ZooKeys 865: 1–20 (2019)
doi: 10.3897/zookeys.865.30705
http://zookeys.pensoft.net

RESEARCH ARTICLE

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Meplitumen aluna gen. nov., sp. nov. an interesting eutardigrade (Hypsibiidae, Itaquasconinae) from the Sierra Nevada de Santa Marta, Colombia

Oscar Lisi[1,2], Anisbeth Daza[2], Rosana Londoño[2],
Sigmer Quiroga[2,3], Giovanni Pilato[1]

1 *Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Catania, Via Androne 81, 95124 Catania, Italy* **2** *Grupo de Investigación MIKU, Universidad del Magdalena, Carrera 32 # 22-08, Santa Marta DTCH, Colombia* **3** *Facultad de Ciencias Básicas, Programa de Biología, Universidad del Magdalena, Carrera 32 # 22-08, Santa Marta DTCH, Colombia*

Corresponding author: *Oscar Lisi* (olisi@unict.it)

Academic editor: *Sandra McInnes* | Received 23 October 2018 | Accepted 11 June 2019 | Published 22 July 2019

http://zoobank.org/DF6A9937-7897-48DD-9CA7-3B866A2892AF

Citation: Lisi O, Daza A, Londoño R, Quiroga S, Pilato G (2019) *Meplitumen aluna* gen. nov., sp. nov. an interesting eutardigrade (Hypsibiidae, Itaquasconinae) from the Sierra Nevada de Santa Marta, Colombia. ZooKeys 865: 1–20. https://doi.org/10.3897/zookeys.865.30705

Abstract

A new genus of Itaquasconinae, *Meplitumen* **gen. nov.**, and a new species, *Meplitumen aluna* **sp. nov.**, are described. The new genus has characters present in other genera of Itaquasconinae but in a unique combination. The spiral thickening of the bucco-pharyngeal tube is also present anteriorly to the insertion point of the stylet supports, excluding only the short portion where the apophyses for the insertion of the stylet muscles (AISM) are present. This character is similar to *Astatumen* Pilato, 1997 but *Meplitumen* **gen. nov.** differs from this genus as stylet furcae are shaped differently and as stylet supports and placoids are present. The presence of a spiral thickening in a portion of the buccal tube anterior to the stylet supports distinguishes the new genus from *Mesocrista* Pilato, 1987, *Platicrista* Pilato, 1987 and *Itaquascon* de Barros, 1939. *Meplitumen* **gen. nov.** also differs from *Mesocrista*, in having the caudal processes of the AISM pointing laterally (instead of posterolaterally), and the apices of the caudal processes of the stylet furcae unswollen. From *Itaquascon* the new genus also differs by having more robust stylet supports, pharyngeal bulb with placoids, stylet furcae differently shaped. *Meplitumen* **gen. nov.** also differs from *Platicrista* in having caudal processes of the AISM more robust and not flexible, and more slender stylet supports. The new species, *Meplitumen aluna* **sp. nov.**, has a cuticle with a very faint roughness at the caudal extremity of the body, and eyes probably absent. The pharyngeal bulb is long, with two long, narrow, rod-shaped macroplacoids; a microplacoid and septulum are absent. The claws are well developed with main branches provided with accessory points, and at the base of the claws, a structure interpretable as a very thin lunule is present. Other cuticular thickenings on the legs are absent.

Keywords
Magdalena, phylogeny, Tardigrada, taxonomy, water bear

Introduction

Rarely in the study of systematics and phylogeny is one so fortunate to encounter a taxon with characters that sheds light on phylogenetic directions within a group. While studying Colombian tardigrades, from the Sierra Nevada de Santa Marta, we found eight specimens and two exuviae with eggs of a new species of Hypsibiidae (Itaquasconinae), for which it is necessary to erect a new genus that we name *Meplitumen* gen. nov. This discovery is further confirmation of the biodiversity richness of this Neotropical region (Myers et al. 2000, Rull 2007), which, at least with regards to the tardigrades, is still far from being thoroughly investigated.

In the framework of the subfamily Itaquasconine Rudescu, 1964, the four genera *Itaquascon* de Barros, 1939, *Mesocrista* Pilato, 1987, *Platicrista*, Pilato, 1987 and *Astatumen* Pilato, 1997 are certainly related to one another, but until now it has not been possible to formulate any hypothesis about the phylogenetic relationships that exist among them. Our new genus shows characters that are present in these other genera but in a unique combination. This encouraged us to hypothesise possible evolutionary pathways that connect these five Itaquasconinae genera, which appear to constitute a homogeneous group within the subfamily.

Material and methods

The present work is part of a revision of the tardigradological collection of the Centro de Colecciones Biológicas de la Universidad del Magdalena (CBUMAG:TAR), Santa Marta, Colombia. The material studied with the new genus and species has been returned to the collection.

The original specimens had been extracted from moss and lichen samples collected in San Lorenzo and El Campano, Colombia. Complete information about localities and samples studied are included in the description of the new species under Material examined.

The studied specimens were mounted in polyvinyl alcohol mounting media (BioQuip #6371A). Measurements are given in micrometres (μm), and photomicrographs made under ×100 oil immersion under phase contrast and differential interference contrast microscopy, using a Leica “DMLB” Microscope equipped with “Canon S40” digital camera and a micrometre, a Zeiss Axio Scope A1 with CCD camera Zeiss AxioCam ICc5, and a Zeiss AxioLab A1 with a Zeiss Axiocam ERc 5s. Images were edited, and plates arranged, using Adobe Photoshop Elements 2.0 digital imaging software.

Notwithstanding the presence of spiral thickening, we refer to the “buccal tube” as the entire portion of the bucco-pharyngeal tube anterior to the stylet supports, which seems to be almost rigid. A problem of interpretation and terminology with regard to that portion of the bucco-pharyngeal tube is discussed in Appendix 1 of this paper. The *pbf* index is the percent ratio between the length of the buccal tube and the total length of the bucco-pharyngeal tube (Pilato et al. 2002). The *pt* index is the percent ratio between the length of a structure and the length of the buccal tube measured from the anterior margin of the stylet sheaths to its posterior end (Pilato 1981): in the new genus, as in others, the posterior end of the buccal tube fixed to assess the *pt* index coincides with the insertion point of the stylet supports. Claws were measured using maximum length of the studied structures and choosing only those with the same, or at least very similar, orientation; we discarded claws with unsuitable orientation for correct measurement. As a consequence, the number of claws measured, reported in Table 1, is limited. When the claw measurement included the basal portion, we chose (Fig. 1A, B) the central point of the claw base and the most distant point of the primary or secondary claw branch, including accessory points. In measuring the main branch of the external claws (Fig. 1B), we were unable to measure only the sclerified portion excluding the very flexible basal section as the precise border was not always clearly defined. That flexible portion may be more or less bent and then its exclusion would be preferable in order to obtain more precise measurements, but it was not possible for us to avoid this problem. The main branch of the internal claws was measured from the junction point of primary and secondary branch (Fig. 1B) to the more distant point of the primary branch.

For comparison, we have examined specimens of: *Mesocrista spitzbergensis* (Richters, 1903) from Tatra Mountains (border between Poland and Slovakia); *Platicrista angustata* (Murray, 1905) from Ligorzano (Modena, Italy); *Itaquascon cambewarrense* Pilato, Binda & Claxton, 2002 from Cambewarra Mountain (Australia),

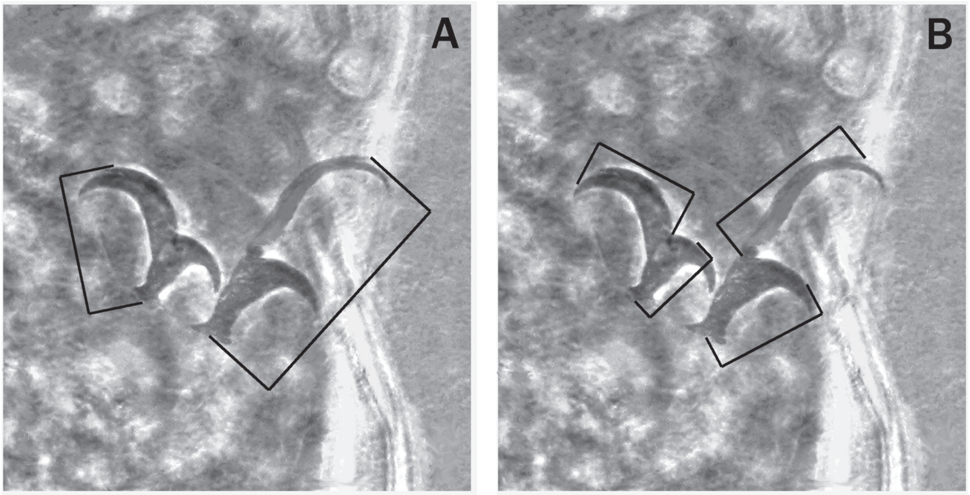

Figure 1. Claws of the *Hypsibius* type showing the criteria used to take the measurements in *Meplitumen aluna* gen. nov., sp. nov. **A** claw orientation chosen for measuring the entire length **B** claw orientation and referring points chosen for various claw portion measurement. The claws shown, used as example, belong to *Platicrista angustata* (Murray, 1905).

and types of *Astatumen trinacriae* (Arcidiacono, 1962) from Nebrodi Mounts (Sicily). All these specimens are deposited in the Binda and Pilato collection (Museum of the Department of Biological, Geological and Environmental Sciences, University of Catania, Italy).

For phylogenetic analysis, a character matrix was prepared and a parsimony analysis applied with a Nearest Neighbor Interchange (NNI) heuristic method, using the software Mesquite (Maddison and Maddison).

Results

Taxonomic account

Phylum Tardigrada Doyère, 1840
Class Eutardigrada Richters, 1926
Order Parachela Schuster, Nelson, Grigarick & Christenberry, 1980
Superfamily Hypsibioidea Pilato, 1969[*]
Family Hypsibiidae Pilato, 1969[**]
Subfamily Itaquasconinae Rudescu, 1964[***]

***Meplitumen* gen. nov.**
http://zoobank.org/4842673D-73A5-459A-A9DC-11C667772F10
Figs 2, 3, 5, 6A–C, 7

Type species. *Meplitumen aluna* sp. nov.

Description. Claws of the *Hypsibius*-type. Bucco-pharyngeal apparatus of Itaquasconinae model but buccal tube with a spiral thickening also present anteriorly to the stylet support insertion point (Fig. 2 A, B arrow ‘a’). Only the very anterior portion of the buccal tube, where the apophyses for the insertion of the stylet muscles (AISM) are present, is the spiral thickening absent. The AISM are wide and flat ridges, symmetrical with respect to the frontal plane (Figs 2A; 3A, B, D) and their caudal processes point sideways. Peribuccal lamellae absent, papulae probably present (needs confirmation). No cuticular thickening is present between buccal and pharyngeal tube (Figs 2A, B; 3A, B). The stylet furcae have the caudal processes with non-swollen apices (Fig. 3D, arrow). The stylet supports are normally developed (Fig. 2A, arrow ‘b’). The pharyngeal bulb is long, without apophyses but with true, long placoids (Fig. 3A–C).

Differential diagnosis. According to the definition in Pilato and Binda (2010), the bucco-pharyngeal tube of Itaquasconinae model, is subdivided into an anterior portion (buccal tube), rigid, without spiral thickening and without ventral lamina, and

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[*] in Marley et al. 2011; amended by Bertolani et al. 2014 and Gąsiorek et al. 2018

[**] amended by Bertolani et al. 2014 and Gąsiorek et al. 2018

[***] sensu Pilato 1987, amended by Bertolani et al. 2014

a posterior portion, generally flexible, provided with a spiral thickening (pharyngeal tube), as in *Astatumen*, *Mesocrista*, *Platicrista* and *Itaquascon*. In *Meplitumen* gen. nov. the bucco-pharyngeal apparatus differs from the above by having a spiral thickening also present anteriorly to the stylet support insertion point (Fig. 2A, B arrow 'a'), which is similar to *Astatumen* but, unlike *Astatumen*, the stylet supports are present (Fig. 2A, arrow 'b'), and mark the border between the posterior flexible portion of bucco-pharyngeal tube and the (almost) rigid anterior section. The sideways pointing caudal processes of the AISM, are similar to *Platicrista*, *Itaquascon* and *Astatumen*, but differ from *Mesocrista* (in which they point postero-laterally). The non-swollen apices (Fig. 3D, arrow) of the stylet furcae caudal processes are more similar to *Platicrista* (Fig. 4B, arrow 'a') than *Mesocrista* where the apices are clearly swollen (Fig. 4A, arrow 'a'). The stylet furcae are clearly larger than those of *Itaquascon* (Fig. 4C, arrow 'a') and *Astatumen* (Fig. 4D, arrow 'a') whose caudal processes are very reduced.

Etymology. Considering that the bucco-pharyngeal apparatus of the new genus has characters present in *Mesocrista*, *Platicrista*, *Itaquascon* and *Astatumen,* we have chosen *Meplitumen* as generic name using the first letters of the name of the first three genera (Me-, Pl-, It-) and the final part of the name *Astatumen* (-umen).

Meplitumen aluna **sp. nov.**

http://zoobank.org/C96630F3-7A49-479E-8B14-5C00788CC0AD
Figs 2, 3, 5, 6A–C, 7; Table 1

Type locality. San Lorenzo, Sierra Nevada de Santa Marta, Magdalena, Colombia.

Material examined. Holotype (slide No. 00462), 5 **paratypes** (slides Nos. 00460, 00461, 00476, 00477), and two exuviae (slide No. 00492) from a sample dominated by lichens (*Usnea*, and *Parmotrema*) mixed with bryophytes (*Sematophyllum*, *Frullania*, *Microlejeunea,* and *Leucolejeunea*), collected in San Lorenzo (Colombia) at 11°06'16.9"N and 74°03'31.2"W, 2517 m a.s.l.. One paratype (slide No. 00545) collected in the type locality but in a different sample containing lichens (*Parmotrema, Heterodermia,* and *Hypotrachyna*) mixed with bryophytes (*Meteoridium*, *Frullania*, and *Archilejeunea*). One additional specimen (slide No. 00376) of a sample of liverworts (*Frullania* and *Cheilolejeunea*), collected in El Campano (Colombia) at 11°6'23.2"N and 74°5'19.2"W, 1368 m a.s.l. The type material was collected by Anisbeth Daza, Rosana Londoño and Sigmer Quiroga on 31 July 2015. The remainder of the material was collected by Anisbeth Daza, Rosana Londoño, Paula Sepúlveda and Sigmer Quiroga, on 21 March 2015.

Type repository. The holotype and paratypes, and the additional specimen, are deposited in the Centro de Colecciones Biológicas de la Universidad del Magdalena (CBUMAG:TAR), Santa Marta, Colombia.

Species description. Body uncoloured. A faint, though difficult to see, cuticular roughness visible on the caudal portion of the body. Eyes probably absent (definitely absent after mounting and no information record when mounted). Bucco-pharyngeal apparatus of the *Meplitumen*-type of Itaquasconinae model (as described above). Mouth terminal. A row of small teeth is present in the caudal portion of the buccal cavity (Fig. 3B, arrow). Pharyngeal bulb long, without apophyses but with two long, narrow rod-shaped macroplacoids (the second more than double the length of the first); microplacoid and septulum absent. Stylet furcae well developed but the caudal processes have non-swollen apices (Fig. 3D, arrow). Claws of the *Hypsibius*-type, with main branches provided with accessory points; these points are well developed on the internal claws, short and thin externally. At the base of the claws, a structure interpretable as a very thin lunule is present; other cuticular thickenings on the legs absent.

Choice of the holotype. We found eight specimens and two exuviae with eggs and it is interesting to note that three specimens were small and of similar body size, whereas the others are markedly longer and certainly adults as demonstrated by the size of the exuviae with eggs. Unfortunately, we have not been able to establish the sex of the specimens; in particular, whether the smallest were three young, or new-born specimens, or were males. It is interesting to note that some metric characters of the smaller specimens are very similar to those of the larger specimens, while others were markedly different (see Table 1). If they are young or new-born examples, we would expect structures to have allometric growth, but these have more marked differences than that we have observed in other species. This makes us suspect the smaller specimens may be males. In any case, we have chosen the holotype among the larger, definitely adult, specimens.

Description of the holotype. Body length 590 µm, uncoloured, cuticle with a faint, very difficult to see roughness in the caudal body portion (Fig. 5A, B, C arrows); as in other species, this cuticular roughness is not visible in some specimens. Eyes probably absent (definitely absent after mounting and no information record when mounted). Bucco-pharyngeal apparatus of the *Meplitumen*-type of Itaquasconinae model (Pilato and Binda 2010) (Figs 2 and 3), i.e., with the bucco-pharyngeal tube subdivided at the junction of the stylet supports into an anterior, almost rigid, buccal tube with spiral thickening (except the very anterior portion) (Fig. 2A, B, arrows 'a'), and a posterior flexible portion with a more obvious spiral thickening. The mouth is terminal without peribuccal lamellae; peribuccal papulae probably present but requires confirmation. A row of small teeth present in the caudal portion of the buccal cavity (Fig. 3B, arrow). The AISM are flat, symmetrical with respect to the frontal plane, and with the caudal processes short and pointing sideways (Fig. 2A, arrow 'c'). Bucco-pharyngeal tube 61.3 µm long, buccal tube 31.8 µm long (*pbf* index = *51.9*) and 8.9 µm wide externally (*pt* index = *28.0*), pharyngeal tube 29.5 µm long. No drop shaped thickening is present between buccal and pharyngeal tube (Figs 2A, B; 3A, B, D). The stylet furcae are well developed but have the caudal processes with non-swollen apices (Fig. 3D, arrow). Pharyngeal bulb (Fig. 3A, C) long, about 2.5 times its width (63.4 µm × 27.0 µm), without apophyses, but with two long, narrow, rod-shaped macroplacoids (Fig. 3A, B, C); first macroplacoid 10.9 µm long (*pt* = *34.3*), the second 29.0 µm long (*pt* = *91.2*); the entire placoid row is 40.7 µm long (*pt* = *128.0*); microplacoid and septulum absent.

Claws of the *Hypsibius*-type well developed (Fig. 6A, B, C) with widened extreme basal portion. Reliable claw measurements, as in many species, are challenging due to

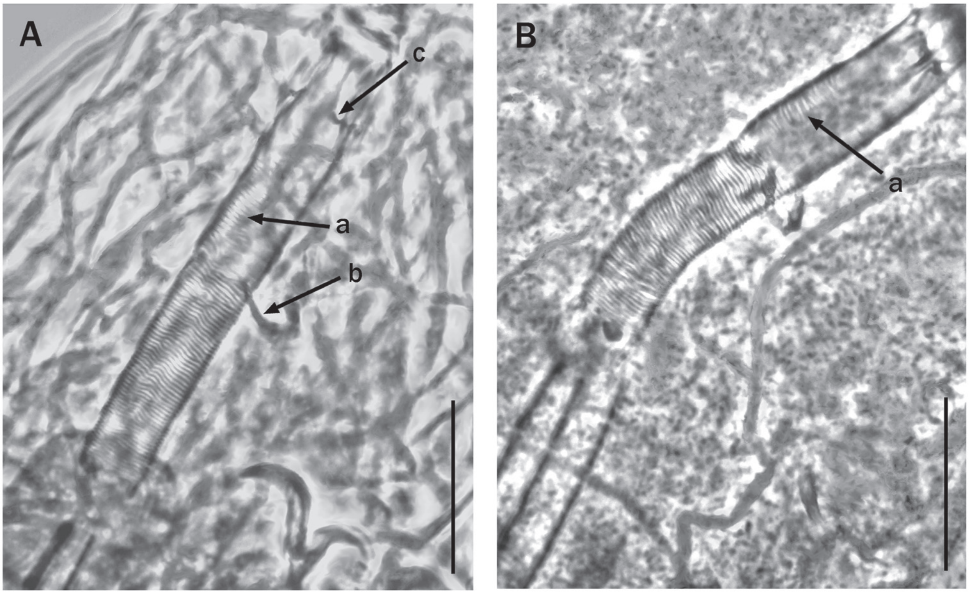

Figure 2. Bucco-pharyngeal tube of *Meplitumen aluna* gen. nov., sp. nov. **A** holotype (slide No. 00462), was invaded by fungal hyphae. The spiral thickening also anterior to the stylet supports insertion point is visible (arrow 'a'). The arrow 'b' indicates the stylet supports. Arrow 'c' indicates the caudal processes pointing sideways of the wide and flat AISM **B** paratype (slide No. 00460). The spiral thickening also anterior to the stylet supports insertion point is visible (arrow 'a'). Scale bar: 20 µm.

the claw orientation affecting the apparent length. The main branch of both external and internal claws with accessory points; more evident in the hind legs. In all legs those points are more developed on the internal claws (Fig. 6A, B, C) and very short and thin on the external claws; particularly those of the first three pair of legs where they are often almost invisible. At the base of the claws a structure interpretable as a very thin lunule is present (Fig. 6A, B, C arrow). Other cuticular thickenings on the legs absent.

Eggs. We found two exuviae, one with 5 and the other with 6 smooth eggs.

Etymology. The specific epithet refers to the term "Aluna" which in Ika, the native language of the Kogui (an Amerindian ethnic group inhabiting the Sierra Nevada de Santa Marta), means the non-visible or spiritual world. Aluna is a name in apposition.

Remarks. As mentioned above, the three smaller specimens (Fig. 7A, B) show some metric differences from the larger. The most obvious differences are the *pt* values relative to the buccal tube width, the placoid length, and claws II, III (less remarkably claw IV) length. In all legs of all specimens the percent ratio between the main branch length and the total claw length are compatible (Table 1). This later character and the high number of important non-variable characters indicate they belong to *Meplitumen aluna* sp. nov., but the differences made us consider they may be very young individuals or males.

Differential diagnosis. The particular combination of the characters of the bucco-pharyngeal apparatus distinguishes the new species from all known species

Table 1. Morphological measurements for the holotype, five paratypes, and the additional specimen of *Meplitumen aluna* sp. nov. The first three small specimens are probably juveniles or males. Measurements given in µm, with **pbf** and **pt** index values for relevant structures, and the percent ratio between the main branch and the total length of the external claw. Specimens ordered by buccal tube length (body length being a less precise measurement). The small specimens differ from the larger in the **pt** values relative to the buccal tube width, the macroplacoid length, and claw II and III length (less remarkably claw IV length), but the percent values of the main branch length with respect to the total claw lengths are compatible.

No. slide	00376	00477	00476	00461	00545	00462 holotype	00460
Body length	303	258	265	482	486	590	515
Bucco-pharyngeal tube length	40.6	41.6	42.4	60.0	61.2	61.3	?
Buccal tube length	21.0	23.3	23.6	30.6	31.7	31.8	33.7
pbf	51.7	56.0	55.7	51.0	51.8	51.9	?
Buccal tube external width	5.2	5.6	5.7	8.7	8.8	8.9	9.5
pt	24.8	24.0	24.2	28.4	27.8	28.0	28.2
pt stylet supports insertion point	100	100	100	100	100	100	100
First placoid	?	5.2	5.2	9.3	10.7	10.9	10.6
pt	?	22.3	22.0	30.4	33.7	34.3	31.4
Second placoid	?	14.3	14.5	25.6	29.0	29.0	29.0
pt	?	61.4	61.4	83.7	91.5	91.2	86.1
Placoid row	?	20.3	20.4	34.5	40.2	40.7	40.7
pt	?	87.1	86.4	112.7	126.8	128.0	120.8
External claw I	14.5	?	15.4	21.4	21.3	?	?
pt	69.0	?	65.3	69.9	67.2	?	?
External claw I - main branch	10.4	?	11.3	15.0	15.2	?	?
pt	49.5	?	47.9	49.0	47.9	?	?
External claw I - main branch % total	71.7	?	73.4	70.1	71.4	?	?
External claw I - base + secondary branch	6.7	?	7.3	11.3	11.8	12.1	?
pt	32.2	?	30.9	36.9	37.2	38.1	?
Internal claw I	?	?	9.7	14.0	13.7	?	?
pt	?	?	41.1	45.7	43.2	?	?
Internal claw I - main branch	?	?	6.1	8.6	8.5	?	?
pt	?	?	25,8	28.1	26.8	?	?
Internal claw I - main branch % total	?	?	62.9	61.4	62.0	?	?
Internal claw I - base + secondary branch	?	6.2	6.1	9.0	9.1	10.2	?
pt	?	26.6	25.8	29.4	28.7	32.1	?
External claw II	15.9	16.9	?	?	?	29.0	?
pt	76.4	72.5	?	?	?	91.2	?
External claw II - main branch	11.5	12.2	?	?	?	20.0	?
pt	55.3	52.4	?	?	?	62.9	?
External claw II - main branch % total	72.3	72.2	?	?	?	69.0	?
External claw II - base + secondary branch	7.1	?	7.4	?	?	13.8	?
pt	34.1	?	31.3	?	?	43.4	?
Internal claw II	?	10.5	10.9	?	?	18.4	?
pt	?	45.1	46.2	?	?	57.9	?
Internal claw II - main branch	?	7.5	7.7	?	?	12.9	?
pt	?	32.2	32.6	?	?	40.2	?
Internal claw II - main branch % total	?	71.4	70.6	?	?	70.1	?
Internal claw II - base + secondary branch	?	6.6	6.3	?	?	11.2	?
pt	?	28.3	26.7	?	?	35.2	?
External claw III	?	18.1	17.4	?	?	28.6	29.0
pt	?	77.7	73.7	?	?	89.9	86.1
External claw III - main branch	?	13.0	11.8	?	18.2	19.5	19.6
pt	?	55.8	50.0	?	57.4	61.3	58.2
External claw III - main branch % total	?	71.8	67.8	?	?	68.2	67.6
pt	?	36.1	33.1	38.6	?	42.1	39.2
Internal claw III	10.3	10.9	?	?	17.4	19.3	?
pt	49.5	46.8	?	?	54.9	60.7	?
Internal claw III - main branch	?	7.3	?	?	11.4	12.7	?
pt	?	31.3	?	?	36.0	39.9	?
Internal claw III - main branch % total	?	67.0	?	?	65.5	65.8	?

No. slide	00376	00477	00476	00461	00545	00462 holotype	00460
Internal claw III - base + secondary branch	?	6.6	6.3	?	?	11.4	11.1
pt	?	28.3	26.7	?	?	35.8	32.9
Posterior claw IV	?	20.0	20.2	?	?	30.5	30.5
pt	?	85.8	85.6	?	?	95.9	90.5
Posterior claw IV - main branch	?	14.5	14.1	?	?	21.8	21.3
pt	?	62.2	59.7	?	?	68.6	63.2
Posterior claw IV - main branch % total	?	69.0	70.1	?	?	71.5	69.8
Posterior claw IV - base + secondary branch	?	9.8	10.1	14.0	?	15.3	14.7
pt	?	42.1	42.8	45.8	?	48.1	43.6
Anterior claw IV	?	10.9	11.0	16.2	?	17.6	18.1
pt	?	46.8	46.6	52.9	?	55.3	53.7
Anterior claw IV - main branch	?	7.7	7.7	11.3	?	12.1	12.2
pt	?	33.0	32.6	36.9	?	38.0	36.2
Anterior claw IV - main branch % total	?	70.6	70.0	69.7	?	68.7	67.4
Anterior claw IV - base + secondary branch	?	?	9.1	12.7	?	13.6	13.0
pt	?	?	38.6	41.5	?	42.8	38.6

of Itaquasconinae. It is possible that the presence of spiral thickening in the "buccal tube", and the lateral orientation of the caudal processes of the AISM was missed in some previously described species. In this case, any species found with these characters should be transferred into the new genus *Meplitumen,* though this may result in *Meplitumen aluna* sp. nov. becoming a junior synonym. Therefore, to avoid this remote risk, we found it opportune to compare the new species with all the similar, known species of Itaquasconinae independently from the extension of the spiral thickening.

It is unnecessary to compare all *Itaquascon* species, as the presence in *Meplitumen aluna* sp. nov. of true macroplacoids, a more developed and differently shaped stylet furca (with longer branches) (Figs 3D arrow, and 4C arrow 'a'), and more robust stylet supports (Figs 2A arrow 'b', and 4C arrow 'b'), clearly separate these species. Also, comparison with *Astatumen* species is unnecessary as the presence in *Meplitumen aluna* sp. nov. of stylet supports, true macroplacoids (lacking in *Astatumen*), and a more developed and differently shaped stylet furcae (with longer branches) (Figs 3D arrow, and 4D arrow 'a'), separates these taxa.

The differences with *Mesocrista* species are also clear, as Gąsiorek et al. (2016) confirmed the lack of spiral thickening in the buccal tube. In addition, in *Meplitumen aluna* sp. nov. the stylet supports are inserted exactly between the buccal and the pharyngel tube (Fig. 2A), whereas in the two known species of *Mesocrista* a short portion of tube without spiral thickening is present after the stylet supports insertion point (see Gąsiorek et al. 2016: fig. 2a, b, figs 5 a, b; 7c). Furthermore, in the new species the caudal processes of the stylet furcae do not have swollen apices (compare Fig. 3D arrow, and Fig. 4A arrow 'a'), the microplacoid is absent, and cuticular bars on the legs are absent.

We carefully examined *Platicrista angustata* (Murray, 1905) and ascertained the absence of spiral thickening in the buccal tube wall (Fig. 4B). In addition, *Meplitumen aluna* sp. nov. differs from *Platicrista angustata* in having thinner stylet supports (Figs 2A arrow 'b', and 4B, arrow 'b'), and thinner and longer claw secondary

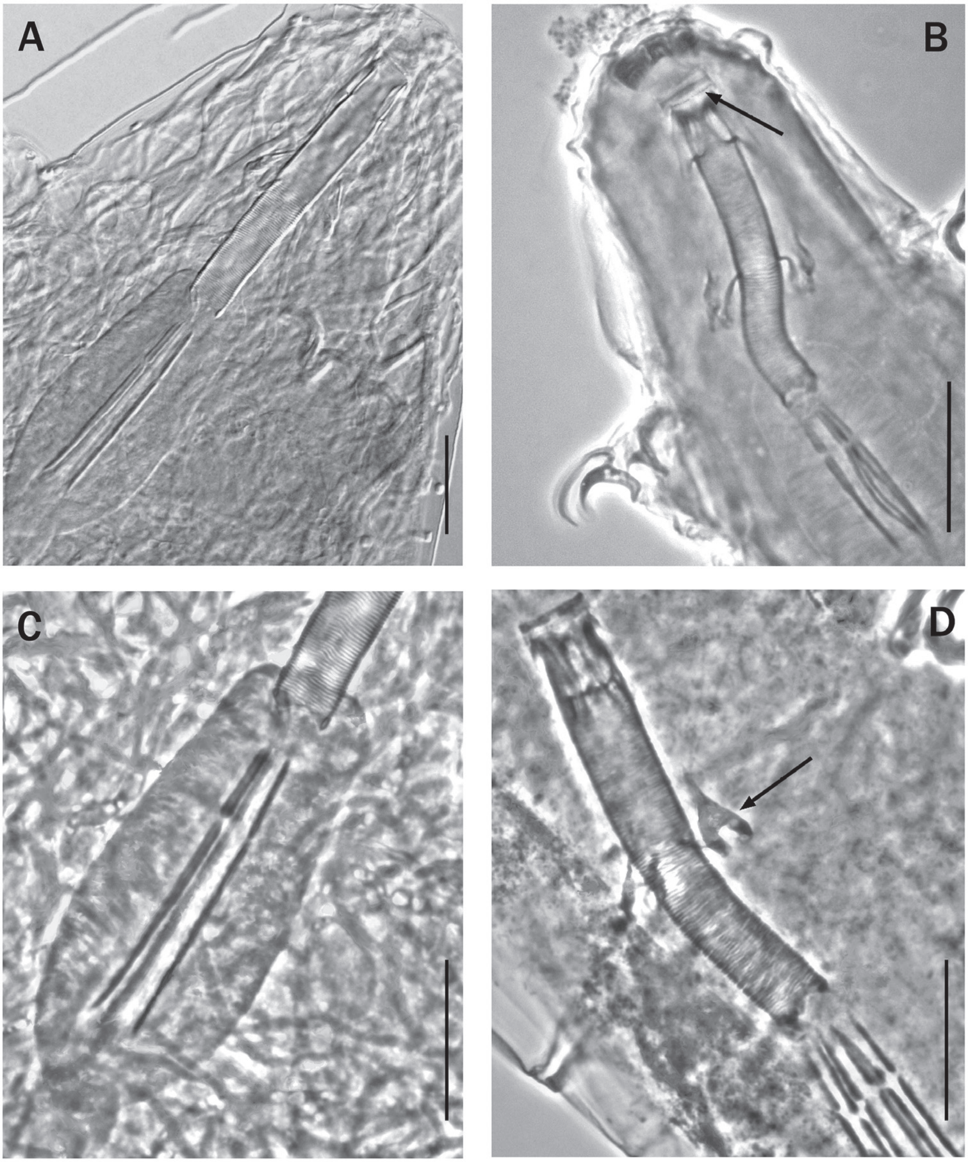

Figure 3. Bucco-pharyngeal apparatus of *Meplitumen aluna* gen. nov., sp. nov. **A** holotype **B** paratype (slide No. 00477); the arrow indicates a row of small teeth **C** detail of the pharyngeal bulb of the holotype with the long placoids well visible **D** bucco-pharyngeal apparatus of a paratype (slide No. 00461); the arrow indicates a stylet furca. Scale bar: 20 μm.

branches (compare Fig. 6A–C with 6D). The new species differs from *Platicrista cheleusis* Kathman, 1990 by the lack of a polygonal pattern on the cuticle, and cuticular bars on the legs. It differs from *Platicrista horribilis* Kaczmarek & Michalczyk, 2003 in the claw shape, and by having the lunules (if they are such) on legs IV less developed

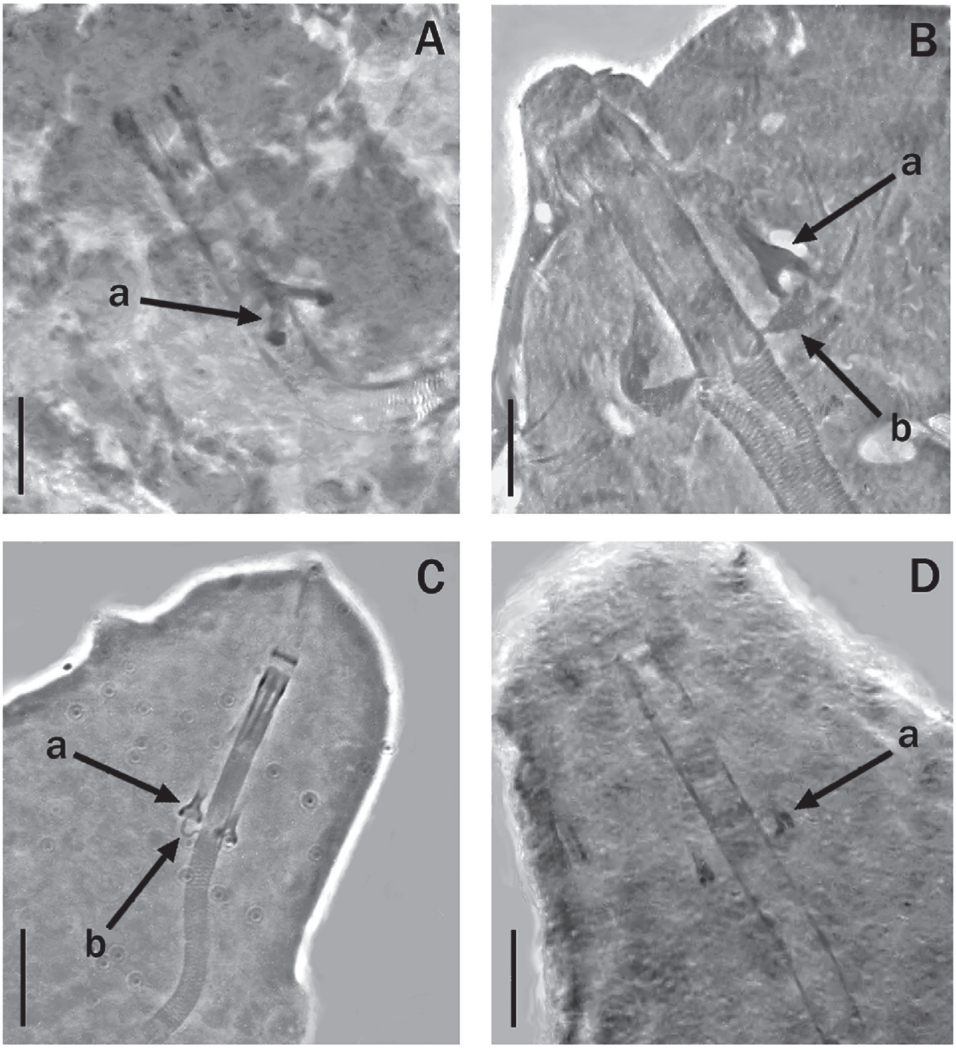

Figure 4. Shape of the stylet furca (arrow 'a') in species of various genera of Itaquasconinae **A** *Mesocrista spitzbergensis* (Richters, 1903) **B** *Platicrista angustata* (Murray, 1905) **C** *Itaquascon cambewarrense* Pilato, Binda & Claxton, 2002 **D** *Astatumen trinacriae* (Arcidiacono, 1962). Arrow 'b' indicates the stylet supports. Scale bar: 10 µm.

and without teeth. *Meplitumen aluna* sp. nov. differs from *Platicrista itaquasconoide* (Durante Pasa & Maucci, 1975) by lacking the microplacoid, and a small basal spur on the claws of the hind legs. It is distinguished from *Platicrista ramsayi* Marley, 2006 by the lack of cuticular bars on the legs.

Platicrista affine (Mihelčič, 1951) is considered a *nomen dubium* (Ramazzotti and Maucci 1983, Dastych 1993, Marley 2006) and as the material studied by Mihelčič no longer exists, a comparison is impossible.

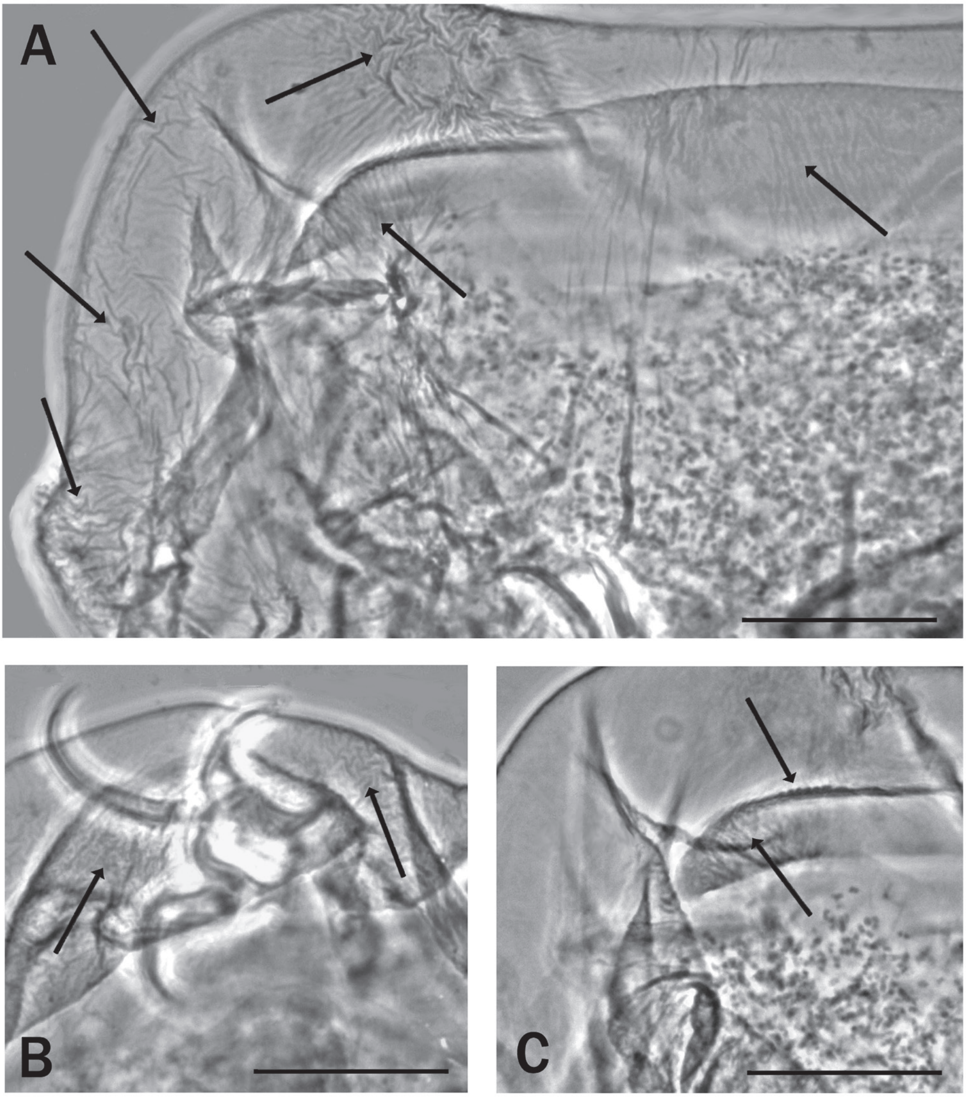

Figure 5. *Meplitumen aluna* gen. nov., sp. nov. Caudal extremity of the body where a faint roughness may be visible (arrows) **A** paratype (slide No. 00477) **B** and **C** paratype (slide No. 00460). Scale bar: 20 µm.

Phylogenetic analysis, discussion and conclusions

Preliminary discussion

The new genus *Meplitumen* is, in our opinion, particularly interesting as it possesses characters which allow us to imagine the evolutionary pathways that connect the five genera of Itaquasconinae *Mesocrista*, *Platicrista*, *Meplitumen* gen. nov., *Itaquascon* and *Astatumen* which constitute a homogeneous group inside that subfamily.

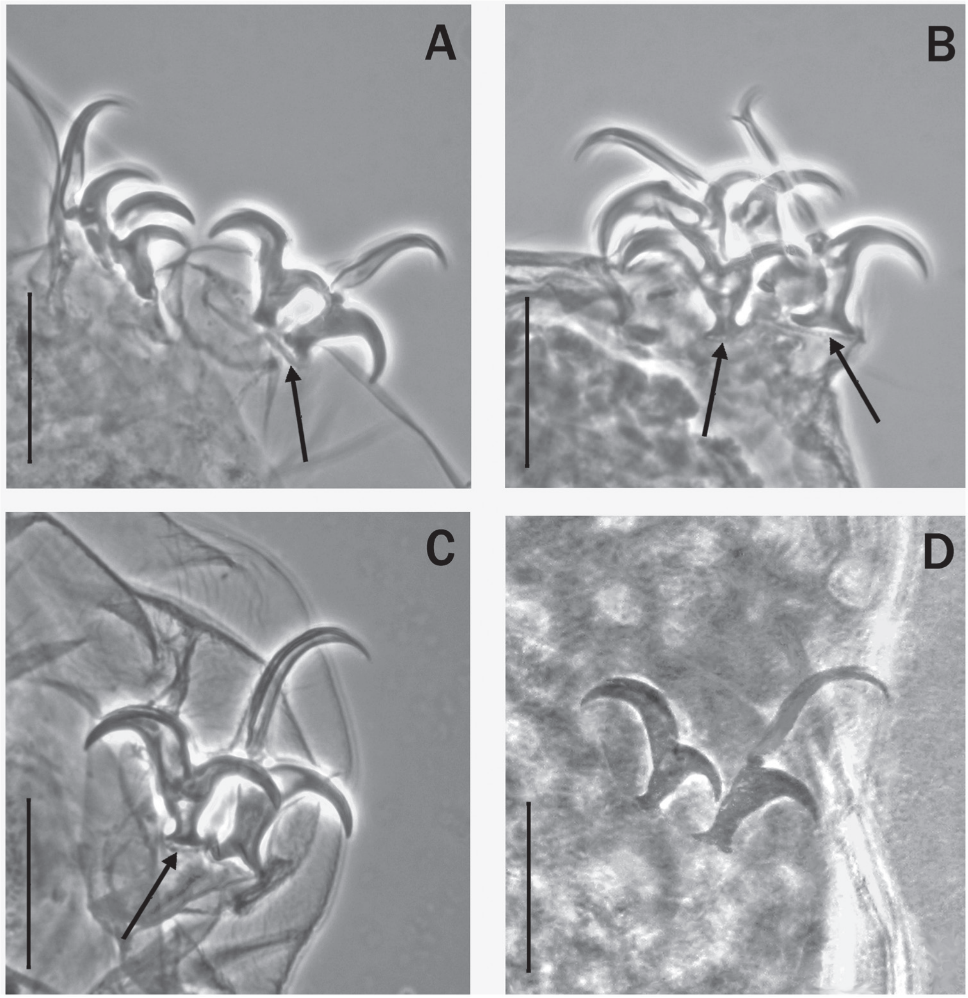

Figure 6. A–C claws of *Meplitumen aluna* sp. nov. (paratypes) **A** claws III **B** and **C** claws IV. The arrow indicates the structure interpretable as a very thin lunule **D** claws III of *Platicrista angustata* (Murray, 1905). It is evident that in *Meplitumen aluna* sp. nov. the claw secondary branches are longer and thinner than those of *Platicrista angustata*. Scale bar: 20 µm.

Bertolani et al. (2014) attributed to the subfamily Itaquasconinae the above four genera (five including our new genus), plus the genera *Adropion* Pilato, 1987; *Parascon* Pilato & Binda, 1987 and *Bindius* Pilato, 2009. Guil et al. (2014) described the genus *Sarascon*, and attributed it to the same subfamily. These additional four genera possess some characters that are similar to the five genera we have studied but differ in key characters. The AISM for *Adropion* and *Bindius* are very different being hock-shaped, very thin in dorso-ventral view and not flat. *Parascon* clearly differs in having a short pharyngeal tube without spiral thickening, and *Sarascon* has the same characters as *Parascon*.

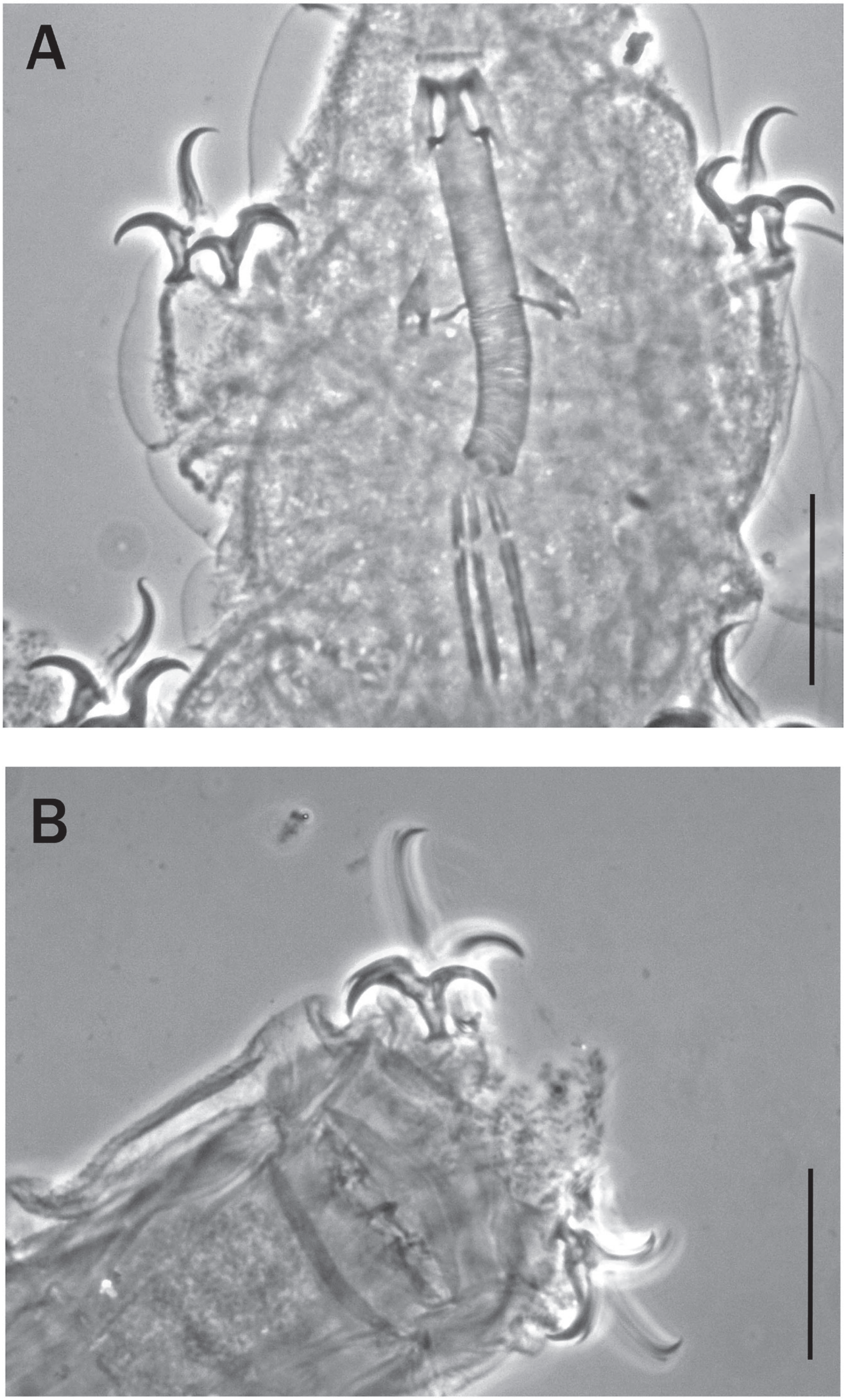

Figure 7. Small specimens (juvenile or male) of *Meplitumen aluna* sp. nov. **A** bucco-pharyngeal apparatus of a paratype (slide No. 00476) **B** claws of the hind legs of another paratype (slide No. 00477). Scale bar: 20 μm.

Above all, the absence of molecular data for *Bindius* and *Parascon* and the fact that we have not had the good fortune to examine *Sarascon*, we prefer in this paper to concentrate our attention on the five genera *Mesocrista*, *Platicrista*, *Meplitumen*, *Itaquascon* and *Astatumen* that certainly, within the family Itaquasconinae, constitute a homogeneous group.

Gąsiorek et al. (2016), in the revision of *Mesocrista*, refrained from putting forward a hypothesis about the phylogenetic relationships between the known genera *Mesocrista*, *Platicrista*, *Itaquascon*, and *Astatumen*. In their defence, they wrote (page 22), "As shown and noted above, the scarcity of molecular data for Itaquasconinae prevents a through phylogenetic analysis of the subfamily". They accepted that, "given that a close morphological similarity of many Itaquasconinae genera might suggest close ancestry, 18S rRNA, being a relatively conservative marker, may not be most suitable for resolving relationships within the subfamily". In conclusion, Gąsiorek et al. (2016) suggested that there was a need to, "test whether analyses of fragments exhibiting higher mutation rates, such as 28S rRNA and COI would produce the same topologies or would corroborate with classic taxonomy". We do not deny the importance of molecular data but we also think that, as a result of the description of *Meplitumen*, morphology already permit us to propose a hypothesis about the phylogenetic relationships between the five above mentioned genera of Itaquasconinae.

Taking into account the morphology of the Hypsibiidae, some easily recognisable evolutionary tendencies can be noticed in the group of genera we are considering.

The first regards the shape of the AISM, which tend to become wide and flat and whose caudal processes only in *Mesocrista* point postero-laterally, as in all the other Parachela, while in *Platicrista*, *Meplitumen*, *Itaquascon* and *Astatumen* they point laterally. These processes are definitely more robust in *Mesocrista* and *Meplitumen*, while they are thinner in *Platicrista*, and extremely thin, even flexible, in *Itaquascon* and in *Astatumen*.

A second evolutionary trend regards the shape and size of the stylet furcae. In *Mesocrista* only are the furca processes well developed and with swollen apices (Fig. 4A, arrow 'a'). However, the furcae general shape is not exactly the same as *Diphascon*, *Adropion*, and the other non-Itaquasconinae Hypsibiidae; the furcae body being slightly reduced in *Mesocrista*. In addition to this, in *Platicrista* (Fig. 4B arrow 'a'), and *Meplitumen* (Fig. 3D, arrow) the apices are not swollen, and in *Itaquascon* and *Astatumen* the whole processes tend to be very short or even almost missing, and as a result the entire furcae are very small (Figs. 4: C arrow 'a'; and D, arrow 'a').

A third evolutionary trend regards the stylet supports, which are normally developed in *Mesocrista*, *Meplitumen* and *Platicrista*, very thin in *Itaquascon* (Fig. 4C, arrow 'b'), and absent in *Astatumen* (Fig 4D).

The fourth evolutionary direction is that of the placoids, which are present, long and slender in *Mesocrista*, *Meplitumen*, and *Platicrista*. In *Itaquascon* and *Astatumen* these are substituted by a long, simple cuticular thickening, which can also be (or seem to be) absent in some species.

Some of these evolutionary tendencies are also recognisable in the genera *Bindius*, *Parascon* and *Sarascon* but, as mentioned above, we are not considering these three genera in this paper.

Phylogenetic analysis and its discussion

A parsimony analysis of the character matrix shown in Table 2 resulted in a best supported tree with consistence index = 0.91 and retention index = 0.83 (Fig. 8).

Mesocrista is the genus which most closely resembles the other Hypsibiidae (i.e. ancestor “0” in Fig. 8). This places it as the most basal of the group we are considering, but already some of the four discussed evolutionary tendencies have started to be expressed (i.e., AISM becoming wide and flat, slight stylet furcae reduction, placoid elongation). The gradual expression of all four evolutionary tendencies in the other four genera make them more derived. It is worth mentioning that our hypothesis about ancestor “0” of Fig. 8, is actually based on the characters present in all the other Hypsibiidae (AISM not wide and flat and with caudal processes pointing postero-laterally, ordinary stylet supports, stylet furcae and placoids), or at least in the *Diphascon*, *Adropion*, Pilatobiinae Bertolani, Guidetti, Marchioro, Altiero, Rebecchi & Cesari, 2014, and Diphasconinae Dastych, 1992 (spiral thickening present only after the stylet supports).

In choosing the proposed phylogenetic tree, we have been careful not to allow character reversals. However, the spiral thickening on the anterior buccal tube is present in two separate branches of the tree (in *Meplitumen* and *Astatumen*), which we hypothesise is not a reversal but the character appearing twice, independently, in those two branches. This is the only weak point of the proposed tree but we want to stress that any change in the tree aimed at correcting this situation invariably produces multiple reversals in other characters. This double appearance of the anterior buccal spiral thickening character in *Meplitumen* and *Astatumen* might be explained by hypothesising that their common ancestor already possessed a genetic prerequisite (to form spiral thickening) thus requiring only one, or few mutations, to express the character.

Table 2. Character matrix used for the phylogenetic analysis. AISM = apophyses for the insertion of the stylet muscles.

	AISM shape	AISM caudal process development	AISM caudal process orientation	Stylet furcae	Stylet furcae apices	Anterior tube spiral thickening	Placoids	Stylet supports
Mesocrista	moderately wide	robust	postero-lateral	“big” with developed branches	swollen	absent	present (slender)	present (normal)
Meplitumen	moderately wide	robust	lateral	“big” with developed branches	non-swollen	present	present (slender)	present (normal)
Platicrista	very wide	thin	lateral	“big” with developed branches	non-swollen	absent	present (slender)	present (normal)
Itaquascon	very wide	extremely thin, even flexible	lateral	small with reduced branches	non-swollen	absent	reduced to a thin bar or absent	present (slendered)
Astatumen	very wide	extremely thin, even flexible	lateral	small with reduced branches	non-swollen	present	reduced to a thin bar or absent	absent

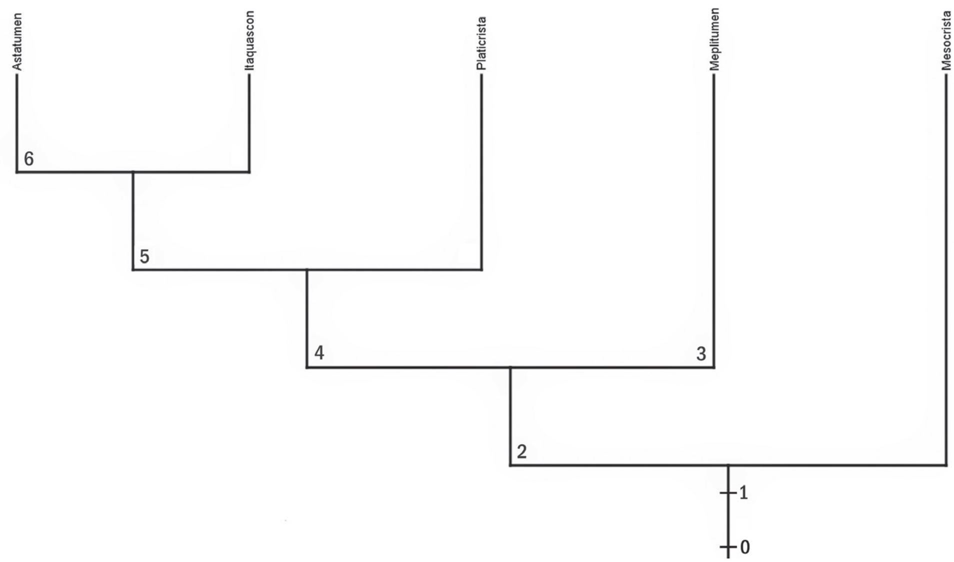

Figure 8. Most supported phylogenetic tree, according to character matrix in Table 2, of the genera *Mesocrista*, *Platicrista*, *Itaquascon*, *Astatumen* and *Meplitumen* (phylogenetic tree values: consistence index = 0.91; retention index = 0.83). **0** = Ancestor with ordinary AISM (i.e. not wide and flat and with caudal processes pointing postero-laterally), stylet supports present, spiral thickening present only after the stylet supports, ordinary stylet furcae and placoids. **1** = AISM became wide and flat, stylet furcae reduced slightly, placoids became elongated; *Mesocrista* maintained these characters. **2** = Caudal processes of the AISM pointed laterally; apices of the caudal processes of the stylet furcae became non-swollen. **3** = appearance of spiral thickening also on the anterior buccal tube; this line gave rise to *Meplitumen*. **4** = AISM became wider and their caudal processes reduced; *Platicrista* maintained these characters. **5** = Stylet furcae small and with reduced caudal processes, placoids reduced to a single, very thin, bar or absent, stylet support slendered; *Itaquascon* maintained these characters. **6** = Stylet supports disappearance, spiral thickening present also on the anterior buccal tube; this line gave rise to *Astatumen*.

In *Mesocrista* the annulation of the pharyngeal tube was described as double (Gąsiorek et al. 2016: fig. 2d). Future investigations may reveal this character is also present in *Platicrista*, *Meplitumen*, *Itaquascon*, and *Astatumen*, in which case the phylogenetic relationship would be reinforced. However, if simple annulation (non-double) were confirmed in these genera, this would not necessarily disprove the affinity. It is possible all these genera were derived from an ancestor similar to *Mesocrista* but with simple annulation, and that *Mesocrista* acquired, individually, the double annulation.

Conclusions

It is clear that in all cases, any attempt to reconstruct phylogenetic relationships requires making a hypothesis that can be tested, and which might give rise to doubts or different opinions. We think that a hypothesis can be proposed when clear characters and evolutionary tendencies are observed in known members of a taxon (for which the phylogeny is totally unknown). Such a proposed hypothesis is justified and needs to be brought to the attention of the scientific community, while awaiting new data to confirm or create a new, more convincing, phylogenetic reconstruction.

The present work also adds value to the biodiversity of Colombia, with a new species and a new genus that, at least for the moment, result endemic for the country. It is worth mentioning that we are about to publish another new genus (of a different family) from Colombia, proving the high potential of these investigations, since to date very little is known about the tardigradological fauna of the country. This encourages us to go on with our studies.

Acknowledgements

We thank Paula Sepúlveda for helping to collecting the samples; Claudia Morales and Kevin R. Roncallo for the identification of the bryophytes and lichens. This work was carried in the framework of the research projects "Diversidad de ositos de agua (Tadigrada) asociados a briófitos y líquenes epífitos en cultivos de café de la Sierra Nevada de Santa Marta, con un enfoque innovador para la apropiación social del conocimiento" and "Ositos de agua (Tardigrada) de la Sierra Nevada de Santa Marta: revisión taxonómica de la colección científica de referencia del Centro del Colecciones Biológicas de la Universidad del Magdalena 'CBUMAG'", supported by the Universidad del Magdalena (FONCIENCIAS 2015–2017 and FONCIENCIAS 2017–2019). This is a scientific contribution number 10 from de Centro de Colecciones Biológicas de la Universidad del Magdalena CBUMAG.

We are also grateful to the colleagues Roberto Bertolani and Barbara Weglarska who donated many years ago, respectively, the specimens of *Platicrista angustata* and *Mesocrista spitzbergensis* for the Binda and Pilato collection. Lastly, we want to thank the anonymous reviewers, and the editor (Sandra McInnes) for improving the quality of the manuscript.

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Appendix

The presence in *Meplitumen* of spiral thickening to almost the whole length of the bucco-pharyngeal tube gives rise to a problem of interpretation, and, as a consequence, terminology of the subdivisions of the *Astatumen* bucco-pharyngeal tube. In all tardigrades with bucco-pharyngeal tube divided into a rigid anterior portion without spiral thickening and a flexible posterior portion with spiral thickening, the spiral thickening and the stylet supports insertion point have been used to differentiate between the anterior buccal tube and the posterior "pharyngeal tube". As a consequence, in *Astatumen*, which lacks stylet supports, only the spiral thickening has been taken as the reference point, and therefore the buccal tube has been considered to be only the very anterior portion where the AISM lie. However, the distinctive situation we have encountered in *Meplitumen* made us realise that the short portion defined buccal tube in *Astatumen* does not correspond to the equivalent buccal tube of the other genera, but only to its anterior part. The absence of stylet supports in *Astatumen* prevents the location of the exact border between what in other genera is defined buccal and pharyngeal tube. Having clarified this situation it would be necessary to modify the terminology, as well as the evaluation of the length of the buccal tube, to redefine the short anterior portion of buccal tube without spiral thickening. Nevertheless, the problem of fixing the exact border between the real buccal tube and pharyngeal tube, and calculating the exact lengths, would remain unsolved. In conclusion, we find it is important to have clarified the morphological and phylogenetic meaning of these structures, but for practical reasons we think that it would not be opportune to change the terminology.

ZooKeys 865: 21–29 (2019)
doi: 10.3897/zookeys.865.35094
http://zookeys.pensoft.net

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A new species of genus *Hoplocryptus* Thomson (Hymenoptera, Ichneumonidae, Cryptinae) and a key to species from Oriental and Eastern Palaearctic regions

Xi-Nan Wang[1], Mao-Ling Sheng[2], Martin Schwarz[3]

1 *Research Institute of Forest Protection, Shandong Academy of Forestry, Jinan, Shandong 250014, China* **2** *General Station of Forest and Grassland Pest Management, National Forestry and Grassland Administration, Shenyang, Liaoning, 110034, China* **3** *Eben 21, A-4202 Kirchschlag, Austria*

Corresponding author: *Mao-Ling Sheng* (shengmaoling@163.com)

Academic editor: *Bernardo Santos* | Received 3 April 2019 | Accepted 12 June 2019 | Published 22 July 2019

http://zoobank.org/3ED6AD85-6C47-49E2-980D-008B4B7730C2

Citation: Wang X-N , Sheng M-L, Schwarz M (2019) A new species of genus *Hoplocryptus* Thomson (Hymenoptera, Ichneumonidae, Cryptinae) and a key to species from Oriental and Eastern Palaearctic regions. ZooKeys 865: 21–29. https://doi.org/10.3897/zookeys.865.35094

Abstract

A new species of Cryptinae, *Hoplocryptus qingdaoensis* Sheng, Wang & Schwarz, **sp. nov.** collected from Qingdao, Shandong Province, in the north border of oriental part of China, is described and illustrated. A key to species known from the Oriental and Eastern Palaearctic regions is provided.

Keywords

Agrothereutina, Cryptini, Eastern Palaearctic region, key, Oriental region, taxonomy

Introduction

Hoplocryptus Thomson, 1873 belongs to the tribe Cryptini of the subfamily Cryptinae (Hymenoptera: Ichneumonidae), and comprises 32 species (Yu et al. 2016), of which three are known from the Oriental Region (Chao 1976, Uchida 1931, 1940, 1956), 14 are from the Eastern Palaearctic Region (Momoi 1963, 1968, 1973, Schwarz 2007, Uchida 1936, 1952, 1956, Yu et al. 2016) (seven of them are found across the Palaearctic), 14 from the Western Palaearctic Region (Schwarz 2007, Yu et al.

2016) and nine from the Nearctic Region (Townes and Townes 1962, Yu et al. 2016). Schwarz (2007) reviewed West Palaearctic species of this genus with a key to these species. Up to now, four species of *Hoplocryptus* Thomson, *H. egregius* (Kokujev, 1909), *H. savioi* Uchida, 1940, *H. sugiharai* Uchida, 1936, and *H. tamahonis* (Uchida, 1931), have been known from China.

In the last two years the first two authors have been exploring the mountains in Qingdao (Laoshan Natural Reserve), Shandong Province, situated along the Yellow Sea in the northern border of the Oriental part of China, and have collected large numbers of ichneumonids. In this article, one new species, collected in this area is described.

Materials and methods

Specimens were collected with interception traps (IT) proposed by Li et al. (2012) in Laoshan Natural Reserve, Qingdao, Shandong Province, P.R. China.

The type specimen of *Hoplocryptus savioi* Uchida, 1940, deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China, was examined. The photos of the types of *Aritranis ohgushii* Momoi, 1963, *A. pini* Momoi, 1973, *Caenocryptus alboanalis* Uchida, 1952, *C. tamahonis* Uchida, 1931, *Hoplocryptus nigripes chinensis* Uchida, 1952, *H. sugiharai* Uchida, 1936 and *H. sumiyona* Uchida, 1956 (deposited in the Hokkaido University Museum and Museum of Nature and Human Activities, Sanda, Hyogo, Japan) taken by Dr. Kyohei Watanabe (Kanagawa Prefectural Museum of Natural History, Odawara, Japan: KPMNH), were checked and compared to the new species by the corresponding author.

Images were taken using a Leica M205A Stereomicroscope with LAS Montage MultiFocus. Morphological terminology is mostly based on Gauld (1991).

Type specimens are deposited in the Insect Museum, General Station of Forest and Grassland Pest Management (**GSFGPM**), National Forestry and Grassland Administration, People's Republic of China.

Hoplocryptus Thomson, 1873

Hoplocryptus Thomson, 1873: 508.

Type-species. *Hoplocryptus binotatulus* Thomson, 1873 (= *murarius* Börner, 1782).

Diagnosis. Ventral margin of clypeus with a more or less distinct tooth or tubercle (rarely somewhat paired). Mesoscutum with distinct punctation on a polished or subpolished background. Fore wing with sides of areolet subparallel or moderately narrowed anteriorly. Often both transverse carinae of propodeum entirely developed. Dorsolateral carina of first metasomal tergite usually distinct basal of spiracle (best seen in dorsal or dorsolateral view), its postpetiole rather weakly convex dorsally and not or only rather weakly wider than petiole. Second tergite with distinct and usually moderate sized punctures. Ovipositor compressed and its tip with rather regular and subvertical ridges on lower valve.

Remarks. This genus morphologically resembles *Aritranis* in having sides of areolet parallel or moderately convergent anteriorly, 2m-cu straight or more or less sinuate, hind wing with M+Cu moderately to strongly arched, lateral longitudinal carina of propodeum absent, first metasomal segment without a lateral tooth basally and with its spiracle at or not very far behind its mid-length; but it can be distinguished from the latter by its dorsolateral carina of first metasomal tergite usually distinct basad of spiracle (best seen in dorsal or dorsolateral view), its postpetiole rather weakly convex dorsally, ventral margin of clypeus with a more or less distinct tooth (rarely paired teeth). Hosts are aculeate Hymenoptera. *Aritranis*: dorsolateral carina of first gastral tergite absent (or more rarely indistinct) basal of spiracle, postpetiole rather distinctly convex dorsally, ventral margin of clypeus without a tooth, except in the *Aritranis nigripes* group. Hosts are Lepidoptera and Coleoptera as far as known.

Key to the species of *Hoplocryptus* known from the Oriental and Eastern Palaearctic Regions (Female only)

This key does not include *H. egregius* (Kokujev, 1909) as its female is unknown.

1 Clypeus with broad, blunt apical median tooth which has often a slight depression medially. Distance from vein 2rs-m to 2m-cu shorter than distance from 2m-cu to 3rs-m. Scutellum and median portion of hind tarsus often white. Tergites 2 and 3 red..***H. confector* (Gravenhorst)**

– Clypeus with a narrow and pointed tooth, more rarely the tooth is somewhat blunt, tooth never with a depression medially. Differ often in other characters. **2**

2 Mesopleuron, mesosternum, propodeum and first tergite black, at most with white flecks **3**

– At least parts of propodeum, mesopleuron, mesosternum, or first tergite red or reddish yellow **13**

3 Areolet convergent anteriorly. Ventral tooth of mandible as long as dorsal tooth. Tergites with dense large punctures. Mesosoma entirely black (sometimes red). Tergites 2–3(4) yellowish red...***H. heliophilus* (Tschek)**

– Not entirely as above; if areolet convergent forward, then ventral tooth of mandible distinctly longer than dorsal tooth. Tergites with weak, fine punctures. Mesosoma with yellowish white spots. Tergites with distinct white spots **4**

4 Lower tooth of mandible distinctly longer than upper tooth. Head posteriorly to eyes as seen from above strongly narrowed. Areolet convergent forward. Ovipositor tip long and comparatively low, about 4 times as long as high..... ***H. murarius* (Börner)**

– Lower tooth of mandible usually as long as upper tooth. Head posteriorly to eyes as seen from above evenly narrowed. Areolet with vein 3rs-m approximately parallel to 2rs-m (except *H. alboanalis*). Ovipositor tip relatively short **5**

5 Apical portion of dorsal valve of ovipositor (Fig. 10) with 6 small tubercles. Transverse carinae of propodeum (Fig. 7) complete and almost transversely straight. Lateral sides of face (Fig. 2) white. Posterior margins of all tergites (Fig. 8) distinctly white. Ventral profiles of hind coxae red brown, dorsal profile black........ .... ***H. qingdaoensis* Sheng, Wang & Schwarz, sp. nov.**

– Apical portion of dorsal valve of ovipositor without tubercles (except *H. ohgushii*), rarely with indistinct one or two swellings. Transverse carinae of propodeum weak, posterior carina vestigial, or median portion of posterior transverse carina strongly bended forwards. Face entirely black. Tergites almost entirely black. Hind coxae unicolor.. **6**

6 Metasomal tergites 2, 3, and hind leg black (hind femur of *H. quadriguttatus* red) **7**

– Tergites 2, 3 and hind femur reddish brown.. **12**

7 Apical portion of scutellum white. Hind femur and tibia red .... ***H. quadriguttatus* (Gravenhorst)**

– Scutellum and hind femur and tibia black (basal portion of *H. sugiharai* white).....**8**

8 Gena, mesosoma and metasomal tergites entirely covered with very dense and relatively large punctures. Hind wing vein 1-cu shorter than cu-a .... ***H. savioi* Uchida**

– Gena, mesosoma and metasomal tergites with fine, relatively sparse punctures, at least tergites 4 to 6 with indistinct, fine punctures. Hind wing vein 1-cu at least as long as cu-a **9**

9 Area basalis large, triangular. Distance between anterior transverse carina and posterior end of propodeum 3 times as long as distance from anterior transverse carina to anterior margin of area basalis. Apical portions of scutellum and first tergite and posterolateral portion of second tergite with white spots.................. .... ***H. ohgushii* (Momoi)**

– Area basalis relatively small, trapezoidal. Length between anterior transverse carina and posterior end of propodeum at least 4 times as long as distance from anterior transverse carina to anterior margin of area basalis. Scutellum, first and second tergites without white spots **10**

10 Posterior transverse carina of propodeum complete, strongly arched forward medially. Area basalis distinctly longer than its width. Fore wing with vein 1cu-a opposite 1/M. Areolet convergent forward. Hind wing vein 1-cu 2.2 times as long as cu-a.. ***H. alboanalis* (Uchida)**

– Posterior transverse carina of propodeum weak, slightly arched forward medially. Area basalis about as long as its width. Fore wing with vein 1cu-a basad of 1/M. Areolet with vein 3rs-m parallel to 2rs-m. Hind wing vein 1-cu at most 1.5 times as long as cu-a **11**

11 Clypeus with dense punctures. Area basalis distinctly trapezoidal, strongly convergent backwardly. Tegula and hind tibia entirely black .... ***H. scorteus* (Momoi)**

– Clypeus smooth, almost without punctures. Area basalis almost quadrate, nearly not convergent backwardly. Tegula and basal portion of hind tibia white .... ***H. sugiharai* Uchida**

12 Ovipositor sheath longer than hind tibia. Basal portion of clypeus with dense fine punctures. Tergites 2 and 3 with dense and large punctures. Hind coxa black ....***H. femoralis*** **(Gravenhorst)**

– Ovipositor sheath distinctly shorter than hind tibia. Clypeus shiny, basal portion with relative sparse fine punctures. Tergites with finely rugate and punctures. Hind coxa sometimes red.. ***H. coxator*** **(Tschek)**

13 Mesosoma 2.0–2.1 times as long as its maximum height. First tergite strongly arched medially. Hind coxa entirely brownish red. Posterior portions of tergites 2 and 3 with wide transverse white bands.........................***H. tamahonis*** **(Uchida)**

– Mesosoma at most 1.9 times as long as its maximum height. First tergite arched distinctly beyond its middle. Hind coxa mainly black, at least apical portion more or less darkish. Tergites 2 and 3 without white bands **14**

14 Antenna without white spot. Mesoscutum and mesopleuron usually orange. Hind coxa mainly black. Tergites 2 and 3 of metasoma almost entirely black ....***H. bellosus*** **(Curtis)**

– Antenna with white spot. Mesoscutum and at least anterior portion of mesopleuron black. Hind coxa mainly brown to reddish brown. Tergites 2 and 3 of metasoma orange to reddish brown... **15**

15 Mesoscutum with distinct punctures. Apical 0.7 of fore tibia strongly swollen. Posterior portion of mesopleuron brown....................................***H. pini*** **Momoi**

– Mesoscutum extensively granulated. Fore tibia evenly, slightly swollen apically. Mesopleuron entirely black.......................................***H. bohemani*** **(Holmgren)**

Hoplocryptus qingdaoensis Sheng, Wang & Schwarz, sp. nov.

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Figures 1–10

Etymology. The specific name is derived from the type locality.

Material examined. Holotype. Female, CHINA: Laoshan, Qingdao, Shandong Province, 12 June 2017, IT. Paratype. 1 female, same data as holotype except 26 June 2017.

Diagnosis. Propodeum rather long. Metasomal tergites 3–6 with even dense and unclear punctures. Second metasomal tergite (Figs 8, 9) slightly longer than its apical width. Ovipositor sheath 0.7 × as long as hind tibia. Apical portion of dorsal valve of ovipositor (Fig. 10) with 6 small tubercles. Face (Fig. 2) with large lateral white spots. All tergites (Fig. 8) with posterior white bands.

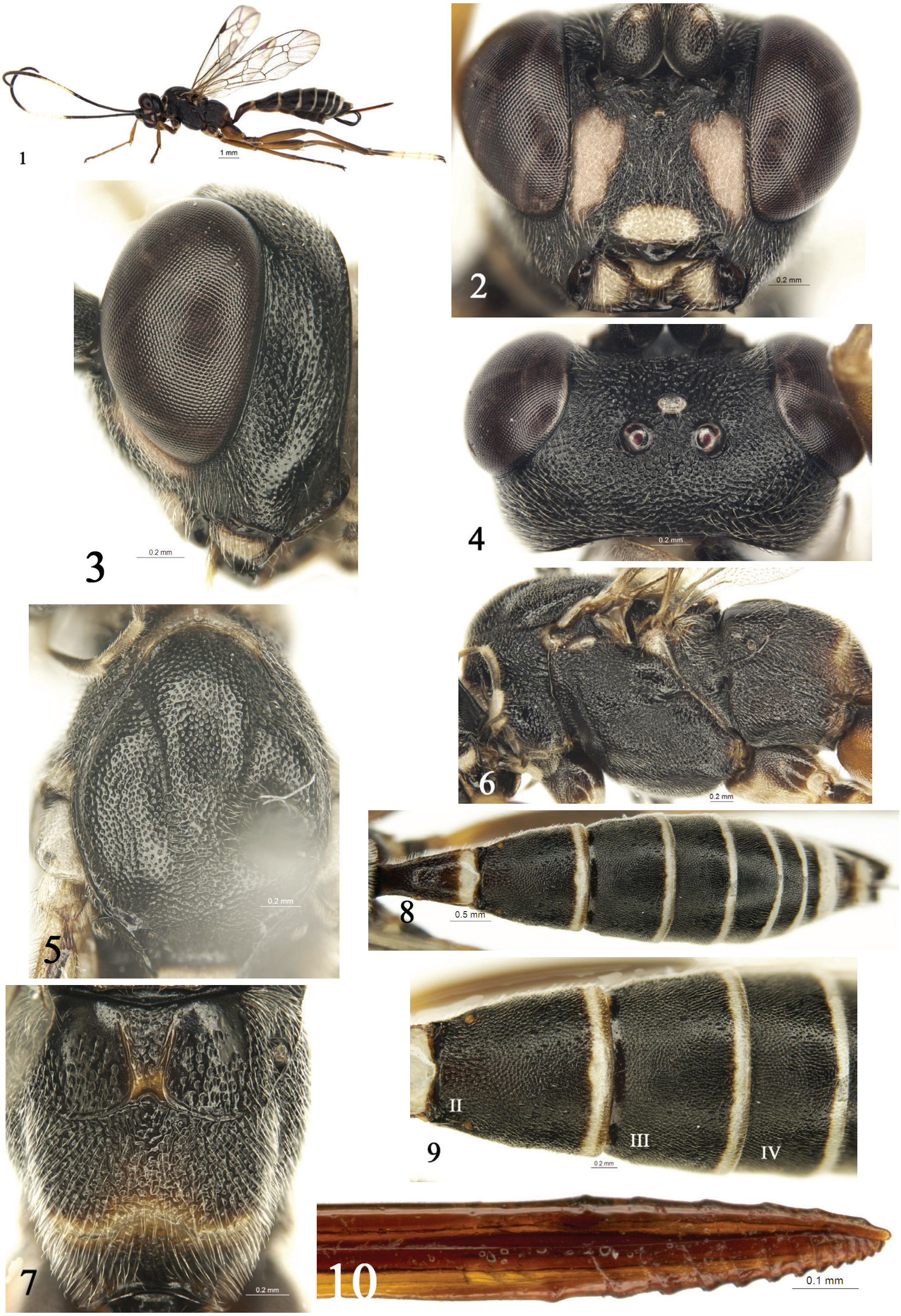
Description. Female. Body length 11.0 to 11.9 mm. Fore wing length 7.0 to 7.4 mm. Ovipositor sheath length 2.3 to 2.4 mm.

Head. Inner margins of eyes slightly convergent ventrally. Face (Fig. 2) medially slightly convex, 1.4 × as wide as long, with dense, indistinct punctures and opalescent hairs; lateral portion shagreened; upper margin with a small median tubercle. Clypeus 1.9 × as wide as long, slightly evenly convex; dorsal portion with dense punctures, ventrally smooth, ventral margin almost truncate, with a strong blunt tubercle medially (Fig. 2). Labrum almost semicircular, ventral margin with long brown hairs. Mandible with dense punctures and yellowish white hairs, upper tooth approximately as long as lower tooth. Malar area finely shagreened. Malar space 0.7 × as long as basal width of mandible. Gena with distinct punctures (Fig. 3). Vertex (Fig. 4) with dense distinct punctures. Postocellar line 0.8 × as long as ocular-ocellar line. Frons with a medio-longitudinal carina. Head behind the eyes in dorsal view weakly narrowed. Antenna with 32 flagellomeres. Ratios of lengths from first to fifth flagellomeres: 1.9:1.7:1.6:1.2:1.0. First flagellomere 8.0 × as long as wide. Occipital carina complete, reaching hypostomal carina distinctly above base of mandible. Hypostomal carina distinctly elevated.

Mesosoma. Anterior portion of pronotum with dense yellowish white hairs, lateral concavity (Fig. 6) wide and shallow, subdorsal posterior portion with oblique rugae, upper and lower portions with dense indistinct punctures. Epomia distinct. Mesoscutum (Fig. 5) shiny, with dense punctures, postero-median portion with short transverse rugae. Notaulus evident on anterior half of mesoscutum. Scutoscutellar groove wide, with weak indistinct longitudinal rugae. Scutellum slightly convex, with sparse fine punctures. Postscutellum transversely slightly convex, shiny. Mesopleuron (Fig. 6) rugose, almost flat, with dense indistinct punctures; upper anterior portion beneath subalar prominence with short indistinct transverse rugae. Epicnemial carina weak, 0.8 × as long as mesopleuron. Speculum almost smooth, with sparse fine punctures. Sternaulus 0.4 × as long as length of mesopleuron. Metapleuron (Fig. 6) evidently rugose, with dense indistinct punctures and short hairs. Hind femur 5.4 × as long as maximum width. Ratio of length of one to fifth hind tarsomeres 6.3:2.9:1.8:1.0:1.8. Wings slightly grey, hyaline. Fore wing with vein cu-a basad of Rs & M by 0.3 times length of cu-a. Areolet receiving vein 2m-cu at its middle (Fig. 1). Vein 3rs-m approximately parallel to 2rs-m. Hind wing cu-a intercepted above middle. Propodeum rather long. Anterior and posterior transverse carinae of propodeum (Fig. 7) complete, median portions weakly bent forward. Area basalis shiny, lateral carinae distinct, strongly convergent caudally. Area externa shiny, anterior portion smooth, posterior portion with distinct punctures. Rest portions of propodeum densely irregularly reticulate, with dense short grey-white hairs. Propodeal spiracle small, almost circular.

Metasoma (Figs 8, 9). First tergite 2.2 × as long as apical width; petiole and anterior portion of postpetiole almost shiny, with fine punctures; apical portion of postpetiole smooth, shiny, posteromedian portion distinctly convex. Median dorsal carina almost reaching to spiracle; dorsolateral and ventrolateral carinae complete. Tergites 2 to 6 with even dense distinct punctures. Second tergite (Figs 8, 9) slightly longer than its apical width. Third tergite 0.6 × as long as apical width. Ovipositor sheath 0.7 × as long as hind tibia. Ovipositor (Fig. 10) compressed; apical portion of dorsal valve with 6 small tubercles; apical portion of ventral valve with 13 edges.

Coloration (Fig. 1). Black, except for the following. Large lateral spots of face, main portion of clypeus, labrum, median portion of mandible, median portions of maxillary and labial palpi, ventral profiles of flagellomeres (5)6 to 10(11), dorsoanterior portion of pronotum, scutellum, postscutellum, tegulae, subalar prominence, apex of fore coxa, ventral profile of mid coxa, apical half of hind tarsomere 1, hind tarsomeres 2–4, basal half of hind tarsomere 5, apical bands of all tergites white. Apical portions of fore femur, fore tibia and tarsus, ventral profiles of mid and hind femora



Figures 1–10. *Hoplocryptus qingdaoensis* Sheng, Wang & Schwarz, sp. nov. Holotype, female **1** habitus, lateral view **2** head, anterior view **3** head, lateral view **4** head, dorsal view **5** mesoscutum **6** mesosoma, lateral view **7** propodeum **8** metasoma, dorsal view **9** second to fourth tergites **10** apical portion of ovipositor, lateral view.

and tibiae, ventral profiles of hind coxae red brown. Basal portion of fore femur, dorsal profiles of mid and hind femora, tibiae, hind coxa, apical portion of hind tibia brownish black. Lateral profile of first tergite slightly brown. Pterostigma and wing veins blackish brown.

Remarks. This new species is similar to *Hoplocryptus alboanalis* (Uchida, 1952) by the characters: Clypeus with a strong smooth blunt tubercle medially; notaulus strong on anterior half of mesoscutum. anterior and posterior transverse carinae of propodeum complete; ventral valve of ovipositor with strong edges; mesosoma almost entirely black; median portion of flagellum, at least ventral profiles, white; and can be distinguished from the latter by the following combination of characters: Fore wing vein cu-a basad of Rs & M by about 0.3 times length of cu-a. Posterior transverse carina of propodeum (Fig. 7) evenly weakly bent forward. Face with large lateral white spots. Clypeus largely white. Ventral profile of hind coxa red brown, dorsal brownish black. Posterior portions of tergites with relative wide transverse white bands. *Hoplocryptus alboanalis*: Fore wing vein cu-a opposite Rs & M. Posterior transverse carina of propodeum almost sharply bent forward. Face, clypeus and hind coxa entirely black. Tergites entirely black.

Acknowledgements

The authors are deeply grateful to Drs Bernardo Santos (Department of Entomology, National Museum of Natural History, Washington, USA), Kyohei Watanabe (KPMNH), and Seung-Ho Oh (Research and Planning Division, National Science Museum, Republic of Korea) for reviewing this manuscript. The authors are also indebted to Drs Kyohei Watanabe (KPMNH) for taking the photos of *Hoplocryptus* types deposited in Japan, Kui-Yan Zhang (Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China) and Masahiro Ohara (Hokkaido University Museum, Hokkaido University, Japan) for their help while the corresponding author was working in their respective museums. This research was supported by the National Natural Science Foundation of China (NSFC, No. 31501887, No. 31310103033) and Forestry Science and Technology lnnovative Program of Shandong (LYCX09-2018-44).

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ZooKeys 865: 31–38 (2019)
doi: 10.3897/zookeys.865.34532
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New record of a parasitising species of *Hydrachna* (Acari, Hydrachnidia) on water beetles *Eretes griseus* (Fabricius, 1781) (Coleoptera, Dytiscidae, Dytiscinae, Eretini)

Elham Arjomandi[1], Andrzej Zawal[2], Hamidreza Hajiqanbar[1],
Ewa Filip[3], Magdalena Szenejko[4]

1 *Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, 14115-336, Tehran, Iran* **2** *Department of Invertebrate Zoology and Limnology, University of Szczecin, Waska 13, 71-415 Szczecin, Poland* **3** *Department of Molecular Biology and Cytology University of Szczecin, Waska 13, 71-415 Szczecin, Poland* **4** *Department of Ecology and Environmental Protection, University of Szczecin, Waska 13, 71-415 Szczecin, Poland*

Corresponding author: *Andrzej Zawal* (andrzej.zawal@usz.edu.pl)

Academic editor: *Vladimir Pesic* | Received 14 March 2019 | Accepted 28 June 2019 | Published 22 July 2019

http://zoobank.org/F32C0055-74B3-4F76-BBE6-2F7F44729227

Citation: Arjomandi E, Zawal A, Hajiqanbar H, Filip E, Szenejko M (2019) New record of a parasitising species of *Hydrachna* (Acari, Hydrachnidia) on water beetles *Eretes griseus* (Fabricius, 1781) (Coleoptera, Dytiscidae, Dytiscinae, Eretini). ZooKeys 865: 31–38. https://doi.org/10.3897/zookeys.865.34532

Abstract

The larvae of water mites of the genus *Hydrachna* parasitise water bugs and water beetles. Larvae of the genus *Hydrachna* attach to the thorax and abdomen sternites and tergites under the elytra. Up to now six species of *Hydrachna* were recorded from Iran, but there are no records on larvae parasitising on water beetles. There is some information about parasitising of *Hydrachna* on water beetles from the genus *Eretes*, which is very well adapted to dry climate. The aim of this paper is to describe the morphology of an unknown larva of the genus *Hydrachna*, found on *Eretes griseus*.

Keywords

Iran, larva, morphological features, taxonomic status, water beetles, water mites

Introduction

Many organisms are dependent on a living host for some part of their life cycle or even the whole life. These symbiotic relationships categorised as mutualistic, commensal or parasitic, while specific specialisation occurs in commensalism and mutualistic symbionts, higher levels of co-evolution can be found in parasitic relationships. Among different groups of mites, larval stages of the cohort Parasitengonina parasitise a wide range of arthropods including terrestrial, freshwater or marine insects (Zawal 2003a; Baker et al. 2008; Normant et al. 2013; Mortazavi et al. 2018).

Beetles of the genus *Eretes* are specifically adapted to desert environments and a dry climate, where they can find small and isolated, warm, standing, water sources such as pools with clayey or sandy bottom and little vegetation (Hájek et al. 2014; Miller 2002). The water mites of subcohort Hydrachnidiae are well known as parasites of water beetles. Larval instars of the genus *Acherontacarus* Angellier attach to the mesosternal region of their host beetles (Aykut et al. 2018), larvae of the genus *Hydrachna* Muller attach to thorax and abdomen sternites and tergites under the elytra (Zawal 2002), while larvae of genus *Eylais* Latreille mostly hide under the beetle's elytra (Zawal 2003b). In this study, we found three larvae of *Hydrachna* sp. attaching to the mesosternal area of the dytiscid host beetle *Eretes griseus* (Fabricius, 1781) (Fig. 2).

Material examined

The larvae were collected as parasites on *Eretes griseus* from a volcanic area on a mountainside, nearly 1,840 meters above the sea level, Badab-e Soort) 36.3549N, 53.8565E (in Mazandarn province, northern Iran (Fig. 1). This natural site comprises two mineral hot springs, one with sour and the other with salty water. Over thousands of years, flowing water from these springs has formed numbers of red, orange, and yellow staircase pools each filled with some amount of mineral water. We collected the host beetle with a net from one of the lower pools as it was swimming.

All small larvae (0.15–0.40 mm) were detached from surfaces of the mesosternum of the beetle body (Fig. 2). They were cleared with lactic acid and mounted in Hoyer's medium. Morphological observations, measurements, and illustrations were made using compound microscopes (Zeiss Axio Scope.A1) equipped with phase contrast optical systems and a camera lucida (Olympus BX51).

Idiosomal setae are named according to Prasad and Cook (1972):

Cx-1-3	coxal plates,
Hu	humeral seta,
L	length,
Lp1, Lp2	lateropropodosomal setae,
Lh3	laterohysterosomal seta,
Mh1, Mh2, Mh3, Mh4	mediohysterosomal setae,

Figure 1. Photographs of sampling site.

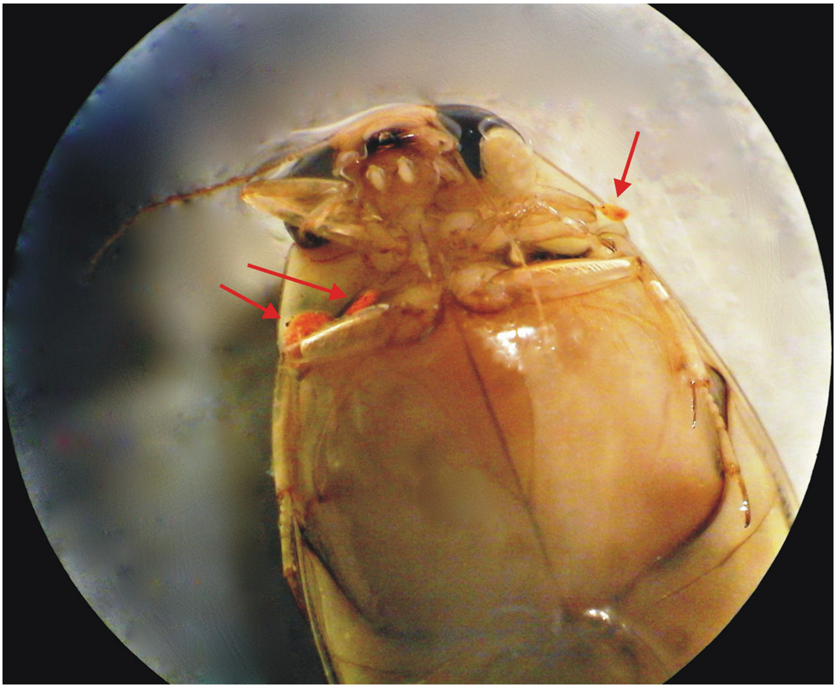


Figure 2. Larvae of *Hydrachna* sp. attached to *Eretes griseus.*

Mp1, Mp2	mediopropodosomal setae,
n	number of specimens measured,
P–1–5	pedipalp segments (trochanter, femur, genu, tibia and tarsus),
I–Leg-1–5	first leg, segments 1–5 (trochanter, femur, genu, tibia and tarsus) i.e.,
III– Leg–3	genu of third leg,
W	width.

All measurements are given in micrometres (μm).

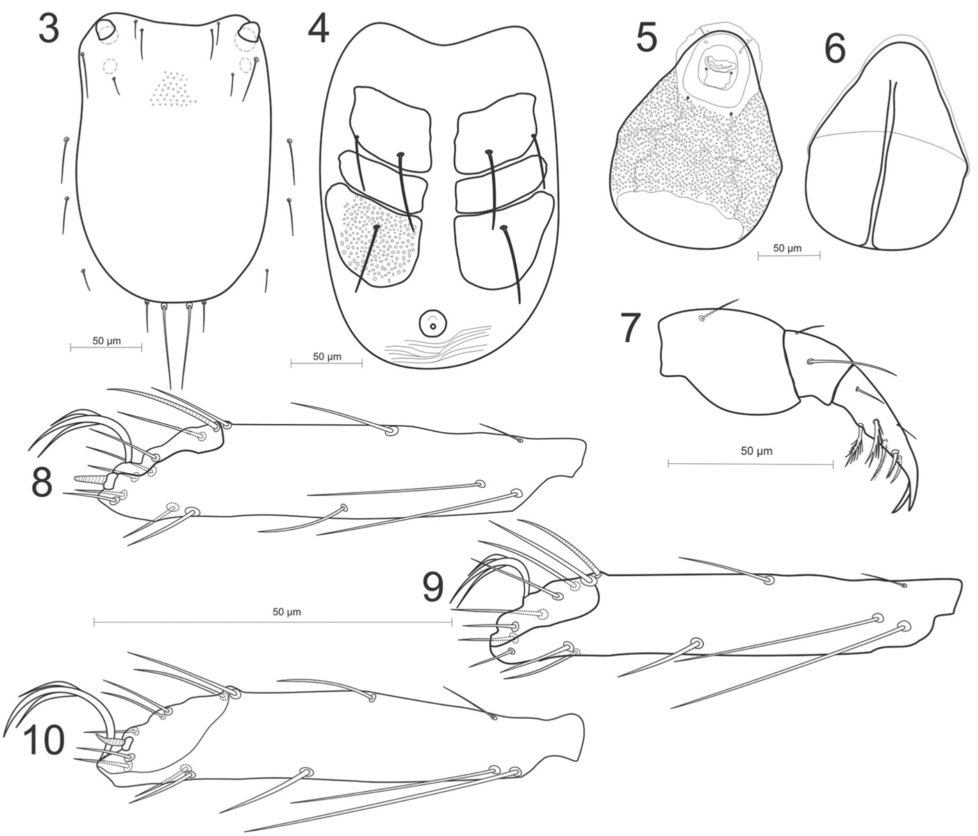
Taxonomic account

Superfamily HYDRACHNOIDEA
Family Hydrachnidae Leach, 1815
Genus *Hydrachna* Müller, 1776

Hydrachna sp.
Figs 3–11

Description. The idiosoma are oval, with the integument striated, and the dorsal plate is very large, covering the whole idiosoma of unengorged larva, the integument pointed and with a concave anterior edge (Figs 3, 11). There are four pairs of setae on the dorsal plate (Mp1, Lp1, Lp2, Hu). The basal bodies of Mp2 on dorsal plate invisible; setae Mh1, Mh2, Mh3 located on soft integument (Fig. 4). There are three pairs of coxal plates located on the proximal half of the idiosoma, and all are wider than long. Median edges of coxa I and III almost the same length and two time longer then coxa II. The anterior coxa bears two setae, the medial coxa is without seta, and the posterior coxa has one seta. The excretory pore plate is very large and is located behind of coxal plates (Figs 5, 11). Gnathosoma short, strongly tapering forward; gnathosomal sucker large, discoid with corrugated borders (Figs 5, 6). Pedipalps relatively short and thin: femur stocky with strongly convex ventral margin and one seta; genu with two setae and concave ventral margin; tibiotarsus relatively long with two claws the same size, weakly bent, five tibiotarsal spines, four of them pinnate (Fig. 7). Trochanters of all legs with one seta, all femora with four setae and with one swimming seta on I and II and two swimming setae on III femora. Genu I with five setae including two swimming setae, genu II and III with four setae including one swimming seta. All tibiae with five setae including one swimming seta, and with one solenidium. Tarsi each have 14 setae including two swimming setae, tarsi I and II have one solenidium, and tarsi I and III have one eupathidium (Figs 8–10).

Measurements. In μm, n = 3. Dorsal plate: L/W 250–254/162–157; coxal plates: Cx-1 L 40–45, Cx-2 L 20–22, Cx-3 42–44; excretory pore plate L/W 17–18/16–17; gnathosoma; L/W 173–176/138–140; diameter of sucker ring 71–73; pedipalpal



Figures 3–10. *Hydrachna* sp. **3** dorsal plate **4** ventral side **5** gnathosoma ventral side **6** gnathosoma dorsal side **7** pedipalp **8** I-leg-5 **9** II-leg-5 **10** III-leg-5.

segments (P-1–3) L: 8–9, 36–38, 39–42; leg segments L: I-leg 1–5: 18–19, 37–39, 32–34, 38–40, 67–69; II-leg 1-5: 20–21, 32–34, 29–30, 37–39, 68–70; III-leg 1-5: 28–29, 29–30, 27–28, 38–40, 61–64.

Remarks. The larva of *Hydrachna* sp. is most similar to larvae of *H. processifera* described by Wainstein (1980) as a *H. inermis* (Aykut et al. 2018). It is similar in the shape of coxal plates, the discoidal hypostomal sucker, the tibiotarsus relatively long with two claws the same size, weakly bent; five tibiotarsal spines the same size. It is different by the presence of a eupathidium on tarsus leg-2; localisation the Mh1, Mh2, and Mh3 setae outside of the dorsal plate on soft integument, and the presence of a very large excretory pore plate. The last two features are very strange and different from all other species of *Hydrachna*. These differences indicate the probability of a separate subgenus to which the described larva would belong.

Thor (1916) split the genus *Hydrachna* into five subgenera: *Hydrachna* s. str., *Anahydrachna*, *Diplohydrachna*, *Schizohydrachna*, and *Monochydrachna*; subsequently he synonymised *Monochydrachna* with *Hydrachna* s. str., and *Schizohydrachna* with *Diplo-*

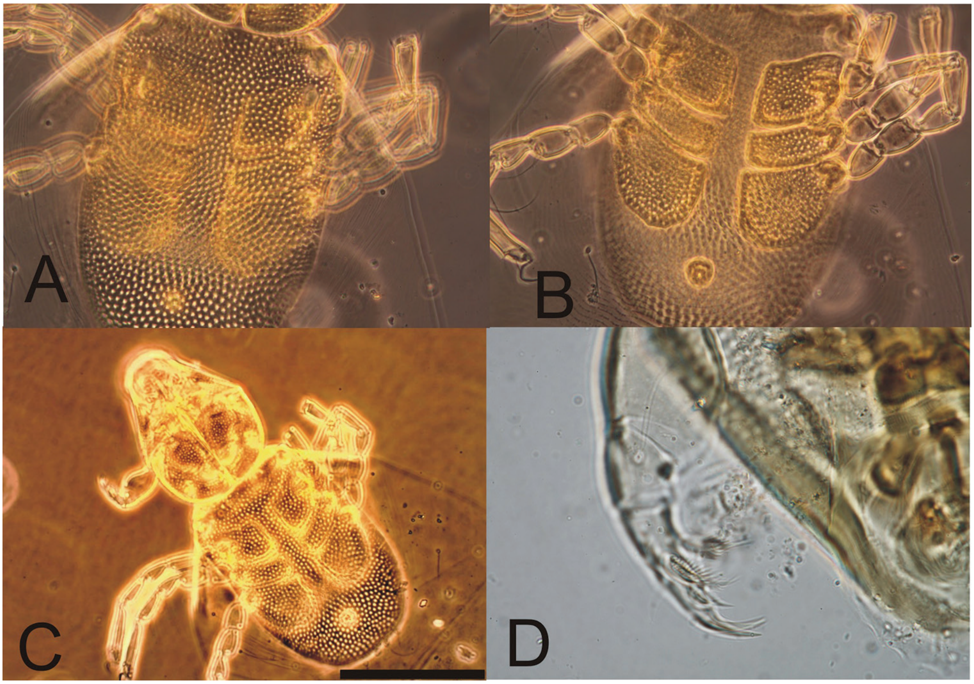


Figure 11. Photographs of *Hydrachna* sp. from *Eretes griseus* **A** dorsal plate **B** ventral side **C** dorsal view **D** pedipalp.

hydrachna, and established two more subgenera: *Rhabdohydrachna* and *Scutochydrachna* (Thor 1925). Davids et al. (2007) stated the differences between these subgenera were not clear and he abolished the division into subgenera.

At the current level of research, we propose to leave the taxonomy of the genus *Hydrachna* without sub-division, indicating the existence of greater morphological differentiation. Relationships within the genus of *Hydrachna* should be recognised on the basis of molecular studies and a decision on the possible splitting the genus into subgenera should be made. Up to now six species of *Hydrachna* were recorded from Iran (*H. cruenta*, *H. skorikowi*, *H. sepasgozariani*, *H.* cf. v*aillanti*, *H. sistanica*, *H. globosa lacerata*), and two of them (*H. sepasgozariani*, *H.* cf. v*aillanti*) belong to the *Hydrachna processifera* group of species (Pešić and Saboori 2007; Pešić et al. 2012, 2014). Larvae were described only for *H. cruenta*, *H. skorikowi*, and *H. globosa* (Wainstein 1980). The morphology of this larva and its parasitism on Dytiscidae show plausible grounds for it belonging to the *H. processifera* group of species and possibly to one of the two species from Iran (*H. sepasgozariani* or *H.* cf. v*aillanti*) for which the larvae are still not described. On the other hand, the differences in morphology (localisation the Mh1, Mh2, Mh3 setae outside of dorsal plate, on soft integument and very large excretory pore plate) indicate that it could belong to another species.

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ZooKeys 865: 39–65 (2019)
doi: 10.3897/zookeys.865.34265
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A new leaf-mining moth from New Zealand, *Sabulopteryx botanica* sp. nov. (Lepidoptera, Gracillariidae, Gracillariinae), feeding on the rare endemic shrub *Teucrium parvifolium* (Lamiaceae), with a revised checklist of New Zealand Gracillariidae

Robert J.B. Hoare[1], Brian H. Patrick[2], Thomas R. Buckley[1,3]

1 *New Zealand Arthropod Collection (NZAC), Manaaki Whenua–Landcare Research, Private Bag 92170, Auckland, New Zealand* **2** *Wildlands Consultants Ltd, PO Box 9276, Tower Junction, Christchurch 8149, New Zealand* **3** *School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

Corresponding author: *Robert J.B. Hoare* (hoarer@landcareresearch.co.nz)

Academic editor: *E. van Nieukerken* | Received 4 March 2019 | Accepted 3 May 2019 | Published 22 Jul 2019

http://zoobank.org/C1E51F7F-B5DF-4808-9C80-73A10D5746CD

Citation: Hoare RJB, Patrick BH, Buckley TR (2019) A new leaf-mining moth from New Zealand, *Sabulopteryx botanica* sp. nov. (Lepidoptera, Gracillariidae, Gracillariinae), feeding on the rare endemic shrub *Teucrium parvifolium* (Lamiaceae), with a revised checklist of New Zealand Gracillariidae. ZooKeys 965: 39–65. https://doi.org/10.3897/zookeys.865.34265

Abstract

Sabulopteryx botanica Hoare & Patrick, **sp. nov.** (Lepidoptera, Gracillariidae, Gracillariinae) is described as a new species from New Zealand. It is regarded as endemic, and represents the first record of its genus from the southern hemisphere. Though diverging in some morphological features from previously described species, it is placed in genus *Sabulopteryx* Triberti, based on wing venation, abdominal characters, male and female genitalia and hostplant choice; this placement is supported by phylogenetic analysis based on the COI mitochondrial gene. The life history is described: the larva is an underside leaf-miner on the endemic divaricating shrub *Teucrium parvifolium* (Lamiaceae), and exits the mine to pupate in a cocoon in a folded leaf of the host plant. The remarkable history of the discovery and rediscovery of this moth is discussed: for many years it was only known from a single sap-feeding larva found in a leaf-mine in a pressed herbarium specimen of the host. The adult was discovered by BHP in Christchurch Botanic Gardens in 2013. Most distribution records of the moth come from a recent search for mines and cocoons on herbarium specimens of *T. parvifolium*. *Sabulopteryx botanica* has high conservation status, and is regarded as 'Nationally Vulnerable' according to the New Zealand Department of Conservation threat classification system, based on the rarity and declining status of its host plant. However, the presence of apparently thriving populations of *S. botanica* on cultivated plants of *T. parvifolium*, especially at the type locality, Christchurch Botanic Gardens, suggests that encouraging cultivation of the plant could greatly improve the conservation status of the moth. A revised checklist of New Zealand Gracillariidae is presented, assigning all species to the currently recognised subfamilies. The Australian *Macarostola ida* (Meyrick, 1880) is newly recorded from New Zealand (Auckland), where it is established on *Eucalyptus*.

Keywords
New species, taxonomy, New Zealand, leaf miners, herbarium, checklist

Introduction

New Zealand has a relatively depauperate fauna of the leaf-mining moth family Gracillariidae: revision and further field work can be expected to increase the number of species, but the fauna is probably relatively well known and genuinely species-poor. Dugdale (1988: 70–72) listed 21 named species, and mentioned two unnamed species. Hoare (2001) added two further adventive species, *Dialectica scalariella* (Zeller, 1850) and '*Acrocercops*' *laciniella* (Meyrick, 1880). The Australian *Macarostola ida* (Meyrick, 1880) was discovered established in Auckland and Northland on planted *Eucalyptus* in January 2019. A further two unnamed endemic species have also been recognised since Dugdale's catalogue, one of which is described in this paper. From this total of 28 species, 22 are endemic to New Zealand, five are adventive from Australia, and one (*Phyllonorycter messaniella* (Zeller, 1846)) is adventive from Europe. Kawahara et al. (2017) recently presented a molecular phylogeny and revised subfamily classification of world Gracillariidae, recognising eight monophyletic subfamilies. In their recent checklist of neotropical Gracillariidae, De Prins et al. (2019) largely followed this revised classification, but reduced Parnornichinae to the status of a tribe (Parornichini) within Gracillariinae and regarded Oecophyllembiini and Marmarini as tribes within an expanded Phyllocnistinae. The classification of De Prins et al. (2019) is consistent with the phylogeny of Kawahara et al. (2017), but the change in ranks was introduced without explicit justification. We note that the nodes supporting the more narrowly defined subfamilies of Kawahara et al. (2017: fig. 2) have stronger bootstrap support values than those supporting each of the expanded subfamilies of De Prins et al. (2019), indicating that the former classification is likely to be more stable, as well as being simpler. We therefore follow the Kawahara et al. (2017) classification here.

Given these recent changes and discoveries, we present an updated New Zealand Gracillariidae checklist here (Appendix 1) and assign the named species as far as possible to the newly defined subfamilies; further changes can be expected once the fauna is revised.

The new species described here was first detected as an early instar larva pressed inside its linear leaf-mine in a herbarium specimen of *Teucrium parvifolium* (Hook. f.) Kattari & Salmaki (Lamiaceae). This larva was collected in the southern North Island at Awahuri Reserve near Feilding WI by the botanist Alan E. Esler on 23 December 1961; it was shown to H. Donner and C. Wilkinson when they were revising the New Zealand Nepticulidae fauna (Donner and Wilkinson 1989). Those authors recognised that the larva was not a nepticulid based on the lack of a spinneret and the lack of anal rods, but they did not assign it to another family. Following this discovery, BHP searched unsuccessfully for mines on *T. parvifolium* at Trotter's Gorge DN, where the plant is common (Donner and Wilkinson 1989). The Awahuri Reserve specimen is still in the ethanol collection in NZAC, and was examined in 1998 by RJBH, who determined it as a sap-feeding early instar gracillariid larva. The associated pressed specimen of *Teucrium parvifolium* from the Esler collection is now in the Auckland Museum herbarium (AK362379; Fig. 1) and was examined by RJBH in June 2018: no further mines were found on this specimen.

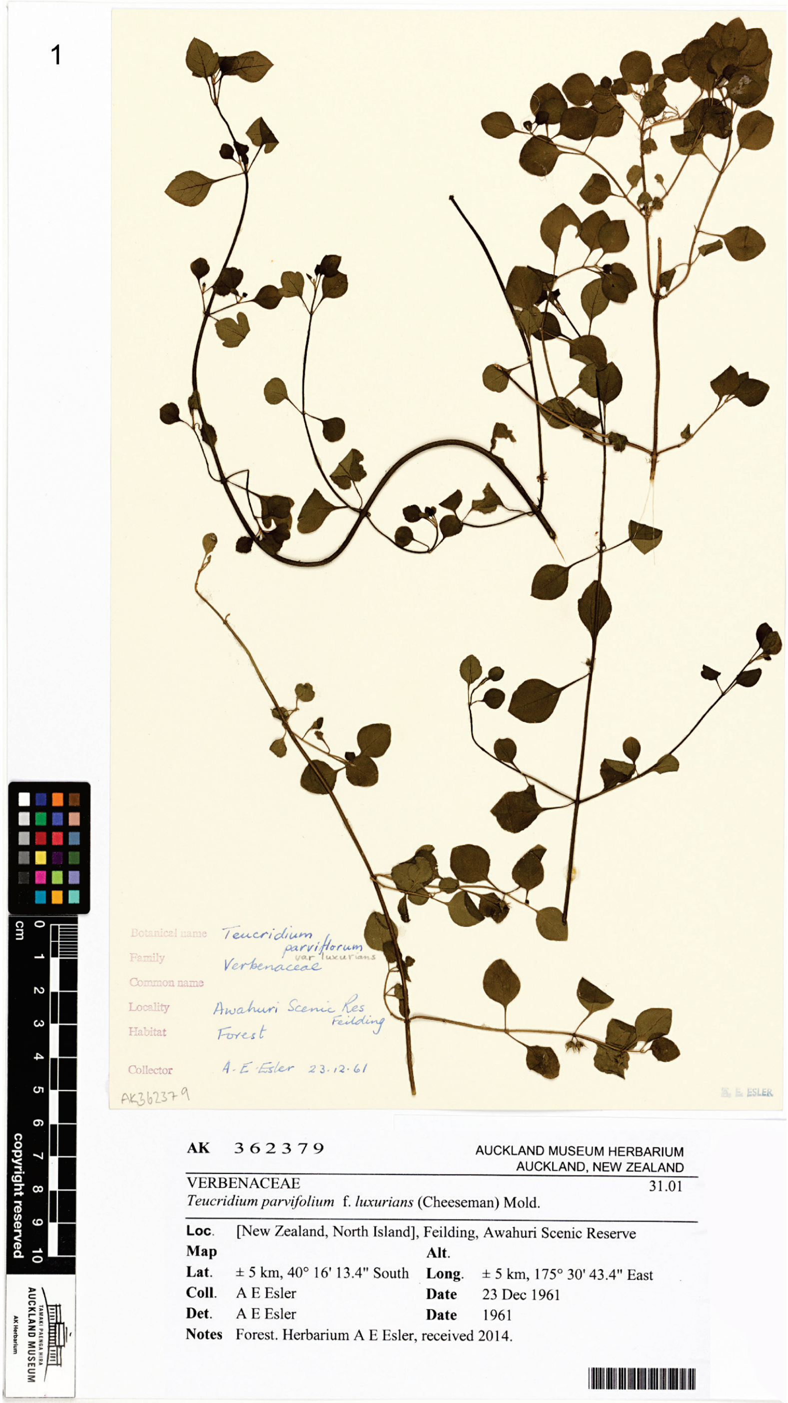
In September 1999, RJBH searched unsuccessfully for *Teucrium* plants at Awahuri Reserve in company with Grace Hall (NZAC). In December 2000, flowering *Teucrium* plants were located at Carters Scenic Reserve near Carterton, WA, but no mines or other damage were discovered. The miner was finally rediscovered on 31 January 2004, when Nicholas Martin collected a sprig of *T. parvifolium* at Paengaroa Scenic Reserve, Mataroa, near Taihape RI, with two mined leaves. He discovered a further mine in a *Teucrium* leaf at Price's Valley, Banks Peninsula MC on 17 August 2005. These pressed mines are in NZAC; no moths were reared.

The matter rested there until 29 January 2013, when BHP disturbed an adult gracillariid from a plant of *Teucrium parvifolium* during a lunchtime walk in the Christchurch Botanic Gardens (Patrick 2018). Further searches revealed the moth to be common amongst its host, and mines and larvae were soon discovered and reared through, confirming that this was the mystery species known for over 50 years only from pressed leaves. Later, the moth was discovered on indigenous remnants of the host plant on Rakaia Island, south-west of Christchurch (Patrick and Grove 2015), rediscovered in Price's Valley, and also found on *Teucrium* amongst the restored native vegetation planted by botanist Carol Jensen at Kennedy's Bush, near Halswell Quarry, Christchurch. Searches of herbarium specimens in the Allan Herbarium (Lincoln) and the Auckland Museum herbarium in 2018 revealed a number of further leaf-mines from almost throughout the plant's range (see Distribution and Remarks under species description below).

The moth is described as new here and assigned to the genus *Sabulopteryx* Triberti, 1985, in the subfamily Gracillariinae (in the sense of Kawahara et al. 2017). *Sabulopteryx* was described as a subgenus of *Aspilapteryx* Spuler, 1910, but removed from synonymy by Pereira et al. (2019) (see under Systematic placement, below). This is the first record of the genus from the southern hemisphere.

Materials and methods

Specimens were reared for this study by enclosing twigs with mined leaves of the host plant in plastic containers lined with absorbent paper. Leaves of *Teucrium parvifolium* dry out readily when picked and care must be taken to maintain sufficient moisture while avoiding mould. Genitalia and wing venation preparations followed the meth-



Botanical name Teucridium parviflorum var luxurians
Family Verbenaceae
Common name
Locality Awahuri Scenic Res Feilding
Habitat Forest
Collector A. E. Esler 23.12.61
AK362379

AK 362379
AUCKLAND MUSEUM HERBARIUM
AUCKLAND, NEW ZEALAND
VERBENACEAE 31.01
Teucridium parvifolium f. *luxurians* (Cheeseman) Mold.
Loc. [New Zealand, North Island], Feilding, Awahuri Scenic Reserve
Map **Alt.**
Lat. ± 5 km, 40° 16' 13.4" South **Long.** ± 5 km, 175° 30' 43.4" East
Coll. A E Esler **Date** 23 Dec 1961
Det. A E Esler **Date** 1961
Notes Forest. Herbarium A E Esler, received 2014.

Figure 1. *Teucrium parvifolium*. The specimen from Awahuri Reserve, Feilding, collected by AE Esler on 23 Dec 1961, on which the first mine of *Sabulopteryx botanica* was detected (Auckland Museum AK362379). Photograph courtesy of Ewen Cameron, Auckland Museum herbarium: the sheet label has been enlarged in this reproduction relative to the specimen.

ods outlined by Hoare (2000) for Nepticulidae, except that the abdominal cuticle was opened up along the side by means of pulling with fine forceps, and a 2% solution of acid fuchsin in 70% ethanol was substituted for the acid fuchsin-azophloxin stain described in that paper. Only the abdominal cuticle and wings and not the genitalia were stained with acid fuchsin; male and female genitalia were stained with Chlorazol Black E. Terminology for the male and female genitalia follows Kumata (1982) and Triberti (1985). Larvae were preserved and examined in 75% ethanol. Pupal exuviae were slide-mounted in Euparal.

All herbarium sheets of *Teucrium parvifolium* in the Auckland Museum herbarium (Auckland) and the Allan Herbarium (Manaaki Whenua – Landcare Research, Lincoln) were searched for preserved leaf-mines by RJBH in June 2018.

All specimens used for drafting the description of the new species are held in NZAC (New Zealand Arthropod Collection, Manaaki Whenua–Landcare Research, Auckland, New Zealand). Additional specimens, including the first adults found of the new species, are in **BPNZ** (Brian Patrick collection, Christchurch, New Zealand).

Plant names for New Zealand plants follow the New Zealand Plant Conservation Network website (NZPCN 2019); readers should refer to this site for authorship of the native host plants listed in Appendix 1; authorities for introduced plants are given in the text. Two-letter area codes for regions of New Zealand are as defined by Crosby et al. (1998).

Molecular systematics

DNA was extracted from legs of two paratype specimens of *Sabulopteryx botanica* using the Qiagen DNeasy Blood & Tissue Kit. The 5’ region of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the primers described by Folmer et al. (1994). Polymerase chain reactions (PCR) were performed using 25 µL volumes containing 2 µL of genomic DNA extract, 2.0 µL PCR Buffer with MgCl2 (Roche, USA), 2.0 µL 2mM dNTP, 0.8 µL BSA (10mg/mL), 0.5 µL of 10 µM each primer, 1.0 µL of Fast start Taq DNA polymerase (Roche, USA). Thermal cycling conditions were an initial denaturation at 95° for 4 minutes, the 38 cycles of 94° for 45 seconds, 55° for 45 seconds and 72° for 45 seconds. This was followed by a final extension of 72° for 5 minutes. PCR products were purified using the Xterminator Purification Kit (Thermo Fisher) and cycle sequenced used BigDye Terminator Version 3.1 (Applied Biosystems). Cycle sequencing conditions followed Platt et al. (2007). The products were run on a 3100-Avant Genetic Analyzer (Applied Biosystems). The two resulting COI sequences have been submitted to Genbank with accession numbers MK797749 and MK797750.

DNA sequences were edited and aligned in Geneious v. 10.2.6 (Kearse et al. 2012). We downloaded the COI sequences from the Gracillariidae phylogeny by Huemer et al. (2016) from NCBI. From Genbank we also downloaded COI sequences for *Aspilapteryx multipunctella* (Chrétien, 1917) (KX042619), *Sabulopteryx limosella* (Duponchel, 1844) (KP253447) and *S. inquinata* (Triberti, 1985) (KP150259). Following Huemer et al. (2016) we rooted the phylogenies using *Anthophila fabriciana*

(Linnaeus, 1767) (Choreutidae). PCR amplification of the histone subunit 3 gene region, included in the Huemer et al. (2016) study, was unsuccessful.

Phylogenetic relationships were reconstructed using MrBayes v. 3.2.6 (Huelsenbeck and Ronquist 2001). We used the GTR+I+Γ model with the following prior distributions; unconstrained branch lengths (gamma parameter = 1.0), among-site rate variation (exponential parameter = 10), proportion of invariable sites (uniform 10 – 1). Each MCMC analysis was run with four chains, five million generations, thinning interval of 1,000, heating temperature of 0.2, and a burnin of 1 million. This analysis was repeated five times to ensure convergence. The MCMC output was summarised in Geneious v. 10.2.6.

Taxonomy

Sabulopteryx botanica Hoare & Patrick, sp. nov.

http://zoobank.org/AE827276-BA17-4BA6-83BC-A62912E77CF2

Figs 2–12, 14–17

Material examined. ***Holotype*:** NEW ZEALAND • ♂; Mid Canterbury [MC], Christchurch Botanic Gardens; 43°31.8'S, 172°37.2'E; emg. 21 Apr. 2014; R.J.B. Hoare, B.H. Patrick leg.; larva in leaf-fold on *Teucridium parvifolium* 31 Mar. 2014; NZAC.

***Paratypes*:** NEW ZEALAND • 1 ♀; same collection data as holotype; emg. 24 Apr. 2014; NZAC • 3 ♂♂; MC, Christchurch Botanic Gardens; 31 Mar. 2014; R.J.B. Hoare, B.H. Patrick leg.; beaten from *Teucrium parvifolium* [as *Teucridium* on labels]; NZAC • 2 ♀♀; same collecting data as preceding; ♀ genitalia on slides NZAC Grac. 2, NZAC Grac. 4; NZAC • 4 ♂♂; MC, Christchurch, Kennedy's Bush Rd, Jensen property; 29 Mar. 2014; R.J.B. Hoare, B.H. Patrick leg.; on and around *Teucrium parvifolium*; ♂ genitalia and wings on slide NZAC Grac. 3; NZAC • 1 ♂; MC, Banks Peninsula, Prices Valley; 1 Apr. 2014, R.J.B. Hoare, B.H. Patrick leg.; beaten from *Teucrium parvifolium*; ♂ genitalia on slide NZAC Grac. 1; NZAC.

Diagnosis. *Sabulopteryx botanica* is distinctive amongst New Zealand gracillariids in its combination of small size (wingspan 10 mm or less) and yellow-ochreous black-speckled forewings. It is perhaps most similar to *Caloptilia selenitis* (Meyrick, 1909), but this species has the centre of the vertex white and has three white spots along the forewing dorsum (there is no white on the vertex or forewing in *S. botanica*). In the male genitalia, the paired processes on the dorsum of the valva are diagnostic, and in the female, the deep invaginations of the S7–S8 intersegmental membrane are characteristic.

Description. Wingspan 8.5–10 mm. *Adult male* (Fig. 2): Head: frons white; vertex yellow-ochreous with some scales tipped darker brown; labial palpus whitish with segments 2 and 3 tipped brown; antenna ochreous, ringed dark brownish (apex of each flagellomere), approximately equal in length to forewing; scape with inconspicuous pecten of ca 5 short bristles (often abraded away). Thorax yellow ochreous with tegulae anteriorly blackish. Forewing: yellow-ochreous, with numerous blackish scales

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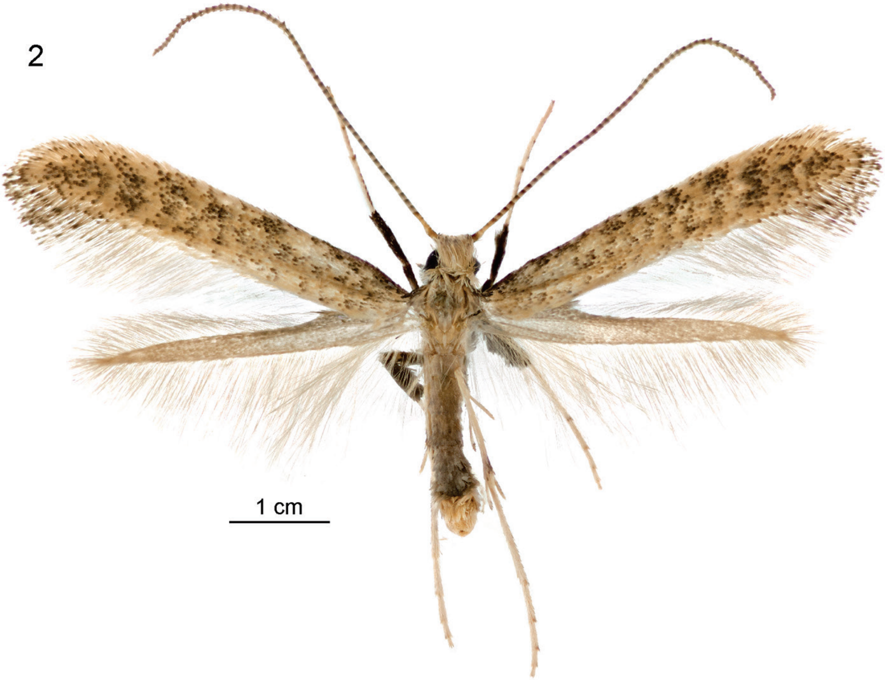

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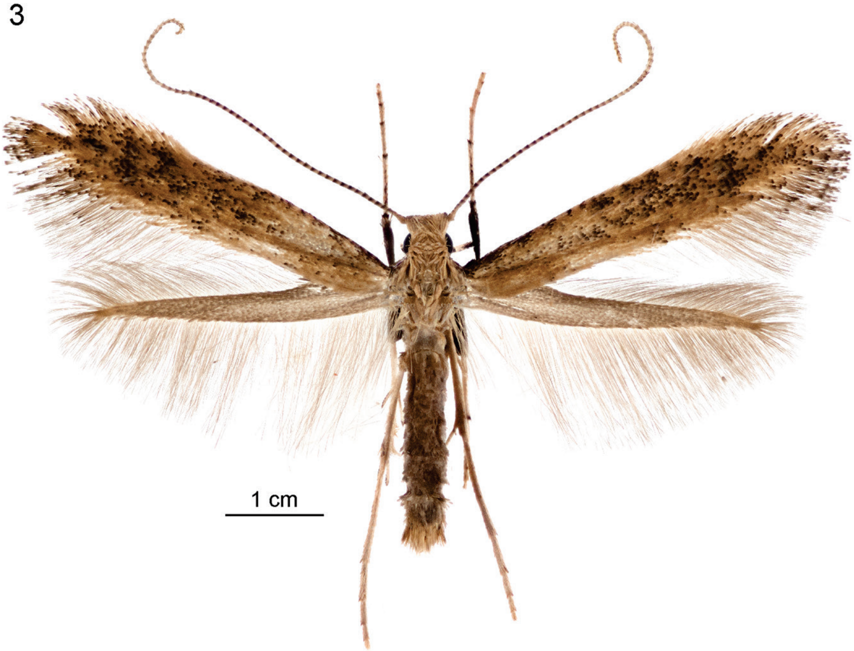


Figures 2, 3. *Sabulopteryx botanica*, adults. **1** Male paratype, Halswell Quarry (Kennedy's Bush Rd), Christchurch MC, 29 Mar 2014 (NZAC) **2** female paratype, Christchurch Botanic Gardens MC, emg. 24 Apr 2014 (NZAC).

forming variable and irregular pattern of broken transverse fasciae; blackish scales often denser towards base of costa and in disc at ca 2/3 length of wing; fringe ochreous whitish, darker around apex to tornus, where dark-tipped scales form three indistinct fringe-lines (in fresh specimens). Hindwing pale greyish; fringe greyish white. Underside: forewing dark brown, paler on dorsum below fold, yellowish around base of cilia; hindwing dark brown on costa and dorsum, pale greyish centrally. Legs: foreleg and midleg with femur and tibia thickened with blackish scales and tarsi yellowish, each tarsomere tipped blackish above; hindleg yellowish, femur with black central patch exteriorly, tibia ringed brownish subapically and each tarsomere with a few brownish apical scales. Abdomen silvery grey, with yellowish anal tuft.

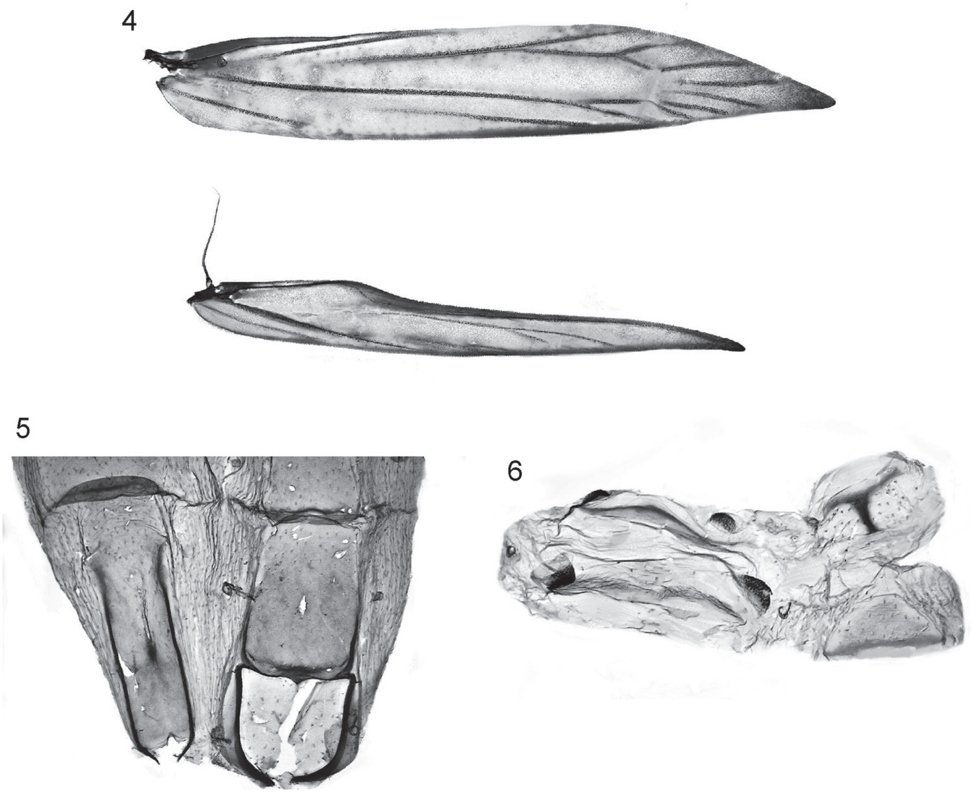
Adult female (Fig. 3). As described for male, but abdomen tipped with glossy ochreous whitish scales.

Wing venation (Fig. 4). Forewing 12-veined, as described for the genus by Triberti (1985), who regarded the 12-veined condition as being due to coincidence of M2 and M3. Discal cell somewhat dilated posteriorly as described by Triberti (1985). Hindwing very narrow (more so than in other *Sabulopteryx* species) with Rs strongly approximated to costa for most of its length; cell open between M2 and M3; M3 and CuA1 closely approximated and parallel.

Male abdomen and genitalia (Figs 5–8). Abdominal base as in Fig. 5. S7 and S8 (Fig. 6) markedly shortened and much wider than long, each with lateral pair of coremata. T8 (Fig. 6) with T-shaped sclerite. Genital capsule (Fig. 7): tegumen rather weakly sclerotised, elongate-triangular with attenuate apex. Tuba analis longer than tegumen; subscaphium slender, weakly sclerotised. Valva oblong, narrowed at base, with rounded apex; apical third bearing numerous long fine setae directed obliquely towards costa. Base of valva complex: costa extended into anteriorly-directed narrow, weakly curved process dorsad of anellus membrane (not fused with process from opposite valva, i.e. forming transtilla broken in the middle); from base of this process sclerotised ridge extends across inner (ventral) face of valva to base of long, sclerotised weakly curved spine that extends from valval dorsum at 1/3 valva length; a second, similar spine (slightly more strongly curved) on valval dorsum at 1/2 valva length. Juxta absent. Vinculum large, oval, saccus not differentiated. Phallus (Fig. 8) very elongated, slender, with sharply pointed apex; basally extending smoothly into ovoid bulbus ejaculatorius; vesica without cornuti; caecum penis absent.

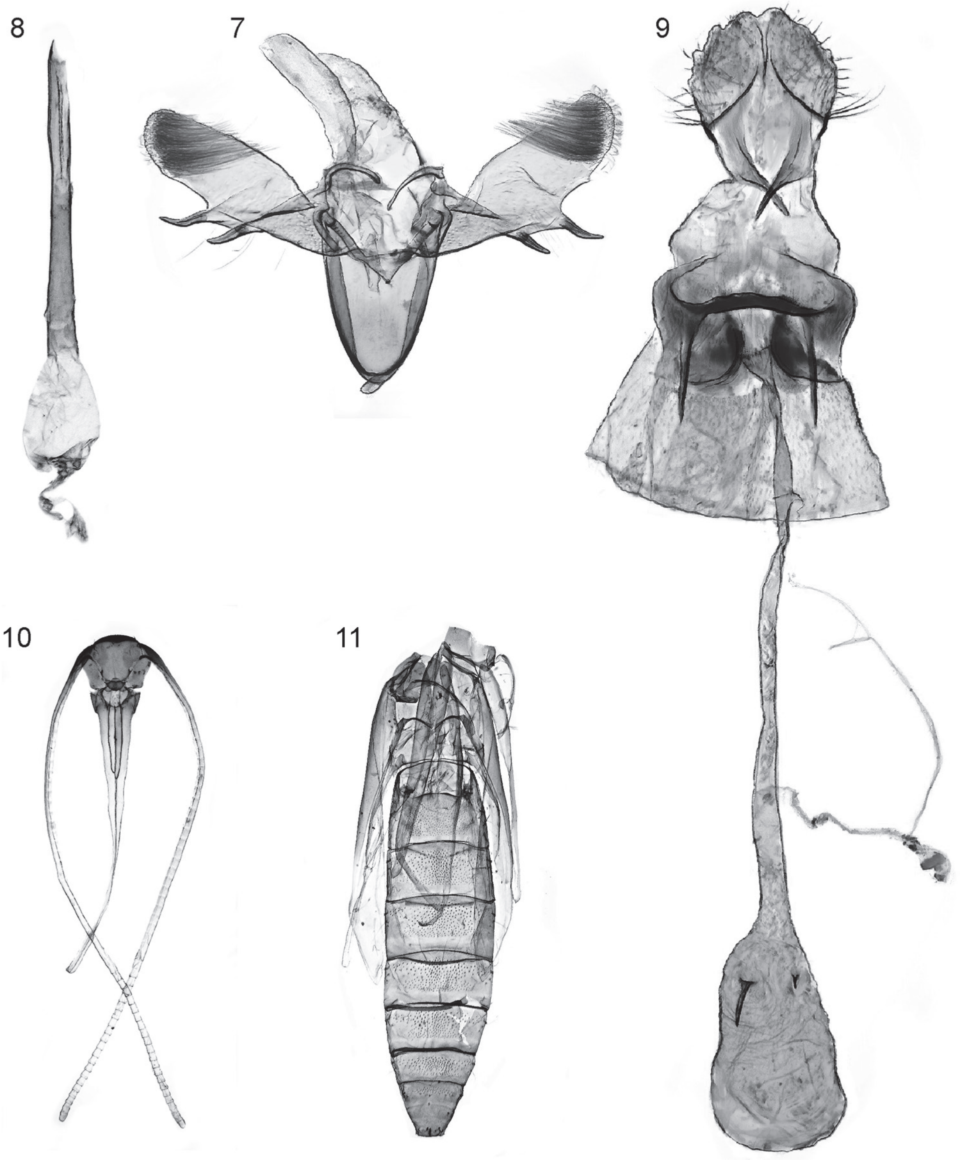
Female genitalia (Fig. 9). Ovipositor lobes rather short, membranous, basally with long setae, setae denser and shorter towards apex of each lobe; apophyses posteriores short. S7-S8 intersegmental membrane with pair of deep membranous sublateral invaginations; ostium lying between these, dorsal wall of ostium extended into T-shaped membranous area bordered posteriorly by strongly sclerotised transverse lamella postvaginalis, which is continuous with and broadens into lateral sclerotisations of S8. Ductus bursae entirely membranous, long and slender, ca 3× length of corpus bursae; corpus bursae more or less ovoid, with pair of spine-like signa, one long and one very short.

Immature stages. Egg. Elongate-oval, flat, showing up as silvery white translucent 'shell' at start of mine, apparently with rather coarsely sculptured chorion (not ob-



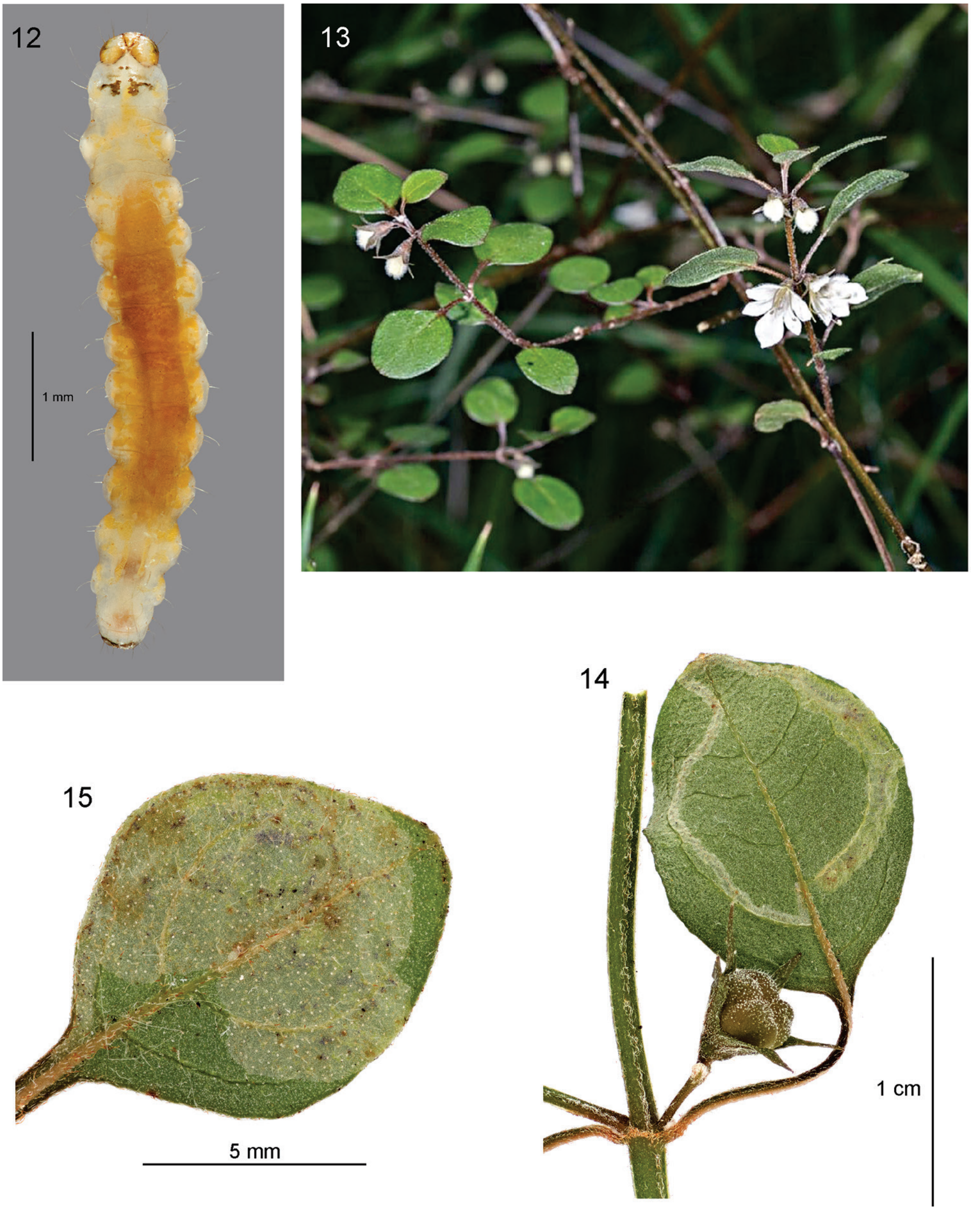
Figures 4–6. *Sabulopteryx botanica*, adult morphology. **4** Wing venation **5** male abdominal base **6** male abdomen, segments 7–8 (sternites on left).

served under SEM). *Larva* (Fig. 12). Head translucent pale yellow-brown, margined dark brown posteriorly and along adfrontal / ecdysial lines; blackish in region of stemmata. Body translucent yellowish white, with the gut contents showing through bright green; prothoracic plate in form of two irregular r-shaped sclerites with outlying smaller sclerites anteriorly. Thoracic legs with sclerotised areas dark grey-brown. Prolegs present on A3–5 and A10; crochets on A3–5 biserial: outer row a complete circle, with anterior crochets reduced, inner row a transverse semicircular band of larger crochets in posterior half of planta; A10 with single transverse band of large crochets in anterior half of planta. Anal plate a small transverse brownish sclerite with poorly defined margins. Chaetotaxy as described and figured for *Aspilapteryx tringipennella* (Zeller, 1839) by Triberti (1985). *Pupa*. Head (Fig. 10): frons smoothly rounded, without processes, without setae near antennal bases; antennae ca 3× as long as labial palpi. Thorax (Fig. 11): mesothorax and metathorax each with one pair of well-developed dorsal setae; forewings reaching to ca 1/2 way along A5, hindwings to A3/A4 junction. Abdomen (Fig. 11): A2–8 each with irregular rows of spinules dorsally, spinules slightly smaller and more widely spaced on A2; A7 not furrowed ventrally; abdominal tip truncate, with 3 pairs of small spinose tubercles.



Figures 7–11. *Sabulopteryx botanica*, adult and pupal morphology. **7** Male genital capsule **8** male phallus **9** female genitalia **10** pupal exuviae, head, ventral view **11** pupal exuviae, thorax and abdomen, dorsal view.

Host plant. The only known host plant is the small-leaved divaricating shrub *Teucrium parvifolium* (Lamiaceae) (Fig. 13), which is endemic to New Zealand and was until recently placed in its own monotypic genus *Teucridium*, and in the family Verbenaceae. Salmaki et al. (2016) showed that *Teucridium* belongs to Lamiaceae and is phylogenetically nested within the large worldwide genus *Teucrium*. The shrub is wide-



Figures 12–15. *Sabulopteryx botanica*, larva, host plant, and early mine. **12** Larva, dorsal **13** *Teucrium parvifolium* in flower, Longbush, Wairarapa (photo courtesy of J. Rolfe) **14** initial linear mine on leaf underside **15** early blotch mine.

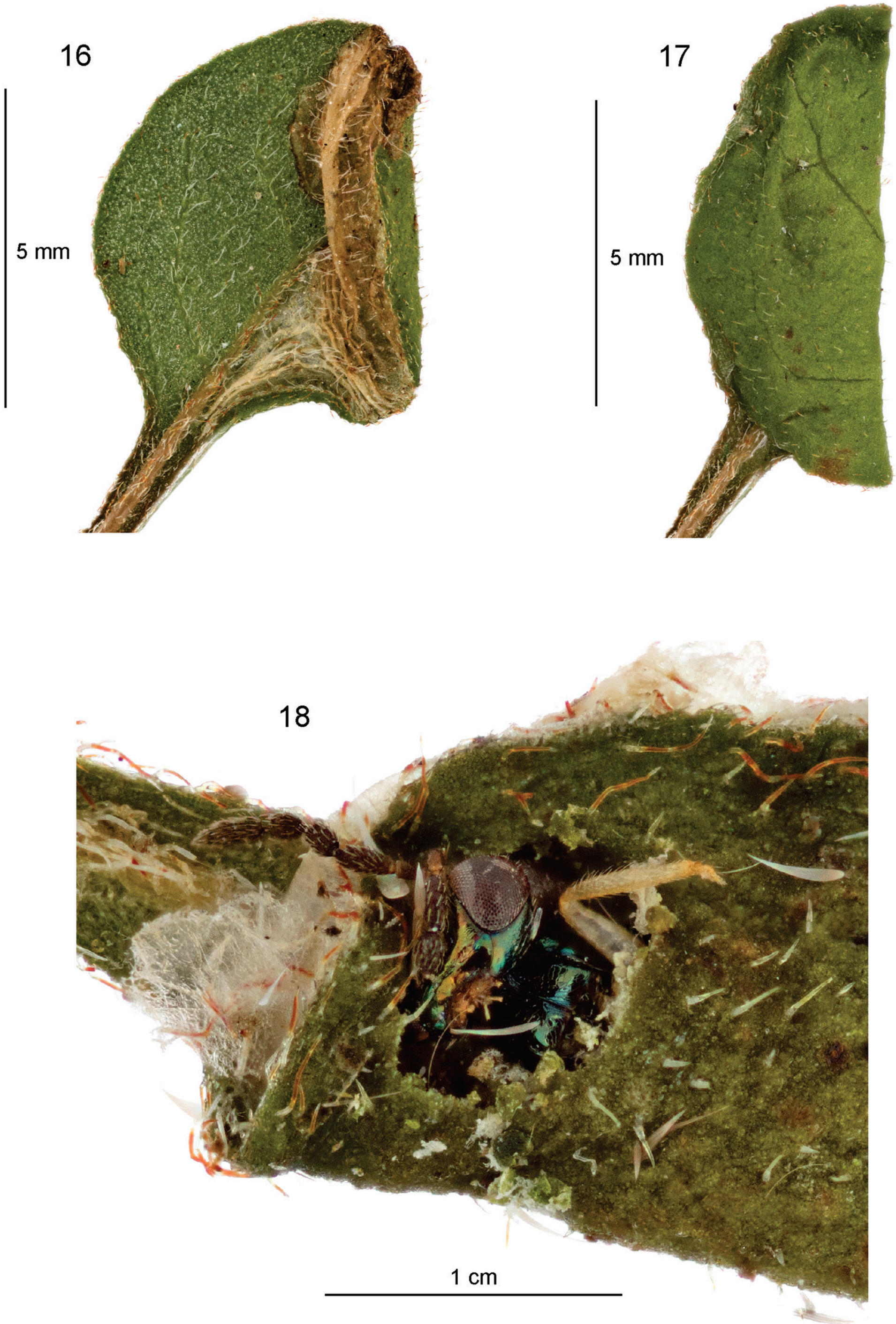
spread on both main islands of New Zealand, but rare and very local, and has a conservation status of 'At Risk – Declining' (de Lange et al. 2018). The habitat is described as 'fertile stream sides and river terraces in lowland dry forest and podocarp-hardwood forest, occasionally on forest margins, clearings and amongst scrub' (NZPCN 2019).

These fertile alluvial habitats have been cleared of forest throughout much of the country. Since no other species of *Teucrium* is native to New Zealand, *Sabulopteryx botanica* must be considered strictly monophagous on *T. parvifolium* (see also Remarks below).

Biology. The egg is laid on a leaf of the host plant, usually on the underside next to the midrib. The young sap-feeding larva forms a linear white mine (Fig. 14), almost invariably on the leaf underside, that extends to the margin of the leaf (on the side of the midrib that the egg was laid), then typically crosses the midrib at the leaf apex and extends for some way down the leaf margin on the other side. A line of blackish frass is more or less visible in the centre of the mine at this stage. The larva then doubles back and begins to expand the mine into a white blotch (Fig. 15), usually concentrated towards the leaf apex or to one side of the midrib, but often taking up the whole leaf on smaller leaves. These early mine stages are often rather hard to see unless the leaf is examined closely from the underside. Occasionally the egg and initial mine are on the upperside. Later the larva expands the mine and spins silk extensively in the interior (on the eroded inner surface of the leaf underside), causing the leaf to fold and creating creases in the underside (Fig. 16), in the typical manner of many gracillariid miners. At this stage, patches of the palisade mesophyll are eaten, leaving small windows of upper epidermis towards the middle of the leaf (appearing like holes), and larger windows (browning with age) towards the leaf margin. Black frass is scattered across the inner surface of the upperside of the leaf. When full-fed, the larva leaves the mine and folds a fresh leaf in half from the underside (Fig. 17), forming a cocoon of dense white silk within, in which it pupates. (In captivity, some larvae spin cocoons in tissue paper at the bottom of the rearing container.)

Parasitoids. One species of hymenopteran parasitoid can be confidently associated with the early stages of *S. botanica*, and one tentatively. A specimen of an unidentified species of *Sympiesis* Förster, 1856 (Eulophidae: Eulophinae) was found partially emerged from a cocoon in a folded leaf on an Auckland Museum herbarium sheet (Fig. 18). The plant specimen (AK285999) was collected at Pareora Scenic Reserve SC on 17 Mar 2004 by P.J. de Lange. (The wasp specimen was removed and mounted, and is now in NZAC, cross-referenced with the herbarium sheet.) Interestingly, two species of *Sympiesis* (*S. euspilapterygis* (Erdös, 1958) and *S. gregori* Boucek, 1959) have been associated with the *Teucrium*-mining *Sabulopteryx limosella* in Europe, but both also attack other leaf-mining Lepidoptera (see references in Noyes 2018, De Prins and De Prins 2018).

One specimen of an unidentified species of *Dolichogenidea* Vierek, 1911 (Braconidae: Microgastrinae) was reared from amongst *Teucrium* leaf-mines collected in Christchurch Botanic Gardens on 23 Jan 2018, emerging on 29 Jan (NZAC). It is thought most likely that this wasp was a parasitoid of *S. botanica*; however, host remains were not found and the sample was discovered later to include one unidentified early instar tortricid larva (preserved, not parasitised). The genus *Dolichogenidea* does not appear to have been associated with *Sabulopteryx* before, but is recorded overseas from other Gracillariinae (*Caloptilia* spp. and *Gracillaria syringella* (Fabricius, 1794)) as well as Lithocolletinae (*Phyllonorycter* spp.), Ornixolinae (*Parectopa ononidis* (Zeller, 1839)) (De Prins and De Prins 2018) and many other Lepidoptera, especially microlepidoptera (Austin and Dangerfield 1992). Most reared material of New Zealand *Sympiesis* and *Dolichogenidea* spp. in NZAC is associated with larvae of Tortricidae (Tortricinae).



Figures 16–18. *Sabulopteryx botanica*, late mine, cocoon, and parasitoid. **16** Fully formed mine with silk spinning causing creases **17** folded leaf with cocoon **18** *Sympiesis* sp. (Hymenoptera: Eulophidae) partially emerged from cocoon of *S. botanica* in leaf from herbarium sheet AK285999 (Auckland Museum).

Distribution. New Zealand, from the following regions: CL, TO, GB, HB, RI, WI, WA / NN, MC, SC, CO.

Note. The adult moth has only so far been found or reared in mid Canterbury (MC), but records of leaf-mines and cocoons on herbarium specimens of the host reveal a much wider range (Fig. 19). In some of these areas the plant is very likely to be severely threatened or even extinct, and renewed searches for plant and moth are desirable throughout the country. Towards the north and south of the plant's range, herbarium records of mines are scarce. The only Coromandel record is from a herbarium specimen collected at Kauaeranga near Thames prior to 1906 by J. Adams (Auckland Museum AK108237); no recent material of the plant from this area was seen. The only Otago record is from Gorge Creek, near Roxburgh CO, where P.N. Johnson found a colony of *Teucrium* in a shaded rock cleft on 24 May 1993 (Allan Herbarium CHR481347; two early mines and one cocoon). It should be noted that only two major herbaria were visited during the course of this research, and there are likely to be preserved mines in other botanical collections that have not yet been visited. The host plant is not known from any offshore islands of New Zealand, so these have been omitted from the map (Fig. 19).

Flight period. Adults have been found in the wild or emerged from wild-collected larvae or pupae in every month of the year in mid Canterbury, and the species is probably more or less continuously brooded here. All stages from early mines to adults can usually be found in the Christchurch Botanic Gardens, where the species is common amongst its host. Phenology in other parts of the range is unknown.

Etymology. The species name refers to the close association of this species with botany and botanists. It was discovered by a botanist (AE Esler) as a pressed larva in a botanical specimen of the host plant, and many further leaf-mines have now been found on herbarium sheets collected as part of botanical surveys. Its discovery by BHP as an adult in the type locality, Christchurch Botanic Gardens, completes the association.

Conservation status and potential management. *Sabulopteryx botanica* was accorded 'Nationally Vulnerable' status in the latest review of the conservation status of New Zealand Lepidoptera, where it was listed as *Caloptilia* sp. "Teucridium" (Hoare et al. 2017). This ranks as the third most critical category assigned to extant species (after 'Nationally Critical' and 'Nationally Endangered'), and was based on the rarity and declining status of the moth's host plant. As noted above, survey for *S. botanica* through most of the range of its host plant has been inadequate; most herbarium records of mines are over 25 years old (Fig. 19), and further field-work is needed to determine the moth's current distribution. *Teucrium parvifolium* is an attractive, easily propagated and cultivated shrub that is tolerant of a wide range of conditions (NZPCN 2019), though relatively short-lived (P. Bellingham, pers. comm.). The moth appears to be thriving in situations where *T. parvifolium* has been planted around Christchurch, for example in the Botanical Gardens and in the native plantings maintained by botanist Carol Jensen at Kennedy's Bush near Halswell Quarry. It should therefore be possible to boost the moth's population substantially by encouraging the propagation and cultivation of the host plant, with due attention to appropriate sourcing and hygiene. This

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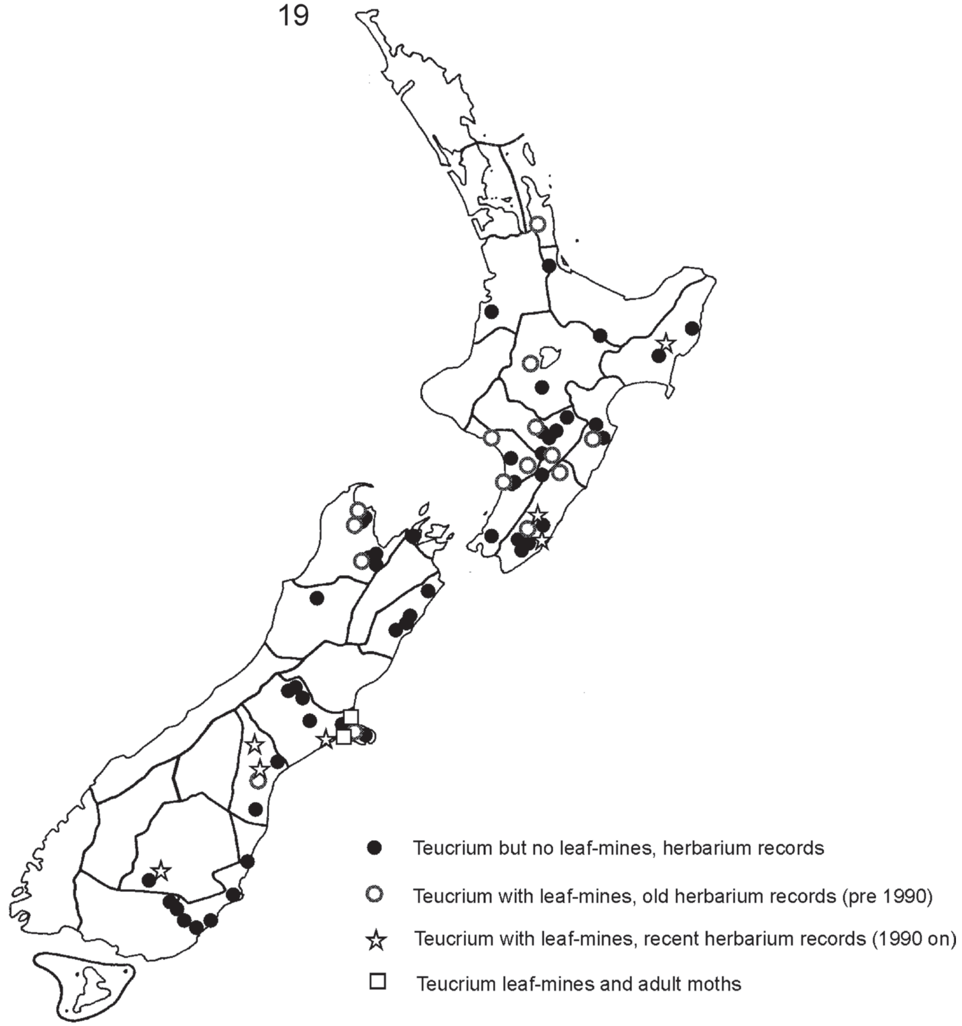

Figure 19. Distribution of *Teucrium parvifolium* and *Sabulopteryx botanica* based mainly on records from herbarium sheets in Allan Herbarium, Lincoln and Auckland Museum herbarium.

could be achieved in natural environments where the plant persists, as well as in public plantings and in suburban gardens.

Systematic placement: morphology. The new species described here can be confidently placed in the *Gracillaria* group of genera (Gracillariinae) as defined by Kumata (1982), based especially on the following characters: mid femur and tibia thickened beneath with rough scales; R1 of forewing arising near base of wing, with upper vein of cell weakened beyond branching point of R1; hindwing R2+3 very short and running parallel with and very close to apical part of Sc+R1; hindwing cell open between M2 and M3; male segments 7 and 8 weakly membranous, with coremata. The following genera belong to the *Gracillaria* group, based on Kumata (1982) and updates from subsequent authors (e.g., Triberti 1985, Huemer et al. 2016, Pereira et al. 2019): *Aspilapteryx*, *Caloptilia* Hübner, 1825, *Calybites* Hübner, 1822, *Ectropina* Vári, 1961, *Eucalybites* Kumata, 1982, *Euspilapteryx* Stephens, 1835 (treated as a synonym of *Calybites* by Kumata (1982)), *Gracillaria* Haworth, 1828, *Mercantouria* Huemer, Lopez-Vaamonde & Triberti, 2016, *Povolnya* Kuznetzov, 1979, *Sabulopteryx* and *Vallissiana* Pereira & Arévolo, 2019.

When first discovered as an adult by BHP in January 2013, and before detailed morphological examination, *S. botanica* was tentatively considered to be a member of the genus *Caloptilia* (Hoare et al. 2017: see above). However, RJBH later noted its remarkable external similarity to some west Palaearctic gracillariids then placed in the genus *Aspilapteryx* (subgenus *Sabulopteryx*), i.e. *S. limosella* from central and southern Europe and *S. inquinata* from southern Europe, Turkey and Lebanon, which it closely resembles in size, wing shape and overall coloration. When describing *Sabulopteryx* as a new subgenus of *Aspilapteryx*, Triberti (1985) anticipated the possibility that it might deserve full genus status. In a recent molecular phylogeny, Pereira et al. (2019) found a 14 to 16% divergence in DNA barcodes between *Aspilapteryx* and *Sabulopteryx* species, and indicated that *Aspilapteryx* is polyphyletic if *Sabulopteryx* is included. Our analysis (see below) also retrieves *Aspilapteryx* and *Sabulopteryx* in separate lineages, so we accept the conclusions of Pereira et al. (2019) and treat *Sabulopteryx* as a genus.

As pointed out by Huemer et al. (2016), morphological comparisons within the *Gracillaria* group are complicated by the apparently homoplasious distribution of character states amongst genera. Based largely on comparison with the descriptions and figures in Vári (1961), Kumata (1982), Triberti (1985), Huemer et al. (2016) and Pereira et al. (2019), the characters listed below in combination lend support for placing the New Zealand *Teucrium*-miner in *Sabulopteryx*:

1. Male abdomen with coremata on both segment 7 and segment 8 (Fig. 6). This conforms with most genera of the *Gracillaria* group, including *Sabulopteryx* and *Mercantouria* (Huemer et al. 2016), but not with *Gracillaria* or *Aspilapteryx*, where there is only one pair of coremata (Kumata 1982; Triberti 1985), nor with *Vallissiana*, where there are no coremata (Pereira et al. 2019).
2. Outline of male valva (beyond sacculus) rounded, not angular, and lacking a ventro-apical lobe. In its rounded / oblong valva, *S. botanica* resembles most genera of the *Gracillaria* group, but not *Mercantouria*, *Calybites* or *Euspilapteryx*, all of which have a distinctly angular valva (Kumata 1982; Huemer et al. 2016), nor *Aspilapteryx* or *Vallissiana*, both of which have a distinct ventro-apical lobe (Pereira et al. 2019).
3. Setae of valva confined to apical area, not extending into basal half. This character does not appear to have been commented on by previous authors: *Caloptilia*, *Gracillaria*, *Povolnya* and *Calybites* all have the valva more extensively setose than the remaining genera of the *Gracillaria* group (including *Sabulopteryx*), perhaps as a result of the relative reduction of the (non-setose) sacculus in these four genera.

4. Valva lacking stout peg-like or spine-like setae distally. This conforms with most genera of the *Gracillaria* group, including *Sabulopteryx*. Short, stout setae are present in the distal part of the valva in *Euspilapteryx* and on the ventrodistal margin in *Calybites* (Kumata 1982), and longer, spine-like setae in *Eucalybites* (Kumata 1982) and *Mercantouria* (Huemer et al. 2016).
5. Male phallus short and straight, without apical processes. The phallus of *S. botanica* (Fig. 8) is similar to those of described species of *Caloptilia*, *Gracillaria*, *Povolnya* and *Sabulopteryx*. It lacks the apical modifications found in *Eucalybites* (Kumata 1982: figs 47 B, C), *Euspilapteryx* and *Vallissiana* (Pereira et al. 2019: fig. 3F) and the rod-like apical sclerite of *Mercantouria* (Huemer et al. 2016: fig. 5). The phallus is long with a helical tip in *Aspilapteryx* (Triberti 1985; Huemer et al. 2016), very long and straight in *Calybites* (Kumata 1982), and curved or sinuous in *Ectropina* (Vári 1961).
6. Female genitalia with two curved, spine-like signa (Fig. 9). This is typical of *Aspilapteryx* and *Sabulopteryx* (Triberti 1985), *Mercantouria* (Huemer et al. 2016), most *Caloptilia* and *Eucalybites* (Kumata 1982), but not of *Gracillaria*, *Caloptilia* subgenus *Minyoptilia* Kumata, 1982, *Calybites*, *Ectropina*, *Euspilapteryx*, or *Vallissiana*, in all of which there is only a single signum (Vári 1961; Kumata 1982; Pereira et al. 2019). *Povolnya* has two signa, but these are short and stout (Kumata 1982).
7. Forewing brownish, without costal streak and with numerous irregularly arranged darker blotches (Figs 1, 2). This wing pattern accords with the description of *Sabulopteryx* by Triberti (1985), and as noted above, there is a strong superficial resemblance between adults of *S. botanica* and the two Palaearctic members of *Sabulopteryx*, *S. limosella* and *S. inquinata*. No other member of the *Gracillaria* group closely approaches this wing pattern.
8. Host-plant genus *Teucrium*. The hostplant genus is shared with *S. limosella*, type species of *Sabulopteryx*, which mines in *Teucrium chamaedrys* L. and *T. montanum* L. in xerothermic localities in central and southern Europe (Triberti 1985). The biology of the two species is also similar. No other gracillariid is known to mine in *Teucrium* (De Prins and De Prins 2018).

In addition, the pupal exuviae of *S. botanica* (Figs 10, 11) match the description and illustrations of the pupa of *Sabulopteryx limosella* in the key to Gracillariidae pupae by Patočka and Turčáni (2005: 75–76). The exuviae readily key out to *Aspilapteryx* in this key, but since the characters of *Aspilapteryx* were based only on *S. limosella*, the name *Sabulopteryx* should be substituted. Characters of *S. botanica* that lead in this key to *Sabulopteryx* are as follows: proboscis long, exceeding prothoracic femora; head without projection and rounded in lateral view; pronotum not disconnected on dorsomeson; frontal setae absent; A7 without longitudinal furrows ventrally; A10 with spine-like tubercles. From the description and illustrations in Pereira et al. (2019), the pupa of *Vallissiana universitaria* Pereira & Arévolo, 2019 shares most of these characters with *Sabulopteryx*.

Sabulopteryx botanica differs strongly in some characters from its Palaearctic congeners. Neither of the other species has two large spine-like processes on the male valva (Fig. 7); in *S. limosella* and *S. inquinata* the single process is on or near the valval costa (Triberti 1985: plate VI B, D); *S. botanica* has the processes on the valval dorsum. The placement of the ostium in the female genitalia in *S. botanica* (in the intersegmental membrane between S7 and S8, Fig. 9) is also atypical of *Sabulopteryx*: in the other species it is at the caudal edge of S7 (Triberti 1985). The invaginations of the intersegmental membrane either side of the ostium (Fig. 9) are apparently unique to *S. botanica*. The male of *S. botanica* has T8 in the form of a T-shaped sclerite (Fig. 6), as in genus *Aspilapteryx*. Given the morphological and molecular support (see below) indicating a close relationship between *S. botanica* and the other species of *Sabulopteryx*, these anomalous characters are tentatively considered autapomorphic.

Systematic placement: molecular phylogenetics. Our molecular analysis, based as it is on a single gene, in no way supplants the much more substantial analysis by Kawahara et al. (2017), but those authors did not include *Sabulopteryx* (or *Aspilapteryx*) in their phylogeny. Our analysis (Fig. 20) provides provisional molecular support for the placement of *Aspilapteryx* and *Sabulopteryx* in Gracillariinae as suggested by the studies of Kumata (1995), Huemer et al. (2016) and Kawahara et al. (2017), and for the placement of *S. botanica* in *Sabulopteryx*, as indicated above from the morphological comparisons.

Despite being only estimated from a single gene, many of the nodes in the phylogeny received posterior probability support values greater than 0.75. Three of the eight subfamilies recovered as monophyletic by Kawahara et al. (2017) are also recovered here, i.e. Gracillariinae (*Caloptilia* to *Mercantouria*, posterior probability 0.75, Fig. 20), Acrocercopinae (*Spulerina* to *Artifodina*, posterior probability 0.53, Fig. 20), and Parornichinae (*Parornix* to *Callisto*, posterior probability 0.68, Fig. 20). Only a single representative each of Marmarinae (*Marmara serotinella* Busck, 1915), Phyllocnistinae (*Phyllocnistis ramulicola* Langmaid & Corley, 2007) and Oecophyllembiinae (*Eumetriochroa hederae* Kumata, 1998) was included. Lithocolletinae (*Macrosaccus*, *Cameraria*, *Phyllonorycter*) were recovered as paraphyletic with respect to Marmarinae, while Ornixolinae (*Parectopa*, *Chileoptila*, *Micrurapteryx*) appeared paraphyletic with respect to Oecophyllembiinae + Phyllocnistinae.

The inconsistencies in our cladogram with respect to Kawahara et al. (2017) are to be expected from a single-gene tree, and suggest the limitations of the current analysis with respect to deeper nodes of the phylogeny. Similarly, there are some inconsistencies with the tree recovered by Huemer et al. (2016), but again these are to be expected and do not undermine the evidence for a close relationship between *Sabulopteryx botanica* and *S. limosella* + *S. inquinata* based on COI, morphology and biology.

The molecular phylogeny supports the treatment of *Aspilapteryx* and *Sabulopteryx* as separate genera (Fig. 20), as proposed by Pereira et al. (2019). The two clades are supported as monophyletic with posterior probabilities of 1 and 0.96 respectively. Though the two clades are separated by two nodes that are only weakly supported (0.51 and 0.65 posterior probabilities, Fig. 20), we consider the evidence from the

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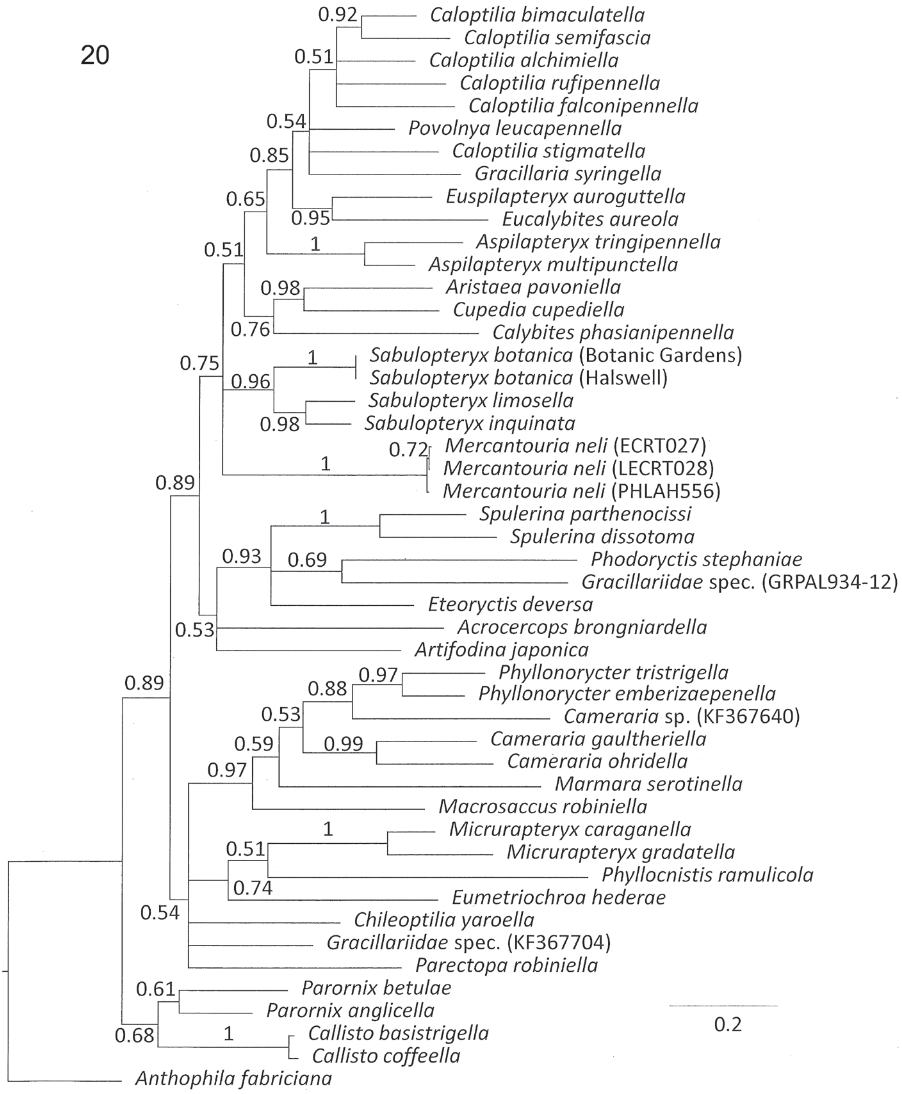

Figure 20. Bayesian consensus phylogeny reconstructed from the COI gene. Numbers above branches represent posterior probabilities. Branch lengths are drawn proportional to the estimated number of substitutions per site, following the scale bar. The tree is rooted with *Anthophila fabriciana* (Choreutidae).

two independent analyses coupled with the differences in morphology and biology outlined by Triberti (1985) and Pereira et al. (2019) as sufficient to warrant full genus status for *Sabulopteryx*.

Remarks. Lees et al. (2011) nicely demonstrated the importance of herbarium specimens as a source of information on insect-plant interactions. They searched herbaria for preserved mines of the horse-chestnut leaf miner *Cameraria ohridella* Deschka

& Dimić, 1986 (Gracillariidae: Lithocolletinae), which has recently become invasive throughout Europe, in spite of remaining undetected by entomologists until 1984. From these pressed mines and the larval remains they contained, they were able to document the historical presence of this species in the native range of its host (*Aesculus hippocastanum* L.) back to 1879, as well as revealing past outbreaks of the moth and novel haplotypes.

Similarly, study of herbarium material (e.g., Fig. 1), in addition to alerting entomologists to the existence of *Sabulopteryx botanica*, has produced many historical records of the moth. It has greatly helped our knowledge of the distribution and also provided a parasitoid record (see above). The mines are not difficult to find on herbarium sheets, though sometimes magnification is required to scan for the earliest stages. Of 159 herbarium sheets examined in Auckland and Lincoln, 32 (20%) had at least one leaf-mine of *S. botanica*. The oldest specimen so far found was a single early mine in a leaf from the Cheeseman collection in the Auckland Museum (AK7584): this was collected at Foxhill near Wakefield NN in January 1882. The plant specimen has been annotated appropriately in the Auckland Museum database and the mined leaf is now arrowed on the sheet (E. Cameron, pers. comm.). These old records of the moth also help to confirm that it is an endemic species on its natural host plant and not a recent adventive that has switched to *T. parvifolium* from an introduced *Teucrium* species. To check this assumption further, RJBH examined all New Zealand specimens of introduced species of *Teucrium* (including cultivated species) in the Allan Herbarium in June 2018, and found no evidence of any mines or cocoons. The following species were examined: *Teucrium betonicum* L'Hér*.*, *T. chamaedrys*, *T. flavum* L., *T. fruticans* L., *T. hircanicum* L., *T. polium* L., *T. pseudochamaepitys* L. and *T. scorodonia* L. Of these, probably only *Teucrium hircanicum* and *T. scorodonia* are established in the wild in New Zealand (NZPCN 2019).

Discussion

The discovery of an endemic species of *Sabulopteryx* in New Zealand is remarkable and unexpected. The close relationship of *S. botanica* to the European *S. limosella* and *S. inquinata* suggests an extraordinary disjunction in distribution within this group. It would be of great interest to elucidate the age of the split between *S. botanica* and its congeners. According to the phylogenetic analysis and molecular dating of Salmaki et al. (2016), *Teucrium* is estimated to have split from its sister-genus *Rubiteucris* about 15.95 mya and to have begun diversifying around 13.13 mya. Presuming that the last common ancestor of *S. botanica* and *S. limosella* was a *Teucrium*-miner, the proposed age of the host-plant genus is far too young to explain the current known distribution of *Sabulopteryx* as a result of vicariance. If we discount extreme long-distance dispersal, either *Sabulopteryx* is a relictual genus that has contracted from a former much wider distribution, or it has been overlooked or misidentified elsewhere (as it was in New Zealand): both could well be true.

In this regard, two taxa that require further study are *Aspilapteryx tessellata* (Turner, 1940) from eastern Australia and *Caloptilia scutellariella* (Braun, 1923) from eastern North America. *Gracilaria* [sic] *tessellata* was transferred to *Aspilapteryx* by Nielsen and Kumata (1996) without further comment and without indicating to which of the then subgenera (*Aspilapteryx* or *Sabulopteryx*) it might belong. The only specimen of *A. tessellata* in ANIC (a syntype from Ebor, N.S.W.) is missing its abdomen. A second syntype is in the Australian Museum, Sydney; this is incorrectly implied to be the holotype on the Atlas of Living Australia website (ALA 2018). Neither specimen was examined for this paper, but a photograph of the ANIC specimen was seen. In wing pattern, *A. tessellata* does not closely resemble *S. botanica* or the other *Sabulopteryx* species; it has much paler forewings with brownish strigulations and lacks distinct blackish speckling. Turner (1940) gives the forewing ground colour as 'white', so the pallid appearance is not due to fading of the specimen. In the Allan Herbarium, there are five specimens of the Australian *Teucrium racemosum*, all collected in South Australia or the Northern Territory; no leaf-mines were found on any of these.

Caloptilia scutellariella is a leaf-miner on *Scutellaria* (Braun 1923), which belongs to the same family as *Teucrium* (Lamiaceae), a rare host-plant family amongst Gracillariidae (De Prins and De Prins 2018). Based on COI, *C. scutellariella* was recovered as the sister-species to their new genus *Mercantouria* by Huemer et al. (2016: figs 9, 10), who therefore suggested that it was probably misplaced in *Caloptilia*; these authors did not include *Sabulopteryx* species in their molecular phylogeny. The life history and leaf-mine of *C. scutellariella* appear to be very similar to those of *Sabulopteryx limosella* and *S. botanica* (see images and text at http://www.microleps.org/Guide/Gracillariidae/Gracillariinae/Caloptilia/index.html), differing from typical *Caloptilia* in that all feeding takes place within the mine, and the larva does not emerge to feed in a rolled or folded leaf, only to pupate. The forewing pattern of *C. scutellariella* could possibly be interpreted as essentially similar to that of *Sabulopteryx* with the area of dark irroration increased so as to obscure the brown ground-colour. The systematic placement of *C. scutellariella* is beyond the scope of this paper and we have not examined specimens.

Acknowledgements

The authors are very grateful to Birgit Rhode (Manaaki Whenua – Landcare Research, Auckland) for the photographs of moths, genitalia, leaf-mines and early stages, and for making up the plates. We thank the staff of the Christchurch Botanic Gardens for permission to collect and study the new species there, and for their enthusiasm for this discovery. RJBH would like to acknowledge the warmth and generosity of the staff at the two herbaria he visited to search for leaf-mines, especially Ewen Cameron and Yumiko Baba at the Auckland Museum herbarium and Sue Gibb, Ines Schönberger and Mary Korver at the Allan Herbarium (Manaaki Whenua – Landcare Research, Lincoln). Peter Bellingham (Manaaki Whenua – Landcare Research, Lincoln) also kindly shared his knowledge of the plant and its ecology in a discussion at Auckland

Museum. We thank Darren Ward (Manaaki Whenua – Landcare Research, Auckland) for identifying the reared parasitoid wasps. Ewen Cameron (Auckland Museum) and Jeremy Rolfe (Department of Conservation) generously allowed us to reproduce the photographs that appear as Figs 1 and 13 respectively. The COI sequences were generated by Talia Brav-Cubitt (Manaaki Whenua – Landcare Research, Auckland). RJBH is indebted to John Dugdale (Emeritus Research Associate, Manaaki Whenua – Landcare Research, Nelson) for his excellent notes and sketches of Gracillariidae in NZAC, which helped greatly in compiling the revised checklist. This manuscript was enormously improved thanks to the reviews of Carlos Lopez-Vaamonde and Paolo Triberti and further comments by Erik van Nieukerken, and we also thank Gilson Moreira (per Erik van Nieukerken) for access to the paper of Pereira et al. (2019) on *Vallissiana*, which was in press at the time of this paper's review. Finally, we acknowledge the dedication of the many botanists past and present who have collected specimens of *Teucrium parvifolium* and (until now unknowingly) added much to our knowledge of the distribution of its associated moth.

This work was funded in part by Strategic Science Investment Funding for Crown Research Institutes from the Ministry of Business, Innovation and Employment's Science and Innovation group.

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Appendix 1

Revised checklist of New Zealand Gracillariidae

This revised checklist places all New Zealand Gracillariidae in the subfamilies defined by Kawahara et al. (2017). We have not followed the newer classification of De Prins et al. (2019) for reasons given in the main text, above.

'*Acrocercops*' has been used as a catch-all genus for otherwise unplaced species; since only '*A.*' *leucocyma* (Meyrick) can be confirmed as belonging to Acrocercopinae, all other species (except the Australian '*A.*' *laciniella* (Meyrick)) are removed from *Acrocercops* and tentatively assigned to other genera that at least belong to the appropriate subfamily. Unpublished notes and drawings by John Dugdale in NZAC (Gracillariidae box-file) have been very helpful in determining the placements adopted here. In most cases, the correct genus placement still needs to be ascertained by further study and some species probably belong to undescribed genera (see note on Oecophyllembiinae below). Therefore these genus placements are qualified with '*sensu lato*' (s.l.). The synonymy remains unchanged from Dugdale (1988) and is not repeated here. Brief notes on host plant and biology are given for all species.

E = endemic to New Zealand. A = adventive in New Zealand.

Gracillariidae
Acrocercopinae

Acrocercops (s.l.) *laciniella* (Meyrick, 1880) A. Leaf-miner on juvenile leaves of *Eucalyptus* spp. (Myrtaceae) (Common 1990). Tentatively retained here in Acrocercopinae in the absence of conflicting evidence.

Acrocercops (s.l.) *leucocyma* (Meyrick, 1889) E. Leaf-miner on *Agathis australis* (Araucariaceae), overwintering in petiole galls (Wise 1952; reared material in NZAC). The expanded A8 of the male (J.S. Dugdale, NZAC notes) confirms placement in Acrocercopinae.

Dialectica scalariella (Zeller, 1850) A. Leaf-miner on Boraginaceae, including *Echium* spp., *Myosotis* spp. and occasionally *Myosotidium hortensia* (Chatham Island forget-me-not) (reared material in NZAC).

Gracillariinae

Caloptilia azaleella (Brants, 1913) A. Leaf-miner and folder on azaleas (*Rhododendron* spp., Ericaceae).

Caloptilia chalcodelta (Meyrick, 1889) E. Leaf-miner and folder on *Nestegis* (Oleaceae) (reared material in NZAC).

Caloptilia chrysitis (Felder & Rogenhofer, 1875) E. Leaf-miner and folder on *Weinmannia* (Cunoniaceae), *Elaeocarpus* (Elaeocarpaceae) and rarely *Knightia excelsa* (Proteaceae) (reared material in NZAC).

Caloptilia elaeas (Meyrick, 1911) E. Leaf-miner and folder on *Coriaria plumosa* and probably other small-leaved *Coriaria* spp. (Coriariaceae) (reared material in NZAC).

Caloptilia linearis (Butler, 1877) E. Leaf-miner and folder on *Coriaria arborea* (Coriariaceae) (reared material in NZAC).

Caloptilia selenitis (Meyrick, 1909) E. Leaf-miner on *Lophozonia menziesii* (Nothofagaceae); cocoon between joined leaves (Watt 1924; reared material in NZAC).

Macarostola ida (Meyrick, 1880) A. Leaf-miner and folder on *Eucalyptus* spp. (Myrtaceae). An Australian species found established locally in east Auckland and Northland in January 2019: adults and numerous larvae. New to New Zealand.

Macarostola miniella (Felder & Rogenhofer, 1875) E. Leaf-miner and folder on *Syzygium maire* (Myrtaceae) (reared material in NZAC).

Sabulopteryx botanica Hoare & Patrick, 2019 E. Leaf-miner and folder on *Teucrium parvifolium* (Lamiaceae) (this paper).

Lithocolletinae

Phyllonorycter messaniella (Zeller, 1846) A. Leaf-miner on *Quercus* spp. (including deciduous species as well as evergreen *Q. ilex* L.) (Fagaceae) and occasionally *Fagus sylvatica* L. (Fagaceae), *Castanea sativa* Mill. (Fagaceae), *Betula pendula* Roth (Betulaceae), *Carpinus betulus* L. (Corylaceae), *Malus pumila* Mill. (= *M.* x *domestica*) (Rosaceae), *Acca sellowiana* (O. Berg) Burret (Myrtaceae) (Wise 1953, 1954). Other New Zealand host plants are also listed by Wise (1953, 1954) based on mines, but only those from which moths were reared are given here.

Porphyrosela hardenbergiella (Wise, 1957) A. Leaf-miner on *Hardenbergia* (Fabaceae) (Wise 1957). Note. This species has not been collected since 1955, and is still not known from Australia, which is almost certainly its country of origin (*Hardenbergia* is endemic to Australia).

Oecophyllembiinae

Note. In this subfamily, species feeding on Araliaceae and Apocynaceae are provisionally assigned to *Eumetriochroa* and the single Rubiaceae-miner is assigned to *Corythoxestis*. These provisional assignments need checking, but are considered for the time being more informative and less misleading than the placement of all species in *Acrocercops*. Male genitalia and wing characters (J.S. Dugdale, NZAC notes) as well as leaf-mining biology and pupal characters (Watt 1920) confirm placement in Oecophyllembiinae, but suggest that all these species may turn out to belong to endemic genera, and further study is required.

Eumetriochroa (s.l.) *aellomacha* (Meyrick, 1880) comb. nov. E. Leaf-miner on *Pseudopanax arboreus* (Watt 1920). Note. Identification of Meyrick's species with subsequently reared material follows Watt (1920) but requires checking.

Eumetriochroa (s.l.) *aethalota* (Meyrick, 1880) comb. nov. E. Leaf-miner and stem-miner on *Parsonsia* (Apocynaceae).

Eumetriochroa (s.l.) *panacicorticis* (Watt, 1920) comb. nov. E. Stem-miner on *Pseudopanax arboreus* (Araliaceae) (Watt 1920; reared material in NZAC).

Eumetriochroa (s.l.) *panacifinens* (Watt, 1920) comb. nov. E. Leaf-miner on *Pseudopanax arboreus* and probably *P. colensoi* (Araliaceae) (Watt 1920; reared material in NZAC).

Eumetriochroa (s.l.) *panacitorsens* (Watt, 1920) comb. nov. E. Leaf-miner (leaf underside) on *Pseudopanax arboreus* and *Raukaua simplex* (Araliaceae) (Watt 1920; reared material in NZAC).

Eumetriochroa (s.l.) *panacivagans* (Watt, 1920) comb. nov. E. Leaf-miner on *Pseudopanax crassifolius* and *P. lessonii* (Araliaceae) (Watt 1920; reared material in NZAC).

Eumetriochroa (s.l.) *panacivermiforma* (Watt, 1920) comb. nov. E. Leaf-miner on *Raukaua edgerleyi* and *R. simplex* (Araliaceae) (Watt 1920; reared material in NZAC).

Corythoxestis (s.l.) *zorionella* (Hudson, 1918) comb. nov. E. Leaf-miner on large-leaved *Coprosma* spp. and sometimes *C. arborea* (Rubiaceae) (Watt 1920; reared material in NZAC).

Ornixolinae

Parectopa (s.l.) *alysidota* (Meyrick, 1880) comb. nov. A. Phyllode-miner and sometimes stem-miner on *Acacia* spp. (Mimosaceae) (Watt 1920; reared material in NZAC). Note. Based on wing venation, this species is related to *Parectopa* (where it was placed by Meyrick, as *P. citharoda* Meyrick, a synonym). The adult resting posture, with the first two pairs of legs spread wide but appressed to each other, resembles that of other genera of Ornixolinae (e.g., *Epicephala* Meyrick*, Cuphodes* Meyrick (Kawahara et al. 2017: fig. 5H). It may require a new genus (J.S. Dugdale, NZAC notes).

Conopomorpha cyanospila Meyrick, 1885 E. Fruit-borer on *Alectryon excelsus* (Sapindaceae) (reared material in NZAC).

Polysoma eumetalla (Meyrick, 1880) A. Miner in surface of *Uromycladium* rust galls on *Acacia* (Mimosaceae) (Common 1990; reared material in NZAC).

ZooKeys 865: 67–85 (2019)
doi: 10.3897/zookeys.865.35111
http://zookeys.pensoft.net

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Three new species of *Herpetogramma* Lederer (Lepidoptera, Crambidae) from China

Xiao-Qiang Lu[1], Ji-Ping Wan[1], Xi-Cui Du[1]

[1] *College of Plant Protection, Southwest University, Chongqing, China*

Corresponding author: *Xi-Cui Du* (duxicui@hotmail.com)

Academic editor: *Bernard Landry* | Received 3 April 2019 | Accepted 1 July 2019 | Published 22 July 2019

http://zoobank.org/F92B5251-0EC4-4737-BBF5-E3BDAC642637

Citation: Lu X-Q, Wan J-P, Du X-C (2019) Three new species of *Herpetogramma* Lederer (Lepidoptera, Crambidae) from China. ZooKeys 865: 67–85. https://doi.org/10.3897/zookeys.865.35111

Abstract

Five species of the genus *Herpetogramma* in China are studied with morphological and DNA barcode data. *Herpetogramma biconvexa* Wan, Lu & Du, **sp. nov.**, *H. longispina* Wan, Lu & Du, **sp. nov.**, and *H. brachyacantha* Wan, Lu & Du, **sp. nov.** are described as new. *Herpetogramma rudis* (Warren) and *H. magna* (Butler) are newly diagnosed. Photographs of the habitus and genitalia of these five species are provided.

Keywords

DNA barcodes, Maximum Likelihood analysis, morphology, Pyraloidea, Spilomelinae, *Syllepte invalidalis*

Introduction

The genus *Herpetogramma* was established by Lederer (1863) for the type species *H. servalis* Lederer, 1863. There are 103 species of *Herpetogramma* recorded in the Global Information System on Pyraloidea (Nuss et al. 2019). Twenty-two species were recorded in China before our study (Wang 1980; Wang and Speidel 2000; Du 2008; He 2014). This genus was well studied in North America (Solis 2010; Handfield and Handfield 2011; Scholtens and Solis 2015). In regions around China, 10 species were recorded in Korea (Bae et al. 2008; Kim et al. 2012; Roh et al. 2014; Park et al. 2016), 18 in Japan (Yamanaka 1960, 1976; Inoue 1982; Yamanaka 2003; Sasaki and Yamanaka 2013), two in Nepal and three in India (Yamanaka 1995; Mathew 2006). Most species found in these surrounding regions are distributed in China. The adults of most species are brown of various shades with distinct wing patterns, so that light brown, brown, and dark brown are used to describe three general major shades. The genitalia structures are very conservative, exhibiting only subtle differences among species (Shaffer and Munroe 1989; Du 2008, 2009; Solis 2010, 2011; Handfield and Handfield 2011). Dissection is essential to identify species of this genus. In this paper, morphological and DNA barcode data were combined to identify three new species of *Herpetogramma*.

Materials and methods

Taxon sampling

Specimens were collected by light trap and killed by ethylacetate or ammonium hydroxide. Genitalia preparation mainly followed the methods introduced by Li and Zheng (1996). Genitalia were examined and described before being mounted on microscope slides. The images of the adults were taken with a digital camera (Nikon P7700) and the images of the genitalia were prepared with a digital camera (Leica DFC 450) attached to a digital microscope (Leica M205 A).

Specimens examined, including types of new species, are deposited in the College of Plant Protection, Southwest University, Chongqing, China (SWUCPP) except for six specimens, including three paratypes, which are deposited in the Insect Collection of the College of Life Science, Nankai University, Tianjin, China (NKU). Among 80 sequences analyzed in the study, 12 sequences were downloaded from the BOLD database at http://v4.boldsystems.org/, two sequences were downloaded from GenBank and 66 newly obtained sequences were deposited in GenBank and can be accessed through the accession numbers listed in Table 1.

DNA extraction, PCR amplification, and sequencing

In total twelve species of *Herpetogramma* were included for PCR analysis and DNA sequencing (Table 1). Total DNA was extracted from legs of fresh or dry specimens using the TIANGEN DNA extraction kit following the manufacturer's instructions. The 658-base pair (bp) barcode region of COI was amplified with the LepF1/LepR1 primers (Hajibabaei et al. 2006). PCR products were sent to Sangon Biotechnology Co., Ltd. (Shanghai, China) for sequencing using the aforementioned primers.

Data analysis

All COI sequences were aligned by MUSCLE 3.8 and corrected by eye after being translated into amino acid sequences (Edgar 2004). Intraspecific and interspecific genetic divergence values were quantified based on the Kimura 2-parameter (K2P)

Table 1. Sample information for the *Herpetogramma* and the outgroup specimens included in the study.

Species	Sequence ID	Location	Accession number
H. basalis (Walker, 1866)	SWU201500270	Guangxi, China	MK950840
	SWU201500271	Guangxi, China	MK950841
	SWU201500273	Guangxi, China	MK950842
	SWU201500275	Yunnan, China	MK950843
	SWU201500276	Yunnan, China	MK950844
	–	Madagascar	MIMAD518-15
H. biconvexa sp. nov.	SWU201600108	Sichuan, China	MK950798
	SWU201600172	Sichuan, China	MK950792
	SWU201600173	Sichuan, China	MK950793
	SWU201600174	Sichuan, China	MK950793
	SWU201500175	Yunnan, China	MK950794
	SWU201600176	Sichuan, China	MK950796
	SWU201600177	Sichuan, China	MK950797
	SWU201600178	Sichuan, China	MK950790
	SWU201600179	Sichuan, China	MK950791
H. bipunctalis (Fabricius, 1794)	SWU201500041	Guangxi, China	MK950820
	SWU201500042	Hainan, China	MK950821
	SWU201500043	Hainan, China	MK950822
H. brachyacantha sp. nov.	SWU201600017	Sichuan, China	MK950819
	SWU201600018	Sichuan, China	MK950808
	SWU201600019	Sichuan, China	MK950809
	SWU201600088	Sichuan, China	MK950810
	SWU201600089	Sichuan, China	MK950811
	SWU201600091	Sichuan, China	MK950812
	SWU201600092	Sichuan, China	MK950813
	SWU201600093	Sichuan, China	MK950814
	SWU201600106	Sichuan, China	MK950815
	SWU201600107	Sichuan, China	MK950816
	SWU201600120	Sichuan, China	MK950817
	SWU201500121	Sichuan, China	MK950818
H. hipponalis (Walker, 1859)	–	Australia	ANICO104-10
	–	Australia	ANICO105-10
H. licarsisalis (Walker, 1859)	SWU201500082	Guangxi, China	MK950830
	SWU201500141	Yunnan, China	MK950828
	SWU201500143	Yunnan, China	MK950829
	SWU201600147	Tibet, China	MK950827
	–	Madagascar	MIMAD522-15
	–	Pakistan	MAMOT958-10
	–	Australia	ANICO091-10
H. longispina sp. nov.	SWU201600090	Sichuan, China	MK950799
	SWU201600095	Sichuan, China	MK950800
	SWU201600096	Sichuan, China	MK950801
	SWU201600097	Sichuan, China	MK950802
	SWU201600115	Sichuan, China	MK950803
	SWU201600116	Sichuan, China	MK950804
	SWU201600117	Sichuan, China	MK950805
	SWU201600126	Sichuan, China	MK950806
	SWU201600127	Sichuan, China	MK950807
H. magna (Butler, 1879)	SWU201600100	Sichuan, China	MK950823
	SWU201600101	Sichuan, China	MK950824
	SWU201200111	Liaoning, China	MK950825
	SWU201700258	Chongqing, China	MK950826

Species	Sequence ID	Location	Accession number
H. moderatalis Christoph, 1881	SWU201600132	Sichuan, China	MK950831
	SWU201500133	Sichuan, China	MK950832
	SWU201200134	Liaoning, China	MK950833
	SWU201500136	Sichuan, China	MK950834
	SWU201200138	Heilongjiang, China	MK950835
	SWU201200139	Jilin, China	MK950836
H. rudis (Warren, 1892)	SWU201200003	Chongqing, China	MK950782
	SWU201400006	Guangxi, China	MK950783
	SWU201600007	Shaanxi, China	MK950785
	SWU201600008	Shaanxi, China	MK950786
	SWU201400011	Hubei, China	MK950787
	SWU201600047	Sichuan, China	MK950784
	SWU201700263	Chongqing, China	MK950788
	SWU201700264	Chongqing, China	MK950789
H. stultalis (Walker, 1859)	SWU201600243	Tibet, China	MK950837
	SWU201500244	Guizhou, China	MK950838
	SWU201500245	Yunnan, China	MK950839
	–	Papua New Guinea	YAWAN352-14
	–	Papua New Guinea	YAWAN347-14
H. thestealis (Walker, 1859)	–	Canada	LBCS753-07
	–	Canada	LBCS752-07
	–	Canada	LBCS404-07
	–	Canada	LBCS402-07
	–	Canada	HM415780
	–	Canada	KT128083
Syllepte invalidalis South, 1901	LXQ1800167	Shanxi, China	MK950779
	LXQ1800168	Hubei, China	MK950780
	LXQ1800234	Chongqing, China	MK950781

distance model (Kimura 1980) and assessed by MEGA 6.0.6 (Tamura et al. 2013). Phylogenetic analysis was performed based on Maximum Likelihood (ML) with the GTR GAMMA model of nucleotide substitution, and with 1000 bootstrap replicates (Stamatakis et al. 2008). *Syllepte invalidalis* South, 1901 was chosen as the outgroup as it was never considered to be congeneric with *Herpetogramma*, but is part of the same subfamily (Spilomelinae).

Results

DNA sequence analysis

A total of 77 COI sequences of *Herpetogramma* were analyzed. The dataset contained no obvious pseudogenes, supporting the assumption that the correct target gene sequence was amplified and sequenced.

We observed 12 monophyletic clades for *Herpetogramma* in the resulting phylogenetic tree (Fig. 1). The pairwise genetic distances within and between these lineages are given in Table 2. Average intraspecific genetic distance ranged from 0.03 to

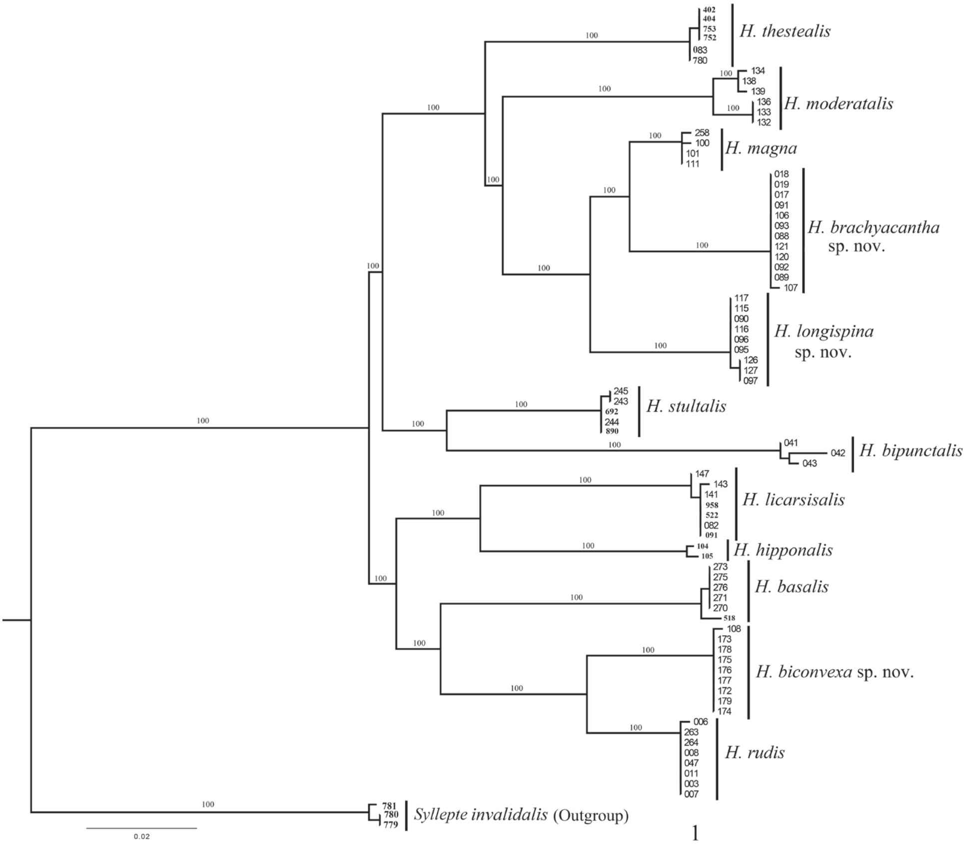


Figure 1. Phylogenetic hypothesis of relationships among 12 species of *Herpetogramma* inferred from a Maximum Likelihood (ML) analysis of the DNA barcode data, with *Syllepte invalidalis* as outgroup.

Table 2. Average Kimura 2-parameter genetic distances in percent, calculated within (in bold) and between species of *Herpetogramma*.

	1	2	3	4	5	6	7	8	9	10	11	12
1. *H. rudis* (*N* = 8)	**0.04**											
2. *H. biconvexa* sp. nov. (*N* = 9)	3.34	**0.03**										
3. *H. basalis* (*N* = 6)	6.96	6.96	**0.15**									
4. *H. longispina* sp. nov. (*N* = 9)	8.31	9.16	8.15	**0.08**								
5. *H. brachyacantha* (*N* = 12)	8.91	9.23	8.08	4.88	**0.03**							
6. *H. magna* (*N* = 4)	8.22	8.35	7.43	3.46	2.93	**0.15**						
7. *H. thestealis* (*N* = 6)	6.39	6.18	6.88	5.78	6.36	5.12	**0.08**					
8. *H. moderatalis* (*N* = 6)	8.98	8.98	8.32	6.64	6.60	6.03	6.93	**0.75**				
9. *H. licarsisalis* (*N* = 7)	7.48	8.40	7.41	9.59	10.02	9.56	8.97	8.58	**0.09**			
10. *H. hipponalis* (*N* = 2)	7.01	8.28	6.93	7.75	8.47	7.95	7.88	7.14	6.29	**0.31**		
11. *H. bipunctalis* (*N* = 3)	8.46	8.53	9.33	9.84	11.17	9.94	8.43	10.41	9.29	9.92	**0.61**	
12. *H. stultalis* (*N* = 5)	7.43	7.94	7.97	8.34	8.54	7.50	7.17	8.01	7.63	8.00	6.98	**0.09**

0.75% while average interspecific genetic distance ranged from 2.93 to 11.17%. The monophyla observed in the phylogenetic analysis were in full congruence with our morphological hypotheses for the investigated species, i.e., our morpho-species hypotheses are in accordance with the barcode clusters recovered (Fig. 1).

Taxonomy

Herpetogramma Lederer, 1863

Herpetogramma Lederer, 1863: 729. Type species: *Herpetogramma servalis* Lederer, 1863, by monotypy.

Pachyzancla Meyrick, 1884: 315. Type species: *Botys mutualis* Zeller, 1852, by monotypy.

Acharana Moore, [1885]. Type species: *Botys otreusalis* Walker, 1859, by original designation.

Stenomeles Warren, 1892: 437. Type species: *Botys agavealis* Walker, 1859, by original designation.

Piloptila Swinhoe, 1894: 142. Type species: *Piloptila nigricornalis* Swinhoe, 1894, by original designation.

Pantoeocome Warren, 1896: 173. Type species: *Pantoeocome deformis* Warren, 1896, by original designation.

Ptiloptila Hampson, 1899: 201. Misspelling.

Stenomelas Hampson, 1912. Misspelling.

Macrobotys Munroe, 1950: 228. Type species: *Botys aeglealis* Walker, 1859, by original designation.

Coremataria Amsel, 1956: 207. Type species: *Botys infuscalis* Guenée, 1854, by original designation and monotypy.

Culcitaria Amsel, 1957: pl. 39, fig. 1. Type species: *Botys infuscalis* Guenée, 1854, by monotypy.

Diagnosis. Frons rounded. Labial palpus obliquely upturned, porrect or upcurved; third segment short and blunt. Maxillary palpi filiform. Male antenna with ventral cilia. Forewing with length of cell ca. half of wing; R from cell at ca. four-fifths above; Rs_1 closely approximated to Rs_2+Rs_3; Rs_4 curved toward Rs_2+Rs_3 at base; M_2, M_3 and CuA_1 from posterior angle of cell, nearly evenly spaced at base; CuA_2 from cell at four-fifths below. Hindwing with length of cell less than half of wing length; discocellulars arcuate curved; Rs anastomosed with Sc+R ca. basal one-fourth beyond cell; M_2, M_3 and CuA_1 from posterior angle of cell, M_2 and M_3 approximated at base; CuA_2 from cell at two-thirds below (Fig. 2). Male genitalia: Uncus basiconic, narrow normally, with hairs distally; valva at base with small lamellate projection or clasper in some species. Female genitalia: Apophysis anterioris longer than apophysis posterioris; ductus bursae shorter than corpus bursae; corpus bursae with a sub-square signum.

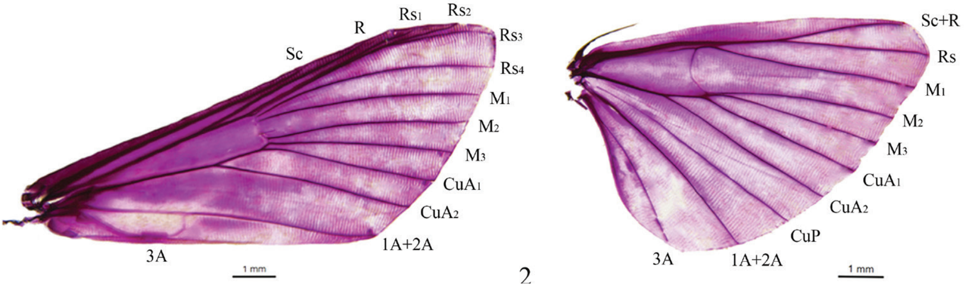


Figure 2. Wing venation of *Herpetogramma rudis* (Warren, 1892).

Checklist of *Herpetogramma* species of China

H. basalis (Walker, 1866)
H. biconvexa sp. nov.
H. bipunctalis (Fabricius, 1794)
H. brachyacantha sp. nov.
H. cynaralis (Walker, 1859)
H. dilatatipes (Walker, 1866)
H. elongalis (Warren, 1892)
H. fuscescens (Warren, 1892)
H. hipponalis (Walker, 1859)
H. hoozana (Strand, 1918)
H. licarsisalis (Walker, 1859)
H. longispina sp. nov.
H. luctuosalis (Guenée, 1854)
H. lulalis (Strand, 1918)
H. magna (Butler, 1879)
H. mimeticalis (Hering, 1901)
H. moderatalis (Christoph, 1881)
H. ochrimaculalis (South, 1901)
H. okamotoi Yamanaka, 1976
H. phaeopteralis (Guenée, 1854)
H. pseudomagna Yamanaka, 1976
H. rudis (Warren, 1892)
H. stultalis (Walker, 1859)
H. subalbescens (Swinhoe, 1894)
H. submarginalis (Swinhoe, 1901)

Key to the new *Herpetogramma* species and their closest relatives based on genitalia

1 Sacculus with a finger-like projection at basal 2/3 on posterior margin, phallus without cornuti; colliculum adjacent to corpus bursae, signum with a distinct lamellate protuberance along diagonal axis 2

– Sacculus without finger-like projection on posterior margin, phallus with cornuti; colliculum adjacent to base of ductus bursae, signum slightly depressed along diagonal axis 3

2 Finger-like projection on posterior margin of sacculus with many tiny protrusions .... ***H. biconvexa*** **sp. nov.**

– Finger-like projection on posterior margin of sacculus without protrusions .... ***H. rudis***

3 Phallus with a cluster of long spinose cornuti (at least 1/4 length of phallus).... 4

– Phallus with a cluster of short spinose cornuti (ca. 1/9 length of phallus) .... ***H. brachyacantha*** **sp. nov.**

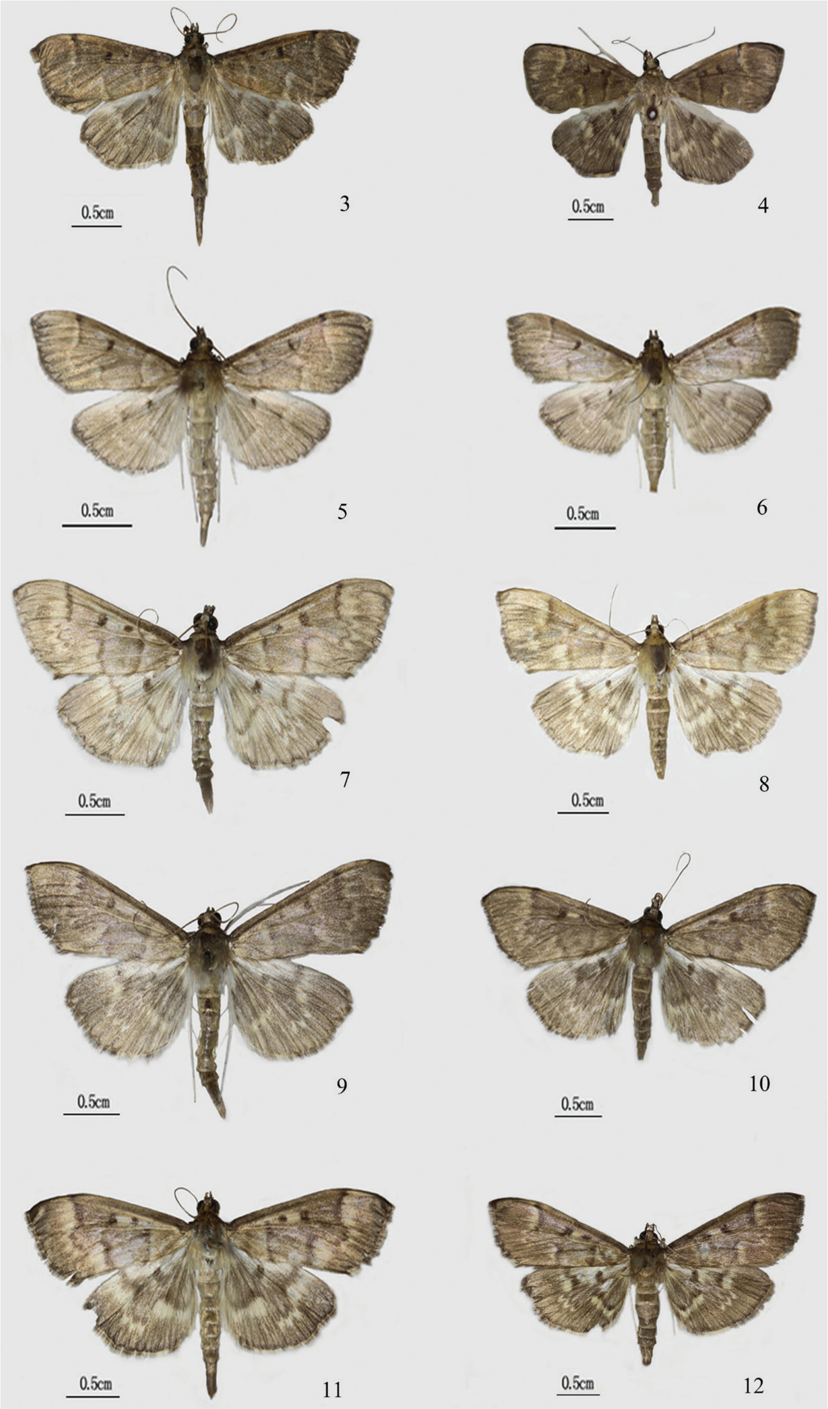
4 Uncus broad, blunt on posterior margin; boundary indistinct between ductus bursae and corpus bursae.. ***H. longispina* sp. nov.**
– Uncus narrowed, pointed on posterior margin; boundary distinct between ductus bursae and corpus bursae.. ***H. magna***

Herpetogramma biconvexa **Wan, Lu & Du, sp. nov.**
http://zoobank.org/2664F46F-0F7C-4DA1-8E39-FBBB1019FE89
Figs 3, 4, 13–16

Type material. Holotype. ♂, pinned, with genitalia in a separate slide. **China**, **Sichuan:** Pingwu, Wanglang Nature Reserve, Baishagou, 103.55°E, 32.49°N, 3100 m, 20.VII.2016, leg. Ji-Ping Wan, genitalia slide no. WJP17419. **Paratypes. China**, **Sichuan:** 1 ♂, 1 ♀, Pingwu, Wanglang Nature Reserve, Changbaigou, 2900 m, 24.VII.2016, leg. Ji-Ping Wan; 11 ♂♂, 7 ♀♀, Ya'an, Baoxing, Fengtongzhai Nature Reserve, 2180 m, 1–3.VIII.2016, leg. Ji-Ping Wan; 2 ♂♂, Anzihe Nature Reserve, 1312 m, 11 & 15.VII.2016, leg. Ji-Ping Wan; **Yunnan:** 1 ♂, Nanjian, Lingbaoshan Forest Park, 2338 m, 26.VIII.2015, leg. Jing-Xia Zhao & Hao Wei; **Tibet:** 6 ♂♂, 1 ♀, Bomi, Tongmai Town, 2100 m, 21.VII.2016, Jian-Yue Qiu. Genitalia slide no.: WJP16178, WJP17371, WJP17383, WJP17389, WJP17422.

Diagnosis. This species is very similar to *H. rudis* (Warren, 1892), but can be distinguished from the latter by the dark brown wings tinged with pale yellow, forewing length 12.5–15.5 mm (wingspan 29.0–34.0 mm), finger-like projection on the posterior margin of the sacculus broad and with many tiny protrusions, and corpus bursae with central depression ca. half depth of diameter of the corpus burse. In *H. rudis* wings are light brown tinged with white, with a forewing length of 9.0–12.0 mm (a wingspan of 21.0–27.0 mm), the finger-like projection on the posterior margin of the sacculus is slender and without tiny protrusions, and the central depression of the corpus bursae is ca. 1/3 the depth of the diameter of the corpus burse.

Description. Adult (Figs 3, 4): Forewing length 12.5–15.5 mm (wingspan 29.0–34.0 mm). Frons rounded, yellowish brown. Vertex with erect brown scales, white close to eye. Antenna dark brown dorsally, yellowish brown ventrally; male antenna with ventral cilia ca. half as long as diameter of flagellomere. Labial palpus obliquely upturned, basal half white and distal half brown. Thorax and abdomen dark brown dorsally, silvery white ventrally. Legs yellowish white; fore tibia brown distally. Wings dark brown tinged with pale yellow, slightly darker in female. Forewing with orbicular spot and discoidal spot black, the latter reniform; antemedial line dark brown, slightly excurved, adjoined by a light-yellow line inside; postmedial line dark brown, from ca. 2/3 of costa, nearly straight to M_1, excurved and serrated from M_1 to CuA_2, then sharply incurved, and nearly vertical to inner margin below posterior angle of cell, adjoined by a light-yellow line outside. Hindwing with pattern of postmedial line similar to forewing; discoidal spot black. Cilia of wings brown, white along anal angle of hindwing. Abdomen long, second segment with two inconspicuous dark spots basally.



Figures 3–12. Habitus of *Herpetogramma* species **3, 4** *H. biconvexa* sp. nov. **3** male, holotype **4** female, paratype **5, 6** *H. rudis* **5** male **6** female **7, 8** *H. longispina* sp. nov. **7** male, holotype **8** female, paratype **9, 10** *H. brachyacantha* sp. nov. **9** male, holotype **10** female, paratype **11, 12** *H. magna* **11** male **12** female.

Male genitalia (Figs 13, 14). Uncus basiconic, distal half narrowed and bearing dorsal hairs, apex pointed and naked. Valva elongate lingulate, densely ciliated, costa slightly widened at middle, and with a small lamellate basal projection bearing hairs distally (Fig. 14A). Sacculus with a broad finger-like projection bearing many tiny protrusions at basal 2/3 of posterior margin, sparse long hairs on top of protrusions (Fig. 14B). Transtilla subtriangular, weakly sclerotized, meeting in middle. Juxta oval, a weakly sclerotized plate. Saccus developed, triangular, distinctly pointed distally. Phallus cylindrical, nearly same length as valva, without cornuti.

Female genitalia (Figs 15, 16). Apophysis anterioris ca. twice length of apophysis posterioris. Ductus bursae very short, ca. 1/8 length of corpus bursae; colliculum as long as diameter of ductus bursae, adjacent to corpus bursae. Corpus bursae elongate, elliptical, broad anteriorly, with a central depression ca. half depth of diameter of corpus bursae. Signum square, near depression of corpus bursae, with a distinct lamellate protuberance along diagonal axis (Fig. 16).

Etymology. The specific name, *biconvexa*, is derived from the Latin *bi* (meaning ‘two’ or ‘double’) and *convexus* (meaning ‘arched outward’), in reference to the finger-like projection bearing tiny protuberances on the posterior margin of the sacculus.

Distribution. China (Sichuan, Yunnan, Tibet).

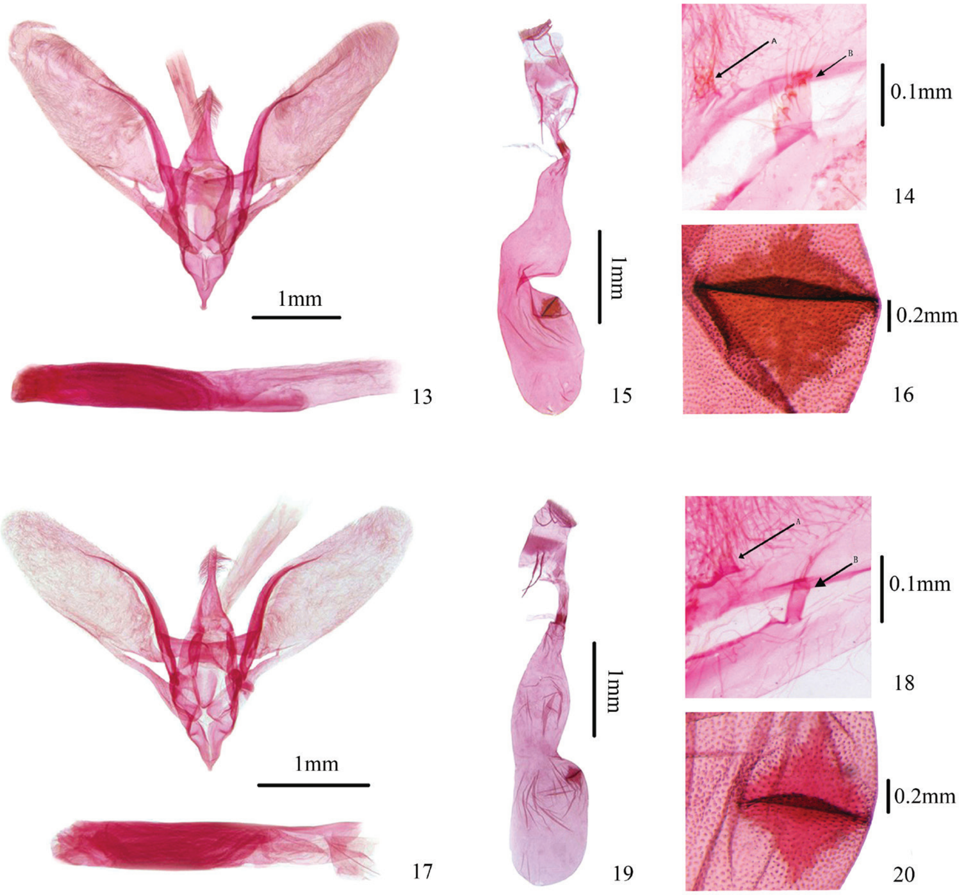
***Herpetogramma rudis* (Warren, 1892)**

Figs 5, 6, 17–20

Acharana rudis Warren, 1892: 435.
Psara rudis: Shibuya 1929: 205.
Pachyzancla rudis: Inoue 1955: 182.
Herpetogramma rudis: Lee and Park 1958: 8.
Herpetogramma rude [sic]: Yamanaka and Yoshiyasu 1992: 88.

Material examined. China, **Chongqing:** 1 ♂, Simian Mountain Nature Reserve, 1000 m, 20.VII.2012, leg. Xi-Cui Du & Li-Fang; 1 ♂, Simian Mountain Nature Reserve, Wangxiangtai, 900 m, 18.VII.2012, leg. Gui-Qing He; 1 ♂, 1 ♀, Jinyun Mountain Nature Reserve, Shamuyuan, 10–11.IX.2009, leg. Xi-Cui Du; **Yunnan:** 2 ♂♂, Malipo, 1098 m, 4.VI.2015, leg. Man-Fei Tao; 1 ♀, Malipo, Chuantou Town, 193 m, 9.VI.2015, leg. Man-Fei Tao; **Guangxi:** 1 ♂, Jingxi, Tengmao, 672 m, 13.VII.2015, leg. Xu Dan; **Hubei:** 2 ♀♀, Enshi, Xingdou Mountain Nature Reserve, Sanxian, 1200 m, 30.VII.2012, leg. Jun Zhang & Xiao-Bin Fu; **Zhejiang:** 1 ♂, 1 ♀, Tianmu Mountain, Zhonglieci, 400 m, 27.VII.2011, leg. Xi-Cui Du & Xiao-Bin Fu. Genitalia slide no.: WJP16066, WJP16067, WJP16077, WJP16085, WJP16103, WJP16140, WJP16141, WJP16142, WJP16143, WJP16180, WJP 16181, WJP16182, WJP16183, WJP17259, WJP17358, WJP17360, WJP17361.

Diagnosis. Adult (Figs 5, 6): Forewing length 9.0–12.0 mm (wingspan 21.0–27.0 mm). Wings light brown tinged with white, lines and spot brown. Forewing



Figures 13–20. Genitalia of *Herpetogramma* species **13–16** *H. biconvexa* sp. nov. **13, 14** male, holotype, genitalia slide no. WJP17419 **15, 16** female, paratype, slide no. WJP17383 **17–20** *H. rudis* **17, 18** male, genitalia slide no. WJP16318 **19, 20** female, genitalia slide no. WJP16316 **14, 18** A: lamellate projection on base of valva; B: projection on posterior margin of sacculus **16, 20** signum of female genitalia.

with postmedial line excurved and serrated from M_1 to CuA_2, adjoined by a light-yellow-white line outside. Male genitalia (Figs 17, 18): Uncus with distal 1/3 narrowed and bearing dorsal hairs, apex pointed and naked. Valva elongate lingulate, with a small basal lamellate projection bearing hairs distally (Fig. 18A). Sacculus with a slender finger-like projection bearing long hairs at basal 2/3 of posterior margin (Fig. 18B). Phallus without cornuti. Female genitalia (Figs 19, 20): Corpus bursae elongate, elliptical, with a central depression ca. 1/3 depth of diameter of corpus bursae. Signum near depression of corpus bursae, with a distinct lamellate protuberance medially along diagonal axis (Fig. 20).

Distribution. China (Chongqing, Sichuan, Guizhou, Yunnan, Shaanxi, Henan, Hubei, Anhui, Zhejiang, Fujian, Guangxi, Hainan, Tibet), Korea, Japan, India (Bae et al. 2008; Du 2008, 2009).

Remarks. The identification of *H. rudis* was based on the description and photographs of external morphology and genitalia (Warren 1892; Inoue 1982; Bae et al. 2008; Sasaki and Yamanaka 2013). Although species of this genus have similar appearance and conservative genitalia, they can be differentiated according to their subtle and definite characteristics. In addition, it was found that the genitalia of *H. biconvexa* and *H. rudis* were different in size, but the ratios between structures were nearly the same, such as the length ratio between the uncus and the valva at ca. 1:4, ca. 4:3 between the uncus and the saccus, ca. 4:5 between the phallus and the transverse distance of the valva, and nearly 1:7 between the ductus bursae and the corpus bursae. We have not observed these characteristics in other similar species studied.

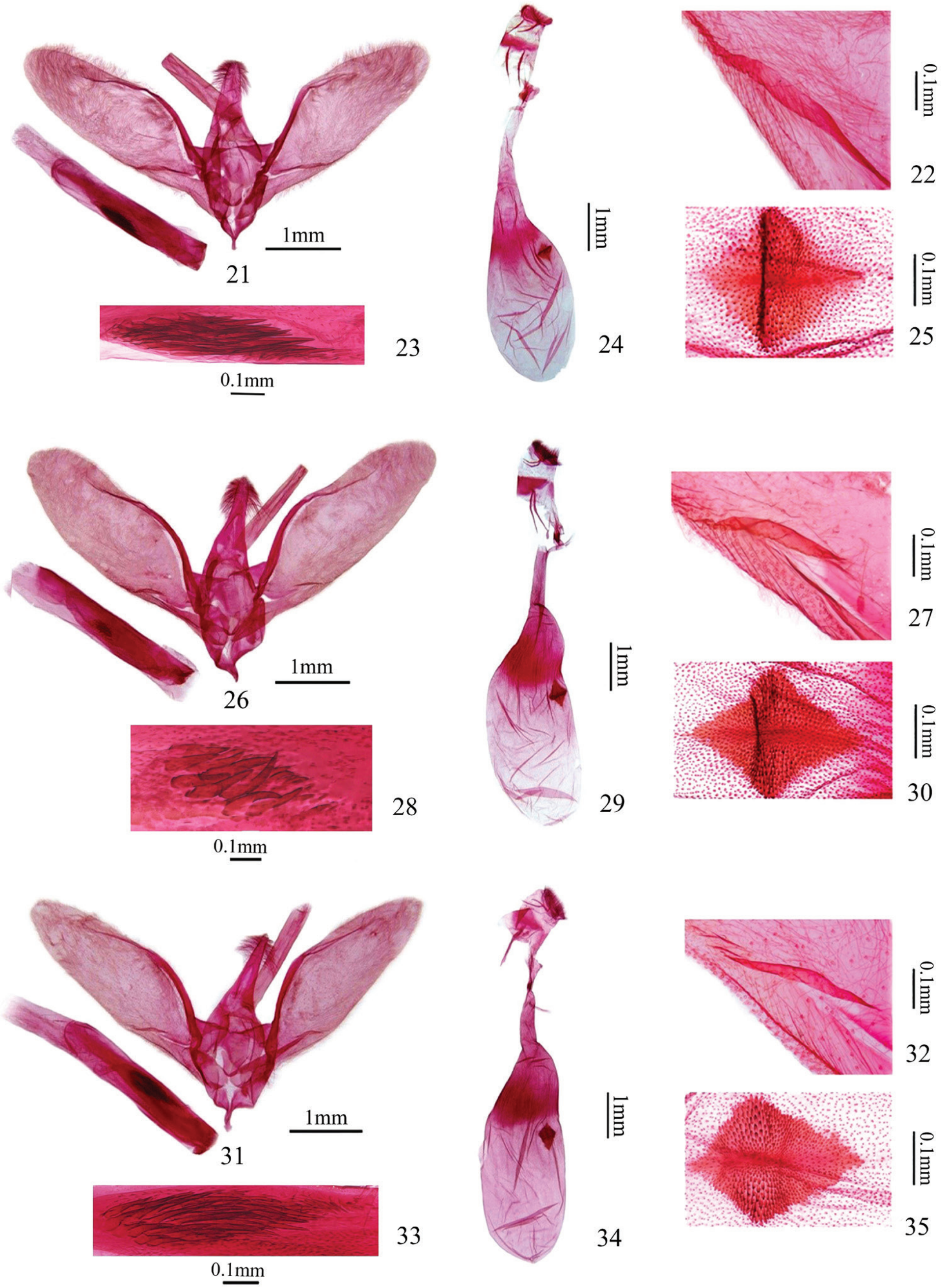
Herpetogramma longispina Wan, Lu & Du, sp. nov.

http://zoobank.org/62F95F4C-2374-4699-96EA-46AB0357F7F4
Figs 7, 8, 21–25

Type material. Holotype. ♂, pinned, with genitalia in a separate slide. **China**, **Sichuan:** Yingjing, Longcanggou, 102°49'22"E, 29°31'5"N, 1610 m, 20.VI.2016, leg. Jian-Yue Qiu, genitalia slide no.: WJP17418. **Paratype. China**, **Sichuan:** 6 ♂♂, 2 ♀♀, Yingjing, Longcanggou, 1610 m, 18–20.VI.2016, leg. Jian-Yue Qiu; 16 ♂♂, 3 ♀♀, Anzihe Nature Reserve, 1312 m, 11–15.VII.2016, leg. Ji-Ping Wan; 1 ♂, 1 ♀, Ya'an, Baoxing, Fengtongzhai, 2180 m, 1.VIII.2016, leg. Ji-Ping Wan; **Hubei:** 1 ♂, 1 ♀, Enshi, Xingdoushan Nature Reserve, Sanxian, 1200 m, 1–2.VIII.2012, leg. Jun Zhang & Xiao-Bin Fu. Genitalia slide no.: WJP17365, WJP17370, WJP17374, WJP17381, WJP17417, WJP17420.

Diagnosis. The species is similar to *H. magna* (Butler, 1879), but can be distinguished from the latter by its light brown wings, the broad uncus blunt at apex and elongate lingulate valva, corpus bursae sharply narrowed posteriorly, and the boundary indistinct between the ductus bursae and corpus bursae. In *H. magna*, wings are brown or dark brown; the narrowed uncus is pointed at apex and the valva is subfusiform, the corpus bursae is slightly narrowed posteriorly, and the boundary is distinct between the ductus bursae and corpus bursae.

Description. Adult (Figs 7, 8). Forewing length 14.5–16.0 mm (wingspan 32.0–34.0 mm). Frons rounded, brown or light brown. Vertex with erect orange-yellow scales. Antenna light brown, male antenna with ventral cilia ca. half as long as diameter of flagellomere. Labial palpus obliquely upturned, basal 2/3 white and distal 1/3 light brown. Thorax and abdomen light brown dorsally, silvery white ventrally. Legs silvery white, fore tibia brown basally. Wings light brown tinged with faint yellow, lines and spots brown, distinct. Forewing with orbicular spot and reniform discoidal spot, faint yellow between orbicular spot and discoidal spot. Antemedial line excurved slightly, adjoined by a light-yellow wider line inside; postmedial line from ca. 2/3 of costa, straight to M_1, excurved and pointedly serrated from M_1 to CuA_2, then sharply



Figures 21–35. Genitalia of *Herpetogramma* species. **21–25** *H. longispina* sp. nov.: **21–23** male, holotype, genitalia slide no. WJP17418 **24, 25** female, paratype, genitalia slide no. WJP17370 **26–30** *H. brachyacantha* sp. nov.: **26–28** male, holotype, genitalia slide no. WJP17421 **29, 30** female, paratype, genitalia slide no. WJP17379 **31–35** *H. magna*: **31–33** male, genitalia slide no. WJP17423 **34, 35** female, genitalia slide no. WJP17416 **22, 27, 32** lamellate protuberance on base of valva **23, 28, 33** cornuti in male genitalia **25, 30, 35** signum of female genitalia.

incurved, and nearly vertical to inner margin below posterior angle of cell, adjoined by a wide and serrated light-yellow line outside. Hindwing with pattern of postmedial line similar to forewing, discoidal spot reniform. Cilia of wings brown, white along anal angle of hindwing.

Male genitalia (Figs 21, 22, 23). Uncus basiconic, broad and shorter, distal 1/3 bearing dorsal setae, apex blunt. Valva elongate lingulate, densely ciliated and bearing a lamellate basal projection (Fig. 22). Juxta cupped, with posterior margin concave and protruding posterolaterally. Saccus subtriangular, short and broad, distinctly pointed distally. Phallus cylindrical; a cluster of long spinose cornuti gathered to subfusiform, ca. 1/4 length of phallus (Fig. 23).

Female genitalia (Figs 24, 25). Apophysis anterioris slightly longer than apophysis posterioris. Ductus bursae short, ca. 1/3 length of corpus bursae; colliculum near base of ductus bursae. Boundary indistinct between ductus bursae and corpus bursae. Corpus bursae nearly pear-shaped, sharply narrowed posteriorly, with shallow depression at basal 1/3. Signum nearly square, slightly depressed along diagonal axis (Fig. 25).

Etymology. The specific name, *longispina*, is derived from the Latin *longus* (meaning 'long') and *spina* (meaning 'thorn'), in reference to a cluster of long spinose cornuti in male genitalia.

Distribution. China (Sichuan, Hubei).

Herpetogramma brachyacantha Wan, Lu & Du, sp. nov.

http://zoobank.org/533445AD-7A9C-4FF0-B6A1-2A3EE9C40D6D
Figs 9, 10, 26–30

Type material. Holotype. ♂, pinned, with genitalia in a separate slide. **China**, **Sichuan:** Anzihe Nature Reserve, 30°N, 1312 m, 15.VII.2016, leg. Ji-Ping Wan, genitalia slide no.: WJP17421. **Paratype. China**: **Sichuan:** 69 ♂♂, 2 ♀♀, Anzihe Nature Reserve, 1312 m, 11–16.VII.2016, leg. Ji-Ping Wan; 1 ♀, Anzihe Nature Reserve, 1690 m, 5.VIII.2015, leg. Xi-Cui Du; 21 ♂♂, 12 ♀♀, Ya'an, Baoxing, Fengtongzhai Nature Reserve, 2180 m, 1–3.VIII.2016, leg. Ji-Ping Wan; 4 ♂♂, Yingjing, Longcanggou, 1610 m, 18–20.VI.2016, leg. Jian-Yue Qiu; 3 ♀♀, Mabian, Yonghong, 1500 m, 23.VII.2004, leg. Ying-Dang Ren (NKU); 1 ♂, Tianquan, Xiaorenyan 1042 m, 9.VII.2012, leg. Jing-Wei Li. Genitalia slide no.: DXC06542, WJP17368, WJP17369, WJP17373, WJP17375, WJP17376, WJP17377, WJP17378, WJP17379, WJP17387, WJP17388, WJP17425.

Diagnosis. The species is similar to *H. longispina* sp. nov. and *H. magna* (Butler). It can be distinguished from them by its wing pattern, which is not as distinct as those of the latter two species. In the male genitalia, its uncus is thinner than that of *H. longispina* and thicker than that of *H. magna*; its cornuti are the shortest, ca. 1/9 of the length of the phallus, while in the latter two species, they are ca. 1/4 of the phallus length. In the female genitalia, the corpus bursae are slightly narrowed posteriorly and the boundary is distinct between ductus bursae and corpus bursae in this species and *H. magna*, while the corpus bursae is sharply narrowed posteriorly and the boundary is indistinct between the ductus bursae and corpus bursae in *H. longispina*.

Description Adult (Figs 9, 10). Forewing length 13.5–15.5 mm (wingspan 31.0–35.0 mm). Frons rounded. Vertex with erect dark brown scales. Antenna brown, male antenna with ventral cilia ca. half as long as diameter of flagellomere. Labial palpus obliquely upturned, basal 2/3 white and distal 1/3 fuscous. Thorax and abdomen dark brown dorsally, silvery white ventrally. Legs silvery white, fore tibia brown distally. Wings brown tinged with faint yellow, lines and spots dark brown and slightly indistinct in female. Forewing with orbicular spot and reniform discoidal spot, faint yellow between orbicular spot and discoidal spot. Antemedial line indistinct, postmedial line from 2/3 of costa, straight to M_1, excurved and pointedly serrated from M_1 to CuA_2, then sharply incurved, and nearly vertical to inner margin below posterior angle of cell, adjoined by a serrated light-yellow line outside. Hindwing with pattern of postmedial line similar to forewing, discoidal spot reniform. Cilia of wings brown, white along anal angle of hindwing.

Male genitalia (Figs 26–28). Uncus basiconic, distal 1/3 bearing dorsal setae, apex slightly blunt. Valva elongate lingulate, densely ciliated and bearing a lamellate basal projection (Fig. 27). Juxta cupped, with posterior margin concave and protruding posterolaterally. Saccus subtriangular, short and broad, pointed distally. Phallus cylindrical; a cluster of short spinose cornuti gathered to subfusiform, ca. 1/9 length of phallus (Fig. 28).

Female genitalia (Figs 29, 30). Apophysis anterioris ca. twice length of apophysis posterioris. Ductus bursae short, ca. 1/3 length of corpus bursae; colliculum near base of ductus bursae. Boundary distinct between ductus bursae and corpus bursae. Corpus bursae nearly pear-shaped, slightly narrowed posteriorly, with shallow depression at basal 1/3. Signum nearly square, near to depression on corpus bursae, slightly depressed along diagonal axis (Fig. 30).

Etymology. The specific name, *brachyacantha*, is derived from the Greek words *brachys* (meaning 'short'), *ake* (meaning 'thorn') and *anthos* (meaning 'flower'), in reference to a cluster of short spinose cornuti in male genitalia.

Distribution. China (Sichuan).

***Herpetogramma magna* (Butler, 1879)**

Figs 11, 12, 31–35

Samea magna Butler, 1879: 74. fig. 2.
Sylepta [sic] *magna*: Hampson 1898: 723.
Syllepte magna: Inoue 1955: 175.
Herpetogramma magna: Yamanaka 1960: 322.

Material examined. China, **Chongqing:** 1 ♂, Chengkou County, Mingzhong Town, 1500 m, 19.VII.2017, leg. Ji-Ping Wan; 2 ♂♂, Chengkou County, Dongan Town, Renhe Village, 1100 m, 28.VI.2013, leg. Gui-Qing He & Li-Jun Xu; 2 ♂♂, Chengkou County, Dongan Town, Xingtian Village, 1300 m, 1.VII.2013, leg. Gui-Qing He & Li-Jun Xu; **Sichuan:** 47 ♂, 7 ♀♀, Nanjiang, Guangwu Mountain, 900 m, 8–9.VII.2013, leg. Gui-Qing He & Li-Jun Xu; 5 ♂♂, 4 ♀♀, Ya'an, Baoxing, Fengtongzhai Nature Reserve, 2180 m, 1–3.VIII.2016, leg. Ji-Ping Wan; 4 ♂♂, Anzihe Nature Reserve, 1312 m, 11–15. VII.2016, leg. Ji-Ping Wan; 2 ♂♂, 1♀, Anzihe Nature Reserve, 1312 m, 4–5.VIII.2015, leg. Xi-Cui Du; 1 ♀, Mabian,Yonghong, 1500 m, 23.VII.2004, leg. Ying-Dang Ren (NKU); 1 ♀, Shimian, Tuanjie Village, 1650 m, 24.VIII.2016, leg. Jian-Yue Qiu & Hao Xu; **Yunnan:** 2 ♂♂, Lijiang, Ninglang, Xichuan Town, 2400 m，31.VII.2013, leg. Gui-Qing He; 1 ♀, Tengchong, Dahaoping Town, 2020 m, 5.VIII.2007, leg. Dan-Dan Zhang; **Liaoning:** 2 ♂♂, 1♀, Huanren, Laotuding, 29.VII.2012, leg. Dan-Dan Zhang & Li-Jun Yang; **Jilin:** 1 ♂, 3 ♀♀, Yanbian, Antu, Wanbao Town, 24.VII.2012, leg. Dan-Dan Zhang; 1 ♂, Linjiang, Huashan Town, Laosandui, 25.VII.2012, leg. Li-Jun Yang; **Hubei:** 1 ♀, Wufeng, Maopin Village, 1175 m, 11.IX.2012, leg. Jin-Wei Li; 2 ♂♂, Xianfeng, Pingbaying, 1280 m, 21.VII.1999, leg. Hou-Hun Li (NKU); 1 ♂, 2 ♀♀, Enshi, Xingdou Mountain, Sanxian, 1200 m, 2.VIII.2012, leg. Jun Zhang; **Shaanxi:** 1 ♀, Yingtou Town, Haopingsi, 1251 m, 17.VII.2012, leg. Jin-Wei Li; 1 ♀, Taibai, Huangbaiyuan Town, 19.VIII.2014, leg. Kai-Li Liu & Jiu-Yang Luo. Genitalia slide no.: DXC06205, DXC06548, WJP17414, WJP17416.

Diagnosis. Adult (Figs 11, 12): Wings brown or dark brown tinged with faint yellow, lines and spots dark brown. Forewing with faint yellow between orbicular spot and discoidal spot, postmedial line excurved and pointedly serrated from M_1 to CuA_2, adjoined by a serrated light-yellow line outside. Male genitalia (Figs 31–33): Uncus narrowed, distal 1/3 bearing dorsal setae, apex pointed. Valva subfusiform, densely ciliated and bearing a lamellate basal projection (Fig. 32). Phallus with a cluster of long spinose cornuti gathered to subfusiform, ca. 1/4 the length of the phallus (Fig. 33). Female genitalia (Figs 34, 35): Corpus bursae nearly pear-shaped. Signum nearly square, slightly depressed along diagonal axis (Fig. 35).

Distribution. China (Chongqing, Sichuan, Guizhou, Yunnan, Liaoning, Jilin, Tianjin, Shaanxi, Hubei, Hunan, Taiwan), Korea, Japan, India, Sri Lanka (Hampson 1898; Bae et al. 2008; Du 2008).

Remarks. The identification of this species was based on the description and photographs of external morphology and genitalia (Butler 1879; Inoue 1982; Bae et al. 2008; Sasaki and Yamanaka 2013).

Discussion

Species of *Herpetogramma* are generally so similar in adult morphology and genitalia that a combined analysis of external morphology, genitalia structures and molecular data is strongly advised for their identification. The three new species described in this paper were discovered by integrating these three sources of data. Over the course of the last decade, an extensive number of specimens of this genus was collected by the members of our laboratory in most regions of China. Judging from preliminary research on these specimens, more new species and/or new records of this genus may be discovered in China in the future. We aim to collect more fresh specimens and obtain more molecular data of *Herpetogramma* species from China and intend to review the whole genus in the future.

Acknowledgements

We are grateful to the other members of our laboratory as well as to Jian-Yue Qiu and Hao Xu for their efforts in collecting specimens, and to Dr Dan-Dan Zhang (Sun Yat-Sen University, China) and Dr Hai-Li Yu (Northwest University, China) for donating specimens to us. Cordial thanks are given to Prof. Hou-Hun Li (NKU) for lending us specimens. We also give our special thanks to Prof. Shu-Xia Wang (NKU), Richard Mally (University Museum of Bergen, Norway), Bernard Landry (Muséum d'histoire naturelle, Geneva, Switzerland), M. Alma Solis (Smithsonian Institution, USA) and an anonymous reviewer for carefully revising our manuscript. The project was supported by the National Natural Science Foundation of China (No. 31772500), by Chongqing Research Program of Basic Research and Frontier Technology (No. cstc2018jcyjAX0544) and by the Insects survey in Anzihe protected area (supported by Conservation International) and the Insects survey in Wanglang Nature Reserve, Sichuan, China.

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ZooKeys 865: 87–102 (2019)
doi: 10.3897/zookeys.865.32878
http://zookeys.pensoft.net

RESEARCH ARTICLE

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A new species of *Hyalella* (Crustacea, Amphipoda, Hyalellidae) from the Puna biogeographic province in Argentina

Marcela Alejandra Peralta[1], Águeda Verónica Isa Miranda[1]

[1] *Instituto de Invertebrados, Fund. M. Lillo. Miguel Lillo 251, T4000JFE-San Miguel de Tucumán, Argentina*

Corresponding author: *Marcela Alejandra Peralta* (maperalta@lillo.org.ar)

Academic editor: *C.O. Coleman* | Received 5 January 2019 | Accepted 4 March 2019 | Published 22 July 2019

http://zoobank.org/1DFAC573-2B4C-4FFF-AF5C-F7304EAAE4BD

Citation: Peralta MA, Isa Miranda ÁV (2019) A new species of *Hyalella* (Crustacea, Amphipoda, Hyalellidae) from the Puna biogeographic province in Argentina. ZooKeys 865: 87–102. https://doi.org/10.3897/zookeys.865.32878

Abstract

A new species of freshwater amphipod belonging to genus *Hyalella* is described from a peatbog at high altitudes (3,650 to 4,400 m above sea level) in the Puna region (Salta province, northwestern Argentina). The new species can be distinguished from other hyalellid species by the following combination of characters: dorso-posterior flanges on pleonites I–III; palp on maxilla 1 reaching almost half of distance between base of palp and base of setae on outer plate, and two papposerrate setae on the inner margin of inner plate of maxilla 2; propodus of gnathopod 1 hammer-shaped, inner face with seven serrate setae; propodus of gnathopod 2 ovate; male uropod 1 without curved seta on inner side of inner ramus; ramus of uropod 3 shorter than peduncle; six pair of sternal gills on pereionites II–VII.

A detailed morphological description and illustrations of the new species are provided. In Argentina, the new species represents the third record for the genus at altitudes greater than 2,000 m a.s.l., after *H. kochi* and *H. fossamancinii* (Dos Santos et al. 2008, González 2003), and the first record above 4,000 m a.s.l. Some comments about distributional and ecological aspects of the new species are included. With this new species, the number of *Hyalella* species known from Argentina and Falkland Islands (Islas Malvinas) rises to 12.

Keywords

Distribution, epigean, freshwater macroinvertebrates, South America, taxonomy

Introduction

Within Amphipoda, the talitroid genus *Hyalella* Smith, 1874 is dominant in the surface freshwaters of South America. This genus is only known within the Neotropical and Nearctic regions. The natural environments inhabited by *Hyalella* include both surface (epigean) and groundwater (hypogean) habitats in a wide range of geographical heights, from sea level to more than 4,000 meters above sea level (a.s.l.).

At present, 73 species of *Hyalella* are known (Bastos-Pereira et al. 2018, Drumm and Knight-Gray 2019), but many remain undescribed. The highest diversity corresponds to Brazil, with 25 species (Streck et al. 2017). The species recorded so far in the freshwater environments of Argentina and the Falkland Islands (Islas Malvinas) are *H. curvispina* Shoemaker, 1942; *H. fossamancinii* Cavalieri, 1959; *H. pampeana* Cavalieri, 1968; *H. neonoma* Stock & Platvoet, 1991; *H. falklandensis* Bousfield, 1996; *H. rionegrina* Grosso & Peralta, 1999; *H. patagonica* Cunningham, 1871; *H. kochi* González & Watling, 2001; *H. bonariensis* Bond-Buckup, Araujo & Santos, 2008; *H. misionensis* Colla & César, 2015; and *H. pseudoazteca* González & Watling, 2003.

The Puna, a region that spans part of northeastern Chile, northwestern Argentina, southeastern Peru, and midwestern Bolivia, is characterized by the presence of endorheic basins at high elevations. In particular, the Puna peatbogs are freshwater bodies that function as natural sponges that hold and slowly release water, and thus help to regulate its transfer to surrounding areas. These peatbogs are ecosystems that hold the greatest biodiversity of the region and are highly vulnerable due to their ecological fragility and climate change (Vuille et al. 2008), which underscores the importance of furthering the knowledge of their biological diversity.

Hyalella is the most conspicuous taxon in the benthic macroinvertebrate communities of the Puna Mountains in Salta province, Argentina (Nieto et al. 2017). As part of the results of a project for the study of freshwater macroinvertebrates (Fundación Miguel Lillo), a new *Hyalella* species is described herein. The new species occurs in the Puna biogeographic region, in wetlands at altitudes greater than 4,000 m a.s.l. in the Argentinian Andes.

Materials and methods

Samples were collected with the aid of a net and fixed in formaldehyde or 96% ethanol in situ. Once in the laboratory, specimens were transferred to 80% ethanol and dissected in Brunson solutions (glycerin, ethanol, and distilled water) under a stereomicroscope, and appendages illustrated using a Leitz Dialux camera lucida. Body measurements were made from the tip of the head to the tip of the telson. Photographs of the male paratype were taken with the aid of a stereomicroscope LEICA M165 C coupled with LEICA DMC 2900 digital camera. The geographic distribution map was digitally generated using the shapefiles from Arana et al. (2017) with the Esri ArcGIS 10.5 desktop software.

The terminology for setae follows Zimmer et al. (2009). The description of the new species was made based on previous taxonomic works on *Hyalella* species (Grosso and Peralta 1999, González and Watling 2001, 2003, Bastos-Pereira and Bueno 2012). Specimens are deposited in the Crustaceans Collection of Fundación Miguel Lillo, Tucumán, Argentina (**FML**).

Taxonomy

Order Amphipoda Latreille, 1816
Family Hyalellidae Bulycheva, 1957
Genus *Hyalella* Smith, 1874

***Hyalella puna* sp. n.**
http://zoobank.org/1EEAB3A6-AF20-4FE2-925D-383AA5E09AC4
Figs 1–7

Type material. ***Holotype:*** Argentina: ♂, 7.42 mm; Salta, La Poma; 24°30'S, 66°47'W; 4,400 m a.s.l.; 11 Nov. 2000; C. Locascio de Mitrovich leg.; peatbog close to Santa Rosa de los Pastos Grandes, depth 5 cm (FML-CRUST 01261). ***Paratypes:*** Argentina: ♀; same data as for holotype (FML-CRUST 01262). 9 ♂♂; same data as for holotype (FML-CRUST 01263). 6 ♀♀ same data as for holotype (FML-CRUST 01264).

Other material. Argentina: 9 ♂♂, 9 ♀♀; Salta, Vega Los Patos; 25°23'S, 66°54'W; 4,120 m a.s.l; 25 Jan 2010; C. Locascio de Mitrovich leg. (FML-CRUST 01265). 9 ♂♂, 8 ♀♀; Jujuy, Cuenca Pozuelos, Pocitos; 22°27'S, 66°00'W; 3,650 m a.s.l.; 23 Jan 2010; A. González Aschem leg.; Physicochemical water parameters, temperature 14 °C, pH 7.5, 0.24 ms conductivity, 157 ppm total solids, 5.2 mg/l OD, sat. O2: 3.2, depth 20 cm (FML-CRUST 01200).

Type-locality. Argentina, Salta, La Poma; 24°30'S, 66°47'W; 4,400 m a.s.l.; peatbog close to Santa Rosa de los Pastos Grandes, depth 5 cm; 11 Nov. 2000; C. Locascio de Mitrovich leg.

Diagnosis. Body with dorso-posterior flanges on pleonites I–III. Eyes pigmented, ovoid. Antenna 1 shorter than antenna 2. Palp of maxilla 1 longer than wide, reaching almost half of distance between base of palp and base of setae on outer plate; inner plate slender, with two strong and papposerrate apical setae. Inner plate of maxilla 2 with two unequal strong papposerrate setae on inner margin. Propodus of gnathopod 1 length less than two times its maximum width, hammer-shaped, inner face with seven serrate setae; comb-scales on disto-posterior and disto-anterior border. Propodus of gnathopod 2 ovate, palm shorter than posterior margin, without notch, slope transverse, anterior edge smooth. Uropod 1 not sexually dimorphic. Peduncle of uropod 3 with three strong and two thin distal setae and other thin marginal setae; ramus shorter than peduncle. Six pair of sternal gills on pereionites II–VII.

Etymology. Species name refers to the Puna biogeographic province.

Habitat. Freshwater, epigean.

Accompanying fauna and algae. Diptera (Chironomidae), Coleoptera (Staphylinidae and Elmidae); Heteroptera; Ephemeroptera; Crustacea Copepoda (Cyclopoida and Harpacticoida); anuran tadpoles; algae *Nostoc.*

Description of Holotype male. Size, 7.42 mm. Head smaller than first two thoracic segments. Eyes pigmented, large, ovoid. Body with dorso-posterior flanges on pleonites I–III. Epimeral plate I round, plates II and III acuminate. Coxae I to III subequal in size and shape, slightly overlapping. Acumination in coxae absent. Coxa III narrower than IV. Coxa IV as wide as deep, excavated posteriorly. Posterior lobe of coxa V deeper than anterior lobe. Anterior lobe of coxa VI small (Figs 1A, 7).

Antenna 1 (Fig. 1B) much shorter than antenna 2 but longer than peduncle of latter; peduncle longer than head, all segments of peduncle with group of plumose or simple setae and microtrichs, first segment ⅓ longer than second one, third segment slightly shorter than second. Flagellum of nine articles, with group of simple setae, 1–2 aesthetascs per article occurring distally between articles 2–7, distal article with group of eight long setae.

Antenna 2 (Fig. 1C) with peduncle longer than head, article 4 slightly shorter than article 5; articles 3–5 with distal groups of simple setae and microtrichs; article 4 and 5 with medial plumose simple setae. Flagellum with 12 articles, distally with group of five simple setae, each article with distal group of simple setae and medial microtrichs.

Labrum (Fig. 1D) ventral margin truncate, covered by short distal setules.

Mandibles basic amphipodan type (sensu Watling 1993); each with well-developed molar large, cylindrical, triturative. Left mandible (Fig. 1F) incisor 6-denticulate (three short, three long); lacinia 5-denticulate; setal row with four pappose setae. Right mandible (Fig. 1E) incisor 8-denticulate (four short, four long); lacinia complex, with multi-denticles, setal row with four pappose setae.

Lower lip (Fig. 2A) outer lobes rounded with distal, internal, and external setules, mandibular projection of outer lobes truncated.

Maxilla 1 (Fig. 2B) palp uniarticulated, reaching almost half of the distance between base of palp and base of setae on outer plate; nine serrate distal setae on outer plate; inner plate slender, shorter than outer plate, bearing two apical papposerrate setae.

Maxilla 2 (Fig. 2C) inner plate subequal in length to outer plate; inner plate with two unequal robust papposerrate setae proximally on inner margin; outer plate with several apical simple setae; outer and inner plates with several setules.

Maxilliped (Fig. 2D) inner plate apically rounded, longer than wide, with three cuspidate setae, apex and inner margins with pappose and simple setae; outer plate with apical and medial simple setae; palp longer than outer plate, with four articles; inner margins of articles 2 and 3 with long simple setae; outer distal face of article 3 with cluster of simple setae, distal margin with simple and serrated setae; article 4 unguiform, shorter than article 3, distal setae simple and shorter than nail.

Coxal gills (Fig. 4A–C) on gnathopod 2 to pereiopod 6, sac-like. Sternal gills tubular on pereionites II–VII (Fig. 4A–D).

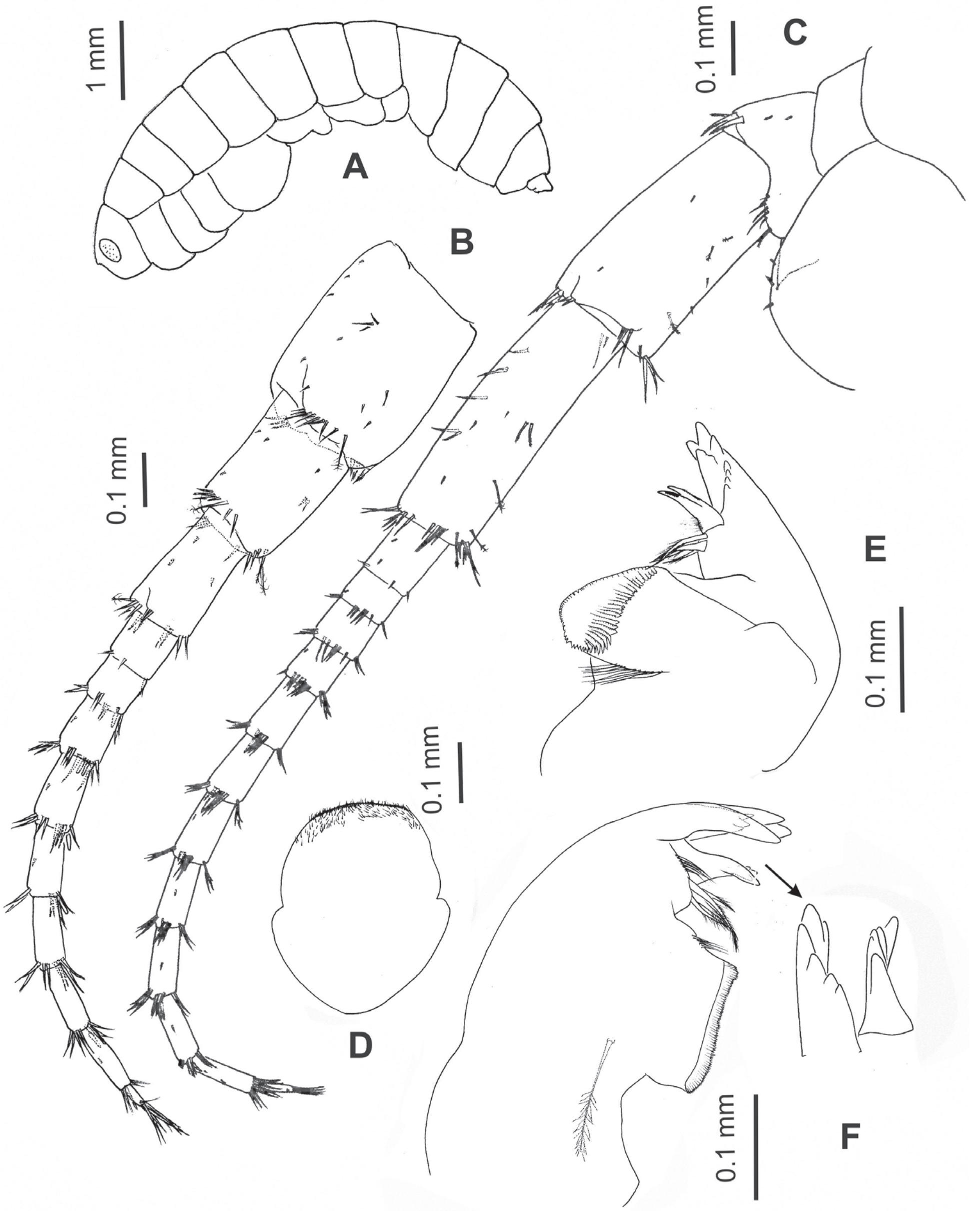

Figure 1. *Hyalella puna* sp. n., male holotype. **A** lateral view of habitus **B** antenna 1 **C** antenna 2 **D** labrum **E** right mandible **F** left mandible, arrow indicates detail of incisor and lacinia.

Gnathopod 1 (Fig. 3A, B) subchelate; basis and ischium with cluster of setae on disto-posterior border; carpus longer than wide, with strong and wide concave posterior lobe, border pectinate and with several serrate setae; propodus length less than two times maximum width, hammer-shaped, without setae on anterior border, inner

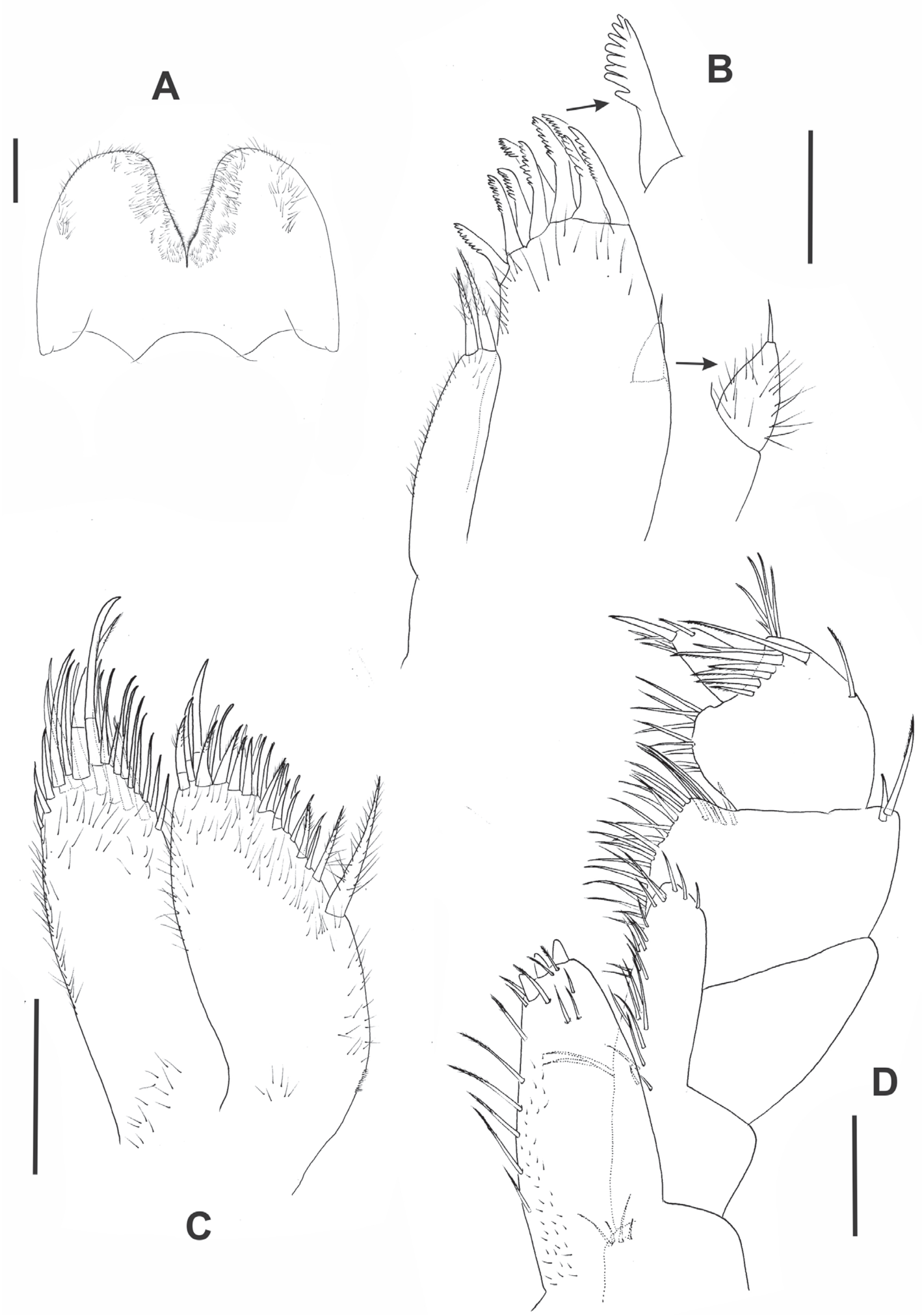

Figure 2. *Hyalella puna* sp. n., male holotype. **A** lower lip **B** maxilla 1, arrows indicate details of seta from outer plate and palp **C** maxilla 2 **D** right maxilliped. Scale bars: 0.1mm.

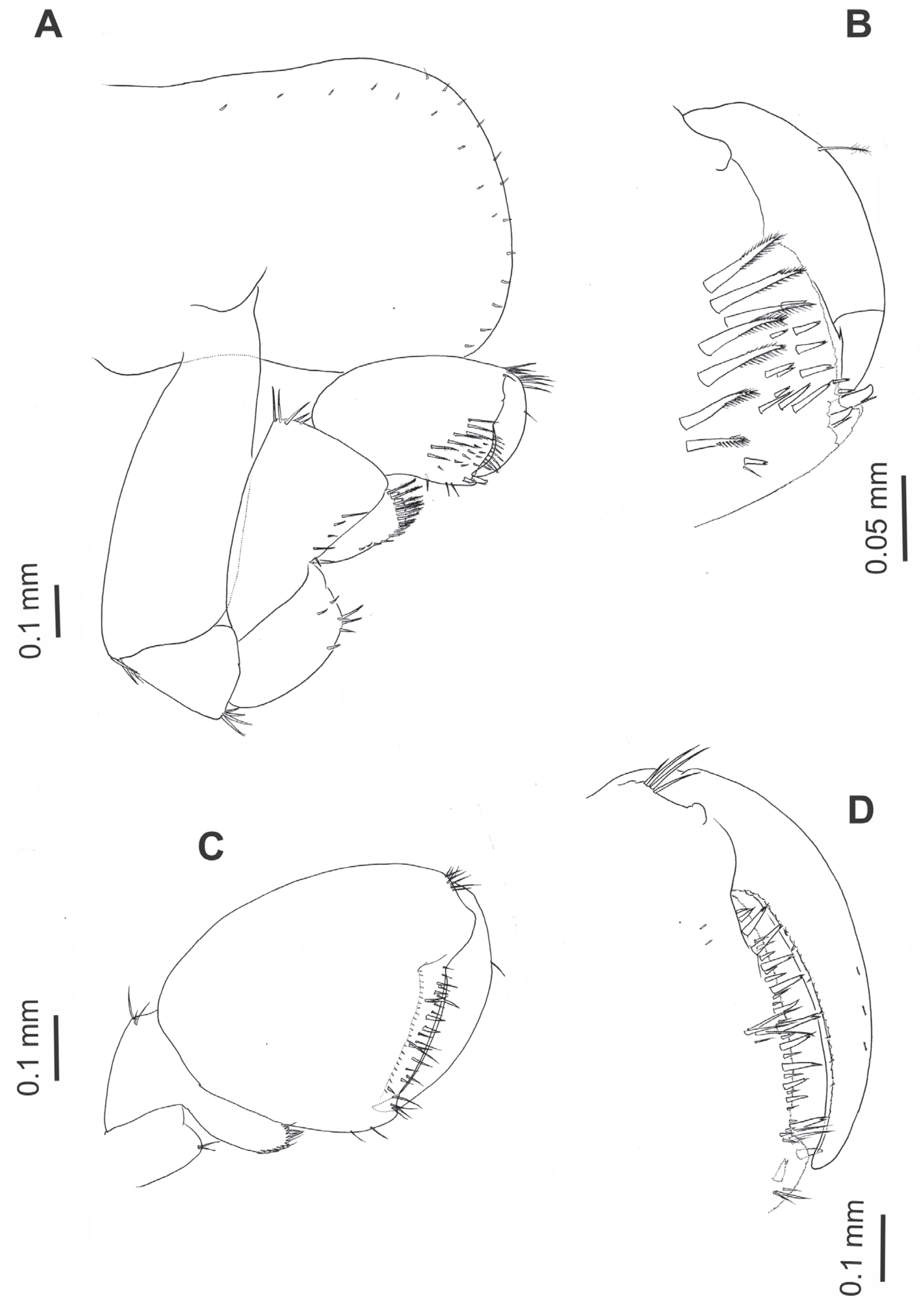

Figure 3. *Hyalella puna* sp. n., male holotype. **A** gnathopod 1 **B** gnathopod 1, detail of propodus and dactylus **C** gnathopod 2 carpus, propodus and dactylus **D** gnathopod 2, detail of propodus, and dactylus.

face with seven serrate setae, and small simple setae, comb-scales on disto-posterior and disto-anterior border, palm slope slightly transverse, margin convex, palm angle with two cuspidate setae with accessory seta; dactylus claw-like, congruent with palm. Palmar Index (sensu Ruffo 1973) = 0.34.

Gnathopod 2 (Fig. 3C, D) subchelate; posterior lobe of carpus elongated, border pectinate with several serrate setae; propodus ovate, comb-scales on disto-posterior margin, palm margin shorter than posterior margin, slope transverse, palm margin straight and regular with several strong short and medium-length setae, few long setae, anterior edge smooth, disto-anterior border with cluster of thin simple setae, palm angle with two cuspidate setae with an accessory seta; dactylus claw-like with several endal setae and comb-scales, congruent with palm, with one thin plumose seta dorsally. Palmar Index (sensu Ruffo 1973) = 0.48.

Pereiopods 3–4 (Fig. 4A, B) similar in size and shape; posterior margins of carpus and propodus with cuspidate and simple setae, posterior margin of merus with simple setae; dactylus less than half the length of propodus, with a plumose seta. Coxal plates: pereiopod 3: longer than wide; pereiopod 4: excavated posteriorly, as long as wide; all coxal plates with small simple setae on margins.

Pereiopods 5–7 (Fig. 4C–E) pereiopod 5 distinctly shorter than 6 and 7, the latter two subequal in length; posterior margin of basis of pereiopods 5–7 expanded (more so in 5 and 7 than in 6) and finely serrate; anterior margins of merus, carpus and propodus with ten marginal clusters of 2–6 cuspidate seta; dactylus less than half the length of propodus, with a plumose seta. Coxal plates: pereiopod 5: wider than long, with two lobes, posterior lobe longer; pereiopod 6: wider than long, with two lobes, anterior lobe much reduced; pereiopod 7: wider than long; all coxal plates with small simple setae on margins.

Pleopods (Fig. 5A) all similar, peduncle shorter than rami, biramous, rami multi-annulated and bearing long plumose setae.

Uropod 1 (Fig. 5B) peduncle longer than rami, with a longitudinal row of three cuspidate setae on dorsal surface and two additional distal setae; rami subequal in length; outer ramus with two dorsal and four distal cuspidate setae; inner ramus with three dorsal and six terminal setae; without curved seta on inner side of inner ramus.

Uropod 2 (Fig. 5C) shorter than uropod 1; peduncle with three setae in a longitudinal row; rami subequal; inner ramus with one dorsal cuspidate seta, apex with six cuspidate setae; outer ramus with two cuspidate setae, one of them shorter, distributed along the ramus, and apex with four cuspidate setae.

Uropod 3 (Fig. 5D) shorter than peduncle of uropod 1, as long as peduncle of uropod 2; peduncle quadrate, wider than ramus, with three strong and two thin distal setae and other thin marginal setae; outer ramus uniarticulated, shorter than peduncle, basal width 1.5 times or less tip of ramus, with four simple distal setae, one very short cuspidate seta with accessory seta.

Telson (Fig. 5E) wider than long, entire, apically rounded, bearing two long simple setae symmetrically distributed on distal margin, and three or four small plumose setae close to each main seta, occasionally with four long simple setae on apical margin (Fig. 5F).

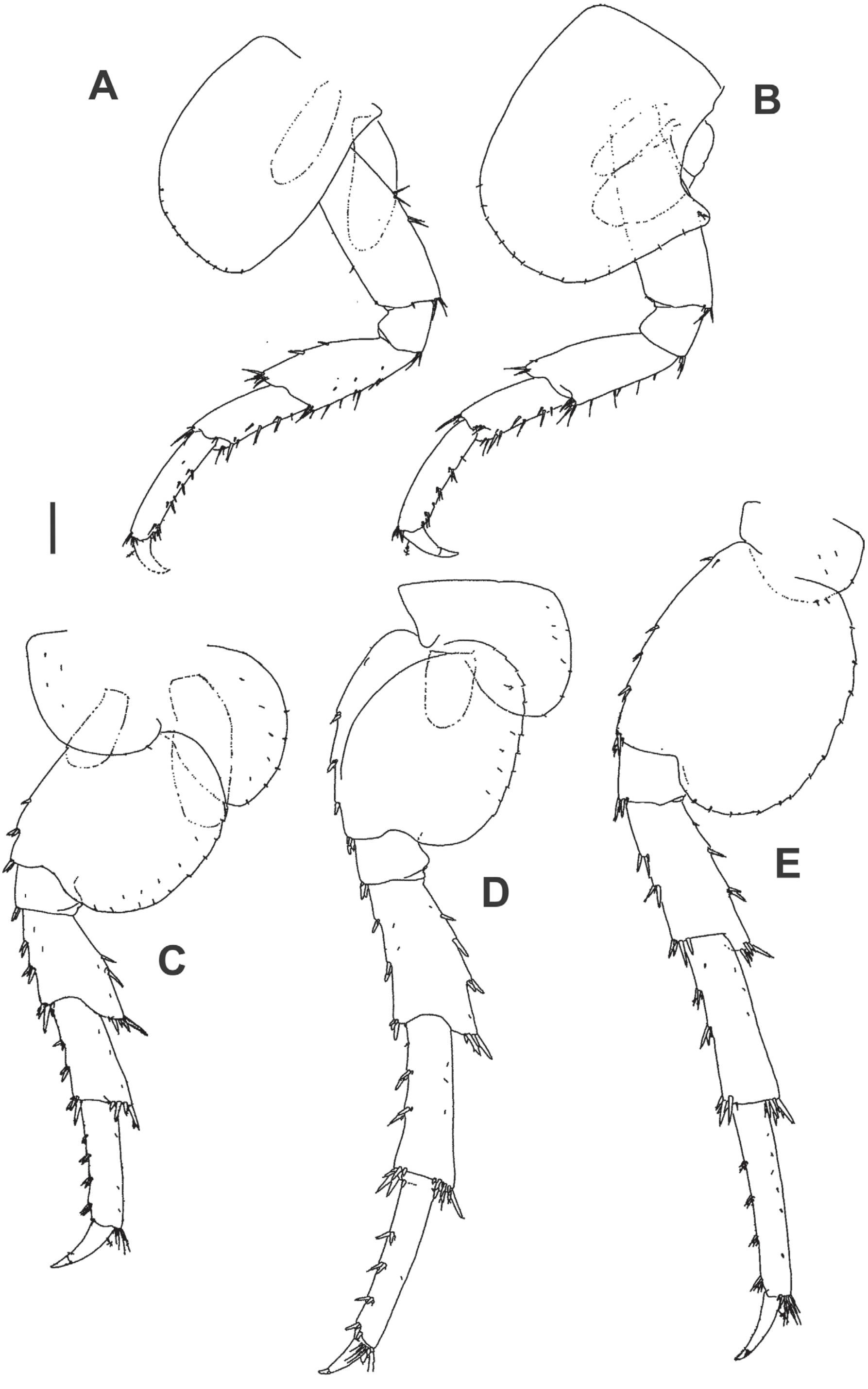

Figure 4. *Hyalella puna* sp. n., male holotype. **A** pereiopod 3 **B** pereiopod 4 **C** pereiopod 5 **D** pereiopod 6 **E** pereiopod 7. Scale bar: 0.2 mm.

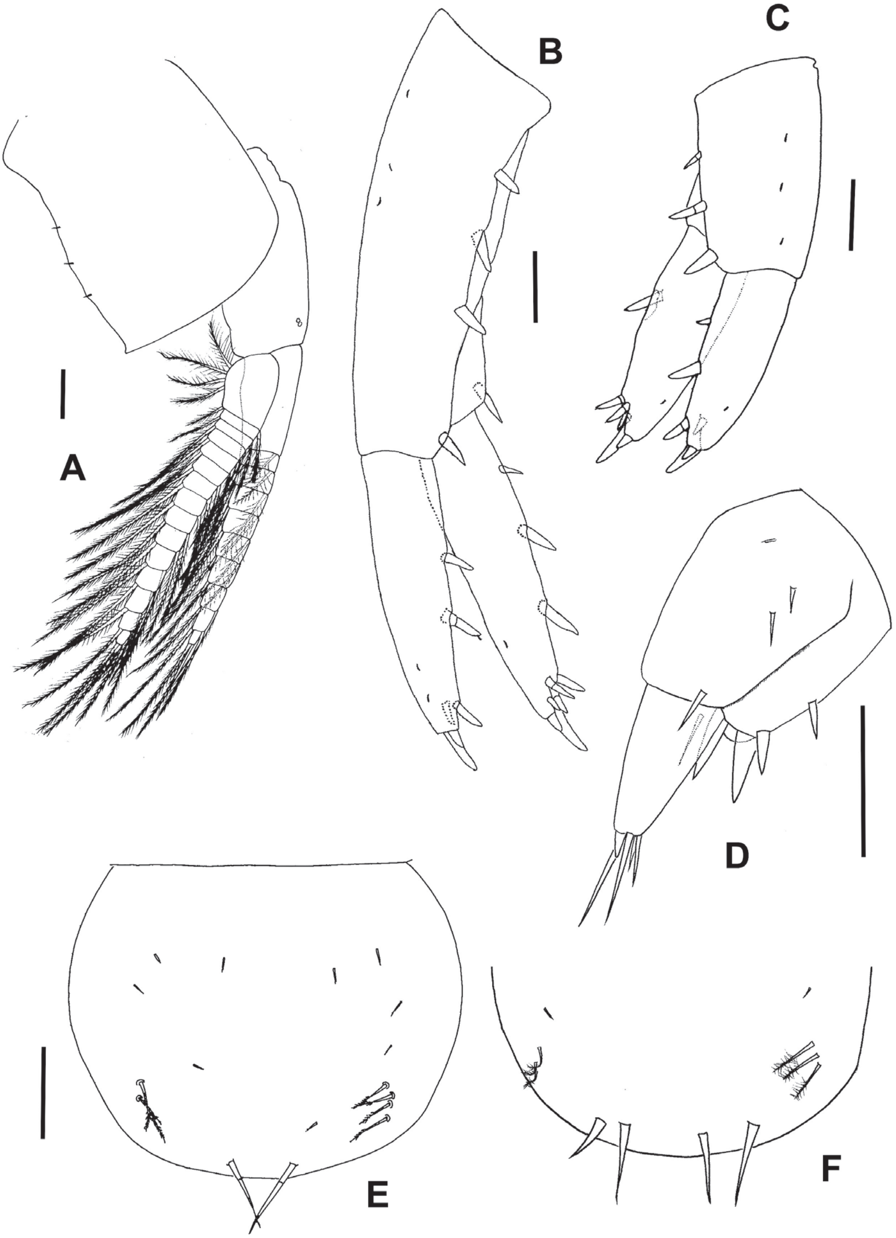

Figure 5. *Hyalella puna* sp. n., male holotype. **A** pleopod 3 **B** uropod 1 **C** uropod 2 **D** uropod 3 **E** telson **F** telson of male paratype. Scale bars: 0.1mm.

Characters of female that differ from male. Female mean body size: 7.78 mm (7 individuals). Presence of foliaceous oostegites, with curl-tipped setae on the margin, on pereionites II–V (Fig. 6B). Both pairs of gnathopods in females similar in size and shape, inner face of propodus of gnathopod 1 with seven serrate setae (Fig. 6A). Gnathopod 2 (Fig. 6B) different from male gnathopod 2 in shape and smaller, propodus

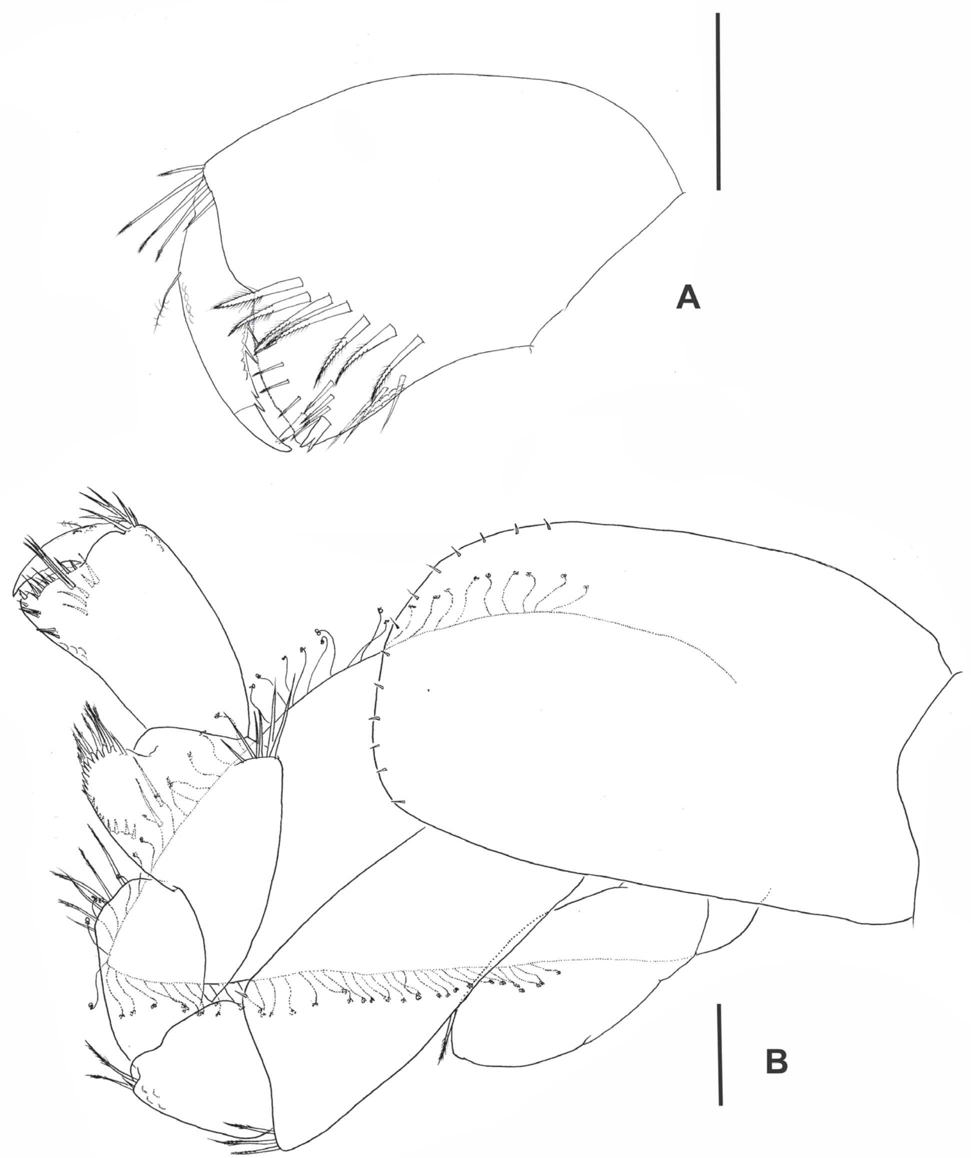

Figure 6. *Hyalella puna* sp. n., female. **A** gnathopod 1, propodus and dactylus **B** gnathopod 2. Scale bars: 0.1mm.

subrectangular, 2–3 times as long as its maximum width, with row of five serrate setae on inner face, palm transverse, subchelate.

Variability. Measurements: Body length ranged from 7.42 to 8.80 mm (males) and 7.33 to 8.13 mm (females). Male mean body size: 7.89 mm (10 individuals). Female mean body size: 7.78 mm (7 individuals). The number of articles of flagellum in antenna 1 varied from 7 to 10 (males) and 7 to 11 (females). In antenna 2 this number ranged from 9 to 14 (males) and 8 to 13 (females).

Taxonomic remarks. This new species can be distinguished from the other Argentine species of *Hyalella* by the flanges on pleonites I, II and III. Beyond this, *Hyalella puna* sp. n. is similar to *H. kochi* in its general morphology, but bearing six sternal gills from pereionites II to VII (*H. kochi*: five sternal gills on pereionites III–VII); ramus of uropod 3 shorter than its peduncle (*H. kochi*: ramus and peduncle equal in length); male uropod 1 without curved seta on inner ramus (*H. kochi:* with curved seta); palp of maxilla 1 longer than wide, reaching almost half of the distance between base of palp and base of setae on outer plate (*H. kochi*: shorter palp); and inner plate of maxilla 2 with two strong pappose setae on inner margin (*H. kochi*: inner plate of maxilla 2 with only one pappose seta).

Table 1 presents a comparison of the main morphological characters of *H. puna* sp. n. and the other Argentinian *Hyalella* species from high elevations, namely *H. kochi* and *H. fossamancinii* (see Fig. 8 for geographical distribution of each species).

Table 1. Male characters of high-altitude *Hyalella* species from Argentina. Key: A1: Antenna 1; A2: Antenna 2; G1: Gnathopod 1; G2: Gnathopod 2; U1: Uropod 1; U3: Uropod 3.

Characters	***H. puna* sp. n.**	***H. kochi* González & Watling, 2001**	***H. fossamancinii* Cavalieri, 1959**
A1: articles of flagellum	9–10	9	9–10
A2: articles of flagellum	10–14	11	9–14
Body length (mm)	7.79 (mean)	6.9	9.42
G1: comb-scales on propodus	present	present	absent
G1: setae on inner face	7	7	more than 10
U1: curved seta on inner ramus	absent	present	absent
Sternal gills on pereionites	II–VII	III–VII	III–VII
Telson: simple apical setae	2–4 long	2 long	12 short
Dorsoposterior flanges on pleonites I–III	present	absent	absent
Maxilla 1: palp length	reaching almost half of the distance between base of palp and base of setae on outer plate	reaching less than a third of the distance between base of palp and base of setae on outer plate	reaching more than half the distance between base of palp and base of setae on outer plate
Maxilla 2: strong paposerrate setae on inner plate	2	1	1
U3: proportion between length of ramus and peduncle	outer ramus shorter than peduncle	subequal	outer ramus shorter than peduncle
Distribution	Salta province: La Poma department, peatbog close to Santa Rosa de los Pastos Grandes (4,400 m). Jujuy province: Cuenca Pozuelos, Pocitos (3,650 m).	Jujuy province: La Quiaca, Yavi Chico River (3,432 m); reservoir near Escuela Agrotécnica in Humahuaca, (2,998 m); Tilcara, a lake north from Tilcara, near Rio Grande, (2,503 m). Tucumán province: Los Sosa River on Route 307 in the direction of Tucumán with Tafí del Valle (1,855m)	San Juan province: Bramadero River, Santa Cruz River (3,500 m), Las Arenas lagoon, Valle Hermoso River, Patillos River.

Figure 7. *Hyalella puna* sp. n., male paratype, habitus.

Ecological and distribution remarks. The type locality of *Hyalella puna* sp. n. is within the Pastos Grandes sub-basin, an area with 90–100 mm total annual rainfall (Nieto et al. 2016). This sub-basin belongs to the "Cuenca Cerrada de la Puna" basin (Nieto et al. 2016). The benthic fauna of the Puna wetlands in Argentina and Chile consists mostly of undetermined *Hyalella* species (Scott et al. 2015, Rodrigues Capítulo et al. 2014, Nieto et al. 2016). In these high Andean wetlands, the macroinvertebrate communities have varying tolerances to different ranges of salinities. Electric conductivity was the second main characteristic associated to compositional changes of the benthic macroinvertebrate communities (Nieto et al. 2017); thus, decreases in the abundance, taxonomic richness, and diversity of various zoobenthic taxa have been attributed to an increase of salinity and conductivity (Rodrigues Capítulo et al. 2014). However, the data reported for physicochemical parameters here (Pozuelos basin, FML-CRUST 01200) and in previous studies of Puna wetlands from northwestern Argentina (Scott et al. 2015; Rodrigues Capítulo et al. 2014; Nieto et al. 2016) have shown that *Hyalella* species are generally more tolerant to greater salinity and conductivity levels (191.15 to 2,203 µS/cm approx.) than other taxa.

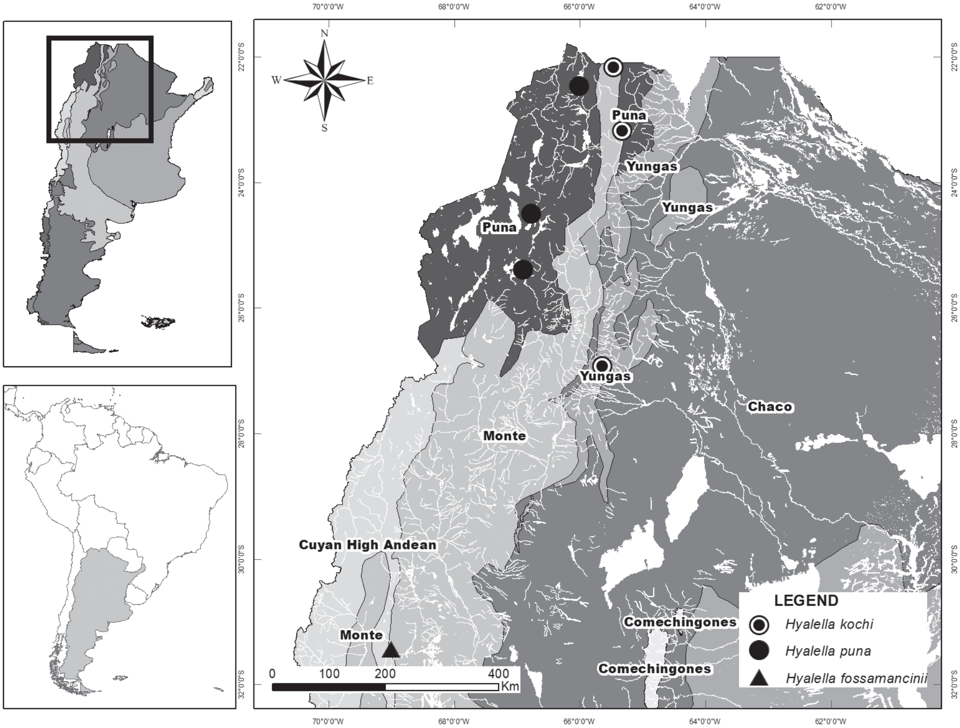

Figure 8. Distribution map of *Hyalella kochi*, *H. fossamancinii,* and *H. puna* sp. n. in northwestern Argentina within a biogeographic scheme.

Little is known about the distribution of benthic macroinvertebrates at high altitudes of the Andean region. *Hyalella puna* sp. n. is known only from high altitude areas, and occurs mostly within the biogeographic Puna province. In Argentina, the new species represents the third record for the genus at altitudes greater than 2,000 m a.s.l., after *H. kochi* and *H. fossamancinii* (Dos Santos et al. 2008, González 2003), and the first record above 4,000 m a.s.l.

If the distribution of the new species is included in a previous panbiogeographic analysis of *Hyalella* species (De Los Ríos Escalante et al. 2012), it is consistent with the 'Central Andes'generalized track. The latter includes inland waters from central Argentina (31°S) to northern Chile (18–26°S). The species assigned to this track are *H. fossamancinii* and *H. kochi* (De Los Ríos Escalante et al. 2012).

Acknowledgements

We thank C. L. de Mitrovich; C. Nieto and Ana Lucía Gonzalez Aschem for donating the specimens, Angela Rodriguez for assistance in elaboration of the distribution map, Marcelo Arana (Universidad Nacional de Río Cuarto, Córdoba, Argentina) for providing the shapefiles, Laura Raquel Figueroa and Programa de Biodiversidad (Secretaría de Ambiente, Salta- Res. 000145, Expte. 0090227-39625-2019-0). Fundación Miguel Lillo provided the institutional support for this research. Pablo Pereyra and Emilia Constanza Perez (FML) helped with the habitus photograph. We also thank Fátima Romero (FML) and two anonymous referees for providing constructive comments.

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ZooKeys 865: 103–121 (2019)
doi: 10.3897/zookeys.865.35827
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Description of *Barathricola thermophilus*, a new species from a deep-sea hydrothermal vent field in the Indian Ocean with redescription of the *Barathricola* type species (Crustacea, Copepoda, Cyclopoida)

Viatcheslav N. Ivanenko[1], Jimin Lee[2], Cheon Young Chang[3], Il-Hoi Kim[4]

1 *Department of Invertebrate Zoology, Biological Faculty, Lomonosov Moscow State University, Leninskie Gory, 1-12, Moscow 119992, Russia* **2** *Marine Ecosystem Research Center, Korea Institute of Ocean Science & Technology, 385 Haeyang-ro, Yongdo-gu, Busan 49111, South Korea* **3** *Department of Biological Science, Daegu University, 201 Daegudae-ro, Gyeongsan 38453, South Korea* **4** *Korea Institute of Coastal Ecology, 397 Seokcheon-ro, Bucheon 14449, South Korea*

Corresponding author: *Viatcheslav N. Ivanenko* (ivanenko.slava@gmail.com)

Academic editor: *D. Defaye* | Received 29 April 2019 | Accepted 28 June 2019 | Published 22 July 2019

http://zoobank.org/9A0189C5-540B-4BF9-87C2-24EB5B74D5A1

Citation: Ivanenko VN, Lee J, Chang CY, Kim I-H (2019) Description of *Barathricola thermophilus*, a new species from a deep-sea hydrothermal vent field in the Indian Ocean with redescription of the *Barathricola* type species (Crustacea, Copepoda, Cyclopoida). ZooKeys 865: 103–121. https://doi.org/10.3897/zookeys.865.35827

Abstract

Re-study of the type species of the genus *Barathricola* Humes, 1999 (Copepoda, Cyclopoida, Schminkepinellidae) described from the Pacific Ocean (Juan de Fuca Ridge), and study of the species *Barathricola thermophilus* **sp. nov.** from a deep-sea hydrothermal vent field on the Central Ridge in the Indian Ocean revealed a derived feature and widespread geographic distribution of this deep-sea genus of cyclopoids. The derived feature of *Barathricola* is the sexually dimorphic third endopodal segment of leg 3 possessing a small outer terminal spine together with spine-like outgrowths on this segment. The new species differs from *Barathricola rimensis* Humes, 1999 in not expressing sexual dimorphism in leg 5, having three spines and one seta on its exopod in both sexes (*B. rimensis* has three spines and one seta on the female exopod but three spines and two setae on the male exopod) and in having broader caudal rami which are 8.9 times longer than wide in the female (this ratio for *B. rimensis* is 11). An amended diagnosis of the genus *Barathricola*, a key and a table of morphological differences for all species of Schminkepinellidae are given.

Keywords

Central Indian Ridge, key, Onnuri vent field, Schminkepinellidae, taxonomy

Introduction

Cyclopoids of the family Schminkepinellidae were discovered in the deep-sea and in marine caves (Martínez Arbizu 2006). The genera initially allocated to Schminkepinellidae were the monotypic genera *Cyclopinella* G.O. Sars, 1913 *Barathricola* Humes, 1999, *Einslepinella* Martínez Arbizu, 2006, *Muceddina* Jaume & Boxshall, 1996, and *Schminkepinella* Martínez Arbizu, 2006. The type species *Cyclopinella tumidula* Sars, 1913 was collected from benthic muds off the Norwegian coast (Sars 1913). *Muceddina multispinosa* Jaume & Boxshall, 1996, the only species of this genus, was collected from anchialine caves on Mediterranean and eastern Atlantic islands (Jaume and Boxshall 1996). Humes (1999) recorded *Barathricola rimensis* Humes, 1999 from a depth of 2254 m at a hydrothermal vent area in the northeastern Pacific. Martínez Arbizu (2006) described *Schminkepinella plumifera* from a depth of 3211 m and *Einslepinella ulrichi* from a depth of 529 m in the Arctic Ocean, both as new genera and species. The family was considered as the sister group of Poecilostomatoida allocated to the order Cyclopoida (Martínez Arbizu 2000). A molecular analysis conducted by Khodami et al. (2017) placed Schminkepinellidae as the sister group of Poecilostomatoida but was not verified by the analysis of Mikhailov and Ivanenko (2019) based on data provided by the authors. Karanovic (2008) described shallow water *Cyclopinella tincanbayensis* Karanovic, 2008 from Queensland in Australia, synonymised *Barathricola* and *Muceddina* with *Cyclopinella* based on characters shared by these two genera and *Cyclopinella.*

In June 2018 the Korea Institute of Ocean Science and Technology (KIOST) made an expedition to deep-sea hydrothermal vent fields on the Central Indian Ridge in the Indian Ocean and sampled benthic habitats, using the research vessel ISABU. Several species of copepods were discovered from this expedition. A new species of the genus *Barathricola*, which is described herein, is among these copepods. In addition, to verify diagnostic characters and the validity of the genus *Barathricola* we restudied the type specimens of the genera *Barathricola* and *Muceddina*.

Materials and methods

Samples of the meiobenthos around the hydrothermal vents of the Onnuri Vent Field (OVF), Central Indian Ridge, Indian Ocean, were made using a TV-grab (Video-Guided Hydraulic Grab, Octopus, Germany) during the deep-sea expedition of the research vessel RV ISABU of the KIOST in June 2018. Sampled sediments were fixed and preserved in 10% formalin for a couple of months. Copepods were sorted out from the sediments and stored in 80% ethanol.

Prior to description of the species, selected copepod specimens were soaked in lactic acid. Dissections were performed using the reversed slide method of Humes and Gooding (1964). The specimens of *Barathricola* and *Muceddina* were studied with a Leica DMR compound microscope using bright-field and differential interference contrast optics. Drawings were made with a camera lucida mounted on the microscope. In the description, the body lengths of the specimens were measured from the anterior margin of the cephalothorax to the end of caudal rami, excluding setae. Type specimens of the new species have been deposited in the Marine Biodiversity Institute of Korea (MABIK), Seocheon, Korea.

Systematic account

Order Cyclopoida Burmeister, 1834
Family Schminkepinellidae Martínez Arbizu, 2006

Genus *Barathricola* Humes, 1999

Amended diagnosis. Cyclopoida. Prosome slender, 5-segmented. Urosome 5-segmented in female, 6-segmented in male, first somite with leg 5. Caudal rami elongate, bearing six or seven setae. Antennule 14-segmented in female and 17-segmented in male; geniculation of male antennules between segments 15 and 16. Antenna 4-segmented, without exopod; armature formula 0-1-5-7. Mandible palp biramous, with elongate basis; endopod 2-segmented, first segment with two, second segment with four setae; exopod small, indistinctly 2-3-segmented, with two terminal setae. Maxillulary coxal endite absent. Maxilla with praecoxa, coxa, basis, and 3-segmented endopod armed with four, two and four setae, respectively. Maxilliped 7-segmented, with syncoxa bearing three (1+2) setae, basis with two setae and 5-segmented endopod with setal formula 1, 1, 1, 1, 3. Legs 1-4 biramous, with 3-segmented rami; armature formula as in Table 1. Leg 1: inner margin of basis bearing long flattened setules. Third endopodal segment of leg 3 with three spines and three setae (1,II,I+2); in male with small outer terminal spine near spine-like outgrowth. Middle endopodal segment of leg 4 with distal inner seta modified into spine. Leg 5 consisting of coxa, basis, and exopod, with intercoxal sclerite; endopod absent; setal formula -0; 1-0; I, I+1+I in female and 0-0; 1-0; I, I+1+I or 0-0; 1-0; I, I+1+I, 1 in male.

Type species. *Barathricola rimensis* Humes, 1999. *Barathricola thermophilus* sp.nov. is the second species of this genus.

Table 1. Spine and setal formulae of legs 1-4 in *Barathricola rimensis* Humes, 1999. Roman numerals indicate spines, and Arabic numerals setae.

	Coxa	Basis	Endopod	Exopod
Leg 1	0-1	1-I	0-1;0-1;1,2,3	I-0;I-1;III,I,4
Leg 2	0-1	1-0	0-1;0-2;1,2,3	I-0;I-1;III,I,5
Leg 3	0-1	1-0	0-1;0-2;1,II,I+2	I-0;I-1;III,I,5
Leg 4	0-1	1-0	0-1;0-1+I;I,II,II	I-0;I-1;II,I,5

***Barathricola thermophilus* sp. nov.**

http://zoobank.org/3AE79CB6-053D-406B-ADA5-69477A4D462A
Figs 1–3

Type locality. The hydrothermal vent field of OVF (11°24'52.97"S, 66°25'25.48"E) on the Central Indian Ridge in the Indian Ocean; sediments at 2022 m in depth.

Type material. Holotype (♀, MABIK CR00244723) and paratypes (6 ♀♀, 6 ♂♂, MABIK CR00244724) have been deposited in the MABIK. Dissected paratypes (2 ♀♀, 1 ♂) are retained in the collection of the last author. All type specimens collected on 23 June 2018 from the type locality.

Description of female. Body (Fig. 1A) slender. Length of dissected and described specimen 776 μm. Other three measured specimens 700, 710, and 715 μm, respectively. Prosome nearly oval, 400 μm long, slightly longer than urosome, consisting of cephalosome and four pedigerous somites. Greatest width of prosome 273 μm across cephalosome. Cephalosome with angular posterolateral corners. First to third pedigerous somites almost equal in length. Fourth pedigerous somite distinctly shorter and narrower than the third. Urosome (Fig. 1B) slender, 5-segmented. Fifth pedigerous somite 38 × 74 μm, broadened distally, with angular posterolateral corners. Genital double-somite 109 × 70 μm, 1.56 times as long as wide, gradually narrowing posteriorly; genital aperture located dorsolaterally at 38% region of double-somite length. Three free abdominal somites 40 × 47, 30 × 42, and 50 × 37 μm, respectively. Anal somite with large anal region and minute spinules along ventrodistal margin. Caudal ramus (Fig. 1C) 116 × 13 μm, 8.92 times as long as wide, more than twice as long as anal somite, armed with six setae and ornamented with row of spinules along ventrodistal margin; outer lateral seta located at 39% region of ramus length; spermatophore (Fig. 1D) attached to female 60 × 27 μm, with thick wall.

Rostrum (Fig. 1E) triangular, with thin-walled lobate distal apex. Antennule (Fig. 1F) 225 μm long, longer than cephalosome, and 14-segmented. Eleventh segment the longest. Armature formula 2-5-4-7-6-(2 + aesthetasc)-0-1-0-1-(2 + aesthetasc)-2-(2 + aesthetasc)-(6 + aesthetasc). Second and third segments each with a trace of one subdivision, and fourth segment with three subdivisions. First segment with two rows of fine spinules. Most of setae naked, except several feebly pinnate ones of proximal two segments. Aesthetascs broad, constricted at region slightly distal to middle, and attenuated distally.

Antenna (Fig. 1G) 4-segmented, consisting of basis and 3-segmented endopod. Basis unarmed, ornamented with several rows of minute spinules. First endopodal segment 36 × 17 μm, with one seta on inner margin and minute spinules proximally and on outer margin. Second endopodal segment narrow proximally and gradually broadened distally, 30 × 18 μm, armed with five setae (three distal and two smaller subdistal) and ornamented with row of minute setules on outer side. Third endopodal segment 23 × 14 μm, armed with seven unequal setae distally, and ornamented with setules on outer side.

Labrum weak, easily destroyed. Mandible (Fig. 1H) consisting of coxa, basis, exopod, and endopod. Coxa with setules on outer margin; cutting margin of gnathobase

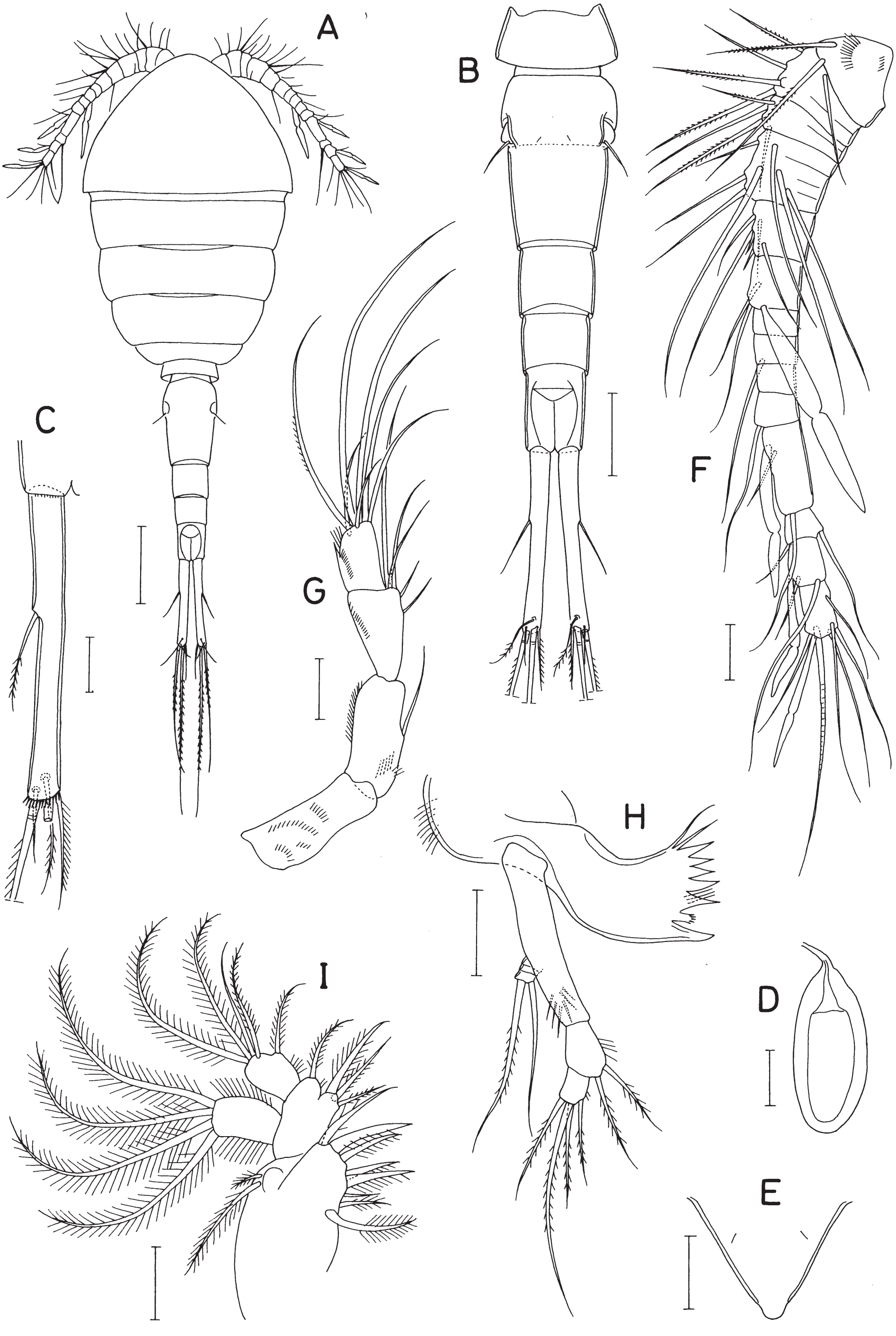

Figure 1. *Barathricola thermophilus* sp. nov., female: **A** habitus, dorsal **B** urosome, dorsal **C** right caudal ramus, ventral **D** spermatophore **E** rostrum **F** antennule **G** antenna **H** mandible **I** maxillule. Scale bars: 0.1 mm (**A**), 0.05 mm (**B**), 0.02 mm (**C–I**).

with six acutely pointed teeth, two thin proximal setae, three setules between distal second and third teeth, and one small, transparent digitiform process bearing fine spinules distally between distal first and second teeth. Basis elongate, 42 × 9 µm, bearing five or six setules subdistally. Exopod small, indistinctly 3-segmented, armed only with two setae on third segment, outer one of these setae sparsely pinnate and slightly longer than inner one. Endopod 2-segmented, armed with two and four setae on first and second segments, respectively, all six setae sparsely pinnate; first segment with several setules on medial margin.

Maxillule (Fig. 1I) with eight setae on praecoxal arthrite; second distal seta spiniform. Coxal endite absent. Epipodite with two unequal setae. Basis with four setae, three proximal and one distal. Exopod with four large setae distally; setae becoming longer from outer to inner margin. Endopod shorter than exopod, armed with five setae, one on medial margin, and four distally.

Maxilla (Fig. 2A) stout, 5-segmented, consisting of syncoxa, basis, and 3-segmented endopod. Syncoxa armed with 11 setae, grouped as four, one, three, and three on first to fourth endites, respectively; third and fourth endites ornamented with two spinules at distal region. Basis armed with three unequal setae (one large, proximally unarticulated, spiniform, one long, and one small setae) and ornamented with one spinule. First endopodal segment with four setae (two proximal and two distal). Second endopodal segment with two long setae; third endopodal segment small, with four setae (one long and three shorter).

Maxilliped (Fig. 2B) slender, 7-segmented, consisting of syncoxa, basis, and 5-segmented endopod. Syncoxa with several scattered rows of minute setules, and armed with three setae, proximal one small and naked. Basis with two setae and rather long setules on medial margin. Endopod armed with one, one, one, one, and three setae on first to fifth segments, respectively; middle seta on terminal segment naked, much longer than other setae, longer than basis and endopod combined. Articulation incomplete between third and fourth endopodal segments.

Legs 1–4 (Figs 2C–E; 3A) with 3-segmented exopod and endopod, lacking inner seta on first exopodal segment; third exopodal segment distinctly broader than proximal segments. All intercoxal sclerites smooth without spinule/setule array along distal margin and on both anterior and posterior surfaces. Endopods of legs 1-3 shorter than exopod, but that of leg 4 distinctly longer than exopod. Leg 1 (Fig. 2C) basis with seven thick setules on inner margin; inner distal spine large, 48 µm long, extending to middle of third endopodal segment, spinulose along both margins. Leg 2 (Fig. 2D) with inner coxal seta characteristically bent at proximal quarter; outer seta on basis shorter than those of legs 1, 3 and 4. Inner distal corner of basis of legs 2-4 with pointed dentiform process. Leg 3 (Fig. 2E) with two distal spines on third endopodal segment (outer spine ca. half as long as inner spine). Leg 4 (Fig. 3A), third endopodal segment elongate, 3.6 times as long as wide; inner distal seta on second endopodal segment and two inner and one outer setae on third endopodal segment transformed to spines. Armature formula for legs 1-4 as in Table 1.

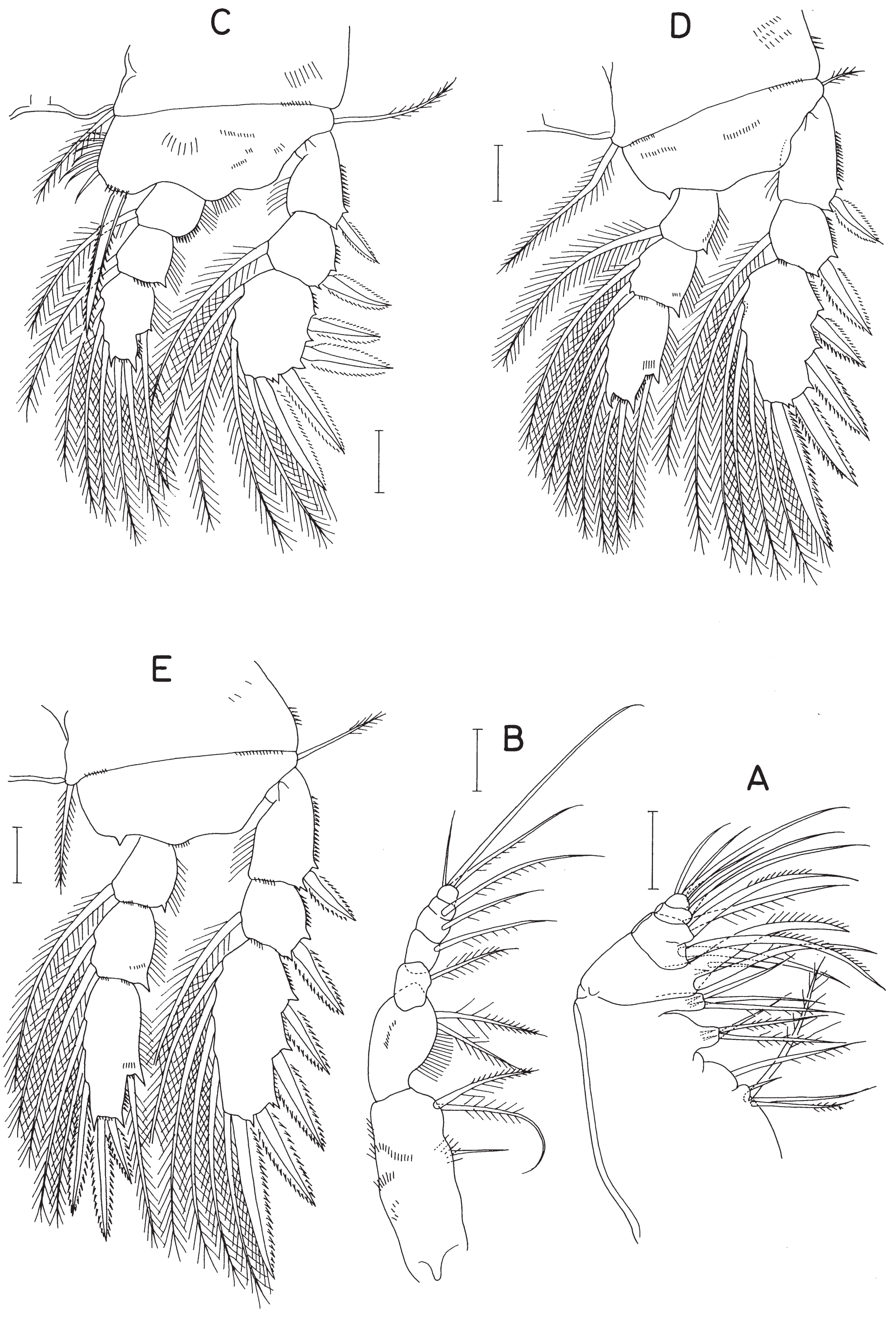

Figure 2. *Barathricola thermophilus* sp. nov., female: **A** maxilla **B** maxilliped **C** leg 1 **D** leg 2 **E** leg 3. Scale bars: 0.02 mm.

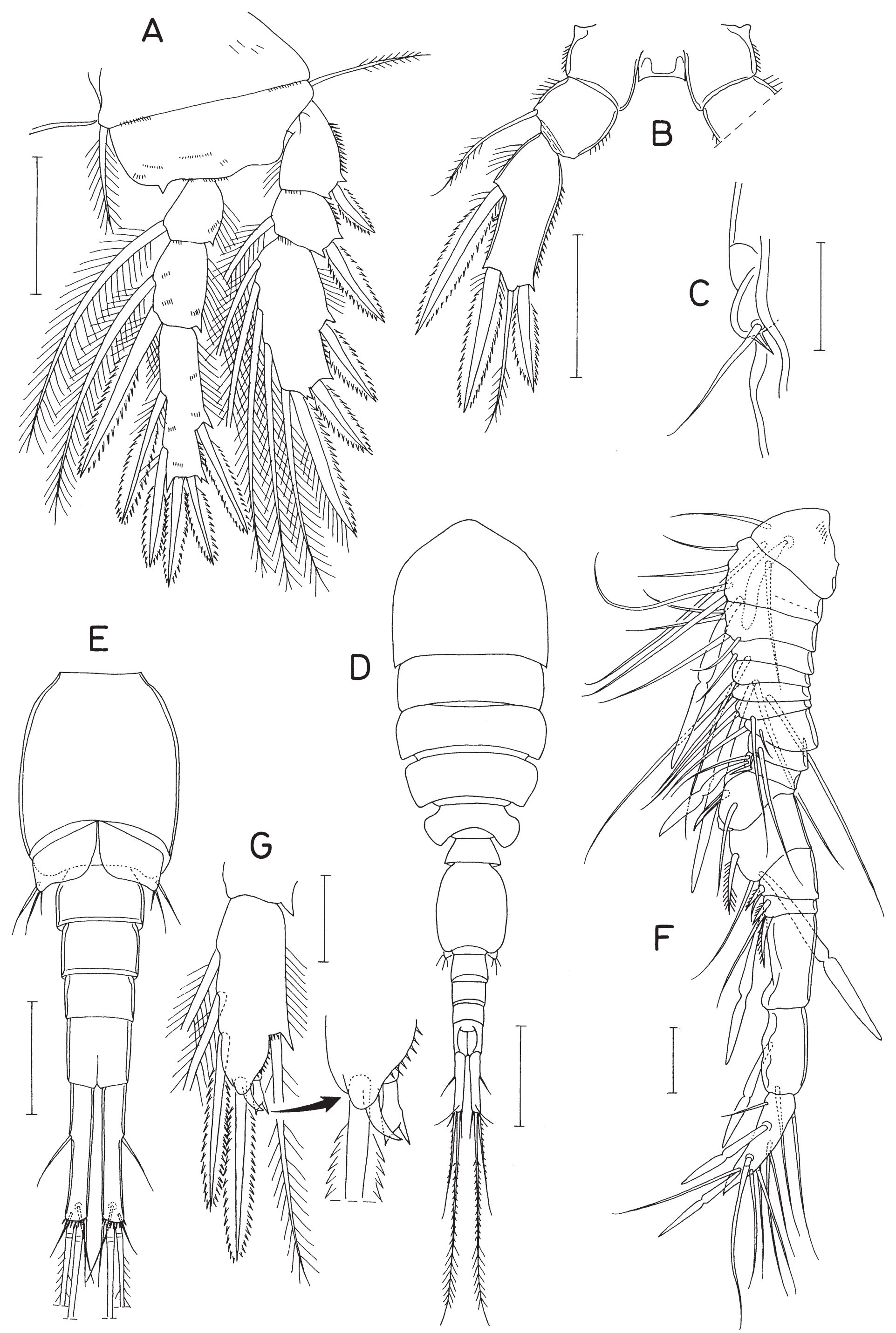

Figure 3. *Barathricola thermophilus* sp. nov. female: **A** leg 4 **B** leg 5 **C** left genital aperture. Male: **D** habitus, dorsal **E** genital somite and abdomen, ventral **F** antennule **G** third endopodal segment of leg 3. Scale bars: 0.05 mm (**A, B, E**), 0.02 mm (**C, F, G**), 0.1 mm (**D**).

Leg 5 (Fig. 3B) 3-segmented, consisting of coxa, basis and exopod; intercoxal sclerite small, narrow, with pointed outer distal corners and slightly concave distal margin. Coxa quadrate, unarmed, not articulated from somite. Basis also quadrate, armed with one pinnate seta outer distally. Exopod 54 × 24 μm, 2.25 times as long as wide, armed with three spines (two distal and one outer) and one pinnate seta; medial margin spinulose and outer margin setulose.

Leg 6 (Fig. 3C) represented by one spinule and one naked seta on genital operculum.

Description of male. Body (Fig. 3D) much narrower than that of female, 582 μm long. Prosome 314 × 153 μm, approximately twice as long as wide. First pedigerous somite slightly narrower than cephalosome and second pedigerous somite. Urosome 6-segmented. Fifth pedigerous somite narrower than genital somite. Genital somite (Fig. 3E) 86 × 72 μm, longer than wide, with well-developed genital operculum. Four abdominal somites 25 × 40, 23 × 34, 20 × 31, and 30 × 28 μm, respectively. Caudal ramus 6.1 times as long as wide (61 × 10 μm); arrangement and locations of caudal setae as in female.

Rostrum as in female. Antennule (Fig. 3F) 17-segmented; armature formula (2 + aesthetasc)-(5 + aesthetasc)-4-2-(2 + aesthetasc)-2-2-2-2-2(2 + aesthetasc)-(1 + spine)-(2 + aesthetasc)-2-[3 + aesthetasc (or 2+aesthetasc)]-[0 (or 1)]-(1 + aesthetasc)-(9 + 2 aesthetascs); eleventh segment with short posterior margin and much longer anterior margin, spine on this segment slender. Antenna as in female.

Mandible and other mouth appendages as in female.

Legs 1, 2, and 4 also as in female. Leg 3 sexually dimorphic; third endopodal segment (Fig. 3G) bearing two spines, three setae, and distally two small specialized elements, one curved, non-articulating, spinule-like element and one straight, distally bifurcate articulating element.

Leg 5 as in female. Leg 6 (Fig. 3E) represented by three naked setae on genital operculum, medial one smaller than other two.

Etymology. The specific name *thermophilus* is a combination of Greek words *therm* (=heat) and *phil* (=loving), referring to the finding of the new species in a hydrothermal vent field.

***Barathricola rimensis* Humes, 1999**

Figs 4–7

Material. Females and males from the type locality dissected by A.G. Humes and marked as *Barathricola rimensis* in the Zoological Museum of Lomonosov Moscow State University (collection numbers: w.cyc.sch.1.1-1.5). The hydrothermal vent field is at Juan de Fuca Ridge (44°08.6'N, 129°42'W) in the northeastern Pacific, 26 August 1996 at 2254 m depth.

Redescription of female. Body as in original description. Differs from *Barathricola thermophilus* sp. nov. in following features.

Caudal ramus (Fig. 4D) elongate, 99 × 9 mm, ratio of length to width 11:1. Outer lateral seta located approximately at junction of first and second thirds of ramus. Dor-

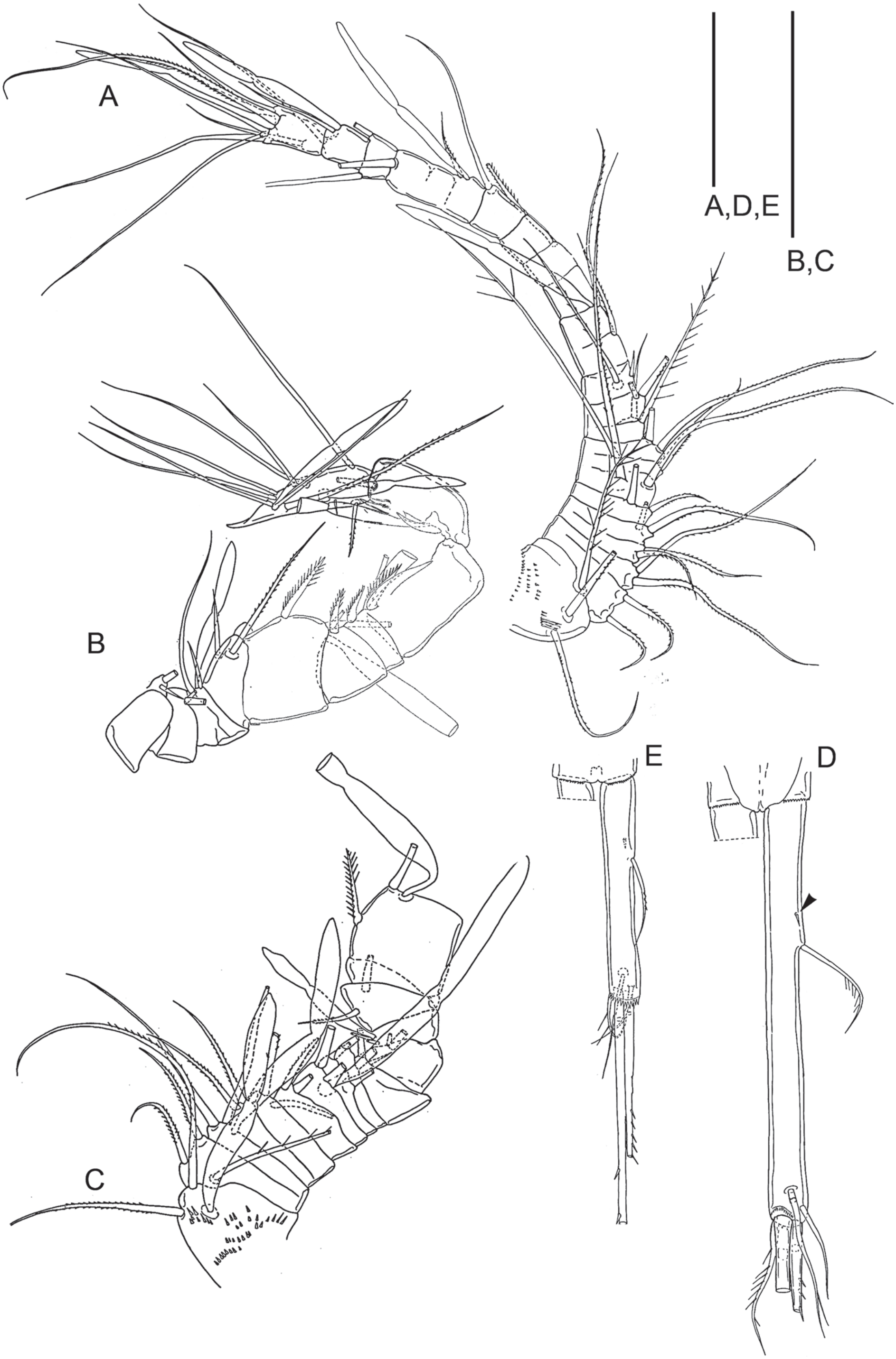

Figure 4. *Barathricola rimensis* Humes, 1999: **A** antennule of female **B** antennule of male, distal segments 8-17 **C** antennule of male, proximal segments 1-12 **D** caudal ramus of female **E** caudal ramus of male. Scale bars: 0.05 mm.

sal seta short. Outermost terminal seta also short, placed dorsally. Innermost terminal seta short. All these setae smooth. Two long median terminal setae 117 mm (outer) and 234 mm (inner), both with lateral setules. Few minute spinules at distal outer corner of ramus.

Antennule (Fig. 4A) 14-segmented with numerous subdivisions. Armature formula: 3-8-8-5-3-0-1-0-1-(2 + aesthetasc)-(2 + aesthetasc)-(2 + aesthetasc)-(6 + aesthetasc).

Antenna (Fig. 5A) four-segmented, with coxa, basis, and two-segmented endopod, armed with 0, 1, 5, and 7 setae. Exopod absent. Length 122 mm without setae.

Mandible (Fig. 5B, C) with coxa having medially directed gnathobase armed distally with row of seven or eight slender teeth. Palp biramous. Basis elongate, with minute exopodal process carrying two long setae, and two prominent setae distally on margin of basis; endopod two-segmented, first segment small, trapezoidal, bearing two setae and row of minute spinules, second segment small with four distal setae and row of minute spinules along anterior edge.

Maxillule (Fig. 6A) with large praecoxa bearing arthrite with eight setae; coxa-basis with 3+1 setae; exopod with two short stout setae and two long slender setae; endopod with five setae.

Maxilla (Fig. 6B) with praecoxa having two endites, proximal endite bearing four setae, distal endite represented by single seta. Coxa with two endites, both with three setae. Basis with endite bearing three setae, one short, one long and slender, and one stout and claw-like, and having few minute subterminal spinules. Endopod three-segmented, with first segment having two endites with two setae each, small second segment bearing two setae, and minute third segment with four setae.

Maxilliped (Fig. 6C) with both coxa and basis swollen medially and bearing three and two setae, respectively; endopod slender, consisting of five segments armed with 1, 1, 1, 1, and 3 setae. Coxae of maxillipeds joined ventrally by one sclerotized line.

Legs 1–4 (Fig. 7A, C, E) biramous with three-segmented rami; armature formula for legs 1-4 as in Table 1. Leg 1 (Fig. 7A), inner side of basis with barbed spine and row of eight slender curved setules. Leg 3 (Fig. 7C) with 2 distal spines on third endopodal segment.

Leg 5 (Fig. 7F). Both legs connected by small quadrangular intercoxal sclerite and consisting of coxa, basis, and one-segmented exopod. Coxa and basis with setules along both sides. Basis with outer seta 44 mm long. Exopod 21 mm in greatest dimensions (15.5 mm wide distally) bearing three spines and one seta. Outer marginal barbed spine 57 mm, two terminal spines 58 mm (outer) and 41 mm (inner), both with minute outer spinules and longer inner fringelike setules. Seta between these two spines smooth, 55 mm. Outer margin of segment proximal to spine with setules; distal to spine and along inner side of segment with shorter setules, inner margin with minute spinules.

Redescription of male. Differs from *Barathricola thermophilus* sp. nov. in following features:

Caudal ramus (Fig. 4E) resembling that of female but shorter, ratio 8.5:1. Antennule (Fig. 4B, C) 17-segmented; armature formula (2 + aesthetasc)-(5 + aesthetasc)-4-2-(2 + aesthetasc)-2-2-2-2(2 + aesthetasc)-(1 + spine)-(2 + aesthetasc)-2-[3

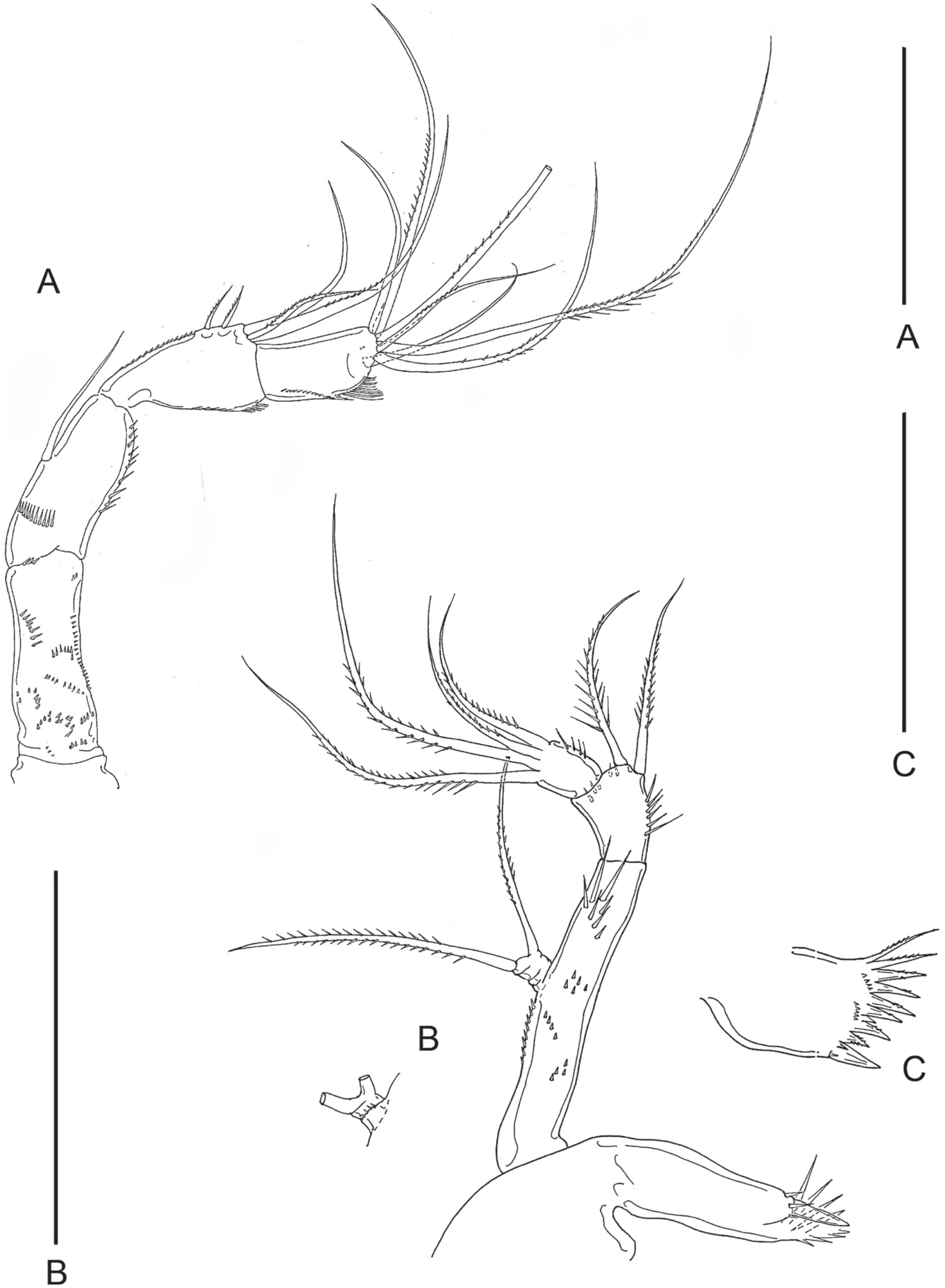

Figure 5. *Barathricola rimensis* Humes, 1999, female: **A** antenna **B** mandible **C** distal part of the mandibular gnathobase. Scale bars: 0.05 mm.

+ aesthetasc (or 2+aesthetasc)]-[0 (or 1)]-(1 + aesthetasc)-(9 + 2 aesthetascs); eleventh segment with short posterior margin and much longer anterior margin, spine on this segment slender. Legs 1 (Fig. 7B) inner side of basis with barbed spine and row of eight slender curved setules. Leg 3 sexually dimorphic; third endopodal segment (Fig. 7D) bearing two spines, three setae, and distally two small specialized elements, one curved, non-articulating, spine-like element and one straight element. Leg 5 (Fig. 7G) different from that of female in having additional seta on inner margin of exopod (armature formula 0-0; 1-0; I, I+1+I, 1).

Remarks. Martínez Arbizu (2006) established the family Schminkepinellidae into which he incorporated five genera, *Cyclopinella*, *Muceddina*, *Barathricola*, and his two new genera *Einslepinella* and *Schminkepinella*. The family is a monophyletic group of genera distinguished from other cyclopoid families by the reduction of a maxillulary coxal endite and the transformation of the distal inner seta on the middle endopodal segment of leg 3 into a spine (Martínez Arbizu 2006). None of the synapomorphies for the order Cyclopoida (a brush-like seta on the exopod of mandible, a brush-like seta on the exopod of maxillule, one or more flange-like setae on the endopod of swimming leg 4, pores with sensory dendrites laterally on the male cephalosome) proposed by Abiahy et al. (2006) are found in Schminkepinellidae. Karanovic (2008) described *Cyclopinella tincanbayensis* as a new species and synonymized two monotypic genera *Muceddina* and *Barathricola* with *Cyclopinella* and included these genera within Cyclopinidae based on the two major characters as synapomorphic shared by these nominal genera and *Cyclopinella*: the third endopodal segment of leg 4 with all armature elements transformed into spines and the three-segmented female leg 5 with an uniform armature and the elongate exopod. Karanovic (2008) recognized the mandibular palp as the most important morphological character differentiating species of *Cyclopinella* and its reduced segmentation and setation is consistent with reductions in other cephalic appendages and in the maxilliped. Our re-examination of the type species of the genus *Muceddina*, confirmed the original description and did not reveal the presence of a sexually dimorphic leg 3. This as well as our re-examination of the type specimens of *Barathricola rimensis* does not provide sufficient support for inclusion of *Muceddina multispinosa* and *Barathricola rimensis* in *Cyclopinella*. Additional data are needed to provide for the proposed taxonomic changes; here *Barathricola* and *Muceddina* are considered valid genera with clear distinctive characters separating them from other genera (see Key and Table 2). *Cyclopinella tincanbayensis* should remain in *Cyclopinella* although its distinctive characters may be significant enough to consider moving it to a new genus after revision. *Barathricola*, *Cyclopinella*, and *Muceddina* should remain in the Schminkepinellidae as was proposed by Martínez Arbizu (2006) until more data are available.

Data here show that the sexual dimorphism in leg 3 occurs in *B. thermophilus* and *B. rimensis*. Thus, the sexually dimorphic leg 3 known from the two species living in the hydrothermal vent environment is clearly the derived character of the genus *Barathricola* as mentioned by Martínez Arbizu (2006). *Barathricola thermophilus* sp.

Table 2. Morphological differences, distributions and habitats among species of the Schminkepinellidae.

Characters\ Species	***Muceddina multispinosa***	***Cycliinella tincanbayensis***	***C. tumidula***	***Barathricola rimensis***	***B. thermophilus* sp. nov.**	***Einslepinella ulrichi***	***E. mediana***	***E. alignatha***	***Schminkepinella plumifera***
♀ Caudal ramus, L/W ratio	7.6	4.0	about 4	11.0	8.9	8	–	8	15.5
Segments of ♀ antennule	15	15	12	14	14	7	8	8	8
Armature of antenna	1-1-5-7	1-1-5-6	0-1-4-7	0-1-5-7	0-1-5-7	0-1-1-5	0-1-1-6	0-1-1-6	1-9
Inner seta on basis of mandible	Present	Present	Present	Absent	Absent	Absent	Absent	Present	Absent
Armature of mandibular exopod	1-1-1-2	1-1-2	1 seta	0-0-2	0-0-2	1-1-1	1-2	1-1-1	2
Armature of mandibular endopod	3-5	2-4	4	2-4	2-4	5	4	4	4
Setae on maxillular basis	4	?	3	4	4	4	–	–	3
Setae on maxillular exopod	4	4	3	4	4	4	–	–	4
Setae on maxillular endopod	6	?	5	5	5	–	–	–	4
Setae on maxilliped segments	5-2-1-1-1-1-4	4-2-1-1-1-1-4	3-2-1-1-3	3-2-1-1-1-1-3	3-2-1-1-1-1-3	0-0-0-1+spine	0-0-0-1	1	1-1
Outer element of 3rd endopodal segment of leg 1	Seta	Seta	Spine	Seta	Seta	Spine	Spine	Spine	None
Armature of 3rd endopodal segment of leg 3	3 spines + 3 setae	2 spines + 4 setae	2 spines + 4 setae	3 spines + 3 setae	3 spines + 3 setae	4 spines + 2 setae	4 spines + 2 setae	–	2 spines + 2 setae
Spines on 3rd exopodal segment of leg 3	4	3	4	4	4	4	4	–	4
Armature on 2nd endopodal segment of leg 4	1 spine + 1 seta	2 setae	2 setae	1 spine + 1 seta	1 spine + 1 seta	1 spine + 1 seta	1 spine + 1 seta	–	1 spine + 1 seta
Armature of exopod of ♀ leg 5	I, I+1+I	II, 1+I	I, I+1+I	I, I+1+I	I, I+1+I	I, I+1+I	I, I+1+I	I, I+1+I	1+1+I
Armature of exopod of ♂ leg 5	I-1; I+1+I, 1	Unknown	As in female	I, I+1+I, 1	As in female	I-1; I+1+I	Unknown	Unknown	I, I+1+I, 1
Distributions (habitats)	Mediterranean & Atlantic (anchihaline caves)	Australia (littoral, interstitial)	Norway (shallow water)	Northeast Pacific (hydrothermal vent area)	Indian Ocean (hydrothermal vent area)	Arctic (depth 8–529 m)	Arctic (depth 156–449 m)	Arctic (depth 256 m)	Arctic (depth 3211 m)
References	Jaume & Boxshall, 1996	Karanovic, 2008	Sars, 1913	Humes, 1998 and this paper	This paper	Martínez Arbizu, 2006	Martínez Arbizu, 2006	Martínez Arbizu, 2006	Martínez Arbizu, 2006

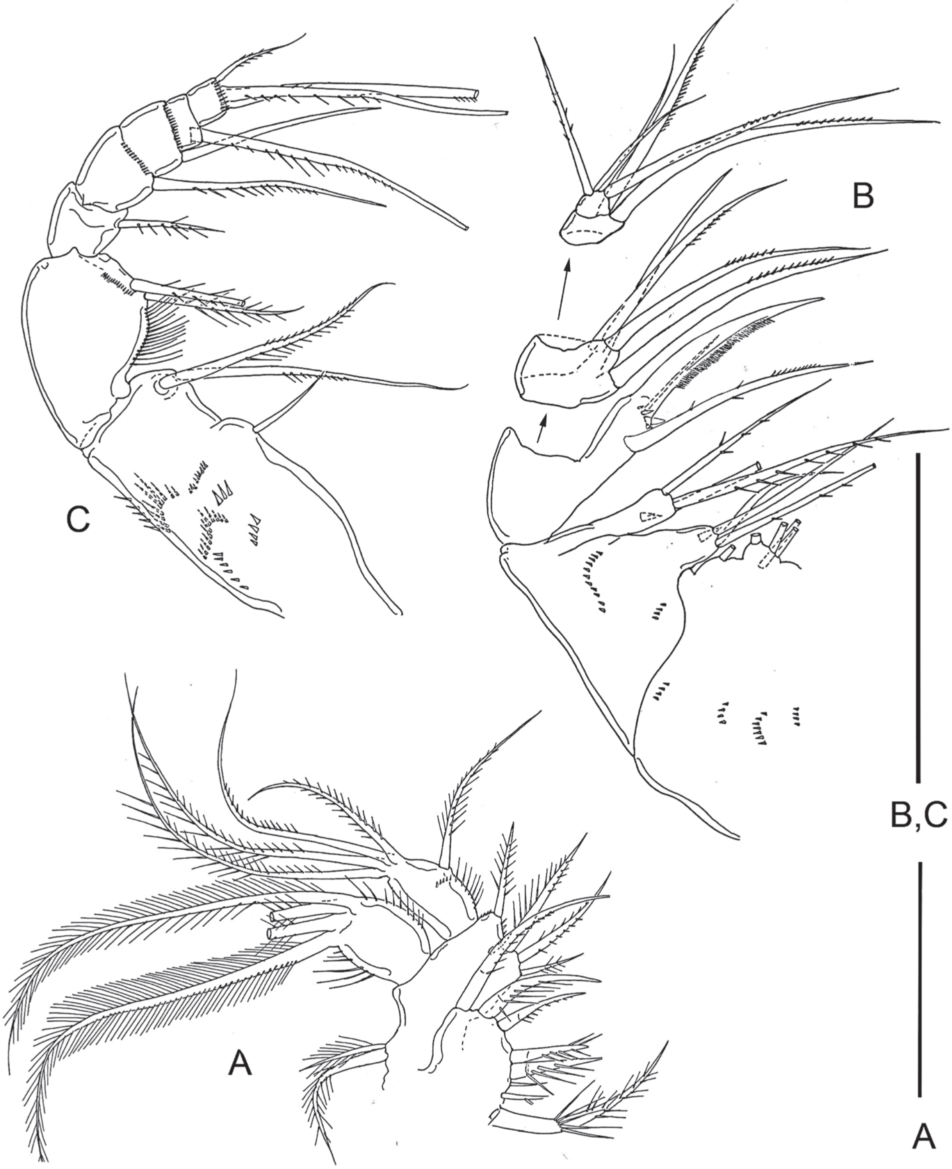

Figure 6. *Barathricola rimensis* Humes, 1999, female: **A** maxillule **B** maxilla **C** maxilliped. Scale bars: 0.05 mm.

nov. shares with *B. rimensis* the shape of the mandibular palp and a number of other characters, e.g., Humes (1999) described the mandibular exopod of *B. rimensis* as "a minute process carrying two long setae", but his illustrations and those here for this appendage show that the exopod is indistinctly 3-segmented, with two setae on the third segment, as in *B. thermophilus* sp. nov. In addition, the two species share the identical armature formula for the antenna (0-1-5-7), the loss of an inner seta on the basis of the

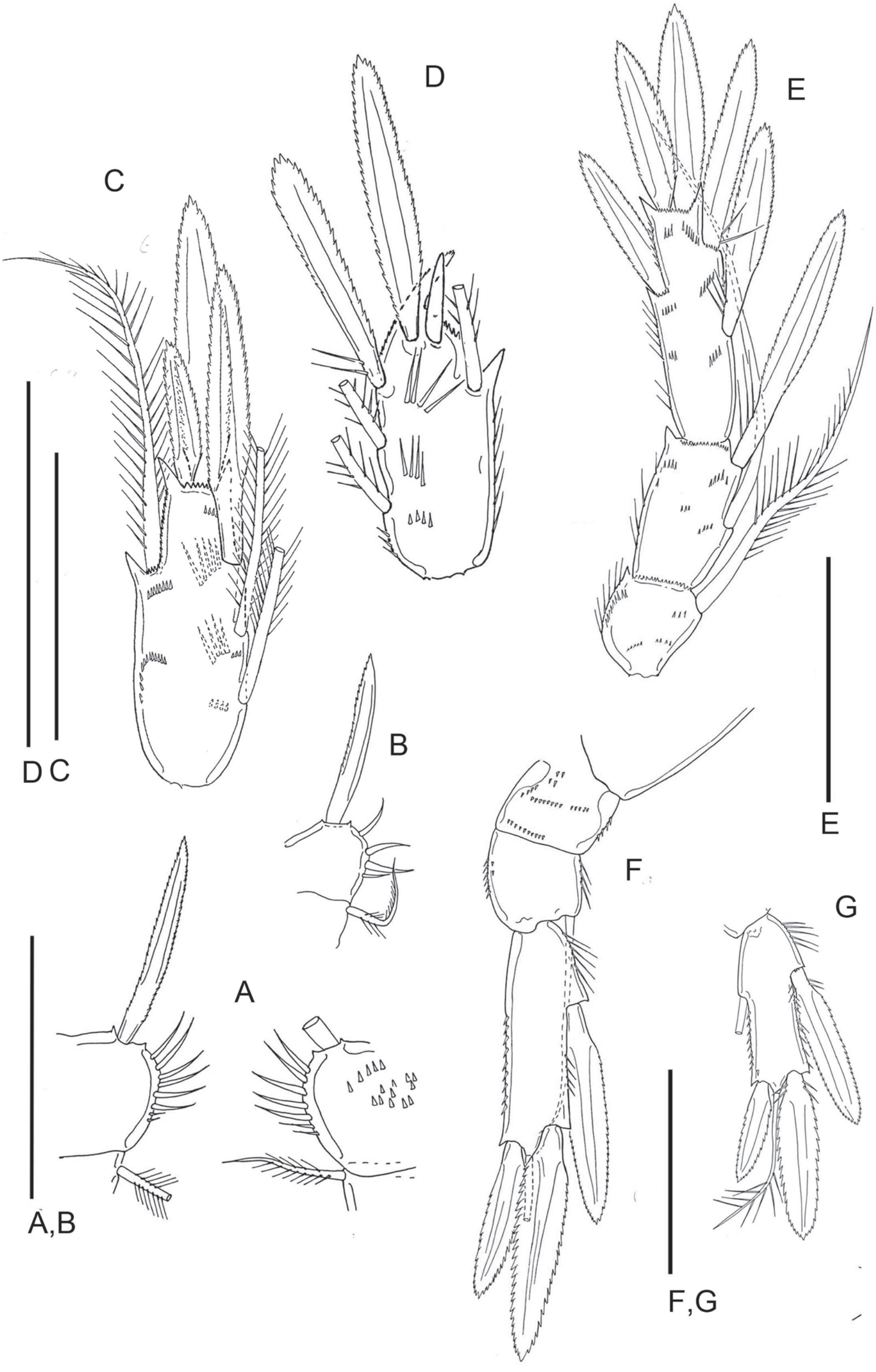

Figure 7. *Barathricola rimensis* Humes, 1999: **A** leg 1 of female, inner part of protopod **B** leg 1 of male, inner part of protopod **C** leg 3 of female, distal endopodal segment, posterior **D** leg 3 of male, distal endopodal segment, anterior **E** leg 4 of female, endopod, anterior **F** leg 5 of female, exopod, anterior **G** leg 5 of male, distal segment. Scale bars: 0.05 mm.

mandible, a two-segmented mandibular endopod bearing two and four setae on the first and second segments, respectively, and elongate caudal rami.

Although the two species of *Barathricola* are very similar to each other, they cannot be treated as conspecific due to a significant difference in leg 5 of the male. The exopod (terminal segment) of leg 5 is armed with three spines and two setae (formula I, I+1+I, 1) in *B. rimensis*, in contrast to three spines and one seta (formula I, I+1+I) in *B. thermophilus* sp. nov. lacking a seta on the inner margin of the exopod. Within the Schminkepinellidae males of six species are known, including *B. rimensis* and *B. thermophilus* sp. nov. In these species a sexual dimorphic leg 5, as in *B. rimensis*, is known in *Muceddina multispinosa*, *Schminkepinella plumifera*, and *Einslepinella ulrichi*. However, Sars (1913) recorded that leg 5 of male *Cyclopinella tumidula* is of exactly the same appearance as in the female. Thus, the sexual dimorphism in leg 5 appears to be a character differentiating species, but not genera, in the Schminkepinellidae. An additional morphological difference between the two species of *Barathricola* is the ratio of the length to the width of the caudal ramus is 11.0:1 in the female and 8.5:1 in the male of *B. rimensis*, which is 8.9:1 in the female and 6.1:1 in the male of *B. thermophilus* sp. nov.

Key to species of the family Schminkepinellidae

1 Antennule of female 7 or 8-segmented; maxilliped 1 to 4-segmented................**2**
– Antennule of female 12 to15-segmented; maxilliped 5 to7-segmented..............**5**
2 Antenna 2-segmented; third endopodal segment of leg 3 armed with 2 spines and 2 setae; third endopodal segment of leg 1 without outer element ....***Schminkepinella plumifera* Martínez Arbizu, 2006**
– Antenna 4-segmented; third endopodal segment of leg 3 armed with 4 spines and 2 setae; third endopodal segment of leg 1 with outer spine....... **3 (*Einslepinella*)**
3 Mandibular basis with inner seta; maxilliped 1-segmented................................ ....***Einslepinella alignatha* Martínez Arbizu, 2006**
– Mandibular basis without inner seta; maxilliped 4-segmented...........................**4**
4 Mandibular endopod armed with 5 setae; terminal segment of maxilliped with 1 spine and 1 seta............................***Einslepinella ulrichi* Martínez Arbizu, 2006**
– Mandibular endopod armed with 4 setae; terminal segment of maxilliped with 1 seta only.................................. ***Einslepinella mediana* Martínez Arbizu, 2006**
5 Second endopodal segment of leg 4 armed with 2 setae; third endopodal segment of leg 3 armed with 2 spines and 4 setae.................................... **6 (*Cyclopinella*)**
– Second endopodal segment of leg 4 armed with 1 spine and 1 seta; third endopodal segment of leg 3 armed with 3 spines and 3 setae **7**
6 Antenna with armature formula 0-1-4-7; mandibular endopod 1-segmented, with 4 setae; maxilliped 5-segmented ....***Cyclopinella tumidula* Sars, 1913**
– Antenna with armature formula 1-1-5-6; mandibular endopod 2-segmented, with 2 and 4 setae on first and second segments, respectively; maxilliped 7-segmented....................................... ***Cyclopinella tincanbayensis* Karanovic, 2008**

7 Antenna with armature formula 1-1-5-7; mandibular basis with inner seta; mandibular endopod with 3 and 5 setae on first and second segments, respectively; first segment of maxilliped with 5 setae ....***Muceddina multispinosa* Jaume & Boxshall, 1996**

– Antenna with armature formula 0-1-5-7; mandibular basis lacking inner seta; mandibular endopod with 2 and 4 setae on first and second segments, respectively; first segment of maxilliped with 3 setae...........................**8 (*Barathricola*)**

8 Leg 5 sexually dimorphic, with exopod bearing 3 spines + 1 seta in female and 3 spines + 2 setae in male; length/width ratio of caudal ramus 11:1 in female and 8.5:1 in male..***Barathricola rimensis* Humes, 1999**

– Leg 5 of both sexes with exopod bearing 3 spines + 1 seta; length/width ratio of caudal ramus 8.9:1 in female and 6.1:1 in male.. ....***Barathricola thermophilus* sp. nov.**

Acknowledgements

We thank the captain and crew members of the R/V ISABU for help and support during the cruises. This research was supported by the project "Understanding the deep-sea biosphere on seafloor Hydrothermal Vent in the Indian Ridge" of the Korea Institute of Marine Science and Technology Promotion (KIMST) funded by Ministry of Oceans and Fisheries, Korea (20170411) and by KIOST(PM60640). Restudy of the type species of the genera *Barathricola* and *Muceddina* was conducted by VNI in Senckenberg Research Institute (DZMB, Wilhelmshaven, Germany) and National Museum of Natural History (Smithsonian Institution, Washington, DC) with support of the Russian Foundation for Basic Research (grants 18-54-45016 and 18-04-01192, respectively). Frank Ferrari commented on a draft of the manuscript; Geoff Boxshall (British Museum of Natural History, London) provided type specimen of *Muceddina.*

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ZooKeys 865: 123–135 (2019)
doi: 10.3897/zookeys.865.35213
http://zookeys.pensoft.net

RESEARCH ARTICLE

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A molecular study of *Neophyllaphis varicolor* (Hemiptera, Aphididae) in Costa Rica

Adonay Zúñiga-Centeno[1], Izayana Sandoval-Carvajal[2], Mauricio Montero-Astúa[1,2], William Villalobos-Muller[2], Nguyễn Bảo Quốc[3], Nicolás Pérez Hidalgo[4]

1 *Universidad de Costa Rica, Escuela de Agronomía, San José, 11501-2060, Costa Rica* **2** *Universidad de Costa Rica, Centro de Investigación en Biología Celular y Molecular, San José, 11501-2060, Costa Rica* **3** *Nong Lam University, Research Institute for Biotechnology and Environment, Ho Chi Minh City, 700000, Vietnam* **4** *Instituto de Biología Integrativa de Sistemas (I²SysBio), Centro Mixto Universidad de Valencia-CSIC, Valencia, 46980, España*

Corresponding author: *Nicolás Pérez Hidalgo* (nipehi@uv.es)

Academic editor: *Roger Blackman* | Received 5 April 2019 | Accepted 29 May 2019 | Published 22 July 2019

http://zoobank.org/2421E9B0-E7B6-431F-8BC2-15131BAB7351

Citation: Zúñiga-Centeno A, Sandoval-Carvajal I, Montero-Astúa M, Villalobos-Muller W, Quốc NB, Pérez Hidalgo N (2019) A molecular study of *Neophyllaphis varicolor* (Hemiptera, Aphididae) in Costa Rica. ZooKeys 865: 123–135. https://doi.org/10.3897/zookeys.865.35213

Abstract

The genus *Neophyllaphis* (Takahashi) (Aphididae: Neophyllaphidinae) is composed of 18 species; however, in the Americas only nine species have been reported previously. A new species, *Neophyllaphis varicolor* Miller & Halbert, was described in 2014 in USA. Colonies resembling those of this new species have been observed in Costa Rica on *Podocarpus* spp. In order to determine if *N. varicolor* is also present in Costa Rica, we sampled *Neophyllaphis* colonies from *Podocarpus falcatus* and *P. chinensis*. Additionally, we sampled individuals from *Podocarpus* sp. in Spain and Vietnam. DNA of each sample was extracted and used to amplify and sequence the cytochrome *c* oxidase subunit I (COI) and elongation factor I (EF-1α) partial regions. According to morphological characteristics, sequences comparisons done in GenBank and BOLD, and phylogenetic analyses, the colonies collected from *Podocarpus* spp. in Costa Rica and the colony from Vietnam corresponded to the species *N. varicolor*. To the best of our knowledge this is the first report of the presence of *N. varicolor* in Central America and Vietnam.

Keywords

Aphids, cytochrome *c* oxidase subunit I, DNA barcoding, elongation factor I, integrative taxonomy, phylogenetic analysis, *Podocarpus*

Introduction

Neophyllaphis Takahashi (Aphididae: Neophyllaphidinae) is a genus occurring predominantly in the southern hemisphere and composed of 18 species characterized by a body covered with pulverulence and waxy secretions, antenna with a short terminal process, siphuncular pores on small cones, cauda with a constriction in the middle and with a caudal knob, and annular secondary rhinaria that are only present in alatae (Quednau 2010). The species of this genus live on species of Podocarpaceae, Araucariaceae, Myrtaceae, and less frequently on Cupressaceae (Hales and Lardner 1988; Miller and Halbert 2014). The genus is divided into two subgenera, a nominotypical subgenus *Neophyllaphis*, and the subgenus *Chileaphis* Essig, 1953 (Hille Ris Lambers 1967). Species of the subgenus *Chileaphis* have a very restricted distribution in South America while the subgenus *Neophyllaphis* is distributed in temperate and tropical regions of Africa, Asia, and Australia, and some species have been introduced in North America (Hille Ris Lambers 1967; Russell 1982; Qiao et al. 2001; Blackman and Eastop 2019). In Europe, only the species *Neophyllaphis podocarpi* Takahashi 1920 has been reported (Aguiar et al. 2013; Pérez Hidalgo et al. 2015).

In the Americas there are nine *Neophyllaphis* species (Mier Durante et al. 2008; Quednau 2010; Miller and Halbert 2014; Blackman and Eastop 2019), six species in the subgenus *Chileaphis* and three invasive species in the subgenus *Neophyllaphis: N. araucariae* Takahashi 1937, *N. podocarpi* (Mier Durante et al. 2008; Quednau 2010), and the newest described as *N. varicolor* Miller and Halbert 2014. The species *N. podocarpi* and *N. varicolor* have been reported in the western hemisphere only in the United States. Both species have been recorded in Florida and Louisiana, while only *N. podocarpi* has been found in California, Mississippi, and Texas (Russell 1982; Skvarla et al. 2017). The species *N. araucariae* is the only one living on the genus *Araucaria* Juss. and it is native of the Oriental region (perhaps native to Norfolk Island or Australia). There are reports of *N. araucariae* in the United States (Florida, California, Hawaii) (Timberlake 1917; Russell 1982), Mexico (Peña-Martínez 1985), Panama (Russell 1982), Venezuela (Cermeli 1990) and Costa Rica (Voegtlin et al. 2003). In Costa Rica, it is the only reported species of *Neophyllaphis* (Voegtlin et al. 2003; Villalobos Muller et al. 2010).

The newly recorded species *N. varicolor*, described by Miller and Halbert (2014), is characterized by a dorsoventrally flattened body and color variations of individuals in the same colony that may be yellow, orange, red or purple. The species was noticed beginning in 2010 in different counties in Florida. There are no records from outside USA. Multi-colored aphid colonies resembling those described by Miller and Halbert (2014), were found infesting the trees *Podocarpus falcatus* (Thunb.) Mirb. and *P. chinensis* Wall. ex J.Forbes Wall. in Costa Rica during 2014 (Figure 1). Morphological identification and molecular analyses were done to determine if the new species, *N. varicolor*, is also present in Costa Rica.

Figure 1. A *Neophyllaphis varicolor* Miller & Halbert, apterous individuals from Costa Rica **B** *Podocarpus chinensis* Wall. ex J.Forbes, and **C** *Podocarpus falcatus* (Thunb.) Mirb.

Materials and methods

Sample collection

Thirteen aphid colonies were sampled for this study. Nine samples from colonies on *Podocarpus* spp. L'Hér.ex Pers. were collected from different localities in San José province and samples from two colonies of *N. araucariae* were collected on *Araucaria* spp. in San José and Cartago provinces (Costa Rica). Additionally, two samples, one of *N. podocarpi* from Gerona (Spain) and one *Neophyllaphis* sp. multicolored colony from a *Podocarpus* sp. shrub in Vietnam were collected for comparison (Table 1). Samples were maintained at -35 °C in 95% ethanol for molecular analyses and at 6 °C in 70% ethanol for morphological analysis.

Morphological identification

Individuals preserved in 70% ethanol were cleared using KOH and acetic acid and mounted in slides with Canada balsam. The morphological identification of the

Table 1. Samples of *Neophyllaphis* spp. colonies (individual aphids per colony indicated by a, b, and c) and the corresponding accession numbers of COI and EF-1α sequences deposited at GenBank.

Species	Colony code	Collection place	Host plant		Accession numbers	
					COI	EF-1α
N. varicolor	CR14-002	Montes de Oca, San José (9.935764°N, 84.057778°W)	*Podocarpus falcatus* (Thunb.) Mirb.	a	MK174294	ND
				b	MK174295	MK174326
				c	MK174296	MK174327
N. varicolor	CR14-004	Montes de Oca, San José (9.934636°N, 84.058056°W)	*Podocarpus chinensis* Wall. ex J.Forbes	a	MK174297	ND
				b	MK174298	ND
				c	MK174299	MK174328
N. varicolor	CR14-005	Montes de Oca, San José (9.934567°N, 84.059167°W)	*Podocarpus falcatus* (Thunb.) Mirb.	a	MK174300	ND
				b	MK174301	MK174329
				c	MK174302	ND
N. varicolor	CR14-012	Goicoechea, San José (9.946283°N, 84.053056°W)	*Podocarpus falcatus* (Thunb.) Mirb.	a	MK174303	MK174330
				b	MK174304	ND
				c	MK174305	ND
N. varicolor	CR14-013	Curridabat, San José (9.923417°N, 84.033056°W)	*Podocarpus falcatus* (Thunb.) Mirb.	a	MK174306	MK174331
				b	MK174307	ND
				c	MK174308	ND
N. varicolor	CR14-033	Montes de Oca, San José (9.943450°N, 84.018889°W)	*Podocarpus chinensis* Wall. ex J.Forbes	b	MK174309	ND
				c	MK174310	MK174332
N. varicolor	CR14-034	Montes de Oca, San José (9.939783°N, 84.009444°W)	*Podocarpus falcatus* (Thunb.) Mirb.	a	MK174311	ND
				b	MK174312	MK174333
				c	MK174313	ND
N. varicolor	CR14-127	San José, San José (9.929947°N, 84.070278°W)	*Podocarpus falcatus* (Thunb.) Mirb.	a	MK174314	ND
				b	MK174315	ND
				c	MK174316	MK174334
N. varicolor	CR14-295	Vázquez de Coronado, San José (9.969086°N, 84.016944°W)	*Podocarpus falcatus* (Thunb.) Mirb.	a	MK174317	MK174335
				b	MK174318	ND
				c	MK174319	ND
N. araucariae	CR14-364	Paraíso, Cartago (9.852750°N, 83.807500°W)	*Araucaria araucana* (Molina) K. Koch	b	MK174320	MK174336
				c	MK174321	MK174337
N. podocarpi	CR14-398	Gerona, Spain (41.676944°N, 2.801944°W)	*Podocarpus neriifolius* D.Don	c	MK174325	MK174341
N. varicolor	CR14-397	Long Dinh, Vietnam (10.384510°N, 106.166800°W)	*Podocarpus* sp. L'Hér.ex Pers.	a	MK463550	MK463553
				b	MK463551	MK463554
				c	MK463552	MK463555
N. araucariae	CR14-423	Vázquez de Coronado, San José (9.970153°N, 84.030833°W)	*Araucaria heterophylla* (Salisb.) Franco	a	MK174322	MK174338
				b	MK174323	MK174339
				c	MK174324	MK174340

specimens was done using a Leica Z16 microscope. We measured structures with taxonomic value and used the keys from Miller and Halbert (2014) and Blackman and Eastop (2019) to identify species of *Neophyllaphis*. The photographs were taken with a Leica Z16 microscope, equipped with a CF500 camera and LAS 4.9 (Leica) image capture. Mounted specimens were deposited at the aphid collection of the Instituto de Biología Integrativa de Sistemas (Centro Mixto Universidad de Valencia-CSIC, Spain) and in the Centro de Investigación en Biología Celular y Molecular (CIBCM), Universidad de Costa Rica.

DNA extraction and molecular identification

DNA was extracted from three individual aphid specimens per colony (preserved in 95% ethanol) using the animal tissue protocol of NucleoSpin Tissue extraction kit (Macherey-Nagel, Germany) following the manufacturer instructions with a modification at the elution step; it was made by duplicate, using 50 µL of elution buffer each time.

For the molecular identification and phylogenetic analysis of the *Neophyllaphis* spp. samples, we amplified the cytochrome *c* oxidase subunit I (COI) and the elongation factor 1α (EF-1α) genes. To amplify COI we used the primer pair C1-J-1490 (= LepF) and C1-N-2198 (= LepR) to obtain an amplicon of 658 bp (Hajibabaei et al. 2006, Miller and Halbert 2014), and the primers C1-J-1718 (Simon et al. 1994) and C1-J-2411 (Lagos et al. 2012) to obtain an amplicon of 868 bp. The EF-1α gene was amplified using the primers EF-3 and EF-6 to generate a fragment of 785 bp (Miller and Halbert 2014). All PCR reactions were done in a final volume of 25 µL with final concentration of 1X Dream Taq Master Mix (2X, Thermo Scientific, Lithuania), 200 nM of each primer, 1% trehalose dehydrated, and 5 µL of DNA. Reactions were run with the following thermocycle profiles: 94 °C x 1 min; 5 x (94 °C x 40 s, 45 °C x 40 s, 72 °C x 1 min); 35 x (94 °C x 40 s, 51 °C x 40 s, 72 °C x 1 min); 72 °C x 5 min (Hajibabaei et al. 2005) for primer pair C1-J-1490 / C1-N-2198; and 96 °C x 2 min; 40 x (95 °C x 30 s, 53 °C x 30 s, 72 °C x 2 min); 72 °C x 10 min for primers C1-J-1718 / C1-J-2411 (Lagos et al. 2012).

Amplicons of COI and EF-1α were directly sequenced after purification in reverse and forward directions by the Sanger method (Macrogen, Korea). The final contigs were obtained using BIOEDIT 7.0 (Hall 1999) and were assigned preliminarily to a species by alignment using the BLAST tool of NCBI (Altschul et al. 1990) and the Identification Engine tool at BOLD (Ratnasingham and Hebert 2007). Sequences obtained are available in GenBank (Table 1).

Phylogenetic analyses

Phylogenetic analyses of *Neophyllaphis* spp. samples were done using partial sequences of the COI gene. Additionally, partial sequences of COI (nucleotide positions from 94 to 570 according to the reference sequence KF199852) and EF-1α (nucleotide positions from 81 to 546 according to the reference sequence KF199851) were concatenated using BIOEDIT tool (Hall 1999) and a phylogenetic tree was generated. Phylogenetic analyses were done using a mixed model of Bayesian phylogenetic inference in MrBayes tool using a Markov Chain Monte Carlo (MCMC) search with ten million generations (Huelsenbeck and Ronquist 2001). The trees were visualized and edited using the tool FigTREE v1.4.2 (Rambaut and Drummond 2012).

Sequences obtained from GenBank (www.ncbi.nlm.nih.gov/Genbank) of the species *N. varicolor* (COI: KF199852; EF-1α: KF199851, USA), *N. podocarpi* (COI: EU701821, Japan and JQ920926, China), *Neophyllaphis brimblecombei* Carver (COI: JF883870, Australia) and from BOLD Systems (http://www.barcodinglife.org) for

Neophyllaphis totarae Cottier (COI: RFBAD211_08, New Zealand) were included for comparison in the phylogenetic analyses. Sequences of *Greenidea anonae* (Pergande) (COI: JQ926000; EF-1α: KF856808, China) and *Greenidea psidii* van der Goot (COI: JQ925937 and EF-1α: KF856814, China, and COI: EU701673, USA) were used as an outgroup because the genus *Greenidea* clustered relatively close to *Neophyllaphis* in a COI phylogeny (Foottit et al. 2008).

Results

Morphological identification

The metric and meristic characters (including color when alive) of the approximately 70 apterous specimens (Figure 2) and of the 12 winged (Figure 3) of *Neophyllaphis* studied in Costa Rica and their comparison with the detailed description of *N. varicolor* by Miller and Halbert (2014), confirmed that the Costa Rican samples belong to this species described from North America. However, the Ant. III/Ant. IV ratios of our apterous specimens varied from 2.32 to 2.88 (•= 2.56). Thus, the antennal ratio character used to separate *Neophyllaphis fransseni* Hille Ris Lambers and *N. varicolor* (ratio greater

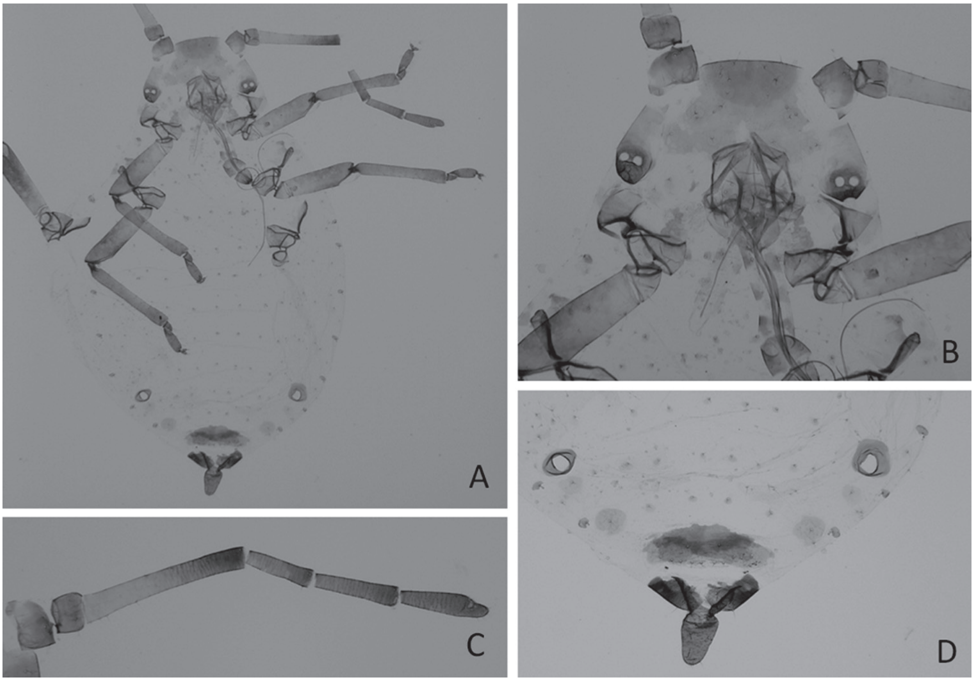

Figure 2. *Neophyllaphis varicolor* Miller & Halbert, apterous **A** body **B** anterior part of the body **C** antennae, and **D** posterior part of body.

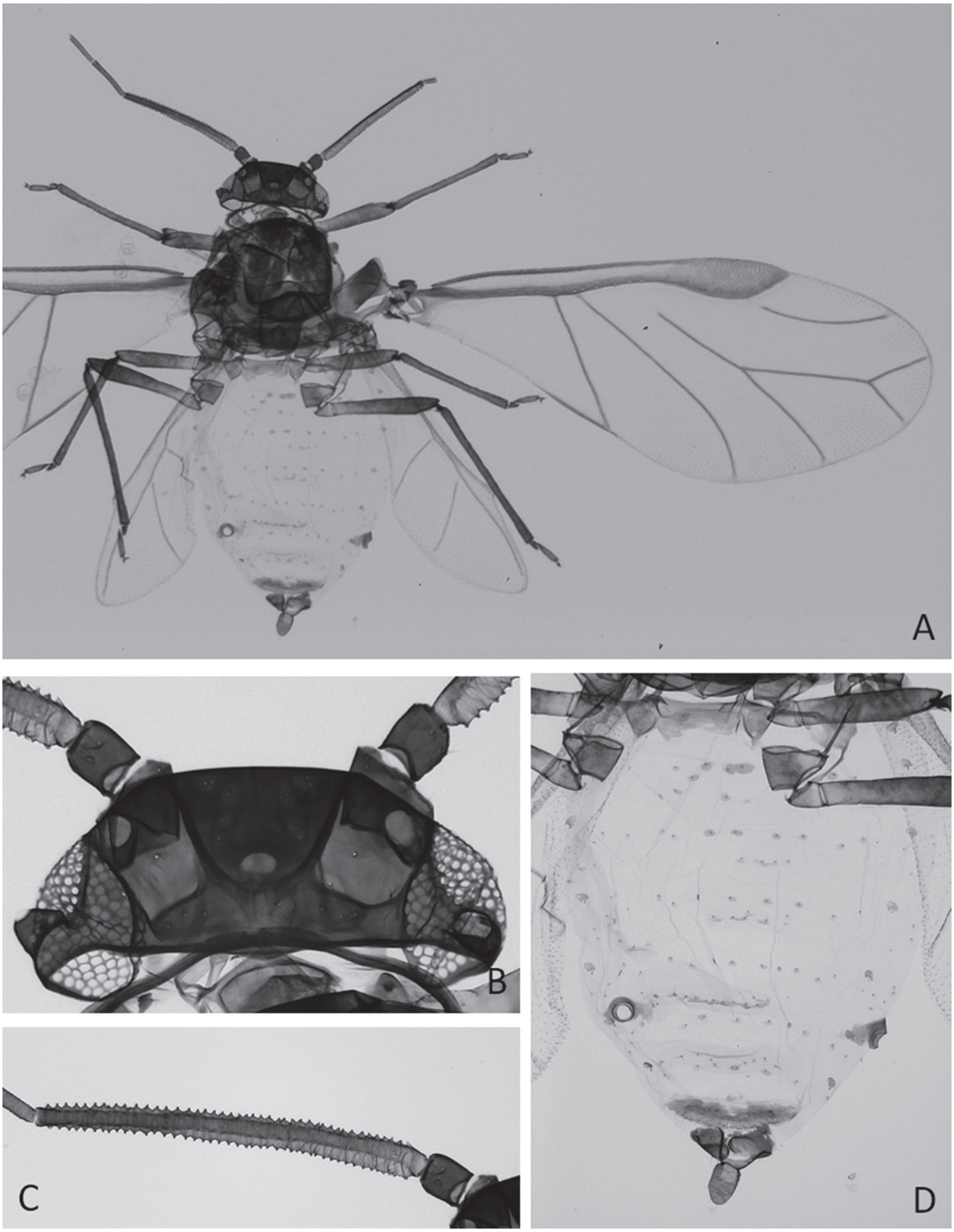

Figure 3. *Neophyllaphis varicolor* Miller & Halbert, alate **A** alate aphid **B** head **C** antennal segment III and **D** abdomen.

than 2.6 in apterae) from *Neophyllaphis gingerensis* Carver, *N. totarae*, *N. brimblecombei*, *Neophyllaphis lanata* Hales & Lardner and *N. podocarpi* (with a ratio shorter than 2.6), should be reevaluated (Miller and Halbert 2014; Blackman and Eastop 2019).

Molecular identification and phylogenetic analysis

A total of 39 individual aphids was analyzed by molecular means; COI sequence data were obtained for 35 individuals and EF-1α sequences for 19 individuals. We did not obtain final sequences for all three aphid individuals processed per colony because amplifications failed for some individuals or the sequencing reaction rendered low quality reads. All COI and EF-1α sequences obtained from samples morphologically identified as *N. varicolor* corresponded to this species according to the identification made in BOLD systems with 99.5% (KF199852.1) and 99.7% (KF199851.1) of similarity, respectively. It was not possible to corroborate the identification of the species *N. araucariae* by sequence identity comparison because data for this species is not available at GenBank or BOLD.

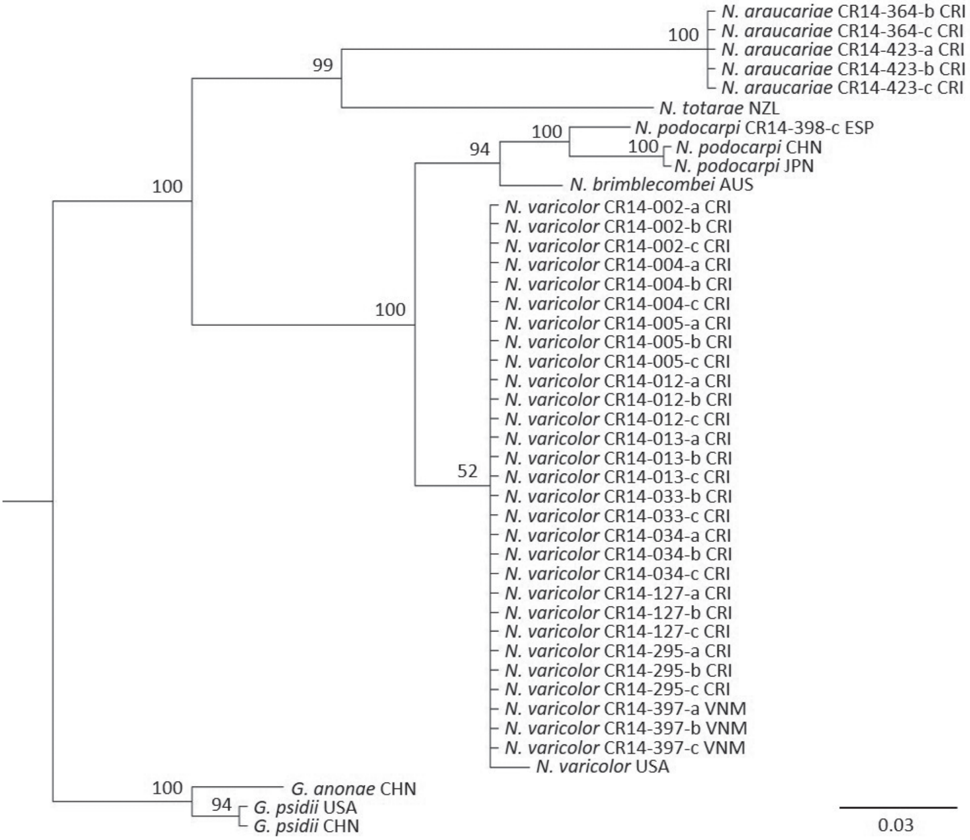

Figure 4. Phylogenetic analysis of *Neophyllaphis araucariae* Takahashi, *Neophyllaphis brimblecombei* Carver, *Neophyllaphis podocarpi* Takahashi, *Neophyllaphis totarae* Cottier, and *Neophyllaphis varicolor* Miller & Halbert, using sequences of cytochrome c oxidase subunit I, made with Bayesian inference and using as outgroup *Greenidea psidii* van der Goot. and *Greenidea anonae* (Pergande). Sequence codes: species name - colony code - (a, b or c represent the specimen sampled) - country code. Key: AUS: Australia, CHN: China, CRI: Costa Rica, ESP: Spain, JPN: Japan, NZL: New Zealand, USA: United States, VNM: Vietnam. Scale bar represents 0.03 changes per site.

All COI sequences of *N. varicolor* from Costa Rica were identical, while, EF-1α sequences showed 0.6% difference. Sequences of COI and EF-1α of *N. araucariae* did not show intraspecific variation. Interspecific genetic variation between *N. varicolor* and *N. araucariae* was of 11.4% in COI sequences and 13.4% in EF-1α sequences.

All 26 partial sequences of COI from individuals morphologically identified as *N. varicolor* were grouped within the same clade, together with the *Neophyllaphis* sp. sample from Vietnam and the reference sequence of *N. varicolor* (GenBank Acc. No. KF199852) from Florida. This clade clustered independently from available sequences for *N. araucariae*, *N. brimblecombei*, *N. podocarpi*, and *N. totarae*. Similarly, all COI sequences of *N. araucariae* were grouped in the same clade, supporting identifications by morphological characters for both species. The *N. araucariae* cluster showed more relatedness to *N. totarae* than to the clade comprising *N. podocarpi*, *N. brimblecombei,* and *N. varicolor*.

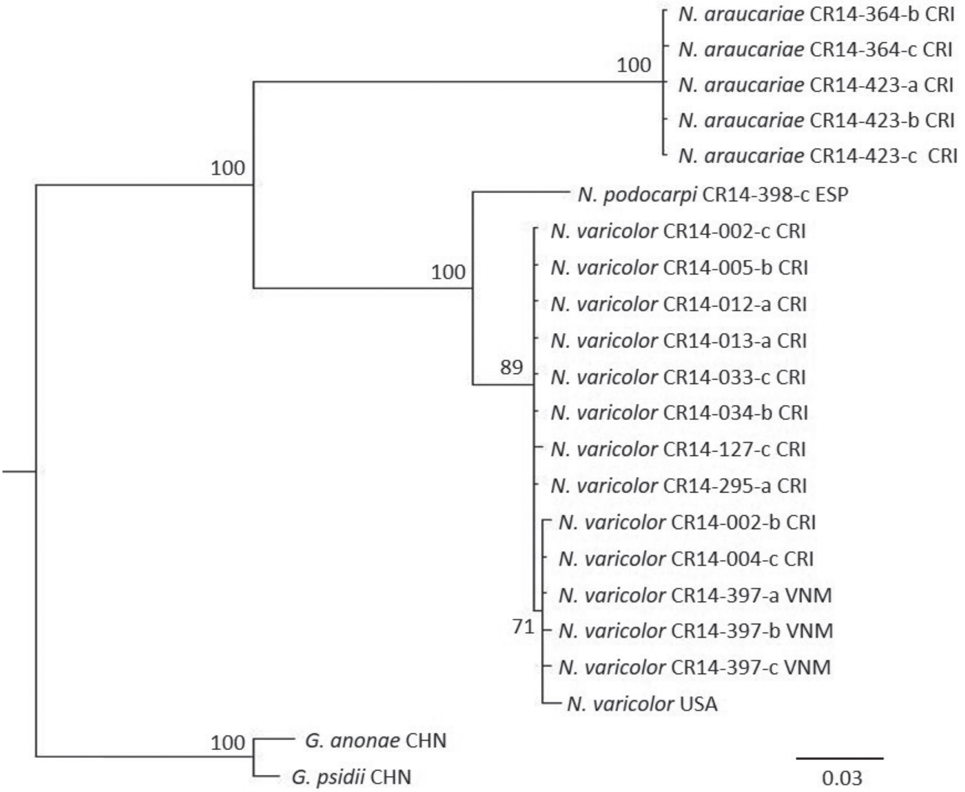

Figure 5. Phylogenetic analysis of *Neophyllaphis araucariae* Takahashi, *Neophyllaphis podocarpi* Takahashi and *Neophyllaphis varicolor* Miller & Halbert, using concatenated sequences of cytochrome *c* oxidase subunit I and elongation factor 1α made by Bayesian inference and using as outgroup *Greenidea psidii* van der Goot. and *Greenidea anonae* (Pergande). Sample names: species name - colony code - (a, b or c represent the specimen sampled) - country code. Key: CHN: China, CRI: Costa Rica, ESP: Spain, USA: United States, VNM: Vietnam. Scale bar represents 0.03 changes per site.

The phylogenetic analysis made with a concatenated sequence composed of partial COI and EF-1α sequences showed a clade grouping all the sequences of *N. varicolor* and another clade with the sequences of *N. araucariae*, in accordance with the COI phylogenetic tree (Figure 4) and with the morphological identification (Figure 5).

Discussion

According to our results, the recently described aphid species *N. varicolor* also is present in Costa Rica and Vietnam. In addition to *N. varicolor* sequence information for Costa Rica and Vietnam, we also generated and submitted partial COI and EF-1α sequences for the species *N. araucariae* to GenBank for the first time. Indeed, we found few sequences available for the genus *Neophyllaphis* in public databases. Publicly available sequence information for all the describe species is important for comprehensive studies of the genus.

The morphological studies and molecular comparisons of COI and EF-1α sequences, supported the description of the new, distinct species, *N. varicolor* on *Podocarpus* spp. (Miller and Halbert 2014). All COI and EF-1α sequences of *N. varicolor* showed less than 0.5% of difference to the reference *N. varicolor* sequences deposited in GenBank. Previous studies have determined that the intraspecific variation in aphids is circa 0.6% in most of the species. There are some exceptions, like *Neomyzus circunflexus* (Buckton), with 3.14%, the species with the highest intraspecific variation in COI out of 300 aphid species included in the study by Foottit et al. (2008).

The COI gene was characterized by a high interspecific variation (Floyd et al. 2009). The level of interspecific variation of COI (11.4%) and EF-1α (13.4 %) observed between sequences of *N. varicolor* and *N. araucariae* from Costa Rica, was congruent with the high genetic divergence expected between different species. However, in some cases, the interspecific variation of COI between congeneric species could be less than 1% (Chen et al. 2012).

According to our phylogenetic analyses made with COI region, the species *N. podocarpi* and *N. brimblecombei* are most related to *N. varicolor*, similar to findings by Miller and Halbert (2014); however, few sequences of *Neophyllaphis* species are available in GenBank or BOLD, so many species are not represented in the phylogenetic analysis. Previous studies have reported that *N. podocarpi* and *N. brimblecombei* have the same number of chromosomes and high morphological similarity, which suggest a recent separation between the species (Hales and Lardner 1988). Therefore, it is plausible to hypothesize that both species also were separated recently from *N. varicolor* in evolutionary time.

Our discoveries of *N. varicolor* in Costa Rica and Vietnam represent the first time that *N. varicolor* is reported outside of the USA. Currently, the genus *Neophyllaphis* is thus represented by two species in Costa Rica: *N. varicolor* and *N. araucariae*.

The presence in Vietnam of *N. varicolor* suggests that it is a species native to Southeast Asia. However, the genus *Neophyllaphis* presents taxonomic problems (Blackman and Eastop 2019) that must be solved with a good taxonomic, bionomic and molecular revisions. A full revision of the genus might shed better light on the geographic origins of the different species (Nibouche et al. 2018).

Acknowledgments

This research, and the scientific visit of NPH to the CIBCM in 2014, were funded by University of Costa Rica (grants 801-B7-169 and 801-A1-801) and the investigation in Spain was conducted in the context of Project CGL2015-68188-P, funded by "Ministerio de Economía, Industria y Competitividad" (MIMECO) of Spain. The authors appreciate the comments and corrections made by Susan Halbert and Roger Blackman.

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ZooKeys 865: 137–154 (2019)
doi: 10.3897/zookeys.865.36296
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RESEARCH ARTICLE

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New insights in *Trichochloritis* Pilsbry, 1891 and its relatives (Gastropoda, Pulmonata, Camaenidae)

Barna Páll-Gergely[1], Eike Neubert[2,3]

1 *Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary* **2** *Natural History Museum of the Burgergemeinde Bern, Bernastr. 15, CH-3005 Berne, Switzerland* **3** *Institute of Ecology and Evolution, University of Bern, 3012 Bern, Switzerland*

Corresponding author: *Barna Páll-Gergely* (pall-gergely.barna@agrar.mta.hu, pallgergely2@gmail.com)

Academic editor: *Frank Köhler* | Received 18 May 2019 | Accepted 23 June 2019 | Published 22 July 2019

http://zoobank.org/EAD1EAB6-70BE-48B1-87E9-DBBFCBEA5EAD

Citation: Páll-Gergely B, Neubert E (2019) New insights in *Trichochloritis* Pilsbry, 1891 and its relatives (Gastropoda, Pulmonata, Camaenidae). ZooKeys 865: 137–154. https://doi.org/10.3897/zookeys.865.36296

Abstract

The genus *Bellatrachia* Schileyko, 2018 was described based on a specimen identified as *Helix* (*Chloritis*) *pseudomiara* Bavay & Dautzenberg, 1909. We concluded that the examined specimen is not that species, but *Helix condoriana* Crosse & Fischer, 1863. Therefore, (1) the type species of *Bellatrachia* must be replaced with *Helix condoriana*; (2) the species *Helix* (*Chloritis*) *pseudomiara* must be re-allocated to the genus *Trichochloritis*; (3) the erroneous treatment of the genus *Trichochloritis* by Schileyko(2007) needs to be corrected through the description of a new genus, *Dentichloritis* **gen. nov.** based on *Helix brevidens* Sowerby I, 1841. In addition, *Chloritis microtricha* Möllendorff, 1898 is treated as a synonym of *Helix condoriana*, and further information on the genitalia of *Chloritis* (?) *bifoveata* (Benson, 1856) is presented.

Keywords

Land snail, nomenclature, Southeast Asia, systematics, taxonomy

Introduction

Almost 20 years ago, the second author of this work became fascinated by the enormously rich shell collection of Colonel Messager (see Breure and Páll-Gergely 2019) from northern Vietnam and Laos housed in the MNHN. While many type specimens taken from Messager's collection were distributed through the activities of the describing authors to other institutions, the main body of the collection remained untouched in Paris. At the suggestion of the first author, we started to systematically compile data on the haired camaenid species of Southeast Asia.

This group was traditionally classified in the genera *Trichochloritis* Pilsbry, 1891 and *Trachia* E. von Martens, 1860 (Richardson 1985; Schileyko2011; Wu et al. 2019); however, it was clear from the beginning that haired and non-haired shells are present in many camaenid genera, that the current classification is rather a paraphyletic "waste-basket taxon", and that only the investigation of the morphology of the genital organs in combination with genetic data will recover the correct phylogenetic relationships. Nonetheless, even current modern research can add to the confusion rather than unravelling some of the old errors.

According to Schileyko(2007), the genus *Trichochloritis* consists of 10–12 species from southern China, Indochina Peninsula, and the Philippines. He published an illustration (drawing) of the shell of the type species, *H. breviseta* (Schileyko2007: fig. 2032a), and added drawings of the reproductive anatomy of *H. brevidens* Sowerby I, 1841 (Schileyko2007: fig. 2032b–c) as representative of *Trichochloritis*. However, the morphology of the genital organs of the latter species differs strongly from the conchologically similar genera as used here (*Trichochloritis*, *Bellatrachia*) from Continental Asia. In 2018, Schileykodescribed the monotypic genus *Bellatrachia*, a genus which was introduced based on conchological characters and traits of the genital anatomy of *Helix* (*Chloritis*) *pseudomiara* Bavay & Dautzenberg, 1909. Unfortunately, the anatomically examined specimen, which was collected in the Cat Tien National Park, southern Vietnam, was misidentified: in fact, Schileyko's (2018) specimen is *Helix condoriana* Crosse & Fischer, 1863.

These misidentifications and errors have nomenclatorial and taxonomical consequences: 1) the type species of *Bellatrachia* must be replaced; 2) the species *Helix* (*Chloritis*) *pseudomiara* Bavay & Dautzenberg, 1909 must be re-allocated in the genus *Trichochloritis*; 3) the erroneous treatment of the genus *Trichochloritis* by Schileyko(2007) needs to be corrected through the description of a new genus, *Dentichloritis* nov. gen. based on *Helix brevidens* Sowerby I, 1841. In addition, the position of two continental species usually confined to *Chloritis* Beck, 1837, is discussed.

Materials and methods

An ethanol-preserved specimen of *Chloritis* (?) *bifoveata* (Benson, 1856) was dissected under a Leica stereo microscope with a camera attachment to provide photographs of the external genital structure, from which drawings were produced. The inner structure of reproductive organs was illustrated from photographs.

Institutional abbreviations:

BOR/MOL BORNEENSIS	collection of Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah

MNHN	Muséum National d'Histoire Naturelle (Paris, France)
NHMUK	The Natural History Museum (London, UK)
RBINS	Royal Belgian Institute of Natural Sciences (Brussels, Belgium)
SMF	Senckenberg Forschungsinstitut und Naturmuseum (Frankfurt am Main, Germany)
ZMH	Zoological Museum, University of Hamburg (Germany)
ZSI	Zoological Survey of India (Kolkata, India).

Abbreviations for anatomical details:

EP	Epiphallus
Fl	Flagellum
MRP	Musculus retractor penis
P	Penis
Pa	Penial appendix
RS	Receptaculum seminis
VD	Vas deferens

Taxonomy

Family Camaenidae Pilsbry, 1893

Genus *Bellatrachia* Schileyko, 2018

Bellatrachia Schileyko, 2018: 169–171.

Type species. *Helix* (*Chloritis*) *pseudomiara* Bavay & Dautzenberg, 1909 by monotypy.

The anatomically-examined specimen (i.e., on which the genus is based) was in fact *Helix condoriana*. Under the provisions of Article 70.3 ICZN, we herewith replace the original type species *Helix* (*Chloritis*) *pseudomiara* Bavay & Dautzenberg, 1909 with *Helix condoriana* Crosse & Fischer, 1863 as the type species of *Bellatrachia* Schileyko, 2018 to serve the stability of nomenclature.

Included species. *Bellatrachia condoriana* (Crosse & Fischer, 1863).

Diagnosis. Shell depressed globular, apex not sunken, hairs or hair scars cover the entire shell. Penis rather long, subcylindrical, its inner surface bears longitudinal pilasters; penial verge absent; penial caecum absent; epiphallus slender, long, convoluted; retractor muscle attached at the penis-epiphallus transition; flagellum thick, with attenuated tip, approximately 2–2.5 times shorter than epiphallus; vagina slender, shorter than penis; stalk of bursa copulatrix long, with thickening at some distance from its origin, shape of bursa unknown (based on Schileyko2018; see Fig. 4).

Description. See *B. condoriana* below.

Remarks. *Bellatrachia* differs from *Trichochloritis* in lacking the penial caecum.

Bellatrachia condoriana (Crosse & Fischer, 1863)

Figs 1–4

Helix condoriana Crosse & Fischer, 1863: 351, pl. 14, fig. 1.
Chloritis microtricha Möllendorff, 1898: 71. syn. nov.
Chloritis (*Trichochloritis*) *microtricha*: Zilch 1966: 304, pl. 9, fig. 23.
Trichochloritis microtricha: Schileyko2011: 47.
Trichochloritis condoriana: Schileyko2011: 47.
Bellatrachia pseudomiara: Schileyko2018: 169–171, figs 1–2 [non *Helix* (*Chloritis*) *pseudomiara* Bavay & Dautzenberg, 1909].

Type specimens. *condoriana*: 1 syntype MNHN-IM-2000-1866, Poulo-Condor, D: 18.3 mm, H: 11.7 mm [this is the syntype figured in the original description]; *microtricha*: lectotype (selected by Zilch 1966) SMF 8540, Vietnam, Annam, Boloven, coll. O. Möllendorff ex coll. Roebelen, D: 24.8 mm, H: 17.7 mm.

Type locality. "insula Poulo-Condor" [Con Son Island], Vietnam.

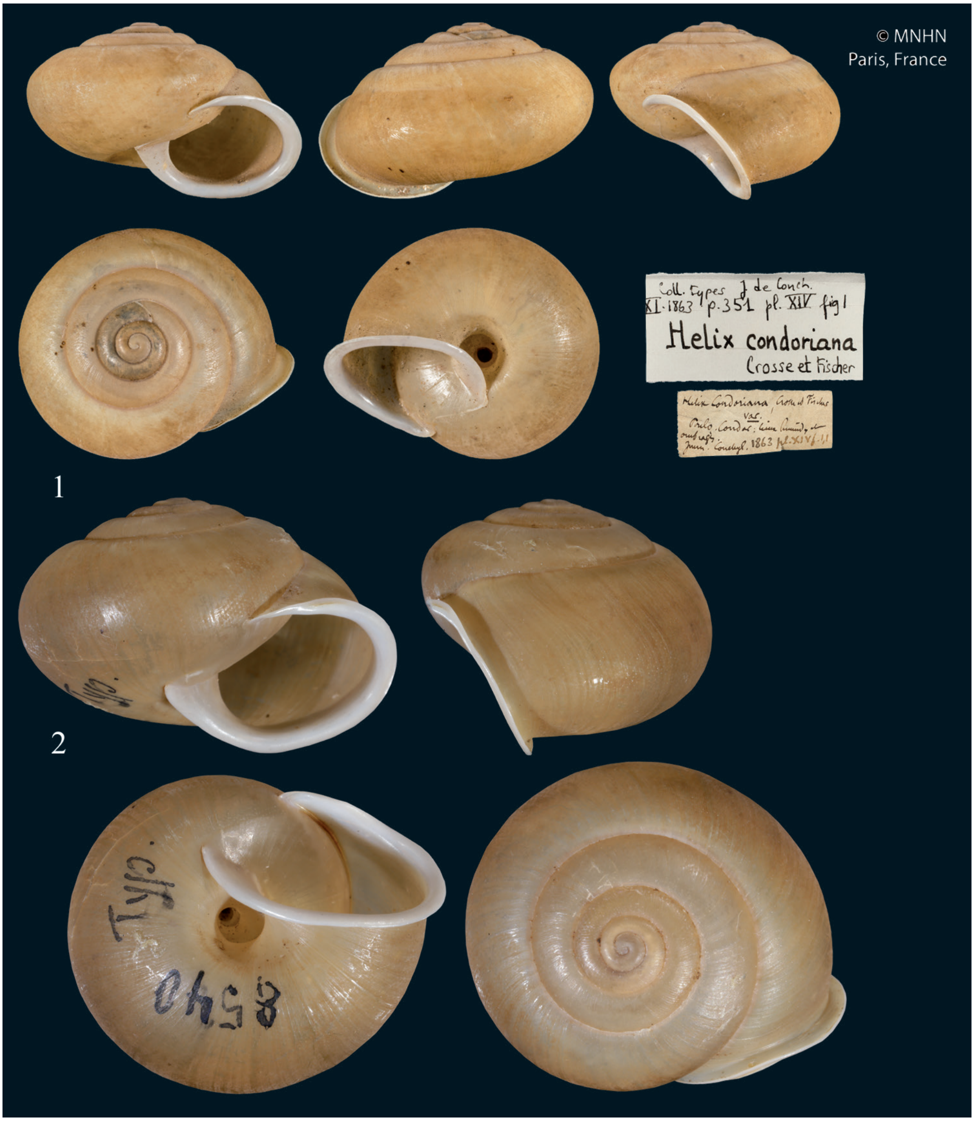
Additional specimens. Vietnam, Bang-Kiang, coll. Denis ex Messager, MNHN-IM-2012-27151 (2 shells).

Diagnosis. Shell biconvex with a whitish subsutural spiral, narrow umbilicus, and hair scars covering the entire surface.

Description. Shell middle sized, biconvex, moderately thin-walled; last whorl only slightly expanding and descending abruptly towards aperture; colour dirty yellowish with a broad pale subsutural spiral band; whorls 4.5–5, separated by a rather shallow suture; body whorl faintly slightly angled; subsutural furrow shallow but present on the complete last whorl; protoconch consists of 1.25–1.5 whorls, very finely squamous, matte; the pattern of hair scars is dense and covers the complete teleoconch; aperture obliquely rounded, and the peristomal rims are close; peristome strongly expanded and somewhat reflected and reinforced by a white lip; parietal side with very thin, inconspicuous light layer; umbilicus open, of medium size, with blunt peripheral angulation, and partly covered by the columellar reflection.

Measurements. D = 18.3–24.8 mm; H= 11.7–17.7 mm (n = 4).

Remarks. The syntype of *B. condoriana* (Fig. 1) is similar to the specimen identified as *Helix* (*Chloritis*) *pseudomiara* by Schileyko(2017) (Fig. 3), but the shell of the latter is somewhat more depressed. The shell of the lectotype of *B. microtricha* (Fig. 2) is larger and somewhat more globular that that of *B. condoriana*. However, both taxa agree quite well in other details such as the relative size of the umbilicus, formation of lip and aperture, and microsculpture of the teleoconch. In contrast, absolute dimensions proved to be insufficient traits for species-level distinction. Therefore, we consider *Chloritis microtricha* as a synonym of *Bellatrachia condoriana*. The subtle conchological differences in the shell morphology shown in Figs 1–3 may be part of the overall variation of *B. condoriana* or may signal a difference at the species level. This question can only be clarified by a revision of a larger number of specimens from the area.

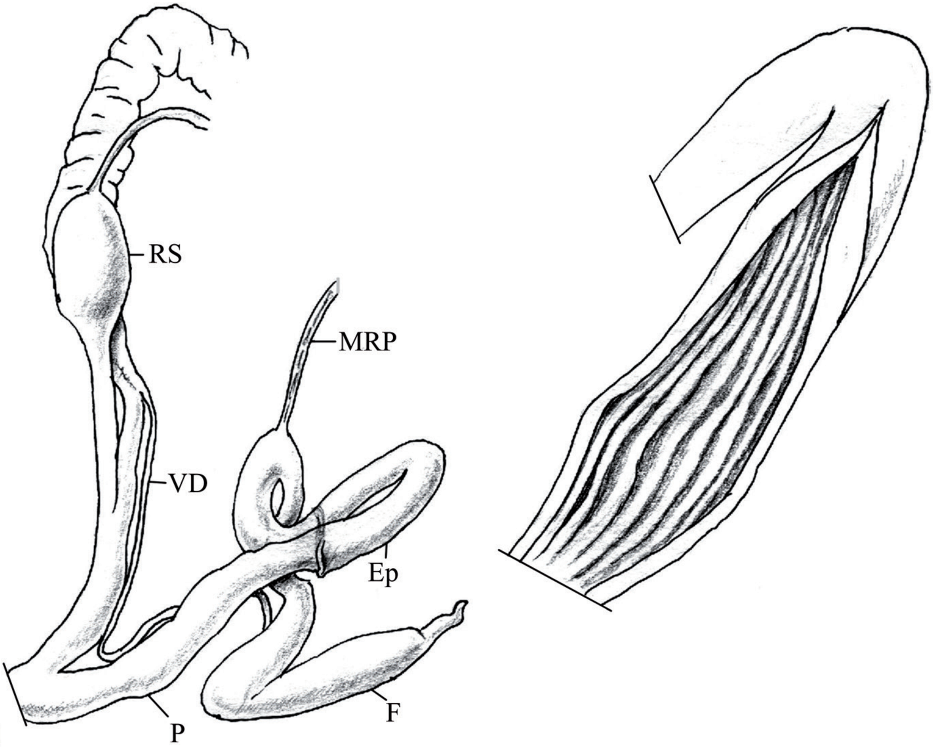


Figures 1–2. *Bellatrachia condoriana* **1** Syntype *Helix condoriana* Crosse & Fischer, 1863, MNHN-IM-2000-1866, D = 18.3 mm, MNHN **2** lectotype *Chloritis microtricha* Möllendorff, 1898, SMF 8540, D = 24.8 mm, S. Hof, Senckenberg. All photographs × 2.

Genus *Trichochloritis* Pilsbry, 1891

Trichochloritis Pilsbry, 1891: 267.
Trichochloritis: Schileyko2007: 2113–2114, fig. 2032 (partim).

Type species. *Helix breviseta* L. Pfeiffer, 1862 by original designation.



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Figures 3–4. Original specimen “*Bellatrachia pseudomiara*” sensu Schileyko**3** shell (Schileyko2018: 170, Fig. 1), D = 23.2 mm, A. Sysoev, photograph × 2 **4** Morphology of the genital organs of “*Bellatrachia pseudomiara*” sensu Schileyko(2018), modified after Schileyko(2018).

Included species. *Helix breviseta* L. Pfeiffer, 1862, *Trachia penangensis* Stoliczka, 1873.

Diagnosis. Shell depressed globular, apex not sunken, hairs or hair scars cover the entire shell. Penis thickened, probably with penial verge (?) and a slender, relatively long penial caecum; epiphallus slender, shorter than penis; retractor muscle attached at the penis-epiphallus transition; flagellum short; vagina slender, shorter than penis; stalk of bursa copulatrix long, with thickened base and oval bursa (based on the drawings of Stoliczka 1873: plate 3, fig. 18 and Collinge 1903: plate 12, fig. 17.).

Remarks. The anatomy of the genital organs of *Helix* (*Trachia*) *malayana* Möllendorff, 1887 (= *Trichochloritis breviseta*; see Maassen 2001) was described by Collinge (1903), and that of *T. penangensis* is known from Stoliczka (1873), here re-drawn and provided in Fig. 8 (*penangensis*) and Fig. 9 (*breviseta*). Both species possess a penial caecum, which is here considered as a diagnostic trait for the genus. Without knowing the full anatomy, it is uncertain how many of the hairy *Chloritis*-like species of continental Asia belong to this group.

Trichochloritis breviseta (L. Pfeiffer, 1862)

Figs 5–7, 9, 10

Helix breviseta L. Pfeiffer, 1862: 41–42, pl. 5, figs 4–5.
Helix (*Trachia*) *malayana* Möllendorff, 1887: 303.
Chloritis malayana Möllendorff, 1891: 335, pl. 30, figs 6–6a.
Helix (*Trachia*) *malayana*: Collinge 1903: 210, pl. 12, fig. 17.
Chloritis (*Trichochloritis*) *malayana*: Pilsbry 1893: 274, pl. 51, figs 34, 35.
Chloritis breviseta (and *Chloritis malayana*, which is considered a synonym): Maassen 2001: 120.
Trichochloritis breviseta: Schileyko2011: 47.
Chloritis breviseta: Foon et al. 2017: 56, fig. 21C.

Type specimens examined. *breviseta*: syntype MNHN-IM-2000-1847, Siam, D: 22.1 mm, H: 12.9 mm; *malayana*: syntypes (2 shells) NHMUK 1891.3.17.3–4, Perak, leg. Hungerford.

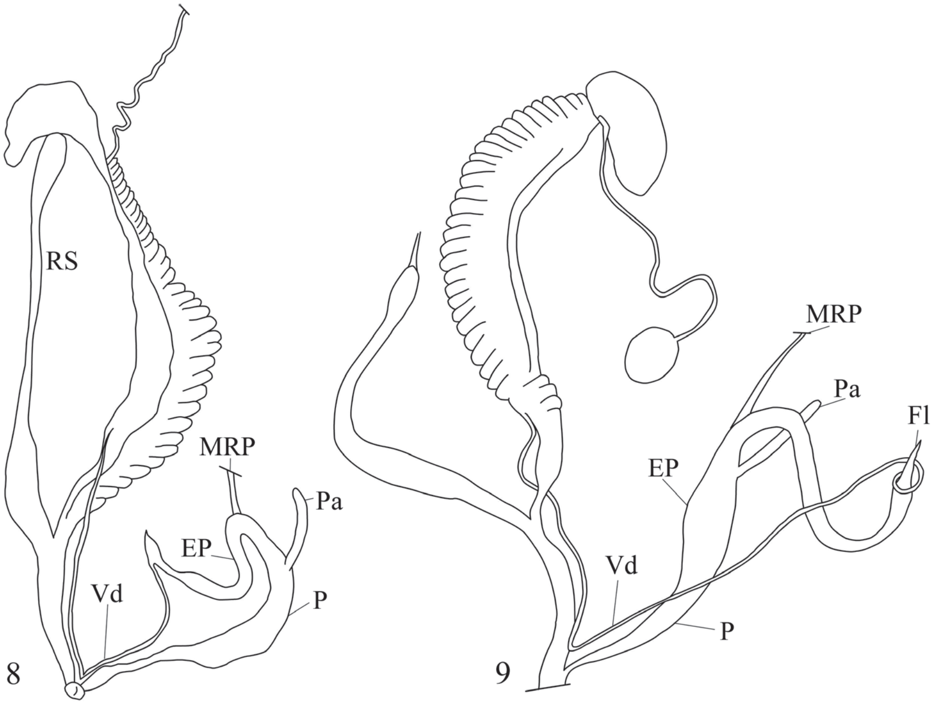
Additional specimens. Perak, leg. Hungerford, NHMUK 1891.3.17.3–4 (2 shells of "*malayana*"); Larut, Malay Peninsula, NHMUK 1897.3.15.7 (1 shell of "*malayana*"); Malakka, Kelantan, Hochland v. Perak, coll. O. Möllendorff ex coll. H. Rolle ex coll. Waterstraat, SMF 8538/1 ("*malayana*").

Type locality. "Siam" (*breviseta*); "Perak" [Perak state, Malaysia] (*malayana*).

Diagnosis. Shell depressed, unicoloured, yellowish, with permanent hairs; umbilicus funnel-shaped with a blunt peripheral angulation.

Description. Spire only slightly elevated, shell depressed, shell thin; last whorl bluntly angled, a subsutural furrow is present but insignificant; colour yellowish, spiral band missing; the 4.5 whorls separated by a rather shallow suture; protoconch consists of slightly more than 1.5 whorls, squamous, bears minute wrinkled hair scars; tel-

Figures 5–7. *Trichochloritis breviseta* **5** syntype *Helix breviseta* L. Pfeiffer, 1862, MNHN-IM-2000-1847, D = 22.1 mm, MNHN **6** syntype *Helix* (*Trachia*) *malayana* Möllendorff, 1887, NHMUK 1891.3.17.3, D = 22.2 mm, NHMUK **7** SMF 8538 ex coll. Möllendorff, D = 20.6 mm, S. Hof, Senckenberg. All photographs × 2.



Figures 8–9. Morphology of the genital organs of *Trichochloritis* species **8** *Trichochloritis penangensis* (Stoliczka, 1873) (redrawn from Stoliczka 1873) **9** *Trichochloritis breviseta* (L. Pfeiffer, 1862) (redrawn from Collinge 1903). Not to scale.

eoconch completely covered by a moderately dense pattern of hairs; bristles stiffy and durable and stick to the shell (their apical part breaks off, but a dark brown conical bristle cone is left making the surface of the shell quite rough); aperture subrectangular with only slightly oblique columella; peristome reflected and covered by a white lip; parietal region with very slight whitish, blunt lime layer, inconspicuous; columellar reflection small; umbilicus wide and funnel-shaped with a blunt peripheral keel.

Measurements. D = 22.9–24.1 mm; H = 12.9–14.7 mm (n = 4).

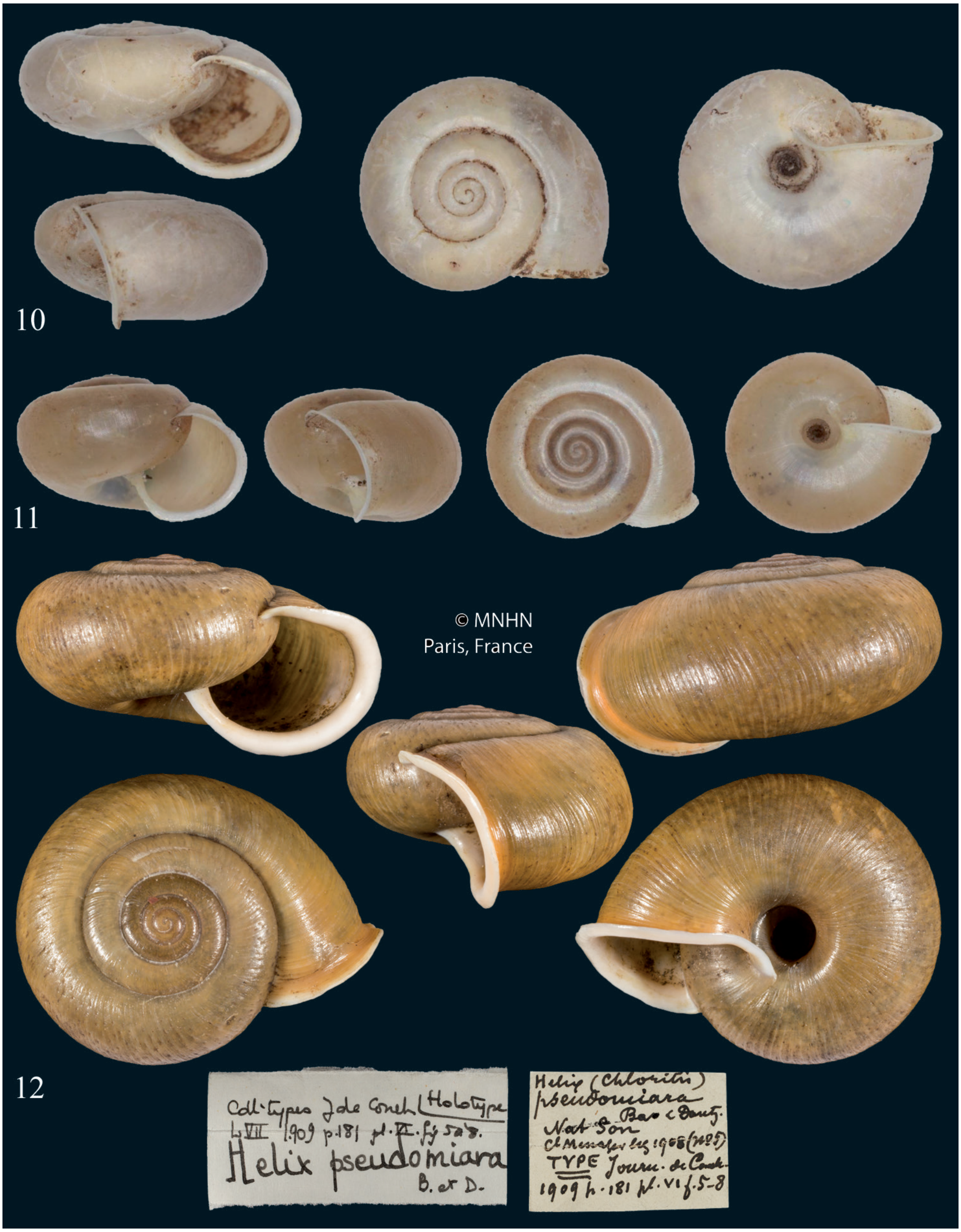
Distribution. Malaysia and Thailand

Trichochloritis penangensis (Stoliczka, 1873)

Figs 8, 11

Trachia penangensis Stoliczka, 1873: 24–26, pl. 3, figs 1, 18–20.
Chloritis penangensis: Foon et al. 2017: 56–57, fig. 21D.

Type specimens. The types should be in the Zoological Survey of India in Kolkata but were not found during a recent search (S.K. Sajan, pers. comm., December 2018). They were likewise not found in the NHM.



Figures 10–12. Shells of *Trichochloritis* species **10** *Trichochloritis breviseta*, BOR/MOL 9091, Perak, Ipoh, Gunung Kanthan plot, D = 19.5 mm **11** *Trichochloritis penangensis*, BOR/MOL 11562, Perak, Ipoh, Gunung Pondok, plot, D = 16.2 mm **12** *Trichochloritis* (?) *pseudomiara*, syntype of *Helix* (*Chloritis*) *pseudomiara* Bavay & Dautzenberg, 1909, D = 24.3 mm, MNHN. **10, 11** Junn Kitt Foon (published in Foon et al. 2017), all photographs × 2.

Type locality. "Penang".

Remarks. "*Chloritis penangensis* has a much more globular shell with less expanded whorls compared to *Chloritis breviseta* which has more expanded (perpendicular to the axis) whorls and thus, "wider" looking shells. These characters appear consistent for each species across Peninsular Malaysia (based on conchological comparisons), although shell size varies within each species." (Junn Kitt Foon, pers. comm., 01 Dec 2018). To illustrate these differences, we illustrated the shells of both species (Figs 10, 11).

Genus uncertain

***Chloritis* (?) *bifoveata* (Benson, 1856)**

Figs 15–17

Helix bifoveata Benson, 1856: 251.
Chloritis bifoveata: Sutcharit and Panha 2010: 278–283, figs 1A, B, 2A–F, 3A–D, table 1.

Specimens examined. Thailand: Krabi: Phanom Benja National Park, Huai To waterfall and surrounding rain forest, 120 m, 08°14'21"N, 098°54'52"E, 08°14'08"N, 098°55'12"E, leg. Hausdorf, 28.07.2010, ZMH 51997/2.

Remarks. For a detailed description of the shell refer to Sutcharit & Panha, 2010. Our data on the reproductive anatomy largely matches that of Sutcharit and Panha (2010), with the following two exceptions: the flagellum is relatively long and slender, and the penial verge is not irregularly shaped but conical and deeply grooved with the folds starting from the epiphallus.

***Trichochloritis* (?) *pseudomiara* (Bavay & Dautzenberg, 1909)**

Fig. 12

Helix (*Chloritis*) *pseudomiara* Bavay & Dautzenberg, 1909a: 236; Bavay and Dautzenberg 1909b: 181, pl. VI, figs 5–8.
Trachia pseudomiara: Schileyko2011: 45.

Type specimens examined. syntype MNHN-IM-2000-31774, Nat Son, leg. Messager, D: 24.3 mm, H: 13.3 mm.

Other specimens examined. Muong-Hum, RBINS/1; Muong-Hum, leg. Messager 1908, RBINS/1; Tonkin, Phong-Tho, RBINS/1 (mixed sample with *Trichochloritis* sp.); Nat-Son, RBINS/3 (mixed sample with *Trichochloritis* sp.); Tonkin, leg. Messager, RBINS/22 (some of them are juveniles); Tonkin, Phong-Tho, NHMUK 1909.7.9.57/1 (photographed for the North Vietnamese Land Snail Guide); Tonkin, Muong-Hum, MNHN-IM-2012-27105/2; Vietnam, Cam Duong, MNHN-IM-2012-27106/2 (probably erroneous locality?); Tonkin, Phong-Tho, MNHN-IM-2012-27107/2; Tonkin, Muong-Hum, MNHN-IM-2012-27108/2; Tonkin, Muong-Hum, leg. Messager, MNHN-IM-2012-27109/2; Tonkin, MNHN-IM-2012-27110/1; Haut Tonkin, MNHN-IM-2012-27111/1.

Type locality. Vietnam, N Vietnam: Nat Son.

Diagnosis. A rather large, usually dark species with rounded body whorl, fine radial growth lines and deep hair scars; umbilicus open, only a small part of it is covered by the columellar reflection.

Description. Shell rather large, almost flat, with relatively thick wall; body whorl rounded; last half whorl with or without very shallow subsutural furrow; the 4.75–5.25 whorls are separated by a shallow suture; colour greyish yellowish, or brown to olive green; protoconch consists of 1.5 whorls, finely granulate, with fine radial lines near the suture of the last half whorl; teleoconch finely, irregularly wrinkled, and covered with very deep hair scars, which are visible to the naked eye as well on the body whorl; hairs not permanent, although we did not have access to live collected specimens; aperture ovoid; peristome expanded and slightly reflected, and reinforced by a thickened whitish/light brown lip; parietal region with an inconspicuous layer, which is often darker than the rest of the shell; umbilicus widely open, concave and funnel-shaped, slightly covered by reflected peristome.

Measurements. D = 21.3 –26.0 mm, H = 11.8–14.4 mm (n = 3).

Distribution. This species is known only from the northernmost part of Vietnam, along the Chinese border.

Remarks. This species can easily be identified based on the dark green-coloured shell and the deep, widely spaced hair scars that cover the entire teleoconch.

Genus *Dentichloritis* gen. nov.

http://zoobank.org/16A52E49-1C90-47D6-B66A-56F12C02B11A

Trichochloritis: Schileyko, 2007: 2113–2114, fig. 2032a–c (partim).

Type species. *Helix brevidens* Sowerby I, 1841: 25 (Puerto Galero, Philippines).

Diagnosis. Shell depressed globular, apex not sunken, hairs or hair scars cover the entire shell, aperture with a basal denticle. Penis very thick-walled, with narrow lumen, internally with very large conic tubercles in main chamber; flagellum and epiphallus absent; vas deferens passes gradually enlarging into penis; retractor muscle inserts at curvature of vas deferens close to its joint with penis; penial sheath thin, surrounds upper two third part of penis; vagina shorter than penis, thick.

Etymology. The name *Dentichloritis* refers to the presence of a denticle on the basal peristomal lip and the conchological similarity to *Chloritis*.

Remarks. There are seven *Trichochloritis* species known from the Philippines (Richardson 1985), and four of them have been photographed by Zilch (1966). They differ from *D. brevidens* in the open umbilicus and the lack of denticle on the basal lip. Therefore, we retain them in *Trichochloritis* until ethanol-preserved specimens become available.

Dentichloritis brevidens **(Sowerby I, 1841)**

Figs 13–14

Helix brevidens Sowerby I, 1841: 25.
Trichochloritis brevidens: Schileyko2007: 2113–2114, fig. 2032b, c.

Type specimens examined. Philippines, m.c. (Museum Cuming), 3 syntypes NHMUK 20190452 (D of photographed shell = 19.5 mm [Fig. 13]).

Type locality. Philippines, Puerto Galero (Municipality of Puerto Galera, municipality in the province of Oriental Mindoro).

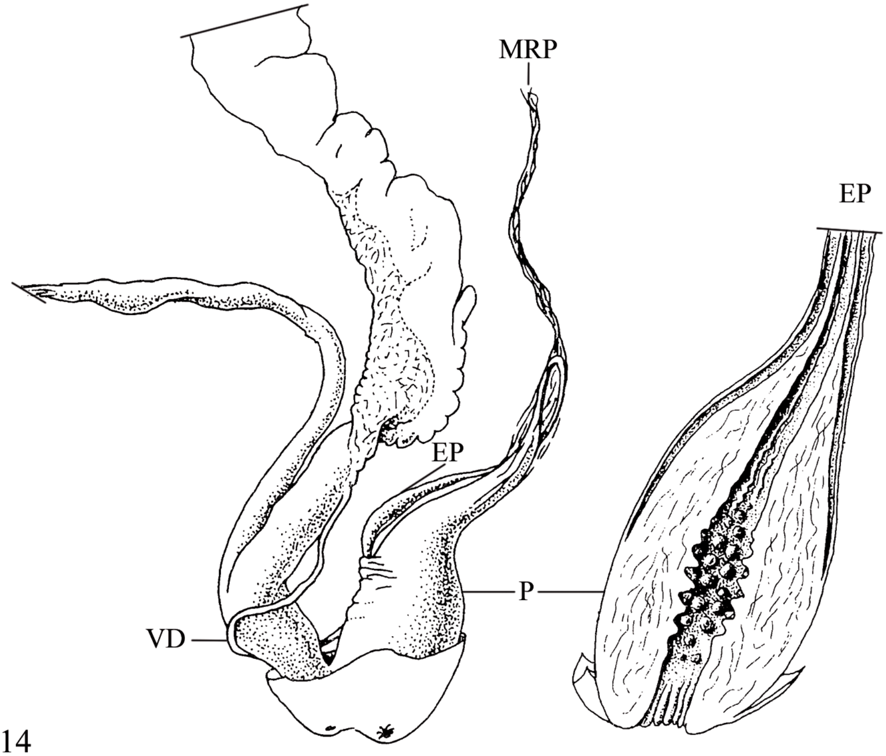
Diagnosis. A middle-sized, yellowish species with a slender reddish peripheral belt, short hairs on the entire shell, nearly closed umbilicus (only visible in oblique view), and a slight thickening (denticle) on the basal part of peristome.

Description. Shell medium sized, depressed globular; body whorl rounded with slight indication of a blunt shoulder; last quarter to half whorl with a very shallow subsutural furrow; the 3.75–4 whorls are separated by a shallow suture; colour yellowish to ochre with a reddish slender belt above shoulder (midpoint of body whorl); protoconch consists of 1.5–1.75 whorls, finely granulate, with fine radial wrinkles; teleoconch covered by short hairs or hair scars, which are visible to the naked eye as well; aperture semilunar; peristome expanded and slightly reflected, and reinforced by a thickened whitish brown lip; a slight swelling (denticle) visible on basal part of peristome, between the midpoint of the basal peristome and the columella; parietal region with an inconspicuous layer, which is matter than the rest of the shell; umbilicus nearly closed by columellar reflection, visible only by oblique view.

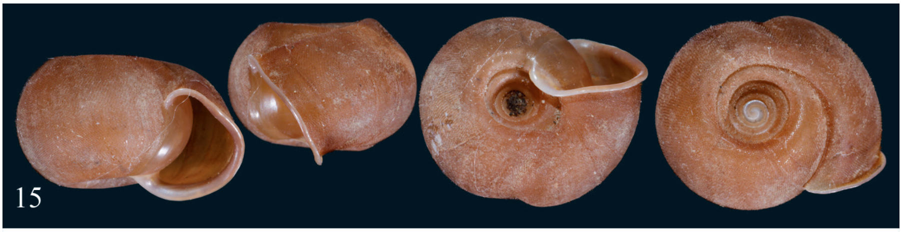
Anatomy: Penis very thick-walled, with narrow lumen, internally with short plicae in basal part and very large conic tubercles in main chamber; flagellum and epiphallus absent; vas deferens rather long, evenly thin down to atrium; approximately one third way up it is attached to penis, and after penis is enlarged and fusiform, then in becomes very thin, thread-like, forming a sharp curvature and passes to penis, gradually enlarging; penial retractor attached to curvature of vas deferens and continues as a fine membrane down to middle part of penis; penial sheath thin, surrounds upper two third part of penis. Vagina shorter than penis, thick; spermatheca without visible division to stalk and reservoir, not attending albumen gland and provided with apical ligament (based on Schileyko2007: 2113–2114, fig. 2032b, c).

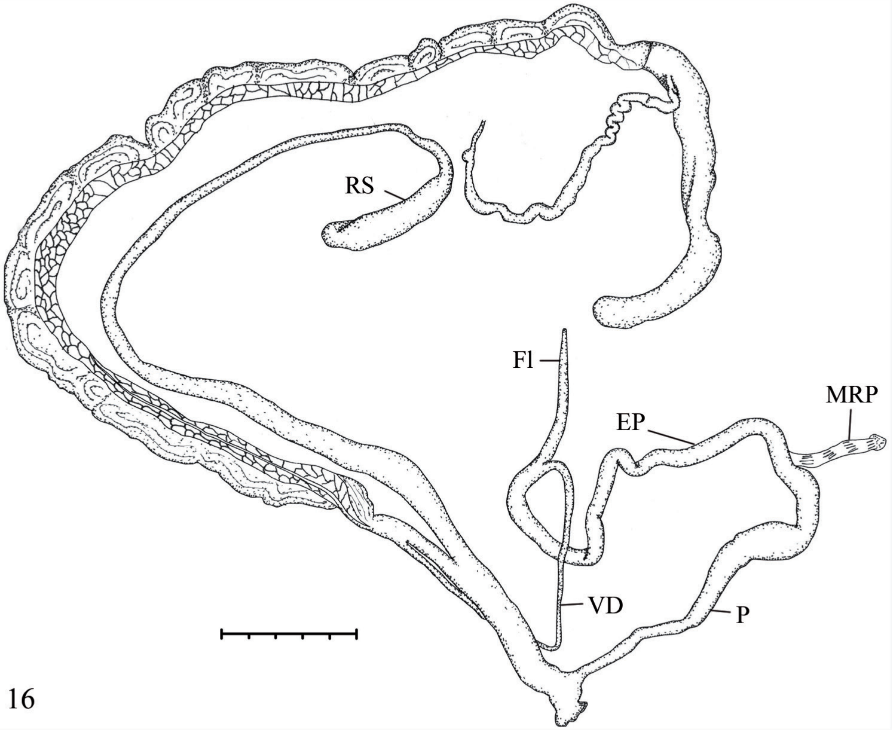
Discussion

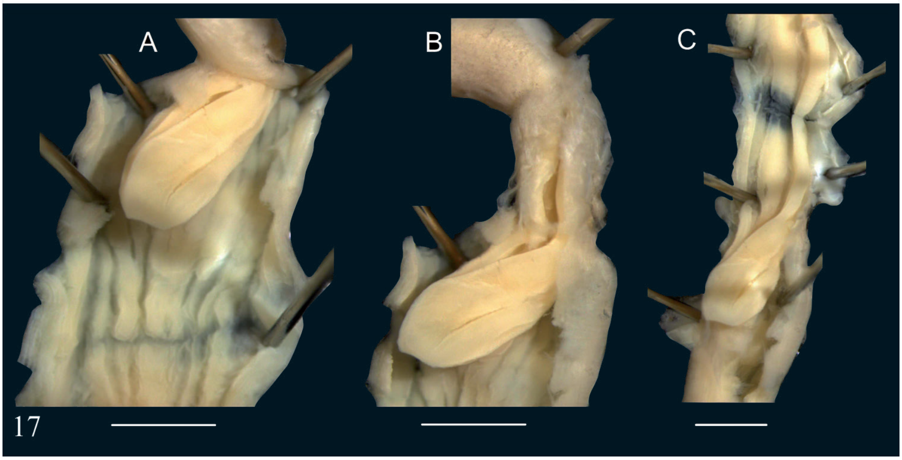
Based on an anatomically examined specimen from southern Vietnam identified as *Helix pseudomiara* Bavay & Dautzenberg, 1909, Schileyko(2018) described the genus *Bellatrachia* Schileyko, 2018. However, that specimen is clearly incorrectly identified. Schileyko's (2018) specimen has a rounded aperture and fine hair scars with fine silky periostracum. Thus, it closely resembles *Helix condoriana* Crosse & Fischer, 1863, also known from southern Vietnam. In contrast, the true *Helix pseudomiara* is known



Figures 13–14. *Dentichloritis brevidens* **13** syntype *Helix brevidens* Sowerby I, 1841, NHMUK 20190452, D = 19.5 mm **14** Morphology of the genital organs of *Dentichloritis brevidens*; modified after Schileyko(2007).







Figures 15–17. *Chloritis* (?) *bifoveata* **15** shell of dissected specimens of *Chloritis bifoveata* (Thailand: Krabi: Phanom Benja National Park, ZMH 51997) **16** Situs of its genital organs **17** Penial verge of *Chloritis bifoveata*. A: verge visible from penis lumen; B: starting to penial verge from epiphallus; C: epiphallus opened until penial verge. Scale bar 1 mm.

only from northern Vietnam, and its shell has characteristic deep and sparsely arranged hair scars. Furthermore, the aperture of the latter is rather oval, not rounded. The reproductive anatomy of type species of *Trichochloritis* Pilsbry, 1891, *Trichochloritis breviseta* (L. Pfeiffer, 1862), was described by Collinge (1903). Although it is not sufficiently detailed (i.e., the inner structure of penis is unknown), it is useful enough to diagnose *Trichochloritis*. The anatomy of *Trichochloritis penangensis* (Stoliczka, 1873) was described in the original generic description, and it largely matches with that of *T. breviseta*. Schileyko(2007) described the genitalia of *Trichochloritis brevidens* (Sowerby I, 1841), originally described from Mindoro Island, the Philippines, as a representative of *Trichochloritis*. The reproductive anatomy of that species, however, differs from those of continental (true) *Trichochloritis* in several important characters. Therefore a new genus, *Dentichloritis* gen. nov. is erected for *T. brevidens*. The largely different anatomy, together with biogeographical reasons, suggest that *Trichochloritis* (continental Asia) and *Dentichloritis* gen. nov. (Philippines) are probably not even closely related.

In the original description of *Trichochloritis*, Pilsbry (1891) claimed that the most closely related genus was *Planispira* Beck, 1837. The anatomy of the type species of that genus (*Helix zonaria* Linnaeus, 1758) was described by Schileyko(2003), and is distinguished from *Trichochloritis* at first sight by the absence of a penial caecum.

It is difficult to interpret the relationship of *Trichochloritis* with *Chloritis*, because the reproductive anatomy of the type species of the latter (*Helix ungulina* Linnaeus, 1758, by subsequent designation of Martens in Albers, 1860, from Ceram Island, Indonesia) is unknown. *Chloritis* is diagnosed conchologically mainly based on the sunken spire and the hairless shell (Schileyko2003). Thus, the two continental species assigned to *Chloritis*, namely *Chloritis bifoveata* (Benson, 1856) and *Chloritis diplochone* Möllendorff, 1898, do not even fit due to their strongly hairy shells. It is very unlikely that the two species inhabiting Thailand and Malaysia would belong to the same group as a species from Ceram Island. However, we refrain from erecting a genus for *C. bifoveata* and *C. diplochone* until we have more information on the anatomy of *C. ungulina*.

Acknowledgements

We are grateful to Sigrid Hof, Ronald Janssen (SMF), Virginie Héros, Philippe Bouchet, Philippe Maestrati (MNHN), Jonathan Ablett (NHM), and Thierry Backeljau (RBINS) for offering access to their museum collections, to Bernhard Hausdorf (ZMH) for loaning specimens of *C. bifoveata*, to Herold Taylor, to Junn Kitt Foon for his information and photographs on *T. penangensis* and *T. breviseta*, to Sheikh K. Sajan (ZSI) for searching for the types of *T. penangensis*, to Yuri Kantor and Alexander Sysoev for sending high quality photographs of *Bellatrachia* cf. *condoriana*. This study was supported by the MTA (Hungarian Academy of Sciences) Premium Post Doctorate Research Program for BPG.

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