ZooKeys 584: 1–23 (2016)
doi: 10.3897/zookeys.584.8171
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Description and life-cycle of *Taenia lynciscapreoli* sp. n. (Cestoda, Cyclophyllidea)

Voitto Haukisalmi[1], Sergey Konyaev[2], Antti Lavikainen[3], Marja Isomursu[4], Minoru Nakao[5]

1 *Finnish Museum of Natural History Luomus, P.O. Box 17, FI–00014 University of Helsinki, Finland* **2** *Institute of Systematics and Ecology of Animals SB RAS, 630091, Frunze str. 11, Novosibirsk, Russia* **3** *Immunobiology Program/Department of Bacteriology and Immunology, Faculty of Medicine, P.O. Box 21, FI–00014 University of Helsinki, Finland* **4** *Finnish Food Safety Authority Evira, Elektroniikkatie 3, FI–90590 Oulu, Finland* **5** *Department of Parasitology, Asahikawa Medical University, Asahikawa, Hokkaido 078–8510, Japan*

Corresponding author: *Voitto Haukisalmi* (voitto.haukisalmi@helsinki.fi)

Academic editor: *B. Georgiev* | Received 18 February 2016 | Accepted 24 March 2016 | Published 25 April 2016

http://zoobank.org/E381D5EF-73A0-43C3-8630-A017869F1473

Citation: Haukisalmi V, Konyaev S, Lavikainen A, Isomursu M, Nakao M (2016) Description and life-cycle of *Taenia lynciscapreoli* sp. n. (Cestoda, Cyclophyllidea). ZooKeys 584: 1–23. doi: 10.3897/zookeys.584.8171

Abstract

A new species of tapeworm, *Taenia lynciscapreoli* sp. n. (Cestoda, Cyclophyllidea), is described from the Eurasian lynx (*Lynx lynx*), the main definitive host, and the roe deer (*Capreolus capreolus* and *C. pygargus*), the main intermediate hosts, from Finland and Russia (Siberia and the Russian Far East). The new species was found once also in the wolf (*Canis lupus*) and the Eurasian elk/moose (*Alces alces*), representing accidental definitive and intermediate hosts, respectively. The conspecificity of adult specimens and metacestodes of *T. lynciscapreoli* sp. n. in various host species and regions, and their distinction from related species of *Taenia*, was confirmed by partial nucleotide sequences of the mitochondrial cytochrome *c* oxidase subunit 1 gene. Morphologically, *T. lynciscapreoli* **sp. n.** can be separated unambiguously from all other species of *Taenia* by the shape of its large rostellar hooks, particularly the characteristically short, wide and strongly curved blade. If the large rostellar hooks are missing, *T. lynciscapreoli* may be separated from related species by a combination of morphological features of mature proglottids. It is suggested that *T. lynciscapreoli* has been present in published materials concerning the tapeworms of *L. lynx* and *L. pardinus* in Europe, but has been misidentified as *Taenia pisiformis* (Bloch, 1780). *Taenia lynciscapreoli* **sp. n.** has not been found in lynx outside the range of roe deer, suggesting a transmission pathway based on a specific predator–prey relationship. The present study applies a novel, simple approach to compare qualitative interspecific differences in the shape of rostellar hooks.

Keywords
Tapeworms, *Lynx*, *Capreolus*, *Alces*, wolf, Finland, Russia, Siberia

Introduction

Morphological differences between independent species of the genus *Taenia* Linnaeus, 1758 and related genera are often limited, and it can be expected that extensive surveys based on molecular methods will reveal unknown, more or less cryptic species. In favour of this idea, at least two probable new species were recently identified in molecular phylogenetic analyses by Terefe et al. (2014) on *Taenia* spp. of the spotted hyena *Crocuta crocuta*. In addition, *Taenia arctos* Haukisalmi, Lavikainen, Laaksonen & Meri, 2011, which uses bears of the genus *Ursus* as definitive hosts (Haukisalmi et al. 2011, Lavikainen et al. 2011, Catalano et al. 2014, 2015), was originally identified as a genetically independent lineage in a cervid intermediate host (*Alces alces*; Lavikainen et al. 2010).

A recent molecular phylogenetic study on *Taenia* spp. in the Eurasian lynx (*Lynx lynx*) from Finland revealed a genetic lineage, which could not be associated with any known species based on sequence data (Lavikainen et al. 2013). In addition, the rostellar hooks of the unknown lineage were shorter than in any *Taenia* species parasitizing felids in the Holarctic region, strongly suggesting presence of a new species. Phylogenetically, the unknown *Taenia* sp. was closely related to *T. hydatigena* Pallas, 1766 and *T. regis* Baer, 1923 from canids and felids (*Panthera* spp.), respectively. At that point, the intermediate hosts of the putative new species were unknown.

Since the report by Lavikainen et al. (2013), we have been able to collect additional molecular and morphological data of the unknown species from felids and cervids, which evidently represent the main definitive and intermediate hosts, respectively, of the new lineage. We here present the new data and describe the previously unknown species as *Taenia lynciscapreoli* sp. n.

Material and methods

The material used in the description of the new species consisted of 14 adult specimens: seven from *L. lynx* from Finland (four host individuals), five from the same host species from the Russian Federation (four host individuals), and two from the wolf (*Canis lupus*) from Russia (one host individual).

In addition, 11 metacestodes (cysticerci) were examined to characterize the rostellar hooks of the new species: two specimens from the European roe deer *Capreolus capreolus* and five specimens from the Eurasian elk/moose *Alces alces* (one host individual each) from Finland, and four specimens from the Siberian roe deer *Capreolus pygargus* (one host individual) from Russia.

Conspecificity of adults and metacestodes in various host species was confirmed using a partial nucleotide sequence (396 bp) of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene as previously described (Lavikainen et al. 2013). This region has been proved to be suitable for DNA barcoding of taeniids including the new species (Lavikainen et al. 2008, 2013). The sequences were compared with selected *cox1* sequences of *Taenia* spp. occurring in felids and/or cervids in the Holarctic region (8 species), and the phylogenetically related *T. regis*. The analyses were performed in MEGA7 (Tamura et al. 2013). The sequence set was aligned using ClustalW (Chenna et al. 2003). Pairwise divergences were calculated by Kimura 2–parameter (K2P) model (Kimura 1980) with a gamma setting 0.5. A phylogeny was constructed by the maximum likelihood method based on evolutionary model HKY+I (Hasegawa et al. 1985), as determined by the Bayesian information criterion. A maximum parsimony tree was used as the initial tree for the heuristic search, and the robustness of the phylogeny was tested by bootstrapping with 1000 replicates.

Adult cestodes were relaxed in water and fixed flat (without pressure) and preserved in 70–75% ethanol. Fragments of each specimen, representing various developmental stages, were stained with alum carmine, cleared in eugenol and mounted in Canada balsam. Hand–cut transverse sections of mature proglottids were prepared to determine the number of dorso–ventral testicular layers and the dorso–ventral position of terminal genital ducts with respect to the longitudinal ventral osmoregulatory canals and the nerve cord.

Cysticerci were fixed and preserved in 70–75% ethanol. The hook crowns extracted from cysticerci were mounted in Berlese's medium for study. Only hooks aligned well in the horizontal plane were used for the morphometric analysis.

Five linear measurements, as defined by Gubányi (1995), were taken from large and small rostellar hooks (Table 1, Fig. 8). The measurements were defined using a longitudinal baseline drawn from the tip of the blade to the furthest point on the tip of the handle. TL (total length) is equal to the length of the baseline. TW (total width) is the distance between two longitudinal lines at the margins of the hook, drawn parallel to the baseline. PL (posterior length) is the distance from the tip of the handle to the tip of the guard. AL (anterior length) is the distance from the tip of the guard to the tip of the blade. GL (guard length) is the distance from the baseline to the tip of the guard, defined by a line drawn perpendicular to the baseline.

The shape of the large rostellar hooks was compared by scaling a representative hook of each species to the same total length, and then aligning a pair of hooks using the outline of the junction between the blade and the guard as an anchor region. The form of the anchor region was almost invariable among the species considered here.

Type and voucher specimens have been deposited in the Finnish Museum of Natural History (MZH) and the Institute of Systematics and Ecology of Animals SB RAS, Novosibirsk, Russia (SVK).

Table 1. Variation in measurements (μm) of large rostellar hooks in *Taenia lynciscapreoli* sp. n. Figures show the range with the mean in parentheses. TL, total length; TW, total width; PL, posterior length; AL, anterior length; GL, guard length (see Fig. 8).

Hosts, region	TL	TW	PL	AL	GL
Lynx, Finland (n=11)	168–228 (195.9)	78–94 (84.5)	114–162 (133.8)	76–97 (86.3)	42–54 (47.7)
Lynx, Russia (n=16)	214–231 (223.4)	79–96 (89.4)	138–162 (152.1)	87–101 (94.9)	40–59 (50.8)
Lynx, combined (n=27)	168–231 (212.2)	78–96 (87.4)	114–162 (144.7)	76–101 (91.4)	42–59 (49.5)
Capreolus, Finland (n=3)	213–222 (216.5)	85–92 (87.5)	136–153 (144.2)	95–98 (96.9)	48–56 (49.9)
Capreolus, Russia (n=15)	215–238 (230.7)	94–109 (103.4)	148–171 (162.7)	92–111 (104.3)	54–88 (65.6)
Alces, Finland (n=7)	213–230 (222.3)	82–97 (90.9)	145–162 (154.8)	86–100 (94.0)	46–60 (52.3)
Cervids, combined (n=25)	213–238 (225.9)	82–109 (97.2)	136–171 (157.2)	86–111 (100.3)	46–88 (59.2)
Lynx + cervids, combined (n=52)	168–238 (219.1)	78–109 (92.3)	114–171 (150.9)	76–111 (95.8)	40–88 (54.4)

Results

Genetic identification

DNA sequences showed unambiguously that the specimens from various host species and regions represent the same species. Four *cox1* haplotypes were identified, the most common of which was identical with the *cox1* haplotype observed by Lavikainen et al. (2013) (GenBank accession number JX860629). The haplotypes formed a well–supported monophyletic entity (Fig 1). Divergence values were 0.3-1.1% between the haplotypes of the new species, whereas between the new species and the most closely related species (*T. hydatigena*, *T.* cf. *kotlani* Murai, Gubányi & Sugar, 1993 and *T. regis*) divergences were clearly higher, 8.3-10.1%. New nucleotide sequence data of the new species is available in the DDBJ/EMBL/GenBank databases under the accession numbers KU324546–KU324548.

Taenia lynciscapreoli sp. n.

http://zoobank.org/559B4067-3FE2-4B35-86B6-03780ED73DDF

Material. *Adult*. Type–material: Holotype MZH 127098 (five slides, including hand–cut transverse sections, and fragments in ethanol). Paratype MZH 127099 (three slides and fragments in ethanol), from the same host individual as the holotype.

Voucher material from *L. lynx*: MZH 127100 (three slides and fragments in ethanol), MZH 127101 (five slides and fragments in ethanol) and MZH 127102 (six slides and fragments in ethanol), Lohja, southern Finland; MZH 127105 (two slides) and MZH 127106 (three slides), Mikhailovskiy raion, Altai Rai, Russia.

Voucher material from *Canis lupus* (wolf): SVK-2265 and SVK-2581, Mikhailovskiy raion, Altai Rai, Russia.

Other museum specimens from *L. lynx* from Finland (in ethanol): MZH 123001, from Hyvinkää; MZH 123002, from Sauvo; MZH 123003 and 123004, from Mustasaari; MZH 123005, locality unknown.

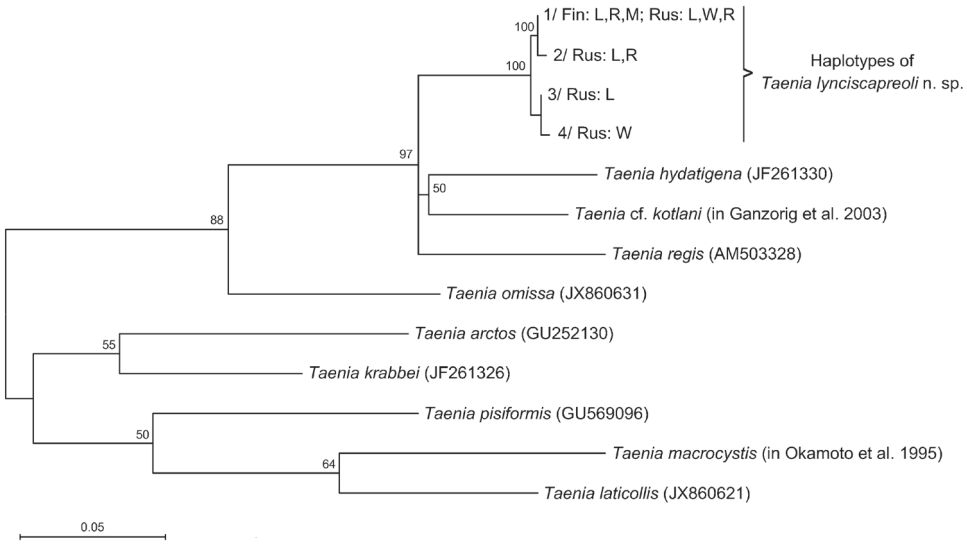


Figure 1. A phylogenetic tree of selected species of *Taenia* inferred from a 396 bp fragment of mitochondrial *cox1* gene by the maximum likelihood method. Bootstrap values >50% are shown. The scale bar represents the estimated number of substitutions per site. Accession numbers or references of the previously published sequences are in parentheses. The haplotypes of *T. lynciscapreoli* sp. n. are designated with numbers 1–4, and their geographical origins and hosts are indicated with abbreviations: Fin, Finland; Rus, Russia; L, lynx; W, wolf; R, European or Siberian roe deer; M, moose.

Other records from *L. lynx*: Kolosovsky and Bolsheukovsky raions, Omskaya oblast', Western Siberia, Russia (morphological identification), coll. Bykova, 2006 [identified as *Taenia pisiformis* (Bloch, 1780)].

Type host. *Lynx lynx* Linnaeus, 1758, the Eurasian lynx. Other hosts: *Canis lupus* Linnaeus, 1758, the wolf.

Type locality. Salo, Perniön Ylikulma (WGS 84: 60°16.948'N; 23°13.288'E), southern Finland.

Site. Small intestine.

Metacestode. Host: European roe deer *Capreolus capreolus* (Finland), Siberian roe deer *Capreolus pygargus* (Russia) and Eurasian elk/moose *Alces alces* (Finland).

Voucher material. MZH 127104 (two specimens in ethanol), *Alces alces* (calf), Hausjärvi, southern Finland; SVK-2344, SVK-2402 (in ethanol), SVK-2458, SVK-2395 (slides), *Capreolus pygargus*, Russian Far East.

Other museum specimens. N16553, Museum of All–Russian K. I. Skryabin Scientific Research Institute of Helminthology (Moscow), *C. pygargus*, Tuva Republic, Southern Siberia, Russia (identified as *T. hydatigena*).

Site. Liver and lungs.

Diagnosis. Adults and metacestodes of *T. lynciscapreoli* sp. n. can be separated unambiguously from all other species of *Taenia* by the shape of their large rostellar hooks,

particularly the characteristically short, wide and strongly curved blade. If the large rostellar hooks are missing in adults, *T. lynciscapreoli* may be separated from related species by a combination of morphological features of mature proglottids (see Discussion).

Description. Measurements are in micrometres if not otherwise stated.

Adult (Figs 2–7; Table 1). Measurements of mature proglottids and scolex are based on specimens from Finland, and other measurements (external features, rostellar hooks, uterine branches, eggs) on combined material from Finland and Russia.

Medium–sized species of *Taenia*; length of fully gravid specimens 55–90 cm (n=4). Maximum width of strobila 5–7 mm (n=4). Scolex 1.1 mm (n=2) wide in specimens mounted in Berlese's medium (BM), 0.85 mm (n=1) wide in specimens mounted in Canada balsam (CB). Maximum diameter of suckers 269–289 in BM (n=7), 213–255 in CB (n=4). Diameter of rostellum 375–425 in BM (n=2), 300–365 in CB (n=2); rostellum larger than suckers. Neck approximately as wide as scolex, of variable length.

Rostellum bearing two rows of hooks; rostellar armature usually incomplete in adult specimens. In combined material, length of large hooks 168–231 (mean=212.2, n=27) and length of small hooks 106–137 (mean=126.2, n=25). Total length and other dimensions of large hooks consistently smaller in specimens from Finland than in those from Siberia and Russian Far East. Large hooks characterized by long, thick and straight handle sometimes provided with apical bulge, relatively short, wide and strongly curved blade and prominent, usually slightly pointed guard. Border between hidden and exposed parts of large hooks marked with distinct oblique ridge. Margin of ridge provided with pits of various sizes at middle of handle; similar but less distinct pits sometimes present at guard portion of ridge.

Proglottids craspedote, but velum poorly developed. Mature proglottids 2.8–5.3 mm (mean=4.3 mm, n=15) wide and 2.0–3.4 mm (mean=2.6 mm, n=15) long, with length/width ratio of 1:1.2–2.6 (mean=1:1.7, n=15) in well–relaxed specimens. Proglottids becoming more elongate posteriorly; fully–gravid proglottids up to 14 mm long, with length/width ratio of 1:4.7.

Genital pores irregularly alternating, positioned in middle of lateral margin of proglottids. Genital atrium weak, usually not protruding, 238–425 (mean=302, n=12) wide at base and 144–264 (mean=186, n=12) deep. Ventral longitudinal osmoregulatory canals 34–110 (mean=75, n=13) wide in mature proglottids, up to 200 in postmature/pregravid proglottids; connected by narrower transverse canals. Dorsal osmoregulatory canals narrow (seen only in transverse sections), running medially to ventral longitudinal canals. Terminal genital ducts positioned between dorsal and ventral longitudinal osmoregulatory canal and dorsal to nerve–cord.

Testes 591–725 (mean=653, n=5) in number, 80–130 in largest diameter, positioned primarily in one dorso–ventral layer. Testicular field widely confluent anteriorly and occupying all parts of median field lacking female organs, except small well–defined region anterior to ovary. Continuous posterior testicular field absent, but sometimes individuals testes positioned posterior to or overlapping vitellarium. Antero–poral testicular field longitudinally as long as postero–poral field (as separated by vas deferens). Testicular field separated from ventral osmoregulatory canals by distinct free

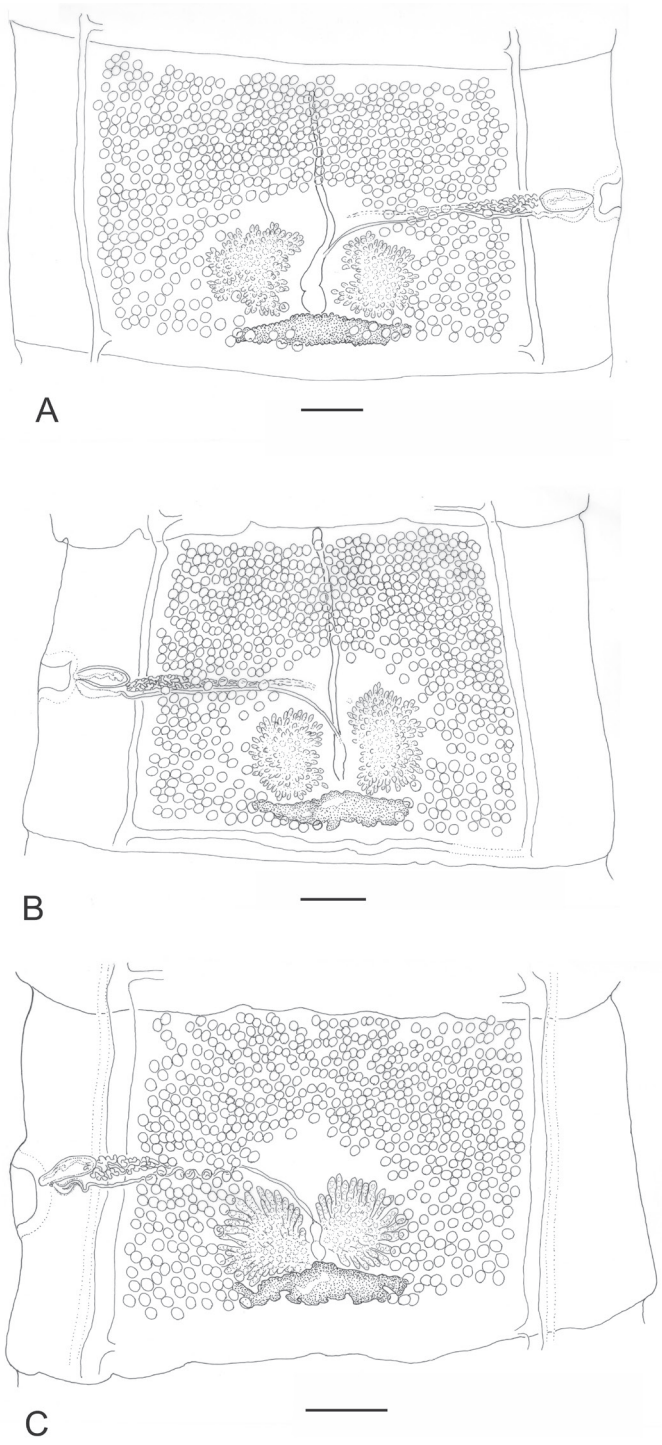


Figure 2. Mature proglottids of *Taenia lynciscapreoli* sp. n. from *Lynx lynx*. **A** holotype **B** paratype **C** voucher. Scale-bars: 500 μm (**A–B**); 300 μm (**C**).

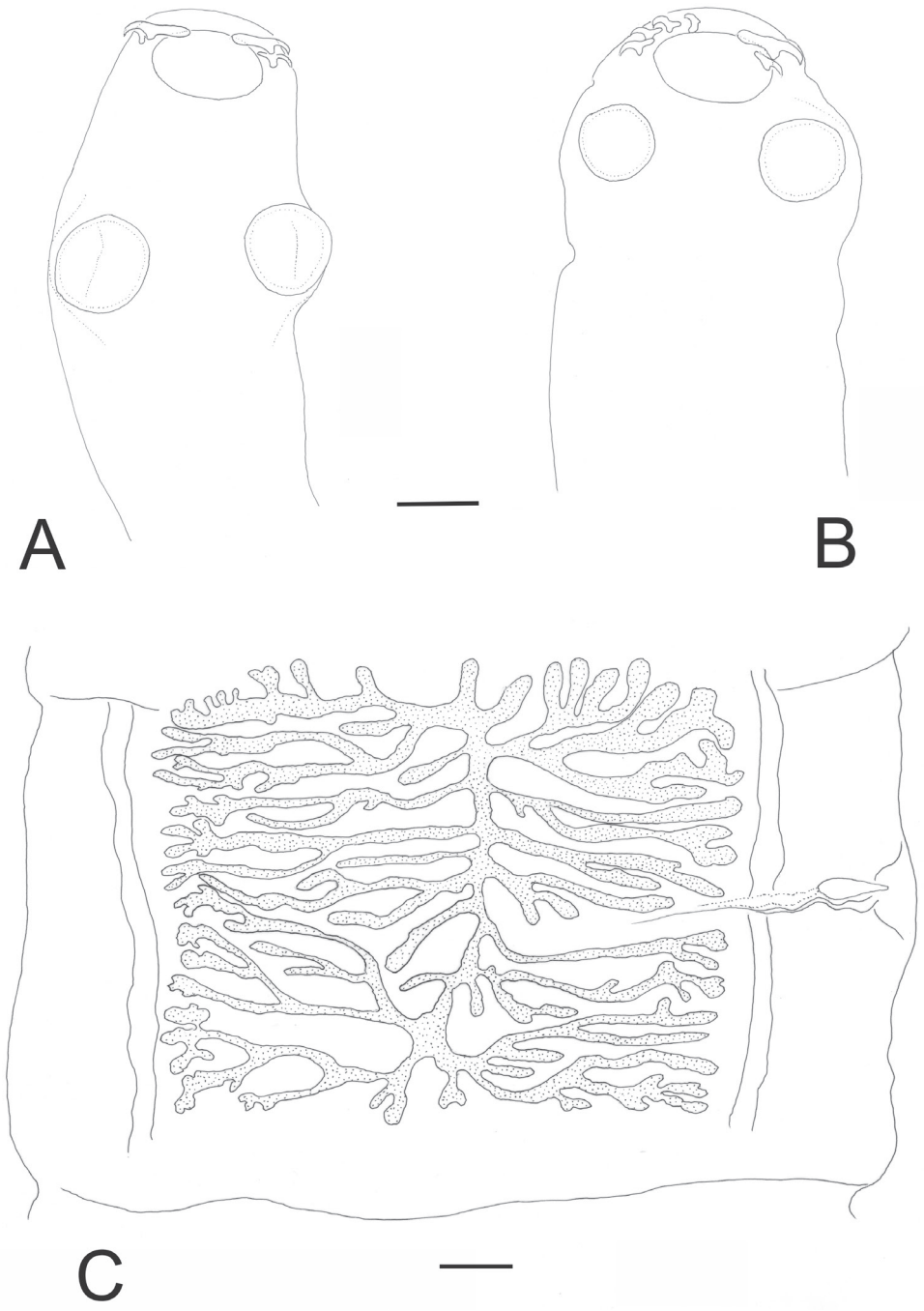


Figure 3. Scolex (**A**, **B**) and a pregravid proglottid with uterus (**C**) of *Taenia lynciscapreoli* sp. n. from *Lynx lynx*. **A, B** paratypes **C** voucher. Scale-bars: 200 μm (**A–B**); 500 μm (**C**).

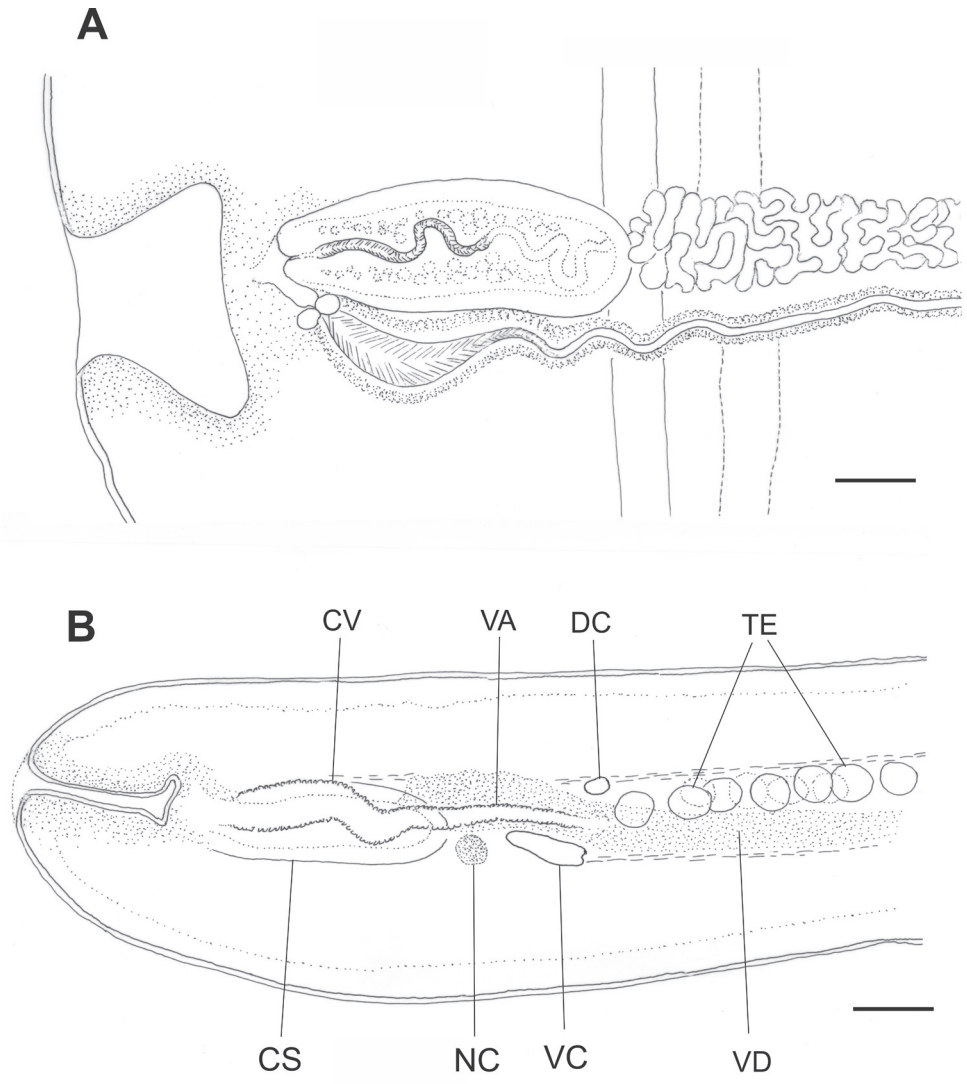


Figure 4. Terminal genital ducts of *Taenia lynciscapreoli* sp. n. (holotype) in whole mount (**A**) and in hand–cut transverse section (**B**). VC, ventral longitudinal osmoregulatory canal; DC, dorsal longitudinal osmoregulatory canal; NC, nerve cord; VA, vagina; CV, copulatory part of vagina; CS, cirrus sac; VD, vas deferens; TE, testes. Scale-bars: 100 μm.

space laterally, anteriorly and posteriorly. Cirrus–sac elongate, 340–425 (mean=382, n=11) long and 153–179 (mean=166, n=11) wide in mature proglottids, usually not extending to longitudinal ventral canal; muscle layers of cirrus–sac well–developed. Distal part of ductus cirri armed with delicate hair–like structures. Vas deferens forming few irregular loops inside cirrus–sac, prominently convoluted outside cirrus–sac.

Ovary bilobed, 98–172 (mean=150, n=15) wide and 57–103 (mean=84, n=15) long; lobes of roughly equal size, but antiporal lobe extending slightly more ante-

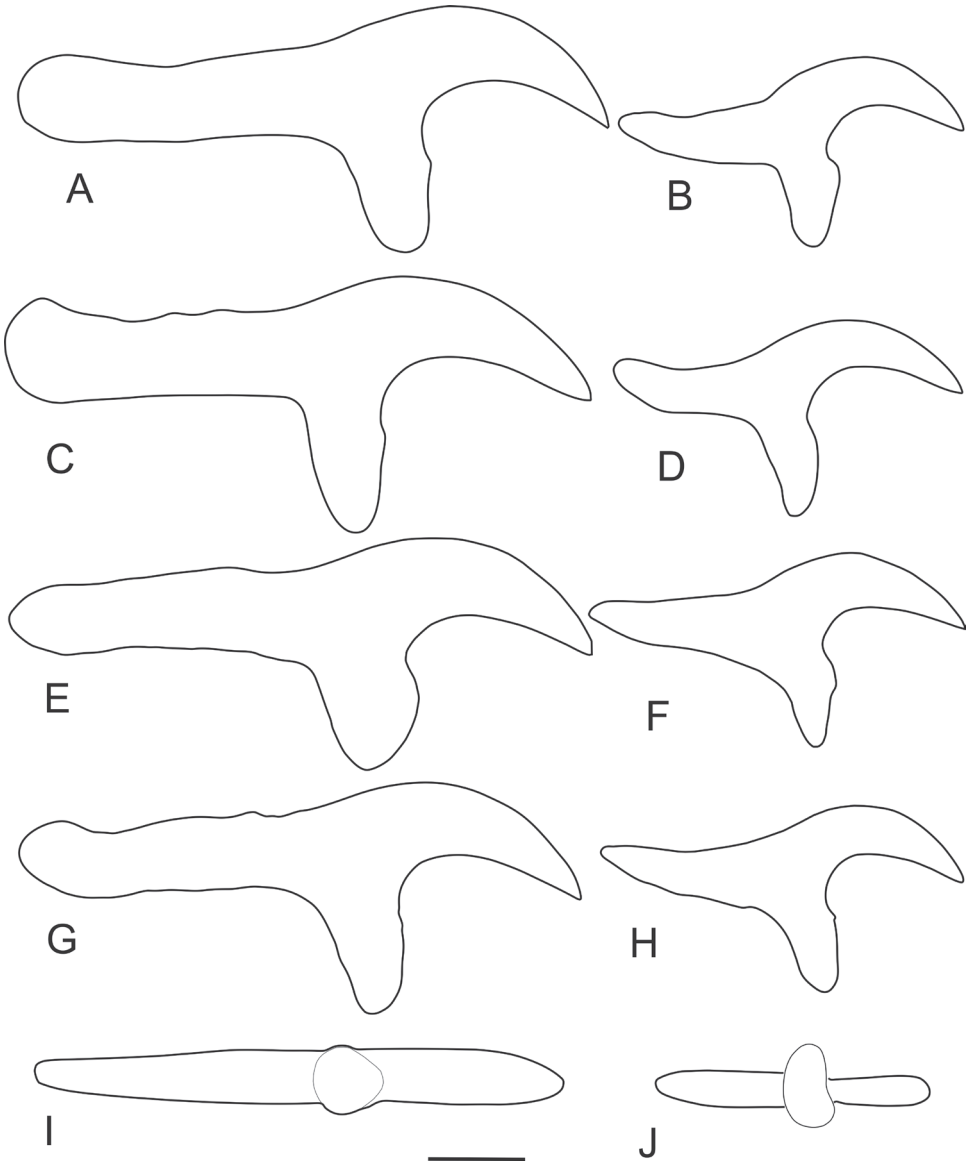


Figure 5. Outline drawings of large and small rostellar hooks of *Taenia lynciscapreoli* sp. n. from various host species. **A–H** side view **I–J** "ventral" view **A–B** *Lynx lynx* (holotype) **C–D** *Canis lupus* **E–F** *Capreolus capreolus* **G–J** *Alces alces*. Scale-bar: 50 μm.

riad than poral lobe; ovary does not reach midline of proglottid longitudinally. Vitellarium distinctly elongated transversely, 80–145 (mean=126, n=15) wide and 19–41 (mean=31, n=12) long, slightly narrower than ovary; lateral extremities usually pointed. Vagina opens posterior to male pore, provided by distinct sphincter ca. 5 from distal end of vagina; sphincter ca. 3 long and 6 wide; sphincter sometimes absent or incom-

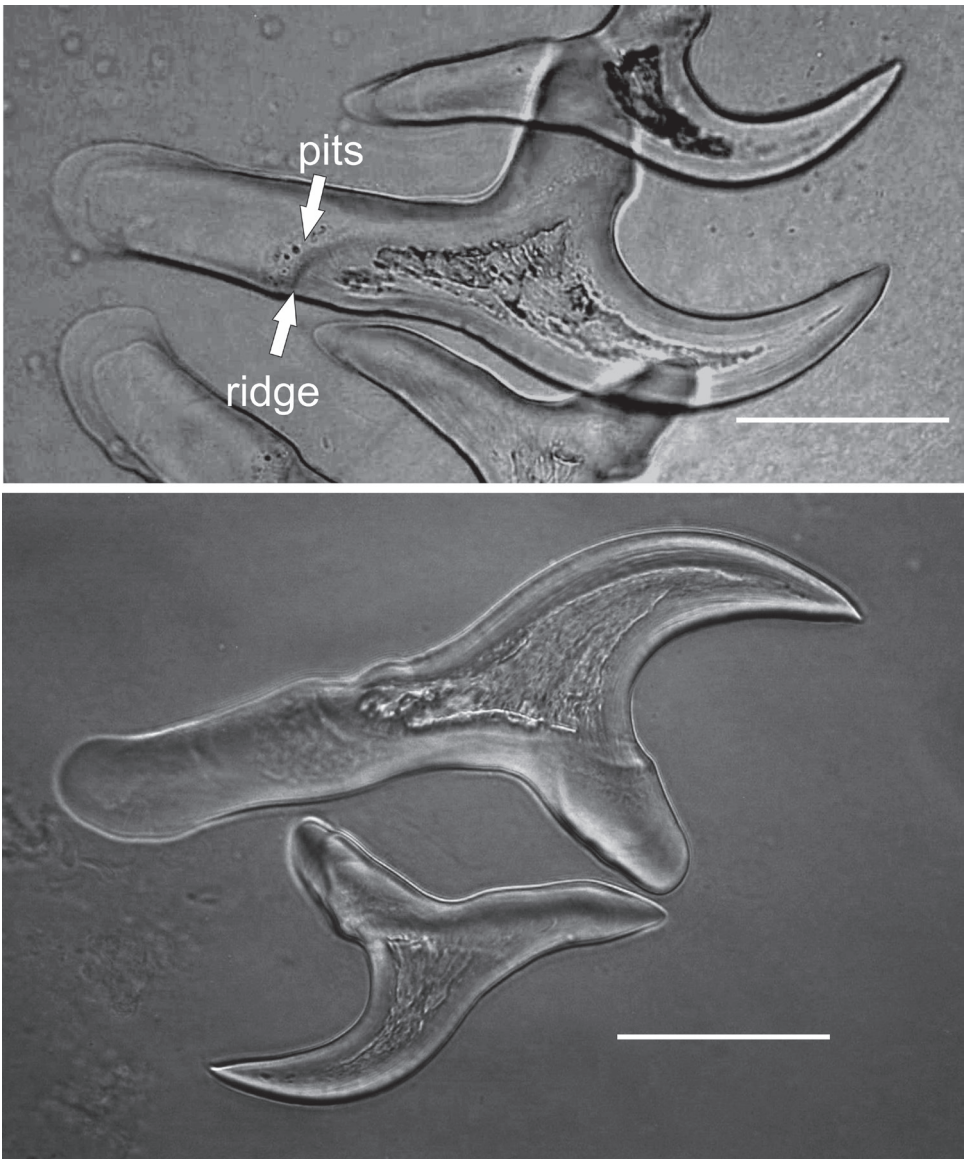


Figure 6. Large and small rostellar hooks of *Taenia lynciscapreoli* sp. n. from *Lynx lynx*, the higher picture showing the characteristic ridge and pits of large hooks. Scale-bars: 50 μm.

plete (present on one side of vagina only). Copulatory part of vagina shorter than cirrus sac, thick–walled, distinctly widened, curved posteriorly; maximum width of copulatory part 94–111 (mean=106, n=10). Proximal vagina narrow, of uniform width, runs posterior to vas deferens, usually slightly undulating, rarely looped. Lumen of vagina lined with delicate hair–like structures almost throughout its length; hairs particularly long in widened copulatory part. Prior to joining seminal receptacle, vagina forms dif-

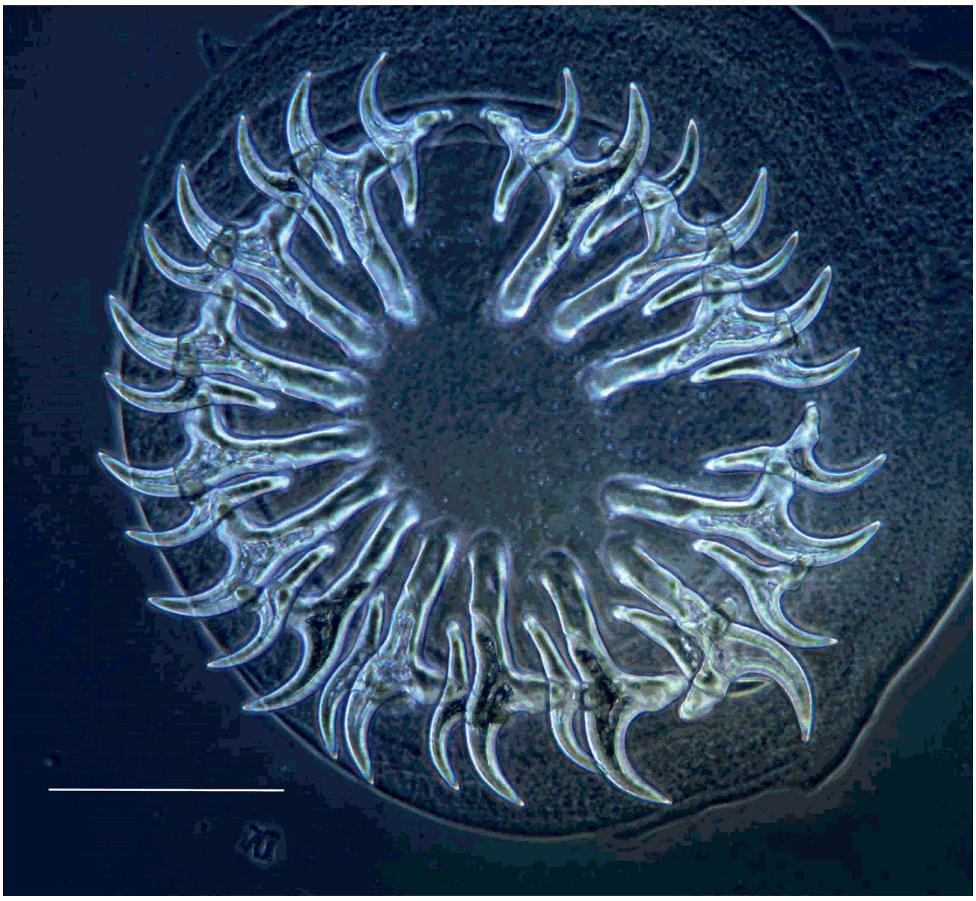


Figure 7. Hook crown of *Taenia lynciscapreoli* sp. n. from *Lynx lynx*. Scale-bar: 200 μm.

ferentiated region, 10–12 long, with tapered lumen lacking hairs. Sperm–filled seminal receptacle elongate, 9–17 (mean=12.4, n=15) long. Mehlis' gland spherical, 18–22 (mean=19.6, n=11) in diameter. Uterus in pre–gravid and early gravid proglottids with 8–11 primary branches on each side, often with secondary and tertiary bifurcations; lateral branches not reaching ventral osmoregulatory canal; terminal branches usually with multiple anterior or posterior sacculations. Eggs spherical or subspherical, with maximum diameter of 34–39 (mean=36.8, n=26) in whole–mounts. Outer egg shell thick (4.0–4.5), distinctly two–layered.

Metacestode (Fig. 5, Table 1). External features of metacestodes are based on specimens from Finland, and measurements of rostellar hooks on combined material from Finland and Russia (Table 1).

Metacestode is cysticercus. Ethanol–fixed cysticerci with fully–developed rostellar hooks 3–14 mm long and 2–5 mm wide; larger cysticerci with elongate or sac–like posterior bladder and, in one case, with short (8 mm) strobila between bladder and scolex

region. Rostellum armed with 30–34 (mean=32.0, n=7) hooks forming two rows. Large hooks 213–238 (mean=225.9, n=27) and small hooks 123–145 (mean=136.7, n=23) long. Average hook dimensions are consistently smaller in specimens from Finland than in specimens from Siberia and Russian Far East. Rostellar hooks of metacestodes are similar in shape to those of adult cestodes.

Distribution. Eurasia, from Finland to Russian Far East.

Etymology. The specific epithet refers to the main definitive and intermediate hosts of the new species.

Discussion

Main morphological differences between *Taenia lynciscapreoli* sp. n. and related species

Taenia lynciscapreoli sp. n. is compared with all congeneric species parasitizing felids (definitive hosts) or cervids (intermediate hosts) in the Holarctic region (12 species), and also with the phylogenetically closely related *T. regis* (see Lavikainen et al. 2013). When compared with the new species (Table 2), *T. arctos*, *T. hydatigena*, *T. kotlani*, *T. pisiformis*, *T. krabbei* Moniez, 1879 and *T. parenchymatosa* Pushmenkov, 1945 showed overlapping numbers and/or lengths of rostellar hooks, and were therefore selected for comparison of the shape of the rostellar hooks. In addition, *Taenia ingwei* Ortlepp, 1938, a parasite of *Panthera pardus* in Africa, was selected for hook shape comparison, because it shows highest overlap in the number and length of rostellar hooks among *Taenia* spp. of African/Asian felids, when compared with *T. lynciscapreoli*.

When aligned using the outline of the junction between the blade and the guard, the large rostellar hooks of *T. lynciscapreoli* have a shorter blade and longer handle, and a wider and more strongly curved blade than those of the other species, with the partial exception of *T. pisiformis* (Fig. 8). The latter species can be distinguished from *T. lynciscapreoli* by its more numerous rostellar hooks and the narrower and less curved blade of the large hooks.

Interspecific differences in the morphology of mature proglottids between *T. lynciscapreoli* and the species showing the highest overlap in hook characteristics are listed in Table 3. All species compared, with the exception of *T. ingwei*, can be unambiguously separated from *T. lynciscapreoli*. The difference in the shape of the large hooks appears to be the only reliable way to distinguish *T. ingwei* and *T. lynciscapreoli*. Additional differences are, however, expected to be found, if the morphology of the mature proglottids of *T. ingwei* were examined in greater detail. It should be noted that there is a disagreement concerning the distribution of testes between the descriptions of *T. ingwei* by Ortlepp (1938) and Verster (1969). The former specifically states that there are no testes posterior to vitellarium, which is shown in his illustration, whereas the latter states that the testicular field is "confluent dorso–posteriorly to the vitellarium". Either this feature is variable in *T. ingwei*, which is

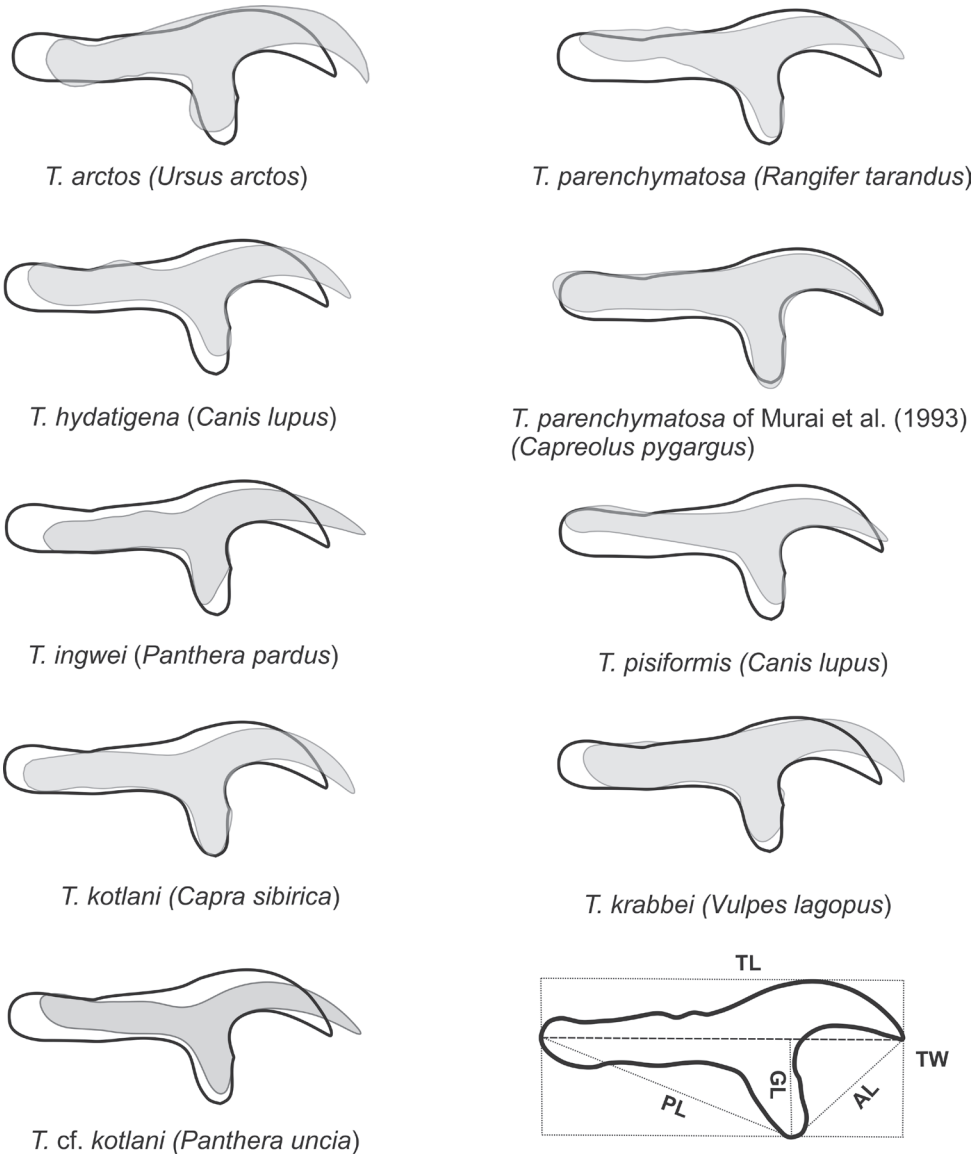


Figure 8. Pairwise comparisons of the shape of the large rostellar hooks in *Taenia lynciscapreoli* sp. n. and related species, using the junction between the blade and the guard as an anchor region for alignment. The hook of *T. lynciscapreoli* sp. n. is indicated by a black outline. A legend for measurements taken from the large hooks of the new species (Table 1) is also shown.

the case in *T. lynciscapreoli*, or the redescription of Verster (1969) is composite (her description was based on the type specimens and "additional adults from the same host and locality").

Table 2. Host species and characteristics of rostellar hooks of *Taenia* spp. compared with *T. lynciscapreoli* sp. n., based on Loos-Frank (2000) and Haukisalmi et al. (2011). Hook characteristics showing highest overlap with those of *T. lynciscapreoli* sp. n. indicated in bold.

***Taenia* spp.**	**Definitive hosts**	**Intermediate hosts**	**Geographic distribution**	**Number of hooks**	**Large hooks, length**	**Small hooks, length**
***T. lynciscapreoli* sp. n.**	**felids (*Lynx*)**	**cervids (*Capreolus*)**	**Eurasia**	**30–34**	**168–238**	**106–145**
T. arctos Haukisalmi, Lavikainen, Laaksonen & Meri, 2011	bears (*Ursus*)	cervids (*Alces*)		**22–36**	**153–180**	**96–130**
T. hydatigena Pallas, 1766	canids	cervids and other ruminants	worldwide	**28–44**	**169–235**	**110–168**
T. ingwei Ortlepp, 1938	felids (*Panthera*)	unknown	Africa	**32–34**	**197–202**	148–151
T. kotlani Murai, Gubanyi & Sugar, 1993	unknown, probably felids (*Panthera*)	bovids (*Capra*)	Central Asia	**30–36**	**187–218**	**118–143**
T. cf. *kotlani* of Ganzorig et al. (2003)†	*Panthera*	unknown, probably cervids	Central Asia	**30–35**	**190–209**	**127–144**
T. krabbei Moniez, 1879	canids	cervids and other ruminants	Holarctic region	**22–36**	**137–195**	**84–141**
T. laticollis Rudolphi, 1819	felids (*Lynx*)	lagomorphs	Eurasia	58–66	370–420	150–247
T. macrocystis (Diesing, 1850)	felids (*Lynx, Leopardus, Puma*)	lagomorphs	America, Asia	54–74	297–430	180–247
T. omissa Lühe, 1910	felids (*Puma, Leopardus*)	cervids (*Odocoileus*)	America	38–44	223–297	165–223
T. parenchymatosa Pushmenkov, 1945	canids	cervids	Russia	**30–34**	**210–240**	**124–160**
T. parenchymatosa of Murai et al. 1993‡	felids (*Lynx*)	cervids (*Capreolus*)	Siberia	**27–34**	**195–234**	**118–149**
T. pisiformis (Bloch, 1780)	canids, occasionally felids including *Lynx*	lagomorphs	worldwide	34–46	**220–300**	**114–177**
T. pseudolaticollis Verster, 1969	felids (*Lynx, Leopardus*)	unknown (probably lagomorphs)	America	38–42	352–415	214–240
T. regis Baer, 1923	felids (*Panthera*)	bovids (antelopes), suids (*Phacocoerus*)	Africa	32–49	223–273	142–199
T. rileyi Loewen, 1929	felids (*Lynx, Puma*)	rodents	America	36–46	238–258	145–198

† *Taenia* cf. *kotlani* of Ganzorig et al. (2003) is considered here to be conspecific with *T. kotlani* Murai, Gubanyi & Sugar, 1993.

‡ *Taenia parenchymatosa* of Murai et al. (1993) is considered here to be conspecific with *T. lynciscapreoli* sp. n.

Table 3. Comparison of morphological features of mature proglottids in *T. lynciscapreoli* sp. n. and species showing highest overlap in the number and length of rostellar hooks. There is no adequate description for the morphology of the adult of *T. kotlani*. Based on Loos-Frank (2000) and Haukisalmi et al. (2011).

Taenia spp.	Vaginal sphincter	Longitudinal extent of ovary	Antiporal lobe of ovary distinctly larger than poral lobe	Free space around testes	Length of poral testicular fields	Width of anterior testicular field	Number of testicular layers
T. lynciscapreoli sp. n.	+	< midline	–	+	A = P†	wide	1
T. arctos	+	> midline	+	–	A = P	wide	2–3
T. hydatigena	–	< midline	+	–	A > P	wide	1
T. ingwei	+	≤ midline	–	?	A = P	wide	1
T. krabbei	+	< midline	+	+	A > P	wide	1–2
T. parenchymatosa	+	= midline	–	+	A < P	narrow	?
T. pisiformis	–	< midline	+	–	A > P	wide	2–4

† A, antero–poral testicular field; P, postero–poral testicular field (as separated by terminal genital ducts).

Rostellar hooks

Gubányi (1995) applied multivariate morphometrics for rostellar hooks of 18 species of *Taenia* s.l., and concluded that "*T. parenchymatosa* and *T. laticollis* can be very well differentiated (100%) from the other species by the small and large hooks". In addition to *T. parenchymatosa* of Murai et al. (1993) and *T. laticollis* Rudolphi, 1819, the analysis of Gubányi (1995) included *T. hydatigena*, *T. kotlani*, *T. pisiformis* (Bloch, 1780) and *T. regis*, all of which are included in our interspecific comparison (Table 2), but also two additional species from African felids (*T. acinomyxi* Ortlepp, 1938 and *T. selousi* Mettrick, 1963). As shown below, *T. parenchymatosa* of Murai et al. (1993) and Gubányi (1995) from *Capreolus pygargus* from Siberia is almost certainly conspecific with *T. lynciscapreoli*, and the results of Gubányi (1995) therefore provide further support for the status of the new species as a morphologically distinct entity.

In practice, the identification of *T. lynciscapreoli* based on rostellar hooks is straightforward; the new species has shorter hooks than other congeneric species parasitizing felids in the Holarctic region, with the possible exception of *T. kotlani*, the definitive host of which is unknown. The identification of metacestodes parasitizing cervids is slightly more challenging, but the present comparison shows that the unique shape of the large hooks of *T. lynciscapreoli*, particularly the short, wide and strongly curved blade, separates it from other species with rostellar hooks of similar length. If properly compared, the characteristic shape of the large hooks of *T. lynciscapreoli* also serves to separate it from all other species of *Taenia*, including those not compared here with the new species (see Gubányi 1995 and the Global Cestode Database; Caira et al. 2012).

Total length has often been the only feature used to characterize the rostellar hooks of *Taenia* spp., although it may be assumed that the shape of the hooks is a taxonomically more informative feature. Interspecific differences in the shape of rostellar hooks have been analysed using multivariate morphometrics (Gubányi 1995, 1996), but such an approach in somewhat unpractical for taxonomical purposes. A more straightforward and practical approach, as applied here, is to scale (large) rostellar hooks to the same total length and align them using an "anchor region" that shows limited variation among species. In this way it is easy to visualize interspecific differences in the shape and proportions of rostellar hooks. Such shape differences should also be easy to quantify, for example, by measuring the overlap between a pair of aligned hooks. This method is naturally most useful when comparing tapeworm species that show overlapping hook dimensions. Intraspecific comparisons of hook shape in *T. lynciscapreoli*, *T. arctos*, *T. hydatigena*, *T. krabbei*, *T. laticollis, T. martis* (Zeder, 1803) and *T. polyacantha* Leuckart, 1856 show that the shape of the blade of the large hooks is very constant within each species, but the shape of the handle and guard are more variable (not shown).

The large hooks of the cestode from *Capreolus pygargus* from Siberia, identified by Murai et al. (1993) as *T. parenchymatosa*, match well with the hook shape of *T. lynciscapreoli* (Fig. 8). Similar hook shape and strong overlap in hook number and length suggest that the *T. parenchymatosa* of Murai et al. (1993) actually represents *T. lynciscapreoli*. Congeneric intermediate hosts (*Capreolus* spp.) support their

conspecificity. The hook comparison also suggests that that *T.* cf. *kotlani* from the snow leopard (Ganzorig et al. 2003) is conspecific with *T. kotlani* from the Siberian ibex *Capra sibirica* (Murai et al. 1993).

Phylogenetics

Besides *T. lynciscapreoli*, there are published DNA sequence data for five species of *Taenia* s.s. parasitizing felids, i.e. *T.* cf. *kotlani* (Ganzorig et al. 2003), *T. laticollis* (Lavikainen et al. 2013, Nakao et al. 2013), *T. macrocystis* (Diesing, 1850) (Okamoto et al. 1995), *T. omissa* Lühe, 1910 (Lavikainen et al. 2013, Gomez–Puerta et al., published only in GenBank), and *T. regis* (Zhang et al. 2007). The present and previously published phylogenetic analyses show unambiguously that none of these can be conspecific with *T. lynciscapreoli*. Although *T. lynciscapreoli* groups with *T.* cf. *kotlani*, *T. regis* and *T. hydatigena*, the latter of which uses canids as definitive hosts, the genetic distances between these species are at an interspecific level (Zhang et al. 2014). The phylogenetic analysis by Lavikainen (2014) included an additional species from felids, i.e. *T. rileyi* Loewen, 1929 (*Lynx* and *Puma*, Nearctic; unpublished sequence), which formed a separate clade with *T. omissa* (Fig. 3 in Lavikainen 2014), clearly distinct from *T. lynciscapreoli*.

Taenia lynciscapreoli was not compared here morphologically with *Taenia* spp. parasitizing felids in Africa and Asia, because, according to present knowledge, their fauna is separate from the corresponding fauna in the Holarctic region. However, *T. regis*, a parasite of the lion in Africa, is included in the present comparison, because it is phylogenetically related to *T. lynciscapreoli*. It is possible that there are more extensive phylogenetic connections between *Taenia* spp. of Holarctic and southern felids, but there are no published DNA sequence data for species of *Taenia* other than *T. regis* parasitizing felids in Africa or Asia. However, our unpublished data suggest that *T. gonyamai* Ortlepp, 1938 and *T. selousi* Mettrick, 1963, parasites of felids in Africa, are phylogenetically distinct entities and therefore not conspecific with *T. lynciscapreoli.*

A group of taeniid cestodes, including two species parasitizing felids [*Hydatigera taeniaeformis* (Batsch, 1786) and *H. krepkogorski* Schulz & Landa, 1934], was recently shown to form a distinct clade by molecular phylogenetic methods, and therefore proposed to represent the resurrected genus *Hydatigera* Lamarck, 1816 (see Nakao et al. 2013). *Hydatigera* spp. can be easily distinguished from *Taenia* spp. by their long rostellar hooks and a strobilocercus–type metacestode.

Life cycle and host specificity

The existing data on *T. lynciscapreoli* strongly suggests that it uses specifically the lynx and the roe deer as definitive and intermediate hosts, respectively. Being small cervids, roe deer are optimal and, where available, preferred prey items for the lynx (Pulliainen 1981, Jedrzejewski et al. 1993, Odden et al. 2006).

The lynx and the roe deer have almost continent–wide, overlapping distributions in Eurasia, although the latter host is represented by two allopatric species (*C. capreolus* and *C. pygargus*). However, the distribution of the Eurasian lynx extends further north than the distribution of the roe deer, and, if the occurrence of the parasite is dependent on the presence of both primary hosts, we would expect to find the parasite in the lynx only in regions inhabited by the roe deer. This seems to be case in Finland, as Lavikainen et al. (2013a) found *T. lynciscapreoli* (referred to as "*Taenia* sp.") in lynx from southern and western Finland, where the roe deer is abundant, but not from the more northern and eastern parts of the country where the roe deer is absent or sporadic. In accordance, the present new findings of *T. lynciscapreoli* in the lynx are from southernmost Finland. Similarly, the present findings of *T. lynciscapreoli* in Russia are located within the range of *C. pygargus*.

However, despite the basically strict host–specificity, accidental infections of other definitive host species are likely to occur, especially with unrelated predators utilizing same intermediate host species. The present finding of *T. lynciscapreoli* in the wolf, confirmed by molecular methods, shows that such spill–over does happen. In this case the obvious explanation is that wolves prey on roe deer, the primary intermediate host of *T. lynciscapreoli*.

The finding of *T. lynciscapreoli* in the Eurasian moose calf, confirmed by molecular methods, shows that the new species is able to infect also cervids other than the roe deer. Although the lynx may succeed in killing a moose calf (Birkeland and Myrberget 1980), the moose is an exceptional prey species and thus cannot be involved in the normal transmission of the parasite.

It may be that infections of larger cervids (*Alces*, *Cervus*) by the metacestodes of *T. lynciscapreoli* occur only in regions where there exists a transmission cycle between the lynx and the roe deer.

Possible misidentifications of *T. lynciscapreoli*

Because *T. lynciscapreoli* is evidently a predictable, wide–spread component in the tapeworm fauna of the lynx and the roe deer, it is probably represented in some previous studies, but has been misidentified or remained unidentified.

A survey of helminths of the lynx in Estonia (Valdmann et al. 2004) showed a rather unexpected result for *Taenia* spp., because every lynx (n=37) was infected with *T. pisiformis* (besides the less prevalent *T. laticollis* and *T. hydatigena*). *Taenia pisiformis* is typically a parasite of canids, particularly the wolf and the dog, with lagomorphs serving as the primary intermediate hosts (Loos-Frank 2000). An experimental study by Beveridge and Rickard (1975) showed that the domestic cat is not a suitable definitive host for *T. pisiformis*, because the worms developed slowly and the infections were lost before the worms became gravid. However, *T. pisiformis* has been reported several times also from other felids, particularly from the wild and domestic cats (*Felis catus*) (see the Host–parasite database of the Natural History Museum, London; Gibson et al. 2005), but also from the Iberian lynx *Lynx pardinus* (see Rodriguez and Carbonell

1998, Torres et al. 1998) and the North American *Lynx canadensis* (see Zyll de Jong 1966, Smith et al. 1986) and *Lynx rufus* (see Tiekotter 1985).

The identification of *Taenia* spp. by Valdmann et al. (2004) was based primarily on the total length of rostellar hooks, although "genital sacs" were also considered. The rostellar hooks of *T. pisiformis* are somewhat longer than those of *T. lynciscapreoli*, but still overlapping (Table 2), and both species can be classified as "short–hooked" among *Taenia* spp. In addition, the relative lengths of the handle and the blade of the large rostellar hooks are very similar in *T. pisiformis* and *T. lynciscapreoli*, although the latter has a wider and more curved blade (Fig. 8). Based on the apparent similarity of hook characteristics in these species, we assume that *T. pisiformis* of Valdmann et al. (2004) was actually *T. lynciscapreoli*. These two species could be separated by the number of rostellar hooks (higher in *T. pisiformis*), but rostellar hooks, particularly the long ones, are easily lost in adult specimens. The roe deer is abundant is Estonia and dominates in the diet of lynx (Valdmann et al. 2005), which should enhance the transmission of *T. lynciscapreoli* and explain its high prevalence.

Rodriguez and Carbonell (1998) reported *T. pisiformis* as a relatively common parasite of *L. pardinus* in south–central Spain, although they could not find its metacestodes in lagomorphs. Torres et al. (1998) also reported *T. pisiformis* in *L. pardinus* from the same region, but at a lower prevalence. The Iberian lynx examined in these studies originated from the Montes des Toledo region, where it co–occurs with the roe deer. The authors do not explain how the tapeworms from the Iberian lynx were identified, but it is again possible that *T. pisiformis* of Rodriguez and Carbonell (1998) and Torres et al. (1998) was actually *T. lynciscapreoli*. However, the reports of *T. pisiformis* in *L. canadensis* most probably do not represent *T. lynciscapreoli*, because there are no roe deer in North America.

It is obvious that some of the existing reports of *Taenia* metacestodes in roe deer, particularly in regions where it co–occurs with lynx, may also be *T. lynciscapreoli*. Three other valid species of *Taenia* using cervids as intermediate hosts in Eurasia, i.e. *T. krabbei* (including the probable junior synonym *T. cervi* Christiansen, 1931), *T. hydatigena* and *T. parenchymatosa*, may all be confused with *T. lynciscapreoli* because of overlapping hook number and dimensions (as shown above, *T. parenchymatosa* of Murai et al. 1993 from *C. pygargus* is actually *T. lynciscapreoli*). The new species could be easily identified by the shape of the hooks, but rostellar hooks have seldom been described in reports concerning *Taenia* metacestodes of roe deer and other cervids. With the exception of Murai et al. (1993), the existing reports on *Taenia* metacestodes of cervids with hook illustrations (Christiansen 1931, Brzheskii 1963, Murai and Sugár 1979, Priemer et al. 2002) do not, however, include *T. lynciscapreoli*.

Acknowledgements

Antti Oksanen (Finnish Food Safety Authority Evira) is acknowledged for support and shared enthusiastic attitude towards parasites of all kinds. We thank Minna Nylund (Evira) for collecting and preserving tapeworms from lynx. Pekka Spets, Esko Huuh-

tanen, Mikko Suomela and Valtteri Söderman kindly submitted the Finnish animal samples from which the parasites were found. Dr Seryodkin provided specimens of *Taenia lynciscapreoli* from Russian Far East and Dr Bondarev helped in the collection samples from Altai Krai. Ian Beveridge, Rodney A. Bray and Eric P. Hoberg kindly checked the language and provided helpful comments on other aspects of the manuscript. This study was supported in part by a Grant-in-Aid for Scientific Research (no. 26460503) from Japan Society for the Promotion of Science (JSPS KAKENHI) to MN.

References

Beveridge I, Rickard MD (1975) The development of *Taenia pisiformis* in various definitive host species. International Journal for Parasitology 5. doi: 10.1016/0020-7519(75)90063-6

Birkeland KH, Myrberget S (1980) The diet of the lynx *Lynx lynx* in Norway. Fauna Norvegica Series A 1: 24–28.

Brzheskii VV (1963) Morfologo-anatomicheskaya kharakteristika tenii *Taenia krabbei* i *Taenia parenchymatosa* [Morphological and anatomical characteristics of *Taenia krabbei* and *Taenia parenchymatosa*]. Helminthologia 4: 115–132.

Caira JN, Jensen K, Barbeau E (2012) Global Cestode Database. World Wide Web electronic publication. http://tapewormdb.uconn.edu/

Catalano S, Lejeune M, Tizzani P, Verocai GG, Schwantje H, Nelson C, Duignanab PJ (2015) Helminths of grizzly bears (*Ursus arctos*) and American black bears (*Ursus americanus*) in Alberta and British Columbia, Canada. Canadian Journal of Zoology 93: 765–772. doi: 10.1139/cjz-2015-0063

Catalano S, Lejeune M, Verocai GG, Duignan PJ (2014) First report of *Taenia arctos* (Cestoda: Taeniidae) from grizzly (*Ursus arctos horribilis*) and black bears (*Ursus americanus*) in North America. Parasitology International 63: 389–391. doi: 10.1016/j.parint.2013.12.012

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research 31: 3497–3500. doi: 10.1093/nar/gkg500

Christiansen M (1931) Die Muskelfinne des Rehes und deren Bandwurm (*Cysticercus* et *Taenia cervi* sp. n. ad interim). Zeitschrift für Parasitenkunde 4: 75–100. doi: 10.1007/BF02122050

Ganzorig S, Oku Y, Okamoto M, Kamiya M (2003) Specific identification of a taeniid cestode from snow leopard, *Uncia uncia* Schreber, 1776 (Felidae) in Mongolia. Mongolian Journal of Biological Sciences 1: 21–25.

Gibson DI, Bray RA, Harris EA (2005) Host-Parasite database of the Natural History Museum, London. http://www.nhm.ac.uk/research-curation/projects/host-parasites/

Gubányi A (1995) Morphometrics of taeniid tapeworms I. Multivariate analysis of distance measurements of the rostellar hooks. Parasitologica Hungarica 28: 21–41.

Gubányi A (1996) Morphometric analysis of microscopic hooks of taeniid tapeworms (Cestoda, Taeniidae). In: Marcus LF, Corti M, Loy A, Naylor GJP, Slice DE (Eds) Advances in morphometrics. Plenum Press, New York, 503–510. doi: 10.1007/978-1-4757-9083-2_43

Hasegawa M, Kishino H, Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22: 160–174. doi: 10.1007/BF02101694

Haukisalmi V, Lavikainen A, Laaksonen S, Meri S (2011) *Taenia arctos* sp. n. (Cestoda: Cyclophyllidea: Taeniidae) from its definitive (brown bear *Ursus arctos* Linnaeus) and intermediate (moose/elk *Alces* spp.) hosts. Systematic Parasitology 80: 217–230. doi: 10.1007/s11230-011-9324-9

Jedrzejewski W, Schmidt K, Milkowski L, Jedrzejewska B, Okarma H (1993) Foraging by lynx and its role in ungulate mortality: the local (Bialowieza Forest) and the Palearctic viewpoints. Acta Theriologica 38: 385–403. doi: 10.4098/AT.arch.93-30

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. doi: 10.1007/BF01731581

Lavikainen A (2014) A taxonomic revision of the Taeniidae Ludwig, 1886 based on molecular phylogenies. University of Helsinki, Helsinki.

Lavikainen A, Haukisalmi V, Deksne G, Holmala K, Lejeune M, Isomursu M, Jokelainen P, Näreaho A, Laakkonen J, Hoberg E, Sukura A (2013) Molecular identification of Taenia spp. in the Eurasian lynx (*Lynx lynx*) from Finland. Parasitology 140: 653–662. doi: 10.1017/S0031182012002120

Lavikainen A, Haukisalmi V, Lehtinen MJ, Henttonen H, Oksanen A, Meri S (2008) A phylogeny of members of the family Taeniidae based on the mitochondrial cox1 and nad1 gene data. Parasitology 135: 1457–1467. doi: 10.1017/S003118200800499X

Lavikainen A, Haukisalmi V, Lehtinen MJ, Laaksonen S, Holmström S, Isomursu M, Oksanen A, Meri S (2010) Mitochondrial DNA data reveal cryptic species within *Taenia krabbei*. Parasitology International 59: 290–293. doi: 10.1016/j.parint.2010.03.003

Lavikainen A, Laaksonen S, Beckmen K, Oksanen A, Isomursu M, Meri S (2011) Molecular identification of *Taenia* spp. in wolves (*Canis lupus*), brown bears (*Ursus arctos*) and cervids from North Europe and Alaska. Parasitology International 60: 289–295. doi: 10.1016/j.parint.2011.04.004

Loos-Frank B (2000) An up-date of Verster's (1969) 'Taxonomic revision of the genus *Taenia* Linnaeus' (Cestoda) in table format. Systematic Parasitology 45: 155–183. doi: 10.1023/A:1006219625792

Murai É, Gubányi A, Sugár L (1993) Examination of taeniid metacestodes from the Far East, with the description of *Taenia kotlani* sp. n. (Cestoda: Taeniidae). Parasitologica Hungarica 26: 27–49.

Murai É, Sugár L (1979) Taeniid species in Hungary (Cestoda, Taeniidae). I. Cysticercisis, coenurosis and hydatidosis. Parasitologica Hungarica 12: 41–52.

Nakao M, Lavikainen A, Iwaki T, Haukisalmi V, Konyaev S, Oku Y, Okamoto M, Ito A (2013) Molecular phylogeny of the genus *Taenia* (Cestoda: Taeniidae): Proposals for the resurrection of *Hydatigera* Lamarck, 1816 and the creation of a new genus *Versteria*. International Journal for Parasitology 43: 427–437. doi: 10.1016/j.ijpara.2012

Odden J, Linnell JDC, Andersen R (2006) Diet of Eurasian lynx, *Lynx lynx*, in the boreal forest of south-eastern Norway: the relative importance of livestock and hares at low roe deer density. European Journal of Wildlife Research 52: 237–244. doi: 10.1007/s10344-006-0052-4

Okamoto M, Bessho Y, Kamiya M, Kurosawa T, Horii T (1995) Phylogenetic relationships within *Taenia taeniaeformis* variants and other taeniid cestodes inferred from the nucleotide sequence of the cytochrome *c* oxidase subunit I gene. Parasitology Research 81: 451–458. doi: 10.1007/BF00931785

Ortlepp RJ (1938) South African helminths. - Part II. Some *Taenias* from large wild carnivores. Onderstepoort Journal of Veterinary Science and Animal Industry 10: 253–278.

Priemer J, Krone O, Schuster R (2002) *Taenia krabbei* (Cestoda: Cyclophyllidea) in Germany and its delimitation from *T. ovis*. Zoologischer Anzeiger 241: 333–337. doi: 10.1078/0044-5231-00076

Pulliainen E (1981) Winter diet of *Felis lynx* L. in SE Finland as compared with the nutrition of other northern lynxes. Zeitschrift für Säugetierkunde 46: 249–259.

Rodriguez A, Carbonell E (1998) Gastrointestinal parasites of the Iberian lynx and other wild carnivores from central Spain. Acta Parasitologica 43: 128–136.

Smith JD, Addison EM, Joachim DG, Smith LM, Quinn NWS (1986) Helminth parasites of Canada lynx (*Felis canadensis*) from northern Ontario. Canadian Journal of Zoology 64: 358–364. doi: 10.1139/z86-057

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. doi: 10.1093/molbev/mst19

Terefe Y, Hailemariam Z, Menkir S, Nakao M, Lavikainen A, Haukisalmi V, Iwaki T, Okamoto M, Ito A (2014) Phylogenetic characterisation of *Taenia* tapeworms in spotted hyenas and reconsideration of the ''Out of Africa'' hypothesis of *Taenia* in humans. International Journal for Parasitology 44: 533–541. doi: 10.1016/j.ijpara.2014.03.013

Tiekotter KL (1985) Helminth species diversity and biology in the bobcat, *Lynx rufus* (Schreber), from Nebraska. Journal of Parasitology 71: 227–234. doi: 10.2307/3281907

Torres J, Garcia-Perea R, Gisbert J, Feliu C (1998) Helminth fauna of the Iberian lynx, *Lynx pardinus*. Journal of Helminthology 72: 221–226. doi: 10.1017/S0022149X00016473

Valdmann H, Andersone-Lilley Z, Koppa O, Ozolins J, Bagrade G (2005) Winter diets of wolf *Canis lupus* and lynx *Lynx lynx* in Estonia and Latvia. Acta Theriologica 50: 521–527. doi: 10.1007/BF03192645

Valdmann H, Moks E, Talvik H (2004) Helminth fauna of Eurasian lynx (*Lynx lynx*) in Estonia. Journal of Wildlife Diseases 40: 356–360. doi: 10.7589/0090-3558-40.2.356

Verster A (1969) A taxonomic revision of the genus *Taenia* Linnaeus, 1758 s. str. Onderstepoort Journal of Veterinary Research 36: 3–58.

Zhang G, Chen J, Yang Y, Liu N, Jiang W, Gu S, Wang X, Wang Z (2014) Utility of DNA barcoding in distinguishing species of the family Taeniidae. Journal of Parasitology 100: 542–546. doi: 10.1645/13-224.1

Zhang L, Hu M, Jones A, Allsopp BA, Beveridge I, Schindler AR, Gasser RB (2007) Characterization of *Taenia madoquae* and *Taenia regis* from carnivores in Kenya using genetic markers in nuclear and mitochondrial DNA, and their relationships with other selected taeniids. Molecular and Cellular Probes 21: 379–385. doi: 10.1016/j.mcp.2007.05.003

Zyll de Jong CG (1966) Parasites of the Canada lynx, *Felis* (*Lynx*) *canadensis*. Canadian Journal of Zoology 44: 499–509. doi: 10.1139/z66-054

ZooKeys 584: 25–48 (2016)
doi: 10.3897/zookeys.584.7173
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Revision of sinistral land snails of the genus *Camaena* (Stylommatophora, Camaenidae) from China based on morphological and molecular data, with description of a new species from Guangxi, China

Hong-Li Ding[1,2], Pei Wang[2], Zhou-Xing Qian[3], Jun-Hong Lin[1,2], Wei-Chuan Zhou[2], Chung-Chi Hwang[4], Hong-Mu Ai[1]

1 *College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China* **2** *Key Laboratory of Molluscan Quarantine and Identification of AQSIQ, Fujian Entry-Exit Inspection & Quarantine Bureau, Fuzhou, Fujian 350001, China* **3** *Zhejiang Museum of Natural History, Hangzhou, Zhejiang, 310014, China* **4** *Department of Life Sciences, National University of Kaohsiung, No.700, Kaohsiung University Road, Nan-Tzu District, Kaohsiung 81148, Taiwan*

Corresponding authors: *Wei-Chuan Zhou* (wczhou@163.com); *Chung-Chi Hwang* (cchwang@nuk.edu.tw); *Hong-Mu Ai* (aihongmu@yahoo.com.cn)

Academic editor: *F. Köhler* | Received 12 November 2015 | Accepted 25 March 2016 | Published 25 April 2016

http://zoobank.org/5E944099-4E3B-4ED2-BF6F-2F68440275D2

Citation: Ding H-L, Wang P, Qian Z-X, Lin J-H, Zhou Z-C, Hwang C-C, Ai H-M (2016) Revision of sinistral land snails of the genus *Camaena* (Stylommatophora, Camaenidae) from China based on morphological and molecular data, with description of a new species from Guangxi, China. ZooKeys 584: 25–48. doi: 10.3897/zookeys.584.7173

Abstract

The camaenid land snail genus *Camaena* is widely distributed throughout Southeast Asia. Thirteen species are found in China alone. Among these, *C. cicatricosa* (Müller, 1774) is the most widely distributed species, including four subspecies, *C. c. ducalis* (Ancey, 1885), *C. c. inflata* (Möllendorff, 1885), *C. c. obtecta* (Fischer, 1898) and *C. c. connectens* (Dautzenberg & Fischer, 1906). The systematics of these taxa is revised herein based on comparative shell morphology and anatomy as well as analyses of DNA sequences of two mitochondrial genes (COI, 16S rRNA) and one nuclear marker, ITS2. We found that all subspecies form well-supported clades in a molecular phylogeny and are well-differentiated from each other by genetic distances that are consistent with amounts of interspecific differentiation. In addition, they clearly differ from each other in reproductive features. Based on these observations, we elevate all four subspecies to the rank of full species. Moreover, based on morphological and mitochdondrial differentiation, we describe a new species, *Camaena poyuensis* sp. n. from Guangxi, China. The new species conspicuously differs from

its sibling species *C. cicatricosa* in having a larger and more depressed shell, a completely covered umbilicus, more or less purplish peristome, an obtuse angle at the junction of the basal and columellar lip, longer pedunculus of the bursa copulatrix, thicker epiphallus and penis, and short conic verge. Previous named species are also redescribed on their shell and anatomical characters, because the original descriptions are uninformative.

Keywords
Land snail, Gastropoda, camaenid, taxonomy, anatomy, molecular phylogeny

Introduction

The genus *Camaena* Albers, 1850, with the type species *Helix cicatricosa* Müller, 1774, is distributed throughout Southeast Asia where it occurs in southern China, Indo-China, Eastern India, the Philippines and Sulawesi (Zilch 1959–1960). In China, this genus is found mainly in the provinces of Hainan, Guangdong, Guangxi, Yunnan, Guizhou, Hunan, Fujian and Sichuan, but has not been recorded from north of the Yangtze River. Thirteen species of *Camaena* have been recorded in China (Pilsbry 1894, Yen 1939, Richardson 1985, Chen and Gao 1987, Chen 1990, Chen and Zhang 1999, Schileyko 2003, Wang et al. 2014).

The shells of all but two Chinese species are dextral. Among the two sinistral species, *C. seraphinica* Heude, 1890 is known only from the type locality, Dingan Town, Tianlin County (formerly "Si-lin"), Guangxi Province. The type locality of the second sinistral species, *Camaena cicatricosa cicatricosa* (Müller, 1774) is unknown, but specimens exhibiting typical features, such as a sinistral, umbilicated, subcarinated, depressed-globular, yellowish shell with numerous chestnut coloured bands, are widely distributed throughout southern China (Möllendorff 1885, Pilsbry 1891). They can be found in Guangdong, Guangxi, Yunnan, Guizhou, Hunan and Hong Kong (Yen 1939, Chen and Gao 1987, Zhang 2008, Wang et al. 2014).

So far, anatomical characters of sinistral camaenids have not been studied except for *C. cicatricosa* (Schileyko 2003). The shell morphology of *C. cicatricosa* is variable. Four infraspecific names have been proposed, these being *C. c. ducalis* (Ancey, 1885) and *C. c. inflata* (Möllendorff, 1885) from Guizhou, China, *C. c. obtecta* (Fischer, 1898), and *C. c. connectens* (Dautzenberg & Fischer, 1906) from northern Vietnam. These subspecies differ from the nominate form in shell features, such as shell dimensions and shape, openness of umbilicus, sharpness of peripheral angle, convexity of whorls and the presence of a hump beside umbilicus. *Camaena c. ducalis* is distinguished from other subspecies by its much larger and stronger malleated shell, a more dilated columellar margin and an almost completely covered umbilicus (Ancey 1885). *Camaena c. inflata* differs from the nominate form in having a much more globular shell, with obsolete peripheral angle and more inflated and gibbous last whorl and nearly closed umbilicus (Möllendorff 1885). *Camaena c. obtecta* is characterized by having a more globular shell, with weak peripheral angle, a completely closed umbilicus and a nearby umbilical hump (Fischer 1898). *Camaena c. connectens* differs from *C. c. cicatricosa* by

having fine and tight granules on shell (Dautzenberg and Fischer, 1906). These taxa have previously been treated either as synonyms, varieties or subspecies of *C. cicatricosa* by different authors based on comparative shell morphology (Pilsbry 1891, 1894, Fischer and Dautzenberg 1904, Dautzenberg and Fischer 1906, Yen 1939, Zilch 1964, Chen and Gao 1987, Schileyko 2011) without reaching a consensus.

Owing to the incongruent delimitation of species in the past, which reflect exclusive reliance on shell characters and the incomplete description of these species, an up-dated revision using modern techniques and species delimitation is required. The mtDNA COI (cytochrome c oxidase subunit I) gene is a commonly used marker for the DNA barcode identification system and is potentially useful for species discovery and identification (Hebert et al. 2003). Additional genetic markers on mitochondrial and nuclear genome have been used alongside COI fragment (e.g., Wu et al. 2008, Criscione and Köhler 2014). The present study aims to resolve the phylogenetic relationships of *C. cicatricosa*, to correctly delimit its closely related allies and to describe a putatively new species based on comparative analyses of morphological and molecular characters.

Material and methods

This study is based on material collected by the authors at several sites in China (Fig. 1). Live adults were drowned in water for 12–24 hours, then boiled briefly in hot water to ensure their death. Soft body was preserved in 95% ethanol and stored at -40 °C. Empty shells were cleaned and preserved at room temperature. Samples have been deposited in the State Key Laboratory of Molluscan Quarantine and Identification, FJIQBC. Shells were measured to 0.01 mm using electronic calipers. Standard shell parameters were measured on 10–74 specimens per species following Dillon (1984). Genitalia of adult snails were dissected under a dissecting microscope (ZEISS Stemi 2000). The terminology used for the reproductive system follows Gómez (2001). More than three specimens of each species have been dissected.

A piece of foot muscle tissue of about 0.05 g was used for DNA extraction. The muscle tissue was bathed in sterile water for 3–6 hours to remove residual alcohol. Genomic DNA was isolated using a DNeasy Blood and Tissue Kit (Qiagen, Beijing), examined by agarose gel electrophoresis, and stored at -20 °C for further use. Three specimens from each sampling locality were used for DNA extraction. Fragments of the partial mitochondrial cytochrome c oxidase subunit 1 (COI) and 16S rRNA (16S), and the internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA were amplified by PCR using the primer pairs and amplification conditions listed in Table 1.

Both strands of PCR products were purified and sequenced by use of the PCR primers. After sequencing, raw sequence files were proof-read based on chromatograms and assembled in BioEdit 7.2 (Hall 1999). ITS2 sequences were annotated by using HMMer (Eddy 1998) and ITS2 Database (Koetschan et al. 2010). Sequence alignments were generated using ClustalW implemented in MEGA6 (Tamura et al. 2013).

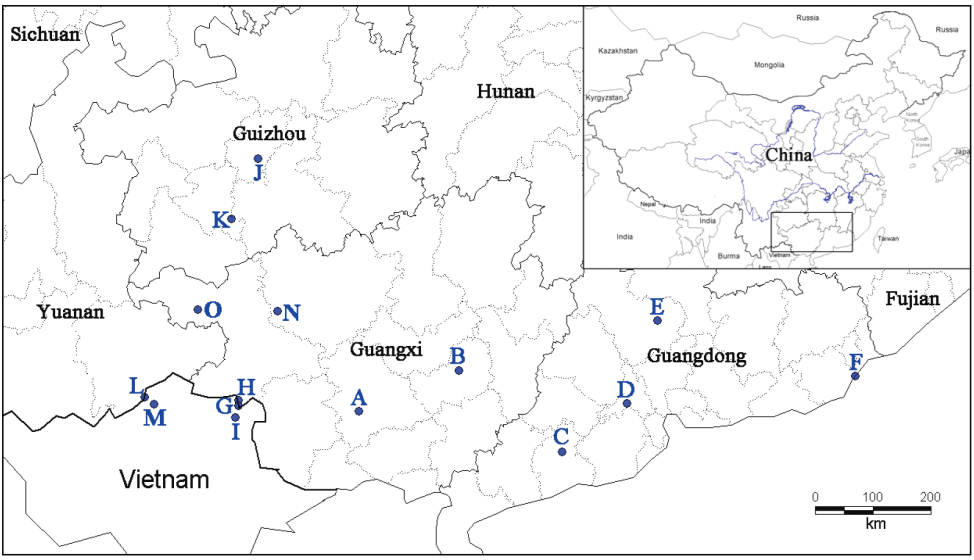


Figure 1. Map of locations of *Camaena* species. *C. cicatricosa*: **A** Nanning, Guangxi, China **B** Guiping, Guangxi, China **C** Yangchun, Guangdong, China **D** Gaoming, Canton, Guangdong, China **E** Yingde, Guangdong, China **F** Shantou, Guangdong, China. *C. obtecta*: **G** Buhaitun, Jinxi, Guangxi, China **H** Longbang, Jingxi, Guangxi, China **I** Cao Bang, Vietnam (type locality). *C. inflata*: **J** Qianlin park, Guiyang, Guizhou, China **K** Ziyun, Guiyang, Guizhou, China. *C. connectens*: **L** Tianbao, Malipo, Yunnan, China **M** Ha Giang, Vietnam (type locality). *C. poyuensis* sp. n.: **N** Poyue, Bama, Hechi, Guangxi, China (type locality). *C. seraphinica*: **O** Dingan, Tianlin, Guangxi, China (type locality).

Table 1. Primer pairs and PCR conditions used in the analysis of the COI, 16S rRNA and ITS2 genes of *Camaena*.

Gene	Primer pairs (5'-3')	Cycling conditions	Reference
COI	LCO:GGTCAACAAATCATAAAGATATTGG HCO:TAAACTTCAGGGTGACCAAAAAATCA	94°: 30s; 94°: 10s, 45°: 50s, 72°: 1min, 40 cycles; 72°: 10min.	Folmer et al. 1994
16S	16SAR: CGCCTGTTTATCAAAAACAT 16SBR: CCGGTCTGAACTCAGATCACGT	94°: 30s; 94°: 10s, 45°: 50s, 72°: 1min50s, 40 cycles; 72°: 10min.	Palumbi et al. 1991
ITS2	FYIT2:CATCGACATCTTGAACGCACAT RYIT2: TCCCAAACAACCCGACTCCT	94°: 30s; 94°: 10s, 55°: 30s, 72°: 1min30s, 40 cycles; 72°: 10min.	Present study

In the 16S alignment, sequences of ambiguous alignment were removed using Gblocks v. 0.91b (Castresana 2000), with the minimum number of sequences for a conserved position set to 22, the minimum number of sequences for a flanking position set to 36, the maximum number of contiguous non-conserved positions set to 8, the minimum length of a block set to 4, and no gap positions are allowed. Sequences were checked for saturation using the test implemented in DAMBE 5.3 (Xia et al. 2003, Xia and Lemey 2009, Xia 2013). Pairwise *p*-distances between taxa were calculated using MEGA6 under the option pairwise deletion of gaps. Prior to the model-based phylogenetic analyses, the best-fitted model of nucleotide substitution was determined for each

gene separately using the Akaike Information Criterion calculated with jModelTest v2.1.7 (Darriba et al. 2012, Guindon and Gascuel 2003). Sequences of the three genes were then concatenated into one partitioned data set. Unique sequences were identified using DAMBE 5.3. Maximum likelihood (ML) analyses were conducted using RaxML v8.2.4 (Stamatakis 2014) by applying the GTRGAMMAI model, with parameters estimated from the data, to separate partitions for each gene. The branch support of the ML tree was estimated by using the bootstrapping criteria autoMRE (Majority Rule Criterion) implemented in RAxML. *Bradybaena sequiniana* (Heude, 1885) (Bradybaenidae) and *Cornu aspersum* (Müller, 1774) (Helicidae) were used as outgroup. Two additional dextral *Camaena* species were also analyzed for comparisons.

Abbreviations

16S, 16S rRNA gene; COI, cytochrome c oxidase subunit 1 gene; FJIQBC, Fujian Entry-Exit Inspection & Quarantine Bureau, Fuzhou, Fujian, China; ITS2, internal transcribed spacer 2 region of nuclear ribosomal DNA; IZCAS, Institute of Zoology, Chinese Academy of Science Museum, Beijing, China; ML, Maximum Likelihood; MNHN, Muséum National d'Histoire Naturelle, Paris, France; SMF, Naturmuseum Senckenberg, Frankfurt/Main, Germany.

Results

Molecular analysis

Molecular phylogenetic analyses were based on DNA sequences from forty-one specimens of *Camaena* from 14 localities as well as sequences from two additional specimens of *Bradybaena sequiniana* and *Cornu aspersum* that were used as outgroup to root the tree (Table 2). Hence, a total of 129 sequences were newly generated and deposited in GenBank (Table 2). The final sequence alignments had lengths of 601 bp (COI), 348 bp (16S) and 586 bp (ITS2), respectively. Poorly aligned segments of the 16S alignments were removed using Gblocks. Gblocks maintained 348 conserved alignment positions in 16S. For phylogenetic analyses, the three sequence data sets were concatenated into one, with a length of 1,535 bp. The concatenated alignment contained 29 unique sequences, which were used for subsequent analyses. Xia's et al. (2003) test indicated no or little saturation in the three fragments, with Iss.c values significantly larger than Iss values ($p < 0.01$). Separate models determined by jModeltest for the three genes revealed the GTR model with gamma distribution and proportions of invariable sites (GTR+G+I) as the best-fit substitution model for COI, TPM2uf with gamma distribution (TPM2uf+G) for 16S, and HKY model with gamma distribution (HKY+G) for ITS2. Hence, the most complex model, GTR+G+I, among the three selected models was used for the maximum likelihood analysis.

Table 2. Sampling information and GenBank accession numbers. Localities are all in China otherwise noted.

Species / Locality	Coordinates	Collection date	COI	16S	ITS2
C. cicatricosa					
Guiping, Guangxi	23°23'58"N; 110°03'44"E	2013.11.02	KU061276 KU061277 KU586516	KU586474 KU586475 KU586476	KU586555 KU586556 KU586557
Yingde, Guangdong	24°09'44"N; 113°24'06"E	2014.09.17	KU586533 KU586534 KU586535	KU586495 KU586496 KU586497	KU586576 KU586577 KU586578
Gaoming, Guangdong	22°54'8"N; 112°53'2"E	2009.10.22	KU586513 KU586514 KU586515	KU586471 KU586472 KU586473	KU586552 KU586553 KU586554
Nanning, Guangxi	22°47'27"N; 108°23'33"E	2013.05.18	KU586521 KU586522 KU586523	KU586483 KU586484 KU586485	KU586564 KU586565 KU586566
Yangchun, Guangdong	22°10'3"N; 111°47'8"E	2014.04.01	KU586530 KU586531 KU586532	KU586492 KU586493 KU586494	KU586573 KU586574 KU586575
Shantou, Guangdong	23°16'60"N; 116°44'23"E	2010.11.03	KU586527 KU586528 KU586529	KU586489 KU586490 KU586491	KU586570 KU586571 KU586572
C. obtecta					
Longbang, Guangxi	22°53'00"N; 106°19'34"E	2015.10.03	KU055610 KU055611 KU586517	KU586477 KU586478 KU586479	KU586558 KU586559 KU586560
Buhaitun, Guangxi	22°52'38"N; 106°19'39"E	2015.10.03	KU586508 KU586509 KU586510	KU586465 KU586466 KU586467	KU586546 KU586547 KU586548
C. inflata					
Guiyang, Guizhou	26°36'8"N; 106°41'15"E	2008.10.16	KU586524 KU586525 KU586526	KU586486 KU586487 KU586488	KU586567 KU586568 KU586569
Ziyun, Guizhou	25°41'42"N; 106°14'31"E	2014.04.18	KU586536 KU586537 KU586538	KU586498 KU586499 KU586500	KU586579 KU586580 KU586581
C. connectens					
Tianbao, Malipo, yunnan	22°57'57"N; 104°49'25"E	2015.04.17	KU586518 KU586519 KU586520	KU586480 KU586481 KU586482	KU586561 KU586562 KU586563
***Camaena poyuensis* sp. n.**					
Poyue town, Bama, Hechi, Guangxi	24°17'30"N; 107°05'32"E	2014.05.25	KU061273 KU586511 KU586512	KU586468 KU586469 KU586470	KU586549 KU586550 KU586551
C. menglunensis					
Xishuangbanna, Yunnan	21°51'36"N; 101°24'53"E	2011.07.24	KU586506 KU586507	KU586463 KU586464	KU586544 KU586545

Species / Locality	Coordinates	Collection date	COI	16S	ITS2
C. jinpingensis					
Jinping, Yunnan	22°53'18"N; 103°20'23"E	2011.07.29	KU586503 KU586504 KU586505	KU586460 KU586461 KU586462	KU586541 KU586542 KU586543
Bradybaena sequiniana **(Family Bradybaenidae)**					
Badong, Hubei	31°02'46"N; 110°22'18"E	2011.06.13	KU586501	KU586458	KU586539
Cornu aspersum **(Family Helicidae)**					
Italy		2010.04.06	KU586502	KU586459	KU586540

The bootstrap support values smaller than 50% were considered poorly supported and are not considered below. Seven *Camaena* clades, which represent candidate species, with terminal clustering of sequences were identified from the reconstructed phylogeny. Although only a limited number of species was sampled, the phylogeny confirmed the monophyly of the five sinistral species from China (Fig. 2).

Genetic distances between the seven *Camaena* clades varied from 13 to 22% (average = 17%) in COI, from 5 to 15% (average = 12%) in 16S, and from 1 to 12% (average = 7%) in ITS2 (Table 3). Within-clade genetic distances were lower than 1.1% (average = 0.4%) in COI, 0.4% (average = 0.1%) in 16S, and 1.3% (average = 0.3%) in ITS2. Genetic distance between the six populations of *C. c. cicatricosa* ranged from 0 to 1.5% (average = 0.9%) in COI, 0% in 16S, and 0 to 0.4% (average = 0.2%) in ITS2. The genetic distance showed no overlap between within-clade and between-clade, with an exception of ITS2 which had slight overlap.

The well supported clades by means of bootstrap values and sufficient differentiation between clades in terms of branch lengths and genetic distances well delimited these clades as species rank. Six of the seven clades were recognized as already described species or subspecies and one clade represented a new taxon, after examining morphological characters (see Systematics part below). The comparative anatomy and nomenclatural act will be assessed in Systematics part. For clarifying, these recognized species have been labeled in figures and tables with the names and ranks of taxa treated or described below.

Systematics

Camaenidae Pilsbry, 1895

Camaena Albers, 1850

Type species. *Helix cicatricosa* Müller, 1774, subsequent designation by Martens, 1860.

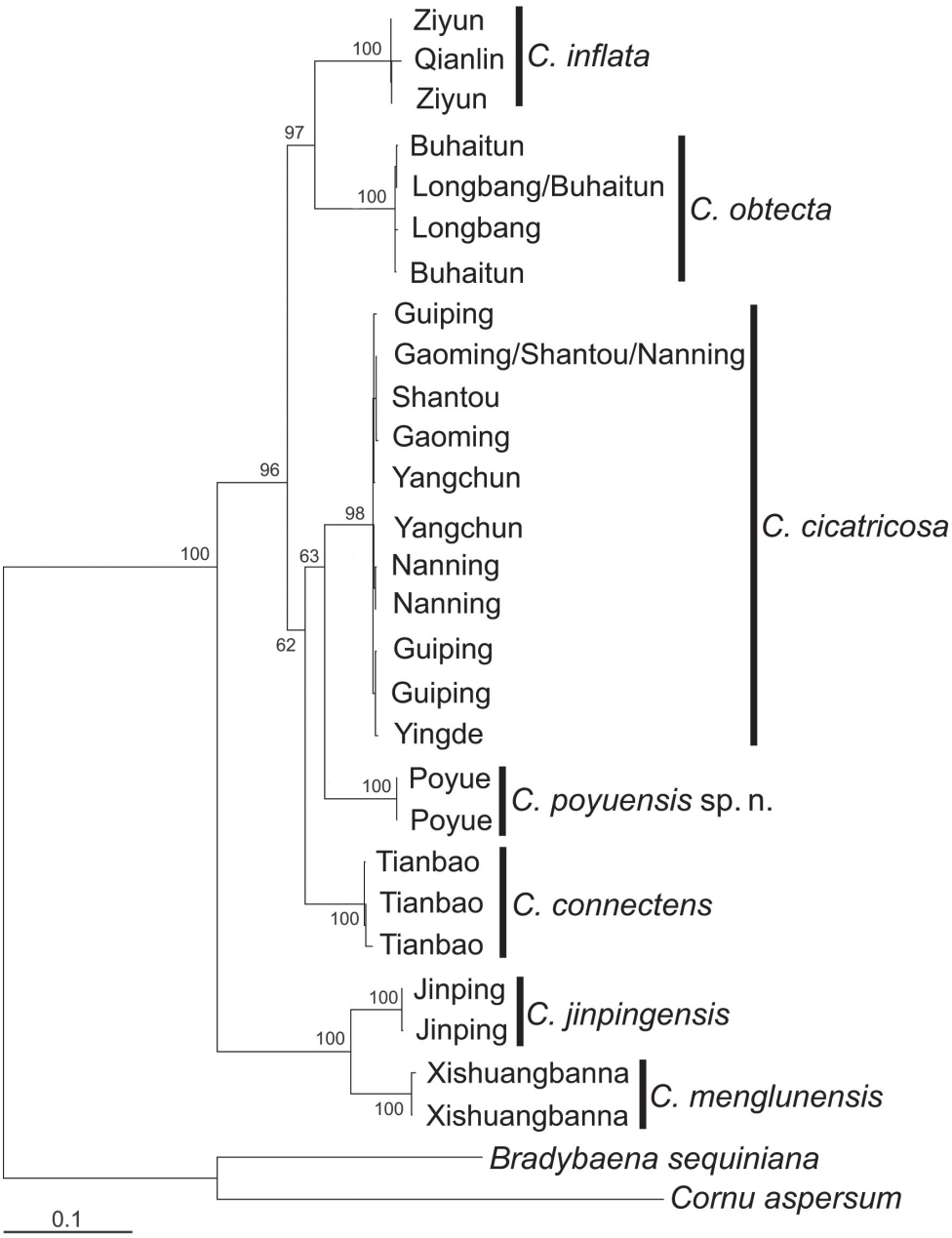


Figure 2. Maximum Likelihood tree based on analysis of the concatenated dataset of COI, 16S and ITS2 sequences. Numbers beside nodes indicate bootstrapping support (%) for main clades. See Table 2 for sampling locations.

Table 3. Average genetic distance of COI, 16S and ITS2 genes between and within (diagonal) species.

	COI	1	2	3	4	5	6	7	8	9
1	*C. cicatricosa*	0.008								
2	*C. inflata*	0.166	0.011							
3	*C. obtecta*	0.164	0.180	0.003						
4	*C. connectens*	0.133	0.184	0.165	0.000					
5	*C. poyuensis* sp. n.	0.135	0.178	0.169	0.126	0.000				
6	*C. jinpingensis*	0.178	0.200	0.189	0.179	0.193	0.001			
7	*C. menglunensis*	0.183	0.200	0.217	0.181	0.181	0.133	0.003		
8	*B. sequiniana*	0.214	0.229	0.220	0.215	0.220	0.230	0.233	-	
9	*Co. aspersum*	0.215	0.220	0.237	0.228	0.223	0.233	0.235	0.240	-
	16S	1	2	3	4	5	6	7	8	9
1	*C. cicatricosa*	0.000								
2	*C. inflata*	0.083	0.003							
3	*C. obtecta*	0.099	0.100	0.004						
4	*C. connectens*	0.072	0.101	0.141	0.000					
5	*C. poyuensis* sp. n.	0.052	0.106	0.114	0.101	0.000				
6	*C. jinpingensis*	0.121	0.138	0.147	0.132	0.144	0.000			
7	*C. menglunensis*	0.129	0.149	0.143	0.132	0.152	0.066	0.000		
8	*B. sequiniana*	0.210	0.227	0.216	0.224	0.230	0.218	0.221	-	
9	*Co. aspersum*	0.230	0.244	0.237	0.244	0.236	0.241	0.239	0.247	-
	ITS2	1	2	3	4	5	6	7	8	9
1	*C. cicatricosa*	0.002								
2	*C. inflata*	0.016	0.000							
3	*C. obtecta*	0.028	0.012	0.001						
4	*C. connectens*	0.034	0.019	0.012	0.013					
5	*C. poyuensis* sp. n.	0.028	0.024	0.027	0.032	0.000				
6	*C. jinpingensis*	0.114	0.113	0.118	0.122	0.104	0.000			
7	*C. menglunensis*	0.116	0.114	0.120	0.123	0.103	0.014	0.006		
8	*B. sequiniana*	0.245	0.242	0.249	0.250	0.243	0.244	0.238	-	
9	*Co. aspersum*	0.291	0.287	0.288	0.291	0.302	0.292	0.285	0.265	-

Abbreviations: B., Bradybaena; Co., Cornu.

***Camaena cicatricosa* (Müller, 1774)**

Figs 3A, 4A, Table 4

Helix cicatricosa Müller, 1774: 42; Chemnitz 1786: 90–91, pl. 109, fig. 923; Albers 1850: 85; Férussac and Deshayes 1850: 168–169, pl. 41, fig. 1–2; Martens 1867: 47.
Nanina (*Ariophanta*) *cicatricosa*, Beck 1837: 5.
Helix (*Camaena*) *cicatrosa*, Adams and Adams 1855: 189 [sic.]
Helix (*Camaena*) *cicatricosa*, Pilsbry 1891: 198, pl. 21, fig. 45–47; Fischer 1898: 314.
Camaena (*Camaena*) *cicatricosa*, Pilsbry 1894: 103, pl. 19, fig. 8; Zilch 1964: 243, 1960 [in 1959–1960]: 606, fig. 2125.

Camaena cicatricosa, Fischer and Dautzenberg 1904: 399; Dautzenberg and Fischer 1906: 353, 355; Yen 1939: 123, pl. 12, fig. 32; Solem 1992: 7–8, figs 1–3; Chen and Gao 1987: 100–101, fig. 129; Schileyko 2003: 1511, fig. 1947, 2011: 41; Hwang 2011: 5, fig. 2; Wang et al. 2014; Qian and Zhou 2014: 123.

Type locality. Unknown.

Material examined. See Table 4.

Diagnosis. Shell sinistral, medium sized, thick, depressed-globular, yellowish brown, with obtuse apex and high dome-shaped spire; with 5 1/2 rapidly increasing and rather flat whorls separated by deep suture; body whorl convex, not descending behind the aperture; periphery bluntly angulate, becoming round behind aperture. Sculpture of fine, dense, irregular and oblique wrinkles and malleation, with low radiate folds below suture. Aperture roundly lunate, white inside, with curved margin. Peristome white, expanded, slightly reflected, thickened and glossy; columellar margin expanded; inner lip thin, callous. Basal lip curved, forming an obtuse angle at junction with straight and oblique columellar lip. Umbilicus half covered by reflected columellar lip. Color pattern of numerous wavy, reddish brown spiral bands of various thickness; subperipheral and subsutural bands much wider (Fig. 3A)

Penis swollen, tapering distally, with a rounded bulge in correspondence of verge. Epiphallus thin with short, thin and wide penis retractor muscle. Flagellum slender, tapering distally. Vas deferens long and thin. Vagina long and thin, thickened proximally. Bursa copulatrix oval with medium-lengthened and thin pedunculus, expanded at base. Verge long, conic, with dense, weak longitudinal grooves. Inner penial wall supporting longitudinal, prominent and narrowly spaced pilasters (Fig. 4A).

Distribution. Guangdong Province to Nanning, Guangxi (Fig. 1).

Ecology. This species is locally found in high densities in a variety of habitats, which include virgin forests, semi-natural woodland, farmlands and even urban parks. Animals breed during April to September, and reach sexual maturity at 7–8 months of age (Xiao 1989).

Comparative remarks. Distinguished from all the other sinistral species of *Camaena* by its smaller size, having half opened umbilicus, and thin shell, as well as by having only longitudinal pilasters on the inner penial wall, and a long conical penial verge with longitudinal wrinkles. The shell is similar in size as *C. inflata*, but the latter differs by having a more globular shape and thicker shell, more convex whorls, and narrowly-spaced transverse wrinkles on the inner penial wall and penial verge.

Camaena obtecta (Fischer, 1898)

Figs 3B, 4B, Table 4

Helix (*Camaena*) *cicatricosa* var. *obtecta* Fischer, 1898: 315, pl. 17, figs 5–6.

Type locality. Vietnam: Luc Chu and Cao Bang

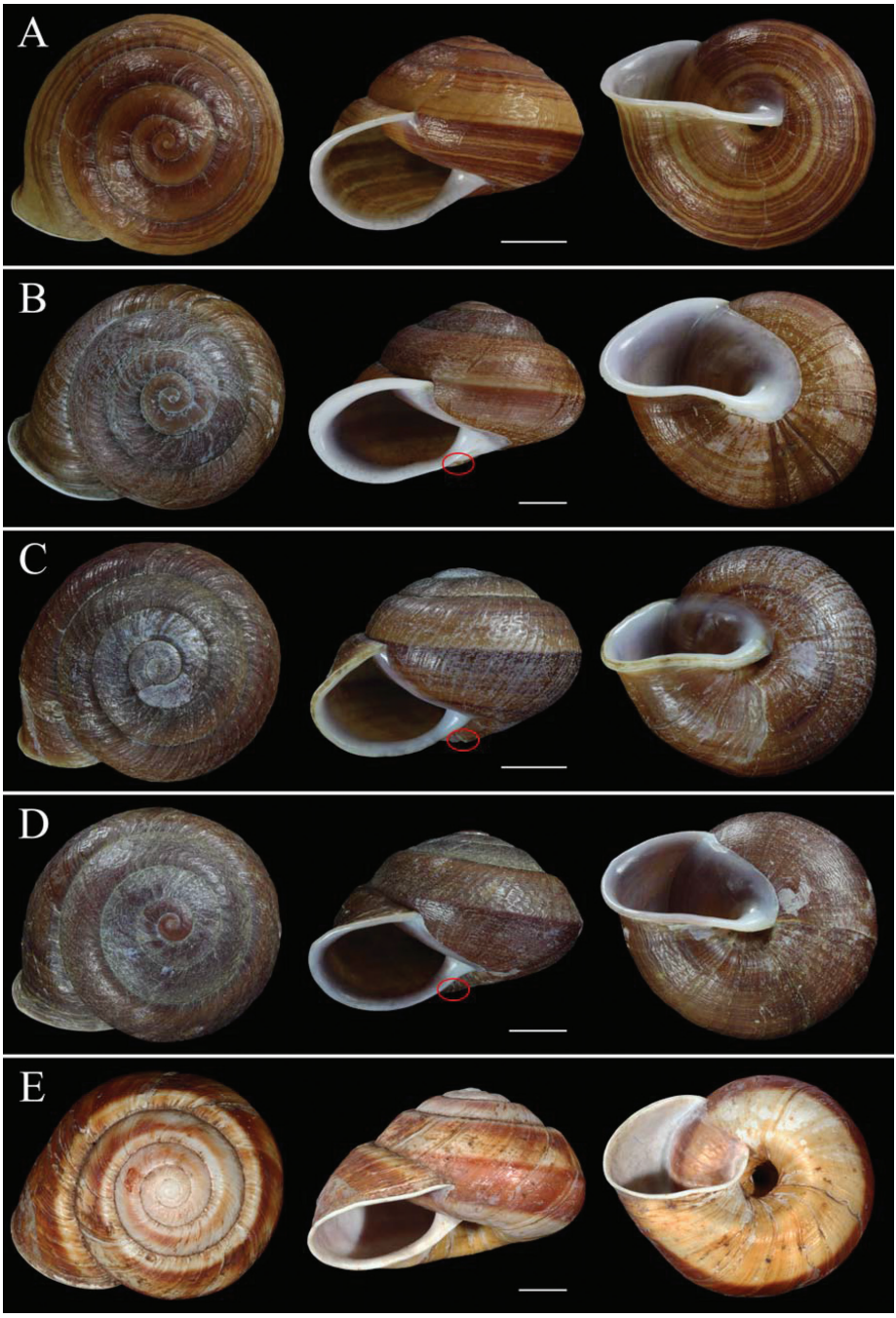


Figure 3. Shells of sinistral Chinese species of *Camaena*. **A** *C. cicatricosa* (FJIQBC 18483, Guiping, Guangxi, China) **B** *C. obtecta* (FJIQBC 18743, Longbang, Jingxi, Guangxi, China) **C** *C. inflata* (FJIQBC 18782, Qianlin park, Guiyang, Guizhou, China) **D** *C. connectens* (FJIQBC 18826, Tianbao, Malipo, Yunnan, China) **E** *C. seraphinica* (syntype, IZCAS HMT-0001a; Dingan, Tianlin, Guangxi, China). Red circle indicates a hump beside the umbilicus. Scale = 10 mm.

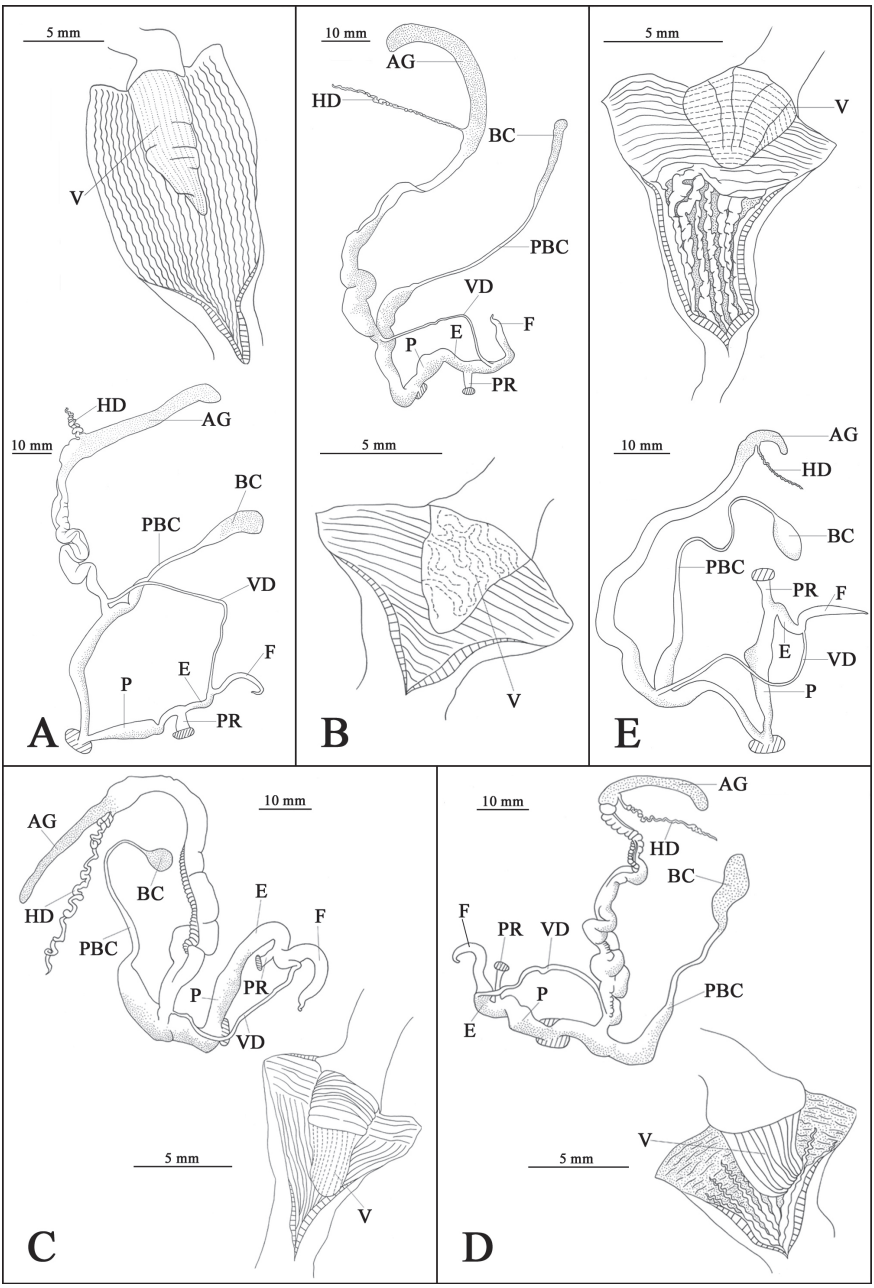


Figure 4. Reproductive system of sinistral Chinese sinistral species of *Camaena*. **A** *C. cicatricosa* (FJIQBC 18483, Guiping, Guangxi, China) **B** *C. obtecta* (FJIQBC 18743, Longbang, Jinxi, Guangxi, China) **C** *C. inflata* (FJIQBC 18797, Qianlin park, Guiyang, Guizhou, China) **D** *C. connectens* (FJIQBC 18832, Tianbao, Malipo, Yunnan, China) **E** *C. poyuensis* sp. n. (FJIQBC 18484, Poyue, Bama, Guangxi, China). Abbreviations: V, verge; AG, albumen gland; BC, bursa copulatrix; E, epiphallus; F, flagellum; HD, hermaphroditic duct; P, penis; PR, penis retractor muscle; PBC, pedunculus of bursa copulatrix; VD, vas deferens.

Table 4. Measurements of shells (mm). Localities are all in China otherwise noted. **n** sample size **SH** shell height **SW** shell width **AH** aperture height **AW** aperture width.

Species / Locality	Voucher	SH	SW	SW/SH	AH	AW	AW/AH
C. cicatricosa							
Guiping, Guangxi	FJIQBC 18503–18542 (n = 40)	21.26–32.86	35.00–42.64	1.28–1.65	16.68–21.44	21.26–27.40	1.18–1.34
		(27.61±3.17)	(39.50±2.21)	(1.44±0.11)	(19.53±1.33)	(24.36±1.74)	(1.25±0.04)
Yingde, Guangdong	FJIQBC 18543–18616 (n = 74)	26.78–36.00	39.10–48.74	1.35–1.56	19.62–24.92	22.10–22.90	1.05–1.26
		(28.66±2.45)	(41.87±2.83)	(1.46±0.05)	(21.28±0.05)	(24.94±2.30)	(1.17±0.06)
Gaoming, Guangdong	FJIQBC 18617–18640 (n = 24)	26.48–33.30	38.20–44.84	1.31–1.56	19.20–21.56	24.64–29.60	1.23–1.37
		(30.53±2.45)	(41.40±2.19)	(1.43±0.22)	(20.61±0.09)	(27.1±0.17)	(1.32±0.05)
Nanning, Guangxi	FJIQBC 18641–18670 (n = 30)	22.10–30.00	36.26–43.76	1.43–1.64	18.08–21.88	22.00–18.64	1.19–1.32
		(26.59±2.12)	(40.95±2.24)	(1.54±0.07)	(20.31±1.13)	(25.48±1.73)	(1.26±0.04)
Yangchun, Guangdong	FJIQBC 18671–18710 (n = 40)	23.44–30.00	33.76–44.26	1.20–1.52	15.86–21.66	19.42–27.06	1.11–1.25
		(27.00±1.94)	(37.76±3.10)	(1.40±0.10)	(18.45±1.61)	(22.07±1.96)	(1.20±0.04)
Shantou, Guangdong	FJIQBC 18711–18742 (n = 32)	23.40–27.26	35.44–39.64	1.44–1.57	17.64–19.36	21.18–24.14	1.19–1.29
		(25.07±1.44)	(37.58±1.53)	(1.50±0.05)	(18.61±0.61)	(22.89±1.04)	(1.23±0.04)
C. obtecta							
Longbang, Guangxi	FJIQBC 18743–18764 (n = 22)	35.76–44.10	53.20–61.74	1.33–1.57	26.70–32.26	33.28–38.80	1.15–1.29
		(39.46±2.22)	(57.93±2.53)	(1.47±0.07)	(29.64±1.70)	(35.54±1.51)	(1.19±0.04)
Buhaitun, Guangxi	FJIQBC 18765–18781 (n = 17)	32.56–40.70	51.64–59.86	1.40–1.59	23.74–30.08	31.54–38.90	1.27–1.37
		(36.90±2.72)	(55.88±2.77)	(1.52±0.06)	(27.12±1.73)	(36.10±2.48)	(1.33±0.03)
C. inflata							
Guiyang, Guizhou	FJIQBC 18782–18813 (n = 32)	25.90–38.60	38.40–46.08	1.19–1.55	20.28–23.02	22.00–38.24	1.09–1.66
		(29.97±3.31)	(42.87±2.18)	(1.44±0.10)	(21.39±0.80)	(26.50±0.40)	(1.24±0.14)
Ziyun, Guizhou	FJIQBC 18814–18825 (n = 12)	31.66–40.40	48.52–56.66	1.40–1.55	21.74–28.62	27.50–33.20	1.11–1.32
		(35.43±3.08)	(52.15±2.96)	(1.48±0.06)	(25.08±2.43)	(30.43±1.88)	(1.22±0.70)
C. connectens							
Tianbao, Malipo, yunnan	FJIQBC 18826–18835 (n = 10)	30.40–37.44	48.18–55.26	1.37–1.59	22.08–27.80	29.20–34.10	1.23–1.32
		(34.47±2.69)	(51.61±2.35)	(1.50±0.08)	(24.37±1.86)	(31.14±1.65)	(1.28±0.40)
***Camaena poyuensis* sp. n.**							
Poyue town, Bama, Hechi, Guangxi	Holotype FJIQBC 18484	38.08	56.08	1.47	27.92	34.72	1.24
	Paratypes FJIQBC 18485–18486, 18489–18502 (n = 16)	34.12–41.00	52.50–58.74	1.43–1.63	23.32–29.00	32.00–37.06	1.21–1.38
		(37.02±2.22)	(55.82±1.74)	(1.51±0.06)	(27.09±1.65)	(34.88±1.32)	(1.29±0.05)

note: Coordinates and collection date see Table 2.

Material examined. See Table 4.

Diagnosis. Shell sinistral, large, thick, solid, depressed-globular, yellowish brown to dark brown, with obtuse apex and low dome-shaped spire; 5 1/2 rapidly increasing and slightly convex whorls separated by deep suture; body whorl expanded, descending in front; periphery bluntly angulate in front of aperture, becoming round behind peristome. Surface with thick growth lines, fine spiral ribs, and weak malleation. Aperture ovate-lunate, white inside, with curved margin. Peristome white, expanded, reflected, thickened and glossy; columellar margin strongly expanded; inner lip thick, callous. Basal lip straight, forming an obtuse angle at junction with straight and oblique columellar lip. Umbilicus completely covered by reflected columellar lip and thickened callus when fully matured. Hump beside umbilicus present. Color pattern of numerous wavy, reddish brown spiral bands of various thickness; subperipheral and subsutural bands much wider (Fig. 3B).

Penis short, swollen, with a rounded bulge in correspondence of verge. Epiphallus medium with short, thin and wide penis retractor muscle. Flagellum elongated, tapering distally. Vas deferens long and thin. Vagina short and thickened. Bursa copulatrix clavate with long and thin pedunculus, apparently expanded at basal one-third its length. Verge conic, with irregular and curly wrinkles. Inner penial wall supporting transverse and narrowly spaced pilasters (Fig. 4B).

Distribution. This species has previously been recorded from Cao Bang and Luc Chu in northern Vietnam. Luc Chu is the area north of Cao Bang to the border with China according to Billet (1898). In addition, it is now recorded from Longbang and Buhaitun, in Jinxi, southwestern Guangxi, China, approximately 20 km north of Cao Bang (Fig. 1).

Ecology. This species inhabits forests on limestone, including degraded forests.

Comparative remarks. This species is characterized in having a hump beside the completely covered umbilicus, thick shell, ovate-lunate aperture, transverse only pilasters on inner penial wall, and a conic verge with irregularly curly wrinkles. It differs from *C. cicatricosa* by having a larger shell, a completely covered umbilicus, humped base beside umbilicus, more convex whorls and ovate-lunate aperture. It forms a well-differentiated clade in the phylogenetic tree (Fig. 2) and exhibits sufficient morphological differences to justify elevation to full species rank.

Camaena inflata (Möllendorff, 1885)

Figs 3C, 4C, Table 4

Helix cicatricosa var. *inflata* Möllendorff, 1885: 393.

Helix (*Camaena*) *cicatricosa* var. *inflata*, Pilsbry 1891: 199, pl. 25, fig. 101.

Camaena cicatricosa var. *inflata*, Fischer and Dautzenberg 1904: 399; Dautzenberg and Fischer 1906: 355–356; Dautzenberg and Fischer 1908: 172.

Camaena cicatricosa cicatricosa, Yen 1939: 123, pl 12, fig. 33; Zilch 1964: 243; Schileyko 2011: 41.

Type locality. Tshien-ti-shan, province of Guidshou [Tshien-te-shan (Yen, 1939)].

Material examined. Holotype. SMF 8092. Paratype. SMF 8093, 26502.

Additional material see Table 4.

Diagnosis. Shell sinistral, medium, thick, solid, globular, yellowish brown to brown, with obtuse apex and dome-shaped spire; 5 rapidly increasing and convex whorls separated by deep suture; body whorl expanded, slightly shouldered, slightly descending in front; periphery weakly angulate in front of aperture, becoming round before peristome. Surface with thick growth lines, and fine spiral ribs. Aperture roundly lunate, white inside, with curved margin. Peristome white, expanded, reflected, thickened and glossy; columellar margin expanded. Upper lip decline quickly; inner lip thickly callous. Basal lip curved, forming a smooth junction with oblique columellar lip. Umbilicus narrow, more than two-third of its area covered by reflected columellar lip. Hump beside umbilicus present. Color pattern of 3–5 reddish brown spiral bands on upper surface and numerous wavy, reddish brown spiral bands of various thickness bands on base; subperipheral and subsutural bands much wider (Fig. 3C).

Penis short, swollen, with a rounded bulge in correspondence of verge. Epiphallus swollen, with short and thin penis retractor muscle. Flagellum thickened, tapering distally. Vas deferens long and thin. Vagina short and swollen. Bursa copulatrix oval with long and thin pedunculus, expanded at basal half its length. Verge long, bluntly conic, with widely-spaced transverse wrinkles basally, dense and weak longitudinal grooves apically. Inner penial wall supporting several weak and dense pilasters: proximally transverse surrounding verge, distally longitudinal (Fig. 4C).

Distribution. Known only from Guizhou, China (Fig. 1).

Ecology. This species inhabits limestone forest. The animals appeared sensitive to environmental condition and can not be observed in farmland. Animals copulate during April to August (May and June mostly), lay eggs in September-October which hatch in 30 to 40 days (Zhang 2008).

Comparative remarks. This species is characterized in having a globular and solid shell, the swelling and gibbous last whorl, a roundly lunate aperture, an almost covered umbilicus, both transverse and longitudinal pilasters on inner penial wall, and a bluntly conic verge with transverse and longitudinal wrinkles. Shell size varied between the two sampled populations (Table 4), but the phylogeny and genetic distances agreed that they are the same species. Comparing with other species, the distinct monophyly on the phylogenetic tree, and sufficient genetic and morphological differences provide enough evidences of species separation. Hence, this taxon is raised to species rank.

Camaena connectens Dautzenberg & Fischer, 1906

Figs 3D, 4D, Table 4

Camaena cicatricosa var. *connectens* Dautzenberg and Fischer, 1906: 356.
Camaena cicatricosa connectens, Schileyko 2011.

Type locality. Vietnam, Ha-Giang

Material examined. Type material. Syntype. MNHN-IM 2000-2020.

Additional material see Table 4.

Diagnosis. Shell sinistral, large, thick, solid, depressed-globular, yellowish brown to brown, with obtuse apex and low dome-shaped spire; 5 1/2 rapidly increasing and slightly convex whorls, separated by shallow suture; body whorl expanded, weakly shouldered, slightly descending in front; periphery bluntly angulate in front of aperture, becoming round behind peristome. Surface with rough growth lines, spiral ribs, and apparent malleation. Aperture roundly lunate, white inside, with curved margin. Peristome white, expanded, reflected, thickened and glossy; columellar margin expanded. Inner lip thickly callous; basal lip curved, forming a smooth junction with oblique columellar lip. Umbilicus narrow, more than two-third of its area covered by reflected columellar lip. Hump beside umbilicus present. Color pattern of a few faint, wavy, reddish brown spiral bands of various thickness bands; subperipheral and subsutural bands much wider (Fig. 3D).

Penis swollen, slightly tapering distally, with a rounded bulge in correspondence of verge. Epiphallus thick, with long and thin penis retractor muscle. Flagellum long, thick basally, tapering distally. Vas deferens short and thin. Vagina short and swollen. Bursa copulatrix elongated-oval with long and thin pedunculus, expanded at basal half. Verge bluntly conic, with dense, deep longitudinal grooves. Inner penial wall supporting proximally transverse, short, weak, narrowly-spaced wrinkles surrounding verge, and distally longitudinal, prominent and widely spaced pilasters (Fig. 4D).

Distribution. This species has previously been recorded from Ha Giang in northern Vietnam only. In addition, it is now recorded from Tianbao, Malipo, southeastern Yunnan, China, approximately 20 km northwest of Ha Giang (Fig. 1).

Ecology. This species inhabits humid limestone forest and can not be found in farmland.

Comparative remarks. *Camaena connectens* can be distinguished from other sinistral *Camaena* species by having a rougher surface, an almost covered umbilicus, fewer and faint spiral bands, a hump beside umbilicus, both transverse and longitudinal pilasters on inner penial wall, and a bluntly conic verge with longitudinal grooves. *Camaena hahni broti* (Dautzenberg & d'Hamonville, 1887) resemble *C. connectens* in having a sinistral shell with rough surface, but the former has a nearly opened umbilicus, and carinate periphery. *Camaena connectens* differs from *C. cicatricosa* in having a larger shell, a narrower umbilicus, a hump beside umbilicus, and both transverse and longitudinal wrinkles on inner penial wall. This species can be distinguished from *C. obtecta* by having a larger shell, a wider umbilicus, a curved basal lip, and both transverse and longitudinal wrinkles on inner penial wall. This taxon is raised to species rank because of the well-differentiation of molecular and morphological characters.

***Camaena poyuensis* Zhou, Wang & Ding, sp. n.**

http://zoobank.org/78C95D9C-A54E-484B-889F-8640CD79DE11

Figs 4E, 5, Table 4

Material examined. Holotype. FJIQBC 18484, specimen preserved in ethanol, China, Guangxi Zhuang Autonomous Region, Hechi City, Bama County, Poyue town, 24°17'30"N; 107°05'32"E (Fig. 1); limestone mountain, coll. WC Zhou, May 25, 2014.

Paratypes. 19 specimens with the same data as holotype but with the following specimen codes: 4 in ethanol (FJIQBC 18485–18488), 2 adults; 15 empty shells (FJIQBC 18489–18503), 9 adults.

Measurements of shells see Table 4.

Diagnosis. Shell sinistral, large, thick, discoidal, with obtuse apex and low dome-shaped spire; 5 1/2 rapidly increasing and slightly convex whorls separated by deep suture; body whorl expanded; peripheral angle blunt. Surface with thick growth lines, and fine spiral ribs. Aperture lunate, angulated by peripheral carina. Peristome expanded, reflected, thickened and glossy. Inner lip thin, forming a smooth, semi-translucent, and purplish callus. Basal lip and columellar lip straight, with obtuse angle at junction. Umbilicus covered completely by reflected columellar lip. Color pattern of several wavy, reddish brown spiral bands of various thickness, peripheral and subsutural bands much wider; spire dark brown. Peristome and callus tinted purplish (Fig. 5A), fading to red-dotted pink on dead-collected shells (Fig. 5B).

Animal light brown, tentacles dark brown, distinct yellowish line, running from the head between tentacles to the collar near the peristome (Fig. 5C). Penis swollen, tapering distally, with a rounded bulge in correspondence of verge. Epiphallus thick, with short, thin and wide penis retractor muscle. Flagellum slender, tapering distally. Vas deferens long and thin. Vagina long and thin, thickened proximally. Bursa copulatrix head oval with long and thin pedunculus, expanded at base. Verge short, conic, with six longitudinal grooves extending from verge base to about three quarters of its length and narrowly-spaced transverse wrinkles. Inner penial wall supporting several pilasters: proximally transverse, weak and dense surrounding verge, distally longitudinal, prominent and widely-spaced (Fig. 4E).

Etymology. For the type locality, adjective of feminine gender.

Distribution. This species is known from the type locality only.

Ecology. The new species habits in a well-preserved subtropical evergreen broad-leaved forest, and is not common. The animal was not found in farmland adjacent to the forest.

Comparative remarks. Diagnostic comparisons of morphological characters of the new species and the other four *Camaena* were summarized in Table 5. The new species and *C. cicatricosa* are sister taxa (Fig. 2) and similar in shell shape, color pattern and absence of a hump beside umbilicus. The shell of *C. cicatricosa* differs from the new species by having a smaller shell, higher spire, a half opened umbilicus, a more dilated columellar lip, peristome white, curved basal lips. Among the sinistral species of the subgenus *Camaena* (*Camaena*), only *C. obtecta* (Fischer, 1898) and the new species have a totally covered umbilicus and, hence, can be distinguished from the others. *Camaena obtecta* shows thick umbilical callus, a hump beside the margin of the callus, white peristome, and a thicker shell and a higher spire than the new species.

The morphology of the reproductive system of *C. poyuensis* is similar to *C. cicatricosa*, but differs in the following characters: The pedunculus of the bursa copulatrix is longer, more than twice as long as the vagina (it is about as long as the vagina in *C. cicatricosa*). The epiphallus and penis are thicker than in *C. cicatricosa,* and the penis has a visible

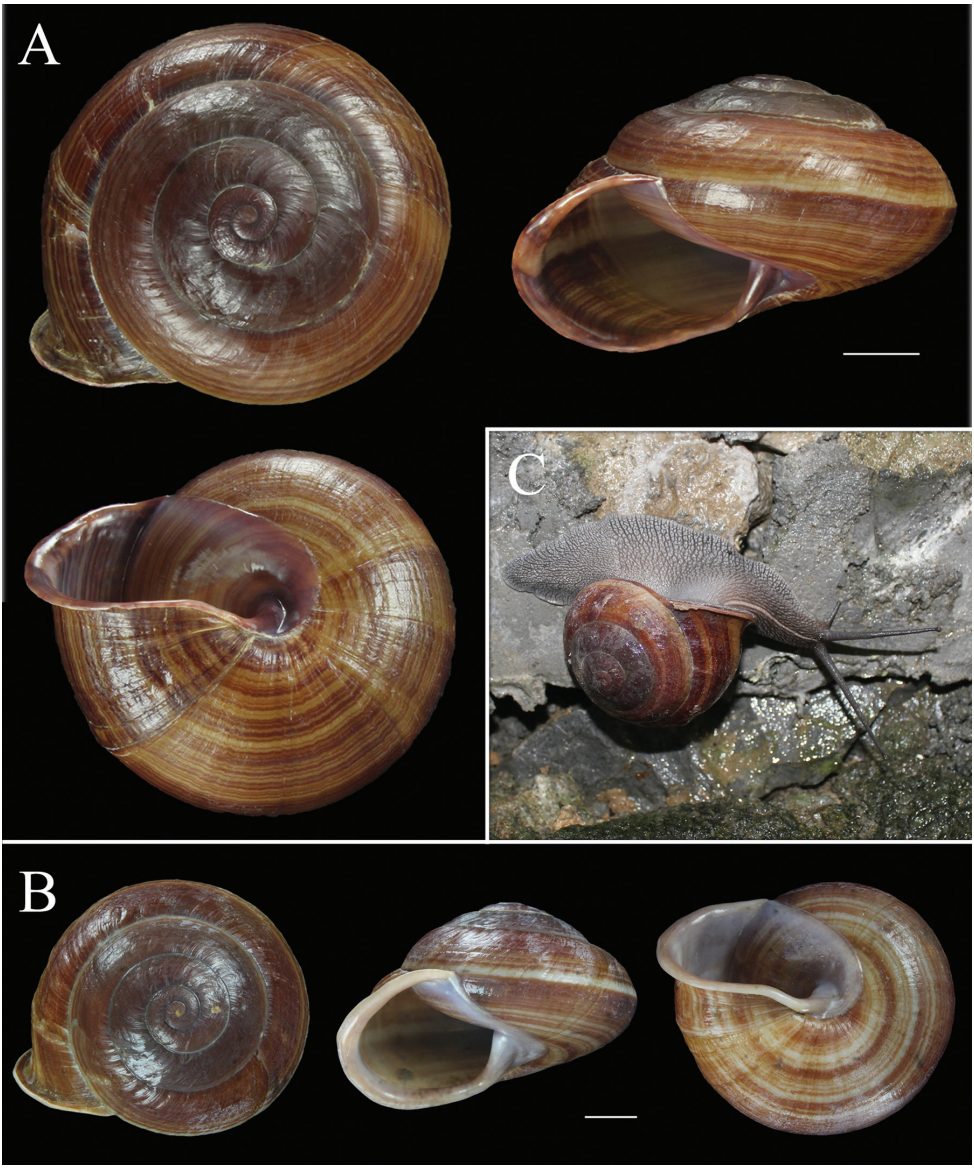


Figure 5. Shell of *Camaena poyuensis* sp. n. **A** Holotype, FJIQBC 18484, Poyue, Bama, Guangxi, China **B** Paratype, FJIQBC18489, from type locality **C** Life photograph of paratype, FJIQBC 18485, from type locality. Scale: 10 mm.

projection. *Camaena poyuensis* sp. n. has shorter verge with both transverse and longitudinal grooves, and transverse pilasters in proximal part of penis. The verge of *C. obtecta* is similar to that of *C. poyuensis* sp. n. in shape, but its surface is covered throughout with irregular, fine and curly wrinkles. Only transverse pilasters are present in the penis of *C. obtecta*, whereas both the transverse and longitudinal pilasters are seen in the new species.

Table 5. Diagnostic comparisons of morphological characters of five sinistral *Camaena* from China.

Character	*C. cicatricosa*	*C. obtecta*	*C. inflata*	*C. connectens*	*C. poyuensis* sp. n.
SW (mm)	33.8–48.7	53.2–61.9	38.4–56.7	48.2–55.3	52.5–58.7
Umbilicus	half covered	completely covered	almost covered	almost covered	completely covered
Shell shape	depressed-globose	depressed-globose	globose	depressed-globose	depressed-globose
Shell thickness	thin	thick	thick	thick	thin
Basal lip	curved	straight	curved	curved	straight
Hump beside umbilicus	absent	humped	humped	humped	absent
Pilaster on inner penial wall	longitudinal, narrowly-spaced	transverse, narrowly-spaced	proximal: transverse, narrowly-spaced distal: longitudinal, narrowly-spaced	proximal: transverse, widely-spaced wrinkles distal: longitudinal, widely-spaced	proximal: transverse, narrowly-spaced distal: longitudinal, widely-spaced
Verge	long conic	conic	bluntly long conic	bluntly conic	short conic
Verge surface	longitudinal, dense, weak wrinkles	irregularly curly, weak wrinkles	Proximal: transverse, deep wrinkles distal: longitudinal, dense, shallow wrinkles	longitudinal, dense, deep grooves	longitudinal, deep grooves

Discussion

The systematics of four of the five subspecies of *C. cicatricosa* has been revised in this study. The genetic distances of the COI barcoding region between *C. cicatricosa*, *C. inflata*, *C. obtecta*, *C. connectens* and the new species agree with the interspecific genetic distances of other camaenid groups, such as, for example, the Australian camaenid *Kimberleytrachia* (0.055–0.161, Criscione and Köhler, 2014), the Japanese camaenid *Luchuhadra* (0.003–0.205, Kameda et al. 2007) and the Taiwanese camaenid *Satsuma* (0.006–0.150, Wu et al. 2008). In addition to genetic distance, phylogenetic topography and morphological differences also support that they are distinct species. Nomenclatural acts were applied to these taxa, which include raises of taxonomic rank of three species and description of a new species.

Live specimens of two Chinese sinistral *Camaena* were not collected in the present study: *C. c. ducalis* and *C. seraphinica.* Their systematic position are not revised and remained as their current taxonomic ranks. *Camaena c. ducalis* was named based on a single specimen collected from Kouy-Yang-Fou (nowadays Guiyang), Guizhou. No further specimens were confirmedly recorded since its publication. The present authors and Prof. Tai-Chang Luo (a malacologist based on Guizhou Normal University, Guiyang, personal communication) have spent decades in biodiversity survey in Guizhou, but no shell fullfil the original descriptions were collected (Luo et al. 2003). This taxon is characterized in its large shell width of 74 mm (Ancey 1885). The shell width of known species of sinistral *Camaena* (*Camaena*) are hardly larger than 62 mm. Owing to its rarity and unusual size, this subspecies is possibly merely a gigantic variation of one of the *Camaena* species or extinct. *Camaena seraphinica* (Fig. 3E) differs

from the *C. cicatricosa* species complex by having a totally open umbilicus, a strongly descending aperture, a non-malleated surface, and a white shell background with few wide bands.

The molecular data also confirms the widely distributed *C. cicatricosa* a well-delineated species by including samples from subtropical lowland and hill region of Guangdong and eastern Guangxi Province. An extreme example is that individuals from Shantou and Nanning, populations that are about 1000 km apart (Fig. 1), shared the same sequences of the three genes. The large distributional range of this species and short genetic distances among populations are probably due to its adaptation to mankind-disturbed environments, such as farmland and forest ecotone. They have the higher probability to be passively transported large distances and to establish a new population, through human activities (Aubry et al. 2006, Guiller and Madec 2010). Besides, species of *Camaena* demonstrate different life history strategies in preliminary field and laboratory observations. *Camaena cicatricosa* laid more but smaller eggs (10–25 eggs each clutch, 0.2–0.25 g each egg) than *C. inflata* (5–9 eggs each clutch, 0.38 g each egg) (Zhang 2008, Xiao 1989). The former also has shorter gestation period (5–36 days) between the last copulation and the first egg-laying than the latter (2 months). Organisms having higher fecundity and, hence, higher abundance tend to be more competitive species (Stearns 1992). This may partly explain the dominance of *C. cicatricosa* in these areas. A thorough sampling for a phylogeographic analysis and comparative studies of life history of *C. cicatricosa* and its allies can provide a considerable insight into the evolutionary processes of *C. cicatricosa.*

Most of *Camaena* (*Camaena*) are distributed in the area of southwestern China and northern Vietnam, where is located at northern part of the Indo-Burma Hotspot and at transition to the Mountains of Southwest China Hotspot (Myers et al. 2000). Three of the species, *C. obtecta*, *C. connectens* and *C. poyuensis* sp. n., studied in the present research are distributed in the Indo-Burma Hotspot. The complex topography, varied physical conditions and a wide diversity of ecosystems in these mountainous areas have likely resulted in allopatric and sympatric speciation (Harl et al 2014, Criscione and Köhler 2016) and hence, a high biodiversity of land snails is expected (Yen 1939, Schileyko 2011).

The present molecular data set using three genetic markers supports the previous separations of *C. cicatricosa* based exclusively on shell morphology. However, more work is needed to sort out some systematic issues: (1) the taxonomy and phylogenetic relationship of all of the *Camaena* species, especially those inhabit around the border between Vietnam and China (2) the mechanism of speciation of *Camaena* in this area (3) the phylogeography of *C. cicatricosa*, the most widely distributed species in China.

Acknowledgements

We sincerely thank associate professor Wei-Hong Zhang of the University of Xinjiang, professor Tai-Chang Luo of Guizhou Normal University, and professor De-Niu

Chen of Institute of Zoology, Chinese Academy of Science for helpful comments on the manuscript. We are grateful to Dr. R. Janssen of SMF for taking photographs of type specimens; to Muséum national d'Histoire naturelle, Paris, France for open access to the digitized photograph of type specimens. We thank anonymous reviewers for their constructive comments on an earlier manuscript. This research is supported by National Natural Science Foundation of China (31372162; 31040072), National Science Council, Taiwan (NSC 98-2621-B-390-001) and Public Science and Technology Research Funds Projects of General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (No. 201410076; 2015IK042).

References

Adams H, Adams A (1855) The genera of Recent Mollusca; arranged according to their organization. Vol. 2. London, 93–284.

Albers JC (1850) Die Heliceen, nach natürlicher Verwandtschaft systematisch geordnet. T. C. F. Enslin, Berlin, 262 pp.

Ancey CF (1885) Nouvelles contributions malacologiques. Bulletins de la Société Malacologique de France 2: 113–164.

Aubry S, Labaune C, Magnin F, Roche P, Kiss L (2006) Active and passive dispersal of an invading land snail in Mediterranean France. Journal of Animal Ecology 75: 802–813. doi: 10.1111/j.1365-2656.2006.01100.x

Beck HH (1837) Index molluscorum praesentis aevi musei principis augustissimi Christian Frederici Hafniae Press, Copenhagen.

Billet A (1898) Deux ans dans le Haut-Tonkin (Région de Gao-Bang). Bulletin scientifique de la France et de la Belgique 28: 1–254.

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540–552. doi: 10.1093/oxfordjournals.molbev.a026334

Chen DN (1990) A new species of land snail from China (Stylommatophora: Camaenidae). Acta Zootxonomica Sinica 15(2): 158–160.

Chen DN, Gao JX (1987) Economic Fauna Sinica of China (Terrestrial Mollusca). Science Press, Beijing, 186 pp.

Chen DN, Zhang GQ (1999) Studies on the genus *Camaena* from China, with description of two new species (Gastropoda: Camaenidae). Transactions of the Chinese Society of Malacology 8: 28–43.

Chemnitz JH (1786) Neues systematisches Conchylien-Cabinet, enthaltened die ausführlichen Beschreibung von den Linksschnecken oder von den verkehrtgewundenen Conchylien welche gegen die Gewohnheit aller übrigen ihre Mundöffnungen nicht auf der rechten, sondern auf der linken Seite haben. Volume 9, Part 1. Gabriel Nicolaus Raspe, Nürnberg, 194 pp, 32 pl.

Criscione F, Köhler F (2014) Molecular phylogenetics and comparative anatomy of *Kimberleytrachia* Köhler, 2011 – a genus of land snail endemic to the coastal Kimberley, Western

Australia with description of new taxa (Gastropoda, Camaenidae). Contributions to Zoology 83(4) 245–267.

Criscione F, Köhler F (2016) Snails in the desert: Assessing the mitochondrial and morphological diversity and the influence of aestivation behavior on lineage differentiation in the Australian endemic *Granulomelon* Iredale, 1933 (Stylommatophora: Camaenidae). Molecular Phylogenetics and Evolution 94: 101–112. doi: 10.1016/j.ympev.2015.08.021

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772. doi: 10.1038/nmeth.2109

Dautzenberg PH, Fischer H (1906) Liste de mollusques récoltés par M. H. Mansuy en Indo-chine et au Yunnan, et description d'espèces nouvelles. Journal de Conchylogie, Paris 53: 343–471.

Dautzenberg PH, Fischer H (1908) Liste des mollusques récoltés par M. Mansuy en Indo-chine et description d'espèces nouvelles II. Journal de Conchylogie, Paris 56: 169–217.

Dillon RT (1984) What shall I measure on my snails? Allozyme data and multivariate analysis used to reduce the nongenetic component of morphological variance in *Goniobasis proxima*. Malacologia 25: 503–511.

Eddy S (1998) Profile hidden Markov models. Bioinformatics 14: 755–763. doi: 10.1093/bioinformatics/14.9.755

Férussac D de, Deshayes GP (1819–1851) Histoire naturelle générale et particulière des mollusques terrestres et fluviatiles. J.-B. Baillière, Paris.

Fischer H (1898) Notes sur la faune du Haut Tonkin. III. Liste des mollusques recueillis par le Dr A. Billet. Bulletin scientifique de la France et de la Belgique 28: 310–338.

Fischer H, Dautzenberg P (1904) Catalogue des Mollusques terrestres et fluviatiles de l'Indo-Chine orientale cites jusqu'à ce jour. Mission Pavie, Etudes diverses 3: 390–442.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Gómez BJ (2001) Structure and functioning of the reproductive system. In: Baker GM (Ed.) The Biology of Terrestrial Molluscs. CABI Publishing, Oxon, UK, 307–330. doi: 10.1079/9780851993188.0307

Guiller A, Madec L (2010) Historical biogeography of the land snail *Cornu aspersum*: a new scenario inferred from haplotype distribution in the Western Mediterranean basin. BMC Evolutionary Biology 10: 18. doi: 10.1186/1471-2148-10-18

Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52: 696–704. doi: 10.1080/10635150390235520

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Harl J, Páll-Gergely B, Kirchner S, Sattmann H, Duda M, Kruckenhauser L, Haring E (2014) Phylogeography of the land snail genus *Orcula* (Orculidae, Stylommatophora) with emphasis on the Eastern Alpine taxa: speciation, hybridization and morphological variation. BMC Evolutionary Biology 14: 223. doi: 10.1186/s12862-014-0223-y

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society B 270: 313–321. doi: 10.1098/rspb.2002.2218

Heude PM (1890) Notes sur les mollusques terrestres de la vallée du Fleuve Bleu. Mémoires Concernant l'Histoire Naturelle de l'Empire Chinois (3): 125–188.

Hwang CC (2011) Anatomy and taxonomy of *Satsuma succincta* (Adams, 1866) and *Satsuma batanica pancala* (Schmacker & Boettger, 1891) (Gastropoda: Camaenidae) from southern Taiwan. Bulletin of Malacology 35: 1–11.

Kameda Y, Kawakita A, Kato M (2007) Cryptic genetic divergence and associated morphological differentiation in the arboreal land snail *Satsuma* (*Luchuhadra*) *largillierti* (Camaenidae) endemic to the Ryukyu Archipelago, Japan. Molecular Phylogenetics and Evolution 45: 519–533. doi: 10.1016/j.ympev.2007.03.021

Koetschan C, Förster F, Keller A, Schleicher T, Ruderisch B, Schwarz R, Müller T, Wolf M, Schultz J (2010) The ITS2 Database III - sequences and structures for phylogeny. Nucleic Acids Research 38: D275–279. doi: 10.1093/nar/gkp966

Luo T, Li DH, Li Y, Chen DN (2003) Investigation on terrestrial mollusk in Guiyang, Guizhou Province, China (Gastropoda: Prosobranchia, Pulmonata). Journal of Guizhou Normal University (Natural Sciences) 21: 53–56.

Martens E von (1860) Die Heliceen, nach natürlicher Verwandtschaft systematisch geordnet von Joh. Christ. Albers. Zweite Ausgabe nach dem hinterlassenen Manuskript besorgt von Eduard von Martens. Wilhelm Engelmann, Leipzig, 359 pp.

Martens E von (1867) Die preussische Expedition nach Ost-Asien. Zoologischer Teil, 2. Die Landschnecken. Königliche Geheime Ober-Hofbuchdruckerei, Berlin, 447 pp.

Möllendorff OF von (1885) Materialien zur Fauna von China. Die Auriculaceen. Jahrbücher der Deutschen Malakozoologischen Gesellschaft 12: 349–398.

Müller OF (1774) Vermivm terrestrium et fluviatilium, v.2. Havniæ,apud Heineck et Faber, 214 pp.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. doi: 10.1038/35002501

Palumbi S, Martin A, Romano S, Mcmillan WO, Stice L, Grabowwski G (1991) The Simple Fool's Guide to PCR. Department of Zoology, University of Hawaii, Honolulu.

Pilsbry HA (1891) Manual of Conchology. Series 2, vol.6. Academy of Natural Sciences, Philadelphia, USA, 308 pp.

Pilsbry HA (1894) Manual of Conchology. Series 2, vol.7. Academy of Natural Sciences, Philadelphia, USA, 366 pp.

Qian ZX, Zhou WC (2014) Illustrated Handbook of Common Terrestrial Mollusks in China. Zhejiang People's Fine Arts Publishing House, Zhejiang, China, 228 pp.

Richardson L (1985) Camaenidae: Catalog of Species. Tryonia 12: 1–479.

Schileyko AA (2003) Treatise on recent terrestrial pulmonate molluscs. Part 11. Trigonochlamydidae, Papillodermidae, Vitrinidae, Limacidae, Bielziidae, Agriolimacidae, Boettgerillidae, Camaenidae. Ruthenica Suppl 2: 1510–1621.

Schileyko AA (2011) Check-list of land pulmonate molluscs of Vietnam (Gastropoda: Stylommatophora). Ruthenica 21: 1–68.

Solem A (1992) Camaenid land snails from Southern and Eastern South Australia, excluding Kangaroo Island. Part I. Systematics, distribution, and variation. Records of the South Australian Museum, Monograph Series 2: 1–338.

Stamatakis A (2014) RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. doi: 10.1093/bioinformatics/btu033

Stearns SC (1992) The Evolution of Life Histories. Oxford University Press, Oxford, 264 pp.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. doi: 10.1093/molbev/mst197

Wang P, Yang HF, Zhou WC, Hwang CC, Zhang WH, Qian ZX (2014) The mitochondrial genome of the land snail *Camaena cicatricosa* (Müller, 1774) (Stylommatophora, Camaenidae): the first complete sequence in the family Camaenidae. ZooKeys 451: 33–48. doi: 10.3897/zookeys.451.8537

Wu SP, Hwang CC, Lin YS (2008) Systematic revision of the arboreal snail *Satsuma albida* species complex (Mollusca: Camaenidae) with descriptions of fourteen new species from Taiwan. Zoological Journal of the Linnean Society 154: 437–493. doi: 10.1111/j.1096-3642.2008.00415.x

Xia X (2013) DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. Molecular Biology and Evolution 30: 1720–1728. doi: 10.1093/molbev/mst064

Xia X, Lemey P (2009) Assessing substitution saturation with DAMBE. In: Lemey P, Salemi M, Vandamme AM (Eds) The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny. 2nd ed. Cambridge University Press, Cambridge, 615–630. doi: 10.1017/cbo9780511819049.022

Xia X, Xie Z, Salemi M, Chen L, Wang Y (2003) An index of substitution saturation and its application. Molecular Phylogenetics and Evolution 26: 1–7. doi: 10.1016/S1055-7903(02)00326-3

Xiao WL (1989) Study on the Bionomics of the *Camaena cicatricosa*. Journal of Jinan University (Natural Science & Medicine Edition) 1: 46–52.

Yen TC (1939) Die chinesischen land-und Süßwasser-Gastropoden des Natur-Museums Senckenberg. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 444: 1–235.

Zhang L (2008) Researches on the individual development and isozyme of *Camaena cicatricosa*. MS Thesis, Guiyang, China: Guizhou Normal University.

Zilch A (1959–1960) Gastropoda. Teil 2: Euthyneura. Handbuch der Paläozoologie, Band 6. Berlin-Nikolassee, Gebrüder Borntraeger, 834 pp.

Zilch A (1964) Die Typen und Typoid des Natur-Museums Senckenberg, 29: Mollusca, Camaenidae (3). Archiv für Molluskenkunde 93: 243–262.

ZooKeys 584: 49–59 (2016)
doi: 10.3897/zookeys.584.6026
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Pseudouroctonus maidu, a new species of scorpion from northern California (Scorpiones, Vaejovidae)

Warren E. Savary[1], Robert W. Bryson Jr.[2]

1 *Department of Entomology, California Academy of Sciences, 55 Music Concourse Drive, Golden Gate Park, San Francisco, CA 94118, USA* **2** *Department of Biology & Burke Museum of Natural History and Culture, University of Washington, Box 351800, Seattle, WA 98195-1800, USA*

Corresponding author: *Warren E. Savary* (wsavary@yahoo.com)

Academic editor: *J.O. Camara* | Received 22 April 2015 | Accepted 18 March 2016 | Published 25 April 2016

http://zoobank.org/F7C82DEE-3477-460B-A72F-4156D910F73F

Citation: Savary WE, Bryson Jr RW (2016) *Pseudouroctonus maidu*, a new species of scorpion from northern California (Scorpiones, Vaejovidae). ZooKeys 584: 49–59. doi: 10.3897/zookeys.584.6026

Abstract

A new species of vaejovid scorpion from northern California, *Pseudouroctonus maidu* **sp. n.**, is named and described. This new species appears to be most similar to *Pseudouroctonus iviei* (Gertsch & Soleglad, 1972) and *Pseudouroctonus glimmei* (Hjelle, 1972).

Keywords

California, *Kovarikia*, *Pseudouroctonus*, taxonomy, Vaejovinae

Introduction

Recent fieldwork in northern California has revealed the presence of a previously undescribed species in the vaejovid scorpion genus *Pseudouroctonus* Stahnke, 1974. To facilitate its inclusion in discussions of ongoing systematic and phylogeographic studies of *Pseudouroctonus* and its near relatives (Francke and Savary 2006, Bryson et al. 2013, Bryson et al. 2014, and others in preparation), the new species is named and described herein. It represents the third species of *Pseudouroctonus* in California, all endemic to the state, and only the fourth new species of scorpion to be described from California in the past twenty years.

Materials and methods

Study specimens were preserved in 70% ethanol and examined and photographed at 6× to 30× magnification with a Wild MP5 stereo microscope. Specimens were photographed under ultraviolet light following Volschenk (2005) for illustrative purposes. Terminology for carination and hemispermatophore follows Williams (1980); trichobothrial terminology follows Vachon (1974). Measurements cited are standard ones used in scorpion systematics, as defined by Williams (1980), unless otherwise noted.

Taxonomy

Family Vaejovidae Thorell, 1876
Subfamily Vaejovinae Thorell, 1876
Genus *Pseudouroctonus* Stahnke, 1974

***Pseudouroctonus maidu* sp. n.**
http://zoobank.org/A7132001-9B73-4DD5-ABDB-2F05CDB235EE
Figs 1–19

Type material. Holotype: Adult ♀ [CASENT 9057357]. Hwy 49 between Auburn and Cool, 1.6 km SE confluence of North and Middle Forks of the American River, El Dorado Co., California (38°54'35.78"N, 121°1'40.66"W; 352 m elevation). 23 September 2013 (R. W. Bryson Jr.). Paratypes: Same locality as holotype, 23 September 2013 (R. W. Bryson Jr.), 2 ♂, 4 ♀ [CASENT 9057358]. Additional material: Same locality as holotype, 6 May 2013 (R. W. Bryson Jr.), 1 ♀ [CASENT 9057358].

Additional material examined. *Pseudouroctonus iviei*: USA: California: Butte County – Pulga Rd nr junction with Hwy 70, above North Fork Feather River, 23 September 2013 (R. W. Bryson Jr.), 1 ♀ [CASENT]; El Dorado County – Hwy 49 between Auburn and Cool, 1.6 km SE confluence of North and Middle Forks of the American River, 6 May 2013 (R. W. Bryson Jr.), 1 ♂ [CASENT]; Ice House Rd, 0.2 mi N junction US 50, 5 May 2013 (R. W. Bryson Jr.), 1 ♂ [CASENT]; Nevada County – San Juan, 15 September 1963 (J. Ivie, W. Ivie), ♀ holotype [AMNH]; Napa County – 0.8 miles N of Robert L. Stevenson State Park, approximately 7 miles N of Calistoga on Highway 29 in log under bark, mixed sclerophyll-conifer community dominated by Ponderosa Pine, 20 April 1968 (E. Bergmark), 1 ♀ [CASENT 9057899]; Shasta County – 200 meters from Bald Mountain Creek, next to The Nature Conservancy McCloud River Preserve, elevation 720 m (2350'), 7 August 1991 (L. H. Simons), 2 ♂ [CASENT 9057878, CASENT 9057898]; ibid., 12 August 1991 [CASENT 9057884], 1 ♂; ibid., 6 September 1991, 1 ♂ [CASENT 9057901]; ibid., 15 September 1991, 1 ♂ [CASENT 9057893]; ibid., 7 September 1991, 1 ♂; ibid., 24 July 1992, 1 ♀ [CASENT 9057897]; Tehama County – 5 meters from Dye Creek upstream from the pond at The Nature Conservancy's Dye

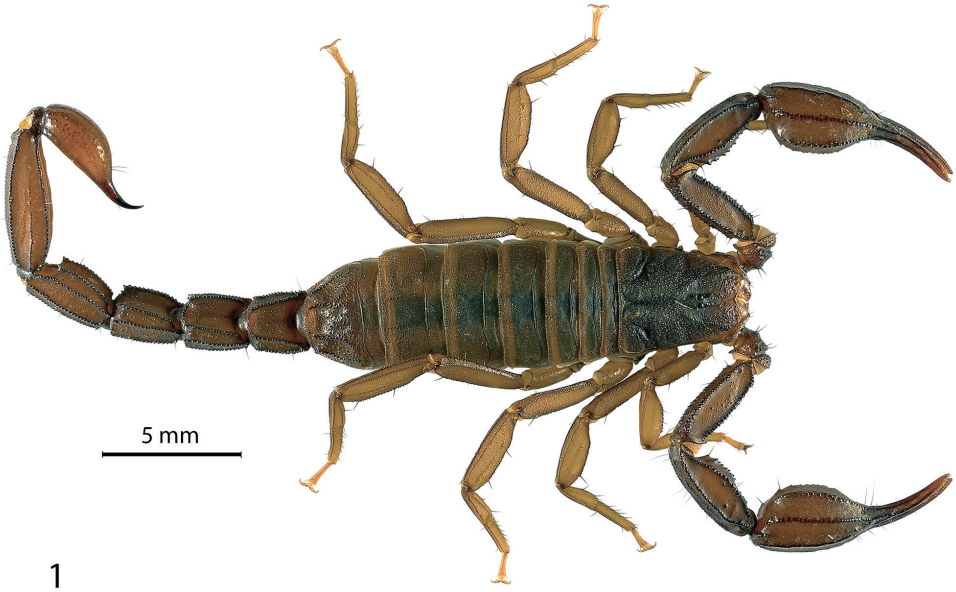


Figure 1. Adult female *Pseudouroctonus maidu* sp. n. in life, dorsal view.

Creek Preserve compound, elevation approximately 110 meters (362 feet), 30 July 1992 (L. H. Simons), 1 ♂ [CASENT 9057886]; 1 meter from Dye Creek upstream from the pond at The Nature Conservancy's Dye Creek Preserve compound, elevation approximately 110 meters (362 feet), 28 August 1992 (L. H. Simons), 3 ♂, 1 ♀ [CASENT 9057881]; 25 meters from Dye Creek upstream from the pond at The Nature Conservancy's Dye Creek Preserve compound, elevation approximately 110 meters (362 feet), 17 April 1992 (L. H. Simons), 1 immature [CASENT 9057900]; ibid., 28 August 1992 (L. H. Simons), 3 ♂ [CASENT 9057887]; ibid., 30 July 1992 (L. H. Simons), 1 ♀ [CASENT 9057895]; ibid., 9 May 1992 (L. H. Simons), 1 immature [CASENT 9057888]; ibid., 23 May 1992 (L. H. Simons), 1 ♀, 1 immature [CASENT 9057891]. *Pseudouroctonus glimmei*: USA: California: Colusa County – Mendocino National Forest, North Fork Campground, under rocks 39°23'N, 122°39'W, elevation 460 meters, 24 February 1997 (J. Schweikert), 1 ♂, 1 ♀ [CASENT 9057862]; between Sites and Maxwell, rocks, 1 June 1966 (K. E. Lucas), 1 immature [CASENT 9057896]; Lake County – approximately 5 miles N of Rayhouse Road at junction of Cache and Davis Creeks, elevation 900 feet, 9 August 1969 (J. T. Hjelle, T. Farris), 1 ♂, 1 ♀, 1 immature [CASENT 9057867]; ibid., 15 June 1969 (J. T. Hjelle, M. Bolander), 3 ♂, 3 ♀ [CASENT 9057877]; Marin County – Bootjack Camp, Mt. Tamalpais, 10 March 1968 (K. E. Lucas), 1 ♀ [CASENT 9057894]; Mendocino County – 13 miles E of Covelo, 30 August 1969 (J. T. Hjelle, B. E. Proeres), 1 ♂ [CASENT 9057868]; 3 miles up Robinson Creek Road, off Highway 253, elevation 1000 feet, 28 August 1969 (J. T. Hjelle, B. E. Proeres), 1 ♂ [CASENT 9057883]; Napa County – .25 miles S of Lake County

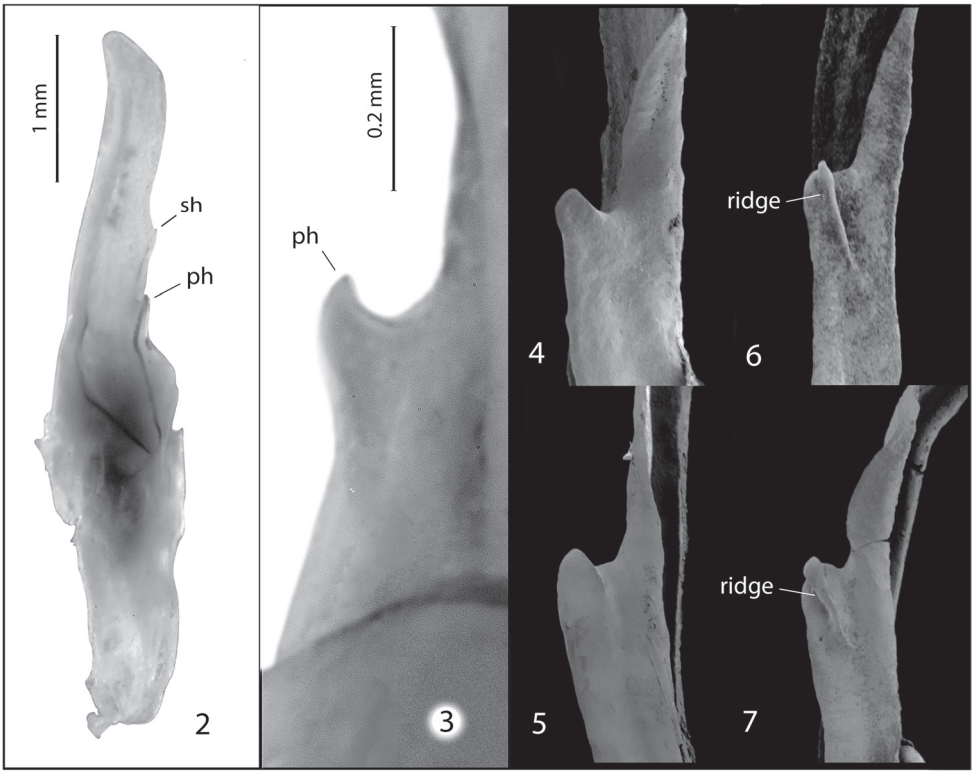
line on Butts Canyon Road, elevation 750 meters, 11 September 1969 (J. T. Hjelle, T. K. Glimme), 1 ♂ [CASENT 9057890]; Stanislaus County – Frank Raines Park, 18 miles W of Patterson, 27 September 1969 (S. C. Williams, J. T. Hjelle, M. M. Bentzien, W. E. Azevedo), 1 ♀.

Comparative diagnosis. Members of this small to medium-sized, darkly pigmented species (Fig. 1) appear to be most closely related to *Pseudouroctonus iviei* (Gertsch & Soleglad, 1972) and *Pseudouroctonus glimmei* (Hjelle, 1972), which together form a distinct group within the genus. All three species can be distinguished from other *Pseudouroctonus* by the lack of a distinct ridge on the primary lamellar hook of the hemispermatophore (Figs 2–7; see also figs 28 and 29 in Williams and Savary 1991). *Pseudouroctonus maidu* differs from *P. iviei* in having a proportionately less elongate and significantly less inflated fifth metasomal segment and in having the second metasomal segment longer than wide (see Figs 8–10 and Table 1). *Pseudouroctonus maidu* differs from *P. glimmei* in having a more granular carapace (Figs 11 and 12), and in having a well-developed, coarsely granular internomedial carina on the pedipalp patella (Figs 13–16).

Unlike other members of *Pseudouroctonus*, *P. maidu*, *P. iviei* and *P. glimmei* bear an elevated secondary lamellar hook on the hemispermatophore (Figs 2–5; see also figs 26 through 29 in Williams and Savary 1991). This feature is also present in members of the related genus *Kovarikia* Soleglad, Fet & Graham, 2014 from southern California, although members of that genus also exhibit a distinct ridge on the primary lamellar hook of the hemispermatophore (Figs 6 and 7; see also figs 22 through 27 in Williams and Savary 1991) that is lacking in *P. maidu*, *P. iviei* and *P. glimmei*.

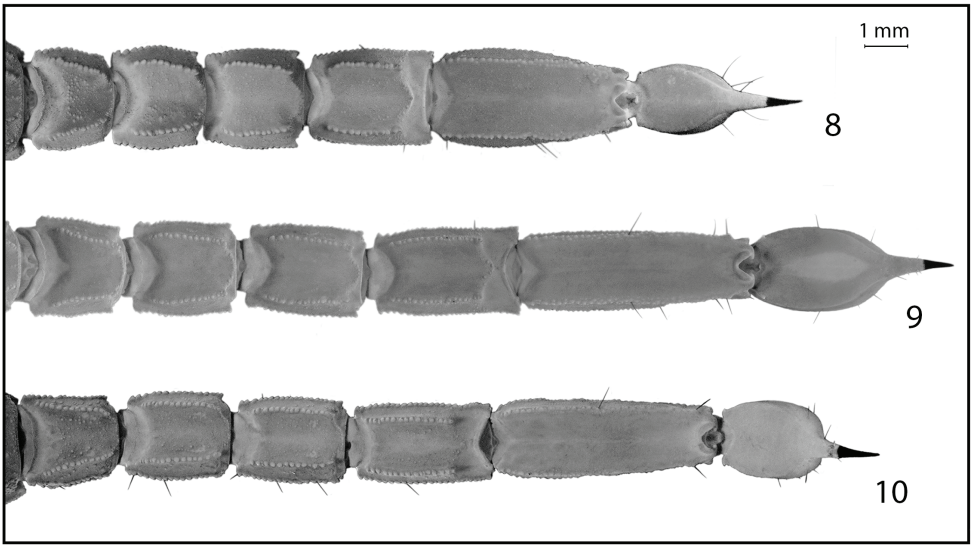
Description. Based on the adult holotype female [CASENT 9057357]. *Color*: Base color uniform dark reddish brown with legs, chelicerae and underside of preabdomen slightly paler.

Morphology: Carapace (Fig. 11): Longer than wide. Median eyes on anterior 35%. Ocular tubercle low, without superciliary crests. Median eyes 0.2 mm in diameter. Three pairs of lateral eyes, posterior-most smallest. Anterior median furrow narrow and distinct. Posterior median furrow shallow, less distinct. Anterior margin broadly bilobed, with 3 pairs of setae. Surface densely granular throughout, with granulation extending into the median furrow. Tergites: Coarsely granular, particularly on lateral and posterior margins. Tergite VII with four well-developed carinae. Sternum (Fig. 17): Subpentagonal, slightly broader than long; median longitudinal furrow deep; with 8 setae (nearly symmetrical in arrangement). Genital opercula (Fig. 17): Well-developed, with ten visible setae or setal sockets each. Pectines (Fig. 17): With 6/7 middle lamellae, of which the proximal appear to be partially fused; 10 teeth on each side, the most distal of which is weakly expanded laterally. Sternites: Anterior (stigmata-bearing) sternites I–VI very finely granular. Sternite VII more coarsely granular, with one pair of weakly developed longitudinal carinae. Stigmata small, elongate, and suboval in shape, about twice as wide as long. Metasoma (Fig. 10): Dorsolateral carinae on I–V strong, denticulate/serrate. Lateral supramedian carinae on I–IV strong, denticulate/serrate. Lateral inframedian carinae on I strong, granular, nearly complete; on II-IV absent. Lateral median carina

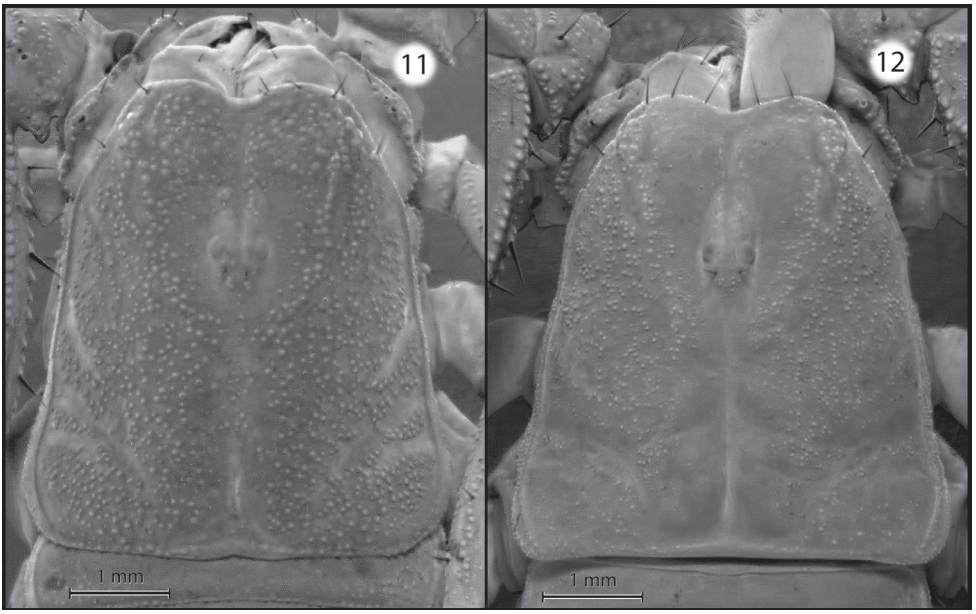


Figures 2–7. Hemispermatophores of California species of *Pseudouroctonus* Stahnke, 1974 and *Kovarikia* Soleglad, Fet & Graham, 2014: **2** hemispermatophore of *Pseudouroctonus maidu* sp. n. (ph = primary hook; sh = secondary hook) **3** primary lamellar hook of *P. maidu* **4** primary lamellar hook of *P. iviei* (Gertsch & Soleglad, 1972) **5** primary lamellar hook of *P. glimmei* (Hjelle, 1972) **6** primary lamellar hook of *Kovarikia bogerti* (Gertsch & Soleglad, 1972) **7** primary lamellar hook of *K. angelena* (Gertsch & Soleglad, 1972).

on V granular on proximal half, obsolete distally. Ventrolateral carinae on I–V, ventral submedian carinae on I–IV and ventral median carina on V strong, denticulate/serrate. Setation on I–IV: Dorsolaterals 0,0,0,1; lateral supramedian 0,1,1,1; lateral inframedian 1,0,0,0; ventrolateral 2,2,2,3; ventral submedian 2,3,3,3. Setation on V: Dorsolateral 2, lateromedian 2, ventrolateral 3 and ventromedian 4. Intercarinal spaces finely granular. Telson (Fig. 10): Vesicle robust, slightly wider than fifth metasomal segment; rugose ventrally, sparsely setose. Aculeus without basal patches of microdenticles. Chelicera: Fixed finger shorter than chela width, movable finger shorter than chela length. Chela with 3 setae dorsally. Fixed finger with basal bicusp nearly symmetrical (distal cusp slightly larger); ventral margin lacking accessory denticles. Movable finger with two dorsal subdistal teeth; with a distinct serrula ventrally, and with ventral carina smooth and strongly delineated. Pedipalp femur: Dorsointernal, externomedial, dorsoexternal, ventrointernal, and internomedial carinae strong, coarsely granular; ventroexternal carina obsolete. Orthobothriotaxia “C”. Internal face with three submedian setae; external face

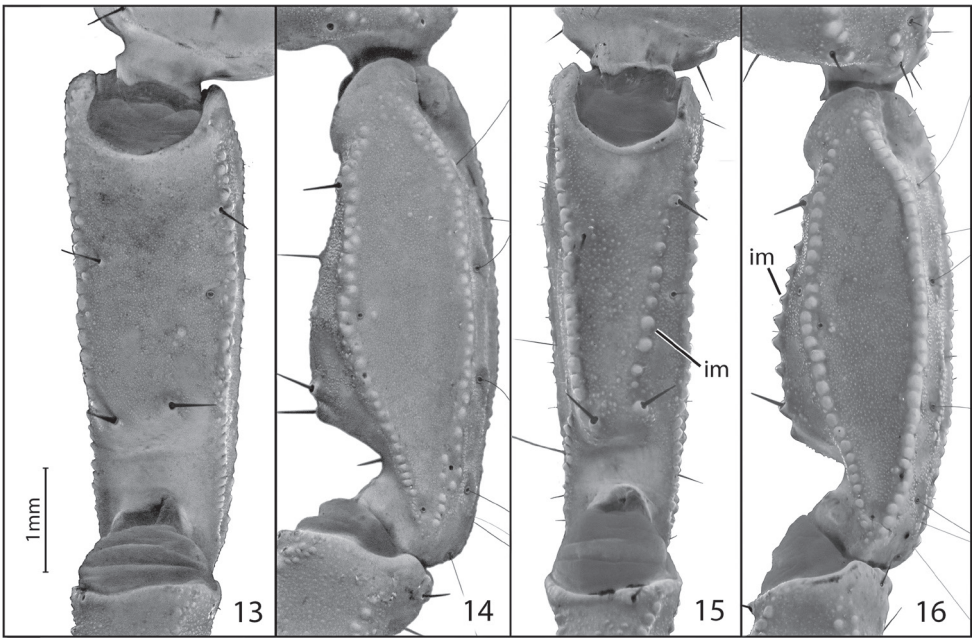


Figures 8–10. Metasomas of California species of *Pseudouroctonus* Stahnke, 1974: **8** female *P. iviei* (Gertsch & Soleglad, 1972) **9** female *P. glimmei* (Hjelle, 1972) **10** female *P. maidu* sp. n.

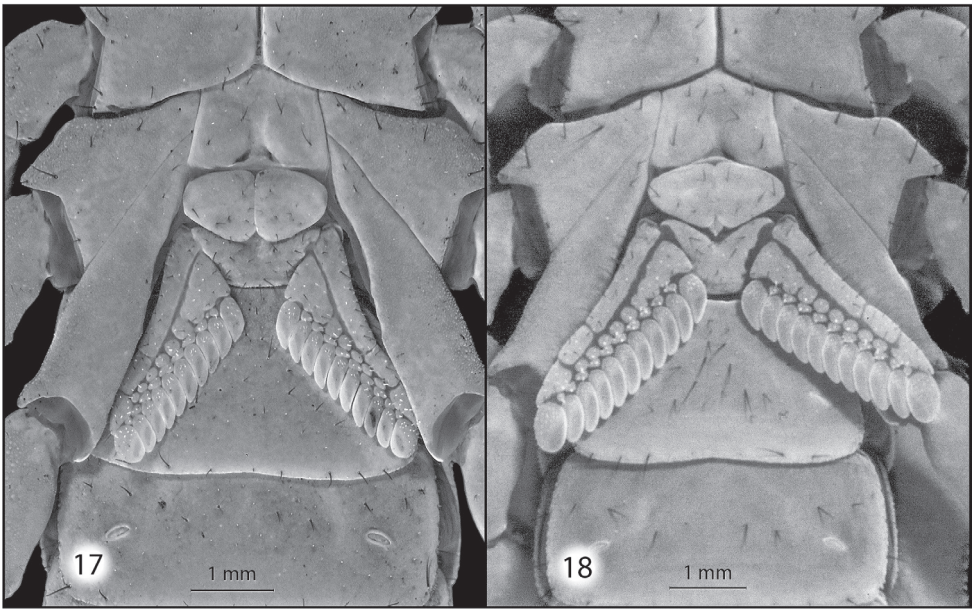


Figures 11–12. Carapaces of *Pseudouroctonus maidu* sp. n. and *P. glimmei* (Hjelle, 1972): **11** female *P. maidu* **12** female *P. glimmei*.

with 3 setae along externomedian keel; all surfaces with moderately dense granulation. Pedipalp patella (Figs 15–16): Dorsointernal, dorsoexternal, ventrointernal, and ventroexternal carinae strong, coarsely granular; internomedian carina well-developed and



Figures 13–16. Pedipalp patellas of *Pseudouroctonus glimmei* (Hjelle, 1972) and *P. maidu* sp. n.: **13** female *P. glimmei*, prolateral (internal) view **14** *P. glimmei*, dorsal view **15** female *P. maidu*, prolateral (internal) view **16** *P. maidu*, dorsal view. The internomedial carina is indicated by "im".



Figures 17–18. Sternum and pectines of *Pseudouroctonus maidu* sp. n.: **17** female **18** male.

Table 1. Morphological measurements (in millimeters) and meristic counts of *Pseudouroctonus maidu* sp. n.

	Holotype (female)	Paratype (female)	Paratype (female)	Paratype (female)	Paratype (female)	Paratype (male)	Paratype (male)
Total Length	40.53	39.01	37.68	38.59	36.06	35.19	31.40
Carapace Length	4.93	4.80	4.67	4.67	4.60	4.33	3.87
Carapace Width at lateral eyes	2.60	2.60	2.47	2.47	2.40	2.47	2.00
Carapace Width at median eyes	3.53	3.60	3.27	3.27	3.20	3.20	2.73
Carapace Width at posterior edge	4.20	4.27	4.00	4.27	3.93	3.87	3.33
Depth of median notch	0.20	0.17	0.17	0.17	0.17	0.17	0.07
Carapace anterior margin to median eyes	1.73	1.67	1.57	1.63	1.57	1.50	0.60
Diameter of median eye	0.17	0.20	0.17	0.20	0.17	0.20	0.10
Distance between median eyes	0.23	0.30	0.30	0.27	0.23	0.27	0.10
Number of lateral eyes	3/3	3/3	3/3	2/2	2/3	3/3	2/2
Mesosoma Length	12.33	11.47	11.27	11.47	11.00	9.33	9.00
Metasoma Length	23.27	22.74	21.74	22.45	20.46	21.53	18.53
Metasoma segment I Length	2.47	2.40	2.33	2.33	2.27	2.27	2.07
Metasoma segment I Width	2.53	2.40	2.33	2.33	2.27	2.27	2.00
Metasoma segment I Depth	2.13	2.00	2.00	2.00	1.93	1.80	1.73
Metasoma segment II Length	2.73	2.67	2.67	2.53	2.60	2.60	2.20
Metasoma segment II Width	2.27	2.27	2.27	2.20	2.13	2.20	1.87
Metasoma segment II Depth	2.07	2.00	1.93	1.93	1.87	1.80	1.67
Metasoma segment III Length	2.87	2.87	2.67	2.73	2.73	2.73	2.33
Metasoma segment III Width	2.20	2.13	2.13	2.20	2.07	2.07	1.87
Metasoma segment III Depth	2.07	2.00	1.93	1.87	1.87	1.93	1.67
Metasoma segment IV Length	3.67	3.60	3.27	3.60	3.33	3.33	2.80
Metasoma segment IV Width	2.07	2.07	2.13	2.07	1.93	2.00	1.73
Metasoma segment IV Depth	1.87	2.00	1.73	1.80	1.73	1.80	1.60
Metasoma segment V Length	5.73	5.53	5.33	5.73	4.13	5.40	4.73
Metasoma segment V Width	2.00	2.00	2.00	2.00	1.80	1.87	1.73
Metasoma segment V Depth	1.80	1.73	1.73	1.73	1.67	1.67	1.60
Telson Length	5.80	5.67	5.47	5.53	5.40	5.20	4.40
Vesicle Length	3.53	3.40	3.47	3.47	3.33	3.20	3.00
Vesicle Width	2.07	1.93	2.00	1.93	1.87	1.80	1.60
Vesicle Depth	1.67	1.53	1.60	1.53	1.60	1.53	1.40
Aculeus Length	1.93	1.80	1.80	1.67	1.67	1.73	1.33
Pedipalp Length	17.67	16.94	16.40	16.6	16.60	15.96	13.41
Pedipalp Femur Length	4.40	4.27	4.13	4.27	4.07	4.00	3.27
Pedipalp Femur Width	1.53	1.47	1.40	1.40	1.47	1.40	1.13
Pedipalp Femur Depth	1.27	1.27	1.20	1.20	1.27	1.00	1.00
Pedipalp Patella Length	4.87	4.60	4.47	4.53	4.53	4.43	3.67
Pedipalp Patella Width	1.80	1.67	1.60	1.80	1.73	1.53	1.33
Pedipalp Patella Depth	1.47	1.47	1.40	1.40	1.53	1.27	1.13
Pedipalp Chela Length	8.40	8.07	7.80	7.80	8.00	7.53	6.47
Palm Length	4.67	4.67	4.53	4.60	4.33	4.33	3.60
Palm Width	2.60	2.40	2.47	2.33	2.27	2.27	1.93

	Holotype (female)	Paratype (female)	Paratype (female)	Paratype (female)	Paratype (female)	Paratype (male)	Paratype (male)
Pedipalp Chela Depth	2.53	2.60	2.47	2.40	2.60	2.60	2.33
Fixed Finger Length	3.60	3.40	3.33	3.33	3.53	3.33	2.60
Movable Finger Length	4.67	4.47	4.47	4.27	4.33	4.20	3.33
Supernumeraries FF	6	6	6	6	6	6	6
Rows FF	6	6	6	6	6	6	6
Supernumeraries MF	7	7	7	7	7	7	7
Rows MF	6	6	6	6	6	6	6
Number of Pectinal Teeth	10/10	11/10	11/11	11/11	11/11	11/11	11/11

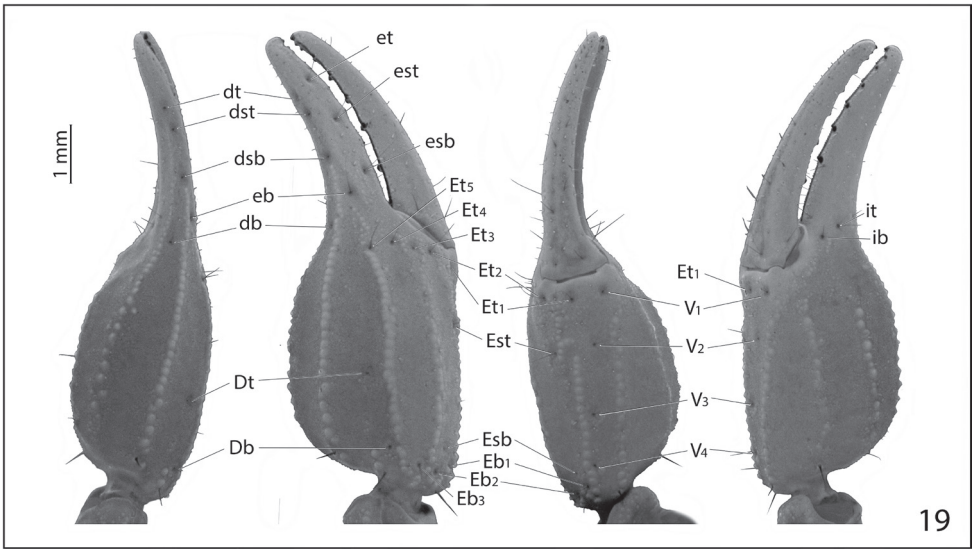


Figure 19. Trichobothrial map of pedipalp chela of female *Pseudouroctonus maidu* sp. n.

granular, extending nearly entire length of patella. Dorsal externomedian carina strong, complete, and granular; ventral externomedian carina weaker, with several interruptions, and not extending entire length of patella. Intercarinal spaces finely granular. Orthobothriotaxia "C". Pedipalp chela: Carinae granulose; intercarinal spaces very finely granular. Fixed finger with 6 rows of granules and 6 inner accessory denticles; movable finger with 6 rows of granules and 7 inner accessory denticles. Orthobothriotaxia "C" (Fig. 19), with trichobothrium *Dt*, located near the midpoint of the chela, conspicuously closer to trichobothrium *Est* than to the palm base, trichobothria Et_1 and V_1 approximately equidistant from the articulation of the movable chela finger, and 4 trichobothria in the ventral series. Walking legs: Typical of the genus. Prolateral and retrolateral pedal spurs present on all legs; tibial spurs absent. Ventral surface of tarsus with single median row of spinules, this terminating distally in two pairs of spinules (one prolateral pair and one retrolateral pair). Unguicular spine well-developed and pointed.

Measurements: See Table 1.

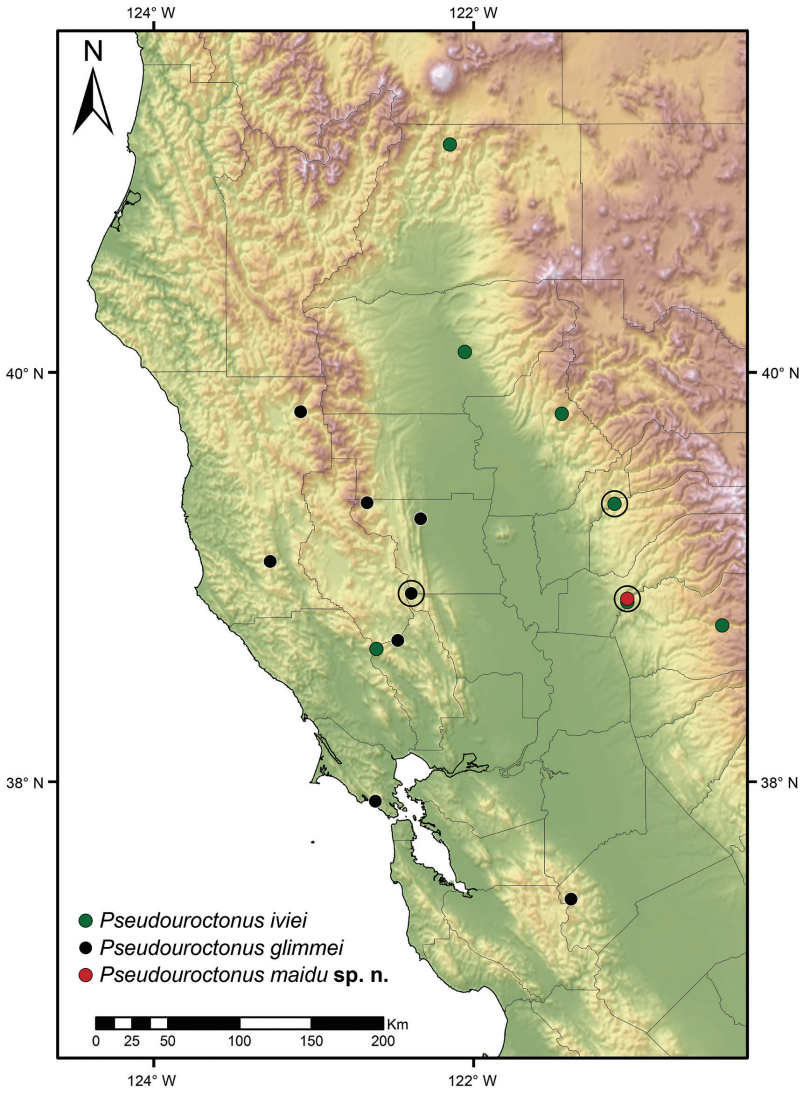


Figure 20. Localities of *Pseudouroctonus iviei* (Gertsch & Soleglad, 1972), *P. glimmei* (Hjelle, 1972), and *P. maidu* sp. n., examined for this study. Circled dots represent type localities.

Male. Similar to female. Genital papillae well-developed, conspicuous (Fig. 18). Hemispermatophore with secondary lamellar hook (Fig. 2); primary hook without raised ridge (Figs 2–7); mating plug present; similar to other members of genus in all other respects.

Variation. See Table 1. The pectinal tooth count was 11/11 in both examined males, and ranged from 10/10 to 11/11 in examined females (n=5).

Etymology. Named after the Maidu people of northern California, in whose historic lands the species occurs.

Distribution. Known only from the type locality near the confluence of North and Middle Forks of the American River in El Dorado County, California (see Fig. 20).

Natural history. Specimens were collected on 6 May 2013 and 23 September 2013. Two were found during the day on 6 May beneath rocks in moist leaf litter along a steep rocky drainage. The remaining specimens were collected by UV detection at night on 23 September. All were found on or near the bottom of a rocky embankment next to the highway. The area is characterized by oak-dominated woodlands on the drier south-facing slopes and mixed-conifer forest on the cooler north-facing slopes. Most *P. maidu* were found on north-facing slopes with scattered patches of moss-covered rocks. *Pseudouroctonus iviei* were common in drier rocky habitat with an abundance of oak leaf litter, and the two species were found in close proximity (within 0.5 m of each other) under rocks in a steep rocky drainage connecting the two types of habitat.

Acknowledgements

We thank D. Ubick and the California Academy of Science for the loan of specimens, and J. A. Ochoa and three anonymous reviewers for suggestions that improved the final version of this manuscript. RWB would like to thank C. A. Harman, M. A. Bryson, and S. E. Bryson for their support and patience. Research was conducted under scientific permits issued by CDFW.

References

Bryson RW Jr, Savary WE, Prendini L (2013) Biogeography of scorpions in the *Pseudouroctonus minimus* complex (Vaejovidae) from south-western North America: Implications of ecological specialization for pre-Quaternary diversification. Journal of Biogeography 40: 1850–1860.

Bryson RW Jr, Prendini L, Savary WE, Pearman PB (2014) Caves as microrefugia: Pleistocene phylogeography of the troglophilic North American scorpion *Pseudouroctonus reddelli*. BMC Evolutionary Biology 14: 1–16. doi: 10.1186/1471-2148-14-9

Francke OF, Savary WE (2006) A new troglobitic *Pseudouroctonus* Stahnke (Scorpiones: Vaejovidae) from northern México. Zootaxa 1302: 21–30.

Soleglad ME, Fet V, Graham MR (2014) *Kovarikia*, a new scorpion genus from California, USA (Scorpiones: Vaejovidae). Euscorpius 185: 1–22.

Vachon M (1974) Étude de caractéres utilisés pour classer les familles et les genres de Scorpions (Arachnides). Bulletin du Muséum National d´Historie Naturelle (Paris) (sér. 3) 104: 857–958.

Volschenk ES (2005) A new technique for examining surface morphosculpture of scorpions. Journal of Arachnology 33: 820–825. doi: 10.1636/S03-047.1

Williams SC (1980) Scorpions of Baja California, México, and adjacent islands. Occasional Papers of the Californinia Academy of Sciences 135: 1–127.

Williams SC, Savary WE (1991) *Uroctonites*, a new genus of scorpion from western North America (Scorpiones: Vaejovidae). Pan-Pacific Entomologist 67: 272–287.

ZooKeys 584: 61–82 (2016)
doi: 10.3897/zookeys.584.8065
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Three new species of mealybug (Hemiptera, Coccomorpha, Pseudococcidae) on persimmon fruit trees (*Diospyros kaki*) in southern Brazil

Vitor C. Pacheco da Silva[1], Mehmet Bora Kaydan[2], Jean-François Germain[3], Thibaut Malausa[4], Marcos Botton[5]

1 *Plant Protection Graduate Program, Plant Protection Department, UFPel, Campus Universitário Capão do Leão s/n, Capão do Leão, Rio Grande do Sul, Brazil* **2** *Imamoglu Vocational School, Çukurova University, 01330, Adana, Turkey* **3** *Laboratoire de la Santé des Végétaux, Unité d'entomologie et plantes invasives, CBGP 755 avenue du campus Agropolis, CS30016, 34988 Montferrier-sur-Lez, Languedoc-Roussillon, France* **4** *Institut Sophia Agrobiotech, UMR INRA, Université Nice Sophia Antipolis, CNRS, 400 Route des chappes, Sophia Antipolis, PACA, France* **5** *Embrapa Grape and Wine, 515 Rua do Livramento, Bento Gonçalves, Rio Grande do Sul, Brazil*

Corresponding author: *Vitor C Pacheco da Silva* (vitorcezar@gmail.com)

Academic editor: *R. Blackman* | Received 7 February 2016 | Accepted 19 March 2016 | Published 25 April 2016

http://zoobank.org/9C7E2192-3D64-455A-89EA-B6BF4B4C9CB8

Citation: Pacheco da Silva VC, Kaydan MB, Germain J-F, Malausa T, Botton M (2016) Three new species of mealybug (Hemiptera, Coccomorpha, Pseudococcidae) on persimmon fruit trees (*Diospyros kaki*) in southern Brazil. ZooKeys 584: 61–82. doi: 10.3897/zookeys.584.8065

Abstract

Brazil has the greatest insect diversity in the world; however, little is known about its scale insect species (Hemiptera: Coccomorpha). Mealybugs (Pseudococcidae) have been found in at least 50% of persimmon orchards *Diospyros kaki* L. in the southern part of the country. In this study three new mealybug species on persimmon trees located in the Serra Gaúcha Region, RS, Brazil, namely, *Anisococcus granarae* Pacheco da Silva & Kaydan, **sp. n.**, *Ferrisia kaki* Kaydan & Pacheco da Silva, **sp. n**. and *Pseudococcus rosangelae* Pacheco da Silva & Kaydan, **sp. n.** are described. In addition, an identification key for the genera occurring on fruit orchards and vineyards in Brazil is provided, together with illustrations and molecular data for the new species.

Keywords

Distribution, Neotropical Region, scale insects, taxonomy

Introduction

Southern Brazil is the third largest fruit-producing region in the country. It produces large amounts of temperate fruits, such as grape, apple, stone fruits and persimmon (Fachinello et al. 2011). Persimmon trees (*Diospyros kaki* L.) (Ebenaceae) were first cultivated in Brazil in the late 19th century, but this crop expanded only after Japanese immigration, around 1920 (Neuwald et al. 2009). Persimmon is currently grown on about 9,000 ha and about 172,000 tons of fruits are produced annually, for domestic consumption and export (Fachinello et al. 2011). The São Paulo and Rio Grande do Sul states are the main producers of persimmon fruits in Brazil (Camargo-Filho et al. 2003). In Rio Grande do Sul, fruit production occurs mostly in the Serra Gaúcha Region, in which mealybugs (Hemiptera: Coccomorpha: Pseudococcidae) have been detected in at least 50% of production areas (Bavaresco et al. 2005), probably due to increases in insecticide application in recent years, leading to a decrease in the population of effective natural enemies.

Ten mealybug species have been recorded in association with persimmon trees worldwide: *Dysmicoccus brevipes* (Cockerell), *Hippeococcus wegneri* Reyne, *Maconellicoccus hirsutus* (Green), *Phenacoccus aceris* (Signoret), *Ph. pergandei* Cockerell, *Planococcus citri* (Risso), *Pl. kraunhiae* (Kuwana), *Pseudococcus cryptus* Hempel, *Ps. longispinus* Targioni Tozzetti and *Ps. viburni* (Signoret) (García et al. 2016).

Live mealybugs are small soft-bodied, sap-sucking insects with an oval, elongated to rounded body, often dorsoventrally compressed, pinkish to grayish in color, covered with a white powdery wax (the source of their common name) (Cox and Pearce 1983). They frequently have waxy filaments, those on the head being shorter than those close to the anus (Gimpel and Miller 1996). These filaments originate from the cerarii – groups of trilocular pores, generally with two conical setae and, in some groups, also auxiliary setae (Williams and Granara de Willink 1992) predominantly found along the margin. The family to which mealybugs belong is the second largest family in infraorder Coccomorpha, in terms of the number of species it contains, almost 2020 species, distributed in 260 genera (García et al. 2016). In the Neotropical Region, only 223 species, from 44 genera, have been recorded (García et al. 2016).

Pseudococcidae can be divided into two subfamilies: Pseudococcinae, characterized by the presence of: (i) apically knobbed tarsal digitules; (ii) claws without a denticle; (iii) antennae generally with eight or fewer segments; (iv) anal ring with setose-like spinules; and (v) absence of quinquelocular pores; and Phenacoccinae, characterized by: (i) setose tarsal digitules; (ii) claws with a denticle; (iii) antennae usually nine-segmented; (iv) anal ring with dome-shaped spinules on the outer ring; and (v) presence of quinquelocular pores (Hardy et al. 2008; Kaydan et al. 2015).

In total, 153 species of *Pseudococcus* (Westwood) have been identified worldwide, 30 of which have been recorded in the Neotropical Region. It can be subdivided into two informal groups according to the presence or absence of simple pores associated with each eye (Gimpel and Miller 1996) — present in *Pseudococcus maritimus* complex.

It includes 33 species, 21 of which are present in the Neotropical Region (García et al. 2016), and is assumed to have originated from the New World, where some species, such as *P. sociabilis* Hambleton, *P. viburni* (Signoret) and *P. maritimus* (Ehrhorn), are considered to be major pests of fruit crops and vineyards (Casco Mila 2012; Correa et al. 2012; Daane et al. 2012; Pacheco da Silva et al. 2014).

The genus *Ferrisia* Fullaway, which is of New World origin, includes 18 species, most (12 species) of which occur in the Neotropics (Kaydan and Gullan 2012). The species from this genus are easily separated from the other genera in the Pseudococcidae by the presence of robust dorsal enlarged tubular ducts opening to the exterior via an irregularly circular sclerotized area bearing one or more setae and, often, one or more minute pores (Gullan et al. 2010). Furthermore, the living insects have long glassy filaments produced by the enlarged tubular ducts, and, depending on the species, may have typical dorsal patterns formed by dark areas of cuticle not covered by white wax (Kaydan and Gullan 2012).

The genus *Anisococcus* Ferris is also believed to have originated from New World and to be closely related to *Ferrisia* on the basis of both molecular phylogenetic studies (Downie and Gullan 2004; Hardy et al. 2008; Gullan et al. 2010) and morphological studies, as it has minute discoidal pores associated with enlarged tubular ducts and oral collar tubular ducts (Kaydan and Gullan 2012). This genus is found exclusively in the Americas, where 11 species have been described, nine in the Nearctic region and two from the Neotropical Region (McKenzie 1967; Williams and Granara de Willink 1992).

Brazil has the greatest biodiversity of any country worldwide and 13% of all species (including animals, plants, fungi and other organisms) are found only in Brazil (Lewinsohn and Prado 2005). Insect diversity is also greater in Brazil than in any other country, with almost 100 thousand species recorded (almost 10% of all insect species worldwide) (Rafael et al. 2009). It has been estimated that almost 11% of hemipteran insects are present in Brazil. However, only 3.8% of mealybug species have been recorded in this country, although this should probably be regarded as an underestimate of the percentage actually present.

In Brazil, 530 species, from 20 families of the infraorder Coccomorpha have been recorded (García et al. 2016). In total, 78 species from 21 genera of Pseudococcidae have been detected in Brazil. The most numerous genera are *Dysmicoccus* Ferris (13) and *Pseudococcus* (13), followed by *Phenacoccus* Cockerell (10), *Nipaecoccus* Sulc (8), *Planococcus* (Ferris) (5) and *Ferrisia* (5) (García et al. 2016). Only one *Anisococcus* species, *A. parasitus* Williams and Granara de Willink, has been recorded from Brazil (Williams and Granara de Willink 1992).

Three new species of mealybugs sampled from persimmon orchards located in the Serra Gaúcha Region, Rio Grande do Sul, Brazil are described, and an identification key for the genera occurring in fruit orchards and vineyards in Brazil is provided, together with illustrations, molecular data and an identification key for the new species of *Anisococcus*, *Ferrisia* and *Pseudococcus* described here.

Methods

Mealybugs were collected from persimmon orchards during the harvest period in the years 2013–2015. Specimens were collected on fruits and leaves of the trees. Insects at all stages of development were collected (nymphs and adult females) and taken to the laboratory for examination. Nymphs were reared until adulthood on persimmon fruits. Labeled specimens were stored in 95% ethyl alcohol.

Molecular characterization

DNA characterization was performed using the nondestructive method described in Malausa et al. (2011). The DNA region studied was a ~ 760 bp fragment within the mitochondrial region of Cytochrome Oxidase Subunit I previously used in molecular studies on mealybugs (Malausa et al. 2011; Pacheco da Silva et al. 2014). DNA was extracted using the Qiagen DNEasy Tissue kit, following the manufacturer's recommendations. For amplification were used the primers 5' CCTTCAACTAATCATAAAAATATYAG 3' (Forward) and 5' TAAACTTCTGGATGTCCAAAAAATCA 3' (Reverse) PCR was performed using the Qiagen Multiplex PCR kit (QIAGEN, Valencia, CA), with a 23 mL reaction mixture and 2 ml of diluted DNA (1–20 ng of DNA matrix). PCR conditions were as follows: initial denaturation at 95°C for 15 mn; 35 cycles of denaturation at 95°C for 30 s, hybridization at 48°C for 90 s, elongation for 60 s; and final extension at 72°C for 10 mn. PCR-amplified fragments were analyzed with the QIAxcel Advanced System with QIAxcel DNA Fast Analysis cartridges (QIAGEN). PCR products were sent to Beckman Genomics (Takeley, United Kingdom) for bidirectional sequencing on ABI automatic sequencers (Applied Biosystems, Foster City, CA, USA). Consensus sequences and alignments were generated and checked with Bioedit version 7.01. We carried out BLAST searches (MEGABLAST method) on the NCBI GenBank database (http://www.ncbi.nlm.gov/BLAST).

The DNA results are shown for each species after the morphological descriptions. Additionally, all sequence data are available as Suppl. material 1 (FASTA format).

Morphological identification

The DNA voucher specimens plus other preserved adult females were slide-mounted and identified by light microscopy in the Plant Protection Department of Çukurova University, Adana, Turkey and ANSES, *Laboratoire de la Santé des Végétaux*, Montferrier-sur-Lez, France, according to a slightly modified version of the method of Kosztarab and Kozár (1988). The mealybugs were examined under a LEICA DM 2500 phase-contrast compound microscope and identified with the keys of Williams and Granara de Willink (1992), Gimpel and Miller (1996) and Kaydan and Gullan (2012).

The slides are stored in the Coccoidea Collection of the Museum Ramiro Gomes Costa (MRGC), Porto Alegre, Brazil (Holotype and some paratypes), Çukurova University Coccomorpha collection, Adana, Turkey (KPTC) and Anses, Laboratoire de la Santé des Végétaux, Montferrier-sur-Lez, France (ANSES/LSV).

Morphometric analysis

Mealybugs were measured and the main taxonomic characters evaluated and quantified under the Leica microscope. Measurements were taken from all the available material. The morphological terms used here are those used by Williams (2004) and Williams and Granara de Willink (1992). All the measurements given are the maximum dimensions (e.g. body width was recorded at the widest part) and are expressed as ranges. Tarsal length excludes the claw. Setal length includes the setal base. Cerarii are numbered as described by Williams and Granara de Willink (1992), with cerarius 1 on the head, anterior to the antenna, and cerarius 17 being on segment VIII.

Illustrations are provided for each species. Each figure represents a generalized individual based on several of the specimens used for description. Each illustration is split longitudinally, with the left half representing the dorsum and the right half the venter. Structural details are shown as enlargements around the central drawing, and are not drawn to the same scale. The translucent pores on the hind legs are mostly found on the dorsal surface, but they are illustrated ventrally on the main figure for convenience. The illustrations and description were prepared by MBK and VCPS.

Results and discussion

Key to identification of Pseudococcinae genera occurring on fruit trees and in vineyards in Brazil, adapted from Williams (2004) and Williams and Granada de Willink (1992).

1 Dorsal tubular ducts large, each with an orifice surrounded by a round, sclerotized area containing 1 or more setae within its borders, or with the setae adjacent to the rim..... ***Ferrisia*** **Fullaway**

– Dorsal tubular ducts, if present, without this combination of characters.....**2**

2 Dorsal tubular ducts each with a small adventitious pore or cell adjoining the main orifice; anal lobe cerarii each with 7-20 conical setae on a sclerotized area; multilocular disc pores always absent.....***Anisococcus*** **Ferris**

– Not with this combination of characters; if there are any pores next to tubular ducts, then each anal lobe cerarius usually with only 2 conical setae; multilocular disc pores present or absent.....**3**

3 Oral rim tubular ducts present somewhere on the body.....**4**

–	Oral rim tubular ducts absent **6**
4	Dorsal surface with setae on posterior segments at least, each broadly lanceolate or conical in shape, sometimes subequal in size and shape to posterior cerarian setae..... ***Nipaecoccus*** **Sulc**
–	Dorsal surface with all setae flagellate, normally much thinner than cerarian setae..... **5**
5	Cerarii anterior to the anal lobe pair mostly with auxiliary setae; with 12–17 distinct pairs of marginal cerarii ..... ***Pseudococcus*** **Westwood**
–	Cerarii anterior to the anal lobe pair without auxiliary setae; with 1–7 distinct pairs of marginal cerarii ..... ***Maconellicoccus*** **Ezzat**
6	With 18 distinct pairs of marginal cerarii; anal lobe bars present .... ***Planococcus*** **Ferris**
–	With 6 to 17 pairs pairs of marginal cerarii; anal lobe bars present or absent .... ***Dysmicoccus*** **Ferris**

Genus *Anisococcus* Ferris

Anisococcus Ferris, 1950

Type species. *Dactylopius crawii* Coquillet by original designation.

Generic diagnosis (adapted from Williams and Granara de Willink 1992; McKenzie 1967). Body narrowly to broadly oval, 2.0–3.8 mm long, 1.1–2.8 mm wide. Labium with three segments, about as long as the clypeolabral shield. Antennae, 8-segmented. Circulus present or absent. Legs well-developed, without translucent pores; apparently with a small denticle on the claw. Both ostioles well developed. Anal lobes well developed. Anal ring rounded, usually large and cellular with six long setae, but sometimes reduced, non-cellular, more or less removed from the posterior apex of the abdomen (*Anisococcus ephedrae* (Coquillett)).

Dorsum. Dorsal tubular ducts with or without a rim, each orifice associated with one or more minute discoidal pores. Cerarii 13–17 pairs. Anal lobe cerarii, each with 7–20 conical setae on a sclerotized area, often with 3–7 auxiliary setae, remaining cerarii smaller, each with two or more conical setae plus an associated cluster of trilocular pores. Preocular cerarius always absent. Dorsal setae, slender and flagellate. Trilocular pores evenly distributed. Discoidal pores scattered and associated with tubular ducts, each smaller than trilocular pores. Multilocular disc pores absent.

Venter. Body setae flagellate. Trilocular pores evenly distributed. Discoidal pores scattered or associated with tubular ducts. Multilocular disc pores absent. Oral collar tubular ducts of one or more sizes, of various lengths and widths, with largest ducts, when present, on body margin, often associated with minute discoidal pores.

Key to adult females of *Anisococcus* found in the Neotropical Region (adapted from Williams and Granada de Willink (1992)).

1 Dorsal oral collar tubular ducts of one size, all large, each about twice the diameter of a trilocular pore, always with a rim..... .....***A. milleri* Williams & Granara de Willink**

– Dorsal oral collar tubular ducts of two sizes, the large ducts with a rim, smaller ducts without a rim.....**2**

2 Ventral oral collar tubular ducts present in rows across medial areas of abdominal segments **3**

– Ventral oral collar tubular ducts on abdomen represented by only 1 or 2, restricted to medially on abdominal segments..... .....***A. parasitus* Williams & Granara de Willink**

3 Oral collar tubular ducts on venter of one size; smaller oral collar tubular ducts on dorsum without a sclerotized area next to duct opening..... .....***A. erbi* Williams & Granara de Willink**

– Oral collar tubular ducts on venter of two sizes; smaller oral collar tubular ducts on dorsum with a sclerotized area next to duct opening..... .....***A. granarae* Pacheco da Silva & Kaydan, sp. n.**

***Anisococcus granarae* Pacheco da Silva & Kaydan, sp. n.**
http://zoobank.org/AB5F7C82-5263-4377-97C7-F98E9FC434F6
Figs 1, 2

Type-locality. Brazil, Farroupilha – Rio Grande do Sul, on fruits in persimmon orchards, *Diospyros kaki*, Apr 2015, VC Pacheco da Silva leg.

Type-specimen. *Holotype* female, Brazil, Farroupilha – Rio Grande do Sul, on *Diospyros kaki*, on fruits, Apr 2015, coll: VC Pacheco da Silva, MRGC: 2263. *Paratypes*: Brazil, 3 ♀♀ (85, 84, 89) - Farroupilha – Rio Grande do Sul, on *D. kaki* 'Fuyu', Apr 2015, coll: VC Pacheco da Silva and ECW Galzer; 1 ♀ (65) - Bento Gonçalves – Rio Grande do Sul, on *D. kaki*, May 2015, coll: VC Pacheco da Silva; 2 ♀♀ (112, 114) - Farroupilha – Rio Grande do Sul, on *D. kaki* 'Kioto', Apr 2015, coll: VC Pacheco da Silva and ECW Galzer; 2 ♀♀ (129, 131) - Caxias do Sul – Rio Grande do Sul, on *D. kaki* 'Fuyu', Apr 2015, coll: VC Pacheco da Silva and ECW Galzer; 1 ♀ (142) - Farroupilha – Rio Grande do Sul, on *D. kaki* 'Kioto', Apr 2015, coll: VC Pacheco da Silva and ECW Galzer; 1 ♀ (166) - Farroupilha – Rio Grande do Sul, on *D. kaki* 'Kioto', Apr 2015, coll: VC Pacheco da Silva and ECW Galzer; 3 ♀♀ (190, 191, 192) - Farroupilha – Rio Grande do Sul, on *D. kaki* 'Kioto', Apr 2015, coll: VC Pacheco da Silva and ECW Galzer. ANSES/LSV 3 slides, MBK 2 slide and MRGC 2 slides (2264 and 2265).

Figure 1. *Anisococcus granarae* Pacheco da Silva & Kaydan, sp. n. Live adult female.

Diagnosis. *Anisococcus granarae* Pacheco da Silva & Kaydan, sp. n. is characterized by the following combination of features: (i) dorsal oral collar tubular ducts of 2 sizes, the large type with an indistinct rim, the small type without a rim (but with a sclerotized area next to the ducts opening); (ii) ventral oral collar tubular ducts of two sizes, smaller ducts present in rows across medial areas of abdominal segments, and larger ducts in body margin.

Description. Adult female.

Appearance in life.

Body oval, up to 4 mm long at maturity, covered in a layer of white wax; with two longitudinal lines of dorsal patches without wax on the intersegmental areas of the abdomen, exposing areas of dark gray-to-black subcutaneous pigment (Fig. 1). The margins have 14 small thin lateral filaments plus a long filament produced by anal lobe cerarii.

Body oval, 2.08–3.28 mm long, 1.06–1.82 mm wide. Eye marginal, 60–80 μm wide. Antennae, 8-segmented, 630–730 μm long, with 4 fleshy setae, each 35–70 μm long; apical segment 120–125 μm long, 35 μm wide, with apical setae 60 μm long. Tentorium 190–200 μm long, 175–210 μm wide. Labium 3-segmented, 220–260 μm long, 135–145 μm wide. Anterior spiracles 95–105 μm long, 50–65 μm wide across atrium; posterior spiracles 115–130 μm long, 70–90 μm wide across atrium. Circulus 145–200 μm wide. Legs well-developed; lengths for posterior legs: coxa 280–330 μm,

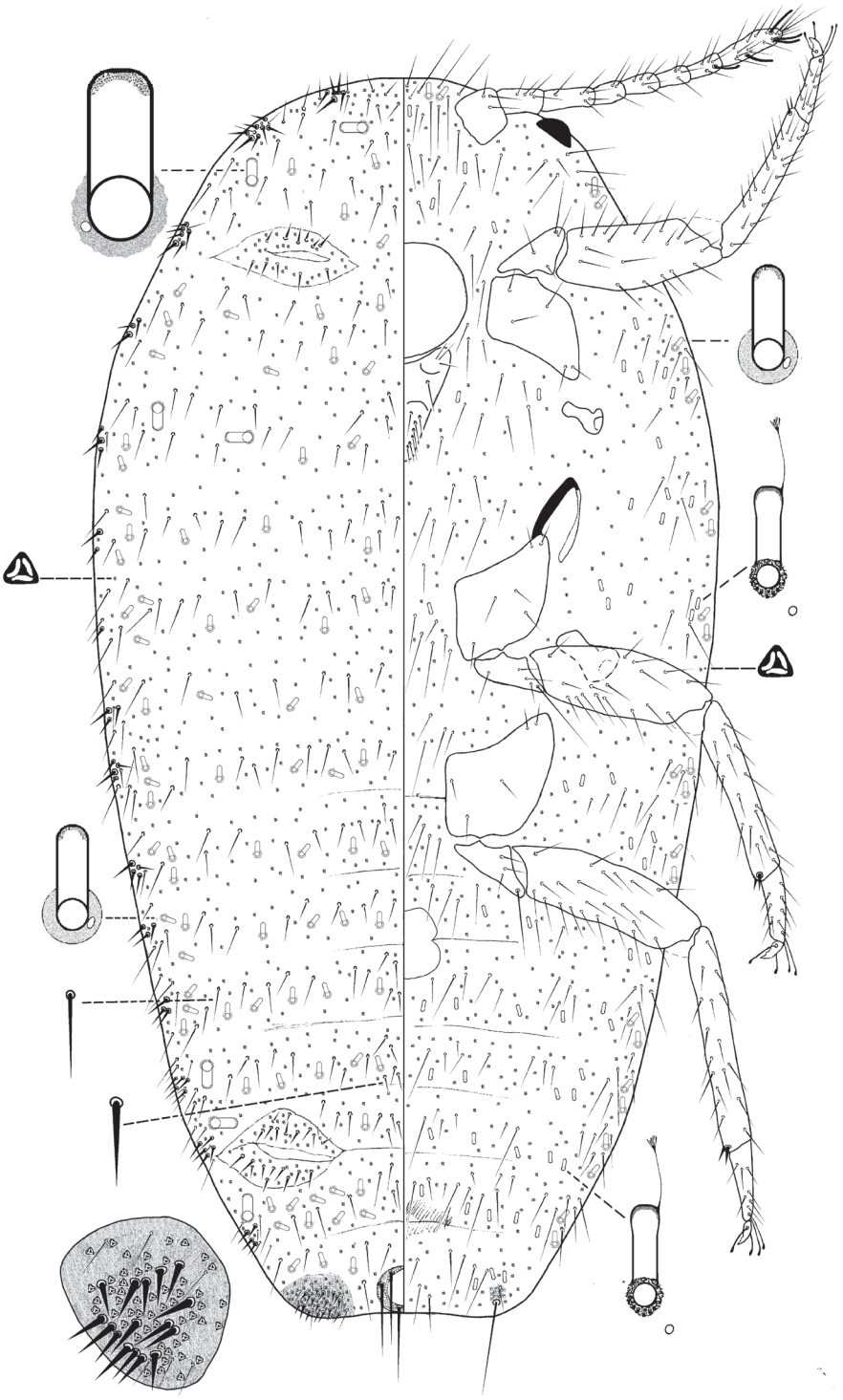


Figure 2. Adult female *Anisococcus granarae* Pacheco da Silva & Kaydan, sp. n. Holotype.

trochanter + femur 490–560 μm, tibia + tarsus 550–590 μm, claw 35–45 μm. Ratio of length of tibia + tarsus to trochanter + femur, 1.03–1.15:1; ratio of length of tibia to tarsus, 3.00–3.38:1; ratio of length of hind trochanter + femur to greatest width of femur, 3.25–3.77:1. Tarsal digitules capitate, each 60.0–72.5 μm long. Claw digitules capitate, each 45–50 μm. Both pairs of ostioles present; anterior ostioles each with a total for both lips of 55–69 trilocular pores and 25–30 setae; posterior ostioles each with a total for both lips of 49–69 trilocular pores and 17–22 setae. Anal ring 120–125 μm wide, with 6 setae, each setae 260–305 μm long.

Dorsum. Derm membranous, with 16 pairs of cerarii around body margin, each cerarius with 1–6 cerarian setae, each 20.0–22.5 μm long, plus 15–20 trilocular pores between cerarian setae and 3–5 spine-like auxiliary setae. Anal lobe cerarii each with about 12–16 conical setae, each 25.0–32.5 μm long, plus 42–54 trilocular pores and 3–5 spine-like auxiliary setae, all on a sclerotized area about the same size as the anal ring. Dorsal body setae of two kinds, (i) short spine-like slightly flagellate setae, each 20–25 μm long, present in middle of body segments, and (ii) hair-like flagellate setae, each 20–50 μm long, scattered on head and thorax and in single rows on abdominal segments. Trilocular pores each 4–5 μm in diameter, scattered over entire body. Minute discoidal pores, each 2.0–2.5 μm in diameter, also scattered throughout the dorsum and associated with oral collar tubular ducts. Oral collar tubular ducts of two kinds, always with at least 1 minute discoidal pore: (i) larger ducts each 20–25 μm long, 9–10 μm wide at mid-width and with an indistinct rim of duct opening 15 μm wide; totaling 14–21 on the dorsum, with 4 on head, 4 or 5 on thorax and on abdominal segments as follows: II 0–2, III 0–2, IV 0–2, V 2, VI 2, VII 2 and (ii) smaller ducts, each duct 10–15 μm long, 4–5 μm wide at mid-width, with sclerotized area next to duct opening 7.0–7.5 μm wide; scattered throughout on head and thorax, and on abdominal segments as follows: I 12–25, II 12–18, III 14–21, IV 11–21, V 9–13, VI 2–6, VII 25–29, VIII 10–14.

Venter. Setae flagellate, each 12.5–225 μm long, longest setae medially on head. Apical setae of anal lobe each 295–360 μm long. Trilocular pores, each 3–4 μm in diameter, frequent throughout the venter. Minute discoidal pores scattered throughout the venter, generally associated with oral collar tubular ducts, each 2–2.5 μm. Oral collar tubular ducts of two sizes: (i) larger ducts concentrated on body margin (same size those on smaller oral collar tubular ducts on dorsum) (2–5 on each side), and (ii) small ducts, each 10.0–12.5 μm long, 2.5–3.0 μm wide, present on head and thorax, and across abdominal segments as follows: I–III 22–31, IV 7–14, V 12–14, VI 6–12, VII 8–10, VIII + IX 0–2.

Comments. *Anisococcus granarae* Pacheco da Silva & Kaydan sp. n. is most similar to *A. erbi* Williams & Granara de Willink and *A. parasitus* Williams & Granara de Willink in having oral collar tubular ducts of two sizes on the dorsum. However, *A. granarae* can be readily distinguished from *A. erbi* in having: (i) oral collar tubular ducts of two sizes on the venter, and (ii) 16 cerarii on body margins (13 –15), and from *A. parasitus* in having: (i) oral collar tubular ducts of two sizes on the venter (*A. parasitus* has oral collar tubular ducts of only one size), and (ii) ventral oral collar tubular ducts present in rows across medial areas of the abdominal segments (not in rows on *A. parasitius*).

Etymology. This species is named after Dr. Maria Cristina Granara de Willink who carried out the most valuable studies on the systematics and taxonomy of mealybugs in Central and South America.

Host plant. *Diospyros kaki*.

Distribution. Brazil (Bento Gonçalves, Caxias do Sul and Farroupilha, Rio Grande do Sul).

Molecular characterization. No intraspecific variation was observed at COI (35 replicates). No BLAST hit with high similarity (> 95%) was obtained with GenBank.

Genus *Ferrisia* Fullaway

Ferrisia Fullaway, 1923
Ferrisiana Takahashi, 1929

Type species. *Dactylopius virgatus* Cockerell, by monotypy and original designation.

Generic diagnosis (adapted from Kaydan and Gullan 2012). Adult female. Body elongate to oval, 1.3–5.5 mm long, 0.5–3.0 mm wide. Antennae almost always 8-segmented (sometimes 7-segmented in *F. milleri* Kaydan & Gullan and *F. pitcairnia* Kaydan & Gullan). Labium 3-segmented, always longer than wide. Posterior pair of spiracles always larger than anterior spiracles. Circulus quadrate, divided by an intersegmental line. Legs well-developed, with or without translucent pores on hind coxa, femur and tibia; claw without a denticle; tarsal and claw digitules both capitate, claw digitules thicker than tarsal digitules. Posterior ostioles well-developed; anterior ostioles usually more weakly developed than posterior pair, or absent. Anal lobes well developed. Anal ring typically with 6 anal ring setae.

Description. *Dorsum*. With long enlarged ducts, each with the orifice surrounded by a circular sclerotized rim, either containing short setae or with setae just outside border. In living insects, these ducts secrete long glassy filaments typical of the genus. Cerarii confined to anal lobes; each anal lobe usually with 2 enlarged conical setae (more on some specimens of *F. dasylirii* Cockerell and *F. virgata* (Cockerell)) plus an associated cluster of trilocular pores and a few auxiliary setae. Body setae slender and flagellate, bluntly tipped to slightly capitate, and of various sizes. Trilocular pores each 3–5 μm in diameter, often slightly larger (4–5 μm diameter) than ventral trilocular pores (typically 3–5 μm), scattered over the dorsum. Minute discoidal pores on the dorsal submargin of the head at base of antennal segment I, usually in a small tight cluster of 3–8 pores (often difficult to see), and also associated with enlarged tubular ducts (generally present within sclerotized area surrounding duct rim). Enlarged tubular ducts present mostly on body margin and submargin in segmental clusters, but often also present medially and submedially; duct opening of each tubular duct with a sclerotized rim surrounded by a circular sclerotized area bearing 0–3 (generally 1 or 2) minute discoidal pores (appearing as clear areas in the cuticle) and with 1–7 (generally 3–5) blunt-tipped to slightly capitated setae. Oral-collar tubular ducts and multilocular pores absent.

Venter. Body setae slender, blunt-tipped to slightly capitate, and of various sizes. Trilocular pores each 2.5–5.0 μm in diameter, scattered over surface. Minute discoidal pores scattered throughout the venter, almost always associated with ventral oral-collar tubular ducts. Enlarged tubular ducts absent. Oral-collar tubular ducts of one or more sizes, of various lengths and widths, shortest ducts often present in marginal clusters, at least on posterior abdominal segments; ducts on anterior abdomen and margins or submargins of posterior abdomen often associated with a minute discoidal pore (rarely 2 pores), usually appearing as a clear circular to oval area in cuticle. Multilocular disc pores generally present (absent in *F. meridionalis* Williams) on posterior abdominal segments, especially around the vulva.

Key to adult females of *Ferrisia* from the Neotropical Region (adapted from Kaydan and Gullan (2012)). The key includes only species displaying the following combination of features: (i) ventral oral-collar tubular ducts of at least 2 sizes, smaller ducts present singly or in segmental clusters on the body margin, at least on the last 2 or 3 abdominal segments, and (ii) minute discoidal pores in sclerotized area of enlarged tubular ducts, touching the sclerotized rim of the duct opening.

1 Translucent pores absent from hind coxae; each anal lobe with ≥60 trilocular pores; small oral-collar tubular ducts usually in tight segmental clusters on ventral margins of posterior 2 or 3 abdominal segments, distributed 0–7 on each side of segment VI, 6–25 on each side of VII, and 8–21 on each side of VIII ..... ***F. kondoi* Kaydan & Gullan**

– Translucent pores present on each hind coxa, >20 in number; each anal lobe with ≤50 trilocular pores; small oral-collar tubular ducts on ventral margins of posterior 2 or 3 abdominal segments either not forming tight clusters or, if perhaps in clusters, these are small, each segment usually with ≤6 ducts on each side **2**

2 Ventral oral-collar tubular ducts on abdominal submargin (not those in posterior marginal clusters) sometimes with 2 contiguous elliptical to elongate triangular discoidal pores in sclerotized rim of duct (check with 100x objective) ..... ***F. williamsi* Kaydan & Gullan**

– Ventral oral-collar tubular ducts on abdominal submargin (not those in posterior marginal clusters) with a circular discoidal pore in sclerotized rim of duct or on nearby derm in at least some ducts **3**

3 Multilocular disc pores only on abdominal segments VII and VII+IX; 87-99 enlarged tubular ducts present on dorsum; translucent pores on hind legs totaling 16–31 on all segments combined; with 11–15 on each hind coxa; small oral collar tubular ducts on last ventral abdominal segments numbering 1–3 on each side of VII; 0–1 on each side of VIII+IX..... ..... ***Ferrisia kaki* Kaydan & Pacheco da Silva, sp. n.**

– Multilocular disc pores only on abdominal segments VI and VII+IX; 95-113 enlarged tubular ducts on dorsum; translucent pores on hind legs totaling 80–93

on all segments combined; with 22–55 on each hind coxa; small oral collar tubular ducts on last ventral abdominal segments numbering 3–6 on each side of VII; 3–6 on each side of VIII+IX.....***F. cristinae*** **Kaydan & Gullan**

Ferrisia kaki **Kaydan & Pacheco da Silva, sp. n.**
http://zoobank.org/47CFCF98-E37E-40BB-B7DD-634F75242FA1
Fig. 3

Type-locality. Brazil, Caxias do Sul – Rio Grande do Sul, on fruits in persimmon orchards, *Diospyros kaki*, Apr 2015, VC Pacheco da Silva leg.

Type-specimen. *Holotype* female, Brazil, Caxias do Sul – Rio Grande do Sul, on *Diospyros kaki*, on fruits, Apr 2015, coll: VC Pacheco da Silva, MRGC: 2266. *Paratypes:* Brazil, 4 ♀♀ Caxias do Sul – Rio Grande do Sul, on *D. kaki* 'Fuyu', iv.2015, coll: VC Pacheco da Silva and ECW Galzer; 1 ♀ Farroupilha – Rio Grande do Sul, on *D. kaki* 'Kioto', iv.2015, coll: VC Pacheco da Silva and ECW Galzer. ANSES/LSV 1 slide, MBK 3 slides and MRGC 1 slide (2267).

Diagnosis. *Ferrisia kaki* Kaydan & Pacheco da Silva, sp. n. is characterized by the following combination of features: (i) ventral oral-collar tubular ducts of two sizes, smaller ducts present singly or in segmental clusters on the body margin, on the last two or three abdominal segments; (ii) minute discoidal pores on the sclerotized area of enlarged tubular ducts, almost always touching the sclerotized duct rim, and (iii) both anterior and posterior pairs of ostioles present and well-developed.

Description. Adult female.

Appearance in life is unrecorded.

Body oval, 2.76–3.74 mm long, 1.26–1.78 mm wide. Eye marginal, 60–70 μm wide. Antennae 8-segmented, 650–700 μm long, with 4 fleshy setae, each 30–55 μm long; apical segment 125–130 μm long, 35.0–37.5 μm wide, apical setae 35–45 μm long. Clypeo-labral shield 160–195 μm long, 135–195 μm wide. Labium 3-segmented, 205–215 μm long, 115–130 μm wide. Anterior spiracles 70–75 μm long, 35–45 μm wide across atrium; posterior spiracles 75–85 μm long, 50–60 μm wide across atrium. Circulus 125–130 μm wide. Legs well-developed; length of posterior legs: coxa 260–300 μm, trochanter + femur 470–500 μm, tibia + tarsus 520–570 μm, claw 37–43 μm. Ratio of length of tibia + tarsus to trochanter + femur, 1.06–1.19:1; ratio of length of tibia to tarsus, 2.82–3.14:1; ratio of length of hind trochanter + femur to greatest width of femur, 3.91–4.70:1. Translucent pores present on the coxa (11–15), femur (3–7) and tibia (2–8). Tarsal digitules capitate, each 55–60 μm long. Claw digitules capitate, each 32–45 μm. Both pairs of ostioles present; anterior ostioles each with a total for both lips of 29–32 trilocular pores and 10–12 setae; posterior ostioles each with a total for both lips of 12–16 trilocular pores and 3–5 setae. Anal ring 100–110 μm wide, with 6 setae, each setae 170–193 μm long.

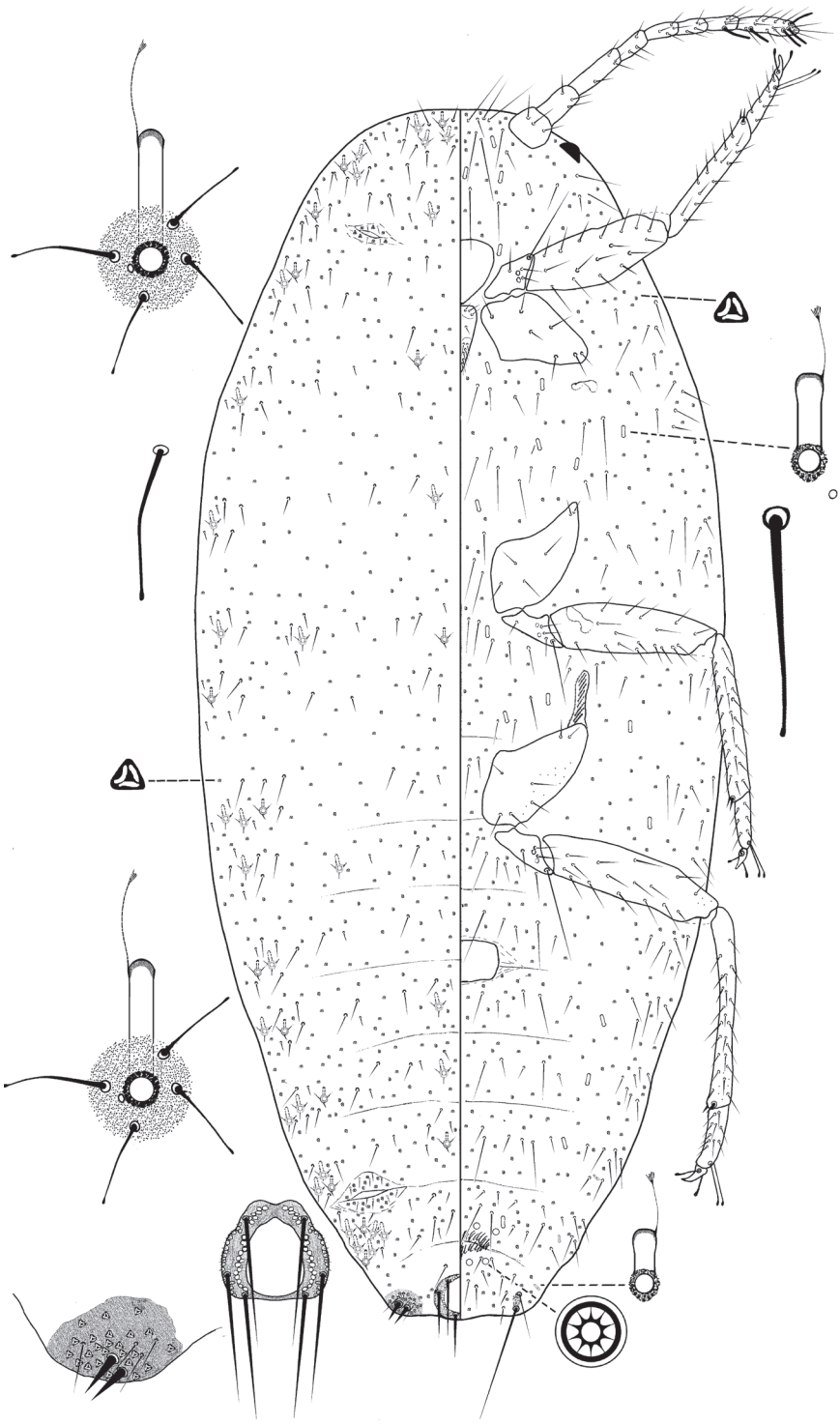


Figure 3. Adult female *Ferrisia kaki* Kaydan & Pacheco da Silva, sp. n. Holotype.

Dorsum. Derm membranous, with only anal lobe pairs of cerarii, each cerarius with 2 cerarian setae, each 30–35 μm long, plus 28–41 trilocular pores between cerarian setae and 3–5 hair-like auxiliary setae. Dorsal body setae hair-like, flagellate, blunt, each 12.5–60.0 μm long, scattered on head and thorax, and in single rows on abdominal segments. Trilocular pores, each 3–4 μm in diameter, scattered over entire body. Minute discoidal pores each 2.0–2.5 μm in diameter, scattered all over body and also associated with enlarged tubular ducts, almost always touching the sclerotized duct rim. Enlarged tubular ducts, each 35.0–42.5 μm long, 6.5–7.5 μm wide at mid-width; rim of duct opening 8–10 μm wide, sclerotized area 20–30 μm wide, bearing 2–7 hair-like setae, each 15–35 μm long; with 87–99 in total, present on head and thorax, and each side of the abdominal segments and also medially on segments IV-VI, numbering as follows: I 1 or 2, II 1 or 2, III 2, IV 1 or 2, V 2 or 3, VI 2 or 3, VII 6–8.

Venter. Setae flagellate, blunt, each 12.5–210 μm long, longest setae medially on head. Apical setae of anal lobe each 280–300 μm long. Multilocular disc pores each 7–9 μm in diameter, in rows on abdominal segments, as follows: VII 2–4, VIII + IX 4. Trilocular pores, each 3–4 μm in diameter, scattered throughout on the venter. Minute discoidal pores, each 2–2.5 μm wide, scattered throughout and associated with oral collar tubular ducts. Oral collar tubular ducts of two sizes, (i) smaller ducts, each 6.5–7.5 μm long, 3 μm width, present on each side of body margin of abdominal segments, as follows: VI 1, VII 1–3, VIII+IX 0–1, and (ii) larger ducts, each14–16 μm long, 3 μm wide, sparse on head and thorax and across abdominal segments, as follows: III 1 or 2, IV 2 or 3, V 2 or 3, VI 2 or 3, VII 2–4, VIII + IX 0.

Comments. *Ferrisia kaki* Kaydan & Pacheco da Silva, sp. n. most closely resembles *F. cristinae* Kaydan & Gullan, in having few ventral oral-collar tubular ducts on the abdominal submargin (not those in posterior marginal clusters), and often with a circular discoidal pore in the sclerotized rim of the duct or on nearby derm. However, *F. kaki* differs from *F. cristinae* in having: (i) multilocular disc pores only on abdominal segments VII and VIII+IX (VI–VIII+IX in *F. cristinae*) and (ii) 87–99 enlarged tubular ducts on the dorsum (95–113 in *F. cristinae*). *F. kaki* is also similar to *F. terani* Williams in having a small number of multilocular disc pores and a slender body shape, but *F. kaki* can be readily distinguished from *F. terani* in having: (i) two sizes of oral collar tubular ducts on the venter (only one size in *F. terani*); (ii) enlarged tubular ducts with a minute discoidal pore touching the sclerotized rim of duct opening.

Etymology. This species was named after its host plant, to reflect the high levels of infestation in persimmon orchards.

Host plant. *Diospyros kaki*.

Distribution. Brazil (Caxias do Sul, Farroupilha, Rio Grande do Sul).

Molecular characterization. No intraspecific variation was observed at COI (7 replicates). A BLAST hit with sequence similarity of 98% was obtained with a sequence assigned to *Ferrisia terani* Williams & Granara de Willink from Pacheco da Silva et al. (2014). The alignment between *F. kaki* and *F. terani* is shown in Figure 4.

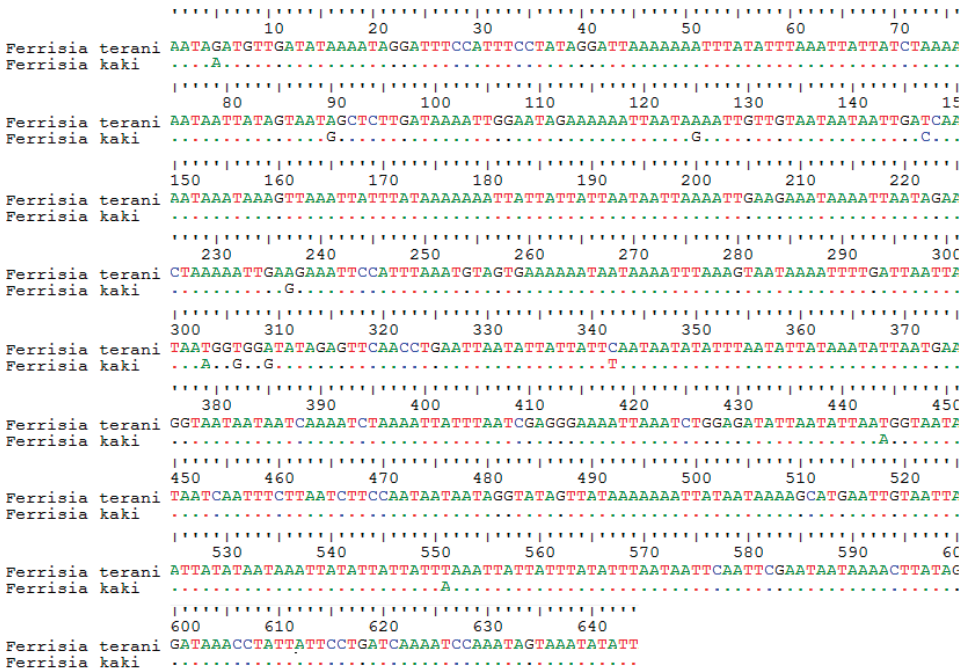


Figure 4. Alignment of the COI DNA sequences obtained for *Ferrisia kaki* Kaydan & Pacheco da Silva, sp. n. and *F. terani* (from Pacheco da Silva et al. 2014). *Ferrisia terani* is used as reference sequence in the alignment and only the differences to this reference are displayed in the sequence of *F. kaki*.

Genus *Pseudococcus* (Westwood)

Pseudococcus (Westwood), 1840
Trechocorys Curtis, 1843
Boisduvalia Signoret, 1875
Oudablis Signoret, 1882

Type species. *Dactylopius longispinus* Targioni Tozzetti.

Generic diagnosis (adapted from Gimpel and Miller 1996; Williams and Granara de Willink 1992). Adult female. Body normally broadly oval, 1.2–4.3 mm long, 0.6–2.6 mm wide. Antennae each normally 8-segmented, occasionally with 7 segments. Labium 3-segmented, always longer than wide. Legs well-developed, claw without a denticle; translucent pores generally present on hind legs, on coxae, and/or femora and/or tibiae, rarely on trochanter; tarsal and claw digitules both capitate, claw digitules thicker than tarsal digitules. Circulus usually present, well-developed and divided by an intersegmental line; rarely small and not divided, usually wider than long. Quinquelocular pores always absent.

Description. *Dorsum*. Dorsal setae flagellate. Anterior and posterior ostioles present, well-developed. Cerarii present, 12–17 pairs, preocular pair always absent, each cerarius normally with two conical setae, except for 1 or 2 on head and thorax, each often with

3 or 4 conical setae plus an associated cluster of trilocular pores; anal lobe cerarii well-developed, each often sclerotized, usually with two enlarged conical setae; usually all cerarii with auxiliary setae, but occasionally auxiliary setae absent anterior to the penultimate pair. Anal ring typically with six setae. Trilocular pores scattered over dorsum. Minute discoidal pores usually present, sometimes situated adjacent to rim of oral rim tubular ducts. Oral rim tubular ducts present on body margins and medially and submedially, or in rows across abdominal segments, sometimes associated with minute discoidal pores and setae. Oral-collar tubular ducts often present. Multilocular pores rarely present on dorsum.

Venter. Body setae flagellate. Trilocular pores scattered over entire surface. Minute discoidal pores scattered throughout the venter, often of two sizes, larger pores frequently present next to eyes and on venter of anal lobes, sometimes also situated adjacent to rim of oral rim tubular ducts. Oral rim tubular ducts occasionally on venter only. Oral-collar tubular ducts of one or more sizes, of various lengths and widths, shortest ducts often present medially on abdominal segments, and larger ducts often present on margins of abdomen. Multilocular disc pores present on posterior abdominal segments, especially around vulva.

Key to adult females of the *Pseudococcus maritimus* complex with multilocular disc pores present on dorsum (adapted from Gimpel and Miller (1996)).

1 Oral collar tubular ducts scattered over dorsum .... ***Pseudococcus rosangelae*** **Pacheco da Silva & Kaydan, sp. n.**

– Oral collar tubular ducts, if present, only on margins or on abdominal segments..... **2**

2 Dorsal multilocular disc pores scarce, restricted to segments V-VII; fewer than 10 ventral multilocular disc pores on head and thorax (in total)..... **3**

– Dorsal multilocular disc pores numerous on segments III-VIII; with more than 20 ventral multilocular disc pores on head and thorax (in total)..... ..... ***P. peregrinabundus*** **Borchsenius**

3 More than 50 translucent pores present on hind tibia; 1–6 large discoidal pores associated with each eye; 137–258 trilocular pores present on segment VI of venter ..... ***P. nakaharai*** **Gimpel & Miller**

– Fewer than 20 translucent pores present on hind tibia; 0–2 small discoidal pore associated with each eye; 42–54 trilocular pores present on segment VI of venter ..... ***P. dasyliriae*** **Gimpel & Miller**

Pseudococcus rosangelae **Pacheco da Silva & Kaydan, sp. n.**
http://zoobank.org/D4E09C38-771A-47A2-BACF-8622154C4726
Fig. 5

Type-locality. Brazil, Farroupilha, Rio Grande do Sul, on fruits in persimmon orchards, *Diospyros kaki*, 15 Apr 2015, VC Pacheco da Silva leg.

Type-specimen. *Holotype* female, Brazil, Farroupilha, Rio Grande do Sul, on *D. kaki*, on fruits, Apr 2015, coll: VC Pacheco da Silva, MRGC: 2262.

Diagnosis. *Pseudococcus rosangelae* Pacheco da Silva & Kaydan, sp. n. is characterized by the following combination of features: (i) multilocular disc pores present on the dorsum, and (ii) dorsal oral collar tubular ducts scattered throughout.

Description. Adult female. Appearance if life is unrecorded.

Body oval, elongate, 2.72 mm long, 1.32 mm wide. Eye marginal, 40 μm wide, each with 3 discoidal pores. Antennae 8-segmented, 560–565 μm long, with 4 fleshy setae, each 25.0–42.5 μm long; apical segment 102.5 μm long, 32.5–35.0 μm wide, with apical setae 45.0–47.5 μm long. Clypeolabral shield 175 μm long, 202.5 μm wide. Labium 3-segmented, 175 μm long, 122.5 μm wide. Anterior spiracles 75–80 μm long, 45 μm wide across atrium; posterior spiracles 82.5–90 μm long, 55–60 μm wide across atrium. Circulus 125 μm long, 135 μm wide. Legs well-developed; lengths for posterior legs: coxa 245.0–252.5 μm, trochanter + femur 405–410 μm, tibia + tarsus 460–475 μm, claw 37.5–40.0 μm. Ratio of length of tibia + tarsus to trochanter + femur, 1.13–1.16:1; ratio of length of tibia to tarsus, 2.80–2.84:1; ratio of length of hind trochanter + femur to greatest width of femur, 3.72–3.85:1; translucent pores absent on legs. Tarsal digitules capitate, each 50–57.5 μm long. Claw digitules capitate, 35 μm long. Both pairs of ostioles present; anterior ostioles each with a total for both lips of 29–32 trilocular pores and 6 setae; posterior ostioles each with a total for both lips of 35–39 trilocular pores and 3–6 setae. Anal ring 72.5 μm wide, with 6 setae, each seta 140.0–167.5 μm long.

Dorsum. Derm membranous, with 17 pairs of cerarii around body margin, each cerarius with 2–4 cerarian setae. Setae on each anal lobe cerarius 25.0–27.5 μm long, 10 μm wide, plus 67–76 trilocular pores and 3–4 spine-like auxiliary setae. Dorsal setae short and flagellate, each 5–20 μm long, scattered throughout the dorsum. Trilocular pores, each 3.7–5.0 μm in diameter, scattered over entire body. A few minute discoidal pores, each 2.5–3.0 μm in diameter, scattered over dorsum. Oral rim tubular ducts, each 12.5 μm long, 3.7 μm wide at mid-width, rim of duct opening 3.7–5.0 μm wide and outer width 7.5–10 μm, seven in total on dorsum, with two ducts on head, two on thorax, and on abdominal segments, as follows: I 2, III 1, IV 1. Oral collar tubular ducts of two sizes: (i) larger ducts, each 5.0 μm long, 3.7–5.0 μm wide, along entire margin of the body; and (ii) smaller ducts, each 5.0–6.2 μm long, 2.5–3.75 μm wide, present throughout the dorsum but in bands on abdominal segments, as follows: I–III 141, IV 88, V 41, VI 32, VII 25, VIII + IX 26. Multilocular disc pores, each 5.0–7.5 μm in diameter, present on abdominal segments, as follows: I–III 6, IV 2, V 6, VI 10, VII 10, VIII + IX 2.

Venter. Setae short and flagellate, each 7.5–145 μm long, longest setae located medially on head. Apical setae of anal lobe each132 μm long. Trilocular pores and minute discoidal pores scattered all over body. Trilocular pores, each 3.7–5.0 μm scattered throughout the venter. Oral collar tubular ducts of 2 sizes: (i) larger ducts, each 5.0–6.3 μm long, 3.7–5.0 μm wide in the margin of the body and throughout, and (ii) smaller oral ducts, each 6.2–7.5 μm long, 2.0–2.5 μm wide, present throughout, and

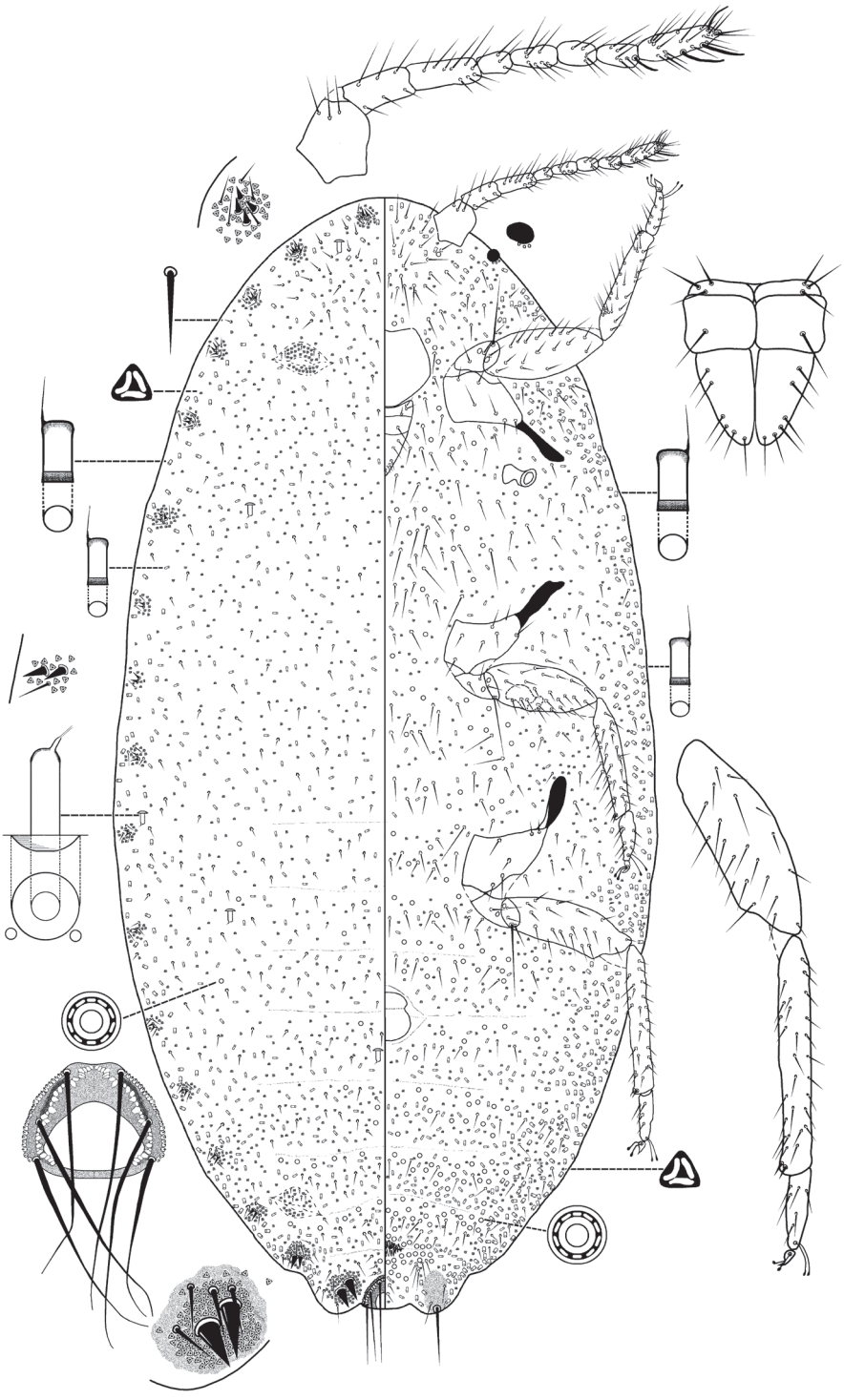


Figure 5. Adult female *Pseudococcus rosangelae* Pacheco da Silva & Kaydan, sp. n. Holotype.

also as bands across abdominal segments, as follows: I–III 110, IV 69, V 81, VI 60, VII 47, VIII + IX 23. Multilocular disc pores, each 5.0–7.5 μm in diameter, present throughout on the venter and on the abdominal segments, as follows: I–III 46, IV 15, V 30, VI 41, VII 29, VIII + IX 21.

Comments. *Pseudococcus rosangelae* Pacheco da Silva & Kaydan most closely resembles *P. peregrinabundus*, *P. nakaharai* and *P. dasyliriae* in having dorsal multilocular disc pores, but *P. rosangelae* can be distinguished from other species in having: (i) oral collar tubular ducts present over the entire dorsum (on other species not scattered all over the dorsum) and (ii) no translucent pores on the hind legs (present in the other species).

Etymology. This species is named after Rosangela Leme do Prado, mother of the author VCPS.

Host plant. *Diospyros kaki*.

Distribution. Brazil (Farroupilha, Rio Grande do Sul).

Molecular characterization. No DNA sequence was obtained for *P. rosangelae*.

Acknowledgment

The first author thanks Ernesto Prado for all his support; Filiz Çalişkan, Alican Kurtuluş and Tair Uulu from Çukurova University and BPI Team from INRA- Institut Sophia Agrobiotech for the help during the internship of VCPS in Turkey and France; to Elisângela Caroline W Galzer for the help during sample collections; and to CNPq for the scholarship of VCPS.

References

Bavaresco A, Botton M, Garcia MS, Nondillo A (2005) Danos e insetos em frutos de caquizeiro em pomares da Serra Gaúcha. Agropecuária Catarinense 18: 56–59.

Camargo-Filho WP, Mazzei AR, Alves HS (2003) Mercado do caqui: Variedades, estacionalidade e preços. Informações Econômicas 33: 81–87. http://www.iea.sp.gov.br/OUT/publicacoes/pdf/seto1-1003.pdf

Casco Mila MN (2012) *Pseudococcus* sp. próximo a *sociabilis* (Hemiptera: Pseudococcidae): Desarrolo estacional y determinación de momentos más apropiados para el control en manzanos y perales. PhD thesis, Universidad de la Repúblic, Montevideo. https://www.colibri.udelar.edu.uy/handle/123456789/1832

Correa MCG, Germain J-F, Malausa T, Zaviezo T (2012) Molecular and morphological characterization of mealybugs (Hemiptera: Pseudococcidae) from Chilean vineyards. Bulletin of Entomological Research 102: 524–530. doi: 10.1017/S0007485312000053

Cox JM, Pearce MJ (1983) Wax produced by dermal pores in three species of mealybug (Homoptera: Pseudococcidae). International Journal of Insect Morphology and Embryology 12: 235–248. doi:10.1016/0020-7322(83)90020-X

Daane KM, Almeida RPP, Bell VA, Walker JTS, Botton M, Fallahzadeh M, Mani M, Miano JL, Sforza R, Walton VM, Zaviezo T (2012) Biology and management of mealybugs in vineyards. In: Bostanian NJ, Vincent C, Isaacs R (Eds) Arthropod Management in Vineyards. Springer Netherlands, Dordrecht, 271–306. doi: 10.1007/978-94-007-4032-7_12

Downie DA, Gullan PJ (2004) Phylogenetic analysis of mealybugs (Hemiptera: Coccoidea: Pseudococcidae) based on DNA sequences from three nuclear genes, and a review of the higher classification. Systematic Entomology 29: 238–259. doi: 10.1111/j.0307-6970.2004.00241.x

Fachinello JC, Pasa MS, Schmitiz JD, Betemps DL (2011) Situação e perspectivas da fruticultura de clima temperado no Brasil. Revista Brasileira de Fruticultura E: 109–120. doi: 10.1590/S0100-29452011000500014

García M, Denno B, Miller DR, Miller GL, Ben-Dov Y, Hardy NB (2016) ScaleNet: A Literature-based model of scale insect biology and systematics. http://www.scalenet.info

Gimpel WF, Miller DR (1996) Systematic analysis of the mealybugs in the *Pseudococcus maritimus* complex (Homoptera: Pseudococcidae). International Contributions on Entomology 2: 1–163.

Gullan PJ, Kaydan MB, Hardy NB (2010) Molecular phylogeny and species recognition in the mealybug genus *Ferrisia* Fullaway (Hemiptera: Pseudococcidae). Systematic Entomology 35: 329–339. doi: 10.1111/j.1365-3113.2009.00513.x

Hardy NB, Gullan PJ, Hodgson CJ (2008) A subfamily-level classification of mealybugs (Hemiptera: Pseudococcidae) based on integrated molecular and morphological data. Systematic Entomology 33: 51–71. doi: 10.1111/j.1365-3113.2007.00408.x

Kaydan MB, Gullan PJ (2012) A taxonomic revision of the mealybug genus *Ferrisia* Fullaway (Hemiptera: Pseudococcidae), with descriptions of eight new species and a new genus. Zootaxa 3543: 1–65. http://www.mapress.com/zootaxa/list/2012/3543.html

Kaydan MB, Kozár F, Hodgson C (2015) A review of the phylogeny of Palaearctic mealybugs (Hemiptera: Coccomorpha: Pseudococcidae). Arthropod Systematics & Phylogeny 73: 175–195. http://www.senckenberg.de/files/content/forschung/publikationen/arthropodsystematics/asp_73_1/09_asp_73_1_kaydan_175-195.pdf

Kosztarab M, Kozár F (1988) Scale Insects of Central Europe. W. Junk Publishers, Dordrecht, 456 pp.

Lewinsohn TM, Prado PI (2005) How many species are there in Brazil? Conservation Biology 19: 619–624. doi: 10.1111/j.1523-1739.2005.00680.x

Malausa T, Fenis A, Warot S, Germain J-F, Ris N, Prado E, Botton M, Vanlerberghe-Masutti F, Sforza R, Couloux A, Kreiter P (2011) DNA markers to disentangle complexes of cryptic taxa in mealybugs (Hemiptera: Pseudococcidae). Journal of Applied Entomology 135: 142–155. doi: 10.1111/j.1439-0418.2009.01495.x

McKenzie HL (1967) Mealybugs of California with Taxonomy, Biology and Control of North American Species (Homoptera: Coccoidea: Pseudococcidae). University of California Press, Berkeley, 526 pp.

Neuwald D, Sestari I, Saquet A (2009) Persimmon production and commercialization in Brazil: An Overview. Acta Horticulturae 833: 51–56. doi: 10.17660/ActaHortic.2009.833.7

Pacheco da Silva VC, Bertin A, Blin A, Germain J-F, Bernardi D, Rignol G, Botton M, Malausa T (2014) Molecular and morphological identification of mealybug species (Hemiptera: Pseudococcidae) in Brazilian vineyards. PLoS ONE 9: 1–7. doi: 10.1371/journal.pone.0103267

Rafael JA, Aguiar AP, Amorim DDS (2009) Knowledge of insect diversity in Brazil: challenges and advances. Neotropical Entomology 38: 565–570. doi: 10.1590/S1519-566X2009000500001

Williams DJ (2004) Mealybugs of Southern Asia. The Natural History Museum, Kuala Lumpur, 896 pp.

Williams DJ, Granara de Willink MC (1992) Mealybugs of Central and South America. CAB International, Wallingford, 635 pp.

Supplementary material 1

COI DNA sequences obtained for *Anisococcus granarae* Pacheco da Silva & Kaydan, sp. n. and *Ferrisia kaki* Kaydan & Pacheco da Silva, sp. n.

Authors: Vitor C. Pacheco da Silva, Mehmet Bora Kaydan, Jean-François Germain, Thibaut Malausa, Marcos Botton

Data type: FASTA file

Explanation note: This supplementary file contais the senquences of a fragment from the mitochondrial region of Cytochrome Oxidase Subunit I of two new species of mealybugs found on persimmon trees in Southern Brazil, *Anisococcus granarae* Pacheco da Silva & Kaydan, sp. n. and *Ferrisia kaki* Kaydan & Pacheco da Silva, sp. n.

ZooKeys 584: 83–94 (2016)
doi: 10.3897/zookeys.584.8129
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Description of three new species of *Arescon* Walker (Hymenoptera, Mymaridae) from China

Xiang-Xiang Jin[1,2,3], Cheng-De Li[4], Jian-Chun Yang[1,2,3]

1 *Guangdong Entomological Institute, Guangzhou, 510260, China* **2** *Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangzhou, 510260, China* **3** *Guangdong Key Laboratory of Integrated Pest Management in Agriculture, Guangzhou, 510260, China* **4** *School of Forestry, Northeast Forestry University, Harbin, 150040, China*

Corresponding author: *Jian-Chun Yang* (yangjc@gdei.gd.cn)

Academic editor: *M. Ohl* | Received 14 February 2016 | Accepted 29 March 2016 | Published 25 April 2016

http://zoobank.org/10972880-6691-49E1-AE4A-BB5E42DBBFF2

Citation: Jin X-X, Li C-D, Yang J-C (2016) Description of three new species of *Arescon* Walker (Hymenoptera, Mymaridae) from China. ZooKeys 584: 83–94. doi: 10.3897/zookeys.584.8129

Abstract

Three new species of *Arescon* Walker, 1846, *A. gaoligongensis* Jin & Li, **sp. n.**, *A. sparsiciliatus* Jin & Li, **sp. n.** and *A. stenopterus* Jin & Li, **sp. n.** are described. A key to the Chinese species is given and photomicrographs are provided to illustrate morphological characters. All the specimens are deposited in the insect collections of Northeast Forestry University, China.

Keywords

Chalcidoidea, Mymaridae, *Arescon*, taxonomy, new species, China

Introduction

Arescon currently contains 22 species according to Noyes (2015). Among them, *A. armata* (Meunier, 1906) and *A. baltica* (Meunier, 1901) are fossils; *A. aspidioticola* (Ashmead, 1879) and *A. peregrina* (Perkins, 1910) are *nomina dubia* according to Schauff (1984) and Beardsley and Huber (2000), respectively. The type material of both species is lost; the Ashmead species likely belongs to Aphelinidae (Schauff 1984) and the Perkins species probably does not belong to *Arescon* but its generic placement within

Mymaridae is uncertain. Since 1990, only one species, *A. zenit* Triapitsyn & Berezovskiy, 2003, has been described as new.

In China, Lin and Xu (2000) keyed *Arescon* in their key to 19 Chinese genera of Mymaridae and briefly summarized its distribution and hosts. Tian (2009) reported *Arescon iridescens* (Enock, 1914) from Hainan Province. In this study, we describe 3 new species and provide a key to the *Arescon* species found in China.

Material and methods

We collected 15 specimens (12 females and 3 males) of *Arescon* in Yunnan Province and Xizang Autonomous Region (= Tibet) by sweeping, Malaise traps (MT) and yellow pan traps (YPT). Specimens were dissected and mounted in Canada balsam on slides following the method described by Noyes (1982) and modified for Mymaridae by Huber (2015). Photographs were taken with a digital CCD camera attached to an Olympus BX51 compound microscope. Most measurements were made from slide-mounted specimens using an eye-piece reticle. Total body length excluding ovipositor was measured with an eye-piece reticle from ethanol-preserved specimens before being dissected. All measurements are given in micrometers (μm). All the specimens listed below are deposited in Northeast Forestry University, Harbin, China (NEFU).

Morphological terminology and abbreviations are adopted from Gibson (1997) and Huber (2012), as follows (with some additions):

Fl_n Flagellar segment
Mps Multiporous plate sensilla

Key to *Arescon* species in China

1 ♀: flagellum clavate, funicle 5-segmented and clava 1-segmented..... 2
– ♂: flagellum filiform, 11-segmented 5
2 Fore wing with venation extending just about half length of wing (Fig. 9); metanotum with dorsellum distinctly triangular (Fig. 8).....***A. stenopterus* sp. n.**
– Fore wing with venation extending about 0.6–0.8× length of wing (Figs 4, 19); metanotum with dorsellum rhomboidal (Figs 3, 18).....**3**
3 Fl_2 longer than fl_3; fore wing with disc densely setose, with at least 6 irregular rows of setae at broadest part of the wing (Fig. 4) **4**
– Fl_2 about as long as or shorter than fl_3 (Fig. 17); fore wing with disc sparsely setose, with at most 3 irregular rows of setae at broadest part of the wing (Fig. 19)..... ***A. sparsiciliatus* sp. n.**
4 Fore wing relatively narrow, length/width 3.9 (Fig. 4); propodeum relatively short, not more than 0.7× length of scutellum (Fig. 3)..... .....***A. gaoligongensis* sp. n.**

– Fore wing relatively broad, length/width 3.2–3.4; propodeum relatively long, about as long as scutellum.....***A. iridescens*** **(Enock)**
5 Fore wing with venation extending just about half length of wing (Fig. 14); metanotum with dorsellum distinctly triangular .....***A. stenopterus*** **sp. n.**
– Fore wing with venation extending just about 0.6–0.8× length of wing (Fig. 25); metanotum with dorsellum rhomboidal (Fig. 24).....**6**
6 Fore wing disc densely setose, with at least 6 irregular rows of setae at broadest part of the wing .....***A. iridescens*** **(Enock)**
– Fore wing disc sparsely setose, with at most 3 irregular rows of setae at broadest part of the wing (Fig. 25)..... ***A. sparsiciliatus*** **sp. n.**

Taxonomy

Arescon gaoligongensis **Jin & Li, sp. n.**
http://zoobank.org/06864F6D-CB04-4258-932B-F0AAE9F17A38
Figs 1–6

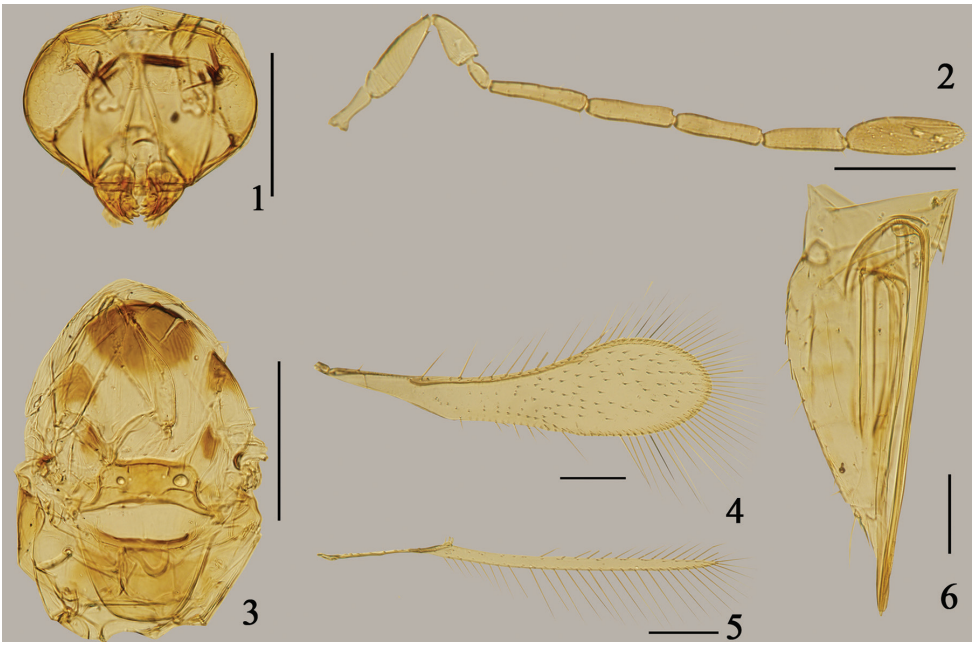
Holotype. ♀ (NEFU) Yunnan Province, Baoshan City, Mt. Gaoligong, Baihualing, 31. VII.2014–2.VIII. 2014, Hui-Lin Han, YPT.

Diagnosis. Clava (Fig. 2) 2.93× as long as wide, longer than scape; metanotum (Fig. 3) with dorsellum rhomboidal; propodeum distinctly shorter than scutellum; phragma broad with posterior margin nearly straight; fore wing (Fig. 4) 3.93× as long as wide, with venation extending 0.7× length of wing; discal setation rather sparse, with about 7 or 8 rows of setae at the broadest part of wing; base of the wing behind submarginal vein asetose; ovipositor (Fig. 6) distinctly exserted, 2.12× as long as metatibia.

Description. Female (Holotype). Body length 756. Head yellowish brown with eye, ocelli, middle part of transverse trabecula, supraorbital trabecula and mandible dark brown. Antenna yellowish brown with radicle, scape, pedicel and fl_1 paler. Mesosoma largely yellowish brown except a large round spot on about anterior two fifths and two relatively small spots on lateral margins of mesoscutum, a small spot on each axilla anteriorly, dark brown. Wings slightly infuscated, with venation brown. Legs brown with basal parts of coxae, apical parts of femora and last tarsal segments paler. Metasoma pale brown with exerted part of ovipositor darker.

Head. Head (Fig. 1) width 168. Vertex and face with faint reticulate sculpture.

Antenna. Antenna (Fig. 2) sparsely setose. Radicle 0.46× as long as scape; scape about 3.5× as long as wide, with distinct striations which are more or less transverse on base and gradually become oblique distad; pedicel with faint longitudinal striations, about 2× as long as wide, and 2× as long as fl_1; all funicular segments much longer than wide, fl_1 distinctly shortest, without mps; fl_2 slightly longer than fl_3, with 1 mps; fl_3 about as long as fl_4 each with 2 mps; fl_5 slightly shorter than fl_4, with 2 mps; clava 2.93× as long as wide, longer than scape, shorter than fl_4 and fl_5 combined, divided



Figures 1–6. *Arescon gaoligongensis* sp. n., holotype female: **1** head, dorsal **2** antenna **3** mesosoma, dorsal **4** fore wing **5** hind wing **6** gaster, lateral. Scale bars: 100 μm.

into 3 segments ventrally by 2 incomplete oblique septa, with 6 mps. Measurements (length/width): radicle 38, scape 84/24, pedicel 48/24, fl_1 24/13, fl_2 82/17, fl_3 72/17, fl_4 72/17, fl_5 67/19, clava 98/34.

Mesosoma. Mesosoma (Fig. 3) length 277. Pronotum entire, with faint longitudinal striations. Mesoscutum with longitudinal reticulate sculpture on mid lobe and isodiametric reticulate sculpture on lateral lobes. Scutellum transverse, distinctly shorter than mesoscutum (30: 51); anterior scutellum (14: 33) subrectangular, with campaniform sensilla a little nearer to lateral margin than to each other. Metanotum with dorsellum rhomboidal. Propodeum smooth, distinctly shorter than scutellum. Phragma broad with posterior margin nearly straight.

Wings. Fore wing (Fig. 4) length 584, width 149, length/width 3.93, with venation extending 0.7× length of wing; longest marginal setae 152, 1.02× as long as greatest wing width. Fore wing base behind submarginal vein without setae, disc behind basal half of marginal vein with 2 or 3 irregular rows of setae, remaining disc distal to middle of marginal vein with 7 or 8 irregular rows of setae and a bare strip present along about distal one third of posterior margin. Hind wing (Fig. 5) length 545, width 17, length/width 32.4, longest marginal setae 101, about 6× as long as greatest wing width.

Metasoma. Metasoma (Fig. 6) distinctly longer than mesosoma. Petiole short. Gaster (376) with ovipositor length 495, distinctly exserted, 2.12× as long as metatibia (233).

Host. Unknown.

Etymology. The specific name is derived from the name of the collection locality of the type species.

Comments. *Arescon gaoligongensis* sp. n. is similar to *A. iridescens*, but can be distinguished from it by the key given above. The new species is also similar to *A. enocki* (Subba Rao & Kaur) in relatively longer fore wing venation and fore wing disc setation, but can be distinguished from it by the relatively shorter clava, 2.9× as long as wide, shorter than fl_4 and fl_5 combined (clava relatively longer, 4.0× as long as wide, much longer than fl_4 and fl_5 combined in *A. enocki*); broader fore wing, 3.9× as long as wide (much narrower, 4.5× as long as wide in *A. enocki*); and the ovipositor characters, ovipositor originated from base of gaster, distinctly exserted (ovipositor originated from distal part of gaster, and slightly exserted in *A. enocki*).

***Arescon stenopterus* Jin & Li, sp. n.**
http://zoobank.org/15741396-703A-4F7D-9DF8-DB83FED7B4A6
Figs 7–16

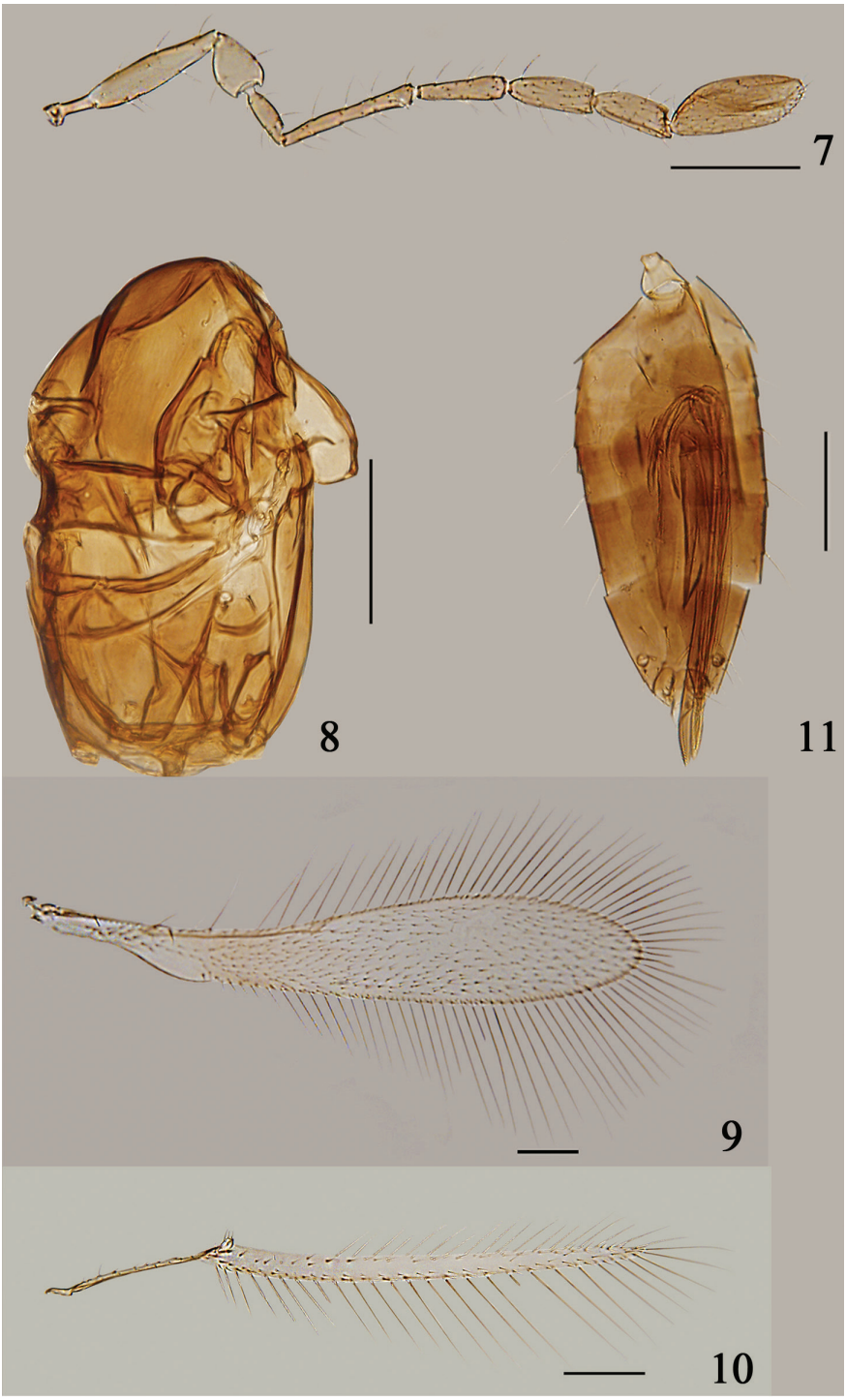
Holotype. ♀ (NEFU) Xizang Autonomous Region (= Tibet), Mt. Sejila, 30.VII. 2013–01.VIII. 2013, Hui-Lin Han, Zhi-Guang Wu, YPT.

Paratypes. 6 females, 1 male. Xizang Autonomous Region (= Tibet): same data as holotype (1♀, NEFU); Linzhi City, 28.VII. 2012–04.VIII. 2012, Zhao-Hui Pan, MT (2 ♀♀, NEFU); Mt. Sejila, 27. VII. 2013, Hui-Lin Han, Zhi-Guang Wu, YPT (1♀, NEFU); Mt. Sejila, 4100 m, 22. VIII. 2014–23. VIII. 2014, Hui-Lin Han, YPT (2 ♀♀ 1♂, NEFU).

Diagnosis. Antenna (Fig. 7) of female with fl_2 distinctly longer than each of fl_3–fl_5; clava 2.2–2.6× as long as wide, slightly shorter than scape; metanotum (Fig. 8) with dorsellum triangular; propodeum longer than scutellum; phragma broad with posterior margin nearly straight; fore wing (Fig. 9) 5.05–5.35× as long as wide, with venation extending just about half wing length; fore wing base behind submarginal vein with 2 or 3 rows of setae, with a small oval bare area behind the basal part of submarginal vein along posterior margin, and disc at the broadest part of wing with about 12 or 13 irregular rows of setae; ovipositor (Fig. 11) 1.03–1.19× as long as metatibia, distinctly exserted.

Description. Female (holotype data in square brackets). Body length 730–980 [780]. Head brown with eye, ocelli, transverse trabecula and part of supraorbital trabecula dark brown. Antenna brown with radicle, scape and pedicel paler. Mesosoma brown with frenum pale yellowish brown. Wings infuscate with base of fore wing brown and largely infuscate behind marginal vein. Legs brown with trochanters and apical parts of femora paler. Metasoma brown with petiole pale yellowish brown and base of gaster and tip of ovipositor pale brown.

Head. Vertex weakly sculptured, ocelli on an almost rectangular stemmaticum; face with faint sculpture.



Figures 7–11. *Arescon stenopterus* sp. n., holotype female: **7** antenna **8** mesosoma, lateral **9** fore wing **10** hind wing **11** gaster, lateral. Scale bars: 100 μm.

Antenna. Antenna (Fig. 7) sparsely setose. Radicle 0.31–0.35 [0.35]× as long as scape; scape with faint longitudinal striations, 4.2–4.9 [4.3]× as long as wide; pedicel with faint longitudinal striations, slightly shorter than fl_1; all funicular segments much longer than wide, fl_1–fl_3 without mps; fl_1 distinctly shortest, fl_2 distinctly longest, more than twice length of fl_1; fl_3–fl_5 slightly shorter and wider distad; fl_4 with 1 mps; fl_5 with 2 mps; clava 2.2–2.6 [2.6]× as long as wide, slightly shorter than scape, shorter than fl_4 and fl_5 combined, with 6 mps. Measurements (length/width): radicle 36–48 [38], scape 108–144/20–31 [113/26], pedicel 48–60/34–60 [50/38], fl_1 46–58/14–19 [46/17], fl_2 91–144/14–17 [110/14], fl_3 60–77/17–22 [70/19], fl_4 60–82/20–24 [67/26], fl_5 58–72/22–26 [65/26], clava 103–118/43–53 [110/43].

Mesosoma. Mesosoma (Fig. 8) with faint reticulate sculpture. Scutellum distinctly shorter than mesoscutum (57: 84); anterior scutellum subrectangular, with campaniform sensilla a little nearer to lateral margins than to each other. Metanotum with dorsellum distinctly triangular. Propodeum smooth, longer than scutellum medially. Phragma broad, with posterior margin nearly straight.

Wings. Fore wing (Fig. 9) length 950–1232 [1000], width 168–244 [188], length/width 5.05–5.35 [5.30], with venation extending about 0.46× length of wing; longest marginal setae 242–300 [242], 1.05–1.34 [1.29]× as long as greatest wing width. Fore wing base behind submarginal vein with 2 or 3 rows of setae, with a small oval bare area behind basal part of submarginal vein along posterior margin; disc at broadest part of wing with 12 or 13 irregular rows of setae. Hind wing (Fig. 10) length 718–990 [750], width 26–43 [33], length/width 23–26 [23], longest marginal setae 182–212 [200], about 6–7 [6]× as long as greatest wing width.

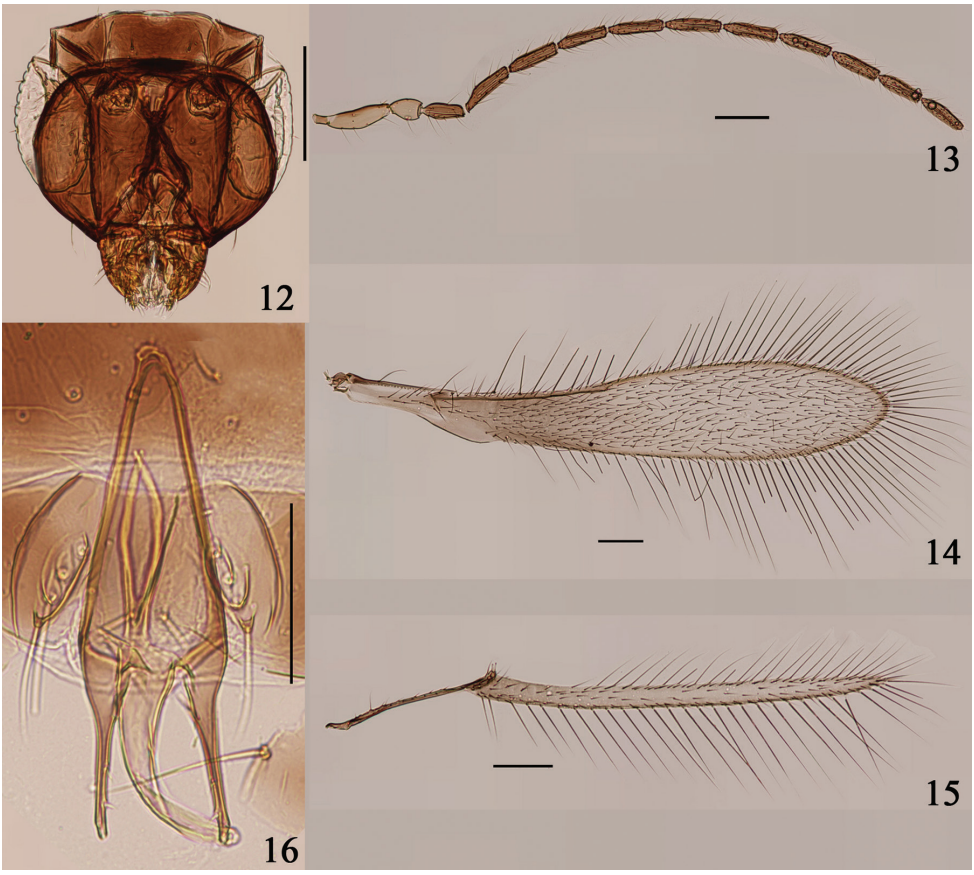
Metasoma. Metasoma (Fig. 11) distinctly longer than mesosoma. Petiole short, trapezoidal. Ovipositor length 300–410[320], distinctly exserted, 1.03–1.19 [1.16]× as long as metatibia (260–400 [275]).

Male. Head (Fig. 12) width 211. Antenna as in Fig. 13. Measurements (length): scape 115, pedicel 55, fl_1 74, fl_2 103, fl_3 96, fl_4 91, fl_5 98, fl_6 101, fl_7 98, fl_8 98. Fore wing (Fig. 14) length 1175, width 210, length/width 5.6, longest marginal setae 260, 1.24× as long as greatest wing width. Hind wing (Fig. 15) length 900, width 36, length/width 25, longest marginal setae 216, 6× as long as greatest wing width. Genitalia (Fig. 16) length 154.

Host. Unknown.

Etymology. From Greek, stenos meaning narrow and pteron meaning wing. The specific name refers to the relatively narrow fore wing.

Comments. *Arescon stenopterus* sp. n., is similar to *A. dimidiatus* (Curtis) in that the fore wing has the venation extending just about half of the wing length and the dorsellum is distinctly triangular, but it can be distinguished from *A. dimidiatus* by the relatively longer fl_3, much longer than fl_1 (about as long as or slightly longer than fl_1 in *A. dimidiatus*); relatively shorter clava, distinctly shorter than fl_4 and fl_5 combined (slightly longer than fl_4 and fl_5 combined in *A. dimidiatus*); and the dimensions of fore wing length and width, 5.05–5.35× as long as wide (6.5× as long as wide in *A. dimidiatus*).



Figures 12–16. *Arescon stenopterus* sp. n., paratype male: **12** head, dorsal **13** antenna **14** fore wing **15** hind wing **16** genitalia. Scale bars: 100 μm.

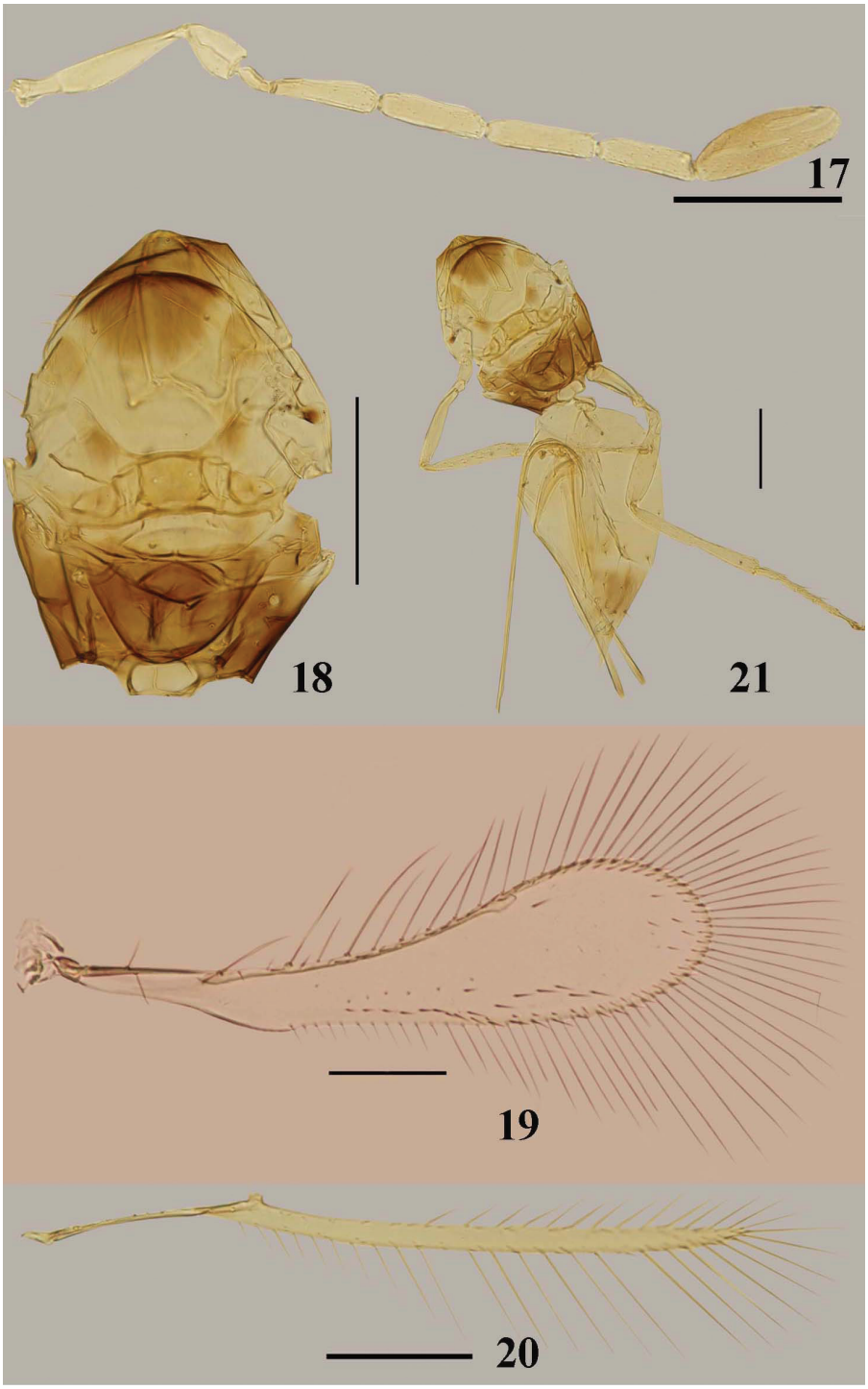
Arescon sparsiciliatus Jin & Li, sp. n.

http://zoobank.org/40F855C9-3379-47C5-B7B4-3AA4C677BBD3
Figs 17–27

Holotype. ♀ (NEFU) Yunnan Province, Ruili City, Mengxiu County, 26–27.IV.2013, Xiang-Xiang Jin, Hui-Lin Han, Guo-Hao Zu, Chao Zhang, YPT.

Paratypes. 5 females, 2 males. Yunnan Province: Longchuan County, 26–27.IV.2013, Xiang-Xiang Jin, Hui-Lin Han, Guo-Hao Zu, Chao Zhang, YPT (3♀♀ 1♂, NEFU); Puer City, Lancang County, 19–20.IV.2013, Xiang-Xiang Jin, Hui-Lin Han, Guo-Hao Zu, Chao Zhang, YPT (1♀, NEFU); Mengla County, Menglun Town, 13.II.2014, Hui-Lin Han, Guo-Hao Zu, Zhong-Ping Xiong, sweeping (1♀ 1♂, NEFU).

Diagnosis. Antenna (Fig. 17) of female with fl_2–fl_5 almost subequal in length; clava 2.67–3.29× as long as wide, shorter than fl_4 and fl_5 combined; metanotum (Fig. 18) with dorsellum rhomboidal; propodeum shorter than scutellum; phragma with



Figures 17–21. *Arescon sparsiciliatus* sp. n., holotype female: **17** antenna **18** mesosoma, dorsal **19** fore wing **20** hind wing **21** body, dorsal. Scale bars: 100 μm.

posterior margin narrowly rounded; fore wing (Fig. 19) 3.94–4.10× as long as wide, with venation extending about 0.7× length of wing; disc nearly asetose, only with a line along apical and posterior margins of wing, 1 or 2 irregular rows of setae near posterior margin and several scattered setae distally; ovipositor (Fig. 21) about 1.6–1.9× as long as metatibia, distinctly exserted.

Description. Female (holotype data in square brackets). Body length 640–700 [655]. Head dark yellowish brown with eyes, ocelli, and transverse trabecula black; mandible brown. Antenna except clava pale brown, clava brown. Mesosoma mostly yellow with middle part of pronotum, about anterior half of mesoscutum except laterally, a small spot on tegula, middle part of metanotum, and propodeum largely except anterior lateral corner, dark brown; anterior internal part of axilla and anterior scutellum pale brown to yellowish brown. Wings uniformly infuscate with venation brown. Legs pale brown with tips of apical tarsomere of all legs brown. Metasoma pale brown with tip of gaster brown.

Head. Vertex and face with faint reticulate sculpture.

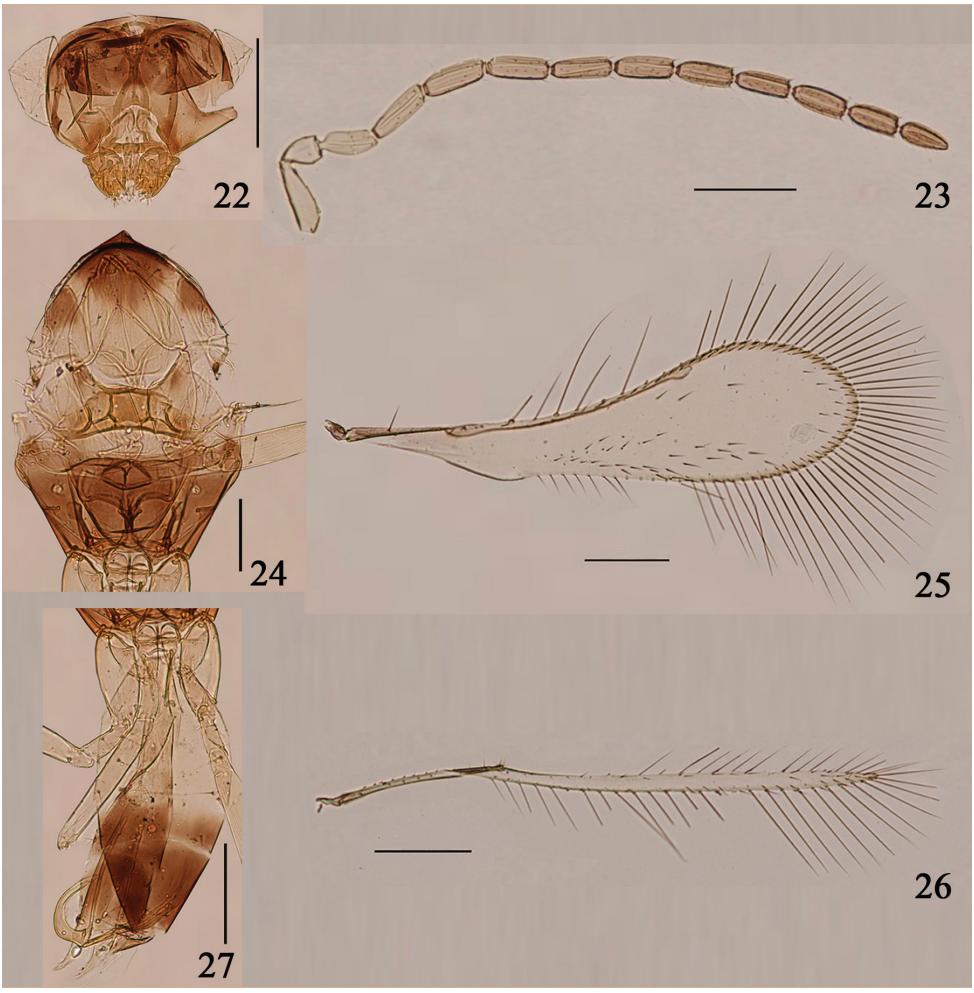
Antenna. Antenna (Fig. 17) sparsely setose. Radicle 0.24–0.31 [0.31]× as long as scape; scape with faint longitudinal striations, 3.63–4.67 [4.42]× as long as wide; pedicel with faint longitudinal striations, 1.6–1.9 [1.6]× as long as wide, longer than fl_1; all funicular segments much longer than wide, fl_1 distinctly shortest, without mps; fl_2–fl_5 each with 2 mps; fl_2 slightly shorter than fl_3; fl_3 about as long as fl_4, slightly longer than fl_5; clava 2.67–3.29 [2.76]× as long as wide, slightly longer than scape, shorter than fl_4 and fl_5 combined, with 7 mps. Measurements (length/width): radicle 24–29 [26], scape 84–106/19–28 [84/19], pedicel 38–46/24–26 [38/24], fl_1 19–24/10 [24/10], fl_2 50–77/17 [62/17], fl_3 62–72/17 [65/17], fl_4 65–74/17 [65/17], fl_5 57–67/18 [60/18], clava 91–110/29–36 [91/33].

Mesosoma (Fig. 18). Mesoscutum longitudinally striate. Scutellum with faint reticulate sculpture distinctly shorter than mesoscutum (27: 45), with campaniform sensilla much nearer to lateral margins than to each other. Metanotum with dorsellum rhomboidal. Propodeum smooth, shorter than scutellum. Phragma with posterior margin narrowly rounded.

Wings. Fore wing (Fig. 19) length 535–560 [535], width 130–142 [135], length/width 3.94–4.10 [3.96], with venation extending 0.7× length of wing; longest marginal setae 144–175 [175], 1.06–1.30 [1.30]× as long as greatest wing width. Discal setation very sparse, only with a line along distal and posterior margins of wing, 1 or 2 irregular rows along near posterior margin, about 5–8 setae scattered on the distal part of wing and sometimes 1–5 seta(e) near stigmal vein. Hind wing (Fig. 20) length 475–530 [475], width 19, length/width 25–28 [25], longest marginal setae 101–119 [119], 5.3–6.3 [6.3]× as long as greatest wing width.

Metasoma. Metasoma (Fig. 21) distinctly longer than mesosoma. Petiole transverse. Ovipositor (340–400 [355]) about 1.6–1.9 [1.8]× as long as metatibia (194–204 [203]), distinctly exserted.

Male. Body length 640. Antenna (Fig. 23). Measurements (length): scape 74, pedicel 36, fl_1 48, fl_2 62, fl_3 60, fl_4 58, fl_5 58, fl_6 55, fl_7 55, fl_8 53, fl_9 53, fl_8 50, fl_9 48.



Figures 22–27. *Arescon sparsiciliatus* sp. n., paratype male: **22** head, dorsal **23** antenna **24** mesosoma, dorsal **25** fore wing **26** hind wing **27** gaster, lateral. Scale bars: 100μm.

Fore wing (Fig. 25) length 600, width 158, length/width 3.8, longest marginal setae 166, 1.05× as long as greatest wing width. Hind wing (Fig. 26) length 550, width 19, length/width 29, longest marginal setae 110, 5.79× as long as greatest wing width.

Comments. *Arescon sparsiciliatus* sp. n. is similar to *A. zenit* in that fore wing venation extends almost 3/4 of the wing length and fl_2–fl_5 are almost subequal in length, but can be distinguished from *A. zenit* by the relatively more sparsely setose fore wing (more densely setose in *A. zenit*); relatively wider fore wing, at most 4.1× as long as wide (about 6.7× as long as wide in *A. zenit*); the longest marginal setae relatively shorter, at most 1.3× greatest wing width (over 2× greatest wing width in *A. zenit*).

Acknowledgements

We are grateful to Dr. J. T. Huber, Canadian Forest Service, Ottawa, Canada, for providing the reference of Beardsley & Huber (2000) reference, and Dr. D. Chesters, Institute of Zoology, Chinese Academy of Sciences, Beijing, P. R. China, for correction of English.

References

Beardsley JW, Huber JT (2000) Key to genera of Mymaridae from the Hawaiian Islands, with notes on some of the species (Hymenoptera: Mymaridae). Proceedings of the Hawaiian Entomological Society 34: 1–22.

Gibson GAP (1997) Morphology and terminology. In: Gibson GAP, Huber JT, Woolley JB (Eds) Annotated keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). NRC Research Press, Ottawa, 16–44.

Huber JT (2015) World reclassification of the *Gonatocerus* group of genera (Hymenoptera: Mymaridae). Zootaxa 3967(1): 1–184. doi: 10.11646/zootaxa.3967.1.1

Huber JT (2012) Revision of *Ooctonus* (Hymenoptera: Mymaridae) in the Nearctic region. Journal of the Entomological Society of Ontario 143: 15–105.

Lin NQ, Xu M (2000) Key to genera of Mymaridae (Hym.: Chalcidoidea) known from China. Journal of Fujian Agricultural University 29(1): 43–49.

Meunier F (1901) Contribution à la faune des Mymaridae ou 'atomes ailés' de l'ambre. Annales de la Société Scientifique de Bruxelles 25: 282–292.

Meunier F (1906) Sur deux Mymaridae de l'ambre de la Baltique. Miscellanea Entomologica, Paris 13: 1–4.

Noyes JS (1982) Collecting and preserving chalcid wasps (Hymenoptera: Chalcidoidea). Journal of Natural History 16: 315–334. doi: 10.1080/00222938200770261

Noyes JS (2015) Universal Chalcidoidea Database. World Wide Web electronic publication. http://www.nhm.ac.uk/chalcidoids

Schauff ME (1984) The holarctic genera of Mymaridae (Hymenoptera: Chalcidoidea). Memoirs of the Entomological Society of Washington 12: 1–67.

Subba Rao BR, Kaur RB (1959) Studies on Indian Mymaridae - part I. Proceedings of the Indian Academy of Sciences (B) 49: 227–238.

Tian HX (2009) Systematic studies on Trichogrammatidae and Mymaridae from Hainan (Hymenoptera: Chalcidoidea). PhD thesis, Fujian Agriculture & Forestry University, China, Fuzhou.

Triapitsyn SV, Berezovskiy VV (2003) Review of the Mymaridae (Hymenoptera, Chalcidoidea) of Primorskii Krai: genera *Arescon* Walker and *Dicopomorpha* Ogloblin. Far Eastern Entomologist 124: 1–15.

Triapitsyn SV, Berezovskiy VV (2004) Review of the genus *Litus* Haliday, 1833 in the holarctic and oriental regions, with notes on the Palaearctic species of *Arescon* Walker, 1846 (Hymenoptera: Mymaridae). Far Eastern Entomologist 141: 1–24.

ZooKeys 584: 95–107 (2016)
doi: 10.3897/zookeys.584.8319
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Keylimepie peckorum gen. n. and sp. n., (Hymenoptera, Braconidae) from southern Florida, U.S., the first known brachypterous member of the subfamily Microgastrinae

Jose Fernandez-Triana[1], Caroline Boudreault[1]

1 *Canadian National Collection of Insects, 960 Carling Ave., Ottawa, ON K1A 0C6 Canada*

Corresponding author: *Jose Fernandez-Triana* (jose.fernandez@agr.gc.ca)

Academic editor: *K. van Achterberg* | Received 1 March 2016 | Accepted 31 March 2016 | Published 26 April 2016

http://zoobank.org/0D009063-FF44-484B-AEF4-03DF98367E72

Citation: Fernández-Triana J, Boudreault C (2016) *Keylimepie peckorum* gen. n. and sp. n., (Hymenoptera, Braconidae) from southern Florida, U.S., the first known brachypterous member of the subfamily Microgastrinae. ZooKeys 584: 95–107. doi: 10.3897/zookeys.584.8319

Abstract

Keylimepie peckorum Fernandez-Triana, **gen. n.** and **sp. n.**, are described from southern Florida, U.S. Females have the shortest wings (0.6–0.7 × body length) of any known microgastrine wasp. The genus can also be recognized on features of the head, propodeum and first three metasomal tergites. All specimens were collected in hammock forests of the Florida Keys and Everglades National Park, but their host caterpillar is unknown. Because its morphology is unique and it is the first new microgastrine genus discovered in North America since 1985, the potential for future conservation of the species is discussed.

Keywords

Microgastrinae, new genus, North America, southern Florida, *Keylimepie*, brachyptery, conservation

Introduction

Microgastrinae (Hymenoptera, Braconidae) is a hyperdiverse and challenging group of parasitoid wasps, with almost 2,700 described species (Yu et al. 2012, Quicke 2015, Fernandez-Triana and Ward 2016). This subfamily is most diverse in tropi-

cal areas, where thousands of species and several new genera remain undescribed. The Nearctic fauna is better studied, although it is far from being completely inventoried, with numerous new species awaiting discovery. The last two new genera of Microgastrinae described from North America were *Pelicope* (Mason, 1981) and *Lathrapanteles* (Williams, 1985), more than 30 years ago. The finding of a new genus in this region is unexpected. As part of ongoing studies on the diversity of Microgastrinae in Florida, a species with unusually short wings was discovered among samples from the Florida Keys and Everglades National Park. They represent the first known brachypterous species of Microgastrinae, and the first new genus found in North America since 1985.

Methods

All 60 specimens studied for this paper were found among unsorted Microgastrinae in the Canadian National Collection of Insects (CNC).

Morphological terms and measurements of structures are mostly as in Mason (1981), Huber and Sharkey (1993), Whitfield (1997), Karlsson and Ronquist (2012), and Fernández-Triana et al. (2014). Mediotergites 1, 2, and so on, are abbreviated as T1, T2, etc.; ocular ocellar line as OOL and posterior ocellar line as POL.

Photos were taken using a Canon EOS 60D with MPE-65 lenses (aperture: 4.0, ISO: 100, CR2 format images) and a 600EX-RT Speedlight (manual) flash. Multiple images through the focal plane were taken of a structure, and these were combined to produce a single in-focus image with Zerene Stacker (http://zerenesystems.com/cms/stacker). Plates for the illustrations were prepared using Adobe Photoshop CS4.

A map with the distribution of all species was generated using SimpleMappr (Shorthouse 2010).

Results

***Keylimepie* Fernandez-Triana, gen. n.**
http://zoobank.org/B1AC9A3C-1368-42AF-9A34-9DDC72867C80

Type species. *Keylimepie peckorum* Fernandez-Triana new species, by present designation.

Diagnosis. Female. Fore wings (0.6–0.7 × body length), shorter than any other species of Microgastrinae (where fore wings are 0.9–1.2 × body length); malar distance more than 2.0 × mandibular width; eye and ocelli small. Both sexes. Propodeum sculpture with a complex pattern that includes partial transverse and longitudinal carinae and a posteriorly defined areola; T1 without median sulcus; T1–T3 with strong longitudinal sculpture.

Description. Female. Head heavily punctured, in lateral view strongly projecting forward below the antennal sockets. Malar distance more than 2.0 × mandibular

width. Anterior tentorial pits very large compared to clypeus width. Eyes and ocelli small. Pronotum with one broad, transverse pronotal sulcus. Propleuron flange sharply defined by carina. Notauli faint, almost invisible. Propodeum with complex sculpture pattern, including partial median longitudinal carina (sometimes obscured by other sculpture), traces of transverse carina, and partial areola with only the posterior carina defined. Fore wing short and narrow, brachypterous (fore wing length 0.6-0.7 × body length, and 1.1–1.3 × metasoma length), with small to almost obliterated quadrangular areolet. Metacoxa of moderate size, not surpassing posterior margin of T2. Inner and outer spurs of metatibia subequal and less than half length of metatarsal segment 1. T1 relatively short, more or less parallel-sided in anterior 0.5, then narrowing towards posterior margin, without median sulcus, with anterior 0.5 rather depressed and concave, and posterior 0.5 with strong transversal striation. T2 trapezoidal. T2 and T3 with strong longitudinal striations, T4–T8 smooth. Hypopygium small, inflexible and unfolded. Ovipositor sheaths mostly smooth, with a few, short setae posteriorly. Ovipositor very short, 0.2 × as long as metatibia. Male as in female but not brachypterous, antenna uniformly brown and body color much darker.

Distribution. Southern Florida: four Florida Keys and a single locality in Everglades National Park (Fig 18).

Hosts and habitat. Hosts are unknown. All specimens were collected in hammock forests from February to November.

Putative autapomorphies. Propodeum carination pattern, shape and sculpture of T1 (without median sulcus), sculpture of T2 and T3, reduced eyes, long malar space, and, in females, short fore wing are treated tentatively as apomorphies.

Etymology. Named after the Key Lime Pie, a typical dessert originated in the Florida Keys –the same area where the new genus was collected. The name not only honors a significant component of the culinary culture in southern Florida, but is also intended to bring attention to and promote conservation efforts for the habitats where the wasps occur. The gender of the genus name is neuter.

Comments. The relationships of *Keylimepie* with other genera of Microgastrinae are hard to assess at present, especially because there are no molecular data available, and nothing is known about potential hosts. Several morphological characters, e.g., head shape and sculpture, mesosoma sculpture, shape and sculpture of T2, and ovipositor, suggest it is related to some species-groups of *Diolcogaster*, part of the Cotesini tribe (sensu Mason 1981). That may be the best placement for the time being and it is the one we favour here. However, several other characters are different from the traditional Cotesini, e.g., the relatively short metacoxa and short metatibial spurs, shape of T1 (and lack of median sulcus), and carination pattern of propodeum. Regardless of affinities to other Microgastrinae genera, *Keylimepie* is a distinctive genus.

The reduced wings in females, relatively small eyes and long malar space present interesting evolutionary questions. More study of additional species of Microgastrinae worldwide would be needed before it can be established if those characters truly are autapomorphies of *Keylimepie* or just an adaptation to local conditions (see Discussion below).

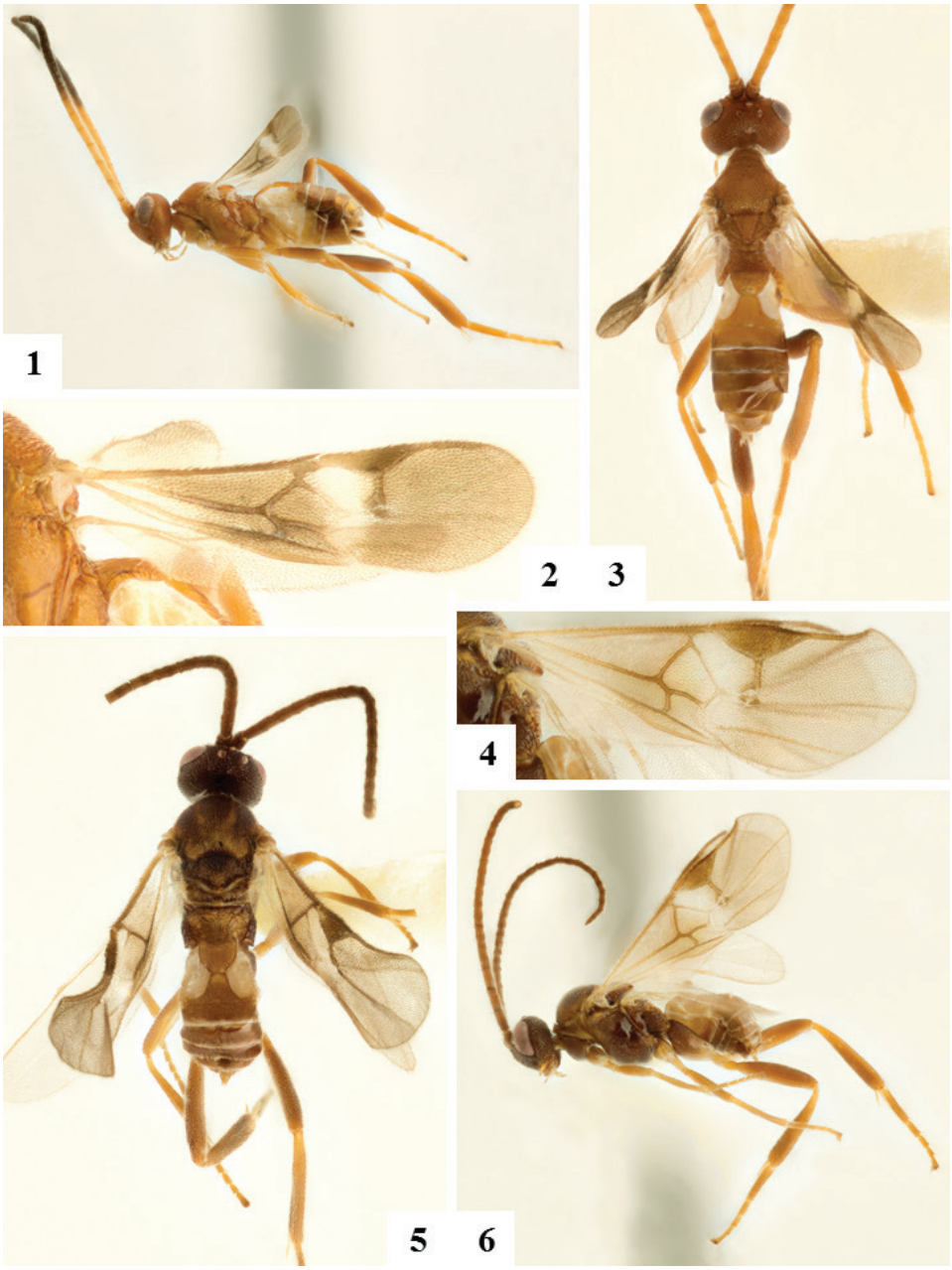
Keylimepie peckorum **Fernandez-Triana, sp. n.**
http://zoobank.org/BFD41F88-9B9F-4088-94D4-200BB7FE9BC1
Figs 1–17

Holotype. Female, CNC. UNITED STATES, Florida, Monroe County, North Key Largo, Sec., 25°17'23"N, 80°18'25"W, 3.IV.1985, Malaise trap, S. & J. Peck (colls). Holotype voucher code: CNC483649.

Paratypes. 3 ♀, 56 ♂ (CNC). United States, Florida, Monroe County. All specimens collected by S. & J. Peck in the following localities and dates: Big Pine Key, Watson's Hammock, 24.716667 -81.304167, 28.viii.1986, voucher code CNC483646; Everglades National Park, Royal Palm Hammock, 25.381667 -80.609722, Malaise trap, 3.iv.1985, coll., voucher codes CNC483647, CNC483648; Fat Deer Key, 24.735344 -81.011853, 18.x.1985-25.ii.1986, voucher codes CNC483464, CNC483465, CNC483468; Malaise trap, 18.xi.1985-25.ii.1986, voucher codes CNC489922, CNC489923, CNC489928, CNC489929, CNC489931–CNC489933, CNC489937, CNC489938, CNC489940, CNC489941, CNC489944, CNC489945, CNC489947; 2.viii-16.xi.1985, voucher codes CNC489955, CNC489959, CNC489962, CNC489965; 24.ii-4.vi.1985, voucher codes CNC483624, CNC483625; 3.iv.1985, voucher codes CNC483627, CNC483630, CNC483634–CNC483636; 4.v-4.viii.1985, voucher codes CNC489874, CNC489876, CNC489877; Malaise trap, iii.1985, voucher codes CNC483626, CNC483628, CNC483629, CNC483631–CNC483633; Fat Deer Key, 24.735344 -81.011853, Hammock, Malaise trap, 24.ii-4.vi.1986, voucher code CNC491265; North Key Largo, Sec., 25.289722 -80.306944, Malaise trap, 3.iv.1985, voucher code CNC483615; N. Key Largo, Sec., 25.289722 -80.306944, Malaise trap, 3.iv.1985, voucher codes CNC483637–CNC483645, CNC483649; North Key Largo, S35, 25.289722 -80.306944, Hammock forest, Malaise trap, 4.v-4.viii.1985, voucher codes CNC483452, CNC483455, CNC483456, CNC483458; Sugar Loaf Key, Kichings, 24.644000 -81.563000, Malaise trap, 3.v-3.viii.1985, voucher codes CNC489899, CNC489907, CNC489913; Vaca Key, Marathon, 24.716936 -81.073308, flight interception trap, 31.viii-15.xii.1986, voucher code CNC483448.

Description. Female. Color. Body mostly orange yellow, with mediotergites 4–8, laterotergites 4–8, sternites 4–8 and hypopygium darker, mostly brown. Antenna with anterior 6–7 flagellomeres yellow, and posterior 9–10 flagellomeres dark brown. Metatibia and metafemur mostly to completely brown. Anteromesoscutum, propodeum and metapleuron varying from entirely orange-yellow to partially dark brown. Wings mostly infumated, except for small central white band; veins mostly brown, pterostigma with a pale spot anteriorly or mostly brown.

Head. Coarsely sculptured, in lateral view strongly projecting forward below antennal sockets. Head in frontal view with maximum width right below the eyes (due to bulging gena). Gena in lateral view wider than eye width. Anterior tentorial pits large, 0.3 × as wide as clypeus width. Malar line long, more than 2.8 × mandibular width. Eye small, its height 0.6 × head height. Ocelli relatively small, diameter of posterior



Figures 1–6. 1–3 Female holotype. **1** Habitus lateral **2** Fore wing **3** Habitus dorsal **4–6** Male paratype **4** Fore wing **5** Habitus dorsal **6** Habitus lateral..

ocelli about half of both OOL and POL. Anatomical line tangent to posterior margin of anterior ocellus, crossing well above the anatomical line tangent to anterior margin of posterior ocelli. Antenna about same length as body.

Mesosoma. Pronotum rather narrow, with one broad, transverse pronotal sulcus. Propleuron flange sharply defined by carina. Notauli very faintly marked by slightly coarser sculpture than rest of anteromesoscutum. Scutoscutellar sulcus with seven carinae. Mesoscutellum with lateral face mostly strongly striated, with polished area (mesoscutellum lunula) very narrow, 0.1 × lateral face height. Scutellum with posteromedian band weakly rugose. Mesopleuron sculptured on anterior half, smooth posteriorly. Mesopleural scrobe extending to almost 0.5 of mesopleuron width. Metapleuron mostly smooth anterior to metapleural scrobe but with strong transverse striation posterior to scrobe. Propodeum with complex sculpture pattern, including partial median longitudinal carina (sometimes obscured by other sculpture), traces of transverse carina and partial areola with only the posterior carinae of areola defined.

Metasoma. T1 relatively short, its medial length 1.2 × its width at anterior margin. T1 more or less parallel-sided for anterior 0.5, then narrowing towards posterior margin (width at anterior margin 2.0 × width at posterior margin). T1 without median sulcus, with anterior 0.5 rather depressed and concave, and posterior 0.5 with strong transversal striation. T2 trapezoidal, its width at posterior margin 1.5 × its medial length. T2 and T3 with strong longitudinal striation; T4–T8 smooth. Hypopygium small, inflexible and unfolded. Ovipositor sheaths mostly smooth, with few, short setae on posterior 0.2–0.3. Ovipositor short, 0.2 × as long as metatibia.

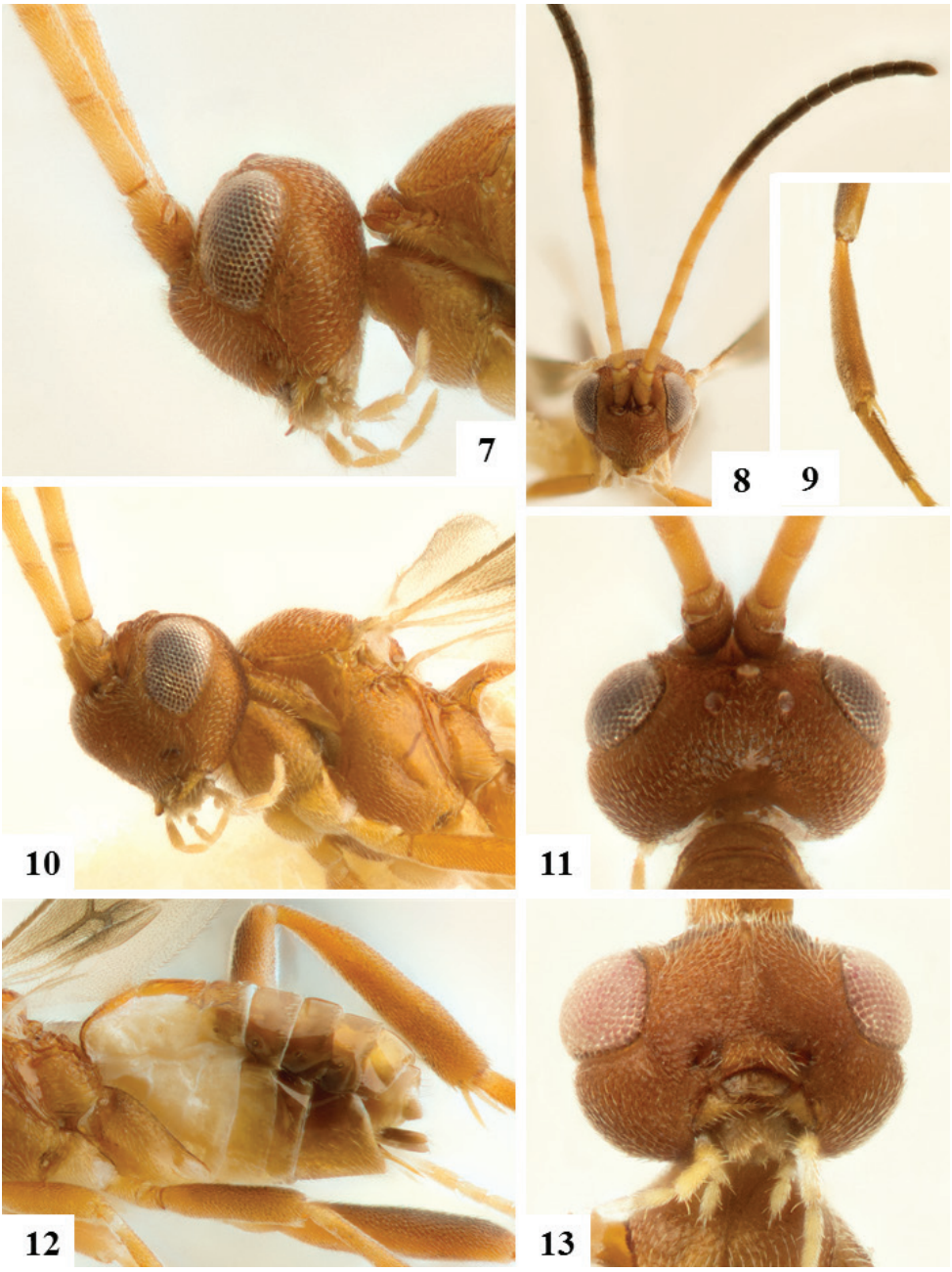
Legs. Metacoxa of moderate size, not surpassing posterior margin of T2; metafemur 5.0 × as long as wide. Metatibia with inner and outer spurs subequal (inner 1.1 × as long as outer) and 0.35–0.40 as long as metatarsal segment 1.

Wings. Fore wing relatively short (0.6–0.7 × body length and 1.1–1.3 × metasoma length), with small to almost obliterated, 4-sided areolet, and with vein R1 shorter than pterostigma length and only 2.0–2.5 × as long as the distance between its posterior end and posterior end of vein M.

Holotype measurements (some measurements of female paratypes between parentheses). Body length: 2.2 mm (1.9– 2.1, 2.4–2.5 mm). Fore wing length: 1.3 mm (1.2, 1.6–1.8 mm). Metasoma length: 1.1 mm (0.9–1.0, 1.2–1.3 mm). Mandible width: 0.06 mm. Malar line: 0.17 mm. Clypeus length/ width: 0.15 mm/0.045 mm. Tentorial pit width: 0.05 mm. Head maximum width: 0.58 mm. Head height: 0.48 mm. Eye height: 0.27 mm. Eye maximum width (lateral view): 0.15 mm. Gena maximum width (lateral view): 0.20 mm. OOL: 0.09 mm; POL: 0.09 mm; diameter of posterior ocellus: 0.05 mm. Flagellomere 2 lenght/width: 0.20 mm/0.065 mm. Flagellomere 14 length/width: 0.14/0.07. T1 width at anterior margin/width at posterior margin: 0.39 mm/0.19 mm. T2 length/width at posterior margin: 0.34 mm/0.52 mm. Metacoxa length: 0.42 mm. Metafemur length/width: 0.75/0.15. Metatibia length: 0.82 mm. Metatibial spurs length, inner/outer: 0.17 mm/0.15 mm. First segment of metatarsus length: 0.40 mm. Ovipositor length: 0.15 mm.

Male. As in female except for uniformly brown antenna, longer fore wing, lighter-coloured wings (hyaline or slightly infumated), and darker body color which in some cases becomes dark brown to black in areas of the head and mesosoma.

Distibution. Southern Florida (Florida Keys and Everglades National Park, Fig 18).



Figures 7–12. Female holotype. **7** Head, pronotum and propleuron, lateral **8** Head and antenna, frontal **9** Metatibia and first segment of metatarsus **10** Head and mesosoma, dorso-lateral **11** Heard, dorsal **12** Metasoma, lateral **13** Female paratype. Head, ventral.

Figures 14–17. 14–16 Female paratypes. **14** and **15** Propodeum and metasomal tergites 1–4, dorsal **16** Mesosoma, dorsal **17** Female holotype. Metasoma, dorsal.

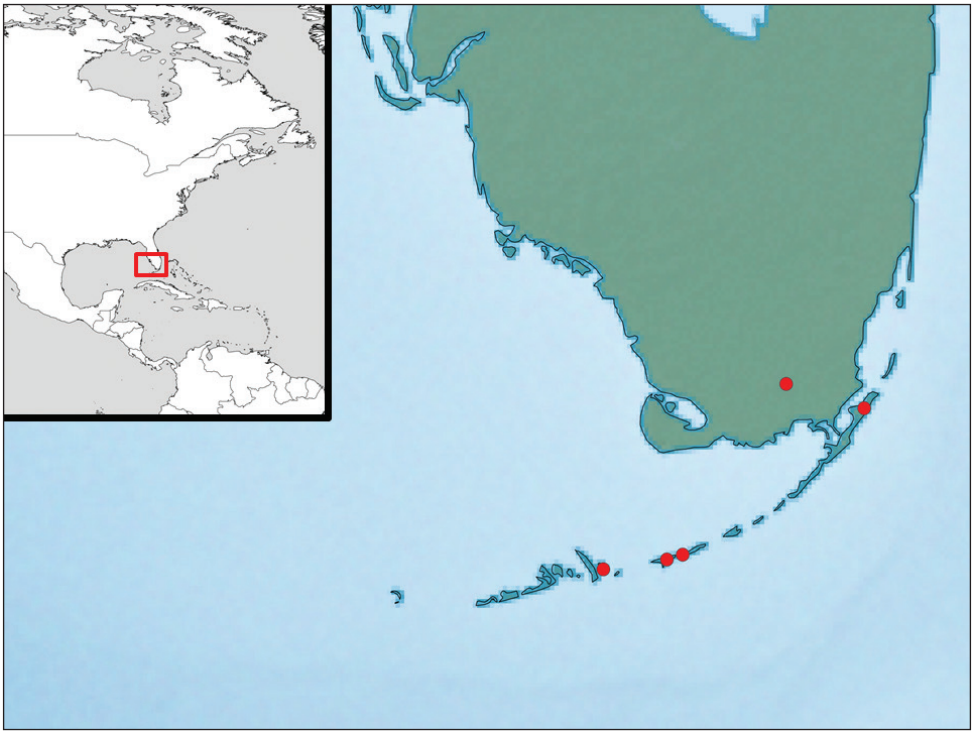


Figure 18. Map showing the distribution of the species.

Biology. Unknown. All specimens were collected in hammock forests from February to November. Based on the labels, the species would seem to be more abundant in April–June and November. However, the collecting device, a Malaise trap, was not emptied regularly (at times running for months), and so the actual flight dates for the species cannot be considered as very precise.

Etymology. Named after Stewart and Jarmila Peck, tireless insect collectors over the last 30+ years and the ones finding the species back in 1985 and1986. Also in recognition of their great knowledge as entomologists and as an appreciation for the important papers published by Stewart on the insect fauna of southern Florida.

Comments. In females, smaller specimens tend to have shorter wings, a smaller areolet and pterostigma in the fore wing, and lighter color than larger specimens. Males also show variation in the extent of darker areas, but are always darker than females. Although numerous specimens (60) were found and are included as part of the type series, it should be noted that all of them were collected during two collecting trips made in 1985 and 1986 (see Peck (1989) for details). Based on the specimens collected, males outnumber females in a proportion of 14: 1. This is an artifact of the collecting method used (a Malaise trap), which is better suited for rather strong fliers. Evidently females do not fly well, if at all.

Discussion

Brachyptery and Microgastrinae?

Belokobylskij and Kula (2012) analyzed the species of Braconidae that could be considered as apterous, micropterous and brachypterous and listed over one hundred species worldwide. Quicke (2015) commented that brachyptery occurs in a few members of virtually all braconid subfamilies within the cyclostome lineage but only in four subfamilies of non-cyclostome, none of them belonging to Microgastrinae; he also speculated that apterygy and brachyptery must have evolved many times (at least 48 times, probably more) within Braconidae.

According to Belokobylskij and Kula (2012: 6) the following parameters characterize specimens of Braconidae as brachypterous: fore wing length greater than 0.25 mesosoma length but less than metasoma length, venation distinct but incomplete, tegula present and usually not reduced in size. Females of *Keylimepie floridaensis* have fore wings just slightly longer than metasoma length (1.1–1.3×), but they do not actually extend beyond T4, i.e., well before the metasoma apex (Fig. 3), and are definitely shorter (0.6–0.7×) than the body length; the venation is complete but some veins are not clearly distinct due to the reduced size of the wing, and the tegula is of normal size. They fulfill two out of three parameters proposed by Belokobylskij and Kula (2012), and thus we consider here that female specimens of *Keylimepie* are indeed brachypterous. Because wing reduction for Braconidae overall is likely continuous rather than discrete, it will be extremely difficult to strictly categorize every species as micropterous, brachypterous, and macropterous.

The phenomenon of wing size reduction in Braconidae has been related to penetration of parasitoids into the microhabitats of their hosts, temporal (seasonal) abundance of preferred hosts in limited space, or unknown causes (see Belokobylskij and Kula 2012). There may also be a tendency for short wings to evolve more on islands and in relatively harsh environments such as arid, arctic or high mountains where it is windy, hosts may be distributed in an exceedingly patchy manner, and/or due to the need to conserve energy (Quicke 2015). Specimens of *K. peckorum* have been almost exclusively collected in small islands with relatively dry environments (Deyrup et al. 1988). Two specimens were collected in mainland Florida (Everglades National Park), in a similar environment to the Florida Keys and very close to them (Fig. 18).

Conservation of *Keylimepie peckorum* in the southern Florida environments

Because of the geographic location of the Florida Keys, its biota includes species that a) arrived naturally from nearby mainland Florida, b) arrived naturally from the nearby West Indies, c) were introduced by humans, or d) are endemic (Snyder et al. 1990, Moreau et al. 2014). Among plants, vertebrates, and butterflies, there are numerous West Indian species whose North American populations are confined just to the Flor-

ida Keys and small nearby areas of mainland Florida (Snyder et al. 1990). However, species diversity is limited by the reduced habitat types and the long dry season during winter and spring (Deyrup et al. 1988).

Conservation efforts in the Florida Keys are mainly focused on marine life, plants such as the Key tree cactus (*Pilosocereus robinii*), and charismatic fauna such as the Key deer (*Odocoileus virginianus clavium*) and the Key Largo woodrat (*Neotoma floridana smalli*). Among insects butterflies and ants have also been studied from a conservation perspective (Deyrup et al. 1988, Moreau et al. 2014, Henry et al. 2015, Jue et al. 2015).

A recent assessment on vulnerability of 300 species in Florida found a number of species from the Keys as of high priority (Reece et al. 2013). The comments made by the authors (p. 8) about conservation of some insect species are significant: "Several species, in particular invertebrates, were ranked as at high risk of extinction, but do not receive high priority for conservation due to a lack of basic life history information. Examples include the Keys scaly cricket (*Cycloptilum irregularis*), the mangrove long-horned beetle (*Heterachthes sablensis*), and the Antillean spreadwing (*Lestes spumarius*). We do not advocate abandoning these and similar species. Nevertheless, conservation actions that target species or groups of species should, whenever possible, be based on knowledge of the life history and ecology of target species... Without life-history information that would indicate potential responses to alternative management actions, we would not give high priority for conservation action to these species, aside from protecting known occurrences. On the other hand, they should receive high priority for basic research. Thus, for many species, additional research must be conducted before conservation measures beyond protecting documented populations can be successfully implemented".

We believe that *Keylimepie peckorum* fits the above scenario perfectly. It is not only the first new genus of Microgastrinae discovered in North America in more than 30 years, but it also represents a distinctive and arguably unique lineage among microgastrine parasitoid wasps, and the species is likely to be endemic to the Florida Keys and Everglades areas. The poor ability to fly may also make *K. peckorum* less adaptable to changes in habitat distribution due to human disturbance, climate change, etc. Based on what is already known, it could easily be considered as a high priority species. However, the lack of basic biological information (host unknown) and the uncertainty of its current presence in the Keys (the only known specimens were collected 30 years ago) is a serious obstacle towards any conservation effort. This paper has only filled the first gap, giving a name to the species and summarizing what is known to date about it. More research is needed, including collecting of fresh specimens (to prove that the species is not extinct) as well as trying to find the host caterpillars of this amazing parasitoid wasp.

Acknowledgements

We are grateful to John Huber, Henri Goulet (CNC) and Robert Kula (National Museum of Natural History, Washington DC), for their valuable comments and suggestions

on early versions of the manuscript. The suggestions from the editor and reviewers, Kees van Achterberg (Naturalis, The Netherlands), Jim Whitfield (University of Illinois, US) and Andrew Austin (University of Adelaide, Australia) are also gratefully acknowledged. Ronald Maclean (CNC) helped with the preparation of specimens.

References

Deyrup MA, Carlin N, Trager J, Umphrey G (1988) A review of the ants of the Florida Keys. Florida Entomologist 71: 163–176. doi: 10.2307/3495364

Fernández-Triana J, Whitfield JB, Rodriguez JJ, Smith MA, Janzen DH, Hallwachs W, Hajibabaei M, Burns JM, Solis MA, Brown J, Cardinal S, Goulet H, Hebert PDN (2014a) Review of *Apanteles* (Hymenoptera: Braconidae, Microgastrinae) from Area de Conservación Guanacaste, northwestern Costa Rica, with keys to all described species from Mesoamerica. ZooKeys 383: 1–565. doi: 10.3897/zookeys.383.6418

Fernandez-Triana J, Ward D (2016) Microgastrinae Wasps of the World. http://microgastrinae.myspecies.info/ [accessed on 2016/02/28]

Henry EH, Haddad NM, Wilson J, Hughes P, Gardner B (2015) Point-count methods to monitor butterfly populations when traditional methods fail: a case study with Miami blue butterfly. Journal of Insect Conservation 19(3): 519–529. doi: 10.1007/s10841-015-9773-6

Huber JT, Sharkey MJ (1993) Structure. In: Goulet H, Huber JT (Eds) Hymenoptera of the world: an identification guide to families. Agriculture Canada Research Branch, Monograph No. 1894E, Ottawa, 13–59.

Jue DK, Daniels JC (2015) A successful model for citizen scientist involvement in building a statewide at-risk butterfly database. Journal of Insect Conservation 19(2): 421–431. doi: 10.1007/s10841-014-9733-6

Karlsson D, Ronquist F (2012) Skeletal morphology of *Opius dissitus* and *Biosteres carbonarius* (Hymenoptera: Braconidae), with a discussion of terminology. PLoS ONE 7(4): e32573. doi: 10.1371/journal.pone.0032573

Mason WRM (1981) The polyphyletic nature of *Apanteles* Foerster (Hymenoptera: Braconidae): A phylogeny and reclassification of Microgastrinae. Memoirs of the Entomological Society of Canada, Ottawa, 147 pp.

Moreau CS, Deyrup MA, Davis LR (2014) Ants of the Florida keys: species accounts, biogeography, and conservation (hymenoptera: formicidae). Journal of Insect Science 14(1): 295. doi: 10.1093/jisesa/ieu157

Peck SB (1989) A survey of insects of the Florida Keys: post-Pleistocene land-bridge islands. Florida Entomologist 72: 603–612. doi: 10.2307/3495034

Quicke DLJ (2015) The Braconid and Ichneumonid Parasitoid Wasps: Biology, Systematics, Evolution and Ecology. Willey-Blackwell, 704 pp.

Reece JS, Noss RF, Oetting J, Hoctor T, Volk M (2013) A vulnerability assessment of 300 species in Florida: Threats from sea level rise, land use, and climate change. PLoS ONE 8(11): e80658. doi: 10.1371/journal.pone.0080658

Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. http://www.simplemappr.net [accessed on 2016/02/28]

Snyder JR, Herndon A, Robertson WB (1990) South Florida rockland. In: Myers RL, Ewel JJ (Eds) Ecosystems of Florida. University of Central Florida Press, Orlando, 230–277.

Whitfield JB (1997) Subfamily Microgastrinae. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) Manual of the New World genera of the family Braconidae (Hymenoptera). Special Publication No. 1, International Society of Hymenopterists, Washington, 333–364.

Williams DJM (1985) The New World genus *Lathrapanteles* n.gen.: Phylogeny and placement in the Microgastrinae (Hymenoptera: Braconidae: Cotesini). Canadian Journal of Zoology 63: 1962–1981. doi: 10.1139/z85-289

Yu DSK, van Achterberg C, Horstmann K (2012) Taxapad 2012, Ichneumonoidea 2011. Database on flash-drive. www.taxapad.com, Ottawa, Ontario.

ZooKeys 584: 109–120 (2016)
doi: 10.3897/zookeys.584.7815
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Major range extensions for two genera of the parasitoid subtribe Facitorina, with a new generic synonymy (Braconidae, Rogadinae, Yeliconini)

Buntika A. Butcher[1], Donald L. J. Quicke[1], Santhosh Shreevihar[3], Avunjikkattu Parambil Ranjith[2,3]

1 *Department of Biology, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, BKK 10330, Thailand* **2** *Insect Ecology and Ethology Laboratory, Department of Zoology, University of Calicut, Kerala, Pin: 673635, India* **3** *Department of Zoology, Malabar Christian College, Kozhikode (Affiliated to University of Calicut), 673001, Kerala, India*

Corresponding author: *Buntika A. Butcher* (buntika.a@chula.ac.th)

Academic editor: *K. van Achterberg* | Received 18 January 2016 | Accepted 22 March 2016 | Published 26 April 2016

http://zoobank.org/69D8DB87-2074-418E-9EDB-E7BDB8CD95EA

Citation: Butcher BA, Quicke DLJ, Shreevihar S, Ranjith AP (2016) Major range extensions for two genera of the parasitoid subtribe Facitorina, with a new generic synonymy (Braconidae, Rogadinae, Yeliconini). ZooKeys 584: 109–120. doi: 10.3897/zookeys.584.7815

Abstract

The genera *Conobregma* van Achterberg and *Facitorus* van Achterberg are recorded from the Afrotropical region and the Indian subcontinent, respectively, for the first time, and two new species are described and illustrated: *Conobregma bradpitti* Quicke & Butcher, **sp. n.** from South Africa and *Facitorus nasseri* Ranjith & Quicke, **sp. n.** from India. *Conobregma bradpitti* **sp. n.** is intermediate between *Conobregma* which was described originally from the New World, and *Asiabregma* Belokobylskij, Zaldivar-Riverón & Maetô, which was coined for the S. E. Asian and East Palaearctic (Japanese) species described under the name *Conobregma*, plus more recently discovered taxa, but the differences between these genera are few and slight. Of the four previously proposed diagnostic characters for separating *Asiabregma* from *Conobregma*, the new species shares two with each, and therefore, the two genera are formally synonymised. *Facitorus* was previously known only from the East Palaearctic region and from S. E. Asia (Japan, Nepal, Taiwan and Vietnam).

Keywords

New distribution record, new species, new synonymy, parasitoid

Introduction

The Facitorini were originally described as a tribe in the subfamily Betylobraconinae Tobias, 1979 based on the genera *Facitorus* van Achterberg from Nepal, China and Taiwan, *Conobregma* van Achterberg from the USA and Dominican Republic, and *Jannya* van Achterberg from Colombia and Costa Rica (van Achterberg 1995a). Despite all taxa placed in the Betylobraconinae being morphologically highly derived with robust femora, and shortened tarsi, moderately to very bulging faces, and curved fore wing vein M+CU (van Achterberg 1995a) they have subsequently been shown not to be monophyletic (Zaldivar-Riverón et al. 2006). The morphological homoplasy of these characters even led van Achterberg (1991) to place the rogadine tribe Yeliconini in the Betylobraconinae, though this arrangement was soon dropped as a result of further consideration of biological and morphological evidence. The Facitorini were transferred to the subfamily Rogadinae as a subtribe of the Yeliconini by Belokobylskij et al. (2008) on the basis of DNA sequence data and this placement has been supported by subsequent studies (Zaldivar-Riverón et al. 2009, Butcher et al. 2014, Quicke and Butcher 2015). Most recently, Butcher and Quicke (2015) formally synonymised Betylobraconinae with the Rogadinae maintaining it as a separate tribe. Unfortunately, nothing is yet known about the biology of the Facitorina though their similarity to *Yelicones*, which is a koinobiont larval endoparasitoid of Lepidoptera larvae concealed to some extent in silk webs, suggests that they may have similar biology.

Shortly after the original description of *Conobregma*, which was based on New World species, van Achterberg (1995b) added a new species from the Indonesian island of Sulawesi, thus extending the apparent distribution of the genus to the Old World tropics. Discovery of additional specimens of another genus, *Aulosaphobracon* Belokobylskij & Long, as well as DNA sequence data led Belokobylskij et al. (2008) to coin a new genus, *Asiabregma*, for the Asian and East Palaearctic species that fell within van Achterberg's concept of *Conobregma*. However, despite their very disjunct distribution, the two genera were only separated by four, rather weak, characters (Table 1), and the new species from S. Africa is rather intermediate. We therefore synonymise *Asiabregma* with *Conobregma*, and treat the new species under the latter name.

Materials and methods

The holotype of *C. bradpitti* sp. n. is deposited in the Hymenoptera Institute Collection, Department of Entomology, University of Kentucky, Lexington, Kentucky. It was imaged using an Olympus SXZ16 microscope with automated multiple image capture at preset focal levels using an Olympus DP72 camera, and image combination using the Cell^D image processing system. The specimen was card-mounted and rather fragile but we successfully remounted it to enable more features to be seen.

The holotype of *F. nasseri* sp. n. is deposited in the Department of Zoology, University of Calicut, Kerala, India. It was imaged using an Leica M205A stereomicro-

Table 1. Differences used by Belokobylskij et al. (2008) to differentiate between *Conobregma* and *Asiabregma*.

Characters	*Conobregma*	*C. bradpitti* sp. n.	'*Asiabregma*'
Claw of middle leg	short, not pectinate	long and pectinate	long and pectinate
Postpectal carina	absent	absent	distinct
Fore wing vein 2CUa	short <= m-cu	short = m-cu	long > twice m-cu
Carina between eye and antennal sockets	absent	present	present

scope with automated multiple image capture at preset focal levels using an Leica DMC 2900 camera, and image combination using the Leica Application Suite image processing system v4.7. All images were edited using Photoshop CS6 (Version 6.1) (Adobe Inc.).

Terminology follows van Achterberg (1988) except for wing venation nomenclature which follows Sharkey and Wharton (1997); see also Figure 2.2 in Quicke (2015) for comparison of wing venation naming systems.

Descriptive taxonomy

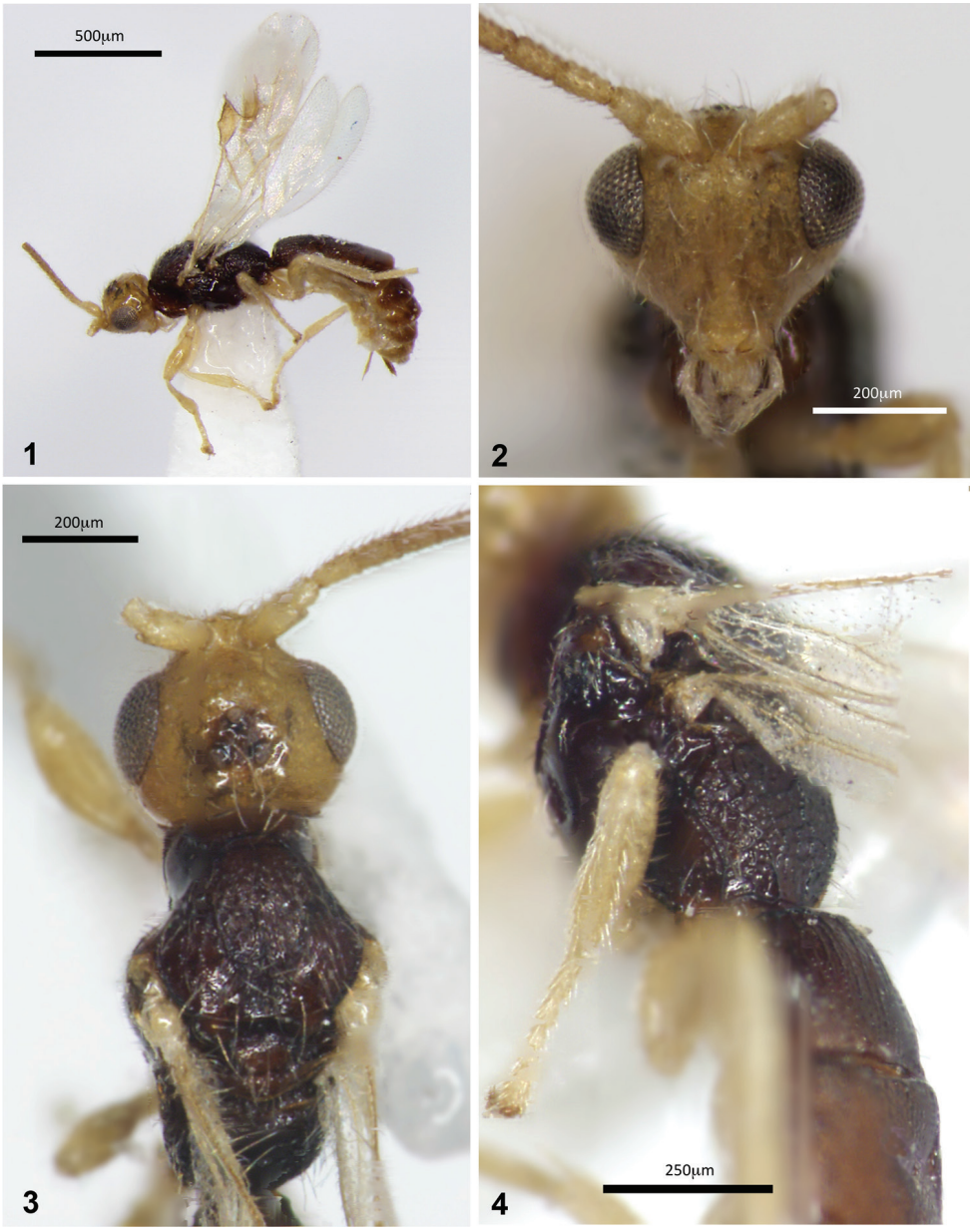
***Conobregma bradpitti* Quicke & Butcher, sp. n.**
http://zoobank.org/4C0937AE-13E0-43F2-B411-1CFFCB881FD6
Figures 1–6

Material examined. Holotype female: "South Africa, Madlangula, Kosi Bay, 14.iii – 30.iv.1985, R. Kyle".

Diagnosis. *Conobregma bradpitti* sp. n. may be distinguished from the East Palaearctic and East Asian species (*C. makiharae* (Belokobylskij, Zaldivar & Maetô, 2008), *C. ryukyuensis* (Belokobylskij, Zaldivar & Maetô, 2008)) and *C. sulaensis* van Achterberg, 1995) by fore wing vein 2CUa being approximately the same length as m-cu rather than approximately twice as long. It may be distinguished from all the New World species except for *C. cometes* van Achterberg, 1995 by having the third metasomal tergite almost entirely smooth. It differs from *C. cometes* in having the mesoscutum coarsely sculptured with deep depressions at the bases of setae rather than being coriaceous, and by having the propodeum distinctly less strongly sculptured antero-laterally.

Description (female). Length of body 1.75 mm, and of fore wing 1.5 mm.

Head. Antennae broken. First flagellomere 1.05 × longer than 2nd and 3rd respectively; approximately 1.8 × longer than apically wide, expanding from base to apex. Width of head: width of face: height of eye = 1.0: 0.5: 0.42. Eyes glabrous, with distinct curving fine ridge between antennal socket and eye. Distance between posterior ocelli: transverse diameter of posterior ocellus: shortest distance between posterior ocellus and eye = 1.0: 1.0: 2.5. Frons and occiput smooth. Occipital carina complete.



Figures 1–4. Montaged light micrographs of *Conobregma bradpitti* sp. n.; **1** habitus **2** face, anterior aspect **3** head and mesosoma, dorsal aspect **4** mesosoma, including propodeum, and anterior half of metasoma, oblique aspect.

Mesosoma. Mesosoma 1.8 × longer than high. Propleuron largely smooth. Mesoscutum irregularly sculptured, with deep pits at bases of setae, these forming very conspicuous submarginal rows; with rugulose sculpture between notauli posteriorly.

Figures 5–6. Montaged light micrographs of *Conobregma bradpitti* sp. n.; **5** mesosoma to tergite 2, dorsal aspect **6** metasoma, dorsal aspect.

Notauli deeply impressed and strongly sculptured. Precoxal sulcus running from anterior margin to just posterior of mid-length of metapleuron, rugulose. Mesopleuron and mesosternum otherwise largely smooth. Median area of metanotum with weak mid-longitudinal ridge. Propodeum largely foveate except for pair of triangular areas anteriorly on either side of mid-line which are finely aciculate; with short mid-longitudinal carina anteriorly.

Wings. Pterostigma 2.1 × longer than its maximum width. Fore wing vein r-rs approximately 0.65 × maximum width of pterostigma. Lengths of fore wing veins r-rs: 3RSa: 3RSb = 1.0: 3.0: 5.5. Lengths of fore wing veins CU1a: CU1b = 1.0:1.25.

Legs. Fore femur: tibia: tarsus = 1.3: 1.25: 1.0. Fore basitarsus 1.5 × longer than next three articles combined. Mid-tibial claw with well-developed, pecten. Hind femur: tibia: tarsus = 1.0: 1.2: 1.2.

Metasoma. Second metasomal tergite with fine longitudinal striation and interconnecting transverse ridges; approximately 1.8 × longer than third metasomal tergite medially. Second suture finely crenulate. Third tergite almost entirely smooth but with traces of longitudinal striation near lateral parts of second suture. Thrid-fifth metasomal tergites distinctly arched in lateral profile. Ovipositor sheath 0.4 × length of hind tibia.

Colour. Stemmaticum and mesosoma entirely dark brown, nearly black; head, antennae (part remaining) and legs pale brown-yellow; metasomal tergites brown. Wings hyaline with pale grey-brown venation.

Etymology. Named after the senior author's favourite film actor Brad Pitt, whose poster adorned the wall of her laboratory during her doctoral studies.

Male. Unknown.

Distribution. South Africa.

Host. Unknown.

Facitorus nasseri Ranjith & Quicke, sp. n.

http://zoobank.org/6739F6C6-62F4-44C4-B5C6-203F8B3895C3
Figures 7–15

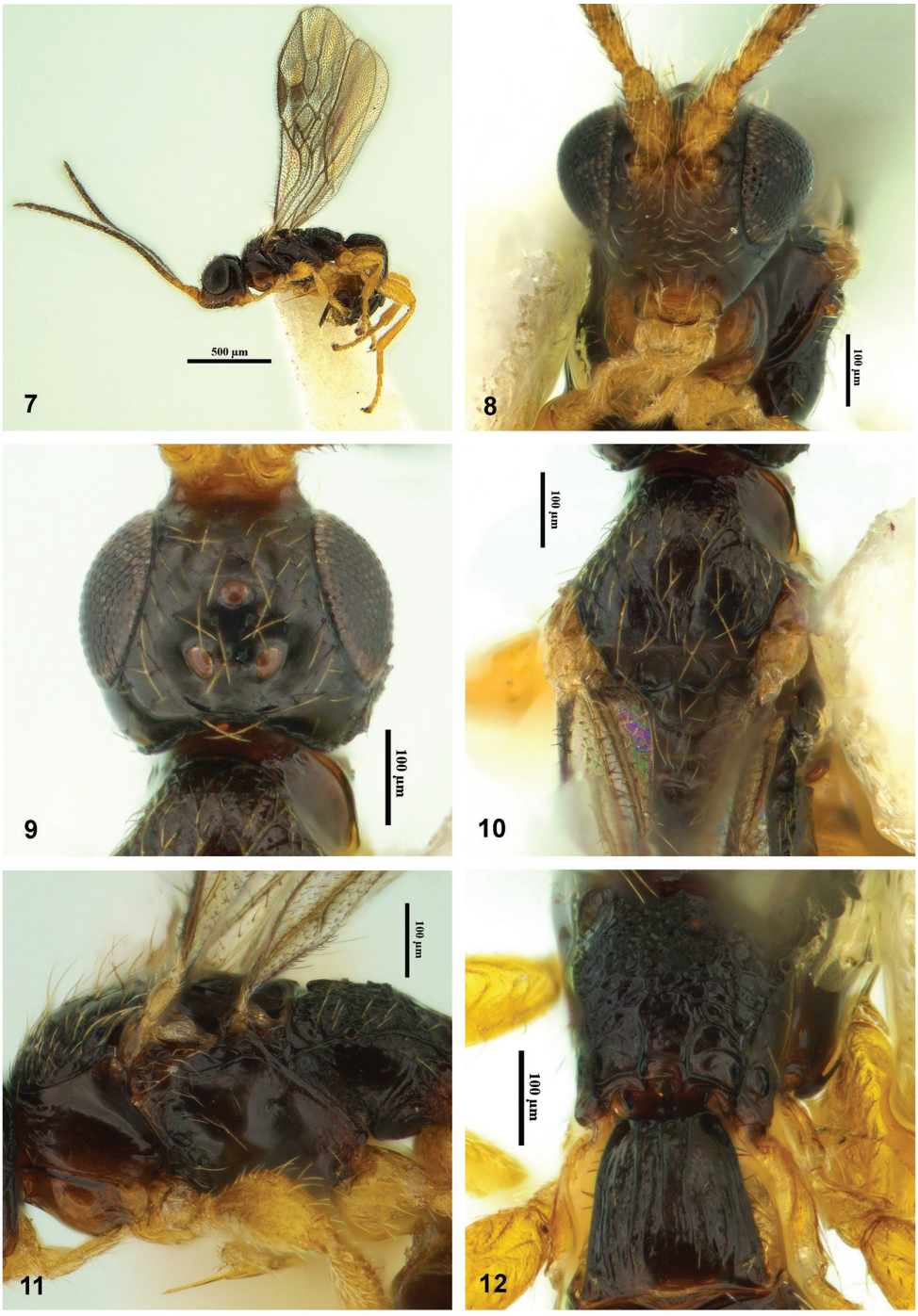
Material examined. Holotype, female, "India, Kerala, Malappuram, Calicut University Botanical Garden, 14–21.xii.2015, Malaise Trap, ex. Ranjith, A.P."

Diagnosis. *Facitorius nasseri* sp. n. is distinguished from *F. brevicornis* van Achterberg and *F. superus* van Achterberg in having occipital carina complete, mesoscutum covered by long setae and scutellum with sub-posterior depression. *Facitorus nasseri* sp. n. comes close to *F. tamdaoensis* Belokobylskij & Long, by its smooth metasomal tergite 2, but it differs from *F. tamdaoensis* by the following characters; mesoscutum sculptured antero-laterally (smooth in *F. tamdaoensis*), frons without shallow pit medially (frons with shallow pit medially in *F. tamdaoensis*), propodeum with 'H' shaped carina posteriorly (smooth in *F. tamdaoensis*), pterostigma 2.9 × longer than maximum wide (3.6 × in *F. tamdaoensis*) and second tergite entirely smooth (densely striate basally in *F. tamdaoensis*). It differs from *F. granulosus* and *F. amamioshimus* by first flagellomere 2.1 × as long as apically wide (3.5–4.2 × in *F. granulosus* and 3.5–4.0 × in *F. amamioshimus*), second metasomal suture not crenulate (crenulate in *F. granulosus* and *F. amamioshimus*), third metasomal tergite entirely smooth (distinctly sculptured at least baso-laterally in *F. granulosus* and *F. amamioshimus*). A key for the identification of *Facitorus* species is presented below.

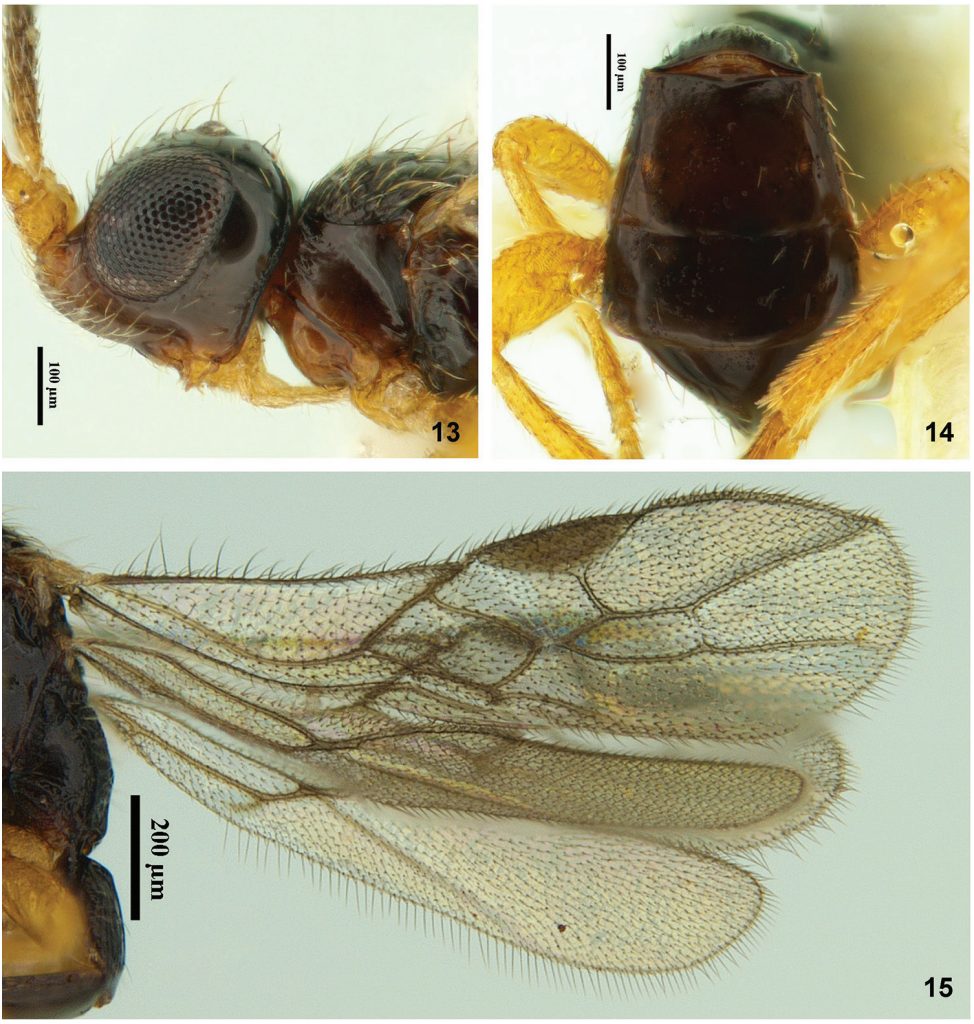
Description (female). Holotype, female (♀), length of body 1.7 mm and fore wing 1.35 mm.

Head. Antennae with 18 segments. First flagellomere 1.2 × as long as second and third respectively, 2.1 × longer than apically wide, distinctly expanded from base to apex. Terminal flagellomere acute, 3.1 × as long as its maximum width. Width of head: width of face: height of eye = 13.4: 6.8: 7.1. Frons and occiput smooth with long setae. Eyes glabrous, with a straight groove between antennal socket and eye margin. Distance between posterior ocelli: transverse diameter of posterior ocellus: shortest distance between posterior ocellus and eye = 13.5: 10.25: 18.8. Occipital carina complete.

Mesosoma. Mesosoma 1.72 × longer than high. Propleuron smooth. Mesoscutum sculptured antero-laterally, smooth medio-posteriorly with long setae. Notauli impressed, meeting posteriorly and finely crenulate. Scutellar sulcus wide, deep and di-



Figures 7–12. Montaged light micrographs of *Facitorus nasseri* sp. n.; **7** habitus **8** head, anterior aspect **9** head, dorsal aspecct **10** mesosoma, dorsal aspect **11** mesosoma, lateral aspect **12** propodeum and first metasomal tergite, dorsal aspect.



Figures 13–15. Montaged light micrographs of *Facitorus nasseri* sp. n.; **13** head & mesosoma (in part), lateral aspect **14** metasomal tergite 2 and following tergites, dorsal aspect **15** wings.

vided by a single carina. Scutellum smooth, sparsely setose with subposterior transverse depression. Median area of metanotum with medio-longitudinal ridge, rest smooth. Precoxal sulcus distinct only anteriorly impressed. Metapleuron medially smooth, rest rugose. Propodeum without medio-longitudinal carina, basal half distinctly foveate and with 'H' shaped carinae and transverse carinae. Pterostigma 2.9 × longer than maximally wide. Fore wing vein r-rs approximately 0.8 × maximum width of pterostigma. Lengths of fore wing veins r-rs: 3RSa: 3RSb = 2.8: 4.4: 12.5. Lengths of fore wing veins CU1a: CU1b = 3.25: 4.37. Fore femur: tibia: tarsus = 4.7: 4.58: 3.34. Fore basitarsus 1.6 × longer than next three articles combined. Mid-tibial claw well-developed, pectinate. Hind femur: tibia: tarsus = 5.4: 7.7: 7.0.

Metasoma. Metasomal tergite 1 distinctly striate, smooth medio-posteriorly, striae reaching posterior margin laterally, slightly convex apically, sparsely setose. Tergite 2 smooth, sparsely setose medially, setose laterally, 1.6 × as long as third tergite. Second metasomal suture slightly impressed, not crenulate. Tergite 3 smooth with a pair of setae medio-basally and postero-laterally. Rest of the tergite smooth, exposed in lateral view and sparsely setose. Ovipositor sheath setose and 0.42 × as long as hind tibia.

Colour. Body dark brown except scape, pedicel, first flagellomere, basal half of second flagellomere, maxillary palp, tegulae, legs and ovipositor yellow; face yellowish brown anteriorly below antennal sockets; propleuron ventrally yellowish brown; wings hyaline; pterostigma and venation light brown.

Etymology. APR dedicates this species to Dr. M. Nasser for his encouragement and sharing his knowledge about the behaviour of parasitoids, and also for the fruitful discussions during the field trips.

Male. Unknown.

Distribution. India (Kerala).

Host. Unknown.

Key to species of *Facitorus* van Achterberg

1 Scutellum without sub-posterior depression; occipital carina interrupted medially; mesoscutum without long setae **2**

– Scutellum with subposterior depression; occipital carina complete; mesoscutum often covered by long setae **3**

2 Fore wing vein r 1.5 × as long as 2-SR; malar space 2.8 × basal width of mandible; face sparsely punctate; second tergite largely smooth ..... ***F. brevicornis* van Achterberg**

– Fore wing vein r almost equal to or shorter than 2-SR; malar space 2 × basal width of mandible; face smooth; second tergite rugose-punctate..... ***F. superus* van Achterberg**

3 Mesoscutum entirely smooth or rugose antero-laterally; third tergite entirely smooth **4**

– Mesoscutum granulate; third tergite distinctly sculptured, at least basolaterally..... **5**

4 Mesoscutum rugose antero-laterally; transverse diameter of eye twice as long as temple; frons without shallow pit near antennal sockets; anterior half of propodeum foveate, with 'H' shaped carina posteriorly and transverse carina; pterostigma 2.9 × as long as its maximum width; hind coxa entirely smooth ..... ***F. nasseri* Ranjith & Quicke, sp. n.**

– Mesoscutum entirely smooth; transverse diameter of eye 2.7 × as long as..... temple; frons with shallow pit near antennal sockets; propodeum densely rugose-reticulate; pterostigma 3.6 × as long as its maximum width; hind coxa rugose-striate laterally ..... ***F. tamdaoensis* Belokobylskij & Long**

5 Hind coxa dorsally striate; second metasomal suture deep; pterostigma enlarged, 1.1–1.2 × as long as R1; third tergite distinctly and widely sculptured; mesoscutum distinctly granulate..... ***F. granulosus*** **Belokobylskij & Long**

– Hind coxa entirely smooth; second metasomal suture shallow; pterostigma not enlarged, 0. 9× as long as R1; third tergite only baso-laterally finely striate or rugulose-strate; mesoscutum finely granulate..... .....***F. amamioshimus*** **Belokobylskij, Zaldivar-Riverón & Maetô**

Discussion

Conobregma bradpitti sp. n. is the first record of the Facitorina from the African continent, the others occurring in the East Palaearctic, East Asia and North America (including Caribbean). The new species keys out easily to *Conobregma* in the generic key to Betylobraconi (as –inae) by van Achterberg (1985), but its characters are intermediate between those of *Conobregma* and the more recently described genus *Asiabregma* established by Belokobylskij et al. (2008). Originating from an intermediate location longitudinally, it may be not surprising that the new species displays a mix of character states between *Conobregma* and *Asiabregma* (Table 1). Differences between *Conobregma* and *Asiabregma* are in any case rather slight and probably would not normally be used to justify separate generic status had they not shown a disjunct distribution. With the discovery of the new species which shares two derived states with each nominal genus, we have to choose whether to arbitrarily assign it to one of them whilst keeping both separate though with reduced differences, creating a new genus for it based only on two small differences, or synonymising them. We have chosen the latter route because of the minimal differences, and therefore, we hereby formally synonymise *Asiabregma* Belokobylskij, 2008, with *Conobregma* van Achterberg, 1995.

Facitorus nasseri sp. n. is the first facitorine recorded from Indian subcontinent. The yeliconine subtribe Facitorina consists of the genera *Facitorus, Conobregma* and *Jannya* and they share the following characters; antennal sockets closer to each other than to eyes, frons without groove, antenna situated on a shelf, fore wing vein M+CU strongly curved apically (van Achterberg 1995a). *Facitorus* differs from the rest in having fore wing vein CU1a arising distinctly below the level of 2-CU1 and with comparatively large dorsope, but it shares a plesiomorphic character with *Conobregma* and *Jannya* of having a subposterior depression at the scutellum (Belokobylskij and Long 2005; Belokobylskij et al. 2008). All *Facitorus* species are distributed in the Oriental and South Palearctic Regions. The new species, *F. nasseri* is different from its closest relative *F. superus* (known from Nepal) in having the scutellum with the sub-posterior depression.

Acknowledgements

BAB and DLJQ are very grateful to Mike Sharkey (Kentucky) for making available for study many interesting braconid specimens. BAB and DLJQ would like to thank Chulalongkorn University Centenary Academic Development Project for support, and the Animal Systematic Research Unit and the Integrative Ecology Lab, Department of Biology, Faculty of Science, Chulalongkorn University for allowing us to use their Cell^D imaging facility. SS and APR financially supported by Department of Science and Technology, Government of India under SERB-Young Scientist Scheme (SB/YS/LS-42/2014).

References

van Achterberg C (1985) Notes on Braconidae V. The systematic position of the genera *Ecnomios* Mason and *Pselaphanus* Szépligeti (Hymenoptera: Braconidae). Zoologische Mededelingen, Leiden 59: 341–348.

van Achterberg C (1988) Revision of the subfamily Blacinae Foerster (Hymenoptera: Braconidae). Zoologische Verhandelingen, Leiden 249: 1–324.

van Achterberg C (1991) Revision of the genera of the Afrotropical and W. Palaearctic Rogadinae Foerster (Hymenoptera: Braconidae). Zoologische Verhandelingen, Leiden 273: 1–102.

van Achterberg C (1995a) Generic revision of the subfamily Betylobraconinae (Hymenoptera: Braconidae) and other groups with modified fore tarsus. Zoologisches Verhandelingen, Leiden 298: 1–242.

van Achterberg C (1995b) New taxa of the subfamilies Betylobraconinae, Cenocoeliinae, Ecnomiinae, Homolobinae, and Sigalphinae (Hymenoptera: Braconidae) from East Indonesia. Zoologische Mededelingen, Leiden 69: 307–328.

Belokobylskij SA, Long KD (2005) The braconid wasps of the subfamily Betylobraconinae (Hymenoptera, Braconidae) in Vietnam. Deutsche Entomologische Zeitschrift 52(1): 155–164. doi: 10.1002/mmnd.200410008

Belokobylskij SA, Zaldivar-Riverón A, Maetô K, Sáez AG (2008) Asian Betylobraconinae (Hymenoptera, Braconidae), with description of a new genus and phylogenetic affinities of the tribe Facitorini. Insect Systematics and Evolution 39: 133–154. doi: 10.1163/187631208788784110

Butcher BA, Quicke DLJ (2015) A remarkable new genus and species of Rogadinae (Hymenoptera: Braconidae) of uncertain tribal placement, from Papua New Guinea, resembling Betylobraconini stat. nov. Journal of Natural History 49: 2045–2054. doi: 10.1080/00222933.2015.1009405

Butcher BA, Zaldivar-Riverón A, van de Kamp T, Dos Santos Rolo T, Baumbach T, Quicke DLJ (2014) Extension of historical range of Betylobraconinae (Hymenoptera: Braconidae) into Palaearctic Region based on a Baltic amber fossil, and description of a new species of *Mesocentrus* Szépligeti from Papua New Guinea. Zootaxa 3860: 449–463. doi: 10.11646/zootaxa.3860.5.4

Quicke DLJ (2015) Biology, Systematics, Evolution and Ecology of Braconid and Ichneumonid Parasitoid Wasps. Wiley Blackwell, Chichester, 688 pp.

Quicke DLJ, Butcher BA (2015) Description of a new Betylobraconini-like parasitoid wasp genus and species (Hymenoptera: Braconidae: Rogadinae) from Chile. Zootaxa 4021(3): 459–466. doi: 10.11646/zootaxa.4021.3.5

Sharkey MJ, Wharton RA (1997) Morphology & terminology. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) Identification Manual to the New World Genera of Braconidae. Special Publication of the International Society of Hymenopterists, 1, Washington, 19–37

Tobias VI (1979) Two new and little known subfamilies of Braconidae (Hymenoptera) from Australia. Entomologicheskoe Obozrenie 58: 128–142. [In Russian]

Zaldivar-Riverón A, Mori M, Quicke DLJ (2006) Systematics of the cyclostome subfamilies of braconid parasitic wasps (Hymenoptera: Ichneumonoidea): a simultaneous molecular and morphological Bayesian approach. Molecular Phylogenetics and Evolution 38: 130–145. doi: 10.1016/j.ympev.2005.08.006

Zaldivar-Riverón A, Shaw MR, Sáez AG, Mori M, Belokobylskij SA, Shaw SR, Quicke DLJ (2009) Evolution of the parasitic wasp subfamily Rogadinae (Braconidae): phylogeny and evolution of lepidopteran host ranges and mummy characteristics. BMC Evolutionary Biology 8: 329. doi: 10.1186/1471-2148-8-329

ZooKeys 584: 121–134 (2016)
doi: 10.3897/zookeys.584.6320
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

A new species of alpine *Apenetretus* Kurnakov from Taiwan: evidences from DNA barcodes and morphological characteristics (Coleoptera, Carabidae, Patrobini)

Yi-Ming Weng[1,2], Wen-Bin Yeh[1], Man-Miao Yang[1]

1 *Department of Entomology, National Chung Hsing University, No. 250 Kuo-Kuang Road, Taichung, Taiwan 402, R.O.C.* **2** *Master Program for Plant Medicine, National Taiwan University, No.1, Sec. 4, Roosevelt Rd., Taipei, Taiwan, 106, R.O.C.*

Corresponding author: *Wen-Bin Yeh* (wbyeh@nchu.edu.tw)

Academic editor: *A. Casale* | Received 24 August 2015 | Accepted 15 March 2016 | Published 26 April 2016

http://zoobank.org/7AC650D5-A682-4D7F-96D9-CF05C02D0368

Citation: Weng Y-M, Yeh W-B, Yang M-M (2016) A new species of alpine *Apenetretus* Kurnakov from Taiwan: evidences from DNA barcodes and morphological characteristics (Coleoptera, Carabidae, Patrobini). ZooKeys 584: 121–134. doi: 10.3897/zookeys.584.6320

Abstract

There are three isolated mountain ranges in Taiwan including Hsueshan Range, Central Mountain Range, and Yushan Range. The rise of these mountains has resulted in the isolation of some species and caused allopatric distribution resulting in divergence and speciation events of high mountain carabids, especially the flightless carabids such as *Epaphiopsis*, *Apenetretus*, and partial *Nebria*. Genus *Apenetretus* Kurnakov (1960) is typically distributed in high mountain areas of Taiwan. Three of the currently known *Apenetretus* species have been described from different mountain ranges. These species include *A. yushanensis* Habu, *A. nanhutanus* Habu, and *A. smetanai* Zamotajlov and Sciaky. In this study, a new species is described from Hsueshan, a mountain separated from the ranges of the previous known species, *Apenetretus hsueshanensis* **sp. n**. A key to the Taiwanese *Apenetretus* is included. A reconstructed phylogeny of the Taiwanese *Apenetretus* is introduced with the use of mitochondrial cytochrome c oxidase subunit I (COI) gene. Molecular data and geographical distribution of *Apenetretus* support the morphological characteristics observed among those mountain-isolated species and confirms the new species as being distinctly different. Moreover, lineage calibration suggests that the southern *A. yushanensis* is the most distant one compared to the other three northern *Apenetretus* at ca. 1.81 million years ago (mya), while the divergence time of *A. hsueshanensis* to its sister group was dated to 0.94 mya.

Keywords

Apenetretus, Carabidae, Hsueshan, mountain island isolation, new species

Introduction

In Taiwan, mountain ranges that have become isolated over time have played a major role promoting divergent events of high mountain dwelling carabids, especially in species with flightless adults. For example, in *Nebria formosana* Habu and *N. niitakana* Kano, morphological variation has been described in populations across mountain ranges (Habu 1972). Ten species of the *Epaphiopsis* Ueno, a genus endemic to Taiwan, are found in the high altitude mountain ranges across Taiwan (Ueno 1989). In addition, the aforementioned species are either allopatrically distributed in specific mountains or have topography-matched divergences. Obviously, these divergent events are highly associated with the effect of mountain isolation.

The genus *Apenetretus* in Taiwan includes three described species, all of which inhabit alpine areas of different mountain ranges (Habu and Baba 1960; Löbl and Smetana 2003; Terada 2006). *Apenetretus yushanensis* (Habu, 1973) and *A. nanhutanus* (Habu, 1973) were first collected and described from Yushan and Nanhudashan, respectively (Habu 1973) (Fig. 1). A third species, *A. smetanai* (Zamotajlov & Sciaky, 1996), was collected by A. Smetana in Mt. Nenggaoshan in 1992. In the original description, *A. yushanensis* and *A. nanhutanus* were considered as members of *Patrobus* Dejean, 1821 and *Apatrobus* was considered a subgenus under *Patrobus*. In 1992, Zamotajlov proposed that *Apatrobus* be given genus status with the rationale that members of *Apatrobus* had both larger eyes and more prominent temples which were sub-equal in length with eyes and therefore distinctly different from species of *Patrobus*. Therefore, based on this definition, *Apenetretus yushanensis* and *Apenetretus nanhutanus* are moved from the genus *Patrobus* to *Apatrobus*, and the third species, *Apenetretus semetanai* (Zamotajlov & Sciaky, 1996), was published as *Apatrobus semetanai* as well. Subsequently, the phylogeny among taxa including genus *Apatrobus* was studied and the taxonomy of *Apatrobus* was rearranged accordingly (Zamotajlov 2002; Zamotajlov and Wrase 2006). Two subgenera of *Apenetretus* and *Parapatrobus* are apparently different from *Apatrobus* by the absence of setae on ventral side of claw segments thus the both genera were proposed to new sense as genera. The three species originally belong to the subgenus *Apenetretus* in Taiwan were consequently changed into *Apenetretus yushanensis*, *Apenetretus nanhutanus*, and *Apenetretus smetanai*, respectively (Zamotajlov 2002). Although Lorenz still treated *Apentetretus* as a subgenus of *Apatrobus* in the recent catalog (Lorenz 2005), we expediently follow the classification of Zamotajlov, using *Apenetretus* as the genus for the four species in this study.

According to Habu's original description, one additional female specimen with larger body and longer, depressed elytra from Mt. Hsueshan (Mt. T'zu-kao) has been collected and was considered by him as a local variety of *A. yushanensis* (Habu 1973). As more specimens were collected, however, we found several stable characters, including male genital characters, which could be used to distinguish the Hsueshan specimens from the other *Apenetretus* species.

In order to further examine the morphologically similar species, molecular barcoding methods were utilized as a practical process to help reveal candidate cryptic species

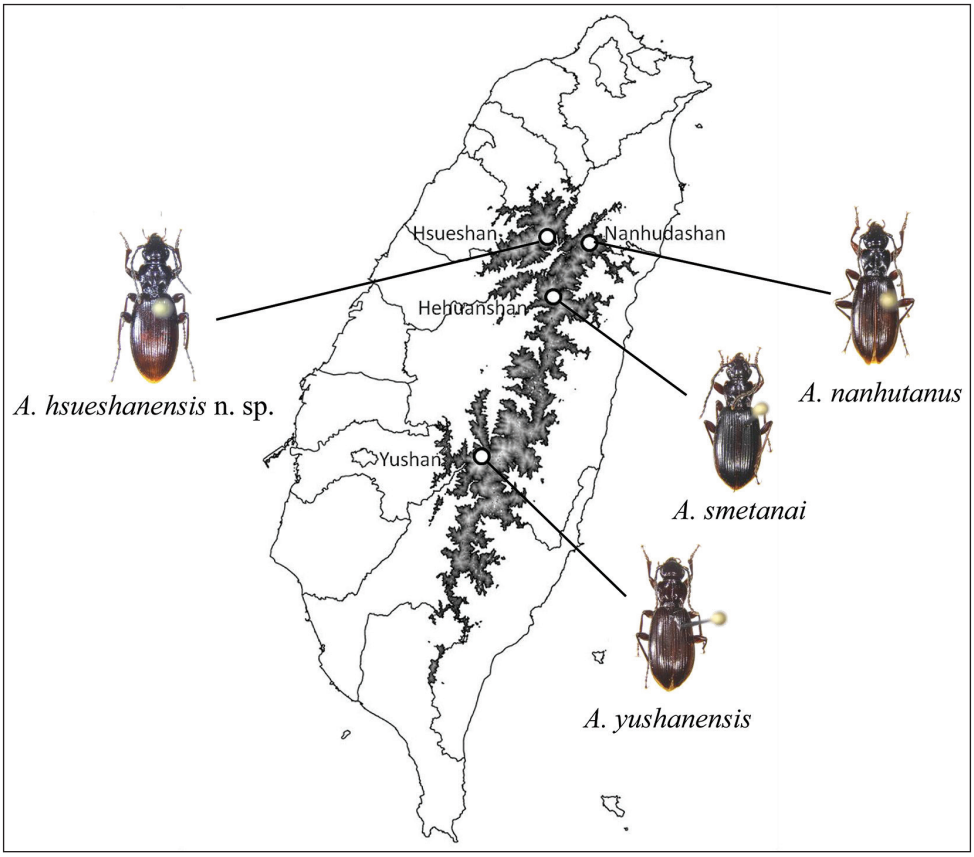


Figure 1. Sample locations of *Apenetretus* spp. *Apenetretus hsueshanensis* sp. n. was collected in Hsueshan; *A. smetanai* was collected in Hehuanshan; *A. nanhutanus* was collected in Nanhudashan; *A. yushanensis* was collected in Yushan. Area of elevation above 2,000 meters is shaded.

among numerous unidentified taxa (Burns et al. 2008; Hebert et al. 2003b; Winterbottom et al. 2014; Yassin et al. 2008). Molecular clock method was also employed to analyze *Apenetretus* genetic divergence times. Here the morphological features of a new *Apenetretus* species are described, including a proposed phylogenetic relationship and divergence time with other species based on mitochondrial cytochrome c oxidase subunit I (COI) gene.

Materials and methods

Study sites and sample collecting

Specimens of *Apenetretus* were collected by hand from various alpine areas across Taiwan. Specimens from the three species preciously described were collected from their respective

Table 1. Sample localities of each species.

	Sample location	latitude	longitude	elevation (m)
A. hsueshanensis sp. n.	Hsueshan	24°23.6N	121°14.7E	3,330
A. yushanensis	Yushan	23°28.5N	120°57.8E	3,369
A. nanhutanus	Nanhutashan	24°22.1N	121°26.5E	3,394
A. smetanai	Hehuanshan	24°8.2N	121°16.5E	3,100

mountain ranges including *A. yushanensis* from Yushan, *A. nanhutanus* from Nanhudashan, and *A. smetanai* from near Nenggaoshan (Table 1; Fig. 1). Twenty one individuals of the new *Apenetretus* species were sampled from Hsueshan in stands of Taiwan white fir (*Abies kawakamii*) forest or along brooks near Sanliujiu cabin (ca. 3,330 m). Eight individuals of *A. yushanensis* were collected from Laonong river campsite (ca. 3,369 m) close to Yushan, twenty *A. nanhutanus* along the stream in Nanhu glacial cirque, (ca. 3,394 m) near Nanhudashan and fifteen individuals of *A. smetanai* from the vicinity of Hehuanshan (ca. 3,100 m, close to Nenggaoshan) were collected.

Morphological measurements

Measurements of morphological characters were done with a Leica S8APO microscope connected to a Canon 600D camera. After taking character photos, images were stacked with software CombineZP (Hadley 2010). Characters were examined and measured with the use of ImageJ 1.48, image analyzing software (Schneider et al. 2012).

DNA extraction, amplification, and sequencing

For molecular work, twelve individuals of *A. hsueshanensis* sp. n., ten of *A. smetanai*, ten of *A. nanhutanus*, and eight of *A. yushanensis* were used for DNA extraction. Following the instructions of BuccalAmp™ DNA Extraction Kit (Epicentre Biotechnologies, Madison, WI), genomic DNA was extracted from one hind tarsus of each individual by glass homogenizer grounding in 50 µl QuickExtract Solution, centrifuging for 15 sec, incubating at 65°C for 10 min, centrifuging for 15 sec again, and then incubating at 98°C for 2 min. Finally, the resultant genomic DNA products were stored at -20°C for polymerase chain reaction (PCR).

Mitochondrial COI barcode region was amplified with forward primer Col46 (5'-AACCATAAAGATATTGGAAC-3') and reverse primer Col731 (5'-CAACAT TTATTTTGATTTTTTGG-3') in PCR (Tsai et al. 2014). The PCR assay was performed in a volume of 25 µl containing 2 µl genomic DNA extraction as template, 2.5 µl 10X Taq buffer, 0.5 µl Prime Taq DNA polymerase (GENET BIO, Korea), 0.4 µl dNTP (25 µM), and 1 µl of each primer (10 µM). After the initial denaturation at 94 °C for 2 min, PCR programming conditions were followed by 35 cycles of 94 °C

for 30 sec, 52 °C for 30 sec and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were purified from 1% agarose gel using QIA quick Gel Extraction Kit (Qiagen, Hilden, German). The resulting DNA product was sequenced in both strands using Taq dye terminator Cycle Sequencing Kit (Applied Biosystems, Foster, CA) and an ABI 377A sequencer. Sequences of COI for the four species have been deposited in GenBank under the accession numbers KR868997–KR869036.

Molecular analyses and phylogeny reconstruction

Sequences were aligned with BioEdit 7.0 software (Hall 1999). Proportional distances among species were conducted using MEGA version 6 (Tamura et al. 2013). The optimal substitution model HKY+I was choice according to jModelTest for Maximum likelihood tree construction and molecular clock calculation (Darriba et al. 2012; Guindon and Gascuel 2003). Phylogenetic inference was performed using maximum likelihood (ML) method with 1,000 bootstrap replications with PhyML version 3.0 (Guindon et al. 2010). The strict molecular clock of the COI gene was calculated under the rate of 3.54% per million years with software BEAST version 1.8.0 (Drummond et al. 2012; Papadopoulou et al. 2010).

Results and discussion

Species description

***Apenetretus hsueshanensis* sp. n.**
http://zoobank.org/AE24C089-561D-4069-B9E2-422AB3B2E67A
Figs 2, 3A, 4A, 5A, 5E, 6

Type locality. Taiwan: Mt. Hsueshan, Hsei-Pa National Park, Black Forest near Sanliujiu Cabin, ca. 3,330 m elevation, 24°23.6N, 121°14.7E.

Type material. Holotype: a male, deposited in National Chung-Hsing University (NCHU) Museum of Entomology, labeled: " TAIWAN, Taichung, Heping District, Hsueshan, Sanliujiu Cabin, 3,330 m, 24°23.6N, 121°14.7E, 08 April 2011, Y. M. Weng collector (red label). Paratypes: A total of 10, 3 males and 4 females with the same collection data as the holotype, 1 male and 2 females labeled: TAIWAN, Taichung, Heping District, Hsueshan, Sanliujiu cabin, 3,330 m, 24°23.6N, 121°14.7E, 01 Oct 2010, Y. M. Weng collector.

Etymology. The new species is named after the original collecting locality, Mt. Hsueshan, where it is likely endemic.

Diagnosis. *Apenetretus hsueshanensis* sp. n. is morphologically similar to the other Taiwanese *Apenetretus* species (*A. yushanensis*, *A. nanhutanus*, and *A. smetanai*). It can be distinguished externally from the other three species by having more slender elytra

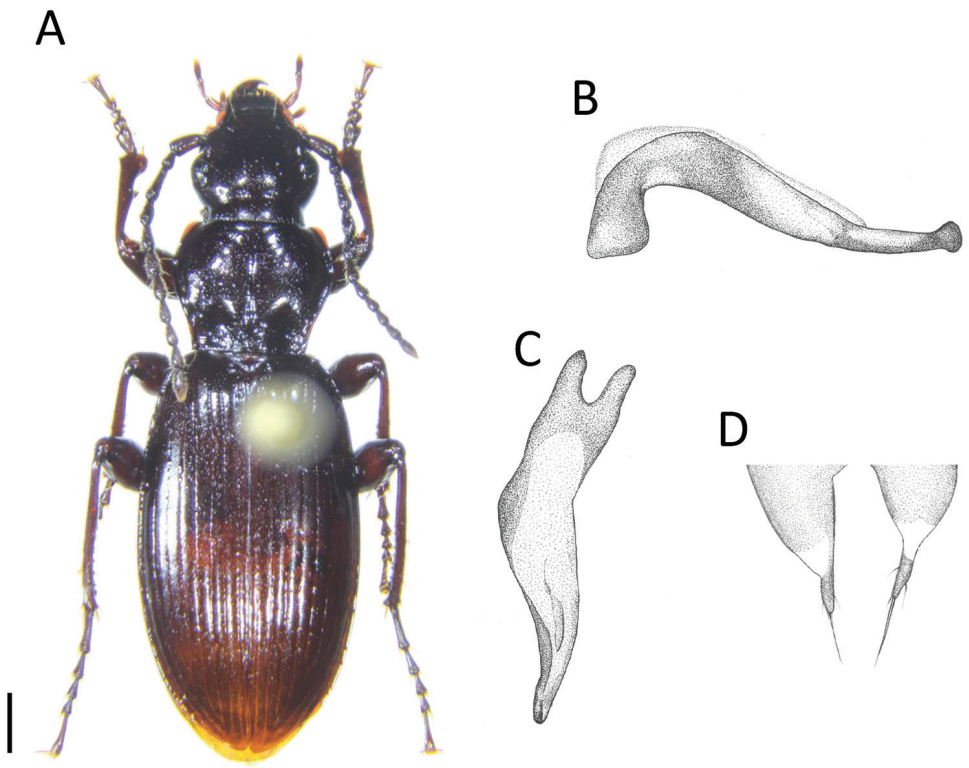


Figure 2. Male of *Apenetretus hsueshanensis* sp. n. (holotype). **A** dorsal view of habitus **B** lateral view of male aedeagus (2×) **C** dorsal view of aedeagus (2×) **D** parameres (3×). Scale bar: 1 mm.

and a ratio of elytral length/width (EL/EW=1.76–1.90) that differs from all other species (Fig. 2A) 1.67–1.75, 1.67, 1.53–1.67, respectively (Habu 1973; Zamotajlov and Sciaky 1996). This character is especially useful in separating male individuals. Male genitalia; aedeagus large (ca. 3 mm in length) and more slender than the other three species (ca. 2.5 mm in length); extremely elongated and twisted after middle (Figs 3, 4). Apical portion of the parameres is prolonged and longer than the other species (Fig. 5) (Habu 1973; Zamotajlov and Sciaky 1996).

Description. Male 10.79–11.77 mm in length, 3.50–3.79 mm in width, female 11.10–12.22 mm in length, 3.71–4.01 mm in width. Color brown to black, ventral surface reddish brown; labrum, mandibles, palpi, legs, and margin of pronotum and elytra lighter in color (Fig. 2A).

Head convex, frontal impression, neck-constriction punctate; microsculpture faint and isodiametric in dorsal view; neck-constriction deep; temporae faintly tumid, longer than eyes, 1.11 (0.88–1.25) times as long as eye in average (only one individual in fifteen individuals has longer eye length than temporae); eye large, convex; with tooth at subapical terminal; palpi truncate at apex; supraorbital setae varied, some individuals

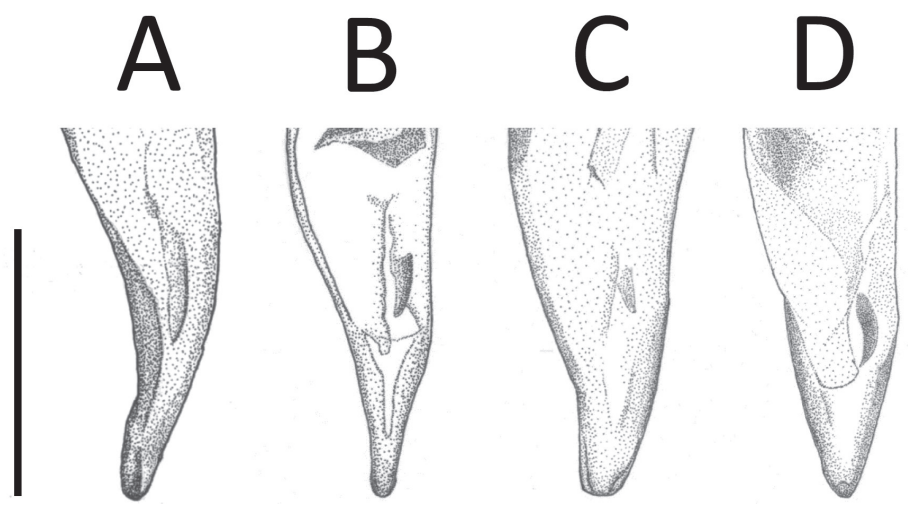


Figure 3. Apical portion of aedeagus of *Apenetretus* spp. in dorsal view. **A** *Apenetretus hsueshanensis* sp. n. holotype **B** *A. smetanai* **C** *A. yushanensis* **D** *A. nanhutanus*. Adapted from Habu 1973; Zamotajlov and Sciaky 1996. Scale bar: 1 mm.

have two closely anterior and one posterior (Fig. 6A), some with only one anterior and one posterior (Fig. 6C), sometimes one between eyes and clypeus, one anterior, and one posterior (Fig. 6B), or one anterior, one between anterior and posterior, and one posterior (Fig. 6D); distance between supraorbital posterior setae rather short, 0.78 (0.73–0.84) times as wide as anterior seta distance; frontal impressions deep, reaching clypeal setae, sometimes divergent posteriorly as *A. smetanai*; third segment of antenna rather long, 1.47 (1.23–1.59) times as long as forth segment; forth segment of antenna longer than fifth segment, 1.09 (1.03–1.15) times as fifth segment; tenth segment 1.78 (1.68–1.94) times as long as wide; eleventh segment rather prolonged, 2.5 (2.29–2.77) times as long as wide.

Pronotum weakly convex, widest at about one third, 1.22 (1.18–1.32) times as wide as head, 1.23 (1.17–1.29) times as wide as long, 1.35 (1.28–1.39) times as wide as posterior margin, anterior generally as wide as posterior margin, 1.00 (0.95–1.06) times as anterior margin as posterior margin; microsculpture faint and isodiametric; anterior margin straight to rounded and protrudingt at angles; surface faintly punctate at apical areas, rather punctate along median line, lateral margins, and basal area; posterior margin straight, shallowly sinuate near hind angles; hind angles acute to rectangular, slightly prominent laterally; lateral margin subsinuate, from front angles to the widest points, rather round from the widest points to the turning points, then prominent to the posterior seta pore; anterior marginal setae located before the widest point; posterior setae in hind angles; median line deep, sometimes reaching both extremities, generally reaching to anterior transverse impression; anterior transverse impression shallow, sometimes deep and forming a Y-shaped impression; posterior impression and basal foveae deep; disk smooth, rather cordate.

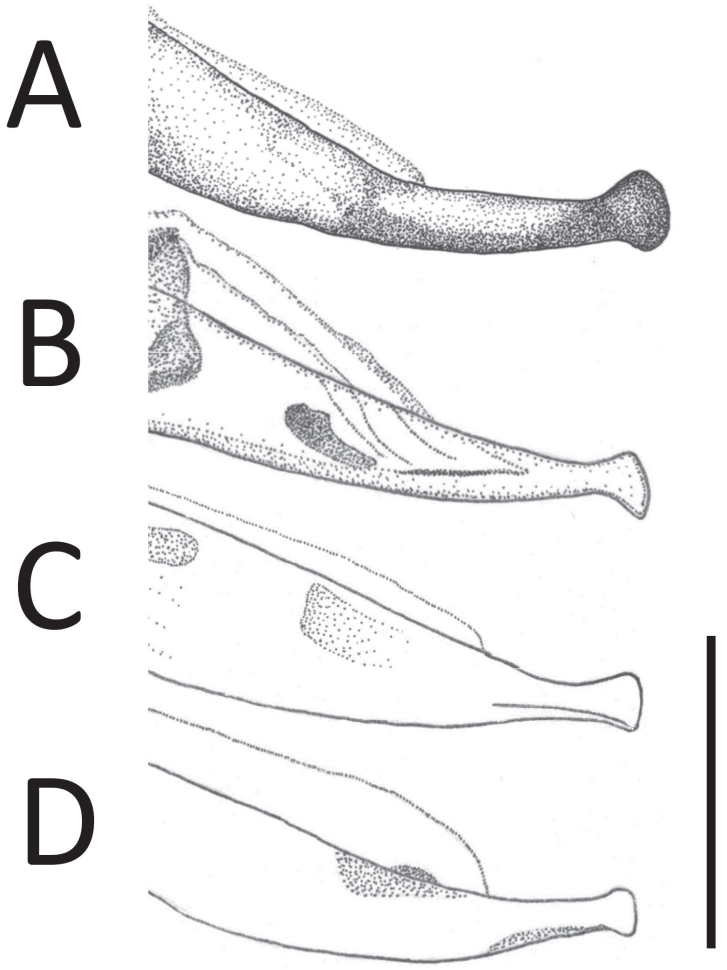


Figure 4. Apical portion of aedeagus of *Apenetretus* spp. in lateral view. **A** *Apenetretus hsueshanensis* sp. n. holotype **B** *A. smetanai* **C** *A. yushanensis* **D** *A. nanhutanus*. Adapted from Habu 1973; Zamotajlov and Sciaky 1996. Scale bar: 1 mm.

Wings atrophied, 0.3 times as long as elytra; elytra rather convex, ovate and more slender than the other three species (Habu 1973; Zamotajlov and Sciaky 1996), 1.82 (1.76–1.90) times as long as wide, widest behind middle, 1.42 (1.30–1.54) times as wide as pronotum, shoulders with one small tooth on each side, wider than posterior margin of pronotum; microsculpture distinct, isodiametric; lateral margin subsinuate before one third, then rounded, apex elongated subapically; striae rather shallow, sometimes finely punctate; scutellary striole punctate; intervals flat, 3rd interval with 3 pores at 0.22 (0.20–0.26), 0.49 (0.41–0.52), and 0.73 (0.69–0.77) times of elytra length; marginal series composed of 10–12 pores.

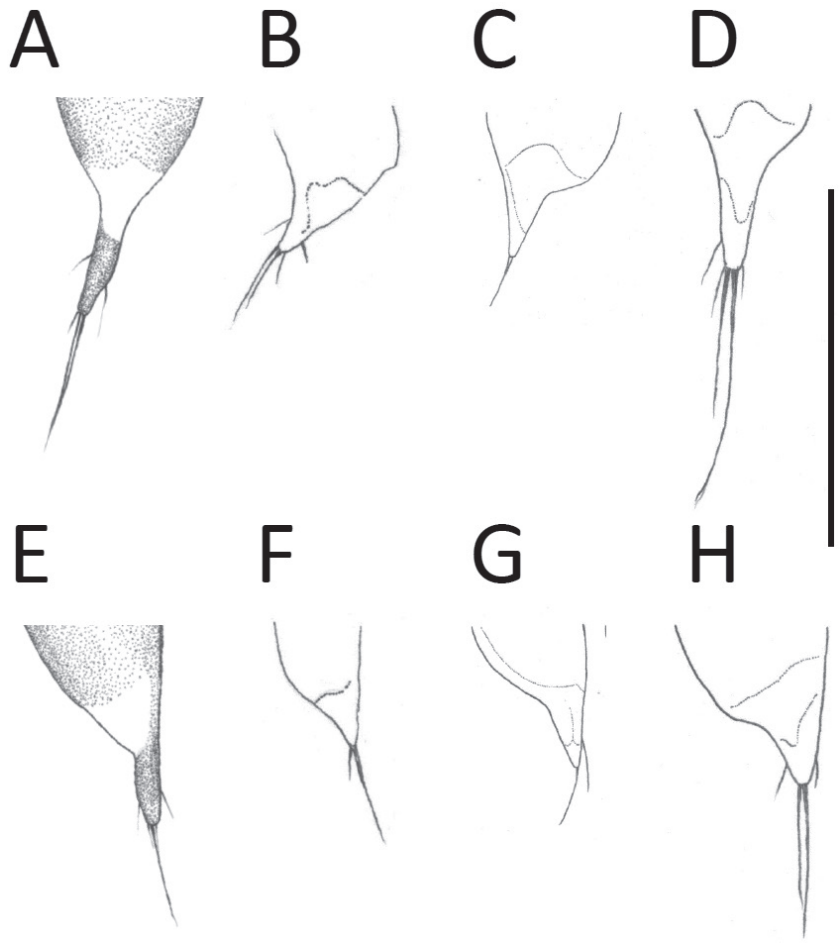


Figure 5. Right parameres (**A**–**D**) and left parameres (**E**–**H**) of *Apenetretus* spp. **A, E** *Apenetretus hsueshanensis* sp. n. holotype **B, F** *A. smetanai* **C, G** *A. yushanensis* **D, H** *A. nanhutanus*. Adapted from Habu 1973; Zamotajlov and Sciaky 1996. Scale bar: 1 mm.

Mesepistern, metepistern, and mesostern, lateral of prostern, metasternum, and pregenital sterna 1 with distinct punctures; ventral side of neck constriction shallowly rugose on each side; metepistern longer than wide.

Aedeagus (Fig. 2B, C) slender, curved to right side in dorsal view, curved and elongate before middle (Fig. 2B); apical lamella extremely twisted toward right side, forming a ridge at middle in dorsal view and hammer shape at apex in lateral view (Fig. 2C); left margin reflexed and sinuate in dorsal view; parameres different in shape and size of left and right, left paramere wider than right one, apical projection extended, much longer than the other three species, apex with two long and one short setae, and two short setae at each subapical margin (Fig. 2D).

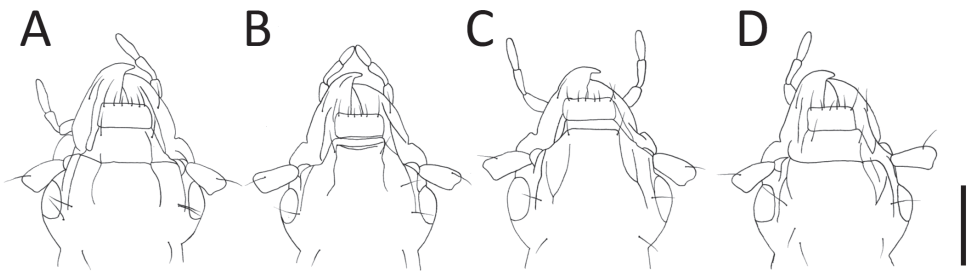


Figure 6. Variation in supraobital setae placement of *Apenetretus hsueshanensis* sp. n. **A** two close anterior setae and one posterior **B** one between eyes and clypeus, one anterior, and one posterior; **C**, one anterior and one posterior **D** one anterior, one between anterior and posterior, and one posterior. Scale bar: 1 mm.

Key to *Apenetretus* species of Taiwan

1 Antenna moniliform, reaching to basal one seventh of elytra; apical part of parameres short, with one short seta at apex and one or no subapical seta (Fig. 5C, G) ..... ***A. yushanensis* Habu**

– Antenna slender, reaching to basal one fifth to one sixth of elytra; apical part of parameres longer, with two long seta and one or no short seta at apex, and two short subapical seta on each side (Fig. 5A, E) **2**

2 Elytra prolonged, more than one and three fourth as long as wide; aedeagus long, (~3mm), extended and extremely twisted toward right side behind middle (Fig. 3A); apical portion of parameres markedly prolonged (Fig. 5A and 5E) ..... ***A. hsueshanensis* sp. n.**

– Elytra not prolonged, one and one half to one and three fourth as long as wide; aedeagus shorter, mostly 2–2.5 mm long, evenly contracted toward apex; apical part of parameres less prolonged (Fig. 5B/F and 5D/H)..... **3**

3 Palpi truncate and depressed apically; temporae longer than eye; front angles of pronotum stronger projected ..... ***A. smetanai* Zamotajlov & Sciaky**

– Palpi not truncate; temporae same length as eye; anterior angles of pronotum weakly projected ..... ***A. nanhutanus* Habu**

Genetic differentiation of *Apenetretus* in Taiwan

Phylogenetic analysis of molecular work with the COI gene (686 bp) shows four distinct lineages within the *Apenetretus* of Taiwan (Fig. 7). *Apenetretus yushanensis* is the most basal lineage; members of *A. hsueshanensis* form a sister group to members of *A. nanhutanus* and *A. smetanai* (Fig. 7). The tree topology is consistent with the results of genetic divergence which informs that the most distinct species is *A. yushanensis* and the least divergent species are *A. smetanai* and *A. nanhutanus* (Table 2). It is worth noting that the genetic p-distance among these *Apenetretus* species are close or higher than 2%, the value defined as the general threshold of species differentiation (Hebert et al.

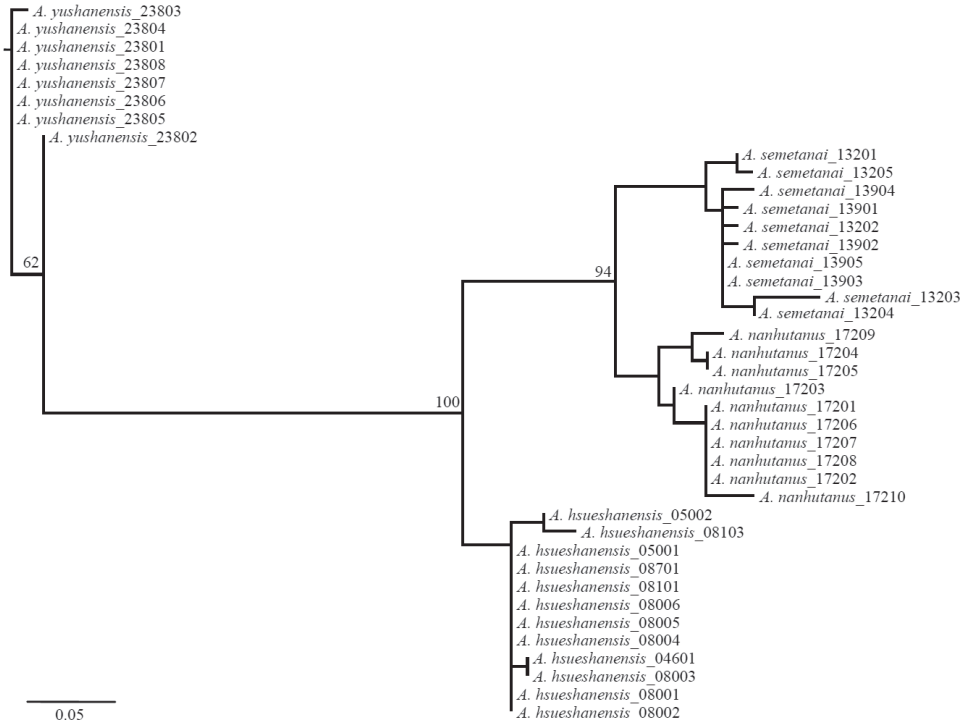


Figure 7. Mitochondrial COI phylogeny of Taiwanese *Apenetretus* constructed with Maximum Likelihood method. One thousand bootstrap values are showed on the branches in percentage.

Table 2. P-distance among species of COI gene.

	***A. hsueshanensis* sp. n.**	***A. smetanai***	***A. nanhutanus***
A. hsueshanensis sp. n.	-	-	-
A. smetanai	0.027	-	-
A. nanhutanus	0.024	0.019	-
A. yushanensis	0.038	0.049	0.048

2003a; Hebert et al. 2003b). The divergent trend among *Apenetretus* species is likely to fit with the geological topology of the mountain ranges in Taiwan, where Yushan and Hsueshan Ranges are distinct from Central Mountain Range including Hehuanshan and Nanhudashan. The southern *A. yushanensis* is the most divergent one to the other three northern *Apenetretus* at ca. 1.81 million years ago (mya). The divergence time between *A. hsueshanensis* and its sister group was dated to 0.94 mya, a period which is sufficient for speciation to occur (Fig. 8), which further supports our findings that there is an independent species occurring in Hsueshan. Therefore, the localized Hsuehsan carabids with >2% COI divergent content have most likely speciated allopatrically due to the effect of mountain-island isolation. Interestingly, the divergent time between

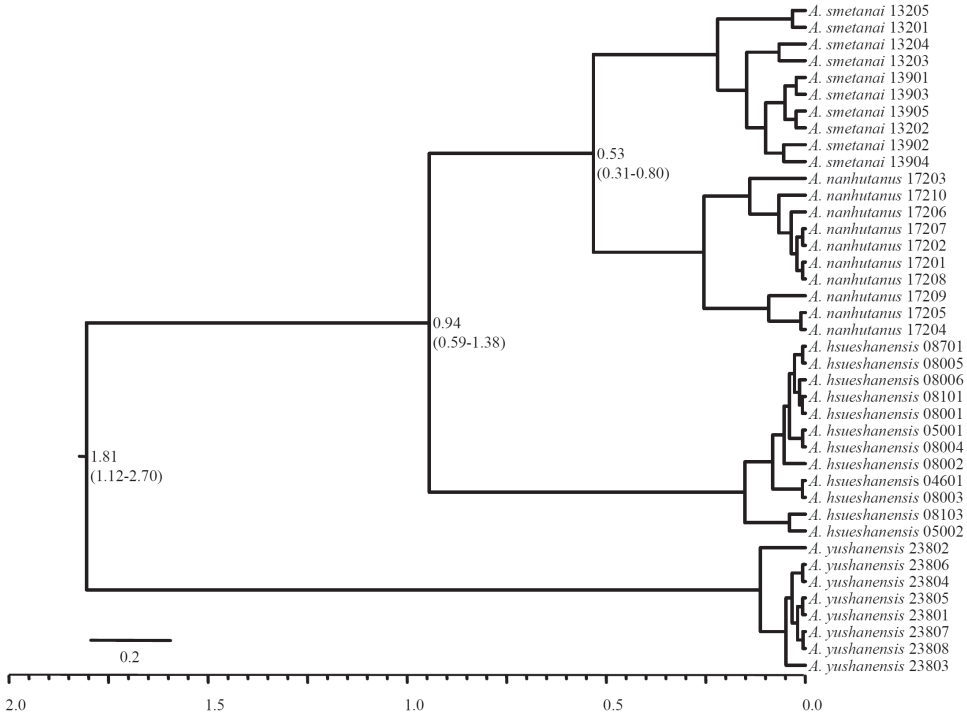


Figure 8. Molecular clock dating of mitochondrial COI gene with BEAST ver. 1.8.0. The oldest divergence between *A. yushanensis* and the other *Apenetretus* species occurred at 1.81 million years ago (mya); the divergence between *A. hsueshanensis* and the group of *A. smetanai* and *A. nanhutanus* occurred at 0.94 mya; and the divergence between *A. smetanai* and *A. nanhutanus* occurred at 0.53 mya.

A. smetanai and *A. nanhutanus*, the two most closely distributed and morphologically similar species appear to have diverged only 0.53 mya. It is yet unclear if there is a geographical barrier between two species, so the possibility is exit that the two species may have other forms of isolated barrier such as isolated by distance or intermittently contact due to glacial cycles. The question can be resolved only by examinations and analyses of series collection along Central Mountain Range.

Acknowledgements

We thank Wesley Hunting, who taught us techniques of collection, preparation and dissection of carabid beetles and provided assistance with an early version of this work. We thank Dr. Dave Kavanaugh, who taught us how to examine carabid genitalia in detail and understand important characters. We also thank Cheng-Lung Tsai, William Chiu, Zong-Han Yang, and Yi-Zhang Liao, who helped us collect the examined specimens from the high mountains of Taiwan. We thank Scotty Yang, who provided im-

portant support both materially and spiritually, and Shih-Tsai Yang who reviewed and corrected our species descriptions. The authors also acknowledge the High-throughput Genome and Big Data Analysis Core Facility, Taiwan (MOST 104-2319-B-010-001), for sequencing. This study was partially supported by the Shei-Pa National Park (SP10102)

References

Burns JM, Janzen DH, Hajibabaei M, Hallwachs W, Hebert PD (2008) DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservacion Guanacaste, Costa Rica. Proceedings of the National Academy of Sciences 105: 6350–6355. doi: 10.1073/pnas.0712181105

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772–772. doi: 10.1038/nmeth.2109

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973. doi: 10.1093/molbev/mss075

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321. doi: 10.1093/sysbio/syq010

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704. doi: 10.1080/10635150390235520

Habu A (1972) Notes and descriptions of Formosan Carabidae taken by Dr. S.-I. ueno in 1961 (Coleoptera: Carabidae). I. on three *Nebria* species. Transactions of the Shikoku Entomological Society 11: 71–80.

Habu A (1973) Notes and descriptions of Formosan Carabidae taken by Dr. S.-I. ueno in 1961 (Coleoptera: Carabidae). II. a new *Broscosoma* and two new *Patrobus* species. Transactions of the Shikoku Entomological Society 11: 99–106.

Habu A, Baba K (1960) A new species of *Patrobus* from Mts. Iide, with the key to the *Patrobus*-species of Japan (Coleoptera, Carabidae). Transactions of the Kyoto Entomological Society 9: 1–10.

Hadley A (2010) Combine ZP Software, new version. http://www.hadleyweb.pwp.blueyonder.co.uk/

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hebert PD, Ratnasingham S, de Waard JR (2003a) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London B: Biological Sciences 270: 96–99. doi: 10.1098/rsbl.2003.0025

Hebert PDN, Cywinska A, Ball SL (2003b) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B: Biological Sciences 270: 313–32. doi: 10.1098/rspb.2002.2218

Löbl I, Smetana A (2003) Catalogue of Palaearctic Coleoptera, Vol. 1: Archostemata-Myxophaga-Adephaga. Apollo Books, Stenstrup 1: 819.

Lorenz W (2005) Systematic list of extant Ground Beetles of the World (Insecta Coleoptera "Geadephaga": Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae). Second Edition. Wolfgang Lorenz, Tutzing, 530 pp.

Papadopoulou A, Anastasiou I, Vogler AP (2010) Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. Molecular Biology and Evolution 27: 1659–1672. doi: 10.1093/molbev/msq051

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671–675. doi: 10.1038/nmeth.2089

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. doi: 10.1093/molbev/mst197

Terada K (2006) A checklist of the Carabidae (Coleoptera) recorded from Taiwan. Miscellaneous Reports of the Hiwa Museum for Natural History 46: 1–72.

Tsai C-L, Wan X, Yeh W-B (2014) Differentiation in stag beetles, *Neolucanus swinhoei* complex (Coleoptera: Lucanidae): Four major lineages caused by periodical Pleistocene glaciations and separation by a mountain range. Molecular Phylogenetics and Evolution 78: 245–259. doi: 10.1016/j.ympev.2014.05.004

Ueno SI (1989) The Taiwanese species of the genus *Epaphiopsis* (Coleoptera, Trechinae). Bulletin of the National Science Museum Series A Zoology 15: 105-137

Winterbottom R, Hanner RH, Burridge M, Zur M (2014) A cornucopia of cryptic species-a DNA barcode analysis of the gobiid fish genus *Trimma* (Percomorpha, Gobiiformes). ZooKeys: 381: 79–111. doi: 10.3897/zookeys.381.6445

Yassin A, Capy P, Madi-Ravazzi L, Ogereau D, David JR (2008) DNA barcode discovers two cryptic species and two geographical radiations in the invasive drosophilid *Zaprionus indianus*. Molecular Ecology Resources 8: 491–501. doi: 10.1111/j.1471-8286.2007.02020.x

Zamotajlov A, Sciaky R (1996) Contribution to the knowledge of Patrobinae (Coleoptera, Carabidae) from south-east Asia. Coleoptera 20: 1–63.

Zamotajlov A (2002) Inferring phylogenetic system of the carabid subfamily Patrobinae (Coleoptera, Carabidae). St-Petersburg: Zoological Institute of Russian Academy of Sciences (Meetings in memory N.A. Cholodkovsky; Iss. 55): 1–145. [In Russian]

Zamotajlov A, Wrase D (2006) New taxa of the tribe Patrobini (Coleoptera, Carabidae) from the East Himalayas. Linzer Biologische Beiträge 38: 991–1008.

ZooKeys 584: 135–164 (2016)
doi: 10.3897/zookeys.584.7882
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

What Azure blues occur in Canada? A re-assessment of *Celastrina* Tutt species (Lepidoptera, Lycaenidae)

B. Christian Schmidt[1], Ross A. Layberry[2]

1 *Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture and Agri-Food Canada, K.W. Neatby Bldg., 960 Carling Ave., Ottawa, ON, Canada K1A 0C6* **2** *6124 Carp Road, Kinburn, Ontario, Canada K0A 2H0*

Corresponding author: *B. Christian Schmidt* (christian.schmidt@agr.gc.ca)

Academic editor: *T. Simonsen* | Received 22 January 2016 | Accepted 24 March 2016 | Published 26 April 2016

http://zoobank.org/3C316B6E-9994-4CDA-A04A-AD66CC0AF685

Citation: Schmidt BC, Layberry RA (2016) What Azure blues occur in Canada? A re-assessment of *Celastrina* Tutt species (Lepidoptera, Lycaenidae). ZooKeys 584: 135–164. doi: 10.3897/zookeys.584.7882

Abstract

The identity of *Celastrina* species in eastern Canada is reviewed based on larval host plants, phenology, adult phenotypes, mtDNA barcodes and re-assessment of published data. The status of the Cherry Gall Azure (*C. serotina* Pavulaan & Wright) as a distinct species in Canada is not supported by any dataset, and is removed from the Canadian fauna. Previous records of this taxon are re-identified as *C. lucia* (Kirby) and *C. neglecta* (Edwards). Evidence is presented that both *Celastrina lucia* and *Celastrina neglecta* have a second, summer-flying generation in parts of Canada. The summer generation of *C. lucia* has previously been misidentified as *C. neglecta*, which differs in phenology, adult phenotype and larval hosts from summer *C. lucia*. DNA barcodes are highly conserved among at least three North American *Celastrina* species, and provide no taxonomic information. *Celastrina neglecta* has a Canadian distribution restricted to southern Ontario, Manitoba, Saskatchewan and easternmost Alberta. The discovery of museum specimens of *Celastrina ladon* (Cramer) from southernmost Ontario represents a new species for the Canadian butterfly fauna, which is in need of conservation status assessment.

Keywords

Voltinism, *Cornus*, *Viburnum*, Eastern Flowering Dogwood, Eriophyidae, Cherry gall, degree-day model, DNA barcode

Introduction

Blues of the genus *Celastrina* Tutt, commonly known as azures, are perhaps the most familiar spring butterflies in Canada, occurring in all ecoregions except the high arctic. Despite their ubiquity, their identification and taxonomy is difficult, with species boundaries and nomenclature having a long history of controversy and confusion. Forty years ago, all North American *Celastrina* taxa were generally considered to represent variation within a single species described from Europe, *C. argiolus* (L.) (Langston 1975). This view remained essentially unchanged for another twenty years, with the exception of a second taxon, *C. nigra* (Forbes) recognized by Miller and Brown (1981) and Scott (1986). A global revision of *Celastrina* and related genera further entrenched the concept of only two North American species (Eliot and Kawazoé 1983). However, with a more detailed study of the genus in North America additional cryptic species were gradually recognized by some (Opler and Krizek 1984, Pratt et al. 1994, Scott and Wright 1998, Wright and Pavulaan 1999, Pavulaan and Wright 2005). *Celastrina* taxonomy is still unsettled, with recent comprehensive North American checklists varying between three (NABA 2001) and nine recognized species (Pelham 2008). A summary of some of the changing concepts, particularly in the historical literature, is given by Pratt et al. (1994) and Pavulaan (2014).

The conservative morphological variation between most *Celastrina* species, coupled with adult seasonal polyphenism, has been a major impediment to *Celastrina* taxonomy and dictated a gradual refinement of species concepts. Comparative data on molecular variation, physiology, development and ecology for sympatric or closely parapatric populations are therefore particularly important in evaluating species concepts, yet such data are largely lacking (but see Pavulaan 2014). To provide a taxonomic reference point for Canada's *Celastrina* populations and to stimulate further study, the identity of Ontario *Celastrina* populations is re-assessed based on published and novel data on phenology, larval host plant use and mtDNA variation. Ontario provides a unique geographic arena where biological and biogeographical attributes of putative species can be examined. Here, three species purportedly occur in sympatry: *C. lucia* (Kirby), *C. serotina* Pavulaan & Wright and *C. neglecta* (Edwards) (Layberry et al. 1998, Pavulaan and Wright 2005). A fourth species, *C. ladon* (Cramer), has been reported from adjacent parts of Ohio and Michigan (Nielsen 1999). With potentially as many as four species present in Ontario, life history traits and diagnostic characters of *Celastrina* were studied and compared among two ecoregions, the Lake Erie region in southernmost Ontario and the Ottawa region in eastern Ontario. These regions were chosen as both have a long history of entomology with a comparatively large data pool on Lepidoptera, and represent separate ecoregions with all three (and potentially four) eastern Canadian *Celastrina* species present.

Current concepts of eastern Canadian *Celastrina*

Four *Celastrina* species are currently attributed to the Canadian fauna, three of them found in the East. The fourth species, *C. echo* (Edwards), is strictly western and al-

though previously ranked as a subspecies of *C. ladon* (Cramer) (e.g. Layberry et al. 1998), it is now recognized as a distinct species by most authors (e.g. Guppy and Shepard 2001, Pohl et al. 2009, Warren 2005, James and Nunnallee 2011, CESCC 2011). The concept of three eastern Canadian species as presented by Layberry et al. (1998) is in current usage (Hall et al. 2014, eButterfly 2015, Macnaughton et al. 2015), with some nomenclatural updates (Table 1).

Celastrina lucia, the Northern Azure (a.k.a. Spring Azure, a name here reserved for *C. ladon*), is the most widespread azure, occurring in every province and territory. In the boreal and subarctic regions it is the only species of the genus. The Northern Azure has been considered to be univoltine throughout its range, flying in early spring (Layberry et al. 1998, Pavulaan 2014). Populations south of the boreal region, where adults are slightly larger and with a more variable ventral wing pattern, have been treated as a separate taxon (*C.* "*lucia*" of authors), also considered to be a univoltine spring-flying species (Pratt et al. 1994, Pavulaan 2014). There is currently no available scientific name for this taxon, nor is it clear that one is needed, as it may merely represent ecophenotypic variation of boreal *C. lucia*. Larvae of *C. lucia* feed on a wide variety of flowering shrubs but, like *C. serotina*, occasionally also on cherry galls in some parts of the range (Pavulaan 2014).

Celastrina neglecta, the Summer Azure, has a more southerly but overlapping distribution with *C. lucia* and is recorded from all provinces except British Columbia, Newfoundland and Labrador. It is distinguished from *C. lucia* by its later flight season, in Canada flying mostly in July, six to eight weeks after the peak flight of spring-flying *C. lucia*. All summer-flying *Celastrina* in southern Canada have been assigned to *C. neglecta* (Layberry et al. 1998, Hall et al. 2014, eButterfly 2015), based on the premise that *C. lucia* is univoltine, and that the time between spring (*C. lucia*) and summer (*C. neglecta*) *Celastrina* flights is not enough for a summer flight to represent a second generation of *C. lucia* (noted as early as Saunders 1875). However, Eberlie (1996, 1997) documented that late-summer larvae from Northumberland County (Ontario), by definition *C. neglecta*, can produce typical early-spring *C. lucia* adults the following year. This phenomenon has also been documented in the Ottawa region (Layberry 2004). The diagnostic value of phenology is complicated further by the possibility that *C. neglecta* sometimes has an earlier flying, spring brood according to Pavulaan (2014), which is difficult or impossible to segregate morphologically from *C. lucia*. Conversely, the possibility of second-generation *C. lucia* has not been adequately evaluated in Canadian populations.

Celastrina serotina, the Cherry Gall Azure, is also a univoltine species but with a late spring flight, between that of *C. lucia* and *C. neglecta*. There is some doubt in the species status of Ontario populations of *C. serotina*, as larvae reared from cherry galls in the spring can produce *C. neglecta*-type adults in the same season (Layberry 2004). The peak flight time of *Celastrina serotina* is from late May to late June in Ontario, about three weeks after that of *C. lucia*, and before the *C. neglecta* peak in July (Pavulaan and Wright 2005). The larvae are said to feed almost exclusively on eriophyid mite galls on black cherry (*Prunus serotina* Ehrh.) and choke cherry (*P. virginiana* L.) leaves.

Table 1. Changing concepts of Canadian *Celastrina* species.

Current concept (Pelham 2011)	Pavulaan and Wright 2005	NABA 2001	Wright and Pavulaan 1999	Layberry et al. 1998	Pratt et al. 1994	Scott 1984
C. lucia	*lucia* (+ *lucia* auct.)	*C. ladon*	*C. ladon lucia*	*C. ladon lucia*	*C. ladon lucia*	*C. argiolus*
C. serotina	*C. serotina*	*C. ladon*	*C. l. ladon*	*C.* sp. n.	*C. ladon* "violacea II"	*C. argiolus*
C. neglecta	*C. neglecta*	*C ladon neglecta*	*C. neglecta*	*C. neglecta*	*C. ladon neglecta*	*C. argiolus*
C. echo	n/a	*C. ladon*	n/a	*C. ladon nigrescens*, *C. ladon echo*	*C. ladon nigrescens, C. ladon echo*	*C. argiolus*
C. ladon	*C. ladon*	*C. ladon*	*C. ladon ladon*	n/a	*C. ladon* "violacea I"	*C. argiolus*

The phenology and larval host plant are key diagnostic features used to distinguish *C. serotina*. Also, the ventral hindwing pattern is stated to be paler whitish grey on average than *C. lucia*, with heavily marked forms being rare. The taxonomy of Ontario *C. serotina* is particularly relevant since life histories and specimens of these populations formed part of the original species description (Wright and Pavulaan 2005). *Celastrina serotina* has also been reported from Québec, New Brunswick, Nova Scotia and Prince Edward Island (Layberry et al. 1998), and recently from Manitoba (based on larval collections, leg. T. Rapati; eButterfly 2015, record #EB-3473).

Celastrina ladon, the Spring Azure, has not been reported in Canada in the sense of the modern concept of the species, where the diagnostic male wing scale morphology (Fig. 1) separates it from all other *Celastrina* (see also Omura 2015; Wright and Pavulaan 1999). Literature reports of *C. ladon* in Canada consist of previous concepts where *C. ladon* and *C. lucia* were considered to be conspecific (e.g. Wright and Pavulaan 1999). *Celastrina ladon* has subsequently not been included in the Canadian fauna (Hall et al. 2014). Older reports of *C. ladon* from southern Ontario may have included true *C. ladon*, but these records cannot be distinguished from *C. lucia* without voucher material. The Spring Azure is known from adjacent parts of southern Michigan (Nielsen 1999) and Ohio (Wright 1998).

Methods and materials

Specimens examined during this study included those deposited in the Canadian National Collection of Insects, Arachnids and Nematodes (CNC), in addition to *Celastrina* records with voucher photographs on eButterfly (2015). Forewing androconial scales of male *Celastrina* were examined using a Leica 205C dissecting scope. Vouchers of reared specimens are deposited in the CNC.

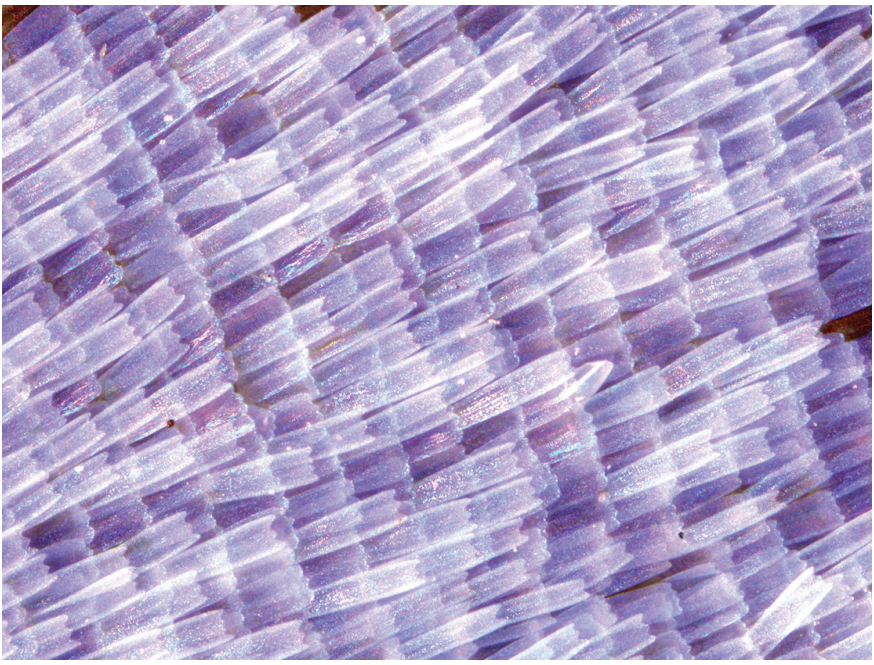


Figure 1a. Male *Celastrina ladon* forewing showing distinctive overlapping scales and lack of androconial scales. Normandale, ON.

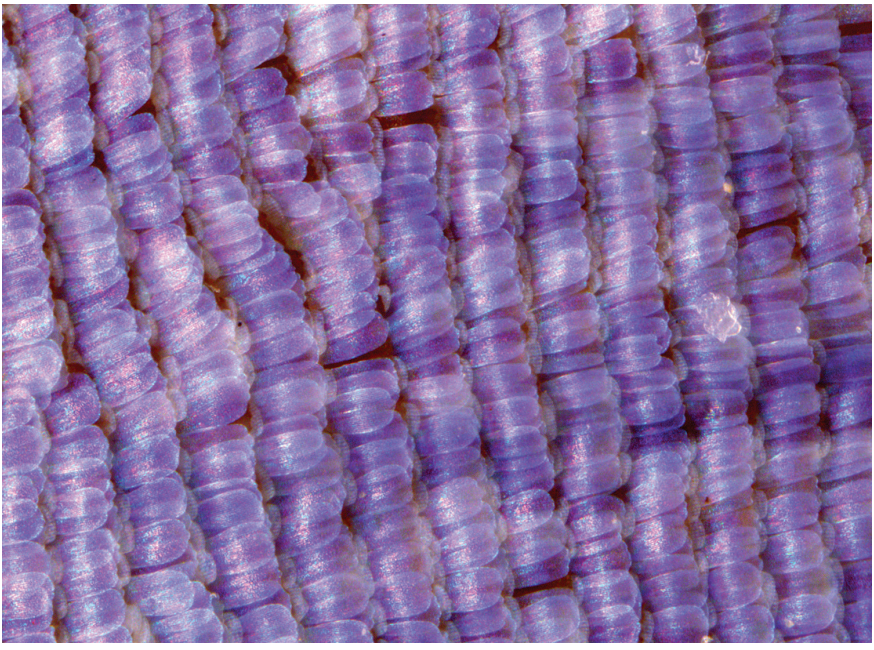


Figure 1b. Male *Celastrina lucia* forewing showing pale, underlying androconial scales typical of this species and *C. neglecta*.

DNA barcodes

Molecular variation of *Celastrina* species was assessed using the COI barcode fragment, with DNA extraction, PCR amplification, and sequencing performed at the Canadian Centre for DNA Barcoding (CCDB), following standard protocols (CCDB 2013). Public barcode sequence records were available for three North American species (*C. lucia*, *C. echo* and *C. neglecta*), and the Eurasian *C. argiolus* and Asian *C. morsheadi* (Evans). Novel sequences were generated for 31 eastern Ontario specimens (Suppl. material 1), initially identified as *C. neglecta* (five wild-collected specimens and five reared from larvae collected in late July to August), *C. serotina* (10 specimens reared from larvae feeding on cherry galls in mid June), and *C. lucia* (four specimens collected in May).

DNA sequences were analyzed on the Barcode of Life Data Systems website (BOLD, www.boldsystems.org). The dataset was filtered to include only records with sequences greater than 600 base-pairs in length, and with voucher specimen photographs and collection data that made independent species identification possible. Sequence variation was analyzed using the Kimura-2-Parameter (K2P) distance model and the neighbor-joining (NJ) algorithm as implemented on BOLD. Voucher specimen data is given in Suppl. material 1.

Larval development and host plants

Larvae were collected from the wild to compare phenology and voltinism of *C. lucia* and *C. serotina*, and to obtain comparative study specimens unambiguously associated with the current concept of *C. serotina*. *Celastrina serotina* is univoltine with a peak flight after that of *C. lucia* (Pavulaan and Wright 2005), so larvae develop later in the season with the resulting pupae entering diapause until the following spring. Larval sampling was carried out in eastern Ontario (Table 2) by directed visual searches and the use of a beating sheet. Numerous species of flowering shrubs were sampled, with most effort directed to sampling *Cornus*, *Viburnum* and *Prunus*. Larvae were reared indoors under natural light:dark conditions and at a constant 20 °C, reflecting the June mean daily temperature of 20.4 °C for Ottawa (Environment and Climate Change Canada 2015).

Flight phenology

As a proxy for mean seasonal abundances of *Celastrina* taxa, observation records spanning from 1895-2014 were compiled from the Ontario Butterfly Atlas (Macnaughton et al. 2015). Each unique location-date record was treated as one observation event, regardless of *Celastrina* abundance during that event. Observation frequency (abundance) by date was assessed for two ecoregions, the Great Lakes-St. Lawrence Mixed Forest of easternmost Ontario and the Carolinian Forest of southernmost Ontario

Table 2. Locality data for study sites mentioned in text.

site #	Locality	Lat	Long
1	CAN: ON, Ottawa, Stony Swamp Conservation Area, Richmond Rd.	45.29	-75.83
2	CAN: ON, Ottawa, Stony Swamp Conservation Area, Timm Dr.	45.315	-75.86
3	CAN: ON, Ottawa, Stony Swamp Conservation Area, Cassidy Rd.	45.323	-75.806
4	CAN: ON, Ottawa, Stony Swamp Conservation Area, Watts Ck.	45.341	-75.869
5	CAN: ON, Ottawa-Carleton Dist., Carp Hills	45.386	-76.075
6	CAN: ON, Hastings Co., Madoc, 3km W	44.5	-77.51
7	CAN: ON, Lanark Co., Pakenham, 4 km W, 9th Concession Rd.	45.304	-76.331
8	CAN: ON, Lanark Co., Pakenham, 12 km SW, Bellamy Rd.	45.276	-76.418

(Scott 1995). The southern Ontario dataset included 1056 records from Brantford, Elgin, Essex, Kent, Lambton, Middlesex, Niagara and Norfolk counties; eastern Ontario data consisted of 2145 records from Ottawa-Carleton, Lanark, Russell, Prescott, Glengarry, Stormont and Dundas counties. Three *Celastrina* taxa were considered to occur in each region, but to avoid *a priori* assumptions about species identities, all *Celastrina* records were combined.

Assessing flight peaks based on phenological data combined for multiple taxa could underestimate the number of taxa, if relative abundance discrepancies are large and flights overlap. Emergence patterns were therefore independently assessed through field surveys of eggs, larvae and adults 1–2 × per week in 2015. These data were supplemented with *Celastrina* records and accompanying voucher photographs available on eButterfly (2015).

To assess between-region differences in adult emergence times due to climatic differences, phenology data were examined using a simple degree-day model (e.g. Kelker et al. 1990; Dearborn and Westwood 2014) using the formula:

$$DD_{LTT} = [((T_{max} - T_{min}) / 2) – LTT]$$

where DD = degree-days, T_{max} and T_{min} = daily maximum and minimum temperatures, respectively, and LTT = the lower threshold temperature of insect development. LTT is the temperature at which physiological development is negligible, for the species and life stage under study. LTT values of 6°C to 10°C are generally implemented for insects, with values in the lower range corresponding to temperate-zone species (e.g. Kelker et al. 1990). As *Celastrina* are cold-adapted and some of the first lepidopterans to emerge from winter-diapausing pupae, LTT was set at 6 °C. A start date of April 1st was chosen as DD accumulation values were zero prior to this date (for all values of LTT between 6 °C and 10 °C). Daily temperature data were obtained for 2009–2015 for two stations, Ottawa (city station) for the eastern Ontario region, and London for the southern Ontario region (Environment Canada 2015). A few instances of missing daily maximum/minimum temperature data were estimated by averaging the corresponding temperature from the preceding and following day. Daily maximum and

minimum temperatures were calculated based on a six-year average from 2009–2015. London was chosen as representative of the Lake Erie region as it is inland from the Lake Erie shoreline and therefore less prone to cooling climatic effects of onshore winds along the immediate shoreline region.

Results and discussion

Wing Scale structure

Examination of forewing scale structure in male *Celastrina* specimens from southern and eastern Ontario led to the discovery of four specimens of *C. ladon*: ON, [Norfolk Co.], Normandale, 22.May.1956, J.R. Lonsway; ON, [Norfolk Co.], St. Williams, 7.May.1977, J.T. Troubridge; ON, Elgin Co., Calton Swamp WMA, 7.May.2000, I. Carmichael. Two female specimens are likely also *C. ladon*, one from Normandale, 28.May.1956, J.R. Lonsway, and one from St. Williams with the same date and collector as the male. All are from the Carolinian forest region of Lake Erie (Fig. 6). *Celastrina ladon* is therefore confirmed as part of the Canadian fauna for the first time. Although other literature and even photo records may exist, voucher specimens are needed to verify identification, at least until phenotypic variation and distribution of *C. ladon* in southern Ontario is better documented. Unvouchered previous records are of little value in ascertaining *Celastrina* identities in southern Ontario, underscoring the importance of voucher study specimens even when a species is thought to be common and well-known.

DNA barcodes

DNA barcode data were available for three North American *Celastrina* species (*C. echo*, *C. neglecta*, and *C. lucia*, based on independent identification), and representative Eurasian *C. argiolus* from seven countries (Fig. 2). Eastern Ontario specimens initially identified as *C. lucia*, *C. neglecta* and *C. serotina* are all considered to represent *C. lucia* based on the larval rearing, adult phenology and wing pattern, as discussed below.

Nearly all samples of *C. lucia*, *C. neglecta* and *C. echo* shared an identical DNA barcode. A single haplotype (h03, Fig. 2) was dominant across the continent, representing 76 of 79 individuals and occurring in all three species. Three additional haplotypes (h01, h02, h04; Fig. 2) differed by only a single base-pair, *i.e.* 0.15% divergence, and were represented by a single individual each (Fig. 2). Comparison of these four North American *Celastrina* haplotypes to others in the BOLD database using the sequence identification search engine showed that the extremely conserved genetic variation was not a sampling artefact, with virtually no variation in samples from across North America including Mexico, and including samples identified as all nine North American species in addition to the Mexican *C. gozora* (Bdvl.). This lack of mtDNA genetic differentiation between distinct species occurs also in other North American blues, as a

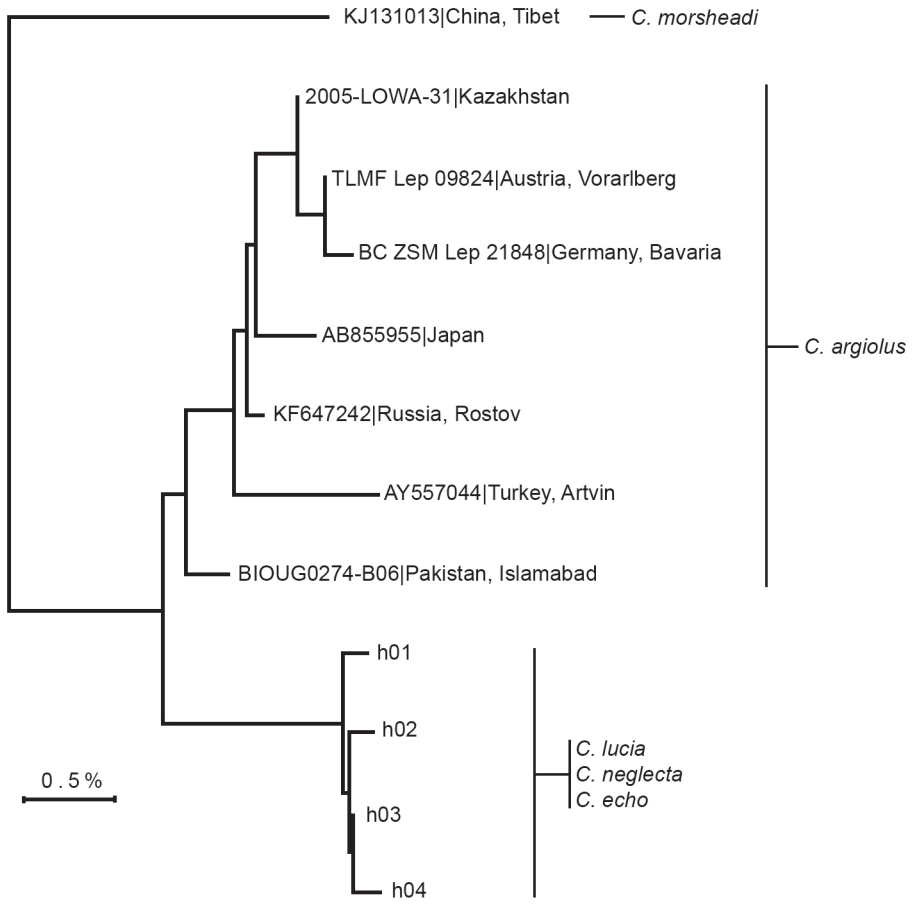


Figure 2. Neighbour-joining tree of DNA barcode sequences for *Celastrina*, with specimen voucher number and country of origin at branch tips. North American samples include 79 samples represented by four haplotypes, with h03 shared among three species (n=76) and remaining three haplotypes with one sample each of *C. lucia* (h01) and *C. neglecta* (h02, h04). Voucher data is given in Suppl. material 1.

result of introgressive hybridization and possibly infection by the endoparasitic bacterium *Wolbachia* (Gompert et al. 2008). Further research with other molecular markers is needed in *Celastrina*. Although the DNA barcode sequence is not taxonomically informative for North American species, it does corroborate separate species status of *C. argiolus*, which differed by a minimum of 1.4% (mean 1.9%).

Larval development and host plants

Eggs and larvae of *C. lucia* were found on flower buds and inflorescences of nine species of shrubs in eastern Ontario (Table 3). Based on correlative adult abundance,

Table 3. Flowering phenology of deciduous shrubs and larval hosts of *C. lucia* in the Ottawa region.

Phenology	Host[1]	Shrub species	Family	Source[2]	Site #
very early spring	N	*Prunus nigra*	Rosaceae	a	2
	N	*Prunus pennsylvanica*	Rosaceae	a	1,2,5
	N	*Amelanchier* spp.	Rosaceae	a	1,2,4,5,8
	N	*Vaccinium* sp.	Ericaceae	a	1,5
early spring	Y	*Prunus serotina*	Rosaceae	a,b	1,2
	Y	*Prunus virginiana*	Rosaceae	a,b	1,2,8
	(Y)	*Cornus sericea*	Cornaceae	c	-
mid to late spring	Y	*Cornus alternifolia*	Cornaceae	a,b	1,3,4
	Y	*Cornus rugosa*	Cornaceae	a	5
	Y	*Viburnum cassinoides*	Caprifoliaceae	a	1,3,4
	Y	*Viburnum lentago*	Caprifoliaceae	a	1,3,4
	(Y)	*Viburnum rafinesquianum*	Caprifoliaceae	a	6,7
	(Y)	*Diervilla lonicera*	Caprifoliaceae	a	5
	(Y)	*Celastrus scandens*	Celastraceae	a	6
mid summer	Y	*Spiraea alba*	Rosaceae	b	-
(2nd generation)	N	*Spiraea latifolia*	Rosaceae	b	-

1. **N** = Not used as a host; **Y** = Commonly used host; **(Y)** = locally or uncommonly used as a host.
2. **a** = this study; **b** = Layberry (2004); **c** = Eberlie (1998).

plant community composition and frequency of larvae on these hosts, *Prunus serotina*, *Cornus alternifolia* L. *C. rugosa* Lam., *Viburnum cassinoides* L. and *V. lentago* L. are the most commonly used larval host plants of spring *C. lucia* in this region. *Prunus pensylvanica* L., *P. nigra* Aiton and *Amelanchier* species were also searched, but these shrubs bloom very early in the spring with flowers already senescing during peak *Celastrina* abundance, and no larvae were found (Table 3). *Viburnum rafinesquianum* Schult. is rarely used, possibly also due to the later flowering phenology; only one larva was found in searches of 20 shrubs at two different sites (#7, 6; Table 2). Three mature larvae were found feeding on flower buds of *Celastrus scandens* L. in open limestone alvar habitat (site #6). This is the first record of *Celastrus* as a host of *Celastrina*, and adds the family Celastraceae to the list of known host plants (Scott 1986).

Other deciduous shrubs flowering during and after the spring flight season of *Celastrina* were sampled opportunistically, but failed to yield larvae, even when larvae were common on other shrub species at the same sites. These included *Ilex verticillata* (L.) A. Gray, *Ilex mucronata* (L.) (both Aquifoliaceae), and *Lonicera tatarica* L. (an introduced invasive shrub), *Cornus racemosa* Lam., *Vaccinium angustifolium* Ait., and *Gaylussacia baccata* (Wangenh.) K. Koch. An extensive search of the introduced *Viburnum lantana* L. at one site (#2) yielded one half-grown larva, which died several days later in captivity feeding on this plant. *Ilex* is the sole host of *Celastrina idella* Wright and Pavulaan, but is thought to be toxic to *C. lucia* (Wright and Pavulaan 2005). *Cornus sericea* L. is a common host of boreal *C. lucia* populations, but searches for larvae in

the study area (site #1) were unsuccessful, despite the patchy but common occurrence of this shrub. Virtually all of the host plants recorded above have completed flowering prior to the onset of summer *C. lucia* flights, which strongly favor *Spiraea alba* Du Roi as oviposition sites and larval hosts (Table 3).

Most of the *C. lucia* host shrubs present in the eastern deciduous forest are absent in the boreal region further north. Within the host genera *Cornus* and *Viburnum*, *C. sericea* and *V. edule* (Michx.) Raf. occur widely in the boreal region, but only sporadically in certain plant communities. By contrast, species of Ericaceae are ubiquitous and constitute the main larval hosts in many parts of the ecoregion, particularly plant communities on acidic substrates such as granite barrens, sand plains and bogs. Host plants documented along the James Bay highway in northern Québec in June 2015 (BCS, unpubl. data) included *Cornus sericea*, *Rhododendron groenlandicum* (Oeder) Kron & Judd and *Kalmia polifolia* Wangenh. Searches on *V. edule* and *Prunus pensylvanica* failed to yield eggs or larvae.

Larvae found on different plant genera exhibited different colour morph frequencies. Larvae on *Cornus alternifolia* were mostly very pale, pastel-green with little patterning (Fig. 3, right), compared to those on *Viburnum lentago*, which were darker green and more patterned (Fig. 3, left). Three larvae from *Celastrus* flowers were very dark green with little patterning. Differences in larval colours and pattern may represent hostplant-induced variation, not previously documented in *Celastrina* but known to occur in other Lepidoptera (e.g. Sandre et al. 2013).

Of approximately 120 gall-feeding larvae found on 18 *Prunus serotina* trees heavily infested with eriophyid galls, 28 were retained for rearing. Based on size and duration to pupation, approximately 75% were penultimate or ultimate instar, but younger instars were present also. A similar age distribution was observed among *Celastrina* larvae on other hosts at the same time and location, based on collection of 30 larvae from *Viburnum lentago* and *Cornus alternifolia*. Twenty-two of 28 larvae from galls survived to pupation, with five adults emerging between June 29th and July 2nd (summer phenotype) and three more emerging within 9 days at room temperature (spring phenotype) after a 95-day treatment of winter diapause conditions at 5°C in a conventional refrigerator. The remaining 14 pupae failed to merge and were dissected, revealing fully developed but desiccated adults, which could be assigned to either summer or spring phenotype by comparison to pinned specimens (Fig. 10). In total, 13/22 (59%) and 9/22 (41%) individuals displayed spring versus summer phenotype.

Similar results were obtained from rearing of cherry-gall feeding larvae collected in June of 2004 (RAL), where some pupae yielded summer-phenotype adults in the same year, and some entered diapause to emerge as spring-phenotype adults the following year (Suppl. material 1).

Phenology of cherry gall-feeding larvae was not notably different from that of larvae on other hosts, contrary to the prediction that larvae should appear later based on a later flight period in late May to late June, after that of *C. lucia* (Pavulaan and Wright 2005). Mature larvae found on 11th June would have to be derived from adults flying at least three weeks earlier, assuming 5d for egg hatch and 16d for larval growth

Figure 3. Variation in larval colour pattern of *C. lucia* found on *Viburnum lentago* (Left column) and *Cornus alternifolia* (right column) at site #3 (Table 2).

even under constant temperatures of 19 °C or more (Table 4). No small larvae were present after June 20th. The gall-feeding larvae showed the same size/age distribution as *C. lucia* larvae collected from *Viburnum* and *Cornus*. Neither the larval phenology

Table 4. Life cycle duration of non-diapausing *Celastrina lucia*.

Stage	Duration (days)				Temp. (deg. C)	Source region	Data source
	min	max	avg	*n*			
egg	3	6	4.5	-	19–22	Washington	James and Nunnallee (2011)
Larva	12	22	16.4	*5*	21	Ontario	This study
	16	25	20.5	-	18–27	Washington	James and Nunnallee (2011)
Pupa	11	14	12.7	*7*	21	Ontario	Layberry (2004)
	8	19	13	*5*	21	Ontario	This study
	7	13	10	-	18–27	Washington	James and Nunnallee (2011)
	7	-	-	-	22	Michigan	Wagner and Mellichamp (1978)

nor the summer-emerging adults resulting from gall-feeding spring larvae support that gall-feeding larvae represent a separate species, i.e. *C. serotina.* Furthermore, both May and August larvae, initially thought to represent *C. lucia* and *C. neglecta* (Suppl. material 1), can yield either summer adults from non-diapausing pupae or spring adults from diapausing pupae.

The alternative taxonomic explanation is that gall-feeding larvae are *C. lucia*, utilizing an unusual plant resource that is, however, similar to a *Prunus* flower bud in size, shape, tissue consistency, and likely phytochemistry. With a relatively long spring flight period and short flowering phenology for a given host species, *C. lucia* must use a suite of hosts to match larval development to host phenology. Galls extend the temporal availability of *Prunus* as they are present longer than flower buds. The total flight season for *C. lucia* is over a month in a given year (Table 6), yet any particular hostplant provides optimal forage for a considerably shorter period. For example *Prunus virginiana* is one of the first hosts to have flower buds, but once flowering begins, females avoid them in favour of other host species.

Degree days

Comparing degree-day accumulation to flight abundances provides a standardized comparison of flight seasons between southern and eastern Ontario (as defined here), where different climatic conditions prevail. In other words, peak adult emergence is expected to have similar degree-day (DD_6) accumulation values (dictated by physiological developmental constraints) in regions with differing climates, even though flight times could have quite different calendar dates. Furthermore, DD_6 accumulation can be used to assess if climatic conditions are amenable to producing multiple yearly generations (multivoltinism).

Cumulative DD_6 during the spring and summer months was greater for southern compared to eastern Ontario (Table 5). In mid- to late April, DD_6 accumulation in eastern Ontario lags behind that of southern Ontario by 4–6 days. As the season progresses, the time lag between the two regions diminishes to 2–3 days, for May to the

Table 5. Comparison of accumulated degree-days (DD_6) on selected dates for Ottawa and London, Ontario, based on daily temperatures averaged for 2009–2015. Time lag represents the number of days that London is ahead of Ottawa, based on DD_6 values averaged for the preceding week.

Date	Ottawa	London	Time lag (d)
01-Apr	0.0	0.0	0.0
10-Apr	0.7	3.4	5.5
20-Apr	10.5	21.5	5.5
30-Apr	35.2	51.4	4.3
10-May	105.3	123.4	2.5
20-May	179.8	200.5	2.5
30-May	288.2	314.4	2.1
10-Jun	403.0	431.2	2.5
20-Jun	528.0	562.6	2.5
30-Jun	675.7	707.0	2.2
10-Jul	830.5	854.7	1.6
20-Jul	987.1	1011.5	1.5
30-Jul	1132.8	1158.3	1.8

Table 6. Phenology of *Celastrina* in the Ottawa region April–July 2015.

Date	adults[1]	eggs	larvae	pupae	Note
Apr 19	**X**	|	|	|	First-of-year (FOY) record for adults; only males present
Apr 28	**X**	|	|	|	adults common, FOY females
May 6	**X**	**x**	|	|	adults common, female oviposition behaviour observed
May 12	**X**	**X**	**X**	|	Hatched and unhatched eggs at site #2
May 14	**X**	**X**	**X**	|	Adults, eggs, and larvae at site #1
May 21	**x**	**X**	**X**		Eggs and larvae present but no adults (site #5)
May 26	**X**	**x**	**X**	|	Adults and mature larvae (site #1)
May 28	**x**	**x**	**X**	|	Mature larvae (site #1)
May 29	**X**	**x**	**x**	|	End of flight period, only 3 worn adults seen in 3h
Jun 2	**X**	**x**	**X**	|	One worn adult
Jun 4	**X**	**x**	**X**	|	One worn adult
Jun 9	|	|	**x**	**x**	FOY pupae predicted[2]
Jun 11	|	|	**X**	**x**	Larvae (site #3,4)
Jun 14	|	|	**X**	**x**	Larvae (site #3)
Jun 18	|	|	**X**	**x**	Larvae (site #3)
Jun 20	**X**	|	|	**x**	FOY summer brood adults - male

1. **X** = presence based on direct observation; **x** = presence inferred based on observation of another life stage; **|** = absent

2. No pupae were found in the field. Presence of pupae is predicted based on degree-day values for a larval stage duration of 17d at 21C (Table 4), subsequent to first observed larval presence on May 12th.

end of July (Table 5). The faster DD_6 accumulation in southern Ontario is correlated with a slightly earlier spring *Celastrina* peak in that region, occurring on average three days earlier (Figs 4, 5). Large differences between abundance peaks (more than one week) observed between regions are therefore not likely attributable to regional variation in development times of the same species, assuming similar development rates and thresholds between regions.

Is it possible that summer abundance peaks represent the offspring of spring *Celastrina*? Currently, spring and summer *Celastrina* are treated as separate species, and some have maintained that *Celastrina* flying subsequent to the spring flight appear too soon for this to be possible (e.g. Saunders 1875). In eastern Ontario, the median abundance dates occur on May 11th and July 12th (Fig. 4; 50% of observations for the period prior to June 12st or after June 20th). In southern Ontario, however, the situation is different, as there is an abundance peak with a mean date of June 15th, after a spring peak on May 8th. The time lag between the first two seasonal peaks is therefore between May 11th - July 12th in Eastern Ontario and May 8th - June 15th in southern Ontario, corresponding to an average degree-day (DD_6) of 750 and 381, respectively (data not shown; degree-day trends in Table 5). These DD_6 values likely represent a slight overestimate of actual degree-days available for completion of a generation, since the between-peak time lag does not account for the fact that most eggs are probably laid after the peak flight period. This is due to females emerging later than males and being less commonly observed, as is true for nearly all butterflies (Scott 1986).

Average life cycle duration of non-diapausing *Celastrina* in Ontario (35d total; egg = 5d, larva = 17d, pupa = 13d at 22 °C; Table 4), has an accumulated DD_6 value of approximately 560, considerably greater than the maximum estimated DD_6 of 381 available in southern Ontario, but less than the DD_6 of 750 in eastern Ontario. Degree-day modelling data therefore indicates that there are enough degree-days between the first and second abundance peaks to permit development of a second generation in eastern Ontario but not in southern Ontario, and the two peaks in the latter region cannot therefore represent the same species.

Adult phenology

Celastrina phenology in eastern Ontario exhibits a bimodal pattern, with a well-defined spring and summer peak. Median spring abundance (*i.e.*, 50% of records) occurs on May 8th and median summer abundance on July 12th (spring and summer periods divided by the trough midpoint at June 21st). *Celastrina* abundance drops sharply between June 5th and June 24th; in other words, azures of any kind are very rarely observed in eastern Ontario during this period (Fig. 4). This is opposite to the pattern seen in southern Ontario, where a June 15–19th abundance peak occurs in addition to a May and July/August peak (Fig. 5).

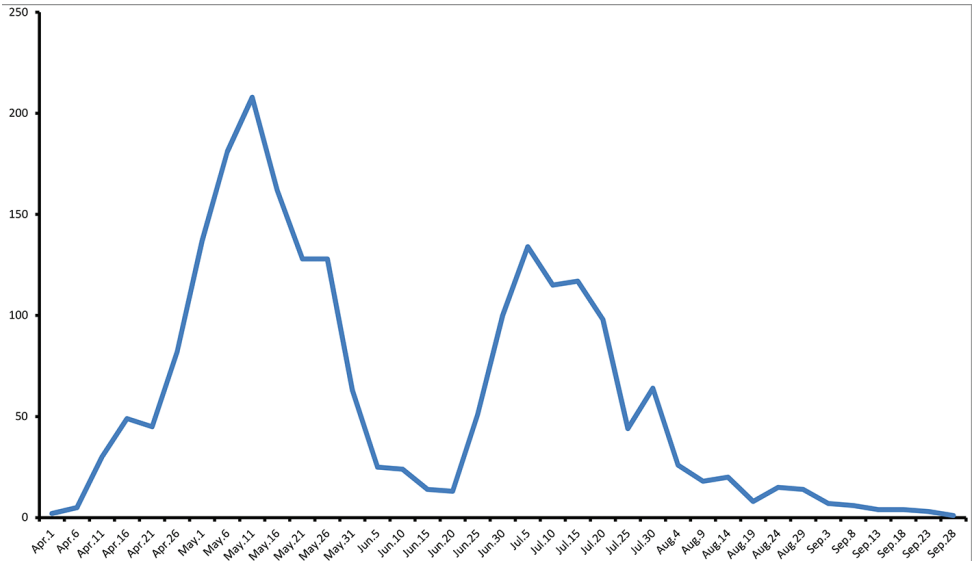


Figure 4. Frequency plot of *Celastrina* adults for eastern Ontario based on cumulative observations from 1899–2014 (n = 2145).

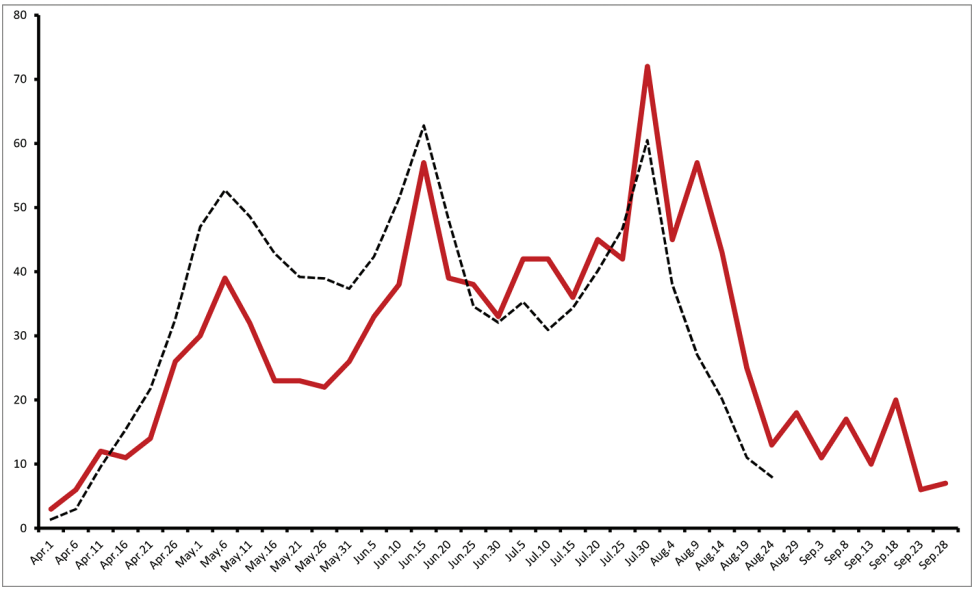


Figure 5. Frequency plot of *Celastrina* adults for southern Ontario (red line) based on cumulative observations from 1895–2014 (n = 1056). Dashed line represents abundance of all *Celastrina* observations assuming hypothetical phenology given in Figure 6.

Another notable difference in *Celastrina* phenology between eastern versus southern Ontario is the magnitude of spring (April–May) versus summer (July onwards) abundance peaks. In southern Ontario, there are considerably fewer spring than summer records, the converse of the pattern in eastern Ontario. *Celastrina* abundance also persists further into the summer in southern Ontario, not declining significantly until after Aug 21st, compared to steady declines after mid-July in eastern Ontario (Fig. 5).

The bimodal abundance pattern in eastern Ontario reflects at minimum two entities, a spring- and a summer-flying *Celastrina*, previously considered to be *C. lucia* and *C. neglecta*, respectively. The time lag between spring and summer emergences, and the rearing results and phenotype comparisons discussed below, indicate that eastern Ontario spring and summer *Celastrina* represent two broods of the same species, *C. lucia*.

Although there is no evidence of a third peak (in eastern Ontario) intercalated between the first and second as would be expected for *C. serotina*, it is possible that such an abundance signature is hidden by virtue of *C. serotina* being much rarer than *C. lucia* and *C. neglecta*. However, the 2015 observations on larval and adult phenology do not support this (Table 6). No "flush" of freshly emerging adults appeared after the peak of *C. lucia* adults, and there was no detectable difference in age (size) of cherry-gall feeding larvae compared to other larvae. What, then, is the true identity of *Celastrina* previously attributed to *C. serotina*? To address this, all eButterfly (2015) *C. serotina* records with voucher photographs were examined, consisting of 28 records with dates ranging from 14 May to 26 June. Both worn *lucia*-like individuals and freshly emerged *neglecta*-like individuals are identified as *serotina*, the primary means of identification apparently being date. Fourteen individuals were visually indistinguishable from either worn individuals of *C. lucia* or fresh, lightly marked (form "violacea") individuals thereof. Ten individuals were fresh with a chalky–white ventrum and small, sharp spots and little to no marginal markings, like those of the June-flying, southern Ontario entity here assigned to *C. neglecta*. Specimens identified as *C. neglecta* tended to occur further south than *C. lucia* (Fig. 8).

In southern Ontario, spring *Celastrina* are rare compared to the abundance of azures seen from June onwards (Layberry 1996). Saunders (1875) noted that *Celastrina* were absent prior to late May in the London area. This pattern is reflected by fewer spring vs. summer observations, and the presence of an additional June flight peak that is absent in eastern Ontario. Comparison of June *Celastrina* from southern Ontario to those from other areas reveals that these also differ phenotypically (Figs 9, 10). As discussed under *C. neglecta* in the Conclusions section, this taxon is here deemed to be *C. neglecta*.

The spring/summer abundance discrepancy in southern Ontario was also noted by Layberry (1996), who stated that spring *Celastrina* were rare and local and could not possibly produce the abundance of ubiquitous summer *Celastrina*. This discrepancy can be explained by the localized occurrence of *C. lucia* (near its southern range limit) and *C. ladon* (restricted to Carolinian woods) in spring, followed by the much more common *C. neglecta* in late May–June and again in Late July–August.

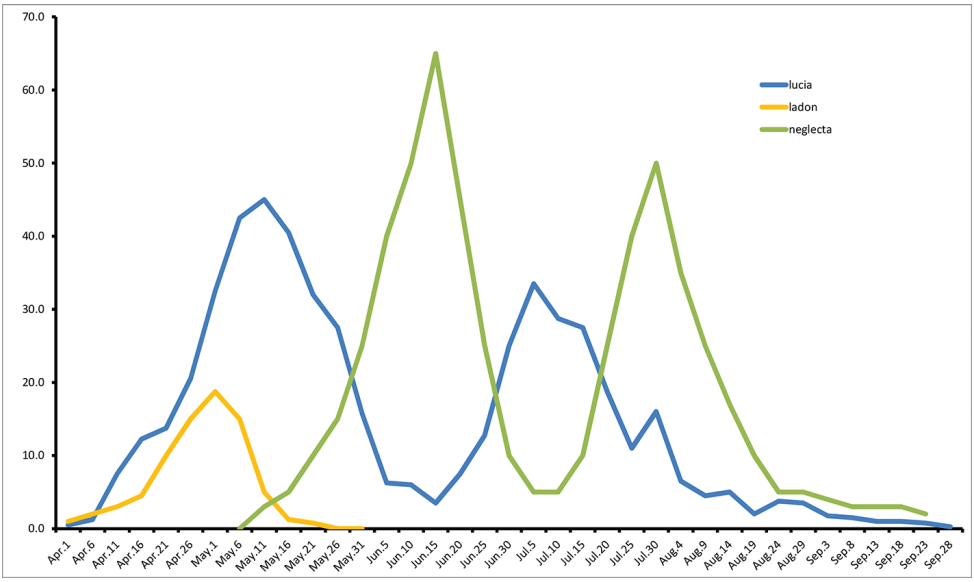


Figure 6. Hypothetical phenology of *Celastrina* species in southern Ontario. *Celastrina lucia* abundance is based on eastern Ontario data (Figure 4), *C. neglecta* data is based on assumption of two annual flights, the first peaking in mid-June and with a generation time similar to that of C. lucia (750 degree-days). *Celastrina ladon* data is based on assumption of a single, earlier flight and lower overall abundance compared to *C. lucia*, but with similar abundance changes and length of flight period. The sum of all predicted *Celastrina* abundances is compared to actual observation frequencies in Figure 5.

Figure 7. Distribution of examined voucher specimens for *C. ladon* in Ontario.

The complex abundance peaks for southern Ontario are at least in part a result of combined data for multiple species. Degree-day modelling can however be used to approximate the apparent abundance peaks. Given a spring peak of *C. lucia* on May 8,

and an average DD_6 accumulation of 750 to reach the second-brood peak (based on the eastern Ontario phenology), summer *C. lucia* would be expected to peak on July 11th on average. A corresponding, although weak, peak occurs in southern Ontario between July 5th and 14th (Fig. 5). Assuming similar physiological development rates and parameters for *C. neglecta*, a summer peak of 750 DD_6 after the June 15th peak would be expected, corresponding to August 5th. This correlates well with the observed peak between July 30th and Aug 3rd (Fig. 5).

Identification and distribution of Canadian *Celastrina neglecta*

To establish comparative phenotypes of *C. neglecta* and summer-brood *C. lucia*, southern Ontario specimens collected during the June flight peak (Figure 5) were compared to July specimens from eastern Ontario (summer peak; Figure 4). This provided a conservative estimate of phenotypic variation in *Celastrina neglecta*, which differs in having darker, smaller and more sharply defined ventral spots, brighter white ventral ground colour, more reduced marginal markings, a solid white dorsal hindwing fringe, and more pronounced dark marginal shading of the forewing apex (compare Fig. 10g–k to 10l–o). To define the distribution of *C. neglecta*, a conservative approach was taken to avoid construing summer *C. lucia* with first or second generation *C. neglecta*. For Ontario and Québec, specimens were identified as *C. neglecta* only if they met two criteria, *i.e.* matching the *C. neglecta* phenotype as above, and a collection date between late May and late June, prior to the onset of the summer *C. lucia* flight. For the Prairies and Maritimes region where flight period is expected to be later compared to Ontario, all available specimens previously identified as *C. neglecta* were evaluated. Specimens previously identified as *C. neglecta* from all parts of the Canadian range revealed that true *C. neglecta* occurs from easternmost Alberta to southern Ontario. Specimens from eastern Ontario, Québec and the Atlantic region match the summer *C. lucia* phenotype, consistent with the notion that Maritimes *Celastrina* all represent a single, partially bivoltine species (Maritimes Butterfly Atlas 2015). Two Nova Scotia specimens reared from *Aralia* (CNC) previously identified as *C. serotina* (Pavulaan and Wright 2005) were also re-identified as summer brood *C. lucia*.

In Canada, *C. neglecta* is sympatric with *C. lucia* in nearly all parts of the *neglecta* range. Most summer records from the Prairie Provinces proved to be *C. neglecta* (Fig. 8), although summer brood *C. lucia* occur also in southern Manitoba (Fig. 10), and are expected in Saskatchewan based on a single recent record from as far west as Edmonton, Alberta. In Ontario, *C. neglecta* has a more restricted southern distribution compared to bivoltine *C. lucia* populations, so far documented to about 44°N (Fig. 8b). The maximum northeastern extent is currently at the eastern edge of the Oak Ridges Moraine (Rice Lake Plains) and the southern Napanee Limestone Plain (Fig. 8b). In southern Ontario it is the most common *Celastrina*, and both *C. lucia* (Fig. 9) and *C. ladon* (Fig. 7) have a more localized occurrence. Field work is needed to definitively establish the northern range limit, especially in the regions of Georgian Bay, Bruce

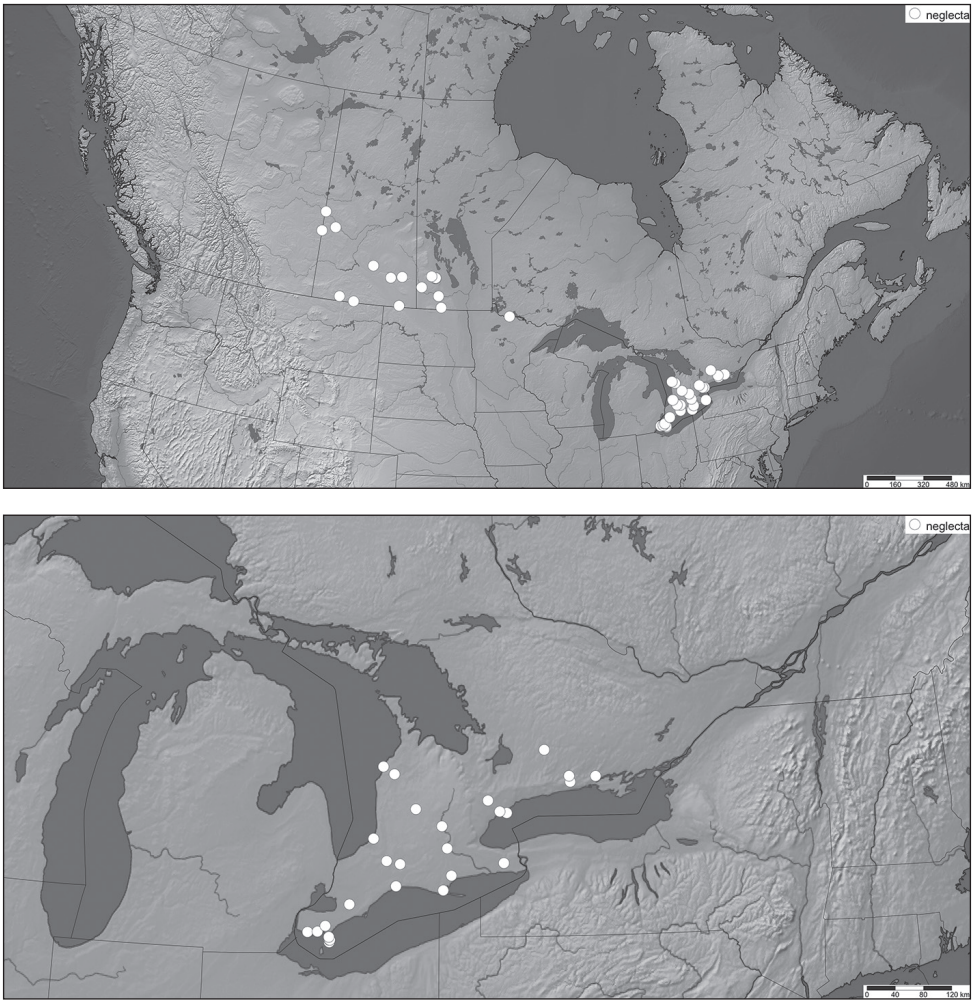


Figure 8. Distribution of examined voucher specimens for *C. neglecta* in Canada (above) and Ontario (below).

Peninsula, and the Frontenac Arch. No Québec vouchers were located but the species could be expected in regions know for southern species, such as the southern Richelieu River valley and the Lake Champlain region.

Conclusions

The Canadian *Celastrina* fauna is revised to consist of four species: *C. lucia* (all provinces and territories), *Celastrina neglecta* (southern Ontario to eastern Alberta), *C. ladon* (Carolinian zone of southernmost Ontario), and *C. echo* (southern British Columbia and southwestern Alberta). From eastern Ontario eastward, what was previously treated as three *Celastrina* species is revised to a single, facultative bivoltine species, *C. lucia*.

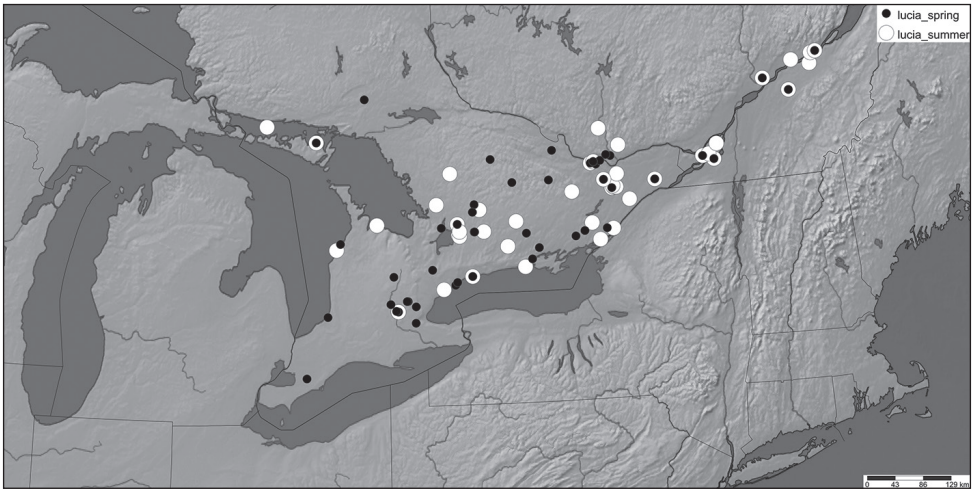


Figure 9. Distribution of examined voucher specimens for spring (black circles) and summer (white circles) *C. lucia* in southern Ontario and adjacent Québec.

Adults of *C. lucia* flying from early to mid-spring, in a relatively prolonged emergence, give rise to a second and possibly a partial third generation in July to September. Larval rearing, phenology, and seasonal emergence patterns show no evidence of *C. serotina* as a separate gall-feeding species distinct from *C. lucia*, and *C. serotina* is therefore removed from the Canadian fauna. Whether or not nominate *C. serotina* (described from Rhode Island) is a valid species, or simply represents late-emerging *C. lucia* that utilize cherry galls, needs to be re-evaluated. Molecular markers such as microsatellites could prove to be particularly valuable in advancing the taxonomy of *Celastrina*, given that the COI barcode marker is taxonomically uninformative here.

Celastrina lucia

Two additional possibilities in the identity of the species here assigned to *C. lucia* warrant comment. It is conceivable that *C. neglecta* is present as a univoltine, summer-flying entity that is phenotypically similar to and unrecognized within summer-brood *C. lucia*. This would require that the June-flying *Celastrina* in southern Ontario be C. *serotina*, and that *Celastrina neglecta* in eastern Ontario overwintering as pupae delay emergence until July. Both of these conditions are improbable; the identity of June *Celastrina* in southern Ontario is most likely *C. neglecta* as discussed below, and there are no known temperate-zone Lycaenidae that overwinter as pupae and delay emergence until July. Eastern Ontario summer *Celastrina* also have the appearance of pale *C. lucia* (Figs 10, 11).

The second possibility is that the eastern Ontario taxon represents a species distinct from nominate *C. lucia*, that is *C. lucia* 'of authors' in the sense of Pratt et al. (1994), based on larger size, wing pattern differences, and differing host plant preferences.

Figure 10. Adult males of *Celastrina*. **a–c** *Celastrina ladon* (Cramer) **a** Normandale, ON, CAN, 22 May 1956, J.R. Lonsway, (CNCLEP 116459) **b** St Williams, ON, CAN, 7 May 1977, J.T. Troubridge (CNCLEP 116460) **c** St Louis, Missouri, United States, 15 April 1979 (CNCLEP 116461) **d–f** *Celastrina lucia* (Kirby), spring generation **d, e, f** Stony Swamp, Richmond Road, Ottawa-Carleton, ON, 45.298°N, 75.828°W, CAN, 28 April 2015, B.C. Schmidt (CNCLEP 116445, 116447, 116446) **g–k** *Celastrina lucia* (Kirby), summer generation **g** Riding Mtns., MB, 12 June 1938, J. H. McDunnough (CNCLEP 116448) **h** Timm Dr., Ottawa, ON, 45.315°N, 75.860°W, CAN, 14 May 2015, B.C. Schmidt (CNCLEP 116451) **i** Bobcaygeon, ON, CAN, 16 July, 1932, J. McDunnough (CNCLEP 116453) **j** Pont Neuf, QC, CAN, 8 July 1973, no collector (CNCLEP 116454) **k** Britannia, Ottawa, ON, CAN, 30 June 1949, R. deRuette (CNCLEP 116455) **l–o** *Celastrina neglecta* (Kirby) **l** Larsson's Camp, One Sided Lake, ON, CAN, 19 June 1960, M.R. MacKay (CNCLEP 116464) **m** Point Erie, ON, CAN, 6 August 1950, T.N. Freeman (CNCLEP 116465). Riding Mountains, MB, CAN, 13 June 1938, J. McDunnough (CNCLEP 116466) **o** Riding Mountains, MB, CAN, 12 June 1938, J. McDunnough (CNCLEP 116467).

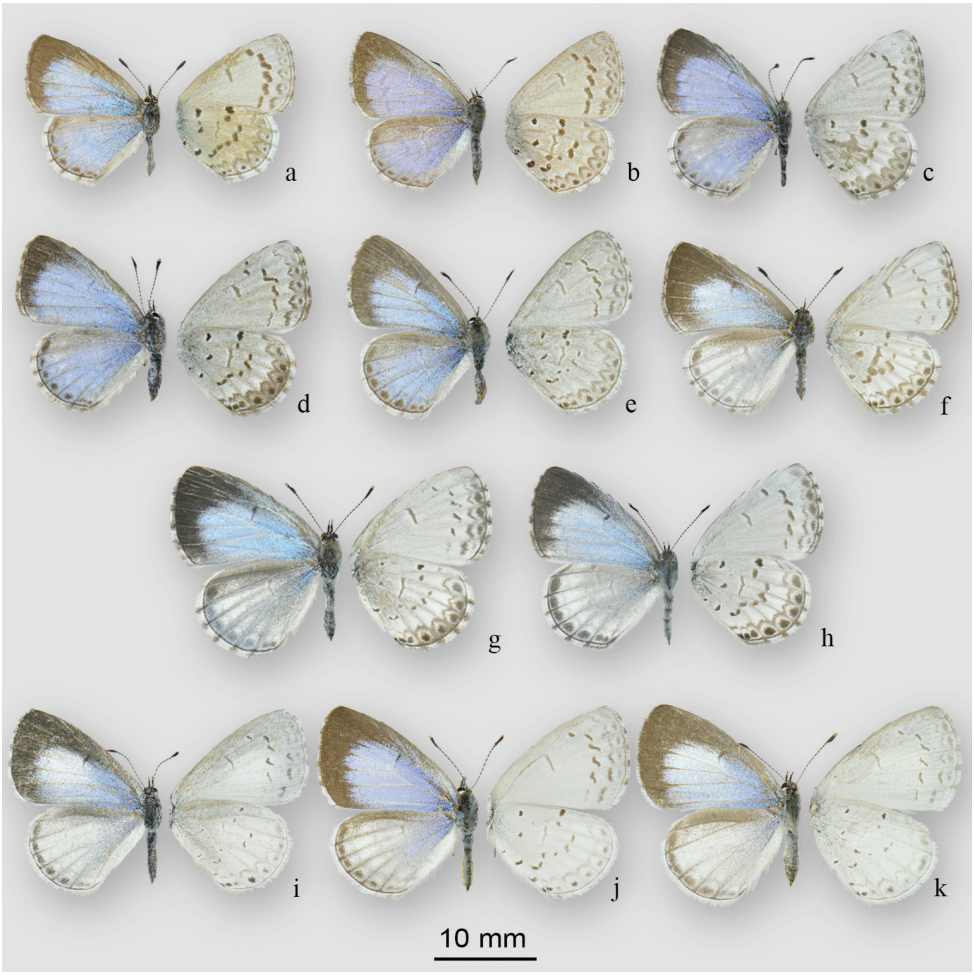


Figure 11. Adult females of *Celastrina*. **a–b** *Celastrina ladon* (Cramer) **a** Normandale, ON, CAN, 28 May 1956, J.R. Lonsway (CNCLEP 116462) **b** Lake Wellington, Washington Co, Arkansas, United States, 12 April 1974 no collector (CNCLEP 116463) **c–e** *Celastrina lucia* (Kirby), spring generation **c** Stony Swamp, Richmond Road, Ottawa-Carleton, ON, 45.298°N, 75.828°W, CAN, 28 April 2015, B.C. Schmidt (CNCLEP 116449) **d** Bells Corners, Timm Road, Ottawa, ON, 45.315°N, 75.860°W, CAN, 14 May 2015, B.C. Schmidt (CNCLEP 116450). **e)** Timm Dr., Ottawa, ON, 45.315°N, 75.860°W, CAN, 14 May 2015, B.C. Schmidt (CNCLEP 116451) **f–g** *Celastrina lucia* (Kirby), summer generation **f** Château-d'Eau, QC, CAN, 21 July 1990, J.-P. Laplante (CNCLEP 116456) **g** *Celastrina lucia* (Kirby): 5kmSE of Fitzroy Harbour, Fitzroy, ON, 45.4348°N, 76.1725°W, CAN, em 20 June 2015, Ross Layberry (CNCLEP 116457) **h** Stony Swamp, Richmond Road, Ottawa-Carleton, ON, 45.297°N, 75.836°W, CAN, 2 July 2015, B.C. Schmidt (CNCLEP 116458) **i–k** *Celastrina neglecta* (Kirby) **i** Harrow, Essex Co., ON, 42.0390°N, 82.9080°W, 28 May 2015, Jeff Larson (CNCLEP 116468) **j** Simcoe, ON, CAN, 26 June 1939, T.N. Freeman (CNCLEP 116469) **k** Bobcaygeon, ON, CAN, 22 June 1932, J. McDunnough (CNCLEP 116470).

This interpretation remains to be thoroughly evaluated, particularly by examining latitudinal gradients of the character traits in question. For now, we favour the simplest taxonomic hypothesis, where this taxon represents *C. lucia* with facultative bivoltine populations, clinally variable phenotypes and regional host plant preferences.

Although consistently stated to be univoltine in the literature, *Celastrina lucia* is here interpreted to be facultatively bivoltine (and possibly trivoltine) in southern Canada (Fig. 9), with northern, boreal populations being univoltine. In addition to climatic conditions, voltinism may be regulated by host plant availability (Shapiro 1975), explaining why more southerly populations of *C. lucia* could be strictly univoltine (Pavulaan 2014). Plasticity in voltinism is perhaps not surprising given that the Eurasian sister species *C. argiolus*, occupying very similar ecological niches, is also well known to be facultatively bivoltine (e.g. Ebert 1993). *Celastrina echo* is well-known to be bivoltine in western North America, and some western *C. lucia* populations can produce a second generation under laboratory conditions (James and Nunnallee 2011). Similar mechanisms of geoclimatically variable voltinism are common and taxonomically widespread in Lepidoptera, although perhaps less prevalent in temperate butterflies. As *Celastrina* is primarily a tropical group, multivoltinism is likely an ancestral evolutionary trait, with univoltinism a derived trait adaptive for climatic or host plant limitations.

Rearing data indicate that a proportion of spring individuals of Ontario *C. lucia* enter diapause the following spring (Eberlie 1997; Layberry 2004; this study). Summer observations are 45% fewer than in spring (Fig. 4), suggesting that roughly half of the individuals resulting from the spring brood enter diapause. 59% of pupae reared in 2015 similarly did so. Triggers for facultative bivoltinism are in part environmental, as flight phenology shifts later into the spring with latitudinal climatic amelioration. Warmer spring temperatures as a result of climate change are expected to favour northward expansion of bivoltinism in *C. lucia*. This was recently documented in Alberta with the first recorded summer brood *C. lucia* (G. Anweiler, pers. comm; photo examined), in an area with a century of butterfly surveying (Pohl et al. 2009). *Celastrina lucia* therefore provides an excellent opportunity to study the effects of climate change on developmental thresholds.

Larvae of *C. lucia* are polyphagous, but show preferences for several genera in different families (Table 3) and feed almost exclusively on flowers and fruits. *Celastrina lucia* uses a variety of host plants with differing flowering phenologies to span the duration of a relatively lengthy flight period. As part of this dietary strategy, *C. lucia* also feeds opportunistically on leaf galls of *Prunus serotina* and *P. virginiana*, which has been documented in Québec, Ontario and Manitoba, but is likely a geographically more widespread phenomenon.

Celastrina neglecta

In southern Ontario, a third *Celastrina* species appears in late spring after an initial May flight of both *C. lucia* and *C. ladon*. The appearance of this species is too soon after the

first flight of *Celastrina* to represent a second annual generation. Adult wing phenotype is similar to the summer brood of *C. lucia*, but differs in having darker, smaller and more sharply defined ventral spots, more reduced marginal markings, a solid white dorsal hindwing fringe, and a less evenly checkered forewing fringe (Table 7). The differences between *C. neglecta* and summer *C. lucia* requires more study, and the diagnosis and accompanying figures given here should be treated as a guideline for further research rather than a definitive diagnostic tool.

In Ontario, this taxon was recognized as distinct from *C. lucia* 140 years ago by Saunders (1875), who considered it to be the most common *Celastrina* in the London area, appearing in late May to early June. Pavulaan and Wright (2005) assigned Saunders' records to *C. serotina* (although Saunders (1869) states that specimens were reared from larvae found on *Cornus*). The abundance of this species in the absence of *Prunus serotina* in southern Ontario (R. Cavasin, pers. comm.), and the larval host plant records discussed below, indicate that this species is not *C. serotina*. What name to apply to this taxon is however not straight-forward. The differential diagnosis of *C. serotina* and *C. neglecta* is based primarily on phenology, voltinism, and to some extent on host plant (Pavulaan and Wright 2005). Pavulaan and Wright (2005) state that *neglecta* has a single summer flight after that of *C. serotina* in Canada, but when *C. neglecta* has a spring flight, it is before that of *C. serotina*. The phenology of *C. neglecta* as proposed by Pavulaan and Wright (2005) seems counterintuitive as it states that *C. neglecta* has a summer flight in the north but then adds an earlier, spring flight southward. Other facultatively bivoltine Lepidoptera generally have additional flights later not earlier in the year. *Celastrina neglecta* is more intense blue with more white suffusion dorsally, and a weaker ventral maculation pattern compared to *C. serotina* (Pavulaan and Wright 2005). Of course the name of this species hinges on the identity of the lectotype specimen of *C. neglecta*, which surprisingly has not been considered in detail. Until this situation can be thoroughly reviewed, the identity of the June/August *Celastrina* of southern Ontario is most parsimonious with the current concept of *C. neglecta*. Many southern Ontario specimens are also very similar to the Manitoba taxon *argentata* (Fletcher), which is currently considered a synonym of *C. neglecta* (Pelham 2011). The distribution, similar phenotype and phenology of Great Lakes *C. neglecta* Great Plains *argentata*, together with Colorado *C. humulus* Scott and Wright 1999 certainly suggest that these taxa all represent the same species.

Canadian host plant records that are probably attributable to *C. neglecta* include *Ceanothus americanus* (based on late June larvae from Northumberland Co., Ontario; Eberlie 1997; 1998; ovipositing female, Northumberland Co., R. Cavasin, photo examined); *Cornus amomum* Mill. (late June oviposition and larvae at Point Pelee, J. Cossey, photo examined), and *Cornus drummondii* C.A. Mey (late June to early July larvae from Essex County, J. C. Lucier, Ontario Butterfly Atlas 2015). Host plants of populations in the prairies are completely unknown; both *Cornus* and *Ceanothus* are sparse or absent where these populations occur.

Table 7. Differential diagnosis of Celastrina species in Ontario, Canada.

Species	Maximum annual # generations	Peak flight times	Distribution	Male forewing androconial scales	Male forewing overlapping scales	Ventral hindwing: confluent discal macules	Ventral Hindwing: confluent marginal markings	Ventral hindwing: expression and colour of marginal markings	Ventral ground colour	Hindwing fringe	Dorsal hindwing	Dorsal forewing
C. lucia	3?	E - L May; E - L Jul	Ubiquitous through most of province; localized south of 43N	present	absent	common	common	spring: well-developed, diffuse, brown to grey. Summer: moderately developed, usually light brown-grey	Spring - grey to greyish white; rarely white. Summer - white	white with black fringe at vein termini	summer: extensive white scasling; ventral pattern usually visible	Fringe and terminal area evenly checkered from apex to tornus, or slightly darker at apex
C. neglecta	2?	L May - L June; L Jul - L Aug	Primarily south of 44.5N, but northern limits uncertain	present	absent	rare	rare	poorly developed, small and dark grey; marginal crescents often absent	white	solid white	limited or sparse white scaling; ventral pattern not usually visible	Fringe and terminal area darker in apical area, with termina lblack line usually widest at apex
C. ladon	1	L Apr - M May	Carolinian zone south of 43N	absent	present	rare	rare	moderately developed, diffuse, brown to grey	grey to greyish white	white with black fringe at vein termini	limited or no white scaling	Margin evenly checkered from apex to tornus, or slightly darker at apex

Celastrina ladon

The Spring Azure, *C. ladon*, is here confirmed as part of the Canadian fauna. It is currently known from only three sites, with the most recent record from 2000. Surveys for this species are urgently needed as the primary larval host, Eastern Flowering Dogwood (*Cornus florida* L.), is endangered in Canada (Environment Canada 2014). This species is experiencing population declines in Ontario caused by dogwood anthracnose fungus, forest succession, habitat loss and herbivory by deer (Environment Canada 2014). Oviposition and suitability of other larval hostplants also needs to be established, as it is possible that *Viburnum* and other *Cornus* may be suitable hosts. Remaining core areas for *Cornus florida* in Ontario include Backus Woods, Wilson Tract, Turkey Point PP, Spooky Hollow Nature Sanctuary (COSEWIC 2007).

Research needs

Surprisingly, there are still many large gaps in our understanding of *Celastrina* taxonomy and biology. The most urgent need for Canadian *Celastrina* research is vouchered surveys for *C. ladon* in southern Ontario, so that potential conservation needs can be established. Regions where *C. neglecta*, *C. lucia* and/or *C. ladon* occur in sympatry provide an excellent opportunity for comparative study, where time series of vouchers are needed to establish diagnostic as well as habitat and host plant differences. Along similar lines, latitudinal transects of voucher series and host use are needed to examine the transition from southern to boreal *C. lucia*.

Lastly, controlled-environment rearing studies of all taxa would establish plasticity in voltinism and developmental requirements and diapause triggers. The use of degree-day modeling could easily be fine-tuned as a useful comparative tool for *Celastrina* taxa and populations, and to model geographic variation of *Celastrina* emergence. Dearborn and Westwood (2014) used a similar approach to predict emergence of an endangered skipper.

Acknowledgments

Rick Cavasin kindly made numerous *Celastrina* photographs available for examination, and provided helpful observations on *Celastrina* behaviour and variation. Gary G. Anweiler provided data and photographs of Alberta and Saskatchewan *Celastrina*. Rick Cavasin, Peter Hall, John Klymko, Max Larrivée and Reggie Webster provided feedback and insightful discussion on eastern *Celastrina*, and Marie-Ève Garon-Labrecque identified eriophyid cherry galls. Alan Macnaughton generously provided additional information on the Ontario Butterfly Atlas database, and Jocelyn Gill provided technical assistance.

References

CESCC (Canadian Endangered Species Conservation Council) (2011) Wild Species 2010: The General Status of Species in Canada. National General Status Working Group, 302 pp.

CCDB (2013) The Canadian Centre for DNA Barcoding. http://www.ccdb.ca/resources.php

Eberlie WJD (1997) Rearing notes from Northumberland County. Butterflies of Ontario and Summaries of Lepidoptera encountered in Ontario in 1996. Toronto Entomologists Association 29: 9–10.

Eberlie WJD (1998) Rearing notes from Northumberland County. Butterflies of Ontario and Summaries of Lepidoptera encountered in Ontario in 1997. Toronto Entomologists Association 30: 15.

eButterfly (2015) An international, data driven project dedicated to butterfly biodiversity, conservation, and education. http://www.e-butterfly.org/ [accessed: 2.Dec.2015]

Eliot JN, Kawazoé A (1983) Blue butterflies of the *Lycaenopsis* group. British Museum (Natural History). BAS Printers, Hampshire, 309 pp.

Environment Canada (2014) Recovery Strategy for the Eastern Flowering Dogwood (*Cornus florida*) in Canada. Species at Risk Act Recovery Strategy Series. Environment Canada, Ottawa, 16 pp.

Environment and Climate Change Canada (2015) Canadian Climate Normals. http://climate.weather.gc.ca/climate_normals/index_e.html [accessed: 10.Dec.2015]

Ferguson DC (1954) The Lepidoptera of Nova Scotia. Proceedings of the Nova Scotia Institute of Science 23(3): 161–375.

Gompert Z, Forister ML, Fordyce JA, Nice CC (2008) Widespread mito-nuclear discordance with evidence for introgressive hybridization and selective sweeps in *Lycaeides*. Molecular Ecology 17: 5231–5244. doi: 10.1111/j.1365-294X.2008.03988.x

Guppy CS, Shepard JH (2001) Butterflies of British Columbia. University of British Columbia Press, Vancouver, 414 pp.

Hall PW, Jones CD, Guidotti A, Hubley B (2014) Butterflies of Ontario. Royal Ontario Museum, Toronto, 488 pp.

James DG, Nunnallee D (2011) Life Histories of Cascadia Butterflies. Oregon State University Press, Corvallis, 448 pp.

Kelker DH, Lee DA, Spence JR (1990) Use of standard temperature thresholds and phenological prediction for the European corn borer (*Ostrinia nubilalis* Hubner) in Alberta. Canadian Entomologist 122: 1247–1258. doi: 10.4039/Ent1221247-11

Langston RL (1975) Genus *Celastrina* Tutt. In: Howe WH (Ed.) The Butterflies of North America. Doubleday and Co., Garden City, NY, 335–337.

Layberry RA (1996) The Spring Azure species complex. In: Butterflies of Ontario and Summaries of Lepidoptera encountered in Ontario in 1995. Toronto Entomologists Association 28: 12–15.

Layberry RA (2004) Rearing lycaenids in 2003–2004. Ontario Lepidoptera 2003–2004. Toronto Entomologists' Association Occasional Publication 36–2006.

Layberry RA, Hall PW, Lafontaine JD (1998) The butterflies of Canada. University of Toronto Press, Toronto, 280 pp.

Macnaughton A, Layberry R, Jones C, Edwards B (2015) Ontario Butterfly Atlas Online. Toronto Entomologists' Association. http://www.ontarioinsects.org/atlas_online.htm [accessed: 15.Dec.2015]

Maritimes Butterfly Atlas (2015) Maritimes Butterfly Atlas. http://www.accdc.com/mba/index-mba.html [accessed 5.Jan.2016]

Miller LD, Brown FM (1981) A catalogue/checklist of the butterflies of America north of Mexico. The Lepidopterists' Society Memoirs 2: 1–280.

NABA (North American Butterfly Association) (2001) NABA Checklist and English Names of North American Butterflies. North American Butterfly Association, Morristown, 41 pp.

Nielsen MC (1999) Michigan Butterflies and Skippers: A Field Guide and Reference. Michigan State University Extension, Michigan State University, East Lansing, Michigan, 248 pp.

Ômura H, Itoh T, Wright DM, Pavulaan H, Schröder S (2015) Morphological study of alar androconia in *Celastrina* butterflies. Entomological Science 1–7. doi: 10.1111/ens.12126

Opler PA, Krizek GO (1984) Butterflies East of the Great Plains. The Johns Hopkins University Press, Baltimore, 294 pp.

Pavulaan H (2014) A case of sympatric *Celastrina ladon* (Cramer), *Celastrina lucia* (W. Kirby) and *Celastrina neglecta* (Edwards) (Lycaenidae: Polyommatinae) in Northern Virginia, with additional records of *C. lucia* in Virginia. Taxonomic Report 7(7): 1–10.

Pavulaan H, Wright DM (2005) *Celastrina serotina* (Lycaenidae: Polyommatinae): a new butterfly species from the northeastern United States and eastern Canada. Taxonomic Report 6: 1–19.

Pelham JP (2008) A catalogue of the butterflies of the United States and Canada: with a complete bibliography of the descriptive and systematic literature. Journal of Research on the Lepidoptera 40: 1–658.

Pohl G, Anweiler GG, Schmidt BC, Kondla N (2010) Annotated List of the Lepidoptera of Alberta, Canada. ZooKeys 38: 1–549. doi: 10.3897/zookeys.38.383

Pratt GF, Wright DM, Pavulaan H (1994) The various taxa and hosts of the North American *Celastrina* (Lepidoptera: Lycaenidae). Proceedings of the Entomological Society of Washington 96(3): 566–578.

Sandre S-L, Kaasik A, Eulitz U, Tammaru T (2013) Phenotypic plasticity in a generalist insect herbivore with the combined use of direct and indirect cues. Oikos 122: 1626–1635. doi: 10.1111/j.1600–0706.2013.00504.x

Saunders W (1969) Entomological Notes. Canadian Entomologist 1(11): 93–101.

Saunders W (1875) [Untitled editor's note]. Canadian Entomologist 7(5): 82–83.

Scott GA (1995) Canada's vegetation: a world perspective. McGill-Queen's University Press, Montreal, 362 pp.

Scott JA (1986) The butterflies of North America: a natural history and field guide. Stanford University Press, Stanford, 581 pp.

Scott JA, Wright DM (1998) A new *Celastrina* from the eastern slope of Colorado. Papilio NS 9: 1–15.

Scudder SH (1876) The relationship of the early spring blues. Canadian Entomologist 8(4): 61–66. doi: 10.4039/Ent861-4

Wagner WH Jr, Mellichamp TL (1978) Foodplant, habitat, and range of *Celastrina ebenina* (Lycaenidae). Journal of the Lepidopterists' Society 32: 20–36.

Warren AD (2005) Butterflies of Oregon: Their Taxonomy, Distribution, and Biology. Contributions of the C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Ft. Collins, 408 pp.

Wright DM (1998) Update on *Celastrina* including notes on Ohio species. The Ohio Lepidopterist 20(2): 18–21.

Wright DM, Pavulaan H (1999) *Celastrina idella* (Lycaenidae: Polyommatinae): a new butterfly species from the Atlantic coastal plain. The Taxonomic Report of the International Lepidoptera Survey 1(9): 1–11.

Supplementary material 1

Data for DNA barcode voucher specimens of *Celastrina*

Authors: B. Christian Schmidt, Ross A. Layberry

Data type: Microsoft Excel Spreadsheet (.xls)