RESEARCH ARTICLE

A new species of *Nebalia* (Crustacea, Leptostraca) from a hydrothermal field in Kagoshima Bay, Japan

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Abstract

A new species of Leptostraca, *Nebalia tagiri* **sp. nov.** is described and illustrated. This species was sampled from 200 m depth at a hydrothermal field in Wakamiko Caldera of Kagoshima Bay, Japan. *Nebalia tagiri* **sp. nov.** is different from known *Nebalia* species as follows: rostral length 2.4 times as long as width; article 4 of antennule with 3–5 robust distal spines; antennular scale approximately twice as long as wide; article 3 of antenna with eight spines and nine spine-like setae along proximal half, two thin setae and six spine-like setae on external lateral face, six spines and four simple setae on distal margin; article 1 of second maxilla longer than article 2; article 2 of mandibular palp with two thin setae; exopod of pleopod 1 with 21 spines along lateral margin; furcal rami longer than combined length of pleonite 7 and telson; rounded denticles of pleonite 6 and 7; anal-plates 'shoulder' not distinct. Furthermore, this specimen is the first genus *Nebalia* found in the hydrothermal vent. The distribution and ecology of this new species is also discussed and a key to all species of *Nebalia* is provided.

Keywords

Introduction

The genus *Nebalia* is a member of the Order Leptostraca, Suborder Nebaliacea, Family Nebaliidae. The family includes four other genera: *Dahlella* (Hessler, 1984), *Nebaliella* (Thiele, 1904), *Sarsinebalia* (Dahl, 1985), and *Speonebalia* (Bowman et al., 1985). *Nebalia* was established by Leach (1814) with type *Nebalia herbstii* from the British Isles. More than 37 species of this genus have been reported thus far from Africa (Barnard 1914; Kensley 1976; Olesen 1999; Bochert and Zettler 2012), Red Sea (Wägele 1983), Adriatic Sea (Dahl 1985), Britain-Celtic Sea (Dahl 1985), Greenland (Dahl 1985), Norway (Dahl 1985), Pakistan (Kazmi and Tirmizi 1989), Antarctic Sea (Dahl 1990), Falkland Islands (Dahl 1990), New Zealand (Dahl 1990), South Atlantic Ocean (Dahl 1990), Mexico (Escobar-Briones and Villalobos-Hiriart 1995; Ortiz et al. 2011), California (Martin et al. 1996; Vetter 1996; Haney and Martin 2000, 2005), Mediterranean Sea (Ledoyer 1997; Moreira et al. 2007, 2012; Koçak and Moreira 2015), New Caledonia (Ledoyer 2000), Northeast Atlantic (Haney et al. 2001; Moreira et al. 2003, 2009), Aegean Sea (Moreira et al. 2007), Hong Kong (Lee and Bamber 2011), South Korea (Song et al. 2012, 2013; Song and Min 2017), and Malaysia (Othman et al. 2016). Although our understanding of the morphology and distribution of the genus *Nebalia* has progressed, further investigation into the ecology of the genus is necessary.

In 2008, a survey was undertaken at the hydrothermal field of the Wakamiko Caldera in Kagoshima Bay, Japan using the deep-sea remotely operated vehicle (ROV) "Hyper-Dolphin" of JAMSTEC. Twenty-eight specimens of a previously undocumented species in the genus *Nebalia* were discovered. In this paper, we describe these specimens as *Nebalia tagiri* sp. nov. Taxonomic keys used to identify all currently known *Nebalia* species are also provided. Comments relating to the ecology and distribution of this species are also noted.

Materials and methods

Samples were collected in 2008 at a hydrothermal vent in the Wakamiko Caldera, Kagoshima Bay, during dive number 886 of the Japanese deep-sea ROV "Hyper-Dolphin" of JAMSTEC. The sampling site was the Wakamiko Caldera, located north of Mt. Sakurajima of Kagoshima Bay at a depth of ca. 200 m (Fig. 1). The water temperature in this field is ca. 10 °C. While bacterial mats were observed, these hydrothermal vents have no documented epifauna occurring around them. Specimens examined in this study were captured using a suction sampler installed on the ROV. Samples were sorted, identified, sexed, and measured. Total length (**TL:** measured from the articulation between the rostrum and the carapace to the posterior end of the caudal furca), carapace length (**CL:** measured from the antero-dorsal margin of carapace to the posterio-median margin of carapace), and rostrum length (**RL:** measured along the midline) were used as size indicators. Illustrations were made with the aid of a camera lucida. The type materials were deposited at the National Science
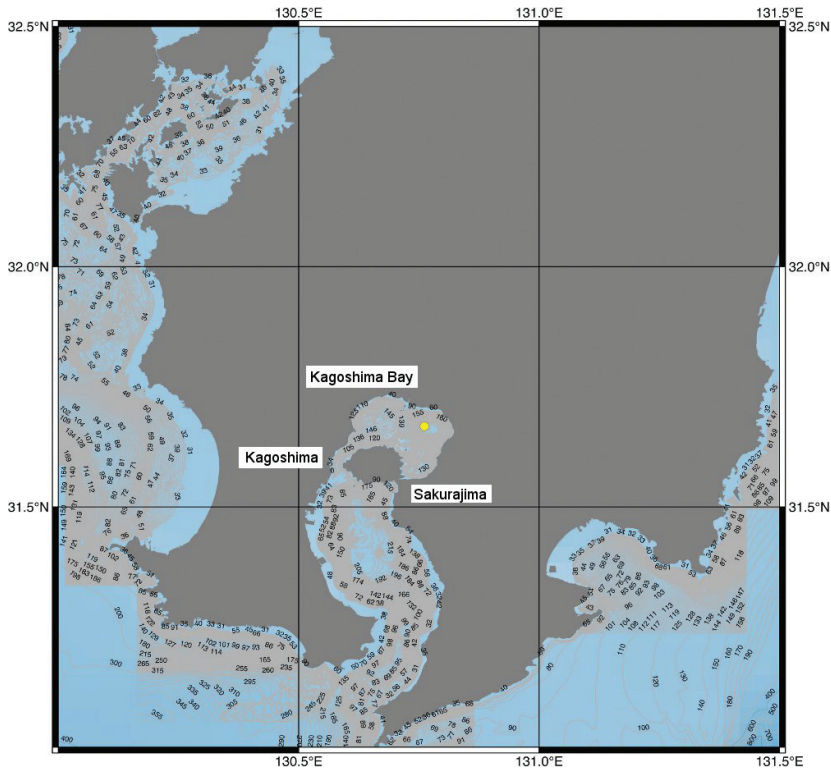
Figure 1. Sampling location (in yellow) of *Nebalia tagiri* sp. nov. in Kagoshima Bay.

Museum (Natural History), Tokyo (**NSMT**), with the remaining material deposited in the JAMSTEC Marine Biological Sample repository.

Systematics

Genus *Nebalia* Leach, 1814

Diagnosis. Carapace almost reaching medial margin of pleonite 4. Rostrum long and narrow, 2.4 times as long as wide. Surface of eyes smooth. Article 4 of antennule with row of four simple setae and four robust distal spines. Article 3 of antenna with eight spines and nine spine-like setae along proximal half, two thin setae and six spine-like setae on external lateral face, six spines and four simple setae on terminal margin. Article 1 of endopod of second maxilla longer than article 2. Exopod of second maxilla longer than article 1 of endopod. Article 2 of mandibular palp with two thin setae. Pleonites 6 and 7 with distally rounded denticles along posterior border. Exopod of pleopod 1 with a single row of approximately 21 stout serrated spines along lateral margin. Anal plates with no distinct lateral 'shoulder'. Furcal rami longer than combined length of pleonite 7 and telson.

Nebalia tagiri sp. nov.

http://zoobank.org/C023E769-AF5A-4421-8671-9BFD96192723
Figs 2–7

Material. Twenty-eight specimens were collected using the JAMSTEC ROV "Hyper-Dolphin" of JAMSTEC during dive 886 in the Wakamiko Caldera during the R/V "Natsushima" NT08-17 Leg-1 cruise. "Hyper-Dolphin" dive 886: the Tagiri Site on the Wakamiko Caldera: 30°40.068'N, 130°45.690'E; 200 m; 7 Aug 2008. 14 ♂♂ (TL: 3.7–6.0 mm), 13 ♀♀ (TL: 2.0–6.1 mm).

Types. *Holotype*: (NSMT-Cr 26758), adult ♀ of 10.7 mm TL. *Allotype*: (NSMT-Cr 26759), adult ♂ of 7.0 mm TL. *Paratypes*: 3 adult ♂♂ (TL: 4.1–6.0 mm) (NSMT-Cr 26760, NSMT-Cr 26761, NSMT-Cr 26762) 3 adult ♀♀ (TL: 3.9–6.0 mm) (NSMT-Cr 26763, NSMT-Cr 26764, NSMT-Cr 26765).

Description. Female holotype. *Carapace* (Fig. 2A) oval, ca. 1.5 times as long as wide, almost reaching pleonite 4.

Rostrum (Fig. 2B) long and narrow, 2.4 times as long as width, with round apex.

Compound eye (Fig. 2C): ommatidial part covering two-thirds of eye-stalk. Supraocular plate reaching to ommatidial part.

Antennule (Fig. 2D): peduncle composed of four articles. Article 2 longer than article 3, with single long plumose seta on anterior margin, 5 long and three short plumose setae arising subterminally and cluster of simple setae on anterior margin, respectively. Article 3 shorter than article 2, widest distally, with terminal cluster of simple setae and long simple seta arising on anterior margin and five long plumose setae and two thin plumose setae on posterio-distal margin. Article 4 much shorter than article 3, with row of four simple setae and four robust spines distally. Antennular scale oval, twice as long as width. Flagellum slightly longer than peduncle, composed of 12 articles.

Antenna (Fig. 2E): peduncle composed of 3 articles. Article 2 2.3 times as long as wide, with stout spine at dorso-distal portion. Article 3 longer than article 2, with different pattern of spines or setae along medial anterior margin as follows:

(1) proximal row of ca. six simple setae and plumose seta on inner surface;
(2) eight spines and nine spine-like setae along proximal half, the distalmost being the longest, respectively;
(3) two thin setae and six spine-like setae on external lateral face;
(4) seven thin plumose setae, three plumose setae and seven simple setae, each associated proximal spines;
(5) six robust spines increasing in length distally and four simple setae at apex, 21 long plumose setae arising from posterior distal margin, and robust plumose seta arising sub-terminally. Flagellum longer than peduncle, composed of 15 articles.

Mandible (Fig. 2F) well developed. Mandibular palp composed of three articles. Article 2 equal in length as article 3, article 2 with two thin setae at mid-length on later-
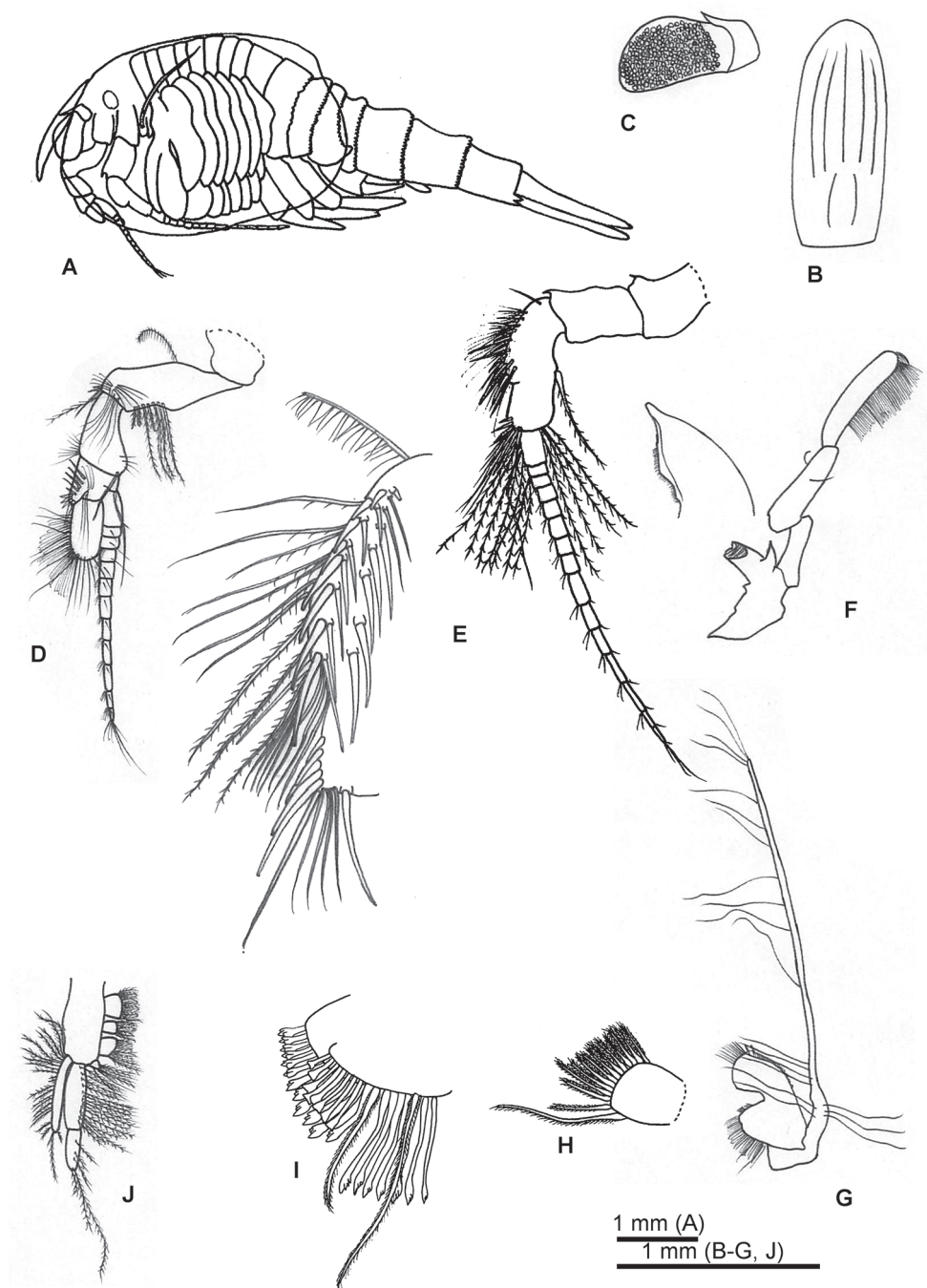
Figure 2. *Nebalia tagiri* sp. nov. **A** female holotype, lateral view **B** rostrum **C** eye **D** antennule **E** antenna and detail of different row of spines and setae of article 3 **F** mandible and detail of incisor process **G** first maxilla **H** detail of proximal endite of first maxilla **I** detail of distal endite of first maxilla **J** second maxilla.

al margin and sub-terminal on superior margin, respectively. Article 3 cylindrical, with marginal setae-row covering four-fifths length of article. Molar process shorter than palp article 1, distal margin with row of teeth forming grinding surface. Incisor process broad basally with acute terminal process and minute tooth along lateral margin.

First maxilla (Fig. 2G): proximal endite (Fig. 2H) with rounded medial margin, bearing short robust plumose setae. Distal endite (Fig. 2I) carrying two rows of stout, spatulate setae and two long plumose seta. Palp long, ca. 4.5 times longer than combined length of both endites, bearing 20 setae.

Second maxilla (Fig. 2J): protopod sub-divided into four endites bearing plumose setae. Endite 1 rectangular and endite 3 rounded approximately equally sized to endite 1; endite 2 oval, smaller than endite 1 and 3; endite 4 smaller than other endites. Endopod composed of two articles, article 1 ca. twice as long as article 2, lateral margin with plumose setae, article 2 with three terminal plumose setae. Exopod reaching beyond apex of endopod article 1, bearing 23 plumose setae on lateral margin.

Thoracopod 1 (Fig. 3A): endopod composed of large article and three small distal articles, with numerous plumose setae along outer margin, terminal setae not reaching the terminal margin of exopod. Exopod oval, not reaching beyond the terminal article of endopod, with single long setae on terminal margin and 21 thin setae along inner margin. Epipod large, proximal lobe beyond the basis, distal lobe reaching beyond the middle of exopod.

Thoracopod 2–6 (Fig. 3B–F): Except for the exopod, shape of each limbs same. Eleven thin setae of inner margin of exopod in thoracopod 2, five in thoracopod 3, seven in thoracopod 4, six in thoracopod 5, seven in thoracopod 6. Shape of exopod gradually changes from oval to triangular from thoracopods 2–6.

Thoracopod 7 (Fig. 3G) endopod composed of one large article and two small distal articles, with numerous plumose setae along outer margin, terminal setae beyond the terminal margin of exopod. Exopod rounded and distal part expanded like a triangle, reaching beyond the terminal article of endopod, with eight thin setae along inner margin. Epipod large and triangular, proximal lobe beyond the basis, distal lobe reaching beyond the middle of exopod, with three thin setae along distal margin.

Thoracopod 8 (Fig. 3H): smaller than other thoracopods. Endopod composed of a large article and three small distal articles, with numerous plumose setae along outer margin, terminal setae not reaching the terminal margin of exopod. Exopod oval, reaching beyond the terminal article of endopod, with five setae along inner margin. Epipod small, proximal lobe beyond the basis, distal lobe not reaching beyond the middle of exopod.

Pleon (Fig. 2A, 5A): composed of seven segments. Posterior margin of pleonite 1 smooth. Pleonite 2 with narrowly triangular denticles on half-length of posterior margin with wide flat margin. Pleonite 3 with round minute denticles. Pleonite 4 with round minute denticles, posterolateral margin expanded and forming narrow acute process. Pleonites 5, 6, and 7 with minute rounded denticles.
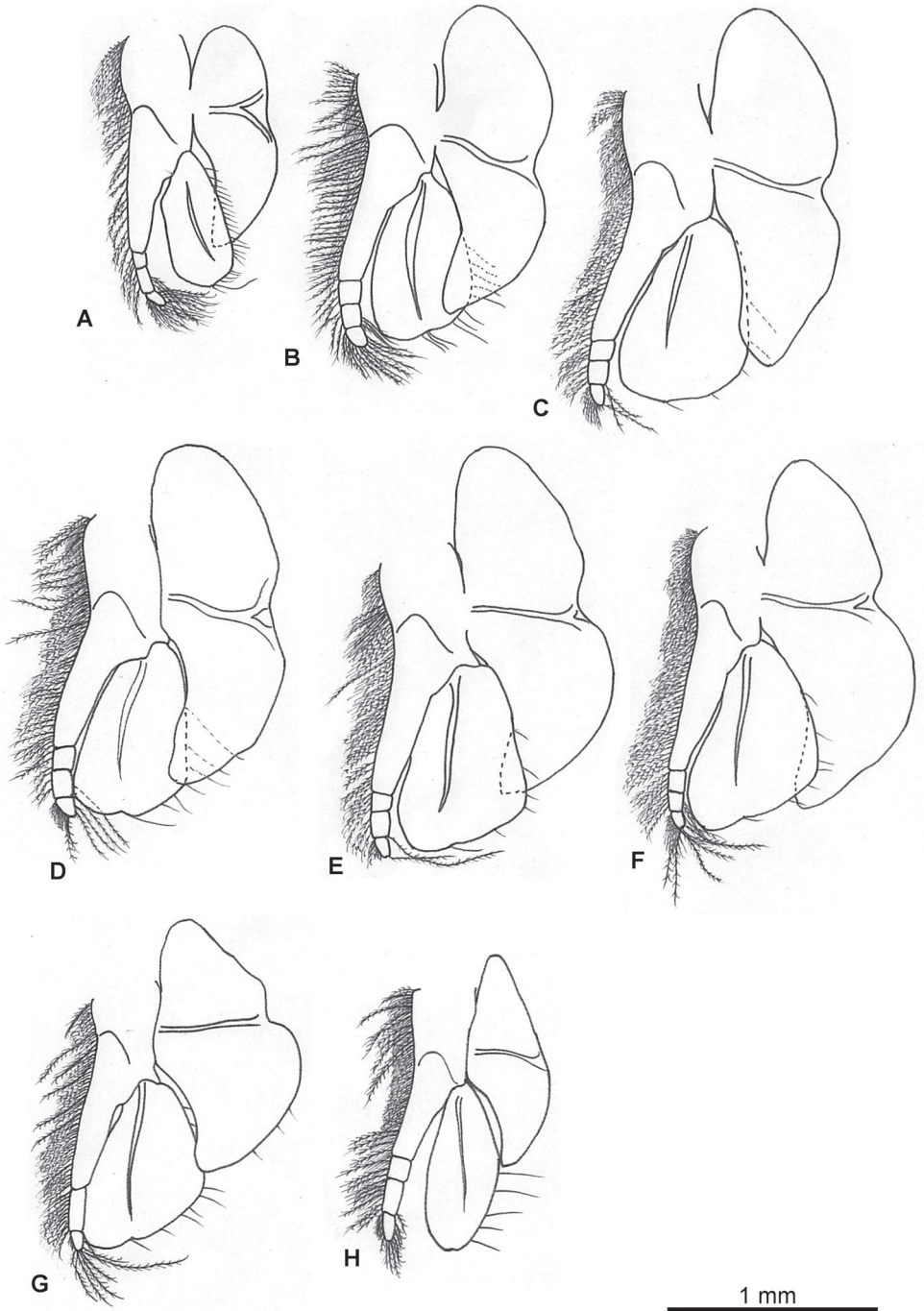
Figure 3. *Nebalia tagiri* sp. nov. **A** thoracopod 1 **B** thoracopod 2 **C** thoracopod 3 **D** thoracopod 4 **E** thoracopod 5 **F** thoracopod 6 **G** thoracopod 7 **H** thoracopod 8.

Pleopod 1 (Fig. 4A): protopod twice as long as width, with three short simple setae arising proximally, simple seta medially, two spine-like setae near base of endopod, spine-like seta near base of exopod. Endopod composed of two segments, longer than exopod, distal segment with acute process at apex, bearing long robust simple spine, lateral and medial margin each with plumose setae, nine short setae on proximal medial margin, appendix interna of proximal segment with three short recurved hooks. Exopod with row of 21 stout serrated spines along lateral margin, five stout simple spines on distolateral margin, distal one longest, plumose setae along distal inner margin.

Pleopod 2 (Fig. 4B): protopod 2.2 times as long as wide, six simple setae and seven simple setae on proximal and distal part of inner side, respectively; simple seta near base of exopod, with blade-like process between exopod and endopod. Endopod composed of two segments, longer than exopod, distal segment with acute process at apex, bearing long robust simple spine, lateral and medial margins each with plumose setae, ten short setae on proximal medial margin, appendix interna of proximal segment with three short recurved hooks. Exopod with row of six pairs of robust spines along lateral margin, three stout simple spines on distal margin, plumose setae along distal inner margin.

Pleopod 3 (Fig. 4C): protopod 2.2 times as long as wide, five simple setae on proximal and distal part of inner side respectively, simple seta near base of exopod, with blade-like process between exopod and endopod. Endopod composed of two segments, longer than exopod, distal segment with acute process at apex, bearing long robust simple spine, lateral and medial margin each with plumose setae, eight short setae on proximal medial margin, appendix interna of proximal segment with three short recurved hooks. Exopod with row of seven pairs of robust spines along lateral margin, three stout simple spines on distal margin, plumose setae along distal inner margin.

Pleopod 4 (Fig. 4D): protopod twice as long as width, bearing five simple setae along lateral proximal border, eleven short simple setae along ventral proximal border and four simple setae along posterior border. Posterolateral corner of peduncle produced as acute point, with blade-like process between exopod and endopod, posterior margin lacking serrations, posterolateral corner with acute projection. Endopod composed of two segments, longer than exopod, distal segment of endopod with acute process at apex, bearing long robust simple spine, lateral and medial margin each with plumose setae, ten short setae on proximal medial margin, appendix interna of proximal segment with three short recurved hooks. Exopod with row of seven pairs of robust spines along lateral margin, three stout simple spines on distal margin, plumose setae along distal inner margin.

Pleopod 5 (Fig. 4E, F): acute process between bases of rami. Protopod composed of two segments. Distal segment 4.5 times as long as wide, bearing five simple spines and ten short setae, lateral margin with ca. 33 simple setae.

Pleopod 6 (Fig. 4G, H): acute process between bases of rami, rami bearing five simple spines and six simple setae.

Telson, anal plates, and furca (Fig. 5B, C): anal plates (Fig. 5C) with medial margin slightly convex, point acute, lateral margin with no distinct 'shoulder'. Furcal rami (Fig. 5B) slightly longer than combined lengths of pleonite 7 and telson, 21 spines
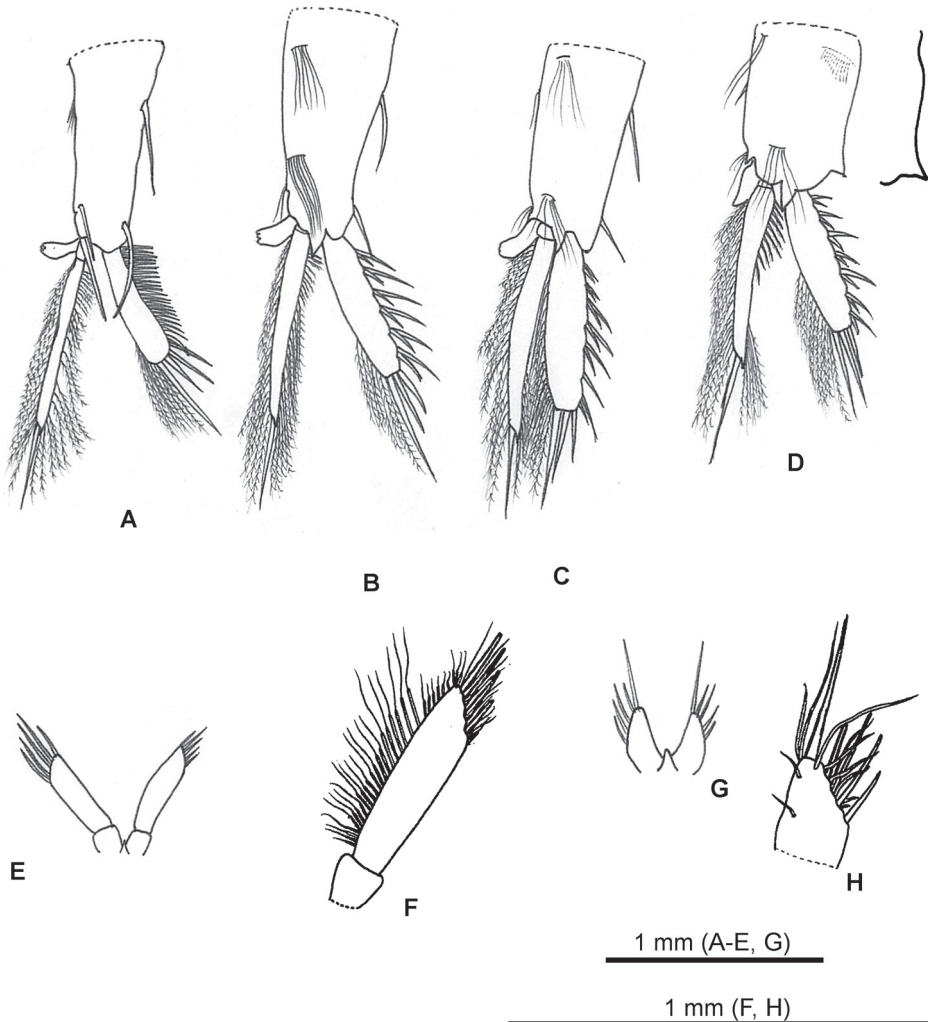
Figure 4. *Nebalia tagiri* sp. nov. **A** pleopod 1 **B** pleopod 2 **C** pleopod 3 **D** pleopod 4 and detail of lateral margin **E** pleopod 5 **F** detail of pleopod 5 **G** pleopod 6 **H** detail of pleopod 6.

along outer edge, 14 robust setae and 16 plumose setae along inner margin, three long robust setae and thin spine on distolateral margin.

Color in life (Fig. 7): living specimens with dark red eyes and most of body transparent.

Allotype (adult male): antennule flagellum more swollen proximally than in female (Fig. 6B). Antenna flagellum composed of more than 50 articles (Fig. 6C). article 2 of endopod palp half-length of article 1 (Fig. 6D). Furcal rami sub-equal in length to combined length of pleonite 7 and telson (Fig. 6E).

Morphological variations. Examination of five female specimens of various sizes shows several morphological variations in the number of robust distal spines of article 4 of antennule and lateral spines of exopod of pleopod 1. Article 4 of antennule with 3–5 spines, exopod of pleopod 1 with 21–24 spines on lateral margin, respectively.
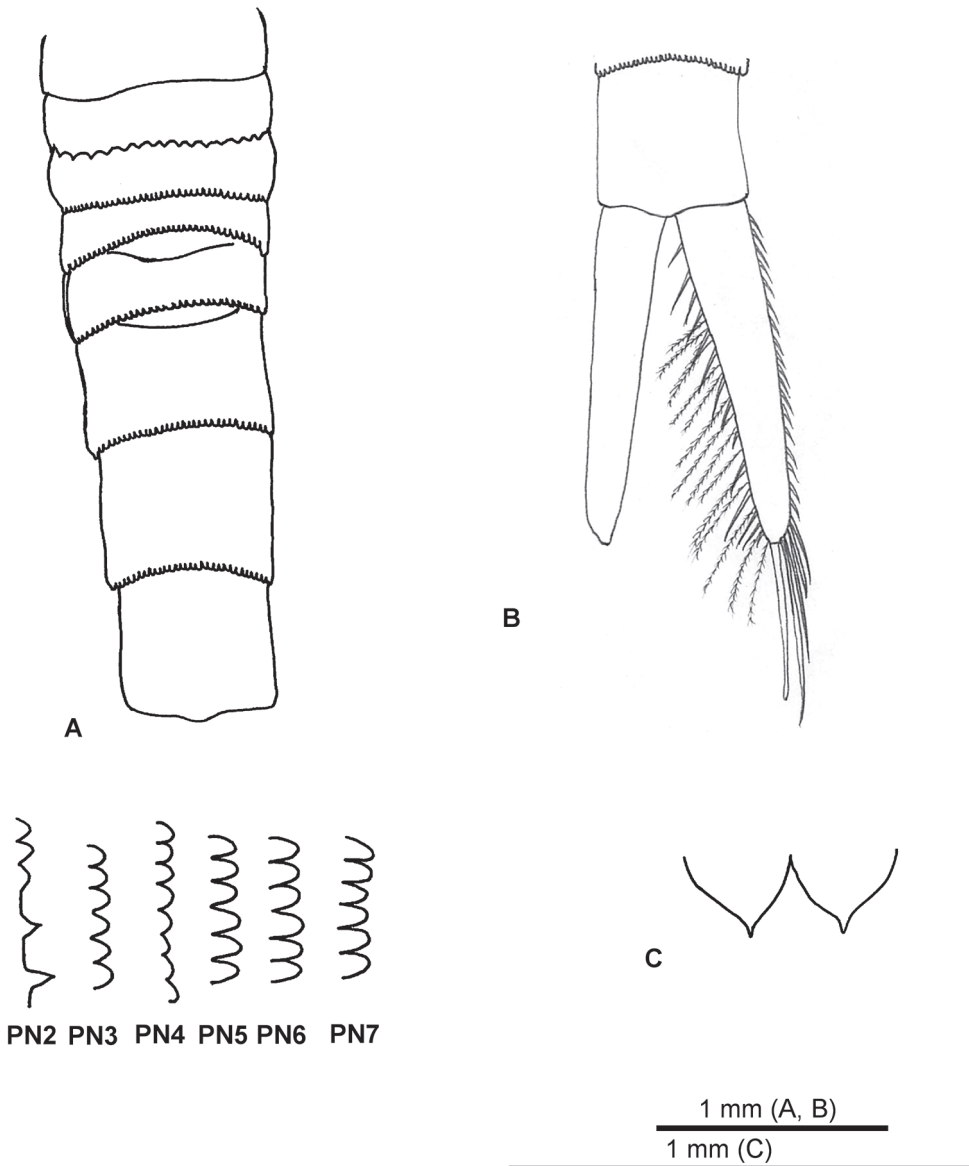
Figure 5. *Nebalia tagiri* sp. nov. **A** female pleonite, dorsal view and detail of denticles **B** furcal rami (setae not illustrated for left limb) **C** anal plates.

Etymology. The specific name *tagiri* originates from the Japanese word 'tagiru' meaning "boiling", a reflection of the hydrothermal venting and bubbling of methane and carbon dioxide as found in the habitat of *N. tagiri* sp. nov.

Remarks. *Nebalia tagiri* sp. nov. differs from the other 34 described species based on four unique characteristics: (1) smooth oval eye-stalk; (2) ommatidial part covering two-thirds of eye-stalk; (3) article 4 of antennule with 3–5 robust distal spines; (4) article 1 of second maxilla endopod longer than article 2. *Nebalia tagiri* sp. nov. can easily
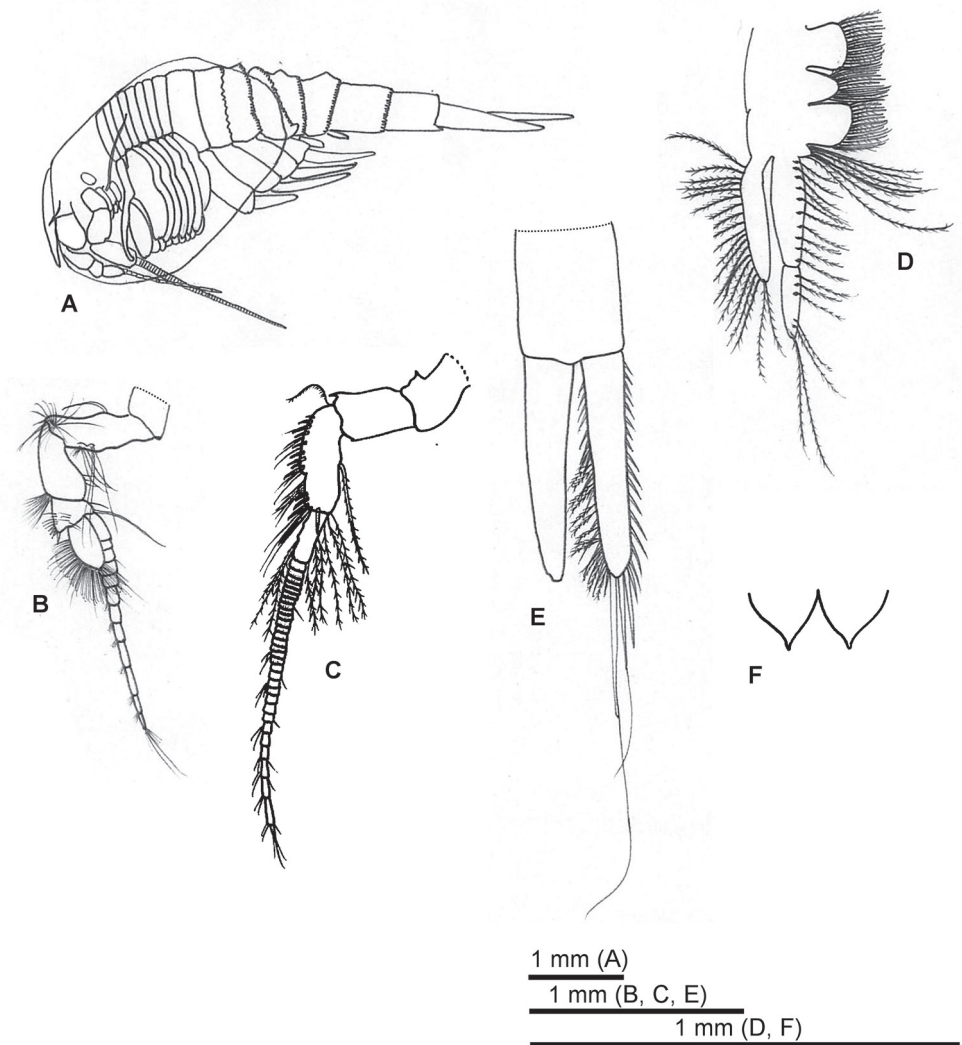
Figure 6. *Nebalia tagiri* sp. nov. **A** male allotype, lateral view **B** antennule **C** antenna **D** second maxilla **E** furcal rami (setae not illustrated for left limb) **F** anal plates.

be distinguished from *N. bipes* (Fabricius, 1780), *N. mortoni* (Lee & Bamber, 2011), and *N. koreana* (Song et al., 2012) based on the following: (1) length of rostrum in *N. tagiri*, *N. koreana*, and *N. mortoni* ca. 2.4 times as long as width, *N. bipes* approximately twice times as long as width; (2) number of distal spines of article 4 of antennule is four in *N. tagiri* sp. nov. and *N. mortoni*, three in *N. bipes* and five in *N. koreana*; (3) length of antennular scale in *N. tagiri*, *N. koreana* and *N. bipes* ca. twice as long as width, *N. mortoni* ca. 2.7 times as long as width; (4) the different pattern of spines or setae of article 3 of antenna among related three species are summarized in Table. 1.

Nebalia tagiri sp. nov. showed different characteristics from *N. koreana* or *N. mortoni* in the following points: (1) number of spines or spine-like setae along proximal

Table 1. Comparison of *Nebalia tagiri* sp. nov. with related species of *Nebalia*. Key: **ro** = Rostrum; **an1** = antennule; **an2** = antenna; **mp** = mandibular palp; **pp** = pleopod; **pn** = pleonite; **a** = article; **exp** = exopod; **sp** = spine; **se** = seta; **sls** = spine-like seta; **ts** = thin seta; **ps** = plumose seta.

|	Habitat	Depth	Ro width	Shape of eye-stalk	Distal sp of an1 a4	Proximal row of an2 a3	Lateral row of an2 a3	Distal row of an2 a3	Ts of mp2 a2	Sp on pp1 exp	Uropod length	Shape of pn6–7 denticles	Presence of 'shoulder' on anal-plates	Reference
N. tagiri sp. nov.	Hydrothermal vent chimney	200 m	2.4 times	Oval	4 sp	8 sp 9 sls	2 ts 6 sls	6 sp 4 se	2 ts	21–24 sp	> Pn7+t	Round	No distinct	This paper
N. abyssicola	Mud	680–820 m	–	Oval	1 sp	8 sp 6 se	2 ts 6 sp 3 sls	5 sp 6 se	2 ts	25–30 sp	> Pn7+t	Round	None	Ledoyer (1997)
N. bipes	Clay and stones	5–13 m	2 times	Oval	3 sp	–	–	–	1 ts	30 sp	= Pn7+t	Round	Distinct	Dahl (1985)
N. borealis	Sand	240 m	2.1 times	Oval	2 sp	–	–	–	1 ts	24 sp	≥ Pn7+t	Round to acute	Distinct	Dahl (1985)
N. koreana	Algal mat	6 m	2.4 times	Oval	5 sp	10 sp 6 sls	2 ts 6 sls	5 sp 4 se	2 ts	30–38 sp	< Pn7+t	Round	No distinct	Song et al. (2012)
N. mortoni	–	17 m	2.4 times	Oval	4 sp	7 sp	2 ts 4 sls	6 sp	2 ts	25 sp	= Pn7+t	Square	No distinct	Lee and Bamber (2011)
N. schizophthalma	–	2886 m	2.6 times	Bilobed	5 ts	10 sls	10 se	6 sp 8 se	2 ts	15 sp	< Pn7+t	Acute	None	Haney et al. (2001)
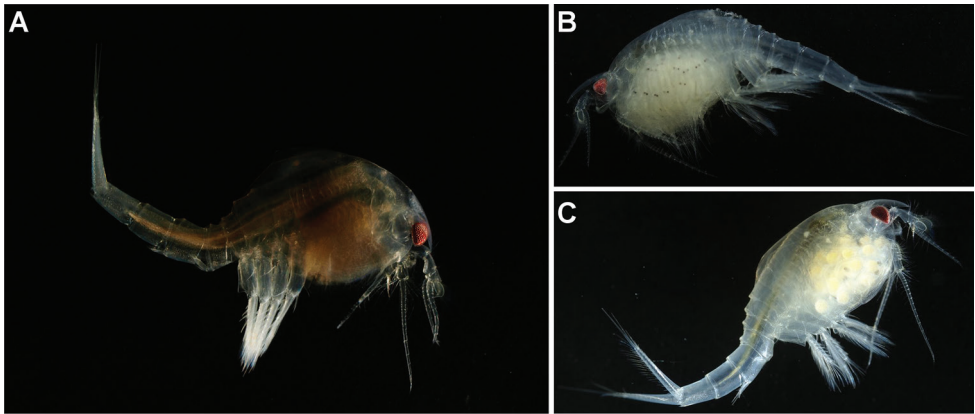
Figure 7. *Nebalia tagiri* sp. nov., photographs **A** female, lateral view **B** female with larvae **C** female with eggs.

half in comparison with *N. koreana* and *N. mortoni*; (2) existence of spine-like setae on external lateral face in comparison with *N. mortoni*; (3) number of spines and setae on terminal margin in comparison with *N. koreana* and *N. mortoni*, *N. bipes* is lacking detailed description; (5) thin seta of article 2 of mandibular palp is two in *N. tagiri* sp. nov., *N. koreana* and *N. mortoni* and one in *N. bipes*; (6) number of lateral spines of exopod of pleopod 1 is 21–24 in *N. tagiri* sp. nov, 25 in *N. mortoni*, and more than 30 in *N. bipes* and *N. koreana*; (7) furcal rami are longer than the combined length of pleonite 7 and the telson in *N. tagiri*. sp. nov. and of the same length in *N. bipes* and *N. mortoni*, while in *N. koreana* they are shorter; (8) denticles of posterior margin of pleonites 6 and 7 are rounded in *N. tagiri* sp. nov., *N. bipes*, and *N. koreana* while in *N. mortoni* are square-shaped.

Most species of *Nebalia* have been reported from shallow water (< 10 m depth) in the world oceans with two exceptions, *i.e.*, *Nebalia abyssicola* (Ledoyer, 1997) and *N. schizophthlma* (Haney et al., 2001) have been reported from ca. 100 m or deeper (Table 1). *Nebalia schizophthalma* was reported at a depth of 2886 m in the North Atlantic Ocean, which is the deepest record of this genus. *Nebalia tagiri* sp. nov. was collected from a hydrothermal vent chimney at a depth of 200 m in Wakamiko Caldera of Kagoshima Bay, Japan (Fig. 1). This depth of 200 m is relatively deep compared to similar species of this genus. The genus *Nebalia* has not been previously reported from any hydrothermal fields to date. The only species reported from a deep-sea hydrothermal field is *Dahlella caldariensis* (Hessler, 1984), observed on the mussel beds of hydrothermal vent areas at the Galapagos and the East Pacific Rise at depths deeper than 2000 m. On the other hand, *N. tagiri* sp. nov. was collected near the interior of chimney walls, which were close to the chimney vents spouting thermal water (ca. 200 °C).

This species was clearly observed on the inner surface of the chimney, suggesting the species is adapted to the hydrothermal environment. For a more accurate understanding of this species, further investigations are needed to determine their ecological and/or physiological aspects in relation to the hydrothermal fields.

Key to species of genus *Nebalia*

1	Pleopod 6 composed of two segments.......... ***N. biarticulata* Ledoyer, 1997**	
–	Pleopod 6 composed of one segment **2**	
2	Entire surface of eye smooth **13**	
–	Eye surface different..**3**	
3	Dorsal margin of eye smooth, with lobes only on antero-distal margin **10**	
–	Eye papillae present on dorsal margin **4**	
4	Eye with several lobes on antero-distal margin	
	....***N. cambodiana* Song et al., 2013**	
–	Eye with antero-distal margin smooth, lacking lobes..............................**5**	
5	Ommatidial part covering most of the eye-stalk.....................................**6**	
–	Ommatidial part not covering most of the eye-stalk................................**7**	
6	Protopod of pleopod 4 with serrations along posterior margin......................	
	.... ***N. cannoni* Dahl, 1990**	
–	Protopod of pleopod 4 lacking serrations along posterior margin..................	
	....***N. longicornis* Thomson, 1879**	
7	Ommatidial part covering four-fifths of eye-stalk.......................................	
	.... ***N. falklandensis* Dahl, 1990**	
–	Ommatidial part not covering four-fifths of eye-stalk..............................**8**	
8	Ommatidial part covering half of eye-stalk......... ***N. capensis* Barnard, 1914**	
–	Ommatidial part covering two-thirds of eye-stalk **9**	
9	Denticles of pleonites 6 and 7 distally acute......... ***N. antarctica* Dahl, 1990**	
–	Denticles of pleonites 6 and 7 rounded...............***N. patagonica* Dahl, 1990**	
10	Anterior margin of eye-stalk with 2 lobes...**11**	
–	Anterior margin of eye-stalk with 3 lobes...**12**	
11	Eye-stalk with flat anterior margin between lobes.... ***N. daytoni* Vetter, 1996**	
–	Eye-stalk no flat margin between lobes..	
	....***N. schizophthalma* Haney et al., 2001**	
12	Denticles of pleonites 6 and 7 acute distally..	
	....***N. troncosoi* Moreira et al., 2003**	
–	Denticles of pleonites 6 and 7 rounded..	
	....***N. pseudotroncosoi* Song et al., 2013**	
13	Shape of the eye-stalk sub-rectangular..............***N. abyssicola* Ledoyer, 1997**	
–	Shape of the eye-stalk oval **14**	
14	Ommatidial part covering half of the eye-stalk......................................**15**	
–	Ommatidial part not covering half of the eye-stalk **20**	
15	Supraocular plate covering proximal portion of eye-stalk **16**	
–	Supraocular plate covering the half of eye-stalk	
	....***N. deborahae* Bochert & Zettler, 2012**	
16	Exopod of second maxilla clearly extend beyond the endopod of article 1.....	
	....***N. clausi* Dahl, 1985**	
–	Exopod of second maxilla subequal length to endopod of article 1 ....**17**	

17   Furcal rami almost the same length as combined length of telson and pleonites 6 and 7 ....*N. marerubri* **Wägele, 1983**
–   Furcal rami shorter than combined length of telson and pleonites 6 and 7....**18**
18   Furcal rami almost the same length as combined length of telson and pleonite 7 ....*N. gerkenae* **Haney & Martin, 2000**
–   Furcal rami longer than combined length of telson and pleonite 7............**19**
19   Article 4 of antennule with single distal spine .... *N. brucei* **Olesen, 1999**
–   Article 4 of antennule with one or two distal spines...
       .... *N. dahli* **Kazmi & Tirmizi, 1989**
20   Ommatidial part covering more than two-thirds of eye-stalk **21**
–   Ommatidial part covering more than three quarters of eye-stalk **32**
21   Article 1 of endopod of second maxilla shorter than article 2...................**22**
–   Article 1 of endopod of second maxilla not shorter than article 2.............**23**
22   Rostrum length ca. 2.4 times as long as width..
       .... *N. lagartensis* **Escobar-Briones & Villalobos-Hiriart, 1995**
–   Rostrum length ca. 1.9 times as long as width..
       .... *N. terazakii* **Othman et al., 2016**
23   Article 1 of endopod of second maxilla subequal in length to article 2 ....
       ....*N. kocatasi* **Moreira et al., 2007**
–   Article 1 of endopod of second maxilla longer than article 2 ....**24**
24   Denticles of pleonites 6 and 7 square .... *N. mortoni* **Lee & Bamber, 2011**
–   Denticles of pleonites 6 and 7 not square..**25**
25   Denticles of pleonites 6 and 7 acute..**26**
–   Denticles of pleonites 6 and 7 rounded..**27**
26   Exopod of pleopod 1 bearing 15 or 16 spines on lateral margin...................
       .... *N. melanophthalma* **Ledoyer, 2000**
–   Exopod of pleopod 1 bearing 26 spines on lateral margin ....
       .... *N. strausi* **Risso, 1826**
27   Anal plates with distinct 'shoulder'............................... *N. bipes* **Dahl, 1985**
–   Anal plates with no distinct 'shoulder'..**28**
28   Protopod of pleopod 4 with serrations along posterior margin.................**29**
–   Protopod of pleopod 4 lacking serration along posterior margin **30**
29   Article 3 of antennal peduncle bearing plumose setae on external lateral face...
       ....*N. mediterranea* **Kocak & Moreira, 2015**
–   Article 3 of antennal peduncle lacking plumose setae on external lateral face...
       ....*N. kensleyi* **Haney & Martin, 2005**
30   Furcal rami shorter than combined length of telson and pleonite 7..............
       ....*N. koreana* **Song et al., 2012**
–   Furcal rami not shorter than combined length of telson and pleonite 7 ....**31**
31   Denticle of pleonite 2 with wide flat margin between denticles.....................
       .... *N. tagiri* **sp. nov., This paper**
–   Denticle of pleonite 2 lacking flat margin between denticles.........................
       ....*N. reboredae* **Moreira et al., 2009**

32   Ommatidial part covering four-fifths of eye-stalk..
        .... ***N. villalobosi* Ortiz et al., 2011**
–   Ommatidial part covering three-quarters of eye-stalk...............................**33**
33   Denticles of pleonites 6 and 7 square-shaped....***N. ilheoensis* Kensleyi, 1976**
–   Denticles of pleonites 6 and 7 not squared...**34**
34   Denticles of pleonite 6 rounded and of pleonite 7 acute ....
        ....***N. borealis* Dahl, 1985**
–   Denticles of pleonites 6 and 7 similar in shape...**35**
35   Denticles of pleonites 6 and 7 distally acute...**36**
–   Denticles of pleonites 6 and 7 rounded..**37**
36   Total length of mature female more than 10 mm, Exopod of pleopod 1 bear-
        ing more than 30 spines on lateral margin ....***N. hessleri* Martin et al., 1996**
–   Total length of mature female less than 5 mm, Exopod of pleopod 1 bearing
        15 or 16 spines on lateral margin ....***N. neocaledoniensis* Ledoyer, 2000**
37   Article 2 of mandibular palp with single thin seta, anal plate with no distinct
        "shoulder"...***N. herbstii* Leach, 1814**
–   Article 2 of mandibular palp with two thin setae, anal plate with distinct
        "shoulder".. ***N. dolsandoensis* Song & Min, 2016**

Acknowledgments

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SHORT COMMUNICATION

The first Elcanidae (Orthoptera, Elcanoidea) from the Daohugou fossil bed of northeastern China

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Abstract

A new species of Elcanidae (Orthoptera, Elcanoidea), *Parelcana pulchmacula* **sp. nov.**, is described based on four new specimens from the Middle Jurassic Jiulongshan Formation of northeastern China. The new species differs from all other known Archelcaninae species by its combination of wing-venation characters. This new finding improves our knowledge of variation on wing venation in elcanid insects and constitutes the first record of Elcanidae from the Daohugou fossil bed (Yanliao Biota) of northeastern China.

Keywords

Archelcaninae, Inner Mongolia, Middle Jurassic, *Parelcana*, systematic paleontology, Yanliao Biota

Introduction

The extinct family Elcanidae is a cryptic group of Orthoptera insects due to their complex anatomical features. The caeliferan-like wing venation, characteristic of this family, groups them close to Caelifera in cladistic analyses (Béthoux and Nel 2002). However, Elcanidae also shares a long, filiform antennae and exerted ovipositors with the suborder Ensifera. The presence of these contrasting anatomical features makes the systematic position of Elcanidae unclear.

The Elcanidae existed from the Upper Triassic to the Cretaceous in Eurasia and America (Handlirsch 1906; Sharov 1968; Martins-Neto 1991; Gorochov et al. 2006; Poinar et al. 2007; Peñalver and Grimaldi 2010; Fang et al. 2015; Fang et al. 2018a, Fang et al. 2018b; Heads et al. 2018; Tian et al. 2019). So far 50 species in 16 genera have been described from compression fossils and ambers. These species have been divided into two subfamilies, the Archelcaninae and the Elcaninae, based on taxonomic characters (Gorochov et al. 2006). Elcanids evolved a unique character among orthopterans, i.e., the presence of various spurs on the distal part of the metatibia. These structures might have been associated with an improved capability to swim (Tian et al. 2019).

The Jurassic elcanids are well known from the UK (Handlirsch 1906; Whalley 1985), Germany (Handlirsch 1906; Ansorge 1996), Kazakhstan (Sharov 1968), and Kyrgyzstan (Sharov 1968). In China, two specimens have been reported, one from the Guangxi Province (from Early Jurassic), and one from the Hebei Province (from Middle Jurassic). Both were attributed to *Elcana reticulata* (Handlirsch, 1939), based on existing highly fragmented forewing sample specimens (Handlirsch 1939; Hong 1983; Lin 1986). Other Orthoptera are commonly discovered from the Daohugou fossil bed, at the widely known and profuse fossil assemblages of Yanliao Biota, northeastern China. Numerous species discovered from this fossil bed have been described, including ensiferans and caeliferans (Ren and Meng 2006; Ren et al. 2010, 2019; Gu et al. 2009, 2011, 2012, 2016; Wang et al. 2017). In this report we describe a new species of Elcanidae, *Parelcana pulchmacula* sp. nov., from the Daohugou fossil bed. The new species is erected based on four isolated but well-preserved forewings, providing new insights into the complex wing-venation patterns of elcanids.

Geological setting

The specimens described here were collected in the Daohugou Bed, located along the boundaries of the provinces of Hebei, Liaoning and Inner Mongolia (Fig. 1). The Daohugou Bed has been previously assigned to the Middle Jurassic Jiulongshan Formation (Ren et al. 2002, 2010, 2019). The Mesozoic section of the Daohugou Bed is mainly composed of tuffaceous conglomerates, tuffaceous siltstones, tuffaceous mudstones, tuffaceous shales, and volcanic breccias. Isotopic radiometric dating of rock samples from the Daohugou area enabled assessment of the age of the Jiulongshan Formation at circa 168–164 million years (Chen et al. 2004; He et al. 2004; Liu et al. 2006; Yang and Li 2008; Chang et al. 2014). This indicates that the age of the Jiulongshan Formation falls within the Bathonian–Callovian boundary interval (Xu et al. 2016).

Materials and methods

The wing specimens were examined with a Nikon SMZ 25 microscope, and photographed with a Nikon DS-Ri 2 digital camera system. Line drawings were prepared using Adobe Illustrator CC 17.0.0 and Adobe Photoshop CC 14.0 graphics software. The measurements were taken using Adobe Illustrator. The lengths of wings were measured
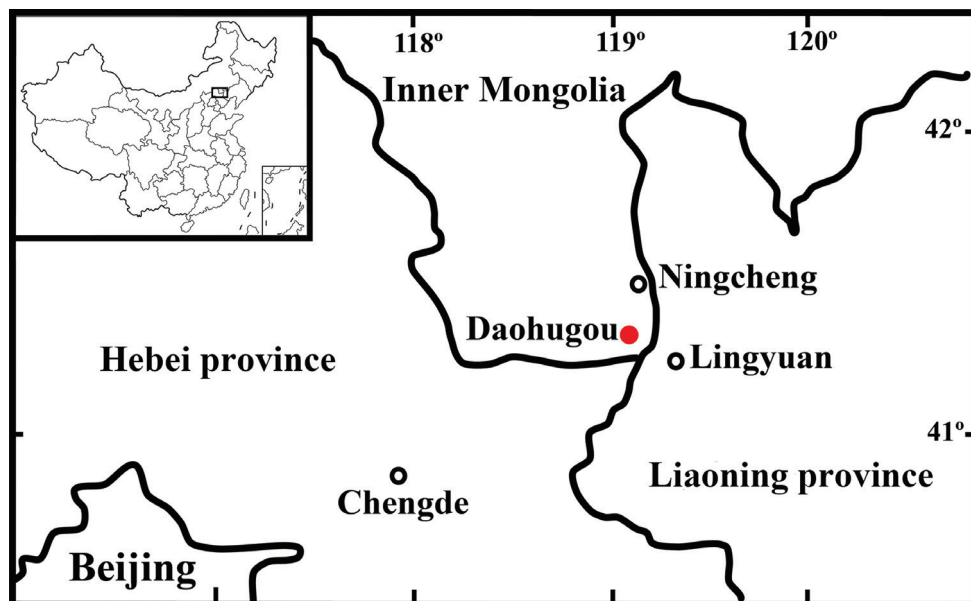
Figure 1. Location map for *Parelcana pulchmacula* sp. nov.

from the apex to the visible base of the wing; the widths of wings refer to the maximum width of the wing. The specimens are deposited in the Key Lab of Insect Evolution & Environmental Changes, Capital Normal University (CNU), Beijing, China.

Wing-venation analyses followed the interpretation proposed by Béthoux and Nel (2001, 2002). Corresponding abbreviations used in taxonomical descriptions are as follows: CP, posterior costa; ScA, ScP, anterior and posterior subcosta, respectively; RA, RP, anterior and posterior radius, respectively; M, media; MA, MP, anterior, posterior media, respectively; CuA, CuP, anterior, posterior cubitus, respectively; CuPaα, the anterior branch of first posterior cubitus; CuPaβ, the posterior branch of first posterior cubitus; CuPb, the second posterior cubitus; AA1, first branch of anterior anal vein.

Systematic paleontology

Class Insecta Linnaeus, 1758
Order Orthoptera Olivier, 1789
Suborder Ensifera Chopard, 1920
Superfamily Elcanoidea Handlirsch, 1906
Family Elcanidae Handlirsch, 1906
Subfamily Archelcaninae Gorochov, Jarzembowski & Coram, 2006

Genus *Parelcana* Handlirsch, 1906

Type species. *Parelcana tenuis* Handlirsch, 1906.

Composition. *Parelcana tenuis* Handlirsch, 1906 (Jurassic, Dobbertin, Germany), *P. anglicana* Handlirsch, 1939 (Jurassic, Binton, UK), probably *P. dubia* Handlirsch, 1939 (Jurassic, Gloucester, UK) and *Parelcana pulchmacula* sp. nov. (Handlirsch 1939; Cigliano et al. 2019).

Revised diagnosis. ScP with numerous branches ending at the anterior margin; M with 3 branches before RP fused with MA1; short CuA; CuPaβ, CuPb, and AA1 detached from each other.

Comments. All species of genus *Parelcana* are based on forewing structure. The forewing of *Parelcana* differs from other genera in Archelcaninae by the presence of 3 branches of M before RP fuses with MA1, and presence a very short and nearly vertical CuA.

Parelcana pulchmacula sp. nov.

http://zoobank.org/8BD8814A-4A53-41C6-8648-00855507D6CF

Fig. 2

Diagnosis. ScP with 6–8 branches ending in anterior margin; CuA very short and fusion with CuPaα before ScA ends in anterior margin, CuA+CuPaα long and S-shaped; occurrence of two big and round dark spots in distal half of wing and one small spot covering the area of CuPa.

Etymology. From the latin 'Pulch-' for beautiful and 'macula' for patches, referring to the beautiful spots and coloration of the forewing.

Type materials. *Holotype*, CNU-ORT-NN2016041; *Paratypes*, CNU-ORT-NN2016035; CNU-ORT-NN2016036; CNU-ORT-NN2016042.

Locality and age. Daohugou Village, Shantou Township, Ningcheng County, Inner Mongolia, China; Jiulongshan Formation, Middle Jurassic.

General description. Forewing 18.4–20.9 mm long and 4.3–5.0 mm wide (maximum width recorded). Costal area long and narrow; CP nearly straight, ending in anterior margin after the forking of M+CuA, generating numerous distinct oblique branches ending in the anterior margin; ScA slightly curved, ending in the anterior margin before 1/3 of total wing length; ScP reaching anterior margin at nearly half-length of wing and generating 6–8 oblique branches ending in the anterior margin; stem R+M+CuA forking into R and M+CuA after the divergence point of CuPa; stem R long and distinctly strong, branched into RA and RP near the mid-length of wing; area between ScP and R narrow; RA slightly curved towards posterior wing margin before its first branch, reaching anterior margin close to apex with 16–18 oblique branches; RP with 10–12 comblike branches reaching wing margin, most of them reaching posterior margin, with several distal terminals dichotomizing and reaching anterior margin; area between RA and RP relatively wide; M forking into MA and MP near to the end of ScA; MA branching into MA1 and MA2 close to the end of ScP; MA1 with 2 branches, with the first fused with RP; MP simple, originates after ScA ends at anterior margin; CuA extremely short, 0.16 to 0.20 mm long, originates before CP ends at anterior margin; CuA almost vertical against the posterior margin; free CuPa short, 0.23 to 0.38 mm long, directed to
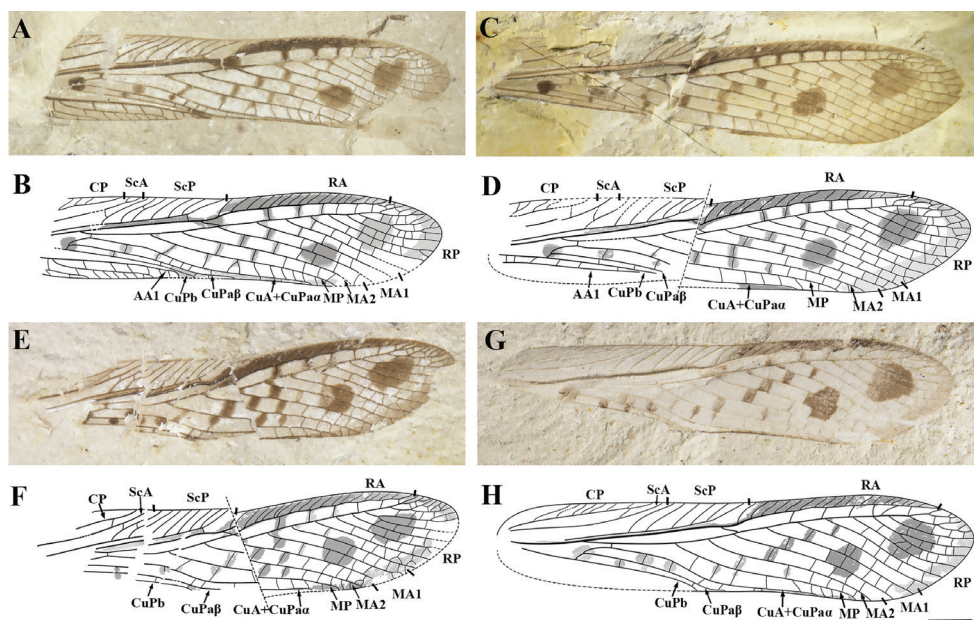
Figure 2. Photo and line drawing of *Parelcana pulchmacula* sp. nov. **A, B** holotype, CNU-ORT-NN2016041 **C, D** CNU-ORT-NN2016035 **E, F** CNU-ORT-NN2016042 **G, H** CNU-ORT-NN2016036. The inclined and dotted lines in the middle of the wings of **D** and **F** represent the cracks in the specimen. The dotted line on the wing venation represents the imaginary line of the wing. Scale bar: 2 mm.

anterior wing margin, forking into CuPaα and CuPaβ before (Fig. 2A–D) or at the level (Fig. 2G, H) of the bifurcation point of M+CuA; free part of CuPaα approximately three times longer than CuPa, then fused with CuA; CuA+CuPaα simple, long and S-shaped, reaching posterior margin at 2/3 of wing length; CuPaβ simple, similar to CuPaα in shape; CuPb simple; areas between CuPaβ–CuPb and CuPb–AA1 narrow; CuPaβ, CuPb, and AA1 detached each other; AA1 strong and straight; area between branches of RP and M covered with simple and straight crossveins. Dark colorations cover the areas between ScP–R and RA–anterior margin, and also along several rows of the crossveins between branches of RP and M; occurrence of two big and round spots in distal half of wing, one located between the branches of RP, one located at the boundary of RP branches and MA1; one small round spot covers the area of CuPa.

Specimen description

CNU-ORT-NN2016041 (Fig. 2A, B). Holotype, forewing nearly complete with only basal and posterior margin partially missing, 18.4 mm long and 4.3 mm wide (the maximum width, the same below). CP with 3 oblique branches preserved; ScA with 2 branches ending in anterior margin; ScP with 7 branches ending in anterior margin; RA with 17 oblique branches; RP with 10 pectinate branches reaching wing margin;

RP fused with anterior branch of MA1 slightly after the ramification point of MA1; CuPaβ reaches the posterior wing margin distally to the end of ScP.

CNU-ORT-NN2016035 (Fig. 2C, D). Paratype, forewing lost anal region and split into two pieces at about mid-length by an oblique crevice, preserved 20.7 mm long and 4.8 mm wide. CP with 3 oblique branches preserved; ScA with 1 branch connected with CP; ScP with 1 branch connected with ScA and 7 branches ending in anterior margin; RA with 16 oblique branches; RP with 12 pectinate branches reaching wing margin; shortly after origination of posterior branch of MA1, RP fused with anterior branch of MA1.

CNU-ORT-NN2016042 (Fig. 2E, F). Paratype, forewing lost basal and anal regions, and an oblique crevice split it into two pieces at about mid-length, remaining part 19.1 mm long and 4.5 mm wide. ScA with 1 branch connected with CP; ScP with 8 branches ending in anterior margin; RA with 18 oblique branches; RP with 12 pectinate branches reaching wing margin; RP fused with anterior branch of MA1 at same level as origination of posterior branch of MA1.

CNU-ORT-NN2016036 (Fig. 2G, H). Paratype, forewing lost anal region, 20.9 mm long and 5 mm wide. CP with 8 oblique branches; ScA with 1 branch connected with CP; ScP with 6 branches ending in anterior margin; RA with 18 oblique branches; RP with 10 pectinate branches reaching wing margin; RP fused with anterior branch of MA1 after the ramification point of MA1; CuPaβ reaches the posterior wing margin basally to the end of ScP.

Discussion

This new species can be assigned to Archelcaninae by its relatively wide area between RA and RP, and free distal part of CuPaβ, CuPb and AA1. Its simple ScA, the presence of 3 branches of M before RP fuses with MA1 and a very short CuA support assignment to the genus *Parelcana*. *Parelcana pulchmacula* sp. nov. shares with *P. tenuis* a short and vertical CuA, but differs from *P. tenuis* in its larger size, greater number of branches of ScP and RP, a long and S-shaped CuA+CuPaα, a wider area between CuPb and anals, and the coloration pattern of the forewing. The new species is notably different from *P. anglicana* in its greater number of branches of ScP, free and vertical CuA, wider area between CuA+CuPaα and anal region, and fusion pattern of CuA and CuPaα. *Parelcana dubia* was erected based on a fragmentary forewing. It differs from *P. pulchmacula* sp. nov. in having a wider area between RA and RP and the branching pattern of RP. *Parelcana pulchmacula* sp. nov. is also different from the other two known Chinese Jurassic elcanids. It is much larger than the specimen from the Early Jurassic with an estimated wing length of approximately 9.5 mm. The other specimen from the Middle Jurassic of Hebei was originally assigned to *Elcana reticulata* based on an isolated forewing with only the distal half. Most of the diagnostic characters were missing, making comparisons with the new species difficult. Based on the line drawing patterns described for the wing (see Hong 1983, fig. 28), it might be an elcanid, but its generic assignment is questionable.
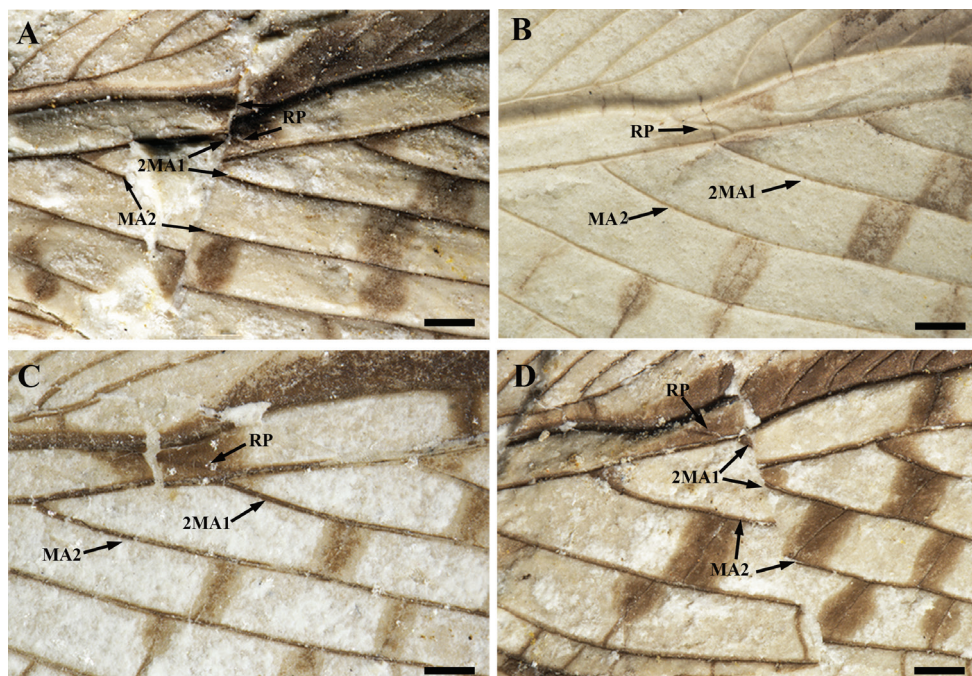
Figure 3. Details of the middle part of the forewings of *Parelcana pulchmacula* sp. nov., showing the variable location of the fusion point of RP and MA1 in the forewing. **A** CNU-ORT-NN2016035 **B** CNU-ORT-NN2016036 **C** holotype, CNU-ORT-NN2016041 **D** CNU-ORT-NN2016042. Scale bar: 0.5 mm.

Variation in forewing size and wing-venation pattern is common in fossil orthopterans and their relatives from the Palaeozoic to the Mesozoic (Prokop and Ren 2007; Gu et al. 2010, 2011, 2014; Béthoux et al. 2012), even between the left and right forewings of the same individual (Gu et al. 2009). For elcanids insects, the documentation on variation of wing venation is scarce since most of the species are described based on limited and often poorly preserved samples. We observed some variation of forewing venation within specimens of *P. pulchmacula* sp. nov.. The number of branches of ScP, RA and RP was not consistent (Fig. 2). Moreover, CuPaβ reaches the posterior wing margin distally to the end of ScP in CNU-ORT-NN2016041(Fig. 2A, B), but basally to the end of ScP in CNU-ORT-NN2016036 (Fig. 2G, H).

Based on the known data of wing venation of fossil orthopterans (Gu et al. 2010, 2011, 2014; Béthoux et al. 2012), the differences found in four *P. pulchmacula* sp. nov. specimens should be considered as variations within a species. The location of the fusion point of RP and MA1 is an important character usually used as a distinctive generic character of Elcaninae (Gorochov et al. 2006). In *P. pulchmacula* sp. nov., three of these fusion points show a clear pattern of M with 3 branches before RP is fused with MA1 (Fig. 3A–C). However in specimen CNU-ORT-NN2016042 (Fig. 3D), 2MA1 branches off at the level of RP when reaching MA1. Although this kind of difference always occurs between different species or genus, the integral similarity of wing venation between the four specimens of *P. pulchmacula* sp. nov. indicates that these specimens belong to the same species.

Acknowledgments

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RESEARCH ARTICLE

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A review of the Indonesian species of the family Signiphoridae (Hymenoptera, Chalcidoidea), with description of three new species

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Citation: Schmidt S, Hamid H, Ubaidillah R, Ward S, Polaszek A (2019) A review of the Indonesian species of the family Signiphoridae (Hymenoptera, Chalcidoidea), with description of three new species. ZooKeys 897: 29–47. https://doi.org/10.3897/zookeys.897.38148

Abstract

The Indonesian species of the family Signiphoridae (Hymenoptera, Chalcidoidea) are revised. Three species of *Chartocerus* are described as new (*Chartocerus kartiniae* Polaszek & Schmidt, **sp. nov.**, *C. sumatrensis* Schmidt & Polaszek, **sp. nov.**, and *C. javensis* Schmidt & Ubaidillah, **sp. nov.**) and four species of *Signiphora*, viz., *S. bennetti* Woolley & Dal Molin, *S. flavella* Girault, *S. perpauca* Girault and *S. bifasciata* Ashmead, are diagnosed.

Introduction

The Signiphoridae are a small family of Chalcidoidea with currently 88 described species in four genera (Noyes 2019). The family has a worldwide distribution, with the majority of species occurring in the tropics. Signiphorids are known primarily

as hyperparasitoids of scale insects, mealybugs, and whiteflies (Hemiptera, Sternorrhyncha) through encyrtid or aphelinid primary parasitoids, while some are obligate primary parasitoids on these sternorrhynchan hosts. Species of *Clytina* Erdös and most of the *Signiphora dipterophaga* species group are primary parasitoids of Diptera puparia (Woolley 1988).

Species of the family Signiphoridae are distinguished from other families of chalcid wasps and can readily be recognised by the following combination of characters: (1) propodeum with a characteristic large triangular median area, (2) antenna with 1 to 4 short, often ring-like flagellomeres, and a long, undivided clava, (3) metasoma broadly attached to the mesosoma, and (4) wings only with marginal setae, the disc bare, occasionally with one large discal seta.

The present revision includes seven species of the family Signiphoridae from Indonesia, three of them described here as new to science, in two genera. The specimens were obtained as part of several capacity building and biodiversity research projects in Indonesia, in particular the IndoBiosSys project that aimed, amongst other goals, at creating inventories of the Indonesian entomofauna using DNA barcoding. Here we present the results for a family of chalcid wasps, the Signiphoridae, that was recorded only recently for the first time in Indonesia, with a single species, *Signiphora bifasciata* Ashmead, in the Bogor Botanic Garden as a parasitoid of the introduced cycad aulacaspis scale (*Aulacaspis yasumatsui* Takagi (Hemiptera, Diaspididae) (Muniappan et al. 2012).

Materials and methods

The specimens were collected during field training courses at the universities in Padang, Sumatra (Andalas University) and Malang, Java (Brawijaya University), as part of capacity building activities for young Indonesian entomologists, and as part of the Indonesian Biodiversity Discovery Project (Cancian de Araujo et al. 2017). The National Park has been recognised as one of the largest remaining tropical rain-forest ecosystems in Java, being designated as a National Park in 2003 with a present area of about 113,357 hectares. During the IndoBioSys Project, Malaise traps and Yellow Pan traps were employed in the Mount Halimun-Salak National Park in West Java. In addition, chalcid wasps were collected using a screen-sweep net (cf. Noyes 1982).

The descriptions of the three new species are based on specimens that were borrowed from the Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences. Data on genetic material contained in this paper and the Barcode of Life Database (BOLD) are published for non-commercial use only, according to the agreements with the providing country of the analysed samples. Use by third parties for purposes other than non-commercial scientific research may infringe the conditions under which the genetic resources were originally accessed and should not be undertaken without obtaining consent from the corresponding author of the paper and/or obtaining permission from the original providers of the genetic material.

DNA sequencing

For DNA studies, whole specimens were sent to the Canadian Centre for DNA Barcoding (CCDB) in Guelph, Canada, for DNA extraction and barcode sequencing, and subsequent recovery of vouchers for preparation and morphological study. DNA extraction, PCR amplification, and sequencing were conducted at the CCDB using standardised high-throughput protocols (Ivanova et al. 2006, deWaard et al. 2008, http://www.ibol-project.org/resources.php). The 658bp target region, starting from the 5' end of the mitochondrial cytochrome c oxidase I (COI) gene, includes the DNA barcode region of the animal kingdom (Hebert et al. 2003). All specimen data are accessible on BOLD through the following doi: dx.doi.org/10.5883/DS-INDOSIG. The specimen data include collecting locality, geographic coordinates, elevation, collector, one or more digital images, identifier, and voucher depository. Sequence data can be obtained through BOLD and include a detailed LIMS report, primer information, and access to trace files. These data are also available through GenBank (Accession nos MH407234–MH407243).

Data analysis

Sequence divergence statistics were calculated using the Kimura two parameter model of sequence evolution (Kimura 1980), as it is commonly applied in the analysis of DNA barcode sequence data because it evaluates the substitution type (i.e., transitions vs transversions) in addition to the number of nucleotide substitutions between sequences. Barcode Index Numbers (BINs) were assigned by the BOLD system, representing globally unique identifiers for clusters of sequences that correspond closely to biological species (Ratnasingham and Hebert 2013). For BIN assignment, a minimum sequence length of 500 bp is required, and sequences between 300 and 500 bp can join an existing BIN but will not create or split BINs. Sequences were aligned using the BOLD Aligner (amino acid-based hidden Markov models). The analyses are based on sequences with a minimum length of 500 bp and <1% ambiguous bases. Genetic distances and summary statistics were calculated using analytical tools in BOLD and are given as mean and maximum pairwise distances for intraspecific variation and as minimum pairwise distances for interspecific variations.

Morphology and imaging

Morphological terminology and the format for species descriptions follow Hayat (2009). Photographs were made using a Leica Ortholux compound microscope with Nomarski Differential Interference Contrast (DIC) illumination and a Leica DM 5000 B with DIC. Images were processed using the stacking software AutoMontage (Synoptics, Cambridge, UK) and Helicon Focus (version 7.0), and further edited using Adobe Photoshop CC 2019. Plates were compiled with Adobe Illustrator CS6.

Acronyms of depositories

MZB   Museum Zoologicum Bogoriense (MZB), Research Center for Biology, Indonesian Institute of Sciences, Cibinong, Indonesia.
NHMUK   Natural History Museum, London, UK
ZSM   SNSB – Zoologische Staatssammlung München, Munich, Germany

Taxonomy

Chartocerus Motschulsky, 1859

Diagnosis. Body dark brown or black, often with metallic luster. Fore tibial spur simple, without comb of setae (cf. Fig. 3H). Propodeum posteriorly without lamelliform process (except in *C. kartiniae* with a suggestion of a lamelliform process on the median sclerite). Female antenna with 4 anelli (except in *C. kartiniae* with 2 anelli), male with 3 anelli. Seta M6 on fore wing marginal vein present, and with additional seta between M2 and M3 (M2b, Fig. 4E). Occipital margin rounded or acute. Mesofemur with 3 or 4 long spines (cf. Fig. 4D).

Chartocerus javensis Schmidt & Ubaidillah, sp. nov.
http://zoobank.org/4BBC4A9C-59D1-4AF0-996D-09931C0F836E
Figs 1A–H, 2A–G

Material examined. *Holotype*. INDONESIA • ♀ (on slide); East Java, Jalan Kandangan, Kasembon; 7.808S, 112.313E; 305 m a.s.l.; 14-Jul-2012; S. Schmidt leg.; screen-sweep net; MZB; specimen ID: BC-ZSM-HYM-20770-C04. *Paratype*. INDONESIA • ♂ (on slide); East Java, Jalan Kandangan, Kasembon; 7.808S, 112.313E; 305 m a.s.l.; 14-Jul-2012; S. Schmidt leg.; screen-sweep net; MZB; specimen ID: BC-ZSM-HYM-20770-C11.

Diagnosis. Head and body dark brown (Fig. 1A, B), fore wing behind marginal vein with infuscation except hyaline area near posterior margin (Fig. 1F). Antenna (in female) with 4 anelli (Fig. 1H). Clava elongate, about 6.7 times as long as broad and 1.5 times as long as scape length (Fig. 1E). Fore wing marginal fringe slightly longer than half the length of the wing disc (Fig. 1F). Midtibial spur subequal in length to corresponding basitarsus (Fig. 1C).

Description (female holotype). *Colour*. Head and body dark brown (Fig. 1A, B), legs brown with tarsi pale (Fig. 1A), antenna brown (Fig. 1E). Fore wing with brown infuscation behind marginal vein, leaving a hyaline area posteriorly in basal half of the infuscation (Fig. 1F), and a brown longitudinal stripe in basal cell.

Morphology. Head 1.78 times as broad as frontovertex width (Fig. 1B), frontovertex width slightly less than length of clava (0.84), distance from mouth margin to facial

Figure 1. *Chartocerus javensis* Schmidt & Ubaidillah, sp. nov., female holotype **A** body in dorsal view **B** head in frontal view **C** middle leg **D** apex of middle femur **E** antenna **F** fore wing **G** hind wing **H** pedicel and flagellum base.
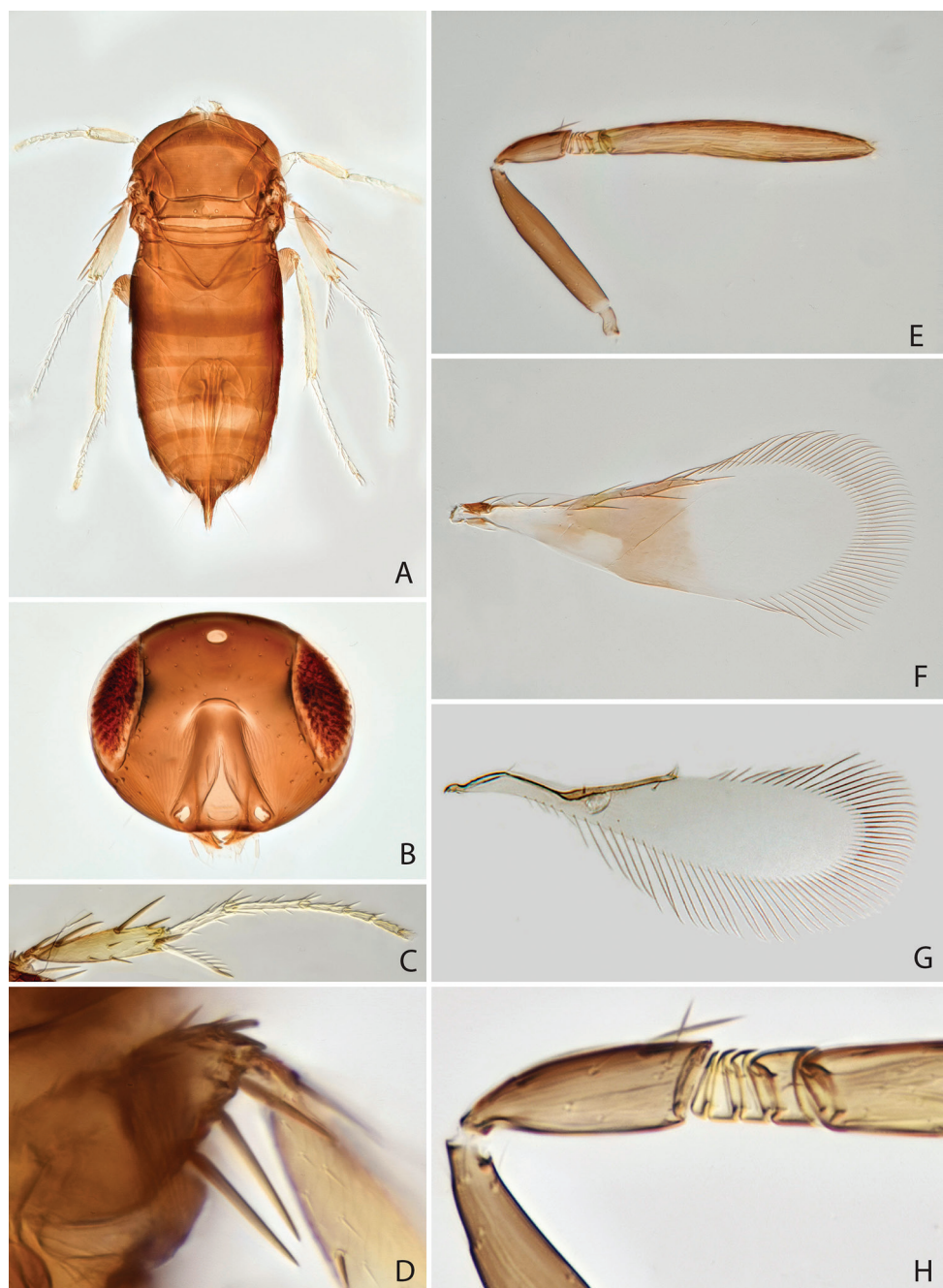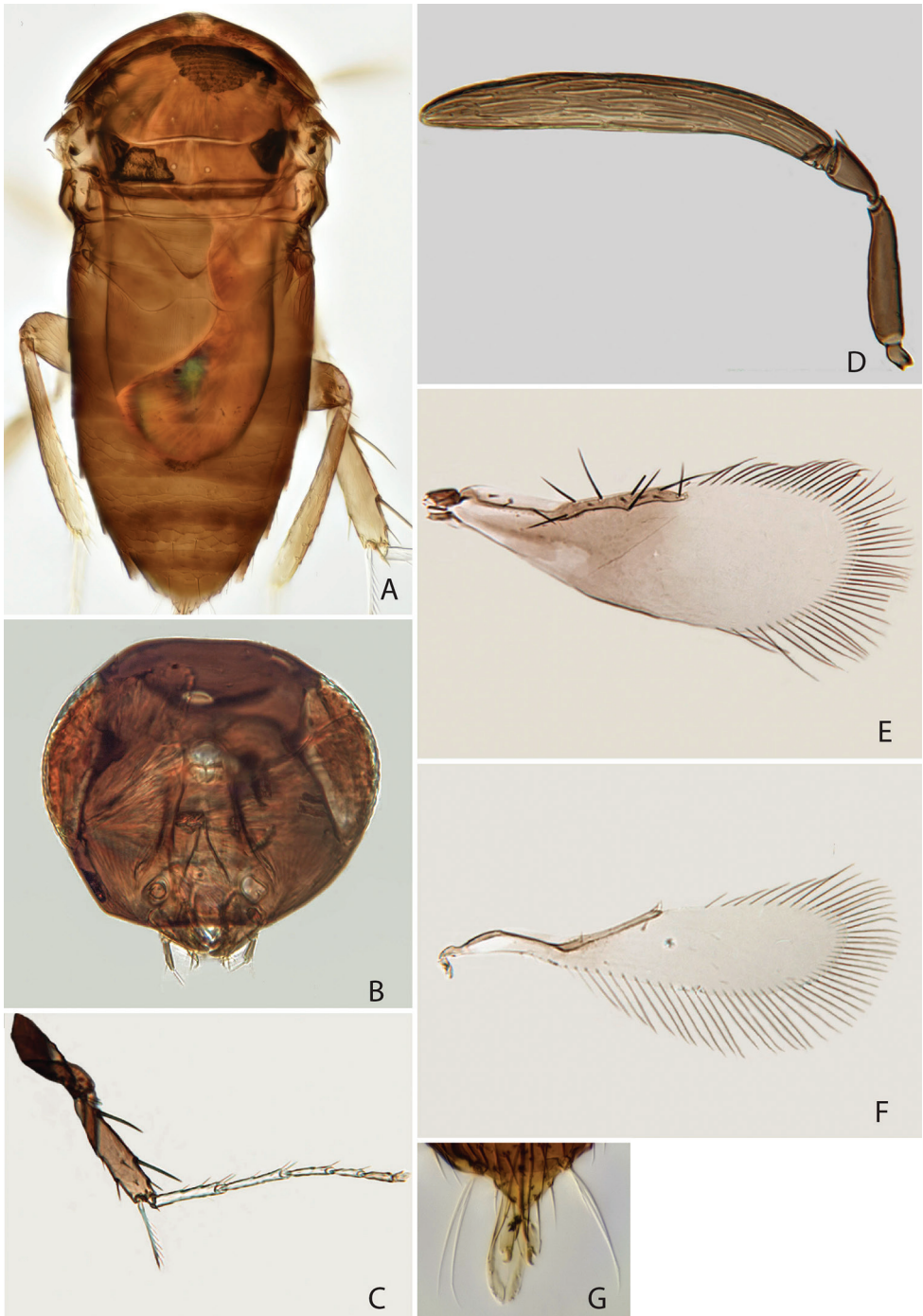
Figure 2. *Chartocerus javensis* Schmidt & Ubaidillah, sp. nov., male paratype **A** body in dorsal view **B** head in frontal view **C** middle leg **D** antenna **E** fore wing **F** hind wing **G** genitalia.

ridge 0.61 times head height. Antenna with 4 anelli, F4 longer than each of the preceding segments, clava 6.7 times as long as broad and 1.51 times as long as scape length, pedicel 0.45 times as long as scape length (Fig. 1E). Midlobe of mesoscutum with 4 setae in anterior half and 6 setae in posterior half, scutellum with 7 setae along posterior margin. Fore wing 2.7 times as long as broad (Fig. 1F), longest setae of marginal fringe 0.56 times as long as width of disc, seta M1 absent, M2b present. Mesofemur ventrally with 3 long spines (Fig. 4D). Midtibial spur subequal in length to corresponding basitarsus (Fig. 1C), the latter 0.55 times as long as midtibia. Ovipositor nearly twice as long as midtibia (1.82) and 1.29 times as long as hind tibia.

Male. Colour and structure similar to female (Fig. 2A, B), but antenna (Fig. 2D) with 3 anelli and longer, clava 7.28 times as long as broad and 2.76 times as long as scape (Fig. 2D). Setae of fore wing (Fig. 2E) stouter than in female. Genitalia as in Fig. 2G, phallobase with a pair of setae, digitus about 3 times as long as broad, strongly curved at apex.

Host. Unknown.

Distribution. Only known from the type locality near Malang, East Java, Indonesia.

Etymology. The species is named after the island of Java.

DNA barcode data. The species exhibits an intraspecific variation of 0.2% and a distance of 12.2% to the nearest neighbour species, *C. sumatrensis* sp. nov. (Suppl. material 1, Genbank Accession Numbers: MH407241, MH407242).

Remarks. The species is similar to *Chartocerus kerrichi* (Agarwal) and *C. sumatrensis* sp. nov. but can be distinguished from the former by the longer clava (6.7 times as long as broad, compared to 4.5 times in *kerrichi*), the longer fore wing marginal fringe (0.56 times as long as width of disc compared to 0.36–0.40 times in *kerrichi*), and the longer midtibial spur (subequal in length to corresponding basitarsus whereas distinctly shorter in *kerrichi*). From *C. sumatrensis* sp. nov. it can be separated by the shorter ovipositor (less than 2.0 times as long as midtibia, compared to 2.9 times in *sumatrensis*) and the longer midtibial spur (subequal in length to corresponding basitarsus, compared to 0.7 times in *sumatrensis*).

Chartocerus kartiniae Polaszek & Schmidt, sp. nov.

http://zoobank.org/FB55530A-5082-40D9-81B8-795DB3F28C44

Fig. 3A–I

Material examined. *Holotype.* INDONESIA • ♀ (on slide); West Java, Mount Halimun-Salak National Park, waterfall; 6.71250S, 106.52305E; 1100 m a.s.l.; 18-Sept-2015; A. Polaszek leg.; screen-sweep net; MZB; specimens ID: DNA 1317.

Diagnosis. *Chartocerus kartiniae* sp. nov. is unique among Signiphoridae and is provisionally placed in *Chartocerus* on a balance of genus-level characters. The female can be quickly diagnosed among all *Chartocerus* species by the following combination of characters: metanotum pale (Fig. 3A); fore wing with discal seta (Fig. 3C); antennal funicle 2-segmented (Fig. 3I).
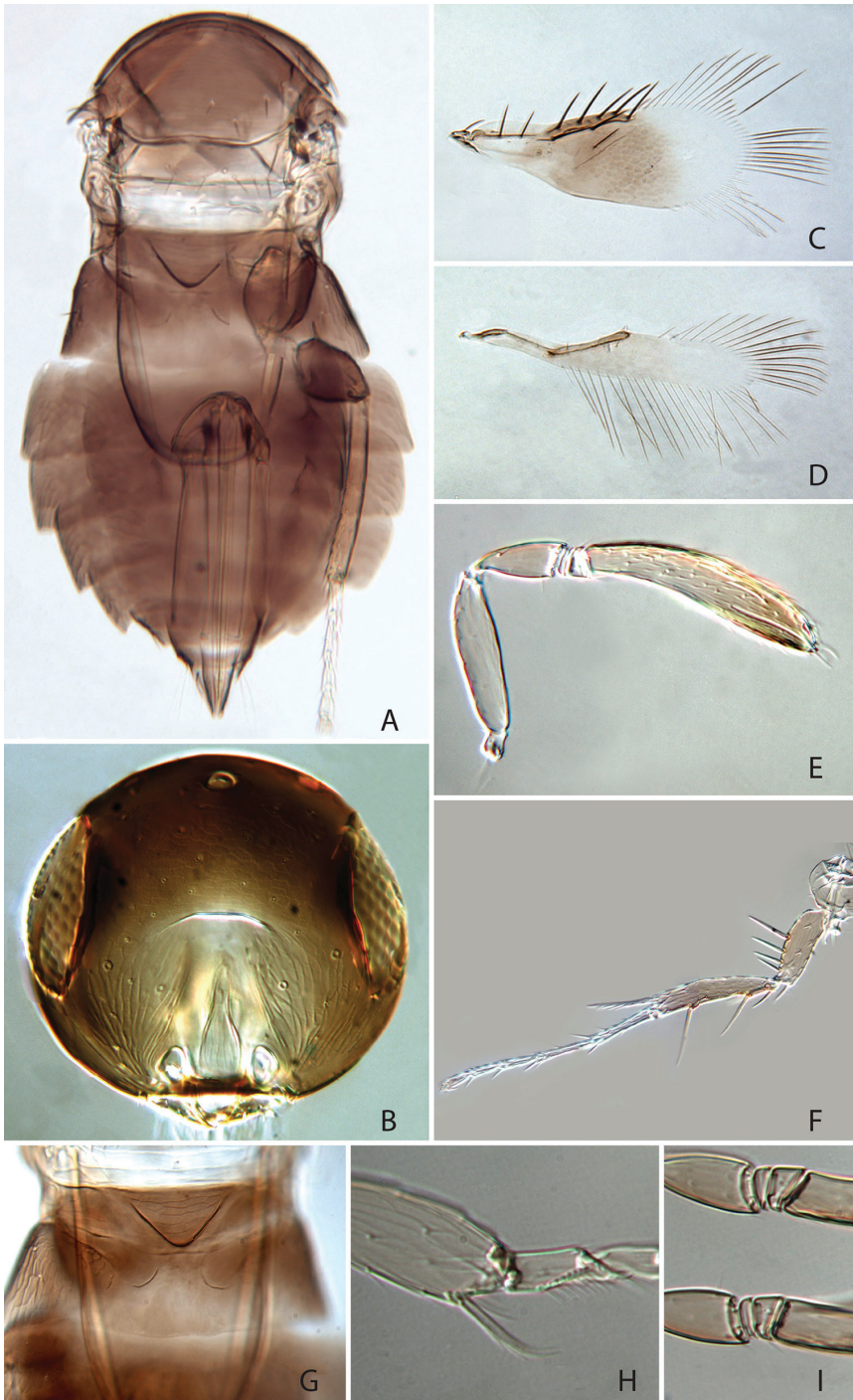
Figure 3. *Chartocerus kartiniae* Polaszek & Schmidt, sp. nov., female holotype **A** body in dorsal view **B** head in frontal view **C** fore wing **D** hind wing **E** antenna **F** middle leg **G** posterior mesosoma and anterior metasoma **H** apex of fore tibia with tibial spur **I** flagellum base.

Description (female holotype). *Colour*. Head and body dark brown (Fig. 3A, B), with metanotum distinctly pale in contrast, legs pale brown with fore tibiae and all tarsi pale yellow (Fig. 3F), antenna yellow-brown with radicle and scape paler (Fig. 3E). Fore wing with brown infuscation centrally and on marginal vein (Fig. 3C).

Morphology. Head 1.52 times as broad as frontovertex width (Fig. 3B), frontovertex width 0.87 times length of clava. Antenna with only 5 antennomeres (excluding radicle); antenna with 2 anelli, clava 5.5 times as long as broad and 1.7 times as long as scape length, pedicel about 0.5 times as long as scape length (Fig. 3E). Midlobe of mesoscutum with 7 setae in posterior half, scutellum with 7 setae along posterior margin. Propodeum with a suggestion of a lamelliform process on the median sclerite. Fore wing 2.8 times as long as broad (Fig. 3C), longest setae of marginal fringe 0.78 times as long as width of disc, all marginal vein setae present, stigmal seta long; discal seta present. Foretibial spur bifid, without a comb (Fig. 3I). Midtibial spur 0.9 times as long as corresponding basitarsus (Fig. 3F), the latter 0.57 times as long as mid tibia. Mesofemur with four long spines anteroventrally. Ovipositor 2.0 times as long as mid tibia and 1.5 times as long as hind tibia.

Host. Unknown.

Distribution. Known only from the type locality at Mount Halimun, West Java, Indonesia.

Etymology. The species is named after Raden Adjeng Kartini.

DNA barcode data. No sequence data were available for analysis.

Remarks. This species is unique for the genus, having only two funicle antennomeres, and a prominent discal seta on the fore wing. The pale metanotum is unusual (or possibly unique) among known species of *Chartocerus* (J.B. Woolley, pers. comm.). The foretibial spur is bifid and without a comb, supporting placement of the species in the genus *Chartocerus.* A full DNA barcode sequence could not be obtained for the single specimen, but analysis of an available DNA fragment suggests membership of the *Chartocerus* lineage (distant from *Signiphora* and *Thysanus* Walker), and a possible sister-group relationship to remaining *Chartocerus* for which DNA data are available.

Chartocerus sumatrensis **Schmidt & Polaszek, sp. nov.**
http://zoobank.org/D19FF62E-3F71-4DFB-A9B7-305C298CC31A
Fig. 4A–F

Material examined. *Holotype*. INDONESIA • ♀ (on slide); West Sumatra, Padang, Universitas Andalas campus; 0.9043S, 100.4802E; 500 m a.s.l.; 17-Jun-2012; S. Schmidt leg.; screen-sweep net; MZB; specimens ID: BC-ZSM-HYM-05406-H10.

Diagnosis. Head and body dark brown (Fig. 4A, B), fore wing basally with brown infuscation (Fig. 4E). Antenna (in female) with 4 anelli (Fig. 4H). Clava elongate, about 6 times as long as broad and 1.5 times as long as scape length (Fig. 4G). Fore

Figure 4. *Chartocerus sumatrensis* Schmidt & Polaszek, sp. nov., female holotype **A** body in dorsal view **B** head in frontal view **C** middle leg **D** apex of middle femur **E** fore wing **F** hind wing **G** antenna **H** pedicel and flagellum base.

wing 2.9 times as long as broad, anteriorly with 4 setae, posterior margin of hind wing disc slightly rounded.

Description (female holotype). *Colour.* Head dark brown (Fig. 4A, B), legs brown with tarsi pale yellow (Fig. 4C), antenna brown with radicle and basal 3 funicle antennomeres pale yellow (Fig. 4G). Fore wing basally with brown infuscation, and a darker patch behind distal part of submarginal vein and proximal part of marginal vein, and longitudinal dark patch along basal part of hind margin (Fig. 4E).

Morphology. Head 1.69 times as broad as frontovertex width (Fig. 4B), frontovertex width subequal to length of clava, distance from mouth margin to facial ridge 0.66. Antenna with 4 anelli, increasing in size towards apex (Fig. 4H), clava about 6 times as long as broad and 1.5 times as long as scape length, pedicel about 0.5 times as long as scape (Fig. 4G). Midlobe of mesoscutum with 11 setae in posterior half, scutellum with 8 setae along posterior margin. Fore wing 2.9 times as long as broad (Fig. 4E), longest setae of marginal fringe 0.38 times as long as width of disc, seta M1 absent, M2b present (Fig. 4E). Hind margin of hind wing disc slightly rounded and nearly parallel to anterior margin (Fig. 4F). Mesofemur ventrally with 3 long spines (Fig. 4D). Midtibial spur 0.7 times as long as corresponding basitarsus (Fig. 4C), the latter 0.53 times as long as midtibia. Ovipositor 2.9 times as long as midtibia and 2.0 times as long as hind tibia.

Host. Unknown.

Distribution. Only known from the type locality near Padang in West Sumatra.

Etymology. The species is named after the island of Sumatra.

DNA barcode data. A single, full-length barcode sequence of the species is separated from its nearest neighbour species, *C. javensis* sp. nov., by 12.2% (Suppl. material 1, Genbank Accession Number: MH407238).

Remarks. The species is similar to *Chartocerus kerrichi* and *C. javensis* sp. nov., but can be distinguished from the former by the longer ovipositor (2.9 times as long as midtibia, compared to 2 times in *kerrichi*) and the more slender clava (about 6 times as long as broad, compared to 4.5 times in *kerrichi*). From *C. javensis* sp. nov. it can be separated by the longer ovipositor (2.9 times as long as midtibia, compared to less than 2 times in *javensis*) and the shorter midtibial spur (0.7 times as long as corresponding basitarsus, compared to subequal in *javensis*).

Signiphora Ashmead, 1880

Diagnosis. Colour variable, pale yellow to completely dark brown or black. Occipital margin acute. Antenna with 3, rarely 1–2 or 4 anelli. Mesoscutum from bare to setose with up to 100 setae. Propodeum posteriorly with lamelliform process. Fore tibial spur with a comb of setae. Fore wing submarginal vein with one or two setae, marginal vein dorsally with (4–)6 setae. Mesofemur with 1 or 2 bristles.

Signiphora bennetti **Woolley & Dal Molin**
Fig. 5A–F

Signiphora bennetti Woolley & Dal Molin, 2017: 19–23.

Material examined. Indonesia • 4 ♀ (on slide); West Java, Mount Halimun-Salak National Park, waterfall; 6.71250S, 106.52305E; 1100 m a.s.l.; 18-Sept-2015; A. Polaszek leg.; Yellow Pan trap; MZB DNA 1321; ZSM DNA 1318; NHMUK DNA 1319-20.

Diagnosis (female). Female with pale band from distal mesoscutum to the proximal propodeal triangle. Antenna with 3 anelli (Fig. 5E). Marginal vein dorsally with 3 setae, seta M1 missing (Fig. 5C), usually with minute seta distal to M2.

Description (female). *Colour*. Head and body dark brown (Fig. 5A, B), with distal half of mesoscutum, scutellum, metanotum and anterior part of propodeal triangle distinctly pale in contrast; legs pale with hind femora brown (Fig. 5A), antenna yellow-brown with clava darker, increasingly so distally (Fig. 5E). Fore wing with brown infuscation centrally and on marginal vein (Fig. 5C).

Morphology. Head 1.5 times as broad as frontovertex width (Fig. 5B), frontovertex width 1.04 times length of clava. Antenna with 6 antennomeres and with 3 anelli, clava 4.6 times as long as broad and 1.7 times as long as scape length, pedicel 0.6 times as long as scape length (Fig. 5E). Midlobe of mesoscutum with 2 fine setae in posterior half, scutellum with 4 fine setae along posterior margin. Fore wing 3.5 times as long as broad (Fig. 5C), longest setae of marginal fringe 1.52 times as long as width of disc, marginal vein seta M1 absent, M5 absent in some paratypes; stigmal seta long (Fig. 5C), vestigial seta base/socket present between M5 and M6 on lower edge of marginal vein, discal seta absent (Fig. 5C). Midtibial spur 0.84 times as long as corresponding basitarsus (Fig. 5F), the latter 0.46 times as long as midtibia. Ovipositor 1.8 times as long as midtibia and 1.4 times as long as hind tibia.

Host. A primary parasitoid, commonly associated with *Melanaspis smilacis* (Comstock) (Hemiptera, Diaspididae) in the New World, a species also widely distributed in SE Asia. Also recorded from *Hemiberlesia oxycoccus* (Woglum); *Melanaspis obscura* (Comstock); *Pseudaulacaspis pentagona* Targioni Tozzetti; *Comstockaspis perniciosa* (Comstock) and *Aspidiella sacchari* (Comstock) (Woolley and Dal Molin 2017).

Distribution. Very widespread in the New World (Woolley and Dal Molin 2017).

DNA barcode data. The species exhibits an intraspecific variation of 0.2% and a distance of 12.5% to the nearest neighbour species, *S. flavella* (Suppl. material 1, Genbank Accession Numbers: MN520843, MN520844).

Remarks. The species belongs to the *Signiphora flavopalliata* Ashmead species group and can be separated from other species in the group by the central dorsal pale band extending from the distal mesoscutum to the proximal propodeal triangle (female), absence of M1 from the marginal vein, and the presence of a minute anterior seta on the marginal vein distal to M2 (fig. 38 in Woolley and Dal Molin 2017), though this seta is not present in every specimen.
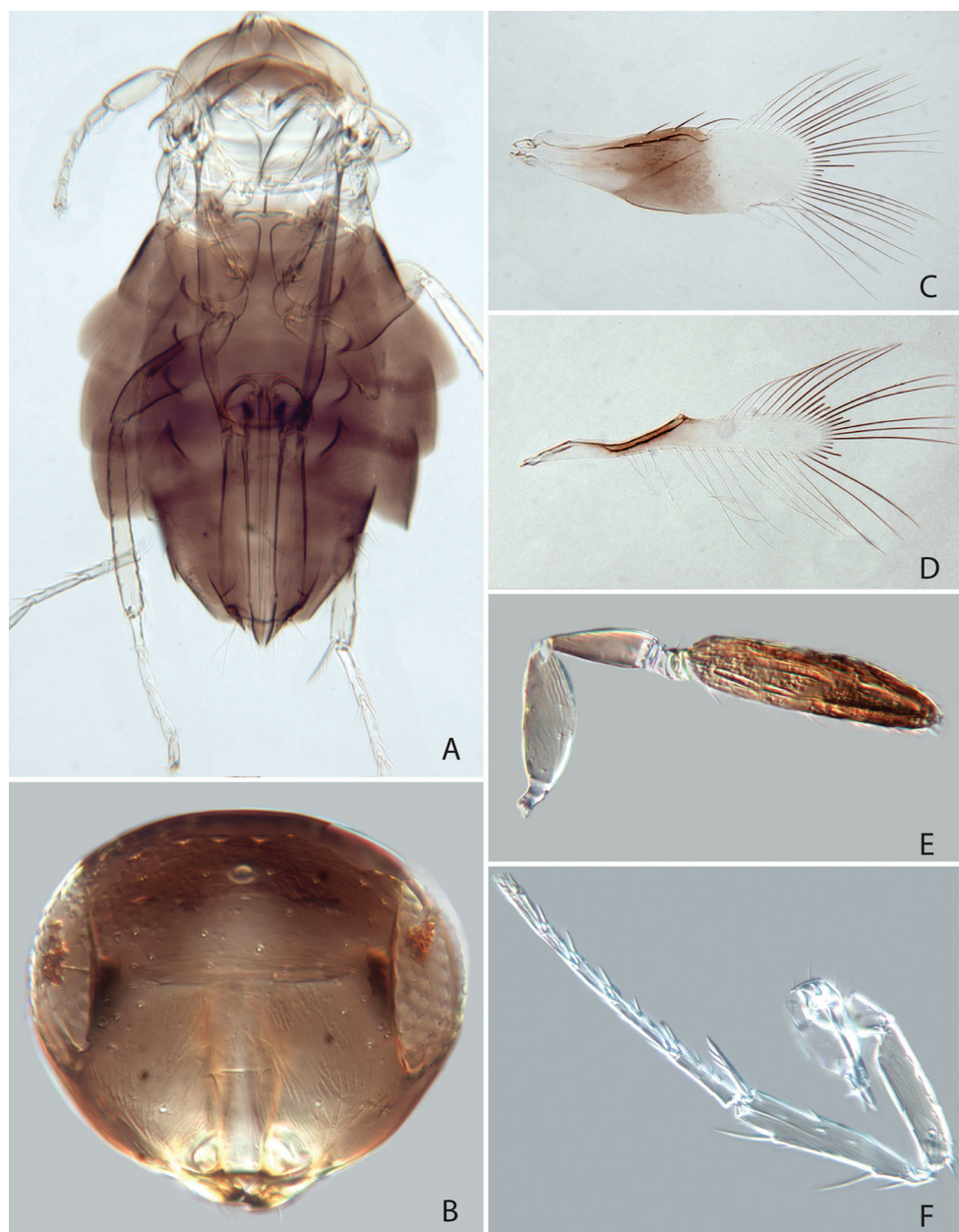
Figure 5. *Signiphora bennetti* Woolley & Dal Molin, female **A** body in dorsal view **B** head in frontal view **C** fore wing **D** hind wing **E** antenna **F** middle leg.

Signiphora flavella Girault
Fig. 6A–F

Signiphora flavella Girault, 1913: 214. Female. For a full list of synonyms see Woolley
    and Dal Molin (2017).

Material examined. Indonesia: • 4 ♀ (on slide); East Java, Malang, Selorejo, Junrejo;
7.940S, 112.529E; 1015 m a.s.l.; 1-Jul-2012; S. Schmidt leg.; screen-sweep net; MZB BC-
ZSM-HYM-20770-G02, BC-ZSM-HYM-20770-E12, BC-ZSM-HYM-20770-G04
(slide with right fore wing only), BC-ZSM-HYM-20770-F12 • 2 ♀; same data as preced-
ing; ZSM BC-ZSM-HYM-20770-F05, BC-ZSM-HYM-20770-F09 • 1 ♀; same data
as preceding; NHMUK BC-ZSM-HYM-20770-F10 • 2 ♀; same locality; 8-Jul-2012;
S. Schmidt leg.; screen-sweep net; MZB • 1 ♀; East Java, Jalan Kandangan, Kasembon;
7.808S, 112.313E; 305 m a.s.l.; 14-Jul-2012; S. Schmidt leg.; screen-sweep net; NHMUK.
    Diagnosis (female). Colour of body yellow (Fig. 6A) with variable extent of
brown markings, clava only apically or completely dusky brown (Fig. 6D). Discal seta
on fore wing missing, seta M1 on marginal vein present (rarely absent) (Fig. 6E). Mt8
in female transverse, without a medial emargination.
    Description (female). *Colour.* Body yellow (Fig. 6A), head yellow except occiput
with curved brown band along occipital carina (Fig. 6B). Antenna with scape yellow
and flagellum brown, apical half of clava darker brown (Fig. 6D). Fore wing with dark
band behind marginal vein that is proximally and distally diffusely delimited (Fig. 6E).
Third valvula brown.
    Morphology. Head 1.76 times as broad as frontovertex width (Fig. 6B), frontover-
tex width 0.77 times length of clava, distance from mouth margin to facial ridge 0.57.
Antenna with 3 anelli, F1 0.4 times as long as broad, F2 0.8 times as long as broad, F3
subquadrate, clava about 6 times as long as broad and 1.7 times as long as scape length
(Fig. 6D), pedicel 0.77 times as long as scape length. Midlobe of mesoscutum with 2
setae on disc and anteriorly with 6 setae, scutellum with 6 setae along posterior margin.
Fore wing 2.9 times as long as broad (Fig. 6E), longest setae of marginal fringe 1.4
times as long as width of disc, seta M1 present, M2b absent. Midtibial spur subequal in
length to corresponding basitarsus (Fig. 6C), the latter 0.56 times as long as midtibia.
Ovipositor 2.1 times as long as midtibia and 1.4 times as long as hind tibia.
    Male. The species reproduces primarily parthenogenetically; males are very rare
(Woolley and Dal Molin 2017) and were not recorded in the study area.
    Host. Polyphagous on many species of Diaspididae (Hemiptera). For a full list of
host records see Woolley and Dal Molin (2017).
    Distribution. Cosmopolitan and, apart from Indonesia, occurring in the follow-
ing countries (after Woolley and Dal Molin 2017): Algeria, Argentina, Australia, Bra-
zil, Chile, Honduras, Honduras, Greece, India, Israel, Mexico, Morocco, New Zea-
land, Peru, Puerto Rico, South Africa, Spain, Trinidad and Tobago, USA, Venezuela.
    DNA barcode data. The sequence data did not reveal any intraspecific variation and a
distance of 9.7% to the nearest neighbour species, *S. perpauca* (Suppl. material 1, Genbank
Accession Numbers: MH407235, MH407237, MH407239, MH407240, MH407243).

Figure 6. *Signiphora flavella* Girault, female **A** body in dorsal view **B** head in frontal view **C** middle leg **D** antenna **E** fore wing **F** hind wing.

Remarks. The species exhibits variation in colour patterns and several similar looking species that had been described mainly based on colour differences were synonymised with *S. flavella* by Woolley and Dal Molin (2017). It resembles *S. perpauca* (see below) but lacks the discal seta in the fore wing.

Signiphora perpauca Girault
Fig. 7A–F

Signiphora perpauca Girault, 1915: 71. Female.
Signiphora woolleyi Hayat: Woolley and Dal Molin (2017).

Material examined. Indonesia • 1 ♀ (on slide); West Java, Mount Halimun-Salak National Park, Sukamantri; 6.682S, 106.751E; 1007 m a.s.l.; 30-Sep-2015; MZB INDOBIOSYS-CCDB25943-H05.
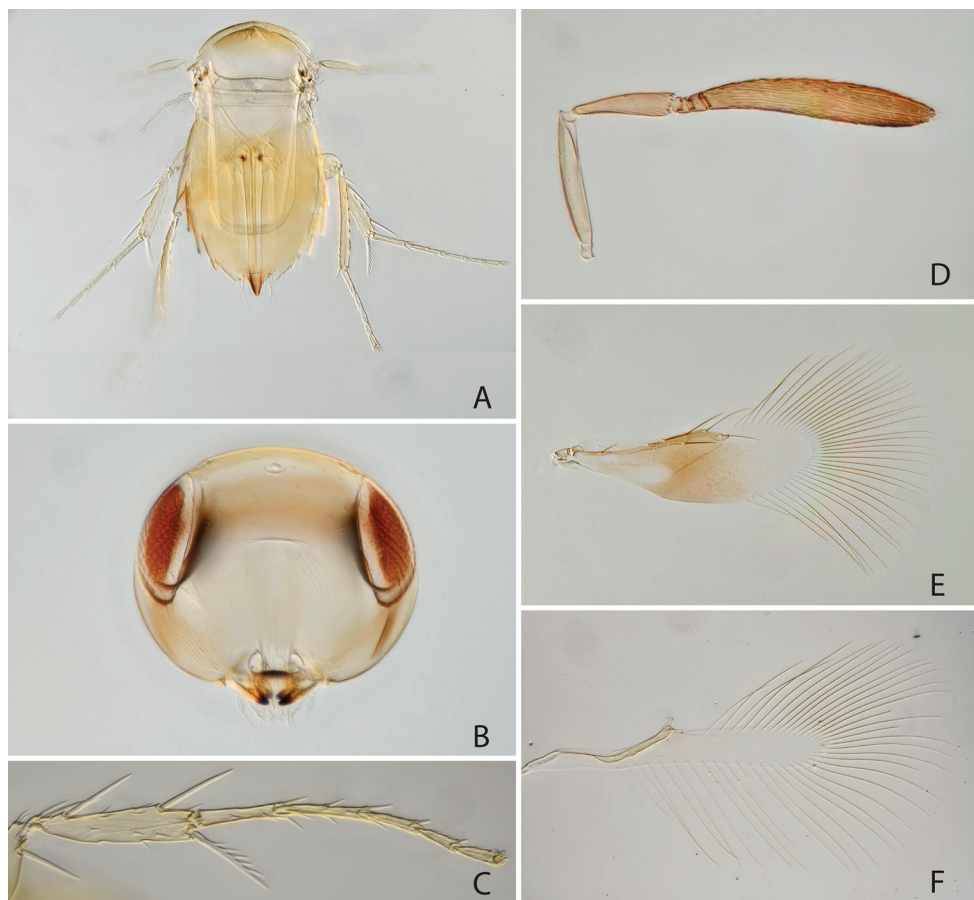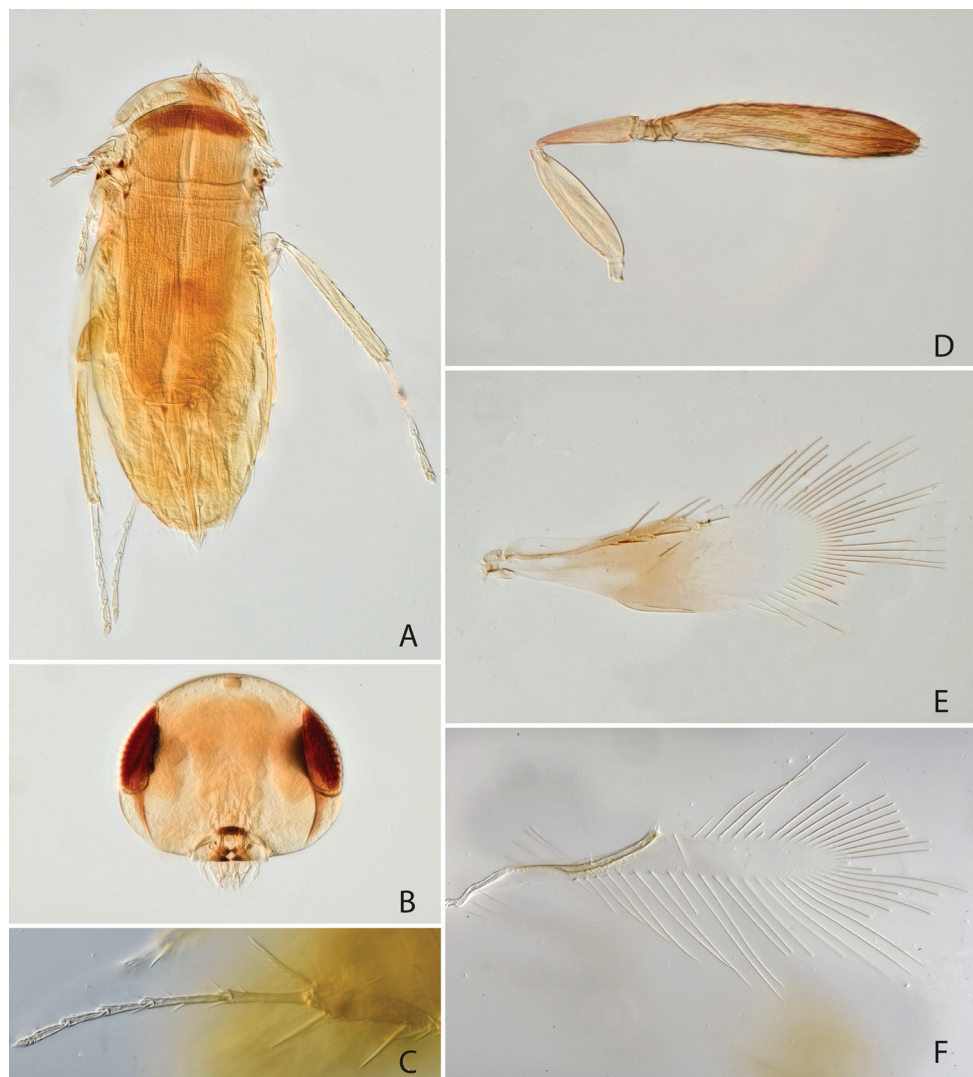
Figure 7. *Signiphora perpauca* Girault, female **A** body in dorsal view **B** head in frontal view **C** middle leg **D** antenna **E** fore wing **F** hind wing.

Description (female). *Colour.* Body yellow (Fig. 7A), head yellow except occiput with curved brown band along occipital carina (Fig. 7B). Antenna with scape yellow and flagellum brown, apical half of clava distinctly darker (Fig. 7D). Fore wing with dark band behind marginal vein that is proximally and distally diffusely delimited (Fig. 7E), discal seta present. Mt8 in female transverse, without a medial incision.

Morphology. Head 1.76 times as broad as frontovertex width (Fig. 7B), frontovertex width 0.77 times length of clava, distance from mouth margin to facial ridge 0.57 times head height. Antenna with 3 anelli (Fig. 4D), F1 0.4 times as long as broad, F2 0.8 times as long as broad, F3 subquadrate, clava about 6 times as long as broad and 1.7 times as long as scape length (Fig. 7D), pedicel 0.77 times as long as scape length. Midlobe of

mesoscutum with 2 setae on disc and anteriorly with 6 setae, scutellum with 6 setae along posterior margin. Fore wing 2.9 times as long as broad (Fig. 4E), longest setae of marginal fringe 1.4 times as long as width of disc, seta M1 present, M2b absent. Midtibial spur subequal in length to corresponding basitarsus (Fig. 4C), the latter 0.56 times as long as midtibia. Ovipositor 2.1 times as long as midtibia and 1.4 times as long as hind tibia.

Host. Diaspididae (Hemiptera, Sternorrhyncha).

Distribution. Widely distributed in the tropics and, apart from Indonesia, recorded from the following countries (after Woolley and Dal Molin 2017): Argentina, Cuba, Brazil, Chile, Egypt, French Polynesia, Haiti, India, Mexico, Panama, Papua New Guinea, Peru, Trinidad and Tobago, South Africa, Taiwan, Thailand, USA.

DNA barcode data. The sequence of the single examined specimen is separated by 9.7% from the nearest neighbour species, *S. flavella* (Suppl. material 1, Genbank Accession Number: MH407234).

Remarks. The species belongs to the *Signiphora flavopalliata* species group and can be separated from other species in the group, among other characters, by its light colour (female) and the presence of a discal seta in the fore wing (fig. 306 in Woolley and Dal Molin 2017).

Signiphora bifasciata Ashmead

Signiphora bifasciata Ashmead, 1900: 411. Female.
Signiphora platensis Bréthes: Woolley (1988).

Diagnosis and remarks. The Neotropical species was recorded as a parasitoid of the cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi (Hemiptera, Diaspididae) in the Bogor Botanic Gardens in 2011 (Muniappan et al. 2012). This represents the first record of the family Signiphoridae from Indonesia. The species can be separated from other *Signiphora* species by the following characters (see Muniappan et al. 2012 and figures therein): discal seta present on both fore wing and hind wing, fore wing infuscate from the wing base to the distal end of the stigmal vein, submarginal vein with 2 setae, marginal vein of the hind wing with 1 seta, female antenna with 2–3 annelli, marginal fringe of fore wing very long, nearly half as long as width of disc, mesosoma brown except scutellum and metanotum which are pale tan, yellow or white, mesoscutum with about 16 setae, scutellum with 6 setae.

Acknowledgments

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Supplementary material 1

BOLD TaxonID Tree
Authors: Stefan Schmidt, Hasmiandy Hamid, Rosichon Ubaidillah, Samantha Ward, Andrew Polaszek
Data type: species data
Link: https://doi.org/10.3897/zookeys.897.38148.suppl1

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The Hydradephaga (Coleoptera, Haliplidae, Gyrinidae, and Dytiscidae) fauna of Cape Breton Island, Nova Scotia, Canada: new records, distributions, and faunal composition

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Citation: Alarie Y (2019) The Hydradephaga (Coleoptera, Haliplidae, Gyrinidae, and Dytiscidae) fauna of Cape Breton Island, Nova Scotia, Canada: new records, distributions, and faunal composition. ZooKeys 897: 49–66. https://doi.org/10.3897/zookeys.897.46344

Abstract

The Haliplidae, Gyrinidae, and Dytiscidae (Coleoptera) of Cape Breton Island, Nova Scotia, Canada were surveyed during the years 2006–2007. A total of 2027 individuals from 85 species was collected from 94 different localities, which brings to 87 the number of species recorded for this locality. Among these, *Heterosternuta allegheniana* (Matta & Wolfe), *H. wickhami* (Zaitzev), *Hydroporus appalachius* Sherman, *H. gossei* Larson & Roughley, *H. nigellus* Mannerheim, *H. puberulus* LeConte, *Ilybius picipes* (Kirby), and *I. wasastjernae* (C.R. Sahlberg) are reported for the first time in Nova Scotia. The Nearctic component of the fauna is made up of 71 species (81.6%), the Holarctic component of 16 species (18.4%). Most species are characteristic of both the Boreal and Atlantic Maritime Ecozones and have a transcontinental distribution but 19 species (21.8%), which are generally recognized as species with eastern affinities. In an examination of the Hydradephaga of insular portions of Atlantic Canada, it was shown that the island faunas of Cape Breton Island and Prince Edward Island are very similar (87 and 84 species, respectively) despite differences in composition suggesting that more Hydradephaga species have yet to be found on Cape Breton Island.

Keywords

biodiversity, faunistic, Hydradephaga, Maritime Ecozone

Introduction

Cape Breton Island is a large (10,311 km^2) rugged and irregularly shaped island, approximately 175 km long by 135 km at is widest, located at 46 degrees latitude, 60 degrees longitude in northern Nova Scotia, Canada at the eastern extremity of the Gulf of St. Lawrence. It lies within the Atlantic Maritime Ecozone along with Québec's Gaspé Peninsula, Magdalen Islands Archipelago and portions of the south shore of the St. Lawrence River. The climate of this ecozone is strongly influenced by the Atlantic Ocean, which produces cooler summers (average 14 °C) and warmer winters (average -5 °C), with coastal areas having slightly warmer winters and cooler summers than inland. Geologically, this region is a mix of sedimentary and igneous bedrock (Alarie 2016). Cape Breton land mass slopes upward from south to north, culminating in the massive highlands of its northern cape, the highest elevation in the Atlantic region.

Water beetles make up a large part of aquatic invertebrates (Jäch and Balke 2008) and as such they play a vital role in terms of biodiversity and ecosystem functioning, and consequently in the stability of ecosystems (Wallace and Webster 1996). Investigating water beetle assemblages may be particularly illuminating considering the potential these groups are demonstrating as bioindicators of aquatic ecosystem viability (Foster et al. 1990; Fairchild et al. 2000; Lundkvist et al. 2001; Arnott et al. 2006). They also allow the diagnosis of alterations causes, the establishment of criteria for protection and restoration of interesting ecosystems and finally the integrated management of watersheds (Abellán et al. 2007). Thus, good knowledge of the species presence and distribution is necessary to protect biodiversity (Millán et al. 2014). Moreover, primary biodiversity data represent the fundamental elements of any study in systematics and evolutionary processes (May 1990; Funk and Richardson 2002; Hortal et al. 2015).

Investigations of the Hydradephaga (Dytiscidae, Haliplidae, Gyrinidae) of the Canadian Maritimes have been sporadic and regionally variable. Recent papers (Majka 2008; Majka and Kenner 2009; Alarie 2009, 2016; Majka et al. 2009; Webster et al. 2016) resulted in a better understanding of the Hydradephaga fauna in some areas. Despite this rapid increase in knowledge of faunal composition, there are still many Hydradephaga species waiting for discovery in eastern Canada. This is amply evidenced by the fact that 30 species were recently added to the list of Prince Edward Island (Alarie 2016).

Little is known about the Hydradephaga fauna of Cape Breton Island. Prior to this study, 51 species were reported as valid records in the faunal list for Cape Breton Island (Alarie 2016), a small proportion of the 118 (43.2%) reported in Nova Scotia (Bousquet et al. 2013). This study was conducted as part of a comprehensive baseline field survey of Hydradephaga biodiversity of Canadian Maritimes Islands (Alarie 2009, 2016). Its main objective is to improve knowledge of the Hydradephaga of Cape Breton Island. Of particular interest was the identification of new species additions to the known fauna of Nova Scotia.

Materials and methods

Study areas

All of Nova Scotia mainland and Cape Breton Island sit within the Acadian Forest region as described by Rowe (1972), which has a mixed-forest species composition consisting predominately of conifers, especially on sites where drainage is impeded. The major conifers include red, white, and black spruce; balsam fir; eastern white and red pine; and eastern hemlock. Common hardwoods include red and sugar maple; white and yellow birch; trembling and largetooth aspen; and beech (Neily et al. 2005).

Although physically separated from the Nova Scotia peninsula by the Strait of Canso, Cape Breton is artificially connected to mainland Nova Scotia by the Canso Causeway. The island is located east-northeast of the mainland with its northern and western coasts fronting on the Gulf of Saint Lawrence; its western coast also forming the eastern limits of the Northumberland Strait. The eastern and southern coasts front the Atlantic Ocean; its eastern coast also forming the western limits of the Cabot Strait. Cape Breton Island is composed mainly of rocky shores, rolling farmland, glacial valleys, barren headlands, mountains, woods and plateaus. The boreal highlands of Cape Breton reach elevations of 300–500 m and represent true boreal forest habitat, which is rare in Nova Scotia (Neily et al. 2005). Geological evidence suggests that at least part of Cape Breton was originally joined with present-day Scotland and Norway (www.newworldencyclopedia.org/entry/Cape_Breton_Island).

Cape Breton Island's hydrological features include the Bras d'Or Lake system, a salt-water fjord at the heart of the island, and freshwater features including Lake Ainslie, the Margaree River system, and the Mira River. Innumerable smaller rivers and streams drain into the Bras d'Or Lake estuary and onto the Gulf of St. Lawrence and Atlantic coasts (www.newworldencyclopedia.org/entry/Cape_Breton_Island).

Geographically, Cape Breton Island is subdivided into four counties: Cape Breton, Inverness, Richmond, and Victoria. More than 70% of the total Cape Breton population live in the industrialized Cape Breton County. The boreal highlands of Cape Breton are located in the northern parts of Victoria and Cape Breton Counties. The climate of this region is influenced by the higher elevations, strong ocean winds and heavy blankets of dense fog that occur during spring and summer. The headwater streams of the highland regions flow over a primarily ancient metamorphic and granitic dominated geologic landscape, originating from cool springs or draining acidic, sphagnum bogs (Ogden et al. 2018). Inverness and Richmond Counties are largely rural and boast Nova Scotia's most pristine areas. Located within Inverness County, the Margaree River is one of the world's most famous fishing rivers. There are almost no lakes in this region, but there are many steep-fast flowing rivers and streams. Inverness County includes some of the most interesting old forests and undisturbed areas in Nova Scotia. Richmond County is the least well-known county in all Nova Scotia in terms of its beetle fauna. There are many lakes, marshes, and bogs in this area, which have been very little investigated.

Collecting methods

Collections were conducted over three periods, 05–10 May 2006, 17–22 August 2006, and 14–19 May 2007. Sampling was unstructured and qualitative with the goal of obtaining a strict inventory of Hydradephaga of Cape Breton Island. Beetles were collected using D-net sweeps in a variety of microhabitats including macrophyte beds, rocky shores, organic-rich sediments, and open water. Overall 94 samples were obtained, which are listed in Table 1, along with locality data and habitat information.

Nomenclature

Nomenclature is based on the classification in Oygur and Wolfe (1991) (Gyrinidae: *Gyrinus* Müller), Vondel (2005) (Haliplidae), Nilsson and Hájek (2019) (Dytiscidae) and Gustafson and Miller (2015) (Gyrinidae: *Dineutus* MacLeay).

Depositories

Voucher specimens are deposited in the author's research collection (Department of Biology, Laurentian University, Sudbury, Ontario).

Results

In total, 2027 specimens representing 85 species of Hydradephaga were collected in this study (Table 2). Among these, eleven species are reported for the first time for Nova Scotia. Details of species added to the Nova Scotia's fauna follow.

Heterosternuta allegheniana (Matta & Wolfe)

Notes. This species is reported from 39 specimens collected in Richmond County and Inverness County (samples R31, R32, R64, I72).

    Habitat. All specimens were collected on pebble substrate or in leaf litter along the margin of cold creek and streams in accordance with Matta and Wolfe (1981).

    Distribution in the Maritime Ecozone. Prior to this study, *Heterosternuta allegheniana* had only been reported from New Brunswick and southern Québec (Bousquet et al. 2013). Its presence in Nova Scotia therefore represents its easternmost distribution in Canada.

Table 1. Cape Breton Island, Nova Scotia (Canada) sampling localities and habitats (2006–2007): letter in sample code refers to the county. Key: C = Cape Breton; I = Inverness; R = Richmond; V = Victoria.

Sample	Locality	Habitat
C01	Cape Breton Co., Leitches creek Road, 1 km off Hwy 223. 14.v.2007	Bog lake with *Carex* and Ericaceae along margin
C02	Cape Breton Co., Quarry Road, off Leitches creek Road, ca. 6 km off Hwy 223. 14.v.2007	Shallow creek in spruce forest; bed with big boulders; swift current; littoral zone with alder, beech, yellow birch
V03	Victoria Co., Mackillop Road off Hwy 105 at exit to Cabot trail. 15.v.2007	Bog pool on *Sphagnum* bed in white spruce forest; dark water; *Carex* along littoral zone
V04	Victoria Co., St. Ann's Provincial Park, Mackillop Road off Hwy 105. 15.v.2007	Pothole along shore of stream; mats of Graminea and *Scirpus*
V05	Victoria Co., Meadow Road, 4 km off Cabot trail N. 15.v.2007	Shallow creek with swift current, ca. 3–4 m wide; cold water
V06	Victoria Co., Meadow Road, 5 km off Cabot trail N. 15.v.2007	Shallow creek
V07	Victoria Co., Oregon Road, 2 km off Cabot trail N. 15.v.2007	Pond covered with dead *Scirpus*
V08	Victoria Co., Cabot trail N., near junction to Meadow Road. 16.v.2007	Shallow creek on rocky bed, with *Sphagnum* and bryophytes; spruce forest with *Fagus*; cold water (6 C)
V09	Victoria Co., West Tarbot Road ca. 1 km off Cabot trail N. 16.v.2007	Ephemeral pot holes on clay bed, in *Scirpus* and Graminea field; shallow with algae; white spruce forest
V10	Victoria Co., West Tarbot Road ca. 1km off Cabot trail N. 16.v.2007	Ephemeral roadside ditch; very eutrophic, with heavy accumulation of *Sphagnum*, black sediments; dark water
V11	Victoria Co., West Tarbot Road ca. 6 km off Cabot trail N. 16.v.2007	Roadside ditch, slowly moving water; very shallow potholes (ca. 6 cm) with emerging vegetation
V12	Victoria Co., Cabot trail N., near junction Tarbot Vale Road and Rear Barachois Road. 16.v.2007	Large pools formed by the river; clear water; rocky bed covered with organic matters (dead wood, dead leaves) in white spruce forest
V13	Victoria Co., Cabot trail N., 4 km south of Little River. 16.v.2007	Man-made pond with mats of *Scirpus*
V14	Victoria Co., Cabot trail N., 4 km S. Little River. 16.v.2007	Fen with Graminea
V15	Victoria Co., Rear Little River Road, off Cabot trail N. 16.v.2007	Brook flowing over rocky bed, ca. 2 m wide; moderately moving water
C16	Cape Breton Co., Morrisson Road, ca. 6 km off Hwy 22 S. 18.v.2007	*Sphagnum* bog in white spruce forest; with Ericacea, *Scirpus* and *Typha*
C17	Cape Breton Co., Morrisson Road, ca. 7 km off Hwy 22 S. 18.v.2007	*Sphagnum* pool with Ericacea and *Scirpus* in white spruce forest
C18	Cape Breton Co., Morrisson Road, ca. 8 km off Hwy 22 S. 18.v.2007	Shallow roadside ditch with Graminea and *Sphagnum*
C19	Cape Breton Co., Broughton Road near junction to Morrisson Rd. 18.v.2007	Shallow eutrophic brook flowing over rocky bed
C20	Cape Breton Co., Broughton Road, 2 km off junction to Morrisson Rd. 18.v.2007	Eutrophic lake
C21	Cape Breton Co., Broughton Road, 3 km off junction to Morrisson Rd. 18.v.2007	*Sphagnum* bog
C22	Cape Breton Co., Broughton Road, 4 km off junction to Morrisson Rd. 18.v.2007	Road-side ditch
C23	Cape Breton Co., South Head, Sailor Dans Lane. 18.v.2007	Brook with heavy accumulation of bryophytes
C24	Cape Breton Co., South Head, Sailor Dans Lane. 18.v.2007	Cattail pond with bryophytes and *Carex*
C25	Victoria Co., West Side Baddek Road, junction Hunter Mtn Road. 19.v.2007	Small eutrophic creek; heavy accumulation of organic debris; margin with dense vegetation including dead Graminea
V26	Victoria Co., West Side Baddek Road, junction Hunter Mtn Rd. 19.v.2007	Fen with dark brown water; heavy accumulation of Graminea
V27	Victoria Co., Baddek Forks. 19.v.2007	Ephemeral woodland pool with *Scirpus*; bed with heavy accumulation of dead leaves
V28	Victoria Co., Baddeck River at Baddek Forks. 19.v.2007	Pools beside river; very eutrophic

Sample	Locality	Habitat
R29	Richmond Co., Road off Hwy 104^E at exit 44 to Port Malcom. 05.v.2006	Roadside bog with *Sphagnum* and *Typha*; in *Picea* and *Larix laricina* forest
R30	Richmond Co., Road off Hwy 104^E near Port Hawksberry. 05.v.2006	Roadside ditch on rocky bed covered with mud; shore with *Typha* and *Alnus*
R31	Richmond Co., Road off Hwy 104^E towards Isle Madame. 05.v.2006	Shallow creek on rocky bed, with mats of algae; shoreline with *Carex* and Graminea
R32	Richmond Co., Road off Hwy 104^E towards Isle Madame. 05.v.2006	Large creek flowing over rocky bed, presence of algae; in *Picea* and *Betula allegheniensis* forest; shoreline covered with dense bryophytes
R33	Richmond Co., Road off Hwy 104^E towards Isle Madame. 05.v.2006	Pond with heavy accumulation of organic debris
R34	Richmond Co., Isle Madame, Lake road off Hwy 206. 06.v.2006	Man-made pond; rocky bed
R35	Richmond Co., Isle Madame, Lake road off Hwy 206. 06.v.2006	Lake on sandy bed
R36	Richmond Co., Isle Madame, Hwy 206 at Anthony road. 06.v.2006	Shallow puddle on muddy bed, in Graminea field
R37	Richmond Co., Isle Madame, Hwy 320 West at bridge, ca. 4 km East of D'Escousses. 06.v.2006	Eutrophic creek
R38	Richmond Co., Isle Madame, Hwy 320 West, ca. 1 km West of D'Escousses. 06.v.2006	Pool with dark brown water, in a vast field of *Carex*; *Larix laricina* present
R39	Richmond Co., Isle Madame, Hwy 320 West, ca. 2 km West of D'Escousses. 06.v.2006	Shallow cattail pond in *Picea* forest
R40	Richmond Co., Sporting Mountain Road, ca. 2 km off Hwy 4 at exit 47. 06.v.2006	*Sphagnum* bog in *Picea* forest.
R41	Richmond Co., Sporting Mountain Road, ca. 3 km off Hwy 4 at exit 47. 06.v.2006	Small creek flowing on rocky bed with dense mats of *Sphagnum*/bryophytes; in *Abies balsamifera* and *Betula allegheniensis* forest
R42	Richmond Co., Sporting Mountain Road, dead end of Hwy 4 at exit 47. 06.v.2006	Man-made shallow pond
R43	Richmond Co., Road off Sporting Mountain Road, towards St Peters lake. 06.v.2006	Lake; shoreline with *Sphagnum*
R44	Richmond Co., Fleur-de-Lis trail, 3 km East of Grand River. 07.v.2006	Road-side bog ditch with slow-moving dark brown water; heavy accumulation of *Sphagnum* and *Scirpus*.
R45	Richmond Co., Fleur-de-Lis trail at junction Barren Hill Road, ca. 6 km East Grand River. 07.v.2006	*Carex* pool with *Saricena purpurea*
R46	Richmond Co., Fleur-de-Lis trail, ca. 9 km E. Grand River. 07.v.2006	Pond in *Picea* forest; littoral margin with abundance of *Scirpus* and Ericacea
R47	Richmond Co., Fleur-de-Lis trail, ca. 6 km East of St. Esprit. 07.v.2006	Inundated *Picea* forest; dark brown slow-moving water
R48	Richmond Co., North Framboise, 5 km W. off Fleur-de-Lis trail. 07.v.2006	Small roadside ditch with Graminea
C49	Cape Breton Co., East Bay, Morrison Road, off Hwy 4. 08.v.2006	Shallow pond fed with flowing water; rocky bed; clear water; dense Graminea along shoreline
C50	Cape Breton Co., East Bay, Morrison Road, off Hwy 4, past bridge. 08.v.2006	Ephemeral pond with accumulation of dead leaves, in *Acer*, *Fagus*, and *Abies* forest
C51	Cape Breton Co., East Bay, Morrison Road, off Hwy 4, at bridge. 08.v.2006	Discharge of lake; pond-like, very eutrophic; dense Graminea along shoreline
C52	Cape Breton Co., East Bay, Morrison Road, off Hwy 4, at bridge. 08.v.2006	Shallow creek flowing over rocky bed; about 1 m wide
C53	Cape Breton Co., East Bay, Chapei Road, off Meadows Road, about 7 km S. of Hwy 4. 08.v.2006	Ephemeral very humic pond with dead leaves; very dark water; shoreline with dense mats of bryophytes
C54	Cape Breton Co., Rear Big Pond Road, 6 km off junction with Chapei Road. 08.v.2006	Small creek flowing over rocky bed
C55	Cape Breton Co., Rear Big Pond Road, 5 km off junction with Chapei Road at Big Pond. 08.v.2006	Muddy creek with cold water; shoreline with Graminea and *Sphagnum*; in *Picea* and *Abies balsamea* forest
C56	Cape Breton Co., Rear Big Pond Road, 1 km off junction with Chapei Road. 08.v.2006	Man-made pond; full of organic debris
C57	Cape Breton Co., Frank Macdonald Road, 9 km off junction with Soldier Cave Road, off Hwy 4. 09.v.2006	*Sphagnum* bog

Sample	Locality	Habitat
C58	Cape Breton Co., Frank Macdonald Road, 9 km off junction with Soldier Cave Road, off Hwy 4. 09.v.2006	Roadside *Typha* pond
C59	Cape Breton Co., Frank Macdonald Road, 8 km off junction with Soldier Cave Road, off Hwy 4. 09.v.2006	*Typha* pond
C60	Cape Breton Co., Frank Macdonald Road, 5 km off junction with Soldier Cave Road, off Hwy 4. 09.v.2006	Shallow narrow creek with bryophytes.
C61	Cape Breton Co., Frank Macdonald Road, 4 km off junction with Soldier Cave Road, off Hwy 4. 09.v.2006	Shallow *Carex* puddles
R62	Richmond Co., Loch Lamond West Road, 11 km of Grand River. 09.v.2006	Emissary of Loch Lamond lake; collecting along river arms; shoreline covered with vegetation
R63	Richmond Co., Loch Lamond Road, 14 km of Grand River. 09.v.2006	Pools covered with bryophytes; dark brown and cold water
R64	Richmond Co., Loch Lamond Road, 14 km of Grand River. 09.v.2006	Small creek flowing over rocky bed
R65	Richmond Co., Loch Lamond Road, 16 km of Grand River. 09.v.2006	Fen; dense accumulation of Graminea and bryophytes; very dark water
I66	Inverness Co., Greignish Mtns Road, 1 Km off junction Hwy 4B. 10.v.2006	Pond
I67	Inverness Co., Road 104E, 2 km off junction to Greignish Mtns Road, off Hwy 19 at Greignish. 10.v.2006	Fen with *Scirpus* and mats of bryophytes; dark brown water
I68	Inverness Co., Road 104E, 5 km off junction to Greignish Mtns Road, off Hwy 19 at Greignish. 10.v.2006	Small brook in *Sphagnum* bog
I69	Inverness Co., Road 104E, 12 km off junction to Greignish Mtns Road, off Hwy 19 at Greignish. 10.v.2006	Shallow pools with mats of Graminea and heavy accumulation of dead maple leaves; higher elevation
I70	Inverness Co., Road 104E, 4 km off junction to Greignish Mtns Road, off Hwy 19 at Greignish. 10.v.2006	Bog with *Carex* and *Saracenia purpurea*
I71	Inverness Co., Road 104E, off junction to Greignish Mtns Road, off Hwy 19 at Greignish. 10.v.2006	Bog with *Carex*
I72	Inverness Co., Graham River, at J. D. MacDonald Road near Judique South Hwy 19. 10.v.2006	Stream flowing over rocky bed
I73	Inverness Co., Margaree River North East, off Cabot Trail. 17.viii.2006	Larger river flowing over rocky bed
I74	Inverness Co., Ingram Charlie Brook, East Big Interval Road at bridge, 7 km of East Margaree Valley. 17.viii.2006	Brook flowing over big boulders covered with bryophytes
I75	Inverness Co., East Big Interval Road, 12 km of East Margaree Valley. 17.viii.2006	Small brook; beetles collected underneath the banks
I76	Inverness Co., East Big Interval Road, ca. 12 km of East Margaree Valley. 18.viii.2006	Beaver dam ditch; dense mats of *Carex* along shoreline
I77	Inverness Co., East Big Interval Road, ca. 21 km of East Margaree Valley. 18.viii.2006	Small creek with very slow-moving water (almost still); muddy bed, dark brown to black sediment
I78	Inverness Co., Kingross Crossing Road, ca. 1 km off East Big Interval Road, ca. 20 km of East Margaree Valley. 18.viii.2006	Small pool with crystal clear water; pool likely formed from a brook
I79	Inverness Co., East Big Interval Road, ca. 2 km off Kingross Crossing Road. 18.viii.2006	Small beaver dam pool, almost still water, fed from a small creek; muddy bottom, dark brown to black sediment
I80	Inverness Co., North of St. Joseph du Moine, Bazile Road, off Cabot trail at bridge. 21.viii.2006	Small eutrophic creek; flowing water at the middle over rocky bed; shoreline with dense vegetation (*Eupatorium maculatum*; *Equisetum* sp.; muddy shoreline; beetles collected along shoreline in shallow water
I81	Inverness Co., North of Saint Joseph du Moine, Bazile Road, ca. 8 km off Cabot trail. 21.viii.2006	Small creek with big boulders; swift currents
I82	Inverness Co., North of Saint Joseph du Moine, Bazile Road, off Cabot trail. 21.viii.2006	Lake; littoral zone with *Typha*; muddy
I83	Inverness Co., North of Gold Brook, Cabot trail. 21.viii.2006	Roadside ditch; shoreline with *Spirea*, *Alnus*, and *Carex*; heavy accumulation of dead leaves
V84	Victoria Co., Middle River, at Cabot trail. 21.viii.2006	Roadside ditch with slow moving water; shoreline with *Alnus* and *Carex*
V85	Victoria Co., Egypt Road, at Cabot trail. 21.viii.2006	Eutrophic brook with swift current; rocky bed; presence of algae

Sample	Locality	Habitat
I86	Inverness Co., Cranton Cross Road, off Margaree Centre. 21.viii.2006	Brook with crystal clear water; almost still water
I87	Inverness Co., Southwest Margaree Road, ca. 4 km south of Cabot trail. 22.viii.2006	Roadside ditch in spruce forest
I88	Inverness Co., Southwest Margaree Road, ca. 8 km south of Cabot trail. 22.viii.2006	Small eutrophic creek with dense vegetation along shoreline; deep, with slow moving water; rocky bed covered with sediments
I89	Inverness Co., Hwy 395 off Hwy 19. 22.viii.2006	Small creek with swift current; cold water, large boulders; abundance of *Mentha* along shoreline
I90	Inverness Co., south west Margaree River at Bridge, Hwy 395 at junction to Kiltarlity Road. 22.viii.2006	Arm of the river looking like a large ditch; shallow with clear water; dense vegetation (*Carex*, *Scirpus*, *Myositis*) along shoreline; accumulation of algae in the middle; extremely beetle rich
I91	Inverness Co., south west Margaree River at Bridge, Hwy 395 at junction to Kiltarlity Road. 22.viii.2006	I have sampled into algae along shoreline of the river
I92	Inverness Co., Kiltarlity Road. 22.viii.2006	Lake with clear water; *Typha* and nenuphar along shoreline
I93	Inverness Co., Scotsville, junction Hwy 395 and Scotsville Road at bridge. 22.viii.2006	Emissary of lake Ainslie
I94	Inverness Co., near junction to Mountain Road and Scotsville Road. 22.viii.2006	Shallow pond in open prairie overlooking lake Ainslie; main vegetation: *Equisetum*, *Typha* and *Carex*

Heterosternuta wickhami (Zaitzev)

Notes. This species is reported from nine specimens collected in Victoria County and Inverness County (samples I78, I79, V85).

Habitat. Like the previous species all specimens were collected in gravel along the margins of streams. Matta and Wolfe (1981) state this species is most common at the margin of medium to small streams.

Distribution in the Maritime Ecozone. This is the first record of *H. wickhami* in the Maritimes. Prior to this study it had only been reported from Ontario and Québec. Its presence on Cape Breton Island represents a significant extension of this species to eastern Canada (Bousquet et al. 2013).

Hydroporus appalachius Sherman

Notes. Several specimens of this distinctive species were collected at two different sites in Inverness County (samples I79, I86).

Habitat. *Hydroporus appalachius* is usually found in habitats where there are some water movements either along the margins of small lakes or in small streams and springs (Larson et al. 2000), which describe exactly the habitats where these beetles were found in Cape Breton Island.

Distribution in the Maritime Ecozone. This species has a wide range in North America east of the Rocky Mountains. It occurs from Labrador and New Hampshire west to the northern Great Plains and north into the boreal zone and southern limits of the low artic (Larson et al. 2000). Its presence in Cape Breton Island represents the first mention of the species in the Canadian Maritimes (Bousquet et al. 2013).

Table 2. Species of Hydradephaga (Dytiscidae, Gyrinidae, Haliplidae) collected in Cape Breton Island, Nova Scotia, Canada in 2006 and 2007 with sample numbers (as in Table 1), absolute (AF) and relative frequencies (%), and relative frequency of occurrence (RFO). Species in **bold** denote new records from Nova Scotia given in the present account.

Taxon	Sample numbers	AF (%)	RFO
Haliplidae			
Haliplus canadensis Wallis	C25, I78, I88	5 (0.25)	3.19
Haliplus connexus Matheson	I79, I80, I93	4 (0.20)	3.19
Haliplus cribarius LeConte	R62, I79	3 (0.15)	2.13
Haliplus fulvus (Fabricius)	R45, C42, I78, I79, I90	11 (0.54)	5.32
Haliplus immaculicollis Harris	V05, V11, V13, C25, V28, R37, R42, R44, C49, C58, R62, I69, I76, I77, I78, I79, I80, V84, I86, I87, I88, I90, I91, I92, I93	112 (5.53)	26.60
Haliplus longulus LeConte	R45, I71	2 (0.10)	2.13
Peltodytes edentulus (LeConte)	I91, I93	28 (1.38)	2.13
Dytiscidae			
Acilius mediatus (Say)	V09, I83, I87	5 (0.25)	3.19
Acilius semisulcatus Aubé	V04, R34, R39, I67, I92	7 (0.35)	5.32
Agabus ambiguuus (Say)	V04, V09, V10, C25, V26, V28, C20, C49, I67, I76, I79, I80, I82, I83, V84, I90	76 (3.75)	17.02
Agabus anthracinus Mannerheim	V03, C21, C22, C24, V26, V28, R29, R40, R46, R47, C49, C57, C59, R61, R63, R65, I67, I69, I76, I80	95 (4.69)	21.28
Agabus erythropterus (Say)	V09, C25, R46, R48, I76, I77, I78, I79, I86; I90	76 (3.75)	10.64
Agabus leptapsis (LeConte)	V12, I75, I76, I79, I80	5 (0.25)	5.32
Agabus phaeopterus (Kirby)	V27	1 (0.05)	1.06
Agabus semipunctatus (Kirby)	V03, C16, C24, V27, R46, C59	6 (0.30)	6.38
Agabus subfuscatus Sharp	R29, R44, C61, R65, I66	13 (0.64)	5.32
Clemnius laccophilinus (LeConte)	I92	1 (0.05)	1.06
Colymbetes paykulli Erichson	V03, C61	2 (0.10)	2.13
Colymbetes sculptilis Harris	I76, I80	2 (0.10)	2.13
Copelatus glyphicus (Say)	V03, V04, V07, V27, C17, C18, C19, C24, R31, R44	64 (3.16)	10.64
Coptotomus longulus LeConte	C21, R33	2 (0.10)	2.13
Desmopachria convexa (Aubé)	C21, C24, R40, R45, R46, C57, C58, C59, I69, I70, I71, I94	70 (3.45)	12.77
Dytiscus fascinventris Say	V84, I88, I92	4 (0.20)	3.19
Dytiscus verticalis Say	R46, I69	2 (0.10)	2.13
***Heterosternuta allegheniana* (Matta & Wolfe)**	**R31, R32, R64, I72**	**39 (1.92)**	**4.26**
Heterosternuta pulchra (LeConte)	R32, I73, V85, I88, I89	28 (1.38)	5.32
***Heterosternuta wickhami* (Zaitzev)**	**I78, I79, V85**	**9 (0.44)**	**3.19**
Hydaticus aruspex Clark	C24, V26, C51, R65, I67, I70, I71, I94	9 (0.44)	8.51
Hydrocolus paugus (Fall)	V03, C17, V27, R40, I67, I75, I80, V84	12 (0.59)	8.51
Hydrocolus stagnalis (G. & H.)	V08, C18, C19, R46, C50, I90	7 (0.35)	6.38
***Hydroporus appalachius* Sherman**	**I79, I86**	**33 (1.63)**	**2.13**
Hydroporus badiellus Fall	R40, C57, I70	13 (0.64)	3.19
Hydroporus dentellus Fall	V09, V28, R40, R45, R46, R47, R65, I92, I93	24 (1.18)	9.58
***Hydroporus gossei* Larson & Roughley**	**V12, V26, V27, C53, C59, I88, I90**	**11 (0.54)**	**7.45**
***Hydroporus nigellus* Mannerheim**	**C21, V28**	**2 (0.10)**	**2.13**
Hydroporus niger Say	C49, C59, I67, I88	7 (0.35)	4.26
Hydroporus notabilis LeConte	V04, C18, C21, C24, V26, V27, V28, R42, C49, C61, I69, I76, I78, I80, I81, I83, V84, I87, I88, I90, I93, I94	52 (2.57)	23.40
Hydroporus obscurus Sturm	I70	1 (0.05)	1.06
***Hydroporus puberulus* LeConte**	**C53, I71**	**9 (0.44)**	**2.13**
Hydroporus rufinasus Mannerheim	R29, R45, R65	4 (0.20)	3.19
Hydroporus signatus Mannerheim	V07, V10, C18, C17, C21, C24, V28, R29, R42, R46, R47, C49, C59, C61	34 (1.68)	14.89
Hydroporus striola (Gyllenhal)	V03, V07, V26, V28, C20, R30, R32, R36, R38, R45, R46, C49, C53, C59, C61, I80, I83, V84, I88, I90	56 (2.76)	21.28

Taxon	Sample numbers	AF (%)	RFO
Hydroporus tenebrosus LeConte	C18, C53, I93	4 (0.20)	3.19
Hydroporus tristis (Paykull)	V07, V09, V14, C17, C18, C21, C24, C27, C50, C53, C59, C61, I77, I80, I83	44 (2.17)	15.96
Hygrotus impressopunctatus (Schaller)	R35	1 (0.05)	1.06
Hygrotus picatus (Kirby)	V04, C20, R46, R47, I76, I94	8 (0.40)	6.38
Hygrotus sayi Balfour-Browne	V14, C25, V26, V28, R45, R47, C58, I80, I87, I88, I94	25 (1.23)	11.70
Hygrotus turbidus (LeConte)	R39; I66	11 (0.54)	2.13
Ilybiosoma seriatum (Say)	C02, V05, V08, C19, C23, C25, R31, C50, C52, C54, C60, R64, I67, I68, I74, I75, V85	76 (3.75)	18.09
Ilybius angustior (Gyllenhal)	R39, R46, I83,	6 (0.30)	3.19
Ilybius biguttulus (Germar)	V03, V04, C21, C24, V28, R34, C49, C59, R64, I75, I76, I77, I80, I87, I88, I90, I92, I93, I94	106 (5.23)	20.21
Ilybius confusus Aubé	I80	1 (0.05)	1.06
Ilybius discedens Sharp	V27, R29, R46, C49, C57, I71, I92	11 (0.54)	7.45
Ilybius erichsoni G. & H.	V07, C53	7 (0.35)	2.13
Ilybius ignarus (LeConte)	V03, C21, V27 R45	6 (0.30)	4.26
Ilybius larsoni (Fery & Nilsson)	V04, V06, V09, C18, C21, V27, R40, R48, C50, C61, I74	22 (1.09)	11.7
***Ilybius picipes* (Kirby)**	**V26, I76**	**6 (0.30)**	**2.13**
Ilybius pleuriticus (LeConte)	C56, C59, I76, I87, I92	15 (0.74)	5.32
***Ilybius wasastjernae* (C.R. Sahlberg)**	**V27**	**1 (0.05)**	**1.06**
Laccophilus m. maculosus Say	V13, C20, C22, C24, V28, R33, R34, R35, R42, C49, I66, I69, I80, I87	32 (1.58)	14.89
Laccornis latens (Fall)	R29	6 (0.29)	1.06
Liodessus affinis (Say)	V04, V26, V28, R34, R35, R42, C49, C59, R62, I90, I93	55 (2.71)	11.70
Meridiorhantus sinuatus (LeConte)	R38, C52	3 (0.15)	2.13
Nebrioporus rotundatus (LeConte)	C25, R32, I73, I88	35 (1.73)	4.26
Neoporus carolinus (Fall)	V09, V12, V13, V15, V28, R40, R41, C49, C50, C52, C55, C57, I68, I75, I76, I77, I78, I79, I80, I86, I88	97 (4.79)	22.34
Neoporus clypealis (Sharp)	R32, R62, I80, I88, I93	14 (0.69)	5.32
Neoporus dimidiatus (G. & H.)	V28, I77, I78, I79, V84, I86, I88, I90, I91	44 (2.17)	9.58
Neoporus spurius (LeConte)	I80, V85, I91	15 (0.74)	3.19
Neoporus sulcipennis (Fall)	R64, 172, I80, I88, I89	28 (1.38)	5.32
Neoporus undulatus (Fall)	C25, V28, R35, R43, R45, R47, I80, I87, I90, I92, I93	84 (4.14)	11.70
Oreodytes s. scitulus (LeConte)	I72, I73	8 (0.40)	2.13
Platambus obtusatus (Say)	V26, R38, R40, C50, C61, R64, I83	9 (0.44)	7.45
Rhantus binotatus (Harris)	V04, V07, V26, R34, I67, I80, I83, V84, I94	15 (0.74)	9.58
Rhantus suturellus (Harris)	V28, R46, R65	4 (0.20)	3.19
Rhantus wallisi (Harris)	C24, R45, C59, C56, I66	11 (0.54)	5.32
Gyrinidae			
Dineutus hornii Roberts	CO1, R35, I93	17 (0.84)	3.19
Dineutus nigrior Roberts	R34, R35, C56	6 (0.30)	3.19
Gyrinus affinis Aubé	R40, C53, C56, I76,	32 (1.58)	4.26
Gyrinus aquiris LeConte	C01, R45, R46; I90	9 (0.44)	4.26
Gyrinus confinis Fall	R45	1 (0.05)	1.06
Gyrinus fraternus Couper	R45; R62	16 (0.79)	2.13
Gyrinus gehringi Chamberlain	V07, V13, R46, C53, I66, I76, V84, V85	48 (2.37)	8.51
Gyrinus latilimbus Fall	V13, R45, C56, R62, I66, I76, I79	44 (2.17)	7.45
Gyrinus pugionis Fall	C01, V14, R45, I92, I93	69 (3.40)	5.32
Gyrinus sayi Aubé	V13, C21, R34, R35, R40, R45, R46, R47, C53, R62, I67, I76	29 (1.43)	12.77
	Total	2027	

Hydroporus gossei Larson & Roughley

Notes. This species is reported for the first time in Nova Scotia from eleven specimens collected in Cape Breton County, Inverness County and Victoria County (samples V12, V26, V27, C53, C59, I88, I90).

Habitat. In Newfoundland and Prince Edward Island, this species has been collected from among flooded grasses and emergent *Carex* along the margins of beaver ponds and roadside ponds, which is similar to the habitats where these beetles were collected in Cape Breton Island which include also eutrophic creeks.

Distribution in the Maritime Ecozone. This large, distinctive *Hydroporus* species has generally been confused with *Hydroporus rectus* Fall. In the Maritime ecozone, *H. gossei* is also reported from the neighboring province New Brunswick and Prince Edward Island (Larson et al. 2000; Bousquet et al. 2013; Alarie 2016).

Hydroporus nigellus Mannerheim

Notes. This species is reported from only two specimens collected in Cape Breton County and Victoria County (samples C21, V28).

Habitat. These beetles are common in small pools with dense emergent vegetation. The two specimens collected in Cape Breton Island were from a sphagnum bog and a eutrophic pool besides a river.

Distribution in the Maritime Ecozone. Prior to this study, this species had only been reported from the neighboring province New Brunswick (Larson et al. 2000; Bousquet et al. 2013). The North American range of this Holarctic species includes most of the boreal zone and extends north to the southern arctic (Larson et al. 2000).

Hydroporus puberulus LeConte

Notes. *Hydroporus puberulus* is reported from nine specimens form two localities in Cape Breton County and Inverness County (samples C53, I71).

Habitat. These beetles were generally collected from small pools where the water is cool, such as small pools in bogs or habitats where the water is densely shaded by *Carex* (Larson et al. 2000), which is similar to the habitats where these beetles were collected in Cape Breton Island.

Distribution in the Maritime Ecozone. In North America this Holarctic species occurs in the boreal zone from western Newfoundland to Alaska (Larson et al. 2000). Prior to this study, this species had only been reported from the neighboring province New Brunswick in the Maritime Ecozone (Larson et al. 2000; Bousquet et al. 2013).

Ilybius picipes (Kirby)

Notes. *Ilybius picipes* is closely similar to *I. angustior* (Gyllehal) from which it can be differentiated by the relative expansion of the protarsal claw (Larson et al. 2000). In Cape Breton Island this species is reported from six specimens from two localities in Victoria County and Inverness County (samples V26, I76).

Habitat. These beetles are generally collected from peatland pools (Larson et al. 2000), which is similar to the habitats where these beetles were collected in Cape Breton Island.

Distribution in the Maritime Ecozone. This species has a Holarctic distribution. In North America it is transcontinental in the boreal region (Larson et al. 2000). Its presence in Cape Breton Island represents the first mention in the Canadian Maritimes (Bousquet et al. 2013).

Ilybius wasastjernae (C.R. Sahlberg)

Notes. *Ilybius wasastjernae* is reported in Cape Breton Island from only one specimen collected in Victoria County (sample V27).

Habitat. These beetles are generally collected from sphagnum pools, usually in, or adjacent to forest (Larson et al. 2000). In Cape Breton Island it was collected in an ephemeral woodland pool covered with *Scirpus.*

Distribution in the Maritime Ecozone. This species has a Holarctic distribution more or less throughout the boreal zone. In North America it is transcontinental in the boreal region (Larson et al. 2000). Prior to this study, this species had only been reported from the neighboring province New Brunswick in the Maritime Ecozone (Larson et al. 2000; Bousquet et al. 2013).

Discussion

A total of 85 Hydradephaga species was recovered from 94 samples during a survey conducted on Cape Breton Island, Canada, between 2006–2007. According to this study and literature (Majka and Kenner 2009) 87 species of Hydradephaga are currently known from Cape Breton Island (Table 3). There are records of 48 species from Cape Breton County, 68 from Inverness County, 56 from Richmond County and 50 from Victoria County. The significantly larger number of species from both Inverness and Richmond Counties is noteworthy knowing that this region includes some of the most undisturbed areas in Nova Scotia.

Included among the species surveyed were eight new Nova Scotia records consisting of the dytiscid species *Heterosternuta allegheniana* (Matta & Wolfe), *H. wickhami* (Zaitzev), *Hydroporus appalachius* Sherman, *H. gossei* Larson & Roughley, *H. nigellus* Mannerheim, *H. puberulus* LeConte, *Ilybius picipes* (Kirby), and *I. wasastjernae* (C.R. Sahlberg) (Table 2). Among these, *Heterosternuta allegheniana* and *H. wickhami* stand out as representing the easternmost reports of these species in Canada.

The Nearctic component of the fauna is made up of 71 species (81.6%), the Holarctic component of 16 species (17.4%). Most species are characteristic of both the Boreal and Atlantic Maritime Ecozones and have a transcontinental distribution except for *Agabus erythropterus* (Say), *Copelatus glyphicus* (Say), *Coptotomus longulus* LeConte,

Table 3. Checklist of species of Hydradephaga recorded from Cape Breton Island, Nova Scotia, Canada, and their provincial and territorial distribution within northeastern North America (NA). Key: asterisk (*), Holarctic species; cross (†), species not collected in this survey but recorded in Majka (2009); C, Cape Breton County; I, Inverness County; R, Richmond County; V, Victoria County; species in **bold** correspond to strict eastern Canada elements; i.e., never recorded west of the province of Ontario.

Taxon	Counties				Distribution in northeastern North America
	C	I	R	V	
GYRINIDAE					
Gyrininae					
Enhydrini					
Dineutus hornii Roberts	1	1	1		CT, MA, ME, MI, NB, NH, NS, NY, ON, PE, QC, RI
Dineutus nigrior Roberts	1		1		CT, MA, ME, MI, NB, NH, NS, ON, PE, QC, RI
Gyrinini					
Gyrinus affinis Aubé	1	1	1		LB, MA, ME, NB, NF, NH, NS, NY, ON, PE, QC, RI, VT
Gyrinis aquiris LeConte	1	1	1		LB, MA, ME, MI, NB, NF, NS, NY, ON, PE, QC, RI
Gyrinus cavatus Atton†		1			LB, MA, ME, NF, NH, NS, NY, ON, QC, RI
Gyrinus confinis Fall			1		CT, LB, MA, ME, NB, NF, NH, NS, NY, ON, PE, QC, SM, VT
Gyrinus fraternus Couper			1		MA, ME, NB, NH, NS, NY, ON, PE, QC, VT
***Gyrinus gehringi* Chamberlain**	1	1	1	1	**NB, NS, NF, NH, ON, PE, QC**
Gyrinus impressicollis Kirby†				1	NS, ON, QC
Gyrinus latilimbus Fall	1	1	1	1	CT, LB, MA, ME, NB, NF, NH, NS, NY, ON, PE, QC, SE
***Gyrinus pugionis* Fall**	1	1		1	**MA, ME, MI, NB, NH, NS, NY, ON, PE, QC. VT**
Gyrinus sayi Aubé	1	1	1	1	CT, MI, LB, MA, ME, NB, NF, NH, NS, NY, ON, PE, QC, RI, SM
HALIPLIDAE					
Haliplus canadensis Wallis	1	1			MA, NB, NS, ON, PE, QC
Haliplus connexus Matheson		1			CT, MA, ME, NB, NH, NS, NY, ON, PE, QC, VT
Haliplus cribarius LeConte		1	1		CT, LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC, SM
Haliplus fulvus (Fabricius)*	1	1	1		ON, MA, ME, NB, NF, NH, NS, NY, ON, QC
Haliplus immaculicollis Harris	1	1	1	1	CT, LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, QC, PE, RI, SM, VT
Haliplus longulus LeConte		1	1		MA, ME, NB, NH, NB, NS, NY, ON, PE, QC, RI
Peltodytes edentulus (LeConte)		1			MA, NB, NH, NS, ON, QC, PE, RI
DYTISCIDAE					
Agabinae					
Agabini					
Agabus ambiguus (Say)	1	1		1	LB, ME, MI, NB, NF, NH, NS, ON, PE, QC, RI, SM
Agabus anthracinus Mannerheim	1	1	1	1	LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC, SM, VT
***Agabus erythropterus* (Say)**	**1**	**1**	**1**	**1**	**CT, LB, MA, ME, NB, NF, NS, NY, ON, PE, QC, RI**
Agabus leptapsis (LeConte)		1		1	LB, ME, NB, NF, NS, NY, ON, QC, VT
Agabus phaeopterus (Kirby)				1	LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC
Agabus semipunctatus (Kirby)	1		1	1	CT, LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, QC, RI
Agabus subfuscatus Sharp	1	1	1		CT, LB, MA, ME, NB, NF, NH, NS, ON, PE, QC, VT
Ilybiosoma seriatum (Say)	1	1	1	1	CT, LB, MA, ME, MI, NB, NF, NH, NS, ON, PE, QC, SM
Ilybius angustior (Gyllenhal)*		1	1		LB, MI, ME, NB, NF, NH, NS, ON, PE, QC, SM, VT
Ilybius biguttulus (Germar)	1	1	1	1	MA, ME, ME, NB, NF, NH, NS, NY, ON, PE, QC, RI, SM, VT
***Ilybius confusus* Aubé**		**1**			**CT, MA, ME, NB, NH, NS, NY, ON, RI. VT**
Ilybius discedens Sharp*	1	1	1	1	LB, ME, MI, NB, NF, NH, NS, ON, PE, QC, SM
Ilybius erichsoni G. & H.*	1			1	LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC
***Ilybius ignarus* (LeConte)**	**1**		**1**	**1**	**CT, MA, ME, NH, NS, NY, ON, QC, RI**
***Ilybius larsoni* (Fery & Nilsson)**	**1**	**1**	**1**	**1**	**NB, NS, ON, PE, QC**
Ilybius picipes (Kirby)*		1		1	LB, NF, NS, ON, QC
Ilybius pleuriticus (Leconte)	1	1			CT, LB, MA, ME, MI, NB, NF, NS, ON, PE, QC, RI, SM, VT
Ilybius wasastjernae (C. R. Sahlberg)*				1	ME, LB, NB, NF, NS, ON, QC
***Platambus obtusatus* (Say)**	**1**	**1**	**1**	**1**	**CT, MA, ME, NB, NH, NS, NY, ON, QC, VT**
Colymbetinae					
Colymbetini					
Colymbetes paykulli Erichson*	1			1	LB, ME, NB, NF, NS, ON, PE, QC

Taxon	Counties				Distribution in northeastern North America
	C	**I**	**R**	**V**	
Colymbetes sculptilis Harris		1			CT, LB, MI, NB, NF, NH, NS, NY, ON, PE, QC, RI
Meridiorhantus sinuatus (LeConte)			1	1	LB, MA, ME, NB, NF, NH, NS, NY, ON, PE, QC
Rhantus binotatus (Harris)			1	1	CT, LB, ME, MI, NB, NF, NH, NS, ON, PE, QC, RI, SM
Rhantus suturellus (Harris)*			1	1	CT, LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC, SM
Rhantus wallisi Hatch	1	1	1		LB, MA, MI, NB, NF, NH, NS, ON, PE, QC, SM
Copelatinae					
Copelatini					
***Copelatus glyphicus* (Say)**	**1**		**1**	**1**	**CT, ME, NF, NH, NB, NS, ON, PE, QC, RI**
Coptotominae					
Coptotomini					
***Coptotomus longulus* LeConte**	**1**		**1**		**MA, ME, MI, NB, NH, NS, NY, ON, QC, NB, PE, RI**
Dytiscinae					
Aciliini					
Acilius mediatus (Say)		1		1	CT, MA, NB, NH, NS, ON, PE, QC, RI
Acilius semisulcatus Aubé		1	1	1	CT, LB, MA, ME, MI, NB, NF, NH, NS, ON, PE, QC, RI, SM
Dytiscini					
Dytiscus fasciventris Say		1		1	CT, LB, ME, NB, NH, NS, ON, PE, QC, RI
Dytiscus verticalis Say		1	1		CT, MA, ME, NB, NH, NS, NY, ON, PE, QC, RI
Hydaticini					
Hydaticus aruspex Clark*	1	1	1	1	CT, LB, MA, ME, NB, NF, NH, NS, ON, PE, QC
Hydroporinae					
Bidessini					
***Liodessus affinis* (Say)**	**1**	**1**	**1**	**1**	**CT, ME, NB, NF, NH, NS, ON, PE, QC, RI**
Hydroporini					
***Heterosternuta allegheniana* (Matta & Wolfe)**		**1**	**1**		**NB, NS, QC**
***Heterosternuta pulchra* (LeConte)**		**1**	**1**	**1**	**CT, ME, LB, NB, NF, NS, ON, QC, SM**
***Heterosternuta wickhami* (Zaitzev)**		**1**		**1**	**NS, ON, QC**
Hydrocolus paugus (Fall)	1	1	1	1	LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC, SM
Hydrocolus stagnalis (G. & H.)	1	1	1	1	CT, MA, ME, NB, NH, NS, NY, ON, PE, QC
Hydroporus appalachius Sherman		1			LB, MA, ME, NH, NS, ON, QC
Hydroporus badiellus Fall		1	1	1	LB, ME, MI, NB, NF, NH, NS, ON, QC
Hydroporus dentellus Fall		1	1	1	LB, MA, ME, MI, NB, NH, NS, NY, ON, PE, QC
***Hydroporus gossei* Larson & Roughley**	**1**	**1**		**1**	**ME, NB, NF, NS, NY, ON, PE, QC**
Hydroporus nigellus Mannerheim*	1			1	LB, NB, NS, ON, QC
***Hydroporus niger* Say**	**1**	**1**			**CT, MA, MI, NB, NF, NH, NS, NY, ON, PE, QC, RI**
Hydroporus notabilis LeConte*	1	1	1	1	LB, MA, ME, MI, NB, NF, NH, NS, ON, PE, QC, SM
Hydroporus obscurus Sturm*		1			LB, NB, NF, NS, ON, PE, QC, SM
Hydroporus puberulus LeConte*	1	1			LB, ME, NB, NF, NS, ON, QC
Hydroporus rufinasus Mannerheim		1			ME, NB, NS, ON, QC
Hydroporus signatus Mannerheim	1		1	1	CT, LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC, RI, SM
Hydroporus striola (Gyllenhal)*	1	1	1	1	LB, ME, MI, NB, NF, NH, NS, ON, PE, QC, RI
Hydroporus tenebrosus LeConte	1	1			MA, ME, MI, NB, NH, NS, NF, ON, PE, QC,
Hydroporus tristis (Paykull)*	1	1		1	LB, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC, RI, SM
Nebrioporus rotundatus (LeConte)	1	1	1		LB, MA, ME, NB, NF, NS, ON, PE, QC, RI
***Neoporus carolinus* (Fall)**	**1**	**1**	**1**	**1**	**LB, MA, ME, NB, NF, NH, NS, NY, ON, PE, QC, SM**
***Neoporus clypealis* (Sharp)**		**1**	**1**		**ME, NB, NH, NS, NY, ON, PE, QC**
Neoporus dimidiatus (G. & H.)		1		1	CT, LB, MA, ME, NB, NF, NH, NS, ON, PE, QC, RI
***Neoporus spurius* (LeConte)**		**1**		**1**	**NB, NS, ON, QC**
***Neoporus sulcipennis* (Fall)**		**1**	**1**		**NB, NH, NS, NY, ON, PE, QC,**
Neoporus undulatus (Say)	1	1	1	1	CT, LB, MA, ME, MI, NB, NF, NS, NY, ON, PE, QC, RI, SM
Oreodytes s. scitulus (LeConte)		1			ME, LB, NB, NF, NH, NS, NY, ON, QC, SM
Hygrotini					
Clemnius laccophilinus (LeConte)		1			ME, NB, NH, NS, ON, PE, QC
Hygrotus impressopunctatus (Schaller)*		1			LB, ME, MI, NB, NF, NH, NS, ON, PE, QC
Hygrotus picatus (Kirby)	1	1	1	1	LB, MI, NB, NF, NH, NS, NY, ON, PE, QC, RI
Hygrotus sayi Balfour-Browne	1	1	1	1	LB, MA, ME, MI, NB, NF, NH, NS, NY, ON, PE, QC, RI, SM, VT

Taxon	Counties				Distribution in northeastern North America
	C	I	R	V	
Hygrotus turbidus (LeConte)		1	1		MA, MI, NB, NH, NS, NY, ON, PE, QC, RI
Hyphydrini					
Desmopachria convexa (Aubé)	1	1	1		ME, MI, NB, NS, ON, PE, QC, RI
Laccornini					
***Laccornis latens* (Fall)**			**1**		**MA, NB, NH, NS, NY, ON, PE, QC,**
Laccophilinae					
Laccophilini					
Laccophilus m. maculosus Say	1	1	1	1	CT, MA, ME, MI, NB, NH, NS, ON, PE, QC, RI
Total	48	68	56	50	

Notes: Regional Distribution information derived from Downie and Arnett (1996), Larson et al. (2000), Majka (2008), Alarie (2009, 2016), Majka et al. (2011), Elder and Abraham (2012), Bousquet et al. (2013), and Webster (2016). Key: CT, Connecticut; LB, Labrador; MA, Massachusetts; ME, Maine; MI, Magdalen Island Archipelago, NB, New Brunswick; NF, insular Newfoundland; NH, New Hampshire; NS, Nova Scotia; NY, New York; ON, Ontario; PE, Prince Edward Island; QC, Québec; RI, Rhode Island; SM, Saint-Pierre et Miquelon; VT, Vermont.

Heterosternuta allegheniana, *H. pulchra* (LeConte), *H. wickhami*, *Hydroporus gossei*, *H. niger* Say, *Ilybius confusus* Aubé, *Ilybius ignarus* (LeConte), *I. larsoni* (Fery & Nilsson), *Laccornis latens* (Fall), *Liodessus affinis* (Say), *Nebrioporus rotundatus* (LeConte), *Neoporus carolinus* (Fall), *Neoporus clypealis* (Sharp), *N. spurius* (LeConte), *N. sulcipennis* (Fall), and *Platambus obtusatus* (Say), which are generally recognized as species with eastern affinities (Larson et al. 2000; Bousquet et al. 2013) (Table 3).

The 87 Hydradephaga species known from Cape Breton Island represent approximately 74% of the fauna known for all Nova Scotia (Bousquet et al. 2013). As is typical of Hydradephaga, the Dytiscidae accounts for the largest share (78.2%) of the fauna, while Gyrinidae (13.8%) and Haliplidae (8.1%) are represented in lesser proportions. Forty-five species were observed at more than 5% of the sites (Table 2). The most common ones (RFO > 15 %) included the haliplid species *Haliplus immaculicollis* Harris and the dytiscid species *Agabus ambiguus* (Say), *A. anthracinus* Mannerheim, *Hydroporus notabilis* LeConte, *H. signatus* Mannerheim, *H. striola* (Gyllenhal), *H. tristis* (Paykull), *Ilybiosoma seriatum* (Say), *Ilybius biguttulus* (Germar), *Laccophilus m. maculosus* Say, and *Neoporus carolinus* (Fall) (Table 2). Considering the relatively large number of species recorded in such a short time, confirm that this region is very important for wetland beetle biodiversity, and its continued conservation.

This research considerably extends the list of reported species in Richmond and Inverness counties both of which were considered the least well-known counties in all Nova Scotia in terms of its beetle fauna prior to this study. Many species recorded in this region are interesting in zoogeographic terms as representing new records for Nova Scotia (see above). The extraordinary species richness of rheophilic species (e.g., *Agabus leptapsis* (LeConte), *A. erythropterus* (Say), *Heterosternuta allegheniana*, *H. pulchra*, *H. wickhami*, *Hydroporus appalachius*, *Neoporus clypealis* (Sharp), *Neoporus dimidiatus* (G. & H.), *N. spurius*, *N. sulcipennis* (Fall), and *Oreodytes s. scitulus* (LeConte)) are worth emphasizing in that they illustrate the high abundance of lotic habitats in this portion of Cape Breton Island. In general, coexisting species may be more closely related than expected by chance if environmental features of a given habitat select for certain traits that are shared

by closely related species (Vamosi and Vamosi 2007), which may explain particularly the highest diversity of *Neoporus* Guignot and *Heterosternuta* Strand in that region.

The 87 species of Hydradephaga reported in this study represent an important richness, proportionally comparable to the number of species found in Prince Edward Island with similar environmental conditions (Alarie 2016). It is worth mentioning, however, that several species (*Agabus leptapsis*, *Dineutus hornii* Roberts, *Dytiscus fasciventris* Say, *Gyrinus cavatus* Atton, *G. fraternus* Couper, *G. gehringi* Chamberlain, *G. impressicollis* Kirby, *Haliplus fulvus* (Fabricius), *Heterosternuta allegheniana*, *H. pulchra*, *H. wickhami*, *Hydroporus appalachius*, *H. badiellus* Fall, *H. nigellus*, *H. puberulus*, *H. rufinasus* Mannerheim, *Ilybius ignarus*, *I. picipes*, *I. wasastjernae*, *Neoporus spurius*, *Oreodytes s. scitulus*, and *Platambus obtusatus*) collected in Cape Breton Island have yet to be discovered in Prince Edward Island. The absence in Cape Breton Island of 12 species listed in the latter province (*Acilius sylvanus* Hilsenhoff, *Agabus punctulatus* Aubé, *Boreonectes griseostriatus* (DeGeer), *Dytiscus dauricus* Gebler, *D. harrisii* Kirby, *Graphoderus liberus* (Say), *G. perplexus* Sharp, *Gyrinus bifarius* Fall, *G. lecontei* (Hope), *Hygrotus compar* Fall, *Peltodytes tortulosus* Roberts, and *Sanfilippodytes planiusculus* (Fall)) is worth mentioning as it suggests that the number of Hydradephaga species on Cape Breton island may be even greater than suggested by this study.

Conclusions

Our study adds considerably to the previous knowledge about Hydradephaga in the Canadian Maritimes, with eight new records for the province of Nova Scotia. The composition of the Cape Breton fauna reflects that of the Maritime Provinces as a whole. Whereas all the species found on Cape Breton Island have also been recorded in New Brunswick (Webster 2016) (except *Gyrinus cavatus* Atton, *Heterosternuta wickhami*, *Hydroporus appalachius*, and *Ilybius picipes*) the presence on Prince Edward Island (Alarie 2016) of 12 species not reported on Cape Breton Island suggests that additional species could potentially still be found.

Acknowledgements

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RESEARCH ARTICLE

Taxonomic study of *Thiotricha* Meyrick (Lepidoptera, Gelechiidae) in Japan, with the description of two new species

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Abstract

A part of Japanese species of the genus *Thiotricha* Meyrick, 1886 are reviewed. Three species described by Omelko (1984) in the genus *Cnaphostola* Meyrick, 1918 are placed in combination with *Thiotricha*; *Thiotricha biformis*, *T. angustella* **comb. nov.** and *T. venustalis* **comb. nov.** These species are redescribed, and two new species, *T. elaeocarpiella* Kyaw, Yagi & Hirowatari, **sp. nov.** and *T. flavitermina* Kyaw, Yagi & Hirowatari, **sp. nov.** are described based on external morphological characters of adults and genitalia of males and females. *Thiotricha chujaensis* (Park, 2016), **comb. nov.** described in *Cnaphostola* from Korea, is newly recorded in Japan feeding on *Mallotus japonica* (Euphorbiaceae). One of the new species, *T. elaeocarpiella* **sp. nov.** has been associated with two different plants, *Elaeocarpus zollingeri* (Elaeocarpaceae) and *Rhaphiolepis indica* (Rosaceae). This paper presents the first comprehensive description of the morphology, pupal morphology and biology of species previously treated in the genus *Cnaphostola* and their relatives in Japan.

Introduction

The family Gelechiidae is one of the largest families of Microlepidoptera in the world and includes more than 4,700 described species belonging to approximately 500 genera (van Nieukerken et al. 2011). In the Palearctic region, there are more than 1,500 species (Piskunov 1990). In Japan, 288 species of this family have been recorded, including 41 unidentified species (Jinbo 2016). There is a great number of species that remain to be described, as Gelechiidae is one of the least studied Lepidoptera families. Currently, this family is thought to consist of seven subfamilies: Anacampsinae, Dichomeridinae, Apatetrinae, Thiotrichinae, Anomologinae, Gelechiinae, and Physoptilinae, mainly based on molecular analyses (Karsholt et al. 2013).

The subfamily Thiotrichinae includes the genera *Thiotricha* Meyrick, 1886, *Macrenches* Meyrick, 1904, *Palumbina* Rondani, 1876 and *Polyhymno* Chambers, 1874. Among them, *Thiotricha* Meyrick, 1886 and *Polyhymno* Chambers, 1874 have a long taxonomic history with various opinions about their separation. The genus *Thiotricha* includes globally nearly 100 described species and is most diverse in Asia (Karsholt et al. 2013). In Japan, 15 species have been recorded and were later treated as *Polyhymno* (Suzuki and Komai 1984; Oku 2003). In 2005, Ueda and Fujiwara described the new species *Thiotricha prunifolivora* Ueda & Fujiwara, 2005 from the host plant *Symplocos prunifolia* (Family: Symplocaceae), with a biological note on the immature stages. Then, this species and the 15 previously recorded species were tentatively treated in the genus *Thiotricha*, together with a taxonomic comment that mainly follows Omelko (1999) and Park (2004). In 2009, *Thiotricha* was synonymized with *Polyhymno* by Ponomarenko (2009), without further comments. Recently, Karsholt et al. (2013) conducted a molecular analysis of the Gelechiidae without *Polyhymno* species in their analysis. However, they compared the type species, *P. longistrigella* Chambers morphologically with species of *Thiotricha*. This resulted in the recognition of both *Thiotricha* and *Polyhymno* as valid genera. Moreover, also their biology differs, the larvae of *Polyhymno* are leaf-spinners and leaf-webbers in Fabaceae (Busck 1900), but larvae of *Thiotricha*, as far as known, make a portable case and feed on flowers and seeds (Robinson et al. 1994, Ueda and Fujiwara 2005).

Another genus, *Cnaphostola* Meyrick, 1918, was described for the single species *C. adamantina* Meyrick, 1918, collected in Assam, North India. Three additional species; *C. biformis* Omelko, 1984, *C. angustella* Omelko 1984, and *C. venustalis* Omelko, 1984, were described from the Primorsky Territory in the Russian Far East (Omelko 1984). These three species have also been recorded from Japan (Oku 2003; Ueda 2013). In 2016, Park described *C. chujaensis* from Chuja Island, Korea, tentatively placing it in *Cnaphostola*. Today, therefore, this genus comprises five species in total (Park and Kim 2016). Kogi (2004, 2008) observed and reported the larval feeding of *C. venustalis* and *C. angustella* on the host plant *Quercus dentata* (Fagaceae), in Hokkaido, Japan. Otherwise, the biology of the immature stages of this genus has not been studied in detail. The members of *Cnaphostola* are very similar to those of *Thiotricha* or *Polyhymno*. Meyrick (1918) noted that the genus *Cnaphostola* probably belongs to the *Thiotricha* group; however, it was not included in the molecular analy-

sis of Karsholt et al. (2013). Then, Sohn et al. (2015) combined *Cnaphostola biformis* as *Thiotricha biformis* without any explanation.

Although the phylogenetic relationship and synonymy of the genera *Cnaphostola*, *Thiotricha* and *Polyhymno* is not fully resolved to date, we place all Japanese species here in *Thiotricha*, we review the Japanese species and compare the morphological characters, including head parts, wing marking, venation, and genitalia, to solve the aforementioned taxonomic problems. Further, we describe two new species with photographs of male and female adults, wing venation, and genitalia. We also report the biology of immature stages of some species, the pupal morphology of *T. chujaensis* (Park, 2016) comb. nov. and *T. elaeocarpiella* Kyaw, Yagi & Hirowatari sp. nov. and discuss the larval feeding mode of this group.

Materials and methods

Dried specimens deposited in the Entomological Laboratory, Kyushu University, Fukuoka (**ELKU**); Osaka Prefecture University, Sakai (**OPU**); the Entomological Laboratory, Kagoshima University, Kagoshima (**KGU**); the National Museum of Nature and Science, Tsukuba, Japan (**NSMT**); and T. Oku's collection, Morioka (**TO**) were examined. Field research was also conducted across Japan from Hokkaido to Okinawa. Larval portable cases were collected from host plants and light traps were used to collect adults. External morphological characteristics were first observed under a stereo microscope (Nikon SMZ-U), and then genitalia slides were prepared as follows: the abdomen was detached, placed in a glass tube with 10% potassium hydroxide (KOH) solution, and boiled in water for ca. 10–15 mins, depending on the size of the genitalia to macerate. After that, the boiled abdomen was neutralized in acetic acid, stained with Chlorazol Black E, and then rinsed with 70% ethanol solution to remove residual scales and internal soft parts. Then, the abdomen was dissected by cutting its intersegmental membrane between T7 and T8 with fine small insect pins. The genitalia were separated and transferred to a glass plate with 50% glycerol solution for observation. After observation, the genitalia and the abdomen were dehydrated in a 70%–100% ethanol series and mounted on a microscope slide in Euparal. Photographs of adults were taken using a Leica S8APO with a digital camera (Canon EOS 7D), and focus stacking was performed in Combine ZP (Hadley, 2010). Photographs of male and female genitalia were taken using a biological microscope (Olympus BX43) with a digital camera (Olympus E5).

Unless otherwise noted, the specimens are deposited in the Entomological Laboratory of Kyushu University (**ELKU**).

Terminology

The descriptive terminology follows Park and Kim (2016) for wing markings, and Karsholt et al. (2013) and Ueda and Fujiwara (2005) for genitalia. The scientific names of plants follow Yonekura and Kajita (2003).

Taxonomy

Genus *Thiotricha* Meyrick, 1886

Type species. *Thiotricha thorybodes* Meyrick, 1886 designated by Meyrick 1925:101.

Thiotricha biformis (Omelko, 1984)
Figs 4A, B, 7A, 8A, B, 9A, 10A

Cnaphostola biformis Omelko, 1984: 32; Omelko 1999: 183; Oku 2003: 65; Park and
     Ponomarenko 2007: 45; Ueda 2013: 298.
Thiotricha biformis: Sohn et al. 2015: 116

Material examined. Japan – **Hokkaido** [Hokkaido] • 1♂,1♀; Katsuranosawa, Uryu-
cho, Uryu-gun; 20 Jul. 2018; S. Yagi leg. • 1 ♂; Katsuranosawa, Ishikari city; 15 Jul.
2007; H. Kogi leg. • 1♂; Manzi, Kurisawa; 8 Jul. 2001; H. Kogi leg.; gen. slide no.
KM-143 • 1 ♂, 1♀; same locality and collector; 28 Jul. 2003; gen. slide no. KM-
144(♂) • 1♂; Kotan Atuta; 10 Jul. 2003; H. Kogi leg. • 1♀; Nopporo (Atsubetsu); 27
Jul. 1993; T. Hirowatari leg. • 2♀♀; Kamisibun, Iwamizawa; 9 Jul 2011; 8 Aug. 2012;
H. Kogi leg.; gen. slide no. KM-145 • 1♀; Hukui, Niseko; 4 Aug. 2006; H. Kogi leg.
• 1♀; Tiyosibetu, Hamamasu; 14 Jul. 2000; H. Kogi leg. • 1♀; Asari Pass, Otaru; 28
Jul. 2012; H. Kogi leg.; gen. slide no. KM-146. – **Honshu** [Iwate] • 1♀; Atei-Sanso
Niisato vill.; 6 Jul. 2002; T. Oku leg. (TO).
     Diagnosis. The forewing is white with broad, dark brown fascia, a rather large yel-
low patch before the apex, and a black rounded apical spot demarcated by a white line.
The anellus lobe of the male genitalia is a short and heavily sclerotized beak-shaped
lobe basally, armed with a small claw-like process apically, which is a unique charac-
teristic of this species. The apopyhsis posterioris of the female genitalia is ca. 1/2 the
length of the papilla analis and approx. two times longer than the apophysis anterioris;
the signum is absent in the corpus bursae.
     Description. Male. (Figs 4A, 7A). Forewing length 3.2–3.7 mm. Wing expanse
7.0–8.1 mm.
     ***Head*:** covered with shiny, creamy white appressed scales. Antennae filiform; basal
segment (scape) elongate without pecten, creamy white; flagellum creamy white on
dorsal surface before middle, then entirely grayish brown beyond on its dorsal and
ventral surface, with rather long and fine cilia ventrally. Labial palpus white, long, and
recurved; first segment shortest with creamy white scales; second segment thickened,
ca. 2.5 the thickness times of first, covered entirely with white scales; third segment
white on dorsal surface, sparse brown scales medially on ventral surface, as long as
second segment, apex sharply acute.
     ***Thorax*:** creamy white. Tegula shiny, creamy white dorsally, with brown scales
along anterior margin.
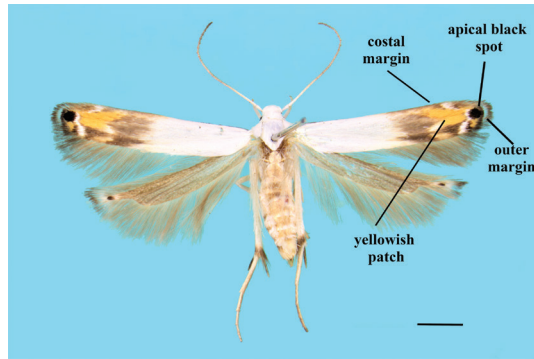
Figure 1. Forewing pattern elements. *Thiotricha biformis*, female. Scale bar: 1 mm.
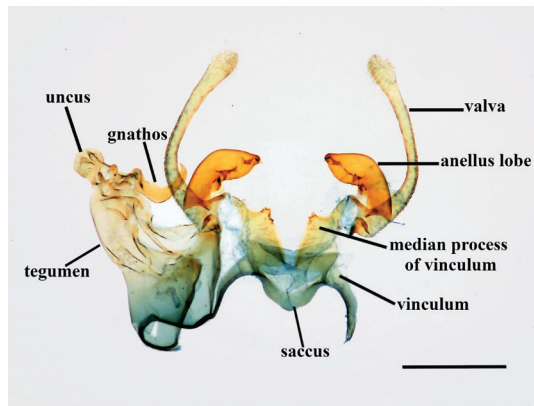
Figure 2. Male genitalia. *Thiotricha biformis*. Scale bar: 0.4 mm.
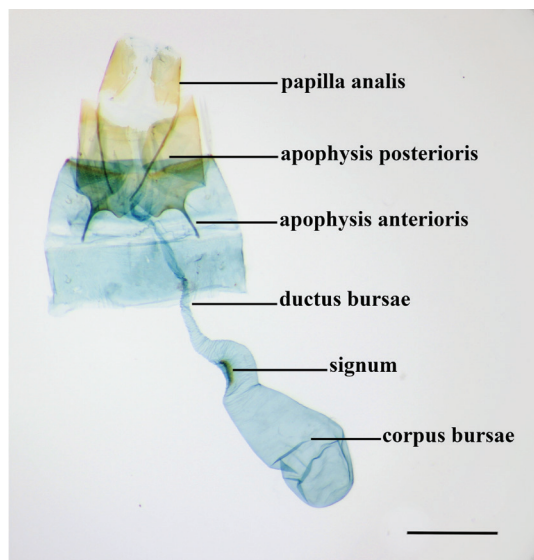
Figure 3. Female genitalia. *Thiotricha angustella* comb. nov. Scale bar: 0.4 mm.
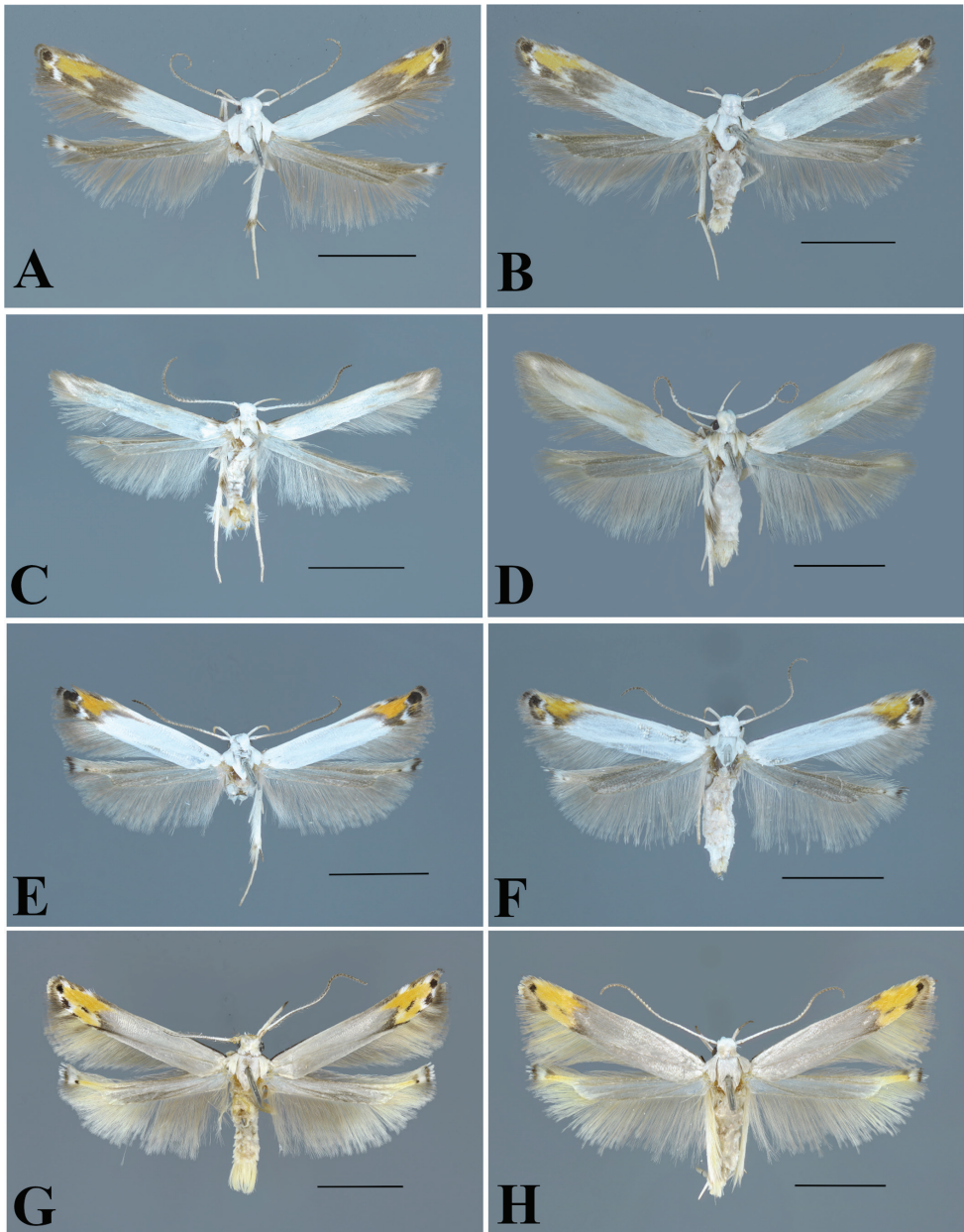
Figure 4. Adults of *Thiotricha* spp. **A** *T. biformis*, male **B** ditto, female **C** *T. angustella* comb. nov., male **D** ditto, female **E** *T. venustalis* comb. nov., male **F** ditto, female **G** *T. chujaensis* (Park, 2016) comb. nov., male **H** ditto, female. Scale bars: 2 mm.

Legs: white; forefemur, tibia, and tarsus suffused inwardly with brownish tinge, white on outer surface; mid legs entirely white; hind femur and tibia white, with a row of long, stiff, stout white bristles on upper and lower surfaces; all tarsal segments brownish gray.

Forewing: eleven veins, $R_3 + R_4$ stalked, M_1 separate, R_5 absent, anal veins furcate (Fig. 7A). Forewing broader in this genus, ground color white to middle, followed by a dark brown fascia, rather large yellow patch before apex not protruding to costal margin; small brown area in costal margin, intercepted by oblique white lines with a rounded black apical spot; small scattering of yellow scales below apical point at outer margin, small brown scale in tornal area; cilia blackish brown at inner margin of apex, outer margin with brown through inner base of wing.

Hindwing: darker grayish brown with tiny black apical dot at apex; cilia well-fringed, grayish brown, with dark brown at tip of wing.

Male genitalia: (Figs 8A, B, 9A) eighth abdominal sternite rather semicircular, broadly concave with strongly sclerotized margin. Uncus semicircular with a few hairs on top. Gnathos sickle-shaped, short and stout, wide and flattened posteriorly, and then curved at middle toward apex. Tegumen long, slightly concave medially, bearing dense hairs on dorsal surface at approx. the midpoint. Anellus lobe, a short and heavily sclerotized beak-shaped lobe, approx. half the length of valva, slightly narrow at base, and then abruptly dilate with short and small claw-like process apically, with a few sclerotized spines at approx. the midpoint on inner dorsal surface. Valva elongate, slender, and curved inwardly, somewhat broad basally, narrowly elongate from base to 2/3 of its length, then distended again at apex, bearing thin and fine setae on its inner surface. Vinculum moderately broad, median process rising to a pair of transverse ridges of short, thorn-like spines on edge of posterior surface, rather long and short fine setae emerging around and below the ridge surface. Saccus broad basally, somewhat triangularly produced. Phallus short and sclerotized, clavate basally and straightly elongate with a round tip distally.

Female. (Figs 1, 4B). Forewing length 2.5–3.9 mm. Wing expanse 5.5–8.2 mm. Similar to male.

Female genitalia: (Fig. 10A) papilla analis long and bilobed with short and long fine setae on its entire surface. Apophyses short; apophysis anterioris shorter, nearly 1/2 length of apophysis posterioris. Ostium opening near anterior margin of 8th sternite. Ductus bursae partially sclerotized, narrow, and slightly elongate, as long as corpus bursae. Corpus bursae large and rounded; signum absent.

Distribution. Japan (Hokkaido, Honshu), Russia, Korea.

Host plant. Unknown.

Remarks. Sohn et al. (2015) treated this species as *Thiotricha biformis*, without explaining the taxonomic reasons.

Thiotricha angustella (Omelko, 1984) comb. nov.
Figs 4C, D, 7B, 8C, D, 9B, 10B

Cnaphostola angustella Omelko, 1984: 32; Omelko 1999: 183; Oku 2003: 65; Park and Ponomarenko 2007: 45; Ueda 2013: 298.

Material examined. Japan – **Hokkaido** [Hokkaido] • 1♀; Yamato, Erimo; 15 Jul. 2002; H. Kogi leg.; Host: *Quercus dentata*; TO • 1♂; same locality and collector; 19

Jul. 2003; Host: *Quercus dentata* • 1♂; Syoya, Erimo; 30 Jun. 2002; H. Kogi leg. • 1♂; Tomikawa, Monbetu; 21 Jul. 2004; H. Kogi leg.; Host: *Quercus dentata*; TO • 3♀♀; same locality and collector; 23–25 Jul. 2006; 6 vii 2007 • 2♀♀; Higasihayakita, Hayakita; 20 Jul. 2005; H. Kogi leg. • 1♂; Tiyosibetu, Hamamasu; 13 Jul. 2002; H. Kogi leg. • 1♂; Katsuranosawa, Atuta; 21 Jul. 2002; H. Kogi leg. • 1♂; Uenai, Tomakomai; 26 Jul. 2002; H. Kogi leg. – **Honshu** [Iwate] • 1♀; Iwayama, Morioka; 2 Jul. 2009; T. Oku leg.; Host: *Quercus mongolica*; 13 Jul. 2009 em.; TO. – **Honshu** [Nagano] • 1♀; Kojiro, Tenryu-mura; N. Hirano leg.; 5 Jul. 2008; gen. slide no. KM-118 • 1♂; same locality and collector; 28 Aug. 2009; gen slide no. KM-106. – **Honshu** [Aichi] • 1♂; Asahikogen, Asahi-cho; 7 Jul. 2001; T. Mano leg.; OPU. – **Honshu** [Mie] • 1♂; Hijiki 250m, Ueno-city; 27 Jun. 1997; T. Mano leg.; gen. slide no. KM-1; OPU • 1♂; Hudodani, Miyama-tyo; 30 Jun. 2001; T. Mano leg.; gen. slide no. KM-96; OPU. – **Honshu** [Kyoto] • 1♂; Mt.Ponpon; 1 Jul. 2000; N. H. Ahn leg.; gen. slide no. KM-2; OPU. – **Honshu** [Nara] • 1♀; Wasamatayama; 9–10 Aug. 1989; S. Moriuti leg.; gen. slide no. KM-51; OPU.

Diagnosis. The forewing is white with pale brown in the distal part, without an apical black spot. The anellus lobe of the male genitalia is a pear-shaped lobe basally, long, strongly sclerotized, and spine-like apically; the valva is narrow and elongate. The apophysis posterioris of the female genitalia is approx. two times of the length of papilla analis and approx. three times longer than the apophysis anterioris; the signum is long, narrow, and arch-shaped.

Description. Male (Figs 4C, 7B). Forewing length 2.7–3.4 mm. Wing expanse 6.0–7.3 mm.

Head: shiny creamy white with appressed scales. Antennae filiform, basal segment elongate without pecten and creamy white; flagellum creamy white on dorsal surface before middle, then grayish brown beyond, with extraordinarily long and fine cilia on its ventral surface. Labial palpus white, long, and recurved; first segment shortest, creamy white suffused with brown scales on outer surface; second segment thickened, up to 2.5 times the length of the first and white; third segment nearly as long as second, creamy white evenly on both surfaces, apex sharply acute.

Thorax: creamy white. Tegula shiny, creamy white dorsally, ornamented with bronze-brown scales along anterior margin.

Legs: white; forefemur, tibia, and tarsus suffused inwardly with brown; hind tibia creamy white, with a row of long, stiff, stout white bristles at approx. the midpoint anteriorly, with dark brown bristles at ca. 1/4 posteriorly on dorsal surface, with white bristles ventrally.

Forewing: eleven veins, $R_4 + M_1$ stalked, R_5 absent, anal vein furcate (Fig. 7B). Forewing ground color shiny creamy white, somewhat rounded and pointed apically, with a brownish hue apically 1/5 of the way beyond costal margin; cilia well-fringed, brown from costal area before apex and brownish white along outer margin to inner base of wing.

Hindwing: narrower than forewing, white to whitish brown; cilia well-fringed, white to brownish white; apex produced conspicuously.

Male genitalia: (Figs 8C, D, 9B) eighth abdominal sternite triangular, long, slightly broadened at base, and then tapered toward posterior with a blunt tip. Uncus swol-

len, like a small tubercle, short and fine hairs on its top. Gnathos short and stout, its posterior margin closer to base with a hump-like outgrowth; closer to top, somewhat expanded, with a blunt tip. Tegumen much longer than uncus, with dense hairs at approx. the midpoint of its length on dorsal surface. Anellus lobe quite long and pear-shaped basally, bearing a rather long and strongly sclerotized spine-like process at apex, slightly acute and curved inward. Valva elongate, slender, slightly expanded basally, narrow to 3/4 of its length, moderately dilated, and lobate with fine, dense hairs on its inner surface apically, moderately curved inwardly, exceeded apex of tegumen. Vinculum narrow, bearing a few rather long spines on median process of vinculum. Saccus broad basally and U-shaped. Phallus long, small, and spherical at base, and extended distally.

Female (Fig. 4D). Forewing length 3.2–3.4 mm. Wing expanse 7.1–7.5 mm. Similar to male.

Female genitalia: (Fig. 10B) papillae anales nearly half the length of the apophysis posterioris, with two lobes and long and short fine setae on its entire surface. Apophysis posterioris as much as three times the length of apophysis anterioris. Ostium opening near posterior margin of 8th sternite. Ductus bursae narrow, nearly equal in length to corpus bursae and moderately sclerotized. Ductus seminalis arising from the posterior third of ductus bursae. Corpus bursae oblong; signum long and a narrow arch shaped at left side wall of posterior end.

Distribution. Japan (Hokkaido, Honshu), Russia, Korea.

Host plant. *Quercus dentata* (Kogi 2008), *Q. mongolica* (Fagaceae) (new host record).

Biology. Kogi (2008) reported that the larvae of *T. angustella* occur in August until the following June and make a portable case with fragments of a host plant leaf. The mature larvae use a larger piece of leaf like a hat. Dr. T. Oku collected a case from *Q. mongolica* in Iwate Prefecture in July.

Thiotricha venustalis (Omelko, 1984) comb. nov.
Figs 4E, F, 7C, 8E, F, 9C, 10C

Cnaphostola venustalis Omelko, 1984: 32; Oku 2003: 65; Park and Ponomarenko 2007: 45; Ueda 2013: 298.

Material examined. Japan – **Hokkaido** [Hokkaido] • 2♂♂; Katsuranosawa, Uryu-cho, Uryu-gun; 20 Jul. 2018; S. Yagi leg. • 1♂, 1♀; Tomuraushi, Shintoku town; 20 Aug. 2000; H. Kogi leg.; gen. slide no. KM-148 (♂) • 2♂♂; Tokachigaoka, Otofuke-cho; 13 Jul. 2000; T. Hirowatari; N.H. Ahn; Y. Miyamoto; H. Okamoto; K. Yamada leg.; gen. slide no. KM-4, 47; OPU • 1♀; Fukuyama, Hobetu Town; 12 Jul. 2005; H. Kogi leg. • 1♀; Siratukari, Atuta; 21 Apr. 2003 em.; H. Kogi leg. • 1♀; Sibi Isikari; 5 Jul. 2016; H. Kogi leg. • 1♂, same locality, 8 Jul. 2007, H.Kogi; 1♂; Ishikari-hama, Ishikari-shi; 18 Jul. 2018; S. Tomura leg. • 3♂♂,1♀; Oyafunebochi, Oyafune-cho, Ishikari; 18 Jul. 2018; S. Yagi leg. • 1♂; Sinkoh, Isikari; 15 Jun. 2003 em.; Host: *Quercus dentata*; H. Kogi leg.; gen. slide no. KM-142 • 1♀; same locality and collector; 25 Jul. 2005 em; Host: *Quercus crispula*; H. Kogi leg. • 1♂; Moiwa, Tomari; 24 Jul. 2006; H. Kogi leg.; gen. slide no. KM-141 • 1♀;

Asari-pass, Otaru; 22 Jul. 2002; H. Kogi leg. • 1♀; Tomakomai shi, Kashiwabara; 21 Jul. 2018; S. Tomura leg. – **Honshu** [Iwate] • 1♂; Dogamori, Morioka; 13 Jul. 1994; N. Doi leg.; TO. – **Honshu** [Chiba] • 1♂; Otake, Narita-shi; 18 Jun. 2016; O. Saito leg. – **Honshu** [Gifu] • 1♀; Oniiwa-onsen, Hiyoshi, Mizumani; 1 Jun. 2017; S. Yagi leg. – **Honshu** [Nagano] • 1♂; Kojiro, Tenryu mura; 5 Jul. 2008; N. Hirano leg.; gen. slide no. KM-108 • 1♀; Reisengoya 2260m, Mt. Norikura, Azumi-vill; 20 Jun. 2001; T. Mano leg.; OPU. – **Honshu** [Aichi] • 1♂; Asahi-highland, Asahi-cho; 13 Jul. 1996; T. Mano leg.; gen. slide no. KM-50; OPU • 1♀; same locality and collector; 7 Jul. 2001; OPU. – **Honshu** [Kyoto] • 1♂; Mt. Ponpon; 1 Jul. 2000; N. H. Ahn leg.; gen. slide no. KM-117; OPU • 2♀♀; same label; gen. slide no. KM-48; OPU – **Honshu** [Osaka] • 1♂; Ikoma, 30 Jun. 1995; S. Kosino leg.; gen. slide no. KM-135; OPU • 1♂; Rokumanji-cho, Higashiosaka-shi; 3 Sep. 2017; H. Shimizu leg. • 1♂, 1♀; Aokaiyama (Toyono-tyo); 7 Jul. 1999; T. Saito leg.; gen. slide no. KM-5(♂), KM-49(♀); OPU • 1♀; same label; 21 Jun. 1999; gen. slide no. KM-52; OPU • 1♀; Iwawaki; 28 Jun. 1952; T. Kodama leg.; OPU • 1♀; Izumi-katuragisan (Kisiwada-si); 17 Aug. 2004; T. Saito leg.; gen. slide no. KM-115; OPU. – **Honshu** [Hiroshima] • 1♂,2♀♀; Yoshiwa, Hatsukaichi-shi; 15–16 Jul. 2017; S. Tomura leg. – **Kyushu** [Fukuoka] • 1♂; Nokonoshima, Fukuoka; 3 Jun. 2013; S. Yagi leg.; gen. slide no. KM-101 • 1♂; Hikosan, Soeda-machi; 1 Jul. 2016; S. Yagi leg.; gen. slide no. KM-13 • 1♂; same label; gen. slide no. KM-28 • 1♂; same label; gen. slide no. KM-97. – **Kyushu** [Kumamoto] • 1♂; Taziri Ubuyama; 14 Jul. 2013; S. Yagi leg.; gen. slide no. SY-1.

Diagnosis. The forewing is white with a small orange patch and a black rounded spot apically. The anellus lobe of the male genitalia is narrow, slender, and longer than the valva, with a sharp and thorn-like spine at the apex, which is a unique characteristic of this species. The apopyhsis posterioris of the female genitalia is ca. 1/3 of the length of papilla analis and approx. half the length of the apophysis anterioris; the signum is absent in the corpus bursae.

Description. Male (Figs 4E, 7C). Forewing length 2.6–3.6 mm. Wingspan 5.5–7.6 mm.

Head: shiny, creamy white with appressed scales. Antennae filiform, basal segment elongate and white; flagellum whitish brown on dorsal surface before middle, then entirely grayish brown beyond, with rather long and fine cilia ventrally. Labial palpus white, long, and recurved; first segment shortest, creamy white; second segment thickened, as much as 2.5 times the length of the first, covered evenly with creamy white scales; third segment as long as second, creamy white, apex sharply acute.

Thorax and tegula: creamy white.

Legs: white; forefemur, tibia, and tarsus suffused inwardly with dark brown, white on outer surface; mid legs entirely white; hind femur and tibia white, dispersed inwardly with brown; with compact ventral and dorsal rows of long, stiff, stout white bristles; all tarsal segments grayish brown.

Forewing: eleven veins, $R_3 + R_4$ stalked, M_1 separate, R_5 absent, anal vein furcate (Fig. 7C). Forewing ground color creamy white to white to ca. 3/4 of the way from base, large orange patch of more or less inverted triangular shape along costal margin, extending to apex of wing; large round black spot apically, bordered by a white line with an orange patch; outer margin blackish brown; small brown scales below api-

cal point; diffused brown scales below orange patch at tornal area; cilia well-fringed, brown-white on costal margin before apex, dark brown with fuscous median band from apex to termen, grayish brown through inner base of the wing.

Hindwing: narrower than forewing, brown with tiny dark brown apical dot; fringe around apex darker in color, long, and brown, cilia well-fringed on inner region of hind wing.

Male genitalia: (Figs 8E, F, 9C) eight abdominal sternite mucronate, short and wide surfaces, anterior 2/3 broadly concave, and then narrow beyond, slightly sharpened basally. Uncus swollen and rounded with short, fine setae on apex. Gnathos sickle-shaped, short and stout, flattened at base posteriorly and then slightly curved toward apex. Tegumen long, nearly three times length of uncus, with dense hairs at approximately its midpoint on dorsal surface. Anellus lobe, a pair of slender processes, slightly longer than valva, slightly broadened and elbowed basally, then narrowly elongate and slightly swollen 1/4 of the way before apex, with rather long and weakly sclerotized thorn-like spine at tip. Valva enlarged at base, with long fine setae on rim of inner surface, gradually narrowing from base to 2/3 of length, slightly curved inwardly, a few setae at corner of anterior margin, rhomboid with a blunt tip apically, short and fine setae on inner and outer surfaces. Vinculum slightly narrow and enlarged surface, a few setae on its median process. Saccus broad basally, somewhat inflate and convex. Phallus large and clavate basally, narrowly elongate in distal half.

Female (Fig. 4F). Forewing length 2.6–3.5 mm. Wing expanse 6.2–7.2 mm. Similar to male.

Female genitalia: (Fig. 10C) papilla analis bilobed, ca. twice length of apophysis anterioris, with long and short fine setae on its entire surface. Apophyses short but apophysis anterioris ca. two times longer than apophysis posterioris. Ostium opening near anterior margin of 8th sternite. Ductus bursae nearly as long as corpus bursae and weakly sclerotized. Corpus bursae slightly oblong; signum absent.

Distribution. Japan (Hokkaido, Honshu, Kyushu), Russia, Korea.

Host plant. *Quercus dentata* (Kogi 2004), *Q. crispula* (Fagaceae) (new host record).

Biology. Kogi (2004) reported that adults of this species fly in July in Hokkaido and larvae live in portable cases in summer until the following spring. In late fall, the larvae move to the underside of twigs of the host plant for hibernation. The next spring, the larvae create triangular cases like hats with fragments of host plant leaves and skeletonize the leaves.

Thiotricha chujaensis (Park, 2016) comb. nov.
Figs 4G, H, 7D, 8G, H, 9D, 10D, 11A–I, 13A–C, 14A–C

Cnaphostola chujaensis: Park and Kim 2016: 172, fig. 1.

Material examined. Japan – **Honshu** [Chiba] • 1♀; Kayano, Orikisawa, Kimitsu-shi; 4 Sep. 2013; O. Saito leg. • 1♂; Otake, Narita-shi; 23 Jul. 2016; O. Saito leg. • 1♀; same locality and collector; 20 Aug. 2016 • 1♂; same locality and collector; 3 Jun. 2017 • 1♀;

same locality and collector; 9 Sep. 2017 • 1♀; same locality and collector; 7 Oct. 2017. – **Honshu** [Ishikawa] • 1♀; Hodatsushimizu cho, Shikinami; 16 Jun. 2018; S. Tomura leg. – **Honshu** [Nagano] • 2♀♀; Kojiro, Tenryu-mura; 19 Jun. 2009; 28 Aug. 2009; N. Hirano leg. • 1♂; Hiraoka, Tenryu-mura; 16 Jun. 2007; N. Hirano leg. [Gifu] • 1♀; Yamagata-gun, Miyama-cho, Iodo; 17 Jun. 1994; T. Mano leg.; gen. slide no. KM-140; OPU. – **Honshu** [Shizuoka] • 1♂; Oonogi Umegasima, Shizuoka-city; 25 Aug. 2001; T. Mano leg.; gen. slide no. KM-32; OPU • 1♀; Shizuoka city, Hirano, Abe river; 23 Aug. 1997; T. Mano leg.; OPU • 1♀; Hirano, Shizuoka city; 26 Aug. 1995; T. Mano leg.; OPU • 1♀; Konya Spa, Shizuoka city; 27 Jul. 2002; T. Oku leg.; TO. – **Honshu** [Aichi] • 3♂♂; Zaikaji-temple, Toyokawa city; 4 Jun. 1994; T. Mano leg.; gen. slide no. KM-18; OPU • 1♂; Somasaka Pass, Toyokawa city; 21 May. 1992; T. Mano leg.; gen. slide no. KM-20; OPU • 2♀♀; same label; gen. slide no. KM-79; OPU • 1♀; Matsumine-cho, Toyota city; 13 Jun. 2001; T. Mano leg.; gen. slide no. KM-77; OPU. – **Honshu** [Mie] • 1♂; Yamadano (Hakusan-tyo); 18 Apr. 2001; Host: *Mallotus japonica* ("shinme"[=a shoot]); 30 Apr. 2001 em.; T. Saito leg.; OPU • 1♂,1♀; same locality and collector; 23 Apr. 2002; gen. slide no. KM-12(♂); OPU • 6♂♂, 2♀♀; same label; 23 Apr. 2002; Host: *Mallotus japonica* ("shinme"[=a shoot]); 30 Apr.–6 May. 2002 em.; gen. slide no. KM-65; OPU • 1♀; Obara-ishiki, Hokusei-cyo; 7 Sep. 1997; T. Mano leg.; OPU • 1♀; Mikuni valley alt 430 m, Hujiwara-cho; 27 Aug. 1998; T. Mano leg; OPU • 1♂; Hijiki 250m, Ueno City; 12 Sep. 1997; T. Mano leg.; gen. slide no. KM-31; OPU • 1♀; Inabe-gun, Fujiwara-cho, Shinodachi; 29 Aug. 1994; T. Mano leg.; OPU. – **Honshu** [Shiga] • 1♂; Makino, Takashima-shi; 13 May. 2015; H. Shimizu leg. – **Honshu** [Kyoto] • 1♂; Mt. Ponpon; 1 Jul. 2000; N. H. Ahn leg.; gen. slide no. KM-34; OPU • 1♂, 1♀; Yamanouchi-cho, Seikanji, Higashiyama-ku, Kyoto-shi; 1 Jun. 2013; H. Shimizu leg. • 4♀♀; Kanmurijima I., Miyazu-shi; 27–28 Sep. 1982; M. Sasakawa; Y. Yoshiyasu; N. Nishida & T. Kamura leg.; gen. slide no. KM-63; OPU. – **Honshu** [Nara] • 1♀; Kasugayama (Nara-si); 26 Aug. 2000; T. Saito leg.; OPU • 1♂; Mt. Takatoriyama (Takatori) ca 580 m; 25 Aug. 1993; Kadohara leg.; OPU • 1♀; Katuragi-shi, Taima; 7 Aug. 2009; H. Shimizu leg. • 1♀; Sannoko, Kawakami village; 18 Jun. 1991; T. Ueda leg.; OPU • 1♂; Kawakami village, Kitamata; 15 Jun. 1993; T. Ueda leg.; gen. slide no. KM-30; OPU. – **Honshu** [Hyogo] • 1♂; Tamida (Inagawa-tyo); 29 Apr. 2002; T. Saito leg.; Host: *Mallotus japonica*; 3 May. 2003 em.; gen. slide no. KM-33; OPU. – **Honshu** [Osaka] • 1♀; Aokaiyama (Toyono-tyo); 10 Jun. 1999; T. Saito leg.; gen. slide no. KM-62; OPU • 1♀; Minou River, Minou City; 5 Aug. 1991; M. Aoyagi & T. Ueda leg.; OPU • 1♂; Higasiosaska-shi, Rokumanji-cho; 23 May. 2015; H. Shimizu leg. • 1♂; same locality and collector; 18 May. 2017 • 1♂; Yao-shi, Kodachi, Jyusan-toge, Fumin no mori; 16 Jul. 2011; H. Shimizu leg. • 1♀; same locality and collector; 17 May. 2016 • 1♀; Hatigamine; 4 Jun. 1993; S. Koshino leg.; gen. slide no. KM-66; OPU • 1♂,1♀; same locality and collector; 15 Jul. 1993; gen. slide no. KM-19 (♂), 126(♀); OPU • 1♀; Minamikawachi-gun, Mizukoshi-toge, Mt. Kongo; 7 Jun. 2015; H. Shimizu leg. • 2♀♀; Tondabayasi Dakeyama ca. 260 m; 4 Sep. 1992; Kadohara leg.; OPU • 1♀; Kawachinagano city, Iwawaki temple; 3 May. 2001; T. Saito leg.; Host: *Mallotus japonica*; 20 May. 2001 em.; OPU • 1♀; Kawati, Iwawakisan; 29 May. 1954; T. Yasuda leg.; OPU • 1♀; Kawakubo; 19 Sep. 1995; S. Kosino leg.; gen. slide no. KM- 72; OPU • 1♀; Tottori

(Hannan); 18 May. 1997; S. Kosino leg.; OPU • 3♀♀; Izumi-katuragisan (Kisiwada-si); 17 Jul. 2004; T. Saito leg.; gen. slide no. KM-42; KM-64; OPU • 1♀; same locality and collector; 21 Jul. 2004; OPU • 1♀; Kyosi, 21 May. 1998; S. Kosino leg.; gen. slide no. KM-128; OPU. – **Honshu** [Wakayama] • 1♀; Wakayama-shi, Nougawa; 11 Jun. 2007; M. Murase leg.; OPU • 1♂, 1♀; Wakayama-shi, Yata; 3 Jul. 2001; T. Hirowatari; B. W. Lee; N. H. Ahn; Y. Miyamoto & K. Yamada leg.; gen. slide no. KM-35(♂),60(♀); OPU. – **Shikoku** [Ehime] • 1♀; Matsuyama; 16 May 1957; M. Okada leg.; gen. slide no, KM-71; OPU. – **Kyushu** [Fukuoka] • 1♂; Orio; 4 Jul. 1958; T. Kawamura leg.; gen. slide no. KM-21 • 2♀♀; Orio; 24 Jun. 1959; T. Kawamura leg.; gen. slide no. KM-78 • 1♂; Kasii, Fukuoka City; 25 May. 1958; K. Yano leg.; gen. slide no. KM-37 • 1♂; Aburayama, Fukuoka; 24 May. 1959; T. Kawarabata leg.; gen. slide no. KM-26 • 1♂; Atago, Nishi-ku, Fukuoka shi; 26 May. 2018 larva; Host: *Mallotus japonica*; 9 Jun. 2018 em.; S. Yagi leg. • 1♀; same locality, collector and host; 9 Jun. 2018 larva; 9 Jul. 2018 em • 2♂♂; Ito campus, Nishi-ku, 26 May. 2017; K.M.M.Kyaw; gen. slide no. KM-43, 44 • 1♂; Mt. Hikosan; 18 Jun.1962; H. Kuroko leg.; OPU • 1♀; same locality and collector; 1 Sep. 1953; OPU • 1♀; same locality; 20 Aug. 2013; S. Yagi leg.; gen. slide no. KM-70 • 1♀; same locality and collector; 25 Jul. 2014; LT; gen. slide no. KM-69 • 1♀; same locality; 31 Jul. 2014; LT; T. Hirowatari & S. Yagi leg. • 1♂; same locality; 4 Sep. 2014; LT, S. Yagi leg.; gen. slide no. KM-15 • 1♂, 4♀♀; same locality and collector; 27 Jul. 2015; LT; gen. slide no. KM-17(♂), 73(♀), 75(♀) • 1♂; same locality and collector; 13 Aug. 2016; LT; gen. slide no. KM-36. – **Kyushu** [Saga] • 1♂; Hokuzan, Saga-shi; 3 Jun. 2017; S. Tomura leg. – **Kyushu** [Kagoshima] • 1♀; Sata; 18 May. 1952; T. Kodama leg.; OPU. – **Ryukyus** [Kagoshima] • 1♀; Nakama, Yakusima Is.; 20 Sep. 1978; S. Moriuti leg.; OPU • 1♂; Okawa Rindo, Kurio, Yakushima, 120m; 21 Jun. 2017; S. Yagi leg. • 2♂♂, 2♀♀; Hatsuno, Amamioshima Is.; 3–5 Apr. 1996; T. Ueda leg.; gen. slide no. TU-751(♂), KM-14(♂), 68(♀); OPU • 1♀; Mt. Yuwan-dake (Lower), Uken-son vill.; 18 May. 2015; S. Sameshima leg.; gen. slide no. KM-57; KGU • 1♀; Akatsuchiyama, Yuwan, Uken-son; 20 Aug. 2014; S. Sameshima leg.; KGU • 1♀; same locality; 6 Jul. 2016; LT 245m; S. Yagi leg.; gen. slide no. KM-83 • 1♂, 3♀♀; Chuorindo, Amamioshima Is.; 6 Apr. 1996; T. Ueda leg.; gen. slide no. KM-25(♂), 67(♀); OPU • 1♀; Nishinakama, Amamioshima Is.; 6 Jun. 1996; T. Ueda leg.; OPU • 1♂; Fureainomori, Uken vill.; 25 Sep. 2002; gen. slide no. KM-27; Host: *Mallotus japonica*; OPU • 1♂; Mt. Yui-dake, Setouchi-cho, Amamioshima Is.; 30 Jun. 2006; U. Jinbo leg.; NSMT-I-L-35780; • 1♀; Tokuno-shima, San Tokunoshima; 9 Jul. 2016; LT 230 m; S. Yagi leg.; gen. slide no. KM-59 • 1♀; Tokuno-shima, Fugusuku; 11 Jul. 2016; S. Yagi leg. – **Ryukyus** [Okinawa] • 1♂; Mt. Terukubi 330 m; Benoki, Kynigami-son; 5 Aug. 2015; L.T; S. Yagi leg.; gen. slide no. KM-29 • 1♀; Yona, Kunigami vill.; 15–18 May. 1998; T. Ueda leg.; OPU • 1♀; Mt. Fuenchijisan, Kunigami vill.; 8 Jun. 1997; T. Ueda leg.; gen. slide no. KM-74. • 1♀; Hentona; 9 Aug. 2016; LT 60 m; S. Yagi leg. • 1♀; Uka, Kunigami-son, Kunigami-gun; 31 May. 2015; 250m LT; S. Yagi leg. • 1♀; Banna Park, Ishigaki Is.; 31 Mar. 2002; B. W. Lee leg.; OPU; gen. slide no. KM-87 • 1♀; Mt Omoto, Ishigaki Is.; 11 Nov. 2003; Host: *Mallotus japonica*; 18 Nov. 2003; S. Shimizu leg.; OPU; gen slide no. KM-76.

Diagnosis. This species is similar to *T. biformis*, which is known in the Russian Far East and Japan but can be distinguished by markings in the distal yellow zone of the

forewing with a distinct black streak below the middle of the yellow zone. The male genitalia is similar to those of *T. epiclista* Meyrick, 1908 described from Khasi Hills, India, but can be distinguished by the basally broadened valva and the presence of a thumb-like basal process bearing numerous setae, the lack of a pre-apical spine on the costal process of the valva, and the slender posterior part of the phallus.

Description. Male (Figs 4G, 7D). Forewing length 2.7–4.3 mm. Wing expanse 5.8–8.8 mm.

Male genitalia: (Figs 8G, H, 9D).

Female (Fig. 4H). Forewing length 3.0–4.4 mm. Wing expanse 5.9–9.1 mm. Similar to male.

Female genitalia: (Fig. 10D) see Park and Kim (2016) for detailed descriptions of the adults and genitalia. In the present study, intraspecific variation observed was the presence or absence of a blackish streak on the yellowish zone of the forewing markings in all examined specimens. Additionally, in contrast, the biggest size of the adult moth of Japanese specimens (wing span 8.8 mm) is quite smaller than that of Korean specimens (wing span 12 mm in Park and Kim's description). In the female genitalia also, Park and Kim described the shape of the signum forming as an elongate plate with dense spicules and located at posterior end; however, that of the Japanese specimens is developing as a pentagonal shape at the posterior end in our observation.

Distribution. Japan (Honshu, Shikoku, Kyushu, Ryukyus), Korea.

Host plant. *Mallotus japonica* (Euphorbiaceae).

Biology (Fig. 11). Although the host plant of *T. chujaensis* is unknown, we found that some specimens of this species preserved in OPU were labeled as having been reared on *Mallotus japonica* (Euphorbiaceae). In the present study, we confirmed that the larvae feed on this plant in the field. The larvae also make portable cases with flower buds or attack the leaf bud (young shoot) of the host plant. The larva uses the flower buds at flowering time to make a case from the plant. After making the portable case, the larva moves from one place to another and attaches the case to the lower surface of the leaf for pupation (Fig. 11C). When there is an early leaf bud (young shoot) on the host plant, the larva penetrates the petiole of the young, newly emerged leaf and feeds inside (Fig. 11E–G). There is no external injury during the feeding period until the leaf is fully grown. Before pupation, the larva cuts the petiole and makes a portable case. After that, it fixes the portable case sideways. Pupation also takes place inside the cases and pupal exuvia is left after the adult emerges.

Pupa (Figs 13A–C, 14A–D). Length ca. 3.2 mm, cylindrical. Color yellowish brown; dark brown before emergence. Vertex armed with many minute spines. Prothorax with a pair of not truly triangular projections on anterolateral corners of tergite. Antenna reaching to posterior margin of 6th abdominal segment. Forewing reaching the mid-way of 6th abdominal segment. Forelegs extending to 3rd abdominal segment; midlegs extending to mid-way of 5th abdominal segment; hindlegs also extending to near posterior margin of 7th abdominal segment. Abdominal segment 7th armed with a row of distinct tergal spines directed posteriorly on anterior margin and indistinct short tergal spines on caudal margin. Seventh abdominal sternite with a pair of oval pads also armed with a row of spines directed anteriorly.

Tenth abdominal segment with a pair of triangular projections at middle, no true cremaster present.

Remarks. This species was described by Park (in Park and Kim 2016) from Chuja Islands which is one of the largest islands among 42 islands in the Jeju Strait, approximately halfway between Jeju Island and the southern coast of the Korean Peninsula. In the present study, it was found that this species is very common and widely distributed in southern Japan.

***Thiotricha elaeocarpiella* Kyaw, Yagi & Hirowatari, sp. nov.**
http://zoobank.org/F05504CD-56BC-4318-855B-5B3640B9D1D2
Figs 5A, B, 6A, B, 7E, 8I, J, 9E, 10E, 12A–I, 13E, F, 14E, F

Cnaphostola sp. 2: Oku et al. 2018: 30, fig. 45.

Type material. *Holotype*: Japan – **Kyushu** • 1 ♂, Fukuoka Pref., Kyushu Univ. Ito Campus, Nishi-ku; 7 Aug. 2017; S. Yagi, T. Hirowatari, K. M. M. Kyaw & C. Tsuji leg.; case on *Rhaphiolepis indica* (case made from flower bud of *Elaeocarpus zollingeri*); 19 Aug. 2017 em.; gen. slide no. KM-88; in ELKU.

Paratypes: Japan – **Kyushu** [Fukuoka] • 1♂; same locality and collectors as holotype; 26 May. 2017; portable case on *Rhaphiolepis indica*; 17 Jul. 2017 em.; gen. slide no. KM-40 • 3♂♂; same locality and collectors as holotype; 31 Jul. 2017; Host: *Rhaphiolepis indica*; 7 Aug. 2017 em.; gen. slide no. KM-104,105 • 2♂♂; same locality and collector as holotype; 22 Jul. 2017; Host: *Rhaphiolepis indica*; 31 Aug. 2017 em. • 1♀; same locality; 22 Jul. 2017; Host: *Rhaphiolepis indica*; 27 Aug. 2017; K.M.M.Kyaw leg.; gen. slide no. KM-132. – **Kyushu** [Kagoshima] • 1♀; Satahetsuka (L), Minamiousumi Town; 9–10 Jul. 2011; T. Terada leg. (KGU). – **Ryukyus** [Kagoshima] • 1♂, 1♀; Amami-Oshima Is., Mt. Yuwan-dake, Uken; 17 Aug. 2012; S. Sameshima leg.; gen. slide no. KM-23(♂), 56(♀); KGU • 1♀; same locality; 4 May. 2013; K. Tsuda leg.; KGU • 1♀; same locality; 4 Aug. 2014; S. Sameshima leg.; KGU • 1♂; same locality; 5 May. 2015; S. Sameshima leg. (KGU); gen slide no. KM-24 • 4♀♀; Akatsuchiyama, Yuwan, Uken-son, 245m; 6 Jul. 2016; LT; S. Yagi leg.; KM-82; 116; 125. – **Ryukyus** [Okinawa] • 1♂; Okinawa ken, Higashi son Kunigami, Takae; T. Hirowatari, S. Yagi, K.M.M.Kyaw leg. • 1♀; Kenmin no-mori, Afuso; 11 Aug. 2017 (larva); Host: *Elaeocarpus zollingeri*; 29 Aug. 2017 em.; same collectors; gen. slide no. KM-89.

Diagnosis. At a glance, the external features are similar to those of *T. chujaensis* (Park, 2016) comb. nov. but it can be distinguished by wing markings in the distal yellow zone of the forewing, which lacks a distinct blackish streak below the middle of the yellow zone and features grayish scales at the costal margin before the apex and the area beyond the tornus. Additionally, it can easily be distinguished based on the male genitalia; the uncus is more rounded apically; the gnathos is U-shaped and acute apically; the valva is narrowly elongate with a sharped pre-apical process ca. 1/4 of the way along its length and the vinculum lacks thumb-like lobes posteriorly; the saccus has a rounded base. The shape of the phallus is also different. However, the male
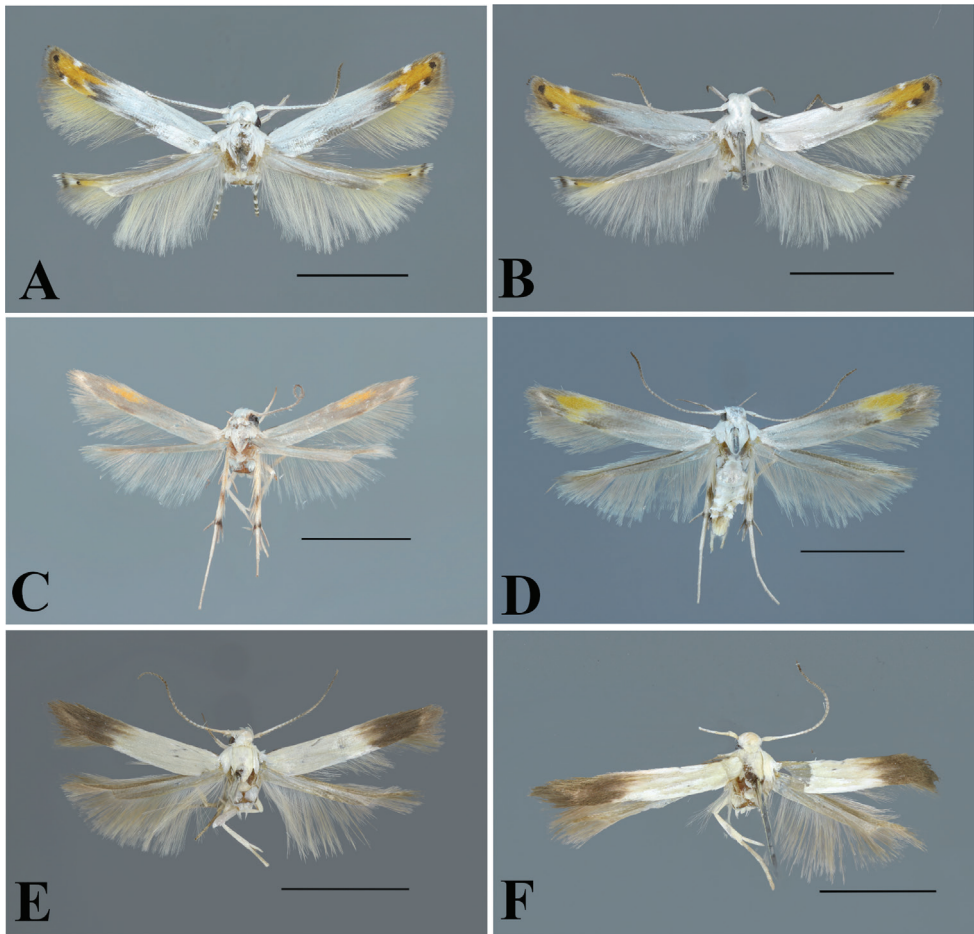
Figure 5. Adults of *Thiotricha* spp. **A** *T. elaeocarpiella* sp. nov., male (holotype) **B** ditto, female (paratype) **C** *T. flavitermina* sp. nov., male (holotype) **D** ditto, female (paratype) **E** ditto, male (paratype) **F** ditto, female (paratype). Scale bars: 2 mm.

genitalia are similar to those of *Thiotricha clidias* Meyrick, 1918, which was described from Khasi Hills, India, although they differ in the shape of the phallus. In *T. clidias* Meyrick, 1918, the phallus is rounded basally, abruptly sinuate and slender in distally but as a cucurbit-shaped in *T. elaeocarpiella* sp. nov.

Description. Male (Figs 5A, 6A, 7E) Forewing length 2.9 mm in holotype, 2.6–3.4 mm in paratypes. Wing expanse 6.4 mm in holotype, 5.2–7.1 mm in paratypes.

***Head*:** shiny creamy white with appressed scales. Antennae filiform, basal segment elongate and creamy white, sparsely speckled with brown scales; flagellum grayish white on dorsal surface before midpoint, then brownish gray beyond on its dorsal and ventral surfaces, with extraordinarily long and fine cilia ventrally. Labial palpus white, moderately long and recurved; first segment approximately half the length of the second, with blackish gray scales on lateral surface; second segment as much as 1.5 times the length of the
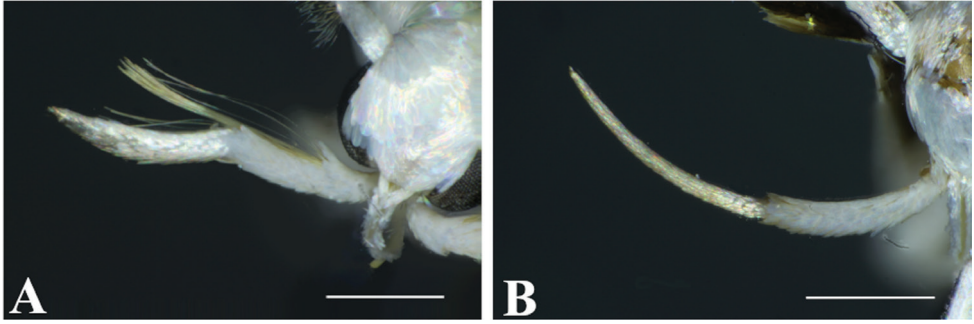
Figure 6. Labial palps of *Thiotricha elaeocarpiella* sp. nov. **A** male with hair pencils, paratype **B** female without hair pencils., paratype. Scale bars: 0.4 mm.

Figure 7. Wing venation of *Thiotricha* spp., male. **A** *T. biformis* **B** *T. angustella* comb. nov. **C** *T. venustalis* comb. nov. **D** *T. chujaensis* (Park, 2016) comb. nov. **E** *T. elaeocarpiella* sp. nov., paratype **F** *T. flavitermina* sp. nov., paratype.

first, creamy white throughout on outer surface; bundle of hair pencils arising from apex of first and second segment, appressed on dorsal surface to near the end of the third segment; third segment as thick as second, with blackish gray scales medially on lower surface ventrally, shiny creamy white evenly on both surfaces, apex sharply acute (Fig. 6A).

Thorax: creamy white. Tegula shiny, creamy white dorsally, ornamented with blackish gray scales along anterior margin.

Legs: white; forefemur, tibia, and tarsus suffused inwardly with blackish brown; scattered with white scales on outer surface; mid femur entirely white; mid tibia and tarsus white but slightly speckled with blackish brown scales on outer surface; hind femur white; hind tibia creamy white, with a row of long, stiff, stout, creamy white bristles above and below, suffused with a small blackish gray scale on lateral outer surface posteriorly; first tarsal segment entirely blackish gray; second and third segment white with blackish gray apical ring; last two segments white.

Forewing: eleven veins, $R_4 + M_1$ stalked, R_5 absent, anal vein furcate (Fig. 7E). Forewing ground color shiny grayish white to white to ca. 3/4 of the way from base; distinct orange zone in distal 1/4, deeply concave along costal margin; costal margin and area beyond tornus grayish colored; small black spot at apex, narrowly connected to another black spot in tornus; cilia before apex to tornus brown, creamy yellow from tornus to inner base of wing.

Hindwing: narrower than forewing, creamy white to grayish white, with pale orange apical zone; apex sharply produced, with small apical black spot; cilia well-fringed to base, fringe around apex creamy white, with broad, dark brown median band.

Male genitalia: (Figs 8I, J, 9E) eighth abdominal sternite more or less triangular, emarginate at the tip, slightly broadened basally with moderately sclerotized margin anteriorly, gradually narrow toward apex. Uncus directed backwards, with broad basal expansion, then narrowly elongate, forming a furrow on lower surface medially, bearing short spines on its lateral margin and abruptly rounded with short and fine setae evenly on its apical dorsal surface. Gnathos U-shaped, stout, strongly bent at basal 1/3, sharply acute apically. Tegumen longer than uncus, slightly concave medially on lateral margins with dense hairs on dorsal surface beyond middle. Anellus lobe, a large process, as much as 1/2 the length of process of valva, ovate membranous pouch at base, with short sclerotized apical spine and short fine setae around apical spine. Valva slender, elongate, broad basally, narrow along 2/3 of length, then dilated apically with dense, long, fine hairs hanging down from its inner surface and developing a sclerotized point, spine-like pre-apical process arising from its base, nearly 1/4 of apex. Vinculum long and slightly narrow, with few rather long setae on its lateral margin. Saccus roundly produced basally. Phallus cucurbit-shaped in basal half, slightly sinuate, slender and recurved upwardly in distal half.

Female (Figs 5B, 6B). Forewing length 2.5–3.3 mm. Wing expanse 5.3–7.1 mm. Similar to male but differs as follows: Labial palps of first segment shortest, with creamy white scales, as thick as second segment; second segment as long as third segment and with white scales on lower surface and grayish or grayish brown scales on upper surface; third segment slender and acute with gray to grayish-brown scales on both surfaces (Fig. 2B)

Female genitalia: (Fig. 10E) papillae anales with long and short fine setae on its entire surface. Apophysis posterioris longer than apophysis anterioris; apophysis anterioris ca. 1/3 the length of posterioris. Ductus bursae rather long, narrow, slightly sclerotized along the posterior half of its length. Corpus bursae clavate in shape; signum rounded at center.
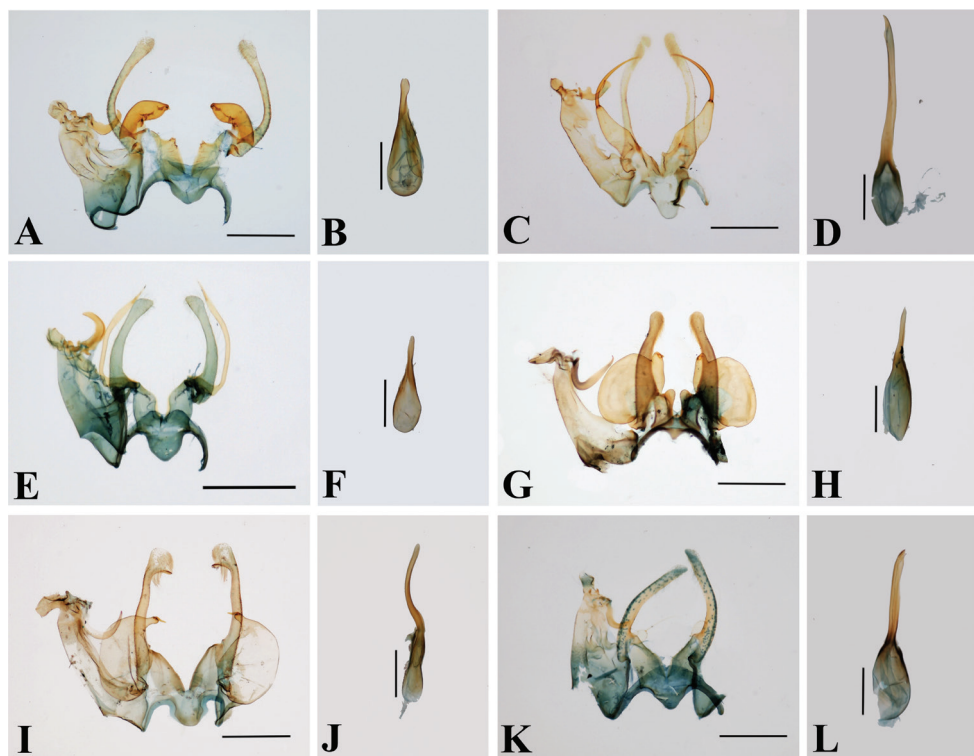
Figure 8. Male genitalia of *Thiotricha* spp. **A, B** *T. biformis* **A** male genitalia, gen. slide no. KM-143 **B** phallus **C, D** *T. angustella* comb. nov. **C** male genitalia, gen. slide no. KM-106 **D** phallus **E, F** *T. venustalis* comb. nov. **E** male genitalia, gen. slide no. KM-1 **F** phallus **G, H** *T. chujaensis* (Park, 2016) comb. nov. **G** male genitalia, gen. slide no. KM-43 **H** phallus **I, J** *T. elaeocarpiella* sp. nov., holotype **I** male genitalia, gen. slide no. KM-88 **J** phallus **K, L** *T. flavitermina* sp. nov., holotype **K** male genitalia, gen. slide no. KM-100 **L** phallus. Scale bars: 0.4 mm (genitalia), 0.2 mm (phallus).

Distribution. Japan (Kyushu, Ryukyus).

Etymology. The name refers to its main host plant, *Elaeocarpus zollingeri*.

Host plant. *Elaeocarpus zollingeri* (Elaeocarpaceae), *Rhaphiolepis indica* (Rosaceae).

Biology (Fig. 12). The larva uses the flower bud or the young shoot of its host plant to construct portable cases. When it utilizes a flower bud, at first, the larva penetrates the bud and then lives and feeds within it. After that, it moves from one flower to another by carrying the bud and attaching it to other flower buds to complete its life cycle (Fig. 12D, E). When the larva is ready to pupate, it attaches the case to the underside of a leaf with silk. When it utilizes a young shoot (Fig. 12C), the larva leaves small dot-like traces of feeding after making cases by the shoot. Pupation also takes place inside the portable cases. After completing development, the adult emerges from the case, leaving the pupal exuvia inside.

Pupa (Figs 13D–F, 14A–D). Length ca. 3.2 mm, cylindrical. Color yellowish brown. Vertex armed with many minute spines. Prothorax with a pair of triangular projections on anterolateral corners of tergite. Antenna and forewing reaching to pos-
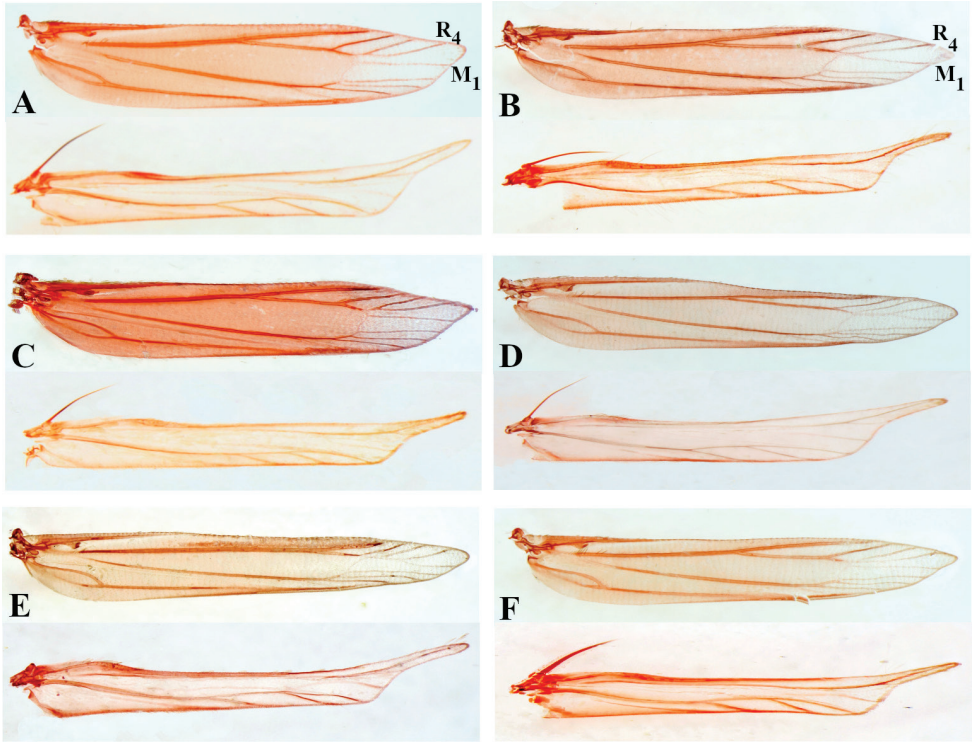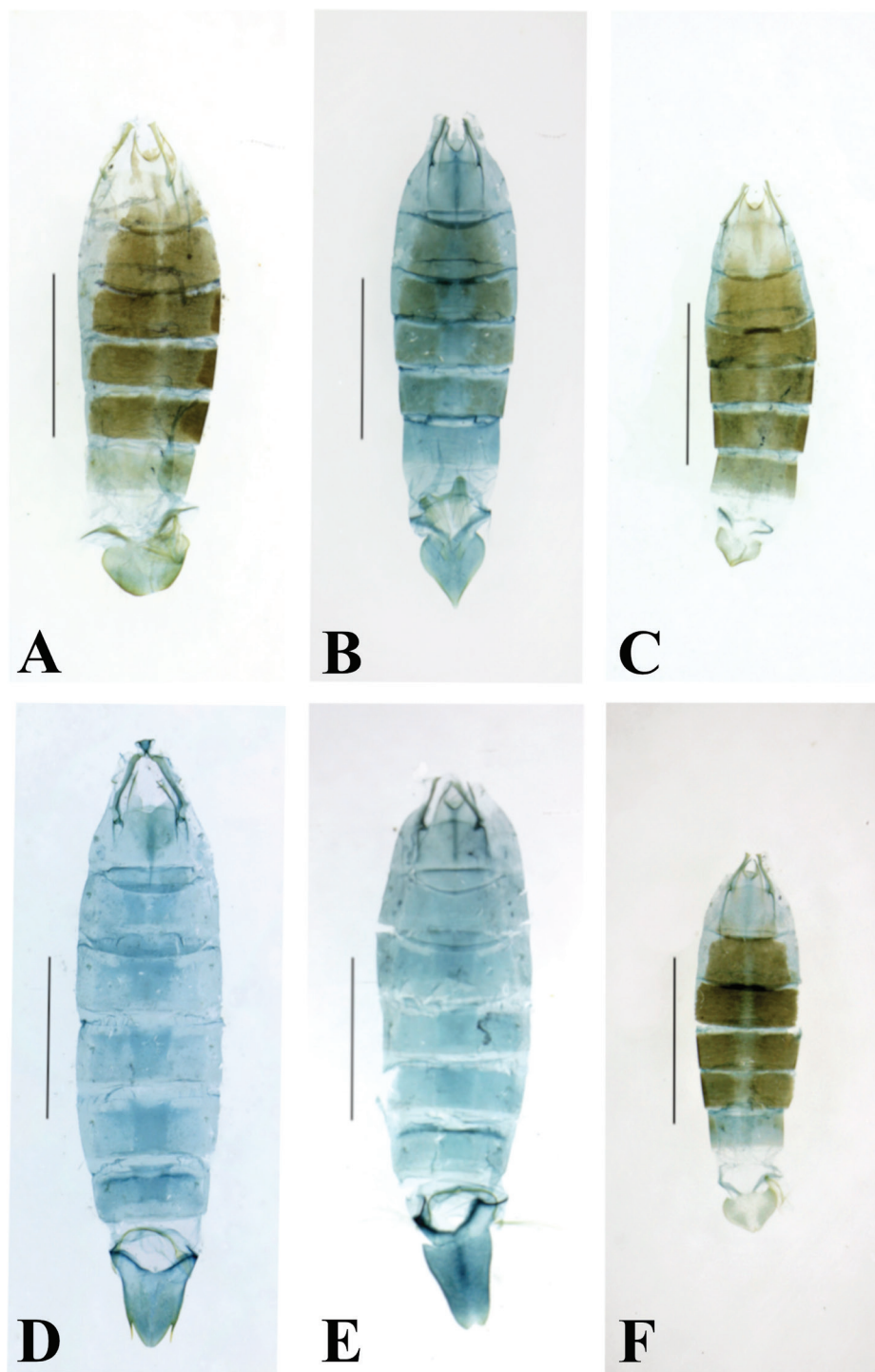
Figure 9. Abdominal segments of *Thiotricha* spp. **A** *T. biformis.* **B** *T. angustella* comb. nov. **C** *T. venustalis* comb. nov. **D** *T. chujaensis* (Park, 2016) comb. nov. **E** *T. elaeocarpiella* sp. nov., paratype **F** *T. flavitermina* sp. nov. Scale bars: 1 mm.
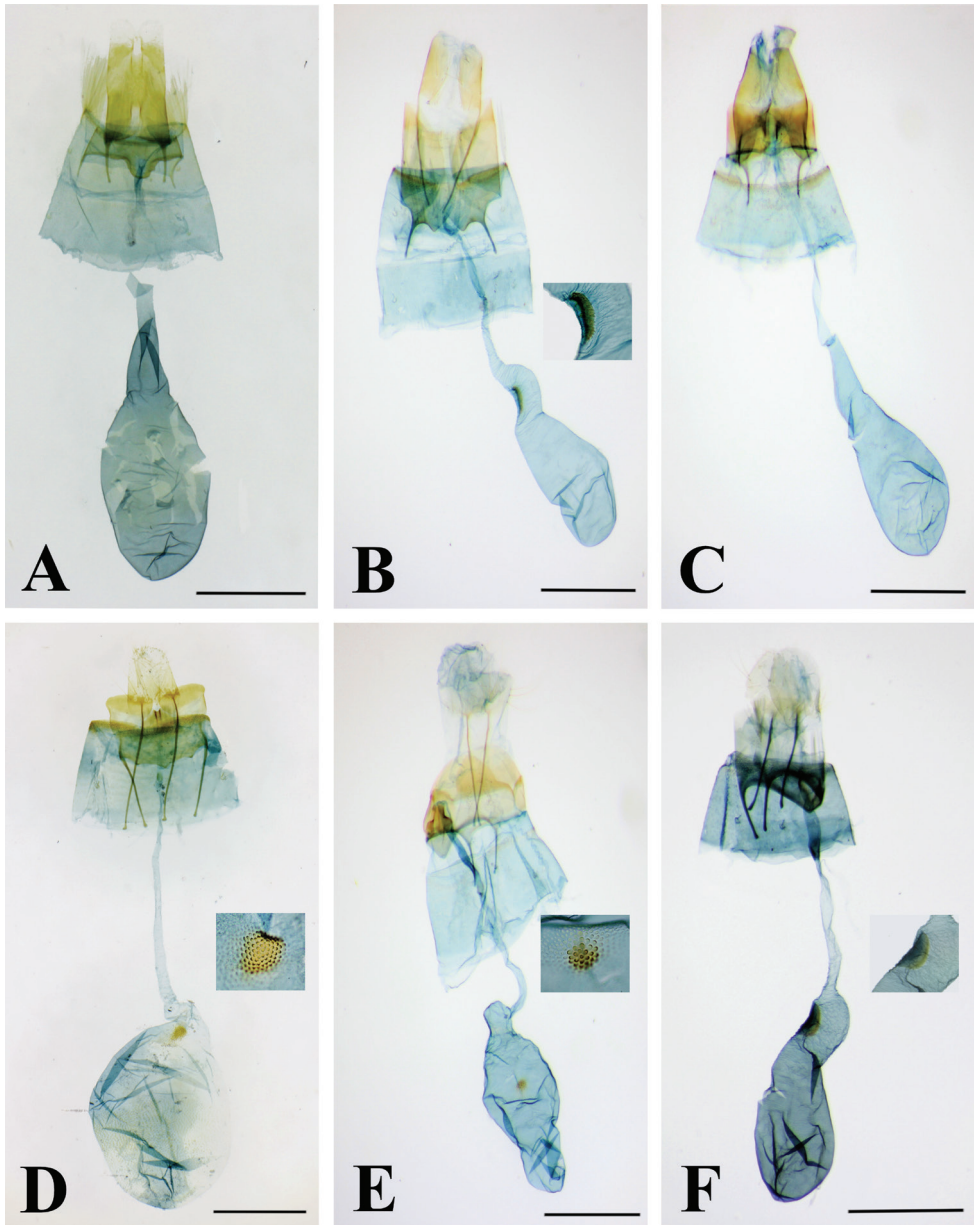
Figure 10. Female genitalia of *Thiotricha* spp. **A** *T. biformis*, gen. slide no. KM-145 **B** *T. angustella* comb. nov., with close up of signum, gen. slide no. KM-118 **C** *T. venustalis* comb. nov., gen. slide no. KM-52 **D** *T. chujaensis* (Park, 2016) comb. nov. with close up of signum, gen. slide no. KM-128 **E** *T. elaeocarpiella* sp. nov. with close up of signum, paratype, gen. slide no. KM-132 **F** *T. flavitermina* sp. nov. with close up of signum, paratype, gen. slide no. KM-55. Scale bars: 0.5 mm.

terior margin of 6th abdominal segment. Forelegs extending to 3rd abdominal segment; midlegs reaching to mid-way of 5th abdominal segment; hindlegs also extending to just beyond the anterior margin of 7th abdominal segment. Seventh abdominal segment

armed with a row of distinct tergal spines directed posteriorly on anterior margin and indistinct short tergal spines on caudal margin. Seventh abdominal sternite with a pair of oval pads also armed with a row of spines directed anteriorly. Tenth abdominal segment with a pair of triangular projections at middle, no true cremaster present.

Remarks. Although this new species was found on two different plants in the present study, it may be that *E. zollingeri* is mainly utilized as the host plant and occasional feeding on *R. indica* occurs when individuals happen to come into contact with this plant. See discussion.

Thiotricha flavitermina Kyaw, Yagi & Hirowatari, sp. nov.
http://zoobank.org/71448E70-4DE6-41EB-A734-F4D5CBEE77F8
Figs 5C–F, 7F, 8K, L, 9F, 10F

Cnaphostola sp. 1: Oku et al. 2018: 29, fig. 44.

Type material. *Holotype*: Japan – **Ryukyu** • 1 ♂; Okinawa Pref., Kunigami vill., Ookunirindo; 26, 27 May. 2000; T. Mano leg.; gen. slide no. KM-100; in OPU.

Paratypes: Japan – **Ryukyus** [Kagoshima] • 1♂; Tokara Island, Nakanoshima Is, Takao; 15 Nov. 2018; K. Sakagami leg. • 1♂; Mt. Akatuti-yama, Uken-son vill., Amamioshima Is.; 21 May. 2013; S. Sameshima leg.; KGU • 5♀♀; same locality and collector; 8–11 Jun. 2013; KGU • 2♂♂, 1♀; same locality and collector; 25–27 May. 2015 (KGU); gen slide no. KM-3,134(♂), KM-46(♀) • 1♂, 1♀; Nankawa path, Amamioshima Is.; 2 Jun. 2013; S. Sameshima leg.; KM-39(♂); KGU • 1♀; Mt. Yuwan-dake, Uken-son vill.; 19 Jun. 2014; S. Sameshima leg. (KGU) [Okinawa] • 6♂♂, 1♀; same locality and collector as holotype; 26–27 May. 2000; KM-6(♂), 38(♂), 55(♀), 99(♂), 102(♂), TU-749(♂); OPU • 1♂; Mt. Nishime, Kunigami-son; 31 May. 2015; S. Yagi leg.; gen. slide no. KM-133.

Diagnosis. The external morphological character of this new species is quite similar to that of *T. angustella*; the wings of both species are shaded brown apically. However, the two can be differentiated based on the brightness of the color of the apical wing markings. In the new species, the wings feature a huge dark brown area distally, so it can be recognized easily at a glance. Likewise, in the male genitalia, the anellus lobe is a small membranous spherical lobe basally with a delicate, thread-like spine apically. Also, the size of apophyses and shape of the signum in the female genitalia are different to those of *T. angustella*. On the other hand, the male genitalia are quite similar to those of *Thiotricha xanthodora* Meyrick, 1923, which was described from Pyinmana, Myanmar, but differ in terms of the uniformly elongate valva, spherical-shaped and straight phallus. In *T. xanthodora*, the shape of the valva is dilated along 1/3 of its length apically whereas the phallus is slightly rounded basally and twisted forward.

Description. Male (Figs 5C, E, 7F). Forewing length 3.3 mm in holotype, 2.5–3.2 mm in paratypes. Wing span 6.2 mm in holotype, 5.6–6.9 mm in paratypes

***Head*:** shiny creamy white with appressed scales. Antennae filiform, basal segment rather large and elongate, white, sparsely speckled with brown scales on dorsal surface;
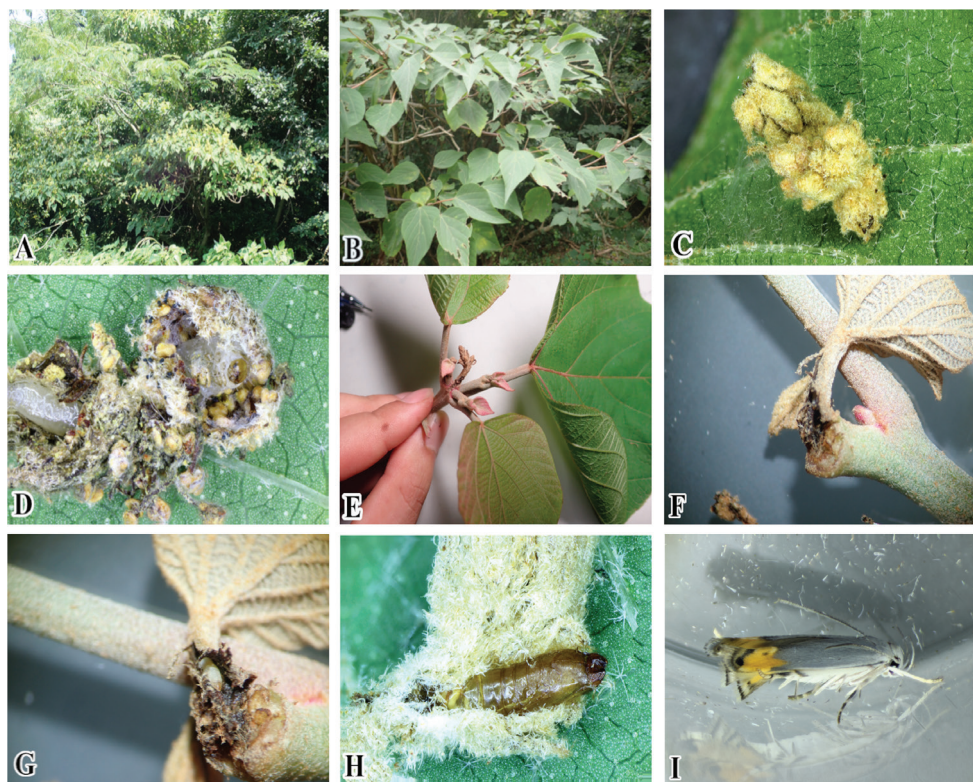
Figure 11. Biology of *Thiotricha chujaensis* (Park, 2016) comb. nov. and its host plant **A** habitat at Kyushu Univ., Fukuoka Pref. **B** host plant, *Mallotus japonica* (Euphorbiaceae) **C** larval portable case made of flower bud attached to the underside of the leaf **D** larva within the portable case **E** a young shoot of host plant **F** Infested part of the shoot **G** Larva inside of the petiole **H** Pupa exuviae **I** Resting posture of adult, lateral view.

flagellum creamy white on dorsal surface before middle, then entirely grayish brown beyond on both surfaces with extraordinarily long and fine cilia ventrally. Labial palps white, long, and recurved; first segment shortest, creamy white with brown scales medially on outer surface; second segment thickened with white scales evenly on both surfaces, as much as 2.5 times the length of the first; third segment as long as second segment, entirely grayish brown, considerably acute and slender.

Thorax and tegula: creamy white.

Legs: white; forefemur and tibia inwardly suffused with brown and white on outer surface; fore tarsus completely brown; mid femur and tibia entirely white; mid tarsus with brown; hind femur and tibia creamy white, with long, stiff, white bristles until the midpoint anteriorly, brown bristles on upper surface at ca. 1/4 beyond half of its length posteriorly, with white bristles ventrally; all tarsal segments dark grayish in color.

Forewing: eleven veins, $R_3 + R_4$ stalked, M_1 separate, R_5 absent, anal veins furcate. (Fig. 7F). Forewing rather broad, rounded and slightly pointed apically, ground color creamy white from base along 2/3 of wing, significantly occupied with a huge dark brown or pale-yellow area along costal margin to apex at ca. 1/3
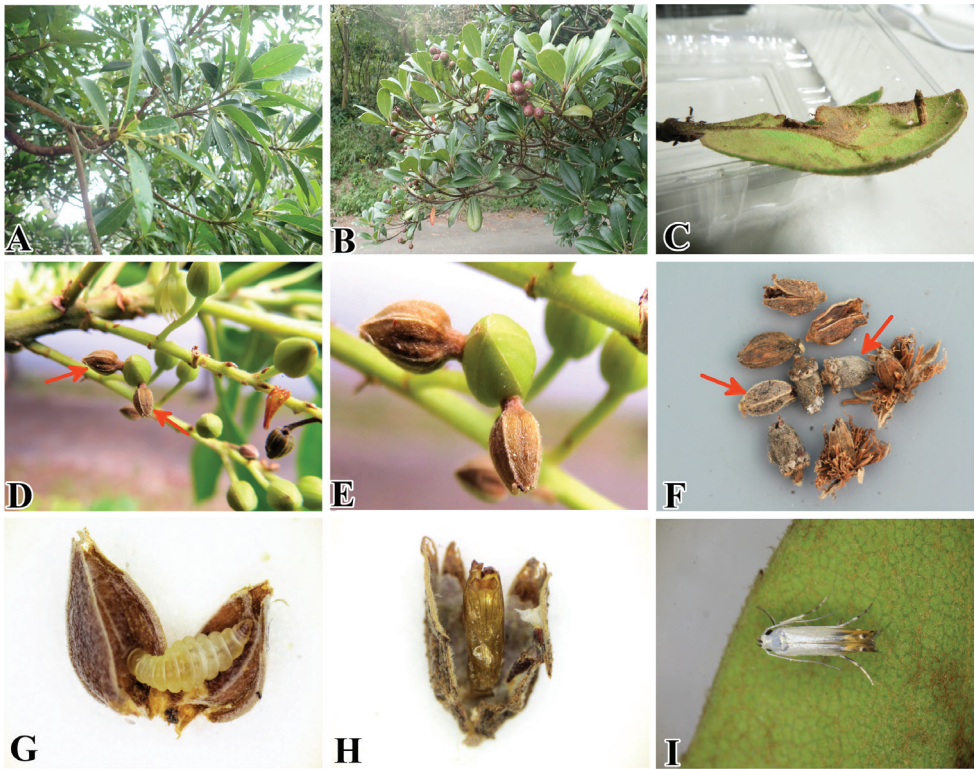
Figure 12. Biology of *Thiotricha elaeocarpiella* sp. nov. and its host plant **A** host plant, *Elaeocarpus zollingeri* (Elaeocarpaceae) **B** host plant, *Rhaphiolepis indica* (Rosaceae) **C** larval portable case made of a young shoot of *R. indica* on the underside of the leaf **D** larval portable cases made with flower buds of *E. zollingeri* **E** close up of larval portable cases **F** different types of portable cases. Left arrow indicates the portable case made of *E. zollingeri*, right arrow indicates the portable case made of *R. indica* **G** larva within the flower bud **H** pupa exuviae **I** resting posture of adult, dorsal view.

apically; cilia well-fringed and dark brown or yellowish brown before apex to inner base of wing.

Hindwing: narrower than forewing, brownish white, pale brown; cilia well-fringed around apex and then white to anterior rim of base.

Male genitalia: (Figs 8K, L, 9F) eighth abdominal sternite obtuse, slightly emarginate at the tip, short, and broadened from base toward apex. Uncus swollen and rather small, like a tubercle, with long and short fine setae on its dorsal surface. Gnathos short and stout, slightly flattened posteriorly, then moderately curved apically. Tegumen extremely long and larger than uncus, with a cluster of dense hairs at approximately its midpoint dorsally. Anellus lobe, a small membranous rounded lobe basally, bearing a flexible and weakly sclerotized thread-like spine, reaching toward nearly 2/3 of valva, slightly curved inwardly, a few spines arising around it. Valva simple, uniformly elongate, broad basally, narrowly elongate from base toward apex, with numerous long fine hairs on its ventral surface. Vinculum narrow and elongate, with long and short fine hairs on rim of its surface posteriorly. Saccus somewhat rounded

Figure 13. Pupa of *Thiotricha* spp. **A–C** *T. chujaensis* (Park, 2016) comb. nov. **D–F** *T. elaeocarpiella* sp. nov. **A, D** dorsal view **B, E** ventral view **C, F** lateral view. Scale bar: 0.1 mm.
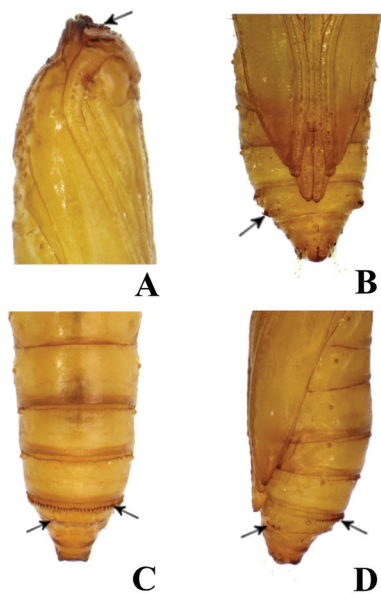
Figure 14. Pupa of *Thiotricha chujaensis* (Park, 2016) comb. nov. **A** head, lateral view Arrow indicates many minute spines on vertex **B** seventh abdominal segment, ventral view Arrow indicates oval pad armed with a row of spines **C** seventh abdominal segment, dorsal view Arrow indicates a row of spines on both anterior and caudal margin **D** seventh abdominal segment, lateral view Arrow indicates oval pad armed with a row of spines and rows of tergal spines. Scale bar: 0.1 mm.
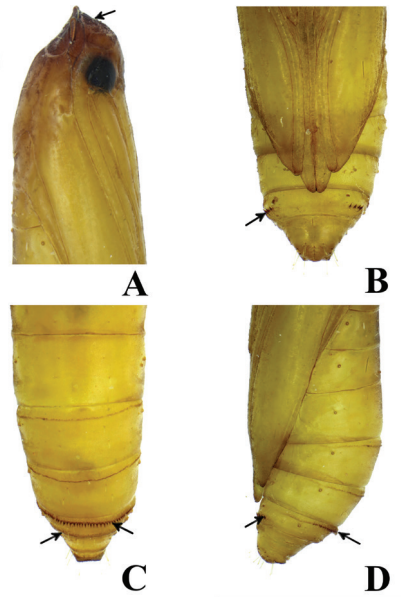
Figure 15. Pupa of *Thiotricha elaeocarpiella* sp. nov. **A** head, lateral view. Arrow indicates many minute spines on vertex **B** seventh abdominal segment, ventral view Arrow indicates oval pad armed with a row of spines **C** seventh abdominal segment, dorsal view Arrow indicates a row of spines on both anterior and caudal margin **D** seventh abdominal segment, lateral view Arrow indicates oval pad armed with a row of spines and rows of tergal spines. Scale bar: 0.1 mm.

and triangular in shape. Phallus spherical basally, becoming narrow and straight, then slender in distal half.

Female (Fig. 5D, F). Forewing length 2.5–3.1 mm. Wing expanse 5.6–6.5 mm. Similar to male.

Female genitalia: (Fig. 10F) papillae anales nearly equal in length to posterior apophysis, with long and short fine setae on its entire surface. Apophysis posterioris longer and apophysis anterioris nearly half the length of posterior. Ostium opening close to anterior margin of 8th sternite. Ductus bursae rather broad, uniformly elongate, nearly same length as corpus bursae. Ductus seminalis arising ca. 1/3 of posterior of ductus bursae. Corpus bursae oblong in shape with narrow; signum short and arch-shaped at left side wall of posterior end.

Distribution. Japan (Ryukyus).

Etymology. The name refers to the coloration of the forewing (yellow apically).

Host plant. Unknown.

Remarks. There are two alternative types of wing markings at the distal portion: the brown form collected from Okinawa-jima Island, and the yellow ones from Amami-oshima Island. As mentioned above, individuals with these wing color variations were separately collected from these two islands in the same season. Therefore, this difference may be due to geographical variation.

Discussion

Morphological characteristics of *Thiotricha* and *Cnaphostola*

Meyrick (1885, 1886) stated that the genus *Thiotricha* is characterized by the extraordinarily long ciliation of the male antennae. However, some *Thiotricha* species (e.g., *T. pontifera* Meyrick, 1932 and *T. attenuata* Omelko, 1993) have minute ciliation in the flagellum. Likewise, in the species previously treated as *Cnaphostola*, the rather long ciliation of male antennae is observed in *T. biformis* and *T. venustalis*, while the antennae of *T. angustella* and *T. chujaensis* exhibit extraordinarily long and fine ciliation.

Meyrick (1886, 1918) also defined *Thiotricha* and *Cnaphostola*, respectively, based on the forewing venation. That is, "vein 8 is absent (coincident with 7) in *Thiotricha*, whereas veins 6 and 8 are stalked, and vein 7 is absent in *Cnaphostola*." However, Meyrick's description of the forewing venation of *Thiotricha* is incongruent with that of its type species, based on the figure provided by Clarke (1969b) in fig.1a, plate 226. It may be that Meyrick overlooked vein 2 (CuA_2) and confused veins 6 (M_1) and 8 (R_4) of the forewing; they were referred as "veins 7 and 6" in 1886, and "veins 8 and 6" in 1918. Regarding wing venation of the examined specimens in the present study (Fig. 7), vein 2 (CuA_2) is indistinct and it is likely that it is usually overlooked. In the species previously treated as *Cnaphostola*, M_1 and R_4 are stalked in *T. angustella*, *T. chujaensis*, and *T. elaeocarpiella*, but not in *T. biformis*, *T. venustalis*, or *T. flavitermina*. This variation was also pointed out by Meyrick (1886) when describing the characteristics of *Thiotricha*, i.e., vein M_1 was stalked with R_4 or separated with R_4 (explained as "6 rising out of 7 or separate"). Therefore, on the basis of the forewing venation it is impossible to distinguish these two genera.

In terms of male genitalia, *T. chujaensis*, *T. elaeocarpiella*, and *T. flavitermina* have a similar-shaped anellus lobe and gnathos, as some other *Thiotricha* species. Omelko (1984) described three species in *Cnaphostola* based on the similarity of their genitalia, but he did not show that they share diagnostic characters with the type species. Huemer (1993), Tanabe (2013), and Karsholt et al. (2013) mentioned the genital characters of the genus *Thiotricha* (e.g., broad uncus, finger-formed anellus lobe, posteriorly bifurcated sternum VIII, and so on). The type species of the two genera share most characteristics of the head, labial palpus, wing venation, and the finger-like anellus lobe in the male genitalia, although we could not observe the condition of sternum VIII.

Judging from figures given by Clarke (1969a), the type species, *Cnaphostola adamantina* Meyrick, 1918, also has the finger-like anellus lobe in the male genitalia. Moreover, in three species described by Omelko (1984), the male genitalia of both *T. biformis* and *T. angustella* have a finger-like anellus lobe while *T. venustalis* has a slender process. Further, the gnathos shape of these three species is short and stout, as in the type species of *Thiotricha*, *T. thorybodes*, whereas it is long and curved in *C. adamantina* as in some other *Thiotricha* species. That is why, on the basis of the genitalia characters, it is inconclusive whether the type species of *Cnaphostola* belongs to *Thiotricha*, and we therefore refrain from synonymizing the genera here.

Table 1. Morphological characters shared by some Japanese *Thiotricha* species.

Species	Ciliation of male antenna (-) minute (+) rather long (++) extraordinarily long	Labial palps 2nd joint thickened with appressed scales, terminal joint as long as 2nd and acute	Wing venation R4 + M1 (6 and 8) stalked	Sternum VIII bifurcate	Anellus lobe in male genitalia
T. biformis	+	+	-	-	+
T. venustalis comb. nov.	+	+	-	-	+
T. pontifera	-	+	-	-	+
T. attenuata	-	+	-	-	+
T. flavitermina sp. nov.	++	+	-	-	+
T. celata	++	+	-	-	+
T. pancratiastis	++	+	-	-	+
T. synodonta	++	+	-	-	+
T. angustella comb. nov.	++	+	+	-	+
T. chujaensis comb. nov.	++	+	+	-	+
T. elaeocarpiella sp. nov.	++	+	+	-	+
T. prunifolivora	++	+	+	-	+

In addition, we studied the pupal morphology, and found that characters of *T. chujaensis* and *T. elaeocarpiella* were congruent with those of *T. prunifolivora* as described by Ueda and Fujiwara (2005). However, we point out here that Ueda and Fujiwara (2005) erroneously described that tergal spines are present on the 6th and 7th abdominal segments of the pupa of *T. prunifolivora.* In contrast, these spines are exactly on the caudal and anterior margins of 7th abdominal segment. This condition was also detected in *T. pancratiastis* Meyrick, 1921 and *T. trapezoidella* (Caradja, 1920) in our observations (Kyaw et al. unpubl. data 2019). Although the pupal morphology of *T. angustella* and *T. venustalis* could not be studied, their pupae may also possess such spines which may assist in protrusion of the pupa from their cases; both species were reported to be case bearers (Kogi 2004, 2008).

Based on the reasoning above, we conclude that Japanese species of *Cnaphostola* should be treated in the genus *Thiotricha*, because they share morphological characters of the antenna, labial palps, wing venation and the anellus lobe in the male genitalia with the type species *Thiotricha thorybodes* Meyrick, 1886 and with some other species in that genus (Table 1).

On the other hand, species of *Thiotricha* are also similar to those of *Polyhymno.* Ueda and Fujiwara (2005) noted a difference in forewing venation: R_5 is present (5 radial veins are present) in the type species of *Polyhymno*, whereas R_5 is absent (4 radial veins are present) in the type species of *Thiotricha.* Therefore, it may be appropriate that all species previously treated as *Cnaphostola* should be combined in *Thiotricha*, having 4 radial veins. According to Karsholt et al. (2013), the type species of *Polyhymno* have posteriorly non-bifurcated sternum VIII, a simple valva with reduced anellus lobe, and shorter ciliae on the male antennae. However, we observed that these characters are also shared by some *Thiotricha* species. For example, the sternum VIII is not bifurcate in some *Thiotricha* species, e.g., *T. prunifolivora*, *T. pancratiastis*, *T. pontifera*, *T. indistincta*

Omelko, 1993, *T. celata* Omelko, 1993, *T. attenuata*, and *T. synodonta* Meyrick, 1936. Therefore, reexamination of the diagnostic characters of these genera is necessary.

In the present study, although we could not find any definite diagnostic character for separating each genus, we confirmed that the presence of anellus lobe in the male genitalia would provide one of the possible characters for *Thiotricha* which is also shared by all examined species. Furthermore, the pupal morphological characters also support the genus *Thiotricha*. In future studies, a molecular analysis would hopefully clarify the phylogenetic relationships between genera and species and solve the taxonomic problems of the generic delimitation.

Host utilization and feeding habits of larva

Although Thiotrichinae are known to utilize plants of ten families (all in eurosids), the host range of each species is usually restricted to one genus or a few related genera (Kuroko 1957; Sattler 1982; Huemer 1993; Omelko 1993; Oku 2003; Ueda and Fujiwara 2005; Kaiser et al. 2008; Fujita et al. 2009; Ueda 2011, 2013). Therefore, the host utilization of *Thiotricha elaeocarpiella* is unusual for this subfamily.

In the present study we observed *T. elaeocarpiella* larvae making portable cases on two different unrelated host plants, *Elaeocarpus zollingeri* (Elaeocarpaceae) and *Rhaphiolepis indica* (Rosaceae), in Okinawa-jima Island and Kyushu. First, we discovered that this species made portable cases with parts of *R. indica* in May 2017 in Fukuoka, Kyushu (Fig. 12C). In the same place in July and August 2017, where *E. zollingeri* grew next to *R. indica*, we also found many differently shaped portable cases made of the flower buds of *E. zollingeri* (N = 14) and some portable cases made of *R. indica* under the leaves of *R. indica* (N = 2). Secondly, on Okinawa-jima Island, we found many portable cases on *E. zollingeri* in August 2017. Although most of the flower buds were already blooming, we found a certain number (N > 20) of portable cases attached to flower buds, most with pupal exuviae inside. We consider *E. zollingeri* to be the main host plant of this species because a great number of portable cases were found on *E. zollingeri* in Kyushu and Okinawa-jima Island.

However, whether the larvae of *T. elaeocarpiella* consumed both plants or accidentally shifted host plants is a controversial matter. Based on shape, the portable case found in May seems to be made of a young shoot of *R. indica*, and we also observed some holes on the leaf near the portable case. These holes are similar to the feeding trace made by *T. prunifolivora*, and the larvae of *T. elaeocarpiella* actually feed on the leaf of *R. indica*. Therefore, we concluded that this species can utilize both of these two plants. In *T. chujaensis*, we found that the larva penetrates the leaf bud (young shoot) of the host plant and then enters entirely and feeds inside (Fig. 11E–G). The last instar larva uses the petiole of the young leaf as a portable case before pupation. Additionally, the larva uses the flower bud at flowering time and makes a portable case on the plant (Fig. 11C, D). After making the portable case, the larva moves and attaches it to the lower surface of a leaf until pupation.

Some *Thiotricha* species showing similar larval feeding habits have been identified. Ueda and Fujiwara (2005) reported that *T. prunifolivora* has three generations per year, and the larvae of each generation have different feeding habits and make a different type of portable case. In the overwintering generation, the hatched larva penetrates the flower bud of the host plant, *Symplocos prunifolia* (Symplocaceae), then uses it as a portable case and attaches the case to the apex of another flower bud with silk. In the first generation, the larva bores into the developing seed and uses it as a portable case, usually accumulating five developing seeds before pupation. In the second generation, the larva bores into the developing seed at first, then after feeding on (usually) two developing seeds, the larva moves to the underside of the host plant and makes several circular holes by feeding. Another species, *T. pancratiastis*, known as a foliage feeder (leaf miner) of *Morella rubra* (Myricaceae), is also reported as a seed predator; the larvae of this species bore into the fruit and, after hollowing it out, utilize it as a portable case. This species probably has two generations annually in Honshu and more in the Ryukyus (Fujita et al. 2009; Ueda 2011, 2013; as *Polyhymno pancratiastis*).

In *T. elaeocarpiella*, *T. chujaensis*, *T. prunifolivora*, and *T. pancratiastis*, the larval feeding mode and behavior are different among generations. These species occur in Honshu, Kyushu, and Ryukyus, have more than two generations a year, and utilize different parts of host plants that grow in temperate climates. On the other hand, *T. venustalis*, *T. angustella*, and some species of *Thiotricha* in the cool climate of the East Palearctic have one generation a year and one mode of feeding (Omelko 1993; Oku 2003; Ueda 2013).

Hence, we presume that the larval feeding mode in *Thiotricha* usually involves the creation of portable cases on host plants, whereas the larvae of *Polyhymno* are leaf-spinners and leaf-webbers (Karsholt et al. 2013). There are some exceptions, such as *T. trapezoidella* and *T. indistincta*; the larvae of *T. trapezoidella* bore into the petiole of Juglandaceae and do not make a portable case (Oku 2003; Ueda 2011, 2013), and *T. indistincta* is a leaf-webber in *Carpinus* spp. (Betulaceae) (Omelko 1993; Oku 2003). In *T. trapezoidella*, however, we confirmed that the larvae make a portable case by cutting the surface of the leaf transversely around the upper tip of the leaf and folding it as a case when the larva is nearly grown to the late instar (Kyaw et al. unpubl. data, 2019). This shows that it is necessary to reinvestigate the larval feeding mode of *Thiotricha* and *Polyhymno* species in detail.

From the results of our taxonomic study together with an exploration of the biology of these species, the evolution of host plant utilization can be elucidated based on species relationships in this genus and its relatives. In future studies, it will be necessary to clarify the biology and DNA sequences of most species.

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RESEARCH ARTICLE

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Five new species of *Mydaea* from China (Diptera, Muscidae)

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Citation: Du J, Hao B, Xue WQ, Zhang CT (2019) Five new species of *Mydaea* from China (Diptera, Muscidae). ZooKeys 897: 101–114. https://doi.org/10.3897/zookeys.897.39232

Abstract

Five new species of *Mydaea* are described from China, namely *M. adhesipeda* Xue, **sp. nov.**, *M. combiniseriata* Xue, **sp. nov.**, *M. qingyuanensis* Xue, **sp. nov.**, *M. quinquiseta* Xue, **sp. nov.**, *M. wusuensis* Xue, **sp. nov.**, and an addendum to the key of the *Mydaea* in China is given.

Keywords

Calyptratae, description, key, Muscoidea, taxonomy

Introduction

Mydaea Robineau-Desvoidy, 1830 is a genus in the subfamily Mydaeinae (Diptera, Muscidae). It comprises approximately 120 species worldwide. About 100 species were recorded from the Palaearctic, 35 from the Nearctic, 26 from the Neotropical, nine from the Oriental, and two species from the Afrotropical regions (Hennig 1957; Huckett 1965; van Emden 1965; Vockeroth 1972; Pont 1977, 1980, 1986, 1989; Shinonaga 2003; Carvalho et al. 2005). Thirty species were reported from China, ap-

proximately one-fifth of the species worldwide (Ma et al. 1986; Xue 1992; Xue and Chao 1996; Feng 2000a, b, 2003; Xue and Tian 2012, 2014). During the study of our collection in recent years, five new species were found in the mountains from Liaoning, Heilongjiang, and Sichuan provinces and from the Ningxia Hui Autonomous Region of China. Descriptions, figures, and an addendum to the key to the Chinese species of the genus *Mydaea* are provided.

Methods and materials

The specimens examined for this study were collected by sweeping. The genitalia were detached from the abdomen, bleached by heating in a 10% NaOH solution (approximately 100 °C) for about 20 min, placed in a droplet of glycerol, and examined using an Olympus SZX7 stereomicroscope. After examination, the genitalia were stored in a small plastic vial filled with glycerin and pinned with the specimen. The type material is deposited in the Insect Collection, Shenyang Normal University, Shenyang, China (**SYNU**).

The morphological terminology follows that of Cumming and Wood (2017). Absolute measurements of the body length are in millimeters (mm). Adhesive hairs are special hair-like setae modified for climbing or gripping. The following abbreviations are used for various morphological structures: *acr*, acrostichal setae; *prst-acr*, presutural acrostichal setae; *dc*, dorsocentral setae; *ial*, intra-alar setae; *pra*, prealar setae; *av*, anteroventral setae; *ad*, anterodorsal setae; *pd*, posterodorsal setae; *p*, posterior setae; *pv*, posteroventral setae.

Results

Mydaea Robineau-Desvoidy, 1830

Mydaea Robineau-Desvoidy, 1830: 479. Type species: *Mydaea scutellaris* Robineau-Desvoidy, 1830 (by subsequent designation of Coquillett 1901).
Xenomydaea Malloch, 1920: 144. Type species: *Xenomydaea buccata* Malloch, 1920 (by original designation).

Generic diagnosis. Meron and anepimeron bare; katepisternal setae 1+2; lower margin of posterior spiracle without row of setae; Sc bent bow-shaped; dorsal and ventral surfaces of radial node with hairs, M_1 straight; lower calypter tongue-shaped; mid tibia with at least 2 *p*; hind coxa bare on posterior surface, hind tibia without *pd*. The cerci of all Chinese species have been divided into seven different kinds. We figure together these seven kinds to show there differences (Fig. 1).
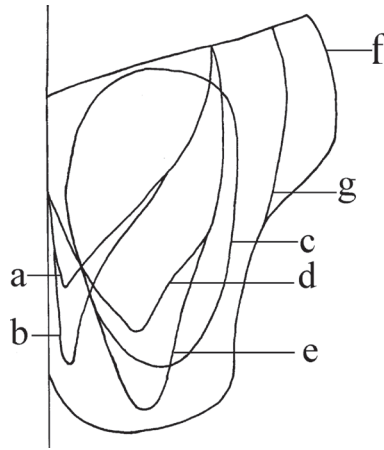
Figure 1. Cerci types of Chinese *Mydaea* **a** *M. subelecta* Feng, 2000; *M. gracilior* Xue, 1992 **b** *M. jiuzhaigouensis* Feng & Deng, 2001; *M. nubila* Stein, 1916; *M. scolocerca* Feng, 2000 **c** *Mydaea ancilloides* Xue, 1992; *M. jubiventera* Feng & Deng, 2001; *M. fuchaoi* Xue & Tian, 2012; *M. flavifemora* Feng, 2000; *M. kangdinga* Xue & Feng, 1992; *M. setifemur* Ringdahl, 1924 **d** *M. brunneipennis* Wei, 1994; *Mydaea glaucina* Wei, 1994; *M. nigra* Wei, 1994; *M. shuensis* Feng, 2003; *M. minor* Ma & Wu, 1986; *M. sinensis* Ma & Cui, 1986; *M. franzosternita* Xue & Tian, 2014; *M. laxidetrita* Xue & Wang, 1992 **e** *M. nigribasicosta* Xue & Feng, 1996; *M. latielecta* Xue, 1992; *M. tinctoscutaris* Xue, 1992 **f** *M. affinis* Meade, 1891; *M. bideserta* Xue & Wang, 1992; *M. urbana* (Meigen, 1826) **g** *M. brevis* Wei, 1994; *M. emeishanna* Feng & Deng, 2001; *M. discocerca* Feng, 2000; *M. minutiglaucina* Xue & Tian, 2012.

Addendum to the key by Xue and Tian (2014) of *Mydaea* (males only)

7	Scutellum yellow or with yellow basal part	**8**
–	Scutellum entirely black	**7a**
7a	Hind femur black	***M. wusuensis* Xue, sp. nov.**
–	Hind femur yellow or fuscous	**11**
8	Hind femur with *pv* in rows	**9**
–	Hind femur with *pv* on the base at most	**8a**
8a	Postpronotal lobe yellow	***M. tinctoscutaris* Xue, 1992**
–	Postpronotal lobe black	***M. quinquiseta* Xue, sp. nov.**
9	Anterior spiracle yellow	**9a**
–	Anterior spiracle fuscous	**10**
9a	2 *prst-acr*	***M. gracilior* Xue, 1992**
–	*prst-acr* absent	***M. qingyuanensis* Xue, sp. nov.**
10	Coxae, trochanters of fore leg and all tarsi fuscous	**10a**
–	All legs yellow	***M. kangdinga* Xue & Feng, 1992**
10a	Ventral surface of fore femur fuscous on basal half	***M. setifemur* Ringdahl, 1924**
–	Ventral surface of fore femur yellow on basal half	***M. combiniseriata* Xue, sp. nov.**

13   Frons subequal with anterior ocellus in width abdomen with shifting patches.....
      ....*M. discocerca* **Feng, 2000**
–   Frons about 2 times as wide as anterior ocellus; abdomen without shifting patch ...**15**
14   *Pra* about 1/2 of posterior notopleural seta in length; wing brown, basal half of
      hind femur with *pv* obviously..**19**
–   *Pra* longer than posterior notopleural seta; wing yellow, hind femur with sparse
      and short *pv* **20**
15   Antennal arista ciliated, the longest hair subequal with antennal postpedicel in
      width **16**
–   Antennal arista short ciliated, the longest hair longer than antennal postpedicel in
      width **17**
16   Parafacial about 1/2 of postpedicel in width......................... *M. brevis* **Wei, 1994**
–   Parafacial subequal with postpedicel in width... **16a**
16a   Basicosta dark-brown, *pra* approximately 1.3 times as long as posterior noto-
      pleural seta; fore tibia without median *p*, ventral surface of fore tarsus without
      adhesive hairs ....*M. fuchaoi* **Xue & Tian, 2012**
–   Basicosta yellow, *pra* shorter than posterior notopleural seta; fore tibia with 1 median
      p , ventral surface of fore tarsus with adhesive hairs .... *M. adhesipeda* **Xue, sp. nov.**

Mydaea adhesipeda Xue, sp. nov.
http://zoobank.org/C84CC490-8266-4ACD-96AC-2FE70E5E5058

Type material. *Holotype.* China, 1 ♂, Ningxia Hui Autonomous Region, Jingyu-
an, Liupan Mountains, 35°39'N, 106°34'E, alt. 2200 m, 3 July 2009, Zhiyuan Yao,
(SYNU). *Paratypes.* 2♂♂, 5 ♀♀, same data as holotype.

Diagnosis. *Post-dc* 4, scutellum dark black; basal and apical scutellar seta approxi-
mately as long as hind tibia; coxae and trochanters brown, tarsi dark brown, ventral
surface of fore tarsus with adhesive hairs; femora and tibiae yellow; hind femur with
av rows on distal half.

Description. Male. Body length 8.0–8.5 mm. *Head:* eyes bare, facets on upper
half not enlarged. Frontal vitta black; lunule brown; genal and postgenal hairs entirely
black; antenna fuscous, palpus black. Frons as wide as postpedicel, and slightly wider
than distance between outer margins of posterior ocelli; fronto-orbital plate narrow, 2/5
of width of frons at middle; fronto-orbital plates, parafacials and genae covered with
sparse gray pruinosity, parafacials as wide as or narrower than the width of postpedicel;
genal height approximately 1/9 of eye height; lower face not projecting, vibrissal angle
situated behind frontal angle in lateral view. Frontal setae with 7 or 8 pairs, upper 2 pairs
short and situated on lower 4/5 of frons; proclinate orbital setae absent; ocellar setae ap-
proximately 2/3 of lower pair of frontal setae in length; postpedicel 3.5 times as long as
wide; arista ciliated and longest hair approximately as long as width of postpedicel; palpi
as long as prementum; prementum 2.5 times as long as wide, and covered with gray
pruinosity; labellum extending to posterior part, and approximately 4/5 as long as palpi.
Thorax: ground color black, covered with sparse gray pruinosity and slightly shining;

scutum with 4 black vittae, and the inner vitta extending to scutoscutellar suture; *acr* 0+1; *dc* 2+4; *ial* 0+2; *pra* slightly shorter than posterior notopleural seta; notopleuron with small hairs; scutellum black, basal and apical scutellar seta strong, approximately as long as hind tibia; basisternum of prosternum, anepimeron, meron, and katepimeron bare; katepisternal setae 1+2; 1 anepisternal seta; anterior and posterior spiracles yellow. ***Wings:*** semi-hyaline; tegula dark brown; basicosta yellow; ventral surface of vein C with hairs; vein Sc bow-shaped; dorsal and ventral surfaces of radial node with hairs; the middle part of crossvein dm-m bent towards base of wing, areas around crossveins r-m and dm-m not clouded; R_{4+5} and M_1 straight and diverging slightly distally; calypter yellowish, lower calypter tongue-shaped; halter knob yellow. ***Legs:*** coxae and trochanters brown, tarsi dark brown, femora and tibiae yellow; ventral surface of fore tarsus with adhesive hairs, fore tibia with 1 median *p*; mid femur without *av* and 6 *pv* on basal half, 2 preapical *ad*, 3 *pd*, mid tibia with 2 or 3 (a few with 4) *pv*; hind femur with *av* rows on distal half, *av* short on basal half, without *pv*, hind tibia with 4 *av*, 2 *ad*, without apical *pv*; tarsi longer than tibiae; fore claws and pulvilli approximately 1.2 times as long as tarsomere 5, mid claws and pulvilli approximately as long as tarsomere 5, hind claws and pulvilli shorter than tarsomere 5. ***Abdomen:*** Black in ground color, ovate in dorsal view, covered with gray pruinosity, both sides without color shifting patch, tergites 3 and 4 with a black median vitta, tergites 4 and 5 each with a complete row of posterior marginal setae, both sides of tergite 5 with 3 or 4 discal setae on anterior half; sternite 1 bare; middle part of lateral margin of cerci invaginated slightly in posterior view.

Female. Body length 8.9–9.5 mm. Frontal vitta 3.5 times as wide as fronto-orbital plate; 7 frontal setae; 2 upper orbital setae; genal height approximately 1/6 of eye height; ventral surface of fore tarsus without adhesive hairs; mid tibia with 3 or 4 *p*; fore claws and pulvilli shorter than tarsomere 5. Other characters as in male.

Remarks. This species is similar to *Mydaea fuchaoi* Xue & Tian, but differs from the latter in the following features: frontal vitta narrower; genae approximately 1/9 of eye height; palpi shorter; fore claws and pulvilli longer, fore tibia with 1 median *p*; ventral surface of fore tarsus with adhesive hairs; hind claws and pulvilli shorter, hind tibia with 4 *av*; middle part of lateral margin of cerci slightly concave in posterior view.

Etymology. The species name refers to ventral surface of the male fore tarsus which has adhesive hairs. It is derived from the Latin words *adhes* meaning adhesive and *ped* meaning leg.

Distribution. China, Ningxia Hui Autonomous Region (Liupan Mountains).

Mydaea combiniseriata Xue, sp. nov.
http://zoobank.org/CD4832B2-CB16-4CB4-9672-F78F044B8C2F

Type material. *Holotype.* China, 1 ♂, Liaoning Province, Qingyuan, 41°81'N, 124°91'E, alt. 800 m, 3 June 2016, Bing Li, (SYNU). ***Paratypes.*** 3♂♂, same data as holotype.

Diagnosis. 5 or 6 frontal setae situated on lower half of frons; scutellum yellow; postpronotal lobe black; anterior spiracle fuscous; legs with femora and tibiae yellow; only distal part of hind femur with distinct *pv* and 5 or 6 small *pv* on basal half.

Figure 2. *Mydaea adhesipeda* Xue, sp. nov. **a** male, sternite 5 in ventral view **b** male, cerci in posterior view **c** male, terminalia in profile **d** female, ovipositor in dorsal view **e** female, ovipositor in ventral view **f** female, spermatheca. Scale bars: 0.50 mm (**a**); 0.20 mm (**b,c**); 0.20 mm (**d,e**); 0.10 mm (**f**).

Description. Male. Body length 7.2–7.4 mm. ***Head***: eyes bare, frontal vitta black, fronto-orbital plates and parafacials brown, mediane red-brown; antennae black, arista brown-yellow; lunule dark brown; genae black, genal and postgenal hairs entirely black; palpi black. Frons less than twice the width of anterior ocellus; fronto-orbital plates contiguous in the middle; frontal triangle on upper 1/3 of frons; fronto-orbital plates and parafacials covered with sparse gray pruinosity, parafacial approximately 1/2 as wide as postpedicel; genal height approximately 1/9 of eye height, genae covered with gray pruinosity; lower face not projecting, vibrissal angle situated behind frontal angle in lateral view. 5or 6 pairs frontal setae situated on lower half of frons; proclinate orbital setae absent; ocellar setae long and strong, slightly longer than the lower frontal setae; postpedicel approximately 3.5 times as long as wide; arista plumose, the longest hairs approximately 4/5 as width of width of postpedicel; palpus black, approximately 1.5 times as long as prementum, prementum short, approximately 1.5 times as long as high, and covered with sparse gray pruinosity; labellum long and big, the length of labellum approximately twice as long as height of prementum. ***Thorax***: fuscous, only scutellum

Figure 3. *Mydaea combiniseriata* Xue, sp. nov. **a** male, sternite 5 in ventral view **b** male, cerci in posterior view **c** male, terminalia in profile. Scale bars: 0.50 mm (**a**); 0.25 mm (**b,c**).

yellow, covered with sparse gray pruinosity; scutum with 4 black vittae, and the inner vittae extending to scutoscutellar suture; *acr* 0+1; *dc* 2+4; *ial* 0+2; *pra* strong, approximately 1.3 times as long as posterior notopleural seta; lateral and ventral surfaces of scutellum without hairs; basisternum of prosternum, anepimeron, meron, katepimeron bare; notopleuron with hairs; katepisternal setae 1+2; anterior spiracle fuscous and posterior spiracle light brown. ***Wings:*** semi-hyaline and slightly brown; base of wing pale yellow; tegula and basicosta yellow; costal spine small; ventral surface of vein C with hairs; vein Sc bow-shaped; crossvein r-m straight, crossvein dm-m bent towards base of wing, areas around crossveins r-m and dm-m not clouded; dorsal and ventral surface of radial node with hairs; R_{4+5} and M_1 straight, apical part of M_1 bent forward slightly; calypter pale yellow, lower calypter tongue-shaped; halter knob yellow. ***Legs:*** femora and tibiae yellow but dorsal surface of fore femur fuscous; coxae, trochanters, and tarsi fuscous; fore tibia without median *p*; mid femur with *pv* row on basal half, 1 apical *ad*, 3 apical *pd*, mid tibia with 3 *p*; hind femur with distinct *av* and *pv* only at distal part, and 5 or 6 small *pv* on basal half; hind tibia with 2 *pd*, 5 *pv*; tarsi slight longer than tibiae; only fore claws longer than pulvilli, mid and hind claws shorter than pulvilli, fore claws approximately as long as tarsomere 5. ***Abdomen:*** black in ground color, covered with light gray pruinosity, both sides of abdomen without color shifting patch, tergite 3 with a complete row of posterior marginal setae, but short and sparse, median pair shorter than half the length tergite 3, tergites 4 and 5 with complete rows of posterior marginal setae and these slightly longer, approximately 3/5 of the length of the tergite, tergite 4 with 3 pairs of discal setae, tergite 5 with 4 pairs of discal setae; posterior margin of sternites 2 and 3 each with a pair of apical setae, posterior margin of sternite 4 with 2 pairs of apical setae, inner margin of lateral lobe at basal part of sternite 5 with a row of 6 or 7 close-set setae, sursyli near rectangle, and inner margin with hairs.

Female. Unknown.

Remarks. This species is similar to *Mydaea corni* (Scopoli, 1763), but differs from the latter in the following features: male eyes bare; 5 or 6 pairs of frontal setae situated on lower half of frons; genal height approximately 1/9 of eye height; *acr* 0+1; *pra* long

and large, approximately 1.3 times as long as posterior notopleural seta; hind tibia without *ad*; both sides of abdomen without color shifting patches.

Etymology. The species name refers to the lobe of sternite 5, which in males have a long row of setae. It is derived from the Latin words *combin* meaning combined and *seriat* meaning rows.

Distribution. China, Liaoning Province (Qingyuan).

Mydaea qingyuanensis Xue, sp. nov.
http://zoobank.org/A9C2CC5B-E88B-4F91-9B6A-275FE9403DB9

Type material. *Holotype.* China, 1 ♂, Liaoning Province, Qingyuan, 41°81'N, 124°91'E, alt. 800 m, 3 June 2016, Bing Li, (SYNU). ***Paratypes.*** 2♂♂, same data as holotype.

Diagnosis. Frons narrower than the width of anterior ocellus; scutellum yellow, *prst-acr* absent, *post-dc* 4, anterior and posterior spiracle yellow; hind femur with complete *pv* row, hind tibia without *ad*.

Description. Male. Body length 7.8–8.0 mm. **Head:** eyes bare; frontal vitta black; antennae black,lunule brown; genae black, genal and postgenal hairs entirely black; palpi black. Frons narrower than the width of anterior ocellus; fronto-orbital plates contiguous in the middle; frontal vitta situated on upper 2/5 of frons; Fronto-orbital plates and parafacials covered with sparse gray pruinosity, parafacials approximately 3/5 as wide as postpedicel; lower face not projecting, vibrissal angle situated behind frontal angle in lateral view; genae covered with gray pruinosity, genal height approximately 1/9 of eye height. Frontal setae in 13 or 14 pairs and extending upwards to anterior ocellus, 5 or 6 strong pairs on lower 2/5 of frons, 7 or 8 extremely small pairs on upper 3/5 and these shorter than eye facets ; proclinate orbital setae absent; ocellar setae strong, slightly longer than the lower frontal setae; postpedicel approximately 3.5 times as long as wide, arista plumose, longest hairs approximately as long as width of postpedicel; palpus approximately 1.5 times as long as prementum, prementum short, approximately 1.5 times as long as high, and covered with sparse gray pruinosity; labellum long and big, the length of labellum approximately twice the height of prementum. **Thorax:** black, but scutellum yellow, covered with sparse gray pruinosity; scutum with 4 black vittae, and inner vitta not extending to scutoscutellar suture; *acr* 0+1; *dc* 2+4; *ial* 0+2; *pra* distinct, slightly shorter than posterior notopleural seta; lateral and ventral surfaces of scutellum without hairs; basisternum of prosternum, anepimeron, meron, and katepimeron bare; notopleuron with hairs; katepisternal setae 1+2; anterior and posterior spiracles yellow. **Wings:** semi-hyaline and pale brown; tegula and basicosta yellow; costal spine slightly short than crossvein r-m; ventral surface of vein C with hairs; vein Sc bow-shaped; areas around crossveins r-m and dm-m not clouded and straight; dorsal and ventral surface of radial node with hairs; R_{4+5} and M_1 straight, apical part of M_1 slightly bent forward; calypter yellow, lower calypter tongue-shaped; halter knob yellow. **Legs:** Tibiae and femora brown-yellow; coxae, trochanters and tarsi dark brown; fore tibia without median *p*; mid femur without distinct *av*, 1 apical *ad*, 3 apical *pd*, complete *pv* rows, mid tibia with 3 *p*; hind femur with complete *av* and
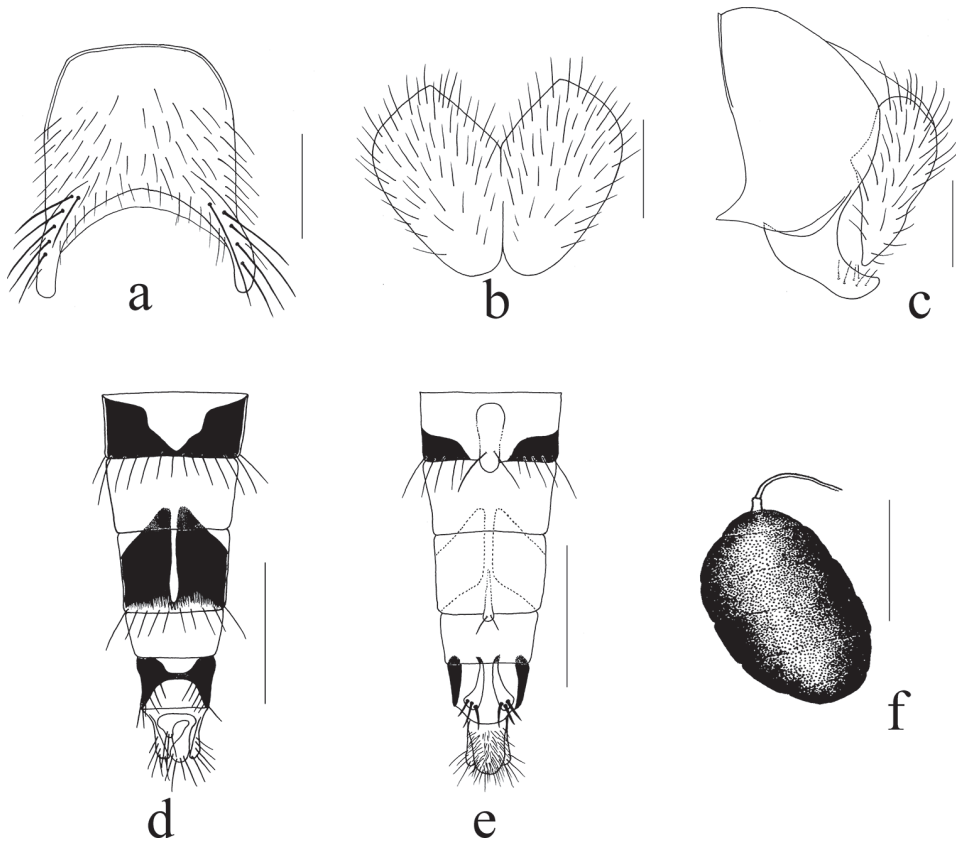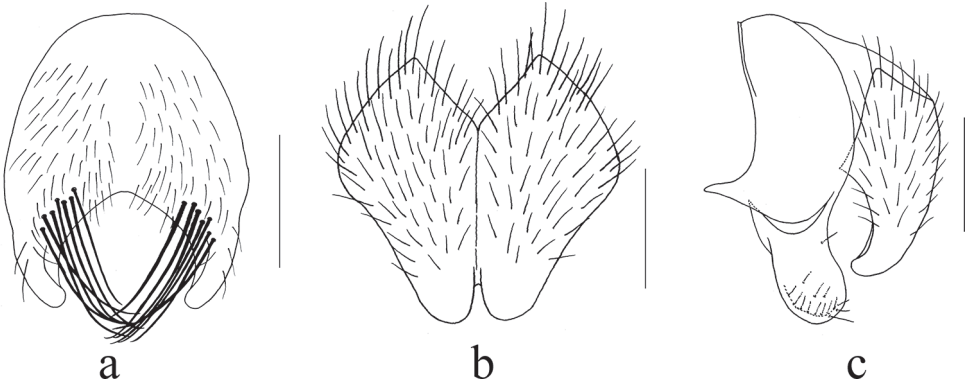
Figure 4. *Mydaea qingyuanensis* Xue, sp. nov. **a** male, sternite 5 in ventral view **b** male, terminalia in posterior view **c** male, terminalia in profile. Scale bars: 0.50 mm (**a**); 0.20 mm (**b**); 0.25 mm (**c**).

pv rows, hind tibia with 2 *pd*, 2 *pv*; tarsi slight longer than tibiae; pulvilli longer than claws, pulvilli approximately 2/3 of tarsomere 5 in length. **Abdomen:** ground color black; ovate in dorsal view, tergites 3–5 with a median black vitta, both sides of tergites 4 and 5 with distinct color shifting patches, tergites 3–5 with a complete posterior marginal rows of setae, tergites 4 and 5 with 4 or 5 pairs of discal setae, sternite 1 bare, apical margin of sternites 2–4 each with a pair of marginal setae; distal part of cerci and sursyli tapering in posterior view, dispart of cerci triangular in posterior view, sursyli narrowed on distal half in profile and bent backwards.

Female. Unknown.

Remarks. This species is similar to *Mydaea corni* (Scopoli, 1763), but differs from it in the following features: male eyes bare; frontal setae in 13 or 14 pairs and extending upwards to anterior ocellus, 5 or 6 strong pairs on lower 2/5 of frons, 7 or 8 extremely small pairs at upper 3/5 and these shorter than facets of eye; postpedicel approximately 3.5 times as long as wide; genal height approximately 1/9 of eye height; *acr* 0+1; *dc* 2+4; *pra* shorter than posterior notopleural seta; posterior spiracle yellow; hind tibia without *ad*.

Etymology. The species is named for the type locality, Qingyuan county.

Distribution. China, Liaoning Province (Qingyuan).

Mydaea quinquiseta **Xue, sp. nov.**
http://zoobank.org/18E8DE94-D3F6-49AB-A66C-C545926A3CC0

Type material. *Holotype.* China, 1 ♂, Sichuan Province, Luding, Yanzigou, 29°38'N, 102°07'E, alt. 2600 m, 17 June 2006, Jiayu Liu, (SYNU). *Paratypes.* 2♂♂, same data as holotype.

Diagnosis. Eyes bare; *post-dc* 4; distal and lateral part of scutellum yellow; anterior and posterior spiracle brown; legs brown-yellow; both sides of abdomen without color shifting patch; cerci circular apically in posterior view.

Description. Male. Body length 7.4–7.6 mm. ***Head:*** eyes bare, facets on upper half not enlarged; frontal vitta black; antennae black, arista brown; lunule brown; genae black, genal and postgenal hairs entirely black; palpi black. Frons approximately 1.5 times as wide as anterior ocellus; fronto-orbital plates contiguous in the middle; frontal vitta triangle on upper 1/4 of frons; fronto-orbital plates and parafacial covered with distinct gray pruinosity, parafacial approximately 3/5 as wide as postpedicel; postpedicel approximately 3.5 times as long as wide; lower face not projecting, vibrissal angle situated behind frontal angle in lateral view; genae covered with gray pruinosity, genal height approximately 1/9 of eye height. Frontal setae in 13 or 14 pairs and these situated on lower half of frons; proclinate orbital setae absent; ocellar setae long and strong, slightly longer than the lower frontal setae; arista short plumose, maximum length of hairs approximately 2/3 of the width of postpedicel; palpus approximately 1.5 times as long as prementum, prementum approximately 2.2 times as long as high, and covered with gray pruinosity; labellum strong, extending to posterior part, and slightly longer than palpi. ***Thorax:*** black, but distal and lateral parts of scutellum yellow, covered with sparse gray pruinosity; scutum with 4 indistinct vittae and the inner vittae not extending to scutoscutellar suture; *acr* 0+1; *dc* 2+4; *ial* 0+2; *pra* long and strong, slightly longer than posterior notopleural seta; notopleuron with small hairs; lateral and ventral surfaces of scutellum without hairs; basisternum of prosternum, anepimeron, meron and katepimeron bare; katepisternal setae 1+2; anterior and posterior spiracles brown. ***Wings:*** semi-hyaline and basal part brown; tegula and basicosta yellow; costal spine small; ventral surface of vein C with hairs; Sc bow-shaped; middle part of crossvein dm-m bent towards base of wing, area around crossveins r-m and dm-m not clouded; dorsal and ventral surfaces of radial node with hairs; R_{4+5} and M_1 straight, apical part of M_1 bent forward slightly; calypter slightly brown; lower calypter tongue-shaped; halter knob yellow. ***Legs:*** entirely black; fore tibia without median *p*; mid femur with a row of *pv* on basal 3/5, and a row of *a* setae on basal half, 1 apical *ad*, 3 apical *pd*, mid tibia with 3 *p*; hind femur with a distinct row of *av* on distal half, and *pv* on basal half shorter than transverse diameter of hind femur, hind tibia with 4 or 5 *av*, 2 *ad*, without *pv*; claws as long as pulvilli, and short than tarsomere 5. ***Abdomen:*** ground color black; ovate in dorsal view, covered with gray pruinosity, both sides without color shifting patch, tergites 3 and 4 with median black vittae, tergites 4 and 5 each with a complete row of posterior marginal setae, tergite 5 with lateral discal setae on anterior half and otherwise devoid of hairs, sternite 1 bare, sternite 5 with 5 strong, median setae; cerci circular apically in posterior view.

Female. Unknown.

Remarks. This species is similar to *Mydaea gracilior* Xue, 1992, but differs from the latter in the following features: facets on upper half of eyes not enlarged; *acr* 0+1; *pv* on basal half of hind femur shorter than transverse diameter of hind femur; cersci circular apically in posterior view; distal parts of cerci and surstyli not bent in opposite directions.

Etymology. The species name refers to the lobe of sternite 5 which in males has 5 strong setae. It is derived from the Latin words *quinqu* meaning five and *seta* meaning setae.

Distribution. China, Sichuan Province (Yanzigou).
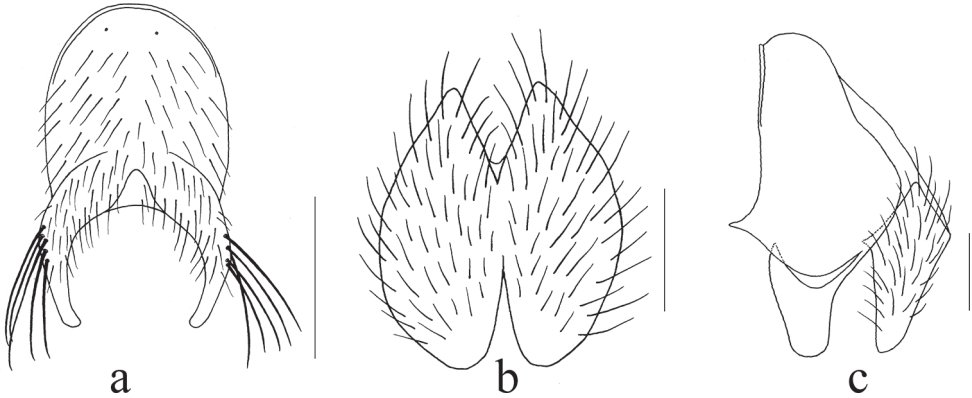
Figure 5. *Mydaea quinquiseta* Xue, sp. nov. **a** male, sternite 5 in ventral view **b** male, cerci in posterior view **c** male, terminalia in profile. Scale bars: 0.50 mm (**a**); 0.20 mm (**b,c**).

Mydaea wusuensis Xue, sp. nov.
http://zoobank.org/5A203D16-965B-4634-BE42-03552051AF28

Type material. *Holotype.* China, 1 ♂, Heilongjiang Province, Jiamusi, Wusuzhen, 48°15'N, 134°12'E, alt. 80 m, 18 May 2017, Bo Hao, (SYNU). *Paratypes.* 2♂♂, same data as holotype.

Diagnosis. Scutellum and legs black; hind femur without *pv*; lateral lobes of sternite 5 short, and basal part near quadrate; cerci distinctly narrowed on distal half in posterior view and rounded on apical part, distal parts of cerci and surstyli bent in opposing directions; in lateral view only surstyli bent posteriorly.

Description. Male. Body length 5.4–5.6 mm. *Head*: eyes bare, frontal vitta black; antenna black, arista brown; lunule brown; genae black, genal and postgenal hairs entirely black; palpi black. Frons approximately 1.5 times as wide as anterior ocellus; fronto-orbital plates contiguous in the middle; frontal vitta triangle on upper 1/3 of frons; fronto-orbital plates and parafacial covered with sparse gray pruinosity, parafacials approximately 2/5 width of postpedicel; lower face not projecting, vibrissal angle situated behind frontal angle in lateral view; genae covered with gray pruinosity, genal height approximately 1/9 of eye height; Frontal setae in 7–8 pairs and these situated on lower 3/5 of frons; proclinate orbital setae absent; ocellar setae long and strong, slightly longer than lower frontal setae; postpedicel approximately 3.0 times as long as wide; arista short plumose, maximum length of hairs approximately 2/3 of width of postpedicel; palpi approximately 1.2 times as long as prementum, prementum approximately 2.2 times as long as high, and covered with gray pruinosity; labellum long and large, extending to posterior part and approximately as long as palpi. *Thorax*: ground color black; covered with sparse gray pruinosity; scutum with 4 black vittae, and the inner vittae not extending to scutoscutellar suture; *acr* 0+1; *dc* 2+4; *ial* 0+2; *pra* approximately as long as posterior notopleural seta; notopleuron with small hairs; scutellum same color as thorax and lateral and ventral surfaces of scutellum with-
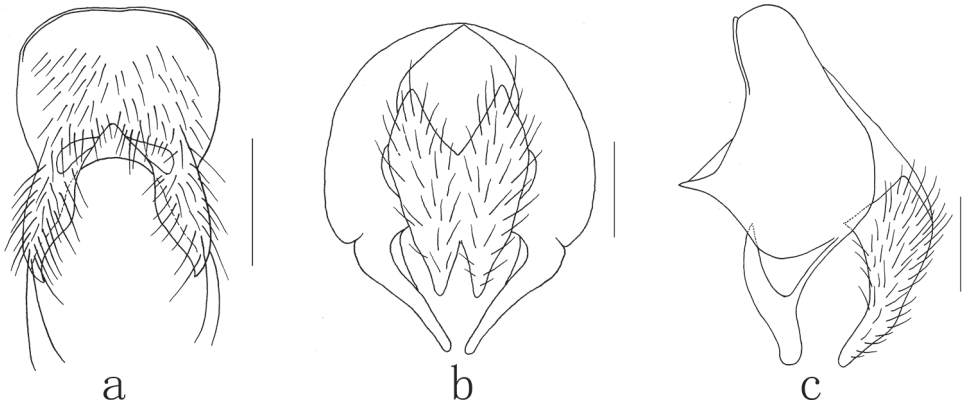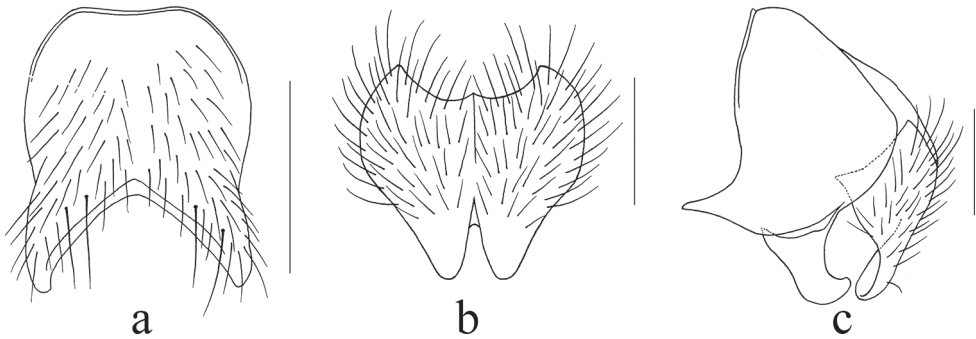
Figure 6. *Mydaea wusuensis* Xue, sp. nov. **a** male, sternite 5 in ventral view **b** male, cerci in posterior view **c** male, terminalia in profile. Scale bars: 0.50 mm (**a**); 0.20 mm (**b,c**).

out hairs; apical scutellar setae strong, slightly longer than posterior notopleural seta; basisternum of prosternum, anepimeron, meron and katepimeron bare; katepisternal setae 1+2; anterior and posterior spiracles brown. *Wings*: semi-hyaline, tegula black; basicosta yellowish brown; costal spine short; vein Sc bow-shaped; crossvein dm-m straight, areas around crossveins r-m and dm-m not clouded; dorsal and ventral surfaces of radial node with hairs; R_{4+5} and M_1 straight; calypter light brown, lower calypter tongue-shaped; halter knob brown-yellow. *Legs*: entirely black; fore tibia without median *p*; mid femur with *pv* and *a* rows of setae on basal half, 1 apical *ad*, 1 *av* on basal part, 2 apical *pd*, mid tibia with 3 *p*; hind femur with a distinct row of *av* on basal 2/5, without *pv*, hind tibia with 2 *av*, 2 *ad*, without apical *pv*; claws as long as pulvilli, and shorter than tarsomere 5. *Abdomen*: ground color black; ovate in dorsal view, covered with gray pruinosity, both sides without color shifting patch, tergites 3–5 with a median black vitta, tergites 4 and 5 each with a complete row of posterior marginal setae, tergite 5 with 4 or 5 discal setae; sternite 1 bare, lateral lobe sternite 5 short, and basal part near quadrate; cerci narrowing distinctly in posterior view and rounded on apical part, distal parts of cerci and surstyli bent in opposing directions; in lateral view only surstyli bent posteriorly.

Female. Unknown.

Remarks. This species is similar to *Mydaea ancilloides* Xue, 1992, but differs from it in the following features: male arista short-plumose, longest hair shorter than width of postpedicel; lower 3/5 of frons without frontal setae; parafacial approximately 2/5 of postpedicel in width; sternite 5 lateral lobe short and basal part near quadrate.

Etymology. The specific name refers to its type locality, Wusu town.

Distribution. China, Heilongjiang Province (Wusuzhen).

Acknowledgements

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RESEARCH ARTICLE

Phenotypic diversity in an endangered freshwater fish *Squalius microlepis* (Actinopterygii, Leuciscidae)

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Abstract

Squalius microlepis was examined from recent and historical collections within the known range of the species with special emphasis on intraspecific variability and variations, and compared to its closest relative species *S. tenellus* (in total, 193 specimens; 33 absolute and 52 proportional measurements and ratios, and 12 counts including vertebrae). *Squalius tenellus* was perfectly differentiated in all statistical analyses and can be diagnosed by 76–95 (vs. 64–80) scales in lateral series, 68–83 (vs. 58–77) lateral-line scales, (17)18–20 (vs. 13–16(17)) scales above lateral line, and (7)8–10 (vs. 4–7) scales below lateral line. *Squalius microlepis* was morphologically heterogeneous, with two phenotypes readily distinguishable (phenotype 1 corresponding to *S. microlepis* s. str. as defined by its lectotype) by a combination of many characters; those contributing most to the discrimination were number of gill rakers, length of lower jaw (% interorbital width), and head length (% SL). Only phenotype 1 was found in the Ričina-Prološko Blato-Vrljika karst system; most of the specimens from the lower Matica and the Tihaljina-Trebižat karst system were identified as phenotype 2; the sample from karstic poljes near Vrgorac contained both phenotype 1 and 2, and individuals of intermediate morphology. As very limited molecular data exist on the two phenotypes of *S. microlepis*, we refrain from any taxonomic conclusions until new molecular approaches (and new markers) are used. We also report on a dramatic reduction of the area of distribution and abundance of *S. microlepis* in recent years.

Introduction

The genus *Squalius* Banaparte is widely distributed throughout Europe and the Middle East, and shows an especially high diversity in the Mediterranean basin. Approximately 50 species are currently recognised in the genus (Kottelat and Freyhof 2007; Turan et al. 2009; Bogutskaya and Zupančič 2010; Zupančič et al. 2010; Özuluğ and Freyhof 2011), and sixteen species are known to occur in Europe (Özuluğ and Freyhof 2011).

Small-scaled chubs, *S. microlepis* Heckel, 1843 and *S. tenellus* Heckel, 1843, are superficially similar but distinguishable based on scale counts according to Bănărescu and Herzig-Straschil (1998): 67–75 vs. 76–85 total lateral line scales, 24–26 vs. 28–32 circumpeduncular scales, 13–15 vs. 15–17 scales in a transverse row between the dorsal-fin origin and the lateral line, 5–6 vs. 6–7 scales in a transverse row between the lateral line and the pelvic-fin origin in *S. microlepis* vs. *S. tenellus.*

Available data on genetic markers for *Squalius microlepis* and *S. tenellus* show that they form a sister-pair in a clade, which is restricted to the Iberian and Apennine Peninsulas and the eastern Adriatic basin (Perea et al. 2010; Geiger et al. 2014; Schönhuth et al. 2018). However, the genetic markers differ in their resolution of phylogenetic relationships between the two species. The CO1 mitochondrial marker do not distinguish them (Perea et al. 2010; Geiger et al. 2014) while mitochondrial cytb, a combined nuclear data set (RAG+S7), and the combined mitochondrial and nuclear data sets CO1+cytb+RAG+S7 (Perea et al. 2010) and CO1+cytb+RAG+S7 (Schönhuth et al. 2018) support some divergence.

Squalius tenellus is distributed in karstic waters of Livanjsko Polje including Buško Blato (Buško Jezero), an accumulation lake, located in the southern part of Livanjsko Polje and northwest of Duvajnsko Polje; Mandečko Lakes and in Blidinje Lake to where it was supposedly introduced over 100 years ago (Bănărescu and Herzig-Straschil 1998; Kottelat and Freyhof 2007; Zupančič 2008). PZ found this species in a stream at Glamoč in Glamočko Polje located in the northeast of Livanjsko Polje and west of Kupreško Polje. Data on distribution presented by Crivelli (2006) and Freyhof and Kottelat (2008) on sympatric distribution of *S. microlepis* and *S. tenellus* in lakes Buško and Mandečko near Livno may probably reflect different taxonomic opinions of the authors on synonymisation of the two species. Ćurčić (1915) reported *S. tenellus* from Mostarsko Blato (repeated by Karaman (1928: 160)) that have been confirmed by recent studies (Šanda et al. 2008, 2010). *Squalius tenellus* was allegedly introduced into the Cetina River drainage and this river is included in the range of this species by some authors (Freyhof and Kottelat 2008; Ćaleta et al. 2015).

Recent summarising publications (Habeković and Pažur 1995; Bănărescu and Herzig-Straschil 1998; Bogut et al. 2006; Mrakovčić et al. 2006, 2016; Kottelat and Frey-

hof 2007; Zupančič 2008; Šanda et al. 2009; Ćaleta et al. 2015, 2019) indicate that the range of *S. microlepis* encompasses the entire karst system of the Culuša – Ričina – Brina – Suvaja – Matica – Vrljika – Tihaljina – Mlade – Trebižat (a single river interrupted by underground sections, a tributary to the Neretva) downstream to the waterfall Kravice. In this karst river system, it occurs in basins of the Prološko Blato Lake and the Ričice Reservoir in the Imotski region in Croatia and in Krenica Lake and the Matica, Vrljika, Tihaljina and Trebižat rivers in Bosnia and Herzegovina. It was found outside the Matica-Vrljika-Tihaljina-Trebižat system further southwards in the Neretva drainage – in the Matica River at Imotski in Polje Jezero [Vrgoraska Matica River, do not be confused with Matica-Vrljika] and reported from Baćina lakes of the lower Neretva. The species is known under a vernacular name 'masnica' or 'mašnica' in western Herzegovina (Bosnia and Herzegovina) and 'makal' ('makali' or 'makalj') in Croatia including in the Vrgorac area (Heckel and Kner 1858: 206, Ćaleta et al. 2019: 168).

An examination of *S. microlepis* samples, deposited in the historical fish collection at Museum of Natural History in Vienna and recent collections, revealed some morphological heterogeneity of the species. The goal of this study was a comparative morphological analysis of the group of the small-scaled Adriatic *Squalius* (*S. tenellus* and *S. microlepis*) to approach issues of its morphological diversity. The study on intraspecific morphological differences was aimed at contributing, in the future, to integrative phylogenetic analyses and species delimitations in the group.

Squalius microlepis was assessed by IUCN at global level as endangered (EN B2ab(ii, iii)) ver. 3.1 (Crivelli 2006), and in Croatian national Red book it was assessed as critically endangered (CR A1ace, C2a(iii)) (Mrakovčić et al. 2006). It is strictly protected by Nature protection Acts in both Croatia and Bosnia and Herzegovina.

Materials and methods

In total, 193 specimens were examined, material see Table 1; examined localities are presented in Fig. 1. Most examined specimens were available in collections. Those specimens collected in the wild using SAMUS 725MP (Samus Special Electronics, Poland) (max. 1000V, 650W) electrofishing device and hand nets were euthanised with etheric clove oil (*Eugenia caryophyllata*) diluted in water (5 drops of oil per 5 l of water) and preserved in 5% formaldehyde and then stored in 70% ethanol.

The fin insertion is the posterior-most point where the last fin ray connects with the body. Measurements follow Kottelat and Freyhof (2007) except that head length (HL), eye diameter, postorbital length and interorbital width include the skin fold. All measurements were made point-to-point with an electronic calliper and recorded to the nearest of 0.1 mm. Standard length was measured from the anteriormost extremity of the upper lip to the posterior margin of the hypurals at midline. Maximum body depth was measured at the deepest section of the body which is about the middle of distance between the nape and the dorsal-fin origin. Body depth was also measured in front of the dorsal-fin origin. Additional measurements of the cranium, jaws and

Table 1. Examined material.

Area	Sample data	Identification (present study)
Ričina-Prološko Blato-Vrljika, Krenica Lake	**Imotsko Polje (Croatia)**	*Squalius microlepis* phenotype 1
	NMW 49413, 2, 84.8–98.2 mm SL, 'Imosky', 1886, no collector;	
	NMW 49415, lectotype, 151.2 mm SL, 'Imosky, Kroatien (Dalmatien), Heckel Reise 1840';	
	NMW 49414, 3 paralectotypes, 75.4–108.6 mm SL, data as lectotype;	
	NMW 49416, 1 paralectotype, 139.6 mm SL, data as lectotype;	
	NMW 49421, 1 paralectotype [not 3 as given by Bănărescu and Herzig-Straschil (1998: 417)], 149.9 mm SL, data as lectotype;	
	NMW 49417, 3, 95.5–98.1 mm SL, Imosky 1886, no collector;	
	NMW 49418, 2, 86.5–92.8 mm SL, same as 49417;	
	NMW 49419, 2, 86.5 mm SL, same as 49417;	
	NMW 49420, 2, 102.3–107.7 mm SL, same as 49417;	
	NMW 49422, 1, 'Prolozac bei Imotski', 1904, Kolombatowitsch;	
	MNCN_ICTIO 291.725–291.729, 4, 147.5–166.8 mm SL, Prološko Blato [Proložac] Lake, 8 May 2008;	
	PZC 283, 3, 160.5–186.2 mm SL, same locality and collector as above, 2 July 2004;	
	PZC 545, 5, 145.2–206.5 mm SL, same locality and collector as above, 16 Aug. 2008.	
	Vrljika River (Croatia)	*Squalius microlepis* phenotype 1
	NMW 12729-732, 4, 119.5–121.7 mm SL, 'Vrlica-Fluss bei Imotski', no date, no collector;	
	NMW 49399, 4, 118.6–149.4 mm SL, Vrlica, Imotski, 1901, coll. Sturany;	
	NMW 49400, 3, 113.3–121.2 mm SL, same data;	
	NMW 49401, 2, 153.2–155.6 mm SL, same data;	
	NMW 49402, 3, 138.4–145.5 mm SL, same data;	
	NMW 49403, 3, 132.2–161 mm SL, same data;	
	NMW 49404, 2, 180.7–215 mm SL, same data;	
	NMW 49405, 3, 142.6–158.1 mm SL, same data;	
	NMW 49406, 3, 133.5–217 mm SL, same data;	
	NMW 49407, 3, 163.7–192.5 mm SL, same data;	
	NMW 49408, 3, 102.6–106.4 mm SL, same data;	
	NMW 49409, 2, 156.6–158.7 mm SL, same data;	
	NMW 49410, 2, 135.3–137.1 mm SL, same data;	
	NMW 49411, 2, 136.6–152.5 mm SL, same data;	
	NMW 49412, 2, 143.3–190.5 mm SL, same data;	
	NMW 49221, 2, 191.2–203.9 mm SL, same data.	
	Ričina River (Croatia)	*Squalius microlepis* phenotype 1
	MNCN_ICTIO 294.784–294.800, 17, 70.9–223.3 mm SL, Ričice Reservoir (Ričina River), coll. Zupančič, 22 Apr. 2004;	
	MNCN_ICTIO 292.541–292.545, 5, 165.7–223.4 mm SL, same locality and collector as above, 16 Aug. 2008.	
	PZC 501, 16, 53.2–135.6 mm SL, same locality and collector as above, 1 May 1999.	
	Krenica Lake (Bosnia and Herzegovina)	*Squalius microlepis* phenotype 1
	MNCN_ICTIO 295.855–295.860, 6, 61.1–116.1 mm SL, Krenica Lake at Drinovci, 43°22'26"N, 17°19'56"E, coll. Zupančič, 17 July 2002;	
	MNCN_ICTIO 296.096–296.097, 2, 71.6, 147.6 mm SL, same locality and collector as above, 7 July 2011.	
Lower Matica-Tihaljina-Trebižat	**Lower Matica River (Bosnia and Herzegovina)**	*Squalius microlepis* phenotype 2
	MNCN_ICTIO 292.120–292.123, 2, 174.9, 177.8 mm SL, Matica River at Drinovci, 43°21'29"N, 17°17'29"E, coll. Zupančič, 4 Aug. 2007;	
	ZISP 54994, 5, 96.3–147.2 mm SL, same locality as above, coll. Zupančič, 7 July 2011.	
	Tihaljina River (Bosnia and Herzegovina)	*Squalius microlepis* phenotype 2
	All from Tihaljina River at bridge in Tihaljina, 43°18'27"N, 17°23'22"E; coll. Zupančič:	
	NMW 95294, 3, 98.6–173.9 mm SL, 4–5 Aug. 2007;	
	MNCN_ICTIO 294.588–294.594, 7, 72.0–194.4 mm SL, 15 Aug. 2001;	
	MNCN_ICTIO 294.548–294.552, 5, 104.2–192.2 mm SL, 16 Aug. 2001;	
	MNCN_ICTIO 293.145–293.147, 3, 126.1–156.3 mm SL, 2 June 2008;	
	MNCN_ICTIO 294.596–294.599, 4, 108.2–158.3 mm SL, 9 July 2008;	
	MNCN_ICTIO 292.129–292.136, 5, 96.4–222.9 mm SL, 4 Oct. 2009;	

Area	Sample data	Identification (present study)
Lower Matica-Tihaljina-Trebižat	PZC 531, 1, 255.7 mm SL, 4–5 Aug. 2007;	*Squalius microlepis* phenotype 2
	uncat., 3, 94.3–153.5 mm SL, 3 June 2000.	
	J: out of MNCN_ICTIO 292.129–292.136, 127.05 mm SL, Tihaljina River at bridge in Tihaljina, 43°18'27"N, 17°23'22"E; coll. Zupančič, 4 Oct. 2009.	*Squalius microlepis* phenotype 1
	K: 149.75 mm SL, as J.	
	Trebižat River: (Bosnia and Herzegovina)	*Squalius microlepis* phenotype 2
	MNCN_ICTIO 294.472–294.473, 2, 140.1, 152.6 mm SL, Trebižat River at bridge between Grabovnik and Vašarovići, 43°12'38"N, 17°29'03"E, coll. Zupančič, 8 July 2011.	
Presumably, from polijes at Vrgorac	**A:** NMW 49428, 1, 165.8 mm SL, 'Lago di Dusino presso Imosky', 1848, coll. Parreyss.	*Squalius microlepis*, intermediate between phenotypes
Neretva drainage, uncertain	**B:** NMW 49427, 1, 140.1 SL mm, 'Narenta, Heckel Reise 1840'.	*Squalius microlepis*, intermediate between phenotypes
Vrgoracko Polje and Polje Jezero karst system (Croatia)	**C:** NMW 49424, 1, 168.1 mm SL, 'Vergoraz [See Jessero], Heckel Reise 1840'.	*Squalius microlepis* phenotype 1
	D: NMW 49425, 1, 178.0 mm SL, 'See zw. Gradač and Vrgorač', 1888, don. Scharfetter.	*Squalius microlepis*, intermediate between phenotypes
	E: NMW 49426, 1, 193.8 mm SL, 'See zw. Gradač and Vrgorač', 1888, don. Scharfetter.	*Squalius microlepis* phenotype 1
	F: NMW 49423:1, 122.5 mm SL, 'Vergoraz [See Jessero], Heckel Reise 1840'.	*Squalius microlepis* phenotype 2
	G: NMW 49423:2, 276.1 mm SL, as F.	*Squalius microlepis* phenotype 2
Presumable not Zadar but Neretva drainage, uncertain	**H:** NMW 49228:1, 165.8 mm SL, Zara [Zadar](see text for discussion on locality), no date, coll. Kolombatović.	*Squalius microlepis* phenotype 1
	I: NMW 49228:2, 205.1 SL, as H.	
	NMW 16001, lectotype, 122.1 mm SL, Livno [Livanjsko Polje], [Heckels Reise, 1840]; NMW 16002, 2 paratypes, 78.6 mm and 73.9 mm SL, data as lectotype;	*Squalius tenellus*
	NMW 49613, 2 paratypes, 94.9 mm and 82.7 mm SL, data as lectotype;	
	MNCN_ICTIO 292.166–292.168, 3, 137.5–183.9 mm SL, stream at Glamoč [Glamočko Polje], ca. 44°1'56"N 16°53'44"E, coll. Zupančič, 17 Aug. 2009;	
	MNCN_ICTIO 293.014–293.016, 4, Žabljak R. at Žabljak, north from Livno [Livanjsko Polje], 43°48'45"N 16°59'51"E, coll. Zupančič, 13 Aug. 2001.	
Buško Reservoir	MNCN_ICTIO 294.142–294.158, 17, 165.0–205.4 mm SL, Buško Blato at Prisoje, ca. 43°40'54"N 17°4'14"E, coll. Zupančič, 22 Apr. 2004.	*Squalius tenellus*
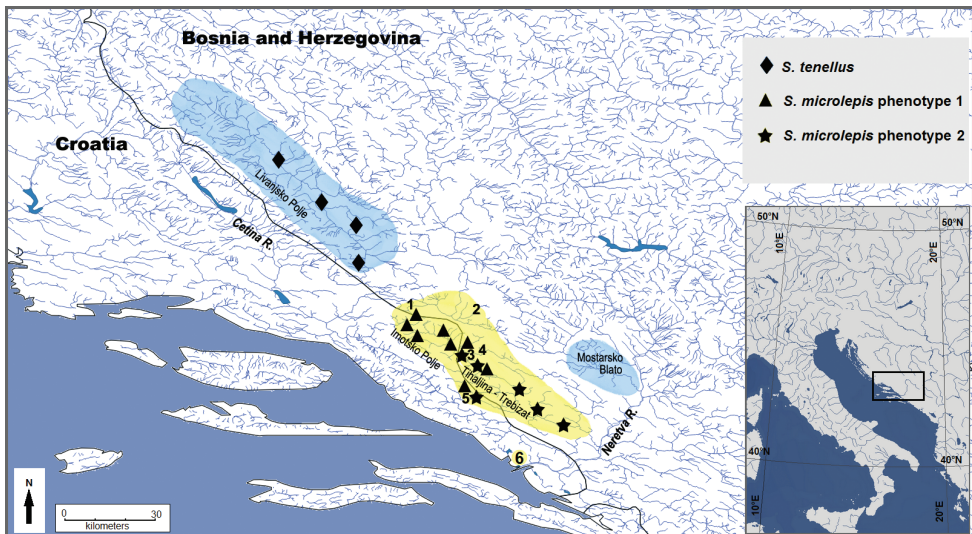
Figure 1. Map showing localities of examined specimens: *S. tenellus*, *S. microlepis* phenotype 1, and *S. microlepis* phenotype 2, shadowed areas showing ranges of *S. tenellus* (blue) and *S. microlepis* (yellow); 1 – Ričice Reservoir, 2 – Ričina River, Posušje, 3 – Lower Matica River, 4 – Krenica Lake, 5 – Vrgoračka Matica River system (Vrgoračko Polje, Polje Jezero), 6 – Baćina lakes.

operculum were made point to point from the anteriormost extremity to the posteriormost extremity (lengths), from the uppermost extremity to the lowermost extremity (depths), and between the lateralmost extremities (widths). Length of the cranial roof was measured from the anterior margin of the supraethmoid to the base of the supraoccipital crest. Characters include 33 absolute and 52 proportional measurements and ratios and 12 counts as given in Tables 2–5. Vertebral counts and terminology follow Naseka (1996). A qualitative character "a point where the dorso-hypural distance, which is taken from the dorsal-fin origin to the end of the hypural complex, falls when reported forward" follows Doadrio et al. (2007) and Kottelat and Freyhof (2007: fig. 2). The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are noted as "1½". Total number of scales in the lateral series (bearing the lateral-line canal or without the canal; equal number of transverse rows of scales) included scales at the caudal-fin base. Total number of lateral-line (pored / bearing the lateral-line canal) scales included scales at the caudal-fin base. Scale counts in a transverse row above and below the lateral line (in transverse row between dorsal-fin origin and lateral line, and in transverse row between lateral line and pelvic-fin origin, respectively) follow Kottelat and Freyhof (2007: fig. 10). Gill rakers count included all gill rakers on both lower and upper limb of the arch. Fin-ray counts and axial skeleton characters were examined from radiographs.

For statistical processing of data, to partly remove the size component from the shape measures, we used: 1) all individual morphometric character measurements standardised following Elliott et al. (1995) and 2) as proportional measurements (as in Tables 2, 3). Taking into account the relatively small sample sizes and the lack of information about the distribution of variables, nonparametric statistic tests (Mann-Whitney and Kruskal-Wallis) were used. Multivariate data analyses included forward stepwise discriminant function analysis (DFA) and cluster analysis (CA; using the unweighted pair-group average method with Euclidean distance). When analysing variables measured at different scales, z-transformation was used. The statistical analyses were performed using Microsoft Excel, Statistica 6.0 (Statistic for Windows. StatSoft) and SPSS Statistics V23.0 (IBM SPSS).

Abbreviations used:

MNCN_ICTIO	Ichthyology Collection, Museo Nacional de Ciencias Naturales, Madrid, Spain;
MZUF	Universita di Firenze, Museo Zoologico e Historia Naturale de la Specola, Firenze, Italy;
NMW	Naturhistorisches Museum, Wien, Austria;
PZC	Collection of P. Zupančič, Dolsko, Slovenia;
ZISP	Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; HL, head length;
SL	standard length;
s. l.	sensu lato;
s. str.	sensu stricto.

Table 2. Morphometric and meristic data of *Squalius microlepis* phenotypes 1 and 2 and *Squalius tenellus* identified based on preliminary examination (see text for explanations).

	S. microlepis phenotype 1, N = 47				*S. microlepis* phenotype 2, N = 46				*S. tenellus*, N = 25			
	min	max	mean	sd	min	max	mean	sd	min	max	mean	sd
SL, mm	61.14	223.44	**142.16**	46.47	72.04	255.72	**146.10**	36.67	122.10	205.39	**171.56**	21.49
Maximum body depth (% SL)	21.05	26.71	**23.67**	1.37	20.91	25.06	**22.87**	1.04	22.03	26.58	**24.38**	1.09
Depth of caudal peduncle (% SL)	9.06	10.61	**9.85**	0.44	9.47	11.01	**10.25**	0.42	9.34	11.30	**10.50**	0.42
Depth of caudal peduncle (% length of caudal peduncle)	45.72	56.58	**51.06**	3.02	45.92	59.48	**51.88**	3.34	49.29	59.24	**53.52**	2.72
Body width at dorsal-fin origin (% SL)	10.54	16.04	**13.47**	1.02	13.09	17.71	**15.06**	1.09	12.91	18.30	**14.48**	1.36
Caudal peduncle width (% SL)	6.80	10.26	**8.41**	0.76	7.75	11.08	**9.37**	0.62	7.77	10.70	**8.98**	0.80
Predorsal length (% SL)	56.11	61.20	**58.49**	1.14	54.33	58.54	**56.46**	1.14	54.96	58.56	**56.75**	0.93
Postdorsal length (% SL)	30.29	35.77	**32.27**	1.28	31.93	35.81	**34.38**	0.83	31.94	36.99	**33.89**	1.20
Prepelvic length (% SL)	50.90	59.63	**55.25**	2.28	49.54	54.27	**51.86**	1.09	53.00	57.03	**54.84**	1.14
Preanal length (% SL)	69.93	78.71	**73.67**	2.21	70.02	74.39	**72.07**	1.13	71.32	77.38	**73.86**	1.18
Pectoral – pelvic-fin origin length (% SL)	22.86	29.33	**26.08**	1.45	22.79	29.14	**26.03**	1.30	25.54	29.44	**27.79**	1.04
Pelvic – anal-fin origin length (% SL)	17.40	23.61	**19.63**	1.18	18.96	23.16	**20.98**	0.98	18.32	21.79	**20.06**	0.85
Length of caudal peduncle (% SL)	16.88	21.77	**19.34**	1.04	17.07	21.62	**19.81**	0.95	17.44	21.68	**19.65**	1.15
Dorsal-fin base length (% SL)	9.38	13.28	**11.09**	0.77	9.23	13.57	**11.37**	0.91	10.73	12.33	**11.66**	0.44
Dorsal fin depth (% SL)	13.94	18.89	**16.19**	1.11	14.32	18.86	**15.94**	1.14	11.42	18.20	**16.06**	1.28
Anal-fin base length (% SL)	8.07	12.38	**10.07**	0.96	9.62	12.34	**10.70**	0.59	9.37	16.07	**10.64**	1.22
Anal fin depth (% SL)	10.21	15.76	**12.42**	0.96	10.42	14.45	**11.99**	0.83	10.14	13.84	**12.17**	0.81
Pectoral fin length (% SL)	15.36	19.09	**17.64**	0.90	14.68	19.97	**17.15**	0.99	15.33	19.26	**17.17**	0.96
Pelvic fin length (% SL)	12.96	15.89	**14.07**	0.61	12.56	15.53	**13.93**	0.74	11.94	15.17	**13.95**	0.77
Head length (% SL)	28.97	33.67	**31.06**	1.34	25.39	29.62	**27.38**	0.95	26.87	29.97	**28.91**	0.77
Head length (% body depth)	113.99	148.29	**131.59**	8.51	108.52	138.39	**119.94**	6.37	104.48	129.69	**118.79**	5.89
Head depth at nape (% SL)	16.34	20.35	**17.92**	0.84	15.84	18.39	**16.99**	0.56	16.60	18.86	**17.69**	0.62
Head depth at nape (% HL)	52.35	61.23	**57.74**	2.21	58.18	66.80	**62.10**	2.29	56.15	65.36	**61.22**	2.35
Head depth through eye (% HL)	36.43	46.06	**41.04**	2.06	40.16	47.39	**43.35**	1.76	38.58	47.87	**43.32**	2.66
Maximum head width (% SL)	13.07	15.90	**14.43**	0.58	13.08	15.56	**14.18**	0.57	12.39	16.68	**14.75**	0.94
Maximum head width (% HL)	40.97	52.29	**46.54**	2.54	45.81	57.88	**51.85**	2.67	42.33	57.60	**51.05**	3.42
Snout length (% SL)	7.90	10.65	**9.18**	0.57	7.75	9.23	**8.41**	0.34	7.70	9.38	**8.75**	0.38
Snout length (% HL)	26.97	32.35	**29.57**	1.52	27.35	33.57	**30.75**	1.48	28.31	32.78	**30.26**	1.05
Eye horizontal diameter (% SL)	4.56	7.94	**5.95**	1.04	4.12	6.62	**5.07**	0.64	4.33	5.98	**4.81**	0.34
Eye horizontal diameter (% HL)	14.09	25.74	**19.13**	3.14	14.96	23.38	**18.51**	2.03	14.94	21.99	**16.66**	1.51
Eye horizontal diameter (% interorbital width)	46.77	86.54	**64.30**	11.65	44.02	69.51	**55.27**	7.27	44.19	69.52	**50.30**	5.02
Postorbital distance (% HL)	49.98	58.10	**54.13**	2.07	51.83	57.43	**54.39**	1.37	53.92	58.84	**56.56**	1.20
Interorbital width (% SL)	8.04	10.25	**9.27**	0.49	8.53	9.80	**9.19**	0.33	8.60	10.46	**9.59**	0.44
Interorbital width (% HL)	26.71	32.92	**29.89**	1.54	30.44	36.57	**33.61**	1.43	30.45	36.58	**33.16**	1.43
Length of upper jaw (% HL)	27.04	34.12	**30.32**	1.59	27.21	33.03	**29.41**	1.01	29.04	33.73	**30.37**	1.09
Length of upper jaw (% SL)	7.92	11.35	**9.42**	0.61	7.42	8.89	**8.05**	0.33	8.01	9.41	**8.78**	0.33
Length of lower jaw (% SL)	11.28	13.70	**12.46**	0.64	9.25	11.08	**10.23**	0.42	10.00	12.04	**11.09**	0.44
Length of lower jaw (% HL)	35.94	43.94	**40.13**	1.65	34.80	40.52	**37.37**	1.05	34.72	40.26	**38.37**	1.37
Length of lower jaw (% interorbital width)	121.44	154.57	**134.50**	6.51	99.74	120.89	**111.35**	4.58	101.98	128.28	**115.92**	6.76
Length of lower jaw (% depth of operculum)	107.34	128.60	**117.57**	4.98	89.15	114.07	**102.71**	4.63	100.00	129.14	**111.63**	7.76
Cranium width between margins of pterotics (% cranium roof length)	60.08	76.79	**68.11**	3.23	58.95	81.63	**71.15**	4.38	64.53	75.25	**69.14**	3.38
Cranium width between margins of sphenotics (% cranium roof length)	49.71	64.45	**56.58**	3.46	51.25	68.40	**61.08**	3.47	54.87	64.87	**59.49**	2.81
Cranium width between margins of supraethmoid (% cranium roof length)	19.74	26.51	**23.63**	1.75	20.05	26.51	**23.65**	1.42	19.38	28.32	**23.66**	2.17
Cranium width between margins of supraethmoid (% cranium width between margins of pterotics)	28.21	40.53	**34.77**	3.04	28.87	37.41	**33.28**	1.74	30.04	41.08	**34.22**	2.68

	S. microlepis phenotype 1, N = 47				*S. microlepis* phenotype 2, N = 46				*S. tenellus*, N = 25			
	min	max	mean	sd	min	max	mean	sd	min	max	mean	sd
Length of lower jaw (% cranium width between margins of pterotics)	95.09	118.46	**105.16**	5.91	81.34	100.86	**89.81**	4.10	88.51	109.80	**98.87**	6.45
Depth of operculum (% HL)	31.35	38.65	**34.18**	1.65	32.11	41.52	**36.45**	1.81	29.91	38.86	**34.48**	1.93
RATIOS:												
Interorbital width/eye horizontal diameter	1.16	2.14	**1.61**	0.28	1.44	2.27	**1.84**	0.24	1.44	2.26	**2.00**	0.17
Snout length/eye horizontal diameter	1.06	2.24	**1.59**	0.31	1.17	2.13	**1.68**	0.22	1.29	2.10	**1.83**	0.17
Head depth at nape/eye horizontal diameter	2.16	4.29	**3.10**	0.55	2.56	4.23	**3.40**	0.42	2.97	4.23	**3.70**	0.29
Head length/caudal peduncle depth	2.77	3.48	**3.16**	0.21	2.35	3.02	**2.68**	0.16	2.41	2.95	**2.76**	0.13
Length of caudal peduncle/caudal peduncle depth	1.77	2.19	**1.97**	0.12	1.68	2.18	**1.94**	0.12	1.69	2.03	**1.87**	0.09
Length of lower jaw/caudal peduncle depth	1.06	1.43	**1.27**	0.09	0.88	1.15	**1.00**	0.06	0.92	1.15	**1.06**	0.06
Pectoral fin length/pectoral – pelvic-fin origin distance	0.60	0.83	**0.68**	0.06	0.56	0.77	**0.66**	0.05	0.53	0.69	**0.62**	0.04
Predorsal length/head length	1.75	2.00	**1.89**	0.07	1.93	2.20	**2.06**	0.07	1.83	2.08	**1.96**	0.06
COUNTS:												
Scales in lateral series	67	78	**72.33**	2.83	64	77	**69.77**	3.22	76	95	**85.48**	4.98
Lateral-line scales	58	77	**68.47**	3.91	58	75	**67.70**	3.99	68	83	**76.72**	4.34
Scales above lateral line	13	16	**14.49**	0.74	13	16	**14.25**	0.78	17	20	**18.80**	0.82
Scales below lateral line	5	7	**6.16**	0.69	4	7	**5.61**	0.72	7	10	**8.76**	0.78
Gill rakers	14	16	**15.21**	0.67	11	14	**12.59**	0.84	14	18	**15.72**	0.98
Number of predorsal vertebrae	15	16	**15.19**	0.39	14	16	**14.86**	0.41	15	16	**15.16**	0.37
Number of abdominal vertebrae	24	25	**24.67**	0.47	24	25	**24.55**	0.50	24	25	**24.40**	0.50
Number of caudal vertebrae	17	19	**17.79**	0.67	17	20	**18.50**	0.59	17	20	**18.64**	0.76
Total vertebrae	42	44	**42.47**	0.67	42	44	**43.05**	0.43	42	44	**43.04**	0.54
Difference between abdominal and caudal numbers	5	8	**6.88**	0.96	4	8	**6.05**	1.01	4	8	**5.76**	1.16

Results

The data presented in Tables 2, 3 and osteological and sensory canal examinations confirmed a traditional concept of *S. microlepis* and *S. tenellus* (Bănărescu and Herzig-Straschil 1998; Kottelat and Freyhof 2007) as a morphologically distinct group different from other *Squalius* species in Europe. This group can be clearly distinguished by having small scales (64–95 in total lateral series and 58–83 in total lateral line) and a reduced or lacking 5th infraorbital.

Squalius tenellus (Fig. 2) has markedly smaller scales than *S. microlepis* s. l. and, respectively, higher numbers of total lateral-line scales, total scales in lateral series and scales above (to the dorsal-fin origin) and below (to the pelvic-fin origin) the lateral line. For *S. tenellus*, ranges of these character states are as follows: 76–95 (80 in lectotype) scales in lateral series, 68–83 (78 in lectotype) in lateral line, 17–20 (19 in lectotype) above lateral line, and 7–10 (9 in lectotype) above lateral line. These numbers are different from those commonly published based on data of Bănărescu and Herzig-Straschil (1998: 420); this may be due to a different method of counting. *Squalius tenellus* can be further distinguished by an often slightly incomplete, interrupted or deformed lateral line and scales somewhat irregularly placed on the back and flanks; these traits have not been found in *S. microlepis*.

Table 3. Frequency of occurrence of diagnostic meristic character states in *Squalius microlepis* phenotypes 1 and 2 and in *S. tenellus*.

	Number of scales in lateral series								Number of scales above lateral line								Number of scales below lateral line							Gill rakers										
	64–67	68–71	72–75	76–79	80–83	84–87	88–91	92–95	13	14	15	16	17	18	19	20	4	5	6	7	8	9	10	8	9	10	11	12	13	14	15	16	17	18
S. tenellus, N = 28				6	5	9	5	3					1	9	13	5				1	9	14	4							1	12	10	3	2
S. microlepis phenotype 1																																		
Ričice Reservoir, N = 38	4	12	17	5					1	16	18	2	1					5	23	10											7	15	16	
Prološko Lake, Imotski N = 30	3	12	13	2					2	12	14	2						7	14	9											8	14	8	
Vrljika, N = 43	3	18	16	4	2				4	18	14	4						8	17	15										1	11	23	9	
Krenica Lake, N = 8		5	3						2	5	1							5	2	1											2	5	1	
Total, N = 119	10	48	49	11	2				9	53	48	9	1					26	58	36										1	28	57	34	
S. microlepis phenotype 2																																		
Tihaljina and Trebižat, N = 39	9	16	9	5					7	21	11							15	19	3							2	22	11	4				
Lower Matica, N = 7		4	1	2						2	3	2						3	3	1								1	3	3				
Total, N = 46	9	20	10	7					7	23	14	2						18	22	4							2	23	14	7				

	Total vertebrae				Abdominal vertebrae		Caudal vertebrae				Predorsal vertebrae				Vertebral formulae						
	41	42	43	44	24	25	17	18	19	20	13	14	15	16	24+17	24+18	24+19	24+20	25+17	25+18	25+19
S. tenellus, N = 25		4	18	3	15	10	1	11	11	2		2	21	4	1			2	1	8	1
S. microlepis phenotype 1, N = 43			23		29	14						3	39	1			19	2	1		9
S. microlepis phenotype 2, N = 44					27	17						5	31	1		3	21	1	1	8	3

Table 4. Morphometric data of *Squalius microlepis* phenotypes in two size classes.

	Phenotype 1, N=20				Phenotype 1, N=27				Phenotype 2, N=15				Phenotype 2, N=31			
	min	max	m	sd	min	max	m	sd	min	max	m	sd	min	max	m	sd
SL, mm	61.1	121.7	94.3		125.3	223.4	178.4		72.0	128.0	98.5		135.7	255.7	182.3	
Maximum body depth (% SL)	21.1	24.6	22.3	0.8	22.8	26.7	24.2	1.1	21.4	24.7	22.7	1.1	20.9	25.1	23.0	1.1
Depth of caudal peduncle (% SL)	9.1	10.4	9.7	0.4	9.1	10.6	9.9	0.5	9.5	10.8	10.2	0.4	9.5	11.0	10.3	0.4
Depth of caudal peduncle (% length of caudal peduncle)	45.7	56.0	50.9	3.4	46.1	56.6	51.1	3.0	45.9	55.8	51.1	3.3	46.0	59.5	52.6	3.5
Maximum body width (% SL)	11.8	15.3	13.5	0.9	12.4	16.0	13.6	0.9	13.1	16.1	13.9	0.8	14.0	17.7	15.6	0.8
Caudal peduncle width (% SL)	7.6	10.3	8.7	0.7	7.2	9.7	8.2	0.7	7.8	10.2	8.8	0.6	8.6	11.1	9.6	0.5
Predorsal length (% SL)	57.0	59.7	58.5	0.9	56.1	60.5	58.2	1.1	54.3	58.5	56.3	1.3	54.5	58.3	56.4	1.2
Postdorsal length (% SL)	30.4	34.6	32.2	1.3	30.3	35.8	32.5	1.5	32.8	35.4	34.4	0.8	33.2	35.8	34.5	0.7
Prepelvic length (% SL)	52.5	57.9	54.6	1.4	50.9	59.4	55.0	2.5	50.7	54.3	52.0	1.0	49.5	54.2	51.8	1.2
Preanal length (% SL)	69.9	78.7	73.6	2.2	70.4	78.7	73.8	2.1	70.9	73.5	72.3	0.8	70.3	74.4	72.1	1.2
Pectoral – pelvic-fin origin length (% SL)	22.9	27.6	25.0	1.3	24.8	29.3	26.5	1.2	23.8	28.5	25.9	1.3	24.0	29.1	26.4	1.3
Pelvic – anal-fin origin length (% SL)	17.4	20.3	18.8	0.9	18.3	23.6	20.1	1.2	19.2	23.2	20.9	1.2	19.0	22.4	21.1	0.9
Length of caudal peduncle (% SL)	16.9	20.9	19.2	1.3	17.3	21.8	19.4	1.1	19.3	20.9	20.0	0.6	17.1	21.6	19.7	1.1
Dorsal-fin base length (% SL)	9.4	11.5	10.9	0.6	9.7	13.3	11.4	0.8	9.2	12.6	11.0	0.9	9.9	13.6	11.5	0.9
Dorsal fin depth (% SL)	15.4	18.9	17.2	1.0	13.9	17.7	15.6	0.9	14.9	18.9	16.9	1.2	14.3	17.1	15.4	0.7
Anal-fin base length (% SL)	8.8	11.5	10.2	0.8	8.1	12.4	10.1	1.0	9.7	11.4	10.6	0.4	9.6	12.3	10.8	0.6
Anal fin depth (% SL)	12.2	15.8	13.2	1.1	10.2	14.0	12.0	0.6	10.7	14.5	12.5	0.9	10.4	12.7	11.7	0.6
Pectoral fin length (% SL)	16.9	19.1	18.0	0.7	15.4	19.1	17.4	1.0	16.5	20.0	17.7	1.0	14.7	18.7	17.0	0.9
Pelvic fin length (% SL)	13.0	15.9	14.4	0.7	13.3	15.2	14.0	0.6	13.3	15.5	14.4	0.6	12.6	15.2	13.8	0.7
Head length (% SL)	29.7	32.6	31.2	0.9	29.0	33.7	30.9	1.5	25.9	29.6	27.8	1.0	25.4	28.2	27.1	0.8
Head length (% body depth)	132.7	148.3	140.4	5.3	114.0	142.6	126.8	6.6	109.2	138.4	122.9	8.0	108.5	126.1	117.8	5.0
Head depth at nape (% SL)	16.3	19.0	17.7	0.6	16.9	20.3	18.0	1.0	16.2	17.8	16.9	0.4	15.8	18.4	17.0	0.7
Head depth at nape (% HL)	52.4	61.2	56.8	2.3	54.3	60.9	58.6	1.7	58.2	64.9	60.7	2.1	59.4	66.8	63.0	2.1
Maximum head width (% SL)	13.5	15.9	14.6	0.6	13.6	15.4	14.5	0.5	13.2	14.4	13.9	0.4	13.1	15.6	14.3	0.6
Maximum head width (% HL)	43.7	50.4	46.8	1.8	43.1	52.3	47.3	2.4	45.8	54.2	49.9	2.2	48.3	57.9	52.8	2.3
Snout length (% SL)	8.2	9.9	9.1	0.5	8.1	10.6	9.3	0.6	7.7	9.2	8.3	0.4	7.8	9.0	8.4	0.3
Snout length (% HL)	27.2	30.8	29.0	1.4	27.5	32.4	30.3	1.3	27.4	32.6	29.8	1.6	29.2	33.6	31.2	1.2
Eye horizontal diameter (% SL)	6.2	7.9	7.1	0.6	4.6	6.4	5.1	0.5	5.0	6.6	5.8	0.5	4.1	5.4	4.7	0.3
Eye horizontal diameter (% HL)	20.0	25.7	22.8	2.0	14.1	19.5	16.7	1.3	18.0	23.4	20.7	1.6	15.0	20.3	17.5	1.4
Eye horizontal diameter (% interorbital width)	61.8	86.5	75.7	8.1	46.8	72.4	55.2	5.6	56.8	69.5	63.0	4.8	44.0	61.0	51.6	4.9
Postorbital distance (% HL)	50.4	54.2	51.9	1.2	50.0	58.1	55.1	1.7	51.9	57.4	54.0	1.3	51.8	56.9	54.5	1.4
Interorbital width (% SL)	8.8	10.3	9.4	0.4	8.3	10.1	9.3	0.5	8.8	9.6	9.1	0.3	8.5	9.8	9.2	0.3
Interorbital width (% HL)	27.2	32.6	30.2	1.4	26.7	32.9	30.3	1.3	31.1	35.4	32.8	1.4	30.4	36.6	34.1	1.2
Length of upper jaw (% HL)	28.0	32.5	30.1	1.1	27.1	34.1	30.8	1.7	27.2	30.0	29.1	0.8	28.3	33.0	29.6	1.0
Length of upper jaw (% SL)	8.6	10.1	9.4	0.4	8.4	11.4	9.4	0.7	7.7	8.5	8.1	0.3	7.4	8.9	8.0	0.3

	Phenotype 1, N=20				Phenotype 1, N=27				Phenotype 2, N=15				Phenotype 2, N=31			
	min	max	m	sd	min	max	m	sd	min	max	m	sd	min	max	m	sd
Length of lower jaw (% SL)	11.4	13.6	12.6	0.5	11.3	13.5	12.4	0.6	10.0	11.1	10.4	0.4	9.3	10.7	10.1	0.3
Length of lower jaw (% HL)	35.9	43.9	40.5	1.7	37.9	43.7	40.3	1.5	35.9	39.7	37.5	1.2	34.8	40.5	37.3	1.0
Length of lower jaw (% interorbital width)	122.3	146.7	134.0	5.3	121.4	142.7	133.5	5.3	109.1	120.9	114.5	3.7	99.7	119.7	109.4	3.4
Length of lower jaw (% depth of operculum)	111.7	128.6	118.7	4.9	107.3	125.9	117.4	4.7	98.9	111.4	103.9	3.8	89.1	114.1	101.9	5.3
Maximum cranial width (% cranium roof length)	63.9	76.8	69.4	4.4	64.0	73.8	68.0	2.6	67.7	77.2	71.6	2.9	69.0	81.6	73.9	4.9
Supraethmoid width (% cranium roof length)	20.0	25.7	23.0	1.8	21.1	26.5	24.1	1.7	20.0	26.1	24.4	1.6	20.5	26.5	23.4	1.4
Length of lower jaw (% maximum cranial width)	95.2	110.9	102.7	5.6	97.9	115.4	106.6	4.9	81.7	94.2	89.5	3.7	81.3	100.9	89.7	4.1
Depth of operculum (% HL)	32.1	36.4	34.1	1.3	32.0	38.7	34.5	1.7	34.0	38.7	36.1	1.7	32.1	41.5	36.6	2.1
RATIOS:																
Interorbital width/eye horizontal diameter	1.2	1.6	1.3	0.2	1.4	2.1	1.8	0.2	1.4	1.8	1.6	0.1	1.6	2.3	2.0	0.2
Snout length/eye horizontal diameter	1.1	1.5	1.3	0.2	1.4	2.2	1.8	0.2	1.2	1.6	1.4	0.1	1.5	2.1	1.8	0.2
Head depth at nape/eye horizontal diameter	2.2	2.9	2.5	0.2	2.8	4.3	3.5	0.3	2.6	3.4	3.0	0.3	3.1	4.2	3.6	0.3
Head length/caudal peduncle depth	2.9	3.5	3.2	0.2	2.8	3.5	3.1	0.2	2.4	3.0	2.7	0.2	2.3	2.8	2.6	0.1
Length of caudal peduncle/caudal peduncle depth	1.8	2.2	2.0	0.1	1.8	2.2	2.0	0.1	1.8	2.2	2.0	0.1	1.7	2.2	1.9	0.1
Length of lower jaw/caudal peduncle depth	1.1	1.4	1.3	0.1	1.1	1.4	1.3	0.1	1.0	1.1	1.0	0.1	0.9	1.1	1.0	0.0
Pectoral fin length/pectoral – pelvic-fin origin distance	0.6	0.8	0.7	0.1	0.6	0.8	0.7	0.0	0.6	0.8	0.7	0.1	0.6	0.7	0.6	0.0
Predorsal length/head length	1.8	1.9	1.9	0.0	1.7	2.0	1.9	0.1	1.9	2.1	2.0	0.1	2.0	2.2	2.1	0.1

Table 5. Morphometric and meristic data of *Squalius microlepis* specimens classified in separate set of analyses (see text for explanations).

Collection	NMW 49428	NMW 49427	NMW 49423.1	NMW 49423.2	NMW 49424	NMW 49425	NMW 49426	NMW 49228.1	NMW 49228.2	MNCN_ICTIO 292.129–292.136	MNCN_ICTIO 292.129–292.136
Stated locality	Lago di Dusino presso Imotsky	Narenta	Vrgoraz [see Jessero]	Vrgoraz [see Jessero]	Vrgoraz [see Jessero]	Vrgoraz	Vrgoraz [see Jessero]	Zara	Zara	Tihaljina	Tihaljina
Specimen	A	B	C	D	E	F	G	H	I	J	K
SL, mm	168.07	140.06	122.52	276.08	269.1	177.98	193.81	165.84	205.13	127.05	149.75
Maximum body depth (% SL)	26.26	21.58	23.20	23.70	22.84	24.74	21.21	24.57	27.75	23.74	23.88
Depth of caudal peduncle (% SL)	11.42	10.35	11.40	10.53	10.62	10.92	9.78	9.86	10.95	9.82	10.09
Depth of caudal peduncle (% length of caudal peduncle)	56.06	51.53	60.40	58.81	55.68	54.27	50.71	50.25	56.83	49.04	47.85
Body width at dorsal-fin origin (% SL)	13.73	11.82	13.64	12.26	11.91	13.02	13.84	12.75	15.37	13.59	16.05
Caudal peduncle width (% SL)	9.64	7.65	9.72	7.38	7.08	8.39	9.40	8.47	8.98	8.26	8.97
Predorsal length (% SL)	58.22	56.55	58.52	57.46	56.85	57.03	58.17	59.35	59.16	55.58	55.26
Postdorsal length (% SL)	33.30	36.58	32.59	33.75	34.40	35.35	34.34	31.08	32.57	34.07	33.92
Prepelvic length (% SL)	52.46	53.59	52.08	55.14	54.83	52.25	53.00	56.22	54.03	54.55	52.73
Preanal length (% SL)	74.87	72.84	73.87	75.86	72.83	74.96	74.22	75.61	73.37	72.61	72.33
Pectoral – pelvic-fin origin length (% SL)	25.97	24.90	24.38	26.80	26.93	27.69	27.77	26.47	24.59	27.63	24.89
Pelvic – anal-fin origin length (% SL)	22.37	18.76	20.66	22.07	20.60	23.49	22.11	20.15	21.19	19.87	19.97
Length of caudal peduncle (% SL)	20.38	20.08	18.88	17.91	19.08	20.11	19.29	19.63	19.28	20.03	21.09
Dorsal-fin base length (% SL)	10.22	10.55	10.10	10.86	11.56	10.41	10.12	11.29	12.12	11.69	11.71
Dorsal fin depth (% SL)	17.56	16.12	16.50	15.81	15.22	15.06	15.53	14.96	14.20	15.93	16.39
Anal-fin base length (% SL)	8.91	10.37	10.55	9.34	10.24	10.39	9.20	10.34	10.95	10.38	11.52
Anal fin depth (% SL)	11.23	12.78	11.57	10.61	12.15	10.60	11.80	11.82	12.61	10.83	11.81
Pectoral fin length (% SL)	16.01	18.14	17.38	17.25	18.12	14.25	14.64	18.31	18.19	16.98	18.01
Pelvic fin length (% SL)	13.74	13.65	13.86	13.35	13.71	12.20	12.49	15.24	15.43	12.96	14.03
Head length (% SL)	27.89	28.67	29.61	29.34	29.49	27.22	27.01	30.37	30.53	28.96	29.47
Head length (% body depth)	106.18	132.82	127.66	123.80	129.12	110.04	127.32	123.61	110.01	122.02	123.41
Head depth at nape (% SL)	17.86	16.92	17.52	18.90	18.31	16.91	18.50	19.09	19.24	16.68	17.32
Head depth at nape (% HL)	64.05	59.03	59.15	64.42	62.06	62.11	68.49	62.85	63.03	57.58	58.78
Head depth through eye (% HL)	43.82	39.78	40.96	47.36	43.79	40.25	47.00	39.25	42.33	39.76	41.58
Maximum head width (% SL)	13.71	12.82	12.59	14.18	13.42	13.41	14.32	13.53	14.17	13.40	14.31
Maximum head width (% HL)	49.18	44.71	42.53	48.35	45.50	49.25	53.04	44.55	46.42	46.25	48.56
Snout length (% SL)	8.67	8.00	8.43	9.18	8.20	7.92	8.19	8.80	8.82	8.32	8.83
Snout length (% HL)	31.11	27.90	28.47	31.30	27.81	29.10	30.32	28.97	28.90	28.72	29.96
Eye horizontal diameter (% SL)	4.19	6.31	6.18	4.37	4.86	4.53	4.27	5.69	5.57	5.31	4.77
Eye horizontal diameter (% HL)	15.02	22.02	20.87	14.90	16.47	16.64	15.80	18.74	18.25	18.34	16.18
Eye horizontal diameter (% interorbital width)	46.25	77.89	69.96	45.91	55.36	46.86	45.62	63.53	60.99	60.70	52.31
Postorbital distance (% HL)	57.33	55.29	52.59	59.59	56.90	56.97	58.54	55.17	54.46	54.18	54.82
Interorbital width (% SL)	9.06	8.10	8.83	9.52	8.77	9.66	9.35	8.96	9.14	8.75	9.12
Interorbital width (% HL)	32.47	28.27	29.82	32.46	29.75	35.50	34.64	29.50	29.93	30.22	30.93

Collection	NMW 49428	NMW 49427	NMW 49423.1	NMW 49423.2	NMW 49424	NMW 49425	NMW 49426	NMW 49228.1	NMW 49228.2	MNCN_ICTIO 292.129–292.136	MNCN_ICTIO 292.129-292.136
Stated locality	Lago di Dusino presso Imotsky	Narenta	Vrgoraz [see Jessero]	Vrgoraz [see Jessero]	Vrgoraz [see Jessero]	Vrgoraz	Vrgoraz [see Jessero]	Zara	Zara	Tihaljina	Tihaljina
Specimen	A	B	C	D	E	F	G	H	I	J	K
Length of upper jaw (% HL)	30.51	24.66	27.73	32.51	32.49	29.37	29.27	30.91	32.35	29.35	29.05
Length of upper jaw (% SL)	8.51	7.07	8.21	9.54	9.58	8.00	7.90	9.39	9.88	8.50	8.56
Length of lower jaw (% SL)	10.37	10.42	10.74	12.50	12.26	10.20	10.33	12.69	12.28	11.14	11.39
Length of lower jaw (% HL)	37.19	36.34	36.27	42.59	41.58	37.46	38.25	41.79	40.21	38.45	38.64
Length of lower jaw (% interorbital width)	114.52	128.55	121.63	131.23	139.77	105.52	110.42	141.66	134.36	127.25	124.91
Length of lower jaw (% depth of operculum)	104.31	96.24	112.48	107.78	111.49	100.33	96.95	113.11	100.12	107.36	101.49
Cranium width between margins of pterotics (% L cranium roof length)	64.68	70.41	68.90	72.54	72.41	78.53	78.75	75.04	73.82	75.76	70.96
Cranium width between margins of sphenotics (% cranium roof length)	59.57	57.79	61.02	61.22	60.14	64.77	69.80	62.63	59.88	62.32	57.52
Cranium width between margins of supraethmoid (% cranium roof length)	28.40	22.60	25.23	22.42	27.38	23.54	27.87	24.64	25.54	26.77	23.51
Cranium width between margins of supraethmoid (% cranium width between margins of pterotics)	43.91	32.10	36.62	30.91	37.81	29.97	35.39	32.83	34.60	35.34	33.13
Length of lower jaw (% cranium width between margins of pterotics)	95.66	89.90	89.58	98.21	105.13	85.53	85.89	102.38	99.68	94.71	94.15
Depth of operculum (% HL)	35.65	37.76	32.25	39.52	37.29	37.34	39.45	36.95	40.16	35.82	38.07
RATIOS:											
Interorbital width/eye horizontal diameter	2.16	1.28	1.43	2.18	1.81	2.13	2.19	1.57	1.64	1.65	1.91
Snout length/eye horizontal diameter	2.07	1.27	1.36	2.10	1.69	1.75	1.92	1.55	1.58	1.57	1.85
Head depth at nape/eye horizontal diameter	4.26	2.68	2.83	4.32	3.77	3.73	4.33	3.35	3.45	3.14	3.63
Head length/caudal peduncle depth	2.44	2.77	2.60	2.79	2.78	2.49	2.76	3.08	2.79	2.95	2.92
Length of caudal peduncle/caudal peduncle depth	1.78	1.94	1.66	1.70	1.80	1.84	1.97	1.99	1.76	2.04	2.09
Length of lower jaw/caudal peduncle depth	0.91	1.01	0.94	1.19	1.15	0.93	1.06	1.29	1.12	1.13	1.13
Pectoral fin length/pectoral – pelvic-fin origin distance	0.62	0.73	0.71	0.64	0.67	0.51	0.53	0.69	0.74	0.61	0.72
Predorsal length/head length	2.09	1.97	1.98	1.96	1.93	2.10	2.15	1.95	1.94	1.92	1.88
COUNTS:											
Scales in lateral series	71	76	68	74	67	66	74	72	71	72	70
Total lateral-line scales	68	74	67	73	66	64	72	70	69	71	70
Scales above lateral line	14	15	14	14	14	13	16	15	14	15	14
Scales below lateral line	7	6	5	6	6	6	7	6	6	6	6
Gill rakers	15	14	15	15	14	12	13	15	15	16	15
Number of predorsal vertebrae	15	15	15	15	15	15	15	15	15	15	15
Number of abdominal vertebrae	24	24	25	24	25	25	25	25	25	25	24
Number of caudal vertebrae	19	19	17	18	17	18	18	17	17	18	19
Total vertebrae	43	43	42	42	42	43	43	42	42	43	43
Difference between abdominal and caudal counts	5	5	8	6	8	7	7	8	8	7	5

Figure 2. *Squalius tenellus*, NMW 16001, lectotype, 122.1 mm SL, 'Livno'.

An examination of the entire set of *Squalius microlepis* examined specimens (Tables 2–5, Figs 3–6) revealed a number of character states that allow to distinguish two phenotypes: phenotype 1 representing *S. microlepis* s. str. as defined by its lectotype (Fig. 3) and phenotype 2 as represented in Fig. 4.

The first step morphological analyses and comparisons excluded specimens A to K as specified in Table 1 and the primary data presented in Table 4. The reasons were as follows:

1　uncertainty of the localities
　–　"Lago di Dusino" (specimen A); we suppose the locality is not 'near Imotski' but the Dusina Polje (Lake of Dusina) formed by some large karstic springs at the village Dusina south of Vrgorac and immediately adjacent to Polje Jezero;
　–　"Narenta" (B); *S. microlepis* is not reliably known from the main stream of the Neretva as discussed below;
　–　"Zara" (H, I), an NMW historic sample, labelled as 'Zara' (Italian name for Zadar), a locality not known for *S. microlepis*-like species and 200 km outside the known range of *S. microlepis* s. str. (probable mislabelling of the sample is discussed below);
2　a high morphological heterogeneity of the sample from karst systems at Vrgorac (C to G); karstic poljes near Vrgorac are geographically distant from the Imotski system though connected to the Tihaljina-Trebižat system; this area is of a special historical importance because no other specimens are extant in collections to our knowledge neither we were able to collect this fish in karts systems near Vrgorac;
3　specimens J and K are the only ones similar to phenotype 1 among the numerous samples of the Tihaljina-Trebižat phenotype 2.

The second step was to run separate statistical analyses for identification of these specimens.

Size-related variability in two phenotypes of *S. microlepis*

Table 4 contains data on a comparison of smaller-sized (SL < 130 mm) and larger-sized (SL > 130 mm) specimens per phenotype. Significantly size-related (p < 0.01000) are

Figure 3. *Squalius microlepis* **A** NMW 49415, lectotype, 150.8 mm SL, "Imosky" **B** phenotype 1: alive specimen, MNCN_ICTIO 296.096-296.097, 147.6 mm SL, Bosnia and Herzegovina: Krenica Lake at Drinovci.

18 characters in *S. microlepis* phenotype 1 and 22 characters in *S. microlepis* phenotype 2. Shared size-related characters are as follows: dorsal fin depth (% SL), anal fin depth (% SL), head length (% SL), head length (% body depth), head depth at nape (% HL), snout length (% HL), eye horizontal diameter (% SL), eye horizontal diameter (% HL), eye horizontal diameter (% interorbital width), interorbital width/eye horizontal diameter, snout length/eye horizontal diameter, head depth at nape/eye horizontal diameter, pectoral fin length/pectoral – pelvic-fin origin distance, predorsal length/head length. Head depth at nape and snout length increase with size while anal- and dorsal-fin depth, head length, and eye diameter decrease.

Difference between two phenotypes of *S. microlepis*

The two phenotypes are readily distinguished (phenotype 1 vs. phenotype 2; external characters on an example of middle-sized specimens see Fig. 5; Tables 2–4) by the following combinations of character states:

1   number of gill rakers: (13)14–16 (15 in lectotype of *S. microlepis*), mean 15.0 vs. 11–14, mean 12.6;
2   total vertebrae: commonly 42 (24+18 or 25+17) and 43 (25+18) vs. commonly 43 (24+19);
3   dorso-hypural distance: commonly falling behind the posterior eye margin (at a considerable distance from the eye margin in large-sized specimens as can be also seen in Kottelat and Freyhof (2007: figure on page 269) vs. commonly falling into the middle or posterior half of the eye when reported forward;

Figure 4. *Squalius microlepis* phenotype 2 **A** SMNH 443, 255.7 mm SL, Bosnia and Herzegovina: Tihaljina River at Tihaljina **B** MNCN_ICTIO 294.472-294.473, 140.1 mm SL, Bosnia and Herzegovina: Trebižat River at Grabovnik-Vašarovići **C** alive specimen, ZISP 54994, 147.2 mm SL, Bosnia and Herzegovina: Matica (Vrljika) River at Drinovci.

4   the back: usually a well pronounced discontinuity behind the head (even in small-sized individuals), a straightened back profile and the maximum body depth located just behind the head vs. smoothly convex lacking a prominent hump behind the head and the maximum body depth located at or slightly in front of the dorsal-fin origin;

5   maximum body depth: the body deepest at a vertical closer to the head than to the dorsal-fin origin and, respectively, maximum body depth exceeds 1.05–1.20 times body depth at the dorsal-fin origin vs. about equal to body depth at the dorsal-fin origin;

6   length of lower jaw (% interorbital width): 121–155% (mean 134.5%) vs. 100–121% (mean 111%); length of lower jaw (% cranium width) 95–118% (mean 105% vs. 81–101% (mean 90%);
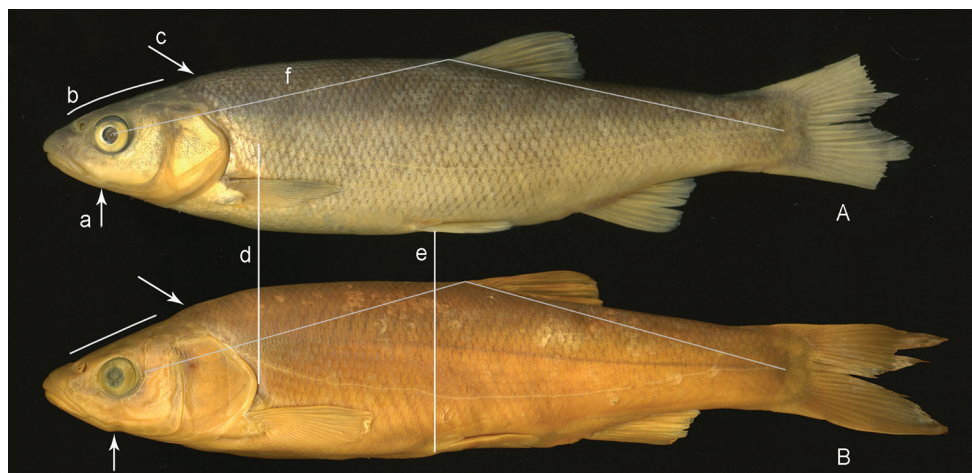
Figure 5. Lateral view to show characters superficially distinguishing phenotypes of *Squalius microlepis* s. l. **A** *Squalius microlepis* phenotype 2: MNCN_ICTIO 294.784-294.800, 128.0 mm SL (Tihaljina) **B** *Squalius microlepis* phenotype 1: NMW 12729-32, 119.5 mm SL ('Imotski'). Key: Arrow a – posterior end of lower jaw, line b – upper head profile, arrow c – body profile just behind head; vertical d – shorter head in *S. microlepis* phenotype 2, vertical e – shorter prepelvic distance in *S microlepis* phenotype 2; line f – dorso-hypural distance if reported forward.

7   head length (% SL): 29–34% (mean 31%) vs. 25–30% (mean 27%); the ranges do not overlap in larger-sized specimens (SL > 130 mm; Table 3);

8   head depth at nape (% HL) in larger-sized specimens (SL > 130 mm; Table 3): 54–61% (mean 59%) vs. 59–69% (mean 63%);

9   the upper head profile: straight vs. commonly slightly convex behind the eyes.

Besides these characters, Fig. 5B illustrates a specimen of *S. microlepis* phenotype 1 having the upper jaw not projecting beyond the lower jaw, the lower jaw-quadrate junction lying on the vertical through the middle of the eye, and a prominent 'angle' formed by the posterior end of the lower jaw; and the mouth cleft is long, straight and oblique. Phenotype 2 (Fig. 5A) is commonly characterised by the upper jaw clearly projecting beyond the lower jaw and including the tip of the lower jaw; the lower jaw-quadrate junction located about at a vertical through or slightly in front of the anterior margin of the pupil; the lower jaw posterior end not forming a prominent angle; and the mouth cleft slightly curved and more horizontal.

Statistical analyses

Comparison of the two phenotypes of *S. microlepis*

I   A DFA based on counts and standardised direct measurements (Fig. 7A) support 100% discrimination for both groups. DFA statistics values are as follows: Wilks'

Figure 6. *Squalius microlepis* phenotype 1, karst systems near Vrgorac **A** phenotype 1, NMW 49423, 276.1 mm SL, 'Vergoraz [See Jessero]' (specimen E) **B** phenotype 2, NMW 49425, 178 mm SL, 'See bei Gradač & Vrgorač' (specimen C); **C**, NMW 49428, 165.8 mm SL, 'Lago di Dusino', intermediate between phenotypes 1 and 2 (specimen A: external appearance as in phenotype 2 but 15 gill rakers as in phenotype 1).

Lambda 0.5525, *appr. F* (19.64) = 60.297, *P* < 0.0000. In this analysis, the lower jaw length, number of gill rakers, head length, upper caudal-fin lobe and maximum head width contribute most to the discrimination between the phenotypes.

II A DFA based on counts and relative measurements (as in Table 2) (Fig. 7B) also support 100% discrimination for both groups (Wilks' Lambda 0.04411, *appr. F* (23.63) = 59.369, *P* < 0.0000), and the most contributing characters are the number of gill rakers, interorbital width (% HL), ethmoid width (% pterotic cranial width), prepelvic length (% SL), and head length (% SL). A CA run for the same set of characters support perfect clusters into two groups (Fig. 8).
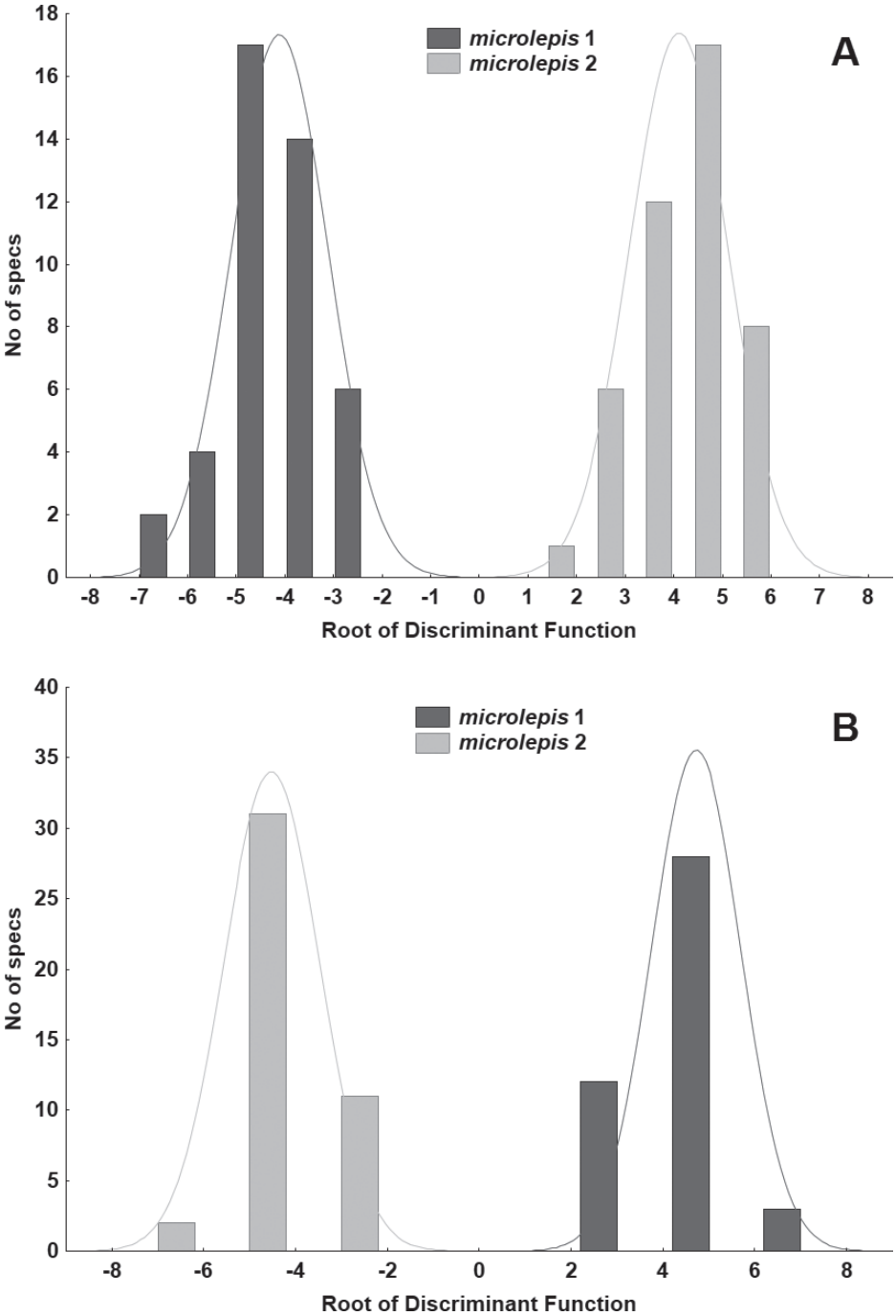
Figure 7. DFA performed for two combined samples of *Squalius microlepis* phenotype 1 and phenotype 2 **A** based on 32 standardised direct measurements and 12 counts **B** based on 52 proportional measurements (as in Table 2) and counts. Specimens A–K in Table 5 not included.
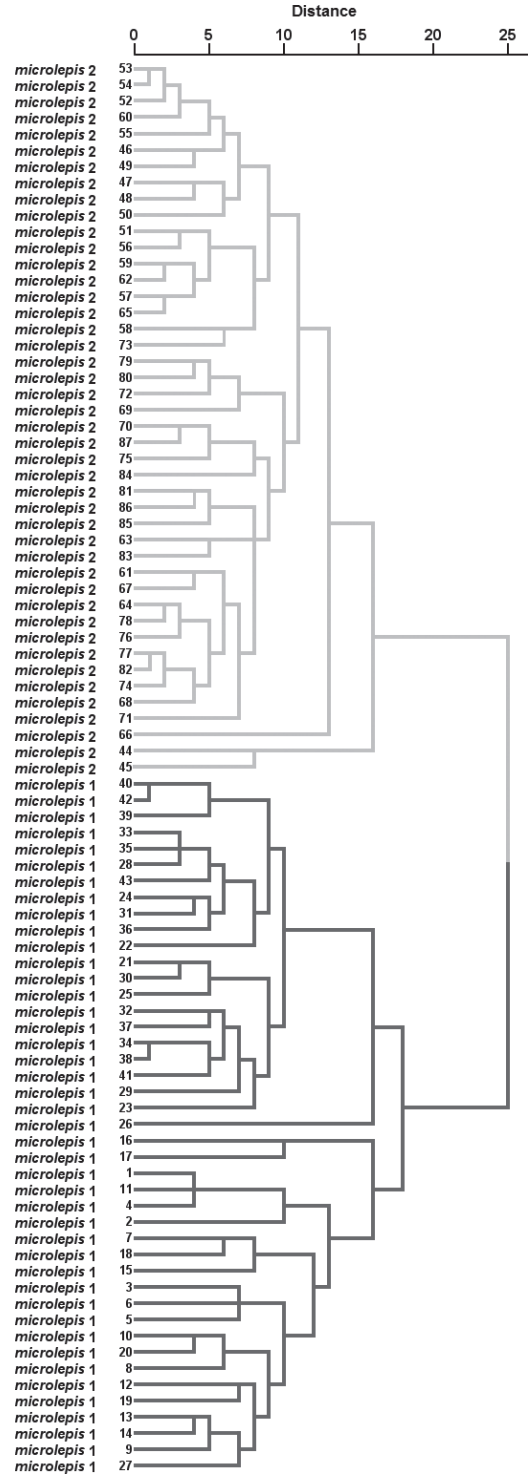
Figure 8. A CA performed for two combined samples of *Squalius microlepis* phenotype 1 and phenotype 2, based on 52 proportional measurements (as in Table 2) and counts. Specimens A–K in Table 5 not included.

Taken together, these two analyses based on differently approached characters, clearly support the primary observations on most influential characters for distinguishing the two phenotypes (1, 6, 7 above): gill rakers count, head length, and length of lower jaw.

Classification of selected specimens A–K between the two phenotypes of *S. microlepis*

Character data for specimens A to K are presented in Table 5.

I   A DFA classification (posterior probabilities and classification functions) based on counts and direct standardised measurements classify these specimens as follows: specimens B, F, and G are identified as phenotype 2 while others as phenotype 1 (Table 6).

II   A DFA analyses based on counts and proportional measurements (as in Table 2) (posterior probabilities and classification functions) unambiguously classified specimens C, E, F (Fig. 6A), G–K as phenotype 2. Classification of specimens A and B is variable and classification of specimen D (Fig. 6B) as phenotype 1 is lower than for other specimens. In a DFA scatter plot (Fig. 9C) they are located between phenotypes 1 and 2.

So, the historical NMW sample from poljes at Vrgorac includes both phenotypes of *S. microlepis*. In the Tihaljina-Trebižat kartic system, most specimens were phenotype 2 while two specimens were clearly classified as phenotype 1 (Fig. 9C).

Discrimination of *S. tenellus* and two phenotypes of *S. microlepis*

I   A DFA performed for three groups of samples (*S. tenellus*, *S. microlepis* phenotype 1 and *S. microlepis* phenotype 2 based on standardised measurements and counts; Fig. 9A) showed a perfect (100%) classification of all three groups (DFA statistics values: Wilks' Lambda 0.00660, *approx. F* (48.172) = 40.519, *P* < 0.0000). The lower jaw length, the number of gill rakers, and the number of scales above the lateral line contribute most to the discrimination between the phenotypes. The closest are two phenotypes of *S. microlepis* and the most distant are *S. tenellus* and *S. microlepis* phenotype 2 (squared Mahalanobis distance equals 52.23712 and 92.95126, respectively).

II   A DFA performed for the same set of samples but based on the proportional measurements and counts (Fig. 9B) also showed a perfect (100%) classification (DFA statistics values: Wilks' Lambda 0.00668, *approx. F* (44.176) = 44.941, *P*<0.0000). Number of gill rakers, number of scales above the lateral line, number of latera-line scale, maximum head width and maximum cranium width contribute most to the discrimination between the three groups. The closest are two phenotypes of *S. microlepis* and the most distant are *S. tenellus* and *S. microlepis* phenotype 2 (squared Mahalanobis distance equals 57.98632 and 84.69049, respectively). When specimens A to K are included into a DFA analysis, specimens A, B, and D are closely
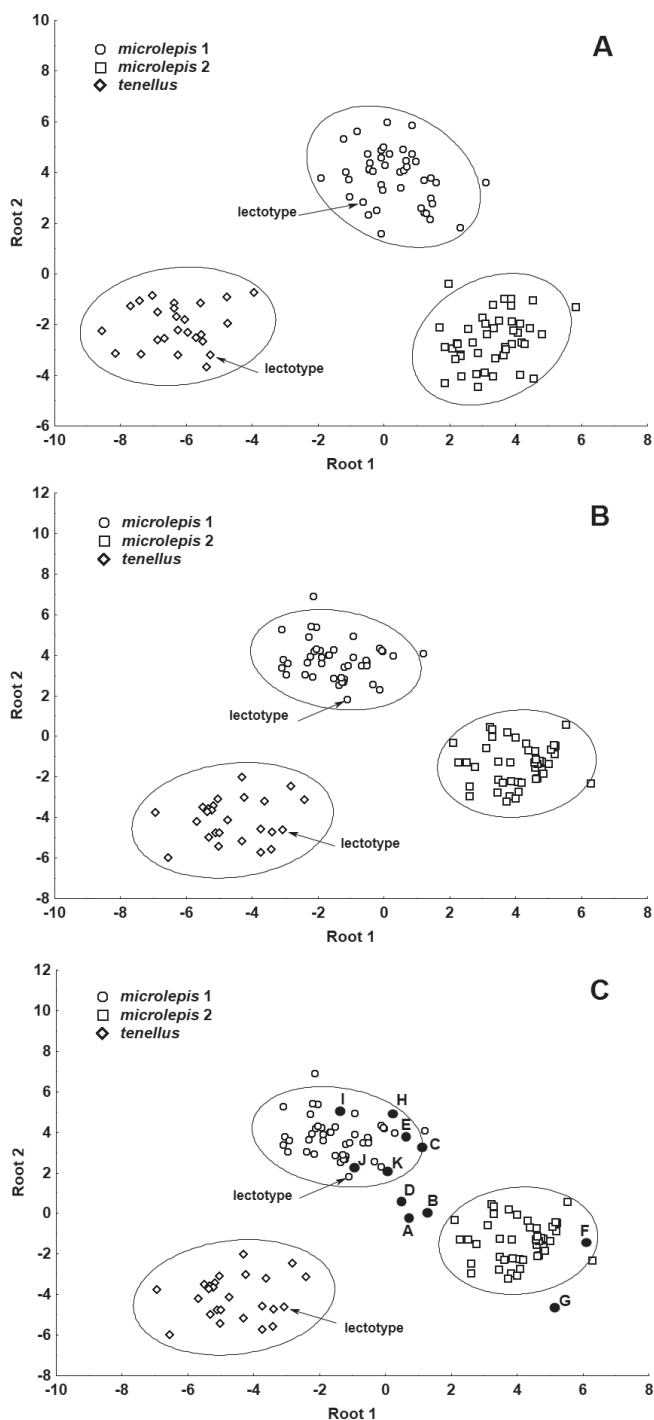
Figure 9. DFA performed for three combined samples, *Squalius tenellus*, *S. microlepis* phenotype 1 and phenotype 2 **A** based on 32 standardised direct measurements and 12 counts (specimens A–K excluded) **B** based on 52 proportional measurements (as in Table 2) and counts (specimens A–K excluded) **C** same analysis as (**B**) but specimens A–K in Table 5 included.

located to each other in the morphological space and intermediate between the two phenotypes (Fig. 9C). Specimens F and G lie within the phenotype 2 while specimens C, E, and H–K lie within the phenotype 1.

Discussion

Distribution of *S. microlepis* phenotypes

Ričina-Prološko Blato-Vrljika karst system

The detailed map of this area at the border between Croatia and Bosnia and Herzegovina, its hydrographic networks, position of main discharge gauging stations and supposed groundwater flow directions are presented by Bonacci and Roje-Bonacci (2008: fig. 1) and Bonacci et al. (2013a: fig. 1). We only found individuals of the phenotype 1 in this karst drainage.

All examined specimens from the Ričina-Prološko Blato-Vrljika karst system belong to *S. microlepis* phenotype 1. The NMW labels and acquisition information for the syntypes (lectotype and paralectotypes by Bănărescu and Herzig-Straschil (1998: 417)) say only 'Imosky, Kroatien (Dalmatien), Heckel Reise 1840' (as well as some other NMW sample, Table 1) ("Gewässer von Imosky" in the original description (Heckel 1843: 52(1042))). We suppose that the syntypes came, most probably, from Prološko Blato, which is a large swampy region in the north-western part of Imotsko Polje in modern Croatia, named after the town of Imotski (also called Imotski field, or valley, or Imotsko-Bekijsko Polje because the Herzegovinian part of the valley is called Bekija). In 19th century, Prološko Blato was part of the year under water, and just one small part was flooded during the whole year (Proložac, or Prološko Lake). The species also occured in three other lakes close to Prološko Blato: Galipovac, Lokvičić and Knezovića lakes (A. Mikulić, pers. comm. 7 May 2008). For the first time *S. microlepis* was reported in the Vrljika by Katurić (1883) but it is not known how far downstream the Vrljika–Matica River this species was distributed in the past. The Vrljika originates by a spring (izvor) east of Prološko Lake and is at present connected to this lake via canal Sija. The historical NMW sample (1901) from the Vrljika is numerous and contains individuals up to 217 mm SL. Recent samples of *S. microlepis* collected by PZ and DJ in Imotsko Polje are only from Prološko Lake itself, at the inflow of the canal that connects it to the Vrljika. Information from local fishermen (more than ten years ago) indicates that 'masnica' had been rarely found in streams of Imotsko Polje but was very abundant in the lake. Further upstream, northwards from Imotsko Polje, *S. microlepis* occured in Ričice Reservoir, a transboundary accumulation lake constructed in the valley of the Ričina River at its confluence with the Vrbica River. It was also found by DJ and PZ in the Ričina River around Posušje (at the village Vir) and in Tribistovo Reservoir north of Posušje (built on a small tributary to the Ričina) in Bosnia and Herzegovina. However, it may be not native there: in 2008, local fishermen claimed that it had been introduced to the Ričina and the Tribistovo Reservoir from Imotski. At present, it

is extremely rare in the entire Imotski area (based on the local population surveys). We failed to collect it in both Imotsko Polje and the Ričina River in 2017–2019.

There are also no recent records of any findings of a small-scaled *Squalius* downstream the Vrljika–Matica at present days, but *S. microlepis* phenotype 1 inhabits a small karstic lake, Krenica, which is located in the south of the Drinovci hill and is fed by underground waters of the Vrljika-Matica. So, it appears that Krenica Lake, populated by *S. microlepis* phenotype 1 and the lower reaches of the Vrljika-Matica, populated by phenotype 2, are the closest known localities of the ranges of the two phenotypes.

Some indications in literature allow to assume that *S. microlepis* of the Imotski area is a lacustrine species rather than a riverine one. Karaman (1928: 159–160) indicated that *S. microlepis microlepis* prefers 'calm' water and was found in Prološko Lake but not in the Vrljika River stream. All individuals ever observed by the authors of this study in the Imotski area were from Prološko Blato. Outside Imotski, Karaman (1928: 159) mentioned that Kolombatović (without an exact reference) had found this fish in Baćina lakes in lower reaches of the Neretva, in stagnant waters only, and never in the Neretva stream. *Squalius microlepis* (as *Leuciscus turskyi microlepis*) was considered as a lacustrine species by Vuković and Ivanović (1971: 150–151).

Matica-Tihaljina-Trebižat karst system

All specimens examined except two found in the karst river system of Matica-Tihaljina-Trebižat of the Neretva drainage belong to *S. microlepis* phenotype 2. The most upstream locality is the lower reaches of the Vrljika-Matica and the Grude Canal at its confluence with the Matica at Drinovci; this locality is close to the terminus of the river. The Vrljika-Matica originates in the northwest of Imotsko-Bekijsko Polje in Croatia. In natural conditions, the river used to go underground in a ponor (swallow hole, or sinkhole) south of the Drinovci hill, now it is accumulated in a lake, and water passes through a tunnel to the Tihaljina River some 150 m below, where a small electric power plant is constructed. The Tihaljina comes from underground very close to this point at the foot of the Jagodnica Mountain south of Drinovci as a strong karst spring, which is a continuation of the Matica underground stream (Bonacci et al. 2013b). It goes southeast to Klobuk Mountain and the spring Klokun, where it changes its name to Mlade, and from Humac to the confluence with Neretva it is called Trebižat. Tihaljina-Mlade-Trebižat is 50 km long. We are not aware of any collection samples of a small-scaled *Squalius* from this river section that we could additionally examine. As *S. microlepis* phenotype 2 is only recorded downstream to Grabovnik-Vašarovići, we suppose that it does not occur below the Kravica Waterfall (ca. 43°9'22"N, 17°36'29"E); this should be checked indeed.

Two specimens (J and K) from the Tihaljina River in the village of Tihaljina (Fig. 10) were unambiguously identified as *S. microlepis* s. str. (phenotype 1) using the diagnosis presented above and clear assigned to this phenotype in statistical analyses (Table 6, Fig. 9C). Our hypothesis is that individuals of phenotype 1 could penetrate from the Imotsko Polje-Vrljika system down to the Tihaljina via existing underground karst flows

Figure 10. A locality where two phenotypes of *Squalius microlepis* co-occur: Tihaljina River at Tihaljina, Bosnia and Herzegovina (7 July 2011).

though the isolation between the two was enough to support the two morphologically distinct groups of populations. A similar phenomenon of migration was discovered in this karst system for sympatric *Delminichthys adspersus* (Palandačić et al. 2012).

According to local fishermen information, after a severe drought some ten years ago, *S. microlepis* has not been found in the Tihaljina near the village of Tihaljina, and *S. tenellus* was introduced to the Tihaljina from Buško Lake but did not establish (N. Ančić, pers. comm. 2011–2019).

Poljes at Vrgorac and Gradac

Historical NMW material includes specimens from at Gradac and Vrgorac, some indicating karst poljes' names (Jezero and Dusina). Polje Jezero is a wetland (blato) with a periodical lake and the sinking stream Matica [Vrgoračka Matica, not to be confused with Vrijeka-Matica in Imotsko Polje] as a part of the right-hand tributary system of the Neretva. The Dusina area, where some karstic streams form temporary lakes, is located near Polje Jezero and belongs to the same karst drainage system. *Squalius microlepis* was often reported from Polje Jezero and 'Lake of Dusina' in the past since its original description based on NMW specimens (e.g., Heckel and Kner 1858: 206, Canestrini 1865: 67, Canestrini 1866: 111, Kolombatović 1886: 16, Car 1911: 64). Mrakovčić et al. (1996) indicate the occurrence of *Squalius microlepis* in the lower part of the Matica in Polje Jezero; however, only one specimen was collected by him many years ago (M. Mrakovčić, pers. comm.).

Table 6. DFA classifications of specimens of *S. microlepis* not identified a priori to phenotype.

Specimen	Based on counts and direct standardised measurements						Based on counts and proportional measurements					
	Posterior probabilities			Classification functions			Posterior probabilities			Classification functions		
	S. tenellus	*S. microlepis* phenotype 1	*S. microlepis* phenotype 2	*S. tenellus*	*S. microlepis* phenotype 1	*S. microlepis* phenotype 2	*S. tenellus*	*S. microlepis* phenotype 1	*S. microlepis* phenotype 2	*S. tenellus*	*S. microlepis* phenotype 1	*S. microlepis* phenotype 2
A	0.000312	0.978473	0.021215	6015	6023	6020	0.000000	0.009082	0.990918	151557	151588	151582
B	0.000000	0.000000	1.000000	6045	6071	6087	0.000000	0.001598	0.998402	150795	150825	150824
C	0.000000	0.999781	0.000219	5865	5900	5891	0.000000	0.999977	0.000023	153984	154039	154019
D	0.000000	0.999995	0.000005	6105	6129	6116	0.000000	0.741566	0.258434	152039	152070	152062
E	0.000000	1.000000	0.000000	5726	5786	5766	0.000000	1.000000	0.000000	152076	152127	152105
F	0.000000	0.000000	1.000000	5771	5799	5833	0.000000	0.000000	1.000000	150471	150500	150535
G	0.000000	0.000000	1.000000	5844	5841	5869	0.000000	0.000000	1.000000	149753	149755	149800
H	0.000000	1.000000	0.000000	5755	5813	5797	0.000000	1.000000	0.000000	152084	152144	152114
I	0.000000	1.000000	0.000000	5839	5900	5883	0.000000	1.000000	0.000000	152894	152945	152909
J	0.000000	0.999998	0.000002	5962	5985	5972	0.000000	1.000000	0.000000	151948	151984	151961
K	0.000000	0.999294	0.000706	5979	6010	6003	0.000000	0.999997	0.000003	151776	151818	151798

There were only five specimens from this area available for examination added by two more specimens we supposedly attribute to it. This sample is quite morphologically heterogeneous. Specimens C and E unambiguously belong to *S. microlepis* s. str. (phenotype 1) and F and G (Fig. 6A) to phenotype 2 (Table 6, Fig. 9C). Specimens A (Fig. 6C), B and D (Fig. 6B) are intermediates between the two phenotypes.

Specimen B (NMW 49427) is labelled as 'Narenta, Heckel Reise 1840' but there is no clarifying information on the exact locality. We failed to find collection specimens or reliable records on *S. microlepis* from the Neretva main stream (a long list of publications checked by us can be requested from the corresponding author). We speculate that "Neretva" as a locality can refer to streams near Vrgorac or the drainage in general; for example, Seeley (1886: 169–170) and Car (1911: 64) mentioned "river Neretva near Vergorac". Karaman (1928) indicated that he had never found *S. microlepis* in the Neretva main stream.

We hypothesise that both phenotypes could co-occur in the poljes near Vrgorac in the past or individuals of the phenotype 2 from the upstream karstic system of the Tihaljina could migrate downstream to the poljes at Vrgorac. They could probably hybridise as some specimens are of intermediate morphology. The Matica [not to be confused with Vrijeka-Matica in Imostko Polje] is a part of the right-hand tributary system of the Neretva and connected to the Tihaljina system in its northernmost (upper) part (Bonacci et al. 2013b). We failed to find this fish during intensive field trips in karst poljes near Vrgorac in 2017–2019.

Two specimens NMW 49228 (as 49227 in Bănărescu and Herzig-Straschil (1998: 419)) labelled 'Zara' (an Italian name for Zadar, a town on the Croatian coast, ca. 44°7'19"N, 15°16'20"E), are also identified by our analyses as *S. microlepis* phenotype 1. Bănărescu and Herzig-Straschil (1998) supposed that these two specimens do not belong to this species but did not offer an alternative hypothesis. However, no other specimens of a small-scaled *Squalius* are known from this area considerably remote from the main range of distribution of *S. microlepis* s. l. In the vicinities of Zadar, there was a lake, Bokanjačko Jezero, dried up long ago. At present, the Baštica River and two artificial lakes in that region are inhabited by *Rutilus aula* (Bonaparte) and a wide range of introduced species (e.g., *Rutilus rutilus* (L.), *Squalius cephalus* (L.), *Lepomis gibossus* (L.), *Carassius gibelio* (Bloch), *Cyprinus carpio* L., *Ameiurus melas* (Rafinesque) (unpublished data of DJ and PZ). Most probably, the label does not refer to Zadar, but the sample might have been sent to NMW from Zadar by Kolombatović. The NMW collection contains two more samples labelled as "Kolombatović, Zara" (no date), of *Chondrostoma knerii* (Heckel, 1843) and *Scardinius plotizza* (Heckel & Kner, 1858) that indicates the Neretva drainage.

The Cetina River *Squalius*

The Cetina River is also sometimes included into the range of *S. tenellus* (Freyhof and Kottelat 2008; Ćaleta et al. 2015), but it is not clear, if the species is considered as

introduced or native. We could not find a published morphological description of the Cetina fish that supports this opinion. On the contrary, the native Cetina *Squalius* was identified as *S. microlepis* by some earlier authors (Kolombatović [Kolombatowitch] 1886, Brusina 1907). Later, it was considered a new undescribed species (Zupančič 2007: *Squalius* sp. 4) but a formal description did not follow. In the most recent review of Croatian freshwater fishes (Ćaleta et al. 2019) the presence of *S. microlepis* in the Cetina is considered as not confirmed.

A historical specimen, collected by Kolombatović in the Cetina (MZUF No. 13512, donated from Kolombatović, June 1880; see Nocita and Vanni 1999: 214) was only examined by us from a photo (Fig. 11). The specimen is damaged, the number of scales in the lateral series can be calculated by the scale pockets and remaining scales, and it is 69. So, it cannot be identified as *S. tenellus* but is similar to *S. microlepis* by this count though being quite different from the latter by its general appearance and may be *Telestes ukliva* (Heckel, 1843) which is a species endemic to the Cetina.

Taxonomy vs. variations and variability

The three small-scaled entities, *S. tenellus*, *S. microlepis* s. str. (phenotype 1) and *S. microlepis* phenotype 2, appear much better morphologically differentiated from each other than species within the *S. cephalus* group (see, e.g., Doadrio et al. 2007; Turan et al. 2009; Bogutskaya and Zupančič 2010; Özuluğ and Freyhof 2011). Four published cytb sequences of *S. microlepis*, two from the Krenica Lake and two from the Trebižat River (Freyhof et al. 2005; Perea et al. 2010; Schönhuth et al. 2018), show some genetic difference between the two localities, 0.53–0.67% (R. Šanda, pers. comm.). We did not have the possibility to examine the voucher specimens, but the Krenica specimens are most probably a true *S. microlepis* (phenotype 1) and the Trebižat specimens might represent a *S. microlepis* phenotype 2. No variability was found between five published sequences of CO1 (Perea et al. 2010, Geiger et al. 2014, Schönhuth et al. 2018) – three from the Krenica-Imotski area and two from the Tihaljina-Trebižat (R. Šanda, pers. comm.).

Readily morphologically diagnosable entities cannot always be taxonomically discriminated using molecular markers due to very rapid events of speciation (i.e., species radiations) and specific factors driving them, such as niche evolution or morphological key innovations (e.g., Bickford et al. 2007; Martin et al. 2016) forming species complexes or polymorphic species. For example, the CO1 marker did not provide resolution in at least 17 complexes of "closely related" conventional (clearly morphologically distinct) species in the subfamily Leuciscinae (Geiger et al. 2014: table S1-C). On the other hand, many intraspecific morphological differences can occur and express themselves, for example, as ecological variability or geographic variation. Polymorphic populations are more the rule than the exception in fish (Skulason and Smith 1995) as differences between habitats of fishes (e.g., related to flow regime or foraging opportunities) create selective pressures resulting in morphological divergence between conspecific populations (Langerhans et al. 2003; Senay et al. 2014).

Figure 11. Specimen MZUF 13512 (identified as *S. microlepis* by Kolombatović), Cetina River. Photo credit: Saulo Bambi, Sistema Museale dell'Università degli Studi di Firenze, Sez. di Zoologia "La Specola", Italy.

The key issue is how to interpret the morphological differentiation in these groups – either as reflecting different nominal species or as representing varieties or (eco-)phenotypes within a single species. As very limited molecular data exist on the two phenotypes of *S. microlepis*, we refrain from any taxonomic and nomenclatural conclusions until new molecular approaches (and new markers) are used, the polymorphism is properly sampled, and much more specimens are available for genetic phylogenetic analyses. However, as shown above, we can hypothesise that the phenotype 1 might represent a lacustrine morph of the species while the phenotype 2 is a riverine one.

Conservation implications

Our study emphasises the fact that *S. microlepis*, either a group of two putative species or two habitat-related phenotypes, has become extirpated or extremely rare in the most part of its range since 2004–2011. A reason of the dramatic decline may be due to introductions of *Perca fluviatilis* Linnaeus, *Squalius cephalus* Linnaeus and *Esox lucius* Linnaeus established throughout the region. Hence, the phenotypic diversity described in the paper has been already largely lost and a critical investigation of its conservation status is severely required based on population genetic data. We applied the IUCN criteria (3.1) and suppose that the Red List status of the species should be Critically Endangered (CR: A2ce) based on 90% population reduction estimated in the last 15 years (ca. three generations). Sub-criteria: (c) population size reduction observed through the decline in the area of occupancy (AOO) and the extent of occurrence (EOO), and (e) effects of introduced taxa, pollutants and competitors are in place. Exact causes of the reduction are not yet known and may have not ceased. Remaining EOO has been estimated as approximately 250 km^2 and AOO only around 20 km^2 (five 2 x 2 km cells), although the lack of data since 2011 makes the situation even more critical.

Acknowledgements

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CORRIGENDA

Corrigenda: Species delimitation of crab-eating frogs (*Fejervarya cancrivora* complex) clarifies taxonomy and geographic distributions in mainland Southeast Asia. ZooKeys 883: 119–153. https://doi.org/10.3897/zookeys.883.37544

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Citation: Yodthong S, Stuart BL, Aowphol A (2019) Corrigenda: Species delimitation of crab-eating frogs (*Fejervarya cancrivora* complex) clarifies taxonomy and geographic distributions in mainland Southeast Asia. ZooKeys 883: 119–153. https://doi.org/10.3897/zookeys.883.37544. ZooKeys 897: 149–150. https://doi.org/10.3897/zookeys.897.48818

In our recent publication (Yodthong et al. 2019), there are errors in Table 1 (Column: GenBank accession numbers). The corrections are as follows:

Page 125:

Accession number of ZMKU AM 01442: "MN453508" should be "MN453513"
Accession number of ZMKU AM 01446: "MN453509" should be "MN453514"
Accession number of ZMKU AM 01451: "MN453510" should be "MN453515"
Accession number of ZMKU AM 01467: "MN453511" should be "MN453516"
Accession number of ZMKU AM 01475: "MN453512" should be "MN453517"
Accession number of ZMKU AM 01479: "MN453513" should be "MN453518"
Accession number of ZMKU AM 01485: "MN453514" should be "MN453519"
Accession number of ZMKU AM 01493: "MN453515" should be "MN453520"

Accession number of ZMKU AM 01498: "MN453516" should be "MN453521"
Accession number of ZMKU AM 01503: "MN453517" should be "MN453522"
Accession number of ZMKU AM 01516: "MN453518" should be "MN453526"
Accession number of ZMKU AM 01520: "MN453519" should be "MN453527"

Page 126:

Accession number of ZMKU AM 01418: "MN453520" should be "MN453508"
Accession number of ZMKU AM 01423: "MN453521" should be "MN453509"
Accession number of ZMKU AM 01425: "MN453522" should be "MN453510"
Accession number of ZMKU AM 01426: "MN453523" should be "MN453511"

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Accession number of ZMKU AM 01430: "MN453524" should be "MN453512"
Accession number of ZMKU AM 01507: "MN453525" should be "MN453523"
Accession number of ZMKU AM 01509: "MN453526" should be "MN453524"
Accession number of ZMKU AM 01511: "MN453527" should be "MN453525"