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A charismatic new species of green lacewing discovered in Malaysia (Neuroptera, Chrysopidae): the confluence of citizen scientist, online image database and cybertaxonomy

Shaun L. Winterton[1,†], Hock Ping Guek[2,‡], Stephen J. Brooks[3,§]

1 *California State Collection of Arthropods, California Department of Food & Agriculture, Sacramento, California, USA* **2** *aman Sri Sinar, Kuala Lumpur, Malaysia* **3** *Department of Entomology, The Natural History Museum, London, Great Britain*

† *urn:lsid:zoobank.org:author:37F5AC48-EC3A-47ED-902B-2BD1467CCA72*
‡ *urn:lsid:zoobank.org:author:36E2F8F7-04F2-4625-B0C8-DB3A6A945818*
§ *urn:lsid:zoobank.org:author:B20666A7-F0FB-460B-958C-FA12250981F0*

Corresponding author: *Shaun L. Winterton* (wintertonshaun@gmail.com)

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Abstract

An unusual new species of green lacewing (Neuroptera: Chrysopidae: *Semachrysa jade* **sp. n.**) is described from Selangor (Malaysia) as a joint discovery by citizen scientist and professional taxonomists. The incidental nature of this discovery is underscored by the fact that the species was initially photographed and then released, with images subsequently posted to an online image database. It was not until the images in the database were randomly examined by the professional taxonomists that it was determined that the species was in fact new. A subsequent specimen was collected at the same locality and is described herein along with another specimen identified from nearby Sabah.

Keywords

citizen scientist, cybertaxonomy, Ankylopterygini

Introduction

New species are increasingly being discovered by the general public with interests in the natural sciences long before they are recognized as new to science by professional taxonomists and formally described. With the rapid development of digital photographic technology, professional and amateur photographers are unknowingly discovering and informally documenting new species of animals and plants by placing images of them in online image databases long before taxonomists can examine them. In some cases the specimen is not collected, so this discovery is effervescent until additional specimens can be subsequently vouchered to enable type designation during the formal descriptive process. Herein we document one such case involving a distinctive new green lacewing (Neuroptera: Chrysopidae) species from Malaysia.

Green lacewings are the second largest family of lacewings, with more than 1200 species in approximately 80 valid genera distributed in all major biogeographical regions worldwide (Brooks and Barnard 1990). Three subfamilies are recognized although number of species in the subfamilies Apochrysinae and Nothochrysinae comprise a relatively minor component of the world fauna. Apochrysinae are represented by six pantropical genera of spectacularly large and delicate species (Kimmins 1952; Winterton and Brooks 2002). Nothochrysinae are represented by nine genera world wide, and contain many species that exhibit putative ancestral characteristics (Adams 1967; Brooks and Barnard 1990; Adams and Penny 1992). The overwhelming majority of the generic and species-level diversity belongs to Chrysopinae, with approximately 97% of all living species. This subfamily is further divided into four tribes: Belonopterygini, Chrysopini, Leucochrysini and Ankylopterygini (Brooks and Barnard 1990; Winterton and de Freitas 2006). Ankylopterygini are a Palaeotropical group thoroughly characterized recently by Brooks (1983, 1986) and Brooks and Barnard (1990) and differentiated from other tribes based on the close proximity of veins Sc and R, narrow mandibles lacking internal teeth, and elongation and apical narrowing of the labial and maxillary palpi. The genus *Semachrysa* Brooks, 1983 contains 14 previously described species from Japan southwards through the Oriental and Australasian regions to Australia (Brooks 1983, 1986). *Semachrysa* is differentiated from other Ankylopterygini genera by the presence of distinct markings (i.e. two or three spots) on the frons, cell *im* present, forewing with an elongate stigma with 3–4 Sc-R crossveins, and male genitalia with two pairs of gonosetae (Brooks and Barnard 1990). A new and distinctive species is described here (*Semachrysa jade* sp. n.) based on two female specimens from Malaysia.

The discovery of a new species of *Semachrysa* described in this paper is a direct result of the incidental interaction of photographer/citizen scientist, online image database and professional scientists. Images of *Semachrysa jade* sp. n. (Figures 2–3) were initially posted by the second author (GHP) on the online image database Flickr ® for comment by the photography and natural history communities. The specimen had been released once it was photographed and at this stage no determination had been made on the taxonomic identity of the species. The online images were then randomly examined by the senior author (SLW) who determined that this distinctive species was not immediately recognizable as any previously described species. Links to the images were forwarded to additional experts in chrysopid taxonomy to elicit comment on its possible taxonomic identity. After extensive discussion it was concluded that the species was likely new to science but its generic placement inconclusive based solely upon the images at hand. The senior author contacted the photographer and an additional specimen to was collected a year later at the same locality (Figure 4). This second specimen was photographed and sent to the senior author for examination and subsequent formal description as a new species of *Semachrysa*. An additional female specimen from Sabah was also located in the entomology collection of the Natural History Museum, London. The manuscript was prepared by the authors based on a cybertaxonomy model using internet cloud based technology (i.e. Google™ Document), while images were archived in Encyclopedia of Life and Morphbank image databases and new taxonomic acts registered in Zoobank (Pyle and Michel 2008).

Materials and methods

Terminology follows Tjeder (1966) and Brooks and Barnard (1990). High-resolution digital images were deposited into Morphbank with embedded URL links within the document between descriptions and Morphbank images. All new nomenclatural acts and literature are registered in Zoobank (Pyle and Michel 2008). Genitalia were macerated in 10% KOH to remove soft tissue, then rinsed in distilled water and dilute glacial acetic acid, and dissected in 80% ethanol and subsequently stained with a saturated solution of Chlorazol Black in 40% ethanol. Genitalia preparations were placed in glycerine in a genitalia vial mounted on the pin beneath the specimen.

Taxonomy

Semachrysa jade **sp. n.**

urn:lsid:zoobank.org:act:2F358345-BE61-46B5-9672-10C1F9730719
http://species-id.net/wiki/Semachrysa_jade
Figures 1–6

Type material. Holotype female, MALAYSIA: Selangor: Road B57, 0.8 km SSW of entrance of Selangor State Park, (3.3057,101.693) closed forest, Guek Hock-Ping, 27.i.2012 (California Academy of Sciences Collection).

Paratype: MALAYSIA: Sabah: Tawau, DR. Lim, 8.vii.1981, on Cocao (Natural History Museum, London).

Diagnosis. Extensive black markings with white fenestrations in basal portion of both wings; forewing with basal four to five crossveins between R and Rs converging

Figure 1. *Semachrysa jade* sp. n., female holotype habitus (Morphbank: 791595). Forewing length: 15.0 mm. Photographer: Guek Hock Ping.

Figure 2. *Semachrysa jade* sp. n. female habitus, specimen that was originally photographed and released (Morphbank: 791596). Forewing length: 15.0 mm. Photographer: Guek Hock Ping.

posteriorly, Rs closely approximating Psm basally; three crossveins between Cu1 and Cu2, 1st posterior marginal crossvein forked with posterior arm joining to Cu2 petiolate to margin; two dark spots across frons below antennae; single marking between antennal bases; dark markings medially on abdominal tergites 2–4; sternite seven with acuminate posteromedial margin with tuft of short dark setae.

Figure 3. *Semachrysa jade* sp. n. female habitus (Morphbank: 791597). Forewing length: 15.0 mm. Photographer: Guek Hock Ping.

Description. Female: Wing length (forewing: 15.0 mm; hindwing: 13.5–14.0 mm). Overall colouration in live specimens bright green and yellow, with dark markings on head, abdomen and both wings (pale yellow in dried specimens). Head. Yellow with black marking on vertex between antennae and small quadrangular marking on frons below antennal base and proximal to eye margin; clypeus with brown suffusion laterally; labrum emarginate medially; antenna pale green but otherwise unmarked, slightly longer than forewing; flagellum with at least 50 flagellomeres; palpi green, unmarked. Thorax. Prothorax green with small brown mark anterolaterally on pronotum; setae short, green, and relatively sparse, darker and more dense laterally; mesonotum and metanotum yellow green, scutum darker in preserved specimen, mesoscutellum pale yellow, setae sparse and pale yellow to white; legs very pale green with white setae, setae shorter and yellowish distally on foretibiae; distal tarsomere and claws brownish on all legs; wings relatively rounded, forewing costal area broad, rounded basally, then straight to wing apex; forewing with Rs sigmoid and closely approximating pseudomedial (Psm) basally; first five r-rs crossveins convergent, remaining crossveins sub parallel; seven inner gradate crossveins, meeting Psm; nine outer gradate crossveins; three crossveins between Cu1 and Cu2, 1st posterior marginal crossvein forked with posterior arm joining to Cu2, petiolate to margin; hind wing with five inner gradate crossveins, seven outer gradate crossveins; wing veins with setae relatively elongate and pedicellate, pedicels longer in basal portion of wing, setae colour corresponding to wing markings and colour of wing venation; wing hyaline with markings as per Figure 5, venation mostly

Figure 4. Type locality of *Semachrysa jade* sp. n., closed forest, 0.8 km SSW of entrance of Selangor State Park, Selangor, Malaysia (GPS: 3.3057,101.693). Photographer: Guek Hock Ping.

pale green, forewing costal crossveins dark anteriorly on crossveins 1–3 and posteriorly on crossveins 8–10; basal subcostal crossvein dark; membrane infuscate and venation dark in medial area of both wings from R vein to posterior margin, markings darker

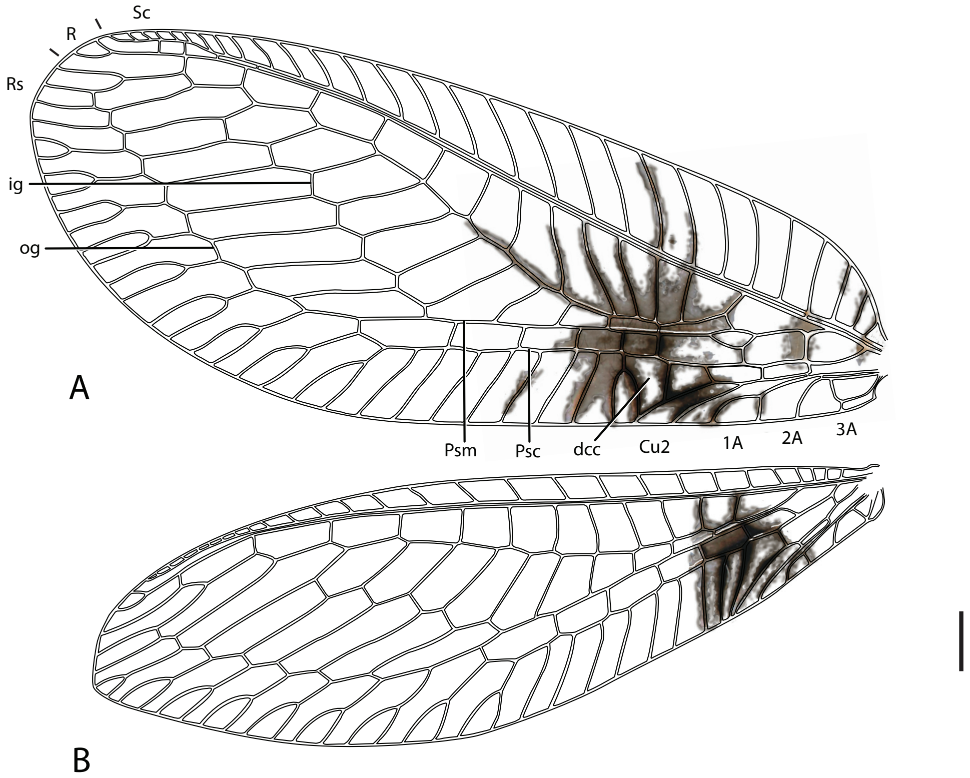

Figure 5. *Semachrysa jade* sp. n. **A** forewing **B** hindwing. Vestiture omitted. Abbreviations: *dcc*, distal cubital cell; *ig*, inner gradate series; *psc*, pseudocubital vein; *psm*, pseudomedial vein; *og*; outer gradate series. Scale line: 1.0 mm.

posteriorly with cells (e.g. dcc, c2 and cu2) with white fenestration (Figs 1–4); basal portion of cell m2 infuscate; spot at base of wing on cubital vein; fourth posterior marginal crossvein dark distally. Abdomen. Yellow green dorsally, white ventrally; dark medial stripe on tergites 2–4; poorly defined dark spot laterally on tergites 3–5; sternite 8 with tuft of short strong setae medially. Female terminalia (Figure 6): Trichobothria ca. 25; sternite seven with broadly acuminate posteromedial margin with tuft of short dark setae; subgenitale relatively broad; spermatheca with vela relatively short and straight; ventral impression relatively shallow and wide; duct with slight coil.

Comments. The unusual new species is easily differentiated from all other species of *Semachrysa* by the distinctive wing venation mark between the antennal bases and only two spots across the frons, as well as the female abdominal sternite 7 being posteromedially acuminate with a tuft of strong setae. Only the female is known at this stage. *Semachrysa jade* sp. n. is similar in appearance to *S. wallacei*, based on head and wing markings. Like *S. jade* sp. n., *S. wallacei* is also only known from the female.

Etymology. This new species is named after the daughter of the senior author, Jade Tanya Winterton.

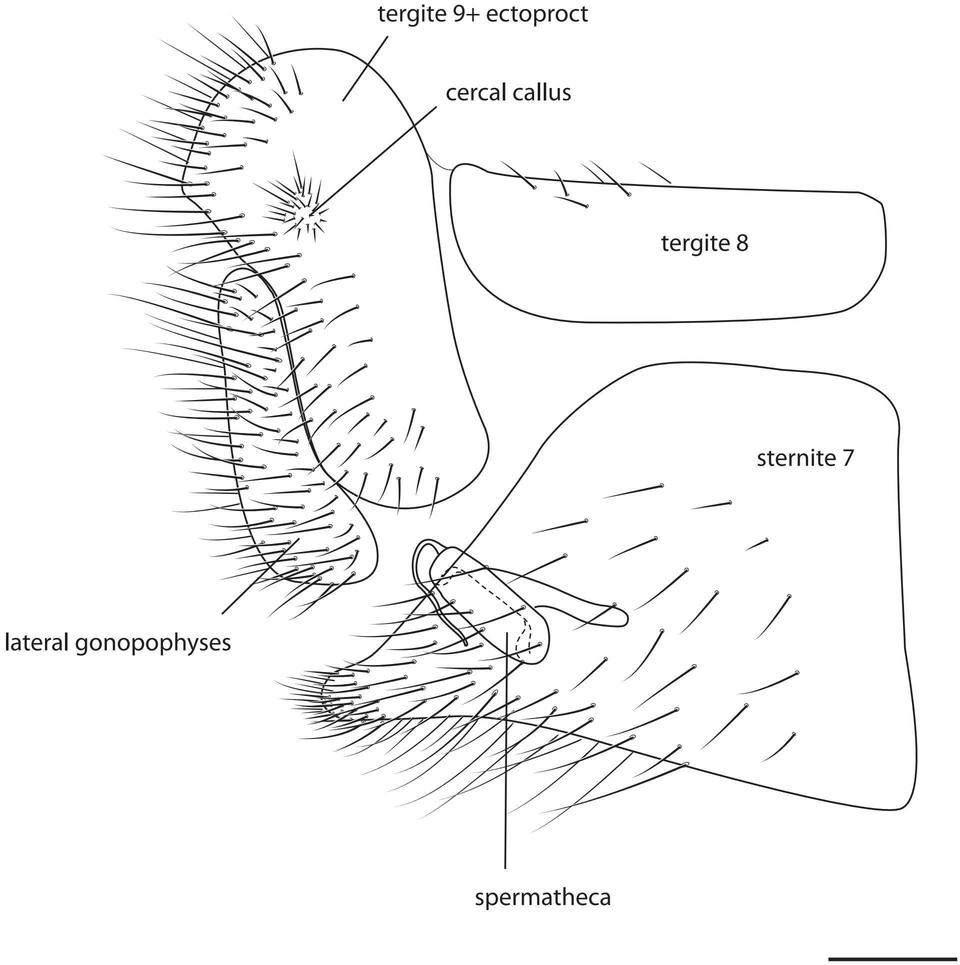

Figure 6. *Semachrysa jade* sp. n. Female terminalia with structures labeled. Scale line: 0.2 mm.

Revised dichotomous key to species of *Semachrysa* Brooks

(Modified after Brooks 1983)

1 Wings unmarked except for indistinct small spot on dcc and black border on costa .... ***S. dammermanni* (Esben-Petersen, 1929)**

– Wings with numerous brown or black markings **2**

2 Forewing with strong dark brown marking at base of outer gradate series ... **3**

– Forewing without dark markings in this position (sometimes very faint)..... **4**

3 Large dark brown marking present on fourth posterior marginal crossvein of forewing; small marking present on Cu2; male genitalia with arcessus narrow, without ridges; female genitalia with duct of spermatheca twisted, vela very long .... ***S. contorta* Brooks, 1983**

– Small mark present on fourth posterior marginal crossvein of forewing, but with large brown mark on dcc; male genitalia with arcessus broad, ridged; female genitalia with duct of spermatheca and vela short .... **_S. matsumurae_ (Okamoto, 1914)**

4 Single median marking on vertex either between, or immediately posterior to antennal bases **5**

– Medial marking absent on vertex between or immediately posterior to antennal bases **7**

5 Extensive dark markings in basal portion of forewing from R vein to posterior margin of wing; basal four to five crossveins between R and Rs converging posteriorly; three crossveins between Cu1 and Cu2, 1st posterior marginal crossvein forked with posterior arm joining to Cu2 petiolate to margin .... **_S. jade_ sp. n.**

– Dark markings in basal portion of forewing restricted to basal cells along posterior margin of forewing; basal four to five crossveins between R and Rs parallel; two crossveins between Cu1 and Cu2, 1st posterior marginal crossvein not forked and separate from Cu2 to wing margin **6**

6 Hindwing with distinct brown marking on dcc and faint shading on posterior marginal crossveins .... **_S. wallacei_ Brooks, 1983**

– Hindwing with only faint markings on dcc or forked posterior forked marginal crossveins, but marking present on fourth posterior marginal crossvein .... **_S. picilabris_ (Kimmins, 1952)**

7 Prominent markings at base of inner gradate series of forewing and on fourth posterior marginal crossvein; male abdominal setae dense and fine .... **_S. papuensis_ Brooks, 1983**

– Forewing lacking above combination of markings, though pale suffusion may be present **8**

8 Two spots on frons below antennae .... **_S. cruciata_ (Esben-Petersen, 1928)**

– Three spots on frons below antennae **9**

9 Brown band extending from along inner margin of eye from postocular lobe to anterior edge of vertex .... **_S. polysticta_ Brooks, 1983**

– Brown band absent; isolated mark on postocular lobe **10**

10 Fore and hindwing lightly suffused with brown, particularly along posterior margins, distinct spots absent .... **_S. minuta_ Brooks, 1983**

– Fore and hind light brown suffusion admixed with dark markings **11**

11 Darkest marking on forewing on dcc **12**

– Darkest marking on forewing on fourth posterior marginal crossvein **13**

12 Forewing dcc with large marking, extending from anal veins to second cubital cell (c2); many crossveins with pale brown suffusion including Rs and inner gradate series .... **_S. sagitta_ Brooks, 1983**

Forewing dcc with small marking, not extending to anal veins or c2; all crossveins pale .... **_S. nigribasis_ (Banks, 1920)**

13 Small species, forewing length 7.5 mm; four inner gradate and five outer gradate crossveins... ***S. hyndi* Brooks, 1983**

– Larger species, forewing length 9.0 mm or greater; at least six inner gradate and outer gradate crossveins.. **14**

14 Forewing length 9.0 mm, faint marking on dcc with extensive brown suffusion; female spermatheca with deep ventral impression .... ***S. decorata* (Esben-Petersen, 1913)**

– Forewing length 13.0 mm, dcc lacking dark markings and brown suffusion posteriorly; female spermatheca with shallow ventral impression.................. .... ***S. claggi* (Banks, 1937)**

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An aberrant species of *Nipponocercyon* from Sichuan, China (Coleoptera, Hydrophilidae, Sphaeridiinae)

Martin Fikáček[1,2], Sergey Ryndevich[3], Fenglong Jia[4]

1 *Department of Entomology, National Museum, Kunratice 1, CZ-148 00 Praha 4, Czech Republic* **2** *Department of Zoology, Faculty of Sciences, Charles University in Prague, Viničná 7, CZ-128 44 Praha 2, Czech Republic* **3** *Baranovichi State University, Voykova ul. 21, Baranovichi 225404, Brest obl., Belarus* **4** *Institute of Entomology, Life Science School, Sun Yat-sen University, Guangzhou, 510275, Guangdong, China*

Corresponding authors: *Martin Fikáček* (mfikacek@gmail.com); *Sergey Ryndevich* (ryndevichsk@mail.ru); *Fenglong Jia* (fenglongjia@yahoo.com.cn)

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Abstract

A detailed examination of specimens of *Cryptopleurum sichuanicum* Ryndevich, 2005 from high altitudes of Sichuan Province, China, revealed that the species belongs in the genus *Nipponocercyon* Satô, 1963 previously endemic to Japan. The species is here transferred in *Nipponocercyon*, and *N. sichuanicus* (Ryndevich, 2005), **comb. n.** is redescribed and compared with *N. shibatai* Satô, 1963. The male genitalia of *N. sichuanicus* is described for the first time. An adapted diagnosis of *Nipponocercyon* is provided, and reasons for the inclusion of *N. sichuanicus* into *Nipponocercyon* and the general distribution of the genus are discussed.

Keywords

Hydrophilidae, Sphaeridiinae, *Nipponocercyon*, taxonomy, morphology, China, Oriental region, Palaearctic region

Introduction

Cryptopleurum sichuanicum Ryndevich, 2005 was described from a few female specimens collected in the mountains of Sichuan Province, China (Ryndevich 2005). Although considered "rather peculiar and isolated with some characters differing from other members of the genus", it was assigned to the genus *Cryptopleurum* based on the combination of large antennal grooves, sculptured dorsal body surface and pentagonal mesoventral plate. Detailed examination of additional material collected more recently revealed that the species shares many characters with the Japanese endemic genus *Nipponocercyon* Satô, 1963 and the superficial similarity with *Cryptopleurum* is due to several unusual apomorphies of the species. *Nipponocercyon* was so far only known from Kyushu, Shikoku and the southern part of the Honshu, where it is represented by a single species, *N. shibatai* Satô, 1963 (Satô 1963, Hoshina and Fikáček 2010). The morphology of the latter Chinese species is compared with *Nipponocercyon shibatai* as well as with the representatives of the genus *Cryptopleurum*, and the reasons for its transfering to *Nipponocercyon* are summarized. The distribution of *Nipponocercyon* is hence extended to the Asian mainland.

Material and methods

Material examined for this study is deposited in the following collections:

CSR	coll. Sergey Ryndevich, Baranovichi, Belarus;
KSEM	Natural History Museum, University of Kansas, Lawrence, USA (A. Short);
NHMW	Naturhistorisches Museum, Wien, Austria (M. A. Jäch, A. Komarek);
NMPC	Department of Entomology, National Museum, Praha, Czech Republic (M. Fikáček);
SYSU	Entomological collection of Sun Yat-sen University, Guangzhou, China (F.-L.Jia).

The current study is largely based on newly collected material of *N. sichuanicus* (18 specimens) which were compared with one paratype of *Cryptopleurum sichuanicum*, and on the specimens of *Nipponocercyon shibatai* and of other megasternine genera deposited in the collection of NMPC.

Selected specimens were dissected, with genitalia embeded in a drop of water-soluble dimethyl hydantoin resin on a piece of transparent plastic pinned below the specimen, or of alcohol-soluble Euparal resin on a small piece of glass attached below the respective specimen. The external morphology was examined using the Hitachi S-3700N environmental electron microscope at the Department of Entomology, National Museum in Prague. Habitus photographs were taken using Canon D-550 digital camera with attached Canon MP-E65mm f/2.8 1–5× macro lens, and subsequently adapted in Adobe Photoshop CS2. Figures of genitalia were prepared with the help of Photoshop CS4. The morphological terminology largely follows Komarek (2004) and Fikáček (2010), the higher-level taxonomic nomenclature follows Hansen (1999) and Short and Fikáček (2011).

Taxonomy

Nipponocercyon Satô, 1963

http://species-id.net/wiki/Nipponocercyon

Adapted differential diagnosis. The inclusion of *Cryptopleurum sichuanicum* into *Nipponocercyon* (see below) requires a modification of the differential diagnosis of the genus as follows:

Head without transverse interantennal ridge; eyes small, separated by 9× of one eye; mentum weakly bisinuate on anterior margin; antennae with 9 antennomeres; maxilla with or without sucking disc in males; maxillary palpomere 2 strongly widened distally; posterior tentorial pits minute; pronotum evenly convex, lateral margin not deflexed or slightly deflexed; transverse row of larger punctures along posterior margin of pronotum absent (large areas without microsculpture in *N. sichuanicus* may actually resemble enlarged punctures on the first view, but the punctures are as large as those in disc when examined in detail, see Fig. 8); median portion of prosternum weakly to distinctly separated from lateral portions, bearing coarse setiferous sculpture; median portion of prosternum carinate medially (carina distinct in *N. shibatai*, partly obliterated by the sculpture but still apparent in *N. sichuanicus*, compare Figs 12 and 15); prosternal process wide, deeply excised; antennal grooves moderately large to large, not reaching lateral margin of hypomeron (Figs 11, 14); anteroventral margin of prothorax with a small denticle on the contact of prosternum and hypomeron; profemur with elongate ventral depression along anterior margin; elytron with 10 punctural series; elytral intervals flat or highly convex; lateral margins of elytra not denticulate nor serrate; mesoventral cavities for reception of procoxae large, reaching mesocoxae; preepisternal elevation subpentagonal, widely contacting metaventral process, median portion of metaventrite slightly to very distinctly elevated; postcoxal ridge lying parallel to posterior margin of mesocoxal cavity, not overlapping to lateral margin of metaventrite; lateral portions of metaventrite with coarse punctation (smaller punctures may be intermixed or absent); metanepisternum narrow, but distinct throughout; abdominal ventrite 1 carinate medially, with coarser punctation than ventrites 2–5; phallobase asymmetrical, much shorter than parameres; gonopore situated in basal half of median lobe; male sternite 9 with median tongue-like projection; male sternite 8 without median projection.

A few characters listed as diagnostic for *Nipponocercyon* by Hoshina and Fikáček (2010) have to be excluded as they are only present in *N. shibatai* but absent from *N. sichuanicus*: antennomeres 7–8 with groups of peg-like sensilla ventrally; metaventrite with two short mesal ridges anteriorly (but remnants of ridges seem to be retained in some specimens of *N. sichuanicus*; see the structure indicated as *mtr* in Fig. 20).

Recognition. By the combination of median portion of the prosternum differentiated from lateral portions, subpentagonal preepisternal elevation of the mesothorax widely contacting the metaventrite, large mesothoracic cavities for reception procoxae (reaching to anterior margin of mesoxocal cavity) and metanepisternum well developed both anteriorly and posteriorly, *Nipponocercyon* is most similar to the genus *Australocyon* Hansen, 1990. It may be easily distinguished from the Australian and Neotropical species of *Australocyon* by the male sternite 9 with tongue-like median portion (Fig. 4c), and male sternite 8 without median projection; from *Australocyon pilocnemoides* group it may be distinguished by the undifferentiated surface of the subpentagonal mesoventral plate (with a semicircular median portion defined by a wide bead in *Australocyon pilocnemoides* group, see Fig. 6 in Hansen (2003)), unmodified antennal morphology (in contrast to long antennae with prolonged antennal club pointed at apex and antennomere 6 not cup-like in shape in *Australocyon pilocnemoides* group), and the excavate ventral surface of profemora (without any sculptured depression in *Australocyon pilocnemoides* group).

When the size of mesoventral cavities for reception of procoxae is not taken into consideration, *Nipponocercyon* may resemble other megasternine genera with small subpentagonal mesoventral plate, clearly defined prosternal plate and male sternite 9 tongue-like medially (characters distinguishing the respective genus from *Nipponocercyon* are listed in parentheses: *Agna* (prosternal plate without deeply excised prosternal process, antennal grooves very small and angular in shape, profemur without sculptured depression); *Bolbonotum* and *Kahanga* (elytral grooves deep and wide, reaching total base of elytra, prosternal plate projecting both anteriad and posteriad, profemur without ventral impression, mesoventral plate rhomboid when examined in detail, gonopore apical), *Deltostethus* (mesoventral plate with wide marginal bead, profemur without ventral depression, gonopore apical), and *Pelocyon* (metavetrite with complete femoral lines, prosternal plate longer than wide). *Nipponocercyon sichuanicus* may resemble some species of the genera *Cryptopleurum*, *Pachysternum* and *Cyrtonion* by its large antennal grooves, large grooves for reception of procoxae, reduced epipleura and strongly sculptured body. See below under that species for characters distinguishing it from the mentioned genera.

Composition and distribution. The genus now includes two species, one distributed in Kyushu, Shikoku and the southern part of the Honshu, the other occuring in high altitudes of the mountain ranges in the Chinese province of Sichuan (Fig. 23).

Key to *Nipponocercyon* species

1 Body uniformly brown (Figs 1–2). Elytral intervals strongly convex (Figs 1–2), whole dorsal surface strongly microsculptured (Figs 7–9). Male maxilla without sucking disc. Antennal club without ventral groups of peg-like sensilla (only seen at high magnifications!). Antennal grooves large, nearly reaching lateral margin of hypomeron (Fig. 11). Prosternal plate with obsolete median carina. Preepisternal elevation of mesothorax slightly wider than long (Fig. 19). Anteromedian portion of metavetrite without (or with at most very weakly developed) two short longitudinal ridges, lateral portions with very coarse punctures (Fig. 20). First abdominal ventrite with setiferous punctures many times larger than on ventrites 2–5 (Fig. 21). Protibia angulate distally.

Median lobe wide apically (Fig. 4b) .... ***N. sichuanicus* (Ryndevich, 2005), comb. n.**

– Elytra pale reddish with darker spots at midlength, head and pronotum dark brown (Hoshina and Fikáček 2010, Figs 1–2), dorsal surface with weak mesh-like microsculpture in elytra in some specimens. Elytral intervals flat (Hoshina and Fikáček 2010, Figs 17–18). Male maxilla with sucking disc. Antennal club with ventral groups of peg-like sensilla (only seen at high magnifications!, see Hoshina and Fikáček 2010, Figs 3–4). Antennal grooves moderately large, not reaching close to lateral margin of the hypomeron (Fig. 14). Prosternal plate with very distinct median carina. Preepisternal elevation of mesothorax as wide as long or slightly longer than wide (Hoshina and Fikáček 2010, Figs 11–14). Anteromedian portion of metaventrite with two short longitudinal ridges (Hoshina and Fikáček 2010, Figs 11–14), lateral portions with coarse punctures intermixed with small ones (Hoshina and Fikáček 2010, Fig. 16). First abdominal ventrite with setiferous punctures slightly larger and denser than punctures on ventrites 2–5 (Hoshina and Fikáček 2010, Fig. 19). Protibia rounded distally. Median lobe narrow apically (Fig. 6b) .... ***N. shibatai* Satô, 1963**

***Nipponocercyon sichuanicus* (Ryndevich, 2005), comb. n.**

http://species-id.net/wiki/Nipponocercyon_sichuanicus
Figs 1–4, 7–12, 19–21, 23–24

Cryptopleurum sichuanicum Ryndevich, 2005: 244
Cryptopleurum sichuanicum: Short and Hebauer (2006: 348, catalogue).

Type material examined. Paratype: 1 female (CSR): 'CH, S Sichuan, near / Bijishan Village, left / tr. of Lianhegou River / 2500–3200 m, 19.6.2000 / Belousov, Kabak, Davidian // Paratype / Cryptopleurum / sichuanicum / Ryndevich S. K. // Coll. / SKR // Cryptopleurum / sp.n. / det HEBAUER'.

Additional material examined. CHINA: Sichuan: 2 males, 1 spec. (CSR, NMPC): 2.1 km N of Dengsheng, SE of Balanguan Pass, elev. 3455 m, 30°53'3"N 102°58'23"E, 29.viii.2004, lgt. Belousov & Kabak; 1 spec. (NHMW): 20 km N Sabdê, elev. 3300 m, 29°35'N 102°23'E, 14.vii.1998, lgt. A. Smetana (C82); 1 spec. (CSR): S of Musu village, elev. 2850 m, 31°56'53"N 103°15'11"E, 19.viii.2007, lgt. Belousov & Kabak; 1 male (NMPC): N Sichuan, SW of Baima, elev. 2980–3040 m [ca. 27°55'N, 101°56'E], 23.vi.2006, lgt. I. Kabak; 5 spec. (CSR, NMPC, SYSU, KSEM): SW of Jiabi, elev. 3240 m, 31°30'40"N 102°43'43"E, 8–13.viii.2007, lgt. Belousov & Kabak; 1 male, 3 spec. (NHMW, NMPC): Ganzi, Daxue Shan, Mugecuo, ca. 26 km NW Kangding, elev. 3200–3400 m, 30°06'36"N 101°31'12"E, 21.v.1997, lgt. A. Pütz.

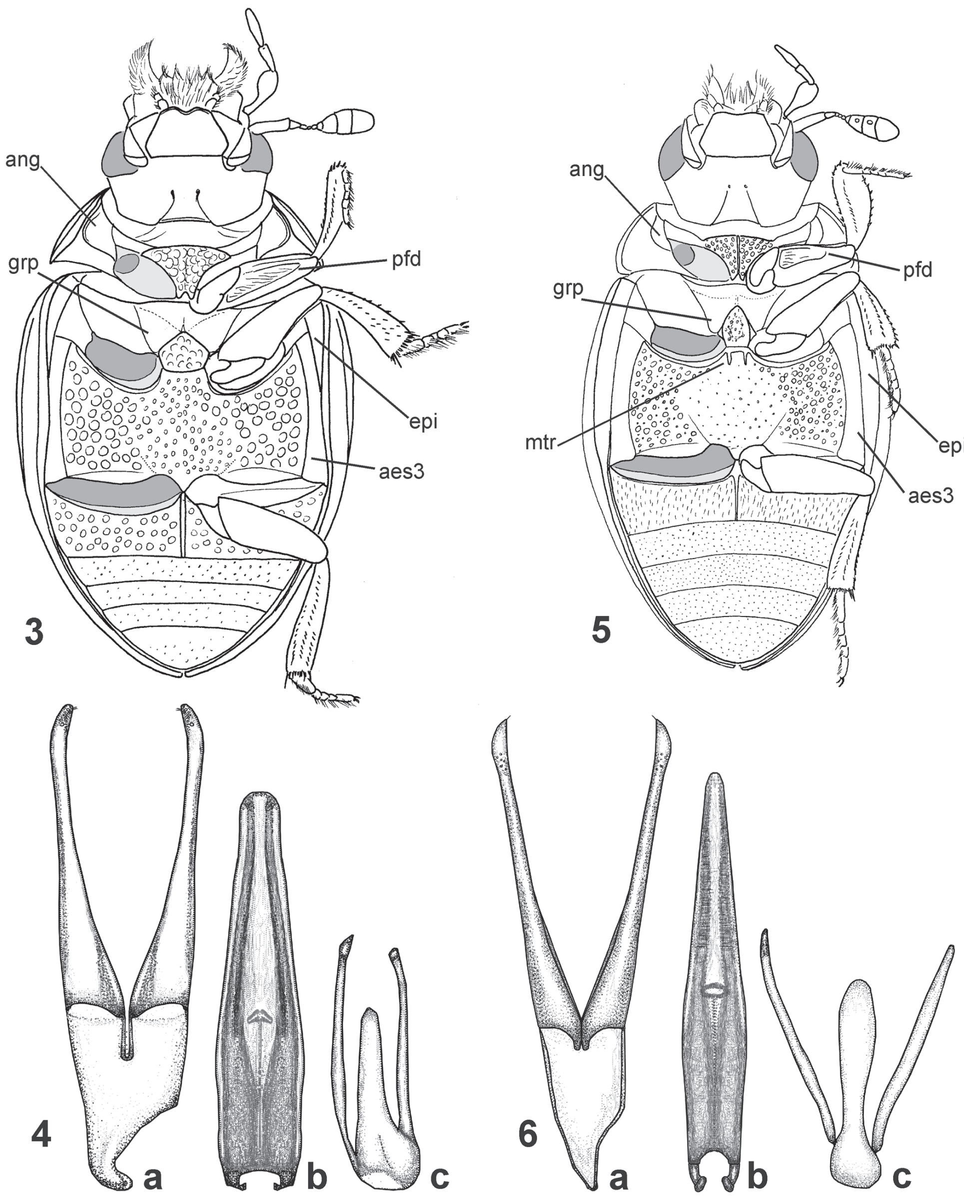
Redescription. Body widely oval, widest in anterior third of elytra. Body length 2.2–2.9 mm, body width 1.4–1.7 mm.

Figures 1–2. General habitus of *Nipponocercyon sichuanicus.* **1** dorsal view **2** lateral view.

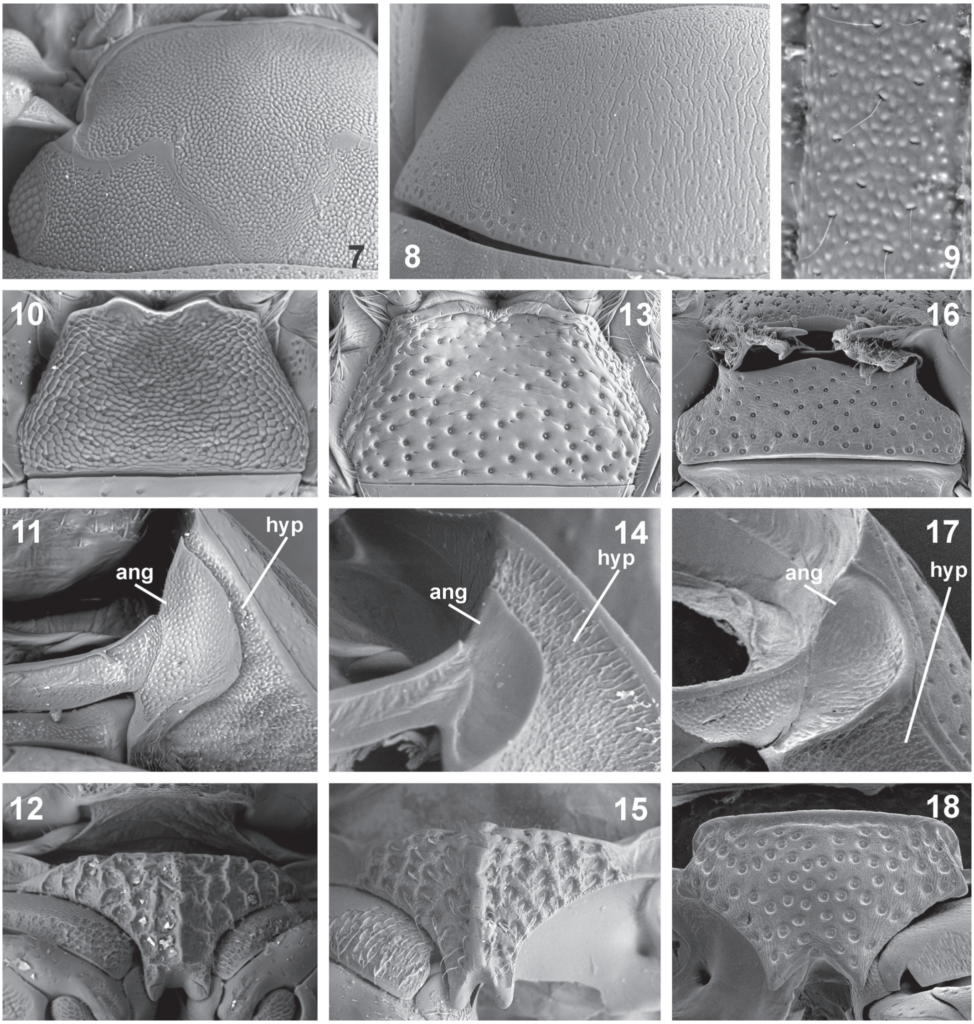
General coloration of dorsal surface dark brown, anterior and anterolateral margins of clypeus and lateral portions of frontoclypeal suture pale reddish, anterior margin of pronotum widely reddish, each elytron slightly paler in humeral area and at elytral apex. Ventral surface dark brown, mentum, mouthparts and posterior portions of temporae reddish brown. Maxillary palpi, antennae and legs pale reddish brown.

Head. Clypeus widely rounded, constricted above antennal bases, with very distinct marginal bead. Dorsal surface of clypeus and frons with strong scale-like microsculpture obscuring the punctation, sparsely arranged punctures not apparent among microsculpture, only evident according to long thin setae arising from punctures. Frontoclypeal suture apparent as a non-sculptured stripe directing mesad, strongly bent posteriad submesally. Mentum with sparsely arranged fine setiferous punctures medially and posteriorly, interstices with strong scale-like microsculpture. Antennal club without distinct groups of peg-like sensilla dorsally or ventrally. Maxilla of male without sucking disc ventrally.

Prothorax. Pronotum with sparsely arranged fine setiferous punctures, larger punctures along posterior margin absent. Whole dorsal surface with mesh-like microsculpture, microsculpture strong along anterior and posterior margins and on lateral portions of pronotum, obsolete on pronotal disc; pronotal disc with

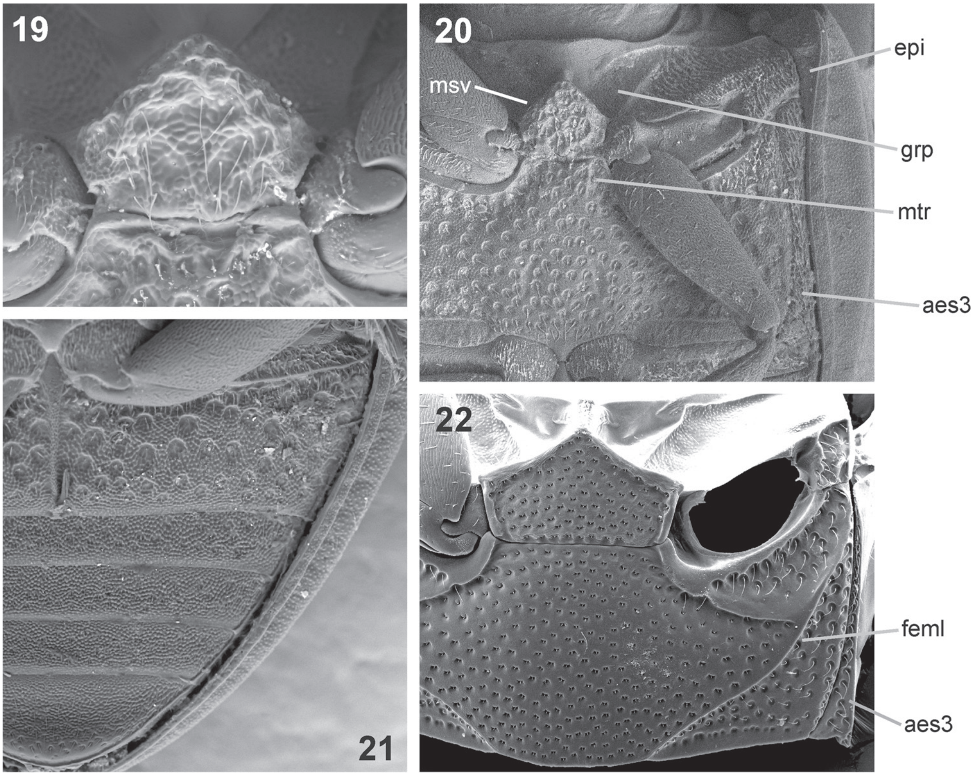


Figures 3–6. Morphological details of *Nipponocercyon* species. **3–4** *N. sichuanicus*: **3** ventral view **4** male genitalia; **5–6** *N. shibatai*: **5** ventral view **6** male genitalia [the drawing is based on that by Hoshina and Fikáček (2010) in combination with the photo sent to us by H. Hoshina, we did not examine any male specimen for this study]). Parts of male genitalia: **a** tegmen, **b** median lobe, **c** sternite 9. Abbreviations: **aes3** metanepisternum, **ang** antennal groove, **epi** epipleuron, **grp** grooves for reception of procoxae, **mtr** anteromesal ridge of metaventrite, **pfd** profemoral depression.



Figures 7–18. Morphological details of *Nipponocercyon sichuanicus* and its comparison with *N. shibatai* and *Cryptopleurum minutum*. **7–12** *Nipponocercyon sichuanicus*: **7** head in dorsal view **8** pronotum **9** superficial microsculpture of elytral intervals **10** mentum **11** antennal groove **12** median portion of prosternum. **13–15** *N. shibatai*: **13** mentum **14** antennal groove **15** median portion of prosternum. **16–18** *Cryptopleurum minutum*: **16** mentum **17** antennal groove **18** median portion of prosternum. Abbreviations: **ang** antennal groove, **hyp** hypomeron.

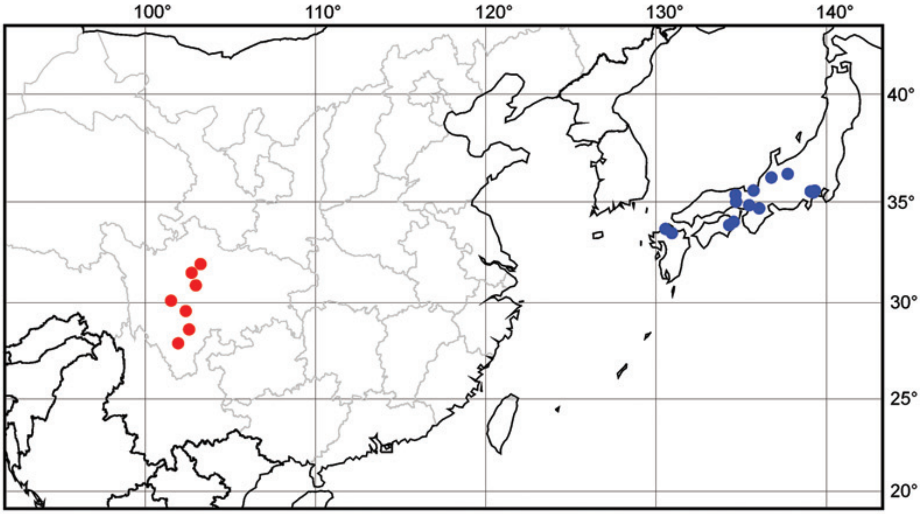
irregular longitudinal striae. Lateral portions of pronotum slightly deflexed (and hence seen in ventral view). Prosternum with well defined median plate 2.0× wider than long, bearing strong rugose sculpture, indistinctly carinate mesally. Anterolateral corners of prosternum (at contact with hypomeron) with small but distinct tooth. Antennal grooves large, but not quite reaching lateral margin of hypomeron. Profemur with a rather shallow sculptured depression on a large portion of ventral surface. Protibia angulate distally.



Figures 19–22. Ventral morphology of *Nipponocercyon sichuanicus* and its comparison with *Cryptopleurum ferrugineum*. **19–21** *Nipponocercyon shibatai*: **19** mesoventral plate **20** meso- and metaventrite **21** abdominal ventrites. **22** meso- and metaventrite of *Cryptopleurum ferrugineum*. Abbreviations: **aes3** metanepisternum, **epi** epipleuron, **feml** femoral line, **grp** groove for reception of procoxae, **msv** mesoventral plate, **mtr** anteromedian ridge of metaventrite.

Mesothorax. Scutellar shield with sparse fine punctation, without microsculpture. Elytra with 10 punctural series, all series deeply impressed, lateral striae deeper than median ones; serial punctures minute and rather inconspicuous; elytral intervals highly convex, bearing sparsely arranged fine setiferous punctation, interstices with strong microsculpture consisting of small bumps; lateral portions of elytra deflexed laterally (hence, visible in ventral view); epipleuron present only on elytral base, reduced to extremelly narrow stripe behind level of mesocoxae. Mesoventrite with pentagonal posteromedian elevation, the elevation 1.3× wider than long, with rugose setiferous sculpture.

Metathorax. Anteromedian process with very weakly developed short longitudinal ridges, in many individuals completely obscured by microsculpture; median portion of metaventrite slightly elevated bearing densely arranged coarse setiferous punctures separated by 0.5–1.2× puncture diameter; lateral portions of metaventrite with extremelly large setiferous punctures; whole surface of metaventrite except its posterome-



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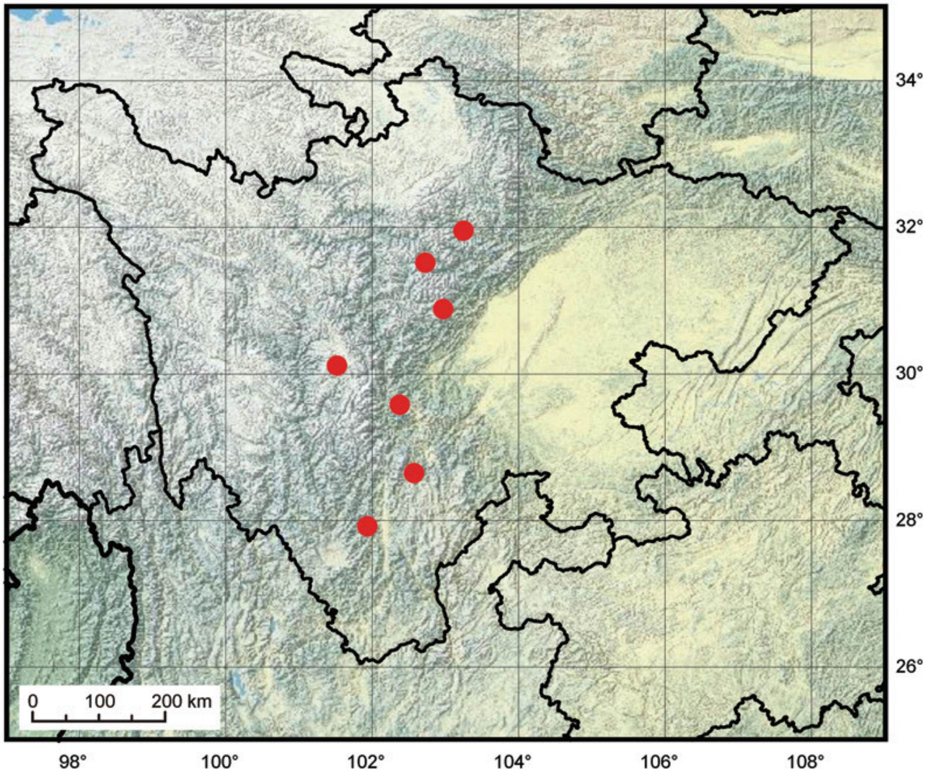

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Figures 23–24. Maps. **23** general distribution of the genus *Nipponocercyon* (data for *N. shibatai* adopted from Hoshina and Fikáček 2010) **24** distribution of *N. sichuanicus* in the Sichuan Province. Symbols: **red dot** *Nipponocercyon sichuanicus*, **blue dot** *N. shibatai*.

dian portion with mesh-like microsculpture on interstices, microsculpture stronger laterally than medially. Hind wings well developed.

Abdomen. All abdominal ventrites with strong scale-like microsculpture, punctation of ventrite 1 consisting of extremelly large setiferous punctures similar to that on lateral portions of metaventrite; punctation of ventrites 2–5 sparse and very fine, nearly completely obscured by microsculpture.

Male genitalia. Parameres slender, 1.8× longer than phallobase. Median lobe robust, very wide and parallel-sided in basal 0.35, slighly and continually narrowing apicad in apical 0.65, apex widely rounded; gonopore situated in basal 0.4 of median lobe. Stenite 9 with slightly asymmetrical median projection.

Differential diagnosis. See the identification key above for characters distinguishing *N. sichuanicus* from *N. shibatai*. *Nipponocercyon sichuanicus* may be confused with some species of *Cryptopleurum*, *Pachysternum* or *Cyrtonion* (the latter not occurring in Asia, however) which are also characterized by large antennal grooves and strongly sculptured dorsal surface. *Nipponocercyon shibatai* may be easily distinguished from them by the combination of following characters: (1) metaventrite without femoral lines (femoral lines present in *Cryptopleurum*, *Pachysternum* and *Cyrtonion*, see e.g. Fig. 22, feml); (2) metanepisternum wide throughout (Fig. 20) (reduced anteriorly and widening posteriad in the above genera as well as in all other genera of the *Megasternum* group characterized by large antennal grooves, see e.g. Fig. 22, aes3); (3) gonopore situated in basal portion of the median lobe (Fig. 4; this character distinguishes both species of *Nipponocercyon* from all other Megasternini); (4) mesoventral plate only slightly wider than long, without acute angles (Fig. 19) (mesoventral plate large and distinctly transverse in *Cryptopleurum*, see e.g. Fig. 22); (5) anterolateral corners of mentum not sharply angulate (Fig. 10) (sharply angulate in *Cryptopleurum*, as in Fig. 16).

Biology. No details on the biology are known. The terrestrial habits of *N. shibatai* (Hoshina & Fikáček 2010) as well as the vast majority of the megasternine taxa suggest that *N. sichuanicus* is a terrestrial species.

Distribution. The species occurs in the mountains of the Sichuan province in South China, at altitudes between 2500–3500 m a.s.l. (Fig. 24).

Discussion

The inclusion of *Cryptopleurum sichuanicum* into the genus *Nipponocercyon* may appear contradictory, as the species differs in its external morphology from *N. shibatai* in many characters, some of which were previously considered as diagnostic at the generic level. However, a detailed comparison reveals that despite many differences, both species are very similar in terms of the morphology of the ventral side and genitalia (compare Figs 3–6) which is crucial for generic assignment of the megasternine taxa. Especially important in this respect are characters which are present in both *Nipponocercyon* species but absent from all other Megasternini: the basal position of the gonopore on the median lobe (Figs 4b, 6b), and the presence of a depression on the ventral surface of profemur (Figs 3, 5, pfd). Another character infrequent in the Megasternini but shared by both *Nipponocercyon* species is the presence of large mesothoracic grooves for the reception of the procoxae (Fig. 20, grp). Except *Nipponocercyon*, large grooves are only present in *Australocyon* and the *Megasternum* group of genera, of which the representatives of the latter generic group and part of *Australocyon* clearly seem unrelated to *Nipponocercyon* based on other external characters (see the differential diagnosis of *Nipponocercyon* for details). Moreover, general morphology of the genitalia of both species is very similar, with principal differences found only in the shape of the apical portion of the median lobe. Eyes with extremelly small dorsal portion are also unusual within the Megasternini but shared by both species. All these characters indicate that both species are more closely related to each other than they are to other megasternine taxa, which justifies the tranfer of *Cryptopleurum sichuanicum* into *Nipponocercyon*.

Characters in which *Nipponocercyon sichuanicus* is contradicting the diagnosis of the genus *Nipponocercyon* used by previous authors (Satô 1963, Hansen 1991, Hoshina and Fikáček 2010) seem now to be autapomorphies of *N. shibatai* or *N. sichuanicus*. For example, the groups of peg-like sensilla on antennomeres 7–8 are autapomorphies of *N. shibatai*; highly sculptured dorsal and ventral surfaces, reduced epipleura (Fig. 20, epi), extremely large antennal grooves (Fig. 11), and the absence of male sucking discs on the maxilla are autapomorphies of *N. sichuanicus*. The reduction of sucking discs in *N. sichuanicus* may be possibly correlated with the strong microsculpture on dorsal surface of this species, which makes the suckers uneffective for male's holding on the female during the copulation. Two diagnostic characters are seemingly absent from *N. sichuanicus*, but a careful examination of multiple specimens revealed that they are present in both *Nipponocercyon* species: remnants of a pair of short anteromesal ridges on the metaventrite (present even in some specimens of *N. sichuanicus*; Fig. 20, mtr) and the median carina of prosternal plate.

The autapomorphies of *N. sichuanicus* make the species similar in habitus to *Cryptopleurum* and related genera, which is the reason why the species was originally described within *Cryptopleurum*. However, a detailed examination shows that this similarity is due to the parallelism, as the body parts which are responsible for the *Cryptopleurum*-like appearance of *N. sichuanicus* differ between *Cryptopleurum* and *N. sichuanicus* in detailed morphology:

(1) The antennal grooves of *N. sichuanicus* are large, but still do not reach the lateral margins of hypomeron, as they do in *Cryptopleurum* and related taxa (compare Figs 11 and 17).

(2) The meso- and metathorax of *N. sichuanicus* resemble *Cryptopleurum* and related genera by their extremely coarse punctation and large grooves for reception

of procoxae, but the overall thoracic morphology is totally different from these genera due to its well developed metepisternum (not reduced anteriorly, Fig. 20: aes3) and the ventral side of mesothorax not steeply declined, possessing unmodified mesepimera.

(3) The prosternal plate of *N. sichuanicus* is feebly carinate even though the carina is partly obscured by the strong microsculpture (ecarinate in *Crypopleurum* and related genera, see Fig. 18).

The above differences indicate that despite its superficial resemblance, *Nipponocercyon sichuanicus* is not related to the *Cryptopleurum* group of genera, but probably represents another example of highly sculptured sphaeridiine taxon derived from a non-sculptured ancestor. The shift from non-sculptured to sculptured phenotype was shown to lead to similar ‚sculptured' morphology in distantly related taxa in the Megasternini (*Oosternum* and *Emmidolium*, see Fikáček 2007), and similar examples also exist in some other groups of the Sphaeridiinae (e.g., the omicrine genera *Noteropagus* and *Peratogonus* resemble the megasternine genus *Cryptopleurum* by the same characters which are responsible for the *Cryptopleurum*-like appearance of *Nipponocercyon sichuanicus*).

The inclusion of *Cryptopleurum sichuanicum* into *Nipponocercyon* extends the range of the genus (previously endemic to Japan) to the mainland Asia. The isolated occurrence of the genus at high altitudes of the mountain ranges in Sichuan and in mountain areas of southern Japan (Fig. 23) suggests that the current distribution may represent relictual remnants of the former wider distribution of the genus. The situation hence resembles that of the myxophagan genus *Satonius* Endrödy-Younga, 1997 which was originally considered as Japanese endemic (Satô 1963; Endrödy-Younga 1997), but was later found to be distributed in various areas in central and southern China (Hájek and Fikáček 2008; Hájek et al. 2011). High altitude areas of other mountain ranges of China need to be sampled in order to test if the occurrence of *Nipponocercyon* on the continent is really restricted to Sichuan, or if the genus is more widely distributed in the mountains of the transitional zone between Palaearctic and Oriental regions.

Hoshina and Fikáček (2008) examined 35 specimens of Japanese *Nipponocercyon* from the entire Japanese range of the genus, including the types of all three taxa described from Japan by Satô (1963) and Nakane (1968): *N. shibarati* Satô, 1963, *N. shibarati omayanum* Nakane, 1968 and *N. monticola* Nakane, 1968. They found a wide variation in several characters among the specimens they examined (especially the shape of mesoventral plate and metaventral ridges, the presence of dorsal sculpture and the impression of elytral series) which was considered as intraspecific variation due to a weak correlation of the characters and extreme uniformity of male geniatalia. The above taxa were therefore synonymized with *N. shibatai*, although the authors expressed the need for more detailed studies based on more extensive material to clarify the taxonomy of Japanese *Nipponocercyon*. The inclusion of *N. sichuanicus* into the genus may bring new insights into the problem. We have examined specimens of *N. sichuanicus* from a rather wide area in Sichuan (Fig. 24), but all of them are very constant in the proportion of the mesoventral plate. This is in contrast to the high variability of the shape of the mesoventral plate of *N. shibatai* proposed by Hoshina and Fikáček (2008). Moreover, *Nipponocercyon shibatai* and *N. sichuanicus* are very similar in the morphology of the aedeagus (the width of the apex of the median lobe is the only relevant difference, in contrast to many differences in external morphology), indicating that genital morphology is very conservative in *Nipponocercyon*. This suggests that no significant differences in aedeagal morphology should be expected among closely related species, whereas the shape of mesoventral plate may be a good indicator of species limits. This further demostrates the need for additional studies of Japanese *Nipponocercyon*, as the existence of more than one species in Japan cannot be excluded.

Acknowledgements

We are indebted to M. A. Jäch for the opportunity to examine the specimens deposited in NHMW, to H. Hoshina (Fukui University, Fukui, Japan) for reexamining several morphological details of *N. shibatai* and taking their photographs for us, to J. Růžička (University of Life Sciences, Prague, Czech Republic) for his help with generating the maps, and to the collectors of the additional specimens presented above for the kind donation of these specimens to the second author. The study was financially supported by the Ministry of Culture of the Czech Republic (DKRVO000023272) and the institutional resources of Ministry of Education, Youth and Sports of the Czech Republic for the support of science and research. Examination of the specimens using scanning electron microscope Hitashi-3700N was possible due to Barrande I Project partially supported by the European Union.

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RESEARCH ARTICLE

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Five new species and three new females of the genus *Endotricha* Zeller from China (Lepidoptera, Pyralidae, Pyralinae)

Yongling Sun[1,2,†], Houhun Li[2,‡]

1 *Department of Biology, Dezhou University, Dezhou 253023, Shandong Province, R. P. China* **2** *College of Life Sciences, Nankai University, Tianjin 300071, P. R. China*

† *urn:lsid:zoobank.org:author:2B88BD41-1722-490D-8267-94D76C4F1384*
‡ *urn:lsid:zoobank.org:author:F6AAB0C0-F312-4035-A08B-3DFE03D02F62*

Corresponding author: *Houhun Li* (lihouhun@nankai.edu.cn)

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Abstract

Five new species of the genus *Endotricha* Zeller are described from China: *Endotricha dentiprocessa* **sp. n.**, *E. unicolor* **sp. n.**, *E. shafferi* **sp. n.**, *E. convexa* **sp. n.** and *E. whalleyi* **sp. n.** Females of three species are described for the first time: *E. hoenei* Whalley, 1963, *E. luteogrisalis* Hampson, 1896 and *E. simipunicea* Wang & Li, 2005. Photographs of the adults and both male and female genitalia are provided.

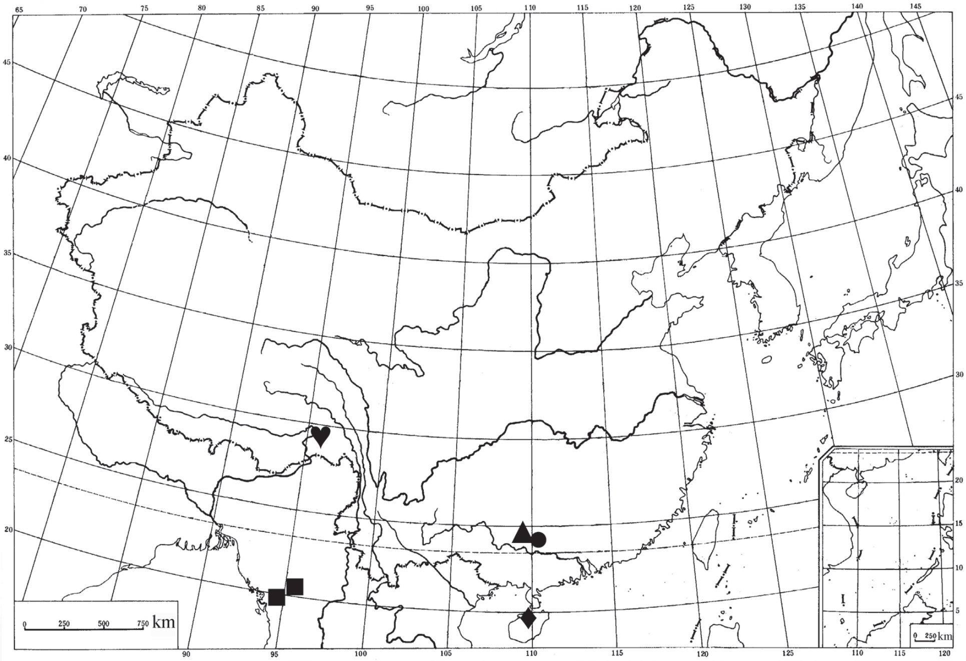
Keywords

Lepidoptera, Pyralidae, Pyralinae, *Endotricha*, taxonomy, new species, China

Introduction

The genus *Endotricha* Zeller, 1847 was erected for the type species *Pyralis flammealis* [Denis & Schiffermüller], 1775. It belongs to the tribe Endotrichini of the subfamily Pyralinae, which includes seven genera and are characterized by having Rs anastomosed with Sc+R_1 in the hindwing (Solis and Shaffer 1999). Solis and co-authors discussed the structures and the systematic position of the tribe, the subfamily and family (Solis and Mitter 1992, Solis and Shaffer 1999, Solis and Metz 2011).

Endotricha is characterized by the forewing usually having a dark-colored ground coloration, the male gnathos being flat and plate-like, and the female corpus bursae having a spined signum. Currently, it comprises over one hundred species worldwide (Whalley 1963, Yoshiyasu 1987 and 1989, Solis and Shaffer 1999, Kirpichnikova 2003, Wang and Li 2005, Lee et al. 2007, Sun and Li 2009, Nuss et al. 2003–2011), occurring throughout the Old World. Thirty-three species were recorded in China prior to this study (Wang and Li 2005, Sun and Li 2009). The aim of the present paper is to describe five new species and report the females of three species for the first time based on the Chinese specimens collected in Fujian, Guangxi, Guizhou, Hainan and Tibet (Map 1).



Map 1. Distribution of new *Endotricha* species in China. ▲ *Endotricha dentiprocessa* sp. n. ■ *E. unicolor* sp. n. ● *E. shafferi* sp. n. ♦ *E. convexa* sp. n. ♥ *E. whalleyi* sp. n.

Material and methods

This study is based on the examination of the specimens collected in mountainous areas or nature reserves in China by using light traps. Terminology mainly follows Whalley (1963). Genitalia dissection and mounting methods follow Li (2002). Images of the adults were taken with a Nikon D300 digital camera plus macro lens, and the genitalia were prepared with an Olympus C-7070 digital camera. All the examined specimens are deposited in the Insect Collection, College of Life Sciences, Nankai University, Tianjin, China.

Descriptions of new species

Endotricha dentiprocessa Li, sp. n.

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http://species-id.net/wiki/Endotricha_dentiprocessa
Figs 1, 9, 14

Type material. Holotype ♂ – **China**, **Guangxi Zhuang Autonomous Region:** Qinmu Village, Yongfu County (24°59'N, 109°59'E), 160 m, 5.V.2008, coll. Li Zhang and Hui Zhen, genitalia slide no. SYL11029. Paratypes: 1 ♂, 2 ♀, same data as for holotype except dated 1–5.V.2008.

Diagnosis. This species is superficially similar to *E. costaemaculalis* Christoph, 1881, but can be distinguished in the male genitalia by the valva having the basal 1/4 ventrally dentate and the sacculus being distally produced into a stout thumb-shaped process, and in the female genitalia by the corpus bursae having basal half wider than distal half. In *E. costaemaculalis*, the valva is not dentate ventrobasally, the sacculus process is narrowing to a point; and the basal half of the corpus bursae is narrower than the distal half.

Description. Adult (Fig. 1): Wing expanse 17.0–18.5 mm. Head blackish brown. Antenna yellowish brown, scape grayish white dorsoapically, flagellum with blackish brown dorsal annuli. Labial palpus blackish brown except second and third segments grayish-white at apices. Thorax and tegula dark yellowish brown. Forewing blackish brown, covered with dense reddish brown scales; costal margin black, interrupted with white dots, with a large ill-defined yellow spot before subterminal line; antemedian line white, slightly arched outward; discal spot inconspicuous; subterminal line purplish red, discontinuous, edged with black; terminal line black, edged with a broad purplish red band along inner margin; fringe reddish brown mottled black at apex, from below apex to anterior 1/4 of termen creamy white, from anterior 1/4 to tornus black mottled purplish red, blackish grey mottled reddish brown on dorsal margin, with a white basal line. Hindwing concolorous to forewing, yellowish white on costal margin; antemedian line white; postmedian line grayish white, edged with black, sinuate; terminal line black; fringe blackish grey mottled purplish-red and white along termen, grayish white on apex and along dorsal margin. Legs pale yellow on dorsal surface, blackish brown on ventral surface; tarsi with brown rings.

Male genitalia (Fig. 9). Uncus inverted triangular, caudal margin gently arched, concave at middle, extending outwards posterolaterally to pointed ends; uncus arm ear-shaped, straight, rounded apically; uncus processes triangular, with dense spines, located at about 2/5. Gnathos narrowly elongate, rounded apically, slightly shorter than arm; arm broad band-shaped. Transtilla large and broad, widened medially, narrowing outwards to both ends. Valva broad tongue-shaped, rounded at apex; costa arched medially; ventral margin concave at base, basal 1/4 with a narrow sclerotized plate bearing large teeth. Sacculus with basal 3/5 broad, with sclerotized narrow edge ventrally, distally produced to a stout thumb-shaped process, slightly hooked backward dorsoapically, reaching beyond middle length of valva apically. Vinculum short

Figures 1–4. Adults of *Endotricha* spp. **1** *E. dentiprocessa* sp. n., paratype, female **2** *E. unicolor* sp. n. **2a** holotype, male **2b** paratype, female **3** *E. shafferi* sp. n., paratype, female **4** *E. convexa* sp. n. **4a** paratype, male **4b** paratype, female.

and broad; saccus short and broad, triangular. Juxta large hexagonal. Phallus slightly shorter than valva, with fine spines; ductus ejaculation from basal 1/3.

Female genitalia (Fig. 14). Ovipositor nearly triangular, narrowing posteriorly to rounded caudal margin. Apophysis posterioris long and slender, about three times length of apophysis anterioris. Ostium bursae broad, trapezoid, weakly sclerotized; antrum heavily sclerotized, funnellike, longer than half length of apophysis anterioris; ductus bursae membranous, shorter than antrum. Corpus burase large and elongate oval, basal half wider than distal half; signum small and rounded, placed at posterior 1/3.

Distribution (Map 1)**.** China (Guangxi).

Etymology. The specific epithet is from the Latin prefix *dent-*, meaning dentate, and *processus*, meaning process, in reference to the valva with narrow sclerotized plate bearing large teeth ventrobasally.

Endotricha unicolor Li, sp. n.

urn:lsid:zoobank.org:act:43C9790A-7B57-4108-95B2-EFACBDC8F9D7
http://species-id.net/wiki/Endotricha_unicolor
Figs 2, 10, 15

Type material. Holotype ♂ – **China, Xizang (Tibet) Autonomous Region:** Milin County (19°10'N, 94°10'E), 2980 m, 30.VII.2010, coll. Houhun Li, genitalia slide no. SYL11147. Paratypes: 2 ♀, same data as for holotype except dated 30–31. VII.2010; 1 ♀, Lulang Town, Linzhi County, 3065 m, 4.VIII.2010, coll. Houhun Li.

Diagnosis. This species is similar in appearance to *E. consobrinalis* Zeller, 1852 from Africa, with some external variation. It can be distinguished in the male genitalia by the apically bluntly rounded uncus arm, the distally dilated gnathos, and by the sacculus produced to a distal-curved process; and in the female genitalia by the oval corpus bursae with signum placed in posterior 1/3. In *E. consobrinalis*, the forewing is ocherous brown, the uncus arm is narrowly rounded, the gnathos is slightly narrowed distally and the sacculus is produced into a distal-straight process; and the corpus bursae is elongate rectangular, and the signum is situated in its posterior 1/4.

Description. Adult (Fig. 2): Wing expanse 20.0 mm. Head yellowish brown. Antenna yellowish brown, with blackish brown dorsal annuli. Labial palpus blackish brown on outer surface, yellowish brown on inner surface, third segment greyish white at apex. Thorax and tegula greyish brown. Forewing brown, irrorate with purple reddish brown scales throughout in female, from base to antemedian line in male; costal margin black, interrupted with white dots, distinct on distal 2/3; antemedian line white, edged with black on inner margin, extending from costal 1/3 obliquely outward to near middle of cell, then inward to fold, and finally arched outward to dorsum; discal spot black, kidney-shaped; postmedian line white, distinctly edged with black on inner margin, extending from about costal 1/8 curved inward to dorsal 3/4; termen with interrupted short black streaks; fringe deep grey. Hindwing yellowish grey, tinged with black distally; ante- and postmedian lines pale silvery grey on dorsal surface, sinuous along both edges, black on ventral surface; termen with black dots and short streaks; fringe greyish brown basally, greyish yellow distally, pale yellow along dorsal margin. Legs yellowish white on dorsal surface, blackish brown on ventral surface.

Male genitalia (Fig. 10). Uncus rectangular, gently arched caudally; uncus arm broad, bluntly rounded apically; uncus processes triangular, situated at about 3/5. Gnathos somewhat racket-shaped, rounded at apex. Valva elongately narrow, arched ventrally, rounded at apex; transtilla a curved narrow band. Sacculus elongate triangular, wide basally, tapering to a long spine-shaped process curved distally, apex reaching middle of ventral margin, curved backward. Vinculum broad; saccus short and broad, rounded anteriorly. Juxta broad basally, narrower and nearly parallel distally; lateral lobe short, about 1/3 of its length. Phallus slender and long; ductus ejaculation from basal 1/4.

Female genitalia (Fig. 15). Ovipositor nearly triangular, narrowly rounded caudally. Apophysis posterioris long and slender, about 2.5 times length of apophysis anterioris. Ostium bursae broad funnel-shaped, weakly sclerotized; antrum heavily sclerotized, lateral sides nearly parallel, slightly longer than half length of apophysis anterioris; ductus bursae membranous, shorter than antrum. Corpus burase oval; signum small, weak, placed at posterior 1/3.

Distribution (Map 1)**.** China (Tibet).

Etymology. This specific name is from the Latin prefix *uni-*, meaning unitary, and the Latin postfix *-color*, meaning color, in reference to the hindwing without distinct patterns.

Endotricha shafferi Li, sp. n.

urn:lsid:zoobank.org:act:8757C5F5-B18B-4B8E-A7E0-AA80384B6C16
http://species-id.net/wiki/Endotricha_shafferi
Figs 3, 11, 16

Type material. Holotype ♂ – **China, Guangxi Zhuang Autonomous Region:** Yinsha Protection Station (24°8'N, 110°11'E), Jinxiu County, 700 m, 27.IV.2008, leg. Hui Zhen and Li Zhang, genitalia slide No. SYL11094. Paratypes: 2 ♀, same data as for holotype.

Diagnosis. This species is similar to *E. hoenei* Whalley, 1963. It can be distinguished by the black or deep blackish brown body; the male genitalia with the sacculus squared basally; and the female genitalia with antrum as thick as ductus bursae and having the signum located at posterior 1/3 of corpus bursae. In *E. hoenei*, the body is purplish red; the sacculus is triangular basally; and the antrum is narrower than the ductus bursae.

Description. Adult (Fig. 3): Wing expanse 21.0 mm. Head yellow. Antenna yellowish brown, with blackish brown dorsal annuli. Labial palpus with basal and second segments blackish brown except second segment pale yellow at apex, third segment pale yellow. Thorax and tegula blackish brown. Forewing blackish brown in male, black in female; costal margin with creamy white dots interrupted by short black streaks along distal 2/3, with an ill-defined ocherous yellow patch at inner side of subterminal line; antemedian line yellow, widened to an inverted triangular spot on anterior 1/3, narrowed to beyond dorsal 1/3; discal spot black, small, placed on outer margin of antemedian line; subterminal line purplish reddish brown, sinuate, extending inward to tornus; terminal line black; fringe orange yellow except black at apex and from 1/2 to 3/4 of termen, with a distinct black line at base from anterior 1/4 to before tornus, reddish brown on dorsal margin. Hindwing black; median line yellow, broad, slightly widened medially, narrowed posteriorly; terminal line black; fringe yellow, with a distinct black basal line, pale yellow along dorsal margin. Legs yellow except fore- and midlegs blackish brown on ventral surface.

Male genitalia (Fig. 11). Uncus wide at base, narrowed to about middle, then widened to blunt apex; uncus arm triangular, blunt apically; uncus processes ciliiform, placed at about middle. Gnathos short, racket-shaped, rounded at apex. Valva elongately narrow, narrowed basally, rounded apically. Sacculus with basal half squared, distal half suddenly narrowed to a slender rod-shaped process. Vinculum broad, narrowed anteriorly; saccus short, rounded anteriorly. Juxta broad, slightly narrowed letaromedially, with fine satae posteriorly. Phallus slender, about 2/3 length of valva; ductus ejaculation from basal 1/4.

Female genitalia (Fig. 16). Ovipositor nearly triangular, narrowly rounded caudally. Apophysis posterioris two times length of apophysis anterioris. Antrum heavily sclerotized, about same size as ductus bursae; ductus bursae membranous, shorter than corpus bursae. Corpus burase elongate oval; signum small, placed at posterior 1/3 of corpus bursae.

Distribution (Map 1)**.** China (Guangxi).

Etymology. This species is named after the late Michael Shaffer (BMNH) in memory of his friendship with the corresponding author as well as for his outstanding work in the taxonomy of Pyraloidea.

Endotricha convexa Li, sp. n.

urn:lsid:zoobank.org:act:17F0E07F-B69F-4B6D-8848-E6FDD3C71D45
http://species-id.net/wiki/Endotricha_convexa
Figs 4, 12, 17

Type material. Holotype ♂ – **China**, **Hainan Province:** Yinggeling (19°02'N, 109°50'E), 620 m, 23.V.2010, coll. Bingbing Hu and Jing Zhang, genitalia slide no. SYL11043. Paratypes: 1 ♂, same data as for holotype; 2 ♂, Mt. Wuzhi, 740 m, 14.IV.2009, coll. Qing Jin and Bingbing Hu; 1 ♀, Yinggeling, 30.IX.2010, coll. Bingbing Hu.

Diagnosis. This species is similar to *E. lobibasalis* Hampson, 1906. It can be distinguished by the forewing in male having a hump from costal 1/5 to 2/5; in the male genitalia by the conspicuous gnathos, the ventroapically right-angled valva, and the oval juxta being deeply concave to mid length on posterior margin. In *E. lobibasalis*, the basal 1/3 of the forewing has a gentle hump; the gnathos is inconspicuous, the valva is bluntly angled ventroapically, and the nearly trapezoidal juxta is not concave.

Description. Adult (Fig. 4): Wing expanse 20.0–22.0 mm. Head reddish brown. Antenna yellowish brown, flagellum with blackish brown dorsal annuli. Labial palpus with basal segment reddish brown, second and third segments blackish brown except second segment pale yellow at apex. Thorax and tegula greyish yellow. Forewing purplish red in male, covered with dense black scales on basal 1/3, scattered with black scales on distal 2/3; costal margin with prominent hump extending from 1/5 to about 2/5, then gently concave to before apex, basal 1/3 orange yellow, interrupted with black dots, distal 3/5 black, with yellow spots; large inverted triangular orange yellow patch placed between middle of costal margin and before subterminal line extending downward, its inner margin obliquely extending to middle of subterminal line, outer margin straight, just adjacent to subterminal line; antemedian line yellowish white, slightly arched outward, not reaching anterior margin; discal spot a short strip, black; subterminal line whitish yellow, thin, curved, edged with black on outer margin; terminal line black; fringe purplish red, tinged with black. Hindwing concolorous to forewing except anteriorly whitish yellow from base to distal 1/5; antemedian line whitish yellow, edged with black on inner margin; postmedian line whitish yellow, both margins edged with black, between two lines pale yellow mixed with dense reddish brown scales; terminal line black; fringe purplish red, with blackish brown dots along basal half, pale yellow along dorsal margin. Legs greyish white dorsally, greyish black ventrally, mid tibia with purplish red scales.

Forewing in female greyish yellow from base to antemedian line, from antemedian line to subterminal line yellow with dense reddish brown scales, from subterminal line to apex purplish red; costal margin straight, interrupted with short blackish brown and yellow streaks. Other characters as in male.

Male genitalia (Fig. 12). Uncus broad at base, slightly narrowing to 3/4, then broadened to blunt apex; uncus arm broad earlike, extending outwards; uncus processes more or less narrow triangular, set at distal 1/4. Gnathos somewhat racket-shaped, rounded at apex; arm extremely narrow-banded. Valva nearly rectangular, truncate apically, right-angled ventroapically; costa protruding at about distal 1/3, bearing three long reflexed hairs; transtilla broad, uniformly narrow-band. Sacculus wide basally, tapering to a long rod-shaped distal process, apex slightly exceeding middle of valva. Vinculum broad; saccus short and broad, with rounded anterior margin. Juxta more or less oval; posterior margin concave to half length at middle, forming two thumb-shaped lateral lobes. Phallus relatively stout, about same length as sacculus; cornutus conspicuous, about 1/2 length of phallus, toothed; ductus ejaculation from basal 1/3.

Female genitalia (Fig. 17). Ovipositor nearly triangular, narrowly rounded caudally. Apophysis posterioris long and slender, about 2.3 times length of apophysis anterioris. Antrum weakly sclerotized, funnel-shaped; bursal ring heavily sclerotized, shorter than half length of antrum; ductus bursae membranous, extremely short. Corpus burase elongate rectangular, length about 3.5 times of width; signum small and rounded, placed medially.

Distribution (Map 1)**.** China (Hainan).

Etymology. The specific epithet is derived from the Latin *convexus*, meaning convex, referring to the forewing hump from costal 1/5 to 2/5.

Endotricha whalleyi Li, sp. n.

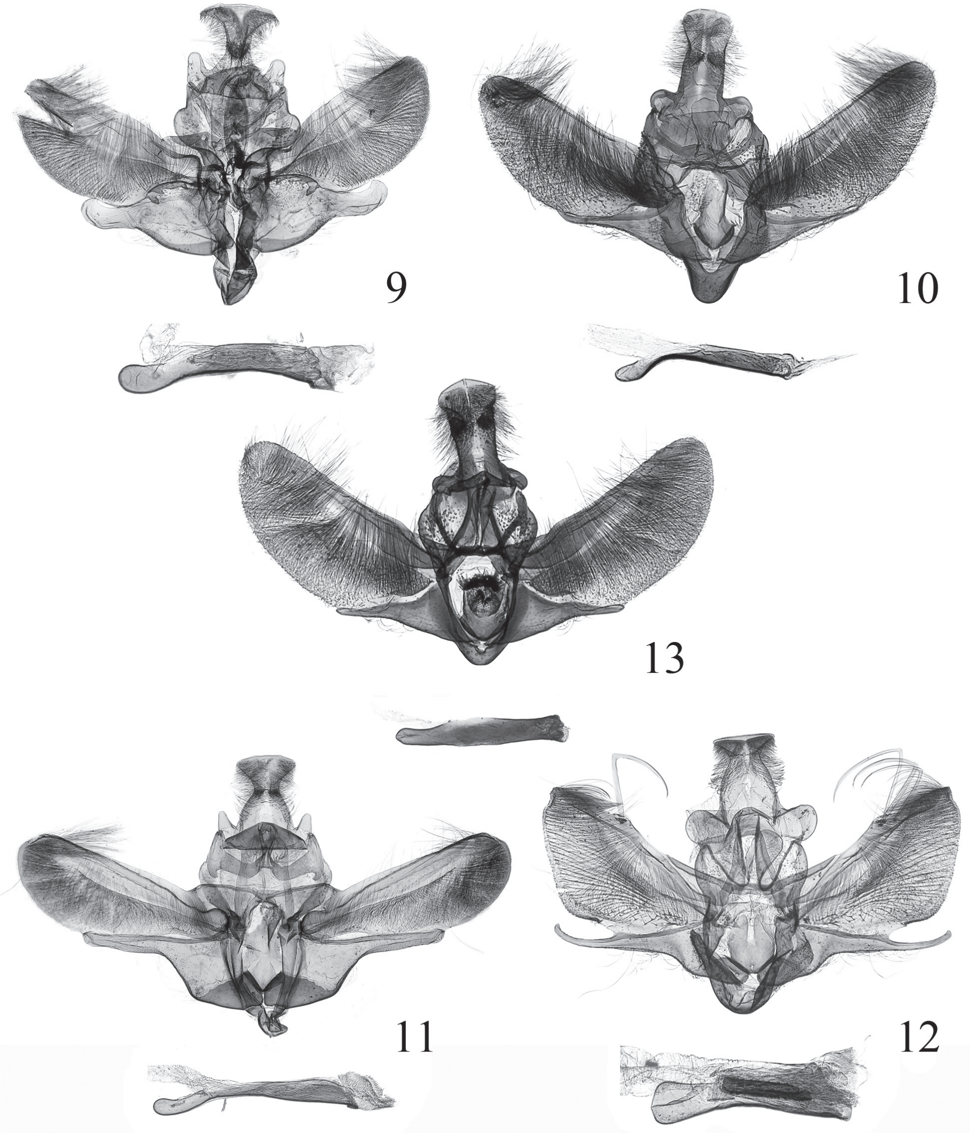
urn:lsid:zoobank.org:act:DB2596C2-912D-41C9-8CBD-79C2E3321647
http://species-id.net/wiki/Endotricha_whalleyi
Figs 5, 13

Type material. Holotype ♂ – **China, Xizang (Tibet) Autonomous Region:** Hanmi, Mêdog County (29°13'N, 95°18'E), 2380 m, 9.VIII.2003, coll. Xinpu Wang and Huaijun Xue, genitalia slide no. SYL11155. Paratypes: 1 ♂, same data as for holotype.

Figures 5–8. Adults of *Endotricha* spp. **5** *E. whalleyi* sp. n., paratype, male **6** *E. hoenei* Whalley, female **7** *E. luteogrisalis* Hampson, female **8** *E. simipunicea* Wang & Li, male.

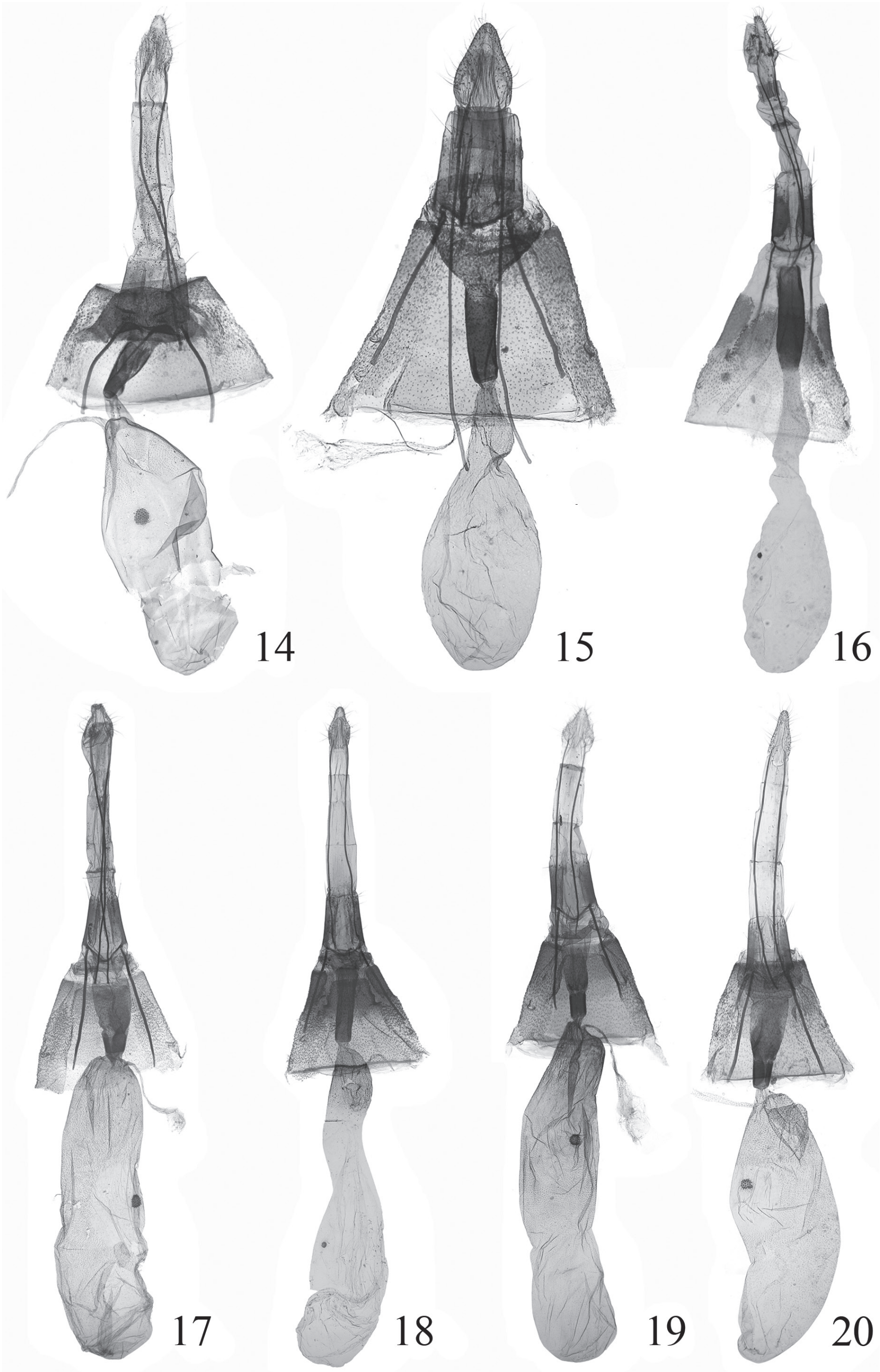
Diagnosis. This new species is superficially similar to *E. metacuralis* Hampson, 1916, but can be distinguished by the triangular uncus process placed more posteriorly, the elongate elliptical gnathos not reaching the base of uncus, the elongate triangular sacculus, and the columniform juxta having clustered strong distal spines. In *E. metacuralis* the nearly squared uncus process is placed medially, the larger racket-shaped gnathos reaches the base of uncus, the sacculus is semicircular, and the juxta has weak distal spines.

Description. Adult (Fig. 5): Wing expanse 16.0 mm. Head greyish brown. Antenna yellowish brown, with blackish brown dorsal annuli. Labial palpus greyish brown except third segment greyish white at apex. Thorax and tegula greyish brown. Forewing greyish brown, irrorated with black and wine reddish scales, almost wine red from postmedian line to termen, costal margin interrupted with yellowish white dots; antemedian line white, edged with black on inner margin posteriorly, extending from beyond costal 1/3 obliquely outward to near middle of cell, then inward to fold, and finally straight to dorsum; discal spot black, conspicuous; subterminal line white, edged with black on inner margin, extending inward from costal margin before apex to the point where M_2 and M_3 separated and a right angle formed, then obliquely straight to near tornus; fringe deep grey. Hindwing concolorous to forewing; antemedian line black, not well defined, inconspicuous anterioly; postmedian line thin, black,



Figures 9–13. Male genitalia of *Endotricha* spp. **9** *E. dentiprocessa* sp. n., holotype, slide no. SYL11029 **10** *E. unicolor* sp. n., holotype, slide no. SYL11147 **11** *E. shafferi* sp. n., holotype, slide no. SYL11094 **12** *E. convexa* sp. n., holotype, slide no. SYL11043 **13** *E. whalleyi* sp. n. holotype, slide no. SYL11155.

ill-defined, curved inward and forming a right-angle at distal 1/4 of 1A, then straight to dorsum; broad yellow fascia placed between ante- and postmedian lines, its posterior 1/4 much narrower, handle-shaped; termen interrupted with dark brown dots and short streaks; fringe greyish black basally, yellow distally, pale yellow along dorsal margin. Legs yellowish white on dorsal surface, blackish brown on ventral surface.



Figures 14–20. Female genitalia of spp. **14** *E. dentiprocessa* sp. n., paratype, slide no. SYL11030 **15** *E. unicolor* sp. n., paratype, slide no. SYL11003 **16** *E. shafferi* sp. n., paratype, slide no. SYL11024 **17** *E. convexa* sp. n., paratype, slide no. SYL11112 **18** *E. hoenei* Whalley, 1963, slide no. SYL11109 **19** *E. luteogrisalis* Hampson, 1896, slide no. SYL11099 **20** *E. simipunicea* Wang & Li, 2005, slide no. SYL11097.

Male genitalia (Fig. 13). Uncus rectangular, slightly widened before apex, length about 2.5 times width, caudal margin gently arched, naked; uncus arm rounded, stretching outward; uncus processes triangular, with short spines, located at about 3/5. Gnathos elongate elliptical, arm band-shaped. Valva slightly widened medially, distally narrowed to rounded apex; transtilla narrow and straight. Sacculus elongate triangular, produced to a long spine-shaped process, reaching beyond 1/3 of valva. Vinculum narrow; saccus short and broad, rounded anteriorly. Juxta columniform, with clustered stout spines distally. Phallus slender, longer than sacculus; ductus ejaculation from basal 1/4.

Female unknown.

Distribution (Map 1)**.** China (Tibet).

Etymology. This species is named after the late Paul E. S. Whalley for his contribution to the revision of the world *Endotricha* species.

Endotricha hoenei Whalley, 1963

http://species-id.net/wiki/Endotricha_hoenei

Figs 6, 18

Endotricha hoenei Whalley, 1963: 430. Holotype ♂, CHINA, Linping, Kwangtung, 18.5.22 (Höne), Brit. Mus. slide No. 6159, deposited in Höne coll, Bonn.

Material examined. 2 ♂, 11 ♀, **China, Fujian Province:** Xianfengling, Mt. Wuyi, 1000 m, 26.V.2004, Haili Yu; 1 ♂, 2 ♀, **China**, **Guangxi Zhuang Autonomous Region:** Yinsha Protection Station, Jinxiu County, 700 m, 27.IV.2008, leg. Hui Zhen and Li Zhang; 2 ♂, 6 ♀, Mt. Daming, 125 m, 20.v.2011, coll. Linlin Yang and Yinghui Mou.

Description. Female adult (Fig. 6) with wing expanse 19.0–21.0 mm.

Female genitalia (Fig. 18). Ovipositor with basal half nearly parallel laterally, distal half narrowed to rounded apex. Apophysis posterioris about 2.2 times length of apophysis anterioris. Antrum heavily sclerotized, uniformly same thickness, almost as long as apophysis anterioris; ductus bursae membranous, very short. Corpus burase elongate, length about five times of width; signum small and rounded, placed at about anterior 1/3.

Distribution. China (Fujian, Guangdong, Guangxi).

Remarks. The female of *Endotricha hoenei* Whalley, 1963 is described here for the first time.

Endotricha luteogrisalis Hampson, 1896

http://species-id.net/wiki/Endotricha_luteogrisalis

Figs 7, 19

Endotricha luteogrisalis Hampson 1896: 136; Whalley, 1963: 414. Holotype ♂, Bhutan, deposited in Natural History Museum London.

Material examined. 4 ♂, 11 ♀, **China, Fujian Province:** Sangang, Mt. Wuyi, 740 m, 25–27.VII.2008, coll. Weichun Li, Yongling Sun and Haiyan Bai; 1 ♀, **China, Hainan Province:** 70 m, 28.V.2007, coll. Zhiwei Zhang and Weichun Li; 1 ♂, Dong'er Work Station, Bawangling, 100 m, 9.IV.2008, coll. Bingbing Hu and Haiyan Bai.

Description. Female adult (Fig. 7) with wing expanse 16.5–20.0 mm.

Female genitalia (Fig. 19). Ovipositor nearly triangular, narrowly rounded caudally. Apophysis posterioris more than twice length of apophysis anterioris. Antrum weakly sclerotized, elongate funnel-shaped, about 3/4 length of apophysis anterioris; bursal ring conspicuous, slightly longer than antrum; ductus bursae membranous, very short. Corpus burase elongate rectangular, length four times of width, about 3.5 times as long as apophysis anterioris, posterior 1/5 spinous and granulous; signum rounded, placed at posterior 1/3.

Distribution. China (Fujian, Hainan, Jiangxi, Yunnan).

Remarks. The female of *Endotricha luteogrisalis* Hampson, 1896 is described here for the first time.

Endotricha simipunicea Wang & Li, 2005

http://species-id.net/wiki/Endotricha_simipunicea

Figs 8, 20

Endotricha simipunicea Wang and Li 2005: 304. Holotype ♂, Mt. Tianmu, Zhejiang Province, alt. 350 m, August 15, 1999, leg. Houhun Li, genitalia slide No. DYL00192, deposited in the Insect Collection, College of Life Sciences, Nankai University.

Material examined. 7 ♂, 3 ♀, **China, Fujian Province:** Sangang, Mt. Wuyi, 740 m, 25–27.VII.2008, coll. Weichun Li, Yongling Sun and Haiyan Bai; 1 ♂, **China, Guizhou Province:** Kuankuoshui Nature Reserves, Suiyang County, 1500 m, 14.VIII.2010-VIII-14, coll. Xicui Du.

Description. Female adult (Fig. 8) with wing expanse 13.5–15.0 mm.

Female genitalia (Fig. 20). Ovipositor narrow triangular, narrowly rounded caudally. Apophysis posterioris about 2.5 times length of apophysis anterioris. Antrum weakly sclerotized, funnel-shaped; bursal ring conspicuous, shorter than half length of antrum; ductus bursae membranous, very short. Corpus burase elongate oval, slightly narrowed anteriorly; signum small and rounded, toothed, placed at posterior 1/3; ductus seminalis arising from corpus bursae posteriorly.

Distribution. China (Fujian, Guizhou, Zhejiang).

Remarks. The female of *Endotricha luteogrisalis simipunicea* Wang & Li, 2005 is described for the first time.

Acknowledgments

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Description of a new species of coral-inhabiting barnacle, *Darwiniella angularis* sp. n. (Cirripedia, Pyrgomatidae) from Taiwan

Yi-Yang Chen[1,†], Hsiu-Chin Lin[2,‡], Benny K.K. Chan[1,2,§]

1 *Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei 106, Taiwan* **2** *Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan*

† *urn:lsid:zoobank.org:author:9F503C9A-CD40-427F-9572-88FBA501CE22*
‡ *urn:lsid:zoobank.org:author:31A34A01-3C15-473F-8817-4131F4802A6D*
§ *urn:lsid:zoobank.org:author:8C3C6E7D-96C0-4E60-BF2B-DF76E03CE0BD*

Corresponding author: *Benny K.K. Chan* (chankk@gate.sinica.edu.tw)

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Abstract

The present study has identified a new species from the previously monotypic genus *Darwiniella* Anderson, 1992. *Darwiniella angularis* **sp. n.** is similar to *D. conjugatum* (Darwin, 1854) in external shell morphology and arthropodal characters. *Darwiniella conjugatum*, however, has a sharper tergal spur and a less obvious adductor plate angle when compared to *D. angularis* **sp. n.** Molecular analyses on mitochondrial DNA 12S rDNA and COI regions also support the morphological differences. Sequence divergences in 12S rDNA and COI between *D. conjugatum* and *D. angularis* **sp. n.** are 5% and 13% respectively, which are equivalent to the inter-specific sequence divergences in other barnacles. Both *Darwiniella* species are common on *Cyphastrea* Milne-Edwards and Haime, 1848 corals and *D. angularis* **sp. n.** is also collected from *Astreopora* de Blainville, 1830 corals in Taiwan.

Keywords

Barnacles, Corals, Pyrgomatidae, cryptic diversity, Symbiosis, host-specificity

Introduction

The coral-inhabiting barnacles of the genus *Darwiniella* Anderson, 1992 is a member of the family Pyrgomatidae, which has a symbiotic relationship with host corals. *Darwiniella* was considered to be a monotypic genus, represented by *Darwiniella conjugatum* (Darwin, 1854).

Darwiniella conjugatum was originally described by Darwin (1854) as *Pyrgoma conjugatum*. The species has a fused shell wall and a pair of fused opercular plates (scutum and tergum). Ross and Newman (1973) erected the genus *Nobia* to accommodate coral-inhabiting barnacles with fused shell plates and fused opercular valves, thus transferred *P. conjugatum* to *Nobia conjugatum*. However, *Nobia conjugatum* has a deep adductor plate and a distinct tergal spur, which is different from all *Nobia* species. Subsequently, Anderson (1992) erected the new genus *Darwiniella* to accommodate *Nobia conjugatum* and defined the genus as " Wall flat, fused; sheath occupying about half inner wall; scutum and tergum calcified together, without visible line of juncture; deep adductor plate and conspicuous rostral tooth; elongate spur; basis oval, deep." However, Anderson (1992) had not formally assigned the type species of *Darwiniella* and Ross (1999) designated the type species of *Darwiniella* as *Darwiniella conjugatum* (Darwin, 1854).

In the present study, we collected a large number of *Darwiniella* specimens from coral reefs in Taiwan. Based on molecular analysis of the mitochondrial 12S rDNA (12S) and DNA barcode gene fragment (COI), we revealed two species of *Darwiniella*, *D. conjugatum* and an undescribed species. We used scanning electron microscopy (SEM) to examine the shell parts and light microscopy (LM) to examine the arthropodal characters of *D. conjugatum* and the *Darwiniella* sp. n. and both species were described herein from Taiwan.

Materials and methods

Specimen sampling and morphological analysis

Sampling of *Darwiniella* specimens was conducted at the main island of Taiwan (Suao and Kenting) and outlying islands (Turtle Island, Green Island and Siaolioucìou Island; see Fig. 1 and on-line Appendix 1: Table 1 for site and location details). Small pieces of coral with barnacles embedded were collected with hammers and chisels at 5–20 m depth by SCUBA diving. All barnacles and host corals were preserved in 95% EtOH. Type and paratype specimens are stored in the National Museum of Natural Science, Taichung, Taiwan (NMNS) and the Biodiversity Museum of the Academia Sinica, Taipei, Taiwan (ASIZCR), and additional specimens in the barnacle collections in the Coastal Ecology Laboratory, Academia Sinica, Taiwan (CEL). Barnacles were isolated from the host corals using forceps and the morphological characters of shell parts (shells, scutum and tergum) and somatic

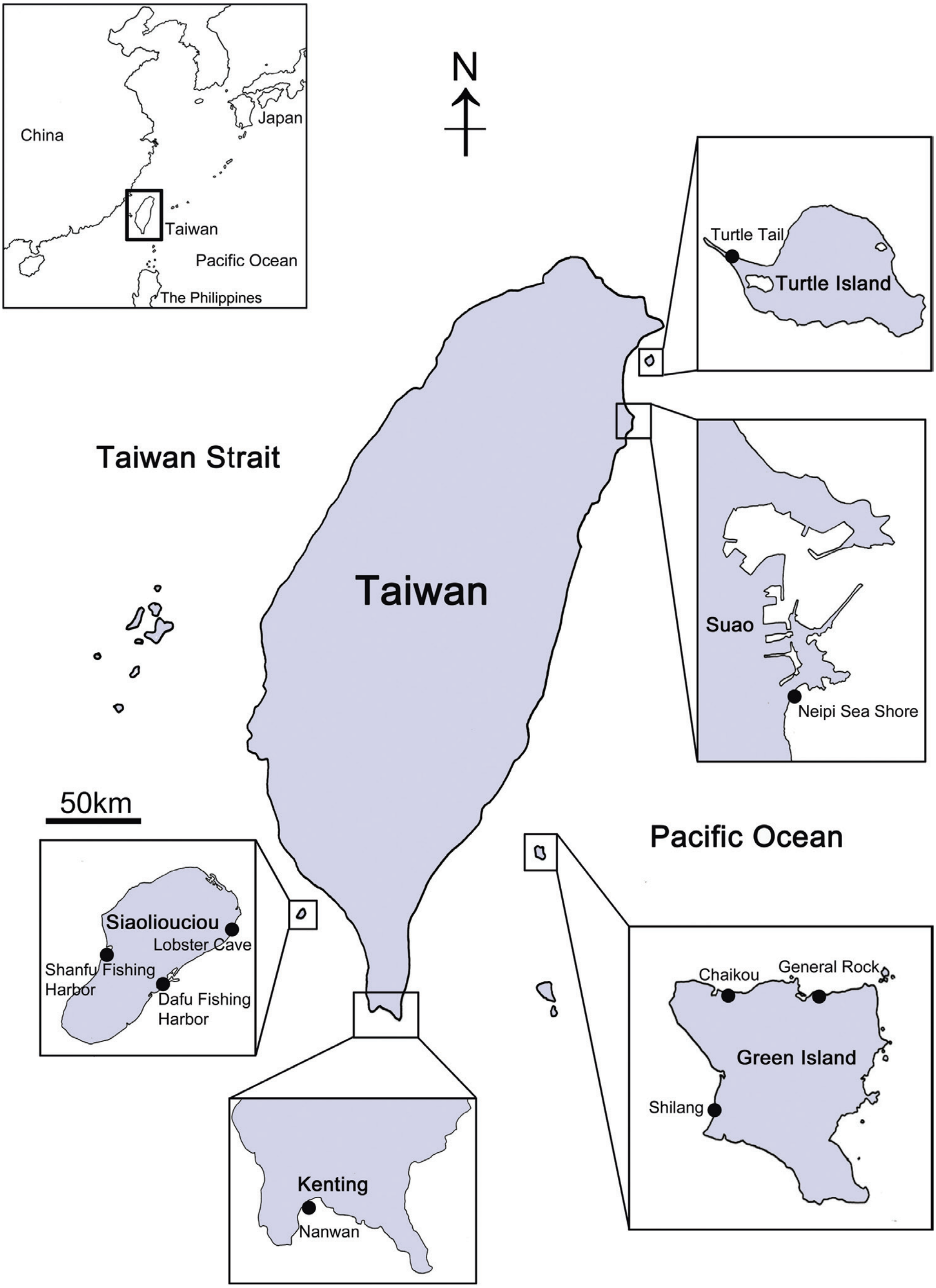

Figure 1. Collection sites of coral-inhabiting barnacle *Darwiniella* in Taiwan.

bodies (6 pairs of cirri, penis and oral cone) were examined. Organic debris and coral tissue on the surface of shells, scutum and tergum were removed with forceps and further cleaned ultrasonically (2–5 seconds). Cleaned shells and opercular valves (scutum and tergum) were immersed in 1.5% bleach for about five hours to completely digest organic tissue and the shells were rinsed by slow running purified water (30 minutes) and air-dried. The shells, scutum and tergum were gold coated and observed under SEM, following the methods of Achituv et al. (2009). Cirri, penis and oral cone were dissected from the somatic bodies and examined under LM (Zeiss Scope A1) with high definition lenses (Zeiss Plan APOChromat 40X/0.95 and ZEISS Plan APO Chromat 100x/1.4 oil), which allowed clear observation of setal types on cirri and mouth parts. Setal descriptions follow Chan et al. (2008).

Molecular analysis

Total genomic DNA was extracted from soft tissue of 110 *Darwiniella* specimens using Qiagen (Chatsworth, CA) QIAquick Tissue Kit following the manufacturer's instructions. Partial sequences of mitochondrial genes 12S rDNA (12S) and cytochrome c oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR) with primers 12S-FB and 12S-R2 (Tsang et al. 2009), and COI-F5 5' AAACCTATAGCCTTCAAAGCT 3' and COI-R4 5' GTATCHACRTCYATWCCTACHG 3', respectively. The PCR solution contained 40 ng of template DNA, 5 µl Taq DNA Polymerase Master Mix (1.5 mM $MgCl_2$; Ampliqon, Denmark), 1 µM of each primer, and ddH_2O with a final volume of 10 µl. The PCR reaction was conducted under the following conditions: 2 min at 95°C for initial denaturing, 35 cycles of 30 sec at 95°C, 1 min at 48°C, 1 min at 72°C with a final extension for 5 min at 72°C. The PCR products were then purified using the DNA Gel purification kit (Tri-I Biotech, Taipei, Taiwan). Direct sequencing of the purified PCR products was performed on an ABI 3730XL Genetic Analyzer with BigDye terminator cycle sequencing reagents (Applied Biosystems, Foster City, California, USA). Sequences were then aligned with CLUSTAL X (Thompson et al. 1997) using default settings and adjusted by eye in MacClade 4.07 (Maddison and Maddison 2005).

The genealogical relationships of specimens based on 12S and COI were inferred using K2P model and 1000-replicate Neighbor-Joining (NJ) method implemented in MEGA v5.05 (Tamura et al. 2011). Two specimens of coral barnacle *Hiroa stubbingsi* Ross & Newman, 1973 (GenBank accession numbers 12S: JQ946198, JQ946212; COI: JQ946237, JQ946244) were applied as the outgroup. The evolutionary distance (number of base differences per site) between sequence pairs were calculated with uncorrected p-distance and Kimura-2-parameter (K2P) models by MEGA.

Results

Systematics
Suborder Balanomorpha Pilsbry, 1916
Family Pyrgomatidae Gray, 1825
Subfamily Pyrgomatinae Gray, 1825
Genus *Darwiniella* Anderson, 1992

Darwiniella angularis sp. n.
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http://species-id.net/wiki/Darwiniella_angularis
Figures 2–9

Material examined. HOLOTYPE. NMNS-6878-001, Lobster Cave, Siaoliouciou Island, Taiwan (22°20'N, 120°23'E), August 2010, coll. B.K.K. Chan, on host coral *Astreopora* sp. PARATYPES. ASIZCR000202, Turtle Tail, Turtle Island, Taiwan (24°50'N, 121°56'E), October 2010, coll. B.K.K. Chan, on coral host *Cyphastrea chalcidicum* (Forskål, 1775). ASIZCR000204, data same as ASIZCR000202. CEL-RYU-13-2 and CEL-RYU-13-3 data same as holotype. CEL-TI-1-7, CEL-TI-5-6, and CEL-TI-9-10 data same as paratype ASIZCR000202.

Diagnosis. Scutum triangular, rostral tooth and obvious adductor plate present, basal margin of adductor plate forming obvious angle. Tergum trapezoid, spur triangular, medial furrow curved.

Description. (Type specimen: 6 mm in basal diameter, rostro-carinal diameter to 5.2 mm). Shell plates fully fused, purple, oval, externally surface with about 24 strip-like projections radiating from subcentral orifice to shell plate margin (Fig. 2A). Bases of shell with about 27 internal ribs radiating from rim of inner operculum (Fig. 2B). Orifice oval, long, narrow, about 1/3 length of rostro-carinal diameter.

Scutum and tergum white, fused without junctions (consistent through 3 specimens, Fig. 2C–H). Scutum triangular, width equal to height, occludent margin slightly curved, rostral tooth basally, 15 teeth along ventral surface of occludent margin, tooth size increasing gradually from apex to base. Ventral view with oval-shaped adductor muscle scar (Fig. 2D, F, H). Dorsal view with obvious adductor plate, convex, extending below basal margin of scutum, plate about 3/5 height of scutum, basal margin with obvious angle (Fig. 2C, E, G). Dorsal surface with horizontal striations, striations with row of small pores.

Tergum trapezoid, lateral depressor muscle crests present. Spur triangular, blunt, curved, height reaching more than 1/2 height of tergum, basal margin not obvious due to curved spur. Dorsal surface with medial furrow, curving from basal margin towards the carinal margin (Fig. 2C, E, G). Dorsal surface with horizontal striations, striations with row of small pores.

Maxilla bilobed (Fig. 3A), serrulate setae distally (Fig. 3C) and along inferior margin (Fig. 3B). Maxillule cutting edge straight, without notch, bearing row of 7 large

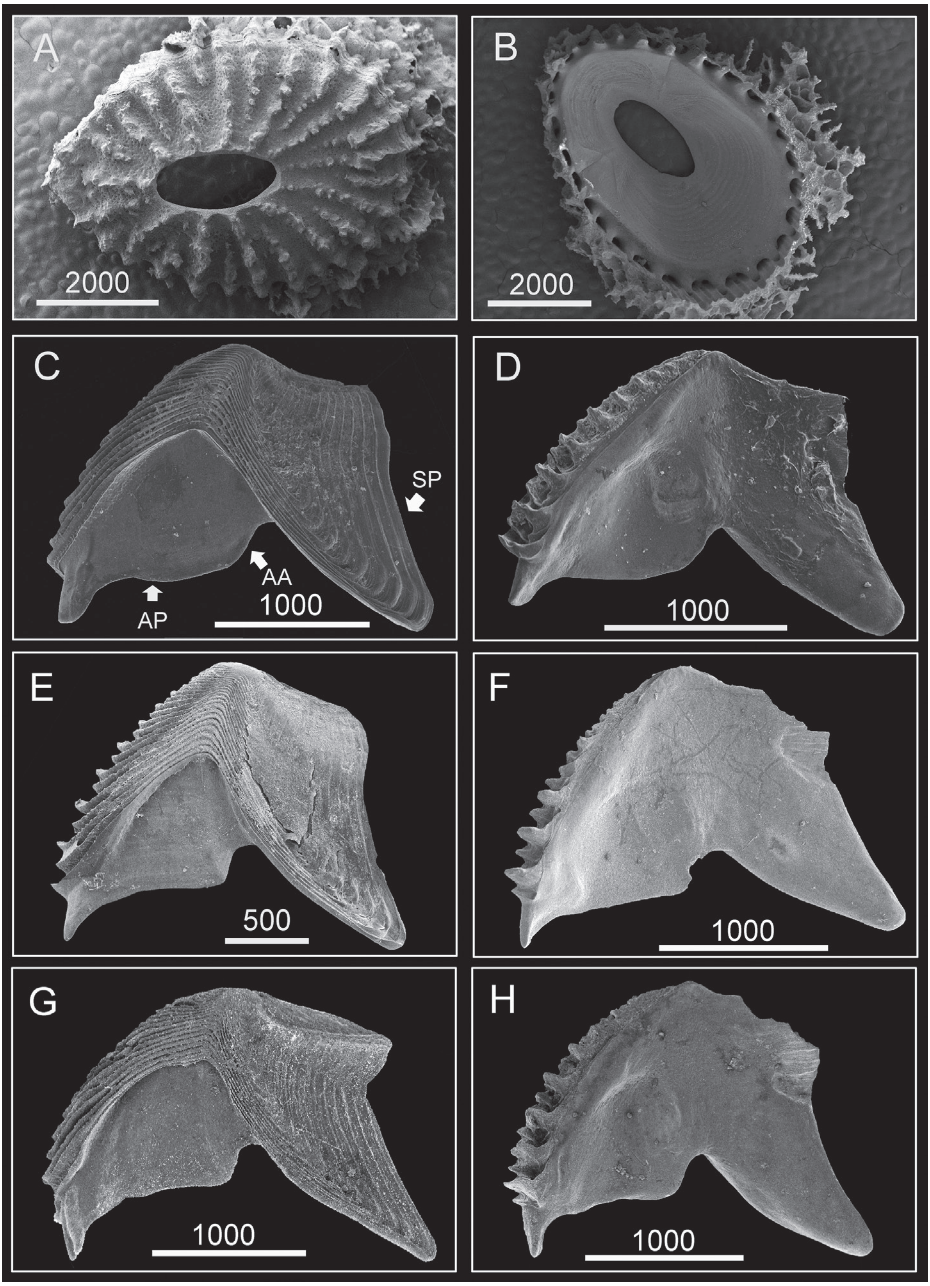

Figure 2. Hard parts of *Darwiniella angularis* sp. n. **A** Dorsal view of shell (NMNS-6878-001) **B** Ventral view of shell (NMNS-6878-001) **C** Dorsal view of fused scutum and tergum (NMNS-6878-001) **D** Ventral view of fused scutum and tergum (NMNS-6878-001) **E** Dorsal view of fused scutum and tergum (ASIZCR000203) **F** Ventral view of fused scutum and tergum (ASIZCR000203) **G** Dorsal view of fused scutum and tergum (additional individual, CEL-RYU-13-3) **H** Ventral view of fused scutum and tergum (additional individual, CEL-RYU-13-3). (AP: adductor plate, AA: adductor plate angle, SP: spur, scale bar: µm). Note the AA and SP of *D. angularis* sp. n. distinguish it from *D. conjugatum* (Darwin, 1854) (see Figure 10).

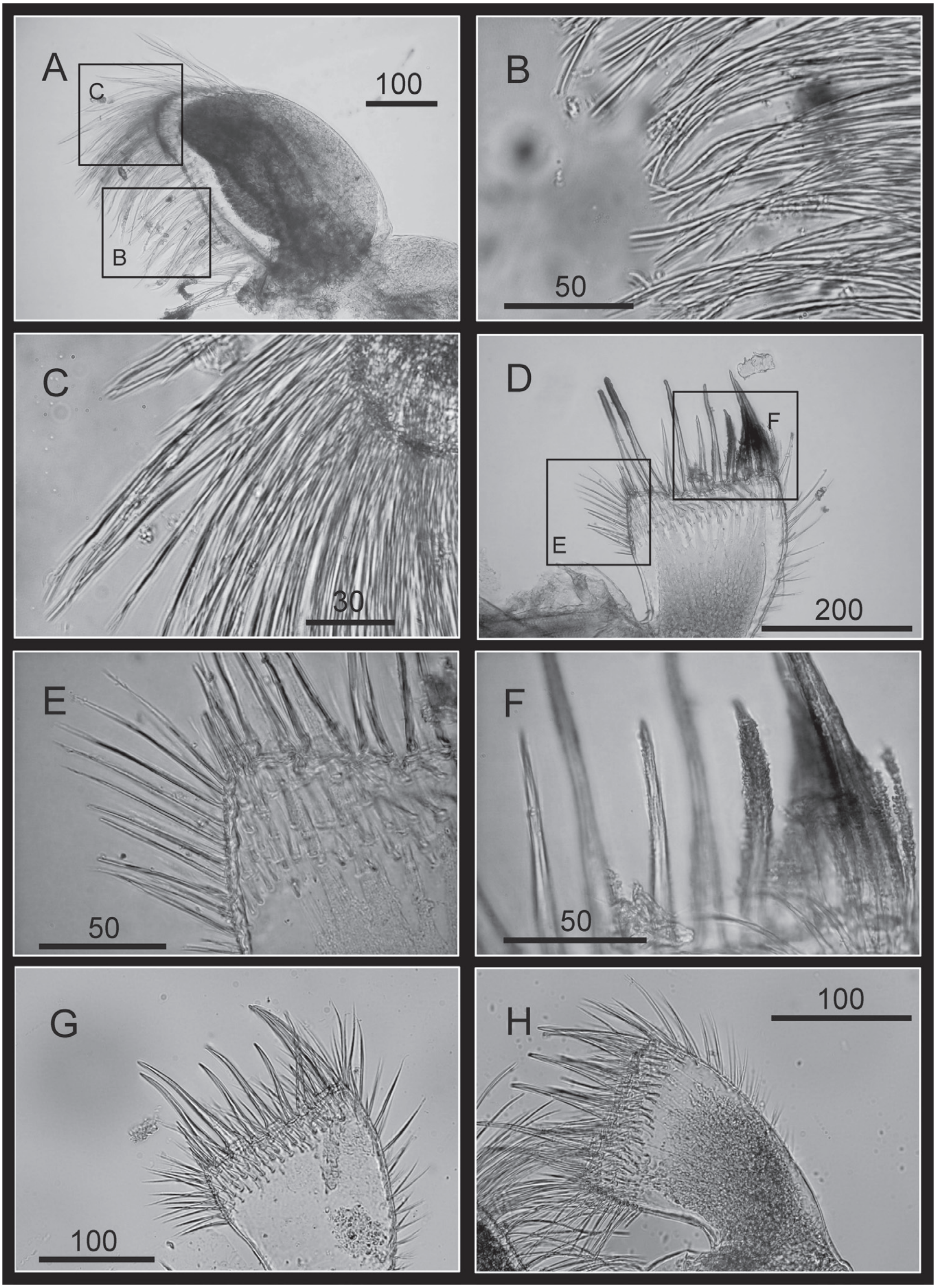

Figure 3. Oral cone of *Darwiniella angularis* sp. n. **A** Maxilla (NMNS-6878-001) **B** Serrulate setae on apex **C** Serrulate setae on inferior margin **D** Maxillule (NMNS-6878-001) **E** Simple setae on posterior margin **F** Large simple setae on cutting edge **G** Maxillule (additional specimen, ASIZCR000202) **H** Maxillule (additional specimen, CEL-TI-9-10). (scale bar: µm)

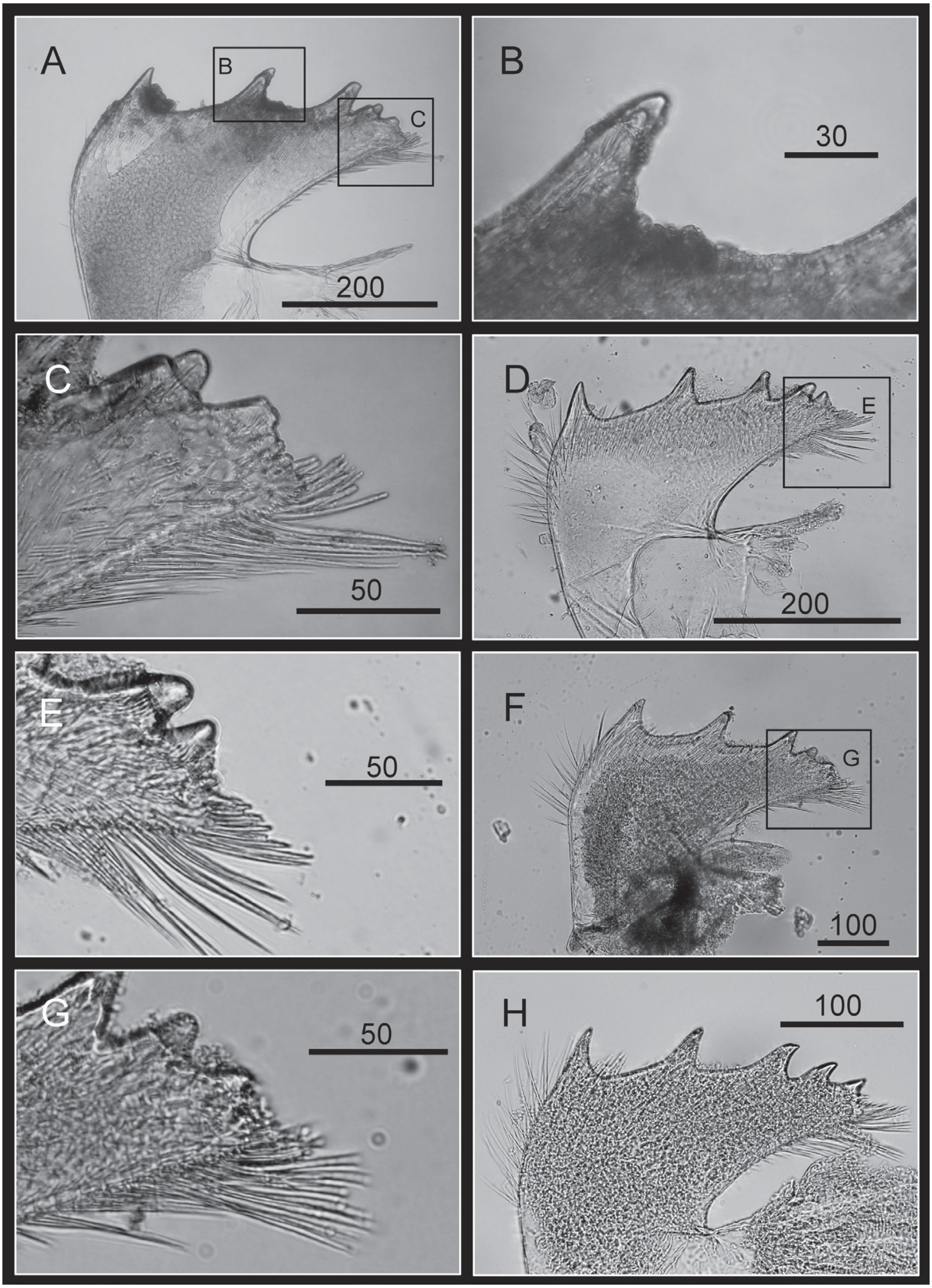

Figure 4. Oral cone of *Darwiniella angularis* sp. n. **A** Mandible (NMNS-6878-001) **B** Bidentate second tooth **C** Inferior angle with simple seta **D** Mandible (additional specimen, ASIZCR000202) **E** Inferior angle with simple seta **F** Mandible (additional specimen, CEL-TI-9-10) **G** Inferior angle with simple seta **H** Mandible (additional specimen, ASIZCR000204). (scale bar: μm)

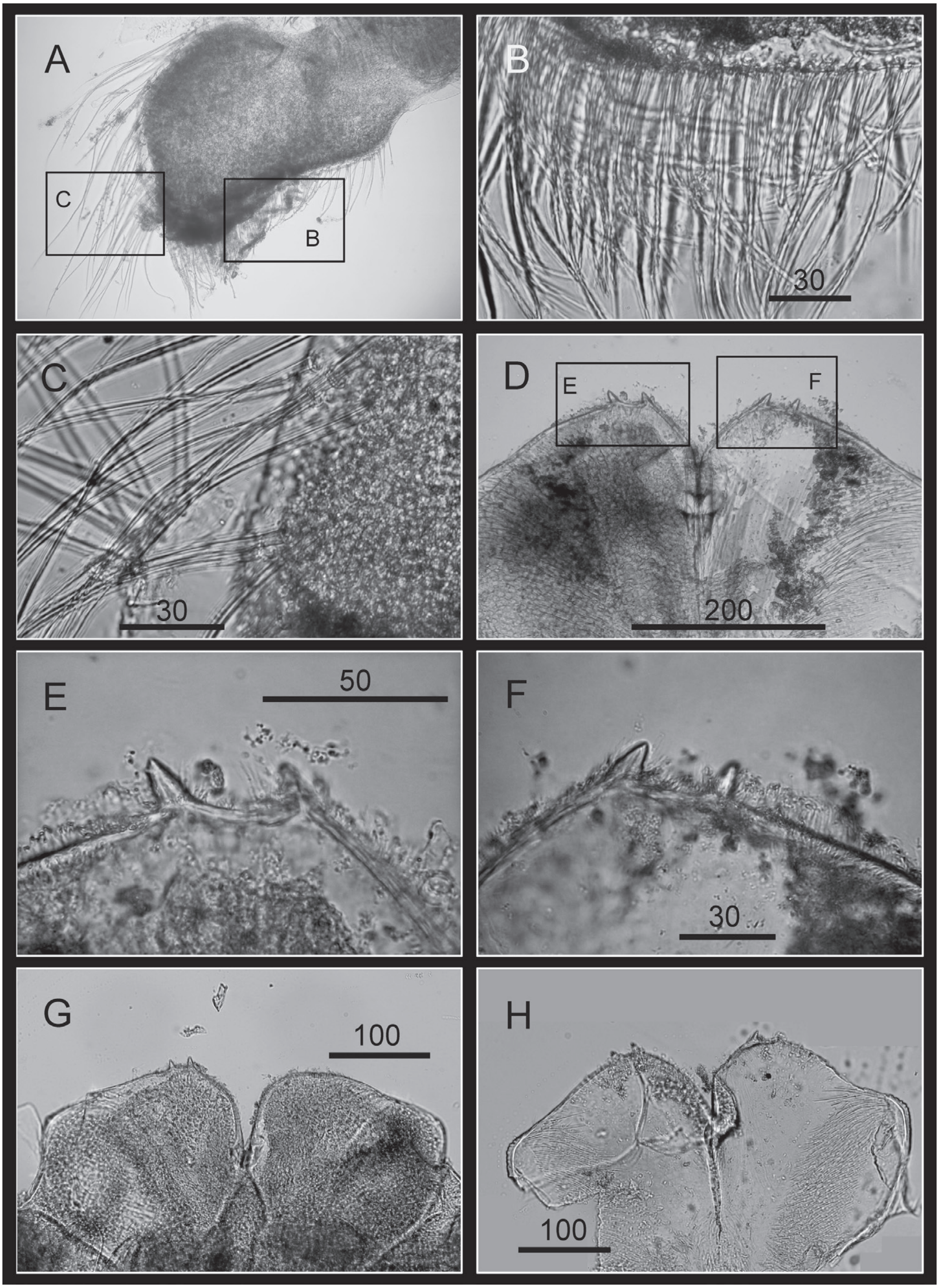

Figure 5. Oral cone of *Darwiniella angularis* sp. n. **A** Mandibular palp (NMNS-6878-001) **B** Serrulate setae on inferior margin **C** Serrulate setae distally **D** Labrum (NMNS-6878-001) **E** Teeth on labrum **F** Teeth on labrum **G** Labrum (additional specimen, CEL-TI-9-10) **H** Labrum (additional specimen, ASIZCR000202). (scale bar: µm)

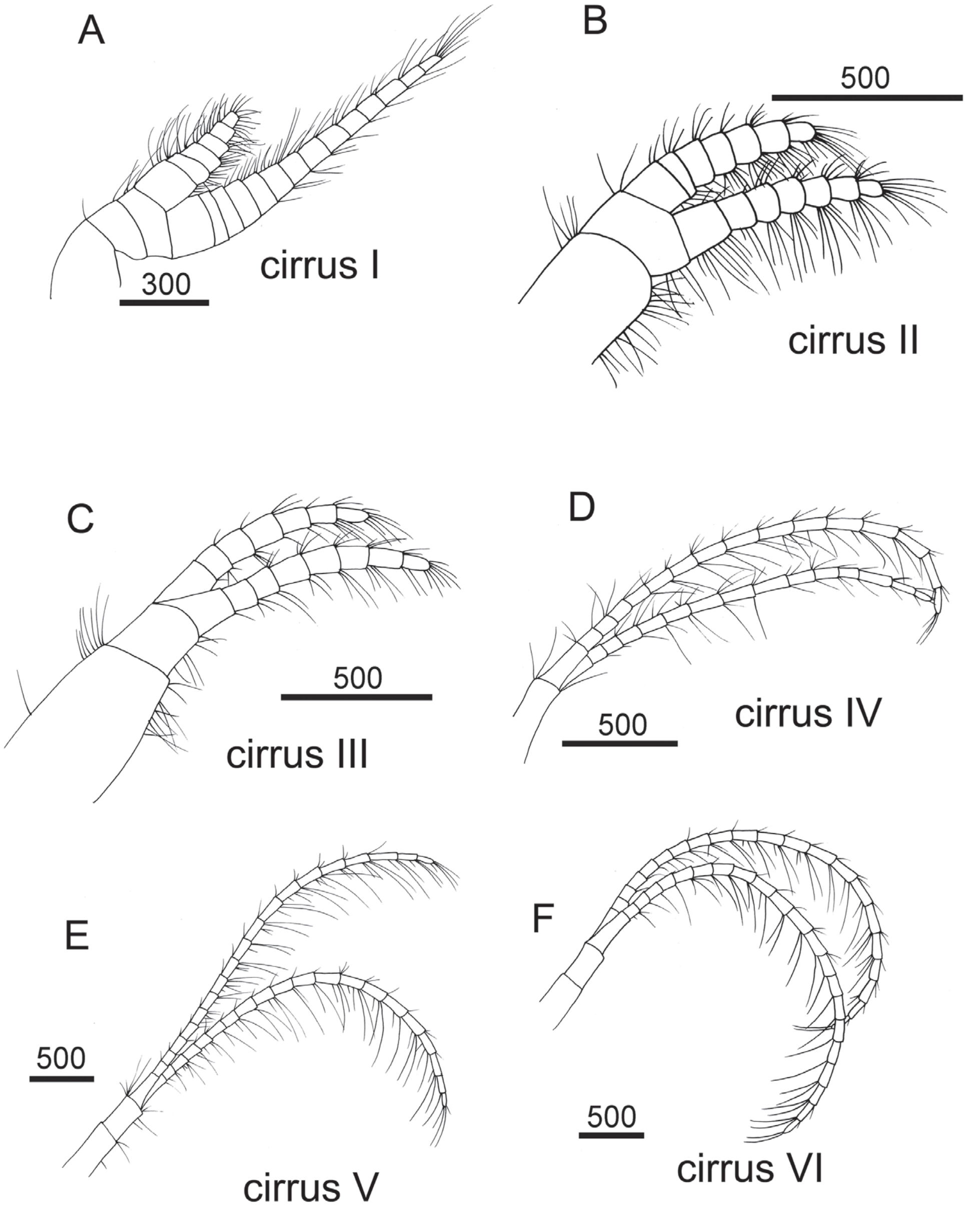

Figure 6. Line drawing of *Darwiniella angularis* sp. n. **A** Cirrus I **B** Cirrus II **C** Cirrus III **D** Cirrus IV **E** Cirrus V **F** Cirrus VI. (scale bar: μm)

and 3 smaller setae (Fig. 3D). Region close to cutting edge with dense fine simple setae (Fig. 3F), anterior and posterior margins with long simple setae (Fig. 3E). Mandible with 4 or 5 teeth, excluding inferior angle (inconsistent in 4 specimens, Fig. 4A, D, F, H). Second, third and fourth teeth bidentate (Fig. 4B), the first 3 teeth occupying most

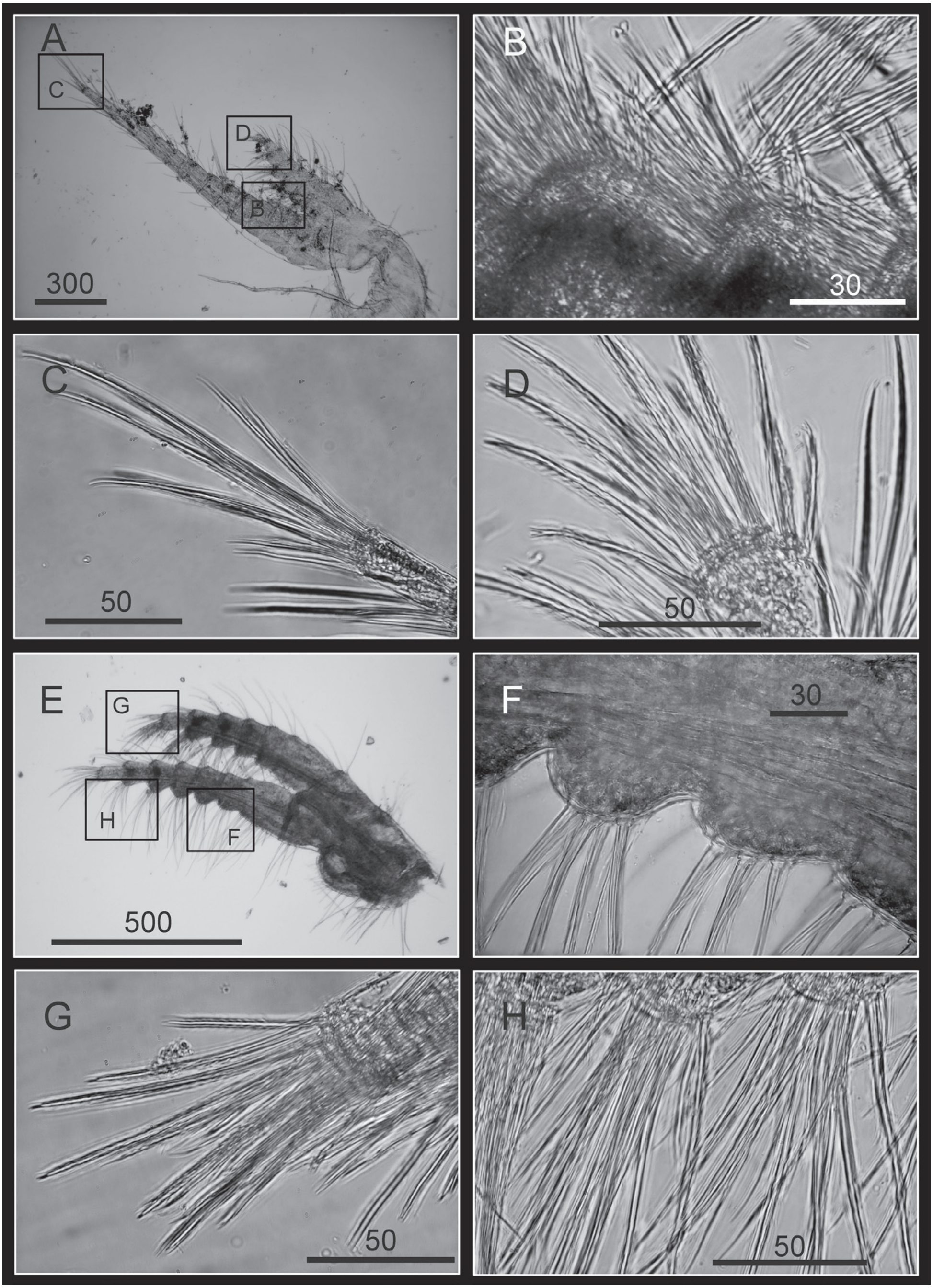

Figure 7. Cirri of *Darwiniella angularis* sp. n. **A** Cirrus I (NMNS-6878-001) **B** Serrulate setae on anterior ramus **C** Serrulate setae on anterior ramus apex **D** Serrulate setae on posterior ramus apex **E** Cirrus II (CEL-TI-1-7) **F** Serrulate setae on posterior ramus **G** Serrulate setae on anterior ramus apex **H** Serrulate setae on posterior ramus base. (scale bar: µm)

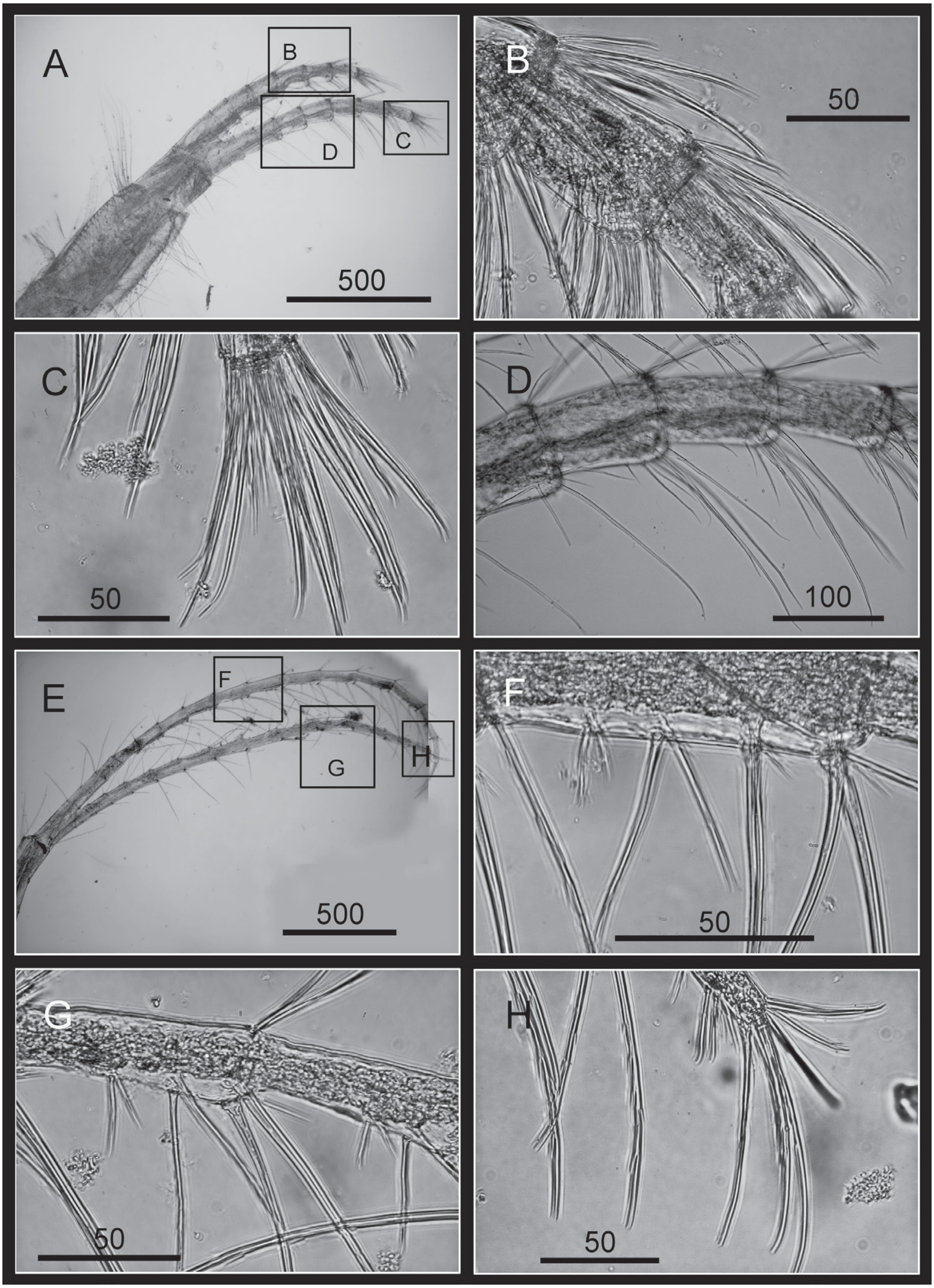

Figure 8. Cirri of *Darwiniella angularis* sp. n. **A** Cirrus III (NMNS-6878-001) **B** Serrulate setae on anterior ramus **C** Serrulate setae distally **D** Serrulate setae on posterior ramus **E** Cirrus IV (NMNS-6878-001) **F** Intermediate segment with 4 pairs of serrulate setae **G** Intermediate segment with serrulate setae **H** Serrulate setae distally. (scale bar: µm)

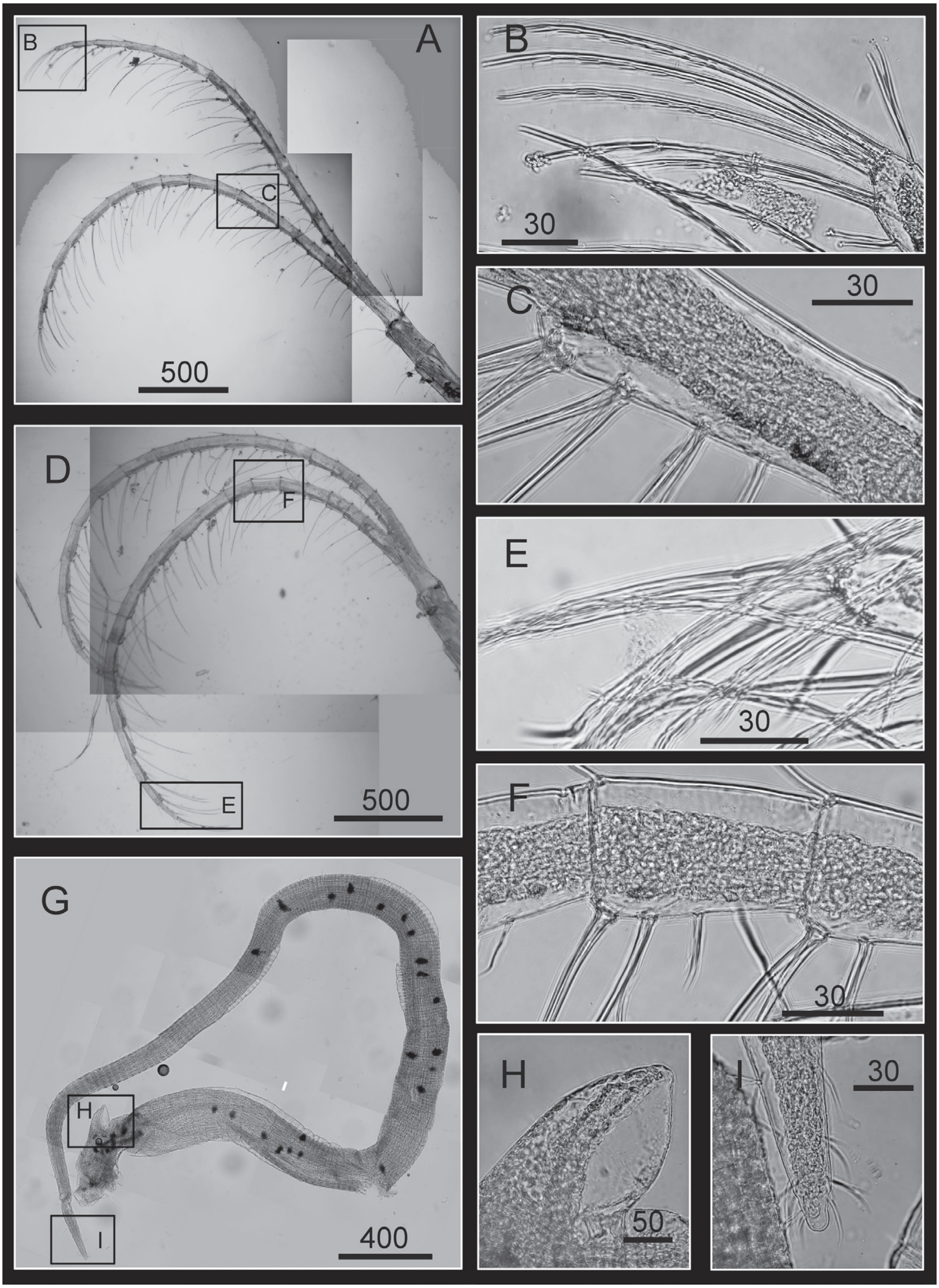

Figure 9. Cirri and penis of *Darwiniella angularis* sp. n. **A** Cirrus V (NMNS-6878-001) **B** Serrulate setae on apex **C** Intermediate segment with 4 pairs of serrulate setae **D** Cirrus VI (NMNS-6878-001) **E** Serrulate setae distally **F** Intermediate segment with 4 pairs of serrulate setae **G** Penis (CEL-TI-9-10) **H** Basi-dorsal point of penis **I** Apex of penis. (scale bar: μm)

of length of cutting edge. Lateral surface, lower margin and cutting edge of mandible bearing simple setae. Lower margin short, about 1/16 length of mandible, inferior angle ending in blunt angle with dense, fine setae (Fig. 4C, E, G). Mandibular palp rectangular, elongated (Fig. 5A), bearing serrulate setae distally (Fig. 5C) and inferior margin (Fig. 5B). Labrum bilobed, V-shaped notch between lobes, no or 2 sharp teeth on each side of notch (inconsistent in three specimens, Fig. 5D, G, H).

Cirrus I with rami unequal, anterior ramus long, slender, 14-segmented, posterior ramus 6-segmented (Figs 6A, 7A), bearing serrulate setae (Fig. 7B, C, D). Cirrus II with rami subequal, both 7-segmented (Figs 6B, 7E), bearing serrulate setae (Fig. 7F, G, H). Cirrus III with rami subequal, anterior ramus 8-segmented, posterior ramus 7-segmented (Figs 6C, 8A), bearing serrulate setae (Fig. 8B, C, D). Cirri IV–VI long, slender, rami equal. Cirrus IV with anterior ramus16-segmented, posterior 15-segmented, Cirrus V (anterior 21-segmented, posterior 19-segmented), Cirrus VI (anterior 22-segmented, posterior 21-segmented) (Figs 6D, E, F, 8E, 9A, D). Intermediate segments of Cirri IV–VI with 4 pairs of serrulate setae (Figs 8F, 9C, F), distal pair longest, proximal pair shortest.

Penis long (1.9 times length of Cirrus VI), annulated, some dark spots present, scattered short simple setae (Fig. 9G). Pedicel with basidorsal point (Fig. 9G, H), apex bearing short simple setae (Fig. 9I).

Distribution. At present only recorded from Taiwan.

Etymology. The name *angularis* denotes the presences of the obvious adductor plate angle, which is a diagnostic character of this species.

Remarks. External shell morphology and arthropodal characters of *D. angularis* sp. n. are similar to *D. conjugatum*. However, *D. angularis* has an obvious adductor plate angle, whereas that of *D. conjugatum* is less obvious (see description below). *Darwiniella conjugatum* also has a sharper spur angle than *D. angularis* - mean ± 1 SD of the spur angle (from 10 specimens) reaching 23.6 ± 4.8° in *D. conjugatum* and 32 ± 4.6° in *D. angularis*, which is significantly different between the two species using the t-test (t value: -4.3, df = 18, $p < 0.05$).

***Darwiniella conjugatum* (Darwin, 1854)**

http://species-id.net/wiki/Darwiniella_conjugatum
Figs 10–17

Pyrgoma conjugatum Darwin 1854: 364–365, pl. 12, fig. 7a–c.; Weltner 1897: 255; Gruvel 1905: 306–309; Annandale 1906: 143; Hoek 1913: 264; Broch 1922: 344; Hiro 1931: 154; Sakakura 1934: tab. II (not seen); Hiro 1935: 47, 59–60, 66, 69, fig. 8a–c; Hiro 1937: 468 (not seen); Nilsson-Cantell 1938: 13, 66, tab. III; Broch 1947: 7; Kolosváry 1947: 427; Utinomi 1949: 69 (not seen).

Pyrgoma (Nobia) conjugatum Baluk and Radwanski 1967: 487.

Nobia conjugatum Ross and Newman 1973: 155, fig. 12d–e; Newman and Ross 1976: 58; Foster 1982: 209, tab. 5, pl. 1G, fig. 7F; Soong and Chang 1983: 244–245, fig. 3; Galkin 1986: 1292; Ren 1986: 146, pl. IV, 15–18; Ogawa and Matsuzaki

1990: tab. I (not seen); Anderson 1992: 306–309, figs 20–21, 37F; Ogawa et al. 1998: 3, fig. 2; Jones et al. 2000: 276; Jones 2003: tabs 6, 8; Poltarukha and Dautova 2004: 111, fig. 66.

Nobia conjugata Ogawa and Matsuzaki 1992: app. tab.

Darwiniella conjugatum Anderson 1992: 329, figs 38F, 39; Simon-Blecher et al. 2007: tabs I–II.

Darwiniella conjugata Asami and Yamaguchi 1997: 13–14, figs 1–2.

Materials examined. CEL-RYU-28-1, Dafu Fishing Harbor, Siaoliouciou Island, Taiwan (22°20'N, 120°22'E), August 2010, coll. B.K.K. Chan, on coral host *Cyphastrea serailia* (Forskål, 1775). CEL-RYU-28-2 data same as CEL-RYU-28-1. CEL-RYU-38-4, Shanfu Fishing Harbor, Siaoliouciou Island, Taiwan (22°21'N, 120°21'E), August 2010, coll. B.K.K. Chan, on coral host *Cyphastrea serailia*. CEL-RYU-47-4 data same as CEL-RYU-38-4. CEL-RYU-66-1, Lobster Cave, Siaoliouciou Island, Taiwan (22°20'N, 120°23'E), August 2010, coll. B.K.K. Chan, on host coral *Cyphastrea* sp.. CEL-RYU-170-1, Shanfu Fishing Harbor, Siaoliouciou Island, Taiwan (22°21'N, 120°21'E), August 2010, coll. B.K.K. Chan, on coral host *Cyphastrea japonica*.

Diagnosis. Scutum subtriangular, rostral tooth and obvious adductor plate present. Adductor plate angle not obvious. Tergum subtriangular, lateral depressor muscle crests, medial furrow and spur present. Spur triangular, long, curved and sharp.

Description. Shell (8 mm in maximum basal diameter, rostro-carinal diameter to 6 mm) plates fully fused, purple, oval, externally surface with about 25 strip-like projections differing in length and radiating from nearly subcentral orifice to plate margin (Fig. 10A). Bases of shell with about 27 internal ribs radiating from the rim of the inner operculum to the basal margin of the shells (Fig. 10B). Orifice oval, long and narrow, about 3/8 length of rostro-carinal diameter.

Scutum and tergum white, fused without any junctions (Fig. 10C, D). Scutum subtriangular, width 1.5 times length of height, occludent margin slightly curved, with a rostral tooth on bottom (Fig. 10F) and continuous teeth along the ventral surface of occludent margin, tooth size increasing gradually from apex to base. Ventral view with an apparent oval-shaped adductor muscle scar (Fig. 10D). Dorsal view with obvious adductor plate, extending below basal margin, plate more than 1/2 height of scutum. Basal margin of adductor plate without obvious adductor plate angle (Fig. 10C). Dorsal surface of scutum with horizontal striations, striations with row of small pores (Fig. 10E).

Tergum subtriangular, lateral depressor muscle crests present. Spur triangular, long, curved and sharp, height reaching more than 1/2 height of tergum, basal margin not obvious due to the curved spur. Dorsal surface with medial furrow, curving from basal margin towards the carinal margin of tergum, width of furrow increased gradually from apex to base (Fig. 10C). Dorsal surface with horizontal striations, striations with row of small pores.

Maxilla oval (Fig. 11A), serrulate setae distally (Fig. 11B) and along inferior margin (Fig. 11C). Maxillule cutting edge straight, without notch, bearing row of 13 large, similar sized setae (consistent through 2 specimens, Fig. 11D, F, and incon-

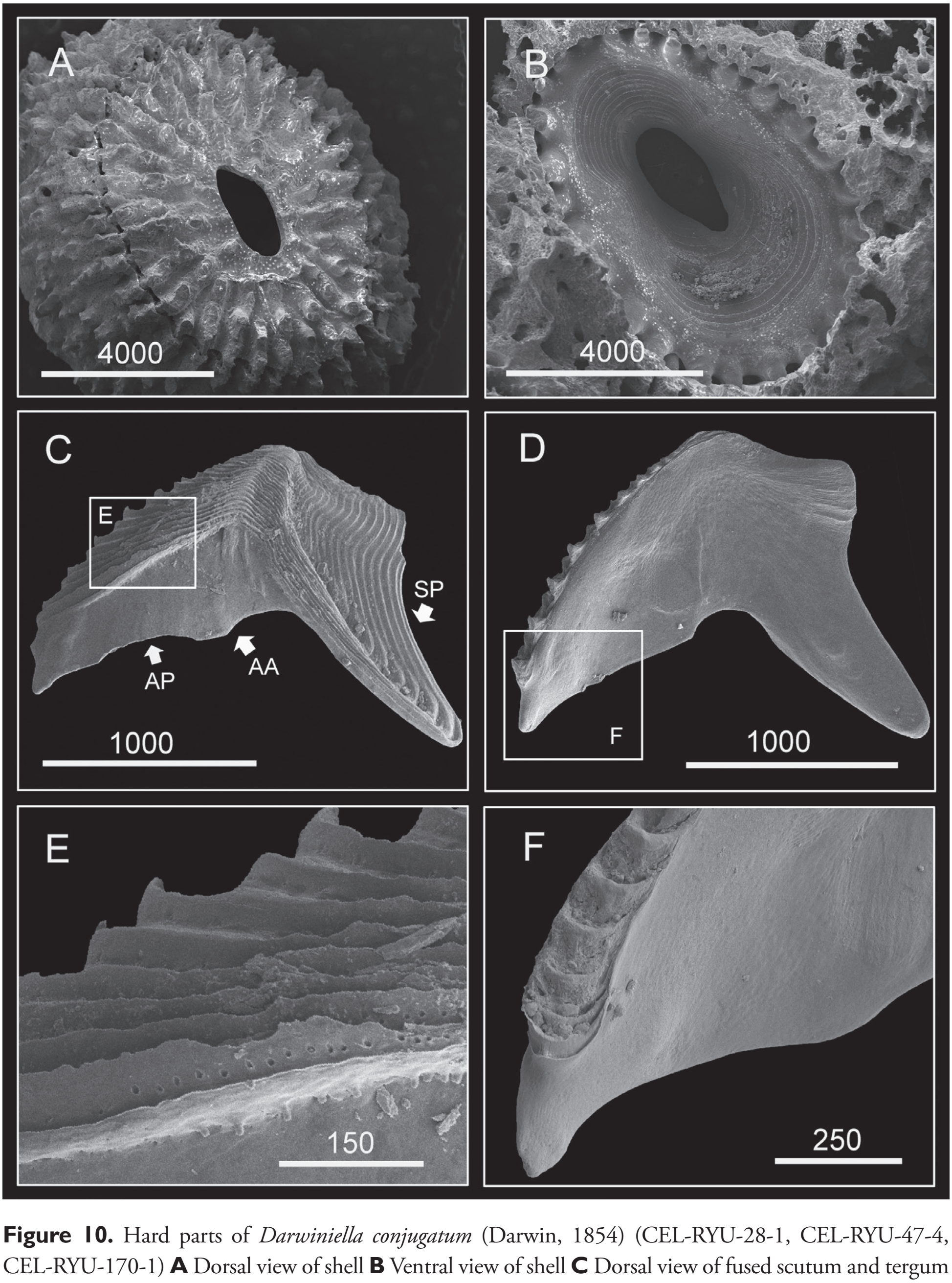

Figure 10. Hard parts of *Darwiniella conjugatum* (Darwin, 1854) (CEL-RYU-28-1, CEL-RYU-47-4, CEL-RYU-170-1) **A** Dorsal view of shell **B** Ventral view of shell **C** Dorsal view of fused scutum and tergum **D** Ventral view of fused scutum and tergum **E** Horizontal striations on dorsal surface of scutum **F** Occludent margin base and rostral tooth. (**AP**: adductor plate, **AA**: adductor plate angle, **SP**: spur, scale bar: μm)

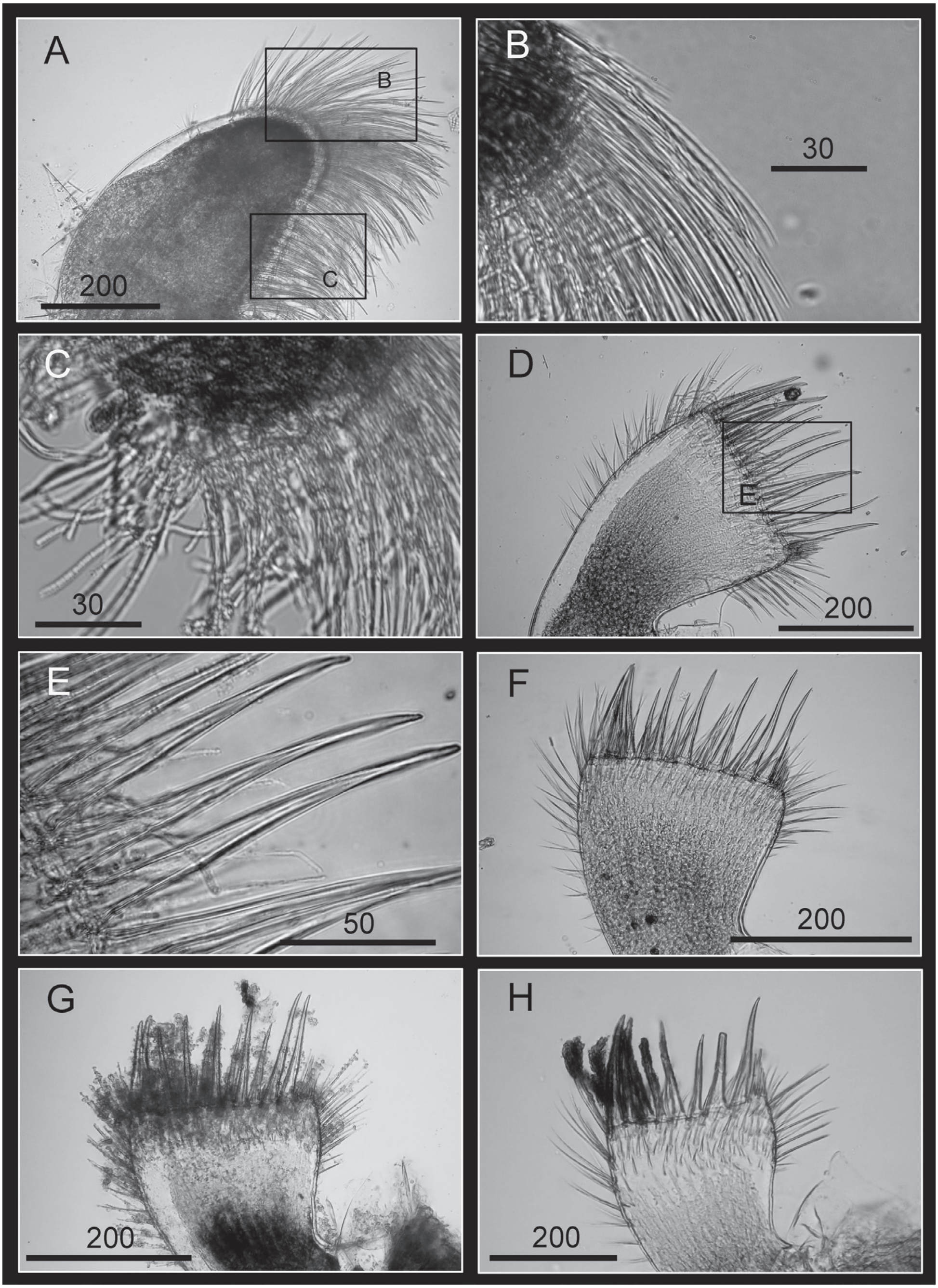

Figure 11. Oral cone of *Darwiniella conjugatum* (Darwin, 1854) **A** Maxilla (CEL-RYU-28-2) **B** Serrulate setae on apex **C** Serrulate setae on inferior margin **D** Maxillule (CEL-RYU-28-2) **E** Large simple type setae on cutting edge **F** Maxillule (additional specimen, CEL-RYU-28-1) **G** Maxillule (additional specimen, CEL-RYU-38-4) **H** Maxillule (additional specimen, CEL-RYU-66-1). (scale bar: μm)

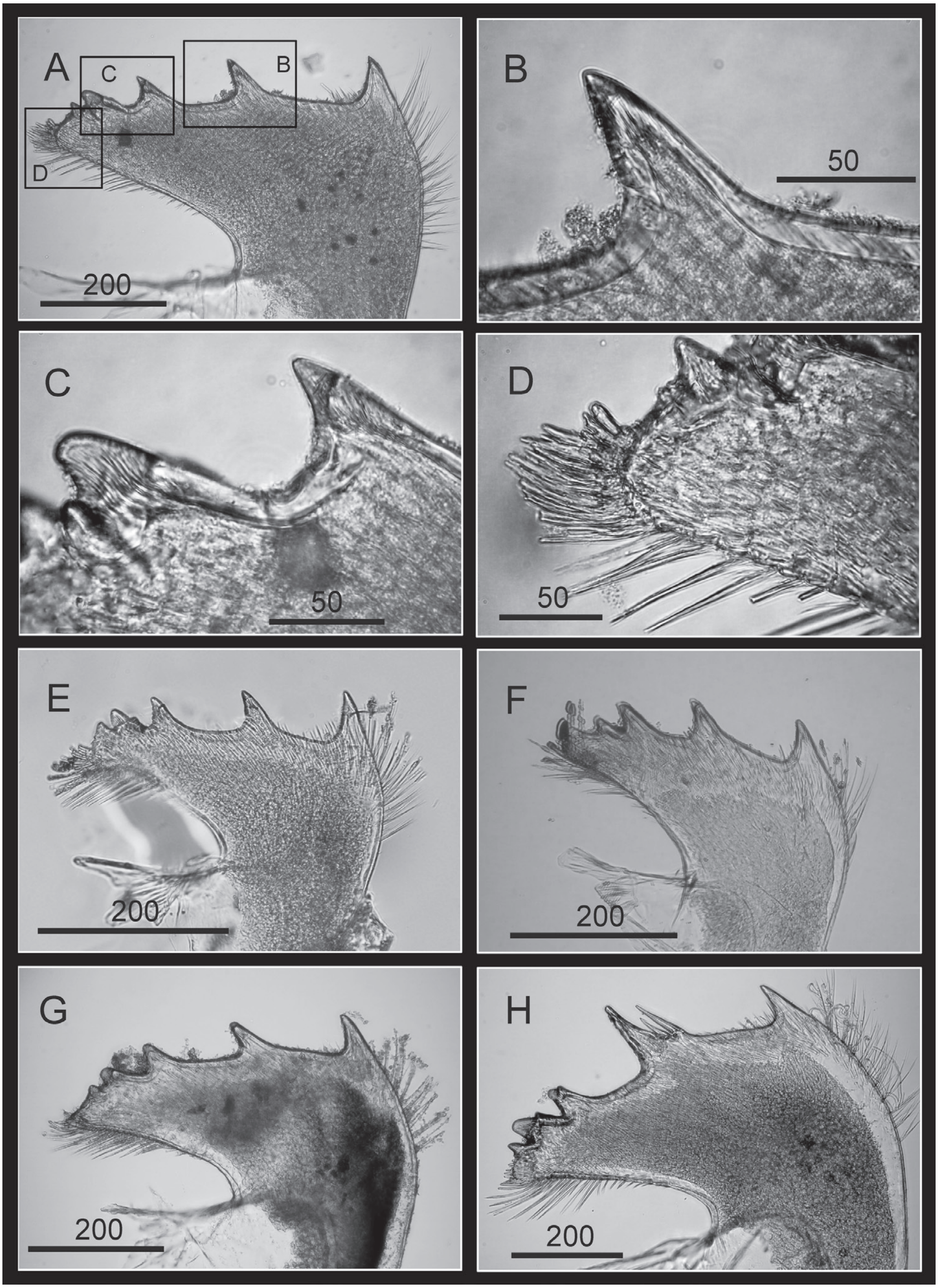

Figure 12. Oral cone of *Darwiniella conjugatum* (Darwin, 1854) **A** Mandible (CEL-RYU-28-1) **B** Bidentate second tooth **C** Bidentate third and fourth tooth **D** Inferior angle with simple type seta **E** Mandible (additional specimen, CEL-RYU-170-1) **F** Mandible (additional specimen, CEL-RYU-66-1) **G** Mandible (additional specimen, CEL-RYU-38-4) **H** Mandible (additional specimen, CEL-RYU-28-2). (scale bar: μm)

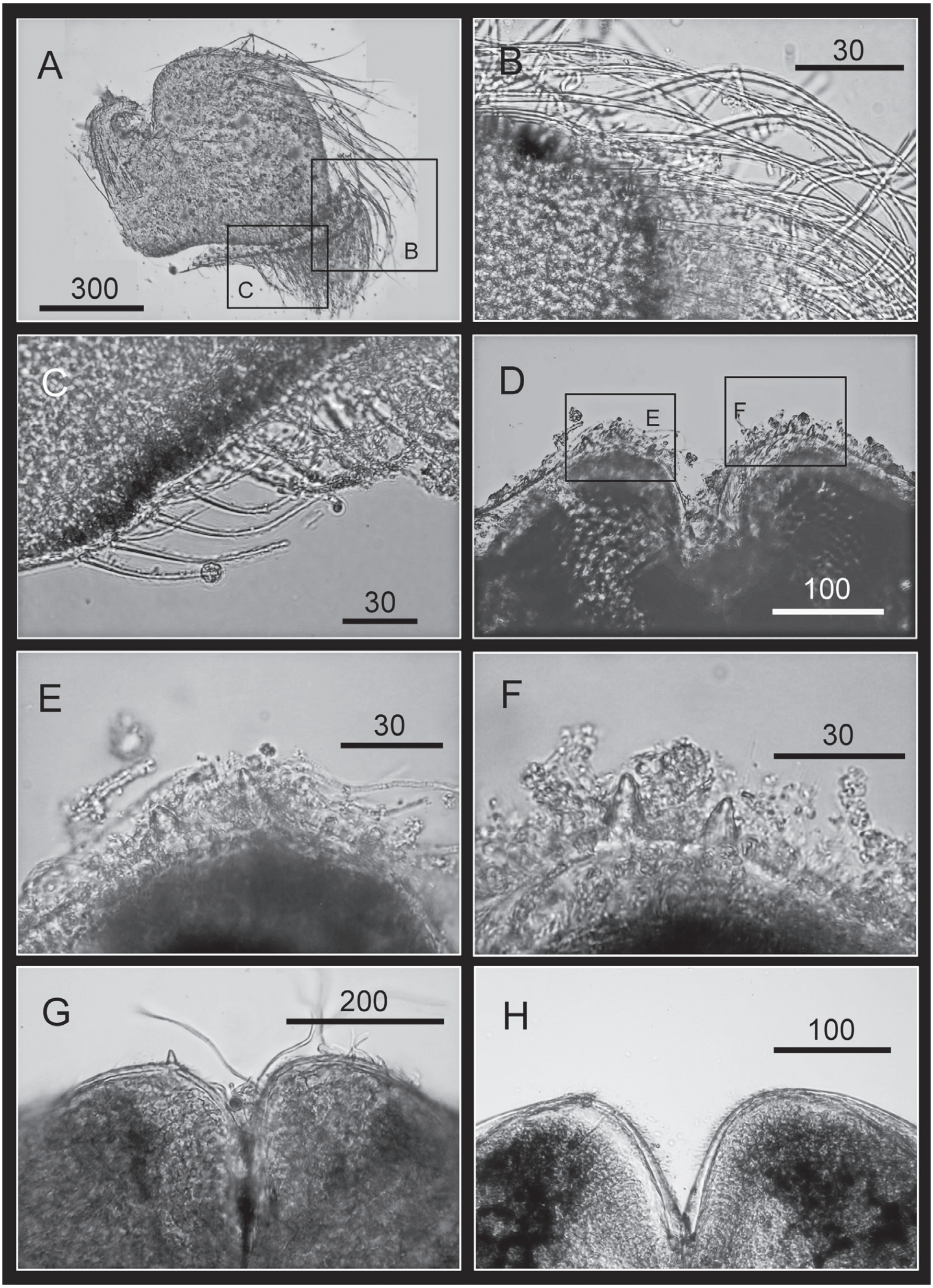

Figure 13. Oral cone of *Darwiniella conjugatum* (Darwin, 1854) **A** Mandibular palp (CEL-RYU-66-1) **B** Serrulate setae distally **C** Serrulate setae on inferior margin **D** Labrum (CEL-RYU-38-4) **E** Teeth on labrum **F** Teeth on labrum **G** Labrum (additional specimen, CEL-RYU-66-1) **H** Labrum (additional specimen, CEL-RYU-28-1). (scale bar: µm)

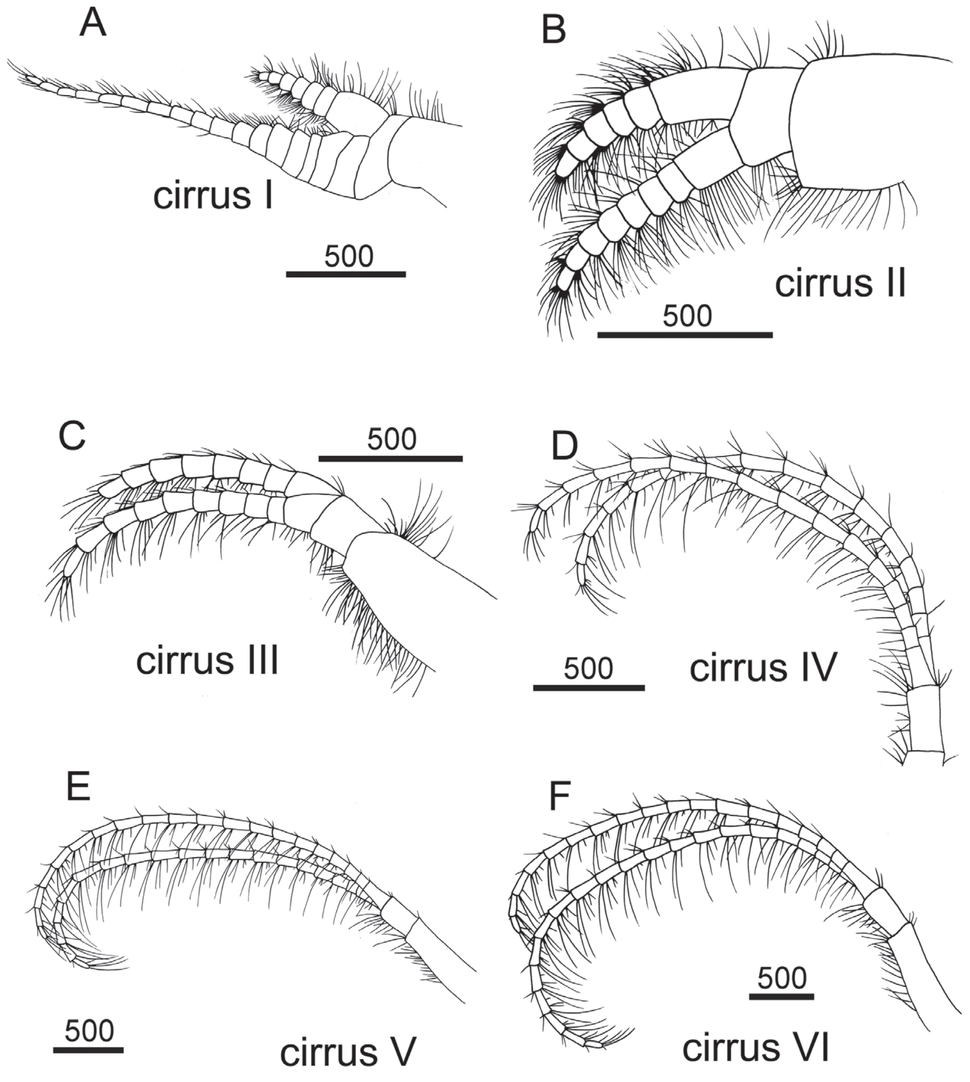

Figure 14. Line drawing of *Darwiniella conjugatum* (Darwin, 1854) **A** Cirrus I **B** Cirrus II **C** Cirrus III **D** Cirrus IV **E** Cirrus V **F** Cirrus VI. (scale bar: μm).

sistent through 2 specimens with 11 and 9 large setae, Fig. 11G, H, respectively). Region close to cutting edge with dense fine simple type setae (Fig. 11E), anterior and posterior margin with long simple type setae. Mandible with five teeth (excluding inferior angle) (teeth number consistent in five specimens but differed in morphologies, Fig. 12A, E, F, G, H). Second, third, fourth and fifth teeth bidentated (Fig. 12B, C), the first 4 teeth occupying most of length of cutting edge. Lateral surface, lower margin and cutting edge of mandible bearing simple type setae. Lower margin short, about 1/20 length of total length of mandible, inferior angle ending in a blunt angle

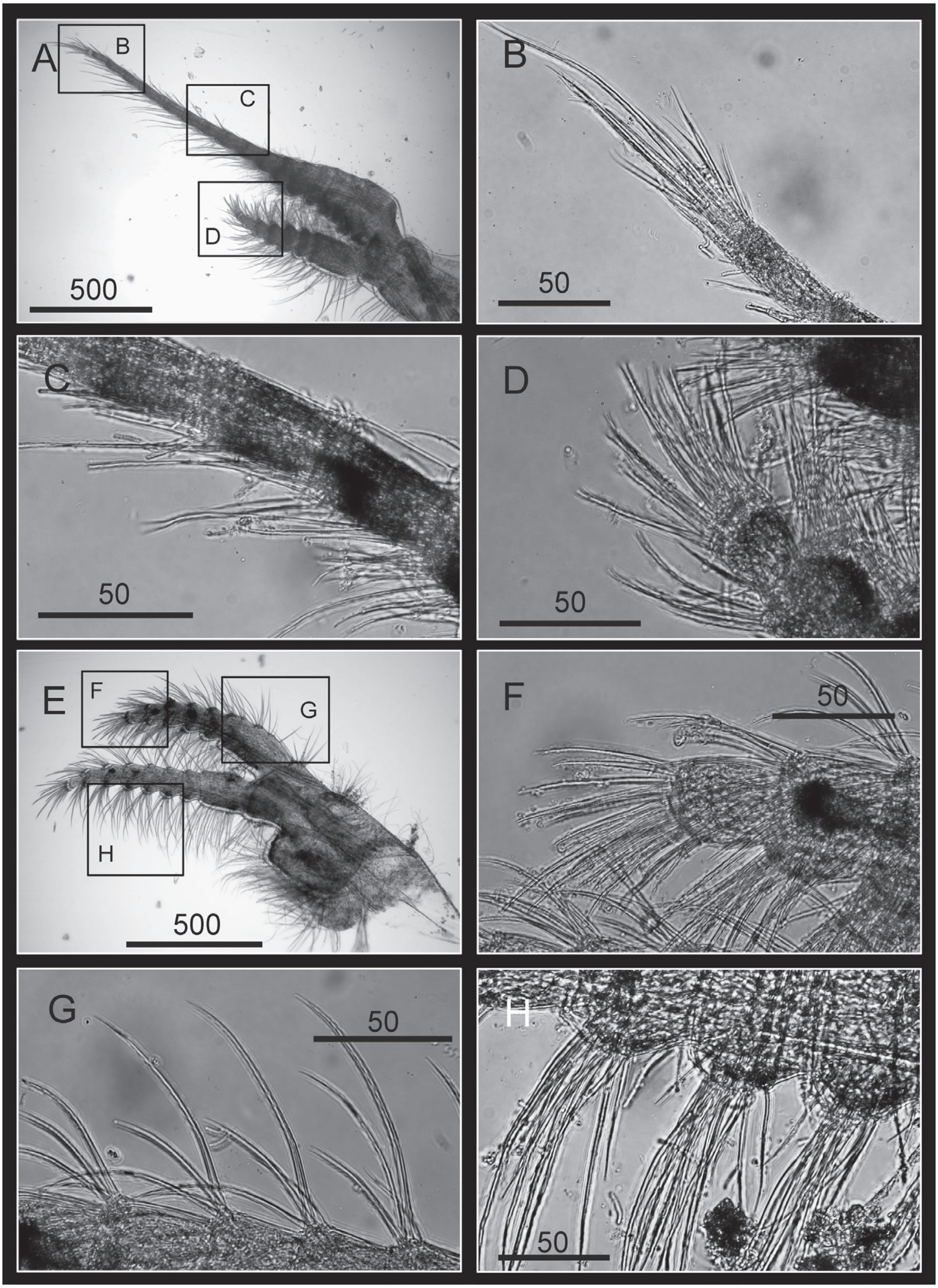

Figure 15. Cirri of *Darwiniella conjugatum* (Darwin, 1854) **A** Cirrus I (CEL-RYU-28-2) **B** Serrulate setae on anterior ramus apex **C** Serrulate setae on anterior ramus **D** Serrulate setae on posterior ramus apex **E** Cirrus II (CEL-RYU-28-2) **F** Serrulate setae on anterior ramus apex **G** Serrulate setae on anterior ramus **H** Serrulate setae on posterior ramus apex. (scale bar: μm)

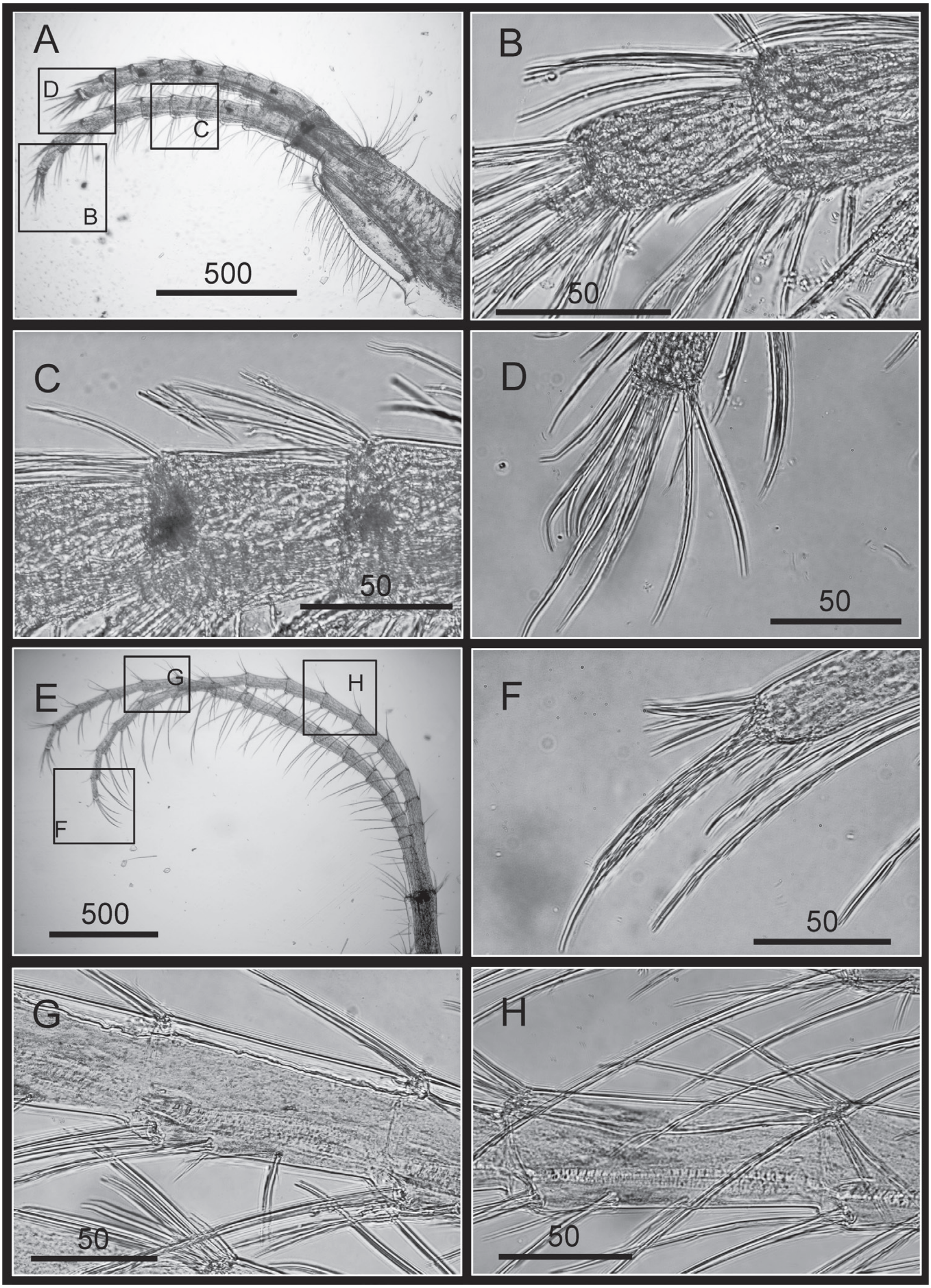

Figure 16. Cirri of *Darwiniella conjugatum* (Darwin, 1854) **A** Cirrus III (CEL-RYU-28-2) **B** Serrulate setae on posterior ramus **C** Serrulate setae on posterior ramus **D** Serrulate setae distally **E** Cirrus IV (CEL-RYU-28-2) **F** Serrulate setae on apex **G** Intermediate segment with 4 pairs of serrulate setae **H** Intermediate segment with serrulate setae. (scale bar: µm)

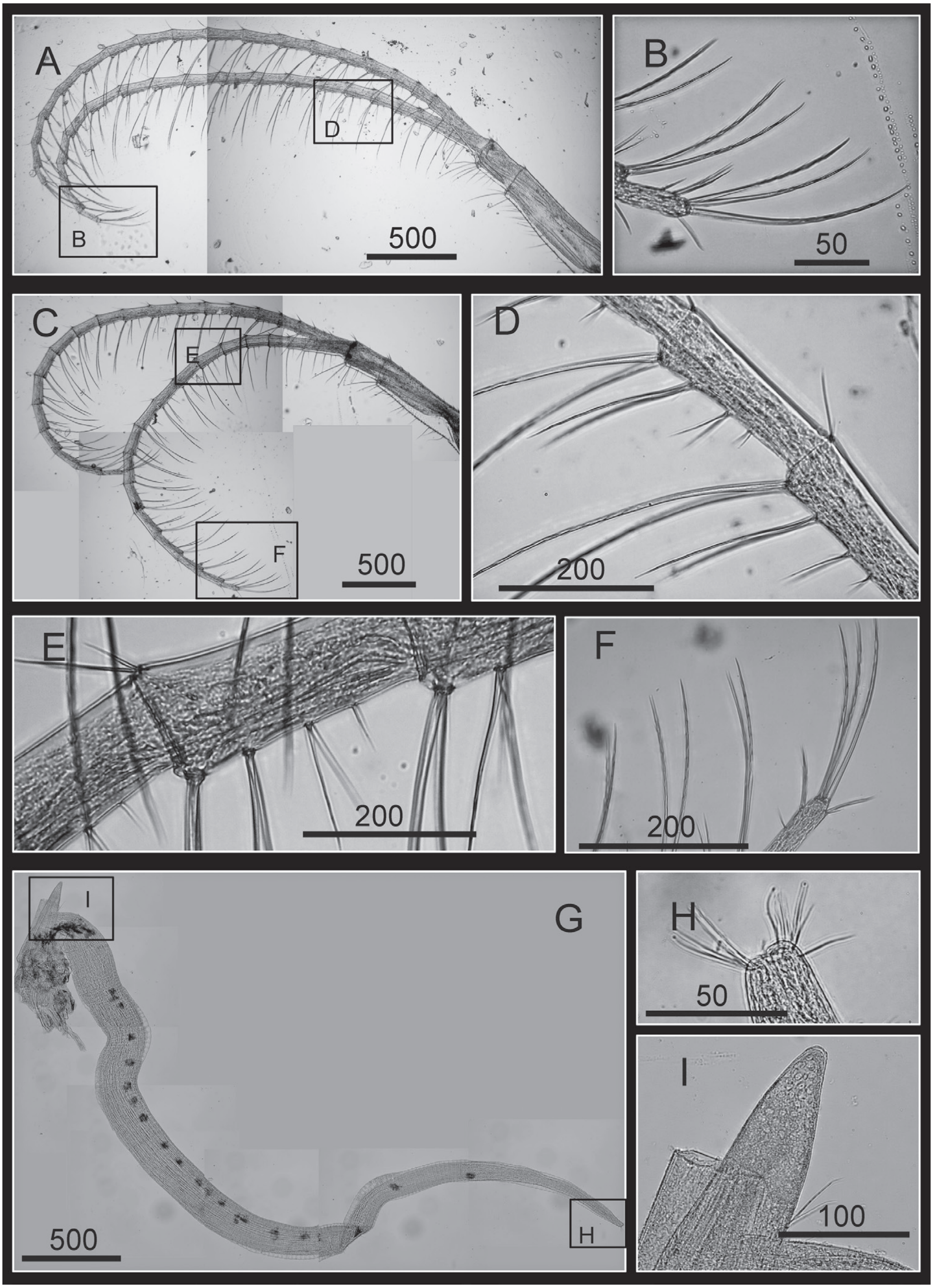

Figure 17. Cirri and penis of *Darwiniella conjugatum* (Darwin, 1854) **A** Cirrus V (CEL-RYU-28-2) **B** Serrulate setae on apex **C** Cirrus VI (CEL-RYU-28-2) **D** Intermediate segment with 4 pairs of serrulate setae **E** Intermediate segment with 4 pairs of serrulate setae **F** Serrulate setae on apex **G** Penis (CEL-RYU-170-1) **H** apex of penis **I** Basidorsal point of penis. (scale bar: μm)

with dense, fine setae (Fig. 12D). Mandibular palp rectangular, elongated (Fig. 13A), bearing serrulate setae distally (Fig. 13B) and on inferior margin (Fig. 13C). Labrum bilobed, with V-shaped notch between 2 lobes, 2 sharp teeth on each side of notch (inconsistent in three specimens, Fig. 13D, G, H).

Cirrus I with rami unequal, anterior ramus long and slender, with 17-segmented, posterior ramus 7-segmented (Figs 14A, 15A), bearing serrulate setae (Fig. 15B, C, D), dark spots exist on each segment of the ramus (Fig. 15A). Cirrus II with rami almost equal, anterior ramus 8-segmented and posterior ramus 6-segmented (Figs 14B, 15E), bearing serrulate setae (Fig. 15F, G, H), dark spots exist on each segment of the ramus (Fig. 15E). Cirrus III rami equal, anterior ramus 10-segmented, posterior ramus 8-segmented (Figs 14C, 16A), bearing serrulate setae (Fig. 16B, C, D), dark spots exist on each segment of the ramus (Fig. 16A). Cirrus IV–VI long, slender, with equal rami size. Number of segments on Cirrus IV (anterior 16, posterior 16), Cirrus V (21, 20), Cirrus VI (21, 18) (Figs 14D, E, F, 16E, 17A, C). Intermediate segments of Cirrus IV–VI with four pairs of serrulate setae (Figs 16G, 17D, E), distal pair longest, proximal pair shortest. Dark spots exist on each segment of the ramus (Figs 16E, 17A, C).

Penis long (1.2 times length of Cirrus VI), annulated, some dark spots present, scattered short simple type setae (Fig. 17G). Pedicel with basidorsal point (Fig. 17G, I), apex bearing short simple type setae (Fig. 17H).

Distribution. South China Sea: Singapore, Mainland China (Hong Kong), Vietnam (Nhatrang Bay). Pacific Ocean: Taiwan (Green Island, Turtle Island, Siaoliouciou Island, Kenting, Suao), Japan, Australia (Western Australia & Great Barrier Reef), Philippine. Indian Ocean: Mauritius (Albion), Bay of Bengal, Thailand (Gulf of Siam), Sri Lanka, Red Sea.

Remarks. *D. conjugatum* is widely reported in the Indo-Pacific Ocean. Note Ogawa and Matsuzaki (1992) and Asami and Yamaguchi (1997) misspelled the species name *Darwiniella conjugatum* as '*conjugata*'.

Molecular analysis

After trimming and aligning the sequences, 472bp of 12S rDNA and 642bp of COI were obtained from 107 and 92 *Darwiniella* specimens without indels, respectively (see on-line Appendix 2: Table 2 for GenBank accession numbers, on-line Appendix 3: alignment Figure). The NJ inferred genealogical relationships based on 12S and COI were congruent to each other (Fig. 18). Both datasets showed the presence of two well-supported distinct lineages, corresponding to *D. conjugatum* and *D. angularis* sp. n.. In 12S, 37 out of the 69 variable nucleotide sites were parsimony informative. Evolutionary distances based on p-distance/K2P-distance were 0.004/0.004 and 0.006/0.006 within *D. conjugatum* and *D. angularis* sp. n., respectively, and 0.056/0.058 between the two species. In COI, 100 out of the 127 variable nucleotide sites were parsimony informative. Evolutionary distances based on p-distance/K2P-distance were

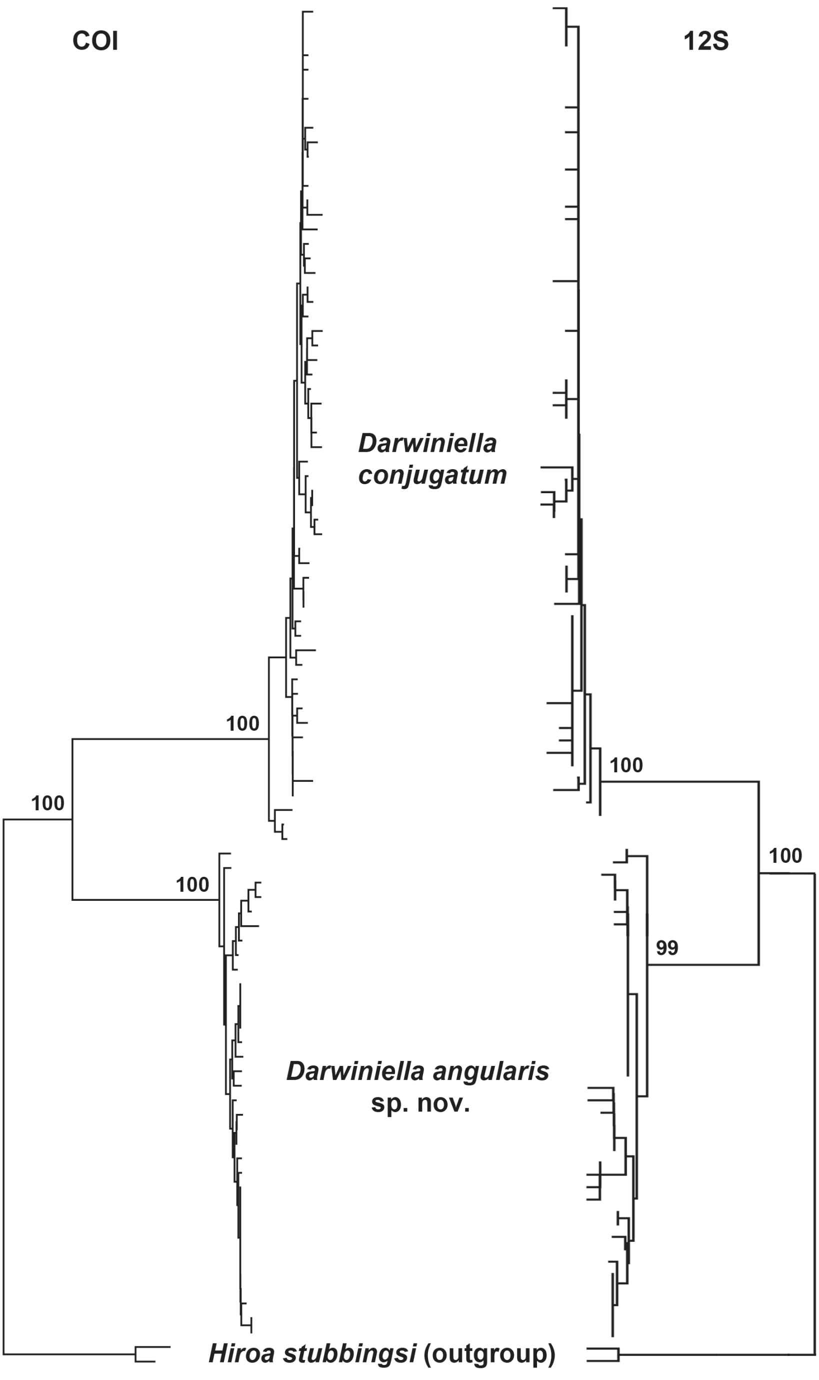

Figure 18. Neighbor-Joining method inferred genealogical relationships of *Darwiniella* specimens based on 472bp 12S and 642bp COI with *Hiroa stubbingsi* as the outgroup. Numbers above the major nodes are bootstrap values of 1000 replicates.

0.010/0.010 and 0.006/0.006 within *D. conjugatum* and *D. angularis* sp. n., respectively, and 0.120/0.132 between species.

Discussion

In the present study, a new species is identified from the previously monotypic genus *Darwiniella* that exhibit diagnostic morphological and molecular differences from *D. conjugatum*. These two species are difficult to distinguish from external shell morphology. The diagnostic characters are of the adductor plate angle of the scutum and the spur angle of the tergum.

Based on molecular analyses, the sequence distance of mitochondrial DNA markers 12S and COI within the two *Darwiniella* species (12S: 0. 4–0.6%, COI: 0.6–1.0%) is much smaller than between species (12S: 5.6–5.8%, COI: 12.0–13.2%). The sequence divergence of these two species has reached the level of congeneric species in another coral barnacle genus, *Cantellius* Ross & Newman, 1973 (12S: 5–7%, COI: 10–11 %) (Achituv et al. 2009), therefore further supporting *D. angularis* sp. n. as a separate species from *D. conjugatum*. Although the application of mitochondrial DNA in delimitating species can be limited by its natural history, e.g. reduced effective population size and introgression, and maternal inheritance (Rubinoff et al. 2006), the species boundary is evident based on the differentiation of 12S, COI and morphology of abundant samples from various localities.

Both *Darwiniella* species show significant host preference for the massive-form coral *Cyphastrea*. In the present study, 21 of 23 coral pieces with *D. conjugatum* embedded are *Cyphastrea* and only one is *Astreopora* (Appendix 2: Table 2). A similar pattern is observed in *D. angularis* where six of eight coral pieces are *Cyphastrea*, and only two are *Astreopora*. In previous studies, *D. conjugatum* has been recorded in *Cyphastrea* corals by Soong and Chang 1983 (Taiwan), Ren 1986 (Mainland China), Ogawa et al. 1998 (Mauritius) and Jones et al. 2000 (South China Sea), agreeing with the current study. However, Ogawa and Matsuzaki (1992) also recorded *Darwiniella conjugatum* from *Cyphastrea*, *Goniopora* de Blainville, 1830 and *Favites* Link, 1807 species in Japan.

Acknowledgements

We would like to thank Pei-Chen Tsai, I-Han Chen, Huai-Chen Hsieh, Hsin-Ying Lee (Academia Sinica) for assisting field collections and DNA analysis; Dr. Ling Ming Tsang (The Chinese University of Hong Kong) and Dr. Chi Chiu Cheang (Academia Sinica) for designing primers for COI. The authors would like to thanks the two reviewers for giving constructive comments on the MS. Thanks to the Yilan, Pingtung, and Taitung County Government, and Kenting National Park of Taiwan R.O.C. for granting permits for collection of corals for research purposes. This project is supported from a grant in National Science Council , Taiwan (NSC-100-2321-B-001-020) and a thematic grant from Academia Sinica, Taiwan (AS-100-TP2-A02-SUB3).

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Appendix 1

Table 1: Site and sampling location details. (doi: 10.3897/zookeys.214.3291.app1) File format: MS Word document(docx).

Explanation note: Study sites and sampling locations for *Darwiniella* in the present study.

Citation: Chen Y-Y, Lin H-C, Chan BKK (2012) Description of a new species of coral-inhabiting barnacle, *Darwiniella angularis* sp. n. (Cirripedia, Pyrgomatidae) from Taiwan. ZooKeys 217: 43–74. doi: 10.3897/zookeys.214.3291.app1

Appendix 2

Table 2: GenBank accession numbers for the barnacle species used in the present study. (doi: 10.3897/zookeys.214.3291.app2) File format: MS Excel spreadsheet (xls).

Explanation note: GenBank accession numbers for the barnacle species used in the present study.

Citation: Chen Y-Y, Lin H-C, Chan BKK (2012) Description of a new species of coral-inhabiting barnacle, *Darwiniella angularis* sp. n. (Cirripedia, Pyrgomatidae) from Taiwan. ZooKeys 214: 43–74. doi: 10.3897/zookeys.214.3291.app2

Appendix 3

Figure 1: Sequence alignment of (a) 472 bp 12S rDNA and (b) 642bp COI. (doi: 10.3897/zookeys.214.3291.app3) File format: MS Word document(docx).

Explanation note: Sequence alignment of (a) 472 bp 12S rDNA and (b) 642bp COI. Matching characters to the first sequence are labeled as dots.

Citation: Chen Y-Y, Lin H-C, Chan BKK (2012) Description of a new species of coral-inhabiting barnacle, *Darwiniella angularis* sp. n. (Cirripedia, Pyrgomatidae) from Taiwan. ZooKeys 214: 43–74. doi: 10.3897/zookeys.214.3291.app3

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The preimaginal stages of *Pnigalio gyamiensis* Myartseva & Kurashev, 1990 (Hymenoptera, Eulophidae), a parasitoid associated with *Chrysoesthia sexguttella* (Thunberg) (Lepidoptera, Gelechiidae)

Ekaterina Yegorenkova[1], Zoya Yefremova[2]

1 *Department of Geography, Ulyanovsk State Pedagogical University, Ulyanovsk, 432700, Russia* **2** *Department of Zoology, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, 69978 Tel-Aviv, Israel*

Corresponding author: *Zoya Yefremova* (eulophids@mail.ru)

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Abstract

The larval instars of *Pnigalio gyamiensis* Myartseva and Kurashev are described in detail for the first time. This species is a larval-pupal ectoparasitoid of *Chrysoesthia sexguttella* (Thunberg) (Lepidoptera, Gelechiidae), which forms leaf mines in the plant *Chenopodium album* L. (Caryophyllales: Amaranthaceae). The female of *P. gyamiensis* lays a single egg on the skin of the host larva or nearby it, without any significant preference for a particular variant. The presence of long hairs on its body provides the newly-hatched first larval instar with high mobility. Some peculiarities in this parasitoid-host relationship are described.

Keywords

Chalcidoidea, Eulophidae, *Pnigalio gyamiensis, Chrysoesthia sexguttella*, preimaginal morphology, physiological larval functions

Introduction

Five species of the genus *Pnigalio* Schrank were reared from larva of *Chrysoesthia sexguttella* (Noyes 2012): *Pnigalio soemius* (Walker) (Triggiani 1978; Hansson 1987; Rizzo and Massa 2002), *P. cristatus* Ratzeburg, *P. incompletus* Bouček, *P. agraules* Walker (Rizzo and Massa 2002) and *P. gyamiensis* Myartseva and Kurashev (Myartseva and Kurashev 1990).

Species belonging to *Pnigalio* are ectoparasitoids, with solitary or gregarious larval development; most of them are polyphagous, feeding on several species of leaf miners (Askew 1971, 1984). They attack 70 species from 21 genera of Lepidoptera, some of which are pests of agricultural crops (Schauff et al. 1998). The *Pnigalio* species are potentially important for biological control of lepidopterous leaf miners.

Several species of *Pnigalio* are poorly morphologically characterized and difficult to identify. Consequently, in 2005 Bernardo et al. (2006, 2007) began to study *P. soemius* species in the laboratory from egg to adult. Bernardo et al. (2008) suggested that *P. soemius* is a complex of at least two cryptic species, and the same authors (Gebiola et al. 2011) later noted four cryptic species belonging to the *P. soemius* complex. All the cryptic species are based on morphological, biological, molecular (Bernardo et al. 2008; Gebiola et al. 2010), karyological (Gebiola et al. 2012a) and endosymbiont data (Giorgini et al. 2010). The *P. soemius* complex is currently considered as a complex of several evolutionary lineages with very little morphological differentiation, but with significant genetic, ecological and biological differences (Gebiola et al. 2012b).

Our reared species is morphologically identical to *P. gyamiensis*. The DNA sequencing of the *P. gyamiensis* paratype was analyzed and revealed to be identical to the DNA sequence of *P. soemius* samples from *C. sexguttella* larvae on *Chenopodium album* and *Atriplex putula. Pnigalio gyamiensis* is genetically and biologically well characterized and its taxonomic validity has been confirmed (Gebiola et al. 2012b). Recent literature (Gebiola et al. 2012b) has shown that, based on ITS2 species-specific sequences, the parasitoids reared on the same host-plant system in Italy were genetically identical to the paratype of *P. gyamiensis* that was described in 1990 by Myartseva and Kurashev and reared on the *C. sexguttella – Atriplex* sp. system in Turkmenistan.

The preimaginal stages of *P. gyamiensis* have never been described. The aim of this work was thus to describe morphologically the preimaginal stages, especially the larval instars; to describe any differences between the physiological functions of each of them; and to elucidate the biological strategies of solitary parasitoids developing inside leaf mines.

Materials and methods

Pnigalio gyamiensis was reared without any other parasitoids from *C. sexguttella* on *Chenopodium album* L. (Caryophyllales: Amaranthaceae). The reared material was studied for the preimaginal stages. Samples of leaf-mines were regularly collected from five different localities in the city of Ul'yanovsk (54°16'N; 48°20'E), (separated from each other by no more than 3 km), between June and mid-September 2009. The food plant of *C. sexguttella* in the Middle Volga region is *C. album*. This plant is widespread, found in fields, gardens, and along roads and paths. *Chrysoesthia sexguttella* has two generations in this area: the first from May to July and the second from August to September.

In total, 500 leaf mines were collected, from which were reared 224 individuals of *C. sexguttella* and 25 specimens of *P. gyamiensis*. There was only one reared parasitoid of *P. gyamiensis*. The first generation of *P. gyamiensis* was reared from mid-June to early July, and the second generation from the end of July to mid-September. Mines from the leaves were stored individually in the laboratory in small glass tubes covered by several layers of wet gauze. In the present study, parasitoids were reared at 25° ± 2°C.

When mines were opened and photographed, this often prevented further development of parasitoid larvae. The total number of observations was 170. The number of observation of each larval instar is given below. In order to assess significant differences we used a non-parametric Fisher's exact test.

Video and photos of larval stages were recorded using a Canon Power Shot A-640. Light microscopy was carried out using a MC-2 ZOOM connected to a digital camera and a Mikromed microscope.

Abbreviation: F1–F4 – length of 1st, 2nd, 3rd and 4th segments of antennal funicle; SMV – submarginal, MV – marginal, PMV – postmarginal and SV – stigmal veins of forewing. Zoological Institute, Russian Academy of Science, St. Petersburg, Russia (ZISP).

Taxonomic survey

Pnigalio gyamiensis Myartseva & Kurashev, 1990

http://species-id.net/wiki/Pnigalio_gyamiensis

Pnigalio gyamiensis Myartseva and Kurashev 1990: 42–43.

Morphology. Our reared specimens were compared with type material (Zoological Institution of Russian Academy of Sciences, St. Petersburg, Russia (ZISP): "Holotype, female, Gami, 3 km W from Ashgabat, ex larva *C. sexguttella* on *Atriplex* sp., 13.10.1986 (Saparmamedova) Myartseva, Kurashev, 1990", two female paratypes with the same label, and one female with label "Gami, 3 km W from Ashgabat, ex larva *C. sexguttella* on *Atriplex* sp., 30.10.1986 (Saparmamedova) Myartseva, Kurashev, 1990".

Morphological diagnosis is based on a study of the type material.

Body length 1.08–1.80 mm; F1 1.1–1.3 times as long as F2; F2 1.1–1.2 times as long as F3; F3 1.0 times as short as F4; F4 1.3 times shorter than clava; callus of propodeum with 2 rows of setae: 1 row with 10–12 setae, 2 with 4 setae; sculpture of mesoscutum areolate and size of seta larger than that in scutellum. Forewing 2.3–3.5 times as long as broad; SMV 1.3–1.6 times shorter than MV; MV 1.8–2.7 times longer than PMV; PMV 2.0–3.3 longer than STV; gaster 1.5–1.8 times as long as broad. Body dark blue, the gaster brown with yellow tick at base, legs completely yellow with dark brown last segment of tarsi, hind coxae yellow with brown bracket (in the base of the coxa).

Seventeen females and eight males of *P. gyamiensis* reared by authors are labelled: "Ul'yanovsk, left bank of the river Volga, Verhnaya Terrasa, 56°49'N; 49°44'E, 15 June–8 August 2009 (Yegorenkova)".

Our species belongs to *P. gyamiensis* and its morphological variability is less high.

Female. Body length 1.35–1.80 mm; F1 1.2–1.3 times as long as F2; F2 1.1 times as long as F3; F3 1.0–1.1 times as short as F4; F4 1.3–1.4 times shorter than clava; forewing 2.5–2.8 times as long as broad; SMV 1.5–1.6 times shorter than MV; MV 1.7–1.8 times longer than PMV; PMV 2.7–2.8 longer than STV; gaster 1.4–1.5 times as long as broad. Body dark green with metallic tint, gaster with yellow tick or spot in the base of the gaster, legs completely yellow with dark brown last segment of tarsi, hind coxae mostly yellow without brown bracket. **Male** (first description): body length 1.25–1.38 mm; thorax 1.6 times as long as broad; pronotum 1.7 times as broad as long; sculpture of scutellum is the same as that of the mesoscutum; propodeum 2.3 times as broad as long; gaster 1.7–1.8 times as long as broad. Colouring is the same as in the female, sometimes hind femur and fourth tarsal segments darkened**.**

Distribution. Turkmenistan (Myartseva, Kurashev, 1990), Italy (Gebiola et al. 2012b). New record for Middle Volga Basin (Russia).

Biology. Larval solitary ectoparasitoid.

Description of preimaginal stages of *Pnigalio gyamiensis*

Egg

The shape of the egg changes during development of the embryo. The egg (Figure 1) just laid by a female of the parasitoid is oblong, both ends are rounded, with one a little broader than the other. The egg is white and shiny without sculpture. As the embryo develops, the egg becomes oval. Such eggs were either found beside the host (larva of dead *C. sexguttella*) (Figure 2) or lying on the surface of the host's cuticle. An egg with a fully developed embryo is elongate (Figure 3).

Eggs of species of the genus *Pnigalio* were previously studied by Delenoue and Arambourg (1967) and Gebiola et al. (2009).The *P. soemius* embryo can also develop successfully within its egg without a host. In 17 cases the egg was found near the host, and in 20 cases on the skin of the host (n=37). The differences are not significant (Fisher's exact test: p>0.05). Development of the egg lasts on average 2.3 ± 0.8 days before hatching of the first larval instar. It is possible that a newly-hatched first larval instar reaches the host using the long hairs on its body to facilitate movement inside the leaf mine (when the egg is laid near to the host).

1st instar larva

Morphology. The larva has 13 distinct segments including the extended head, which is 1.3 times as long as the second thoracic segment. The head capsule is dark yellow with one brown mandibular tooth that is used to puncture the cuticle of the host (Figure 4). The shape of the mandible is triangular. Chaetotaxy: the head capsule is

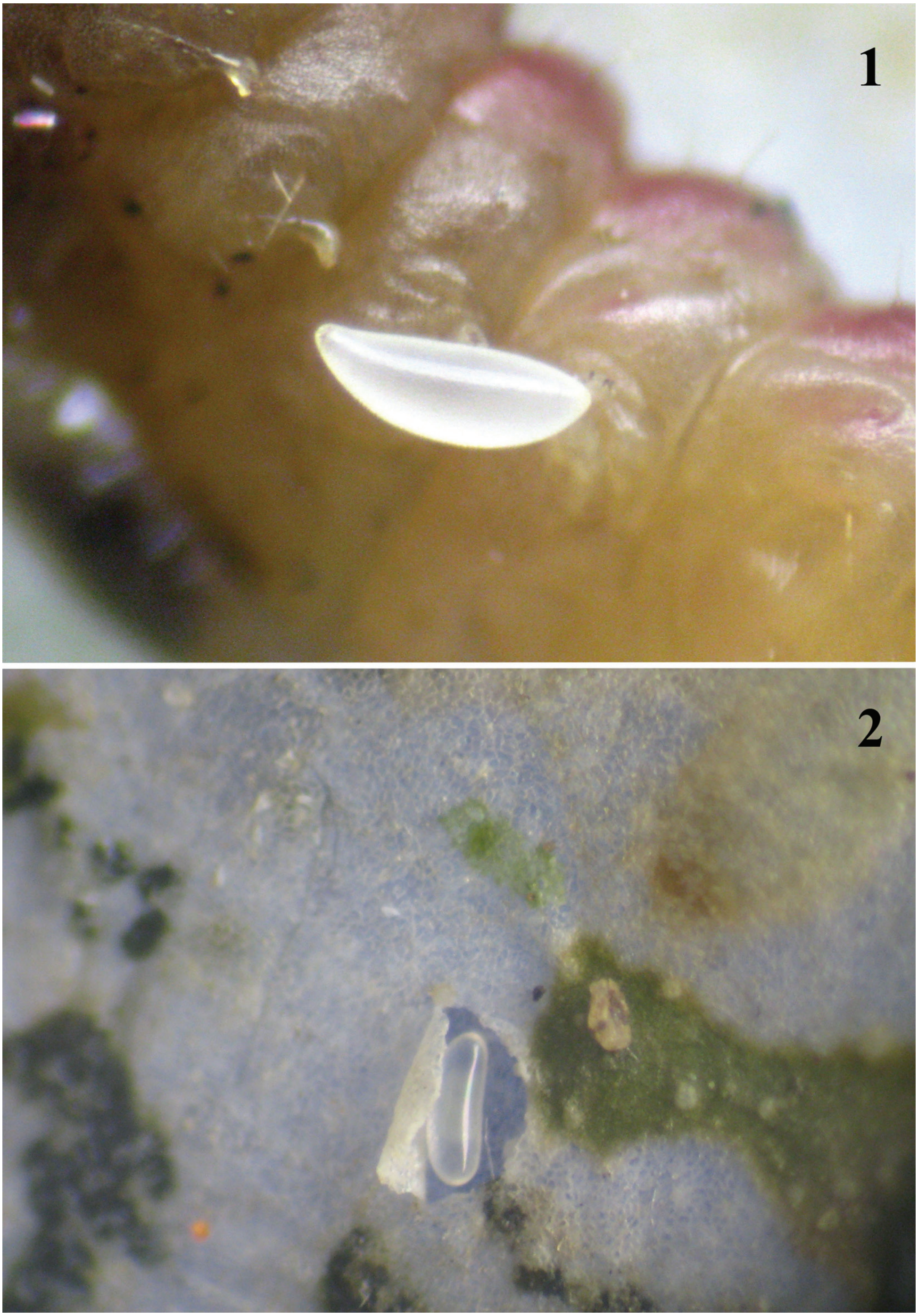

Figure 1–2. 1 A recently laid egg of the female of *Pnigalio gyamiensis*, on the IV–V segments of the second larval instar of *C. sexguttella* (ventral view) **2** *Pnigalio gyamiensis* egg with developing embryo in an opened mine of *C. sexguttella* beside the host remains.

covered by hairs. The body has 2 lateral rows of protuberances on segments II, III, IV, VI, VIII, X, XII and XIII, each with long hairs (total 32 hairs). Length of the hairs is equal to the diameter of the abdominal segments. Long hairs are at an angle of 45° to the body and at an obtuse angle to each other. The first dorsal hairs bend towards the head while the last ones bend back towards the anus.

Behaviour. The larva is very active and quickly moves in the mine by means of muscle contractions, which are clearly visible. The parasitoid larva may feed on the haemolymph but does not do so solely. During observation of this instar it was noted that the larva punctured (drilled into) the cuticle of the host anticlockwise, thereby gaining access to the haemolymph. We observed such larva externally on the host larva and in a mine without a host, where it was probably searching for a host. At the end of this instar the larva becomes less active and moults to the second instar on the surface of the host's body (33 observations).This stage lasts on average 3.8 ± 0.7 days.

2nd instar larva

Morphology. The second instar larva is larger than the first, less active, and the body is segmented (Figure 5). Pulsation of the gut becomes distinct, and the food (firstly pale yellow and later on darkened) is moved to the anal part of gut. This larva moults to the 3rd instar on the surface of the host and the larva's head loses any distinctive shape as well as its hairs.

Behaviour. In contrast to larva of the 1st instar that leaves the host several times, this 2nd larva stays on the host, feeding almost entirely on the haemolymph. The 2nd larval instar spends much longer periods feeding than that of the 1st instar larva, resulting in a rapid increase in size. We observed siblicide behavior between the 2nd larval and 4th larval instar of *P. soemius* (Figure 7). It means the 2nd larval instar begins to feed on the haemolymph of 4th instar larva (28 observations).This stage lasts on average 2.9 ± 0.6 days.

3rd instar larva

Morphology. The 3rd instar larva (Figure 6) has distinct protuberances in some segments (II–IV thoracic, VI, VIII, X and XII abdominal, and XIII anal). Length of a protuberance is equal to its width. Each protuberance has hair 1.5 times as long as length of protuberance.

Behaviour. The larva is actively feeding at this stage and its gut reveals a visible pulsation. We did not observed siblicide behavior between two larvae of 3rd instar. This stage lasts on average 2.5 ± 0.7 days (20 observations).

4th instar larva / prepupa

Morphology. The 4th larval instar (Figure 8) lacks mobility and has nine visible pairs of spiracles of the respiratory system on the II and III thoracic and I–VII abdominal seg-

Figure 3–4. 3 *Pnigalio gyamiensis* egg with developing embryo on IV–V segments of *C. sexguttella* larva (ventral view) **4** 1st instar *P. gyamiensis* larva in the mine of *C. sexguttella*

Figure 5–6. Larva of *C. sexguttella* (dorsal view) parasitized by 3rd instar larva of *P. gyamiensis* **6** 3rd instar *P. gyamiensis* larva (protuberances are visible, ventral view).

Figure 7–8. 7 Siblicide behavior exhibited by 2nd and 4th larval instars of *P. gyamiensis* on a larva of *C. sexguttella* (dorsal view) **8** 4th larval instar of *P. gyamiensis* on a larva of *C. sexguttella* inside its mine.

ments. The fully fed larva has a dark brown gut that pulsates in one direction for 40 seconds and then in the opposite direction for 40 seconds. It is important to note that as the larva develops the frequency of pulsation decreases to a rate of 60 seconds in one direction and 60 seconds back. The prepupa loses segmentation and the body instead forms two sections between head and thorax and thorax and abdomen.

Behaviour. At the end of this stage the parasitoid larva leaves the host, stops feeding and loses mobility; its gut is full and equal to 75% of body weight. Antagonistic behavior by larvae against larvae of the same species was not observed; larvae of 4th instar may feed on the same host independently of each other (Figure 9) but might be attack by larvae of the 2nd instar (see Figure 7). This stage lasts on average 2.9 ± 0.6 days (24 observations).

Pupa

Morphology. The pupa attaches to the leaf epidermis (Figure 10).The pupa is initially white or slightly yellow and then begins to darken to dark brown or black. The fully developed pupa of *P. gyamiensis* (Figure 11) has a metallic tint but the adult is never visible through the chitinized exuviae of pupa. Female pupa is recognizable by their large gaster and ovipositor visible through the light coloured cuticle of the gaster in the early stage of pupation, whereas the male pupa has a smaller gaster and darker colour.

The pupa is situated inside the mine ventrally to the leaf's surface. It develops on average 5.4 ± 0.8 days (28 observations).

The total duration of development is 19.8 ± 1.2 days.

Behaviour. The adult exits through the oral cavity of the pupa often in the early morning. The adult begins to clean its antennae and head and then leaves the mine.

Conclusion

Pnigalio gyamiensis presents four larval instars and the three moults are easy recognizable. The 1st instar larva is clearly visibly by the presence of long hairs on its body. The mandibles are very small and curved, and used to hook onto the cuticle of the host (Figure 12). Some authors have noted a difficulty in differentiating larval instars, such as in *Hyssopus pallidus* Askew (Tschudi-Rein and Dorn 2001), with the only discernible differences being in the shape and size of the mandibles. They did not report the long hairs on the body that the larva uses for moving across the surface of the host or inside the mine. The 2nd larval instar loses these long hairs and moves slowly; it is recognizable by its mandibles. The mandibles of 2nd, 3rd and 4th instar larvae (Figure 12) differ in size but the 4th instar has one large, well-development tooth.

The emerged adults (both sexes) are shown in Figure 13, 14. The female parasitoid *P. gyamiensis* paralyses the larva *C. sexguttella*, which loses mobility, stops feeding and

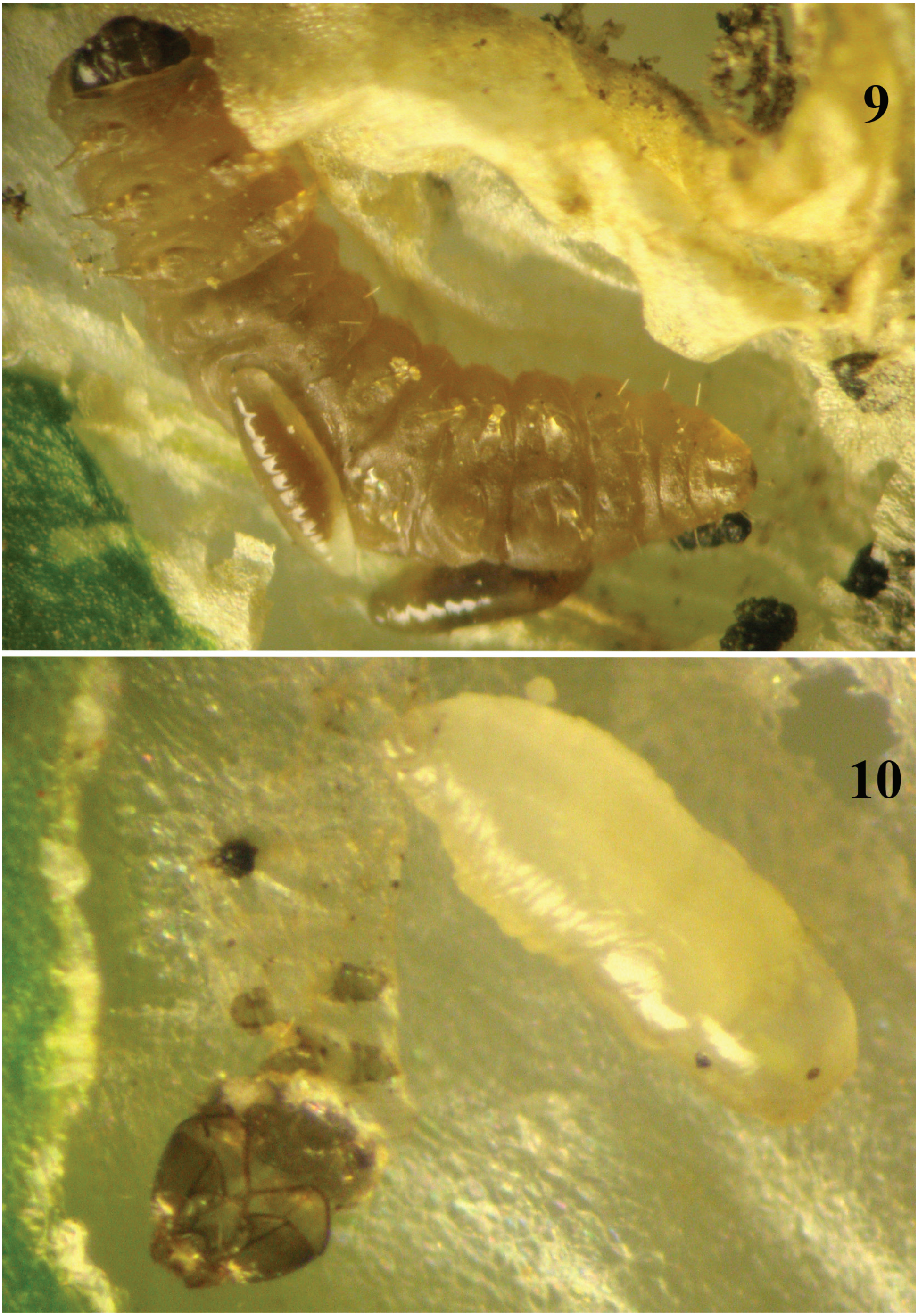

Figure 9–10. 9 Two 4th larval instar *P. gyamiensis* on a larva of *C. sexguttella* inside its mine **10** Prepupa of *P. gyamiensis* in an opened mine of *C. sexguttella*.

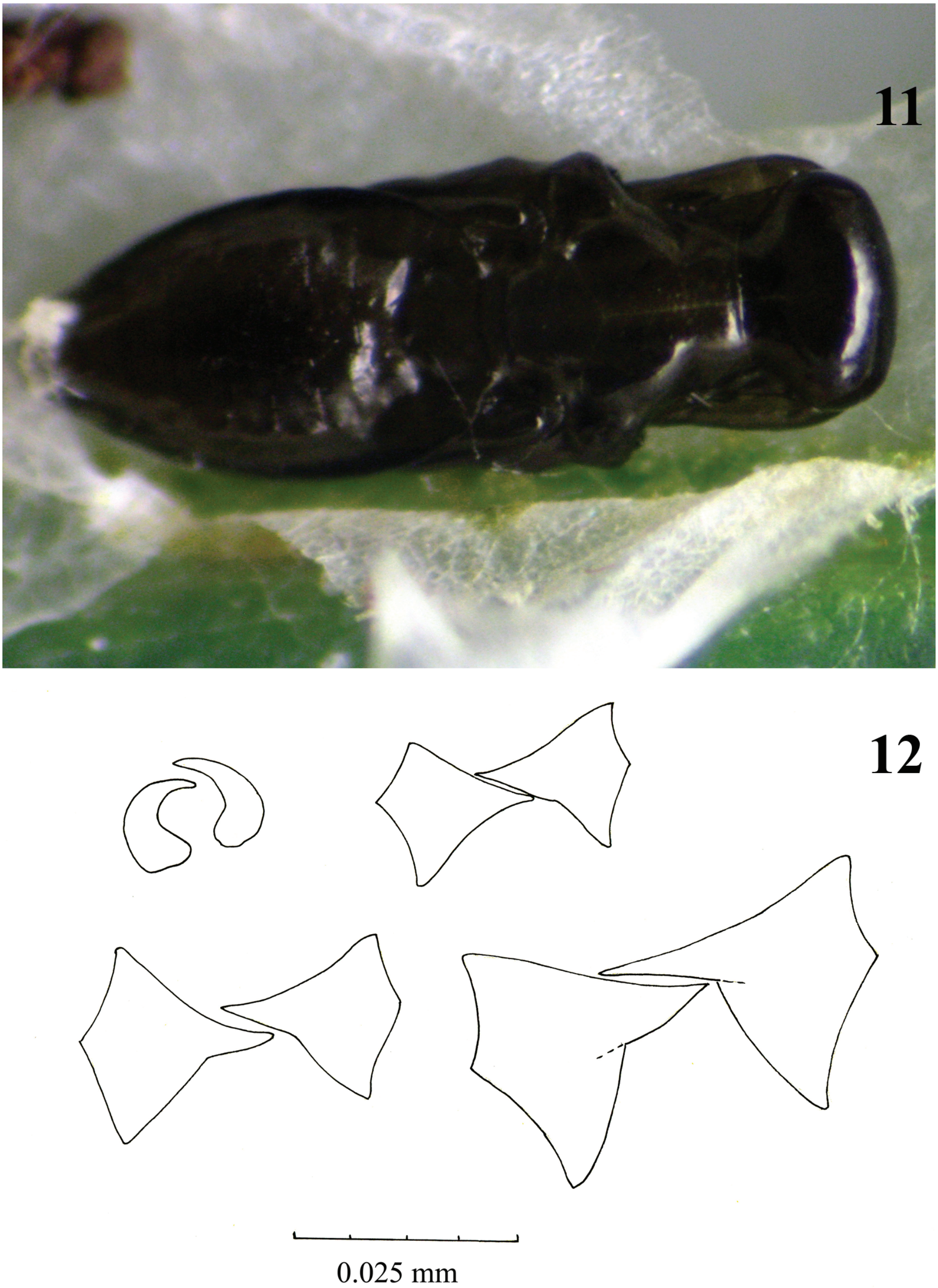

Figure 11–12. 11 Fully developed pupa of *P. gyamiensis* in a mine of *C. sexguttella* (dorsal view) **12** Mandibles of larval instars of *P. gyamiensis*: top row mandibles of 1st and 2nd larval instars, bottom row mandibles of 3rd and 4th larval instars.

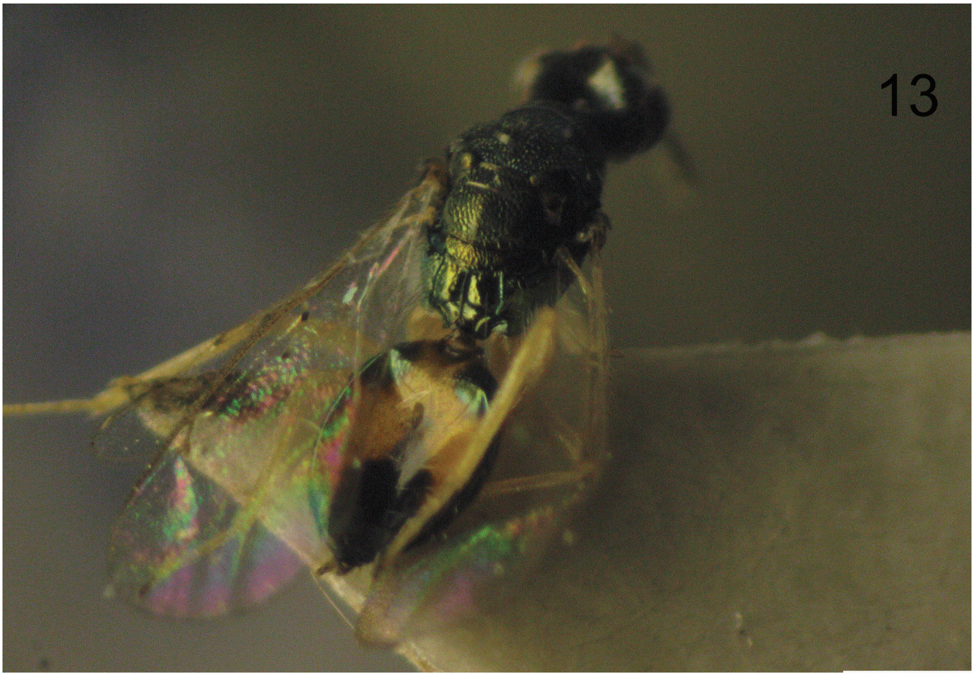

Figure 13–14. 13 Emerged female of *P. gyamiensis* **14** Emerged male of *P. gyamiensis*.

dies. The parasitoid larva then feeds on the killed host. Only a few cases were observed of the parasitoid female having laid an egg on the skin of a dead host larva; but in each case the parasitoid larva developed successfully. The female hid her egg on the skin of the host larva or near it without significant preference for any of the variants. The high mobility of the 1st instar allows the larva to find a host quickly and begin to feed.

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