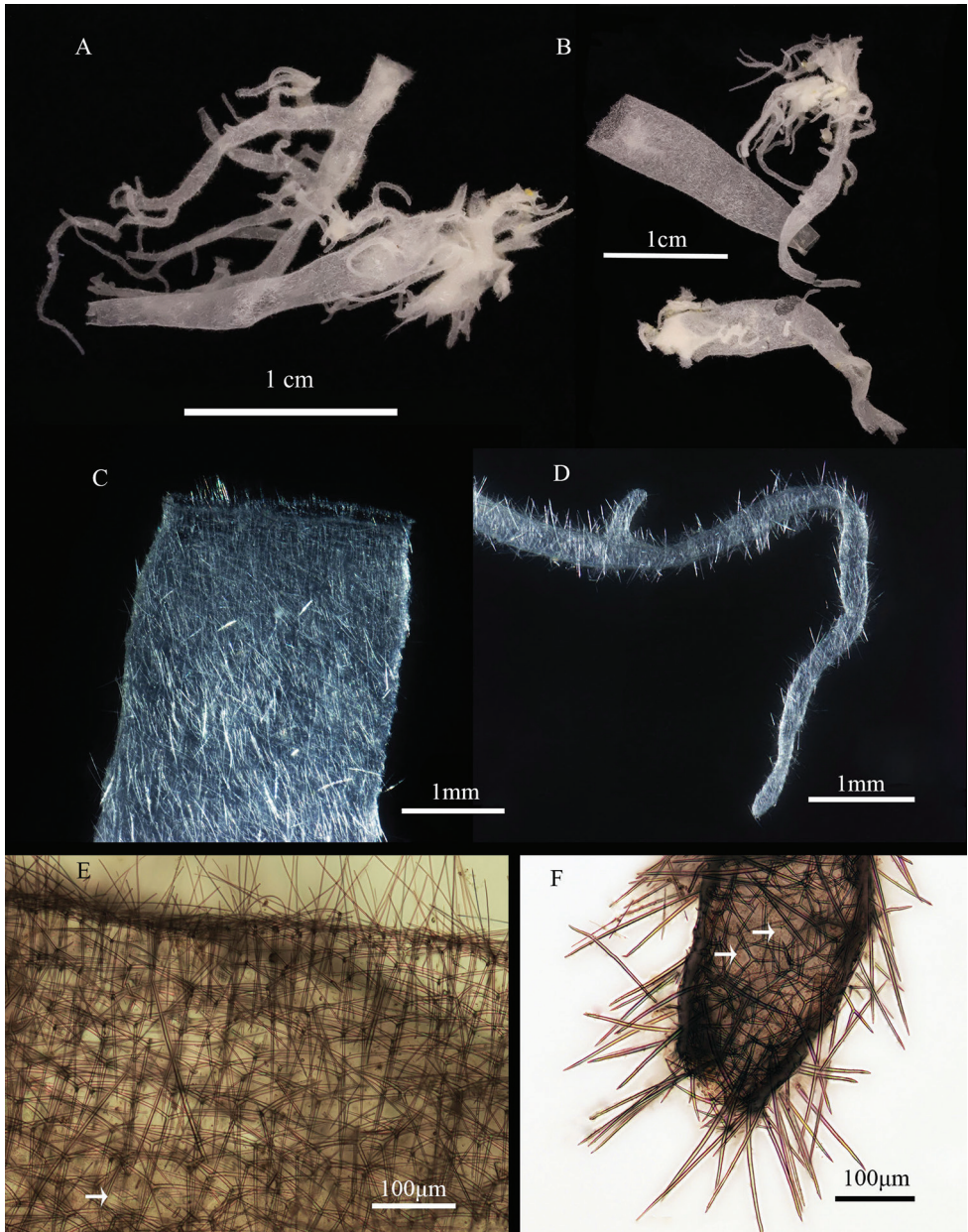


Figure 2. *Leucosolenia qingdaoensis* sp. nov. **A** holotype **B** paratype **C** detail of oscula (stereo microscope) **D** detail of root-like structures (stereo microscope) **E** detail of oscula (optical microscope) **F** detail of root-like structures (optical microscope); arrowhead pointing at the ostium.

By observing the sponge tissue taken from different parts, it is clear that as the diameter of the tubes decreases, the contents of small diactines and small triactines increase. This observation can suggest that in the growth zone spiculogenesis is more intense.

Spicules. Diactines. There is only one type of diactine (Fig. 3A1–3), though the diactines vary in size and shape, their width varies from 24 μm to 61 μm , the length of diactines vary from 43 μm to 421 μm but half of the diactines present a length of 200–300 μm (Fig. 4). The shapes of the diactines are straight or slightly curved in different directions. The variation in *Leucosolenia* is very common and considerable.

Triactines. Two types of triactines are present, with actines straight or undulated. Their ends are generally sharp or asymmetrical (Fig. 3B1–2). The paired actines are slightly curved. Some deformations are present.

Type 1: triactines with paired actines longer than unpaired actines (Fig. 3B1): unpaired actines 42–105 \times 3–5 μm ; paired actines 63–105 \times 3–5 μm .

Type 2: triactines with unpaired actines longer than paired ones (Fig. 3B2): unpaired actines 76–129 \times 3–4 μm ; paired actines 60–104 \times 3–4 μm .

Tetractines. A relatively small number of tetractines are observed, approximately 10 per 100 spicules, with straight and fusiform actines (Fig. 3C1–2). The tetractines are similar to triactines but with the addition of apical actines, the apical actines are fairly stout and short, sharply pointed and curved: unpaired actines 93–119 \times 2–5 μm ; paired actines 50–93 \times 2–5 μm ; apical actines 11–29 \times 2–5 μm .

Remarks. Three species described by Tanita (*L. minuta*, *L. pyriformis*, and *L. serica*) exhibit only regular (equiangular and equiradiate) spicules. This characteristic does not fit the description of *Leucosolenia*, *L. qingdaoensis* sp. nov. can be easily differentiated from the 12 species of *Leucosolenia* reported from the Pacific Ocean. The skeletal compositions of these species are shown in Table 1.

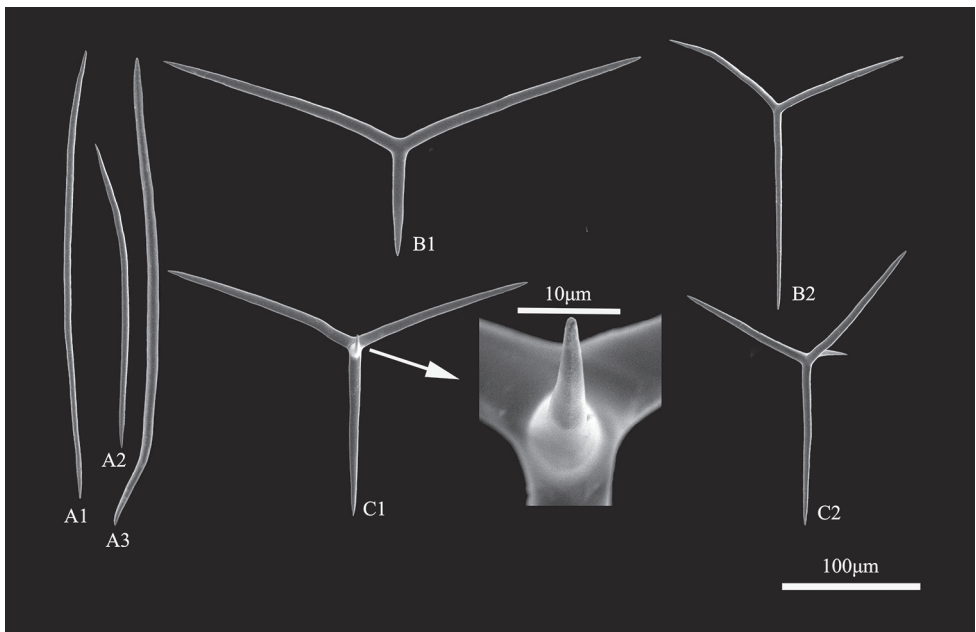


Figure 3. Spicules of *Leucosolenia qingdaoensis* sp. nov. (holotype) A1–3 = diactines; B1 = triactines of type 1; B2 = triactines of type 2; C1–2 = tetractines.

Table 1. Spicules dimensions of *Leucosolenia* Bowerbank, 1864 in the Pacific Ocean. Measurements are reported in μm .

	Triactines		Tetractines			Diatines	References
	Unpaired	Paired	Unpaired	Paired	Apical		
	Length/Width	Length/Width	Length/Width	Length/Width	Length/Width	Length/Width	
<i>L. albatrossi</i>	70–90/8	80–100/8	70–90/8	80–100/8	40–60/6	70–90/8	Hózáwa 1918
	60–90/8	130–240/8	60–90/8	130–240/8	40–60/6–8	–	
<i>L. australis</i>	69–122/6	66–106/6	66–119/6	69–99/7	27–41/4	41–49/1	Azevedo et al. 2009
	–	–	–	–	–	63–347/7	
<i>L. echinata</i>	100/10	130/10	130/15	150/15	70/15	240–730/10–5	Kirk 1893
<i>L. eleanor</i>	80/7	80/7	140/9	140/9	140/9	105/4	Laubenfels 1932
	140/7	140/7	–	–	–	434/9	
<i>L. feuerlandica</i>	50–70/12–18	70–95/12–18	60–70/8–10	75–90/8–10	40–50/6–8	70–90/4–6	Tanita 1942
	60–70/8–10	75–90/8–10	–	–	–	–	
<i>L. lucasi</i>	100/5	70/5	100/5	70/5	<70/5	160/5	Dendy 1891
<i>L. macquariensis</i>	980/9	980/9	980/9	980/9	–	140/6	Dendy 1918
	–	–	–	–	–	90/5	
<i>L. minuta</i>	130–175/14–18	130–175/14–18	60–75/8–10	60–75/8–10	50–60/7–10	–	Tanita 1943
	60–75/8–10	60–75/8–10	–	–	–	–	
<i>L. mollis</i>	70–130/6–8	90–140/6–8	70–130/6–8	90–140/6–8	35–55/6	230–400/7–10	Tanita 1941
<i>L. nautilia</i>	140/9	140/9	140/9	140/9	30/8	400/10	Laubenfels 1932
	–	–	–	–	–	140/4	
	–	–	–	–	–	1000/20	
<i>L. pyriformis</i>	180–190/12–18	180–190/12–18	180–190/12–18	180–190/12–18	150–260/8–15	630–800/40–55	Tanita 1943
<i>L. rosea</i>	300/70	300/70	140/10	140/10	110/8	–	Kirk 1896
	200/18	200/18	–	–	–	–	
<i>L. serica</i>	140–210/7–8	140–210/7–8	140–210/7–8	140–210/7–8	90–135/8–10	–	Tanita 1942
<i>L. tenera</i>	80–180/7–10	90–210/7–10	80–180/7–10	90–210/7–10	30–10/6–8	200–530/8–12	Tanita 1940
<i>L. ventosa</i>	100–120/10	85–100/10	–	–	–	–	Hózáwa 1940
	150–180/20–25	140–150/20–25	–	–	–	–	
	100–120/10–14	70–90/10–14	–	–	–	–	
<i>L. qingdaoensis</i>	42–104/3–5	63–105/3–5	93–119/2–5	50–93/2–5	11–29/2–5	43–422/4–7	Present paper
sp. nov.	76–129/3–4	60–104/3–4	–	–	–	–	

Table 2. Spicules measurements of *Leucosolenia qingdaoensis* sp. nov. (holotype).

	length(μm)				width(μm)				n
	min	mean	max	sd	min	mean	max	sd	
Diatines	43	219	422	93	1	4	7	1.7	50
Triactines 1									
paired	63	83	105	9	3	4	5	0.8	50
unpaired	42	66	105	13	–	–	–	–	–
Triactines 2									
paired	60	79	104	11	3	3	4	0.4	50
unpaired	76	102	129	15	–	–	–	–	–
Tetractines									
paired	50	77	93	12	2	4	5	0.8	20
unpaired	93	104	119	11	–	–	–	–	–
apical	11	21	29	6	–	–	–	–	–

The new species exhibits one type of diactine. In *L. ventosa* and *L. rosea*, there is no record of diactines, and in *L. mollis* and *L. nautilia*, there are two types of diactines. The triactines of *L. ventosa* are 2–8 times thicker than those in the new species; the triactines of *L. rosea* are 10–35 times thicker than in the new species; and *L. mollis* only has one type of triactine and all rays being nearly equally thick. The diactines

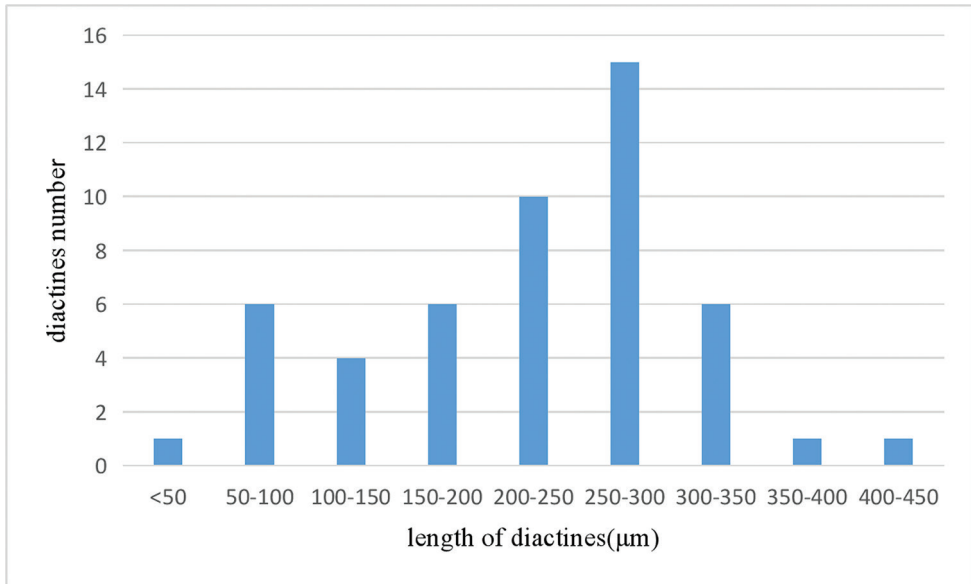


Figure 4. *Leucosolenia qingdaoensis* sp. nov. Size-class distribution of diactines (holotype).

of *L. nautilia* are extremely large, with a length of 1 mm and a thickness of 20 μm (Laubenfels 1932), while in the new species the diactines are less than 8 μm thick. Laubenfels (1932) gave few details on the actines, but *L. nautilia* differs from the new species by having only one type of triactine.

The difference between *L. albatrossi* and the new species is obvious. The diactines of *L. albatrossi* are club-shaped, while the diactines of the new species are spindle-shaped.

The sagittal triactines of the new species distinguish it from *L. macquariensis*, *L. tenera*, and *L. eleanor*. The new species have two types of sagittal triactines, while *L. macquariensis* and *L. tenera* only have one type of sagittal triactine, with rays of approximately equal length. *Leucosolenia eleanor* have both sagittal and regular triactines.

The new species, with slender and long diactines, the longest diactines 5 times longer than those of *L. feuerlandica*, is distinct from that species. Additionally, the triactines of the new species are sagittal, and the actines straight or undulated. However, the triactines of *L. feuerlandica* are pseudoderm sagittal and are tripod-shaped.

Leucosolenia echinata, *L. lucasi*, and *L. qingdaoensis* sp. nov. have many features in common, including their body shape, colour in alcohol, general arrangement, shape of diactines, and apical ray, but they show important differences in the shape of their triactines. The new species has two types of triactines; *L. lucasi* and *L. echinata* only have one type of triactine. The triactines of *L. lucasi* are sagittal, but the three angles are roughly equal; the triactines of *L. echinata* are generally regular, and frequently slightly sagittal, with the oral angle largest and the basal ray longest.

Key to the species of *Leucosolenia* in the Pacific Ocean

1	Skeleton contains only regular spicules.....	2
1a	Skeleton contains sagittal spicules	4
2	Skeleton including diactines	<i>L. pyriformis</i>
2a	Skeleton without diactines	3
3	Rays are stout.....	<i>L. minuta</i>
3a	Rays are relatively thin	<i>L. serica</i>
4	Skeleton contains diactines, triactines, and tetractines.....	5
4a	Skeleton contains triactines and tetractines	<i>L. rosea</i>
4b	Skeleton contains only triactines	<i>L. ventosa</i>
5	Skeleton contains one type of diactine	8
5a	Skeleton contains two types of diactines	6
6	Diactines are club-shaped	<i>L. macquariensis</i>
6a	Diactines are spindle-shaped.....	7
7	Skeleton without large diactines.....	<i>L. mollis</i>
7a	Skeleton including large diactines	<i>L. nautilia</i>
8	One tip of diactines has spines	<i>L. australis</i>
8a	Diactines have no spines	9
9	Skeleton contains one type of triactine.....	10
9a	Skeleton contains two types of triactines	11
10	Sagittal triactines with rays are of approximately equal in length....	<i>L. tenera</i>
10a	Sagittal triactines with rays are of different lengths.....	<i>L. lucasi</i>
10b	Triactines are generally regular, slightly sagittal.....	<i>L. echinata</i>
11	Skeleton including tripod type of triactines.....	<i>L. feuerlandica</i>
11a	Skeleton without tripod type of triactines	12
12	Diactines have one 'lance head' type ends	<i>L. albatrossi</i>
12a	Diactines have two smooth and sharply pointed ends	13
13	Skeleton contains both sagittal and regular triactines	<i>L. eleanor</i>
13a	Skeleton contains only sagittal triactines	<i>L. qingdaoensis</i> sp.nov.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (no. 41706188), the Science and Technology Basic Work Program (2014FY110500), Biological Resources Programme, Chinese Academy of Sciences KFJ-BRP-017-37, and the Scientific and Technological Innovation Project Financially Supported by Qingdao National Laboratory for Marine Science and Technology (no. 2015ASKJ01). We thank Dr Yuanyuan Sun for assistance with SEM operations, and we appreciate Dr Qinghe Liu for the help with photography. We also thank Mr Hongfa Wang, Dr Lin Ma, Dr Jixing Sui, and Mr Youwei Tzeng for providing important references needed for this study.

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On *Araniella* and *Neoscona* (Araneae, Araneidae) of the Caucasus, Middle East and Central Asia

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Academic editor: G. Blagoev | Received 3 November 2019 | Accepted 18 December 2019 | Published 22 January 2020

<http://zoobank.org/EF6D38B4-B1A3-402B-B764-2DACC4490AC7>

Citation: Zamani A, Marusik YM, Šestáková A (2020) On *Araniella* and *Neoscona* (Araneae, Araneidae) of the Caucasus, Middle East and Central Asia. ZooKeys 906: 13–40. <https://doi.org/10.3897/zookeys.906.47978>

Abstract

New taxonomic data for species belonging to *Araniella* Chamberlin & Ivie, 1942 and *Neoscona* Simon, 1864 occurring in the Caucasus, Middle East and Central Asia are provided. Three species are described as new to science: *A. mithra* **sp. nov.** (♂♀, northwestern, central and southwestern Iran), *A. villanii* **sp. nov.** (♂♀, southwestern Iran, eastern Kazakhstan and northern India) and *N. isatis* **sp. nov.** (♂♀, central Iran). *Neoscona spasskyi* (Brignoli, 1983) **comb. nov., stat. res.** is removed from the synonymy of *N. tedgenica* (Bakhvalov, 1978), re-described and recorded from Iran and Turkmenistan for the first time. New combinations are established for this species, as well as for *Araniella nigromaculata* (Schenkel, 1963) **comb. nov.** (♀, north-central China) (both ex. *Araneus*). Two new synonymies are proposed: *Araniella tbilisiensis* Mcheidze, 1997 **syn. nov.** is synonymized with *A. opisthographa* (Kulczyński, 1905), and *Neoscona sodom* Levy, 1998 **syn. nov.** is synonymized with *N. theisi* (Walckenaer, 1841); the latter is recorded from Iran, Georgia, and Russia (Northern Caucasus) for the first time.

Keywords

Aranei, new species, new combination, new record, new synonymy, orb-web spiders, redescription

Introduction

Araneidae Clerck, 1757 with 3072 valid species (WSC 2019) is the third largest family of spiders. At least in the Palaearctic, it is the best-studied family of spiders due to numerous publications dealing with the survey of regional fauna, or revisions of Euro-

pean and Far East (China, Japan, Korea) species. However, the Central Palaearctic is not well studied in comparison to other parts. Several species described by Bakhvalov (1970, 1974, 1978, 1981) remain known only from the original publications supplied with very schematic figures and brief descriptions. In order to fill this gap, we decided to study all available material from Iran and Central Asian countries and provide step by step reviews of different genera. Among material examined, we recognized two new species of *Araniella* Chamberlin & Ivie, 1942 and one new species of *Neoscona* Simon, 1864. While comparing new species with species occurring in the region, we recognized two new synonyms and two new combinations in both genera. The goals of this paper are to provide illustrated descriptions of new species and redescriptions of poorly known species, along with new combinations, synonymies, and distribution records.

Materials and methods

Specimens were photographed using an Olympus Camedia E-520 camera attached to an Olympus SZX16 stereomicroscope or to the eye piece of an Olympus BH2 transmission microscope, and a JEOL JSM-5200 scanning electron microscope (SEM) at the Zoological Museum of University of Turku, Finland. Digital images were prepared using CombineZP image stacking software. Illustrations of internal genitalia were made after clearing them in a 10% KOH aqueous solution. Lengths of leg segments were measured on the dorsal side. Measurements are provided for leg I only (IV, if missing) and listed as: total length (femur, patella, tibia, metatarsus, tarsus). All measurements are given in millimeters.

Abbreviations not explained in the text: **ALE** – anterior lateral eye, **AME** – anterior median eye, **PLE** – posterior lateral eye, **PME** – posterior median eye.

Depositories: **MHNG** – Muséum d'histoire naturelle, Genève, Switzerland, **MMUE** – Manchester Museum of the University of Manchester, England, **ZMMU** – Zoological Museum of Moscow University, Moscow, Russia, **ZMUT** – Zoological Museum of University of Turku, Finland, **PPC** – A.V. Ponomarev's personal collection, Rostov on Don, Russia.

Taxonomy

Family Araneidae Clerck, 1757

Genus *Araniella* Chamberlin & Ivie, 1942

Type species. *Epeira displicata* Hentz, 1847 from Alabama, USA.

Comments. Currently, this genus includes 12 species distributed exclusively in the Holarctic (WSC 2019). Only two species, the generotype and *A. proxima* (Kulczyński, 1885), are known in both parts of the realm (Palaearctic and Nearctic); all other spe-

cies are restricted to the Palaearctic. Although the genus has never been the subject of a global revision, it is well studied, and all species are known by both sexes, with the exception of *A. tbilisiensis* (Mcheidze, 1997). This species was described on the basis of both sexes, but the male palp has never been illustrated.

Diagnosis. The genus well differs from all Holarctic genera of Araneidae by large (as long as embolus and terminal apophysis), claw- or spine-like median apophysis directed mesally (vs. not claw- or spine-like but having at least 2 arms).

***Araniella mithra* sp. nov.**

<http://zoobank.org/DC0D034A-4554-4C01-B641-D2C6731DE77F>

Figs 1A, C; 2A, B; 4A, B; 6A, B; 7C; 8C; 9C; 10C; 18

Araniella proxima: Zamani et al. 2017: 58 (misidentification).

Type material. Iran: **Holotype** ♂ and **paratypes** 1♂ 2♀ (MHNG), Isfahan Province: Nowgahan, 33°11'N, 50°04'E, 22.06.1974 (A. Senglet); 1♀ (MHNG), Falavarjan, 32°34'N, 51°31'E, 14.06.1974 (A. Senglet); 2♂12♀ (MHNG), Chaharmahal & Bakhtiari Province: Dimeh, 32°29'N, 50°16'E, 21.06.1974 (A. Senglet); 1♂ 1♀ (MHNG), West Azarbayjan Province: Maku, 39°08'N, 44°30'E, 23.06.1973 (A. Senglet), 1♂ (MMUE), no label.

Comparative material. *Araniella opisthographa* (Kulczyński, 1905). FINLAND: 1♂ (ZMUT); Åland Islands: Lemland, Rörstorp, 27.06.1971 (P. Lehtinen); IRAN: 1♂1♀1sub♂ (ZMMU): Mazandaran Province: Barseh Vil., 36°37'N, 50°41'E, 10.06.2000 (Y.M. Marusik). TURKEY: 1♂5♀1sub♂ (ZMMU): Kastamonu Province: Azdavay Dist., 41°41'N, 33°25'E, 975 m, 30.05.2009 (Y.M. Marusik).

Etymology. The specific epithet is a noun in apposition, and refers to Mithra, the god of light in ancient Indo-Iranian mythology.

Diagnosis. Male palp and epigyne resemble those of *A. opisthographa*, but the two species can be differentiated by the following characters: 1) the embolus is slimmer in *A. mithra* sp. nov., vs. triangular-shaped and with a wider base in *A. opisthographa*; 2) the terminal apophysis in *A. mithra* sp. nov. is almost as wide over its entire length, vs. wider near the peak in *A. opisthographa*; 3) the conductor in *A. mithra* sp. nov. has three distinct spikes, vs. one spike and one more rounded process in *A. opisthographa*; 4) the tegulum in *A. mithra* sp. nov. is higher with a short pointed tip, vs. the slender tegulum with a longer tip in *A. opisthographa*; 5) male carapace unicolor in *A. mithra* sp. nov., vs. presence of broad dark marginal bands in *A. opisthographa*; 6) epigyne with slightly longer scape, and the sclerotized bulges are rounded around the base of scape in *A. mithra* sp. nov., vs. more incised triangular bulges in *A. opisthographa*.

Description (colors and pattern seem faded). **Male** (holotype). Habitus as in Fig. 1A. Total length 5.04. Carapace 2.36 long, 2.19 wide in pars thoracica, 0.91 in pars cephalica. Eye sizes and interdistances: AME: 0.09, ALE: 0.09, PME: 0.11, PLE: 0.12, AME–AME: 0.13, PME–PME: 0.12. Carapace, sternum, labium, chelicerae,

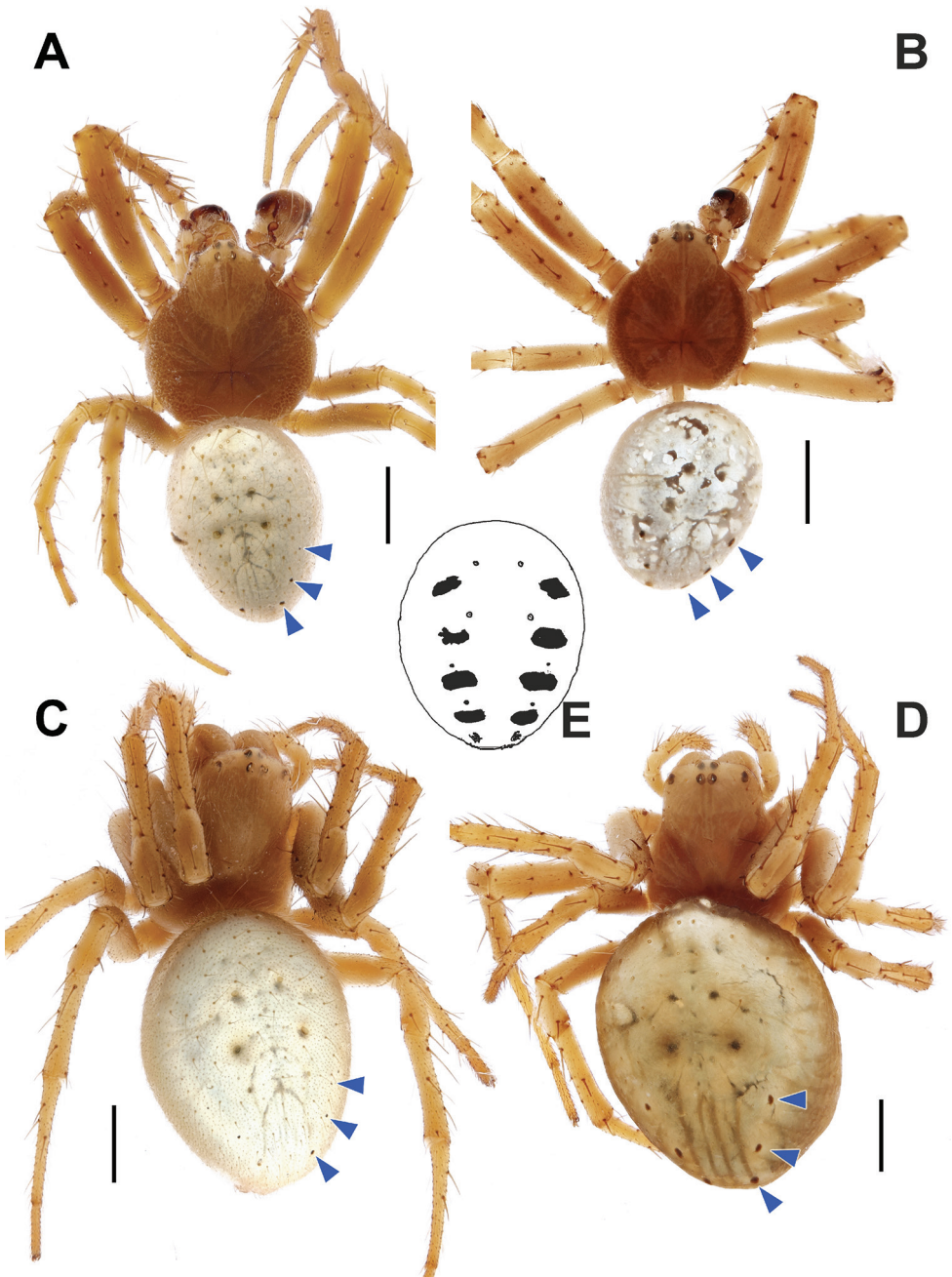


Figure 1. Dorsal habitus of *Araniella mithra* sp. nov. (**A, C**) and *A. villanii* sp. nov. (**B, D**) and abdomen of *A. nigromaculata* (**E**). **A, B** Males **C, D** females. Blue triangles point on black dots on opisthosoma. Scale bars: 1 mm.

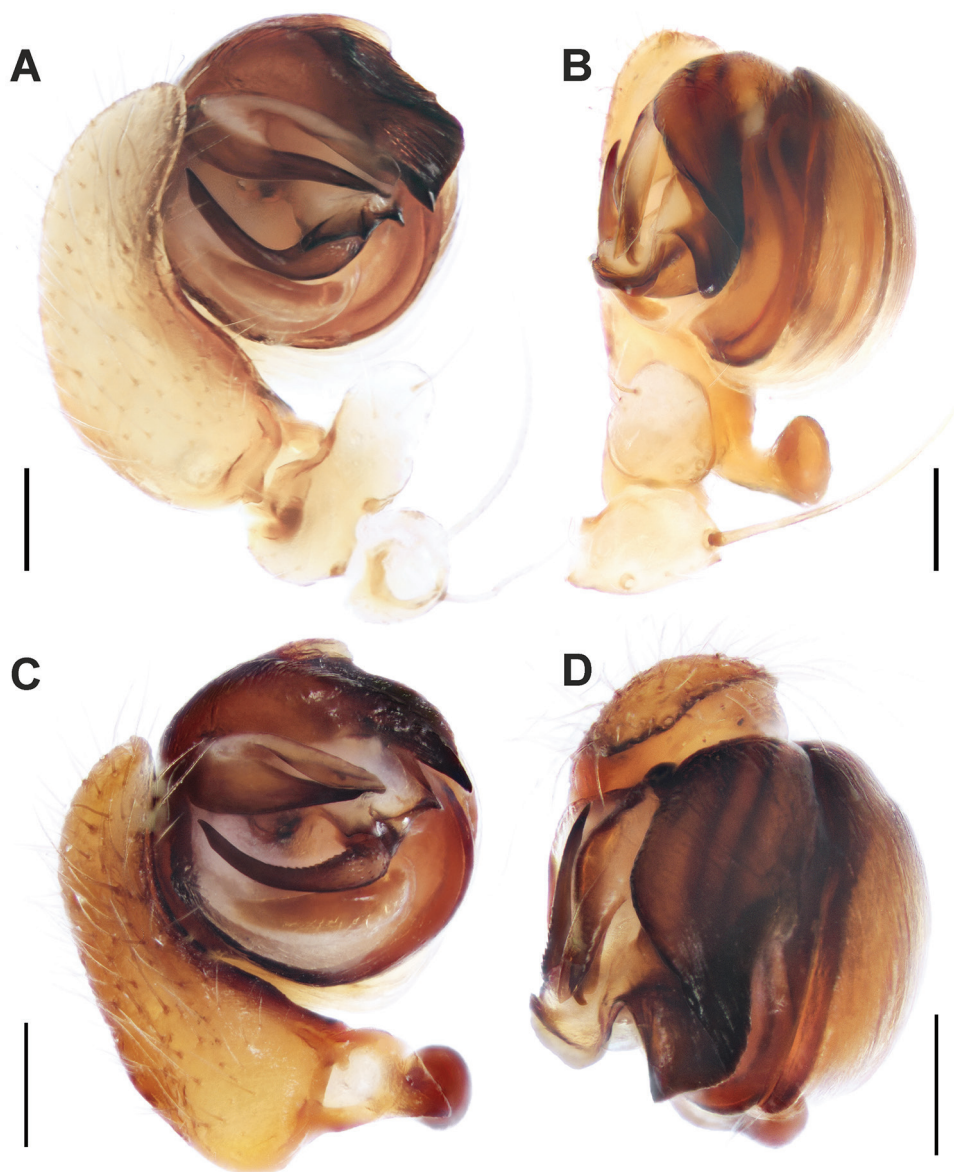


Figure 2. Male palps of *Araniella mithra* sp. nov. (**A, B**) and *A. opisthographa* (**C, D**). **A, C** Retrolateral **B, D** ventral. Scale bars: 0.2 mm.

and maxillae reddish brown, lighter ventrally and in pars cephalica, without any patterns. Legs the same color as the carapace. Abdomen pale (stored in alcohol, most probably green in live specimens) dorsally, dark gray ventrally, with three pairs of black lateral spots on dorsum posteriorly. Spinnerets light brown, apical segment lighter. Leg I measurements: 7.46 (2.21, 0.93, 1.75, 1.75, 0.82).

Palp as in Figs 2A, B; 4A, B; 6A, B. Tegulum with low round ridge and terminally with short pointed tip; terminal apophysis with blunt end and almost equally wide along its length; embolus pointed, sickle-shaped bent; median apophysis sickle-shaped bent upwards, covered by small denticles (less visible via stereomicroscope), with pointed tip ended near base of embolus; conductor with three distinct spikes.

Female. Habitus as in Fig. 1C. Total length 5.65. Carapace 2.40 long, 1.87 wide in pars thoracica, 1.19 in pars cephalica. Eye sizes and interdistances: AME: 0.11, ALE: 0.12, PME: 0.12, PLE: 0.09, AME–AME: 0.14, PME–PME: 0.11. Coloration as in male, slightly lighter. Leg I measurements: 7.30 (2.08, 1.02, 1.60, 1.75, 0.85).

Epigyne as in Figs 7C, 8C, 9C, 10C. Scape longer than wide, slightly wider at its base, reaching distinctly beyond epigyne. Copulatory ducts visible through epigynal cuticle. Receptacles oval, entrance ducts touching each other. Median plate (posterior view), between lateral sclerotized copulatory bulges, round and widest in its center.

Phenology. Adult males and females were collected in mid and late June.

Distribution. Known only from the type localities in northwestern, central and southwestern Iran. It is possible that some of the previous Iranian records of *A. opisthographa* refer to this species.

Araniella villanii sp. nov.

<http://zoobank.org/067356F5-0F8F-4F5D-A3D9-604EE3AEDC12>

Figs 1B, D; 3A, B; 4C, D; 5A, B; 7A; 8A; 9A; 10A; 18

Type material. IRAN: *Holotype* ♂ and *paratypes* 1♀ (MHNG), Chaharmahal & Bakhtiari Province: Kuhrang, 32°28'N, 50°08'E, 19.06.1974 (A. Senglet). KAZAKHSTAN: 2♂ 4♀ (ZMMU), East Kazakhstan Region: Urzhar Distr., Tarbagatai Mt. Range, 5 km NE of Alekseevka, Urzharka river canyon, left bank, 47°17'N, 81°37'E, 1050–1200 m, 23.06.2001 (A.V. Gromov); 3♂ 4♀ (ZMMU), Urzhar Distr., 7–8 km NE of Karatuma [=Kirovka], Tarbagatai Mt. Range, Sholakterek river canyon, left bank, 47°10'N, 82°06'E, 1200–1250 m, 23.06.2001 (A.V. Gromov); 1♂ 2♀ (ZMMU), Urzhar Distr., ca. 4 km NE of Kyzylbulak [=Petrovskoye], Kyzylbulak river canyon, left bank, 47°03'N, 82°18'E, 1100–1150 m, 21.06.2001 (A.V. Gromov). INDIA: 6♂ 2♀ (MMUE), Himachal Pradesh State: Tandi Vill., 5 km S of Keylong, 2700 m, 11.06.1999 (Y.M. Marusik); 1♂ 1♀ (MMUE), Jahalman Vill., 32°38'N, 76°51'E, 3000–3100 m, 13.06.1999 (Y.M. Marusik).

Comparative material. *Araniella proxima* (Kulczyński, 1885). RUSSIA: 1♂ 1♀ (ZMMU): SE Tuva, Tere-Khol Lake, Sharlaa Stand and vicinity, 50°01'N, 95°03'E, 1050 m, 6–14.07.1996 (Y.M. Marusik).

Etymology. This species is named after French mathematician Cédric Villani (born 5.10.1973), winner of the Fields Medal in 2010 and the former director of Sorbonne University's Henri Poincaré Institute, for his “mysterious love” for spiders.

Diagnosis. Male palp and epigyne resemble those of *A. proxima* and *A. opisthographa*. Both species, compared to *A. villanii* sp. nov., have similar shape of embolus, and ter-

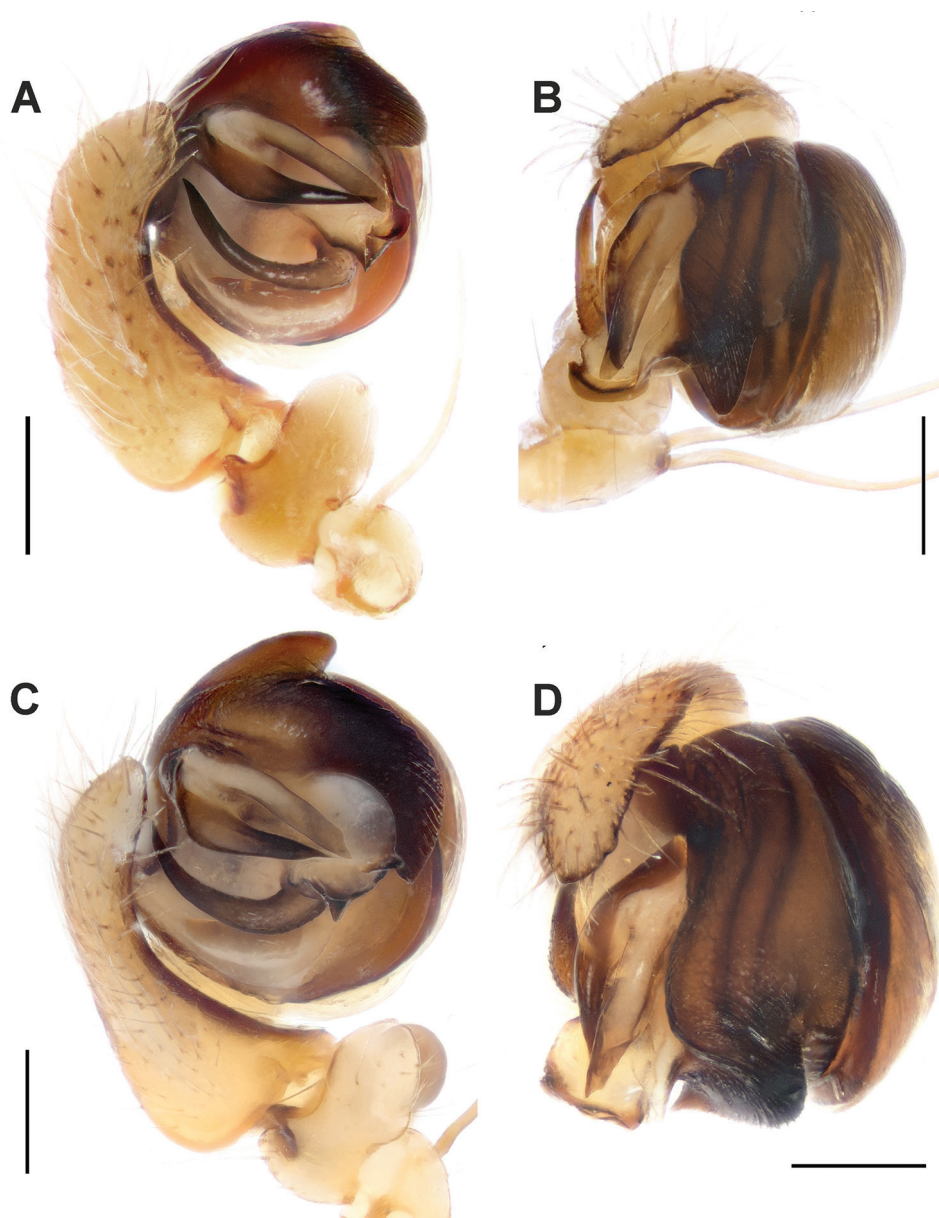


Figure 3. Male palps of *Araniella villanii* sp. nov. (**A, B**) and *A. proxima* (**C, D**). **A, C** Retrolateral **B, D** ventral. Scale bars: 0.2 mm.

minal apophysis is identical to that of *A. proxima* and conductor is identical to that of *A. opisthographa*. However, the new species can be diagnosed by the following characteristics: 1) the tegulum in *A. villanii* sp. nov. is markedly shorter, higher, protruding and rounded, vs. more compact non-protruding tegulum with distinctly higher ridge in *A. proxima*, and slender with pointed tip in *A. opisthographa*; 2) the terminal apophysis

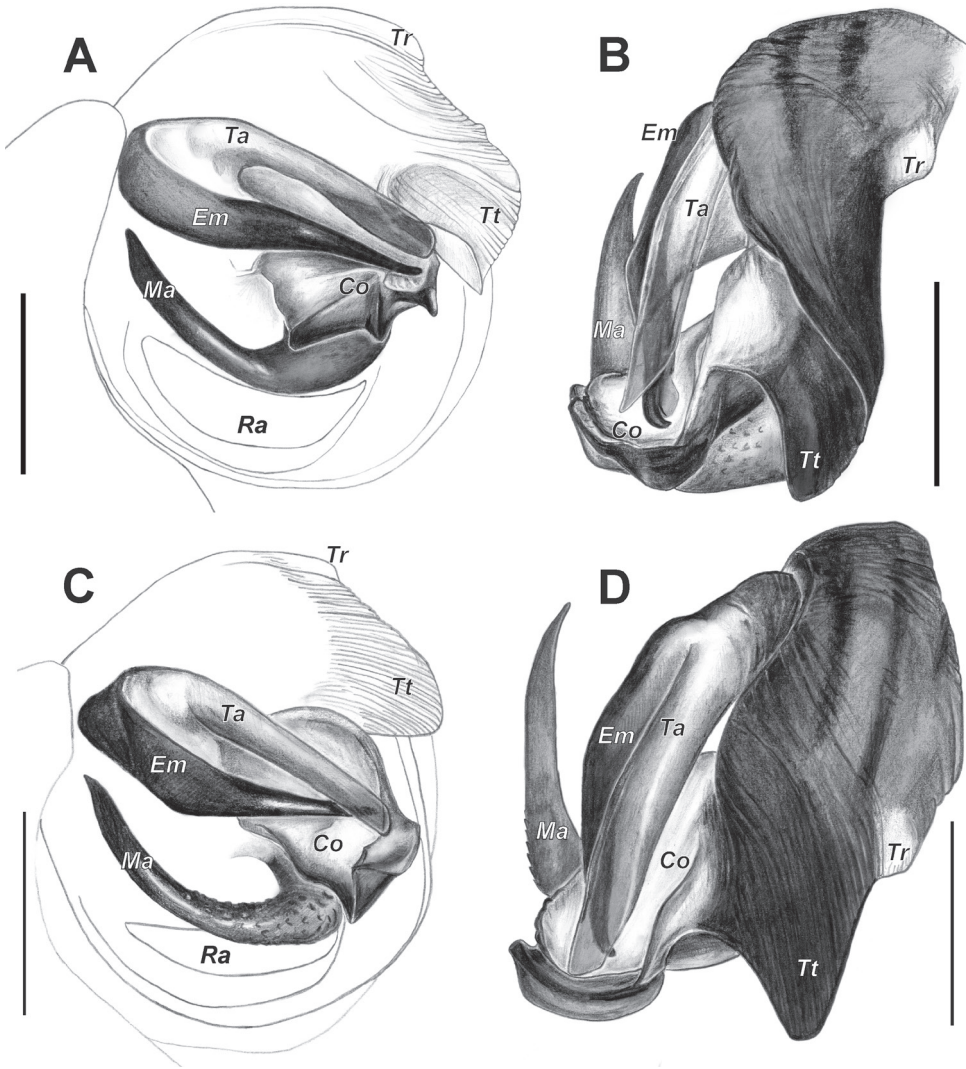


Figure 4. Male palps of *Araniella mithra* sp. nov. (**A,B**) and *A. villanii* sp. nov. (**C,D**). **A,C** Retrolateral **B, D** ventral. Abbreviations: *Co* conductor, *Em* embolus, *Ma* median apophysis, *Ra* radix, *Ta* terminal apophysis, *Tr* tegular ridge, *Tt* tip of tegulum. Scale bars: 0.2 mm.

in *A. villanii* sp. nov. is almost as wide throughout its length, vs. wider at the tip in *A. opisthographa*; 3) the conductor in *A. villanii* sp. nov. has one spike and one more rounded process connected to each other, vs. two independent spikes in *A. proxima*; 4) the median apophysis in *A. villanii* sp. nov. is longer in comparison to both mentioned species; 5) epigyne of *A. villanii* sp. nov. has a distinctly broader scape, vs. slender in *A. proxima* and *A. opisthographa*; 6) the median plate is narrower and more rectangular in the new species, vs. wider and rounded plate in *A. opisthographa* and triangular plate in *A. proxima*; 7) receptacles and entrance ducts in *A. villanii* sp. nov. do not touch

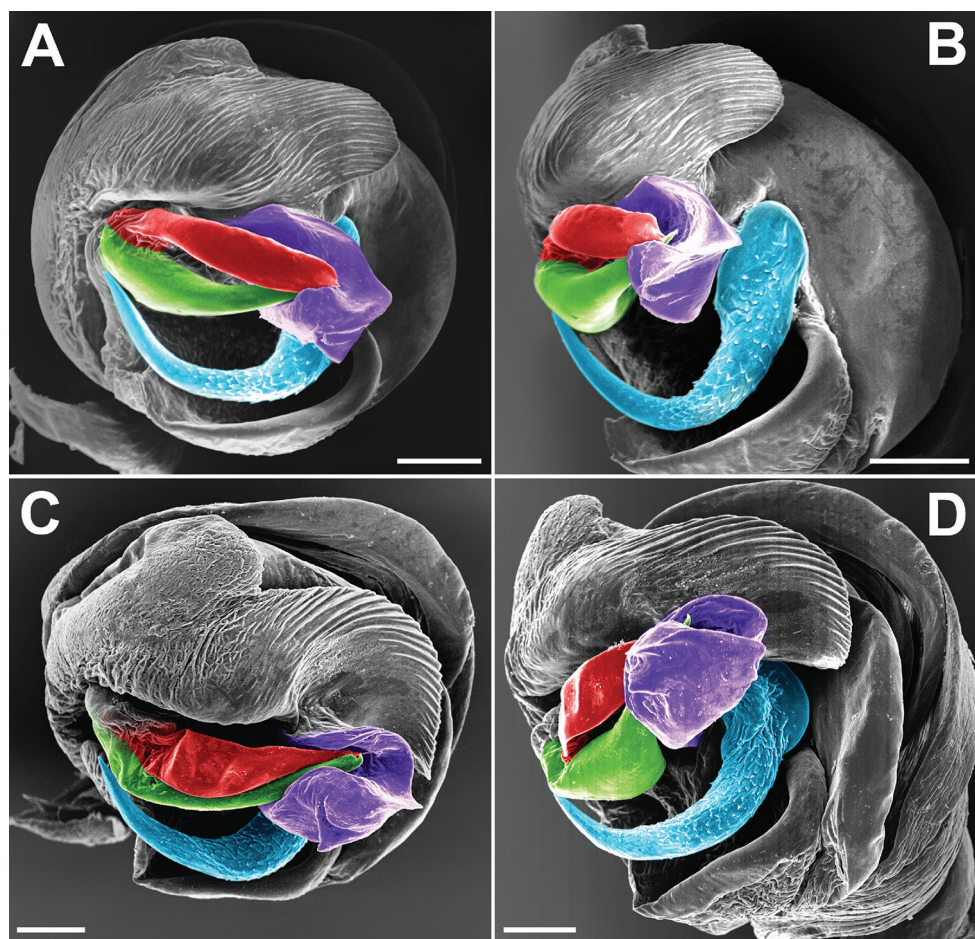


Figure 5. SEM graphs of the bulbs of *Araniella villanii* sp. nov. (**A, B**) and *A. proxima* (**C, D**). **A, C** Retrolateral **B, D** ventro-retrolateral. Blue – median apophysis, green – embolus, red – terminal apophysis, violet – conductor. Scale bars: 0.1 mm.

each other, but in *A. opisthographa* both structures touch each other, and in *A. proxima* only receptacles touch each other.

Description (colors and pattern seem faded). **Male** (holotype). Habitus as in Fig. 1B. Total length 4.37. Carapace 1.91 long, 1.69 wide in pars thoracica, 0.76 in pars cephalica. Eye sizes and interdistances: AME: 0.08, ALE: 0.07, PME: 0.09, PLE: 0.09, AME–AME: 0.12, PME–PME: 0.11. Carapace, sternum, labium, chelicerae, and maxillae reddish brown, lighter ventrally, carapace with two broad dark marginal bands. Legs lighter in color than the carapace, distally with dark broad annulations. Abdomen pale (stored in alcohol, most probably green in live specimens) dorsally, dark gray ventrally, posterodorsally with three pairs of black lateral spots. Spinnerets light brown, apical segment lighter. Leg I measurements: 6.43 (1.97, 0.82, 1.50, 1.46, 0.68).

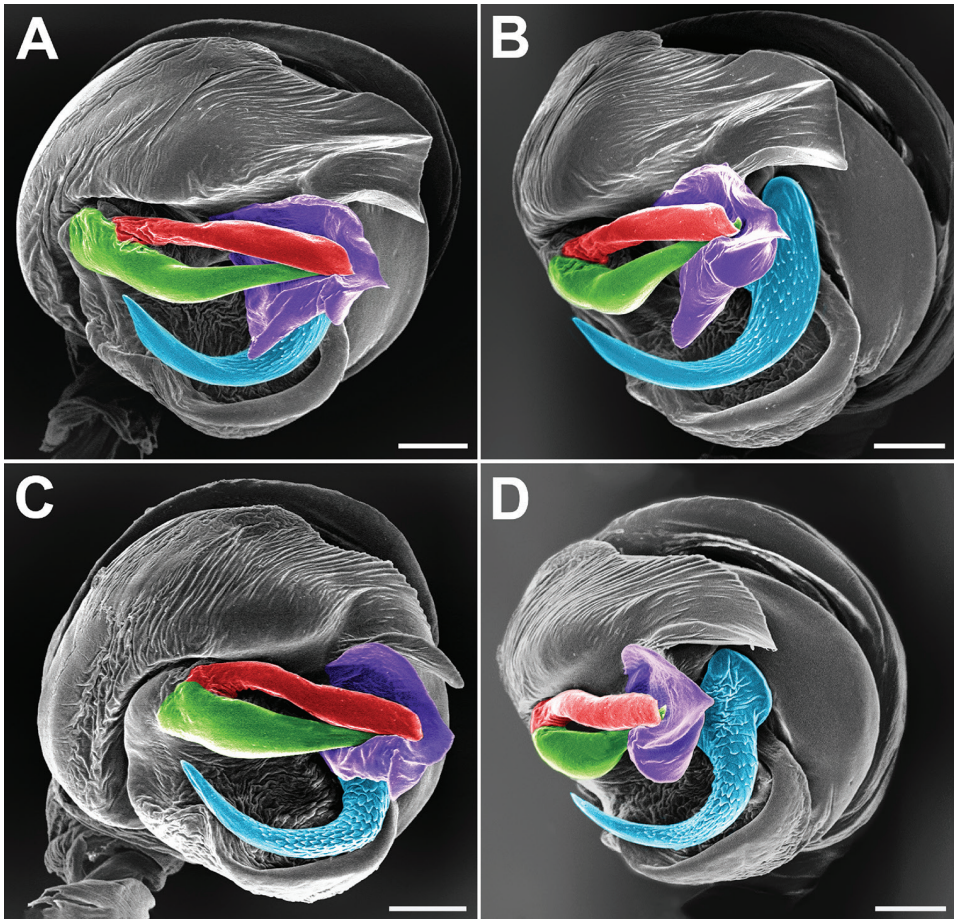


Figure 6. SEM graphs of the bulbs of *Araniella mithra* sp. nov. (**A, B**) and *A. opisthographa* (**C, D**). **A, C** Retrolateral **B, D** ventro-retrolateral. Blue – median apophysis, green – embolus, red – terminal apophysis, violet – conductor. Scale bars: 0.1 mm.

Palp as in Figs 3A, B; 4C, D; 5A, B. Tegulum terminally blunt with round ridge; terminal apophysis with blunt end and almost equally wide along its length; embolus triangular-shaped, with wider base; median apophysis sickle-shaped bent upwards with pointed tip ending near base of embolus and covered by many small denticles; conductor with one distinct spike and one more rounded process.

Female. Habitus as in Fig. 1D. Total length 6.00. Carapace 2.58 long, 2.15 wide in pars thoracica, 1.29 in pars cephalica. Eye sizes and interdistances: AME: 0.09, ALE: 0.08, PME: 0.10, PLE: 0.09, AME–AME: 0.14, PME–PME: 0.12. Coloration as in male. Leg I measurements: 6.78 (1.93, 0.98, 1.49, 1.51, 0.87).

Epigyne as in Figs 7A, 8A, 9A, 10A. Scape wider in the middle, extending beyond epigynal plate. Copulatory ducts not clearly visible through epigyne cuticle. Oval receptacles are about half their diameter apart; entrance ducts a similar distance apart. Median plate (posterior view), between lateral sclerotized copulatory bulges, slender, slightly wider in the middle.

Phenology. All adult specimens were collected in mid and late June.

Distribution. Known only from the type localities in southwestern Iran, eastern Kazakhstan and northern India. Potentially widely distributed in the Middle East and Central Asia.

Araniella opisthographa* (Kulczyński, 1905)

Figs 2C, D; 6C, D; 7D; 8D; 9D; 10D

Araniella opisthographa: Blanke 1982: 289, fig. 3c–d, 5c–d, 6c–d, 8b (♂♀); Roberts 1995: 328, fig. (♂♀); Almquist 2005: 154, fig. 162a–g (♂♀).

Araneus tbilisiensis Mcheidze, 1997: 280, fig. 642–644 (♂♀). **syn. nov.**

Comments. *Araneus tbilisiensis* was described based on one male and four females from the environs of Tbilisi, Georgia. There is no indication which specimen/sex was selected as the holotype. Mcheidze (1997) provided figures of male and female habitus, as well as epigyne, but the male palp was not illustrated. Judging from the figure of epigyne and distribution, it is most likely a junior synonym of *A. opisthographa*, which is already known from the surroundings of Tbilisi (Otto 2019). We tried to obtain the type material for this study, but we have been informed that the single male specimen is most probably lost (V. Pkhakadze, pers. comm.).

***Araniella nigromaculata* (Schenkel, 1963), comb. nov.**

Figs 1E, 8E

Araneus nigromaculatus Schenkel, 1963: 154, fig. 91a–c (♀).

Araneus nigromaculatus: Yin et al. 1997: 204, fig. 122a–c (♀); Song et al. 1999: 240, fig. 139e, f, 148l (♀).

Comments. The female holotype was collected in southern Gansu (ca. 33°40'N, 104°20'E), north-central China. Figures of Yin et al. (1997) and Song et al. (1999) are reproduced after Schenkel (1963). The holotype (in Muséum National d'Histoire Naturelle, Paris) was examined in 1980 by Yuri Marusik and illustrated, but no data have been copied from the label. Abdominal pattern and shape of epigyne indicates its belonging to *Araniella* and therefore we provide a new combination.

Genus *Neoscona* Simon, 1864

Neoscona Simon, 1864: 261.

Neoscona: Berman and Levi 1971: 469; Grasshoff 1986: 4; Tanikawa 1998: 134.

* For complete list of references see WSC (2019)

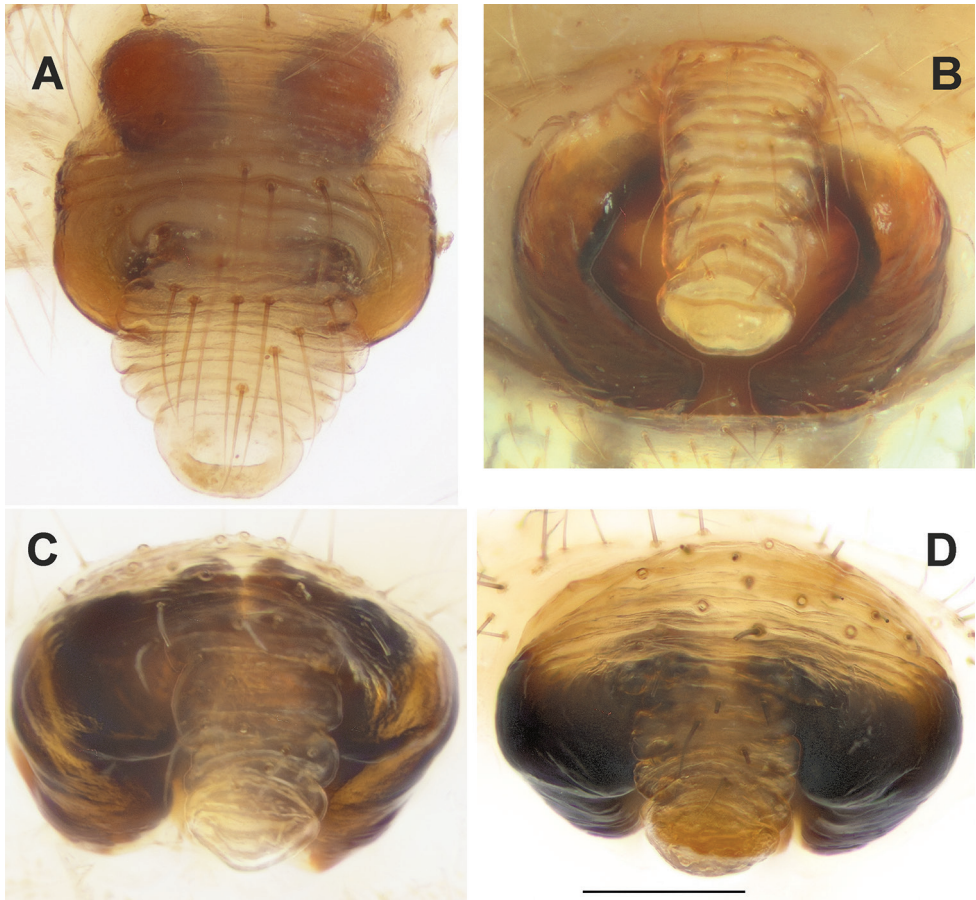


Figure 7. Ventral view of epigynes of *Araniella villanii* sp. nov. (A), *A. proxima* (B), *A. mithra* sp. nov. (C) and *A. opisthographa* (D). Scale bar: 0.2 mm.

Type species. *Epeira arabesca* Walckenaer, 1841, fixed by F. O. Pickard-Cambridge 1904: 466.

Note. Simon (1864) proposed this genus for nine species currently considered in *Larinioides* Caporiacco, 1934, *Araneus* Clerck, 1757 and *Neoscona*. Although type species were not fixed for any genera described in Simon's book [there were no rules for type fixation at that time], the author used the term 'espèces principales' (=main species). Simon (1864) considered "*L'épéire scalaire (neoscona)*" (= *A. marmoreus* Clerck, 1757) as the "main species".

Comments. With 124 valid species (WSC 2019), *Neoscona* is the third largest genus in Araneidae. Only *Araneus* Clerck, 1757 (595 spp.) and *Cyclosa* Menge, 1866 (180 spp.) are more speciose. At the same time, it has the highest number of synonyms (114) and *nomina dubia* (10) (WSC 2019) in comparison to the valid names. The genus has an almost global distribution, unknown only in South America. It is relatively well studied in North America, Africa, China, and Japan due to the revisions by

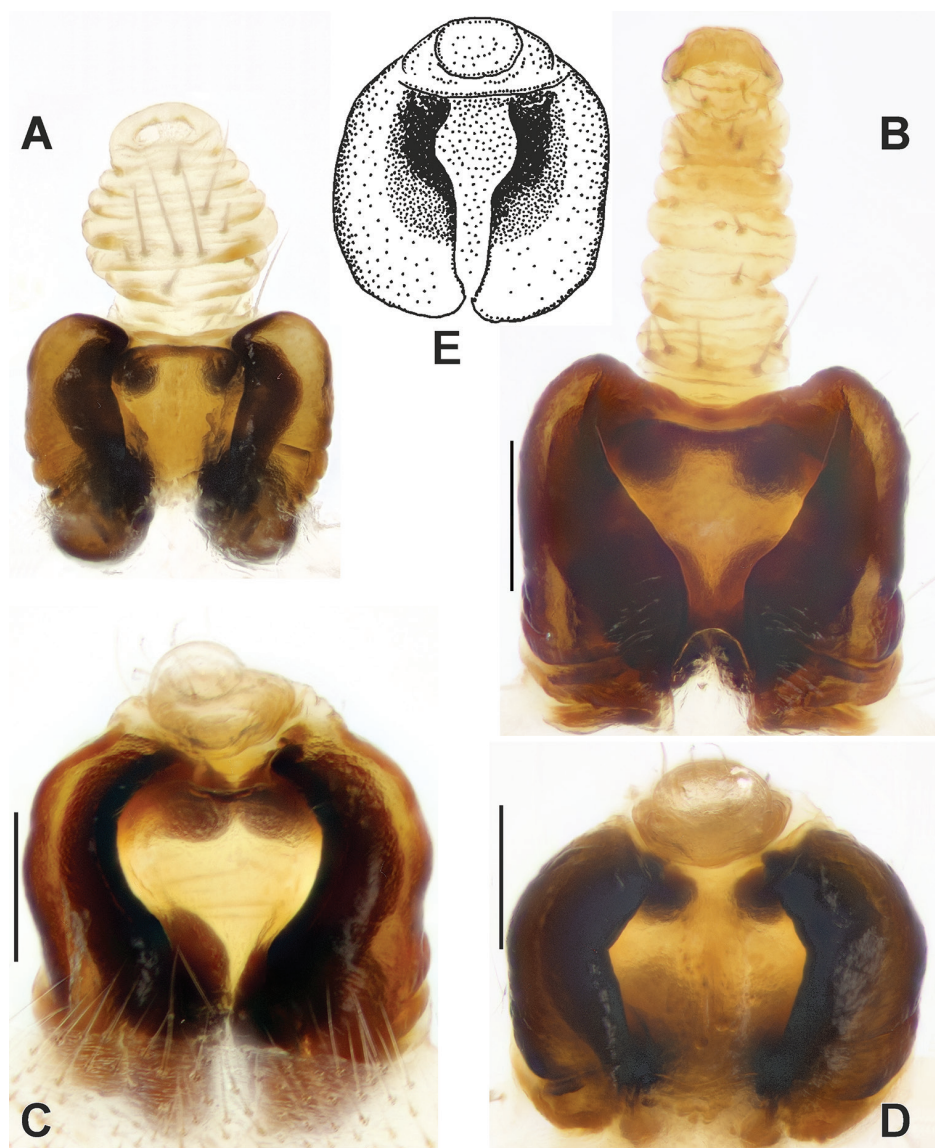


Figure 8. Posterior view of epigynes of *Araniella villanii* sp. nov. (A), *A. proxima* (B), *A. mithra* sp. nov. (C), *A. opisthographa* (D) and *A. nigromaculata* (E). Scale bars: 0.2 mm.

Berman and Levi (1971), Grasshoff (1986), Yin et al. (1997) and Tanikawa (1998), respectively, but remains poorly known in the Central Asia, India, South East Asia and Australia. Although the male palp is rather uniform in shape across the genus, epigynes can be split into two morphotypes, with inflexible scape (*Neoscona* s. str.) and with flexible scape (*Afraranea* Archer, 1951, a genus currently considered as a synonym of *Neoscona* in WSC (2019) with reference to Grasshoff (1986), although the latter author considered *Afraranea* as a subgenus of *Neoscona*).



Figure 9. Anterior view of epigynes of *Araniella villanii* sp. nov. (A), *A. proxima* (B), *A. mithra* sp. nov. (C) and *A. opisthographa* (D).

Currently, six species of *Neoscona* are known in the region: *N. adianta* (Walckenaer, 1802), *N. subfusca* (C.L. Koch, 1837), *N. theisi* (Walckenaer, 1841) (all throughout the region), *N. spasskyi* (Brignoli, 1983) (Tajikistan, Kyrgyzstan, Turkmenistan, Iran), *N. tedgenica* (Bakhvalov, 1978) (Turkmenistan) and *N. isatis* sp. nov. (Iran).

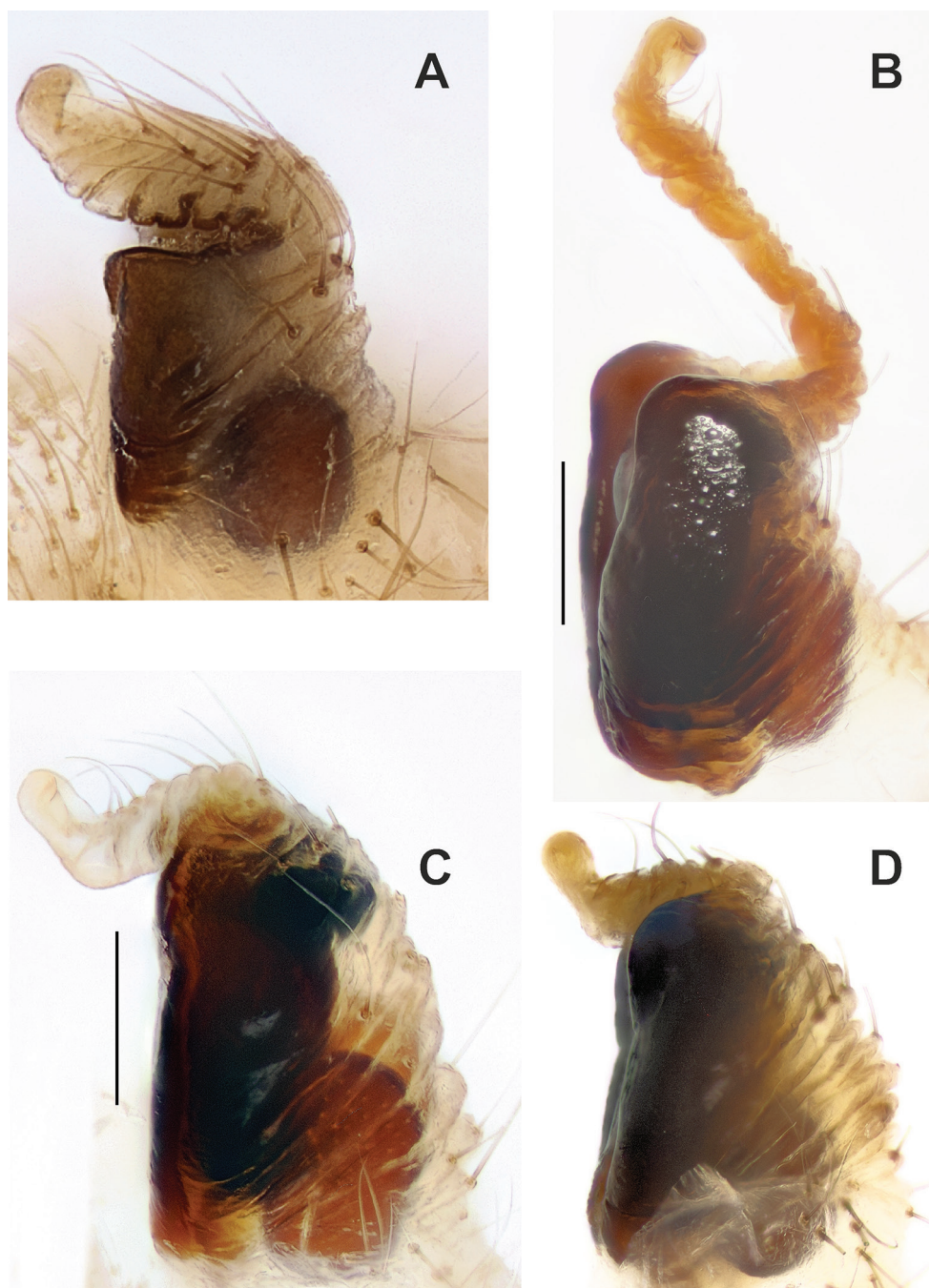


Figure 10. Lateral view of epigynes of *Araniella villanii* sp. nov. (A), *A. proxima* (B), *A. mithra* sp. nov. (C) and *A. opisthographa* (D). Scale bars: 0.2 mm.

Neoscona adianta* (Walckenaer, 1802)

Neoscona adiantum: Grasshoff 1986: 66, fig. 85–89 (♂♀).

Neoscona adianta: Levy 1998: 339, fig. 108–116 (♂♀); Tanikawa 1998: 140, fig. 9, 18–24 (♂♀); Tanikawa 2007: 68, fig. 160–161, 575–577 (♂♀).

Diagnosis. Both sexes of this species well differ from other congeners occurring in Central Asia, Iran and Caucasus by the absence of a white median band on the sternum.

Description. See above-cited literature.

Distribution. Transpalaeartic, known throughout the region: Armenia, Azerbaijan, Georgia, Iran, Turkmenistan, Uzbekistan, Kazakhstan, Kyrgyzstan, Tajikistan, and Altai in South Siberia (Mikhailov 2013, Zamani et al. 2019).

***Neoscona isatis* sp. nov.**

<http://zoobank.org/06094E25-00A6-473B-9AC7-4319CD43F833>

Figs 11D, E; 13E, F; 14B, F; 15E–G; 16D–F; 17G–I; 18

Type material. IRAN: **Holotype** ♂ and **paratype** 1♀ (MHNG), Yazd Province: Ahmabad, 32°20'N, 53°59'E, 15.08.2018 (A. Zamani).

Etymology. The specific epithet is a noun in apposition, and refers to the historic name of Yazd, the type locality of the species.

Diagnosis. The new species is similar to *N. theisi* and *N. spasskyi* in having a white median band on sternum (Fig. 13A, C, E), but well differs by having a broad white median band on the venter of abdomen (vs. venter with lateral white band, and dark median band). Males of *N. isatis* sp. nov. can be easily distinguished from the species occurring in the region by numerous small spines on tibia II (Fig. 14F) lacking in other species (Fig. 14D–E) and median apophysis lacking prolateral extension (*Me*) (vs. present). Epigyne of this species well differs from other species occurring in Central Asia by having prominent lateral extensions (*Le*) as long as wide and long scape (*Sc*) almost 2 times longer than wide (vs. lateral extensions absent or poorly developed and scape almost as wide as long, cf. Fig. 17A, D, G).

Description. Male. Habitus as in Fig. 11E. Total length 9.62. Carapace 4.14 long, 3.90 wide in pars thoracica, 1.28 in pars cephalica. Eye sizes and interdistances: AME: 0.20, ALE: 0.19, PME: 0.14, PLE: 0.14, AME–AME: 0.25, PME–PME: 0.10. Carapace, labium, chelicerae, and maxillae light brown, carapace with distinct and relatively long foveal mark, slightly darker in submarginal and without any patterns. Sternum with light median band. Legs the same color as the carapace, with annulations and numerous spines. Tibia II ventrally with about 90 spines of three types, fine – over 50, medium-sized – over 30, and few macrospines. Abdomen light yellowish, with scattered long white setae, dorsally with a horizontal gray line anteriorly, and a gray longitudinal branched pattern medially, with a brown dot on each side; ventrally with a white patch

* For complete list of references see WSC (2019)

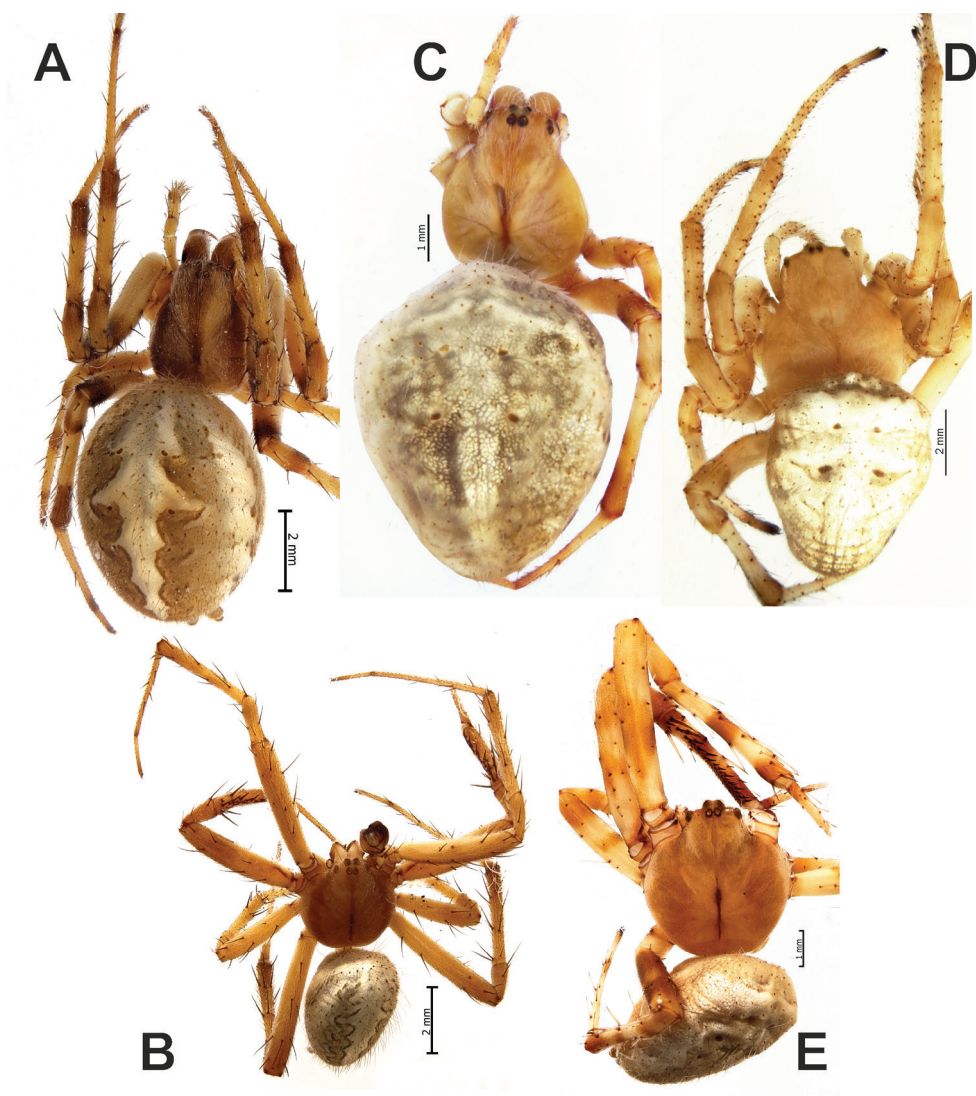


Figure 11. Dorsal habitus of *Neoscona theisi* (A, B), *N. spasskyi* (C) and *N. isatis* sp. nov. (D, E). A, C, D Females B, E males.

between epigastric furrow and spinnerets area. Spinnerets light brown, apical segment lighter. Leg IV (leg I incomplete) measurements: 14.39 (4.84, 1.91, 3.07, 3.47, 1.10).

Palp as in Figs 14B, 15E–G, 16D–F. Tegulum without distinct ventral extension; median apophysis (*Ma*) without prolateral extension, stipes of median apophysis (*Sm*) as long as apophysis; lamella (*La*) weakly sclerotized; conductor club-like.

Female. Habitus as in Fig. 11D. Total length 11.56. Carapace 5.02 long, 3.49 wide in pars thoracica, 1.74 in pars cephalica. Eye sizes and interdistances: AME: 0.17, ALE: 0.17, PME: 0.18, PLE: 0.19, AME–AME: 0.31, PME–PME: 0.12. Coloration

generally as in male, slightly lighter and more uniform, with less distinct patterns and markings, and abdomen with an additional two brown dots on dorsum, without any distinct patterns. Leg I measurements: 18.11 (5.01, 2.69, 4.27, 4.53, 1.61).

Epigyne as in Figs 13F, 17G–I. Long, with scape (Sc) as long as base; lateral extensions (Le) prominent, as long as wide, originates dorsally; scape almost twice longer than wide.

Distribution. Known only from the type locality in Yazd Province, central Iran.

***Neoscona spasskyi* (Brignoli, 1983), comb. nov., stat. res.**

Figs 11C; 12A, B; 13C–D; 14C, E; 15A–C; 16A–C; 17D–F; 18

Araneus cruciferoides Spassky, 1952: 203, fig. 6, 10 (♂♀).

Araneus spasskyi: Brignoli 1983: 258 (replacement name for *A. cruciferoides*).

Neoscona tedgenica: Marusik et al. 1991: 20 (misidentified).

Material examined. IRAN: 1♂ 3♀ (ZMMU): Golestan Province: Ramiyan, 36°59'N, 55°07'E, 29.07.74 (A. Senglet); 1♀ (MHNG): Razavi Khorasan Province: route to Amirabad, 36°47'N, 59°54'E, 1100 m, 23.07.74 (A. Senglet); 3♂ 9♀ (MHNG): North Khorasan Province: Bojnurd, 37°29'N, 57°26'E, 26.07.74 (A. Senglet); TURKMENISTAN: 1♂ 2♀ (ZMMU): Balkan Province: Magtymguly (formerly Garrygala, Kara-Kala), in house, 02.08.79 (V. Fet).

Diagnosis. *Neoscona spasskyi* differs from the similar *N. theisi* by having a thinner dark median band on the carapace and wider white lateral bands (cf. Figs 11C and 11A, B, D, E). Some specimens of this species have a pyramid-type pattern (Fig. 12A, B) lacking in other species. Males of this species differ from the congeners known in the region by having about 40 ventral spines on tibia II (vs. ca. 90, 20 or 10). *Neoscona spasskyi* differs from *N. isatis* sp. nov. by having prolateral extension of median apophysis. Epigyne of this species has the scape almost as wide as long vs. about twice longer than wide in *N. isatis* sp. nov. It differs from those in *N. theisi* by having distinct constriction (vs. lacking).

Description. Male. Habitus as in Figs 11B, 12A. Total length 7.47. Carapace 3.60 long, 2.98 wide in pars thoracica, 1.19 in pars cephalica. Eye sizes and interdistances: AME: 0.20, ALE: 0.14, PME: 0.15, PLE: 0.13, AME–AME: 0.19, PME–PME: 0.12. Carapace, labium, chelicerae, and maxillae reddish brown, carapace with distinct and relatively long foveal mark, slightly darker in submarginal and without any patterns. Sternum with dark frontal edges, and a light median band. Legs the same color as the carapace, with annulations and numerous spines. Abdomen grayish green, dark gray in frontal, and with a distinct dark green patch on dorsum, and two light bands with a dark gray patch between them ventrally. Spinnerets light brown, apical segment lighter. Leg I measurements: 16.48 (5.11, 1.83, 4.11, 4.51, 1.28).

Palp as in Figs 14C, 15A–C, 16A–C. Tegulum without distinct ventral extension; median apophysis (Ma) with prolateral extension (Me) subequal in length to spur (Ms) of median apophysis; stipes of median apophysis (Sm) as long as apophysis; lamella (La) weakly sclerotized; conductor club-like.

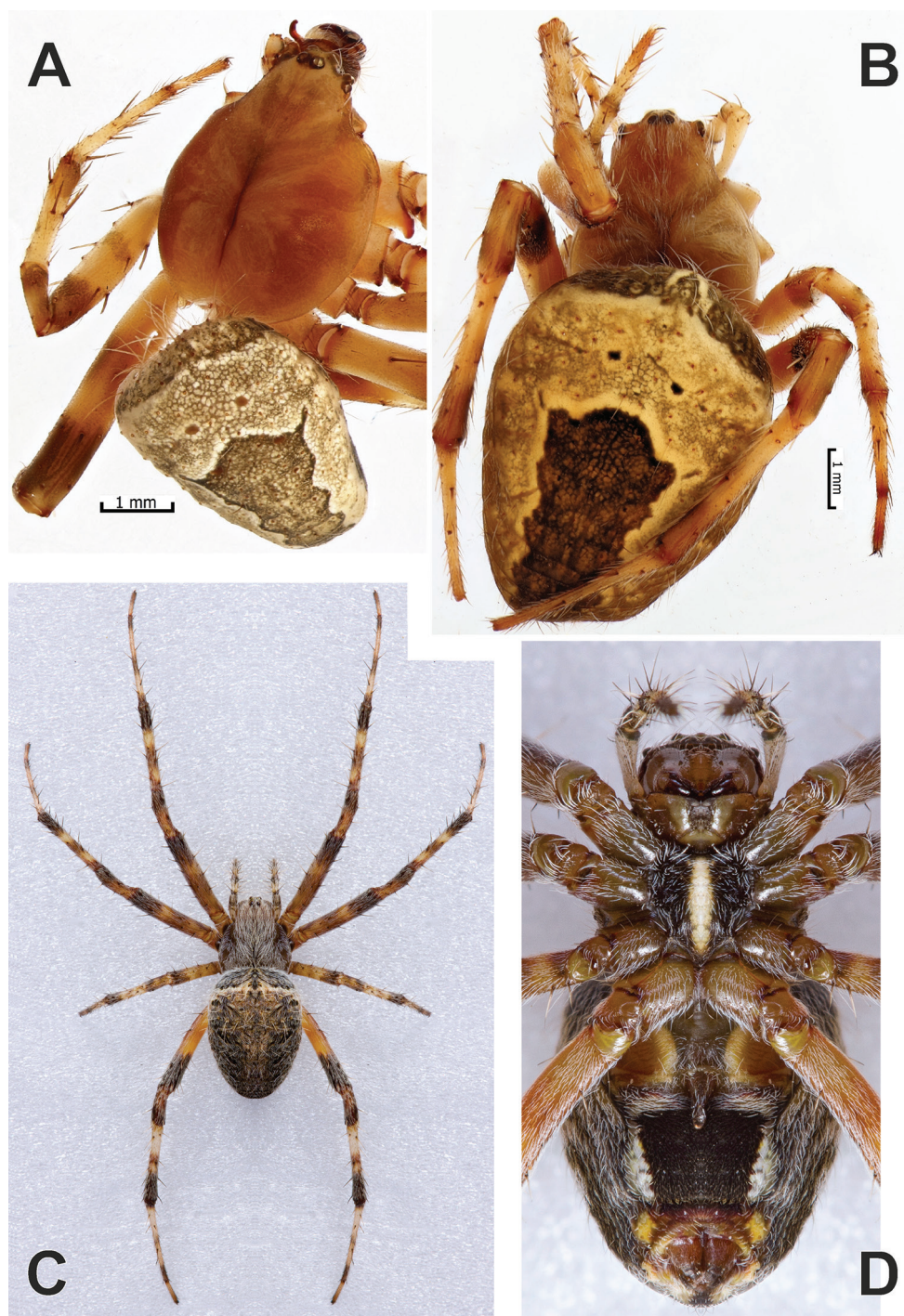


Figure 12. Habitus of *Neoscona spasskyi* (A, B) and *N. theisi* (C, D). A–C Dorsal D ventral C, D showing variations in comparison to specimens depicted in Figure 11. Photos C, D courtesy of A. Seropian.

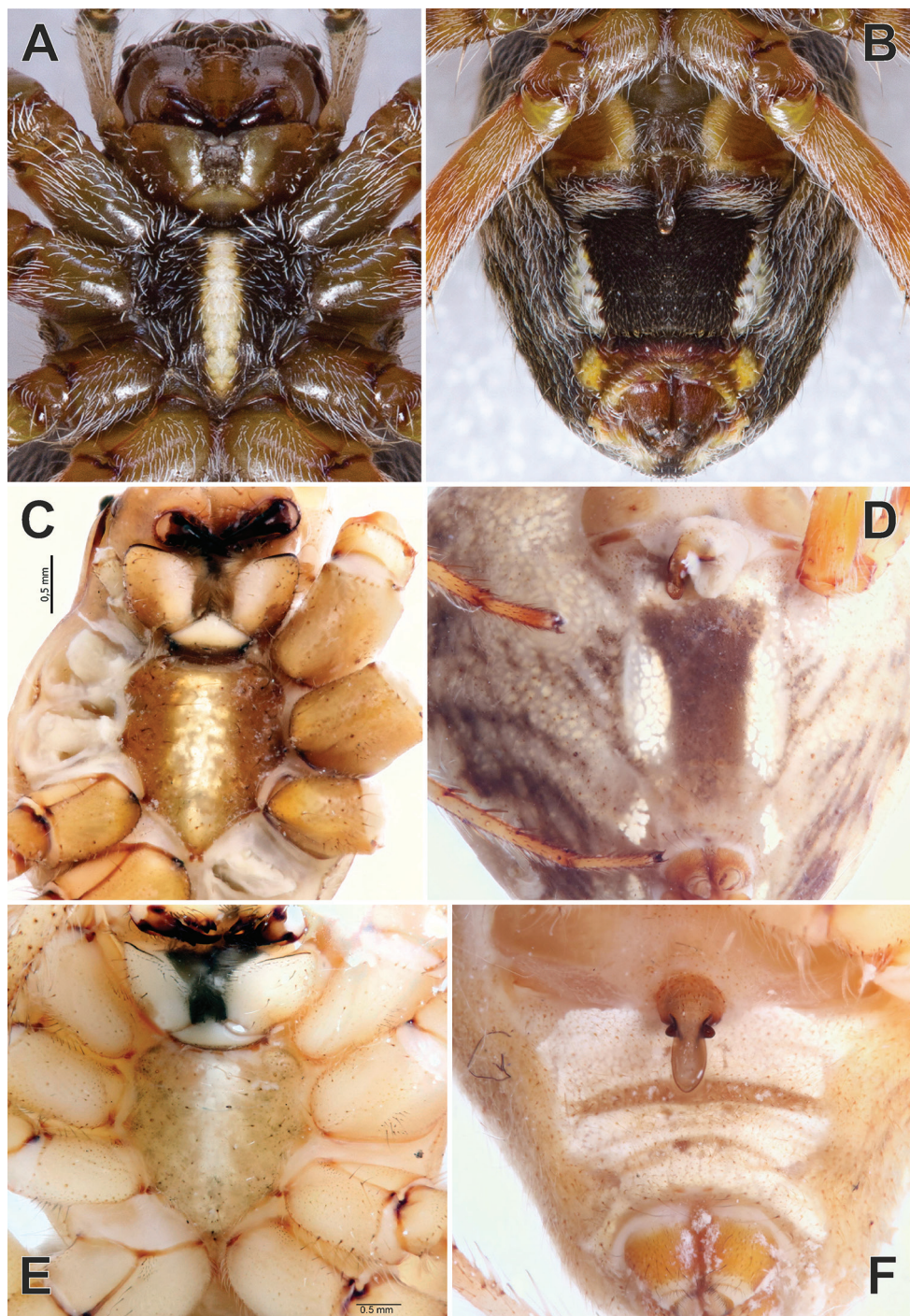


Figure 13. Females of *Neoscona theisi* (**A, B**), *N. spasskyi* (**C, D**) and *N. isatis* sp. nov. (**E, F**). **A, C, E** Prosoma, ventral **B, D, F** abdomen, ventral. Photos **A, B** courtesy of A. Seropian.

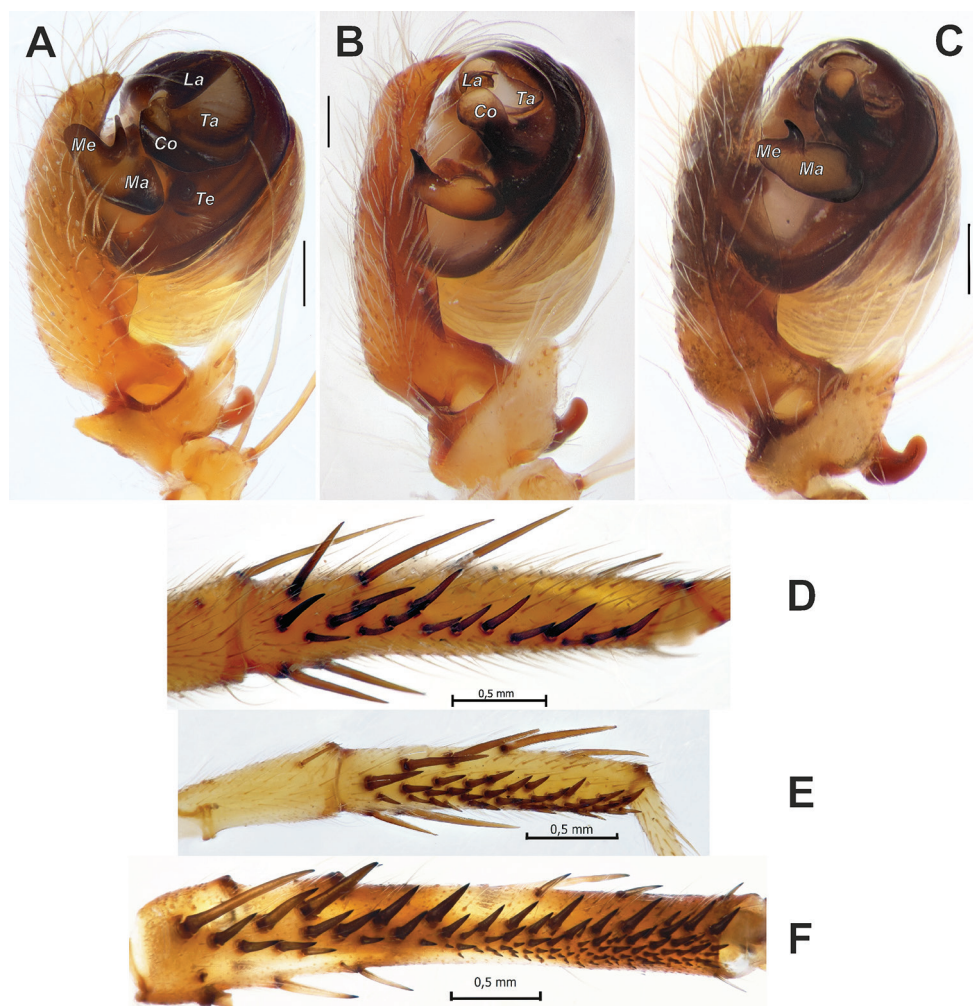


Figure 14. Male palps and tibiae II of *Neoscona theisi* (**A, D**), *N. isatis* sp. nov. (**B, F**) and *N. spasskyi* (**C, E**). **A–C** Male palp, prolateral **D–F** male tibia II, ventral. Abbreviations: *Co* conductor, *La* lamella, *Ma* median apophysis, *Me* extension of median apophysis, *Ta* terminal apophysis, *Te* tegulum. Scale bars: 0.2 mm, unless stated otherwise.

Female. Habitus as in Figs 11C; 12B; 13C, D. Total length 8.75. Carapace 3.98 long, 2.97 wide in pars thoracica, 1.50 in pars cephalica. Eye sizes and interdistances: AME: 0.21, ALE: 0.14, PME: 0.15, PLE: 0.13, AME–AME: 0.21, PME–PME: 0.13. Coloration as in male. Leg I measurements: 7.30 (2.08, 1.02, 1.60, 1.75, 0.85).

Epigyne as in Figs 13D, 17D–F. Epigyne with distinct constriction; lateral extensions distinct, wider than long; scape almost as wide as long.

Comments. Types of this species have not been found among the Spassky's collection in the Zoological Museum, St. Petersburg (Nekhaeva, pers. comm.). Spassky (1952) described this species as *Araneus cruciferoides*, a name preoccupied by Tullgren

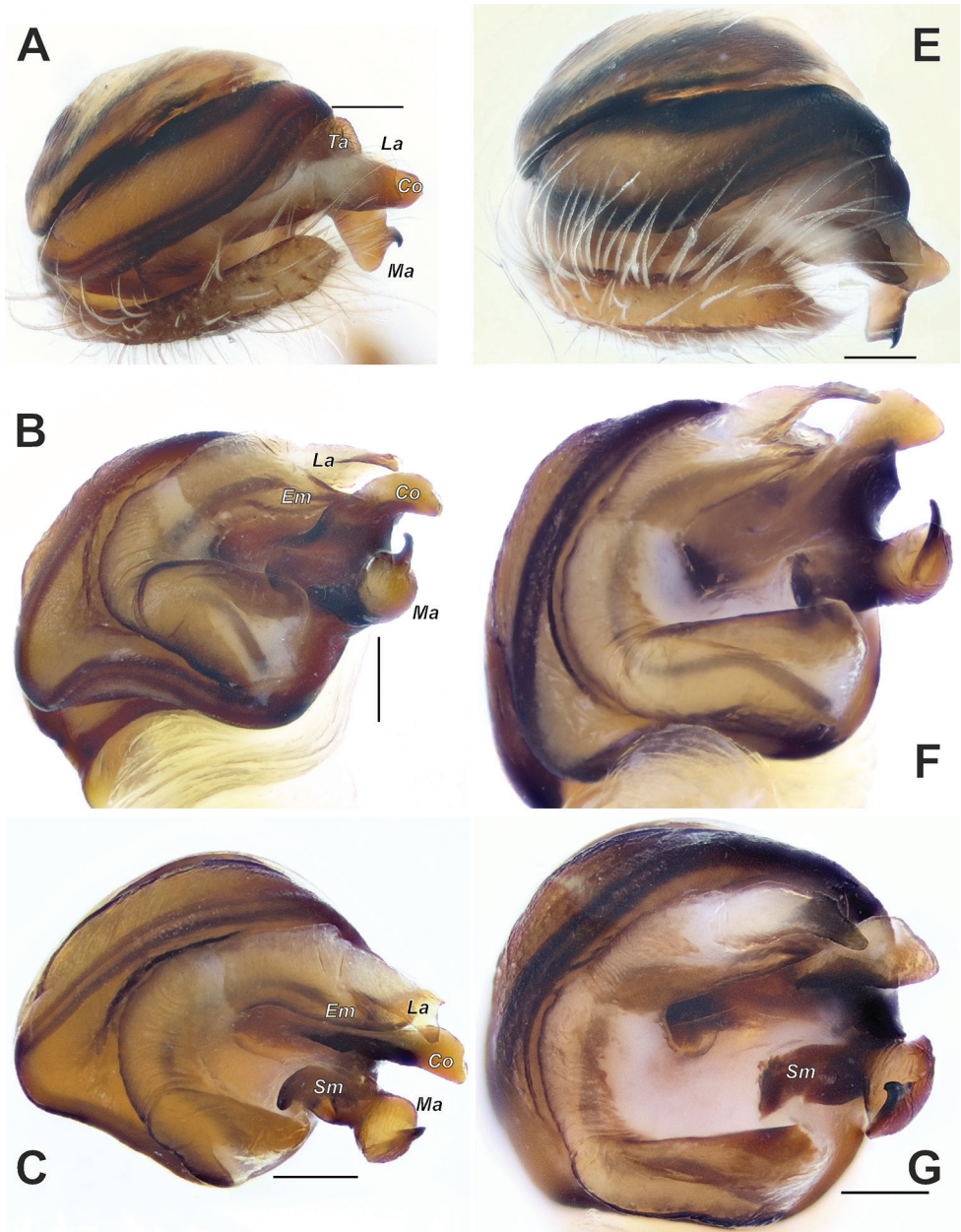


Figure 15. Male palps of *Neoscona spasskyi* (A–C) and *N. isatis* sp. nov. (E–G). A, C, E, G Anterior; B, F ventral. Abbreviations: Co conductor, Em embolus, La lamella, Ma median apophysis, Sm stipes of median apophysis, Ta terminal apophysis. Scale bars: 0.2 mm.

(1910) on the basis of both sexes. Later, a replacement name, *Araneus spasskyi*, was provided by Brignoli (1983). Marusik et al. (1991) erroneously synonymized it with *Neoscona tedgenica* (Bakhvalov, 1978), a species known only from a female and a juvenile

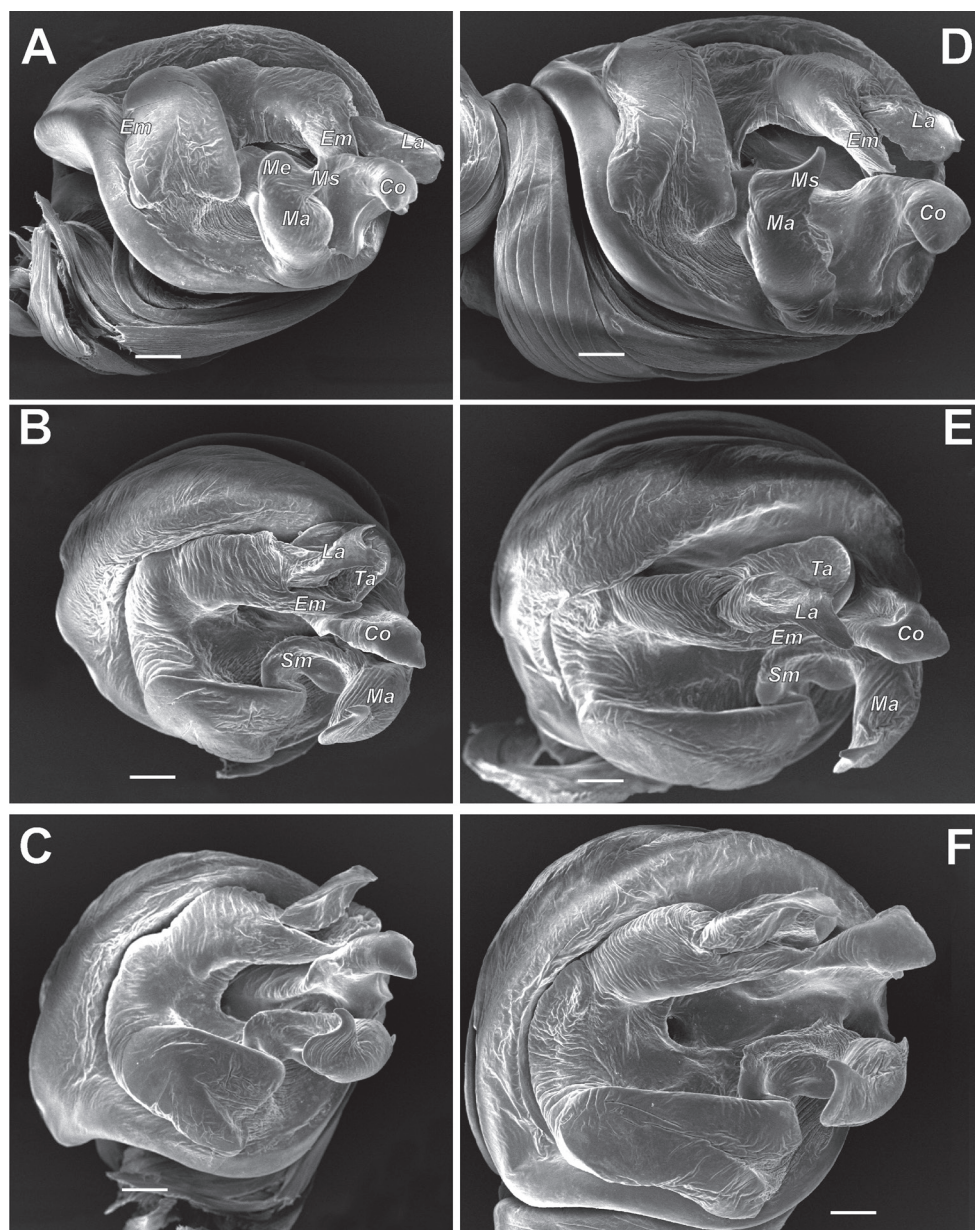


Figure 16. SEM graphs of the bulbs of *Neoscona spasskyi* (A–C) and *N. isatis* sp. nov. (D–F). A, C, D, F Prolateral B, E anterior. Abbreviations: Co conductor, Em embolus, La lamella, Ma median apophysis, Ms spur of median apophysis, Sm stipes of median apophysis, Ta terminal apophysis. Scale bars: 0.1 mm.

specimen collected in Turkmenistan (Bakhvalov 1978), and transferred to *Aculepeira* by Brignoli (1983). Comparing available figures in Spassky (1952) and Bakhvalov (1978) and the newly studied material, these two species differ in the shape of the posterior scape (rounded vs. triangulate) and the dorsal abdominal pattern (white “true” folium

on a dark background in *N. tedgenica*, vs. dark “incomplete” folium on a light background in the other species). For these reasons, we now revalidate the name ‘*spasskyi*’ and establish a new combination for it: *Neoscona spasskyi* (Brignoli, 1972) comb. nov.

Distribution. Tajikistan, Kyrgyzstan (Spassky 1952), Turkmenistan, Iran (first records for both).

Neoscona subfusca (C. L. Koch, 1837)*

Neoscona subfusca: Grasshoff 1986: 15, fig. 2, 4, 11–24 (♂♀); Levy 1998: 336, fig. 96–107 (♂♀).

Diagnosis. This species well differs from other species occurring in the region by the abdomen being as wide as long in the female and with small horns in the male (vs. abdomen longer than wide and lacking horns).

Description. See above-cited literature.

Distribution. Entire Africa, Mediterranean (Grasshoff 1986) to Turkmenistan (Mikhailov 2013).

Neoscona tedgenica (Bakhvalov, 1978)

Fig. 18

Araneus tedgenicus Bakhvalov, 1978: 790, figs 1–4 (♀).

Aculepeira tedgenica: Brignoli 1983: 255.

Diagnosis. *Neoscona tedgenica* differs from the closely similar *N. spasskyi* in the shape of the posterior area of the scape (triangulate vs. rounded) and the dorsal abdominal pattern (white “true” folium on a dark background in *N. tedgenica*, vs. dark “incomplete” folium on a light background in *N. spasskyi*).

Comments. See under *Neoscona spasskyi* (Brignoli, 1983). Types of this species are lost along with the rest of the private collection of Bakhvalov.

Distribution. Turkmenistan (Bakhvalov 1978).

Neoscona theisi (Walckenaer, 1841)*

Figs 11A, B; 12C, D; 13A, B; 14A, D; 17A–C; 18

Neoscona theisi: Grasshoff 1986: 69, fig. 90–100 (♂♀); Tanikawa 1998: 137, fig. 1–8, 11–17 (♂♀); Tanikawa 2007: 67, fig. 150–159, 572–574 (♂♀).

Neoscona sodom Levy, 1998: 340, fig. 117–126 (♂♀). **syn. nov.**

Neoscona sodom: Bosmans et al. 2019: 9, fig. 1a–e (♂).

* For complete list of references see WSC (2019)



Figure 17. Epigynes of *Neoscona theisi* (A–C), *N. spasskyi* (D–F) and *N. isatis* sp. nov. (G–I). A, D, G Ventral B, E, H posterior C, F, I lateral. Abbreviations: *Le* lateral extension, *Sc* scape. Scale bars: 0.2 mm.

Material examined. IRAN: 2♂ 5♀ (MHNG), Mazandaran Province: around Amol, 36°18'N, 52°21'E, 18.07.1973 (A. Senglet); 3♂ 6♀ (MHNG), Babol, 36°33'N, 52°42'E, 19.07.1973 (A. Senglet); 5♀ (MHNG), Gilan Province: Rudbar, 36°49'N,



Figure 18. Distribution records of *Araniella mithra* sp. nov. (blue circle), *A. villanii* sp. nov. (violet star), *Neoscona isatis* sp. nov. (green pentagon), *N. spasskyi* (black square), *N. theisi* (gray triangle, only new records) and *N. tedgenica* (brown asterisk).

49°25'E, 4.09.1973 (A. Senglet). Russia: Daghestan: 1♂ (PPC), Sergokalinski Dist., Sergokala Vil., 31.07.2008 (A.V. Alieva); 1♀ (PPC), Makhachkala, 08.2009 (S.V. Alieva); 1♀ (PPC), same locality, 08.2008 (S.V. Alieva); 3♀ (PPC), Magaramkentski Dist., Tselegyun Vil., 8.08.2008 (S.V. Alieva); 1♀ (PPC), Kizilyurtovski Dist., Sultan-Yangiyurt Vil., 18.05.2009 (M.A. Aliev, Z.A. Shavlukov); 1♀ (PPC), Karabudakhkentkiy Dist., 07.2008. (N.M. Gasanova). GEORGIA: 1♀ (photographed specimen), Tbilisi, 41.767986N, 44.767779E, 17.09.2019 (A. Seropian). INDIA: 1♀ (MMUE), Himachal Pradesh State: Patlikuhl Town, 32°07'N, 77°08'E, 1200 m, 28–29.5.1999 (Y.M. Marusik); 4♂ 2♀ (MMUE), Punjab State: Patiala, University campus, 30°21'N, 76°27'E, 24–25.6.1999 (Y.M. Marusik); 4♂ 1♀ (MMUE) and 5♀ (MMUE), same data.

Diagnosis. *Neoscona theisi* differs from the congeners occurring in the region by the presence of a wide black median band on the venter of abdomen and thin white lateral stripes (Fig. 12D). Males of this species have tibia II with fewer ventral spines (ca. 20) than *N. spasskyi* (ca. 40) and *N. isatis* sp. nov. (ca. 90) and more than in *N. adianta* (ca. 10). Males of *N. theisi* can be recognized also by the palp with pointed dorsal extension/projection of the tibia (Fig. 14A) (vs. absent), distinct ventral conical projection of the tegulum (*T_e*) lacking in other species, broad and well sclerotized lamella and wide conductor (vs. lamella thin and weakly sclerotized, conductor club-like), and long prolateral extension of median apophysis, longer than spur of median apophysis (vs. extension absent or as long as spur). The epigyne of *N. theisi* differs from those of *N. isatis* sp. nov. and *N. spasskyi* by the lack of constriction. Females of *N. theisi* well differ from those of *N. adianta* by having a white median band on carapace, darker abdominal pattern and the epigyne being almost twice longer than wide (vs. white band absent, epigyne almost as wide as long).

Description. See Grasshoff (1986) and Tanikawa (1998).

Comments. *Neoscona theisi* is a widely distributed species, with a current natural range covering Pakistan to Japan. Levy (1998) described *N. sodom* on the basis of both sexes from Israel. Judging by the figures provided in the original description, there are no significant differences in the copulatory organs and habitus of *N. sodom* and *N. theisi*. Therefore, the former name is synonymized with the latter.

Distribution. Pakistan, India, Philippines, China to Indonesia, Japan. Introduced to Seychelles, Pacific Is. (WSC 2019). The westernmost localities of this species (sub *N. sodom*) are Cyprus (Bosmans et al. 2019) and Israel (Levy 1998). New records for Iran, Georgia, and Russia.

Acknowledgments

We are grateful toward Peter J. Schwendinger (MHNG) for sending us the material collected by Antoine Senglet deposited in their museum. Veriko Pkhakadze (Tbilisi, Georgia), Alexander Ponomarev (Rostov on Don, Russia) and Armen Seropian (Tbilisi, Georgia) kindly provided us with their material and photographs. We also thank Anna A. Nekhaeva (Moscow, Russia) for her help in searching for the type specimens in the collection of the Zoological Institute, Russian Academy of Sciences (St. Petersburg). Special thanks to Seppo Koponen and Ilari Sääksjärvi (Zoological Museum, University of Turku) for the permission to use museum facilities and arranging the visit of Yuri Marusik to Turku.

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Notes on the green lacewing subgenus *Ankylopteryx* Brauer, 1864 (s. str.) (Neuroptera, Chrysopidae) from China, with description of a new species

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Academic editor: Benjamin Price | Received 9 September 2019 | Accepted 24 December 2019 | Published 22 January 2020

<http://zoobank.org/35E0E026-6C39-4DF7-8C39-3EA9DD1F9440>

Citation: Ma Y, Yang X, Liu X (2020) Notes on the green lacewing subgenus *Ankylopteryx* Brauer, 1864 (s. str.) (Neuroptera, Chrysopidae) from China, with description of a new species. ZooKeys 906: 41–71. <https://doi.org/10.3897/zookeys.906.46438>

Abstract

A taxonomic study of the green lacewing subgenus *Ankylopteryx* Brauer, 1864, from China is presented. Eight species of this subgenus are recorded from China. A new species, namely *Ankylopteryx* (A.) *yangi* **sp. nov.**, is described. *Ankylopteryx* (A.) *delicatula* Banks, 1937, and *Ankylopteryx* (A.) *ferruginea* Tsukaguchi, 1995 are recorded from China for the first time. Four new junior synonyms are proposed for *Ankylopteryx* (A.) *octopunctata candida* Fabricius, 1798: i.e. *Ankylopteryx* (A.) *fraterna* Banks, 1939, *Ankylopteryx* (A.) *laticosta* Banks, 1939, *Ankylopteryx* (A.) *lii* Yang, 1987, and *Ankylopteryx* (A.) *tibetana* Yang, 1987. A revised key to the Chinese species of the subgenus *Ankylopteryx* is provided.

Keywords

Description, new species, synonym, key

Introduction

The green lacewing genus *Ankylopteryx* (Chrysopidae, Chrysopinae, Ankylopterygini) was established by Brauer (1864), with *Chrysopa venusta* Hagen, 1864 as its type species by subsequent designation by Tjeder (1966). This genus is characterized by the strongly

broadened, usually immaculate basal part of the forewing costal space and the presence of the pseudopenis in the male genitalia. This genus consists of two subgenera, i.e. *Ankylopteryx* (*s. str.*) Brauer and *Sencera* Navás (Brooks and Barnard 1990). The subgenus *Sencera*, which occurs in the Oriental and Australian regions (Breitkreuz et al. 2015), was firstly established as a genus by Navás (1925) and subsequently treated as a subgenus of *Ankylopteryx* (*s. l.*) by Brooks and Barnard (1990) on account of striking similarity on external and genital characters proposed by Brooks (1983). *Sencera* differs from *Ankylopteryx* (*s. str.*) by the absence of the forewing intramedian cell (Brooks and Barnard 1990). However, *Sencera* was synonymized with *Ankylopteryx* (*s. str.*) by Tsukaguchi (1995), but it was still treated as a valid subgenus by New (2003), Yang et al. (2005), and Breitkreuz et al. (2015). Breitkreuz et al. (2015) revised the subgenus *Sencera* but questioned its subgeneric status. The subgenus *Ankylopteryx* (*s. str.*), containing 44 described species, is relatively poorly studied compared to *Sencera*. Previously, 10 species of *Ankylopteryx* (*s. str.*) were recorded from China (Yang et al. 2005). Recently, we examined about 100 specimens of *Ankylopteryx* (*s. str.*) from China, including several type specimens of the Chinese species described by Chikun Yang (i.e. *A. lii* Yang, 1987, *A. magnimaculata* Yang, 1987, and *A. tibetana* Yang, 1987). Accordingly, we present an overview of the species of *Ankylopteryx* (*s. str.*) from China and describe a new species. *Ankylopteryx* (*s. str.*) *delicatula* Banks, 1937 and *A. ferruginea* Tsukaguchi, 1995 are newly recorded from China. A revised key to the Chinese species of *Ankylopteryx* (*s. str.*) after Yang et al. (2005) is provided.

Material and methods

Terminology of wing venations in Neuroptera was proposed in a number of studies, such as Tillyard (1916), Comstock (1918), Adams (1967), Kukalová-Peck (1991), Kukalová-Peck and Lawrence (2004), and Breitkreuz et al. (2017), but with different interpretations on certain veins. The terminology of wing venation used in this paper mainly follows the previous studies on green lacewings, e.g. Tillyard (1916), Tauber (2003), and Tauber et al. (2017). Terminology of genitalia in Neuroptera was comprehensively studied by Acker (1960) and subsequently modified in a series of works (e.g. Tjeder 1966, 1970; Adams 1969; Principi 1977; Aspöck 2002; Aspöck and Aspöck 2008). In particular, Aspöck and Aspöck (2008) provided homology interpretation on the genital segments 8–11 based on the gonocoxite concept. Nevertheless, the terminology of genitalia used in this paper still follows some major works on systematics of green lacewings (e.g. Tjeder 1970; Principi 1977; Adams and Penny 1985; Brooks and Barnard 1990; Tauber 2003; Tauber et al. 2017).

Measurement of head width was made across the widest part of the dorsum of head including the compound eyes; the ratio of head width : eye width used the distance between middle of vertex and the maximum width of the compound eye; prothoracic length and width was respectively measured along the dorsal midline and at the widest position (straight line distance across the posterior margin) of prothorax; the wing length and width was respectively measured at the longest and widest portion of wing.

The genitalia were macerated in 10% KOH, then washed twice in dH₂O and stained with Chlorazol Black in 80% ethanol. The dissected genitalia from dried specimens were placed in glycerine in a tube pinned beneath the specimen. The genitalia from specimens preserved in alcohol were placed in 95% ethanol in a tube, placed with the remaining part of specimen in a larger tube filled with 95% ethanol.

Specimens herein examined are deposited in the Entomological Museum of China Agricultural University (CAU), Beijing except for the type of *Ankylopteryx doleshalii* Brauer, 1864. Other collections with primary types of relevant species cited in this paper are listed below.

CLMX	Collection of Liang Minxuan, Hong Kong, China
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA
MNHN	Muséum National d'Histoire Naturelle, Paris, France
NHMV	Naturhistorisches Museum, Wien, Austria
NSMT	National Science Museum (Natural History), Tokyo, Japan
UOP	Osaka Prefecture University, Osaka, Japan
ZMUK	Universität Kiel, Zoologisches Museum, Kiel, Germany

Taxonomy

Subgenus *Ankylopteryx* Brauer, 1864

Ankylopteryx: Brauer 1864: 899; Hagen 1866: 377; Kuwayama 1924: 7; Banks 1938: 225; Tjeder 1966: 497; Hölzel 1970: 50; Hölzel 1973: 382; New 1980: 15; Brooks 1983: 6; Tsukaguchi 1985: 505; Brooks and Barnard 1990: 125, 155; Tsukaguchi 1995: 122; Yang et al. 2005: 49.

Type species. *Chrysopa venusta* Hagen, 1853, by subsequent designation by Tjeder (1966).

Synonym. *Ethiochrysa* Fraser 1952: 57; Brooks and Barnard 1990: 155 (synonymized *Ethiochrysa* Fraser, 1952 with *Ankylopteryx* Brauer, 1864). Type species: *Ethiochrysa polychlora* Fraser, 1952, by monotypy.

Diagnosis (adapted from Brooks and Barnard 1990). Small to medium-sized green lacewings, body generally pale green. Head narrow (head width : eye width = 1.9–2.6 : 1), marked with black or red stripes on clypeus, gena or frons; maxillary palp and labial palp narrow, elongate apically; antenna nearly as long as forewing. Pronotum narrow, sometimes marked with black lateral spot, and with pale long fine setae; meso- and metanotum sometimes with broad black markings. Legs with protibia and mesotibia often marked with spots at median portion; metatibia seldom marked. Forewing broad (length : width = 2.1–2.5 : 1); marked with large black or brown spots or suffusion; costal space broad near wing base; costal vein with erect long setae; Sc very short; pterostigma often with black spots; Subcosta (Sc) and Radius (R) closely spaced;

first intramedian cell present; two gradate series of crossveins present, slightly divergent anteriad, basal inner gradate series meeting Psm; veins not crassate in male. Hind wing narrow (length : width = 3.0–4.0 : 1). Abdomen with sparse, long setae, with terga often marked; callus cerci ovoid; both male and female ectoprocts fused dorsally with slight dorsal invagination; male sterna 8+9 fused, microtholi absent. Female sternum 7 posteriorly truncate in ventral view with small setose apical tubercle.

Distribution. Afrotropical, Australian, and Oriental regions.

Ankylopteryx (A.) *delicatula* Banks, 1937

Figs 1, 10–14, 15–17, 112

Ankylopteryx (A.) *delicatula*: Banks 1937: 280 (original: *Ankylopteryx*; type locality: Ryukyu (Japan, Okinawa); syntypes in MCZ); Kuwayama 1964: 42 (*Ankylopteryx*); Brooks and Barnard 1990: 265 (*Ankylopteryx* (*Ankylopteryx*)); Tsukaguchi 1995: 123 (key to Japanese species), 126, fig. 100 (*Ankylopteryx*).

Material examined. China: 1 ex, Yunnan, Jinghong, Sanchahe, 620 m, 1981.IV.12, Yang Chikun (CAU); 1 ♀, Yunnan, Yingjiang, Tongbiguan, Jinzhuzhai, 2012.V.2, Liang Feiyang (CAU); 1 ♂, Hong Kong, 2015.V, Liang Minxuan (CLMX).

Diagnosis. Frons with two brownish small spots between antennae, two brownish stripes below toruli; frontal markings more or less curved posteriorly and contiguous with clypeal markings anteriorly; gena with a long brownish stripe. Protibia and mesotibia with median markings. Both wings distinctly marked with brownish vittae along posterior margins near base and medial fork to apex of distal cubital cell (*dcc*). Abdomen with brownish markings on terga 2–8.

Supplemental description. Female: Sternum 8 distinctly convex at median part of posterior margin, with setae apically. Subgenitale stubby, bilobed apically; spermatheca round, as wide as long; spermatheca coiled, about three times as long as spermatheca.

Distribution. China (Yunnan, Hong Kong); Japan (Okinawa).

Remarks. The frontal spots between antennae and vittae on both wings in our examined specimens from Yunnan are same with that in the type of *A. delicatula*, as originally described. Accordingly, we identified the above two specimens from Yunnan to be *A. delicatula*, which is newly recorded from China.

Ankylopteryx (A.) *doleschalii* Brauer, 1864

Figs 2, 18–22, 23–29, 112

Ankylopteryx (A.) *doleschalii*: Brauer 1864: 901 (original: *Ankylopteryx*; type locality: “Amboina” [Ambonia] (Indonesia, Maluku Prov.); holotype in NHMV); Brauer 1866: 37 (*Ankylopteryx*); van der Weele 1909: 60 (*Ankylopteryx*); Banks 1937: 280 (*Ankylopteryx*); Banks 1939: 473 (key to Chinese species); Kuwayama 1964: 42

(*Ankylopteryx*); Brooks and Barnard 1990: 265 (*Ankylopteryx* (*Ankylopteryx*)); New 2003: 163 (*Ankylopteryx* (*Ankylopteryx*)); Yang et al. 2005: 51 (*Ankylopteryx* (*Ankylopteryx*)).

Material examined. Holotype ex, Indonesia, Amboina, 1950 (NHMV). Paratype 1 ♂, same data as holotype (NHMV).

Diagnosis. Stripes below toruli absent; frontal markings not curved posteriorly, anteriorly contiguous with clypeal markings and genal markings. Protibia and mesotibia with median markings. Both wings with brownish marking patterns. First intramedian cell very long and narrow.

Distribution. China (Hainan); Indonesia (Maluku).

Remarks. This species was recorded from Hainan by Banks (1937). Unfortunately, we have not examined any specimen of this species from Hainan. Considering the greatly disjunct distribution records of this species (i.e. Ambonia and Hainan), there is a possibility that *A. doleschalii*, from Hainan was a misidentification of *A. gracilis* (a morphologically similar species widely distributed in eastern and southeastern Asia).

Ankylopteryx (*A.*) *ferruginea* Tsukaguchi, 1995

Figs 3, 30–32, 33–35, 106, 110, 112

Ankylopteryx (*A.*) *ferruginea*: Tsukaguchi 1995: 127 (original: *Ankylopteryx*; type locality: Iriomote (Japan, Okinawa); holotype in UOP).

Material examined. China: 1 ♂, Guangxi, Longzhou, Nonggang, 240 m, 1982.V.19, Li Fasheng (CAU); 1 ♂, Guangxi, Longzhou, Nonggang, 240 m, 1982.V.20, Yang Chikun (CAU); 1 ♂, 1 ♀, Guangxi, Longzhou, Nonggang, 240 m, 1982.V.21, Li Fasheng (CAU); 1 ♀, Guangxi, Ningming, Longrui, 180 m, 1984.V.16, Li Fasheng (CAU); 1 ♀, Guangxi, Ningming, Longrui, 180 m, 1984.V.17, Li Fasheng (CAU); 1 ♀, Yunnan, Jinghong, Yexianggu, 2015.IV.17, Liang Feiyang (CAU); 1 ♂, Yunnan, Lancang, Yunxian, Xingtuo, 2017.VII. 20, Yang Mengxian (CAU); 1 ♀, Hainan, Yinggeling, Wang Jianyun (CAU). **Japan:** 2 ♂, Okinawa, Iriomote-jima, Airagawa, Komi, 2012.VI.2, Liu Xingyue (CAU).

Diagnosis. Stripes below toruli absent; clypeal markings indistinct or absent, if present, contiguous with indistinct genal markings. Pro-, meso-, metibia with median markings. Both wings with yellowish marking patterns. First intramedian cell very long and narrow. General width of gonarcus narrow; entoprocessus attached at basal fourth of gonarcus, about 3/4 times as long as gonarcus; pseudopenis long and straight, about two times as long as entoprocessus.

Distribution. China (Guangxi, Yunnan); Japan (Okinawa).

Remarks. This species is newly recorded from China. Tsukaguchi (1995) stated that two forewing m-cu crossveins are present before first intramedian cell. However, this char-

acter is variable among individuals of this species based on the specimens we examined. Other external diagnostic characters and genital characters in the Chinese specimens fit well with the original description, which confirms our identification of this species.

This species is similar to *A. gracilis*, based on the long and narrow first intramedian cell, but differs from the latter two species by the absence of markings on frons between antennae (present in *A. doleschalii*, and *A. gracilis*), the yellowish wing marking pattern (wing marking pattern much darker in *A. doleschalii*, and *A. gracilis*) and the narrow general width of gonarcus (strongly broad in *A. gracilis*).

***Ankylopteryx (A.) gracilis* Nakahara, 1955**

Figs 4, 36–40, 41–43, 44–47, 107, 111, 113

Ankylopteryx (A.) gracilis: Nakahara 1955: 143, pl. 21 fig. 1 (original: *Ankylopteryx*; type locality: “Formosa” [Taiwan] (China); holotype in NSMT); Kuwayama 1964: 42 (*Ankylopteryx*); Tsukaguchi 1985: 505 (*Ankylopteryx*); Brooks and Barnard 1990: 265 (*Ankylopteryx (Ankylopteryx)*); Tsukaguchi 1995: 123 (key to Japanese species and a key to the third instar larvae), 129 (*Ankylopteryx*); New 2003: 164 (*Ankylopteryx (Ankylopteryx)*); Yang et al. 2005: 51 (key to Chinese species, *Ankylopteryx (Ankylopteryx)*).

Material examined. China: 1 ♀, Guangxi, Longzhou, Nonggang, 240 m, 1982.V.19, Yang Chikun (CAU); 1 ♂, 1 ex, Guangxi, Longzhou, Nonggang, 240 m, 1982.V.20, Yang Chikun (CAU); 1 ♂, 2 ♀, Guangxi, Longzhou, Nonggang, 240 m, 1982.V.20, Li Fasheng (CAU); 13 ♂, 2 ♀, Yunnan, Jinghong, Sanchahe, 620 m, 1981.IV.12, Yang Chikun (CAU); 1 ex, Hainan, Baisha, Hongmao, 430 m, 2007.V.18, Liu Xingyue (CAU); 1 ♀, Hainan, Baisha, Yinggeling, Hongxin, 2007.V.23–24, Liu Jingxian (CAU); 2 ♂, Taiwan, Nantou, Yüchih, Lienhuachih, 675 m, 2010.XI.11, Yang Ding (CAU). **Japan:** 3 ♂, Okinawa, Iriomote-jima, Airagawa, Komi, 2012.VI.29, Liu Xingyue (CAU). **Vietnam:** 1 ♀, Quang Nam, Phuoc Son, Phue My, 580 m, 2012.V.6, Liu Xingyue (CAU). **Laos:** 1 ♀, Vientiane Prov., Phou Panang NBCA, 260 m, 2016.III.27, Liu Xingyue (CAU).

Diagnosis. Frons with three spots between antennae, stripes below toruli absent; frontal markings not curved posteriorly contiguous with clypeal markings anteriorly; gena with long brownish stripe. First intramedian cell very long and narrow, general width of gonarcus very broad; entoprocessus attached at about median part of gonarcus, about half as long as gonarcus; pseudopenis long and straight, about twice as long as entoprocessus.

Distribution. China (Guangxi, Yunnan, Hainan, Taiwan); Japan (Okinawa); Vietnam (Quang Nam); Laos (Vientiane).

Remarks. The frontal spots between antennae, the long and narrow intramedian cell, and the genital characters assigned the specimens examined to *A. gracilis*.

In the original description of *A. gracilis*, Nakahara (1955) stated that the legs of this species are immaculate, and this feature was followed by Tsukaguchi (1995) as a diagnostic character of this species. However, among the specimens examined here, we found that the markings on foretibia and mesotibia are either absent or present. After

examination of the male genitalia of these specimens, no significant differences among them could be found, indicating that they belong to a same species. Therefore, we consider the presence/absence of markings on tibia to be intraspecific variation, which is common in some other species of this subgenus.

***Ankylopteryx* (A.) *magnimaculata* Yang, 1987**

Figs 6, 58–63, 64–66, 102, 112

Ankylopteryx (A.) *magnimaculata*: Yang 1987: 204 (original: *Ankylopteryx*; type locality: Dongjiong (China, Xizang, ä); holotype in CAU); Yang et al. 2005: 51 (key to Chinese species), 54 (*Ankylopteryx* (*Ankylopteryx*)).

Material examined. Holotype ♂, Xizang, Zäyu, Dongjiong, 1570 m, 1978.VI.26, Li Fasheng (CAU).

Diagnosis. Two brownish vittae on frons, clypeus, and labrum; gena with long brownish stripes contiguous with above vitta. Protibia and mesotibia with median markings. Forewing with pterostigma brown, extending over four crossveins; large brownish vittae present along posterior margins at basal third, enclosing over five veins on wing margin; first intramedian cell short and wide. General width of gonarcus narrow; entoprocessus attached near base of gonarcus, slightly longer than gonarcus; pseudopenis short and curved, about 1.5 times as long as entoprocessus.

Distribution. China (Xizang).

Remarks. This species can be distinguished from all the other species of *Ankylopteryx* (s. str.) from China by the large brownish vittae along posterior margin at basal third of both wings.

***Ankylopteryx* (A.) *octopunctata candida* (Fabricius, 1798)**

Figs 5, 7, 8, 58–63, 64–66, 67–72, 73–76, 77–82, 83–85, 99–101, 108, 113

Ankylopteryx (A.) *octopunctata candida*: Fabricius 1798: 202 (original: *Hemerobius*; type locality: “India orientali” [E. India]; holotype in ZMUK); Schneider 1851: 161 (*Chrysopa*); Walker 1853: 274 (*Chrysopa*); Brauer 1864: 900 (*Ankylopteryx*); van der Weele 1909: 58 (*Ankylopteryx*); Banks 1939: 473 (key to Chinese species, *Ankylopteryx*); Brooks and Barnard 1990: 265 (*Ankylopteryx* (*Ankylopteryx*)).

Ankylopteryx (A.) *fraterna* Banks, 1939: 473 (key to Chinese species; original: *Ankylopteryx*; type locality: Guangdong and Hainan (China); syntypes in MCZ); Yang et al. 2005: 51 (A key to Chinese species, *Ankylopteryx* (*Ankylopteryx*)). **syn. nov.**

Ankylopteryx (A.) *laticosta* Banks, 1939: 472 (original: *Ankylopteryx*; type locality: Guangdong and Hainan (China); syntypes in MCZ), 473 (key to Chinese species); Brooks and Barnard 1990: 265 (*Ankylopteryx* (*Ankylopteryx*)); Yang et al. 2005: 51 (key to Chinese species), 53 (*Ankylopteryx* (*Ankylopteryx*)). **syn. nov.**

Ankylopteryx (A.) *lii* Yang 1987: 204 (original: *Ankylopteryx*; type locality: Shajiong (China, Xizang, Zäyu); holotype in CAU); Yang et al. 2005: 51 (key to Chinese species), 54 (*Ankylopteryx* (*Ankylopteryx*)). **syn. nov.**

Ankylopteryx (A.) *tibetana* Yang 1987: 205 (original: *Ankylopteryx*; type locality: Dongjiong (China, Xizang, Zäyu); holotype in CAU); Yang et al. 2005: 51 (key to Chinese species), 56 (*Ankylopteryx* (*Ankylopteryx*)). **syn. nov.**

Material examined. China: 1 ♂, Fujian, Shaowu, 1943.IX.1 (CAU); 1 ♂, Fujian, Dehua, Shuikou, 1974.XI.6, Li Fasheng (CAU); 3 ♂, 1 ♀, Fujian, Dehua, Shuikou, 1974.XI.6, Yang Chikun (CAU); 1 ♂, Fujian, Dehua, Shuikou, 1974.XI.13, Yang Chikun (CAU); 1 ♀, Jiangxi, Shangrao, 1978.IV.30, Yang Chikun; 1 ♂, Guangxi, Longzhou, Nonggang, 240 m, 1982.V.18, Yang Chikun (CAU); 1 ♂, Guangxi, Longzhou, Nonggang, 240 m, 1982.V.19, Li Fasheng (CAU); 2 ♂, Guangxi, Longzhou, Nonggang, 240 m, 1982.V.18, Li Fasheng (CAU); 1 ex, Guangxi, Ningming, Longrui, 180 m, 1984.V.16, Li Fasheng (CAU); 2 ♀, Guangxi, Ningming, Longrui, 180 m, 1984.V.17, Li Fasheng (CAU); 1 ♂, Guangxi, Jinxiu, 720 m, 1982.VI.11, Li Fasheng (CAU); 3 ♀, Sichuan, Leshan, 1978.IX.20, Li Fasheng (CAU); 1 ♂, Chongqing, Liangping, Luoia, Shapingba, Li Zhifei (CAU); 1 ♀, Guizhou, Libo, Maolan, Banzhai, 2013.X.13, Liu Xingyue (CAU); 1 ♂, 1 ♀, Guizhou, Libo, Maolan, Limingguan, 2013.X.14, Liu Xingyue (CAU); 1 ♂, 1 ♀, Guizhou, Libo, Maolan, Limingguan, 2013.X.14, Liang Feiyang (CAU); 2 ♀, Yunnan, Puer, Taiyanghe National Forestry Park, 1450 m, E101.3 N22.68, 2016.VIII.12, Jiang Yunlan (CAU); 1 ♀, Guangdong, Huizhou, Xiangtoushan, Wang Mengqing (CAU); 1 ♂, 1 ♀, Guangdong, Zhaoqing, Dinghushan Nature Reserve, Lyu Yanan (CAU); 1 ♂, Hainan, Diaoluoshan, 2014.V.4, Lu Xiumei (CAU); 1 ♂, Xizang, Zäyu, Shajiong, 1570 m, 1978.VI.26, Li Fasheng (holotype of *Ankylopteryx lii* Yang, 1987) (CAU); 1 ♂, Xizang, Zäyu, Dongjiong, 1570 m, 1978.VI.26, Li Fasheng (holotype of *Ankylopteryx tibetana* Yang, 1987) (CAU); 1 ♂, Xizang, Zäyu, Shajiong, 1700 m, 1978.VI.25, Li Fasheng (paratype of *Ankylopteryx tibetana* Yang, 1987) (CAU); 1 ♂, 5 ♀, Taiwan, Pingtung, Lilungshan, 2013.VI.18, Liang Feiyang (CAU). **Japan:** 5 ♀, Okinawa, Iriomote-jima, Airagawa, Komi, 2012.VI.29, Liu Xingyue (CAU). **Laos:** 1 ♂, 2 ♀, Luang Namtha, Nam Ha NBCA, Along Route 3, 690–750 m, 2016.III.22, Liu Xingyue (CAU).

Diagnosis. Stripes below toruli from absent to visibly present; frontal markings contiguous with clypeal markings and genal marking. Scape usually with brownish stripe. First intramedian cell short and wide. General width of gonarcus normal; entoprocessus attached at basal fifth to fourth of gonarcus, about as long as gonarcus; pseudopenis short and curved, about twice as long as entoprocessus.

Distribution. China (Fujian, Guangxi, Sichuan, Chongqing, Guizhou, Yunnan, Guangdong, Hainan, Xizang); Japan (Okinawa); Laos (Luang Namtha); India (eastern India).

Remarks. Seven subspecies hitherto are placed under the species *A. octopunctata*, which are separately distributed. According to the known distribution of *A. octopunctata candida* and the character mentioned above, we assigned the materials examined to this subspecies.

Synonyms. *Ankylopteryx fraterna* and *A. laticosta* were first recorded by Banks (1939). In the original literature, he mentioned that the key difference between *A. fraterna* and *A. octopunctata candida* was the presence or absence of brownish stripe on scape (absence in *A. fraterna* versus presence in *A. octopunctata candida*); differences between *A. laticosta* and *A. octopunctata candida* are the coloration of the costal vein (black for a short distance in *A. laticosta* versus not black in *A. octopunctata candida*), costal area (unusually broad in *A. laticosta* versus normal in *A. octopunctata candida*), and the number of lower Banksian cells (two in *A. laticosta* versus three in *A. octopunctata candida*). We have studied the specimens of *A. octopunctata candida* from various localities and found the difference mentioned above is continuous in this species. We, therefore, synonymize *A. fraterna* and *A. laticosta* with *A. octopunctata candida*. We have also studied the holotypes of *A. lii* and *A. tibetana*. The external characters (frontal stripes and markings, and markings on both wings) of three nominal species are similar. We dissected specimens of each of the three species, found no significant difference among them, and therefore confirmed the synonymization of *A. lii* and *A. tibetana* with *A. octopunctata candida*.

***Ankylopteryx* (A.) *quadrimaculata* (Guérin-Ménéville, 1844)**

Ankylopteryx (A.) *quadrimaculata*: Guérin-Ménéville 1844: 388 (original: *Hemerobius*; type locality: “Chine” [China]; type in MNHN); Schneider 1851: 162 (*Chrysopa*); Hagen 1866: 380 (*Ankylopteryx*); Brooks and Barnard 1990: 265 (*Ankylopteryx* (*Ankylopteryx*)); Yang et al. 2005: 56 (*Ankylopteryx* (*Ankylopteryx*)).

Distribution. China.

Remarks. The original description of this species (Guérin-Ménéville 1844) is too simple, and the only informative descriptions are the body length (= 12 mm), forewing span (= 36 mm), and the presence of stripes below toruli. Nevertheless, the stripes below toruli are also present in *Ankylopteryx octopunctata* (Fabricius, 1793), *A. tessellata* Needham, 1909, *A. nonelli* Navás, 1913, *A. nepalensis* Hölzel, 1973, and *A. yangi* **sp. nov.** Thus, the validity of *A. quadrimaculata* is doubtful and this species may be a synonym of those species mentioned above except *A. yangi* **sp. nov.** (differences between *A. yangi* **sp. nov.**, and *A. quadrimaculata* are outlined below in the Remarks for *A. yangi* **sp. nov.**). This species is not included in the present key, but still treated as a valid species until the type is examined.

***Ankylopteryx* (A.) *yangi* sp. nov.**

<http://zoobank.org/5E1748C9-4C93-4DAC-9C9D-66C63FAA8EC8>

Figs 9, 86–90, 91–94, 95–98, 103, 105, 112

Material examined. Holotype ♂, China, Guizhou, Libo, Maolan, Limingguan, 2013.X.14, Liu Xingyue (CAU). Paratypes 1 ♀, China, Guizhou, Libo, Maolan

Limingguan, 2013.X.14, Liang Feiyang (CAU); 1 ♀, Taiwan, Pingtung, Lilungshan, 2013.VI.18, Liang Feiyang (CAU).

Diagnosis. Frons with three brownish small spots between antennae, and anteriorly with two arcuate markings, which are more or less connected with each other at posterior ends; gena with long brownish stripe; clypeal markings contiguous with frontal markings posteriorly and labial markings anteriorly; maxillary palp and labial palp pale green. Thorax with mesonotum entirely brown; protibia and mesotibia with median markings. Forewing with intramedian cell short and wide. Abdomen with brownish markings on terga 2–8.

Description. Measurements: Head width 0.7–1.0 mm; ratio of head width/eye width 1.72–1.74; prothorax 0.7–0.9 mm long and 0.85–1.0 mm wide. Forewing 11.5–13.9 mm long, 5.0–6.0 mm wide; length of first intramedian cell 0.98 mm; 12 radial cells; 4–5 Banksian cells (b cells), 4–5 lower Banksian cells (b' cells); 7–8 inner gradates, 7–10 outer gradates. Hind wing 10.0–13.5 mm long, 3.2–4.0 mm wide; 11 radial cells; 4–6 Banksian cells (b cell), 5–6 lower Banksian cell (b' cells); 4–6 inner gradates, 5–7 outer gradates.

Male. Head with vertex creamy yellow, immaculate; frons creamy yellow, with three small spots between antennae, brownish stripes below toruli, and a pair of median arcuate markings anteriorly, more or less connected with each other posteriorly; gena with long brownish stripe extending along inner ocular margin to posterolateral part of clypeus; tentorial pits with brownish margins; scape with brownish stripe; clypeus with brownish arcuate markings contiguous with frontal markings anteriorly and labial markings posteriorly; maxillary palp and labial palp pale green.

Prothorax almost pale green, with wide, brownish lateral stripe, and with white long setae. Mesothorax entirely brown dorsally, with sparse white long setae. Metathorax pale green, with sparse white long setae. Legs pale green, tarsomere 5 and pretarsal claws dark brown; protibia with a brownish median marking; mesotibia with a smaller median brownish marking.

Forewing broad, slightly tapering apically, hyaline; pterostigma brownish, extending over four crossveins; setae almost whitish; veins mostly pale green; costal crossveins at junctions with wing margin, radial crossveins at junctions with R and dcc brown; Radical sector (Rs) sinuated; transverse veins pale green; gradate series of crossveins almost brown; dcc closed. Hind wing narrow, more acutely tapering apically than forewing, hyaline; pterostigma faint, extending over three crossveins; transverse veins pale green; gradate series of crossveins almost brown.

Abdomen pale green, with brownish markings on terga 2–8, and tergal markings slightly wider than that on anteriorly neighbouring terga. Abdominal setae white, microsetae dense, and long setae sparse.

T9+ectoproct about half as long as tergum 8, with dorsal invagination shallow; ectoproct with rounded dorsal and posterior margins; callus cerci rounded, trichobothria densely ranged. S8+9 fused, as long as wide, with line of fusion not demarcated; lateral margin almost straight, posterior margin rounded. Only gonarcus, entoprocessus, and pseudopenis present. Gonarcus broadened at apex of lateral arms. Gonarcus with the

general width normally broad; entoprocessus attached at about basal fourth of gonarcus, about half as long as gonarcus, medially fused forming an arch over pseudopenis; pseudopenis about twice as long as entoprocessus, broadened subapically, long and distinctly curved, and pointed apically; gonosaccus with sparse setae.

Female. External characters same as male. Sternum 7 distinctly convex postero-medially, setose posteriorly. Subgenitale and spermatheca with spermaduct present; subgenitale bilobed apically; spermatheca round, as wide as long; spermaduct coiled, much longer than spermatheca.

Distribution. China (Guizhou, Taiwan).

Etymology. This new species is dedicated to Professor Yang Chikun, who made tremendous contributions to the taxonomy of Chrysopidae from China.

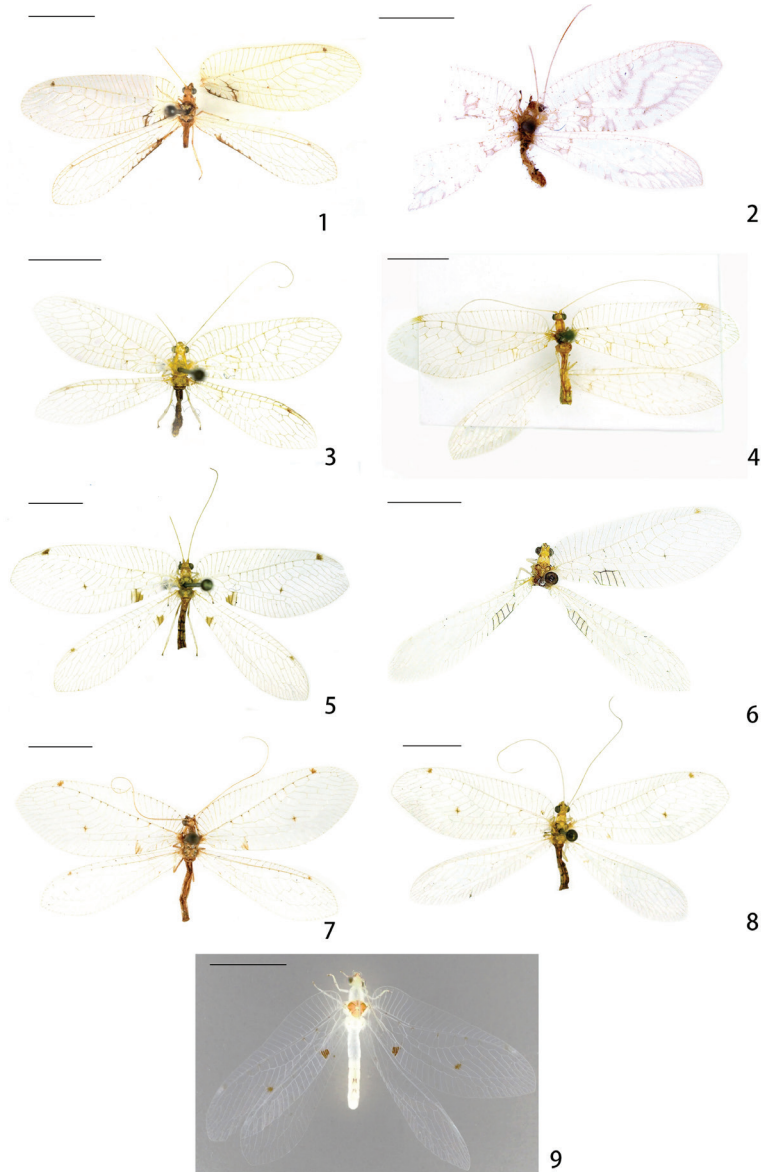
Remarks. This new species appears to be closely related to *A. octopunctata candida* in having similar frontal markings, but it differs from the latter species by the presence of median arcuate frontal markings (absent in *A. octopunctata candida*), the ratio of gonarcus/entoprocessus (2.0 in *A. yangi* versus 1.0 in *A. octopunctata candida*), and the distinctly curved pseudopenis (moderately curved in *A. octopunctata candida*).

Key to Chinese species of *Ankylopteryx* (s. str.) (revised after Yang et al. 2005)*

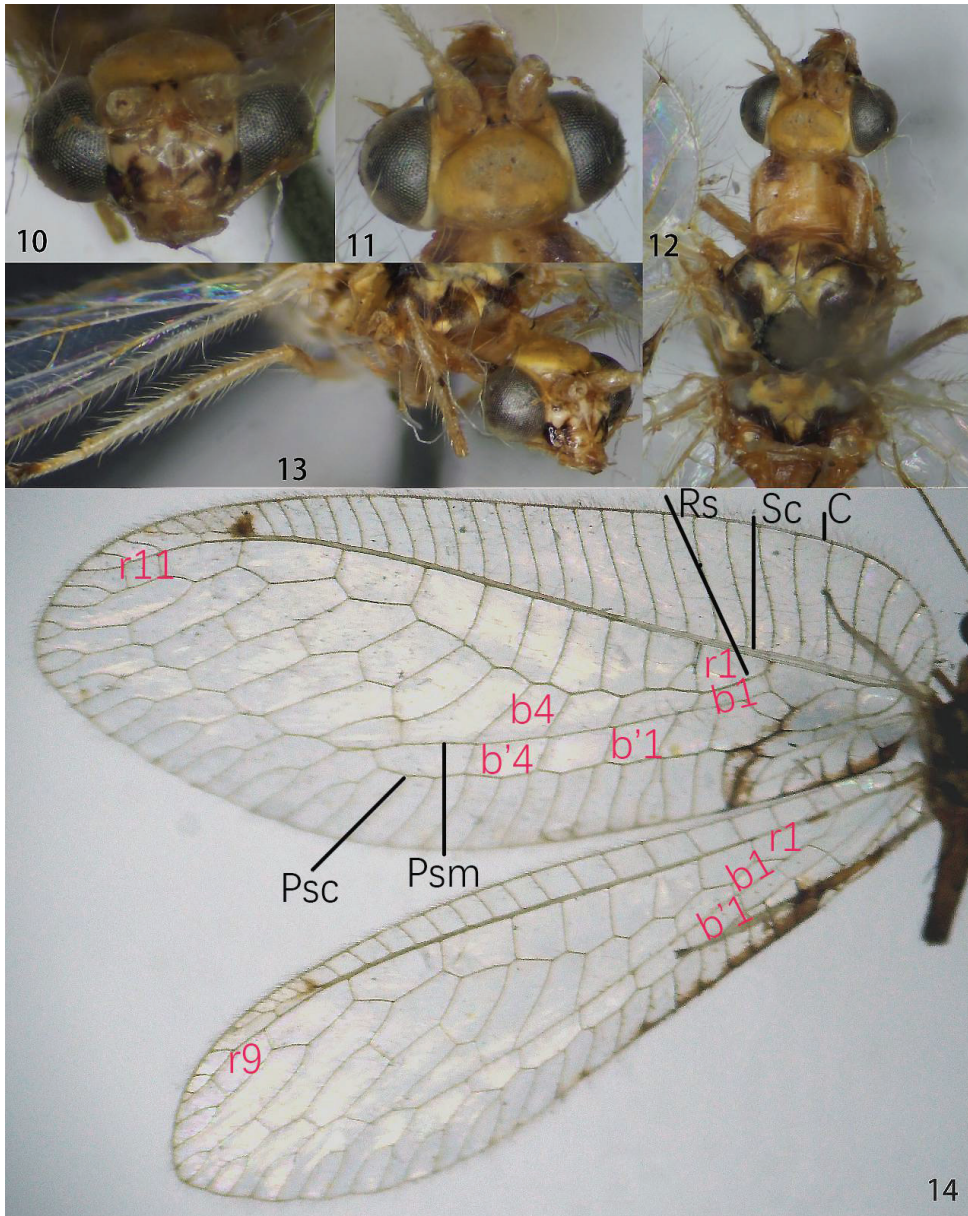
- 1 First intramedian cell very long and narrow 2
- First intramedian cell short and wide 4
- 2 Frontal area between antennae with three black spots (Fig. 36); scape and pedicel with brownish stripes (Fig. 37)..... *A. gracilis* Nakahara, 1955
- Frontal area between antennae immaculate; scape and pedicel immaculate . 3
- 3 Both wings with yellowish marking patterns (Figs 28, 29) *A. ferruginea* Tsukaguchi, 1995
- Both wings with brownish marking patterns (Figs 21, 22) *A. doleschalii* Brauer, 1864
- 4 Frontal area between antennae with brownish spots 5
- Frontal area between antennae immaculate 6
- 5 Three spots present (Fig. 86); both wings without large brownish vittae along posterior margins (Fig. 90)..... *A. yangi* sp. nov.
- Two spots present (Fig. 10); both wings with large brownish vittae along posterior margins near base (Fig. 14) *A. delicatula* Banks, 1937
- 6 Both wings with brownish vittae along posterior margins (Figs 62, 63); general width of gonarcus narrow (Figs 65, 66, 102) *A. magnimaculata* Yang, 1987
- Both wings without brownish vittae along posterior margins (Figs 71, 72); general width of gonarcus normal (Figs 75, 76, 100) *A. octopunctata candida* (Fabricius, 1798)

* *A. quadrimaculata* is not included in the key. See Remarks for this species.

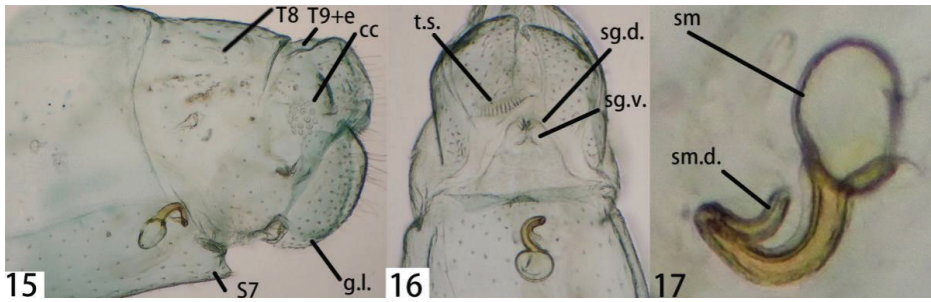
Plates



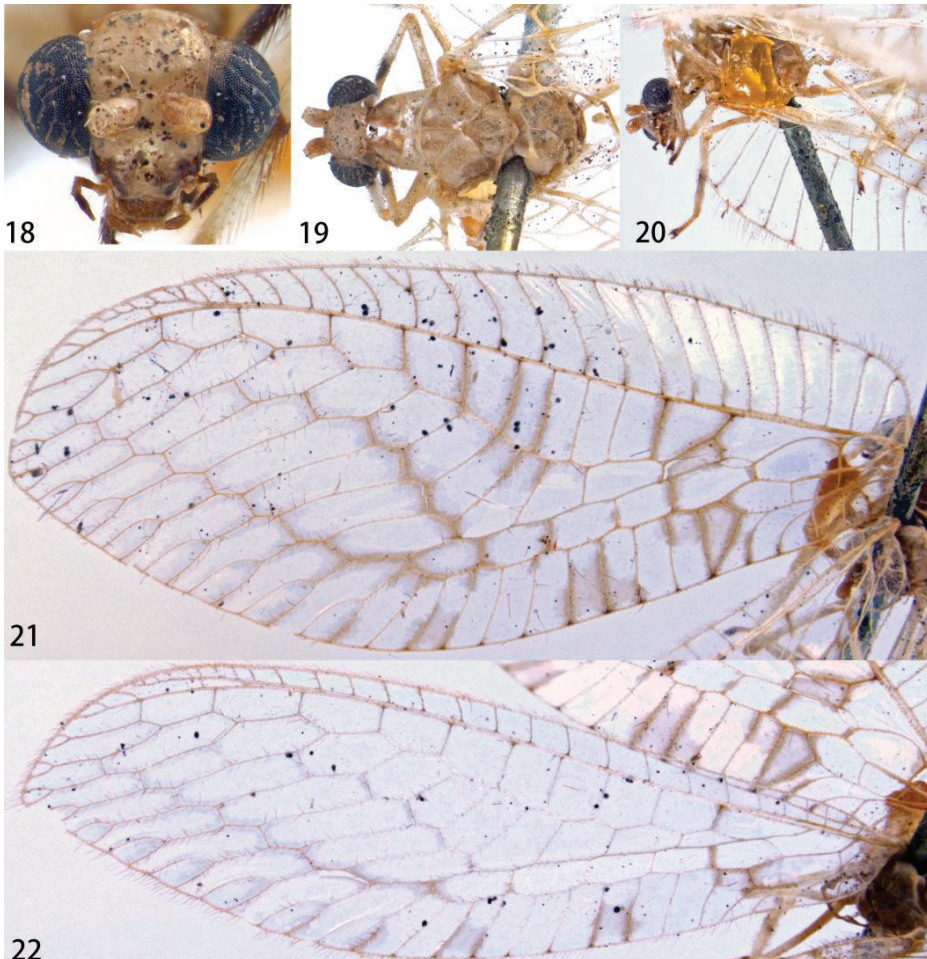
Figures 1–9. Habitus photos of the *Ankylopteryx* species. **1** *Ankylopteryx* (*A.*) *delicatula* Banks, 1937 (Yunnan, Jinghong, sex unknown, CAU) **2** *Ankylopteryx* (*A.*) *doleschalii* Brauer, 1864, paratype (Indonesia, Ambonia, paratype, male, provided by NHMV) **3** *Ankylopteryx* (*A.*) *ferruginea* Tsukaguchi, 1995 (Guangxi, Longzhou, female, CAU) **4** *Ankylopteryx* (*A.*) *gracilis* Nakahara, 1955 (Guangxi, Ningming, female, CAU) **5** *Ankylopteryx* (*A.*) *lii* Yang, 1987 (Xizang, Zäyu, paratype, male, CAU) **6** *Ankylopteryx* (*A.*) *magnimaculata* Yang, 1987 (Xizang, Zäyu, holotype, male, CAU) **7** *Ankylopteryx* (*A.*) *octopunctata candida* (Fabricius, 1798) (Guangxi, Ningming, female, CAU) **8** *Ankylopteryx* (*A.*) *yangi* sp. nov. (Taiwan, Pingtung, paratype, female, CAU) **9** *Ankylopteryx* (*A.*) *tibetana* Yang, 1987 (Xizang, Zäyu, holotype, male, CAU). Scale bars: 5.0 mm.



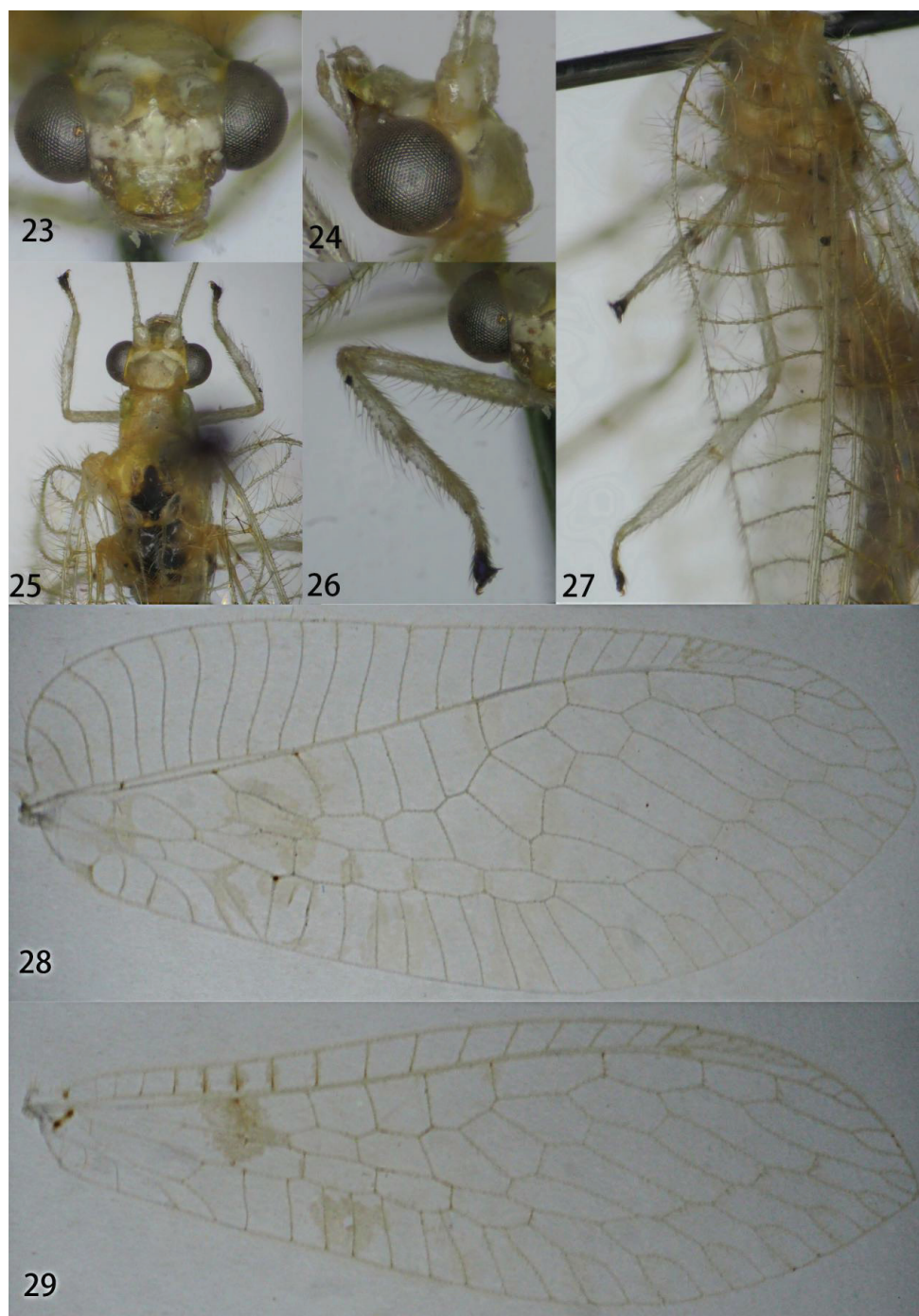
Figures 10–14. *Ankylopteryx* (*A.*) *delicatula* Banks (Yunnan, Jinghong, sex unknown, CAU). **10** head, frontal **11** head, dorsal **12** thorax, dorsal **13** protibia and mesotibia **14** forewing and hind wing. Veins (black lettering): C Costa Sc Subcosta R Radius Rs Radial sector Psc Pseudocubitus Psm Pseudomedia. Cells (red lettering): **b1**, **b4** first, fourth upper Banksian cells **b'1**, **b'4** first, fourth lower Banksian cells **r1**, **r9**, **r11** first, ninth, eleventh radial cell.



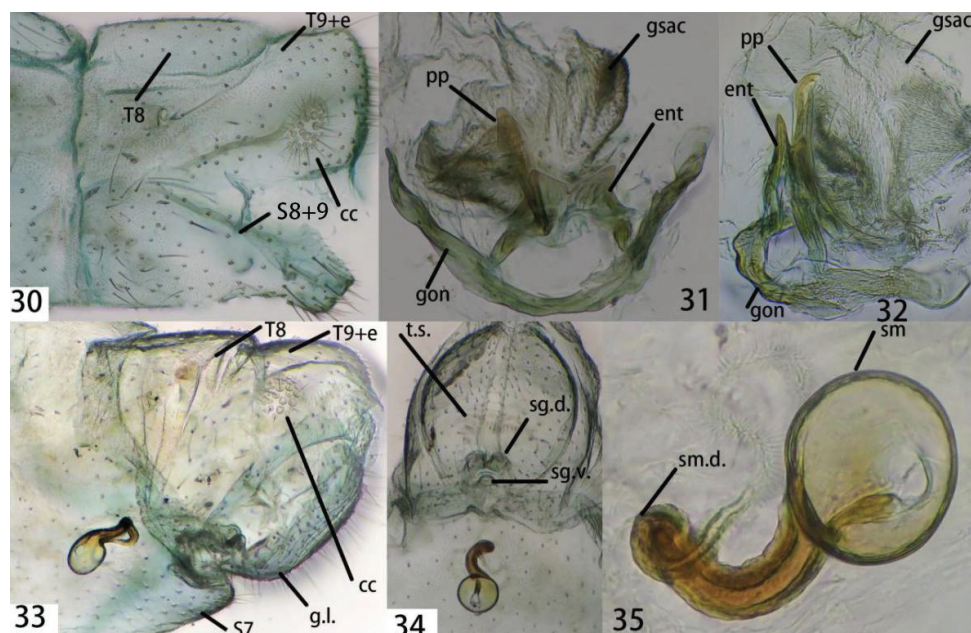
Figures 15–17. *Ankylopteryx* (*A.*) *delicatula* Banks, female abdomen (Yunnan, Jinghong, sex unknown, CAU). **15** segment A7-terminus, lateral **16** terminalia, ventral **17** spermatheca. **cc** callus cerci **g.l.** gonapophyses lateralis **S7** seventh sternum **sg.d.** dorsal lobe of subgenitale **sg.v.** ventral lobe of subgenitale **sm** spermatheca **sm.d.** spermathecal duct **t.s.** transverse sclerite **T8** eighth tergum **T9+e** ninth tergum + ectoproct.



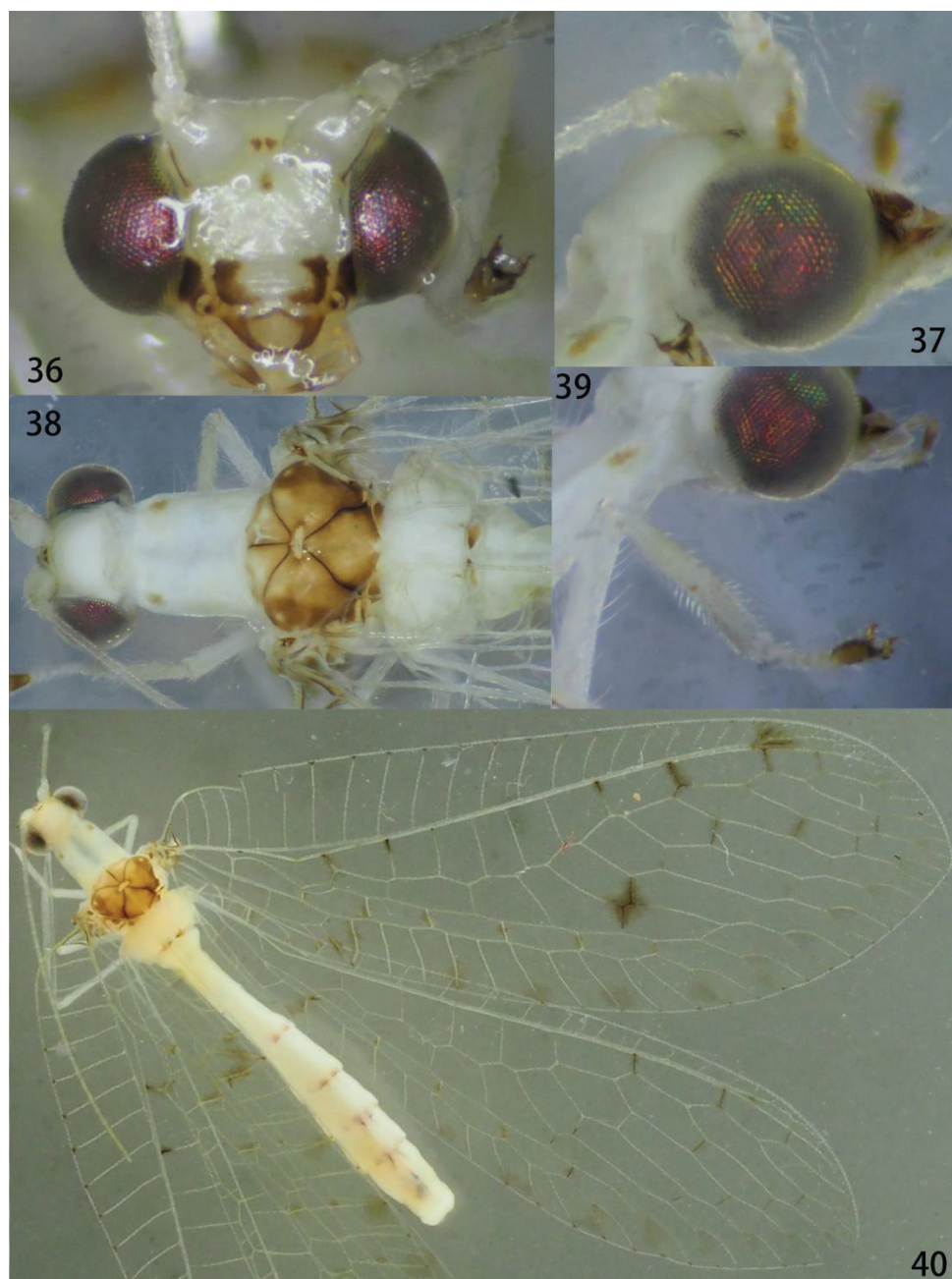
Figures 18–22. *Ankylopteryx* (*A.*) *doleschalii* Brauer (Indonesia, Ambonia, holotype, ex, provided by NHMV). **18** head, frontal **19** head and thorax, dorsal **20** protibia and mesotibia **21** forewing **22** hind wing.



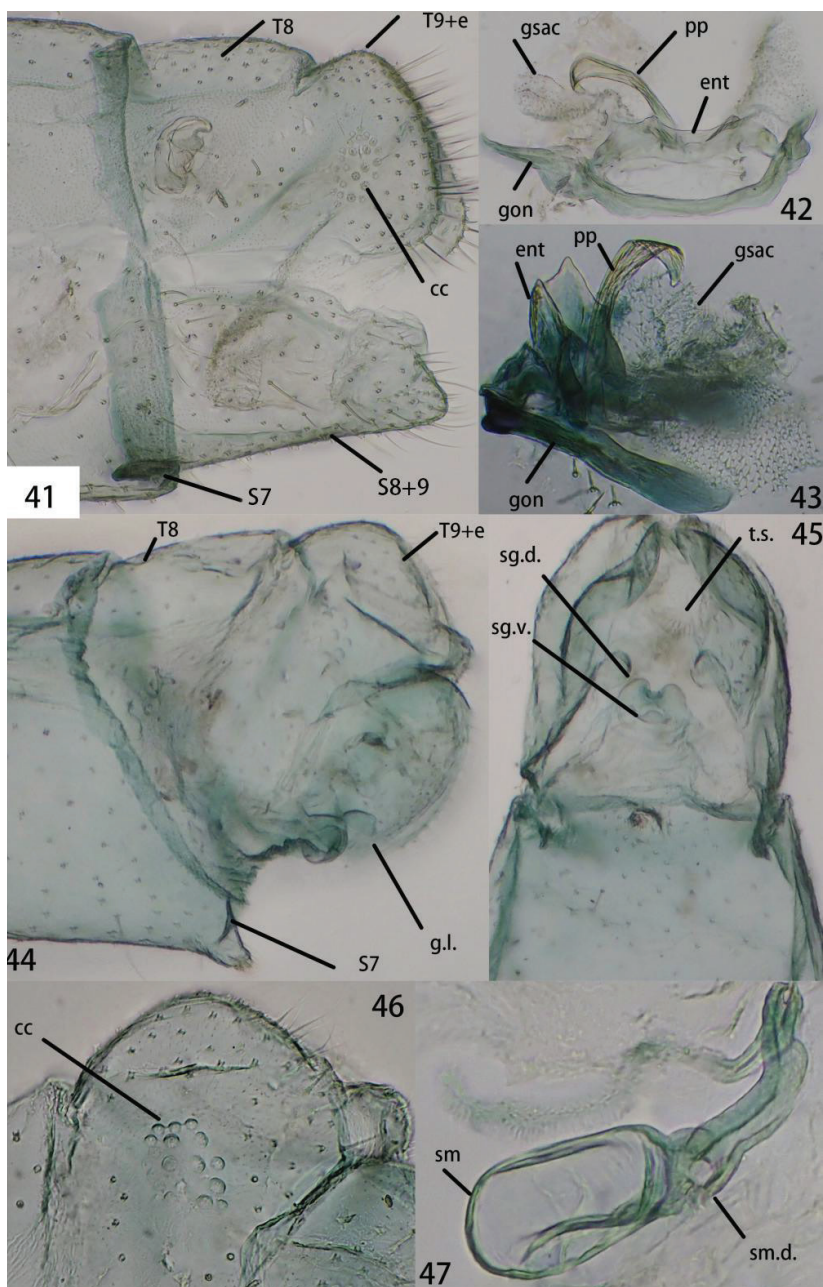
Figures 23–29. *Ankylopteryx (A.) ferruginea* Tsukaguchi. **23** head, frontal **24** head, lateral **25** thorax, dorsal **26** protibia **27** mesotibia and metatibia **28** forewing **29** hind wing (23–27: Yunnan, Lancang, male, CAU; 28, 29: Hainan, Yinggeling, female, CAU).



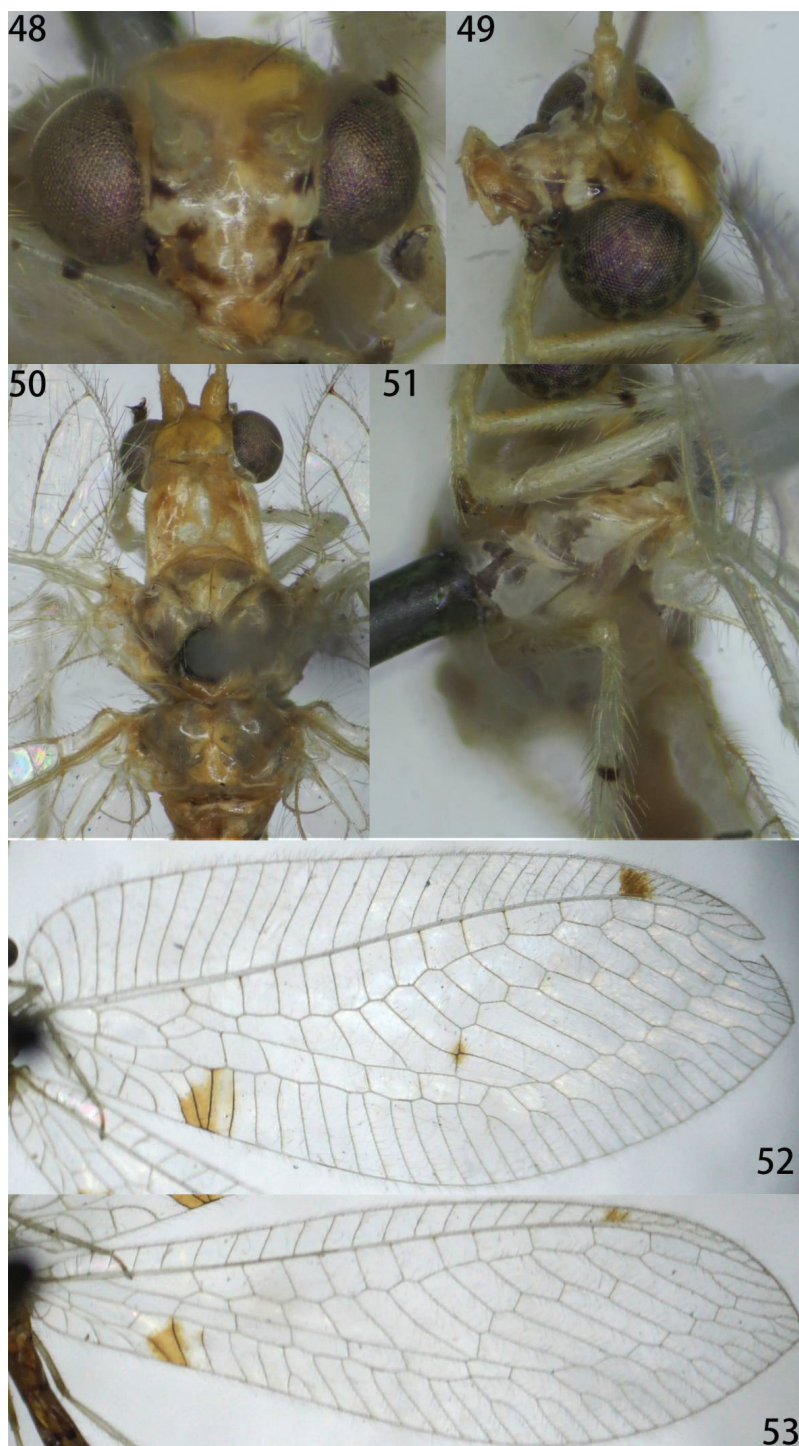
Figures 30–35. 30–32 *Ankylopteryx (A.) ferruginea* Tsukaguchi, male abdomen (Yunnan, Lancang, male, CAU). 30 segment A7-terminus, lateral 31 gonarcal complex, dorsal 32 gonarcal complex, lateral. 33–35 *Ankylopteryx (A.) ferruginea* Tsukaguchi, female abdomen (Hainan, Yinggeling, female, CAU). 33 segment A7-terminus, lateral 34 terminalia, ventral 35 spermatheca. cc callus cerci ent entoprocessus g.l. gonapophyses lateralis gsac gonosaccus gon gonarcus pp pseudopenis S7 seventh sternum S8+9 fused eighth+ ninth sternum sg.d. dorsal lobe of subgenitale sg.v. ventral lobe of subgenitale sm spermatheca sm.d. spermathecal duct t.s. transverse sclerite T8 eighth tergum T9+e ninth tergum + ectoproct.



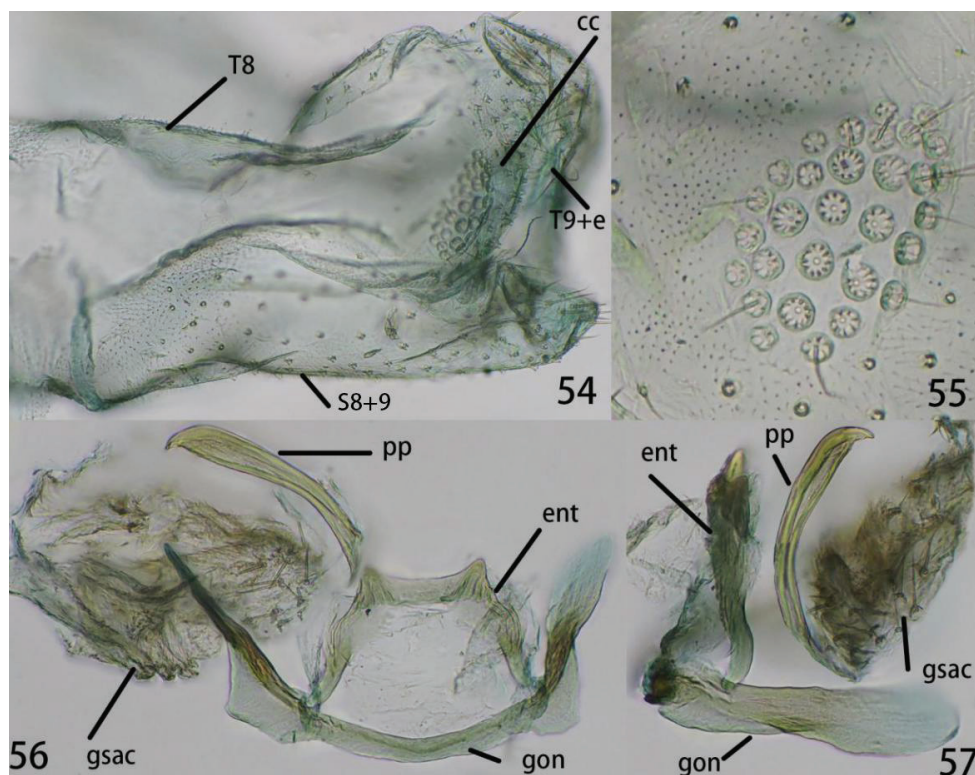
Figures 36–40. *Ankylopteryx* (*A.*) *gracilis* Nakahara (Japan, Okinawa, Iriomote-jima, male, CAU).
36 head, frontal **37** head, lateral **38** head and thorax, dorsal **39** protibia **40** forewing and hind wing.



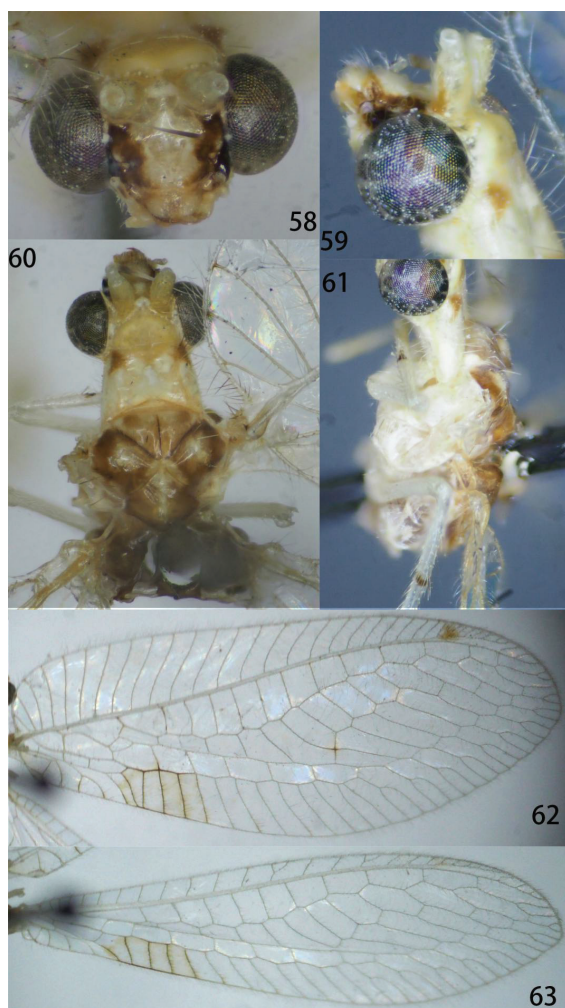
Figures 41–47. 41–43 *Ankylopteryx (A.) gracilis* Nakahara, male abdomen (Japan, Okinawa, Iriomotejima, male, CAU). 41 segment A7-terminus, lateral 42 gonarcal complex, dorsal 43 gonarcal complex, lateral. 44–47 *Ankylopteryx (A.) gracilis* Nakahara, female abdomen (Guangxi, Ningming, female, CAU). 44 segment A7-terminus, lateral 45 terminalia, ventral 46 callus cerci 47 spermatheca. cc callus cerci ent entoprocessus g.l. gonapophyses lateralis gsac gonosaccus gon gonarcus pp pseudopenis S7 seventh sternum S8+9 fused eighth + ninth sternum sg.d. dorsal lobe of subgenitale sg.v. ventral lobe of subgenitale sm spermatheca sm.d. spermathecal duct t.s. transverse sclerite T8 eighth tergum T9+e ninth tergum + ectoproct.



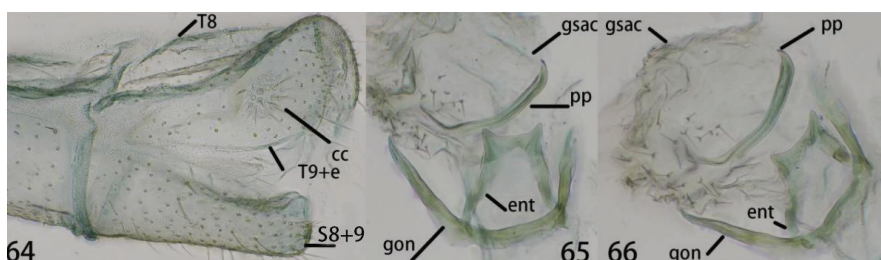
Figures 48–53. *Ankylopteryx* (A.) *lii* Yang (Xizang, Zāyu, holotype, male, CAU). **48** head, frontal **49** head, lateral **50** head and thorax, dorsal **51** protibia and mesotibia **52** forewing **53** hind wing.



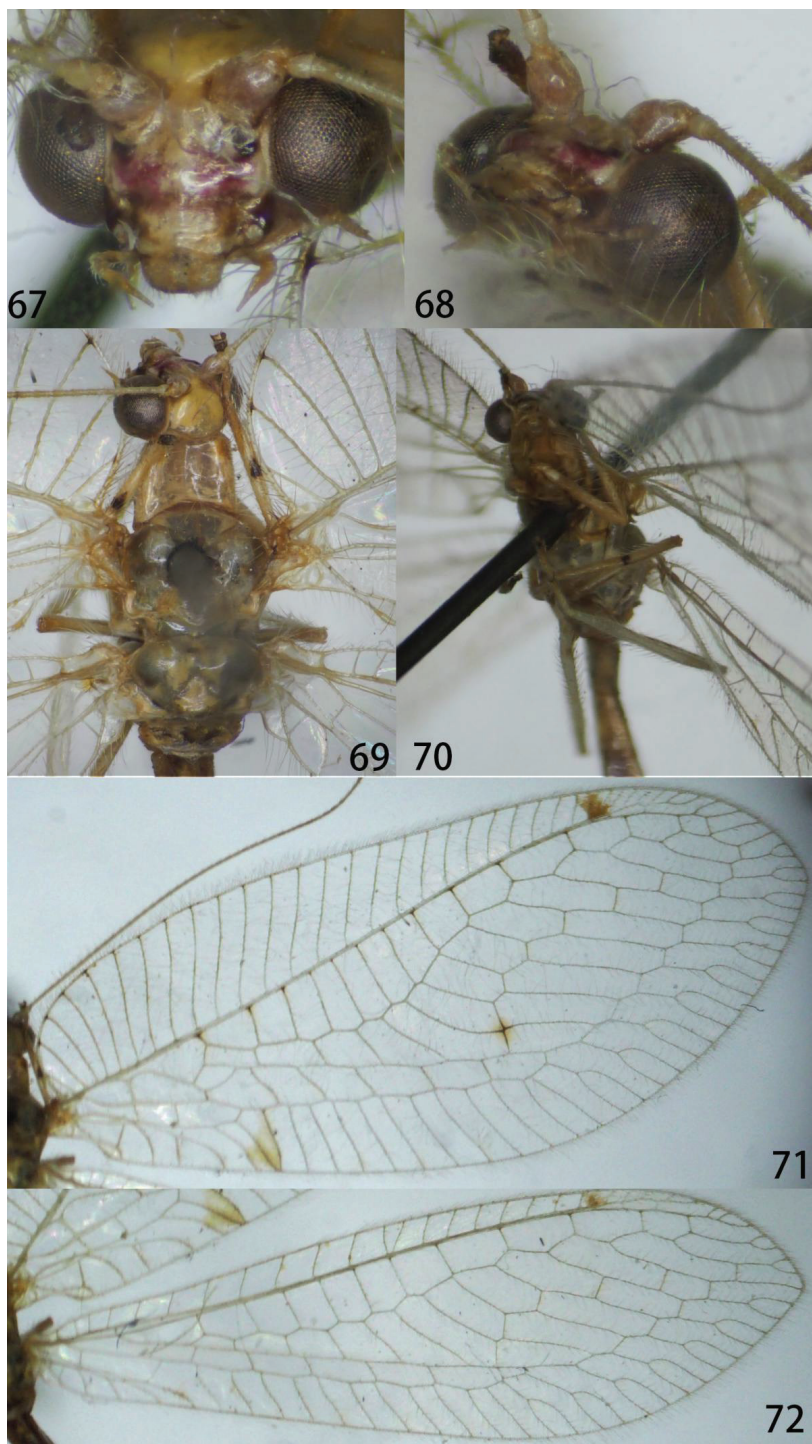
Figures 54–57. *Ankylopteryx (A.) lii* Yang, male abdomen (Xizang, Zāyü, holotype, male, CAU). **54** segment A7-terminus, lateral **55** callus cerci **56** gonarc complex, dorsal **57** gonarc complex, lateral. **cc** callus cerci **ent** entoprocessus **gsac** gonosaccus **gon** gonarcus **pp** pseudopenis **S8+9** fused eighth + ninth sternum **T8** eighth tergum **T9+e** ninth tergum + ectoproct.



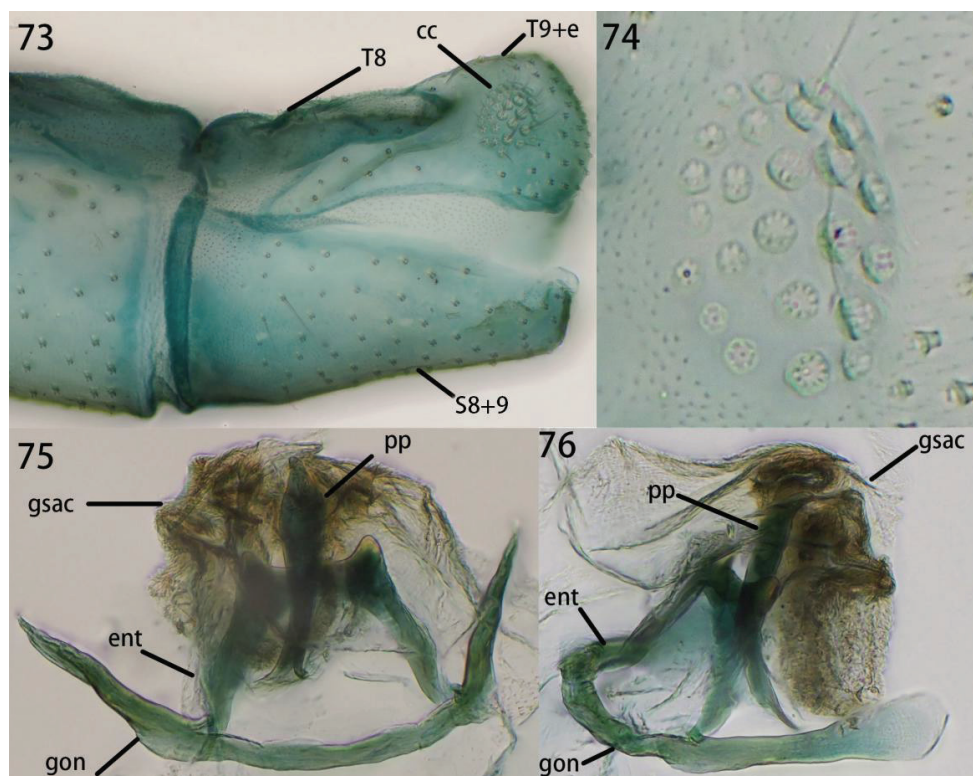
Figures 58–63. *Ankylopteryx* (*A.*) *magnimaculatus* Yang (Xizang, Zāyu, holotype, male, CAU). **58** head, frontal **59** head, lateral **60** head and thorax, dorsal **61** protibia and mesotibia **62** forewing **63** hind wing.



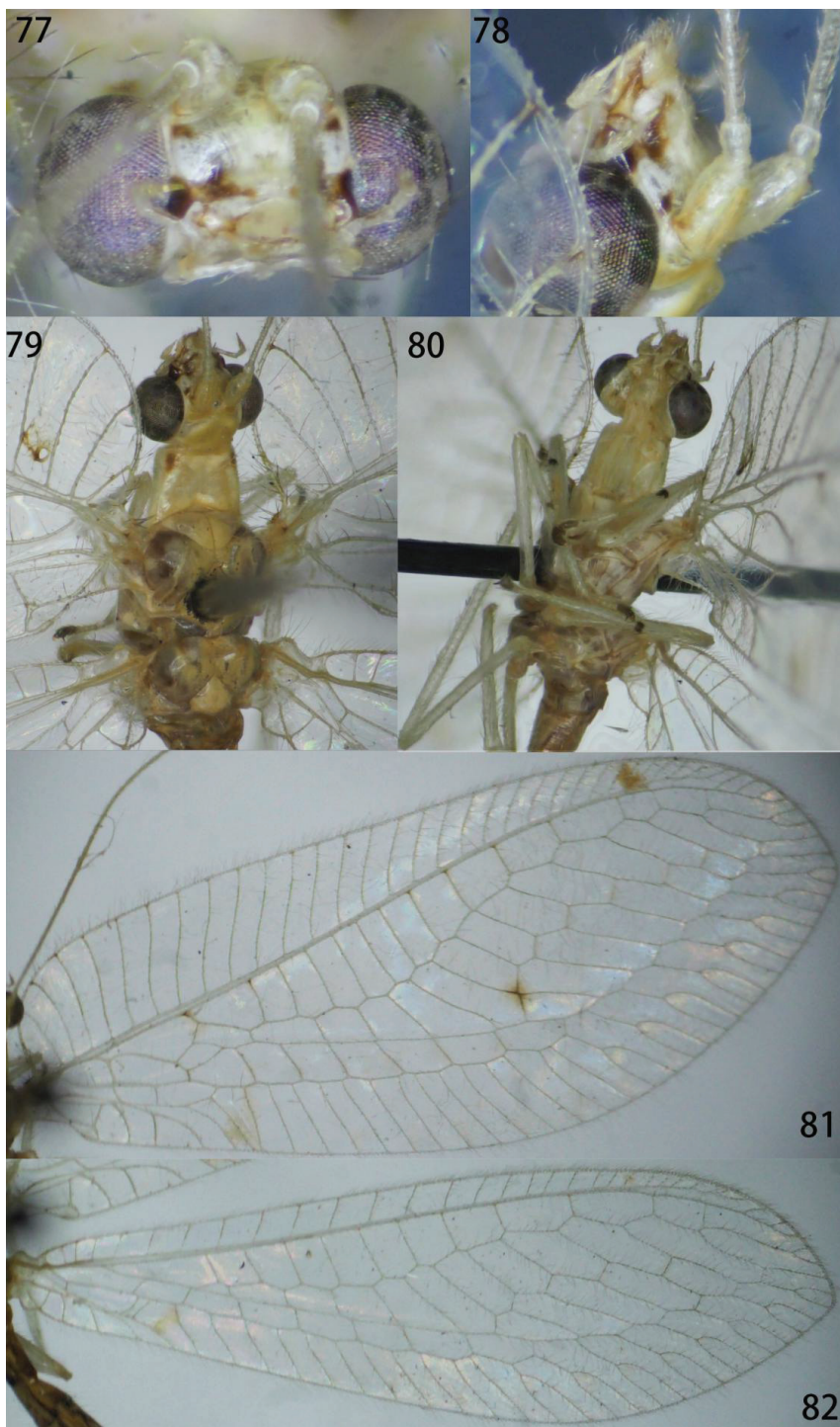
Figures 64–66. *Ankylopteryx* (*A.*) *lii* Yang, male abdomen (Xizang, Zāyu, holotype, male, CAU). **64** segment A7-terminus, lateral **65** gonarcual complex, dorsal **66** gonarcual complex, dorsolateral. **cc** callus cerci **ent** entoprocessus **gsac** gonosaccus **gon** gonarcus **pp** pseudopenis **S8+9** fused eighth + ninth sternum **T8** eighth tergum **T9+e** ninth tergum + ectoproct.



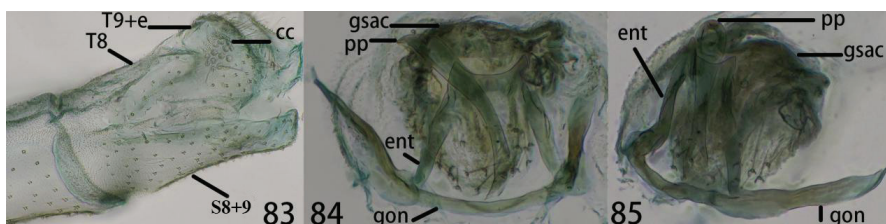
Figures 67–72. *Ankylopteryx (A.) octopunctata candida* (Fabricis) (Guangxi, Ningming, male, CAU). **67** head, frontal **68** head, frontolateral **69** head, thorax and protibia, dorsal **70** mesotibia **71** forewing **72** hind wing.



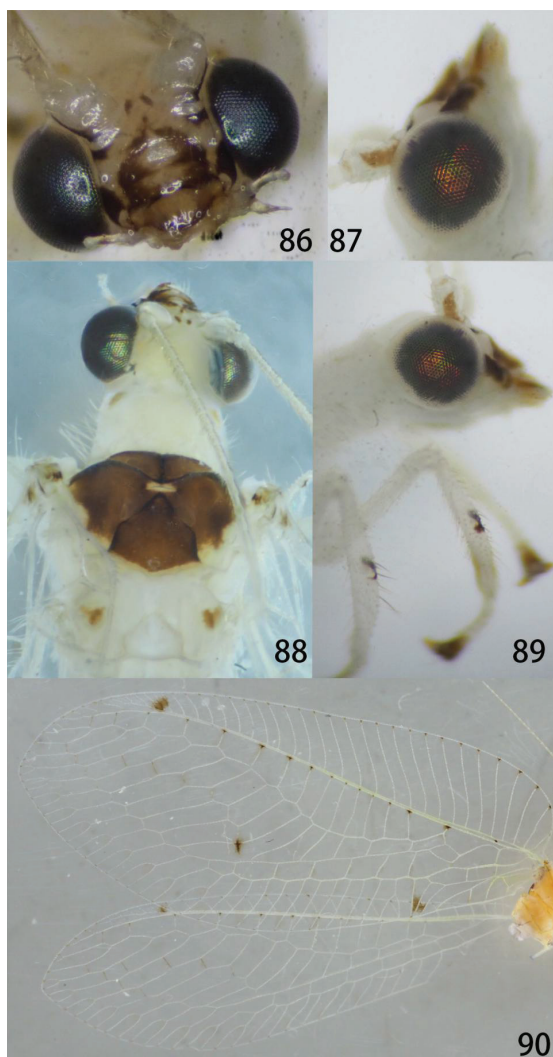
Figures 73–76. *Ankylopteryx* (*A.*) *octopunctata candida* Fabricius, male abdomen (Laos, Luang Namtha, male, CAU). **73** segment A7-terminus, lateral **74** callus cerci **75** gonarc complex, dorsal **76** gonarc complex, lateral. **cc** callus cerci **ent** entoprocessus **gsac** gonosaccus **gon** gonarcus **pp** pseudopenis **S8+9** fused eighth + ninth sternum **T8** eighth tergum **T9+e** ninth tergum + ectoproct.



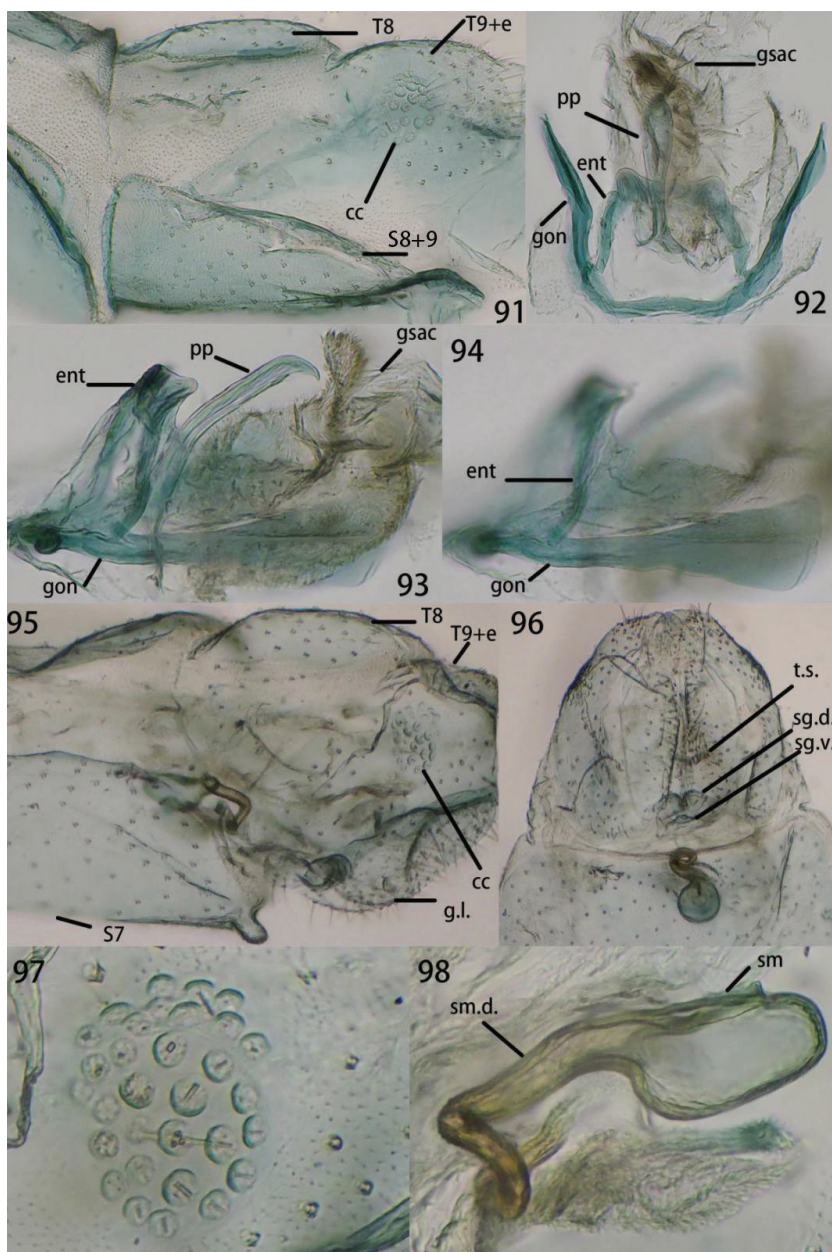
Figures 77–82. *Ankylopteryx (A.) tibetana* Yang (Xizang, Zāyu, paratype, male, CAU). **77** head, frontal **78** head, lateral **79** head, thorax and protibia, dorsal **80** protibia and mesotibia **81** forewing **82** hind wing.



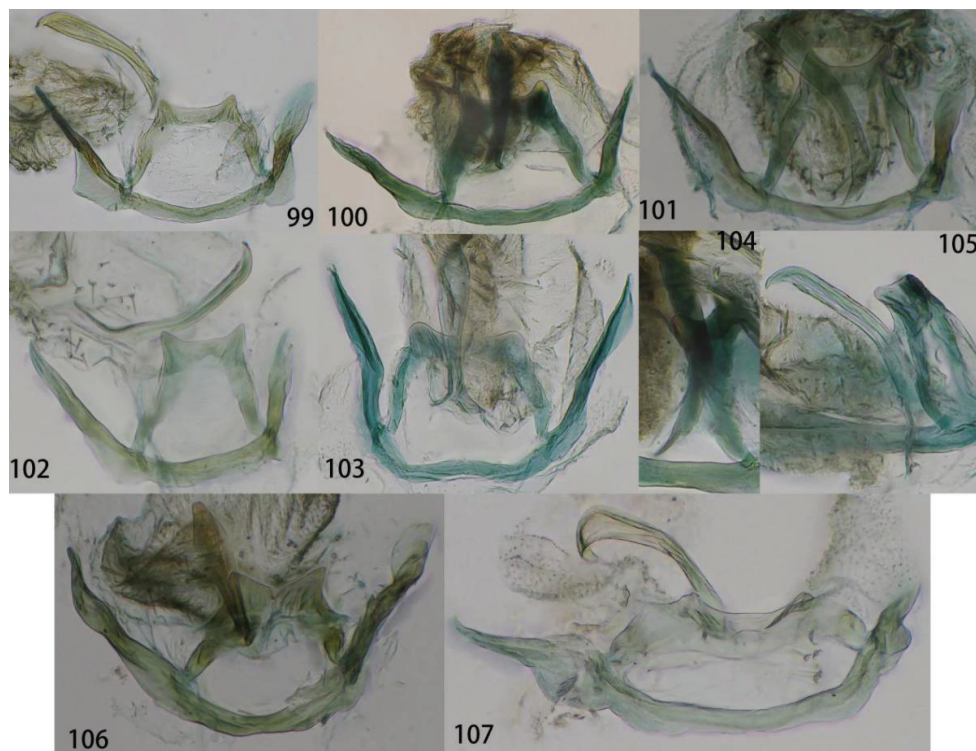
Figures 83–85. *Ankylopteryx* (*A.*) *tibetana* Yang, male abdomen (Xizang, Zāyu, holotype, male, CAU). **83** segment A7-terminus, lateral **84** callus cerci **85** gonarc complex, dorsal. **cc** callus cerci **ent** ento-processus **gsac** gonosaccus **gon** gonarcus **pp** pseudopenis **S8+9** fused eighth + ninth sternum **T8** eighth tergum **T9+e** ninth tergum + ectoproct.



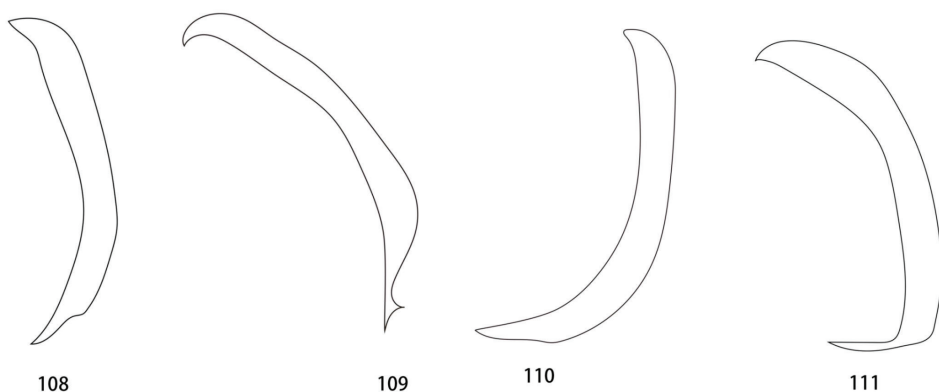
Figures 86–90. *Ankylopteryx* (*A.*) *yangi* sp. nov. (Guizhou, Libo, holotype, male, CAU). **86** head, frontal **87** head, lateral **88** head and thorax, dorsal **89** protibia and mesotibia **90** forewing and hind wing.



Figures 91–98. 91–94 *Ankylopteryx (A.) yangi* sp. nov., male abdomen (Guizhou, Libo, holotype, male, CAU). 91 segment A7-terminus, lateral 92 gonarcus complex, dorsal 93 gonarcus complex, lateral 94 gonarcus. 95–98 *Ankylopteryx (A.) yangi* sp. nov., female abdomen (Guizhou, Libo, paratype, female, CAU). 95 segment A7-terminus, lateral 96 terminalia, ventral 97 callus cerci 98 spermatheca. cc calculus cerci ent entoprocessus g.l. gonapophyses lateralis gsac gonosaccus gon gonarcus pp pseudopenis S7 seventh sternum S8+9 fused eighth + ninth sternum sg.d. dorsal lobe of subgenitale sg.v. ventral lobe of subgenitale sm spermatheca sm.d. spermathecal duct t.s. transverse sclerite T8 eighth tergum T9+e ninth tergum + ectoproct.



Figures 99–107. Gonarc complex. **99** *Ankylopteryx* (*A.*) *lii* Yang, dorsal **100** *Ankylopteryx* (*A.*) *octopunctata candida* (Fabricius), dorsal **101** *Ankylopteryx* (*A.*) *tibetana* Yang, dorsal **102** *Ankylopteryx* (*A.*) *magnimaculatus* Yang, dorsal **103** *Ankylopteryx* (*A.*) *yangi* sp. nov., dorsal **104** *Ankylopteryx* (*A.*) *octopunctata candida* (Fabricius), lateral **105** *Ankylopteryx* (*A.*) *yangi* sp. nov., lateral **106** *Ankylopteryx* (*A.*) *ferruginea* Tsukaguchi, dorsal **107** *Ankylopteryx* (*A.*) *gracilis* Nakahara, dorsal.



Figures 108–111. Pseudopenis, line drawings in lateral view. **108** *Ankylopteryx* (*A.*) *octopunctata candida* (Fabricius) **109** *Ankylopteryx* (*A.*) *yangi* sp. nov. **110** *Ankylopteryx* (*A.*) *ferruginea* Tsukaguchi **111** *Ankylopteryx* (*A.*) *gracilis* Nakahara



Figure 112. Known distribution of *Ankylopteryx* (*s. str.*) species from China and neighboring countries. *Ankylopteryx* (*A.*) *delicatula* Banks (white triangle); *Ankylopteryx* (*A.*) *doleschalii* Brauer (black triangle); *Ankylopteryx* (*A.*) *ferruginea* Tsukaguchi (circle); *Ankylopteryx* (*A.*) *magnimaculata* Yang (white square); *Ankylopteryx* (*A.*) *yangi* sp. n. (black square).



Figure 113. Known distribution of *Ankylopteryx* (*s. str.*) species from China and neighboring countries. *Ankylopteryx* (*A.*) *gracilis* Nakahara (white diamond); *Ankylopteryx* (*A.*) *octopunctata candida* Fabricius (black diamond).

Acknowledgements

We are very grateful to Dr Susanne Randolph and Mag. Harald Bruckner of NHMV for providing the detailed photos of *A. doleschalli* types. This work was supported by the Biodiversity Survey and Assessment Project of the Ministry of Ecology and Environment, China (no. 2019HJ2096001006) and the Key Project of Science – Technology Basic Condition Platform from The Ministry of Science and Technology, China (no. 2005DKA21402).

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A novel leaf-rolling chironomid, *Eukiefferiella endobryonia* sp. nov. (Diptera, Chironomidae, Orthocladiinae), highlights the diversity of underwater chironomid tube structures

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Academic editor: F. L. da Silva | Received 4 November 2019 | Accepted 17 December 2019 | Published 22 January 2020

<http://zoobank.org/A511F89A-4BBB-408B-A77B-D8CD694AD519>

Citation: Imada Y (2020) A novel leaf-rolling chironomid, *Eukiefferiella endobryonia* sp. nov. (Diptera, Chironomidae, Orthocladiinae), highlights the diversity of underwater chironomid tube structures. ZooKeys 906: 73–111. <https://doi.org/10.3897/zookeys.906.47834>

Abstract

The non-biting midges, Chironomidae (Diptera), are dominant components of most freshwater ecosystems. Many chironomids construct tubes or cases as larvae out of various materials bound together with silk. The structures of tubes show a wide range of variation, and some are morphologically comparable to those of caddisflies. Herein a new species is described, *Eukiefferiella endobryonia* **sp. nov.**, which exhibits a very unusual behavior in which it constructs tubes from aquatic mosses. This species' fourth-instar larvae construct their cases exclusively from the leaves of *Fontinalis* mosses (Hypnales: Fontinalaceae) and exhibit a stereotyped behavior in which they remain attached to the apical shoot of the moss stem. The larvae then pupate within the case. The case of *E. endobryonia* **sp. nov.** represents one of only a few examples of chironomid tubes made exclusively out of plants. Based on the species delimitation analyses using the partial COI sequences, together with some morphological and behavioral characteristics, this species is hypothesized to be a member of *devonica* group, and especially may have a close affinity to *E. dittmari* (Lehman). A provisional typology for the diversity of chironomid tube structures is provided, with a summary of different tube structures, which can be used for future research.

Keywords

bryophytivore; freshwater; Orthocladiinae; tubicolous; *Eukiefferiella*

Introduction

Many aquatic animals build biogenic structures, such as burrows, tubes, and cases (Chamberlain 1975). Construction behavior has evolved in a taxonomically diverse array of animals, including Protozoa, Mollusca, Annelida, Polychaeta, Crustacea, Echinodermata, fishes, and Nematoda (Nehring et al. 1990; Dudgeon 1994; Charbonneau and Hare 1998; Nehring 1993; Merz 2015). Dwelling tubes can be preserved as ichnofossils and provide evidence for activities by organisms over geological time (Chamberlain 1975; Gall 1983; Minter et al. 2016). In the freshwater realm, three insect orders contain notable numbers of species with such tube construction behaviors (Wallace and Merritt 1980): Trichoptera, Diptera (family Chironomidae), and Ephemeroptera (family Polymitarcyidae). Tube morphology and ultrastructure vary significantly among taxa, and these tubes may serve various functions (Dudgeon 1994).

Chironomidae is a diverse nematocerous family of Diptera, to which ca. 7290 described species belong (Pape et al. 2011; Courtney et al. 2017). Larval chironomids inhabit a broad spectrum of habitats, especially in permanent and temporal freshwater environments ranging from running and standing water, to macrolimnetic zones, temporary pools, and phytotelmata; a substantial number of species also occur in terrestrial habitats (Oliver 1971; Pinder 1995). Their modes of feeding are also diverse (Thienemann 1954). They have been classified as belonging to most functional feeding groups (Cummins 1974), including collector-gatherers, collector-filterers, scrapers, shredders, engulfers, and piercers (predators and parasites) (Berg 1995).

Most larvae of Chironomidae construct dwelling tubes or cases by combining various particles together with silk (Oliver 1971) that they secrete from their labial gland (Sehnal and Sutherland 2008). In many cases, chironomid tubes are soft, flexible, and cryptic in sediments (Heckman 2018). Several functions have been hypothesized for the tubicolous habit of these species, including improved respiration (Walshe 1950; Kon and Hidaka 1983; Stief et al. 2005), feeding (Walshe 1947, 1951), anti-predator defense (Hershey 1987), and protection against physiological stress (e.g., toxicity and desiccation) (Hinton 1951; Kikawada et al. 2005; Vedamanikam and Shazili 2009). Tube-making chironomids can be important ecosystem engineers (Lawton and Jones 1995). Tube-dwelling chironomids can be pioneer species, as they often colonize newly submerged substrata first and in greater numbers than other colonists (Nilsen and Larimore 1973). Tube-dwelling, deposit-feeding chironomids play important roles in the bioturbation of organic detritus (Dudgeon 1994; Svensson and Leonardson 1996; Chapin 2011). Consequently, they impact the physical properties of sediments and drive biogeochemical processes in lake ecosystems (Ólafsson and Paterson 2004; Hölker et al. 2015). The presence and feeding activities of chironomids also have impacts on the structures of meiofaunal and protozoan communities (Ptatscheck et al. 2017; Webert et al. 2017). Additionally, tube structures, by themselves, can exert considerable effects on the periphytic diatom flora living on river rocks (Pringle 1985; Hershey et al. 1988; Herren et al. 2017).

In lotic habitats, aquatic mosses harbor various benthic invertebrates (Suren 1993). In many stream habitats, Chironomidae can be abundant on clumps of aquatic

fountain mosses, *Fontinalis* spp. (Linhart et al. 2002; Vlčková et al. 2002; Bogut et al. 2009). Aquatic bryophytes can interact with aquatic arthropods by providing them with space and shelter from predators (Stream Bryophyte Group 1999). However, it is unclear whether aquatic bryophytes are an important food source for invertebrates (Winterbourn et al. 1986; McWilliam-Hughes et al. 2009), or not (Bunn et al. 1989; Suren and Winterbourn 1991). The inhibition of invertebrate feeding on bryophytes is often explained as likely resulting from the low nutritional value and presence of secondary chemicals in aquatic mosses (Parker et al. 2007).

While searching for arthropods that interact with aquatic bryophytes in North America, I discovered chironomid larvae that were notably distinct from other tubicolous chironomids due to their unique tube-constructing behavior. Specifically, the fourth-instar larvae of these chironomids make cases exclusively using the leaves of *Fontinalis* mosses. Although a number of chironomid larvae were found attached to moss shoots, some larvae could be clearly distinguished by their construction behavior at the shoot tips of *Fontinalis* mosses. This species turned out to be a new species belonging to the genus *Eukiefferiella* Thienemann (Orthocladiinae). The life history of this species was clarified with the aid of DNA barcoding, and a description of it is given herein, including an account of its larval tube construction behavior. As the taxonomy of *Eukiefferiella* can be problematic, the genetic differentiation of the new species in comparison to some congeners which are hypothetically closely related is estimated using methods for delimitation of species. Additionally, a provisional typology of chironomid tube morphology is provided, to highlight the diverse morphology of tube structure among chironomids.

Materials and methods

Study sites

Chironomids were collected during 24–27 February and on 10 November in 2018 in a stream connected to Mountain Lake, Virginia, USA. Mountain Lake is an oligotrophic lake that is located at an elevation of 1181 m above sea level near the summit of Salt Pond Mountain, and is the only natural lake of substantial size in the unglaciated part of the Southern Appalachian Mountain Range (Sharp 1933). Mountain Lake possesses an unusually high diversity of aquatic plants and invertebrates (reviewed by Parker 2003). Two species of *Fontinalis* have been recorded from the lake itself and the brooks in its vicinity, *F. dalecarlica* B. S. G. and *F. novae-angliae* Sull. (Studler 1982). *F. dalecarlica* is common in gently flowing water bodies, including in the brook that I sampled. Additional samples were collected during 4–9 April and 11–13 November in 2018 in a flowing-water stream, Rowans River, along Sparks Lane in Cades Cove, in the northwest part of the Great Smoky Mountains National Park (GRSM), Tennessee, USA, ca. 430 km away from Mountain Lake. *Fontinalis novae-angliae* with rigid stems and concave leaves occur from the streambed. Samples were collected in GRSM under research permit GRSM-2017-SCI-2389.

Collections, rearing, and observations

Insects were searched for underwater in the sampled streams and brooks and were collected together with the host plants occurring in their habitats. At Mountain Lake Biological Station, the clumps of mosses were detangled from detritus, and sediments were washed out of them. The larvae were placed in small plastic cases and observed with a microscope. The plastic chambers were constantly cooled with a refrigerant to keep their temperature in the range between 10–22 °C. Fourth-instar larvae were observed for 27 h in total, between 08:00 hrs and 18:00 hrs during 4–25 March 2018. Rearing and observations of chironomid larvae were performed at the National Museum of Natural History, Smithsonian Institution.

Molecular analyses

To compare and differentiate the chironomids of different stages and sexes occurring at the study sites, their partial COI (cytochrome c oxidase subunit I) gene sequences were determined. Total genomic DNA was extracted from 23 specimens at different stages, including adults (from a single adult leg or abdomen), larvae (two or three abdominal segments), and pupae (the whole abdomen) or pupal exuviae, using a NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany) and following the protocol provided by the manufacturer, with some modifications. The protocol was modified as follows: (i) tissue was digested for 48 h at 58 °C; (ii) after digestion with proteinase K, tissues were removed, washed in distilled water and used for morphological assessments; and (iii) the final elution volume was 30 µL. The primer pair used for the COI region consisted of primers 911 and 912 of Folmer et al. (1994), as used in some previous DNA barcoding and phylogenetic studies of Chironomidae (e.g., Guryev et al. 2001; Stur and Ekrem 2011; Cranston et al. 2012). Amplifications of the COI region were performed in a thermocycler, with an initial denaturation step of 94 °C for 4 min, followed by 40 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, and one cycle at 72 °C for 10 min. Amplification products were purified with ExoSAP-IT, according to the manufacturer's instructions.

Direct sequencing of polymerase chain reaction (PCR) products was performed using the ABI Big Dye Terminator 3.1 cycle sequencing kit (Applied Biosystems, Lennik, Belgium) while following the manufacturer's instructions and was carried out in an ABI 3130 Capillary Electrophoresis Genetic analyzer. Both DNA strands were sequenced. Sequences were deposited in the GenBank database (Table 1).

Phylogenetic analyses

Sequence trace files were edited with 4PEAKS v. 1.8 (by A. Griekspoor and Tom Groothuis, nucleobytes.com). Nucleotide sequences were aligned with CLUSTAL W implemented in MEGA 7 (Kumar et al. 2016). To evaluate if the new species is phyloge-

netically exclusive among hypothetically closely related species (particularly the species in 'devonica' group, as discussed later) and to assess the intra- and interspecific genetic distances, the species delimitation plug-in in the software Geneious Prime 2019.2.3 (www.geneious.com) was used (Masters et al. 2011; Kearse et al. 2012).

The COI sequences of a rich record of *Eukiefferiella* species were found in GenBank with 1052 fragment sequences (accessed on October 10th, 2019), although the sequence data identified at the species level were available only for five species (i.e., *E. devonica* (Edw.), *E. ilkleysensis* (Edw.), *E. claripennis* (Lundbeck), *E. minor* (Edw.), *E. dittmari* (Lehman)). In the dataset, 23 sequence data representing five species were included (Table 1), as well as five sequences obtained in this study. Phylogenetic trees were inferred by Bayesian inference (BI). Trees were rooted with two species of the genus *Cardiocladius* (i.e., *C. capucinus* (Zetterstedt), *C. fuscus* Kieff.). Evolutionary model was selected with MrModeltest v. 4.0b10 (Nylander 2004). The best fitting models were chosen with the Akaike Information Criterion (Akaike 1973). For the COI dataset, GTR + Gamma model was selected and used for the following Bayesian phylogenetic analysis. BI trees were constructed with MrBAYES v.3.2.6 (Huelsenbeck and Ronquist 2001), using the plug-in of Geneious Prime, based on a cold chain and four heated chains with $T = 0.2$, running for 1,100,000 generations with a sample frequency of 200. The first 100,000 trees were discarded and the remaining trees were used to build a consensus tree, with estimated Bayesian posterior probabilities (PP). At the point of burn-in, the chains had all converged to a stable standard deviation of split frequencies lower than 0.01.

The species delimitation plug-in in the Geneious Prime 2019.2.3 (Kearse et al. 2012; Masters et al. 2011) was used (1) to measure the phylogenetic support of new species described herein, and (2) to evaluate the genetic differentiation among and within species, among other congeners which are hypothetically closely related to this species. For these purposes, Rosenberg's P_{AB} (Rosenberg 2007) Rodrigo's P (Randomly Distinct) (Rodrigo et al. 2008) were calculated. Rosenberg's P_{AB} is a test for taxonomic distinctiveness of a clade based on the null hypothesis that monophyly is a chance outcome of random branching. Rodrigo's P(Randomly Distinct) ('Rodrigo's P (RD)') is the probability that a focal clade has the observed degree of distinctiveness (i.e., the ratio between the distance from a species-defining node to the tips of the tree, and the distance from that same node to its immediate ancestor) due to random coalescent processes (Rodrigo et al. 2008). Focal groups with values between 0.05 and 1 represent groups that have branching events that would be expected under the coalescent model in a Wright-Fisher population and a strict molecular clock. Additionally, six statistics which are useful to species delimitation were presented, along with Rosenberg's P_{AB} and Rodrigo's P (RD): the average pairwise tree distance, among members of the focal species/populations ('D Intra') and between the members of the focal species and members of the next closest species ('D Inter'); as a measure of genetic differences between the focal species and its closest neighboring species, the ratio of D Intra to D Inter ('Intra/Inter'); as the measures for evaluating diagnosability of each species/population, the mean probability of making a correct identification of a hypothetical sample of the focal species using placement on a tree under two different criteria, 'P ID(Strict)' (the sample

Table 1. Specimen and collection information used for the DNA barcoding analysis. Summary of specimens used for the COI analyses. Species names identified by morphology.

Species	Voucher ID	Accession number	Collection locality	Coordinates	Reference
<i>Cardiocladius capucinus</i> <i>Cardiocladius fuscus</i> <i>E. claripennis</i>	ATNA466	HM421556.1	Norway: Rondane National Park	61.9935N, 9.80343E	GenBank
	NIESH0714	LC329044.1	Japan: Nagano, River Chikuma	–	GenBank
	ATNA247	HM421358.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
	ATNA260	HM421370.1	Norway: Rondane National Park	61.9835N, 9.80384E	GenBank
	ATNA354	HM421455.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
<i>E. devonica</i>	Finnmark412	JN275486.1	Norway: Fállevejajohka	69.6779N, 30.4494E	GenBank
	BIOUG05490-D09	KR175110.1	Canada: Ontario, Rouge National Urban Park	43.8223N, 79.1897W	GenBank
	BIOUG10589-G08	KR276839.1	Canada: Gros Morne National Park	49.5686N, 57.8302W	GenBank
	BIOUG09943-A01	KR276908.1	Canada: Quebec: Forillon National Park	48.857N, 64.376W	GenBank
	BIOUG09308-C08	KR282929.1	Canada: Ontario: Georgian Bay Islands National Park	44.7418N, 79.8501W	GenBank
	BIOUG09943-C02	KR283193.1	Canada: Quebec: Forillon National Park	48.857N, 64.376W	GenBank
	ATNA239	HM421351.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
	ATNA241	HM421353.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
	ATNA246	HM421357.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
	ATNA499	HQ551492.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
<i>E. dittmari</i> <i>E. endobryonia</i> sp. nov.	Finnmark194	JF870841.1	Norway: Masi	69.4482N, 23.7576E	GenBank
	YI-CR-001	LC505506	USA: TN: Great Smoky Mountains National Park	35.600894N, 83.794004W	This study
	YI-CR-006	LC505507	USA: TN: Great Smoky Mountains National Park	35.600894N, 83.794004W	This study
	YI-CR-008	LC505508	USA: TN: Great Smoky Mountains National Park	35.600894N, 83.794004W	This study
	YI-CR-009	LC505509	USA: VA: Mountain Lake	37.357627N, 80.534448W	This study
<i>E. ilkleyensis</i>	YI-CR-015	LC505510	USA: VA: Mountain Lake	37.357627N, 80.534448W	This study
	ATNA348	HM421450.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
	ATNA497	HQ551490.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
	ATNA498	HQ551491.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
	ATNA512	HQ551503.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
<i>E. minor</i> <i>E. sp.</i>	ATNA513	HQ551504.1	Norway: Rondane National Park	61.9819N, 9.80454E	GenBank
	Finnmark570	JF870931.1	Norway: Rafsbotn	70.0137N, 23.5547E	GenBank
	BIOUG01648-H02	KR660601.1	Canada: Ontario: Elizabethtown-Kitley	44.618N, 75.775W	GenBank

must fall within the species clade) and ‘P ID(Liberal)’ (the sample is allowed to fall sister to or within a species clade); the mean distance between the most recent common ancestor of a species and its members (‘Av(MRCA)’). Accession number, voucher ID, and information on the localities for each specimen are shown in Table 1.

Morphological analyses

All specimens of adult abdomens, pupae, and larvae were digested with proteinase K, which made it relatively easy to examine the specimens morphologically. When necessary, the apical portion of the adult abdomen was macerated with warm (ca. 90 °C) 5% KOH and rinsed with distilled water. Each body part sample was mounted on a microscopic slide with Euparal.

The terminology of morphological features used herein followed Sæther (1971, 1977, 1980). The antennal ratio (AR), leg ratios (LR, BV, SV), wing ratio (L/WR) and hypopygium ratio (HR), and other morphological features were measured for adult male specimens following Sæther (1968), Schlee (1966) and Soponis (1977). Abbreviations that are used in this work:

AR	antennal ratio: length of last flagellomere / length of remaining flagellomeres;
LR	leg ratio: length of first tarsal segment/ length of tibia;
BV	“Beinverhältnis”: length of femur, tibia plus first tarsal segment/ length of tarsal segments 2–5;
SV	length of femur plus tibia/ length of tarsal segments 1–3;
L/WR	wing length/ wing width ratio;
HR	hypopygium ratio: length of gonocoxite/length of gonostylus.

The type specimens are deposited in **NMNH** (National Museum of Natural History, Washington DC, USA). For each specimen, voucher ID is given as ‘YI-CR-##’.

Taxonomy

Eukiefferiella endobryonia sp. nov.

<http://zoobank.org/2EFD6644-44B9-4CF2-8C5F-6E2523DE6CF6>

Figs 1, 2

Diagnosis. Adult male with squama with few (two or three) setae; gonostylus with crista dorsalis; hind tibial comb and tibial spurs reduced, outer spur absent. Pupa lacks precorneal setae and respiratory horns; three anal macrosetae consisting of two thinner inner macrosetae and a normal outer macroseta. Larval body setae short; seta interna with five branches deeply divided to the base; mentum with four pairs of lateral teeth and single, wide, truncate median tooth.

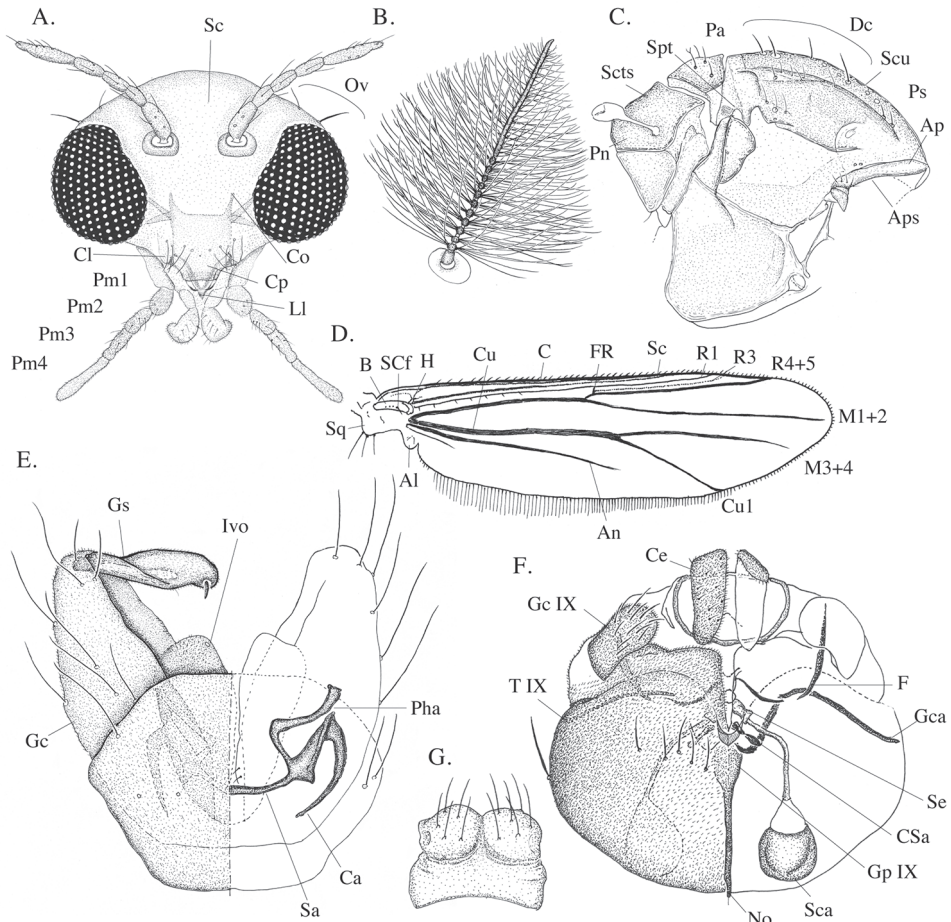


Figure 1. *Eukiefferiella endobryonia* sp. nov., adult. **A** Female head **B** male antenna **C** thorax **D** right wing **E** hypopygium with tergite IX and with left gonocoxite and gonostylus, in dorsal view with gonostylus (left) and in ventral view without gonostylus (right) **F** female genitalia, dorsal (left) and ventral view (right) **G** female tergum IX. Abbreviations (adult). Al: alula; An: anal vein; Ap: antepronotum; Aps: anteprenotals; B: brachiolium; C: costa; Ca: coxapodeme; Ce: cercus; Cl: clypeus; Co: cornua; Cp: cibarial pump; Csa: coxosternapodeme; Dc: dorsocentrals; F: fulcrum; Gc: gonocoxite; Gca: gonocozapodeme; Gc IX: gonocoxite IX; Gp IX: gonapophysis IX; Gs: gonostylus; H: humerals; Ivo: inferior volsella; Ll: labial lonchus; No: notum; Pa: prealars; Pha: phallapodeme; Pm: palpal segments; Pn: postnotum; Ps: pseudospurs; Sa: sternapodeme; Sc: subcosta; Sca: seminal capsule; Scts: scutellars; Scu: scutum; Se: spermathecal eminence; Spt: scopula thoracalis; Sq: squama; T IX: tergum IX.

Material examined. Holotype: USA, VA • 1 adult male (YI-CR-013); Mountain Lake (37.357627 N 80.534448 W); 24-II-2018 (as larva); Y. Imada leg; emerged as adult on 12-III-2018; NMNH.

Paratypes: USA, VA • 2 adult males (YI-CR-009, YI-CR-016) and 3 adult females (YI-CR-010, YI-CR-011, YI-CR-015); Mountain Lake (37.357627N 80.534448W);

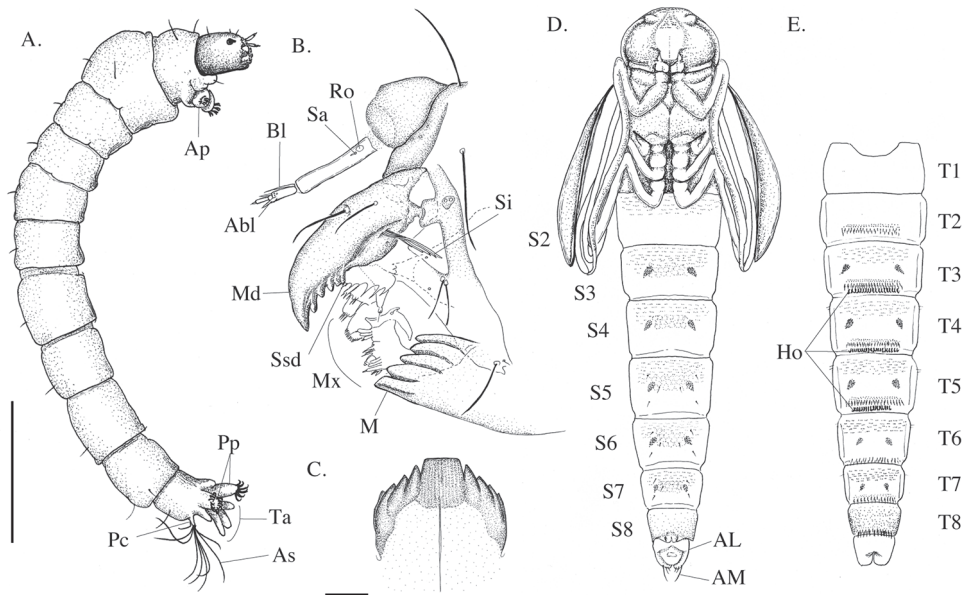


Figure 2. *Eukiefferiella endobryonia* sp. nov., fourth-instar larva and pupa. Larva (A–C): **A** general appearance of larva **B** larval antenna, maxilla and mandible, lateral view **C** mentum. Pupa (D, E): **D** pupa, ventral aspect **E** ditto, dorsal aspect. Abbreviations (larva). Abl: accessory blade; Ap: anterior parapods; As: anal seta; Bl: blade; M: mentum; Mx: maxilla; Pc: procercus; Pm: premandible; Pp: posterior parapods; Ro: ring organ; Sa: supraanal seta; Si: seta interna; Ssd: seta subdentalis; Ta: anal tubules. Abbreviations (pupa). Al: anal lobe; Am: anal macroseta; Ho: orally curved hooklets. Scale bars: 0.1 mm (C), 1 mm (A).

24-II-2018 (as larvae); Y. Imada leg; emerged as adults between 12-III-2018 and 28-IV-2018; NMNH.

Other material. USA, TN • 2 female pupae (YI-CR-001, YI-CR-002), 2 larvae (YI-CR-006, YI-CR-007); Sparks Lane (35.600894N, 83.794004W); 13-XI-2018 (as larvae); Y. Imada leg; NMNH; VA • 1 male pupa (YI-CR-012), 2 female pupa (YI-CR-005, YI-CR-015), 1 pupal exuviae (no voucher), 4 larvae (YI-CR-003, YI-CR-023, YI-CR-024, YI-CR-025); Mountain Lake (37.357627N, 80.534448W); 9-XI-2018; Y. Imada leg, NMNH.

Egg. Unknown.

First instar larva. Unknown.

Fourth instar larva. ($N = 4$) Body length 3.0 mm. Head capsule dark brown. Body yellowish. Head capsule with frontoclypeal apotome with clypeus without divided by strong suture. Antenna nonretractile, 5-segmented; fourth segment twice as long as third segment; lauterborn organ small; blade as long as flagellum; ring organ in basal third. Premandible with one broad, blunt apical tooth. Mandible with apical tooth longer than first lateral tooth; inner margin smooth, without serrations; seta subdentalis short, peg-like; five very long seta interna with five branches divided nearly to the base, each branch similar in length and width to each other; mola with four long

spines. Maxilla without pecten galearis; chaetulae of palpiger lacking; lamellae of galea short; anterior lacinial chaeta apparently short, broad-based, more or less differentiated from other chaetae. Mentum with single median tooth and four pairs of lateral teeth; ventromental plates inconspicuous, without beard beneath. Parapods well developed. Claws of anterior parapods all smooth. Procercus unsclerotized, less than 1.5 times as long as wide, without tooth, spur, or seta; anal setae 5–7. Supraanal seta absent. Anal tubules developed, longer than posterior parapods. Body setae very short and inconspicuous, shorter than one-quarter the length of abdominal segments.

Pupa. ($N = 8$) Frontal apotome without frontal seta and warts. Thoracic horn and precorneal seta absent. Dorsocentrals four. Thorax nearly smooth. Wing sheath smooth, without pearl row. T I–II, T VIII, S I and S VIII without shagreen. T II–IX with strong anterior shagreen. S II–VII with weak posterior shagreen. Pedes spurii A and B absent. Caudal spines absent on T II–VIII. S IV–VII female at most with very weak caudal spines. Orally curved hooklets present in uninterrupted rows posterior to caudal spines on T III–V. Apophyses and O setae absent. Segments IV–VIII with very short and weak L-setae. Anal lobe with three unequal anal macrosetae, consisting of two, thinner inner macrosetae and a normal outer macroseta; without median seta, fringe, apical spine.

Adult male. ($N = 3$, if not mentioned) Body length 2.9–3.0 mm without antenna. Body color dark brown. Antennal length 0.8 mm. Flagellum plumose, with 13 flagellomeres; apex spatula-shaped, without a strong straight seta; antennal groove in male reaching flagellomere 3; AR 1.1. Eye bare. Temporal setae 2, not clearly separated into inner and outer verticals and postorbitals. Postocular setae present in a single row, only behind eyes. Palpus 5-segmented; palpomere lengths: 55–72, 86–90, 96, 159–159 ($N = 1$); palpomeres with 3, 4, 5, 0 setae, respectively. Antep pronotum well developed with lobes meeting medially at anterior margin of scutum; dorsal antep pronotals absent; four lateral antep pronotals; acrostichals absent; six dorsocentrals in a single row. Approximately three prealars. Scutellum smooth with nine scutellars in single row. Supraalar setae present. Wing length 2.3 mm; L/WR 3.01. Wing membrane glabrous, unmarked. Anal lobe small. Costa not extended. Crossvein m-cu absent. Cu_1 straight. R_{4+5} only fused with C at apex. R_{2+3} present, ending at middle of distance between R_1 and R_{4+5} . Cu_1 very slightly curved apically at wing margin. Squama with two or three setae. Sensilla campaniformia ca. eight at base of brachiolum, three above setae and eight at apex of brachiolum; 1 on Sc, one basally on R, one near base of R1; and one on FR. Calypter without marginal setae; calyptal fringe absent. First tarsomere of foreleg shorter than fore tibia. Fore coxa not enlarged. Hind tibial comb and tibial spurs reduced; outer spur absent. Pulvilli very faint. Gonostylus hinged to gonocoxite and folded inward. Anal point absent. Anterior margin of transverse sternapodeme convex, phallapodeme and aedeagal lobe normal. Virga absent. Gonocoxite with well-developed inferior volsella. Gonostylus with crista dorsalis; apical spine absent. HR 1.99. Lengths of leg segments and leg ratios as in Table 2.

Adult female. ($N = 3$, if not mentioned) Body length 2.8 mm. Antenna with five flagellomeres; flagellomere lengths (in μm): 56.7, 35.8, 38.2, 45.2, 101.2; with 2, 3, 2, 3, 3 setae, respectively ($N = 1$). Eye bare. Clypeus with 8 setae. R with two setae, squama with 4–6 setae. Scutellum as in male. Gonocoxapodemes not jointed me-

Table 2. Leg segment lengths of adult male specimens of *E. endobryonia* sp. nov. Data are provided in μm (N = 1). Abbreviations. Fe: femur; Ti: tibia; Ta1-5: tarsal segments 1-5; P1-3: front, mid and hind legs, respectively.

	Fe	Ti	Ta1	Ta2	Ta3	Ta4	Ta5	LR	BV	SV
P1	737.01	675.68	538.6	300.14	216.45	98.12	113.63	0.79	2.67	1.33
P2	831.89	788.23	427.12	292.56	218.61	110.38	124.81	0.54	2.74	1.72
P3	736.29	736.29	540.4	200.21	180.73	91.99	104.25	0.73	3.48	1.59

sally, well sclerotized. Gonocoxite long, with long and short setae. Tergite IX with two unseparated distinct lobes. Triangular floor under vagina present. Gonapophysis VIII pointed caudally, with two apodeme lobe. Membrane T-shaped. Labia small, bluntly quadrangular, void of microtrichia. Seminal capsule ovoid, darker sclerotized in oral half, without microtrichia. Spermathecal ducts with triangular bulb before separate openings. Cercus normal, length twice as long as width.

Distribution. North America (US: Tennessee, Virginia).

Etymology. The species name is a compound word in which three words from Ancient Greek are combined, *endo-* (ἔνδον), a prefix meaning within, *bryon* (βρύον), meaning moss, and the suffix *-ia* (-ία), forming abstract nouns of feminine gender. It alludes to the biology of this species, which live within the case made of mosses.

Remarks. This species is unique among species of *Eukiefferiella* in that its pupae lack the precorneal seta. This species can also be distinguished from others in the genus by the following combination of traits: pupa lacks respiratory horns, and has the unique configuration of pupal anal macrosetae (two thinner inner macrosetae, a normal outer macroseta); and larva has a mentum with four pairs of lateral teeth and a single, wide, and truncate median tooth. Any geographic variation in this species' characters was detectable between the populations sampled in VA and TN.

DNA barcoding. The results of the species delimitation analyses are summarized in Table 3. First, BLAST search using the partial COI sequence of voucher YI-CR-001 was executed. This resulted in 98.7 % identical to '*Eukiefferiella* sp. voucher BIOUG01648-H02' in GenBank (accession No. KR660601.1) (Telfer et al. 2015); thus, this sequence was included in the following phylogenetic analyses on the assumption that this specimen may belong to *E. endobryonia* sp. nov. (Table 1). Second, the intra- and inter-specific genetic differentiations were estimated using Bayesian inferences, with the dataset for 28 OTUs. Monophyly of each five species of *Eukiefferiella* was recovered in the Bayesian phylogeny (Fig. 3), as well as *E. endobryonia* sp. nov. (95 % BPP) together with the above-mentioned sequence data. A Bayesian tree indicated that *E. endobryonia* sp. nov. was sister to *E. dittmari* among four species of *Eukiefferiella* in the dataset with significantly high BPP support (Fig. 3). Values of P ID(Strict) for *E. endobryonia* sp. nov. moderately supported the prediction that this species is correctly identified based on the COI sequence (Table 3). Likewise, P(RD) value exceeded 0.05 and hence the clade distinctiveness was supported (Table 3). However, Rosenberg's P_{AB} value was not significant ($P = 0.05$) and thus the reciprocal monophyly of the clade of *E. endobryonia* sp. nov. was not supported. Two geographic populations sampled,

Table 3. Summary of the results of species delimitation analysis based on COI. Measures of phylogenetic support and diagnosability of species calculated by species delimitation plug-in in Geneious bioinformatics software are summarized for the species of the genus *Eukiefferiella* included in the dataset, as well as two geographic populations (GRSM and ML) of *E. endobryonia* sp. nov. Monophyly (‘Mono’) was supported for all species/populations. For the other measures, see Materials and methods.

Species	Closest species	Mono	D Intra	D Inter	Intra/Inter	P ID(Strict)	P ID(Liberal)	Av(MRCA)	P(RD)	Rosenberg's P _{AB}
<i>E. ilkleyensis</i>	<i>E. devonica</i>	yes	0.002	0.152	0.01	0.93 (0.80, 1.0)	0.98 (0.88, 1.0)	9.33E-04	0.05	1.20E-04
<i>E. devonica</i>	<i>E. ilkleyensis</i>	yes	0.002	0.152	0.01	0.86 (0.72, 1.0)	0.98 (0.87, 1.0)	9.38E-04	0.05	5.20E-05
<i>E. claripennis</i>	<i>E. devonica</i>	yes	0.024	0.206	0.12	0.91 (0.83, 1.0)	0.97 (0.92, 1.0)	0.0172	0.05	2.50E-08
<i>E. endobryonia</i>	<i>E. ilkleyensis</i>	yes	0.02	0.163	0.12	0.85 (0.73, 0.98)	0.97 (0.86, 1.0)	0.0131	0.1	0.05
<i>E. endobryonia</i> (GRSM)	<i>E. endobryonia</i> (ML)	yes	0.008	0.026	0.3	0.59 (0.41, 0.77)	0.84 (0.69, 0.98)	0.0039	0.05	0.02
<i>E. endobryonia</i> (ML)	<i>E. endobryonia</i> (GRSM)	yes	0.015	0.026	0.57	0.41 (0.23, 0.59)	0.68 (0.53, 0.83)	0.0096	0.11	0.02

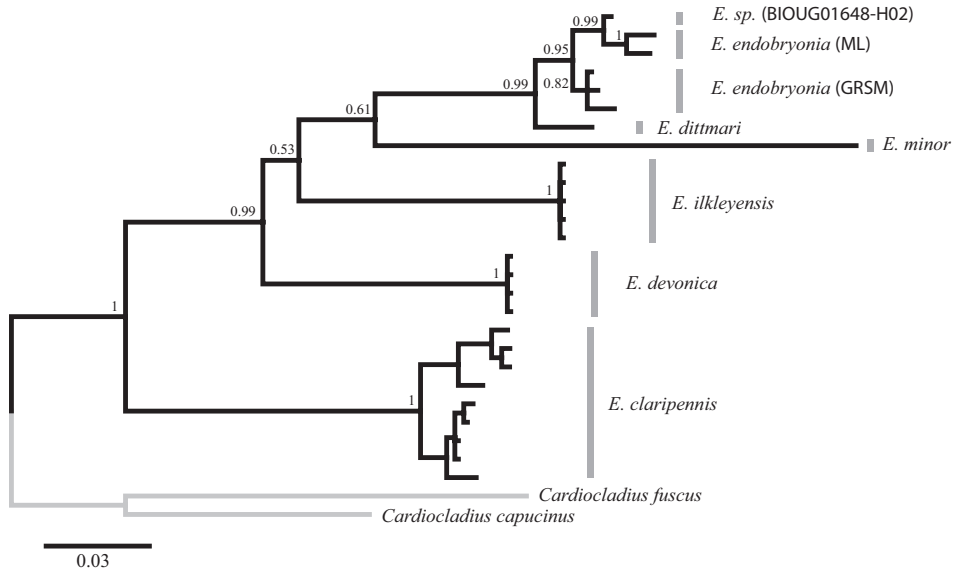


Figure 3. A Bayesian phylogeny based on the COI dataset. Information on the sequences used for this analysis is shown in Table 1. Bayesian Posterior Probabilities were given on each node. Caution is needed to interpret the phylogenetic relationship between and among the members of the species groups (e.g., *devonica* group, consisting of *E. devonica* and *E. ilkleyensis*) included herein, due to the scarcity of available genetic data of the species belonging to the genus *Eukiefferiella*.

Great Smoky Mountains (GRSM) and Mountain Lake (ML), formed separate clades and between which genetic divergence among population was substantial (Intra/Inter = 0.12) (Table 3), of which values were equivalent to those of the species clade of *E. claripennis*, composed by the specimens from Europe and Canada (Table 3).

Habitat. Larvae of this species occupied slightly different microhabitats in Mountain Lake, VA (Fig. 4A) and Sparks Lane, TN (Fig. 4B). At Mountain Lake, they inhabited a shallow inlet brook flowing into the sink water of the lake. Some leafy and thallose liverworts, including *Chiloscyphus* (Jungermanniales: Geocalycaceae), as well as some pleurocarpous moss species, such as *Brachythecium* spp. (Hypnales: Brachytheciaceae), were abundant there on the upper sides of boulders and cobbles that were exposed to spray and occasionally submerged in water. *Fontinalis dalecarlica*, a host plant species of *E. endobryonia* sp. nov., occurred at high densities on the lateral sides of submerged boulders in the stream. As a matter of fact, this seemed to be the only aquatic moss species of which conspicuous amounts were found in this particular stream. I was able to find some white-bodied insect larvae occupying some of the apical shoots of *Fontinalis* moss plants (Fig. 4C) simply by looking in the surface layer of slow-moving, shallow water. Interestingly, these larvae apparently resembled the moss capsules enclosed within the bracts of intact plants at first glance (Fig. 4D). At another locality in TN, the larvae occurred in a stream with fast-flowing water. Some clumps of *F. novae-angliae* were found growing in this rapidly flowing stream, which were

anchored to the sediment of the streambed. The plants bend 50 cm below the water surface in riffle habitats. Similar to observations in the other population, larval and pupal cases occurred at the terminal ends of moss shoots of *F. novae-angliae*.

Life history. The life cycle of this new species between late spring and early autumn (May–October) is unknown. This species is likely multivoltine because fourth-instar larvae and pupae were found together at both sites in both April and November. It appears that the larvae were collector-gatherers at the third instar, but became scrapers at the fourth instar (*sensu* Berg 1995). The third-instar larva restlessly gathered diatoms, which grew on the rims or surfaces of moss leaves. During the later period of the third larval instar, the larva started to dwell on the apical moss shoots and undulated its body among the terminal leaves. *Fontinalis* leaves are slender, with enrolled margins, and are closely appressed at the tip, forming a semi-enclosed space. At the early stage, a larva showed sinusoidal swimming or undulation behavior (Brackenbury 2000) within the terminal leaves, where it would later make its case. Approximately five days after colonizing the terminal moss leaves, it developed into the fourth larval instar stage (Fig. 4E). The fourth-instar larva seemed incapable of living detached from the case due to its limited locomotory habits. When it was removed from its case, the larva attempted to crawl using the anterior half of its body, but was not able to move forward. It spent most of its time feeding on moss leaves. It extended its head and the anterior part of its body outside of the tube to feed, while using its posterior prolegs to maintain contact with a part of its own tube. It grasped the marginal tissues of moss leaves with its mandibles and dragged them back toward its case (Fig. 4F); simultaneously, silk threads produced from the mouth were extruded with the assistance of the serrated claws of the anterior parapods. The partly grazed leaves were therefore pulled toward the case, which made it easier for the larva to access the surrounding leaf area. The larva repeatedly cut out and fed on the leaves in the bore of the plant in proximity to its case; as a result, ca. 12–20 leaves occurring more or less within ca. 13 mm from the base of the tube were completely consumed ($N = 6$). The foraging areas were therefore mainly restricted to the region immediately surrounding the tube. This territorial feeding behavior has been reported for many tube-dwelling chironomids (e.g., Darby 1962; Jónasson and Kristiansen 1967; Edgar and Meadows 1969; McLachlan 1977; McAuliffe 1984; Leuchs and Neumann 1990). The larva occasionally defecated, and subsequently immediately ejected the fecal pellet from the end of the tube, which is similar to the behavior of *Cladotanytarsus atridorsum* (K.) Edw. (Mackey 1976). The larval fecal pellets were long, ca. half of the body length of the larva, and were loose and cylindrical, which allowed them to easily be released into the water. Under laboratory conditions, younger larvae often used the particles originating from the fecal pellets of mature larvae as tube materials (Fig. 4G), which means these fecal pellets may also be a source of tube material for younger larvae (Hirabayashi and Wotton 1999). Judging from the composition of fragments in the fecal pellets, at this stage, the larva largely relied on moss leaves as a food source, which is supplemented with fine amorphous detritus and epiphytic diatoms. Under laboratory conditions, the larva only occasionally withdrew into the tube and engaged in lateral undulations of the body therein.

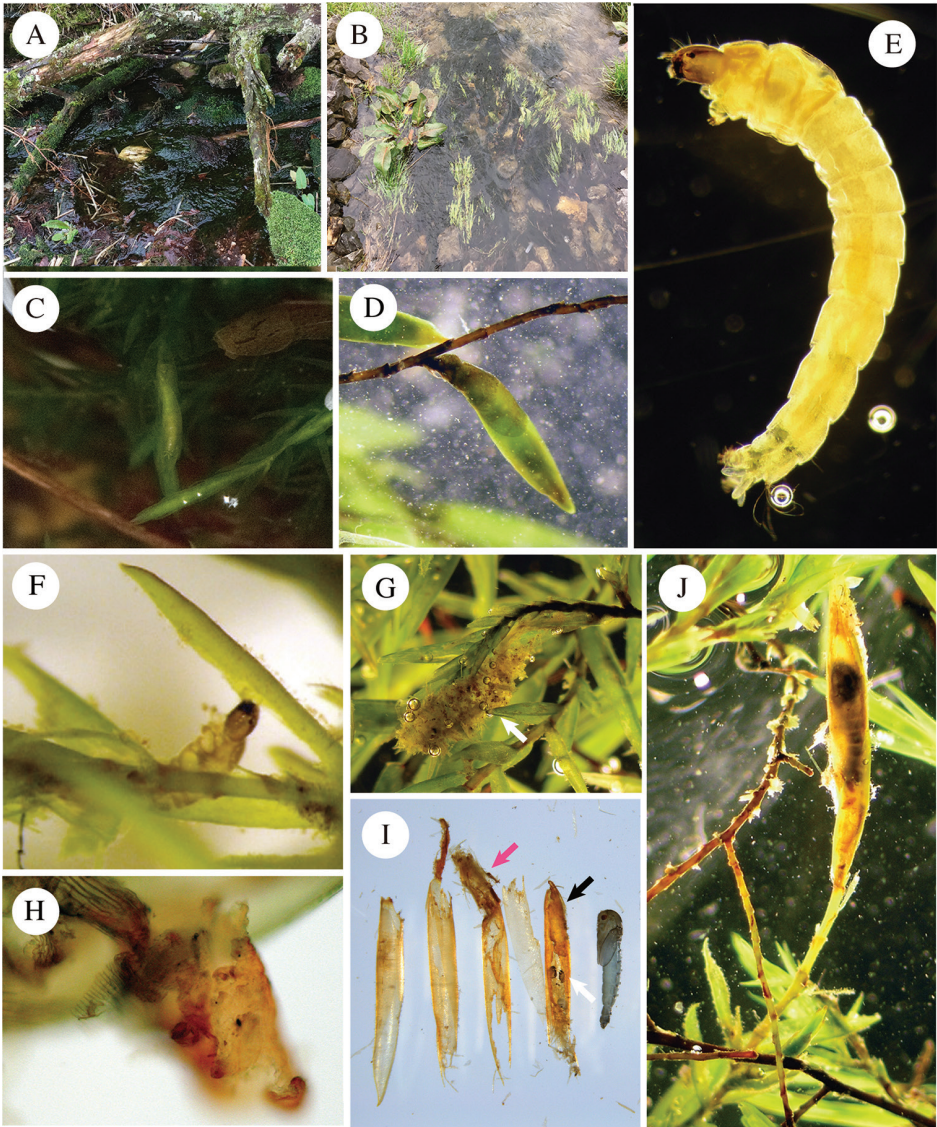


Figure 4. Biology of *Eukiefferiella endobryonia* sp. nov. **A** a colony of *Fontinalis dalecarlica* growing on the sides of pebbles in a gently flowing inlet connected to Mountain Lake, VA, USA (type locality) **B** a colony of *Fontinalis novae-angliae* occurring in a rapidly flowing stream at Sparks Lane, TN, USA **C** early fourth-instar larva, undulating its body in the tube **D** immature capsule of *Fontinalis dalecarlica* attached to the stem underwater **E** fourth-instar larva **F** fourth-instar larva feeding on a leaf margin of *F. dalecarlica* **G** a tube structure of the third-instar larva, which was mainly built from particles from the feces of mature larvae **H** amorphous, jelly-like silk mass spotted with detritus and diatoms, ripped off of the inner wall of the inner end of the pupal case **I** a dissected leaf-rolling case, consisting of five leaves and the resident pupa; the leaves used as the case materials are placed in the order of leaf arrangement, with the outermost leaf at the left-most; the innermost leaf (right next to the pupa) contains the head capsule (white arrow) and exuvium of the fourth-instar larva; some debris (pink arrow) and silk mass (black arrow) stuffed in at both ends can be seen **J** a pupa in its case: the pupal head is oriented toward the distal end of the shoot tip; most of the leaves near the pupal case were consumed by the larva early in the fourth instar stage.

The larva became less active in the later period of the fourth instar. It scratched the inner surfaces of the leaf margins, not for consuming the leaves, but presumably for strengthening the case wall. As a result of this intensive fabrication behavior, the tissues of the leaves comprising the case became light brown to red in color due to reactions in the plant tissues, whereas undamaged leaves and stems remained green. Approximately half of the larva's time was spent spinning silk at this point, and the other half was spent staying still. The spinning behavior was stereotyped, regular, and persisted for more than 5 h at a time. The larva lined the interior of the case with silk, which provided a surface with which the claws of the anal prolegs could engage, anchoring the insect within the case. Due to the fabrication and feeding behavior performed in the earlier stages, there were some apertures in the rolled leaf case on the stem-end side. The larva frequently turned around inside the case to strengthen the case's inside wall. The innermost leaves in the wall, especially at both ends, thus included a thick layer of silk (Fig. 4H) as a consequence of continuous silk fabrication. Before entering the prepupal stage, debris containing various particles (fecal pellets, diatoms, and strips of moss leaves) was squashed and accumulated at both ends of the case together with a silk mass to seal the end of the tube (Fig. 4I). At the prepupa stage or later, the case consisted of five or six moss leaves, which were firmly enclosed in silk. The larva molted inside the case and casted off the cuticle; the head capsule and exuvium were thus packed into the posterior end of the pupal case (Fig. 4I). Pupation occurred with the head oriented toward the distal end of the case, without exception ($N = 7$) (Fig. 4J). The pupa rested inside the case throughout most of its development. The pupa then swam toward the surface water and emerged as an adult when its development was completed.

Discussion

Taxonomic placement of *E. endobryonia* sp. nov.

Eukiefferiella represents a large and widespread genus of Orthoclaadiinae (Moubayed-Breil and Ashe 2013). This genus was erected by Thienemann (1926), and the independence of this genus from others, as well as the validity of its type species, has been debated in many studies (Edwards 1929; Thienemann 1936; Zavřel 1939; Brundin 1956; Lehmann 1972; Sublette and Wirth 1980). This genus is closely related to some orthoclaidine genera in the *Cardiocladius* group (Sæther and Halvorsen 1981): *Cardiocladius* Kieff., *Tokunagaia* Sæth., *Tvetenia* Kieff., 1922 (Sæther and Halvorsen 1981), *Nanocladius* Kieff. (Sæther 1977; Lehmann 1972), and *Hydrobaenus* Fries. Keys for European species of this group are available for larvae (Thienemann 1936; Zavřel 1939; Chernovskii 1949; Bode 1983), pupae (Thienemann 1936; Lehmann 1972), adults (Brundin 1956; Lehmann 1972), and all these stages combined (Sæther and Halvorsen 1981).

The study of the taxonomy of *Eukiefferiella* is far from complete. Most existing records from the Nearctic are at the genus, or at most the species-group, level (Epler 2001). Ten species have been recorded from North America (Sæther 1969; Sublette

1970; Simpson and Bode 1980; Oliver et al. 1990; Bode et al. 1996; Patrick 1996; Sandlund and Aagaard 2004; Egan and Ferrington 2015): *E. brevinervis* (Malloch), *E. brevicealcar* (Kieff.), *E. claripennis*, *E. coerulea* (Kieff.), *E. cyanea* Thienemann, *E. devonica*, *E. graeci* (Edw.), *E. minor*, *E. unicalcar* (Sæth.), and *E. pseudomontana* Goetghebuer. However, there is also evidently a large number of undescribed species (Cranston et al. 1983; Bode 1983; Merritt and Cummins 1996).

Eukiefferiella endobryonia sp. nov. was assigned to *Eukiefferiella* on the basis of diagnostic characters proposed by previous studies (Zavřel 1939; Lehmann 1972; Sæther and Halvorsen 1981; Cranston et al. 1983; Bode 1983; Sasa and Kikuchi 1995). Lehmann (1972) suggested that the possession of orally curved hooklets in a row located posteriorly on T III–V is a diagnostic character of the pupae of this genus, although he concluded that there is no single character by which this genus can be defined. Sæther and Halvorsen (1981) emended the definition of *Eukiefferiella* by comparing it to the other genera of the *Cardiocladius* group, and distinguished it based on the following features: lack of a male anal point; presence of a hind tibial comb; female gonapophysis VIII undivided; pupa lacking frontal setae, pearl rows, and a median anal seta; and larvae having a simple S I, seta interna, chaetae, and spinulae. Cranston et al. (1983) noted that *Eukiefferiella* is distinguished from *Tvetenia* based on a combination of the following larval characters: procercus less than 1.5 times as long as wide; abdominal setae shorter than 1/2 the length of an abdominal segment; and a simple S I.

Eukiefferiella endobryonia sp. nov. is unique in its lack of a precorneal seta, as most of its congeners have three precorneal setae present in a row or triangle (Lehmann 1972; Coffman et al. 1986); also, the configuration of pupal anal macrosetae (inner two macrosetae reduced, and outer one macroseta regular) is apparently unique for the species of *Eukiefferiella*. The affinity of this new species to others in this genus can thus be problematic. For example, *E. endobryonia* sp. nov. does not key out into any of the species groups proposed by previous authors (Brundin 1956; Lehmann 1972; Cranston et al. 1983; Bode 1983; Coffman et al. 1986). When compared to the species in Japan, this species may have affinity to *chuzeoctava* group defined in Sasa and Kikuchi (1995), although squama has only a few setae in this species (as opposed to squama fringed in the species of *chuzeoctava* group). Some morphological and behavioral traits of *E. endobryonia* sp. nov. show affinity to those of *E. ilkeleyensis* in the *E. devonica* group, specifically: the larval mentum medially truncate with four lateral teeth; and the larva of *E. ilkeleyensis* is reported to make a case at the shoot tips of the moss *Eurhynchium riparoides* (Hedw.) (Brennan and McLachlan 1980). *E. ilkeleyensis* has been recorded from Palaearctic sites (Chernovskii 1949; Brennan and McLachlan 1979; Moller Pillot 2013; Erbaeva and Safronov 2016) but is not known from Nearctic sites. Hence, it is hypothesized that *E. endobryonia* sp. nov. can be included in the *devonica* group; importantly, however, this species morphologically contradicts with the distinguishing characters of the *devonica* group: the pupa of this species does not possess either median anteprenotal setae or the row of hooks at the posterior edge of sternite VII (Lehmann 1972); deeply branched seta interna of the larva extending nearly to the base, although shallowly branched seta interna is said to be characteristic of the *E.*

devonica group (Epler 2001); further, the larval thoracic setae of the new species are less than one-third as long as the segment from which they arise, as opposed to being more than half as long as the corresponding segment in *E. ilkleyensis* (Chernovskii 1949).

The molecular phylogeny (Fig. 3) clarified that *E. endobryonia* sp. nov. is genetically well-differentiated from *E. ilkleyensis*. This species is suggested to be relatively closely related to *E. dittmari*, which has not been assigned to any species group (Lehmann 1972) but may be incorporated in *devonica* group (Moller Pillot 2013). *E. endobryonia* sp. nov. is morphologically distinguished from *E. dittmari* based on the diagnostic character (i.e., the absence of precorneal setae and unequal length of pupal macrosetae in pupa). The immature stages of *E. dittmari* is not described, but the larvae live in mosses (Moller Pillot 2013). Based on the species delimitation analyses, the species clade of *E. endobryonia* sp. nov. was estimated to be distinctive and a significant level of genetic differentiation is detected between the geographic populations sampled (ML and GRSM). However, Rosenberg's P_{AB} for each species used herein suggests that the null hypothesis that monophyly of each taxonomic group occurs due to the random coalescent process is not rejected. To clarify the phylogenetic status of the species and to trace the evolutionary history of biology of this group, especially the specialized relationship with mosses, it is necessary to elucidate the biology for the species of which immature stages are unknown and to accumulate morphological and genetic data of the related groups.

Biology of *Eukiefferiella*

Eukiefferiella species are generally abundant in the riffles of rivers and streams (Madder et al. 1977; Brennan and McLachlan 1980; Egan et al. 2014), as well as in the littoral zones of lakes (Thienemann 1936). The species of this genus show a broad spectrum of life histories. Brennan and McLachlan (1980) suggested that the larval feeding or tube-dwelling behaviors of species in this group may be affected by their habitats (i.e., riffle zones), as these are locations where the supplies of particles and sediments for tube construction are not always abundant.

Several species of *Eukiefferiella* are reported to be ectosymbionts of various freshwater invertebrates, including acting as parasites and commensals (Tokeshi 1995), although in many cases these ectosymbionts are not identified at the species level, or may not even have been described yet. An unidentified species in western North America makes silken tubes within the cases of the caddisfly *Brachycentrus occidentalis* Banks (Trichoptera: Brachycentridae), which may result in a higher mortality rate of the host (Gallepp 1974). *E. aneyla* Svensson and *E. brulini* Moubayed-Breil & Ashe are commensal on limpets of the genus *Ancylus* Müller (Gastropoda: Ancyliidae) (Svensson 1986; Moubayed-Breil and Ashe 2013; Schiffels 2014; Fig. 4E). This genus also includes some species that are commensal on naucorid beetles (Roback 1977; Fig. 4H) or mayflies (Winterbourn 2004). Some species use rocks as substrates, including: *E. verrallii* (Edw.) and *E. calvescens* (Edw.), which construct loose tubes incorporating rocky particles (Brennan and McLachlan 1980); and *E. clypeata* Thienemann, which inhabits

the exposed regions of rocks swept by water currents and builds solid sheets of silk (Brennan and McLachlan 1980) or cobweb-like tubes (Thienemann 1936; Chernovskii 1949) on the surfaces of stones, similarly to the filter net of hydrosychid caddisflies. Cocoon-making is only known in one species, *E. claripennis* (Madder et al. 1977).

The larvae of *Eukiefferiella* are frequently found among aquatic mosses, including *Fontinalis* spp. (Zavřel 1939; Bode 1983; Suren 1993; Pinder 1995; Callisto et al. 2007). Interestingly, the fourth-instar larvae and pupae of *E. ilkleyensis* build tubes among the terminal leaves of the long-beaked water feathermoss *Eurhynchium riparoides* (Hedw.) (Hypnales: Brachytheciaceae) (Brennan and McLachlan 1980). *E. ilkleyensis* previously showed a preference for *Fontinalis* moss leaves as case materials (Brennan and McLachlan 1979). Feeding on living and decomposing leaves has been reported for some species (e.g., Callisto et al. 2007). For example, *E. ilkleyensis* is facultatively phytophagous on *Ranunculus calcareus* Butcher (Ranunculaceae) in its later larval instars (Berg 1995; Pinder 1995). The moss-feeding and leaf-rolling behaviors of *E. endobryonia* sp. nov. are apparently related to this species' biological background. Some morphological traits of this species may be associated with their biology, including the secondary loss of the respiratory horns in the pupa. Respiratory horns represent a major morphological adaptation to oxygen-poor environments (Marziali et al. 2006; Marziali and Rossaro 2006), and tubicolous species often have variously shaped respiratory organs that lack a plastron plate (Cranston 1995). Therefore, the pupa of *E. endobryonia* sp. nov. lacks these additional respiratory adaptations and is dependent on cuticular respiration, although it should be noted that the tubicolous habit itself can be advantageous to respiration (Milne 1938; Walshe 1950; Williams et al. 1987; Kon and Hidaka 1983; Stief et al. 2005).

Examining the diversity of chironomid tube morphotypes

Building diverse, elaborate tubes is a characteristic behavior of the larvae of Chironomidae. Tube morphology is determined by construction behaviors, which are stereotypical for specific lineages (Chaloner and Wotton 1996; Charbonneau and Hare 1998). Tube construction behavior can therefore be seen as an example of these species' "extended phenotype" (Dawkins 1982). Importantly, spinning in many chironomids is tightly linked with their feeding activity (Wallace and Merritt 1980; Dudgeon 1994; McLachlan and Ladle 2009). In addition, the case morphology of a species is determined by its habitat and the space, substrata, and particle sources available therein (McLachlan and Cantrell 1976; McLachlan 1977; McLachlan et al. 1978; Brennan and McLachlan 1979; Pinder 1986). However, the features of tubes that result from taxon-specific construction behaviors have often been studied with more emphasis on their food types and functional feeding group (Pinder 1986; Berg 1995; Ferrington et al. 2009). Virtually no previous study has ever given a comprehensive account of the diversity of chironomid tube morphology. Herein, I provide a provisional classification of chironomid tube structures based on their morphotypes (see below), with a sum-

mary of species' biology and some examples. This enables us to give a brief overview of the biology of this group from the perspective of their construction behaviors to deepen our understanding of their ecology and evolution.

Chironomid tube structures can be categorized broadly based on their transportability and the substratum to which the tube is attached. First, tubes can be divided into those with fixed shelters (i.e., the larva cannot move around with the tube) and transportable cases (i.e., the larva freely moves while carrying the tube). Fixed shelters are much more common than transportable cases. Second, fixed shelters can be categorized into three groups (see below) based on the substratum to which the tube is attached. In fact, chironomids as a whole are able to colonize a broad spectrum of substrates. Third, these tube morphotypes can be further subdivided based on the materials out of which the tube is made, but only if the larva has a preference for certain types of particles (e.g., Brennan and McLachlan 1979; Xue and Ali 1997), and most tend to show low specificity for particular tube materials (Brennan and McLachlan 1979).

Tube structure is proximately influenced by the spinning mechanisms of larvae (Webb et al. 1981; Leuchs and Neumann 1990). The silk formation processes differ within the subfamily Chironominae (Webb et al. 1981; Tönjes 1989; Leuchs and Neumann 1990; Ólafsson 1992). Differences in silk properties exist between two subfamilies (i.e., Orthocladiinae and Chironominae) due to the influence of the striated ventromental plates, which are only present in the Chironominae (Churney 1940; Patrick 1966; Webb et al. 1981; Kullberg 1988). Orthocladiine larvae form separate threads of a soft, jelly-like substance, with which they spin loosely woven tubes that are normally attached to hard substrates (Webb et al. 1981). On the contrary, Chironominae manufacture a harder silk that comprises an amorphous liquid secretion while passing the ventromental plates and then becomes a sticky mass in water (Webb et al. 1981). They normally spin compact tubes, in which sediment particles are embedded in a silk matrix (Webb et al. 1981). In addition, some species can produce silk threads with different diameters (Walshe 1951; Leuchs and Neumann 1990), which may be achieved by shearing forces exerted by the ventromental plates or their tensile powers (Leuchs and Neumann 1990).

Chironomid tubes can most commonly be found bound to rocky materials in virtually all types of streams or lake ecosystems. These represent the first major morphotype of tubes: (1) *rock material-bound tubes*. Rock materials with various grain sizes are used to make tubes, ranging from very coarse (e.g., pebbles, cobbles) to fine (e.g., sand, silt, clay, mud). This type can therefore be subdivided by the grain size of particles used. Soil-dwelling chironomids often occur in lentic or lake environments, where they make soft, cryptic tubes with various lengths and forms, from short, cylindrical tubes to meandering, non-blindly ending tubes, in mud. The shapes of the silk-laden burrows or tubes of species dwelling in soft sediments are associated with their feeding strategies, as these are often deposit- and filter-feeding animals (McLachlan and Cantrell 1976; Leuchs and Neumann 1990; Stief et al. 2005). For example, a mud-dwelling species, *Chironomus plumosus* (Linn.) (Chironominae: Chironomini) (Fig. 5A), makes tubes with a variety of forms, including U- or J-shaped tubes, or

horizontal tubes (e.g., McLachlan and Cantrell 1976; McLachlan 1977; LeSage and Harrison 1980; Hodkinson and Williams 1980).

It is noteworthy that three different spinning behaviors are known for species belonging to the genus *Chironomus*, which correspond to the different feeding strategies used by these species. The first method is found in the filter-feeding *C. plumosus*: the larva spins a funnel-shaped net across the lumen of the tube for filtering fine organic particles out of the water (Walshe 1947, 1951). The second method is another filter-feeding strategy, but is more common than the first type: the larva spins a thin layer of silk on the tube wall and then grazes on the food particles that attach to it inside the tube, together with the old net; the tube walls are thus renewed by the larva spinning new bundles of silk threads (Leuchs and Neumann 1990). The third method is deposit-feeding: the larva builds a cylindrical tube and reaches out of their tube to scrape up food particles from the surrounding sediments (Leuchs and Neumann 1990; Stief et al. 2005).

Some species of this type exhibit an aberrant, more elaborate construction behavior than the others. For example, larvae of *Orthocladius* (*Euorthocladius*) *rivulorum* Kieff. (Fig. 5B) make a suspended tube attached by one end to a riffle rock and feed on diatoms growing on the tube (Hershey et al. 1988; Taylor 1903; Peterson et al. 1993). They convert this tube into a pupal tube by binding the basal part with silk to form a long anchoring strand (Taylor 1903), which is similar to those structures made by limnocentropodid caddisflies (Wiggins 2005). The tubes of *O. (E.) rivulorum* are coated with diatoms, including *Hannaea* Patrick and Reimer (Fragilariaceae), and exert a considerable effect on the periphytic diatom flora (Pringle 1985; Hershey et al. 1988; Herren et al. 2017). Some Tanytarsini (Chironominae) make rigid tubes, such as *Rheotanytarsus* Thienemann & Baus and *Lithotanytarsus* Thienemann. *Rheotanytarsus* (Fig. 5C), which make tubes with arms on which the larvae spin silk strands attached to various substrates (Thienemann 1954; Scott 1967; McLachlan 1977; Kullberg 1988; Spies et al. 2009). *Lithotanytarsus* spp. and *Rheotanytarsus reissi* (Fig. 5D) spin serpentine tubes on the surfaces of travertine rocks, which become encrusted by carbonate mineral deposits, creating tufa over time (Thienemann 1934, 1936, 1954; Pinder 1995; Burmeister and Reiss 2003). Importantly, some ichnofossils have been interpreted as burrowing tubes from lentic environments (Chamberlain 1975; Rodríguez-Aranda and Calvo 1998; Sanz-Montero et al. 2013), with the oldest example of meandering tubes found in Triassic lake deposits (Voigt and Hoppe 2010). Calcareous columnar tufas resembling the biogenic constructions of *Lithotanytarsus* spp. have also been recorded in deposits from the Lower Cretaceous or later (Edwards 1936; Pentecost 2005; Brasier et al. 2011).

Chironomids can also colonize a wide range of organisms, which include motile (animals) or sessile (e.g., plants, cyanobacteria) organisms. Information on the tubes formed by symbiotic chironomids is often limited, and their construction behaviors were not described in many previous studies. The second major group of tubes is: (2) *tubes on symbiotic animals*. Various chironomid lineages (Buchonominae, Podonominae, Chironominae, and Orthocladiinae) exhibit a wide range of symbiotic associations with aquatic animals, ranging from ectoparasitism to phoresis and commensalism, which can be either obligate or facultative associations (Steffan 1968; Tokeshi 1993, 1995;

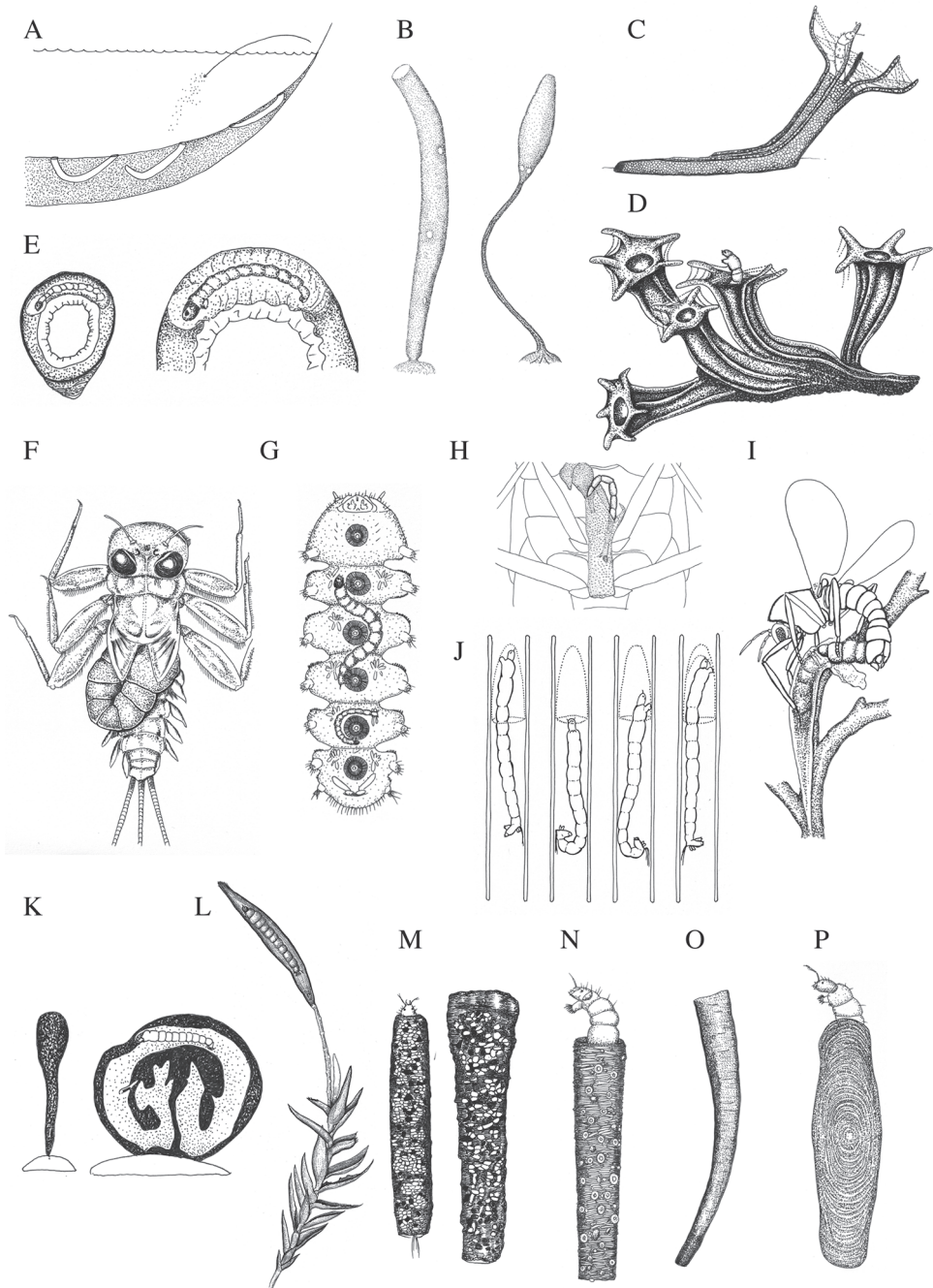


Figure 5. Summary of the biology of chironomids, with special focus on their tube structures. Some taxa without apparent tube structures (**F**, **G**) are included to give some accounts of their biology. Rock material-bound type (**A–D**): **A** three types of tubes, U-shaped (left), J-shaped (middle), and open-ended (right), built by the mud-dwelling species *Chironomus plumosus*, redrawn from McLachlan and Cantrell (1976) **B** larval (left) and pupal (right) tubes of *Orthocladius (Euorthocladius) rivulorum*, redrawn from Taylor (1903).

Schiffels 2014). Their hosts are mainly aquatic invertebrates, including snails (Prat et al. 2004; Fig. 5E), caddisflies (Ashe et al. 2015), mayflies (Claassen 1922; Tonnoir 1922; Codreanu 1939; Thienemann 1954; Jacobsen 1995; Fig. 5F), net-winged midges (Cranston 2007; Fig. 5G), naucorid beetles (Fig. 5H), sponges (da Silva Fernandes et al. 2019), and bryozoans (Moller Pillot 2009; Schiffels 2014). Host selectivity and the site of attachment to the host's body are taxon-specific, and are likely determined by the behavioral patterns of the hosts and the ecological requirements of the chironomids, such as their feeding guilds (Tokeshi 1993). Some species that are tentatively categorized in this group herein presumably do not produce silk (e.g., *Symbiocladius*, Fig. 5F; *Tonnoirocladius*, Fig. 5G). The level of dependency on tube construction seems to differ greatly among taxa. For example, species of *Symbiocladius* (Orthoclaadiinae) (Fig. 5F) are obligate parasites of mayflies, and their larvae drill under the wing buds of the host and feeds on the host's hemolymph. *Symbiocladius* spp. are not known to dwell in tubes, but may use other types of attachment mechanisms (Schiffels 2014). Some symbiotic chironomids, particularly facultative symbionts or those that are commensal to the host, tend to make tubes by weaving particles accumulated or flowing in the water on the body surface or shelters of hosts (Schiffels 2014). For example, Winterbourn (2004) gave an account of the tubes of an *Eukiefferiella* sp. in New Zealand, a commensal midge with a mayfly host, throughout its immature stages, and reported that it used silk thread to attach to the host; however, at the third and fourth larval instars, it built an open-ended silken tube above the mayfly's gills that prevented them from beating, and the wall of this tube incorporated sand grains and fine organic particles.

Similar to the above, the third major category of tubes is: (3) *tubes on/within plants, algae, cyanobacteria*. Some chironomids in the Orthoclaadiinae and Chironominae often facultatively make tubes on the external surfaces of plants or algae (McGaha 1952;

Figure 5. Continued. **C** *Rheotanytarsus rivulorum*, redrawn from Walshe (1951) **D** *Rheotanytarsus reissi*, encrusted to form tufa, redrawn from Burmeister and Reiss (2003). Tubes on symbiotic animals (**E–H**): **E** *Eukiefferiella brulini* on *Ancylus fluviatilis*, redrawn from Moubayed-Breil and Ashe (2013) **F** *Symbiocladius* sp., an obligate parasite of the heptageniid mayfly *Heptagenia lateralis*, redrawn from Codreanu (1939) **G** *Tonnoirocladius commensalis* (Tonnoir), which is commensal to the larvae of the net-winged midge (Blephariceridae), redrawn from Tonnoir (1922) **H** silk tube of a Neotropical species of *Eukiefferiella* phoretically attached to *Cryphocricos peruvianus* De Carlo (Naucoridae), in which the open-ended tube is made between the meso- and meta-thoracic coxae of the host, redrawn from Roback (1977). Tubes on/within plants, algae, cyanobacteria (**I–L**): **I** silk tube made among algae by the marine species *Clunio takahashii*, shown in copula, redrawn from Hashimoto (1976) **J** a cone-shaped net constructed in the leaf mine of *Endochironomus*, redrawn from Walshe (1951) **K** a tunnel made by *Cricotopus nostocicola* in a spherical colony of cyanobacteria, *Nostoc parmelioides*, redrawn from Brock (1960) **L** a case formed by leaf-rolling on the shoots of *Fontinalis* mosses by *Eukiefferiella endobryonia* sp. nov., based on this study. Portable cases (**M–P**): **M** a solid tube composed of fine detritus constructed by *Stempellinella minor*, redrawn from Brundin (1948) **N** a tubular, firmly constructed case armored with diatoms and rhizopod shells constructed by *Zavrelia pentatoma*, redrawn from Lauterborn (1905) **O** a slightly curved, conifer needle case of *Micropsectra pharetrophora*, redrawn from Fittkau and Reiss (1998) **P** a laterally flattened purse case with concentric growth strips constructed by *Lauterborniella agrayloides*, redrawn from Lauterborn (1905).

Berg 1995). For example, species of *Telmatogon* Schiner (Telmatogetoninae) inhabit intertidal zones and make their tubes within the green algae occurring on rocks (Cranston 2010). *Clunio* spp. (Orthocladiinae) are marine and make irregular silk tubes dotted with debris on seaweeds, and feed on encrusting diatoms and organic matter (Hashimoto 1976; Archer-Thomson and Cremona 2019). The female adult of *Clunio takahashii* (Fig. 5I) has a degenerated body and copulates with the male without fully extending her body out of her tube, and subsequently lays eggs in the tube (Hashimoto 1976). Many species in Chironominae and Orthocladiinae live inside plant tissues (Berg 1995), mainly as miners, but also rarely as gall-inducers (Jäger-Zürn et al. 2013). Some leaf-mining genera (*Glyptotendipes* Kieff., *Endochironomus* Kieff., and *Polypedilum* (*Pentapedilum*) Kieff.) are filter-feeders and spin a conical net inside the mine to filter out floating particles (Fig. 5J), similarly to *Chironomus plumosus* (Walshe 1951). Species of *Stenochironomus* Kieff. feed either on living or sequestered plant leaves and tend to show weak preferences for host plants (Kato 2015). *Cricotopus nostocicola* Wirth (Orthocladiinae) (Fig. 5K) has a symbiotic relationship with colonial cyanobacteria in the genus *Nostoc*. The larva makes a tube by feeding on globular colonies of *Nostoc*. The feeding activity of the larva exposes the host to more sunlight, and consequently facilitates photosynthesis by the host (Brock 1960; Dodds and Marra 1989). For many miners (internal-feeders) of plants or algae, silk production has not been confirmed or described. This may be because they are more dependent on their substrates than other taxa. The tube of *E. endobryonia* sp. nov. (Fig. 5L), as described herein, exemplifies one of the few examples of a chironomid that make a tube exclusively using plant materials with silk. This study showed that the larva of this species dwells among the terminal leaves of moss plants, and draws their body out from the tube to consume all of the leaves occurring within a distance reachable by the larva; this species also forms a pupal case consisting solely of the apical five or six leaves of the moss, which are firmly attached together and internally lined by silk. It is interesting that a congener, *E. ilkleyensis*, has been reported to make a tube at the shoot terminus of mosses, yet without using silk for construction (Brennan and McLachlan 1979).

The fourth major group of tubes is: (4) *portable cases*. Among chironomids, transportable cases are far less common than fixed shelters. This is in sharp contrast to the myriad examples of portable cases seen among the Trichoptera (Wiggins 2005). Transportable cases have been found to be made by members of the Chironominae, mainly those in the tribe Tanytarsini, and less commonly in the tribe Chironomini (Walshe 1951; Reiss 1984; Ferrington 1995; Cranston 2010; Stur et al. 2005). *Heterotanytarsus apicalis* (Kieff.) is the only species so far known to make portable cases among the species in the Orthocladiinae (Moog 1995; Moller Pillot 2013). Many genera of the subtribe Zavreliina in the tribe Tanytarsini, such as *Stempellina* Thienemann & Bause, *Stempellinella* Brundin (Fig. 5M), and *Zavrelia* Kieff. (Fig. 4N), live in cool mountain streams and construct heavy cases with hard shells composed of sand or silt (Lauterborn 1905; Hudson et al. 1990; Stur et al. 2005; Lenz 1924; Ekrem and Stur 2009; Namayandeh et al. 2016). Lauterborn (1905) described a cylindrical case made by *Zavrelia pentatoma* Kieffer & Bause (Fig. 5N), the surface of which was armored

with diatoms and rhizopod shells; this species can also build cases exclusively out of rocks (Thienemann 1954). In the subtribe Tanytarsina (Tanytarsini), *Calopsectra* Kieff. and *Micropsectra pharetrophora* Fittkau & Reiss (Chironominae: Tanytarsini) (Fig. 5O) build 'conifer-needle' shaped case with spirally arranged sand grains, silt, and long pieces of detritus, especially diatom frustules (Fittkau and Reiss 1998). A few genera of Chironomini, such as *Lauterborniella* Thienemann & Bause and *Zavreliella* Kieff., also make motile cases with sand or silt particles (Thienemann 1954; Moller Pillot 2009). *Lauterborniella agrayloides* (Kieff.) (Fig. 5P) makes a soft purse- or spindle-shaped case that is slightly widened in the middle, strongly pressed together laterally, and rounded at both ends; it also contains characteristic concentric growth strips composed of numerous materials. This case resembles those of hydroptilid caddisflies (Lauterborn 1905). The construction behaviors and preferences for case materials of chironomids that make motile cases have largely not been investigated.

As seen above, many chironomid tubes are comparable to those of caddisflies. Not only are the forms of portable cases made by caddisflies and chironomids similar, but there are also notable shared characters of the pupal cases, known as 'silken closures' in caddisflies, between these groups (Wiggins 2005). Diverse forms of silken closures are known among many species of caddisflies in the suborder Integripalpia (Wiggins 2005). These silken closures are disc-shaped masses of silk that are used for closing an open end of the case, and are spun by the final-instar larva before entering pupation. Analogous structures, called 'Verschlußdeckel (closure cap)' or 'Vorderdeckel (front cover)' by Thienemann (1954), were described in some chironomids by previous studies (Lauterborn 1905; Lenz 1924). This represents a remarkable example of convergent evolution in portable case-makers in two distantly related insect clades (chironomids and caddisflies).

Tube morphotypes can be a tool for use in research in the taxonomy, ecology, and evolution of tube-building animals. The morphotypes of tubes should be assessed if they are useful for taxonomy. The tube, as a functional trait of a specific taxonomic group, may also be useful for examining different species' ecological niches within a community (McGill et al. 2006). From an ecological perspective, tube functions have been and will continue to be an important subject at various scales, ranging from their adaptive significance for tube-dwelling individuals or species (e.g., Walde and Davies 1984; Williams et al. 1987) to their ecological impacts at the community or ecosystem scales (e.g., Ólafsson and Paterson 2004).

Herein, I made the simplistic assumption that the evolution of case-construction behavior in chironomids could be estimated based on the results of a previous molecular phylogenetic study (Cranston et al. 2012), although the phylogeny of this group has historically been contentious. Buchonominae was recovered as the most plesiomorphic subfamily (Murray and Ashe 1985; Cranston et al. 2012), although this contradicted some previous studies' conclusions (e.g., Sæther 2000). Importantly, *Buchonomyia* spp. (Buchonominae) are ectoparasites of caddisflies, and supposedly do not manipulate silk (Ashe et al. 2015). The early diverging subfamilies identified, including Podonominae and Tanypodinae, are free-living (Moller Pillot 2013). Among extant lineages, Telmatogetoninae is likely to be one of the earliest-diverging clades of case-builders (Cranston

et al. 2012). Tube diversity and construction behavior have evidently diversified in two more derived subfamilies, Chironominae and Orthoclaadiinae, which split during the mid-Jurassic. Interestingly, the spinning mechanism and silk properties are apparently different among the three clades of tube-making chironomids (Leuchs and Neumann 1990). The different ecological settings and evolutionary backgrounds of these groups have led to them developing a wide range of construction behaviors that have not yet been addressed within a geochronological framework. The emergence of two currently dominant and diverse clades of decomposers (i.e., caddisflies and chironomids) is estimated to have occurred in the Early Triassic (Cranston et al. 2012; Malm et al. 2013). Further, it has been suggested that the major clades of case-builders in both clades appeared contemporaneously during or after the Late Jurassic (Malm et al. 2013). The behavioral convergence of these tube-making insects may have been associated with major changes in freshwater ecosystems that occurred through geological time, such as increased allochthonous inputs of coarse plant materials and fine detritus into these systems (Lancaster and Downes 2013). It would be interesting to examine how these clades of ecosystem engineers have contributed to, or been affected by, the drastic changes in the trophic structures of stream and lake ecosystems that occurred in the Mesozoic Era (Lancaster and Downes 2013; Minter et al. 2016; Buatois et al. 2016; Savrda 2019).

The tube morphology of chironomids does indeed show significant diversity. It is likely that lability in silk production, particle and substrate selection, and construction behavior has made it possible for chironomids to use a broad array of ecological niches, as is the case for the Trichoptera (Weaver and Morse 1986; Wiggins and Mackay 1978; Mackay and Wiggins 1979; Wiggins 2005; Morse et al. 2019). Future investigations of the evolution and diversification of construction behaviors in chironomids in relation to changes in available habitat types will be important to improve our understanding of the evolution of aquatic ecosystems.

Acknowledgements

I sincerely wish to thank Dr. Conrad C. Labandeira for his thoughtful support throughout my tenure at the National Museum of Natural History, for providing the laboratory supplies and equipment used, and for assisting with field sampling. I also gratefully acknowledge the assistance of the interlibrary loan staff of Ehime University Library for collecting literature. Thanks are also due to Dr. Michael Donovan for helping take care of the insects in the laboratory; Dr. Hiroki Hata for initial assistance of using the software packages for analysis of genetic data; Drs. Koichiro Kawai and Athol McLachlan for providing valuable information on chironomid taxonomy and tube structures, respectively; Drs. Charles Watson, Athol McLachlan, and Fabio Laurindo da Silva for reviewing the manuscript and for providing constructive comments; the staff at Mountain Lake Biological Station and of the National Park Service at Great Smoky Mountains National Park for allowing sampling in these areas. I would also like to thank Editage (www.editage.com) for English language editing. This study was

made possible with support from the Yoshida Scholarship Foundation, a research grant for Environmental Field Research by the Asahi Glass Foundation, and Grant-in-Aid for Research Activity Start-up Grant Number JP18H06077.

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A new species of Baikal endemic sponges (Porifera, Demospongiae, Spongillida, Lubomirskiidae)

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Academic editor: Roberto Pronzato | Received 29 August 2019 | Accepted 16 December 2019 | Published 22 January 2020

<http://zoobank.org/2EC18723-4B0F-43BF-B27A-AA5A9D752E27>

Citation: Bukshuk NA, Maikova OO (2020) A new species of Baikal endemic sponges (Porifera, Demospongiae, Spongillida, Lubomirskiidae). ZooKeys 906: 113–130. <https://doi.org/10.3897/zookeys.906.39534>

Abstract

This paper reports on a new species of the Baikal endemic sponge (fam. Lubomirskiidae) *Swartschewskia khanaevi* **sp. nov.** The description of this species is based on morphological and molecular data (ITS and mitochondrial IGRs). Morphologically, *S. khanaevi* **sp. nov.** differs from *S. papyracea* by loose tracts arranged in an irregular network as well as the presence on strongyles of compound spines looking like tubercles densely ornamented with simple spines. Moreover, specimens of *S. khanaevi* **sp. nov.** show a peculiar structure of the aquiferous system at the body surface that may be an adaptive trait for environmental conditions. Phylogenetic analysis has revealed that *S. khanaevi* **sp. nov.** forms a well-supported (0.99) monophyletic clade with *S. papyracea* and is allocated as its sister taxa.

Keywords

ITS, mitochondrial IGRs, morphological analysis, *Swartschewskia*

Introduction

Baikal is the most ancient and deepest lake on the Earth with the huge water volume. The lake is considered to be 25–30 million years old (Mazepova 1995); its maximum depth is 1641 m, and the volume of water body exceeds 23 000 km³ (Mats et al. 2001). Due to these facts, the lake is characterised by minor environmental oscillations. The

family Lubomirskiidae represents the most spectacular example of endemic radiation in freshwater sponges under the specific conditions of the great lake. According to molecular phylogeny the endemic family Lubomirskiidae was monophyletic and diverged from Spongillidae (Itskovich et al. 2008; Maikova et al. 2012; Maikova et al. 2017). Existence in a stable environment over a long period of time resulted in the disappearance of gemmulation from the life cycle of Lubomirskiidae (Efremova and Goureeva 1989; Manconi and Pronzato 2002).

At present, 14 species are allocated to the family Lubomirskiidae (Efremova 2001, 2004; Manconi and Pronzato 2019). The actual number of species is most likely underestimated. Comprehensive morphological study of the Baikal sponges revealed at least five forms that showed a constant set of morphological characteristics but could not be related to any known species (Khanaev et al. 2018). In this regard, these forms were suggested to be new species. Additionally, some specimens with uncommon morphology have been described (Weinberg 2005). We also observed several sponge specimens having unusual or transitional traits that interfered with precise species identification in our previous study (Khanaev et al. 2018).

The gaps in our knowledge of Lubomirskiidae morphology and taxonomy concern some aspects in the biology of the Baikal sponges. The absence of gemmules, gemmuloscleres, and parenchymal microscleres, which often contribute to taxonomy, complicates species identification (Manconi and Pronzato 2002). Moreover, the majority of the Lubomirskiidae species were described in the late 19th – early 20th century. The descriptions were limited to the classical taxonomy based on diagnostic morphological characters and were often very brief.

The genus *Swartschewskia* Makuschok, 1927 is clearly segregated from other Lubomirskiidae genera (Dybowski 1880, Swartschewsky 1901, Makushok 1927a). Only *Swartschewskia* is characterised by cortex: an ectosomal skeleton, tangential arrangement of primary tracts and stout bent strongyles as megascleres. The genus includes two species: *S. papyracea* (Dybowski, 1880) and *S. irregularis* (Swartschewsky, 1902). *Swartschewskia papyracea* is widely distributed in the depth range of 1–80 m in Baikal. *Swartschewskia papyracea* morphology was reported in a number of works (Swartschewsky 1902, Makushok 1927a, Rezvoy 1936, Manconi and Pronzato 2002, 2015, 2019; Weinberg 2005, Masuda 2009). On the contrary *S. irregularis* is extremely rare species inhabiting sublittoral zone of Baikal (70–150 m). The species was described based on the single specimen that was not preserved (Swartschewsky 1902). During the next 120 years only one sponge specimen with a similar morphology was found but no data on its morphology were published (Efremova 2001).

Molecular approach is also limited for phylogenetic studies of the Baikal sponges due to low variability of markers (COI, ITS, rRNA-genes) usually applied for this purpose throughout the world (Itskovich et al. 2008, Hargett et al. 2010). Recently (Maikova et al. 2015, 2016), the protein coding sequences of mitochondrial DNA were used to study the phylogenetic relationship of Baikal sponges only at the genera taxonomic level. This study showed that the nucleotide substitution rate of intergenic regions (IGRs) of the Baikal sponge mtDNA is significantly higher than coding sequences,

which makes them very promising for phylogenetic reconstructions of closely related species (Lavrov 2010, Maikova et al. 2012). However, only concatenated nuclear (ITS-regions) and mitochondrial (IGRs) data allowed us to separate closely related species of the family Lubomirskiidae (Maikova et al. 2017). Therefore, in this study we use ITS and mitochondrial IGRs sequences to investigate the phylogenetic position of a new species within the family Lubomirskiidae.

During the 2016 expeditions, unusual sponges were sampled in Olkhonskiye Vorota Strait. These sponges were identified as a separate species based on their morphological and molecular phylogenetic data. The paper describes a new species of *Swartschewskia* and we present additional data on the morphology of *Swartschewskia papyracea* (Dybowski, 1880) and provide diagnostic keys for the species belonging to the genus *Swartschewskia*.

Materials and methods

Study site and sample collection

The Olkhonskiye Vorota is a narrow strait that connects the Maloye More Strait with the main part of Baikal. The bottom of the Maloye More and the Olkhonskiye Vorota straits consists of different types of ground: rock debris, boulders, pebbles, various sand fractions, and silt (Kozhov 1947). The samples were collected at the three study sites (Fig. 1). At the study site 1 and 2, an extensive multi-layered bank of rock debris is located along the shore from the shoreline to the depth of 4–10 m. Stone fragments have rather large interstices between each other. The interstices are not filled with smaller fractions of ground (such as gravel, sand or silt); hence, unhampered water movements can take place there. Below 10 m, the bottom is sandy with rare boulders submerged in the sand with their lower side. At the study site 3, the bottom mainly consists of sand with detached boulders and rocks, which can be partially submerged in sand.

Samples were collected by SCUBA divers. All specimens were photographed and fixed in 96 % ethanol or frozen at -20 °C.

Holotype and four paratypes of the new species (specimens in ethanol and microscopic slides with tissue-free spicules preparations) have been deposited in Zoological Institute of the Russian Academy of Sciences, St Petersburg (ZIN). One paratype has been deposited in Porifera collection of Laboratory of Analytical and Bioorganic Chemistry, Limnological Institute of the Russian Academy of Sciences, Irkutsk (LIN).

DNA extraction, amplification, and sequencing

Total DNA was extracted from approximately 0.1 g of fixed tissue by modified phenol-chloroform method (Maniatis et al. 1984). For molecular analysis, two internal transcribed spacers (ITS1 and ITS2), as well as intergenic regions (IGRs) between tRNA^{Tyr}



Figure 1. Sampling sites in Lake Baikal: 1, 2 samples of *S. khanaevi* sp. nov, 3 samples of *S. papyracea*.

and tRNA^{Met} genes of mitochondrial DNA (mtDNA), were sequenced. Amplification of ITS and IGRs sequences was performed using specific primers and parameters described previously (Itskovich et al. 2008; Maikova et al. 2012). Each PCR reaction was purified by electrophoresis in 0.8 % agarose gels and eluted by freezing and thawing. DNA sequencing was carried out using the BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, United States) with subsequent analysis of the reaction products on an Applied Biosystems 3130xl Genetic Analyzer sequencer (USA) at Synmol Company (Moscow, Russia).

Sequence alignments and tree reconstructions

PCR-fragments were assembled and aligned using MAFFT (Katoh and Toh 2008) and BioEdit 5.09 (Hall 1999). Bayesian reconstructions were performed using MrBayes v. 3.2.1. (Ronquist and Huelsenbeck 2003). For concatenated data (ITS and IGRs), the nucleotide substitution model GTR+I+G was used for ITS and “mixed” parameter for IGRs. The Markov chain Monte Carlo search was run twice (default parameter) on four chains for 20 000 000 generations. Trees were sampled every 1000 cycles after the first 10 000 burn-in cycles. Genetic distances in pairwise comparisons between all analysed sequences were calculated according to the Kimura’s two-parameter model using MEGA7 (Kumar et al. 2016).

Morphological analysis*

Two variants of skeleton preparations were made for each specimen. In the first case, the small pieces of specimens were saturated with water and frozen. Vertical sections of frozen pieces (0.3–0.5 mm thickness) were made manually to investigate the ectosomal and choanosomal skeleton (Efremova 2004). In the second case, the small piece of the ectosomal skeleton was detached from the choanosomal one, washed with water and oriented upwards with the superior surface. Both kinds of skeleton preparations were mounted on slides and on stubs. Tissue-free spicules preparations were made by dissolving small pieces of sponge in sodium hypochlorite with a subsequent rinse with water and transfer to 96 % ethanol. Clean spicules and skeleton pieces were mounted on slides and aluminium stubs and air-dried. Samples on slides were placed in Canada balsam for the observation with Light Optical Microscope Olympus CX-21. Samples on aluminium stubs were coated with gold for further investigation with a Scanning Electron Microscope Philips SEM 525 (Collective Instrumental Center "Ultramicroanalysis" at LIN SB RAS). Digital images of both kinds of skeleton preparations and spicules were made using an integrated camera by SEM. Skeleton preparations were also photographed using the light optical microscope with an ocular camera Toupcam 5.1. Spicules of six samples (length and width; 50 spicules in every sample) were measured in several fields of view using Olympus CX-21 and ocular micrometre. Spicules dimensions were listed as three values: minimum–(mean)–maximum.

Cortex thickness and dermal pores dimensions were measured by Philips SEM 525 digital images in every investigated specimen ($N = 9$). Dermal pores ranged from rounded (diameter was measured) to ovoid or elliptic (width and length were measured). Dimensions were listed as three values: minimum–(mean)–maximum. Light optical microscope photographs were used for pore fields and oscula measurements. Apertures in the sieve-like osculum of *S. papyracea* were measured by photograph ($N = 1$).

The percentage of sponge surface lacking in both oscula and pores was calculated in SpongeArea (original software is available at <https://gitlab.com/bukshuk-sci/spongearea>). Macro photographs for the analysis were taken using Canon EOS 450D with LPL Copy Stand CS-40.

For the taxonomy of genus and species level and name validity the World Porifera Database was considered as reference (Van Soest et al. 2019).

Results

Phylogenetic analysis

For phylogenetic analysis, the ITS and mitochondrial IGRs sequences were obtained from six specimens of *S. khanaevi* sp. nov. and two specimens of *S. papyracea*, which were deposited into GenBank (Table 1). Additionally, we used previously published sequences of Lubomirskiidae and Spongillidae species (Maikova et al. 2017). *Ephydatia*

* In situ photograph of sponges is provided by Viktor Lyagushkin (scale bar is approximate)

Table 1. Sample numbers in the collection and sequence numbers in GenBank.

Species	Number in the collection	GenBank number	
		Sequences of ITS-regions	Sequences of mtDNA intergenic regions (IGRs)
<i>S. papyracea</i>	LIN-BS-1837	MH133907	MH257749
	LIN-BS-2360	MH133908	MH257750
<i>S. khanaevi</i> sp. nov.	ZIN 11990	MH133901	MH257748
	LIN-BS-1740-2	MH133902	MH257744
	ZIN 11986	MH133903	MH257746
	ZIN 11987	MH133904	MH257743
	ZIN 11988	MH133905	MH257745
	ZIN 11989	MH133906	MH257747

fluviatilis (Linnaeus, 1759) (fam. Spongillidae) was used as an outgroup (Itskovich et al. 2008). The length of the aligned concatenated sequences was 1266 bp, the ITS and IGRs partitions were 734 bp and 532 bp in the length respectively. The overall variability (K2P) for ITS-regions was 1.9 % and for IGRs – 0.9 %. Within the family Lubomirskiidae the intraspecific genetic distances of the ITS-regions varied from 0 to 0.6 %, while the interspecific ones varied from 0.1 to 4.7 % (average 1.5 %). The intraspecific genetic distances of the IGRs varied from 0 to 0.8 % and the interspecific ones varied from 0 to 4.9 % (average 2.2 %). Based on concatenated data the intraspecific genetic variability was from 0 to 1.6 % and between species ones was from 0.3 to 4.2 % (average 1.7 %). The pairwise genetic distances between the sequences of *S. khanaevi* sp. nov. varied from 0 to 1.5 % (average 0.7 %), and the ones between the sequences of *S. khanaevi* sp. nov. and *S. papyracea* ranged from 1.4 to 2.6 % (average 1.9 %).

In the phylogenetic tree, the specimens of *S. papyracea* and *S. khanaevi* sp. nov. form a well-supported (0.99) monophyletic clade named A (Fig. 2). Within clade A, we recognise two well-supported monophyletic groups named B and C. Clade B contains the specimens of *S. khanaevi* sp. nov.; *S. papyracea* was allocated as its sister group (clade C). Thus, *S. khanaevi* sp. nov. was named as a separate species.

Systematics

Phylum Porifera Grant, 1836

Class Demospongiae Sollas, 1885

Subclass Heteroscleromorpha Cárdenas, Pérez & Boury-Esnault, 2012

Order Spongillida Manconi & Pronzato, 2002

Family Lubomirskiidae (Weltner, 1895)

Genus *Swartschewskia* Makushok, 1927

Included species. *Swartschewskia papyracea* (Dybowski, 1880), *Swartschewskia irregularis* (Swartschewsky, 1902).

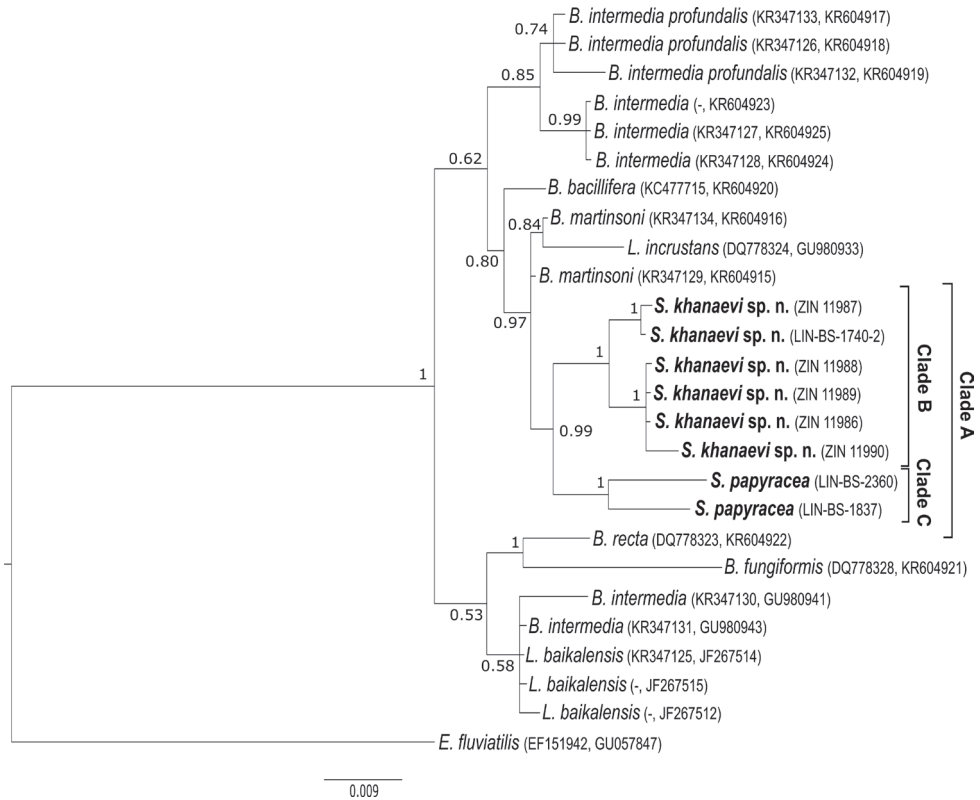


Figure 2. Phylogenetic tree based on concatenated nuclear (ITS1 and ITS2) and mitochondrial (IGRs) sequences: Bayesian posterior probabilities are shown at the bases of the clusters. Taxon names and collection numbers of sponges analysed in this study are marked in bold. Scale bar denotes substitutions per site.

Type species. *Swartschewskia papyracea* (Dybowski, 1880).

Genus diagnosis. Body shape encrusting to globose or branched. Ectosomal skeleton hard and well developed as more or less regular alveolar network of thick tangential spicular fibres. Choanosomal skeleton sparsely developed with scarce spicules irregularly arranged in few weak fibres. Abundant spongin. Megascleres strongyles, from spiny to smooth (modified from Manconi and Pronzato 2002).

***Swartschewskia khanaevi* sp. nov.**

<http://zoobank.org/40B1B85A-7E8E-4C09-8DD7-15CE4BB56A28>

Figs 1, 3, 4; Tables 1, 2

Type material. Holotype: ZIN 11986 (specimen in ethanol), ZIN 11986A (slide), Lake Baikal, the Olkhonskiye Vorota Strait, sampling site 1 (52°59.42'N 106°55.53'E), depth 10 m, SCUBA divers, September 9, 2016, collected by I. V. Khanaev, 1 specimen. **Paratypes:** ZIN 11987 (specimen in ethanol), ZIN 11987A (slide): ibid, 1 specimen.

Table 2. *Swartschewskia khanaevi* sp. nov. spicules length and width (*N* = 50).

Specimen	Length (μm)	Width (μm)
	min–(mean)–max	
Holotype		
ZIN 11986	111–(128)–141	11–(15)–20
Paratypes		
LIN-BS-1740-2	99–(127)–146	11–(15)–19
ZIN 11987	108–(125)–138	10–(15)–19
ZIN 11988	106–(128)–140	11–(14)–18
ZIN 11989	104–(123)–138	13–(17)–21
ZIN 11990	109–(128)–149	9–(14)–18

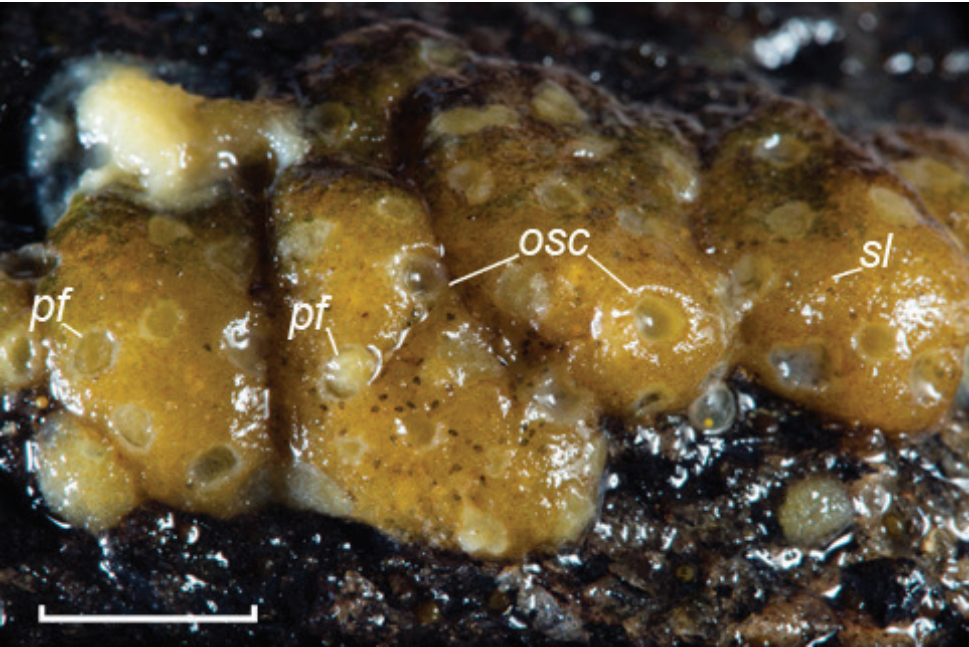


Figure 3. *Swartschewskia khanaevi* sp. nov., external view. Abbreviations: **osc** oscula, **pf** pore fields. Scale bar: 5 mm.

men; ZIN 11988 (specimen in ethanol), ZIN 11988A (slide): ibid, 1 specimen; ZIN 11989 (specimen in ethanol), ZIN 11989A (slide): ibid, 1 specimen; LIN-BS-1740-2 (specimen in ethanol, slide): ibid, 1 specimen. ZIN 11990 (specimen in ethanol), ZIN 11990A (slide): the Olkhonskiye Vorota Strait, sampling site 2 (53°01'03.40"N 106°55'47.00"E), depth 2.5 m, SCUBA divers, June 7, 2016, collected by I. V. Khanaev, 1 specimen.

Etymology. Named after Dr Igor V. Khanaev, scientist and diver who organised a dive program and collected type material.

Description. Thin encrusting sponge. Sponge thickness is maximal in the centre of the body (0.5–1 mm) and minimal at the edge (0.05–0.3 mm).

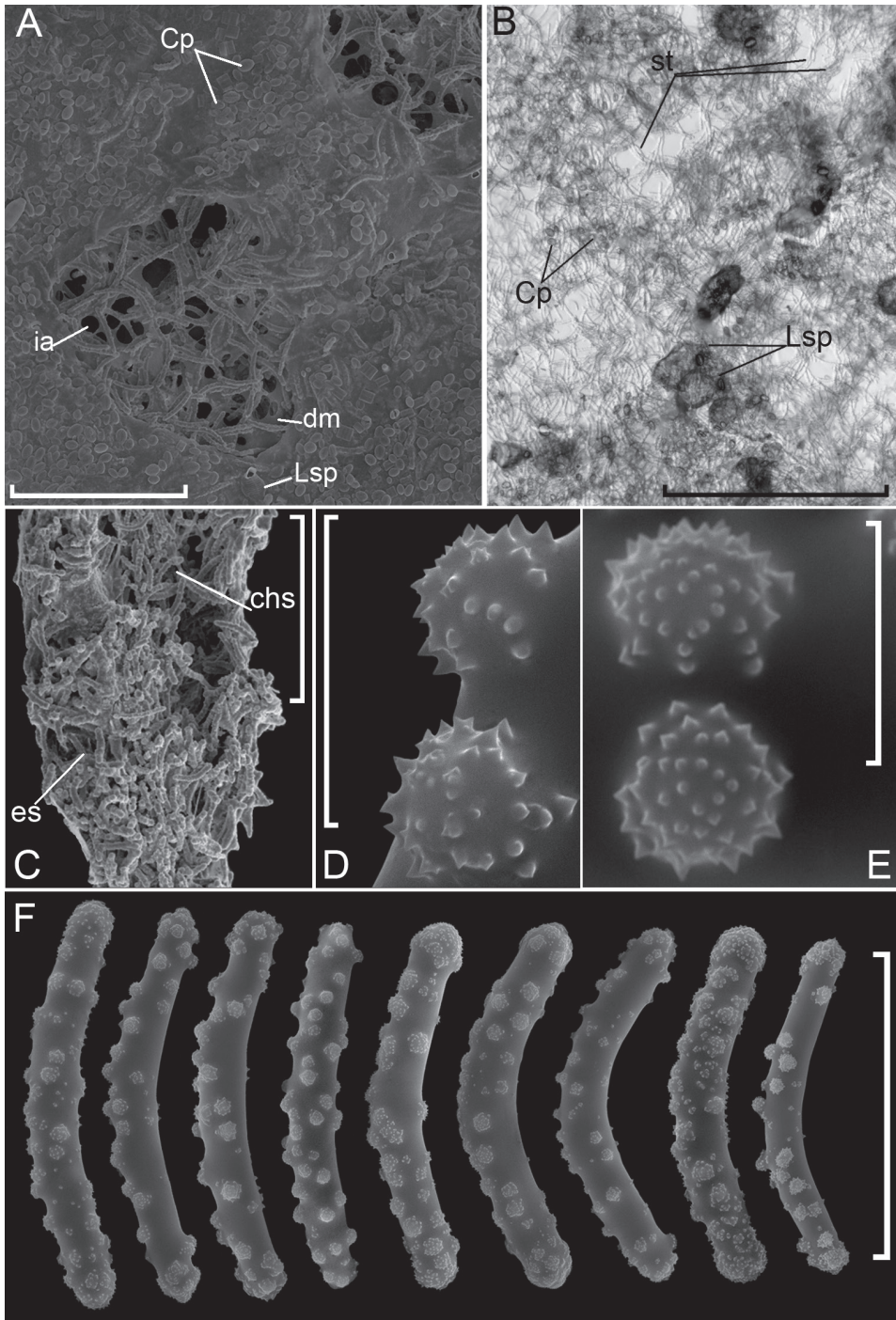


Figure 4. *Swartschewskia khanaevi* sp. nov. **A** sponge surface **B** ectosomal skeleton **C** cross section of skeleton **D, E** secondarily microspined tuberculated spines on strongyles **F** strongyles. Abbreviations: **chs** choanosomal skeleton, **Cp** Cocconeis placentula, **dm** dermal membrane, **es** ectosomal skeleton, **ia** inhalant apertures, **Lsp** *Lagenophrys* sp., **st** spicular tracts. Scale bars: 10 μ m (**D, E**), 100 μ m (**F**), 500 μ m (**A**), 1 mm (**B**).

The natural colour is yellowish beige and almost white in ethanol with brown areas on the surface. Usually, sponges have from one to three oscula, and only paratype ZIN 11990 has six oscula. Oscula are almost round, deepened, edged with well-developed spicular vallum. Oscula size is $146\text{--}(585)\text{--}978 \times 235\text{--}(663)\text{--}1148 \mu\text{m}$. Dermal pores are non-uniformly distributed on sponge surface. They are mostly aggregated in pore fields. Those are not deepened relatively to sponge surface, diverse in shape and can join to each other. Round or ovoid inhalant apertures of $7\text{--}(42)\text{--}106 \times 7\text{--}(54)\text{--}140 \mu\text{m}$ in size perforate dermal membrane. The apertures are located in meshes of ectosomal skeleton network. Pore fields size varies significantly: $0.07\text{--}(0.5)\text{--}1.5 \times 0.09\text{--}(0.7)\text{--}2 \text{ mm}$. One field usually contains 4–40 pores; the maximum number of pores is 78. There are also isolated pores.

Up to 70–80 % of sponge surface is lacking in both oscula and pores and covered with dense accumulations of *Cocconeis placentula* Ehrenberg, 1838 and sporadic exemplars of other diatoms (identified by Dr N.A. Bondarenko). Additionally, some ciliated protozoa of genus *Lagenophrys* von Stein, 1851 (identified by Dr T.Ya. Sitnikova) were observed on all specimen of *Swartschewskia khanaevi* sp. nov.

Sponge surface is a hard but fragile crust, i.e., ectosomal skeleton; the inner part of the body is soft and can be easily detached from the crust. The ectosomal skeleton has a form of a cortical layer (cortex) of tangentially arranged tracts forming an alveolar network. Meshes are disordered; size and shape vary. In some parts of the cortex, meshes are indistinguishable; tracts cross irregularly. Megascleres in tracts are arranged in loose bundles, 2–8 megascleres in every bundle. The thickness of cortex varies significantly from 44 to $307 \mu\text{m}$. The thickest cortex is observed near oscula, the thinnest one in the areas of pore fields. The choanosomal skeleton is weak; it consists of separated spicules and thin disordered fibres.

Megascleres are exclusively strongyles of $99\text{--}(127)\text{--}149 \times 9\text{--}(15)\text{--}21 \mu\text{m}$ with different sorts of spines: simple spines, rosette spines, and a peculiar sort of spine, secondarily microspined tuberculated spines. The latter look like tubercles ($4\text{--}9 \mu\text{m}$ in diameter and $1\text{--}5 \mu\text{m}$ in height) densely ornamented with simple spines (number 13–58) and these are the most abundant sort of spines. Rosette spines are comparatively rare ($0.8\text{--}(1.4)\text{--}3.2 \times 1\text{--}(1.6)\text{--}3.6 \mu\text{m}$ in size, contain 3–9 simple spines). The length of isolated simple spines and simple spines in both kinds of complex spines is similar: $0.1\text{--}(0.4)\text{--}0.9 \mu\text{m}$. Microscleres absent.

Swartschewskia papyracea (Dybowski, 1880)

Figs 1, 5; Tables 1, 2

Note. The morphology of three specimens of *S. papyracea* sampled in the Olkhonskiye Vorota Strait was examined.

Description. Body shape is globose. The sponge often has a single osculum but several oscula are also possible. Mostly the oscula look like round pits with 3–5 exhalant apertures on the bottom. One specimen bears a sieve-like osculum that consists of a number of exhalant apertures not deepened relatively to sponge surface. Distribution

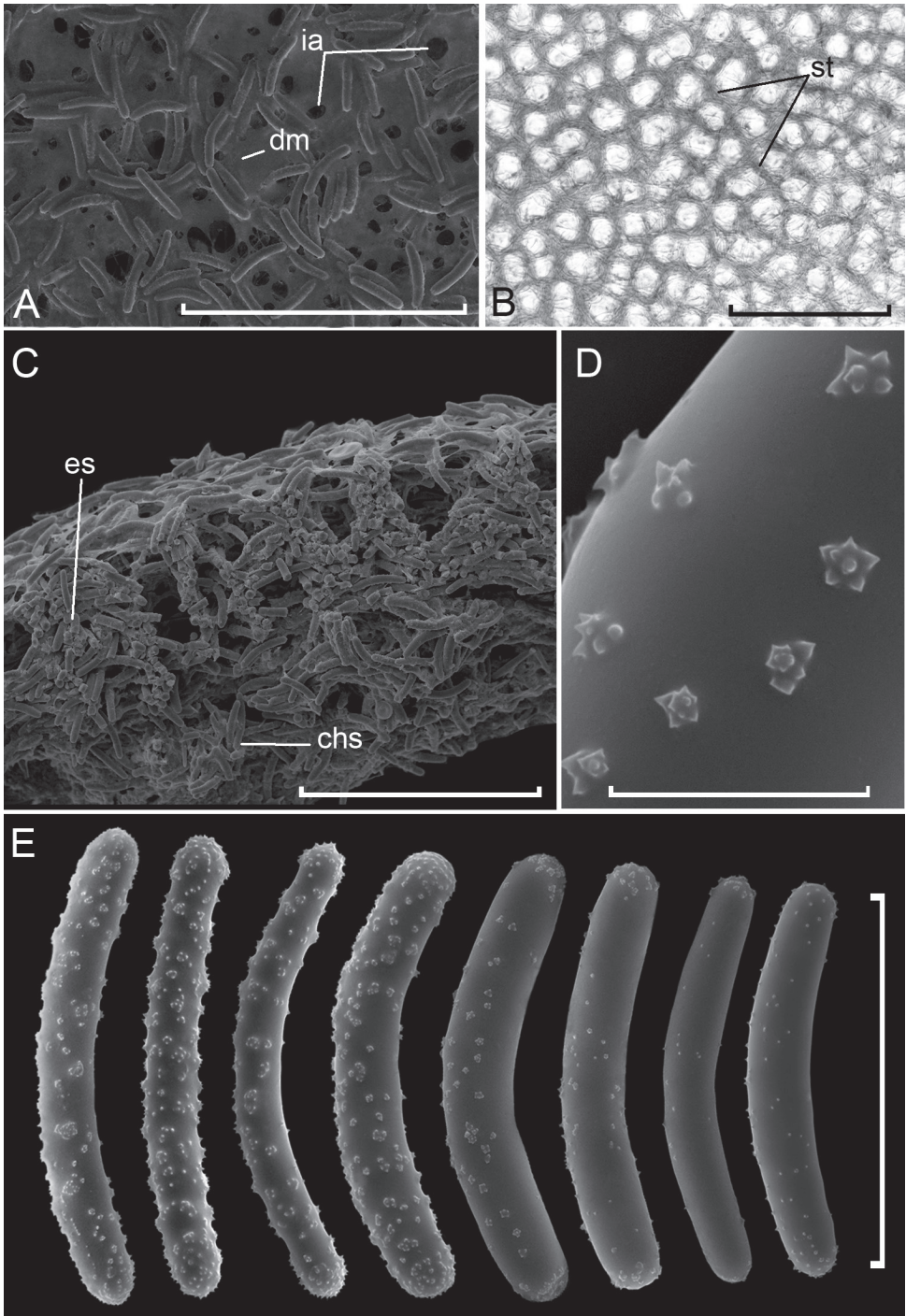


Figure 5. *Swartschewskia papyracea* **A** sponge surface **B** ectosomal skeleton **C** cross section of skeleton **D** rosette spines on strongyles **E** strongyles. Abbreviations: **chs** choanosomal skeleton, **dm** dermal membrane, **es** ectosomal skeleton, **ia** inhalant apertures, **st** spicular tracts. Scale bars: 10 µm (**D**), 100 µm (**E**), 500 µm (**A**, **C**), 1 mm (**B**).

of dermal pores is uniform. Inhalant apertures are observed almost in every meshes of ectosomal skeleton network. One mesh contains 1–5 round or ovoid apertures, 5–(28)–87 × 6–(35)–102 µm in size. Exhalant apertures in the sieve-like osculum have elongated or round shape, 214–(281)–357 × 178–(219)–286 µm in size.

The ectosomal skeleton is a high ordered alveolar network, mesh shape resembles a convex polygon. There are no parts with a disordered network structure. Megascleres in tracts are arranged in dense bundles, 6–12 megascleres in every bundle.

Megascleres are stout and bent strongyles of 93–(117)–138 × 13–(17)–22 µm. Analysis of the fine morphological structure of *S. papyracea* spicules indicated the presence of only two sorts of spines: rosette spines and isolated simple spines. Rosette spines are slightly elongated, 0.5–(1.4)–3.2 × 0.6–(1.6)–4.1 µm, and contain 4 – 18 simple spines. Isolated spines and simple spines in rosettes have a similar size of 0.1–(0.4)–0.9 µm.

Discussion

Two species, *S. papyracea* and *S. irregularis*, were included in the genus *Swartschewskia* before the present study. We used the following sources for comparative analysis of diagnostic morphotraits. The original description of *S. papyracea* was made by W. Dybowski (1880) on several exemplars. Afterwards the type material was lost (Efremova 2001). Due to the impossibility of comparing our data with type material we were guided by the generally accepted recent descriptions of Manconi and Pronzato (2002, 2019). Additionally, we studied the morphology of *S. papyracea* specimens from the type locality of *S. khanaevi* sp. nov. Data on *S. irregularis* morphology are extremely poor. For more than 200 years of studies of the Baikal sponges, only two specimens of *S. irregularis* were collected. Both specimens are no longer available; therefore, we relied on the original description of the species (Swartschewsky 1902).

Based on molecular data, the new species belongs to the genus *Swartschewskia* (fam. Lubomirskiidae). The limitations of the molecular approach were previously shown for phylogenetic studies of the Baikal sponges due to low variability of markers (COI, ITS, rRNA-genes) usually applied for this purpose throughout the world (Itskovich et al. 2008, Harcet et al. 2010). The protein coding sequences of mtDNA allowed phylogenetic relationships within Lubomirskiidae to be resolved only at the genera taxonomic level (Maikova et al. 2015, 2016). The mtDNA intergenic regions, as we suggested, could be suitable for the separation of closely related species of Baikal sponges (Maikova et al. 2012) due to their increased rate of substitution accumulation (Lavrov 2010). But on the phylogenetic tree based on mtDNA intergenic regions, the species of Baikal sponges did not form separate clades at the species level, with some exceptions (deep-sea sponges, for example) (Maikova et al. 2012). The concatenated nuclear (ITS-regions) and mitochondrial (IGRs) data were most suitable for studying phylogenetic relationships within the family Lubomirskiidae at the moment (Maikova et al. 2017). The limitation of the molecular approach is apparently related to

the low evolutionary rate of both nuclear and mitochondrial DNA of Baikal sponges (Lavrov 2010), which is enhanced by the relatively recent divergence of many species. An exception is the *S. papyracea*, which shows the acceleration of the accumulation of nucleotide substitutions in mtDNA to be twice relative to other species of the family Lubomirskiidae (Maikova et al. 2015). We hypothesise that this species is one of the most ancient of the existing species. On the phylogenetic tree based on protein-coding mtDNA genes, *S. papyracea* is closer to a common ancestor than all other species of the Baikal sponges (Maikova et al. 2016). In this study, based on concatenated nuclear (ITS) and mitochondrial (IGRs) data, the maximum interspecific genetic distances were between *S. papyracea* and other Lubomirskiidae species. Within the genus *Swartschewskia* the intraspecific and interspecific genetic distances do not overlap. This shows the genetic subdivision of the species within the genus and the genetic isolation of the genus *Swartschewskia* within the family Lubomirskiidae. The division of the new species into two groups inside the Clade B is not reflected in their morphology.

Swartschewskia khanaevi sp. nov. has skeleton structure and spicules typical for the genus (Manconi and Pronzato 2002, 2019). *Swartschewskia khanaevi* sp. nov. differs from *S. papyracea* by the clustering of pores in pore fields, less ordered structure of the ectosomal skeleton and unusual secondarily microspined tuberculated spines on strongyles. In *S. papyracea* distribution of dermal pores is uniform, ectosomal skeleton is highly ordered alveolar network with polygonal meshes, spicules bear spines grouped in rosettes (Manconi and Pronzato 2002, 2019). Generally, oscula are similar in both species, but *S. papyracea* has an alternative rather rare kind of osculum, which is sieve-like. It consists of a number of exhalant apertures inside rounded vallum (Manconi and Pronzato 2002). Pore fields of *S. khanaevi* sp. nov. could hardly be misinterpreted as sieve-like oscula. The total shape of the latter is always roundish; apertures are packed very closely and are noticeably larger than dermal pores (mean size $281 \times 219 \mu\text{m}$ vs $42 \times 54 \mu\text{m}$). Ectosomal skeleton of *S. irregularis* even less ordered than of *S. khanaevi* sp. nov. It lacks polygonal network and looks like randomly arranged spicules. Strongyles of *S. irregularis* are smooth.

Previous data on the morphology of *Swartschewskia* species do not contain records of strongyles ornamented with tuberculated spines or pore fields (Dybowski 1880; Swartschewsky 1901, 1902; Makushok 1927a; Rezvoy 1936; Manconi and Pronzato 2002, 2019). Weinberg (2005) mentioned an unusual specimen of *S. papyracea* as a thin encrusting sponge with numerous oscula and bearing: (a) spicules as stout and bent strongyles thinner than usual in the species and ornamented with massive complex spines; (b) skeleton of clearly divided ectosomal and choanosomal parts, but intensive study of the skeleton was not carried out. The sponge was collected from the Maloye More Strait (the precise locality was unknown). Based on these facts, we suppose that Weinberg met a specimen of *S. khanaevi* sp. nov. Taking into account sites, where *S. khanaevi* sp. nov. was collected, the species is most likely a local endemic of the Olkhonskiye Vorota Strait or Maloye More Strait as a whole.

Fossil spicules similar to *S. khanaevi* sp. nov. were found in the Late Pliocene sediments (interval of 3.2–2.8 Ma) of Lake Baikal (Veynberg 2009). They were described

as spicules of extinct species *Palaeoswarschewskia* sp. 1, they were some thinner and longer than in *S. khanaevi* sp. nov. and had complex spines. These spines, with a smaller number of simple spines than tuberculated spines and less expressed tubercles (see Veynberg 2009), represent intermediate variant between rosette spines of *S. papyracea* and tuberculated spines of *S. khanaevi* sp. nov. In this regard, we cannot ascertain the unambiguous identity of *Palaeoswarschewskia* sp. 1 and *S. khanaevi* sp. nov., but these two species are doubtlessly morphologically close.

Non-uniform localisation of pores in *S. khanaevi* sp. nov. is uncommon amongst the Baikal sponges. Normally, in lubomirskiids pores are evenly distributed throughout the sponge surface. The bottom at the study site consists of stones (a substrate for sponges) and sandy areas located nearby. The latter saturate the water with suspended grains of sand. The number of suspended particles combined with hydrodynamic activity can lead to clogging of the aquiferous system (Bell et al. 2015). The concentration of inhalant pores at restricted areas of the body surface was previously described as an adaptive trait of some sponge species living under the conditions of high sedimentation (Rützler 1974; Werding and Sanchez 1991; Pronzato et al. 1998). A larger size of inhalant apertures of *S. khanaevi* sp. nov. in comparison with *S. papyracea* can also prevent clogging.

The presence of sessile ciliates and dense aggregation of diatom algae on the sponge surface is not common for Lubomirskiidae. Isolated diatom algae can be observed sometimes on the lubomirskiids surface. There are no descriptions of mass diatom accumulations on the surface of a number of specimens. Any attached ciliates on sponges in Baikal also have never been mentioned. However, ectosymbiotic sessile ciliates of the *Lagenophrys* genus were described on Baikal endemic amphipods cuticle (Khalzov et al. 2018). Probably the emergence of a unique epibiotic community on *S. khanaevi* sp. nov. is possible due to unusual structure of aquiferous system. Permanent exhalant and inhalant water currents are normally presented at the sponge surface and prevent its colonisation. In *S. khanaevi* sp. nov., exhalant and inhalant apertures are concentrated in restricted areas. Therefore, up to 80 % of the body surface has no currents, which is a favourable substrate for epibiotic organisms.

Key to *Swarschewskia* species

The key to Lubomirskiidae genera and species was offered by Manconi and Pronzato (2019) and was the basis for the present key.

Spongillida: Lubomirskiidae: Genera

- | | | |
|---|---|---------------------------|
| 1 | Growth form massive (globular) to encrusting with digitiform outgrowths; consistency firm to hard; surface smooth | 2 |
| – | Growth form encrusting to massive, branching; consistency soft; densely conulose, variably long conules | <i>Rezinkovia</i> |
| 2 | Megascleres typically strongyles variably spiny | 3 |
| – | Megascleres typically spiny oxeads | <i>Lubomirskia</i> |

- 3 Megascleres typically smooth/spiny, stout, bent strongyles **with compound spines**, rare spiny oxeas*Swartschewskia*
- Megascleres typically smooth strongyles with spiny tips; spiny strongyles and/or smooth/spiny oxeas also present.....*Baikalosporgia*

Spongillida: Lubomirskiidae: *Swartschewskia*: Species

Three species are endemic to Lake Baikal.

- 1 Massive, rounded or encrusting, bent **spiny** strongyles; rare oxeas regularly spiny **2**
- Massive, irregular; bent smooth strongyles
..... *Swartschewskia irregularis* (Swartschewsky, 1902)
- 2 Strongyles with spines in rosettes
..... *Swartschewskia papyracea* (Dybowski, 1880)
- Strongyles with tubercles densely ornamented with simple spines
..... *Swartschewskia khanaevi* sp. nov.

Conclusions

A new species *Swartschewskia khanaevi* sp. nov. was described based on morphological traits and sequences of nuclear (ITS1 and ITS2) and mitochondrial (IGRs) markers. In the molecular phylogeny the specimens of *S. khanaevi* sp. nov. are clustered within a well-defined group containing *S. papyracea* as the most closely related species. Indeed, the specimens' morphological traits clearly indicate their belonging to *Swartschewskia*: well-developed ectosomal skeleton of tangential spicular fibres and sparsely developed choanosomal skeleton, stout bent strongyles as megascleres. The major morphological traits that distinguish *S. khanaevi* sp. nov. from other congeners are the structure of ectosomal skeleton and compound spines on strongyles. *Swartschewskia khanaevi* sp. nov. was sampled only from the Olkhonskiye Vorota Strait, and we assumed it to be a local endemic of this strait. We suggest the non-uniform localisation of pores on the sponge surface may be an adaptation to biotope conditions.

Acknowledgements

This study was performed within the framework of the State Tasks Nos. 0345-2019-0002 and 0345-2019-0009 and supported by the RFBR grant No. 19-04-00787A. The authors are grateful to Dr Igor V. Khanaev and Valery I. Chernykh for samples collection. Authors thank Dr Tatyana Ya. Sitnikova (Limnological Institute SB RAS, Russia), who organised the expedition in 2003 and identified ciliates on the sponge surfaces, and Dr Nina A. Bondarenko (Limnological Institute SB RAS, Russia) for identification of diatom algae on sponges. SEM analyses were carried out in the Center 'Ultramicroanalysis' (Limnological Institute SB RAS).

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Review and new species of *Tiferonia* Darlington, 1962 (Carabidae, Abacetini)

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Academic editor: B. Guéorguiev | Received 6 November 2019 | Accepted 16 December 2019 | Published 27 January 2020

<http://zoobank.org/AD702E75-3A08-481E-81B1-0203AEF362BE>

Citation: Will KW (2020) Review and new species of *Tiferonia* Darlington, 1962 (Carabidae, Abacetini). ZooKeys 906: 131–140. <https://doi.org/10.3897/zookeys.906.48255>

Abstract

Darlington described *Tiferonia* based on *T. parva* from New Guinea. In this review, *Tiferonia leytensis* **sp. nov.** is described from Leyte Island, Philippines, *Tiferonia schoutedeni* (Straneo, 1943) **comb. nov.** is transferred from *Melanchrous* Andrewes, and inclusion of *Tiferonia brunnea* (Jedlička, 1935) in the genus is confirmed. Characteristics of *Tiferonia* and genera that have been proposed as closely related to *Tiferonia* are discussed and a unique character, the post-ocular sulcus, shared among species of *Tiferonia* and *Holconotus* is proposed as a synapomorphy for these two genera. A key to identify adults of the four species of *Tiferonia* is provided.

Keywords

New Guinea, Africa, The Philippines, ground beetles, *Holconotus*, *Melanchrous*

Introduction

Darlington (1962) described the genus *Tiferonia* for two species from New Guinea and the Philippines but subsequently there have been no publications dealing with any additional specimens, species or taxonomic issues in the genus. Outside of check-lists or catalogs, only a few papers have mentioned the genus as part of some larger

study or peripheral to the principal paper topic. These typically only note the genus to distinguish it from of other genera, note its inclusion in abacetines or Loxandrinini auctorum, or remark on its presence in the New Guinea fauna (Darlington 1962, 1971; Allen and Ball 1979; Allen 1982; Will and Park 2008; Will and Kavanaugh 2012). Specimens are quite rare in collections and there are no current efforts to collect in areas where species may exist using methods likely to obtain more material. This creates the very familiar problem of having only small numbers of specimens for study. During my recent investigations of various Abacetini genera and other Harpalinae that may have a relationship to abacetine taxa, including Darlington's carabid specimens at the Museum of Comparative Zoology, it became apparent that there were several issues that need to be addressed in order to improve the state of the taxonomic understanding of *Tiferonia* with regard to species membership and possible phylogenetic relationships of this genus to other genera.

Material and methods

Material examined. Specimens were examined from the following collections:

ANIC	Australian National Insect Collection, Canberra;
CMNH	Carnegie Museum of Natural History, Pittsburg, PA;
CSCHM	J. Schmidt collection, Admannshagen, Germany;
EMEC	Essig Museum of Entomology, Berkeley, CA;
MCZ	Museum of Comparative Zoology, Cambridge, MA;
NHM	The Natural History Museum, London.

Locality information for holotypes of the species described here is verbatim. Text as it appears on the labels is contained in quotation marks. The text for each label is delimited by double forward slash marks.

Images. Habitus photos of beetles were taken as image stacks that were aligned and assembled with Helicon Focus version 5.3 and image files were edited to enhance clarity using standard image editing software.

Dissection and measurements. Male genitalia were prepared using the same methods as Will (2002). Measurements were made using an ocular reticle. Standard body length (sbl) is the sum of the distance from the base of the labrum to just anterior of the occipital suture + the length of the pronotum along its midline + the length of the left elytron from basal margin where it meets the scutellum to the apex of the elytron. The width of the elytra is the widest point viewed dorsally. The ocular ratio is the width over the eyes divided by the width between the eyes measured at the level of the anterior supraocular setae, viewed dorsally. Measurements and ratios are given for the type specimen and then a range of all specimens measured is given in brackets.

Taxonomic treatment

Abacetini Chaudoir, 1873

Tiferonia Darlington, 1962

Tiferonia Darlington, 1962: 560.

Type species. *Tiferonia parva* Darlington, 1962: 562, by original designation.

Generic diagnosis. With a combination of typical abacetine characters such as clearly defined frontal impressions on the head; deeply impressed, linear basolateral pronotal impressions; no angular base of stria 1 on elytra; setose puncture at the base of elytral stria 2; well-developed elytral plica; metacoxal sulcus sinuate; abdominal ventrites without transverse sulci; ostium of aedeagus dorsal; and aedeagus left side dorsal in repose. Recognizable from other abacetine genera that share the character states listed above by the combination of deep post-ocular sulcus (Fig. 1), smooth elytral margins, and lack of elytral discal setae.

Genus characteristics. Small size beetles (3.8–4.3 mm), castaneous or darker, nearly piceous colored, parallel sided, somewhat convex body form; apical segment of labial palpi elongate and fusiform. Mentum narrow triangular, shallowly emarginate; epilobes long and narrow, not prominent; median tooth prominent and entire, not reaching tips of lobes, mentum paramedial pits absent; paraglossae short, glabrous; submentum narrow, posteriorly sculpted; antennae of moderate length, somewhat thickly filiform, three basal segments glabrous except for apical ring of setae; postocular orbits moderately pronounced, with deep post-ocular sulcus (Fig. 1). Elytra free, lateral margin smooth; border entire across base; parascutellar stria present, joined to stria 1; 13–15 umbilicate setae in stria 8; hind wings fully developed; humeri obtusely angled with very small, usually sharp denticle; anterior tarsi of male with three basal segments narrowly dilated and squamose beneath. Aedeagus (Fig. 2) with orifice on dorsum; parameres conchoid, the right smaller than the left.

Tiferonia parva Darlington, 1962

Figs 3, 5, 6

Material examined. Holotype: NEW GUINEA • ♂, (M.C.Z. No. 30,231). //“Aitape Brit. N.G. Aug 1944”// “det: Darlington at B.M. 1947-48 Notes p.”// “Genus.? Det. Darlington”// “Meas. ♂”// “gen. Melanchrous Andr. det. S.L.Straneo 1953”// “*Tiferonia parva* Darl.”// “M.C.Z. Type 30231”//.

Paratypes: NEW GUINEA • 1♂, 1♀, same data as holotype • 1♂, // “vic. Hollandia Dutch N.G. July-Sept 1944 Darlington”// [ANIC].

Diagnosis. Very similar to *T. leytenensis* but distinct from that species by the form of the pronotum, which is broad and straight onto the base and the form of the male genitalia (Figs 2, 3).

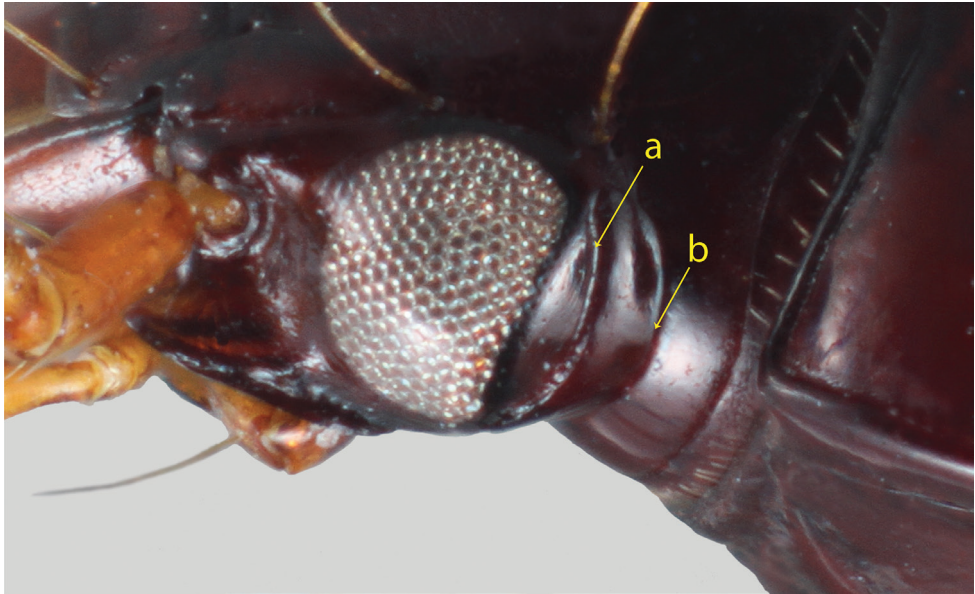


Figure 1. *Tiferonia leytenensis* sp. nov. head, left lateral view. Arrows indicate **a** post-ocular sulcus and **b** posterior edge of the orbit.

***Tiferonia brunnea* (Jedlička, 1935)**

Fig. 9

Fouquetius brunneus Jedlička, 1935: 108.

Holconotus brunneus: Lorenz 2005: 259.

Tiferonia brunneus: Darlington 1962: 561.

Material examined. Holotype: PHILIPPINES • ♂, // “Mt. Makiling, Luzon Baker”// “H.E.Andrewes Coll. BM 1945-97.”// [red label, black border, printed] // “TYPUS”// // [pink label, handwritten and print] “Fouquetius brunneus type sp. nov. DET H. ANDREWES”// [NHM].

Type locality. Estimated to be centered on 14.1346N, 12.1955E, south east of Calamba.

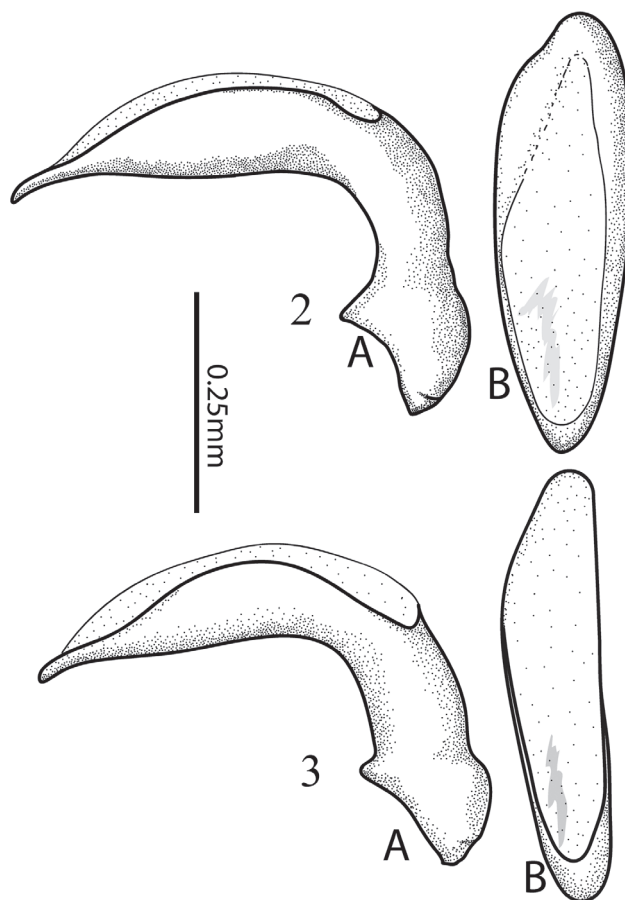
Diagnosis. The single pair of supraorbital setae distinguishes this species from all other species of *Tiferonia*.

***Tiferonia schoutedeni* (Straneo, 1943) comb. nov.**

Fig. 8

Patellus schoutedeni Straneo, 1943: 5.

Melanchrous schoutedeni: Straneo 1962: 54; Lorenz 2005: 328.

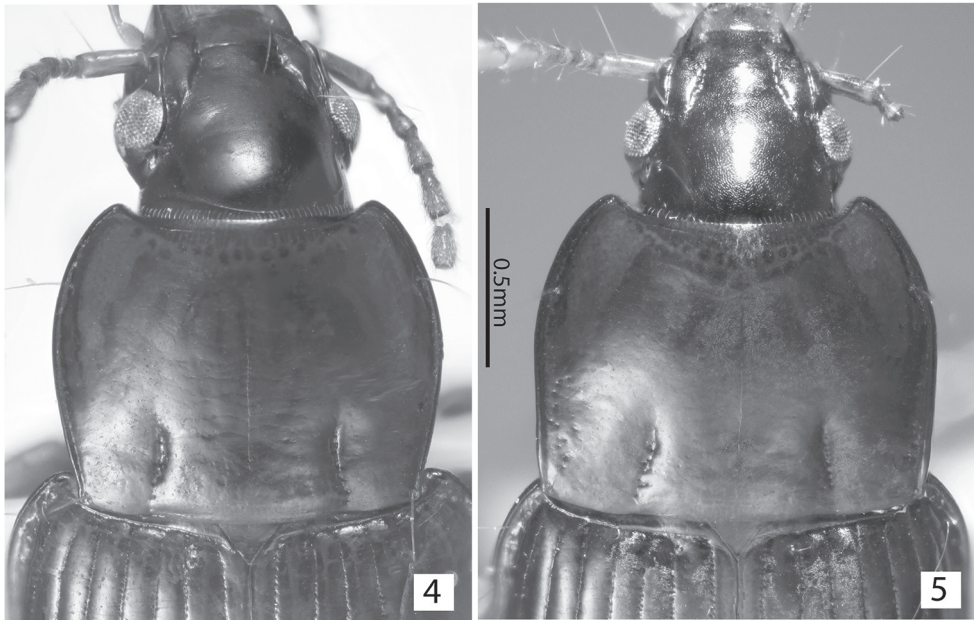


Figures 2, 3. *Tiferonia* species aedeagi, right lateral view (A) and dorsal view (B). **2** *Tiferonia leytensis* sp. nov. **3** *Tiferonia parva*.

Notes. According to Straneo (1943) the type was intended to be deposited in Tervuren. However, the specimen was not located (S. Hanot, Musée Royal de l'Afrique Centrale, Tervuren, Belgium, in litt.). Straneo stated that the specimen is labelled "Congo Belge, Eala (17-I-1921, Dr. H. Schouteden)."

Material examined. CENTRAL AFRICAN REPUBLIC • 1♀, // "R[epublic] C[entral] A[frika], P[ark]. N[ational]. [Dzanga]-Ndoki, Camp1 02 28 51.0N 016 13 04.5E, 9–11. II.2012, piège UV canopée 35m, Exp. Sangha 2012, P. Moretto leg. -70-"/[CSCHM] • ♂ // "Bot. N°69 Humus dans résidu forestier"// "I[nstitut pour la]. R[cherche]. S[cientifique en]. A[frique]. C[entrale]. –Mus. Congo Kwango: terr. de feshi, rive dr. Kwenge III-1959 B. 69 Mme J. Leleup"// "*Melanchrous schoutedeni* S.L. Straneo det. 1960"//[CMNH].

Diagnosis. Having only the first three elytral intervals impressed and a relatively large eyes (gena is only half the width of antennomere 1) distinguishes this species from all other *Tiferonia*.



Figures 4, 5. *Tiferonia* species pronota. **4** *Tiferonia leytensis* sp. nov. **5** *Tiferonia parva*.

***Tiferonia leytensis* sp. nov.**

<http://zoobank.org/939FDB9B-0561-473C-921D-D9AD409E87BF>

Figs 1, 2, 4, 7

Material examined. Holotype: PHILIPPINES • ♂, // “Plains of NE Leyte Is., P.I. Nov ’44-Jan’45 Darlington”// “MCZ Holotype 36215”// [deposited MCZ].

Paratypes: PHILIPPINES • 1♂, 1♀, same data as holotype [MCZ].

Type locality. As listed on locality label, type locality is estimated to be roughly centered on 11.221N, 124.828E.

Diagnosis. The combination of two pairs of supraorbital setae, all elytral striae impressed, and the pronotum (Fig. 4) basally narrowed with slightly sinuate lateral margins separates this species from all other species of *Tiferonia*.

Description. Size. Overall length (sbl) 3.96 mm [3.96–4.21 mm]; greatest width over elytra 1.65 mm [1.62–1.79 mm]. **Color.** Dorsal and ventral surfaces piceous to dark rufous, head slightly darker, elytral interval 1 and apical fourth of elytra paler; legs, mouthparts, and antennae pale brunneous, tibiae darker rufous. **Luster.** Dorsally and ventrally distinctly shiny. Iridescence not evident on head, spectral iridescence slightly apparent on pronotum, distinctly evident on elytra, slightly apparent on ventral surface of body. **Head.** Dorsal microsculpture evident nearly isodiametric mesh. Clypeal-ocular impressions clearly impressed, narrow, divergent, and extended to anterior supraocular seta. Ocular ratio 1.61 [1.55–1.58]. Eyes moderate size, prominent, with large posterior orbital area; gena slightly narrower than width of antennomere 1. Labrum with anterior



Figures 6–9. *Tiferonia* species dorsal habitus. **6** *Tiferonia parva*, male paratype **7** *Tiferonia leytenis* male holotype **8** *Tiferonia schoutedeni* **9** *Tiferonia brunnea*, male holotype.

margin straight. Mentum median tooth entire, triangular. Antennae, overall length moderately long, antennomeres 10–11 surpassing pronotal base. **Thorax.** Pronotum dorsally impunctate, microsculpture not evident at 50× magnification in anterior half, irregular, very transverse mesh slightly evident near base; widest at middle, lateral margins with short, slight sinuation near hind angles; lateral marginal bead uniformly evident and narrow throughout; basal margin smooth, not beaded; anterior angles moderately produced; anterior submarginal sulcus broadly interrupted medially; hind angles right angled, denticulate; basal impressions linear, deeply impressed, slightly crenulate, shallowly reaching basal margin; seta at hind angle touching marginal bead. Elytra parallel sided; plica large and externally visible. Elytral striae well impressed, deeply, densely crenulate-punctate; intervals nearly flat, slightly convex basally. Elytral microsculpture scarcely evident as very transversely stretched sculpticells. Prosternal process rounded, margin not marked with bead; prosterna and proepisterna smooth. Mesosterna with few, coarse punctures. Metasternum laterally and metepisternum with shallow, coarse punctures. **Abdomen.** Abdominal ventrites irregularly, coarsely punctate laterally, impunctate medially. **Male genitalia** (Fig. 2), ostium dorsal, endophallus with light spine field in left apical position in repose. **Female ovipositor** moderately long, slightly curved, two large ensiform setae, one dorsal one ventral, two long nematoform setae in well-developed groove.

Etymology. The specific epithet *leytensis* is based on the type locality and is treated as an adjective.

Notes. In his discussion of the species of *Tiferonia*, Darlington (1962: 561) states that *T. brunnea* from the Philippines is distinguished by having only a single pair of supraorbital setae and then states that he has a series of that species from Leyte Island. However, this appears to be an error. Among Darlington's specimens at the MCZ he has a series from Leyte Island, but they all have two pairs of supraorbital setae and are otherwise distinctly different from *T. brunnea*. These specimens comprise the holotype and paratypes of *T. leytensis*.

Possible evolutionary relationships of *Tiferonia*

Darlington (1962) noted that *Tiferonia* was “superficially similar to *Melanchrous*.” *Melanchrous* was treated as a member of Oodini by Chaudoir (1883) but then moved to Melanchitonini by Straneo (1962) and has remained in that tribe in recent catalogs (Lorenz 2005a, b; Bousquet 2012). The holotype of *T. parva* bears a determination label written by Straneo from 1953 with “gen. *Melanchrous* Andr.,” which is likely what suggested this comparison to Darlington. He then points out that *Melanchrous* from southeast Asia and the Malay Archipelago have protarsomeres with densely pubescent pads ventrally, similar to what is found in some melanchitonines and oodines, not biseriately squamulose as in *Tiferonia* and other abacetines. I have examined types or confidently identified specimens of all *Melanchrous* species except for one of the three the African species, *Melanchrous celisi* Straneo, 1962. All examined *Melanchrous* spe-

cies differ from *T. schoutedeni* by having protarsomeres with densely pubescent pads ventrally, not squamulose biseriate. Additionally, no species of *Melanchrous* has the post-ocular sulcus found in *Tiferonia* and *Holconotus* (Fig. 1). The type specimen of *M. celisi* could not be located (S. Hanot in litt.) and I have not seen any specimens that agree with Straneo's description of the species. Straneo described *M. celisi* in comparison to *T. schoutedeni*, to which it is similar in having a reduced number of impressed striae, but no character states were reported that can verify or refute its placement in *Melanchrous*.

Tiferonia and *Holconotus* are both abacetine genera that appear to be close relatives. Darlington (1962) included Jedlička's *brunneus* in *Tiferonia* while noting that *Holconotus* (= *Fouquetius*) has "dentate humeri and serrate elytral margins," which he states *Tiferonia* does not. While it is correct that all *Holconotus* have these states, it is not the case that the humeral tooth is lacking in *Tiferonia*. The tooth is slightly smaller and, in some cases, more rounded than typically observed in *Holconotus*, but always present. The humeri in *Melanchrous* (see above) is fully rounded, with no suggestion of a tooth. The presence of the serrate elytral margin is likely a synapomorphy for *Holconotus* species, excluding *Tiferonia*. The shared post-ocular sulcus appears to be a good synapomorphy for a sister-group relationship for *Tiferonia* and *Holconotus*. No other genera of Abacetini, and to my knowledge no other carabids, have the post-ocular sulcus as in these two genera.

Key to adults

- 1 Elytron with eight striae impressed from the apex to or nearly to the base...2
- Elytron with only the first three striae impressed from the apex to, or nearly to the base (Fig. 8). Africa..... ***Tiferonia schoutedeni* (Straneo, 1943)**
- 2 Two pairs of supraorbital setae3
- One pair of supraorbital setae. The Philippines
..... ***Tiferonia brunnea* (Jedlička, 1935)**
- 3 Pronotum lateral margins slightly sinuate in the basal third, base notably narrower than elytra (Fig. 4). Male aedeagus wide and sharply narrowing at tip in ventral view (Fig. 2). The Philippines..... ***Tiferonia leytensis* sp. nov.**
- Pronotum lateral margins nearly straight in the basal third, base nearly as wide as elytra (Fig. 5). Male aedeagus narrow and blunt at tip in ventral view (Fig. 3). New Guinea ***Tiferonia parva* Darlington, 1962**

Acknowledgements

This study was partially funded by a grant from the US National Science Foundation to the author, DEB0444726.

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A new species of *Eumerus* (Diptera, Syrphidae) from the Kingdom of Bhutan, the easternmost representative of the *bactrianus* subgroup

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Academic editor: X. Mengual | Received 15 November 2019 | Accepted 16 December 2019 | Published 27 January 2020

<http://zoobank.org/46D77D87-0F79-40CD-A661-0E545EEA42F1>

Citation: Smit J, Zeegers T, Dorji P (2020) A new species of *Eumerus* (Diptera, Syrphidae) from the Kingdom of Bhutan, the easternmost representative of the *bactrianus* subgroup. ZooKeys 906: 141–151. <https://doi.org/10.3897/zookeys.906.48501>

Abstract

A new species of *Eumerus*, *Eumerus druk* Smit **sp. nov.**, is described from Bhutan. This species belongs to the *bactrianus* subgroup of the *strigatus* species group. Seven species are currently known within this subgroup: four European, one of which is also found in the Near East, and three more only known from Tajikistan. The new species extends this disjunct distribution to the east by at least 2,000 km, stretching far beyond the reported Turano-Mediterranean region and into the Himalayas. A diagnosis and a key to all Central and Eastern Palearctic species of the *Eumerus bactrianus* subgroup are provided.

Keywords

Central Palearctic, Eastern Palearctic, flower flies, hover flies, Himalaya, identification key

Introduction

Hover flies are often large and attractively coloured insects that are frequently found on flowers and play a vital role in ecosystem services as pollinators (Biesmeijer et al. 2006; Inouye et al. 2015; Ssymank et al. 2008). In contrast, the majority of the species from the very speciose genus *Eumerus* Meigen, 1822 are inconspicuously dark coloured,

sometimes with metallic bronze-green or even golden luster or bluish sheen, and they are more often than not found on the ground or on rocks. With the wings folded over the abdomen, the metallic luster is obscured when in rest. For the same reason, the bright-red abdomen of some species is less conspicuous when in rest (J. Smit pers. obs.). The genus is widespread in the Old World and Australia and introduced to the Americas (Johnson 1910; Davidson 1915; Smith 1928; Gerding et al. 1999; Marinoni and Morales 2007; Speight et al. 2013). There are over 300 valid species of *Eumerus* described (Evenhuis and Pape 2019) and taxonomical difficulties abound, mainly due to the large number of species as well as the lack of comprehensive keys. Fortunately, in recent times more and more species groups have been treated integrally, where morphology is often supplemented by molecular characters (Grković et al. 2015, 2017, 2019; Chroni et al. 2017, 2018). One such species cluster is the *bactrianus* subgroup of the *Eumerus strigatus* group as defined by Grković et al. (2019). This subgroup has four Western Palaearctic described species (Markov et al. 2016; Grković et al. 2019) and three species only known from the Hissor mountain range ('Gissar' in Russian) in Tajikistan (Stackelberg 1952). In their work, Grković et al. (2019) treated the Western Palaearctic representatives from this subgroup and redescribed *Eumerus bactrianus* Stackelberg, 1952, one of the species from Tajikistan. In this paper we describe a new Eastern Palaearctic species from Bhutan. The discovery of this new species from Bhutan, a small kingdom in the eastern Himalayas, stretches the known distribution of the *bactrianus* subgroup some 2,000 km to the east, well beyond the reported Turano-Mediterranean region by Grković et al. (2019). Diagnosis and an identification key to all four Central and Eastern Palaearctic species are presented.

This new species was collected during an expedition in spring 2018 as part of the Bhutan Biodiversity Project. This project is a cooperation between the National Biodiversity Center (Bhutan), Naturalis Biodiversity Center (Netherlands), and five other Bhutanese organizations aiming to generate knowledge on Bhutanese invertebrates. The main goal was to make a survey of several invertebrate groups and make this knowledge available through publications and field guides.

Material and methods

Material from the following collections has been studied or is deposited therein, introducing the abbreviations: National Biodiversity Center, Thimphu, Bhutan (NBCB), Naturalis Biodiversity Center, Leiden, the Netherlands (NBC), and Zoological Institute in St Petersburg, Russia (ZIN). Male genitalia were removed and macerated in an aqueous 10% KOH solution at ambient temperatures for 12–24 hours and stored in glycerol. Photos of the terminalia were taken through a Bresser Biolux NV microscope with a MicrOculair and CamLabLite software, and subsequently stacked using Combine ZP 1.0 software. The remaining photos were made using an Olympus Tough TG-5 camera with built-in focus stacking software. The male holotype and the female paratype of *Eumerus druk* Smit sp. nov. had one leg removed for DNA barcoding (He-

bert et al. 2003a, 2003b). DNA barcodes, sequences and collection data were uploaded to the Barcode of Life Database (BOLD: <http://www.boldsystems.org>). Specimens are linked through their specimen code to their respective entry on BOLD. Terminology of morphological characters follows Thompson (1999), with the exception of the terminology for the genitalia, which follows Doczkal (1996) and Hurkmans (1993). Abdominal tergites and sternites are abbreviated with a 't' or 's' respectively.

Taxonomy

The *Eumerus strigatus* group was first defined by Speight et al. (2013) for a group of species closely related to *E. strigatus* (Fallén, 1817), i.e., *E. consimilis* Simic & Vujic, 1996, *E. funeralis* Meigen, 1822, *E. narcissi* Smith, 1928, *E. sogdianus* Stacklberg, 1952, and *E. strigatus*. Later Chroni et al. (2017) added *E. amoenus* Loew, 1848 based on molecular data and Grković et al. (2017) included another two species (i.e., *E. montanum* Grković, Radenković & Vuić, 2017 and *E. pannonicus* Ricarte, Vujic & Radenković, 2016). Grković et al. (2017) provided a description and a diagnosis for the group, stating that it comprises relatively small, inconspicuous species with usually a bronze shine and without coloured markings on the tergites, simple sternites, and s4 in males differently shaped but always with a v-shaped notch at the posterior margin. The main diagnostic character is the shape of the male genitalia, particularly the epan-drium with an elongated, posterior surstyle lobe of a species-specific shape.

The *Eumerus bactrianus* subgroup within the *strigatus* group was defined by Grković et al. (2019), and its members are very similar to the other species of the *strigatus* group but share the apomorphic character of the bifurcate posterior lobe of the surstylus in the male terminalia. Furthermore, the shape of s4 is more complex in the *bactrianus* subgroup than in the other species of the *strigatus* group. All species of the *bactrianus* subgroup are easily recognised; all have a unique shape of their antennae, s4, and the male terminalia. Females of all species of the *bactrianus* subgroup as well as the females of the *strigatus* group are extremely similar. The females of all species from the Central Palaearctic are known but have not been examined; therefore, the identification key presented here is only for the males.

The *bactrianus* subgroup is represented by four Western Palaearctic species (i.e., *E. banaticus* Nedeljković, Grković & Vujic, 2019, *E. bicornis* Grković, Vujic & Hayat, 2019, *E. bifurcatus* van Steenis & Hauser, 2019, and *E. pannonicus*), three Central Palaearctic species described from the Hissor Mountains in Tajikistan (Stackelberg 1952) (i.e., *E. bactrianus*, *E. turanicola* Stackelberg, 1952, and *E. turanicus* Stackelberg, 1952), and one Eastern Palaearctic species, *E. druk* Smit sp. nov. from Bhutan. Of the Western Palaearctic species, only *E. bicornis* is also found outside Europe, more precisely in Turkey in the Near East.

The Palaearctic Region can be divided into subregions. Semenov-Tian-Shanskij (1936) made a first division in four subregions based on the distribution of Coleoptera, combined with the geological history as well as the fossil fauna. This only ap-

peared in Russian, but an English summary was published in Nature that same year (Anonymous 1936). Kozár (1995) modified it and now the current subdivision into three regions, Western, Central, and Eastern, is widely applied (Kodandaramaiah and Wahlberg 2009; Sanmartin et al. 2001; Simonsen et al. 2010).

Eumerus bactrianus Stackelberg, 1952

Material examined. Paratype Tajikistan • male; у. Копдара 1100 м, д. ВарзобаТадж., Гуссаковский [Kopdara 1100 m, d. VarzobaTadž., Gussakovskii]; 15 May [19]39 (ZIN).

Diagnosis. Body golden- or bronze-green, often with purple tinge. Legs bronze-green, tip of femora and basal third of tibia as well as tarsomeres 1–4 brightly brownish yellow, apical tarsal segment dark. Metaleg with basotarsomere expanded and short, as longs as second and third segment combined. Basoflagellomere trapezoid (Fig. 2F). s4 rectangular, roughly wrinkled, posterolateral narrowly rounded corners with long pile, posteromedially with a deep and sharp notch. Male terminalia figured in Grković et al. (2019: fig. 7A–D), anterior sustyle lobe elongated, ventral margin of posterior surstyle lobe greatly produced.

Eumerus druk Smit, sp. nov.

<http://zoobank.org/5BBC6AD2-F2BF-4726-A26E-6055791AFE79>

Figs 1A–F, 2A–E, G

Type locality. Bhutan, Thimphu.

Diagnosis. Body golden-coppery, except t2 and t3 medially and t4 basomedially: shiny black amplified by short adpressed black pile. Basoflagellomere rectangular, with a rounded posterior corner. Male: abdomen t3 and t4 laterally with long, silvery, ventrally directed pile; s4 without an incision posteriomedially but medial part of sternite less sclerotized. Basotarsomere of metaleg simple, equal in length to the rest of the tarsomeres. Male terminalia: posterior surstyle lobe with a tuft of long pili just anterior to the bifurcation.

Description. Male. Length of body (excluding antennae) 7.5–8.5 mm, length of the wing 5.5–6.5 mm. **Head.** Eyes holoptic, eye contiguity 9–10 ommatidia long, ommatidia near eye contiguity conspicuously larger than those in the posterior part (Fig. 1E). Eye margins ventrally slightly divergent. Eye covered with dense white pile; posterior eye margin bare. Face with dense, silvery-white pollinosity and white pile. Frons with golden-yellow pile, intermixed with black pile or even predominantly black pilose on the ocellar triangle. Ocellar triangle isosceles; distance between anterior ocellus and posterior ocelli compared to the distance between both posterior ocelli 1:0.55. Frons with a small pollinose macula anterior to anterior ocellus. Occiput with dense white pollinosity up to about 3/4 dorsally; dorsal part shiny black, with coppery luster. Antenna black; basoflagellomere rectangular (Fig. 2D), with a rounded posterior corner. Arista entirely black. Scape and pedicel black, with white pile; black pile dorsally; dorsal pile much shorter than ventral pile.

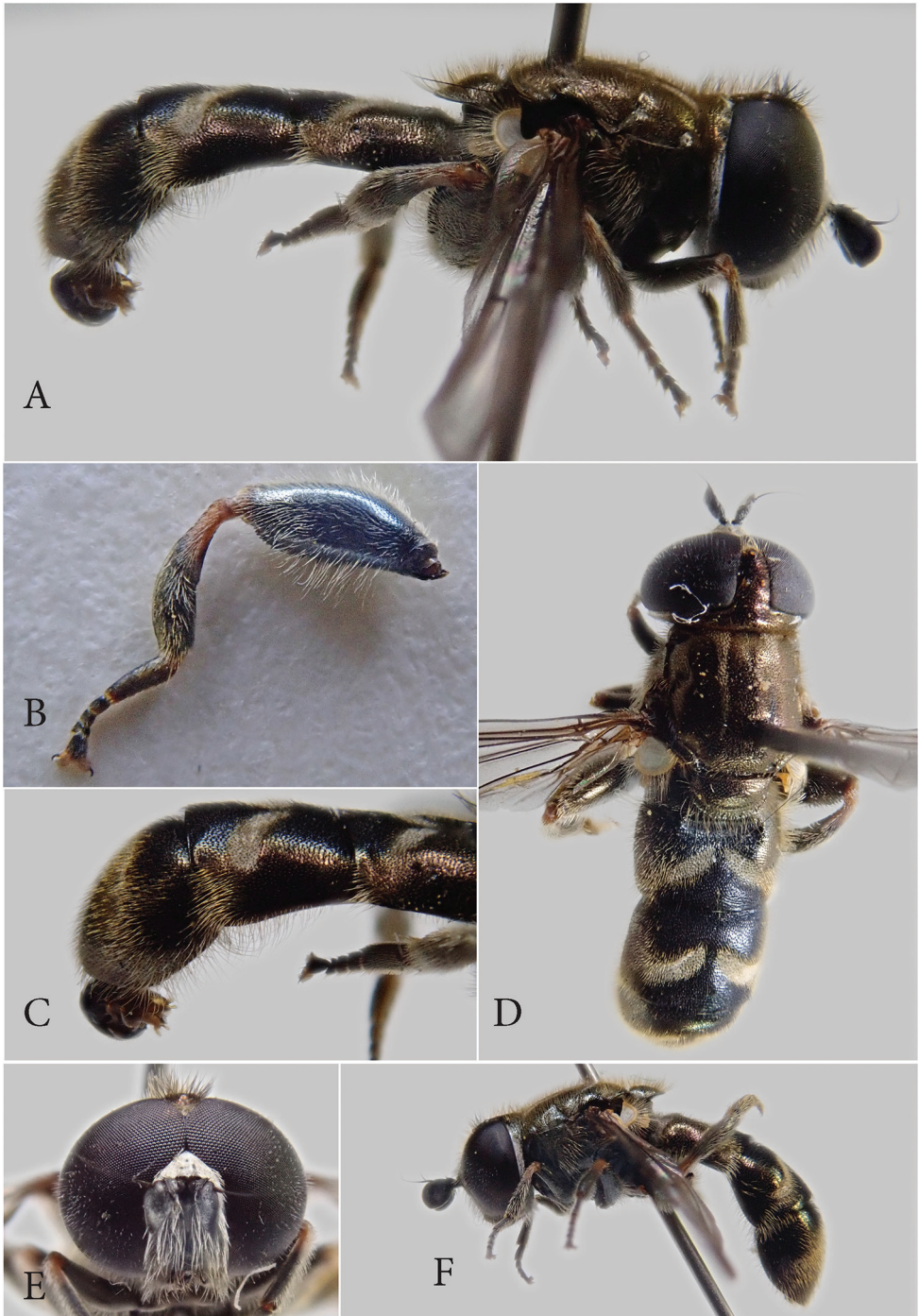


Figure 1. *Eumerus druk* Smit, sp. nov., male holotype **A** lateral view **B** metaleg, lateral view **C** abdomen, lateral view **D** dorsal view **E** head, frontal view. *Eumerus druk* Smit, sp. nov., female paratype **F** lateral view.

Thorax. Entirely shiny black, with golden luster (Fig. 1D). Mesonotum with a pair of white pollinosity vittae covering $3/4$ of scutal length. Mesonotum and scutellum covered with golden-yellow pile; clearly longer near the posterior margin of the mesonotum and scutellum. Notopleural suture absent. Scutum next to wing base with a row of strong black setae. Scutellum with a broad rim, somewhat granular. Anepisternum and anepimeron with the same golden luster; katepisternum pollinose, with a small shiny spot dorsally, posterior to tuft of long white pile, ventrally with a few long white pili. **Legs.** All black, except for the tibiae, which are red on the basal third. Tarsi black, claws bicoloured, red basally, and black apically. Metafemur moderately swollen, slightly curved, with two rows of black setae apicoventrally, 11 on anterior ridge and 11–13 on posterior ridge, long white pile dorsally, about half as long as the maximum width of the femur and even longer white pile ventrally, the longest ones slightly more than $3/4$ the maximum width (Fig. 1B). Metatibia with a flange of adpressed setae on the basal half, ventrally, followed by a shallow notch, apicoposteriorly with a single row of long light pile, longer than the maximum width of the metatarsus. Basotarsomere of metaleg simple, equal in length to the rest of the tarsomeres. **Wings.** Hyaline, pterostigma light brown, entirely microtrichose.

Abdomen. Entirely black, parallel sided, t2–4 with oblique maculae of white pollinosity, those on t3 and t4 longer and clearly lunulate (Fig. 1A). t2 and t3 shiny black medially, as well as t4 basomedially, laterally with golden-coppery luster (Fig. 1D). The black colour in the middle of the tergites is amplified by the short adpressed black pile, light on the pollinose maculae as well as on the lateral sides and the majority of the t4. Abdomen with conspicuous long, silvery, ventrally directed, white pile on the lateral sides of the t3 and t4 (Fig. 1C). s4 with long silvery-white pile laterally, distinctly shorter medially, posteromedially without incision, but medial part of sternite less sclerotized (Fig. 2E).

Terminalia (Fig. 2A–D). Posterior lobe of sursylus bifurcate, with a tuft of long light pile just anterior to bifurcation.

Description of female. Similar to male except for the normal sexual dimorphism (Fig. 1F). Length of body (excluding antennae) 7 mm, length of the wing 6 mm.

Head. Frons with some pollinosity alongside the eye-margin, from the antennae up to the anterior ocellus. Ocellar triangle isosceles, distance between anterior ocellus and posterior ocelli compared to the distance between both posterior ocelli 1:0.88. **Abdomen.** t3 and t4 laterally with slightly longer, silvery and ventrally directed, pile.

Etymology. The specific epithet ‘druk’ is Dzongkha (the Sino-Tibetan language spoken in Bhutan) for dragon and refers to the official name of the kingdom: *Druk yul* (country of the Dragon people, or the Land of the Thunder Dragon). It should be treated as a noun in apposition.

Distribution. This species is only known from the type series collected at the Royal Botanical Garden in Thimphu, Bhutan, but it likely has a wider distribution in the Himalayas. This is the only Eastern Palearctic species of the *bactrianus* subgroup of the *strigatus* species group.

Examined material. Type material. **Holotype** Bhutan • male; Thimphu, Royal Botanical garden; 27.425N, 89.650E, 2400 m a.s.l.; 26 April 2018; J.T. Smit & Th. Zeegers leg.; RMNH.INS1092470.

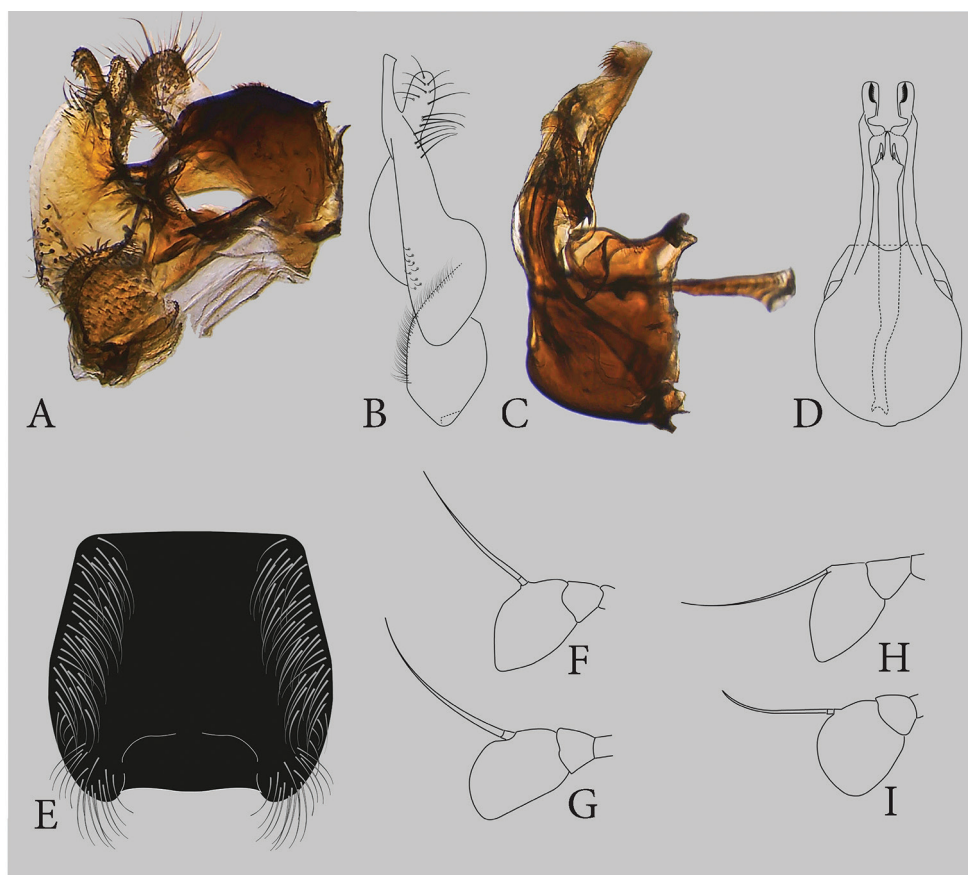


Figure 2. *Eumerus druk* Smit, sp. nov., male holotype **A** epandrium, lateral view **B** surstyle lobe, ventral view **C** hypandrium, lateral view **D** hypandrium, ventral view **E** fourth sternum. *Eumerus bactrianus* Stackelberg, 1952, male **F** antenna, lateral view. *Eumerus druk* sp. nov. **G** antenna, lateral view. *Eumerus turanicola* Stackelberg, 1952, male **H** antenna, lateral view, after (Stackelberg 1952). *Eumerus turanicus* Stackelberg, 1952, male **I** antenna, lateral view, after (Stackelberg 1952).

Paratypes Bhutan • 4 males; same collection data as for holotype • 1 female; same data as for holotype; RMNH.INS1092471.

The holotype is in good condition and is deposited, together with one male and female paratype in the National Biodiversity Center, Bhutan (NBCB). The remaining three paratype males, as well as the DNA material are stored in the collection of Naturalis Biodiversity Center, the Netherlands (NBC).

Remarks. The male of *Eumerus druk* Smit, sp. nov. is easily distinguished from all other species in the *bactrianus* subgroup by the long, silvery, ventrally directed, pile on the lateral sides of t3 and t4. *Eumerus banaticus* has some longer pile on the lateral sides of t4, but this is shorter, not ventrally directed, and not present on t3. Furthermore *E. banaticus* is easily distinguished by the lack of pollinose maculae on t4 and by the shape of st4 and the terminalia. *Eumerus hungaricus* Szilády, 1940 and *E. pulchellus*

Loew, 1848, which have similar long, ventrally directed pile on t3 and t4, are superficially similar but the pile is much more dense. *Eumerus druk* Smit sp. nov. can easily be distinguished by the bifurcate posterior surstyle lobe. *E. hungaricus* and *E. pulchellus* furthermore lack the golden-coppery luster on the thorax and abdomen of *E. druk*. *Eumerus pulchellus* is a more slender built species, with a more bluish luster, a relatively slender metafemur, the pro- and mesotarsi predominantly light brown, the basoflagellomere orange. *Eumerus hungaricus* is a more black species with less luster, especially on the abdomen, which is predominantly black pilose; s3 is very slender, about 2.5 times longer than wide, and t4 has a yellow posterior margin, medially.

***Eumerus turanicola* Stackelberg, 1952**

Diagnosis. (based on Stackelberg 1952). Body golden- or bronze-green. Legs dark bronze-green, tip of the femora, basal half as well as the tips of the tibia and tarsi reddish yellow. Metaleg with basotarsomere not expanded nor shortened, longer than second and third segment combined. Basoflagellomere triangular (Fig. 2H). s4 figured by Stackelberg (1952, 1961), gradually widening posteriorly, with a broad incision posteriomediaally, with two sharp angles well below the apex of S4 and two rounded lobes on both sides of the incision. Male terminalia figured by Stackelberg (1952, 1961), posterior surstyle lobe with a ventral triangular extension.

***Eumerus turanicus* Stackelberg, 1952**

Diagnosis. (based on Stackelberg 1952). Body bronze-green. Legs dark bronze green, tip of the femora, basal half as well as the tips of the tibia and tarsi reddish yellow. Metaleg with basotarsomere not expanded nor shortened, longer than second and third segment combined. Basoflagellomere oval-shaped (Fig. 2I). s4 figured in Stackelberg (1952, 1961), gradually narrowing, with an incision posteriomediaally, with two rounded, densely pilose angles at the apex of s4, with two rounded, spatulate lobes on both sides of the incision. Male terminalia figured in Stackelberg (1952, 1961), with dense pilosity on the main branch of the posterior surstyle lobe, cerci with distinct thorn-like projections.

An identification key to the males of the Central and Eastern Palearctic species of the *Eumerus bactrianus* subgroup

- 1 All tarsi entirely black. Basoflagellomere rectangular (Fig. 2G), with a rounded posterior corner. Abdomen with t2 and t3 shiny black in the middle, amplified by the short adpressed black pile, continuing on the basal part of t4. Abdominal t3 and t4 with long, silvery, ventrally directed, white pile on the lateral sides, s4 without an incision posteriomediaally, but middle part of ster-

- nite less sclerotized. Terminalia: posterior surstyle lobe with a tuft of long pile just anterior to the bifurcation ***E. druk* Smit, sp. nov.**
- Tarsi predominantly reddish-yellow. Basoflagellomere trapezoid, oval or triangular shaped. Abdomen with t2–4 with the same bronze-green luster as laterally. Male: s4 with a clear incision posteromedially and posterior surstyle lobe lacking the tuft of long pili prior to the bifurcation **2**
- 2 Basoflagellomere trapezoid (Fig. 2F). s4 without lobes on both sides of the posteromedially incision, with a deep and sharp notch. Terminalia: anterior surstyle lobe elongated, ventral margin of posterior surstyle lobe greatly produced..... ***E. bactrianus* Stackelberg**
- Basoflagellomere not trapezoid but oval or triangular. s4 with a deep, angular incision posteromedially **3**
- 3 Basoflagellomere triangular (Fig 2H). s4 gradually widening posteriorly, with a broad incision posteromedially, with two sharp angles well below the apex of s4 and two rounded lobes on both sides of the incision. Terminalia: posterior surstyle lobe with a ventral triangular extension..... ***E. turanicola* Stackelberg**
- Basoflagellomere oval (Fig. 2I). s4 gradually narrowing posteriorly, with an incision posteromedially, with two rounded, densely pilose angles at the apex, with two rounded, spatulate lobes on both sides of the incision. Terminalia: with dense pilosity on the main branch of the posterior surstyle lobe, cerci with distinct thorn-like projections..... ***E. turanicus* Stackelberg**

Acknowledgements

Naturalis Biodiversity Center, Leiden and the Uyttenboogaart-Eliasen stichting are kindly thanked for their financial support of the collecting trip for JS (SUB.2017.12.07) and TZ (SUB.2017.12.06). We thank Dr. Tashi Yangzome Dorji, Program Director National Biodiversity Centre, Ministry of Agriculture and Forests, Choki Gyeltshen from the National Biodiversity Center, Bhutan and Tshelthrim Zangpo Plant Protection Officer, Entomology for their support with permits, logistics, and help in the field. We thank Jeroen van Steenis for providing the male paratype of *Eumerus bactrianus* held in ZIN. Lastly, we thank Ana Grković, Martin Hauser, and Jeroen van Steenis for their valuable comments on the manuscript.

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