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Taxonomic review of the genus *Empria* Lepeletier & Serville (Hymenoptera, Tenthredinidae) in South Korea: morphological and molecular identification of two new species

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Abstract

The sawfly genus *Empria* Lepeletier & Serville (Hymenoptera: Tenthredinidae: Allantinae) is reviewed in South Korea and four species are reported as new in the country: *Empria lycroi* Prous & Park, **sp. nov.** (also Russia); *E. nigroterga* Park & Lee, **sp. nov.** (also Russia); *E. wui* Wei & Nie (also Japan); and *E. zhangi* Wei & Yan (also Russia). *Tenthredo magnicornis* Eversmann, 1864, **syn. nov.** is treated as a synonym of *E. candidata* (Fallén, 1808). A key to the six species currently known from South Korea and photographs of the diagnostic characters for each species are presented. In addition, phylogenetic analysis based on one mitochondrial (COI) and two nuclear (NaK, POL2) genes are reported for 20 species of *Empria*.

Keywords

Allantinae, COI, key, new record, nuclear genes, Symphyta

Introduction

The genus *Empria* Lepeletier & Serville, a member of the subfamily Allantinae, currently includes 56 described world species. They are separated into six species groups: *candidata* group, *hungarica* group, *immersa* group, *longicornis* group, *quadrimaculata* group, and *wui* group. 40 species have been recorded in the Palaearctic, 11 species in the Nearctic, four species in the Oriental, two species in the Neotropic, and one fossil species (Taeger et al. 2018). The native habitat of the genus is the Northern Hemisphere and most occur at higher latitudes (mostly north of 30–40°N) (Prous and Heidemaa 2012). The larvae of *Empria* species feed on species of the families Betulaceae, Ericaceae, Rosaceae, and Salicaceae (Shinohara et al. 2015). The general morphology of *Empria* is very similar to that of *Monsoma*, but it can be easily distinguished by the following key characters: clypeus incised with distinct middle tooth or terga with pairs of pale patches. Identification of *Empria* species using only external morphology can often be difficult and members of some species groups can hardly be separated. It is essential to dissect female ovipositors and male genitalia for reliable identification (Prous et al. 2011a, b).

Although this group is widely distributed in the Holarctic, very little is known about the South Korean species. Until recently, only two species (*E. candidata* and *E. tridentis*) had been identified (Lee and Ryu 1996; Prous et al. 2011a). Here we report four additional species, two of them described as new: *Empria lycroi* Prous & Park, sp. nov., *E. nigroterga* Park & Lee, sp. nov., and *E. zhangi* Wei & Yan from South Korea and Russia, and *E. wui* Wei & Nie from South Korea and Japan. *Tenthredo magnicornis* Eversmann, 1864, syn. nov. is synonymized under *E. candidata* (Fallén, 1808). A key is provided to distinguish the six species from South Korea. In addition, phylogenetic analysis based on one mitochondrial (COI) and two nuclear (NaK, POL2) genes are reported for 20 species of *Empria*.

Materials and methods

Morphological terminology follows Viitasaari (2002). Ratios for species identifications were based on measurements following Prous et al. (2011b). The parenthesis used in the species descriptions indicate character states for variations between specimens in the new species, and character states for color variations in other species. Names of the mentioned host plants follow 'The Plant List' (http://www.theplantlist.org/). Photos were taken with a digital camera attached to a microscope. Composite images were created from stacks of images using AxioVision40AC, IMT iSolution Lite software, or CombineZP (Alan Hadley; http://www.hadleyweb.pwp.blueyonder.co.uk/), and final plates were prepared in Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, CA, USA).

Newly obtained sequences of cytochrome oxidase subunit I (COI), nuclear sodium/potassium-transporting ATPase subunit alpha (NaK) and DNA dependent RNA polymerase II subunit RPB1 (POL2) (sequenced as described in Liston et al. 2019 and references therein) were submitted to NCBI GenBank (accession numbers MN294991–MN295013). Nuclear internal transcribed spacer (ITS) sequences mentioned for *Empria wui* were published by Prous and Heidemaa (2012). To facilitate comparison with data in BOLD (http://www.boldsystems.org/) and unless otherwise stated, COI p-distance values are based on the barcode region (658 bp), but in the phylogenetic analyses in combination with nuclear genes, 1078–1089 bp COI fragment was used instead. Nuclear distances are based on the combined NaK and POL2. Distances were taken from BOLD or calculated in R (R Core Team 2018) with the package *ape* (Paradis and Schliep 2018). Phylogenetic analyses using maximum likelihood (ML) were performed using IQ-TREE 1.5.6 (http://www.iqtree.org/) (Nguyen et al. 2015). By default, IQ-TREE runs ModelFinder (Kalyaanamoorthy et al. 2017) to find the best-fit substitution model and then reconstructs the tree using the model selected according to the Bayesian information criterion (BIC). We complemented this default option with an SH-like approximate likelihood ratio (SH-aLRT) test (Guindon et al. 2010) and ultrafast bootstrap (Hoang et al. 2018) with 1000 replicates to estimate robustness of reconstructed splits. Analyzed sequences are from Prous and Heidemaa (2012), Liston et al. (2019), Prous et al. (2019), and this study.

Pinned specimens including the studied types come from the following institutional collections: CSCS, Central South University of Forestry and Technology (Changsha, China); NIBR, National Institute of Biological Resources (Incheon, South Korea); NNIBR, Nakdonggang National Institute of Biological Resources (Sangju, South Korea); NSMT, National Museum of Nature and Science (Tsukuba, Japan); SDEI, Senckenberg Deutsches Entomologisches Institut (Müncheberg, Germany); USNM, National Museum of Natural History, Smithsonian Institution (Washington DC, USA); YNU, Yeungnam University (Gyeongsan, South Korea); ZIN, Russian Academy of Sciences, Zoological Institute (St. Petersburg, Russia).

The following abbreviations are used throughout the text: OOCL (ocellar-occipital carina line), the shortest distance between posterior margin of a lateral ocellus and the hind margin of the head; POL (postocellar line), the shortest distance between medial margins of the two lateral ocelli; MT, Malaise trap.

Results and discussion

Genus *Empria* Lepeletier & Serville, 1828

Key to the species from South Korea

1 Head largely pale in female; clypeus mostly pale without median keel (Fig. 1I, J); posterior margin of sternum 9 with a notch or truncated (Prous et al. 2011a; fig. 7); valviceps about 1.6 times as long as valvura (Fig. 7A) .... ***E. candidata*** **(Fallén)**

– Head mostly black in both sexes; clypeus entirely black with median keel (Figs 1K, L, 2I–L); posterior margin of sternum 9 rounded or truncated (Figs 3J, 4J); valviceps shorter or almost equal in length to valvura (Fig. 7B–F) **2**

2 Vein 2A+3A in fore wing incomplete (Prous and Heidemaa 2012; fig. 14); cell M in hind wing open; posterior margin of sternum 9 truncated .... ***E. wui* Wei & Nie**

– Vein 2A+3A in fore wing complete; cell M in hind wing closed (Fig. 4A, B); posterior margin of sternum 9 rounded or truncated **3**

3 Tegula mostly black; abdominal terga with indistinct pairs of whitish (pale) patches (Fig. 4A, B) .... ***E. nigroterga* Park & Lee, sp. nov.**

– Tegula usually entirely white; abdominal terga with distinct pairs of whitish (pale) patches (Figs 1C, D, 2C, D, 3A, B) **4**

4 Malar space 1.7–1.9 times as long as diameter of median ocellus in female, 1.4–1.6 times in male (Fig. 1K, L); flagellum 2.3–2.5 times as long as head width in female, 3.6–3.7 times in male; ovipositor sheath not extending or slightly extending beyond apex of abdomen; each serrula subtriangularly protruding basally (Fig. 6B) .... ***E. tridentis* Lee & Ryu**

– Malar space 1.1–1.7 times as long as diameter of median ocellus in female, 0.8–1.0 times in male (Figs 2K, L, 3F, I); flagellum 1.4–2.0 times as long as head width in female, 1.8–2.4 times in male; ovipositor sheath not extending to distinctly extending beyond apex of abdomen; each serrula subtriangularly or circularly protruding basally (Fig. 6D, E) **5**

5 All trochanters and trochantelli black; ovipositor sheath distinctly extending beyond apex of abdomen (Fig. 2F); each serrula circularly protruding basally (Fig. 6D).. ***E. zhangi* Wei & Yan**

– All trochanters and trochantelli pale; ovipositor sheath not extending or slightly extending beyond apex of abdomen (Fig. 3C); each serrula subtriangularly protruding basally (Fig. 6E)........... ***E. lycroi* Prous & Park, sp. nov.**

Empria candidata (Fallén, 1808)

Figs 1A, B, E, G, I, J, 6A, 7A

Tenthredo candidata Fallén, 1808: 105–106.
Tenthredo (*Allantus*) *repanda* Klug, 1816: 77–78.
Tenthredo magnicornis Eversmann in Kawall, 1864: 297, syn. nov.

Diagnosis. ***Female*** (Figs 1A, E, I, 6A). Body length 7.2–8.0 mm. Head and thorax black, except clypeus, labrum, basal half of mandible, facial orbit, gena, temple, genal orbit, labial and maxillary palps, pronotum dorsally, tegula, mesepisternum medially, postspiracular sclerite, all outer coxae, all trochanters and trochantelli, all femora, tibiae and tarsi anteriorly pale; rest of mandible reddish brown with black margins apically; supraclypeal area, scape and pedicel (brown to black), cenchrus, all tibiae and tarsi posteriorly (entirely) brown; abdomen dark brown, segments with posterior pale margins (sterna without pale margins, but 4–6 with pale patches medially) and abdominal terga with 3–5 pairs of pale patches (Fig. 1A, E, I). Clypeus weakly rugulose without median keel. Malar space 1.2–1.4 times as long as diameter of median ocel-

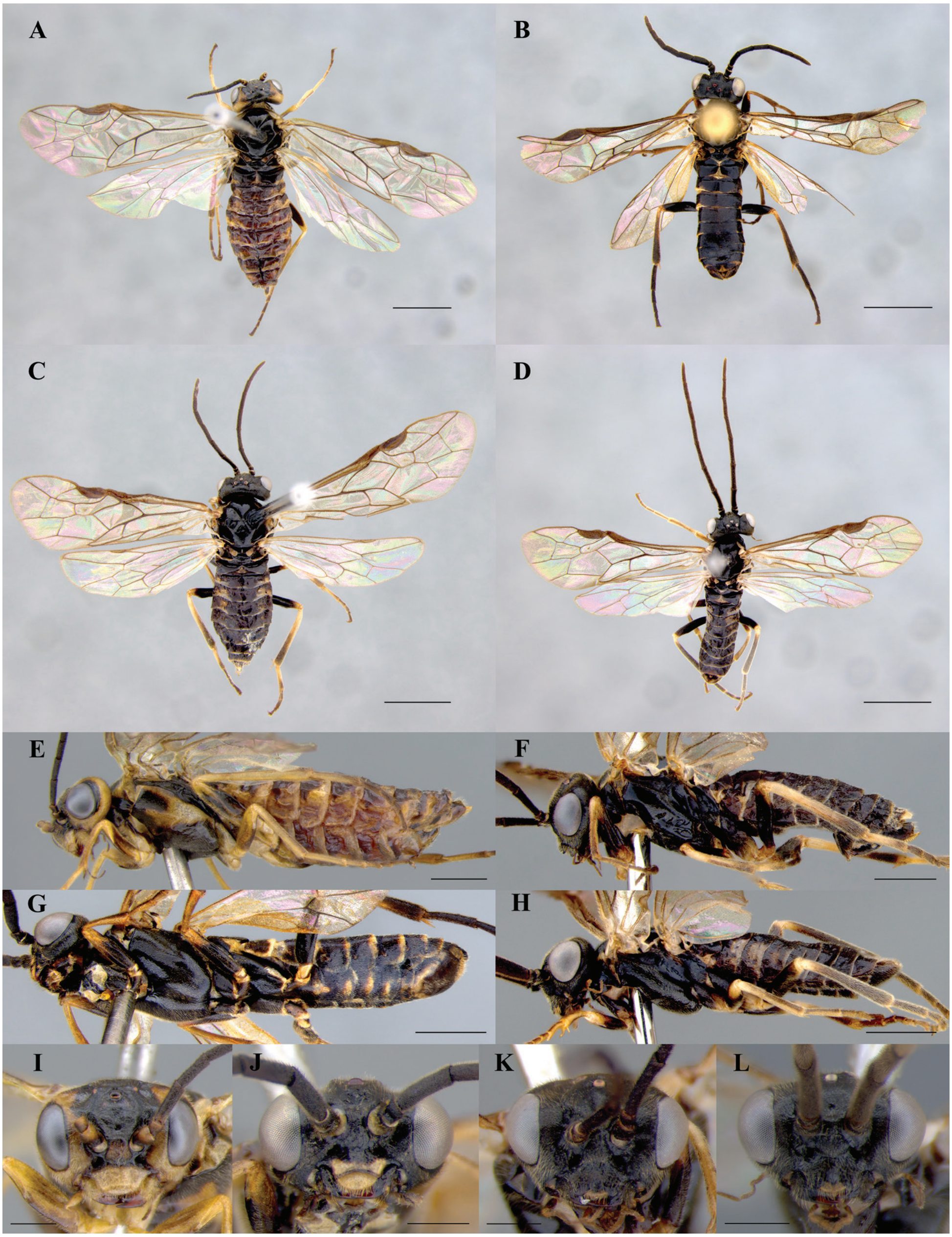

Figure 1. *Empria* spp.: **A, B, E, G, I, J** *E. candidata* from Gangwon, South Korea **C, D, F, H, K, L** *E. tridentis*, female from Jeonnam and male from Gangwon, South Korea **A, C** dorsal habitus, female **B, D** dorsal habitus, male **E, F** lateral habitus, female **G, H** lateral habitus, male **I, K** frontal head, female **J, L** frontal head, male. Scale bars: 2 mm (**A–D**); 1 mm (**E–H**); 0.5 mm (**I–L**).

lus (Fig. 1I, J). Flagellum 1.3–1.4 times as long as head width. Lancet with about 19 serrulae, membrane between serrulae flat; each serrula weakly protruding basally (Fig. 6A), with a basal denticle and 8–11 distal denticles.

Male (Figs 1B, G, J, 7A). Body length 5.5–6.4 mm. Similar to female, but body black, except lower half of clypeus and labrum pale (white); patches of abdominal terga fading towards the apex; posterodorsal margin of pronotum, cenchrus, all tibiae and tarsi posteriorly, and basal 2/3 of hind tibia posteriorly yellowish brown; all femora at apex and all tibiae at base with yellowish brown ring (Fig. 1B, G, J). Flagellum about 2.0 times as long as head width. Posterior margin of sternum 9 weakly concave at middle (truncated). Penis valve as in Fig. 7A, valviceps about 1.6 times as long as valvura.

Genetic data. Based on the mitochondrial COI barcode sequences available in BOLD (15 specimens within BOLD:ABV8001, BOLD:ADS7820, and BOLD:AAG3534), the maximal distance between the specimens is 3.5%. The nearest neighbour, diverging by a minimum of 7.4%, is the North American *E. multicolor* (Norton). Based on the nuclear data of two specimens (Sweden and Russia, Liston et al. 2019), the distance between them is 1.0%. The nearest neighbour, diverging by a minimum of 5.2%, is *E. multicolor* (Norton).

Specimens examined. South Korea: 1♀, Gangwon-do, Mt. Odaesan, Mirugam (Pugdaesa), 1300 m, 31.V.1991, A. Shinohara (CSCS; NSMT187), specimen in exchange from NSMT; 1♀1♂, same locality, 28.V.1992, A. Shinohara (NSMT; 1♀, NSMT-HYM65594/NSMT186; 1♂, NSMT-HYM65597/NSMT191); 1♂, same locality, 29.V.1992, A. Shinohara (NSMT; NSMT-HYM65596/NSMT190); 1♂, same locality, 28.V.1993, A. Shinohara (CSCS; NSMT221), specimen in exchange from NSMT; 1♂, same data (NSMT; NSMT-HYM65598/NSMT192); 1♀1♂, same data (YNU; 1♀, NSMT-HYM65591/NSMT189; 1♂, NSMT-HYM65599/NSMT196), specimens in exchange from NSMT VI/2018; 1♀, same data (NSMT; NSMT-HYM65592/NSMT188); 1♀, same locality, 29.V–1.VI.1996, A. Shinohara (NSMT; NSMT-HYM65595/NSMT185); 1♀, Gangwon-do, Taebaek-si, Sododong, Mt. Taebaeksan, Danggol, 6.V.1999, S.M. Ryu (YNU); Czech Republic: 1♂, Bohemia, Decinsky Sneznik, 50.8N, 14.11666E, 29.IV.1994, E. Kula (SDEI; DEI-GISHym88921); 1♂, same locality, 5.V.1995 (SDEI; DEI-GISHym21037); 1♀, same locality, 26.V.1995 (SDEI; DEI-GISHym21036); 1♂, same locality, 20.IV.2007 (SDEI; DEI-GISHym21032); 1♀, same locality, 24.IV.2007 (SDEI; DEI-GISHym21033); 1♀, same locality, 27.IV.2007 (SDEI; DEI-GISHym21034); Finland: 1♀, Northern Ostrobothnia, Hailuoto, Keskikylae, 65.022N, 24.743E, 10 m, 31.V.2018, A. Liston & M. Prous, (SDEI; DEI-GISHym84426); 1♀, Northern Ostrobothnia, Hailuoto, Potti, 65.059N, 24.884E, 1 m, 31.V.2018, A. Liston & M. Prous, (SDEI; without ID number); 1♀, Lapin Laeaeni, Leppaelae 20 km S, 69.52N, 27.22E, 10.VI.2001, A. Taeger & Ch. Kutzscher (SDEI; without ID number); 1♂, Lapin Laeaeni, Nuorbenjarga 46 km SW Utsjoki, 69.685N, 25.94E, 9.VI.2001, A. Taeger & Ch. Kutzscher (SDEI; without ID number); 1♀, Lapin Laeaeni, Nuorbenjarga 46 km SW Utsjoki, 69.685N, 25.94E, 13.VI.2001, A. Taeger & Ch. Kutzscher (SDEI; DEI-GISHym14907); 1♀, Lapin Laeaeni, Nuorgam 17 km SSE, 69.944N, 28.041E, 12.VI.2001, A. Taeger & Ch. Kutzscher (SDEI; DEI-GISHym4835); 1♀, Lapland, Tolva 7 km W, 66.223N, 28.552E, 330–450 m, 9.VI.2018, SDEI Hym-group (SDEI; DEI-GISHym84427); GERMANY: 1♂, Sachsen, Erzgebirge, Altenberg Umg., 13.V.1986, S. Walter (SDEI; DEI-GISHym20803); 1♀, Sachsen, Syrau, NSG Syrau-Kauschwitzer Heide, 50.53121N, 12.07119E, 21.V.2008, F. Burger (SDEI; DEI-GISHym19044); 1♀, Brandenburg, Waldsieversdorf, 52.55N, 14.03333E, 24.IV.2005, A.D. Liston (SDEI; DEI-GISHym19068); Japan: 1♀, Hokkaido, Nissho-toge, Hidaka Mts., Hidaka/Tokachi, 1100 m, 23.VI.2004, A. Shinohara (NSMT; NSMT-HYM69256/NSMT184); 1♂, Hokkaido, Nisshotoge, Hidaka Mts. Hidaka, 42.971N, 142.752E, 1050 m, 2–12.VI.2010, N. Kuhara (USNM; USNM2057434_02); Portugal: 1♀, Viana do Castelo, Paredes de Coura 6 km NNE, 41.947N, 8.50619W, 480 m, 13.V.2012, Blank, Jacobs, Liston & Taeger (SDEI; DEI-GISHym15216); Russia: 2♀♀, Khabarovskiy Kray, Boitsovo N Bikin, Bolshoi Solntsepyok Hill SE Boitsovo, 47.03333N, 134.35E, 300 m, 26.V.1993, A. Taeger (SDEI; without ID number, DEI-GISHym14908); 1♀, Primorsky Krai, Anisimovka, Gribanovka, 1 km N, 43.126N, 132.797E, 450 m, 8.V.2019, M. Prous (SDEI; DEI-GISHym80656); 3♀♀, same locality, 9.V.2019, M. Prous (SDEI; DEI-GISHym80795, DEI-GISHym80661, DEI-GISHym80665); 5♀♀2♂♂, same locality, 2 km N, 43.14N, 132.791E, 360 m, 10.V.2019, M. Prous (SDEI; 5♀♀, two without ID numbers, DEI-GISHym80682, GISHym80691, DEI-GISHym80819; 2♂♂, DEI-GISHym80839, DEI-GISHym80854); 1♀, Primorskiy Kray, Biological station 30 km SE Chuguyevka (Sichote Alin), 44.08333N, 134.2E, 650 m, 31.V.1993, A. Taeger (SDEI; DEI-GISHym14909); 1♀, Primorskiy Kray, Gornotajozhnoe, 1 km E, 43.694N, 132.168E, 150 m, 11.V.2019, M. Prous (SDEI; DEI-GISHym80694); 1♂, Primorskiy Kray, Gornotajozhnoe, Observatorium, 43.699N, 132.166E, 270 m, 18.V.2016, K. Kramp, M. Prous & A. Taeger (SDEI; DEI-GISHym80252); Sweden: 1♂, Norrbotten, Överkalix, 22 km W, 140 m, 66.295N, 22.356E, 2.VI.2014, leg. A. Liston & M. Prous (YNU; S343), specimen presented from SDEI XII/2014; 1♀, Norrbotten, Råneå, 10 SW, 10 m, 65.788N, 22.156E, 30.V.2014, leg. A. Liston & M. Prous (YNU; S334), specimen presented from SDEI XII/2014; 1♂, Norrbottens Laen, Abisko National Park, E10, 68.353N, 18.815E, 390 m, 16.VI.2012, A.D. Liston, A. Taeger & S.M. Blank (SDEI; without ID number); 1♂, Norrbottens Laen, Aengestraesk 11 km NEE, 66.052N, 22.386E, 140 m, 28.V.2014, A. Liston & M. Prous (SDEI; without ID number); 2♀♀, same locality, 3 km N, 66.035N, 22.16E, 40 m, 28.V.2014, A. Liston & M. Prous (SDEI; without ID numbers); 1♀, same locality, 6 km NE, 66.048N, 22.239E, 140 m, 28.V.2014, A. Liston & M. Prous (SDEI; without ID number); 3♀♀1♂, Norrbottens Laen, Bjoerkfors, 65.92N, 23.46E, 10 m, 1.VI.2014, A. Liston & M. Prous (SDEI; without ID numbers); 1♀, Norrbottens Laen, Gaellivare 18 km SEE, 67.082N, 21.051E, 320 m, 11.VI.2014, A. Liston & M. Prous (SDEI; without ID number); 1♀, Norrbottens Laen, Gaellivare Dundret, 67.105N, 20.641E, 450 m, 11.VI.2014, A. Liston & M. Prous (SDEI; without ID number); 1♀, Norrbottens Laen, Haparanda 13 km NW, 65.926N, 23.918E, 60 m, 27.V.2014, A. Liston & M. Prous (SDEI; without ID number); 1♀, same locality, 5 km W, 65.82N, 24.033E, 2 m, 3.VI.2014, A. Liston & M. Prous (SDEI; without ID number); 1♂, Norrbottens Laen, Hietaniemi 10 km SW, 66.166N, 23.495E, 90 m, 1.VI.2014, A. Liston & M. Prous (SDEI; without ID number); 1♀, Norrbottens Laen, Kalix, Palaenge, 65.815N, 22.975E, 50 m, 26.V.2014, A. Liston & M. Prous (SDEI; without ID number); 1♀, Norrbottens Laen, Kamlunge, 65.993N, 22.858E, 40 m, 28.V.2014, A. Liston & M. Prous (SDEI; without ID number); 1♀1♂, Norrbottens Laen, Kiruna nr. airport, 67.84N, 20.35E, 450 m, 21.V.2012, A.D. Liston, A. Taeger & S.M. Blank (SDEI; without ID numbers); 3♀♀1♂, Norrbottens Laen, Kitkioejaervi, 6 km SE, 67.77N, 23.265E, 240 m, 7.VI.2014, A. Liston & M. Prous (SDEI; 3♀♀, without ID numbers; 1♂; DEI-GISHym80016); 3♂♂, Norrbottens Laen, Lantjaerv, 4 km N, 65.89N, 23.347E, 30 m, 1.VI.2014, A. Liston & M. Prous (SDEI; without ID number, DEI-GISHym31012, DEI-GISHym31064); 2♀♀, Norrbottens Laen, Oeverkalix 22 km W, 66.295N, 22.356E, 140 m, 2.VI.2014, A. Liston & M. Prous (SDEI; without ID numbers); 2♀♀, Norrbottens Laen, Pajala, 67.204N, 23.409E, 150 m, 5.VI.2014, A. Liston & M. Prous (SDEI; without ID numbers); 1♀, Norrbottens Laen, Ranea 10 SW, 65.788N, 22.156E, 10 m, 30.VI.2014, A. Liston & M. Prous (SDEI; without ID number); 1♂1♀, Norrbottens Laen, Taerendoe, 67.166N, 22.642E, 160 m, 6.VI.2014, A. Liston & M. Prous (SDEI; without ID numbers); 1♀, Norrbottens Laen, Tornetraesk station, 68.215N, 19.74E, 400 m, 21.VI.2012, A.D. Liston & A. Taeger (SDEI; without ID number); 1♀, Torne Lappmark, Abisko, 6 km E, 68.348N, 18.969E, 400 m, 4.VII.2016, A. Liston & M. Prous (SDEI; without ID number); 1♀, Vaesterbottens Laen, Loevanger 3 km N, 64.402N, 21.301E, 60 m, 25.V.2014, A. Liston & M. Prous (SDEI; without ID number); United Kingdom: 1♀, Scotland, Claybokie, near Braemar, 56.99237N, 3.50222W, 4.VI.2010, A.D. Liston (SDEI; DEI-GISHym19067).

Host plants. Betulaceae: *Betula pendula* Roth, *Betula* sp. (Lorenz and Kraus 1957; Verzhutskii 1966, 1981).

Distribution. Austria, Belgium, Bulgaria, Canada, China, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Hungary, Japan, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, South Korea, Sweden, Switzerland, United Kingdom, USA (Prous et al. 2011a; Taeger et al. 2018).

Discussion. Belongs to *E. candidata* group. This species was first recorded from South Korea by Prous et al. (2011a). Significant sexual dimorphism is represented in the body color as noted above.

Macek and Kula (2015) distinguished two forms, *E. candidata* (Fallén, 1808) and *E. magnicornis* (Eversmann, 1864) which we are unable to confirm. Color of mesepisternum in females varies from almost completely black (small round or vaguely triangular posterior fleck) to almost completely pale. Commonly there are two, anterior and posterior triangular or vaguely triangular flecks, which are clearly separated, or connected in various degrees. The vague differences in shape of head, thorax, and body mentioned by Macek and Kula (2015) vary continuously too and in various combinations. No clear correlation was detected between these characters and coloration. In males, there is no clear correlation between coloration of clypeus and head shape. Penis valves vary significantly (partly because of variations in slide preparations) and not in correlation with coloration or head shape. For example, a specimen from Russian Far

East with nearly completely pale clypeus and with head shape more like *E. candidata* sensu Macek and Kula (2015) has a penis valve more like *E. magnicornis* (https://sdei.de/ecatsym/ecat_bild.php?NameNr=1003677&DateiName=24088.JPG).

Although two specimens at the extremes of variation can look strikingly different, most specimens cannot be identified as belonging to one or the other form identified by Macek and Kula (2015). The variation is too large and the characters too ill-defined by Macek and Kula (2015) to have any practical value in separating two forms in *E. candidata*. There are various other and equally legitimate starting points to divide morphological variation in *E. candidata* into more than one group (e.g. the presence or absence of pale flecks in the middle of abdominal terga), but it is not clear if any of them have taxonomic value. Because morphological variation in *E. candidata* is better explained by variation in widely distributed species, we treat *E. magnicornis* (Eversmann, 1864) as a synonym of *E. candidata*.

***Empria tridentis* Lee & Ryu, 1996**

Figs 1C, D, F, H, K, L, 6B, 7B

Empria tridentis Lee & Ryu, 1996: 23.

Diagnosis. *Female* (Figs 1C, F, K, 6B). Body length 5.8–6.1 mm. Head and thorax black (clypeus pale in lower margin), except labrum, posterodorsal margin of pronotum, tegula, apex of all femora, basal 1/3 of fore and middle tibiae, basal 2/3 (mostly) of hind tibia, basal half of middle and hind tarsomere 1 white (pale); cenchrus, all trochanters and trochantelli yellowish brown; apical 2/3 of fore tibia, fore tarsus and middle tibia anteriorly brown; mandible at apex reddish dark brown; labial and maxillary palps dark brown; abdomen dark brown, abdominal terga with 3–4 pairs of whitish (pale) patches (the tergum 5 with small-sized or indistinct patch) (Fig. 1C, F, K). Malar space 1.7–1.9 times as long as diameter of median ocellus (Fig. 1K). Flagellum 2.3–2.5 times as long as head width. Ovipositor sheath only reaching beyond apex of abdomen; dorsal and ventral margins slightly convergent and apex obliquely truncated (Fig. 1F). Lancet with about 17 serrulae, membrane between serrulae weakly convex; each serrula subtriangularly protruding basally (Fig. 6B), with a basal denticle and 2–7 distal denticles.

Male (Figs 1D, H, L, 7B). Body length 4.7–5.1 mm. Similar to female, but fore leg much paler than female; middle tibia anteriorly and hind tibia at base white (pale); middle and hind tarsomere 1 entirely dark brown; sometimes abdominal segments with narrow posterior whitish margins (Fig. 1D, H, L). Malar space 1.4–1.6 times as long as diameter of median ocellus (Fig. 1L). Flagellum 3.6–3.7 times as long as head width. Posterior margin of sternum 9 rounded. Penis valve as in Fig. 7B, valviceps almost equal in length to valvura.

Genetic data. Based on the barcode region of two available COI sequences (Japan), the distance between them is 0.2%. The nearest neighbours are species of the

E. longicornis group, diverging by a minimum of 4.9%. Based on the nuclear data of one specimen (Japan), the nearest neighbours are species of the *E. longicornis* group, diverging by a minimum of 2.4%.

Type specimens examined. South Korea: ♀, holotype of *Empria tridentis* (YNU-Sym-0011), "Gangwon-do, Goseong-gun, Hyangnobong, 38°19'N, 128°18'E, 13.V.1992, S.M. Ryu" (YNU); 8♀♀, paratypes of *Empria tridentis* (YNU-Sym-1115~1121, 1123), "same data as holotype" (1♀ in CSCS, 2♀♀ in NNIBR, 5♀♀ in YNU).

Additional specimens examined. South Korea: 1♂, Gangwon-do, Pyeongchang-gun, Mt. Balwangsan, 11.VI.2000, J.W. Lee (YNU); 1♀4♂♂, Gangwon-do, Wonju-si, Mt. Baegunsan, 37°15'30.5"N, 127°58'55.11"E, 19.V–6.VI.2011, H.Y. Han (YNU); 2♂♂, same data, J.W. Lee (YNU); 2♂♂, Gangwon-do, Wonju-si, Panbu-myeon, Mt. Baegunsan, 28.IV–24.V.2012, H.Y. Han (YNU); 1♀1♂, Jeollanam-do, Jangseong-gun, Bukha-myeon, Hyangnobong, 37°27'00"N, 126°51'00"E, 13.V.1992, J.W. Lee (YNU); 3♀♀1♂, same locality, 13.VI.1992, J.W. Lee (1♀1♂ in NSMT, 2♀♀ in YNU).

Host plants. Rosaceae: *Filipendula camtschatica* (Pall.) Maxim., *Geum japonicum* (Shinohara et al. 2015).

Distribution. Japan, Russia (Prous et al. 2011a), South Korea (Lee and Ryu 1996).

Empria wui **Wei & Nie, 1998**

Figs 2A, B, E, G, I, J, 5A–G, 6C, 7C

Empria wui Wei & Nie, 1998: 363–386.

Diagnosis. ***Female*** (Figs 2A, E, I, 5A–G, 6C). Body length 5.2–6.0 mm. Head and thorax black, except cenchrus white; posterodorsal margin of pronotum, apical 1/3 of fore femur, apex of middle and hind femora slightly, fore and middle tibiae anteriorly, basal 2/3 or less of hind tibia yellowish brown; labrum, labial and maxillary palps dark brown at apex; apical half of mandible reddish dark brown; abdomen dark brown, segments with posterior whitish (pale) margins and abdominal terga with 3–4 pairs of large pale patches (Figs 2A, B, E, G, I, J, 5B, C, E). Malar space about 1.2 times as long as diameter of median ocellus (Figs 2I, J, 5E). Flagellum 1.5–1.8 times as long as head width. Vein 2A+3A in fore wing incomplete; vein m-cu in hind wing absent, cell M open (Fig. 2A, B). Claws simple or with small denticle (Fig. 5A). Ovipositor sheath extending beyond apex of abdomen (Figs 2E, 5D). Lancet with 16–18 serrulae, membrane between serrulae weakly convex; each serrula subtriangularly protruding basally (Figs 5G, 6C), with a basal denticle and roughly 4–10 distal denticles.

Male (hitherto undescribed) (Figs 2B, G, J, 7C). Body length 4.5–5.0 mm. Similar to female, but flagellum 1.9–2.1 times as long as head width. Posterior margin of sternum 9 truncated. Penis valve as in Fig. 7C, valviceps almost equal in length to valvura.

Genetic data. Based on the barcode region of two COI sequences available in GenBank, the distance between the specimens from China and Japan is 2.6% (same distance based on the complete COI). The nearest neighbour, diverging by 2.3–2.7%

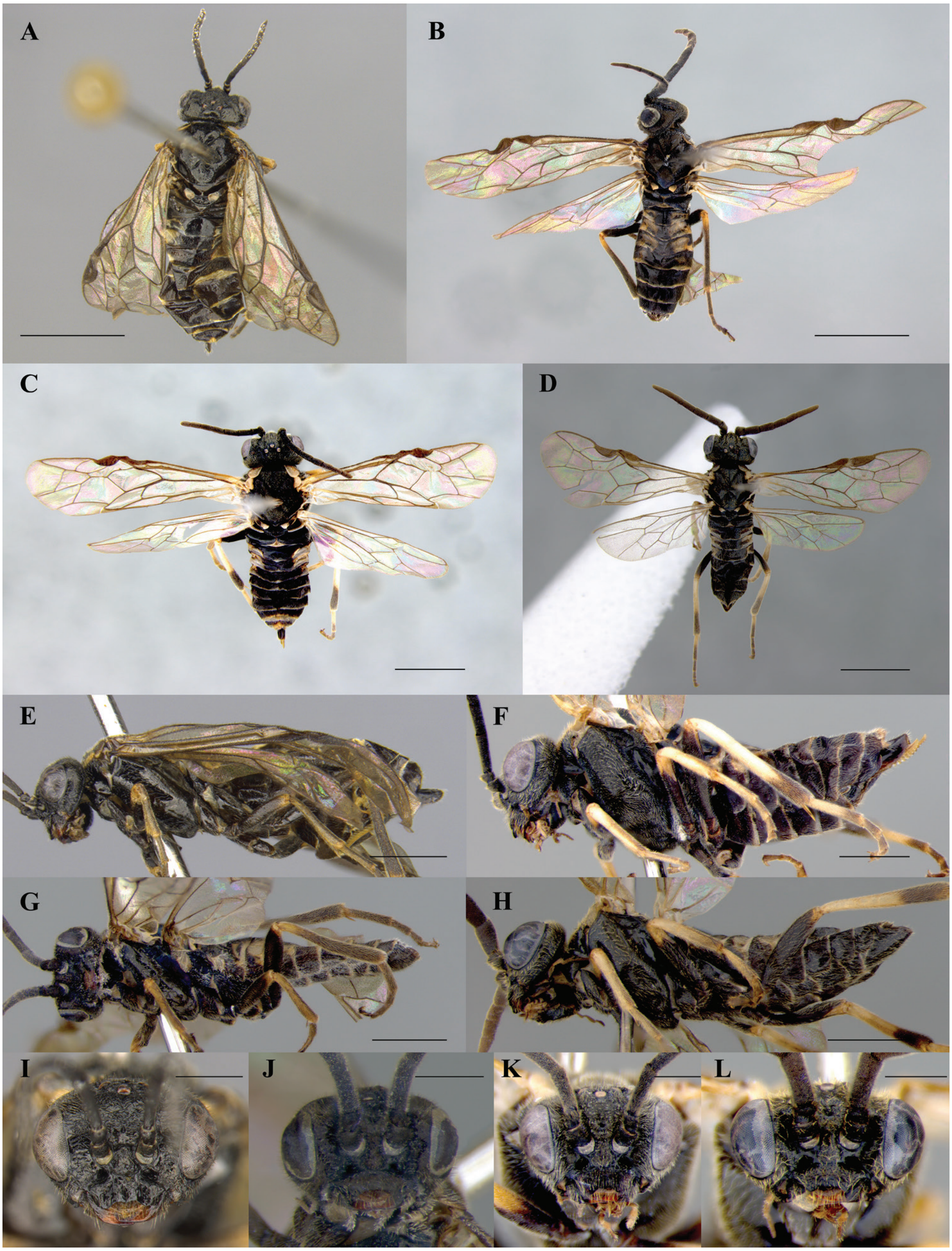

Figure 2. *Empria* spp.: **A, B, E, G, I, J** *E. wui*, female from Shanxi, China and male from Gyeongbuk, South Korea **C, D, F, H, K, L** *E. zhangi* from Gangwon, South Korea **A, C** dorsal habitus, female **B, D** dorsal habitus, male **E, F** lateral habitus, female **G, H** lateral habitus, male **I, K** frontal head, female **J, L** frontal head, male. Scale bars: 2 mm (**A–D**); 1 mm (**E–H**); 0.5 mm (**I–L**).

(or 2.5–3.1% based on the complete COI), is possibly an undescribed species from China (sp. 2 in Prous and Heidemaa 2012). Based on the nuclear ITS, the distance between the specimens from China and Japan is 0.7%. The nearest neighbour based on nuclear ITS, diverging by 1.4%, is sp. 2 in Prous and Heidemaa (2012). Nuclear NaK and POL2 are only available for one specimen from Japan and the nearest neighbour being *E. liturata* (Gmelin), diverging by 5.3%, but this data is unavailable for sp. 2 from Prous and Heidemaa (2012).

Type specimen examined. China: ♀, holotype of *Empria wui* (CSCSEmp6), “Zhejiang, Longwangshan, 30.4N, 119.4E, 7.IV.1996, Hong Wu” (CSCS).

Additional specimens examined. South Korea: 1♂, Gyeongsangbuk-do, Yeongju-si, Dansan-myeon, Marak-ri, San 46-5, Mt. Sobaeksan, Euipungji, 5.IV–3.V.2016, E.J. Hong (NIBR); China: 1♀, Shanxi, Alt. 1703 m, 37.8314N, 111.4861E, 30.V.2008 (CSCS; W08-03a); Japan: 1♂, Ibaraki, Mt. Tsukuba, Alt. 800 m, 36.2256N, 140.1038E, 20–21.IV.1989 (MT), M.J. Sharkey (USNM; USNM2051678_022); 2♂♂, Tochigi, Tamozawa, Nikkô-shi, 2–13.IV.2009 (MT), T. Nakamura (USNM; 1♂, USNM2057434_01; 1♂, USNM2057434_27).

Host plant. Unknown.

Distribution. South Korea (new record), Japan (new record), China (Wei and Nie 1998).

Remarks. Belongs to *E. wui* group (Prous and Heidemaa 2012). Prous and Heidemaa (2012) already treated this species (along with related species), but they suggested that some of the specimens treated here might represent species different from *E. wui*. Because the differences are similar to intraspecific variation in other *Empria* species, we consider the sp. 1 and sp. 3 mentioned in Prous and Heidemaa (2012) to be conspecific with *E. wui*. The previously unknown male of *E. wui* is described for the first time. No females were collected in Korea and Japan, so the description is based on CSCS specimens (holotype of *E. wui*, Fig. 5, and sp. 3) from China. This species has an incomplete vein 2A+3A in the fore wing, which is unusual among *Empria* species.

Empria zhangi Wei & Yan, 2009

Figs 2C, D, F, H, K, L, 6D, 7D

Empria zhangi Wei & Yan in Yan et al. 2009: 248–250.

Diagnosis. ***Female*** (Figs 2C, F, K, 6D). Body length 6.0–8.0 mm. Head and thorax black, except pronotum dorsally, tegula, cenchrus, apical half of fore femur, middle femur at apex, fore and middle tibiae anteriorly, basal 2/3 of hind tibia, basal half of hind tarsomere 1 white; labrum, labial and maxillary palps, fore and middle tibiae posteriorly (sometimes at apex), fore and middle tarsi brown; mandible at apex reddish brown; apical half of hind tarsomere 1 and tarsomeres 2–5 dark brown; abdomen black, segments with narrow posterior whitish (pale) margins, and abdominal terga with 3 pairs of whitish patches (the tergum 4 with small-sized or indistinct patch) (Fig. 2C, D, F, H, K, L). Malar space 1.2–1.7 times as long as diameter of median ocellus (Fig. 2K). Flagellum 1.7–2.0 times as long as head width. Claws simple or with small denticle. Ovipositor sheath extending beyond apex of abdomen; dorsal and ventral margins subparallel and apex truncated (Fig. 2F). Lancet with about 18 serrulae, membrane between serrulae weakly convex; each serrula circularly protruding basally (Fig. 6D), with a basal denticle and 3–6 distal denticles.

Male (Figs 2D, H, L, 7D). Body length 4.8–6.0 mm. Similar to female, but malar space 0.8–1.0 times as long as diameter of median ocellus (Fig. 2L). Flagellum 1.8–2.4 times as long as head width. Posterior margin of sternum 9 rounded. Penis valve as in Fig. 7D, valviceps almost equal in length to valvura.

Genetic data. Based on the COI barcode region of four specimens (China and Russia), the maximal distance between them is 3.8%. The nearest neighbour, diverging by a minimum of 5.0%, is *E. nigroterga*. Based on the nuclear data of three specimens (China and Russia), the maximal distance between them is 0.7%. The nearest neighbour, diverging by a minimum of 1.4%, is *E. nigroterga*.

Type specimens examined. China: ♀, holotype of *Empria zhangi* (CSCSEmp7), “Hunan, Nantianmen, Mt. Heng, Alt. 1000–1110 m, 27.2333N, 112.85E, 11.IV.2004, Wei-Xing Liu” (CSCS); 1♀2♂♂, paratypes of *Empria zhangi* (zhangi_paratypus_01-03), “1♀, same locality as holotype, Alt. 1050 m, 10.IV.2004, Shao-Bing Zhang”, “1♂, same data as holotype”, “1♂, Hunan, Mt. Mufu, Pingjiang, Alt. 1200 m, 28.9667N, 113.8167E, 7.V.2001, Meicai Wei” (CSCS).

Additional specimens examined. South Korea: 1♀1♂, Gangwon-do, Hongcheon-gun, Bukbang-myeon, Gwangwon Prov. Environment Research Park, Alt. 220 m, 37°45'15.6"N, 127°51'1.7"E, 30.IV.2012, S.J. Jang (YNU); 1♂, Gangwon-do, Jeongseon-gun, Mt. Hambaeksan, Sanaegol, 6.V.1999, S.M. Ryu (YNU); 1♂, Gangwon-do, Taebaek-si, Mt. Taebaeksan, 14.V.1992 (NIBR); 1♂, Jeollanam-do, Jangseong-gun, Bukha-myeon, Cheongryangwon (s-25), 8–26.IV.2006 (MT), J.W. Lee (YNU); 1♂, same locality, 26.IV–4.VI.2006 (MT) (YNU); 1♀, Jeollanam-do, Jangseong-gun, Buki-myeon, Jahadonggol (s-23), 8–26.IV.2006 (MT), J.W. Lee (YNU); China: 1♀, Hunan, Mt. Yunshan, near Wugang, Alt. 1250 m, 26.65N, 110.61666E, 14.IV.2012, A. Shinohara (NSMT; NSMTHYM2012111005); 1♂, Zhejiang, Tianmushan, Kaishan Laodian, Alt. 1150 m, 30.33331N, 119.43335E, 9.IV.2014, A. Shinohara (NSMT; NSMTHYM20141216-17); Russia: 1♀, Primorsky Krai, Biological station 30 km SE Chuguyevka (Sichote Alin), Alt. 650 m, 44.083N 134.2E, 31.V.1993, A. Taeger (SDEI); 1♀, Primorsky Krai, Gornotajozhnoe, 1 km E, Alt. 150 m, 43.694N, 132.168E, 19.V.2016, K. Kramp, M. Prous & A. Taeger (SDEI; DEI-GISHym86113); 3♀♀, Primorsky Krai, Gornotajozhnoe, Dendrarium, Alt. 150 m, 43.691N, 132.153E, 21.V.2016, K. Kramp, M. Prous & A. Taeger (SDEI; 1♀, DEI-GISHym86163; 2♀♀, without ID numbers); 1♀1♂, Primorsky Krai, Ussuri Nature Reserve, Alt. 150 m, 43.644N, 132.346E, 20.V.2016, K. Kramp, M. Prous & A. Taeger (SDEI; 1♀, without ID number; 1♂, DEI-GISHym86136); 1♀, same locality, 23.V.2016, K. Kramp, M. Prous & A. Taeger (SDEI; DEI-GISHym86235); 1♀, Primorsky Krai, Vladivostok, Sedanka, Alt. 100 m, 43.21N, 131.973E, 17.V.2016, K. Kramp, M. Prous & A. Taeger (SDEI; DEI-GISHym86082).

Host plant. Unknown.

Distribution. South Korea (new record), Russia (new record), China (Yan et al. 2009).

Remarks. Based on morphology, Prous (2012) suggested that this species belongs to *E. quadrimaculata* group, which is here confirmed based on the genetic data. Compared to specimens from South Korea and Russia (Primorsky Krai), specimens studied from China (Hunan and Zhejiang) have longer antenna (flagellum about 2.0 times as long as head width in female, 2.3–2.4 times in male) and are larger (females 6.5–8.0 mm, males about 6.0 mm), but this could be due to geographic differences rather than indicating the presence of different species.

Empria lycroi Prous & Park, sp. nov.

http://zoobank.org/2081DF3F-61FC-404D-A52D-21AA6739B22D
Figs 3, 6E, 7E

Type specimens examined. [Holotype] Russia: ♀, Primorsky Krai, Vladivostok, Sedanka, Alt. 100 m, 43.21N, 131.973E, 17.V.2016, K. Kramp, M. Prous & A. Taeger (ZIN; DEI-GISHym86081); [Allotype] Russia: ♂, Primorsky Krai, Novonezhino 4 km W, Alt. 70 m, 43.227N, 132.544E, 7.V.2019, M. Prous & S. Tuerk (SDEI; DEI-GISHym80769); [Paratypes] South Korea: 1♀, Gangwon-do, Mt. Odaesan, Mirugam (Pugdaesa), Alt. 1300 m, 37.8N, 128.56667E, 30.V.1992, A. Shinohara (NSMT; NSMT231); 1♀, same locality, 28.V.1998, A. Shinohara (NSMT; NSMT207); Russia: 1♀, Primorsky Krai, Anisimovka, Alt. 300 m, 43.16666N, 132.8E, 1.VI.1994, A. Shinohara (NSMT; NSMT232); 1♀, same locality, 3.VI.1995, A. Leleij (NSMT; NSMT141); 1♂, same locality, 10 km NW, Alt. 150 m, 43.195N, 132.665E, 9.V.2019, M. Prous & S. Tuerk (SDEI; DEI-GISHym80812); 1♀, Primorsky Krai, Gornotajozhnoe, 1 km E, Alt. 150 m, 43.694N, 132.168E, 19.V.2016, K. Kramp, M. Prous & A. Taeger (SDEI; DEI-GISHym80068); 1♀, same locality, 12.V.2019, M. Prous (SDEI; DEI-GISHym80704); 1♀, Primorsky Krai, Kamenushka 5 km E, Alt. 140 m, 43.636N, 132.294E, 13.V.2019, M. Prous (SDEI; DEI-GISHym80712); 1♀1♂, Primorsky Krai, Novonezhino 4 km W, Alt. 70 m, 43.227N, 132.544E, 7.V.2019, M. Proshchalykin (ZIN; without ID numbers); 4♀♀3♂♂, same data, M. Prous & S. Tuerk (3♀♀, 3♂♂ in SDEI; 1♀, DEI-GISHym80776; 2♀♀, without ID numbers; 3♂♂, DEI-GISHym80642, DEI-GISHym80770, DEI-GISHym83873; 1♀ in YNU; without ID number); 1♀, Primorsky Krai, Tigrovoy, Alt. 300 m, 43.18333N, 132.9E, 5.VI.1995, A. Shinohara (NSMT; NSMT233).

Description. ***Female*** (holotype, DEI-GISHym86081) (Figs 3A, C, E–G, K, 6E). Body length 5.9 (5.2–6.8) mm.

Color. Body black, except labial and maxillary palps, posterodorsal margin of pronotum, tegula, and cenchrus white; labrum yellow (yellowish brown); mandible at apex reddish brown; apex of all coxae slightly, all trochanters and trochantelli, fore and middle femora anteriorly and posteriorly, apex of hind femur slightly (black to extensively pale), fore and middle tibiae anteriorly and posteriorly, basal 1/3 (1/4–2/3)

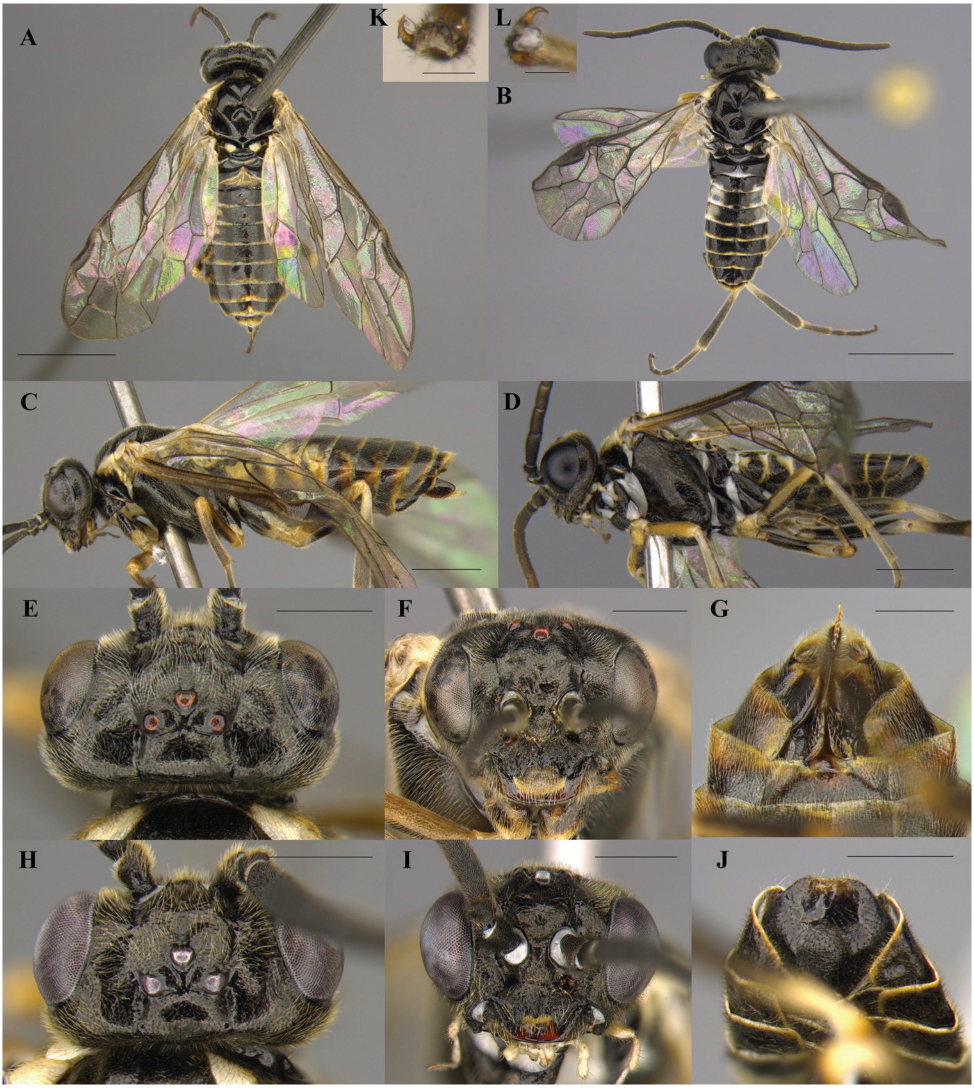

Figure 3. *Empria lycroi* sp. nov. from Primorsky Krai, Russia: **A, C, E–G, K** holotype, DEI-GISHym86081 **B, D, H–J, L** allotype, DEI-GISHym80769. **A, B** dorsal habitus **C, D** lateral habitus **E, H** dorsal head **F, I** frontal head **G** ventral abdomen at apex **J** sternum 9 **K, L** claw. Scale bars: 2 mm (**A, B**); 1 mm (**C, D**); 0.5 mm (**E–J**); 0.1 mm (**K, L**).

of hind tibia, fore and middle tarsi anteriorly, and basal 1/3 (1/4–2/3) of hind tarsomere 1 pale; abdominal segments with narrow posterior whitish (pale) margins, and abdominal terga with 4 (3–5) pairs of whitish (pale) patches. Wings hyaline; venation brown; body with yellowish setae (Fig. 3A, C, F, G).

Head. Length of postocellar area much shorter than width, 2.9 (2.6–2.9) times as long as diameter of lateral ocellus, and POL: OOCL = 1: 0.9 (0.9–1.1) (Fig. 3E). Clypeus weakly rugulose with median keel. Malar space 1.1 (1.1–1.4) times as long as diameter of median ocellus (Fig. 3F). Maximal temple length 1.3 (1.2–1.3) times as long as minimal temple length in lateral view. Flagellum 1.4 (1.4–1.6) times as long as head width.

Thorax. Propleura not meeting in front. Vein 2A+3A in fore wing complete; vein m-cu in hind wing present, cell M closed. Claws without denticle (Fig. K, L).

Abdomen. Ovipositor sheath not or slightly extending beyond apex of abdomen; dorsal and ventral margins parallel basally and ventral margin apically narrowing towards dorsal margin (Fig. 3C). Lancet with 16–17 serrulae, membrane between serrulae flat; each serrula subtriangularly protruding basally (Fig. 6E), and with indistinct denticles apically.

Male (allotype, DEI-GISHym80769) (Figs 3B, D, H–J, L, 7E). Body length 4.4 (4.4–5.2) mm. Similar to female, but abdominal terga with 3 pairs of whitish patches (Fig. 3B, D, I). Length of postocellar area 2.4 (2.2–2.4) times as long as diameter of lateral ocellus (Fig. 3H). Malar space 0.9 (0.8–0.9) times as long as diameter of median ocellus (Fig. 3I). Flagellum 1.8 (1.7–1.9) times as long as head width. Posterior margin of sternum 9 truncated (Fig. 3J). Penis valve as in Fig. 7E, valviceps shorter than valvura.

Genetic data. The COI sequences of three specimens are identical. The nearest neighbour, diverging by a minimum of 7.0%, is *E. liturata* (Gmelin). Based on the nuclear data of three specimens (Russia), the maximal distance between them is 0.03% (0.07% when counting heterozygous positions in the holotype female). The nearest neighbour, diverging by a minimum of 2.0%, is the North American *E. coryli* (Dyar).

Host plant. Unknown.

Distribution. South Korea, Russia.

Etymology. The species name is an arbitrary combination of letters, to be treated as a noun.

Remarks. The most similar species is *E. coryli* from North America. Females of *E. coryli* (based on Smith 1979) are even paler than *E. lycroi* (character states for this species in the parenthesis): mesepisternum partly pale (completely black) and abdominal terga with 5–6 pairs of whitish patches (3–5). Males of *E. coryli* apparently also have more pairs of whitish patches (Smith 1979) than *E. lycroi*. The penis valve of *E. coryli* has large irregular spines on the dorsal margin of valviceps that are lacking in *E. lycroi*.

Empria nigroterga Park & Lee, sp. nov.

http://zoobank.org/62C8E656-35EE-4614-9A6F-33CC56D0B714
Figs 4, 6F, 7F

Type specimens examined. [Holotype] South Korea: ♀, Chungcheongnam-do, Seosan-si, Haemi-myeon, Daegok-ri 880, Hanseo Univ., 36°41'30"N, 126°34'50"E, 22.IV–6.V.2009 (MT), J.W. Lee (YNU; YNU-Sym0023); [Allotype] South Korea: ♂, same locality as holotype, 14–22.IV.2009 (MT), J.W. Lee (YNU; YNU-Sym1160); [Paratypes] South Korea: 1♂, Chungcheongbuk-do, Danyang-gun, Danyang-eup, Cheondong-ri, 36°57'25.1"N, 128°25'47.6"E, 22.IV–4.V.2009,

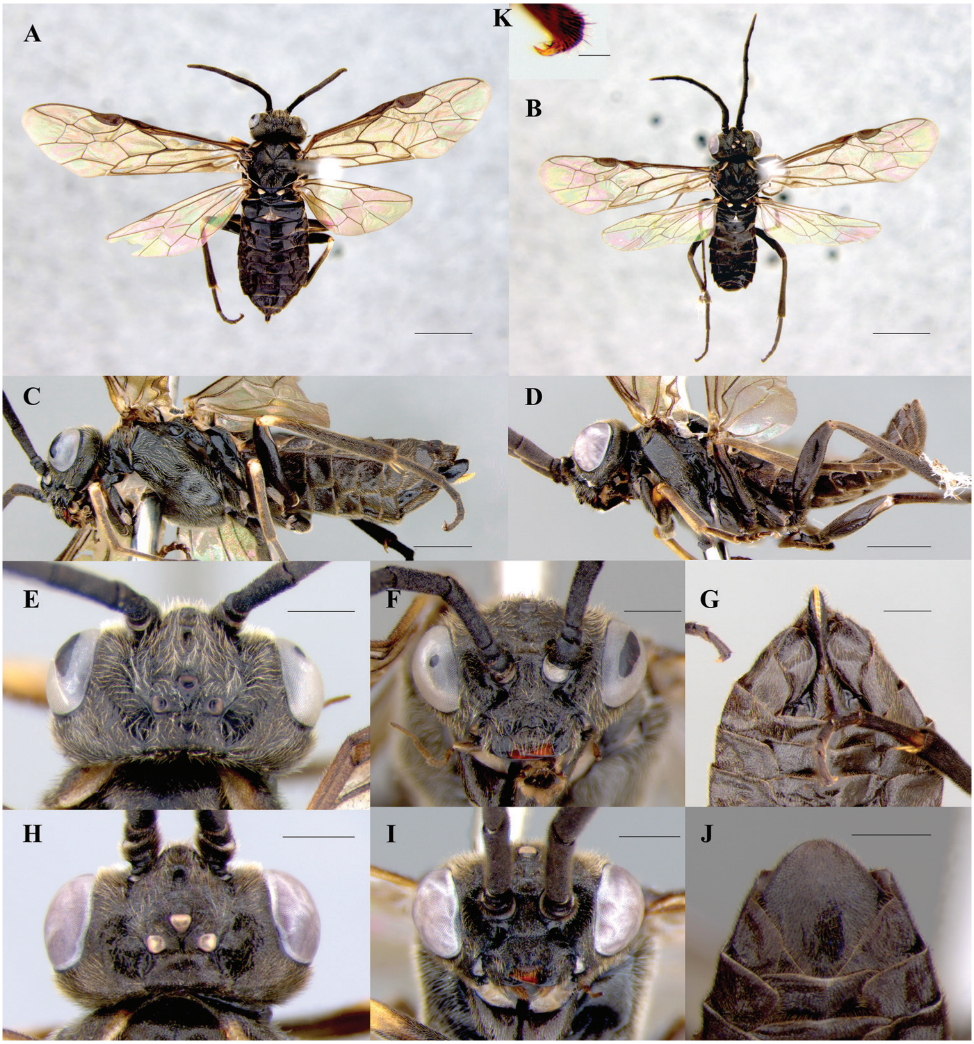

Figure 4. *Empria nigroterga* sp. nov. from Chungnam, South Korea: **A, C, E–G** holotype, YNU-Sym0023 **B, D, H–K** allotype, YNU-Sym1160 **A, B** dorsal habitus **C, D** lateral habitus **E, H** dorsal head **F, I** frontal head **G** ventral abdomen at apex **J** sternum 9 **K** claw. Scale bars: 2 mm (**A, B**); 1 mm (**C, D**); 0.5 mm (**E–J**); 0.1 mm (**K**).

J.W. Lee (YNU; YNU-Sym1161); 1♀1♂, same locality, Mt. Sobaeksan, Cheondong valley, 36°57'00"N, 128°26'00"E, 19–30.IV.2007 (MT), J.W. Lee (YNU; 1♀, YNU-Sym1162; 1♂, YNU-Sym1163); 1♀, Chungcheongnam-do, Seosan-si, Haemi-myeon, Daegok-ri 880, Hanseo Univ., 36°41'30"N, 126°34'50"E, 2–14.IV.2009 (MT), J.W. Lee (YNU; YNU-Sym1164); 1♀, same locality, 22.IV–6.V.2009 (MT), J.W. Lee (YNU; YNU-Sym1165); 1♀, Daegu-si, Nam-gu, Mt. Apsan, 18.V.1996, Y.J. Jang (YNU; YNU-Sym1166); 1♀, Daejeon-si, Dong-gu, Daejeon Univ., 36°20'05"N, 127°27'38"E, 1–17.V.2006 (MT), J.W. Lee (YNU;

YNU-Sym1176); 2♀♀, Gangwon-do, Chuncheon nr. Chongpyongsa, 23.V.1992, A. Shinohara (NSMT; 1♀, NSMT240; 1♀, NSMTHYM2012111008); 1♂, Gangwon-do, Hongcheon-gun, Seo-myeon, Magok-ri, 37.7162N, 127.5908E, 3–17.V.2006 (MT), P. Tripotin (USNM; USNM2051678_017); 1♀, Gangwon-do, Mt. Odaesan, Mirugam (Bukdaesa), Alt. 1300 m, 28.V.1991, A. Shinohara (NSMT; NSMTHYM2012111007); 1♀, same locality, 29.V.1992, A. Shinohara (NSMT; NSMTHYM2012111009); 1♀, same locality, 25.V.1993, A. Shinohara (NSMT; NSMT237); 1♂, same locality, 1.VI.1993, A. Shinohara (NSMT; NSMT142); 1♀, same locality, 22.V.2002, A. Shinohara (NSMT; NSMT238); 1♂, same locality, 25.V.2002, A. Shinohara (NSMT; NSMT236); 1♀, same locality, 28.V.2002, A. Shinohara (NSMT; NSMT239); 1♀, same locality, 25.V.2008, A. Shinohara (NSMT; NSMTHYM2012111006); 1♂, Gangwon-do, Sokcho-si, Mt. Seoraksan National Park, Misiryeong services (Sinseongbong direction), 1.IV–24.VI.2010 (MT), J.C. Jeong (YNU; YNU-Sym1167); 3♂♂, Gyeonggi-do, Gapyeong-gun, Cheongpyeong-myeon, Goseong-ri, Mt. Homyeongsan, Alt. 168 m, 37°43'15.0"N, 127°29'18.9"E, 8–31.IV.2009 (MT), J.W. Lee (YNU; YNU-Sym1168~1170); 1♀, Gyeonggi-do, Yangpyeong-gun, Yongmun-myeon, Yeonsu-ri, Mt. Youngmunsan, Alt. 324 m, 37°31'49.5"N, 127°34'18.8"E, 1–26.V.2009 (MT), J.W. Lee (YNU; YNU-Sym1171); 1♀, Gyeongsangbuk-do, Birosa, 5.V.1999, S.M. Ryu (YNU; YNU-Sym1172); 2♂♂, Gyeongsangbuk-do, Chilgok-gun, Dongmyeong-myeon, Hakmyeong-ri, San 25 (Site-15), 36°02'46.08"N, 128°33'45.41"E, 8.V.2014, J.W. Lee (1♂, YNU-Sym1174 in NIBR; 1♂, YNU-Sym1175 in YNU); 2♂♂, Gyeongsangbuk-do, Gunwi-gun, Bugye-myeon, Dongsan-ri, San75, Odoam, 36°01'29.04"N, 128°41'31.11"E, 25.IV–9.V.2015 (MT), J.W. Lee (YNU; YNU-Sym1173, 1177); 1♀, Gyeongsangbuk-do, Mt. Sobaeksan, Huibang valley, 3.V.1997, S.H. Park (YNU; YNU-Sym1178); 1♀, Jeollabuk-do, Muju-gun, Mt. Deokyusan, 16.V.1999, S.M. Ryu (YNU; YNU-Sym1179); 1♀, Jeollabuk-do, Wanju-gun, Dongsang-myeon, Daea-ri, San 1-2, Daea Arboretum, 35°58'24.24"N, 127°18'13.53"E, 16–30.IV.2013, J.M. Park (YNU; YNU-Sym1180); Russia: 1♂, Primorsky Krai, Anisimovka, Alt. 300 m, 1.VI.1994, A. Shinohara (NSMT; NSMT234); 2♀♀, Primorsky Krai, Anisimovka, 70 km E Vladivostok, Alt. 250 m, 43.16666N, 132.8E, 7.VI.1993, A. Taeger (SDEI; without ID numbers); 1♂, Primorsky Krai, Foot, Mt. Litvoka, Anisimovka, Alt. 400 m, 30.V.1994, A. Shinohara (NSMT; NSMT235); 1♀, same data (NSMT; NSMT241); 1♀, Primorsky Krai, Tikhoye nr. Razdolnoye, 36 km S Ussuriysk, Alt. 100 m, 43.6N, 131.86667E, 22.V.1993, A. Taeger (SDEI; DEI-GISHym15184); 2♀♀, Primorsky Krai, Ussuri Nature Reserve, Alt. 150 m, 43.644N, 132.346E, 20.V.2016, K. Kramp, M. Prous & A. Taeger (SDEI; 1♀, DEI-GISHym86132; 1♀, without ID number); 1♀, Primorsky Krai, Ussurijskij Res., 9–12.VI.1995, A. Lelej (NSMT; NSMT205); 1♀, Primorsky Krai, Vladivostok, Sedanka, Alt. 100 m, 43.21N, 131.973E, 17.V.2016, K. Kramp, M. Prous & A. Taeger (SDEI; DEI-GISHym86721).

Description. ***Female*** (holotype, YNU-Sym0023) (Figs 4A, C, E–G, 6F). Body length 7.4 (7.0–8.7) mm.

Figure 5. *Empria wui*, holotype (CSCSEmp6). **A** claw **B** dorsal habitus **C** lateral habitus **D** lateral abdomen **E** frontal head **F** labels **G** ovipositor. Scale bars: 2 mm (**B**); 1 mm (**C, D**); 0.5 mm (**E**); 0.1 mm (**A, G**).

Color. Body black, except posterodorsal pronotum, cenchrus, apical half of fore femur anteriorly, most of fore and middle tibiae anteriorly white; all tibiae with white ring at base; mandible at apex reddish brown; labial and maxillary palps dark brown; abdominal terga with 1 (0–2) pairs of whitish (pale) patches. Wings subhyaline; venation dark brown; body with yellowish setae (Fig. 4A, C, F, G).

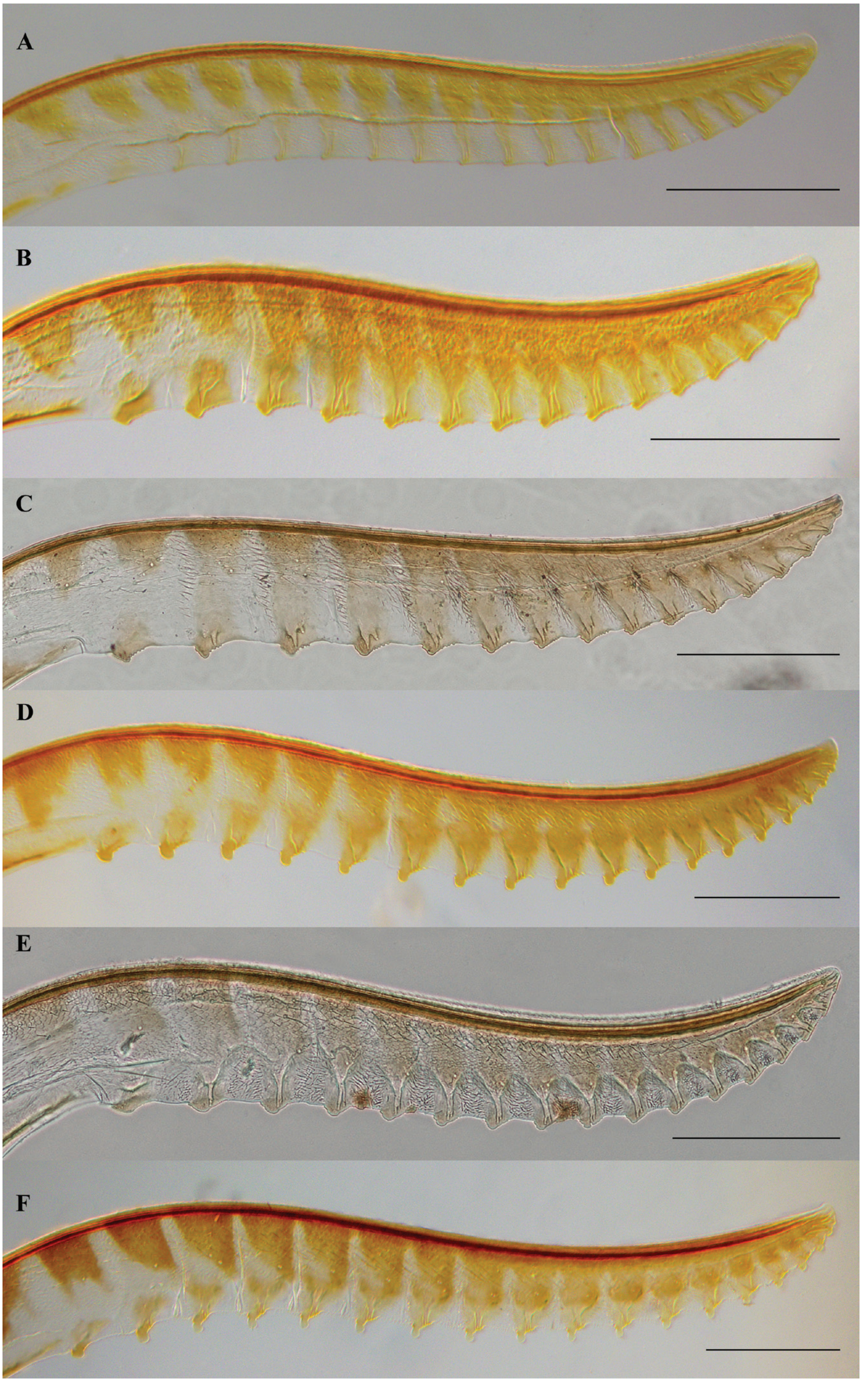

Figure 6. Female lancets. **A** *Empria candidata* from Gangwon, South Korea **B** *E. tridentis* from Gangwon, South Korea **C** *E. wui* from Shanxi, China (W08-03a) **D** *E. zhangi* from Jeonnam, South Korea **E** *E. lycroi*, sp. nov. from Primorsky Krai, Russia (paratype, NSMT232) **F** *E. nigroterga*, sp. nov. from Daejeon, South Korea (paratype, YNU-Sym1176). Scale bars: 0.1 mm (**A–F**).

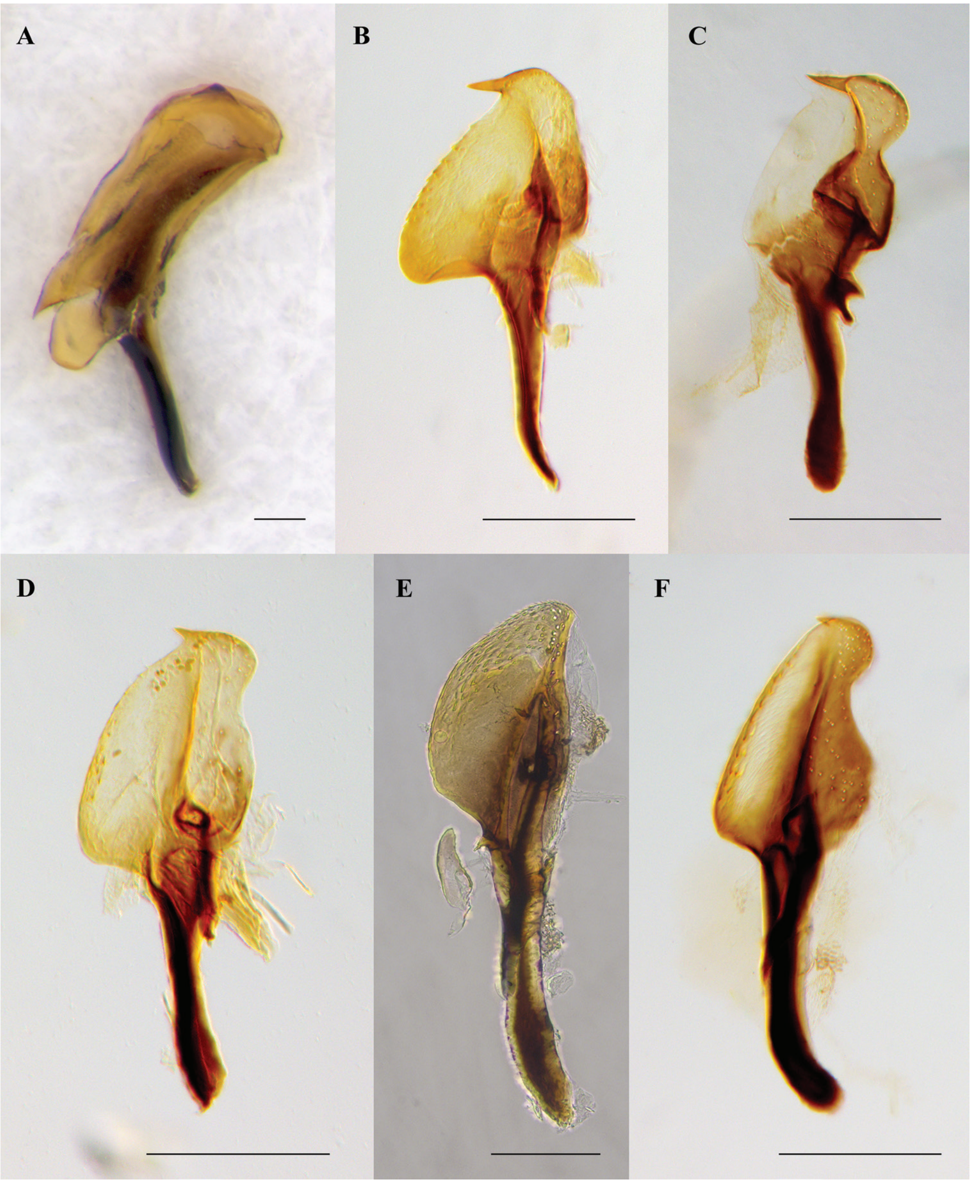

Figure 7. Male penis valves. **A** *Empria candidata* from Gangwon, South Korea **B** *E. tridentis* from Gangwon, South Korea **C** *E. wui* from Gyeongbuk, South Korea **D** *E. zhangi* from Jeonnam, South Korea **E** *E. lycroi*, sp. nov. from Primorsky Krai, Russia (paratype, DEI-GISHym83873) **F** *E. nigroterga*, sp. nov. from Gyeongbuk, South Korea (paratype, YNU-Sym1173). Scale bars: 0.1 mm (**A–F**).

Head. Length of postocellar area much shorter than width, 1.8 (1.8–2.3) times as long as diameter of lateral ocellus, and POL: OOCL = 1: 0.9 (0.8–1.0) (Fig. 4E). Clypeus strongly punctate with median keel. Malar space 1.5 (1.3–1.7) times as long as diameter of median ocellus (Fig. 4F). Maximal temple length 1.3 (1.1–1.3) times as long as minimal temple length in lateral view. Flagellum 2.0 (1.9–2.0) times as long as head width.

Thorax. Propleura meeting (not meeting) in front. Vein 2A+3A in fore wing complete; vein m-cu in hind wing present, cell M closed. Claws with small denticle (Fig. 4K).

Abdomen. Ovipositor sheath extending beyond apex of abdomen; dorsal and ventral margins parallel and apex rounded (obliquely truncated) (Fig. 4C). Lancet with 18–19 serrulae, membrane between serrulae flat basally and weakly convex apically; each serrula circularly protruding basally (Fig. 6F), and with a basal denticle and 3–5 distal denticles.

Male (allotype, YNU-Sym1160) (Figs 4B, D, H–K, 7F). Body length 6.4 (5.1–6.6) mm. Similar to female, but abdominal segments entirely black (with narrow posterior whitish margins) (Fig. 4B, D, I). Length of postocellar area 1.8 (1.8–2.0) times as long as diameter of lateral ocellus (Fig. 4H). Malar space 1.0 (0.9–1.3) times as long as diameter of median ocellus (Fig. 4I). Maximal temple length 1.3 (1.3–1.4) times as long as minimal temple length in lateral view. Flagellum 2.6 (2.5–2.7) times as long as head width. Posterior margin of sternum 9 rounded (Fig. 4J). Penis valve as in Fig. 7F, valviceps almost equal in length to valvura.

Genetic data. Based on the COI barcode region of two specimens (Russia and South Korea), the distance between them is 0.5%. The nearest neighbour, diverging by a minimum of 5.0%, is *E. zhangi* Wei & Yan. Based on the nuclear data of one specimen (Russia), the nearest neighbour, diverging by a minimum of 1.4%, is *E. zhangi* Wei & Yan.

Host plant. Unknown.

Distribution. South Korea, Russia.

Etymology. The species name, a noun, is formed from the Latin *nigro* and *terga*, and refers to black color of abdomen.

Remarks. Belongs to *E. quadrimaculata* group (as sp7 in Prous 2012). Numerous additional specimens (36 females, 76 males in YNU) studied from South Korea were not included in the type series. Although most *Empria* species have pairs of pale patches on the abdominal terga (one of the general morphological characters of *Empria*), this new species has the abdominal terga with reduced pale patches.

Phylogenetic analyses

A result of maximum likelihood analysis of 20 species (including all species treated here) of *Empria* combining all three genes (COI, NaK, and POL2) is shown in Fig. 8. Even with data of more than 5000 bp of sequences, most of the relationships are poorly supported. Well supported are separation of *E. candidata* and *E. multicolor* from other species, monophyly of *quadrimaculata* group (*E. nigroterga* and *E. zhangi*), *hungarica* group (*E. granatensis*, *E. testaceipes*, and *E. pumiloides*), grouping of *E. coryli* and *E. lycroi*, and grouping of *E. gelida*, *E. immersa*, *E. tridentis*, and *E. tridens* (see more detailed results regarding *immersa* and *longicornis* groups in Prous et al. 2019).

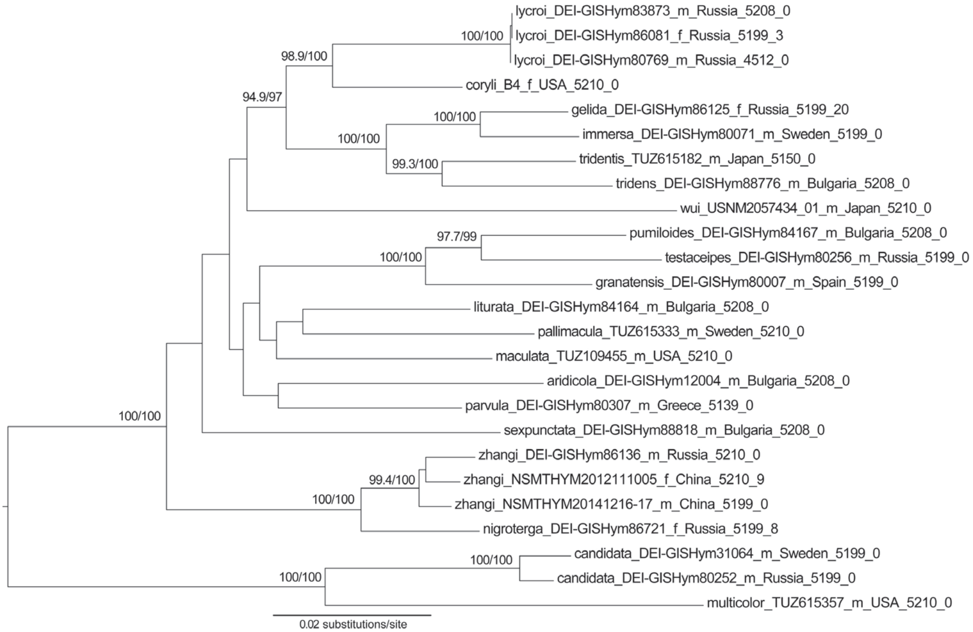

Figure 8. Maximum likelihood tree of *Empria* based on mitochondrial COI and nuclear NaK and POL2. Best-fit model chosen according to the Bayesian information criterion was GTR + R3. Numbers above nodes show SH-aLRT support (%) / ultrafast bootstrap support (%) values. Support values for weakly supported branches (< 90) are not shown. Letters "f" and "m" stand for "female" and "male". Numbers at the end of the tip labels refer to the length of the sequence and the number of heterozygous positions in nuclear DNA. *Empria candidata* and *E. multicolor* were used to root the tree.

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SHORT COMMUNICATION

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Plant associations for three sawfly species (Hymenoptera, Tenthredinidae) in the Pacific Northwest

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Abstract

Plant associations are newly recorded for three tenthredinid species in the Pacific Northwest. A single *Monardis pulla* D.R. Smith, 1969 emerged from a chamber inside a cynipid gall on *Rosa nutkana* C. Presl. (Rosaceae). This is the first plant association record for *M. pulla*. Two *Aphilodyctium fidum* (Cresson, 1880) emerged from a stem and cynipid gall of *Rosa rugibinosa* Linnaeus, 1758, respectively. Several *Rhogogaster lateraria* (Cresson, 1880) eggs were discovered on *Castilleja* sp., which has no previously recorded sawfly associations.

Keywords

Allantinae, Blennocampinae, Tenthredininae, Symphyta, host plant, *Rosa*, *Castilleja*

Introduction

Tenthredinidae is the most species-rich family of Symphyta, found on every continent excluding Antarctica. The family comprises a prominent group of herbivorous insects that can inflict negligible to significant damage on their host plants, and as such there is value in recording plant association data. Host plant associations and feeding approaches within the group are diverse (eg. gall induction, leaf mining, external defoliation), but are unknown for many taxa. This paper presents host plant associations for three species of sawfly, each collected incidentally while conducting research on other insect groups.

Methods and results

All adult sawflies were mounted and identified using keys in Goulet (1992) and Smith (1969, 1979). Molecular data were used to identify one series of larval specimens. All specimens and two associated galls are deposited in the Washington State Department of Agriculture Collection in Olympia, WA.

Tenthredinidae
Blennocampinae

Monardis pulla D.R. Smith, 1969

Notes. A single female was reared from a *Periclistus*-modified gall of *Diplolepis oregonensis* (Beutenmüller, 1918) (Hymenoptera: Cynipidae) on *Rosa nutkana* C. Presl. hand-collected in February, 2017. Galls were collected by visually searching *R. nutkana* stands, taken to the lab, and held at room temperature until insects emerged. Galls were checked several times a week and insects were pinned or transferred to vials of 70% EtOH as they emerged. This specimen emerged from a smooth, spherical gall, measuring about 15 mm in diameter and attached to the stem near the point of the spine node (Fig. 1). The adult emerged from the gall, at the point farthest from the stem, on 1–2 March, 2017. The emergence hole measured 1.5 mm in diameter and led to a gallery about 5 mm in length. At the end of the gallery was the discarded pupal case of the sawfly. The gallery does not lead into the center of the gall, and there was no evidence of feeding activity in the gall, suggesting that the gall may only have served as an overwintering site. Two specimens of *Periclistus* (Hymenoptera: Cynipidae) also emerged from the gall.

Specimen data. United States; 1♀; Oregon, Lane County, 10 km N of Corvallis; 44°38.66'N, 122°19.39'W; 12 Feb. 2017, em. 1–2 Mar. 2017; C. Looney & R. Chappel leg.; ex: *Rosa nutkana*.

Tenthredinidae
Allantinae
Empriini

Aphilodyctium fidum (Cresson, 1880)

Notes. One male *A. fidum* was reared from a stem of *Rosa rubiginosa* Linnaeus, 1758, hand-collected in March 2018 and connected to a *Diplolepis rosae* (Linnaeus, 1758) gall measuring about 29 mm in diameter. The gall was from a previous season, and all gall wasps and the associated component community had already emerged. The

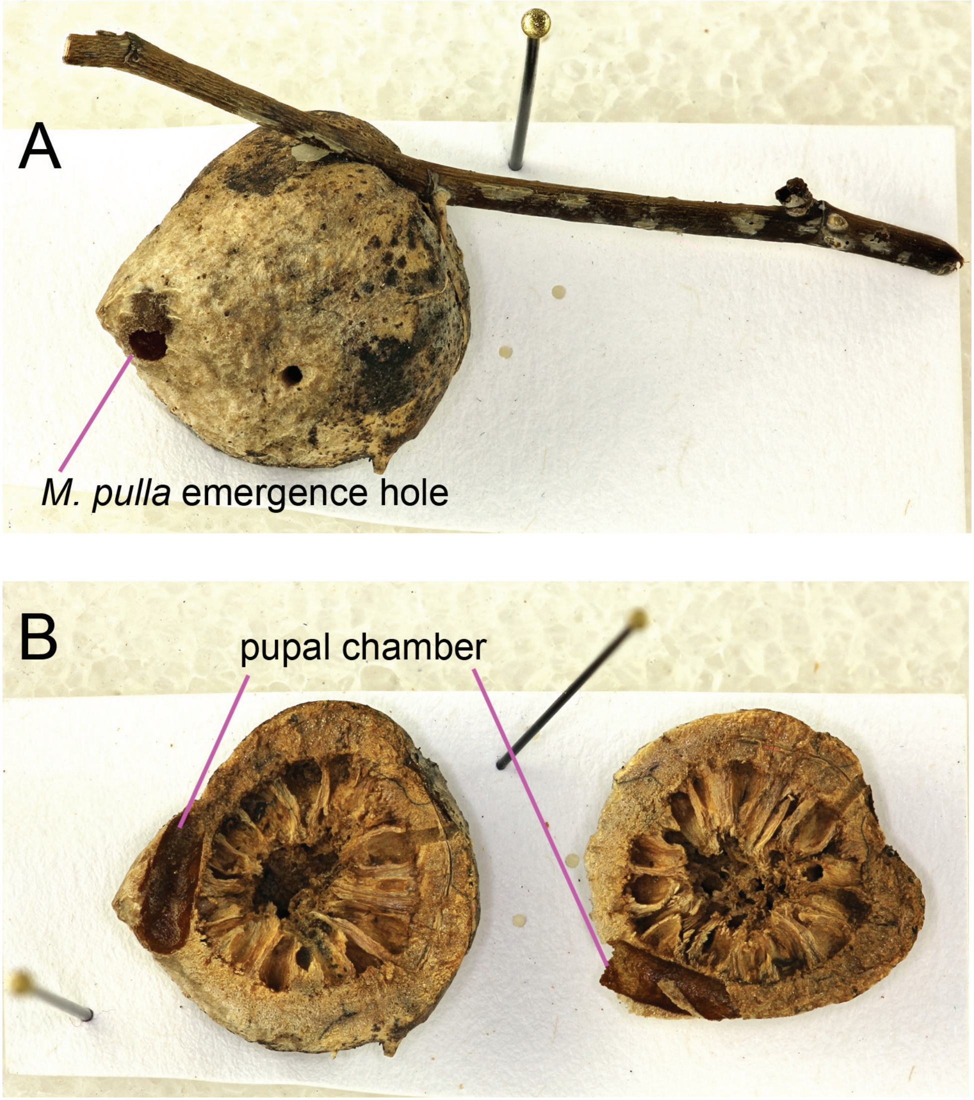

Figure 1. *Periclistus*-modified gall of *Diplolepis oregonensis* (**A**), dissected to reveal the pupal chamber of *Monardis pulla* (**B**).

sawfly emerged from the apex of the cut stem, leaving a hole that measured about 3.5 mm in diameter leading to a frass-filled gallery through the stem pith, about 23 mm in length. The discarded larval skin of the sawfly was discovered at the end of this gallery, as well as a partial adult head and some exoskeletal remnants of a different unidentified hymenopteran.

One male *A. fidum* was reared from a gall of *D. rosae* also collected from *R. rubiginosa*. The gall was collected in the fall and stored at 0 °C for 90 days to simulate

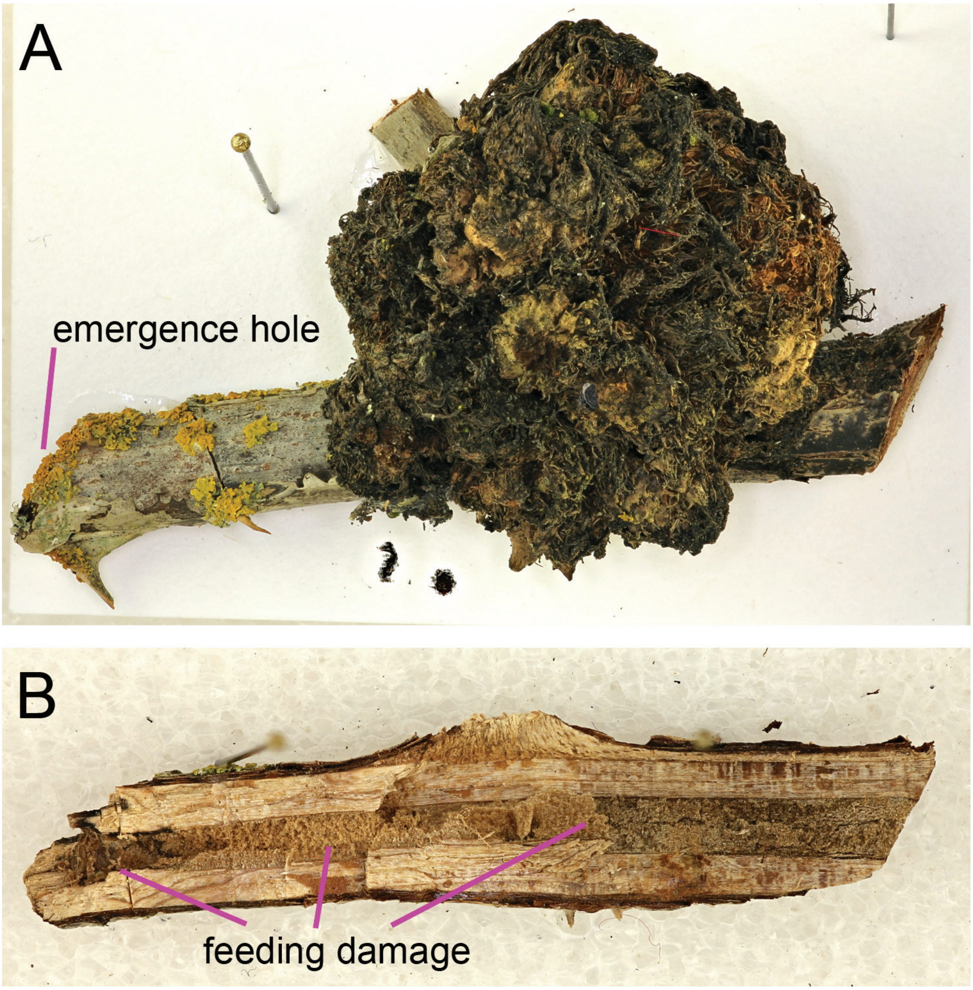

Figure 2. *Diplolepis rosae* gall (**A**), and dissected stem showing feeding damage of *Aphilodyctium fidum* (**B**).

overwintering conditions (Williams et al. 2003). After this the gall was transferred to room temperature to allow insects to emerge. Galls were checked several times a week, with insects pinned or transferred to vials of 70% EtOH as they emerged. This gall was not retained, but was instead dissected as part of a different research project. It therefore isn't clear if the insect was feeding within the gall or if, like the *M. pulla* specimen from Oregon, it was feeding or overwintering in other associated plant tissue.

Specimen data. United States; 1♂; Washington, Clark County, Port of Vancouver; 45°38.60'N, 122°42.33'W; Mar. 2018, em. unknown; C. Looney leg.; ex: *Rosa rubiginosa*. United States; 1♂; Idaho, Ada County, Boise; 43°34.36'N, 116°8.78'W; 26 Dec. 2006, em. Feb. 2007; E. Poor leg.; ex: *Rosa rubiginosa*.

Tenthredinidae
Tenthredininae
Tenthredinini

Rhogogaster lateraria (Cresson, 1880)

Notes. During a Bioblitz on property adjacent to the North Cascades National Park, what were thought to be insect mines were observed on four individual *Castilleja* sp. near the Stehekin Landing Strip. No damage was observed other than the putative mines on any of the plants nearby. Leaves from two plants were collected and taken to the lab in Olympia, WA, in hopes of rearing the associated insects. Upon examination in the lab we determined that the supposed mines were actually small groups of insect eggs (Fig. 3), from which multiple sawfly larvae hatched on 23 and 24 May, 2016. The first instar sawfly larvae did not commence feeding upon their natal leaves, instead began crawling about the container. The larvae were provided with fresh *C. levisecta* Greenm. leaves from surplus greenhouse material provided by the Center for Natural Lands Management, in Olympia, WA. None of the larvae fed upon these leaves, and all died within a few days after emerging. DNA was extracted from two larvae, and the COI "barcode" region was sequenced and compared with data in the Barcode of Life

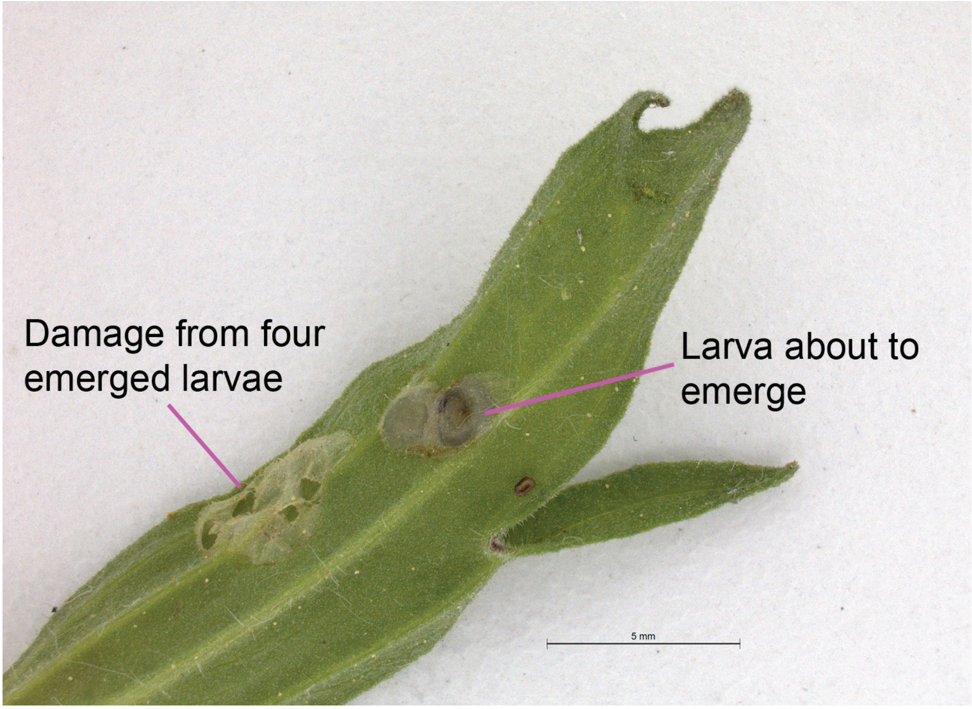

Figure 3. *Rhogogaster lateraria* eggs in *Castilleja* sp. leaf.

Data System and on Genbank. DNA from one of the larvae was successfully amplified and sequenced, and was a 99.85% match with three sequences of *R. lateraria* from adult specimens identified by D.R. Smith, all collected from Washington State. The sequence derived from the larvae was submitted to GenBank (accession number MN545964).

Specimen data. United States; 9 1st instar larvae; Washington, Chelan County, Stehekin State Airport, Lake Chelan National Recreation Area; 48°20.86'N, 120°43.38'W; 20 May 2016, em. 23–23 May 2016; C. Looney leg.; ex: *Castilleja* sp.

Discussion

Monardis is represented in North America only by *M. pulla*, which is broadly distributed in western states and provinces. No host plants have been previously recorded for this species (Smith 1969). Several species of *Monardis* in Asia and Europe feed on *Rosa* (Smith 1969; Scheibelreiter 1972). *Monardis plana* (Klug, 1817) larvae in Europe have been observed feeding on *Rosa* leaves and boring into flower stalks and soft shoots, subsequently pupating in the soil (Scheibelreiter 1972; Gibbs 2006). The species is occasionally a pest, and is known as the "rose bud sawfly" in parts of Europe (Scheibelreiter 1972; Gibbs 2006).

Aphilodyctium is monotypic in North America, and has been previously associated with unidentified *Rosa* (described only as "prairie rose") and galls of *Diplolepis* (=*Rhodites*) *arefacta* (Gillette, 1894), although Smith (1979) posited that the latter was likely an overwintering site. *Aphildyctium fidum* has also been reared from *Rosa californica* Cham. & Schldl. in California (https://bugguide.net/node/view/576002; also P. Bryant in litt. 2019). Other plant associations include *Quercus* and *Sambucus*, although Smith (1979) again suggests that these only represent overwintering sites.

The genus *Rhogogaster* in North America is associated with many plant genera in several families, including *Populus* (cottonwood), *Filipendula* (meadowsweet), *Alnus* (alder), *Stellaria* (chickweed), *Circaea* (evening primrose) and *Ranunculus* (buttercup) (Goulet 1992). However, no other *Rhogogaster*, and in fact no other sawfly to our knowledge, has been previously associated with *Castilleja*. *Rhogogaster* may, like the closely related genus *Tenthredo*, oviposit in plants that are not actually food sources. For example, *Tenthredo koehleri* Klug, 1817 is known to oviposit in *Myosotis*, but has not been observed feeding on the plant (Beneš 2008). The absence of feeding observed in the field and on the provided plant material suggests this is similarly the case with the *R. lateraria* larvae we encountered.

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DNA barcoding of rhopalosomatid larvae reveals a new host record and genetic evidence of a second species of *Rhopalosoma* Cresson (Hymenoptera, Rhopalosomatidae) in America north of Mexico

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Abstract

Rhopalosomatidae are unusual wasps whose larvae develop as ectoparasitoids on crickets. In America north of Mexico, three genera and six species are recognized. Host species are known only for *Rhopalosoma nearcticum* Brues and include *Hapithus agitator* Uhler, *H. brevipennis* (Saussure), and *H. saltator* (Uhler) (Gryllidae: Hapithinae). Here we report a new host species: the *Anaxipha exigua* (Say) species-group (Trigonidiidae: Trigonidiinae) discovered by barcoding nine rhopalosomatid larvae collected from Cypress Grove Nature Park, Jackson, Tennessee. *Rhopalosoma nearcticum* is currently the only documented species of *Rhopalosoma* Cresson in America north of Mexico, but our phylogenetic analyses recovered two genetically distinct clades of *Rhopalosoma* and thus reveal the presence of at least two species of *Rhopalosoma* in America north of Mexico.

Keywords

Parasitoid wasps, molecular phylogenetics, Vespoidea, Orthoptera

Introduction

Rhopalosomatidae (Hymenoptera) are unusual aculeate wasps that apparently develop as ectoparasitoids on crickets (Grylloidea) (Perkins 1908; Hood 1913; Gurney 1953; Lohrmann et al. 2014; Lohrmann and Engel 2017). Of the 72 extant species in the family (Aguiar et al. 2013), only six have been recorded in America north of Mexico. Whereas *Liosphex* Townes and *Olixon* Cameron are represented by two and three species respectively, *Rhopalosoma* Cresson (Fig. 1) is represented solely by the widespread species *Rhopalosoma nearcticum* Brues (Townes 1977; Lohrmann and Ohl 2010; Lohrmann et al. 2012).

Within the Rhopalosomatidae, *R. nearcticum* is the only species whose biology and life cycle has been investigated in detail (Lohrmann and Engel 2017). Hood (1913) was the first to document observations of a larval *R. nearcticum* (originally misidentified as *R. poeyi* Cresson) attached to a *Hapithus saltator* (Uhler) (Orthoptera: Gryllidae: Hapithinae) host. Later, Gurney (1953) provided detailed descriptions of the immature stages of *R. nearcticum* and added *Hapithus agitator* Uhler and *H. brevipennis* (Saussure) as hosts.

In contrast to other hymenopteran parasitoids of crickets, *R. nearcticum* oviposit without relocating the host (Melo et al. 2011). The egg is laid behind the coxa of the host's hind leg. With its mandibles imbedded in the abdomen of the host, the larva develops from first to fourth instar. As it grows, the hind leg of the cricket is forced outward at an unnatural angle (Gurney 1953). The fifth instar detaches from the host, usually killing it in the process (JDB, pers. obs. 2017), and borrows in the soil where it spins a cocoon and pupates (Gurney 1953; fig. 2). The following spring it emerges as an adult (Hood 1913; Gurney 1953).

Although rhopalosomatids are rarely collected, they can be locally abundant (e.g., Smith 2008), in particular in floodplain forests where hosts are numerous (Barrows 2013). Adults are most often collected passively in Malaise traps (e.g., Townes 1977; Smith 2008; Barrows 2013), especially in the summer and early fall (Freytag 1984). Gurney (1953) observed a group of ~10 adults flying over shrubbery at twilight "until no longer visible in the gathering dark", and this, coupled with their unusual large ocelli, their inconspicuous pale brown color, and their occasional appearance in light trap samples (e.g., Stange 1991), indicates that *R. nearcticum* is a crepuscular or nocturnal species.

Morphological phylogenies have been reconstructed for the closely related *Paniscomima* Enderlein (Guidotti 2007), the brachypterous *Olixon* Cameron (Krogmann et al. 2009), and the family as a whole (Guidotti 1999). However, no comprehensive molecular study has focused on the Rhopalosomatidae itself, nor thoroughly examined any individual genus within it. However, *Rhopalosoma nearcticum* has been included in several molecular analyses of Hymenoptera (Carpenter and Wheeler 1999; Hines et al. 2007; Pilgrim et al. 2008; Szafranski 2009*; Heraty et al. 2011; Klopfstein et al.

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* A crosscheck of Szafranski's sequence of *R. nearcticum* (GenBank: EU567206.1) against the data in BOLD and GenBank reveals the sequence belongs to *Drosophila melanogaster*.

Figure 1. Adult female *Rhopalosoma* cf. *nearcticum* attracted to a mercury-vapor lamp in Fairfax County, VA, USA on July 29, 2018. Photo by Ashley Bradford, initially posted on bugguide.net.

2013; Branstetter et al. 2017), and several rhopalosomatids have been sequenced for the Barcode of Life Database (BOLD) (Ratnasingham and Hebert 2007).

In September, 2016, a bush cricket of the *Anaxipha exigua* (Say) species-group (Trigonidiidae) was collected at Cypress Grove Nature Park (CGNP) in Jackson, TN. Attached to the abdomen directly behind the right hind leg was a dark brown sac-like protuberance that was identified as a potential rhopalosomatid larva. Given the rarity of documented rhopalosomatid larvae and the novelty of the host record, our objectives were to 1) collect additional larvae and hosts from CGNP, 2) attempt to rear larvae to adulthood, and 3) sequence the barcoding gene COI of each specimen for molecular identification. Here, we report the *Anaxipha exigua* species-group as a new host record for *Rhopalosoma* and identify two genetically distinct clades of *Rhopalosoma* in America north of Mexico.

Materials and methods

Collection and rearing attempts

Cricket specimens were collected from CGNP using sweep nets in 2017 and 2018 from July–October when *Rhopalosoma* seems to be at peak abundance (Barrows 2013).

Parasitized crickets were retained at the Blaschke Lab at Union University for observation and attempted rearing of parasitoids. Crickets were maintained at 26 °C with a 16:8 photoperiod and were supplied apple slivers and raisins for food. Soil from CGNP was provided for burrowing/cocoon-formation when the larva detached from its host. If the cricket expired before the larva was mature enough to detach, the parasitoid and its host were stored in a freezer at 4 °C for subsequent molecular analysis. Photographs of parasitized crickets and larvae were taken using a camera phone mounted on a Fisher Stereomaster microscope and the images were stacked and edited using Helicon Focus 6 (v.6.8.0) software. Voucher specimens are retained at Union University, Jackson, TN.

Barcoding

Genomic DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Venlo, Netherlands). Due to their small size, rhopalosomatid specimens were extracted whole using whatever material was available (larvae, pupae, and/or exuviae). A BIO-RAD T100 Thermal Cycler was used to perform 50 µL PCR reactions modeled after Hebert et al. (2003). The following settings were used for the PCR reaction: 30 s denaturation at 98 °C followed by five cycles of 98 °C for 5 sec, 45 °C for 5 sec, and 72 °C for 15 sec; 35 cycles of 98 °C for 5 sec, 51 °C for 5 sec, and 72 °C for 15 sec; and a final extension at 72 °C for 1 min. The traditional invertebrate primers for COI were used for barcoding (LCO1490/HCO2198; Folmer et al. 1994). Difficult templates were amplified using a custom rhopalosomatid-specific forward primer (5’-CYATATGATCAGGAATAGTAGGWT-3’). Successful amplifications were confirmed via thin gel electrophoresis and the samples were sent to GeneWiz (South Plainfield, New Jersey) for post-PCR clean-up and sequencing.

Sequence quality was determined by the quality scores provided by GeneWiz and by examining the chromatograms visually using Geneious Prime (v.2019.0.4). The novel rhopalosomatid COI barcodes were uploaded to GenBank (Table 1). Sequences were aligned manually with a *R. nearcticum* reference sequence from GenBank (ID# GQ374638.1). Additional sequences from BOLD included barcodes from two unidentified *Rhopalosoma* specimens from Florida (initially assumed to be *R. nearcticum*), two unidentified *Rhopalosoma* specimens from Costa Rica, and an *Olixon* specimen as the outgroup (max = 657bp, min = 456bp, avg = 602.3pb) (Table 1).

Phylogenetic relationships were reconstructed using maximum likelihood (ML) and neighbor joining (NJ). ML trees were generated using RAxML (v.8.2.12) (Stamatakis 2014) through the CIPRES Science Gateway (Miller et al. 2010) with default parameters and analyzed statistically using 1000 bootstrap (BS) replicates. A strict consensus neighbor joining (NJ) tree was constructed using Geneious Prime with default parameters and one million bootstrap replicates. All phylogenies were visualized and clades compared in Geneious Prime. The Species Delimitation plug-in (Masters et al. 2011) was used to assess species boundaries and diversity using the Intra Dist, Inter Dist, P ID(Liberal) and P(Randomly Distinct) calculations.

Results

Collection and parasitoid rearing

In total, 12 parasitized crickets were collected and nine rhopalosomatid larvae were successfully barcoded (Table 1). Seven larvae were found on *H. agitator* hosts (five of these larvae were barcoded) (Fig. 2G), one on an *H. saltator* (larva barcoded) (Fig. 2H), and four were discovered attached to crickets from the *A. exigua* species-group (three larvae barcoded) (Fig. 2I).

Six parasitoid larvae detached from their host and four of these successfully spun cocoons to begin pupation. One specimen from an *H. agitator* host developed into an adult, but failed to eclose properly from the cocoon, resulting in the death of the wasp (Fig. 2C). Because so little is known about the phenology of these wasps, two cocoons from *A. exigua* hosts were dissected to observe the pupae *in situ* after attempting to activate the final molt to adulthood by incubating the pupae at 26 °C for >4 weeks. One larva had failed to develop into a pupa and the only identifiable tissue inside the cocoon was a disarticulated mandible (Fig. 2D). The other specimen had not pupated either, but was alive and appeared to be in a suspended animation "pre-pupa" state (Fig. 2E). The final cocoon is currently still in incubation (Fig. 2F).

Barcoding and phylogenetics

The topologies from the ML and NJ analyses were identical and statistically robust (Figs 3–4). Both trees recovered two distinct clades of Nearctic *Rhopalosoma*. Clade 1 contained the reference *R. nearcticum* sequence from GenBank along with seven of the

Table 1. Specimens used in phylogenetic analyses of Rhopalosomatidae with GenBank accession numbers. *ID numbers from BOLD.

Tree ID	Species	Host	Sample	Accession #
Parasitoid_1_TN	*R. ?nearcticum* sp. 1	*H. agitator*	Larva	MK991300
Parasitoid_2_TN	*R. ?nearcticum* sp. 1	*A. exigua* s.g.	Pupa	MK991301
Parasitoid_3_TN	*R. ?nearcticum* sp. 1	*A. exigua* s.g.	Larva	MK991302
Parasitoid_4_TN	*R. ?nearcticum* sp. 1	*H. agitator*	Larva	MK991303
Parasitoid_5_TN	*R. ?nearcticum* sp. 2	*H. agitator*	Larva	MK991304
Parasitoid_6_TN	*R. ?nearcticum* sp. 1	*H. agitator*	Larva	MK991305
Parasitoid_7_TN	*R. ?nearcticum* sp. 1	*A. exigua* s.g.	Larva	MK991306
Parasitoid_8_TN	*R. ?nearcticum* sp. 1	*H. saltator*	Larva	MK991307
Parasitoid_9_TN	*R. ?nearcticum* sp. 2	*H. agitator*	Larva	MK991308
*Rhopalosoma*_BBHYA1357_FL	*R. ?nearcticum* sp. 2	–	Adult	BBHYA1357-12*
*Rhopalosoma*_BBHYA1345_FL	*R. ?nearcticum* sp. 2	–	Adult	BBHYA1345-12*
*Rhopalosoma*_JICAX021_CR	*Rhopalosoma* sp.	–	Adult	JICAX021-16*
*Rhopalosoma*_JIAAG042_CR	*Rhopalosoma* sp.	–	Adult	JIAAG042-16*
*Olixon*_BBHY2946_TX	*Olixon* sp.	–	Adult	BBHYA2946-12*
*R. nearcticum*_GQ374638.1	*R. ?nearcticum* sp. 1	–	Adult	GQ374638.1

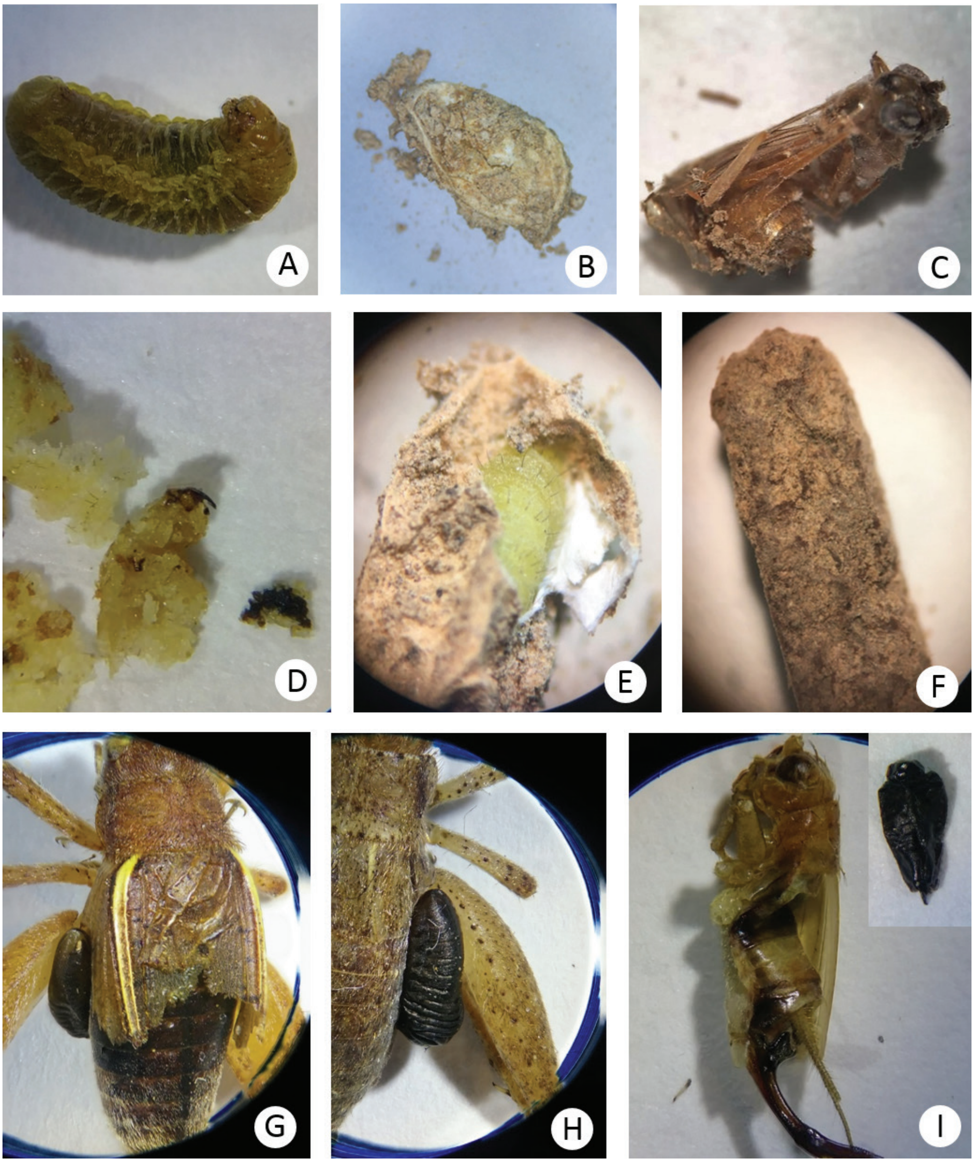

Figure 2. Life stages and representative specimens of *Rhopalosoma* **A** 5th instar larva prior to burrowing (MK991305) **B** pupal case extracted from soil (MK991302) **C** adult after failing to emerge properly from cocoon (MK991303) **D** disarticulated mandible from pupal case (MK991302) **E** pupal case extracted from dirt showing still living pre-pupa (MK991301) **F** pupal case awaiting adult emergence (MK991300) **G–I** early instar larvae attached to: **G** *Hapithus agitator* adult (larva: MK991304) and **H** *H. saltator* nymph (larva: MK991307) **I** *Anaxipha exigua* species group (inset: detached larva: MK991302).

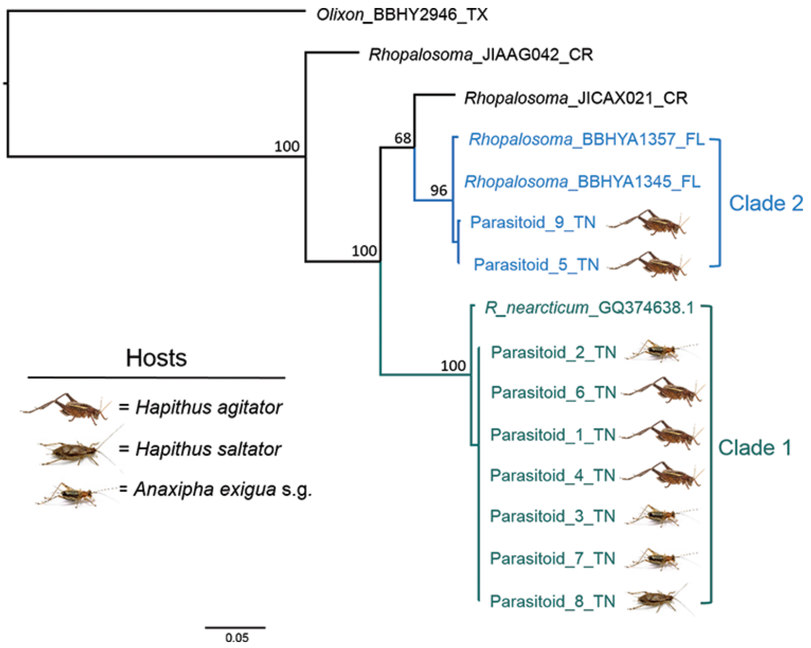

Figure 3. Maximum Likelihood phylogeny of *Rhopalosoma* with *Olixon* sp. as the outgroup. Bootstrap support shown for important nodes. Cricket photos by Carl Strang (*Hapithus agitator*), and Wil Hershberger/Lang Elliott (*H. saltator* and *Anaxipha exigua* s.g.).

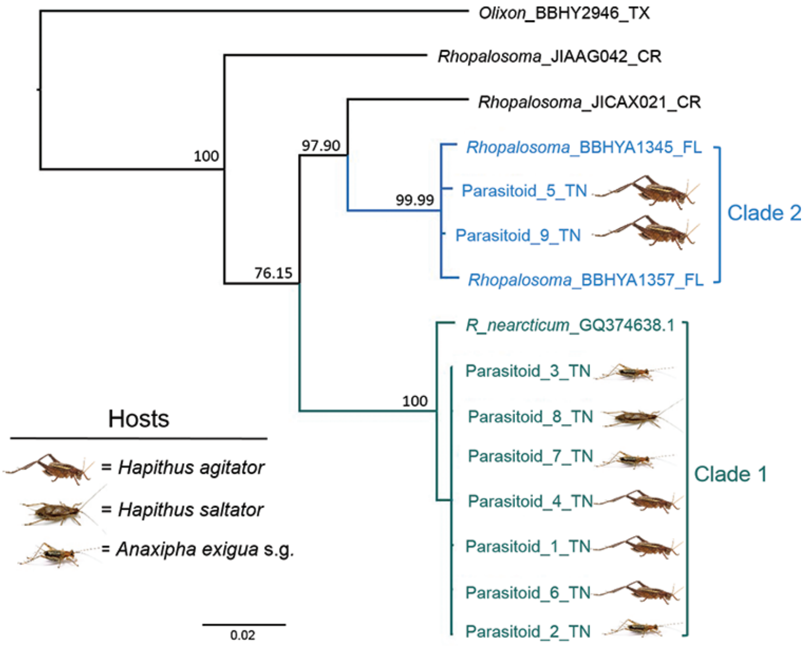

Figure 4. Neighbor Joining phylogeny of *Rhopalosoma* with *Olixon* sp. as the outgroup. Consensus support shown for important nodes. Cricket photos by Carl Strang (*Hapithus agitator*), and Wil Hershberger/Lang Elliott (*H. saltator* and *Anaxipha exigua* s.g.).

new barcodes, representing three larvae from *H. agitator*, one larva from *H. saltator*, and three larvae from *A. exigua* (BS = 100). Clade 2 included the two specimens from Florida along with two new barcodes representing two larvae from CGNP, both from *H. agitator* hosts (BS = 96). These clades were not sister to each other. Instead, Clade 2 was recovered sister to a specimen from Costa Rica (BS = 68).

The intraspecific distance was low within each clade (Clade 1 = 0.002; Clade 2 = 0.006), while the interspecific distance between these clades was high (0.148). Similarly, the P ID(Liberal) calculations, which serve as predictions of the utility of the gene for species delimitation (Ross et al. 2008), were high (Clade 1 = 0.97; Clade 2 = 1.00). The P(Randomly Distinct) values were <0.05 indicating a high probability that these two clades represent two distinct lineages (Rodrigo et al. 2008).

Discussion

The 12 Nearctic *Rhopalosoma* specimens included in the analyses (nine novel, plus two from BOLD and one from GenBank) were recovered in two distinct clades with convincing statistical support (BS = 98 and BS = 100). Clade 1 establishes one species of *Rhopalosoma* to be a generalist parasitoid of Grylloidea by expanding the known hosts to include the *Anaxipha exigua* species group. Unfortunately, even after barcoding the crickets, the exact species of the new host *Anaxipha* was not possible to determine. Six nominal species are included in the *A. exigua* species-group, only reliably distinguishable by the cadence of their mating calls (Walker and Funk 2014). However, *A. exigua* is the only member of the group whose range would include CGNP, indicating the host is most likely *A. exigua*, but we leave confirmation of the new host beyond the species-group level to future researchers.

Clade 2 reveals a second distinct genetic lineage of *Rhopalosoma* in the Nearctic. This clade includes specimens from Tennessee and Florida, and was recovered sister to a specimen from Costa Rica. This implies that this species has a closer evolutionary relationship with at least one Neotropical species than with its sympatric species in North America, even while developing on the same host as its Tennessee relative (*H. agitator*).

Although a *R. nearcticum* reference sequence was recovered within Clade 1, it is not possible at this time to determine if the sequence actually belongs to true *R. nearcticum*. Now that it is apparent that two species of *Rhopalosoma* inhabit America north of Mexico, adults of each clade need to be compared with the type specimen of *R. nearcticum* and with other members of the genus, *Rhopalosoma simile* Brues and the Caribbean species in particular. Either clade could be *R. nearcticum*, or neither group could be. Morphologically, *Rhopalosoma simile* is quite similar to *R. nearcticum* and apart from the color of the scape, pedicel, and flagellomeres I–V there is no character known that distinguishes these two taxa (Townes 1977). Gauld (1995) interpreted *R. nearcticum* and *R. simile* as conspecific and reported the occurrence of *R. nearcticum* in Costa Rica. However, this assumption has never been tested and a more detailed study should address the question of whether the two clades revealed in the study might represent these two species. Unfortunately, no high quality adult specimens were obtained during this study to compare with type specimens of *Rhopalosoma* spp.

The relatively few barcodes of *Rhopalosoma* generated here reveal the desperate need for thorough revision of this genus and phylogenetic analysis of intrageneric relationships. Although a species-level identification key for *Rhopalosoma* exists (Townes 1977), accurate identification remains a challenge. Other genera within Rhopalosomatidae (i.e., *Liosphex*, *Olixon* and *Paniscomima*) have received major taxonomic updates since Townes' family revision in 1977 and many new species have been discovered (e.g., Guidotti 2007; Krogmann et al. 2009; Lohrmann and Ohl 2010; Lohrmann 2011; Lohrmann et al. 2012). *Rhopalosoma*, however, has remained almost untouched apart from minor, mostly faunistic notes (e.g., Freytag 1984; Coronado Blanco and Cancino 2002; Smith 2008) with the exception of the description of the first fossil species in the genus (Lohrmann et al. 2019).

The evidence that two non-sister clades of *Rhopalosoma* develop on *Hapithus* hosts indicates that other members of the genus may do so as well. There is a striking similarity in the overall distribution range of *Rhopalosoma* and *Hapithus* (Townes 1977; Cigliano et al. 2019), and future studies could explore the hypothesis that *Hapithus* served as the ancestral host for a larger clade within *Rhopalosoma* or even the genus as a whole.

Cypress Grove Nature Park has shown to be an excellent site for observing and collecting these unusual wasps. Future studies should focus on describing adult and larval morphologies of the rhopalosomatids of CGNP and correlating them with the two genetic clades discovered here.

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RESEARCH ARTICLE

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Homonymy in Pompilidae: The case of *Balboana* Banks (Pompilinae, Priochilini)

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Abstract

The pompilid genus *Balboana* Banks, 1944 is preoccupied by Uvarov, 1939. A new replacement name, *Braunilla* Wasbauer and Kimsey is proposed and a species checklist included.

Keywords

Balboa, Tettigoniidae, Rhyparochromidae

Introduction

In 1925, Nathan Banks published the generic name *Balboa* for a spider wasp species from "America Meridionalis". Later he became aware that *Balboa* already been used by Distant (1893) for a species of rhyparochromid bug (*Balboa germana* Distant), and he proposed the replacement name *Balboana* for the pompilid genus (Banks 1943). Unfortunately, we were recently informed that the name *Balboana* is itself a homonym, preoccupied by *Balboana* Uvarov, a tettigoniid orthopteran. Thus, we propose the replacement name *Braunilla* below to honor the discoverer of the homonymy, Dr. Holger Braun, Division Entomologia, Museo de La Plata, La Plata, Argentina.

Type repositories listed below include:

CAMBRIDGE	Museum of Comparative Zoology, Cambridge, Massachusetts, USA;
COPENHAGEN	Zoologisk Museum, Copenhagen, Denmark;
ITHACA	Cornell University Insect Collection, Ithaca, New York, USA;
LONDON	The Natural History Museum, London, England;
WASHINGTON	U. S. National Museum, Washington, D.C., USA.

Taxonomy

Genus *Braunilla* Wasbauer & Kimsey, new replacement name

Balboa Banks 1925: 336. Type species: *Priocnemis barbouri* Banks (=*Priocnemis auripennis* Fabricius).

Balboana Banks 1944: 102. Replacement name for *Balboa* Banks 1925. Nec Distant 1893 (*Balboa variabilis* Distant, Rhyparochromidae).

Braunilla Wasbauer & Kimsey. Replacement name for *Balboana* Banks, 1944. Nec Uvarov, 1939 (*Balboana tibialis* (Brunner von Wattenwyl, 1895, Tettigoniidae).

Braunilla is a small genus of Neotropical pompilids in the subfamily Pompilinae, tribe Priochilini (Waichert et al. 2015). The species are strongly sexually dimorphic and range in size from 5–23 mm in length. Ten species have been attributed to the genus, although only the Central America and Mexican species have been reviewed (Evans 1966). There is also some confusion about the author of the species, *Braunilla manifestata.* The species was originally published as *Agenia manifestata* by F. Smith, but was later attributed to Banks by Fernández (2000).

Nothing is known about the biology of any of the species in the genus. Evans (1968) suggested they might be parasites of *Priochilus* because the ranges of the two genera are coincident and *Braunilla* females have the compressed apical metasomal sternum typical of some parasitic pompilids.

Checklist of *Braunilla* species

Braunilla auripennis (Fabricius). Panama, Guyana.

- *Pompilus auripennis* Fabricius 1804: 192. Syntype females; America Meridionalis (=South America) (COPENHAGEN).
- *Pompilus chiriquensis* Cameron 1893: 201. Holotype female; Panama: Bugaba (LONDON). Synonymized by Evans 1966.
- *Pompilus moorei* Cameron 1912: 424. Holotype female; British Guyana: Demerara (LONDON). Synonymized by Banks 1944.
- *Procnemis* (*Balboa*) *barbouri* Banks 1925: 333. Holotype female; Panama (CAMBRIDGE). Synonymized by Evans 1966.

Braunilla cameroni (Evans). Costa Rica, Panama.
  Balboana cameroni Evans 1966: 179. Holotype male; Costa Rica: Turrialba (WASHINGTON).

Braunilla elegans (Banks). Bolivia.
  Balboana elegans Banks 1946: 509. Holotype female; Bolivia: Sara Prov., Buenavista (CAMBRIDGE).

Braunilla fenestralis (Banks). Bolivia?
  Balboana fenestralis Banks 1946: 508. Holotype female; Bolivia: Santa Cruz, Buenavista (ITHACA).

Braunilla fulvipes (Banks). Guyana.
  Balboana fulvipes Banks 1944; Holotype female; Guyana: Cuyni-Mazaruni, Kartabo (CAMBRIDGE).

Braunilla manifestata (F. Smith). northern South America.
  Agenia manifestata F. Smith 1866: 264. Type: "The Amazons: Tunantius" (LONDON).
  Balboana manifestata Banks, 1944. Incorrectly attributed to Banks (Fernández 2000). Nomen nudum.

Braunilla nayaritana (Evans). Mexico.
  Balboana nayaritana Evans 1966: 181. Holotype female; Mexico: Nayarit, Vic. Compostela (CAMBRIDGE).

Braunilla nigrina Banks. Brazil.
  Balboana nigrina Banks 1946: 507. Holotype female; Brazil: Santa Catarina, Nova Teutonia (CAMBRIDGE).

Braunilla pulchella (Evans). Panama.
  Balboana pulchella Evans 1966: 182. Holotype female; Panama: Barro Colorado Is. (WASHINGTON).

Braunilla tarsalis (Cameron). Guatemala.
  Pseudagenia tarsalis Cameron 1897: 372. Holotype female; Guatemala: Purula, Vera Paz (LONDON?).

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We are grateful to Holger Braun for pointing out the homonymy in this name, which led to the entertainment of discovering how many homonyms this small obscure genus had accrued. Thanks also to the reviewers for their suggestions.

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RESEARCH ARTICLE

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Taxonomic notes on the paper wasps of the subgenus *Polistes* (*Gyrostoma*) (Hymenoptera, Vespidae, Polistinae) occurring in Vietnam, with description of a new species

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Abstract

Taxonomy of the paper wasps of the subgenus *Gyrostoma* of the polistine genus *Polistes* from Vietnam is treated, with a key to all the five species occurring in Vietnam, including one described herein as new to science under the name *Polistes longus* **sp. nov.** Nests of *P. gigas* and *P. longus* **sp. nov.** are also described.

Keywords

Polistes, *Gyrostoma*, distribution record, key, nest description, new species

Introduction

The paper wasp genus *Polistes*, with more than 200 species worldwide, is divided into four subgenera (Carpenter 1996a). The subgenera *Gyrostoma* Kirby 1828, *Polistella* Ashmead 1904, and *Polistes* Latreille 1802 are distributed in the Old World (including

Australasia), and a few species of the subgenera *Gyrostoma* and *Polistes* s. str. have been accidentally introduced into the New World; the subgenus *Aphanilopterus* Meunier 1888 is distributed exclusively in the New World. Of the three Old World subgenera, *Gyrostoma* presently has 22 species, of which four have been recorded from Vietnam: *P. gigas* Kirby, 1826, *P. olivaceus* (DeGeer, 1773), *P. rothneyi* Cameron (1900) and *P. tenebricosus* Lepeletier, 1836 (Nguyen and Kojima 2013).

In this paper, based on specimens deposited in the Institute of Ecology and Biological Resources (IEBR), a taxonomic study on the subgenus *Gyrostoma* from Vietnam is presented: a species is described as new to science together with its nest, and the nest of another species is also described.

Methods

All material including the holotype of the new species is deposited in the Institute of Ecology and Biological Resources (**IEBR**), Hanoi, Vietnam. The adult morphological and color characters were observed using pinned and dried specimens under a stereoscopic microscope. Measurements of body parts were made with an ocular micrometer attached to the microscope. "Body length" indicates the length of head, mesosoma and the first two metasomal segments combined. The parts measured for the morphometric characters referred to in the descriptions are defined as in Nguyen et al. (2011). Photographic images were made with Nikon SMZ 800N Digital Stereo Microscope, using Helicon Focus 7 software; the plates were edited with Photoshop CS6.

In the descriptions of adult morphology, the following abbreviations are used: The abbreviations F, S and T refer to numbered flagellomeres, metasomal sterna and metasomal terga, respectively; POD, distance between the inner margins of the posterior ocelli; OOD, distance between the outer margin of the posterior ocellus and the inner margin of the eye at vertex; Od, transverse diameter of a posterior ocellus; IED-c and ISD-c refer to collectors of the Insect Ecology Department and Insect Systematic Department, IEBR.

Taxonomic accounts

Subgenus *Gyrostoma* Kirby

(1) *Polistes gigas* (Kirby)

Figs 1–3, 19, 20

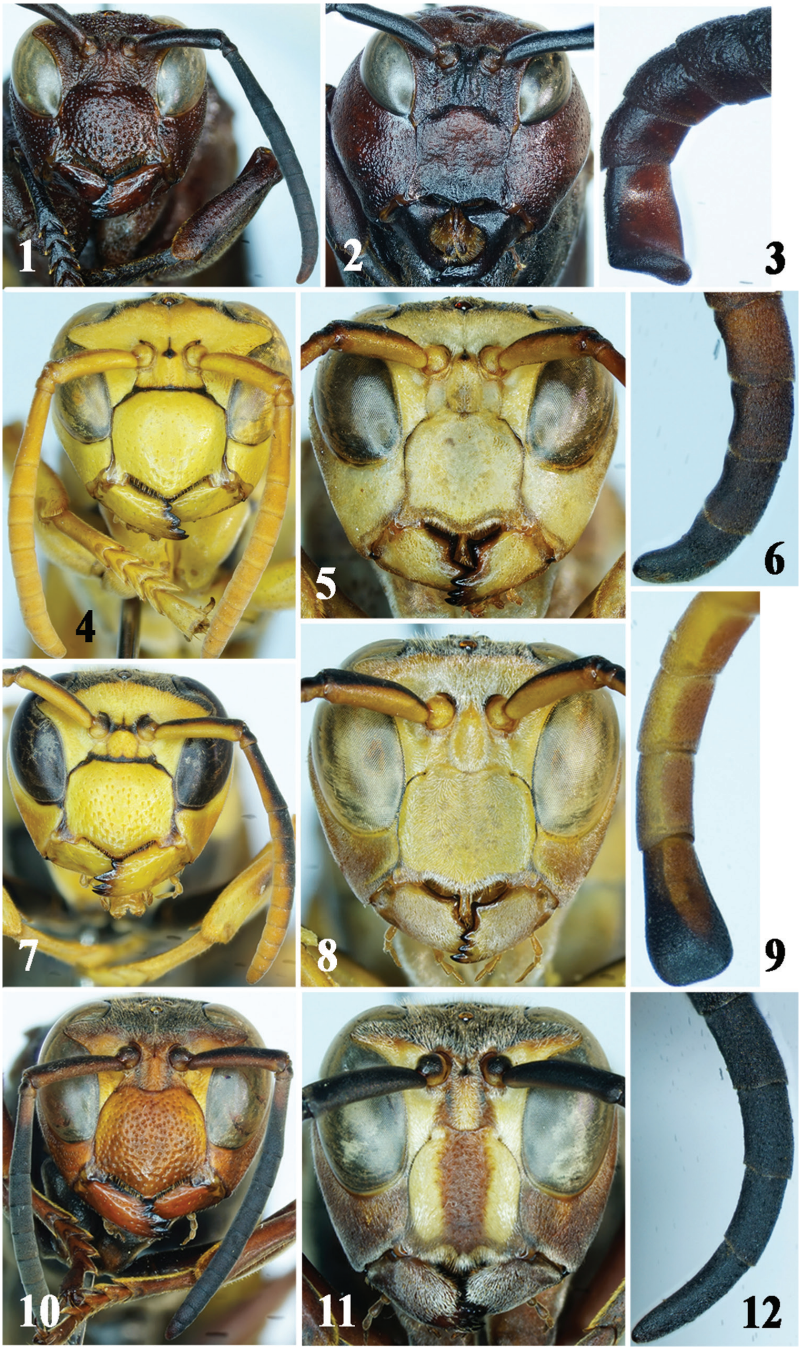
Cyclostoma gigas Kirby, 1826, 1, 3: 36.
Polistes gigas: Sonan, 1943, 33: 469.

Notes. This is the biggest species among polistine wasps, with female body length being about 25–30 mm, and male body length even bigger, about 32–35 mm. Its distribution is restricted to one area in the northeastern part of India (Sikkim), southern China including Hong Kong, and Taiwan (Das and Gupta 1989; Tan et al. 2014). In Vietnam, this species has been recorded from many localities in the northern part of the country (Nguyen et al. 2005). In this study, some new localities in the northern part are added to the distribution range of the species, and a record of one locality in the southern part is also added.

Material examined. Vietnam. Cao Bang [2 ♀, Nguyen Binh, Thanh Cong, 22°34'14.2"N, 105°52'51.7"E, 9 Aug. 2012, J Kojima, H Nugroho & IED-c leg.; 2 ♀, Nguyen Binh, Thanh Cong, 23°32'29"N, 105°52'52.7"E, 8 Aug. 2012, J Kojima, H Nugroho & IED-c leg.; 1 ♀, Nguyen Binh, Thanh Cong, 23°32'37"N, 105°52'10.4"E, 7 Aug. 2012, J Kojima, H Nugroho & IED-c leg.; 6 ♀, Nguyen Binh, Tam Kim, Na Va, 22°36'17"N, 106°01'47"E, alt. 320m, 18 Oct. 2015, LPT Nguyen, DD Nguyen, MP Nguyen leg.; 1 ♀, Nguyen Binh, Thanh Cong, Phia Oac, 11 Aug. 2012, ISD-c leg.; 2 ♀, Trung Khanh, Dam Thuy, Nguom Ngao cave, 28 Apr. 2015, LPT Nguyen leg.]; 1 ♀, Bac Kan. Na Ri, Lan San, Kim Hy NR, 3 Jun. 2014, 22°14'N, 106°5'E, SV Tran leg.; 1 ♀, Thai Nguyen, Dai Tu, Phuc Xuyen, 23 Apr. 2012, LD Khuat leg.; 1 ♀, Thai Nguyen, Dai Tu, 29 Sep. 2006, LD Khuat leg.; 3 ♀, Phu Tho, Xuan Son NP, alt. 500–600 m, 12–13 Jun. 2004, LTP Nguyen leg.; 1 ♀, Thai Nguyen, Xuan Dai, Tan Son, 20 May 2011, HP Pham leg.; Bac Giang [5 ♀, Son Dong, An Lac, Keo Vang, 21°18'46"N, 106°56'E, 4 Jun. 2014, LTP Nguyen, DD Nguyen, DD Tran leg.; 1 ♀, Son Dong, Tuan Dao, Khe Dan, Tay Yen Tu NR, 4 Jul. 2010, DD Tran leg.; 1 ♀, Son Dong, Khe Vang, 16 May 2013, DD Nguyen leg.; 1 ♀, Son Dong, An Lac, Dong Bay, 12 Aug. 2012, J Kojima, H Nugroho & IED-c leg.]; 1 ♀, Quang Ninh, Yen Tu mountain, 4 Mar. 2002, LPT Nguyen leg.; Hoa Binh [1 ♀, Yen Thuy, Da Phuc, 27 Apr. 2012, HT Dang leg.; 1 ♀, Mai Chau, Chieng Chau, Lac village, alt. 600m, 10 Jun. 2008, LPT Nguyen & PH Pham leg.; Ha Noi [1 ♀, Ba Vi, Van Hoa, 03 Jun. 2001, LPT Nguyen leg.; 3 ♀, Ba Vi, Yen Bai, Suoi Mo, alt. 100 m, 01 Jun. 2001, LPT Nguyen leg.]; Hai Phong [4 ♀, Cat Ba NP, 20°47'58"N, 106°59'93"E, Nest#-CB-2013-Po-01, 26 Jul. 2013, LTP Nguyen leg.; 1♀, Cat Ba NP, 10 Jun. 2003, DL Khuat leg.]; Vinh Phuc [1 ♀, Me Linh, Ngoc Thanh, 02 Aug. 2000, LX Truong leg.; 6 ♀, Tam Dao NP, 2005, 21°26'N, 105°37'E, alt. 400 m, 20 Aug., J. Kojima leg.; 1 ♀, Me Linh, Ngoc Thanh, 13 Jun. 2001, LX Truong leg.; 8 ♀, Tam Dao, alt. 900–1200m, 30 Jul.-3 Aug. 2012, DT Tran leg.; 6 ♀, 21 ♂, Phuc Yen, Me Linh, Nest# 2012-ML-P-01, 11 Nov. 2012, J Kojima, DT Dang & LPT Nguyen leg.]; Thanh Hoa, Thuong Xuan, Van Xuan, Hon Can, Xuan Lien NR [1 ♀, 23–24 Aug. 2012, LPT Nguyen & TV Hoang leg.; 1 ♀, 19°51'41.2"N, 105°14'6"E, alt. 175 m, 27 Aug. 2012, LPT Nguyen leg.]; 1 ♀, Nghe An, Con Cuong, Khe Bu, 16 Apr. 2006, ISD-c leg., light trap; 1 ♀, Ha Tinh, Huong Son, Son Kim, alt. 400 m, 06 May 2004, LPT Nguyen leg.; 1 ♀, Quang Binh, Quang Ninh, Truong Son, U Bo, alt. 650–800 m, 10 Jun. 2006, LTP Nguyen leg.; 1 ♀, Thua Thien Hue, Phu Loc, Bach Ma NP, 30 May 2001, LD Khuat leg.; 1♀, Quang Nam, Dong Giang, Macoih, 27 May 2006, ISD-c leg.

Distribution. India: Sikkim; southern China; Vietnam.

Nest. A nest (Nest# 2012-ML-P-01) collected in Me Linh station, Vinh Phuc on 11 November 2012 was examined. The nest (Fig. 19) was collected inside an old toilet, on the wall about 2 m from the ground, together with 8 females and 25 males.



Figures 1–12. *Gyrostoma* species. **1–3** *Polistes gigas* **1** female head, frontal view **2** male head, frontal view **3** antennal flagellomeres 1–4. **4–6** *Polistes olivaceus* **4** female head, frontal view **5** male head, frontal view **6** antennal flagellomeres 1–4. **7–9** *Polistes rothneyi* **7** female head, frontal view **8** male head, frontal view **9** antennal flagellomeres 1–4. **10–12** *Polistes tenebricosus* **10** female head, frontal view **11** male head, frontal view **12** Antennal flagellomeres 1–4.

Nest structural characters are as follows. Single comb, nearly round in shape with diameter of the comb nearly 10cm and 10cm in height, tough paper-like in texture, bicoloured, almost all dark brown mixed with reddish gray, expanded from the petiole to form fan-shape in ventral view, with dorsal surface slightly concave. One-third of the cell length from the bottom at outside covered with sharp thorns (Fig. 20). The cell where the petiole is placed is longest, about 88 mm in length, and the length of the other two next cells is 71 mm (while only 51.8 mm and 45.5 mm deep respectively). Outside of those cells with a fold at about one-third of the length from the bottom implying that the wasps may have reused the nest. Petiole single, terminal, with thick central core of plant fibers, 8.7 mm long and 4.6 × 4.7 mm thick. Cells generally arranged in regular rows, round at open end; cell gradually expanded towards open end, 8.4 mm (range 7.0–10.3; n=10) wide at bottom and 14.8 mm (rang 13.5–16.7; n = 10) wide at open end, 49.7 mm (range 43.6–52.9; n=10) deep; cell wall about 0.25 mm thick; cocoon cap strongly domed beyond level of cell opening, whitish gray.

(2) *Polistes olivaceus* (DeGeer)

Figs 4–6

Vespa olivacea DeGeer, 1773, 3: 582.
Polistes olivaceus: de Saussure, 1853: 89.

Notes. This species has been recorded from Vietnam (Das and Gupta 1984; Carpenter 1996b) but the details of localities were not given. Later, the species was recorded from provinces in the northeastern part of the country such as Ha Giang, Cao Bang, Tuyen Quang, Bac Kan, Thai Nguyen, Lang Son and Bac Giang (Nguyen et al. 2015, Nguyen 2016). The present study gives records from some localities in the northern part including the northwestern part, and a single locality in the southern part of the country.

Material examined. Vietnam. 1♀, Dien Bien, Dien Bien city market, 25 Aug. 2006, LTP Nguyen, F Saito & J Kojima leg.; Lao Cai [10 ♀, Bao Yen, Thuong Ha, Nest # VN-P-2006-1, 18 Aug. 2006, LTP Nguyen, F Saito & J Kojima leg.; 11 ♀, Nam Cuong, alt. 120 m, 19 Aug. 2006, LTP Nguyen, F Saito & J Kojima leg.]; 2 ♀, Yen Bai, Yen Bai city, 18 Aug. 2006, LTP Nguyen, F Saito & J Kojima leg.; 1 ♀, Son La, To Hieu farm, 7 Sep. 1993, XL Truong leg.; Vinh Phuc [1 ♀, Tam Dao NP, 21°27'N, 105°38'E, alt. 890 m, 4 May 2000, JM Carpenter leg.; Tam Dao, Me Linh, Ngoc Thanh [2 ♀, 20 Jul. 2000, DL Khuat leg.; 1 ♀, 24 May 2000, LTP Nguyen leg.]; 1 ♀, Tam Dao, Tam Dao NP, alt. <50 m, 21 Aug. 2005, LTP Nguyen & J Kojima leg.; Hanoi [2 ♀, Cau Giay, Nghia Do, 29 May 2000, LTP Nguyen leg.; 2 ♂, 23 ♀, Cau Giay, Nghia Do, Nest# VN-P-2005-1, 1 Aug. 2005, LTP Nguyen & J Kojima leg.; 9 ♀, Thong Nhat park, 7 Sep. 2006, J Kojima leg.; 1 ♀, Quoc Oai, 14 Aug. 1998, XL Truong leg.; 1 ♀, Ba Vi, Van Hoa, Yen Bai, 15 Aug. 2006; ISD-c leg.; 1 ♀, Thuong Tin, 11 Jul. 2005, XT Nguyen leg.]; Hoa Binh, Yen Thuy [1 ♀, Lac Thinh, 30 Apr. 2002, DL Khuat leg.; 4 ♂, Da Phuc, 30 Aug. 2000, LTP Nguyen; 5 ♀, Da Phuc, 5 Jul. 2000, XL Truong leg.; 1 ♀, Bao Hieu, 9 Aug. 2000, LTP Nguyen leg.; Lac Thinh, 6 May 2002, VT Hoang leg.]; Nghe An [2 ♀, Khe Bo, 19°03'N, 104°43'E, alt. 120 m, 25–28 Apr. 1998, JM Carpenter leg.; 2 ♀, Con Cuong, Mon Son, Pha Lay, 11 Mar. 2002; 5 ♀, Con Cuong, Mon Son, Bung village, 7–9 Sep. 2005; 2 ♀, Pu Mat NP office, 17 Nov. 2005; 1 ♀, Con Cuong, Khe Bu, 16 Jul. 2006; 1 ♀, Co Phat, alt. 200 m, 22 Jul. 2006; 2 ♀, Chau Khe, Khe Choang, 14 Jul. 2005, leg. ISD-c; 4 ♀, Pu Mat NP office, 21 Jul. 2004], LTP Nguyen leg.; 1♀, Con Cuong, Mon Son, 22–24 Jul. 2004, LTP Nguyen leg.; 4 ♀, Quang Binh, Bo Trach, 17 Aug. 2005, LTP Nguyen & J Kojima leg.; 9 ♀, Thua Thien Hue, Hue City, 16 Aug. 2005, LTP Nguyen & J Kojima leg.; 1 ♀, Kien Giang, U Minh Thuong NP, 30 Sep. 2003, LTP Nguyen leg.

Distribution. Madagascar; Réunion; Mauritius (including Chagos Archipelago); Tanzania: Zanzibar; Seychelles (including Amirantes); Egypt; Oman; Iran; Afghanistan; India: Gujarat, Rajasthan, Jammu and Kashmir, Jharkhand, Assam, Himachal Pradesh, Arunachal Pradesh, Karnataka, Andhra Pradesh, Madhya Pradesh, Chhattisgarh, Odisha, Manipur, Meghalaya, Nagaland, Sikkim, Tripura, Uttarakhand, Uttar Pradesh, West Bengal; Sri Lanka; Nepal; Myanmar; China; ? Japan: Okinawa; Vietnam; Laos; Cambodia; Thailand; Malaysia; Singapore; ? Indonesia: Kalimantan; Marianas; New Caledonia; Fiji; Tonga; Samoa; French Polynesia: Tahiti, Tuamotu Archipelago, Marquesas; adventive in Chile: Easter Is.; U.S.A.: Hawaii; Australia: Queensland; New Zealand.

(3) *Polistes rothneyi* Cameron

Figs 7–9, 23, 24

Polistes rothneyi Cameron, 1900, (7) 6: 410.

Notes. In Vietnam, van der Vecht (1968) recorded this species from a single locality in the southern part of the country. The species has been recorded from some provinces in the northern part such as Ha Giang, Cao Bang, Bac Kan, and Lang Son (Nguyen et al. 2015, Nguyen 2016). This study adds records from other localities in the northern part of the country, and one southern record has been also added.

In previous study in Vietnam, this species has only one color form and it agrees with the color form that van der Vecht (1968: 106) referred to as subspecies *quatei* (Fig. 23). In this study, the specimens from the southern part of the country have ground color darker than the specimens from the northern part (Fig. 24).

Material examined. Vietnam. Cao Bang [2 ♀, Nguyen Binh, Phia Oac NR, alt. 1590 m, 23°02'49"N, 104°59'35.6"E, 5 Nov. 2016, NN Tran, MP Nguyen, TV Luong leg.; 1 ♀, Nguyen Binh, Thanh Cong, Phia Oac, 11 Aug. 2012, leg. ISD-c leg.; 3 ♀, Nguyen Binh, Thanh Cong, 22°32'37"N, 105°52'10.4"E, 7 Aug. 2012, J Kojima, H Nugroho & IED-c leg.; 1♀, Bang Giang river, 21 Aug. 2001, XL Truong leg.]; Lang Son [1 ♀, Bac Son, 1 Jul. 2003, XL Truong leg.; 1 ♀, Huu Lung, Cai Kinh, alt. 85m, 16 Jul. 2016, 20°31'37.6"N, 105°00'24.2"E, LPT Nguyen, DD Nguyen, NT Tran leg.]; Bac Giang [1 ♀, Son Dong, Khe Ro, 18 May 2013, DD Nguyen leg.; 1 ♀, Son Dong, Thanh Son, alt. 300m, 1 Jul. 2010, HP Pham leg.]; 3 ♀, Ha Noi, Ba Vi, 16 Jun. 2016, TV Tran leg.; 4 ♀, Phu Tho, Xuan Son NP, alt. 400 m, 12–13 Jun. 2004, LTP Nguyen leg.; Vinh Phuc [3 ♀, Tam Dao NP, alt. 800 m, 01 Jul. 2003, LTP Nguyen leg.; 6 ♂, 7 ♀, Tam Dao NP, alt. 900 m, 21 Aug. 2005, LTP Nguyen & J Kojima leg.; 26 ♂, Tam Dao NP, alt. 800 m, Nest# VN-P-2006-19, 1 Sep. 2006, F Saito & J Kojima leg.; Hoa Binh, Mai Chau, Pa Co [1 ♀, alt. 1100 m, 28 Apr. 2002; ISD-c leg.; 1 ♀, alt. 900 m, 1 Aug. 2004, XL Truong leg.]; 1 ♀, Hoa Binh, Yen Thuy, 1 May 2002, VT Hoang leg.; 1 ♀, Ninh Binh, Cuc Phuong NP, 8 May 2002, VT Hoang leg.; 1 ♀, Nghe An, Quy Hop, Chau Thanh, 16 Jul. 2004, XH Le leg.; 4 ♀, Gia Lai, Kon Chu Rang NR, Son Lang, Kbang, 14°28'27"N, 108°32'24.3"E, alt. 830 m, 13 Apr. 2015, LTP Nguyen leg.

Distribution. Pakistan; Nepal; India: Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh; Uttarakhand, West Bengal, Sikkim, Bihar, Assam, Delhi, Karnataka, Kerala, Tamil Nadu, Manipur, Meghalaya; Laos; Malaysia; Myanmar; Vietnam; Indonesia: Sumatra, Java; China; Japan; Korea.

(4) ***Polistes tenebricosus*** **Lepeletier**

Figs 10–12, 25, 26

Polistes tenebricosa Lepeletier, 1836, 1: 529.

Notes. This species has been recorded from the northern part of Vietnam (Nguyen et al. 2005) (Fig. 25). Specimens from the southern part are examined, which are new records for that region of the country. Those specimens have the metasoma more yellow than the specimens from the northern part (Fig. 26).

Material examined. Vietnam. 1 ♀, Bac kan, Ba Be NP, 17 Jul. 2004, DH Nguyen leg.; Bac Giang [2 ♀, Son Dong, Khe Vang, 16 May 2013, DD Nguyen leg.; 1 ♀, Son Dong, Thanh Son, alt. 300 m, 1 Jul. 2010, HP Pham leg.]; 5 ♀, Phu Tho, Xuan Son NP, alt. 400–600 m, 12–16 Jun. 2004, LTP Nguyen leg.; Vinh Phuc [1 ♀, Tam Dao NP, alt. 1000 m, 3 Jul. 2003, LTP Nguyen leg.; 1 ♀, Tam Dao NP, alt. 900 m, 19 Aug. 2005, LTP Nguyen & J Kojima leg.; 7 ♀, Tam Dao NP, Nest # VN-P-2006-18, alt. 800 m, 31 Aug. 2006, F Saito & J Kojima leg.]; Hoa Binh [1 ♀, Yen Thuy, Lac Thinh, alt. 130 m, 30 Apr. 2002, DL Khuat leg.; 3 ♀, Kim Boi, Thuong Tien, 5 Aug. 2012, LD Khuat leg.]; 2 ♀, Thanh Hoa, Thuong Xuan, Van Xuan, Hon Can, Xuan Lien NP, 23 Aug. 2012, LTP Nguyen leg.; Nghe An [1 ♀, Con Cuong, Mon Son, 22–24 Jul. 2004, LTP Nguyen leg.; 1 ♀, Quy Hop, Chau Cuong, 14–19 Jul. 2004, XH Le leg.; 1♀, Ha Tinh, Huong Son, 18°21N 106°15E, alt. 450 m, 14–21 Apr. 1998, Malaise Trap, J M Carpenter, DL Khuat, Grimaldi, Herman, Silva leg.; 1 f#, Quang Nam, Dong Giang, Prao district, alt. 500–600 m, 28 May 2006, ISD-c leg.; 1 ♀, Gia Lai, Mang Yang, A Yun, Kon Ka Kinh NP, alt. 881 m, 04 Jun. 2011, ISD-c leg.; 2 ♀, Ninh Thuan, Ngoan Muc pass, 4 Aug. 2005, LTP Nguyen & J Kojima leg..

Distribution. India: Jammu and Kashmir, Uttarakhand, Sikkim, West Bengal, Arunachal Pradesh, Assam, Meghalaya, Mizoram, Nagaland, Tripura; Nepal; China; Vietnam; Myanmar; Indonesia: Sumatra, Java, Bali, Lombok, Flores, Sumba, Kalimantan; Sulawesi; Philippines: Negros, Sibuyan.

(5) *Polistes longus* Nguyen & Carpenter, sp. nov.

http://zoobank.org/AEFFE8F9-6207-4C73-9BE6-175D9E45C8F1
Figs 13–18, 21, 22

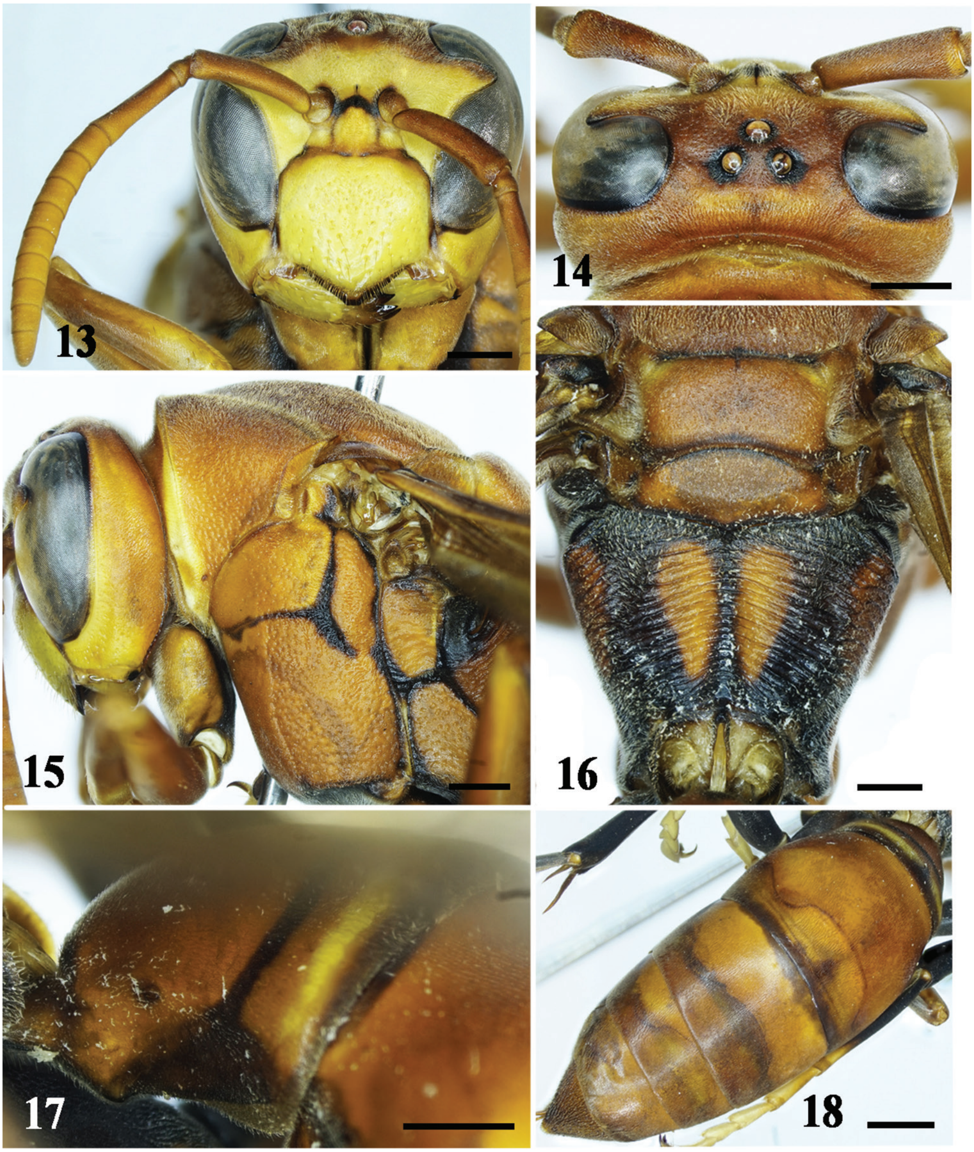
Material examined. Holotype, ♀, Vietnam: Ia Pal, Chu Se, Gia Lai, 13°39'46.2"N, 108°08'04.2"E, alt. 370 m, Nest#2012-TN-P-04, 25 Jul. 2012, LTP Nguyen leg., deposited in IEBR. Paratypes: 30 ♀, same data as holotye.

Other material examined. 3 ♀, Binh Thuan, Ham Thuan Nam, 10°51'07.7"N, 109°53'59.8"E, 11 Aug. 2005, LTP Nguyen & J Kojima leg.; 2 ♀Kon Tum, Kon Tum City, 20 Jul. 2012, LPT Nguyen leg.; Gia Lai [10 ♀, Ia Pal, Chu Se, 13°39'46.2"N, 108°08'04.2"E, alt. 370 m, Nest#2012-TN-P-04, 3 ♀, Ia Pal, Chu Se, 21–25 Jul. 2012, ISD-c leg.; 3 ♀, Chu Se, 14 Apr. 2013, LTP Nguyen leg.; 1 ♀, Ayun Pa, Ia Rto, 13°21'49.6"N, 108°30'06.4"E, alt. 200 m, 1 May 2016, LPT Nguyen, DD Nguyen, NT Tran leg.; 8 ♀, Chu Se, Ia Pal, 13°39'46.2"N, 108°08'04.2"E, alt. 370 m, 21 Jul. 2012, LTP Nguyen leg.]; 1 ♀, Dak Lak, Nam N Jang, 24 Jul. 2012, ISD-c leg.

Description. Female. Body length 17.7–19.5 mm (holotype 19 mm); fore wing length 19.5–21.5 mm (holotype 21 mm).

Head in frontal view about 1.12 × as wide as high (Fig. 13); in dorsal view weakly swollen laterally behind eyes, then narrowed posteriorly, with posterior margin shallowly and broadly emarginate. Vertex (Fig. 14) slightly raised in area among ocelli, slightly sloped down behind posterior ocelli towards occipital carina; POD: OOD = about 1:1.7; POD about 1.5 times Od. Gena wider than eye, in lateral view 1.2 × as wide as eye (Fig. 15). Occipital carina complete, clearly present along entire length of gena. Inner eye margins slightly convergent ventrally, in frontal view distance of inner eye margins at clypeus 1.1 × than those at vertex (Fig. 13). Antennal sockets closer to inner eye margin than to each other; anterior tentorial pit slightly further apart from inner eye margin than from antennal socket; interantennal space strongly raised. Clypeus in frontal view (Fig. 13) nearly as wide as high, produced ventrally into blunt angle, in lateral view weakly swollen anteriorly (Fig. 15); length of lateral margin of clypeus lying along inner eye margin longer than diameter of antennal socket and slightly shorter than the length of malar space. Antennal scape 3 × as long as its maximum width; FI 3.1 × as long as its maximum width, FII–IV as long as wide, FV-IX wider than long, FX bullet-shaped, 1.3 × as long as its basal width.

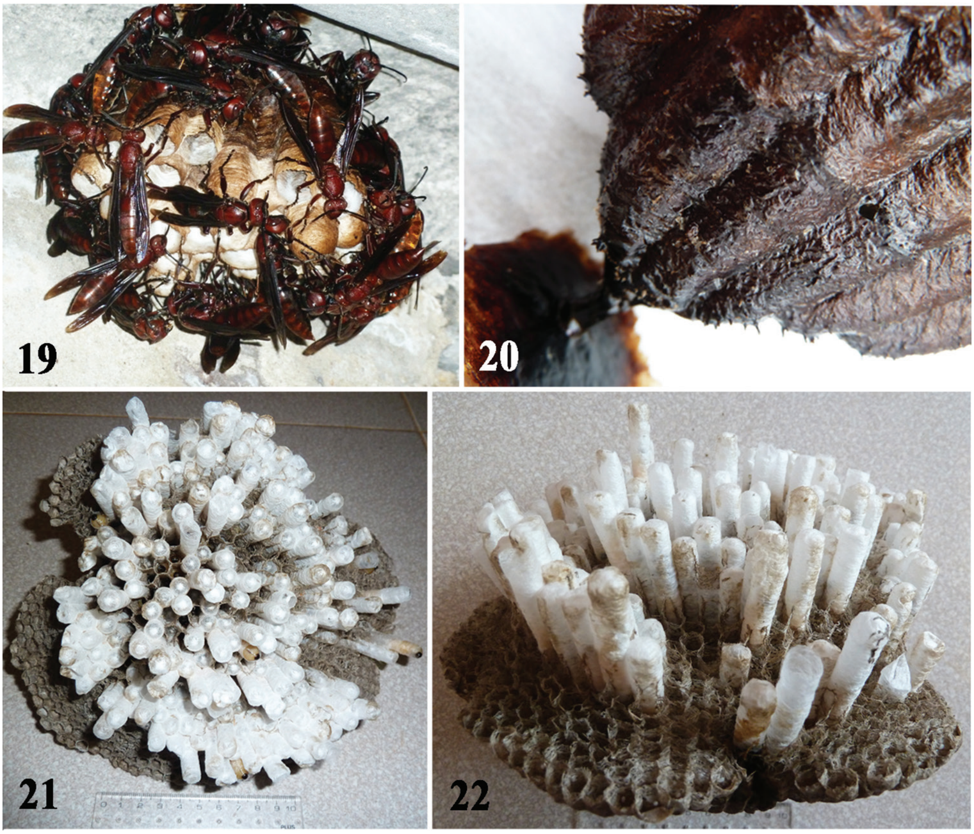
Pronotal carina raised, produced dorsally into lamella in dorsal part, slightly sinuate backward on lateral side, reaching ventral corner of pronotum. Mesoscutum in lateral view weakly convex, about 1.16 × as long as wide between tegulae; anterior margin broadly rounded. Scutellum flattened, slightly depressed in the middle, in lateral view slightly higher at the level of mesoscutum, strongly depressed along anterior margin. Metanotum weakly convex, disc nearly flat but strongly depressed anterior margin. Propodeum short (Fig. 16); posterior face narrowly (about one third the maximum width of propodeum) and strongly excavated medially, more or less smoothly passing into lateral faces; propodeal orifice elongate, about 2.8 times as long as wide (measured at widest part), somewhat narrowed in dorsal half. Wings hyaline.



Figures 13–18. *Polistes longus* sp. nov. , holotype female **13** head, frontal view **14** head, lateral view **15** vertex, dorsal view **16** propodeum, dorsal view **17** T1, lateral view **18** abdomen, dorsal view. Scale bars: 1 mm.

TI short and thick, about 0.9 times as long as its apical width, in lateral view abruptly swollen dorsally just behind basal slit for reception of propodeal suspensory ligament; corner between anterior and dorsal faces bluntly angled (Fig. 17). SII in lateral view swollen ventrally in smoothly curved line from base to apical margin.

Clypeus with scattered large punctures, each bearing sharply pointed golden bristle. Mandible with several small and shallow punctures at base and deep punctures at anterior margin. Frons covered with small and shallow punctures. Vertex and gena with minute punctures, area around ocelli with minute punctures; ventral one third



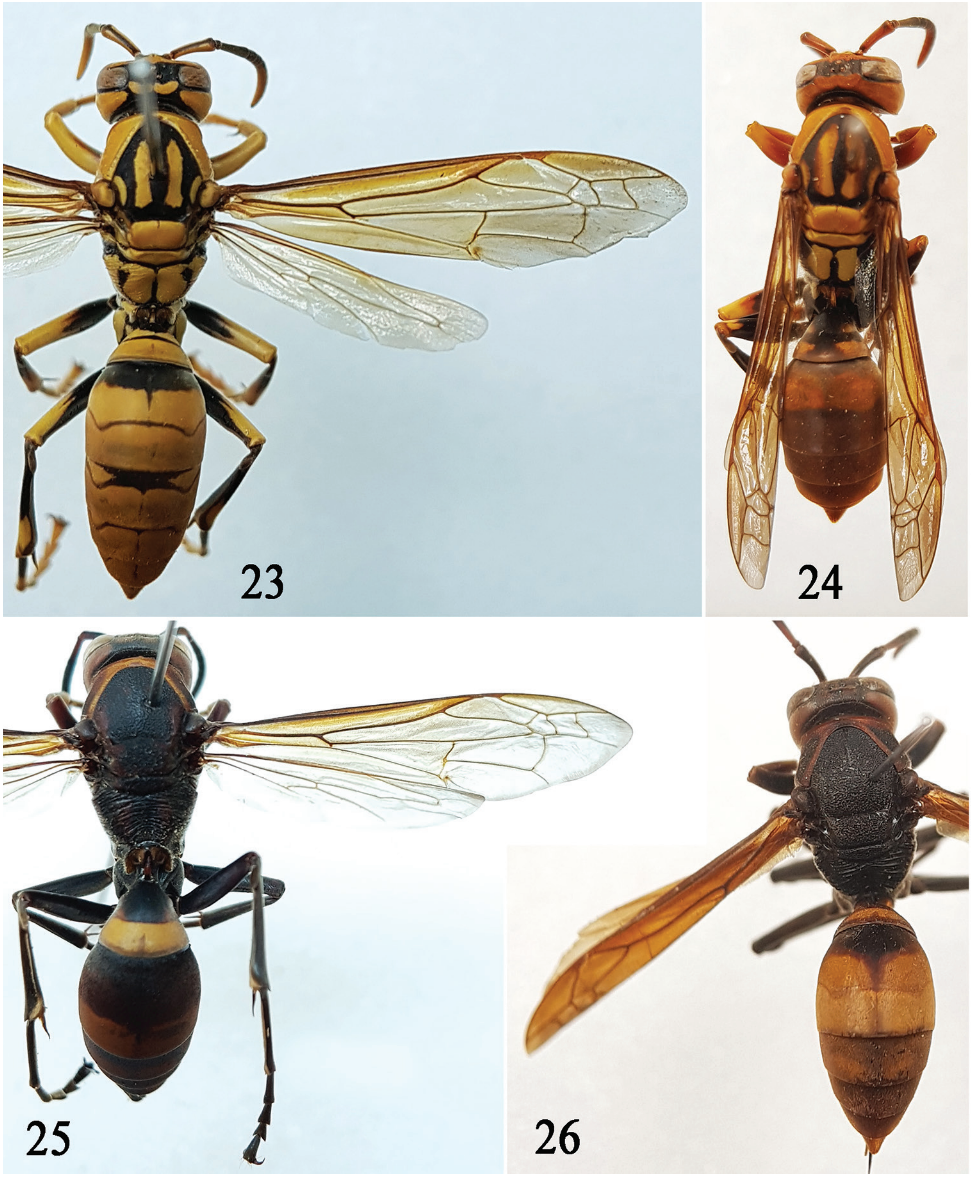
Figures 19–22. Nest of *Gyrostoma* species. **19, 20** Nest of *P. gigas* **21, 22** Nest of *P. longus* sp. nov.

of gena with large and shallow punctures. Pronotum with medium deep punctures. Mesoscutum sparsely with deep punctures; punctures on scutellum and metanotum similar to those on mesoscutum. Mesepisternum with sparse large and deep punctures in posterodorsal part, scattered shallow punctured in anteroventral part; border between posterodorsal and anteroventral parts indistinct. Dorsal metapleuron with some strong striae and shallow large punctures; ventral metapleuron with sparse strong punctures. Propodeum with strong transverse striae. Metasomal segments (Fig. 18) covered with minute punctures. Dark reddish brown; following parts yellow to orange-yellow: clypeus up to front except black strip between antennal socket, mandible except apical and lateral margins, lower part of gena near mandible; apical margin of pronotum along pronotal carina, a narrow transverse band near apical margin of T1, all tarsae; following parts black: propodeum except 4 large reddish brown mark, valvula, a narrow transverse strip at two third from base of T1, some mark on T2-6; hind leg (except yellow tarsa).

Male. Unknown.

Distribution. South Vietnam.

Remarks. This species comes close to *P. rothneyi* but it is different from the latter by the female clypeus nearly as wide as high (slightly wider than high in *P. rothneyi*),



Figures 23–26. Color variation in *Gyrostoma* species. **23, 24** *P. rothneyi* **23** typical form **24** darker form in the southern part **25, 26** *P. tenebricosus* **25** typical form **26** brighter form in the southern part.

dorsal two thirds of the gena without medium punctures (dorsal two thirds of the gena with sparse and medium punctures in *P. rothneyi*), propodeum with posterior face narrowly and strongly excavated medially (propodeum with posterior face widely and more shallowly excavated medially in *P. rothneyi*).

Etymology. The specific name refers to the very long cells of the nest.

Nest. A nest (Nest#2012-TN-P-04) (Figs 21, 22) collected in Ia Pal, Chu Se, Gia Lai was examined.

The nest was attached to a thin branch of a shrub about 0.5 m above the ground. The nest characteristics are as follows: Petiole lost during collecting, but the remaining part showed that it is single, nearly central. Comb rather tough, pliable paper-like in texture, more or less uniformly brown in cell walls, subcircular (about 22 mm × 20 mm) in view from side of cell opening, dorsal surface dome shape. Cells arranged in regular rows and round at open end; cell expanded towards open end, 6.3 mm (range 6.0–6.9 mm; n = 10) wide at bottom and 7.3 mm (range 7.0–7.5 mm; n = 10) wide at open end, 16.3 mm (range 16.1–16.9 mm; n = 10) deep; cell wall about 0.03 mm thick. Cocoon caps very long (range 20–42mm), white.

Key to species of *Gyrostoma* from Vietnam

The characters used are applicable to both sexes unless the sex is specified.

1 Prestigma longer than the length of the pterostigma measured along ventral part; mesepisternum without dorsal episternal groove; interantennal carina toothed; pronotum with striations; hind trochanter toothed. In male, clypeus as wide as high, separated from eye by a gap on both sides, mandible tridentate. Body color entirely dark brown, clypeus brown.................................. ***Polistes gigas* (Kirby)**

– Prestigma about equal to or shorter than the length of the pterostigma measured along ventral part; mesepisternum with dorsal episternal groove; interantennal carina blunt; pronotum without striations; hind trochanter smooth................. **2**

2 Mesepisternum without epicnemial carina. Clypeus dark brown, metasomal terga yellow with black or dark brown bands or entirely dark brown ....
....***Polistes tenebricosus* Lepeletier**

– Mesepisternum with epicnemial carina.. **3**

3 Mesopleuron weakly and sparsely punctate medially. In male, clypeus touching eye, wider than high, SVII with lateral process. Clypeus yellow, metasomal terga yellow with reddish-brown bands............................***Polistes olivaceus* (DeGeer)**

– Mesopleuron strongly and densely punctate medially...................................... **4**

4 Female clypeus nearly as wide as high. Prestigma shorter than the length of the pterostigma; dorsal two thirds of gena without medium punctures; propodeum with posterior face narrowly (about one third the maximum width of propodeum) and strongly excavated medially. Body color dark reddish brown, clypeus yellow ....***Polistes longus* sp. nov.**

– Female clypeus slightly wider than high. Prestigma about equal to the length of the pterostigma; dorsal two thirds of gena with sparse and medium punctures; propodeum with posterior face widely (about half the maximum width of propodeum) and shallowly excavated medially. Body color yellow with black marks, clypeus yellow ....***Polistes rothneyi* Cameron**

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RESEARCH ARTICLE

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Flower use by late nineteenth-century orchid bees (*Eufriesea surinamensis*, Hymenoptera, Apidae) nesting in the Catedral Basílica Santa María la Antigua de Panamá

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Abstract

A recent restoration of the Basilica Cathedral in Casco Viejo, Panamá, revealed that prior to 1871–1876 female orchid bees (*Eufriesea surinamensis*) built large nesting aggregations high above the main altar, based on physical evidence dating to a nineteenth-century restoration. Bees constructed cells in approximately 120 clusters in six different aggregations on the reredos ("altarpiece"). Palynological analyses of cell contents showed that bees visited 48 species of plants, representing 43 genera and 23 families. Contents of bee cells reflect elements of floristic diversity surrounding Panama City that are seen in historical contemporaneous photographs of the nesting site and environs.

Resumen

Una restauración reciente de la Catedral Basílica en el Casco Viejo de la ciudad de Panamá reveló que las hembras de abejas solitarias (*Eufriesea surinamensis*) nidificaron en lo alto de su retablo mayor previo a 1871–1876, basada en evidencia física de una restauración del siglo XIX. Las abejas construyeron celdas en aproximadamente 120 grupos de seis agregaciones diferentes en este retablo. Un análisis de los granos de polen dentro de estos nidos demostró que las abejas visitaron 48 especies de plantas que representan 43 géneros y 23 familias. Los elementos de la diversidad florística en la ciudad de Panamá se pueden observar en fotografías históricas de esta época del sitio de anidación y sus alrededores.

Keywords

Euglossini, pollination, floral utilization, nesting sites, historical reconstruction

Introduction

The Euglossini is a diverse Neotropical taxon comprised of five genera and nearly 200 species of beautiful, often brilliantly colored and metallic bees (Dressler 1982; Ramírez et al. 2002; Cameron 2004; Roubik and Hanson 2004). Males are well-known for their behavior in collecting chemical fragrances, mainly from orchids and thereby pollinating them (Dressler 1982). Females are more secretive. Their nests generally are dispersed and difficult for us to locate (reviewed by Ramírez et al. 2002; Wcislo et al. 2012). Consequently, relatively little is known about females' behavior, including which plants they visit to collect floral resources for food (pollen and nectar) and nest-building materials (resin and wood fragments), and which plants they pollinate. Orchid bees tend to be denizens of natural forests and adjacent biomes (Dressler 1982; Roubik and Hanson 2004). Some species tolerate disturbances due to human activities (e.g., López-Uribe et al. 2008; Brosi 2009), and one species established itself in Florida USA, well outside its native range (Pemberton and Wheeler 2006). Based on limited information, females tend to gather pollen and nectar from many flowers, and do not specialize on specific host plant taxa (e.g., Cortopassi-Laurino et al. 2009; Villanueva-Gutierrez et al. 2013). For most euglossine species, however, there are no data on pollen preference and utilization.

The genus *Eufriesea* contains about 52 species of large bees (13–27 mm long), many of which are brilliantly metallic in color, while others have striking bands of yellow and black hairs (Michener 2007). They are mainly neotropical-forest-loving creatures, though species extend to southern Argentina and north to Sinaloa and Chihuahua, México (Kimsey 1982). *Eufriesea surinamensis* is widely distributed from central México to southern Brazil, occurring in forested, cultivated and disturbed habitats, up to 1100 m (Kimsey 1982; Roubik and Hanson 2004). Nests consist of exposed, adjacent brood cells (~13 × 22 mm) constructed of plant resin mixed with numerous small pieces of tree bark wood (Bennett 1972; Myers and Loveless 1976; Kimsey 1982). Isolated cells or aggregated clusters are situated on natural sheltered cavities and crevices, such as under overhanging rocky banks or in cliff caves, within rotten hollows in termite nests, under dead bark, or inside bamboo internodes (*op. cit.*). Females also readily use sheltered human-built structures (e.g., under flooring, eaves, and trestles). A female constructs a cell, in which she places a cache of pollen mixed with nectar, lays an egg, and seals the cell. The larva feeds on the pollen and nectar, and later defecates the undigested pollen remains on an interior wall of the cell, coincident with the development of pupal features within the larval exoskeleton (Rozen 2018). Multiple females may build cells in close proximity to cells built by others, re-use the cells, and defend them from take-over, but they do not cooperate and are aggressive to neighbors (Myers and Loveless 1976). Nesting sites are likely re-used over a number of years by successive generations (Bennett 1972; Myers and Loveless 1976; Young 2012).

Floral resources used by *E. surinamensis* are little known, and we know of no published palynological studies of brood cell contents. Males are readily attracted by the scent vanillin in forested regions, and visit the orchids *Notylia*, *Pterostemma*, *Peristeria*, and *Sievekingia* (Orchidaceae) (Roubik and Hanson 2004). Based on specimen label data, Kimsey (1982) reports that females visited *Solanum* sp. (Solanaceae) and *Cassia* sp. (Fabaceae) for pollen, and also visited *Psidium guajava* (Myrtaceae), *Petastoma patelliferum* (Bignoniaceae), and *Argyeria speciosa* (Convolvulaceae) for unknown purposes.

Here we provide an analysis of pollen grains recovered from old brood cells of *E. surinamensis* nesting in an urban setting in late nineteenth-century Panamá. The nesting sites were within the Basilica Cathedral, which was consecrated in 1796 (Calvo 1999). An extensive restoration was completed in 2018, in preparation for the consecration of a new altar by Pope Francis. While restoring the reredos ("altarpiece"; Fig. 3A), workers discovered large numbers of bee cell clusters in numerous locations; many cells were covered with gold leaf and other golden material applied during an earlier restoration more than a century ago. These golden bee cells reliably date the time of nest cell construction prior to the years 1871–1876, when restoration workers repaired damage from an 1870 fire (Calvo 1999; Lobo and Tribaldos unpublished MS).

In the face of changing environments, and recognizing that bees play key roles in most ecosystems as primary pollinators, we looked to the past to analyze historical data on pollen use by orchid bees, *E. surinamensis*, living in a human-modified environment. Our study provides a baseline for comparative studies with contemporary populations in natural environments. The unusual nesting site—within the first Cathedral on *tierre firme* in the Americas—helps call attention to Francis' urgent plea in *Laudato Si* (2015, §42), for the need to increase investment in research to better understand the functions of natural ecosystems and their component species, and how these are being shaped by significant environmental modifications resulting from collective human behavior.

Material and methods

Bee nests or cell clusters were discovered during restoration work of the Basilica Cathedral in Casco Viejo, Panama (8°57'N, 79°32'W) in 2018 by S. Lobo and other restorers working for Dalmática Conservação e Restauro. W. Tribaldos then brought them to the attention of scientists at the Smithsonian Tropical Research Institute (**STRI**). These cells were carefully removed using chisels, spatulas and related restoration tools, and were sent to STRI. Twenty-seven brood cells were selected for analyses from the recovered cells.

Following chemical washes to isolate pollen grains at STRI's Center for Tropical Paleoecology & Archaeology (**CTPA**), we carried out standard palynological procedures (methods in Roubik and Moreno 1991). Subsequently, permanent microscope preparations were made using glycerin jelly as a mounting medium and paraffin as a sealant. Transects of all preparations were made at 40× magnification using a Nikon Eclipse-Ni binocular scope in order to identify all pollen types. Both biological bright light and differential interference contrast (DIC) microphotographs were obtained at 100× magnification using a Nikon DS-Ri1. The magnification of the camera lens of the Nikon scope was 0.7×, yielding a final magnification of 700 times unless indicated otherwise (Plates 1–5). Each photograph displays its own scale (in microns), but photographs in each plate are not to the same scale. Photographs are alphabetically sorted in families, genera and species. Palynology and pollen grain recognition was based on Moore et al. (1991) and Punt et al. (2007). Botanical names were established at the species level if possible, by comparisons with local and regional pollen atlases, STRI collections and botanical inventories (e.g., Croat 1978; Roubik and Moreno 1991; Moreno et al. 2014). The taxonomic status of botanical names was updated using the Tropicos on-line database (Missouri Botanical Garden 2019), following the new angiosperm phylogeny classification (APG IV 2016), and a new subfamily classification of the Leguminosae (LPWG 2017; Banks and Lewis 2018).

External fragments of nests, mainly small pieces of bark, were subjected to chemical analyses to obtain the siliceous fraction containing phytoliths for possible identification [methods from Piperno (2006) and using a reference collection at STRI's CTPA].

Voucher specimens of the bee cells and pupae are in the STRI Dry Reference Collection, which also houses the remaining unprocessed nest cells.

Results

The nesting site was surrounded by a mosaic of disturbed forest and open land (Figs 1, 2). Brood cells (N = ~550) of *Eufriesea surinamensis* were distributed among approximately 120 clusters or aggregations on the six capitals behind and above the altar (Fig. 3A), built in the crevices and scrolls of the capitals of the columns (Fig. 3B, C), at heights comparable to upper understory and lower canopy in adjacent natural forests. Nests were found in clusters (Fig. 4A, D) or in isolation. (Fig. 4E). Wood fragments were used extensively in nest construction (Fig. 4C, E); three different phytoliths were recovered from these fragments, but they are common to many species of woody plants, including *Vismia* (Hypericaceae) and could not be identified further (D. Piperno *in litt.* 2019). Nest cells must have been constructed prior to restoration work to repair fire damage (see Introduction). The nineteenth-century restorers left the bee

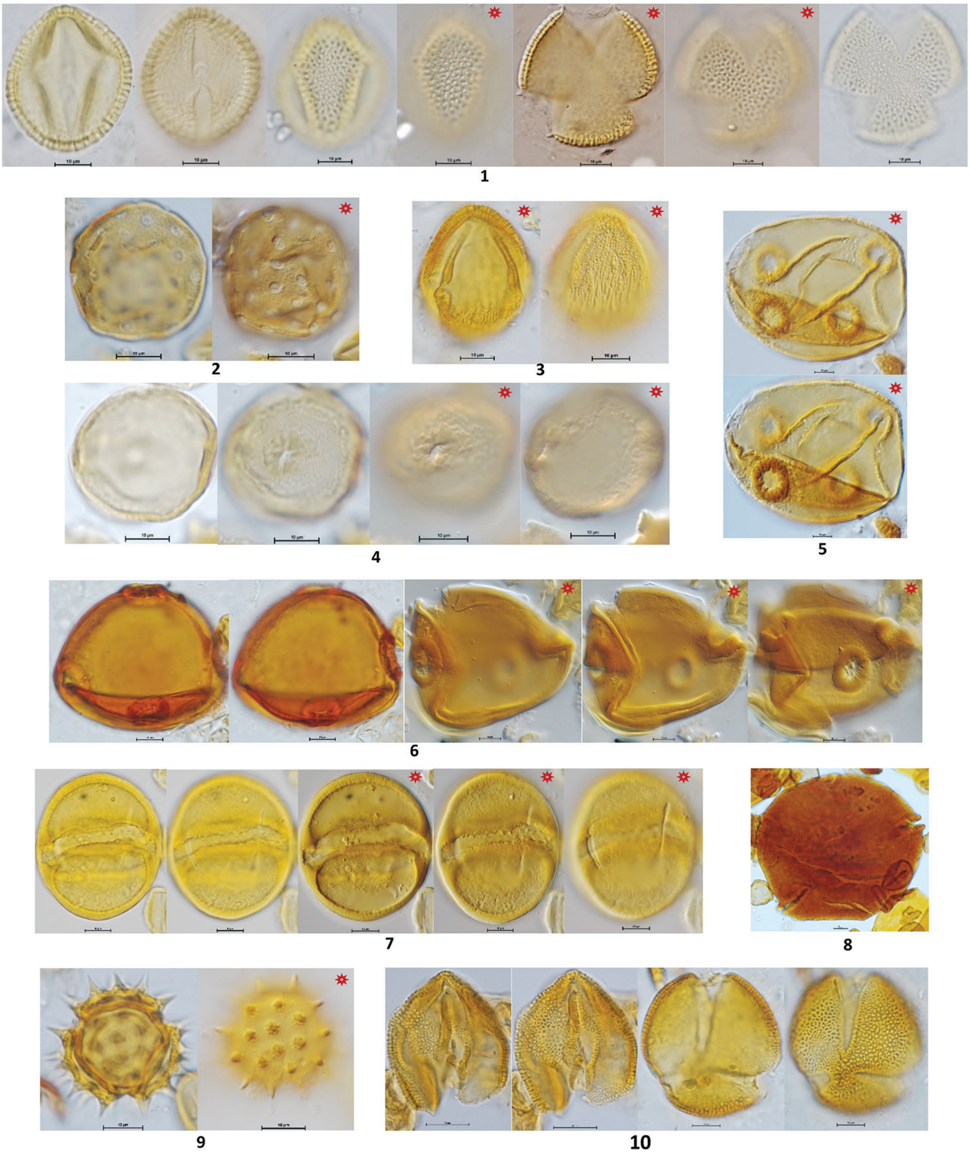

Plate 1. Eudicots. Acanthaceae: *Avicennia germinans* (**1**) Amaranthaceae: aff. *Chenopodium* sp. (**2**) Anacardiaceae: *Spondias* sp. aff. *S. mombin* (**3**) Apocynaceae: *Malouetia guatemalensis* (**4**) *Mandevilla* sp. aff. *M. villosa* (**5**) *Prestonia* sp. (**6**) *Stemmadenia grandiflora* (**7**) *Thevetia ahouai* (**8**) Asteraceae: undetermined (**9**) Bignoniaceae: *Arrabidaea* sp. (**10**) (×100) (Red circle = DIC photo).

cells in place and covered them with gold leaf and golden paint (Figs 3C, 4). Pupal remains were found in seven cells (Fig. 5A), which allowed us to identify the bee species (Fig. 5B). Taxonomic identification of pollen grains showed that bees used 48 species of plants, representing 43 genera and 23 families (Table 1 and Plates 1–5).

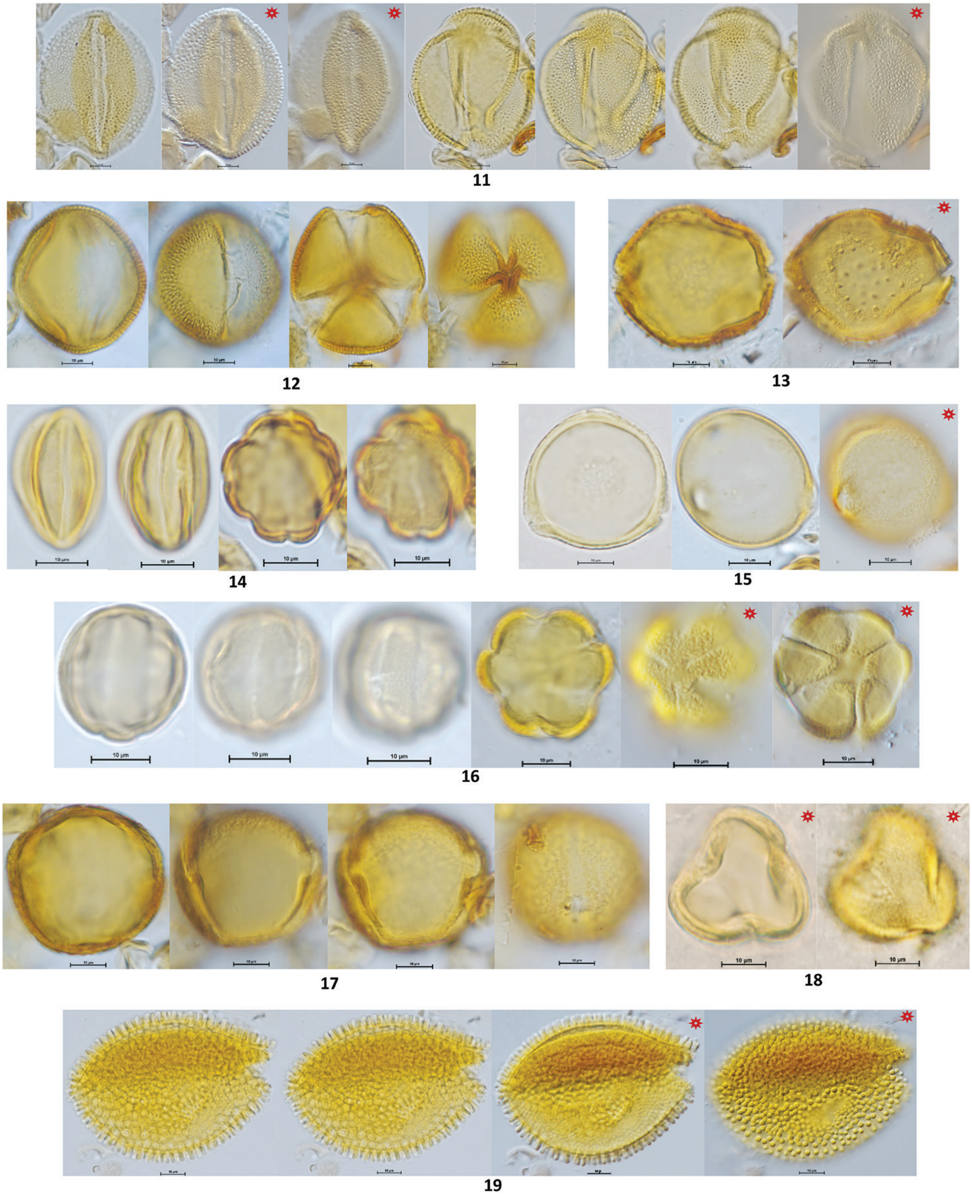

Plate 2. Bignonaceae: aff. *Ceratophytum tetragonolobum* (**11**) *Tabebuia* sp. (**12**) Boraginaceae: *Cordia* sp. aff. *C. spinescens* (**13**) *Heliotropium procumbens* (**14**) Cannabaceae: *Celtis* sp. (**15**) Combretaceae: *Conocarpus erectus* (**16**) *Laguncularia racemosa* (**17**) Euphorbiaceae: *Alchornea* sp. aff. *A. latifolia* (**18**) *Croton* sp. (**19**) (×100) (Red circle = DIC photo).

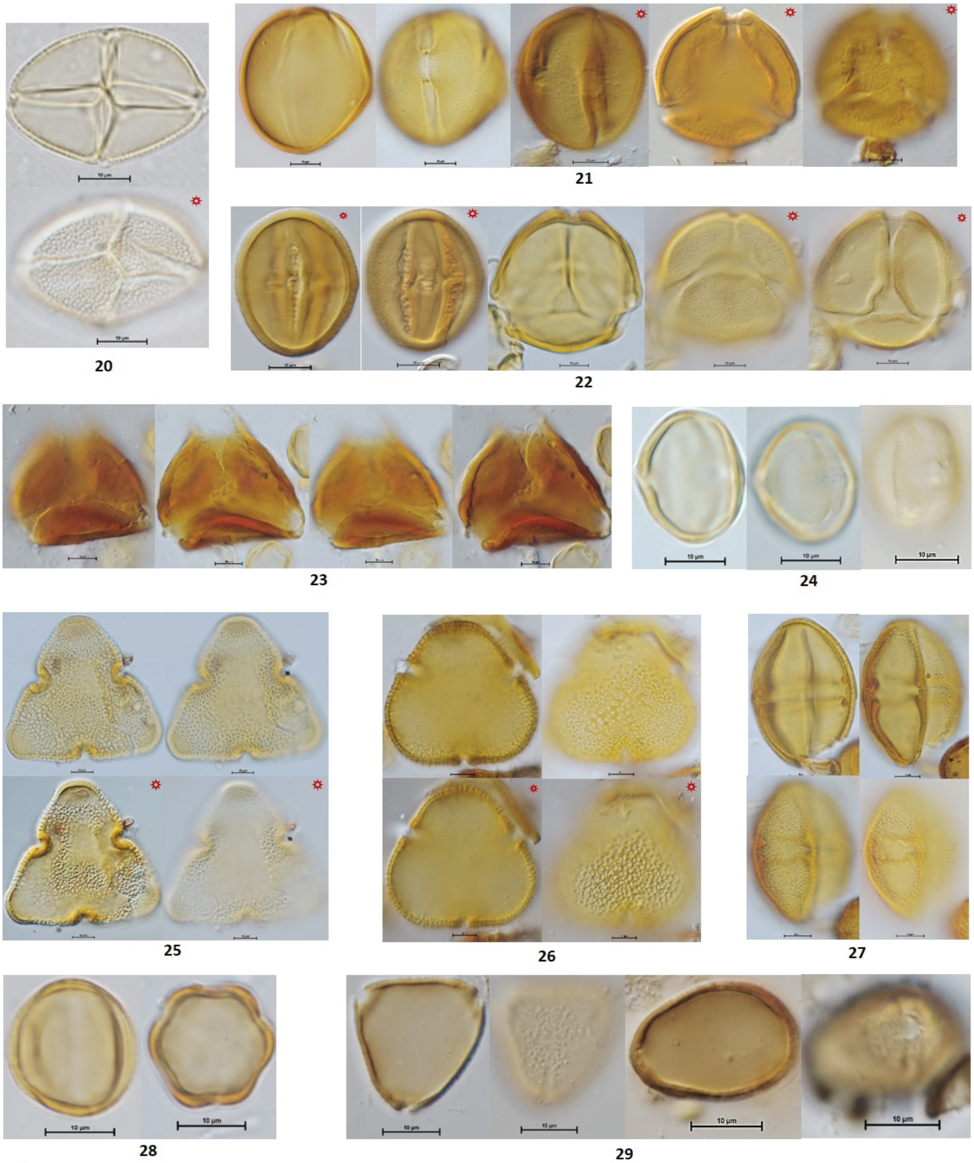

Plate 3. Fabaceae-Caesalpinioideae: *Mimosa* sp. (**20**) Fabaceae-Cercidoideae: *Bauhinia guianensis* (**21**) *Bauhinia reflexa* (**22**) Fabaceae-Papilionoideae: *Dioclea reflexa* (**23**) *Machaerium* sp. (**24**) Malvaceae-Bombacoideae: *Bombacopsis quinata* (**25**) *Pseudobombax septenatum* (**26**) Malvaceae-Grewioideae: aff. *Heliocarpus* sp. (**27**) Melastomataceae: *Miconia* sp. (**28**) Myrtaceae: *Eugenia* sp. (**29**) (×100) (Red circle = DIC photo).

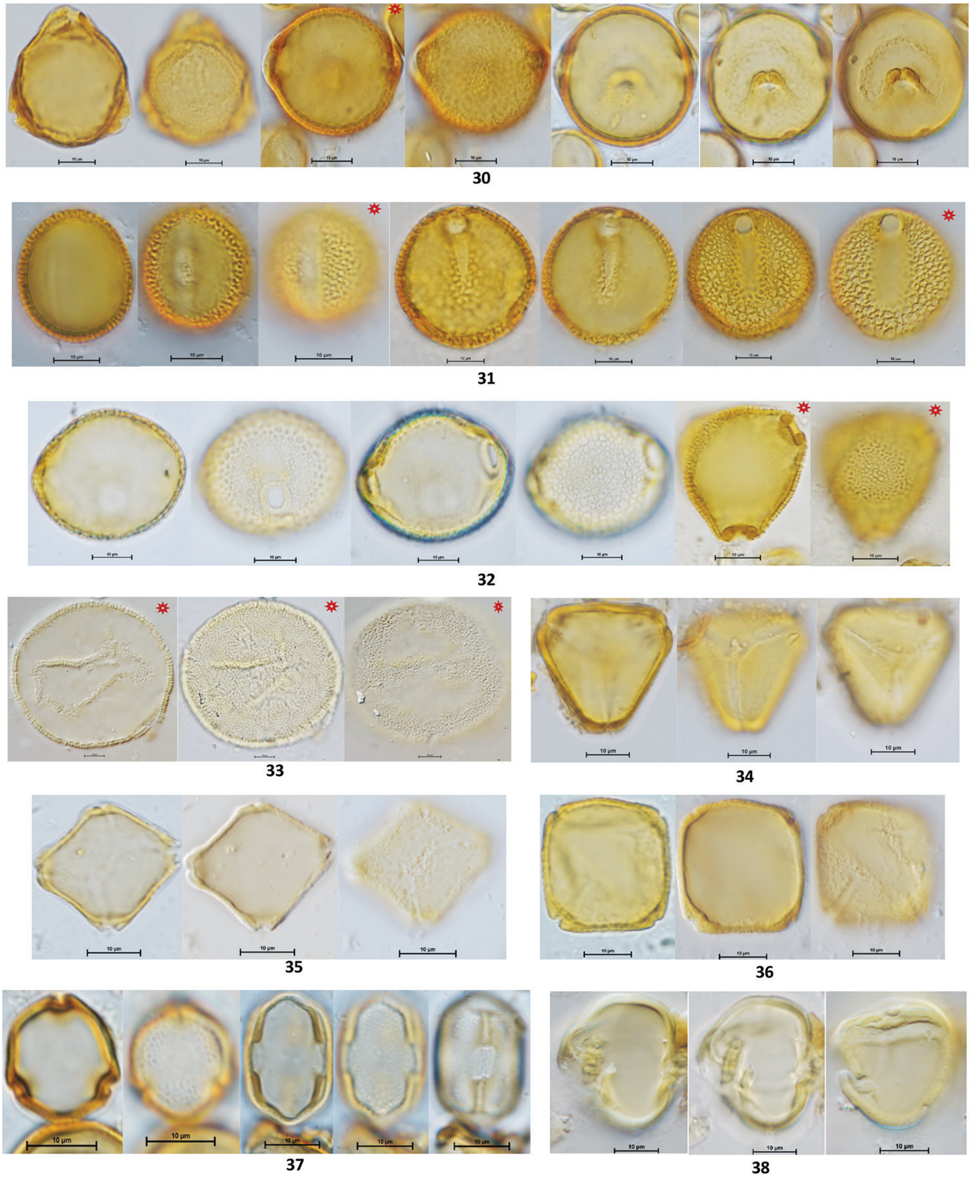

Plate 4. Rubiaceae: aff. *Faramea* sp. (**30**) *Genipa americana* (**31**) *Macrocnemum glabrescens* (**32**) *Psychotria* sp. (**33**) Sapindaceae: *Cupania* sp. (**34**) *Serjania* sp.1 (**35**) *Serjania* sp.2 (**36**) Sapotaceae: *Pouteria* sp. (**37**) Solanaceae: *Solanum* sp. (**38**) (×100) (Red circle = DIC photo).

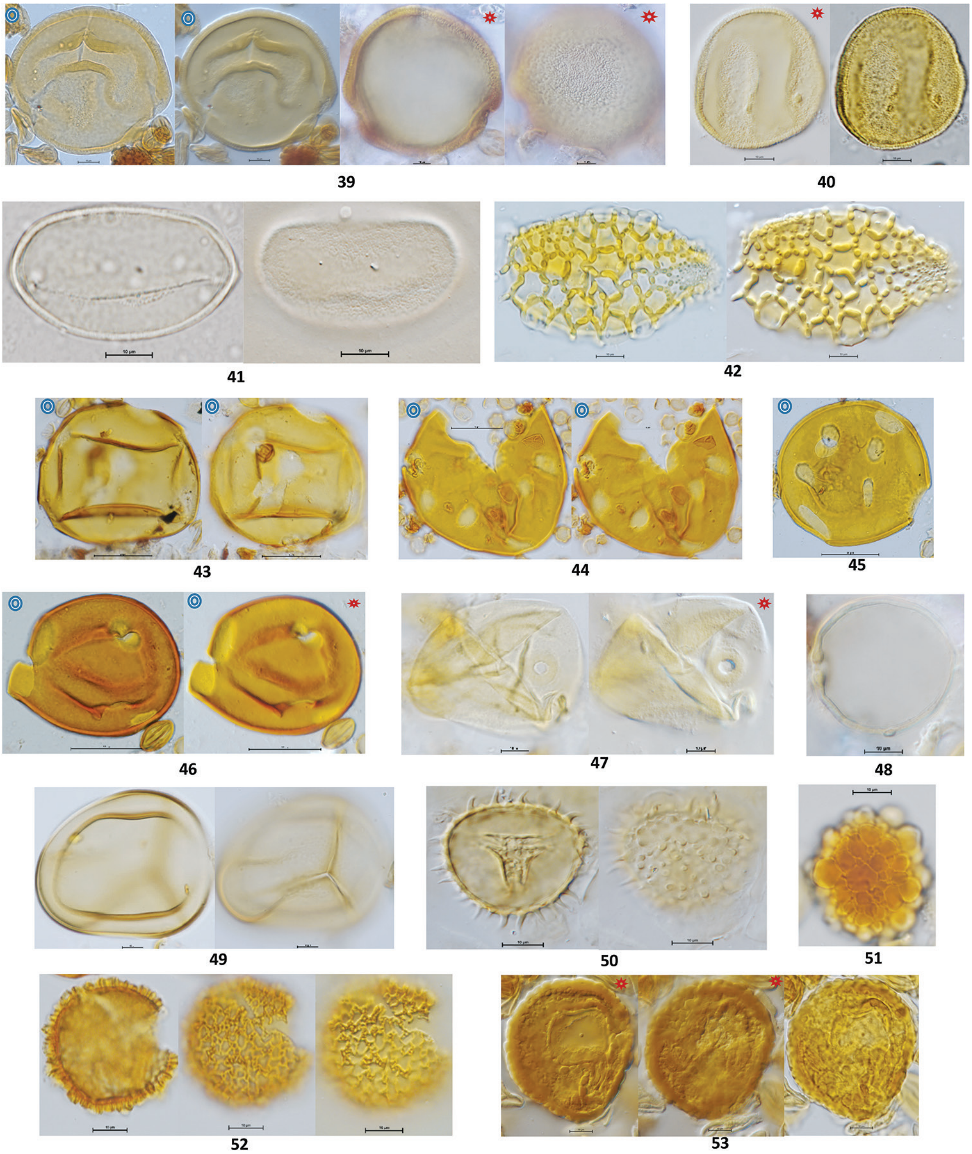

Plate 5. Tetrameristaceae: *Pelliciera rhizophorae* (**39**) Monocots. Arecaceae: Undetermined sp.1 (**40**) Undetermined sp.2 (**41**) Bromeliaceae: *Vriesea* sp. (**42**) Costaceae: *Costus* sp.1 (**43**) *Costus* sp.2 (**44**) *Costus* sp.3 (**45**) *Costus* sp.4 (**46**) Poaceae: aff. *Zea mays* (**47**) Undetermined sp. (**48**) FERN SPORES. Cyatheaceae: *Cyathea* sp. (**49**) Selaginellaceae: *Selaginella* sp. (**50**) UNDETERMINED. Fungal sp.1 (**51**) Fungal sp.2 (**52**) Fungal sp.3 (**53**) (×100) (Blue circle = 60X) (Red circle = DIC photo)

Table 1. List of plants identified from pollen grains recovered of nest cells of *Eufriesea surinamensis* L. from Casco Viejo, Panamá, and sujective estimates of their relative occurrence.

Family	Genus/Species	Relative occurrence
Eudicots		
Acanthaceae	*Avicennia germinans*	Common
Amaranthaceae	aff. *Chenopodium* sp.	Rare
Anacardiaceae	*Spondias* sp. aff. *S. mombin*	Rare
Apocynaceae	*Malouetia guatemalensis*	Scarce
Apocynaceae	*Mandevilla* sp. aff. *M. villosa*	Scarce
Apocynaceae	*Prestonia* sp.	Common
Apocynaceae	*Stemmadenia grandiflora*	Rare
Apocynaceae	*Thevetia ahouai*	Rare
Asteraceae	Undetermined	Rare
Bignoniaceae	*Arrabidaea* sp.	Common
Bignoniaceae	aff. *Ceratophytum tetragonolobum*	Common
Bignoniaceae	*Tabebuia* sp.	Common
Boraginaceae	*Cordia* sp. aff. *C. spinescens*	Scarce
Boraginaceae	*Heliotropium procumbens*	Common
Cannabaceae	*Celtis* sp.	Abundant
Combretaceae	*Conocarpus erectus*	Abundant
Combretaceae	*Laguncularia racemosa*	Scarce
Euphorbiaceae	*Alchornea* sp. aff. *A. latifolia*	Rare
Euphorbiaceae	*Croton sp.*	Rare
Fabaceae-Caesalpinioideae	*Mimosa* sp.	Rare
Fabaceae-Cercidoideae	*Bauhinia guianensis*	Common
Fabaceae-Cercidoideae	*Bauhinia reflexa*	Common
Fabaceae-Papilionoideae	*Dioclea reflexa*	Rare
Fabaceae-Papilionoideae	*Machaerium* sp.	Rare
Malvaceae-Bombacoideae	*Bombacopsis quinata*	Rare
Malvaceae-Bombacoideae	*Pseudobombax septenatum*	Rare
Malvaceae-Grewioideae	aff. *Heliocarpus* sp.	Rare
Melastomataceae	*Miconia* sp.	Frequent
Myrtaceae	*Eugenia* sp.	Frequent
Rubiaceae	*Faramea* sp.	Common
Rubiaceae	*Genipa americana*	Scarce
Rubiaceae	*Macrocnemum glabrescens*	Scarce
Rubiaceae	*Psychotria* sp.	Rare
Sapindaceae	*Cupania* sp.	Scarce
Sapindaceae	*Serjania* sp.1	Common
Sapindaceae	*Serjania* sp. 2	Common
Sapotaceae	*Pouteria* sp.	Rare
Solanaceae	*Solanum* sp.	Rare
Tetrameristaceae	*Pelliciera rhizophorae*	Scarce
Monocots		
Arecaceae	Undetermined sp. 1	Rare
Arecaceae	Undetermined sp. 2	Rare
Bromeliaceae	aff. *Vriesea* sp.	Rare
Costaceae	*Costus* sp. 1	Common
Costaceae	*Costus* sp. 2	Common
Costaceae	*Costus* sp. 3	Common
Costaceae	*Costus* sp. 4	Common
Poaceae	aff. *Zea mays*	Rare
Poaceae	Undetermined	Rare
Others		
Cyatheaceae	*Polypodium* sp.	Contaminant
Selaginellaceae	*Selaginella* sp.	Contaminant
Fungal?	Undetermined 1	
Fungal?	Undetermined 2	
Fungal?	Undetermined 3	

Discussion

Eufriesea surinamensis females nesting in a Cathedral in an urban area in nineteenth-century Panamá were catholic in their flower preferences, visiting a diverse array of flowering trees, shrubs and grasses to collect food and nest-building materials, consistent with the known behavior of other orchid bees. They visited more than twice the number of plant species used by related *Euglossa* in a present-day urban area of Brazil (Pinto et al. 2019), comparable to the 45 plant species used by two species of *Euglossa* in Yucatán, México (Villanueva-Gutierrez et al. 2013). The species composition of the collected pollen reflects a mixed-age Neotropical secondary forest, with a mosaic landscape of forest and open areas, as seen in Muybridge's landscape photographs (Figs 1, 2). The bees visited understory species such as *Costus* (Costaceae) that do well in full sunlight, such as gaps or along forested roads or openings, as well as canopy trees like *Pseudobombax* (Malvaceae-Bombacoideae) ("barrigón") that typically are found in more mature forests (Croat 1978).

Orchid bees are long-tongued bees (Michener 2007), a trait that facilitates the collection of nectar from flowers that have deep and fused corollas (Endress 1994), providing them with a rich nectar source (e.g., Apocynaceae of Table 1). Additionally, other flowers are structured such that the bee must grab the flower while buzzing to vibrate free the pollen (Buchmann 2015), which provide a rich protein source. Other important nectar flowers were likely *Avicennia germinans* (Acanthaceae), *Heliotropium procumbens*

Figure 1. Environs of the *Eufriesea surinamensis* nesting site in Casco Viejo, Panamá in 1875, as seen from the summit of Cerro Ancón. A white tower of the Cathedral where bees were nesting is visible in the distant background in the center of the peninsula. Photo by Eadweard Muybridge, courtesy of the Smithsonian American Art Museum; gift of Mitchell and Nancy Steir.

Figure 2. Panama City's waterfront and surrounding area as seen from the shoreline of Casco Viejo, Panamá in 1875. The large building in the left foreground is *La Casa de la Marina*, near *El Palacio de las Garzas* (Presidential Palace). The bees were nesting approximately 160 m in-land. The peak of Cerro Ancón is approximately 1.6 km distant. Photo by Eadweard Muybridge, courtesy of the Smithsonian American Art Museum; gift of Mitchell and Nancy Steir.

Figure 3. Locations of nest cell aggregations of *Eufriesea surinamensis* within the Cathedral in Casco Viejo, Panamá **A** restored reredos showing the capitals above the columns where the historical bee cells were found (black arrows) **B** a scroll removed during the contemporary restoration, showing bee cells within its crevices and golden material applied during the nineteenth-century restoration **C** close-up of scrolls on a capital showing painted bee cells from the prior restoration.

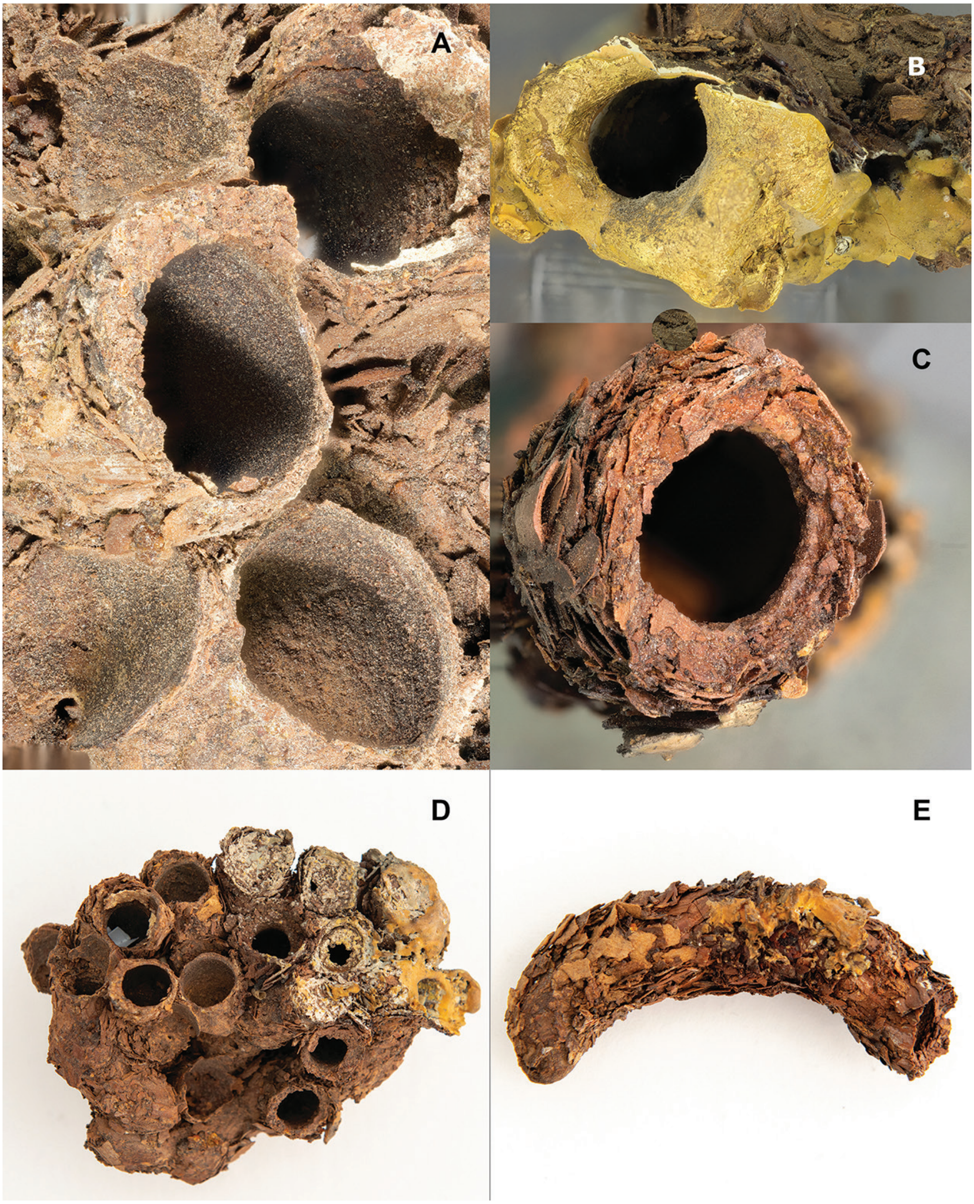

Figure 4. *Eufriesea surinamensis* cells painted during the nineteenth-century restoration **A** close-up of a cell cluster recovered from a capital **B** isolated cell entrance that was painted over **C** isolated cell entrance showing the abundance of bark fragments as a main resource for nest construction **D** cell cluster covered in golden paint **E** exterior view of an isolated cell, covered with golden leaf; the cell entrance faces right.

(Boraginaceae), *Bauhinia* spp. (Fabaceae- Cercidoideae), *Eugenia* sp. (Myrtaceae), *Faramea* sp. (Rubiaceae) and *Serjania* spp. (Sapindaceae). Plants that were likely important protein sources include *Costus* (Costaceae), *Celtis* sp. (Cannabaceae), *Conocarpus erectus* (Combretaceae), *Miconia* sp. (Melastomataceae), and species of Bignoniaceae (Table 1).

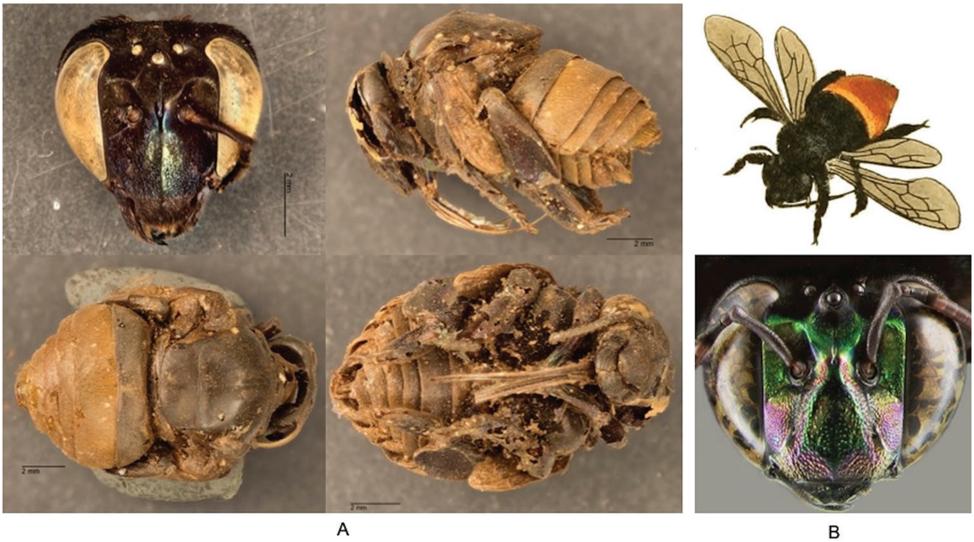

Figure 5. *Eufriesea surinamensis* reared from cells **A** head, lateral, dorsal and ventral views of recovered bees **B** habitus drawing and head of exemplar (STRI-Portal; https://www.stricollections.org/portal/taxa/index.php?taxon=48960).

Together, these species comprise about half of the total taxa reported. Some of the species, though rare in this sample, likely contributed to the diet of *E. surinamensis*, while other species may be present from visits to plants collecting resin or nesting materials, or be contaminants (e.g., the two fern spores and fungal species reported). The pollen list also contains elements of a coastal forest with mangroves, including one species (*Pelliciera rhizophorae*, Tetrameristaceae) that today is extremely rare in central Panamá (Castillo-Cárdenas et al. 2016). The precise foraging range of these bees is unknown, but *E. surinamensis* females in Costa Rica's Osa Peninsula were capable of navigating home from distances as far as 23 km (Janzen 1971). Thus, the foraging range of the Cathedral bees likely extended far beyond Cerro Ancón (Fig. 2), approximately to present-day towns of Tocumen, La Chorrera, and Gamboa, depending on outbound flight direction.

Today in central Panamá, including the Panama City metropolitan region, these bees are confined to remote forested areas of the central isthmus (Roubik and Ackerman 1987), and there have been no detailed studies of female nesting biology. Accordingly, we now need to collect comparative data on the biology and distribution of contemporary populations of *E. surinamensis* in central Panama and elsewhere in the Neotropics, to better understand how these bees are responding to changing environments.

Conclusions

Palynological studies of the contents of orchid bee brood cells indicate that late-nineteenth century Panama City was surrounded by a patchwork of tropical forests sufficient to sustain nesting populations of what today is a forest-dwelling species of bee.

Acknowledgements

We are grateful to the entire group of collaborators from Dalmática Conservação e Restauro-represented here by Sofia Lobo-for their interest in understanding which biological agents cause damage to our cultural heritage. The staff of the Comité Arquidiocesano Amigos Iglesias Casco Antiguo and the Consorcio la Antigua helped in providing access to the samples and to the site. Current and former STRI colleagues contributed as follows: Jorge Ceballos helped with microscopy; Marleny Rivera and Jorge Aleman took photos of the bees and the nest cells, respectively; Carlos Jaramillo, Rick Condit, Stanley Heckadon, Joe Wright, and Jeff Hall provided helpful botanical or historical information; Dolores Piperno and Irene Holst analyzed phytoliths and provided related information; Lina Gonzalez helped with graphics; and Annette Aiello first recognized the recovered materials as hymenopteran nest cells, and brought them to the attention of WTW. We are especially grateful to our Smithsonian colleagues, John Jacob and Richard Sorensen of the Smithsonian American Art Museum, who promptly provided high-resolution copies of the original Muybridge photographs in their collection, and related information. Margarita López-Uribe, Santiago Ramírez, and Jack Neff provided many helpful and thoughtful comments, and corrections, which improved the manuscript.

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Plagiolepis alluaudi Emery, 1894, a globally spreading exotic ant (Hymenoptera, Formicidae) newly recorded from Tenerife (Canary Islands, Spain)

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Abstract

Exotic ants have been a prevalent ecological problem, particularly in tropical and subtropical islands. Here *Plagiolepis alluaudi* Emery, 1894 is recorded from the island of Tenerife (Canary Islands, Spain) for the first time, where it was commonly encountered in the town Puerto de la Cruz. This is the first Canary Islands record of this species that is presumably native to Madagascar and surrounding islands. Whether or not *P. alluaudi* will be able to spread into natural Canary ecosystems that have a high share of endemic species is unknown.

Keywords

biogeography, conservation, endemic species, exotic species, invasive ants, Macaronesia, Oceanic island

Introduction

Many species of ants have been spread by human activities outside their native distribution ranges, where they are ecologically successful particularly in subtropical and tropical islands and disturbed habitats (McGlynn 1999). A subset of these exotic ant species have become invasive and threaten native species when they reach high densities in invaded natural ecosystems (Holway et al. 2002). Like all other non-native species (Banks et al. 2015), exotic ants are spread by humans, for example when colonies or mated queens are unintentionally moved over long distances within cargo, soil or plant material (Holway et al. 2002). Realized distributions of exotic ant species are not static and new locality records of exotic ants are constantly being reported (e.g. Gotzek et al. 2012; Guénard et al. 2018; Schifani et al. 2018; Schifani 2019).

Plagiolepis alluaudi Emery, 1894 is a tiny yellowish ant species in the subfamily Formicinae that is invasive in tropical and subtropical islands (Wilson and Taylor 1967; Wetterer 2014) and also increasingly occurring in continental localities, for example in Florida (Chouvenc et al. 2018). The type locality of *P. alluaudi* is the Seychelles (Emery 1894) and the species is originally likely native to Madagascar and neighboring islands (Wetterer 2014). In the past, *P. alluaudi* has been widely spread by human commerce (especially within living plant material, Smith 1957) and can be a nuisance in greenhouses (Smith 1957; Wetterer 2014; Blatrix et al. 2018). The distribution of the species was comprehensively mapped by Wetterer (2014), but since then it became known from additional locations (e.g. Chouvenc et al. 2018) and it is likely that *P. alluaudi* will spread further (Smith 1957).

Tenerife is the largest island of the Canary Island Archipelago located in the Atlantic Ocean west of Morocco. As oceanic islands of volcanic descent, the Canary Islands were never connected to continental land and have evolved diverse native biota with many endemic species (Juan et al. 2000). At least 50 native ant species (including subspecies) are known from the Canary Islands (Wheeler 1927; Barquín 1981; Högmo 2003; Espadaler 2007; Guénard et al. 2017), of which the majority also occurs on Tenerife. Like on other oceanic islands with a favorable climate, non-native ants are now common in the Canary Islands and at least 15 exotic ant species have been recorded from Tenerife (Espadaler and Bernal 2003; Espadaler and Fernández 2014; Schifani et al. 2018), among them the globally invasive *Linepithema humile* (Mayr, 1868) and *Paratrechina longicornis* (Latreille, 1802). Exotic ants continue to be introduced to Tenerife, as the recent finding of *Lepisiota frauenfeldi kantarensis* (Forel, 1911) exemplifies (Schifani et al. 2018), and are currently estimated to account for up to a third of the ant fauna of the entire archipelago.

Here the first record of the invasive ant species *P. alluaudi* for Tenerife is reported, where the species was commonly encountered in gardens in the town Puerto de la Cruz. This is the first record of *P. alluaudi* for the Canary Islands and for any island of Macaronesia.

Methods

In August 2018 ants were collect from the ground by hand with featherweight forceps without following a standardized collection protocol. Small yellowish ants were repeatedly noticed in gardens in Puerto de la Cruz and subsequently collected in two locations.

Specimens were stored in 95% ethanol, mounted on points and identified with http://www.antkey.org (an online tool for the identification of introduced ants, Sarnat and Suarez 2012) and by comparison with the original description (Emery 1894), a redescription (Smith 1957), and images of type specimens that are illustrated on the https://www.AntWeb.org database (AntWeb 2019).

To illustrate a worker and a dealate queen, raw image stacks were recorded with a Leica M165 C microscope that was equipped with a Leica MC190 HD camera (Leica Microsystems, Wetzlar, Germany) and the final montage images were assembled with Helicon Focus (version 7) software (Helicon Soft Ltd., Kharkiv, Ukraine). Voucher specimens will be deposited in the Museum für Naturkunde der Humboldt-Universität (Berlin, Germany) and the personal collection of the author.

Results

The specimens from Puerto de la Cruz (Tenerife, Canary Islands) were identified as *Plagiolepis alluaudi* (Fig. 1) and agree in all aspects with the original description, the redescription, the type series (CASENT0101699, CASENT0102083, CASENT0102084) and further syntypes (CASENT0912413, CASENT0912414). Even though the species superficially resembles yellow *Brachymyrmex* species, *P. alluaudi* can be easily distinguished by having eleven antennal segments (nine in *Brachymyrmex*). *Plagiolepis alluaudi* was found in a garden in the town center (28°24'54"N, 16°33'19"W, 20 m asl, 26-VIII-2018, label 'MS1884', leg. Michael Staab) and in a second more suburban garden approximately 1.1 km away from the first location (28°24'49"N, 16°32'40"W, 60 m asl, 29-VIII-2018, label 'MS1886', leg. Michael Staab). Ants with the general appearance of *P. alluaudi* were seen in several further locations in Puerto de la Cruz but not collected. For sample MS1886, several dealate queens (Fig. 2) were moving on a linear trail together with workers that were carrying brood, presumably during a nest relocation. Co-occurring species were the exotic *Cardiocondyla obscurior* (Wheeler, 1929) (MS1886), *Linepithema humile* (MS1884), *Nylanderia jaegerskioeldi* (Mayr, 1904), *Paratrechina longicornis* (MS1884, MS1886), *Tapinoma melanocephalum* (Fabricius, 1793) (MS1886), and *Tetramorium caldarium* (Roger, 1857) (MS1884, MS1886), the possibly native *Lasius grandis* Forel, 1909 (MS1886) and *Plagiolepis pygmaea* (Latreille, 1798) (MS1886), and the native *Plagiolepis schmitzii canariensis* Santschi, 1920 (MS1886) (see locality data in Suppl. material 1: Table S1).

Discussion

The globally spreading *P. alluaudi* is here recorded from Tenerife for the first time. The species was in August 2018 found in several locations in Puerto de la Cruz, a tourist town in the north of the island, where the species appears to be firmly established. Albeit no systematic search was performed, *P. alluaudi* was not collected outside Puerto de la Cruz.

Figure 1. *Plagiolepis alluaudi* worker (MS1866) from Puerto de la Cruz, Tenerife **A** body in profile **B** body in dorsal view **C** head in full-face view.

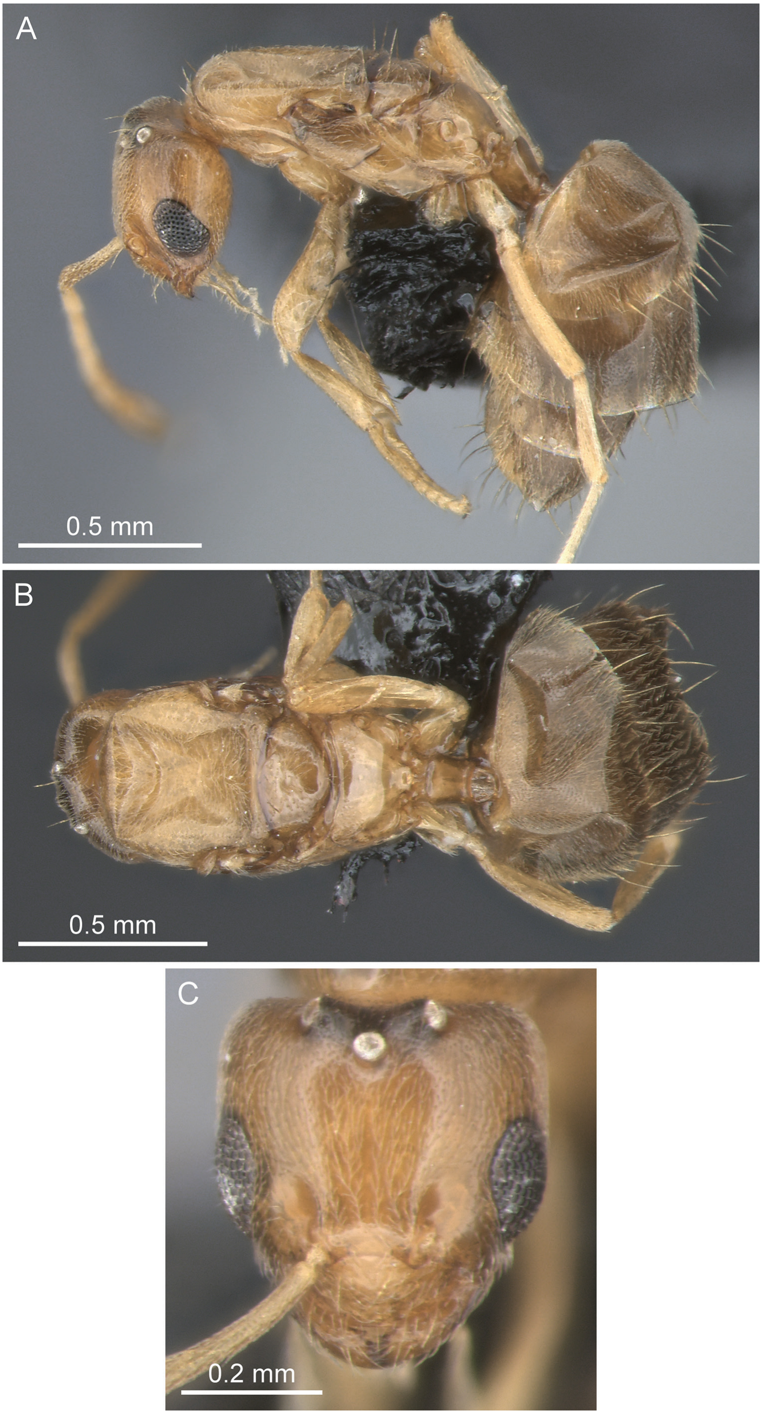

Figure 2. *Plagiolepis alluaudi* dealate queen (MS1866) from Puerto de la Cruz, Tenerife **A** body in profile **B** body in dorsal view **C** head in full-face view. The specimen was collected from the same colony as the worker in Fig. 1.

As the species was not detected in previous surveys of ants on Teneriffe (e.g. Stitz 1917; Wheeler 1927; Barquín 1981; Espadaler and Bernal 2003; Schifani et al. 2018), this may suggest a rather recent introduction event, potentially with plant material (Smith 1957; Wetterer 2014; Blatrix et al. 2018). Because *P. alluaudi* is widespread in tropical and subtropical islands and commonly occurs in greenhouses around the world, it is at present not possible to determine the source of the population in Tenerife. The Canary Islands are over 8,000 km from the native distribution range and over 2000 km from the closest exotic records from greenhouses in France (Wetterer 2014; Guénard et al. 2017).

Being an oceanic island in a subtropical climate, diverse endemic biota have evolved on Tenerife for example in plants and beetles (Emerson et al. 1999; Juan et al. 2000). Regarding ants, endemic species likely account for over 30% of the Canary Islands myrmecofauna (Schifani et al. 2018). Competition between exotic and native species is likely and can have far-reaching consequences for native biota. For example, Valido et al. (2019) recently showed that in Tenerife managed exotic honeybees reduce the diversity of wild pollinators and change pollination networks. Concerning *P. alluaudi*, it is currently unknown if and how the species will integrate into undisturbed native ecosystems. Most exotic ants are not imposing threats to native ecosystems and only a relatively small subset of species can establish outside disturbed areas (McGlynn 1999; Holway et al. 2002). However, *P. alluaudi* has in several places spread into relatively undisturbed forests, for example in Hawaii, where it is now the most common ant species in some areas (Krushelnycky 2015).

Being a generalist forager that frequently tends Hemiptera for honeydew (Smith 1957; Krushelnycky 2015), it can live in mutualistic association with many honeydew producers (compare Staab et al. 2015), many of which are also exotic and can facilitate the ecological success of exotic ants (Lach 2003). Thus, in case *P. alluaudi* will become established in native habitats, it may compete with native ants for resources. When an ant species spreads on an island, the species often replaces other native and exotic ants (Wilson and Taylor 1967). Even though *P. alluaudi* has a very small body size (~ 1.2 mm total length in workers), it can vigorously defend resource and is resistant to larger competitively superior ants (Burwell et al. 2012). This also explains why the species was in Puerto de la Cruz thriving in direct proximity to the Argentine ant *L. humile*, an invasive species that is notoriously known for displacing other ants (Holway 1999) and is a pest in Tenerife since the early 20th century (Stitz 1917; Wheeler 1927; Espadaler and Bernal 2003).

The present record of *P. alluaudi* is the first record for the Canary Islands. As the species combines a small body size, opportunistic foraging and polygyny, which are all traits facilitating spread of exotic ants (McGlynn 1999; Wetterer 2014), it is not unlikely that *P. alluaudi* will soon be found on other islands of the Canary Archipelago (see also Espadaler and Fernández 2014; Schifani et al. 2018).

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Supplementary material 1

Table S1. Locality data for all ant species reported in this study
Authors: Michael Staab
Data type: Occurrence data

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A new species of *Bicurta* Sheng et al. from China (Hymenoptera, Ichneumonidae, Collyriinae), a parasitoid of *Stenocephus fraxini* Wei (Hymenoptera, Cephidae)

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Abstract

A new species of the genus *Bicurta* Sheng, Broad & Sun, 2012, is described and illustrated, *B. hejunhuai* sp. nov., from North and Northeast China. The new species was reared from the stem-sawfly *Stenocephus fraxini* Wei (Hymenoptera, Cephidae), which is the first host record for the genus *Bicurta*.

Keywords

Parasitoid, new species, biological control, China

Introduction

Collyriinae is small subfamily of Ichneumonidae, including only three genera (*Aubertiella* Kuslitzky & Kasparyan, *Bicurta* Sheng et al., and *Collyria* Schiødte) with 11 described species, all distributed in the Palaearctic region (Gürbüz and Kolarov 2006; Wahl et al. 2007; Kuslitzky and Kasparyan 2011; Sheng et al. 2012; Yu et al. 2016; Broad et al. 2018), although with *Bicurta sinica* Sheng et al. found in both the Palaearctic and Oriental regions (Sheng et al. 2012, 2013; Shang et al. 2016). The systematics of this subfamily, based on morphological characters, were discussed by Sheng et al. (2012).

The genus *Bicurta* is monotypic, with only the type species *B. sinica* described from Jiangxi Province of China (Sheng et al. 2012). Shang et al. (2016) reported a male specimen of *B. sinica* from Liaoning Province in Northeast China. The biology of the genus *Bicurta* was not known until this study.

During a survey of pests of *Fraxinus chinensis* Roxb. and *F. mandshurica* Rupr. (Lamiales, Oleaceae) in Northeast China (Yan et al. 2018, 2019), the second author reared about 40 specimens of *Bicurta* from larvae of the stem-sawfly *Stenocephus fraxini* Wei, 2015 (Hymenoptera, Cephidae) which is a very common pest of urban greening trees, *Fraxinus* spp. (Lamiales, Oleaceae), in Northeast China (Fig. 26) (Yan et al. 2018), mainly from the downtown of Shenyang City, Liaoning Province.

The aim of this study is to describe a new species of *Bicurta* parasitizing *S. fraxini* Wei.

Materials and methods

Parasitoids were reared in the laboratory from larvae of *Stenocephus fraxini* collected in branches of *Fraxinus* spp. from North and Northeast China (Inner Mongolia, Liaoning and Heilongjiang), mainly from the downtown of Shenyang City. Photographs were taken using a KEYENCE VHX-5000 Digital Microscope imaging system and processed with Photoshop CS software.

Morphological terms follow Broad et al. (2018). Abbreviations used in the text are as follows: POL = the shortest distance between posterior ocelli; OD = diameter of a posterior ocellus; OOL = the shortest distance between a posterior ocellus and a compound eye. Type material of the new species is deposited in South China Agricultural University, Guangzhou, Guangdong (**SCAU**).

Taxonomy

Genus *Bicurta* Sheng, Broad & Sun, 2012

Type species. *Bicurta sinica* Sheng et al., 2012.

Diagnosis. This genus is distinguished from *Collyria* by the epicnemial carina indistinct because of sculpture on the mesopleuron; ovipositor straight and smooth (Fig. 12), lacking teeth on the ventral valve; and the fore and mid tarsal claws each having an acutely lobed tooth (Figs 10, 24) (Sheng et al. 2012), while in the other two other collyriine genera, *Aubertiella* and *Collyria*, fore and mid tarsal claws with a median tooth, rather than a lobe.

Biology. Adults of the new species emerged from larvae of *Stenocephus fraxini* Wei (Hymenoptera, Cephidae) from March to May 2019 in Northern China. This is the first report of a host of *Bicurta*, which is consistent with the known biology of *Collyria*, as parasitoids of stem-sawflies (Hymenoptera, Cephidae).

Based on the field work survey during 2018 to 2019, the parasitism rate of this species on *S. fraxini* was 59.3% on average in the downtown of Shenyang city (J.H. Yan, unpublished data).

Bicurta hejunhuai Liu, Yan & Broad, sp. nov.

http://zoobank.org/FCDA6C05-C21E-4FE1-AD15-B5B0B79C55B7

Figs 1–26

Material examined. ***Holotype*** female, China: Liaoning Province, Shenyang City, 22–30 March 2019, leg. Jia-He Yan, reared from larva of *Stenocephus fraxini* Wei (SCAU). ***Paratypes*** 2♀♀ and 21 ♂♂, same data as holotype (SCAU); 1 female, Inner Mongolia Autonomous region of China, Huhehot, Wulanfu Park, 24 March 2019, leg. Jia-He Yan (SCAU); 2♀♀, Heilongjiang Province, Harbin, Botany Garden of Harbin, reared from *Fraxinus mandshurica* Rupr. (Lamiales, Oleaceae), 7 May 2019, leg. Jia-He Yan (SCAU); 4♀♀ and 9♂♂, Liaoning Province, Shenyang, collected from *Fraxinus chinensis* Roxb. and *F. pennsylvanica* Marsh., 25 April 2019, leg. Jia-He Yan (SCAU).

Description. Holotype, female (Fig. 1). Body length 10.0 mm, fore wing length 6.2 mm, antenna length 3.8 mm, ovipositor length 1.6 mm.

Head. Face flat (Fig. 2), 1.2× as wide as high, centrally with sparse punctures, distance between punctures of central area 1.0 to 5.0× diameter of punctures, punctures close below antennal sockets and near inner orbits; face next to inner orbit with fine granular texture. Clypeus (Fig. 1) 2.2× as wide as high, finely and sparsely punctate, apical margin with an obtuse median tubercle, impunctate. Mandible weakly narrowed to apex, middle width of mandible 0.57× as wide as basal width of mandible, with lower tooth slightly longer than upper tooth. Labrum not exposed. Malar space short (Fig. 4), finely wrinkled and with fine leathery texture in between, 0.33× as long as basal mandibular width. Gena (Fig. 3) evenly convergent posteriorly, finely punctulate and pubescent, 0.63× as long as eye in dorsal view. Vertex (Fig. 3) with posterior portion finely punctulate, between lateral ocellus and eye with fine leathery texture. POL = 1.0, OD = 1.25, OOL = 1.0. Interocellar area flat with a short longitudinal groove. Frons finely punctate above antennal sockets, centrally with a weak longitudinal carina extending between antennal sockets to median ocellus, frons slightly rugose along carina sides. Antenna (Fig. 8) with 19 flagellomeres, ratio of length of basal five flagellomeres as follows: 1.42 : 1.25 : 1.17 : 1.08 : 1.0, first flagellomere 2.83× as long as its apical width, apical flagellomere 2.4× as long as its basal width, slightly shorter than fourth flagellomere (12 : 14). Occipital carina sharp and strong. Distance from hypostomal carina to mandible 1.25× longer than basal mandibular width.

Mesosoma. Propleuron finely and densely punctate, pubescent. Pronotum (Fig. 4) strongly sculptured, central depressed portion with anteriorly arched longitudinal wrinkles, dorsal half irregularly strongly rugose, with epomia indistinctly

Figure 1. *Bicurta hejunhuai* sp. nov. (female holotype), habitus, lateral view.

differentiated from these rugae. Mesoscutum (Fig. 5) with median lobe slightly convex in lateral view, median lobe with irregularly distributed fine punctures which are sparser centrally; punctures on lateral lobe sparser than those on median lobe; notaulus deep notaulus deep and crenulate, mesoscutum centrally with a short longitudinal groove behind median lobe and between meeting point of notauli; posterior median portion of mesoscutum polished, with sparse punctures. Scutellum (Fig. 5) evenly and slightly convex, sparsely punctate, distance between punctures 1.0 to 2.0× diameter of puncture. Metanotum rugose punctate. Epicnemial carina (Fig. 7) distinct ventrally and obscure dorsally, not prominent in front of median longitudinal suture. Mesopleuron (Figs 7, 13, 23) largely densely punctate reticulate and pubescent; dorsal anterior portion in front of subtegular ridge with short irregular longitudinal wrinkles; below subtegular ridge and in front of speculum slightly ru-

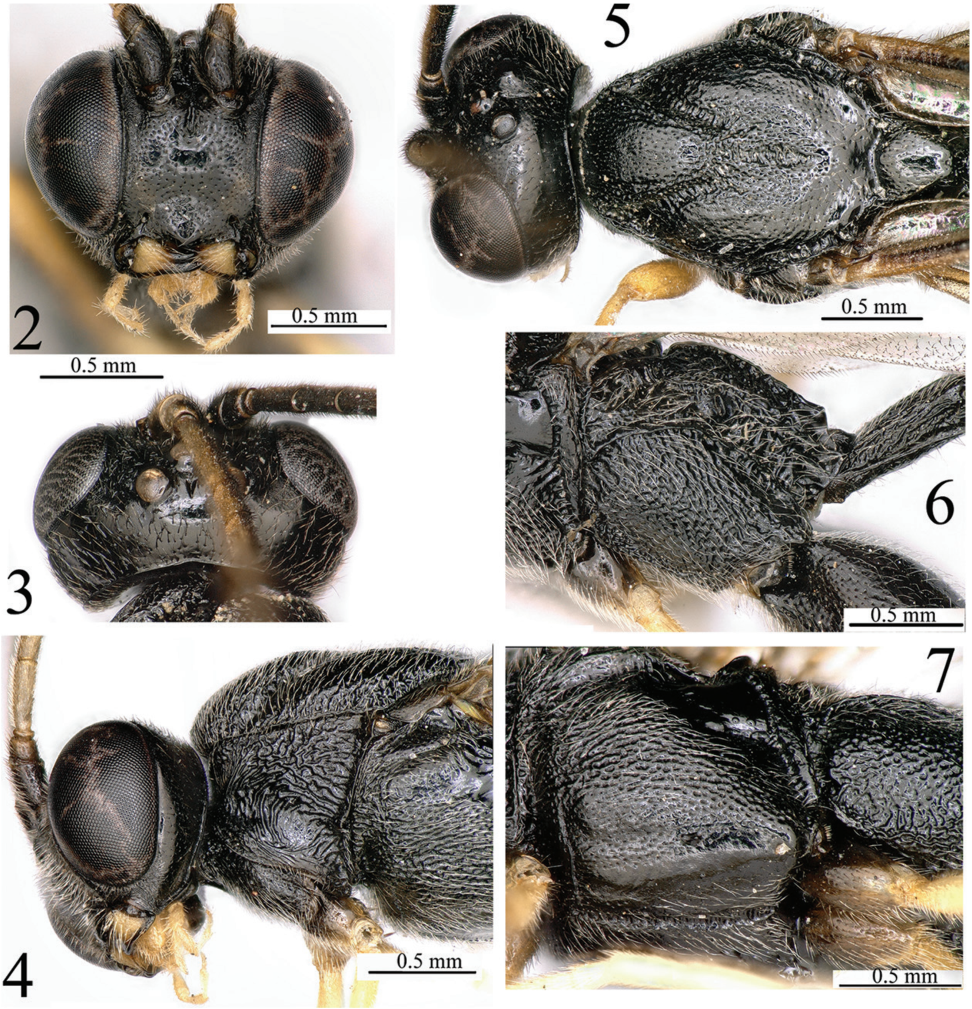
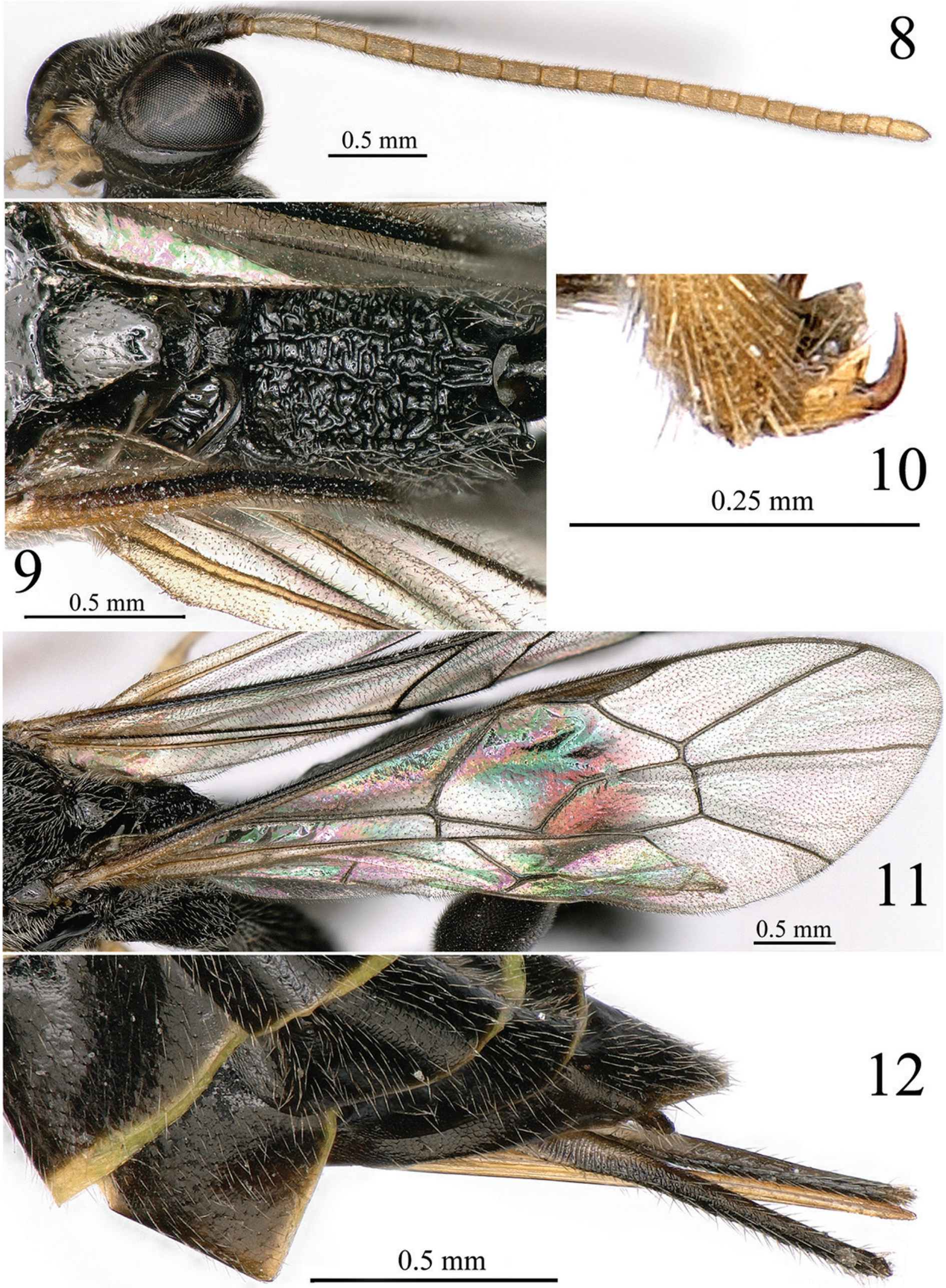


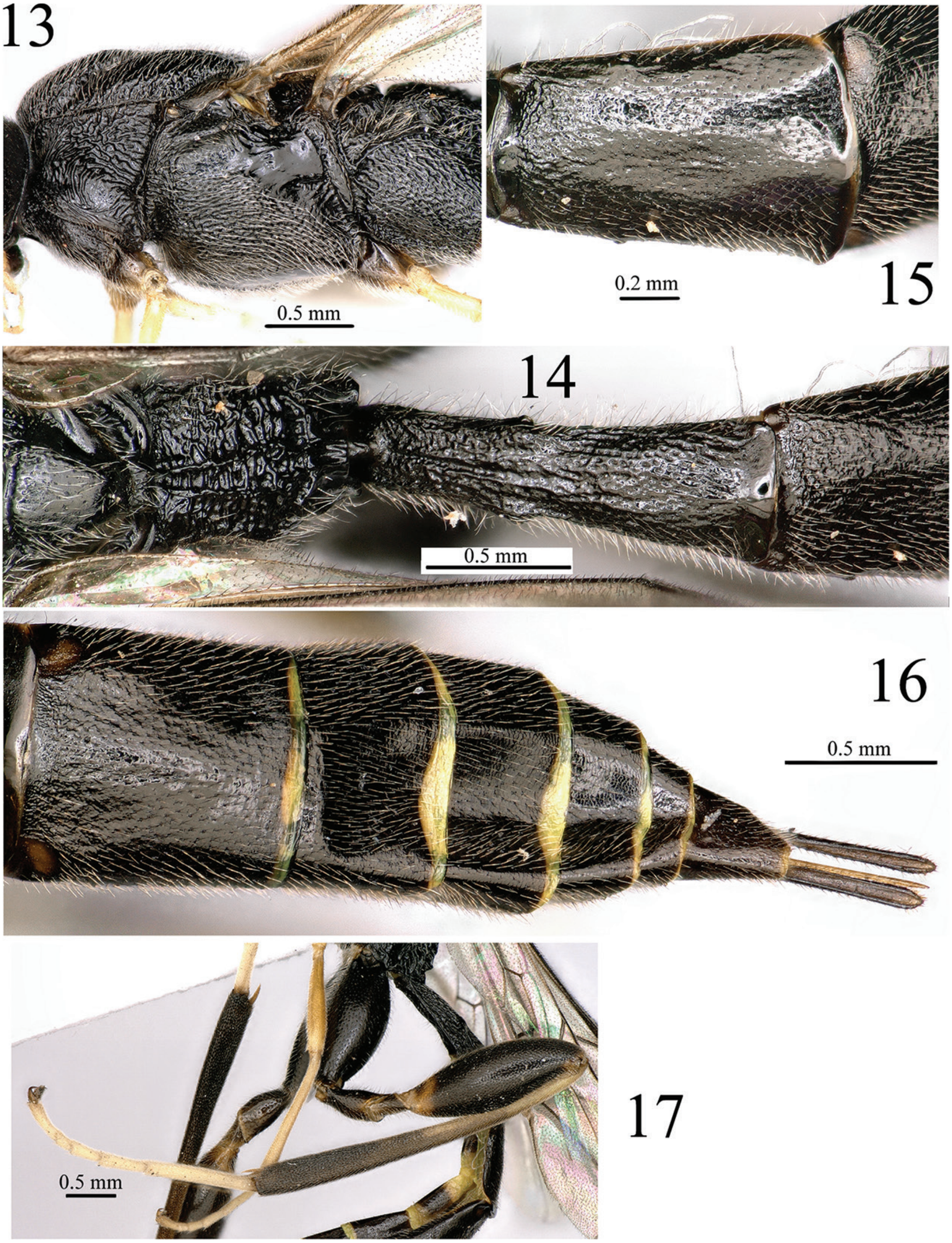
Figure 2–7. *Bicurta hejunhuai* sp. nov. (female holotype) **2** face, frontal view **3** head, dorsal view **4** head and frontal half of mesosoma, lateral view **5** head and mesoscutum, dorsal view **6** metapleuron, propodeum and base of first tergite, lateral view **7** mesopleuron and mesosternum, lateroventral view.

gulose; ventroposterior portion below episternal scrobe with short wrinkles, more or lese shiny. Speculum (Fig. 13) relatively large, as long as (or 0.6× as wide as) length of tegula, polished and glabrous. Mesopleural furrow distinctly foveolate. Mesosternum (Fig. 7) along medial sternal groove more or less polished, with a few sparse punctures laterally and centrally. Median longitudinal suture of mesosternum distinct, inner side with short transverse carinae. Metapleuron (Fig. 6) scabrous, evenly convex, dorsal portion strongly rugose, central portion punctate reticulate, ventral portion irregularly rugulose. Submetapleural carina present on anterior 0.4 of metapleuron, weakly convex, posterior 0.6 of submetapleural carina replaced by wrinkles. Metasternum 0.8× as long as mesosternum, with irregular transverse wrinkles. Pro-



Figures 8–12. *Bicurta hejunhuai* sp. nov. (female holotype) **8** head and antenna, lateral view **9** scutellum and propodeum, dorsal view **10** mid claw, lateral view **11** fore wing **12** apex of metasoma and ovipositor, lateral view.

podeum elongate (Figs 6, 9, 14), strongly rugose; median longitudinal carinae strong and reaching to posterior end of propodeum, anteriorly convergent, with transverse wrinkles in between; dorsal area with irregular wrinkles; pleural area with transverse



Figures 13–17. *Bicurta hejunhuai* sp. nov. (female holotype) **13** mesosoma, lateral view **14** scutellum, propodeum, first tergite and base of second tergite, dorsal view **15** second tergite, dorsolateral view **16** third to eighth tergites and ovipositor sheath, dorsal view **17** hind leg lateral view.

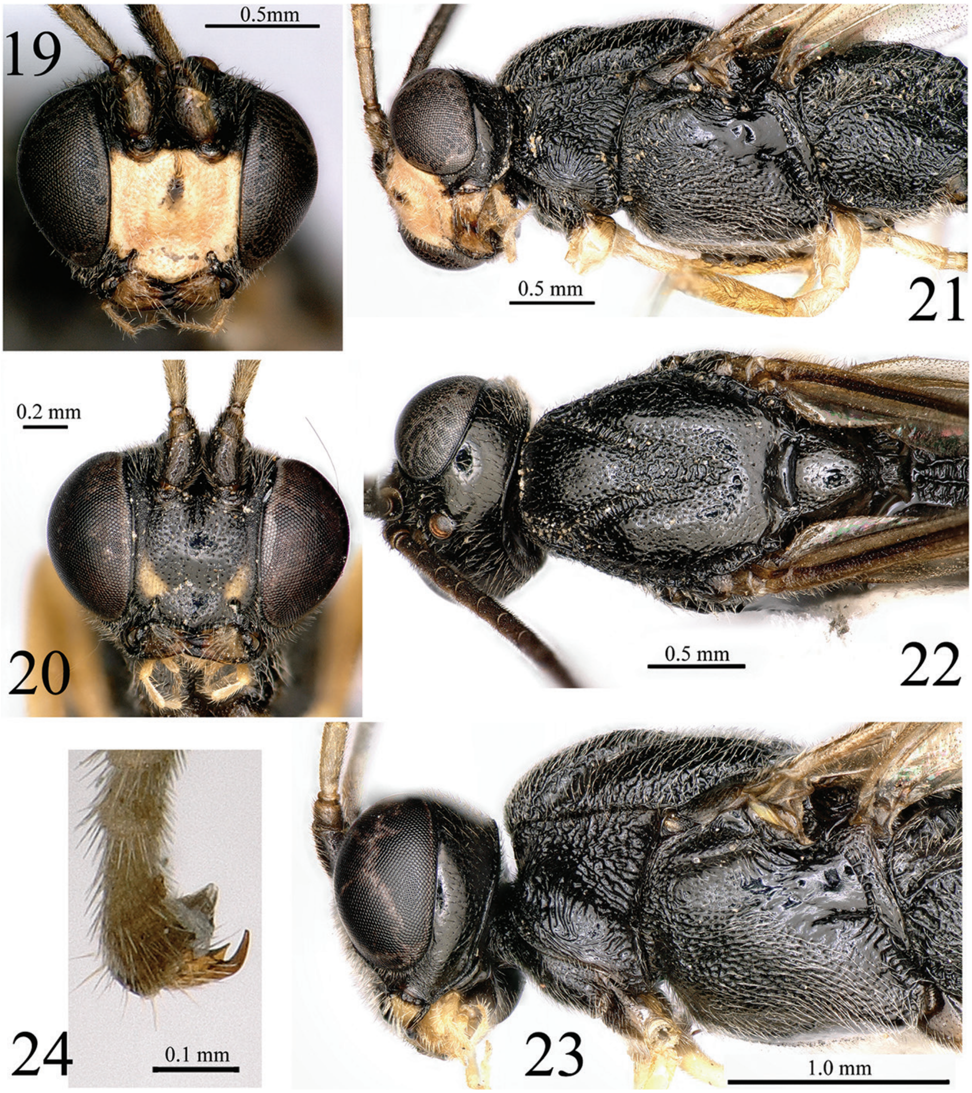
wrinkles; pleural carina complete and sharp. Propodeal spiracle elongate, 2.5× as long as wide, separated from pleural carina by 1.0× its own longest diameter.

Figure 18. *Bicurta hejunhuai* sp. nov. (male, paratype) habitus, lateral view.

Wings. Fore wing (Fig. 11) with vein 1cu-a distad of M&RS by 0.2× length of 1cu-a; 2rs-m basad 2m-cu by 0.83× length of 2rs-m. Hind wing with 8 distal hamuli; CU & cu-a reclivous, distinctly intercepted at upper 0.27; distal abscissa of CU weakly pigmented.

Legs. Fore and mid legs slender. Hind coxa elongate, 3.2× as long as its maximum width, sparsely punctulate, punctures on dorsal area sparser. Hind femur (Fig. 17) 2.8× as long as its maximum width. Hind tibia 1.9× as long as hind femur; tibial spurs very short, inner spur equal to outer spur, 0.18× length of hind basitarsus. Ratio of length of first to fifth hind tarsomeres as follows: 5 : 2.3 : 1.5 : 1.0 : 2.1. Tarsal claw strongly bent (Fig. 10).

Metasoma. First tergite (Fig. 14) 3.0× as long as its apical width, dorsally rugose punctate, with a longitudinal median furrow on anterior 0.4, laterally strongly rugose; sclerotized part of first sternite extending behind spiracle, 0.63× as long as tergite; spiracle convex, located at anterior 0.4. Second tergite (Fig. 15) 1.42× as long as pos-



Figures 19–24. *Bicurta hejunhuai* sp. nov. **19** male face, front view **20** face of female paratype **21** head and mesosoma of male paratype, lateral view **22** head and mesosoma of male paratype, dorsal view **23** head and mesosoma of female type, lateral veiw **24** mid claw of female paratype.

terior width, anterior 0.2 of tergite granulate punctate, remainder with fine punctures. Third tergite (Fig. 16) finely punctulate, anteriorly with fine leathery texture, anterior lateral corner with a small thyridium on each side. Ovipositor sheath (Fig. 12) 0.25× as long as hind tibia.

Colour. Body mainly black. Head black, face with a pair of obscure yellow marks laterally just above tentorial pits; these yellow marks are very distinct in female paratypes (Fig. 20) and hardly discernible in holotype (Fig. 2). Mandible testaceous with

Figure 25. *Bicurta hejunhuai* sp. nov., female paratype on a leaf of *Fraxinus pennsylvanica* in Shenyang City, Liaoning province (cell phone photo by Jia-He Yan).

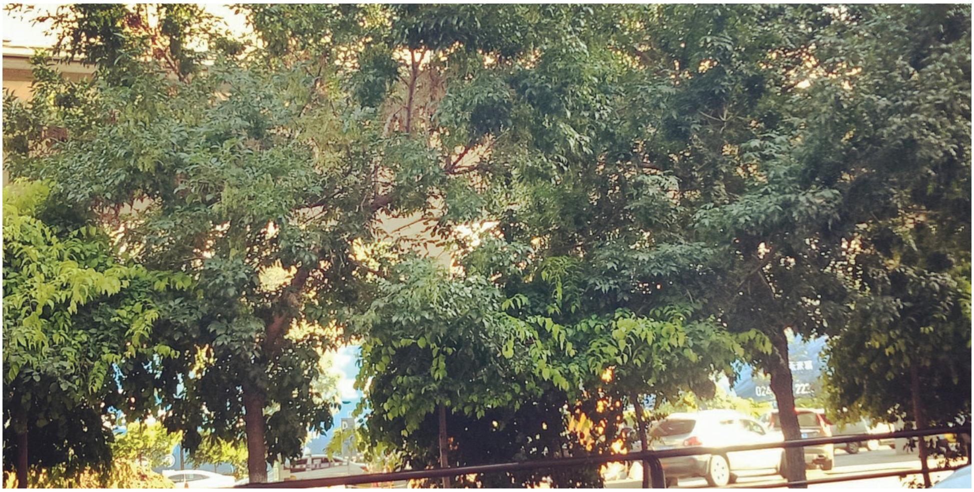


Figure 26. One habitat of *Bicurta hejunhuai* sp. nov.: greening trees *Fraxinus pennsylvanica* in the street of Downtown Shenyang City, Liaoning province, China (cell phone photo by Jia-He Yan).

lower margin and apical teeth black. Stipes and prementum black. Labial and maxillary palpi yellow. Antenna with scape and pedicel black, flagellum dorsally blackish brown and ventrally yellowish brown. Fore and mid legs buff with coxae black; hind leg black, apex of trochanter yellow, trochantellus blackish brown, proximal base of hind femur ventrally buff, proximal half of hind tibia ventrally dull yellow and dorsally dark brown to blackish brown, apical half of hind tibia black. Hind margins of tergites 1–7 narrowly yellow. Tegula black. Wings hyaline, with veins and pterostigma blackish brown.

Variation. Body length 7.0–10.0 mm. Antenna with 19–20 flagellomeres; yellow spots on face obscure to distinct (Fig. 2, 20).

Male (Fig. 18, 19, 21, 22). Body length 8.2 mm, fore wing 5.4 mm. similar to female. Differences from female as follows: antenna ventrally yellow to yellowish brown; face and clypeus (except lower margin blackish) yellow (Fig. 19), sometimes with a small blackish spot on face centrally; frons with several transverse wrinkles just above antennal sockets; first tergite centrally with two distinct carinae which extend to posterior 0.7 of tergite, posterior tips of carinae irregularly branched. Paramere apically truncate.

Variation. Body length 6.2–8.2 mm, fore wing length 4.6–5.2 mm. Fore wing with vein 1cu-a opposite or slightly distad to M&RS.

Etymology. The new specie is named in honour of Prof. He Junhua from Zhejiang University in recognition of his years of dedicated and conscientious performance in the study of Chinese Hymenoptera, and also for the celebration of his 90th birthday.

Comparison. The new species is similar to the genotype, *B. sinica*, in its overall appearance and colour pattern. But it can be distinguished from *B. sinica* by the face having two obscure or distinct yellow marks (the face of *B. sinica* has the ventral inner orbits, clypeus and a stripe passing through the anterior tentorial pits yellow); the mandible weakly narrowed from middle toward the apex, with middle width of mandible 0.57× as wide as the basal width of mandible (*B. sinica* with the mandible strongly narrowed from middle toward the apex, with middle width of mandible 0.26× as wide as the basal width of mandible, measurements based on the figure of Sheng et al. (2012); the central part of the face with sparse punctures (with dense punctures in *B. sinica*); the mesosternum polished, with sparse punctures (*B. sinica* has the mesosternum densely punctate); and the fore wing vein 1cu-a usually distinctly distad of M&RS (1cu-a opposite M&RS in *B. sinica*).

Biology. The species was reared from the larvae of *Stenocephus fraxini* Wei (Hymenoptera, Cephidae) in Northern China.

Discussion

Knowledge of the biology of the subfamily Collyriinae has been limited to life history studies of just two species of *Collyria* (Salt 1931; Wahl et al. 2007). Both *Collyria coxator* (Villers) and *C. catoptron* Wahl have been shown to be koinobiont endoparasitoids of *Cephus* (Hymenoptera: Cephidae), ovipositing in the host egg and emerging from the cocooned larva. Sheng et al. (2012) presented detailed morphological evidence that placed the genus *Bicurta* in the subfamily Collyriinae, despite the very different morphology of the ovipositor. Confirmation that *B. hejenhuai* sp. nov. is also a parasitoid of larval Cephidae, but in a different habitat (tree twigs as opposed to grass stems) suggests that all collyriines may be koinobiont endoparasitoids of larval cephid sawflies, including the poorly known *Aubertiella nigricator* (Aubert), which has never been reared. The fine ovipositor of *Bicurta* species suggests that oviposition will be into host eggs or early instar larvae, although this has not been confirmed.

Acknowledgements

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RESEARCH ARTICLE

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Fighting injuries, fig exit, and dimorphism in two species of sycoryctine fig wasp (Chalcidoidea, Pteromalidae)

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Abstract

Similar to many vertebrate and invertebrate species, many fig wasp species are fighting other members of their species for mates. Fighting between the males of many non-pollinating fig wasp species involves injuries and fatalities. Studies have shown that large males fight for mates, whereas conspecific small males tend to adopt nonfighting, sneaky behaviors. To analyze male morphs in two non-pollinating fig wasps (*Philotrypesis taida* Wong & Shiao, 2018 and *Sycorycteridea taipeiensis* Wong & Shiao, 2018) associated with the fig tree *Ficus benguetensis*, the head and mandible allometry and injuries were examined as well as the morphologies of their heads and mandibles. Male fig wasps of these two nonpollinating species can be divided into two morphological groups according to their head and mandible shapes. Approximately 88% of the *Philotrypesis* and 62% of the *Sycorycteridea* males were injured and no males belonging to largest morphs were decapitated. Moreover, nearly 31% of the *Philotrypesis* and 45% of the *Sycorycteridea* males left their natal figs. No difference in injury level or male exit rate between the male morphs was observed. This study reveals slight morphological and behavioral differences that may hint towards different mating strategies among morphs.

Keywords

Ficus benguetensis, fatal fighting, male dimorphism, male dispersal, *Philotrypesis*, *Sycorycteridea*

Introduction

Fighting is ubiquitous in the animal kingdom (Hardy and Briffa 2013; Huntingford 2013; Vieira and Peixoto 2013), and intraspecific fighting is mainly caused by the need to defend for food and territory (Backwell and Jennions 2004) or for mating opportunities (Baxter et al. 2015). For example, many Cervidae stags fight for the control of the females (Clutton-Brock et al. 1979; McElligott et al. 1998); such activity is common in mammals (Packer 2001; Casey et al. 2015). Fighting is also extremely common in invertebrate species such as cephalopods (Schnell et al. 2015) and weaponless butterflies (Kemp and Wiklund 2001). Hymenopterans, such as ants, wasps, and bees, are well-known to engage in intraspecific fighting, either to protect their colonies (Gloag et al. 2008; Rudolph and McEntee 2015) or to compete for the queen position (Berthelot et al. 2017). Fighting often causes many casualties, especially in reproductive castes of social insects (Kärcher et al. 2013; Heinze et al. 2016). Fatal fighting may occur when the following two conditions are met (Enquist and Leimar 1990): the value of the contested resource (e.g. a limited number of mating partners) is particularly high and the value of future reproduction is extremely low (e.g., the organism's lifespan is short). Many examples of fatal fights between males have been documented in the hymenopteran superfamily Chalcidoidea: in the genus *Melittobia* (Hartley and Matthews 2003) and in numerous fig wasp species (Cook 2005). For example, the males of several fig wasp species can only live for up to one day, and the numbers of females within a mating arena in these species are often low (Cook et al. 2017). Thus, the non-pollinating fig wasp model fits the conditions required for the evolution of fatal fighting (Enquist and Leimar 1990).

Fig wasps are small insects that are obligately associated with plant of genus *Ficus* (Kjellberg et al. 2005). As larvae, all fig wasp species (pollinators and non-pollinating fig wasps) are confined inside closed inflorescences called figs. Because of the following aspects of their peculiar life cycle, non-pollinating fig wasps provide a unique opportunity for studying non-pollinating male fig wasp fights: (1) Each fig is a spherical enclosed inflorescence containing hundreds of flowers with one single exit (the ostiole), and (2) limited variation in environmental factors, and the number of figs produced by each tree enable easy replication of experiments in a known environment. In each fig, one or a few female pollinators enter receptive fig, pollinate the flowers inside the fig and lay eggs into the fig ovaries. Wasp larvae then develop inside galled flowers over several weeks. Non-pollinating fig wasps mostly colonize figs after pollination and parasitize fig ovules or pollinating or non-pollinating fig wasp larvae; they do not enter the fig but rather use their long ovipositor to lay eggs through the fig wall into ovules (Kerdelhué and Rasplus 1996). Male pollinating fig wasps hatch first and mate with unhatched pollinating female fig wasps.

Fighting among male fig wasps occurs in both pollinating and non-pollinating species (Bean and Cook 2001; Nelson and Greeff 2009) and tend to be fatal (Bean and Cook 2001; Pereira and Prado 2008; Dunn et al. 2015). The fights take place in the fig: more precisely in the central cavity (the lumen) of the fig and during the short period before the fig opens (or is opened by male fig wasps) to let the female fig wasps disperse. A considerable proportion of fighting occurring between individuals results in severe injuries or death (Bean and Cook 2001). Fig wasp males belonging to the same species can exhibit a range of morphologies, including armored males bearing large falcate mandibles that fight for mates and flattened males that stealthily seek mates (Murray 1990; Cook et al. 1997; Jousselin et al. 2004). Moreover, some species have dispersing morphs such as winged males (Jousselin et al. 2004) and apterous dispersing males (Greeff and Ferguson 1999); however very little is known about the dispersal rate and behavior of the male fig wasps. Theoretically, a male reaching a fig other than its native fig would be able to mate with female wasps that likely are genetically very different.

The aim of this study was to document morphological variations, the fighting outcomes, and the exit rates of two species of non-pollinating fig wasp living on *Ficus benguetensis*. Preliminary observations of *Philotrypesis taida* Wong & Shiao, 2018 and *Sycorycteridea taipeiensis* Wong & Shiao, 2018 males had revealed that: (1) males are fighting inside the figs, (2) fighting may result in death by decapitation, (3) males vary in size, and (4) males may exit figs. On the basis of these observations, this study raised the following questions concerning male interactions among the studied species: (1) How different in size and morphology are these males? (2) How common are injuries and fatal injuries? (3) How often do males exit their natal figs? (4) What are the factors (e.g., fig diameter linked with the fighting space, sex ratio linked with the number of fighters) affecting the level of injury and the exit rate?

Methods

Study species

Philotrypesis taida and *Sycorycteridea taipeiensis* belong to the Sycoryctinae subfamily (Chalcidoidea: Pteromalidae) and are the two non-pollinating fig wasp species associated with the gynodioecious *Ficus benguetensis* Merrill (Berg 2011; Wong et al. 2018). The trees bear either seed-producing female or wasp-producing "male" figs. Until recently (Segar et al. 2012), the genus *Sycorycteridea* and three other genera (*Arachonia*, *Sycoryctes*, and *Sycoscapteridea*) were synonymized under the *Sycoscapter* genus (Bouček 1988).

Sample collection and behavior observation

Thirty ripe unexited figs were haphazardly collected from five *F. benguetensis* trees in Taiwan from October 2011 to July 2012 (see Suppl. material 1: Table S1). Unripe figs are dark green and firm to the touch, whereas ripening figs gradually change to yellowish green and yield to gentle pressure. The diameters of all sampled figs were measured before the figs were stored at room temperature in transparent plastic pots covered with mesh for 24 h with their ostioles facing upward. A fig's diameter is an indicator of its size and correlates with the size of the lumen, i.e. the space available for the male fig wasps to fight. Onset of wasp exit was identified by the presence of a hole chewed through the fig-wall. All wasps that exited from their natal figs were collected and stored in 75% ethanol. The figs were then dissected and the wasps inside them were collected. All the collected wasps were then identified, sexed, and counted under a stereomicroscope at 6.3× magnification. Images were captured using a digital reflex camera. Head width (across the eyes) and mandible length were measured using the 1.45a image processing software (National Institutes of Health, USA). Head width, rather than the hind tibia length, was used to estimate body size because limbs are often lost during fighting.

Fighting injuries and exit

The collected *Philotrypesis* and *Sycorycteridea* males were examined for their fighting injuries. All observed lacerations and amputations were noted. The injury level of an individual male was estimated according to a rating scale developed for fighting fig wasps (Murray 1987). However, because the behavior of male fig wasps is specialized to the dark environment of a fig, the scale was modified in the present study; loss of antenna was changed to from 0.5 point to 2 points (Table 1). The original scale developed by Murray (1987) focused on the fluid loss, whereas the modified scale considers the necessity for the male wasps to locate females in dark environments by using only their antennae. Mate-seeking ability is severely impaired when an individual loses part or all of its antennae. Hence, it was assigned a heavier weight in the present study. Injury level per fig was estimated according to the following three variables proposed by Murray (1987): (1) lifetime extent of injury (LEI), the injury scores of all males from the same fig divided by the total number of males; (2) injury frequency (IF), the proportion of injured males; and (3) severe injury frequency (SIF), the proportion of injured males with injury scores greater than 8 points.

Male exit was estimated as the proportion of males exiting their natal fig, namely the "male exit proportion", and was calculated as the number of exited males divided by the total number of males per fig.

Table 1. Criteria used in scoring injuries of male fig wasps.

Score	Description
1	Loss of part or whole tarsus
1	Loss of part or whole mandible
2	Loss of part or whole antenna
2	Loss of part or whole tibia
3	Loss of part or whole femur
4	Loss of part or whole coxa
4	Laceration in abdomen
8	Decapitation

Data analysis

The sex ratio for each species was calculated as the number of male wasps divided by the total number of wasps within a fig. All statistical tests were performed using the R 3.6.1 statistical software (R Development Core Team 2019). The dataset was not normally distributed so the used tests were nonparametric. To investigate the factors affecting male exit proportion, multiple linear regression (which do not require a normally distributed dataset) was performed, with five variables: average head width, fig diameter, LEI, morph ratio, and wasp sex ratio.

Results

Male morphology and sex ratio

One hundred eighty-two *Philotrypesis* and 42 *Sycorycteridea* male wasps were collected. The average sex ratio of the sampled figs was 0.36 ± 0.03 for *Philotrypesis* and 0.47 ± 0.09 for *Sycorycteridea*, which was significantly different from the 1:1 ratio for *Philotrypesis* (t = 4.44, df = 48, P < 0.001) but not for *Sycorycteridea* (t = 0.39, df = 22, NS). The sex ratio of the two species was not different (t = -1.16, df = 14.03, NS).

Two *Philotrypesis* male morphologies, namely "atypical" and "typical" morphs, were identified. They were distinguished by the shapes of their mandibles (Fig. 1; alternative images in Suppl. material 2: Fig. S1); the atypical males (7% of the males) have scythe-shaped mandibles, whereas the typical males have three-tooth mandibles. In addition, atypical males were significantly larger than the typical males (Table 2). The largest *Philotrypesis* male was approximately twice the size of the smallest, and its mandible was approximately three times as long (Table 2; Fig. 2a). The sizes of the two morphs overlapped; the smallest *P. taida* atypical males were smaller than many of the typical males (Fig. 2a). The *Sycorycteridea* males were divided into two morphs according to their size (Table 2): the "small" morph, having head widths narrower than 0.425 mm (57% of the individuals), and the "large" morphs, having heads wider than 0.425 mm (Fig. 2b). The largest *Sycorycteridea* male was approximately thrice the size of the smallest and had a mandible approximately four times as long. *Sycorycteridea* mandible length and head width were discontinuously distributed. No male with a

Table 2. Head width and mandible length of each male morph.

	Morph	N	Mean head width	Head width range	Mean mandible length	Mandible length range
Philotrypesis	Atypical	13	0.673 ± 0.187 ^{a}	0.550–0.719	0.323 ± 0.089	0.242–0.370
	Typical	169	0.576 ± 0.044 ^{b}	0.366–0.675	0.233 ± 0.018	0.134–0.310
Sycorycteridea	Large	18	0.502 ± 0.118 ^{c}	0.453–0.562	0.290 ± 0.068	0.243–0.326
	Small	24	0.336 ± 0.069 ^{d}	0.215–0.388	0.153 ± 0.031	0.078–0.230

Means are given ± SE. Numbers with different letters indicate significant differences.

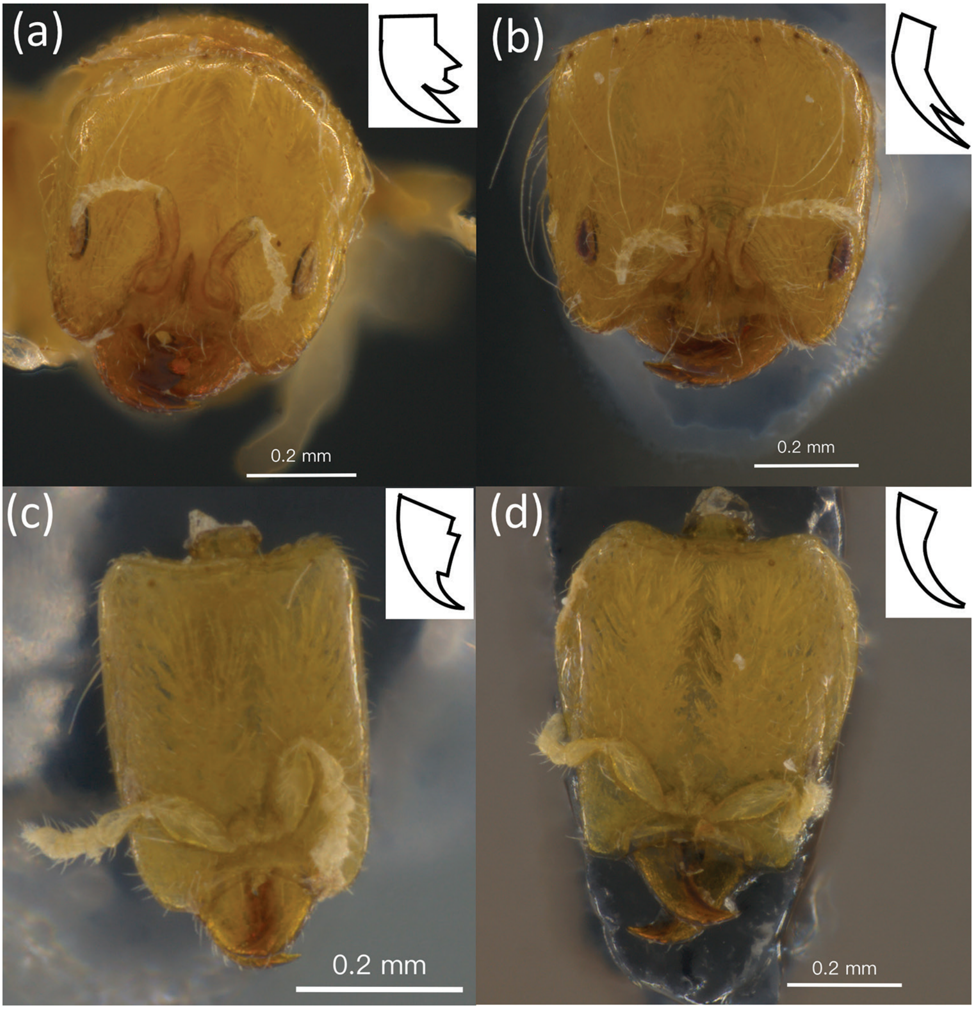

Figure 1. Frontal view of the males of the two studied species and mandible outline (**a**) a "typical" *Philotrypesis* male: rounded back of the head and broad mandibles (**b**) "atypical" *Philotrypesis* male: head more square and falcate mandibles (**c**) *Sycorycteridea* small male, rectangular head (**d**) *Sycorycteridea* large male, lantern-shaped head.

head width between 0.400 and 0.450 mm was found. The measurement gap between the two morphs represents approximately 12% and 19% of the average male head width and mandible length, respectively.

The mandible length of *Philotrypesis* males correlated positively with head width (Spearman rank correlation test: ρ = 0.759, df = 180, P = 2.58E-35; Fig. 2a). In addition, significantly positive correlations were independently observed in the atypical morph (Spearman rank correlation test: ρ = 0.714, df = 167, P = 1.09E-27) and typical morph (Spearman rank correlation test: ρ = 0.828, df = 11, P = 0.000473). The mandible length of the *Sycorycteridea* males exhibited a positive relationship with their

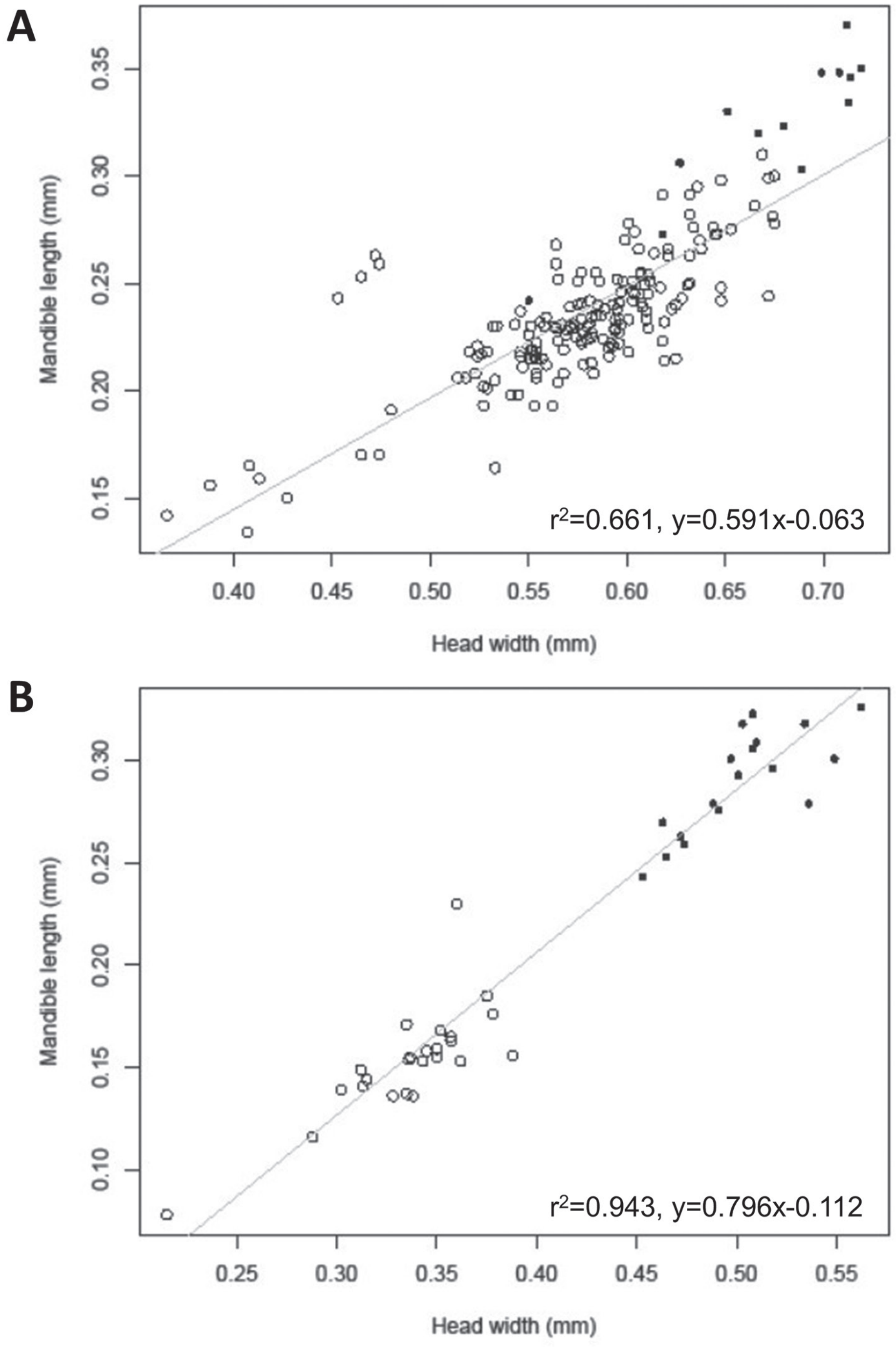

Figure 2. Relationship between head width and mandible length in **a** *Philotrypesis* and **b** *Sycorycteridea*. Typical *Philotrypesis* and small *Sycorycteridea* are represented by the unfilled circles whereas atypical *Philotrypesis* and large *Sycorycteridea* are represented by filled circles.

head width (Spearman rank correlation test: $\rho = 0.939$, df = 40, P = 3.82E-20; Fig. 2b). Furthermore, the same positive correlation existed independently for the large males (Spearman rank correlation test: $\rho = 0.765$, df = 22, P = 1.35E-05) and small males (Spearman rank correlation test: $\rho = 0.765$, df = 22, P = 1.35E-05).

Fighting injuries

The number of *Philotrypesis* males of each morph in each injury level was not different ($\chi^2_1 = 0.48$, NS), same for the *Sycorycteridea* morphs ($\chi^2_1 = 0.00$, NS; Table 3). Additionally, no difference was observed in average injury score between the atypical and typical *Philotrypesis* males (Wilcoxon rank sum test: W = 1096.5, P = 0.99; Table 4) or between the *Sycorycteridea* male morphs (Wilcoxon rank sum test: W = 197.5, P = 0.64; Table 4). Finally, half of the *Sycorycteridea* large males were uninjured (Table 3; Fig. 3b), whereas more than half of the atypical *Philotrypesis* were severely injured (Table 3).

Philotrypesis had a significantly higher LEI value (t = 3.11, df = 35, $P < 0.01$; Table 5) and a higher proportion of injured males than did *Sycorycteridea* ($\chi^2_1 = 15.58$, $P < 0.01$; Table 5); however, the proportion of severe injury was not different between the two species ($\chi^2_1 = 7.86$, NS). Rates of decapitation were low in *Philotrypesis* and *Sycorycteridea* males and all decapitated specimens were small *Sycorycteridea* and typical *Philotrypesis* males (Table 4; Fig. 3). The femur and tarsus were the most commonly injured body parts for the *Philotrypesis* males, whereas for the *Sycorycteridea* males, the antenna and tarsus were the most commonly injured parts (Table 4).

The calculation of the injury score differs from Murray's scoring (1987) from the points allotted to the antenna injuries, increasing their score from 1.5 or 3 points from

Table 3. Proportion of injured males.

	Philotrypesis		*Sycorycteridea*	
	typical (N = 169)	**atypical (N = 13)**	**small (N = 24)**	**large (N = 18)**
Uninjured (injury score = 0)	0.11	0.15	0.29	0.50
Minor (injury score <8)	0.46	0.31	0.50	0.33
Severe (injury score ≥8)	0.43	0.54	0.21	0.17

Means are given ± SE.

Table 4. Mean injury score per individual and injury proportion distributed among different body parts.

	Philotrypesis		*Sycorycteridea*	
	typical (N = 169)	**atypical (N = 13)**	**small (N = 24)**	**large (N = 18)**
Mean injury score	7.4 ± 0.6 ^{a}	7.1 ± 2.0 ^{a}	3.8 ± 0.8 ^{b}	3.5 ± 0.8 ^{b}
Abdomen	0.01	0.00	0.00	0.00
Antenna	0.37	0.62	0.25	0.28
Coxa	0.31	0.23	0.21	0.11
Decapitation	0.08	0.00	0.04	0.00
Femur	0.49	0.46	0.25	0.22
Tarsus	0.42	0.38	0.29	0.22
Tibia	0.32	0.46	0.17	0.22

Means are given ± SE. Numbers with different letters indicate significant differences.

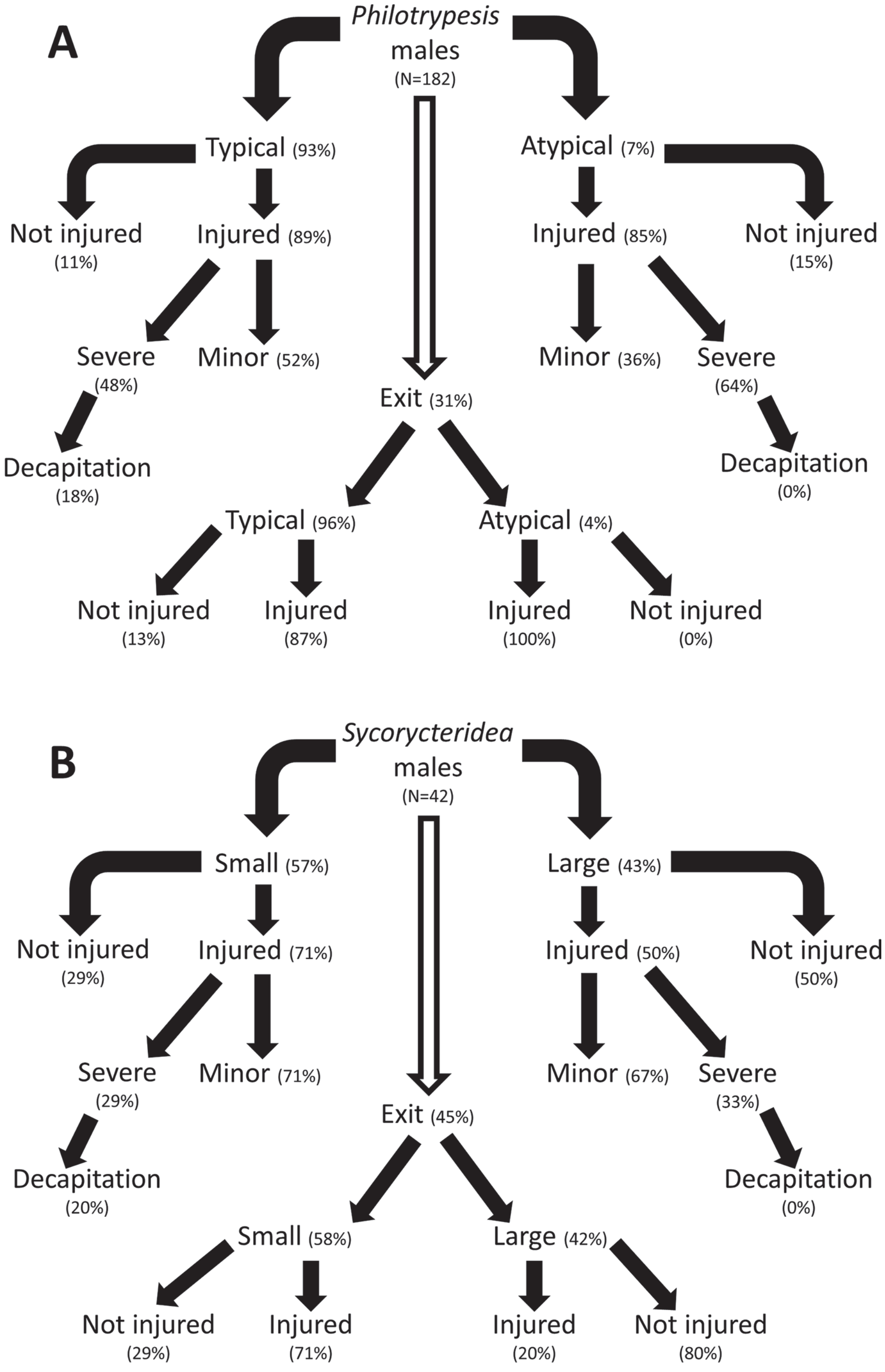

Figure 3. Proportions of **a** *Philotrypesis* and **b** *Sycorycteridea* exited and injured males.

Table 5. Average numbers per fig and injury rates for the two non-pollinating fig wasp species (N = 30 figs).

	Philotrypesis	*Sycorycteridea*
Mean wasp number	21 ± 5	12 ± 3
Male exit rate	0.31 ± 0.06	0.45 ± 0.13
Lifetime Extent of Injury (LEI)	5.6 ± 0.6 ^{a}	2.5 ± 0.7 ^{b}
Proportion of injured males (IF)	0.88 ± 0.02 ^{a}	0.62 ± 0.07 ^{b}
Severe Injury Frequency (SIF)	0.23 ± 0.03	0.10 ± 0.05

Means are given ± SE. Numbers with different letters indicate significant differences.

Table 6. Result of the multiple linear regression model with the male exit proportion as dependent variables.

	Philotrypesis **(R^2 = 0.47* F = 3.37)**		***Sycorycteridea*** **(R^2 = 0.52 F = 1.28)**	
	β	t	β	t
Average head width	4.05	2.36*	1.15	0.28
Fig diameter	-0.02	-1.40	-0.01	-0.22
LEI	-0.02	-0.85	-0.07	-1.12
Morph ratio	-0.49	-1.09	-0.48	-0.67
Wasp sex ratio	-0.32	-0.90	-0.93	-1.61

*P < 0.05.

Murray's score. Seventy-one (39.0%) *Philotrypesis* males and 11 (26.2%) *Sycorycteridea* males had antenna injured. Moreover, 12 *Philotrypesis* males and two *Sycorycteridea* males have seen their injury level increased using our scoring method (from minor to severe). The new injury scores were significantly higher than Murray's scores for *Philotrypesis* (Wilcoxon signed rank test: V = 2556, P = 3.548e-15) and for *Sycoscapteridea* (Wilcoxon signed rank test: V = 66, P = 0.001586).

Male exit

Most of the male fig wasps exited within 2 h after the first wasp had emerged from the fig. No difference in the proportion of exited males was observed between the two non-pollinating species (χ^2_1 = 0.00, NS; Table 5). However, the two species differed in terms of the injury scores of exited males. First, the average injury scores of exited and philopatric *Philotrypesis* males were 3.1 (range: 0–8) and 4.2 (range: 0–18), respectively, indicating significant variation between the two groups (Wilcoxon rank sum test: W = 4329.5, P = 0.001145). The test for *Sycorycteridea* values was not significant (Wilcoxon rank sum test: W = 229.5, P = 0.1595): 1.33 (range: 0–5) for exited males and 2.33 (range: 0–11) for philopatric males. Moreover, the severe injury proportion was lower for exited males than for philopatric males among *Philotrypesis* males (χ^2_1 = 6.25, P < 0.05) but not among the *Sycorycteridea* males (χ^2_1 = 0.00, NS).

The multiple linear regression model contained five variables (average head width, fig diameter, LEI, morph ratio, and sex ratio) was significant, showing that the five variables collectively explain the exit rate. In addition, the average *Philotrypesis* male head width was the main contributor of the model (Table 6).

Discussion

Our results show that the males of each of the two studied species had two clearly defined morphs. The *Philotrypesis* males could not be categorized according to their size only because some of the atypical males were smaller than the largest typical male: The shapes of the heads and mandibles were the discriminative morphological features. The shapes of the head and the mandibles were also discriminative for the morphs of the *Sycorycteridea* males but these males could also be segregated according to their size only. Such dimorphism (discontinuous distribution of male size) has never been observed before in male fig wasps. Moreover, injuries were found in every morph of both species, and it seems that belonging to a specific morph does not affect the probability of injury. Nevertheless, morph type may affect the severity of injury; no decapitated males belonging to the atypical *Philotrypesis* or large *Sycorycteridea* morphs were found in this study. However, the absence of decapitated males could be a random effect of the sampling as only the little number of atypical *Philotrypesis* has been found.

In contrast to other *Philotrypesis* species (Cook et al. 1997; Jousselin et al. 2004), no winged males have been found in *P. taida*. The dimorphism of the *Philotrypesis* morphs in the present study is similar to that of congeneric species living on *F. septica* in Australia (Cook and Bean 2006) and *F. rubiginosa* (Moore et al. 2009). In other words, in the three *Philotrypesis* species whose morphometries have been analyzed, atypical males have longer mandibles than typical males (for a given head size). The main difference between the Taiwanese species and Australian species is that the Australian *Philotrypesis* morphs are more clearly separated by their head and mandible morphometry (Cook and Bean 2006; Moore et al. 2009) (Fig. 2a). To the best of our knowledge, no other study has described the morphometry of the *Philotrypesis* male morphs.

The *Sycorycteridea* morphs in this study were uniquely morphometrically defined; no continuous distribution of mandible length or head width was noted, in contrast to Australian *Sycoscapter* species living on *F. macrophylla* (Bean and Cook 2001) or *F. rubiginosa* (Moore et al. 2008). Genera *Sycorycteridea* and *Sycoscapter* are closely related but phylogenetically distinct (Segar et al. 2012). Dimorphism seems to be expressed differently among the genera of a single hymenopteran group, with three distinct morphometric manners to be dimorphic according to Cook and Bean (2006). Our study added a fourth manner among *Sycorycteridea* morphs, namely discrete size-dependent dimorphism. The diversity of the allometric dimorphism has also been documented in other insect genera such as *Onthophagus* dung beetles (Emlen et al. 2005), *Oxyporus* rove beetles (Hanley 2001), and hymenopteran insects (Danforth and Desjardins 1999).

The injury frequencies (IF) of *P. taida* and *S. taipeiensis* in this study are the highest ever documented in any non-pollinating fig wasp species (88% and 62% for *Philotrypesis* and *Sycorycteridea* respectively). In congeneric species, very few fight winners were injured, whereas 22% of *Philotrypesis* losers and 73% of *Sycorycteridea* losers were injured (Moore et al. 2008). As examples for other genera, in *Walkerella*, the IF was approximately 17% (Wang et al. 2010), and in *Idarnes* wasps, it was approximately 40% (Pereira and Prado 2008). In our study, morph type had no effect on IF for either of the studied species; however, for another species of *Philotrypesis* for a given mandible size, atypical males were less injured (Moore et al. 2009). The IF of non-pollinating wasps living in *F. benguetensis* figs is higher than that of other species; thus, environmental factors might be key for determining the intensity of fights. Indeed, IF is often affected by the number of conspecific males (Murray 1987; Pereira and Prado 2005, 2008; Wang et al. 2010), fight duration (Moore et al. 2008, 2009), fig diameter (Pereira and Prado 2005, 2008), male size (Moore et al. 2008; Pereira and Prado 2008), and number of females (Pereira and Prado 2008). Furthermore, injuries can occur only after a certain number of males have hatched (Wang et al. 2010). On the basis of our data, we cannot discriminate between hypothetical explanatory factors. The severe injury frequency (SIF) values of *P. taida* and *S. taipeiensis* in this study are within the ranges presented in the literature; SIF was low (1–3%) in *Idarnes* wasps (Pereira and Prado 2008), approximately 25% in *Sycoscapter* wasps (Bean and Cook 2001), and approximately 44% in *Sycoscapter* fight losers (Moore et al. 2008). Also, 11% of *Philotrypesis* losers-four times less than the SIF of our studied *Philotrypesis* species-were severely injured (Moore et al. 2008). This difference may be due to the changes we made to Murray's injury score (1987), increasing the value of damaged antennae.

Although studies on the dispersal (exit from a natal fig) of non-pollinating wingless male fig wasps are extremely limited, the few published studies have revealed a wide range of exit rates; 27%–71% of males of non-pollinating fig wasp species living in *F. ingens* figs exit their native figs (Greeff and Ferguson 1999), as do 17% of *Sycoscapter australis* (Bean and Cook 2001) and 29% of *Walkerella* sp. ex. *F. benjamina* male wasps (Wang et al. 2010). The exit rate of the *Philotrypesis* males from *F. benguetensis* (31%) is very close to that of *Philotrypesis* (35%) from *F. ingens* (Greeff and Ferguson 1999). In addition, species from the Sycoryctini tribe exhibit a wide range of exit rates: 17% for *Sycoscapter australis* on *F. macrophylla* in Australia (Bean and Cook 2001), 45% for *Sycorycteridea* sp. 1 on *F. benguetensis* in Taiwan; and 71% for *Sycoryctes* sp. on *F. ingens* in South Africa (Greeff and Ferguson 1999). These numbers reveal highly variable exit rates among studied species in the literature, and even among species living in the same figs (*F. benguetensis* and *F. ingens* wasps). This feature may be a strong indicator that exit rate is not primarily driven by environmental factors but by genetic or maternal factors. Even in a single wasp species, different morphs can have very distinct exit rates; almost all typical *P. taida* typical males exit figs, whereas very few of atypical male ones do. This situation is similar to that of the non-pollinating wasp species *Otitesella longicauda*, which has two morphs. The dispersing morph (called digitata) always exits figs, whereas the other morph (called religiosa) almost never leaves its natal fig (Greeff and Ferguson 1999). The exit rates of different morphs in a single species do not always differ extremely; for example, in contrast to the *Otitesella rotunda* digitata and religiosa morphs having an exit rates of 70% and 27%, respectively, the small and large *Sycorycteridea* sp. 1 have exit rates of 58% and 42%, respectively, and the black and pale morphs of *Walkerella* sp. ex. *F. benjamina* have low exit rates of 36% and 23%, respectively (Wang et al. 2010). Differences in exit rates between intraspecific morphs show that dispersal is likely not influenced by environmental cues but by maternal choices, and these choices may be influenced by environmental factors. Nevertheless, some exit rate values fit the morphological adaptations of the males, such as the digitata males (attracted to light and strong legs) and black *Walkerella* males (darker males may better resist to dehydration). However, the smaller morphs of *P. taida* and *S. taipeiensis* exited most frequently; for *Philotrypesis*, it may be that the largest males of the typical morph exited (i.e., the head width is the only significant factor to explain the exit rate). The fact of smaller morphs exiting more frequently is somewhat counterintuitive because figs of *F. benguetensis* are patrolled by numerous ants (Lin et al. 2016) and are, on average, more frequently and more severely injured (for *Philotrypesis*) than the larger morphs; thus, their chances of successful dispersal are low. Smaller males may exit as an alternative strategy to avoid more competitive fighting males inside figs, and because figs can be closely clustered together (Suppl. material 2: Fig. S2), exited males may find females in neighboring figs. Finally, it seems that males with severe injuries are less inclined to exit figs than are males with minor injuries.

Considering the high probability of being captured by ants (Lin et al. 2016), the cost of dispersal seems very high; however, because a fig regularly contains few mothers (in three *Sycoscapter* species) and because 10%–22% of females are born in figs without conspecific males (Cook et al. 2017), mating in one's non-natal fig would be extremely rewarding from a genetic perspective. The dispersal strategy could be adopted by less successful fighting males, but not by the smallest males.

Conclusion

The findings of this study confirm the type of dimorphism that has been described in other *Philotrypesis* species and reveal a new dimorphism type in the Sycoryctinae subfamily. Some intermorph values found in this study could be used as insights for behavioral differences between morphs related to aspects such as fighting or exiting figs. Our research provides basic information that could lead to behavioral studies of these males and the environmental factors that may affect the choices of fig wasp mothers to produce any morph at an oviposition site.

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Supplementary material 1

Table S1. Thirty sampled figs were haphazardly collected from *Ficus benguetensis* trees within five natural habitats of Taiwan

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Data type: species data

Link: https://doi.org/10.3897/jhr.74.36461.suppl1

Supplementary material 2

Figures

Authors: Da-Mien Wong, Anthony Bain, Shiuh-Feng Shiao, Lien-Siang Chou

Data type: multimedia

Explanation note: **Figure S1.** The heads from two *Philotrypesis taida* individuals. **Figure S2.** Cluster of *Ficus benguetensis* figs in the National Taiwan University campus, Taipei, Taiwan.

Link: https://doi.org/10.3897/jhr.74.36461.suppl2

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Surveys of stink bug egg parasitism in Asia, Europe and North America, morphological taxonomy, and molecular analysis reveal the Holarctic distribution of *Acroclisoides sinicus* (Huang & Liao) (Hymenoptera, Pteromalidae)

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Abstract

Halyomorpha halys is an invasive, widespread stink bug for which only short-term solutions are currently available for pest control worldwide. The need for long-term management solutions for *H. halys* has driven studies on augmentative and classical biological control of this species, especially by its egg parasitoids. Numerous investigations in Asia, USA, and Europe on native and exotic egg parasitoids of *H. halys*, and the effects on non-target pentatomids, have improved the global knowledge of parasitoid-host relationships, uncovered new associations, and led to the discovery of new species. This trend continues with *Acroclisoides sinicus*, a pteromalid that was described in the 1980's from Asia. In this work we report recent findings of this species in North America and Europe. Moreover, we propose that *Acroclisoides solus* **syn. nov.**, a species described originally from the USA, is conspecific with *A. sinicus* based on morphological and molecular analysis.

Keywords

brown marmorated stink bug, egg parasitoids, exotic species, biological control

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is a stink bug originating from eastern Asia, which in the last three decades has invaded many regions worldwide: North America in the mid-1990s, followed by Europe in the mid-2000s and, more recently, South America (Hoebeke and Carter 2003; Wermelinger et al. 2008; Faúndez and Rider 2017). In its native range, natural enemies, including egg parasitoids, are among the primary factors controlling its populations (Yang et al. 2009; Lee 2015; Leskey and Nielsen 2018). Egg parasitoids of *H. halys* have followed this species in its spread to new areas, likely transported as they develop in eggs laid on plants or other materials. *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), commonly known as the samurai wasp, was first detected in 2014 in the eastern USA, followed by a second discovery in the northwestern USA in 2016 (Talamas et al. 2015; Milnes et al. 2016). It has since spread north to Canada, where it has been present since at least 2017 (Abram et al. 2019; Gariepy and Talamas 2019). *Trissolcus japonicus* was recently detected in Europe, first in Switzerland in 2017 and then in Italy in 2018 (Sabbatini Peverieri et al. 2018; Moraglio et al. 2019; Stahl et al. 2019). Moreover, *T. japonicus* is not the only species that has followed *H. halys* in its worldwide expansion: the Asian egg parasitoid *Trissolcus mitsukurii* (Ashmead) (Hymenoptera: Scelionidae) was detected for the first time in Europe (northeastern Italy) in 2018 (Sabbatini Peverieri et al. 2018).

These examples of egg parasitoids following their host in the colonization of new areas are not isolated cases. There are many examples, including *Aprostocetus fukutai* Miwa & Sonan (Hymenoptera: Eulophidae) that recently followed its host *Anoplophora chinensis* (Förster) (Coleoptera: Cerambycidae) from eastern Asia to Europe (Delvare et al. 2004; Hérard et al. 2017).

However, not all adventive parasitoids are beneficial for controlling populations of *H. halys*. *Acroclisoides* Girault and Dodd (Hymenoptera: Pteromalidae) was established in 1915 and currently comprises just over a dozen species in the Afrotropics, Australia, and South Asia. A single species with a disjunct distribution, *Acroclisoides solus* Grissell & Smith, 2006 (Hymenoptera: Pteromalidae), was described from North America (Grissell and Smith 2006; see Noyes 2019) (Table 1). *Acroclisoides* includes species that are apparently facultative or obligate hyperparasitoids, in most cases of pentatomid eggs parasitized primarily by scelionids, but also by eupelmids (Clarke and Seymour 1992; Sureshan and Narendran 2002; Grissell and Smith 2006).

We present the records of *Acroclisoides sinicus* (Huang & Liao, 1988) (Hymenoptera: Pteromalidae) from central Europe and the USA. We treat *A. solus* syn. nov. as a new junior synonym of *A. sinicus* based on morphological and molecular comparisons, revealing that *A. sinicus* is a widespread Holarctic species. Our analysis of *COI* diversity, and the distribution of other species in the genus, suggest that the European and North American populations are recent introductions.

Material and methods

Surveys in Friuli Venezia Giulia region (northeastern Italy)

Field surveys were conducted in 2018 by personnel of the local Plant Protection Service (ERSA Friuli Venezia Giulia) in Cordenons commune (46.0082N, 12.6713E) as a part of routine monitoring of *H. halys* in the region. During these surveys, more than a dozen egg masses were found to be parasitized by *T. mitsukurii* (Sabbatini Peverieri et al. 2018). In Cordenons, on August 7, three *H. halys* egg masses collected on a *Robinia pseudoacacia* L. hedgerow near an IPM apple orchard appeared to be parasitized (visually detected by the dark color of the eggs). Collected egg masses were reared in a climatic chamber (26 °C, 65% RH, 16:8 L:D) until emergence of parasitoids. Emerged parasitoid specimens were stored in ethanol for further taxonomic and molecular analysis.

Surveys in Veneto region (northeastern Italy)

In the Veneto region, field pest surveys were conducted by personnel of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, during the summers of 2017 and 2018. At three sites in the vicinity of Povegliano (45.7575N, 12.1872E), Montebelluna (45.7586N, 12.0174E) and Riese Pio X (45.7170N, 11.9397E), dozens of *H. halys* egg masses were collected in apple and kiwi orchards and vineyards implementing integrated pest management and in surrounding hedgerows of *R. pseudoacacia*, *Acer campestris* L., *Sambucus nigra* L. and *Prunus* sp. Collected egg masses were reared in a climatic chamber (26 °C, 65% RH, 16:8 L:D) until emergence of parasitoids. Emerged parasitoid specimens were stored in ethanol for further taxonomic and molecular analysis.

Table 1. Worldwide *Acroclisoides* species[1].

Species	Distribution	Citations of primary host (phytophagous)	Citations of primary parasitoid host	References
Acroclisoides africanus Ferrière, 1940	Central Africa, Madagascar	*Atelocera notatipennis* Stål; *Antestiopsis facetoides* Greathead; *Antestiopsis intricata* (Ghesquire & Carayon); *Antestiopsis orbitalis* (Westwood); *Antestiopsis thunbergii* (Gmelin); *Bathycoelia rodhaini* Schouteden; *Bathycoelia thalassina* (Herrich-Schaeffer); Pentatomidae species	*Trissolcus* sp. *Asolcus* sp.	Ferrière 1940; Grissell and Smith 2006; see Noyes 2019[2]
Acroclisoides bicolor Luo & Qin, 1991	China	*Halyomorpha picus* (Fabricius)	n.a.	Luo and Qin 1991; Xiao and Huang 2000
Acroclisoides indicus Ferrière, 1931	China, India, Sri Lanka, Myanmar	*Placosternum dama* (Fabricius); *Erthesina* sp.; Pentatomidae species	n.a.	Ferrière 1931; Luo and Qin 1991; Xiao and Huang 2000; Sureshan and Narendran 2002; Grissell and Smith 2006; Sureshan 2007; see Noyes 2019[2]
Acroclisoides laticeps Girault & Dodd, 1915	Australia	n.a.	n.a.	Girault and Dodd 1915; see Noyes 2019[2]
Acroclisoides luzonensis Gahan, 1920	China, Philippines	*Tectocoris lineola* (Fabricius); Pentatomidae species; Scutelleridae species	*Trissolcus banksi* (Gahan) *Trissolcus* sp.	Gahan 1920; Grissell and Smith 2006; Xiao and Huang 2000; see Noyes 2019[2]
Acroclisoides maculatus Sureshan & Narendran, 2002	India	n.a.	n.a.	Sureshan and Narendran 2002
Acroclisoides major Girault & Dodd, 1915	Australia	n.a.	n.a.	Girault 1915; see Noyes 2019[2]
Acroclisoides megacephalus Girault & Dodd, 1915	Australia	*Axiagastus cambelli* Distant	*Anastatus* sp. *Trissolcus painei* (Ferrière)	Girault 1915; Grissell and Smith 2006; see Noyes 2019[2]
Acroclisoides quintus Xiao & Huang, 2000	China	n.a.	n.a.	Xiao and Huang 2000
Acroclisoides sinicus (Huang & Liao, 1988)	China, Korea, Italy, Switzerland, USA	Pentatomidae species (China, Korea); *Halyomorpha halys* (Stål); *Palomena prasina* L.; *Chinavia hilaris* (Say); *Euschistus* sp.; *Brochymena* sp.	n.a. (China, Korea); *Anastatus bifasciatus* (Geoffroy); *Anastatus reduvii* (Howard); *Trissolcus edessae* Fouts; *Trissolcus euschisti* (Ashmead); *Trissolcus japonicus* (Ashmead); *Trissolcus mitsukurii* (Ashmead)	Huang and Liao 1988; Xiao and Huang 2000; Grissell and Smith 2006; Ko et al. 2018; present work
Acroclisoides sativa Kumar & Khan, 2012	India	*Phytomyza atricornis* Meigen	n.a.	Kumar and Khan 2012
Acroclisoides solus Grissell & Smith, 2006	USA, Canada, Italy	n.a. (USA); *Acrosternum hilare* Say (Canada); *Arma custos* Fabricius (Italy)	n.a. (USA and Italy), *Trissolcus* sp. (Canada)	Grissell and Smith 2006; Gariepy et al. 2014; Moraglio et al. 2019
Acroclisoides spilopterus (Masi, 1917)	Seychelles	n.a.	n.a.	Masi 1917; Grissell and Smith 2006; see Noyes 2019[2]
Acroclisoides tectacorisi (Girault, 1924)	Australia	*Biprorulus bibax* Breddin; *Oechalia consocialis* (Boisduval); *Tectocoris banksii* (Donovan); *Tectocoris lineola* (Fabricius)	*Trissolcus biproruli* Girault[3]; *Anastatus biproruli* Girault	Girault 1924; Grissell and Smith 2006; James 1990; see Noyes 2019[2]

[1] *Acroclisoides borocerae* (Risbec 1957) is not listed here since it needs to be transferred to another genus (Mircea-Dan Mitroiu, unpublished data).

[2] see Noyes 2019 for additional references.

[3] possible hosts based on the presumption that *A. sinicus* is a hyperparasitoid of *Trissolcus* and *Anastatus*.

Surveys in Trentino-Alto Adige/Südtirol region (northern Italy)

In 2018, during monitoring of *H. halys*, naturally laid egg masses of *H. halys* were collected in a parking area in the municipality of Ora (46.3620N, 11.2985E), in the province of Bolzano. Several species of maple trees (*Acer platanoides* L., *Acer negundo* L. and *Acer pseudoplatanus* L.), ailanthus [*Ailanthus altissima* (Mill.) Swingle] and linden (*Tilia platyphyllos* Scop.) were sampled for egg masses once or twice per week. Egg masses were collected between September 10 and October 26. Field-collected eggs were reared in a climatic chamber at 25 ± 1 °C, 65 ± 5% RH, 16:8 L:D until *H. halys* nymphs or parasitoids emerged. Emerged parasitoids were stored in 70% ethanol for further analysis.

Surveys in Zurich city (northeastern Switzerland)

In 2019, natural egg masses of *H. halys* (n = 11) and *Palomena prasina* L. (Hemiptera: Pentatomidae) (n = 6) were collected in an urban park area at the lake of Zurich (district Seefeld, 47.355912N, 8.550674E), where adventive *H. halys* populations were first detected in Europe in 2007 (Wermelinger et al. 2008). Trees inspected for eggs included *Catalpa bignonioides* Walter, *Paulownia tomentosa* (Thunb.) Steud., *Tilia platyphyllos*, and *Liriodendron tulipifera* L. Egg masses were collected on three occasions (June 28, July 2 and 10). Field collected egg masses were kept in small Petri-dishes and stored at 25 ± 1 °C, 60% RH, 16:8 L:D until nymphs and parasitoids had emerged. Emerged parasitoid specimens were stored in ethanol for further taxonomic and molecular analysis.

Surveys in Alabama and Georgia (southeastern USA)

In 2017 and 2018, during monitoring activities of stink bugs and their egg parasitoids, two sites yielded naturally laid stink bug egg masses from which *Acroclisoides* emerged. One was an egg mass of *H. halys* collected from a pecan tree [*Carya illinoinensis* (Wangenh.) K. Koch] close to a commercial organic blueberry farm in Auburn (32.5325N, 85.4316W), Lee County, Alabama. Three egg masses of *Chinavia hilaris* (Say) (Hemiptera: Pentatomidae) were collected from a mimosa tree (*Albizia julibrissin* Durazz) in Irwin County, Georgia (31.3339N, 83.1926W). The egg masses were reared in a walk-in environmental chamber at 25 ± 2.0 °C, 50 ± 10% RH, and 12:12 L:D photoperiod for parasitoid emergence.

Surveys in Maryland (mid-Atlantic USA)

In 2017 and 2018, Project ‘Stink-be-Gone’, a citizen science project in collaboration with University of Maryland Extension and Master Gardeners, was established in central and western Maryland to monitor for stink bug egg masses, including *H. halys*. Sampling occurred for an average of one hour per week for six weeks (July and August 2017; late June to early August 2018). Participants recorded the time spent searching and collected GPS coordinates and general habitat characteristics (e.g., "private yard" or "park") for all survey periods regardless of collection results. If egg masses were found, the host plant was identified to genus or species. Following collection, all egg masses and collection data were immediately sent to researchers at the University of Maryland, College Park for processing. Samples were placed in a growth chamber (Model 36LLVL, Percival Scientific, Perry, Iowa, USA) at 25 °C and 16:8 L:D. Egg masses were monitored daily for emergence of either bug nymphs or parasitoids. Parasitoids were transferred to 70% ethanol for later identification to species (all *Trissolcus* Ashmead and *Telenomus* Haliday and females of *Anastatus* Motschulsky) or genus (*Anastatus* males, see Burks 1967). After six weeks, unhatched eggs were dissected to ascertain their fate (e.g., unemerged bug nymph or parasitoid adult, partially developed parasitoid, infertile bug egg). Egg masses were identified to genus (unpublished key by Dieckhoff and Hoelmer). Master Gardeners collected egg masses of eight stink bug genera throughout both summers.

Origin of other material examined

Other samples of *Acroclisoides* specimens used in the molecular analysis were collected in China and South Korea by Kim Hoelmer from 2014–2017 and by Lucian Fusu during field trips conducted in 2016 (see results, Table 4). Paratypes of *A. solus* were provided on loan from the National Museum of Natural History, Smithsonian Institution, Washington, D.C. The remaining sequences analyzed here were mined from GenBank.

Morphological analysis and material examined

The following keys and taxonomic works were used for the identification of *Acroclisoides* species: Grissell and Smith (2006), Sureshan and Narendran (2002), Xiao and Huang (2000), Huang and Liao (1988), Masi (1917), Girault and Dodd (1915).

The examined material listed below (all initially unidentified Pteromalidae, except paratypes of *A. solus*), including vouchers used in the molecular analyses, is deposited in the following institutions: CABI, Delémont (Switzerland); CREA, Florence (Italy); DAFNAE, University of Padua, Padova (Italy); EBCL, Montferrier le Lez (France); ERSA, Udine (Italy); FSCA, Florida State Collection of Arthropods, Florida (USA); Laimburg RC, Laimburg Research Centre, Vadena (Italy); MICO, Mitroiu Collection, Iași (Romania); NHMB, Natural History Museum Bern (Switzerland).

Italy: 19♀♀, 9♂♂, Cordenons, Friuli V. G., 46.0082N, 12.6713E, 8.viii.2018, Iris Bernardinelli, Giorgio Malossini & Luca Benvenuto leg., on *Halyomorpha halys* eggs on *Robinia pseudoacacia* (6♀♀, 3♂♂, CREA; 9♀♀, 6♂♂, ERSA; 1♀, FSCA; 3♀♀, MICO); 1♀, 8 unsexed, Riese Pio X, Veneto, 29.viii.2017, Paola Tirello and Davide Scaccini leg., on *Halyomorpha halys* eggs on *Vitis vinifera* (1♀, MICO; 8 unsexed DAFNAE); 3♀♀, 3 unsexed, Montebelluna, Veneto, 29.viii.2017, Paola Tirello and Davide Scaccini leg., on *Halyomorpha halys* eggs on *Vitis vinifera* (1♀, 3 unsexed, DAFNAE; 2♀♀, MICO); 4♀♀, 5 unsexed, Povegliano, Veneto, 16.viii.2017, Paola Tirello and Davide Scaccini leg., on *Halyomorpha halys* eggs on *Actinidia* sp. (1♀, 5 unsexed, DAFNAE; 3♀♀, MICO); 12♀♀, 2 unsexed, Ora, Trentino-Alto Adige/Südtirol, 46°21'43.3"N, 11°17'54.6"E, 27.ix.2018, Martina Falagiarda, on *Halyomorpha halys* eggs on *Acer* sp. (10♀♀, 2 unsexed, Laimburg RC; 2♀♀, MICO).

Switzerland: 5♀♀, 3♂♂, Zurich city, Canton Zurich, Lat. 47.351708 Long. 8.559493, 2.vii.2019, Tim Haye and Emily Grove leg., ex eggs of *Halyomorpha halys* on *Tilia platyphyllos* (ZP4), (1♀, MICO; 1♀, NHMB, 3♀♀, 3♂♂, CABI); 9♀♀, 6♂♂, Zurich city, Canton Zurich, Lat. 47.353213 Long. 8.553968, 10.vii.2019, Emily Lemke and Emily Grove leg., ex eggs of *Halyomorpha halys* on *Liriodendron tulipifera* (ZP6), (1♀, MICO; 3♀, NHMB; 5♀♀, 6♂♂, CABI); 12♀♀, 8♂♂, Zurich city, Canton Zurich, Lat. 47.353213 Long. 8.553968, 10.vii.2019, Emily Lemke and Emily Grove leg., ex eggs of *Palomena prasina* on *Liriodendron tulipifera* (ZP7), (1♀, 1♂, MICO; 1♀, 1♂, NHMB; 10♀♀, 6♂♂, CABI); 6♀♀, 6♂♂, Zurich city, Canton Zurich, Lat. 47.354905 Long. 8.535045, 10.vii.2019, Emily Lemke and Emily Grove leg., ex eggs of *Palomena prasina* on *Catalpa bignonioides* (ZP8), (1♀, 1♂, MICO; 1♀, 1♂, NHMB; 4♀♀, 4♂♂, CABI).

South Korea: 5♀♀ [GB] Gyeongsan-si, Daehak-ro, 280, Yeungnam Univ., 35°49'11.6"N, 128°45'53.6"E, 14.viii.2016, L. Fusu (MICO); 1♀ S. Korea: Chungbuk, Okcheon-gun, Bougimyeon, Soesan-li, 150 m, Malaise trap, 10.ix–03.x.2004, 36°16.594'N, 127°36.742'E, Tripotin rec. (MICO).

USA: 2♀♀ paratypes of *A. solus*, VA: Fairfax Co. near Annandale 38°50'N, 77°12'W Aug. 17–20 2004 Malaise trap David R. Smith; 2♀♀ Alabama, Lee Co., Auburn, ex BMSB eggs on pecan, R. Balusu and G. Tillman 25.vii.2018 (FSCA); 1♀ Georgia, Irwin Co., ex *Chinavia hilaris* eggs on mimosa tree, G. Tillman 24.vii.2017 (FSCA); 3♀♀ Georgia, Irwin Co., ex *Chinavia hilaris* eggs on mimosa tree, G. Tillman 21.vii.2017 (FSCA).

Abbreviations of morphological terms: F, funicular segment; GT, gastral tergite; MT, metasomal tergite; MV, marginal vein; OOL, ocello-ocular line; PV, postmarginal vein; POL, posterior ocellar line; SM, submarginal vein; SV, stigmal vein.

Molecular analysis

The DNA extraction, PCR amplification and sequencing of all the specimens listed in Table 4 were conducted in three different laboratories: the USDA-ARS – European Biological Control Laboratory (EBCL), the Florida Department of Agriculture and Consumer Service – Florida State Collection of Arthropods (FSCA), Division of Plant Industry (FDACS-DPI), and the Research Group in Invertebrate Diversity and Phylogenetics at the University of Iasi (UAIC). Genomic DNA was nondestructively isolated from the entire specimen using the Qiagen DNeasy kit (Hilden, Germany) at EBCL and FSCA as described in Sabbatini Peverieri et al. (2018) and at UAIC following the protocol developed by Cruaud et al. (2019). The barcode region of the mitochondrial Cytochrome Oxidase Subunit I (*COI*) was amplified using the universal barcoding primers LCO1490 and HCO2198 (Folmer et al. 1994). Amplification and sequence editing were done at EBCL and FSCA as described in Sabbatini Peverieri et al. (2018) and at UAIC as reported in Fusu and Ribes (2017).

Two *A. solus* samples required troubleshooting for successful *COI* barcoding. The samples USNMENT01335770 (MN018863.1) and FSCA00090246 (MN018864.1) both yielded *Wolbachia COI* sequences when using the universal barcoding primers (Folmer et al. 1994) and LEP-F1/LEP-R1 (Hebert et al. 2004) (see Smith et al. 2012 for an explanation of this phenomenon). These samples were alternatively amplified and sequenced with the primer pair C1-J-1632/C1-N-2191 (Kambhampati and Smith 1995; Simon et al. 1994). The thermocycling conditions for C1-J-1632/C1-N-2191 were: 1) initial denaturing at 95 °C for 2 minutes, 2) 98 °C for 20 seconds, 3) 40 °C for 30 seconds, 4) 72 °C for 30 seconds [steps 2–4 repeated for 30 cycles], and 5) final extension at 72 °C for 7 minutes. Slightly shorter (432 bp and 484 bp) *COI* barcode sequences were generated from these samples using this primer pair.

All sequences generated from this study are deposited in GenBank and all residual DNAs are archived at the place of the DNA extraction (Table 4). Voucher specimens which have been reexamined following the molecular analysis are presently deposited in public collections or in laboratory collections (Table 4). All barcode sequences were translated into amino acids to check for stop codons. The 45 sequences obtained were compared with sequences present in GenBank using the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLASTn) and aligned with the four barcode sequences of *A. solus* previously available. The alignment was performed using Clustal Omega (Sievers and Higgins 2014), implemented in Seaview version 4 (Gouy et al. 2010). Polymorphism information, haplotype diversity (*Hd*), nucleotide diversity (π) and number of haplotypes were determined using DnaSP version 6 (Rozas et al. 2017). The phylogenetic relationships among haplotypes were depicted using statistical parsimony in TCS (Clement et al. 2000) implemented in PopART (Leigh and Bryant 2015) which allows visualization of the frequency and geographical distribution of haplotypes.

The molecular distances between sequences of all individuals from different countries were calculated by the standard Kimura 2-parameter (K2P) measure (Kimura 1980) using Mega 6 (Tamura et al. 2013). The range of *COI* K2P distances found between *A. sinicus* haplotypes was compared to distances in the subfamily Pteromalinae as a whole using i) intraspecific distances, and ii) congeneric distances. The Pteromalinae DNA sequences used in this study were downloaded from GenBank and searched locally using software from the Blast+ toolkit. To create the Pteromalinae database used in this study, we targeted only genera represented by at least two identified species, and each species was represented by at least two sequences. The qualifying sequences were then extracted and aligned with the barcode sequence MK188331.1 of *A. solus.* The aligned Pteromalinae sequences were checked by eye and the edges trimmed using Seaview version 4 and imported into Mega 6 to calculate the molecular distances. The molecular distances were then split into intraspecific observations and interspecific (congeneric) observations.

Results

Surveys in Friuli Venezia Giulia region (northeastern Italy)

Our taxonomic studies determined that the *Acroclisoides* specimens found in Cordenons in 2018 belong to the species *A. sinicus*. From the three collected *H. halys* egg masses a total of 28 specimens of *A. sinicus* emerged (Table 2). Specimens of *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae), a well-known European native parasitoid that can parasitize eggs of *H. halys* (Haye et al. 2015; Roversi et al. 2016), also emerged. Detailed observation of the parasitized eggs revealed consistent differences in the exit holes produced by *A. sinicus* and *An. bifasciatus*, enabling eggs to be associated with the species that parasitized them.

Males of *An. bifasciatus* are visibly smaller than females (Bolivar y Pieltain 1935), and the circular exit holes that they produce are smaller than those of the larger females (Fig. 1A, B). *Acroclisoides sinicus*, independent of sex, produced exit holes on *H. halys* eggs of similar size to those of females of *An. bifasciatus*, but with much more irregular margins (Fig. 1C, D). *Trissolcus mitsukurii* (associated with *A. sinicus* in the Veneto region), produced round exit holes similar in size to females of *An. bifasciatus*, but with less serrate margins and a conspicuous dark ring on the upper margin of the parasitized eggs, just below the exit hole and the operculum (Fig. 1E). These observations permitted us to assess the number and species of parasitoids that emerged prior to the field collection of the egg masses (see Table 2). Emergence holes produced by *T. japonicus* (associated with *A. sinicus* in Zurich city, see results of Switzerland) are similar to those produced by *T. mitsukurii*, except there is no dark ring below the operculum (Fig. 1F).

Surveys in Veneto region (northeastern Italy)

Acroclisoides specimens found at the three localities of Povegliano, Montebelluna and Riese Pio X belong to the species *A. sinicus* as confirmed by our taxonomic study. In 2017, 24 *A. sinicus* specimens emerged from three collected *H. halys* egg masses. Egg masses parasitized by *A. sinicus* were collected on August 16 (one egg mass from Povegliano site on *Actinidia* sp.) and August 29 (two egg masses, one from Montebelluna

Table 2. Parasitoids that emerged from stink bug eggs collected in Cordenons and Ora (Italy) and in Zurich city (Switzerland).

Site	Pentatomid host species	Plant host genus/ species	N of eggs/ mass	N of eggs hatched	N of *A. sinicus* emerged	N of other parasitoids emerged	N of eggs unhatched, with no parasitoids emerged
Cordenons	*H. halys*	*Robinia pseudoacacia*	15	0	8 (5♀♀, 3♂♂)	2 *An. bifasciatus* (2♂♂)	5 (unidentified content[1])
		Robinia pseudoacacia	28[2] 14[2]	0 0	20 (14♀♀, 6♂♂)	3 *An. bifasciatus* (2♂♂, 1♀♀)	12 (unidentified content[3]) 0
Ora	*H. halys*	*Acer* sp.	14	0	14 (12♀♀, 2 unsexed)	0	0
Zurich city	*H. halys*	*Tilia platyphyllos*	28	0	8 (5♀♀, 3♂♂)	1 *T. japonicus* (1♀)	19 (2 parasitized by *T. japonicus*, 1 scelionid pupa, 16 unidentified content)
		Liriodendron tulipifera	20	0	15 (9♀♀, 6♂♂)	0	5 (1 parasitized by *T. japonicus*, 4 unidentified content)
	P. prasina	*Liriodendron tulipifera*	27	0	20 (12♀♀, 8♂♂)	0	7 (1 parasitized by *A. sinicus*, 6 unidentified content)
		Catalpa bignonioides	26	0	12 (6♀♀, 6♂♂)	12 *T. japonicus* (1♂, 11♀♀)	2 (unidentified content)

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[1] One egg was suspected to be parasitized by *T. mitsukurii* due to the presence of a black ring below the operculum of the egg.

[2] The two egg masses were found on the same leaf and reared together in the same vial. From these two egg masses, 7 parasitoids emerged in the field prior to collection; an estimation of total number of egg parasitoids emerged assessed through analysis of exit holes revealed that from the two egg masses collected, the following egg parasitoids emerged: 12 *A. sinicus* and 4 *An. bifasciatus* (1♀, 3♂♂) in the first egg mass, and 10 *A. sinicus* and 4 *An. bifasciatus* (3♀♀, 1♂) in the second egg mass.

[3] Suspected to be all parasitized by *T. mitsukurii* due to the presence of a black ring below the operculum of the eggs.

and one from Riese Pio X both on *Vitis vinifera* L.). Parasitism of single egg masses by different parasitoid species was also observed: from the egg mass containing six *A. sinicus* individuals in Montebelluna, 15 individuals of *T. mitsukurii* also emerged; from the egg mass in Povegliano from which nine *A. sinicus* individuals emerged, 10 *An. bifasciatus* also emerged; but from the *H. halys* egg mass collected in Riese Pio X, only *A. sinicus* emerged (nine individuals). No *Acroclisoides* individuals were found in the monitoring campaign of 2018.

Surveys in Trentino-Alto Adige/Südtirol region (northern Italy)

From the *H. halys* egg masses collected in Ora, only one egg mass (collected on September 27, 2018) containing 14 eggs produced 14 specimens of *A. sinicus*. No other parasitoid species or *H. halys* nymphs emerged from the egg mass.

Surveys in Zurich city (northeastern Switzerland)

Acroclisoides individuals were reared from two *H. halys* egg masses and two egg masses of the native stink bug, *P. prasina* (Table 2). The first *A. sinicus* emerged from a single *H. halys* egg mass collected from *Tilia platyphyllos* on July 2, 2019. From the same egg mass a single female of *T. japonicus* was reared (Fig. 1F). On July 10, 2019 single *H. halys* and *P. prasina* egg masses were found on the same *L. tulipifera* tree, both generat-

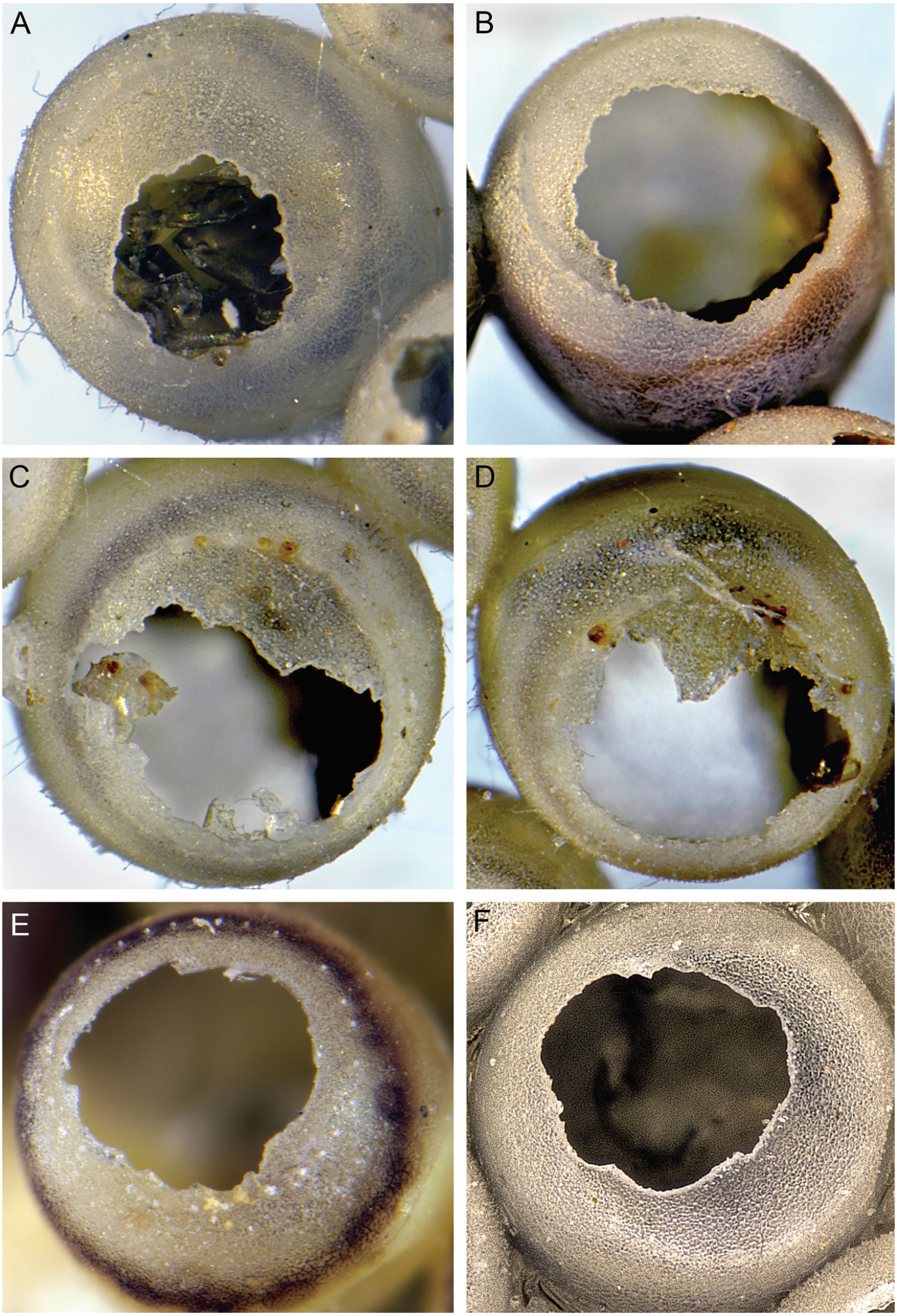

Figure 1. Typical shapes of exit holes produced by egg parasitoids of *Halyomorpha halys* in Europe: *Anastatus bifasciatus* male (**A**) and female (**B**) (NE-Italy); *Acroclisoides sinicus* (**C, D**) (NE-Italy); *Trissolcus mitsukurii* (**E**) (NE-Italy) and *Trissolcus japonicus* (**F**) (Switzerland).

ing *Acroclisoides* individuals. From an additional *P. prasina* egg mass collected on the same date on *C. bignonioides*, 12 *A. sinicus* emerged along with 12 *T. japonicus.* No *Acroclisoides* individuals emerged from egg masses collected June 28, 2019.

Surveys in Alabama and Georgia (southeastern USA)

Of the three *H. halys* egg masses collected in Auburn, AL, during the summer of 2018, one egg mass collected from pecan on July 25 produced six specimens of *A. sinicus* (Table 3). Nine *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae) and seven *Anastatus reduvii* (Howard) (Hymenoptera: Eupelmidae), both of which are common indigenous parasitoids that sometimes parasitize *H. halys* in the USA (Cornelius et al. 2016), also emerged from this egg mass. One scelionid pupa died; four eupelmid late instars died. Of the nine *C. hilaris* egg masses collected in Irwin County, GA, during the summer of 2017, three egg masses collected from mimosa produced *A. sinicus*. Fifteen *A. sinicus* emerged from one *C. hilaris* egg mass collected from mimosa on July 21, 2107, and from this egg mass emerged 49 *Trissolcus edessae* Fouts (Hymenoptera: Scelionidae) and two *An. reduvii*. The second *C. hilaris* egg mass collected from this plant on the same date produced 31 *A. sinicus* and no other parasitoids. From the third egg mass of *C. hilaris*, collected on mimosa on July 24, 2017, one *A. sinicus* and 53 *T. edessae* emerged.

Surveys in Maryland (mid-Atlantic USA)

Acroclisoides sinicus emerged from two egg masses of *Euschistus* sp. (Hemiptera: Pentatomidae) collected on August 15 and 27, 2017 in Montgomery (39.2063N, 77.2079W)

Table 3. Parasitoids that emerged from stink bug egg masses collected in Alabama and Georgia (southeastern USA) and in Maryland (mid-Atlantic USA).

Site	Pentatomid host species	Plant host genus/ species	N of eggs/ mass	N of eggs hatched	N of *A. sinicus* emerged	N of other parasitoids emerged	N of eggs unhatched, with no parasitoids emerged
Auburn, AL	*H. halys*	*Carya illinoinensis*	32	5	6 (4♀♀, 2♂♂)	9 *T. euschisti* (9♀♀); 7 *An. reduvii* (3♀♀, 4♂♂)	5 (1 scelionid pupa, 4 eupelmid late instars)
Irwin Co., GA	*C. hilaris*	*Albizia julibrissin*	71	5	15	49 *T. edessae*; 2 *An. reduvii* (2♀)	0
			56	22	31	0	3 (unidentified content)
			56	2	1	53 *T. edessae*	0
Montgomery Co., MD	*Euschistus* sp.	*Cornus* sp.	14	0	5 (3♀♀, 2 unsexed)	0	9 (all eggs parasitized but only 2 unemerged *A. sinicus* identified)
Frederick Co., MD	*Euschistus* sp.	*Cercis canadensis*	14	0	2	0	12 (9 eggs parasitized, 7 by *Telenomus* sp. and 2 unidentified, 3 eggs predated)
			13	0	12 (8♀♀, 2♂♂, 2 unsexed)	0	1 (parasitized, unidentified)
Allegany Co., MD	*Brochymena* sp.	*Lonicera japonica*	12	0	6 (4♀♀, 2 unsexed)	0	6 (5 parasitized by *A. sinicus*)

and Frederick (39.4760N, 77.2703W) Counties, respectively (Table 3). In Montgomery County, five *A. sinicus* emerged from the egg mass collected on *Cornus* sp. in a private home site with a wooded landscape of mixed deciduous trees, shrubs and herbaceous plants. In Frederick County, 14 *A. sinicus* emerged from two egg masses collected from *Cercis canadensis* L. located in the Fountain Rock Park and Nature Center (landscape has a mix of deciduous trees and shrubs and herbaceous plants). Six *A. sinicus* emerged from a *Brochymena* sp. (Hemiptera: Pentatomidae) egg mass collected in Allegany County (39.6786N, 78.7372W) (July 7, 2018) on *Lonicera japonica* Thunb. in the demonstration garden of the University of Maryland Extension (landscape with fruiting trees, managed and unmanaged trees, flowering herbaceous plants and shrubs, vegetable plants and mown turf). Fifteen of the emerged specimens were females, two were males, and the sex of the remainder could not be determined. No other parasitoids emerged during rearing and no stink bug nymphs emerged from any of the three egg masses.

Morphological analysis and redescription of the species

Acroclisoides Girault & Dodd, 1915

Acroclisoides Girault & Dodd, 1915: 344.
Neocoruna Huang & Liao, 1988: 426; synonymy by Xiao and Huang (2000): 94.

Diagnosis. BOTH SEXES: antenna 11263 (Figs 2E, 3B, D), inserted very high on face (Figs 2B, 3B, D); head much wider than high (Figs 2B, 3B, D), with strong occipital carina (Fig. 2C); gena with very large hollow at mouth margin (Fig. 2G); mandibles large (Figs 2B, 3B); notauli complete (Fig. 2F); fore wing with MV slightly thickened (Fig. 2D); metasoma with petiole subquadrate, smooth; gaster with GT1 narrow (Fig. 3A). FEMALE: ovipositor sheaths short, mostly concealed under terminal gastral tergites (Fig. 3A).

Acroclisoides sinicus (Huang & Liao, 1988)

Neocoruna sinica Huang & Liao, 1988: 427.
Acroclisoides sinicus (Huang & Liao, 1988); new combination by Xiao and Huang (2000): 95.
Acroclisoides solus Grissell & Smith, 2006: 925; syn. nov.

Diagnosis. BOTH SEXES: clypeal margin emarginate (Figs 2B, 3B, D); antenna with F6 whitish, occasionally also F5, the latter especially in males (Figs 2E, 3B, D); MV 0.9–1.1× SV and distinctly shorter than PV (Fig. 2D). FEMALE: fore wing usually with brownish infuscated spot, ranging from faint to pronounced, behind SV; spot

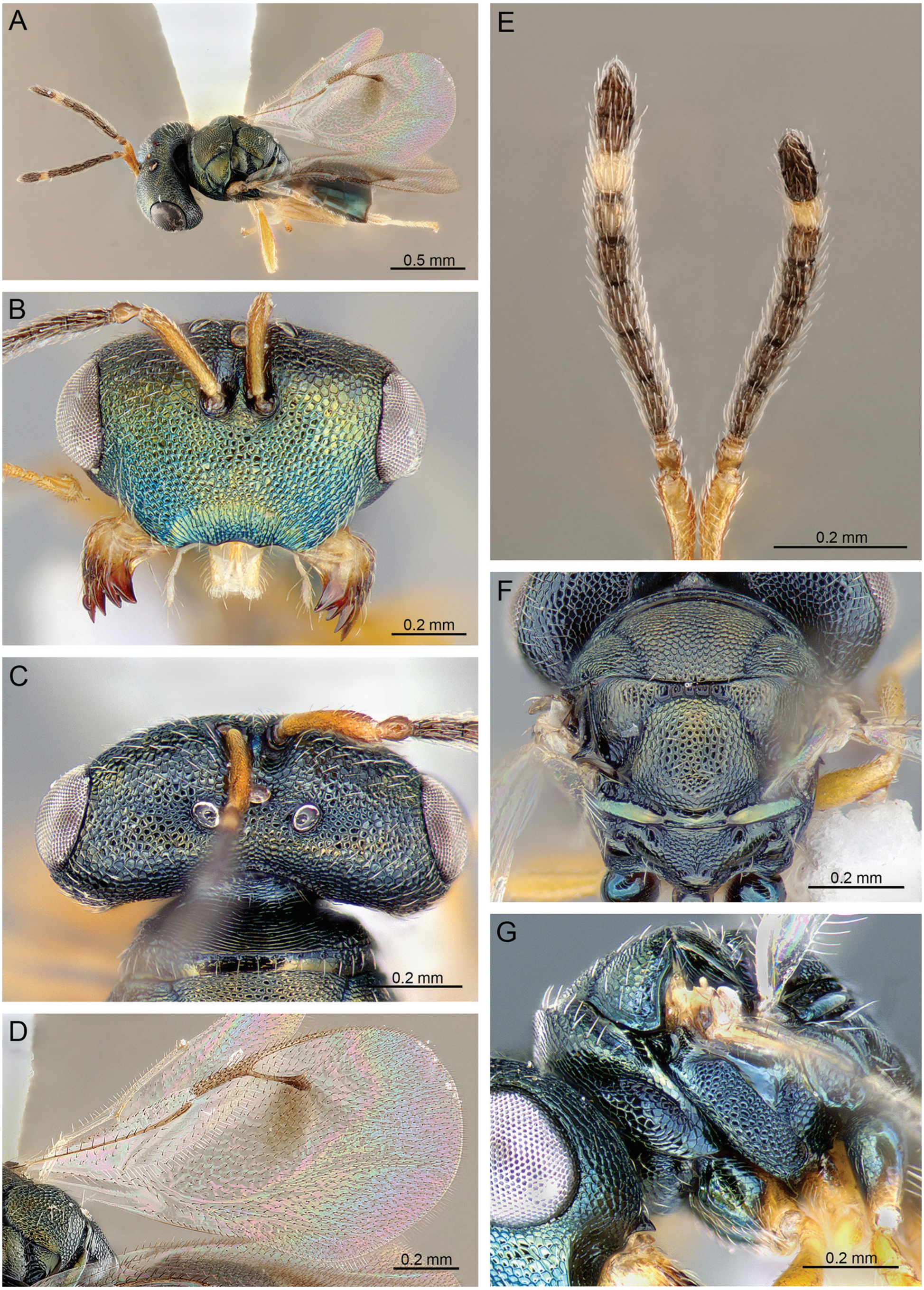

Figure 2. *Acroclisoides sinicus*, ♀ (Italy, Cordenons): habitus in dorso-lateral view (**A**); head in frontal view (**B**); head in dorsal view (**C**); fore wing (**D**); antennae (**E**); mesosoma in dorsal view (**F**); mesosoma in lateral view (**G**).

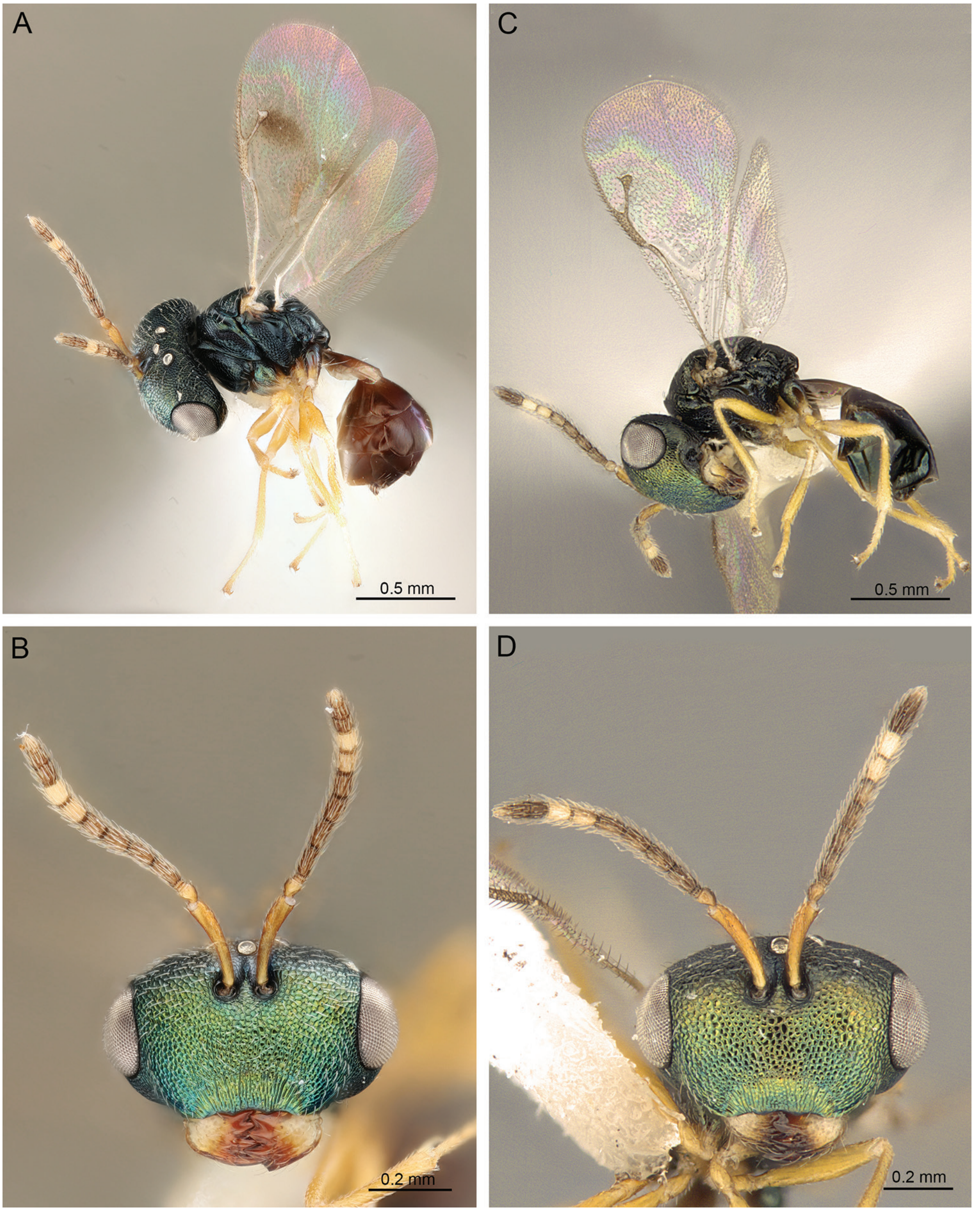

Figure 3. *Acroclisoides sinicus*: paratype of *A. solus* ♀ (U.S.A.), habitus in lateral view (**A**); idem, head in frontal view (**B**); ♂ (Switzerland), habitus in lateral view (**C**); idem, head in frontal view (**D**).

round to oval and not projecting beyond SV (Figs 2D, 3A). MALE: fore wing always hyaline (Fig. 3C).

Description. Female (Figs 2A–G, 3A, B). ***Body length.*** 1.7–2.5 mm (n = 10). ***Color.*** Head in frontal view bright green, with golden reflections (Figs 2B, 3B); frons, vertex and occiput dark olive-green (Fig. 2C, F). Antenna (Figs 2E, 3B) with scape and pedicel yellowish-brown; funicle and clava brown to dark brown, except F6 and sometimes ventral side of F5 whitish. Mandible with basal half whitish-yellow to yellowish-brown, distal half including teeth reddish-brown (Figs 2B, 3B). Mesosoma in dorsal view mainly dark olive-green (Fig. 2F); mesosoma in lateral view dark blue-green (Fig. 2G). Legs (Fig. 3A) with fore and hind coxae dark green at least dorsally, apices and sometimes ventral part yellowish to yellowish-brown; mid coxa mainly yellowish-brown, basally and dorsally at least slightly darker; the rest of leg parts yellowish-brown, except tarsal apices dark brown. Fore wing hyaline, usually with distinct brownish spot behind SV, which may be very faint or absent to very distinct (Figs 2D, 3A); hind wing hyaline (Fig. 3A); tegula and venation dark brown. Metasoma with petiole black; gaster mainly dark metallic green to brown (Figs 2A, 3A).

Head. Clypeus broadly emarginate (Fig. 2B). Occipital carina (Fig. 2C) ventrally terminating in a conspicuous tooth, visible in lateral view of the head (Fig. 2G). Antennal scrobes conspicuous, separated by interantennal crest not reaching median ocellus (Fig. 2B). Clypeal region striate-reticulate; face reticulate, alveolae getting smaller towards vertex (Fig. 2B). Width of head in frontal view about 1.6× height; in dorsal view width 2.05–2.40× length. OOL 1.4–1.6× POL. Minimum distance between eyes 2.3–2.4× eye height. In lateral view eye height 1.1–1.2× length and 1.6–1.7× malar space. In dorsal view of the head temple about 1/3 eye length or slightly less. Mandibles with 4 teeth each (Fig. 2B). Antenna with scape in lateral view gradually widening distally and reaching above level of vertex; both anelli transverse; all funicular segments longer than wide (Fig. 2E). Scape length 4.8–5.0× width. Pedicel length 1.2–1.4× width in lateral view. F1 length 1.6–1.8× width, length 1.1–1.6× pedicel length; F6 length 1.3–1.4× width; clava length 2.7–3.0× width, slightly longer than F5+F6.

Mesosoma. Pronotal collar posterior to setal row smooth. Mesoscutum and scutellum strongly and uniformly reticulate (Fig. 2F). Axillae with fine reticulation. Anterior margin of mesoscutellum separated from posterior margin of mesoscutum by several deep pits (Fig. 2F). Metascutellum virtually smooth (Fig. 2F). Propodeum (Fig. 2F) with small round basal foveae and median carina vaguely indicated, the latter extending to distinct, almost smooth nucha; median area centrally reticulate and almost smooth laterally, adjacent to conspicuous postspiracular sulci, the latter strongly convergent towards nucha; callus mainly smooth except superficially reticulate above hind coxa, with few setae. Mesepisternum reticulate; upper mesepimeron smooth, lower mesepimeron reticulate (Fig. 2G). Metapleuron reticulate. Hind coxa with dorsal basal setae extremely long. Mesosoma length 1.10–1.15× width, length 1.4–1.5× height. Mesoscutum width 2.3–2.4× length. Scutellum width 1.0–1.1× length. Propodeum median length about 0.60× scutellum length. Fore wing (Fig. 2D) with MV moderately thickened; parastigma with hyaline break; costal cell on dorsal side of the wing with single row of setae in distal half, on ventral side with several rows of setae in distal half and single anterior row extending to base; basal cell with several scattered setae and delimited by completely setose basal and cubital folds; speculum moderate, not extending beyond parastigma and closed below. Fore wing length 1.9–2.0× width. SM 3.0–3.9× MV. MV 0.9–1.1× SV. PV about 1.6× MV.

Metasoma. Dorsally flat or convex (Figs 2A, 3A). Petiole (MT1) subtriangular, about as long as wide; GT1 (MT2) long and narrow, occupying 1/3–1/4 of gaster length (Fig. 3A); GT2–4 trapezoidal, GT4 the largest; GT2 longer than GT3 and shorter than GT4; GT5–7 very short, partly to completely retracted; hypopygium extending to about 0.8–0.9× of gaster length; ovipositor sheaths short and visible only on ventral side of gaster (Fig. 3A). Gaster length 1.4–2.0× width.

Male (Fig. 3C, D). Similar to the female, it differs in having the fore wing entirely hyaline (see also the remarks below).

Remarks. *Acroclisoides sinicus*, together with *A. maculatus* Sureshan & Narendran, *A. megacephalus* Girault & Dodd and *A. spilopterus* (Masi) (Hymenoptera: Pteromalidae), belongs to a group normally having maculate fore wings in females. *Acroclisoides sinicus* can be separated from the other species cited above by the whitish color of F6, sometimes also of F5, the latter especially in males (color also slightly variable, but at least on the ventral side of the antenna the segment is slightly to distinctly lighter than other segments) and different shape, size and position of the brownish spot on the fore wing in females (usually at least slightly visible, of small to moderate size, behind the stigmal vein and not projecting beyond it). According to the original description of *A. solus* and the paratypes we examined, this species is extremely close to *A. sinicus* in most characters, including the color of the funicle and fore wing. According to Grissell and Smith (2006), both sexes of *A. solus* differ from *A. sinicus* only in having a longer PV as compared with the MV (over 1.6× versus less than 1.4× in *A. sinicus*); in addition, the female of *A. solus* has a longer F1 as compared with the pedicel (2× versus slightly longer than pedicel in *A. sinicus*), clava slightly longer than F5+F6 versus clava longer than F4+F6 in *A. sinicus*, and the flagellar setae are depressed versus outstanding in *A. sinicus*. In our specimens from Italy and South Korea (MICO), PV was about 1.6× as long as MV; F1 was 1.1–1.6× as long as the pedicel; clava was slightly longer than F5+F6; flagellar setae were moderately depressed. All examined specimens fit well with the original description of *A. sinicus* (Huang & Liao, 1988).

Males and females of *A. sinicus* are very similar. The brownish infuscation of the fore wing is found only in females but is not always present. This character can thus be used to confirm that a specimen is female, but the absence of the infuscation cannot be used to reliably determine that a specimen is male. The presence of an ovipositor, ovipositor sheaths or a projecting aedeagus can be used to confirm the sex. In cases where the terminal gastral tergites are retracted and the wings are hyaline, unambiguous determination of sex may require dissection to expose the genitalia.

Distribution. China (Huang and Liao 1988; Xiao and Huang 2000), South Korea (Ko et al. 2018), Canada, USA, Italy, Switzerland; previously recorded for Italy, USA and Canada as *A. solus* (Grissell and Smith 2006; Gariepy et al. 2014; Moraglio et al. 2019).

Table 4. Sample information, GenBank accession numbers, barcode length, DNA and voucher collection information for the *Acroclisoides* specimens included in this study.

Specimens	Country	Site/State or Region or Province	Year of Collection, Name of Collector[a]	Pentatomid host (host plant)	GenBank Accession Number	Barcode length in bp	Haplotype	DNA collection code[b]	Voucher and Collection code[c]
A. sinicus	Italy	Cordenons/Friuli Venezia Giulia	2018, IB, LB & GM	*H. halys* (*Robinia pseudoacacia*)	MN395435 (this study)	619	H1	FSCA00033132, EBCL	FSCA00033132, FSCA
		Cordenons/Friuli Venezia Giulia	2018, IB, LB & GM	*H. halys* (*Robinia pseudoacacia*)	MN413502 (this study)	621	H1	AcIt0103, UAIC	AcIt0103, MICO
		Montebelluna/ Veneto	2017, PT & DS	*H. halys* (*Vitis vinifera*)	MN395436 (this study)	652	H1	Acro1, EBCL	n.a., DAFNAE
		Povegliano/ Veneto	2017, PT & DS	*H. halys* (*Actinidia* sp.)	MN395437 (this study)	652	H1	Acro2, EBCL	n.a., DAFNAE
		Montebelluna/ Veneto	2017, PT	*H. halys* (*Vitis vinifera*)	MN413501 (this study)	622	H1	AcIt0101, UAIC	AcIt0101, MICO
		Ora, Trentino-Alto Adige/Südtirol	2018, MF	*H. halys* (*Acer* sp.)	MN395438 (this study)	619	H1	Acro31, EBCL	n.a., Laimburg RC
		Ora, Trentino-Alto Adige/Südtirol	2018, MF	*H. halys* (*Acer* sp.)	MN395439 (this study)	652	H1	Acro32, EBCL	n.a., Laimburg RC
A. sinicus (*A. solus*)	Italy	Cavour/Piemonte	2017, na	*Arma custos* (*Acer* sp.)	MH521285 (Moraglio et al. 2019)	718	H1	DISAFA	2017/23, DISAFA
A. sinicus	Switzerland	Zurich, Canton Zurich	2019, EG & EL	*H. halys* (*Liriodendron tulipifera*)	MN395440 (this study)	652	H1	Acro33, EBCL	SAMPLECH00000002, NHMB
		Zurich, Canton Zurich	2019, EG & EL	*H. halys* (*Liriodendron tulipifera*)	MN395441 (this study)	652	H1	Acro34, EBCL	SAMPLECH00000002, NHMB
		Zurich, Canton Zurich	2019, EG & EL	*P. prasina* (*Liriodendron tulipifera*)	MN395442 (this study)	652	H2	Acro36, EBCL	SAMPLECH00000003, NHMB
		Zurich, Canton Zurich	2019, EG & EL	*P. prasina* (*Catalpa bignonioides*)	MN395443 (this study)	652	H1	Acro37, EBCL	SAMPLECH00000004, NHMB
		Zurich, Canton Zurich	2019, EG & EL	*P. prasina* (*Catalpa bignonioides*)	MN395444 (this study)	652	H1	Acro38, EBCL	SAMPLECH00000004, NHMB
		Zurich, Canton Zurich	2019, EG & TH	*H. halys* (*Tilia platyphyllos*)	MN395445 (this study)	652	H1	Acro39, EBCL	SAMPLECH00000001, NHMB
		Zurich, Canton Zurich	2019, EG & TH	*H. halys* (*Tilia platyphyllos*)	MN395446 (this study)	652	H1	Acro40, EBCL	SAMPLECH00000001, NHMB
		Zurich, Canton Zurich	2019, EG & EL	*P. prasina* (*Liriodendron tulipifera*)	MN395447 (this study)	652	H1	Acro41, EBCL	SAMPLECH00000003, NHMB
		Zurich, Canton Zurich	2019, EG & EL	*P. prasina* (*Liriodendron tulipifera*)	MN395448 (this study)	652	H1	Acro42, EBCL	SAMPLECH00000003, NHMB
A. sinicus (*A. solus*, paratype)	USA	Fairfax Co/Virginia	2004, DRS (Grissell and Smith 2006)	Malaise trap	MN018863 (this study)	432	H3	n.a., FSCA	USNMENT01335770, FSCA
A. sinicus	USA	Auburn/Alabama	2018, RB & GT	*H. halys* (*Carya illinoinensis*)	MN018864 (this study)	484	H4	n.a., FSCA	FSCA 00090246, FSCA
A. sinicus (2 specimens)	USA	Montgomery Co./ Maryland	2017, RW & CR	*Euschistus* sp. (*Cornus* sp.)	MN395449–MN395450 (this study)	619	H4	Acro5-6, EBCL	Acro5-6, BIIR

Specimens	Country	Site/State or Region or Province	Year of Collection, Name of Collector [a]	Pentatomid host (host plant)	GenBank Accession Number	Barcode length in bp	Haplotype	DNA collection code[b]	Voucher and Collection code[c]
A. sinicus (2 specimens)	USA	Frederick Co./ Maryland	2017, RW & LR	*Euschistus* sp. (*Cercis canadensis*)	MN395451–MN395452 (this study)	619	H3	Acro7-8, EBCL	Acro7-8, BIIR
A. sinicus (4 specimens)	USA	Frederick Co./ Maryland	2017, RW & LR	*Euschistus* sp. (*Cercis canadensis*)	MN395453–MN395456 (this study)	619	H4	Acro9-12, EBCL	Acro9-12, BIIR
A. sinicus (2 specimens)	USA	Allegany Co./Maryland	2017, RW & SF	*Brochymena* sp. (*Lonicera japonica*)	MN395457–MN395458 (this study)	652	H4	Acro13-14, EBCL	Acro13-14, BIIR
A. sinicus (*A. solus*)	Canada	Hamilton/Ontario	2012, TG (Gariepy et al. 2014)	n.a.	MK188333	652	H4	Asco-0004, AAFC	Asco-0004, AAFC
		Hamilton/Ontario	2012, TG (Gariepy et al. 2014)	n.a.	MK188332	652	H4	Asco-0003, AAFC	Asco-0003, AAFC
		Hamilton/Ontario	2012, TG (Gariepy et al. 2014)	n.a.	MK188331	652	H4	Asco-0005, AAFC	Asco-0005, AAFC
A. sinicus (3 specimens)	China	Beijing, National Botanical Garden	2017, KH	*H. halys* (on apples)	MN395459–MN395461 (this study)	619	H5	Acro16-18, EBCL	Acro16-18, BIIR
A. sinicus (2 specimens)	China	Beijing, National Botanical Garden	2017, KH	*H. halys* (*Sophora japonica*)	MN395462–MN395463 (this study)	619	H6	Acro19-20, EBCL	Acro19-20, BIIR
A. sinicus	China	Nanjing, Zhongshan Botanical Garden	2014, KH	*Erthesina fullo*	MN395464 (this study)	619	H5	Acro21, EBCL	Acro21, BIIR
A. sinicus (2 specimens)	China	Nanjing, Zhongshan Botanical Garden	2014, KH	*Erthesina fullo*	MN395465–MN395466 (this study)	619	H7	Acro22-23, EBCL	Acro22-23, BIIR
A. sinicus (4 specimens)	South Korea	Suwon Arboretum (SNU)/ Gyeonggi	2014, KH	*Plautia stali* (*Magnolia* sp.)	MN395467–MN395470 (this study)	619	H4	Acro24-27, EBCL	Acro24-27, BIIR
A. sinicus (2 specimens)	South Korea	Anyang/ Gyeonggi	2014, KH	Undet. egg masses (*Acer palmatum*)	MN395471–MN395472 (this study)	619	H3	Acro29-30, EBCL	Acro29-30, BIIR
A. sinicus	South Korea	Gyeongsan-si/ Gyeongsangbuk-do	2016, LF	Sweep net	MN413503 (this study)	652	H3	AcKo0101, UAIC	AcKo0101, MICO
		Gyeongsan-si/ Gyeongsangbuk-do	2016, LF	Sweep net	MN413504 (this study)	652	H3	AcKo0102, UAIC	AcKo0102, MICO
		Gyeongsan-si/ Gyeongsangbuk-do	2016, LF	Sweep net	MN413505 (this study)	652	H3	AcKo0103, UAIC	AcKo0103, MICO

[a] Name of collectors: RB, Rammohan Balusu, LB, Luca Benvenuto, IB, Iris Bernardinelli, MF, Martina Falagiarda, SF, Sherry Frick, LF, Lucian Fusu, TG, Tara Gariepy, EG, Emily Grove, TH, Tim Haye, KH, Kim Hoelmer, EL, Emily Lemke, GM, Giorgio Malossini, CR, Cindy Reilly, LR, Laura Robinson, DS, Davide Scaccini, DRS, David R. Smith, ET, Elijah Talamas, PT, Paola Tirello, GT, Glynn Tillman, RW, Rebeccah Waterworth.

[b] DNA collections: AAFC, Agriculture and Agri-Food Canada, Canada; DISAFA, University of Turin, Torino, Italy; EBCL, European Biological Control Laboratory – USDA-ARS, France; FSCA, Florida Department of Agriculture and Consumer Service, Florida, USA; UAIC, University of Iasi, Romania.

[c] Voucher collections: AAFC, Agriculture and Agri-Food Canada, Canada; BIIR, Beneficial Insects Introduction Research Unit-USDA-ARS, Newark, USA; DAFNAE, University of Padua, Padova, Italy; DISAFA, University of Turin, Torino, Italy; FSCA, Florida Department of Agriculture and Consumer Service, Florida, USA; Laimburg RC, Laimburg Research Center, Vadena, Italy; MICO, Mitroiu Collection, Iasi, Romania; NHMB, National History Museum of Bern, Switzerland.

Molecular analysis

The 5'-*COI* barcode fragment was successfully obtained from 45 individuals collected in numerous countries and from different hosts, plus four sequences from GenBank (Table 4). The lengths of these sequences varied from 432 bp for the paratype of *A. solus* to 652bp for three Italian, all Swiss, two American and three South Korean specimens. After edge trimming, the final data matrix of 49 sequences including the four GenBank accessions consisted of 429 characters with seven parsimony informative sites. As is typical for insect mitochondrial genes, the AT content in *Acroclisoides* was high (74.8%), similar to the AT content reported in Cynipidae (74.8%) at the higher end of the range observed for parasitic wasps (Rokas et al. 2002). A total of 7 haplotypes were recorded among the analyzed individuals. In Italy, we detected one unique haplotype (H1) which is shared with Switzerland. In Switzerland where H1 predominates (8 out of 9 specimens), we also found a second haplotype (H2) in one specimen. Although H1 and H2 did not match any of the 5 haplotypes found outside Europe, the network analysis showed that they are closely related as H1 differed only by one substitution from the haplotype H6 found in China and by two substitutions from the haplotype H3 found in South Korea and North America (Fig. 4). The two haplotypes found in North America, H3, which includes the *A. solus* paratype and H4, were shared with South Korea. Three haplotypes (H5, H6 and H7) were identified in China, H6

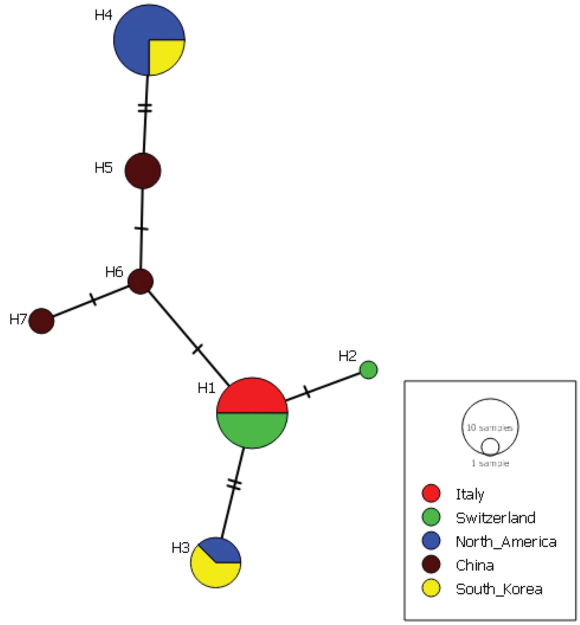

Figure 4. Haplotype network of the 49 *Acroclisoides sinicus* barcodes analyzed in this study. Each circle corresponds to one haplotype; circle size gives the proportion of individuals belonging to the haplotype. The color of the circles represents the geographical origin. Numbers correspond to the haplotype numbers. Hash marks symbolize the number of mutations between haplotypes.

being only one mutational step away from the other two. The degree of divergence was found to be very low at the sampling level, with a mean K2P distance of 0.64%, ranging from 1.41% between the two haplotypes H3 and H4 both detected in North America and South Korea to 0.23% between H1 from Europe and H6 found in China or H2 in Switzerland. Haplotype diversity (*Hd*) was 0.118 (SD of 0.101) and 0.343 (SD of 0.128) in Europe and North America respectively. Nucleotide diversity (π) was 0.00027 (SD of 0.00024) and 0.0048 (SD of 0.00179) in Europe and North America respectively. In Asia, *Hd* was 0.824 (SD of 0.047), and π was 0.00662 (SD of 0.0007). In order to confirm that the range of divergence is what is expected at the intraspecific level and given that there are no data available at the genus level, we compared these data with other species in the subfamily. A total of 432 Pteromalinae barcodes representating of a minimum of two species in four genera (*Lyrcus* Walker, *Mesopolobus* Westwood, *Pachyneuron* Walker and *Pteromalus* Swederus) were downloaded and aligned. From this 283bp dataset (available upon request to authors), K2P distances for two classes (intraspecific and interspecific) were calculated. Figure 5 plots K2P values for the Pteromalinae, along with the divergence observed between *Acroclisoides* haplotypes, clearly confirming that divergence between *Acroclisoides* haplotypes is not significantly different from the Pteromalinae intraspecific divergence (Kruskal-Wallis test, p=0.294), but significantly different from the Pteromalinae congeneric divergence (Kruskal-Wallis test, p<0.0001). Species boundaries between *A. solus* and *A. sinicus* are not supported by the present mitochondrial data.

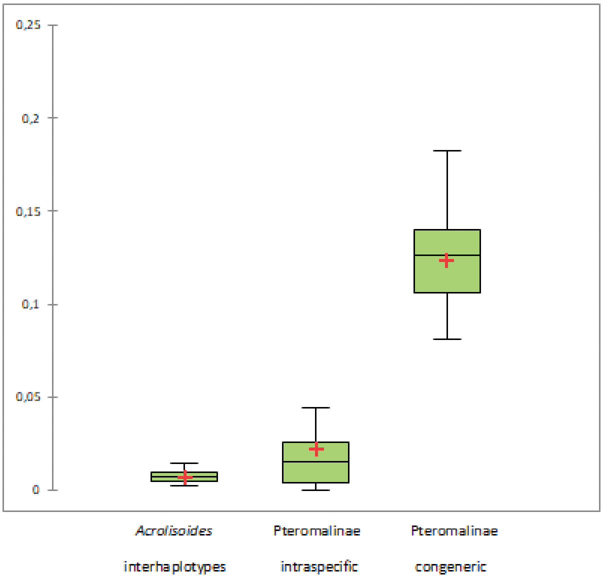

Figure 5. Boxplot of pair-wise molecular distances between (left) the *Acroclisoides* haplotypes evidenced in this study, (center) conspecific individuals in the Pteromalinae, (right) individuals from different species of the same genus in the Pteromalinae.

Discussion

Grissell and Smith (2006) stated that *A. solus* was very similar to *A. sinicus* and gave a few morphological differences that they considered enough to separate the two species. They compared *A. solus* with two Chinese specimens of *A. sinicus* as mentioned in their acknowledgments section. No further information was provided and we could not locate those specimens. After examining the specimens mentioned in the above sections and finding intermediate features between *A. solus* and *A. sinicus*, we conclude that they comprise a single species.

More samples of *A. sinicus* and other *Acroclisoides* species are required to more accurately assess the intraspecific and interspecific variability, respectively, necessary to compute the "barcoding gap" (Meyer and Paulay 2005). However, our analysis of the barcode region showed that the level of divergence was less than 1% among specimens from Italy, Switzerland, South Korea, China, USA, Canada and the paratypes of *A. solus*. This level of divergence is consistent with that observed at the intraspecies level within the subfamily Pteromalinae, and hence supports the conspecificity of *A. solus* and *A. sinicus*. This study highlights the value of DNA barcoding in the identification and delineation of species in Hymenoptera (Stahlhut et al. 2013). Overall, this research expands the barcoding sequence database for parasitoids and hyperparasitoids and provides a foundation for additional molecular studies aimed to a better understanding of the biocontrol services provided by parasitoids (Gariepy et al. 2007; Ye et al. 2017; Halim et al. 2018).

There were no prior reports in the literature on the associations of *A. sinicus* and its hosts, with the exception of Xiao and Huang (2000) who reported that this species was reared from pentatomid eggs (Huang and Liao 1988; Xiao and Huang 2000; Grissell and Smith 2006; Ko et al. 2018). In our study, *A. sinicus* was found to be associated with *H. halys* in Italy and *P. prasina* and *H. halys* in Switzerland; in the USA it was associated with *Euschistus* sp., *Brochymena* sp., *C. hilaris*, and *H. halys*; and in Asia it is associated with *H. halys*, *Plautia stali* Scott and *Erthesina fullo* (Thunberg) (Hemiptera: Pentatomidae). In addition, in Italy *A. sinicus* (formerly identified as *A. solus*) was recently found associated with *Arma custos* (Fabricius) (Hemiptera: Pentatomidae) eggs (Moraglio et al. 2019).

We consider it likely that *A. sinicus* is a hyperparasitoid of both *Anastatus* and *Trissolcus*. In the Friuli Venezia Giulia region *A. sinicus* only emerged from egg masses associated with the egg parasitoid *An. bifasciatus*, even though egg masses parasitized by *T. mitsukurii* were present in the vicinity (Sabbatini Peverieri et al. 2018). However, in the Veneto region, *A. sinicus* emerged from two egg masses parasitized by *T. mitsukurii* and in Switzerland from two egg masses parasitized by *T. japonicus*, suggesting hyperparasitism might occur on species in both genera as previously reported by Clarke and Seymour (1992). In the southeastern USA, one egg mass of *C. hilaris* from Georgia produced specimens of *A. sinicus* and *T. edessae*; another egg mass produced specimens of both these parasitoid species as well as *An. reduvii*. Molecular analysis of remnants of parasitized host eggs [*Acrosternum hilare* (Say) (Hemiptera: Pentatomidae)] led to the suspicion that *A. sinicus* (formerly identified as *A. solus*) was associated with *Trissolcus* sp. (Gariepy et al. 2014). Preliminary laboratory no-choice experiments have found that *A. sinicus* does not accept unparasitized *H. halys* egg masses as a host (Sabbatini Peverieri unpublished data). Similar observations were made by Clarke and Seymour (1992), who exposed two undetermined *Acroclisoides* species collected in Australia to *Nezara viridula* L. (Hemiptera: Pentatomidae) eggs, showing that only eggs parasitized previously by *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) were accepted. James (1990) also found that the Australian species *Acroclisoides tectacorisi* (Girault) (Hymenoptera: Pteromalidae) is "difficult to rear" in laboratory conditions on eggs of *Biprorulus bibax* Breddin (Hemiptera: Pentatomidae). To date, there are no other published studies that test the host range and preference of *A. sinicus* in a laboratory setting.

The ability to identify emerged parasitoid species from host eggs collected in the field is a valuable tool for research. For example, the three parasitoid species that emerged in Italy from *H. halys* eggs produce distinct and diagnostic exit holes that can be used to assess parasitism if only empty parasitized egg masses are found. The distinct shape of the margin of the exit hole correlates well with the distinct shape of the mandibles in the three species of wasps. *Anastatus bifasciatus* has bidentate mandibles characteristic for the genus (Gibson 1995), with one small and one large tooth and the exit hole has serrated margins (Fig. 1A, B). *Trissolcus mitsukurii* and *T. japonicus* have tridentate mandibles with teeth of similar size (Talamas et al. 2017) and the exit hole has smaller serrations compared to *An. bifasciatus* (Fig. 1E, F). *Acroclisoides sinicus* has large mandibles with four long sharp teeth (Fig. 2B), and they were directly observed to cut and tear the egg chorion (Fig. 1C, D) while pushing it outward. Molecular analysis will be another useful tool to evaluate the impact of *Acroclisoides* on the parasitoid guild (Gariepy et al. 2014; Gariepy et al. 2018).

It is unknown how *A. sinicus* arrived in Europe or in the USA, but this could have happened via the same pathways as with adventive *T. japonicus* and *T. mitsukurii*. Another possibility is that *A. sinicus* is a Holarctic species, and its presence was only detected recently due to increased interest in field collection of pentatomid eggs as a result of *H. halys* biocontrol research. The latter hypothesis can be tested by thorough examination of insect collections that may contain specimens collected prior to the arrival of *H. halys*. It is noteworthy that we currently have no records of *A. sinicus* from North America or Europe that predate the arrival of *H. halys*, with the earliest detection in the USA in 2002 (Grissell and Smith 2006) and the earliest European record in 2016 (Moraglio et al. 2019). It should also be noted that one of us (MDM) has studied Pteromalidae in Europe for decades without detecting any specimens of *A. sinicus* in entomological collections. Moreover, Dieckhoff et al. (2017) have extensively surveyed parasitoids of *H. halys* and native pentatomids in Delaware, USA, since 2007 without any recoveries of *Acroclisoides* from natural or sentinel egg masses. This apparent absence is congruent with the hypothesis that the populations of *A. sinicus* in North America and Europe are adventive and recent.

Analysis of *COI* sequences revealed that all samples collected in Italy and all samples except one collected in Switzerland belong to the same *A. sinicus* haplotype (H1), while in the USA two haplotypes (H3 and H4) are present, both of which are shared with South Korea. The lower haplotype and nucleotide diversities observed in North

America and Europe compared to Asia are congruent with a mild population genetic bottleneck subsequent to an introduction (Hufbauer et al. 2004). Also, values of *Hd* (0.118 and 0.343) and π (0.00027 and 0.0048) in Europe and North America respectively are low for the scenario of a species with a long-established Holarctic distribution. For example *Aedes caspius* (Pallas) (Diptera: Culicidae), which persisted through the last glacial period across the Mediterranean Basin, has *Hd* and π of 0.971 and 0.0067 respectively (Porretta et al. 2011). The genetic similarity between North American and South Korean specimens suggests that either the North American specimens originated from South Korea or populations of *A. sinicus* in both countries originated from the same area. All specimens from Europe except one have the same haplotype (H1) but are different from those in the USA or Asia. This suggests that the European *Acroclisoides* did not originate in the USA but from an unknown region. In any case, *A. sinicus* appears to be a species that is not commonly found in the field but could become more prevalent in the future as a result of invasive *H. halys* populations.

Hyperparasitoids have traditionally been regarded as detrimental to biological control, although some authors have suggested they may provide stabilizing influences on populations depending on the model assumptions (Kidd and Jervis 2007; Snyder and Ives 2008). The effect that *A. sinicus* might have on the eventual biological control of *H. halys*, as well as on non-target species of Pentatomidae and their parasitoids in the environment, is the subject of ongoing studies.

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