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Two new species of *Parapharyngodon* parasites of *Sceloporus pyrocephalus*, with a key to the species found in Mexico (Nematoda, Pharyngodonidae)

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Abstract

Two new species of *Parapharyngodon* collected from the intestine of the Mexican boulder spiny lizard *Sceloporus pyrocephalus* are described. This study increases to 49 the number of valid species assigned to *Parapharyngodon* worldwide, 11 of them distributed in Mexico. Males of the two new species share the presence of four pairs of caudal papillae, an anterior echinate cloacal lip and the presence of lateral alae; however, both differ from each other in lateral alae extension and echinate cloacal anterior lip morphology. Females of both species have a prebulbar uterus and eggs shell punctuate with pores, characteristics shared with few other species of *Parapharyngodon*. Both new species differ from other congeneric species in the papillar arrangement, the anterior cloacal lip morphology, the lateral alae extension and total length/spicule ratio. A taxonomic key for the species of *Parapharyngodon* distributed in Mexico is provided.

Keywords

Helminth, Enteric nematode, Reptile, Lacertilia, Phrynosomatidae, Michoacán, Guerrero

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Introduction

Mexico has a species-rich reptile fauna, with 864 species (8.7% of the worldwide total); 57% of them are endemic (Flores-Villela and García-Vázquez 2014). Although Mexico has a long tradition of helminthological and herpetological research, there are large gaps in the knowledge of the species diversity of helminths associated with these vertebrates (Pérez-Ponce de León et al. 2011). *Sceloporus* Wiegmann is a reptile genus distributed in the Americas; it inhabits a wide range of environments, and it is the most representative lizard taxon of the Mexican herpetofauna with 92 of 97 species that composed the genus, with the majority of them endemic (Flores-Villela 1993). *Sceloporus pyrocephalus* Cope is an oviparous endemic lizard to Mexico. It is associated with streams and rivers within tropical deciduous and semi-deciduous forest, and it is distributed from the Southwestern Pacific coast of Jalisco and Colima to Michoacán, Guerrero, South-western Mexico State, and Southern Morelos (Uetz and Hošek 2013). There are scarce studies on this phrynosomatid lizard; the majority of these are focused in taxonomy, conservation, and reproductive research (Ramírez-Bautista and Olvera-Becerril 2004, Calisi et al. 2007, Leaché 2010).

Recently, the validity of species of *Parapharyngodon* Chatterji, 1933 was reviewed (Velarde-Aguilar et al. 2015, Bursey and Goldberg 2015). In accordance with these authors, of the 78 *Parapharyngodon* species assigned to this genus, only 47 have been properly described, and recognized as valid species. Nine of these species are distributed in the Panamanian realm, seven in Oriental, seven in Palearctic, six in Neotropical, five in Afrotropical, five in Nearctic, five in Saharo-Arabian, one Australian, one in Sino-Japanese realm, and one species in Madagascan region. In this paper, two new species of *Parapharyngodon* parasites of *S. pyrocephalus* collected from Michoacán and Guerrero states, Mexico, are described, increasing the basic knowledge about helminths of Mexican lizards.

Materials and methods

During the breeding season months of June-July in 2003, 2004 and 2005, 16 *S. pyrocephalus* were captured (under the collection permit SEMARNAT FAUT-0056 issued to VLR) by noosing or hand in ten different locations (seven from Michoacán and three from Guerrero states, Mexico, Table 1). Hosts were killed by an intraperitoneal injection of sodium pentobarbital overdose. The mouth, peritoneal cavity and all internal organs were examined for helminths with the use of stereoscope. Nematodes found were counted, fixed in hot 4% formaldehyde solution and stored in alcohol 70%. For morphological study, specimens were cleared in glycerin-alcohol 70% solution at 1:1 ratio, and mounted on temporary slides for examination under a light microscopy. Original drawings were made with an Olympus BX53 microscope equipped with camera lucida. For scanning electron microscopy (SEM), worms were dehydrated through ethanol series, dried with a K850 Critical Point Drier (Emitech, Ashford, England),

Table 1. Sampling sites for *S. pyrocephalus* analyzed in this study.

Locality (reviewed host) geographic coordinates	Nematode species (specimens obtained)	Collecting date
Michoacán		
Aquila (1) 18.5911 N, 103.5667 W	*P. ayotzinapaensis* sp. n. (17)	07/2003
Arteaga (3) 18.6468 N, 101.9684 W	*P. ayotzinapaensis* sp. n. (2) *P. tikuinii* sp. n. (5)	07/2005
La Huacana (1) 18.6734 N, 101.9951 W	*P. tikuinii* sp. n. (1)	07/2005
Tepalcatepec (1) 19.0758 N, 102.8936 W	*P. tikuinii* sp. n. (1)	06/2004
Álvaro Obregón (1) 19.0386 N, 102.9744 W	*P. tikuinii* sp. n. (1)	06/2004
Buenavista (2) 19.1766 N, 102.6635 W	*P. tikuinii* sp. n. (5)	07/2005
Apatzingan (1) 19.1247 N, 102.4014 W	*P. tikuinii* sp. n. (1)	07/2003
Gabriel Zamora (3) 19.1764 N, 102.0633 W	*P. ayotzinapaensis* sp. n. (8) *P. tikuinii* sp. n. (4)	06/2004
Guerrero		
Tecpan de Galeana (2) 17.2967 N, 101.0467 W	*P. ayotzinapaensis* sp. n. (3) *P. tikuinii* sp. n. (1)	07/2004
El Patio (1) 17.177 N, 100.5953 W	*P. ayotzinapaensis* sp. n. (12)	07/2005

coated with gold using a Q150R Modular Coatin System (Quórum, Ashford, England), and examined in a Hitachi S-2460N (Hitachi, Tokyo, Japan) and SU1015 SEM (Hitachi) SEM. Measurements are provided in millimeters, including the range, followed by average and standard deviation, and the sample size. Host specimens collected were deposited in the Herpethology Collection of the Museo de Zoología, Facultad de Ciencias (MZFC), UNAM, and helminths were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, UNAM.

Results

Family Pharyngodonidae Travassos, 1920
Genus *Parapharyngodon* Chatterji, 1933

Parapharyngodon ayotzinapaensis sp. n.

http://zoobank.org/E06EF8E0-3F05-4A60-A599-4DAEDC81353A
Figs 1A–E; 2A–H

Type material. Holotype, male, CNHE 9432. Allotype, female, CNHE 9433. Paratypes (4 males, 7 females), CNHE 9434-9438.

Etymology. The species is named in honor of the 43 missing students from "Escuela Normal Rural Raúl Isidro Burgos" in Ayotzinapa, Guerrero, Mexico; in solidarity with their families and the Mexican people.

Diagnosis. Robust, small and white fusiform nematodes, males smaller than females. Cuticle with prominent transverse striations along the whole body except tail. Triangular oral opening surrounded by three simple lips in males and bilobed in females, in both sexes ventrolateral lips have an amphid each one, in females it is located on the dorsal lobe. Within buccal cavity, both sexes have three transverse plates, bilobed in males and complete in females. Esophageal bulb with sclerotized apparatus. Excretory pore evident, it is located at level of posterior edge of esophageal bulb. A vesicular body surrounds excretory duct. Males with lateral alae covering the last third of body, females lacking lateral alae. Males without caudal alae. Four pairs of caudal papillae. Caudal filament sub-terminal and directed dorsally in males. Females with a conical posterior end. Vulva located at middle region. Eggs no shown alae, with a punctuated shell and subpolar operculum, embryo in early stage of cleavage.

Description of male. Distinctly truncate posterior end (Fig. 1E), total body length 2.72–3.92 (3.458 ± 0.458, n = 5), maximum width 0.6–1.88 (1 ± 0.505, n = 5) at middle region. Cuticle with wide transverse striae 0.05–0.08 (0.062 ± 0.011, n = 5) maximum width at middle region. Triangular oral opening surrounded by three simple lips at whose internal bases are located three transverse bilobed plates, ventrolateral lips with one amphid each one (Fig. 2D). Esophagus total length 0.7–0.84 (0.79 ± 0.054, n = 5) and maximum width 0.04–0.06 (0.05 ± 0.01, n = 5), esophageal bulb length 0.12–0.17 (0.148 ± 0.023, n = 5) and width 0.13–0.16 (0.146 ± 0.013, n = 5). Nerve ring and excretory pore 0.1–0.24 (0.172 ± 0.064, n = 4) and 0.74–1.39 (1.046 ± 0.237, n = 5) from anterior end, respectively. Testis extends from middle body region to level of anterior end of intestine. Lateral alae start abruptly from level of the beginning of third caudal region of body (Fig. 1E). The left one at 2.24–3.01 (2.573 ± 0.395, n = 3), and the right one at 2.31–2.88 (2.565 ± 0.253, n = 4) from anterior end, both with a maximum width of 0.06–0.07 (0.062 ± 0.005, n = 4); extending to posterior end of body, the left one at 0.02–0.07 (0.046 ± 0.025, n = 3) and the right one at 0.03–0.09 (0.056 ± 0.023, n = 5) from the base of corresponding paracloacal papillae. Four pairs of caudal papillae distributed as follows: one precloacal pedunculate, one paracloacal pedunculate, one lateral sessile at the central lobe apex of postcloacal lip and one mammilliform at 0.06–0.07 (0.065 ± 0.006, n = 4) from posterior end of caudal filament (Fig. 2A and B). Top of pedunculate and mammilliform papillae in a rosette-like structure (Fig. 2A, B and E). Echinate precloacal lip with rough appearance, at base of posteriorly directed finger-like ornamentation, which vary in simple or bifurcate outgrowths disposition (Figs 1B; 2B). In SEM pictures morphology is distinguished as follows: cloacal lip rise up from anterior cloacal edge, at both end sides it has a rough thickening region longer that remainder of lip (1 and 5 in Fig. 2B), adjacent to these, at both sides of lip and towards central axis of body, there are finger-like outgrowths that start from rough regions (2 and 4 in Fig. 2B). On the central axis of body is located a rough region (3 in Fig. 2B) upon

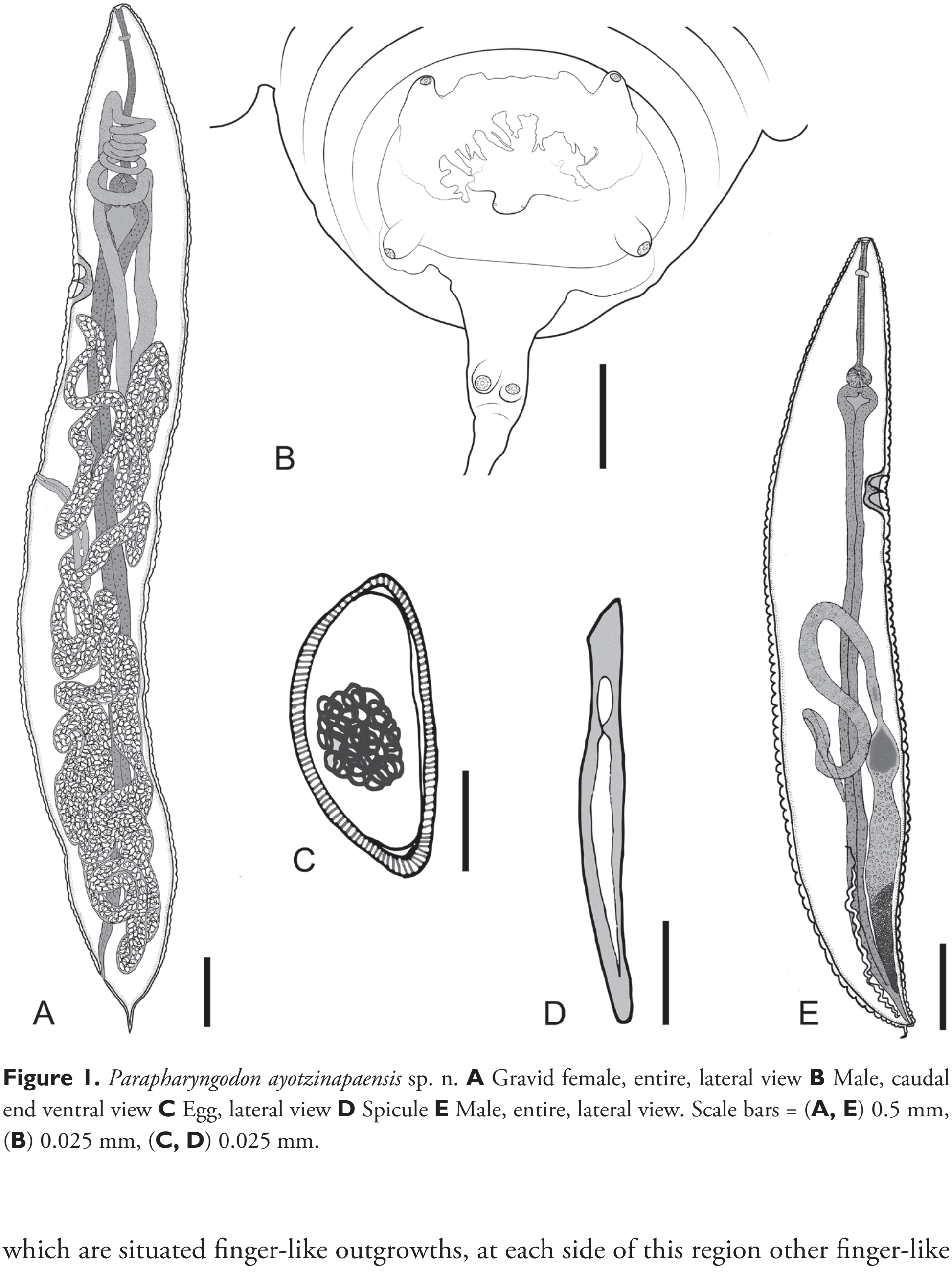

Figure 1. *Parapharyngodon ayotzinapaensis* sp. n. **A** Gravid female, entire, lateral view **B** Male, caudal end ventral view **C** Egg, lateral view **D** Spicule **E** Male, entire, lateral view. Scale bars = (**A, E**) 0.5 mm, (**B**) 0.025 mm, (**C, D**) 0.025 mm.

which are situated finger-like outgrowths, at each side of this region other finger-like outgrowths are situated. Posterior cloacal lip is divided into three lobes, the middle one is more developed than lateral lobes, it is 0.03–0.05 (0.04 ± 0.008, n=4) length and has a pair of tiny simple papillae (Fig. 2C). Caudal filament 0.09–0.1 (0.093 ± 0.006, n = 3) length. Phasmids situated on caudal filament base laterally. Spicule length 0.11–0.13 (0.12 ± 0.01, n = 3) 3.614% of body length, distal end obtuse and thinnest than proximal (Fig. 1D).

Description of female. Round anterior end and conical posterior end (Fig. 1A), total body length 6.41–9.32 (7.45 ± 1.149, n = 8) and maximum width 0.76–1.26 (0.94 ± 0.189, n = 8) at middle body region. Cuticle with wide transverse striae 0.07–0.09 (0.08 ± 0.01, n = 8) maximum width at esophageal bulb level. Triangular oral opening surrounded by three bilobed lips whose internal base are located three transverse complete plates, ventrolateral lips with one amphid each one located at dorsal lobe (Fig. 2F). Esophagus total length 1.17–1.35 (1.269 ± 0.073, n = 8) and maximum width 0.07–0.09 (0.076 ± 0.007, n = 8), esophageal bulb length 0.15–0.21 (0.177 ± 0.019, n = 7) and width 0.21–0.28 (0.24 ± 0.024, n = 7). Nerve ring and excretory pore 0.15–0.21 (0.181 ± 0.023, n = 8) and 1.71–2.4 (1.937 ± 0.237, n = 8) from anterior end, respectively. Sclerotized vulva at 2.95–4.13 (3.45 ± 0.396, n = 8) from anterior end. Vagina transversely directed and posteriorly flexed to posterior region of body. Didelphic, prodelphic, ovaries reach esophagus region coiling around prebulbar esophagus. Uterus reach caudal region in gravid individuals. Anus 0.4–0.48 (0.435 ± 0.039, n = 6) from posterior end. Phasmids 0.14–0.25 (0.204 ± 0.042, n = 8) from posterior end, located laterally at the base of the conical tail (Fig. 2F). Tail 0.2–0.26 (0.237 ± 0.026, n = 8) length. Eggs containing embryo in early stage of cleavage, oval, without alae, asymmetric, slightly flattened on one side and convex on the other side in lateral view, 0.07–0.09 (0.08 ± 0.006, n = 16) length by 0.03–0.05 (0.04 ± 0.005, n=16) width, shell with pores that cross the uppermost layer, radial striations in lateral view, subpolar operculum without pores (Figs 1C; 2H).

Distribution. Técpan de Galeana, Guerrero, Mexico (coordinates, see Table 1; elevation 22 m).

Biology. Nematode species parasite of the intestine of *Sceloporus pyrocephalus* Cope, collected on July 6, 2005.

Remarks. *Parapharyngodon ayotzinapaensis* sp. n. is the 79th species assigned to *Parapharyngodon* and the 48th valid species of the genus (Velarde-Aguilar et al. 2015; Bursey and Goldberg 2015). It is distinguished from the other *Parapharyngodon* species by a combination of characters including the possession of 4 pairs of caudal papillae, an echinate anterior cloacal lip, lateral alae covering the last third of body, spicule length representing 3.614% of body length, prebulbar ovaries coiling around prebulbar esophagus and eggs with punctuated shell and without alae. Of the 47 valid species before this study, 12 species have cloacal papillar arrangement similar to *P. ayotzinapaensis* (four pairs of caudal papillae: one precloacal, one paracloacal, one at postcloacal lip and one at caudal filament), as well as echinate precloacal lip and lateral alae, namely: *P. adramitana* Adamson & Nasher, 1984, *P. almoriensis* (Karve, 1949) Freitas, 1957, *P. anomalus* Hobbs, 1996, *P. brevicaudatus* Bogdanov & Markov,1955, *P. colonensis* Bursey, Goldberg & Telford, 2007, *P. dolgieli* (Markov & Bogdanov, 1965) Adamson & Nasher, 1984, *P. echinatus* (Rudolphi, 1819) Freitas, 1957, *P. grenadaensis* Bursey, Drake, Cole, Sterner, Pinckney & Zieger, 2013, *P. margaritiferi* Hering-Hagenbeck, 2001, *P. meridionalis* (Chabaud & Brygoo, 1962) Adamson, 1981, *P. micipsae* (Seurat, 1917) Freitas, 1957 and *P. rousseti* (Tcheprakoff, 1966) Adamson & Nasher, 1984. Only 2 of these species (*P. grenadaensis* and *P. colonensis*) shares with

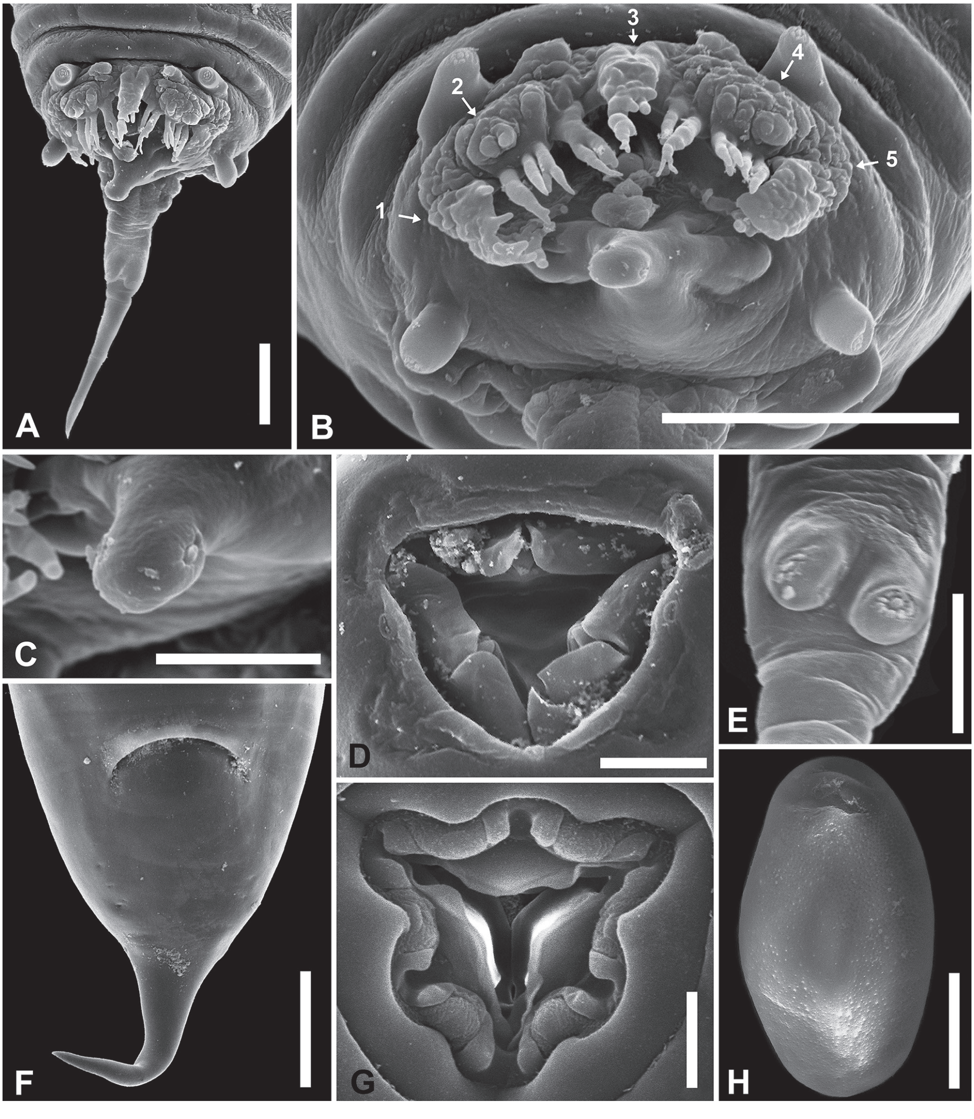

Figure 2. *Parapharyngodon ayotzinapaensis* sp. n. SEM microphotographs. **A** Male, ventral view of posterior end **B** Male, ventral view of posterior end showing cloacal lip and papillae (1-5 outgrowths ornamentation disposition) **C** Male, posterior end of post cloacal lip showing right papillae **D** Male, oral opening **E** Male, pair of papillae at caudal filament **F** Female, ventral view of posterior end **G** Female, oral opening **H** Egg. Scale bars = (**A**) 0.025 mm, (**B**) 0.04 mm, (**C, D, E, F, G**) 0.01 mm, (**H**) 0.02 mm.

the new species the egg shell punctuated and prebulbar ovaries in females; however, these two species differ in several morphological features with *P. ayotzinapaensis* sp. n.: *P. grenadaensis* and *P. colonensis* have three bilobed lips, which are simple in *P. ayotzinapaensis*; lateral alae extension covers from nerve ring level to precloacal papillae in *P. grenadaensis* and *P. colonensis*, meanwhile in *P. ayotzinapaensis* begins abruptly at last third of caudal region ending before paracloacal papillae. Finally, spicule length/body length ratio is greater in *P. grenadaensis* (4.488%) and *P. colonensis* (3.765%) than in *P. ayotzinapaensis* (3.614%). Therefore, we consider *P. ayotzinapaensis* to represent a new species, the 10th recorded in Mexico (Bursey and Goldberg 2005, Bursey et al. 2013, Velarde-Aguilar et al. 2015, Bursey and Goldberg 2015).

Parapharyngodon tikuinii **sp. n.**

http://zoobank.org/C9D2FD3C-F12E-47A8-A755-E06EEEE9502C
Figs 3A–D; 4A–F

Type material. Holotype, male, CNHE 9439. Allotype, female, CNHE 9440. Paratypes (8 males, 6 females), CNHE 9441–9447.

Etymology. The species is named after the Purepecha word "tikuini" which means lizard, referring to the host (Lathrop 1973).

Diagnosis. Fusiform and robust nematodes, males smaller than females. Cuticle with thin transverse striations constant in width along the whole body except tail. Triangular oral opening surrounded by three lips simple in males and bilobed in females, in both sexes ventrolateral lips with an amphid each one. Within buccal cavity, both sexes have three transverse plates, bilobed in males and complete in females. Esophageal bulb with sclerotized apparatus. Excretory pore evident, it is located posterior to esophageal bulb-intestine junction. A vesicular body surrounds excretory duct. Males with lateral alae covering almost of body length, females lacking lateral alae. Males without caudal alae. Four pairs of caudal papillae. Caudal filament subterminal and directed dorsally in males. Females with a conical posterior end. Vulva located at middle region. Eggs without alae with punctuated shell and a subpolar operculum, embryo in early stage of cleavage.

Description of male. Truncated at posterior end (Fig. 3D), total body length 1.9–3.575 (2.62 ± 0.5, n = 9) and maximum width 0.237–0.475 (0.326 ± 0.09, n = 6) at excretory pore level. Cuticle with transverse striae 0.015–0.03 (0.02 ± 0.005, n = 7) maximum width at esophageal bulb level. Triangular oral opening surrounded by three simple lips, at whose internal bases are located three transverse bilobed plates, ventrolateral lips with one amphid each one (Fig. 4A). Esophagus total length 0.342–0.53 (0.416 ± 0.056, n = 8) and maximum width 0.022–0.04 (0.0325 ± 0.006, n = 8), esophagus bulb length 0.075–0.105 (0.09 ± 0.01, n = 8) and width 0.08–0.11 (0.09 ± 0.012, n = 8). Nerve ring and excretory pore at 0.1–0.19 (0.147 ± 0.038, n = 6) and 0.71–1.02 (0.857 ± 0.11, n = 6) from anterior end, respectively. Testis extends from middle body region to level of anterior end of intestine. Hyaline lateral alae start at anterior region between nerve ring and excretory pore at 0.25–0.45 (0.334 ± 0.07, n = 6) from anterior end, with a maximum width of 0.06–0.075 (0.065 ± 0.008, n = 7) and extending to posterior end of body terminating abruptly before cloacal region at 0.12–0.435 (0.263 ± 0.11, n = 7) from the base of corresponding paracloacal papillae (Figs 3D; 4D). Four pairs of caudal rosette papillae as follows: one ventral precloacal

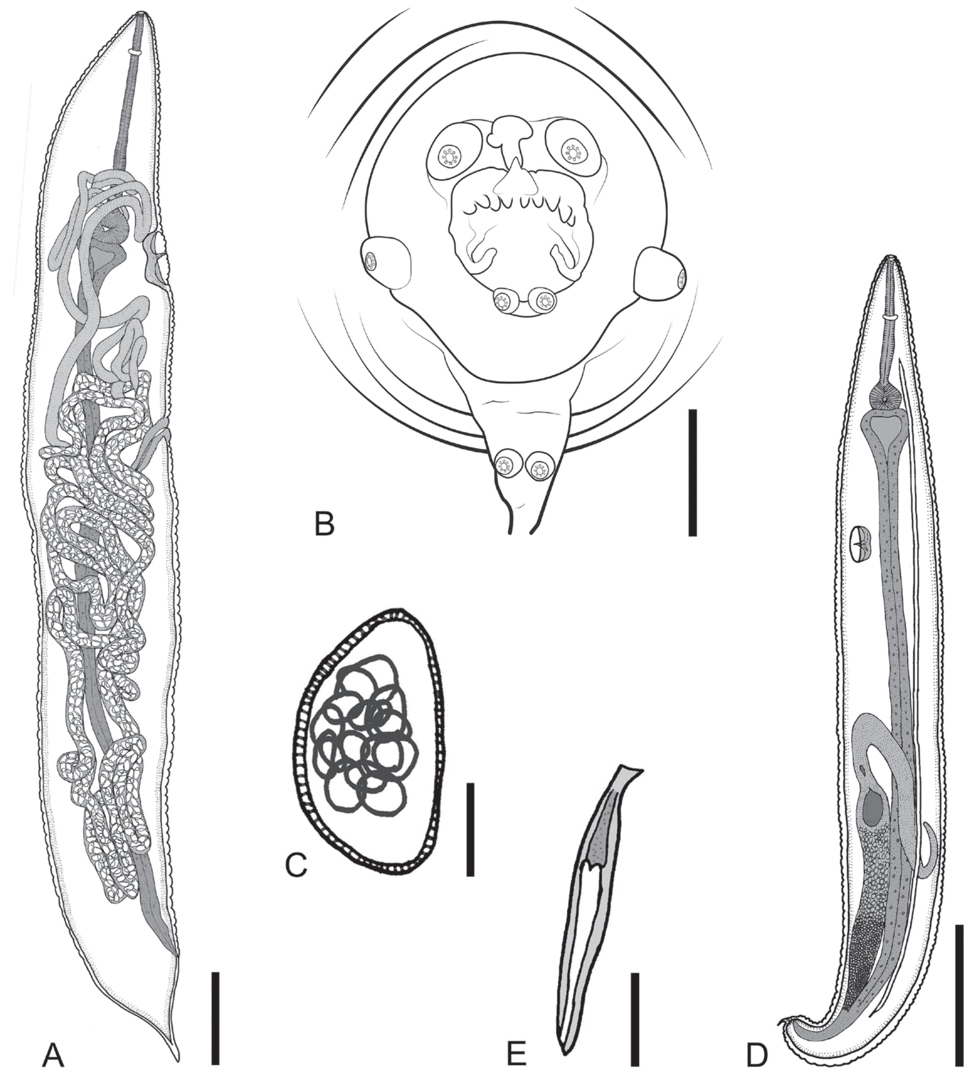

Figure 3. *Parapharyngodon tikuinii* sp. n. **A** Gravid female, entire, lateral view **B** Male, caudal end, ventral view **C** Egg, lateral view **D** Male, entire, lateral view **E** Spicule. Scale bars = (**A**) 0.5 mm, (**B**) 0.02 mm, (**C**) 0.025 mm, (**D**) 0.5 mm, (**E**) 0.0125 mm.

pedunculated, one lateral postcloacal pedunculated, one postcloacal mammilliform on the posterior base of postcloacal lip, and one mammilliform on the caudal filament (Figs 3B; 4C). Anterior cloacal lip echinate, with symmetrical ornamentation consisting of a smooth outgrowth with V-form at each side of cloacal opening, in the middle of the lip are located small equidistant simple finger-like outgrowths which vary in number (Figs 3B; 4C). Thick and smooth posterior cloacal lip, with a cuticular outgrowth at its base. This structure has two papillae at the top. Phasmids located at

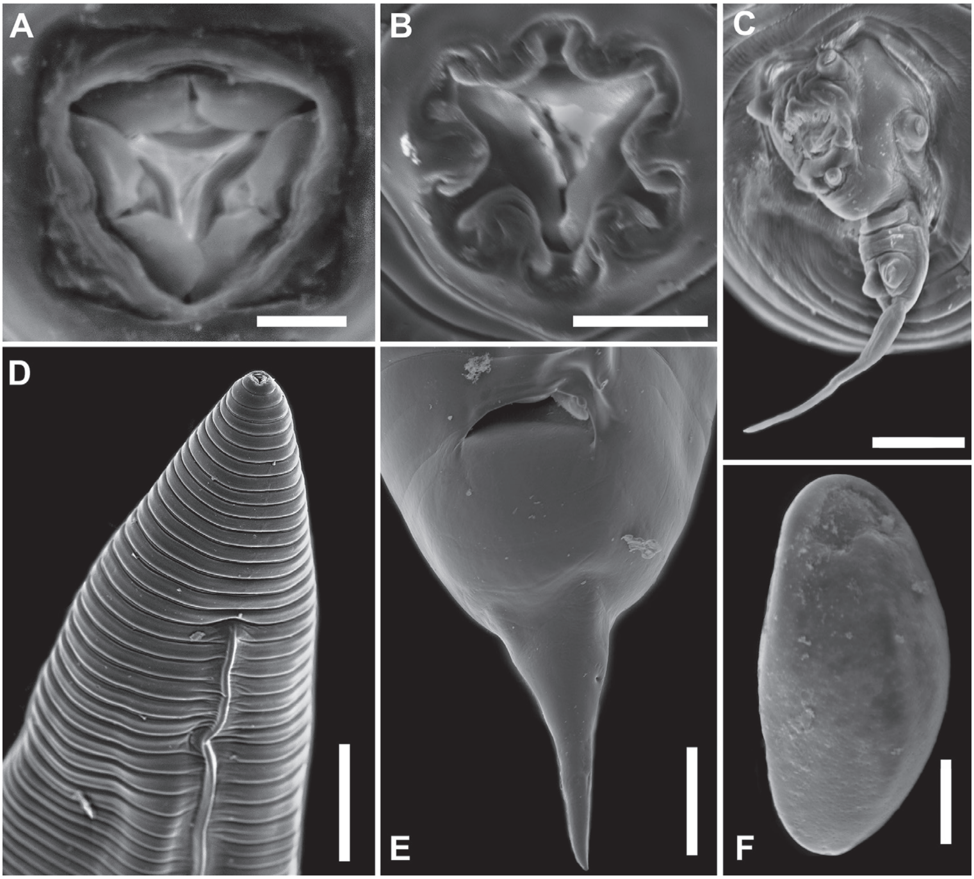

Figure 4. *Parapharyngodon tikuinii* sp. n. SEM microphotographs. **A** Male, oral opening **B** Female, oral opening **C** Male, posterior end, ventrolateral view **D** Male, anterior end showing lateral alae, lateral view **E** Female, posterior end, ventral view **F** Egg. Scale bars = (**A**) 0.05 mm, (**B**) 0.015 mm, (**C**) 0.025 mm, (**D**) 0.1 mm, (**E**) 0.1 mm, (**F**) 0.015 mm.

caudal filament base, whose length is 0.045–0.0775 (0.064 ± 0.013, n = 7). Spicule length 0.07–0.11 (0.093 ± 0.014, n = 7), 3.287% of total body length, proximal end wider than distal obtuse tip (Fig. 3E).

Description of female. Round anterior end, conical posterior end (Fig. 3A). Total body length 4.47–7.57 (6 ± 1.213, n = 7) and maximum width 1.04–1.3 (1.18 ± 0.09, n = 7) at middle body level. Cuticle with transverse striae 0.05–0.08 (0.06 ± 0.01, n = 7) maximum width. Triangular oral opening surrounded by three bilobed lips with an amphid located at dorsal lobe of each ventrolateral lip, at whose internal bases are located three transverse complete plates (Fig. 4B). Esophagus length 1.25–1.56 (1.367 ± 0.134, n = 4) and maximum width 0.07–0.085 (0.075 ± 0.005, n = 6), esophageal bulb length 0.187–0.257 (0.219 ± 0.029, n = 4) by 0.21–0.25 (0.236 ± 0.02, n = 4) width. Nerve ring and excretory pore at 0.15–0.28 (0.21 ± 0.065, n = 3) and 1.17–1.97 (1.658 ± 0.31, n = 5) from anterior end, respectively. Sclerotized vulva at 1.33–3.32 (2.61 ± 0.716, n = 7) from anterior end. Vagina transversely directed and posteriorly flexed to posterior region of body (Fig. 3A). Didelphic, prodelphic, ovaries reach esophagus region coiling around prebulbar esophagus. Uterus reaching caudal region in gravid individuals. Anus 0.44–0.64 (0.566 ± 0.065, n = 6) from posterior end. Phasmids 0.09–0.28 (0.182 ± 0.095, n = 3) from posterior end (Fig. 4E), located laterally at the base of the conical tail. Tail 0.182–0.285 (0.232 ± 0.04, n = 7) length. Eggs containing embryo in early stage of cleavage, oval, without alae, asymmetric, slightly flattened on one side and convex on the other side in lateral view, 0.067–0.087 (0.078 ± 0.006, n = 13) length by 0.02–0.05 (0.04 ± 0.008, n = 12) width, shell egg with pores that cross the uppermost layer, radial striations in lateral view, subpolar operculum smooth without pores (Figs 3C; 4F).

Distribution. Gabriel Zamora, Michoacán, Mexico (19°10'35"N, 102°03'48"W, elevation 752 m).

Biology. Nematode species parasite of the intestine of *Sceloporus pyrocephalus* Cope, collected on June 21, 2004.

Remarks

Parapharyngodon tikuinii sp. n. is the 80^{th} species assigned to *Parapharyngodon* and the 49^{th} valid species of the genus. It is characterized by the presence of a cuticular outgrowth at base of posterior cloacal lip. In addition, the following composition of traits allow us to differentiate the new species described herein: four pairs of caudal papillae, an echinate anterior cloacal lip, lateral alae covering almost the length of the body, spicule length 3.287% of total body length, prebulbar ovaries coiling around prebulbar esophagus and eggs shell punctuate. Nineteen of the 48 valid species described before (including *Parapharyngodon ayotzinapaensis*), share with *P. tikuinii* the arrangement of caudal papillae (four pairs of caudal papillae: one precloacal, one paracloacal, one at postcloacal lip and one at caudal filament). Thirteen of these species have echinate precloacal lip and lateral alae as the second new species described herein; of these, only *P. grenadaensis*, *P. colonensis* and *P. ayotzinapaensis* share the presence of prebulbar ovaries and a punctuate egg shell with *P. tikuinii*. Nonetheless, *P. tikuinii* differs from *P. grenadaensis*, *P. colonensis* and *P. ayotzinapaensis* in the lateral alae extension (which start at level of nerve ring and end at level of precloacal papillae in these three species, whereas in *P. tikuinii* lateral alae cover exclusively the last portion of the body); in addition, spicule length-total body length ratio is greater in *P. grenadaensis* (4.488%), *P. colonensis* (3.765%) and *P. ayotzinapaensis* (3.614%) than in *P. tikuinii* (3.287%) (Bursey and Goldberg 2005, Bursey et al. 2013, Velarde-Aguilar et al. 2015, Bursey and Goldberg 2015). Finally, males of *P. grenadaensis* and *P. colonensis* have three bilobed lips which are simple in *P. tikuinii*. Consequently, *P. tikuinii* is proposed as new species for the genus and the 11th recorded in Mexico.

Discussion

Parapharyngodon includes species parasitizing ectothermic vertebrates (mainly reptiles), few species of amphibians [*Rhinella marina* Linnaeus (Anura: Bufonidae) parasitized by *P. grenadaensis*, *Phrynohyas venulosa* Laurenti (Anura: Hylidae) parasitized by *P. duniae*, *Onychodactylus japonicus* Houttuyn (Caudata: Hynobiidae) parasitized by *P. japonicus*, *Triprion petasatus* Cope (Anura: Hylidae) parasitized by *P. hylidae*, and *Diaglena spatulata* Günther (Anura: Hylidae) parasitized by *P. chamelensis*] and one ancestral mammal species [*Tachyglossus aculeatus* Shaw (Monotremata: Tachyglossidae) (Irwin and Raharison 2009)]. Only two species of *Parapharyngodon* have been described as parasites of phrynosomatid lizards: *P. grismeri* and *P. iguanae* in *Petrosaurus repens* Van Denburgh and *Petrosaurus mearnsi* Stejneger, respectively (Paredes-León et al. 2008, Velarde-Aguilar et al. 2015). In this study, we describe two additional species infecting phrynosomatid lizards: *P. ayotzinapaenis* and *P. tikuinii*.

Some authors had emphasized the relationship between food habits and composition of helminths richness in reptiles (Martin et al. 2005, Pereira et al. 2013, Roca 1999, Roca et al. 2005). In this sense, Petter (1966) and Petter and Quentin (1976) recognized an evolutionary trend within Pharyngodonidae and distinguished two groups of genera: 1) Parasites of herbivorous iguanids and testudines, and 2) Parasites of omnivorous and insectivorous reptiles. In Mexico, nine pharyngodonid genera have been registered, five in herbivorous iguanids and testudines (*Ozolaimus* Dujardin, 1845; *Tachygonetria* Wedl, 1862; *Alaeuris* Thapar, 1925; *Thaparia* Ortlepp, 1933; *Gopheruris* Petter & Douglas, 1976), and four in omnivorous and insectivorous reptiles (*Parapharyngodon*; *Pharyngodon*, Diesing, 1861; *Spauligodon* Skrjabin, Schikhobalova & Lagodovskaja, 1960, and *Skrjabinodon* Inglis, 1968). In this sense, the presence of *Parapharyngodon* species infecting *S. pyrocephalus* confirm indirectly the feeding habits observed in this lizard, which is considered an omnivorous and insectivorous species by Alvarado-Diaz et al. (2009). On the other hand, there are scarce studies focused on evolutionary history of Pharyngodonidae and only include few genera from the same region (Jorge et al. 2011, Jorge et al. 2012) and some species parasitic in herbivores (Bouamer and Morand 2003). This information is not enough to attempt to relate the trend observed among pharyngodonids to parasitize hosts based on its different feeding habits with their evolutionary history. Robust analyses based on molecular and morphological information about the relationship of pharyngodonid species and their hosts will allow analysis of the patterns and process involved in their evolutionary history.

Key to Mexican species of *Parapharyngodon*

1	Lateral alae present	**2**
–	Lateral alae absent	***P. californiensis***
2	Lateral alae covering almost total body length	**3**

–	Lateral alae confined to posterior region of the body	**9**
3	With three pairs of caudal papillae	**4**
–	More than three pairs of caudal papillae	**7**
4	Lateral alae start at level of esophageal bulb	***P. guerreroensis***
–	Lateral alae start at half the length of the esophagus	**5**
5	Lateral alae extend to level of the first pair of precloacal papillae	***P. alvarengai***
–	Lateral alae extend to four fifth of the length body	**6**
6	Gubernaculum present	***P. hylidae***
–	Gubernaculum absent	***P. maestro***
7	With four pairs of caudal papillae	***P. tikuinii* sp. n.**
–	With three pairs of caudal papillae and one extra papilla	**8**
8	Protuberance in posterior cloacal lip present	***P. chamelensis***
–	Protuberance in posterior cloacal lip absent	***P. lamothei***
9	With four pairs of caudal papillae	***P. ayotzinapaensis* sp. n.**
–	With 3 pairs of caudal papillae	**10**
10	Middle pair of caudal papillae mammilliform	***P. grismeri***
–	Middle pair of caudal papillae digitiform	***P. iguanae***

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RESEARCH ARTICLE

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A new interstitial ostracod species of the genus *Paracobanocythere* from Vietnam, with mitochondrial *CO1* sequence data of three Asian species

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Abstract

This study is a first report of an interstitial ostracod from Southeast Asia. The ostracod species, *Paracobanocythere vietnamensis* sp. n., was found in the marine interstitial environment of Phu Quoc Island, Vietnam. Thus far, three species of this genus have been described. The morphology of the carapace as well as the appendages of this new species are quite similar to *P. hawaiiensis* and *P. watanabei*. However, we found that they could be easily distinguished according to the morphology of the male copulatory organ. Additionally, we estimated the evolutionary distances among these three species based on nucleotide and amino acid sequences of the mitochondrial *CO1* gene. Similar morphologies of carapaces and appendages, and relatively small evolutionary distances according to *CO1* between *P. vietnamensis* **sp. n.** and *P. watanabei* suggest that these two species are very closely related.

Keywords

DNA barcode, interstitial animals, meiofauna, Southeast Asia

Introduction

Ostracods are small bivalve crustaceans that inhabit various aquatic environments. They are one of the major constituents of the meiobenthos, especially interstitial animals inhabiting the pore space in sediment (Giere 2009). Interstitial ostracods are found in the Atlantic, Indian and Pacific Oceans (Rao 1972, Danielopol and Hartmann 1986). Although a number of taxonomic studies have been performed on extant and fossilized marine ostracods from the marginal sea located in Southeast Asia (e.g. Brady 1880, Müller 1906, Kornicker 1970, Keij 1974, 1975, Hanai et al. 1980, Tanaka et al. 2009), interstitial species have, thus far, not been reported.

This study is the first description of an interstitial ostracod species from Southeast Asia. The new species belongs to the genus *Paracobanocythere*, which shows typical features of interstitial taxa, including a small and dorso-ventrally depressed carapace inhabiting the interstices between grains of coarse sand (Gottwald 1983, Hartmann 1991, Higashi and Tsukagoshi 2011). Thus far, three species of this genus have been described; these include *Paracobanocythere hawaiiensis* Gottwald, 1983 (type species), from the island of O'ahu in Hawaii, as well as *P. grandis* Higashi & Tsukagoshi, 2011 and *P. watanabei* Higashi & Tsukagoshi, 2011, from the sand beach in Shizuoka Prefecture, on the Pacific coast of central Japan. Here, we describe a new species from Vietnam and supply DNA sequence data of the mitochondrial *cytochrome c oxidase subunit 1* (*CO1*) gene from the three described Asian species.

Materials and methods

Very coarse sand was collected from the Dăm Ngoài Island, in the Phu Quoc Marine Protected Area of Phu Quoc Island, from southwest Vietnam, 9°59'42"N, 104°02'17"E (Fig. 1) approximately 10 cm below the shoreline sand surface at low tide. The samples were washed five times in a bucket with sea water, and the supernatant was strained through a mesh with a 40-μm pore size. The living specimens were isolated from the deposits using a stereo-binocular microscope (SZ-60, OLYMPUS, Japan). The collected specimens were fixed in 80% ethanol and preserved at room temperature for description and DNA extraction. The soft parts of the organisms were separated from the valves and dissected using fine needles. The valves were preserved on a cardboard cell slide and the soft parts mounted in a gum-chloral medium, Neo-Shigaral (Shiga Konchu Fukyusha, Japan), on glass slides using a stereo-binocular microscope. The specimens were then observed and sketched using a transmitted-light binocular microscope (BX-53, OLYMPUS, Japan) with a differential interference contrast system and a camera lucida. The valves were washed with distilled water and gold-coated by an Ion sputtering device (JFC-1100, JEOL, Japan). The materials were then observed by SEM (JSM-6510LV, JEOL, Japan).

The type series was deposited in the collection of the National Museum of Nature and Science, Tokyo (NSMT), with the prefix 'NSMT-Cr'.

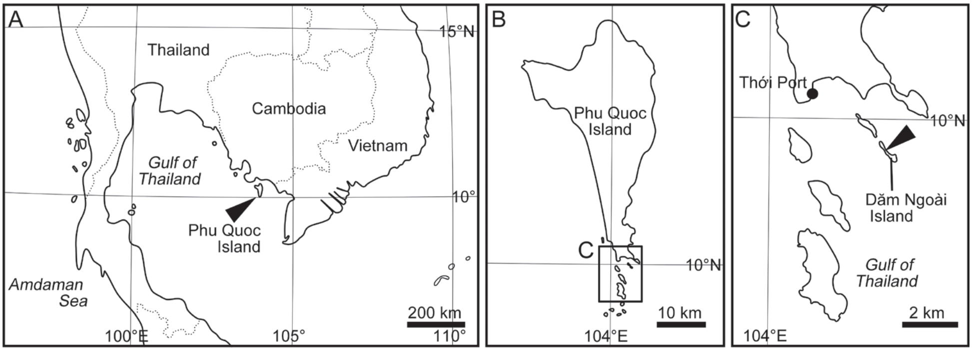

Figure 1. Map showing sampling locality of *Paracobanocythere vietnamensis* sp. n. **A** western part of Southeast Asia **B** Phu Quoc Island, Vietnam **C** The Dăm Ngoài Island. Arrowhead indicates the type locality.

DNA experiment and analyses

The specimens of both *Paracobanocythere grandis* and *P. watanabei* used in DNA extractions were collected from the type localities (See Higashi and Tsukagoshi 2011, fig. 1): Mochimune Beach (34°55'04"N, 138°21'43"E), Shizuoka, central Japan, on 1 Aug. 2015 and Oura Beach (34°40'05"N, 138°56'28"E), Shizuoka, central Japan, on 30 Sep. 2012. Details of the specimens from the three species used for DNA experiment are found in Table 1. Total DNA extraction was performed using the DNeasy Blood and Tissue Kit (Qiagen, USA) following the manufacturer's protocol, except that the elution volume used was changed to 100 µl. To prepare samples for DNA extraction individuals were dissected as follows: the carapace and gut content was removed before DNA extraction, and after the protein digestion step the chitinous soft parts were retrieved from the microtube and mounted in the same manner as method of dissected soft parts samples. The valves and chitinous soft parts were preserved as morphological voucher specimens, and deposited in the NSMT with serial numbers.

Partial sequences of the mitochondrial *CO1* gene were PCR amplified using the following primers: a degenerate forward primer (COIO_F 5'- CNACNAAYCAYAARGATATTGG -3') designed in this study and the universal reverse primer HCO2198 (Folmer et al. 1994). This region is the most commonly used for DNA barcoding to identify animals (Hebert et al. 2003, Bucklin et al. 2011). The 25 µl reaction contained 0.125 µl of *TaKaRa Ex Taq* HS (TAKARA BIO Inc., Japan), 2.5 µl of 10×*Ex Taq* buffer, 2 µl of dNTP mix, 2 µl of each primer (5 pmoles each), 2 µl of template DNA, and 14.375 µl sterilized distilled water. The PCR protocol consisted of an initial denaturation step at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 40 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Quantity and length of the PCR products were checked by 1% agarose S (Nippon Gene, Japan) gel electrophoresis and stained with ethidium bromide. The products were purified for sequencing using a FastGene Gel/PCR Extraction Kit (NIPPON Genetics Co,Ltd, Japan), according to the manufacturer's protocol. Sequencing

Table 1. List of *CO1* sequences and vouchers for the three *Paracobanocythere* species.

Species	Specimen catalog number	GenBank number
P. vietnamensis sp. n.	NSMT-Cr 24323 (paratype)	LC101962
P. vietnamensis sp. n.	NSMT-Cr 24324 (paratype)	LC101963
P. vietnamensis sp. n.	NSMT-Cr 24325 (paratype)	LC101964
P. grandis	NSMT-Cr 24335(topotype)	LC101965
P. grandis	NSMT-Cr 24336(topotype)	LC101966
P. grandis	NSMT-Cr 24337(topotype)	LC101967
P. watanabei	NSMT-Cr 24338(topotype)	LC101968
P. watanabei	NSMT-Cr 24339(topotype)	LC101969
P. watanabei	NSMT-Cr 24340(topotype)	LC101970

(of both the forward and reverse reads) was performed by the Macrogen Japan Corp. (Tokyo) with the same primers as were used for PCR amplification. A homology search of *CO1* sequences was performed by BLAST (Altschul et al. 1990, 1997) with the discontiguous megablast program from the National Center for Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The *CO1* sequences were converted to amino acids based on the invertebrate mitochondrial genetic codon using MEGA6 (Tamura et al. 2013). The evolutionary distances of both the nucleotide and amino acid sequences were estimated with MEGA6 (Tamura et al. 2013) using Kimura's two parameter model (Kimura 1980) and the Poisson model (Nei and Kumar 2000), respectively. Standard error estimates were obtained by a bootstrap procedure (1000 replicates).

Taxonomy

Order Podocopida Sars, 1866
Superfamily Cytheroidea Baird, 1850
Family Cobanocytheridae Schornikov, 1975
Genus *Paracobanocythere* Gottwald, 1983

Paracobanocythere vietnamensis Tanaka & Le, sp. n.
http://zoobank.org/EFD9A861-5477-488C-BC70-4B6861FFEB86

Type series. Holotype: adult male (NSMT-Cr 24314), right valve length 323 μm, height 107 μm, left valve length 337 μm, height 111 μm, soft parts mounted on a slide and valves preserved in a cardboard cell slide. Paratypes: 11 adult males (NSMT-Cr 24315–24325) and 9 adult females (NSMT-Cr 24326–24334). All specimens were collected by Hayato Tanaka on 21 November 2014.

Type locality. The holotype specimen was collected from Dăm Ngoài Island, Phu Quoc Marine Protected Area in Phu Quoc Island, the southwest Vietnam, 9°59'42"N, 104°02'17"E (Fig. 1C); in an interstitial environment at 10 cm below the shoreline sand surface. The substrate consisted mainly of very coarse sand (median grain size is about 2 mm).

Diagnosis. Carapace elongate in lateral view and depressed dorsoventrally. Anterior and posterior margins rounded. Carapace surface smooth but with small granular texture visible at high magnification. Sieve-type normal pores with recessed sieve plates and thick rims on carapace surface. Left hemipenis bearing one additional pincer-like structure and one hooked process.

Description of adult male. Carapace (Figs 2A–D, G–H, K, M–O; 3; 4). Length and height of left valves greater than those of right valve (Table 2). Carapace elongated in lateral view and depressed dorso-ventrally. In anterior view, carapace rounded triangular (Fig. 2K). Left valve slightly overlapping right valve along anterior and posterior margins. Anterior and posterior margin rounded in lateral view. Marginal infold broad along anterior margin and narrow in posterior area (Fig. 2C, D). Anterior vestibulum occupying most of area in marginal infold, containing five and six marginal pore canals in left and right valve, respectively (Fig. 3). In both valves a thick, irregular ridge runs diagonally upward across the anterior infold, and three short buttress-like ridges or wrinkles run anteriorly from the upper part of this ridge for additional strength. Adductor muscle scar pattern consisting of row of four elongate closely spaced scars and three frontal scars (Figs 2O; 3). Carapace surface with faint granular texture visible at high magnification, resulting from close-packed, tiny tubercules (Fig. 4A). Sieve type normal pores with recessed sieve plates and thick rims on carapace surface (Fig. 4). Pore systems with one bristle. Hingement adont type (Fig. 2M, N). Color translucent white; living individuals have brown granular patterns.

Antennula (Fig. 5A). Consists of six articulated podomeres, of which fourth and fifth are incompletely separated. First podomere bare. Second podomere about two and a half times as long as first podomere, with one long posterodistal seta and short setulae on distal end and eight coarser setulae on anterior margin. Third podomere same length as first podomere and bare. Fourth podomeres twice as long, with one long posterodistal seta. Fifth podomere almost as long, with three anterodistal setae of staggered lengths and one posterodistal seta. Sixth podomere long, slender, with three long anterodistal setae and long distal seta fused at its base with distal aesthetasc.

Antenna (Fig. 5B). Four articulated podomeres. First podomere (basis) bare and slightly triangular, tapering distally, with a long, thick, three-segmented exopodite (spinneret seta) reaching beyond distal claws. Second (first endopodial) podomere with one short seta on posterodistal end. Third podomere with one short and one medium anterodistal setae, one short posteromedial seta, and one short posterodistal seta. Fourth podomere with one long stout posterodistal seta and one curved stout distal claw.

Mandibula (Fig. 5C). Coxa with one short setulous seta on anterior margin. Coxal endite consisting of seven teeth, two short setae and one short claw-like seta. Palp consisting of four indistinct podomeres. Basis with one long setulous seta (exopodite) near proximal end and medium setulous seta on posterodistal end. First podomere of endo-

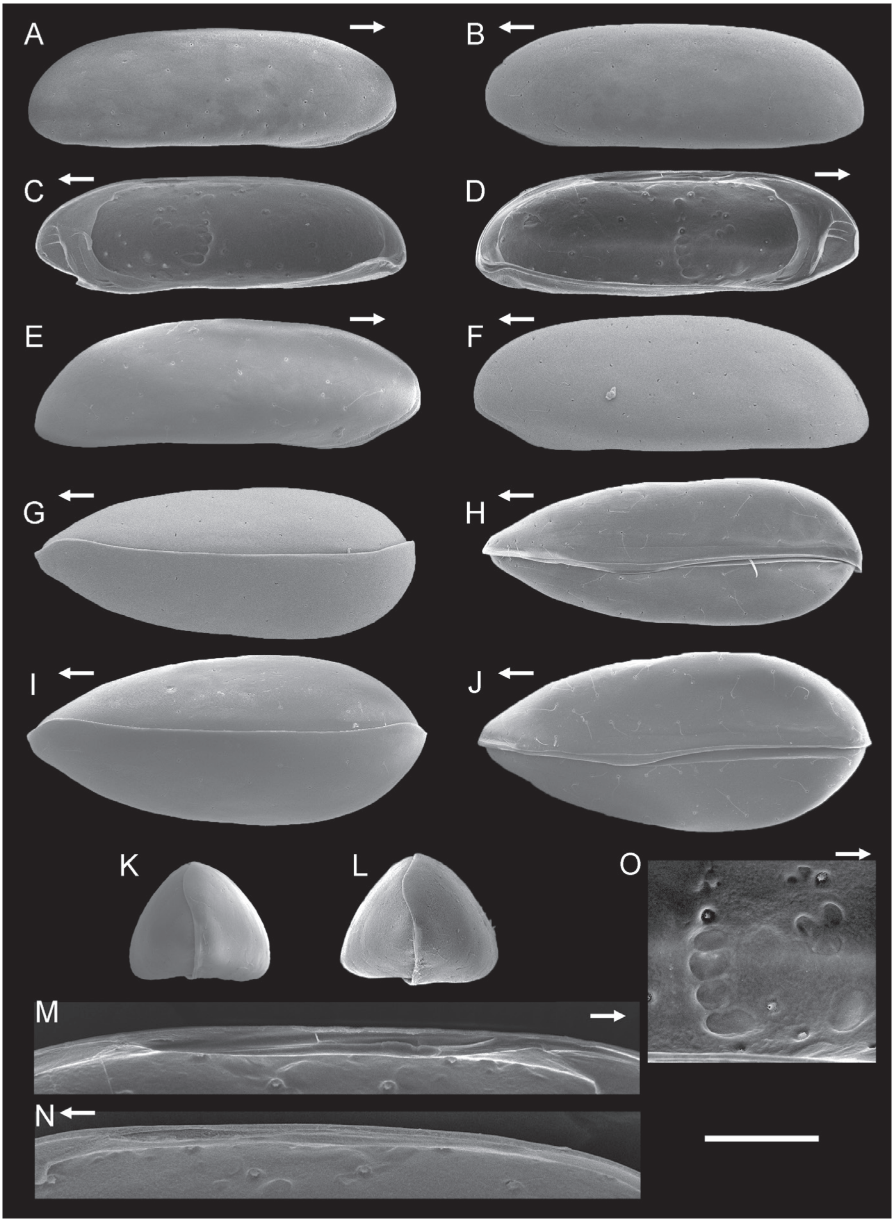

Figure 2. SEM images of valves and carapace of *Paracobanocythere vietnamensis* sp. n. **A** and **B** male paratype (NSMT-Cr 24315) **C, D, M–O** male, paratype (NSMT-Cr 24316) **E** and **F** female, paratype (NSMT-Cr 24327) **G** male, paratype (NSMT-Cr 24317) **H** male paratype (NSMT-Cr 24318) **I** female, paratype (NSMT-Cr 24328) **J** female, paratype (NSMT-Cr 24329) **K** male, paratype (NSMT-Cr 24319) **L** female, paratype (NSMT-Cr 24330). **A** right external lateral view **B** left external lateral view **C** right internal lateral view, anteroventral margin is slightly damaged **D** left internal lateral view **E** right external lateral view **F** left external lateral view **G** dorsal view **H** ventral view **I** dorsal view **J** ventral view **K** anterior view **L** anterior view **M** hingement part of left valve **N** hingement part of right valve **O** adductor muscle scars of left valve. Scale bar indicates 100 μm (**A–N**) and 40 μm (**O**). Arrows indicate anterior direction.

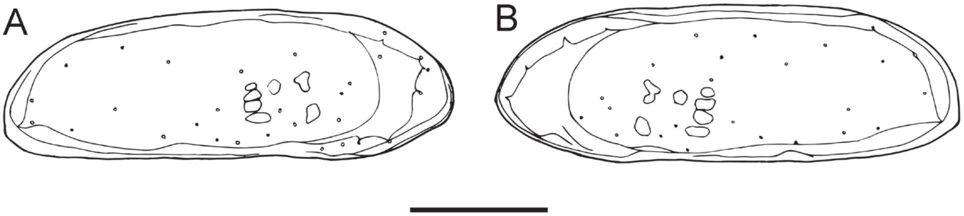

Figure 3. Valves of *Paracobanocythere vietnamensis* sp. n. Male, holotype (NSMT-Cr 24314). **A** left internal lateral view **B** right internal lateral view. Scale bar indicates 100 μm.

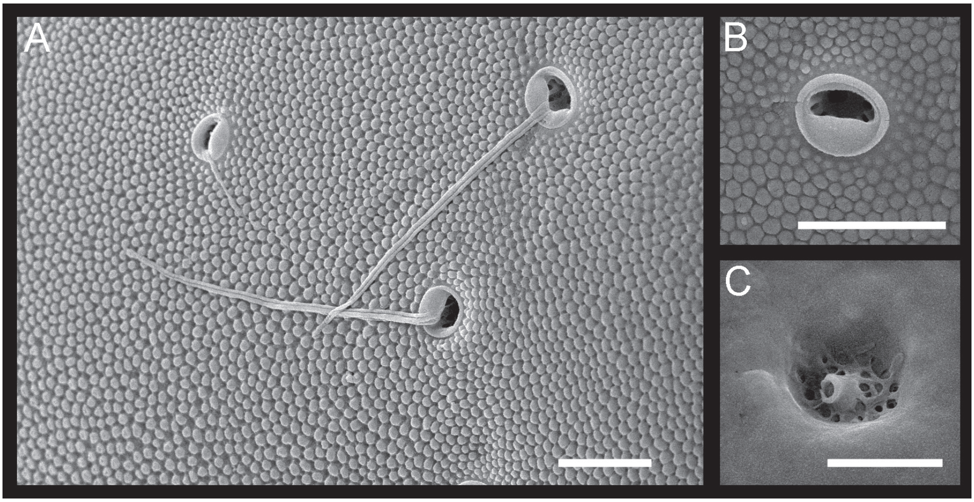

Figure 4. SEM images of the detailed structure of *Paracobanocythere vietnamensis* sp. n. **A** and **B** male paratype (NSMT-Cr 24315) **C** male paratype (NSMT-Cr 24316) **A** three pore systems and small granular texture in external lateral view **B** sieve type normal pore with recessed sieve plate and thick rim **C** internal view of sieve plate of sieve type normal pore. Scale bars indicate 5 μm.

podite about one and a half times as long as basis, with one medium anterodistal seta and setulae on anterior margin. Second podomere half as long as first podomere, with two long and one medium setulous setae on middle of anterior margin, one medium mediodistal seta, and one medium posterodistal seta. Third podomere small, with four medium setae on distal end.

Maxillula (Fig. 5D). Thin branchial plate (exopodite) with ten plumose setae. Basal podomere with one palp (endopodite) and three endites. Palp consisting of two distinct podomeres: first podomere with five long setae on distal end; second podomere two-thirds as long as first podomere, with one long and one medium setae on distal end. Outer two endites with five setae, and posteriormost one with four setae.

Male brush-shaped organ (Fig. 5E). Consisting of two branches (right and left) each with 16 setae on distal margin.

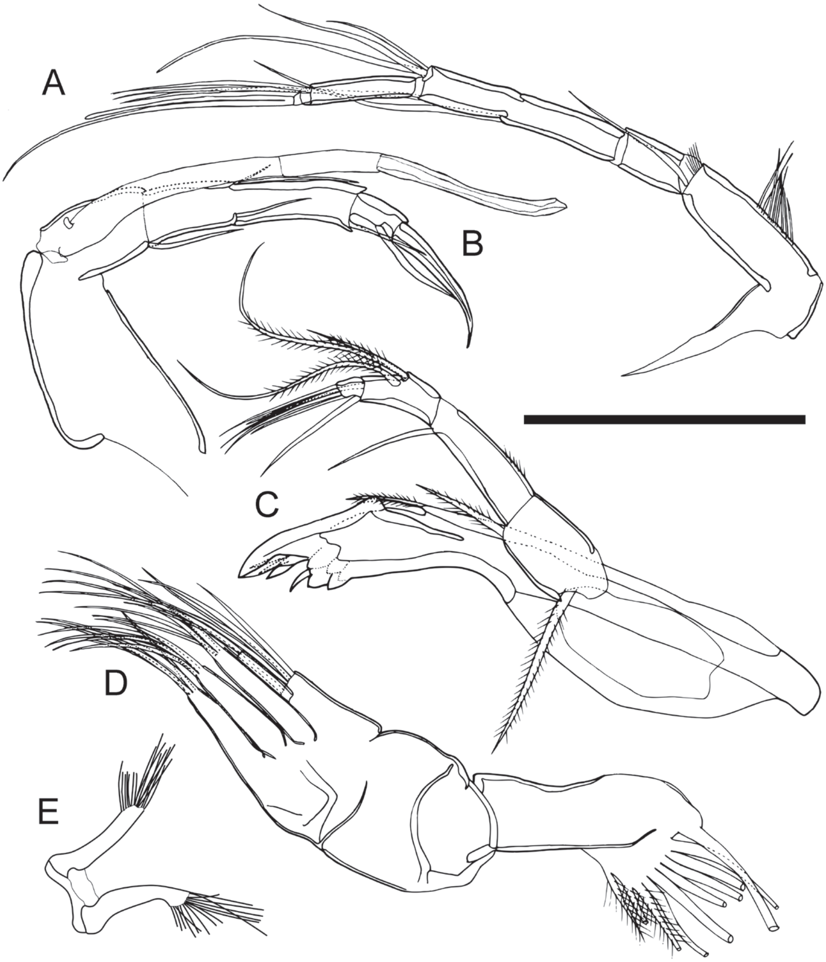

Figure 5. Appendages of *Paracobanocythere vietnamensis* sp. n. Male, holotype (NSMT-Cr 24314). **A** antennula **B** antenna **C** mandibula **D** maxillula **E** brush-shaped organ. Scale bar indicates 50 µm (**A–D**) and 25 µm (**E**).

Table 2. Dimensions of valves of *Paracobanocythere vietnamensis* sp. n.

		Length (µm)			Height (µm)		
		Mean	Observed range	N	Mean	Observed range	N
Male	Right valve	332	322–338	7	107	100–111	7
	Left valve	337	325–347	7	111	105–117	7
Female	Right valve	349	342–359	5	115	113–119	5
	Left valve	354	346–361	5	119	115–120	5

Fifth limb (Fig. 6A). Four articulated podomeres; two distal podomeres and claw somewhat thickened. First podomere with one medium setulous anterodistal seta, one long setulous posteroproximal seta and setulae along both margins. Second podomere

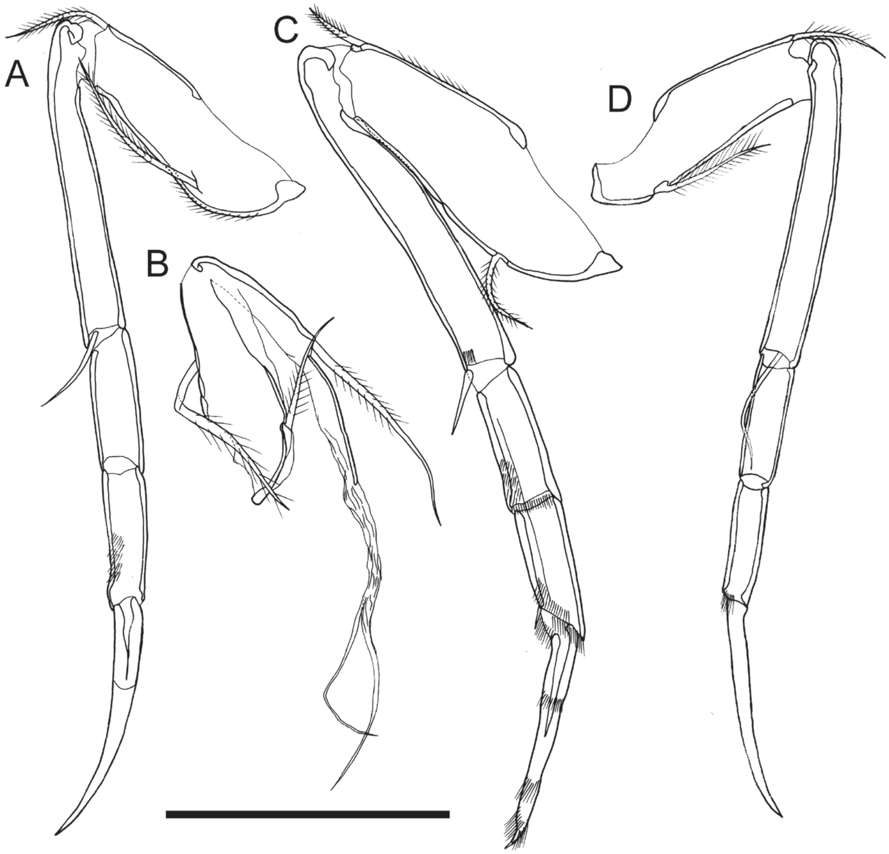

Figure 6. Appendages of *Paracobanocythere vietnamensis* sp. n. **A** and **C** male paratype (NSMT-Cr 24320) **B** male holotype (NSMT-Cr 24314) **D** female paratype (NSMT-Cr 24326) **A** fifth limb **B** sixth limb **C** seventh limb **D** sixth limb. Scale bar indicates 50 μm.

five-fourths as long as first podomere, with one short anterodistal seta. Third podomere bare and half length of second podomere. Fourth podomere same length as third podomere with rows of setulae on anterior surface and one stout distal claw.

Sixth limb (Fig. 6B). Three podomeres, of which two are flimsy and weakly developed. First podomere with one medium setulous seta on middle of anterior margin and one long setulous seta on middle of posterior margin. Second podomere two-thirds as long as first podomere, posterior margin flabby, with one long setulous seta on antero-distal end. Basal part of third podomere same length as second podomere, with flabby elongated sheet distally and two weakly developed long branches.

Seventh limb (Fig. 6C).Four articulated podomeres, all very large. First podomere with one short setulous seta on antero-distal end, one medium setulous seta on near postero-proximal part, and setulae along anterior margin. Second podomere five-fourths as long as first podomere, with one short seta and row of setulae on antero-distal end. Third podomere one-third as long as second podomere, with rows of setulae on

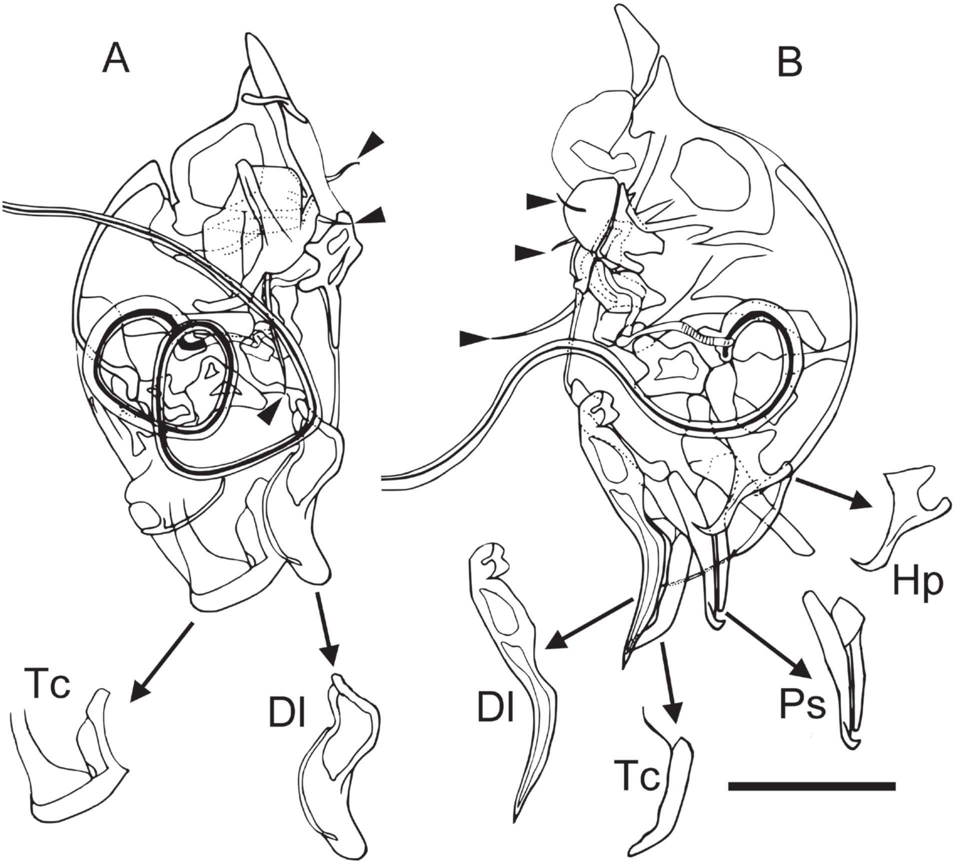

Figure 7. Copulatory organ of *Paracobanocythere vietnamensis* sp. n. Male, paratype (NSMT-Cr 24320) **A** internal view of right organ **B** internal view of left organ. Abbreviations: **Dl** distal lobe **Hp** hooked process **Ps** pincer-like structure **Tc** tip of capsule. Scale bar indicates 50 μm.

anterior surface and distal margin. Fourth podomere same length as third podomere, with rows of setulae on anterior surface and distal margin, with one stout, club-shaped distal claw with rows of setulae on middle part, near distal part, and around distal end.

Male copulatory organ (Fig. 7). Copulatory duct very long, more than length of capsule. Tip of capsule (Tc) and distal lobe (Dl) asymmetric in right and left hemipenes. Right hemipenis (Fig. 7A): Tc almost square distal part with rounded corner; Dl finger-shaped, bending ventrally at half. Left hemipenis (Fig. 7B): Tc slender, bending ventrally near the tip; Dl acute-angle triangular, curving ventrally at half; additional pincer-like structure (Ps) and hooked process (Hp) exist. Both hemipenes bearing one long and two short setae on ventral margin (vestigial furca).

Eye. Present.

Description of adult female. Carapace (Fig. 2E, F, I, J, L). Both left and right valve of female slightly greater than valves of male (Table 2, Fig. 9). In dorsal view,

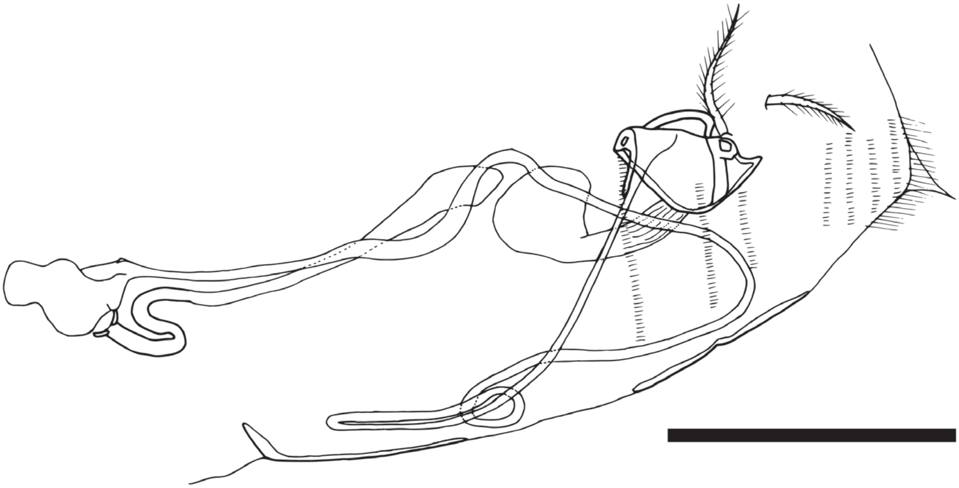

Figure 8. Posterior body and genitalia of *Paracobanocythere vietnamensis* sp. n. Female, paratype (NSMT-Cr 24326). Scale bar indicates 50 µm.

width of carapace slightly greater than that of male (Fig. 2I, J). Anterior and posterior margins more tapered rather than those of male (Fig. 2E, F).

Sixth limb (Fig. 6D). Four articulated podomeres with slender, more normal proportions than the male limb. First podomere with one medium setulous anterodistal seta and one medium setulous posteroproximal seta. Second podomere four-thirds as long as first podomere, with one short anterodistal seta. Third podomere bare and half as long as first podomere. Fourth podomere same length as third podomere, with row of setulae on anterior distal surface, with one tapering distal claw.

Posterior part of body and female genitalia (Fig. 8). Sclerotized framework of paired genital openings trapezoidal in shape. Spermathecal duct very long, connecting with genital opening and receptaculum seminis. Two setulose setae (vestigial caudal rami) situated near each genital opening. Five rows of tiny setulae on abdominal end.

Dimensions. See Table 2 and Fig. 9.

Occurrence. So far known only from type locality.

Etymology. Named in recognition of this being the first record of *Paracobanocythere* from Vietnam.

Remarks. *Paracobanocythere grandis* and other three species including *P. vietnamensis* sp. n. are easily distinguishable by the length of carapace (Table 3). While *P. grandis* has an exceptionally large carapace (approximately 500 µm) for this genus (Higashi and Tsukagoshi 2011), those of the other three species are relatively smaller (roughly 300 µm) (Gottwald 1983, Higashi and Tsukagoshi 2011). Furthermore, the female carapace larger than that of the male in *P. grandis*, and that is opposite to the status in the other three species (Table 3). The carapace shape as well as appendage morphologies including chaetotaxy and the number of podomeres, of *P. vietnamensis*

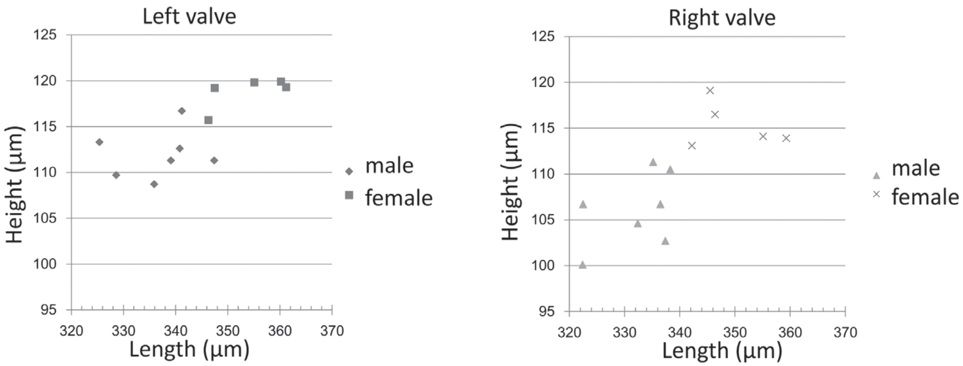

Figure 9. Scatter plots of valves of *Paracobanocythere vietnamensis* sp. n.

Table 3. Morphological difference among four species of *Paracobanocythere*.

Character	*P. hawaiiensis*	*P. grandis*	*P. vietnamensis* sp. n.	*P. watanabei*
Male				
Carapace, length (left; right) [μm]	256–290	497–519; 481–503	325–347; 322–338	240–254; 236–250
height (left; right) [μm]	84–97	166–175; 157–165	105–117; 100–111	67–82; 72–80
granular texture on surface	–	–	present	–
Antennula, one seta on middle of anterior margin of fourth podomere	present	present	absent	absent
Mandibula, one seta on antero-distal end of basis	absent	present	present	present
Maxillula, seta number on endites (anterior; middle; posterior)	5 to 6	(6; 5; 4)	(5; 5; 4)	(5; 5; 4)
Sixth limb, one short seta on antero-distal end of 1st podomere	present	present	absent	present
third podomere	weakly developed	clearly segmented	weakly developed	clearly segmented
Seventh limb, proximal spines and hook-shaped structure on distal claw	absent	presnt	absent	absent
Brush-shaped organ, seta number	12	16	16	16
Copulatory organ, pincer-like structure and hooked process on left hemipenis	absent	absent	present	absent
Female				
Carapace, length (left; right) [μm]	260–344	466–486; 457–471	346–361; 342–359	252–266; 246–259
height (left; right) [μm]	92–117	162–171; 151–158	115–120; 113–119	81–89; 81–86
length and height larger than male	no	yes	no	no

sp. n. are quite similar to those in *P. hawaiiensis* and *P. watanabei* (Table 3). One slight difference is found in the chetotaxy of the sixth limb of male, i.e., the first podomere lacks the one short seta on antero-distal end, which is present in *P. hawaiiensis* and *P.*

watanabei but absent in *P. vietnamensis* sp. n. Moreover, the faintly granular texture of carapace surface of *P. vietnamensis* sp. n. has never been reported from *P. hawaiiensis* and *P. watanabei*. As for the morphology of the male coplatory organ, the new species can be easily distinguished from these two species. Specifically, the left hemipenis of *P. vietnamensis* sp. n. possesses Ps and Hp, whereas these structures are not observed in the original description of either of the two other species (Table 3).

Evolutionary distances of nucleotide and amino acid sequences among three Asian species

The *CO1* sequences from *Paracobanocythere vietnamensis* sp. n., *P. watanabei* and *P. grandis* were obtained in this study. The lengths of the *CO1* barcoding region were 661 bp and the alignment of each sequence contained no indels. From this barcoding region, the first nucleotide was removed as it was not a complete codon, and the remaining 660 bp of the aligned sequence were translated into amino acid sequences. The evolutionary distances of both the nucleotide and amino acid sequences are shown in Table 4. The distances between *P. vietnamensis* sp. n. and *P. watanabei* are the least since this is the most closely related pair according to both the nucleotide and amino acid sequences.

Discussion

Paracobanocythere vietnamensis sp. n. closely resembles *P. watanabei* and *P. hawaiiensis* according to the appendage morphology, including chaetotaxy and shapes of the podomeres; however, they have divergent male copulatory organ morphologies (Table 3). Some interstitial ostracods, e.g., species from the genera *Microloxoconcha* and *Parapolycope*, are distinguished by specific differences only in the characters

Table 4. Evolutionary distances of *CO1* among three Asian species of *Paracobanocythere*. Standard error estimate are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates). **A** the result of nucleotide sequences with Kimura's two parameter model **B** the result of amino acid sequences with Poisson model.

A		1	2	3
	1 *P. vietnamensis* sp. n.		0,03	0,03
	2 *P. watanabei*	0,31		0,03
	3 *P. grandis*	0,41	0,37	
B		1	2	3
	1 *P. vietnamensis* sp. n.		0,02	0,04
	2 *P. watanabei*	0,12		0,03
	3 *P. grandis*	0,26	0,21	

associated with mating or courtship; these are not highly divergent in carapace morphology, and the other appendages have almost no differences (see Higashi and Tsukagoshi 2008, Tanaka and Tsukagoshi 2013). Therefore, most diagnostic characters appear in reproductive features such as the male copulatory organ, which can be used to identify interstitial species rather than surface-dwelling ostracods. This is possibly due to the simplification of appendage morphologies driven by the adaptation to the interstitial environment (Hartmann 1973, Maddocks 1976, Danielopol 1976) and the relatively large size of reproductive characters such as the male copulatory organ (Polilov and Beutel 2009). The *Paracobanocythere* species are likely not an exception to this observed taxonomic tendency.

We discovered that the evolutionary distances among three Asian species of *Paracobanocythere* ranged from 0.37±0.03 to 0.41±0.03 according to the nucleotide sequences (Table 4). This value is almost identical to the interspecies genetic distances of other podocopid ostracods (Yamaguchi 2000, Higashi et al. 2011) or somewhat larger (Martens et al. 2005, Brandăo et al. 2010, Karanovic 2015). The distance revealed by the nucleotide and amino acid sequences demonstrated that the distance between *P. vietnamensis* sp. n. and *P. watanabei* is smaller than that between *P. vietnamensis* sp. n. and *P. grandis* or *P. watanabei* and *P. grandis* (Table 4). It is highly possible that *P. vietnamensis* sp. n. and *P. watanabei* are phylogenetically closely related. The similar carapace and appendage morphologies (see Table 3) and small evolutionary distance between these lineages also supports this suggestion. In the future, the discoveries of additional undescribed species and molecular phylogenetic analyses with summarizing their fossil records will shed light on the evolutionary story of the interstitial genus *Paracobanocythere*.

This new species is the first marine interstitial ostracods described from Southeast Asia. Since there have been no taxonomic studies of interstitial ostracods in this region, their biodiversity has largely remained unknown. The Southeast Asian region (the Oriental realm) is known as a marine biodiversity hotspot (see Roberts et al. 2002), likely harboring an abundance of undescribed species. Therefore, we can expect that there are also highly diverse interstitial ostracods in the region since the majority of interstitial genera are distributed globally (Rao 1972, Hartmann 1973, Gottwald 1983, Higashi and Tsukagoshi 2008). Finally, we suggest that more intensive studies are needed in this area, which could reveal cryptic diversity of interstitial ostracods in this underexplored geographic location.

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A black-and-red stick insect from the Philippines – observations on the external anatomy and natural history of a new species of *Orthomeria*

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Abstract

A new stick insect of the genus *Orthomeria* Kirby, 1904 (Phasmatodea, Aschiphasmatidae) is described from the Philippines. *Orthomeria* (*O.*) *kangi* **sp. n.** is readily distinguished from all other congeners by the distinctive blood red colouration of the costal region of the hind wings. Major features of the external morphology of adults, eggs, and first-instar nymphs are illustrated. Locomotory attachment pads are of the smooth type with irregular microgrooves on the contact surface. An unusual condition of male terminalia is the absence of tergal thorn pads on segment 10. The male clasping organs are represented by an elongated vomer terminating in a prominent spine, and by incurved cerci featuring a bilobed apex equipped with a sharp blade-like ridge. Intraspecific variation in body colouration and hind wing length occurs in females. The new species lives at 400-650 m elevation in the surroundings of the Sablang and Tuba regions, in the Benguet Province of Luzon island. Host plants include *Ficus* spp. (Moraceae), and *Pipturus* spp. and *Leucosyke* spp. (Urticaceae). Observations on the mating and defensive behaviour are presented. *Orthomeria* (*O.*) *catadromus* (Westwood, 1859) is recognised as a junior synonym of *O.* (*O.*) *pandora* (Westwood, 1859), **syn. n.** A lectotype is designated for both species. Finally, an updated identification key to the species of the subgenus *Orthomeria* is provided.

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[*] Both authors contributed equally to this work and are considered co-first authors

Keywords
Insecta, Phasmatodea, Aschiphasmatidae, stick insects, new species, taxonomy, morphology

Introduction

The stick insect genus *Orthomeria* Kirby, 1904 belongs to the South-east Asian family Aschiphasmatidae (Bragg 2001), and includes ten species divided into two subgenera (Bragg 2006). The nominal subgenus *Orthomeria*, characterised by a relatively thickened body with dark brown or black basic colouration, comprises seven species found in Borneo, Seram, Sulawesi, Sumatra, and the Philippines (Bragg 2001). The subgenus *Parorthomeria*, characterised by a slim green body and setose eggs, contains the remaining three species that are endemic to Borneo (Bragg 2006).

As part of our research on the Philippine stick and leaf insect fauna (Gottardo 2007; Gottardo 2008; Hennemann et al. 2009; Bresseel 2012), a field expedition was carried out to the Benguet province of Luzon island during which several specimens of *Orthomeria* were found. After a careful examination of the general anatomy, the specimens were assumed to represent a new species of the subgenus *Orthomeria* showing an original set of morphological traits, including a distinctive red colouration on the wings which was not previously reported within the genus (see Bragg 2001).

The aim of this study is to provide a formal description of the new species. Some features of the external anatomy such as attachment devices and male terminalia are characterised in detail for the first time in *Orthomeria*. The morphological data are integrated with observations on the habitat and various life traits. We also provide an updated identification key to the species of the subgenus *Orthomeria* adapted from Bragg (2001).

Material and methods

Orthomeria specimens were collected at night by searching the vegetation along road sides. Specimens were euthanized in glass jars with fumes of ethyl acetate, and subsequently preserved dried and pinned. Some adult females were kept alive to obtain eggs. Linear body dimensions were taken with digital calipers (to the nearest 0.1 mm). The description of chromatic characters was based on live specimens. Observations on the external morphology were carried out with a Zeiss Stemi DV4 stereo light microscope. Photomicrographs were taken with a Nikon D200 SLR digital camera equipped with Nikon Micro-Nikkor AI-s 105 mm f/2.8 lens or with Nikon 24 mm f/2.8 AI-s lens. For scanning electron microscopy (SEM) observations, samples were dehydrated through a graded ethanol series and dried with CO_2 at the critical point (Balzers CPD 030). Dried samples were mounted on aluminium stubs, sputter coated with gold

(Balzers MED 010), and observed with a Philips XL20 scanning electron microscope operating at an accelerating voltage of 10 kV.

The wing venation nomenclature follows Ren (1997). Terminology of eggshell features follows Clark Sellick (1997).

The following acronyms are used to designate the collections: BMNH - The Natural History Museum, London, United Kingdom; MSNG – Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy; OC - Private collection Oskar V. Conle, Oberstaufen, Germany; RBINS - Royal Belgian Institute of Natural Sciences, Brussels, Belgium; UMO - University Museum, Hope Entomological Collections, Oxford, United Kingdom; UPLBM - Museum of Natural History, University of the Philippines at Los Banos, Laguna, Philippines.

Results

Orthomeria (*Orthomeria*) *kangi* sp. n.

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Holotype. 1 ♂, Philippines, Luzon Island, Benguet, Sablang, Barangay Bayabas, 5.VI.2014, leg. T. Heitzmann (MSNG) (Fig. 1).

Paratypes. 1 ♂, 2 ♀♀, and eggs (MSNG); 1 ♂, 1 ♀, and eggs (OC); 1 ♂, 1 ♀, and eggs (RBINS); 1 ♂, 1 ♀, and eggs (UPLBM), same data as for holotype.

Additional material examined. 3 ♂♂, 3 ♀♀, eggs (reared by D. Vallotto 2015; origin, same locality as for holotype; used for macrophotography); 2 ♂♂ (reared by M. Gottardo 2015; origin, same locality as for holotype; used for SEM).

Etymology. This species is named after Albert Kang (Manila) who discovered the first specimens during a photographic trip down the Benguet province of the Philippines.

Diagnosis. A new species of *Orthomeria* (*Orthomeria*) characterised by (1) relatively small body size, ♂♂ ca. 40 mm in length or shorter, ♀♀ less then 50 mm in length; (2) tegmina blood red; (3) costal area of hind wings with blood red markings; (4) tergum 7 of ♀♀ pale brown with a definite black longitudinal line centrally; (5) relatively short hind wings, only slightly projecting over abdominal tergum 5.

Description of the male. A full set of measurements is presented in Table 1.

Colouration: Body, compound eyes, antennae and legs black. Tegmina red. Costal region of hind wings blood red with a longitudinal black narrow stripe on posterior margin. Anal region of hind wings dark brown (Figs 1, 2A).

Head: In lateral view (Fig. 3), head capsule prognathous, almost as long as the pronotum. Vertex relatively flat. Compound eye large and circular, distinctly protruding hemispherically. Gena narrower than the diameter of the compound eye. Paraglossae not distinctly surpassing the anterior margin of the labrum. In dorsal view (Fig. 4A), head capsule slightly wider than long; dorsal surface with four shallow median depression between the compound eyes. Coronal suture barely recognizable. Ocelli lacking. In ventral view (Fig. 4B), frontal convexity ventrad the antennal base present.

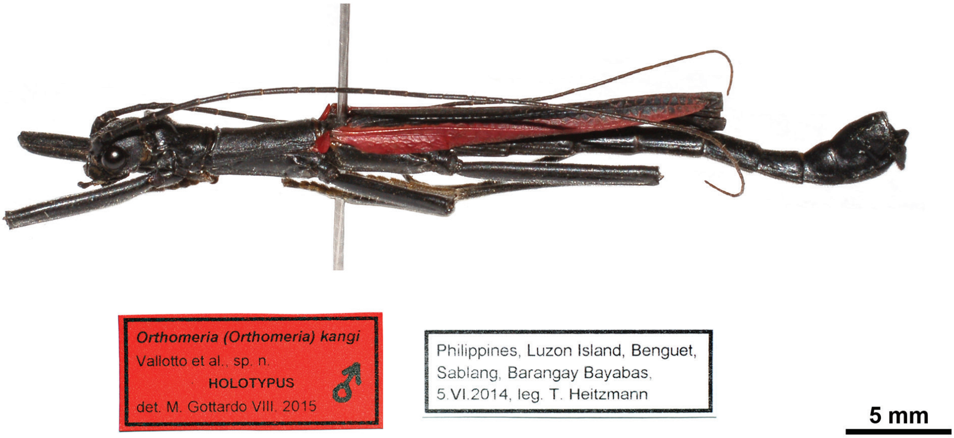

Figure 1. *Orthomeria* (*Orthomeria*) *kangi* sp. n. holotype ♂, habitus, lateral view.

Table 1. Morphometric data for the type specimens of *Orthomeria* (*Orthomeria*) *kangi* sp. n. from Benguet, Philippines.

Parameter	♂ holotype	♂♂ paratypes	♀♀ paratypes
Measurement (*mm*)			
Body length	38.8	37.3–40.1	41.8–46.2
Antenna length	38.4	34.3–35.3	38.7–41.0
Head length	3.0	2.8–3.3	3.8–5.5
Pronotum length (PL)	3.6	2.8–3.2	3.9–4.5
Mesonotum length (MOL)	4.9	4.4–4.8	5.7–6.9
Metanotum length (MAL)	2.2	1.9–2.3	2.6–2.8
Median segment length (MSL)	3.7	3.5–4.0	4.1–4.5
Tegmina length	1.3	1.1–1.3	0.9–1.6
Hind wing length	19.0	17.0–18.4	14.9–18.9
Fore femur length	6.9	6.9–7.0	6.8–7.9
Fore tibia length	6.0	6.0–6.1	6.4–7.4
Mid femur length	6.4	5.7–6.0	6.3–6.7
Mid tibia length	5.8	5.4–5.7	6.1–6.7
Hind femur length	9.6	8.6–9.6	8.7–10.1
Hind tibia length	9.4	9.0–9.3	9.4–10.2
Cercus length	1.9	1.9–2.3	1.2–1.4
Morphometric ratios			
MOL divided by PL	1.36	1.50–1.57	1.46–1.53
MAL divided by MOL	0.45	0.43–0.48	0.41–0.46
MSL divided by MAL	1.68	1.74–1.84	1.58–1.61

Labrum notched anteromedially. Median gular sclerite present and small (not shown). Palpomeres of labial and maxillary palps cylindrical. Antenna filiform, slightly shorter than body length (Fig. 2A), consisting of ca. 58 antennomeres; scapus roughtly rec-

Figure 2. *Orthomeria* (*Orthomeria*) *kangi* sp. n. habitus, dorsal view **A** Adult ♂ **B** Adult ♀.

tangular; pedicellus cylindrical, shorter than scapus (Fig. 4); first flagellomere twice as long as pedicel. Antennifer absent.

Thorax: Prothorax longer than head. Pronotum longer than wide (Fig. 4A); anterior half approximately one-third higher than posterior half (Fig. 3); front margin very moderately concave with narrow elliptical glandular field laterally (Fig. 3); hind margin rather straight. Paranota well-developed. Prothoracic coxopleurite subtriangular;

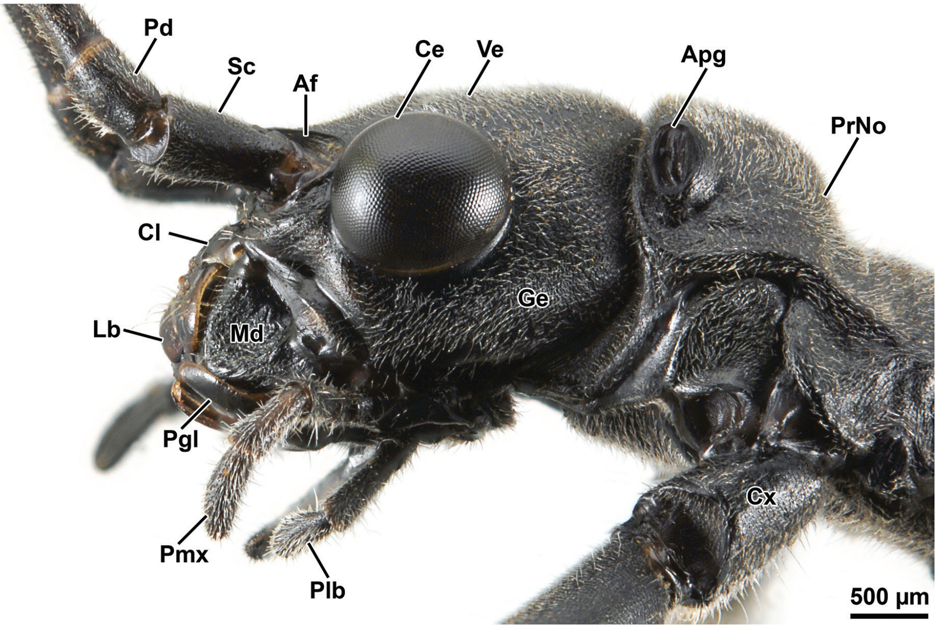

Figure 3. *Orthomeria* (*Orthomeria*) *kangi* sp. n. ♂ head and prothorax, lateral view; Apg, aperture of pronotal gland; Af, antennal field; Ce, compound eye; Cl, clypeus; Cx, coxa; Ge, gena; Lb, labrum; Md, mandible; Pd, pedicellus; Pgl, paraglossa; Plb, labial palpus; Pmx, maxillary palpus; PrNo, pronotum; Sc, scapus.

prothoracic basisternum with bell-shaped outline (Fig. 4B). Mesothorax very moderately constricted anteriorly, then slightly widening posteriorly. Mesonotum with concave front and hind margins, about 1.70 times length of pronotum. Basisternum flat; precoxale narrow with subtriangular outline; furcasternum with clearly demarcated furca (Fig. 4B). Metathorax only slightly shorter then mesothorax. Metanotum with globose and strongly setose prescutum (Fig. 5A). Metathoracic pleural and sternal regions as in the mesothorax but shorter.

Wings: Tegmina very small, oval, without shoulder pads (Fig. 5A–B). Hind wing slightly extending beyond the fifth abdominal tergum (Figs 1, 2A). Area between anterior wing margin and posterior Subcosta bent laterally; posterior Subcosta weak, not reaching the wing apex; Radius strongly sclerotized and unbranched, parallel to posterior Subcosta; anterior Media and posterior Media simple and straight; Cubitus + first anterior Analis straight; hind wing fan oval (Fig. 5C).

Legs: Hind leg distinctly projecting beyond the abdomen (Fig. 2A). Coxa unarmed; trochanters small and fused to femora (Fig. 4B). Femora semicircular in cross section with weakly developed carinae. Fore femur straight basally, unarmed (Fig. 2A). Mid femur with 3 minute spines on the ventro-anterior carina. Hind femur with 4–7 small spines on the ventro-anterior carina and about 3 minute spines on the ventro-posterior carina. Tibiae unarmed and circular in cross section, lacking carinae, with V-shaped area apicalis (Fig. 6A). Tarsus more than two-thirds the length of the cor-

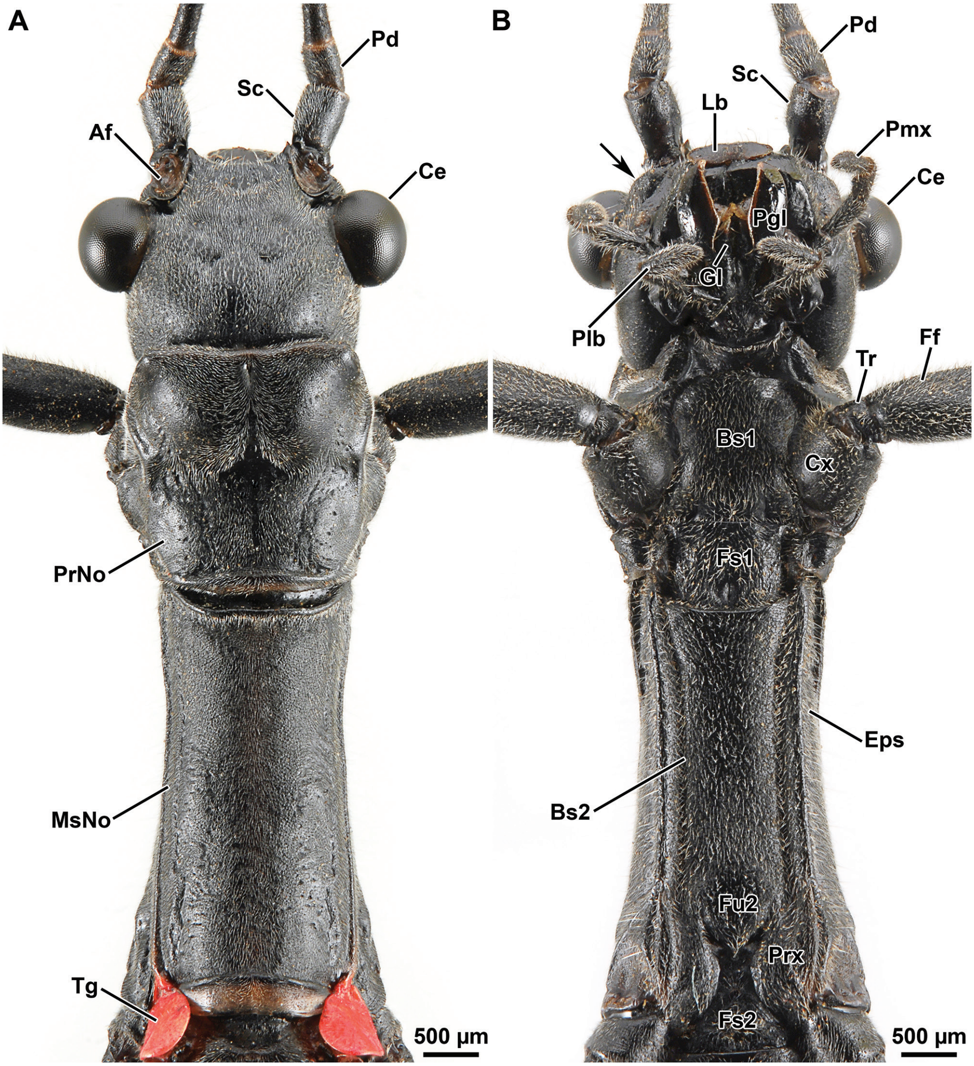

Figure 4. *Orthomeria* (*Orthomeria*) *kangi* sp. n. ♂ head and pro- and mesothorax **A** dorsal view **B** ventra view; Af, antennal field; Bs1-Bs2, pro- and mesothoracic basisterna; Ce, compound eye; Cx, coxa; Eps, episternum; Ff, fore femur; Fs1-Fs2, pro- and mesothoracic furcasternites; Fu2, mesothoracic furca; Gl, glossa; Lb, labrum; Pd, pedicellus; Pgl, paraglossa; Plb, labial palpus; Pmx, maxillary palpus; PrNo, pronotum; Prx, precoxale; Sc, scapus; Tg, tegmina; Tr, trochanter; Arrow, frontal convexity.

responding tibia (Fig. 2A). Proximal tarsomere (1) elongated, about as long as combined length of tarsomeres 2–4; tarsomeres 1–4 progressively shorter; distal tarsomere (5) distinctly shorter than combined length of tarsomeres 1–4 (Fig. 6A). Tarsomeres 1–4 each with a small euplantula, absent on tarsomere 5 (Fig. 6A). Pretarsus with well developed unguitractor plate; arolium large with broad outer band covered with oval or rounded outgrowths (Fig. 6B–C); pretarsal claws distinctly pectinate (Fig. 6D).

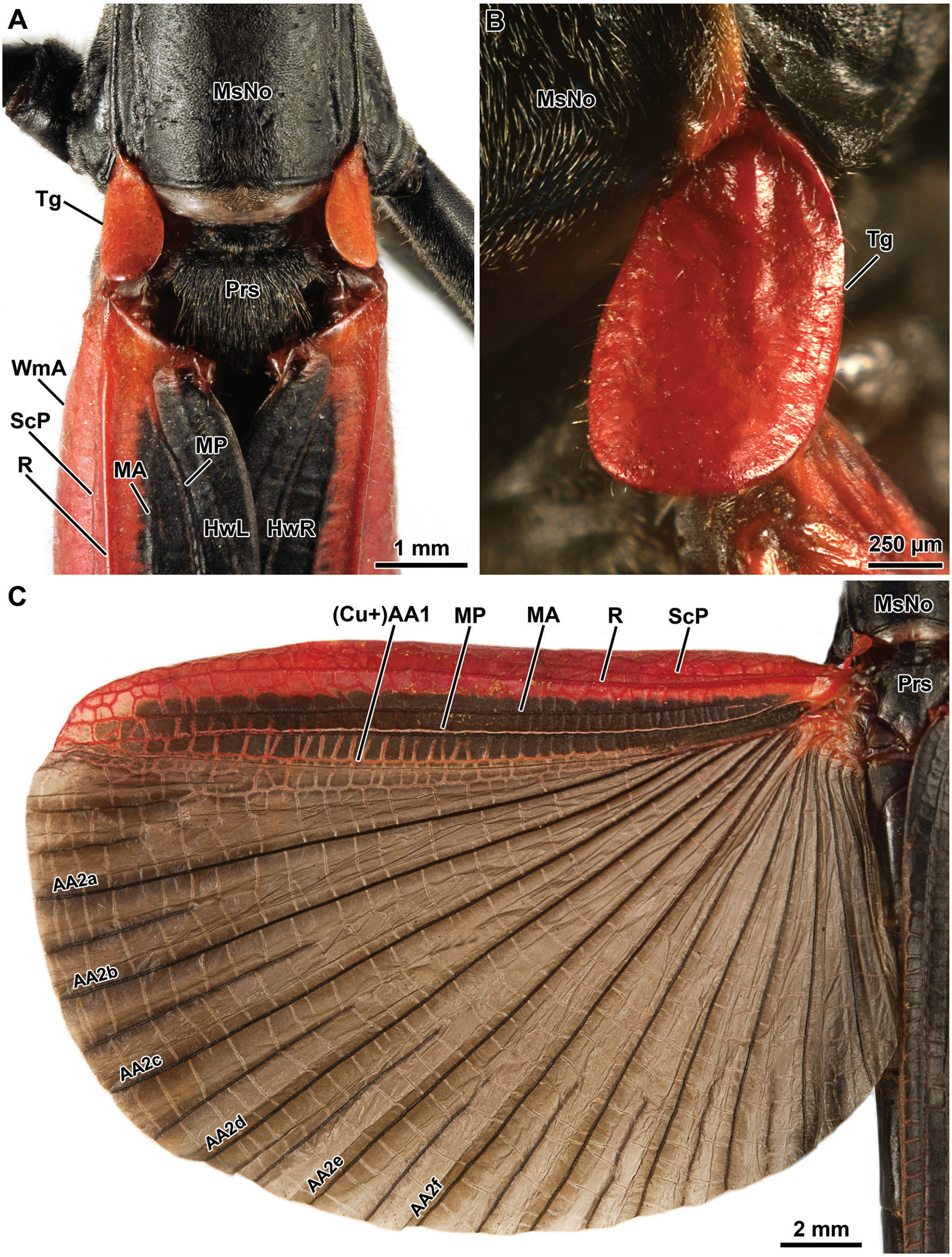

Figure 5. *Orthomeria* (*Orthomeria*) *kangi* sp. n. ♂ **A** Pterothorax, dorsal view **B** Right tegmina, lateral view **C** Left hind wing, dorsal view; AA2a-f, second anterior Analis; (Cu+)AA1, Cubitus + first anterior Analis; HwL, Left hind wing; HwR, right hind wing; MA, anterior Media; MP, posterior Media; MsNo, mesonotum; Prs, prescutum; R, Radius; ScP, posterior Subcosta; Tg, tegmina; WmA, anterior margin of the hind wing.

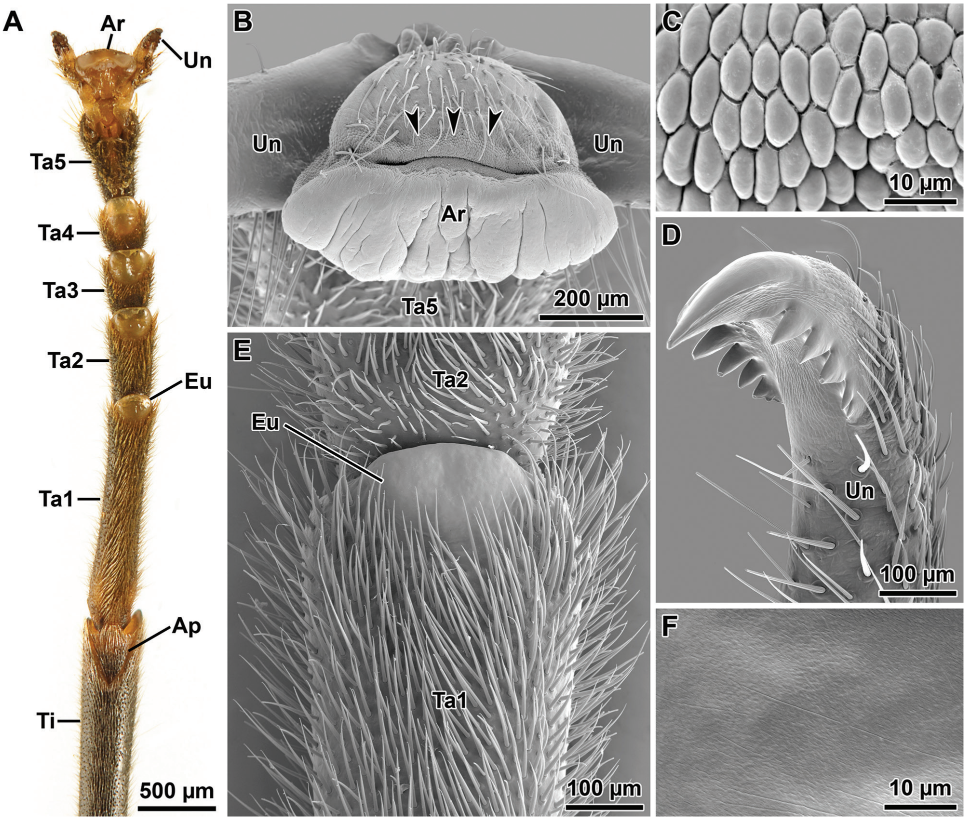

Figure 6. *Orthomeria* (*Orthomeria*) *kangi* sp. n. tarsal and pretarsal attachment devices **A** Fore tarsus, ventral view **B** Arolium, frontal view, arrowheads indicate the outer band **C** Arolium's micropattern at the outer band level **D** Pretarsal claw, lateral view **E** Tarsal euplantula, ventral view **F** Euplantula's micropattern; Ap, area apicalis; Ar, arolium; Eu, euplantula; Ta1-Ta5, tarsomeres 1-5; Ti, tibia; Un, peretarsal claws.

Euplantulae without transverse furrows and lacking sensory bristles (Fig. 6E); surface microstructure smooth with irregular patterns of microgrooves (Fig. 6F).

Abdomen: About 1.60 times length of head and thorax combined. Segments 2–5 increasing in length, 6–9 decreasing in length, 10 about 1.40 times length of 9. First tergum (= median segment) longer than wide, and longer than metanotum, fused to the metanotum. Terga 2–6 longer than broad; terga 7–10 broader than long (Fig. 7A). Sternum 1 fused with the metasternum. Sterna without carinae. Sternum 9 undivided, upcurving and slightly boat shaped in lateral view (Fig. 7B), about 2.50 times length of sternum 8 (Fig. 7C), apex rounded distinctly projecting beyond segment 10. Tergum 10 slightly longer than tergum 9, hind margin concave dorso-medially (Fig. 7A). Tergal thorn pads lacking ventrally (Fig. 8A–B). Epiproct very short, triangular; paraprocts sub-triangular with a straight inner side (Fig. 8A). Cerci about 1.30 times length of tergum 10 (Fig. 7B), slightly flattened and gently incurved, clasper-like in appearance (Figs 7A–C, 8A). Distal tip of cerci broadened, vaguely bilobed; outer

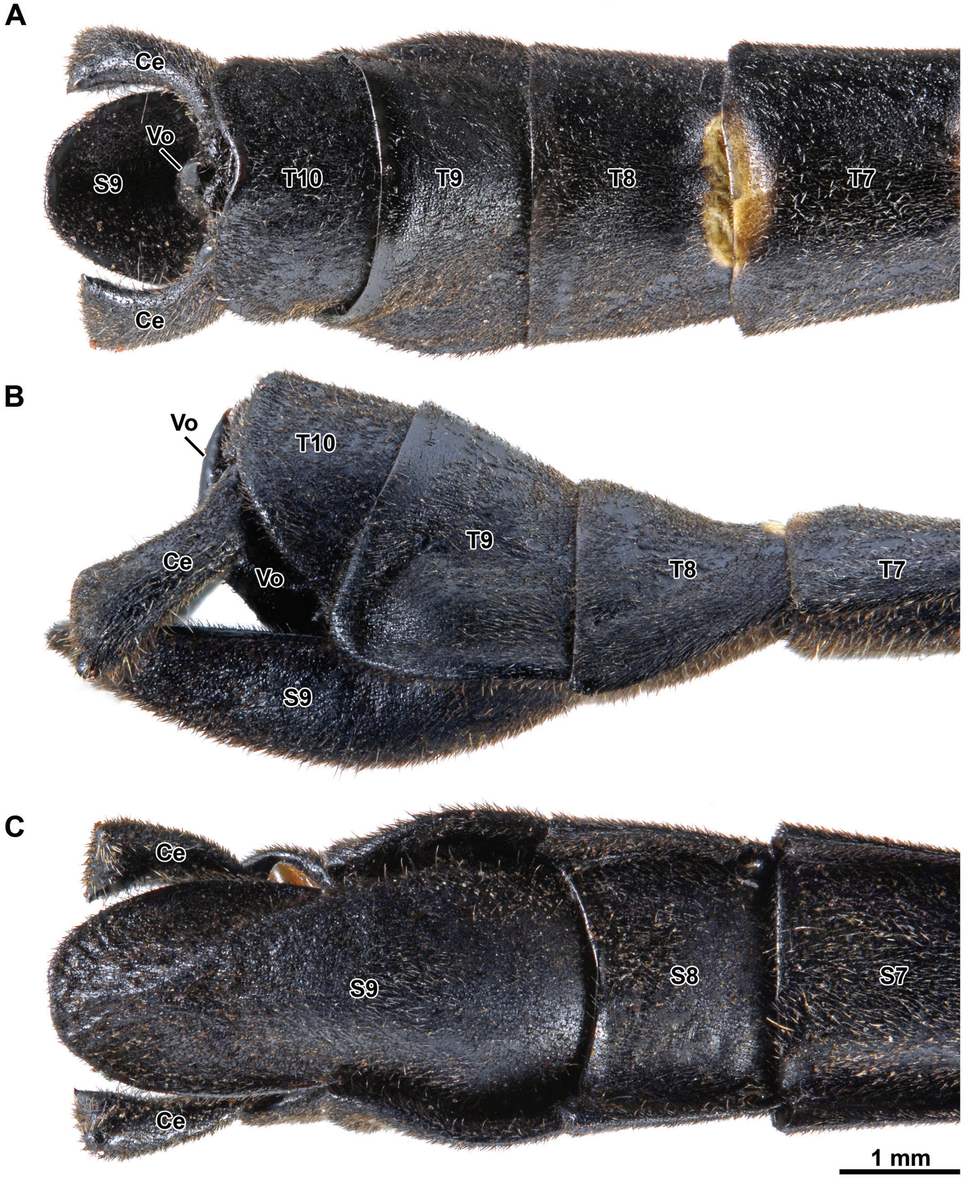

Figure 7. *Orthomeria* (*Orthomeria*) *kangi* sp. n. ♂ postabdomen **A** dorsal view **B** lateral view **C** ventral view; Ce, cercus; T7-T10, terga 7-19; S7-S9, sterna 7-9; Vo, vomer.

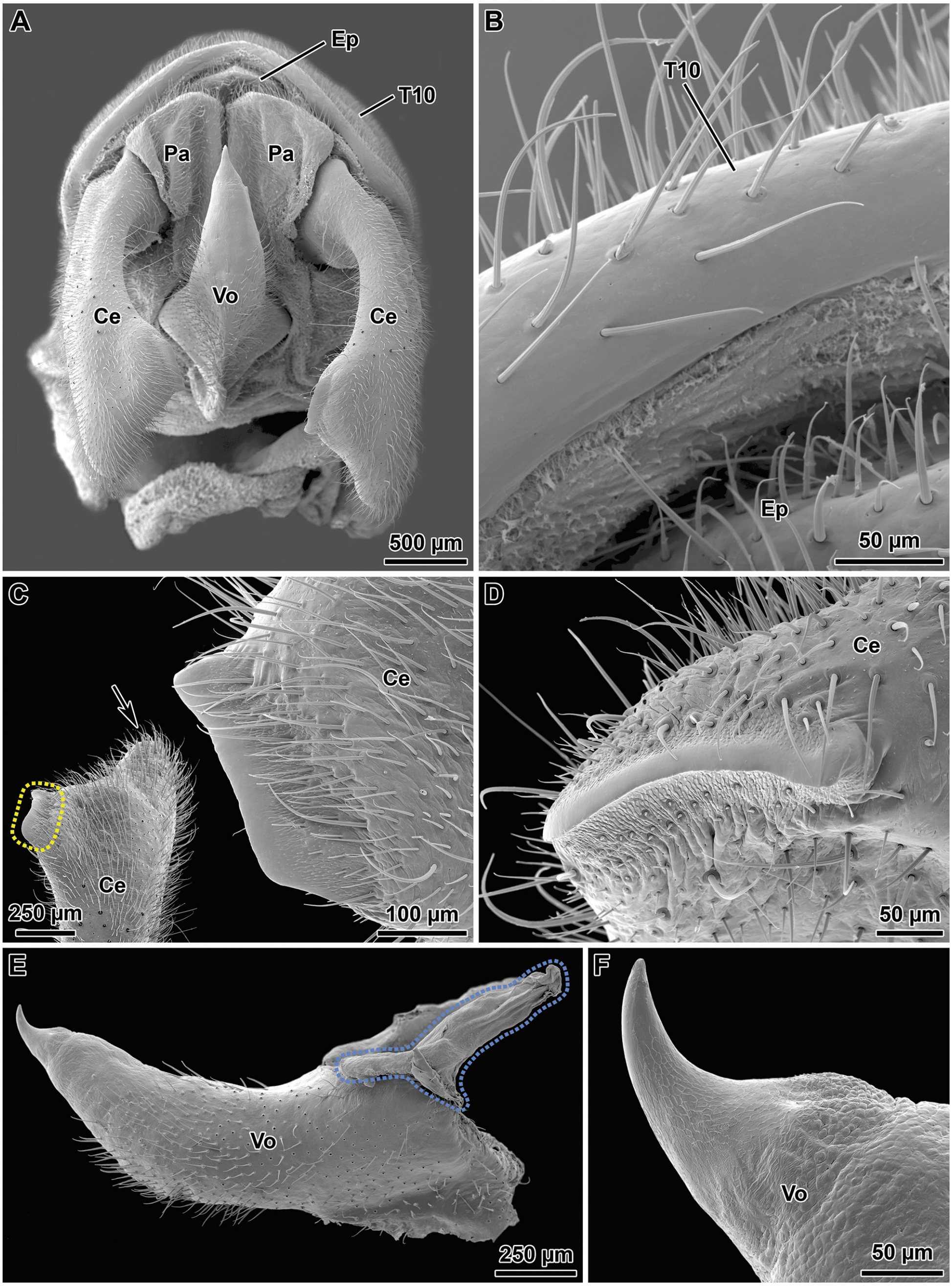

Figure 8. *Orthomeria* (*Orthomeria*) *kangi* sp. n. ♂ details of tenth abdominal segment **A** Whole segment X, ventral view **B** Hind margin of tergum 10, ventral view **C** Distal tip of right cercus with highlight (hatched area) and detail of the inner blade-like ridge, dorsal view, arrow indicates the outer pointed lobe **D** Blade-like ridge of right cercus, lateral view **E** Vomer, lateral view, hatched area indicates the proximal arm-like process **F** Spine-like apex of vomer, lateral view; Ce, cercus; Ep, epiproct; Pa, paraproct; T10, tergum 10; Vo, vomer.

Figure 9. *Orthomeria* (*Orthomeria*) *kangi* sp. n. ♀ postabdomen **A** dorsal view **B** lateral view **C** ventral view; Ce, cercus; T6–T10, terga 6–10; S6–S8, sterna 6–8.

lobe roundly pointed, inner lobe differentiated into a sclerotized blade-like ridge ca. 340–360 µm in length (Fig. 8C–D). Vomer acutely triangular and setose (Figs 7A–B, 8A), inserted into sternum 10 through two proximal arm-like processes (Fig. 8E); proximal two-thirds almost straight, distal third strongly curved upwards with smooth spine-like apex (Figs 7A–B, 8E–F).

Description of the female. The female differs from the male in the following characters. Body slightly larger (Table 1) and more robust (Fig. 2B). Colouration variable, typically similar to male, except abdominal tergum 7 pale brown with a definite dark longitudinal line medially and lateral margins of abdominal terga with yellowish rim (Figs 2B, 9A–B). Alternatively, body, compound eyes, antennae and legs brown; costal

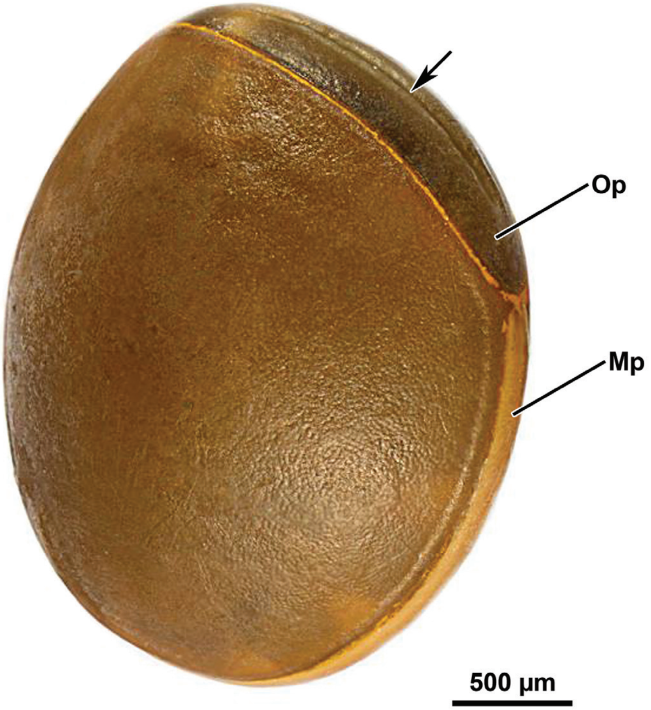

Figure 10. *Orthomeria* (*Orthomeria*) *kangi* sp. n. egg; Mp, micropylar plate; Op, operculum; Arrow, medial longitudinal furrow.

region of hind wings dark brown with pale brown venation, anterior margin with a blood red tinge near wing articulation (Suppl. material 1). Mesothorax parallel-sided. Length of hind wing variable, reaching to posterior margin of the fourth tergum up to extending midway on to the fifth tergum. Abdomen about 1.50 times length of head and thorax combined. Abdominal segments 2–3 increasing in length, 4 shorther than 3, 5–6 increasing in length, 7–10 progressively shorter, 10 ca. 0.90 times length of 9. Abdominal terga 2–10 broader than long in full-grown females. Hind margin of tergum 10 rounded (Fig. 9A). Cerci terete and straight (Fig. 9A–B). Sternum 8 (= operculum) folded in two along the middle, covering completely the ovipositor, apex roundly pointed (Fig. 9B–C).

External eggshell morphology. Capsule light brown, oval in outline, laterally flattened, surface minutely pitted (length, 2.6–2.7 mm; height, 2.3 mm; width, 1.6–1.7 mm) (Fig. 10). Operculum mid brown, elongate-oval with a medial longitudinal furrow, slightly convex in lateral aspect (height, 1.8 mm; width 0.7 mm). Opercular angle negative. Micropylar plate visible in lateral aspect, structured as pale brown stripe surrounding the entire capsule and delimited by a thin yellow rim extending also along the opercular opening area. Micropylar cup close to the posterior pole.

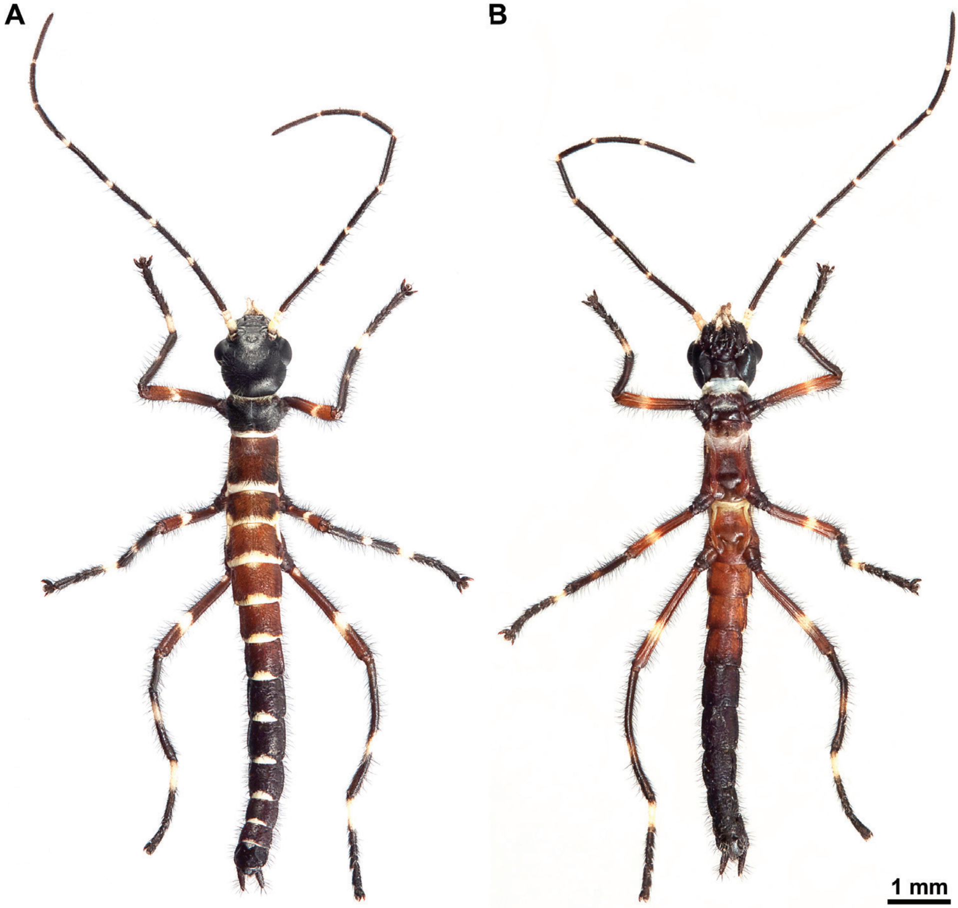

Figure 11. *Orthomeria* (*Orthomeria*) *kangi* sp. n. first instar nymph **A** Dorsal view **B** ventral view.

Description of the first-instar nymph. Body length ca. 8.9 mm. Head, prothorax, and abdominal segments V–X black; meso- and metatorax, and abdominal segments I–IV brown (Fig. 11A–B). Scape and pedicel white; flagellomeres black with white distal dot. Palpomeres of labial and maxillary palps white. Hind margin of thoracic and abdominal terga white. Femora brown with a white spot in the middle. Tibiae black with a central white band. Tarsi with proximal tarsomere white, remainder black.

Geographic distribution. The new species is so far reported only from the Benguet province, Luzon island, Northern Philippines (Fig. 12). Specimens have been found in the Sablang region (Barangay Bayabas, Mt. Bilbil) and in the Tuba region (Mt. Calugong).

Natural history observations. The studied locality, Sablang, is a mountainous region (400-650 m elevation) of the Benguet Province, in the north-west Philippines. It is characterised by the presence of small communities scattered around a main provincial road, with several pockets of secondary vegetation (Fig. 13A) and some areas

Figure 12. Distribution of *Orthomeria* (*Orthomeria*) *kangi* sp. n. in Luzon island.

of primary forests. Adults of *O. kangi* sp. n. were first observed at night on the leaf underside of *Ficus* spp. trees (Moraceae). The host plants were mainly big trees of an unidentified *Ficus* sp. ca. 8–10 m height and up to 10–15 m wide located on forested slopes. The stick insects have been found on the low hanging branches (< 4 m height), where the number of observed individuals varied from 1 to 10 per tree (Fig. 13B). The species was found also in smaller fig trees (e.g. *Ficus septica*) ca. 2–3 m height (Fig. 13C), and occasionally on shrubs of *Pipturus* spp. and *Leucosyke* spp. (Urticaceae) (Fig.

Figure 13. *Orthomeria* (*Orthomeria*) *kangi* sp. n. **A** Secondary vegetation in the type locality, Sablang, Benguet, Philippines **B** Adults on *Ficus* sp. **C** Adult females on *Ficus septica* **D** Adult female on *Pipturus arborescens*; arrowheads indicate the insects on branches. Photographs by Albert Kang.

13D), with usually 2–5 individuals present on the same plant. Daytime search revealed the presence of fewer individuals, mainly juveniles at different nymphal stages. Searching over a wide area, we noted that the distribution of the species on host trees was markedly discontinuous, with individuals concentrated on single larger plants and apparently absent from *Ficus* trees in the immediate vicinity.

Eggs were dropped to the ground and needed ca. 40 days to hatch at 23 °C. Newly hatched nymphs were reared to adulthood using the hauili tree (*Ficus septica*) or stinging nettle (*Urtica dioica*) as food plants. Under rearing conditions, the nymphal development lasted for ca. 60 days, and the average life span for males was ca. 45 days compared with ca. 140 days for females. The insects were active both during the day and night.

The mating was observed under rearing conditions. On day before the final moult, the subadult female starts to be guarded by 3–4 competing males, usually with one male mounted on the female's back and facing into the same direction as the female. Copulation starts immediately after the female has completed the last nymphal moult. Generally, we found that when presented with a receptive virgin female, the male

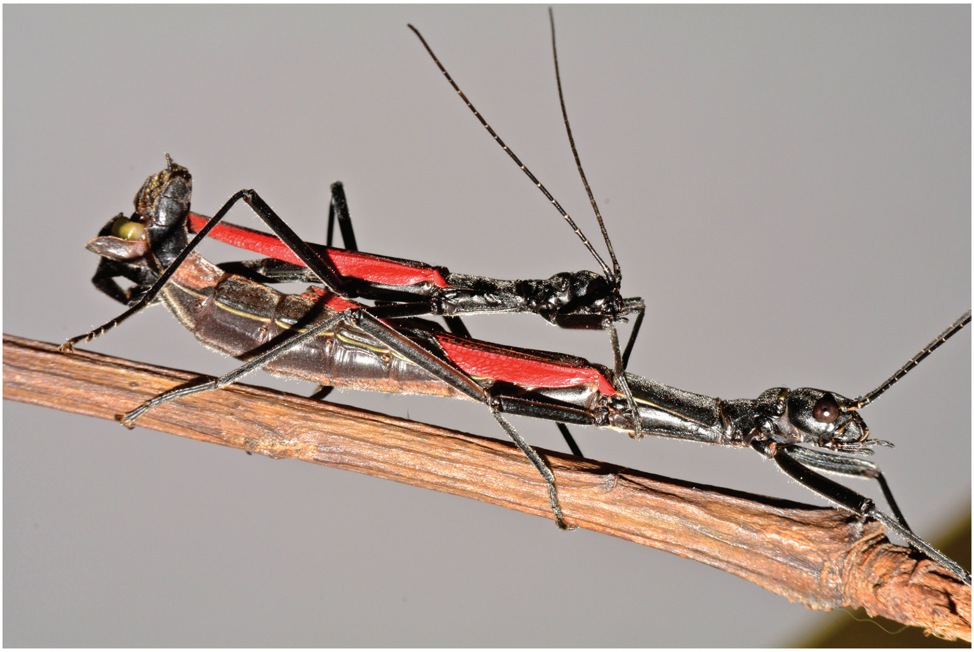

Figure 14. *Orthomeria* (*Orthomeria*) *kangi* sp. n. mating pair. Photograph by Albert Kang.

quickly mounts her and starts a series of abdominal bending movements apparently searching for the appropriate mating position. The female curve the abdominal tip upwards thereby exposing her terminalia, while the male bends its abdomen on the left side with his terminalia directed forward. The clasping cerci of the male grasp the female at the base of her eighth sternum, and at the same time a bulb-like phallic organ comes into contact with the female genitalia (Fig. 14). Copulation lasted approximately three hours. The presence of a spermatophore has not been ascertained. Mated females showed an aggressive behaviour towards potential mates, and multiple matings were rarely observed. They chase away approaching males through quivering movements of the body, beating legs on the substrate, and flashing their wings for a few seconds. This behaviour is also practiced by resting individuals when disturbed by conspecifics.

If threatened, adults and nymphs of *O. kangi* sp. n. spray a milky defensive secretion from the prothoracic exocrine glads and inevitably let themselves fall to the floor and quickly run away.

Taxonomic notes on some species of *Orthomeria* (*Orthomeria*)

During our comparative analysis of the type material and additional specimens of *Orthomeria* spp., we recognised that *Orthomeria catadromus* (Westwood, 1859) represents a junior synonym of *O. pandora* (Westwood, 1859), syn. n. Both species were published in the same publication (Westwood 1859), but *O. pandora* has page priority.

The type series of *O. catadromus* consists of three syntypes, two of them in BNHM and one in UMO. A specimen in BNHM bearing the following data is here designated as lectotype: the locality Sumatra was handwritten by Westwood on the identification label under *Aschiphasma catadromus*; the specimen has a white database label with the code BMNH(E) #845139. There is no separate label with a locality, however there is a small round grey label with a question mark. Although *O. catadromus* was originally recorded from Sumatra, there is reasonable doubt that this is in error, and subsequent reports of this species refer only to the Philippines (Bruner 1915, Bragg 2001). In fact, various species described by Westwood have doubtful locality data and several have since been recorded from different localities. Another example concerning the Philippine phasmid fauna is that of *Theramenes olivaceus* (Westwood, 1859), a species of Eubulidini (Heteropterygidae: Obriminae) originally described from Sri Lanka, but afterwards recorded from the Philippines (Bruner 1915) in accordance with the fairly restricted distribution of the tribe, not including Sri Lanka and continental Asia.

A lectotype is here designated also for *O. pandora* (Westwood, 1859). The original syntype series in BMNH originates from different localities. Two specimens originate from the Philippines and a third one from “ceram”. The specimens from the Philippines do not bear more precise data than “Philippine Islands”. The specimen from the Philippines still having one of its forelegs is hereby designated as lectotype.

Identification key to the species of *Orthomeria* (*Orthomeria*)

♂♂

1 Hind area of wings with at the base a large sky blue area.......................... **2**
– Hind area of wings uniformly dark or turning pale at the base without a distinct large sky blue area.......................... **3**
2 Wings long, sky blue region not circular, beginning at A6; base of A 1–5 not blue .... ***Orthomeria* (*O.*) *superba* (Redtenbacher, 1906)**
– Wings short, base with an almost circular sky blue area which crosses all the anal veins.......................... ***Orthomeria* (*O.*) *versicolor* (Redtenbacher, 1906)**
3 short winged species, wings projecting slightly over abdominal tergum 5.... **4**
– long winged species, wings projecting over abdominal tergum 7.......................... **5**
4 Costal area of wings with definite orange area.......................... ***Orthomeria* (*O.*) *forstenii* (Haan, 1842)**
– Costal area of wings with a definite blood red area.. ***Orthomeria* (*O.*) *kangi* sp. n.**
5 Mesonotum more than one and a half times as long as the pronotum.......................... ***Orthomeria* (*O.*) *pandora* (Westwood, 1859)**
– Mesonotum not one and a half times the length of pronotum.......................... **6**
6 Anterior portion to radial vein of hind wing uniformly yellow. Base of antennae black, thereafter rust coloured.......................... ***Orthomeria* (*O.*) *smaragdinum* (Redtenbacher, 1906)**

– Anterior portion to radial vein of hind wing yellow with black cells. Antennae uniformly black .... ***Orthomeria* (*O.*) *xanti* (Redtenbacher, 1906)**

♀♀*

1 Hind area of wings with at the base a large sky blue area............................ **2**

– Hind area of wings uniformly dark or turning pale at the base without a distinct large sky blue area.. **3**

2 Wings long, sky blue region not circular, beginning at A6; base of A 1–5 not blue .... ***Orthomeria* (*O.*) *superba* (Redtenbacher, 1906)**

– Wings short, base with an almost circular sky blue area which crosses all the anal veins...................... ***Orthomeria* (*O.*) *versicolor* (Redtenbacher, 1906)**

3 Body > 50mm, tegmina brown, costal area of hind wings without red markings, tergum 7 pale without black longitudinal line...................................... .... ***Orthomeria* (*O.*) *pandora* (Westwood, 1859)**

– Body < 50mm , tegmina red, costal area of hind wings with red markings, tergum 7 pale with a definite black longitudinal line centrally .... ***Orthomeria* (*O.*) *kangi* sp. n.**

* Females of *Orthomeria* (*O*) *forstenii*, *O.* (*O.*) *smaragdinum*, and *O.* (*O.*) *xanti* are unknown.

Discussion

Phylogenetic interpretation of morphological characters

O. kangi sp. n. shows some phylogenetically informative characters that are helpful to find its placement among the subgroups of Euphasmatodea. The unbranched radial vein (= absence of the radial sector), the undivided sternum 9 and the incurved cerci with an apical spine or tooth in the male, represent key synapomorphies of Aschiphasmatidae (Bradler 2009). The latter specialized character is definitely homologous with the sharp blade-like ridge found in the male cerci of the new species. *O. kangi* sp. n. also shows distinctly pectinate claws, a putative derived character of the tribe Aschiphasmatini (Aschiphasmatidae excluding *Dajaca*) (Bragg 2001, Bradler 2009). Within Aschiphasmatidae, a brightly coloured (yellow, orange, or red) costal region of the hind wings occurs only in *Orthomeria*. This character can be interpreted as an autapomorphy that provides evidence for the monophyly of the genus.

Attachment structures of Aschiphasmatidae has been previously analysed in *Dallaiphasma eximius* (Gottardo 2011b). A feature shared between this species and *O. kangi* sp. n. is the absence of an euplantula on tarsomere V. In *Timema*, a small euplantula is present on the distal tarsomere (Beutel and Gorb 2008). Within Euphasmatodea the condition appears variable: the euplantula is present and generally small compared to the size of tarsomere V in *Eurycantha calcarata* and *Conlephasma enigma* (Gottardo and Heller 2012, Gottardo et al. 2015), while it is absent in several taxa including *Carausius morosus*, *Medauroidea extradentata*, *Ophicrania conlei*, and *Hermarchus leytensis* (Gottardo 2011a, Bußhardt et al. 2012, Gottardo and Vallotto 2014). The two species of Aschiphasmatidae have also smooth euplantulae on tarsomeres I-IV. Interestingly, while *D. eximius* has a honeycomb micropattern, *O. kangi* sp. n. shows only faint microgrooves on the contact surface. It has been hypothesized that nubby euplantulae covered with acanthae are a groundplan feature of Phasmatodea (Beutel and Gorb 2008), implying secondary modification of these surface structures in Aschiphasmatidae. It would be interesting to analyze the attachment structures of additional species of Aschiphasmatidae, since the character system could be more diverse within the family.

The male terminalia of *O. kangi* sp. n. show a number of specific modifications. An unusual feature is the complete absence of clasping devices (the tergal thorn pads) on the hind margin of the tergum X. Within Aschiphasmatidae these structures have been described in *D. eximius* as a single row of ca. 12 tooth-like projections (see Gottardo 2011b: fig. 5), and are usually present and well developed in Euphasmatodea (Bradler 2009). However, they are absent for example in *Timema*, *Agathemera*, in the taxon Sermyleformia sensu Bradler (2009), and their secondary loss has been established in extant leaf insects (partim) (Wedmann et al. 2007). It is conceivable that in *O. kangi* sp. n the reduction of tergal thorn pads has been compensated by the acquisition of the specialized cerci, that together with the vomer form the male clasping organs of this species. The structure of male terminalia has been described here in detail for the first time in *Orthomeria*, and for future studies these characters may represent important diagnostic features of males of species of this genus, as also exemplified in other euphasmatodean taxa by Bradler (2009) and Buckley et al. (2014).

Intraspecific morphological variations

The captive rearing showed the presence of substantial intraspecific colour variation in the females of *O. kangi* sp. n. All wild individuals, both males and females, had the chromatic characters of the typical black colour morph. When the species was reared at cool temperatures (ca. 16 °C) all females developed the brown colour morph, while males were invariably black. Interestingly, the offspring of the brown females reared at warmer temperatures (ca. 23 °C) consisted of only black females.

A certain amount of variation was found also as to the length of the hind wings of females, regardless of the two colour morphs. In some females the hind wings reach the hind margin of the abdominal segment IV, whereas in other they can extend up to the hind margin of segment V. Intraspecific trends of variation in wing length have been rarely documented in Phasmatodea. A different example is that of *Asceles margaritatus*

Redtenbacher, 1908 (Necrosciinae), where two separated macropterous and micropterous forms involving both sexes have been described (Bragg 2002).

Foodplants of *Orthomeria*

Information about *Orthomeria* foodplants are mainly available for two species of the subgenus *Parothomeria*. Both *Orthomeria* (*Parothomeria*) *alexis* and *O.* (*P.*) *cuprinus* use trees of the genus *Macaranga* (Euphorbiaceae) as foodplants (Junker et al. 2008, Shimizu-kaya and Itioka 2015). For members of the subgenus *Orthomeria*, Bragg (2001) reported *Oreocnide rubescen* (Urticaceae) as foodplant of *Orthomeria* (*Orthomeria*) *superba* from Borneo. *O. kangi* sp. n also feeds on members of Urticaceae, but seems to prefer *Ficus* spp. (Moraceae) which represents a new foodplant association for *Orthomeria*.

The species of *Orthomeria* from the Philippines

As a result of this study, seven species of *Orthomeria* (*Orthomeria*) are recognised, two of which occur in the Philippines: *O.* (*O.*) *pandora* and *O.* (*O.*) *kangi* sp. n. Both species seem to be restricted to Luzon island. *O.* (*O.*) *pandora* is found in the Sierra Madre mountain range in east Luzon, while *O.* (*O.*) *kangi* sp. n. occurs in the Benguet province in west Luzon. It is likely that other species of *Orthomeria* will be discovered in the other island of the Philippine archipelago.

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Supplementary material 1

Intraspecific colour variations

Authors: Davide Vallotto, Joachim Bresseel, Thierry Heitzmann, Marco Gottardo

Data type: multimedia

Explanation note: Brown colour morph of a female of *Orthomeria* (*Orthomeria*) *kangi* sp. n.

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Revision of *Paranastatus* Masi (Eupelmidae, Eupelminae) with descriptions of four new species

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Abstract

Paranastatus Masi, 1917 (Eupelmidae, Eupelminae) was originally described based on two species from Seychelles: *P. egregius* and *P. violaceus*. Eady (1956) subsequently described *P. nigriscutellatus* and *P. verticalis* from Fiji. Here, four new species of *Paranastatus* are described: *P. bellus* Scallion, **sp. n.** and *P. pilosus* Scallion, **sp. n.** from Indonesia, and *P. halko* Scallion, **sp. n.** and *P. parkeri* Scallion, **sp. n.** from Fiji. A key to all *Paranastatus* species based on females is included and lectotypes are designated for *P. egregius* and *P. violaceus*. Finally, previously unobserved colour variation from newly collected material of *P. verticalis*, distribution patterns of species, and possibilities for future research are discussed.

Keywords

Graeffea crouanii, *Anastatus*, South Pacific, Indian Ocean, dispersal mechanisms, biodiversity, lectotye designation, identification key

Introduction

Paranastatus Masi, 1917 (Eupelmidae, Eupelminae) is one of 33 currently recognized genera within Eupelminae (Gibson 1995). This genus was initially established for two species based primarily on the distinctive triangular shape of the head of females. Four species have been described to date: *P. egregius* Masi, 1917 and *P. violaceus* Masi, 1917 from Seychelles, and *P. verticalis* Eady, 1956 and *P. nigriscutellatus* Eady, 1956 from Fiji (Masi 1917, Eady 1956). No new specimens of either *P. egregius* or *P. violaceus* have been reported since their original description and their biology remains unknown. However, O'Conner et al. (1955) and Rapp (1995) subsequently reared *P. nigriscutellatus* and *P. verticalis* from the eggs of the walking stick, *Graeffea crouanii* Le Guillou (Phasmatodea: Phasmatidae). Males are known only for *P. egregius*, *P. nigriscutellatus*, and *P. verticalis*. A key to these males was provided by Eady (1956).

Eupelmidae is likely a grade-level taxon (Gibson 1989) rather than being monophyletic (Heraty et al. 2013), though Eupelminae is supported as monophyletic (Gibson 1989). The subfamily is characterized in part by its extreme sexual dimorphism, and species and higher level taxonomy is based primarily on female morphology. Eupelmines are parasitoids or predators of eggs and primary or hyperparasitoids of other immature stages of various arthropods, including Blattaria, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Orthoptera and Phasmida, as well as Araneae and even Pseudoscorpionida (Gibson 1995, Austin et al. 1998). Gibson (1995) hypothesized that *Paranastatus* and five other genera, including *Anastatus* Motschulsky, formed a monophyletic clade, though with unresolved relationships and with *Paranastatus* possibly rendering *Anastatus* paraphyletic. Like known *Paranastatus*, members of *Anastatus* are mostly egg parasitoids and have been recorded as endoparasitoids of the eggs of Phasmida (Gibson 1995).

More recent collections from the South Pacific revealed new specimens of *Paranastatus*, including what appeared to be undescribed species. The purpose of this study was to differentiate and describe these new species and provide observations on variation observed among new *P. verticalis* material. An illustrated key to the world species of female *Paranastatus* is also included.

Methods

Type material of *P. verticalis*, *P. nigriscutellatus*, *P. egregius*, and *P. violaceus* was examined as part of a loan from The Natural History Museum, London, England (BMNH). Paratypes of one female of *P. nigriscutellatus* and a male and female of *P. verticalis* were also examined on loan from the U.S. National Museum of Natural History, Washington, DC, USA (USNM). Other material was borrowed from the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, ON, Canada (CNC). Some of the latter material was collected in projects requiring primary type material to be returned to the Bernice P. Bishop Museum, Honolulu, HI, USA (BPBM).

Two systems were used to image specimens, a Nikon D5200 camera mounted on an Olympus SZX16 stereomicroscope, and a Canon DSLR 7D Mark II camera with a MP-E 65mm macro lens attached to a motorized rail. Images were taken at multiple levels of focus, and stacked into a single image using the program Helicon Focus 6 (Helicon Soft Ltd, 2014). Images were processed and enhanced using Adobe Photoshop CC 2014. Scanning electron microscope images were obtained using a Hitachi Tabletop Microscope TM-1000. Measurements were taken using a Motic SMZ-168 microscope with an Olympus G10x micrometre eyepiece. Body length was measured in millimetres a total of three times and averaged. Excluding primary types, imaged specimens are labelled with a "JBWM Photo 2015-X" specimen number label. This is cited with other label data given for the respective specimen, in the Suppl. material 1: "*Paranastatus* Label Data", and in the figure captions.

Structure and sculpture terminology follows Gibson et al. (1997), but additional clarification on sculpture terminology is provided below. Images are provided for clarity where necessary in the keys and descriptions. Alutaceous and coriaceous are similar in that they both mean leather-like (Harris 1979). Here, alutaceous refers to sculpturing where fine grooves create elongated cells, whereas coriaceous refers to sculpturing where the fine grooves create more square but irregularly-shaped cells. Coriaceous-imbricate refers to cells that appear overlapping. Reticulate refers to cells that are delineated by ridges. Pustulate refers to a bumpy texture, whereas granulate refers to many fine bumps, like granules. Rugose means wrinkled, whereas rugulose means very finely wrinkled.

Facial structuring can be divided into the lower face (region below toruli to clypeus and between malar sulci), scrobes (depressions rising above toruli and joining anterior to frontovertex), and interantennal area (region between scrobes and toruli). Gena refers to the region delineated by the malar sulcus and occipital margin, and extends to halfway along posterior margin of the eye. The vertex lies between the eyes from the frontovertex to the posterior margin of the eyes, where the temple begins. The temple runs between the posterior occipital margin and eyes to the genae.

Colouration of the antennomeres is often a quick identifier to species because females of four species have unique antennal colouration, though females of two species have overlapping colour patterns and two have the same colour pattern. The sculpture of the mesoscutum in combination with the extent of its concavity can be used to separate species with similar antennal colouration.

Due to the number of specimens examined, paratype and other material label data has been condensed for a few species to include localities, dates collected, and collector. A number in brackets corresponds to the number of specimens from each locality. For verbatim label data, see the Suppl. material 1: "*Paranastatus* Label Data". A double line, ‖, represents a new line on the label, and ++ represents a separate label.

Taxonomy

For a key to the world species of known *Paranastatus* males (*P. egregius*, *P. nigriscutellatus*, and *P. verticalis*), see Eady (1956).

Key to world species of *Paranastatus* Masi based on females

Note: When viewing mandible dentition, a dorsolateral view with the teeth directed forward is best for visualizing dentition (see Fig. 3).

1 Mandible tridentate (Fig. 1). Flagellum mostly white or, if mostly brown, then flagellomere 7 entirely brown or white only apically. Lower face reticulate (Fig. 1). Gena mostly reticulate, or coriaceous to coriaceous-imbricate along occipital margin toward temple (Fig. 2)..**2**

– Mandible quadridentate (Fig. 3). Flagellum mostly brown basally but flagellomeres 7 and 8 entirely white or light yellow-brown and sometimes flagellomere 6 white. Lower face smooth to alutaceous or coriaceous (Fig. 3). Gena alutaceous or coriaceous (Fig. 4) **5**

2(1) Head in lateral view with vertex raised between eyes, and temple flat such that temple and occiput at almost a right angle (Fig. 5). Temple smooth. Lower face with fringe of setae below toruli (Fig. 6). Flagellum brown except flagellomere 8 and club white (Figs 15, 17)........... ***Paranastatus verticalis* Eady**

– Head in lateral view with vertex and temple slightly to distinctly convex between eyes, and temple and occiput at an obtuse angle (Figs 7, 8). Temple variably sculptured. Lower face with setae, but not arranged as a fringe (Figs 1, 3). Flagellum colour variable..**3**

3(2) Vertex smooth posterior to ocelli towards temple. Antenna with scape blue (Fig. 8) and flagellum brown except flagellomere 8, club, and usually apex of flagellomere 7 white (Fig. 19). Mesoscutum smooth to slightly rugulose. Gaster with tergites brown except apex of gaster green; sternites brown except white at very base.............................. ***Paranastatus halko* Scallion, sp. n.**

– Vertex rugose or reticulate posterior to ocelli towards temple. Antenna mostly white except basal half of scape brown and club variable in colour. Mesoscutum reticulate (Fig. 9). Gaster brown except tergites 1 and 2 white; sternites variable..**4**

4(3) Vertex rugose posterior to ocelli (Fig. 10). Temple coriaceous and brownish-green to blue-green laterally. Gena blue-green, coriaceous to reticulate along malar sulcus. Antennal club brown. Mesoscutum purple-brown medially, straw yellow laterally, and reticulate. Fore wing hyaline behind distal half of submarginal vein, but deeply infuscate basally, lightly infuscate (tinted brown) in patch behind base of marginal vein, and infuscate from behind postmarginal vein to wing apex. Gaster brown beyond basal white sternites.. ***Paranastatus bellus* Scallion, sp. n.**

– Vertex reticulate posterior to ocelli (Fig. 11). Temple reticulate and dark blue-purple. Gena blue-purple, reticulate. Antennal club white except slightly darkened apically. Mesoscutum blue-purple medially, brown laterally, and reticulate. Fore wing hyaline except lightly infuscate in apical half. Gaster purplish-brown beyond basal white sternites......***Paranastatus pilosus* Scallion, sp. n.**

5(1) Temple smooth, and in dorsal view occipital margin straight. Flagellum brown except flagellomeres 7, 8 and club white. Pronotum smooth dorsally or coriaceous only along anterior edge. Mesoscutum distinctly and deeply concave posteromedially (Fig. 12). Fore wing infuscate with hyaline band behind distal half of submarginal vein..**6**

– Temple coriaceous or pustulate, and in dorsal view occipital margin concave. Flagellum variable in colour, but club brown. Pronotum coriaceous dorsally. Mesoscutum slightly concave posteromedially (Fig. 13). Fore wing variable.. **7**

6(5) Head with face green to coppery-green, lower face alutaceous to coriaceous centrally (Fig. 3), and interantennal area and scrobes coriaceous. Frontovertex with blunt teeth projecting towards vertex. Mesoscutum mostly alutaceous, except coriaceous posteromedially (Fig. 12). Legs with profemur black-brown except for light brown patch ventroapically, mesofemur black-brown dorsally and yellow ventrally and basally, and metafemur yellow basally and darkening to brown apically. Gaster brown except green apically, basal tergites white centrally and sternites 1–4 white......... ***Paranastatus nigriscutellatus* Eady**

– Head with face dark purple-brown and entirely smooth to alutaceous. Frontovertex smooth with a few small bumps. Mesoscutum smooth, except slightly coriaceous posteromedially. Legs with all femora straw yellow. Gaster green apically, but tergites otherwise dark coppery-green and sternites brown .... ***Paranastatus parkeri* Scallion, sp. n.**

7(5) Vertex coriaceous and dull black-brown. Flagellum with apical two funiculars light yellow-brown. Mesoscutum dark purple-brown, and mostly alutaceous except coriaceous posteromedially (Fig. 13). Fore wing evenly infuscate. Gaster dark brown except apical tergites green and sternites 1 and 2 light brown ....***Paranastatus violaceus* Masi**

– Vertex pustulate, purple except for coppery sheen between ocelli (Fig. 14). Flagellum with apical three funiculars white. Mesoscutum light brown, and slightly coriaceous. Fore wing with hyaline band behind distal half of submarginal vein, lightly infuscate behind base of marginal vein and behind postmarginal vein, hyaline between infuscate regions and apically (Fig. 27). Gaster dark brown except tergites 1 and 2 and sternites 1–3 white ....***Paranastatus egregius* Masi**

***Paranastatus bellus* Scallion, sp. n.**

http://zoobank.org/65D79CA1-0DA8-4483-9884-9F3694D5ED5B
Figs 10, 20, 21

Material examined. Holotype female, dry pinned, deposited in BMNH (Hym Type 5.4813, barcode NHMUK010198566). Label data: "SULAWESI UTARA: Dumoga-Bone Nat. Pk, edge of rainforest, 0°34'N, 123°54'E. A.D. Austin June 1985, M.T."

Paratype female, dry pinned, deposited in CNC. Label data: "INDONESIA. Sulawesi Utara, Dumoga Bone Nat. Pk, Toraut IV.1985, JS Noyes, forest edge, MT."

Diagnosis. Females of *P. bellus* are differentiated by the following combination of features: vertex rugose (Fig. 10); antenna mostly white except base of scape and club brown (Fig. 20); mandible tridentate; mesoscutum purple-brown medially, straw-yellow laterally and reticulate.

Description. Female. Length: 2 mm.

Colour. Head with vertex dull black-brown (Fig. 10); temple brownish-green dorsally, blue-green laterally (Fig. 10); gena and face metallic blue-green (Fig. 20); frontovertex dull black-brown. Antenna mostly white, except base of scape and club brown (Fig. 20). Pronotum light brown; mesoscutum purple-brown medially, straw-yellow laterally; scutellar-axillar complex dark orange-brown; mesopleuron light brown to white anteriorly (Fig. 21). Legs white with mesofemur darkened along posterior apical edge and metafemur darkening to brown apically. Fore wing hyaline behind distal half of submarginal vein, but deeply infuscate basally, lightly infuscate patch behind base of marginal vein, and infuscate from behind postmarginal vein to wing apex; hind wing hyaline. Gastral tergites 1 and 2 white, remaining tergites dark brown; gastral sternites 1–4 white, remainder brown. Colour of setae on various body regions discussed in appropriate sections below.

Head. Vertex rugose (Fig. 10); temple coriaceous (Fig. 10); gena coriaceous except reticulate along malar sulcus (Fig. 20); face reticulate; frontovertex with blunt teeth projecting posteriorly towards vertex. Mandible tridentate. Vertex, temple and gena with sparse, very light brown setae; face with sparse white setae except scrobes bare; eyes with dense, short white setae.

Mesosoma. Pronotum coriaceous; mesoscutum reticulate, distinctly concave posteromedially; scutellar-axillar complex reticulate; mesopleuron coriaceous. Pronotum with white setae, setae longer along posterior edge; mesoscutum with many white setae; scutellar-axillar complex with few white setae along edges; mesopleuron with white setae anteriorly, remainder bare. Fore wing with dense, short brown setae; hind wing with relatively fewer short, light brown setae.

Metasoma. Entirely coriaceous with white setae ventrally, the setae very sparse dorsally and long at apex of gaster.

Male. Unknown.

Etymology. From the Latin *bellus*, meaning handsome, in memory of Melanie Scallion's dog Beau (French: handsome). This is an adjective in the nominative singular.

Distribution. Sulawesi Island, Indonesia.

Biology. Unknown.

Remarks. Holotype deposited in the BMNH at the request of Dr. Andrew Austin, University of Adelaide, Australia. Both specimens are in poor condition. The head of the holotype is glued to the point, and the paratype is contorted with the body curled up on itself.

***Paranastatus egregius* Masi, 1917**
Figs 14, 26, 27

Paranastatus egregius Masi, 1917: 165–166.

Material examined. Lectotype female, here designated; dry pinned, deposited in BMNH (Hym Type 5.1,035a). Label data: "Mahe, '08–9 Seychelles Exp. Percy Sladen Trust Exped. B.M. 1913-170."

Paralectotype male, here designated; dry pinned, deposited in BMNH. Label data: "Mahe, '08–9 Seychelles Exp. Percy Sladen Trust Exped. B.M. 1913-170."

Diagnosis. Females of *P. egregius* are differentiated by the following combination of features: vertex behind ocelli and temple pustulate (Fig. 14); antenna brown except flagellomeres 6–8 white (Fig. 26); mandible quadridentate; mesoscutum light brown, slightly coriaceous and only slightly concave posteromedially. Males of *P. egregius* are differentiated by the following combination of features: vertex weakly reticulate; mandible bidentate; mesoscutum convex; colouration similar to females.

Distribution. Mahé Island, Seychelles.

Biology. Unknown.

Remarks. Masi (1917) established *P. egregius* based on one female and two males, but without designating a holotype. Of the three specimens, the BMNH only has the female and one of the males in its collection (Dale-Skey, pers. comm.). Here we designate the female as lectotype and the male as paralectotype, and have labelled the specimens accordingly. The location of the second male is presently unknown.

***Paranastatus halko* Scallion, sp. n.**
http://zoobank.org/6881140A-142F-48F1-89A6-9748FB3361FE
Figs 1, 2, 8, 19

Material examined. Holotype female, dry pinned, deposited in BPBM (Type No. 17540). Label data: "FIJI: Viti Levu, Vuda Prov., Koroyanitu Pk, 1 km E Abaca Vlg., Savuione Trl, 800m, 22.IV–6.V.03 Malaise 1, Schlinger, Tokota'a. 17.667°S, 177.55°E. FBA 180165."

Paratype females (24), dry pinned, deposited in BPBM and CNC. Collecting data for all specimens examined are listed below. However, date ranges are provided when multiple specimens were collected from the same locality with the full label data for each specimen listed in Suppl. material 1: "*Paranastatus* Label Data".

(14). **FIJI**. Viti Levu, Vuda Prov., 1 km E Abaca Vlg., Koroyanitu Ntl. Pk, Savuione Trl. Dates collected range from 7.X.2002–6.V.2003 by E. Schlinger and M. Tokota'a.

(4, includes JBWM Photo 2015-02). **FIJI**. Viti Levu, Vuda Prov., 0.5 km N Abaca Vlg., Koroyanitu Eco Pk, Mt Evan's Range. Dates collected range from 26.X–3.XII.2002 by E. Schlinger and M. Tokota'a.

(2). **FIJI**. Viti Levu, Naitasiri Prov., 4 km WSW Colo-i-Suva Vlg., Mt Nakobalevu, 300m. Collected 12.IV.2004 by E. Schlinger and M. Tokota'a.

(4, includes JBWM Photo 2015-01). **FIJI**. Viti Levu, Naitasiri Prov., 4 km WSW Colo-i-Suva Vlg., Mt Nakobalevu, 372m. Dates collected range from 25.II–14.XI.2003 by E. Schlinger and M. Tokota'a.

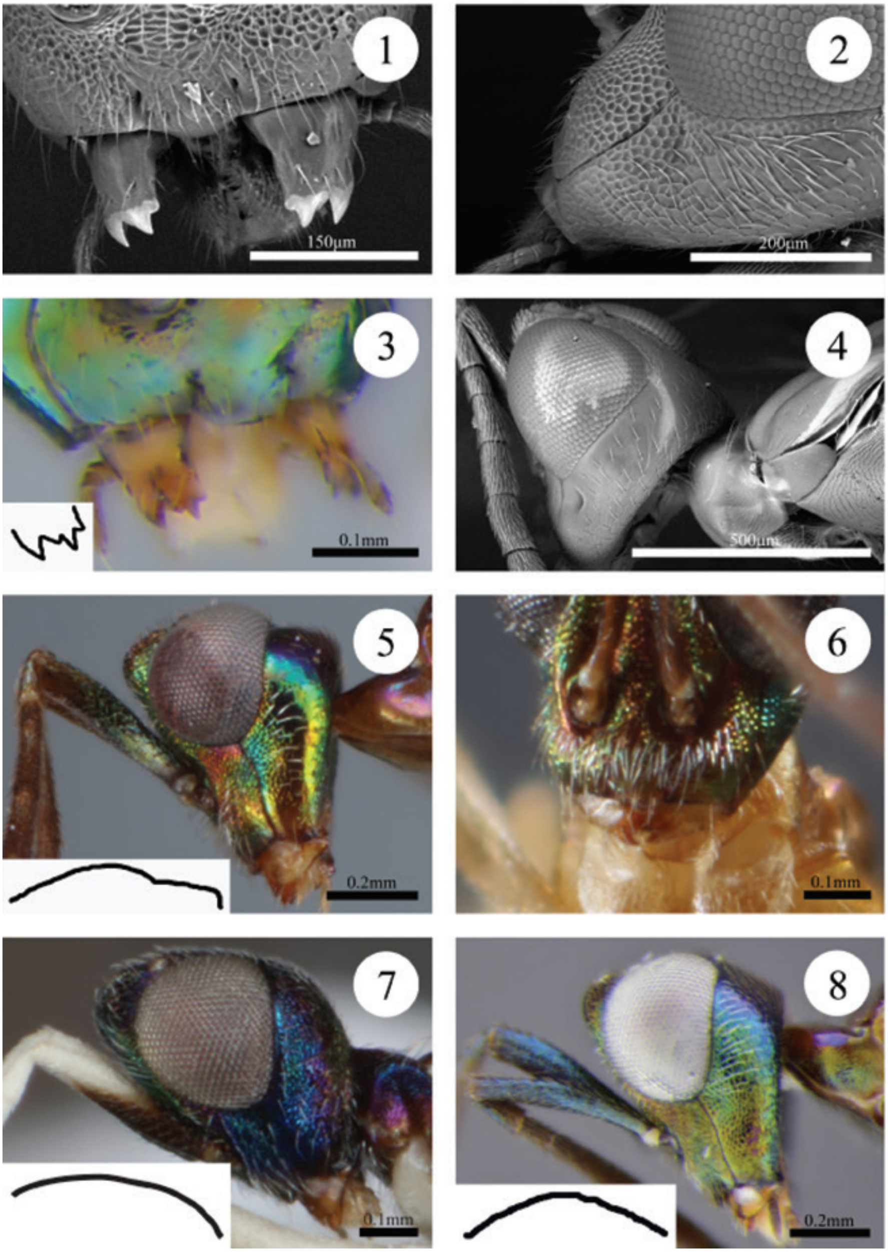
Diagnosis. Females of *P. halko* are differentiated by the following combination of features: vertex granulate between ocelli and smooth posterior to ocelli; temple smooth; scape and pedicel blue (Fig. 8); mandible tridentate (Fig. 1); mesoscutum smooth or very slightly rugulose.

Description. Female. Length: 2.6–2.95 mm.

Colour. Head with vertex dull black-brown between ocelli and metallic green changing to blue-purple posterior to ocelli towards temple; temple shining metallic blue-purple; gena and face metallic coppery-green (Figs 8, 19); frontovertex usually brown, sometimes blue-green with brown centrally (5/25 specimens). Antenna with scape and pedicel blue (Fig. 8), anellus (flagellomere 1) and flagellomeres 2–6 brown, tip of 7 usually white but sometimes brown, 8 and club all white (Fig. 19). Pronotum metallic blue-purple dorsally, coppery-green laterally; mesoscutum reddish-brown to more orange-brown; scutellar-axillar complex dull black; mesopleuron brown. Legs with procoxa light yellow-brown although sometimes dark brown (3/25 specimens), protrochanter dark brown, sometimes light yellow-brown (8/25), and profemur dark black-brown, sometimes with light spot apically on ventral surface (1/25); mesocoxa light yellow-brown, mesotrochanter light brown, mesofemur black-brown dorsally with a lighter streak ventrally, and mesotibia white; metacoxa white, sometimes with brown spot basoventrally (1/25), metatrochanter light brown, sometimes white (2/25), and metafemora dark black-brown; remaining tibiae and tarsomeres light yellow-brown to straw-yellow. Fore wing infuscate with hyaline band behind distal half of submarginal vein; hind wing hyaline. Gastral tergites brown, apex of gaster green; sternites brown except white at very base. Colour of setae on various body regions discussed in appropriate sections below.

Head. In lateral view, vertex distinctly convex between eyes, and temple sloping toward occiput to create a strongly obtuse angle (Fig. 8); vertex granulate between ocelli and smooth posterior to ocelli, sometimes appearing pustulate due to setae; temple smooth; gena coriaceous-imbricate to reticulate along malar sulcus (Figs 2, 8); face reticulate; frontovertex usually with blunt teeth projecting posteriorly towards vertex. Mandible tridentate, possibly appearing quadridentate in some views due to slight bump on ventral edge of large middle tooth (Fig. 1). Vertex and temple with evenly dispersed light brown setae; gena with brown setae, but with a patch of thick white setae on upper part of gena below eye; parascrobal region and region between toruli and clypeus with thick white setae; eye with short white setae; face otherwise with thinner brown setae.

Mesosoma. Pronotum coriaceous; mesoscutum usually smooth, sometimes very slightly rugulose, and only slightly concave posteromedially; scutellar-axillar complex reticulate; mesopleuron coriaceous. Pronotum with few brown setae; mesoscutum with sparse light brown setae posteromedially and along margins; scutellar-axillar com-



Figures 1–8. 1 Scanning electron micrograph (SEM) of *Paranastatus halko*, lower face in anterior view showing mandibular dentition (JBWM Photo 2015-01) **2** SEM of *P. halko*, gena in lateral view (JBWM Photo 2015-02) **3** *P. nigriscutellatus*, lower face in frontolateral view showing mandible dentition (JBWM Photo 2015-03). Inset: outline of mandible dentition showing four teeth **4** SEM of *P. nigriscutellatus*, head and anterior part of mesosoma in lateral view (JBWM Photo 2015-03) **5** *P. verticalis*, head in lateral view. Inset: outline of vertex-temple shape (JBWM Photo 2015-04) **6** *P. verticalis* holotype, lower face in anterior view showing fringe of setae below toruli **7** *P. pilosus*, head in lateral view. Inset: outline of vertex-temple shape (JBWM Photo 2015-05) **8** *P. halko* holotype, head in lateral view. Inset: outline of vertex-temple shape.

plex with few light brown setae; mesopleuron bare. Fore wing with dense, short brown setae; hind wing with relatively fewer short, light brown setae.

Metasoma. Entirely coriaceous; short to long brown setae evenly distributed ventrally; very sparse, short brown setae dorsally.

Male. Unknown.

Etymology. Named in honour of Ed and Eliz Halko from Winnipeg, Manitoba, Canada. Their daughter, Gail Halko, also from Winnipeg, has made a donation to the Wallis-Roughley Museum of Entomology at the University of Manitoba to honour her parents, who both celebrated their 85th birthdays in 2015. This is a noun in apposition to retain integrity of the name Halko in the species name.

Distribution. Viti Levu, Fiji.

Biology. Unknown.

Remarks. Vertex may appear pustulate under a stereomicroscope due to the setae. Care should be taken when using antennal colouration as a guide to species since flagellomere 7 is sometimes completely brown instead of white at apex, thus resembling the antennae of *P. verticalis*.

Paranastatus nigriscutellatus **Eady, 1956**

Figs 3, 4, 12, 23

Paranastatus nigriscutellatus Eady, 1956: 61–64.

Material examined. Holotype female, dry pinned, deposited in BMNH (Hym Type 5.1624a). Label data: "HY 976 FIJI Savu Savu II.1954 B.A. O'Connor. Ex *Graeffea crouani* eggs. Com. Inst. Ent Coll. No. 13599."

Allotype male, dry pinned, deposited in BMNH. Label data: "HY 976 FIJI Savu Savu II.1954 B.A. O'Connor. Ex *Graeffea crouani* eggs. Com. Inst. Ent Coll. No. 13599."

Paratype females (6), dry pinned.

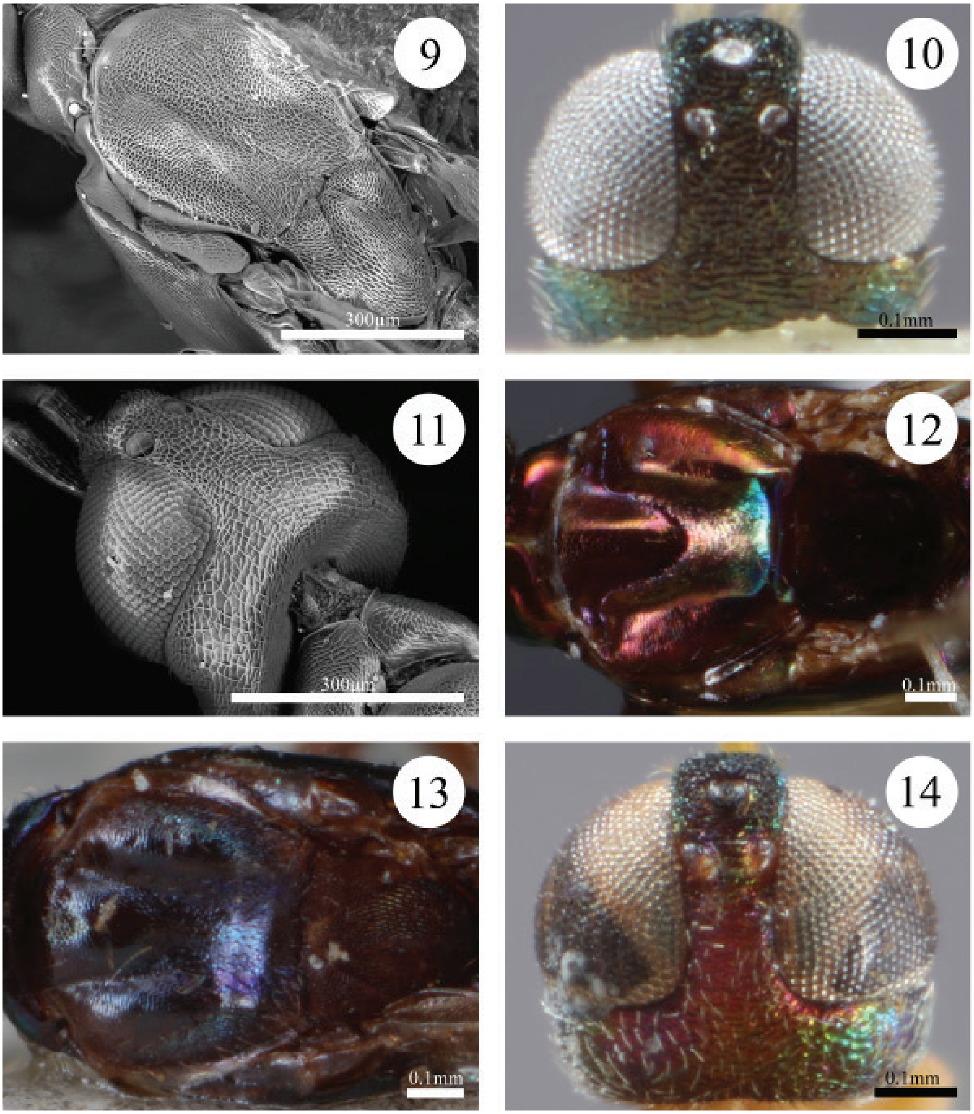
(2, deposited in BMNH). HY 975 **FIJI** Taveuni XI.1953 B.A. O'Connor Ex eggs of *Graeffea crouani* in coconut crowns.

(3, deposited in BMNH). HY 976 **FIJI** Savu Savu II.1954 B.A. O'Connor Ex *Graeffea crouani* eggs.

(1, deposited in USNM). HY 975 **FIJI** Taveuni XI.1953 B.A. O'Connor Ex eggs of *Graeffea crouani* in coconut crowns.

Other material. Females (11), dry pinned, deposited in BPBM and CNC. Collecting data for all specimens examined are listed below. However, date ranges are provided when multiple specimens were collected from the same locality with the full label data for each specimen listed in Suppl. material 1: "*Paranastatus* Label Data".

(5). **FIJI**, Viti Levu, Namosi Prov., 2 km SE Nabukavesi Vlg., Ocean Pacific Rsrt, 40m. Dates collected range from 13.III–11.XII.2003 by E. Schlinger, M. Tokota'a and W. Naisilisili.



Figures 9–14. 9 Scanning electron micrograph (SEM) of *Paranastatus pilosus*, mesonotum in dorsal view (JBWM Photo 2015-05) **10** *P. bellus* holotype, head in dorsal view **11** SEM of *P. pilosus*, head and pronotum in dorsal view (JBWM Photo 2015-05) **12** *P. nigriscutellatus* holotype, mesonotum in dorsal view **13** *P. violaceus* lectotype, mesonotum in dorsal view **14** *P. egregius* lectotype, head in dorsal view.

(2, includes JBWM Photo 2015-03). **FIJI**: Viti Levu, Vuda Prov., Koroyanitu Pk, 1 km E Abaca Vlg., SavuioneTrl, 800m. 22.IV–6.V.2003 by E. Schlinger and M. Tokota'a.

(1). **FIJI**: Kaduva I., 0.25 km SW Solodamu Vlg., Moanakaka Bird Sanctuary, 60m. Collected 9–30.V.2003 by E. Schlinger and M. Tokota'a.

(1). **FIJI**: Viti Levu, Sigatoka Prov., Sigatoka Sand Dunes Nat. Pk, 44m. Collected 12.II–12.III.2003 by E. Schlinger and M. Tokota'a.

(1). **FIJI**: Levu Is., Maitaesiri Prov., Hakobalevu Mt, 340m. Collected 12–24.III.2003 by M. Irwin et al.

(1). **FIJI**: Viti Levu, Naitasiri Prov., Bakobalevu logging rd. Collected 17.III–9.IV.2003 by E. Schlinger and M. Tokota'a.

Diagnosis. Females of *P. nigriscutellatus* are differentiated by the following combination of features: face alutaceous to coriaceous centrally; mandible quadridentate (Fig. 3); pronotum smooth dorsally (sometimes coriaceous along anterior margin), alutaceous laterally; mesoscutum purplish-coppery, slightly bluish-green posteriorly, alutaceous to coriaceous posteromedially, distinctly concave posteromedially (Fig. 12). Males of *P. nigriscutellatus* are differentiated by the following combination of features: head entirely coriaceous; mandible quadridentate; scutellar-axillar complex coriaceous; colouration darker than that of females.

Distribution. Islands of Fiji, Tonga, Western Samoa (Noyes 2015).

Biology. Parasitoids of *Graeffea crouanii* eggs.

Paranastatus parkeri Scallion, sp. n.

http://zoobank.org/536F9418-3E12-4D39-AD29-051A59D1FEAF

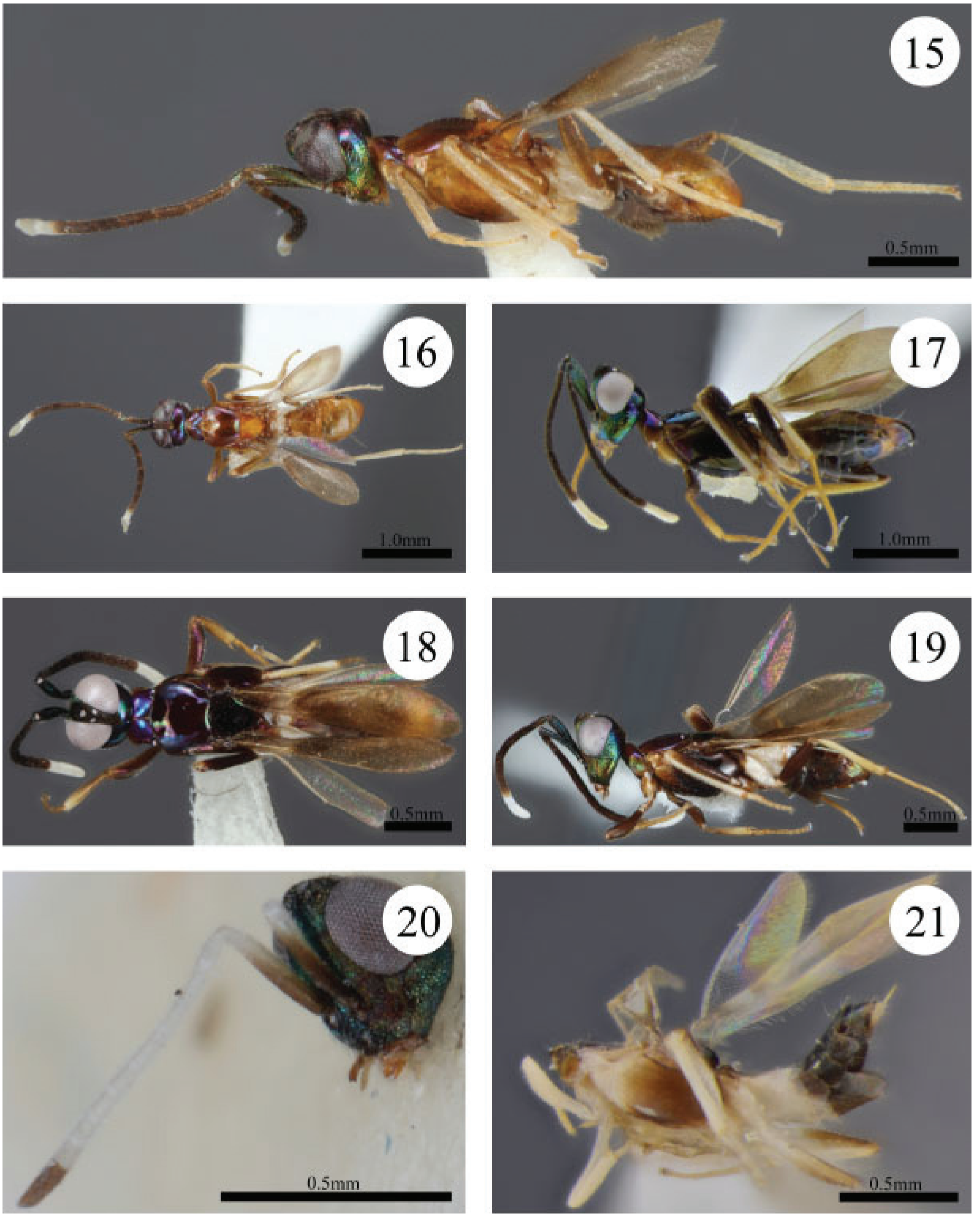
Fig. 24

Material examined. Holotype female, dry pinned, deposited in BPBM (Type No. 17541). Label data: "FIJI: Viti Levu, 3.5 km N Veisari Stlmt, logging rd to Waivudawa, 14.II–8.III.03, 300m, Malaise 3, coll. E. Schlinger, M. Tokota'a 18.068°S, 178.367°E. FBA 136331."

Diagnosis. The unique female of *P. parkeri* is differentiated by the following combination of features: vertex and temple smooth; frontovertex smooth with a few small bumps; face smooth to alutaceous; mandible quadridentate; mesoscutum smooth except faintly coriaceous in posteromedial concavity.

Description. Female. Length: 2.2 mm.

Colour. Head with vertex coppery between ocelli, metallic green to blue-purple posterior to ocelli; temple shining metallic green-purple dorsally to metallic blue-purple laterally; gena shining metallic coppery-green; entire face metallic dark purple-brown; frontovertex blue-green with brown centrally. Antenna with scape lightly shining green; pedicel, anellus (flagellomere 1), and flagellomeres 2–6 brown, 7, 8 and club white. Pronotum coppery-green; mesoscutum purplish-coppery, slightly bluish-green posteriorly; scutellar-axillar complex dull black; mesopleuron purple-coppery. Legs with procoxa dark brown, protrochanter light brown; mesocoxa light yellow-brown; metacoxa brown basally and white apically; remaining leg segments straw-yellow. Fore wing very lightly infuscate with hyaline band below distal half of submarginal vein; hind wing hyaline. Gaster green apically, tergites otherwise dark coppery-green and sternites brown. Colour of setae on various body regions discussed in appropriate sections below.



Figures 15–21. 15 *Paranastatus verticalis* holotype, lateral habitus **16** *P. verticalis* holotype, dorsal view **17** *P. verticalis* (Taveuni, Fiji), lateral habitus (JBWM Photo 2015-06) **18** *P. verticalis* (Taveuni, Fiji) in dorsal view (JBWM Photo 2015-06) **19** *P. halko* holotype, lateral habitus **20** *P. bellus* holotype, head in frontolateral view and antenna **21** *P. bellus* holotype, body in lateral view.

Head. Vertex and temple smooth; gena smooth to alutaceous along occipital margin; lower face smooth to alutaceous centrally, scrobes smooth to weakly alutaceous, interantennal area alutaceous; occipital margin straight in dorsal view; frontovertex smooth with a few small bumps. Mandible quadridentate. Entire head with sparse brown setae; eyes with sparse, very short white setae.

Mesosoma. Pronotum smooth; mesoscutum smooth to slightly coriaceous posteromedially, distinctly concave posteromedially; scutellar-axillar complex reticulate; mesopleuron coriaceous. Pronotum, mesoscutum, and scutellar-axillar complex with very few brown setae; mesopleuron with few short white setae anteriorly, remainder bares. Fore wing with dense, short brown setae; hind wing with relatively fewer short, brown setae.

Metasoma. Entirely coriaceous with long, brown setae sparsely distributed.

Male. Unknown.

Etymology. Named in honour of Parker Brant, nephew of Barb Sharanowski, born November 2, 2012 in Australia to Julie and Billy Brant. This is a noun in the genitive case.

Distribution. Viti Levu, Fiji.

Biology. Unknown.

Remarks. Abdomen was broken and lost after description and imaging had been completed. Antennae cannot be used as an identifying character in this species because the antennal colouration is the same as that of *P. nigriscutellatus*.

***Paranastatus pilosus* Scallion, sp. n.**

http://zoobank.org/57CABD16-BA30-4BDD-A5E8-74A0780A4A41

Figs 7, 9, 11, 22

Material examined. Holotype female, dry pinned, deposited in BMNH (Hym Type 5.4814, barcode NHMUK010198567). Label data: "INDONESIA: Seram, Solea VIII.1987, MT M. Day, forest."

Paratype females (8), dry pinned, deposited in BMNH and CNC.

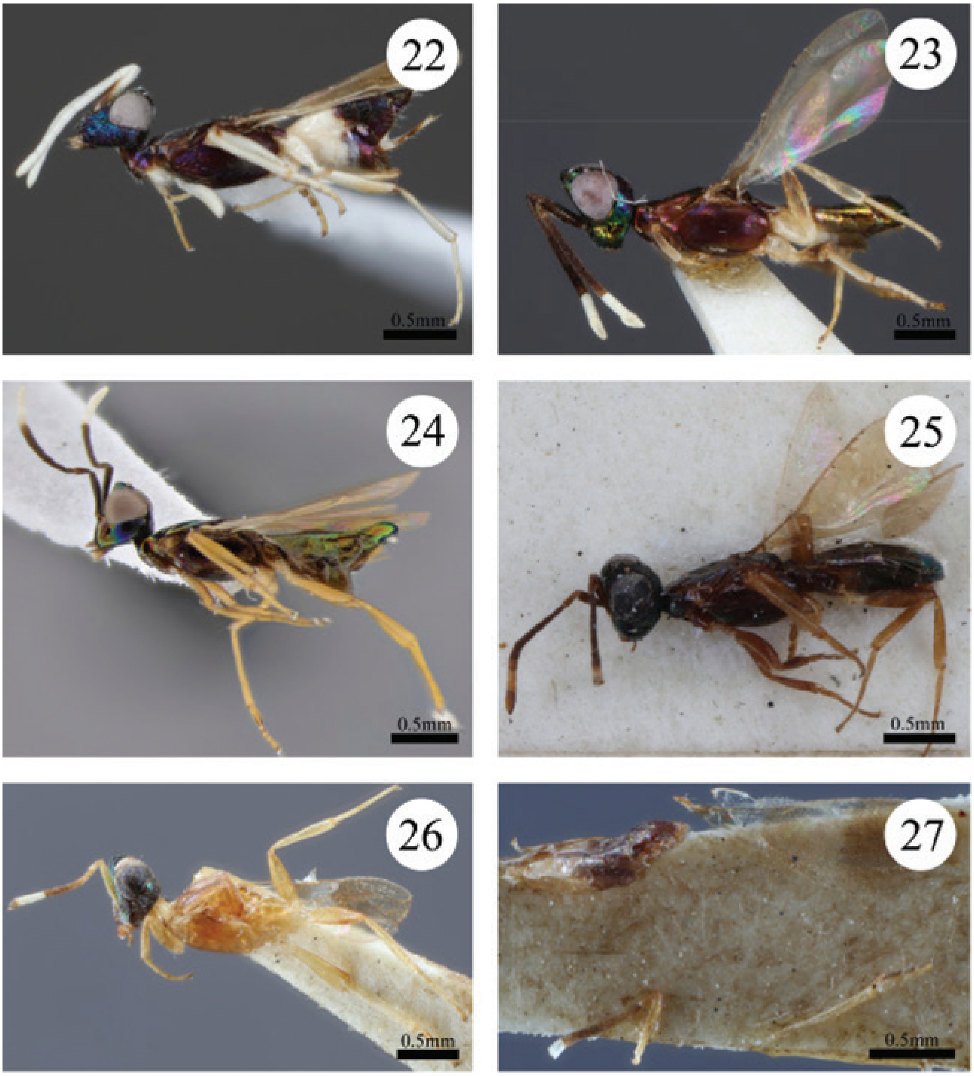
(4, includes JBWM Photo 2015-05). **INDONESIA**. Seram, Solea. IX.1987, M. Day.

(4). **INDONESIA**. Seram, Solea. VIII.1987, M. Day, forest.

Diagnosis. Females of *P. pilosus* are differentiated by the following combination of features: vertex granulate between ocelli, reticulate posterior to ocelli (Fig. 11); temple reticulate (Fig. 11); antenna mostly white except scape brown basally and club lightly darkened apically (Fig. 22); mandible tridentate; mesoscutum blue-purple medially, brown laterally, and reticulate (Fig. 9).

Description. Female. Length: 2.6 mm.

Colour. Head with vertex dull black-brown, sometimes purple-brown posterior to ocelli; temple dark blue-purple; gena blue-purple (Fig. 7); lower face mostly blue-purple but brown centrally below toruli; scrobes and interantennal area green or coppery-green; frontovertex dull black-brown or with blue centrally. Antenna white, except basal half of scape brown and very tip of club slightly darkened, and sometimes club completely white (Fig. 22). Pronotum metallic purple-blue, sometimes purple-brown laterally; mesoscutum blue-purple medially, brown laterally; scutellar-axillar complex dull black; mesopleuron purple. Legs with profemur white; mesofemur white with



Figures 22–27. 22 *Paranastatus pilosus* holotype, lateral habitus **23** *P. nigriscutellatus* holotype, lateral habitus **24** *P. parkeri* holotype, lateral habitus **25** *P. violaceus* lectotype, lateral habitus **26** *P. egregius* lectotype, partial body in lateral view **27** *P. egregius* lectotype broken body parts glued to the point.

darkened posterior apical edge; metafemur white becoming yellow-brown apically; rest of legs white. Fore wing lightly infuscate in apical half, hyaline in basal half with small infuscate patch at base; hind wing hyaline. Gastral tergites 1–2 white, rest dark brown; gastral sternites 1–4 white, remainder purplish-brown. Colour of setae on various body regions discussed in appropriate sections below.

Head. Vertex granulate between ocelli, reticulate posterior to ocelli (Fig. 11); temple reticulate (Fig. 11); gena and face reticulate; occipital margin concave in dorsal view; frontovertex with blunt teeth projecting posteriorly towards vertex or sometimes granulate. Mandible tridentate. Head with white setae except scrobes bare; eyes with dense, short white setae.

Mesosoma. Pronotum coriaceous (Figs 9, 11); mesoscutum reticulate, distinctly concave posteromedially (Fig. 9); scutellar-axillar complex reticulate (Fig. 9); mesopleuron coriaceous. Pronotum with white setae, setae longer along posterior edge; mesoscutum with dense white setae; scutellar-axillar complex with few long white setae; mesopleuron with few white setae anteriorly, remainder bare. Fore wing with dense, short brown setae; hind wing with relatively fewer short, light brown setae.

Metasoma. Entirely coriaceous with white setae evenly distributed ventrally, setae sparser and shorter dorsally, and longer at apex of gaster.

Male. Unknown.

Etymology. From Latin *pilosus*-hairy, in reference to the females having noticeably more setae than the other species. This is an adjective in the nominative case.

Distribution. Seram Island, Indonesia.

Biology. Unknown.

Paranastatus verticalis **Eady, 1956**

Figs 5, 6, 15–18

Paranastatus verticalis Eady, 1956: 64–65.

Material examined. Holotype female, dry pinned, deposited in BMNH (Hym Type 5.1625a). Label data: "HWY 976 FIJI Suva Suva VI.1954 B.A. O'Connor. ex eggs of *Graeffea crouani* C.I.E.Coll. 13792."

Allotype male, dry pinned, deposited in BMNH. Label data: "HWY 976 FIJI Suva Suva VI.1954 B.A. O'Connor. ex eggs of *Graeffea crouani* C.I.E.Coll. 13792."

Paratype females (7), dry pinned.

(6, deposited in BMNH, includes JBWM Photo 2015-04). HY 976 **FIJI** Suva Suva VI.1954.

(1, deposited in USNM). HY 976 **FIJI** Suva Suva VI.1954 B.A. O'Connor.

Other material. Females (2), dry pinned.

(1, deposited in BPBM). **FIJI**. Taveuni, Cakaudrove Prov., 5.5 km SE Tavuki Vlg., Devo Pk. 1188m, 30.VI–14.VIII.2004 Malaise 1, Schlinger, M. Tokota'a. 16.843°S, 179.966°W. FBA 152624. JBWM Photo 2015-06.

(1, deposited in CNC). **FIJI**. Vanua Levu, Bua Prov., 6 km NW Kilaka, 15.VI–24.VI.2004 Batiqere Range. Malaise. 98m ‖ Schlinger, Tokota'a FJVN58b_M05_07 _16.8067, 178.9914 FBA174462.

Diagnosis. Females of *P. verticalis* are differentiated by the following combination of features: vertex raised between eyes, and temple flat such that temple and occiput form almost a right angle (Fig. 5); vertex granulate; lower face with fringe of setae below toruli (Fig. 6); mandible tridentate; mesoscutum smooth and convex or flat, not concave (Fig. 16). Males of *P. verticalis* are differentiated by the following combination of features: vertex granulate; mandible tridentate; in dorsal view occipital margin deeply excavate; colouration darker than that of females.

Distribution. Islands of Fiji, Tonga, Western Samoa (Noyes 2015).

Biology. Parasitoids of *Graeffea crouanii* eggs.

Variation. The two specimens collected in 2004 differ in several features compared to the type series described by Eady (1956). The specimen from Taveuni Island has a body length of 2.85 mm, whereas all specimens in the type series range from 2.4–2.5mm. Unfortunately, an accurate measurement of body length was not possible in the specimen from Vanua Levu because the body is contorted. The scutellar-axillar complex in the new material is dark black-brown (Fig. 18) not light orange-brown (Fig. 16), and the legs are darker than in the type series (Fig. 17). Other slight variations in colour include: temple green-purple laterally, not blue-purple; lower face metallic blue-green, not coppery-green; and pronotum purple-blue dorsally, not purple-coppery. The new material has gastral tergite 1 white, tergite 6 light orange-brown, and remaining tergites dark brown, not gastral tergite 1 white and remaining tergites light brown, or gastral tergite 1 white, tergites 2 and 3 dark brown, and remaining tergites grading to light brown at gastral apex.

Paranastatus violaceus Masi, 1917

Figs 13, 25

Paranastatus violaceus Masi, 1917: 166–167.

Material examined. Lectotype female, here designated; dry pinned, deposited in BMNH (Hym Type 5.1,036). Label data: "Silhouette, '08. Seychelles Exp. Percy Sladen Trust Exped. B.M. 1913-170."

Diagnosis. Females of *P. violaceus* are differentiated by the following combination of features: vertex and temple coriaceous; antenna brown except flagellomeres 7 and 8 light yellow-brown (Fig. 25); mandible quadridentate; fore wing evenly infuscate (Fig. 25). Males unknown.

Distribution. Silhouette Island, Seychelles.

Biology. Unknown.

Remarks. Masi (1917) established *P. violaceus* based on three females, one of which was stated as lacking its gaster. Of the three females, the BMNH only has one complete specimen in its collection (Dale-Skey, pers. comm.). We here designate this female as lectotype and have labelled it accordingly. The location of the other two females is presently unknown.

Discussion

During the last 100 years, *Paranastatus* has been recorded throughout the South Pacific and from one location in the Indian Ocean (Masi 1917, Eady 1956, Rapp 1995, O'Connor 1955). *Paranastatus egregius* and *P. violaceus* were described from Seychelles and additional specimens have not been reported since. This may be because these two species are extremely rare, now extinct, or most likely collecting efforts have been insufficient to recover them. Differentiating between these possible reasons would require more intensive sampling of biodiversity, an issue that is important worldwide because of climate change, habitat destruction, and species extinctions. *Paranastatus nigriscutellatus* and *P. verticalis* have been recorded subsequent to their description, mostly from the islands of Fiji, but also from Tonga (Rapp 1995) and only through rearing rather than collecting. Most of the new material described here was obtained through passive collecting by Malaise traps. This and other passive collecting methods may provide the best way to obtain specimens of *Paranastatus* other than through rearing.

Graeffea crouanii, the coconut stick insect, is a pest of coconut palms and is found on many islands throughout the South West Pacific, including Fiji (Deesh et al. 2013). Deesh et al. (2013) hypothesized that *G. crouanii* dispersed in one of three ways: (1) by eggs that fell into canoes from overhanging palms on the beach; (2) by eggs floating across the ocean to other islands because they are saline-tolerant; or (3) simply by the adults being carried on coconut leaves by humans to new locations. The dispersal of eggs of *G. crouanii* could account for the dispersal of *P. verticalis* and *P. nigriscutellatus*, suggesting that wherever this stick insect is found, these two species of *Paranastatus* could be found as well.

Gibson (1995) proposed a hypothesis to explain dispersal ability of females in Eupelminae. Because of structural modifications to improve jumping ability, females appear to have reduced flight capabilities that reduce their ability to disperse. He used this hypothesis to explain why better known eupelmine species often have several to numerous hosts, it being advantageous to develop on a wide array of hosts within a limited dispersal area for survival of the parasitoid population. This suggests that species of *Paranastatus* could have a wider host range than is currently recorded.

The current known distribution of *Paranastatus* is perplexing because there are large distances between localities, which leaves the question of how the wasps dispersed through time. One hypothesis that could explain *Paranastatus* distribution is wind dispersal as aerial plankton. Insects have been collected far from land in both the Indian and South Pacific Ocean through aerial netting (Holzapfel and Harrel 1968), and since *Paranastatus* wasps are very small it is possible that they were carried across the ocean on the wind. Another hypothesis is that the wasps dispersed passively through their hosts, such as what may have occurred with parasitized *Graeffea crouanii* eggs (Deesh et al. 2013). If other hosts are discovered similar dispersal mechanisms might also be discovered. It is also possible that the true distribution of this genus has yet to be discovered. Because two of the new species as well as *P. nigriscutellatus* and *P. verticalis* are from Fiji, and the other two new species are from Indonesia, it is conceivable that more species of *Paranastatus* exist in other regions of the South Pacific and Indian Ocean.

Another possible explanation for the distribution of *Paranastatus* is that it was once larger than it is now. *Paranastatus* species are basically confined to geographical clusters: the Fijian species have not been found in Indonesia and vice versa. *Paranastatus* *nigriscutellatus* and *P. verticalis* have been found on several other South Pacific islands, but only east of Fiji. The two Seychelles species (*P. egregius* and *P. violaceus*) have never been found since their original capture. It may be that species of *Paranastatus* did exist in other regions, but have since become extinct. Extinction, if it has happened, may have occurred through habitat fragmentation that reduced the genus to its current number of species and localities. Fragmented habitats can lead to extinction by decreasing available habitat and causing smaller population sizes, and parasitoids tend to be more sensitive to habitat fragmentation than other trophic levels (Kruess and Tscharntke 2000).

It is interesting that the newly collected female specimens of *P. verticalis* are darker in colour than the original specimens collected in 1954. One possibility for this is that the type specimens have faded over the course of 50 years; however, this does not seem likely as the specimens still conform to Eady's (1956) description. The two newly collected specimens of *P. verticalis* are from localities east and west of the original type locality. The slightly more western specimen is from Bua province on Vanua Levu, whereas the eastern specimen is from Cakaudrove province, Taveuni Island. The type specimens were from Savusavu, Vanua Levu, which is centered between Bua province and Taveuni Island. It is possible that continual colour variation would be found across the entire distribution of this species, if sampled, or that females vary in colour pattern based on a specific niche, host, environment or some other difference affecting development.

One complication that arises when studying the taxonomy of Eupelminae is their extreme sexual dimorphism. Non-chalcidologists are likely to identify male eupelmines as Pteromalidae rather than Eupelmidae, and most identification keys to species of eupelmine genera are based only on females, which makes it difficult to correctly identify males unless they are reared together with females. There is a chance that *Paranastatus* males have been collected before, but were misidentified or unidentified. This may account for the lack of males recorded in this genus.

Future work on *Paranastatus* could include a closer examination of the biogeography of the different species. The disparate distribution between *P. egregius* and *P. violaceus* from Seychelles, and the remaining species from islands in the South Pacific, suggests that locations between these regions may have additional species of *Paranastatus*. Therefore, it could be worthwhile collecting in regions between Seychelles and Indonesia to improve knowledge of the distribution of the genus or to discover new species.

Acknowledgments

We would like to thank the staff of BMNH and USNM for lending us type material of all previously described species. Dr. Erwin Huebner, Andre Dufresne, Leanne Peixoto, and Derek Eyer were all greatly appreciated for their help in obtaining scanning electron microscope images. We would also like to thank Amber Bass and Jordan Bannerman for their help with wasp photo shoots, and Miles Zhang for offering lots of support and ideas through the course of this research project as well as patiently putting up with all sorts of questions from MLS. We are also grateful to the two reviewers for their valuable comments. This research was funded by the NSERC Discovery program from a grant awarded to BJS and a NSERC Undergraduate Student Research Award to MLS.

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Supplementary material 1

Paranastatus Label Data

Authors: Melanie L. Scallion, Gary A.P. Gibson, Barbara J. Sharanowski

Data type: Label data

Explanation note: This file contains complete label data for all material examined, including localities

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RESEARCH ARTICLE

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Revision of the subgenus *Tinotus* Sharp, stat. n., of the parasitoid rove-beetle genus *Aleochara* Gravenhorst (Coleoptera, Staphylinidae, Aleocharinae) from Japan, Taiwan, and the Russian Far East

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Abstract

The subgenus *Tinotus* Sharp, 1833, **stat. n.**, of the genus *Aleochara* Gravenhorst, 1802 (Aleocharini: Aleocharina) from Japan, Taiwan, and the Russian Far East is revised. *Tinotus* is a new record from the latter two regions. Three species are recognized: *Aleochara* (*Tinotus*) *morion* Gravenhorst, 1802, **comb. n.** [Japan (new record), the Russian Far East (new record)], *A.* (*T.*) *eoa* **nom. n.** [replacement name for *Tinotus japonicus* Cameron, 1933; Japan, Taiwan (new record)], and *A.* (*T.*) *takashii* **sp. n.** (central Honshû, Japan). The systematic position of *Tinotus* is discussed. All species are (re-)described, keyed, and figured. A world checklist of *Tinotus* species, comprising 40 valid species, is provided in an appendix. Additional taxonomic changes are proposed, including a new synonymy, a revalidation, 13 new replacement names, and 27 new combinations.

Keywords

Aleocharini, new species, new combinations, revalidation, replacement names, checklist, East Asia, Palaearctic Region

Introduction

The rove-beetle genus *Aleochara* Gravenhorst, 1802 (Aleocharinae: Aleocharini, Aleocharina) is distributed worldwide, except in Antarctica (Klimaszewski 1984). This genus is the most speciose genus of the tribe Aleocharini, with approximately 500 species in 18 subgenera. *Aleochara* is likely a monophyletic group based on extensive molecular analyses (Maus et al. 2001).

On the other hand, the genus *Tinotus* Sharp, 1883 has recently been recognized as phylogenetically close to, or a possible member of, *Aleochara* (Maus et al. 2001; Hanley 2002; Osswald et al. 2013). Its taxonomic placement, including its tribal assignment, has been controversial for a long time, mainly due to its tarsal formula (4-5-5; see Hanley 2002). According to Maus et al. (2001), *Tinotus* is a taxon that should be considered within the genus *Aleochara*. The distributional range of *Tinotus* is quite wide, as it has been recorded from every zoogeographic region, except the Australian Region, with approximately 40 species (Hanley 2002; Klimaszewski et al. 2002).

Larvae of *Aleochara* and *Tinotus* act as ectoparasitoids on cyclorrhaphous Diptera, and the adults prey upon dipteran eggs and larvae (e.g., Klimaszewski 1984; Maus et al. 1998). Thus, they have been considered potential candidates for classic biological control of commercial crops against pest flies in Europe and North America (e.g., White and Legner 1966; Fournet et al. 2000).

Taxonomic knowledge of *Tinotus* in East Asia is still incomplete. In Japan, Taiwan, and the Russian Far East, just one species, *Tinotus japonicus* Cameron, 1933, has been originally described from Japan. In this study, we (re-)describe three *Tinotus* species distributed in these regions. We discuss the systematic position of *Tinotus*. We also provide a complete *Tinotus* species list, reflecting recent species additions, synonyms, and corrections.

Material and methods

We used the technical procedures and methods used by Maruyama (2006) and Yamamoto and Maruyama (2012). The terminology used for this study generally follows that of Hanley (2002), Klimaszewski et al. (2002), and Yamamoto and Maruyama (2012, 2013). For chaetotaxy of the mouthparts we followed Sawada (1972, 1987). We followed Welch (1997) for genital terminology, especially that of females. In the descriptions, the number of macrosetae on tergite VIII and sternite VIII refers to one side of the body. Furthermore, minute setae were omitted or depicted only for one side of the body.

Abbreviations for measurements: BL, length of the body from clypeus to apex of the abdomen; EW, maximum width both elytra combined; HL, maximum length of the head; HW, maximum width of the head; PL, maximum length of the pronotum; PW, maximum width of the pronotum along midline.

Other abbreviations: BRL, blue round label pinned by a curator; HW, handwritten.

The following acronyms of museums and private collections are used throughout the text:

BMNH	Natural History Museum, London, U.K. (R. Booth);
FMNH	Field Museum of Natural History, Chicago, U.S.A. (R. Baquiran);
HUM	Hokkaido University Museum (M. Ôhara);
KUM	Kyushu University Museum, Fukuoka, Japan (M. Maruyama);
PCTW	Private collection of Mr. Takashi Watanabe (Kanagawa, Japan).

Taxonomy

Genus *Aleochara* Gravenhorst, 1802

Subgenus *Tinotus* Sharp, 1883, stat. n.

Tinotus Sharp, 1883: 170. Type species: *Tinotus cavicollis* Sharp, 1883. Fixed by Fenyes 1918: 25, by subsequent designation.

Exaleochara Keys, 1907: 102. Type species: *Tinotus morion* Gravenhorst, 1802. Fixed by Klimaszewski et al. 2002: 284, by monotypy. As synonym of *Tinotus*: e.g., Bernhauer & Scheerpeltz 1926: 713; Blackwelder 1952: 163; Ashe 2000: 360; Hanley 2002: 457; Klimaszewski et al. 2002: 284; Gouix and Klimaszewski 2007: 30; Schülke and Smetana 2015: 505.

Acrimea Casey, 1911: 14. Type species: *Acrimea resecta* Casey, 1911. Fixed by Fenyes 1918: 20, by subsequent designation. Synonymized by Gusarov 2003: 353.

See further references in Hanley (2002), Klimaszewski et al. (2002), and Gusarov (2003).

Diagnosis. This subgenus is rather easily distinguished from the other congeneric taxa by 1) compact, small (< 4 mm), and 2) strongly spindle-shaped body; 3) 4-5-5 tarsal formula (5-5-5 in the other subgenera of *Aleochara*); 4) fully carinate mesoventrite; 5) wide and 6) truncate apex of intercoxal process of mesoventrite, 7) and its apex reaching to apex of intercoxal process of metaventrite; 8) median lobe of aedeagus with developed flagellum; 9) female spermatheca without apical invagination of spermathecal head (*sensu* Welch 1997) and, 10) coiled basally. Minute characters on mouthparts probably define the subgenus as well, e.g., setula *a* on the first segment of labial palpi located at nearly apical margin of the segment (Fig. 5; see also Sawada 1987).

Remarks. See other characters mentioned in detail by Hanley (2002) and Klimaszewski et al. (2002).

Systematic position. Sharp's (1833) original description of *Tinotus* placed this taxon in the group Myrmedoniina (= Lomechusini) due to its 4-5-5 tarsal formula. Since, *Tinotus* has also been placed in Hoplandriini (e.g., Seevers 1978) or Aleocharini (e.g., Lohse 1974), mainly based on the presence of a pseudosegment on the maxillary and labial palpi (see Hanley 2002 for a historical review).

In contrast to these ambiguities, recent studies have refuted all tribal placements other than Aleocharini. Hanley (2002) recognized *Tinotus* within Aleocharini, suggesting a close relationship with the genus *Aleochara*, based on the seven shared morphological characteristics of the genus, e.g., bifid to crescent-shaped apex of the ligula. According to the extensive molecular study of *Aleochara* by Maus et al. (2001), *Tinotus* was fully resolved within the "*bilineata* clade" of *Aleochara*. Similarly, Osswald et al. (2013), who used significantly fewer species (only four species of *Aleochara*, one of which is *Tinotus*) but analyzed them with much more molecular markers (4599 bp), also supported the assignment of *Tinotus* to *Aleochara*.

In our morphological study of *Tinotus* and *Aleochara* species, we found numerous morphological similarities between these genera, including a long intercoxal process of mesoventrite, except for the 4-5-5 tarsal segmentation in *Tinotus* (5-5-5 in *Aleochara*). Among the subgenera of *Aleochara*, *Tinotus* shares characters with the subgenus *Xenochara* Mulsant & Rey, 1874, i.e., carinate mesoventrite and fusiform body (including convexed pronotum). Remarkably, the subgenus *Coprochara* Mulsant & Rey, 1874 seems to be significantly more closely related to *Tinotus*. In fact, they share some important characters, including a completely carinate mesoventrite and a coiled spermatheca (Yamamoto and Maruyama 2013). Maus et al. (2001) also implied that both subgenera are phylogenetically close to *Tinotus*. Reduction of the antennal segment or tarsal segmentation in Aleocharinae is associated rather frequently with miniaturization of their body size (e.g., tribes Hypocyphtini and Mesoporini), and *Tinotus* species are possibly no exception. Therefore, no significant character exists to distinguish *Tinotus* from *Aleochara* at the genus level. We herein transfer *Tinotus*, as the 19th subgenus, to the genus *Aleochara*.

***Aleochara* (*Tinotus*) *morion* Gravenhorst, 1802, comb. n.**

Figs 1, 4–19, 36

Aleochara morion Gravenhorst, 1802: 97 (original description).

Tinotus morion: Seevers 1978: 196 (male genitalia figured); Hanley 2002: 463 (catalogue of world species of *Tinotus*); Klimaszewski et al. 2002: 285 (key to Nearctic species of *Tinotus*), 294 (redescription); Smetana 2004: 362 (catalogue of Palearctic species of Aleocharinae); Gouix and Klimaszewski 2007: 30 (catalogue of Canadian and Alaskan species of Aleocharinae), 149 (dorsal habitus photographed); Klimaszewski et al. 2013: 16 (catalogue of Canadian Staphylinidae), 60 (redescription), 247 (dorsal habitus photographed), 273 (male and female genitalia figured); Schülke and Smetana 2015: 506 (catalogue of Palearctic species of Aleocharinae).

See other references and synonymies in Hanley (2002) and Klimaszewski et al. (2002).

Type locality. Braunschweig, Germany.

Non-type material examined. JAPAN: Hokkaidô: 1 male, Nemuroshibetsu, Shibetsu-chô, 18.vii.1977, S.-I. Naomi leg. (KUM); 1 male, Lake Toro, Shibecha, 27.vii.1986, S. Nomura leg. (KUM); 1 male, 3 spec., Kamishumbetsu, Betsukai-chô, 20.vii.1977, S.-I. Naomi leg. (KUM); 1 male, Mt. Mashû-dake (just below the summit), 820 m, Teshikaga-chô, 15.vii.1990, sweeping of *Carex*-grass, K. Haga leg. (KUM); 1 female, Shiretoko-tôge Pass, Rausu-chô, 3.viii.1989, bottom of gutter on roadside, K. Haga leg. (KUM); 1 male, 1 spec., Sakae-machi, Oshidomari, Rishiri-fuji-chô, S.-I. Naomi leg. (KUM); 2 females, 1 spec., Nukanan Dam (right bank), Memuro, Ashoro-chô, 30.vii.1988, human excrement, K. Haga leg. (KUM); 1 male, 1 female, 7 spec., Shihoro, Kamishihoro-chô, GPS 43°32'03.9"N, 143°09'58.5"E, 13.vii.2014, bear dung, S. Yamamoto leg. (KUM); 2 males, 1 female, 1 spec., Obihiro-shi, 6.vi.1980, H. Togawa leg. (KUM); 2 spec., Obihiro-shi, 7.vii.1980, H. Togawa leg. (KUM); **Honshû:** 1 female, Inashiki, Ibaraki-ken, 29.iv.1983, S. Ohmomo leg. (KUM); 1 male, Sugaya, Ranzan-machi, Saitama-ken, 10.iv.1994, K. Toyoda leg. (KUM); 1 female, Mt. Gagyû-san, Takahashi-shi, Okayama-ken, 29.v.1977, S.-I. Naomi leg. (KUM). **RUSSIA: Far East:** 1 male, Maltsevskaya Cape, Churkin, Vladivostok, Primorsky, 22.vi.1997, human excrement, M. Ôhara leg. (HUM).

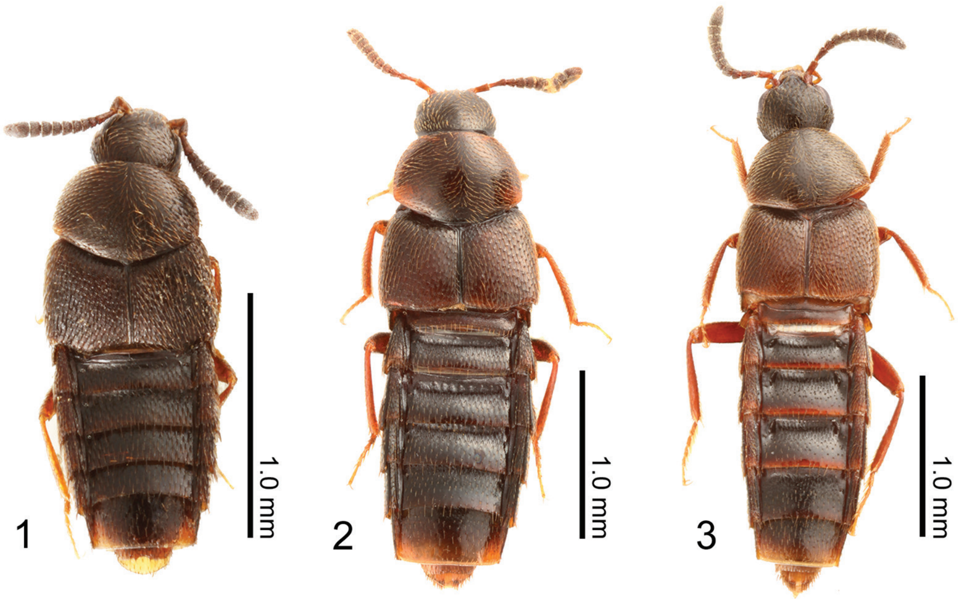
Reference material examined. AUSTRIA: Niederösterreich: 1 spec., "Ulrichskirchen / N. Ö., J. Spurny // morion [HW] / grh. [HW] // Chicago NHMus / M. Bernhauer / Collection" (FMNH); **ITALY: Calabria:** 1 spec., "Calabria / Cimina / lg. Paganetti // morion [HW] / grh. [HW] / det. Bernh. // Chicago NHMus / M. Bernhauer / Collection" (FMNH).

Diagnosis (see Klimaszewski et al. 2002). This species can be distinguished from most members of the subgenus *Tinotus* by the following combination of characters: body entirely black, rarely light brown (Fig. 1); median lobe of aedeagus of male with a basal protuberance in lateral view (Fig. 16: arrow); spermatheca with a simple and oblong spermathecal head, equally serrate inner walls inside spermathecal head, and with four coils at base (Fig. 19). *Aleochara morion* is extremely similar to *A.* (*T.*) *rougemonti* (Pace, 1993), comb. n. from China, including male-female genital structures, but the former species is discriminated from the latter by having longer sclerites inside a median lobe of the male aedeagus (Pace 1993: Fig. 160), and by having four coils of the female spermatheca (*A. rougemonti* with two coils; Pace 1993: fig. 162).

Redescription. *Measurements* (in mm, n = 30): BL = 2.448 (1.777–2.996); HL = 0.382 (0.315–0.453); HW = 0.392 (0.332–0.451); PL = 0.421 (0.355–0.485); PW = 0.632 (0.518–0.724); EW = 0.737 (0.595–0.853).

Body (Fig. 1): fusiform, compact, and robust; dorsal surface moderately glossy and pubescent, covered with large micro-reticulation. Color (Fig. 1): usually uniformly black to blackish brown; antennomeres I–III dark brown, but segments IV to X darker with numerous minute whitish setae; mouthparts and legs yellowish brown to brown; pubescence yellowish brown to brown.

Head (Fig. 1): subquadrate, as long as width (HW/HL = 1.03, n = 30), widest at just behind eyes; setae on vertex rather dense, directed anteriomedially. Eyes: small, occupying approximately one third of head length, very slightly protruding laterally.



Figures 1–3. Dorsal habitus of Japanese species of *Aleochara* (*Tinotus*): **1** *Aleochara* (*Tinotus*) *morion* **2** *A.* (*T.*) *eoa* **3** *A.* (*T.*) *takashii*.

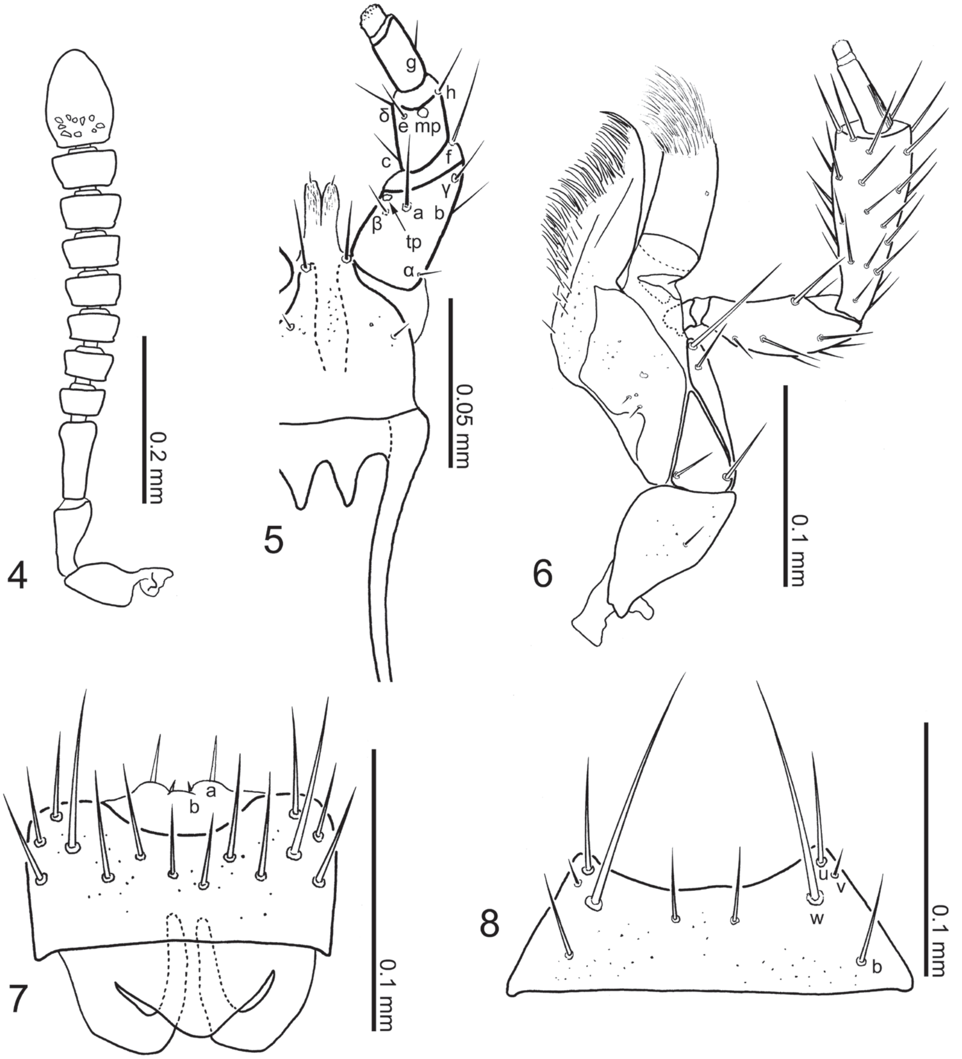
Mouthparts (Figs 5–8): labrum (Fig 7) moderately transverse, approximately 1.70 times as wide as long (excluding basal apodeme), anterior margin slightly emarginate medially, basal apodeme semi-transparent; surface with pseudopores scattered scarcely. Labial palpus (Fig. 5): setula *a* well-developed, situated near apical margin of labial palpomere I, while that of *b* and *c* strongly reduced. Maxilla (Fig. 6): lacinia with a distal comb consisting of dense-thin spines; galea rather short, as long as maxillary palpomere II; maxillary palpomere IV narrow and short, less than half length of that of III. Mentum (Fig. 8): anterior margin broadly emarginate.

Antennae (Fig. 4): short, moderately shorter than head and pronotum combined; thick, setaceous, becoming gradually and slightly broaden apically in segments IV to X, with segments IV to X clearly transverse; segment XI symmetrical, obtusely pointed at apex; approximate relative length of segments from basal to apex: 22: 14: 16: 5: 5: 5: 6: 6: 7: 7: 19.

Pronotum (Fig. 11): convex above dorsally, transverse (PW/PL = 1.50, n = 30), moderately longer than sutural length of elytra, widest around below of basal half, basal margin weakly rounded; pubescence rather long, dense, directed laterally and posterolaterally; micro-reticulation conspicuous.

Mesoventrite (Fig. 9): completely carinate along midline; inter coxal process broadly elongate, with truncate apex, completely reaching to inter coxal process of metaventrite.

Elytra (Figs 1, 10): together, transverse, rather small, widest at middle; pubescence short, finely scattered densely, diverging posterolaterally in each elytron; dorsal surface rough, somewhat deeply impressed; posterolateral corner of each elytron moderately sinuate.

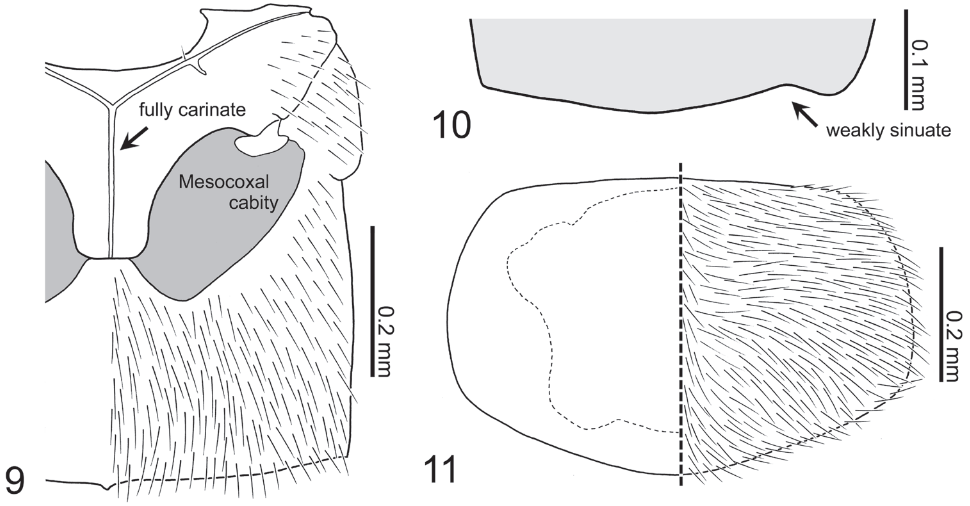


Figures 4–8. Body parts of *Aleochara* (*Tinotus*) *morion* of male: **4** right antenna **5** labium **6** maxilla **7** labrum **8** mentum.

Legs (Fig. 1): simple, short and, moderately slender; fore and midtibia with dozens of undeveloped spines, respectively.

Abdomen (Fig. 1): first three visible tergites rather shallowly impressed transversely at base; dorsal and ventral surface covered with setae densely.

Male. Tergite VIII (Fig. 12): basal suture fully developed (see Maruyama 2006: 20); posterior margin very weakly serrate, insignificantly emarginate medially; dorsal surface covered with setae rather densely, with five macrosetae. Sternite VIII (Fig. 14): basal suture fully developed; posterior margin very weakly pointed; ventral surface cov-

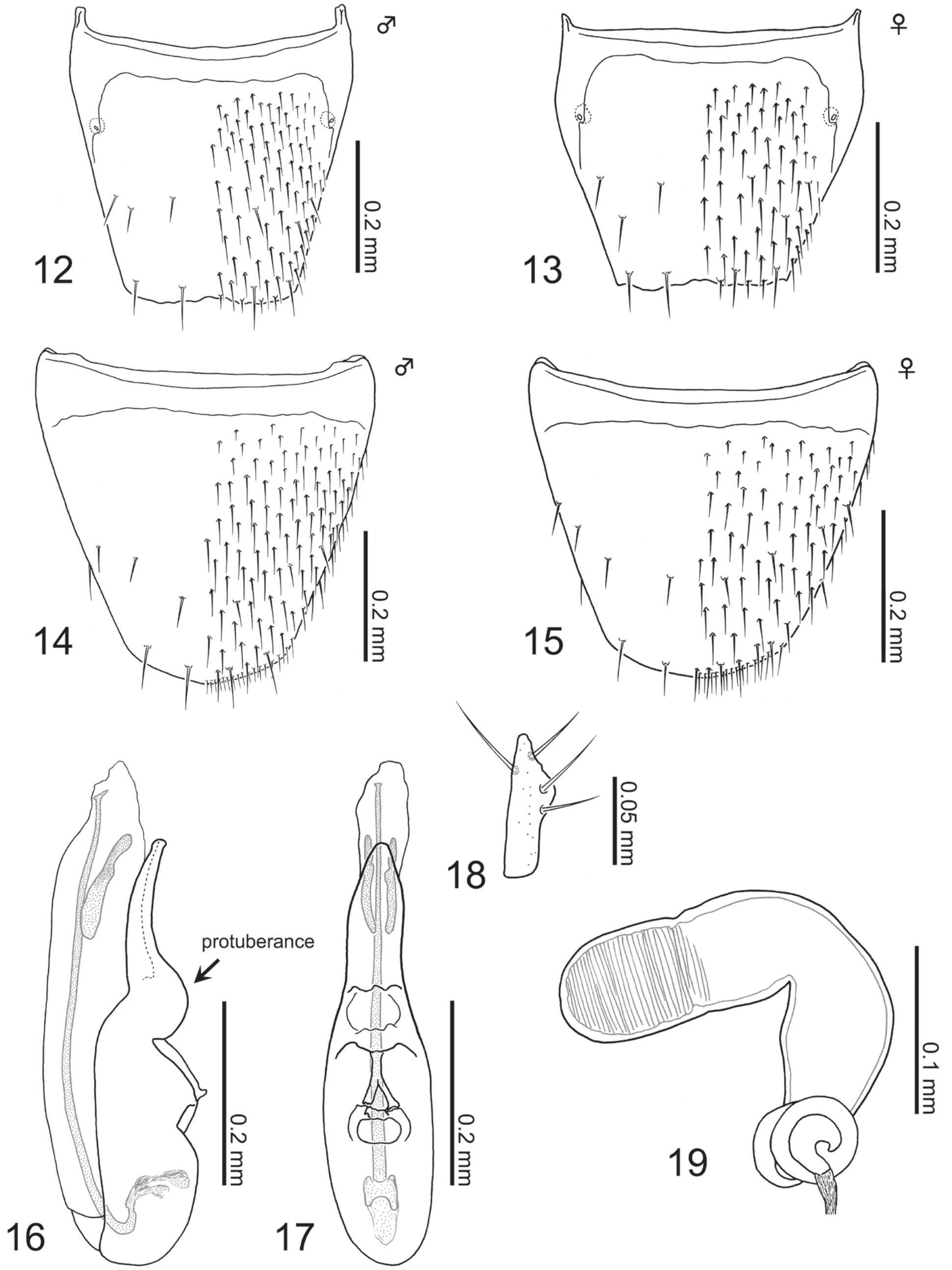


Figures 9–11. Body parts of *Aleochara* (*Tinotus*) *morion* of male: **9** mesoventrite and metaventrite **10** right elytron, posterior margin **11** pronotum.

ered with setae densely, with approximately six macrosetae. Median lobe of aedeagus (Figs 16, 17): very slender in parameral view; apical lobe slender, weakly narrowing apically, and gently curved paramerally in lateral view; a conspicuous protuberance present at base of apical lobe (see arrow); a pair of simple sclerites, narrowly elongate, longer than half length of apical lobe; flagellum well developed, slightly shorter than median lobe, sharply curved near basal plate. Apical lobe of paramerite (Fig. 18): narrowly elongate, widest just above middle, with sharply pointed apex.

Female. Tergite VIII (Fig. 13): basal suture fully developed; posterior margin very weakly serrate, insignificantly emarginate medially; dorsal surface covered with setae rather sparsely, with five macrosetae. Sternite VIII (Fig. 15): basal suture fully developed; posterior margin rounded; ventral surface covered with setae densely, with approximately seven macrosetae. Spermatheca (Fig. 19): L-shaped; spermathecal head and neck fused together, forming a narrowly elongate capsule; attachment of spermathecal duct inconspicuous; basal part of spermathecal stem moderate in size, slightly longer than spermathecal neck, with four coils attached at base; each part of spermatheca entirely and very weakly sclerotized; inner wall of spermathecal head and neck, along border with head, finely and densely striate.

Distribution. This species has a wide range in distribution covering the entire Holarctic region, mainly Europe and North Africa (Schülke and Smetana 2015). The records in the Nearctic region are regarded as those species introduced from Europe (Klimaszewski et al. 2002). In Russia, the easternmost record reported is from West Siberia (Schülke and Smetana 2015). We provide new distributional records of *A. morion* as follows: new country record, Japan (Hokkaidô, Honshû); new regional record, the Russian Far East.



Figures 12–19. Terminalia of *Aleochara* (*Tinotus*) *morion*: **12** tergite VIII of male **13** tergite VIII of female **14** sternite VIII of male **15** sternite VIII of female **16** median lobe of male aedeagus, lateral view **17** ditto, parameral view **18** apical lobe of paramerite **19** female spermatheca.

Bionomics. SY collected eight specimens from one Hokkaido brown bear (*Ursus arctos*) dung found on the roadside of a mixed needleleaf and broadleaf forest in Hokkaidô, Japan (Fig. 36). *Aleochara morion* has been found among various habitats, such as decaying organic matter, including fungi, compost, animal excrement, and carrion (Horion 1967). In addition, this species is found in moss, bark debris, straw, hay, and on sandy soil (Klimaszewski et al. 2002).

Host records. Three dipteran families are known as its host (Maus et al. 1998): Sepsidae, Drosophilidae, and Sarcophagidae.

Remarks. Whether this species is native to East Asia or just an introduction from Europe is unknown, although the records from North America suggest this species has been introduced (Klimaszewski et al. 2002). Recently, Pace (2013) recorded *A. morion* on the mainland of China.

***Aleochara* (*Tinotus*) *eoa* nom. n.**

Figs 2, 20–26, 35

Tinotus japonicus Cameron, 1933: 217 (original description).

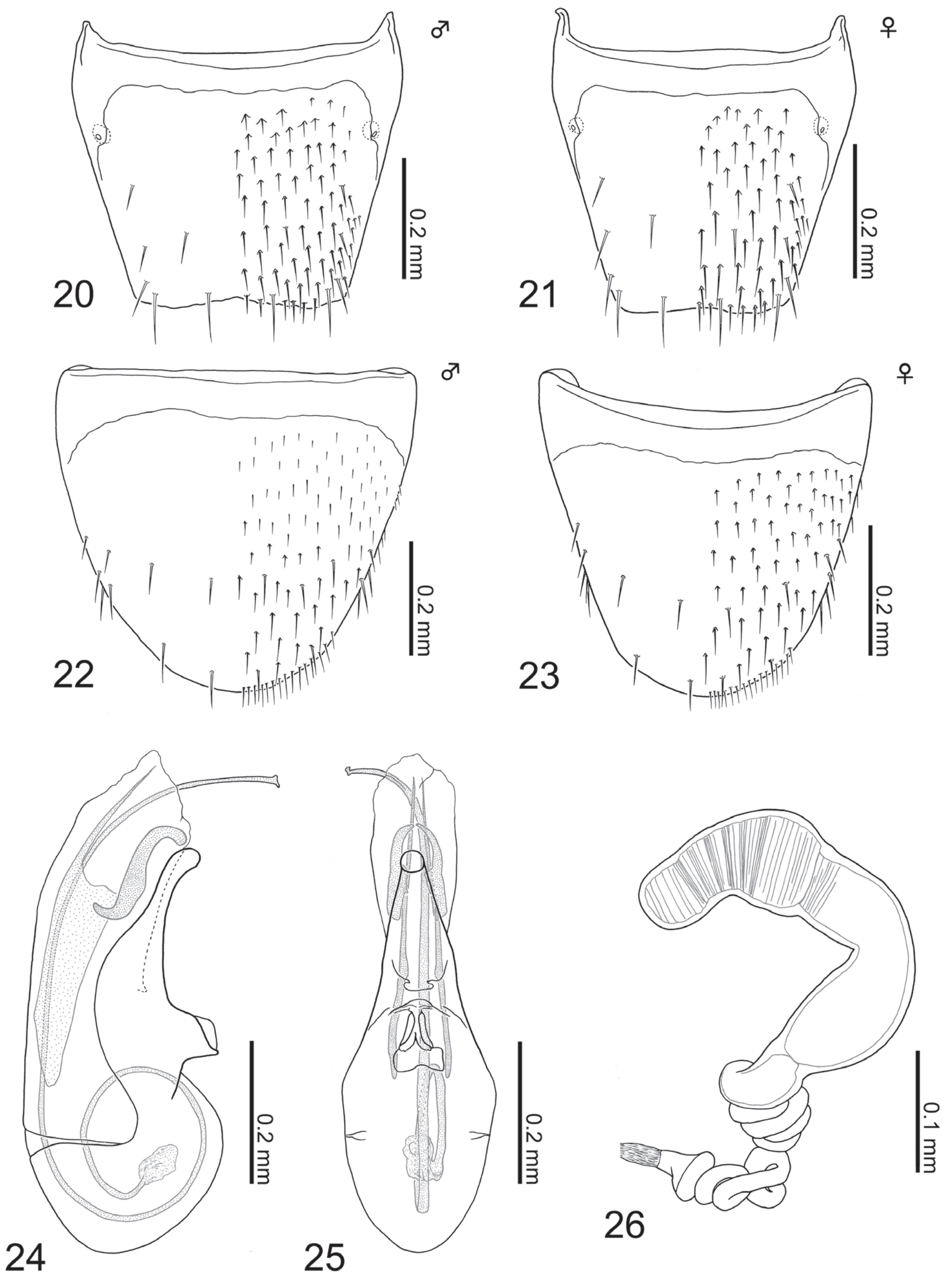
Tinotus japonicus: Smetana 2004: 362 (catalogue of Palearctic species of Aleocharinae); Shibata et al. 2013: 106 (catalogue of Japanese species of Staphylinidae); Schülke and Smetana 2015: 505 (catalogue of Palearctic species of Aleocharinae).

Type locality. Kobe, Japan.

Type material examined. *Tinotus japonicus*: Lectotype (here designated): male, “SYN- / TYPE [BRL] // JAPAN / Kobe // J. E. A. Lewis // M. Cameron / Bequest. / B. M. 1955-147 // Tinotus / japonicus / TYPE Cam [HW] // Tinotus / japonicus / P. M. Hammond / det. 1973 / SYNTYPE // Lectotype / Tinotus japonicus / Cameron, 1933 / des. Maruyama, 2011” (abdominal segments VIII-X and aedeagus were dissected and mounted in Euparal by MM) (PL, 0.42 mm; PW, 0.59 mm; Hind tibial length, 0.40 mm) (BMNH). Paralectoypes: 3 males, 1 female, same original labels as lectotype but without the label “Tinotus / japonicus / TYPE Cam [HW]” (abdominal segments VIII-X and spermatheca were dissected and glued on paper card together with body by MM) (BMNH).

Additional material examined. JAPAN: Honshû: 1 female, Shigasaka-tôge Pass, Kanna-machi, Gunma-ken, 17-19.vi.2008, Flight Interception Trap, T. Watanabe leg. (KUM); 1 male, 2 females, Sugaya, Ranzan-machi, Saitama-ken, 10.iv.1994, K. Toyoda leg. (KUM). **TAIWAN: Nantou:** 1 male, 3 females, 5 spec., Songkang, 2000m, 14.iv.1986, M. Ôhara leg. (KUM).

Diagnosis. This species is distinguished from the other congeneric species of the subgenus by a following combination of character states: body reddish brown to dark brown (Fig. 2); median lobe of aedeagus of male with a coiled flagellum, and with two pairs of characteristic sclerites (Figs 24, 25); spermatheca with a curved spermathecal



Figures 20–26. Terminalia of *Aleochara* (*Tinotus*) *eoa*: **20** tergite VIII of male **21** tergite VIII of female **22** sternite VIII of male **23** sternite VIII of female **24** median lobe of male aedeagus, lateral view **25** ditto, parameral view **26** female spermatheca.

head, unequally serrated inner walls inside spermathecal head, and with multiple coils at base (Fig. 26). *Aleochara eoa* is the most similar externally to *A.* (*T.*) *rougemontiana* (Pace, 1999a), comb. n., from mainland China, differing from it additionally by having much less coiled spermatheca in the female (Pace 1999a: Fig. 183).

Redescription. *Measurements* (in mm, n = 13): BL = 2.709 (2.288–3.011); HL = 0.427 (0.358–0.511); HW = 0.439 (0.380–0.486); PL = 0.466 (0.368–0.565); PW = 0.666 (0.514–0.758); EW = 0.780 (0.605–0.948).

Body (Fig. 2): fusiform, compact, and robust; dorsal surface somewhat strongly glossy and pubescent, covered with small and inconspicuous micro-reticulation.

Color (Fig. 2): usually uniformly dark reddish brown to dark brown; antennomeres I–IV much lighter, but segments V to XI darker with numerous minute whitish setae; mouthparts and legs light-yellowish brown to reddish brown; pubescence yellowish brown to brown.

Head (Fig. 2): subquadrate, as long as width (HW/HL = 1.03, n = 13), widest at base of eyes; setae on vertex rather dense, directed anteriomedially. Eyes: small, occupying approximately one third of head length, very slightly protruding laterally.

Antennae (Fig. 2): short, moderately shorter than head and pronotum combined; relatively thick, setaceous, becoming gradually and slightly broaden apically in segments IV to X, with segment V spherical and segments VI to X clearly transverse; segment XI symmetrical, obtusely pointed at apex; approximate relative length of segments from basal to apex: 21: 17: 14: 6: 7: 7: 7: 7: 7: 7: 18.

Pronotum (Fig. 2): strongly convex above dorsally, transverse (PW/PL = 1.43, n = 13), moderately longer than sutural length of elytra, widest around below of basal half, basal margin weakly rounded; pubescence rather long, rather dense but thin, directed laterally and posterolaterally; micro-reticulation inconspicuous.

Elytra (Fig. 2): together, transverse, rather small, widest at middle; pubescence short, finely scattered densely, diverging posterolaterally in each elytron; dorsal surface moderately rough, shallowly impressed; posterolateral corner of each elytron moderately sinuate.

Abdomen (Fig. 2): first three visible tergites rather shallowly impressed transversely at base; dorsal and ventral surface covered with setae densely.

Male. Tergite VIII (Fig. 20): basal suture fully developed; posterior margin very weakly serrate, insignificantly emarginate medially; dorsal surface covered with setae rather sparsely, with six macrosetae. Sternite VIII (Fig. 21): basal suture fully developed; posterior margin rounded to only weakly produced; ventral surface covered with short setae sparsely, with approximately nine macrosetae. Median lobe of aedeagus (Figs 24 & 25): ovular in lateral and limuloid in parameral view; apical lobe rather slender, gently curved paramerally, weakly narrowing apically in parameral but with weakly dilated apex in lateral view; without a protuberance at base of apical lobe; a pair of sclerites S-shaped, longer than half length of apical lobe; flagellum strongly developed, much longer than median lobe, coiled 1.5 times at base.

Female. Tergite VIII (Fig. 21): basal suture fully developed; posterior margin very weakly serrate or almost truncate; dorsal surface covered with setae rather sparsely, with six macrosetae. Sternite VIII (Fig. 23): basal suture fully developed; posterior margin rounded; ventral surface covered with setae rather sparsely, with approximately nine macrosetae. Spermatheca (Fig. 26): deformed M-shaped; spermathecal head curved at middle; attachment of spermathecal duct inconspicuous; basal part of spermathecal stem moderate in size, clearly longer than spermathecal neck, with approximately ten coils attached complicatedly at base; each part of spermatheca entirely and very moderately sclerotized; inner wall of spermathecal head and neck, along border with head, finely and densely striate irregularly.

Etymology. The replacement name is derived from "Eos" of the Greek mythology which is a Titaness and the goddess of the dawn because "Nippon" (= Japan, type locality) means a country of the dawn.

Distribution. Japan, Taiwan (new record).

Bionomics. One individual was caught with a flight interception trap (FIT).

Host records. No host record is available.

Remarks on type materials. Five syntypes were found. Among them, a male specimen (Fig. 35) labeled "Tinotus / japonicus / TYPE Cam [HW]" is designated as the lectotype herein.

Comments. Since the name *Aleochara japonica* was already preoccupied by Sharp (1874), a new replacement name, *Aleochara* (*Tinotus*) *eoa* nom. n., for *Tinotus japonicus* Cameron, 1933 [nec. Sharp, 1874: 8 (*Aleochara*)], is proposed herein. No record of this species exists since its original description.

***Aleochara* (*Tinotus*) *takashii* sp. n.**

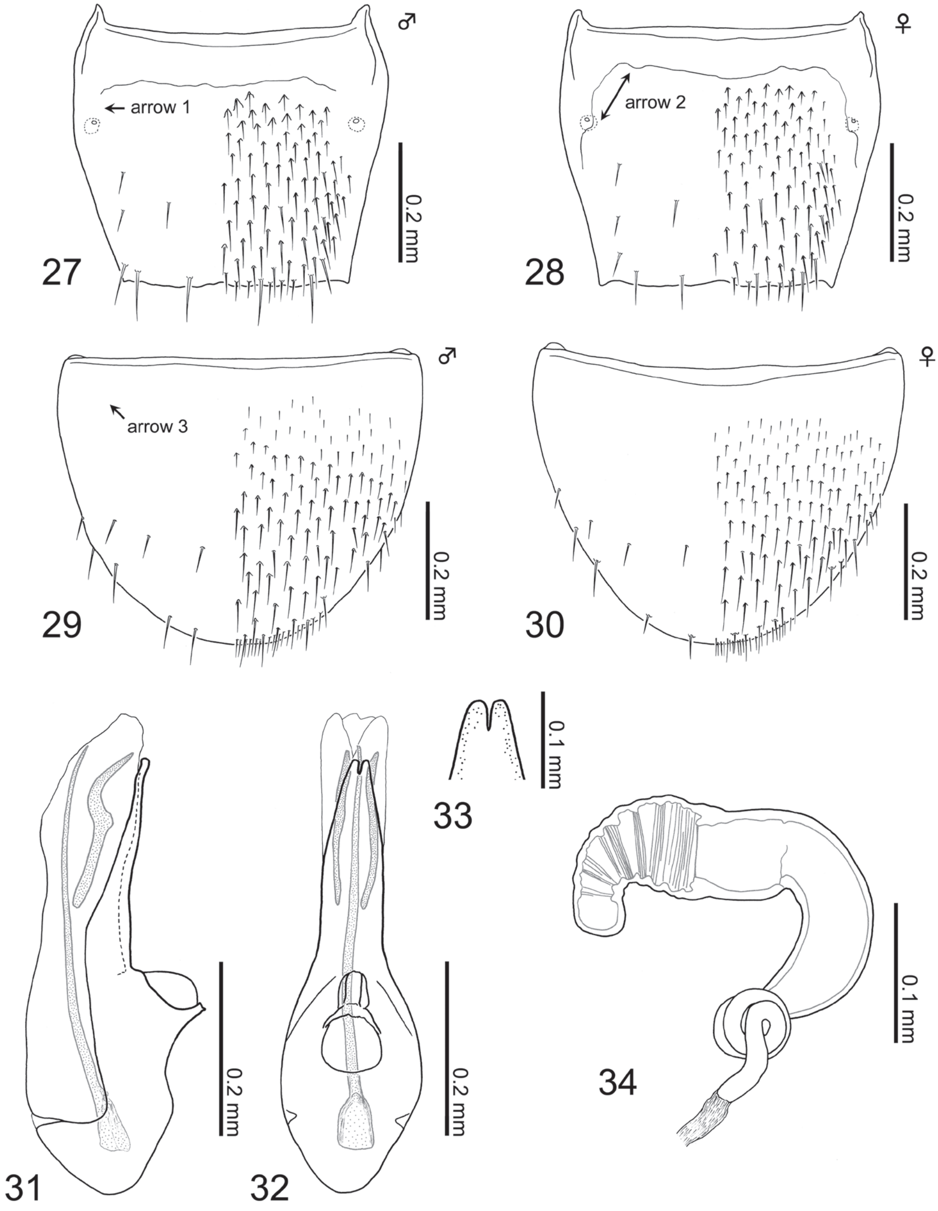
http://zoobank.org/79D2ADA2-E87B-4F16-867C-904F9A438C20

Figs 3, 27–34

Type locality. Japan, Honshû: Takahachiyama, Fujinomiya City, Shizuoka Prefecture.

Type material. Holotype: male, "Takahachiyama / Fujinomiya-shi / Shizuoka, JAPAN / 17-24. VIII. 2010 / T. Watanabe leg. [printed] // Flight / Intercept. / Trap [printed] // Aleocharini / Gen. / sp. / det. T. Watanabe 2013 [yellow square paper card, printed]" (KUM).

Paratypes: 1 male, "Teppogino-atama / Nishitanzawa / Kanagawa, Japan / 5-12. VII. 2007 / T. Watanabe leg. // Flight / Intercept. / Trap" (KUM); 1 male, "Teppogino-atama / Nishitanzawa / Kanagawa, Japan / 5-12. VII. 2007 / T. Watanabe leg. // Flight / Intercept. / Trap // *Aleochara* / sp. / det. T. Watanabe 2007" (KUM); 1 male, "Teppogino-atama / Nishitanzawa / Kanagawa, Japan / 5-12. VII. 2007 / T. Watanabe leg. // Flight / Intercept. / Trap // *Aleochara* / sp. / det. T. Watanabe 2008" (KUM); 1 male, "Idenzawa / Nishitanzawa / Kanagawa, Japan / 31. V – 6. VI. 2006 / T. Watanabe leg. // *Aleochara* / sp. / det. T. Watanabe 2007" (KUM); 2 spec., "Yanagisawa-toge / Enzan-shi / Yamanashi, Japan / 2-9. VIII. 2006 / T. Watanabe leg. // Flight / Intercept. / Trap // *Aleochara* / sp. / det. T. Watanabe 2007" (KUM); 1 female, "Yanagisawa-toge / Enzan-shi / Yamanashi, Japan / 9-15. VIII. 2006 / T. Watanabe leg. // Flight / Intercept.



Figures 27–34. Terminalia of *Aleochara* (*Tinotus*) *takashii*: **27** tergite VIII of male **28** tergite VIII of female **29** sternite VIII of male **30** sternite VIII of female **31** median lobe of male aedeagus, lateral view **32** ditto, parameral view **33** ditto, apex of apical lobe, parameral view **34** female spermatheca.

/ Trap // *Aleochara* / sp. / det. T. Watanabe 2006" (PCTW); 2 females, "Karumizu-rindo / Narusawa-mura / Yamanashi, JAPAN / 30. VIII-14. IX. 2010 / T. Watanabe leg. // *Aleochara* / sp. / det. T. Watanabe 2012" (KUM); 1 female, "Karumizu-rindo / 1600 m, Narusawa / Yamanashi, JAPAN / 3-10. VIII. 2011 / T. Watanabe leg. // Flight / Intercept / Trap // *Aleochara* / sp. / det. T. Watanabe 2012" (KUM); 1 male, "Aokigahara, Fuji- / Kawaguchiko / Yamanashi, JAPAN 11-17. V. 2012 / T. Watanabe leg. // Flight / Intercept. / Trap / *Aleochara* / sp. / det. T. Watanabe 2013" (KUM); 1 male (head mounted on slide), 1 spec., "Fujisan 1-gome / Subashiri (1400 m) / Shizuoka, JAPAN / 20-26. V. 2011 / T. Watanabe leg. // Flight / Intercept. Trap // *Aleochara* / sp. / det. T. Watanabe 2012" (KUM); 1 male, 1 female, "Ohbuchi (alt. 950m) / Fuji-shi / Shizuoka, JAPAN / 13-18. V. 2010 / T. Watanabe leg. // Flight / Intercept. / Trap // *Aleochara* / sp. / det. T. Watanabe 2012" (KUM); 1 spec., "Ohbuchi (alt. 950m) / Fuji-shi / Shizuoka, JAPAN / 16-22. VII. 2010 / T. Watanabe leg. // Flight / Intercept. / Trap // *Aleochara* / sp. / det. T. Watanabe 2012" (KUM); 1 spec., "Ohbuchi (alt. 950m) / Fuji-shi / Shizuoka, JAPAN / 24. X. 2012 / T. Watanabe leg. // Aleocharinae" (KUM); 1 male, "Takahachiyama / Fujinomiya-shi / Shizuoka. JAPAN / 28. VIII. 2012 / T. Watanabe leg. // Aleocharinae" (KUM); 1 male, "Nishiusuzuka / Fujinomiya-shi / Shizuoka, JAPAN / 22-31. V. 2013 / T. Watanabe leg. // Flight / Intercept. / Trap // *Aleochara* / sp. / det. T. Watanabe 2013" (PCTW); 1 male, "Nishiusuzuka / Fujinomiya-shi / Shizuoka, JAPAN / 8-16. VII. 2010 / T. Watanabe leg. // Flight / Intercept. / Trap // *Aleochara* / sp. / det. T. Watanabe 2010" (PCTW); 1 male, 1 spec., "Nishiusuzuka / Fujinomiya-shi / Shizuoka, JAPAN / 16-22. VII. 2010 / T. Watanabe leg. // Flight / Intercept. / Trap // *Aleochara* / sp. / det. T. Watanabe 2011" (KUM); 1 spec., "Nishiusuzuka / Fujinomiya-shi / Shizuoka, JAPAN / 17-24. VIII. 2010 / T. Watanabe leg. // Flight / Intercept. / Trap // *Aleochara* / sp. / det. T. Watanabe 2012" (KUM).

Diagnosis. This species can be easily distinguished from the other members of the subgenus by a following combination of characters: body entirely reddish brown (Fig. 3); abdominal segments III-V (first three visible terga) deeply impressed laterobasally; both tergite and sternite VIII with weakly developed basal sutures (Figs 27–30); median lobe of aedeagus with long apical lobe, notched deeply and medially at apex in ventral view (Fig. 33); spermatheca with a curved spermathecal head, unequally serrated inner walls inside spermathecal head, and with approximately three coils at base (Fig. 34). *Aleochara takashii* is the most similar to the North American species, *A.* (*T.*) *imbricata* (Casey, 1894), comb. n., of which shares including the similar configuration of male genitalia. From *A. imbricata*, it can be distinguished additionally by having much more developed sclerites inside the median lobe of the aedeagus and by overall shape of the spermatheca (Klimaszewski et al. 2002: 289: Figs 13–16, 31–33).

Description. *Measurements* (in mm, n = 23): BL = 3.156 (2.637–3.669); HL = 0.419 (0.361–0.483); HW = 0.488 (0.418–0.760); PL = 0.528 (0.421–0.605); PW = 0.756 (0.599–0.866); EW = 0.880 (0.693–1.019).

Body (Fig. 3): fusiform, compact, and robust; dorsal surface moderately glossy and pubescent, covered with small and inconspicuous micro-reticulation.

Color (Fig. 3): usually uniformly dark reddish brown to dark brown; elytra lighter; antennomeres I–IV lighter, but segments V to XI darker with numerous minute whitish setae; mouthparts and legs yellowish brown to dark reddish brown; apices of tergites III-V pale reddish brown transversely; pubescence yellowish brown to brown.

Figures 35–36. Type material and habitat of *Aleochara* (*Tinotus*): **35** lectotype of *Tinotus japonicus* (= *A. eoa*) **36** Habitat of *A.* (*T.*) *morion* at Shihoro (Hokkaidô, Japan), red star indicating bear dung where eight specimens were collected.

Head (Fig. 3): subquadrate, slightly transverse (HW/HL = 1.17, n = 23), widest just behind base of eyes; setae on vertex rather dense, directed anteriomedially. Eyes: small, occupying approximately one third of head length, very slightly protruding laterally.

Antennae (Fig. 3): short, moderately shorter than head and pronotum combined; relatively thick, setaceous, becoming gradually and moderately broaden apically in segments V to X, with segment V elongate and segments VI to X clearly transverse; segment XI symmetrical, obtusely pointed at apex; approximate relative length of segments from basal to apex: 23: 16: 17: 10: 9: 9: 9: 9: 9: 9: 22.

Pronotum (Fig. 3): strongly convex above dorsally, transverse (PW/PL = 1.43, n = 23), moderately longer than sutural length of elytra, widest around below of basal half, basal margin weakly rounded; pubescence in moderate length but thin, directed laterally and posterolaterally; micro-reticulation inconspicuous.

Elytra (Fig. 3): together, transverse, rather small, widest at middle; pubescence short, finely scattered densely, diverging posterolaterally in each elytron; dorsal surface moderately rough, shallowly impressed; posterolateral corner of each elytron moderately sinuate.

Abdomen (Fig. 3): first three visible tergites deeply impressed transversely at base; dorsal and ventral surface covered with setae rather sparsely.

Male. Tergite VIII (Fig. 27): basal suture not fully developed, suture partially disappeared laterally (Fig. 27: arrow 1); posterior margin very weakly serrate, insignificantly emarginate medially or truncate; dorsal surface covered with setae rather densely, with six macrosetae. Sternite VIII (Fig. 29): basal suture completely lost (Fig. 29: arrow 3); posterior margin rounded; ventral surface covered with short setae densely, with approximately eight macrosetae. Median lobe of aedeagus (Figs 31–33): narrowly elongate in lateral, and limuloid narrowly in parameral view; apical lobe slender and long, as long as basal capsule, moderately narrowing apically in lateral view, with deeply notched apex medially in parameral view (Fig. 33); without a protuberance at base of apical lobe; a pair of sclerites curved just above middle, long, approximately 2/3 length of apical lobe; flagellum developed, shorter than median lobe, without any coils at base.

Female. Tergite VIII (Fig. 28): basal suture not fully developed, suture partially disappeared laterally like male (see Fig. 27: arrow 1) or at most weakly developed (Fig. 28: arrow 2); posterior margin very weakly serrate or almost truncate; dorsal surface covered with setae densely, with six macrosetae. Sternite VIII (Fig. 30): basal suture completely lost like male (see Fig. 29: arrow 3); posterior margin rounded; ventral surface covered with setae densely, with approximately eight macrosetae. Spermatheca (Fig. 34): curved semi-circularly in lateral view; spermathecal head curved at middle; attachment of spermathecal duct inconspicuous; basal part of spermathecal stem moderate in size, clearly longer than spermathecal neck, with approximately three coils; each part of spermatheca entirely and very moderately sclerotized; inner wall of spermathecal head, coarsely striate irregularly.

Etymology. The species name is dedicated to its collector, Mr. Takashi Watanabe (Kanagawa, Japan).

Distribution. Only known from central Honshû, Japan (Kanagawa, Yamanashi, and Shizuoka Prefectures).

Bionomics. Most specimens were caught with flight interception traps (FIT).

Host records. No host record is available.

Remarks. This new species is distinct among the species of *Tinotus*. In particular, reduced or non-developed basal sutures on tergite and sternite VIII of both sexes are notable character states (Figs 27–30). Furthermore, the apical lobe of the median lobe of male aedeagus, [i.e., deeply notched medially at apex in parameral view (Fig. 33)], is also a remarkable character state even among the subfamily. Since other morphological characters correspond fully to that of the subgenus *Tinotus*, we assign this species to *Tinotus* without hesitation.

Key to species of the subgenus *Tinotus* (genus *Aleochara*) from Japan, Taiwan, and the Russian Far East

1 Body black to blackish brown including pronotum and elytra (Fig. 1); median lobe of aedeagus of male with a basal protuberance in lateral view (Fig. 16); spermatheca coiled four times basally, with oblong and simple spermathecal head (Fig. 19) .... ***Aleochara* (*Tinotus*) *morion* Gravenhorst, 1802, comb. n.**

– Body dark brown to reddish brown (Figs 2, 3); median lobe of aedeagus of male without a basal protuberance in lateral view (Figs 24, 31); spermatheca with curved and deformed spermathecal head (Figs 26, 34) **2**

2 Abdominal segments III-V (first three visible terga terga) deeply impressed laterobasally; tergite and sternite VIII with reduced basal sutures (Figs 27–30); median lobe of male aedeagus with short flagellum (Figs 31–32); spermatheca with approximately three coils at base (Fig. 34)............***A.* (*T.*) *takashii* sp. n.**

– Abdominal segments III-V (first three visible terga terga) rather shallowly impressed laterobasally; tergite and sternite VIII with complete basal sutures (Figs 20–23); median lobe of male aedeagus with long and coiled flagellum (Figs 24, 25); spermatheca with complex multiple coils at base (Fig. 26) ....***A.* (*T.*) *eoa* nom. n.**

Discussion

We recognize three *Tinotus* species from Japan. This species count is clearly lower than those for mainland China (6 spp.: Pace 2013a; Schülke and Smetana 2015) and the United States (6 spp.: Klimaszewski et al. 2002; Gusarov 2003). In contrast, it surpasses the counts of adjacent countries, e.g., South and North Korea (0 sp.: Schülke and Smetana 2015) and Russia, including the Far East (1 sp.; Schülke and Smetana 2015; present study). Surprisingly, all of the continental European countries has no, or at most, only one *Tinotus* species (*A. morion*; Schülke and Smetana 2015). Within central Honshû, Japan, we found all of the three species. Therefore, Japan, especially central part of the country, is considered to possess a relatively high species diversity of *Tinotus*.

Only one species has been confirmed in Taiwan and the Russian Far East, respectively, which may reflect potentially low species diversity in these regions or merely insufficient accumulation of materials. Since taxonomic studies and records of *Tinotus* in East Asia, including these two regions, are still lacking, further discussions are avoided here. The finding of *A. eoa* in Taiwan implies a wide distributional range of this species, and it may be discovered on mainland China.

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Appendix

Checklist of the world species of the subgenus *Tinotus* of the genus *Aleochara*. Forty species are recognized in total. See annotated checklist of world species of *Tinotus* by Hanley (2002) for further details.

Genus *Aleochara* Gravenhorst, 1802

Subgenus *Tinotus* Sharp, 1883: 170, stat. n.

1. *acerba* (Casey, 1911: 15), **comb. n.** (*Acrimea*). Distribution: Canada, USA.
   = *resecta* (Casey, 1911: 14) (*Acrimea*).
2. *andensis* (Pace, 2008a: 365), **comb. n.** (*Tinotus*). Distribution: Ecuador.
3. *arawakorum* (Pace, 1990: 74), **comb. n.** (*Tinotus*). Distribution: Brazil (São Paulo).
4. *boreoindica* **nom. n.** [for *indica* (Cameron, 1939: 557) (*Tinotus*), nec. Fauvel, 1904: 66 (*Maseochara*), nec. Cameron, 1939: 635 (*Aleochara*)]. Distribution: India, China (Hubei, Shaanxi).
   Etymology. Meaning northern India where the type locality is located.
5. *caelatimas* (Pace, 2008b: 177), **comb. n.** (*Tinotus*). Distribution: Malaysia (Sabah).
6. *carnivora* (Cameron, 1920: 270), **comb. n.** (*Paratheta*), nec. Gravenhorst, 1806: 171 (*Aleochara*). Distribution: Singapore.
7. *caviceps* (Casey, 1894: 316), **comb. n.** (*Tinotus*). Distribution: Canada, USA.
   = *parata* (Casey, 1911: 64) (*Tinotus*).
8. *cavicollis* (Sharp, 1883: 170), **comb. n.** (*Tinotus*). Distribution: USA (FL), Guatemala, Nicaragua, Brazil, Paraguay, Argentina, Cuba.
9. *clavicornuta* **nom. n.** [for *clavicornis* (Cameron, 1945: 729) (*Tinotus*), nec. L. Redtenbacher, 1849: 822 (*Aleochara*)]. Distribution: South Africa.
   Etymology. Small change of the older specific epithet for this species, *clavicornis*, meaning clavate antenna.
10. *densula* Wasmann, 1900: 240, **comb. n.** Distribution: South Africa.
11. *eoa* **nom. n.** [for *japonica* (Cameron, 1933: 217) (*Tinotus*), nec. Sharp, 1874: 8 (*Aleochara*)]. Distribution: Japan, Taiwan (new record).
    Etymology. See, the redescription section above.
12. *flavescens* (Sharp, 1883: 171), **comb. n.** (*Tinotus*). Distribution: Mexico, Guatemala.
13. *frontalis* (Pace, 1997: 45), **comb. n.** (*Tinotus*), nec. Stephens, 1832: 111 (*Aleochara*). Distribution: Colombia.
14. *globicollis* (Bernhauer, 1934b: 19), **comb. n.** (*Tinotus*). Distribution: China (Sichuan), Philippines.
15. *imbricata* (Casey, 1894: 317), **comb. n.** (*Tinotus*). Distribution: USA.
    = *texana* (Casey, 1911: 67) (*Tinotus*), nec. Casey, 1906: 137 (*Aleochara*).
    = *coelebs* (Casey, 1911: 68) (*Tinotus*).
    = *fusina* (Casey, 1911: 68) (*Tinotus*).
    = *pectinella* (Casey, 1911: 69) (*Tinotus*).
    = *ampla* (Notman, 1920: 723) (*Tinotus*).
    = *brunnipes* (Notman, 1920: 724) (*Tinotus*), nec. Stephens, 1832: 133 (*Aleochara*).
16. *janklimaszewskii* **nom. n.** [for *klimaszewskii* (Pace, 2008a: 365) (*Tinotus*), nec. Maus, 1999: 358 (*Aleochara*)]. Distribution: Ecuador.
    Etymology. Dedicated to Dr. Jan Klimaszewski, as the older specific epithet for this species.

17. *kashmirica* (Cameron, 1939: 559), **comb. n.** (*Tinotus*). Distribution: India (Kashmir, Himachal Pradesh).
18. *malaya* **nom. n.** [for *antennalis* (Cameron, 1950a: 128) (*Tinotus*), nec. Fenyes 1914: 54 (*Aleochara*)]. Distribution: Malaysia (Malay Peninsula).
    Etymology. An old name of Peninsular Malaysia where the type locality is located.
19. *morion* Gravenhorst, 1802: 97, **comb. n.** Distribution: Holarctic region (native to Palaearctic region).
    = *exigua* Mannerheim, 1930: 68 (*Aleochara*).
20. *namibiensis* (Pace, 1999b: 207), **comb. n.** (*Tinotus*). Distribution: Namibia.
21. *natalensis* (Pace, 1986: 107), **comb. n.** (*Tinotus*). Distribution: Kenya, Zimbabwe, South Africa.
22. *ndogo* **nom. n.** [for *minuta* (Bernhauer, 1915b: 158) (*Tinotus*), nec. Casey, 1906: 161 (*Baryodma*)]. Distribution: Zaire, Tanzania, Kenya, South Africa.
    = *suffusa* (Cameron, 1950b: 76) (*Tinotus*), nec. Casey, 1906: 162 (*Baryodma*).
23. *neocaledonica* (Pace, 1991: 165), **comb. n.** (*Tinotus*). Distribution: New Caledonia.
    Etymology. Swahili adjective ndogo meaning small in referring to the minute body of this species.
24. *nepalensis* (Pace, 1984: 338), **comb. n.** (*Tinotus*). Distribution: Nepal.
25. *olivosensis* **nom. n.** [for *densissima* (Bernhauer, 1934a: 512) (*Tinotus*), nec. Bernhauer, 1906: 345 (*Aleochara*)]. Distribution: Brazil (Rio de Janeiro), Argentina.
    Etymology. Derived from the type locality of *densissima*, Olivos, Buenos Aires of Argentina.
26. *papuana* (Pace, 2000: 161), **comb. n.** (*Tinotus*). Distribution: Papua New Guinea.
27. *planula* (Notman, 1920: 724), **sp. rev., comb. n.** (*Tinotus*). Distribution: USA.
    = *parvicornis* (Casey, 1911: 69), **syn. n.** (*Tinotus*), nec. Fauvel, 1900: 248 (*Aleochara*).
    = *densiventris* (Casey, 1911: 70) (*Tinotus*), nec. Bernhauer, 1906: 346 (*Aleochara*), nec. Casey, 1906: 158 (*Baryodma*).
28. *refusa* (Hanley, 2002: 454), **comb. n.** (*Tinotus*). Distribution: Mexico.
29. *riodejaneirensis* **nom. n.** [for *eidmanni* (Scheerpeltz, 1936: 528) (*Tinotus*), nec. Scheerpeltz, 1929: 137 (*Aleochara*)]. Distribution: Brazil (Rio de Janeiro).
    Etymology. Derived from Rio de Janeiro, the capital city of Brazil and the type locality of this species.
30. *robertopacei* **nom. n.** [for *major* (Pace, 1986: 107) (*Tinotus*), nec. Fairmaire, 1858: 737 (*Aleochara*), nec. Eichelbaum, 1912: 176 (*Aleochara*)]. Distribution: South Africa, Tanzania, Kenya.
    Etymology. Dedicated to Mr. Roberto Pace who first described this species.
31. *rougemonti* (Pace, 1993: 116), **comb. n.** (*Tinotus*). Distribution: China (Sichuan, Xinjiang).
32. *rougemontiana* (Pace, 1999a: 152), **comb. n.** (*Tinotus*). Distribution: China (Shaanxi, Sichuan, Yunnan).
33. *surrubicunda* **nom. n.** [for *rufipennis* (Cameron, 1939: 558) (*Tinotus*), nec. Stephens, 1832: 161 (*Aleochara*), nec. Erichson, 1839: 162 (*Aleochara*)]. Distribution: India (Uttar Pradesh, Uttarranchal), Nepal.

Etymology. Combination of the Latin prefix *sur* meaning above or upper and the Latin adjective *rubicunda* meaning red in referring to the reddish body of this species.

34. *takashii* **sp. n.** Distribution: Japan (central Honshû).
35. *taprobanensis* (Likovský, 1984: 4), **comb. n.** (*Tinotus*). [replacement name for *Aleochara minutissima* Kraatz, 1859: 19]. Distribution: Sri Lanka, Malaysia (Penang), Himalaya.
    = *minutissima* Kraatz, 1859: 19 (*Aleochara*).
36. *thailandensis* (Pace, 1992: 260), **comb. n.** (*Tinotus*). Distribution: Thailand.
37. *trisecta* (Casey, 1906: 321), **comb. n.** (*Tinotus*). Distribution: Canada, USA.
    = *fimbriata* (Casey, 1911: 15) (*Acrimea*).
    = *pallida* (Casey, 1911: 65) (*Tinotus*).
    = *brunnea* (Casey, 1911: 65) (*Tinotus*), nec. Motschulsky, 1860: 582 (*Caladera*).
    = *binaria* (Casey, 1911: 66) (*Tinotus*).
    = *lateralis* (Notman, 1821: 154) (*Tinotus*).
38. *uttariana* **nom. n.** [for *castanea* (Cameron, 1939: 559) (*Tinotus*), nec. Motschulsky, 1858: 239 (*Aleochara*)]. Distribution: India (Uttar Pradesh), Nepal, China (Sichuan).
    Etymology. Derived from the type locality of *castanea*, Uttar Pradesh, India.
39. *vietnamensis* (Pace, 2001: 143), **comb. n.** (*Tinotus*). Distribution: Vietnam.
40. *zairensis* (Pace, 1986: 107), **comb. n.** (*Tinotus*). Distribution: Zaire.

Genus *Aleochara* Gravenhorst, 1802

Subgenus *Aleochara* Gravenhorst, 1802: 67.

1. *derougemonti* **nom. n.** [for *rougemonti* Pace, 2011: 68 (*Aleochara*), nec. Pace, 1993: 116 (*Tinotus*)]. Distribution: Venezuela.
   Etymology. Dedicated to Mr. Guillaume de. Rougemont as the older specific epithet for this species.
2. *vietnamiana* **nom. n.** [for *vietnamensis* Pace, 2013b: 376 (*Aleochara*), nec. Pace, 2001: 143 (*Tinotus*)]. Distribution: Vietnam.
   Etymology. Small change of the he older specific epithet for this species, meaning "of Vietnam".

Subgenus *Xenochara* Mulsant & Rey, 1874: 60.

1. *natalicola* **nom. n.** [for *natalensis* Klimaszewski, 1993: 67 (*Aleochara*), nec. Pace, 1986: 107 (*Tinotus*)]. Distribution: South Africa.
   Etymology. Combination of Natal, the type locality of this species, and the Latin suffix *cola* meaming inhabitor.

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Drusus sharrensis sp. n. (Trichoptera, Limnephilidae), a new species from Sharr National Park in Kosovo, with molecular and ecological notes

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Abstract

In this paper we describe *Drusus sharrensis* **sp. n.**, from the Sharr Mountains in Kosovo. Males of the new species are morphologically most similar to *Drusus krusniki* Malicky, 1981, *D. kerek* Oláh, 2011 and *D. juliae* Oláh, 2011 but differ mainly in exhibiting (1) a differently shaped spinose area on tergite VIII; (2) intermediate appendages anteriorly curved in lateral view with broad tips in dorsal view; (3) inferior appendages with a distinct dorsal protrusion in the proximal half. Females of the new species are morphologically most similar to *D. krusniki*, *D. kerek*, *D. juliae*, and *D. plicatus* Radovanovic, 1942 but mainly differ in (1) segment X that is longer than the supragenital plate with distinctly pointed tips; (2) supragenital plate quadrangular with a distinct round dorsal protrusion; (3) a vulvar scale with a small median lobe. Results of phylogenetic species delimitation support monophyly of *Drusus sharrensis* sp. n. and recover it as sister to a clade comprising (*D. pelasgus* Oláh, 2010 + *D. juliae* + *D. arbanios* Oláh, 2010 + *D. plicatus* + (*D. dacothracus* Oláh, 2010 + *D. illyricus* Oláh, 2010)). The new species is a micro-endemic of the Sharr Mountains, a main biodiversity hotspot in the Balkan Peninsula. Main threats to the aquatic ecosystems of this part of the Balkan Peninsula are discussed.

Keywords

Caddisfly, Drusinae, Europe, Sharr Mountains, taxonomy, freshwater biodiversity

Introduction

The genus *Drusus* Stephens contains the greatest number of species within the Drusinae. Members of the genus mostly inhabit the European continent with a few additional species known from Asia Minor. Within Europe, the Balkan Peninsula is recognized as one of the most important diversity hotspots of this genus (e.g., Kumanski 1973, Kučinić et al. 2011, Malicky 2004, Oláh 2010, Oláh and Kovács 2013, Previšić et al. 2014a, 2014b, Sipahiler 1999, 2002, Vitecek et al. 2015b, 2015c, Waringer et al. 2015).

The Sharr Mountains represent the border area of three countries, i.e., the Republic of Kosovo, Macedonia, and a small portion extending into north-eastern Albania. This region is characterized by substantial forest ecosystems, diverse geomorphological and hydrological features, and high numbers of endemic and relict species. The name of this mountain range appears in antiquity as "Scardus" "Scordus" or "Scodrus" (Smith 1870) and is reflected in several scientific names, mostly of plants (e.g. *scardicus*, *scardicum*, *scardica*, *scardicolum*, *schariensis*) (Anonymous 2010). The Sharr National Park covers five municipalities (Dragash, Prizren, Suharekë, Shtërpce and Kaçanik) in Kosovo with 36% of its total area covered by forest.

Due to the lack of systematic inventories, biodiversity data for the Sharr Mountains in all three countries are incomplete and are mostly limited to several plant groups, or large mammals. Data for reptiles, amphibians, small mammals, fish, and particularly insects are scarce and outdated (Hajredini et al. 2013). Among insects, the butterfly fauna of the Sharr Mountains is best known, with 147 species registered (Jakšić 1998).

In an ongoing project on the caddisfly fauna of Kosovo (e.g., Ibrahimi et al. 2012a, 2012b, 2013), we sampled caddisfly communities in the Sharr National Park. In this contribution we describe a new *Drusus* species from the Sharr Mountains.

Materials and methods

We collected adult caddisflies with entomological nets and handpicking from the riparian vegetation near the streams, and nocturnal light trapping in the vicinity of the streams. Nocturnal light trapping followed Malicky's (2004) protocols. All collected specimens were stored directly in 96% ethanol. The collected material is deposited in the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Prishtina "Hasan Prishtina", Prishtinë, Republic of Kosovo (DBFMNUP), Croatian Natural History Museum, Zagreb, Croatia (coll. Kučinić-Trichoptera) (CNHM), and Biologiezentrum des Oberösterreichischen Landesmuseums, Linz, Austria (BDOL).

Morphological characteristics of male terminalia were examined in cleared specimens. Specimens were cleared using either the Qiagen Blood and Tissue Kit for

DNA-extraction according to the manufacturer's recommendation and subsequent KOH-treatment (Böhm et al. 2011), or KOH-treatment. Nomenclature of male terminalia follows Nielsen (1957, for *Limnephilus flavicornis* Fabricius) using the simplifying terms "superior appendages" for the lateral processes of segment X (cerci *sensu* Snodgrass 1935), and "intermediate appendages" for the sclerite and the anterior process of segment X (paraproct *sensu* Snodgrass 1935). Illustrations were prepared according to Thomson and Holzenthal (2010) in which pencil drawings made with a camera lucida were digitized, edited, and inked in Adobe Illustrator (v. 16.0.4, Adobe Systems Inc.).

Whole genomic DNA was extracted from the abdomen or the thorax of adult or larval specimens using the DNEasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol. Standard PCR procedures and primers were used to amplify three mitochondrial gene regions (mtCOI5-P, mtCOI3-P, 16SrDNA) and three nuclear gene regions (CADH, WG, 28SnrDNA) (Table 1). PCR reactions were set up in 10µl reactions. Unpurified PCR products were sequenced on an ABI 3177XL capillary sequencer at the Biodiversität und Klima-Forschungszentrum (BiK-F, Frankfurt am Main, Germany) using the PCR primers and two additional internal primers for 28SrDNA (D2UP-4 and D2DN-B, Zhou et al. 2007).

Sequences were edited in Geneious R6 (http://www.geneious.com, Kearse et al. 2012) and aligned using MAFFT v7 (Katoh and Standley 2013) as implemented in Geneious R6. Nucleotide substitution models for each partition were selected according to the Bayesian Information Criterion in the model test module of Mega v5.1 (Tamura et al. 2007) (Table 2). For phylogenetic analysis, the 16SrDNA and 28SnrDNA fragments were not partitioned.

To examine species delineation and association of morphologically similar species of Western Balkan Drusinae, we inferred a phylogeny using all available sequences of the new species (Table 3). As outgroup taxa we used *Drusus discolor* (Rambur, 1842) (Limnephilidae: Drusinae), *Anisogamus waringeri* Graf & Vitecek, 2015 and *Melampophylax austriacus* Malicky, 1990 (Limnephilidae: Stenophylacini) (Table 3).

To assess potential conflicts or incongruence among gene fragments, B/MCMCMC single gene analyses were conducted in MrBayes 3.2 (Ronquist et al. 2012), implementing the respective substitution models. Four parallel runs with twelve chains each were performed (10×10^6 generations, sampling every 5000th generation). Stationary distribution of runs in the same optimal tree space was assumed if the average standard deviation of split frequencies reached values below 0.01. Additionally, MrBayes parameter files were examined in Tracer v1.8 (Rambaut et al. 2014) to assess if runs had reached a stationary phase and converged on model parameters. For each partition, a majority clade credibility tree was estimated based on trees sampled by MrBayes after discarding the first 600 trees of each run as burn-in. Datasets were concatenated as no conflicts among data sets were found, indicating homogeneity of phylogenetic signal from each partition.

Bayesian inference of the concatenated dataset (mtCOI5-P + mtCOI3-P + 16SrDNA + CADH + WG + 28SnrDNA) was performed in MrBayes 3.2, implementing the

Table 1. PCR primers and PCR cycling conditions.

Fragment	Primers & Primer Concentration		PCR Cycling conditions	Taq Kit	Additional Reagents
mtCOI5-P	HCO2198 & LCO1490 (Folmer et al. 1994)	0.25 µM	5'95°C, 5 x (30"95°C, 1'44°C, 1'72°C), 15x (30"95°C, 30"48°C, 1'72°C), 20 x (30"95°C, 30"50°C, 1' + (10'' * n) 72°C)	peqGOLDHotTaq	-
mtCOI3-P	Jerry & S20 (Pauls et al. 2006)	0.25 µM	5'95°C, 35 x (45"95°C, 30"45°C, 45"72°C), 5'72°C	peqGOLDHotTaq	-
16SrDNA	Lepto-F &Lepto-R (Malm and Johanson 2008)	0.75 µM	3'95°C, 35 x (30"95°C, 30"52°C, 40"72°C), 5'72°C	peqGOLDHotTaq	4 mg BSA
WG	WGbDrrev (5'-ACCCTCTCCCGCARCACTTGAG) &WGbDrfwd (5'-CTTGCTGGATGCGTCTGCC)[1]	0.5 µM	5'95°C, 35 x (45"95°C, 45"60°C, 90"72°C), 7'72°C	QiagenHotstarTaq plus Master mix	-
CADH	1028r-ino &743nF-ino (Johanson and Malm 2010)	0.25 µM	5'95°C, 35 x (45"95°C, 30"50°C, 45"72°C), 5'72°C	peqGOLDHotTaq	-
28SnrDNA	D1-3up1 (5'-CGAGTAGCGGCGAGCGAACGGA) & D3-TRIC-DN (5'-ATTCCCCTGACTTCGACCTGA)[2]	0.25µM	3'95°C, 35 x (45"95°C, 45"60°C, 60"72°C), 5'72°C	peqGOLDHotTaq	2 mg BSA, 5% DMSO

1: unpublished primer sequence by M. Bálint

2: unpublished primer sequence by K. Kjer

Table 2. Substitution models used in phylogenetic analysis.

Fragment	unpartitioned	codon position 1	codon position 2	codon position 3
mtCOI5-P	GTR+G+I	TN93+G	TN93+G	HKY
mtCOI3-P	GTR+G+I	TN93+G+I	K2+G	HKY
16SrDNA	T92+G	-	-	-
WG	T92+G	T92	JC+G	JC
CADH	T92+G+I	HKY+G	TN93	T92
28SnrDNA	T92+G+I	-	-	-

respective substitution models. Four parallel runs with twelve chains each were carried out (10×10^6 generations, sampling every 5000th generation). Analytical parameters were examined as stated above. A majority clade credibility tree was estimated based on trees sampled by MrBayes after discarding the first 600 trees of each run as burn-in.

Results

Species description

***Drusus sharrensis* Ibrahimi, Vitecek & Previšić, sp. n.**
http://zoobank.org/0DBB5862-13D4-40FB-98B5-D78288318B1C

Material examined. Holotype. 1 male: Republic of Kosovo, Shtërpce Municipality, Sharr Mountains, tributary of the Lepenc River, 2 km above the main road Prizren – Shtërpce, 1558 m, 42.17228°N, 20.98823°E, 21.v.2014, leg. Halil Ibrahimi (DBFMNUP). **Paratypes:** same collection and locality data as holotype, 6 males, 3 females (DBFMNUP), 2 males, 1 female (CNHM), 2 males, 1 female (BDOL); same except 8.v.2014, 2 males, 1 female (CNHM); same except 15.vi.2013, leg. Halil Ibrahimi and Joachim Milbradt, 3 males (DBFMNUP); Shtërpce Municipality, Sharr Mountains, small spring, a branch of the Lepenc River 50 meters above the main road Prizren – Shtërpce, 1410 m, 42.17506°N, 20.97593°E, 08.vi.2010, leg. Halil Ibrahimi, 2 males (DBFMNUP); Shtërpce Municipality, Sharr Mountains, Lepenc River on the main road Prizren – Shtërpce, 1465 m, 42.1813°N, 20.9781°E, 18.v.2010, leg. Halil Ibrahimi, 2 males (DBFMNUP); Prizren Municipality, Sharr Mountains, Lumbardhi i Prizrenit River, Prevallë village 1664 m, 42.161°N, 20.99533°E, 08.vi.2009, leg. Halil Ibrahimi, 1 male (DBFMNUP); Prizren Municipality, Sharr Mountains, first small lake above Prevallë village, 2142 m, 42.152402°N, 20.995024°E, 18.ix.2010, leg. Halil Ibrahimi, 3 males, 1 female (DBFMNUP).

Distribution. Republic of Kosovo, Sharr Mountains.

Diagnosis. Males of the new species are most similar to *Drusus krusniki*, *D. kerek* and *D. juliae* but differ in exhibiting (1) a dorsally distinctly indented tergite VIII; (2) a narrow, laterally suboval, caudally protruding spinose area of tergite VIII that is me-

Table 3. Collection data of specimens and length of partial gene sequences used in phylogenetic inference. Abbreviations: Speciment ID, unique study-specific specimen identifier; BOLD ID, BOLD process ID – a unique Barcode of Life Database-specific specimen identifier. Numbers in square parentheses after fragment length indicate number of missing positions. Collectors: AC - Andela Ćukusić, AP – Ana Previšić, BS - Boštjan Surina, DD – Dejan Dmitrović, GS – Goran Šukalo, HI – Halil Ibrahimi, IM – Iva Mihoci, MK – Mladen Kučinić, VK – Vladimir Krpać,WG – Wolfram Graf.

Specimen ID	BOLD ID	28SnrDNA	COI-5P	CADH	COI-3P	16Sr-DNA	Wnt1	Collectors	Coll. date	Latitude (N)	Longitude (E)	Elevation	Taxon
fAns0101L	SPDRU147-14	1038[0n]	658[0n]	848[0n]	541[0n]	360[0n]	0	WG	09.vi.2013	42,4851	2,4134	1888	*Anisogamus waringeri*
fDar0106M	SPDRU163-14	923[84n]	658[0n]	848[0n]	541[0n]	360[0n]	346[0n]	MK, AC	02.vi.2013	40°31.614'	20°25.021'	1920	*Drusus arbanios*
fDar0107M	SPDRU164-14	1040[0n]	658[0n]	848[0n]	541[0n]	360[0n]	346[0n]	MK, AC	02.vi.2013	40°31.614'	20°25.021'	1920	*Drusus arbanios*
fDda0204M	SPDRU227-14	1038[0n]	658[0n]	0	541[0n]	360[0n]	346[0n]	MK, HI, IM, AC	07.vi.2013	41°38.792'	20°11.390'	980	*Drusus dacothracus*
fDda0208M	SPDRU230-14	1036[2n]	658[0n]	848[0n]	541[0n]	360[0n]	346[0n]	MK, HI, IM, AC	07.vi.2013	41°38.792'	20°11.390'	980	*Drusus dacothracus*
fDdd0801M	SPDRU231-14	1038[0n]	658[0n]	848[0n]	541[0n]	362[0n]	346[0n]	AP	10.vii.2013	42,6859	19,7364	960	*Drusus discolor*
fDdd0802F	SPDRU232-14	1038[0n]	658[0n]	848[0n]	541[0n]	362[0n]	346[0n]	AP	10.vii.2013	42,6859	19,7364	960	*Drusus discolor*
fDds0110M	SPDRU243-14	1038[0n]	658[0n]	848[0n]	474[0n]	360[0n]	346[0n]	MK, VK, AC	29.v.2013				*Drusus discophorus*
fDds0111M	SPDRU244-14	1038[0n]	658[0n]	848[0n]	0	360[0n]	346[0n]	MK, VK, AC	29.v.2013				*Drusus discophorus*
fDil0109M	SPDRU268-14	1038[0n]	658[0n]	847[1n]	541[0n]	360[0n]	346[0n]	MK, AC	06.vi.2013	41,5358	20,2279	1830	*Drusus illyricus*
fDju0103M	SPDRU277-14	1038[0n]	658[0n]	848[0n]	541[0n]	362[0n]	346[0n]	MK, HI, IM, AC	04.vi.2013	41°51.848'	20°07.088'	1175	*Drusus juliae*
fDju0104M	SPDRU278-14	1038[0n]	658[0n]	848[0n]	541[0n]	362[0n]	346[0n]	MK, HI, IM, AC	04.vi.2013	41°51.848'	20°07.088'	1175	*Drusus juliae*
fDke0105M	SPDRU280-14	1038[0n]	658[0n]	847[1n]	541[0n]	362[0n]	346[0n]	MK, HI	13.ix.2013	42°31.326'	20°05.919'	2010	*Drusus kerek*
fDke0106M	SPDRU281-14	1036[1n]	658[0n]	848[0n]	541[0n]	362[0n]	346[0n]	MK, HI	13.ix.2013	42°31.326'	20°05.919'	2010	*Drusus kerek*
fDkr0101M	SPDRU294-14	1037[1n]	658[0n]	848[0n]	541[0n]	362[0n]	346[0n]	WG	30.v.2009	42,6438	19,8692		*Drusus krusniki*
fDkr0102M	SPDRU295-14	0	658[0n]	0	541[0n]	362[0n]	346[0n]	WG	30.v.2009	42,6438	19,8692		*Drusus krusniki*
fDpc0106M	SPDRU330-14	1038[0n]	658[0n]	847[1n]	0	360[0n]	346[0n]	MK, VK	31.v.2012	41,7902	20,6348	1279	*Drusus plicatus*
fDpe0105M	SPDRU334-14	1038[0n]	658[0n]	848[0n]	541[0n]	360[0n]	346[0n]	MK, HI, IM, AC	28.vii.2012	41°48.143'	20°33.285'	2300	*Drusus pelasgus*
fDpe0106F	SPDRU335-14	1038[0n]	658[0n]	845[3n]	541[0n]	327[0n]	346[0n]	MK, HI, IM, AC	28.vii.2012	41°48.143'	20°33.285'	2300	*Drusus pelasgus*
fMelaus0101M	SPDRU496-14	1038[0n]	658[0n]	842[6n]	541[0n]	361[0n]	0	WG	20.x.2013	46,8106	14,9931		*Melampophylax austriacus*

Specimen ID	BOLD ID	28SnrDNA	COI-5P	CADH	COI-3P	16Sr-DNA	Wnt1	Collectors	Coll. date	Latitude (N)	Longitude (E)	Elevation	Taxon
fMelaus0102F	SPDRU497-14	1038[0n]	658[0n]	843[5n]	0	361[0n]	0	WG	20.x.2013	46,8106	14,9931		*Melampophylax austriacus*
fDsp4403F	SPDRU545-15	1002[0n]	658[0n]	850[0n]	541[0n]	360[0n]	345[0n]	HI	21.v.2014	42,17228	20,98823	1558	*Drusus sharrensis* sp. n.
fDsp4402M	SPDRU544-15	1002[0n]	454[0n]	848[2n]	541[0n]	360[0n]	345[0n]	HI	21.v.2014	42,17228	20,98823	1558	*Drusus sharrensis* sp. n.
fDsp4401M	SPDRU543-15	1002[0n]	658[0n]	849[1n]	541[0n]	360[0n]	345[0n]	HI	21.v.2014	42,17228	20,98823	1558	*Drusus sharrensis* sp. n.
fDsp4501M	SPDRU546-15	1038[0n]	658[0n]	0	542[0n]	362[0n]	345[0n]	DD, GS	01.x.2014	44,5489	17,3927	393	*Drusus crenophylax*
fDsp4502L	SPDRU547-15	1037[0n]	658[0n]	850[0n]	542[0n]	362[0n]	345[0n]	DD, GS	19.x.2014	44,55	17,393	456	*Drusus crenophylax*

dially indented; (3) anteriorly curved intermediate appendages with broad tips; (4) inferior appendages with a distinct dorsal protrusion in the proximal half; (5) parameres with 3 distinct medial spines. *Drusus krusniki* males have (1) a flat, caudally depressed tergite VIII lacking a distinct indentation; (2) a laterally broad, subtriangular, almost straight spinose area of tergite VIII lacking an indentation; (3) intermediate appendages straight, with narrow tips, in lateral view protruding somewhat dorsocaudad; (4) inferior appendages with a slight dorsal protrusion in the proximal half; (5) parameres with a single, dorsal spine in the posterior half and several medial small spines. *Drusus kerek* males have (1) a flat tergite VIII lacking a distinct indentation; (2) a laterally narrow, suboval, almost straight spinose area of tergite VIII lacking an indentation; (3) straight intermediate appendages, with narrow tips; (4) inferior appendages subconical, curved dorsad; (5) parameres with 3 distinct medial spines. *Drusus juliae* males have (1) a rounded tergite VIII lacking a distinct indentation; (2) broad, subtriangular, spinose area of tergite VIII lacking an indentation, lateral parts of spinose area protrude caudad; (3) straight intermediate appendages, tips in dorsal view narrow, in lateral view somewhat pointed posteriad; (4) inferior appendages subconical, curved dorsad; (5) parameres with a single, dorsal spine in the posterior third and several medial small recumbent spines.

Females of the new species are most similar to *D. krusniki*, *D. kerek*, *D. juliae*, and *D. plicatus* but differ in exhibiting (1) segment X longer than the supragenital plate with distinctly pointed tips, distally tall in lateral view, caudal margin shallowly concave in dorsal view; (2) a quadrangular supragenital plate with a distinct round dorsal protrusion; (3) a vulvar scale with a small median lobe. *Drusus krusniki* females have a more-slender segment X that is shorter than the supragenital plate in dorsal view and has round tips and a deeply concave caudal margin. *Drusus kerek* females have a ventrally curved segment X shorter than the supragenital plate, a dorsally irregularly rounded supragenital plate, and a vulvar scale lacking the median lobe. *Drusus juliae* females have round tips of segment X and lack a distinct dorsal protrusion of the supragenital plate. *Drusus plicatus* females have a more-slender segment X that is shorter than the supragenital plate in dorsal view and has round tips and a deeply concave caudal margin, and a rounded supragenital plate in ventral view that lacks a distinct dorsal protrusion in lateral and caudal views.

Description. *General appearance.* Habitus dark; sclerites and tergites dark brown; cephalic and thoracic setal areas pale; cephalic, thoracic and abdominal setae blond; legs brown to fawn, proximally darker; haustellum and intersegmental integument pale, whitish. Wings dark brown with dark setae. Male maxillary palp 3-segmented. Forewing length 11–12.5 mm, spur formula 1–3–3 in males; forewing length 11.5–13 mm, spur formula 1–3–3 in females.

Male genitalia (Fig. 1A–E; Fig. 2A–C). Tergite VIII dark brown, in dorsal view distinctly incised anteriorly (*arrow 1*, Fig. 1); setation concentrated laterally; spinose area divided into two suboval laterocaudal lobes medially connected by band of spines, embracing distinct medial, indented, weakly sclerotized (translucent in cleared specimens) oval area with few spines (*arrow 2*, Fig. 1). Ninth abdominal segment in caudal

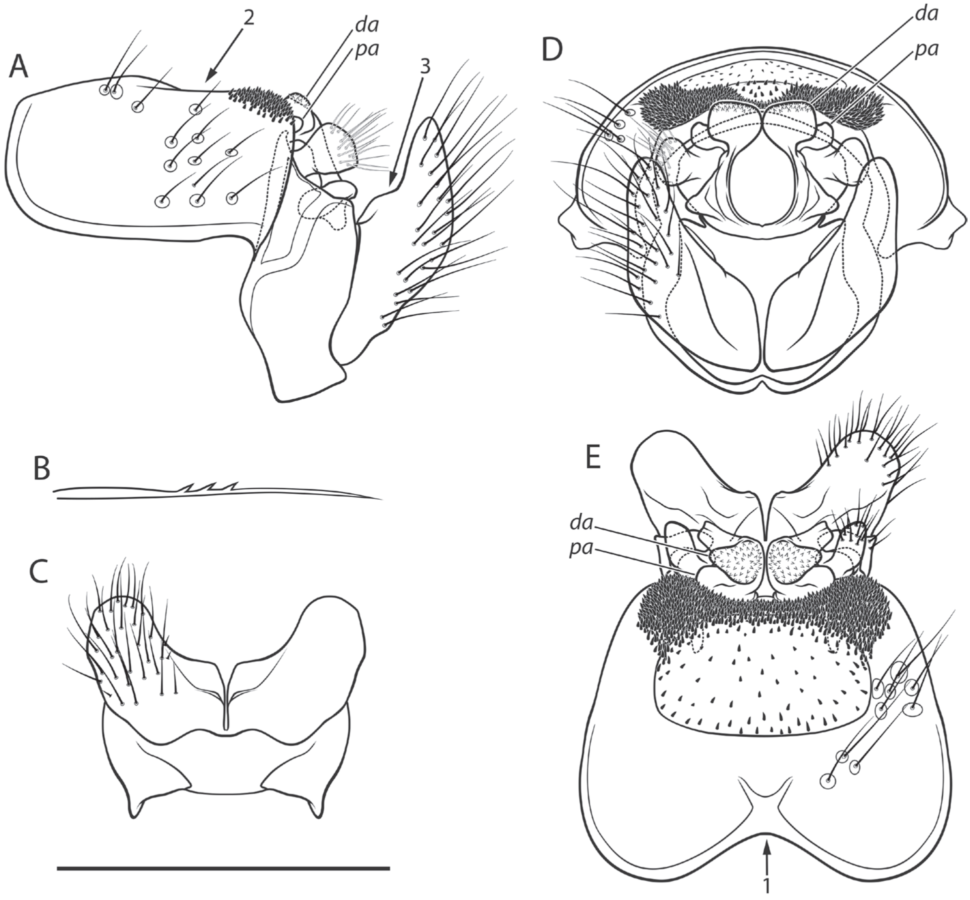

Figure 1. Male genitalia of *Drusus sharrensis* sp. n.: **A** left lateral view **B** paramere left lateral view **C** ventral view **D** caudal view **E** dorsal view. Small letters and numbers indicate structures referred to in the description. Scale bar 1 mm. Illustrations by S. Vitecek.

view widest ventrally; in lateral view with rounded apical protrusion at the base of the intermediate appendages, medially widest mid-height, apical margin ventrally concave with slight ventral protrusion embracing ventral base of inferior appendages (gonopods *sensu* Snodgrass 1935). Superior appendages in lateral view short, proximally constricted, suboval, ventroposterially somewhat pointed. Intermediate appendages in lateral view dorsally curved anterad, dorsal tip of each with proximal and distal aspect separated by distinct indentation: proximal aspect (*pa*, Fig. 1) rounded, flat, distal aspect (*da*, Fig. 1) curving anterad, rough; tips in dorsal view approximately parallel, proximal section rounded, extending laterad, the distal end subtriangular with rounded corners, medially somewhat dilated, rough; in caudal view approximately trapezoidal, proximal tips wider than distal tips and slightly pointed dorsad, distal tips rounded. Inferior appendages in lateral view curved dorsad, proximally with distinct dorsal protrusion (*arrow 3*, Fig. 1), ventral margin proximally slightly indented; in dorsal and ventral views

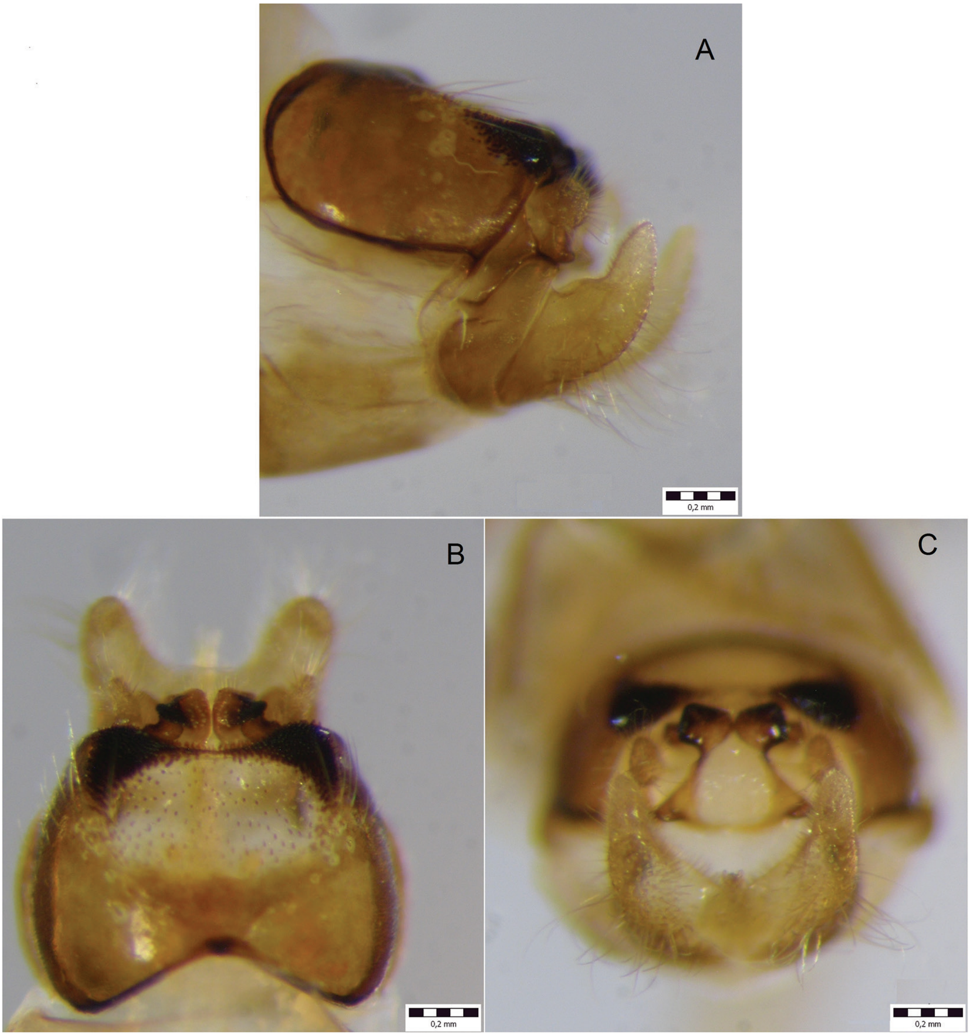

Figure 2. Male genitalia of *Drusus sharrensis* sp. n. **A** left lateral view **B** dorsal view **C** caudal view.

with subtriangular median lobe separated by longitudinal grooves; in dorsal, ventral, and caudal views proximally laterally protruding, distally approximately straight in dorsoventral plane; in caudal view inferior appendages suboval; in ventral view inferior appendages seemingly medially fused proximally. Parameres simple, with 3 distinct median spines.

Female genitalia (Fig. 3A–D; Fig. 4A–D). Segment IX setation abundant, concentrated in caudal half; lateral lobe (*LL*, Fig. 3) of segment IX membranous, in lateral view right-angled triangular, the assumed adjacent angle about twice as long as the assumed opposite angle with dorsal sclerotized setose lobe protruding caudad; in dorsal and ventral views slender, projecting caudad; in caudal view dorsal sclerotized setose part rounded, well separated from membranous part. Segment X longer than supra-

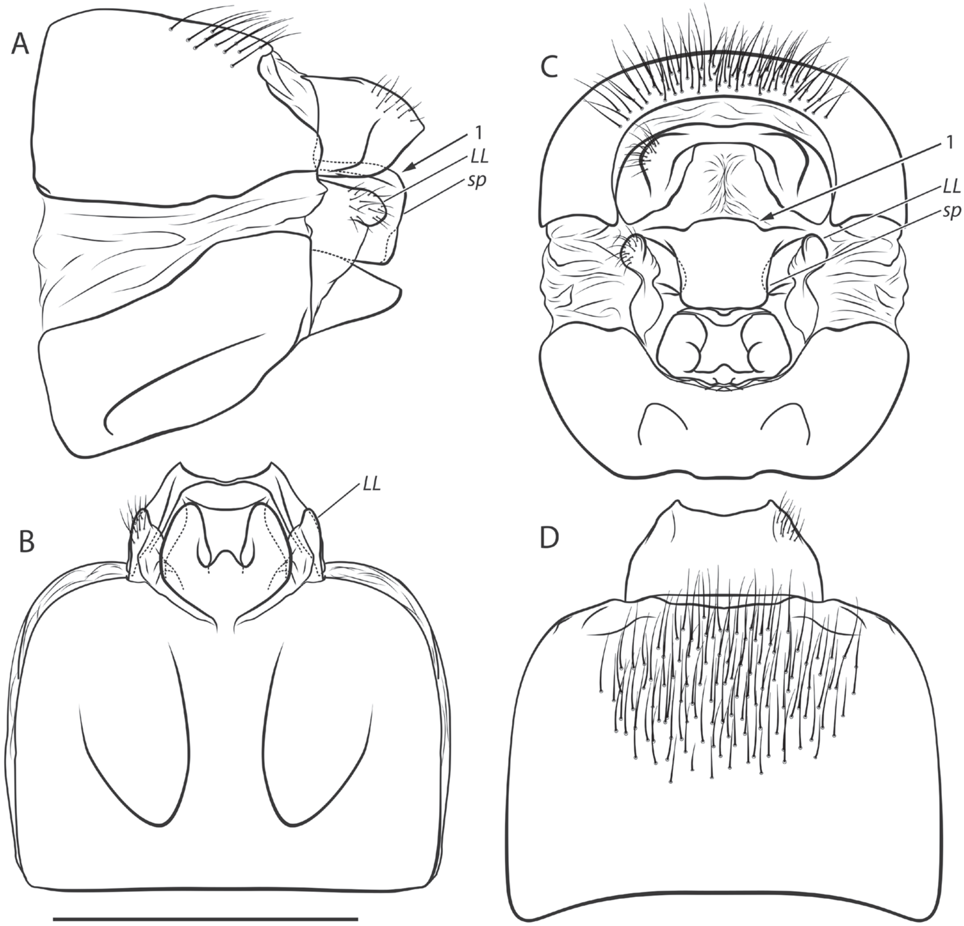

Figure 3. Female genitalia of *Drusus sharrensis* sp. n.: **A** left lateral view **B** ventral view **C** caudal view **D** dorsal view. Scale bar 1 mm. Small letters and numbers indicate structures referred to in the description. Illustrations by S. Vitecek.

genital plate, in lateral view distally higher than proximally with distinct posterior tip; in dorsal view medially widest, caudally tapering, with 2 small round setose lateral protrusions and distinct tips, apical margin irregularly concave; ventrally unsclerotized, open. Supragenital plate (*sp*, Fig. 3) in lateral view quadrangular with distinct, rounded dorsal protrusion (*arrow 1*, Fig. 3), apical margin ventrally slightly protruding; in ventral view quadrangular, medially concave; in caudal view quadrangular, wider dorsally than ventrally, with distinct rounded dorsal protrusion. Vulvar scale in lateral view subtriangular, slightly curved ventrad, longer than supragenital plate; in ventral view separated from sternite IX by proximal constriction, with 3 lobes: 2 lateral lobes, roundly oval, tapering caudad; median lobe short, wider than long.

Etymology. The species epithet *sharrensis* translates to 'from [the] Sharr [mountains]', and was formed by appending the Latin suffix '-ensis' to the actual name of the moun-

Figure 4. Female genitalia of *Drusus sharrensis* sp. n.. **A** left lateral view **B** ventral view **C** caudal view **D** dorsal view.

tain range where the new species is found. Note: In Albanian 'Sharr' also refers to the city of Dragash (Kosovo), the municipality of a large proportion of Sharr Mountains.

Ecological notes and distribution. During our field survey in the Sharr Mountains we found *Drusus sharrensis* at five locations within a 20 km perimeter, between 1410 and 2141 m above sea level. The new species was collected from one spring, two spring brooks and two mid-stream locations of the Lumbardhi i Prizrenit and Lepenc rivers. Substrate of streams close to the sampling sites was dominated by meso- to macrolithal. The highest number of specimens was collected at spring brooks surrounded by dense riparian vegetation. The species was mostly collected during the day with entomological nets – only one male specimen was collected by nocturnal light trapping although the weather was suitable and light trapping effort was considerable, indicating a diurnal activity pattern. The species was collected during May, June, July, and September.

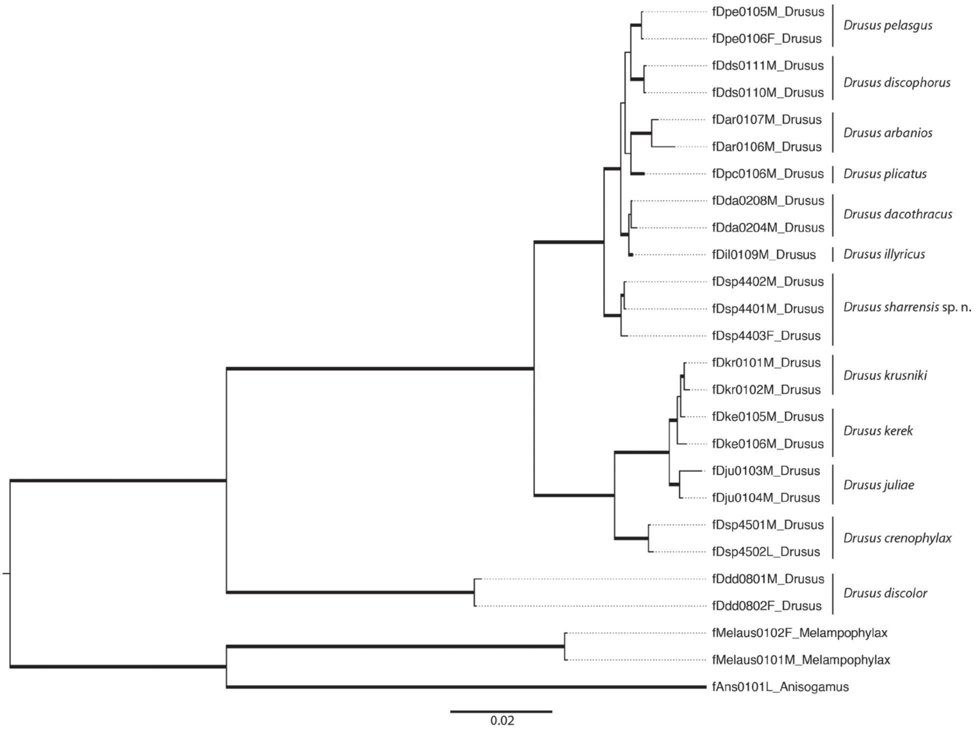

Figure 5. Results of phylogenetic inference. B/MCMC species tree analysis for nine *Drusus* species (26 terminal taxa) based on 3805bp-long sequence from 6 loci (mtCOI5-P, mtCOI3-P, CADH, 16SrDNA, WG, 28SnrDNA).

Results of phylogenetic species delimitation. In a B/MCMCMC phylogeny based on partial sequence data from six loci, monophyly of *Drusus sharrensis* was highly supported (Fig. 5). However, relationships between species were not resolved. The new species *Drusus sharrensis* was recovered, with high support, as sister to a clade comprising (*D. pelasgus* + *D. discophorus* Radovanovic, 1942 + *D. arbanios* + *D. plicatus* + (*D. dacothracus* + *D. illyricus*)). The clade (*Drusus sharrensis* + (*D. pelasgus* + *D. discophorus* + *D. arbanios* + *D. plicatus* + (*D. dacothracus* + *D. illyricus*))) is a derived sister to a clade composed of (((*D. krusniki* + *D. kerek*) + *D. juliae*) + *D. crenophylax* Graf & Vitecek, 2015) in which (*D. krusniki* + *D. kerek*) is recovered as a polytomy, and this relationship was highly supported.

Discussion

Systematic position

The combination of the gene fragments mtCOI3-P, 16SrDNA, and WG was previously demonstrated to successfully resolve phylogenetic relationships of Drusinae (Pauls et al. 2008), and was used to delineate species of Western Balkan Drusinae (Previšić et al. 2014a). In the present study, a set of six gene fragments was used to infer phylogenetic relationships of taxa in a Bayesian framework to discriminate species. These genes were successfully employed by Vitecek et al. (2015a) to assess other relationships among Drusinae. Bayesian phylogenetic inference based on the combination of six gene fragments (mtCOI5-P, mtCOI3-P, CADH, 16SrDNA, WG, 28SnrDNA) recovers the new *Drusus* species as monophyletic, and sister to a clade comprising (*D. pelasgus* + *D. discophorus* + *D. arbanios* + *D. plicatus* + (*D. dacothracus* + *D. illyricus*)).

Ecological notes

Data on the ecology of species closely related to *Drusus sharrensis* are incomplete. From what is known, the emergence pattern of the new species corresponds to that of a related species from Bjeshkët e Nemuna, *Drusus krusniki*. The sex ratio of the new species ranges from 1:2 to1:3 in favour of males at the different sampling locations, similar to sex ratios recorded in *Drusus krusniki* (Ibrahimi et al. 2014b).

Aquatic insect diversity of Sharr Mountains and main threats

There are currently about 30 stonefly (Dauti 1980) and about 50 caddisfly species (Ibrahimi et al. 2012a, 2012b) known from the Sharr Mountains. Many of these species are rare and/or endemics of the Balkan Peninsula. This number of known aquatic insect species is surely far below the real number inhabiting this range of mountains. The stonefly *Nemoura zwicki* Sivec, 1980 is an endemic species of this mountain range described from a streamlet only a few kilometers away (Sivec 1980) from type locality of the new *Drusus* species. The caddisfly *Limnephilus petri* Marinković-Gospodnetić, 1966 is also an endemic species of the Sharr Mountains (Marinković-Gospodnetić 1966).

The biodiversity of the Sharr Mountains is threatened by illegal logging, water extraction from springs, expansion of touristic activities and several other anthropogenic factors (Flores and Selimi 2013). Several limestone and rock quarries operate in the Sharr Mountains in the vicinity of aquatic ecosystems potentially causing severe siltation. Additionally, recent development of a winter tourism facility at Brezovicë, close to the type locality of *Drusus sharrensis*, may enhance local degradation of terrestrial and, particularly, aquatic ecosystems in the Sharr Mountains through water intake, habitat deterioration, and discharge of sewage effluents. The Brezovica Touristic Centre Development Project was designed by the Government of the Republic of Kosovo with support from the European Union to promote the touristic appeal and thus economic importance of the area. This project will impact a total area of roughly 3,700 ha (Flores and Selimi 2013).

The description of *Drusus sharrensis* is a contribution to the faunistic list of Kosovo caddisflies (Gashi et al. 2015, Malicky 1986, 1999, Marinković-Gospodnetić 1975, 1980, Oláh 2010, Oláh et al. 2013, 2014, Radovanović 1931, Ibrahimi and Gashi

2008, Ibrahimi et al. 2012a, 2012b, 2013, 2014a, 2014b, 2015). Further, the description of the new species highlights the importance of this rapidly changing area to local and regional biodiversity.

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RESEARCH ARTICLE

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Ectomyelois Heinrich, 1956 in China, with descriptions of two new species and a key (Lepidoptera, Pyralidae, Phycitinae)

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Abstract

Only three species belonging to the genus *Ectomyelois* Heinrich, 1956 are recorded from China, of which two species, *E. bipectinalis* **sp. n.** and *E. furvivena* **sp. n.** are described as new. We discuss the status of *Ectomyelois* that has been treated as a junior synonym by previous authors; we treat it as a valid genus, **revised status**, based on characters of the venation and female genitalia. Photographs of the adults and illustrations of the genitalia are given, along with a key to the three known Chinese species.

Keywords

Lepidoptera, Pyralidae, Phycitinae, *Ectomyelois*, *Ectomyelois ceratoniae*, new species, key, China

Introduction

Ectomyelois was established by Heinrich (1956) with *Myelois decolor* Zeller, 1881 as the type species. It is a small genus consisting of six species: *E. ceratoniae* (Zeller, 1839), *E. decolor* (Zeller, 1881), *E. furvidorsella* (Ragonot, 1888), *E. muriscis* (Dyar, 1914), *E. zeteki* Heinrich, 1956, and *E. austrella* Neunzig & Goodson, 1992. Most are Neotropical, but *E. ceratoniae* also occurs in the Oriental region. All but one species was described in detail by Heinrich (1956). *Ectomyelois* was once treated as a junior synonym of *Spectrobates* Meyrick by Roesler (1968) and subsequently of *Apomyelois* Heinrich by Roesler & Küppers (1981). A few authors followed these treatments (e.g. Neunzig 1979; Roesler 1983; Heppner and Inoue 1992; Leraut 2002), but most authors (Munroe 1983; Inoue 1982; Palm 1986; Sinev 1986; Goater 1986; Neunzig 1990; Balinsky 1994; Yamanaka 2013) treated *Ectomyelois* as a valid genus. Indeed, there is little to separate *Ectomyelois* from *Apomyelois* in the male genitalia, but the two genera can be distinguished by the venation and the different place of inception of the ductus seminalis from the corpus bursae in the female genitalia. Here, we agree that *Ectomyelois*, revised status, does indeed represent a valid genus.

Ectomyelois was only represented by the common carob moth *E. ceratoniae* in China before this study. Herein the three species are described, including two new species: *Ectomyelois bipectinalis* sp. n. and *E. furvivena* sp. n.

Material and methods

Genitalia dissections were carried out following the methods described by Li (2002). The photographs of the adults and venation were taken with a Leica M205A, and photographs of the genitalia and details of head were taken with a Leica DM750, with Leica Application Suite 4.2 software to capture images. All the specimens examined are deposited in NKUM unless otherwise noted.

Abbreviations

BMNH	Natural History Museum, London, UK.
HAASM	Insect Collection, Institution of Plant Protection, Henan Academy of Agricultural Sciences, Zhengzhou, China.
NKUM	Insect Collection, College of Life Sciences, Nankai University, Tianjin, China.
USNM	National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, U.S.A.
ZMHB	Museum für Naturkunde, Universität Humboldt, Invalidenstrasse 43, 104 Berlin, Germany.
TD	Type depository.
TL	Type locality.

Systematic part

Ectomyelois Heinrich, 1956

Ectomyelois Heinrich, 1956: 43. Type species: *Myelois decolor* Zeller, 1881, by original designation.

Diagnosis. Antenna of male usually shortly ciliate (bipectinate in *E. bipectinalis* sp. n.), basal shaft without notch or other modifications, of female simple. Labial palpus upturned, nearly reaching apex, third segment distinctly shorter than second. Forewing with R_2 closely approximate to the stalk of R_{3+4}+R_5, M_2 and M_3 stalked for less than half of their length. Hindwing with Sc + R_1 and Rs strongly anastomosed for most of their lengths, M_2 and M_3 stalked for not over half of their length. Male genitalia with uncus subtriangular to bell-shaped, apical projection of gnathos simple, slightly bent and furcated at apex, transtilla well developed, juxta U-shaped, with lateral lobes stout, vinculum U-shaped, more truncate and less tapering, phallus without cornutus. Female genitalia with signum consisting of an elongate patch of scobinations (absent in *E. furvidorsella*) and ductus seminalis from corpus bursae near junction of corpus bursae and ductus bursae.

Ectomyelois is similar to *Apomyelois*, but can be distinguished from the latter by the forewing with R_2 closely approximate to the stalk of R_{3+4}+R_5, and the female genitalia with the ductus seminalis arising from the corpus bursae near junction of the corpus bursae and ductus bursae. In *Apomyelois*, the forewing with R_2 shortly stalked with R_{3+4}+R_5, and the female genitalia with the ductus seminalis arising from anterior end of the corpus bursae.

Distribution. China (Gansu, Guangdong, Guangxi, Hainan, Taiwan, Yunnan), India, Sri Lanka, Sikkim, Israel, Mediterranean, Central Europe, Norway, United Kingdom, North Africa, Australia, Argentina, United States, Cuba, Haiti, Puerto Rico, Jamaica, Bahamas, Guatemala, Costa Rica, Colombia, Panama, Venezuela, Guiana, Surinam, Bolivia, Guyana, French Guiana, Brazil.

Key to species of *Ectomyelois* from China

1 Forewing with narrow grayish white, distinctly notched antemedial line (Fig. 1)..***E. ceratoniae***
– Forewing with antemedial line invisible..**2**
2 Male flagellum bipectinate (Fig. 3b); transtilla trefoiled (Fig. 7); corpus bursae three times as long as wide (Fig. 11)........................***E. bipectinalis* sp. n.**
– Male flagellum simple, not bipectinate (Fig. 5b); transtilla inverse-goblet shape (Fig. 9); corpus bursae twice as long as wide (Fig. 12)..***E. furvivena* sp. n.**

Ectomyelois ceratoniae (Zeller, 1839)

Figs 1, 2, 7, 10

Myelois ceratoniae Zeller, 1839: 176. TL: Laibach, Austria. TD: BMNH.

Phycis ceratoniella Fischer von Röeslerstamm, 1839: 147. TL: Laibach, Austria. TD: unknown.

Trachonitis pryerella Vaughan, 1870: 130. TL: London, England. TD: BMNH.

Myelois tuerckheimiella Sorhagen, 1881: 103. TL: Berlin, Germany. TD: ZMHB.

Euzophera zellerella Sorhagen, 1881: 104. TL: Berlin, Germany. TD: unknown.

Phycita dentilinella Hampson, 1896: 91. TL: Manipur, India. TD: BMNH.

Hypsipyla psarella Hampson, 1903: 30. TL: Sikhim, India. TD: BMNH.

Heterographis rivularis Warren & Rothschild, 1905: 31. TL: Nakheila, Sudan. TD: unknown.

Myelois oporedestella Dyar, 1911: 30.TL: Florida, USA. TD: USNM.

Myelois phoenicis Durrant, 1915: 303. TL: Constantine, Algeria. TD: BMNM.

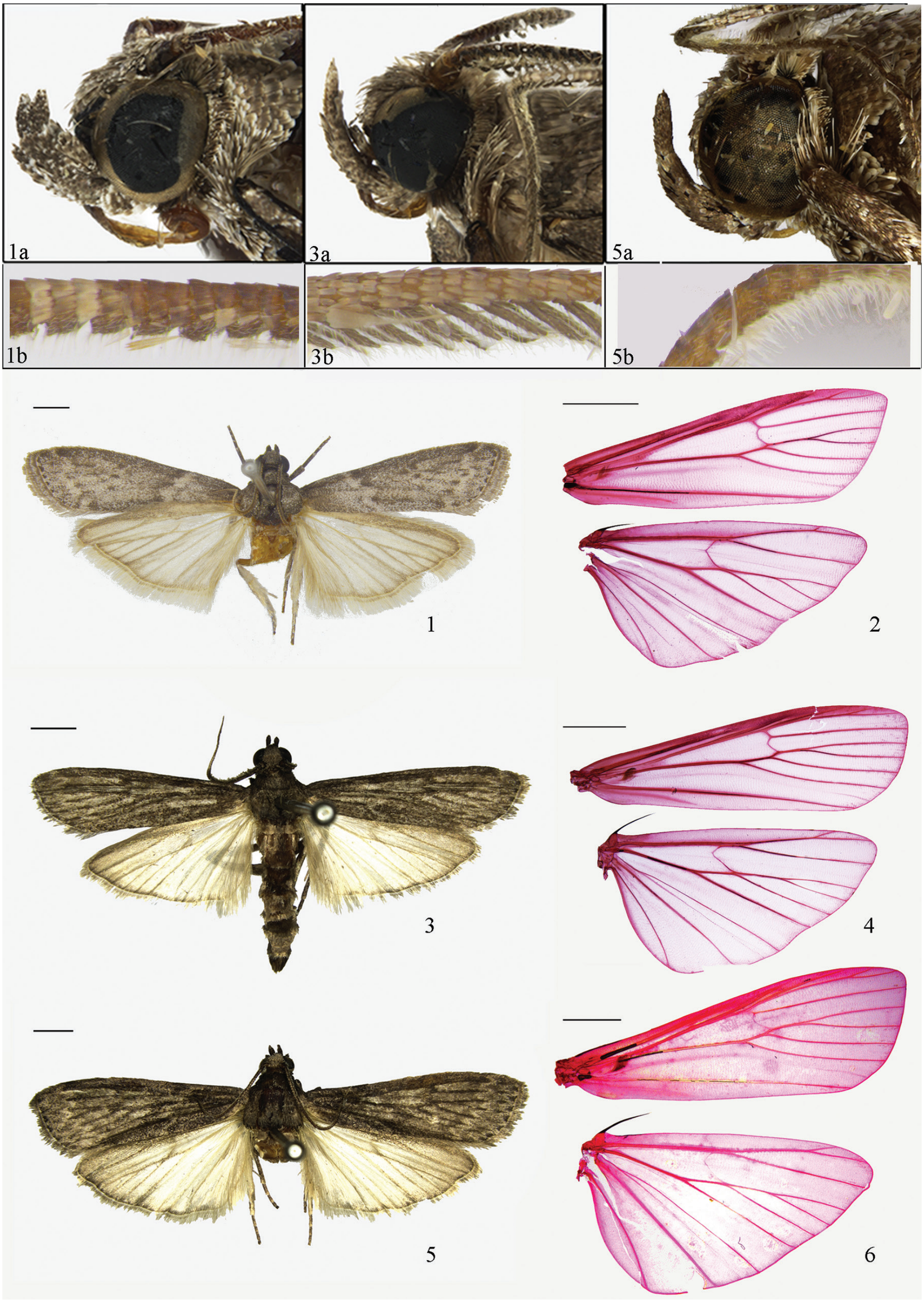
Laodamia durandi Lucas, 1950:142. TL: Tunisia. TD: unknown.

Apomyelois ceratoniae (Zeller): Roesler and Küppers 1981: 80.

Ectomyelois ceratoniae (Zeller): Heinrich 1956: 44.

Material examined. CHINA: **Guangdong**: 9 ♂♂, 1 ♀, Mt. He (22°45'N, 112°57'E), 09–10-X-2002, coll. Guilin Liu & Binglan Zhang, gen. slide nos. RYD04529m, RYD04530f; 1 ♂, same data as former except dated 6-XI-2002. **Guangxi**: 1 ♀, Milv (21°59'N, 107°52'E), Nanping, Shangsi, 770 m, 3-IV-2002, coll. Shulian Hao & Huaijun Xue, gen. slide no. RYD04658; 1 ♂, Yachang Yard, Leye County (24°47'N, 106°33'E), 665 m, 24-VII-2004, coll. Jiasheng Xu, gen. slide no. KDH05263; 1 ♀, Longrui (22°45'N, 110°55'E), 18-VIII-2011, coll. Muchun Cheng, gen. slide no. RYD20120185 (deposited in HAASM); 1 ♂, 1 ♀, Nonggang (23°14'N, 108°10'E), 20-VIII-2011, coll. Dandan Zhang, gen. slide no. RYD2014237 (deposited in HAASM). **Hainan**: 1 ♂, Mt. Diaoluo (18°39'N, 109°54'E), 29-V-2007, 80 m, coll. Zhiwei Zhang & Weichun Li, gen. slide no. LJY10595. **Yunnan**: 1 ♂, 1 ♀, Ganlanba (22°45'N, 101°08'E), Xishuangbanna, 19-IV-1995, coll. Guangyun Yan, gen. slide nos. RYD04615m, LJY10107f; 1 ♂, 4 ♀♀, Mt. Yunpan (23°44'N, 100°39'E), Puer, 1600 m, 6-VII-2013, coll. Linlin Yang, gen. slide nos. RYD2014219m, RYD2014221f (deposited in HAASM).

Diagnosis. Wingspan 15.0–22.00 mm (Fig. 1). *Ectomyelois ceratoniae* can be recognized by the following characters: the forewing with a narrow, distinctly notched antemedial line, the hindwing with free element of Sc+R$_1$ very short (Fig. 2); the uncus is bell-shaped, basally protruded on both sides, the apical projection of gnathos is stout, gently curved, about same length of the uncus, the trefoiled transtilla includes a pair of inflated bases and a more constricted central projection, the basally rectangular juxta with a pair of stout lateral lobes in the male genitalia (Fig. 7); and the ovate corpus bursae with signum is an elongate patch of scobinations, the ductus seminalis from junction of corpus and ductus bursae in the female genitalia (Fig. 10). It is quite similar to *E. bipectinalis* sp. n., but with differences as mentioned in the diagnosis of the latter.



Figures 1–6. Adults of *Ectomyelois* spp. **1** *E. ceratoniae*, male (1a, head; 1b, antenna) **2** *E. ceratoniae*, venation, slide No. RYD04529w **3** *E. bipectinalis* sp. n., paratype, male (**3a** head **3b** antenna) **4** *E. bipectinalis* sp. n., venation, slide No. RYD04718w **5** *E. furvivena* sp. n., paratype, male (**5a** head **5b** antenna) **6** *E. furvivena* sp. n., venation, slide No. RYD04529w. Scale bars: 2.0 mm.

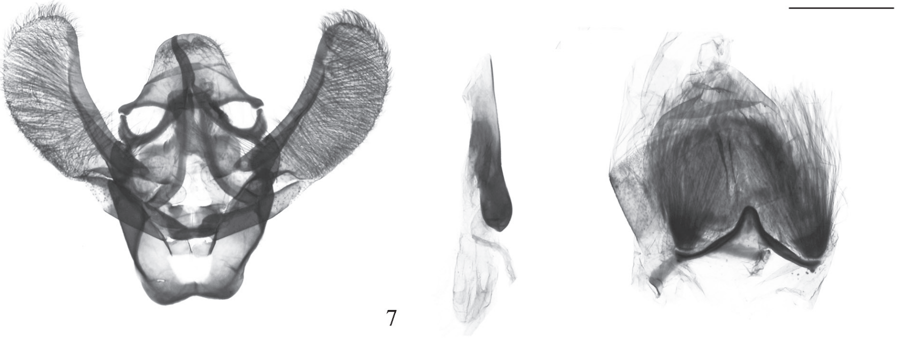
Distribution. China (Guangdong, Guangxi, Hainan, Taiwan, Yunnan), Japan, India, Sri Lanka, Sikkim, Israel, Mediterranean, Central Europe, Norway, United Kingdom, North Africa, Australia, Argentina, United States, Puerto Rico, Jamaica.

Ectomyelois bipectinalis sp. n.

http://zoobank.org/BF7291BA-0081-45D5-B1F4-6980247E5C59
Figs 3, 4, 8, 11

Type material. Holotype ♂, **CHINA**: Guanping (22°14'N, 100°53'E), Xishuangbanna, Yunnan, 1200 m, 19-VIII-2005, coll. Yingdang Ren. **Paratypes**: **Fujian**: 2 ♀♀, Mt. Tianzhu (24°35'N, 117°55'E), 220 m, 8,14-IX-2010, coll. Yinghui Sun & Jing Zhang. **Gansu**: 1 ♂, Fanba (32°44'N, 105°07'E), Wenxian, 718 m, 18-VII-2005, coll. Haili Yu, gen. slide no. RYD04745. **Guangxi**: 1 ♂, Yachang Yard, Leye County (24°47'N, 106°33'E), 665 m, 24-VII-2004, coll. Jiasheng Xu; 1 ♂, Mt. Yuanbao (25°14'N, 109°07'E), 500 m, 10-VIII-2006, coll. Weichun Li, gen. slide no. LYJ10112; 1 ♂, Pingxincun, Yizhou (24°30'N, 108°40'E), 150 m, 16-VIII-2011, coll. Shulian Hao & Yinghui Sun; 1 ♂, 1 ♀, Shaoping Yard, Pingxiang (22°03'N, 106°55'E), 190 m, 24,28-VII-2011, coll. Bingbing Hu; 1 ♀, Qingshan Yard, Pingxiang(22°03'N, 106°55'E), 300 m, 20-VII-2011, coll. Bingbing Hu. **Hainan**: 1 ♂, Mt. Diaoluo (18°39'N, 109°54'E), 70 m, 27-V-2007, coll. Zhiwei Zhang & Weichun Li, gen. slide no. LJY10104; 1 ♀, Mt. Duowen (19°48'N, 109°45'E), 120 m, 2-V-2009, coll. Qin Jin & Bingbing Hu, gen. slide no. LJY10105; 1 ♀, Wuzhishan (18°46'N, 109°30'E), 740 m, 14-IV-2009, coll. Qin Jin & Bingbing Hu, gen. slide no. LJY10097; 1 ♂, Shuimanxiang (18°53'N, 109°40'E), Wuzhishan, 620 m, 19-IV-2014, coll. Tengteng Liu, Wei Guan & Xuemei Hu; 1 ♂, Mt. Limu (19°10'N, 109°44'E), Qiongzhong, 640–700 m, 4-V-2014, coll. Tengteng Liu, Wei Guan & Xuemei Hu; 1 ♂, Wuzhishan (18°40'N, 109°29'E), 500 m, 12-IV-2013, coll. Yingdang Ren & Xiaoguang Liu, gen. slide no. RYD2013046 (deposited in HAASM). **Yunnan**: 4 ♂♂, Rare Botanical Garden, Ruili (24°00'N, 97°50'E), 1000 m, 5–8-VII-2005, leg. Yingdang Ren, gen. slide nos. RYD04718, RYD04718w; 1 ♂, Guanping (22°15'N, 100°53'E), Xishuangbanna, 1200 m, 17-VIII-2005, coll. Yingdang Ren, gen. slide no. RYD04717; 1 ♂, Botanical Garden, Menglun (21°52'N, 101°18'E), 570 m, 13-VIII-2005, coll. Yingdang Ren, gen. slide no. RYD04721; 4 ♂♂, Bubang (21°36'N, 101°35'E), Xisuangbanna, 650 m, 22–24-VIII-2005, coll. Yingdang Ren, gen. slide nos. LHX14081, LHX14081w; 1 ♂, Botanical Garden (21°55'N 101°16'E), Xishuangbanna, 560 m, 1-VIII-2010, coll. Yinghui Sun & Lixia Li; 2 ♂♂, Bakaxiaozhai (21°58'N, 101°12'E), Mengla, Xisuangbanna, 630 m, 7-VIII-2010, coll. Yinghui Sun & Lixia Li; 7 ♂♂, 1 ♀, Mengyuan (21°42'N, 101°23'E), Mengla, Xishuangbanna, 640 m, 10~13-VIII-2010, coll. Yinghui Sun & Lixia Li, gen. slide nos. LJY10351, LJY10352; 20 ♂♂, 16 ♀♀, Bubang (21°36'N, 101°35'E), Mengla, 650 m, 12~14-VII-2013, coll. Linlin Yang, gen. slide nos. RYD20120106m, RYD20120107m, RYD20120167f, RYD20120168m (deposited in HAASM).

Diagnosis. This new species is notable superficially for its bipectinate male flagellum. It is much more similar to *E. ceratoniae* in genitalic structures, but can be distin-



7

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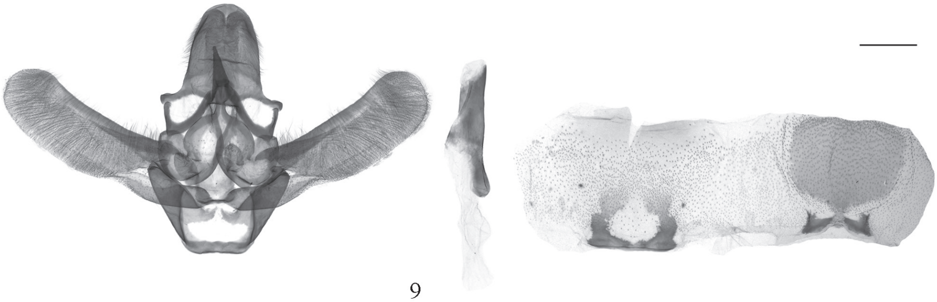


9

Figures 7–9. Male genitalia of *Ectomyelois* spp. **7** *E. ceratoniae*, slide No. LJY10595 **8** *E. bipectinalis* sp. n., paratype, slide No. RYD04718 **9** *E. furvivena* sp. n., paratype, slide No. LHX14084. Scale bars: 0.5 mm.

guished from the latter by the narrower uncus with width almost equaling length, the widest part is at basal 2/5, the apical projection of gnathos approximately 3/5 length of uncus in male, and the elongate corpus bursae three times as long as wide in female. In *E. ceratoniae*, the uncus is more wide than long, the widest part at base, the apical projection of gnathos nearly the same length as uncus in male, and the corpus bursae is twice as long as wide in female.

Description. Wingspan 19.5–28.0 mm (Figs 3, 4). Vertex brown, with individual scales tipped with grayish white. Antenna (Fig. 3a, b) brown; bipectinate, with pecten about twice length of width of shaft in male, shortly ciliate in female. Labial palpus brown, individual scales white-tipped. Maxillary palpus brown. Occiput, patagium, tegula and thorax brown. Forewing dark grayish fuscous, blackish brown along veins; antemedial line invisible; discal spots blackish brown, separated; postmedial line faint, grayish white, serrated; terminal line black, interrupted; cilia brown. Hindwing grayish white, pale brown along costa, termen and veins; cilia grayish white. Legs brown, mottled with grayish white, spurs grayish white. Abdomen yellowish brown.

Male genitalia (Fig. 8). Uncus bell-shaped, width almost equals length, rounded at apex, triangularly protruded laterally at basal 2/5, arched on basal margin. Apical projection of gnathos about 3/5 length of uncus, clubbed, slightly bent and furcated at apex. Transtilla trefoiled, including a pair of triangularly inflated bases, and a tongue-shaped central projection with rounded apex posteriorly. Valva three times as long as wide, evenly curved toward rounded apex, costal margin almost parallel with ventral margin except slightly convex at basal 2/3; costa strongly sclerotized, broad at base, slightly narrowed and extending to end of valva, without process apically; sacculus strongly sclerotized, stout, 2/5 length of valva. Juxta U-shaped, base an arched belt, with a pair of wide, stout, incurved lateral lobes, expanded and bearing sparse setae apically. Vinculum U-shaped, length almost equal to the widest posterior margin, slightly concave at middle of anterior margin. Phallus slightly shorter than valva, with membranous crimples internally; cornutus absent. Eighth tergite fan-shaped, 4/5 length than width, with a pair of spoon-like sclerites anteriorly; eighth sternite with a pair of triangular plates narrowly connected anteriorly. Culcita (sensu Amsel 1956) simple, one pair of fine scale tufts.

Female genitalia (Fig. 11). Anal papillae triangular, with a few setae, blunt apically. Eighth tergite slightly concave on posterior margin, trapezoidally convex on anterior margin; eighth sternite with membranous part inverse-funneled. Antrum twice as wide as length. Ductus bursae membranous, twice length of apophyses posteriores. Corpus bursae membranous, elongate, slightly shorter than ductus bursae, three times as long as wide; signum an elongate patch of microspines, placed at posterior 2/5. Ductus seminalis from junction of corpus bursae and ductus bursae.

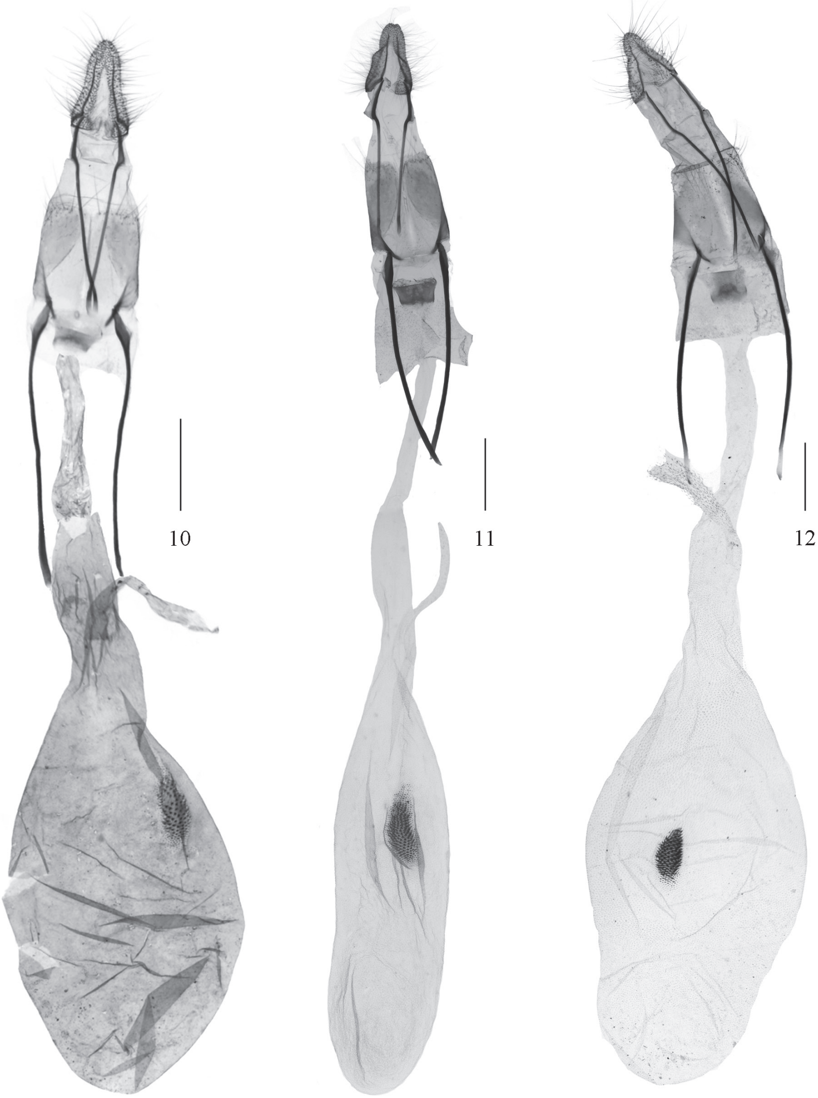
Distribution. China (Fujian, Gansu, Guangxi, Hainan, Yunnan).

Etymology. The specific name is derived from the Latin prefix *bi-*, meaning two, and the Latin *pectinalis*, meaning pectinate, referring to the bipectinate male flagellum.

Ectomyelois furvivena sp. n.

http://zoobank.org/72178A69-8258-4F83-A8C0-D1153B7E86CE
Figs. 5, 6, 9, 12

Type material. Holotype ♂, **CHINA**: Rare Botanical Garden, Ruili (24°00'N, 97°50'E), Yunnan, 1000 m, 8-VII-2005, leg. Yingdang Ren, gen. slide no. RYD04737.



Figures 10–12. Female genitalia of *Ectomyelois* spp. **10** *E. ceratoniae*, slide No. RYD04658 **11** *E. bipectinalis* sp. n., paratype, slide No. LJY10107 **12** *E. furvivena* sp. n., paratype, slide No. RYD04744. Scale bars: 0.5 mm.

Paratypes: **Gansu**: 1 ♂, Fanba (32°44'N, 105°07'E), Wenxian, 718 m, 18-VII-2005, coll. Haili Yu, gen. slide no. RYD04744. **Yunnan**: 2 ♂♂, same data as for holotype, gen. slide nos. LHX14084, LHX14084w, LHX14085; 1 ♀, Botanical Garden, Menglun (21°52'N, 101°18'E), 570 m, 13-VIII-2005, coll. Yingdang Ren, gen. slide no. RYD04720; 1 ♂, Baihualing, Mt. Gaoligong (25°31'N, 98°32'E), 1470 m, 30-VII-2013, coll. Linlin Yang, gen. slide no. RYD20120181 (deposited in HAASM).

Diagnosis. This new species is similar to *E. bipectinalis* sp. n., but can be recognized by the male antenna is not bipectinate, the uncus is rather abruptly narrowed beyond its broad base, tapered apical projection of the gnathos is about half length of the uncus and the inverse-goblet transtilla in the male genitaia. In *E. bipectinalis* sp. n., the antenna is bipectinate, the uncus protrudes triangularly at basal 2/5, the apical projection of gnathos is about 3/5 length of the uncus and the transtilla is trefoiled in the male genitalia. There is little difference in the female genitalia except the corpus bursae is much broader and the signum is smaller than in *E. bipectinalis* sp. n.

Description. Wingspan 25.0–30.0 mm (Figs 5, 6). Vertex brown, with individual scales grayish white-tipped. Antenna (Fig. 5a, b) brown, scales dark-tipped. Labial palpus brown, first segment with scales grayish white-tipped. Maxillary palpus brown. Occiput, patagium, tegula and thorax grayish brown, with scales tipped with grayish white. Forewing dark grayish brown with some white powdering, black along veins; antemedial line invisible; discal spots blackish brown, separated; postmedial line faint, grayish white, serrated, gently curved inwardly from costal 1/5 to dorsum 1/5; terminal line black, interrupted; cilia brown. Hindwing grayish white, light brown along costa and veins; cilia white. Foreleg blackish brown; mid- and hind legs brown with grayish white powdering, spurs yellowish brown. Abdomen with each tergite gray basally and grayish white distally, sternite yellowish brown.

Male genitalia (Fig. 9). Uncus bell-shaped, length longer than wide, abruptly narrowed beyond its broad base, rounded at apex. Apical projection of gnathos about half length of uncus, tapered, slightly bent and furcated at apex. Transtilla inverse-goblet shaped; deeply concaved in U shape on anterior margin, a rounded plate protruding on posterior margin. Valva three times as long as wide, evenly curved toward rounded apex, costal margin almost parallel with ventral margin, ventral margin concave at basal 1/3; costa strongly sclerotized, broad at base, narrowed and extending to near end of valva, without process apically; sacculus strongly sclerotized, stout, 2/5 length of valva. Juxta a broad, quadrate plate; lateral lobes ovate, 1.5 times as long as wide, bearing sparse setae in distal half. Vinculum trapezoid, widest posterior margin about 1.6 times of its length, straight on anterior margin. Phallus about 2/3 length of valva, smooth inside; cornutus absent. Eighth tergite cupped, with a pair of triangular sclerites anteriorly; eighth sternite with a pair of boot-like sclerites narrowly connected anteriorly. Culcita simple, one pair of fine scale tufts.

Female genitalia (Fig. 12). Anal papillae triangular, with a few setae, blunt apically. Eighth tergite slightly concave on posterior margin, convex W-shaped on anterior margin; eighth sternite with membranous part inverse-funneled. Antrum somewhat quadrate. Ductus bursae membranous, 1.5 times length of apophyses posteriores. Corpus bursae membranous, about same length as ductus bursae, twice as wide; signum a spindle-like patch of scobinations, at middle of corpus bursae. Ductus seminalis from junction of corpus bursae and ductus bursae.

Distribution. China (Gansu, Yunnan).

Etymology. The specific name is derived from the Latin prefix *furv-*, meaning black, and the Latin *vena*, vein, referring to the forewing with black scales along its veins in this species.

Discussion

The genus *Ectomyelois* is characterized by the wing venation, a signum with a patch of microspines and the inception of the ductus seminalis in the female genitalia. Two new species are assigned to this genus based on these characters. *Ectomyelois bipectinalis* sp. n. is unique for its bipectinate male flagellum, but the other characters, especially the genitalia, are in accord with the generic characters.

Neunzig and Goodson (1992) described one new species, *Ectomyelois austrella* Neunzig & Goodson, 1992, from Argentina. However, the male genitalia has a basal protuberance on the valva not found in *Ectomyelois* species, and the female genitalia bears a narrowly and deeply invaginated signum on the corpus bursae also not found in *Ectomyelois* species. Although we retain this species in *Ectomyelois*, the characters indicate that *austrella* might not be suitably placed in this genus.

Acknowledgments

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RESEARCH ARTICLE

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Two new and one newly recorded species of Gracillariidae from China (Lepidoptera)

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Abstract

The paper presents four Chinese species belonging to the genera *Metriochroa* Busck, *Eumetriochroa* Kumata, and *Gibbovalva* Kumata & Kuroko (Lepidoptera, Gracillariidae), including two new species: *Metriochroa alboannulata* Bai, **sp. n.** and *Gibbovalva clavata* Bai, **sp. n.** *Eumetriochroa hiranoi* Kumata, 1998, is newly recorded from China. Photographs of adults and figures of the genital structures are provided, along with keys to the Chinese species of *Metriochroa*, *Eumetriochroa*, and *Gibbovalva*.

Keywords

China, Gracillariidae, new species, new record, taxonomy

Introduction

Eumetriochroa Kumata, 1998 and *Metriochroa* Busck, 1900 are small genera of Gracillariidae Oecophyllembiinae (Kobayashi et al. 2013; De Prins and De Prins 2015). The genus *Eumetriochroa* contained four new species worldwide when it was erected (Kumata, 1998), namely *E. hederae* Kumata, 1998, *E. hiranoi* Kumata, 1998, *E. kalopanacis* Kumata, 1998 and *E. miyatai* Kumata, 1998. A new species, *E. araliella* Kobayashi, Huang & Hirowatari, 2013, was subsequently added to the genus (Kobayashi et al.

2013). Accordingly, five species are currently recognized in *Eumetriochroa* worldwide, all of them originally recorded from Japan. Larvae are leaf-miners on Aquifoliaceae, Araliaceae, and Styracaceae. To date eleven plant species in seven genera have been recorded as host plants of *Eumetriochroa* (Kumata 1998; Kobayashi et al. 2011, 2013; De Prins and De Prins 2015). Prior to this study, *Eumetriochroa* was represented in China by only one species, *E. hederae*, firstly reported there by Kobayashi et al. (2011).

The genus *Metriochroa* contains twelve described species worldwide. There are seven species in the Afrotropical region, three in the Palearctic region, and one each in the Oriental and Nearctic regions. *Metriochroa* was not recorded in China until *M. symplocosella* Kobayashi, Huang & Hirowatari, 2013 was described on the basis of Chinese material (Kobayashi et al. 2013). A total of twenty plant species in twelve genera of six families are known as host plants of *Metriochroa*. Eleven species in five genera of the family Oleaceae serve as the most common host plants for the larvae of *Metriochroa* (Kumata 1998; Kobayashi et al. 2013; De Prins and De Prins 2015).

The genus *Gibbovalva* Kumata & Kuroko, 1988 is one of the smallest genera of the subfamily Gracillariinae, and is represented by eight species worldwide. The majority of them (five) occur in the Palearctic and Oriental regions, the remaining two species were recorded from the Australasian region and one from the Afrotropical region. Host plants of *Gibbovalva* comprise thirty-four species in ten genera under four families, the majority of which (twenty species in six genera) belong to the family Lauraceae, followed by the Magnoliaceae (ten species in two genera), the Typhaceae (three species in one genus), and the Apocynaceae (one species) (Kumata et al. 1988; Bai and Li 2008; Bai et al. 2009; Triberti and Jaworski 2014, De Prins and De Prins 2015). Six species of *Gibbovalva* were recorded in China, mainly distributed in Guangdong, Guangxi, Hainan, Fujian, Guizhou, Zhejiang, Anhui, Yunnan, Hunan, Liaoning and Hong Kong (Bai and Li 2008).

Of the four gracillarid moth species treated in the present paper, *Eumetriochroa hiranoi* is newly recorded from China, and *Metriochroa alboannulata* sp. n. and *Gibbovalva clavata* sp. n. are new to science.

Methods

All adult specimens were obtained after by rearing from immature stages. Adult external morphology was examined by using a Leica M-205C stereomicroscope, and photographs were taken with a Leica DFC-450 digital camera connected to a Leica M-205C stereomicroscope. Genitalia were prepared following the methods of Li and Zheng (1996). Dissections of genitalia were conducted under an Olympus SZX-7 stereomicroscope. Genital morphology was examined with an Olympus BX-53 microscope, and the illustrations were prepared by using an Olympus DP-26 digital camera connected to the Olympus BX-53 microscope. Terminology follows Kumata (1998) and Kumata et al. (1988).

All specimens studied are deposited in the Insect Collection, Department of Bioscience and Biotechnology, Changzhi College, Changzhi, Shanxi, China (ICCC).

Taxonomy

Eumetriochroa **Kumata, 1998**

Eumetriochroa Kumata, 1998, *Insecta Matsumurana* (N.S.) 54: 83.

Type species. *Eumetriochroa hederae* Kumata, 1998.

References. Kumata (1998: 85, figs 1, 2A, 12A, B, 14A, 17, 18A, 22A, 24A, B).

Key to the Chinese species of *Eumetriochroa*

1 Forewing snow white, with five ochreous brown fasciae; (♂) valva with a trapezoid lobe on disc, vesica with a weakly sclerotized tubular part; (♀) signum blade-shaped, with a laterally elongated, triangular basal plate .... ***E. hiranoi*** **Kumata, 1998**

– Forewing ochreous brown, with five white fasciae; (♂) valva with a finger shaped process basally, vesica with a cornutus which has three to four transverse dentils; (♀) signum thorn-shaped, with a small triangular basal plate... .... ***E. hederae*** **Kumata, 1998**

Eumetriochroa hiranoi **Kumata, 1998**

Figs 1, 5, 9

Eumetriochroa hiranoi Kumata, 1998, *Insecta Matsumurana* (N.S.) 54: 96.

References. De Prins & De Prins (2005: 185), Kobayashi et al. (2013: 119).

Adult (Fig. 1). Wing expanse 4.7–5.1 mm.

Material examined. 2♂♂, 2♀♀, China. Feng Shan, Ganzhou, Jiangxi Province, 8 September 2012, leg. Jiasheng Xu and Chengqing Liao; genitalia slide Nos B13087, B13088, B13089, B13090; all in ICCC.

Host plant. Styracaceae: *Styrax japonicus* Siebold & Zucc. (Kumata, 1998).

Distribution. China (Jiangxi), Japan.

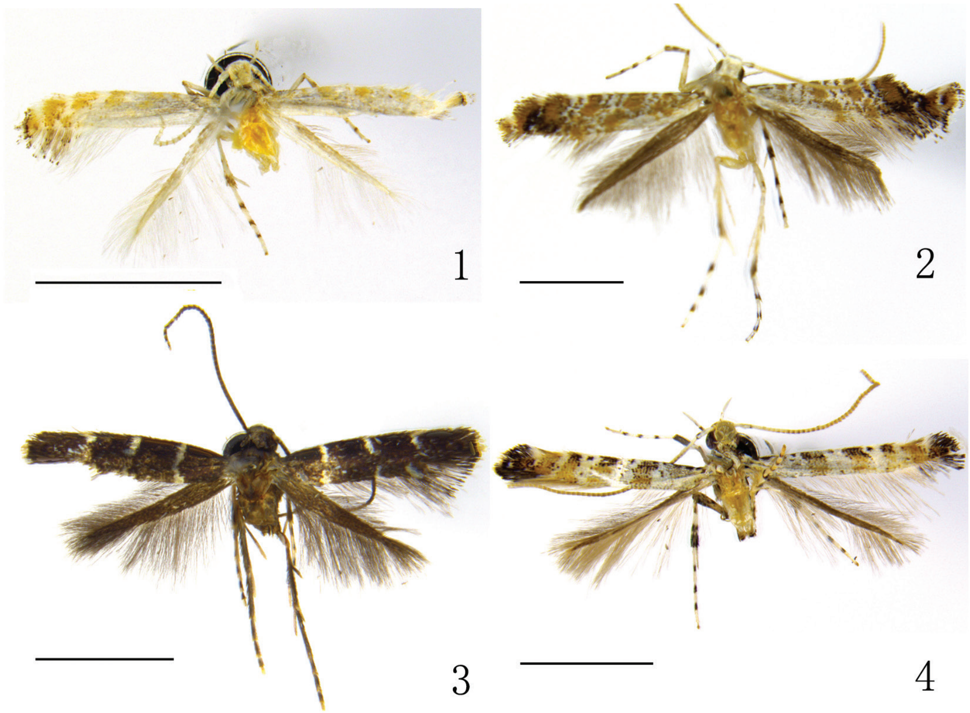
Remarks. In all four specimens examined the fore wing markings were poorly preserved, but the genital characters (Figs 5, 9) well agree with the description given by Kumata (1998). First record from China.

Eumetriochroa hederae **Kumata, 1998**

Figs 2, 6, 10

Eumetriochroa hederae Kumata, 1998, *Insecta Matsumurana*. (N.S.) 54: 85.

References. De Prins and De Prins (2005: 185), Kobayashi et al. (2011: 28).



Figures 1–4. Adults. **1** *Eumetriochroa hiranoi* Kumata **2** *Eumetriochroa hederae* Kumata **3** *Metriochroa alboannulata* Bai, sp. n. **4** *Gibbovalva clavata* Bai, sp. n. Scale bar 2000 μm.

Adult (Fig. 2). Wing expanse 8.1~8.7 mm.

Material examined. China. 1♂, Daqiutian, Jiulian Mountain, Jiangxi Province, 18 January 2013, leg. Xiaohua Dai; 2♀♀, Yangling National Forest Park, Chongyi County, Jiangxi Province, 700 m, 10 March 2012, leg. Jinshui Liang; genitalia slide Nos B12011, B12012, B13057; all in ICCC.

Host plants. Araliaceae: *Hedera sinensis* (Tobler) Hand.-Mazz.; *H. rhombea* (Miq.) Bean (Kumata 1998; Kobayashi et al. 2011).

Distribution. China (Hunan, Jiangxi), Japan.

Remarks. Specimens from China do not fully agree with the original description (Kumata 1998), especially in fore wing markings. Their fore wing has a white stripe situated between the third and fourth fasciae which extends from the dorsal edge of the third fascia towards costa to the middle of the fourth fascia. This character was not recorded by Kumata (1998) in the original description based on Japanese specimens. In addition, instead of the fourth fascia as described by Kumata, it is the apex of the fifth fascia which is edged with remarkable darker spots. However, the structures of the male (Fig. 6) and the female genitalia (Fig. 10) are in accordance with the original description, which provides us confidence to assign the specimens reared in China to this species.

Metriochroa **Busck, 1900**

Metriochroa Busck, 1900, *Proceedings of the United States National Museum* 23: 244.

Type species. *Metriochroa psychotriella* Busck, 1900.

References. Busck (1900: 245, pl. 1, fig. 13).

Key to the Chinese species of *Metriochroa*

1	Fore wing fuscous, with three white fasciae; (♂) vesica with a clavate cornutus	***M. alboannulata* sp. n.**
–	Fore wing pure to ochreous white, with three fuscous obscure specks; (♂) vesica with a bundle of spines at the middle	***M. symplocosella* Kobayashi, Huang & Hirowatari, 2013**

Metriochroa alboannulata **Bai, sp. n.**

http://zoobank.org/40AAAD7F-773E-4932-9C33-BD34A83D068D

Figs 3, 7, 11

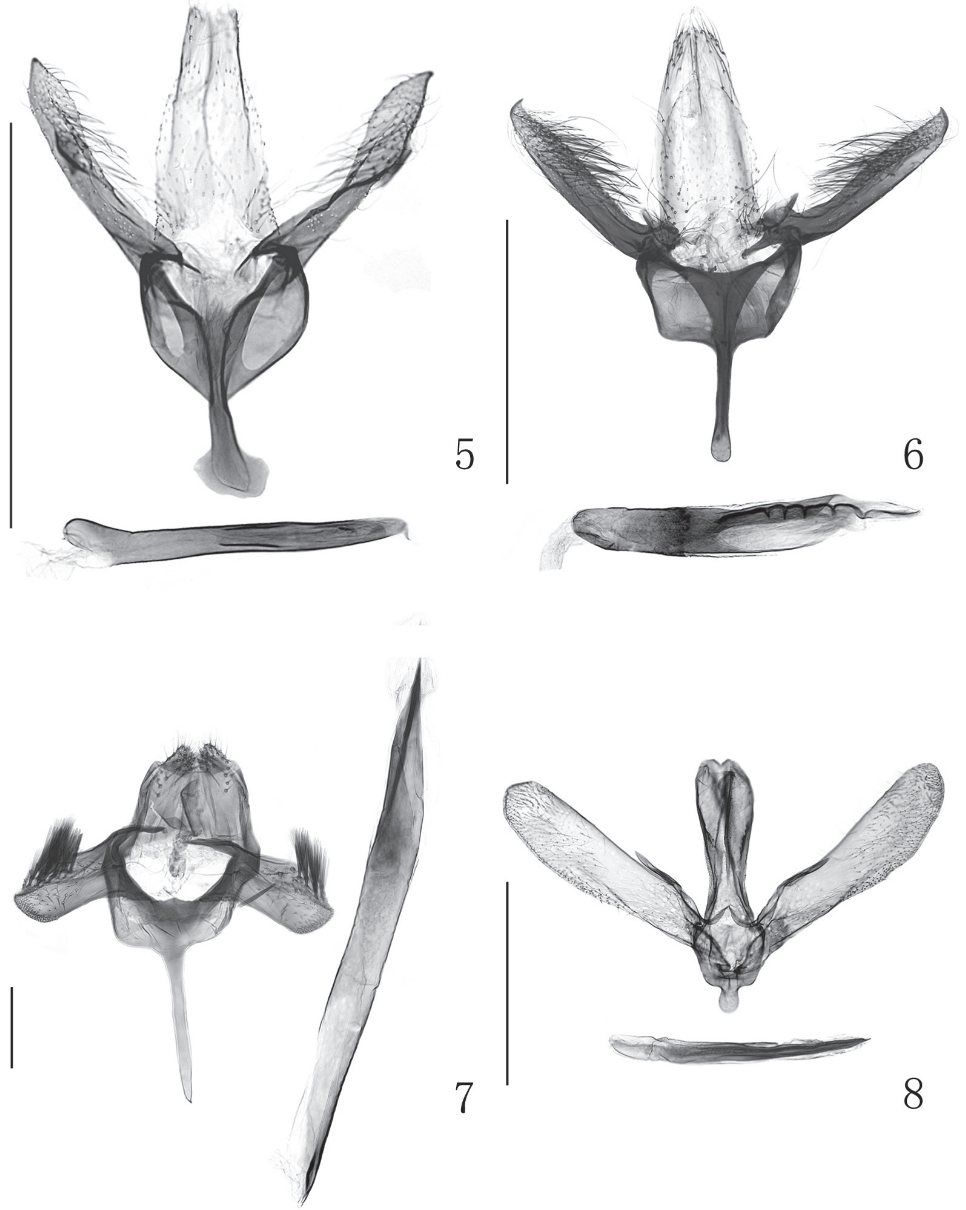
Diagnosis. The new species is a member of *Metriochroa* by the characteristics of venation and male genitalia. Fore wing of *M. alboannulata* sp. n. has nine veins (Fig. 11), M_3 and CuA are absent, M_1 is stalked with R_5, R_4 is connate or shortly stalked with stalk of M_1 and R_5; the valva is covered with partite scales.

Flagellum of *Metriochroa alboannulata* sp. n. has six white rings on distal part. Forewing has two silvery white fasciae: one placed at the basal 1/4 and is slightly outwardly angulate on wing fold, the other situated preapically; forewing possesses white costal and dorsal specks, two of them at the middle, and opposite each other, and one near the tornus. Valva is divided into dorsal and ventral portions by a sclerotized ridge, the former shorter than the ventral one. Aedeagus is tubular, and with a clavate cornutus on vesica.

Forewing markings of *Metriochroa* vary notably. *M. alboannulata* sp. n. is similar to *M. argyrocelis* Vári, 1961 and *M. celidota* Bradley, 1965 in forewing with obvious white or silvery white markings. These characteristics easily distinguish these species from other members of the genus.

Metriochroa alboannulata is close to *M. celidota* in forewing with two silvery white fasciae, especially as the first fascia is present at the basal 1/4 in both species. However, in *M. alboannulata* the first fascia is evident and joins with dorsum, and the second fascia is closer to the apex of forewing than in *M. celidota*; in addition, *M. alboannulata* has a silvery white speck near tornus, which does not occur in *M. celidota*.

Both *Metriochroa alboannulata* and the female of *M. argyrocelis* (forewing markings of male *M. argyroscelis* are clearly dissimilar from those of *M. alboannulata*) have a



Figures 5–8. Male genitalia. **5** *Eumetriochroa hiranoi* Kumata **6** *Eumetriochroa hederae* Kumata **7** *Metriochroa alboannulata* Bai sp. n. **8** *Gibbovalva clavata* Bai, sp. n. Scale bar 500 µm.

silvery white fascia at the basal 1/4 of forewing, and a silvery white speck near tornus, but they differ in the following characteristics: in *M. alboannulata*, the fascia is of uniform width, and is narrower than that of *M. argyrocelis*, in which it gradually widens towards dorsum; in addition, in place of the fascia near the apex of forewing and the silvery white bar-shaped specks at the middle of costa and dorsum present in *M. alboannulata*, *M. argyrocelis* has two silvery white specks at the middle and basal 3/4 of costa, respectively.

Description. Adult (Fig. 3). Wingspan 6.5–7.5 mm. Head fuscous with metallic luster. Antenna fuscous, flagellum with six white rings on distal part. Labial palpus whitish-yellow, with the outer side of second and third segments fuscous. Thorax, tegula, and fore wing fuscous. Fore wing shining with purple; two silvery white fasciae present, first fascia at the basal 1/4, and slightly outwardly angulate on wing fold, second fascia at subapex and outwardly oblique; costa and dorsum with an outwardly oblique bar-shaped silvery white speck each at the middle, costal speck longer than the dorsal one; dorsum with a silvery white speck near tornus; cilia grayish-brown, those on termen with median and apical fringe lines of black spots, which run parallel with termen. Hindwing and its cilia fuscous. Legs fuscous. External surface of profemur and mesofemur, internal surface of metafemur ochreous white; protibia basally, mesotibia and extremities of metatibia ochreous white; both ends of first tarsomeres, apical tarsomeres and the apex of other tarsomeres white. Abdomen dorsally fuscous, ventrally ochreous white, anterior margin of each sternite fuscous.

Male genitalia (Fig. 7). Tegumen *ca*. 100 µm in length, with widely rounded apex. Tuba analis bilobed apically, with setae on each lobe. Vinculum Y-shaped; saccus *ca*. 180 µm in length, clavate, with pointed apex. Valva *ca*. 160 µm in length, about three times as long as wide; inner surface with a sclerotized longitudinal ridge which divides the valva into dorsal and ventral portions; dorsal portion slightly shorter than ventral one, with obliquely truncated apex, and covered with a group of partite scales on its distal part; ventral portion with spine-like setae on its rounded apex. Aedeagus tubular, *ca*. 700 µm long, obliquely truncated along apical 2/7, pointed apically; vesica with a clavate cornutus, which is approximately 160 µm long.

Female. Unknown.

Type material. Holotype ♂. China. Wuzhifeng, Shangyou County, Jiangxi Province, 2 January 2013, leg. Chengqing Liao; genitalia slide No. B13051, in ICCC. Paratypes 2♂♂. China, with same data as holotype; genitalia slide Nos B13050, BX15001, in ICCC.

Etymology. The specific name is composed of "*albus*" and "*annulatus*", meaning "with white ring", referring to the flagellum of antenna with white rings on its distal part.

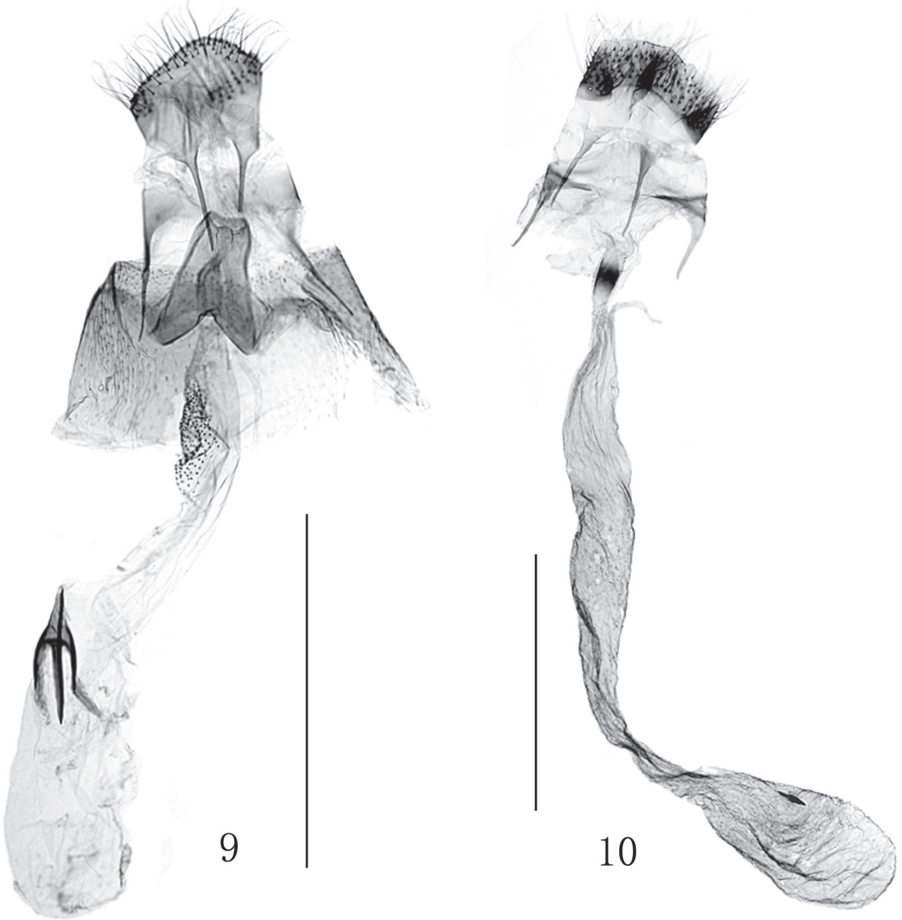
Distribution. China (Jiangxi).

Gibbovalva Kumata & Kuroko, 1988

Gibbovalva Kumata & Kuroko, 1988, In: Kumata, Kuroko and Ermolaev, 1988, *Insecta Matsumurana* (N.S.) 40: 3

Type species. *Gracilaria* (sic) *quadrifasciata* Stainton, 1862.

Reference. Stainton (1862: 295, pl. 10, fig. 5).



Figures 9–10. Female genitalia. **9** *Eumetriochroa hiranoi* Kumata **10** *Eumetriochroa hederae* Kumata. Scale bar 500 µm.

Key to the Chinese species of *Gibbovalva*

1 Forewing with five white or ochreous yellow fasciae....................................**2**
– Forewing with four white fasciae...**5**
2 Forewing fuscous with ochreous yellow fasciae ... ***G. civica* (Meyrick, 1914)**
– Forewing ochreous yellow with white fasciae **3**
3 Antenna with flagellum white in several basal segments; aedeagus without cornutus .... ***G. urbana* (Meyrick, 1908)**
– Antenna with flagellum ochreous yellow to ochreous brown; aedeagus with cornutus **4**
4 Forewing with fifth fascia intercalated by a black narrow line in centre; aedeagus with a flap like process at basal 1/3 ....***G. kobusi* Kumata & Kuroko, 1988**

– Fifth fascia without the aforementioned characteristic; flap like process of aedeagus absent............................***G. magnoliae*** **Kumata & Kuroko, 1988**

5 Forewing fuscous, white fasciae with large evident fuscous spots, third fascia interrupted by ground color................ ***G. quadrifasciata*** **(Stainton, 1862)**

– Forewing ochreous yellow or distal 2/3 ochreous, fuscous spots in white fasciae obscure or absent, fasciae uninterrupted..**6**

6 First fascia at base of forewing; three basal white fasciae wider than the fourth one, and almost equal in width; forewing with a black speck on dorsum between third and fourth fasciae, and a white speck above the black one......... ....***G. singularis*** **Bai & Li, 2008**

– First fascia nearly at the middle of forewing, the first and the second fasciae wider than the third and the fourth fasciae; space between third and fourth fasciae without spots .... ***G. clavata*** **sp. n.**

Gibbovalva clavata Bai, sp. n.

http://zoobank.org/C81BC6DD-CFDF-4DC9-85BF-EEA4FFCC83EF
Figs 4, 8

Diagnosis. A new species of *Gibbovalva*, with antennal scape bearing a ventral flap and valva with a costal process as for other members of the genus, with which it also shares the fore wing markings and characteristics of vinculum and saccus. The basal 1/3 of the forewing of *Gibbovalva clavata* sp. n. is white in ground color and has four black costal specks; the distal 2/3 is ochreous yellow in ground color and has four white fasciae. The valva is blade-shaped, the costa possesses a clavate process at the basal 1/6; saccus is thumb-shaped with rounded apex; the aedeagus does not have a flap-like process, and its thorn-like cornuti are arranged in rows from basal 1/3 to subapex.

Gibbovalva clavata sp. n. is similar to *G. magnoliae* and *G. tricuneatella* in the aedeagus lacking a flap-like process, but it is distinguishable by the forewing markings. In *G. clavata*, basal 1/3 of forewing is white with four black costal specks; apical 2/3 of forewing has four white fasciae, whereas in *G. magnoliae* forewing has a V-shaped speck at base and five white fasciae and in *G. tricuneatella* forewing has three white fasciae which markedly dilate towards wing fold. In addition, *G. clavata* resembles *G. quadrifasciata* (Stainton) in the male genitalia, as in both species the ventral surface of valva is covered with lanceolate setae, but it is distinguishable by other characters.

Description. Adult (Fig. 4). Wingspan 7.0 mm. Head white, with frons fuscous. Labial palpus white, second segment apically and third segment basally with a fuscous spot on their outer side. Thorax white, its sides edged with fuscous line; tegula fuscous with white apex. Basal 1/3 of forewing white, with four black specks along costa, of which the last one smallest; distal 2/3 of forewing ochreous yellow with fuscous band along costa and four white, nearly equally spaced fasciae which obliquely extend outwards from costa to dorsum; two basal fasciae, approximately twice the width of the two distal ones, enclose a black spot on costa. Cilia black from dorsal third fascia to

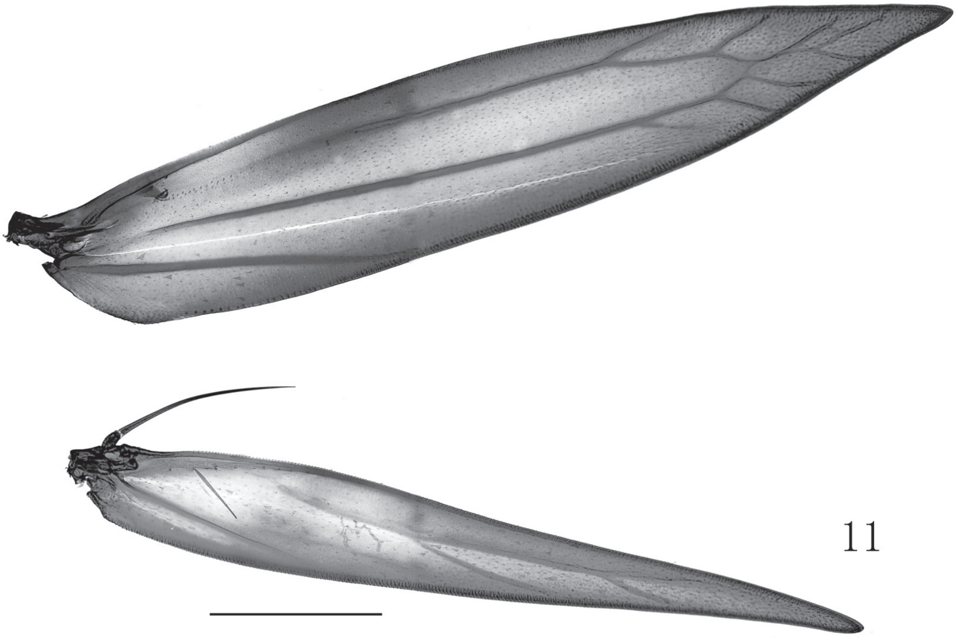


Figure 11. Wing venation of *Metriochroa alboannulata* Bai, sp. n. Scale bar 500 μm.

costal fourth fascia, white at the apical angle, the remaining cilia pale grey. Hind wing and its cilia pale grey. Legs with coxae and tarsi white; tarsi with three fuscous rings, the last tarsomere ochreous yellow apically. Profemur fuscous; protibia white in basal 1/3, the remaining part fuscous. Mesofemur with external surface fuscous, internal surface ochreous white; mesotibia white, with three fuscous rings, of which one at the basal 1/3, two at the distal part. Metafemur white, external side with a fuscous spot at base and middle respectively; metatibia white, with a median fuscous ring, the last tarsomere fuscous apically.

Male genitalia (Fig. 8). Tegumen approximately 400 μm long, tongue-like, slightly wider on apical half, densely covered with fine setae on ventral and dorsal surfaces and with a sparse row of longer setae on each side. Valva approx. 600 μm long, blade-shaped, slightly narrowed at base, obliquely truncated at apex and almost parallel-sided; costa straight with a clavate process at the basal 1/6, dorsum slightly upcurved near apex; inner surface covered with usual setae except for lanceolate setae clustered on distal part. Saccus thumb-shaped, rounded apically. Aedeagus nearly 650 μm long, tapering to a pointed apex from around the distal 1/4; vesica with acute, thorn-like cornuti arranged in rows from basal 1/3 to aedeagus subapex, some cornuti arranged between apical 1/5 and apex being larger than others. Antero-dorsal apodeme of the eighth tergite approx. 150 μm long, with slender sclerotization extending caudad to the middle of the eighth tergite; eighth sternite with a pair of very slender invaginations, nearly equal in length to dorsal apodeme.

Female. Unknown.

Type material. Holotype ♂. China, Jiulian Mountain, Longnan, Jiangxi Province, 600 m, 30 March 2012, leg. Jiasheng Xu; genitalia slide No. B12020, in ICCC.

Etymology. The species name is derived from the Latin "*clavatus*", meaning "clavate", in reference to the costal process of valva.

Distribution. China (Jiangxi).

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On the synonymy of two *Acantholycosa* species (Araneae, Lycosidae) from the Altai

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Abstract

Two species previously known from East Kazakhstan, *Acantholycosa katunensis* Marusik, Azarkina & Koponen, 2004, known from the holotype male, and *A. kurchumensis* Marusik, Azarkina & Koponen, 2004, **syn. n.** known from females, are synonymized, and priority is given to *A. katunensis*. *Acantholycosa katunensis* is reported for the first time in the Russian Altai. Both sexes of this species are illustrated, and a distribution map is provided.

Keywords

Aranei, wolf spider, South Siberia, Russia, new record, new synonymy

Introduction

Acantholycosa Dahl, 1908 is one of the best studied among species-rich Pardosinae genera in the Holarctic due to a revision (Marusik et al. 2004), a regional review (Marusik and Omelko 2011) and several species surveys (Kronestedt and Marusik 2002; Fomichev and Marusik 2011; Marusik and Logunov 2011). Nevertheless, its taxonomy has remained improperly studied. Over one third of *Acantholycosa* species are known from only a single sex (two from females and eight from males). All these species are either endemic to the Altai-Sayan Mountain region (nine species) or the Sikhote-Alin' Mountain Range (one species). Such a high number of species known only from one sex may be because of the habitat preference of *Acantholycosa* species. With one exception (*A. lignaria* (Clerck, 1757)), all species inhabit stony screes in mountains, and this habitat is difficult to reach and study. So far, 29 species are known in the genus (World Spider Catalog 2015), most of which (20) occur in the Altai-Sayan Mountain region (Marusik et al. 2004).

While studying material from the Russian Altai, the first author found two species from nearby localities, *A. katunensis* Marusik, Azarkina & Koponen, 2004 and *A. kurchumensis* Marusik, Azarkina & Koponen, 2004, both previously known from East Kazakhstan. The former species, assigned to the *azyuzini*-group, was known from the holotype male from Rakhmanovskiye Klyuchi Village, and the latter species, assigned to the *dudkorum*-species group, was known from three females from two localities, the Kurchum River and Rakhmanovskiye Klyuchi. Marusik et al. (2004) suggested that these two species found in a single locality (Rakhmanovskiye Klyuchi) could be conspecific. The occurrence of these two species *A. katunensis* and *A. kurchumensis* in another locality led us to the conclusion that they are conspecific, and the tentative placement of these species into different species groups by Marusik et al. (2004) was incorrect. Additional arguments in support of the conspecificity of two species are the same spination of tibia I (5-6 proventral and 4-5 retroventral spines) and also that these species in South-Western Altai are the only known species by opposite sexes. The goal of this paper is to synonymize the two species, provide an illustrated redescription of both sexes and new data about species distribution.

Material and methods

Specimens were photographed with a Canon EOS 7D camera attached to an Olympus SZX16 stereomicroscope at the Zoological Museum, University of Turku, Finland. Digital images were montaged using "CombineZP" image stacking software. Epigynes were cleared in a KOH/water solution until soft tissues were dissolved. Photographs were taken in dishes with paraffin on the bottom to hold the specimens in position. Background maps are from Microsoft Encarta Premium 2009. All material examined is deposited in the Institute for Systematic and Ecology of Animals, Novosibirsk (ISEA).

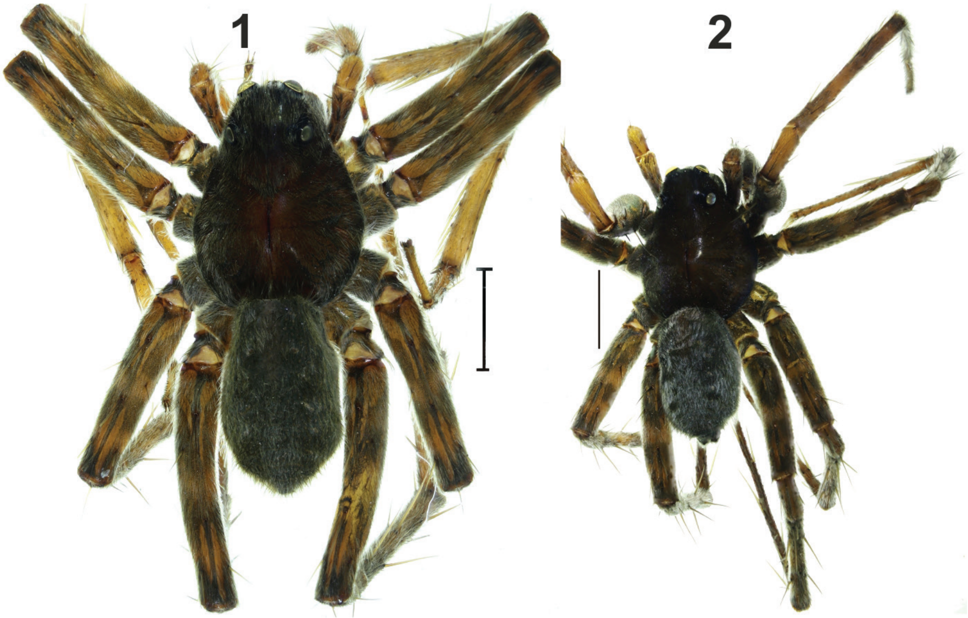
Taxonomy

Acantholycosa katunensis **Marusik, Azarkina & Koponen, 2004**
Figs 1–10

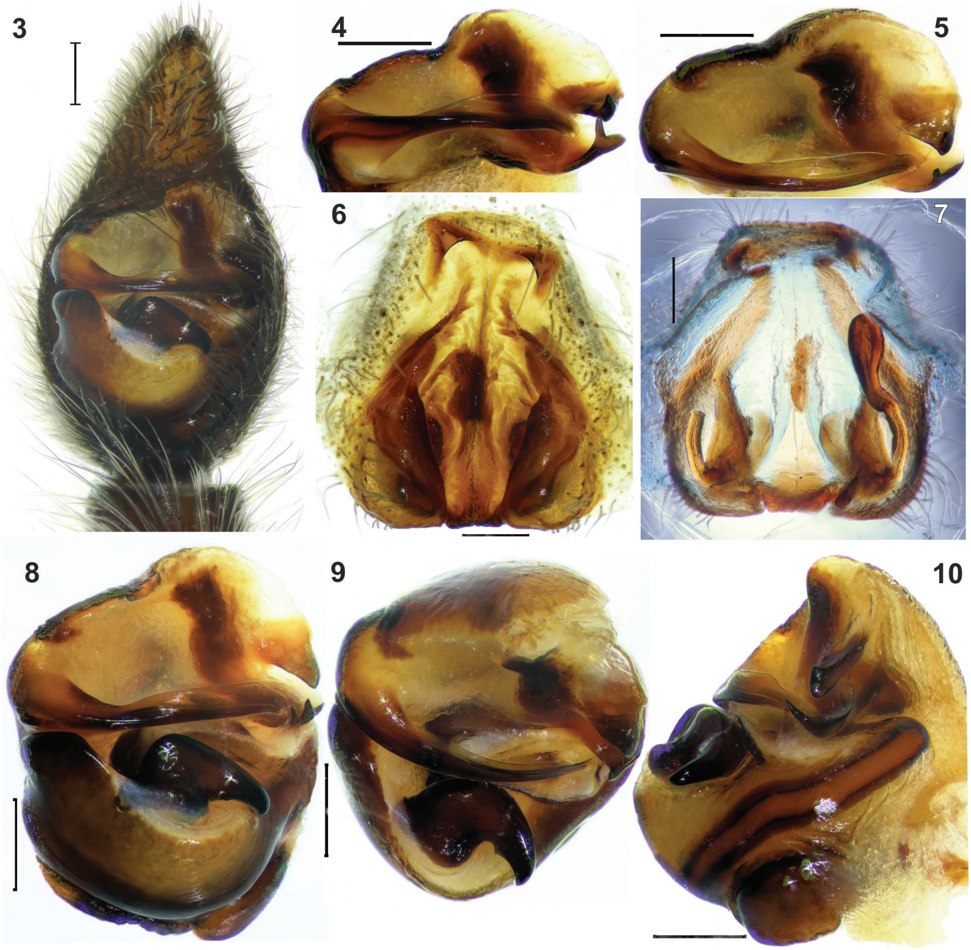
A. katunensis Marusik, Azarkina & Koponen, 2004: 107, figs 21–23 (♂).
A. kurchumensis Marusik, Azarkina & Koponen, 2004: 119, figs 82–83 (♀). **Syn. n.**

Material examined. RUSSIA, ***Altai*** Republic, Kosh-Agach District: 3♂ (ISEA), Karagemskyi Mt. Range (49°52'N; 87°07'E), 2500–2900 m, "kurums" (=scree formed by huge boulders) and alpine meadow, 27.06.2014 (A.A. Fomichev); 1♀ (ISEA), Karagem River valley (49°53'N; 87°11'E), 1360 m, stony steppe slope, 28.06.2014 (A.A. Fomichev); KAZAKHSTAN, ***East Kazakhstan*** Area: 1♂ (holotype of *A. katunensis*) (ISEA) South Altai, south part of Katun' Mt. Range, 5 km SE of Rakhmanovskiye Klyuchi (=Springs), 2100–2500 m, alpine zone, 26.06.1997 (R.Yu. Dudko and V.K. Zinchenko); 1♀ (holotype of *A. kurchumensis*) (ISEA) Kurchum Mt. Range, Kurchum River, upper flow, 23.08.1990 (V.K. Zinchenko).

Diagnosis. *Acantholycosa katunensis* is most similar to *A. dudkorum* Marusik, Azarkina & Koponen, 2004 by having a similarly shaped tegular apophysis that lacks an apical arm, a similar conductor, a wide apical pocket and a thin septum. The two species can be separated by the shape of the embolus, which tapers toward the tip in *A. katunensis* (Figs 3–5) and widens in *A. dudkorum* (cf. Marusik et al. 2004: figs 73, 75); the shape of



Figures 1–2. Habitus of *Acantholycosa katunensis*. **1** female **2** male. Scale: 2 mm.

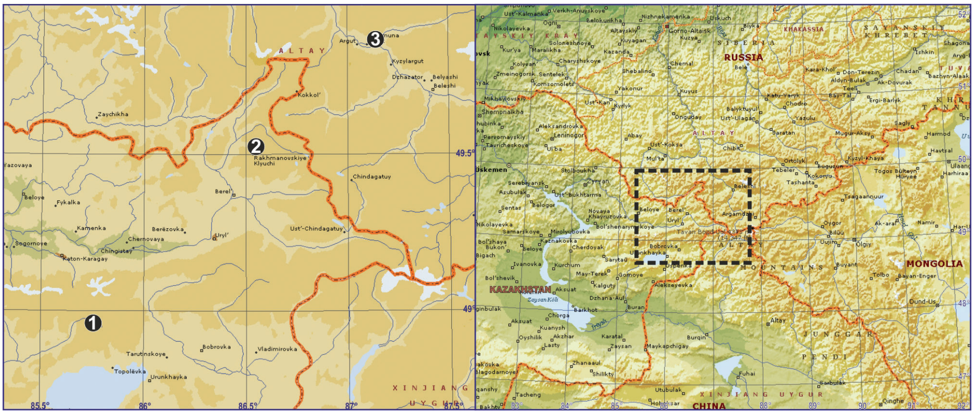


Figures 3–10. Copulatory organs of *Acantholycosa katunensis*. **3** male palp, ventral **4–5** embolic division, ventral and anterior **6–7** epigyne, ventral and dorsal (left head of receptacle is broken) **8–10** bulb, ventral, anterior and retrolateral. Scale: 0.2 mm.

the palea: in *A. dudkorum* (cf. Marusik et al. 2004: figs 73, 75), the paleal process is thin with a hollow on the prolateral side, and it is unmodified in *A. katunensis* (Figs 4–5, 9). Females of the two species can be distinguished by the shape of septum: long (starting from the pocket) with a subparallel base in *A. katunensis* and short (starting from fovea) and widened at the base in *A. dudkorum* (cf. Marusik et al. 2004: fig. 78).

Description. See Marusik et al. (2004).

Distribution. So far, this species is known from three localities. The most distant localities, the upper reaches of the Kurchum River (locality 1, Map 1) and the Karagemskyi Mt Range (locality 3) are approximately 190 km apart (Map 1). Rakhmanovskiye Klyuchi Village (locality 2), the type locality of *A. katunensis*, is located between two extreme distribution records (Map 1). Listings for the species by Platnick



Map 1. Distribution records of *Acantholycosa katunensis*. **1** Kurchum River **2** Rakhmanovskiye Klyuchi Village **3** Karagemskyi Mt. Range.

(2004–2014) and the World Spider Catalog (2015) for Russia were erroneous based on wrong country data provided by Marusik et al. (2004). Type locality of *A. katunensis* was mistakenly assigned to Russia (Altai) by Marusik et al (2004) instead of Kazakhstan (East Kazakhstan Area). The new record from the Karagemskyi Mt Range is the first locality record of this species in Russia.

Discussion

Synonymizing these two names reduced the number of species known in the genus to 27. Although the number of *Acantholycosa* species in the Altai-Sayan Mountain Region decreased from 20 to 19, this region still has the highest species diversity and endemism (59%) in the entire range of the genus. The record of *A. katunensis* from the Russian Altai increased the number of *Acantholycosa* species found in Russia from 21 to 22. Judging from the high level of endemism in the Altai-Sayan Mountain Region, the limited distribution of species in that area, habitat preferences (stony screes) and the number of unexplored mountain ranges in the West and East Sayan Mountains (cf. Marusik et al. 2004: map 1) and the lack of material from adjacent Xinjiang (China) and Mongolia, many new species are expected.

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