RESEARCH ARTICLE



# A new species of Agelas from the Zanzibar Archipelago, western Indian Ocean (Porifera, Demospongiae)

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# Abstract

A new sponge species (Demospongiae: Agelasida: Agelasidae) is described from the eastern coast of Unguja Island in the Zanzibar Archipelago. *Agelas sansibarica* **sp. n**. is compared to all other *Agelas* species described so far. The new species differs from its congeners mainly in its three categories of verticillate spicules (acanthostyles, acanthostrongyles, and acanthoxeas) and their sizes. Acanthostrongyles, well represented in the spicular complement, are an exclusive trait of the new species widening the morphological range of the genus. Summarizing on spicular complement and spicular morphotraits of 36 species belonging to the genus *Agelas*: i) 32 species show only acanthostyles from Indo-Pacific (n = 14), Atlantic (n = 17), and Mediterranean (n = 1); ii) three Indo-Pacific species show acanthostyles and acanthoxeas; iii) one species *A. sansibarica* **sp. n.** from the western Indian Ocean is characterised by the unique trait of three categories of verticillate spicules (acanthostyles, acanthostrongyles and acanthoxeas). A key for the Indo-Pacific species is supplied together with short descriptions, illustrations, and geographic range; literature on chemical bioprospecting of the genus *Agelas* is also provided.

# Keywords

Biodiversity, sponges, morpho-taxonomy, diagnostic key, geographic range, Unguja Island

# Introduction

The sponge fauna of the Zanzibar Archipelago is poorly studied and data are reported almost exclusively in very old papers (Lendenfeld 1897, Baer 1906, Jenkin 1908, Sollas 1908, Thomas 1976). In none of these papers species belonging to the genus *Agelas* Duchassaing & Michelotti, 1864 (Porifera: Demospongiae: Agelasida: Agelasidae) are reported. The presence of *Agelas mauritiana* (Carter, 1883) along the Zanzibar coasts was recently recorded (Said et al. 2010) as producer of bioactive compounds.

The widespread genus *Agelas*, including until now 35 valid species, was established by Duchassaing and Michelotti (1864: 76) describing the type species *Agelas dispar* from the Caribbean Sea. *Agelas oroides* is the only Mediterranean species, and is endemic. The western Atlantic (Gulf of Mexico, Caribbean, and Brazil) hosts 17 species. The majority of the latter (13) were recently revised while the remaining four species were considered dubious or suggested as synonyms (Van Soest 2002, Muricy et al. 2011, Parra-Velandia et al. 2014).

The Indo-Pacific species of *Agelas* number 18, including the new species here described. The most widespread species is *Agelas mauritiana* (including its *oxeata* variety) recorded in the Australian western Pacific, and the Indian Ocean from the Mascarenes Archipelago (type locality), Seychelles Archipelago, Madagascar, and Mozambique Channel to the southern Red Sea and east to Sri Lanka.

Several species (14) are reported only once from the type locality i.e. Agelas axifera Hentschel, 1911; A. bispiculata Vacelet, Vasseur & Lévi, 1976; A. braekmani Thomas, 1998 (1997); A. carpenteri (Gray, 1867); A. cavernosa Thiele, 1903; A. citrina Gotera & Alcolado, 1987; A. dendromorpha Lévi, 1993; A. inaequalis Pulitzer-Finali, 1986; A. linnaei de Voogd, Parra-Velandia & Van Soest, 2008; A. nakamurai Hoshino, 1985; A. nemoechinata Hoshino, 1985; A. novaecaledoniae Lévi & Lévi, 1983; A. robusta, Pulitzer-Finali 1982; A. semiglaber Pulitzer-Finali, 1996.

In the framework of sponges, applied research on bioactive compounds at a global level (e.g. Murray et al. 2013) focuses on *Agelas* species as producers of interesting molecules e.g. *A. clathrodes, A. linnaei, A. mauritiana, A. nakamurai, A. oroides,* and *A. sceptrum* (Walker et al. 1981, Fathi-Afshar et al. 1989, Keifer et al. 1991, Braekman et al. 1992, Bernan et al. 1993, König and Wright 1993, Chanas et al. 1996, König et al. 1998, Eder et al. 1999, Assmann et al. 2000, 2001, 2004, Fattorusso and Taglialatela-Scafati 2000, Assmann and Köck 2002, Fujita et al. 2003, Bickmeyer et al. 2004, 2005, 2008, Bickmeyer 2005, Costantino et al. 2006, Meketa and Weinreb 2006, Ferretti 2006, Vik et al. 2010, Said et al. 2010, Regalado et al. 2011, Mordhorst et al. 2015). In this scenario of intensive bioprospecting, research knowledge of systematics and taxonomy in depth is a key tool to identify and define the status of specimens/biomaterial to be processed.

The present paper aims to report the discovery of a new species of *Agelas* from the Zanzibar Archipelago comparing it to all species belonging to the genus. To support global sharing of information on faunistics and taxonomy of Porifera from not widely accessible data sources an updated overview on the morphology and geographic distribution of

*Agelas* species from the Indo-Pacific area is also provided together with a brief description and exhaustive iconography, as well as a dichotomous key to Indo-Pacific species.

# Materials and methods

Representative fragments of six sponge specimens from the Unguja Island were studied. Growth form, surface traits, skeletal architecture, shape and size of the spicules are considered diagnostic morphotraits. Spicule dimensions are given as maximum, minimum, and medium lengths and widths of *ca*. 400 spicules.

The skeletal architecture was investigated by hand-cut sections of the ectosome and choanosome. To study the three-dimensional arrangements of fibres and spicules in the skeleton, fragments *ca.*  $10 \times 10 \times 2$  mm were cut, cleaned with 5% sodium hypochlorite (NaClO) for 24 h in a warm temperature (35–40 °C), then washed and stirred five times in abundant double distilled water, washed and stirred twice in ethanol 95%, and finally allowed to air dry and gold-sputtered or mounted in Eukitt. The skeletal samples were than studied by light microscope (LM) and scanning electron microscope (SEM). Spicule preparations were made by dissolving a small fragment of the specimen in 65% boiling nitric acid (HNO<sub>3</sub>)and cleaned spicules were rinsed four times with water, once with 95% ethanol. The spicules were air-dried on slides, mounted in Eukitt, and observed by a Leitz Dialux 20 EB (LM), as well as on aluminium stubs and coated with gold for the observation with a Vega3 TESCAN type LMU (SEM).

Specimens were deposited at the Museo civico di Storia Naturale "Giacomo Doria" of Genoa, Italy (acronym MSNG). For the taxonomy of genus and species level the Systema Porifera (Hooper and Van Soest 2002) and the World Porifera Database (Van Soest et al. 2015) are considered as reference.

# Systematic account

Phylum Porifera Grant, 1835 Class Demospongiae Sollas, 1885 Order Agelasida Hartman, 1980 Family Agelasidae Verrill, 1907 Genus Agelas Duchassaing & Michelotti, 1864 Chalinopsis Schmidt, 1870 (junior synonym) Ectyon Gray, 1867 (junior synonym) Oroidea Gray, 1867 (junior synonym) Pachychalinopsis Schmidt, 1880 (nomen nudum) Siphonochalinopsis Schmidt, 1880 (nomen nudum)

**Diagnosis** (emended from Van Soest 2002, p. 820). Massive-lobate, encrusting, tubular, branching or flabellate sponges, often of considerable size, with smooth to finely

conulose surfaces provided with small rounded and/or key-hole shaped apertures. Colour usually orange or brownish-orange. Consistency toughly compressible, firm. No ectosomal specialization. Choanosomal skeleton isotropic or anisotropic, occasionally irregular, network of primary ascending spongin fibres and secondaries. Main fibres mostly cored by megascleres. Main and interconnecting fibres echinated by megascleres in most cases. Spicules as verticillate styles, or styles and oxeas, or styles, oxeas and strongyles. Biogeographic pattern of 36 nominal species mostly matches tropical waters, a single species occurs in the Mediterranean. The genus has not been recorded from the eastern Pacific, West Africa, and the northern Atlantic European coasts.

#### Agelas sansibarica Perino & Pronzato, sp. n.

http://zoobank.org/7F8E3929-DD8D-4991-A8E0-C391C89AC1D0 Figs 1–5

**Material examined.** Holotype: MSNG 57991 (A30), 70% ethanol, Jambiani (06°18'44.8"S, 39°33'32"E), eastern coast of Unguja Island, Zanzibar Archipelago, United Republic of Tanzania, SCUBA diving, 4.vii.2010, leg. Mr. Okala. Paratypes: MSNG 57992, MSNG 57993, MSNG 57994, MSNG 57995, MSNG 57996 (A12, A26, A27, A28, A29, respectively) ibid.

**Diagnosis.** *Agelas* with unique spicular complement composed of three spicular categories, oxeas, styles and strongyles with spines arranged in a variable number of verticilles.

Etymology. The speciphic epithet refers to the Zanzibar Archipelago.

**Habitat.** Coral reef, quite common at 7–12 m of depth. Water temperature 28–31 °C. Salinity 20–36‰ (Fryday 2011). As reported by the Swiss Marine NGO manager of the local sponge farming facility (Jambiani Lagoon) the new species is massively farmed (Christian Vaterlaus, pers. comm., 2010).

**Geographic distribution.** Western Indian Ocean, but only recorded from the type locality to date.

**Description.** Growth form massive, thick, rounded, 6–10 cm in diameter. Colour in life purple to orange and light brown. Consistency firm and elastic. Surface rough to the touch, finely hispid, finely conulose for tips of ascending fibres supporting the dermal membrane, with regularly scattered circular and convoluted depressions (0.5 cm in diameter) very similar to those of *A. cerebrum*. Oscules few, small, irregularly scattered. Choanosomal skeleton as an irregularly reticulate network of spongin fibres. Primary fibres 50–110 (71.67 ±17.63) µm in diameter, strongly echinate by single, scattered spicules to groups of diverging spicules; ascending primary fibres cored by spicules also present. Secondary fibres 20–50 (35 ± 9) µm in diameter notably echinate and cored by spicules. Tertiary network not observed.

Megascleres as three categories of monaxons mostly with acute spines. Acanthostyles 90–250 (180.72 ± 28.66) × 7.5–20 (13.46 ± 2.59)  $\mu$ m ornate by verticillate spines arranged as 11–27 (17.8 ± 2.86) whorls. Acanthoxeas 130–295 (195 ± 43.09)



**Figure 1.** Genus *Agelas*. Biogeographic pattern (grey areas). The red dot indicates the type locality of the new species *Agelas sansibarica* sp. n. at Jambiani (06°18'44.8"S, 39°33'32"E), eastern coast of Unguja Island, Zanzibar Archipelago, United Republic of Tanzania.



**Figure 2.** *Agelas sansibarica* sp. n. Type specimen (alcohol preserved, colour different from *in vivo*) from the Zanzibar Archipelago.



Figure 3. Agelas sansibarica sp. n. Spicular complement of verticillate acanthostyles, acanthoxeas and acanthostrongyles (SEM).

× 7.5–15 (12.17 ± 1.89)  $\mu$ m ornate by verticillate spines arranged as 14–26 (19.24 ± 3.47) whorls. Acanthostrongyles 80–245 (148.18 ± 36.82) × 4–17 (11.09 ± 4.24)  $\mu$ m ornate by verticillate spines arranged as 9–26 (15.76 ± 3.85) whorls. Annulate spicules apparently young.

**Remarks.** The new species is characterized by the co-presence of three categories of spicules never recorded in other *Agelas* species. Acanthostrongyles are abundant, *ca*. 20–30 % of the total number of spicules.

# Discussion

# Geographic range of Indo-Pacific Agelas species

Madagascar, Mozambique Channel, Seychelles and Mascarene archipelagos (Western Indian Ocean province) harbour four species, whereas Japan (Ryukyu Archipelago) and New Caledonia enumerates two species each. Only one species each is recorded



Figure 4. Agelas sansibarica sp. n. Spicular complement of verticillate acanthostyles, acanthoxeas and acanthostrongyles (LM).

from Philippines, Papua New Guinea, and Funafuti. Only one species each is harboured in the Red Sea/Gulf of Aden, Sri Lanka, Moluccas, Sunda Shelf/Java Sea (Indonesia), Hong Kong, Funafuti, and Australia (Fig. 1).

# Diagnostic morphotraits comparative analysis of Agelas Indo-Pacific species

To discriminate between all 36 *Agelas* species by diverging diagnostic morphotraits is notably difficult, as highlighted in the previous section. Morphotraits of the genus are extremely conservative and different species appear very similar. Focusing on the Indo-Pacific species our attempt was not as completely successful as is also the case for the Atlantic species by Parra-Velandia et al. (2014).

Atlanto-Mediterranean *Agelas* species (18) seems to possess only acanthostrongyles, including the uncertain *A. fascicularis*, *A. flabelliformis*, *A. inaequalis*, and *A. rudis* not redescribed by Parra-Velandia et al. (2014).



**Figure 5.** *Agelas sansibarica* sp. n. **a** skeletal network of spongin fibres echinated by spicules (SEM) **b** detail of fibres surface echinated by verticillate spicules (SEM) **c** section of a primary fibre cored by a verticillate acanthostrongyle **d–e** skeletal network (LM).

Among the 17 previously known Indo-Pacific *Agelas* species, the spicular complement of 14 species is composed of acanthostyles in a single or two-dimensional classes (see Appendix 1, Figs 6–21 for details).

The most common and studied Indo-Pacific species, i.e. *A. mauritiana*, are characterized by a single spicular type acanthostyles, which are extremely variable in morphology, abundance of spines, and dimensional range (sometime more than three times in length) (see Table 1). The Atlantic *A. dispar* and the Mediterranean *A. oroides* show a similar size variability of acanthostyles. Only the Indo-Pacific *A. axifera*, *A. mauritiana* var. *oxeata*, and *A. novaecaledoniae* show two different categories of spicules, i.e. acanthostyles and acanthoxeas.

The new species *A. sansibarica* is characterized by the unique morphotrait of three categories of megascleres, i.e. acanthostyles, acanthoxeas, and acanthostrongyles with spines arranged in verticilles regularly scattered along the entire spicule. No other *Agelas* species exhibit this spicular combination. Acanthostrongyles, well identifiable and abundant, represent an exclusive diagnostic trait of the new species. The functional

References	Acanthostyles µm	Whorls n <sup>o</sup>	Colour	Habitus size (cm)	Consistency
Carter 1883	132	15–18	-	-	-
Thiele 1903	200 × 14–15	16	-	-	-
Dendy 1905	176 × 16	_	dark brown	tubular 3.1 × 1.6 length × diameter	firm resilient
Laubenfels 1954	170–180 × 10–14	12–18	-	-	-
Lévi 1961	150–160 × 8–12	16–20	brown	massive 6–10 × 4	firm resilient soft
Lévi 1964	275 × 12–13	15–17	-	-	-
Lévi 1967	140–230 × 8–10	10-15	-	-	-
Vacelet and Vasseur 1965	135–250	13–20	-	-	-
Vacelet and Vasseur 1971	80–180 × 7.5–12.5	18–23	brown	encrusting 1.6–1.5 thickness	firm resilient coriaceus
Thomas 1979	112–212 × 6–8	6–8	pale yellow	encrusting cavernous	firm resilient compressible

**Table 1.** Agelas mauritiana. Morphometries and morphotraits by different authors.

role of acanthostrongyles is doubled since echinanting spicules arm both the fibres surface and the core of the axial part of fibres.

Summarizing spicular complements and spicular morphotraits of 36 Agelas species: i) 32 species show only acanthostyles from Atlantic (17), Mediterranean (1), and the majority (14) of the Indo-Pacific areas; ii) three Indo-Pacific species show acanthostyles and acanthoxeas; iii) only one species *A. sansibarica* sp. n. from the western Indian Ocean show a spicular component composed of acanthostyles, acanthoxeas, and acanthostrongyles.

#### Key to the Indo-Pacific Agelas species

The present key is an attempt to discriminate between the Indo-Pacific species, but the scenario appears very intricate mainly because morphotraits from many descriptions and illustrations are overlapping (see Table 1). A similar attempt, on the Atlantic species, was performed by Parra-Velandia et al. (2014) emphasizing that: "Caribbean *Agelas* taxonomy rests heavily on the external morphology"; as a consequence their key is essentially based on growth form and colour. Since this is the situation, our key is not simply dichotomous and allows the disctintion of only 13 of the 17 Indo-Pacific species (see Appendix 1). The remaining four species have acanthostyles with length ranges which are widely overlapping (from less than 150 to more than 250 µm). Three of these (*A. carpenteri*, *A. marmarica*, and *A. robusta*) are known only from the original descriptions; on the contrary, *A. mauritiana* is reported by several authors but with discordant descriptions (Table 1).

1	Spicular complement composed by 1 or 2 spicular types (acanthoxeas, acan-
	thostyles)
-	Spicular complement composed by 3 spicular types (acanthoxeas, acantho-
	styles, acanthostrongyles) A. sansibarica sp. n.
2	Spicular complement composed by 2 spicular types (acanthoxeas and acan-
	thostyles)
_	Spicular complement composed by 1 spicular type (acanthostyles)4
3	Sponge body cup-shaped
_	Sponge body blade-shaped
_	Sponge body lobed
_	Sponge body digitate
_	Sponge body as slim cylindrical erected axis (branched or unbranched)
	A. gracilis
4	Acanthostyles of 2-dimensional categories
_	Acanthostyles of 1-dimensional category7
5	Long acanthostyles spiny only at the tips
_	Long and short acanthostyles almost entirely spiny
7	Primary and secondary fibres uncored
_	Primary and/or secondary fibres cored
8	Acanthostyles longer than 300 µm
_	Acanthostyles length no more than 200 µm
9	Primary and secondary fibres cored
_	Primary fibres cored and secondary uncored10
10	Acanthostyles $(185-265 \times 8-15 \mu m)$ with 15-23 whorls A. nakamurai
_	Acanthostyles $(130-220 \times 4-21 \ \mu m)$ with 8–18 whorls
_	Acanthostyles $(80-370 \times 5-24 \mu m)$ with 11–33 whorls

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# Appendix I

Indo-Pacific species belonging to the genus Agelas reported from the literature.

# Agelas axifera Hentschel, 1911

Fig. 6

**Description** (modified from Hentschel 1911). Growth form club-shaped ( $12 \times 6$  cm), walls 0.5 cm in thickness. Surface irregular. Colour orange. Ectosomal skeleton not reported. Choanosomal skeleton irregularly reticulate network (meshes 320 µm in diameter) of fibres echinated by few spicules. Primary fibres (80 µm in diameter) cored by spicules. Secondary fibres ( $40 \mu$ m in diameter) not cored. Megascleres verticillate of two categories. Acanthostyles and acanthoxeas of similar size ( $112-152 \times 5-7 \mu$ m) ornate by 7–15 regular whorls with 5–6 acuminate thorns each one. **Habitat.** Not reported. **Geographic distribution.** Pacific Ocean, Australia. **Remarks.** Type specimen stranded on beach at Champion Bay, Geraldton, Western Australia.



**Figure 6.** *Agelas axifera*. **a** living specimen **b** skeleton fragment with two spicular types, axially embedded in a fibre and arming the surface **c** acanthostyles **d** acanthoxea (**a** modified from an original underwater shot by J. Hooper **b–d** modified from Hentschel 1911).

# Agelas bispiculata Vacelet, Vasseur & Lévi, 1976

Fig. 7

**Description** (modified from Vacelet et al. 1976). Growth form massive; hemispherical fragments (5–6 cm in diameter). Surface hispid. Oscules and pores not evident.



**Figure 7.** *Agelas bispiculata*. Spicular complement of acanthostyles of two size categories (modified from Vacelet et al. 1976).

Consistency elastic. Colour yellowish in spirit. Ectosomal skeleton not reported. Choanosomal skeleton as reticulate network of thick, armed spongin fibres. Primary fibres (70  $\mu$ m in diameter) axially cored by large acanthostyles type I and echinated by small acanthostyles type II. Secondary fibres (40–50  $\mu$ m in diameter) less abundant, without spicules. Megascleres of one category, acanthostyles of two dimensional classes. Acanthostyles type I (320–400 × 14–17  $\mu$ m) ornate by 20 not accentuate whorls. Acanthostyles type II (55–120 × 6–10  $\mu$ m) ornate by 11–15 accentuate whorls. **Habitat.** Coral reefs, shade zone (caves, cavities). **Geographic distribution.** Western Indian Ocean. Recorded only from Mozambique Channel (eastern Madagascar). **Remarks.** de Voogd et al. (2008) focused on larger acanthostyles localized in the axial skeleton, and the smaller ones echinanting the fibres. Known only from the type locality, Grand Recife, Entsetreky (Tulear), Madagascar.

# Agelas braekmani Thomas, 1998

Fig. 8

**Description** (modified from Thomas 1998). Growth form tubular and irregularly branched 10 mm in diameter, branches tips blind or with apical oscule 2–4 mm in diameter, or sometime bearing a funnel-shaped opening with an orifice 30 mm in diameter. Consistency stiff, cork-like. Foreign particles as shell pieces embedded on the wall at place. Surface hispid. Ectosomal skeleton as erected spicules supporting the dermal membrane. Choanosomal skeleton as reticulate network of spiculous spongin fibres, mesh size 180–560  $\mu$ m. Primary fibres echinate and thick (up to 132  $\mu$ m in diameter) feeble cored by acanthostyles. Secondaries not cored, fairly common echinated by spicules (42–76  $\mu$ m). Megascleres verticillate of a single category. Acanthostyles 130–220



**Figure 8.** *Agelas braekmani.* **a** schematic drawings of two specimens **b** spicular complement of verticillate acanthostyles **c** skeleton architecture with echinate fibres, sponge surface on the right (modified from Thomas 1998).

 $(159) \times 4-21 \ \mu m \ (15 \ \mu m \ excluding spines; up to 25 \ \mu m \ with spines) ornamented by verticillate spines arranged as 8–18 whorls. Younger spicules partly annulated, each spine tubercled at the base.$ **Habitat.**Shallow water.**Geographic distribution.**Pacific Ocean. Recorded from Hansa Bay (type locality), Madang Province, Bismarck Sea.**Remarks.**This species was not considered in the revision by de Voogd et al. (2008).

# Agelas carpenteri (Gray, 1867)

Fig. 9

*Halichondria* (?) Carpenter, 1856 in Gray (1867) *Ectyon carpenteri* Gray, 1867

**Description** (modified from Gray 1867). Growth form massive. Ectosomal skeleton not reported. Choanosomal skeleton as a reticulate network of cylindrical spongin fibres echinated by single scattered spicules or groups of diverging spicules. Spicules fusiform, verticillate with *ca.* 10 whorls. **Habitat.** Not reported. **Geographic distribution.** Western Indian Ocean. Recorded only once from Madagascar. **Remarks.** This species is poorly described and illustrated. The spicule typology (oxeas or styles) are not reported by the author. Gray erected this species on the basis of what is shown by Carpenter (1856) in his book on microscopy.



Figure 9. Agelas carpenteri. Drawing of the reticulate network with spongin fibres echinated by verticillate spicules perpendicularly arranged (modified from Carpenter 1856).

# Agelas cavernosa Thiele, 1903

Fig. 10

**Description** (modified from Thiele 1903). Growth form irregularly massive with basal portion bearing digitiform outgrowths with a single, large oscule at the tips. Surface grainy with small stones or other foreign particulate. Colour blackish. Cavernous structure. Ectosomal skeleton not reported. Choanosomal skeleton as a dense network of brownish fibres. Fibres uncored, echinated by spicules. Megascleres of a single category. Acanthostyles ( $200 \times 15 \mu m$ ) verticillate, ornamented by 16 whorls of 15 spines each. **Habitat.** Tropical; not reported in detail. **Geographic distribution.** Ternate (type locality), Halmahera, Maluku Islands (Moluccas), eastern Indonesia. **Remarks.** The original description and particularly the illustration (only one spicule) are not exhaustive.



**Figure 10.** *Agelas cavernosa*. The original illustration of verticillate acanthostyles ornate by spiny whorls by Thiele (1903).



**Figure 11.** *Agelas ceylonica*. **a** very low quality image of the specimen studied by Dendy 1921 **b** schematic drawing of a branched specimen by Van Soest 1989 **c** skeleton architecture with echinate fibres **d–e** spicular complement of verticillate acanthostyles (**c** modified from Thomas 1981 **d** modified from Dendy 1921 **e** modified from Lévi 1961).

# Agelas ceylonica Dendy, 1905

Fig. 11

**Description** (modified from Dendy 1905, 1921, Lévi 1961, Thomas 1981). Growth form notably variable from ramose with slender subcylindrical digitiform branches with blunt tips (2–5 mm in diameter; 27 mm total height) to bushy, very irregular 60 × 41 ×

40 mm (Lévi 1961) or encrusting (20 × 15 mm, 1–3 mm in thickness) with oscules and pores not evident (Thomas 1981). Surface minutely hispid by spicules, irregular, conulose due to sharp, blunt, minute conules resulting from the protruding tips of ascending fibres. Consistency compressible, quite soft, elastic, resilient, fibrous and fairly tough in spirit. Colour brown, dark brown in spirit (Dendy 1905, 1921), orange (Lévi 1961), pale gray (Thomas 1981). Ectosomal skeleton as erect spicules (Thomas 1981). Choanosomal skeleton irregular network of pale-coloured spongin fibres (30 µm in diameter) cored by spicules and abundantly echinated by acanthostyles. Ascending primary and secondary fibres not well defined (20-60 µm in diameter), never cored by spicules (Thomas 1981). Megascleres asbelonging to two categories of styles: spiny, very variably verticillate and smooth. Acanthostyles 240  $\times$  20  $\mu$ m (Dendy 1905, 1921), 80 –275  $\times$  5–15  $\mu$ m with 16-21 verticilles and 100-300 × 6-15 µm with 13-23 verticilles (Lévi 1961), acanthostyles  $(240 \times 2 \,\mu\text{m})$  straight to slightly curved, with small, conic spines (Thomas 1981). Smooth styles  $(320 \times 24 \ \mu\text{m})$  less frequently present (Dendy 1921). Habitat. Not reported. Geographic distribution. Indian Ocean. Recorded from south India, Sri Lanka, Indonesia, and Seychelles. **Remarks.** Recently recorded from Indonesia (de Voogd et al. 2008). The latter authors report that acanthostyles in the Manaar Gulf (type locality) specimens have a maximum dimension of  $240 \times 20 \,\mu\text{m}$ .

#### Agelas dendromorpha Lévi, 1993

Fig. 12

**Description** (modified from Lévi 1993). Growth form bush-like (30–35 × 15–40 mm) branched, with main stem 9–10 mm in height, 3 mm in diameter. Branches with terminal buds (2–3 mm in diameter). Dermal membrane hispidate. Ectosomal skeleton as small acanthostyles in the dermal membrane. Choanosomal skeleton dense with spongin fibres echinated by spicules at the end of the main column or branches. Megascleres of a single category with two dimensional classes. Acanthostyles I (130–260 × 10–12  $\mu$ m) abundant, slightly curved, verticillate by 12–18 irregular whorls. Acanthostyles II (60–100 × 3–4  $\mu$ m) from the ectosome less abundant, with 8–9 less organized whorls. **Habitat.** Deep water, 245–275 m of depth. **Geographic distribution.** Western Pacific Ocean. Recorded only from the type locality in New Caledonia. **Remarks.** Also small oxea-like spiny spicules reported in original illustrations but not in descriptions. de Voogd et al. (2008) reported that verticillation is absent in smaller acanthostyles.

#### Agelas gracilis Whitelegge, 1897

Fig. 13

**Description** (modified from Whitelegge 1897, Lévi and Lévi 1989). Growth form subcylindrical, thin (2–3 mm in diameter; 25–75 mm in length) unbranched, settled



**Figure 12.** *Agelas dendromorpha*. **a** the sponge specimens of the type series **b** spicular complement of two spicular types, acanthostyles and acanthoxeas; small oxea-like spicules (bottom, right) are not reported in the original description (modified from Lévi 1993).



**Figure 13.** Agelas gracilis. **a** ramose slim dry specimen **b**–**c** spicular complement of verticillate acanthostyles of different dimensional categories (**a–b** modified from Lévi and Lévi 1983 **c** modified from Whitelegge 1897).

on shells fragments. Surface uneven, hispid, with numerous minute conules (0.2–0.5 mm in height, 2–5 mm apart). A few minute pores between the conules. Consistency soft, spongy but also a bit tough. Colour grevish-vellow in spirit. Ectosomal skeleton

soft, spongy but also a bit tough. Colour greyish-yellow in spirit. Ectosomal skeleton not reported. Choanosomal skeleton network irregularly reticulate of spongin fibres, with oval or oblong mesh, rarely angular. Primary fibres (70  $\mu$ m in diameter) with an axial plexus from which secondary (45  $\mu$ m in diameter) and tertiary fibres (25  $\mu$ m in diameter) are given off. Megascleres of single category. Acanthostyles (100–220 × 7–13  $\mu$ m) verticillate by 16 to 24 whorls of small spines (Whitelegge 1897). **Habitat.** Coral reef, 72–125 m of depth. **Geographic distribution.** Western Pacific Ocean. Recorded from Funafuti and Philippines. **Remarks.** Lévi and Lévi (1989) reported branched growth form, fibres (20–30  $\mu$ m in diameter), acanthostyles of two dimensional classes, type I 90–120 × 7–8  $\mu$ m with 9–12 verticilles, and type II 190–290 × 8–13  $\mu$ m with 17–21 verticilles. de Voogd et al. (2008) report that the specimen from the deeper subtidal (85–90 m) in the Philippines (Lévi and Lévi 1989) was ascribed to this species despite the divergence in spicular size.

# Agelas linnaei de Voogd, Parra-Velandia & Van Soest, 2008

Fig. 14

Description (modified from de Voogd et al. 2008). Growth form roundly lobate to thickly flabellate (8 cm in height, 2.5 cm in diameter). Consistency very soft, spongy. Colour bright orange at the surface to cream-orange internally. Surface with dense conules (1-3 mm in height) supported by tips of ascending fibres covered by a bright easily distinguishable membrane. Small apertures (< 2 mm) scattered on the surface, bigger pores (2-3 mm) connected to internal axial canals sometime present between some lobes. Choanosome dense with narrow canals (primary canals 200  $\mu$ m–2.00 mm in diameter; secondary canals 100 µm-1.00 mm in diameter). Ectosomal skeleton not reported. Choanosomal skeletal network irregularly and densely reticulate; primary fibres (35-80 µm in diameter) aggregated in packs, more or less undulated, heavily cored (1–7 spicules in cross section) and echinate; secondary fibres (25–40  $\mu$ m in diameter) not cored and less echinate. Megascleres of a single category. Acanthostyles  $(78.7-(187)-372.3 \times 5.2-(12.1)-24 \ \mu m)$  straight, a few slightly curved, ornate by 11-(19.3)-33 whorls with 5–12 spines each; whorls conspicuous in the spicule centre but sometimes faint and irregular at the spicule tip and head. Habitat. Coral reef, overgrowing other reef invertebrates. Geographic distribution. Recorded from the Thousands Islands Reef complex, off Jakarta, West Java, Indonesia. Type locality: Peniki Island and Payang Island. Remarks. de Voogd et al. (2008) focused on the whorl measurements performed in the middle third of the spicule because spine abundance is dependent upon the width. This is a useful rule to perform uniform measurements of whorls in verticillate spicules.



**Figure 14.** *Agelas linnaei.* **a** type series specimens (liquid preserved) **b** a living shallow water specimen **c** spongin skeleton with spicules **d** verticillate acanthostyles (modified from de Voogd et al. 2008).

# Agelas marmarica Lévi, 1958

Fig. 15

**Description** (modified from Lévi 1958, Vacelet and Vasseur 1965, Vacelet and Vasseur 1971). Growth form not recorded. Colour orange to/or bright red. Ectosomal skeleton not reported. Choanosomal skeleton as a reticulate network of fibres <30  $\mu$ m in diameter. Megascleres of a single category. Acanthostyles (230 × 10  $\mu$ m) verticillate by 19–21 whorls of spines (Lévi 1958). Acanthostyles type I 100–270 × 7–14  $\mu$ m with 21–25 whorls; acanthostyles type II 160–215 × 7–20  $\mu$ m with 16–24 whorls (Vacelet and Vasseur 1965). **Habitat.** Coral reef, 20–30 m of depth. **Geographic distribution.** Indian Ocean. Recorded from Mozambique Channel (Madagascar) and Red Sea. Type locality: Saudi Arabian Red Sea. **Remarks.** Spicules morph and their sizes are very variable in descriptions and illustrations of different authors.

# Agelas mauritiana (Carter, 1883)

Fig. 16, Table 1

Ectyon mauritiana Carter, 1883

**Description** (modified from Carter 1883, Thiele 1903, Dendy 1905, Laubenfels 1954, Lévi 1961, 1964, 1967, Vacelet and Vasseur 1965, 1971, Thomas 1979, 1998). Growth form highly variable: encrusting, tubular, massive, sometimes cavernous (Table 1) also ramose or massively branched 4.5 cm in height bearing five blunt branches 2 cm in di-



**Figure 15.** *Agelas marmarica.* **a–b** spicular complement (**a** modified from Vacelet and Vasseur 1965 **b** modified from Lévi 1958).



**Figure 16.** *Agelas mauritiana*. **a** drawing of a massive specimen **b** skeleton fragment **c**–**g** spicular complement **b**–**d** *A. mauritiana* var. *oxeata* **a** modified from Van Soest 1989; **b**–**d** modified from Thomas 1979 **c** modified from Carter 1883 **e** modified from Vacelet and Vasseur 1965 **f** modified from Lévi 1964 **g** modified from Lévi 1961).

ameter with a circular oscule at the tip. Surface with raised oscules 2–3 mm in diameter (Lévi 1961, Vacelet and Vasseur 1971). Consistency firm, resilient coriaceus, but also soft, compressible. Colour notably variable (Table 1). Surface distinctly conulose at growing portions, older ones smooth and glabrous (Thomas 1979). Ectosomal skeleton not reported. Choanosomal skeleton as a dense, irregularly reticulate network of stout fibres very abundantly echinated (Dendy 1905, Lévi 1961, Thomas 1979). Primaries and secondary fibres amber -coloured and not differentiated from each other (Thomas 1979). Megascleres as verticillate acanthostyles of two size classes. Acanthostyles 130–275 × 8–20  $\mu$ m with 10–20 whorls (Table 1). Smaller acanthostyles also reported (Table 1). **Habitat.** Not reported. **Geographic distribution.** Indian Ocean and West Pacific. Mascarene Archipelago (Mauritius is the type locality) and Seychelles Archipelago (Aldabra), Madagascar, Mozambique, Gulf of Aden, southern Red Sea, Gulf of Aqaba, Sri Lanka, and Australia. **Remarks.** Variable morphometries are reported by different authors (Table 1).

#### Agelas mauritiana var. oxeata Lévi, 1961

Fig. 16

**Description** (modified from Lévi 1961). Growth form lobed, each of the two lobes measures 20 mm in length and 8 mm in diameter. Colour brownish-red. Surface velvety. Choanosomal skeleton of primaries fibres cored by oxeas. Megascleres (100–220  $\times 4$ –18  $\mu$ m) of two categories. Acanthostyles verticillate with 14–16 whorls. Acanthoxeas verticillate with 20–24 whorls. **Habitat.** Shallow water, 12 m of depth. **Geographic distribution.** Indian Ocean. Recorded only from Aldabra Island (type locality) in the Seychelles Archipelago. **Remarks.** This variety was not considered in the revision by de Voogd et al. (2008).

# Agelas nakamurai Hoshino, 1985

Fig. 17

**Description** (modified from Hoshino 1985). Growth form massive, rounded or thickly encrusting. Colour orange rufous to brick red at the surface in dry specimens. Surface smooth, uneven, with irregularly meandering surface grooves. Consistency firmly spongy, resilient when wet; very hard, hardly compressible when dry. Ectosomal skeleton armed by tangential acanthostyles irregularly distributed. Choanosomal skeleton as a reticulate network with elliptical meshes (40–200  $\mu$ m in diameter) of primary ascending spongin fibres and secondaries. Primary fibres (70–100  $\mu$ m in diameter) cored by 4–8 acanthostyles, and echinated by the same acanthostyles. Secondary fibres (20–70  $\mu$ m in diameter) not cored, in places echinated by acanthostyles. Megascleres of a single category. Acanthostyles 185–(226)–267 × 8–(12)–15  $\mu$ m, straight to slight-



**Figure 17.** *Agelas nakamurai.* **a** skeleton architecture **b** close up of spicules insertion in the spongin fibres **c** acanthostyles (modified from Hoshino 1985).

ly curved, ornate by 15–23 whorls with eight spines each. **Habitat.** Shallow water, 20 m depth. **Geographic distribution.** Pacific Ocean. South Kuroshio, east Japan. Zamami Island (type locality), Ryukyu Archipelago, Japan. **Remarks.** Also recorded from Indonesia (de Voogd et al. 2008).

# Agelas nemoechinata Hoshino, 1985

# Fig. 18

**Description** (modified from Hoshino 1985). Growth form massive or thickly encrusting.

Consistency spongy, resilient when wet, hard and difficult to compress when dry. Colour madder brown at the surface to rufous-orange in the interior when dry. Oscules from 1–3 to 5–8 mm in diameter. Ectosomal skeleton not reported. Choanosomal skeleton reticulate network with elliptical meshes (50–250  $\mu$ m in diameter) of spongin fibres. Primary and secondary fibres almost indistinguishable (20–50  $\mu$ m in diameter) and cored by 1–3 acanthostyles, occasionally not cored, only slightly echinated. Megascleres verticillate of a single category. Acanthostyles 170–(189)–210 × 9–(11)–13  $\mu$ m, straight to gently curved, ornate by 16–23 regular whorls, each with eight spines. **Habitat.** Shallow water, 20 m depth. **Geographic distribution.** North Pacific Ocean. Recorded from south Kuroshio (east Japan). Type locality: Zamami Island, Ryukyu Archipelago. **Remarks.** Also recorded from Indonesia (de Voogd et al. 2008). These authors report acanthostyles occasionally sharply pointed at both ends (oxeas), as previously reported also in the original figures.



Figure 18. Agelas nemoechinata. a skeleton architecture b spicular complement (modified from Hoshino 1985).

#### Agelas novaecaledoniae Lévi & Lévi, 1983

#### Fig. 19

**Description** (modified from Lévi and Lévi 1983). Growth form as a thick blade (18 cm in height, 12.5 cm in diameter, 1–4 cm in thickness). Consistency elastic. Colour ochre brown. Surface irregularly cavernous and strongly hispid. Oscules (2–3 mm in diameter) numerous, 3–8 mm apart. Ectosomal skeleton armed by spicules. Choanosomal skeleton as a reticulate network of fibres (100 × 20  $\mu$ m in diameter) laminar and fibrillar, rarely echinate at the sponge basal portion. Megascleres of two categories. Acanthostyles 100–190 × 5–8  $\mu$ m (7–10 with spines) straight or slightly curved, verticillate by 12–17 whorls of spines. Acanthoxeas slightly curved (120–250 × 5  $\mu$ m) verticillate by 12–18 whorls of spines. Habitat. Deep water, 390–395 m of depth. Geographic distribution. Pacific Ocean. Recorded from New Caledonia. Remarks. Known only from the type locality.

# Agelas robusta Pulitzer-Finali, 1982

Fig. 20

**Description** (modified from Pulitzer-Finali 1982). Growth form encrusting (5 mm in thickness) to massive, roundish ( $6 \times 3$  cm in diameter). Cavernous (clathrous) structure,



Figure 19. Agelas novaecaledoniae. **a** type specimen **b** spicular complement with two spicular types (modified from Lévi and Lévi 1983).

with narrow channels through the entire sponge. Consistency tough and resilient. Colour dull orange, dull yellowish-brown. Ectosomal skeleton not reported. Choanosomal skeleton reticulate with irregular meshes of pale spongin fibres (38–80  $\mu$ m in diameter) abundantly echinate by acanthostyles. Megascleres of a single category. Acanthostyles very stout, verticillate (170–250 × 14–30  $\mu$ m spine included) with 11–12 whorls of short, blunt spines. **Habitat.** Not reported. **Geographic distribution.** Pacific Ocean. Recorded from Hong Kong, Southern China. **Remarks.** Known only from the type locality. At present for this species only three slides are available after damaging of type materials during the recent Genova flood in 2014 (October).

# Agelas semiglabra Pulitzer-Finali, 1996

Fig. 21

Agelas semiglaber Pulitzer-Finali, 1996



**Figure 20.** *Agelas robusta.* **a** type specimen (dry) **b** spicular complement of acanthostyles very stout, verticillate by blunt spines (modified from Pulitzer-Finali 1982).

**Description** (modified from Pulitzer-Finali 1996). Growth form encrusting, very small. Skeleton architecture not reported. Megascleres of single category and two size classes. Acanthostyles type I verticillate  $(230-375 \times 11-16 \ \mu\text{m})$ , spiny only towards the tips. Acanthostyles type II (75–100 × 3.5  $\mu$ m) verticillate, entirely spiny. **Habitat.** Shallow water. **Geographic distribution.** Pacific Ocean. Recorded from Bismarck Sea, known only from Papua New Guinea (type locality). **Remarks.** The specific epithet ending with *–er* is masculine despite the gender *Agelas* is feminine, as a consequence the ending must be changed into *–ra*. Because of the overlap in spicule dimension de Voogd et al. (2008) report that is not possible to distinguish different size categories.



Figure 21. Agelas semiglabra. Spicular complement with two dimensional categories of acanthostyles; long acanthostyles spiny only at the tips (modified from Pulitzer-Finali 1996).

# Agelas spp.

Several findings from the Seychelles Archipelago (Thomas 1973) and Kenya (Pulitzer-Finali 1993). The revisitation of the 11 *Agelas* specimens slides of the Pulitzer-Finali collection from East Africa highlighted the presence of only styles in the spicular complement with notable variability of thickness, length, and spinosity. This confirms his opinion: 'It would be inappropriate at the moment to try to identify some of them with established species and to propose new species.' Also in this case the original material is not available after damaging of types during the recent Genova flood in 2014 (October).

RESEARCH ARTICLE



# A new quadrannulate species of Orobdella (Hirudinida, Arhynchobdellida, Orobdellidae) from western Honshu, Japan

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# Abstract

A new quadrannulate species of *Orobdella* Oka, 1895, *Orobdella naraharaetmagarum* **sp. n.**, from the mountainous region of western Honshu, Japan is described. *Orobdella naraharaetmagarum* is a small species with a body length of less than 5 cm. Phylogenetic analyses using nuclear 18S rRNA and histone H3, as well as mitochondrial cytochrome *c* oxidase subunit I, tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, 12S rRNA, tRNA<sup>Val</sup>, 16S rRNA, tRNA<sup>Leu</sup> and NADH dehydrogenase subunit 1 markers indicated that the present new species is the sister species of the quadrannulate *Orobdella esulcata* Nakano, 2010. Furthermore, mitochondrial DNA genealogy within *O. naraharaetmagarum* demonstrated that this new species is divided into eastern and western lineages.

# **Keywords**

Hirudinea, Orobdella, new species, gastroporous, molecular phylogeny, Japan

# Introduction

The terrestrial macrophagous leech genus *Orobdella* Oka, 1895 contains 12 species that are distributed throughout the Japanese Archipelago, Korean Peninsula, and Taiwan (Nakano 2014, Nakano and Lai 2012, Nakano and Seo 2014). These 12 species are split into three groups based on their mid-body somite annulation: seven species in the quadrannulate (four annuli) group, four in the sexannulate (six annuli) group, and one octannulate (eight annuli) species.

*Orobdella* leeches had been considered large species, with body lengths reaching to 10 cm or greater (Sawyer 1986). In recent years, however, small mature leeches belonging to this genus have been discovered in Japan and described as new species: *Orobdella koikei* Nakano, 2012b from Hokkaido, and *Orobdella masaakikuroiwai* Nakano, 2014 from central Honshu. The bodies of mature individuals of these two species are shorter than 4 cm. Both species possess mid-body somites that are quadrannulate. Nakano (2014) suggested that differences in the body lengths of mature leeches might enable more than one species of *Orobdella* to coexist in the same region.

Additional small *Orobdella* leeches were collected from Chugoku District, western Honshu, Japan. The body lengths of the specimens were less than 5 cm. Nevertheless, a few individuals were regarded as mature leeches because they possessed an obvious clitellum. These specimens are described here as a new species. In addition, the phylogenetic position of this new species was estimated using nuclear 18S rRNA and histone H3, as well as mitochondrial cytochrome *c* oxidase subunit I, tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, 12S rRNA, tRNA<sup>Val</sup>, 16S rRNA, tRNA<sup>Leu</sup>, and NADH dehydrogenase subunit 1 sequence data.

# Materials and methods

#### Sampling and morphological examination

Leeches were collected from five localities in Chugoku district, western Honshu, Japan (Fig. 1). When possible, elevation and geographical coordinates for localities were obtained using a Garmin eTrex<sup>®</sup> GPS unit.

Almost all of the specimens were relaxed by the gradual addition of absolute ethanol (EtOH) to freshwater. For DNA extraction, botryoidal tissue was removed from the posterior part of the body around the caudal sucker of every specimen, and then preserved in absolute EtOH. The remainder of the body was fixed in 10% formalin and preserved in 70% EtOH. Four measurements were taken: body length (BL) from the anterior margin of the oral sucker to the posterior margin of the caudal sucker, maximum body width (BW), caudal sucker length (CL) from the anterior to the posterior margin of the sucker and caudal sucker width (CW) from the right to the left margin of the sucker. Examination, dissection, and drawing of the specimens were conducted using a stereoscopic microscope with a drawing tube (Leica M125). Specimens used in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).



**Figure 1.** Map showing the collection localities of the specimens examined in this study. Open circle (**5**) indicates the type locality of the new species, *Orobdella naraharaetmagarum* sp. n., and closed circles (**1–4**) indicate additional localities.

The numbering convention is based on Moore (1927): body somites are denoted by Roman numerals, and the annuli in each somite are given alphanumeric designations.

# PCR and DNA sequencing

The extraction of genomic DNA from botryoidal tissues preserved in absolute EtOH followed Nakano (2012b). Primer sets for the PCR and cycle sequencing (CS) reactions used in this study were as follows: for 18S rRNA, A and L (PCR and CS), C and Y (PCR and CS), as well as O and B (PCR and CS) (Apakupakul et al. 1999); for histone H3 (H3), H3aF and H3bR (PCR and CS) (Colgan et al. 1998); for cytochrome *c* oxidase subunit I (COI), LCO 1490 and HCO 2198 (PCR and CS) (Folmer et al. 1994), and LCO-in (Nakano 2012b) and HCO-outout (Nakano 2012a) (PCR and CS), or LCO-inerpo2 (5'-GCTATTACAATATTACTTACAGATCG-3'; this study) and HCO-out (Nakano 2012b) (PCR and CS); for tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, 12S rRNA, tRNA<sup>Val</sup> and 16S rRNA (tRNA<sup>Cys</sup>–16S), 12SA-out and 12SB-in (PCR and CS), and 12SA-in and 12SB-out (Nakano 2012b) or 12SB-outin (5'-AAAGGTACGAATATATTTAC-3'; this study) (PCR and CS); for tRNA<sup>Leu</sup> and NADH dehydrogenase subunit 1 (ND1) (tRNA<sup>Leu</sup>–

ND1), LDN3000 and HND1932 (PCR and CS) (Light and Siddall 1999). The PCR reactions and DNA sequencing were performed using the modified method mentioned in Nakano (2012a). The PCR reactions were performed using a GeneAmp PCR System 2700 and a GeneAmp PCR System 9700 (Applied Biosystems) as well as a T100 Thermal Cycler (Bio-Rad). The PCR mixtures were heated to 94 °C for 5 min, followed by 35 cycles at 94 °C (10 s each), 52 °C for 18S and H3, 60 °C, and 44 °C, respectively, for the anterior, and posterior parts of tRNA<sup>Cys</sup>–16S or 42 °C for COI and tRNA<sup>Leu</sup>–ND1 (20 s), and 72 °C (42 s each), and a final extension at 72 °C for 6 min. The sequencing mixtures were heated 96 °C for 2 min, followed by 40 cycles at 96 °C (10 s each), 50 °C (5 s each) and 60 °C (48 s each). The obtained sequences were edited using DNA BAS-ER (Heracle Biosoft S.R.L.). The DNA sequences listed in Table 1 were newly obtained in this study and were deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan (DDBJ).

# Molecular phylogenetic and genetic distance analyses

Eighty published sequences were obtained from the INSDC for use in molecular phylogenetic analyses (Table 1). In addition to 12 known *Orobdella* species, the following four erpobdelliform species were used as outgroup taxa: *Erpobdella japonica* Pawłowski, 1962 (Erpobdellidae), *Gastrostomobdella monticola* Moore, 1929 (Gastrostomobdellidae), *Mimobdella japonica* Blanchard, 1897, and *Odontobdella blanchardi* (Oka, 1910) (both Salifidae).

The phylogenetic position of the newly identified *Orobdella* species within the genus was estimated based on 18S, H3, COI, tRNA<sup>Cys</sup>–16S, and tRNA<sup>Leu</sup>–ND1 sequences. The alignments of H3 and COI were trivial, as no indels were observed. 18S, tRNA<sup>Cys</sup>–16S, and tRNA<sup>Leu</sup>–ND1 were aligned using MAFFT v. 7.245 L-INS-i (Katoh and Standley 2013). The lengths of the 18S, H3, COI, tRNA<sup>Cys</sup>–16S, and tRNA<sup>Leu</sup>–ND1 sequences were 1,844, 328, 1,267, 1,120, and 633 bp, respectively. The concatenated sequences yielded 5,192 bp of aligned positions.

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). ML phylogenies were constructed using RAxML v. 8.1.5 (Stamatakis 2014) with the substitution model set as GTRCAT, immediately after nonparametric bootstrapping (Felsenstein 1985) conducted with 1,000 replicates. The best-fit partitioning scheme for the ML analyses was identified with the Akaike information criterion (Akaike 1974) using PartitionFinder v. 1.1.1 (Lanfear et al. 2012) with the "greedy" algorithm: 18S/the 1<sup>st</sup> and 2<sup>nd</sup> positions of H3/the 3<sup>rd</sup> position of H3/the 1<sup>st</sup> position of COI/the 2<sup>nd</sup> position of COI/the 3<sup>rd</sup> positions of COI and ND1/the 1<sup>st</sup> position of ND1/the 2<sup>nd</sup> position of ND2/12S/16S/tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, tRNA<sup>Val</sup> and tRNA<sup>Leu</sup>.

BI and Bayesian posterior probabilities (BPPs) were estimated using MrBayes v. 3.2.5 (Ronquist et al. 2012). The best-fit partition scheme and models for each partition were selected based on the Bayesian information criterion (Schwarz 1978) using PartitionFinder with the "greedy" algorithm: for 18S and the 1<sup>st</sup> position of H3, K80+I; for the 2<sup>nd</sup> position of H3, JC69; for the 3<sup>rd</sup> position of H3, HKY85; for the
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Species	Voucher (locality number)	185	Histone H3	COI	tRNA <sup>Cys</sup> -16S	tRNA <sup>Leu</sup> –ND1
Orobdella naraharaetmagarum sp. n.	KUZ Z1360 (4)			LC087131*	LC087130*	LC087132*
Orobdella naraharaetmagarum sp. n.	KUZ Z1380 (2)			LC087134*	LC087133*	LC087135*
Orobdella naraharaetmagarum sp. n.	KUZ Z1535 (1)			LC087137*	LC087136*	LC087138*
Orobdella naraharaetmagarum sp. n.	KUZ Z1582 Paratype (5)			LC087140*	LC087139*	LC087141*
Orobdella naraharaetmagarum sp. n.	KUZ Z1652 Holotype (5)	LC087143*	LC087145*	LC087144*	LC087142*	LC087146*
Orobdella naraharaetmagarum sp. n.	KUZ Z1654 Paratype (5)			LC087148*	LC087147*	LC087149*
Orobdella naraharaetmagarum sp. n.	KUZ Z1655 (3)			LC087151*	LC087150*	LC087152*
Orobdella dolichopharynx Nakano, 2011b	KUZ Z120 Holotype	AB663665	AB698876	AB679680	AB679681	AB828558
Orobdella esulcata Nakano, 2010	KUZ Z29 Holotype	AB663655	AB698873	AB679664	AB679665	AB828555
Orobdella ijimai Oka, 1895	KUZ Z110 Topotype	AB663659	AB698877	AB679672	AB679673	AB828559
Orobdella kawakatsuorum Richardson, 1975	KUZ Z167 Topotype	AB663661	AB698878	AB679704	AB679705	AB828561
Orobdella ketagalan Nakano & Lai, 2012	KUZ Z208 Holotype	AB704785	AB704786	AB704787	AB828582	AB828563
<i>Orobdella koikei</i> Nakano, 2012b	KUZ Z156 Holotype	AB698883	AB698882	AB679688	AB679689	AB828560
Orobdella masaakikuroiwai Nakano, 2014	KUZ Z694 Holotype	AB938003	AB938013	AB938006	AB937997	AB938016
Orobdella mononoke Nakano, 2012a	KUZ Z224 Holotype	AB698868	AB698869	AB698866	AB698867	AB828564
Orobdella octonaria Oka, 1895	KUZ Z181 Topotype	AB698870	AB698871	AB679708	AB679709	AB828562
Orobdella shimadae Nakano, 2011b	KUZ Z128 Holotype	AB663663	AB698875	AB679676	AB679677	AB828557
Orobdella tsushimensis Nakano, 2011a	KUZ Z134 Holotype	AB663653	AB698872	AB679662	AB679663	AB828554
Orobdella whitmani Oka, 1895	KUZ Z45 Topotype	AB663657	AB698874	AB679668	AB679669	AB828556
Erpobdella japonica Pawłowski, 1962	KUZ Z178	AB663648	AB698879	AB679654	AB679655	AB828542
Gastrostomobdella monticola Moore, 1929	UNIMAS/A3/BH01/10	AB663649	AB698880	AB679656	AB679657	AB828543
Mimobdella japonica Blanchard, 1897	KUZ Z179	AB663650	AB698881	AB679658	AB679659	AB828544
Odontobdella blanchardi (Oka, 1910)	KUZ Z180	AB663651	AB938012	AB938004	AB937995	AB938014

1<sup>st</sup> position of COI, GTR+G; for the 2<sup>nd</sup> positions of COI and ND1, HKY85+I; for the 3<sup>rd</sup> positions of COI and ND1 plus 16S, HKY85+I+G; and for the 1<sup>st</sup> position of ND1, and 12S, tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, tRNA<sup>Val</sup> and tRNA<sup>Leu</sup>, GTR+I+G. Two independent runs of four Markov chains were conducted for 12 million generations, and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Tracer v. 1.6.0 (Rambaut and Drummond 2009) and the first 30,001 trees were discarded based on these results.

The phylogenetic relationships within the available Orobdella materials were estimated based on sequences of COI, tRNA<sup>Cys</sup>-16S and tRNA<sup>Leu</sup>-ND1. tRNA<sup>Cys</sup>-16S and tRNA<sup>Leu</sup>–ND1 were aligned using MAFFT L-INS-i. The lengths of the COI, tRNA<sup>Cys</sup>– 16S, and tRNA<sup>Leu</sup>-ND1 sequences were 1,267, 634, and 1,107 bp, respectively. The concatenated sequences yielded 3,008 bp of aligned positions. ML phylogenies were constructed in RAxML with the substitution model set as GTRCAT, immediately after nonparametric bootstrapping conducted with 1,000 replicates. The best-fit partitioning scheme was identified with the Akaike information criterion using PartitionFinder with the "greedy" algorithm: the 1st position of COI/the 2nd positions of COI and ND1/the 3<sup>rd</sup> positions of COI and ND1/the 2<sup>nd</sup> position of ND1/the 1<sup>st</sup> position of ND1/tRNA-<sup>Cys</sup>, tRNA<sup>Met</sup>, tRNA<sup>Val</sup>, tRNA<sup>Leu</sup>/12S/16S. BI and BPPs were estimated using MrBayes. The best-fit partition scheme and models for each partition were selected based on the Bayesian information criterion using PartitionFinder with the "greedy" algorithm: for the 1<sup>st</sup> positions of COI and ND1, GTR+I+G; for the 2<sup>nd</sup> positions of COI and ND1, F81+I; for the 3<sup>rd</sup> positions of COI and ND1 plus 16S, HKY+G; tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, 12S, tRNA<sup>Val</sup> and tRNA<sup>Leu</sup>, GTR+I+G. Two independent runs of four Markov chains were conducted for 10 million generations and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Traced, and the first 25,001 trees were discarded based on these results.

Nodes with bootstrap support (BS) values higher than 70% were considered sufficiently resolved (Hillis and Bull 1993). Nodes with BPPs higher than 95% were considered statistically significant (Leaché and Reeder 2002).

Pairwise comparisons of uncorrected *p*-distances for seven COI sequences (1,266 bp) obtained from specimens of the studied species and *Orobdella esulcata* Nakano, 2010 were calculated using MEGA6.06 (Tamura et al. 2013).

#### Taxonomy

Family Orobdellidae Nakano et al., 2012

http://zoobank.org/5F5BABE8-BD26-4FC7-9593-F73E62E26122

#### Genus Orobdella Oka, 1895

http://zoobank.org/FA8333ED-8C17-41FD-AFC1-62A4F98D4AC1

#### Orobdella naraharaetmagarum sp. n.

http://zoobank.org/5A831984-50F6-433A-A058-ED2ECFF2DFDC Figs 2–5

**Diagnosis.** Body length of mature individual less than 5 cm. Somite IV uniannulate, somites VIII–XXV quadrannulate. Male gonopore in middle of XI b6, female gonopore in middle of XIII a1, behind gastropore, gonopores separated by 1/2 + 4 + 1/2 annuli. Clitellum in XI b5 to XIII a2. Pharynx reaching to XIII b5/b6–XIII/XIV. Gastropore conspicuous, in middle of XIII a1. Gastroporal duct bulbous, slightly winding at junction with gastropore. Paired epididymides in XIV b6–XV b5 to XVIII b6–XX a2/b5, occupying 16–20 annuli (four to five somites). Atrial cornua developed, ellipsoid or ovate.

**Type materials. Holotype.** KUZ Z1652, dissected, collected from under a rock along a mountain trail at Mt. Kanmuriyama, Hatsukaichi, Hiroshima Pref., Japan (34.47325°, 132.10362°; Elev. 757 m; locality number 5), by TN on 25 April 2015. **Paratypes.** Two specimens from near the type locality, along a forest road, "Japan National Route 488", Hatsukaichi, both dissected: KUZ Z1582, under a rock (34.50118°, 132.08933°; Elev. 790 m; locality number 5), by Yoshiko Yamane on 10 August 2014, and KUZ Z1654, under a rotten tree (34.50182°, 132.08961°; Elev. 791 m; locality number 5), by TN on 16 June 2015. For locality numbers, see Fig. 1.

Additional materials. In total four specimens were examined, all dissected. Three specimens collected from Hiroshima Pref., Japan: KUZ Z1360, from Hosomi, Kitahiroshima (34.685°, 132.292°; Elev. 470 m; locality number 4), by Yukiko Narahara on 9 July 2011; KUZ Z1380, from Mt. Azumayama, Hiwacho, Shobara (35.0639°, 133.0268°; Elev. 1010 m; locality number 2), by Ayane Maga on 3 October 2011; and KUZ Z1655, from under a rock along a mountain trail at Mt. Izaiyama, Hiwacho-Mitsugaichi, Shobara (35.00143°, 133.04640°; Elev. 906 m; locality number 3), by TN on 17 June 2015. KUZ Z1535, collected from under a rock along a mountain trail at Mt. Iimoriyama, Noigura, Kotoura, Tottori Pref., Japan (35.37603°, 133.59953°; Elev. 619 m; locality number 1), by TN on 11 December 2013. For locality numbers, see Fig. 1.



**Figure 2.** Orobdella naraharaetmagarum sp. n., holotype, KUZ Z1652. **A** Dorsal and **B** ventral views. Scale bar: 5 mm.



**Figure 3.** Orobdella naraharaetmagarum sp. n., holotype, KUZ Z1652. **A** Dorsal and **B** ventral views of somites I–VIII. **C** Dorsal and **D** ventral views of somites XXIV–XXVII and caudal sucker **E** Ventral view of somites XI–XIII **F** Ventral view of gastropore and female gonopore **G** Ventral view of gastroporal duct. Scale bars: 1 mm (**C–E, G**), 0.5 mm (**A, B**) and 0.25 mm (**F**). Abbreviations: af, annular furrow; an, anus; cp, crop; fg, female gonopore; gd, gastroporal duct; gp, gastropore; mg, male gonopore; np, nephridiopore; and ph, pharynx.

**Etymology.** The specific name is a noun in the genitive case formed directly from the names of Ms Yukiko Narahara and Ms Ayane Maga, who collected specimens of this new species. Its stem is determined as "naraharaetmag" herein.

**Description of holotype.** Body firm and muscular, elongate, with constant width in caudal direction, dorsoventrally compressed, BL 40.0 mm, BW 5.3 mm (Fig. 2). Caudal sucker ventral, elliptic, CL 2.4 mm, CW 3.0 mm (Figs 2B, 3D).

Somite I completely merged with prostomium (Fig. 3A). Somites II–IV uniannulate, II not separate from I (Fig. 3A). Somite V biannulate, (a1 + a2) > a3; a3 forming posterior margin of oral sucker (Fig. 3A, B). Somites VI and VII triannulate, a1 = a2 = a3 (Fig. 3A, B). Somites VIII–XXV quadrannulate, a1 = a2 = b5 = b6 (Fig. 3A–E). Somite XXVI dorsally triannulate, a1 > a2 < a3, a3 with slight furrow; ventrally biannulate, (a1 + a2) > a3, (a1 + a2) with slight furrow; (a1 + a2) being ventrally last complete annulus (Fig. 3C, D). Somite XXVII uniannulate with slight dorsal furrow; anus behind it with no post-anal annulus (Fig. 3C).

Male gonopore in middle of XI b6 (Fig. 3E). Female gonopore slightly anterior to middle of XIII a1, inconspicuous, located posterior to gastropore (Fig. 3E, F). Gonopores separated by 1/2 + 4 + 1/2 annuli (Fig. 3E).

Anterior ganglionic mass in VI a2 and a3. Ganglion VII in a2. Ganglia VIII–X, of each somite, in a2. Ganglia XI and XII, of each somite, in a2 (Fig. 4A). Ganglion XIII in a2 and b5 (Fig. 4A). Ganglion XIV in a2 (Fig. 4A). Ganglia XV–XX, of each somite, in a1 and a2 (Fig. 4A). Ganglia XXI–XXIII, of each somite, in a2. Ganglion XXIV in a1. Ganglion XXV in b6 of XXIV and a1 of XXV. Ganglion XXVI in b5 and b6 of XXV. Posterior ganglionic mass in (a1 + a2) of XXVI.

Eyes in three pairs, first pair dorsally on posterior margin of II, second and third pairs dorsolaterally on posterior margin of V (a1 + a2) (Fig. 3A). Papillae numerous, minute, hardly visible, one row on every annulus.

Nephridiopores in 17 pairs, one each situated ventrally at posterior margin of a1 of each somite in VIII–XXIV (Fig. 3B, D, E).

Pharynx agnathous, euthylaematous, reaching to XIII b5/b6 (Fig. 3G). Crop tubular, acecate, reaching to XIX b5/b6. Gastropore conspicuous, ventral, slightly anterior to middle of XIII a1 (Fig. 3E, F). Gastroporal duct bulbous, slightly winding at junction with gastropore, joining with crop in XIV a1 (Fig. 3G). Intestine tubular, acecate, reaching to XXIV a1. Rectum tubular, thin-walled, straight.

Testisacs multiple (Fig. 4A); on right side, in XVIII b6 to XXIV a2, in total app. 28 testisacs, 1 in XVIII, 3 in XIX, 5 in XX, 4 in XXI, 6 in XXII, 7 in XXIII, 2 in XXIV; on left side, in XIX a1 to XXV a2, in total app. 27 testisacs, 5 in XIX, 6 in XX, 4 in XXI, 4 in XXII, 4 in XXIII, 3 in XXIV, 1 in XXV. Paired epididymides; right epididymis in XIV b6 to XVIII b6, occupying 17 annuli; left epididymis in XIV/XV to XVIII b6, occupying 16 annuli (Fig. 4A). Ejaculatory bulbs absent. Paired ejaculatory ducts; right duct in XI a2/b5 to XIV b6; left duct in XI a2/b5 to XIV/XV; coiled in position posterior to ovisacs; each duct crossing ventrally beneath each ovisac, then curved in position anterior to ovisacs; each widening from respective junction with epididymis, narrowing at junction with atrial cornua, then turning sharply inward toward atrial cornua without pre-atrial loop (Fig. 4A). Pair of muscular atrial cornua ellipsoid, in XI b5 and b6 (Fig. 4A–D). Atrium short, muscular, globular in XI b5 and b6 (Fig. 4B–D). Penis sheath and penis absent.

Paired ovisacs globular; right ovisac in XIII a2 and b5; left ovisac in XIII a1 and a2 (Fig. 4A, E). Oviducts, thin-walled, left oviduct crossing ventrally beneath nerve cord; both oviducts converging into common oviduct in XIII a2 (Fig. 4A, E). Common oviduct thin-walled, short, directly descending to female gonopore (Fig. 4E).

Variations. BL 48.2 (KUZ Z1654)–33.0 (KUZ Z1360) mm, BW 4.4 (KUZ Z1535)–2.7 (KUZ Z1380) mm, CL 2.5 (KUZ Z1654)–1.2 (KUZ Z1380) mm, CW



**Figure 4.** Orobdella naraharaetmagarum sp. n., holotype, KUZ Z1652. **A** Dorsal view of reproductive system including ventral nervous system. **B** Dorsal (including positions of ganglia XI and XII), **C** lateral, and **D** ventral views of male atrium. **E** Dorsal view of female reproductive system including position of ganglion XIII. Scale bars: 2 mm (**A**), 0.5 mm (**B–D**) and 0.25 mm (**E**). Abbreviations: ac, atrial cornua; at, atrium; cod, common oviduct; ed, ejaculatory duct; ep, epididymis; gp, gastropore; od, oviduct; ov, ovisac; and ts, testisac.

3.0 (KUZ Z1654)-1.8 (KUZ Z1360, Z1380) mm. Somite XXVI triannulate, a1 = a2 < a3 (KUZ Z1380, Z1582); KUZ Z1360, Z1535, Z1654, Z1655, a3 with slight dorsal furrow. Somite XXVII uniannulate or biannulate (KUZ Z1360). Male gonopore generally in middle of XI b6, rarely slightly anterior or posterior to middle of XI b6. Female gonopore in middle of XIII a1, slightly anterior or posterior to middle of XIII a1. X b5 and XIII a2, respectively, being first and last annuli of clitellum. Eyes generally three pairs; KUZ Z1654, Z1655, first pair dorsally on anterior margin of III; KUZ Z1582, multiple eyes detected, one eye on left dorsal of II/III, one small eyespot on right dorsal of III, one small eyespot on left dorsal of III/IV, one small eyespot on right dorsal of IV, and two pairs of eyes dorsolaterally on posterior margin of V (a1 + a2). Pharynx reaching to XIII b5/b6-XIII/XIV. Crop reaching to XIX b5/b6-XX a1. Gastropore in middle of XIII a1, slightly anterior or posterior to middle of XIII a1. Gastroporal duct generally bulbous; KUZ Z1360, Z1582, tubular. Intestine reaching to XXIII/XXIV–XXIV/XXV. Testisacs multiple; right side app. 11–24 sacs in XIX b6– XX b5 to XXIV b5-b6; left side app. 11-23 sacs in XIX b6-XX a2 to XXIII b6-XXV a1. Paired epididymides; right epididymis in XV a1-XV b5 to XIX a2-XX a2/b5, occupying 17–20 annuli; left epididymis in XV a1–XV b5 to XIX b5–XX a2, occupying 17-20 annuli. Paired ejaculatory ducts, curved, loosely curved, or straight in position posterior to ovisacs. Atrial cornua ovate, fusiform, or ellipsoid in XI b5 and b6; KUZ Z1535 in XI b5-XII a. Atrium generally in XI b5 and b6; KUZ Z1535, Z1582 in XI b6. Paired ovisacs generally in XIII a2 and b5; KUZ Z1535, undeveloped, in XIII a2. Right or left oviduct crossing ventrally beneath nerve cord; KUZ Z1380 both oviducts converging into common oviduct in XIII a1/a2.

**Coloration.** In life, dorsal surface bluish gray (Fig. 5A, B), or gray; ventral surface reddish white or ash gray; clitellum, when obvious, whitish gray (Fig. 5B). Color faded in preservative; KUZ Z1535 with one dorsal black line from VIII b5 to XXVI a2.

**Distribution.** This species was primarily collected in Hiroshima Prefecture in Chugoku District, Honshu, Japan, and in Tottori Prefecture. The lowest elevation among the localities was 470 m, and the highest was 1010 m. The locality data for this species suggested that it is distributed in mountainous regions in Chugoku District, Honshu, Japan.

**Natural history.** This species was generally found curled up under rocks or rotten trees in moist mountainous habitats (Fig. 5 C, D). Oligochaete worms were sometimes observed in the digestive tract during specimen dissection. Therefore, this species is an earthworm-eater, as are the other known *Orobdella* leeches.

A mature leech with an obvious clitellum was collected on 16 June. Moreover, the holotype, which appeared to have a clitellum (Fig. 5A), was collected on 25 April. These findings suggest that the reproductive season of the new species begins in May and then continues at least to mid-to-late June.

**Remarks.** The new species unambiguously belongs to *Orobdella* as it has all the generic diagnostic characteristics defined in Nakano et al. (2012): post-anal annulus absent; pharynx agnathous, euthylaematous; gastropore in XIII; gastroporal duct lying on female organ; gonopores separated by more than one full somite; testisacs multiple;



**Figure 5.** Orobdella naraharaetmagarum sp. n., holotype, KUZ Z1652 (**A**, **D**) and paratype, KUZ Z1654 (**B**). Habitat of holotype (**C**). **A** and **B** Dorsal views of live animals. **D** Live animal found curled up under a stone at the type locality. Scale bars: 5 mm.

male atrium in XI without penis sheath and penis; ovisacs globular in XIII; female median reproductive system essentially lacking.

The specimens were small (up to 48 mm). However, one leech (KUZ Z1654) was determined to be mature because it possessed an obvious clitellum. The holotype, which had a body length of 40 mm, also possessed a slightly developed clitellum and developed testisacs. Two specimens (KUZ Z1360, Z1582) possessed a tubular gastroporal duct. This tubular gastroporal duct was thought to be observed in immature individuals because these two specimens had undeveloped, undetectable testisacs.

Taxonomic studies (Nakano 2010, 2012b, 2014, Nakano and Gongalsky 2014, Nakano and Lai 2012, Nakano and Seo 2014) indicate that the new species differs from the seven other quadrannulate species (i.e., *O. esulcata, O. kawakatsuorum* Richardson, 1975, *O. ketagalan* Nakano & Lai, 2012, *O. koikei, O. masaakikuroiwai, O. tsushimensis* Nakano, 2011a, and *O. whitmani* Oka, 1895) by the following combination of characteristics (Table 2): body length less than 5 cm, IV uniannulate, gonopores separated by 1/2 + 4 + 1/2 annuli, XXV quadrannulate, gastroporal duct bulbous, epididymides in XIV to XX, atrial cornua developed, ellipsoid or ovate. Among the above seven quadrannulate species, two species, *O. koikei* and *O. masaakikuroiwai*, are known to have body lengths shorter than 5 cm. *Orobdella naraharaetmagarum* can be distinguished from these two species by the annulation of XXV and the length of the epididymides.

The new species is distinguishable from the four sexannulate species *O. dolichopharynx* Nakano, 2011b, *O. ijimai* Oka, 1895, *O. mononoke* Nakano, 2012a, and *O. shimadae* Nakano, 2011b and the octannulate species *O. octonaria* Oka, 1895, since *O. naraharaetmagarum* possesses mid-body somites that are quadrannulate.

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Number of annuli between $1/2 + 4 + 1/2$ $2/3 + 4 + 1/3$ $6$ $1/2 + 4 + 1/2$ $1/2 + 4 + 1/2$ $1/2 + 4 + 1/2$ $1/2 + 4 + 1/2$ $1/2 + 4 + 1/2$ $1/2 + 5 + 1/2$ Annulation of the tubulation of XIVXVI to XVIIXVI to XVIIXVI to XVIIXVI to XVII $1/2 + 5 + 1/2$ $1/2 + 5 + 1/2$ $1/2 + 1/2$ Annulation of XIVAnnulation of XIVXVI to XVIIAnnulation of XVII	Annulation of IV	uniannulate	uniannulate	biannulate	uniannulate	uniannulate	uniannulate	uniannulate	uni- or biannulate
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EpididymidesXV (posterior of XIV) to XXXVI to XXXVI to XVIIXVI to XVIIXVI to XXAtrial cornuadeveloped, ellipsoid or ovatedeveloped, ovateundevelopedundevelopeddeveloped, ovatedeveloped, ovatedeveloped, ovate	Gastroporal duct	bulbous	tubular, but bulbous at junction with gastropore	simple tubular	simple tubular	snoqlud	pulbous	bulbous	snoqlud
Atrial cornua developed, ellipsoid or developed, ovate undeveloped undeveloped developed, ovate developed, o	Epididymides	XV (posterior of XIV) to XX	XVI to XX	XVI to XVII	absent	XV to XX	XVI to XVIII	XVII to XIX	XVI to XVIII
	Atrial cornua	developed, ellipsoid or ovate	developed, ovate	undeveloped	undeveloped	developed, ovate	developed, ovate	developed, ovate	developed, ovate

#### Molecular phylogenies and genetic distances

The BI tree (Fig. 6) for estimating the phylogenetic position of the new species had an identical topology to that of the ML tree ( $\ln L = -24617.61$ ; not shown). The monophyly of *Orobdella naraharaetmagarum* and *O. esulcata* was strongly supported (BS = 100%, BPP = 1.0).

The ML tree (ln L = -15057.02) (Fig. 7) used to construct the phylogenetic relationships of the new species had an almost identical topology to that of the BI tree (not shown). The monophyly of the specimens identified as *O. naraharaetmagarum* was recovered (BS = 100%, BPP = 1.0). This clade was divided into two subclades (hereafter lineages 1 and 2). The monophyly of lineage 1 was strongly supported (BS = 99%, BPP = 1.0). Lineage 1 comprised three specimens: KUZ Z1380 (locality number 2 in Fig. 1), Z1535 (locality number 1), and Z1655 (locality number 3). KUZ Z1380 and Z1655 formed a monophyletic group (BS = 99%, BPP = 0.99). The monophyletic lineage 2 (BS = 100, BPP = 1.0) contained four individuals: KUZ Z1380 (locality number 4), Z1582, Z1650, and Z1652 (locality number 5). In the ML phylogeny, the three specimens KUZ Z1582, Z1650, and Z1652 formed a monophyletic lineage, although this relationship was not supported (BS = 33%). The monophyly of KUZ Z1582 and Z1652 was also not fully supported (BS = 63%, BPP = 0.76).

The pairwise COI uncorrected *p*-distance within *O*. *naraharaetmagarum* was 0.6-4.7% (mean = 3.3%) (Table 3). The genetic distance between lineages 1 and 2



**Figure 6.** Bayesian inference tree for 5,192 bp of nuclear 18S rRNA and histone H3 and mitochondrial COI, tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, 12S rRNA, tRNA<sup>Val</sup>, 16S rRNA, tRNA<sup>Leu</sup> and ND1 markers. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities. A species name of *Orobdella* in red indicates a quadrannulate species; in green, sexannulate; and in blue, octannulate.



**Figure 7.** Maximum likelihood tree (ln L = -15057.02) for 3,008 bp of mitochondrial COI, tRNA<sup>Cys</sup>, tRNA<sup>Met</sup>, 12S rRNA, tRNA<sup>Val</sup>, 16S rRNA, tRNA<sup>Leu</sup> and ND1 markers. Numbers on nodes represent bootstrap values for maximum likelihood and Bayesian posterior probabilities.

Specimen (locality number)	1	2	3	4	5	6	7
1: KUZ Z1535 (1)							
2: KUZ Z1380 (2)	0.042						
3: KUZ Z1655 (3)	0.041	0.031					
4: KUZ Z1360 (4)	0.045	0.043	0.041				
5: KUZ Z1654 (5)	0.042	0.042	0.042	0.011			
6: KUZ Z1582 (5)	0.047	0.043	0.042	0.014	0.009		
7: KUZ Z1652 (5)	0.047	0.046	0.044	0.013	0.010	0.006	

**Table 3.** Uncorrected *p*-distances for the 1266 bp for the COI sequences of *Orobdella naraharaetmagarum* sp. n. specimens, with associated collection locality numbers (see Fig. 1).

was 4.1–4.7% (mean = 4.4%). The genetic divergences within lineages 1 and 2 were 3.1–4.2% (mean = 3.8%) and 0.6–1.4% (mean = 1.1%), respectively. In addition, that between *O. naraharaetmagarum* and *O. esulcata* was 8.4–9.1% (mean = 8.9%)

## Discussion

The obtained molecular phylogenies showed that the present specimens formed a wellsupported clade. In addition, the mean value of the COI uncorrected *p*-distance among the individuals was 4.4%. This value indicated a clear gap between the present specimens and the closest congener, *Orobdella esulcata*. Therefore, all of the specimens examined can be considered to belong to the same species, *Orobdella naraharaetmagarum*.

Although the precise phylogenetic position of *O. tsushimensis* from the Korean Peninsula and the adjacent islets could not be determined in the obtained phylogenies (see Fig. 6), they showed that the genus *Orobdella* comprises three clades: a Hokkaido lineage including *O. kawakatsuorum* and *O. koikei*; an eastern lineage consisting of four species, *O. ijimai*, *O. masaakikuroiwai*, *O. octonaria*, and *O. whitmani*, distributed in eastern Honshu; and a western lineage containing five previously described species, *O. mononoke*, *O. dolichopharynx* and *O. shimadae* from the Nansei Islands, *O. ketagalan* from Taiwan, and *O. esulcata* recorded in Kyushu, Japan. The present phylogenies demonstrated that *O. naraharaetmagarum* was a member of the last clade, and formed a monophyly with *O. esulcata* with strong support. Therefore, the range of the western lineage group covers the area from Chugoku District, at the western tip of Honshu, to Taiwan.

As indicated in Figure 7, Orobdella naraharaetmagarum was divided into eastern (lineage 1; locality numbers 1–3) and western (lineage 2; locality numbers 4, 5) phylogroups. The COI uncorrected *p*-distances within lineage 1 were higher than those within lineage 2. The calculated genetic divergences between the three specimens collected from Mt. Kanmuriyama (locality number 5; KUZ Z1582, Z1652, and Z1654) and one individual, KUZ Z1360, from Kitahiroshima (locality number 4) was 1.1–1.4%. The geographic distance between these two collection localities is ca. 28 km. In comparison, the genetic distance between KUZ Z1380 collected on Mt. Azumayama (locality number 2) and KUZ Z1655 from Mt. Izaiyama (locality number 3) was greater than this value (3.1%), although these two localities are separated only by ca. 7 km. These phylogenetic relationships and genetic divergences implied that leeches belonging to lineage 2 dispersed more recently and rapidly than those of lineage 1. Such discordance between the COI genetic divergences and geographical distances was also seen in the small species O. masaakikuroiwai (Nakano 2014). Further molecular phylogenetic studies will help to reveal the biogeographical history of the Orobdella leeches.

The phylogenetic position of *O. naraharaetmagarum* also indicated that the small size of the mature leeches evolved in parallel within *Orobdella*, as mentioned in Nakano (2014). According to the obtained molecular phylogenies and the phylogenetic trees from studies (Nakano 2012b, 2014), each of the three small species, *O. koikei*, *O. masaakikuroiwai*, and *O. naraharaetmagarum* may have diverged from a single large quadrannulate species. As with the other two small species (Nakano 2012b, 2014), *O. naraharaetmagarum* is also distributed sympatrically with undescribed large quadrannulate species in Chugoku District (Nakano, unpublished data). Therefore, further systematic studies should be carried out to reveal the species diversity and evolutionary history of the genus *Orobdella*.

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RESEARCH ARTICLE



# Endonura Cassagnau in Iran, with a key to species of the genus (Collembola, Neanuridae, Neanurinae)

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## Abstract

Three new species of *Endonura* are described from Iran. *Endonura dichaeta* sp. n. can be recognized by an ogival labrum, head without chaetae O and E, chaeta D connected with tubercle Cl, tubercle Dl with five chaetae on head, absence of tubercles Di on thorax I and tubercle (Di+Di) of thorax V with 2+2 chaetae. *Endonura ceratolabralis* **sp. n.** is characterized by large body size, reduction of labral chaetotaxy, ogival labrum, head without chaeta O and fusion of tubercles Di and De on first thoracic segment. *Endonura persica* sp. n. is distinguished from its congeners by a nonogival labrum, absence of chaeta O, tubercles Dl and (L+So) with five and eight chaetae respectively and claw with inner tooth. The key to all species of the genus is given.

## Keywords

Springtails, taxonomy, new species

## Introduction

Endonura was established by Cassagnau (1979) as one of four subgenera within the genus Neanura MacGilliwray, 1893. Later, Deharveng (1982) raised it to the generic level. At present, Endonura is one of the largest (37 valid species) and most accurately studied genera within the subfamily Neanurinae (Dallai 1983, Deharveng 1979, 1982, Fanciulli and Dallai 2008, Pomorski and Skarżyński 2000, Pozo and Simón 1982, Smolis and Kaprus' 2003, 2009, Smolis 2006, Smolis et al. 2007, 2011). It is mostly a Palaearctic genus and only one species, E. reticulata (Axelson, 1905), is known from the Nearctic (Alaska, Smolis et al. 2011). According to a recent definition (Smolis 2008), Endonura is characterized by the following characters: 0-2 ocelli, reduced mouth parts with a thin mandible and a styliform maxilla, separate tubercles Di and De on the head, the non-cross-type of chaetotaxy on the head and three or two tubercles on abdomen V. The highest species diversity is observed in Europe (32 from among the 37 known species). However, this may be a false picture because many areas of the Palaearctic have been poorly studied by collembologists. Undoubtedly, one of such regions is Central Asia, but in this case the situation is rapidly and positively changing (Arbea and Kahrarian 2015, Kahrarian 2014, Kahrarian et al. 2013, Mayvan et al. 2015, Shayanmehr et al. 2013, Smolis et al. 2012). In the present paper, three new non-European Endonura from the western part of Iran are described. An updated key to all species of the genus is included.

## Terminology

Terminology for the description follows that given in Deharveng (1983), Deharveng and Weiner (1984), Smolis and Deharveng (2006) and Smolis (2008).

## Abbreviations used:

General morphology: abd. – abdomen, ant. – antenna, AOIII – sensory organ of antennal segment III, Cx – coxa, Fe – femur, Scx2 – subcoxa 2, T – tibiotarsus, th. – thorax, Tr – trochanter, VT – ventral tube.

Groups of chaetae: Ag – antegenital, An – chaetae of anal lobes, ap – apical, ca – centroapical, cm – centromedial, cp – centroposterior, d – dorsal, Fu – furcal, vc – ventrocentral, Ve or ve – ventroexternal, Vea – ventroexternoanterior, Vem – ventroexternomedial, Vep – ventroexternoposterior, Vel – ventroexternolateral, Vec – ventroexternocentral, Vei – ventroexternointernal, Vi or vi – ventrointernal, VI – ventrolateral.

Tubercles: Af – antenno–frontal, Cl – clypeal, De – dorsoexternal, Di – dorsointernal, Dl – dorsolateral, L – lateral, Oc – ocular, So – subocular.

Types of chaetae: Ml – long macrochaeta, Mc – short macrochaeta, Mcc – very short macrochaeta, me – mesochaeta, mi – microchaeta, ms – s–microchaeta or micro-sensillum, S or s – chaeta s, bs – border s–chaeta on ant. IV, miA – microchaetae on

ant. IV, iv – ordinary chaetae on ventral ant. IV, or – organite of ant. IV, brs – border s–chaeta on ant. IV, i – ordinary chaeta on ant. IV, mou – cylindrical s–chaetae on ant. IV ("soies mousses"), x – labial papilla x, L' – ordinary lateral chaeta on abd. V, B4, B5 – ordinary chaetae on tibiotarsi.

### Materials and methods

The specimens were cleared in Nesbitt's fluid, subsequently mounted on slides in Swan's medium and observed using a phase contrast microscope Nikon E600. Photographs were made using a camera Nikon D5100 mounted on a microscope mentioned above. Photographs were stacked using Helicon Focus 6.2.2. and prepared for publication using Adobe Photoshop CS3. Material is deposited in the Department of Invertebrate Biology, Evolution and Conservation, Institute of Environmental Biology, University of Wrocław, Poland.

#### Taxonomy

#### Endonura dichaeta sp. n.

http://zoobank.org/4CBE64B2-069B-4254-AF20-43D26E6CFE10 Figs 1–4, Table 1

**Type material.** Holotype: adult female on slide, Iran, Osmanevand area, near Golestan village (N33°55', E47°06', 1241 m a.s.l.), litter in oak forest, 13.XII.2013, leg. M. Kahrarian. Paratypes: female, two males and two juveniles on slides, same data as holotype.

**Other material.** Two females and male on slide, Iran, Osmanevand area, near Chelkooshk village (N34°03', E47°12', 1516 m a.s.l.), litter in oak forest, 31.I.2014, leg. M. Kahrarian; three juveniles on slide, Iran, Paveh county, near Shabankereh village (N34°52.978', E46°30.760', 1632 m a.s.l.), litter in oak forest, 20.I.2014, leg. M. Kahrarian; two females and juvenile, Iran, Kermanshah county, near Chahar zebra-e-oliya village (N34°13', E46°40', 1592 m a.s.l.), litter in oak forest, 24.I.2014, leg. M. Kahrarian.

**Etymology.** The species name refers to rare feature within the genus - only two chaetae Di on each side of tubercle (Di+Di) of abdomen V.

**Diagnosis.** Habitus typical of the genus *Endonura*. Dorsal tubercles present and well developed, except tubercles Di on th. I. 2+2 unpigmented eyes. Buccal cone long, labrum ogival. Head with chaetae A, B, C, D, F and G. Chaetae O and E absent. Tubercles Cl and Af separate. Tubercle Cl with chaetae D. Tubercles Dl and (L+So) on head with five and eight chaetae respectively. Tubercles De on th. II and III with three and four chaetae respectively. Tubercles L on abd. III and IV with three and six chaetae respectively. Abd. IV and V with eight and three tubercles respectively. Claw without inner tooth. Tibiotarsi with chaetae B4 and B5 short.



**Figures 1–4.** *Endonura dichaeta* sp. n.: I head (holotype), dorsal and lateral chaetotaxy **2** ventral sclerification of labrum **3** dorsal chaetotaxy of thorax **4** dorsal chaetotaxy of abdomen III–VI. Arrows indicate the position of eyes.

**Description.** Habitus typical of the genus. Body length (without antennae): 0.75–1.55 mm (holotype 1.30 mm). Colour of the body white. 2+2 medium unpigmented eyes (Fig. 1).

Types of dorsal ordinary chaetae. Macrochaetae Ml relatively long, strongly thickened, almost cylindrical, arc-like or straight, narrowly sheathed, feebly serrated, apically pointed (Figs 1, 3–4); macrochaetae Mc and Mcc thickened, straight and pointed; mesochaetae and microchaetae short, thin, feebly serrated and pointed.

Head. Labrum ogival, with ventral sclerifications as in Fig. 2. Labrum chaetotaxy 2/2, 4. Labium with four basal, three distal and four lateral chaetae, papillae x absent. Maxilla styliform, mandible thin tridentate. Chaetotaxy of antennae as in Table 1c. Apical vesicle distinct, trilobed. S–chaetae of ant.IV long and moderately thickened. Chaetotaxy of head as in Table 1a, b, and Fig. 1. Chaeta D connected with tubercle Cl. Tubercle Af on head longer than tubercles Oc. Tubercle Dl with five chaetae, chaeta Dl3 absent, chaeta Dl6 as minute microchaeta and hard to detect (Fig. 1). Tubercle (L+So) with eight chaetae, chaetae So2 and L3 absent, chaeta So6 as Mc (Fig. 1). Elementary tubercles BE and CD present. Chaeta A shorter than B.

Thorax, abdomen, legs. Body s-chaeta thin and smooth, shorter than nearby macrochaetae (Figs 3, 4). Chaetotaxy of th. and abd. as in Table 1d and in Figs 3, 4. Tubercles Di on th.I not differentiated. Chaetae De3 on th. III and abd. I–III as Mcc. Chaetae De2 on th. II–III and De3 on th. III connected with tubercle De. Chaetae De3 on abd. I–III connected with tubercle De (Fig. 4). The line of chaetae De1–chaeta

Tubercle	Number of chaetae	Types of chaetae	Names of chaetae
$C^{\dagger}$	(	Ml	F
CI	6	Mc	D, G
٨£	6	Ml	A
AI		Mc	B, C
0-	2	Ml	Ocm, Ocp
Üč	3	Mc	Oca
D:	2	Ml	Di1
Di	<i>L</i>	Mc	Di2
D.	2	Ml	De1
De	2	Mc	De2
Dl		Ml	Dl1, Dl5
	F	Mc	Dl4
	)	Мсс	Dl2
		mi	Dl6
		Ml	L1, L4, So1
(L+So)	8	Mc	L2, So6
		me	So3-5

Table 1a. Chaetotaxy of Endonura dichaeta sp. n.: Cephalic chaetotaxy-dorsal side.

Table 1b. Chaetotaxy of Endonura dichaeta sp. n.: Cephalic chaetotaxy-ventral side.

Group	Number of chaetae
Vi	6
Vea	3
Vem	3
Vep	4
labium	11, 0×

Segment, Group	Number of chaetae	Segment, Group	Number of chaetae adult
Ι	7		0.6 : 10 (1
II	11	IV	or, $8$ S, 1, 12 mou, 6 brs,
III	5 sensilla AO III	ap	2 10
ve	5		8 bs, 5 miA
VC	4	са	2 bs, 3 miA
vi	4	cm	3 bs, 1 miA
d	5	ср	8 miA, 1 brs

Table Ic. Chaetotaxy of Endonura dichaeta sp. n.: Chaetotaxy of antennae.

		Terga					Legs		
	Di	De	Dl	L	Scx2	Сх	Tr	Fe	Т
th. I	1	2	1	-	0	3	6	13	19
th. II	3	2+s	3+s+ms	3	2	7	6	12	19
th. III	3	3+s	3+s	3	2	8	6	11	18
							Sterna		
abd. I	2	3+s	2	3	VT: 4				
abd. II	2	3+s	2	3	Ve: 4–5	Ve1 -	present		
abd. III	2	3+s	2	3	Vel:4–5			Fu:5–6me	2–4mi
abd. IV	2	2+s	3	6	Vel: 4	Vec: 2	Vei: 2	Vl: 4	
abd. V	(2+2)		5+s		Ag: 2			Vl: 1	Ľ: 1
abd. VI		7			Ve:13-14			An: 2mi	

Table Id. Chaetotaxy of *Endonura dichaeta* sp. n.: Postcephalic chaetotaxy.

s not perpendicular to the dorsomedian line on abd I–III. Furca rudimentary with 2–4 microchaetae. Tubercles Di on abd. V fused, with chaetae Di2 as Mc or Mcc, chaetae Di3 absent (Fig. 4). Chaetae L' and Vl on abd. V present. IV abd. with 2+2 chaetae Ag. No cryptopygy. Chaetotaxy of legs as in Table 1d.

**Remarks.** In general appearance (shape of dorsal chaetae, chaetotaxy of central area of head and dorsal side of thorax and abdomen, complete absence of pigmentation and absence of cryptopygy), *E. dichaeta* sp. n. strongly resembles *E. tartaginenis* Deharveng, 1980 described from Corsica. Nevertheless, both taxa differ in some essential characters, important from taxonomic point of view: presence/absence of chaetae E on head (*dichaeta* sp. n. absent, *tartaginenis* present), number of chaetae Dl on head (*dichaeta* sp. n. five, *tartaginenis* six), number of chaetae (L+So) (*dichaeta* sp. n. eight, *tartaginenis* nine), presence/absence of elementary tubercle EE on head (*dichaeta* sp. n. absent, *tartaginenis* present), number of chaetae Di on abd. V (*dichaeta* sp. n. 2+2, *tartaginenis* 3+3) and presence/absence of tooth on claw (*dichaeta* sp. n. absent, *tartaginenis* 3+3) and ogival labrum (unknown in *tartaginenis*), characters rarely observed within the genus.

#### Endonura ceratolabralis sp. n.

http://zoobank.org/FC09DDF3-EB60-416D-B31C-A290A4E812D5 Figs 5–9, Table 2

**Type material.** Holotype: adult female on slide, Iran, Osmanevand area, near Markhor village (N33°53', E47°05', 1389 m a.s.l.), litter in oak forest, 13.XII.2013, leg. M. Kahrarian. Paratypes: 3 females on slide, same data as holotype.

**Other material.** Three females on slide, Iran, Osmanevand area, near Ghader marz village (N34°01.030', E47°12.415', 1682 m a.s.l.), litter in oak forest, 31.I.2014, leg. M. Kahrarian.

**Etymology.** The species name refers to sharp labral apex which looks like a horn ("cera" in latin).

**Diagnosis.** Habitus typical of the genus *Endonura*. Dorsal tubercles present and well developed. 2+2 eyes darkly pigmented. Buccal cone long. Head with chaetae A, B, C, D, E, F and G. Chaeta O absent. Tubercles Cl and Af separate. Tubercles Dl and (L+So) on head with six and nine chaetae respectively. Tuberles Di and De on th. I fused. Tubercles De on th. II and III with three and four chaetae respectively. Tubercles L on abd. III and IV with three and 6–7 chaetae respectively. Abd. IV and V with eight and three tubercles respectively. Claw without inner tooth. Tibiotarsi with chaetae B4 and B5 short.

**Description.** Habitus typical of the genus. Body length (without antennae): 2.25–2.55 mm (holotype: 2.55 mm). Colour of the body bluish grey. 2+2 medium dark-pigmented eyes (Fig. 5).

Types of dorsal ordinary chaetae. Macrochaetae Ml thickened, relatively long, arclike or straight, narrowly sheathed, feebly serrated, apically pointed or rarely rounded (Figs 5, 7–9); macrochaetae Mc and Mcc thickened, straight, pointed or apically rounded; mesochaetae and microchaetae short, thin and pointed.

Head. Buccal cone very long. Labrum ogival, with ventral sclerifications as in Fig. 6. Labrum chaetotaxy 0/2, 2. Labium with four basal, three distal and four lateral chaetae, papillae x absent. Maxilla styliform, mandible thin with two basal and two apical teeth. Chaetotaxy of antennae as in Table 2c. Apical vesicle distinct, trilobed. S-chaetae of ant.IV of medium length and moderately thickened. Chaetotaxy of head as in Table 2a, b, and Fig. 5. Tubercles Cl and Af separate. Tubercle Af on head longer than tubercles Oc. Chaeta O absent. Chaeta D free. Tubercle Dl with six chaetae, chaeta Dl3 present. Tubercle (L+So) with nine chaetae, chaeta So2 absent and chaeta So3 as Mc (Fig. 5). Elementary tubercles BE and CD present. Chaeta A shorter than B.

Thorax, abdomen, legs. Body s-chaetae thin and smooth, shorter than nearby macrochaetae (Figs 7–9). Chaetotaxy of th. and abd. as in Table 2d and in Figs 7–9. Tubercles Di on th.I differentiated and fused with De (Fig. 7). Dorsal side of th. and abd. without free chaetae De. The line of chaetae De1–chaeta s perpendicular to the dorsomedian line on abd I–III. Furca rudimentary with two or without microchaetae. Tubercles Di on abd. V fused, with chaetae Di2 and Di3 as Mc (Fig. 9). Chaetae L' and VI on abd. V present. No cryptopygy. Chaetotaxy of legs as in Table 2d.



**Figures 5–9.** *Endonura ceratolabralis* sp. n.: **5** head (holotype), dorsal and lateral chaetotaxy **6** ventral sclerification of labrum **7** dorsal chaetotaxy of thorax **8** dorsal chaetotaxy of abd. II **9** dorsal chaetotaxy of abdomen IV–VI. Arrows indicate the position of eyes.

**Remarks.** Because of the very characteristic long and pointed labrum, *E. cera-tolabralis* sp. n. seems to be most similar to *E. cretensis* (Ellis, 1976) (Crete) and *E. gracilirostris* Smolis et al. 2007 (Crimea). Nevertheless, the new species can be easily distinguished from these two taxa by the following combination of characters: maximum length of the body without antennae (*ceratolabralis* sp. n. 2.55 mm; *gracilirostris* 

Tubercle	Number of chaetae	Types of chaetae	Names of chaetae
C1	6	Ml	F
CI	4	Mc	G
٨£	10	Ml	А
Al		Mc	B, C, D, E
0-	2	Ml	Ocm, Ocp
00	3	me	Oca
D:	2	Ml	Di1
Di	Z	Mc	Di2
Da	2	Ml	De1
De	Z	Mc	De2
		Ml	Dl1, Dl5
Dl	6	Mc	Dl2, Dl3, Dl4
		mi	Dl6
		Ml	L1, L4, So1
(L+So)	9	Mc	L2, L3, So3
		me	So4-6

Table 2a. Chaetotaxy of Endonura ceratolabralis sp. n.: Cephalic chaetotaxy-dorsal side.

Table 2b. Chaetotaxy of Endonura ceratolabralis sp. n.: Cephalic chaetotaxy-ventral side.

Group	Number of chaetae
Vi	6
Vea	3-4
Vem	3
Vep	4
Labium	11, 0x

Table 2c. Chaetotaxy of Endonura ceratolabralis sp. n.: Chaetotaxy of antennae.

Segment, Group	Number of chaetae	Segment, Group	Number of chaetae adult
Ι	7		
II	12-14	IV	an 85 i 12 may 6 hm 2 in
III	5 sensilla AO III	ap	01, 8 5, 1, 12 1100, 0 018, 2 1
ve	5		8 bs, 5 miA
VC	4	са	2 bs, 3 miA
vi	4	cm	3 bs, 1 miA
d	5	ср	8 miA, 1 brs

1.45 mm; *cretensis* 0.8 mm), labral formula (*ceratolabralis* sp. n. 0/2, 2; *gracilirostris* 0/2, 4; *cretensis* 2/2, 4), presence/absence of chaeta O on head (*ceratolabralis* sp. n. absent, in others present), number of chaetae Dl on head (*cretensis* three, in others six), number of chaetae (L+So) on head (*cretensis* seven, in others nine), fusion/separation of tubercles Di and de on th. I (*gracilirostris* separate, in others fused), number of ordinary chaetae De on th. III (*cretensis* two, others three), presence/absence of free chaetae on thorax (*ceratolabralis* sp. n. absent, in others present) and number of chaetae Di on abd. V (*cretensis* 1-2, others three).

		Terga					Legs		
	Di	De	Dl	L	Scx2	Сх	Tr	Fe	Т
th. I	1	2	1	-	0	3	6	13	19
th. II	3	2+s	3+s+ms	3	2	7	6	12	19
th. III	3	3+s	3+s	3	2	8	6	11	18
							Sterna		
abd. I	2	3+s	2	3	VT: 4				
abd. II	2	3+s	2	3	Ve: 5–6	Ve1 -	Present		
abd. III	2	3+s	2	3	Vel:4–5			Fu:3–6me	0–2mi
abd. IV	2	2+s	3	6–7	Vel: 4	Vec: 2	Vei: 2	Vl: 4	
abd. V	(3+3)		5+s		Ag: 3			Vl: 1	Ľ: 1
abd. VI		7			Ve: 1	3–14		An: 2mi	

Table 2d. Chaetotaxy of Endonura ceratolabralis sp. n.: Postcephalic chaetotaxwy.

#### Endonura persica sp. n.

http://zoobank.org/9CFE5947-62CC-4A3E-ABF7-5B84EA69A21A Figs 10–13, Table 3

**Type material.** Holotype: adult female on slide, Iran, Kermanshah area, near Ghaleh shahin village (N34°25.590', E05°12.415', 566 m a.s.l.), litter in willow shrubs, 7.IV.2014, leg. M. Kahrarian. Paratypes: two females, four males and four juveniles on slides, same data as holotype.

**Other material.** Female on slide, Iran, Kermanshah Province, Halashi County, near Sarfiroozabad village (N34°02', E47°10', 1624 m a.s.l.), litter in oak forest, 15.II.2014, leg. M. Kahrarian; female and male on slide, Iran, Osmanevand area, near Sarjoob village (N33°56', E47°08', 1240 m a.s.l.), litter in oak forest, 13.XII.2013, leg. M. Kahrarian.

Etymology. The species name refers to the historic name of Iran, Persia.

**Diagnosis.** Habitus typical of the genus *Endonura*. Dorsal tubercles present and generally well developed, only tubercles Di on th. I weakly differentiated. 2+2 large dark-pigmented eyes. Buccal cone rather short. Head with chaetae A, B, C, D, E, F and G. Chaeta O absent. Tubercles Cl and Af separate. Tubercles Dl and (L+So) on head with five and eight chaetae respectively. Tubercles De on th. II and III with three and four chaetae respectively. Tubercles L on abd. III and IV with four and 6–7 chaetae respectively. Abd. IV and V with eight and three tubercles respectively. Claw with inner tooth. Tibiotarsi with chaetae B4 and B5 long.

**Description.** Habitus typical of the genus. Body length (without antennae): 0.75–1.90 mm (holotype: 1.10 mm). Colour of the body bluish grey. 2+2 large dark pigmented eyes (Fig. 10).

Types of dorsal ordinary chaetae. Macrochaetae Ml thickened, relatively long, arc– like or straight, narrowly sheathed, feebly serrated, apically rounded or rarely pointed (Figs 10, 12); macrochaetae Mc and Mcc thickened, straight and not pointed; mesochaetae and microchaetae short, thin and pointed.



**Figures 10–13.** *Endonura persica* sp. n.: **10** head and th. I, dorsal and lateral chaetotaxy **11** ventral sclerification of labrum **12** dorsal chaetotaxy of abdomen III–VI (holotype) **13** tibiotarsi and claw of leg III. Arrows indicate the position of eyes.

Head. Buccal cone short. Labrum rounded, with ventral sclerifications as in Fig. 11. Labrum chaetotaxy 4/2, 4. Labium with four basal, three distal and four lateral chaetae, papillae x absent. Maxilla styliform, mandible thin with two basal and two apical teeth. Chaetotaxy of antennae as in Table 3c. Apical vesicle distinct trilobed. S-chaetae of ant.IV of medium length and moderately thickened. Chaetotaxy of head as in Table 3a, b, and Fig. 10. Chaetae D and E free. Tubercles Cl and Af separate.

Tubercle	Number of chaetae	Types of chaetae	Names of chaetae
CI	4	Ml	F
CI	4	Мс	G
		Ml	В
Af	10	Mc	A, C, E
		Mc or Mcc	D
		Ml	Ocm
Oc	3	Mc	Оср
		mi	Oca
D:	2	Ml	Di1
Di	2	Mcc	Di2
D	2	Ml	De1
De	Z	Mcc	De2
DI	E	Ml	Dl1, Dl5
DI	)	Mc or Mcc	Dl2, Dl4, Dl6
		Ml	L1, L4, So1
(L+So)	8	Мс	L2
		me or mi	So3–6

**Table 3a.** Chaetotaxy of *Endonura persica* sp. n.: Cephalic chaetotaxy–dorsal side.

Table 3b. Chaetotaxy of Endonura persica sp. n.: Cephalic chaetotaxy-ventral side.

Group	Number of chaetae
Vi	6
Vea	3-4
Vem	3
Vep	4
labium	11, 0x

Table 3c. Chaetotaxy of *Endonura persica* sp. n.: Chaetotaxy of antennae.

Segment, Group	Number of chaetae	Segment, Group	Number of chaetaev adult
Ι	7		
II	12–14	IV	or, 8 S, i, 12 mou, 6 brs, 2 iv
III	5 sensilla AO III	ap	
ve	5		8 bs, 5 miA
VC	4	са	2 bs, 3 miA
vi	4	cm	3 bs, 1 miA
d	5	ср	8 miA, 1 brs

Tubercle Af on head longer than tubercles Oc. Tubercle Dl with five chaetae, chaeta Dl3 absent. Tubercle (L+So) with eight chaetae, chaetae So2 and L3 absent (Fig. 10). Elementary tubercle BE absent. Chaeta A shorter than B.

Thorax, abdomen, legs. Body s-chaetae fine and smooth, distinctly shorter than nearby macrochaetae (Fig. 12). Chaetotaxy of th. and abd. as in Table 3d and in Figs

		Terga					Legs		
	Di	De	Dl	L	Scx2	Cx	Tr	Fe	Т
th. I	1	2	1	-	0	3	6	13	19
th. II	3	2+s	3+s+ms	3	2	7	6	12	19
th. III	3	3+s	3+s	3	2	8	6	11	18
							Sterna		
abd. I	2	3+s	2	3	VT: 4				
abd. II	2	3+s	2	3	Ve: 5–6	Ve1 -	present		
abd. III	2	3+s	2	4	Vel: 5			Fu:5–10me	0 mi
abd. IV	2	2+s	3	6–7	Vel: 4	Vec: 2	Vei: 2	Vl: 4	
abd. V	(3+3)		8+s		Ag: 3			Vl: 1	Ľ: 1
abd. VI		7			Ve: 1	3–14		An: 2mi	

Table 3d. Chaetotaxy of Endonura persica sp. n.: Postcephalic chaetotaxy.

10, 12. Tubercles Di on th.I differentiated or not. Chaetae De2 on th. II–III and De3 on th. III free. Chaetae De3 on abd. I–III free (Fig. 12). The line of chaetae De1– chaeta s parallel to the dorsomedian line on abd. I–III. Furca rudimentary without microchaetae. Tubercles Di on abd. V fused, with chaetae Di2 as Mcc and Di3 as mi (Fig. 12). Chaetae VI on abd. V present. Cryptopygy slightly developed. Chaetotaxy of legs as in Table 3d. Tibiotarsi with rather long chaetae B4 and B5. Claw with inner tooth (Fig. 13).

**Remarks.** In general appearance and presence of inner tooth on claw, characters rarely observed within the genus, *E. persica* sp. n. strongly resembles to *E. dentifera* Smolis et al. 2007 (described from Crimea). However, the new species can be reliably separated from Crimean species with the following characters: number of chaetae Dl on head (*persica* sp. n. five, *dentifera* six), number of chaetae (L+So) on head (*persica* sp. n. eight, *dentifera* ten), presence/absence of tubercles Di on the first thoracic segment (*persica* sp. n. present, *dentifera* absent) and number of chaetae L of abd. IV (*persica* sp. n. 6–7 chaetae, *dentifera* 8–9).

## Key to the genus Endonura

In 1982, Deharveng, in his PhD thesis, elevated *Endonura* to the generic level and prepared a key to the genus that comprised 23 species. Nowadays, including the taxa described herein, the genus contains 40 members and is the second largest of the tribe Neanurini, after *Deutonura* Cassagnau, 1979. Moreover, after the publication of Deharveng's paper (date), a few species were redescribed and one taxon was synonymised

(Smolis and Kaprus' 2003, Smolis 2008, Smolis et al. 2007, 2011). Considering these facts, the preparation of an updated key to all species of the genus seemed to be highly recommended.

1	Head with fusion of tubercles Af and Cl2
_	Head with separation of tubercles Af and Cl7
2.	Chaeta O on head present
	Chaeta O on head absent
3	Tubercles Di on th. I present and fused with De, tubercle (Di+Dl+L) on abd.
	V with nine chaetae
_	Tubercles Di on th. I absent, tubercle (Di+Dl+L) on abd. V with seven chae-
	tae <i>E. ichnusae</i> Dallai, 1983 (Italy, Sardinia)
4	Tubercles De on abd. I–III with four chaetae
_	Tubercles De on abd. I–III with three chaetae
	<i>E. granulata</i> (Cassagnau & Delamare Deboutteville, 1955) (Lebanon)
5	Tubercles Di and De on th. I fused, cryptopygy strongly developed
_	Tubercles Di and De on th. I separate, cryptopygy absent or weakly devel-
	oped
6	Chaeta E on head present, Tubercle Dl on head with four chaetae
_	Chaeta E on head absent, Tubercle Dl on head with six chaetae
7	Tubercle Af on head equal or shorter than tubercles Oc
_	Tubercle Af on head longer than tubercles Oc9
8	Labrum with ventral sclerifications ogival and without prelabral chaetae
_	Labrum with ventral sclerifications nonogival and with prelabral chaetae
	<i>E. taurica</i> (Stach, 1951) (Crimea)
9	Chaeta O on head present10
_	Chaeta O on head absent
10	Eyes completely absent11
_	Eyes present
11	Tubercles Di on th. I present <i>E. arbasensis</i> Deharveng, 1979 (France, Spain)
_	Tubercles Di on th. I absent <i>E. caeca</i> (Gisin, 1963) (Bosnia and Herzegovina)
12	Anterior eye present and located outside tubercle Oc
	<i>E. asiatica</i> Smolis et al., 2011 (Kyrgyzstan)
_	Anterior eye present or absent, if present located within tubercle Oc13
13	Anterior eye present14
_	anterior eye absent <i>E. immaculata</i> Deharveng, 1980 (France, Corsica)
14	Claw with inner tooth, tibiotarsi with long chaetae B4 and B515
_	Claw without tooth, tibiotarsi with short chaetae B4 and B516

15	Tubercle Dl on head with three chaetae, tubercles Di on th. II-III with two
	chaetae <i>E. tetrophtalma</i> (Stach, 1929) (Hungary)
	Tubercle Dl on head with five chaetae, tubercles Di on th. II-III with three
	chaetae
16	Chaeta E on head absent
_	Chaeta E on head present
17	Tubercle Cl on head with chaetae D, elementary tubercle DF present
	<i>E. colorata</i> (Gama, 1964) (Portugal)
_	Tubercle Cl on head without chaetae D, elementary tubercle DF absent
18	Tubercle Dl on head with six chaetae
_	Tubercle Dl on head with less number of chaetae
19	Tubercles Di on head present
_	Tubercles Di on head absent
	<i>E. dalensi</i> Deharveng, 1979 (Andorra, France, Spain, Italy)
20	Body white 21
_	Body hue or bluish-grev 22
21	Tubercle $(I + S_0)$ on head with nine chaetae macrochaetae thin and pointed
21.	<i>E deharmengi</i> Cassagnan & Péia 1979 (Greece)
_	Tubercle (I+So) on head with eight chaetae macrochaetae thickened and
_	blunt <i>E langantica</i> Smolis et al 2011 (Israel)
22	Tuberde De on the III with two ordinary chaetae
	<i>E aladiolifu</i> (Cosserve 1054) (Algoria Spain)
	Tubarala Da on the III with three ardinary chaster
-	Tubercle De on in. In with three ordinary chaetae
23	Tubercie Ci on nead with chaetae D, furca rudimentary with microchaetae
	The and Class had a with and a base of the star D. former and incontent are in the star miner
_	iubercie CI on nead without chaetae D, furca rudimentary without micro-
2/	$\begin{array}{c} \text{Chaetae} \\ \text{The let} (L, \mathbb{C}) \\ \text{The let} (L, \mathbb{C}) \\ \text{The let} (L, \mathbb{C}) \\ \text{Chaetae} \\ Chaet$
24	Iubercle (L+So) on head with nine chaetae, free chaeta L on abd. IV present
	<i>E. quadriseta</i> Cassagnau & Peja, 19/9 (Greece, Turkey, Crimea)
_	Iubercle (L+So) on head with ten chaetae, free chaeta L on abd. IV absent
	<i>E. reticulata</i> (Axelson, 1905) (Finland; Russia; Sweden; United States, Alaska)
25	Tubercle DI on head with four chaetae, tubercles Di and De on th. I sepa-
	rate <i>E. occidentalis</i> (Deharveng, 1979) (Spain)
-	Tubercle Dl on head with three chaetae, tubercles Di and De on th. I fused
	<i>E. cretensis</i> (Ellis, 1976) (Greece, Israel)
26	Cryptopygy strong and complete, tubercles of abd. VI invisible in dorsal
	view
-	Cryptopygy absent or weak, tubercles of abd. VI well or partially visible in
	dorsal view27
27	Body bluish–grey28
_	Body white

28	Claw with inner tooth, labrum chaetotaxy 4/2, 4
_	Claw without inner tooth, labrum chaetotaxy 0/2, 2
	<i>E. ceratolabralis</i> sp. n. (Iran)
29	Tubercle Dl on head with five chaetae, tubercles Di on th. I present
	<i>E. persica</i> sp. n. (Iran)
-	Tubercle Dl on head with six chaetae, tubercles Di on th. I absent
30	Chaeta C on head absent
_	Chaeta C on head present
31	Macrochaetae Di1 on abd. V distinctly thickened and club-like
	<i>E. baculifer</i> Deharveng, 1979 (Portugal)
-	Macrochaetae Di1 on abd. V slightly thickened and cylindrical
32	Eyes present, tubercles Di on th. I present
	<i>E. transcaucasica</i> (Stach, 1951) (Georgia)
-	Eyes absent, tubercles Di on th. I absent <i>E. carpatica</i> Smolis, 2006 (Poland)
33	Tubercle Cl on head with chaetae D, elementary tubercle DF present34
-	Tubercle Cl on head without chaetae D, elementary tubercle DF absent35
34	Chaeta E on head present, tubercle Dl on head with six chaetae
	<i>E. tartaginenis</i> Deharveng, 1980 (France, Corsica)
-	Chaeta E on head absent, tubercle Dl on head with five chaetae
35	Chaeta E on head present
-	Chaeta E on head absent
36	Chaeta L4 on head free, eyes absent or present unpigmented
-	Chaeta L4 within tubercle (L+So), eyes present and pigmented <b>39</b>
37	Abd. V with two tubercles
	<i>E. incolorata</i> (Stach, 1951) (Poland, Ukraine, Romania)
-	Abd. V with three tubercles
38	Abd. IV with eight tubercles, macrochaetae Ml relatively short
	<i>E. tatricola</i> (Stach, 1951) (Poland, Slovakia)
-	Abd. IV with five tubercles, macrochaetae Ml long
	<i>E. dudichi</i> (Loksa, 1967) (Hungary, Poland, Slovakia)
39	Tubercle Dl on head with six chaetae, tubercle L on abd. III with three chae-
	tae E. centaurea Cassagnau & Péja, 1979 (Greece)
-	Tubercle Dl on head with five chaetae, tubercle L on abd. III with four chae-
	tae <i>E. saleri</i> Fanciulli & Dallai, 2008 (Italy)

## Discussion

Considering the data presented here and those obtained from the literature (Mayvan et al. 2015, Shayanmehr et al. 2013, Smolis et al. 2012), Neanurinae fauna of Iran

comprises ten species and seven genera: Bilobella aurantiaca (Caroli, 1912), Cryptonura persica Smolis et al., 2012, C. maxima Smolis et al., 2012, Deutonura decolorata (Gama & Gisin, 1964) (Gisin 1964), Endonura ceratolabralis sp. n., E. dichaeta sp. n., E. persica, sp. n., Neanura muscorum (Templeton, 1835), Persanura hyrcanica Mayvan et al. 2015, Thaumanura echinata (Kos, 1940). It should be noted, however, that until now only the western part of Iran has been roughly studied. Although future research may change the present picture of the subfamily diversity in the studied country and region, some preliminary conclusions can be drawn. The first is related to the higher systematic pattern and composition of Neanurinae of Iran. This fauna consists almost exclusively of members of the tribe Neanurini, the most diverse and dominant among Neanurinae in the western Palaearctic. To date, none of the Lobellini and Paranurini genera have been found in Iran, although they are numerous and widely distributed in south, south-east and east Asia. The second conclusion seems to be more expected, Endonura species from Iran resemble those known from south-east Europe. It suggests their close affinity and the historical connection between these faunas. The third conclusion sheds light on the distribution and the history of this genus. Most Endonura species were recorded from Mediterranean and temperate zones of Europe, where they live predominantly in forests. It is worth saying that the greatest diversity of the genus through the continent is more or less correlated to the areas of land that have never been subjected to glaciations. Till now, the occurrence of only a few species is documented outside Europe, especially in the Middle East (Smolis and Kaprus 2003, 2009; Smolis et al. 2011). The recent and present discoveries of Endonura species in Kyrgyzstan (Smolis et al. 2011) and Iran significantly expand the list of species and also our knowledge on the genus. Undoubtedly, diverse forest habitats of the coastal and montane regions of Iran and adjacent countries hide a rich fauna of Neanurinae. We therefore hope that a more comprehensive study in the future will allow us to present a better picture of the distribution of *Endonura* in Iran and the near East.

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RESEARCH ARTICLE



# A new Chinese Pseudoogeton species and key to the species of the genus (Coleoptera, Tenebrionidae, Amarygmini)

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# Abstract

*Pseudoogeton maoxianum* **sp. n.** is described from Sichuan, China. A key to the males of the species of *Pseudoogeton* Masumoto, 1989 is presented.

# Keywords

Tenebrionidae, Pseudoogeton, new species, China, key to species

# Introduction

*Pseudoogeton* (Tenebrionidae: Amarygmini) was established by Masumoto (1989) with *P. amplipennis* (Fairmaire, 1897) as the type species. The genus is similar to *Plesioph-thalmus* Motschulsky, 1858, but it can be distinguished from the latter by the absence of hind wings or shortened hind wings.

Although Bremer and Lillig (2014) are of the opinion that the absence of hind wings is not a satisfactory character state to separate the genus from *Plesiophthalmus*; still, *Pseudoogeton* is retained as a separate genus until other characters are found to justify its status or to merge it with *Plesiophthalmus*.

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At present the genus contains six valid species described by Fairmaire (1891, 1897) and Masumoto (1981, 1989, 1996, 2010). They are distributed in the mountains of China (*P. amplipenne* (Fairmaire, 1897), *P. gebieni* Masumoto, 1989, *P. ovipenne* (Fairmaire, 1891) and *P. uenoi* (Masumoto, 1981)), the Ryukyu Island (*P. kimurai* Masumoto, 1996) and Laos (*P. endoi* Masumoto, 2010).

Working on Chinese specimens of the genus deposited in the Museum of Hebei University (MHBU), Baoding, a new species was found, which was collected by the authors in Sichuan Province of China in 1999. In this paper the new species is described and a key to males of the species of *Pseudoogeton* is presented.

# Material and methods

The photos were taken with a Leica DFC 450 digital microscope camera attached to Leica M205A stereomicroscope. The aedeagus was dissected, cleared in 5% NaOH solution, and placed in glycerin for observation and imaging. Images were edited using Adobe Photoshop CS6. The terminology follows Masumoto (2010). All measurements given are in millimeters. The type specimens are deposited in MHBU, Baoding, China.

# Results

# Key to the species of *Pseudoogeton* (males)

*P. ovipenne* from Hubei whose holotype is female is not in the key.

1	Hind wings shortened
_	Hind wings absent
2	Pronotum approx. as wide as long; apicale of aedeagus arrowheaded (Figs
	25-26, in Masumoto 1989). China (Taiwan) P. uenoi
_	Pronotum approx. 1.4 times as wide as long; apicale of aedeagus simply short
	fusiform. Japan
3	Body blackish brown, with ferruginous tinge, dorsal surface strongly shin-
	ing; apicale of aedeagus elongated equilateral triangular, lateral sides gradually
	narrowed forward. Laos P. endoi
_	Black, or with violet and sericeous tinge; lateral sides of apicale of aedeagus
	abruptly narrowed forward4
4	Pronotum widest in middle (Fig. 3); aedeagus stouter, apicale length/width <
	1.5 (Figs 5–7). China (Sichuan)
_	Pronotum widest behind middle; aedeagus slender, apicale length/width
	> 1.5
5	Dorsal surface of body with violet tinge; pronotum hemispherical; striae on elytra
	fine and shallow, intervals almost not punctate. China (Sichuan) P. gebieni

Dorsal surface of body black, pronotum moderately convex and transverse;
striae on elytra strong and deep, intervals with clear punctures. China (Sichuan)

#### Pseudoogeton maoxianum sp. n.

http://zoobank.org/C3E7E346-28DE-4EBC-A8A6-62CBED3F2AAB

**Type specimens.** Holotype  $\mathcal{F}$  (MHBU): CHINA, Sichuan, Maoxian County, Mt. Xiaomiaoshan, 1600m, 22.viii.1999, leg. Guo-Dong Ren & Sai-Hong Dong. Paratype:  $1^{\circ}$  (MHBU): same data as holotype [Transliterated from Chinese labels].

Distribution. China (Sichuan).

**Diagnosis.** The new species is characterized by the following: pronotum nearly hemispherical, widest in middle; elytra strongly convex, with fine strial puncture; apicale of aedeagus stouter, ratio of length/width = 1.4; basale 1.9 times longer than apicale.

**Description.** *Male.* Wingless; body oblong oval (Fig. 1), dorsum strongly convex, black; elytra with sericeous tinge, pronotum and legs more lustrous than elytra.

Clypeus transverse, with dense punctures. Frontoclypeal suture fine and straight. Genae relatively small, roundly protruded laterad. Eyes small, reniform, distance between them approximately 2.3 times their own diameter. Mentum trapezoidal. Gula widely triangular. Terminal maxillary palpomeres securiform. Antennae filiform (Fig. 9), reaching over half of elytra; length ratio of antennomeres 1 to 11 as 0.33: 0.20: 1.24: 0.59: 0.78: 0.69: 0.69: 0.64: 0.57: 0.61: 0.85.

Pronotum (Fig. 3) convex, almost hemispherical, 1.3 times as wide as long, widest in middle, roundly narrowed anteriorly and posteriorly, anterior margin slightly arcuate, finely beaded, posterior margin noticeably produced, lateral margins finely beaded, visible in dorsal view throughout their whole length, anterior and posterior corners obtuse in lateral view; disc with tiny and sparse punctures.

Scutellum widely triangular, with a few punctures.

Elytra ovate, approximately 1.5 times as long as wide, approximately 2.6 times longer and 1.3 times wider than pronotum. Dorsum convex, maximum height at basal third, widest at basal 2/5, lateral sides roundly narrowed anteriorly and posteriorly. Disc with rows of tiny and sparse punctures, their distance equal to 3–4 times of puncture diameter; intervals flat and wide, transversely micro-aciculate, with extremely tiny punctures; lateral margins finely beaded.

Prosternum with a deep median groove between coxae. Prosternal process (Fig. 4) strongly bent upwards, with apophysis near apex.

Legs slender and rather long. Anterior edge of profemora (Fig. 8) with acute spine in apical third. Protibiae moderately curved, slightly widened and haired in apical half. Ratio of length of pro-, meso- and metatarsomeres 1 to 5 (or 4, metatarsomeres) as 0.60: 0.42: 0.42: 0.29: 1.13; 1.13: 0.63: 0.45: 0.28: 1.14; 1.93: 0.71: 0.46: 1.18. Claws sharp, falciform.



Figures 1–9. *Pseudoogeton maoxianum* sp. n. 1 Habitus, male 2 Habitus, female 3 Pronotum 4 Prosternal process 5 Aedeagus in dorsal view 6 Aedeagus in lateral view 7 Aedeagus in ventral view 8 Foreleg 9 Antenna. Scale bars: 1 mm.

Abdominal ventrites with microscopic punctures and setae, ventrite V slightly emarginate at apex.

Aedeagus fusiform (Figs 5–7), 3.5 mm long. Apicale elongate, lateral sides serrate, ratio of length/width = 1.4. Basale 1.9 times longer than apicale.

*Female*: Body stouter than male (Fig. 2), pronotum 1.5 times as wide as long, elytra ovate, about 1.4 times as long as wide, about 3.0 times longer and 1.4 times wider than pronotum; prosternum groove between the coxae shallow.

Body length:  $\mathcal{J}$ : 12.9 mm;  $\mathcal{Q}$ : 15.2 mm. Body width:  $\mathcal{J}$ : 5.7 mm;  $\mathcal{Q}$ : 7.8 mm.

**Etymology.** This specific epithet is derived from the type locality, Maoxian County, Sichuan Province, China.

# **Acknowledgments**

We thank Dr. Ottó Merkl, Mrs. Aranka Grabant and Mr. Tamás Németh (Hungarian Natural History Museum, Budapest) for providing the photographs of the holotype of *P. gebieni*, and Mrs. Cai-Xia Yuan and Mrs. Shan-Shan Liu (College of Life Sciences, Hebei University) for taking photographs of the holotype of *P. uenoi* from National Science Museum (Nat. Hist.), Tokyo, Japan, and *P. amplipenne* and *P. ovipenne* from Muséum National d'Histoire Naturelle, Paris, France. The research was supported by the National Natural Science Foundation of China (No. 31572309, No. 31402003), the Foundation of the Key Laboratory of Zoological Systematics and Application in Hebei Province (No. 14967611D).

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RESEARCH ARTICLE



# Revision of the wingless Sikkimia Duvivier (Coleoptera, Chrysomelidae, Galerucinae) from Taiwan, including a new generic synonymy and four new species descriptions

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# Abstract

The genus *Taiwanolepta* Kimoto, 1989 (type species *T. babai* Kimoto, 1989) is proposed as a junior synonym of *Sikkimia* Duvivier, 1891. *Sikkimia* species from Taiwan form a group characterized by the reduction of their hind wings. Most of them cannot be distinguished using external morphology, except by the structure of last two antennomeres in males. Diagnoses are made by using distribution, aedeagal, and gonocoxal morphology. The group includes one previously described species, *Sikkimia babai* (Kimoto, 1989), **comb. n.**, and four new species, *S. meihuai* **sp. n.**, *S. sufangae* **sp. n.**, *S. tsoui* **sp. n.**, and *S. yuae* **sp. n.** Speciation models, supporting the high diversity of *Sikkimia* species in Taiwan, are discussed. *Sikkimia metallica* Jacoby, 1903 and *S. tamra* Maulik, 1936, both from southern India, are transferred to the genus *Cerophysa* Chevrolat, 1836.

# Keywords

Leaf beetles, Polygonum chinense, nocturnal behavior, taxonomic revision

# Introduction

Subsequent to the original description of the genus, several new genera have been proposed for *Sikkimia* species. Based on the study of type specimens, the genera *Yunomela* Chen, 1964 and *Vietocerus* Lopatin, 2003 were synonymized with *Sikkimia* by Bezděk and Zhang (2006). Another genus, *Taiwanolepta* Kimoto, 1989, is here synonymized. While continental *Sikkimia* species have well developed wings and are capable of flying, the Taiwanese species have, until now, been classified in *Taiwanolepta* and are wingless and nocturnal. In Taiwan, *Sikkimia* species appeared to be rare as no recent records had been reported.

The basic bionomics of Taiwanese *Sikkimia* populations can be summarized as follows: adults are nocturnal and closely associated with these host plants: *Polygonum chinense* L., *P. posumbu* Buch.-Ham. ex Don, and *P. thunbergii* Sieb. & Zucc. (Polygonaceae); *Rubus swinhoei* Hance and *R. corchorifolius* L. f. (Rosaceae); and *Dumasia miaoliensis* Y. C. Liu & F. Y. Lu subsp. *bicolor* (Hayata) Ohashi & Tateishi (Fabaceae); mainly feed on the host plant *P. chinense*. This plant is widely distributed and grows on the edges of forests, roads, walking trails, and rivers. As these environments are easily accessible, adults can be collected by searching for adults on host plants at night. Approximately 350 specimens have been collected throughout Taiwan by members of the Taiwan Chrysomelid Research Team (TCRT) led by author Lee.

# Materials and methods

Larvae were put into small glass containers (diameter 142 mm × height 50 mm) with cuttings from their host plants at average 20.8 °C, 74%RH, with a photoperiod of 12:12 (L:D) for laboratory rearing. When mature larvae began searching for pupation sites, they were transferred to smaller plastic containers (diameter 90 mm × height 57 mm) filled with moist soil (about 80% of container volume).

The abdomen was separated from the body and boiled in a 10% KOH solution, followed by washing in distilled water to prepare genitalia for drawing purposes. The genitalia were then dissected from the abdomen, mounted on slides in glycerin, and studied and drawn using a Leica M165 stereomicroscope. For detailed examination a Nikon ECLIPSE 50i microscope was used.

At least three pairs from each species were examined to delimit the variability of diagnostic characters,. When a species was collected from more than one locality, at least one pair from each locality was examined. Females are associated with a distinct species based on localities where they were collected. Length is measured from the anterior margin of the eye to the elytral apex, and width at the greatest width of the elytra.

Specimens studied herein are deposited at the following institutes: The Natural History Museum (BMNH), London, UK; Jan Bezděk collection (JBCB), Brno, Czech Republic; Ehime University (EUMJ), Matsuyama, Japan; Kitakyushu Museum of

Natural History and Human History (KMNH), Kitakyushu, Japan; TARI: Taiwan Agricultural Research Institute, Taichung, Taiwan. Depositions are indicated with their recognized abbreviations except for those deposited at TARI.

# Taxonomy

#### Genus Sikkimia Duvivier, 1891

- *Sikkimia* Duvivier, 1891: 154 (type species: *Sikkimia antennana* Duvivier, 1891, by monotypy); Maulik 1936: 520 (redescription).
- *Yunomela* Chen, 1964: 201 (type species: *Yunomela rufa* Chen, 1964, by original designation); Bezděk and Zhang 2006 (as synonym of *Sikkimia*).
- *Taiwanolepta* Kimoto, 1989: 73 (type species: *Taiwanolepta babai* Kimoto, 1989, by original designation). **New Synonym**
- *Vietocerus* Lopatin, 2003: 103 (type species: *Vietocerus kabakovi* Lopatin, 2003, by original designation); Bezděk and Zhang 2006 (as synonym of *Sikkimia*).

**Remarks.** The diagnostic characters for the genus *Sikkimia*, as indicated by Bezděk and Zhang (2006), are modified and extended as follows: body large (6.1–12.0 mm), robust, orange, red or brown; last two segments of antennae strongly enlarged in most males (Figs 1–2); frontal tubercles large, sub-quadratic; pronotum with antebasal transverse impression, limited on sides by short longitudinal furrows, and an additional longitudinal groove half way between short longitudinal furrows and lateral margin, running parallel to the lateral margin (Fig. 1); all pronotal margins bordered; procoxal cavities closed; apical ventrite trilobed in male, with internal anterior margin extended (Figs 7, 48 & 49); and claws appendiculate.

As all the main diagnostic characters are shared by both the continental and Taiwanese species, *Taiwanolepta* is here synonymized with *Sikkimia*. Taiwanese species differ from the continental species in having a shorter body (6.1–9.0 mm) reduced hind wings and consequently reduced humeral calli. The apical antennomere in the male is spear-shaped and more or less symmetrical in continental species (Fig. 2, see also the drawings in Bezděk and Zhang (2006)), while strongly asymmetrical in Taiwanese species (Figs 24–47). Outer longitudinal grooves on pronotum are deeper in Taiwanese species while more feeble in continental species. Aedeagus sclerotized ventrally in Taiwanese species, but membranous in continental species. Internal sclerite divergent apically in almost all Taiwanese species (Figs 19, 53, 66, 86), while the continental *S. rufa* has the sclerite divergent basally (Fig. 3). On the other hand, the structure of the spermatheca, gonocoxae, ventrite VIII and extended internal part of male abdominal ventrite V in the male are very similar (these structures of *S. rufa* as in Figs 5–8, and for the other Taiwanese species as in Figs 21–23, 48, 49, 55–57, 68–70, 81–83).

**Biology.** Taiwanese species of *Sikkimia* appear to be univoltine, based on field observations. Larvae are nocturnal and found on the underside of the host plant's leaves



**Figures 1–2.** *Sikkimia rufa* (Chen). **I** Male, dorsal view **2** Three apical antennomeres of right antenna, ventral view.

between February and April. Larval development takes about 20–22 days, based on laboratory rearing. Mature larvae leave the host plant and burrow into the soil where they build underground chambers for pupation. The pupal stage lasts for 22 days, and adults begin to emerge after April. The adults are also nocturnal and live for more than



Figures 3–8. *Sikkimia rufa* (Chen). 3 Aedeagus, dorsal view 4 Aedeagus, lateral view 5 Apices of gonocoxae 6 Eigth abdominal ventrite 7 Male abdominal ventrites III–V 8 Spermatheca.

three months, a lengthy longevity for chrysomelids. Females deposited single eggs on leaves under laboratory conditions, but these failed to hatch. Presumably *Sikkimia* species overwinter as adults, as some females were collected during winter.



Figures 9–16. Field photography. 9 Larva of *Sikkimia sufangae* sp. n. feeding on *Polygonum chinense* 10 Larva of *S. tsoui* sp. n. feeding on *Rubus corchorifolius* 11 Larva of *S. tsoui* sp. n. feeding on *Dumasia miaoliensis* subsp. *bicolor* 12 Larva of *Gallerucida singularis* feeding on *P. chinense* 13 Female of *S. tsoui* sp. n. feeding on stem of *R. corchorifolius* 14 Female of *S. sufangae* sp. n. 15 Male of *S. tsoui* sp. n. feeding on leaves of *R. swinhoei* 16 Female of *S. sufangae* sp. n. feeding on flowers of *P. posumbu*.

All known *Sikkimia* species feed on the leaves of *P. chinense* L. (Polygonaceae) (Fig. 9). However, some populations of *S. tsoui* sp. n. also feed on other plants in different areas. For example, populations from Yangminshan National Park (including Hsiaoy-uken, Erhtzuping, Lengshuiken, Tatunshan) have been observed feeding on *R. swinhoei* (Fig. 15) and *R. corchorifolius* (Fig. 10) (Rosaceae), and members of populations from Tahunshan feed on *Dumasia miaoliensis* subsp. *bicolor* (Fig. 11) (Fabaceae). Specimens from these populations will feed on *P. chinense* if switched from their original host plant.

In Taiwan, leaf beetles from three genera are known to feed on *P. chinense*. These include *Altica birmanensis* (Jacoby, 1896) (Lee and Cheng 2007), *Gallerucida singularis* Harold, 1880 (Lee and Bezděk 2013), and *Sikkimia* species. *Altica birmanensis* inhabits lowlands, at elevations below 1200 m. *Gallerucida singularis* occurs at slightly higher elevations, ranging between 1000 m and 1500 m. *Sikkimia* species occupy the higher elevations, and are found from 1000 m to 2500 m in central and southern Taiwan. Thus *G. singularis* is sympatric with *Sikkimia* species in some areas. Although members of both taxa are nocturnal, their larvae prefer different sites on the host plant. Larvae of *G. singularis* always appear on the upper surface of leaves, their body segments lack lateral expansions, and the apical posterior tergites are narrower (Fig. 12). *Sikkimia* larvae occur on the underside of leaves, each body segment has lateral expansions, and the apical posterior tergites are wider (Figs 9–11).

Distribution. China, India (Sikkim), Laos, Myanmar, Taiwan, and Vietnam.

# Revision of Taiwanese Sikkimia

# Sikkimia babai (Kimoto, 1989), comb. n.

Taiwanolepta babai Kimoto, 1989: 74.

**Type locality.** Taiwan: Kaoshiung county, Shinanshan (溪南山), 23°05'36"N, 120°48'18"E, 2600 m.

**Type material.** Deposition of type specimens (holotype and one paratype) was not indicated by the original paper. The paratype ♂was found at the KMNH, labeled: "Thu Yun Shan [出雲山], near Liu Kui [六龜], S-Taiwan 23.VII.1986 Col. K Baba / *Taiwanolepta babai* n. sp. Det. S. Kimoto, 1989 / PARATYPE (printed on blue paper) / PHOTO (printed on red paper)".

**Other material examined** (n= 18). **Kaoshiung:** 733, 799, Tengchi (藤枝), 23°04'02"N, 120°45'21"E, 2.VI.2008, leg. C.-F. Lee (2 spec. in JBCB); 13, same locality, 26.V.2009, leg. C.-F. Lee; 19, Shihshan logging trail (石山林道, =Tengchi), 1.X.2008, leg. M.-H. Tsao; 13, 399, same locality, 2.X.2008, leg. M.-H. Tsou.

**Description.** *Male.* Length 7.1–7.5 mm; width 3.9–4.1 mm. Coloration reddishbrown, head dark brown, legs and antennae black. Antenna (Fig. 17) elongate, about as long as body; antennomeres I to VIII filiform; IX widening slightly towards apex; × and XI extremely swollen (Figs 24–26, 36–38), × with a deep groove, from middle to apex,



Figures 17–23. *Sikkimia babai* (Kimoto). 17 Antenna, male 18 Antenna, female 19 Aedeagus, dorsal view 20 Aedeagus, lateral view 21 Gonocoxae 22 Eighth abdominal ventrite 23 Spermatheca.

of mesal surface; apex of XI pointed, weakly concave in apical 1/3 of mesal surface and in basal 1/4 of outer surface; dorsal surface with two longitudinal ridges, one centrally located, curved, from middle to basal 1/5; other longitudinal ridge along mesal surface from middle to basal 1/4; one deep groove between the two longitudinal ridges; one transverse groove near base; small process at apical 1/3 near outer margin; length ratios



Figures 24–35. Photographs of male antennomeres X–XI. 24 *S. babai* (Kimoto), outer view 25 Ventral view 26 Inner view 27 *S. meihuai* sp. n., outer view 28 Ventral view 29 Inner view 30 *S. sufangae* sp. n., outer view 31 Ventral view 32 Inner view 33 *S. yuae* sp. n., outer view 34 Ventral view 35 Ditto, inner view.

of antennomeres II to XI about 1.0: 1.2: 2.0: 1.9: 1.9: 1.9: 1.7: 1.9: 3.2: 3.5, and length to width ratios of antennomeres II to XI about 1.4: 1.5: 2.4: 2.1: 2.1: 2.3: 2.2: 1.8: 2.4: 2.0. Pronotum transverse,  $1.7 \times$  wider than long; anterior and posterior mar-

gins almost straight; lateral margins weakly rounded or straight; disc finely punctured. Elytra narrow, about  $1.3 \times longer$  than wide; densely and randomly punctuate, humeri reduced. Abdominal ventrite V (Fig. 48) trilobed, internal anterior margin extended, reaching ventrite III; median longitudinal, internal ridge running from base to apex of extension. Abdominal tergite I with only spiracles sclerotized; tergites II–V with sclerotized spiracles and transverse weakly sclerotized areas; most of tergite VI and spiracles strongly sclerotized; tergite VII entirely and strongly sclerotized. Aedeagus (Figs 19–20) narrow in dorsal view, about  $6.2 \times longer$  than wide, parallel-sided in basal 1/3, becoming slightly narrower towards apex; apex subtriangular and pointed; ventral surface well sclerotized and smooth; narrow and moderately curved in lateral view; endophallic sclerite longitudinal and slender, bifurcate apically, about  $0.3 \times as long as aedeagus.$ 

*Female.* Length 8.1–8.4 mm; width 5.3–5.8 mm. Similar to males, but dark brown ventrally; antennae (Fig. 18) filiform, antennomeres × and XI not swollen; length ratio of II to XI about 1.0 : 1.7 : 2.2 : 2.2 : 2.0 : 1.8 : 1.9 : 2.2 : 2.8, and length to width ratios of II to XI about 1.9 : 2.4 : 3.4 : 3.6 : 3.8 : 3.6 : 3.2 : 3.3 : 3.7 : 4.4. Elytra wider than in male, length equal to width. Gonocoxae (Fig. 21) slender, together about 4.0 × longer than wide, joined from base almost to middle, base strongly narrowed in basal 1/3 with a long medial groove, apices tubular and sub-parallel, inner margins slightly indented medially, apex with nine setae. Ventrite VIII (Fig. 22) with extremely long spiculum; apical margin widely rounded, weakly sclerotized basally, disc with long scattered setae along apical margin. Abdominal tergites I–III membranous, only spiracles sclerotized, tergites IV–VII entirely and strongly sclerotized. Receptacle of spermatheca (Fig. 23) strongly swollen and transverse, pump elongate and moderately curved, proximal spermathecal duct long and wide.

**Diagnosis.** Sikkimia babai is similar to S. sufangae sp. n. They share a slender aedeagus (more than 5.9× longer than wide), but in S. babai it is parallel-sided (aedeagus wider basally in S. sufangae sp. n. (Fig. 66, 67)). Antennomere XI in male S. babai has one process on the inner antero-lateral surface and the outer antero-lateral surface is flat (process absent on inner antero-lateral surface and outer antero-lateral surface depressed in S. sufangae sp. n.). The gonoxae are sub-parallel in S. babai (diverging in S. sufangae sp. n.).

Host plant. Polygonum chinense L. (Polygonaceae).

Distribution. Tengchi (Kaoshiung county) (Fig. 50) and its surrounding areas.

#### Sikkimia meihuai sp. n.

http://zoobank.org/DD3EE7B6-4DD2-4CD7-AAF7-F2FCD115C6CC Figs 27–29, 39–41, 50–57

**Type locality.** Taiwan: Taitung county, Liyuan (栗園), 23°13'17"N, 121°00'40"E, 1800 m.

**Type material** (n= 19). Holotype 3: **Taitung:** Liyuan (栗園), 23.VI.2010, leg. M.-H. Tsou. Paratypes: 333, 299, same data as holotype; 299, same locality,



Figures 36–47. Illustrations of male antennomeres X–XI. 36 *S. babai* (Kimoto), outer view 37 Ventral view 38 Inner view 39 *S. meihuai* sp. n., outer view 40 Ventral view 41 Inner view 42 *S. sufangae* sp. n., outer view 43 Ventral view 44 Inner view 45 *S. yuae* sp. n., outer view 46 Ventral view 47 Inner view.

19.VI.2013, leg. C.-F. Lee; 7♀♀, same locality, 24.VII.2013, leg. C.-F. Lee; 1♂, 3♀♀, Motien (摩天), 23°11'41"N, 121°01'18"E, 20.VI.2011, leg. C.-F. Lee (1♂, 2♀♀ in JBCB).



Figures 48-49. Male abdominal ventrites III-V, dorsal view. 48 Sikkimia babai 49 S. tsoui sp. n.

Description. Male. Length 7.3–7.5 mm; width 4.0–4.2 mm. Coloration brown, head dark brown, legs and antennae black. Antenna (Fig. 51) long, about as long as body; antennomeres I–VII filiform; VIII-IX widening slightly;× and XI extremely swollen (Figs 27-29, 39-41),× with a shallow groove from middle to apex of mesal surface, XI moderately concave in basal 1/4 of outer surface, weakly concave in apical 1/3 of mesal surface and pointed apically; dorsal surface with two longitudinal ridges, one close to mesal margin, extending from middle and abbreviated near base, other longitudinal ridge along mesal margin extending from apical 1/3, projecting in middle, and ending in basal 1/4, with a deep groove between the longitudinal ridges, and a transverse groove near the base; length ratio of II to XI about 1.0: 1.3: 1.8: 1.6:1.8 : 1.6 : 1.4 : 1.3 : 1.8 : 2.8, and length to width ratios of II to XI about 2.0 : 2.0 : 2.7 : 2.4 : 2.7 : 2.2 : 2.3 : 1.9 : 2.8 : 2.8. Pronotum transverse, 1.5× as wider than long; anterior and posterior margins almost straight, slightly concave medially; lateral margin weakly rounded; disc with finely punctured. Elytra narrow, about 1.2× longer than wide; densely and randomly punctuate, humeri reduced. Abdominal ventrite V trilobed, internal anterior margin extended, reaching ventrite III; median longitudinal internal ridge running from base to apex of extension. Abdominal tergite I with only spiracles sclerotized; tergites II-V with sclerotized spiracles and transverse weakly sclerotized areas; most of tergite VI and spiracles strongly sclerotized; tergite VII entirely and strongly sclerotized. Aedeagus (Figs 53–54) wide in dorsal view, about 4.8× longer than wide, base strongly incised medially, wide in basal 1/3, becoming slightly narrower towards the subtriangular apex; ventral surface well sclerotized and smooth; broad and moderately curved in lateral view; endophallic sclerite longitudinal and slender, bifurcate apically, about  $0.3 \times$  as long as aedeagus.

*Female.* Length 7.5–8.2 mm; width 4.5–4.8 mm. Similar to male, but antennae (Fig. 52) filiform, antennomeres× and XI not swollen; length ratio of II to XI about 1.0 : 1.6 : 2.1 : 2.0 : 2.1 : 2.0 : 1.8 : 1.9 : 2.1 : 2.6, and length to width ratios of II to XI about 1.9 : 2.6 : 3.3 : 3.1 : 3.3 : 3.2 : 2.9 : 3.0 : 3.3 : 4.2. Elytra relatively wide, about  $1.1 \times$  longer than wide. Gonocoxae (Fig. 55) wide, together about  $2.7 \times$  longer than wide and joined from base almost to middle, basal margin deeply indented medially narrowing strongly in basal 1/3 with a short medial groove, apices tubular and parallel, narrowing slightly in apical 1/3 and curving inward, with nine setae. Ventrite VIII (Fig. 56) weakly sclerotized; with extremely long speculum; apex transverse, api-



Figure 50. Distribution map of Sikkimia species of Taiwan, solid line: 1000 m, broken line: 2000 m. Brown dots: S. sufangae sp. n., green dots: S. babai, pink dots: S. yuae sp. n., red dots: S. tsoui sp. n., blue dots: S. meihuai sp. n.



Figures 51–57. *Sikkimia meihuai* sp. n. 51 Antenna, male 52 Antenna, female 53 Aedeagus, dorsal view 54 Aedeagus, lateral view 55 Gonocoxae 56 Eighth abdominal ventrite 57 Spermatheca.

cal margin widely rounded, with scattered long setae along apical margin. Abdominal tergites I–III membranous with only spiracles sclerotized, tergites IV–VII entirely and strongly sclerotized. Receptacle of spermatheca (Fig. 57) strongly swollen and transverse; pump elongate and strongly curved; spermathecal duct short but extremely wide.

**Diagnosis.** *Sikkimia meihuai* sp. n. is similar to *S. yuae* sp. n. in greatest width of the aedeagus ( $4.8 \times$  longer than wide), but differs in having the aedeagus narrowing very slightly towards the apex (distinctly narrower in apical 1/3 in *S. yuae* sp. n.); short median ridge on antennomere IX in males (long median ridge in *S. yuae* sp. n.); and wider gonocoxae,  $2.7 \times$  longer than wide (slender gonocoxae in *S. yuae* sp. n.,  $4.4 \times$  longer than wide).

Host plant. Polygonum chinense L. (Polygonaceae).

**Etymology.** This new species is named after Mr. Mei-Hua Tsou, who is a member of TCRT and the first to collect this new species.

Distribution. East half of South Cross-Island Highway (南横公路) (Fig. 50).

#### Sikkimia sufangae sp. n.

http://zoobank.org/9AD84610-CAB7-4517-934E-689E4A8F3393 Figs 9, 14, 16, 30–32, 42–44, 50, 58–70

**Type locality.** Taiwan: Pingtung county, Tahanshan (大漢山), 22°24'27"N, 120°45'23"E, 1400 m.

**Type material** (n= 75). Holotype  $\mathcal{J}$ : **Pingtung:** Tahanshan (大漢山), 6.VI.2012, leg. C.-F. Lee. Paratypes:  $5\mathcal{J}\mathcal{J}$ ,  $9\mathcal{Q}\mathcal{Q}$ , same data as holotype ( $2\mathcal{J}\mathcal{J}$ ,  $2\mathcal{Q}\mathcal{Q}$  in JBCB); 1 $\mathcal{J}$ , same locality, 18.VII.2007, leg. C.-F. Lee; 1 $\mathcal{J}$ , 1 $\mathcal{Q}$ , same locality, 22.I.2009; leg. S.-F. Yu; 1 $\mathcal{J}$ , same locality, 25.V.2009, leg. M.-L. Jeng; 1 $\mathcal{Q}$ , same locality, 21.I.2012, leg. S.-F. Yu; 1 $\mathcal{Q}$ , same locality, 19.VII.2012, leg. C.-F. Lee;  $2\mathcal{J}\mathcal{J}$ , same locality, 29.IV.2013, leg. Y.-T. Chung; 1 $\mathcal{J}$ , same locality, 2.VI.2013, leg. J. Yamasako (EUMJ); 1 $\mathcal{Q}$ , same locality, 29.VI.2013, leg. B.-X. Guo;  $3\mathcal{Q}\mathcal{Q}$ , same locality, 8.VII.2013, leg. B.-X. Guo;  $3\mathcal{Q}\mathcal{Q}$ , same locality, 11.VII.2013, leg. B.-X. Guo; 1 $\mathcal{Q}$ , same locality, 12.VII.2013, leg. Y.-T. Chung; 1 $\mathcal{Q}$ , same locality, 19.VII.2013, leg. M.-H. Tsou; 1 $\mathcal{Q}$ , 28.VIII.2014, leg. Y.-T. Chung; 3 $\mathcal{Q}\mathcal{Q}$ , 4.X.2014, leg. Y.-T. Chung; 8 $\mathcal{J}\mathcal{J}$ , 1 $\mathcal{Q}$ , same locality, 1.V.2015, leg. Guo & Chung; 1 $\mathcal{J}$ , same locality, 19.V.2015, leg. Y.-T. Chung; 3 $\mathcal{J}\mathcal{J}$ , same locality, 27.V.2015, leg. Y.-T. Chung; 1 $\mathcal{J}$ , same locality, 29.V.2015, leg. Y.-T. Chung; 4 $\mathcal{J}\mathcal{J}$ , 3 $\mathcal{Q}\mathcal{Q}$ , same locality, 6.VI.2015, leg. Y.-T. Chung; 3 $\mathcal{J}\mathcal{J}$ , Peitawushan (t大式thu), 22°37'47"N, 120°45'41"E, 22.IV.2015, leg. J.-C. Chen; 7 $\mathcal{J}\mathcal{J}$ , 7 $\mathcal{Q}\mathcal{Q}$ , same locality, 24.IV.2015, leg. J.-C. Chen.

**Description.** *Male.* Length 7.8–9.0 mm; width 4.0–4.3 mm. Coloration (Figs 58–60) brown, head dark brown, legs and antennae black. Antenna (Fig. 64) long, about as long as body; antennomeres I to VII filiform; VIII and IX widening slightly;× and XI (Figs 30–32, 42–44) extremely swollen,× with shallow groove from middle to apex of mesal surface; apex of XI pointed, moderately concave in basal 1/4 of outer surface and in apical 1/3 of mesal surface, dorsal surface with two longitudinal ridges, one close to mesal margin, from base to near middle, strongly curved; other longitudinal ridge along mesal margin extending from basal <sup>1</sup>/<sub>4</sub> to apical 1/3, projecting medially, with a deep groove between the longitudinal ridges, and a transverse groove near the base, shallowly depressed on outer antero-lateral surface; length ratio of II to XI about 1.0 : 1.4 : 1.8 : 2.2



Figures 58–63. Habitus of *Sikkimia sufangae* sp. n. 58 Male, dorsal view 59 Male, ventral view 60 Male, lateral view 61 Female, dorsal view 62 Female, ventral view 63 Female, lateral view.

: 2.0 : 1.9 : 2.1 : 1.9 : 1.8 : 2.8, and length to width ratios of II to XI about 1.6 : 1.9 : 2.9 : 2.7 : 2.6 : 2.8 : 2.8 : 2.5 : 1.9 : 2.2. Pronotum transverse, 1.5× wider than long; anterior and posterior margins almost straight; lateral margin weakly rounded; disc with small punctures. Elytra narrow, about 1.4× longer than wide; densely and randomly punctuate, humeri reduced. Abdominal ventrite V trilobed, internal anterior margin extended, reaching ventrite III; median longitudinal, internal ridge running from base to apex of extension. Abdominal tergite I with only spiracles sclerotized; tergites II-V with sclerotized spiracles and transverse weakly sclerotized areas; most of tergite VI and spiracles strongly sclerotized; tergite VII entirely and strongly sclerotized. Aedeagus (Figs 66–67)



Figures 64–70. *Sikkimia sufangae* sp. n. 64 Antenna, male 65 Antenna, female 66 Aedeagus, dorsal view 67 Aedeagus, lateral view 68 Gonocoxae 69 Eighth abdominal ventrite 70 Spermatheca.

slender, about 5.9× longer than wide, base moderately incised medially, basal 1/3 wide, narrowing considerably towards the rounded apex, ventral surface well sclerotized and smooth; moderately curved in lateral view; endophallic sclerite longitudinal and extremely slender, bifurcate apically, about 0.3× as long as aedeagus.

*Female.* Length 7.8–8.1 mm; width 5.2–5.3 mm. Similar to males (Figs 61–63), but antennae filiform (Fig. 65), antennomeres× and XI not swollen; length ratio of II to XI about 1.0 : 1.5 : 2.0 : 1.9 : 2.0 : 1.9 : 1.8 : 1.9 : 2.0 : 2.6, and length to width ratios of II to XI about 1.9 : 2.3 : 3.3 : 3.2 : 3.4 : 3.1 : 3.0 : 3.1 : 3.4 : 4.0. Elytra as wide as long. Gonocoxae (Fig. 68) slender, about 3.6× longer than wide, joined from base to middle, base rounded, strongly narrowed in basal 1/3, apices tubular curved slightly inwards, diverging, apex with seven or eight setae. Ventrite VIII (Fig. 69) weakly sclerotized; apex extremely transverse, apical margin widely rounded, disc with long scattered long setae towards apex. Abdominal tergites I–III membranous with only the spiracles sclerotized, IV–VII entirely and strongly sclerotized. Receptacle of spermatheca (Fig. 70) strongly swollen and transverse, pump long and strongly curved; proximal spermathecal duct short and swollen distally.

**Diagnosis.** *Sikkimia sufangae* is similar to *S. babai*. See diagnosis of *S. babai* for a summary of the differentiating characteristics of these two species.

**Host plant.** *Polygonum chinense* L.; *P. posumbu* Buch.-Ham. ex Don (Polygonaceae) (Fig. 16).

**Etymology.** This new species is named after Mrs. Su-Fang Yu, who is a member of TCRT and the first to collect this new species.

Distribution. Southern Taiwan (Fig. 50).

# Sikkimia tsoui sp. n.

http://zoobank.org/385E17EE-64F8-4B24-AF8B-E7D2C948731C Figs 10, 11, 13, 15, 49, 50, 71–83

**Type locality.** Taiwan: Taipei city, Hsiaoyuken (小油坑), 25°10'38"N, 121°32'50"E, 800 m.

**Type material** (n= 229). Holotype **⑦**: **Taipei:** Hsiaoyuken (小油坑), 22.VI.2008, leg. M.-H. Tsou. Paratypes:  $1^{\circ}$ , same as holotype;  $1^{\circ}$ , same locality, 21.IV.2008, leg. M.-H. Tsou; 233, same locality, 24.IV.2008, leg. M.-H. Tsou; 6, same locality, 22.VI.2008, leg. S.-F. Yu; 1Å, same locality, 24.V.2008, leg. M.-H. Tsou; 1Å, 3°, 9 same locality, 5.IV.2009, leg. M.-H. Tsou; 833, 1322, same locality, 8.V.2010, leg. M.-H. Tsou;  $2 \bigcirc \bigcirc$ ,  $8 \bigcirc \bigcirc$ , same locality, 15.V.2011, leg. M.-H. Tsou;  $1 \bigcirc$ , Erhtzuping (二子坪), 25°11'01"N, 121°31'07"E, 14.VIII.2011, leg. M.-H. Tsou; 7♂♂, 4♀♀, same locality, 3.VI.2011, leg. M.-H. Tsou; 299, Lengshuiken (冷水坑), 25°10'03"N, 121°33'46"E, 07.IV.2009, leg. H. Lee; 13, same locality, 08.IV.2009, leg. H. Lee; 1<sup>♀</sup>, Tatunshan (大屯山), 25°11'12"N, 121°31'22"E, 22.V.2010, leg. M.-H. Tsou; Hsinchu: 1<sup>Q</sup>, Lupi (魯壁), 24°39'56"N, 121°16'47"E, 19.VII.2008, leg. M.-H. Tsou; 1♂, 2♀♀, Mamei (馬美), 24°40'13"N, 121°19'13"E, 10.VII.2010, leg. M.-H. Tsou; 1♂, Tahunshan (大混山), 24°41′20″N, 121°16′29″E, 08.IV.2009, leg. M.-H. Tsou; 13, same locality, 11.IV.2009, leg. M.-H. Tsou; 12, same locality, 13.IV.2009, leg. M.-H. Tsou; 1♂, Talu logging trail (大鹿林道), 24°32'06"N, 121°07'01"E, 1.VIII.2015, leg. Y.-L. Lin; Ilan: 19, Mingchi (明池), 24°39'01"N, 121°28'22"E,



Figures 71–76. Habitus of *Sikkimia tsoui* sp. n. 71 Male, dorsal view 72 Male, ventral view 73 Male, lateral view 74 Female, dorsal view 75 Female, ventral view 76 Female, lateral view.

2.VII.2008, leg. H.-J. Chen; 1♂, Taipingshan (太平山), 24°29'53"N, 121°32'06"E, 5.VIII.2015, leg. Y.-T. Chung; 16♀♀, Yuanyanghu (鴛鴦湖), 24°34'36"N, 121°24'09"E, 22.VIII.2011, leg. C.-F. Lee; 10♂♂, 5♀♀, same locality, 22.VIII.2011, leg. M.-H. Tsou (2♂♂, 2♀♀ in JBCB); 15♂♂, 5♀♀, same locality, 22.VIII.2011, leg. H. Lee; 3♂♂, Tatung (大同, = Yuanyanghu), 19.VIII.2010, leg. H.-H. Lee; **Miaoli:** 1♂, Luchang (鹿場), 24°32'26"N, 121°01'38"E, 1.VI.2014, leg. Y.-M. Weng; **Nantou:** 15♂♂, 35♀♀, Hsitou (溪頭), 23°40'20"N, 120°47'53"E, 14.VI.2011, leg. C.-F. Lee; 10♂♂, 7♀♀, same locality, 9.VIII.2011, leg. M.-H. Tsou; 1♂, Shanlinhsi (



Figures 77–83. *Sikkimia tsoui* sp. n. 77 Antenna, male 78 Antenna, female 79 Aedeagus, dorsal view 80 Aedeagus, lateral view 81 Eighth abdominal ventrite 82 Gonocoxae 83 Spermatheca.

杉林溪), 23°38'22"N, 120°47'32"E, 10.IX.2009, leg. Y.-T. Wang; 2♂♂, 3♀♀, same locality, 12.VIII.2015, leg. S.-P. Wu; **Taichung:** 7♂♂, 7♀♀, Anmashan (鞍馬山), 24°14'41"N, 120°58'30"E, 19.X.2011, leg. C.-F. Lee; 2♂♂, Tahsuehshan (大雪山, = Anmashan), 7.VI.2010, leg. C.-F. Lee; 2♀♀, same locality, 4.VI.2012, leg. J.-C. Chen; **Taoyuan:** 1♂, Hsuanyuan (萱源), 24°39'11"N, 121°24'17"E, 13.V.2010, leg. S.-F. Yu; 4♀♀, same locality, 1.VI.2010, leg. W.-T. Liu; 1♂, Lalashan (拉拉山), 24°40'47"N, 121°23'02"E, 20.IV.2008, leg. C.-F. Lee.

Description. Male. Length 6.1-6.5 mm; width 3.7-3.8 mm. Coloration brown (Figs 71–73), legs and antennae black. Antenna (Fig. 77) long, about long as body; filiform; length ratio of antennomeres II to XI about 1.0: 1.3: 1.8: 1.8: 1.7: 1.7: 1.7 : 1.7 : 1.7 : 2.1 and length to width ratios of II to XI about 2.1 : 2.6 : 3.6 : 3.6 : 4.0: 4.0: 4.0: 4.0: 4.0: 4.9. Pronotum transverse, 1.6× wider than long; anterior and posterior margins almost straight; lateral margin weakly rounded; disc with reduced punctures. Elytra wide, about 1.2× longer than wide; densely and randomly punctuate, humeri reduced. Abdominal tergite I membranous with sclerotized spiracles; II-VI with medial transverse patch sclerotized as well as area surrounding spiracles; VII mostly sclerotized, with spiracle inside sclerotized area. Abdominal ventrite V (Fig. 49) trilobed, internal anterior margin extended, reaching ventrite III; median longitudinal, internal ridge extending from base to mid-length of extension. Aedeagus (Figs 79-80) wide in dorsal view, about 4.5× longer than wide, base shallowly incised medially; greatest width in basal 1/3, becoming very slightly narrower towards the subtriangular apex; ventral surface well sclerotized, concave medially; narrow and moderately curved in lateral view; endophallic sclerite longitudinal, slender, joined from base to near apex, about  $0.4 \times$  as long as aedeagus (Fig. 79).

*Female.* Length 8.0–8.3 mm; width 4.9–5.7 mm. Similar to male (Figs 74–76). Antenna (Fig. 78) about long as body; filiform; length ratio of antennomeres II to XI about 1.0 : 1.3 : 1.8 : 1.8 : 1.7 : 1.7 : 1.8 : 1.8 : 2.1 and length to width ratios of II to XI about 2.2 : 3.0 : 3.9 : 3.8 : 4.0 : 4.0 : 4.0 : 4.2 : 4.3 : 6.0. Elytra as long as wide, wider than in male. Gonocoxae (Fig. 82) extremely wide, about 2.9× longer than wide, joined from basal 1/5 to middle, with several long and many short dense setae on tubular apices; greatest width at base incised medially, narrowing slightly in basal 1/3 before widening slightly again. Abdominal tergites I and II membranous, only area surrounding of spiracles sclerotized; III with one pair of transverse sclerotized areas near middle; IV with one transverse sclerotized area at middle; V and VI with sclerotized areas larger than on IV; VII mostly sclerotized with spiracles lying inside sclerotized area. Ventrite VIII (Fig. 81) strongly sclerotized; apex transverse, apical margin weakly emarginate, with long dense setae along apical margin. Receptacle of spermatheca (Fig. 83) swollen, pump long and strongly curved, spermathecal duct long and slender.

**Variation.** Specimens collected from Hsiaoyuken have more robust antennae (length ratio of antennomeres II to XI about 1.0 : 1.5 : 2.2 : 2.1 : 2.2 : 2.1 : 2.1 : 2.2 : 2.3 : 2.3 : 2.7 and length to width ratios of II to XI about 1.6 : 2.4 : 3.2 : 3.4 : 3.5 : 3.3 : 3.3 : 3.7 : 3.7 : 4.3).

**Diagnosis.** This species is easily distinguished from other Taiwanese species of *Sikkimia* using a combination of the following characters: filiform antennae in males (swollen antennomeres× and XI in other species), reduced median ridge on internal anterior margin extension of abdominal ventrite V (well developed internal median ridge in other species), and the endophallic sclerite of aedeagus that is joined from the base almost to the apex (endophallic sclerite of aedeagus bifurcate apically in other species); abdominal tergites IV-VI largely membranous in female, and gonocoxae much wider with numerous setae on their apices (other species with entirely sclerotized abdominal tergites, slender gonocoxae with few setae on their apices in other species).

Host plant. Polygonum chinense L.; P. thunbergii Sieb. & Zucc. (Polygonaceae); Rubus swinhoei Hance; R. corchorifolius L. f. (Rosaceae); Dumasia miaoliensis Y. C. Liu & F. Y. Lu subsp. bicolor (Hayata) Ohashi & Tateishi (Fabaceae).

**Etymology.** This new species is named after Mr. Mei-Hua Tsou, who is a member of TCRT and the first to collect this new species.

**Distribution.** North and Central Taiwan (Fig. 50). The distribution extend northwards to Yamingshan National Park (陽明山國家公園) and southwards to Hsitou (溪頭).

#### Sikkimia yuae sp. n.

http://zoobank.org/288CCEA4-D157-43B8-A523-ED8B454EEDFF Figs 33–35, 50, 84–90

**Type locality.** Taiwan: Kaoshiung county, Chungchihkung (中之關), 23°17'10"N, 120°53'51"E, 2300 m.

**Type material** (n= 16). Holotype 3: **Kaoshiung:** Chungchihkung (中之關), 10.VI.2015, leg. C.-F. Lee. Paratypes: 533, 799, same data as holotype (233, 299 in JBCB); 13, 299, Taoyuan (桃源= Chungchihkung), 1.VII.2009, leg. S.-F. Yu.

Description. Male. Length 7.1–7.5 mm; width 3.9–4.1 mm. Coloration reddishbrown, head dark brown, legs and antennae black. Antenna (Fig. 84) long, about long as body; antennomeres I to VII filiform; VIII and IX slightly widened;× and XI extremely swollen (Figs 33-35, 45-47),× with deep groove from middle to apex of mesal surface, apex of XI pointed, moderately concave in apical 1/3 of mesal surface and in basal 1/4 of outer surface, dorsal surface with two longitudinal ridges, one close to mesal margin extending from basal ¼ to apical 1/3, other longitudinal ridge along mesal margin from basal <sup>1</sup>/<sub>4</sub> to the middle, deep groove between longitudinal ridges, transverse groove near base; length ratio of antennomeres II to XI about 1.0: 1.3: 1.5: 1.5: 1.6 : 1.6 : 1.5 : 1.6 : 2.5 : 3.1, length to width ratios of antennomeres II to XI about 1.8 : 1.8 : 2.4 : 2.2 : 2.3 : 2.3 : 2.1 : 2.0 : 2.7 : 2.7. Pronotum transverse, 1.6× wider than long; anterior and posterior margins sinuate, weakly concave medially; lateral bordermargin weakly rounded; disc with fine scattered punctures. Elytra narrow, about 1.2× longer than wide; punctuate densely, reduced humeri, lateral margin rounded, widest just posterior of the middle. Abdominal ventrite V trilobed, internal anterior margin extended, reaching ventrite III; median longitudinal, internal ridge running from base



Figures 84–90. *Sikkimia yuae* sp. n. 84 Antenna, male 85 Antenna, female 86 Aedeagus, dorsal view 87 Aedeagus, lateral view 88 Gonocoxae 89 Eighth abdominal ventrite 90 Spermatheca.

to apex of extension. Abdominal tergite I with only spiracles sclerotized; II-V with spiracles sclerotized and transverse weakly sclerotized areas; most of VI and spiracles strongly sclerotized; whole of VII strongly sclerotized. Aedeagus (Figs 86–87) wide

in dorsal view, about 4.8× longer than wide, base shallowly incised medially; greatest width in basal 1/3, narrowing slightly towards apical 1/3, widening slightly subapically before the subtriangular apex with a pointed tip; ventral disc well sclerotized and smooth; aedeagus wide and moderately curved in lateral view; endophallic sclerite longitudinal and slender, bifurcate apically, about 0.4× as long as aedeagus.

*Female.* Length 7.8–8.2 mm; width 5.3–5.6 mm. Similar to male, but underside dark brown; antenna (Fig. 85) filiform, antennomeres× and XI not swollen; length ratio of antennomeres II to XI about 1.0 : 1.2 : 1.9 : 1.8 : 1.9 : 1.9 : 1.7 : 1.9 : 2.1 : 2.6, and length to width ratios of II to XI about 2.0 : 2.0 : 3.2 : 3.4 : 3.5 : 3.4 : 3.3 : 3.5 : 4.8 : 4.3. Elytra wider than in male, length and width the same. Gonocoxae (Fig. 88) slender, about 4.4× longer than wide, joined from base to just before middle, apices tubular, straight and subparallel, with 9–10 apical setae, base rounded and slightly narrower than greatest width at middle, narrowing slightly at basal 1/3. Ventrite VIII (Fig. 89) with extremely long spiculum; apex very small and oval in shape, weakly sclerotized basally, disc with long scattered setae. Abdominal tergites I–III membranous with only the spiracles sclerotized, IV–VII entirely and strongly sclerotized. Receptacle of spermatheca (Fig. 90) slightly swollen, pump short and moderately curved, spermathecal duct wide and long.

**Diagnosis.** This new species can be distinguished from others by the following combination of characters: apical 1/3 of aedeagus narrowing slightly before widening slightly again subapically (aedeagus parallel in *S. babai* and widening basally in *S. sufangae* sp. n. and *S. meihuai* sp. n.), and straight subparallel apices of gonocoxae (curved apices of gonocoxae in *S. meihuai* sp. n., *S. babai*, and *S. sufangae* sp. n.).

Host plant. Polygonum chinense L. (Polygonaceae).

**Etymology.** This new species is named after Mrs. Su-Fang Yu, who is a member of TCRT and the first person to collect this new species.

Distribution. West half of South Cross-Island Highway (南横公路) (Fig. 50).

### Key to species of Sikkimia in Taiwan

2 Aedeagus narrowing slightly towards apical 1/3, widening slightly subapically (Fig. 86); median anterior ridge on antennomere XI extending into apical 1/3 in males (Figs 33-35, 45-47); apices of gonocoxae straight and subparallel (Fig. 88) ......S. yuae sp. n. Aedeagus parallel-sided or widened basally (Figs 19, 53, 66); median ridge of antennomere XI abbreviated or curved outwards at middle in males (Figs 3 Aedeagus slender, more than 5.9× longer than wide (Figs 19, 66); median ridge of antennomere XI curved medially in males (Figs 24-26, 30-32); gonocoxae slender, more than 3.6× longer than wide (Figs 21, 68)......4 Aedeagus wide, 4.8× longer than wide (Fig. 53); median ridge of antennomere XI abbreviated medially in males (Figs 27–29, 39–41); gonocoxae wide, Aedeagus parallel-sided (Fig. 19); antennomere XI in males with a small pro-4 cess on inner antero-lateral surface and flat on outer antero-lateral surface (Figs 24–26, 36–38); gonocoxae sub-parallel (Fig. 21).... S. babai (Kimoto) Aedeagus wide basally (Fig. 66); antennomere XI in males without processes on inner antero-lateral area and depressed on outer antero-lateral area (Figs 

# Species excluded from Sikkimia

As mentioned by Maulik (1936) and subsequently also by Bezděk and Zhang (2006), the descriptions of *S. metallica* Jacoby, 1903 and *S. tamra* Maulik, 1936 are very different to other *Sikkimia* and their position in *Sikkimia* was regarded as doubtful. In 2007, one of us (JB) examined the type specimens of both species. They are here moved from *Sikkimia* and to *Cerophysa* Chevrolat, 1836.

The reason why Jacoby (1903) classified his new species in *Sikkimia* is unknown to us. Probably he misinterpreted or overlooked some important characters like colour of uthe venter, impressions on the pronotum, or structure of the antennae. Duvivier (1891: 154) described the pronotal impressions as "présentant de chaque côté une profonde impression oblique" what probably allows some misinterpretations. The pronotum of *S. metallica* has transverse impression in the middle more or less interrupted medially. The differences in the structure of antennae Jacoby (1903) attributed to the sexual dimorphism as the specimens of *S. metallica* are females.

Maulik (1936) also did not examined true *S. antennata* as he published only the English translation of Duvivier's description of *Sikkimia*. It is evident that he compared *S. tamra* with Jacoby's *S. metallica* and thus mistakenly classified his species also in *Sikkimia*.

The main differencies between true *Sikkimia* species and *S. tamra* with *S. metallica* can be described as follows: true *Sikkimia* are large (6.1–12.0 mm), robust and convex species of orange, red or brown upperside, last two antennomeres in males are strongly

modified (except *S. tsoui* sp. n.), pronotum with antebasal transverse impression limited on sides by short longitudinal furrows and additional longitudinal groove parallel to lateral margin and procoxal cavities closed behind. The same characters of *S. tamra* and *S. metallica* (which simultaneously allow us to transfer both species to *Cerophysa*) are: body 5.5–6.0 mm long, narrow, subparallel, flat, with upperside metallic green, antennae without modifications; pronotum with transverse impression in the middle of pronotum and procoxal cavities open behind. The structure of antennae is variable throughout *Cerophysa*. In some species one, two or three antennomeres can be modified, but never last two antennomeres.

# Cerophysa metallica (Jacoby, 1903), comb. n.

Sikkimia metallica Jacoby, 1903: 122.

# Type locality. Nilgiri hills.

**Type material.** Syntype ( $\bigcirc$ , BMNH), labeled: "Nilgiri Hills (printed on white label) / 482 (handwritten on white label) / Type (printed on red label) / Sikkimia metallica Jac. (handwritten on blue label) / Andrewes Bequest B. M. 1922–221. (printed on white label)".

# Cerophysa tamra (Maulik, 1936), comb. n.

Sikkimia tamra Maulik, 1936: 523.

#### Type locality. Nilgiri hills.

**Type material.** Syntype (unsexed, BMNH), labeled: "Type (printed on white round label with red collar) / Nilgiri Hills. G. F. Hampson 94–89. (printed on white label) / Sikkimia tamra M. S. Maulik TYPE 1935 (handwritten and printed on white label)".

#### Catalogue of Sikkimia

Sikkimia antennata Duvivier, 1891	Sikkim
Sikkimia babai (Kimoto, 1989), comb. n.	Taiwan
Sikkimia kabakovi (Lopatin, 2003)	Vietnam
Sikkimia meihuai sp. n.	Taiwan
Sikkimia miranda (Lopatin, 2003)	Vietnam
Sikkimia rufa (Chen, 1964)	China (Yunnan), Laos, Myanmar
<i>Sikkimia sufangae</i> sp. n.	Taiwan
Sikkimia tsoui sp. n.	Taiwan
<i>Sikkimia yuae</i> sp. n.	Taiwan

# Discussion

Lee (2015) proposed a possible cause of brachelytry of leaf beetles for tropical forest habitats. Reduction of hind wings may result from the production of physogastric females. Nocturnal behavior increases survival since natural enemies are less of a threat. Males actively search for mates. As like survival at adverse environments such as islands, deserts and alpine regions, flight is not essential at night and energy can be diverted to egg production (Beenen and Jolivet 2008). Thus, brachelytry is a predictable evolutionary trend. Although no related reports for this hypothesis, wingless chrysomelids at tropical forest habitats can be used to test. Like Taiwanese populations of *Paraplotes*, those of *Sikkimia* are nocturnal with brachelytrous females. Moreover, the elytral calli of both sexes, and hind wings of males, are reduced. Thus Taiwanese populations of *Sikkimia* support this hypothesis of brachelytry in leaf beetles.

Species richness of *Sikkimia* in Taiwan (five species) is lower than that of *Paraplotes* (ten species) (Lee 2015), possibly due to several causes. All *Sikkimia* species are allopatric on the same mountain ranges and not separated by elevation. Only one *Sikkimia* species, *S. tsoui* sp. n., occupies northern and central Taiwan, whereas five species of *Paraplotes* are recorded from the same area. In addition to its wider distribution, *S. tsoui* sp. n. is abundant in some areas. For example, there were so many adults at Hsitou (溪頭) and Yuanyanghu (鴛鴦湖) that during one night 50 adults were collected at Hsitou and 51 at Yuanyanghu. Both features may be the result of some autamorphic characters in *S. tsoui* sp. n. Males of *S. tsoui* sp. n. have no enlarged apical antennomeres, a character that may be involved in courtship behavior. Lack of this secondary sexual character may result in low speciation. The ability to feed on a wide range of host plants, weak sclerotization of abdominal tergites, and the unique shape of gonocoxae may increase the fitness of this species.

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RESEARCH ARTICLE



# Denopelopia amicitia, a new Tanypodinae from Brazil (Diptera, Chironomidae)

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# Abstract

A new species of *Denopelopia* from Brazil is described based on adult male and pupa. The male of the new species can be distinguished from all other species of the genus by the genitalia and fore-tibial spur morphology. The pupa is very similar to those of *D. atria*, but it can be distinguished by the absence of distinct constrictions in the respiratory atrium of the thoracic horn. Generic diagnosis to male and pupa of *Denopelopia* is emended and keys to male and pupae of known species are provided.

# Keywords

Tanypodinae, Pentaneurini, Amazonian, Neotropical Region, taxonomy, aquatic Insects

# Introduction

The tanypod genus *Denopelopia* was erected by Roback and Rutter (1988) based on a single species (*D. atria*) from the Nearctic region. According to these authors, *Denopelopia* is closely related to *Telmatopelopia* Fittkau and *Zavrelimyia* Fittkau, these suppositions were recently corroborated by Silva and Ekrem (2015).

After the genus description, one undescribed species was reported from Panama based on adult male (Spies and Reiss 1996). So far, *Denopelopia* is composed of five described species, three of them with Asian distribution; Cheng and Wang (2005) described *D. diaoluonica*, *D. bractea* and *D. viridula*, from China, Kobayashi and Endo (2008) transferred *Yaequintus irioquereus* Sasa & Suzuki to the genus *Denopelopia* as senior synonym of *D. bractea*. The last species described on the genus, *D. moema*, was collected in Midwestern Brazil (Silva et al. 2014). Only one species, *D. atria*, has immature stages described, the remaining are known only as adult males.

In the present study, a new species from the Brazilian Amazon rainforest is described based on adult male and pupa, the generic diagnosis to male and pupa of *Denopelopia* (Roback and Rutter 1988, Murray and Fittkau 1989) is emended and keys to males and pupae are provided.

### Materials and methods

A pupa was collected in a small pond using a hand net. It was reared in laboratory isolated in a vial to obtain the associated adult; for further details on rearing techniques see Mendes (2002). The material examined was slide-mounted in Euparal, following the procedures outlined by Pinder (1986, 1989). The colour is described as observed in specimen conserved in alcohol. The general terminology follows Sæther (1980). The holotype of the named species was deposited in the Invertebrates collections of the Instituto Nacional de Pesquisas da Amazônia (INPA), Amazonas, Manaus, Brazil.

# Taxonomy

# Denopelopia Roback & Rutter, 1988

Denopelopia Roback & Rutter, 1988: 117.

### Type species. Denopelopia atria Roback & Rutter, 1988.

**Emended diagnosis.** Based on the additional characters found in *D. amicitia* sp. n., the generic diagnosis for the pupa and male of *Denopelopia* given by Roback and Rutter (1988) and Murray and Fittkau (1989) must be emended. **Male:** scutal tubercle absent; wing densely covered with macrotrichia, costa not produced beyond  $R_{4+5}$ , ending clearly before  $M_{1+2}$ ; tibial spurs with elongate apical tooth, more than half the length of the entire spur; tergite IX straight or rounded, with a transverse row of setae; gonocoxite with or without internal lobe. **Pupa:** wholly brown; thoracic horn elongated, with an apical nipple, a small plastron plate and respiratory atrium with or without constrictions; thoracic comb present; TVII with 3 lateral filaments; anal lobe with spines on the outer margin only; genital sac not surpassing apex of anal lobe.
#### Denopelopia amicitia sp. n.

http://zoobank.org/09A94352-BDD3-423A-A61B-1740C1452F0C

Type material. Holotype male with pupal exuviae, Brazil, Amazonas State, Presidente Figueiredo, pisciculture pond, BR 174-Km 121, 01°55'50.2" S, 60°03'02.0" W, 10/ xii/2012, G.P.S. Dantas, (INPA).

Diagnosis. Male: AR 1.92; wing with well-developed anal lobe; spur of the fore tibia with the most basal tooth longer and slender than the other lateral teeth and strongly curved backwards; tergite IX rounded; gonocoxite with a well-developed setose lobe at the base. Pupa: with a distinct apical nipple, 38 µm long and 42 µm wide, L/W 0.9; absence of distinct constrictions in the respiratory atrium of the thoracic horn.

Etymology. From Latin, *amicitia*, meaning friends, referring to friends who helped during fieldwork.

Male (n = 1). Total length 2.90 mm. Wing length 1.7 mm. Total length/wing length 1.67. Wing length/length of profemur 2.34.

General coloration brown. Head yellow, occipital area brown; maxillary palp yellow; pedicel yellow, brown near the insertion of the flagellum; flagellomere I-XII light brown, VIII-VIV yellow. Thorax dark brown, pleura light brown. Legs yellow. Wings with membrane transparent, veins yellow. Abdomen: T I yellow, with light brown pigmentation on the anterolateral margin, T II-III yellow with clear brown band close to anterior margin; TIV with 1/3 anterior brown; TV-VIII brown, genitalia yellow.

Head (Fig. 1A). AR 1.92. Antenna with 14 flagellomere; thirteenth flagellomere 585 μm long. Apical flagellomere 92 μm long; 23 μm wide at base; with a subapical setae 65 µm long. Temporal setae 14. Clypeus 99 µm long, 82 µm wide, with 19 setae. Cibarial pump with anterior margin concave, 215 µm long and with orifice 95 from apex. Tentorium 165 µm long. First palpomere reduced. Palpomere lengths (1-5 in μm): 30; 65; 156; 168; 265.

Thorax (Fig. 1B). Scutal tubercle absent. Acrostichals 30, biserial, starting close to antepronotum and reaching half of scutum; dorsocentrals 14, biserial anteriorly and uniserial posteriorly; prealars 6, in a single irregular row; supraalar 1. Antepronotum with 3 setae. Scutellum with 22 setae, in three rows. Postnotum without setae.

Wing (Figs. 1D, 2A). 1.7 mm long, 0.4 mm wide. VR 0.91 (Cu = 510 µm, M = 560  $\mu$ m). Membrane with covering of macrotrichia, denser at the 1/3 distal; costa 1.6 mm long, not produced beyond apex of R<sub>4+5</sub>. R<sub>2+3</sub> present. MCu proximal to the RM. Brachiolum with 3 setae. Squama with 12 setae. Anal lobe well-developed.

Legs. Fore leg: tibia with an apical, pectinate spur, 47 µm long, with three lateral teeth and one elongate apical tooth, the most basal tooth is longer and slender than the other lateral teeth and strongly curved backwards (Fig. 2B); two preapical setae 138 µm long; width at apex of tibia 40 µm; ta, with one preapical stout setae, 86 µm long; ta, with one preapical stout setae, 75 µm long; ta, with two preapical stout setae, 72 µm long. Mid leg: tibia with two apical, pectinate spurs, 33 and 59 µm long, longest spur with three lateral teeth and one elongate apical tooth, shortest spur with three lateral teeth and one apical tooth (Fig. 2C); two preapical setae 119  $\mu$ m long; width at apex of tibia 38  $\mu$ m; ta, with a preapical stout setae, 71  $\mu$ m long; ta, with two preapical stout



Figure I. Denopelopia amicitia sp. n. Adult male: A head B thorax C wing.

setae, 65  $\mu$ m long; ta<sub>3</sub> with two preapical stout setae, 48  $\mu$ m long. Hind leg: tibia with two apical, pectinate spurs, 39 and 78  $\mu$ m long, longest spur with three lateral teeth and one elongate apical tooth, shortest spur with three lateral teeth and one apical tooth (Fig. 2D); one preapical setae 182  $\mu$ m long; width at apex of tibia 44  $\mu$ m; ta<sub>1</sub> with one preapical stout setae, 90  $\mu$ m long; ta<sub>2</sub> with two preapical stout setae, 61  $\mu$ m long. Tibial comb on hind leg with 7 bristles, the lateral longer than the medial ones (Fig. 2E). Claws of all legs normal, curved, sharply pointed; pulvilli absent. Lengths (in  $\mu$ m) and proportions of leg segments as in Table 1.

*Hypopygium* (Figs. 2F-G). Tergite IX rounded, with 7 posterior setae. Anal point conical. Phallapodeme indistinct. Sternapodeme with triangular anterior process. Gonocoxite subcylindrical, 130  $\mu$ m long, 40, 55, 65  $\mu$ m wide at apex, at mid and at base respectively; with an internal setose lobe at the base, as in figure 2G. Gonostylus simple and slightly curved, 70  $\mu$ m long; megaseta 10  $\mu$ m long. HR 1.83; HV 4.10.

	Fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
<b>P</b> <sub>1</sub>	729	850	743	422	341	204	109	0.87	2.16	2.12
<b>P</b> <sub>2</sub>	717	1016	758	412	294	184	109	0.75	2.49	2.29
<b>P</b> <sub>3</sub>	782	797	550	315	220	132	93	0.80	2.80	2.87

**Table 1.** Lengths (in  $\mu$ m) and proportions of leg segments in *Denopelopia amicitia* sp. n., male (n = 1).



**Figure 2.** *Denopelopia amicitia* sp. n. Adult male: **A** wing **B** fore tibial spur **C** mid tibial spur **D** hind tibial spur **E** hypopygium in dorsal view **F** hypopygium with tergite IX removed.

**Pupa** (n = 1). Dimensions. Male abdomen 2.63 mm long.

*Coloration*. Cephalothorax brownish; thoracic horn dark brown, apical nipple transparent, plastron plate yellowish. Tergite I light brown, scar brown, T II-AL brown (Fig. 3C).

*Cephalothorax.* Frontal apotome somewhat triangular (Fig. 4A). Wing sheath smooth, 1.1 mm long and 0.4 mm wide. Thoracic horn elongate and narrow (Fig. 3A-B, 4B-C), 390  $\mu$ m long and 48  $\mu$ m wide; with a distinct apical nipple, 38  $\mu$ m long and 42  $\mu$ m wide; plastron plate rounded, 28  $\mu$ m long; aeropyle tube bended at the base, 53  $\mu$ m long. Horn sac tubular, filling the respiratory atrium, except at the base; respiratory atrium without distinct constriction (Fig. 3A-B, 4B). Thoracic horn with small surface spines at the base (Fig. 4C). Basal lobe well developed, as in figure 4C. Thoracic comb with 12 conical teeth.

Abdomen (Fig. 3C). Tergite I without shagreen, T II–TVIII with shagreen composed by scattered fine spinules. Sternite II with a large field of shagreen composed by spinules arranged in combs (Fig. 4D). T I with a distinct and elongate scar, 170  $\mu$ m long. T VII with 3 lateral filaments, 252  $\mu$ m long; filaments placed at 132, 210 and 275  $\mu$ m from base to apex of segment. T VIII with 5 lateral filaments, 372  $\mu$ m long. Anal lobe as in figures 3D and 4E, 350  $\mu$ m long, 213  $\mu$ m wide at base and with two lateral macrosetae with sticky sheaths; outer margins with 12 spinules; inner margins without spinules. Genital sac smaller than anal lobe, 233  $\mu$ m long, 162  $\mu$ m wide at base. GS/AL 0.67.

#### Systematic remarks

To date, *Labrundinia* is the only genus in the Tanypodinae which features a rounded male tergite IX, considered a synapomorphy for the group (Silva et al. 2015). The presence of this trait in D. amicitia n. sp. might suggest that a new genus should be erected to accommodate this species. However, considering that many tanypod genera are not properly studied, with only few described species, in many of these descriptions, this trait might have been overlooked by peers. Moreover, the pupa of *D. amicitia* sp. n. is very similar to that of D. atria, which leaves no doubt that the two species are congeneric. The adults of Denopelopia can be easily distinguished from those of Lab*rundinia* by morphology of tibial spurs. In *Denopelopia* the spurs are well-developed with an elongated apical tooth and two spurs are present in the posterior tibia, while in Labrundinia the spurs are small, with subequal teeth and are absent in the posterior tibia. According to Roback and Rutter (1988), Denopelopia is closely related to Telmatopelopia and Zavrelimyia based on the overall morphology of adults and immature stages. However, adults of Denopelopia possess costa (C) not produced beyond  $R_{4+5}$  and ending clearly before  $M_{1+2}$ , while in *Telmatopelopia* and *Zavrelimyia* costa is slightly produced and ends above or slightly beyond  $M_{1+2}$ . The pupae of *Denopelopia* has anal macrosetae with adhesive sheath and spines only at the outer margin of the



**Figure 3.** *Denopelopia amicitia* sp. n. Pupa: **A** thoracic horn, in frontal view **B** thoracic horn, in lateral view **C** abdomen, in dorsal view **D** T VII–VIII and Anal lobe.



**Figure 4.** *Denopelopia amicitia* sp. n. Pupa: **A** frontal apotome **B** thoracic horn **C** base of thoracic horn, basal lobe and thoracic comb **D** shagreen of sternite II **E** T VII–VIII and Anal lobe.

anal lobe, which contrast to *Telmatopelopia* with no adhesive sheath and *Zavrelimyia* that possesses spines both in the outer and inner margins of the anal lobe. In addition, *Denopelopia* has spines restricted to the base of the external membrane of the thoracic horn and possesses three lateral filaments in tergite VII, in contrast to *Telmatopelopia* and *Zavrelimyia*, which have the external membranes of the thoracic horn covered with spines and possess four lateral filaments in tergite VII. The larva of *Denopelopia* can be recognized by the presence of a trifid paraligula and elongated labial vesicles, in contrast to *Telmatopelopia* and *Zavrelimyia* where the paraligula is bifid and the labial vesicles are more or less rounded.

The male of *D. amicitia* sp. n. can be distinguished from all other species of the genus by the hypopygium morphology. It has a well-developed setose lobe at the base of the gonocoxite, which is absent or reduced in other species of the genus. In addition, the male of *D. amicitia* sp. n. has the anal lobe of the wing well-developed, which sets it apart from *D. viridula* and *D. diaoluonica*, that have it reduced and absent, respectively; the absence of two scale-shaped bristles at the apex of the anterior tibia, the well-developed anal lobe of the wing and the sternapodeme with a pointed anterior process, distinguishes *D. amicitia* sp. n. from *D. irioquerea*; the morphology of the anterior tibial spur and the rounded tergite IX distinguishes *D. amicitia* sp. n. from *D. moema* and *D. atria*. The pupal stage is similar to that of *D. atria* due to the morphology of the thoracic horn and the number of lateral filaments on tergite VII, but can be distinguished by the absence of distinct constrictions in the respiratory atrium and by the low length/width ratio of the apical nipple.

#### **Ecological notes**

There is little information on the biology of *Denopelopia*, since only *D. atria* has its immature stages described. This species was described based on material collected and reared from a shallow drainage ditch amongst *Typha* sp., with low dissolved oxygen and relatively high iron concentrations (Roback and Rutter 1988) in Florida (USA). This species has also been collected in lentic environments in Costa Rica (Epler 2001) and an unreared larva of the genus was recorded from Southeastern Brazil by Trivinho-Strixino and Strixino (1995).

The pupa of *D. amicitia* sp. n. was collected in a small disabled pisciculture pond, associated with marginal vegetation, in a eutrophic environment where a carcass of a large animal, in advanced stages of decomposition, was observed.

Several attempts to collect additional material were made; however, the pond where it was collected was drained and sampling in the adjacent areas, such as streams and wetlands, was not successful.

# Key to adult males of *Denopelopia* (adapted from Cheng and Wang (2005) to include the Brazilian species)

1	Apex of fore tibia with two large scale-like setae
	D. irioquerea (Sasa & Suzuki, 2000)
_	Fore tibiae without scale-like setae
2	Gonocoxite with a well-developed setose lobe at the base <i>D. amicitia</i> sp. n
_	Gonocoxite without a well-developed setose lobe at the base
3	Wing with well-developed anal lobe
_	Wing with reduced or absent anal lobe
4	Anterior margin of abdominal segment I and IV with distinctive brown
	spots D. moema Silva, Wiedenbrug & Oliveira, 2014
_	Anterior margin of abdominal segment I and IV without brown spots
	D. atria Roback & Rutter, 1988
5	AR > 0.9; wing with anal lobe reduced <i>D. viridula</i> Cheng & Wang, 2005
_	AR < 0.6; wing with anal lobe absent

# Key to pupae of Denopelopia

1	Respiratory atrium without distinct constriction (Fig. 3A-B, 4C); proportion
	L/W of apical nipple of thoracic horn 0.9 D. amicitia sp. n
_	Respiratory atrium with distinct constriction; proportion L/W of apical nip-
	ple of thoracic horn about 2.0D. atria Roback & Rutter, 1988

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RESEARCH ARTICLE



# A review of Cyclidiinae from China (Lepidoptera, Drepanidae)

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#### Abstract

The subfamily Cyclidiinae from China is reviewed: two genera and seven species are reported from China. One new subspecies, *Cyclidia fractifasciata indistincta* **subsp. n.**, is described. Two new synonyms are established: *Cyclidia substigmaria* (Hübner, 1831) (= *Cyclidia substigmaria brunna* Chu & Wang, 1987, **syn. n.** = *Cyclidia tetraspota* Chu & Wang, 1987, **syn. n.**). One misidentification in Chu & Wang (1987) is corrected. Identification keys and diagnoses for all discussed Chinese species are provided. External features and genitalia are depicted. In addition, results of DNA barcoding for five taxa of *Cyclidia* are briefly discussed.

#### Keywords

DNA barcodes, morphology, new subspecies, new synonymy, taxonomy

#### Introduction

The subfamily Cyclidiinae Warren, 1922, is the smallest subfamily within four subfamilies (besides Drepaninae, Oretinae, and Thyatirinae) of Drepanidae. This subfamily was first proposed as Eucherinae by Strand (1911) based on the genus *Euchera* Hübner, 1825. Later, it was treated as a separate family (Inoue 1962), followed by other authors (Fletcher 1979, Chu and Wang (1987, 1991) (Yan et al. 2009). However, Minet (1983) regarded Cyclidiinae as a subfamily of Drepanidae, based on the study of the tympanal organs. This treatment was later followed by many researchers, e.g. Holloway (1998) and Minet (2002), and was also supported by molecular data (Wu et al. 2010).

Recently, Chen (2011) performed a phylogenetic analysis of Cyclidiinae, based on morphological characters. In his study, the monophyly of respectively Cyclidiinae, *Cyclidia* and *Mimozethes* was supported, and most synapomorphies for Cyclidiinae proposed by previous studies were shown to be plesiomorphies. Three major synapomorphies of Cyclidiinae were given (Chen 2011): 1) the developed anterotergal syndeses (a paired of semi-translucent structure, see Yan et al. 2005) at the anterior margin of the 2<sup>nd</sup> tergum; 2) a pair of androconial hair-pencils on the 2<sup>nd</sup> pleuron of the male and 3) the short and robust gnathos in the male genitalia.

Species of Cyclidiinae are distributed in the Palearctic Asia and Oriental regions. Up to the present, two genera (*Cyclidia* Guenée, 1858 and *Mimozethes* Warren, 1901) have been recognized in Cyclidiinae. Ten species and eight subspecies are included in *Cyclidia*, with six species and four subspecies (*C. substigmaria substigmaria* (Hübner, 1831), *C. substigmaria brunna* Chu & Wang, 1987, *C. substigmaria intermedia* Prout, 1918, *C. tetraspota* Chu & Wang, 1987, *C. rectificata rectificata* (Walker, 1862a), *C. fractifasciata* (Leech, 1898), *C. sericea* Warren, 1922, *C. orciferaria* Walker, 1860) recorded in China (Moore 1886, Aurivillius 1894, Swinhoe 1899, Strand 1911, Warren 1914, Bryk 1943, Inoue 1962, Chu and Wang 1991, Chang 1989, Holloway 1998, Lutz and Kobes 2002). Three species are included in *Mimozethes*, with two species recorded in China, *M. lilacinaria* (Leech, 1897) and *M. angula* Chu & Wang, 1987. However, the taxonomy of some Chinese taxa remained unclear (e.g. the subspecies delimitation of *C. substigmaria*; the taxonomic status of *C. tetraspota* and the puzzling distribution of *C. sericea*) (Yan et al. 2009, Chen 2011). It is obviously that further research is needed and molecular markers could be used to clarify these problems.

The DNA barcoding method using a 658 bp base pair fragment of the cytochrome c oxidase subunit I gene (COI) as a tool for species discrimination was first put forward based on two hundred closely related species of Lepidoptera (Hebert et al. 2003). It has since been successfully used for species delimitation in lepidopteran species that are difficult to separate morphologically (see Hajibabaei et al. 2006, Yang et al. 2012). The barcoding gap between intra- and inter-specific variation was used for species

discrimination (Hebert et al. 2004a, Meier et al. 2006, Meier et al. 2008, Sihvonen et al. 2014, Jiang et al. 2014).

In the present study an overview of the Chinese Cyclidiinae is given with diagnostic characters for each genus and species, one new subspecies is described, two new synonyms are established, and one misidentification in Chu and Wang (1987) is revised. Also photos of external features and genitalia are provided of all Chinese species discussed. In addition, we discuss the application of the results of DNA barcoding for delimitation of five taxa of *Cyclidia*. As a result of this study five species and five subspecies of *Cyclidia* and two species of *Mimozethes* are regarded as valid for the fauna of China.

#### Materials and methods

*Morphology.* Studied specimens mainly belong to the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZCAS) and the Natural History Museum, London, United Kingdom BMNH. Terminology for wing venation follows the Comstock-Needham System (Comstock 1918), and that of the genitalia is based on Klots (1970), Nichols (1989) and Kristensen (2003). Photographs of the moths were taken with digital cameras. Composite sharp images were generated using Auto-Montage software version 5.03.0061 (Synoptics Ltd). The plates were compiled using Adobe Photoshop software.

DNA-Barcoding. Prior to DNA sequencing, one or two legs were removed from several specimens of each of five examined taxa (C. substigmaria substigmaria, C. rectificata rectificata, C. fractifasciata fractifasciata, C. fractifasciata indistincta, C. orciferaria). DNA extraction was done using Qiagen DNeasy Blood and Tissue Kit (Qiagen, Beijing, China). The primers for the amplification of the 658 bp fragment were LepF1 (5'-ATTCAACCAATCATAAAGATATTGG-3'), LepR1 (5'-TAAACTTCTGGAT-GTCCAAAAAATCA-3') (Hebert et al. 2004a). The PCR reactions were performed using the standard procedure described by Hebert et al. (2004a). The PCR products were detected by 1% agarose gel electrophoresis and directly sequenced with ABI PRISM 3730xl capillary sequencers. The amplification and sequencing for some dried material (Sequence ID begins with "DB") were carried out in BGI-Shenzhen, (China) using standard protocols described in Hebert et al. (2004a). Forward and reverse nucleotide sequences were assembled in SeqMan 5.01 (DNASTAR, Inc. 1996). The assembled sequences were aligned and manually edited in MEGA 5.0 (Tamura et al. 2011). The neighbor-joining (NJ) tree (Saitou and Nei 1987) was reconstructed based on Kimura 2-parameter (K2P) distances (Kimura 1980) using MEGA 5.0. All the sequences have been deposited in GenBank under accession numbers, and their full data including images and are in the Barcode of Life Database (http://www.boldsystems.org; see Ratnasingham and Hebert 2007) (Table 1).

Taxa	Sequence ID	Collecting locality	Collecting	GenBank	BOLD process ID
	DB00162	West Tianmushan, Zheijang	Jul. 2003	KR872896	CLDC001-15
	DB00173 DB00174	Wuzhishan, Hainan Lingshui, Hainan	May 2007 May 2007	KR872897 KR872898	CLDC002-15 CLDC003-15
	DB00181	Baotianman, Henan	Aug. 2008	KR872899	CLDC004-15
	DB00182	Luovang, Henan	Aug. 2006	KR872900	CLDC005-15
	DB00184	Baoshan, Yunnan	innan Aug. 2007 KR87		CLDC006-15
	DB00189	Yanling, Hunan	Jul. 2008	KR872902	CLDC007-15
	IOZ LEP M 01129	Mengla, Yunnan	Jul. 2013	KR872903	CLDC008-15
	IOZ LEP M 01134	Tengchong, Yunnan Aug. 20		KR872904	CLDC009-15
C. substigmaria substigmaria	IOZ LEP M 01304	West Tianmushan, Zhejiang	Jul. 2011	KR872905	CLDC010-15
	IOZ LEP M 08961	Mengla, Yunnan Jul. 2013		KR872906	CLDC011-15
	IOZ LEP M 09195	Qushi, Yunnan	Aug. 2013	KR872907	CLDC012-15
	IOZ LEP M 16605	Kangxian, Gansu	Aug. 2014	KR872908	CLDC013-15
	IOZ LEP M 16606	Kangxian, Gansu	Aug. 2014	KR872909	CLDC014-15
	IOZ LEP M 16607	Kangxian, Gansu	Aug. 2014	KR872910	CLDC015-15
	IOZ LEP M 16608	Kangxian, Gansu	Aug. 2014	KR872911	CLDC016-15
	IOZ LEP M 17993	Liuku, Yunnan	Sep. 2014	KR872912	CLDC017-15
	IOZ LEP M 17994	Liuku, Yunnan	Sep. 2014	KR872913	CLDC018-15
	IOZ LEP M 02790	Guilin, Guangxi	Apr. 1952	KR872914	CLDC019-15
	DB00226	Bomi, Tibet	Aug. 2005	KR872923	CLDC020-15
	DB00228	Mêdog, Tibet	Aug. 2006	KR872924	CLDC021-15
<i>C</i>	DB00229	Mainling, Tibet	Aug. 2006	KR872925	CLDC022-15
C. rectificata	IOZ LEP M 03475	Zayü, Tibet	Aug. 2014	KR872926	CLDC023-15
recujicaia	IOZ LEP M 03476	Zayü, Tibet	Aug. 2014	KR872927	CLDC024-15
	IOZ LEP M 03477	Zayü, Tibet	Aug. 2014	KR872928	CLDC025-15
	IOZ LEP M 16015	Zayü, Tibet	Aug. 2014	KR872929	CLDC026-15
	IOZ LEP M 00657	Pianma, Yunnan	May 2011	KR872930	CLDC027-15
C. fractifasciata	IOZ LEP M 00683	Pianma, Yunnan	May 2011	KR872931	CLDC028-15
fractifasciata	IOZ LEP M 07012	Pianma, Yunnan	May 2011	KR872932	CLDC029-15
	IOZ LEP M 07013	Pianma, Yunnan	May 2011	KR872933	CLDC030-15
	IOZ LEP M 16601	Kangxian, Gansu	Aug. 2014	KR872934	CLDC031-15
	IOZ LEP M 16602	Kangxian, Gansu	Aug. 2014	KR872935	CLDC032-15
C. fractifasciata	IOZ LEP M 16603	Kangxian, Gansu	Aug. 2014	KR872936	CLDC033-15
inaistincia	IOZ LEP M 16604	Kangxian, Gansu	Aug. 2014	KR872937	CLDC034-15
	IOZ LEP M 09387	Wushan, Chongqing	Jul. 2013	KT250118	CLDC035-15
	DB00202	Bawangling, Hainan	May 2007	KR872915	CLDC036-15
	DB00203	Wuzhishan, Hainan	Apr. 2008	KR872916	CLDC037-15
	DB00210	Yanling, Hunan	Jul. 2008	KR872917	CLDC038-15
	DB00211	Yanling, Hunan	Jul. 2008	KR872918	CLDC039-15
C orciferaria	DB00213	Shixing, Guangdong	Jun. 2008	KR872919	CLDC040-15
S. C. C. feranda	DB00216	Baoshan, Yunnan	Aug. 2007	KR872920	CLDC041-15
	IOZ LEP M 01208	West Tianmushan, Zhejiang	Jul. 2011	KR872921	CLDC042-15
	IOZ LEP M 01324	West Tianmushan, Zhejiang	Jul. 2011	KR872922	CLDC043-15

Table 1. Cyclidia species included in this study with GenBank accession numbers and BOLD process ID.

#### Results

#### Taxonomy

#### Cyclidiinae Warren, 1922

Cyclidiinae Warren, 1922: 444.

#### Cyclidia Guenée, 1858

*Cyclidia* Guenée, 1858: 62. Type species: *Cyclidia substigmaria* (Hübner, 1831), by monotypy.

*Nelcynda* Walker, 1862a: 1142. Type species: *Nelcynda rectificata* Walker, 1862, by monotypy.

Ciclidia Chou & Xiang, 1984: 159. [Incorrect spelling of Cyclidia Guenée.]

Generic characters. Head. Antennae lamellate, partly unipectinate, rami very short (Fig. 1a). Frons not protruding. Labial palpi with third segment distinct, up-curved. Thorax. Hind tibia with two pairs of spurs. Apex of forewing often rounded, sometimes pointed and protruding. Wing colour usually white or grey (except C. orciferaria); antemedial and postmedial lines of forewing double; medial line of forewing broad; terminal lines of both wings usually double, sometimes single (e.g. C. substigmaria, C. rectificata, and C. diehli Lutz & Kobes, 2002). Venation (Fig. 3a). Forewing with  $R_1$  separate,  $R_{2-4}$  and  $R_5$  stalked,  $R_2$  and  $R_{3+4}$  stalked,  $R_5$  and  $M_1$ separate, M<sub>2</sub> arising from middle of discocellulars; Hind wing with Sc+R<sub>1</sub> close to Rs beyond distal cell, then far from Rs, M, arising from middle of discocellulars. Anterotergal syndeses developed at anterior margin of 2<sup>nd</sup> tergum (Fig. 2). A pair of androconial hair-pencils present on 2<sup>nd</sup> sternum of male (Fig. 2). *Male genitalia.* Uncus triangular; socii developed, often sclerotized (except C. orciferaria), sometimes with small setose process at base (e.g. C. pitimani (Moore, 1886), C. sericea and C. *diehli*); gnathos connected at middle and with median process narrow and triangular; valva simple and broad; juxta deeply concaved posteriorly; saccus short and broad, rounded terminally; phallus slightly curved; vesica without cornuti. *Female genitalia.* Papillae anales broad and rounded; lamella postvaginalis usually well developed; ductus bursae very long and narrow, with a colliculum; corpus bursae oval, with a paired band-like spinose signa.

**Diagnosis.** *Cyclidia* is quite different from *Mimozethes* externally and in the genitalia. For example, externally, the rami of the antennae are much shorter; the species of *Cyclidia* are much larger, and the postmedial lines of forewing are often double, while in *Mimozethes*, it is single and forms a ">" shaped protrusion near R<sub>5</sub>; in the male genitalia, the socii are well developed in *Cyclidia*, but absent in *Mimozethes*; the sacculus unmodified in *Cyclidia* but forming a process in *Mimozethes*; in the female genitalia, the signa are a paired band-like sclerotization in *Cyclidia*, but absent in *Mimozethes*.

Distribution. China, Japan, Korean Peninsula, south and southeast Asia.



**Figures 1–3.** I Male antennae **a** *Cyclidia substigmaria* **b** *Mimozethes angula* **2** Anterotergal syndeses and androconial hair-pencils **3** Wing venation (from Chu and Wang, 1991) **a** *C. substigmaria* **b** *M. angula*.

# Key to Chinese Cyclidia species

1	Wings colour white or grey
_	Wings colour blackish brown
2	Discal spots on hind wing distinct
_	Discal spots on hind wing indistinct
3	Discal spots on hind wing dark grey C. substigmaria substigmaria, Figs 4-8
_	Discal spots on hind wing black
4	Terminal lines of both wings single C. rectificata rectificata, Figs 10-11

_	Terminal lines of both wings double
5	Outer margin of forewing medial line forming an right angle below $M_3$
_	Outer margin of forewing medial line not forming an right angle below M <sub>3</sub> .
6	Outer line of antemedial line and inner line of postmedial line of forewing
	distinct C. fractifasciata fractifasciata, Figs 14–15
_	Outer line of antemedial line and inner line of postmedial line of forewing
	invisible <i>C. fractifasciata indistincta</i> , Figs 16–17

# Cyclidia substigmaria (Hübner, 1831)

Euchera substigmaria Hübner 1831: 29. pl. 90, figs 519, 520. Syntypes, China. Cyclidia substigmaria: Guenée 1858: 63.
Abraxas capitata Walker, 1862a: 1121. Holotype ♀, China: Hong Kong (BMNH). Euchera capitata: Strand 1911: 196.
Cyclidia substigmaria brunna Chu & Wang, 1987: 205. Holotype ♂, China: Sichuan: Emeishan, Qingyinge (IZCAS). Syn. n.
Cyclidia tetraspota Chu & Wang, 1987: 206. Holotype ♂, China: Yunnan: Xishuangbanna, Yunjinghong (IZCAS). Syn. n.

**Diagnosis.** In external appearance, this species is distinguishable from other congeners by the following characters: the discal spots of hind wing are very distinct on the upper side and the underside; the discal spot of the forewing is covered with white scales on the upper side; two greyish brown markings are present inside the anal angle of the forewing. The male genitalia of the species are close to those of *C. rectificata*, but the terminal part of the uncus and the socii are narrower; the vesica is much more scobinate. In the female genitalia, the two signa are close to each other posteriorly, while in *C. rectificata*, they are almost parallel.

**Remarks.** There are five subspecies of *C. substigmaria*:

C. s. substigmaria (Hübner, 1831), most parts of China and Vietnam;

C. s. intermedia Prout, 1918 in Tibet;

C. s. nigralbara Warren, 1914 in Japan and Korean Peninsula;

C. s. modesta Bryk, 1943 in Myanmar;

C. s. superstigmaria Prout, 1918 in India and Nepal.

**Distribution.** China, Japan, Korean Peninsula, India, Nepal, Myanmar, Vietnam. **Biological notes.** Sugi (1987) and Holloway (1998) mentioned that larval Cyclidiinae may be uniquely associated with the plant family Alangiaceae (now incorporated in Cornaceae). However, *Cyclidia substigmaria* also has been recorded from Malvaceae (*Hibiscus cannabinus* L.) (Chu 1981, Chu and Wang 1987, 1991, Kadoorie Farm and Botanical Garden 2004). The morphology of the eggs, larva, pupa and life history of *C. substigmaria* were described in detail by Zhou and Wang (1985), Chu and Wang (1991) and Yan et al. (2009).

### Cyclidia substigmaria substigmaria (Hübner, 1831)

Figs 4-8, 24-28, 37-39, 48-50

**Diagnosis.** The subspecies is very similar to *C. substigmaria intermedia*, but differs externally by the paler discal spot of the hind wing and the two less distinct markings inside the anal angle of the forewing.

**Type material examined. CHINA: Sichuan** (IZCAS): 1Å (Holotype of *C. substigmaria brunna*), Emeishan, Qingyinge, 800–1000 m, 17.V.1957, coll. Wang Zongyuan. **Zhejiang** (IZCAS): 1 $\bigcirc$  (Allotype of *C. substigmaria brunna*), Hangzhou, 4.V.1975, coll. Zhang Baolin. **Fujian** (IZCAS): 3Å (Paratypes of *C. substigmaria brunna*), Wuyishan, 6–21.V.1983, coll. Wang Linyao. **Yunnan** (IZCAS): 1 $\bigcirc$ (Paratypes of *C. substigmaria brunna*), Liuku, 2500 m, 23.V.1981, coll. Liao Subai; 1Å (Holotype of *C. tetraspota*), Xishuangbanna, Yunjinghong, 650 m, 22.VI.1959, coll. Meng Xuwu; 1 $\bigcirc$  (Allotype of *C. tetraspota*), Yiwubanna, Menglun, 650 m, 23.VII.1959, coll. Zhang Facai; 1Å (Paratype of *C. tetraspota*), ibidem, 28.V.1958, coll. Wang Shuyong. **Hainan** (IZCAS): 1Å (Paratype of *C. tetraspota*), Wanning, 10 m, 9.IV.1960, coll. Li Zhenfu. **Guangxi** (IZCAS): 1Å (Paratype of *C. tetraspota*), Guilin, Liangfeng, 20.IV.1952. **Hongkong** (BMNH): 1 $\bigcirc$ , collector and collecting date unknown (Holotype of *C. substigmaria capitata*).

Additional material examined. CHINA: Henan (IZCAS): 13, Luoyang, Huaguoshan, 4.VIII.2006, coll. Song Hao; 12, Baiyunshan, 1400 m, 27.VII.2003, coll. Lu Yanan;  $1\overline{\partial}$ , Jigongshan, 25.VI.1984. **Shaanxi** (IZCAS):  $2\overline{\partial}1\overline{\mathcal{Q}}$ , Ningshan, Guanghuojie, 1189 m, 28.VII.2014, coll. Liu Shuxian and Ban Xiaoshuang; 13, Zhashui, Yingpanzhen, 980 m, 31.VII.2014, Liu Shuxian and Ban Xiaoshuang; 1 Xunyang, Bailiuzhen, 386 m, 3.VIII.2014, coll. Liu Shuxian and Ban Xiaoshuang. Gansu (IZCAS): 13, Wenxian, Qiujiaba, 2200-2350 m, 29.VI.1998, coll. Yuan Decheng; 12, Kangxian, Baiyunshan, 1250–1750 m, 12.VII.1998, coll. Wang Shuyong; 1∂7♀, Kangxian, Yangba, Meiyuangou, 1000 m, 13.VIII.2014, coll. Xue Davong & Ban Xiaoshuang; 19, Wenxian, Lukou, 22.V.1987. Jiangsu (IZCAS):  $7\sqrt[3]{4}$ , Chemo, 22.IV–2.V.1935, coll. O. Piel. Anhui (IZCAS): 12, Linzongchang, IX.1970, coll. Mai Weiqiang; 2<sup>Q</sup>, Yuexi, Linyeju, 11.IX.1982, coll. Zhou Tiying. **Zhejiang** (IZCAS):  $5^{\circ}_{\circ}3^{\circ}_{\circ}$ , Lin'an, West Tianmushan, 400–1506 m, 6.IX.1981, 26–30.VII.2003, 27.VII.2011, coll. Xue Dayong et al.; 15Å1<sup>Q</sup>, Tianmushan, 15-25.VI.1936, 25-30.VIII.1947, 22.VIII.1972, 28-31.VII.1998, coll. O. Piel et al.; 1312, Hangzhou, 4.V.1975, 1981, coll. Zhang Baolin; 13, Qingyuan, Fengyangshan, Datianping, 1290 m, 6-10.VIII.2003, coll. Han Hongxiang. **Hubei** (IZCAS): 1Å, Shennongjia, Muyu, 22.VII.1998, coll. Zhou Hongzhang;  $1^{\circ}$ , Shennongjia, Dalongtan, 2700 m, 27.VII.1998, coll. Zhou Haisheng;  $1^{\circ}_{\circ}$ , Xingshan, Longmenhe, 1300 m, 12.IX.1994, coll. Song Shimei;  $4^{\circ}$ , Xuan'en, 650 m, 25.V.1989, coll. Li Wei; 12, Hefeng, Fenshuiling Linchang, 31.VII.1989, coll. Li Wei. Jiangxi (IZCAS): 1<sup>o</sup>, Yifeng, Yuanqian, 8.IX.1959. Hunan (IZCAS):  $1^{\circ}$ , Yanling, Taoyuandong, 631 m, 4–8.VII.2008, coll. Chen Fuqiang;  $1^{\circ}$ , Fenghuang, 15.IX.1988, coll. Song Shimei; 1<sup>Q</sup>, Cili, 3.IX.1988, coll. Song Shimei.



**Figures 4–11.** Adults. **4–9** *Cyclidia substigmaria substigmaria* **4** male (with dot-like and wavy submarginal line of the forewing, Yunnan) **5** ditto, underside **6** male (with faint, broad and interrupted submarginal line of the forewing, Zhejiang) **7** male (holotype of *C. substigmaria brunna*, Sichuan) **8** male (holotype of *C. tetraspota*, Yunnan) **9** *C. substigmaria intermedia*, male (Tibet) **10–11** *C. rectificata* **10** male (Tibet) **11** ditto, underside. Scale bar: 1 cm.

**Fujian** (IZCAS): 1139, Wuyishan, 26.IV–14.VI.1983, coll. Wang Linyao and Zhang Baolin; 13, Xinkou, 15.VI.1981, coll. Lin Yibiao; 231, Jianyang, Huangkeng, 270–950 m, 23.IV–1.V.1960, coll. Jiang Shengqiao and Zuo Yong; 1, Chong'an, Xingcun, Guadun, 840–1210 m, 25.VIII.1960, coll. Ma Chenglin; 1, Chong'an, Xingcun, Sangang, 740 m, 17.V.1960, coll. Zhang Yiran.

**Guangdong** (IZCAS): 13, Guangzhou, 8.VI.1973, coll. Zhang Baolin; 435, Guangzhou, Sanyuanli, 27.IV.1958, coll. Wang Linyao. Hainan (IZCAS): Wanning, 10 m, 14.IV.1960, coll. Li Changqing; 3Å, Xinglong, 24.III.1963, IV.1963, coll. Zhang Baolin; 33, Lingshui, Diaoluoshan, 4–5.V.2007, coll. Han Hongxiang; 1 $\mathcal{Q}$ , Wuzhishan, Shuiman, 600 m, 12.V.2007, coll. Han Hongxiang; 1 $\mathcal{Q}$ , Baisha, Yinggeling, 434 m, 3–4.XII.2007, coll. Li Jing; 19, Jianfengling, Tianchi, 3.III.1982, coll. Long Yongcheng. **Guangxi** (IZCAS):  $1 \Diamond 1 \heartsuit$ , Jinxiu, Luoxiang, 200–400 m, 1–16.V.1999, coll. Huang Fusheng and Han Hongxiang;  $1^{\circ}_{\circ}$ , Jinxiu, Yonghe, 500 m, 12.IV.1999, coll. Han Hongxiang; 12, Jinxiu, Jinzhong Gonglu, 1100 m, 12.V.1999, coll. Li Wenzhu; 2<sup>3</sup>, Guilin, Yanshan, 26.IX.1958, 19.XI.1959; 1♂5♀, Fangcheng, Fulong, 240–260 m, 1.III.1998, 19–20.IV.1998, coll. Li Wenzhu and Wu Chunsheng; 1Å, Napo, Nianjing, 900 m, 11.IV.1998, coll. Wu Chunsheng; 19, Napo, Defu, 1350 m, 19.VI.2000, coll. Yao Jian; 19, Napo, Nonghua, 990 m, 13.IV.1998, coll. Li Wenzhu;  $1^\circ$ , Napo, Baihe, 540 m, 8.IV.1998, coll. Qiao Gexia; 13, Pingxiang, 230 m, 8.VI.1976, coll. Zhang Baolin; 2<sup>Q</sup>, Longsheng, 10–11.VI.1980, coll. Zhong Tiesen and Song Shimei;  $2^{\circ}$ , Daxin, Xialei, 680 m, 31.III.1998, coll. Li Wenzhu;  $2^{\circ}$ , Longzhou, Nonggang, 195 m, 15-17.VII.2013, coll. Liu Shuxian and Li Xinxin. Sichuan (IZ-CAS): 1<sup>Q</sup>, Emeishan, Baoguosi, 550–750 m, 8.IV.1957, coll. Wang Zongyuan; 1∂, Emeishan, 580–1100 m, 22.VI.1955, coll. Zi Yunzhen; 36∂34♀, Emeishan, Qingyinge, 800–1000 m, 17.IV–20.V.1957, 19.IX–28.X.1957, coll. Zhu Fuxing et al.;  $1^{\circ}$ , Yanyuan, Jinhe, 2.VII.1984, coll. Chen Yixin. **Guizhou** (IZCAS):  $1^{\circ}$ , Sinan, 350 m, 9.V.1983, coll. Liu Yanxian; 13, Koei-Yang, 5.IX.1935. Yunnan (IZCAS): 2∂7♀, Xishuangbanna, Mengna, 550 m, 22–30.VI.1959, coll. Zhang Yiran and Li Zhenfu; 1∂3♀, Xiaomengyang, 850–1000 m, 6.V.1957, 12.VII–22. VIII.1957, 10.X.1957, coll. Wang Shuyong et al.; 1Å12, Xishuangbanna, Menghun, 160–750 m, 4.VI.1958, coll. Meng Xuwu et al.; 1♀, Xishuangbanna, Yunjinghong, 650 m, 3.VII.1957, coll. Wang Shuyong; 2369, Xishuangbanna, Mengla, 620–650 m, 2.V–6.VI.1959, coll. Zhang Yiran et al.;  $639^{\circ}$ , Mengla, Menglun, 650-665 m, 22-29.X.1958, 3.IV-18.V.1964, 29.VII.2013, coll. Wang Shuyong et al.; 13, Xishuangbanna, Menghai, 1200-1600 m, 18.VII.1958, coll. Wang Shuyong; 2♀, Xishuangbanna, Ganlanba, 560 m, 9–10.VII.1958, coll. Li Chuanlong; 1Å, Xishuangbanna, Bubang, 700 m, 14.IX.1993, coll. Yang Longlong; 1¢, Xishuangbanna, Yiwu, 800–1300 m, 13.VII.1959, coll. Pu Fuji; 6312, Baoshan, Baihualing, 1520 m, V.11–13.VIII.2007, coll. Wu Chunguang and Lang Songyun; 2∂1♀, Baoshan, Bawan, 1040–1100 m, 19–23.1992, 8–10.VIII.2007, 8–10. VIII.2013, coll. Wu Chunguang et al.; 232, Baoshan, Xinujiang Hegu, 800-1000 m, 10–11.V.1955, coll. Xue Yufeng; 1♂, Tengchong, Qushi, Dabacun, 1873 m, 4.VIII.2013, coll. Liu Shuxian and Li Xinxin;  $731^{\circ}$ , Tengchong, Zhengding, 1833 m, 6–7.VIII.2013, coll. Liu Shuxian and Li Xinxin;  $2^{\circ}$ , Tengchong, Heinitang, 1824 m, 26–27.VI.2014, coll. Li Xinxin and Pan Xiaodan; 1<sup>Q</sup>, Cheli, 620 m, 18.IV.1957, coll. Zang Lingchao; 2♂, Yuanyang, Nansha, 1100 m, 26.V.1979,

coll. Luo Kezhong;  $1 & 1 \\cite{1}$ , Lushui, Liuku, 860–1220 m, 18–19.IX.2014, coll. Liang Hongbin;  $2 & 4 \\cite{2}$ , Lushui, Pianma, 1750–1980 m, 7.V.1981, 8–12.V.2011, 3–4.VII.2014, coll. Zhang Xuezhong et al.;  $1 \\cite{2}$ , Jinping, Mengla, 500 m, 2.V.1956, coll. Huang Keren;  $1 \\cite{2}$ , Jinping, Chang Potou, 1200 m, 23.V.1956, coll. Huang Keren. **Vietnam** (IZCAS):  $1 \\cite{2}$ , Tonkin, Hoa-Binh, leg. A. de Cooman.

**Variation.** The submarginal line of the forewing varies from dot-like and wavy to faint, broad and interrupted between veins. In the male genitalia, the terminal half of the costa vary from smooth (Fig. 27, IOZ LEP M 01129) to strongly protruding (Fig. 28, IOZ LEP M 08961) among the material on the same region.

**Genetic data.** The distance to the nearest neighbour *C. rectificata* is 8.92%. The intrasubspecific divergence of the barcode region of *C. substigmaria substigmaria* ranges from 0%–2.6% (average distance 1%) (n = 19). Some specimens from Yunnan cluster together at some distance from all other specimens (Fig. 58). Despite the high divergence, no morphological characters were found which separate these populations.

**Remarks.** After examining the types of *C. substigmaria brunna*, *C. tetraspota* and a long series of material collected near their type localities, it was found that the external and genital features of *C. substigmaria brunna* and *C. tetraspota* are nearly identical to those of *C. substigmaria substigmaria*. Barcodes of one paratype of *C. tetraspota* (IOZ LEP M 02790) and two specimens from type locality of *C. substigmaria brunna* (IOZ LEP M 17993 and 17994) were clustered within *C. substigmaria substigmaria* in the Neighbour Joining (NJ) tree with the genetic distances from 0.015%–2.6% (see fig. 58). Thus, *C. tetraspota* and *C. substigmaria brunna* are considered as junior synonyms of *C. substigmaria substigmaria*.

**Distribution.** China (Henan, Shaanxi, Gansu, Jiangsu, Anhui, Zhejiang, Hubei, Jiangxi, Hunan, Fujian, Taiwan, Guangdong, Hainan, Hong Kong, Guangxi, Sichuan, Guizhou, Yunnan), Vietnam.

#### Cyclidia substigmaria intermedia Prout, 1918

Figs 9, 29, 40, 51

*Cyclidia substigmaria intermedia* Prout, 1918: 416. Holotype *A*, China: Tibet (BMNH).

#### Diagnosis. See under C. substigmaria substigmaria.

**Type material examined. CHINA: Tibet** (BMNH): 1<sup>(3)</sup> (Holotype), Tibet, collector and collecting date unknown, ex. Joicey Collection.

Additional material examined. CHINA: Tibet (IZCAS):  $13^{\circ}$ , Mêdog, Yarang, 1091 m, 20–23.VIII.2006, coll. Lang Songyun;  $1^{\circ}$ , Mêdog, Beibung, 850 m, 24.VI.1983, coll. Han Yinheng;  $2^{\circ}$ , Mêdog, 2750 m, 22.VIII.1982, coll. Han Yinheng;  $1^{\circ}$ , Zayü, Dongyan, 1600 m, 17.VII.1973.

Genetic data. No genetic data available.

Distribution. China (Tibet).

#### Cyclidia rectificata (Walker, 1862)

*Nelcynda rectificata* Walker, 1862a: 1142. Holotype 1∂, India: Sikkim (BMNH). *Cyclidia muricolaria* Walker, 1862b: 1483. Holotype 1♀, India: Darjeeling (BMNH). *Cyclidia patulata* Walker, 1866: 1537. Holotype ♀, India: Darjeeling (BMNH). *Chorodna rectificata*: Cotes and Swinhoe 1888: 475. *Enchera rectificata*: Hampson 1893: 328. *Cyclidia rectificata*: Warren 1922: 445.

**Diagnosis.** The species is very similar to *C. diehli* Lutz & Kobes, 2002 (Sumatra) externally, but can be distinguished by the blackish brown and more distinct forewing submarginal line. The most distinct differences are in the male genitalia: the terminal part of the uncus is much narrower and longer; a rounded process with short setae is absent on the basal part of each socius, while *C. diehli* has this character; the terminal part of the valva is much broader than that of *C. diehli*. The male and female genitalia are also similar to those of *C. substigmaria*, the diagnosis can be seen under *C. substigmaria*.

**Remarks.** There are two subspecies of *C. rectificata*. *C. rectificata rectificata* (Walker, 1862) is distributed in China and India, and *C. rectificata malaisei* Bryk, 1943 is distributed in Myanmar.

Distribution. China, India, Myanmar.

Cyclidia rectificata rectificata (Walker, 1862)

Figs 10, 11, 30, 41, 52

#### Diagnosis. See under C. rectificata.

Material examined. CHINA: Yunnan (IZCAS): 1Å, Tengchong, Heinitang, 1930 m, 28–30.V.1992, coll. Xue Dayong. Tibet (IZCAS): 7 $\bigcirc$ , Nyalam, Zham, 2250 m, 12–20.V.1974, coll. Zhang Xuezhong; 1Å, Cona, 2800 m, 8.VIII.1974, coll. Huang Fusheng; 1Å, Zham, 2200 m, 25.VI.1975, coll. Wang Ziqing; 1 $\bigcirc$ , Gyirong, 2800 m, 26.VIII.1975, coll. Wang Ziqing; 3 $\bigcirc$ , Bomi, Yi'ong, 2300 m, 23–29.VIII.1983, coll. Han Yinheng; 2Å5 $\bigcirc$ , Nyingchi, Bomi, Tangmai, 2100 m, 29–31.VIII.2005, coll. Wang Xuejian; 3Å1 $\bigcirc$ , Nyingchi, Pélung, 2115 m, 1–2. IX.2005, coll. Wang Xuejian; 4Å3 $\bigcirc$ , Zayü, Shang Zayü, 1812–1960 m, 21–23. VIII.2005, 10–11.VIII.2014, coll. Wang Xuejian, Cheng Rui and Cui Le; 1Å1 $\bigcirc$ , Zayü, Rongcheng Binguan, 2178 m, 8–12.VIII.2014, coll. Cheng Rui and Cui Le; 2 $\bigcirc$ , Mainling, Pai, 2883 m, 4–6.VIII.2006, coll. Lang Songyun; 8Å11 $\bigcirc$ , Mêdog, Lage, 3213 m, 7–8.VIII.2006, coll. Lang Songyun; 3Å2 $\bigcirc$ , Mêdog, Dayandong, 2880 m, 9.VIII.2006, coll. Lang Songyun; 2Å, Mêdog, Hanmi, 2095 m, 10–11. VIII.2006, coll. Lang Songyun; 2 $\bigcirc$ , Mêdog, Pomo Gonglu 80K, 2118 m, 24–25. VIII.2006, coll. Lang Songyun. **Genetic data.** The intraspecific divergence of the barcode region of *C. rectificata rectificata* is 0% (average distance 0%) (n = 7). The distance to the nearest neighbour *C. substigmaria* is 8.92%.

Distribution. China (Yunnan, Tibet), India.

#### Cyclidia pitimani (Moore, 1886)

Figs 12, 13, 31, 42

*Euchera pitimani* Moore, 1886: 99. Syntypes including 1<sup>(2)</sup>, Burma: Tenasserim, Tavoy (BMNH).

Cyclidia pitimani: Warren, 1922: 445.

Cyclidia sericea Warren sensu Chu & Wang, 1987: 206. (Misidentification)

Cyclidia sericea Warren sensu Chu & Wang, 1991: 64, fig. 24, pl. 1: 4. (Misidentification)

**Diagnosis.** This species is very similar to *C. sericea* (Borneo, Sumatra), but can be distinguished by the following characters: smaller than *C. sericea* (the average forewing length of the male is 32 mm, against *ca* 40 mm in *C. sericea*); in *C. pitimani*, the doubled antemedial line form almost right angles anteriorly, especially the inner line, while in *C. sericea*, the protrusions of the antemedial lines are more rounded; the anterior part of the median band is much narrower in *C. pitimani*; the terminal spots are less distinct than those of *C. sericea*. In the male genitalia, the terminal part of the valva is broader and more rounded.

Material examined. CHINA: Yunnan (IZCAS): 2♂, Xishuangbanna, Xiaomengyang, 850 m, 6–7.IX.1957, coll. Zang Lingchao and Zhang Yiran (one male was originally incorrectly recorded as "Qinghai, Gonghe"); 2♂, Xishuangbanna, Bubang, 700 m, 14.IX.1993, coll. Yang Longlong.

Genetic data. No genetic data available.

**Remarks.** After examining the types of *C. pitimani* and *C. sericea*, and studying the descriptions and figures of the two species (Moore 1886, Warren 1922, Holloway 1998, Chen 2011), we found that the specimens from Yunnan which were identified as *C. sericea* by Chu and Wang (1987, 1991) well agree with *C. pitimani*. Thus, *C. sericea* in Chu and Wang (1987, 1991) is considered to be a misidentification of *C. pitimani*.

Chu and Wang (1987, 1991) recorded one male specimen from "Qinghai, Gonghe, 3150 m, 6.IX.1957, coll. Zang Lingchao". After examination, it was noted that the locality on the label of this specimen was incorrect. According to the collecting records of IZCAS, the collector (Zang Lingchao) went to Xiaomengyang of Xishuangbanna in Yunnan on September 6th, 1957, and no collector went to Qinghai on that date. We also found another specimen of *C. pitimani* which was collected at the same locality on September 7th, 1957. So, the locality on label should be written as Yunnan, Xishuangbanna, Xiaomengyang, 850 m. Qinghai should be deleted from the range area of *C. sericea* and the species should be deleted from the fauna of China.

Distribution. China (Yunnan), Myanmar.



Figures 12–23. Adults. 12–13 *Cyclidia pitimani* 12 male (Yunnan) 13 ditto, underside 14–15 *C. frac-tifasciata fractifasciata* 14 male (Yunnan) 15 ditto, underside 16–17 *C. fractifasciata indistincta* subsp. n. 16 male (holotype, Gansu) 17 ditto, underside 18–19 *C. orciferaria* 18 male (Hainan) 19 ditto, underside 20–21 *Mimozethes angula* 20 male (holotype, Sichuan) 21 ditto, underside 22–23 *M. lilacinaria* 22 male (holotype, Sichuan) 23 ditto, underside. Scale bar: 1 cm.



Figures 24–29. Male genitalia of *Cyclidia*. 24 *C. substigmaria substigmaria* (Baoshan, Yunnan, slide no. 41) 25 ditto (holotype of *C. substigmaria brunna*, Emeishan, Sichuan, slide no. 12) 26 ditto (holotype of *C. tetraspota*, Xishuangbanna, Yunnan, slide no. 10) 27 ditto (Xishuangbanna, Yunnan, slide no. 681) 28 ditto (Xishuangbanna, Yunnan, slide no. 683) 29 *C. substigmaria intermedia* (Tibet, slide no. 311). Scale bar: 1 mm.

#### Cyclidia fractifasciata (Leech, 1898)

*Euchera fractifasciata* Leech, 1898: 360. Syntypes 1∂, 1♀, China: Western China (BMNH).

Cyclidia fractifasciata: Gaede 1931: 2.

**Diagnosis.** The species can be distinguished by the following characters: a black broad subbasal line is present on the forewing; the forewing medial line is broad at anterior half and very narrow and dot-like at posterior half; outer margin of the forewing medial line forms an almost right angle below  $M_3$ ; the phallus of the male genitalia forms a small protrusion posteriorly; the lamella postvaginalis of the female genitalia is rectangle.

**Remarks.** Chu and Wang (1991) did not record this species. The specimens from Yunnan should be identified as *C. fractifasciata fractifasciata*, and the specimens from Gansu and Chongqing should be identified as a new subspecies, *C. fractifasciata indistincta* subsp. n., based on adult morphology and DNA barcodes.

Distribution. China.

#### Cyclidia fractifasciata fractifasciata (Leech, 1898)

Figs 14, 15, 32, 43, 53

#### Diagnosis. See under C. fractifasciata indistincta.

**Material examined. CHINA: Yunnan** (IZCAS): 1 $\stackrel{\circ}{\circ}$ , Dulongjiang, 1500 m, 29.V.2006, coll. Xiao Ningnian;  $3\stackrel{\circ}{\circ}1\stackrel{\circ}{\Rightarrow}$ , Lushui, Pianma, 8–12.V.2011, coll. Yang Xiushuai and Wang Ke.

Distribution. China (Yunnan).

**Genetic data.** The intrasubspecific divergence of the barcode region in *C. fractifasciata fractifasciata* is 0% (n = 4).

#### Cyclidia fractifasciata indistincta Jiang, Han & Xue, subsp. n.

http://zoobank.org/8BC2D3BA-389A-43E9-991E-CA93B3DE1837 Figs 16, 17, 33, 44, 54

**Description.** *Head.* Antennae blackish brown dorsally, flat and unipectinate, basal half without rami, rami very short. Frons blackish grey, not protruding. Labial palpi black with third segment distinct, extending beyond frons. Vertex black scattered with grey scales.

*Thorax.* Patagia white at basal half and blackish grey at terminal half. Tegula blackish grey. Dorsal side of thorax white with two pairs of blackish grey patches medially. Hind tibia with two pairs of spurs in both sexes. Forewing length: 37–40 mm. Apex of forewing rounded, not falcate; outer margin of both wings smooth. Wings white, transverse lines black. Forewing with a blackish brown patch basally; subbasal line broad; antemedial lines double, outer line indistinct and often invisible; medial line broad band-like at anterior half, very narrow and dot-like at posterior half; outer margin of medial line forming an almost right angle below M<sub>3</sub>; discal spot white, almost rhombic; postmedial lines double, wavy, inner line very obscure; submarginal line double, broad, and invisible between M<sub>3</sub> and CuA<sub>1</sub>; terminal lines double and discontinuous on each vein, inner line composed of oval markings, outer line appearing as series of short strips, inner markings often fused with outer ones; fringes white mixed with blackish grey. Hind wing with indistinct submarginal line; terminal lines and fringes similar to those of forewing. Underside white, striations indistinct than those of upperside.

*Abdomen.* Abdominal segments diffused with white scales. Pairs of black quadrate markings on first to seventh abdominal segments. Anterotergal syndeses developed at anterior margin of 2<sup>nd</sup> tergum. A pair of androconial hair-pencils present on 2<sup>nd</sup> pleuron of male.

*Male genitalia.* Uncus triangular. Socii sclerotized, about four-fifths the length of uncus. Gnathos with median process small and triangular. Valva narrow terminally; costa sclerotized and almost straight. Juxta formed a pair of forcipiform processes posteriorly. Saccus semicircular, about two-fifths length of basal width. Phallus slightly curved, with a small triangular lateral process posteriorly; vesica without cornuti.

*Female genitalia.* Lamella postvaginalis rectangle. Ductus bursae with a colliculum, long and narrow, striate longitudinally. Corpus bursae oval, with a paired slender signa; signa separated and parallel.

**Diagnosis.** The subspecies is very similar to the nominate subspecies, but differs externally by the following characters: the outer line of the antemedial line and the inner line of the postmedial line on the forewing are invisible, while in the nominate subspecies, they are much more distinct; the forewing discal spot is larger; the inner terminal markings of the forewing are larger and fused with the outer ones partly, while in *C. fractifasciata fractifasciata*, they are often smaller and separated from the outer ones.

**Type material examined.** Holotype,  $\mathcal{O}$ , **CHINA: Gansu** (IZCAS): Kangxian, Yangba, Meiyuangou, 1000 m, 13.VIII.2014, coll. Xue Dayong and Ban Xiaoshuang. Paratypes:  $3\mathcal{O}2\mathcal{Q}$ , same data as holotype. **Chongqing** (IZCAS):  $1\mathcal{Q}$ , Wushan, Wulipo, Dangyang, Congping, 1773 m, 25.VII.2013, coll. Cheng Rui.

**Genetic data.** The intrasubspecific divergence of the barcode region in *C. fractifasciata indistincta* is 1%. The intraspecific divergence of the barcode region between *C. fractifasciata fractifasciata* (n = 4) and *C. fractifasciata indistincta* (n = 5) is 2.3%. The distance between *C. fractifasciata* with the nearest neighbour species *C. substigmaria* is 12.5%.

Distribution. China (Gansu, Chongqing).

**Etymology.** The subspecies is named on the basis of the Latin adjective *indistinctus*, referring to the transverse lines of the forewing.



**Figures 30–34.** Male genitalia of *Cyclidia*. **30** *C. rectificata* (Tibet, slide no. 2) **31** *C. pitimani* (Yunnan, slide no. 9) **32** *C. fractifasciata fractifasciata* (Yunnan, slide no. 724) **33** *C. fractifasciata indistincta* subsp. n. (paratype, Gansu, slide no. 721) **34** *C. orciferaria* (Hainan, slide no. 728). Scale bar: 1 mm.



Figures 35–47. 35–36 Male genitalia of *Mimozethes.* 35 *M. angula* (paratype, Sichuan, slide no. 34) 36 *M. lilacinaria* (Sichuan, BMNH, slide No. 304) 37–47 Phallus 37 *Cyclidia substigmaria substigmaria* (Tengchong, Yunnan, slide no. 682) 38 ditto (holotype of *C. substigmaria brunna*, Emeishan, Sichuan, slide no. 12) 39 ditto (holotype of *C. tetraspota*, Xishuangbanna, Yunnan, slide no. 10) 40 *C. substigmaria intermedia* (Tibet, slide no. 311) 41 *C. rectificata* (Tibet, slide no. 727) 42 *C. pitimani* (Yunnan, slide no. 9) 43 *C. fractifasciata fractifasciata* (Yunnan, slide no. 724) 44 *C. fractifasciata indistincta* subsp. n. (paratype, Gansu, slide no. 721) 45 *C. orciferaria* (Hainan, slide no. 728) 46 *M. angula* (holotype, Sichuan, slide no. 19) 47 *M. lilacinaria* (Sichuan, BMNH, slide No. 304). Scale bars: 1 mm.

#### Cyclidia orciferaria Walker, 1860

Figs 18, 19, 34, 45, 55

*Cyclidia orciferaria* Walker, 1860: 56. Syntypes, China: North China. *Cyclidia ociferaria* Kirby, 1892: 725. [Incorrect spelling of *Cyclidia orciferaria* Walker.]

**Diagnosis.** This species is different from other congeners in the following external characters: the apex of the forewing is falcate; the wing colour is blackish brown; two bands covered with greyish blue scales are present on the forewing, and the inner band is narrower and less distinct than the outer band; the discal spot of the forewing is yellowish brown, oblong, with a blackish brown narrow line medially; greyish blue scales are covered on the submarginal lines of both wings, and often absent on the middle part of the hind wing. There are also differences in the male genitalia: the socii are weakly sclerotized and much shorter than the uncus; the valva is short. In the female genitalia, the posterior margin of the lamella postvaginalis is slightly concaved; the two signa are tapered at posterior half and situated very close to each other.

Material examined. CHINA: Zhejiang (IZCAS): 2<sup>(2)</sup>, Tianmushan, 20–23. VII.1973, coll. Zhang Baolin; 1319, Lin'an, West Tianmushan, 400–1500 m, 26.VII-29.VIII.2003, coll. Xue Dayong et al.; 18, West Tianmushan, Zhonglieci, 363 m, 24.VII.2011, coll. Yan Keji; 13, West Tianmushan, Xianrending, 1506 m, 27.VII.2011, coll. Yan Keji;  $2\sqrt[3]{2}$ , Taishun, Wuyanling, Shuangkengkou, 680 m, 28–29.VII.2005, coll. Lang Songyun; 19, Taishun, Siqianzhen, 250 m, 4.VIII.2005, coll. Lang Songyun; 1♂, Ningbo, V.1981. **Jiangxi** (IZCAS): 1♀, Huzhi, 28.VII.1990. Hunan (IZCAS): 2<sup>3</sup>, Yanling, Taoyuandong, 631 m, 4–8.VII.2008, coll. Chen Fuqiang; 1∂, Tianpingshan, 25.VI.1981. Fujian (IZCAS): 1∂, Jiangle, Longqishan, 800 m, 15.IX.1990, coll. Yang Bin; 8<sup>3</sup>, Wuyishan, 24.IV-21.V.1983, coll. Wang Linyao; 1, Wuyishan, Sangang, 24.VII.1980; 1, Nanping, Shangyang, 9.VI.1963, coll. Zhang Youwei. **Guangdong** (IZCAS): 1<sup>(2)</sup>, Ruyuan, Nanling, Baohuzhan, 1020 m, 16–20.VII.2008, coll. Chen Fuqiang; 19, Shixing, Chebaling, 365–401 m, 22–26. VII.2008, coll. Chen Fuqiang. **Hainan** (IZCAS): 4Å2, Nankai, Nanmaola, 1261 m, 10–14.V.2009, coll. Chen Fuqiang and Yan Keji;  $631^{\circ}$ , Jianfengling, Tianchi, 828 m, 1–5.V.2007, 18.V.2009, coll. Chen Fuqiang;  $1\sqrt[3]{2}$ , Bawangling, Dong'er Linchang, 1004–1015 m, 8.V.2007, 7.IV.2008, coll. Chen Fuqiang and Lang Songyun; 113, Wuzhishan, Shuiman, 730-900 m, 7-11.V.2007, 1-3.IV.2008, coll. Lang Songyun and Han Hongxiang;  $1\sqrt[3]{3}$ , Lingshui, Diaoluoshan, 190–920 m, 3–7.V.2007, coll. Han Hongxiang and Lang Songyun; 1<sup>Q</sup>, Qiongzhong, Limuling, 620 m, 15.V.2007, coll. Han Hongxiang; 1♀, Xinglong, 24.IV.1963, coll. Zhang Baolin. **Guangxi** (IZCAS): 3Å2, Fangcheng, Fulong, 200–550 m, 23–26.V.1999, coll. Yuan Decheng et al.; 1312, Napo, Defu, 1350 m, 19.VI.2000, coll. Zhu Chaodong; 1, Jinxiu, Linhai Shanzhuang, 1100 m, 2.VII.2000, coll. Li Wenzhu; 1, Jinxiu, Jinzhong Gonglu, 1000 m, 10.V.1999, coll. Han Hongxiang; 1♀, Daxin, Xialei, 680 m, 31.III.1998, coll. Li Wenzhu. **Yunnan** (IZCAS):  $1 \swarrow 1 \subsetneq$ , Hekou, Xiaonanxi, 200 m, 10–11.VI.1956, coll. Huang Keren et al.; 1♀, Pingbian, Daweis-



Figures 48–57. Female genitalia. 48 Cyclidia substigmaria substigmaria (Henan, sldie. no. 726) 49 ditto (Jiangsu, slide. no. 33) 50 ditto (paratype of *C. tetraspota*, Xishuangbanna, Yunnan, slide no. 36) 51 *C. substigmaria intermedia* (Tibet, slide no. 685) 52 *C. rectificata* (Tibet, slide no. 3) 53 *C. fractifasciata fractifasciata* (Yunnan, slide no. 725) 54 *C. fractifasciata indistincta* subsp. n. (paratype, Gansu, slide no. 722) 55 *C. orciferaria* (Hainan, slide no. 729) 56 *M. angula* (Henan, slide no. 288) 57 *M. lilacinaria* (Sichuan, slide no. 280). Scale bars: 1 mm.

han, 1500 m, 20.VI.1956, coll. Huang Keren et al.; 1Å, Xishuangbanna, Mengla, Menglun, 650 m, 1.VI.1964, coll. Zhang Baolin; 1Å1Q, Mengla Linchang, 550 m, 20.IV.1982, coll. Wang Yongxian; 1Å, Mengla, 20.VI.1982, coll. Chen Yixin; 1Q, Mengla, Lengku, 623 m, 10.VI.1980, coll. Guo Zuyun; 1Å, Xishuangbanna, Bubang, 700 m, 14.IX.1993, coll. Yang Longlong; 1Å, Xishuangbanna, Damenglong, 650

m, 1.VIII.1958, coll. Zheng Leyi; 1♂, Xishuangbanna, Dameng'a, 1050–1080m, 15.VIII.1958, coll. Wang Shuyong; 2♂, Cangyuan, 790–1100 m, 19–22.V.1980, coll. Song Shimei and Shang Jinwen; 1♂, Xiaomenglun, 21.IV.1982, coll. Wang Linyao; 1♂, Ruili, Dengga, 6–8.VI.1992, coll. Xue Dayong; 1♂, Baoshan, Baihualing, 1520 m, 11–13.VIII.2007, coll. Wu Chunguang.

**Genetic data.** The intraspecific divergence of the barcode region of *C. orciferaria* is ranges from 0%–1.7% (average distance 1.09%) (n = 8). The distance to the nearest neighbour *C. substigmaria* is 11.5%.

**Distribution.** China (Jiangsu, Zhejiang, Jiangxi, Hunan, Fujian, Guangdong, Hainan, Guangxi, Sichuan, Yunnan), Myanmar, Vietnam, Indonesia.

**Biological notes.** The morphology of the larva of *C. orciferaria* was illustrated in Chen (2011).

#### Mimozethes Warren, 1901

*Mimozethes* Warren, 1901: 190. Type species: *Euchera nana* Warren, 1897, by original designation.

Generic characters. Head. Antennae lamellate and shortly unipectinate, basal part of antennae without rami (Fig. 1b). Frons not protruding. Labial palpi with second segment slightly curved, third segment oval. Thorax. Hind tibia with two pairs of spurs. Apex of forewing falcate; outer margin of forewing protruding. Wing. Wings colour dark brown. Forewing with silver grey antemedial line, sometimes indistinct; discal spot black and small; postmedial line silver grey, forming a ">" shaped protrusion near R<sub>s</sub>. Hind wing with medial line and postmedial line silver grey and almost straight. Black brown patches present near anal angle of both wings. Terminal lines of both wings composed of a series of blackish brown strips covering silver grey scales, very distinct towards apex. Underside with distinct discal spot, costa, apex and outer margin suffused with pale yellowish brown scales. Vein (Fig. 3b). Forewing with  $R_1$  separate,  $R_2$  and  $R_5$  stalked,  $M_2$  arising from middle of discocellulars; Hind wing with Sc+R<sub>1</sub> close to Rs beyond distal cell, then far from Rs, M<sub>2</sub> arising from middle of discocellulars. Anterotergal syndeses developed at anterior margin of 2<sup>nd</sup> tergum (Fig. 2). A pair of androconial hair-pencils present on 2<sup>nd</sup> sternum of male (Fig. 2). *Male genitalia*. Uncus triangular, acute terminally; socii undeveloped; gnathos connected at middle and with median process small and acute apically; sacculus forming a long process; juxta short and broad, concaved posteriorly; saccus broad and rounded terminally; Phallus short; vesica without cornuti. Female genitalia. Papillae anales broad and rounded; lamella postvaginalis large and oval, with many tiny spines; ductus bursae long and narrow, with a colliculum; corpus bursae oval, without a signum.

Diagnosis. See under Cyclidia.

**Remarks.** According to Inoue (1962), *M. argentilinearia* (Leech, 1897) occurs in Japan and Taiwan. However, it has not been recorded from Taiwan in later studies (Inoue 1992, Yan et al. 2009, Chen 2011). Thus, following that, we do not include the species in this paper.

Distribution. China, Japan.

#### Key to Chinese Mimozethes species

Outer margin of forewing weakly protruding; ventral margin of valva forming a small triangular protrusion apically in male genitalia.....*M. angula*, Figs 20–21
 Outer margin of forewing strongly protruding; ventral margin of valva not forming a small triangular protrusion apically in male genitalia ......*M. lilacinaria*, Figs 22–23

# Mimozethes angula Chu & Wang, 1987

Figs 20, 21, 35, 46, 56

Mimozethes angula Chu & Wang, 1987: 207. Holotype &, China: Sichuan: Mt. Emei (IZCAS).

**Diagnosis.** This species is very similar to *M. lilacinaria* (Leech, 1897) and *M. argentilinearia*, but it can be distinguished by the following characters: the outer margin of the forewing is less strongly protruding than that of *M. lilacinaria* and *M. argentilinearia*; the black patch inside the anal angle of the forewing is less distinct than that of *M. argentilinearia*; the yellowish brown patch on the underside of the forewing is much smaller and less distinct than that of *M. lilacinaria* and *M. argentilinearia*. In the male genitalia, the uncus is shorter; the ventral margin of the valva forms a small triangular protrusion apically, but *M. lilacinaria* and *M. argentilinearia* lack this character; the sacculus process is much longer than that of *M. lilacinaria*.

**Type material examined. CHINA: Sichuan** (IZCAS): 13 (Holotype), Emeishan, Qingyinge, 800–1000 m, 15.IX.1957, coll. Zhu Fuxing; 12 (Allotype), same locality, 22.IX.1957, coll. Zhu Fuxing; 4322 (Paratype), same locality, 22.VI.1957, 15–19. IX.1957, coll. Zhu Fuxing *et al.* 

Additional material examined. CHINA: Henan (IZCAS): 1 $\bigcirc$ , Baiyunshan, 13–15.VIII.2008, 1550 m, coll. Jiang Nan. Hubei (IZCAS): 1 $\bigcirc$ , Shennongjia, Daji-uhu, 1800 m, 1.VIII.1981, coll. Han Yingheng. Sichuan (IZCAS): 9 $\bigcirc$ 2 $\bigcirc$ , Emeishan, Qingyinge, 800–1000 m, 20.VI.1957, 15–22.IX.1957, coll. Zhu Fuxing et al.; 1 $\bigcirc$ , Qingchengshan, 1000 m, 4.VI.1979, coll. Shang Jinwen; 1 $\bigcirc$ , Emeishan, 1288 m, 31.VII.2013, coll. Cheng Rui.

Genetic data. No genetic data available.

Distribution. China (Henan, Hubei, Sichuan).

#### Mimozethes lilacinaria (Leech, 1897)

Figs 22, 23, 36, 47, 57

- Decetia lilacinaria Leech, 1897: 184. Holotype 3, China: Sichuan: Emeishan (BMNH).
- Heteromize lycoraearia Oberthür, 1912: 269. Holotype &, China: Sichuan: Mou-pin (BMNH).

Mimozethes lilacinaria: Beccaloni et al. 2003 [accessed 26 November 2015].

#### Diagnosis. See under *M. angula*.

**Type material examined. CHINA: Sichuan** (BMNH): 1Å (Holotype), Omei-Shan, 3620 ft., Native coll. July & Aug. 1890, Leech Coll. 1900-64, BMNH (E) 1377104.

**Additional material examined. CHINA: Sichuan** (BMNH): 1∂, Chasseurs indigènes, de Tà-tsien-lou, Récolle de 1910, Ex Oberthür Coll. Brit. Mus. 1927-3, Drepanidae genitalia slide No. 304; 1♀, Siao-Lou, 1900, Chasseurs indigènes, Ex Oberthür Coll. Brit. Mus. 1927-3. **Yunnan** (IZCAS): 1♀, Xishuangbanna, Menghai, 21.VII.1958, coll. Wang Shuyong.

Genetic data. No genetic data available.

**Remarks.** Chu and Wang (1991) did not record this species. The specimens from Yunnan should be identified as *M. lilacinaria* based on adult morphology.

Distribution. China (Sichuan, Yunnan).

#### DNA barcoding results and discussion

Forty-three DNA barcode sequences of lengths 658bp were obtained for *Cyclidia* species. The nucleotide composition of *Cyclidia* species COI genes was 30.60% of A, 38.54 of T, 16.06% of C, 14.80% of G. The interspecific distance within the genus was range from 8.8%–13.9%. The maximum intraspecific distances was 2.6% in *C. substigmaria*, 1.7% in *C. orciferaria*, 0.0% in *C. rectificata*, and 2.3% in *C. fractifasciata*. The maximum genetic distances observed within species (2.6% at COI) were less than the minimum distances observed between the species (8.8%). There is a clear barcoding gap between intra and interspecific variation; furthermore, NJ tree also provided strong support for the separation of *Cyclidia* species (Fig. 58).

In recent revisionary work of Drepanidae, Song et al. (2011, 2012) and Park et al. (2011) found many new taxa, synonyms and misidentifications in earlier studies. However, when dealing with some morphologically similar taxa, it is difficult to discriminate only using the subtle diagnostic characters. The present study utilizing morphological and molecular characters revised some Chinese *Cyclidia* species. The morphological analysis indicated that some structures of the genitalia were found to be less diagnostic than the external characters between some species (i.e. *C. substigmaria* and *C. rectificata*). Sihvonen et al. (2014) also mentioned this trait in the Geometridae.



**Figure 58.** Neighbour joining tree (Kimura 2-parameter distance model for 658bp COI marker) for Chinese *Cyclidia* species. Terminals with sequence ID and collecting locality.

Additionally, some structures of the male genitalia (e.g. the shape of the valva) sometimes varied among individuals of *C. substigmaria*. Therefore, species have been delineated on the basis of a combination of data from morphology and DNA barcodes. In the molecular analysis, DNA barcodes proved to be very helpful. The interspecific divergence of *Cyclidia* species (minimum distance 8.8%, maximum distance 13.9%) was much larger than the 2% or 3% of the threshold for species diagnosis (Hebert et al. 2003, Hebert et al. 2004a, Hebert et al. 2004b). The remarkably high interspecific divergence and low intraspecific divergence on average 1% (minimum distance 0.0%, maximum distance 2.6%) fully supports the morphological species concept.

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CORRIGENDA



## Corrigenda: Breedy O, Guzman HM (2015) A revision of the genus *Muricea* Lamouroux, 1821 (Anthozoa, Octocorallia) in the eastern Pacific. Part I: Eumuricea Verrill, 1869 revisited. ZooKeys 537: 1–32. doi: 10.3897/ zookeys.537.6025

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Several errors came to our attention after our manuscript was published, which we address here. First, Figure 9 corresponds to the colour plate under Figure 10; Figure 10 corresponds to the SEM plate under Figure 9. Second, Figure 13 corresponds to the SEM plate under Figure 14. Third, Figure 14 is a new plate that was missing. Finally "Figure 12" that is written under **Genus** *Swiftia* **Duchassaing & Michelotti, 1864**, in page 21 is a typo.



The correct, whole Figures and captions are reproduced here below.

Figure 9. *Muricea tubigera* Verrill, 1869a YPM 807. **A** Colony **B** Detail of branches **C** Sclerites, light micrograph.



**Figure 10.** *Muricea tubigera* Verrill, 1869a YPM 807. **A**, **B** Calycular and coenenchymal spindles **C**, **D** Axial sheath spindles.



Figure 13. Astrogorgia splendens (Thomson & Simpson, 1909), BM 1933.05.03.094. SEM sclerites.



**Figure 14.** *Leptogorgia ruberrima* (W. Koch, 1886), BM 1933-03-13-024. **A** Fragment of the holotype **B** Sclerites, light micrograph **C** SEM sclerites.

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