

Hymenoptera functional groups' shifts in disturbance gradients at Andean forests in Southern Ecuador

Marina Mazón^{1,2}, Ximena López³, Oscar Romero⁴

1 Biodiversity and Ecosystem Services Research Program, Universidad Nacional de Loja, Ciudadela Universitaria, sector La Argelia, EC 110101 Loja, Ecuador **2** Departamento de Ciencias Ambientales y Recursos Naturales, Universidad de Alicante, Apdo. Correos 99, 03080 Alicante, Spain **3** Carrera de Ingeniería en Manejo y Conservación del Medio Ambiente, Universidad Nacional de Loja, Ciudadela Universitaria, sector La Argelia, EC 110101 Loja, Ecuador **4** Freelance consultor, Av. Pío Jaramillo, EC 110101 Loja, Ecuador

Corresponding author: Marina Mazón (marinamazonmor@gmail.com)

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Abstract

Ecosystems under ecological restoration should be monitored in order to investigate if the ecosystem is being functionally recovered, especially in highly vulnerable biodiversity hotspots like Andean forests. Here we sampled Hymenoptera families in four Andean forest reserves above 1800 masl from Southern Ecuador, in three conservation levels in each forest: low (degraded), medium (10–15 years of recovery) and high (well-conserved forest). All Hymenoptera families were classified into four functional groups: predators, herbivores, pollinators and parasitoids. A total of 32 hymenopteran families were collected, with parasitoids clearly dominating in the samples. Family assemblages were not statistically different, neither in abundance nor family richness. Assemblages were more similar between them in the high and medium areas than in low conservation areas, where assemblages were very variable and showed a higher functional diversity in two of the reserves. The low presence of pollinators may be due to the high humidity during the sampling and the sampling method. Although some results are promising for the restoring trajectory, especially for parasitoids, we should keep in mind that this is at family level, so it would be interesting to know if these patterns persist at lower taxonomic levels.

Keywords

Herbivores, Hymenoptera families, Parasitoids, pollinators, predators

Introduction

Ecosystems provide a wide range of services that are at risk because of biodiversity loss derived from demographic growth and land use changes (De Groot et al. 2012). Ecosystems do have an ability to recover previous biodiversity up to a certain point, beyond which they need some assistance. The process of “assisting the recovery of an ecosystem that has been degraded, damaged or destroyed” is called ecological restoration (SER 2004). However, since ecosystems may progress in different ways after restoration efforts, depending on many different environmental variables (Bullock et al. 2011), monitoring is essential to know how ecosystem attributes are recovering.

Andean forests are considered as a highly vulnerable biodiversity hotspot (Myers et al. 2000; Mathez-Stiefel et al. 2017; Murcia et al. 2017), with most of its plant species already experiencing shifts in their distribution because of global warming (Fadrique et al. 2018). Although there are some initiatives for restoring Andean forests, they are not being monitored comprehensively (Mazón et al. 2019), with plant structure and diversity being evaluated in most cases (Yepes et al. 2010; Szejner et al. 2011; Camelo et al. 2017). Therefore, little is known about whether many other ecosystem services are being recovered in these forests.

Hymenoptera is one of the most important and diverse insect orders, including representative groups of two of the most essential ecosystem services: pollination (i.e., bees) and natural pest control (i.e., parasitoid wasps). Both pollinators and parasitoids (whose hosts are mostly herbivorous insects), because of their close relationship with plants, have been used as indicators for ecosystem disturbances (Vos et al. 2001; Requier 2019) and their recovery (Maeto et al. 2008; Ferronato et al. 2018). Pollinators have received much attention in recent years because of their worrying decreasing populations and their high vulnerability to climate change (Potts et al. 2010; Powney et al. 2019). However, other hymenopterans like parasitoid wasps and ants are also sensitive to ecosystem degradation and fragmentation (de Sassi and Tylianakis 2012). Since hymenopteran families may have different responses to habitat degradation (Banks et al. 2013), they may also have different responses when being monitored in ecological restoration projects, as well as the roles they play in the ecosystem. For instance, bee and parasitoid diversity has been shown to be higher in restored areas than in disturbed ones (Barbieri Junior and Pentead-Dias 2012; Marrec et al. 2018) but the species interaction and functions appear to take a longer time to recover (Albrecht et al. 2007). Therefore, species diversity *per se* may not be the best predictor of ecosystem services, but, rather, diversity at the functional level (Griffin et al. 2013). In consequence, measuring functional diversity may predict ecosystem functioning better than the traditional species diversity measures (Loreau et al. 2001).

In this study we aim to 1) identify the Hymenoptera families present in different disturbance levels in Andean forests, and 2) evaluate how functional groups within Hymenoptera are shifting as disturbance increases.

Methods

Study area

The research was done in four protected areas of Andean forest located in Loja and Zamora Chinchipe provinces, in the buffer area of Podocarpus National Park, at southern Ecuador (Fig. 1), with altitudes ranging 1840–2630 masl (Table 1). Andean forests are ecosystems located between 1200 and 3600 masl at Los Andes mountains (Quintero et al. 2017), characterized by their elevated humidity and a high diversity of ferns, lichens, mosses, palms and orchids (Bravo 2014; Kattan 2017).

Sampling and identification

In each reserve, we identified three areas of different conservation status with the help of their personal staff: a well-conserved area, an area under about 10–15 years of recovery, either naturally or assisted, and a degraded area (Table 1). In each area, we installed a white Townes style Malaise trap (Townes 1972) for six weeks, from December 2015 to January 2016 in most reserves, except for Tapichalaca, which was sampled from March to April 2018; in all cases, sampling was carried out during the rainy season. We filled pots with ethanol 70% and replaced them every two weeks, i.e., having three samples per area.

We sorted and identified all hymenopteran specimens to family level, and then we classified them into four major functional groups: predators, herbivores, pollinators and parasitoids, following Fernández and Sharkey (2006).

Data analyses

We compared the family assemblages in the three conservation levels by a non-metric multidimensional scaling (NMDS) and a PERMANOVA with 9999 permutations, using Jaccard index for similarity, which considers presence/absence of families, regardless of the relative abundances of each one. We did the same analyses to check for differences in the hymenopteran families assemblages related to the reserves.

Regarding the functional groups, we calculated both richness (i.e., number of families) and abundance (i.e., number of individuals) for every functional group, and we compared them across the conservation levels by means of a KRUSKAL-WALLIS test and a post-hoc DUNN test.

Additionally, we evaluated functional diversity with the Shannon index, considering the abundance of individuals belonging to every functional group. Since some samples were damaged and lost, we considered the mean abundance values for the two or three samples in every sampling site. Then, we compared Shannon indices in two ways: by the KRUSKAL-WALLIS with the four reserves as replicates, and in the four reserves treated as independent samples, compared by a randomization test with 1000 random partitions (Solow 1993).

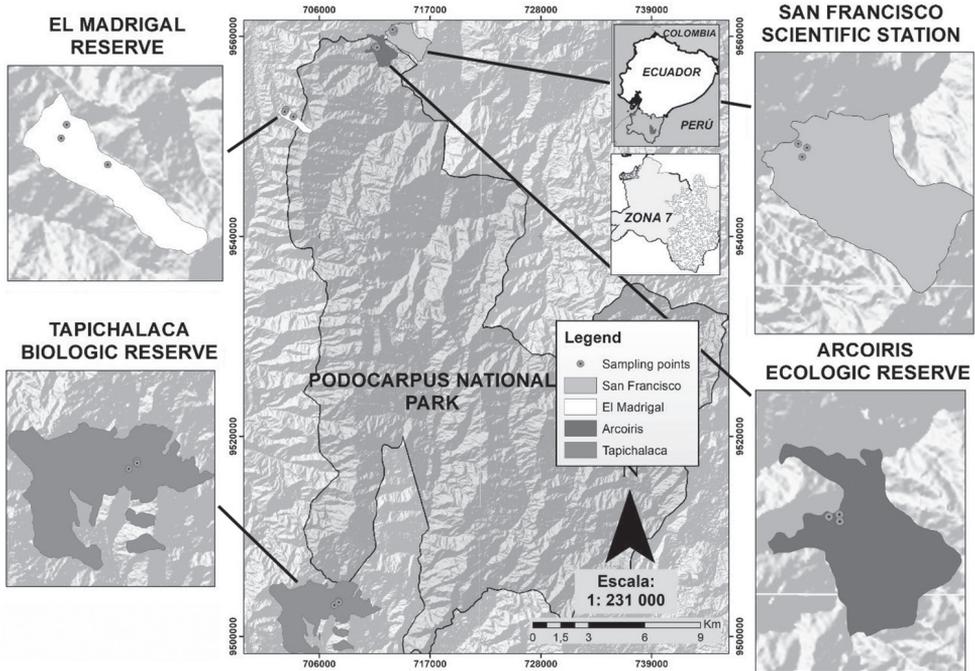


Figure 1. Localities of the four Andean forest reserves and the sampling points where the Malaise traps were placed in each one for collecting Hymenoptera.

The NMDS, PERMANOVA, KRUSKAL-WALLIS and post-hoc tests were run with software Past version 3.0 (Hammer et al. 2001). Shannon diversity index and randomization tests were performed with software Species Diversity and Richness 3.02 (Pisces Conservation, Ltd., Pennington, Lymington, UK).

Results

We collected a total of 32 hymenopteran families, mostly belonging to the parasitoid functional group, with family Ichneumonidae clearly dominating the sampling (1930 individuals). We only collected seven specimens belonging to three families from the pollinator functional group (Table 2).

Families assemblages were not statistically different, neither regarding the conservation level ($F = 1.354$, $p = 0.156$) nor the reserve ($F = 1.382$, $p = 0.118$). In the NMDS, although the samples were distributed from high to low conservation level (Fig. 2A), those from the low level were the most variable, especially the sample from Madrigal reserve, which was more different than the others in terms of hymenopteran family composition (Fig. 2B). In both cases, axis 1 explained more of the data (50.14% and 49.87%, respectively) than axis 2.

Table 1. Location of trapping sites in the three conservation levels of the four Andean forest reserves.

Reserve	Conservation level	Coordinates X	Coordinates Y	Altitud (masl)
ECSF	high	-79.077	-3.973	ca 1870
	medium	-79.078	-3.975	ca 1860
	low	-79.079	-3.972	ca 1840
Arcoiris	high	-79.095	-3.988	ca 2160
	medium	-79.093	-3.988	ca 2160
	low	-79.093	-3.989	ca 2160
Madrigal	high	-79.168	-4.051	ca 2520
	medium	-79.175	-4.045	ca 2350
	low	-79.176	-4.047	ca 2400
Tapichalaca	high	-79.126	-4.489	ca 2570
	medium	-79.130	-4.493	ca 2520
	low	-79.126	-4.490	ca 2620

Table 2. Abundances and functional groups of every collected hymenopteran family in the three conservation levels (high, medium and low) from the four Andean forest reserves in southern Ecuador. PRED = predators, HERB = herbivores, PAR = parasitoids, POL = pollinators.

Family	Functional groups	ECSF			Arcoiris			Madrigal			Tapichalaca			Total
		high	medium	low	high	medium	low	high	medium	low	high	medium	low	
Pompilidae	PRED	0	0	0	1	0	0	0	0	0	1	5	1	8
Sphécidae	PRED	0	0	0	0	0	0	0	0	0	0	1	0	1
Vespidae	PRED	1	0	0	0	1	4	1	1	0	0	14	5	27
Formicidae	PRED	19	4	4	24	2	1	3	1	0	0	2	3	65
Pergidae	HERB	0	0	0	0	0	0	4	0	0	1	1	0	6
Tenthredinidae	HERB	0	0	0	2	0	0	3	0	0	1	2	4	12
Xiphidiidae	HERB	0	0	0	2	1	0	0	0	0	2	0	0	5
Bethylidae	PAR	10	0	0	1	0	1	1	1	0	0	1	6	21
Braconidae	PAR	167	9	12	60	15	6	134	25	2	38	31	76	577
Diapriidae	PAR	48	1	1	6	1	1	14	3	0	6	17	36	134
Dryinidae	PAR	0	1	1	0	0	0	0	1	0	2	10	4	19
Embolemyidae	PAR	2	0	0	0	0	0	0	0	0	0	0	0	2
Eucharitidae	PAR	0	0	0	0	1	0	0	0	0	0	0	0	1
Eulophidae	PAR	5	0	0	0	0	0	1	0	0	1	2	2	11
Eupelmidae	PAR	0	1	1	0	0	0	0	0	0	1	1	0	4
Eurytomidae	PAR	3	0	0	0	0	0	0	0	0	0	0	0	3
Evanidae	PAR	25	2	0	14	4	2	11	3	0	1	14	9	92
Figitidae	PAR	1	0	0	2	0	0	3	0	0	4	2	5	17
Ichneumonidae	PAR	300	33	6	188	72	23	248	101	6	195	233	401	1930
Liopteridae	PAR	0	0	0	0	0	0	0	0	0	0	2	0	2
Mutillidae	PAR	5	0	0	0	0	0	0	0	0	0	0	0	5
Mymaridae	PAR	0	0	0	0	0	0	0	0	0	0	0	1	1
Orussidae	PAR	0	0	0	0	1	0	0	1	0	2	1	0	5
Perilampidae	PAR	3	0	0	0	0	0	1	0	0	0	0	0	4
Platygastridae	PAR	13	2	2	5	0	0	3	0	0	5	6	8	44
Proctotrupidae	PAR	2	0	0	0	3	0	0	0	0	2	1	2	10
Pteromalidae	PAR	6	0	0	2	0	0	1	0	0	0	6	0	16
Sapygidae	PAR	1	0	0	1	4	1	0	1	0	0	1	0	9
Tiphidae	PAR	5	0	0	0	0	0	0	3	0	0	12	3	23
Agaonidae	POL	0	0	0	1	0	0	0	0	0	0	0	0	1
Apidae	POL	0	1	1	0	0	1	0	0	0	0	0	1	4
Halictidae	POL	0	0	0	1	0	0	0	0	0	0	2	0	3

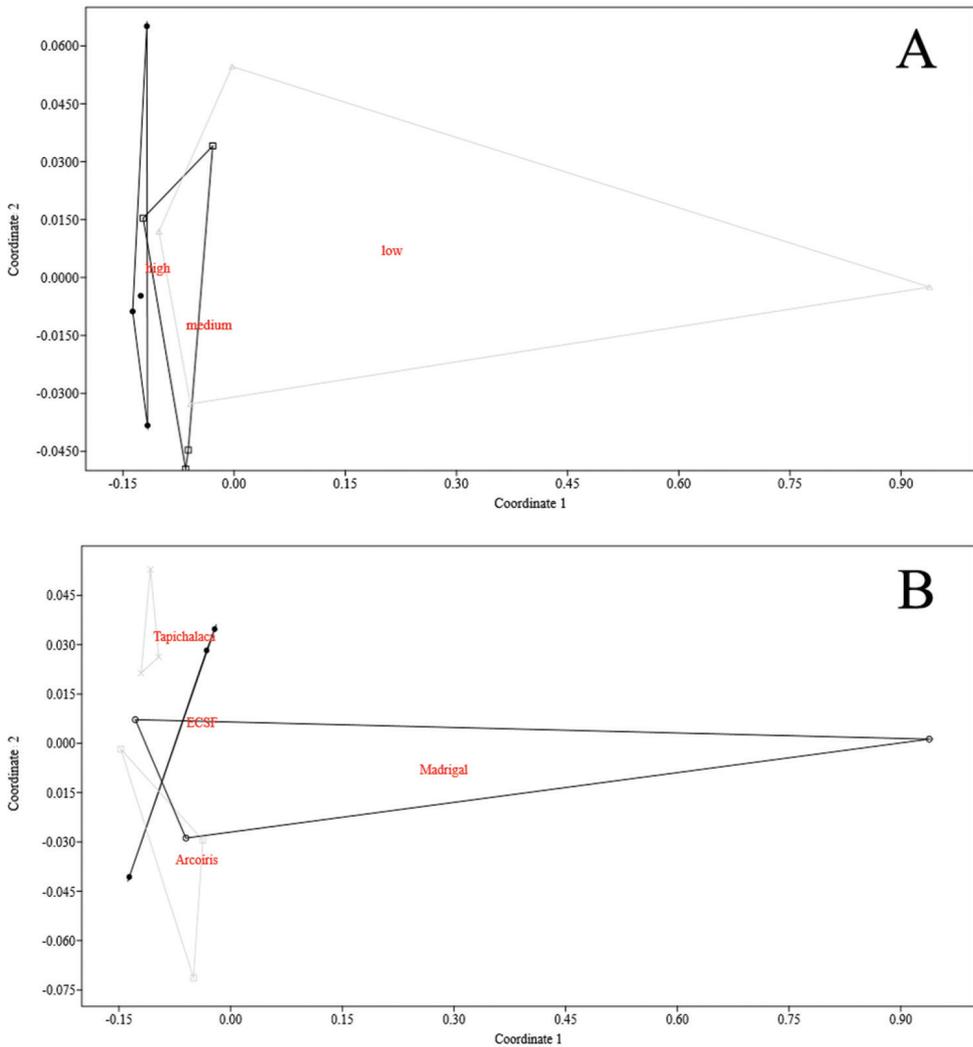


Figure 2. NMDS plots for hymenopteran families assemblages grouped by conservation level **(A)** and by reserve **(B)**. Samples from the same group are gathered by convex hulls.

When comparing mean abundance and total richness of every functional group with the conservation levels no significant differences were found either (Table 3).

Regarding functional diversity, no significant results were obtained in the KRUSKAL-WALLIS test ($H = 0.3462$, $p = 0.841$). However, when treating every reserve independently, permutational tests gave very different results in the four reserves (Fig. 3). Surprisingly, functional diversity was significantly higher in the more perturbed areas in ECSF and Arcoiris. However, diversity in the areas under restoration (i.e., medium conservation level) was more similar to those in the conserved areas than in the highly perturbed ones.

Table 3. Results from Kruskal-Wallis test (H) and p-value (p) when comparing number of families (S) and number of individuals (Ab) from all Hymenoptera and every functional group (PRED = predators, HERB = herbivores, PAR = parasitoids, POL = pollinators) in the three conservation levels of Andean forests.

	H	p
S_total Hymenoptera	3.298	0.19
Ab_total Hymenoptera	3.962	0.138
S_PRED	0.183	0.903
Ab_PRED	0.269	0.872
S_HERB	3.010	0.174
Ab_HERB	3.151	0.207
S_PAR	3.537	0.171
Ab_PAR	3.962	0.138
S_POL	0.644	0.671
Ab_POL	0.5	0.74

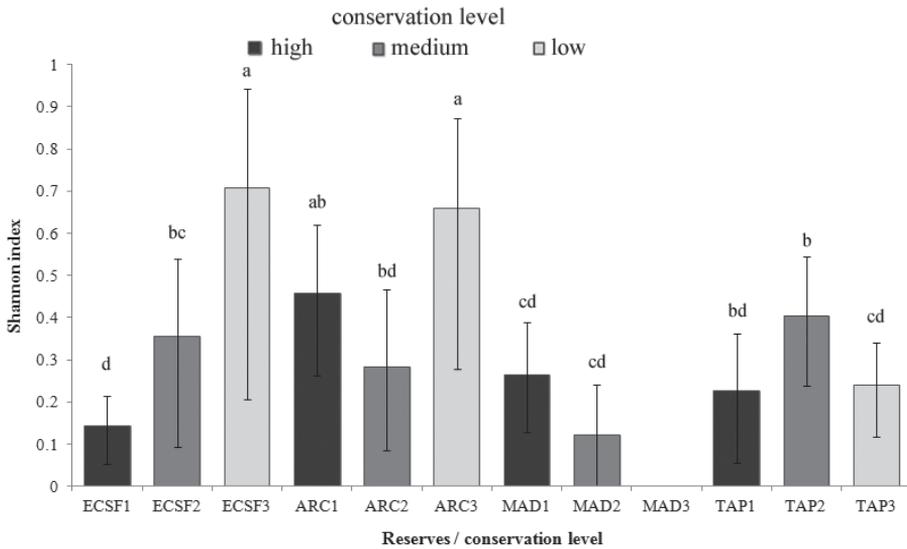


Figure 3. Mean values of Shannon index for functional diversity of Hymenoptera collected in the three conservation levels (high, medium, low) from the four reserves of Andean forests. Vertical bars denote 95% confidence intervals. Different letters indicate statistically significant differences.

Discussion

In the present research we found 32 hymenopteran families, which represents 36.31% of Neotropical families (Fernández and Sharkey 2006). Ichneumonidae and Braconidae were the most abundant, which are usually amongst the main hymenopteran families collected by Malaise trap in many ecosystems, as in preserved sandbanks (Oliveira et al. 2009), open pastures (Castiglioni et al. 2017) or cacao plantations (Mazón 2016). The high abundance of these two families is also reflected in the dominance registered for parasitoid functional groups. Actually, parasitoids are the most abundant and diverse hymenopteran group (Fernández and Sharkey 2006) and are able to adapt

to different environmental conditions, with a rather wide range of potential hosts (Santos and Quicke 2011).

However, the low occurrence of hymenopteran pollinators was unexpected. Bees are abundant in Andes even above 2500 masl (Gonzalez and Engel 2004), and at least 51 bee species have been recorded from Southern Ecuador (Rasmussen 2004). Since one of the main factors limiting bee nesting is high humidity (Michener 2007; Reyes-Novelo et al. 2009), the season when sampling was done may have influenced these results. Furthermore, Malaise traps may not be the most appropriate sampling method for bees. Although showed as highly effective for parasitoids (Mazón and Bordera 2008) and aculeates in general (Volpato et al. 2020), with some reported bias either towards males (Aguir and Santos 2010) or females (Mazón et al. 2020), scent-baited traps or entomological nets seem to work better for bees (Santos Júnior et al. 2014; Ferronato et al. 2018; Alvarenga et al. 2020). Other sampling methods should be used to complement Malaise traps, especially when time for field work is brief (McGravy et al. 2016; Saunders and Ward 2018). Sampling should also include different weather seasons in order to have a better representation of all Hymenoptera families and to clarify if bees have reduced populations in these forests.

No differences among conservation levels were found. Restoration may favour the presence of wild bees (Araújo et al. 2018; Taki et al. 2018; Alvarenga et al. 2020) and parasitoids (Marrec et al. 2018), but some hymenopteran assemblages from restored areas may be functionally similar to those from forests (Montoya-Pfeiffer et al. 2020), and their diversity has been found to be lower in forests compared to more open areas (Pardo and Gonzalez 2007). Therefore, it seems that, even when the areas labeled as low conservation presented a high degree of perturbation, being embedded into a preserved area matrix helped to increase diversity in these areas, since the hymenopteran diversity is positively affected by the proximity to forest (Banks et al. 2013). Furthermore, not all types of forest restoration will equally favour Hymenopterans, with ecological restoration, as applied in all the studied reserves, being more effective than monoculture tree plantations (de Araújo et al. 2019). This indicates that ecological restoration in these areas has a positive effect on hymenopteran diversity, and the more disturbed areas within the reserve matrix do not significantly impact these assemblages, at least at family level.

Another aspect that may have masked the effect of conservation level on Hymenoptera richness and abundance are the altitudinal differences amongst some of the reserves. Parasitoids (van Noort 2004; Veijalainen et al. 2014; Hall et al. 2015), wasps and bees (Perillo et al. 2017; Widhiono et al. 2017) and ants (Guerrero and Sarmiento 2010; Burwell and Nakamura 2011) have been found to be sensitive to altitude in tropical and subtropical ecosystems. However, to see more clearly how Hymenoptera are responding to both altitude and conservation level it would be necessary to use lower taxa. Higher taxa have been used as surrogates for species to assess biodiversity in highly species-rich ecosystems or when sampling time is limited. For this purpose, genus (Derraik et al. 2010; Vieira et al. 2012) or subfamily level (Mazón 2016) have proved to be useful surrogates in Hymenoptera. Although family level may not be such

a good surrogate for species diversity than other lower taxonomic levels (Balmford et al. 1996), it can provide important ecological information, especially when grouped into functional groups (Bragança et al. 1998).

When looking at assemblage composition, samples were grouped according to conservation level rather than to reserves, although samples were very separated one from another, showing that assemblages, even in the same conservation level, were highly variable. The type of disturbance may be influencing these results, since it will affect the way the ecosystem responds to it (Jones and Schmitz 2009; Pandit et al. 2018), and therefore insect communities will also change accordingly. However, that variable was not considered in this study, so it is difficult to draw those conclusions. On the other hand, most of the assemblages from conserved areas were very similar amongst themselves except for the Tapichalaca reserve, that was clearly separate from the others, indicating the Andes may be acting as an ecological barrier for these insects, even at a local scale.

Regarding overall functional diversity, it was significantly higher in the low conservation areas of two reserves. Although it can not be seen as a consistent pattern, open areas may serve as corridors for insects (Mazón and Bordera 2014; Gutiérrez-Chacón et al. 2020) connecting well-conserved forests, and therefore these more disturbed areas would have more diverse assemblages. Also, by being embedded in a conserved matrix, these areas may be acting as ecotones, which may harbor a higher diversity of Hymenoptera than the forests, as seen for ants (Coelho and Ribeiro 2006), bees (Polatto and Alves 2020; Theodorou et al. 2020) and parasitoids (Rossetti et al. 2013). However, the occurrence of insects in these ecotones would be limited by some traits as the presence of trees (especially for nesting bees and wasps, Polatto and Alves 2020) or the dispersal ability of each species (Hamm and Drossel 2017), therefore functional diversity based on the genus or species level, with more detailed functional groups, would provide a better information.

Ecological restoration is an effective way to recover the structure and function of ecosystems, but comprehensive monitoring should be carried out in order to investigate if its functions are becoming similar to those from a healthy well conserved reference ecosystem. Here we show promising results for parasitoid wasps, but not so for bees. It would be interesting to see what happens when identification goes to genus or species level, and whether trends observed for families are persisting for these lower taxonomic levels.

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Review of the genus *Syzeuctus* (Hymenoptera, Ichneumonidae, Banchinae) from South Korea

Gyu-Won Kang¹, Janko Kolarov², Jong-Wook Lee³

1 Department of Life Sciences, Yeungnam University, Gyeongsan, South Korea **2** Faculty of Pedagogy, University of Plovdiv, Plovdiv, Bulgaria **3** Georim Entomological Institute, Nature And People Co., Ltd, Daegu, South Korea

Corresponding author: Jong-Wook Lee (jwlee1@ynu.ac.kr)

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Abstract

Only two species (*Syzeuctus coreanus* and *S. sambonis*) of the genus *Syzeuctus* were known to inhabit South Korea. In the present study, four species of this genus are newly recorded from South Korea. Among them, two species, *S. albopictus* Kang & Lee, **sp. nov.** and *S. flavofacialis* Kang & Lee, **sp. nov.**, are described as new. Of the other species, the male of *S. takaozanus* was hitherto unknown and is described here, and *S. apicifer* is also new to South Korea. A key to these South Korean species along with the descriptions of the two newly recorded species and digital images are provided.

Keywords

Atrophini, endoparasitoids, ichneumonid wasps, key to species, new record, taxonomy

Introduction

The genus *Syzeuctus* Förster is one of the large genera of the tribe Atrophini, subfamily Banchinae, and comprises 123 species (Yu et al. 2016). Among them, only 14 species inhabit the Eastern Palearctic region, and two species (*Syzeuctus coreanus* Uchida and *S. sambonis* Uchida) were recorded in South Korea. This genus can be distinguished from other genera of the tribe by the following characteristics: frons often with a horn, ridge, or tooth above each antennal socket; genal carina reaching base of mandible; small and triangular areolet with a long stalk. Species of this genus are koinobiont endoparasitoids of Lepidoptera larvae, especially Pyralidae (Yu et al. 2016).

In the present study, we report two species new for science (*Syzeuctus albopictus* Kang & Lee, sp. nov. and *S. flavofacialis* Kang & Lee, sp. nov.) and two species newly recorded from South Korea (*S. apicifer* and *S. takaozanus*). Digital images of the new species and newly recorded species along with a key to the South Korean species of *Syzeuctus* are provided.

Materials and methods

The wasps investigated in this study were collected by sweep net and by Malaise traps and deposited in the Georim Entomological Institute (Daegu, South Korea). Distributional data follow Yu et al. (2016). Abbreviations used in the text are as follows.

GEI	Georim Entomological Institute, Daegu, South Korea	GB	Gyeongsangbuk-do
HU	Hokkaido University, Faculty of Agriculture, Entomological Institute, Sapporo, Japan	GG	Gyeonggi-do
NHMUK	The Natural History Museum, London, United Kingdom	GW	Gangwon-do
CB	Chungcheongbuk-do	GN	Gyeongsangnam-do
CN	Chunagcheongnam-do	JB	Jeollabuk-do
		JN	Jeollanam-do
		TD	Type depository
		TL	Type locality

Specimens were examined using an AxioCam MRC5 camera attached to a stereo microscope (Zeiss SteREO Discovery. V20; Carl Zeiss, Göttingen, Germany), processed using the AxioVision SE64 software (Carl Zeiss), and optimized with a Delta imaging system (i-solution, IMT i-Solution Inc., Vancouver, Canada). Morphological terminology is applied according to the American Entomological Institute website (<http://www.amentinst.org/GIN/morphology.php>).

Taxonomy

Order Hymenoptera

Family Ichneumonidae

Subfamily Banchinae Wesmael, 1845

Genus *Syzeuctus* Förster, 1869

Syzeuctus Foerster, 1869: 167. Type species: *Ichneumon maculatorius* Fabricius.

Diceratops Foerster, 1869: 167. Type species: *Pimpla bicornis* Gravenhorst.

Meyva Cameron, 1899: 19 1. Type species: *Meyva villosa* Cameron.

Rhynchotrevoria Cameron, 1906: 125. Type species: *Rhynchotrevoria rostrata* Cameron.

Ephialtina Szépligeti, 1908: 74. Type species: *Ephialtina apicalis* Szépligeti.

Leptoglyphis Brèthes, 1927: 319. Type species: *Leptoglyphis minasensis* Brèthes.

Paratanera Rao, 1953: 171. Type species: *Paratanera indica* Rao.

Diagnosis. Fore wing 5.0–14.0 mm. Body of moderate proportion to rather slender, the mesosoma usually short. Apex of clypeus in general convex but often truncate or less strongly convex apically. Occipital carina reaching base of mandible. Epomia long and strong. Areolet rather small, triangular with a long stalk, receiving second recurrent vein distad of middle. First tergite rather short, polished with rather sparse to very sparse punctures, moderately tapered toward base. Ovipositor sheath 1.5 to 3.0 times as long as hind tibia (Townes 1970).

Key to the South Korean species of *Syzeuctus*

- 1 Head, mesosoma, and first tergite covered with dense, long, white setae. Face swollen (as in metopiines). Lower ridge of mandible strong, lamelliform **2**
- Body with short setae. Face not swollen. Lower ridge of mandible not lamelliform **3**
- 2 Body length 13–14 mm. Sides of first tergite strongly convergent anteriorly. Face evenly convex. Hind tarsal segments entirely black..... ***S. sambonis***
- Body length about 10 mm. Sides of first tergite very weakly convergent anteriorly. Face with median swelling and two lateral swellings above the clypeal fovea. Hind tarsal segments with white bases and black tips ... ***S. flavofacialis* sp. nov.**
- 3 Malar space more than 1.0 times as long as the basal mandibular width. Fore wing without spots apically ***S. albopictus* sp. nov.**
- Malar space less than 1.0 times as long as the basal mandibular width. Fore wing with dark spot apically **4**
- 4 Posterior transverse carina of propodeum complete..... ***S. takaozanus***
- Posterior transverse carina of propodeum absent **5**
- 5 The first to fourth tergites entirely red with a pair of brown spot, sometimes each tergite with yellow band anteriorly and posteriorly..... ***S. apicifer***
- First to fourth tergites black with yellow posterior bands ***S. coreanus***

***Syzeuctus albopictus* Kang & Lee, sp. nov.**

<http://zoobank.org/83536F9D-12CA-4C95-BD34-04F625C8DE3A>

Fig. 1

Material examined. *Holotype*, Male, South Korea: [Ulsan] Ulju-gun, Sangbuk-my-eon, Mt. Sinbulsan, 9 Oct. 2006, S. J. Park (GEI) leg.

Description. Male. Body 13.0 mm in length (Fig. 1A), fore wing 8.0 mm in length.

Head in dorsal view with temple narrowed at an angle of more than 45° (Fig. 1C), strongly rounded. Face strongly convex, densely and coarsely punctate, with strong median swelling and two lateral swellings above clypeal fovea. Clypeus convex, with

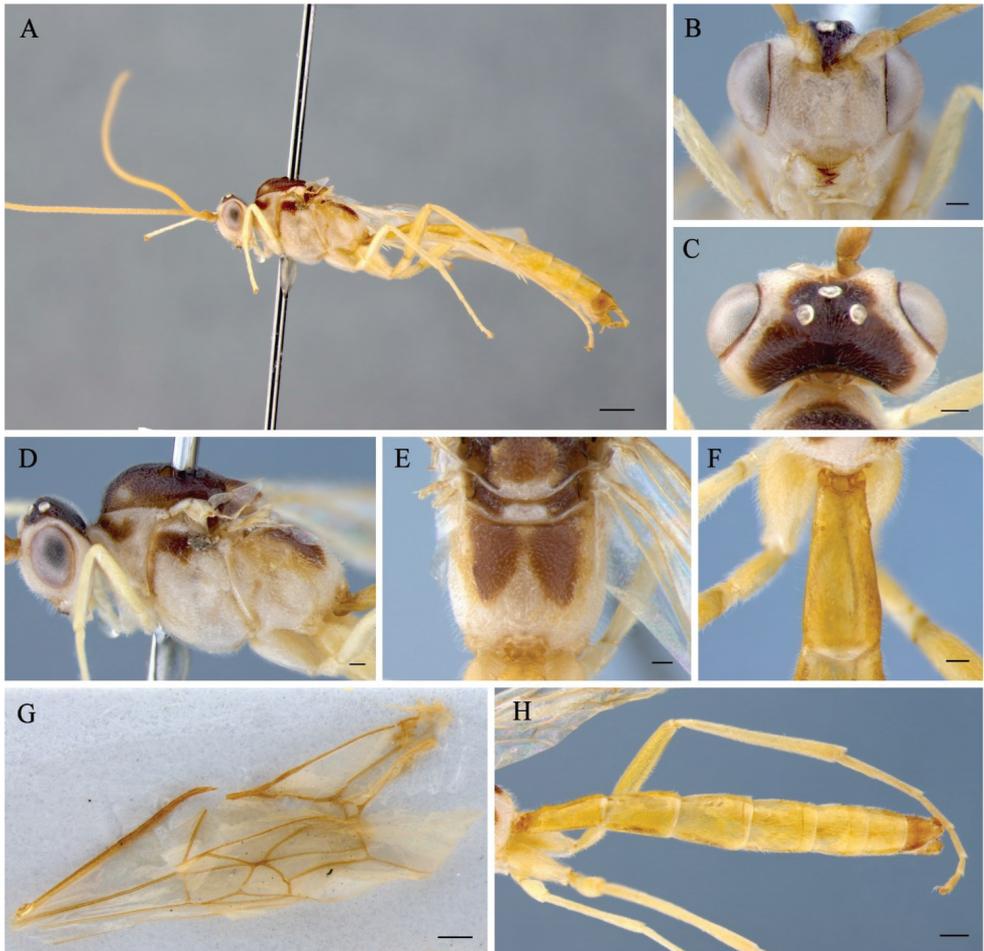


Figure 1. *Syzeuctus albopictus* Kang & Lee, male **A** habitus in lateral view **B** head in frontal view **C** head in dorsal view **D** thorax in lateral view **E** propodeum in dorsal view **F** first tergite in dorsal view **G** wings **H** abdomen in dorsal view. Scale bars: 1.0 mm (**A**), 0.2 mm (**B–F**), 0.5 mm (**G, H**).

long and thin setae; convex apically; clypeal fovea small, open. Mandible slightly tapered, upper tooth slightly longer than lower tooth; lower ridge lamelliform. Maxillary palp with five segments. Frons smooth and concave with small dense punctures. Vertex more sparsely punctate than face. Occipital carina complete, slightly sinuous, joining hypostomal carina at mandibular base. Temple polished and convex with fine punctures. Malar space about 1.3 times as long as basal mandibular width. Inner margin of eye parallel. Antenna filiform with 49 flagellomeres; first flagellomere 3.8 times as long as its width, 1.5 times as long as second flagellomere.

Mesosoma with dense and long setae. Pronotum with fine dense rugoso-punctures. Mesopleuron uniformly punctate except medially where it is polished and impunctate, punctures bigger than those on pronotum; postpectal carina absent. Metapleuron with dense and coarse punctures. Submetapleural carina complete, anterior half very

strongly developed, rectangular. Mesoscutum densely irregularly punctate, notaulus weakly present (Fig. 1D). Scutellum, in profile, strongly convex, densely punctate. Propodeum in dorsal view rather elongate, closely punctate tending to rugose medially, with long and dense setae (Fig. 1E); spiracle of propodeum oval; without posterior transverse carina. Legs slender. Fore tibial spur sinuate. Fore and mid tarsal claws pectinate. Ratio of hind tarsal segments are 5.0:2.5:1.7:1.0:1.3. Hind tarsal claw not pectinate. Hind wing with eight hamuli.

Metasoma elongate with shallow and fine punctures (Fig. 1H). First tergite 2.5 times as long as posterior width, without lateral longitudinal carina (Fig. 1F). Second tergite coriaceous, 1.3 times as long as posterior width.

Color whitish yellow.

Head whitish yellow with brown marks. Mandibular teeth brown (Fig. 1B). Frons and vertex with brown mark around ocellar triangle. Antenna yellow. Mesoscutum brown with triangular whitish yellow spot on its anterior on both sides. Scutellum yellowish brown. Postscutellum whitish yellow. Anterior margin of pronotum dorsally with brown mark. Mesopleuron with yellowish brown mark. Propodeum in dorsal view with elongate brown mark on each side of its anterior part (Fig. 1E). Legs entirely whitish yellow. Hind tarsal claw bright brown. Wings hyaline (Fig. 1G). Metasoma whitish yellow with yellowish brown coloration posteriorly (Fig. 1H).

Female. Unknown.

Distribution. South Korea (new record).

Remark. This species is easily distinguished by its very bright body color and very long malar space, 1.3 times the basal mandibular width.

Syzeuctus apicifer (Walker, 1874)

Fig. 2A

Macrus apicifer Walker, 1874: 305. Type: ♀, TL: Japan, TD: NHMUK.

Diagnosis. Female. Face convex, densely and coarsely punctate. Clypeus truncate apically. Temple polished and convex, with fine punctures. Malar space about 0.7 times as long as basal mandibular width. Antenna filiform with 43 flagellomeres; first flagellomere 1.8 times as long as second flagellomere. Mesosoma stout with dense pubescence. Epicnemium with a developed lamelliform projection apically. Propodeum in dorsal view stout; closely punctate tending to rugose, partly with dense setae; without posterior transverse carina. Fore and mid tarsal claws pectinate. Ratio of hind tarsal segments are 4.5: 2.5: 1.6: 0.8: 1.0; hind tarsal claw simple. Hind wing with eight hamuli. First tergite rectangular, 2.5 times as long as posterior width, without lateral longitudinal carina. Second tergite coriaceous, 1.5 times as long as posterior width. Ovipositor sheath with dense, short setae; approximately as long as metasoma.

Color. Black with brownish metasoma. Frontal orbit yellow, interrupted medially. Clypeus and mandible yellow with black mandibular teeth. Genal orbit yellow. Malar space yellow apically. Antenna brown with black scape and pedicel. Mesoscutum black

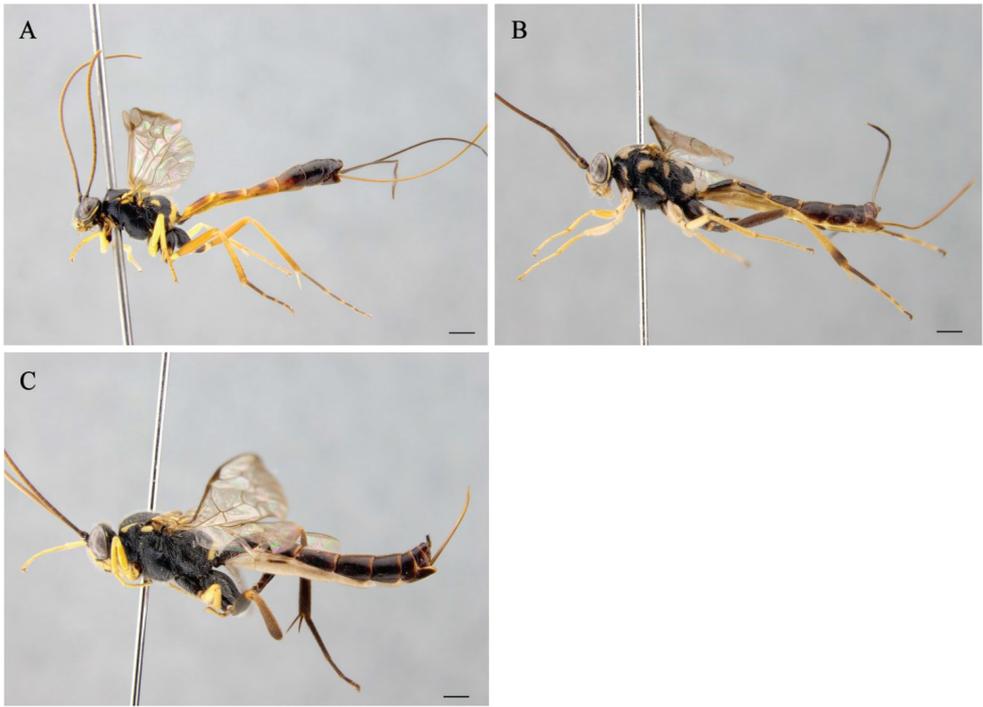


Figure 2. Habitus in lateral view **A** *Syzeuctus apicifer* (Walker) **B** *S. coreanus* Uchida **C** *S. sambonis* Uchida. Scale bars: 1.0 mm.

with a small anterior triangular yellow spot on each side. Scutellum yellow except black triangular spot anteriorly. Tegula yellow. Mesopleuron black with small yellow mark just below tegula. Upper projection of mesepimeron yellow. Propodeum in dorsal view with small yellow spot on each side of anterior part; strongly pointed triangular mark posteriorly. Fore wing with brown spot apically. Fore and mid legs entirely yellow with brown tarsal claw. Hind coxa and trochanter black with brown apically; trochantellus yellow; femur yellowish brown; tarsus and tarsal claw tan brown. Metasoma brown with black posteriorly. First tergite with yellow anteriorly and posteriorly.

Male. Male has more yellow spots; face, pronotum with longitudinal mark anteriorly and upper and posterior corner, mesopleuron with a longitudinal mark, posterior half of metapleuron. The color of genal orbit, mesoscutum, scutellum, propodeum are same with female. First and Second tergite black with yellow anteriorly and posteriorly. Third and fourth tergites brown with yellow posteriorly. Fore wing a bit infuscate apically, without spot.

Material examined. South Korea: 1♀, [CN] Sijang-ri, Ibjang-myeon, 10 Sep. 2010, H. S. Lee leg.; 1♀, [GB] Cheongdo-gun Gakbuk-myeon Namsan-ri, 20 Jun. 2007, J. W. Lee leg.; 2♀♀, ditto, 27 Jul. – 14 Aug. 2008, J. W. Lee leg.; 1♀, ditto, 23 Aug. 2008, J. W. Lee leg.; 1♀, ditto, 25 Jun.–8 Jul. 2012, J. W. Lee leg.; 2♀♀,

ditto, 2 Sep.–18 Nov. 2012, J. W. Lee leg.; 1♀, [GB] Cheongdo-gun Maejeon-myeon Mt. Seonuisan, 21 Sep. 2008; 1♂, [GB] Cheonmunsa, 1 Jul. 1981, S. S. Kim leg.; 1♀, [GB] Goryang-gun Osilnaru, 6 Sep. 1997, J. W. Lee leg.; 1♂, [GB] Gunwi-si Bugye-myeon Dongsan-ri San75 Odoam, 17 Aug. 2015, J. W. Lee leg.; 1♂, [GB] Gyeongju-si Sinwon2-gyo, 17 Jun. 2016, J. W. Lee leg.; 1♀1♂, [GB] Gyeongsan-si Apryang-myeon Sinwol, 21 Jun. 1986, J. M. Choi leg.; 1♀, [GB] Gyeongsan-si Daehak-ro 280 Yeungnam Univ., 27 Jun. 1986, M. L. No leg.; 12♀♀, ditto, 3 Jul. 1986, J. W. Lee leg.; 3♀♀, ditto, 10 Jul. 1986, J. W. Lee leg.; 3♀♀1♂, ditto, 8 Sep. 1986, J. W. Lee leg.; 1♂, ditto, 9 Sep. 1986, J. W. Lee leg.; 2♀♀1♂, ditto, 14 Oct. 1986, J. W. Lee leg.; 1♂, ditto, 29 Jun. 1987, J. W. Lee leg.; 2♀♀, ditto, 3 Jul. 1987, J. W. Lee leg.; 1♂, ditto, 6 Jul. 1987, J. W. Lee leg.; 1♀2♂♂, ditto, 8 Nov. 1987, J. W. Lee; 1♀, ditto, 18 Sep. 1987, J. W. Lee leg.; 1♀, ditto, 26 Aug. 1988, K. I. Suh leg.; 1♂, ditto, 27 Aug. 1988, J. W. Cha leg.; 1♀1♂, ditto, 30 Aug. 1988, J. Y. Cha leg.; 1♀, ditto, 31 Aug. 1988; 2♂, ditto, 1 Sep. 1988, J. W. Lee leg.; 2♀♀3♂♂, ditto, J. W. Lee leg.; 3♀♀1♂, ditto, 3 Sep. 1988, E. S. Kim leg.; 2♂♂, ditto, 7 Sep. 1988; 1♂, 3 Jul. 1989, J. G. Kim leg.; 1♀, ditto, 5 Aug. 1989; 2♀, ditto, 5 Sep. 1989; 1♂, ditto, 6 Sep. 1989; 1♀, ditto, 20 Jun. 1992, S. M. Lee leg.; 1♀, ditto, 20 Jun. 2008, Y. S. Choi leg.; 1♂, ditto, 13 Jul. 2008; 1♀, ditto, 8 Jul. 2008; 1♀, ditto, 28 Aug. 2008, J. Chen leg.; 1♀, ditto, 20 Sep.–3 Oct. 2015, J. W. Lee leg.; 1♂, [GB] Gyeongsan-si Namcheon-myeon Hado-ri Hadojeosiji, 18 Jun. 2016, J. W. Lee leg.; 3♀♀1♂, [GB] Gyeongsan-si Nammaeji, 7 Jul. 1986, J. Y. Cha leg.; 1♂, [GB] Mt. Baekamsan Subi, 10 Jul. 1999; 1♂, [GB] Mt. Hakgasan, 6 Sep. 1998; 1♀1♂, [GB] Mt. Sonamsan, 5 Sep. 1998, S. J. Suh leg.; 2♀♀, [GB] Sangju-si Gongseong-myeon, 18 Jul. 2007, S. K. Lee leg.; 1♀, [GB] Yeongju-si Bonghyeon-myeon Mt. Sobaeksan Ongnyeobong, 27 Jul. 2003; 1♀, [GG] Gwangcheon-ri, 23 Apr. 1984, J. W. Lee leg.; 1♂, [GN] Jinju-si Gajwa-dong, 29 Jul. 1989, J. S. Park leg.; 1♀, [GW] Goseong-gun Ganseong-eup Tapdong-ri Scheonsa, 10 Sep. 2008, H. S. Lee, S. W. Suk, & J. S. Lim leg.; 1♀, [GW] Hoengseong-gun Gapcheon-myeon Hadae-ri Holocene, 8–14 Jul. 2009, J. W. Lee leg.; 1♀, [GW] Hongcheon-gun Bukbangmyeon Seongdong-ri Jayeonhwangyeongyeongu park, 1419 Sep. 2011; 1♀, [GW] Wonju-si Heungeop-myeon Maeji-ri Yonsei Univ. Wonju Campus, 16 Aug.–16 Oct. 2008, H. Y. Han leg.; 1♀, ditto, 523 Sep. 2008, H. Y. Han leg.; 1♀, ditto, 16 Aug.–16 Oct. 2009, H. Y. Han leg.; 1♀5♂♂, ditto, 29 Jun.–15 Jul. 2011, H. Y. Han leg.; 1♀, ditto, 1 Jun.–30 Jul. 2012, H. Y. Han leg.; 1♀, ditto, 20 Jun.–30 Jul. 2013, H. Y. Han leg.; 1♀, ditto, 523 Sep. 2015, H. Y. Han leg.; 1♂, ditto, 17 Jul.–5 Sep. 2015, H. Y. Han leg.; 1♂, [JB] Jinan-gun Baegun-myeon Nochon-ri, 10 Jul. 2013, S. H. Oh leg.; 1♀1♂, [JB] Mt. Unjangsan, 5 Sep. 1998; 1♀, [JB] Wanju-gun Dongsang-myeon Daea Arboretum, 1630 Jun. 2012, J. M. Park leg.; 1♀, [JJ] Noruoreum, 26 Jun. 2003; 1♀1♂, [Daegu] Dalseo-gu Daegok-dong Daegu Arboretum, 20 Jun.–4 Jul. 2012, S. G. Kang leg.; 1♂, [Seoul] Noweon-gu Sanggye4-dong Mt. Suraksan, 18 Jul.–24 Aug. 2007, J. O. Lim leg. China: 1♂, Jirin, Yanbian Hunchun, 21 Jul. 2010, J. W. Lee leg.

Distribution. South Korea (new record), China (Sichuan), Japan.

***Syzeuctus coreanus* Uchida, 1928**

Fig. 2B

Syzeuctus coreanus Uchida, 1928: 93. Lectotype: ♀, TL: Korea, TD: HU.

Diagnosis. Body with short setae. Face not swollen. Malar space less than 1.0 times as long as the basal mandibular width. Antenna with 44 flagellomeres; first flagellomere 1.8 times as long as second flagellomere. Propodeum in dorsal view with yellow spot on each side of anterior part; strongly pointed triangular mark in posteriorly; posterior transverse carina absent. Ratio of hind tarsal segments are 5.0: 2.5: 1.5: 0.8: 1.0. Fore wing with dark spot apically. First to fourth tergites with thin yellow apically.

Material examined. South Korea: 1♀, [CB] Danyang-gun Cheondong-ri Mt. Sobaeksan, 2 Aug. 1994, J. I. Kim leg.; 1♀, ditto, 2 Aug.–14 Sep. 2005; 1♀, ditto, 28 Jul.–13 Aug. 2006; 1♀, ditto, 25 Jun.–9 Aug. 2007; 1♀, [CB] Mt. Sobaeksan Chondonggyegok, 22 May 1997, J. W. Lee leg.; 1♂, [CN] Gyeryong-si Sindoan-myeon Bunam-ri Mt. Gyeryongsan Donghaksa upper, 14 Mar.–28 Aug. 2012, J. C. Jeong leg.; 1♀, [Daejeon] Seopgu Wolpyeong Park, 20 Jun.–10 Aug. 2008; 2♂♂, [GB] Cheongdo-gun Unmun-myeon Haksodaepokpo, 2228 Jun. 2014, J. W. Lee leg.; 1♀, [GB] Yeongcheon-si Sinyeong-myeon Chisan-ri San 141-4, 15 Jul.–29 Aug. 2014, J. W. Lee leg.; 1♀, [GG] Gapyeong Cheongpyeong Goseong Mt. Homyeongsan, 16–30 Jul. 2009, J. O. Lim leg.; 1♀, ditto, 31 Jul.–17 Aug. 2009, J. O. Lim leg.; 1♀, [GG] Yangpyeong Yongmun Yeonsu Mt. Youngmunsan, 31 Jul.–17 Aug. 2009, J. O. Lim leg.; 1♀, [GN] Changyeong-gun Yueo-myeon Daedae-ri Uponeup, 5 May 2015, J. W. Lee leg.; 1♀, [GN] Mt. Sokrisan, 23 May 1981; 1♀, [GN] Yeongju-si Punggi-eup Jungyeong, 12 Jun.–23 Jul. 2008, J. M. Kwon leg.; 1♀, [GW] Donghaesi Samhwadong Mureung valley, 917 Aug. 2005; 1♀, [GW] Wonju-si Gwirae-myeon from Cheoneunsa Temple to Sibjabong, 5 May 2005, H. W. Kim & S. R. Lee leg.; 1♀, [JB] Namwon-si Sannae-myeon Mt. Jinisan Baemsagol, 11 Jun.–7 Jul. 2001, J. W. Lee leg.; 1♀, [JN] Choeung-gun Yeongnam-myeon Paryeong-ro Geumsa-ri Mt. Paryeongsan Forest Resort, 13 Apr. 2012; 1♂, [JN] Gurye-gun Sandong-myeon Simwon, 30 Jul. 1992; 1♀, [JN] Jeongeup-si Jangseong-gun Buka-myeon Sajabong, 31 Aug. 2005; 1♀, [JN] Mt. Jirisan, 15 Jul. 1979, S. H. Jeong leg.

Distribution. South Korea, China (Qinghai), Japan.

***Syzeuctus flavofacialis* Kang & Lee, sp. nov.**

<http://zoobank.org/C927F7B7-455B-46F9-BE8A-BEC0773D1CED>

Fig. 3

Description. Female. Body 10.0 mm in length. Fore wing 7.0 mm in length (Fig. 3A).

Head in dorsal view flat with temple narrowed at an angle of more than 45°, round (Fig. 3C). Face convex, densely and coarsely punctate; with median swelling and two lateral swellings above clypeal fovea (Fig. 3B). Clypeus flat, with long and strong setae;

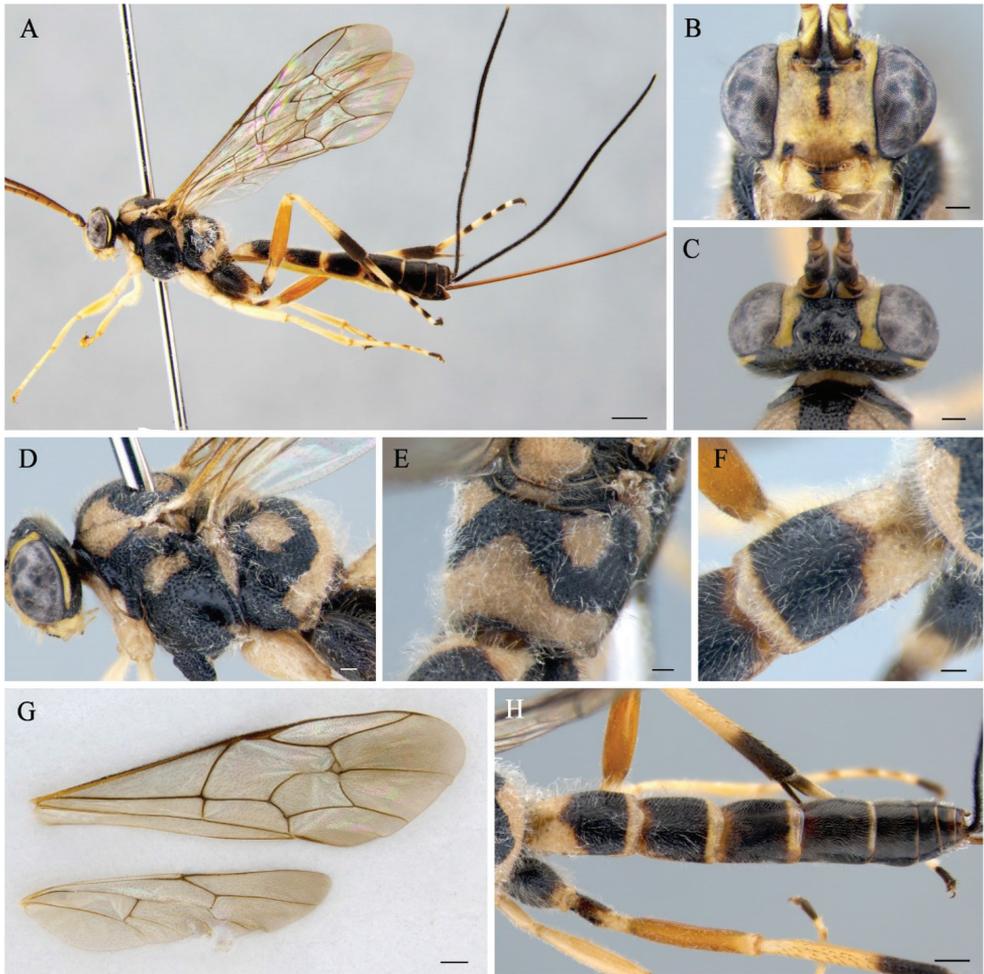


Figure 3. *Syzeuctus flavofacialis* Kang & Lee, female **A** habitus in lateral view **B** head in frontal view **C** head in dorsal view **D** thorax in lateral view **E** propodeum in dorsal view **F** first tergite in dorsal view **G** wings (left) **H** abdomen in dorsal view. Scale bars: 1.0 mm (**A**), 0.2 mm (**B–F**), 0.5 mm (**G, H**).

apically truncate; clypeal fovea open. Mandible moderately stout, strongly tapered, upper tooth slightly longer than lower tooth; lower ridge strongly developed, lamelliform. Maxillary palp with five segments. Frons smooth, convex, with small dense punctures. Vertex more sparsely punctate than face. Occipital carina complete, slightly sinuous, joining hypostomal carina at mandibular base. Temple polished and convex, with fine punctures. Malar space about 0.5 times as long as basal mandibular width. Inner margins of eyes parallel. Antenna filiform with 42 flagellomeres; first flagellomere 4.5 times as long as its width, 1.6 times as long as second flagellomere.

Mesosoma with dense and long setae. Pronotum with rugoso-punctures; upper posterior margin hook-shaped. Mesopleuron with epicnemial carina laterally, uniformly punctate except medially where it is polished and impunctate; punctures bigger

than those on pronotum; postpectal carina absent. Metapleuron with dense and coarse punctures. Submetapleural carina complete, anterior half strongly developed. Mesoscutum irregularly densely punctate, notaulus absent. Scutellum, in profile, slightly convex, densely punctate. Propodeum in dorsal view very stout, closely punctate towards rugose, partly with long and dense setae (Fig. 3E); spiracle of propodeum oval; without posterior transverse carina. Legs slender. Fore tibial spur sinuate. Fore and mid tarsal claws pectinate. Ratio of hind tarsal segments 2.5:1.4:1.0:0.5:0.6; hind tarsal claw not pectinate. Hind wing with eight hamuli.

Metasoma depressed with shallow and dense punctures (Fig. 3H). First tergite rectangular, 1.8 times as long as posterior width, without lateral longitudinal carina (Fig. 3F). Second tergite coriaceous, 1.2 times as long as posterior width. Ovipositor sheath with dense, short setae, 1.6 times as long as metasoma, 4.0 times as long as hind tibia.

Color black with yellow marks.

Head black. Face yellow with black median longitudinal mark (Fig. 3B); area around clypeal fovea black. Mandibular teeth black. Frontal orbit and genal orbit broadly yellow. Malar space yellow. Antenna brown; scape black with brown ventrally; pedicel yellow. Mesosoma black with yellow marks (Fig. 3D). Mesoscutum black with anterior triangular yellow spot on each side; medially with big yellow spot. Scutellum entirely yellow. Upper margin of pronotum broadly yellow; lower anterior margin with yellow spot. Tegula yellow. Mesopleuron black with yellow upper margin and lower posterior margin, slightly above middle with short yellow short boomerang-shaped mark. Propodeum in dorsal view with yellow spot on each side of anterior part; triangular yellow mark posteriorly (Fig. 3E). Fore and mid legs bright yellow with brown apex of mid tarsus and tarsal claw. Hind coxa and trochanter black; trochantellus yellow; femur bright brown; all tarsal segments yellow with black each apically; tarsal claw black. Wings weakly infusate (Fig. 3G). Metasoma black. First tergite black with yellow anterior third; first to fourth tergites each with a posterior yellow line (Fig. 3H).

Material examined. Holotype. ♀, South Korea, [GW] Wonju-si Heungeop-myeon Maeji-ri Yensei Univ., 18 Jul.–4 Sep. 2015, H. Y. Han (GEI) leg. **Paratypes** (GEI). South Korea: 1♀, [CB] Cheongwon-gun Miwon-myeon Miwon-ri, 2229 Jul. 2005, J. H. Han leg.; 1♀, [CB] Sobaeksan Danyang-gun Danyang-eup, 25 Jun.–9 Aug. 2007; 1♀, [CN] Mt. Deoksungsan Sudeoksa, 11 Aug. 1983, J. W. Lee leg.; 1♀, [Daejeon] Dong-gu Daejeon Univ., 15 Aug.–30 Sep. 2006, J. W. Lee leg.; 1♀, [Daejeon] Seogu Wolpyeong-dong Wolpyeon park, 20 Jun.–10 Aug. 2008; 1♀, [GB] Yeoncheon-si Cheongtong-myeon Temp. Eunhaesa, 21 Jul.–10 Aug. 2015, J. W. Lee leg.; 1♀, [GB] Yeonju-si Dansan-myeon Jwaseok-ri Mt. Sobaeksan Yeonhwa 2 bridge, 6 Aug.–8 Sep. 2016, Y. J. Kim leg.; 2♂, [GG] Annyang-si Manan-gu Gwanaksan, 519 Jul. 2007, J. O. Lim leg.; 2♀♀, [GG] Anyang-si Kwanak, 26 Jul.–7 Aug. 2008; 1♀, [GG] Goyang-si Deog-yang-gu Goyang-dong, 24 Aug.–6 Sep. 2007, J. O. Lim leg.; 1♀, [GW] Donghae-si Samhwa-dong Mureung valley, 15 Jul.–1 Aug. 2005; 1♀, [GW] Wonju-si Heungeop-myeon Maeji-ri Yensei Univ., 11 Aug.–8 Sep. 2007, H. Y. Han leg.; 1♀, ditto, 31 Jul.–5 Sep. 2014, H. Y. Han leg.; 1♀, ditto, 18 Jul.–4 Sep. 2015, H. Y. Han leg.; 1♀, [Incheon] Ongin-gun Daechong-myeon daechongri [natural habitat

of *Camellia japonica*] Daecheongdo, 9 Aug. 2017, J. W. Lee leg.; 1♀, [JB] Buan-gun Jinseo-myeon Unho-ri San1-1, 7 Jul.–18 Aug. 2016, E. J. Hong leg.; 2♀♀, [Seoul] Noweon-gu Sanggye4-dong Mt. Suraksan, 18 Jul.–24 Aug. 2007, J. O. Lim leg.

Distribution. South Korea (new record).

Remark. This species is similar to *S. sambonis* in that its face is convex, but unlike *S. sambonis*, which has a face that is convex overall, *S. flavofacialis* has median and lateral swellings.

Syzeuctus sambonis Uchida, 1928

Fig. 2C

Syzeuctus sambonis Uchida, 1928: 94. Type: ♂, TL: Japan, TD: HU.

Diagnosis. Body entirely covered with dense, long, white setae. Face swollen medially with two lateral swelling above clypeal fovea. Malar space about 0.3 times as long as basal mandibular width. Lower ridge of mandible strong, lamelliform. Posterior transvers carina of propodeum present. Pleural carina present. Ratio of hind tarsal segments are 2.5:1.2:1.0:0.3:0.5. Sides of first tergite strongly convergent anteriorly. Hind tarsal segments entirely black.

Material examined. South Korea: 1♂, [CB] Cheongju-si Chungbuk National Univ., 19 Jul. 2003, G. H. Park & O. B. Kwon leg.; 1♀, [CB] Cheongwon-gun Miwon-myeon Miwon-ri, 22–29 Jul. 2005, J. H. Han leg.; 1♀, [CN] Onyang, 19 Jun. 1983, J. W. Lee leg.; 2♀♀, [CN] Seosan-si Haemi-myeon Daegok-ri Hanseo Univ., 11 Jun.–8 Jul. 2011, J. W. Lee leg.; 1♀, [GB] Bonghwa-gun Myeongho-myeon Mt. Cheongyangsan, 5 May 2000, J. W. Lee leg.; 1♀, [GB] Gyeongju-si Hyeongok-myeon Namsa-ri, 30 Jun.–14 Jul. 2005, J. T. Mun leg.; 1♀, ditto, 28 Jul.–11 Aug. 2005, J. T. Mun leg.; 1♀, ditto, 1825 Aug. 2005, J. T. Mun leg.; 1♀, [GG] Ongjin Mungapdo, 4 Aug. 1982, J. I. Kim leg.; 1♀, [GM] Mt. Jirisan Jangdan, 2124 Jul. 1975; 1♀, [Incheon] Nam-gu Jamsu-dong, 5 Jul. 1986, H. M. Park leg..

Distribution. South Korea, China (Henan, Shandong), Japan.

Syzeuctus takaozanus Uchida, 1928

Fig. 4

Syzeuctus takaozanus Uchida, 1928: 95. Lectotype: ♀, TL: Japan, TD: HU.

Description. Male. Body 13.0 mm in length. Fore wing 8.0 mm in length (Fig. 4A).

Head in dorsal view flat with temple narrowed at an angle of about 45°, round (Fig. 4C). Face convex, densely and coarsely punctate, without swelling. Clypeus flat, with long and thin setae, apically convex (Fig. 4B); clypeal fovea open. Mandible moderately stout, strongly tapered, upper tooth slightly longer than lower tooth; lower ridge

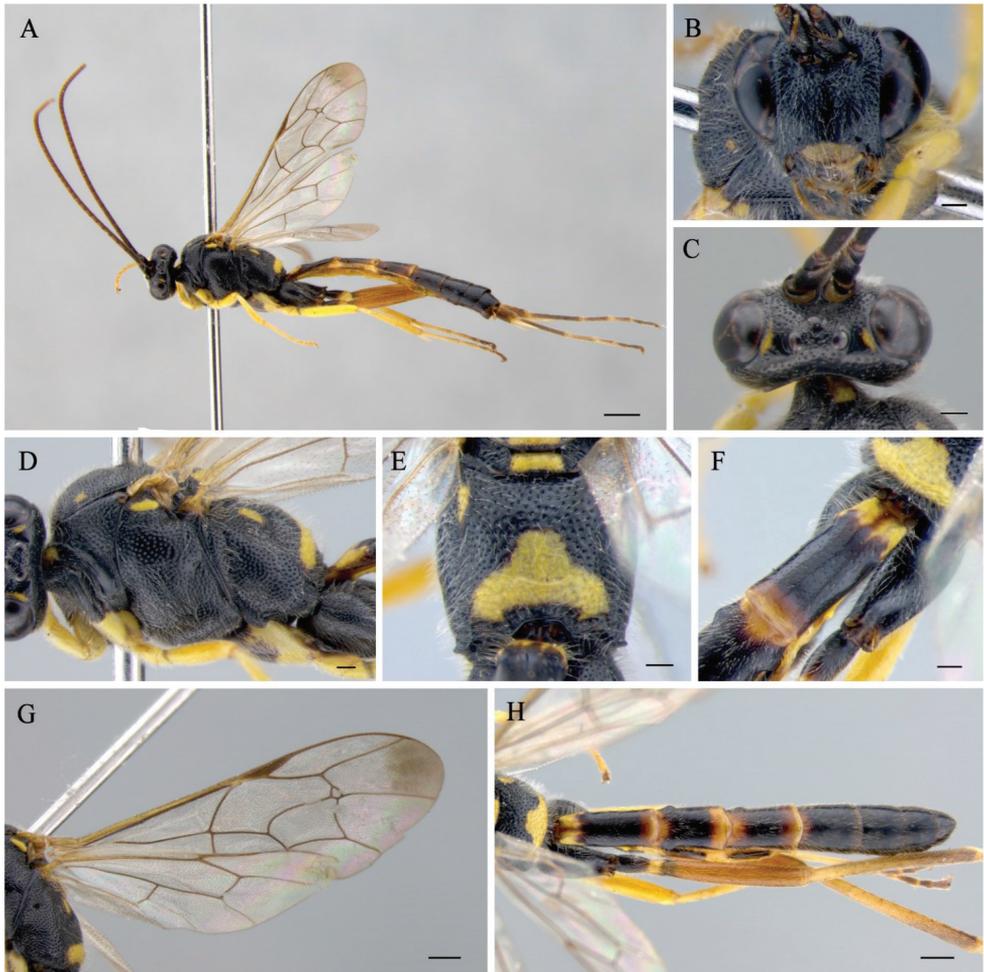


Figure 4. *Syzeuctus takaozanus* Uchida, male **A** habitus in lateral view **B** head in frontal view **C** head in dorsal view **D** thorax in lateral view **E** propodeum in dorsal view **F** first tergite in dorsal view **G** wings (left) **H** abdomen in dorsal view. Scale bars: 1.0 mm (**A**), 0.2 mm (**B–F**), 0.5 mm (**G, H**).

not developed. Maxillary palp with five segments. Frons smooth and convex with dense punctures. Vertex more sparsely and finely punctate than face. Occipital carina complete, slightly sinuous, joining hypostomal carina at mandibular base. Temple polished and convex with fine punctures. Malar space about 0.6 times as long as basal mandibular width. Inner margin of eye parallel. Antenna filiform with 42 flagellomeres; first flagellomere 3.0 times as long as its width, 2.0 times as long as second flagellomere.

Mesosoma with dense and short setae (Fig. 4D). Pronotum with punctures entirely, not fused; upper posterior margin hook-shaped. Mesopleuron with epicnemial carina reaching medially; uniformly punctate, except medially where it is polished and impunctate; punctures bigger than on pronotum; postpectal carina absent. Metapleuron with dense punctures. Submetapleural carina complete, anterior half strongly de-

veloped. Mesoscutum polished, irregularly densely punctate, notaulus absent. Scutellum, in profile, slightly convex, densely punctate. Propodeum in dorsal view stout; closely punctate with long and dense setae; only posterior transverse carina completely developed; spiracle of propodeum oval. Legs slender. Fore tibial spur sinuate. Fore and mid tarsal claws pectinate. Ratio of hind tarsal segments 4.8:2.5:1.7:1.0:1.3; hind tarsal claw simple. Hind wing with seven hamuli.

Metasoma elongate with shallow and dense punctures (Fig. 4H). First tergite 3.1 times as long as posterior width (Fig. 4F), without lateral longitudinal carina. Second tergite coriaceous, 1.6 times as long as posterior width.

Color black with yellow marks.

Head black. Clypeus yellow. Frontal orbit with yellow spot. Genal orbit medially yellow. Antenna brown with black scape and pedicel. Tegula yellow. Upper margin of mesopleuron with yellow longitudinal mark. Mesoscutum with small anterior yellow spot on each side. Scutellum yellow, except black triangular mark anteriorly. Propodeum, in dorsal view, with small yellow spot on each side of anterior part; \square -shaped yellow mark posteriorly (Fig. 4E). Fore wing infusate apically (without any spots) (Fig. 4G). Fore leg yellow with brown tarsal claw. Mid leg yellow with black coxa ventrally and tarsal claw. Hind coxa and trochanter black; trochantellus yellow; femur brown; tibia and tarsus yellow with black apically; tarsal claw black.

Material examined. South Korea: 1 ♀, [CN] Daejeon Dong-gu yongundong Daejeon Univ., 18 May–14 Jun. 2007, J. W. Lee leg.; 1 ♀, [GB] Gyeongsansi Daedong Yeungnam Univ., 1125 Jun. 2009, J. W. Lee leg.; 1 ♀, [GG] An-yang-si Man-an-gu Kwan-ag [Arb], 924 Jun. 2007, J. O. Lim leg.. China: 1 ♂, Jirin, Yanbian Hunchun, 21 Jul. 2010, J. W. Lee leg.

Distribution. South Korea (new record), China (Henan, Liaoning), Japan.

Remarks. The male specimen is newly described based on the Chinese specimen. *Syzeuctus takaozanus* and *S. sambonis* are similar because of the entirely black body, and easily distinguished from other *Syzeuctus* by the complete posterior transverse carina of the propodeum (Sheng et al. 2013; Uchida 1928). These two species are easily distinguished from each other by the color of the face: female *S. takaozanus* has an entirely black face, while *S. sambonis* has a yellow face with a black spot (Sheng et al. 2013). Furthermore, these two species differs by the presence (*S. sambonis*) or absence (*S. takaozanus*) of the pleural carina. By these characters, females and males of *S. takaozanus* are easily associated. Unlike the male, the female of *S. takaozanus* has an entirely black body and weakly infusate wings apically.

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Revision of *Neorthostigma* Belokobylskij, 1998 (Hymenoptera, Braconidae, Alysiinae) with description of a new species from Papua New Guinea

Francisco Javier Peris-Felipo¹, Julia Stigenberg²,
Donald L. J. Quicke³, Sergey A. Belokobylskij^{4,5}

1 Bleichstrasse 15, Basel CH–4058, Switzerland **2** Department of Zoology, Swedish Museum of Natural History, Stockholm S-114 18, Sweden **3** Department of Biology, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, BKK 10330, Thailand **4** Zoological Institute, Russian Academy of Sciences, St Petersburg, 199034, Russia **5** Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, Warszawa 00–679, Poland

Corresponding author: Francisco Javier Peris-Felipo (peris.felipo@gmail.com)

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Abstract

The status of the genus *Neorthostigma* Belokobylskij, 1998 is re-established as a result of additional morphological studies. A new species, *N. braeti* **sp. nov.**, from Papua New Guinea is described and illustrated. *Aspilota brachychypeata* Fischer 1978 is transferred to *Neorthostigma*, hence *N. brachychypeata* (Fischer, 1978), **comb. nov.** A new synonym is suggested, *Aspilota macrops* Stelfox & Graham, 1951 = *Neorthostigma eoum* Belokobylskij, 1998, **syn. nov.**; *A. macrops* is transferred to *Neorthostigma*. This genus is additionally recorded in the Western Palaearctic (Norway) and Australasian (Papua New Guinea) regions for the first time. A key for the determination of the three known species of *Neorthostigma* is provided.

Keywords

Australasia, Braconidae, endoparasitoid of Diptera, key, new species, new synonym, redescription, Western Palaearctic

Introduction

Neorhostigma Belokobylskij, 1998 is a rare genus, previously known exclusively from the Eastern Palaearctic, based only on its type species, *N. eoum* Belokobylskij, 1998. It belongs to the *Aspilota* genus group of the subfamily Alysiinae (Hymenoptera, Bracconidae). *Neorhostigma* appears to be closely related with *Orthostigma* Ratzeburg, 1844 because its peculiar mandible structure with a distinct transverse, curved carina and an enlarged lobe-shaped lower (third) tooth. *Neorhostigma* was synonymised with *Orthostigma* by Wharton (2002) but retained it here as a subgenus. However, the combination of its particular diagnostic characters widely used and generally considered important in the subfamily Alysiinae, leads us to reinstate *Neorhostigma* as a genus in its own right. Notable among these are absence of an oblique sulcus between eye and antennal socket, enlarged anterior tentorial area almost reaching margin of eye and lack of medio-posterior mesoscutal pit (Belokobylskij 1998; Belokobylskij and Tobias 2007; Belokobylskij et al. 2019).

After study of the photos and description of the holotype of *Aspilota macrops* Stelfox & Graham as well as additional material from the Russia (Far East and north-west of the European part), a new synonymisation is here suggested, *Aspilota macrops* Stelfox & Graham, 1951 = *Neorhostigma eoum* Belokobylskij, 1998, syn. nov. As result of this synonymy the known geographic distribution of *Neorhostigma* is greatly extended here from Eastern [Japan (Kyushu Island), Russia (Primorskiy Territory and Sakhalin Island)] till Western Palaearctic [Ireland, Netherlands, Norway (new record), Russia (Leningrad Province), Slovakia and United Kingdom]. From the Australasian region a new species, *N. braeti* sp. nov., from Papua New Guinea is here described, and additionally *Aspilota brachychypeata* Fischer, 1978 from Papua New Guinea is also transferred to *Neorhostigma*. This genus now comprises three species all of which are here described or re-described and illustrated.

Materials and methods

New Australasian specimens were collected with Malaise traps during the expedition “Our Planet Reviewed – Papua New Guinea” carried out from 25 October to 10 November 2012 at eight sampling sites at 500 m altitudinal intervals. The sampling transect was located on the north-eastern face of Mt Wilhelm and at Wanang (Swire) Research Station (175 m asl) terminating in lowland forest 63 km north of Mt Wilhelm. Four Malaise traps were set up at each elevation at intervals of 100 m following the same contour line. Catches were preserved with 90% ethyl alcohol (Robillard et al. 2016).

For morphological terminology, sculpture and measurements see Peris-Felipo et al (2014); for wing venation nomenclature see van Achterberg (1993); for measurements of the marginal cell see Peris-Felipo and Belokobylskij (2017).

For molecular methods, DNA from the specimens was extracted from five ethanol-preserved specimens using the Thermo Labsystems KingFischer extraction robot at the

Swedish Museum Natural History (NHRS) laboratory facility. A 658-bp fragment from the 5' region of CO1 was amplified using the LCO and HCO primers (Folmer et al. 1994) using Ready-To-Go PCR beads (Amersham Pharmacia Biotech, Amersham, UK) on the following program: 5 min 94 °C hot-start; 40 cycles: denature 94 °C for 15 s, anneal 46 °C for 15 s, extend 72 °C for 15 s; final extension 72 °C for 10 min. This gene has been used in previous studies of braconid phylogenetics (Belshaw et al. 2000; Belshaw and Quicke 2002; Dowton et al. 2002; Zaldivar-Riverón et al. 2006; Sharanowski et al. 2011; Stigenberg et al. 2015). PCR products were purified with EXO1 and FastAP. The product was sequenced using both the forward and reverse primers and were assembled and edited using Geneious Pro v.9.1.8. The Voseq v.1.7.3 (Peña and Malm 2012) database was used for storing voucher and DNA sequence data. All sequences obtained as part of this study were deposited in GenBank under accession numbers: MW191729–MW191733.

Material was imaged using a Digital Microscope Keyence VHX-2000 and Adobe Photoshop imaging system. The studied material including the types of described species are deposited in the collection of the Bishop Museum (Honolulu, USA; BPBM), Muséum national d'Histoire naturelle (Paris, France; MNHN), the Natural History Museum (Oslo, Norway; NHMO), the Zoological Institute of the Russian Academy of Sciences (St Petersburg, Russia; ZISP), and the F.J. Peris-Felipo Entomological Collection (Basel, Switzerland; PFEC)

Taxonomic part

Class Hexapoda Blainville, 1816

Order Hymenoptera Linnaeus, 1758

Family Braconidae Nees, 1811

Subfamily Alysiinae Leach, 1815

Tribe Alysiini Leach, 1815

Genus *Neorhostigma* Belokobylskij, 1998

Belokobylskij 1998: 9; Fischer 2001: 65; Wharton 2002: 91 (as subgenus); Belokobylskij and Tobias 2007: 10 (as valid genus); Yu et al. 2016; Belokobylskij et al. 2019: 215.

Type species. *Neorhostigma eoum* Belokobylskij, 1998 (= *Aspilota macrops* Stelfox & Graham, 1951).

Diagnosis. Mandibles small, tridentate, with distinct complete transverse and curved submedian carina. Upper tooth very small; median tooth narrow and rather short; lower tooth more or less wide, lobe-shaped, rounded or with distinct angle ventro-distally, with several long outstanding curved setae. Paraclypeal fovea oval, long, almost reaching inner margin of eyes. Oblique sulci between margins of

antennal socket and eye always absent. Antenna thickened; first flagellar segment distinctly longer than second segment. Mesoscutum without medio-posterior pit; notauli present only on anterior (vertical) part of mesoscutum; precoxal sulcus present, oblique and sculptured; propodeum variable, with distinctly delineated large areola and with different types of sculpture and longitudinal or transverse carinae. Fore wing: marginal cell not shortened; vein 2-SR always present and rather distinctly sclerotized; veins m-cu and cu-a strongly postfurcal; first subdiscal cell closed postero-apically by vein CU1b; vein CU1a arising from vein 3-CU1 before its middle. Metasoma more or less distinctly compressed laterally. Ovipositor sheath much shorter than metasoma.

Hosts. Unknown.

Remarks. Wharton (2002) treated this genus only as a subgenus of *Orthostigma* on the basis of similarity of mandibular characteristics. However, re-evaluation of the morphological diagnostic features widely applied for separation and diagnosis of genera within the Alysiinae (see also: Zhu et al. 2017), provides additional evidence of the distinction between *Neorthostigma* and closed genera (see above).

***Neorthostigma brachyclypeata* (Fischer, 1978), comb. nov.**

Figs 1, 2

Aspilota brachyclypeata Fischer 1978: 487; Wharton 1982: 294; Yu et al. 2016.

Type material. Holotype: female, Papua New Guinea, NE. Swart Val.: Karubaka, 1400 m, 21.xi.1958, Coll. by J. L. Gressitt [12233] (BPBM).

Re-description. Female (holotype).

Length. Body 1.8 mm, fore wing 2.0 mm, hind wing 1.4 mm.

Head. In dorsal view, 1.9 times as wide as long, 1.3 times as wide as mesoscutum, smooth, with temple rounded behind eyes. Antenna 22-segmented, 1.2 times as long as body. Scape 2.0 times as long as pedicel. First flagellar segment 2.2 times as long as its apical width, 1.3 times as long as second segment. Second flagellar segment 1.8 times, third to 20th (apical) segments 1.6–1.8 times as long as their maximum width. Eye in lateral view 1.6 times as high as wide and 1.8 times as wide as temple medially. POL 0.9 times OD; OOL 2.6 times OD. Face 1.4 times as wide as high, with sparse setae medially, punctate-rugulose medially and smooth laterally; inner margins of eyes subparallel. Clypeus 2.9 times as wide as high, slightly concave ventrally. Mandible almost parallel-sided, 1.2 times as long as its maximum width. Upper tooth very small and obtuse; middle tooth rather narrow and longer than lower tooth, weakly directed upwards; lower tooth narrow, round, with several long outstanding curved setae.

Mesosoma. In lateral view 1.4 times as long as high. Mesoscutum (dorsal view) 0.7 times as long as its maximum width, smooth, sparsely setose. Notauli mainly absent

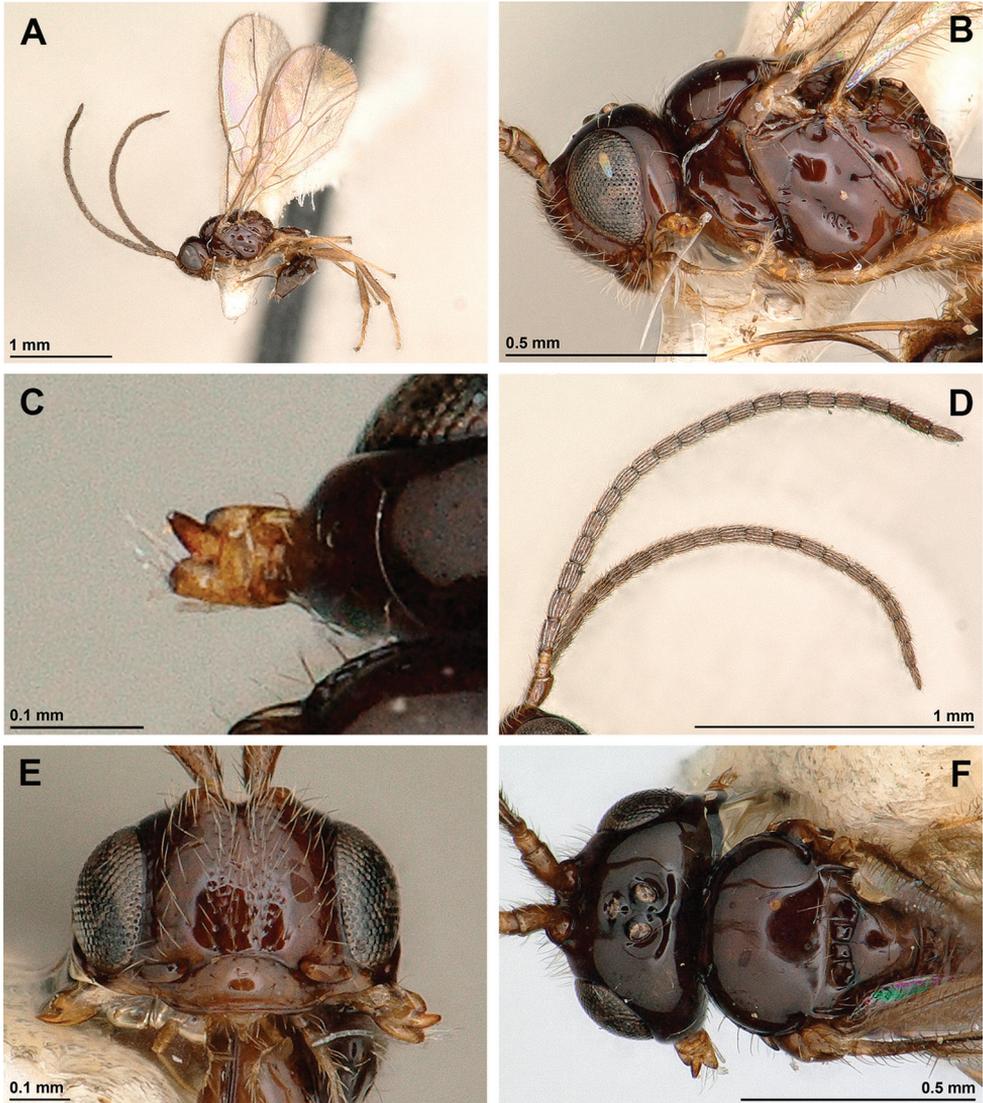


Figure 1. *Neorthostigma brachyclypeata* (Fischer, 1978) (female, holotype) **A** habitus, lateral view **B** head and mesosoma, lateral view **C** mandible **D** antenna **E** head, frontal view **F** head and mesonotum, dorsal view.

on horizontal surface of mesoscutum. Mesoscutal pit absent. Prescutellar depression smooth, with median and lateral carinae, 1.7 times as long as its maximum width. Precoxal sulcus present, crenulate, short, not reaching anterior and posterior margins of mesopleuron. Posterior mesopleural furrow smooth. Propodeum with large, wide and mainly smooth but partly rugulose pentagonal areola; basolateral areas smooth. Propodeal spiracles medium-sized, diameter 0.3 times distance from spiracle to anterior margin of propodeum.



Figure 2. *Neorthostigma brachychypeata* (Fischer, 1978) (female, holotype) **A** propodeum, dorsal view **B** first metasomal tergite, dorsal view **C** hind leg, metasoma and ovipositor, lateral view **D** fore and hind wings.

Wings. Fore wing 2.5 times as long as its maximum width. Marginal cell ending at apex of wing, 3.9 times as long as its maximum width. Vein 3-SR 1.8 times as long as vein 2-SR. Vein SR1 2.3 times as long as vein 3-SR. Vein 3-SR 3.7 times as long as vein r. Vein r much longer than pterostigma width. First subdiscal cell 2.5 times as long as its maximum width. Hind wing 5.3 times as long as its maximum width.

Legs. Hind femur 3.8 times as long as its maximum width. Hind tibia weakly widened to apex, 6.4 times as long as its maximum subapical width, 1.1 times as long as hind tarsus. First segment of hind tarsus 1.7 times as long as second segment.

Metasoma. First tergite widened towards apex, 1.5 times as long as its apical width, sparsely striate laterally but rugose in medio-basal half. Ovipositor 1.6 times as long as first tergite, 0.5 times metasoma, approximately as long as hind femur.

Colour. Body, flagellar segments of antenna dark brown. Hind femur and tibia, mandible, pterostigma, fore and middle legs and hind tarsus light brown to reddish brown. First metasomal tergite similar colour to second and third tergites. Wings almost hyaline.

Male. Unknown.

Comparative diagnosis. This species is similar to *N. braeti* sp. nov.; the differences between them are indicated in the key to species (see below).

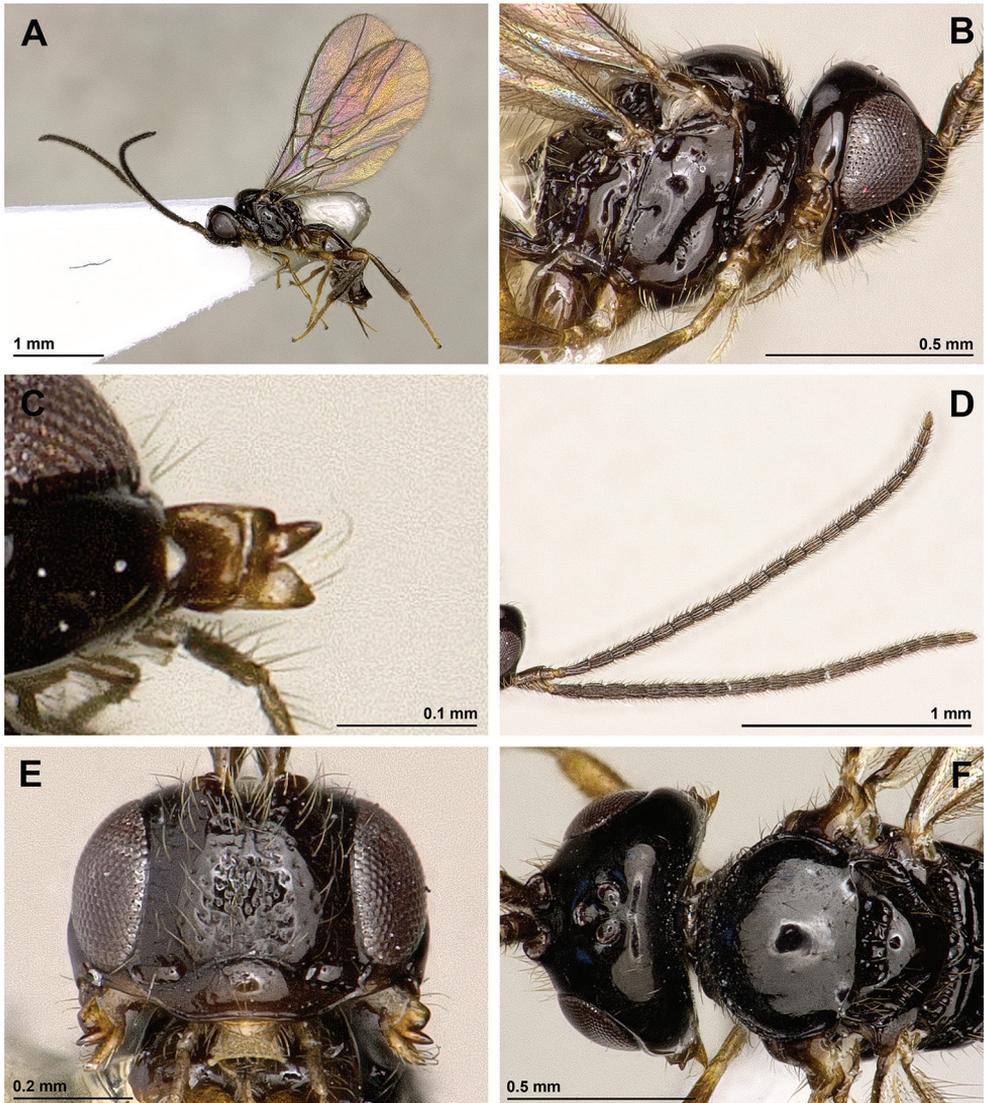


Figure 3. *Neorthostigma braeti* Peris-Felipo, sp. nov. (female, holotype) **A** habitus, lateral view **B** head and mesosoma, lateral view **C** mandible **D** antenna **E** head, frontal view **F** head and mesonotum, dorsal view.

***Neorthostigma braeti* Peris-Felipo, sp. nov.**

<http://zoobank.org/A3F4AABD-4E96-4705-A31D-31FAA627E16E>

Figs 3, 4

Type material. *Holotype*: female, Papua New Guinea, Mt Wilhelm, UTM (-5.731961, 145–2522), 700 m, 17–18.v.2013, understorey, Coll. by Sam et al., site: MW0700–01, P4705, vial: 20833, MAL–MW0700’A–05/16–d05 (MNHM).

Paratypes: 19 females, same location as holotype but: 2 females, 200 m, 1–2.11.2012; MAL–MW0200’C–08/16–d08 (MNHM); 1 female, MW0700–03,



Figure 4. *Neorhostigma braeti* Peris-Felipo, sp. nov. (female, holotype) **A** propodeum, dorsal view **B** first metasomal tergite, dorsal view **C** hind leg, metasoma and ovipositor, lateral view **D** fore and hind wings.

P4737, vial: 20837, MAL–MW0700°C–05/16–d05, voucher ID: JP_110, GenBank ID: MW191729 (MNHM); 1 female, MW0700–03, P4753, vial: 20839, MAL–MW0700'D–05/16–d05, voucher ID: JP_113, GenBank ID: MW191732 (MNHM); 2 females, MW0700–04, P4737, vial: 20837, MAL–MW0700'C–05/16–d05, voucher ID: JP_112, GenBank ID: MW191731 (MNHM); 3 females, MW0700–04, P4737, vial: 20839, MAL–MW0700'D–05/16–d05 (MNHM, ZISP); 2 females, 200 m, 20–21.v.2013; MAL–MW0200'B–08/16–d08 (MNHM); 2 females, 22–23.v.2013, P4742, vial: 20873, MAL–MW0700'C–10/16–d10 (PFEC); 1 female, 22–23.v.2013, P4758, vial: 20624, MAL–MW0700'D–10/16–d10, voucher ID: JP_111, GenBank ID: MW191730 (MNHM); 1 female, 22–23.v.2013, P4742, MAL–MW0700D–09/16–d09, voucher ID: JP_127, GenBank ID: MW191733 (MNHM); 5 females, 175 m, 20–25.xi.2012; MAL–MAN–D–07/16–d07 (leg. Basset) (MNHM; ZISP).

Description. Female (holotype).

Length. Body 2.0 mm, fore wing 2.4 mm, hind wing 1.5 mm.

Head. In dorsal view, 1.9 times as wide as long, 1.3–1.4 times as wide as meso-scutum, smooth, with temple rounded behind eyes. Antenna 24-segmented, 1.2 times as long as body. Scape 2.0 times as long as pedicel. First flagellar segment 2.3 times

as long as its apical width, 1.3 times as long as second segment. Second flagellar segment 1.8 times, third to 22th (apical) segments 1.6–1.8 times as long as their maximum width. Eye in lateral view 1.6 times as high as wide and 1.6 times as wide as temple medially. POL 0.9 times OD; OOL 2.7 times OD. Face 1.4 times as wide as high, with sparse setae, punctate-rugulose medially; inner margins of eyes subparallel. Clypeus 2.8 times as wide as high, slightly concave ventrally. Mandible almost parallel-sided, 1.4 times as long as its maximum width. Upper tooth very small and obtuse; middle tooth rather narrow and as long as lower tooth, weakly directed upwards; lower tooth narrow, distinctly angulated ventro-distally, with several long outstanding curved setae.

Mesosoma. In lateral view 1.1 times as long as high. Mesoscutum (dorsal view) 0.9 times as long as its maximum width, smooth, sparsely setose. Notauli mainly absent on horizontal surface of mesoscutum. Prescutellar depression smooth, with median and lateral carinae, 1.3–1.4 times as long as its maximum width. Precoxal sulcus present, crenulate, long, almost reaching anterior margin but not reaching posterior margin of mesopleuron. Posterior mesopleural furrow almost smooth. Propodeum with large, wide and mainly smooth pentagonal areola; basolateral areas smooth, sparsely rugose close to carinae. Propodeal spiracles medium-sized, diameter 0.3 times distance from spiracle to anterior margin of propodeum.

Wings. Fore wing 2.3 times as long as its maximum width. Marginal cell ending at apex of wing, 3.9 times as long as its maximum width. Vein 3-SR 2.0 times as long as vein 2-SR. Vein SR1 2.4 times as long as vein 3-SR. Vein 3-SR 3.1 times as long as vein r. Vein r much longer than pterostigma width. First subdiscal cell 2.5 times as long as its maximum width. Hind wing 5.0 times as long as its maximum width.

Legs. Hind femur 4.0 times as long as its maximum width. Hind tibia weakly widened to apex, 7.8 times as long as its maximum subapical width, about as long as hind tarsus. First segment of hind tarsus 1.7 times as long as second segment.

Metasoma. First tergite widened towards apex, 1.8 times as long as its apical width, sparsely striate mainly but rugose in medio-basal half. Ovipositor 1.5 times as long as first tergite, 0.5 times metasoma, approximately as long as hind femur.

Colour. Body, hind femur and tibia, flagellar segments of antenna and pterostigma dark brown to black. Mandible, fore and middle legs and hind tarsus light reddish brown to reddish brown. First metasomal tergite similar colour to second and third tergites. Wings almost hyaline.

Variation. Body length 1.9–2.3 mm, fore wing length 2.1–2.6 mm, hind wing length 1.4–1.6 mm. Antenna 24–27-segmented. First flagellar segment 2.2–2.4 times as long as its maximum width. Hind femur 3.9–4.0 times as long as its maximum width. Hind tibia 7.5–8.0 times as long as its maximum subapical width. First metasomal tergite 1.8–1.9 times as long as its apical width.

Male. Unknown.

Etymology. Named in honor of Dr Yves Braet, Belgian braconidologist.

Comparative diagnosis. Differs from *N. brachyclypeata* as indicated in the key to species (see below).

***Neorhostigma macrops* (Stelfox & Graham, 1951), comb. nov.**

Figs 5–7

Aspilota macrops Stelfox and Graham 1951: 3; Tobias 1962: 106; 1986: 123; Fischer 1972: 409; Shenefelt 1974: 976; Yu et al. 2016; Belokobylskij et al. 2019: 208.

Neorhostigma macrops: Belokobylskij and Tobias 2007: 10.

Neorhostigma eoum Belokobylskij 1998: 9, syn. nov.; Fischer 2001: 65; Belokobylskij and Tobias 2007: 10; Yu et al. 2016; Belokobylskij et al. 2019: 215.

Orthostigma (*Neorhostigma*) *eoum*: Wharton 2002: 91.

Type material of *Aspilota macrops* (Figs 5A, 5B). *Holotype*: female, Ireland, Sligo, S. shore of Lough Gill near Doonee Rock, 15.x.1937 (AWS leg.) [USNM #76022; USNMMENT 01569377] (NMNH).

Type material of *Neorhostigma eoum* (Figs 5C–7A). *Holotype*: female, Russia, Primorskiy kray, Anisimovka, forest, 16.viii.1979 (S. Belokobylskij leg.) (ZISP).

Paratypes: **Russia:** 2 females, Primorskiy kray, Spassk-Dal'niy, forest, glades, 16 and 22–23.viii.1995 (S. Belokobylskij leg.) (ZISP); 1 female, Sakhalin Island, 10 km W of Aniva, mixed forest, 15.viii.1981 (S. Belokobylskij leg.) (ZISP). **Japan:** 1 male, Fukuoka, Nogochi, Fukuoka-shi, 28.viii.1992 (V. Makarkin leg.) (ZISP).

Additional studied material. Norway. 1 female, Oslo [AK], Maridalen, Dausjøen, Spruce forest, 5.vi–16.x.2010, 60.01234 N 10.787665 E, 160 m, Malaise trap, river outlet (Lars Ove Hansen leg.) (NHMO).

Russia. Leningradskaya Province: 1 female, Tolmachevo, mixed forest, 22.VIII.1960 (V. Tobias leg.), “*Aspilota macrops* Stelf., Tobias det. 1961” (ZISP). Primorskiy kray: 1 female, 30 km E of Spassk-Dal'niy, forest, glades, 4.vi.1984 (S. Belokobylskij leg.) (ZISP); 1 female, Nadezhdinskiy District, 15 km SSW of Nezhino, forest, 16–18.vii.1993 (S. Belokobylskij leg.) (ZISP); 1 female, 30 km SE of Ussuriysk, forest, border of forest, 12–17.vii.2001 (S. Belokobylskij leg.) (ZISP); 1 female, Vladivostok, Okeanskaya, forest, 25.vii.2001 (S. Belokobylskij leg.) (ZISP); 1 female, Vladivostok, Sedanka, forest, border of forest, 30.vii.2001 (S. Belokobylskij leg.) (ZISP).

Re-description. Female (holotype).

Length. Body 2.6 mm, fore wing 3.3 mm, hind wing 2.1 mm.

Head. In dorsal view, 1.9 times as wide as long, 1.2 times as wide as mesoscutum; smooth, with temple rounded behind eyes. Antenna 27-segmented, 1.3 times as long as body. Scape 3.0 times as long as pedicel. First flagellar segment 2.6 times as long as its apical width, 1.3 times as long as second segment. Second to 25th (apical) segments 1.6–2.0 times as long as their maximum width. Eye in lateral view 1.6 times as high as wide and 1.9 times as wide as temple medially. POL 1.1 times OD; OOL 2.7 times OD. Face 1.5 times as wide as high; inner margins of eyes subparallel, with numerous setae, with numerous sparse punctation. Clypeus 2.5 times as wide as high, slightly concave ventrally. Mandible almost parallel-sided, 1.4 times as long as its maximum width; upper tooth very small; middle tooth rather wide and short, directed forwards,

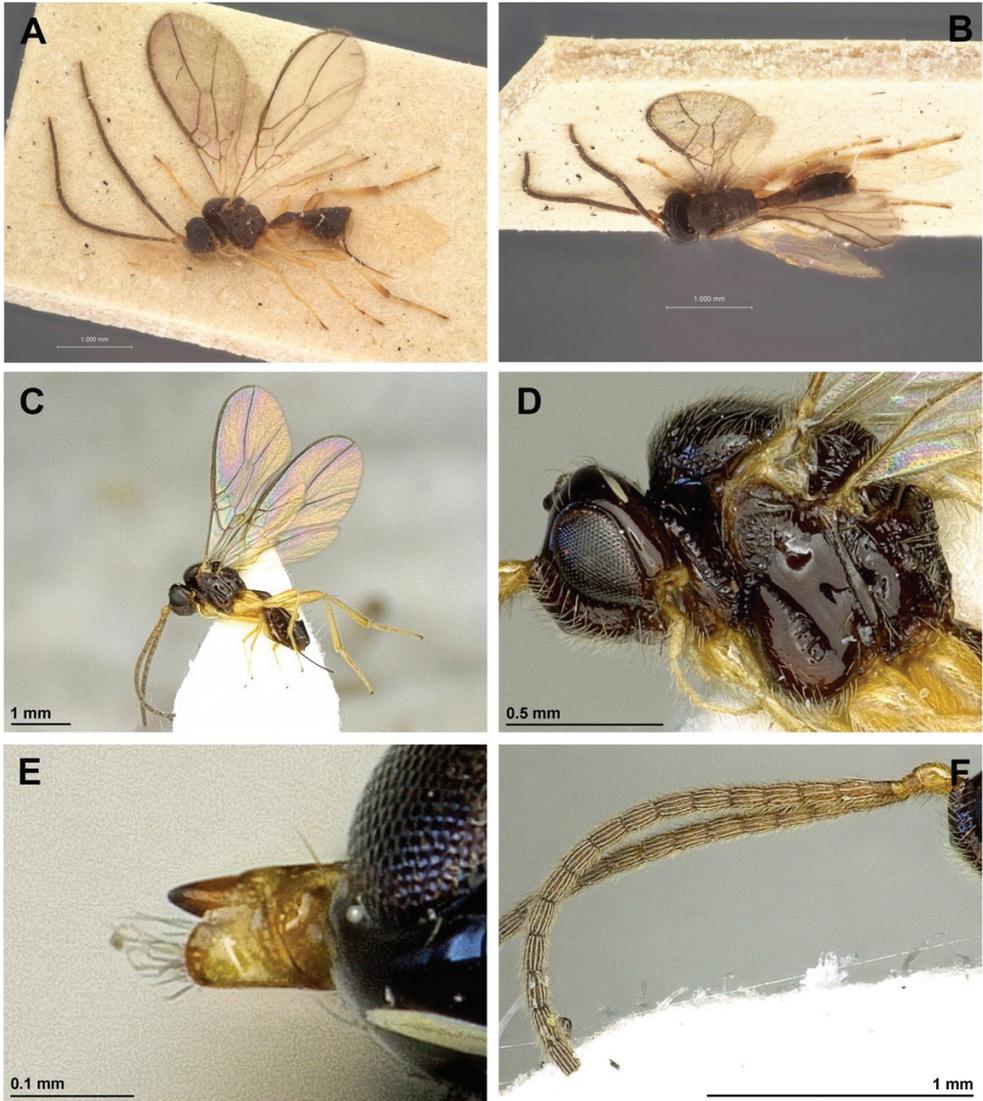


Figure 5. *Neorthostigma macrops* (Stelfox & Graham, 1951) (**A, B** female, holotype of *A. macrops*; **C–F** female, holotype of *Neorthostigma eoum*) **A, C** habitus, lateral view **B** habitus, dorsal view **D** head and mesosoma, lateral view **E** mandible **F** antenna.

longer than lower tooth; lower tooth wide and rounded distally, not angulated ventro-distally, with several long outstanding curved setae.

Mesosoma. In lateral view 1.1 times as long as high. Mesoscutum (dorsal view) 0.9 times as long as its maximum width, entirely densely setose. Notauli mainly absent on horizontal surface of mesoscutum. Mesoscutal pit absent. Prescutellar depression smooth, with median and lateral carinae, almost twice as long as its maximum width. Precoxal sulcus present, crenulate, reaching anterior margin and not reaching

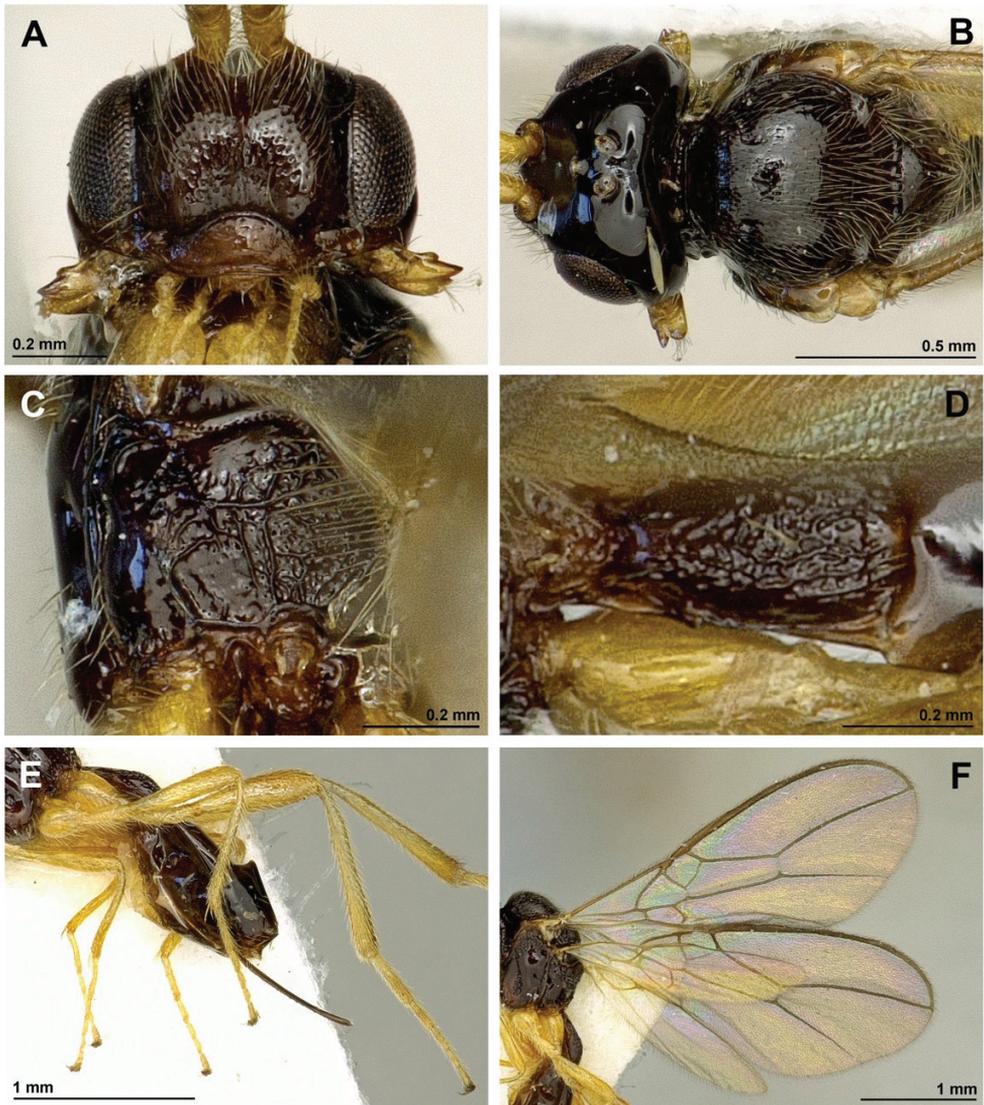


Figure 6. *Neorthostigma macrops* (Stelfox & Graham, 1951) (A–F: female, holotype of *Neorthostigma eoum*) **A** head, frontal view **B** head and mesonotum, dorsal view **C** propodeum, dorsal view **D** first metasomal tergite, dorsal view **E** hind leg, metasoma and ovipositor, lateral view **F** fore and hind wings.

posterior margin of mesopleuron. Posterior mesopleural furrow smooth. Propodeum largely rugulose, smooth anteriorly and postero-laterally, with some longitudinal and transverse carinae, with wide rugose areola delineated by distinct carinae. Propodeal spiracles relatively small, its diameter 0.2 times distance from spiracle to anterior margin of propodeum.

Wings. Fore wing 2.3 times as long as its maximum width. Marginal cell ending at apex of wing, 4.4 times as long as its maximum width. Vein 3-SR 2.5 times as long as vein 2-SR. Vein SR1 2.0 times as long as vein 3-SR. Vein 3-SR 4.7 times as long as

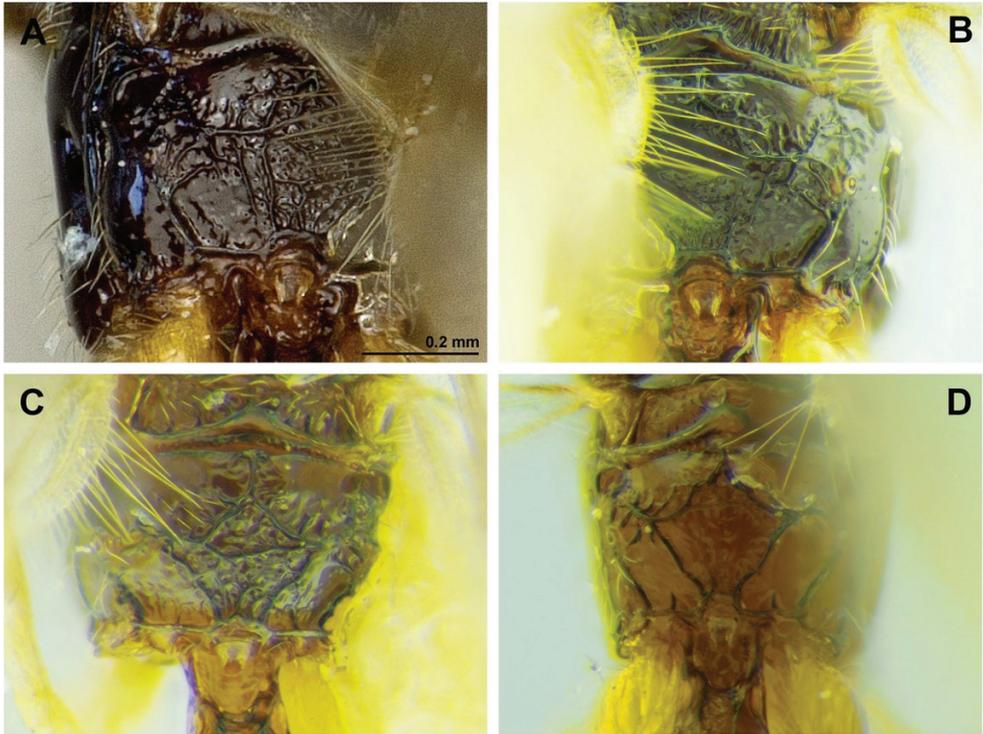


Figure 7. Propodeum variation of *Neorthostigma macrops* (Stelfox & Graham, 1951) (**A** female, holotype of *Neorthostigma eoum*; **B–D** additional material).

vein r. Vein r much longer than width of pterostigma. First subdiscal cell 1.9 times as long as its maximum width. Hind wing 5.0 times as long as its maximum width.

Legs. Hind femur 3.9 times as long as its maximum width. Hind tibia weakly widened to apex, 8.8 times as long as its maximum (subapical) width, 1.1 times as long as hind tarsus. First segment of hind tarsus 1.7 times as long as second segment.

Metasoma. First tergite weakly and evenly widened towards apex, 1.7 times as long as its apical width, entirely densely rugose-reticulate. Ovipositor 1.5 times as long as first tergite, 0.5 times as long as metasoma, approximately as long as hind femur.

Colour. Body black or dark reddish brown. Antenna black, paler basally; two basal segments light brown. Legs entirely light brown, but hind tibia finely infuscate apically. Wings hyaline.

Variation. Body length 1.9–2.7 mm; fore wing length 2.3–3.3 mm; hind wing length 1.8–2.1 mm. Antenna 20–27 segments, 0.9–1.2 times as long as body. First flagellar segment in lateral view 2.7–3.5 times as long as maximum subapical width, 1.4–1.7 times as long as second segment. Face 1.2–1.5 times as wide as high, often with weak medial vertical carina in upper half, but sometimes this carina rather distinct; laterally usually with scattered row of orbital setae, but sometimes these setae very sparse; clypeus sometimes with only a few setae. Mandible 1.3–1.5 times as long as its maximum width. Mesoscutum almost entirely in dense, long and white setae, but often

sublaterally with narrow or rather wide glabrous areas. Propodeum with areola often entirely densely and rather coarsely rugose-striate, but sometimes this sculpture fine to very fine, and as exception areola entirely almost smooth; basolateral areas smooth at least in basal half or two thirds, but rarely at most part; propodeum postero-laterally often with small smooth areas. Precoxal sulcus often distinctly crenulate, but sometimes crenulae fine and anteriorly almost indistinct. In fore wing, vein 3-SR 3.2–4.8 times as long as vein r, 2.0–2.6 times as long as vein 2-SR. First submarginal cell 2.7–2.9 times as long as maximum width; First subdiscal cell 1.9–2.6 times as long as its maximum width. Vein 3-CU1 usually straight, but sometimes weakly and evenly curved. Hind femur 3.7–4.1 times as long as its maximum width. First metasomal tergite 1.45–1.70 times as long as apical width. Ovipositor 1.3–1.6 times as long as first tergite. Body often brown or reddish brown, rarely almost entirely black with scape and pedicel of antenna darkened; legs may be infuscate, light reddish brown to almost reddish brown.

Male. Body length 2.3 mm; fore wing length 2.0 mm. Otherwise similar to female.

Comparative diagnosis. This species is similar to *N. brachyclypeata* (Fischer, 1978) and *N. braeti* sp. nov., but differs from them in having precoxal sulcus reaching anterior margin of mesopleuron (not reaching in *N. brachyclypeata* and *N. braeti*), mesoscutum almost entirely densely setose (glabrous laterally and very sparsely setose medially in *N. brachyclypeata* and *N. braeti*), and lower (third) tooth of mandible rather wide (narrow in *N. brachyclypeata* and *N. braeti*).

Distribution. Ireland, Japan (Kyushu Island), Netherlands, Norway (new record), Russia (Leningradskaya Province, Primorskiy Territory, Sakhalin Island), Slovakia, United Kingdom.

Remarks. We studied the photos of the holotype of *Aspilota macrops* (female, “Ireland, Sligo, S. shore of Lough Gill near Doonee Rock, 15.X.1937, A.W. Stelfox; Smithsonian Institute, Washington) (<http://n2t.net/ark:/65665/36930ade8-ba26-4c84-967b-7484f2b81346>) (Figs 5A, 5B). These illustrations together with originally well described and figured species (Stelfox and Graham 1951) and additional material from Norway and Russia (Far East and North West of the European part) showed morphological identity of *A. macrops* and *N. eoum* together with distinct variability of some their morphological characters (see Variation section in re-description). As result of this study *Neorthostigma eoum* Belokobylskij 1998 is here synonymised with *Aspilota macrops* Stelfox and Graham 1951 (syn. nov.).

Key to *Neorthostigma* species

- 1 Precoxal sulcus reaching anterior margin of mesopleuron (Fig. 5D). Mesoscutum entirely covered by dense numerous setae (Fig. 6B). Lower (third) tooth of mandible relatively wide (Fig. 5E). Vein 3-SR 4.0–4.7 times as long as vein r (Fig. 6F). Body length 1.9–2.7 mm. Ireland, United Kingdom, Netherlands, Norway, Slovakia, Russia, Japan..... ***N. macrops* (Stelfox & Graham)** (♀♂)
- Precoxal sulcus not reaching anterior margin of mesopleuron (Figs 1B, 3B). Mesoscutum very sparsely setose only medially (Figs 1F, 3F). Lower (third)

- tooth of mandible narrow (Figs 1C, 3C). Vein 3-SR 3.1–3.7 times as long as vein r (Figs 2D, 4D) **2**
- 2 First metasomal tergite 1.5 times as long as its apical width (Fig. 2B). Mesosoma in lateral view 1.4 times as long as high (Fig. 1B). Mesoscutum in dorsal view 0.7 times as long as its maximum width (Fig. 1F). Vein 3-SR 3.7 times as long as vein r (Fig. 2D). Hind tibia 6.4 times as long as its maximum (subapical) width (Fig. 2C). Prescutellar depression 1.7 times as long as its maximum width (Fig. 1F). Middle tooth longer than rounded apically lower tooth. Body length 1.8 mm. Papua New Guinea
 *N. brachylypeata* (Fischer) (♀)
- First metasomal tergite 1.8–1.9 times as long as its apical width (Fig. 4B). Mesosoma in lateral view 1.1 times as long as high (Fig. 3B). Mesoscutum in dorsal view 0.9 times as long as its maximum width (Fig. 3F). Vein 3-SR 3.1 times as long as vein r (Fig. 4D). Hind tibia 7.5–8.0 times as long as its maximum (subapical) width (Fig. 4C). Prescutellar depression 1.3–1.4 times as long as its maximum width (Fig. 3F). Middle tooth as long as acuminate apically lower tooth. Body length 1.9–2.3 mm. Papua New Guinea
 *N. braeti* Peris-Felipo, sp. nov. (♀)

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First report and integrated analysis of two native *Trissolcus* species utilizing *Bagrada hilaris* eggs in California

Fatemeh Ganjisaffar¹, Elijah J. Talamas², Marie Claude Bon³, Thomas M. Perring¹

1 Department of Entomology, University of California, Riverside CA 92521, USA **2** Florida State Collection of Arthropods, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL 32608, USA **3** USDA-ARS European Biological Control Laboratory, 810 Avenue du Campus Agropolis, 34980 Montferrier le Lez, France

Corresponding author: Elijah J. Talamas (billy.jenkins@gmail.com)

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Abstract

Surveys with sentinel eggs of *Bagrada hilaris* (Hemiptera: Pentatomidae) in southern California retrieved two parasitoids that were not previously known to be associated with this stink bug, *Trissolcus hullensis* and *T. utahensis* (Hymenoptera: Scelionidae). Molecular and morphological analysis of these specimens is used to modify the concept of *T. utahensis* and assess the factors that contribute to intraspecific variation. We provide an updated couplet to separate *T. utahensis* from a morphologically similar species, *T. cosmopeplae*.

Keywords

Bagrada bug, egg parasitoids, painted bug, Scelionidae, sentinel eggs

Introduction

Bagrada hilaris (Burmeister) (Hemiptera: Pentatomidae) is native to Africa, Asia, and the Middle East (Howard 1907; Husain 1924). This stink bug first was reported in the United States in Los Angeles County, California, in 2008 (Arakelian 2008). By 2015, it had spread to 21 other counties in California, and six other states (Nevada, Arizona,

Utah, New Mexico, Texas, and Hawaii) (Palumbo and Natwick 2010; Palumbo et al. 2016; Bundy et al. 2012, Vitanza 2012; Perring et al. 2013; Reed et al. 2013; Matsunaga 2014). *Bagrada hilaris* also has been reported from six states of Mexico (Sánchez-Peña 2014; Torres-Acosta and Sánchez-Peña 2016; Hernández-Chávez et al. 2018) and Chile (Faúndez et al. 2016; Faúndez et al. 2017). *Bagrada hilaris* attacks various vegetable crops, weedy mustards, and several ornamental plants within the mustard family (Brassicaceae). In the United States, *B. hilaris* has been a serious pest of cole crops (Perring et al. 2013; Reed et al. 2013; Palumbo et al. 2016). The bugs are particularly damaging to young seedlings, but they also feed on leaves, stems, flowers, and seeds of older plants (Palumbo and Natwick 2010; Huang et al. 2014).

Chemical applications have been the main approach used against this pest (Palumbo 2015) and efforts to establish a biological control program were initiated in 2014. Three egg parasitoids of *B. hilaris* were collected in Pakistan (Mahmood et al. 2015) and brought to the United States to be evaluated as biological control candidates: *Trissolcus hyalinipennis* Rajmohana & Narendran (Rajmohana 2006), *Gryon gonikopalense* Sharma (Platygastridae: Scelionidae) (Sharma 1982), and *Ooencyrtus mirus* Triapitsyn & Power (Chalcidoidea: Encyrtidae) (Triapitsyn et al. 2020). Laboratory evaluations of these parasitoids are still ongoing, and no release permits have been issued to date (Sforza et al. 2017; Martel et al. 2019; Power et al. 2020a, b). Meanwhile, monthly surveys using *B. hilaris* sentinel eggs have been conducted in California since the fall of 2017 to identify potential native or introduced parasitoids. Through these surveys, two scelionid species, *T. hyalinipennis* and *Trissolcus basalis* (Wollaston) (Ganjisaffar et al. 2018), and a new encyrtid species, *Ooencyrtus lucidus* Triapitsyn & Ganjisaffar (Triapitsyn et al. 2020), have been identified. This study reports the discovery and identification of two additional scelionid species that parasitized *B. hilaris* sentinel eggs in southern California: *Trissolcus hullensis* (Harrington) and *Trissolcus utahensis* (Ashmead).

Our analysis includes *T. utahensis* reared from sentinel eggs of *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) in British Columbia, Canada, to assess the limits of this species and determine its intraspecific variation using molecular and morphological data. Previous identification of the Canadian specimens brought attention to problems with the couplet that separates *T. utahensis* and *Trissolcus cosmopeplae* (Gahan) in the key to Nearctic *Trissolcus* of Talamas et al. (2015). This key has been used in annual workshops to identify stink bug egg parasitoids throughout North America, during which this couplet has proven to be problematic as well. We here employ molecular phylogenetics to determine which characters are variable, and which are sufficiently stable to be used for identification in these species, and we provide an updated couplet for *T. utahensis* and *T. cosmopeplae*.

Materials and methods

Survey locations

The Agricultural Operations of the University of California, Riverside, was the main site for the surveys. Fields that had been planted for various research were used for



Figure 1. Survey locations are displayed in red dots. The black rectangle shows the Agricultural Operations of the University of California, Riverside, where most of our surveys were conducted. Five *Trissolcus hullensis* were recovered from an alfalfa field (33.96508°N, 117.34084°W), one *Trissolcus utahensis* was recovered from a squash field with mustard weeds (33.96611°N, 117.34230°W), and eleven *T. utahensis* were recovered from roadside mustard weeds (33.99105°N, 117.33360°W).

our sentinel egg deployments. A mixed vegetable field available from October 2017 to March 2018, an alfalfa field (*Medicago sativa* L.) available from October 2017 to January 2019, and a squash field (*Cucurbita moschata* L., variety black futsu) available from January 2018 to September 2019 were used. The mixed vegetable field was selected particularly because of its two rows of broccoli (*Brassica oleracea* L., variety Italica), a favorable host for *B. hilaris*. The alfalfa field hosted several stink bug species during spring and summer, serving as a potential source of stink bug egg parasitoids. The squash field was selected because *B. hilaris* adults were found on shortpod mustard weeds, *Hirschfeldia incana* (L.) Lagr.-Foss., within the field. Surveys also were conducted at other locations on the Agricultural Operations property where mustard weeds were found. In addition, various locations in the urban area with brassicaceous weeds surrounding the University of California, Riverside, were surveyed (Fig. 1). Previous monthly samplings from January to December 2011 by Reed et al. (2013) had shown that the primary weeds supporting *B. hilaris* in this area were London rocket (*Sisymbrium irio* L.), shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.), and shortpod mustard. *Bagrada hilaris* also were found occasionally on Russian thistle (*Salsola australis* R.Br.) and telegraph weed (*Heterotheca grandiflora* Nutt.) when they were in close proximity to senesced shortpod

mustard. According to Reed et al. (2013), these weeds were available following fall and winter rains through June and the peak *B. hiliaris* abundance was during the spring on these host plants. We used the same locations for our sentinel egg deployments, and surveys were conducted monthly from 21 October 2017 through 27 September 2019.

Sentinel egg card preparation and parasitoid recovery

Bagrada hiliaris eggs (≤ 24 hours old) were used for the surveys. Adult mating pairs of greenhouse-grown *B. hiliaris* (Reed et al. 2017) were maintained on organic broccoli florets in plastic containers (15 cm diameter \times 6.5 cm height) with 2 screen openings for air circulation in an insectary room at 30 ± 1 °C, 40–50% humidity and 14:10 (L:D) photoperiod. White paper towels were cut in circles to fit the bottom of each container to provide a substrate for oviposition. Approximately 15 mating pairs were placed into each container. *Bagrada hiliaris* eggs were collected daily and glued (Gorilla Super Glue Gel, The Gorilla Glue Co., Ohio, USA) on a 3×5 grid of squares on a weatherproof card so that each card contained 15 eggs (Ganjisaffar et al. 2018). For each location, a sentinel card was taped to the wire of a landscape flag, positioning it about 30 cm from the ground. Previous studies showed that eggs placed on cards on the soil surface were eaten by predators (Ganjisaffar et al. 2018). The number of cards used for each survey date varied from 10 to 20, depending on the availability of *B. hiliaris* eggs. Cards were left in the field for 3–5 days (3.7 days on average) to avoid *B. hiliaris* eggs from hatching in the field. According to Reed et al. (2017), *B. hiliaris* eggs hatch after 5 days at temperatures approximating the warmest temperatures on the days of the field surveys. Once collected, the grid was cut and placed in glass vials plugged with cotton. The vials were maintained in the same insectary room that was used for the *B. hiliaris* colony and were examined for parasitism and wasp emergence. Emerged wasps were transferred to vials containing 95% ethanol for identification.

Molecular analysis

Genomic DNA was non-destructively isolated from the entire specimen using the Qia-gen DNeasy Blood and Tissue kit (Hilden, Germany) as described in Sabbatini Peverieri et al. (2018). A comprehensive list of all samples extracted with author and year, host, and locality data is given in Table 1 in Supporting Information. The barcode region of the mitochondrial Cytochrome Oxidase Subunit I (*COI*) was amplified using the universal barcoding primer set LCO1490/HCO2198 (Folmer et al. 1994) (Table 1). The primer set LCO1490puc (Cruaud et al. 2009)/C1-N-2353 (Simon et al. 2006) was used in six samples to amplify a longer region (~850 bp) than the classical barcode region (~710 bp), providing more than enough coverage for the barcode that is not always obtained when using universal primer sets (Table 1). All PCRs were performed as described in Ganjisaffar et al. (2018), except the PCR conditions. The thermocycling conditions were as follows: 1 cycle of denaturation at 94 °C for 3 min, 35 cycles at 94 °C for 30 s, 52 °C (LCO1490/HCO2198) or 50 °C (LCO1490puc/C1-N-2353) for 30 s, 72 °C for 1 min with a final extension step of 10 min at 72 °C. All samples were

Table 1. Collection data on samples of *Trissolcus utahensis*, *T. hullensis* and *T. colemani* obtained in this study, and corresponding *COI* information.

Sample ID	Collection Unit identifier	Species (Hymenoptera: Scelionidae)	Host (Hemiptera: Pentatomidae)	Location	Date collected; name of the collector	Primers for mt-CO1 PCR and sequencing	DNA depository	COI Genbank Accession number
TSP274	FSCA 0000302	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	July 2017; W. Wong & Paul Abram	LCO1490/HCO2198	EBCL	MT804738
TSP276	FSCA 00033040	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	June 2017; W. Wong & Paul Abram	LCO1490/HCO2198	EBCL	MT804739
TSP277	FSCA 00033041	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	June 2017; W. Wong & Paul Abram	LCO1490/HCO2198	EBCL	MT804740
TSP278	FSCA 00033042	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	August 2017; W. Wong & Paul Abram	LCO1490/HCO2198	EBCL	MT804741
TSP279	FSCA 00033043	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	August 2017; W. Wong & Paul Abram	LCO1490/HCO2198	EBCL	MT804742
TSP280	FSCA 00033044	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	June 2017; W. Wong & Paul Abram	LCO1490/HCO2198	EBCL	MT804743
TSP281	FSCA 00033045	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	June 2017; W. Wong & Paul Abram	LCO1490/HCO2198	EBCL	MT804744
TSP288	FSCA 00033239	<i>Trissolcus utahensis</i>	<i>Bagrada hilaris</i>	Riverside California, USA	October 2018; Fatemeh Ganjisaffar	LCO1490/HCO2198	EBCL	MT804745
PL141	FSCA 00091859	<i>Trissolcus utahensis</i>	<i>Bagrada hilaris</i>	Riverside California, USA	October 2018; Fatemeh Ganjisaffar	LCO1490/HCO2198	FSCA	MT804746
PL142	FSCA 00091872	<i>Trissolcus utahensis</i>	<i>Bagrada hilaris</i>	Riverside California, USA	October 2018; Fatemeh Ganjisaffar	LCO1490/HCO2198	FSCA	MT804747
PL259	FSCA 00094712	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	July 2017; W. Wong & Paul Abram	LCO1490/HCO2198	FSCA	MT804748
PL260	FSCA 00094711	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	May 2017; W. Wong & Paul Abram	LCO1490/HCO2198	FSCA	MT804749
PL261	FSCA 00094713	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	August 2017; W. Wong & Paul Abram	LCO1490/HCO2198	FSCA	MT804750
PL262	FSCA 00094714	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	August 2017; W. Wong & Paul Abram	LCO1490/HCO2198	FSCA	MT804751
PL263	FSCA 00094715	<i>Trissolcus utahensis</i>	<i>Podisus maculiventris</i>	Peniticon, BC, Canada	August 2017; W. Wong & Paul Abram	LCO1490/HCO2198	FSCA	MT804752
TSP291	FSCA 00090585	<i>Trissolcus hullensis</i>	<i>Halyomorpha halys</i>	Sr. Helena, CA, USA	August 2017; Kent Daane	LCO1490/HCO2198	EBCL	MT804753
PL143	FSCA 00091873	<i>Trissolcus hullensis</i>	<i>Bagrada hilaris</i>	Riverside, CA, USA	March 2019; Fatemeh Ganjisaffar	LCO1490/HCO2198	FSCA	MT804754
PL144	FSCA 00091874	<i>Trissolcus hullensis</i>	<i>Bagrada hilaris</i>	Riverside, CA, USA	March 2019; Fatemeh Ganjisaffar	LCO1490/HCO2198	FSCA	MT804755
TSP398	FSCA 00094753	<i>Trissolcus colemani</i>	<i>Piezodorus lituratus</i>	Assas, France	June 2011; Marie Roche	LCO-1490puc/C1-N-2353	EBCL	MT804756
TSP400	FSCA 00094754	<i>Trissolcus colemani</i>	<i>Eurydema ventralis</i>	Monferrtier le Lez, France	May 2019; Marie Roche	LCO-1490puc/C1-N-2353	EBCL	MT804757
TSP401	FSCA 00094755	<i>Trissolcus colemani</i>	<i>Eurydema ventralis</i>	Monferrtier le Lez, France	May 2019; Marie Roche	LCO-1490puc/C1-N-2353	EBCL	MT804758
TSP403	FSCA 00094756	<i>Trissolcus colemani</i>	<i>Dolyrois baccharum</i> (Hemiptera: Pentatomidae)	Monferrtier le Lez, France	July 2010; Marie Roche	LCO-1490puc/C1-N-2353	EBCL	MT804759
TSP409	FSCA 00094757	<i>Trissolcus colemani</i>	<i>Graphosoma italicum</i>	Monferrtier le lez, France	July 2019; Marie Roche	LCO-1490puc/C1-N-2353	EBCL	MT804760
TSP410	FSCA 00094758	<i>Trissolcus colemani</i>	<i>Graphosoma italicum</i>	Monferrtier le lez, France	July 2019; Marie Roche	LCO-1490puc/C1-N-2353	EBCL	MT804761

sequenced bidirectionally using the BigDye chemistry by Genoscreen (Lille, France) or the ABI SeqStudio Platform at FDACS-DPI in Florida (USA). A *COI* consensus sequence was established for each specimen. All sequences generated from this study are deposited in GenBank, and all residual DNAs are archived at EBCL or FSCA (Table 1).

Voucher specimens which have been reexamined following the molecular analysis are presently archived at FSCA (Table 1). All sequences were translated into amino acids to check for stop codons and frame shifts. All sequences obtained were compared with sequences present in GenBank using the Basic Local Alignment Search Tool (<http://www.ncbi.nlm.nih.gov/BLASTn>). BOLD identification engine (Ratnasingham and Herbert 2007) was similarly datamined for barcodes of *Trissolcus* species and evaluated for barcode identification success. The 28 sequences obtained in this study were aligned with 28 barcode sequences of *Trissolcus* retrieved from GenBank. The final alignment of 56 sequences was performed using the default settings of CLUSTAL W (Thompson et al. 1994) as implemented in MEGA X (Kumar et al. 2018) and resulted in 576 characters with 174 parsimony informative sites. The phylogenetic relationships among specimens were reconstructed following a Bayesian analysis as implemented in MrBayes v. 3.2 (Ronquist et al. 2012). Searches were run for 1 million generations, in two independent runs, using default priors and the GTR+I+G substitution model that was selected using the Bayesian information criterion (BIC) in MEGA X. One sequence of *Trissolcus thyantae* Ashmead (GenBank MN615574.1) was specified as the outgroup based on the results of Talamas et al. (2019). Because a network approach is well adapted to infer intraspecific genealogical relationships, a haplotype network was built for *T. utahensis* using TCS 1.21 (Clement et al. 2000). As the resulting clades were not connected under 90% statistical parsimony limits, we reran the TCS analysis by fixing connection limits at 50 steps. To estimate the divergence within and between terminal taxa and clades, we calculated the uncorrected p-distance using MEGA X, since the generally used K2P distance (Kimura 1980) could be inappropriate when applied to closely related taxa (Srivathsan and Meier 2012).

Morphology

Terminology follows that of Talamas et al. (2017). Following non-destructive DNA extraction, six specimens used in the molecular analysis were photographed (1 *T. hullensis* and 7 *T. utahensis*) to document morphology of specimens from different haplogroups and those reared from different hosts. Images were produced with a Macropod imaging system. Image stacks were rendered with Helicon Focus and further processed in Adobe Photoshop CS6.

Abbreviations and characters annotated in the figures

aem	anteroventral extension of the metapleuron (Figs 6, 13)
gc	genal carina (Fig. 16)
msct	metascutellum (Figs 4–5)
mshs	mesoscutal humeral sulcus (Figs 6, 21)
mtnm	metanotum (Figs 4–5)

- mtpm** metapostnotum (Figs 4–5)
not notaulus (Figs 7, 20, 22)
oc occipital carina (Figs 22, 25)
ppm propodeum (Figs 4–5)

Results

Field surveys

Our survey period of March 6–9, 2018, in the alfalfa field, yielded a sentinel card with 6 parasitized eggs from which 5 specimens of *T. hullensis* and 1 specimen of *T. basalis* wasps emerged. A month later during our April 6–9, 2018, survey in the roadside mustard weeds (33.99105N, 117.33360W), two sentinel cards were parasitized; one of them had 5, and the other one had 7 parasitized eggs, which yielded 11 *T. utahensis*. One *T. utahensis* wasp was recovered from a sentinel card that was deployed in the squash field with mustard weeds on October 12–16, 2018.

Molecular analysis

The phylogenetic analysis based on the *COI* barcode data revealed a relatively well-resolved and supported topology identifying six terminal taxa (Fig. 2). The deepest node corresponds to the split between *T. utahensis* and the other species, including *T. hullensis*. The *T. hullensis* clade comprised two specimens reared from *B. hilaris* eggs and one specimen reared from frozen sentinel eggs of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in Napa County, California. The *T. utahensis* cluster contains four main clades. The TCS analysis yielded four haplotype sub-networks, which were not connected under the 90% statistical parsimony limits, corresponding to these four clades of which clade 2 and clade 3 are each represented by a single haplotype, and clade 1 and clade 4 by three haplotypes each (Fig. 3). Clades 1, 3 and 4 contained specimens from British Columbia that were reared from *P. maculiventris* eggs. Clade 1 contained specimens from Canada and southern California, reared from the eggs of *P. maculiventris* and *B. hilaris*, respectively, indicating that they are conspecific. Interestingly, clade 2 included only Californian specimens reared from *B. hilaris*. Genetic distance between all clades ranged from 2.8% (clade 1/clade 2) to 9.9% (clade 1/clade 4) but remained lower than the range (from 10.6% to 14.3%) of interspecific distances obtained with the five other species in our analysis (Table 2).

Trissolcus hullensis (Harrington)

Remarks. The identification of *Trissolcus hullensis* is straightforward using the characters presented in Johnson (1985) and repeated in Talamas et al. (2015). Characters of the posterior mesosoma are particularly useful for determining this species: In

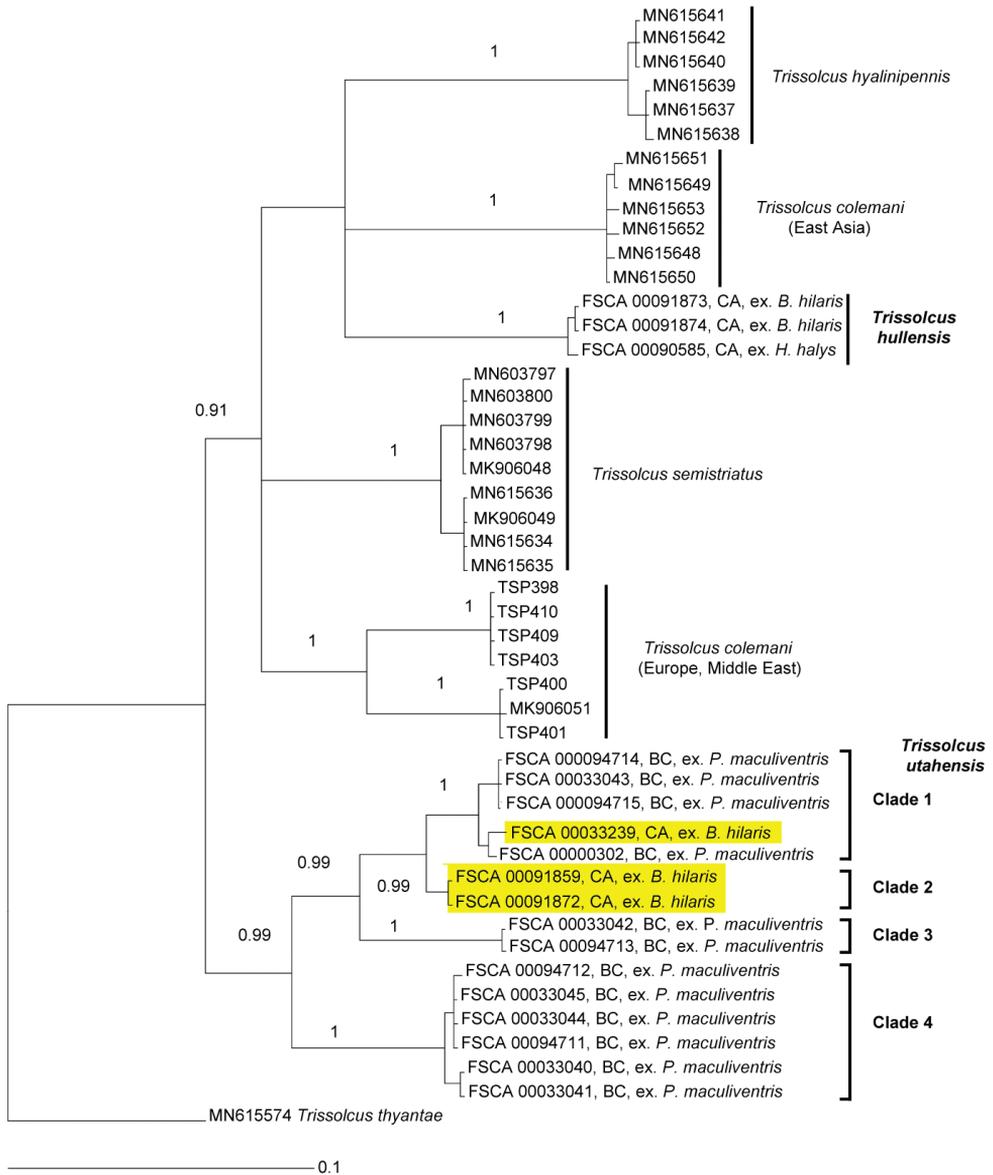


Figure 2. The Bayesian 50% majority rule consensus tree inferred from the 56 *COI* sequences of the six *Trissolcus* species including *T. hullensis* and *T. utahensis*. Only posterior probabilities >90% are indicated on the nodes. The tree is rooted with the outgroup *Trissolcus thyantae* (GenBank MN615574). The scale bar corresponds to 0.1 estimated substitutions per site.

T. hullensis the propodeum and metanotum are directly adjacent between the metapostnotum and metascutellum (Fig. 4), whereas in other species of Nearctic *Trissolcus*, the metapostnotum extends medially toward the metascutellum, and separates the propodeum from the metanotum (Fig. 5).

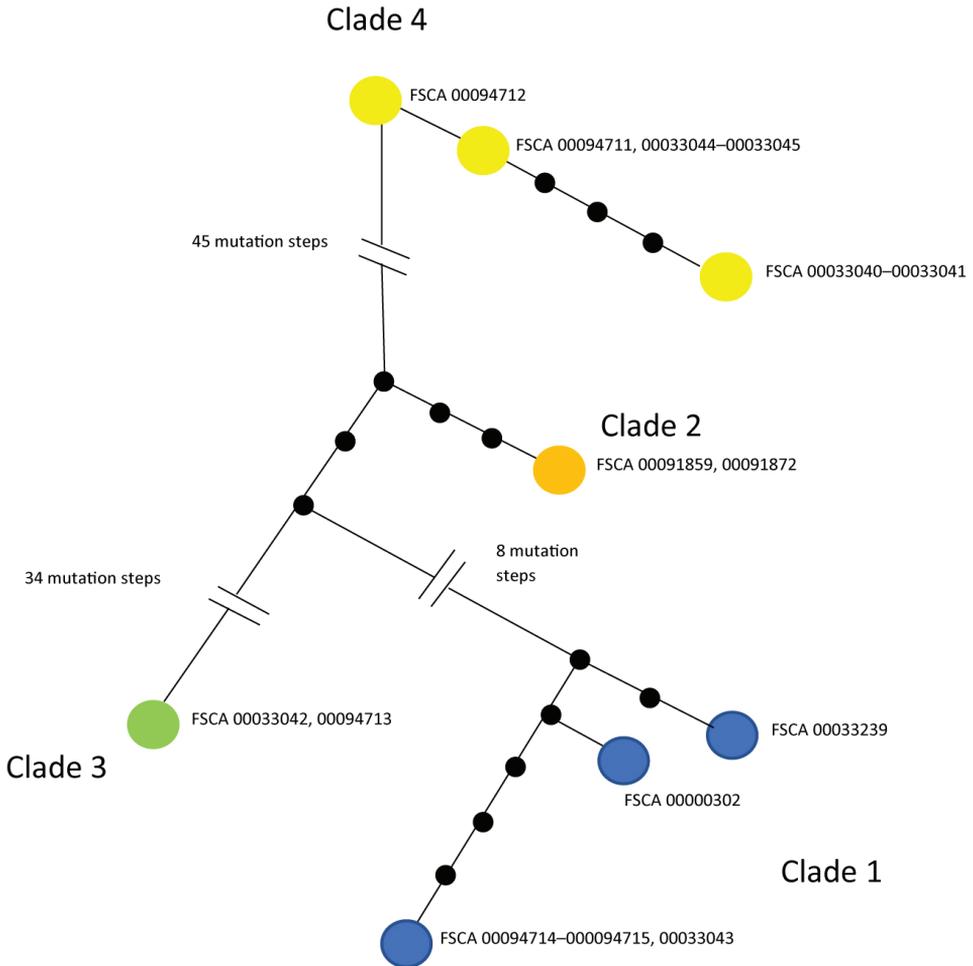


Figure 3. TCS *COI* haplotype network for the four clades of *T. utahensis* by fixing connection limits at 50 steps. Each haplotype is represented by a colored circle. Lines represent one mutational step between haplotypes, and dark circles represent unsampled haplotypes inferred from the data. Interrupted lines were used when haplotypes were separated by a long branch of more than 7 mutation steps.

Material Examined. 5 females, FSCA 00091873–00091874, 00091886–00091888 (deposited in FSCA) USA: CA: UC Riverside Agricultural Operations, 33.96508N, 117.34084W, alfalfa field, ex. *Bagrada hilaris* sentinel eggs deployed 6–9. III.2018, parasitoids emerged 23.III.2018, Coll. Ganjisaffar.

Trissolcus utahensis (Ashmead)

Remarks. The concept of *Trissolcus utahensis* was most recently treated in a revisionary context by Johnson (1985). This treatment separated *T. utahensis* from *T. cosmopeplae*

Table 2. Genetic distances (p-distances %, 1,000 bootstrap replications) for the *COI* barcode at the levels of intra-species, inter-species and clades of *T. utahensis*. Data are expressed as mean ± S.E.

	<i>T. colemani</i> (East Asia)	<i>T. semistriatus</i>	<i>T. hyalinipennis</i>	<i>T. colemani</i> (Europe, Middle East)	<i>T. hullensis</i>	<i>T. utahensis</i> Clade 1	<i>T. utahensis</i> Clade 2	<i>T. utahensis</i> Clade 3	<i>T. utahensis</i> Clade 4
<i>T. colemani</i> (East Asia)	4.2 ± 0.6								
<i>T. semistriatus</i>	10.6 ± 1.18	0.7 ± 0.2							
<i>T. hyalinipennis</i>	13.3 ± 1.2	12.1 ± 1.3	0.4 ± 0.2						
<i>T. colemani</i> (Europe, Middle East)	12.7 ± 1.31	14.1 ± 1.4	14.3 ± 0.1	0.4 ± 0.1					
<i>T. hullensis</i>	12.5 ± 1.3	12.4 ± 1.3	13.1 ± 1.3	12.3 ± 1.4	0.2 ± 0.1				
<i>T. utahensis</i> Clade 1	11.4 ± 1.2	10.8 ± 1.3	14.2 ± 1.4	14.1 ± 1.4	13.1 ± 1.4	0.7 ± 0.2			
<i>T. utahensis</i> Clade 2	11.6 ± 1.2	10.8 ± 1.3	14.1 ± 1.4	14.3 ± 1.4	12.8 ± 1.3	2.8 ± 0.6	0		
<i>T. utahensis</i> Clade 3	12.6 ± 1.3	11.8 ± 1.3	16 ± 1.5	14.5 ± 1.4	12.2 ± 1.3	7.78 ± 1.1	7.12 ± 1.1	0	
<i>T. utahensis</i> Clade 4	11.9 ± 1.2	12.9 ± 1.3	12.6 ± 1.3	14.7 ± 1.5	11.9 ± 1.3	9.97 ± 1.1	8.65 ± 1.1	9.61 ± 1.2	0.4 ± 0.1

based on the length of the anteroventral extension of the metapleuron, the absence of a genal carina, the shape of the gena in lateral view, and if notauli could be distinguished from the surface sculpture of the posterior mesoscutum. These characters were used again in the key to Nearctic *Trissolcus* by Talamas et al. (2015), with the addition of the form of the mesoscutal humeral sulcus, which was treated as variable within *T. cosmopeplae*. Talamas et al. (2015) also treated the anteroventral extension of the metapleuron as variable within *T. cosmopeplae* and emphasized the shape of the gena in lateral view to separate these species. This modification to the key sought to reconcile variability in the shape of the gena with other, seemingly variable characters. The shape of the gena has proven to be one of the more difficult characters to interpret because there is not a discrete boundary between “narrow” and “bulging”. Because the variation in these characters does not correspond to clades in our phylogeny, we treat them as intraspecifically variable and the *T. utahensis* clade as a single species (Fig. 2). Based on the morphological analysis provided below we propose the following replacement for couplet 14 in Talamas et al. (2015):

- 14 Anteroventral extension of the metapleuron long, extending to base of mesocoxa (Fig. 6); mesoscutal humeral sulcus comprised of cells (Figs 6–7)..... *T. cosmopeplae* (Gahan)
- Anteroventral extension of the metapleuron short, not approaching base of mesocoxa (Figs 12–19); mesoscutal humeral sulcus indicated by a smooth furrow (Figs 20–22)..... *T. utahensis* (Ashmead)

Sculpture of the dorsal frons. Figs 8–11 illustrate variation in the size of the smooth area directly below the preocellar pit, and the striation that radiates from the

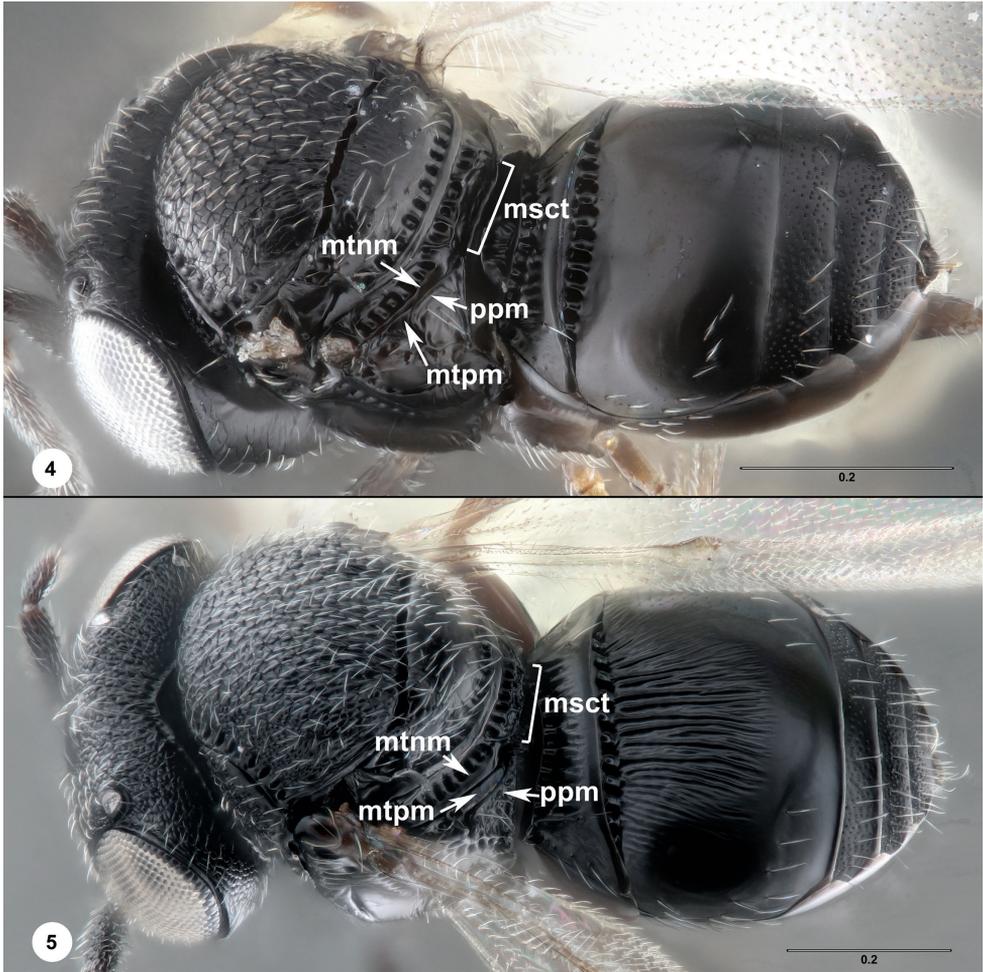
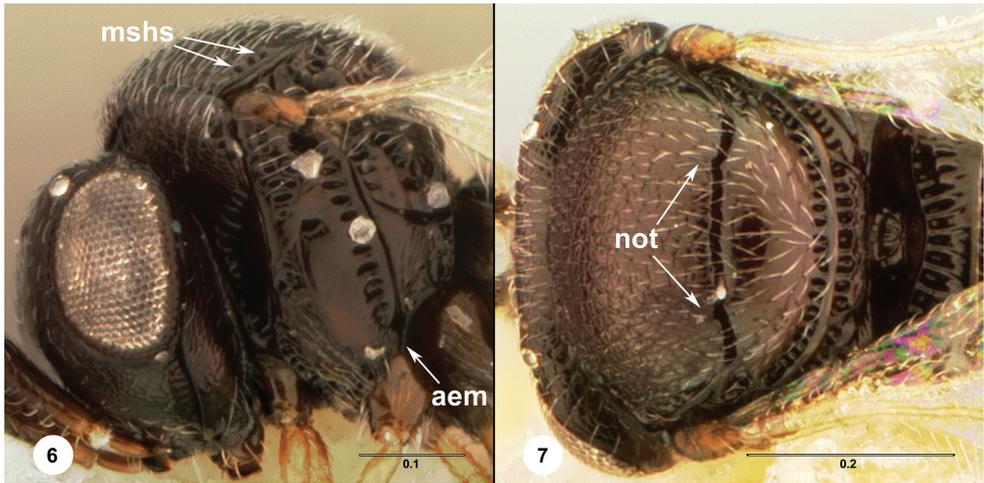


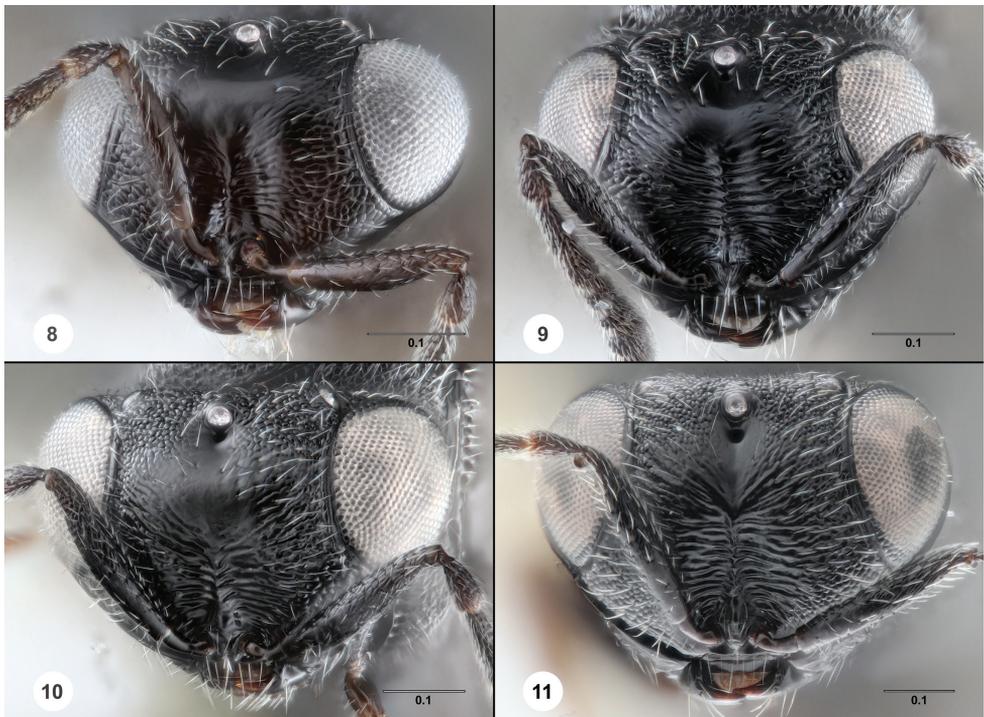
Figure 4–5. 4 *Trissolcus bullensis* (FSCA 00091886), head, mesosoma, metasoma, dorsolateral view
 5 *T. utahensis* (FSCA 00000302), head, mesosoma, metasoma, dorsolateral view. Scale bars in millimeters.

antennal scrobe. Fig. 8 illustrates a specimen that emerged from a *B. hilaris* egg. As was found in *T. basalis*, specimens that developed in *B. hilaris* eggs have reduced sculpture relative to those that developed in other hosts (Ganjisaffar et al. 2018). The specimens in Figs 9, 11 were both reared from the eggs of *P. maculiventris* in British Columbia, and have identical *COI* barcode sequences, yet the size of smooth area on the dorsal frons differs between them. The specimen in Fig. 11 is the largest (1.35 mm) among these, and the specimen in Fig. 8 is the smallest (0.93 mm). The specimens in Figs 9, 10 have the smooth area on the frons about equal in size and these specimens are also approximately equal in length (1.11 and 1.07 mm, respectively). These two specimens were retrieved in different haplogroups (clades 1 and 4), and we thus postulate that sculptural differences on the frons are size dependent.

Variation on the gena. The shape of the gena varies between and within the four clades of *T. utahensis*. In clades 1 and 3, the specimens have a rather narrow gena, and

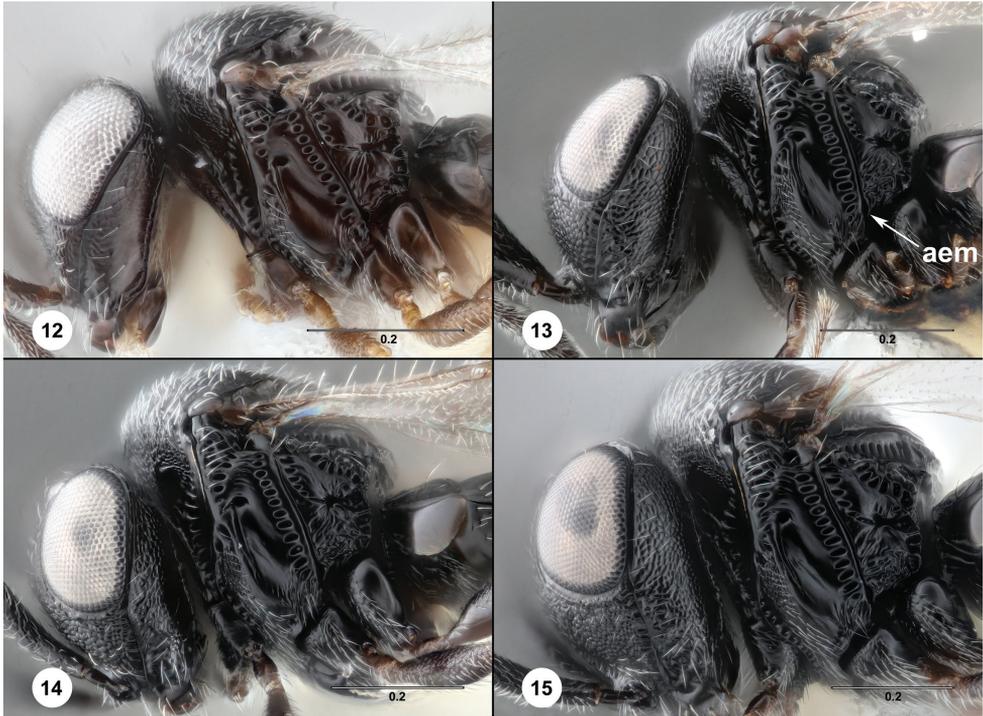


Figures 6–7. *Trissolcus cosmopeplae*, holotype female (USNMENT00989096) **6** head and mesosoma, lateral view **7** head and mesosoma, dorsal view. Scale bars in millimeters.



Figures 8–11. *Trissolcus utabensis*, head, anterior view **8** DPI_FSCA00033239 (ex. *B. hilaris*) **9** FSCA 00033041 (ex. *P. maculiventris*) **10** FSCA 00000302 (ex. *P. maculiventris*) **11** FSCA 00033040 (Ex. *P. maculiventris*). Scale bars in millimeters.

in clades 2 and 4 the gena is moderately to distinctly bulging in lateral view. Figs 12–15, 24 and 27 illustrate this variation. The degree to which the gena is bulging in lateral view does not appear to be host or size related. Specimens reared from *B. hilaris* eggs



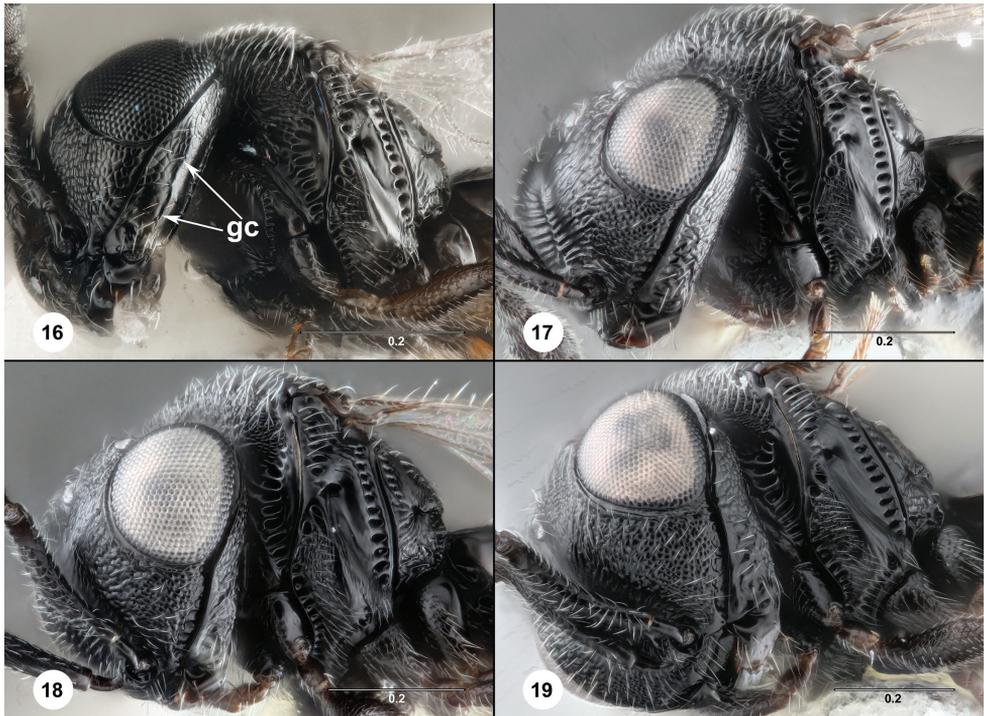
Figures 12–15. *Trissolcus utahensis*, head and mesosoma, lateral view **12** DPI_FSCA00033239 (ex. *B. hilaris*) **13** FSCA 00033041 (ex. *P. maculiventris*) **14** FSCA 00000302 (ex. *P. maculiventris*) **15** FSCA 00033040 (ex. *P. maculiventris*). Scale bars in millimeters.

are the smallest and have the gena moderately (Fig. 12) to distinctly (Fig. 24) bulging. The specimens with the most distinctly bulging gena (clade 4, Figs 15, 19) and the narrowest gena (clade 3, Figs 27, 28) were both reared from eggs of *P. maculiventris* and the specimens are larger than those reared from *B. hilaris* eggs.

Specimens with a bulging gena tend not to have the genal carina indicated, whereas specimens with a narrow gena often have it clearly expressed, but this is not an exact correlation. The specimen in Figs 12, 16 has a moderately bulging gena and the genal carina is distinctly present.

Microsculpture on the poster gena is less developed in specimens reared from *B. hilaris* eggs (Fig. 12), and this area is noticeably smoother than in specimens reared from *P. maculiventris* eggs (Figs 13–15). This phenomenon is consistent with a general pattern of reduced sculpture in smaller specimens.

Occipital carina. Tortorici et al. (2019) presented a new character to distinguish *T. semistriatus* (Nees von Esenbeck) from closely related species: the form of the occipital carina in dorsal view. In most species of *Trissolcus* the occipital carina is evenly convex, but in a few species, including *T. semistriatus* and *T. utahensis*, the occipital carina forms a distinct angle and the vertex of this angle may have a short carina directed toward the median ocellus (Figs 5, 22). In some specimens that emerged from *B. hilaris* eggs, the vertex of the angle formed by the occipital carina is less sharp, perhaps due to



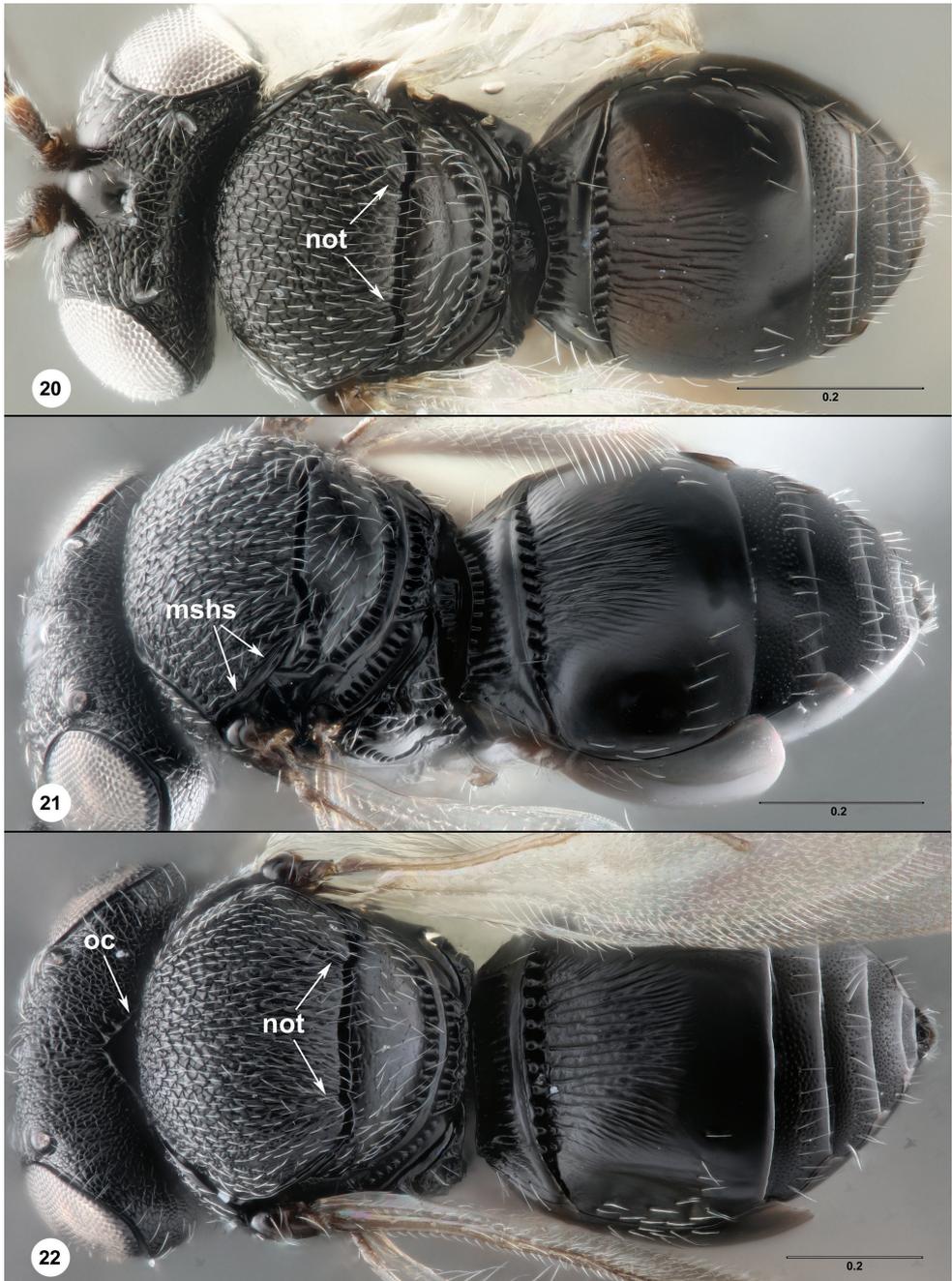
Figures 16–19. *Trissolcus utahensis*, head and mesosoma, ventrolateral view **16** DPI_FSCA00033239 (ex. *B. hilaris*) **17** FSCA 00033041 (ex. *P. maculiventris*) **18** FSCA 00000302 (ex. *P. maculiventris*) **19** FSCA 00033040 (ex. *P. maculiventris*). Scale bars in millimeters.

its diminutive size (Fig. 25). The occipital carina is not visible in the available images of *T. cosmopeplae*, and it is obscured by glue in the holotype specimen of *T. utahensis*. This character deserves further attention in Nearctic *Trissolcus* although we are not presently able to determine if it can separate *T. cosmopeplae* and *T. utahensis*.

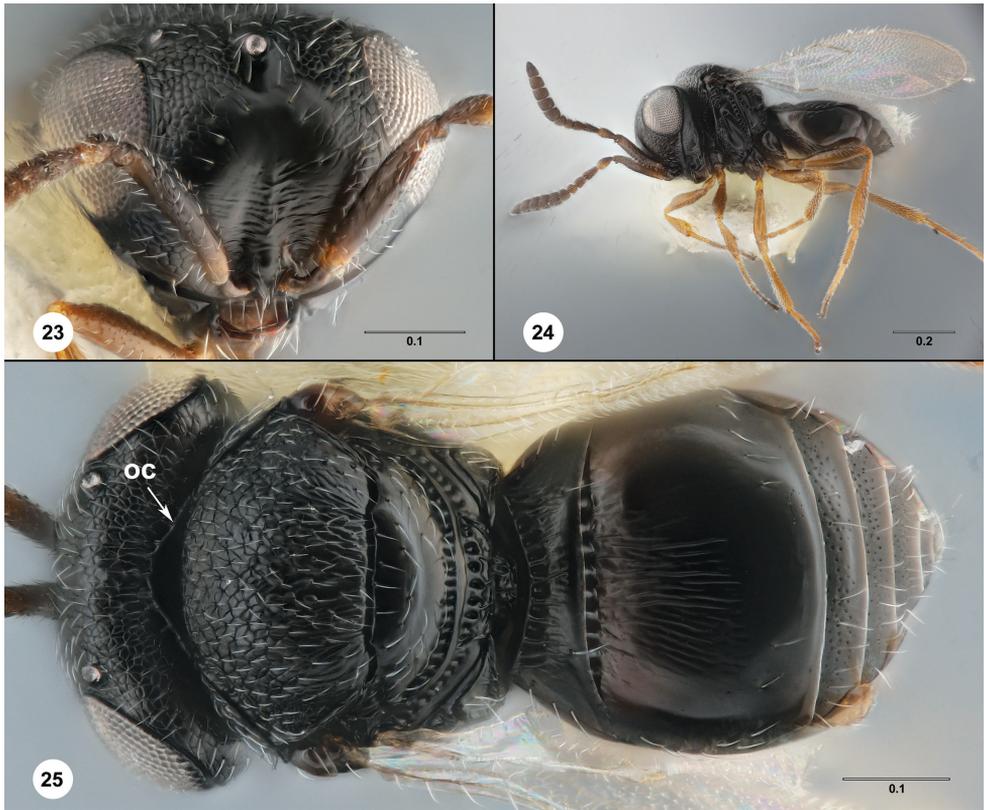
Notaulus. Specimens of *T. utahensis* reared from both *B. hilaris* and *P. maculiventris* have the notaulus indicated by short, shallow grooves present at the posterior margin of the mesoscutum (Figs 5, 20–22). These are visible in the holotype of *T. utahensis* (see fig. 100 in Talamas et al. (2015)), and they have the same form in the holotype of *T. cosmopeplae* (Fig. 7). Based on re-examination of images of the types and the specimens at hand, we conclude that this character does not separate these species.

Mesoscutal humeral sulcus. The form of the mesoscutal humeral sulcus was used by Tortorici et al. (2019) to separate very similar Palearctic *Trissolcus* species. This sulcus is clearly indicated by cells in the holotype of *T. cosmopeplae* (Figs 6, 7) and it is present as a smooth furrow in all specimens of *T. utahensis* that we have examined (Figs 5, 20–21), including the holotype.

Anteroventral extension of the metapleuron. The length of this structure, reaching to the mesocoxa in *T. cosmopeplae* (Fig. 6) and very short in *T. utahensis* (Figs 12–19),



Figures 20–22. *Trissolcus utahensis* **20** FSCA 00033239 (ex. *B. hilaris*), head, mesosoma, metasoma, dorsal view **21** FSCA 00033041 (ex. *P. maculiventris*), head, mesosoma, metasoma, dorsolateral view **22** FSCA 00033040 (ex. *P. maculiventris*), head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

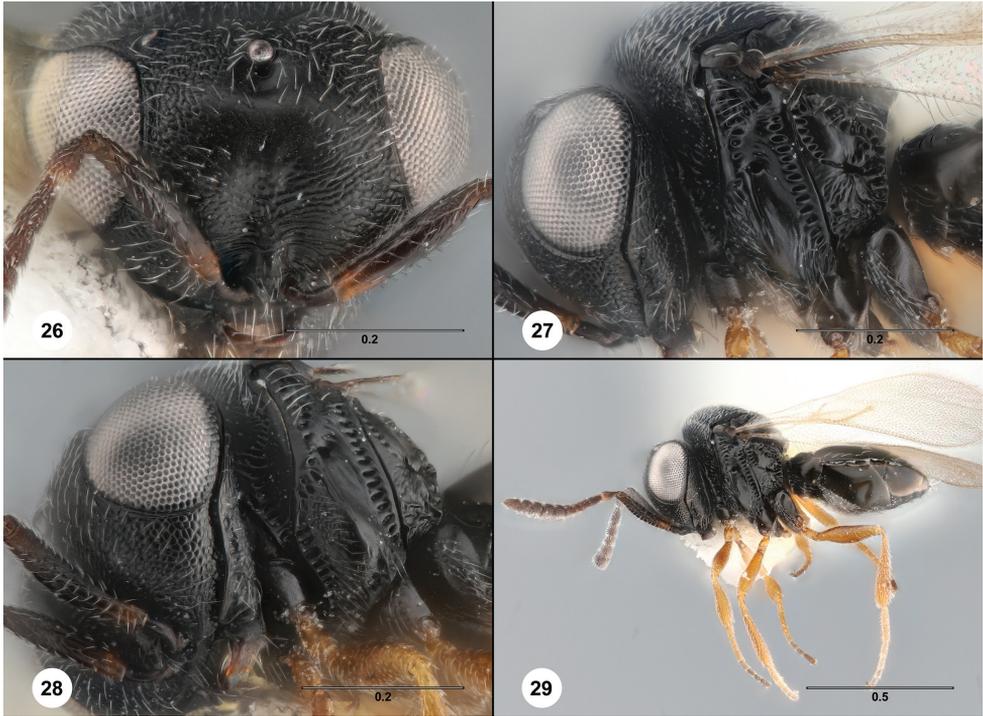


Figures 23–25. *Trissolcus utahensis* (FSCA 00091872, ex. *B. hilaris*) **23** head, anterior view **24** lateral habitus **25** head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

was the first character listed in the couplet that separates these species in Johnson (1985). Our analysis of specimens in this study lends weight to the reliability of this character.

Color. Most species in *Trissolcus* have a black metasoma. The most notable exception is a Palearctic species, *T. rufiventris* Mayr, in which T2–T7 vary from bright yellow to dark brown. The specimens reared from *B. hilaris* eggs have T2–T7 notably lighter in color than the head and mesosoma (Figs 20, 25), and the body overall is lighter in color than in specimens reared from *P. maculiventris* eggs (compare Fig. 8 to Figs 9–11, Fig. 12 to Figs 13–15).

The most obvious color variation in *T. utahensis* is in the legs. Specimens that parasitized *B. hilaris* eggs have legs that are pale brown to orange distal to the coxae (clades 1, 2; Figs 12, 24). Brightly colored legs were found in *T. utahensis* that emerged from *P. maculiventris* eggs (clade 3, Fig. 29), although the clear majority of specimens reared from *P. maculiventris* eggs in British Columbia had dark brown legs. Interestingly, FSCA 00033239, with pale brown legs, in clade 1 is sister to FSCA 00000320, with dark brown legs. The antennae vary in color in accordance with the legs, although the variation is less pronounced and ranges from medium to dark brown. These data do not indicate that *T. utahensis* exhibits a direct correlation of appendage color with host, size, or lineage.



Figures 26–29. *Trissolcus utahensis* (FSCA 00033042, ex. *P. maculiventris*) **26** head, anterior view **27** head and mesosoma, lateral view **28** head and mesosoma, ventrolateral view **29** lateral habitus. Scale bars in millimeters.

Material Examined. 11 females, FSCA 00091859, 00091872, 00094741–00094749, USA: CA: 33.99105N, 117.33360W, roadside mustard weeds, ex. *Bagrada hilaris* sentinel eggs deployed 6–9.IV.2018, parasitoids emerged 22–23.IV.2018, Coll. F. Ganjisaffar; 1 female, FSCA 00033239, USA: CA: UC Riverside Agricultural Operations, 33.96611N, 117.34230W, squash field with mustard weeds, ex: *Bagrada hilaris* sentinel eggs deployed 12–16-X-2018; parasitoid emerged 27–28-X-2018, Coll. F. Ganjisaffar; 9 females, 2 males, CANADA, BC, Penticton, reared from *Podisus maculiventris*, JUN–AUG-2017, Coll. W. Wong & P. Abram. Egg mass #181: FSCA 00094713–00094715, 00033042–00033043; Egg mass #144: FSCA 00000302; Egg mass #160: FSCA 00094712; Egg mass #92: 00033044–00033045; Egg mass #102; FSCA 00033040–00033041. 1 female, FSCA 00094711, CANADA, BC, Kelowna, reared from *Podisus maculiventris*, 23.V.2017, Coll. W. Wong & P. Abram. Egg mass #171.

Discussion

The reports of this study indicate that at least four species of *Trissolcus* (*T. basalis*, *T. hyalinipennis*, *T. hullensis*, and *T. utahensis*) are actively parasitizing *B. hilaris* eggs

in southern California (Ganjisaffar et al. 2018). Three other scelionids have been reported parasitizing *B. hilaris* eggs in Mexico: *Telenomus podisi* (Ashmead), *Gryon myrmecophilum* (Ashmead), and *Idris elba* Talamas (Felipe-Victoriano et al. 2019, Lomeli-Flores et al. 2019). This suggests that *B. hilaris* eggs may be broadly suitable for Nearctic parasitoids. We conducted surveys in the same locations as Reed et al. (2013) did monthly samplings of *B. hilaris* in 2011, and the fact that we did not find any *B. hilaris* in those areas during our two-year survey, suggests that populations of *B. hilaris* have steadily declined in recent years, and it is likely that parasitoids are part of the cause.

Trissolcus utahensis exhibits a striking degree of variation in *COI* among specimens from only a few localities. An expansion of this analysis with additional samples is certain to expand our understanding of genetic diversity in this species. It is worth noting that specimens FSCA 00094713–00094715 emerged from the same egg mass and were retrieved in different clades (1 and 3), indicating that interbreeding opportunities exist between these maternally definable populations. Future work should include nuclear genes to provide a broader view of population dynamics and genetic diversity in this species.

The systematics of *Trissolcus* is undergoing perpetual improvement as it continues to receive attention for the species that attack the eggs of economically important stink bugs. This study demonstrates how a seemingly routine activity of rearing and identifying specimens can require a multifaceted research endeavor to reach a satisfactory answer while also providing new lines of inquiry. For example, our molecular analysis retrieved *T. colemani* in two clades, one comprised of specimens from East Asia and one from Europe and the Middle East. This study also demonstrates how ongoing parasitoid surveys continue to be productive by providing fresh specimens with host association data. These specimens and data are instrumental for an integrated approach to systematics in which morphological, molecular, and behavioral data are combined to provide robust and holistic species concepts.

Acknowledgements

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The great greenbriers gall mystery resolved? New species of *Aprostocetus* Westwood (Hymenoptera, Eulophidae) gall inducer and two new parasitoids (Hymenoptera, Eurytomidae) associated with *Smilax* L. in southern Florida, USA

Michael W. Gates¹, Y. Miles Zhang¹, Matthew L. Buffington¹

¹ *Systematic Entomology Laboratory, USDA-ARS, c/o National Museum of Natural History, Washington, DC, 20013, USA*

Corresponding author: Michael W. Gates (michael.gates@usda.gov)

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Abstract

Aprostocetus smilax Gates & Zhang, **sp. nov.**, is described from stem and leaf galls on *Smilax havanensis* Jacq. in southern Florida, USA. It is the third species of *Aprostocetus* Westwood known to induce plant galls. Two parasitoids of *A. smilax* are also described: *Phylloxeroxenus smilax* Gates & Zhang **sp. nov.** and *Sycophila smilax* Gates & Zhang, **sp. nov.** We conclude that *A. smilax* is the true gall inducer on *Smilax* L., and thus the host records of *Diastrophus smilacis* Ashmead and its inquiline *Periclistus smilacis* Ashmead, both from *Smilax*, are erroneous.

Keywords

Chalcidoidea, Cynipidae, *Diastrophus*, *Sycophila*, *Phylloxeroxenus*, *Periclistus*

Introduction

Gall induction in Chalcidoidea was summarized by La Salle (2005) wherein he noted its occurrence in six families, representing at least 15 independent origins. Within Eulophidae

he reported 11 genera across two subfamilies, Opheliminae (two genera) and Tetrastichinae (nine genera), with documented gall induction behaviors. Since then, an additional six genera have been added to the list of gall inducers (Fisher et al. 2014; Kim et al. 2004; Kim and La Salle 2008; Kim et al. 2005; Mendel et al. 2004; Rasplus et al. 2011), including serious invasive pests of *Eucalyptus* L'Hér worldwide. In Tetrastichinae, the Neotropical gall associates and inducers tend to have heavier sclerotization and be larger in size than other members of the subfamily (La Salle 2005).

Aprostocetus Westwood is the largest genus within the subfamily Tetrastichinae, containing >800 species distributed worldwide that are most frequently associated with insect galls induced by four insect orders and Acari as parasitoids or inquilines (Graham 1987; La Salle 1994). Gall induction is somewhat rare in *Aprostocetus*, with only five documented cases worldwide: (1) *A. colliguayae* (Philippi) in flower buds of *Colliguaja* Molina (Euphorbiaceae) in Chile (Martinez et al. 1992); (2) *A. gallicolus* Nieves-Aldrey & Askew on stems of *Hedysarum boveanum* Bunge ex Basiner (Fabaceae) in Spain (Nieves-Aldrey and Askew 2011); (3) *Aprostocetus monacoi* Viggiani described from stem galls in *Melilotus indicus* L. (Fabaceae) from Italy; (4) *Aprostocetus* sp. on leaf midribs of *Corymbia citriodora* (Hook.) (Myrtaceae) reported from California and Hawaii (Beardsley and Perreira 2000); and (5) *Aprostocetus* sp. on stems of *Melilotus* Mill. infested with wound tumor virus *Aureogenus* Black in the US (Teitelbaum and Black 1954). In this paper we describe *Aprostocetus smilax*, sp. nov. (Hymenoptera: Eulophidae), a gall inducer on *Smilax havanensis* Jacq. and the second recorded case of gall induction for the genus in North America. We also describe two parasitoids of *A. smilax*, *Phylloxeroxenus smilax*, sp. nov., and *Sycophila smilax*, sp. nov. (Fig. 1).

Smilax L. are monocots in the family Smilacaceae, with ~350 species found mostly in tropical and subtropical regions of the world (Ferrufino-Acosta 2014). A variety of gall midges (Diptera: Cecidomyiidae) and fungi in the genus *Synchytrium* de Bary & Woronin are known to induce galls on *Smilax* (Cook 1951; Uechi et al. 2012; Urso-Guimarães and Scareli-Santos 2006). The only record of a *Smilax* gall induced by Hymenoptera in North America is *Diastrophus smilacis* Ashmead (Cynipidae: Diastrophini), collected from Illinois and Florida (Ashmead 1896a). This host record is somewhat puzzling, as other members of *Diastrophus* Hartig exclusively induce galls on Rosaceae (*Fragaria* L., *Rubus* L., and *Potentilla* L.), and all other known cynipids have been recorded from dicots (Ronquist and Liljeblad 2001; Schick et al. 2003).

Methods

Dissection

Freshly collected stem and leaf galls of *S. havanensis* were dissected during field work in the Miami area in 2010 by MWG and MLB. A Nikon 20× Mini Field stereoscope, fine forceps, and GEM Blue Star Super Single Edge razors were used. Slices of galls were successively removed, gradually exposing individual locules. We dissected ~20

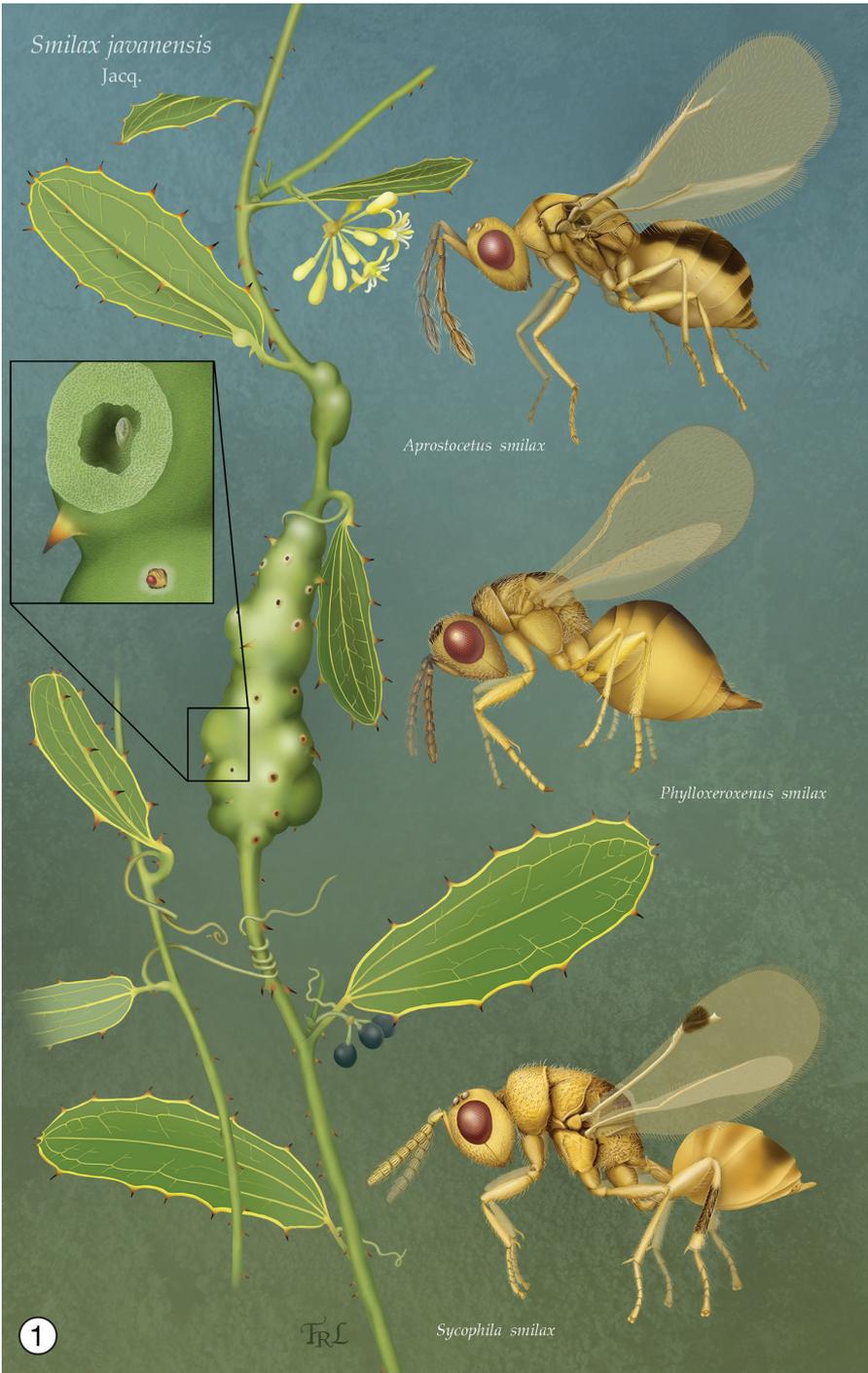


Figure 1. Illustration of the stem gall on *Smilax javanensis* induced by *Aprostocetus smilax* (top right), with the inset showing the internal structure and an egg. Two eurytomid parasitoids, *Phylloxeroxenus smilax* (middle right), and *Sycophila smilax* (bottom right) are included. Illustration by Taina Litwak.

multilocular galls and notes were made about the contents of each locule in terms of its condition and occupant prior to each occupant being assigned a code and preserved in 80% ethanol. We noted six ectoparasitoid specimens. Pertinent taxon-specific notes are included in results below.

Imaging

Ethanol-preserved specimens were dehydrated through increasing concentrations of ethanol, and transferred to hexamethyldisilazane (HMDS) (Heraty and Hawks 1998) before point-mounting. MWG identified parasitoids using a Leica M205C stereomicroscope with 10X oculars and a Leica LED ring light source for point-mounted specimen observation. We took scanning electron microscope (SEM) images with a Hitachi TM3000 (Tungsten source). Body parts of disarticulated specimens were adhered to a 12.7 × 3.2 mm Leica/Cambridge aluminum SEM stub by a carbon adhesive tab (Electron Microscopy Sciences, #77825-12). Stub-mounted specimens were sputter coated with gold-palladium using a Cressington Scientific 108 Auto from multiple angles to ensure complete coverage (~20–30 nm coating). Habitus images were obtained using a Visionary Digital imaging system. The system consists of a Canon EOS 5D Mark II digital SLR camera with a 65 mm macro lens. A Dynalite MP8 power pack and lights provided illumination. Image capture software was Visionary Digital's proprietary application with images saved as TIF with the RAW conversion occurring in Canon Digital Photo Professional software. Image stacks were montaged with Helicon Focus 6.2.2. Image editing was done in Adobe Photoshop and plate layout in Adobe Illustrator. The painting was made from pinned and live insect specimens, plant herbarium sheets and photographs. Additional structural details of the insects were obtained from SEM photographs. The final image was painted using Adobe Photoshop.

We used several species keys to determine whether our material belonged to any described species (Balduf 1932; Graham 1987) with details below under each specific treatment. Where possible, all species identifications were corroborated by comparison with authoritatively identified specimens in the Smithsonian National Museum of Natural History.

Terminologies used for surface sculptures follow Harris (1979), while the morphology follows Gibson (1997), La Salle (1994), Lotfalizadeh et al. (2007), and Gates and Pérez-Lachaud (2012). Abbreviations for museums are: ABS, Archbold Biological Station, Archbold, FL, USA; FSCA, Florida State Collection of Arthropods; USNM, United States National Museum of Natural History, Washington, D.C., USA.

Molecular protocol

Specimens were extracted, amplified, and sequenced at USDA Beltsville Agricultural Research Center (BARC) using the DNeasy™ Tissue Kit protocol (Qiagen, Valencia, CA, USA). Specimens were digested for circa three hours using 20 µL of 20 mg/

mL Proteinase K at 55 °C. The DNA was resuspended with 150 µL of Qiagen elution buffer. Fragments of mtDNA *COI* (620 bp) were amplified using LCO1490 5'-GGT-CAACAAATCATAAAGATATTGG-3' and HCO2198 5'-TAAACTTCAGGGT-GACCAAAAAATCA-3' (Folmer et al. 1994). Amplifications for rDNA *28S* (820 bp) used 28S_D1F 5'-ACCCGCTGAATTTAAGCATAT-3' (Harry et al. 1996) and 28S_D2R 5'-TTGGTCCGTGTTTCAAGACGGG-3' (Campbell et al. 1994). All PCRs were performed using approximately 2 µL DNA extract, 1.25 µL 10× Buffer, 1 µL dNTP, 1 µL of each primer, 1 unit of Taq DNA polymerase (TaKaRa Bio, Mountain View, CA, USA), and purified water for a final volume of 25 µL. Amplicons of *COI* were generated with an initial denaturation of 1 min at 95 °C, followed by 35 cycles of 95 °C for 15 s, 49 °C for 15 s and 72 °C for 45 s, and a final elongation period of 4 min at 72 °C. The thermocycler setting for *28S* is similar to *COI*, with the exception of annealing temperature being at 55 °C. Sequencing was conducted using a ABI 3730xl DNA sequencer following manufacturer's instructions. Contigs were assembled and edited using Sequencher version 4.5 (Gene Codes). DNA sequences were then compared with available sequences in the Barcode of Life Database (BOLD, Ratnasingham and Hebert 2007) and the Basic Local Alignment Search Tool (BLAST) for nucleotides in GenBank. All sequences are uploaded onto GenBank (see Table 1).

Phylogenetic analysis

COI was aligned using default MAFFT v7.45 settings (Katoh et al. 2002) and checked by eye, for *28S* the Q-INS-I strategy (Katoh and Toh 2008) was implemented to account for secondary RNA structures. Each gene was analyzed separately, and concatenated using SequenceMatrix (Vaidya et al. 2011) in IQ-TREE v2.0.5 (Minh et al. 2020). Best models of evolution were determined using ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE, with 1000 ultrafast bootstrap pseudoreplicate support (Hoang et al. 2017). The output trees were visualized in R v4.0 (R Core Team 2020) using the packages ggtree v2.2.0 (Yu et al. 2017) and treeio v1.12.0 (Wang et al. 2020).

Results

Taxonomy

Eulophidae

Aprostocetus smilax Gates & Zhang, sp. nov.

<http://zoobank.org/D29A7AF7-E6D5-488D-996A-1228BD8F320F>

Figs 2–18

Diagnosis. This species keys to *Aprostocetus* subgenus *Aprostocetus*, couplet 103 in Schauff et al. (1997) and 53 in La Salle (1997). This is the most biologically diverse

Table I. Voucher identification and associated GenBank accession numbers.

ID	Voucher	COI	28S	
<i>Aprostocetus smilax</i>	G0006A	MT576085	MT560740	
	G0008A	N/A	MT560741	
	G0008B	MT576086	MT560742	
	G0009	MT576087	MT560743	
	G0010A	MT576088	MT560744	
	G0010C	MT576089	MT560745	
	G0010E	MT576090	MT560746	
	G0010F	MT576091	MT560747	
	G0013A	MT576092	MT560748	
	G0014	MT576093	MT560749	
	<i>Phylloxeroxenus smilax</i>	G0015	MT576094	MT560750
	<i>Aprostocetus smilax</i>	G0016	MT576095	N/A
		G0017	MT576096	MT560751
		G0018	MT576097	MT560752
G0019		MT576098	MT560753	
G0021		MT576099	MT560754	
G0022		MT576100	MT560755	
G0023		MT576101	MT560756	
G0024		MT576102	MT560757	
G0025		MT576103	MT560758	
G0026		MT576104	MT560759	
G0030		MT576105	MT560760	
G0031		MT576106	MT560761	
G0034		MT576107	MT560762	
G0036		MT576108	MT560763	
G0041		MT576109	MT560764	
<i>Tetrastichinae</i> sp.		G0042	MT576110	MT560765
<i>Aprostocetus smilax</i>		G0043	MT576111	MT560766
	G0044	MT576112	MT560767	
<i>Sycophila smilax</i>	G0045	N/A	MT560768	
<i>Aprostocetus smilax</i>	G0046	MT576113	MT560769	
	G0047	MT576114	MT560770	
	G0049	MT576115	MT560771	
	G0050	MT576116	N/A	
	<i>Phylloxeroxenus smilax</i>	G0051	N/A	MT560772
<i>Brasema</i> sp.	G0052	MT576117	MT560773	
<i>Sycophila smilax</i>	G0053	N/A	MT560774	
<i>Phylloxeroxenus smilax</i>	G0054	MT576118	MT560775	
<i>Sycophila smilax</i>	G0055	N/A	MT560776	
	G0056	MT576119	MT560777	
<i>Phylloxeroxenus smilax</i>	G0057	N/A	MT560778	
	G0058	N/A	MT560779	
<i>Aprostocetus smilax</i>	G0059	MT576120	MT560780	
	G0061	MT576121	MT560781	
	G0063	MT576122	MT560782	
	G0064	MT576123	MT560783	
	G0065	N/A	MT560784	
	G0066	MT576124	MT560785	
	G0070	MT576125	MT560786	
	<i>Phylloxeroxenus smilax</i>	G0071	N/A	MT560787
	<i>Sycophila smilax</i>	G0076	MT576126	MT560788
<i>Aprostocetus smilax</i>	G0077	MT576127	MT560789	
	G0078	MT576128	MT560790	
<i>Aprostocetus smilax</i>	G0080	MT576129	MT560791	
	<i>Phylloxeroxenus smilax</i>	G0082	N/A	MT560792

and speciose of the five *Aprostocetus* subgenera, often associated with insects inhabiting plant galls such as Diptera (Cecidomyiidae), Hymenoptera (Cynipoidea), Hemiptera (Coccoidea), Coleoptera, and eriophyid mites (La Salle 2005). Burks (1967) published a key to 13 North American species, which is dated, and a comparative diagnosis of all 58 species is beyond the scope of this paper. Nevertheless, this species keys to couplet 2 of Burks' key, and differs from the two species with coriaceous mesoscutum (*A. coelioxydis* Burks and *A. granulatus* Ashmead) which are both metallic blue/black in coloration. Recent phylogenomic study of Eulophidae has shown *Aprostocetus* to be paraphyletic (Rasplus et al. 2020), and some of these subgenera might be elevated to genus level in the future.

Material examined. Holotype, female: USA • **FL**: Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 19.Dec.2001, C. Rodriguez & T. Smith leg.; USNMENT01735185 (deposited at USNM). Paratypes (4♀, 7♂): Same information as holotype; USNMENT01735186, 01735187 (1♀, 1♂, USNM). **FL**: Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 18.Apr. 2010; M. Gates & M. Buffington leg.; USNMENT01735188–01735196 (3♀, 6♂, USNM).

Description. Female. Body length 1.7 mm (Fig. 2).

Color. Mostly whitish-yellow, pedicel, flagellomeres, clava, axillula, and marginal vein, sides of gastral tergites brown. Fore and midlegs white (Fig. 2).

Head. Squareish in dorsal view, 1.2× as wide as long in dorsal view (Fig. 4). Lower face coriaceous, clypeus bilobed, mandible tridentate (Fig. 5). Malar sulcus present, malar space 0.7× eye height. Genal carina absent. Toruli positioned slightly below median of compound eyes, diameter of torulus equal that of the intertorular space. Frons striate, scrobal depression converging towards the clypeus with a row of setae along depression (Fig. 4). Vertex coriaceous, ratios of POL:OOL:LOL equal to 2.8:2.1:1 (Fig. 6). Ratio of scape (minus radicle):pedicel:A1:A2: F1:F2: F3:club as 72:33:3:1:53:40:35:68; pedicel conical expanding distally; funicle cylindrical; anellus two segmented, funicular segments with single row of longitudinal sensilla and one whorl of setae, shorter than its bearing segment; clava trisegmented (Fig. 8). Head posteriorly coriaceous with a ring of setae around the outer edge, smooth with two setae. Postgenal bridge ornamentation narrow. Postgenal sulci, postgenal groove, and hypostomal bridge absent (Fig. 7). Labium square-shaped.

Forewing. Three setae on submarginal vein, 7 setae on marginal vein. Ratio of marginal vein:postmarginal vein:stigmatal vein as 22.5:1:6.

Mesosoma. Mesosoma coriaceous, 1.14× as long as broad (Fig. 9); notauli complete, shallow. With 2 adnotaular seta on the midlobe of mesoscutum, and two setae on the lateral lobes (Fig. 10). Scutellum with two setae on each side, submedian groove deep, complete. Lateral panel of axilla strigate, axillula coriaceous dorsally and strigate ventrally. Prepectus coriaceous. Mesopleuron coriaceous, dorsally delimited from femoral depression raised ridge. Epicnemium flat and ventral shelf not projected forward (Fig. 11). Propodeum coriaceous and divided by median carina that diverges into



Figure 2–3. *Aprostocetus smilax* **2** female habitus **3** male habitus.

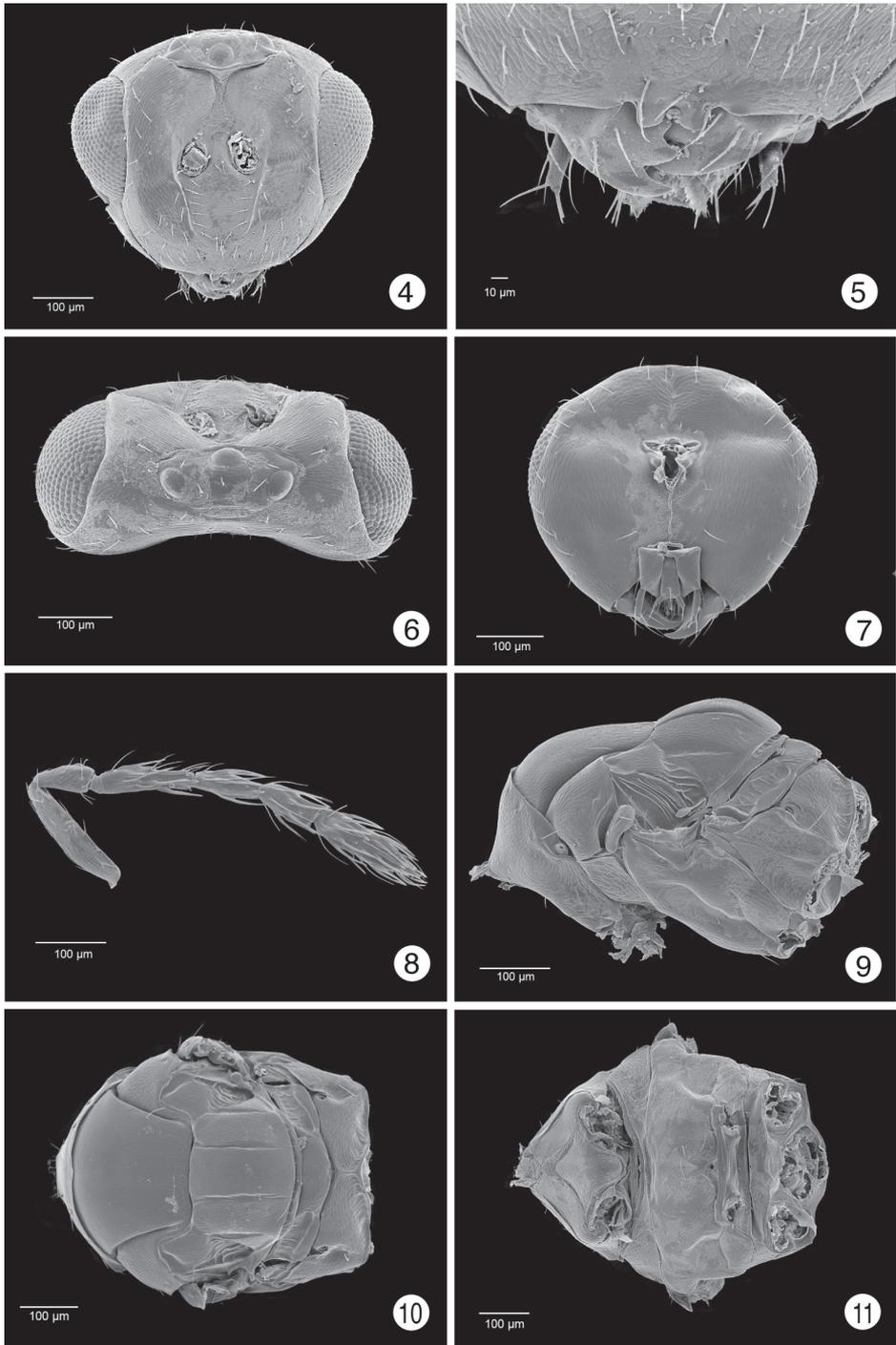


Figure 4–11. *Aprostocetus smilax* **4** frontal view of head **5** frontal view of lower face **6** dorsal view of head **7** posterior view of head **8** female antenna **9** lateral view of mesosoma **10** dorsal view of mesosoma **11** ventral view of mesosoma.

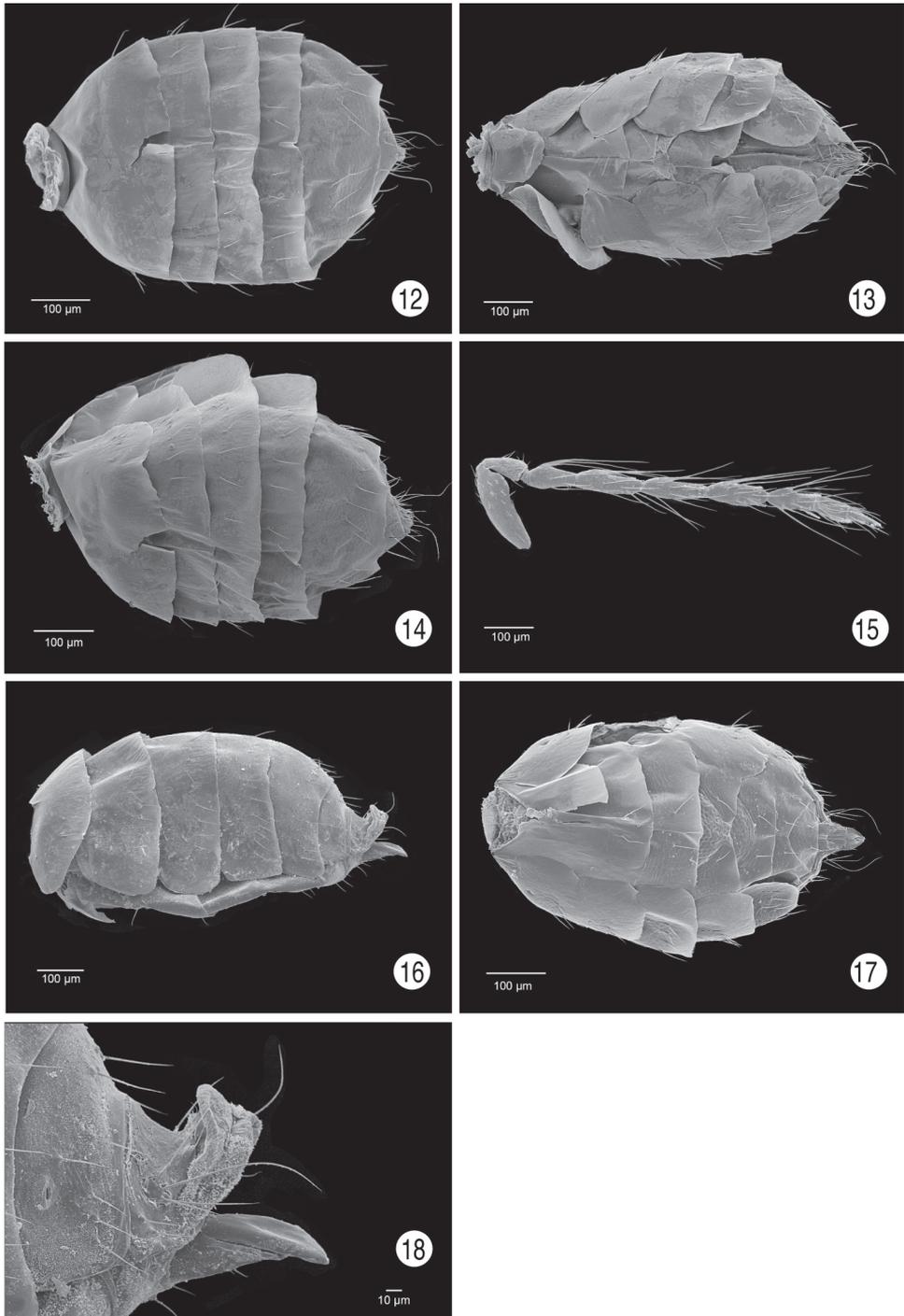


Figure 12–18. *Aprostocetus smilax* **12** dorsal view of female metasoma **13** ventral view of female metasoma **14** lateral view of female metasoma **15** male antenna **16** lateral view of male metasoma **17** ventral view of male metasoma **18** closeup of male genital opening.

raised, scalloped ridges posteriorly. Spiracle within a depression. Callus with a single seta, raised and partly overhanging outer rim of conspicuous spiracle (Fig. 10).

Metasoma. Metasoma smooth, Gt1 and Gt2 dorsally glabrous (Fig. 12), subsequent tergites each with a ring of setae (Fig. 13). Cercus with 1 seta distinctly longer ($>1.5\times$) than others (Fig. 14).

Male. 1.1 mm. Color and sculpture as described for female (Fig. 3). Antennae with setae $>1.5\times$ as long as width of segment (Fig. 15). Gt7 curves up to form genital opening (Figs 16, 17), with a pair of long and three pairs of shorter cercal setae (Fig. 18).

Variation. Size ranges from 1.6–1.8 mm for females, and 1.1–1.2 mm for males. The number of setae on marginal vein ranges from 6–8.

Biology. It induces round galls on the stems of *Smilax havanensis*, often coalescing to form irregularly rounded, polythalamous swellings. Individual galls can also be found on the edge of leaves. Green when fresh and of a pithy structure (Figs 1, 63 inset), tissues around the emergence hole often form a black ring.

Distribution. Southern Florida, USA.

Eurytomidae

Phylloxeroxenus smilax Gates & Zhang, sp. nov.

<http://zoobank.org/6D5262E0-7A91-4A3A-9E1A-C219C1F5EBA3>

Figs 19–34

Diagnosis. *Phylloxeroxenus smilax* can be easily distinguished from the only other known North American species, *Phylloxeroxenus phylloxerae* (Ashmead), which is suspected to be a parasitoid of the cecidomyiid inquiline within *Phylloxera* Boyer de Fonscolombe galls on hickory (*Carya* Nutt.) (Ashmead 1881). The lower face is strigose and the ventral half of the body is yellow in *P. smilax*, while in *P. phylloxerae* the lower face is striate and the body is completely black. There are at least 50 undescribed species in at least three species groups for the Neotropical region that exhibit a range of variation in diagnostic generic characters such as the propodeum in lateral view forming a 90° angle with mesosoma; long/short petiole and resultant effect on striate part of S1 (Fig. 30), with the striae on S1 being a reliable diagnostic though expressed to varying degrees; and lower face with/without striae (Gates, unpublished data).

Material examined. **Holotype**, female: USA • **FL**: Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 18.Apr. 2010; M. Gates & M. Buffington leg.; USNMENT01735174 (deposited at USNM). Paratypes (5♀, 6♂): Same information as holotype; USNMENT01735175–01735178 (3♀, 1♂, USNM). **FL**: Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 19.Dec.2001, C. Rodriguez & T. Smith leg.; USNMENT01735179–01735184 (2♀, 5♂, USNM). Additional material: **FL**: Dade Co.: Coral Gables, Deering Estate Pineland, Ex *Smilax havanensis* stem gall; 23.Feb.1995, G. Melika leg.; (3♀, 4♂, ABS).

Description. Female. Body length 1.88 mm (Fig. 19).



Figure 19–20. *Phylloxeroxenus smilax*: **19** female habitus **20** male habitus.

Color. Orange-yellow; antennal segments light brown; edges of ocelli, scutellum, metasoma mediadorsally with black band, eyes pinkish red (Fig. 19).

Head. Rounded in dorsal view, 1.3× as wide as long in dorsal view, umbilicate with appressed setae (Fig. 21). Lower face strigose, clypeus emarginate, mandible tridentate and step-like, supraclypeal area smooth, glabrous, slightly raised, and extending to the toruli (Fig. 22). Malar sulcus present, malar space 0.7× eye height. Genal carina present. Toruli positioned slightly above lower ocular line, diameter of torulus 4.4× that of the intertorular space. Scrobal depression carinate laterally, fading apically. Vertex imbricate, ratios of POL:OOL:LOL equal to 2.5:1:1 (Fig. 23). Ratio of scape (minus radicle):pedicel:anellus: F1:F2: F3:F4:F5:club as 19:7.3:1:7:6.6:6.6:6.4:6:18; pedicel chalice-shaped; funicle fusiform; funicular segments with single row of longitudinal sensilla and one whorl of setae, as long as its bearing segment; clava bisegmented (Fig. 25). Head posteriorly lacking postgenal lamina, postgenal groove straight and not converging in their lower part, extending ventrally to lower margin of eyes. Postgenal bridge ornamentation narrow and delicate (Fig. 24). Postgenal sulci small.

Forewing. Eight submarginal setae, 3 on parastigma. Ratio of marginal vein:postmarginal vein:stigmatal vein as 2:1:1.

Mesosoma. Mesosoma umbilicate, 1.45× as long as broad; notauli complete, shallow (Fig. 27); lateral surface of prepectus triangular, smooth, ventral surface of prepectus without median tooth, subventral carina diverging strongly (Fig. 26). Mesopleuron reticulate ventrally, dorsally delimited from femoral depression by fine carina. Epicnemium flat and ventral shelf not projected forward. Propodeum in lateral view forming a 90° angle with mesosoma, broadly flattened and apically arcuate, with numerous carinae forming irregular asetose cells, these bordered laterally by setose cells; cluster of setae anterolaterad nucha (Fig. 29). Metaplural-precoxal carina complete (Fig. 28).

Metasoma. Metasoma smooth, Gt4–syntergum setose, Gt6 and syntergum microreticulate; petiole 0.78× as long as broad in dorsal view, with ventral anterior groove and carina (Fig. 31); gaster S-shaped in lateral view, ovipositor angled at about 30° dorsad horizontal axis (Fig. 30); Gt4 emarginate posteriorly in dorsal view.

Male. 1.51 mm. Color and sculpture as described for female (Fig. 20). Antennal with funicular segments pedicellate, each with 2 or more rows of erect setae and about 1.5× as long as width of segment. Four funicular segments and a trisegmented clava (Fig. 32). Gastral petiole in lateral view cylindrical with projecting lateral teeth and mediadorsal prong (Fig. 34), in dorsal view length about 2.5× as long as greatest width, 1.6× as long as the length to metacoxa; evenly reticulate dorsally and ventrally (Fig. 33), smooth laterally.

Variation. Size ranges from 1.76–1.91 mm for females, and 1.45–1.52 mm for males. The coloration on the body can range from almost completely yellow, to mostly black on the dorsolateral surfaces, particularly in males.

Biology. Associated with galls of *Aprostocetus smilax*, likely a parasitoid of the gall inducer.

Distribution. Southern Florida, USA.

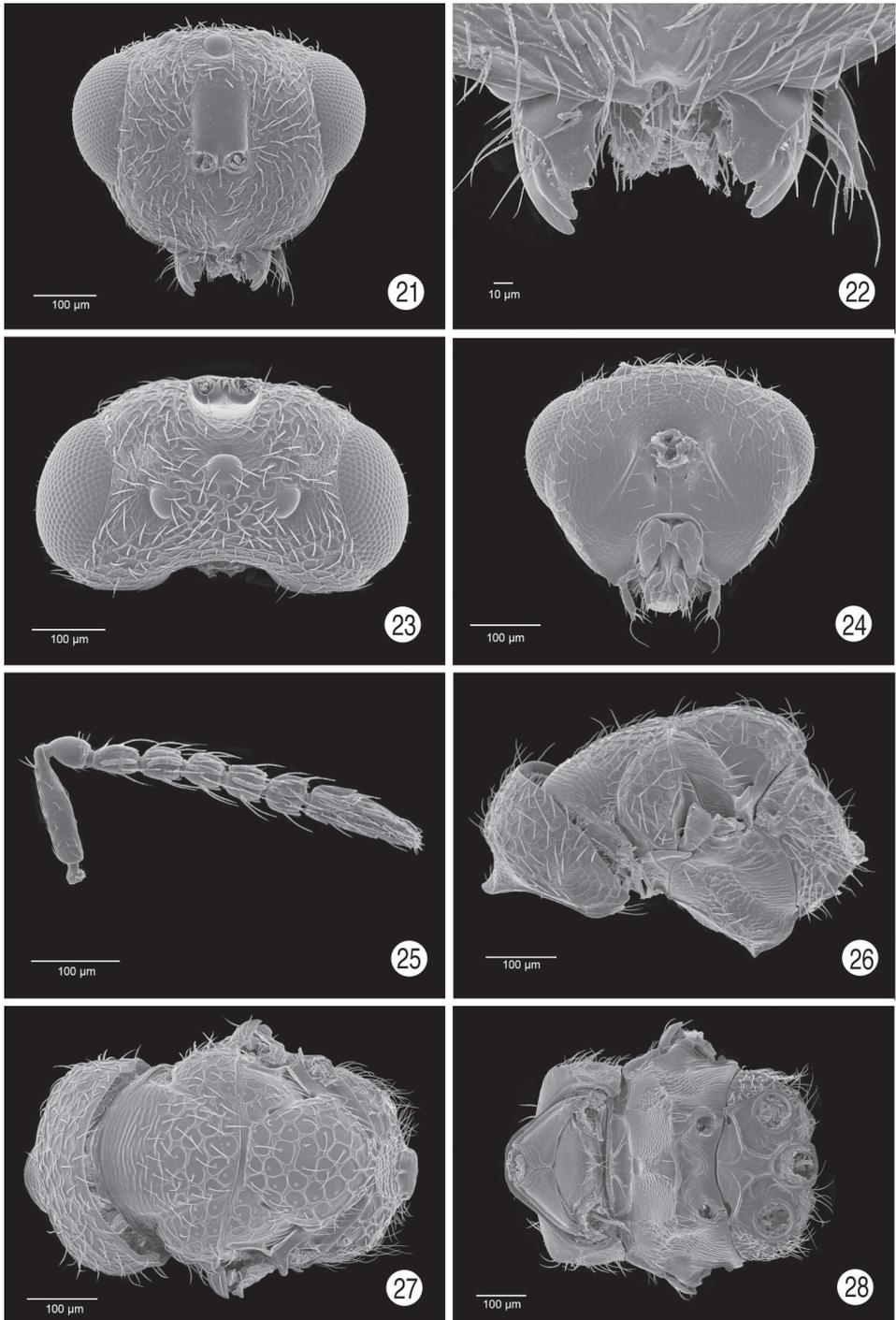


Figure 21–28. *Phylloxeroxenus smilax* **21** frontal view of head **22** frontal view of lower face **23** dorsal view of head **24** posterior view of head **25** female antenna **26** lateral view of mesosoma **27** dorsal view of mesosoma **28** ventral view of mesosoma.

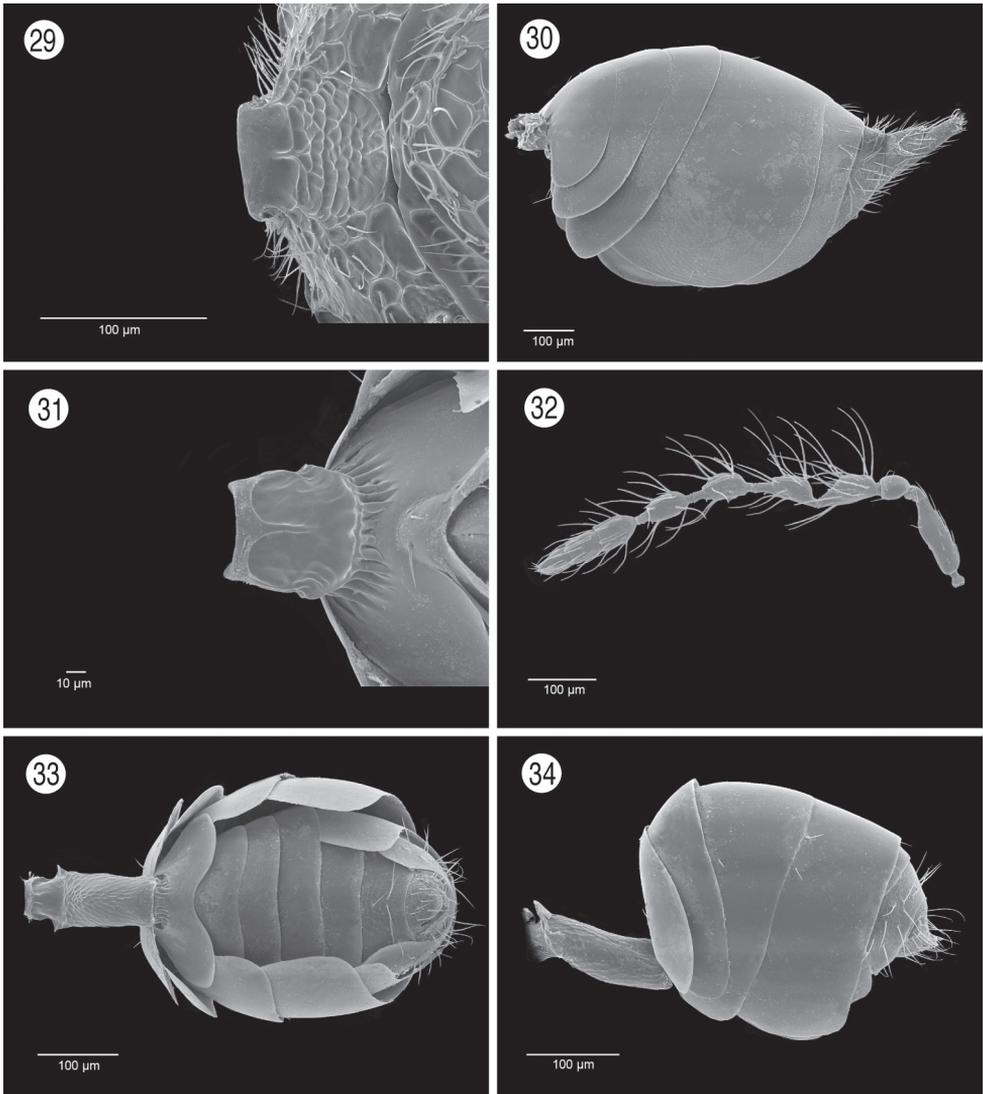


Figure 29–34. *Phylloxeroxenus smilax* **29** dorsal view of paropodeum **30** lateral view of female metasoma **31** ventral view of female petiole **32** male antenna **33** ventral view of male metasoma **34** lateral view of male metasoma.

***Sycophila smilax* Gates & Zhang, sp. nov.**

<http://zoobank.org/AC0B37C6-F069-4E19-91EF-946EE0466F1B>

Figs 35–53

Diagnosis. This species is recognized by its small size, pale yellow coloration and small/faint stigmal band. It keys to couplet 9 of Balduf (1932)'s key of North American *Sycophila*, but differs from the other mostly yellow species *Sycophila mimosae* Balduf

by the lack of a constricted marginal vein. The Central and South American *Sycophila* fauna is poorly known, and no current key exists.

Material examined. Holotype, female: **FL**: Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 19.Dec.2001, C. Rodriguez & T. Smith leg.; USNMENT01735197 (deposited at USNM). Paratypes (36♀, 8♂): Same information as holotype; USNMENT01735198–01735206 (6♀, 2♂, USNM). **FL**: Dade Co.: SE Miami, Rockdale Pineland, Ex *Smilax havanensis* stem gall; 18.Apr. 2010; M. Gates & M. Buffington leg.; USNMENT01735207–01735238 (27♀, 5♂, USNM). **FL**: Dade Co.: South Miami, Quail Roost Pineland, Em 1.VI.2000 from galls of *Smilax* sp.; 8.V.2000; USNMENT01735239–01735242 (3♀, 1♂, USNM). Additional material: **FL**: Dade Co.: Coral Gables, Deering Estate Pineland, Ex *Smilax havanensis* stem gall; 23.Feb.1995, G. Melika leg. (3♀, 2♂, ABS). **FL**: Dade Co.: Coral Gables, Ex. *Dias-trophus smilacis* on *Smilax havanensis*; 8.Nov.1977, R. Schimmel leg. (1♀, 1♂, FSCA).

Description. Female. Body length 1.8 mm (Fig. 35).

Color. Mostly pale yellow; antennal segments dark yellow; edges of ocelli, scutellum, hindtibia laterally, tarsal claw, tip of ovipositor black, pterostigma dark brown, wing band light brown, eyes pinkish red (Fig. 35).

Head. Rounded in dorsal view, 1.22× as wide as long in dorsal view, umbilicate with appressed setae (Fig. 37). Lower face strigose, clypeus bilobate, mandible tridentate with supraclypeal area smooth, glabrous, slightly raised and extending to toruli (Fig. 38). Malar sulcus present, malar space 0.59× eye height. Genal carina absent. Toruli positioned on lower ocular line, diameter of torulus 1.2× that of the intertorular space. Interantennal projection narrow, 1.5× that of the diameter of torulus. Scrobal depression carinate laterally, slightly diverging basally. Vertex imbricate, ratios of POL:OOL:LOL equal to 2.7:1:1 (Fig. 39). Ratio of scape (minus radicle):pedicel:anellus: F1:F2: F3:F4:F5:club as 17:6.7:1:5:5:4.7:4.7:4.7:13; pedicel chalice-shaped; funicle fusiform; funicular segments with single row of longitudinal sensilla and two whorls of setae, as long as its bearing segment; clava bisegmented (Fig. 41). Head posteriorly lacking postgenal lamina, postgenal groove faint, straight and not converging in their lower part, extending ventrally to $\frac{4}{5}$ the lower margin of eyes (Fig. 40). Postgenal sulci small.

Forewing. Dark brown band on the wing about the same width as pterostigma and does not reach uncus, faint, reaching about $\frac{1}{2}$ down the wing width, 8 submarginal setae, 3 on parastigma, 1 in basal cell, surrounded by basal and costal setal lines. Pterostigma covering marginal, postmarginal, and stigmal vein.

Mesosoma. Mesosoma umbilicate, 1.52× as long as broad; notauli complete, shallow (Fig. 43); lateral surface of prepectus triangular, smooth, ventral surface of prepectus without median tooth (Fig. 42). Mesopleuron reticulate ventrally, dorsally delimited from femoral depression by fine carina. Epicnemium flat and ventral shelf not projected forward. Propodeum with median furrow bordered mediolaterally by numerous carinae forming irregular asetose cells, these bordered laterally by setose cells (Fig. 44). Metaplural-precoxal carina interrupted by rugose carinae (Fig. 45).



Figure 35–36. *Sycophila smilax* **35** female habitus **36** male habitus.

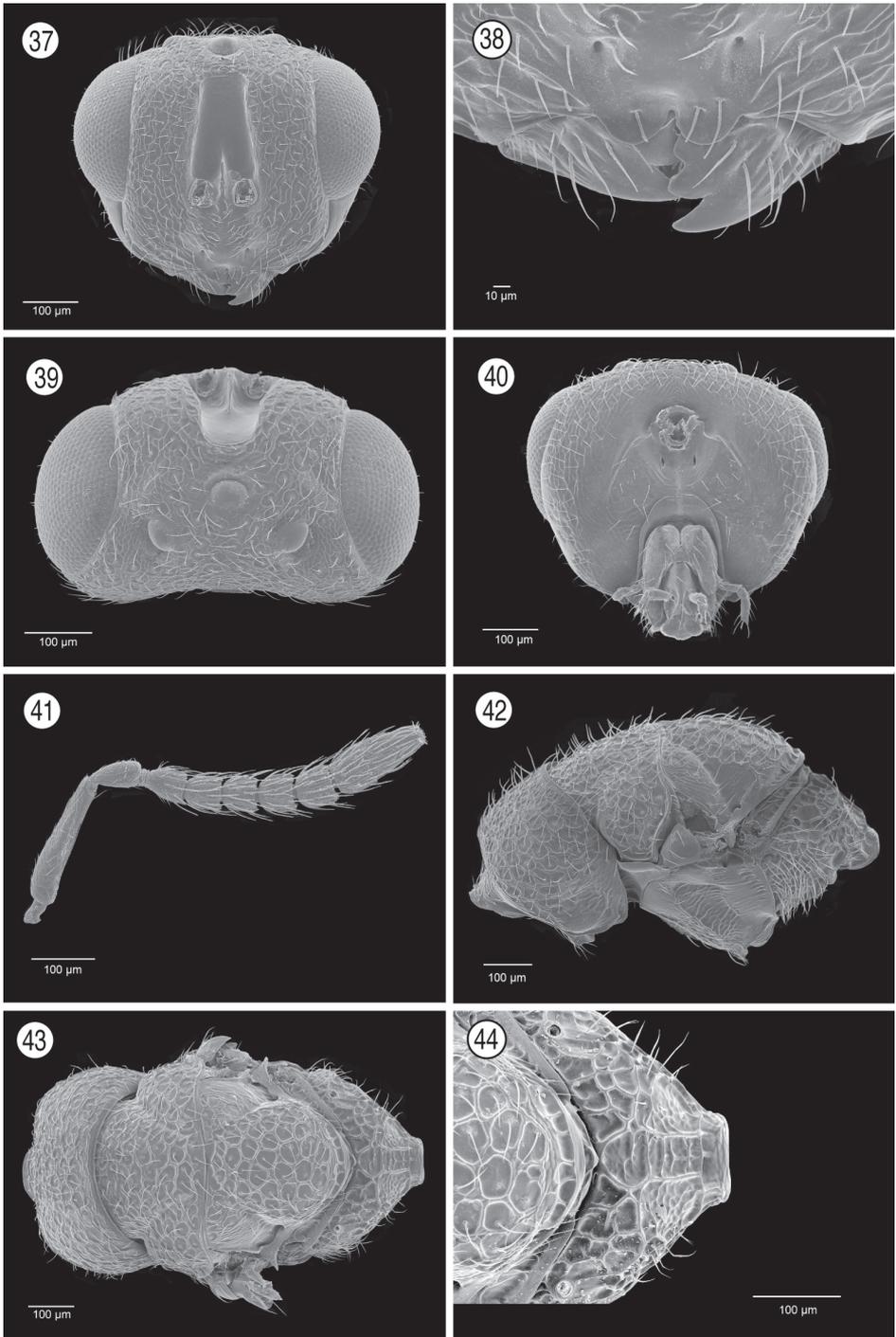


Figure 37–44. *Sycophila smilax* **37** frontal view of head **38** frontal view of lower face **39** dorsal view of head **40** posterior view of head **41** female antenna **42** lateral view of mesosoma **43** dorsal view of mesosoma **44** dorsal view of propodeum.

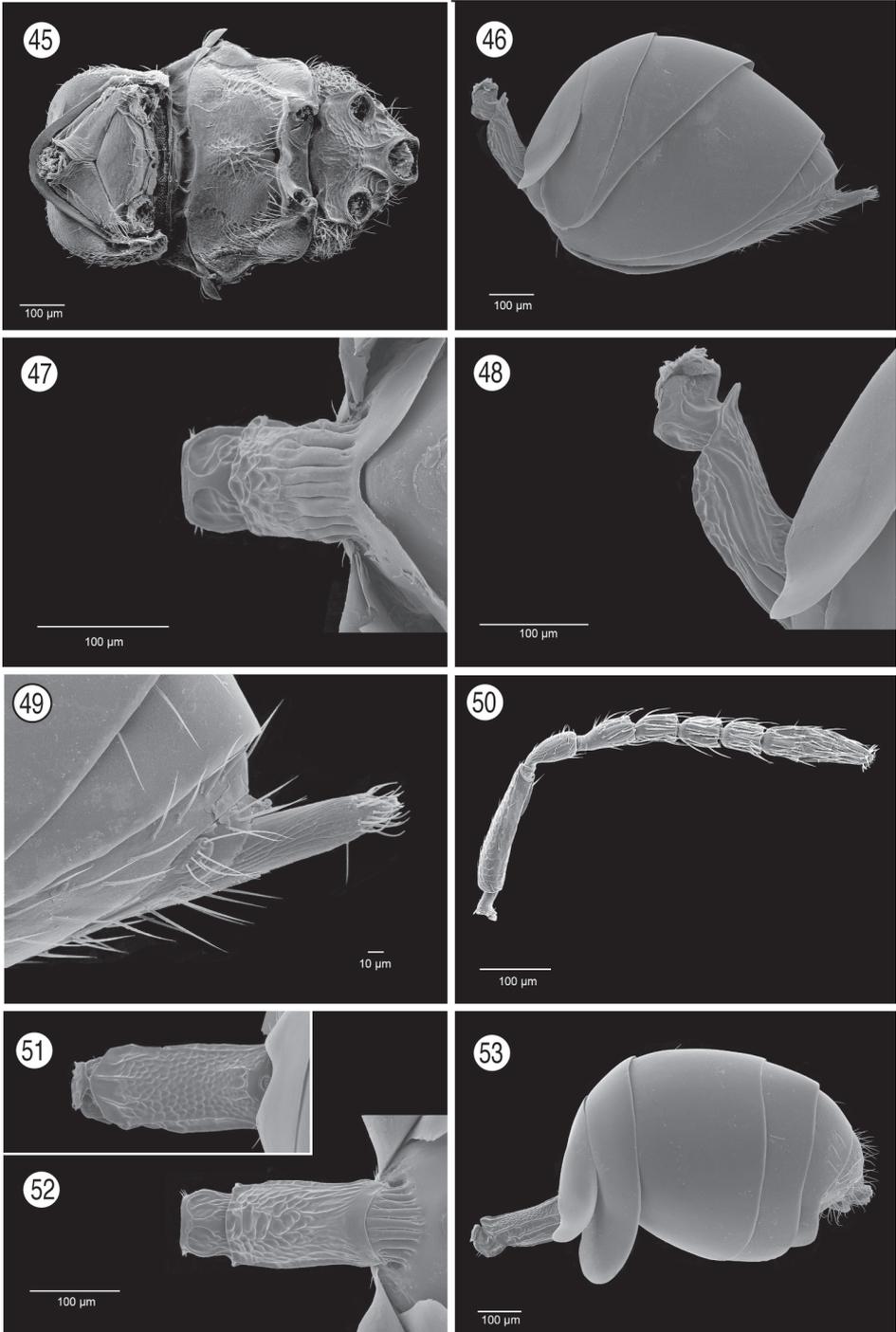


Figure 45–53. *Sycophila smilax* **45** ventral view of mesosoma **46** lateral view of female metasoma **47** ventral view of female petiole **48** lateral view of female petiole **49** closeup of female ovipositor **50** male antenna **51** dorsal view of male petiole **52** ventral view of male petiole **53** lateral view of male metasoma.

Metasoma. Metasoma smooth, ovipositor sheath microreticulate (Figs 46, 49); petiole 2.3× as long as broad in dorsal view, with ventral anterior groove, carina, and mediodorsal prong (Figs 47, 48); gaster teardrop-shaped in lateral view, ovipositor angled at about 30° dorsad horizontal axis (Fig. 49).

Male. 1.88 mm. Mediodorsal of Gt3–5 black, wing band very faint. Otherwise color and sculpture as described for female (Fig. 36). Antenna with four funicular segments (Fig. 50). Gaster cylindrical, petiole 3× as long as wide (Figs 51, 52). Gt4 emarginate posteriorly in dorsal view (Fig. 53).

Variation. Body ranges 1.7–1.8 mm for females, 1.65–1.88 mm for males. The wing band can range from very faint, mesosoma and metasoma dorsally can be yellow or with a tinge of black.

Biology. Associated with galls of *Aprostocetus smilax*, likely a parasitoid of the gall inducer.

Distribution. Southern Florida, USA.

Molecular analyses

A total of 55 individuals had both or at least one of the two genes sequenced. BLAST and BOLD search results confirmed the family and sometimes genus level identification, but did not return any hits at the species level. This *Smilax* gall contains 3 different families of chalcidoids: the majority of the gall inhabitants consisted of the suspected gall inducer *Aprostocetus smilax* (n = 40), and two eurytomid parasitoids *Phylloxeroxenus smilax* (n = 7) and *Sycophila smilax* (n = 6) (Fig. 63). Specimen G0042 was identified as an unknown tetrastichine eulophid that was destructively sampled, while G0052 was identified as *Brasema* Cameron (Eupelmidae) (Fig. 63). This *Brasema* specimen was never reared as an adult from this system, we noted it encircling another larva, presumably the gall inducer, and characterized by large mandibles and erect setae.

Validity of Cynipidae associated with *Smilax*

As the result of this study, the validity of *Diastrophus smilacis* (Figs 54, 55) inducing galls on *Smilax* was also investigated. The resulting fieldwork revealed *Aprostocetus smilax* is the true gall inducer in Florida, after some 400 galls never yielded any cynipids. Further, dissections of the galls from which the type specimen of *D. smilacis* was reared from (collected in Illinois) revealed vascular tissue patterns consistent with dicots and not monocots (Fig. 56). As no additional material of *D. smilacis* has been found since its original description, despite extensive searches in Illinois (Zhiwei Liu, pers. comm.) and other parts of North America (Weld 1959), we can safely conclude *Smilax* is not the host of *Diastrophus smilacis*.

Working with the type material of both *D. smilacis* and *Periclistus smilacis* Ashmead (Figs 59, 62, the putative inquilline of *D. smilacis*) revealed additional curiosities that require mentioning here. Ashmead (1896a) reports specimens of *D. smilacis* were apparently sent to C.V. Riley from Chicago, Illinois (Figs 57, 58), and that Ashmead



Figure 54–58. *Diastrophus smilacis* **54** lateral habitus of holotype **55** dorsal habitus of holotype **56** gall of holotype **57** label of holotype **58** label of other specimens collected by C.V. Riley.

intended to describe them but the publication of the manuscript was delayed due to C.V. Riley's untimely death. Ergo, time passed, and in the same year (1896), in two different publications, we find the descriptions of *D. smilacis* (Ashmead 1896a) and *P. smilacis* (Ashmead 1896b). While this is not an entirely foreign set of circumstances, the specimens referred to in these two publications are quite confusing.

Ashmead (1896a) reports 13 specimens (females) for the description of *D. smilacis*, but the taxon is only known from the type specimen in the USNM and there is no record of additional specimens being loaned out; one cotype of this taxon is in AMNH, for a total of two specimens. The gall with the same type specimen number as the holotype wasp in the USNM (No. 3096, Fig. 57) has the label '86x' affixed to the pin, and it is mentioned in Ashmead (1896a) that a gall was collected for this species in Florida, but no wasp. Hence, Illinois is the origin of all material associated with *D. smilacis*. Ashmead (1896b) describes *P. smilacis* from 17 specimens and goes on to say the collection data for 13 specimens (same number as *D. smilacis*, above) is labeled 'No. 864, reared April 28, 1871 and four numbered 1010, reared February 4, 1884, from *Diastrophus smilacis*'. However, the type specimen of *P. smilacis* (Fig. 61) in the USNM has label data consistent with the label data of *D. smilacis*, suggesting Ashmead (1896b) erroneously read '86x' as '864' and that the same gall that yielded the type



Figure 59–62. *Periclistus smilacis* 59 lateral habitus of lectotype 60 series of specimens and gall of lectotype 61 label of lectotype 62 dorsal habitus of lectotype.

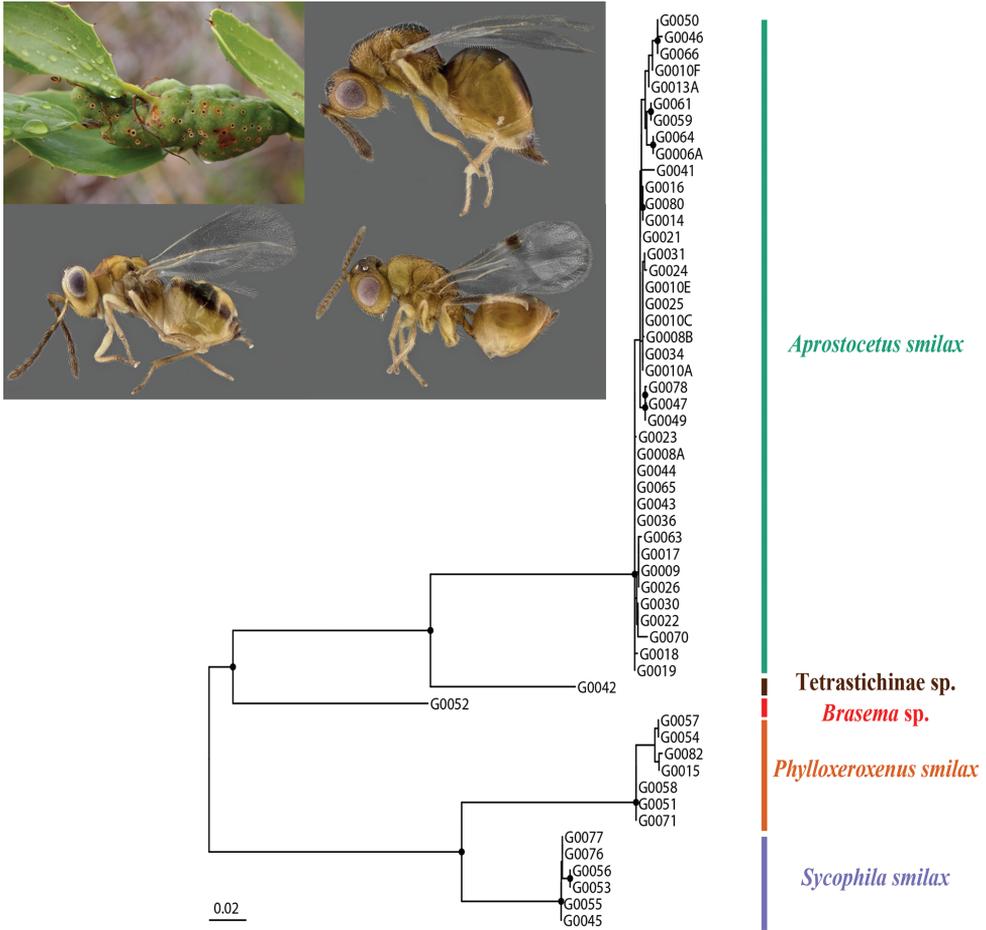


Figure 63. Concatenated 28S and COI phylogram of the *Smilax* gall inhabitants estimated using Maximum Likelihood framework in IQ-TREE2. Black dots at the nodes indicate $\geq 90\%$ ultrafast bootstrap support. Inset images in counterclockwise order: Stem gall induced by *Aprostocetus smilax* on *Smilax havanensis*, with emergence holes (photo by MWG); *A. smilax*, *Sycophila smilax*, *Phylloxeroxenus smilax*.

specimens of *D. smilacis* yielded the type specimens for *P. smilacis*; there is no date on the '86x' specimens and it is not clear how the collection date in Ashmead (1896b) was obtained. The four specimens labeled '1010' cannot be located and are presumed either lost or in another, unreported museum.

Adding to this confusing picture is that it appears A. Ritchie intended to include *P. smilacis* in his dissertation work on *Periclistus* in 1984, and even went so far as to designate a lectotype for this species (Fig. 61). The series of specimens seen in Fig. 60 is the source of the specimen that Ritchie intended as the lectotype, making the total number of specimens for *P. smilacis*, in the USNM, 11 specimens. When we consider *D. smilacis* is represented by two specimens (holotype in USNM, cotype in AMNH), we have

a grand total of 13 specimens. Our conclusion from all of this is that the original 13 specimens mentioned in Ashmead (1896a) for *D. smilacis* turned out to be a mixture of gall inducer and inquilline, and further, the host plant for this gall was mis-identified in the field as *Smilax rotundifolia* L. The US Forest Service Fire Effects Information System indicates *S. rotundifolia* and *Rubus* spp. co-occur in old fields throughout the range of *Smilax* and it is possible that the galls of *Diastrophus smilacis* are actually collected from a *Rubus*, and the two host plants were confused when the original collection was made.

The original collections made in Florida in 2010 that led to the chalcidoids described herein were also focused on the (now) erroneous records of *D. smilacis* on *Smilax havanensis* mentioned in Beutenmüller (1909) collected around Miami by Dr. E. Bessey. When looking closely at the *D. smilacis* gall figured in Beutenmüller (1909), it is clear that gall matches exactly what was collected in this project and illustrated in Fig. 1. No gall material from *S. havanensis* is in the cynipid gall collection, and indeed, there are no galls in this collection that look like the one figured in Beutenmüller (1909). As no cynipids apparently emerged from the Miami gall reported and figured in Beutenmüller (1909), we consider this an erroneous host record as well.

Lastly, the USNM has a specimen labeled as lectotype for *Periclistus smilacis*, yet this taxon lacks a published lectotype designation. We presume the team of Ritchie and Shorthouse, whose names appear on the purported lectotype labels, planned to publish these designations (as mentioned above), but were not able to. In order to stabilize the name of *Periclistus smilacis*, we hereby designate USNMENT00802336, type number 3287, as lectotype of this taxon, deposited in the USNM (Figs 59, 61, 62).

Conclusion

Here we describe the new eulophid species *Aprostocetus smilax*, the second recorded case of gall induction by *Aprostocetus* in North America. This new species is the true gall inducer on *Smilax*, and previous records of cynipid species *Diastrophus smilacis* and the inquilline *Periclistus smilacis* associated with this host plant are erroneous. Additionally, we described two eurytomid parasitoids associated with this *Smilax* gall. The distribution of all three new species is on the southern tip of mainland USA, but it is likely that they are also found in the Caribbean region in which the host plant *S. havanensis* is found (Ferrufino-Acosta 2014). A comprehensive taxonomic revision of these incredibly diverse but understudied minute wasps will undoubtedly reveal additional ecological associations and new species.

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staff from Miami-Dade County (C. Rodriguez and T. Smith) provided specimens and access to Rockdale Pineland for gall collecting. We would also like to thank Gary Oullette for performing all DNA extractions and amplifications, Taina Litwak for the illustration and image editing, and Zhiwei Liu for discussion on the validity of *Diastrophus smilacis*. Finally, we would like to thank Paul Hanson and an anonymous reviewer that have provided comments that improved the manuscript. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer.

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Taxonomic revision of *charon*-, *floridanum*- and *muscaeforme*-groups of *Gryon* Haliday, 1833 (Hymenoptera, Scelionidae) from Japan, with descriptions of two new species and host information

Yoto Komeda^{1*}, Toshiharu Mita², Yoshimi Hirose³, Kenzo Yamagishi⁴

1 Entomological Laboratory, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Motoooka 744, Fukuoka, 819–0395, Japan **2** Entomological Laboratory, Faculty of Agriculture, Kyushu University, Motoooka 744, Fukuoka, 819–0395, Japan **3** Kyushu University, Motoooka 744, Fukuoka, 819–0395, Japan **4** Entomological Laboratory, Faculty of Agriculture, Meijo University, Shiogamaguchi 1-501, Nagoya, 468–8502, Japan

Corresponding author: Yoto Komeda (kome123k123@gmail.com)

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Abstract

Japanese species of the *muscaeforme*-group, *charon*-group, and *floridanum*-group of *Gryon* have been revised. Among the species of the *muscaeforme*-group, *G. fulvicoxa* **sp. nov.** is newly described. *Gryon misha* Kozlov & Kononova, **syn. nov.** is considered a junior synonym of *G. japonicum* (Ashmead). *Gryon maruzsae* Mineo, **syn. nov.** and *G. sugonjaevi* Kozlov & Kononova, **syn. nov.** are considered junior synonyms of *G. yamagishii* Mineo. Among the species of the *charon*-group, *G. shisa* **sp. nov.** is newly described. *Gryon hakonense* (Ashmead) **syn. nov.** is considered a junior synonym of *G. philippinense* (Ashmead). Among the species of the *floridanum*-group, *G. pennsylvanicum* (Ashmead) is recognized. Host records of the three species groups are also revised.

Keywords

Alydidae, Coreidae, egg parasitoids, natural enemy

* Present affiliation: Teiso Kasei co. Ltd., Nishijima 164-1, Shizuoka, 422-8045 Japan.

Introduction

Gryon Haliday, 1833 is one of the largest genera in Scelioninae with 332 species known in the world (Johnson 2019). Almost all of them are egg parasitoids of Heteroptera, mainly Pentatomidae, Reduviidae, and Coreidae (Masner 1983). In Japan, 23 species are known (Ashmead 1904; Mineo 1979; 1980a, b, 1981, 1990a, b, 1991; Kozlov and Kononova 1989; Yasuda 1990; Kononova and Fursov 2005; Kononova and Kozlov 2008) and four of them are recorded as natural enemies of pests of rice, soybeans, vegetables and fruits (Appendix 1). Some members of Scelionidae, including *Gryon*, are important natural enemies of agricultural pests. The life history of *G. japonicum* was partially provided by Noda (1993), however, life cycles of other species are almost unknown. Owing to the potential of *Gryon* as biocontrol agents, some biological and ecological studies have also been conducted (Noda 1993; Dasilao and Arakawa 2004, 2005; Nakajima and Fujisaki 2010; Nakajima et al. 2012).

Gryon is divided into 22 species groups based on morphological characters such as the sculpture of the frons and occiput, and the ratios of fore wing vein lengths (Masner 1975, 1979, 1983; Mineo 1980b, 1981, 1983a, b, 1990b, 1991; Mineo and Caleca 1987b). Among Palearctic and Oriental regions, six species groups (*charon*, *insulare*, *misellum*, *muscaeforme*, *myrmecophilum* and *pubescens*) are known from both regions, two groups (*hungricum* and *subfasciatum*) are known only from the Palearctic region and two groups (*floridanum* and *leptocorisae*) are known only from the Oriental region (Masner 1975, 1983; Mineo 1980b, 1981, 1983a, 1991). Among Japanese species, seven species groups have been recognized (Appendix 1).

In the present study, we taxonomically revised three species groups (*muscaeforme*-group, *charon*-group, and *floridanum*-group) of Japanese *Gryon*, which include important natural enemies of pests. We examined not only field-collected specimens but also voucher specimens of previous ecological, biological and applied studies in order to confirm identifications.

Methods

Specimens examined in the present study are deposited in collections which are abbreviated as follows: Entomological Laboratory, Kyushu University, Fukuoka, Japan (**ELKU**); Entomological Laboratory, Meijo University, Nagoya, Japan (**ELMU**); Ehime University Museum, Matsuyama, Japan (**EUMJ**); Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization, Tsukuba, Japan (**NIAES**); Hokkaido University Museum, Sapporo, Japan (**SEHU**); I.I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kiev, Ukraine (**IZAN**); Zoological Institute, Russian Academy of Sciences, Saint Petersburg, Russia (**ZIN**). We also visited Institute of Ecology and Biological Resources, Hanoi, Vietnam (**IEBR**) to examine type specimens of Vietnamese species. The following abbreviations are used for the collecting methods: Malaise trap (MT) and yellow pan trap (YPT).

Photographs were taken using a Canon MP-E65 mm micro lens or an Olympus SZX10 stereomicroscope mounted on a Canon EOS 60D, combined by CombineZM, and processed in GIMP 2.8.14. SEM images were taken using a Hitachi S-3000N.

Morphological terminology and measurements follow Mineo and Villa (1982), Masner (1980), and Mikó et al. (2007, 2010). Description of surface sculpturing follows Eady (1968) and Harris (1979). Abbreviations used for measurements are as follows: head height (HH); head width (HW); head length (HL); length of interorbital space (IOS); width of antennal depression (WAD); ocular-antennal depression distance (OAD); length of posterior ocellar line (POL); length of ocular-ocellar line (OOL); lateral ocellar line (LOL); maximum length of mesoscutum (ML); length of transscutal line (TSL); maximum length of mesoscutellum (SL); maximum width of mesoscutellum (SW). The ratio FCI (frontal cephalic index) is the ratio of HW/HH, the ratio LCI (lateral cephalic index) is the ratio of HH/HL, and the ratio DCI (dorsal cephalic index) is the ratio of HW/HL.

Taxonomy

Gryon Haliday, 1833

Gryon muscaeforme-group Mineo, 1981

Diagnosis. Frons reticulate with setae; frontal depression weakly developed without enclosing strong carina. Clypeus almost rounded. Eyes with sparse setae or without. Occipital carina complete, angular point of occipital carina developed; postoccipital carina discontinuity present; postgena weakly costate along postoccipital carina; postgenal pit located near fossa.

Gryon fulvicoxa Komeda & Mita, sp. nov.

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Figs 1A, 2A, 3A, 4A, 5A, G, 6A

Diagnosis. Frontal depression transversely costate by strong irregular carinae. Horizontal portion of occipital carina straight, short, reaching longitudinal extension line of outer margin of lateral ocelli. Legs entirely yellow.

Description. Female. Length = 1.1–1.3 mm.

Color (Figs 1A, 2A). **Body** mainly dark brown-black. A2–6, forecoxa brown. A1, mandibles, and legs (including mid and hind coxae) yellow.

Head. FCI = 1.20–1.33; LCI = 1.48–1.64; DCI = 1.86–2.05; HW/IOS = 1.76–1.83; head about 1.3 times as wide as mesosoma. (HW/TSL = 1.25–1.34). Frons (Fig. 3A) reticulate with setae; central carina present ventrally; frontal depression weakly developed, transversely costate by strong irregular carinae. Vertex reticulate

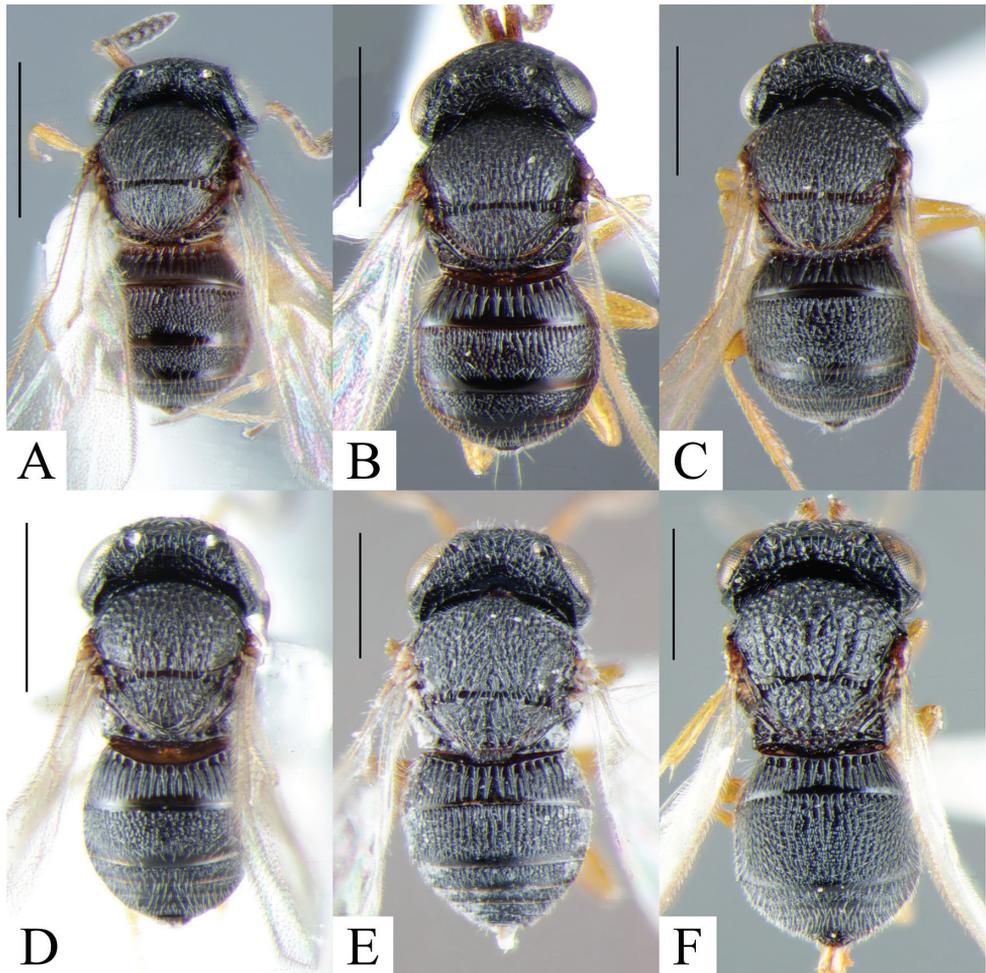


Figure 1. Japanese *Gryon* spp., dorsal view **A** *G. fulvicoxa* sp. nov., holotype **B** *G. japonicum* **C** *G. yamagishii* **D** *G. philippinense* **E** *G. shisa* sp. nov., holotype **F** *G. pennsylvanicum*. Scale bars: 1 mm.

with setae; intercellular space reticulate; hyperoccipital carina absent; POL 3.1 times as long as OOL (POL/OOL = 2.84–3.32); OOL half times as long as LOL (OOL/LOL = 0.48–0.63). Clypeus rectangular, with rounded corners. Gena coriaceous with setae; medial genal carina absent. Occiput (Fig. 4A) transversely costate with setae; occipital carina complete; angular point of occipital carina developed; horizontal portion of occipital carina straight, short, reaching longitudinal extension line of outer margin of lateral ocelli; postoccipital carina discontinuity present; postgena weakly costate along postoccipital carina; postgenal sulcus curved toward hypostoma; postgenal bridge smooth, weakly longitudinally costate beside median sulcus. Antennae (Fig. 5A) clavate; A1 about 3.2 times longer than radicle, as long as clava; clava with five segments; claval sensilla formula A8–12/2–2–2–1; claval length about 3.7 times longer than width. Mandible thin, tridentate, anterior tooth longer than other teeth.

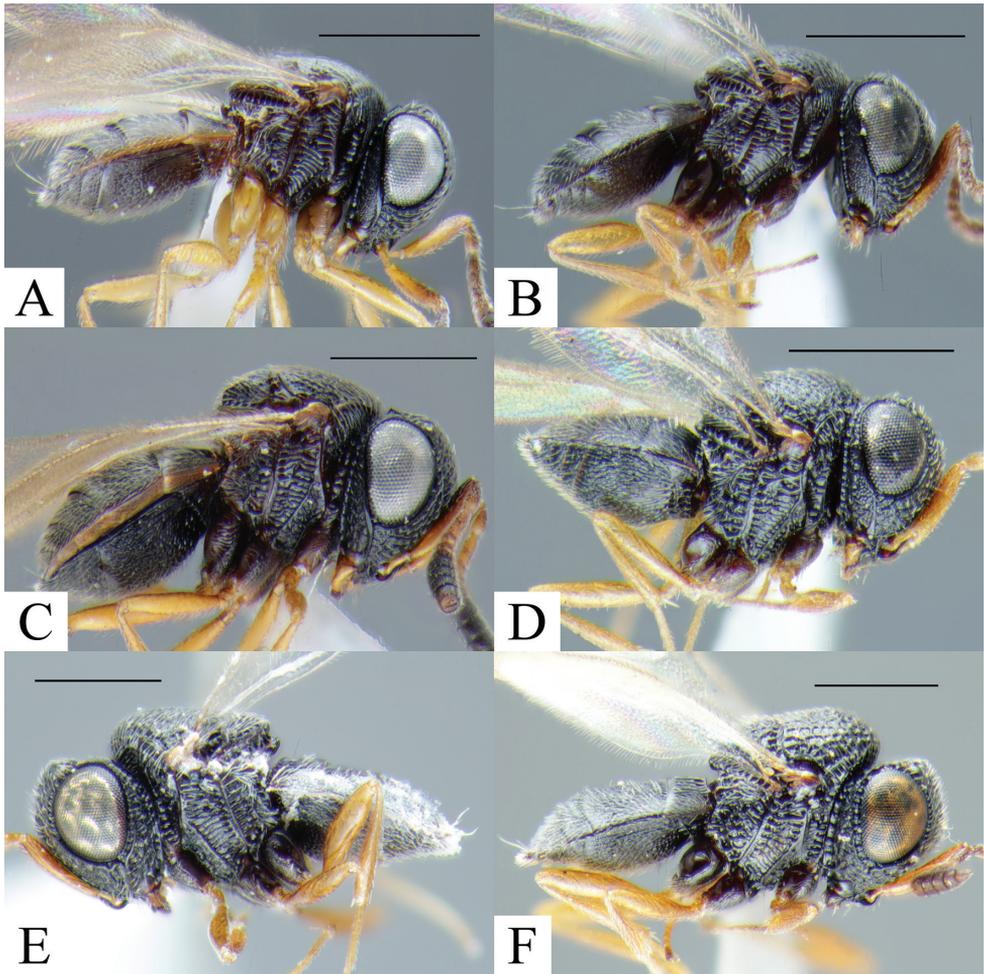


Figure 2. Japanese *Gryon* spp., lateral views **A** *G. fulvicoxa* sp. nov., holotype **B** *G. japonicum* **C** *G. yamagishii* **D** *G. philippinense* **E** *G. shisa* sp. nov., paratype **F** *G. pennsylvanicum*. Scale bars: 1 mm.

Mesosoma. Cervical pronotal area granulate with dense setae; epomial carina strongly present, not reaching dorsal edge; pronotal suprahumeral sulcus foveolate, unclear mesad; lateral pronotal area smooth with transverse sparse carinae. Propleuron smooth with imbricate sculpture. Mesoscutum about 1.4 times as wide as long (TSL/ML = 1.35–1.49), with dense setae, reticulate anteriorly, longitudinally costate posteriorly; parascutal carina absent; notaulus absent. Mesoscutellum about 2.3 times as wide as long (SW/SL = 2.10–2.43) with dense setae, longitudinally costate mesad, granulate laterad. Mesopleuron smooth with transverse dense carinae above mesopleural canina, smooth with sparse setae below mesopleural canina; prespecular and mesepisternal sulci foveolate; prespecular sulcus with setae; mesopleural carina strongly present; postacetabular sulcus foveolate. Metascutellum weakly produced, longitudinally striate. Dorsal metapleural area smooth with setae dorsad; ventral metapleural area weakly

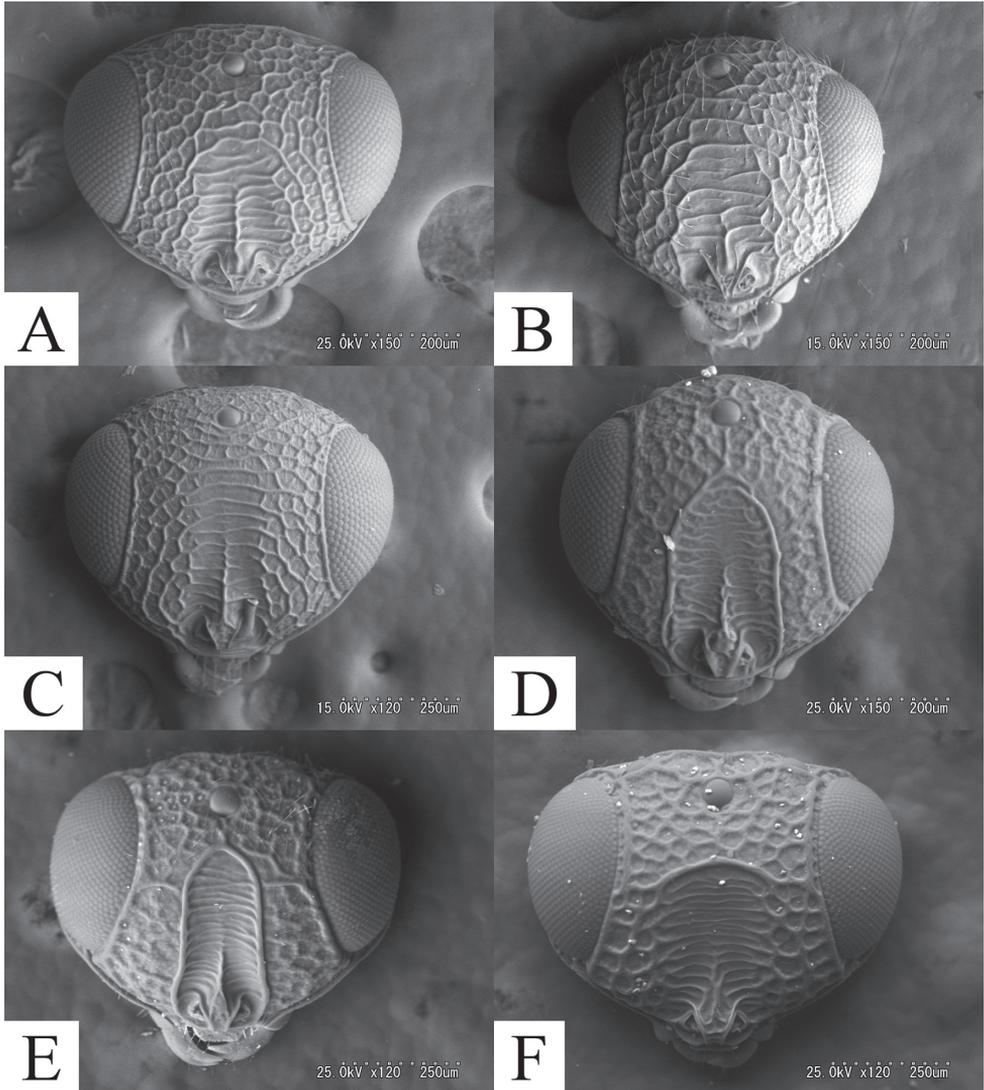


Figure 3. Frons of Japanese *Gryon* spp. **A** *G. fulvicoxa* sp. nov. **B** *G. japonicum* **C** *G. yamagishii* **D** *G. philippinense* **E** *G. shisa* sp. nov. **F** *G. pennsylvanicum*.

rugulose with setae; anterior part of metapleural sulcus and upper paracoxal sulcus with setae. Propodeum foveolate with setae. Fore wing (Fig. 7A), stigmal vein about 1.6 times longer than marginal vein; postmarginal vein about 3.5 times longer than marginal vein.

Metasoma. T1 longitudinally striate, setose laterally. S1 longitudinally striate. T2 longitudinally striate anteriorly, reticulate posteriorly, setose laterally. S2 with setae, granulate mesad, striate laterad. T3 reticulate, with setae laterad and posteriorly. S3–6 punctate with setae. T4 punctate-striate with setae. T5–6 punctate with setae.

Male. Almost same as female, but antennae (Fig. 5G) filiform; A1 yellow, A2–11 brown.

Host. Unknown.

Material examined. Holotype: Hokkaido, Sapporo city, Toyohira ward, Hitsujigaoka. 43.008°N, 141.415°E; alt. 100 m, 8–15.VI.2010, Kazuhiko Konishi leg. (MT) 1♀ [ELKU]. Paratypes. Same locality as holotype, 1–8.VI.2010, Kazuhiko Konishi leg. (MT) 1♀ [ELKU]; 8–15.VI.2010, Kazuhiko Konishi leg. (MT) 1♀ [ELKU]; 22–29.VI.2010, Kazuhiko Konishi leg. (MT) 1♀ [ELKU]; 6–13.VII.2010, Kazuhiko Konishi leg. (MT) 1♀ [ELKU]; 27.VII.–3.VIII.2010, Kazuhiko Konishi leg. (MT) 1♂ [ELKU]; 1♂1♀ [EUMJ]; Tokyo pref., Minami-Tama dist., Asakawa town, Mt. Takao-san, 19.V.1935, H. Ise leg. 1♀ [NIAES]; Nagano pref., Ueda city, Sugadaira-Kôgen, Tsukuba University, 26. VII–1.VIII.2015. So Shimizu leg. (MT) 1♀ [ELKU]; Gifu pref., Kani city, Katabira, 10–16.IV.2004, Kenzo Yamagishi leg. (MT) 1♀ [ELMU]; 26.VI.–2.VII.2004. Kenzo Yamagishi leg., 1♀ [ELMU]; 24–30.VII.2004. Kenzo Yamagishi leg., 2♀ [ELMU]; Tottori pref., Saihaku dist., Daisen town, Mt. Daisen, 25.VIII.1970. 1♂1♀ [ELKU]; Fukuoka pref., Fukuoka city, Sawara ward, Mt. Sefuri-san, 1.VIII.1992, Yoshimitsu Higashiura leg. 1♀ [ELKU]; Tagawa dist., Soeda town, Mt. Hiko-san. 12.IX.1968, (MT) 1♀ [ELKU]; 11.VII.1969. Kenkichi Kanmiya leg. 3♂3♀ [ELKU]; 12–19.V.2008, Toshiharu Mita and Sinsuke Sato leg. (MT) 1♀ [ELKU]; Kumamoto pref., Yatsushiro dist., Izumi vill., Shiratori rindô, 9.VIII.1992. 1♂ [ELKU].

Distribution. Japan (Hokkaido; Honshu: Tokyo, Nagano, Gifu, and Tottori; Kyushu: Fukuoka and Kumamoto)

Etymology. The species name refers to the yellow coxae.

Remarks. Among Japanese species, *G. fulvicoxa* sp. nov. is very similar to *G. japonicum* (Ashmead, 1904) in the sculpture of the head but differs from it in the shape of the horizontal portion of the occipital carina (*G. fulvicoxa* (Fig. 4A): straight, short; *G. japonicum* (Fig. 4B): curved, relatively long), sculpture of mesopleuron (carinae of *G. fulvicoxa* (Fig. 2A) stronger than *G. japonicum* (Fig. 2B)) and the color of the coxae (*G. fulvicoxa* (Fig. 2A): yellow; *G. japonicum* (Fig. 2B): brown). The sculpture of the frons in *G. fulvicoxa* (Fig. 4A) is finer than that of *G. japonicum* (Fig. 4B). Russian Far Eastern species, *G. amissum* Kozlov & Kononova, 1990, is also similar to *G. fulvicoxa* in the shape of the horizontal portion of the occipital carina. However, in *G. amissum*, the sculpture of frons is regularly arranged like *G. yamagishii*, the sculpture of mesopleuron is transversely costate with granulate sculpture between lower costae, mesopleural carina is weak, and the color of coxa is dark brown to black. Talamas and Pham (2017) provided images of type specimens of Vietnamese Scelionidae deposited in Institute of Ecology and Biological Resources, Hanoi, Vietnam, and we also examined these type specimens. According to these images and our examination, *G. alames* Kozlov & Lê, 1996, *G. avanus* Kozlov & Lê, 1996, and *G. cromion* Kozlov & Lê, 1997 have a horizontal portion of the occipital carina similar to *G. fulvicoxa*. The color of coxa of these species are dark brown to black, however, that of *G. fulvicoxa* is yellow.

***Gryon japonicum* (Ashmead, 1904)**

Figs 1B, 2B, 3B, 4B, 5B, H, 6B, 7A, B, 8A, B, 9A, B

Hadronotus japonicus Ashmead, 1904b: Kieffer 1926. *Gryon japonicus* (Ashmead): Masner and Muesebeck 1968; Mineo 1979. *Gryon japonicum* (Ashmead): Mineo 1981; Noda and Hirose 1989; Noda 1989, 1990a, b; Johnson 1992; Noda 1993; Kikuchi et al. 1995; Teraoka and Numata 1997; Lê 2000; Kononova and Kozlov 2008.

Hadronotus hakonensis Ashmead, 1904b. *Gryon hakonensis* (Ashmead): Masner and Muesebeck 1968. *Gryon hakonensis* (Ashmead): Mineo 1981; Kikuchi and Kuranouchi 1985 (misidentification); Kikuchi et al. 1986 (misidentification).

Telenomus orestes Dodd, 1913. *Liophanurus orestes* (Dodd): Kieffer 1926. *Gryon orestes* (Dodd): Johnson 1988; Mineo 1990a (misidentification); Johnson 1992; Kononova and Kozlov 2008 (misidentification).

Hadronotus flavipes Ashmead, 1905: Kieffer 1926; Baltazar 1966. *Gryon ferus* Masner & Muesebeck, 1968. *Gryon flavipes* (Ashmead): Mineo 1979, 1981, 1990a (syn.); Johnson 1992.

Telenomoides flavipes Dodd, 1913. *Hadronotus rufipes* (Dodd): Dodd 1914. *Plastogryon rufipes* (Dodd): Dodd 1915. *Hadronotus rufipes* (Dodd): Kieffer 1926. *Gryon rufipes* (Dodd): Galloway 1976; Mineo 1990a (syn.); Johnson 1992.

Telenomoides giraulti Dodd, 1913. *Hadronotus giraulti* (Dodd): Dodd 1914; Dodd 1915; Kieffer 1926; Galloway 1976; Mineo 1990a (syn.); Johnson 1992.

Telenomoides bicolor Dodd, 1913. *Hadronotus affinis* Dodd, 1914: Dodd 1915. *Hadronotus doddi* Kieffer, 1926. *Hadronotus affinis* Dodd: Galloway 1976; Mineo 1990a (syn.); Johnson 1992.

Plastogryon fuscus Dodd, 1915. *Gryon fuscus* (Dodd): Galloway 1976; Mineo 1990a (syn.); Johnson 1992.

Hadronotus leptocorisae Nixon, 1934. *Gryon nixonii* Masner, 1965: Mineo 1979 (syn.); Mineo 1981; Mineo 1990a; Johnson 1992.

Gryon mischa Kozlov & Kononova, 1989: Kozlov and Kononova 1990; Johnson 1992; Kononova 1995; Kononova and Petrov 2002; Kononova and Kozlov 2008. syn. nov.

Diagnosis. Frontal depression with strongly transversely irregular costate sculpture. Horizontal portion of occipital carina curved, reaching longitudinal extension line of inner margin of lateral ocelli. Coxae brown-black.

Description. Female. Length 1.1–1.7 mm.

Color. (Figs 1B, 2B). **Body** mainly black. A2–6 brown. A1, mandibles, and legs (excluding coxae) yellow.

Head. FCI = 1.06–1.27; LCI = 1.43–1.61; DCI = 1.76–1.94; HW/IOS = 1.67–1.87; head about 1.3 times as wide as mesosoma (HW/TSL = 1.16–1.34). Frons (Fig. 3B) reticulate with setae; central carina present ventrally; frontal depression weakly developed, with strongly transverse irregularly costate sculpture. Vertex reticulate with setae; interocellar space reticulate; hyperoccipital carina absent; POL about

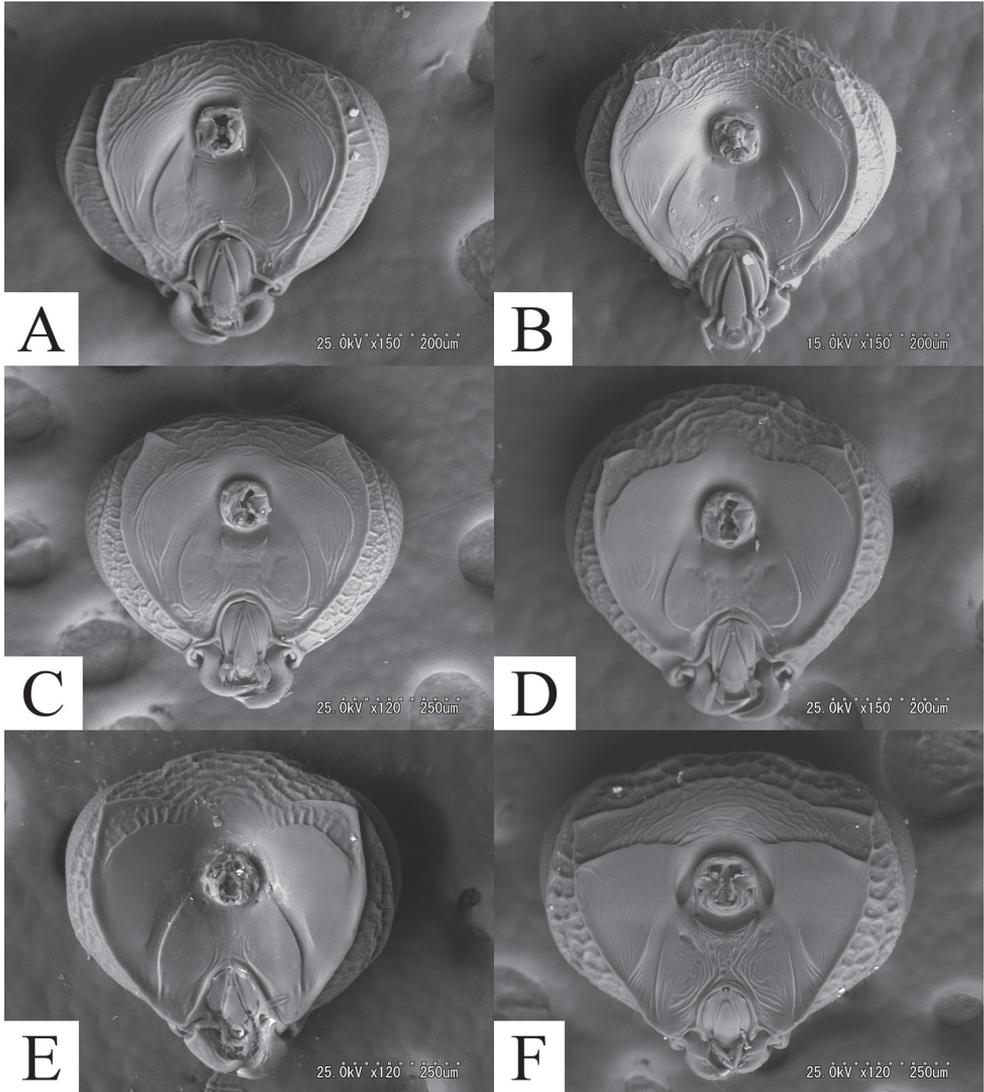


Figure 4. Occiput of Japanese *Gryon* spp. **A** *G. fulvicoxa* sp. nov. **B** *G. japonicum* **C** *G. yamagishii* **D** *G. philippinense* **E** *G. shisa* sp. nov. **F** *G. pennsylvanicum*.

4.5 times as long as OOL ($POL/OOL = 4.23-4.74$); OOL about 0.4 times as long as LOL ($OOL/LOL = 0.37-0.47$). Clypeus trapezoidal, with rounded corners. Gena coriaceous with setae; medial genal carina absent. Occiput (Fig. 4B) transversely costate with setae; occipital carina complete; angular point of occipital carina developed; horizontal portion of occipital carina curved, reaching longitudinal extension line of inner margin of lateral ocelli; postoccipital carina discontinuity present; postgena weakly costate along postoccipital carina; postgenal sulcus curved toward hypostoma; postgenal bridge smooth, weakly longitudinally costate beside median sulcus. Antennae

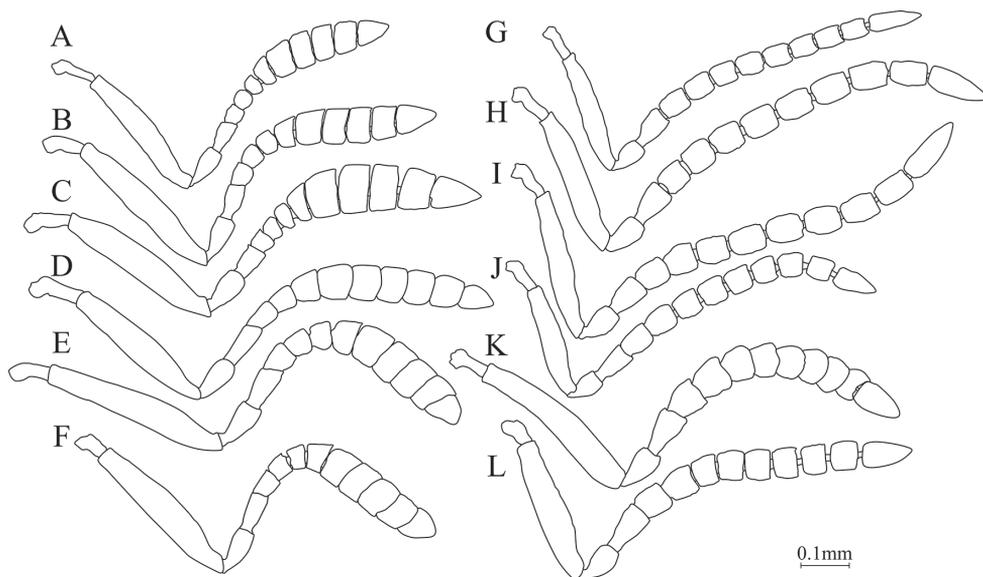


Figure 5. Antennae of Japanese *Gryon* spp. **A** *G. fulvicoxa* sp. nov., female **B** *G. japonicum*, female **C** *G. yamagishii*, female **D** *G. philippinense*, female **E** *G. shisa* sp. nov., female **F** *G. pennsylvanicum*, female **G** *G. fulvicoxa* sp. nov., male **H** *G. japonicum*, male **I** *G. yamagishii*, male **J** *G. philippinense*, male **K** *G. shisa* sp. nov., male **L** *G. pennsylvanicum*, male.

(Fig. 5B) clavate; A1 about 4.3 times longer than radicle, as long as clava; clava with five segments; claval sensilla formula A8–12/2–2–2–1; claval length about 3.6 times longer than width. Mandibles thin, tridentate, anterior tooth longer than other teeth.

Mesosoma. Cervical pronotal area granulate with dense setae; epomial carina strongly present, not reaching dorsal edge; pronotal suprahumeral sulcus foveolate, unclear mesad; lateral pronotal area smooth with transverse sparse carinae. Propleuron weakly transversely costate. Mesoscutum (Fig. 7A) about 1.5 times as wide as long (TSL/ML = 1.39–1.67) with dense setae, reticulate anteriorly, longitudinally costate posteriorly; parascutal carina absent; notaulus absent. Mesoscutellum about 2.2 times as wide as long (SW/SL = 1.98–2.35), with dense setae, longitudinally costate mesad, granulate laterad. Mesopleuron (Fig. 7B) smooth with weak transverse dense carinae above mesopleural canina, smooth with sparse setae below mesopleural canina; prespecular and mesepisternal sulci foveolate; prespecular sulcus with setae; mesopleural carina strongly present; postacetabular sulcus foveolate. Metascutellum (Fig. 8B) weakly produced, longitudinally striate. Dorsal metapleural area (Fig. 7B) smooth with setae dorsad; ventral metapleural area weakly rugulose with setae; anterior part of metapleural sulcus and upper paracoxal sulcus with setae. Propodeum foveolate with setae. Fore wing (Fig. 6B): stigmal vein about 1.8 times longer than marginal vein; postmarginal vein about 3.3 times longer than marginal vein.

Metasoma. T1 (Fig. 9A) longitudinally striate, setose laterally. S1 (Fig. 9B) longitudinally striate. T2 longitudinally striate anteriorly, reticulate posteriorly, setose

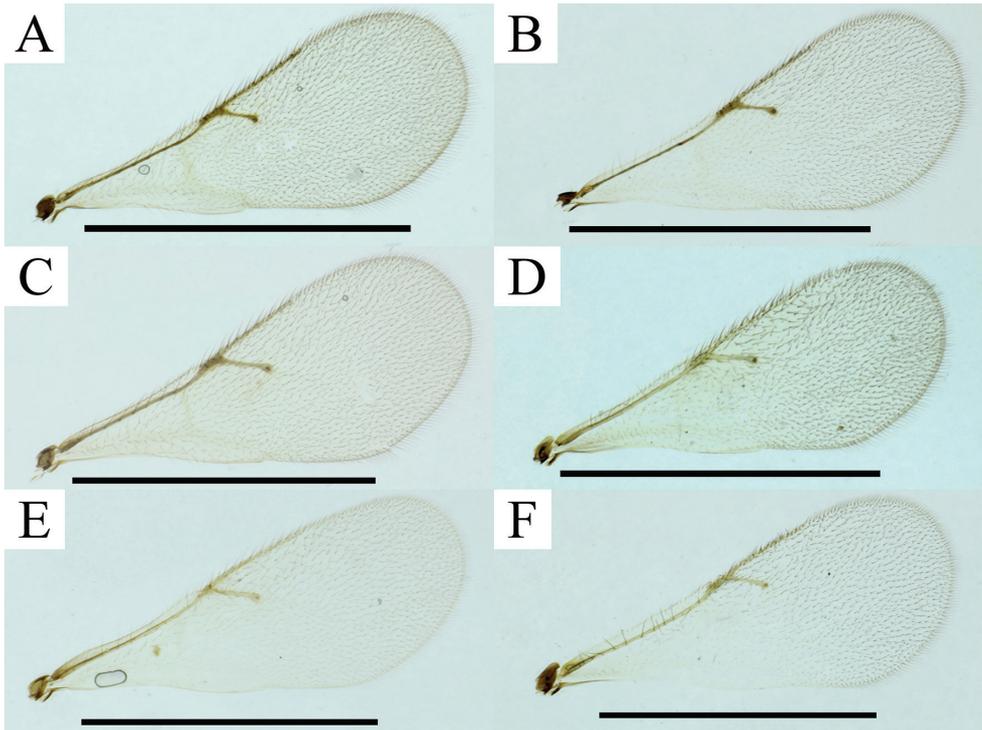


Figure 6. Forewing of Japanese *Gryon* spp. **A** *G. fulvicoxa* sp. nov. **B** *G. japonicum* **C** *G. yamagishii* **D** *G. philippinense* **E** *G. shisa* sp. nov. **F** *G. pennsylvanicum*.

laterally. S2 with setae, granulate mesad, striate laterad. T3 reticulate, with setae laterad and posteriorly. S3–6 punctate with setae. T4 punctate-striate with setae. T5–6 punctate with setae.

Male. Almost same as female, but antennae (Fig. 5H) filiform; A1 yellow, A2–11 brown.

Variation. This species has a correlation between the size of specimens and the convexity of the frons: in small specimens the frons is more convex than in large specimens. This correlation is also known in *G. pennsylvanicum* (Ashmead, 1893) (Masner 1983). Small (or minute) specimens of *G. japonicum* emerged from *Riptortus pedestris* (Linnaeus, 1758), *Leptocorisa chinensis* Dallas, 1852, *Cletus punctiger* (Dallas, 1852), and *Acanthocoris sordidus* (Thunberg, 1783). Hosts of large specimens are still unknown.

Host. Coreidae: *A. sordidus*, *Cletus trigonus* (Thunberg, 1783), and *C. punctiger* new record; Alydidae: *R. pedestris*, *Leptocorisa varicornis* (Fabricius, 1803), *L. acuta* (Thunberg, 1783), and *L. chinensis*. Noda (1990b) reported that *G. japonicum* also emerged from sentinel eggs of *C. schmidtii* (Kritshenko, 1916) (Coreidae).

Biology. In spring, the female is found on blossoms of *Acer palmatum* (Sapindaceae).

Material examined. Holotype. *Gryon mischa*: Япония, Каганихара. [= Japan: Gifu pref., Kakamigahara city] 19.X.1981. E. Sugonjaev leg. ♀ [ZIN].

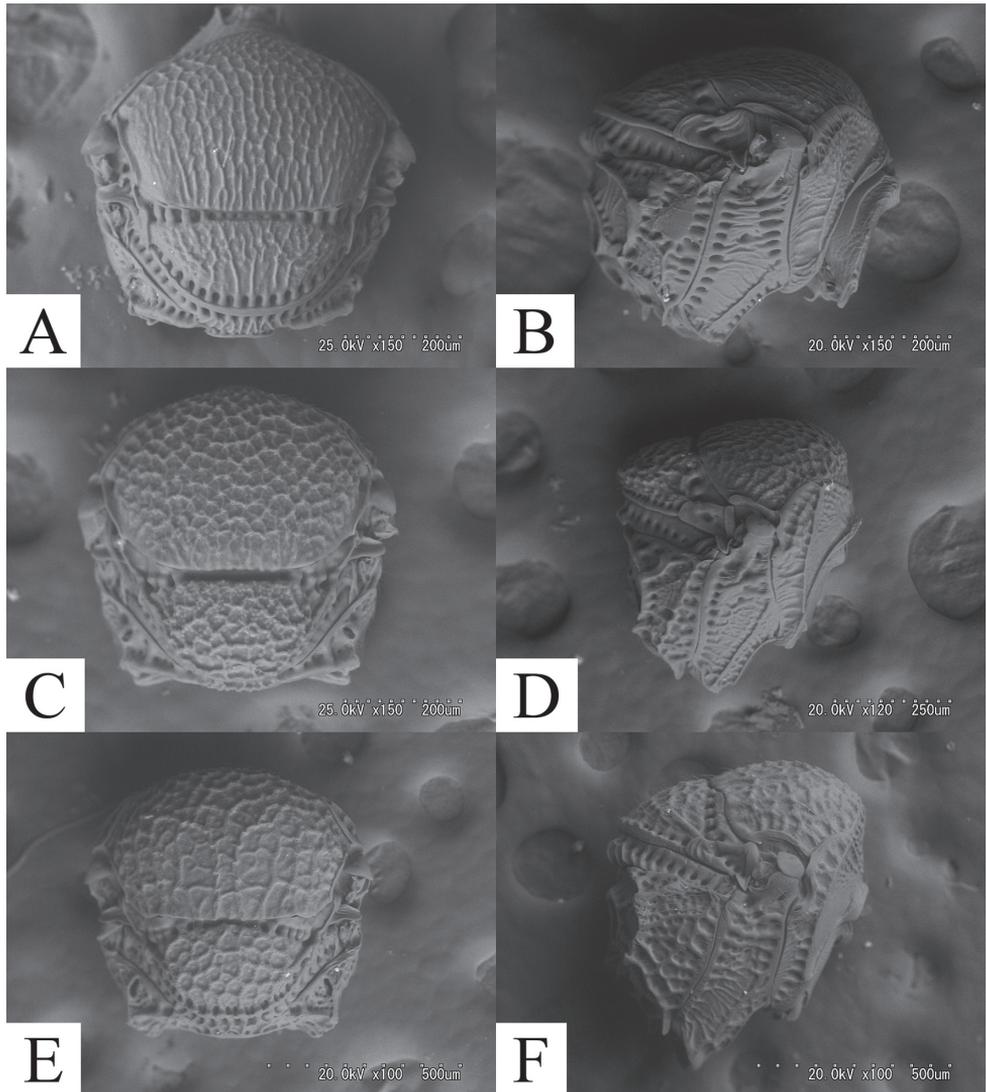


Figure 7. Mesosoma of Japanese *Gryon* spp. **A** *G. japonicum*, dorsal view **B** lateral view **C** *G. philippinense*, dorsal view **D** lateral view **E** *G. pennsylvanicum*, dorsal view **F** lateral view.

Other materials. Iwate pref., Morioka city, Yamagishi. 8.VIII.1985. Yoshimi Hirose leg. (emerged from eggs of *Riptortus pedestris* on soybean plant) 2♂2♀ [ELKU]; Ibaraki pref., Mito city. Kami-kunii-chô. 25.VII.1986. Ryutaro Komori leg. (emerged from an egg of *R. pedestris*) 1♀ [ELKU]; Hitachi-Ôta city, Chinone-chô. 2-.VII.1986. Ryutaro Komori leg. (emerged from eggs of *R. pedestris*) 3♂18♀ [ELKU]; Hitachi-Ôta city, Zuiryû-chô. 2-.VII.1986. Ryutaro Komori leg. (emerged from eggs of *R. pedestris*) 2♂7♀ [ELKU]; Tsuchiura city, Hitana. 2*.VII.1986. Ryutaro Komori leg. (emerged from eggs of *R. pedestris*) 3♂12♀ [ELKU]; Tsuchiura city (near Tsukuba),

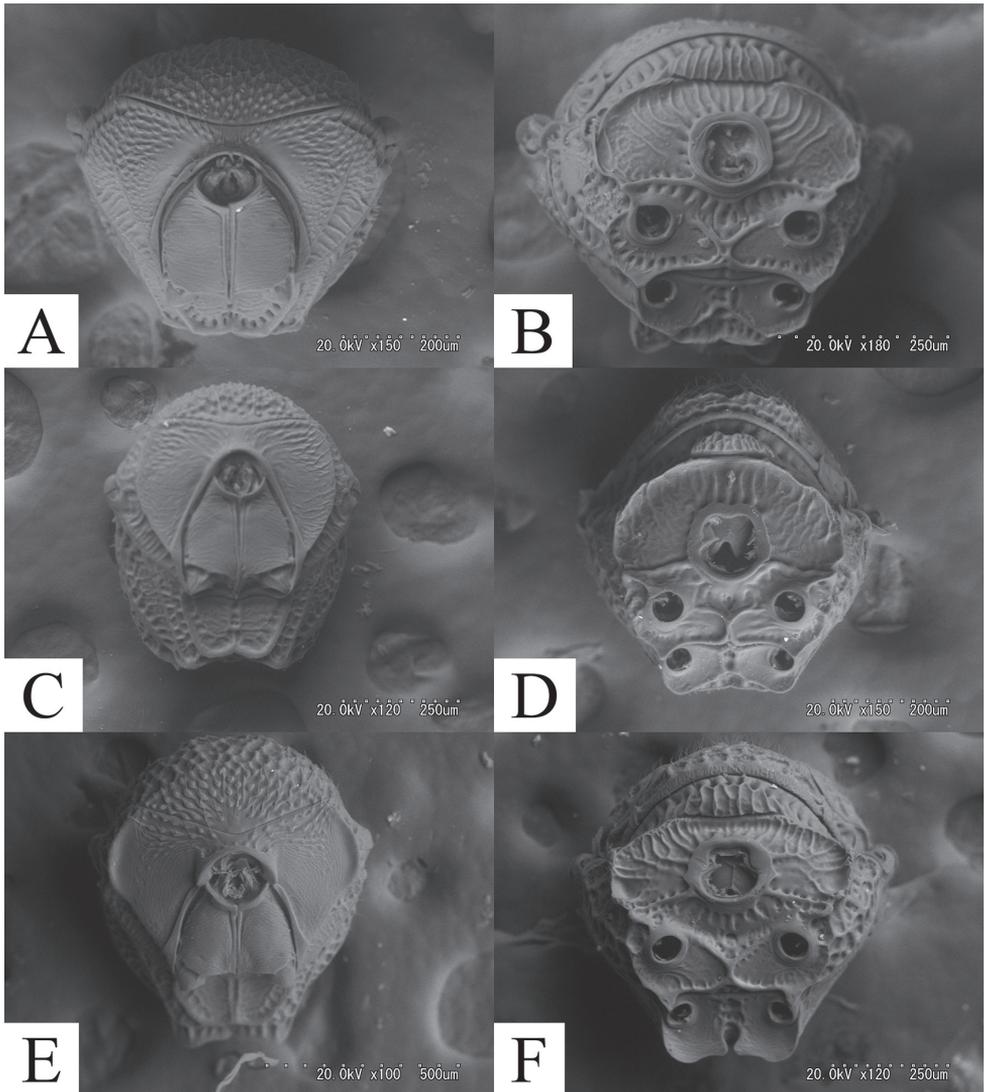


Figure 8. Mesosoma of Japanese *Gryon* spp. **A** *G. japonicum*, anterior view **B** posterior view **C** *G. philippinense*, anterior view **D** posterior view **E** *G. pennsylvanicum*, anterior view **F** posterior view.

Pond Shishituka-Ôike. 15.VII.1997. Victor Fursov leg. 2♀ [IZAN]; Tsukuba dist., Yatabe town, Kan'nondai, National Institute for Agro-Environmental Sciences. 22–24.VIII.1983. Takashi Noda leg. (emerged from eggs of *R. pedestris*) 1♂1♀ [ELKU]; 16–23.VIII.1985. Takashi Noda leg. (emerged from eggs of *R. pedestris*) 3♂8♀ [ELKU]; 12.VIII.1989. Takashi Noda leg. (emerged from eggs of *L. chinensis*) 4♂8♀ [ELKU]; Tsukuba dist., Yatabe town, Kan'nondai, National Agriculture Research Center. 27–28.VII.1984. Atsushi Kikuchi leg. (emerged from eggs of *R. pedestris*) 1♂5♀ [ELKU]; Tsukuba dist., Yawara vill., rice fields. 29.VII.1997. Victor Fursov

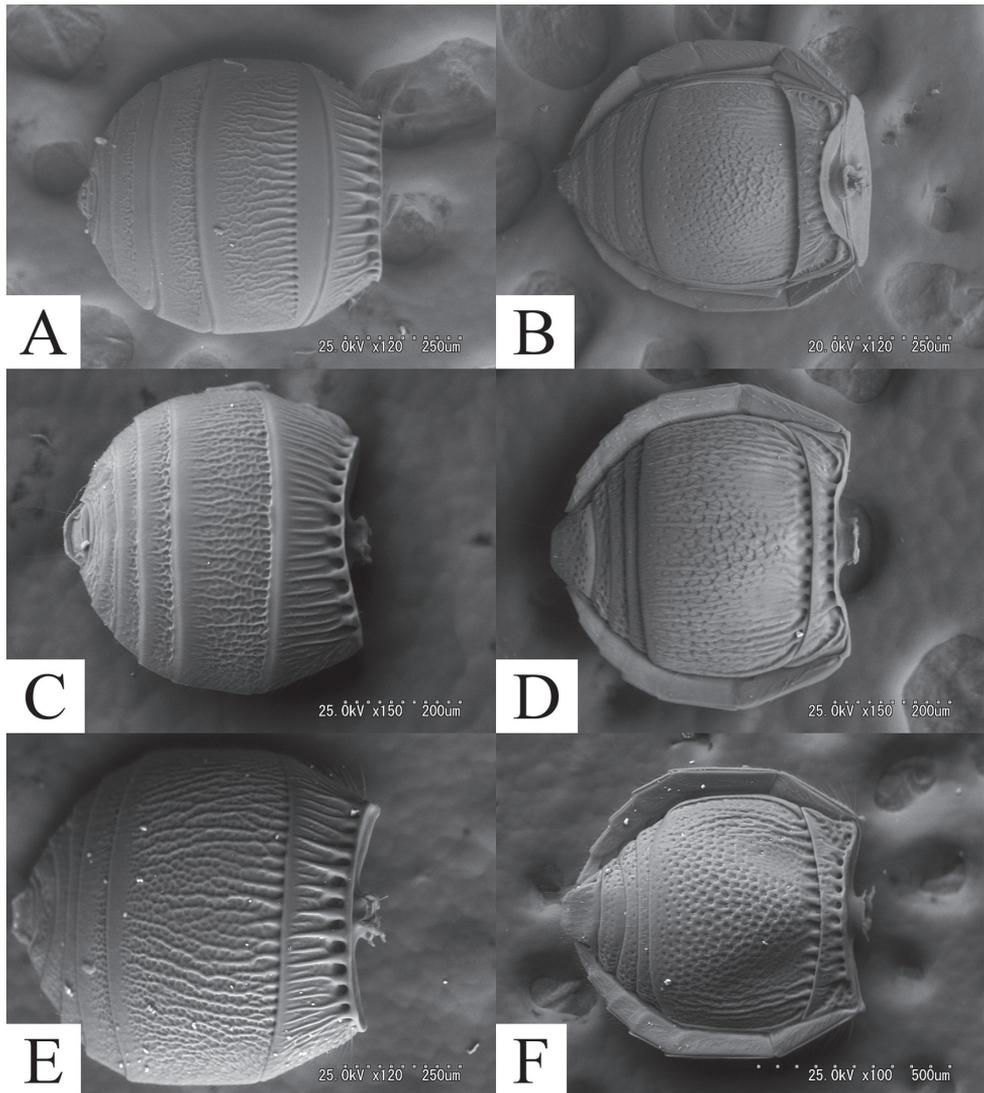


Figure 9. Metasoma of Japanese *Gryon* spp. **A** *G. japonicum*, dorsal view **B** ventral view **C** *G. philippinense*, dorsal view **D** ventral view **E** *G. pennsylvanicum*, dorsal view **F** ventral view.

leg. 2♀ [IZAN]; 22.VI.1999. Victor Fursov leg. 1♂2♀ [IZAN]; Inashiki dist. (near Tsukuba), Kukizaki town. 14.VI.1997. Victor Fursov leg. 1♂ [IZAN]; Saitama pref., Kitamoto city. 5–13.VI.1979. Kiyomitsu Ito leg. (emerged from eggs of *R. pedestris*) 6♂12♀ [ELKU]; IX.1980. Kiyomitsu Ito leg. (emerged from eggs of *Cletus punctiger*) 1♂8♀ [ELKU]; Chiba pref., Chiba city, Daizen'nochô, Chiba-Ken Agricultural Experiment Station (Soybean field) 3.X.1985. Masaaki Sawada leg. (emerged from eggs of *Acanthocoris sordidus*) 5♂5♀ [ELKU]; Ishikawa pref., Nomi city, Mitsukuchi (paddy field) 28.V–8.I.2011, H. Fukutomi and R. Ishiguro leg. (MT) 2♀ [ELMU];

19. VIII–7.IX.2011, H. Fukutomi and R. Ishiguro leg. (MT) 1♀ [ELMU]; 22.IX–5.X.2011, H. Fukutomi and R. Ishiguro leg. (MT) 2♂ [ELMU]; Fukui pref., Fukui city, Ryômachî, Fukui Agricultural Experiment Station. 6.IX.1983. Kazuo Imamura leg. (emerged from eggs of *R. pedestris*) 1♂3♀ [ELKU]; Mt. Tenjosau. 24.VII.1956. Yozo Murakami leg. (with identification label; *Gryon japonicus* (Ashm.) det. G. Mineo, 1978); Gifu pref., Kani city, Katabira, 3–9.IX.2004. Kenzo Yamagishi leg. (MT) 1♀ [ELMU]; Aichi pref., Nagoya city, Chikusa ward, Higashiyama Park. IV–VI. 1997, Victor Fursov leg. 1♂22♀ [IZAN]; Tempaku ward, 24.IX.2012. Kenzo Yamagishi leg. 1♂ [ELKU]; Ichinomiya city, Tomida, Kiso river. 26.IX.2013. Y. Miyata leg. (YPT) 1♂1♀ [ELMU]; Kasugai city, Takagi. 29.VIII.2000. C. Mizuno and M. Suzuki leg. (MT) 1♀ [IZAN]; 20.IX.2000. C. Mizuno and M. Suzuki leg. (MT) 1♂ [IZAN]; 21.IX.2000. C. Mizuno and M. Suzuki leg. (MT) 1♂ [IZAN]; 22.IX.2000. C. Mizuno and M. Suzuki leg. (MT) 1♀ [IZAN]; 16.VII.2013. Y. Kamiya leg. (YPT) 1♂2♀ [ELMU]; 31.VII.2013. Y. Kamiya leg. (YPT) 4♂ [ELMU]; 13.VIII.2013. Y. Kamiya leg. (YPT) 1♂3♀ [ELMU]; 17.IX.2013. Y. Kamiya leg. (YPT) 1♀ [ELMU]; Nisshin city, Komenogi, 28. V–3.VI.2011. H. Seo and R. Mizutani leg. (MT) 1♀ [ELMU]; 3–10.IX.2011. H. Seo and R. Mizutani leg. (MT) 1♂ [ELMU]; 17–25. IX.2011. H. Seo and R. Mizutani leg. (MT) 1♂ [ELMU]; 16–22.X.2011. H. Seo and R. Mizutani leg. (MT) 1♀ [ELMU]; Nisshin city, Nokata, 3–10.IX.2011. H. Seo and R. Mizutani leg. (MT) 1♀ [ELMU]; 2–9.X.2011. H. Seo and R. Mizutani leg. (MT) 1♀ [ELMU]; Aichi dist., Nagakute town, Yazako, Sagamine, Aichi Agricultural Research Center. 15–21.VIII.2008. Kenzo Yamagishi and K. Fukushima leg. (MT) 3♀ [ELMU]; 29.VIII–4.IX.2008. Kenzo Yamagishi and K. Fukushima leg. (MT) 3♀ [ELMU]; 12–18.IX.2008. Kenzo Yamagishi and K. Fukushima leg. (MT) 1♂6♀ [ELMU]; 26. IX–2.X.2008. Kenzo Yamagishi and K. Fukushima leg. (MT) 2♂2♀ [ELMU]; 9–16.X.2008. Kenzo Yamagishi and K. Fukushima leg. (MT) 1♂ [ELMU]; 23–29.X.2008. Kenzo Yamagishi and K. Fukushima leg. (MT) 2♂ [ELMU]; Nagakute town, near water channel. 7.VII.2004. Victor Fursov leg. 1♀ [IZAN]; Shiga pref. 1.VIII.1974. Y. Hasegawa leg. (emerged from an egg of *Leptocorisa chinensis*) 1♂ [NIAES]; 2.VIII.1974. Y. Hasegawa leg. (emerged from an egg of *C. punctiger*) 1♀ [NIAES]; 5.VIII.1974. Y. Hasegawa leg. (emerged from eggs of *L. chinensis*) 1♂8♀ [NIAES]; 10.VIII.1974. Y. Hasegawa leg. (emerged from eggs of *L. chinensis*) 6♀ [NIAES]; Ôtsu city, Dô. 10.VI.2018. Hiroya Higuchi leg. (emerged from sentinel eggs of *C. punctiger*) 1♂1♀ [ELKU]; Kyoto pref., Kyoto city, Kurama. 13.VII.1992. Takeshi Teraoka leg. (emerged from eggs of *R. pedestris*) 2♂2♀ [ELKU]; Tokushima pref., Zen'nyuji-toh (River island in Yoshino River), 13–22.V.2003. K. Ohara and H. Otsuka leg. (MT) 3♀ [ELMU]; 6–18.VI.2003. K. Ohara and H. Otsuka leg. (MT) 4♀ [ELMU]; 5–17.IX.2003. K. Ohara and H. Otsuka leg. (MT) 3♂2♀ [ELMU]; Ehime pref., Matsuyama city, Tarumi, Ehime University, 8.II.2016. Yu Hisasue leg. (sweeping of trees) 1♀ [ELKU]; Kôchi pref., Shimanto city, Tosa-Nakamura, Nyûta, Hiramoto. 33.004°N, 132.898°E; alt. 10 m. 6–9.IX.2017. Y. Komeda leg. (YPT) 1♀ [ELKU]; Fukuoka pref., Fukuoka city, Hakozaki. 26.V.1969. Osamu Yata leg. 1♀ [ELKU]; Fukuoka city, Hakozaki, Tsuya-Honmachi. (paddy field) 15.VIII.1973. Chiyoko

Okuma leg. 1♂ [ELKU]; Fukuoka city, East ward, Hakozaki, Kyushu Univ. Matsubara Farm. 16.X.1993. Hiroshi Honda leg. (YPT) 1♂ [ELKU]; 23.X.1993. Hiroshi Honda leg. (YPT) 3♂ [ELKU]; 30.X.1993. Hiroshi Honda leg. (YPT) 3♂ [ELKU]; Fukuoka city, Mt. Tachibana-yama, 6.V.1968. N. Yoshida leg. 1♀ [ELKU] (with identification label; *Gryon japonicus* det. G. Mineo); 25.VI.1994. Hiroshi Honda leg. (YPT) 1♂ [ELKU]; Fukuoka city, East ward, Tatara. 2.IX.1996. Yoshimitsu Higashimura leg. 1♀ [ELKU]; Fukuoka city, Ropponmatsu, Gokoku-Jinja shrine. 18.VI.1969. Minoru Miyazaki leg. 1♀ [ELKU]; Kasuya dist., Sasaguri town, Mt. Wakasugi-yama. 13.V.1969. Minoru Miyazaki leg. 1♀ [ELKU]; Tagawa dist., Soeda town, Mt. Hikosan. 26.VII.1966, Kôichi Takeno leg. 2♀ [ELKU]; 17.VII.1967, Kôichi Takeno leg. 1♀ [ELKU]; 18.VII.1968. Kenkichi Kanmiya leg. 2♀ [ELKU]; 11.VII.1969. Kenkichi Kanmiya leg. 2♂1♀ [ELKU]; 6.VIII.1969. Kenkichi Kanmiya leg. 1♀ [ELKU]; 16.X.1969, Kôichi Takeno leg. (MT) 1♀ [ELKU]; 25.VII.1970, Kôichi Takeno leg. (MT) 1♂ [ELKU]; 20.V.1971, Michitaka Chujo leg. 1♀ [ELKU]; 22–26.V.1979, Katoru Maeto leg. 1♀ [NIAES]; Nagasaki pref., Minami-Takaki dist., Kuchinotsu town. 23.IV.1954. S. Kato leg. 1♀ [NIAES]; Kumamoto pref., Aso dist., Takamori town. 11.X.1993. N. Wasano leg. 1♂ [ELKU]; Hondo city, Nishinoku Lake, 5.VI.1999. Victor Fursov leg. 3♀ [IZAN]; Miyazaki pref., Koyu dist., Takanabe town, Minami-Takanabe, Horinouchi. 16.VII.1964. K. Yasumatsu and T. Nishida leg. 1♀ [ELKU] (with identification label; *Gryon flavipes* (Ashm.) det. G. Mineo); Kagoshima pref., Kagoshima city, 18.VII.1969, Osamu Yata leg. 1♂1♀ [ELKU]; 20.VII.1969, Minoru Miyazaki leg. 2♀ [ELKU]; Kumage Dist., Yaku-shima Isl., Yaku town, Kurio, 8.VII.1975. Kenzo Yamagishi leg. 1♂2♀ [ELKU]; Ôshima dist., Toku-no-shima Isl., Amagi town, Nishi-Agina, Mikyo. 27.VII.1963. J. L. Gressitt leg. 1♀ [ELKU]; Okinawa pref., Okinawa Isl., Kunigami dist., Kunigami town, Yona. 18–21.X.1973. M. Owada leg. 1♀ [SEHU]; Yaeyama dist., Iriomote Isl., Taketomi town, Shirahama, 21.VII.1963, Yorio Miyatake leg., 1♀ [ELKU]; Taketomi town, Sonai. 12.X.1963. Shoichi Miyamoto leg. 1♀ [ELKU] (with identification label; *Gryon flavipes* (Ashm.) det. G. Mineo); Taketomi town, Komi (Paddy field). 24.330°N, 123.912°E; alt. 10 m, 22–25.VI.2015, Yoto Komeda leg. (YPT) 1♀ [ELKU].

Distribution. Japan (Honshu: Iwate, Ibaraki, Saitama, Chiba, Kanagawa, Ishikawa, Fukui, Gifu, Aichi, Shiga, and Kyoto; Shikoku: Tokushima, Ehime, and Kochi; Kyushu: Fukuoka, Nagasaki, Kumamoto, Miyazaki, and Kagoshima; Ryukyus: Yaku-shima Isl., Toku-no-shima Isl., Okinawa Isl., and Iriomote Isl.), South Korea (North Gyeongsang: Andong), Vietnam (Hanoi).

Remarks. Talamas et al. (2017) provided photographs of the holotype of *G. japonicum*. Based on these photographs, morphological characters of specimens examined in this study are the same as the holotype. *Gryon orestes* (Dodd, 1913) is recorded from Japan as *G. flavipes* (Ashmead, 1905) in Mineo (1979) and *G. nixonii* (Masner, 1965) in Mineo (1981). We examined the voucher specimen determined by Mineo, and found that the specimen is a small individual of *G. japonicum*. We also examined the holotype of *G. mischa* held at ZIN, and confirmed that it is also *G. japonicum*.

***Gryon yamagishii* Mineo, 1981**

Figs 1C, 2C, 3C, 4C, 5C, I, 6C

Gryon yamagishii Mineo, 1981: Johnson 1992; Kononova and Petrov 2002; Kononova and Kozlov 2008.

Gryon maruzsae Mineo, 1981: Johnson 1992; Kononova and Kozlov 2008. syn. nov.

Gryon sugonjaevi Kozlov & Kononova, 1989: Kozlov and Kononova 1990; Johnson 1992; Kononova 1995; Kononova and Petrov 2002; Kononova and Kozlov 2008. syn. nov.

Diagnosis. Frontal depression transversely costate by strong regular carinae. Angular points of occipital carina modified as short sharp horns; horizontal portion of occipital carina straight, expanding inwardly. Coxae brown-black.

Description. Female. Length = 1.4–1.6 mm.

Color. (Figs 1C, 2C). **Body** mainly black. A2–6 brown. A1, mandibles, and legs (excluding coxae) yellow.

Head. FCI = 1.16–1.28; LCI = 1.66–1.62; DCI = 2.03–2.23; HW/IOS = 1.64–1.78; head about 1.3 times as wide as mesosoma (HW/TSL = 1.24–1.30). Frons (Fig. 3C) reticulate with setae; central carina present ventrally; frontal depression weakly developed, transversely costate by strong regular carinae. Vertex reticulate with setae; interocellar space reticulate; hyperoccipital carina absent; POL about 4.6 times as long as OOL (POL/OOL = 3.67–4.83); OOL about 0.4 times as long as LOL (OOL/LOL = 0.33–0.50). Clypeus semi-elliptical. Gena coriaceous with setae; medial genal carina absent. Occiput (Fig. 4C) transversely costate with setae; occipital carina complete; angular points of occipital carina well-developed, as short sharp horns; horizontal portion of occipital carina straight, expanding inwardly; postoccipital carina discontinuity present; postgena weakly costate along postoccipital carina; postgenal sulcus curved toward hypostoma; postgenal bridge smooth, weakly longitudinally costate beside median sulcus. Antennae (Fig. 5C) clavate; A1 about 3.5 times longer than radicle, as long as clava; clava with five segments; claval sensilla formula A8–12/2–2–2–1; claval length about 3.4 times longer than width. Mandibles thin, tridentate, anterior tooth longer than other teeth.

Mesosoma. Cervical pronotal area granulate-punctate with dense setae; epomial carina strongly present, not reaching dorsal edge; pronotal suprahumeral sulcus foveolate, unclear mesad; lateral pronotal area smooth with transverse dense carinae. Propleuron weakly transversely costate. Mesoscutum about 1.4 times as wide as long (TSL/ML = 1.35–1.50), with dense setae, reticulate in anterior, longitudinally costate in posterior; parascutal carina absent; notaulus absent. Mesoscutellum about 2.2 times as wide as long (SW/SL = 2.07–2.35), with dense setae, longitudinally costate mesad, granulate laterad. Mesopleuron smooth with transverse dense carinae above mesopleural canina, rugulose with setae below mesopleural canina; prespecular and upper mesepisternal sulci foveolate; prespecular sulcus with setae; mesopleural carina strongly

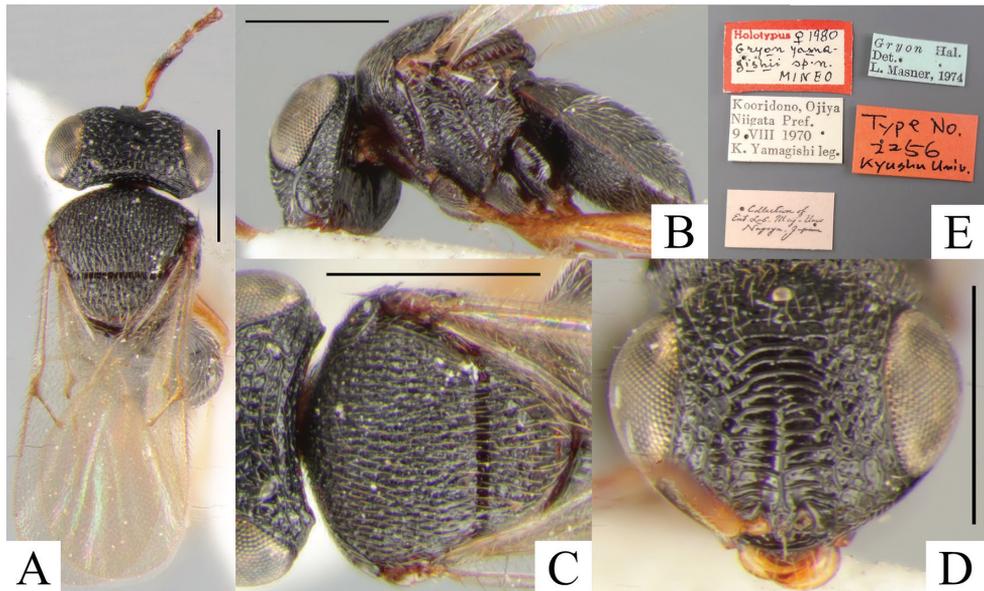


Figure 10. The holotype of *G. yamagishii* **A** dorsal view **B** lateral view **C** mesosoma, dorsal view **D** frons **E** labels.

present; postacetabular sulcus foveolate. Metascutellum weakly produced, longitudinally striate. Metapleuron weakly convex, foveolate-rugulose, with dense setae posteriorly; anterior part of metapleural sulcus and upper paracoxal sulcus with setae. Propodeum foveolate with setae. Fore wing (Fig. 7C); stigmal vein about 2.5 times longer than marginal vein; postmarginal vein about 3.7 times longer than marginal vein.

Metasoma. T1 longitudinally striate, setose laterally. S1 longitudinally striate. T2 longitudinally striate anteriorly, reticulate posteriorly, setose laterally. S2 with setae, granulate mesad, striate laterad. T3 reticulate, with setae laterad and posteriorly. S3–6 punctate with setae. T4 punctate-striate with setae. T5–6 punctate with setae.

Male. Almost same as female, but antennae (Fig. 5I) filiform; A1 yellow, A2–11 brown.

Host. Unknown.

Biology. In winter, the female overwinters under the bark of *Zelkova serrata* (Thunberg) Makino (Ulmaceae).

Material examined. Holotype. *G. yamagishii*: Kooridono, Ojiya, Niigata Pref. [Japan: Niigata pref., Ojiya city, Nishi-Yoshidani, Kōridono.] 9.VIII.1970. K. Yamagishi leg. ♀ [ELKU] (Fig. 10). *G. maruzzae*: (Kyushu) Okinoshima (Chikuzen). [Japan: Fukuoka pref., Munakata dist., Munakata town, Chikuzen Oki-no-shima Isl.] 25–28.VII.1958, Hirashima, Murakami and Y. Miyatake leg. 1♂ [ELKU] (Fig. 11). *G. sugonjaevi*: Япония, Инуяма, 40 км СЗ Нагоя. [=Japan: Aichi pref., Inuyama city.] 4.X.1981. E. Sugonjaev leg. ♀ [ZIN].

Paratypes. *G. yamagishii*: same locality as holotype, 1.VIII.1970. K. Yamagishi leg. ♀ [ELKU]; Nisshin, Aichi-gun, Aichi pref. [Aichi pref., Aichi dist., Nisshin town], 5.V.1970. K. Yamagishi leg. ♀ [ELKU]. *G. sugonjaevi*: Япония, Каганихара. [= Japan: Gifu pref., Kakamigahara city] 19.X.1981. E. Sugonjaev leg. ♀ [IZAN].

Other material. JAPAN: Hokkaido, Sapporo city, Toyohira ward, Hitsujigaoka. 43.008°N, 141.415°E; alt. 100 m, 8–15.VI.2010, Kazuhiko Konishi leg. (MT) 5♀ [ELKU]; 15–22.VI.2010, Kazuhiko Konishi leg. (MT) 2♀ [ELKU]; 22–29.VI.2010, Kazuhiko Konishi leg. (MT) 2♀ [EUMJ]; 29.VI.–6.VII.2010, Kazuhiko Konishi leg. (MT) 2♀ [EUMJ]; 27.VII.–3.VIII.2010, Kazuhiko Konishi leg. (MT) 2♀ [EUMJ]; 10–17.VIII.2010, Kazuhiko Konishi leg. (MT) 2♀ [EUMJ]; Fukushima pref., Aizu-Wakamatsu city, Monden-machi, Kuroiwa, Minami-Aoki. 6.I.2017. Keisuke Narita leg. (Collected from under the bark of *Zelkova serrata*) 1♀ [ELKU]; Ibaraki pref., Tsuchiura city (near Tsukuba), Pond Shishituka-Ôike. 15.VII.1997. Victor Fursov leg. 1♀ [IZAN]; Tochigi pref., Nikko city, Tamozawa. 20–25.VI.2008. Takeyuki Nakamura leg. (MT) 1♀ [ELKU]; Niigata pref., Nagaoka city, Urase-machi, 37.464°N, 138.907°E, alt. 40 m, 2015.V.28.–VI. 7, Ryo Shimizu and So Shimizu leg. (MT), 6♀ [ELKU]; 2015. VI.7.–VI.18, Ryo Shimizu and So Shimizu leg. (MT), 8♀ [ELKU]; 2015.VII.6–25, Ryo Shimizu and So Shimizu leg. (MT), 5♂17♀ [ELKU]; 2015. VII.25–VIII.7, Ryo Shimizu and So Shimizu leg. (MT), 2♂16♀ [ELKU]; 2015. VIII.7–VIII. 22, Ryo Shimizu and So Shimizu leg. (MT), 5♂2♀ [ELKU]; 2015. VIII.22–IX.5, Ryo Shimizu and So Shimizu leg. (MT), 7♂2♀ [ELKU]; 2015. IX.27–X. 12, Ryo Shimizu and So Shimizu leg. (MT), 6♀ [ELKU]; 2016.VI.11–23, Ryo Shimizu and So Shimizu leg. (MT), 3♀ [ELKU]; 2016. VII.13–VIII.1, Ryo Shimizu and So Shimizu leg. (MT), 2♂12♀ [ELKU]; Ishikawa pref., Mt. Hakusan. 29–21. VIII.1960. Terunobu Hidaka leg. 1♂ [ELKU]; Hakusan city, San'nomiya, 9–22. VIII.2009, H. Fukutomi and S. Nakagawa leg. (MT) 1♀ [ELMU]; Nomi city, Mitsukuchi (paddy field) 30.IV–13.V.2011, H. Fukutomi and R. Ishiguro leg. (MT) 1♀ [ELMU]; 13–26.V.2011, H. Fukutomi and R. Ishiguro leg. (MT) 3♀ [ELMU]; 9–21. VI.2011, H. Fukutomi and R. Ishiguro leg. (MT) 3♀ [ELMU]; 17.VII.–4.VIII.2011, H. Fukutomi and R. Ishiguro leg. (MT) 1♂ [ELMU]; 19.VIII.–7.IX.2011, H. Fukutomi and R. Ishiguro leg. (MT) 1♂ [ELMU]; 8–21. IX. 2011, H. Fukutomi and R. Ishiguro leg. (MT) 1♀ [ELMU]; 22.X.–2.XI.2011, H. Fukutomi and R. Ishiguro leg. (MT) 1♀ [ELMU]; Gifu pref., Kani city, Katabira, 1–7.V.2004, K. Ito leg. (MT) 2♀ [ELMU]; 6–12.VIII.2004, Kenzo Yamagishi leg. (MT) 1♀ [ELMU]; 20–26. VIII.2004, Kenzo Yamagishi leg. (MT) 1♂1♀ [ELMU]; 2–8.X.2004. Kenzo Yamagishi leg. (MT) 3♀ [ELMU]; Shizuoka pref., Iwata city, dike of Ohta river, 29.VII.2001, M. Ura leg. (YPT) 1♀ [IZAN]; 13.VIII.2001, M. Ura leg. (YPT) 1♂ [IZAN]; 27. VIII.2001, M. Ura leg. (YPT) 1♂ [ELMU]; 1♂1♀ [IZAN]; Aichi pref., Nagoya city, Chikusa ward, Higashiyama Park. 1–10.V.2001. M. Watanabe leg. (MT) 1♀ [IZAN]; IV–VI. 1997, Victor Fursov leg. 25♀ [IZAN]; 11.VI.1997. Victor Fursov leg. 1♂ [IZAN]; Tempaku ward, Yagoto-Urayama, 21.IX.2002. Kenzo Yamagishi leg. 1♀ [ELMU]; Tempaku ward, 29.IX.2012. Kenzo Yamagishi leg. 1♀ [ELMU]; Tempaku

ward, Meijo University, 17.IV.2013. N. Kusuda leg. 1♀ [ELMU]; Tempaku ward, Meijo University, 31.VII.2013. N. Kusuda leg. 1♂1♀ [ELMU]; Ichinomiya city, Tomida, Kiso River, 10.V.2013, Y. Miyata leg. (YPT) 5♀ [ELMU]; 2.VII.2013, Y. Miyata leg. (YPT) 1♀ [ELMU]; 22.VIII.2013, Y. Miyata leg. (YPT) 1♀ [ELMU]; Kasugai city, Takagi (Grassland), 14.V.2013. Y. Kamiya leg. (YPT) 1♀ [ELMU]; 31.VII.2013. Y. Kamiya leg. (YPT) 1♂ [ELMU]; Nisshin city, Akaike, 30.IX.2006. Kenzo Yamagishi leg. 1♀ [ELMU]; Nisshin city, Nokata, 28.V–3.VI.2011. H. Seo and R. Mizutani leg. (MT) 1♀ [ELMU]; 10–17.VI.2011. H. Seo and R. Mizutani leg. (MT) 1♀ [ELMU]; 2–9.VI.2011. H. Seo and R. Mizutani leg. (MT) 1♀ [ELMU]; [ELMU]; 16–22.VI.2011. H. Seo and R. Mizutani leg. (MT) 1♂ [ELMU]; Toyota city, Obara, Kajiyashiki-chô, Nishinohara, 12.VI.–6. 2014. Hiroaki Iketake leg. (MT) 1♀ [ELKU]; 12–26.X.2014. Hiroaki Iketake leg. (MT) 1♀ [ELKU]; Osaka pref., Sen'nan Dist., Misaki town, Kyôshi, 16.IV.1975. 3♀ [ELKU]; Wakayama pref., Wakayama city, Sandô, 16.IV.1975. 1♀ [ELKU]; Shimane pref., Izumo city, Mt. Kita-yama, 11.VI.1969. Minoru Miyazaki leg. 1♀ [ELKU]; Tokushima pref., Zen'nyuji-toh (River island in Yoshino River), 13–22.V.2003. K. Ohara and H. Otsuka leg. (MT) 3♀ [ELMU]; 5–17.IX.2003. K. Ohara and H. Otsuka leg. (MT) 3♀ [ELMU]; Ehime pref., Matsuyama city, Sugitake. 26.V.1976. N. Takaki leg. 1♀ [EUMJ]; Matsuyama city, Komenono. 1.VI.1978. N. Takaki leg. 1♀ [EUMJ]; Matsuyama city, Jikiba-chô. 33.51°N, 132.42°E; 14.V.2016. K. Kuroda and K. Sogoh leg. 1♀ [EUMJ]; Matsuyama city, Suemachi, Wakigafuchi Park. 33.52°N, 132.49°E; 20.V.2017. K. Kuroda leg. 1♀ [EUMJ]; Ochi dist., Kamijima town, Iwagi, Akahone Isl., 29–30.XI.2004. Jirô Ogawa leg. 1♀ [EUMJ]; Kami-Ukena dist., Yanadani vill., Yokono (alt. 650 m). 7.V.1994. M. Sakai leg. 1♀ [EUMJ]; Kami-Ukena dist., Kuma-Kougen town, Saragamine. 4.VI.2006. Yûgo Satô leg. 1♀ [EUMJ]; Kami-Ukena dist., Kuma-Kougen town, Yurano. 5.VI.2007. Eiji Yamamoto leg. 1♀ [EUMJ]; 17.VII.2007. Eiji Yamamoto leg. 1♀ [EUMJ]; 24–26.VII.2008. Eiji Yamamoto leg. 1♀ (MT) [EUMJ]; Kita dist., Uchiko town, Hongawa, Hirose shrine. 21.V.2014. Eiji Yamamoto leg. 2♀ [EUMJ]; Kita dist., Uchiko town, Hiraoka. 11–15.V.2014. Eiji Yamamoto leg. (MT) 1♀ [EUMJ]; 16–20.V.2014. Eiji Yamamoto leg. (MT) 2♀ [EUMJ]; Kôchi pref., Nankoku city, Estuary of Monobe river. 1.VII.2002. M. Sakai leg. 1♀ [EUMJ]; Kami city, Kami-anauchi. 30–31.VII.2016. Kazuhiko Konishi leg. (MT) 1♀ [EUMJ]; Hata dist., Nishi-Tosa vill., Oku-Yanai, Kuroson, 29.IV.1956, Yozo Murakami leg., 2♀ [ELKU]; Fukuoka pref., Fukuoka city, Mt. Tachibana-yama, 14.V.1970. Minoru Miyazaki leg. 3♀ [ELKU]; 13.V.1994. Hiroshi Honda leg. (YPT) 1♀ [ELKU]; 2.VII.1994. Hiroshi Honda leg. (YPT) 5♂ [ELKU]; 16.VII.1994. Hiroshi Honda leg. (YPT) 2♂4♀ [ELKU]; 23.VII.1994. Hiroshi Honda leg. (YPT) 1♂1♀ [ELKU]; 30.VII.1994. Hiroshi Honda leg. (YPT) 1♀ [ELKU]; 7.VIII.1994. Hiroshi Honda leg. (YPT) 1♂1♀ [ELKU]; Fukuoka city, Mt. Abura-yama, 21.VI.1959. Shoichi Miyamoto leg. 1♀ [ELKU]; Fukuoka city, East ward, Tatara. 8.IX.1996. Yoshimitsu Higashiura leg. 1♀ [ELKU]; Fukuoka city, Sawara ward, Mt. Sefuri-san, 1.VIII.1992, Yoshimitsu Higashiura leg. 2♀ [ELKU]; Kasuya dist., Sasaguri town, Mt. Wakasugi-yama. 12.V.1969. Masako Honda leg. 1♀ [ELKU]; 13.V.1970. Masako Honda leg. 1♀ [ELKU]; Dazaifu

city, Kitadani. 5.III.2016. Yu Hisasue leg. 1♀ [ELKU]; Iizuka city, Ae, 33.564°N, 130.640°E, 5.V.2018. Yu Hisasue leg., (collected from blossoms of *Acer palmatum*) 1♀ [ELKU]; Tagawa dist., Soeda town, Mt. Hiko-san. 28.IX.1966, Masako Honda leg. 3♀ [ELKU]; 8.V.1967, Masako Honda leg. 1♀ [ELKU]; 10.V.1967, Masako Honda leg. 2♀ [ELKU]; 20.IV.1967, Kenkichi Kanmiya leg. 1♀ [ELKU]; 20.VI.1967, Kenkichi Kanmiya leg. 1♀ [ELKU]; 20.IX.1967, Kenkichi Kanmiya leg. 3♀ [ELKU]; 25.VII.196–, Kôichi Takeno leg. (MT) 1♂ [ELKU]; 5.VI.1970, Kenkichi Kanmiya leg. 3♀ [ELKU]; 15.IV.1971, Michitaka Chujo leg. 1♀ [ELKU]; 24.VII.1972, Michitaka Chujo leg. 1♀ [ELKU]; 22–26.V.1979, Kaoru Maeto leg. 1♀ [NIAES]; 25–26.VII.1979, Kaoru Maeto leg. 3♂1♀ [NIAES]; 24–26.X.1979, Kaoru Maeto leg. 3♀ [NIAES]; Miyako dist., Saigawa town, Hobashira, Mt. Hiko-san, No-tôge pass. 29.VIII.1993, N. Wasano leg. 1♂ [ELKU]; Saga pref., Kishima dist., Yamauchi town. 22.X.1991. Yoshimitsu Higashiura leg. 2♀ [ELKU]; Higashi-Matsuura dist., Genkai town, Kariya. 23.VIII.1993. Yoshimitsu Higashiura leg. 3♀ [ELKU]; Nagasaki pref., Minami-Takaki dist., Obama town. 25.II.1960. Terunobu Hidaka leg. 1♀ [ELKU]; Kumamoto pref., Hondo city, Nishinoku Lake, 5.VI.1999. Victor Fursov leg. 1♀ [IZAN]; Miyazaki pref., Higashi-Usuki dist., Shiiba vill., Shimo-Fukura, Ohira. 29.VIII.1993, N. Wasano leg. 3♀ [ELKU]; Kagoshima pref., Aira dist., Kirishima town, Taguchi, Kirishima-jingû shrine, 15.VII.1969. Osamu Yata leg. 1♂ [ELKU]; Kimotsuki dist., Sata town, Magome, Tajiri, Kape Sata, 17.VII.1969. Osamu Yata leg. 2♀ [ELKU]; Kumage Dist., Yaku-shima Isl., Yaku town, Kurio, 8.VII.1975. Kenzo Yamagishi leg. 2♂1♀ [ELKU]; Kagoshima Dist., Toshima vill., Tokara Isles., Kuchino-shima Isl., 2.V.1993, Hiroshi Honda leg., 2♀ [ELKU]; Satoshi Kamitani leg., 2♀ [ELKU]; Ôshima dist., Amami-ôshima Isl., Uken vill., Mt. Yuwan-dake, 30.VII.1963, J. L. Gressitt leg. 1♂1♀ [ELKU]; Okinawa pref., Okinawa Isl., Nago city, Mt. Nago-dake, 26.587°N, 128.000°E, 140 m, 3–14.VII.2016, Keisuke Narita leg. 1♀ [ELKU]; Tokyo Met., Hachijo Isl., Hachijo town, Kamogawa. 27.V.1964, Y. Hirashima and M. Shiga leg. 3♀ [ELKU]; Hachijo town, Mitsune-Kantoyama. 30.V.1964, Y. Hirashima and M. Shiga leg. 1♀ [ELKU]; Fukuoka pref., Munakata dist., Munakata town, Chikuzen Oki-no-shima Isl. 25–28.VII.1958, Yoshihiro Hirashima, Yozo Murakami and Yorio Miyatake leg. 1♂ [ELKU].

Distribution. Japan (Hokkaido; Honshu: Fukushima, Ibaraki, Tochigi, Niigata, Ishikawa, Gifu, Shizuoka, Aichi, Osaka, Wakayama, and Shimane; Shikoku: Tokushima, Ehime, and Kochi; Kyushu: Fukuoka, Saga, Nagasaki, Kumamoto, Miyazaki, and Kagoshima; Ryukyus: Yaku-shima Isl., Naka-no-shima Isl., Amami-ôshima Isl., and Okinawa Isl.; Hachijo Isl; and Chikuzen Oki-no-shima Isl.)

Remarks. Among Japanese species, *G. yamagishii* is similar to *G. japonicum* and *G. fulvicoxa*, but *G. yamagishii* differs from these species in the sculpture of the antennal depression (*G. yamagishii* (Fig. 3C): transversely costate by strong regular carinae; *G. fulvicoxa* (Fig. 3A) and *G. japonicum* (Fig. 3B): transversely costate by strong irregular carinae) and the presence of the short sharp horn on the angular point of occipital carina (Fig. 4C). Russian Far Eastern species, *G. amissum* Kozlov & Kononova, 1990 and *G. sibiricum* Kononova, 2001 are also similar to *G. yamagishii* in the sculpture of

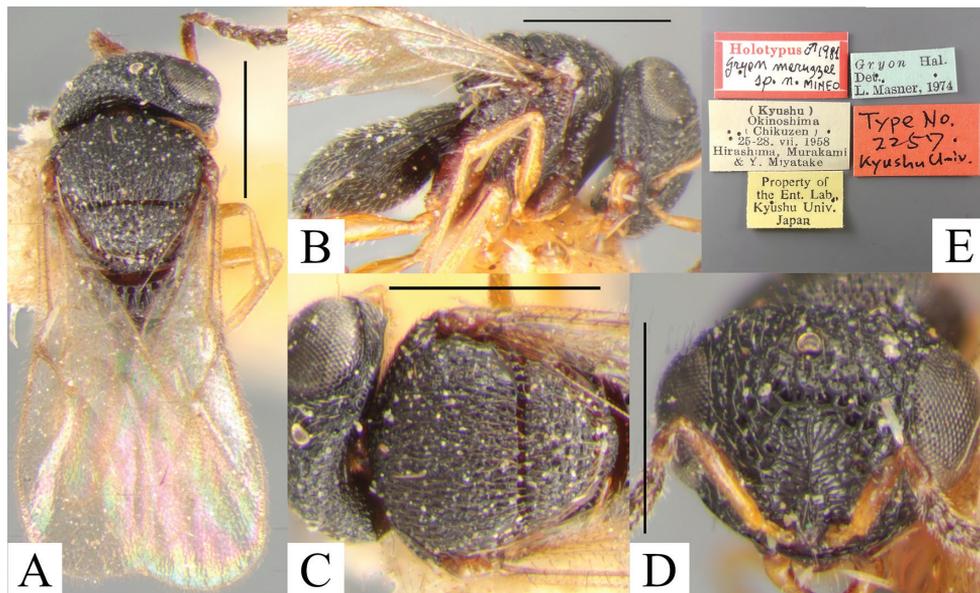


Figure 11. The holotype of *G. maruzzae* syn. nov. **A** dorsal view **B** lateral view **C** mesosoma, dorsal view **D** frons **E** labels.

the frons. In *G. amissum* and *G. sibiricum*, however, the angular points of occipital carina are not developed as horns, and the mesopleural carina is weak (*G. amissum*) or absent (*G. sibiricum*). We examined the holotype of *G. maruzzae* at ELKU (Fig. 11) and *G. sugonjaevi* at ZIN, and they belong to *G. yamagishii*.

Gryon charon-group (Mineo, 1983)

Diagnosis. Frons reticulate with setae; frontal depression developed with enclosing strong carina. Clypeus almost rounded. Eyes with sparse setae or without. Occipital carina complete, angular point of occipital carina developed; postoccipital carina present, at least laterally; medial genal carina present; postgena weakly almost smooth; postgenal pit located near fossa.

Gryon philippinense (Ashmead, 1904)

Figs 1D, 2D, 3D, 4D, 5D, J, 6D, 7C, D, 8C, D, 9C, D

Hadronotus philippinensis Ashmead, 1904a: Ashmead 1904c; Kieffer 1926; Baltazar 1966. *Gryon philippinensis* (Ashmead): Masner and Muesebeck 1968. *Gryon philippinense* (Ashmead): Mineo 1983; Mineo 1990b; Johnson 1992; Lê 2000; Dasilao and Arakawa 2004, 2005; Nakajima and Fujisaki 2010; Nakajima et al. 2012.

Hadronotus hakonensis Ashmead, 1904b; Kieffer 1926; Watanabe 1951. *Gryon hakonensis* (Ashmead): Masner and Muesebeck 1968. *Gryon hakonense* (Ashmead): Mineo 1981; Johnson 1992; Kononova and Kozlov 2008. syn. nov.

Hadronotus homoeoceri Nixon, 1934; Mineo 1979 (syn.). *Hadronotus homoceri* Nixon: Mani 1941. *Gryon homoeoceri* (Nixon): Masner 1965; Mani and Sharma 1982; Sharma 1982; Johnson 1992.

Diagnosis. Horizontal portion of occipital carina short, reaching longitudinal extension line of outer margin of lateral ocelli or shorter. Postgena almost smooth, longitudinally costate by weak furrows along postoccipital carina; postgenal sulcus curved toward hypostoma; postgenal bridge smooth, weakly punctate-costate beside of median sulcus.

Description. Female. Length = 1.2–1.7 mm.

Color. (Figs 1D, 2D). **Body** mainly black. A1–6, mandibles, and legs (excluding coxae) yellow.

Head. FCI = 1.05–1.18; LCI = 1.53–1.71; DCI = 1.69–1.98; HW/IOS = 1.78–1.87; head about 1.3 times as wide as mesosoma (HW/TSL = 1.20–1.29). Frons (Fig. 3D) reticulate with setae; central carina weakly present in lower half of frontal depression, absent in upper half of frontal depression, present between enclosing carina of frontal depression and anterior ocellus, frontal depression weakly developed, with enclosing carina; antennal depression width about 1.8 times wider than distance between eye and antennal depression (WAD/OAD = 1.61–2.32). Vertex reticulate with setae; interocellar space reticulate-granulate; hyperoccipital carina present; POL about 5.7 times as long as OOL (POL/OOL = 5.04–6.02); OOL about 0.3 times as long as LOL (OOL/LOL = 0.27–0.38). Clypeus rectangular, with rounded corners. Gena coriaceous with setae; medial genal carina present. Occiput (Fig. 4D) transversely semi-elliptically costate, with setae; occipital carina complete; angular point of occipital carina developed; horizontal portion of occipital carina short, reaching longitudinal extension line of outer margin of lateral ocelli or shorter; postoccipital carina present, weak mesad; postgena almost smooth, longitudinally costate by weak furrows along postoccipital carina; postgenal sulcus curved toward hypostoma; postgenal bridge smooth, weakly punctate-costate beside median sulcus. Antennae (Fig. 5D) clavate; A1 about three times longer than radicle, as long as clava; clava with six segments; claval sensilla formula A7–12/1–2–2–2–1; claval length about 4.8 times longer than width. Mandibles thin, tridentate, anterior tooth longer than other teeth.

Mesosoma. Cervical pronotal area (Fig. 8C) granulate dorsad, smooth-imbricate ventrad, with dense setae; epomial carina strongly present, reaching dorsal edge; pronotal suprahumeral sulcus foveolate with setae, unclear mesad; lateral pronotal area rugulose dorsad, smooth with transverse dense carina ventrad. Propleuron weakly transversely costate. Mesoscutum (Fig. 7C) about 1.4 times as wide as long (TSL/ML = 1.33–1.45) reticulate, inside of cell coriaceous, with setae; parascutal carina absent; notaulus absent. Mesoscutellum about 1.8 times as wide as long (SW/SL = 1.65–1.86), reticulate, inside of cell coriaceous, with setae, slightly produced posteriorly. Mesopleuron (Fig. 7D)

costate-reticulate above mesopleural canina, reticulate with setae below mesopleural canina; prespecular and upper mesepisternal sulci foveolate; prespecular sulcus with setae; mesopleural carina strongly present; postacetabular sulcus foveolate. Metascutellum (Fig. 8D) weakly produced, rugose. Metapleuron (Fig. 7D) foveolate anteriorly, glanulate with dense setae posteriorly, with longitudinal carina modified as weak ridge; anterior part of metapleural sulcus and upper paracoxal sulcus with setae. Propodeum foveolate, with setae laterad. Fore wing (Fig. 6D); stigmal vein about 3 times longer than marginal vein; postmarginal vein about 4.9 times longer than marginal vein.

Metasoma. T1 (Fig. 9C) longitudinally striate, setose laterally. S1 (Fig. 9D) longitudinally striate. T2 reticulate, with setae laterally. S2 reticulate-granulate, with setae. T3 reticulate with setae. S3–6 punctate with setae. T4 reticulate-rugose with setae. T5–6 rugose with setae.

Male. Almost same as female, but antennae (Fig. 7J) filiform; A1 yellow, A2–11 brown.

Variation. The sculpture of frons and postgena of the small specimens is weaker than that of the large specimens. In the smallest specimens collected in Kôchi University, the sculpture of the frons is reticulate-granulate with puncture and the sculpture of postgena is barely costate. In contrast, the sculpture of the frons in the larger specimens is clearly reticulate, and that of the postgena is also clear. The number of sulci is large in large specimens. The pronotal cervical sulcus is weakly foveolate in the large specimens, however, the foveolae are lacking in small specimens. Owing the smaller host egg size, specimens that emerge from *A. sordidus* are smaller and the sculpture is weaker than those that emerge from the larger eggs of *H. unipunctatus*.

Host. Coreidae: *Acanthocoris sordidus*, *Homoeocerus marginellus* (Herrich-Schäffer, 1840), *H. unipunctatus* (Thunberg, 1783) new record; and *Leptoglossus membranaceus* (Fabricius, 1781). Watanabe (1951) also recorded *Homoeocerus marginiventris* Dohrn, 1860 (Coreidae) and *Riptortus pedestris* (Alydidae), but the identification of wasps is problematic (see remarks).

Biology. Females of *G. philippinense* are found in the “Komomaki”, rice straw belts wrapped around trees during winter. Some females are also found on the underside of leaves of evergreen broad-leaved trees. In spring, females can be collected from blossoms of *Acer palmatum* Thunberg (Sapindaceae).

Material examined. Ibaraki pref., Tsukuba city, near Mt. Tsukuba-san, 13.IX.1984, Takashi Noda leg., emergence from eggs of *Acanthocoris sordidus* on pod of soybeans, 2♂8♀ [ELKU]; Niigata pref., Nagaoka city, Urase-machi, 37.464°N, 138.907°E, alt. 40 m. 25. VII–7.VIII.2015, Ryo Shimizu and So Shimizu leg. (MT), 1♀ [ELKU]; 23. VI–13.VII.2016, (MT), 1♀ [ELKU]; 13. VII–1.VIII.2016, (MT), 1♀ [ELKU]; Wakayama pref., Nishi-Muro dist., Shirahama town, Tonda-cho, 13.IX.1984, Takashi Noda leg., emergence from eggs of *Acanthocoris sordidus*, 2♂9♀ [ELKU]; Yamaguchi pref., Yamaguchi city, Ôuchi-Nagano. 34.167°N, 131.523°E. 29.XI.2014. Yoshimitsu Higashiura leg. 1♀ [ELKU]; Ehime pref., Matsuyama city, Tarumi, Ehime University, 23.I.2016. Yu Hisasue leg., (collected from Komomaki) 2♀ [ELKU]; 15–16.IV.2016, 2♀ (YPT) [ELKU]; Kita dist., Uchiko town, Hongawa, Hirose shrine. 27.V.2014.

Eiji Yamamoto leg. 1♀ [EUMJ]; 1.III.2017. Hiroyuki Yoshitomi leg. (collected from back of the leaves of *Quercus gilva*) 10♀ [EUMJ]; Kôchi pref., Hata dist., Nishi-Tosa vill., Oku-Yanai, Kuroson, 29.IV.1956., Yozo Murakami leg., 2♀ [ELKU]; Nankoku city, Kôchi University, Ryo Arakawa leg., emergence from eggs of *Acanthocoris sordidus*, 7♂14♀ [ELKU]; Fukuoka pref., Tagawa dist., Soeda town, Ochiai, Kajiya, 24.VIII.1972, Michitaka Chujo leg., 2♀ [ELKU]; 12.IX.1972, 1♀ [ELKU]; 5.X.1972, 1♀ [ELKU]; Tagawa dist., Soeda town, Mt. Hiko-san, Kaoru Maeto leg., 24–26.X.1979, 1♀ [NIAES]; Fukuoka city, Sawara ward, Ishigama, 6.V.2014, Taisuke Kawano leg., 1♀ [ELKU]; Yanagawa city, Shin'hokamachi, 33.158°N, 130.399°E, 12.III.2016, Yu Hisasue leg., (sweeping of trees) 1♀ [ELKU]; Kasuya dist., Hisayama town, Ino, 10.IV.2018, Taisuke Kawano leg., (collected from blossoms of *Acer palmatum*) 1♀ [ELKU]; Iizuka city, Ae, 33.564°N, 130.640°E, 5.V.2018. Yu Hisasue leg., (collected from blossoms of *Acer palmatum*) 2♀ [ELKU]; Kumamoto pref., Hondo city, Jôshita, 22.VII.1996. Hiroshi Honda leg., (emergence from eggs of *Acanthocoris sordidus*) 2♀ [ELKU]; Kagoshima pref., Kimotsuki dist., Sata town, Magome, Tajiri, Kape Sata, 17.VII.1969, Minoru Miyazaki leg. 1♀ [ELKU]; Kagoshima city, 18.VII.1969, Osamu Yata leg. 1♀ [ELKU]; Kagoshima City, Mt. Taga-yama, 1.VI.1987, Yoshimi Hirose leg. (emergence from egg of *Homoeocerus unipunctatus* on *Pueraria montana* var. *lobata*) 1♂1♀ [ELKU]; Hioki dist., Ijûin town, 1.VI.1987, Yoshimi Hirose leg. (emergence from egg of *Homoeocerus unipunctatus* on *Pueraria montana* var. *lobata*) 2♂7♀ [ELKU]; Kumage Dist., Yaku-shima Isl., Yaku town, Kurio, 8.VII.1975. Kenzo Yamagishi leg. 2♂2♀ [ELKU]; Kagoshima Dist., Toshima vill., Tokara Isles., Kuchino-shima Isl., 2.V.1993, Hiroshi Honda leg., 2♀ [ELKU]; Ôshima dist., Amami-ôshima Isl., Tatsugo town, Ankiyaba, 22.V.2002, Kenji Fujisaki leg., (emergence from egg of *Acanthocoris sordidus*) 18♂13♀ [ELKU]; Okinawa pref., Okinawa Isl., Kunigami dist., Motobu town, Izumi. 21.X.1963, Yoshihiro Hirashima leg. 1♀ [ELKU]; Ôgimi vill., Ôgimi, 27.VII.1995, Yoshimitsu Higashiura leg. (emergence from coerid egg on papaya) 7♂5♀ [ELKU]; Nago city, Mt. Nago-dake, 26.587°N, 128.000°E, 140 m, 3–14.VII.2016, Keisuke Narita leg. 1♀ [ELKU]; Miyako Isl., Miyako-jima city, Ueno, Mt. Nobaru-dake, 27.XII.2017, Hiraku Yoshitake leg. 1♀ [ELKU]; 29.XII.2017, 1♀ [ELKU]; Miyako-jima city, Hirara, Ôno-sanrin, near Miyako-seishônen-no-ie, 29.XII.2017, Hiraku Yoshitake leg. 6♀ [ELKU]; Ishigaki Isl., Ishigaki city, Kawaradake, 28.X.1963, Yoshihiro Hirashima leg. 1♀ [ELKU]; Yaeyama dist. Iriomote Isl., Taketomi town, Ôhara-komi, 17.VII.1963, Yorio Miyatake leg., 1♀ [ELKU]; Taketomi town, Ôtomi, 15–23.III.1995, Takeshi Matsuura leg. 1♀ [NIAES]; Nagasaki pref., Tsushima Isl., Shimo-Agata dist., Izuhara town, Mt. Tatera, 27.IX.1959, 1♀ [ELKU] (with identification label; *Gryon hakonensis* (Ashm.) det. G. Mineo, 1978).

Distribution. Japan (Honshu: Ibaraki, Niigata, Wakayama, and Yamaguchi; Shikoku: Ehime and Kôchi; Kyushu: Fukuoka, Saga, Kumamoto, and Kagoshima; Ryukyus: Yaku-shima Isl., Naka-no-shima Isl., Amami-ôshima Isl., Okinawa Isl., Miyako Isl., Ishigaki Isl., and Iriomote Isl.; Tsushima Is.), South Korea (North Gyeong-sang: Mt. Sudosan) Philippines (Luzon Isl.: Manila), Indonesia (Java Isl.: Bogor), India (Kerala), Vietnam (Ho Chi Minh City).

Remarks. Among the *charon*-group species, *G. philippinense* differs from other species in the sculpture of the median sulcus of the postgenal bridge (Fig. 4D). Talamas et al. (2017) provided some pictures of holotypes of *G. philippinense* and *G. hakonense*. Also, we examined the voucher specimen of Mineo (1979) deposited in ELKU. Based on these pictures and the voucher specimens, *G. hakonense* is a junior synonym of *G. philippinense*. Watanabe (1951) redescribed *G. hakonense* based on specimens that emerged from eggs of *Homocercus marginiventris* and *Riptortus pedestris*. We could not, however, find the voucher specimens in SEHU and the redescription is insufficient to identify the species properly, therefore, we excluded the two host records.

***Gryon shisa* Komeda & Mita, sp. nov.**

<http://zoobank.org/45003564-D0AB-4E7B-A91A-E6495DE3ADB1>

Figs 1E, 2E, 3E, 4E, 5E, K, 7E

Diagnosis. Horizontal portion of occipital carina curved, reaching central longitudinal line of lateral ocelli. Postgena smooth; postgenal sulcus straight; postgenal bridge smooth.

Description. Female. Length = 1.6–1.7 mm.

Color. (Figs 1E, 2E). **Body** mainly black. Mandibles brown. A1–6 and legs (excluding coxae) yellow.

Head. FCI = 1.15–1.22; LCI = 1.51–1.62; DCI = 1.75–1.88; HW/IOS = 1.88–2.14; head about 1.2 times as wide as mesosoma (HW/TSL = 1.10–1.19). Frons reticulate with setae, with transverse carina between eye and frontal depression; central carina weakly present in lower half of frontal depression, absent in upper half of frontal depression, present between enclosing carina of frontal depression and anterior ocellus; frontal depression weakly developed, with enclosing carina; antennal depression about 1.3 times wider than distance between eye and antennal depression (WAD/OAD = 1.19–1.40). Vertex reticulate with setae; interocellar space reticulate-granulate; hyperoccipital carina present; POL about six times as long as OOL (POL/OOL = 5.54–6.55); OOL about 0.3 times as long as LOL (OOL/LOL = 0.26–0.32). Clypeus trapezoidal, with rounded corners. Gena coriaceous with setae; medial genal carina present. Occiput transversely semi-elliptically costate, with setae; occipital carina complete; angular point of occipital carina developed; horizontal portion of occipital carina curved, reaching central longitudinal line of lateral ocelli; postoccipital carina present laterally; postgena smooth; postgenal sulcus straight; postgenal bridge smooth. Antennae (Fig. 5E) clavate; A1 about 4.8 times longer than radicle, about 1.2 times longer than clava; clava with six segments; claval sensilla formula A7–12/1–2–2–2–1; claval length about 4.1 times longer than width. Mandibles thin, tridentate, anterior tooth longer than other teeth.

Mesosoma. Cervical pronotal area costate dorsad, smooth-imbricate ventrad, with sparse setae; epomial carina strongly present, not reaching dorsal edge; pronotal suprahumeral sulcus foveolate with setae, unclear mesad; lateral pronotal area with transverse dense carina; pronotal cervical sulcus foveolate. Propleuron weakly

transversely costate. Mesoscutum about 1.5 times as wide as long (TSL/ML = 1.44–1.61), reticulate; parascutal carina absent; notaulus absent. Mesoscutellum about 2.1 times as wide as long (SW/SL = 1.84–2.22), reticulate with setae, slightly produced posteriorly. Mesopleuron mesopleuron costate-reticulate above mesopleural canina, reticulate with setae below mesopleural canina; prespecular and upper mesepisternal sulci foveolate; prespecular sulcus with setae; mesopleural carina strongly present; postacetabular sulcus foveolate. Metascutellum weakly produced, striate. Metapleuron foveolate anteriorly, rugulose with dense setae posteriorly, with longitudinal irregular carina; anterior part of metapleural sulcus and upper paracoxal sulcus with setae. Propodeum foveolate, with setae laterad. Fore wing (Fig. 7E); stigmal vein about four times longer than marginal vein; postmarginal vein about 7.3 times longer than marginal vein.

Metasoma. T1 longitudinally striate, setose laterally. S1 longitudinally striate. T2 reticulate with setae laterally. S2 reticulate-granulate, with setae. T3 reticulate with setae. T4 reticulate-rugose with setae. T5–6 rugose with setae. S3–6 punctate with setae

Male. Almost same as female, but antennae (Fig. 5K) filiform; A1–11 yellow.

Host. Coreidae: *Paradasynus spinosus* Hsiao, 1963.

Material examined. Holotype: Okinawa pref., Okinawa Isl., Kunigami Dist., Ôgimi vill., Janagusuku. 30.VII.2002, Yasutsune Sadoyama leg. (emergence from an egg of *Paradasynus spinosus*) 1♀ [ELKU]. **Paratypes.** Same data as holotype. 3♂7♀ [ELKU].

Distribution. Japan (Ryukyus; Okinawa Is.)

Etymology. The species name refers to Shisā, the Okinawan traditional statue of the guardian lion, because this species defends the shequasar (*Citrus × depressa* Hayata), a kind of citrus fruit, from the important pest (*Paradasynus spinosus*) on Okinawa Island. (Zukeyama et al. 2007).

Remarks. Among the species of the *charon*-group, *G. shisa* is the only species without sculpture on the postgena, except for the postgenal sulcus (Fig. 4E). This species also differs from *G. philippinense* in the shape and length of the postgenal sulcus (*G. shisa* (Fig. 4E): straight; *G. philippinense* (Fig. 4D): curved toward hypostoma) and the length of horizontal portion of occipital carina (*G. shisa* (Fig. 4E): long, reaching central longitudinal line of lateral ocelli; *G. philippinense* (Fig. 4D): short, reaching longitudinal extension line of outer margin of lateral ocelli or shorter). Among Vietnamese species, four species, *G. ancinla* Kozlov & Lê, 1996, *G. clavaerus* Kozlov & Lê, 1996, *G. drunoris* Kozlov & Lê, 1996, and *G. sponus* Kozlov & Lê, 1996 seem to belong to *charon* group, but their horizontal portions of occipital carinae is also short like *G. philippinense*.

Gryon floridanum-group (Masner, 1983)

Diagnosis. Frons reticulate with setae; Clypeus almost rounded. Eyes without setae. Occipital carina complete, horizontal part of occipital carina well-developed, each

arms fused; postoccipital carina well-developed, subparallel under horizontal part of occipital carina; medial genal carina absent; postgenal pit located near fossa.

***Gryon pennsylvanicum* Ashmead, 1893**

Figs 1F, 2F, 3F, 4F, 5F, L, 6F, 7E, F, 8E, F, 9E, F

Telenomus pennsylvanicus Ashmead, 1893: *Hadronotus pennsylvanicus* (Ashmead): Kieffer 1926: *Gryon pennsylvanicus* (Ashmead): Masner 1961: *Gryon pennsylvanicum* (Ashmead): Masner 1983b; Mineo and Caleca (1987a); Yasuda 1990; Yasuda and Tsurumachi 1995; Kononova and Kozlov 2008.

Hadronotus ajax Girault, 1920: *Gryon ajax* (Girault): Muesebeck and Masner in Krombein and Burks 1967; Masner and Muesebeck 1968; Mineo 1980a; Masner 1983 (syn.); Johnson 1992.

Hadronotus atriscapus Gahan, 1927: *Gryon atriscapus* (Gahan): Muesebeck and Masner in Krombein and Burks 1967; Masner and Muesebeck 1968; Mineo 1980a (syn.); Masner 1983; Johnson 1992.

Gryon sp. affinis *pennsylvanicum* (Ashmead): Mineo 1990b.

Diagnosis. Horizontal part of occipital carina well-developed, curved mesad. Postoccipital carina weakly curved.

Description. Female. Length 1.6–1.8 mm.

Color. (Figs 1F, 2F). **Body** mainly black. A1–6, and legs (excluding coxae) yellow.

Head. FCI = 1.23–1.32; LCI = 1.46–1.65; DCI = 1.87–2.13; HW/IOS = 1.90–1.94; head about 1.3 times as wide as mesosoma (HW/TSL = 1.22–1.32). Frons (Fig. 3F) reticulate, setose, with transverse carina above frontal depression; central carina absent; frontal depression developed, transversely costate by weak irregular carinae. Vertex reticulate; interocellar space reticulate; hyperoccipital carina present; POL about seven times as long as OOL (POL/OOL = 6.59–7.73); OOL about 0.2 times as long as LOL (OOL/LOL = 0.22–0.26). Clypeus rectangular, with rounded corners. Gena reticulate, inside of cell coriaceous, with setae; medial genal carina absent. Occiput (Fig. 4F) transversely costate with setae; occipital carina complete, with angular point; horizontal portion of occipital carina well-developed, curved mesad, straight laterad, each arms fused; postoccipital carina well-developed, weakly curved, reaching fossa; postgena weakly striate longitudinally; postgenal sulcus straight; postgenal bridge smooth, weakly curvedly costate beside median sulcus. Antennae (Fig. 5F) clavate; A1 about 6.4 times longer than radicle, as long as clava; clava with five segments; claval sensilla formula A8–12/2–2–2–1; claval length about 3.9 times longer than width. Mandibles tridentate, anterior tooth longer than other teeth.

Mesosoma. Cervical pronotal area (Fig. 8E) smooth-imbricate; epomial carina strongly present, as redge; pronotal suprahumeral sulcus foveolate with setae; lateral

pronotal area narrow, smooth with transverse dense carina; pronotal cervical sulcus foveolate. Propleuron weakly imbricate. Mesoscutum (Fig. 7E) about 1.5 times as wide as long (TSL/ML = 1.45–1.59), with dense setae, reticulate-rugose; parascutal carina absent; notaulus absent. Mesoscutellum about 1.6 times as wide as long (SW/SL = 1.49–1.66), reticulate, with dense setae. Mesopleuron (Fig. 7F) costate above mesopleural canina, rugulose with sparse setae below mesopleural canina; prespecular and upper mesepisternal sulci foveolate; prespecular sulcus with setae; mesopleural carina present; postacetabular sulcus weakly foveolate. Metascutellum (Fig. 8F) weakly produced, longitudinally striate. Metapleuron randomly foveolate; anterior part of metapleural sulcus and posterodorsal metapleural sulcus with setae. Propodeum foveolate-rugulose. Fore wing (Fig. 6F): stigmal vein about 2.3 times longer than marginal vein; postmarginal vein about 4.3 times longer than marginal vein.

Metasoma. T1 (Fig. 9E) longitudinally striate, setose laterally. S1 (Fig. 9F) longitudinally striate. T2 longitudinally striate anteriorly, reticulate posteriorly, setose laterally. S2 with setae, granulate-punctate mesad, striate laterad. T3 reticulate, with setae laterad and posteriorly. S3–5 punctate with setae. T4 punctate-striate with setae. T5–6 punctate with setae. S6 smooth with setae.

Male. Almost same as female, but antennae (Fig. 5L) filiform; A1–11 yellow.

Host. Coreidae: *Anasa tristis* (De Geer, 1773), *Narnia femorata* Stål, 1862, *Leptoglossus corculus* (Say, 1832), *L. fulvicornis* (Westwood, 1842), *L. gonagra* (Fabricius, 1775) new record, *L. phyllopus* (Linnaeus, 1767), *L. occidentalis* Heidemann, 1910, *Chelinidea* sp.

Material examined. Okinawa pref., Ishigaki Isl., Ishigaki city, Maesato. 17.VII.1989. Koji Yasuda leg. emerged from an egg of *Leptoglossus gonagra* on 2.VIII.1989. 8♂38♀ [ELKU]. 1♂4♀ [IZAN].

Distribution. Japan (Ryukyus: Ishigaki Isl.): Canada (British Columbia), Italy (Tuscany: introduced), USA (Alabama, Arizona, Arkansas, California, Columbia D.C., Florida, Georgia, Louisiana, Maryland, Missouri, North Carolina, Pennsylvania (?), South Carolina, Tennessee, Texas), tropical areas of New World include Colombia, Dominican Republic, Brazil.

Remarks. Before this study, the known distribution of *G. pennsylvanicum* covered the Eastern, Midwest, Western and Southern USA, British Colombia, tropical areas of the New World (Masner 1983) and the Northern Italy (introduced as a natural enemy of *Leptoglossus occidentalis* Heidemann, 1910: Roversi et al. 2011). These localities are very far from Ishigaki Island. Hayashi and Kogure (2013) recorded *Xyphon reticulatum* (Signoret, 1854), a leafhopper originally distributed in the Southern Nearctic and the Northern Neotropical regions (Catanach et al. 2013), from the Southern Ryukyus including Ishigaki Island. This leafhopper is considered to introduced with some poaceous pastures (Hayashi and Kogure 2013). Japanese *G. pennsylvanicum* could have been accidentally introduced from the New World as well as *X. reticulatum*.

Key to species of Japanese *muscaeforme*-group of *Gryon*

- 1 Sculpture of frontal depression (Fig. 3A, B) irregular. Angular point of occipital carina (Fig. 4A, B) weakly projected **2**
- Sculpture of frontal depression regular (Fig. 3C). Angular point of occipital carina (Fig. 4C) modified as short sharp horn; horizontal portion of occipital carina straight, expanding inwardly..... ***G. yamagishii* Mineo, 1981**
- 2 Horizontal portion of occipital carina (Fig. 3A) curved, reaching longitudinal extension line of inner margin of lateral ocelli. Coxae (Fig. 2A) brown-black, same as body..... ***G. japonicum* (Ashmead, 1904)**
- Horizontal portion of occipital carina (Fig. 3B) straight, short, reaching longitudinal extension line of outer margin of lateral ocelli. Legs (including coxae yellow (Fig. 2A), same as other parts of legs..... ***G. fulvicoxa* sp. nov.**

Key to species of Japanese *charon*-group of *Gryon*

- 1 Horizontal portion of occipital carina (Figs 1D, 4D) short, reaching longitudinal extension line of outer margin of lateral ocelli or shorter. Postgena (Fig. 4D) almost smooth, longitudinally castate by weak furrows along postoccipital carina; postgenal sulcus curved toward hypostoma ***G. philippinense* (Ashmead, 1904)**
- Horizontal portion of occipital carina (Figs 1E, 4E) curved, reaching central longitudinal line of lateral ocelli. Postgena (Fig. 4E) smooth; postgenal sulcus straight; postgenal bridge smooth ***G. shisa* sp. nov.**

Discussion

Mineo (1981) established the *muscaeforme*-group based on the body sculpturing, the form and sculpturing of the frontal depression and mesosoma, and the form of the carinae on the occiput. Kononova and Kozlov (2008) provided another concept for the *muscaeforme*-group based on the presence of the hyperoccipital carina. As a result of our study, however, some members of *muscaeforme*-group sensu Kozlov and Kononova (*G. misha* (synonymized with *G. japonicum*), *G. japonicum*, and *G. yamagishii*) do not have the hyperoccipital carina. Species group concepts by Mineo are based on multiple well-defined characters, therefore, at least for the Palearctic species, the concept by Mineo is more practical.

While the hosts of some species are known, the life history of most Scelionidae in the field is unknown. Some specimens examined in this study were collected in winter. They were collected from under the bark of *Zelkova serrata* (Fig. 12), in the “Komomaki” (see above), and the underside of leaves of evergreen broad-leaved trees. Also, sometimes they showed overwintering aggregation with other species of Scelionidae such as *Trissolcus corai* Talamas, 2017, *T. cultratus* (Mayr, 1879), *T. gonopsidis*



Figure 12. Overwintering aggregation of *Gryon* and *Trissolcus* under the bark of *Zelkova serrata* in Akita prefecture (provided by J. Kobayashi).

(Watanabe, 1951), *T. japonicus* (Ashmead, 1904), *T. plautiae* (Watanabe, 1954), *Idris* sp., *Psilanteris* sp., and *Telenomus* sp. Therefore, natural cracks such as narrow slit under the bark or artificially created cracks such as “Komomaki” and corrugated fiberboards could provide suitable winter habitat for not only predators (Fye 1985; Togashi et al. 1988; Yoshimura et al. 1995; Korenko and Pekár 2010; Band Trap research group of Abiko Bird Museum 2012) but also parasitoids. In spring, some females collected from maple blossoms. Nectar of small blossoms could work as one of important energy sources for adults in this season. Further investigation on their life cycles is required to understand and enhance their functions as natural enemies.

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Appendix I

Checklist of Japanese *Gryon* with its host records.

Species group and species	Host species	Reference
<i>charon</i> -group		
<i>G. philippinense</i> (= <i>G. hakonese</i> syn. nov.)	<i>Acanthocoris sordidus</i> (Thunberg) <i>Homoeocerus marginellus</i> (Herrich-Schäffer) <i>Leptoglossus membranaceus</i> (Fabricius)	Mineo 1990b Nixon 1934 Kieffer 1926
<i>G. shisa</i> sp. nov.	<i>Paradasynus spinosus</i>	The present study
<i>floridanum</i> -group		
<i>G. pennsylvanicum</i>	<i>Anasa tristis</i> (De Geer) <i>Narnia femorata</i> Stål <i>Leptoglossus corculus</i> (Say) <i>L. fulvicornis</i> (Westwood) <i>L. gonagra</i> (Fabricius) † <i>L. phyllopus</i> (Linnaeus) <i>L. occidentalis</i> Heidemann <i>Chelinidea</i> sp.	Masner 1983 Masner 1983 Masner 1983 Masner 1983 Yasuda 1990 Masner 1983 Roversi et al. 2011 Masner 1983
<i>insulare</i> -group		
<i>G. hidakae</i>	Unknown	–
<i>G. insulare</i>	Unknown	–
<i>G. janus</i>	Unknown	–
<i>G. viggianii</i>	Unknown	–
<i>misellum</i> -group		
<i>G. misellum</i>	Unknown	–
<i>muscaeforme</i> -group		
<i>G. ruficoxa</i> sp. nov.	Unknown	–
<i>G. japonicum</i> (= <i>G. misha</i> syn. nov.) (= <i>G. orestes</i>) ‡	<i>Acanthocoris sordidus</i> (Thunberg) <i>Cletus trigonus</i> (Thunberg) <i>C. schmidti</i> Kiritshenko § <i>C. punctiger</i> (Dallas) § <i>Leptocoris chinensis</i> (Dallas) <i>Riptortus pedestris</i> (Linnaeus) ¶	Mineo 1990a Mineo 1990a Noda 1990b Noda 1990b Noda 1990b Noda 1989
<i>G. yamagishii</i> (= <i>G. marruzzae</i> syn. nov.) (= <i>G. sugonjaevi</i> syn. nov.)	Unknown	–
<i>myrmecophilum</i> -group		
<i>G. remotum</i>	Unknown	–
<i>pubescens</i> -group		
<i>G. nigricorne</i>	<i>Riptortus pedestris</i> (Linnaeus) ¶ <i>R. linearis</i> (Fabricius)	Higuchi et al. 1999 Noda 1989 #
Incertae sedis		
<i>G. ennius</i>	Unknown	–
<i>G. excertum</i>	Unknown	–
<i>G. marina</i>	Unknown	–
<i>G. tardum</i>	Unknown	–

† as *L. australis* (Fabricius). ‡ record based on misidentification. § result of sentinel egg tests in the field. ¶ as *R. clavatus* (Thunberg). # as *Gryon* sp.

First record of *Leptoomus janzeni* Gibson (Hymenoptera, Chalcidoidea) from Rovno amber

Serguei A. Simutnik¹, Evgeny E. Perkovsky¹, Dmitry V. Vasilenko^{2,3}

1 I.I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kiev, 01601 Ukraine
2 Borissiak Paleontological Institute, Russian Academy of Sciences, Moscow, 117647 Russia **3** Cherepovets State University, Lunacharsky Avenue, 5, Cherepovets, 162600 Russia

Corresponding author: Serguei A. Simutnik (simutnik@gmail.com)

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Abstract

The large and distinctive chalcidoid wasp *Leptoomus janzeni* Gibson, 2008, originally described from late Eocene Baltic amber, is newly recorded from coeval Rovno amber (Ukraine) based on a single well-preserved female specimen. Only 66 species of Rovno hymenopterans (49%) are also known from Baltic amber. High resolution photomicrographs and measurements of the specimen are given. Some character states of the new specimen, such as a green metallic coloration, a bare and flat prepectus, location and number of multiporous plate sensillae on the flagellum, sclerotized spur vein of the hind wing, and two metatibial spur are reported in this species for the first time.

Keywords

Baltic amber, Eocene, prepectus, spur vein, Ukraine

Introduction

Chalcidoidea (Hymenoptera) are currently divided into 23 extant families (Heraty et al. 2013; Janšta et al. 2018). Members of the families Cynipencyrtidae, Encyrtidae, Eupelmidae (except male Eupelminae), and Tanaostigmatidae share an enlarged, convex mesopleuron (acroleuron *sensu* Gibson 1986) and several other correlated

adaptations hypothesized to enhance jumping ability (Gibson 1986). It has sometimes been suggested that these taxa constitute a monophyletic group based on this enlarged mesopleuron (Trjapitzin 1968, 1989; LaSalle 1987). However, Gibson (1989) did not find any putative synapomorphies for the group as he defined it, and the morphological analysis of Heraty et al. (2013) retrieved the group as a monophyletic only if *Oodera* Westwood (Pteromalidae: Cleonyminae) was included. The combined morphological-molecular results of Heraty et al. (2013) did not retrieve this group as monophyletic, nor did the transcriptome-based phylogenies of Peters et al. (2018) and Zhang et al. (2020), suggesting that adaptations for jumping evolved independently.

Here, we report a large and well-preserved female from Rovno amber that we treat as conspecific with *Leptoomus janzeni* Gibson, 2008 (Chalcidoidea), originally described from Baltic amber. Gibson (2008) did a detailed analysis of the morphology of *L. janzeni* and compared it with that of *Cynipencyrtus* Ishii, 1928 (Cynipencyrtidae), Encyrtidae, Eupelmidae, and Tanaostigmatidae. This suggested that *Leptoomus* is likely in or near *Clade E*, sensu Heraty et al. (2013), the chalcid “jumpers” with an enlarged acropleuron. Gibson (2008, p. 24) proposed: “*until evolutionary relationships of the treated taxa are established more confidently by such studies it seems prudent to classify L. janzeni along with Cynipencyrtus in Tanaostigmatidae*”. Members of Tanaostigmatidae are distinguished in particular by an enlarged, bulbous prepectus projecting anteriorly beside the pronotum that resembles the prepectal structure of *L. janzeni* (Figs 1B, C, 2A: pre).

Material and methods

Ukrainian Rovno amber (Priabonian stage, 33.9–37.8 Mya) is the southern coeval of Baltic amber, from which *L. janzeni* was described. The amber containing the specimen of *L. janzeni* was found at the village of Velyki Telkovichi (Vladimirets Distr., Rovno Region, Ukraine) and is housed at the Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine, Kiev (SIZK). The localities and composition of the Rovno amber fauna were recently characterized in a series of reviews by Perkovsky et al. (2010), Jałoszyński and Perkovsky (2016), Perkovsky (2016, 2018) and Martynova et al. (2019). Including *Ektopicercus punctatus* Simutnik (Simutnik and Perkovsky 2020), and *L. janzeni*, 135 species of Hymenoptera are now known from Rovno amber, with 66 (49%) in common with Baltic amber (Radchenko and Perkovsky 2020; this paper).

Nearly all studied Rovno amber inclusions from Rovno Region were collected from Klesov and the Horyn River Basin (Perkovsky et al. 2010; Perkovsky 2017) except new collections from the more western basins of the Styr and Stokhod rivers and especially the Veselukha River floodplain between them (Lyubarsky and Perkovsky 2020). These new collections (mostly from Voronki and Velyki Telkovichi) revealed a number of new species of beetles, neuropterans and snakeflies (Jałoszyński and Perkovsky 2019; Legalov et al. 2019; Colombo et al. 2020; Perkovsky and Makarkin 2019,

2020; Makarkin and Perkovsky 2020; Lyubarsky and Perkovsky 2020; Radchenko and Khomich 2020; Perkovsky et al. 2020) as well as new mosses and liverworts (Mamontov et al. 2020 and references therein), including the first named amber *Sphagnum* from Velyki Telkovichi and some species previously recorded from Baltic amber (Perkovsky and Olmi 2018; Martynova et al. 2019; Mamontov et al. 2020) or from Baltic and Bitterfeld ambers (Radchenko and Perkovsky 2018, 2020).

Photographs were taken using a Leica Z16 APO stereomicroscope with a Leica DFC 450 camera and processed with LAS V3.8 software. To improve imaging, we applied sucrose syrup of approximately the same refractive index as the amber and placed a glass coverslip on top; after photography, the syrup was removed using warm water. Some images were then enhanced (brightness and contrast only) using Adobe Photoshop.

Terminology and abbreviations follow Gibson (1997), Noyes et al. (1997), and Heraty et al. (2013). The following abbreviations are used in the text and illustrations:

OOL	minimum distance between an eye margin and the adjacent posterior ocellus;
POL	minimum distance between the posterior ocelli;
OCL	minimum distance between a posterior ocellus and the occipital margin;
LOL	minimum distance between the anterior ocellus and a posterior ocellus;
F1, F2, etc.	funicular segments 1, 2, etc.;
mps	multiporous plate sensilla;
mspl	mesopleuron;
pre	prepectus;
spv	spur vein.

Results

Taxonomy

Chalcidoidea Latreille, 1817

Leptoomus janzeni Gibson, 2008

Figs 1, 2A–E

Material examined. SIZK VT-95, 1 ♀, Velyki Telkovichi, Rovno amber; late Eocene. The inclusion is in a clear amber piece (about of 30 × 14 × 8 mm) of irregular shape (Fig. 1A). A syninclusion consists of a precariously preserved small insect with only the legs visible.

Measurements. Body length 2.45 mm; other reported measurements are relative (one micrometer division = 0.014 mm) and are approximate because of optical effects in the amber.

Head. Head length 26, width 46, height 38; eye height 15; malar space 15; posterior ocellus diameter 3; OOL 1.5; POL 7; OCL 6; LOL 5; distance between toruli 7,

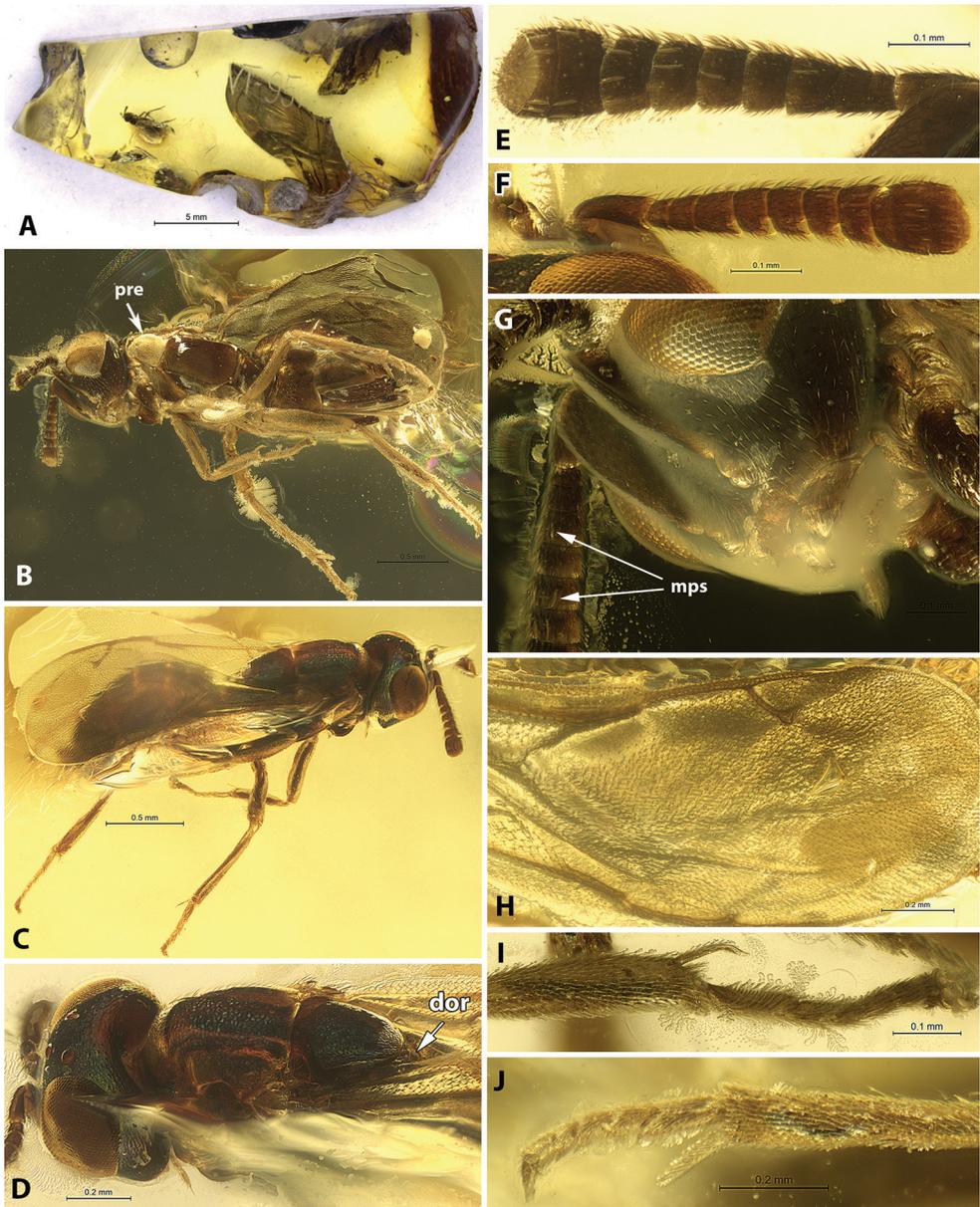


Figure 1. *Leptoomus janzeni*, female VT-95 from Rovno amber (deposited in SIZK) **A** piece of amber containing the specimen **B** habitus ventrolateral (left side) **C** habitus dorsolateral (right side) **D** head and mesosoma dorsolateral, dorsellum **E** antenna ventral **F** antenna dorsal **G** head frontolateroventral, part of antenna with mps arrowed **H** forewing **I** protibial spur, protarsus **J** mesotibial spur, mesotarsus.

between torulus and eye 9, from torulus to mouth margin 6; length to width ratio of scape 25:7, pedicel 8:4, F1 2:4, F2 3:4.5, F3 4:5, F4 3:5, F5 3:5.5, F6 3:6, F7 3:7 (very approximately), clava 7.5:10, micropilose sensory region 5:7.5.

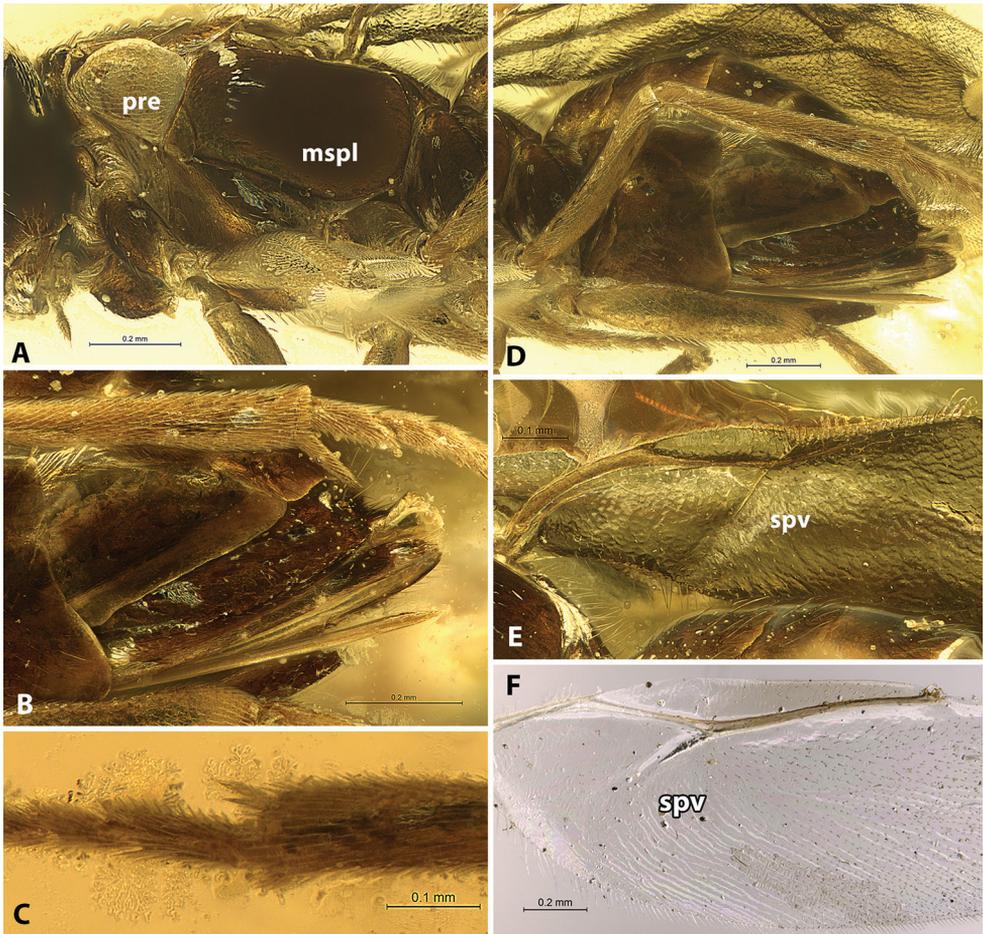


Figure 2. A–E *L. janzeni*, female **A** mesosoma ventrolateral **B** apex of metasoma lateroventral **C** metatibial apex with two spurs **D** metasoma lateral **E** venation of hind wing with spur vein **F** *Pentacladia* sp., female, venation of hind wing.

Mesosoma. Mesosoma length 68; length to width ratio of mesoscutum 30:30, scutellum 26:21; prepectus maximal length 16, height 15.

Appendages. Length to width ratio of fore wing 120:55; mv:pmv:stv about 22:18:15; length to width ratio of hind wing 77:25; protibia 30, protibial spur (calcar) 8; mesotibia 48, mesobasitarsus 12, mesotibial spur 11.

Metasoma. Length 77, height 45 (lateral view), width 45; ovipositor stylet from hypopygium margin 41.

Comparison with *L. janzeni* type material. The Rovno amber specimen differs from the Baltic amber material by having slightly infuscate, brownish, rather than hyaline forewings. The head and thorax have a distinct green metallic sheen not seen in the Baltic specimen (Figs 1C, D). Multiporous plate sensilla on the flagellum were not described by Gibson (2008), but are visible in the Rovno specimen on F3–F7, and on

the apical two segments of the 3-segmented clava (Figs. 1E, F, G); F4 appear to have only a single mps but the others have multiple mps in a single row per segment that does not fully encircle the segment.

Also, in the Rovno specimen the spur vein originating from the marginal venation of the hind wing is visible (Fig. 2E: spv). In addition to Tanaostigmatidae, some Pteromalidae (for example, *Nasonia*), and some Eupelmidae (e.g. *Pentacladia*, Fig. 2F: spv) also have a similar sclerotized spur vein.

The only uniquely shared feature of *L. janzeni* and Tanaostigmatidae is that in both the prepectus extends anteriorly, exterior to pronotum, though in *L. janzeni* it is flatter and its lateral panel is bare (Figs 1B, 2A: pre).

The metatibia of the new specimen has two spurs (Fig. 2C). This character *L. janzeni* shares the with many other chalcidoid taxa.

The metanotum is not clearly visible because the wings are positioned over the gaster. The dorsellum (Fig. 1D: dor) appears to taper posteriorly to fit into a broadly incised anterior margin of the propodeum such that the medial length of the dorsellum is greater than the medial length of the propodeum.

Conclusions

The set of morphological features possessed by *L. janzeni* places the taxon in the “jumpers” Clade E sensu Heraty et al. (2013). As previously shown by Gibson (2008), *L. janzeni* appears to be close to Tanaostigmatidae. To establish the position of *L. janzeni* on the chalcidoid tree, further research is needed with additional fossil and molecular data.

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A study of the biology of *Epicharis (Epicharoides) picta* using emergence-traps

Hugo de Azevedo Werneck^{1,2}, Lucio Antonio de Oliveira Campos¹

1 Departamento de Biologia Geral, Universidade Federal de Viçosa, Avenida Ph Rolfs s/n, 36.570-000, Viçosa, MG, Brazil **2** Departamento de Entomologia, Universidade Federal de Viçosa, Avenida Ph Rolfs s/n, 36.570-000, Viçosa, MG, Brazil

Corresponding author: Hugo de Azevedo Werneck (beehugo@gmail.com)

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Abstract

This study investigates the nesting habits of *Epicharis picta* in a nest aggregation located in a fragment of the Atlantic forest in Southeastern Brazil. Ten emergence-traps were set up in this nest aggregation to standardize data collection of phenology, natural enemies, and sex ratio. *Epicharis picta* nests were in an area of 160 m² with a density of 41 nests/m². Nest and cell architecture are described. *Epicharis picta* is a protandrous, univoltine species with its emergence in this study occurring between 28 January and 15 April. We provide direct evidence of parasitism on *E. picta* by *Rhathymus friesei*, *Tetraonyx sexguttata* and *T. aff. lycoides*. The predator *Apiomerus lanipes* was found to prey *Epicharis* for the first time. We suggest the use of emergence-traps as tools to support studies of ground-nesting bees. In addition, we compile, update, and discuss data on the nesting biology of all *Epicharis* subgenera.

Keywords

Cleptoparasitism, Emergence-trap, Ground-nesting bees, Nest architecture, Solitary bees

Introduction

Solitary bees use a wide variety of nesting substrates, digging their nests in soil or wood, constructing freestanding nest, or using pre-existing cavities (Michener 1974, 2007). In fact, most of them nest in the ground (Linsley 1958; Batra 1984; Roubik 1989; Michener 2007), which may be a plesiomorphic condition among bees

(Michener 1964, 2007; Radchenko 1996). This variety of nesting habits gives rise to a wide diversity of life cycles, nest architecture, nesting behaviors, and relationships with their natural enemies.

Centridini is a neotropical tribe of oil-collecting bees, composed of only two well-supported monophyletic genera (Moure et al. 2012; Bossert et al. 2019) of solitary bees: *Epicharis* Klug, 1807, which dig their nests in the soil; and *Centris* Fabricius, 1804, composed mostly of some species that dig their nests in the soil or sometimes in termite nests (e.g., Rozen and Buchmann 1990; Gaglianone 2001; Aguiar and Gaglianone 2003; Rozen et al. 2011) and some that use pre-existing cavities (e.g., Costa and Gonçalves 2019). Even though nesting habits tend to be constant in some species, *Centris*' nesting behavior has some plasticity (Vinson and Frankie 1991; Martins et al. 2014).

Epicharis has nine subgenera (*Anepicharis* Moure, 1945, *Cyphepicharis* Moure, 1945, *Epicharana* Michener, 1954, *Epicharis* Klug, 1807, *Epicharitides* Moure, 1945, *Epicharoides* Radoszkowski, 1884, *Hoplepicharis* Moure, 1945, *Parepicharis* Moure, 1945 and *Triepicharis* Moure, 1945), with a total of 36 species described (Moure et al. 2012; Laroca and Nery 2018). However, there are available data on nesting biology for only 11 of them. Most species studied dig their nests exclusively in sandy soils (Roubik and Michener 1980; Raw 1992; Hiller and Wittmann 1994; Gaglianone 2005; Rocha-Filho et al. 2008; Rozen 2016; Dec and Vivallo 2019; Martins et al. 2019; Vivallo 2020a), except for *E. (Epicharana) rustica* (Olivier, 1789) (Michener and Lange 1958), *E. (Epicharana) flava* Friese, 1900 (Camargo et al. 1975), *E. (Hoplepicharis) fasciata* Lepeletier & Serville, 1828 (Vesey-FitzGerald 1939; Rozen 1965; Vivallo 2020b), *E. (Epicharitides) obscura* Friese, 1899 (Laroca et al. 1993), and *E. (Parepicharis) metatarsalis* Friese, 1899 (Thiele and Inouye 2007).

Univoltinism is the phenological pattern observed for most species of *Epicharis* (Roubik and Michener 1980; Raw 1992; Hiller and Wittmann 1994; Gaglianone 2005; Gaglianone et al. 2015; Vivallo 2020a, b), except multivoltinism for *E. flava* (Camargo et al. 1975) and bivoltinism for *E. (Epicharis) bicolor* Smith, 1854 (Rocha-Filho et al. 2008). Adult short-term activity may be a strategy that minimizes exposure to attacks by parasitic species (Wcislo 1987).

The compilation made by Gaglianone (2005) on the data known for *Epicharis*' nesting biology, does not point to any clear patterns among its nine subgenera. Hence there is a need for an increase in the quantity of species studied, particularly with regard to number of generations per year, presence or absence of diapause in the immature stages, type of soil used as substrate, depth of brood cells, number of cells per nest, plus other biological data that may aid us comparisons within and between subgenera of *Epicharis*. In addition, a phylogenetic approach of these characters compared to phylogenetic studies on Centridini (e.g., Martins and Melo 2016) can elucidate evolutionary aspects of these bees.

The natural enemies of *Epicharis*, include parasitoids, cleptoparasitic, and predatory insects. Bees of the genus *Rhathymus* (Apidae, Rhathymini) are known to be specialized cleptoparasites of *Epicharis*' nests (compiled by Werneck et al. 2012),

although there are also more generalized cleptoparasites, such as those of the genus *Mesoplia* Lepeletier, 1841 (Apidae, Ericrocidini) which also attack nests of *Centris* (Gaglianone 2005; Rocha-Filho et al. 2008; Rocha-Filho et al. 2009; Vivallo 2020a). Besides bees, there are records of *Epicharis* as a host for both cleptoparasitic species of Meloidae (Coleoptera) and parasitoid species of Conopidae (Diptera) and Mutillidae (Hymenoptera) (Gaglianone 2005; Rocha-Filho et al. 2008; Gaglianone et al. 2015). Despite the scarcity of information in the literature, species of Hemiptera, especially those of the genus *Apiomerus* (Reduviidae), are known to be predators of adult bees (Silva and Amaral 1973; Cane 1986; Amaral-Filho et al. 1994; Marques et al. 2003, 2006; Silva and Gil-Santana 2004).

Studies on the nesting biology, relationships with natural enemies, and phenology of *Epicharis* species have been performed using direct observations of nest aggregations. Nonetheless, there is a need for methods that provide standardization for data collection. To this aim, emergence-traps have been used in ground-nesting solitary bees and wasps studies and have shown to be effective in answering key questions on the biology of these insects (Hiller and Wittmann 1994; Sardiña and Kremen 2014; Rocha-Filho and Melo 2017; Cope et al. 2019).

Epicharis picta occurs in Uruguay, Paraguay, Argentina, and Brazil (Federal District and the states of Espírito Santo, Minas Gerais, Paraná, Paraíba, Rio de Janeiro, Santa Catarina, and São Paulo – Moure et al. 2012). Its life cycle is univoltine (Gaglianone et al. 2015) and it is oligolectic on Malpighiaceae (Werneck et al. 2015). However, data on its nesting behavior, nests and cell architecture, and natural enemies are scarce in the literature.

In this study, we investigated the nesting habits of *Epicharis picta* for two years and provide information on its biology, natural enemies, nest and cell architecture, sex ratio, and phenology, based on direct observations and emergence-traps data. An updated compilation of nesting biology and nest architecture data of the *Epicharis* genus is also provided.

Material and methods

Study site

The nest aggregation of *Epicharis picta* studied was in a fragment of semideciduous, montane and submontane Atlantic Forest (Veloso et al. 1991), in the municipality of Viçosa, Minas Gerais-Brazil, on the access road to the Estação de Pesquisa, Treinamento e Educação Ambiental Mata do Paraíso (20°47'56"S, 42°52'07"W) (see Gaglianone et al. 2015). The climate of the region is temperate, rainy (mesothermal), with hot and rainy summers, and cool and dry winters (type Cw, according to the classification of Köppen – Kottek et al. 2006). The climatological data of the region between 2009 and 2011 are shown in Fig. 1.

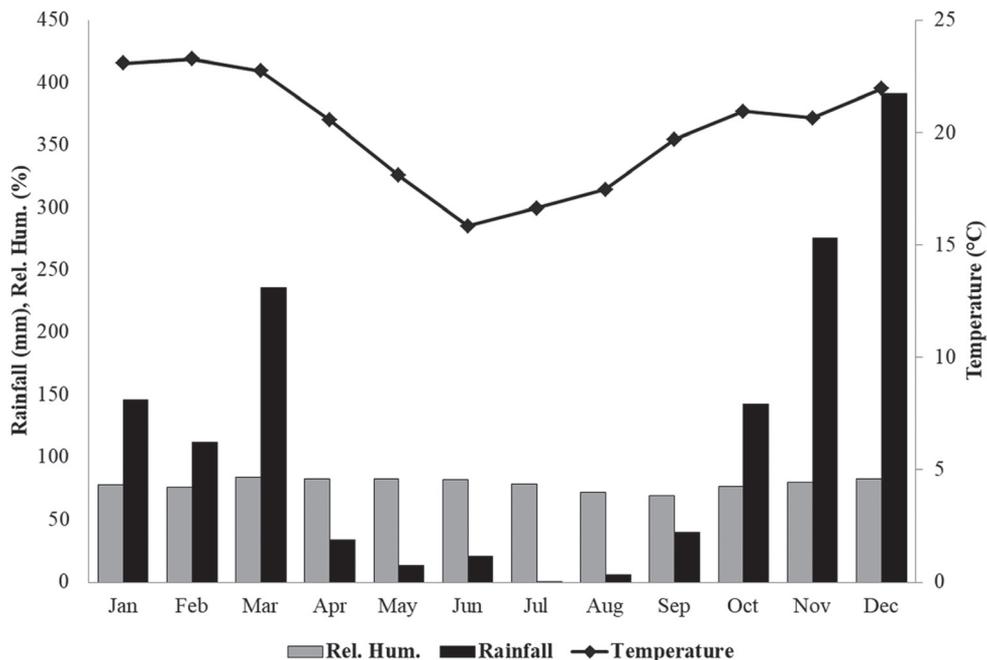


Figure 1. Climatological data from Viçosa, Minas Gerais-Brazil, between 2009 and 2011 (Source: Estação Climatológica Principal de Viçosa, Departamento de Engenharia Agrícola – UFV).

Nesting biology, nest architecture, and brood cells

The fieldwork was carried out for two years. In 2010, visits took place monthly; and in 2011, daily, during the period of activity of the adult bees. The nesting habits of *E. picta* were obtained from direct observations throughout each day from 5:30 h to 19:00 h. To study the brood cells, eight excavations were made in 50 cm × 50 cm plots. The brood cells found in the soil were collected, placed in plastic pots with substrate from the nesting site, and kept in the laboratory to assess their content and dimensions. For nest architecture, as the main burrow remains open after a nest is completed, we injected plaster in five nests. Ten plots of 1 m² were randomly set up in the aggregation to estimate nest density.

Emergence-traps, natural enemies, and associated species

To standardize the sampling method when studying phenology, sex ratio, and parasite-host synchrony, 10 emergence-traps were randomly set up at the nesting site from 20 January to 20 May 2011 (Fig. 2A). The emergence-traps consisted of a pyramid-shaped wooden frame covered with thin nylon mesh, with the following dimensions: 50 cm × 50 cm base, 10 cm × 10 cm top end, and 50 cm height (Fig. 2B). On one side, a 12 cm long opening was made in the longitudinal direction to install a Velcro



Figure 2. Emergence-traps set up on aggregate of *Epicharis (Epicharoides) picta* (A); Detail of emergence-trap (B).

tape, forming a “window” that allowed our access to its interior. (Fig. 2B). A piece of PVC pipe attached to a plastic container was placed at the upper end with its bottom facing the interior of the trap and its top inside the plastic container, which was filled with a 1:1 alcohol and water solution (Fig. 2B). Inspections on emergence-traps were conducted daily.

Data on potential natural enemies and associated species that were seen visiting the nest aggregation area were collected throughout the study period. Vouchers from the specimens studied are deposited at Museu Regional de Entomologia, Departamento de Entomologia-Universidade Federal de Viçosa (MEUFV). This study follows the classification system of Moure et al. (2007), which treats the whole group of bees as a single family (Apidae).

Results

Nesting biology, nest architecture, and brood cells

Epicharis picta nests were aggregated in an area of approximately 160 m² of exposed slopes of about 45°, with an average nesting density of 41 entrances/m². Females began their activities between 6:00 h and 6:30 h and ended between 18:00 h and 18:30 h, daily. The activity peak occurred between 7:30 h and 11:30 h. The females rested inside the nests at night (Fig. 3A). Male activity period was shorter, between 6:00 h and 16:00 h. The mating male behavior is being dealt in a separate study. Every day at the beginning of activities (~6:00 h), females took about five minutes at the nest entrance before making their first flight. After their first trip, their scopae were still clean when they returned, with no evidence of oil or pollen having been collected (Fig. 3B). For digging new nests, they selected a new nest site, excavated the soil vertically, using their mandibles, anterior and middle legs, and deposited the material from the excavation around the nest entrance, forming a circular tumulus (Fig. 3C, D).

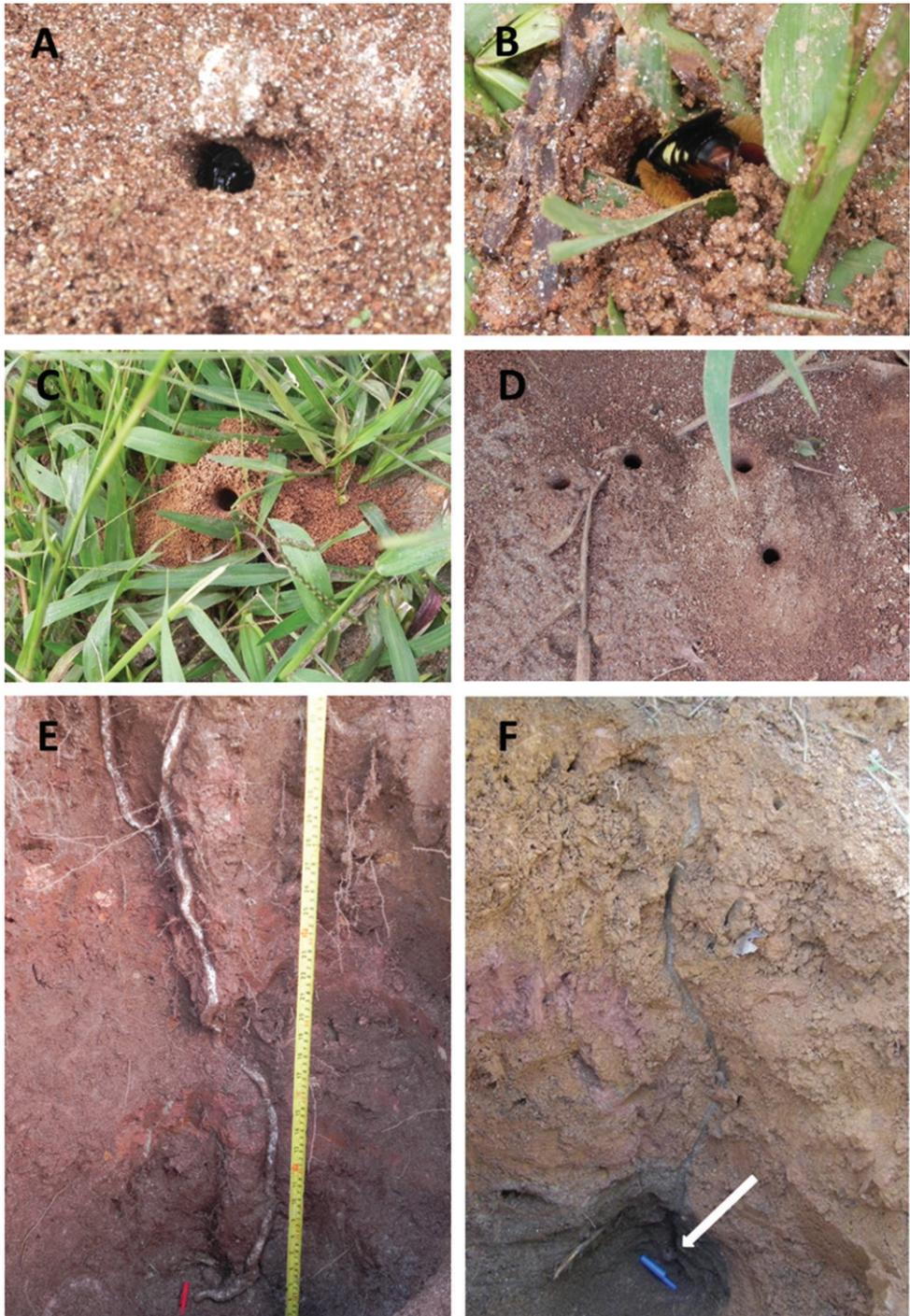


Figure 3. Nesting habits and nest architecture of *Epicharis (Epicharoides) picta* **A** female resting inside the nests at night **B** female of *E. picta* excavating nest **C, D** entrance of the nests showing the presence of a tumulus **E, F** architecture of 110 cm deep nests.

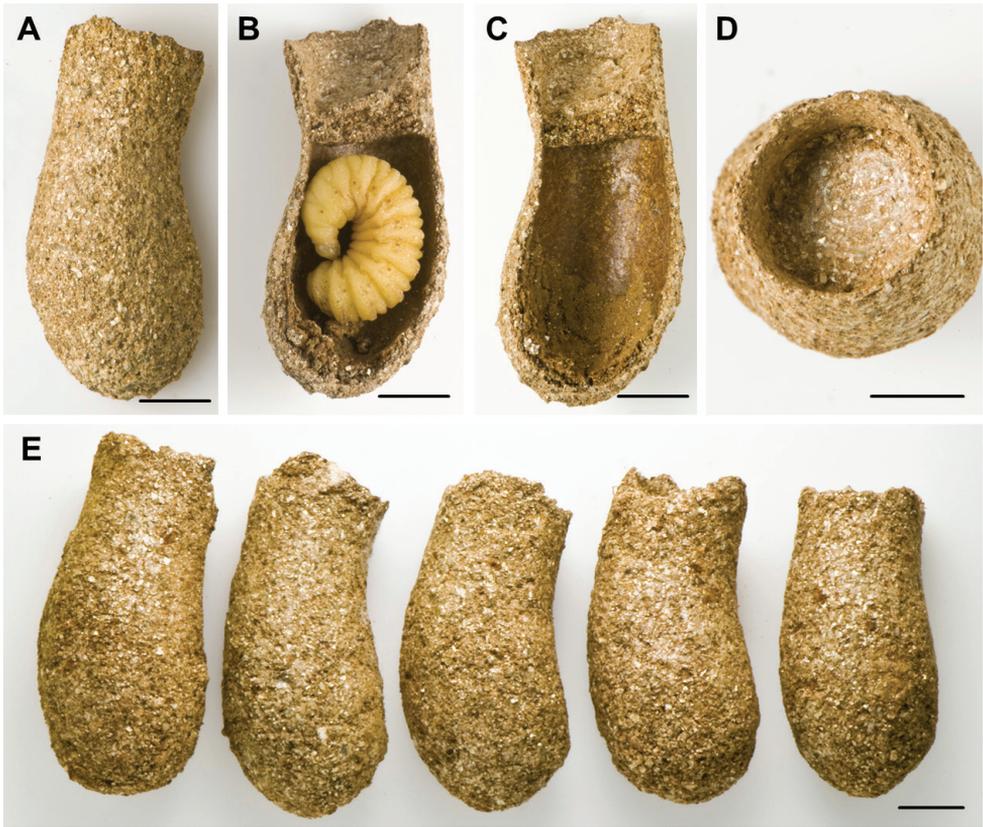


Figure 4. Brood cells of *Epicharis (Epicharoides) picta* **A** outer cell wall surface **B** mature larva inside the cell **C** inner cell wall surface **D** the cell cap inserted below the apex of the cell wall **E** variation in brood cell shape. Scale bars: 5 mm.

The excavated nests ($N = 8$) contained one to two cells each. In nests with only one cell ($N = 6$), there was a single tunnel (Fig. 3E, F), whereas in nests containing two cells at the end of the main tunnel ($N = 2$), a branch with no cell was also found. The diameter of these tunnels ranged from 10 mm to 12 mm ($N = 20$). The nests were perpendicular to the surface, ranging from 50 cm to 110 cm deep.

The brood cells were slightly curved (Fig. 4A–E) and built at an angle of about 45° to the ground surface. Their outer walls are rough and rigid, whereas inner walls are smooth, shiny, and hydrophobic (Fig. 4A–C). The cell cap is inserted below the apex of the cell wall and is slightly inclined (Fig. 4D). The cells size ranged from 20 mm to 27 mm ($\bar{X} = 24.57$ mm; $N = 27$) in length, between 10 mm and 12 mm ($\bar{X} = 11.12$ mm; $N = 27$) in diameter at cell cap height, and the base diameter between 12 mm and 14.5 mm ($\bar{X} = 13.74$ mm; $N = 27$).

Females of *Epicharis (Epicharoides) albofasciata* were observed founding nests ($N = 14$) in the aggregation of *E. picta*. Only one nest of *E. albofasciata* was excavated, and it consisted of a single 35 cm deep tunnel with one cell at its end. Agonistic

behavior among females of *E. picta* and *E. albofasciata* was observed when females returned from the field. *E. albofasciata* males were neither observed nor collected on the nest aggregation during fieldwork.

Emergence-traps, phenology, and sex ratio

Ten emergence-traps were set in the aggregation for 121 days, between 20 January and 20 May 2011. The emergence period in the traps was from 28 January to 15 April 2011. The emergence peak, encompassing all species, occurred from 19 February to 18 March (Fig. 5A). From the 271 individuals that emerged in the emergence-traps, *E. picta* was the most abundant species (211, 78%), followed by *Rhathymus friesei* (23, 8.5%), *Tetraonyx sexguttata* (Meloidae) (18, 6.65%), *Physocephala* sp. (Conopidae) (7, 2.55%), *Tetraonyx* aff. *lycoides* (5, 1.85%), *E. albofasciata* (3, 1.10%), *Augochlora thalia* (Apidae, Augochlorini) (2, 0.71%), *Acamptopoeum prinii* (Apidae, Calliopsini) (1, 0.32%), and *Epinysson* sp. (Crabronidae, Nyssonini) (1, 0.32%).

Epicharis picta first appeared in the emergence-traps on 29 January, and males were the first to emerge (Fig. 5B), indicating protandry. From a total of 211 individuals, 111 were male (52.6%), and 100 female (47.4%). Thus, the sex ratio of *E. picta* was 1.11 males to 1 female. *Rhathymus friesei* emerged from 5 February to 18 March, peaking from 26 February to 11 March. Males were the first to emerge, on 5 February, whereas females emerged from 19 February, which also indicates protandry (Fig. 5C). From the 23 emerged individuals, 13 were males (56.53%) and 10 were females (43.47%), resulting in a sex ratio of 1.3 males to 1 female.

Natural enemies and associated species sampled from direct observations

In addition to species sampled from the emergence-traps, we collected another 24 species of insects found in the area, which were then identified and classified according to their association with the nesting aggregation (Table 1). *Rhathymus friesei* was the most abundant natural enemy (Fig. 6A); in some moments, up to four individuals could be seen inspecting the nest aggregation at the same time. *Tetraonyx sexguttata* was observed walking on the aggregation, landing on vegetation, and emerging from *E. picta* nests (Fig. 6B). Individuals of *Apiomerus lanipes* were observed five times near the entrances of the nests capturing females of *E. picta* when those entered or left their nest. The predator attacked the prey with its forelegs, inserting the stylet between the thorax and the head (Fig. 6C). Twenty-seven individuals of Mutillidae were collected in the aggregation. However, only one female of *Traumatomutilla* sp. was observed directly inspecting *E. picta* nests (Fig. 6D). *Physocephala* sp. specimens were also observed flying over the nest site between 5 February and 22 March.

Females of *Augochloropsis* cf. *cupreola* (Apidae, Augochlorini), *Hypanthidium nigrifulum* (Apidae, Anthidiini), and *Colletes petropolitanus*, (Apidae, Colletini) were observed performing inspection flights over the soil and branches of vegetation in the aggregation, but they neither nested nor interacted with *E. picta* females. *Trigona spinipes*

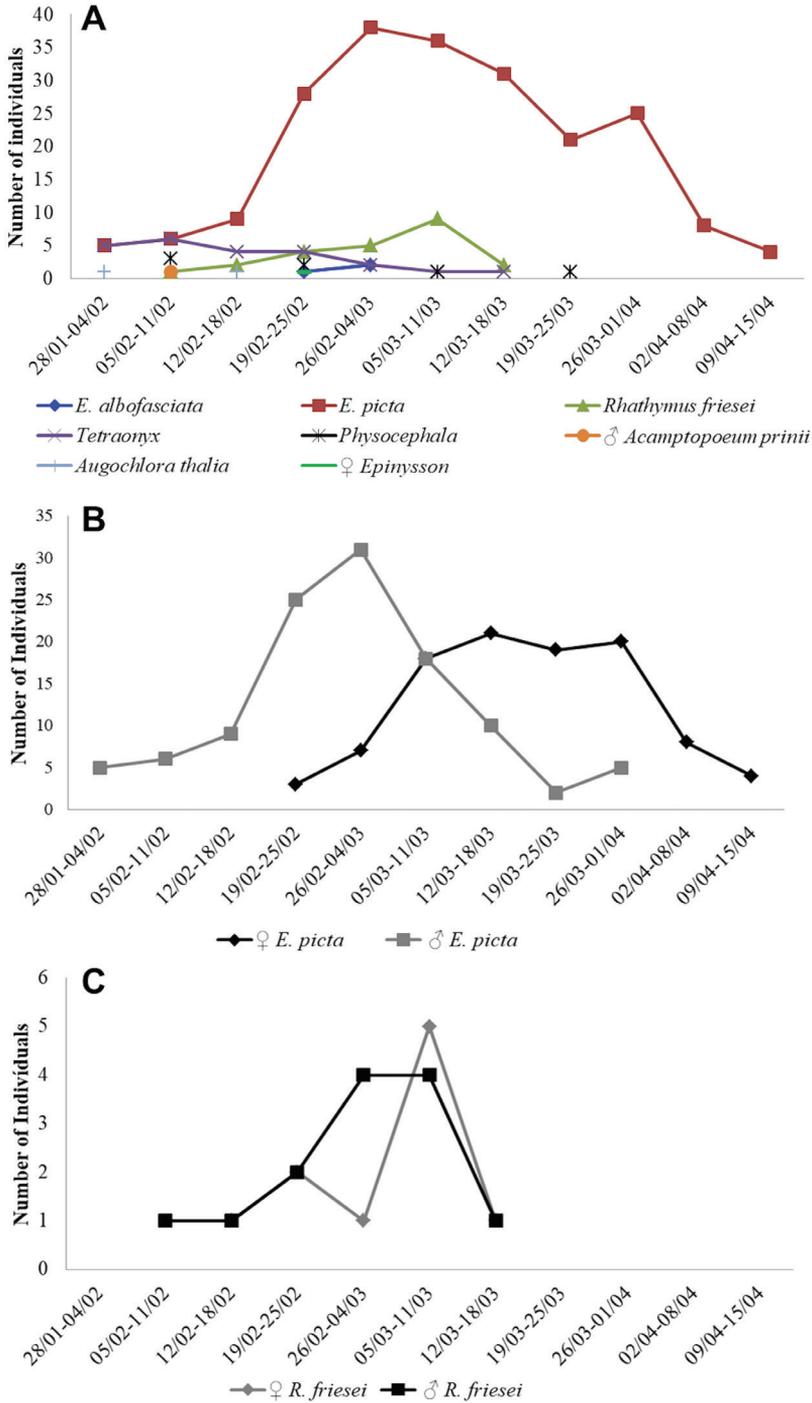


Figure 5. Emergence patterns in the emergence-traps set up on the nest aggregation of *Epicharis (Epicharoides) picta* **A** emergence of all species sampled in the traps **B** emergence of the males and females of *E. picta* **C** emergence of the males and females of *Rhathymus friesei*.

Table 1. Natural enemies and associated species from a nesting aggregation of *Epicharis* (*Epicharoides*) *picta*. Type of evidence for potential parasitoids, claptoparasites or predators: *Indirect evidence; **Direct evidence.

Order	Family	Tribe	Species	Type of association		
Diptera	Conopidae	Physocephalini	<i>Physocephala</i> sp.	Parasitoid*		
Coleoptera	Meloidea	Tetraonycini	<i>Tetraonyx</i> (<i>Tetraonyx</i>) <i>sexguttata</i> (Olivier, 1795)	Cleptoparasite**		
			<i>Tetraonyx</i> aff. <i>lycoides</i>	Cleptoparasite**		
Hemiptera	Reduviidae	Apiomerini	<i>Apiomerus lanipes</i> (Fabricius, 1803)	Predator**		
Hymenoptera	Apidae	Anthidiini	<i>Hypanthidium nigrifulum</i> Urban, 1998	No direct association		
		Augochlorini	<i>Augochlora thalia</i> Smith, 1879	No direct association		
			<i>Augochloropsis</i> cf. <i>cupreola</i> (Cockerell, 1900)	No direct association		
		Calliopsini	<i>Acamptopoeum prinii</i> (Holmberg, 1884)	No direct association		
		Centridini	<i>Epicharis</i> (<i>Epicharoides</i>) <i>albofasciata</i> Smith, 1874	No direct association		
		Colletini	<i>Colletes petropolitanus</i> Dalla Torre, 1896	No direct association		
		Ericrocidini	<i>Mesoplia rufipes</i> (Perty, 1833)	Cleptoparasite*		
		Meliponini	<i>Trigona spinipes</i> (Fabricius, 1793)	No direct association		
		Rhathymini	<i>Rhathymus friesei</i> Ducke, 1907	Cleptoparasite**		
		Crabronidae	Larrini	<i>Tachysphex</i> sp. 1	No direct association	
				<i>Tachysphex</i> sp. 2	No direct association	
				<i>Tachysphex</i> sp. 3	No direct association	
		Mutillidae	Nyssonini	<i>Epinysson</i> sp.	No direct association	
				Ephutini	<i>Ephuta</i> sp. 1	Parasitoid*
					<i>Ephuta</i> sp. 2	Parasitoid*
				Sphaerophthalmini	<i>Hoplocrates cephalotes</i> (Swederus, 1787)	Parasitoid*
					<i>Hoplomotilla spinosa</i> (Swederus, 1784)	Parasitoid*
					<i>Pseudomethoca macropis</i> (Gerstaecker, 1874)	Parasitoid*
					<i>Pseudomethoca</i> sp. 1	Parasitoid*
					<i>Pseudomethoca</i> sp. 2	Parasitoid*
<i>Pseudomethoca</i> sp. 3	Parasitoid*					
<i>Traumatomutilla inermis</i> (Klug, 1821)	Parasitoid*					
<i>Traumatomutilla</i> sp.	Parasitoid*					
<i>Traumatomutilla trochantera</i> (Gerstaecker, 1874)	Parasitoid*					
Sphecidae	Ammophilini			<i>Ammophila</i> sp.	No direct association	
		Sphecini	<i>Sphex</i> sp.	No direct association		
Vespidae	Eumenini		<i>Pirhosigma superficiale</i> (Fox, 1899)	No direct association		

(Apidae, Meliponini) workers landed on the nest site and collected soil material removed by *E. picta* females during the excavation of their nests. A female of *Mesoplia rufipes* was collected on 20 March 2010. This bee flew over the aggregation and periodically approached some entrances of *E. picta* nests. However, it was not seen entering any nest.

Brood cells collected during excavations

In the two years of studies, a total of 121 cells were collected, ranging between 30 cm and 110 cm deep. From these, 45 were already open, containing only soil in their interior. Six cells were taken by fungi, one of which contained a dead *E. picta* female pupae (Fig. 7A). Another six cells containing only fungi on the food (Fig. 7B) with no evidence of dead larvae or egg, nor parasitic traces. In one cell there was an exoskeleton of *T. sexguttata* (Fig. 7C). In the 46 cells, there were 38 mature larvae (Fig. 4B) and eight pupae of *E. picta*. From the 17 cells kept in the laboratory throughout



Figure 6. Natural enemies and associated species of *Epicharis (Epicharoides) picta* **A** *Rhathymus friesei* inspecting at the nest aggregation **B** *Tetraonyx sexguttata* emerging from a nest of *E. picta* **C** *Apiomerus lanipes* preying on a female of *E. picta* **D** *Traumatotutilla* sp. inspecting the entrance of a nest of *E. picta*.

the year, four males (Fig. 7D) and six females of *E. picta*, two *T. sexguttata*, and one *T. aff. lycoides* emerged. In the remaining four cells, there were mature larvae of *R. friesei*. Larvae of *R. friesei* were easy to identify due to the presence of their cocoon (see Rozen 1969; Camargo et al. 1975; Werneck et al. 2012).

Discussion

Studies reporting biological data about *E. picta* are recent in the literature (Werneck et al. 2012; Werneck et al. 2015; Gaglianone et al. 2015). These, however, do not address aspects related to their nesting habits, natural enemies, and associated species. Therefore, this present study is the first to bring such data, in addition to using emergence-traps as a model.

Nesting biology and notes on patterns among *Epicharis* subgenera

Our data on *E. picta* reinforce the hypothesis that all species of *Epicharis* nest gregariously in the soil (Vesey-FitzGerald 1939; Michener and Lange 1958; Rozen 1965;

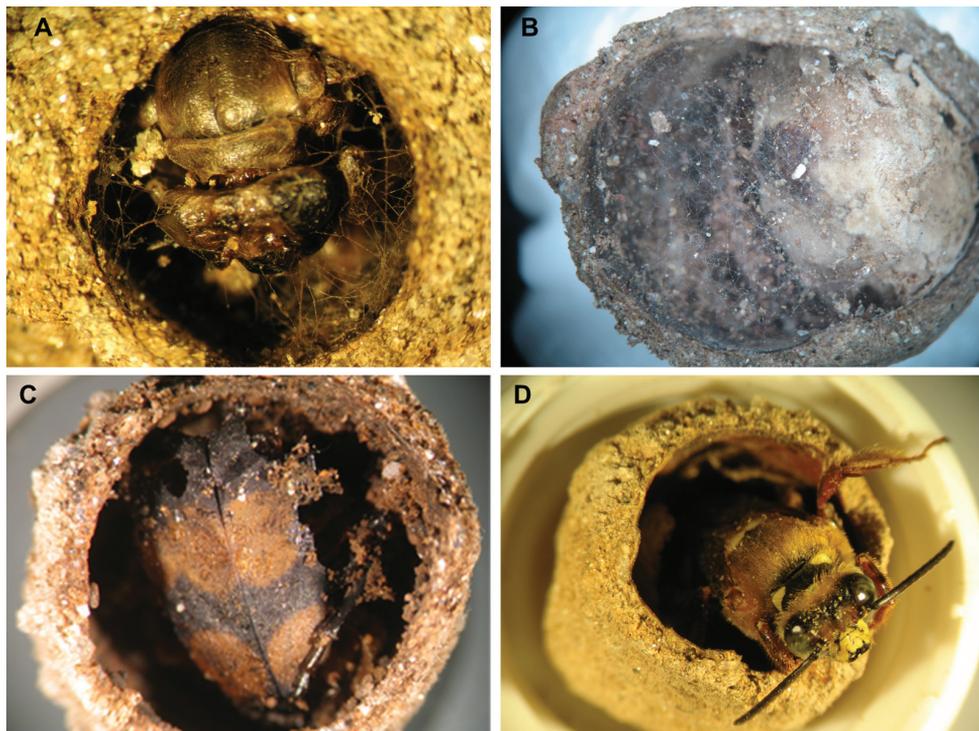


Figure 7. Content of *Epicharis (Epicharoides) picta* brood cells **A** dead *E. picta* female pupa with fungus **B** food in *E. picta* brood cell taken by fungus **C** exoskeleton of *Tetraonyx sexguttata* **D** male of *E. picta* emerged from brood cell maintained in laboratory.

Camargo et al. 1975; Roubik and Michener 1980; Raw 1992; Laroca et al. 1993; Hiller and Wittmann 1994; Gaglianone 2005; Thiele and Inouye 2007; Rocha-Filho et al. 2008; Rozen 2016; Dec and Vivallo 2019; Martins et al. 2019; Vivallo 2020a, b). In spite of the low quantity of species studied, it is possible to point to a pattern on the exclusive use of sandy soil for nesting by the subgenera *Epicharoides*, *Epicharis*, *Triepicharis* and *Anepicharis* (Table 2). Although *Parepicharis* also uses sandy soil, there are records of *E. metatarsalis* nesting in clay (Thiele and Inouye 2007). For *Epicharana*, there seems to be some preference over nesting in low light places, as recorded for *E. flava* (Camargo et al. 1975), *E. rustica*, and *E. elegans* (Michener and Lange 1958). Despite that, it is not possible to point to any patterns regarding nest depth, which can vary according to the characteristics of each site used for nesting (Cane 1991), nest density, and the number of brood cells per nest. A study of phylogenetic reconstruction based on nesting behavior characters encompassing the whole Centridini tribe is under way (Werneck HA, unpublished data). As a result, we expect a better understanding of the patterns and evolution of these characteristics. For a comparison of the characteristics regarding nesting habits amongst the species of *Epicharis* studied, see Table 2.

Table 2. Compilation of comparative data on nesting biology of the genus *Epicharis*.

	<i>E. (Epicharoides) picta</i> ^a	<i>E. (Epicharoides) albofasciata</i> ^{b,c}	<i>E. (Epicharis) bicolor</i> ^d	<i>E. (Epicharis) nigrita</i> ^{b,c}	<i>E. (Parepicharis) metatarsalis</i> ^e	<i>E. (Parepicharis) zonata</i> ^f	<i>E. (Triepicharis) analis</i> ^{g,h}	<i>E. (Anepicharis) dejeanii</i> ^{i,j,k}	<i>E. (Epicharvana) flavu</i> ^l	<i>E. (Hoplepipharis) fasciata</i> ^o	<i>E. (Epicharritides) obscura</i> ^m
Phenology	univoltine	univoltine	bivoltine	univoltine	univoltine	univoltine	univoltine	univoltine	multivoltine	univoltine	?
Nesting place	slanted	slanted	Flat	flat	flat	flat	flat	flat	vertical	vertical/flat	flat
Soil type	sandy soil	sandy soil	sandy soil	sandy soil	clay and sandy soil	sandy soil, seasonally swamp aggregated	sandy soil	sandy soil	basal afloration	earth bank/sandy soil	wet soil
Nest arrangement	aggregated	aggregated	aggregated	aggregated	aggregated	aggregated	aggregated	aggregated	aggregated	aggregated	?
Nest type	one tunnel, branched	one tunnel	one tunnel	one tunnel	one tunnel, branched	branched	one tunnel, branched	one tunnel, branched	branched	one tunnel, branched	one tunnel
Nest density	41/m ²	1/m ²	40/m ²	3–32/m ²	25/m ²	12/m ²	0.31/m ²	1–25/m ²	?	1.5/m ²	?
Cell arrangement	isolated, end of tunnels	isolated, end of tunnels	linear	isolated, end of tunnels	isolated, end of tunnels	isolated, end of tunnels	isolated, end of tunnels	isolated, end of tunnels	linear	isolated, end of tunnels	isolated, end of tunnels
Cell position	slanted	slanted	transversal, horizontal or vertical	slanted	slanted	vertical	vertical	slanted, horizontal	horizontal	vertical	vertical
Cell per nest	1–2	1–6	1–2	1–5	1	1–7	2–5	1–10	1–3	1–2	1
Cell length	20–27 mm	15–23 mm	21 mm	20–25 mm	?	24–30 mm	30 mm	29–36 mm	23–25 mm	28 mm	16.7 mm
Cell diameter	10–14.5 mm	9.5–11.5 mm	14.5 mm	20–25 mm	?	14–20 mm	13–18 mm	19–20 mm	13–15 mm	13–15 mm	12.5 mm
Cell depth	30–110 cm	16–35 cm	10–25 cm	16–60 cm	62–120 cm	15–52 cm	30–45 cm	25–140 cm	110 cm	30 cm	10–30 cm

^aTable adapted and updated from Gaglianone (2005);

^bThis study; ^cGaglianone (2005); ^dRozen (2016); ^eRocha-Filho et al. (2019); ^fThiele and Inouye (2007); ^gRoubik and Michener (1980); ^hRaw (1992); ⁱHiller and Wirtmann (1994); ^jDec and Vivallo (2019); ^kCamargo et al. (1975); ^lVisey-FitzGerald (1939); ^mLaroca et al. (1993); ⁿVivallo (2020a); ^oVivallo (2020b)

^{***}The data on *E. (Triepicharis) analis* were joined with those on *E. (Triepicharis) schratkyi*, described by Gaglianone (2005), due to the proposal of *E. schratkyi* as a junior synonym for *E. analis* by Véllez and Silveira (2006).

^{****}Cited by Raw (1992) as *E. (Anepicharis) melanoxantha*. Werneck et al. (2012) reported that *E. melanoxantha* corresponds to *E. dejeanii*. Here, we joined the data from Raw (1992) with the available data for *E. dejeanii*;

Species that nest in the soil in aggregations might also build their nests in the nest aggregations of other species (Michener 1974). For instance, there are reports of *E. albofasciata* building their nests in *E. nigrita* (Gaglianone 2005) and *E. picta* (this study) nest aggregations. However, these bees can build their own nest aggregation, as described by Rozen (2016). The characteristics described by Rozen (2016), such as nest depth, presence of a tumulus around the entrance of each nest, female preference for inclined sites (about 45°), and shape and composition of the brood cells, corroborate the findings and allow us to point out that these characteristics may be diverse within *Epicharoides*.

The development of immature stages seems to be more constant within *Epicharis*. Both *E. picta* and *E. albofasciata* present the same hatching pattern, with the presence of a pharate first instar, which is also recorded for *E. flava* and *E. nigrita* (Camargo et al. 1975; Gaglianone et al. 2015). Pharate first instar larvae were also observed for *Centris flavofasciata* Friese, 1899 (Rozen et al. 2011), in addition to other groups of solitary bees that nest in the soil, such as *Monoeca haemorrhoidalis* (Smith, 1854) (Apidae: Tapinotaspidini) (Rozen et al. 2006).

Emergence-traps and phenology

Emergence-traps have been effective in collecting data on species of bees and wasps nesting in the soil (Hiller and Wittmann 1994; Sardiña and Kremen 2014; Rocha-Filho and Melo 2017; Cope et al. 2019; Martins et al. 2019). In the present study, these emergence-traps were useful for sampling data on natural enemies that emerged in the nest aggregation as well as in measuring the phenology and sex ratio of *E. picta*. We were able to measure the emergence patterns of both the host and its natural enemies.

Our data from emergence-traps corroborate the hypothesis that most *Epicharis* species are univoltine (see Gaglianone et al. 2015). The beginning of the emergence of *E. picta* occurred during the period of high temperatures and rainy season. The adults remained active until the middle of April, when the temperatures decreased and the rainfall declined drastically (see Fig. 1). These abiotic factors combined with other biotic features may influence in the diapause of *Epicharis*. A discussion about the biotic and abiotic factors that can influence the diapause process, and, consequently, the phenology of *Epicharis* species, can be seen in Gaglianone et al. (2015).

The emergence of *Rhathymus friesei* occurred about one week after the beginning of the *E. picta* adult activity. As cleptoparasitic species require provisioned brood cells from their hosts to oviposit (Wcislo 1987; Rozen 2001; Michener 2007), this emergence pattern was expected. Another fact that reinforces this pattern is that *Rhathymus* females lay their eggs in cells closed by the host female (Camargo et al. 1975; Rozen 1991). The strategy of parasitizing cells closed by the host is a plesiomorphic feature among the cleptoparasitic species that might have evolved from nest-building species, which could have five independent origins within bees (Litman et al. 2013).

Tetraonyx species reported in this study emerged in the same period of *E. picta*. These claptoparasites, unlike *Rhathymus*, are not considered specialists of *Epicharis* species. *Tetraonyx* spp. parasitize bees that nest in the soil (Roubik 1989; Gaglianone

2005; Rocha-Filho et al. 2008; Rocha-Filho and Melo 2011; Gaglianone et al. 2015), in addition to other bees that nest in trap-nests (Morato et al. 1999; Parizotto 2019).

Augochlora thalia and *Acamptopoeum prinii*, and *Epinysson* sp. were collected in emergence-traps. These species nest in the soil and presumably were just opportunistically using the *Epicharis* nest site. The issue of nesting in nest aggregations of other species may be related to the strategy of inhibiting parasite attacks (Wcislo 1987; Wcislo and Cane 1996). *Physocephala* sp., also collected in the emergence-traps, is a parasitoid of bees and is discussed in the next section of this study.

Some natural enemies do not emerge in the same nest aggregation in which they attack their hosts. This can be a problem when the inference about the relationships between natural enemies and hosts is conducted by direct observations. Therefore, the use of emergence-traps allowed us to determine which species of natural enemies actually emerged from the nest aggregation. On the other hand, as in this study we have a mixed species nest aggregation, there were limitations to determine the type of association among some species that emerged in the emergence-traps (see Table 1).

In summary, we suggest that emergence-traps are tools that can aid in studies of solitary bees nesting in the soil. This method allows the measurement of phenology, parasite-host synchrony, parasitism rate, and sex ratio.

Natural enemies and species associated with the nest aggregation of *E. picta*

Many natural enemies are reported for *Epicharis* species, but there is direct evidence only for *Rhathymus* spp. and *Tetraonyx* spp. (Werneck et al. 2012; Gaglianone et al. 2015; this study). Indirect evidence, however, is reported for species of *Mesoplia* and *Mesonychium* (Apidae, Ericrocidini), *Physocephala* spp. and several species of Mutillidae (Camargo et al. 1975; Hiller and Wittmann 1993; Gaglianone 2005; Rocha-Filho et al. 2008; Luz et al. 2016). Regarding cleptoparasitism on *E. picta*, our data show that there is direct evidence only for *R. friesei*, *T. sexguttata* and *T. aff. lycoides*. *Rhathymus friesei* was the most abundant cleptoparasitic species observed in this study. Even though it is not possible to determine specificity relationships between *Rhathymus* species and *Epicharis* subgenera, there is specificity in the cleptoparasite-host relationship between the genera *Rhathymus* and *Epicharis* (Werneck et al. 2012). On the meloid beetles, there are data on the relationship of *Tetraonyx* spp. to *E. dejeanii* Lepeletier, 1841 (Hiller and Wittmann 1994), *E. nigrita* (Gaglianone 2005; Martins et al. 2019), *E. bicolor* (Rocha-Filho et al. 2008), and *E. picta* (Gaglianone et al. 2015).

Physocephala is a genus composed of parasitoid species that mainly attack adult Hymenoptera. Among neotropical bees, the host records of these Conopidae are for Bombini, Centridini, Euglossini, Megachilini, Tapinotaspidini, and Xylocopini (Rasmussen and Cameron 2004; Melo et al. 2008; Santos et al. 2008; Rocha-Filho and Melo 2011; Stuke et al. 2011; Almada et al. 2020). For *Epicharis*, there are records from indirect evidence that *P. bipunctata* may parasitize *E. bicolor* (Rocha-Filho et al. 2008; Santos et al. 2008). Although data from emergence-traps record *Physocephala* sp., our evidence is indirect, not corroborating this relationship of parasitism on *E. picta*.

The Mutillidae is composed of parasitic wasps that attack Hymenoptera in general, with records for bees as hosts (Brothers et al. 2000; Luz et al. 2016). Although some studies report the parasite-host relationship between these wasps and *Epicharis*, there is no direct evidence of this relationship (Rocha-Filho et al. 2008). Luz et al. (2016) compiled the data known for host bees of these wasps and these authors consider Apidae to be the main hosts of Mutillidae in the Neotropical region. For *Centris*, nine records of Mutillidae species as parasitoids are known (see Luz et al. 2016), whereas for *Epicharis*, the only record is for *Hoplomutilla myops myops* (Burmeister, 1854), considered as a potential parasitoid of *E. bicolor* (Rocha-Filho et al. 2008). In the present study, it was only possible to observe *Traumatomutilla* sp. approaching the nests of *E. picta*, although it was not seen entering nor leaving the nests.

Apiomerus are predators, some species being reported as common predators of bee species, such as stingless bees (Apidae, Meliponini) (Silva and Gil-Santana 2004), and *Apis mellifera* Linnaeus, 1758 (Apidae, Apini) (Silva and Amaral 1973; Amaral-Filho et al. 1994; Marques et al. 2003, 2006). In this study, we show direct evidence that this hemipteran preys on *E. picta*. Until now, no species of *Epicharis* had been associated with this predator. Data on the biology of *A. lanipes* and its mode of predation are still scarce in the literature and it will be necessary to study whether it is a predator specialized in bees.

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First record of the carpenter bee *Xylocopa pubescens* (Hymenoptera, Apidae) in the Canary Islands confirmed by DNA barcoding

Carlos Ruiz¹, Daniel Suárez^{2,3}, Manuel Naranjo⁴, Pilar De la Rúa⁵

1 Departamento de Biología Animal, Edafología y Geología, Sección de Biología, Facultad de Ciencias, Universidad de La Laguna, 38206, La Laguna, Spain **2** Island Ecology and Evolution Research Group, Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), 38206, La Laguna, Tenerife, Spain **3** Escuela de Doctorado y estudios de Posgrado, Universidad de La Laguna, 38206, La Laguna, Spain **4** Sociedad Entomológica Canaria Melansis, C/Guaydil 3-1A, 35016, Las Palmas de Gran Canaria, Gran Canaria, Spain **5** Departamento de Zoología y Antropología Física, Facultad de Veterinaria, Universidad de Murcia, 30100, Murcia, Spain

Corresponding author: Carlos Ruiz (cruizcar@ull.edu.es)

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Abstract

Island ecosystems are particularly vulnerable to the introduction of exotic species that can have an impact on local fauna and flora. Here, the carpenter bee *Xylocopa pubescens* is reported in Gran Canaria (Canary Islands, Spain) for the first time. This species is native to North Africa and the Near East and shows a rapid dispersion across the city of Las Palmas de Gran Canaria, together with a single record in the southernmost tip of the island. Different hypotheses about its arrival to the island are discussed.

Keywords

Canary Islands, Exotic species, range expansion, new record, social media, wood nesting bees

Introduction

Bees of the genus *Xylocopa* are characterised by a large size (13–30 mm) and robust jaws used for cutting the corolla of tubular flowers to extract the nectar, and also for their nesting habits, mainly in trees and wooden structures. The genus has 469 species grouped into 38 subgenera (Ascher and Pickering 2020) and is distributed throughout the World with a high level of diversification in tropical and subtropical areas (Michener 2007). In Europe, the genus has seven species whereas in North Africa there are six known species (Terzo and Rasmont 2014).

Several species of *Xylocopa* have been introduced into both continental (Dahlberg et al. 2013) and island ecosystems (Okabe 2010), mainly due to wood imports. For example, *Xylocopa sonorina* Smith, 1874 has been introduced into several Pacific islands (Hurd 1958; Barrows 1980). More recently *Xylocopa tranquebarorum* (Swederus, 1787) has been reported for the first time in Japan (Okabe 2010).

In the Canary Islands, despite their proximity to the African continent (Fuerteventura is 96 km off the northwest coast of Morocco), there were no representatives of the genus until 2013–2014, when *Xylocopa violacea* (Linnaeus, 1758) was recorded for the first time on the south of the island of Gran Canaria (Ortiz et al. 2016). This species has spread throughout the island in the last years (C. Ruiz *pers. obs.*).

The arrival of an exotic species can lead to negative impacts on ecosystems such as competition with native pollinators for floral and nesting resources, or the introduction of new pathogens (Kawazoe et al. 2010). They can also have negative effects on native flora (Barrows 1980), as damaging flowers due to nectar robbery without pollinating them (Dedaj and Delaplane 2004). In addition, carpenter bees can be a nuisance to humans, due to their ability to nest in human structures, such as fences or wooden roofs. In insular ecosystems, these impacts can be exacerbated due to the vulnerability of island ecosystems to invasive species (Reaser et al. 2007), which are the primary threat to island invertebrates (Leclerc et al. 2018).

Early detection of invasive species is therefore one of the most important actions to ensure the success of control or eradication campaigns, as it reduces costs and potential damage. In this context, citizen science has proven as an effective tool for early detection of exotic species irrespective of their invasive potential (Thomas et al. 2017; Poland and Rassati 2019). Herein, we report the first record of a second exotic *Xylocopa* species for the Canary Islands, *Xylocopa pubescens* Spinola, 1838, based on data published on social media and confirmed using DNA barcoding for the species identification.

Methods

From June to October 2020, several observations were made of a new species of *Xylocopa* by the authors and in social networks such as Facebook ('Fauna Vertebrada e Invertebrada de las islas Canarias' and 'Sociedad Entomológica Canaria Melansis') and Whatsapp groups. For each species observation (captures included), we obtained

photographic evidence from authors and asked them to provide the following information: date/period, number of individuals observed and location with GPS coordinates.

In order to confirm the identification, DNA from a single leg of a captured individual was extracted and amplified in a single step using Phire Animal Tissue Direct PCR Kit (Thermo Scientific) and standard barcoding primers (LCO1490, HCO2198).

Results

New records

Xylocopa (Koptortosoma) pubescens Spinola, 1838

New records. SPAIN: Canary Islands, Gran Canaria, **Las Palmas de Gran Canaria.** 1 ♀ Ciudad Jardín, 11 Jun. 2020, Kings Ruly *obs.*; several *ex.* Parque Doramas, 28.120, -15.428, Jun. 2020, Pablo Martínez Darve *obs.*; 1 ♀ Mercado central, 06 Jul. 2020, 28.133, -15.432, Manuel Betancor *obs.*; 1 ♀ Playa de las Canteras, 28.142, -15.432, 18 Aug. 2020; 1 ♀, 1 ♂ Parque Doramas, 28.120, -15.428, 2 Oct. 2020, Manuel Arechavaleta *obs.*; 4 ♀, 5 ♂, Parque de La Ballena, 28.1052, -15.4424, 30 Sept. 2020, M. Naranjo *obs.*; 1 ♀, 1 ♂ Urbanización Las Filipinas, 28.0761, -15.4198, 25 Sept. 2020, M. Naranjo *leg.*

SPAIN: Canary Islands, Gran Canaria, **Maspalomas.** 1 ♀ Playa del Inglés, 27.749, -15.578, 05 Sep. 2020, Carlos Velázquez Padrón *leg.*

Identification. *Xylocopa pubescens* was identified at subgenus level (*Koptortosoma*) by its external morphology. It is morphologically unique among Canary Island bees; the only species of *Xylocopa* on the archipelago is *X. violacea*. From this last species, *X. pubescens* can be easily differentiated by the mesosoma covered dorsally with yellow hairs. Males are smaller than females, and they can be distinguished by a narrow head and yellow pubescence covering their entire body (Fig. 1). However due to morphological similarities with related species such as *X. modesta* Smith, 1854 from Cape Verde, *X. aestuans* (Linnaeus, 1758) from Southeast Asia or *X. appendiculata* Smith, 1852 (introduced in North America), a molecular identification through barcode was accomplished. An individual from Las Palmas de Gran Canaria city was successfully sequenced for the barcode region of mitochondrial *cox1*. Sequence length was 658 bp (0% ambiguities) with no evidence of stop codons or NUMTs. Comparison with the DNA barcode library using the BOLD ID Engine resulted in a 99.35% of similarity with an exemplar of *X. pubescens* from Fes-Boulemane Region (Rif region, North of Morocco). Individuals from Cyprus and Israel showed lower similarity (99.23–99.07%). NCBI Blast resulted in 97.26% of similarity with the related species *Xylocopa aestuans*.

Distribution

Xylocopa pubescens is naturally distributed in Near-East and North Africa. The species is expanding its native range, as it has been recently detected in the Balcanic Peninsula



Figure 1. Pictures of *Xylocopa pubescens* (male left; female right) in the Parque de La Ballena (Las Palmas de Gran Canaria). Photos: M. Naranjo.

(Greece; Terzo and Rasmont 2014) and the Iberian Peninsula (South Spain; Ortiz and Pauly 2016). In Gran Canaria, all the new records are restricted to the North in Las Palmas de Gran Canaria city, in the surroundings of the port and large urban gardens; additionally, there is one record from the southern tip of Gran Canaria in La Playa del Inglés (Fig. 2). A social network member reported a previous sighting in 2019 in the area where the current sightings occurred, which suggests that the species has been on the island for at least one year.

Discussion

The occurrence of *Xylocopa pubescens* in the island of Gran Canaria can be explained by two alternative scenarios. It may have arrived in a shipment of wood in the port of Las Palmas, with a secondary expansion up to Maspalomas. This hypothesis is supported by its wide distribution around the port area of the capital (Fig. 1). The port receive more than one million containers a year (<http://www.palmasport.es/es/puerto-de-las-palmas/>). The fact that other *Xylocopa* species have been commonly intercepted in wood shipments elsewhere (e.g. San Francisco: Hurd 1955; Japan: Maidl 1912), and

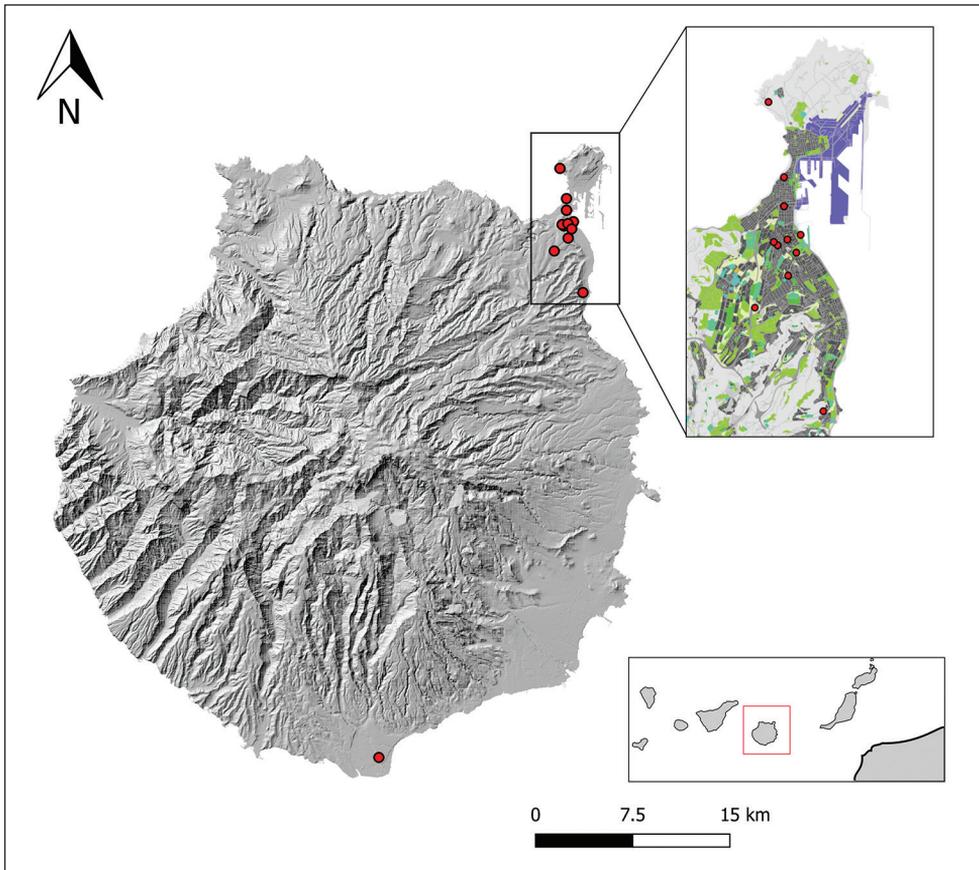


Figure 2. Localities where *Xylocopa pubescens* has been recorded in Gran Canaria island (red dots). A detailed distribution in Las Palmas de Gran Canaria city is presented, showing urban areas (grey) and the port of Las Palmas (in blue).

this large volume of containers trading-off, make possible the accidental arrival and posterior dispersal of this species across the urban area of Las Palmas de Gran Canaria. Wood- or stem-nesting bees, such as those of the family Megachilidae or the genus *Xylocopa*, are known to disperse further by transported nests to isolated islands than by flight (Michener 1979; Poulsen and Rasmussen 2020). Globalization has accelerated this process, thus favouring the introduction of species as carpenter bees that nest in wood or other commercial substrates. In the last decade, several exotic wood nesting bees have been reported in the Canary Islands such as *X. violacea* (Ortiz et al. 2016) or *Megachile otomita* Cresson, 1878 (Strudwick and Jacobi 2018).

Alternatively, *X. pubescens* may have arrived by its own means, transported by the warm east-wind from the Moroccan desert (locally known as ‘calima’). *X. pubescens* is adapted to xeric conditions, and it is likely expanding its native range by colonizing southern Europe in two independent events during the last decade, probably in

relation with the global climate change. Therefore, a natural expansion to the Canary Islands cannot be ruled out. This process may also explain the record of the species in an isolated locality at the very south of Gran Canaria. Future genetic analysis including continental populations should be conducted to discern between both hypotheses.

The species is conspicuous with local abundance in the surroundings of Las Palmas de Gran Canaria and it has been widely observed during the summer and autumn of 2020. The potential negative effects on native bees has not been evaluated yet, therefore it is important to continue monitoring its spread on the island and to assess its possible impacts on island ecosystems. These results provide an example of the potentiality of social media and citizen science for exotic species early detection and monitoring.

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Nesting ecology of the Pacific cicada killer, *Sphecius convallis* Patton (Hymenoptera, Crabronidae), in the Sonoran Desert

Joseph R. Coelho¹, Jon M. Hastings², Charles W. Holliday³

1 Biology Program, Quincy University, 1800 College Ave., Quincy, IL 62301, USA **2** Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099, USA **3** Department of Biology, Lafayette College, Easton, PA 18042, USA

Corresponding author: Joseph R. Coelho (coelhoj@quincy.edu)

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Abstract

Factors affecting the ecology of a large population of Pacific cicada killers (*Sphecius convallis*) occupying a field of mine tailings in Ruby, AZ, were examined. Burrows were quite dense in certain areas around the periphery of the mine tailings, but were dispersed randomly within these areas. Approximately 1600 females (based on burrow counts) and 2500 males (based on mark-recapture) were recorded, yielding a total population estimate of 5000–6000 adults. Female wasps were able to dig much more rapidly in the mine tailings than their congeners *S. speciosus* in soils from PA, suggesting that the habitat suitability was a large factor in this robust population. Provisioning rate was comparatively slow, however, suggesting that cicada abundance in that year was not a contributor to the high population density. The presence of a sap-producing tree may have eased the energetic and thermoregulatory demands of the wasps. Although excavations revealed that the number of burrows and cells could easily maintain the population size, the lack of cicadas probably resulted instead in a population crash the following season.

Keywords

Wasp, soil, digging, dispersion, population, provisioning

Introduction

Four species of cicada killers (genus *Sphecius*) occur in North America (Coelho et al. 2011). The Eastern species (*S. speciosus* Drury) is best known, having received considerable attention from biologists as early as the late 19th century (Riley 1892). The other species, however, have been relatively neglected until recently. Pacific cicada killers (*Sphecius convallis* Patton, 1879; Hymenoptera: Crabronidae) have nested at Ruby, Arizona (Santa Cruz County) probably at least since 1940, and perhaps since the sandy galena ore mine tailings were deposited in the valley by the operations of the Montana and Ruby mines between the late 1800s and the 1930s (Ring et al. 2005). Their presence was recorded in a documentary film (BBC 1993), which prompted us to visit the site. In 2009, when we found a huge population. Prior to our work there were only treatments of the species' taxonomy (Krombein 1979; Bohart 2000; Holliday and Coelho 2006) and distribution (Coelho et al. 2011), but not its biology. We found that, like other cicada killers, *S. convallis* is endothermic, and regulates its body temperature (Coelho et al. 2016). The only prey species used by *S. convallis* at this site was *Hadoa parallela* (Davis, 1923)(Hemiptera: Cicadidae). The flight muscle ratio of *S. convallis* loaded with *H. parallela* is nearly optimal, contributing to a high foraging efficiency (Coelho et al. 2012). However, many prey cicadas are stolen by birds and conspecific females, which enter the burrows of others and lay eggs on their already-provisioned cicada (Coelho et al. 2019).

While these studies contributed greatly to our understanding of the biology of *S. convallis*, factors contributing to its large population size are not fully explained. Schmidt (2013) describes the general characteristics of the Ruby site, and the diversity of digger wasps and their parasites that thrive there. The goal of the present study is to examine some ecological factors potentially affecting the success of Pacific cicada killers in Ruby, Arizona.

Methods

Burrow density

We described the field site previously (Coelho et al. 2012), a pile of sand-like mine tailings centered on approximately 31°27'28"N, 111°14'00"W. We measured burrow density in three areas containing the highest density of cicada killer burrows, which we designate as Areas A, B and C (Fig. 1). The areas were measured using a series of rectangles with a large measuring tape. The number of burrows within each area was counted.

Burrow dispersion

Nest burrows were generally clustered around the periphery of the mine tailings; however, we wanted to determine the dispersion pattern of burrows within these aggregations. We

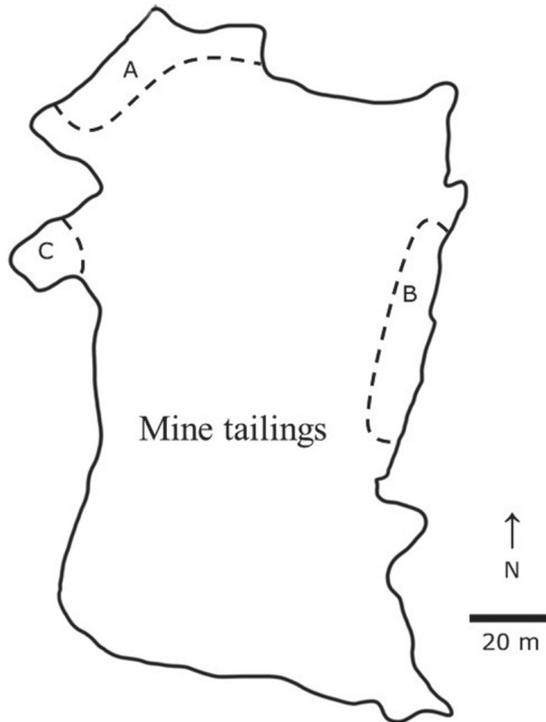


Figure 1. Map of burrow aggregation sites on the Ruby mine tailings.

had predicted that the burrows would be uniformly arranged in space, as that is the general impression they make to the eye, especially in high density areas. Known aggression between cicada killer females at high densities (Lin 1963; Hastings et al. 2008) should lead to the regular (uniform) dispersion pattern (Molles and Sher 2018). The nearest neighbor technique was performed (Clark and Evans 1954) using data from 09/08/2009, which was slightly past the peak of burrow density (see below). Two sites that appeared to be high (Willow and Hammock within Area A) and low (Areas B and C) density were examined.

Mark-recapture

We used a mark-recapture method to estimate the male population size of *S. conval-lis* in Area B, which had the highest apparent density of male wasps. We captured all of the male wasps that we could (195) on three successive days (7–9 August 2009), marked them on the scutum with an approximately 3-mm diameter dot of blue nail polish, confined them in a 2.43 × 2.43 m (8 × 8 feet) screen tent with a shaded roof for up to 4 hours during the capture period to prevent recaptures, and released them at the capture site (Fig. 2). On the morning of the fourth day (10 August) we captured all of the marked and unmarked male wasps that we could in Area B, confined them in the screen tent for up to 3 hours during the recapture period, and then counted them as



Figure 2. Screen tent used in estimating male cicada killer population size. Field of mine tailings in the background.

they were released. We used the Chapman (1951) estimator to calculate male population size. This method ignores the effect of any differential wasp mortality, which we expect to be minimal in the 3-d marking period.

Male population density (wasps/m²) was calculated as the number of males estimated by the mark-recapture method divided by the area occupied by males in Area B. The total number of males in all three areas was crudely estimated by multiplying the density of males in Area B by the total number of square meters in all three areas.

Estimation of female population size and density

To estimate the sizes of the female *S. convallis* populations in the three areas, we counted the number of nest entrances in Areas A and B up to August 29 and in Area C up to August 31 as a proxy for female numbers. This method is a rough approximation and a minimum population estimate, as we observed many females moving from burrow to burrow and entering other females' burrows in apparent attempts at cleptoparasitism (see below). Female population density (wasps/m²) was calculated as the number of burrows in each area divided by the area occupied by burrows in each area.

Phenology

We used our field notes and data sheets from our observations to track the abundance of males, females and burrows, as well as the frequency of matings through the season. For each data sheet, we recorded the number of males and females observed or used in experiments on that day. For mating observations, one may assume that one female and a variable number of males were in attendance.

Digging

We undertook measurements to determine whether the substrate was easier to dig in Ruby than in other locations. We located females that were in the act of digging or expanding their burrows. Digging behavior was essentially identical to that observed in *S. speciosus*, and digging rate was measured largely as described previously (Coelho and Holliday 2008). In brief, a pit was excavated in front of the burrow entrance and a small, plastic condiment cup was placed in front of it. Each load of soil was captured by the cup as the wasp backed out of the burrow and replaced with another cup while time was recorded for each event. Cups were individually marked and placed on the dashboard of an automobile placed facing the sun with one side window open and the other equipped with a solar-powered fan. In this way, the soil sample was dried to constant mass and weighed on an Ohaus Adventurer-Pro electronic balance to the nearest 0.001 g.

Sap tree census

Cicada killers frequently eat sap exuded from wounded trees, presumably as a source of carbohydrate fuel (CWH, personal observations). We examined the use of sap from a willow (*Salix* sp.), over the course of a day to determine how this type of foraging fit into the cicada killers' daily routine. On 11 August 2009, beginning at 05:30 h, we counted the number of cicada killers visiting the sap tree and measured the ambient temperature in the shade at the height of the sap-producing would every 15 min until 20:00 h.

Provisioning effort

Focal sites were chosen in which several burrows could be observed at once. We closely observed the provisioning activities of female *S. convallis* at two sites in Area A. Nesting females were marked, then observed so that provisioning behavior, and burrow entries and exits could be recorded along with the time they occurred.

Excavations

In order to estimate the total seasonal provisioning success of a subsample of nests, we dug up an area with 10–13 active burrows in Area A as the site of our studies of cicada

provisioning success down to 0.7 m, below which the wasps did not dig because of a layer of gravel. To make sure that we found all nest cells, the area excavated included extended for an area of 1 m outside the area occupied by nest entrances.

Results

Burrow density

The nest aggregation areas had maximum densities of burrows as follows: Area A, 1213 burrows on 8/29 on 1505 m², or 0.81 burrows/m²; Area B, 299 burrows on 8/29 on 3221 m², or 0.09 burrows/m²; Area C, 76 burrows on 8/31 on 240 m², or 0.032 burrows/m². There were many other burrows spread out much more thinly on the central area of the sandy dune. Thus, a total of 1588 burrows were recorded for the high density sites alone on the dates we recorded them.

Burrow dispersion

All sites were relatively flat and unvegetated. Burrow density varied from just under 0.5 to 1.3 burrows/m². At all four sites, the ratio (R) of observed to expected distances was not significantly different from 1 (Table 1). Hence, all were consistent with a random arrangement of burrow entrances on the surface of the soil.

Mark-recapture

On August 10, the mark-recapture method indicated that there were 2556 male *S. convallis* in Area B (Table 2), yielding a population density of 0.79 wasps/m². Using this density and the total area of Areas A, B and C, this indicates that nearly 4000 males were present in the three areas. On August 29–31, burrow counting indicated a minimum total female population of nearly 1600 wasps in the three areas and an average density of 0.403 females/m². Near the end of the breeding season (September 17), the presence of fresh burrow excavation indicated that there were only 206 active burrows in Area A, 50 in Area B, and 36 in Area C, for a minimum total female population of 292 wasps (data not shown in Table 2).

Phenology

Males were first observed emerging at the site 7 August (Fig. 3). Females began to appear in small numbers and matings were observed. Male numbers began to fall around 10 August, and males became hard to find for the last set of experiments in which they were used on 3 September. We left the site on 17 September; while female foraging and burrow digging was still underway, no males had been seen for 14 days. Female density and activity seemed to be decreasing. Mating was observed as soon as females began

Table 1. Dispersion pattern of burrows in four nesting colonies of Pacific cicada killers.

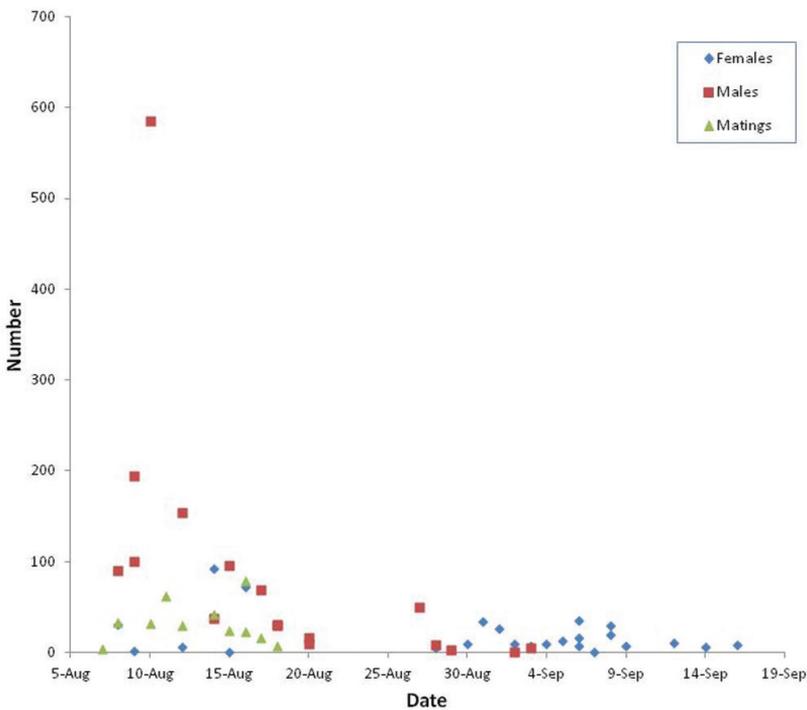
Site name	Area C	Area B	Willow	Hammock
Number	114	171	118	87
Area (m ²)	240	200	88.6	66.1
Density (burrows/m ²)	0.475	0.855	1.33	1.31
R	1.04	0.973	0.94	0.957
K*	0.75	-0.667	-1.25	-0.772

*As the critical value of K is 1.96 at the 5% level, none of the values of R are significantly different from 1, which is consistent with random dispersion.

Table 2. Male and female population density estimates in three areas at Ruby, AZ.

Area	Male population	Male density (males/m ²)	Female population	Female density (females/m ²)
A	–	–	1213	0.806
B	2556	0.794	299	0.093
C	–	–	76	0.317
Total	3943*	0.794	1588	0.403

*estimated based on density of Area B and surface area of Areas A and C.

**Figure 3.** The timing of male emergence, female emergence and mating in *Sphecius convallis*.

to emerge on 7 August, peaked approximately on 13 August, then fell off quickly, no further matings being observed after 18 August.

The first burrow was observed on 14 August (Fig. 4). Their numbers increased rapidly to a peak of nearly 1600 by 30 August, but decreased steadily afterward.

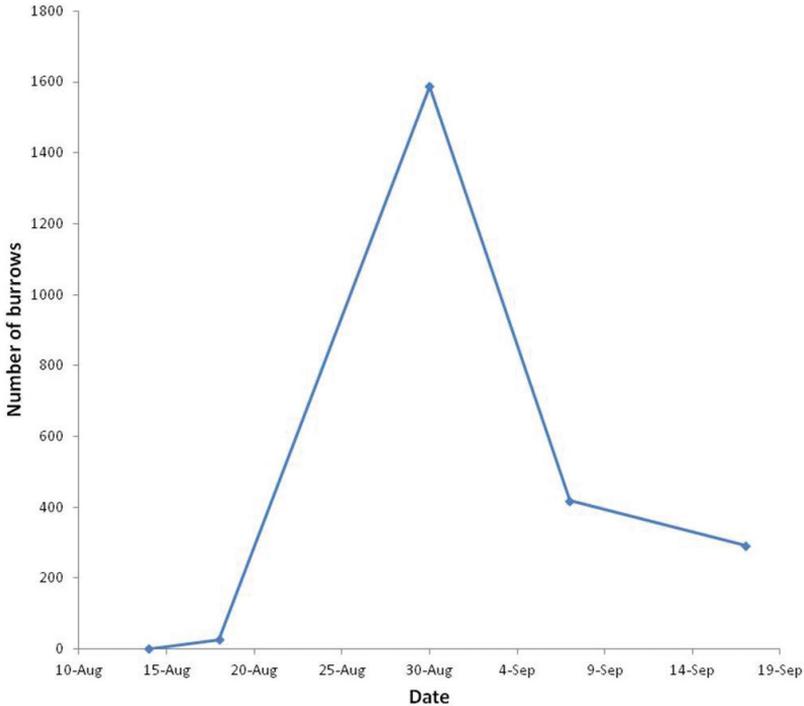


Figure 4. The timing of burrow construction in *Sphecicus convallis*.

Digging

S. convallis at Ruby, AZ, removed soil at a rate of 1.46 g/min dry mass, while *S. speciosus* in Easton, PA, digs at a rate of 0.98 g/min (Coelho and Holliday 2008). These data exclude lapses when wasps were assumed to be loosening soil in the burrow. While the Ruby wasps took longer between digging bouts, their gross digging rate (1.33 g/min dry mass, which includes such intermissions) was still greater than even the net rate of Easton wasps with intermissions excluded. Hence, the rate of soil removal at Ruby is 48% higher than at Easton.

Sap tree census

There was considerable activity among cicada killers feeding at sap trees. A great deal of bumping and brief fighting between individuals, and, occasionally, other species, occurred as insects attempted to access the site of the sap flow (Fig. 5).

No cicada killers were observed at the sap tree until 0615 h, when 17 suddenly appeared. This event marked the early peak in wasp sap tree attendance, which plunged to 4 by 0715 h. The numbers climbed afterward to 22 at 1030 h. Thereafter, the number of cicada killers varied dramatically until late in the day, as attendance ranged as high as 26 at 1400 h, and plunged to zero between 1900 and 1945 h. As many wasps



Figure 5. *Sphecius convallis* feeding on sap at a willow tree, along with several flies. Note the foam in the wound in the tree's bark, probably made by yeast sugar fermentation.

were individually identifiable, having been marked in the course of other studies, we found that some wasps returned to the sap tree repeatedly during the day.

There was a weak but significant relationship between ambient temperature ($^{\circ}\text{C}$) and sap tree attendance (number of wasps = $1.04(\text{temperature}) - 16.1$, $R^2 = 0.33$). Attendance increased linearly with temperature even though the data from the early peak ran counter to this trend.

Provisioning effort

During 348 hours spent observing an isolated group of 10–13 active burrows (the number of active burrows varied from day to day over the observation period) at one site in the picnic area, a total of 43 paralyzed cicadas were placed into the burrows by female *S. convallis*.

Excavations

Nest architecture was essentially the same as that observed in *S. speciosus*. We located a total of 104 nest cells, of which 14 were empty. Females use the soil from a new cell to backfill the previously completed cell (Dambach and Good 1943). Since the number of empty cells is approximately equal to the number of burrows, we can reasonably assume that all of the cells containing cicadas were completed. Of the remaining cells, 68 held one cicada, 20 had two cicadas and 2 had three cicadas. Of the 90 nest cells with fresh cicadas, 68 contained male eggs (male cicada killers are smaller than females because they get only one cicada on which to feed [Lin 1979]) and 22 had 2–3 cicadas and were females, yielding an apparent male:female ratio of 68:22 or 3:1.

In digging up the 10–13 burrows noted above, we also found nest cells from 2008 and perhaps earlier seasons (old nest cells): a total of 235 older nest cells with decayed, moldy and obviously old cicada and cicada killer cocoon remains in them were found.

Discussion

Density

Several estimates of population density of cicada killers can be extracted from the existing literature to compare to those here. The overall nest density in the largest colony of *S. speciosus* studied by Dambach and Good (1943) was ca. 0.1 nests/m²; local areas of high density had ca. 0.75 nests/m². Eason et al. (1999) indicate a territorial male density of ca. 0.5 males/m². The density of nests in three aggregations in Easton, PA, ranged from 0.301–0.345 nests/m² (CWH, unpublished data). At a remarkably high population site in Channahon (Will Co.), IL, Hastings et al. (2008) estimated the density of nests at \sim 1.07/m² overall, and 1.64/m² within a focal area. The density of males active within the focal berm was greater than 1.64/m². Burrow densities of our nesting aggregations of *S. convallis* exceed typical estimates and approach those of *S. speciosus* in Channahon. Hence, the data corroborate our observations that the Ruby site held a large population with high densities of burrows during the field season under study.

Several factors probably contribute to the abundance of cicada killers at the site. One is obviously the availability of cicadas, although long hunt times, low hunting success, and the low frequency of males singing (Coelho et al. 2019) suggest that the cicada population size was low during the year of this study. We can infer that the

density of cicadas was much higher during the previous nesting season. Another factor appears to be the availability of nesting habitat. The mine tailings create sandy substrate that appears to be ideal for burrowing. Roots and rocks that could interfere with burrow construction are absent; whereas, the surrounding substrate is very rocky and unsuitable. The mine tailings have a large fraction of fine particles which hold water very well. As a result, just a few inches below the surface, the sand is moist and cohesive, so it does not have a tendency to collapse as would a coarser or drier sand. Also, an almost complete lack of vegetation resulted an open habitat, which cicada killers apparently prefer (Coelho et al. 2011), perhaps because it is easier to reach their nest entrances unhindered, especially while carrying a cicada.

Dispersion

Nesting aggregations were clumped within the field of mine tailings as a whole; however, within nesting aggregations, burrows were shown to have a random arrangement in space. A uniform pattern would have reflected territoriality and/or competition, which we did observe to some extent between females. Although one female was actually observed to steal a cicada from another, prey theft more often takes the form of a female sneaking into an unoccupied burrow and laying an egg on an already provisioned cicada while the burrow owner is away (Coelho et al. 2019). Hence, the direct agonistic encounters that might result in a uniform dispersion pattern are likely to be rare. In *S. speciosus*, nearby burrows tend to be owned by relatives (Pfennig and Reeve 1989), which may ease antagonism. A clumped arrangement might have reflected a preference for particular microhabitats, which appeared to be the case with higher densities around mesquite trees. The random dispersion pattern suggests that our perceptions were mistaken, and the wasps make no particular choice in where to dig their burrows.

Phenology

As has been observed in *S. speciosus* (Dambach and Good 1943) and *S. grandis* (Hastings 1989b), *S. convallis* is protandrous. Males emerge well before females and establish territories where virgin females are likely to emerge from their natal nests. The mating season occurs as females begin to emerge, but once all females have done so, opportunities for mating are rare (as females mate only once) and males begin to die off. Females continue provisioning through the end of summer, but begin to die off with the onset of cooler fall weather.

Role of soil type

Not only does the mine tailing field at Ruby provide a large area of suitable habitat relative to the surrounding rocky terrain, it appears to provide excellent conditions for digging. *S. convallis* at Ruby removed soil at a rate of ~1.5x that of *S. speciosus* in Easton, PA, (0.98 g/min, Coelho and Holliday 2008). The soft, easily dug, yet cohesive

mine tailings appear to be ideal for cicada killer burrow construction. Soft soil can be dug much faster, while moisture retention maintains humidity in the nest cell. Hence, several hymenopterans have been shown to have nesting preferences for soil humidity and particle composition (see Potts and Willmer 1997). The slightly smaller great golden digger wasp (*Sphex ichneumoneus* (Linnaeus, 1758) also chooses flat, soft soil that is unlikely to collapse (Brockmann 1979). Less time spent digging translates into more time available for foraging. An analysis in our previous paper indicates that the fitness gains of such temporal advantages are large, in part because life span is relatively short (Coelho and Holliday 2008). This measure stands out strongly as a potential factor leading to the high density of *Sphex convallis* at Ruby.

We expect that this population is isolated to a high degree from other Pacific cicada killer populations. The mine tailings provide an unnaturally favorable habitat. Although we searched the surrounding region (the southwest corner or “panhandle” of Santa Cruz County) extensively during this period, we never observed burrows or adult wasps in other places. It has long been our contention that cicada killers thrive on disturbance. In the eastern US, this means areas of open soil or very low vegetation, such as suburban lawns (Tashiro 1987) or sports fields (Lin 1967). Thus we suggest that cicada killers are a synanthropic species that do not require the presence of humans, but thrive where they occur.

Sap tree

The early peak in sap tree attendance is likely attributable to the wasps arising from nocturnal inactivity and requiring carbohydrate energy. The timing is strikingly similar to the early perching of males on basking trees (Coelho et al. 2016). The sap tree attendance drops quickly as the refueled wasps turn to the important activities of territory defense by males and nest construction and provisioning by females. As these activities use up energy and water, wasps return to the tree during the day. Fluctuations in attendance likely occur as individuals become unsynchronized. All abandon sap foraging as the sun sets, females returning to their burrows and males to night perches.

The direct relationship between sap tree attendance and ambient temperature is subject to a number of interpretations. It may be that, as activity increases with temperature, energy needs increase. However, as male territorial activity decreases during the afternoon (Coelho et al. 2016), this explanation fails. It is possible that increasing ambient temperature increases foraging on sap as the wasps require more water. Both males and females use evaporative cooling as part of their thermoregulatory strategy (Coelho et al. 2016). Although tree sap is fairly concentrated, it may provide a rare source of both fuel for activity and water for evaporative cooling. Preliminary measurements of crop fluid spontaneously released by Easton, PA, *Sphex speciosus* under CO₂ anesthesia showed the presence of reducing sugars and yielded osmotic pressures of 1500–2000 mOsm/kg H₂O (CWH, unpublished data). Hence, the presence of the sap-producing tree may have eased both the energetic and thermoregulatory demands of the wasps, perhaps contributing to the size of the local population.

Provisioning success

The rate of provisioning, 8.1 h per cicada, is very low compared to that of *Sphecius speciosus* in Easton, PA, and is consistent with very low cicada availability in the local environment (we heard very few *H. parallela* males calling at Ruby). Hence, at least during our season of observation, it does not appear that high cicada density contributed to the high density of *Sphecius convallis*. It is likely, however, that cicada density was much higher during the prior nesting season, resulting in the high population observed in 2009. We suspect that *Sphecius convallis* and *H. parallela* are a case of predator and prey cycling out of phase in population size, but this possibility requires further investigation. There was, in fact, a much smaller cicada killer population the following year of approximately 150 individuals in the entire area (J.O. Schmidt, personal communication), as one might predict from such a model.

Sex ratios

The strongly male-biased offspring sex ratio may well have been caused by the low availability of cicadas, particularly when the high incidence of birds stealing the provisioners' cicadas and cleptoparasitism (Coelho et al. 2019) is taken into account. A cicada killer laying an egg on a "stolen" cicada must, of necessity, lay a male egg, as a female egg given a single cicada to eat would be too small to carry cicadas when she eclosed the next summer. Cells with two cicadas are unavailable to cleptoparasitic females, as they are immediately sealed with soil by the nest owner upon provisioning with the second cicada.

Estimates of total nest and cell numbers

The 104 nest cells in 13 burrows from 2009 average 8.0 cells per burrow, assuming the empty ones would have eventually received cicadas and eggs. Let us assume that another cell per burrow would have been added by the time all of the females were dead, making the average 9 nest cells per burrow. Using the highest values above for burrow densities in Area A (1213), Area B (299) and Area C (22), we have a total of 1534 known cicada killer burrows at Ruby, each with 9 nest cells, making an estimated 13,806 nest cells. Allowing for about 50% mortality (probably not a bad guess, given all of the parasitic velvet ants that were present and the high rate of dead/moldy grubs and cocoons we found in the nest cells we dug up), this gives us about 6900 wasps to appear the next summer at Ruby. The true number should be perhaps 25% higher, as we did not count all of the burrows present, but only those in the Areas A, B and C. Though these calculations suggest the potential to maintain the population at the same high level during the following year, it seems unlikely that the low cicada supply would support it. There was a much smaller cicada killer population the following year, as noted above. Hence, one should expect the cicada killer population to vary considerably from year to year, not to be as large as we encountered in 2009 every year.

Conclusion

In summary, it seems likely that the greatest factor drawing Pacific cicada killers to the Ruby site is the mine tailings themselves. The open habitat and the favorable soil make accessing nest entrances and digging substantially easier than in the surroundings. We took numerous hikes throughout the region during the period of the study, but we never saw any cicada killer burrows in other places; this absence was also reported to us by S. Hunter, the caretaker of the site for several years before our visit. In spite of substantial prey theft (Coelho et al. 2019), cicada killers were abundant there. Other factors contributing to the success of cicada killers might also be the presence of sap-exuding trees to provide them carbohydrate fuel and water. The abundance of cicadas in the trees surrounding the site, at least during previous years, likely also contribute to the high density of *Sphecius convallis* there. These factors, among others, combined to produce one of the largest populations of cicada killers ever described. It is not surprising that this is a human-altered site, as the high-density site in Channahon, IL, was for *Sphecius speciosus* (Hastings et al. 2008). Cicada killers thrive on disturbance, in part because it removes vegetation and changes soil structure, creating open areas for burrow digging and making burrow entrances more accessible to females returning with prey.

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Michael Charles Day (1942–2020)

John S. Noyes¹, George R. Else²

1 *Life Sciences, Natural History Museum, London SW7 5BD, UK* **2** *Northcroft, St Peter's Road, Hayling Island, Hampshire PO11 0RX, UK*

Corresponding author: John S. Noyes (j.noyes@nhm.ac.uk)

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Abstract

This article, in memoriam, provides details of the professional life of Michael Charles Day, researcher at the Natural History Museum, London and specialist in the taxonomy of aculeate Hymenoptera especially Pompilidae. It also includes a short account of his lifelong interest in caving.

Keywords

Caving, Obituary, Pompilidae, Taxonomy

Michael (“Mick”) Charles Day died on 13 September 2020. He is survived by his wife Judy and two daughters, Caitlin and Cainwen.

Mick was born on 1 July 1942 in Coulsdon, Surrey. He was educated at Reigate Grammar School and developed an early interest in collecting Lepidoptera and stamps. After working for a year at Legal and General Insurance Society he returned to education and took his first degree in Zoology at Cardiff, University of Wales, from 1962 to 1965. During the following three years he remained at Cardiff where he studied for a PhD under the supervision of Michael Claridge and John Edington. His topic was the parasitoid complex of Cecidomyiidae (Diptera) galling stems of common reed (*Phragmites australis*) on nearby coastal areas. Unfortunately, this study was never completed.



Figure 1. Hymenoptera researchers at the British Museum (Natural History) (c.1977). Left to right: John Noyes, Ian Gauld, Mike Fitton, Mick Day, Barry Bolton (Photo Kyaw Than).

Mick joined the British Museum (Natural History) (now Natural History Museum, London, UK) in September 1969 as a curator and researcher on aculeate Hymenoptera. Initially he dressed in a smart, dark suit and wore a tie, but soon converted to the informal dress of a museum taxonomist. Mick joined an amazing team of Hymenopterists that included Owain Richards (retired), John Perkins (Ichneumonidae), Ian Yarrow (bees, retired), Colin Vardy (lower aculeates), John Quinlan (Cynipoidea) and George Else (bees) and Commonwealth Institute of Entomology (CIE) taxonomists Ron Eady (parasitoid Hymenoptera), Gilbert Nixon (parasitoid Hymenoptera), Geoffrey Kerrich (parasitoid Hymenoptera) and B.R. Subba Rao (parasitoid Hymenoptera). This team was very soon to be joined by museum researcher Barry Bolton (Formicidae) and CIE researcher Zdenek Bouček (Chalcidoidea). A few years later the group was joined by museum researcher Michael Fitton (Ichneumonidae) and CIE researcher Ian Gauld (Icheumonidae), and then by museum researcher John Noyes (Chalcidoidea) when John Perkins, Gilbert Nixon, Geoffrey Kerrich and Ron Eady retired. Early on, Mick decided to concentrate his attention on lower aculeates and especially the spider-hunting wasps Pompilidae, and soon became one of the World's authorities on the group. He also acquired a very impressive general knowledge of Hymenoptera. Unfortunately Mick was also a bit of perfectionist when it came to publishing and consequently his publications were, by current standards, relatively few and far between. He always regretted this but seemed unable to rectify it.



Figure 2. Participants of the southwestern-Africa expedition. Left to right: Brian Cogan, Dave Hollis, Peter Hammond, Mick Day, Dick Vane-Wright (Photo by permission of the Trustees of the Natural History Museum).

During 1971 and 1972 Mick joined four other members of entomology department (Dave Hollis, Brian Cogan, Dick Vane-Wright and Peter Hammond) in planning and undertaking a five-month expedition to Namibia and adjacent parts of South Africa, Botswana and Angola, including the Namib and Kalahari deserts. This was a huge undertaking and as usual, the expedition was undertaken on a shoe-string budget. Mick and his colleagues decided that the expedition needed a mobile laboratory that was capable of handling the terrain and variable weather. For this they bought an ex-army, four-wheel drive, Bedford “gun tractor” and fitted it with the cab of an army fire engine (“green goddess”) for less than £350. During evenings and weekends, over a period of several months, the team designed and built a laboratory on the back of the truck. This space provided for research as well as storage space for collecting and personal equipment, food and living quarters. The truck was shipped from England to Cape Town South Africa. The expedition travelled 7,500 miles through south western Africa and material was collected at over 150 sites resulting in millions of specimens being obtained. At that time it had probably been the most productive entomological expedition in any tropical or subtropical environment. This material is now housed

in the Natural History Museum, London, and includes at least 20 species of insects bearing the specific epithet '*dayi*' in Mick's honour. The total cost of the expedition in 1971–1972 was £3,000 and when the truck returned to the UK it was used for a while as a training vehicle and then later for two trans-Sahara expeditions by the museum's Palaeontology Department, It finally gave up the ghost after crossing the Sahara for the fourth time.

In 1979 Mick, George Else and Dave Morgan travelled overland to Olympia in Greece in the hope of collecting fresh specimens of *Proscolia* (Hymenoptera: Scoliidae). This genus of primitive scoliid wasps was known from only a single male collected in Armenia in 1961 and described later by Rasnitsyn in 1977 as *Proscolia archaica*. At that time, the species was the only known representative of the subfamily Proscoliinae. However, in 1976 a single male of a second species had been collected by Piers Baker in the Olympia archaeological site and donated to the Natural History Museum by his father, Donald Baker, a frequent visitor to the Hymenoptera section. Mick, George and Dave set out for Greece in Mick's old Rover car but nearly had to terminate the trip when Mick, who was driving, nodded off whilst descending a steep road in the Austrian mountains. The car veered off the road, down a culvert and blew out the nearside front tyre, badly bending the rim of the wheel in the process. Amazingly, Mick produced a sledgehammer from the boot of the car and this was used to straighten the wheel rim. The burst tyre was replaced by the spare and a replacement was purchased in Belgrade. The trip was successful with more than 80 specimens of both sexes of a new species (*Proscolia spectator*) being collected. The first specimen was found by Mick amongst the ruins at Olympia and his resulting shriek attracted the attention of several puzzled tourists. The observations of the three entomologists and descriptions of both sexes of this primitive wasp were published two years later (Day et al. 1981).

Mick visited Brunei in February and March 1982 with Ian Gauld. They joined up with the Gurkhas and were lucky enough to be flown to several collecting localities in a Puma helicopter.

In 1983 Mick and John Noyes visited Madagascar to collect Hymenoptera. The trip was initially planned to last nine weeks from the beginning of February but, because of problems obtaining science visas, it was curtailed to four weeks from mid-April. The British Embassy did offer some help, but only after a Telex was sent to the Embassy from the Department of Education (the museum did not possess a Telex machine) a day or two before departure, thus proving they were not merely students trying their luck but serious scientists undertaking a research project. As a result, Mick and John were met at Tananarive airport by the deputy British Ambassador and were invited later to the Ambassador's house for lunch. During the first few days Mick and John organised research permits with the Department de l'Eaux et Forêts and met up with John Williams, a British microclimatologist based in Madagascar. He was a great help by obtaining a small supply of alcohol (for preserving insects) and ferrying them to and from Andasibe. Mick, John and Jean-Pierre, a member of the Academie Malgache, then travelled to Jean de Heaulme's famous, private reserve at Berenty, about 3 hours drive from Fort Dauphin. Here they lived off a diet of potato and lobster for

a week. Mick nearly gave Jean de Heaulme a heart attack when he made a move to pass Jean's prized *Aepyornis* egg to John rugby style, pulling out of the pass at the last second. From Berenty they travelled to another of Jean de Heaulme's reserves, Bereboka, about 60 km northwest of Morondava. For security reasons, they had to retain accommodation at the Hotel Bougainvillea in Morondava, with Mick and John taking it in turn to travel to the reserve and spend the night there with Jean-Pierre. John and Jean-Pierre had one scary night at Bereboka when it was rumoured that cattle-rustlers were in the area stealing cattle and killing anyone who got in the way.

During the trip to Madagascar Mick collected several specimens of three strange species of wasp which he later decided belonged to *Heterogyna*. This enigmatic hymenopteran genus had been described by Nagy in 1969 but Mick was unable to examine the unique holotype of the type species, *Heterogyna protea* Nagy, collected in Rhodes and which was held in Nagy's collection. In 1984 Mick published a revision of five species of this genus confident that he had identified it correctly. The revision included the three new species collected in Madagascar and one from Botswana. A year later, one of the new species was designated type species of *Daycatinca* in Mick's honour. Sadly, this name is now regarded as a synonym of *Heterogyna* and no longer valid.

Soon after the revision was published, Mick managed to borrow the unique male holotype of *Heterogyna protea* and was able to confirm that his interpretation of the genus was correct. Unfortunately, the specimen was destroyed in a freak accident in the environmental chamber of a scanning electron microscope. Luckily, Mick had managed to capture several images and make notes on the specimen before it was destroyed and was able to publish these in 1985. In the hope of rediscovering the species and perhaps collect some females, Mick managed to convince his family to take several holidays on Rhodes, the type locality of *Heterogyna protea*. He was extremely successful and collected about 150 males and two females of the species at Ixia in August 1984 and August 1989. He subsequently distributed specimens to most major collections in the world including several to Nagy, the discoverer of the species. Unfortunately, Mick never managed to publish descriptions of the brachypterous females, but passed on his unfinished manuscript and notes to Michael Ohl for use in a forthcoming revision of the genus. Mick's suspicion that the species occurs on the mainland was confirmed in 2019 when the Russian hymenopterist Mikhail Mokrousov found a female in Turkey.

In July and August 1987, Mick joined Operation Raleigh in Seram, Indonesia, in order to collect insects for the museum. The trip was beset by several problems, not least his equipment and clothing being delayed on the way to Seram. This resulted in him having to walk several kilometers to base camp through streams and along trails carrying only what was contained in his cabin bag. On the way, one of the porters who was working for Operation Raleigh died of a heart attack. Mick and a few companions carried the body back to the deceased's home village where they joined in the ensuing wake. Otherwise, the trip was extremely successful and Mick made an excellent collection of Hymenoptera, including many new genera and species.

In 1988 Mick completed a major work on the 41 species of British Pompilidae. This was published as a Royal Entomological Society Handbook for the Identification

of British Insects. In this work there is a short discussion with regards Day's Organ which was named in honour of Mick by Arnold Menke (1982, see *Sphecos* 6:5). The function of this structure is unknown, but it is an exocrine gland found near the base of the metasoma and is likely to produce a sex pheromone. The handbook is a major work for anyone interested in this group of insects in Britain and Europe. The only recently published work on this group of insects elsewhere in Europe is clearly based on it, even occasionally using direct extracts.

In the late 1980s Mick realised that computing was becoming increasingly important in storing information useful to our work and decided to spend a greater proportion of his time and effort becoming the Entomology Department's computer expert. Mick thought that by doing this he could make himself indispensable and therefore safe from any possibility of losing his post. This decision was a mistake and he paid the price for this when, in 1990, he was made redundant during the museum's reorganisation. He was poorly treated in the process and never really recovered his confidence in the years that followed.

For a few years after his redundancy Mick continued to spend some time in the museum. He hoped to use some of his redundancy compensation to build a machine that would automatically cut data labels to size, but the technological challenge was too great and the project was put on hold indefinitely. In 1993 Mick was invited to work with Ian Naumann and Andrew Austin as senior collaborator on a two-year project to write a guide to the genera of Australian Pompilidae. The project was funded by the Australian Biological Resources Study. He accepted and during that time he visited Australia for several weeks to study material of this group held in the major museums. On his return to London most of the material was sent to Mick in London for further investigation. Unfortunately, the project ground to a halt when Ian Naumann left CSIRO in 1997–1998. It was revived by Andrew Austin in 2005 who was awarded ABRS funding to hire a postdoc. Lars Krogmann was appointed and visited Mick in London for some initial training and in 2007 Mick visited Australia for two weeks to advise further on the project. Regrettably, Mick was unable to carry the borrowed material to Australia with him and this was posted back to Adelaide in 2008 with the help of Mike Wilson at the National Museum of Wales in Cardiff. At about this time, Mick lost interest in the project, possibly because he and Judy had moved in 2006 from their home in Harrow not far from the Natural History Museum to Pen y Cae about 15 miles southwest of Brecon in Wales, more than one hundred miles away. It was left to Lars Krogmann to complete the project some years later.

During his career Mick became very good friends with Raymond Wahis, a Belgian entomologist and specialist on Pompilidae taxonomy. The two families spent many holidays together, in Harrow and Liege, where Mick and Raymond lived respectively. Judy and Josaine and girls would holiday together. The families remained close over the years and Mick considered the Wahis as family.

Mick spent most of his later life as an active member of the South Wales Caving club based at Penwyllt near Pen y Cae and only a short distance from his final home. Mick had been a gifted climber and caver from an early life and as a student he had



Figure 3. Mick outside his home in Pen y Cae, April 2015 (Photo George Else).

been active with Cardiff University Climbing and Caving Club. He and his wife Judy joined the South Wales Caving Club in October 1966. Mick was a central figure in the South Wales Caving Club and also nationally. He had helped in the exploration of the Dan yr Ogof and Ogof Ffynnon Ddu cave systems in South Wales and discovered several new large passages. It was here that he introduced willing museum colleagues to the delights of these cave systems, ensuring the survival of several total novices. He had also been part of an expedition that had discovered new passages and a huge

chamber in Grotta di Monte Cucco in Italy in 1969. Mick was chairman of the South Wales Climbing Club from 1970–1976 and from 1976–1984 was Biological Recorder of the British Cave Research Association. Then, for 20 years, he was Chairman of the National Caving Association until he oversaw its transformation into the British Caving Association, continuing as its Chairman for five years until he stood down in 2009. From 2011 until his death he was Honorary President of the British Caving Association. Mick contributed a huge amount to caving and to the organisation of caving UK wide, and is assured of a prominent place in caving history.

Mick will be remembered fondly by those who knew him at the Natural History Museum, as well as those in the South Wales Caving Club and British Caving Association. Above all he was a gentle, approachable, amusing person, and extremely good company with a love for debate. He also had a love of old Rover cars with a prowess for converting, dismantling and reassembling them. His knowledge and understanding of motor vehicles was fundamental to the success of the 1972 south-western Africa expedition which could not have happened without him. His caving associates will remember him for his bizarre caving garb of long johns, Aran sweater and cardboard helmet that became soft when wet!

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