

The species of *Eucera* Scopoli, subgenus *Tetralonia* Spinola from Sardinia (Italy) with new records and *E. gennargentui* sp. nov. (Hymenoptera, Apidae)

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Academic editor: Michael Ohl | Received 29 June 2021 | Accepted 25 November 2021 | Published 30 December 2021

<http://zoobank.org/510FBE37-39CF-4332-B105-59DF9EC9F65D>

Citation: Catania R, Nobile V, Bella S (2021) The species of *Eucera* Scopoli, subgenus *Tetralonia* Spinola from Sardinia (Italy) with new records and *E. gennargentui* sp. nov. (Hymenoptera, Apidae). Journal of Hymenoptera Research 88: 1–16. <https://doi.org/10.3897/jhr.88.70819>

Abstract

In this paper, an update of the species of the genus *Eucera* Scopoli, 1770, subgenus *Tetralonia* Spinola, 1838 from Sardinia is reported, based on data collection as well as on recent survey carried out at Gennargentu Massif. Seven species are recorded, four of which are newly added: *Eucera fulvescens* (Giraud, 1863), *E. gennargentui* sp. nov. Nobile, Catania & Bella, *E. julliani* (Pérez, 1879), and *E. nana* (Morawitz, 1873). The new species, *Eucera* (*Tetralonia*) *gennargentui* Nobile, Catania & Bella is described from the high altitude of Gennargentu Massif. Details on distributions, host plants, and other biological aspects are given for each species treated. The taxonomic comparison of the taxa belonging to the subgenus *Tetralonia* from Sardinia, including *E. gennargentui* sp. nov., are discussed taking into account both morphological and COI barcode sequences. An identification key to *Eucera* (*Tetralonia*) species from Sardinia is also provided.

Keywords

Apidae, *Eucera* (*Tetralonia*), new taxon, Mediterranean bees, Sardinia

Introduction

Belonging to the Eucerini tribe, *Eucera* Scopoli, 1770 is a genus of bees widespread in the world (Michener 2007), with a relevant number of species in the Palaearctic region and especially in the Mediterranean Basin (Tkalcu 1979, 1984; Risch 1997, 1999, 2001, 2003; Michener 2007; Dorchin et al. 2018). In their recent taxonomic studies Dorchin et al. (2018) treated the genus *Tetraloniella* Ashmead, 1899 within *Tetralonia* Spinola, 1838, and this last as a subgenus of *Eucera*.

The subgenus *Tetralonia* includes different oligolectic species, some of them closely associated and specialised with plants of the Asteraceae family (Müller 2008). The species of this subgenus have been revised for the Afrotropical region by Eardley (1989), for the New World by LaBerge (2001), and by Tkalcu (1979, 1984) for the Palaearctic region.

Eucera (*Tetralonia*) includes eleven species in Italy and some endemic subspecies of the main Italian islands, such as *E. dentata amseli* (Alfken, 1938) described from Sardinia and *E. alticincta bindai* (Nobile, 1993) described from Sicily (Alfken 1938; Nobile 1993).

To date, the knowledge on Sardinian bees is still limited, and only three species of *Eucera* (*Tetralonia*) are known: *E. dentata amseli*, *E. graja* (Eversmann, 1852) and *E. malvae* (Rossi, 1790) (Comba 2019; Nobile et al. 2021). This last species was recently found 78 years after its last report (Nobile et al. 2021).

In this paper, we add a further four species of *Eucera* (*Tetralonia*) as new for Sardinia and describe a new endemic species *E. gennargentui* sp. nov. Nobile, Catania & Bella from Gennargentu Massif, based on both morphological and molecular data.

Materials and methods

The material examined belongs partly to the collection of Vittorio Nobile and partly comes from field collecting efforts carried out by the two other authors in Sardinia during the year 2020.

Species identification was based on the works of Tkalcu (1979, 1984) and Scheuchl (2000). Morphological terminology and taxonomic classification follow Michener (2007) and Dorchin et al. (2018). Reference was made to the online ‘Check-list of Western Palaearctic Bees’ by Kuhlmann et al. (2018) and of ‘Hymenoptera: Apoidea: Anthophila of Italy’ by Comba (2019).

Observations in the field on *Eucera gennargentui* sp. nov. were made in the first week of August 2020 in the locality of Bruncu Spina, near the town of Fonni (40°01'48.0"N, 9°17'56.6"E), in the central area of Gennargentu Massif, at 1540 m a.s.l., characterized by several endemic high mountain species plants (Bacchetta et al. 2013) (Fig. 1); observations were carried out from 09:30 to 16:30, on the flowers of the Asteraceae family.

Gennargentu Massif is located near the centre of the island, almost directly at 40° N. Bruncu Spina, at 1829 m a.s.l., is the second highest mountain in Sardinia,

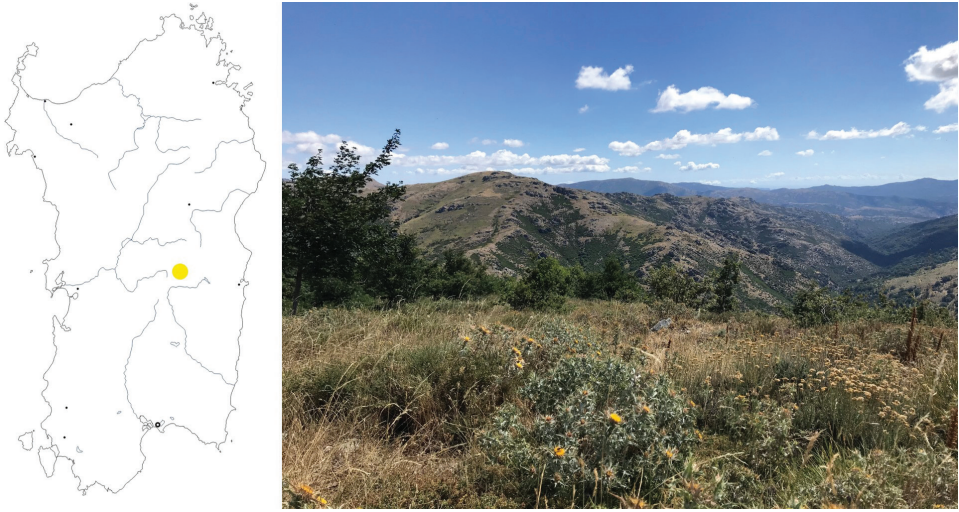


Figure 1. Sardinia and Gennargentu Massif, locality of Bruncu Spina, 1540 m a.s.l., the site of the samplings of *Eucera gennargentui* sp. nov.

after Punta La Marmora (1834 m a.s.l.), and is the northernmost summit of the main Gennargentu ridge.

In order to collect biological data on the new taxon, some specimens were marked using a dye specific to bees. The date of capture, general and Italian distribution, number of specimens observed, and the plants visited are reported for all species.

Each specimen was killed by freezing at -20°C for use in DNA analysis and was dried and identified to species level after the observation of genital structures. The specimens were preserved in the collection of the authors, and in the collection of the Department of Agriculture, Food and Environment, sect. Applied Entomology, University of Catania (Di3A).

The specimens were studied with a stereomicroscope Leica EZ4HD, the photos taken with a Leica Application Suite vs. 4.13.

Total DNA was extracted from one posterior leg using the NucleoSpin Tissue Kit (Macherey-Nagel, Germany) following the manufacturer's instructions. Subsequently, the enzymatic amplification took place using primers LCO1490F and HC02198R (Folmer et al. 1994). PCR products were purified using ExoSAP-IT (ThermoFisher Scientific) and were sequenced at BMR genomics (Padua, Italy), and the sequences obtained were analysed using MEGA X 10.2.4 (Kumar et al. 2018).

The sequences were aligned with seven COI sequences of *Eucera* (*Tetralonia*) available in GenBank, and we also included two sequences of *Habropoda tarsata* (Spinola, 1838) (accession numbers MN919536, MN919537) as an outgroup (Table 1). Sequence divergences were calculated using the *p*-distance model (Srivathsan and Meier 2012), and a neighbour-joining (NJ) tree (Saitou and Nei 1987), as implemented in MEGA X, was used to visualize the distance matrix among taxa.

Table 1. Analyzed sequences with accession number and origin countries.

Species	Accession number	Country
<i>Eucera alticincta alticincta</i>	MNHNL005-20	Luxembourg
<i>Eucera alticincta bindai</i>	MZ437367	Italy, Sicily
<i>Eucera dentata</i>	FBAPD711-11	Germany
<i>Eucera fulvescens</i>	ABEE208-17	Austria
<i>Eucera gennargentui</i> sp. nov.	MZ437082	Italy, Sardinia
<i>Eucera graja</i>	MG251108	-
<i>Eucera malvae</i>	FBAPC604-11	Germany
<i>Eucera nana</i>	ABEE206-17	Austria
<i>Eucera salicariae</i>	FBAPC749-11	Croatia
<i>Habropoda tarsata</i>	MN919536	Italy, Sicily
<i>Habropoda tarsata</i>	MN919537	Italy, Sicily

Acronyms

Di3A	Department of Agriculture, Food and Environment, Catania, Italy;
RC	Roberto Catania, Catania, Italy;
SB	Salvatore Bella, Catania, Italy;
VN	Vittorio Nobile, Ragusa, Italy.

Results

Detected species

Genus *Eucera* Scopoli, 1770

Subgenus *Tetralonia* Spinola, 1838

Eucera dentata amseli (Alfken, 1938)

Fig. 2

Tetraloniella dentata amseli Alfken, 1938, Mem. Soc. Entomol. It., 16: 97–114.

Data from literature. Alfken 1938.

Material examined. • 1 ♀; ITALY Sardinia, Iglesias, San Pietro (Cagliari province); 12.VII.1976.

Distribution. Sardinia and Corsica.

Range in Italy. Sardinia.

Eucera fulvescens (Giraud, 1863)

Tetralonia fulvescens Giraud, 1863, Verh. zool.-bot. Ges. Wien, 13: 42–43.

Material examined. • 1 ♀; ITALY Sardinia, Busachi (Oristano province); 10.VI.1975; coll. C. Meloni – D. Sechi.



Figure 2. Male of *E. dentata amseli* Alfken. Sardinia, Ittiri (Photo by P. Niolu).

Distribution. South Europe, North Africa, West and Central Asia (Tkalcu 1979).

Range in Italy. This species is discontinuously present in the Italian peninsula and Sicily.

New record for Sardinia

***Eucera gennargentui* Nobile, Catania & Bella sp. nov.**

<http://zoobank.org/B12C779B-9542-4C49-ADE2-3E1F07D99A43>

Material examined. 10 ♀, 1 ♂: **Holotype:** • 1 ♀; ITALY, Sardinia, Fonni (Nuoro province), Gennargentu Massif, Bruncu Spina, 1540 m a.s.l.; 6–8.VIII.2020; S. Bella and R. Catania leg.; Di3A. **Paratypes:** • 9 ♀, 1 ♂; paratypes identical data to previous; Di3A, RC, VN, SB.

Description. Female (Fig. 3). Body length: 10 mm. Body with black integument with fine and evident punctation, grey setae. Head. Head with wrinkled punctation, finer at the edges and with white setae; clypeus black, with the lower half light yellow; this spot, extending upwards, and in the middle part, ends with a triangulated tip; labrum and mandibles black, antennae dorsally black, ventrally orange, with scape and

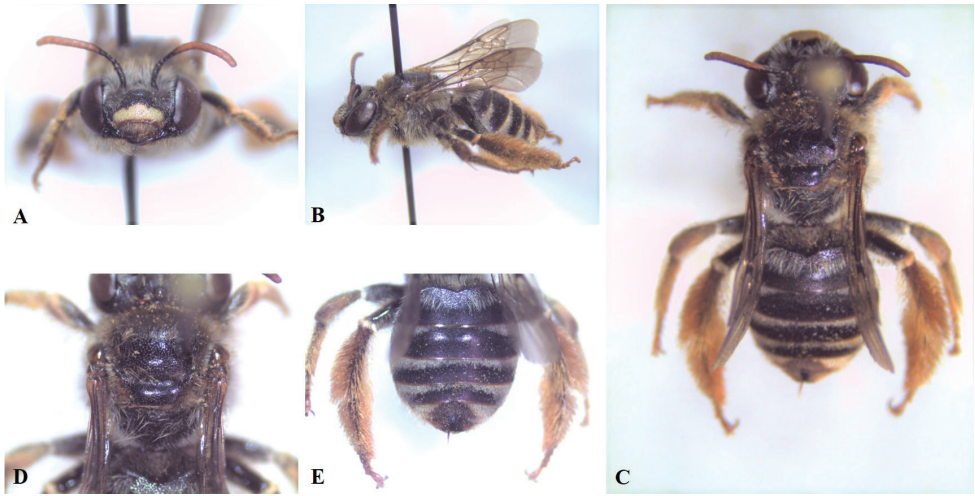


Figure 3. *Eucera gennargentui* sp. nov., female (**A-E**) **A** head, anterior view **B** habitus, lateral view **C** dorsal view **D** mesosoma, dorsal view **E** metasoma, dorsal view. (Photos by R. Catania).

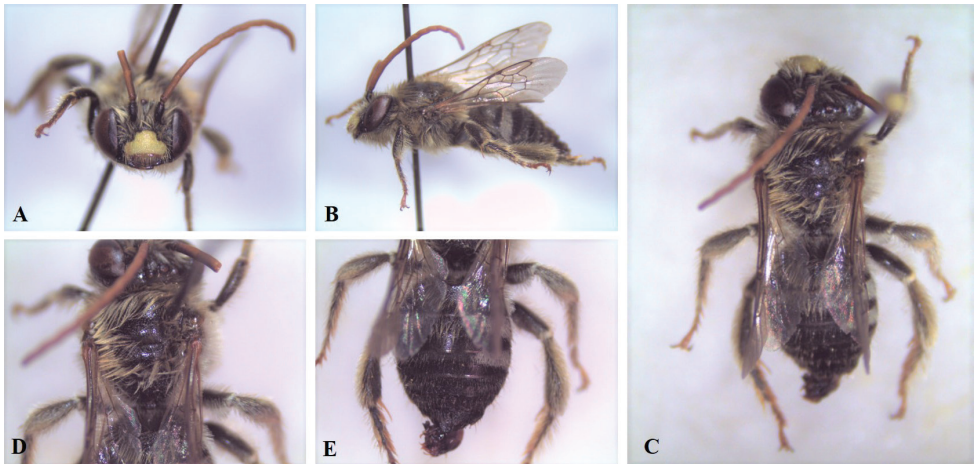


Figure 4. *Eucera gennargentui* sp. nov., male (**A-E**) **A** head, anterior view **B** habitus, lateral view **C** dorsal view **D** mesosoma, dorsal view **E** metasoma, dorsal view. (Photos by R. Catania).

first two flagellar articles black. Mesosoma. Mesothorax with greyish setae, median part of the metathorax with dense and large punctation. Black legs with greyish setae and the last four tarsi brown; the hind legs are equipped with a uniformly yellow-rust-coloured pollen-collection system. Slightly darkened wings, with black veins and dark brown regulae. Metasoma. Dark brown, almost black, with dense and fine punctation. T1 with long and sparse white setae, T2–4 with basal bands of white felt, T5 with white felt on the sides and dark brown in the middle, and T6 almost completely covered with dark-brown felt. Dark-brown sternites with thick punctation and long golden-yellow fringes.

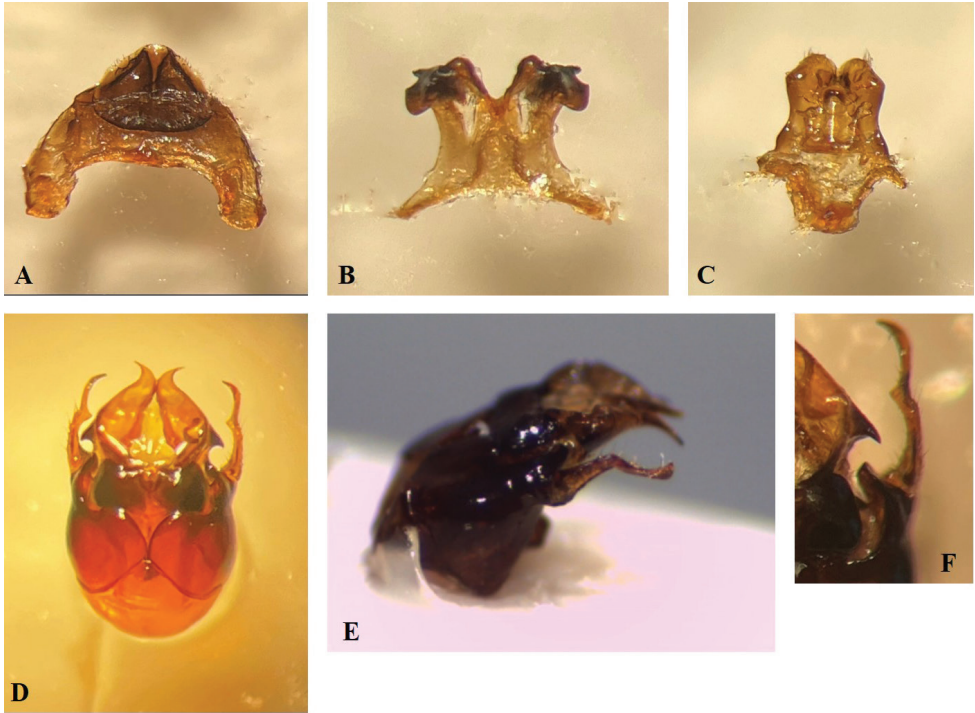


Figure 5. *Eucera gennargentui* sp. nov., genitalia and hidden sterna (A-F) **A** S6 ventral view **B** S7 ventral view **C** S8 ventral view **D** genitalia, dorsal view **E** genitalia, lateral view **F** vision of gonostylus and posterodorsal projection of gonocoxa (Photos by R. Catania).

Male (Figs 4–5). Body length: 9 mm. General appearance similar to the female, with yellow clypeus and labrum, antennae are more than half of the body length long and reddish, except for a black streak on the whole dorsal part; black scape; mandibles are black, but reddish in the distal half. Mesosoma. With long silky whitish setae. Black legs with whitish setae. The hind femur, in the lower median part, has a small pointed tubercle equipped with a tuft of brownish bristles. Metasoma. As in the female, is dark brown, but all segments are covered with white felt, and T6 on the sides has 2 prominent teeth, one on each side. The last sternites are provided with a long and deep longitudinal median sulcus. Genitalia and hidden sterna. S6 without anterolateral marginal projections; posterolateral carina curved laterally at each side. S7 with medial process rounded and attached at the posterior lobes of lateral processes; the posterior lobes of lateral processes are relatively short with straight superior margins; anterior lobes of lateral processes wide with straight superior margins and rounded lateral-inferior margins. S8 with a wide concavity between rounded apical lobes; apicomedian ventral process well defined and saddle shape, with a large depressed area at the base. Genital capsule with the apex of the gonostylus remarkably arched, and inward-facing triangular protuberance placed slightly over the central part of the gonostylus.

Table 2. Morphological comparison between *Eucera gennargentui* sp. nov. and the closest related taxa.

<i>E. alticincta alticincta</i>	<i>E. alticincta bindai</i>	<i>E. gennargentui</i> sp. nov.
Female		
1) slim overall appearance	1) compact and robust overall appearance	1) slim overall appearance
2) mesosoma with sparse punctation	2) mesosoma with dense punctation	2) mesosoma with denser punctation
3) metasoma ovoidal	3) metasoma subspherical	3) metasoma ovoidal
4) 3° and 4° metasomal tergites with white, broad, median tomentose band, slightly narrowing in the middle	4) 3° and 4° metasomal tergites with white, broad, median tomentose band, deeply narrowing in the middle	4) 3° and 4° metasomal tergites with white, broad, median tomentose band, deeply narrowing in the middle
5) white long fringes on the metasomal sternites	5) white long fringes on the metasomal sternites	5) yellow-gold long fringes on the metasomal sternites
6) scopa whitish externally and brownish internally	6) scopa whitish externally and yellowish internally	6) yellow-rust scopa
7) pigidial plate normally thinned	7) less thinned pigidial plate	7) pigidial plate normally thinned
8) body length = 7 mm	8) body length = 8 mm	8) body length = 9–10 mm
Male		
1) compact overall appearance	1) less compact overall appearance	1) less compact overall appearance
2) brownish-yellow long silky mesosomal setae	2) brownish-yellow long silky mesosomal setae	2) whitish long silky mesosomal setae
3) metasomal ovoidal (longer than wide)	3) metasomal subspherical (as long as wide)	3) metasomal ovoidal (longer than wide)
4) 3° and 4° metasomal tergites with white, broad, median tomentose band, slightly narrowing in the middle	4) 3° and 4° metasomal tergites with white, broad, median tomentose band, deeply narrowing in the middle	4) 3° and 4° metasomal tergites with white, broad, median tomentose band, deeply narrowing in the middle
5) 1° – 4° metasomal sternites distally with fringe of dense, bristly, short and protruding light ivory setae (more developed those on the 2° and 3°)	5) 1° – 4° metasomal sternites without fringe	5) 3° – 5° metasomal sternites distally with fringe of short and protruding yellow setae
6) normal pigidial plate	6) less wide pigidial plate	6) normal pigidial plate
7) body length = 8 mm	7) body length = 7 mm	7) body length = 9 mm

Difference with the closest related taxa. The new species *Eucera gennargentui*, is similar to *E. alticincta* (Lepeletier, 1841), including the nominate subspecies and *E. alticincta bindai*.

In the female of *E. gennargentui*, the long fringes of the sternites are yellow-gold, whereas in the closest related species *E. alticincta alticincta*, they are white; the scopa is yellow-rust in the new species, whitish externally and brown internally in *E. alticincta alticincta*.

In the male of the new species, the long silky hairs of the mesosoma are whitish, while they are brownish-yellow in *E. alticincta alticincta*. Genitalia of *E. gennargentui* is very similar to that of *E. alticincta alticincta*, however, in the gonostylus the inward-facing triangular protuberance, that in *E. alticincta* is located in the middle part, in the new species is placed slightly over half of the gonostylus, slightly closer to the apex. Further differences can be noted in the morphology of the hidden sterna, especially for the medial and lateral processes in S7, and for apical lobes and apicomedian ventral process in S8.

In both sexes of the new species, the integument and wings are considerably darker than *E. alticincta alticincta*, and the body length of both sexes of *E. gennargentui* is greater than that of the related species.

Further morphological and bio-ecological comparisons between *E. alticincta alticincta*, *E. alticincta bindai*, and *E. gennargentui* are reported in Tables 2 and 3.

Derivatio nominis. We named the new species after Gennargentu Massif, where the specimens were found.

Table 3. Geographic and bio-ecological comparison between *Eucera gennargentui* sp. nov. and the closest related taxa.

<i>E. alticincta alticincta</i>	<i>E. alticincta bindai</i>	<i>E. gennargentui</i> sp. nov.
Distribution		
Widespread in Europe, North Africa, and western Asia (Varnava et al. 2020)	Sicily (Nobile, 1987; 1993), Nebrodi Mts (Messina province), northern slope of Mt Etna (RC, pers. observ.), and Syracuse province	Central Sardinia, Gennargentu Massif
Host plants		
oligolectic on <i>Inula</i> and <i>Pulicaria</i> (Tkalcu, 1979; Müller, 2008)	observed on <i>Pulicaria</i> (RC, pers. observ.)	oligolectic on <i>Carlina</i> , <i>Helichrysum</i> , and <i>Tanacetum</i>
Environments		
Temperate and Mediterranean	Mediterranean and Mediterranean mountains (100 – 1350 m a.s.l.)	Mediterranean high mountains (1540 m a.s.l.)
Phenology (generation and months)		
probably univoltine; VII–VIII	probably univoltine; VIII–IX	probably univoltine; VIII

Distribution. Sardinia (Italy).

Flower choices. Specimens of the new taxon were observed on *Carlina macrocephala* Moris subsp. *macrocephala*, *Tanacetum audibertii* (Req.) DC. (Sardo-Corsican endemisms), and *Helichrysum saxatile* Moris subsp. *saxatile* (Sardinian endemism) (Asteraceae) (Fig. 6).

Bio-ecological aspects. A total of 22 specimens (half captured and half marked) were observed in the sampling site: 20 females and 2 males. Specimens of *E. gennargentui* were observed in activity in a restricted area of about 300 m², from 10:30 to 15:00; in particular, the females visited the flowers from 10:30 to 14:30, and the two males were observed from 13:30 to 15:00 on *Carlina macrocephala* subsp. *macrocephala*. The female specimens visited the same flowers at intervals of about ten minutes, with a preference for the flowers of *Carlina macrocephala* subsp. *macrocephala*.

To know better the flights period of *E. gennargentui*, further surveys in the same site were conducted during mid-July 2021, however no specimens were found. This confirms the relationship between the new species and its main host plant, in fact *Carlina macrocephala* subsp. *macrocephala* was at the beginning of its flowering.

**Figure 6.** Female of *Eucera gennargentui* sp. nov. Sardinia, Gennargentu Massif, locality of Bruncu Spina (Photos by P. Niolu - R. Catania).

***Eucera graja* (Eversmann, 1852)**

Macrocera graja Eversmann, 1852, Bull. soc. natural. Moscou. 25: 124.

Data from literature. Grandi 1962 (Ozieri, Sassari province); Comba 2019.

Distribution. South and Central Europe, and West Asia (Banaszak & Ortiz-Sánchez 1993).

Range in Italy. This species is discontinuously present in the Italian peninsula, Sardinia and Sicily.

***Eucera julliani* (Pérez, 1879)**

Macrocera julliani Pérez, 1879, Act. Soc. Linn., 33: 150.

Material examined. • 1 ♀; ITALY Sardinia, Assemini (Cagliari province); 3.IX.1990; L. Fancello leg. • 1 ♂; Sardinia, Rio Flumini Mannu (Cagliari province), 2.VII.1992; C. Meloni leg.; on *Ammi visnaga* (L.) Lam. (Apiaceae).

Distribution. Turanic-South-European (Nobile 1987).

Range in Italy. This species is discontinuously present in the Italian peninsula, and Sicily.

New record for Sardinia

***Eucera malvae* (Rossi, 1790)**

Apis malvae Rossi 1790, Fauna Etrusca II, p. 107.

Data from literature. Nobile et al. (2021).

Material examined. • 1 ♀, 1 ♂; ITALY Sardinia, Capoterra, Rio S. Lucia (Cagliari province); 4.VI.1985; C. Meloni leg.

Distribution. Widespread in Europe, Eastern Mediterranean (Cyprus) and Western Asia (Turkey).

Range in Italy. This species is discontinuously present in the Italian peninsula, and Sicily.

***Eucera nana* (Morawitz, 1873)**

Tetralonia nana Morawitz, 1873, Horae soc. entom. Ross. X, p. 144.

Material examined. • 1 ♀; ITALY Sardinia, Teulada, Tuerru (South Sardinia province); 16.VII.1973; • 3 ♂♂; Sardinia, Rio Flumini Mannu (Cagliari province); 2.VII.1992; C. Meloni leg.; on *Ammi visnaga* (L.) Lam. (Apiaceae).

Distribution. Widespread in Europe, and Western Asia (Turkey).

Range in Italy. This species is known in the regions of

.New record for Sardinia.

Identification key to *Eucera* (*Tetralonia*) from Sardinia

Female:

- 1 Clypeus more or less yellow 2
- Clypeus black 5
- 2 Antennae ventrally dark brown. 10,5–11,5 mm..... *E. dentata amseli*
- Antennae ventrally light red, except for the scape and 1–2 flagellar articles that are black 3
- 3 Scopa dark brown externally and even darker internally. 12–14 mm.....
..... *E. graja*
- Different colour of the scopa, smaller body size 4
- 4 Scopa whitish externally and brown internally. 8–9 mm *E. julliani*
- Scopa uniformly rusty-yellow. 9–10 mm..... *E. gennargentui* sp. nov.
- 5 Orange scopa. 11–12 mm..... *E. fulvescens*
- White or whitish scopa 6
- 6 Scopa with long, sparse, thin, and white setae. 8 mm..... *E. nana*
- Scopa with long, sparse and whitish setae. 11–13 mm *E. malvae*

Male:

- 1 Antennae ventrally more or less dark brown..... 2
- Antennae ventrally light red except for the scape black..... 5
- 2 Labrum and clypeus yellow..... 3
- Labrum and the lower part of the clypeus yellow. 11–13 mm *E. malvae*
- 3 T6 laterally with short and stocky teeth, one on each side, facing backwards. 8 mm..... *E. nana*
- More tergites with teeth, larger body size 4
- 4 In addition to clypeus and labrum, the base of the mandibles is yellow, T 5–6 with short and stocky teeth. 10 mm..... *E. dentata amseli*
- Mandibles black, T5–7 with slightly longer and sharper teeth. 11–12 mm ...
..... *E. fulvescens*
- 5 Clypeus, labrum, supraclypeal space and mandible yellow. 11–13 mm
..... *E. graja*
- Mandibles and supraclypeal space black..... 6
- 6 In the genital capsule the apex of the gonostylus facing inwards are almost straight. 9–10 mm *E. julliani*
- In the genital capsule the apex of the gonostylus are remarkably arched. 9 mm *E. gennargentui* sp. nov.

Table 4. *p*-distance and nucleotide divergences (expressed as percentages) of *Eucera gennargentui* sp. nov., *E. alticincta bindai*, and *Eucera* (*Tetralonia*) species with *Habropoda tarsata* used as the outgroup. Average distance: 12.25.

1	<i>Eucera gennargentui</i> sp. nov.										
2	<i>Eucera alticincta bindai</i>	2.21									
3	<i>Eucera alticincta alticincta</i>	2.08	1.21								
4	<i>Eucera fulvescens</i>	4.09	3.55	3.41							
5	<i>Eucera graja</i>	7.65	7.31	7.47	7.47						
6	<i>Eucera dentata</i>	8.34	8.0	8.05	8.68	8.34					
7	<i>Eucera salicariae</i>	7.30	6.95	6.68	6.82	7.65	8.20				
8	<i>Eucera nana</i>	7.69	8.84	8.07	8.84	9.61	8.84	6.92			
9	<i>Eucera malvae</i>	7.82	8.0	8.51	7.44	6.43	8.81	6.38	6.92		
10	<i>Habropoda tarsata</i> MN919536	22.61	23.29	24.73	24.92	23.29	23.96	23.05	24.61	23.05	
11	<i>Habropoda tarsata</i> MN919537	22.61	23.29	24.06	24.24	22.76	23.44	22.36	24.61	22.51	0

Molecular evidences

High-quality DNA sequences were obtained for the two specimens analysed, one sequence of *E. gennargentui* sp. nov. from Sardinia (GenBank accession number MZ437082) and one of *E. alticincta bindai* from Sicily (1 ♀; ITALY, Sicily, Mount Etna, Gurrida Lake, 25.VIII.2020 RC leg., det., and coll.) (GeneBank accession number MZ437367). Both COI sequences obtained from the samples were used as queries in BOLD-IDS tools, and the system returned a match (sequence of *E. gennargentui*: 97.88%; sequence of *E. alticincta bindai*: 98.77%) with a sequence of *E. alticincta* present in GenBank.

Sequence divergences calculated using the *p*-distance model are reported in Table 4, and the neighbor-joining tree showing the relationships between the new species and other related species of *Eucera* (*Tetralonia*) are reported in Fig. 7.

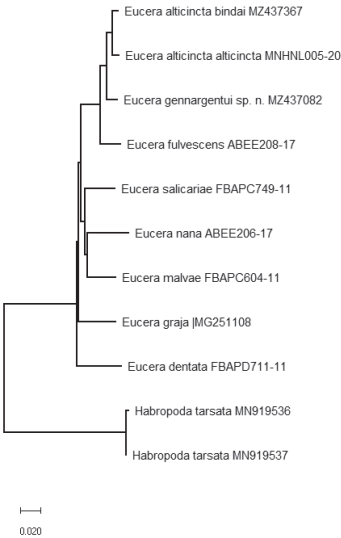


Figure 7. Neighbor-Joining tree showing relationships among *Eucera gennargentui* sp. nov., and *Eucera* (*Tetralonia*) species with *Habropoda tarsata* used as the outgroup.

Conclusion

In the current study, *Eucera fulvescens*, *E. julliani*, and *E. nana* are reported for the first time for Sardinia, and *E. gennargentui* sp. nov. is described from Gennargentu Massif.

This new species shows a larger body size and a darker appearance than the closest related taxa *E. alticincta alticincta* and *E. alticincta bindai*, and other different characters reported in this work. Furthermore, following our field observations, we ascertained how the new species was linked to three Sardo-Corsican and Sardinian endemic botanical species of the flora of Gennargentu Massif.

The barcode sequence of the new species *Eucera gennargentui* diverged from that of *E. alticincta alticincta* from 2.08% and from that of *E. alticincta bindai* by 2.21%.

In Europe, wild bees are threatened by habitat changes resulting from human activities, e.g., urban expansion, agricultural practices, habitat destruction or changes, and climate changing (Matheson et al., 1996; Steffan-Dewenter et al., 2005; Nieto et al. 2014; Bella et al. 2020; Fisogni et al. 2020). There was poor information for the majority of the species (56.7%) to determine their conservation status (Nieto et al. 2014).

In Italy, the availability of information on bees is particularly scarce, especially in southern and insular regions, so it is important to increase research to better understand the distribution and the population tendencies, especially for localised taxa.

More observations are necessary to understand the conservation status of this new species and of the other *Eucera* (*Tetralonia*) species present in Sardinia. However, the area of Gennargentu where *Eucera gennargentui* was found is rather restricted and subject to disturbing factors due to strong grazing regimes and reckless reforestation that inexorably damage the grasslands where the endemic host plants live. The frequency of numerous fires that devastate natural ecosystems and rural areas in Sardinia (Capra et al. 2018; Salis et al. 2021) represents a further important threat to the diversity of flora and consequently of wild bees.

The present study greatly improves the Sardinian bee fauna, showing that this Mediterranean island will surely deserves further new acquisitions, in order to increase our knowledge of its still underestimated pollinator fauna.

Acknowledgements

We thank Pietro Niolu (Alghero, Italy) for guiding us in the territory of Gennargentu Massif, and Silvia Di Silvestro (CREA, Acireale, Italy) for helping us in the genetic investigations. We also wish to thank the two reviewers, whose detailed comments and suggestions helped improve this manuscript.

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Phenological and social characterization of three *Lasioglossum* (*Dialictus*) species inferred from long-term trapping collections

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Academic editor: C.K. Starr | Received 20 August 2021 | Accepted 29 November 2021 | Published 30 December 2021

<http://zoobank.org/A852B543-9336-493E-8C8D-4D489B97424E>

Citation: Corbin LA-J, Awde DN, Richards MH (2021) Phenological and social characterization of three *Lasioglossum* (*Dialictus*) species inferred from long-term trapping collections. Journal of Hymenoptera Research 88: 17–38. <https://doi.org/10.3897/jhr.88.73220>

Abstract

Detailed social and phenological data collected from nesting aggregations exist for relatively few sweat bee species because nesting aggregations are rarely found in large numbers, even when local populations are highly abundant. This limits researchers' abilities to assess the social status of many species, which in turn, limits our ability to trace the sequence of evolutionary steps between alternative social states. To address this problem, we demonstrate the utility of rehydrated, pinned specimens from pan trap and netting collections for generating inferences about the phenology and social status of a well-studied sweat bee species, *Lasioglossum* (*Dialictus*) *laevissimum*. A detailed comparison of phenology and reproductive traits, between pinned specimens and those in a previous nesting study, produced similar results for bivoltine foraging activity and eusocial colony organization typical in this species. We then used pinned specimens from monitoring studies to describe, for the first time, the foraging phenology and social behaviour of two additional *Dialictus* species, *L. hitchensi* and *L. ellisiae*. Both *L. hitchensi* and *L. ellisiae* each exhibited two peaks in abundance during their breeding seasons, indicating two periods of foraging activity, which correspond to provisioning of spring and summer broods. Differences in body size, wear, and ovarian development of spring and summer females indicated that *L. hitchensi* is most likely eusocial, while *L. ellisiae* is either solitary or communal. This study demonstrates that analyses of specimens obtained from flower and pan trap collections can be used for assessing the phenology and social organization of temperate sweat bees in the absence of nesting data. The phenological and social lability of many sweat bee species make them ideal for studying geographic and temporal variability in sociality, and analyses of pan trap collections can make these studies possible when direct observations are impossible.

Keywords

Eusociality, pan traps, social reversal, solitary, sweat bees

Introduction

In temperate zone sweat bees (Hymenoptera: Halictidae), flight phenology is closely linked to sociality. Univoltine species are virtually always solitary or communal, whereas the necessity of producing a worker brood requires that eusocial species are bivoltine, or multivoltine, if multiple worker broods are produced. While the term “voltinism” technically refers to the number of generations per year, in studies of halictid bees, it has taken on an additional usage in phenology, and is frequently used to refer to the number of broods produced each year (Richards et al. 2010). In this study, we use voltinism to refer to the latter. Facultative social sweat bees exemplify the strong connection between phenology and sociality. Some species, like *Lasioglossum calceatum* and *Halictus rubicundus*, exhibit a bivoltine, eusocial life history at low altitudes and a univoltine, solitary life cycle at high altitudes (Sakagami and Munakata 1972; Eickwort et al. 1996). Others exhibit social and phenological polymorphism within populations, with solitary nest foundresses provisioning a single brood of reproductives, while eusocial foundresses produce a worker brood that subsequently provisions the second brood (Packer et al. 1989; Packer 1990). The strength of the link between a bivoltine phenology and eusociality is well known phylogenetically, leading to the hypothesis that in halictine bees evolutionary transitions to eusociality involved two major steps: an initial transition from univoltinism to bivoltinism, and a subsequent step from solitary breeding to eusociality (Brady et al. 2006).

The subgenus *Lasioglossum* (*Dialictus*) is well known as a vast and socially diverse group of halictid bees, with over 250 species existing in North America (Ascher and Pickering 2020). *Dialictus* includes species categorized as solitary, communal, semisocial, eusocial, and socially parasitic (Batra 1966; Breed 1976; Eickwort 1986; Wcislo 1997; Gibbs 2010, 2011). Phylogenetic analyses suggest that *Lasioglossum* as a whole is ancestrally eusocial, implying considerable evolutionary lability in social traits and multiple reversions from social to solitary behaviour (Gibbs et al. 2012). However, the behaviour of many *Dialictus* remains unstudied. The social status of some species has been broadly categorized based on presumed similarities among closely related taxa, but the lability of halictid sociality means that closely related species might exhibit very different types of social behaviour. Moreover, broad social categories are limited in their usefulness for precisely tracing the sequence of evolutionary changes that must have occurred in transitions between alternative social states, such as caste-based or casteless sociality (Dew et al. 2016; Richards 2019). More detailed studies of solitary and social halictine bees are required to provide this kind of behavioural data.

Ideally, to study colony social organization in sweat bees, large numbers of colonies are observed from start to finish of at least one complete breeding season (Rehan et al. 2013;

Onuferko et al. 2015; Awde and Richards 2018). The activity of foragers at nest entrances established flight phenology and the timing of brood provisioning phases. Nests are excavated at various times to obtain ‘snapshots’ of colony development and brood production, as well as to collect adult females. Adult females are examined to investigate body size, wing wear, mandibular wear, and ovarian development status, traits that relate to caste and reproductive status. However, a major challenge is that sweat bee nests are rarely found together in large enough numbers that biologists are likely to devote the time to studying them, even in areas where local populations are highly abundant. Museum collections provide an alternative source of specimens that can be used to investigate both phenology and reproductive activities of halictid and other bees. Passive collection methods, such as regular pan-trapping or sweep-netting, provide large numbers of halictids specimens (Portman et al. 2020) that can be counted and used for estimating the relative abundance of foraging females from spring to autumn. Preserved specimens, including pinned specimens, can be rehydrated in water, allowing females to be dissected and their ovarian development measured (Packer et al. 2007; Richards et al. 2010, 2015).

In this study, we used pinned specimens previously collected and identified for a long-term monitoring study in the Niagara region of southern Ontario, Canada (Richards et al. 2011; Onuferko et al. 2015) to evaluate the social status of three sympatric *Dialictus* populations. Of 33 *Lasioglossum* (*Dialictus*) species present locally, only seven are known to be eusocial (including *L. laevisissimum*), 17 are predicted to be eusocial (including *L. hitchensi* and *L. ellisiae*), one is possibly communal, four are cleptoparasites or social parasites, and four have undescribed social statuses (Onuferko et al. 2015). None of the species are described as solitary. Thus our primary objective was to describe the phenology and social behaviour of two species, *L. (Dialictus) hitchensi* and *L. (D.) ellisiae*, both predicted to be eusocial on phylogenetic grounds (Gibbs et al. 2012; Onuferko et al. 2015). Our second objective was to further validate the use of pinned specimens collected using passive collecting methods, such as pan traps and sweep nets, when nests are not available. We do this by explicitly comparing social trait inferences from pan trap collections of *L. (D.) laevisissimum*, to those from a study based on detailed behavioural observations and nest excavation from a sympatric population (Awde and Richards 2018).

Methods

Study sites and specimen collections

All specimens were collected from grassy meadows as part of a monitoring study from 2003 to 2006 and from 2008 to 2013 at four sites in southern Ontario: Brock University (43.1178°N, 79.2473°W) and the adjacent Glenridge Quarry Naturalization Site in St. Catharines (43.1197°N, 79.2390°W), the Elm Street Naturalization Site in Port Colborne (42.9235°N, 79.2579°W), and the Station Road Naturalization Site in Wainfleet (42.8847°N, 79.376°W; Onuferko et al. 2015). Descriptions of collecting

sites and the local bee assemblage, which includes 33 *Dialictus* species, are available in Richards et al. (2011) and Onuferko et al. (2015).

We used specimens collected via pan traps, netting from flowers, and sweep-netting to examine the social status of *L. laevisissimum*, *L. hitchensi* and *L. ellisiae*. *Lasioglossum laevisissimum* was used for a comparison of social trait inferences from monitoring studies with the ‘gold standard’ of nest-based observations from a previous study in the same region (Awde and Richards 2018). *Lasioglossum hitchensi* and *L. ellisiae* were selected because large numbers of specimens had been identified and collected, and because these two species are likely to be phylogenetically informative in future studies.

Collection methods were compared and described in detail in Richards et al. (2011). Pan trapping and sweep netting were carried out each year either weekly or biweekly from spring (April or May) to fall (September or October), for a total of 10 years of collections, covering the entire flight season for the local bee community. Specimens were preserved in 70% ethanol before being pinned and identified using taxonomic keys by Gibbs (2010, 2011) for *Lasioglossum* (*Dialictus*) in combination with online keys on Discover Life (Onuferko et al. 2015; Ascher and Pickering 2020). Specimens caught in 2003 were identified by Dr. Jason Gibbs (Richards et al. 2011) and those caught from 2004–2013 were identified by Dr. Thomas Onuferko (Onuferko et al. 2015), with additional confirmations by Ms. Nora Romero. All specimens are currently in the collection of the Brock Bee Lab, at Brock University, Canada.

Flight phenology

In the Niagara region of southern Ontario, halictid bees generally begin spring brood provisioning activity in late April or early May and summer flight activity peaking in July (Richards et al. 2010, 2015; Proulx 2020). Locally, univoltine sweat bee populations can be identified in weekly (or biweekly) pan trap collections as those showing a single peak in female abundance (pan traps are presumed to mainly attract foragers) from spring to summer, whereas bivoltine sweat bee populations exhibit two peaks, one in spring and one in summer.

Flight phenology (whether bees were univoltine or bivoltine) was inferred solely from weekly abundances of female and male bees caught in pan traps, pooling samples over the ten years from 2003 to 2013 (bees were not collected in 2007). Total numbers of pan trapped specimens were 51 females and 1 male for *L. laevisissimum*, 1473 females and 7 males for *L. hitchensi*, and 54 females and 13 males for *L. ellisiae*. For each species, we decided which week to designate as the end of the spring provisioning and the beginning of summer provisioning periods, based on several key factors: the re-appearance of unworn females suggesting emergence of Brood 1, the appearance of males (most eusocial species produce at least a few males in Brood 1), and whether population sizes seemed to be increasing (reviewed in Awde and Richards 2018; Breed 1976; Packer 1992; Richards et al. 2010). We also plotted mean weekly abundance of females and males (the number of bees caught per calendar week divided by the total number of collections per calendar week, pooling over years), and then used a local

polynomial regression (Loess) smoother to evaluate the number of peaks in female bee abundance and foraging activity from spring to summer (geom_smooth command, package: ggplot2). Since data were pooled over ten years of collections over which the timing of spring activity might differ considerably, the designated cutoff dates between spring and summer provisioning are approximate.

Female traits related to social behaviour

Female traits were examined in females collected by all three methods (Table 1). We examined female traits frequently used to evaluate colony social organization in halictid bees, including body size, mandibular and wing wear, and degree of ovarian development (Packer 2007; Richards et al. 2010, 2015). Pinned female specimens were measured and dissected using a Zeiss stereomicroscope, equipped with an ocular micrometer at 8–66× magnification. Head width (HW) was measured as the widest distance across the head, including the compound eyes. Costal vein length (CVL) was measured on the right forewing, from the base of the costal vein to the stigma (Suppl. material 1). If the right wing was damaged, the left wing was used instead. Since HW and CVL were positively correlated in all species (Pearson’s product-moment correlation; *L. laevisissimum*: $t=4.75$, $df=91$, $p=7.322e-06$; *L. hitchensi*: $t=21.65$, $df=120$, $p<2.2e-16$, *L. ellisiae*: $t=15.93$, $df=78$, $p<2.2e-16$), HW was used for size comparisons. Size differences between spring and summer females were calculated using the formula:

$$[(\text{mean spring HW} - \text{mean summer HW}) / \text{mean spring HW}] \times 100$$

We assessed the amount of burrow digging and flight activity performed by each female by examining the level of wear that accumulated on their mandibles and forewings respectively (Suppl. material 2). Mandibular wear (MW) and wing wear (WW) were scored on a scale from 0 to 5 (i.e., unworn to completely worn), using whole numbers only. A total wear (TW) score for each female was obtained by summing MW + WW. We classified ‘worn’ bees as those having MW or WW scores ≥ 2 (Richards et al. 2010); we rarely observed bees with distinctly different levels of wear on their mandibles and wings among all species.

Ovarian development score was assessed to distinguish sterile and reproductive females (Suppl. material 3). Pinned specimens were rehydrated in a jar of distilled water for 24 hours, then dissected. Thin or only slightly thickened ovaries were

Table 1. Numbers of pinned specimens used for examining female social traits.

Species	Collection years	Collection method			Total
		Pan traps	Netting from flowers	Sweep-netting	
<i>L. laevisissimum</i>	2003–2013*	34	43	18	95
<i>L. hitchensi</i>	2009	124	0	0	124
<i>L. ellisiae</i>	2003	40	9	33	82

* Bees were not collected in 2007.

given a score of 0. For all females with developing oocytes, each oocyte was scored by its size relative to a fully developed oocyte (0.25, 0.50, 0.75, or 1), and then scores for all oocytes within an individual were summed to create a total ovarian development (OD) score. This scoring method has frequently been used to assess potential reproductivity of halictid bees (e.g. Richards et al. 2010, 2015). Following Breed (1976), any female whose largest oocyte was at least 1/2 size (scored as 0.5), was classified as “fecund”.

While rehydrated ovaries from pinned specimens appeared similar in size to those of specimens stored in liquid preservative (Packer 2007; Richards et al. 2010), it is possible that the ovaries of pinned specimens do not return to their pre-desiccation size, which could result in pinned specimens appearing to have lower OD. We avoid this potential bias by using fractional scores to calculate total OD, which is, in effect, a relative ranking of females’ ovarian development, and by avoiding comparisons of absolute oocyte size across studies.

Inferring colony social organization

Univoltine populations of non-parasitic halictids are most likely to be either solitary or communal. In both solitary and communal species, the distributions of body size and ovarian development are expected to be unimodal, so nest observations are required to distinguish between these possibilities.

Bivoltine populations of non-parasitic halictids are most likely to be either solitary or eusocial. Locally, the only known communal sweat bee (*Agapostemon virescens*) is univoltine (MH Richards, unpub. data), whereas at least one solitary species (*Lasioglossum zonulum*) is bivoltine (Proulx 2020). We use the term foundress to refer to overwintered females that establish nests and forage in spring after emerging from hibernation (Eickwort et al. 1996). Based on previous studies of solitary and eusocial sweat bees (Danforth et al. 2003), in solitary bivoltine species, foundresses and their daughters (summer females) are predicted to be similar in size, to accumulate similar levels of wear, and to have similar levels of ovarian development (Packer 1994; Eickwort et al. 1996). In contrast, eusocial foundresses (spring queens) are predicted to be significantly larger than most of their daughter workers (summer females), to accumulate more wing and mandibular wear, and to have significantly higher ovarian development (Packer 1986; Richards et al. 2010; Awde and Richards 2018). Moreover, the distributions of ovarian development should differ between the summer foragers of solitary and eusocial species. In solitary species, summer foragers should be provisioning their own brood cells and so all females are expected to have high levels of ovarian development, consistent with laying eggs. In eusocial species, many or most summer workers provision queen-laid eggs and exhibit little or no ovarian development. Across eusocial *Dialictus* populations, the proportion of foraging workers with developed ovaries can vary from 9 to 63% (summarized in Awde and Richards 2018).

Data analyses and software

Data is available on the Brock University repository (<https://dr.library.brocku.ca/>). All statistical comparisons were performed in R, version 3.4.3 (through RStudio, version 1.1.383). Figures were created using *ggplot2*. Initial comparisons of HW, TW, and OD between spring and summer females were based on analysis of variance. Where the error term was not normally distributed, we used Kruskal-Wallis tests.

Results

Lasioglossum laevisissimum

Phenology: The phenology based on pan trapped specimens of *L. laevisissimum* is shown in Figure 1, with additional details presented in Table 2. Spring females (foundresses) were mostly caught from April to early May (weeks 0 to 3). Two large, worn females collected in week 6 were classed as foundresses. Thus, the spring brood-provisioning phase for Brood 1 extended to about the end of May. Summer females (females produced in Brood 1) were first pan-trapped in week 8, suggesting that the Brood 2 provisioning phase began in mid-June. No males were caught until week 19, suggesting that males were not produced in Brood 1. The single male caught in week 19 likely was produced in Brood 2, so its capture suggested the onset of Brood 2 emergence. A slight increase in the numbers of foragers caught during weeks 23–25 suggests the possibility that late foragers (possibly produced in Brood 2) might be provisioning a third brood (Brood 3). Females were caught in pan traps until October (week 25).

Colony social organization: Traits of spring and summer females are shown in Table 2. Spring females were 3.6% larger than workers based on mean head width (ANOVA, $F=4.62$, $df=1,93$, $p=0.034$). Spring and summer females had similar wear scores (ANOVA, $F=0.62$, $df=1,92$, n.s.). Spring females, on average, had significantly higher OD scores than summer females (Kruskal-Wallis, $H=5.45$, $df=1$, $p=0.020$) and were more likely to be fecund (Figure 2).

The bivoltine or multivoltine phenology inferred from pan trapped specimens, as well as the larger size and greater ovarian development of spring females, are consistent with eusociality, in agreement with the results from nest observations and excavations for a sympatric population (Table 2).

Lasioglossum hitchensi

Phenology: The spring provisioning period began in early April and lasted until late June (weeks 0 to 8). The summer provisioning period lasted from late June to September. The capture of a male in week 6 (2011) indicates that nests initiated very

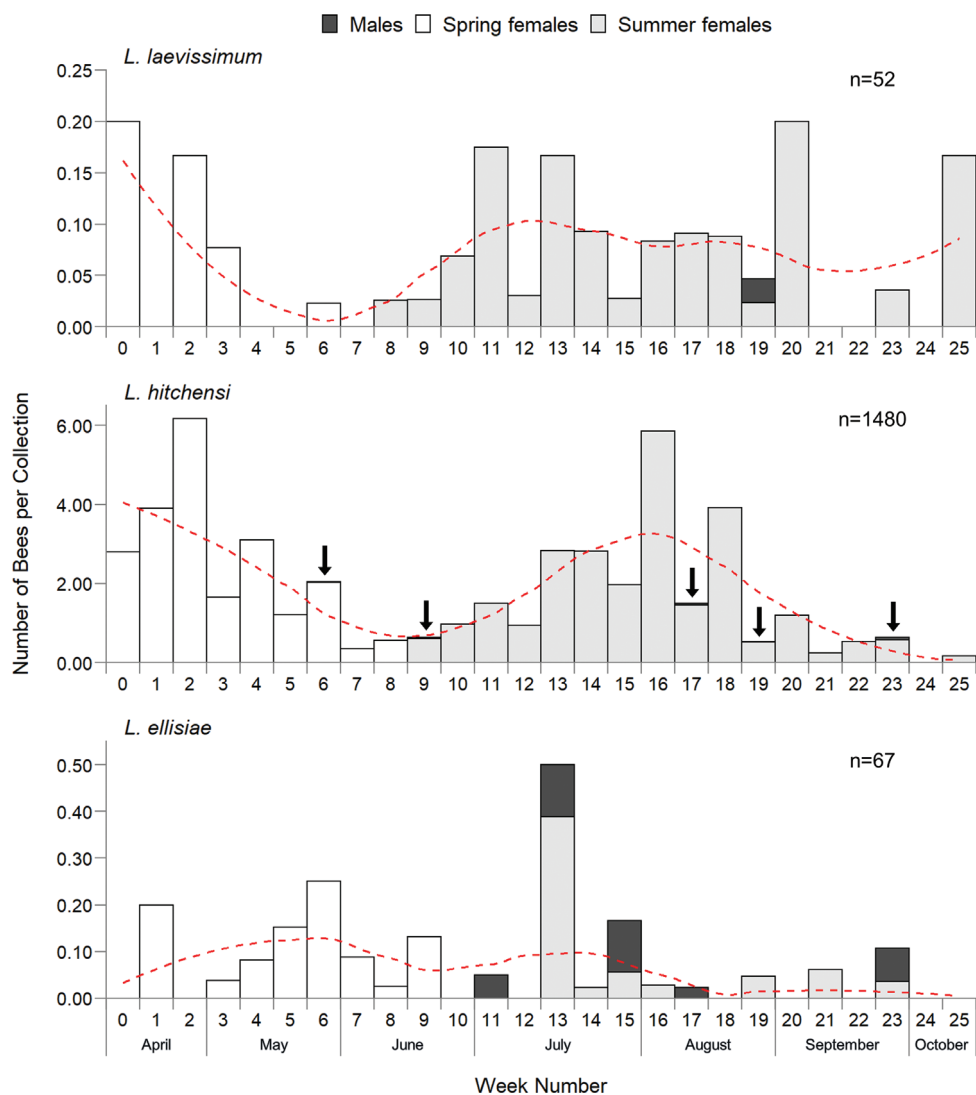


Figure 1. Bivoltine flight phenologies of *L. laevissimum*, *L. hitchensi* and *L. ellisiae*, inferred from pan trap collections, 2003–2013. The number of bees collected per week represents the average number of bees collected in pan traps, across sites and years, from 2003 to 2013. A total of 52 *L. laevissimum* bees, 1480 *L. hitchensi* bees, and 67 *L. ellisiae* bees were collected. Dark grey bars represent males, white bars represent spring females and light grey bars represent summer females. The red polynomial regression (drawn using the *geom_smooth* function, Loess method, in R) was used to help visually identify the number of abundance peaks for each species. Black arrows indicate the weeks when *L. hitchensi* males were collected in pan traps.

early in spring may produce Brood 1 offspring several weeks earlier than the population average. The males caught in week 17 would likely have been produced in Brood 2 emergence. Thus, *L. hitchensi* exhibits a bivoltine phenology.

Table 2. Phenological events and social traits of *L. laevisimum* females inferred from passive collections and nest excavations. Spring females from Awde and Richards (2018) were queens collected from nests in summer that would have been foraging in spring, while summer females were workers collected from nests in summer. Statistical comparisons are for pinned females assessed in this study versus females collected from nests in Awde and Richards (2018).

	Pan traps, sweeps, and flower collections (this study)	Nest excavations and observations (Awde and Richards 2018)	Statistical comparisons
Phenology			
First foraging trip by spring female	late April (week 0)	late April (week 1)	–
Quiescent period between spring and summer foraging	mid-May – early June (week 4 – 7)	mid-May – late June (week 6 – week 10)	–
First foraging trip by summer female	mid-June (week 7 or 8)	late June (week 10)	–
First adult male	late August (week 19)	mid-July (week 13)	–
First adult gyne	mid-July (week 12)	mid-July (week 12)	–
Last foraging trip by summer female	early October (week 25)	early October (week 25)	–
Spring female traits			
Head width (mm) (mean \pm SD, n)	1.65 \pm 0.06, n=10	1.67 \pm 0.08, n=24	KW=0.07, df=1, p=0.79
Median total wear score (range, n)	4 (1 – 8, n=10)	4.5 (2 – 9, n=24)	KW=0.27, df=1, p=0.60
Median OD score (range, n)	1.125 (0 – 2.5, n=10)	2.5 (0.75 – 3.5, n=24)	KW=6.88, df=1, p=0.009
Proportion fecund (largest oocyte at least 1/2 size)	5/10 (50%)	21/24 (87.5%)	$\chi^2=0.36$, df=1, p=0.5474
Proportion mated	NA	24/24 (100%)	–
Summer female traits			
Head width (mm) (mean \pm SD, n)	1.59 \pm 0.09, n=85	1.61 \pm 0.89, n=135	KW=4.34, df=1, p=0.037
Median total wear score (median, range, n)	4 (0 – 8, n=84)	3 (0 – 10, n=132)	KW=11.66, df=1, p<0.001
Median OD score (range, n)	0 (0 – 2.25, n=85)	0 (0 – 3.25, n=133)	KW=0.78, df=1, p=0.38
Proportion fecund (largest oocyte at least 1/2 size)	11/85 (12.9%)	23/133 (17.3%)	$\chi^2=0.3$, df=1, p=0.5813
Proportion mated	NA	52/133 (39.1%)	
Queen-worker size difference	3.6%	4.6% (n=21 comparisons within colonies)	

Colony social organization: Reproductive traits of spring and summer females are presented in Table 3. Spring females on average were 3.2% larger than summer females based on head width. Wear did not differ significantly between spring and summer females. Median ovarian development scores were substantially higher in spring compared to summer females, as most spring females had at least one $\frac{3}{4}$ or fully developed oocyte, while most summer females had undeveloped oocytes.

The average size difference between spring and summer females belied a curious pattern, evidenced by a distinct drop in summer female head width in week 16 (Suppl. material 4). We therefore divided the summer females into early (collected from weeks 10 to 15) and late groups (collected from week 16 onward) for further comparisons (Figure 3). Early summer females were as large as spring females, and significantly larger than late summer females (ANOVA, $F=24.107$, $df=2, 119$, $p=1.62e-09$). However, all three groups had exhibited a similar degree of wear (TW: ANOVA, $F=1.70$, $df=2, 119$, $p=0.188$). Spring females had significantly higher OD scores than both early and late summer females (Kruskal-Wallis, $H=21.27$, $df=2$, $p=2.41e-05$).

The combination of a bivoltine phenology, greater size, and higher ovarian development of spring than summer females suggests that *L. hitchensi* is eusocial.

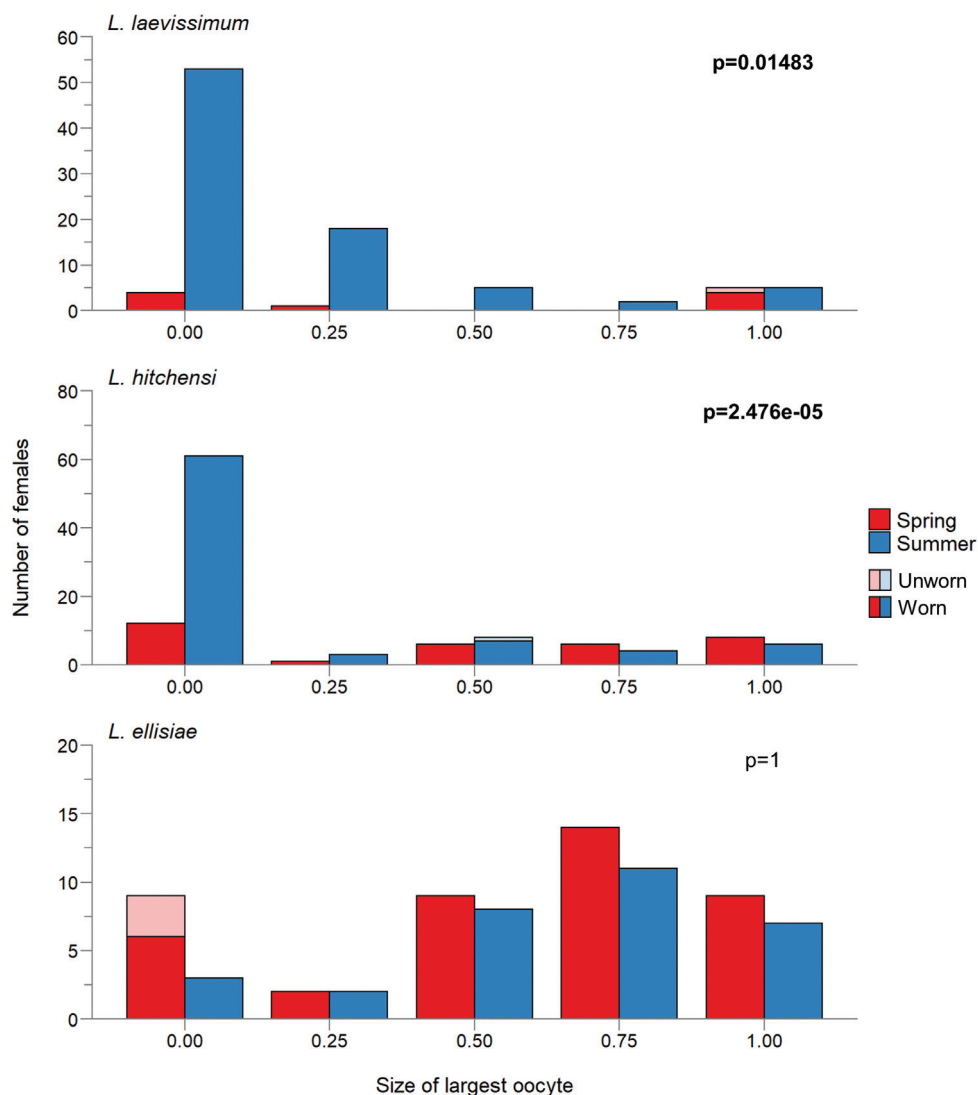


Figure 2. Wear and ovarian development of adult females caught in spring and summer. Fisher exact tests were used to compare the proportions of spring and summer females in each ovarian category. Unworn summer females with no ovarian development were excluded, as these females likely were newly eclosed. In *L. laevissimum* and *L. hitchensi*, spring females were significantly more likely to be fecund (largest oocyte at least 1/2-developed), while in *L. ellisiae*, spring and summer females showed similar levels of ovarian development (see Table 3 for statistics).

Lasioglossum ellisiae

Phenology: The brood-provisioning phase for Brood 1 began in mid-April and continued until mid-June (weeks 1 to 9; Figure 1). The second brood-provisioning phase began in mid-July (week 13) and continued until late September (week 23).

Table 3. Body size and reproductive traits of *L. hitchensi* and *L. ellisiae* females with statistical comparisons between spring and summer. Head width is given as the mean and standard deviation. Total wear and ovarian development scores are given as the median and range. Statistical comparisons are for spring versus summer females.

Species and trait	Spring females	Summer females	Statistical comparisons
<i>L. hitchensi</i>			
No. specimens	34	90	
Head width (mm)	1.58 ± 0.09	1.53 ± 0.09	ANOVA, F=7.40, df=1,120, p=0.007
Total wear score	4.0 (2–8)	4.0 (0–10)	ANOVA, F=0.385, df=1,120, p=0.536
OD score	0.75 (0–3.25)	0.0 (0–2)	Kruskal-Wallis, H=19.99, df=1, p=7.80e-06
Proportion fecund	21 / 34 (62%)	18 / 88 (20%)	Fisher test, p=2.48e-05
<i>L. ellisiae</i>			
No. specimens	46	36	
Head width (mm)	1.43 ± 0.05	1.38 ± 0.04	ANOVA, F=21.05, df=1,79, p=1.66e-05
Total wear score	4.0 (0–7)	5.0 (0–10)	ANOVA, F=3.88, df=1,78, p=0.052
OD score	0.75 (0–2.25)	1.0 (0–1.75)	Kruskal-Wallis, H=0.01, df=1, p=0.923
Proportion fecund	32 / 43 (74%)	26 / 35 (74%)	Fisher test, p=1

Males first appeared in week 11 so Brood 1 was definitely emerging by this time. Thus, *L. ellisiae* females exhibit a bivoltine phenology.

Colony social organization: Reproductive traits of spring and summer females are presented in Table 3. Spring females were 3.5% larger than summer females based on head width. However, spring and summer females had similarly high wear and OD scores and 76% of females from each group were fecund (Figure 2, Table 3). The similar wear and ovarian development of spring and summer females suggest that both spring and summer females were engaged in nest construction, brood provisioning, and egg-laying (Table 3). Thus, *L. ellisiae* likely is solitary or communal.

Discussion

The value of monitoring studies for inferring colony sociality: evidence from *L. laevisimum*

Observations of bees at nests are the ‘gold standard’ for investigating colony social organization. However, the social status of many sweat bee species has remained unstudied because nests have not been found in large enough numbers to persuade biologists to spend time studying them. The widespread growth of monitoring studies based on collections of foragers, provides an alternative source of social information. In this study, one of our objectives was to demonstrate that specimens collected in monitoring studies provide detailed information about sweat bee social behaviour that compares well with studies based on nest observations.

Table 2 provides a detailed comparison of the phenology and sociality of *L. laevisimum* in southern Ontario inferred from nest and non-nest-based observations (for convenience, we refer to specimens from the current study as ‘trapped’). Based on

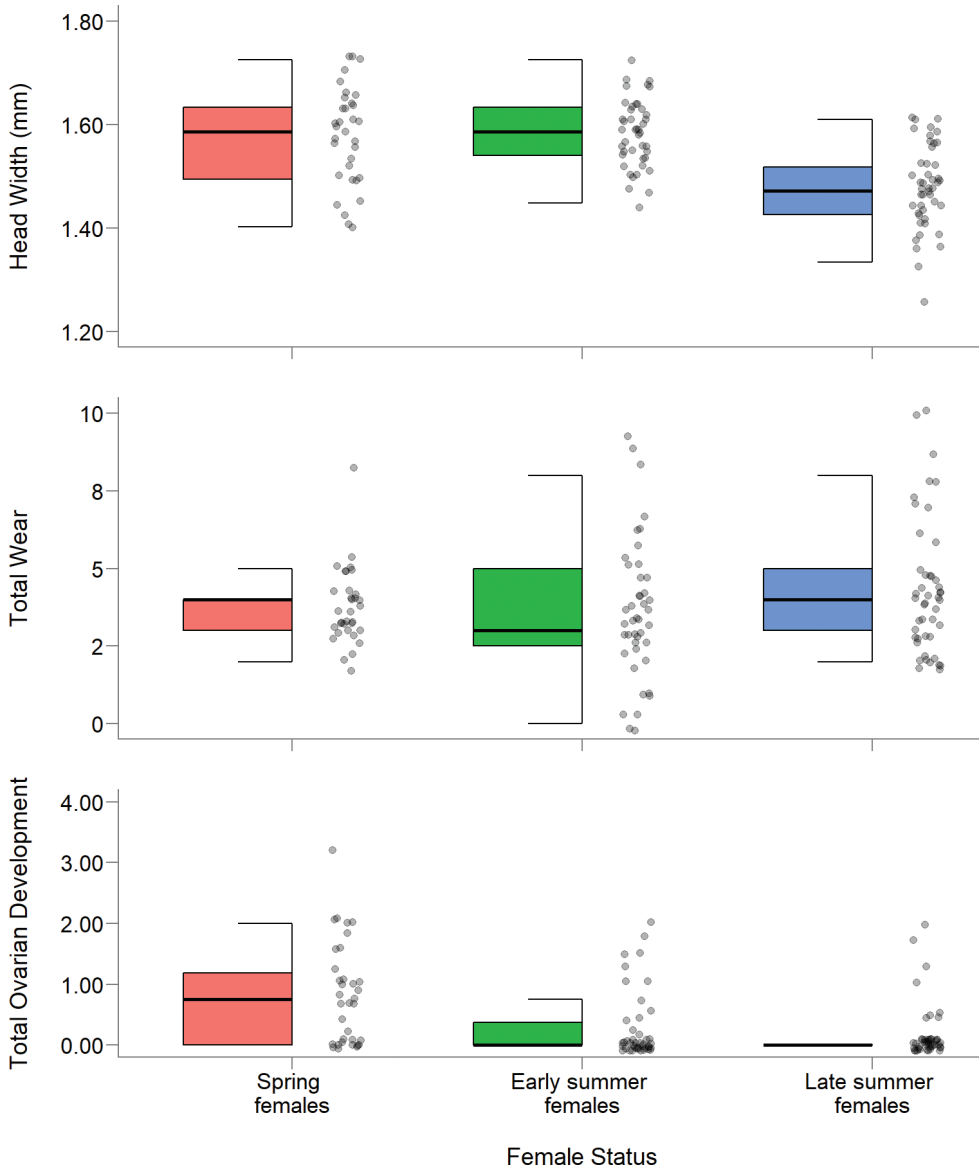


Figure 3. Social trait comparisons among spring, early summer and late summer females of *L. hitchensi*. Spring females were caught in weeks 0 to 8, early summer females in weeks 10 to 15, and late summer females from week 16 onward. Early summer females were larger than late summer females but showed similar signs of wear and ovarian development. See Table 2 for statistical analyses.

three years of nest observations and excavations, Awde and Richards (2018) found that spring foundress provisioning lasted from late April to late June, worker provisioning activity began in late June, and males and gynes were first collected from nests in July. The timing of brood-provisioning by queens and workers inferred from the pan trap data agrees well with this colony phenology inferred from nest data (Awde and

Richards 2018). In fact, the pan trap phenology for *L. laevissimum* even confirmed subtle, but repeated observations from multiple populations, that there is a third phase of female flight activity, as previously suggested by finding newly provisioned brood cells produced in nests excavated in late summer and autumn (Packer et al. 1989; Packer 1992; Awde and Richards 2018). Discrepancies between pan traps and nest data in the timing of major events likely stem from two causes: first, sweat bees exhibit considerable variation in the timing of spring and summer foraging in response to weather and climate variation (Richards et al. 2015). Second, pan traps likely do not attract all flying bees equivalently; at our research sites, male sweat bees are captured in pan traps far less frequently than females (MH Richards, unpub. data). The lack of males in pan traps probably accounts for the large discrepancy in timing of male emergence between pan traps and nest data (Table 2). Thus, female phenology based on pan traps matched exceedingly well with nest observations, but male phenology did not. Fortunately, most conclusions about colony social organization are based on the activities of females.

Social trait inferences based on examination of female specimens from trapping and nesting studies were largely in accordance, in that both clearly point to eusocial colony organization. While several traits were similar between trapped and nest specimens (e.g., spring female size, wear, and proportion of fecund females), there were also some differences. Trapped queens had significantly lower OD scores than nest queens; this difference reflects the fact that trapping began in April when some foundresses had not yet begun ovarian development, whereas nest queens were mostly collected after the onset of Brood 2 egg-laying (Awde and Richards 2018). Trapped workers were on average slightly smaller and more worn than nest workers, which suggests that smaller workers were more likely to leave the nest as foragers, while larger workers may be more likely to remain inside the nests, waiting for reproductive opportunities (Awde and Richards 2018). Another important difference was in the queen-worker size difference, which was smaller in trapped than nest specimens. Ideally, queen-worker size difference should be calculated based on comparisons between queens and the workers in their own nests; population-level averages, whether from pan traps, netting, or nest observations, consistently underestimate nest-based estimates, because of wide overlap in the sizes of queens and workers (Dunn et al. 1998; Richards et al. 2010, 2015). This would be an important consideration in cross-species comparisons that use queen-worker size differences as a metric (Breed 1976; Packer and Knerer 1985).

Some variables important in comparative analyses of the strength of eusocial colony organization cannot be inferred without nest data, including colony size, queen longevity, the frequency of queen replacement, and the proportion of males in Brood 1 (Breed 1976; Packer and Knerer 1985; Awde and Richards 2018). The proportion of mated workers could probably be reliably estimated from trapped specimens stored in liquid; finding the spermatheca and distinguishing whether it contains sperm is difficult when specimens have been pinned and dried for years before being rehydrated and dissected (Packer et al. 2007). Although rehydrated *L. laevissimum* females were assessed for matedness, we were not confident in the results (D.N. Awde, pers. obs.). One variable that we did not report here is the timing of gyne emergence. In our

experience, it can be difficult to distinguish between gynes and late-emerging workers, even when using specimens derived from nest excavations. In fact, *L. laevissimum* presents a particular puzzle with respect to gyne behaviour. In our nest-based study, we found females that appeared to be newly emerged gynes, but which had developing ovaries. We also found newly provisioned brood cells far too late in the season for brood to complete development, an odd phenomenon also noted for a Calgary population of this species (Packer 1992; Packer and Owen 1994). In general, late-emerging workers of eusocial sweat bees, especially those that emerge into queenless nests, may frequently overwinter as gynes (Danforth et al. 2003).

The social status of *Lasioglossum hitchensi* and *L. ellisiae*

Reproductive division of labour is the crucial variable distinguishing halictine eusociality (Batra 1966; Richards 2019). In *L. hitchensi*, eusociality was indicated by the significantly higher ovarian development of spring females. Small summer foragers with low ovarian development fit the classic eusocial phenotype of workers provisioning eggs to be laid by queens. The low proportion of fecund summer foragers (20%) is comparable to that observed in *L. laevissimum* (Table 2) and suggests fairly strong queen control of worker behaviour, as measured by this criterion (reviewed in Awde and Richards 2018). A novel finding was that foragers collected in early summer (weeks 10 to 15) were as large as spring foundresses. In contrast, small body size predominated among summer foragers a few weeks later. The large size of these early summer females suggests that they could be more resistant to queens' efforts to coerce them into becoming workers (Richards and Packer 1996), but on average, the early summer workers did not have higher ovarian development scores than late summer workers, so the significance of their large body size awaits nest-based observation.

We concluded that *L. ellisiae* is either solitary or communal because summer foragers were just as likely to have highly developed ovaries as spring foragers, and because summer foragers of *L. ellisiae* had significantly more ovarian development than those of the two eusocial species, *L. hitchensi* and *L. laevissimum*. Without nest data, females of solitary and communal species are indistinguishable, because the variance among females in their degree of ovarian development should be similar to the variance in solitary species. However, we suggest that solitary behaviour is more likely for two reasons. First, the only other communal halictid at our sites, *Agapostemon virescens*, is univoltine, and univoltinism is thought to be typical of communal halictids (Abrams and Eickwort 1980). Second, evolutionary reversion from eusocial to solitary behaviour has occurred frequently in halictid bees, whereas transitions between communal and eusocial behaviour are thought to be far rarer (Richards et al. 2003).

Body size patterns in eusocial and secondarily solitary sweat bees

Comparisons between eusocial and secondarily solitary sweat bees should help us to understand trait changes hypothesized to have been important in evolutionary tran-

sitions between solitary and social behaviour (Plateaux-Quénu and Plateaux 1985; Plateaux-Quénu et al. 1989). Body size differences between queens and workers are almost ubiquitous in eusocial Hymenoptera. In eusocial sweat bees, the larger size of queens enables them to aggressively manipulate the behaviour of their smaller daughters, forcing them to behave as workers (Richards and Packer 1996; Smith et al. 2019). Thus, in eusocial species there is clearly a selective advantage for queens in producing workers smaller than themselves.

Although solitary halictines are not expected to produce spring and summer females of different sizes, since they are all the same caste (Lin and Michener 1972), there may be reasons why spring and summer females of solitary species differ in size. For instance, larger body size of spring females might be an advantage in surviving hibernation, while smaller body size might be an advantage for summer females that forage in higher temperatures (Richards and Packer 1994, 1995). We found that in *L. ellisiae*, spring females were larger, as is also true in another secondarily solitary species, *L. (D.) villosulum* (Plateaux-Quénu et al. 1989). Secondarily solitary species might advantageously retain a spring-summer size difference inherited from a eusocial ancestor. In contrast, spring and summer females of two largely solitary species in another subgenus, *L. (Lasioglossum) scitulum* and *L. (L.) mutilum*, do not differ in size (Miyana et al. 1998, 2000). In these two species, the reversion to solitary behaviour may be associated with a switch to similarly sized spring and summer females, which would depend on whether ancestrally eusocial halictines always displayed this size difference. In the most strongly eusocial halictine, *Lasioglossum marginatum*, queens and workers are the same size (Plateaux-Quénu 1959, 1962).

Linkages between phenology and social behaviour

Bivoltinism is necessary for eusociality in sweat bees, because in virtually all known species, daughters born in the first brood, remain in their natal nest to help rear a second brood during the same brood-rearing season, after which colonies die out. The only known exception to this rule is *Lasioglossum marginatum*, which lives in perennial colonies that produce a single brood of workers each year for several years, before producing reproductives only in the last year of a colony's life (Plateaux-Quénu 1959, 1962); the phylogenetic position of *L. marginatum* clearly indicates that its univoltine, perennial life history is derived. While it seems that halictine eusociality requires bivoltinism, the converse is not true. There have been reversions to solitary behaviour occurring in *Dialictus* (Danforth 2002; Danforth et al. 2003; Gibbs et al. 2012), and species like *L. ellisiae* and *L. villosulum* show social reversion is not always associated with reversions to univoltinism. Indeed, phylogenetic relationships within *Dialictus* indicate that there may have been multiple evolutionary changes back and forth in both phenology and sociality. *Lasioglossum ellisiae* is closely related to *L. vierecki*, which is also bivoltine and solitary, but other closely related species are eusocial (*L. parvum*, *L. umbripenne* and *L. gundlachii*; Wille and Orozco 1970; Eickwort and Eickwort 1971; Eickwort 1988; Gibbs et al. 2012), so either solitary or eusocial behaviour might

be secondary in this group. *Lasioglossum villosulum* is related to the solitary univoltine bees *L. lucidulum* and *L. lustrans*, implying that it might be secondarily bivoltine (Daly 1961; Knerer 1981; Plateaux-Quénu 2008; Gibbs et al. 2012).

Conclusions

The first study to compare specimens from pan trap and nest data in assessing colony social organization, focused on the eusocial behaviour of *Halictus confusus* (Richards et al. 2010). Systematic pan trap collections have now been used to describe solitary and eusocial behaviour of five species for which nests were not available: *Halictus tripartitus* (Packer et al. 2007), *H. ligatus* (Richards et al. 2015), *L. ellisiae* and *L. hitchensi* (current study), and *L. zonulum* (Proulx 2020). Given the phenological and social lability of many sweat bee species, it has long been a goal of social insect biologists to examine geographic and temporal variability in sociality, within and among species (Danforth et al. 2013; Kocher et al. 2014). Using specimens collected in monitoring studies, including dissections of rehydrated, pinned specimens, can make achieving this goal realistic.

Acknowledgements

We thank Dr. Alan Castle and Alex Proulx for their comments and members of the Brock Bee Lab for their input. We also thank the reviewers, Dr. Bill Wcislo and Dr. Zach Portman, for their insightful reviews, which substantially improved our manuscript.

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Supplementary material I

Figure S1. Right forewing of an *L. hitchensi* female.

Authors: Lyllian A-J Corbin

Data type: Image

Explanation note: Forewing of a *Lasioglossum (Dialictus) hitchensi* adult female with labels indicating the stigma, costal vein, and costal vein base. Image was captured using a Ziess stereomicroscope, with an AmScope camera attachment, at 40× magnification.

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Link: <https://doi.org/10.3897/jhr.88.73220.suppl1>

Supplementary material 2

Figure S2. Scoring systems for mandibular wear (MW) and wing wear (WW).

Authors: Lyllian A-J Corbin

Data type: Image

Explanation note: Diagram showing wear scores assigned to *Lasioglossum (Dialictus)* females based on the amount of accumulated wear on their mandibles and wing margins.

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Link: <https://doi.org/10.3897/jhr.88.73220.suppl2>

Supplementary material 3

Figure S3. Scoring systems for ovarian development scores.

Authors: Lyllian A-J Corbin

Data type: Image

Explanation note: Diagrams illustrating oocyte sizes (0, 0.25, 0.5, 0.75, and 1) scored in female specimens to assess ovarian development

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Link: <https://doi.org/10.3897/jhr.88.73220.suppl3>

Supplementary material 4

Figure S4. Head width comparisons among weeks suggest a mix of social traits in *L. hitchensi* summer females

Authors: Lyllian A-J Corbin

Data type: Image

Explanation note: Head width comparisons in *Lasioglossum (Dialictus) hitchensi* spring and summer females based on week collected. Figure shows boxplots, coloured by spring and summer female groups, on weeks that females were collected in pan traps in 2009. The black arrow indicates a decrease in summer female head width started on week 16, in which early summer females (collected in weeks 10 to 15) were larger than late summer females (collected in weeks 16 to 23).

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Link: <https://doi.org/10.3897/jhr.88.73220.suppl4>

Higher developmental temperature increases queen production and decreases worker body size in the bumblebee *Bombus terrestris*

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Academic editor: Jack Neff | Received 9 September 2021 | Accepted 19 October 2021 | Published 30 December 2021

<http://zoobank.org/E40A6966-CF3A-4B44-A0A9-629C1A1028CE>

Citation: Guiraud M, Cariou B, Henrion M, Baird E, Gérard M (2021) Higher developmental temperature increases queen production and decreases worker body size in the bumblebee *Bombus terrestris*. Journal of Hymenoptera Research 88: 39–49. <https://doi.org/10.3897/jhr.88.73532>

Abstract

Climate change and increasing average temperatures are now affecting most ecosystems. Social insects such as bumblebees are especially impacted because these changes create spatial, temporal and morphological mismatches that could impede their ability to find food resources and mate. However, few studies have assessed how the colony and life cycle are affected when temperatures rise above optimal rearing temperature. It has become imperative to understand how heat stress affects the life history traits of insect pollinators as well as how changes in life history interact with other traits like morphology. For example, a decrease in the number of foraging workers could be balanced by producing larger workers, able to forage at longer distances and gather more resources. Here, we investigated the impact of temperature on colony production and body size in the bumblebee *Bombus terrestris*. Colonies were exposed to two temperatures: 25 °C, which is around the optimal temperature for larval development and 33 °C, which is slightly above the set-point that is considered stressful for bumblebees. Although the production of males and workers wasn't significantly affected by these different temperatures, queen production and reproductive investment were much higher for colonies placed in 33 °C than in 25 °C. We also found that, in agreement with the temperature-size rule, workers were significantly smaller in the higher temperature. The decrease in worker body size could affect resource collection and pollination if their foraging distance and

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the quantity of food they are taking back to the colony decreases. While in our controlled conditions the bumblebees were fed *ad libitum*, the decrease of resource collection in field conditions could prevent colonies from producing as many queens as in our study. Together with the decrease of worker body size, our results suggest that elevated temperatures could ultimately have a negative impact on bumblebee colony fitness. Indeed, smaller workers are known to have weaker flight performance which could affect foraging performance and consequently colony development.

Keywords

Bees, climate change, colony development, ITD, Hymenoptera

Introduction

Over the past few decades, climate change has led to increasingly unpredictable weather patterns (Thibeault and Seth 2014) like heat waves (Perkins-Kirkpatrick and Lewis 2020), and will raise the global average surface temperature by 0.3 °C to 4.8 °C before the end of this century (Pachauri et al. 2014). These changes have a wide range of consequences on many animal species by affecting geographical range and phenology, disrupting ecological interactions and potentially altering their ability to perform ecosystem services (Bale et al. 2002; Buckley et al. 2017). Life-history traits related to life cycle and reproduction are central for the fitness of organisms and are particularly useful in helping us to understand how species will respond to a warming world. For example, insects are known to develop faster under warmer conditions, potentially leading to an increase in the number of generations (Altermatt 2010; Hamann, et al. 2020). Amongst insects, pollinators are particularly important both for the ecosystem services they provide us, as well for the central role they play in the pollination of many wild plant species (Gallai et al. 2009; Ollerton et al. 2011). While the effects of climate change on insect pollinators are still mostly theoretical and based on model predictions in the early 21st century, recent studies have shown that it is already having a negative effect on them (Kammerer et al. 2020; Soroye et al. 2021). For instance, global warming affects plant-pollinator interactions, creating potential spatial, temporal and morphological mismatches (Hegland et al. 2009; Miller-Struttmann et al. 2015; Pyke et al. 2016; Gérard et al. 2020). While informative, these studies do not consider the potential impact of warming on life history traits, such as the number of individuals produced – particularly sexuals (*i.e.* queens and males in social bees) – or the timing of their production, which is important for understanding the impact on reproduction, pollination and life cycles. In bees, for example, warmer rearing conditions can decrease development time (Radmacher and Strohm 2010), advance emergence date (Duchenne et al. 2020) or increase mortality before emergence (O'Neill et al. 2011). Strong evidence that increased temperatures can affect the life cycle of bee colonies comes from Mediterranean populations of bumblebees (Rasmont et al. 2005, 2008; Goulson 2010). Mediterranean *Bombus terrestris* colonies aestivate during the warm summer instead of hibernating during winter (Rasmont et al. 2008) and this same species is increasingly active during the winter in UK. Moreover, while most of bumblebee species have one colony cycle *per* year (Goulson, 2010), Mediterranean populations of *B. terrestris* tend to have two colony

cycles *per year* (Rasmont et al. 2005). Further evidence that elevated temperatures affect bumblebee life cycles comes from the UK, where *B. terrestris* are becoming increasingly active during the winter (Edwards 2006; Farmer 2006; Hart et al., 2021). In addition to this field-based work, several studies have also assessed the impact of rearing temperature on colony development in controlled conditions. Favourable temperatures for colony development vary between species and studies. Indeed, different studies identified 25 °C (Holland and Bourke 2015), 27–29 °C (Weidenmüller et al. 2002) or 28–30 °C (Nasir et al. 2019) as optimal temperatures for colony longevity, individual longevity, colony productivity as well as thermoregulatory behaviour. The temperature at which bumblebees increase fanning intensity (indicating heat stress) also differs between studies – from 29 °C (Weidenmüller et al. 2002) to 32 °C (Grad and Gradisek 2018) – leading to changes in the allocation of energy towards thermoregulation, which may affect other aspects of colony life. As bumblebees are social insects, the colony itself is characterized by its own development, notably in terms of number of individuals or sexuals produced (Wilson 1985; Holland et al. 2013). However, the effect of increased developmental temperature on colony productivity and life history traits remains unclear. Here, we address this knowledge gap by experimentally investigating how elevated developmental temperatures affect the life history traits of *B. terrestris* colonies. We assess how caste production (*i.e.* queens, workers and males), as well as reproductive investment, are affected by high rearing temperature and how individual body size from these castes varies depending on the temperature. Taking body size into consideration is crucial, as lower worker production could be compensated by larger body size. Indeed, larger individuals can have higher foraging performance and larger foraging ranges, which might compensate for the potential decrease of foraging efficiency due to lower worker production (Spaethe and Weidenmüller 2002; Greenleaf et al. 2007; Klein et al. 2017). We assess the variation of these features at two temperatures: 25 °C – which is a temperature commonly experienced by bumblebees in temperate regions during spring and summer and can be an optimal temperature for colony development (Holland and Bourke 2015) – and 33 °C, which is slightly above the set-point at which bumblebees increase fanning behaviour and is considered as a stressful condition (Vogt 1986; Weidenmüller et al. 2002; Grad and Gradisek 2018). With the increase of heat wave frequency (*i.e.* prolonged periods of excessive heat; Perkins-Kirkpatrick and Lewis 2020), this stressful temperature is also becoming ecologically relevant during summer in many European countries. We hypothesized that colonies reared at 33 °C may have lower production due to stressful temperature and that the individuals produced will have smaller body size than bumblebees reared at 25 °C, as predicted by the temperature-size rule (TSR; Atkinson 1994; Angilletta and Dunham 2003).

Methods

Biological models

The experiments were conducted over two sessions, each lasting two months: session 1 occurred during winter 2020 and session 2 during spring 2021. In each session, eight

colonies of *Bombus terrestris audax* (Koppert, Berkel en Rodenrijs, The Netherlands) were used (making 16 colonies in total). The colonies were kept in the dark at 50% humidity, in temperature-controlled incubators (Panasonic MIR, 123L) at the Department of Zoology in Stockholm (Sweden) – four colonies were incubated at 25 °C and four at 33 °C. The experimental colonies were placed in wooden nest boxes (28 cm × 16 cm × 11 cm). Pollen was delivered every two to three days (Naturprodukter, Rawpowder Bipollen) inside the colony. *Ad libitum* 30% sucrose solution (w/w) was available all times *via* a gravity feeder. After 25 days of development, all individuals in each colony were marked. Thus, at day 26, each newly emerged individual had experienced the temperature treatment throughout the entirety of its development, as 25 days corresponds to the duration of worker development (Duchateau and Velthuis 1988). All males and queens included in the analysis emerged after day 26, so that they also experienced the full temperature treatment during their development. One of the colonies reared at 25 °C during session 1 was removed from the analysis because the queen died at the beginning of the experiment, thus inducing a bias in the number of individuals produced by this colony. In total, we gathered a dataset of 2834 workers ($n = 1460$ at 25 °C from seven colonies, $n = 1374$ at 33 °C from eight colonies), 182 males ($n = 65$ at 25 °C from seven colonies, $n = 117$ at 33 °C from eight colonies) and 182 queens ($n = 2$ at 25 °C from seven colonies, $n = 180$ at 33 °C from eight colonies).

Body size measurements

The inter-tegular distance (ITD, *i.e.* the minimal distance between the tegulae; the coverings over the wing bases) was used as a proxy for body size (Cane 1987) and was measured using a digital calliper (Cocraft, Insjön, Sweden).

Statistical analyses

First, we used separate Wilcoxon tests to assess if there were differences in (i) the total number of individuals, (ii) the total number of individuals in each caste separately and finally (iii) the reproductive investment between the colonies. Reproductive investment is defined as the percentage of sexuals (males and queens) on the total number of individuals produced in a colony. If the ratio is higher, it thus means that the proportion of sexuals is higher.

After checking assumptions, we built linear mixed models (LMM; lmer4 R package) to understand the impact of rearing temperature on body size. We computed two different models for males and workers, as their body size differs significantly. If these assumptions were not verified even when using log- or rank transformation, we built Generalized Linear Mixed Model with a Gamma distribution (GLMM). This distribution is adapted for non-normal positive and continuous data. We fitted the models with body size (ITD) as a response variable, included temperature as a fixed effect, and colony ID and session number as random effects. We selected the best model using AIC criteria (Burnham and Anderson 2004) after testing all possible combinations.

Results

Colony development

First, the session did not have any significant impact on any parameter of the colony development (all p -values > 0.05). The temperature treatment did not affect the total number of individuals ($p = 0.96$), males ($p = 0.24$) or workers ($p = 0.34$) produced by each colony (Fig. 1). However, the number of queens produced was significantly higher at 33 °C ($p = 0.001$; Fig. 1). While the first queen appeared after 28 days of development at 33 °C, the first queens from 25 °C appeared after 39 days. In both temperature treatments, the first males appeared after 26 days of development. The reproductive investment was also significantly higher for bumblebees reared at 33 °C ($p = 0.02$).

Body size

The model that best explained the variation in body size of males included temperature and colony ID (Marginal R-squared = 0.02; Conditional R-squared = 0.27). No significant impact of the rearing temperature was observed ($p = 0.53$; Fig. 2). The random factor colony ID explained 25.7% of the variance that remained in the residuals after the variance explained by the fixed factors was removed. The model that best explained the variation in body size of workers included temperature, session and colony (Marginal R-squared = 0.1; Conditional R-squared = 0.28). The body size of workers reared at 33 °C was significantly smaller than those reared at 25 °C ($p = 0.006$; Fig. 2). The random factors colony ID and session number explained 6.3% and 14.1% of the variance that remained in the residuals, respectively. Variance in body size in males and workers was not affected by rearing temperature ($p = 0.73$ and $p = 0.49$ respectively). Due to the low number of queens produced at 25 °C, we were not able to assess the impact of temperature on their body size.

Discussion

Here, we investigated the effect of elevated developmental temperatures on bumblebees by measuring the production and body size of colonies kept at two different developmental temperatures – one that is optimal for larval development (25 °C) and one that causes heat stress (33 °C). Overall, we found that only queen production and reproductive investment were significantly affected by the elevated temperature. We also found that workers that developed under the elevated temperature had a smaller body size, an effect that was not observed among males. Like in the present study, Nasir et al. (2019) highlighted an increase of queen production when rearing temperature increased from 24 °C to 30 °C. Although we observed a similar trend, the absolute number of queens produced was much lower in our colonies, suggesting that the pace of queen production was slower and that additional weeks of colony development could

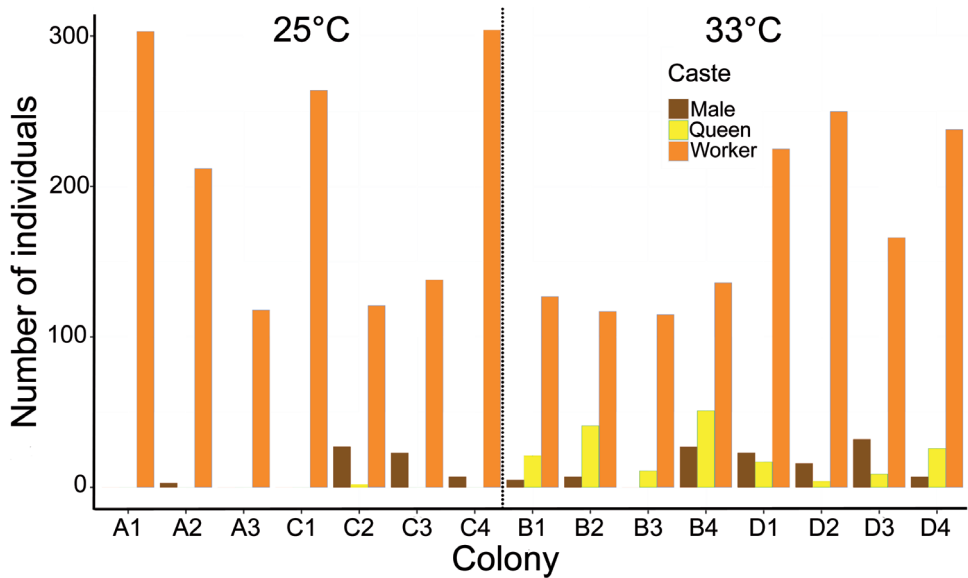


Figure 1. The effect of temperature on the total number of individuals of each caste produced in each colony Colonies A1-A3 and B1-B4 were from session 1, colonies C1-C4 and D1-D4 were from session 2. No significant effect of the session for any caste ($p > 0.05$). No significant impact of the temperature on the total number of individuals ($p = 0.96$), neither on the number of males ($p = 0.24$) or workers ($p = 0.34$) The number of queens produced was significantly higher at 33°C ($p = 0.001$).

have increased the total number of sexuals. In contrast with our findings, Nasir et al. (2019) found that queen production decreased at 32 °C. The higher investment in the production of queens in warmer conditions may counteract the detrimental impacts of high stressful temperatures during punctual events like heat waves. Indeed, if the mortality of bumblebees increases during prolonged periods of excessively hot weather (Rasmont and Iserbyt, 2012), higher queen production may increase the probability of a population to have successful colonies at the next generation. Our results on male and worker production are consistent with previous studies that exposed colonies to more optimal rearing temperatures. Gurel and Gosterit (2008) did not find any significant impact of temperature (*i.e.* 24 °C, 27°C and 30 °C) on worker production, and neither did Yoon et al. (2002) which used 23 °C, 27°C and 30 °C. Our findings add to this body of work by showing that the number of workers/males produced also seems relatively constant even at higher, more stressful temperatures. In addition, in our study, the queen production was also advanced at the higher developmental temperature, starting several weeks before the colonies reared at 25 °C. This is congruent with the results of Nasir et al. (2019) which also find the shortest queen emergence time at the higher temperature. This switch point is a key event during bumblebee life cycle: it marks the investment transition from growth of the colony (*i.e.* worker production) to reproduction (*i.e.* queen and male production; Lopez-Vaamonde et al. 2009). It is known that climate change is already advancing the phenology of a lot of bee species

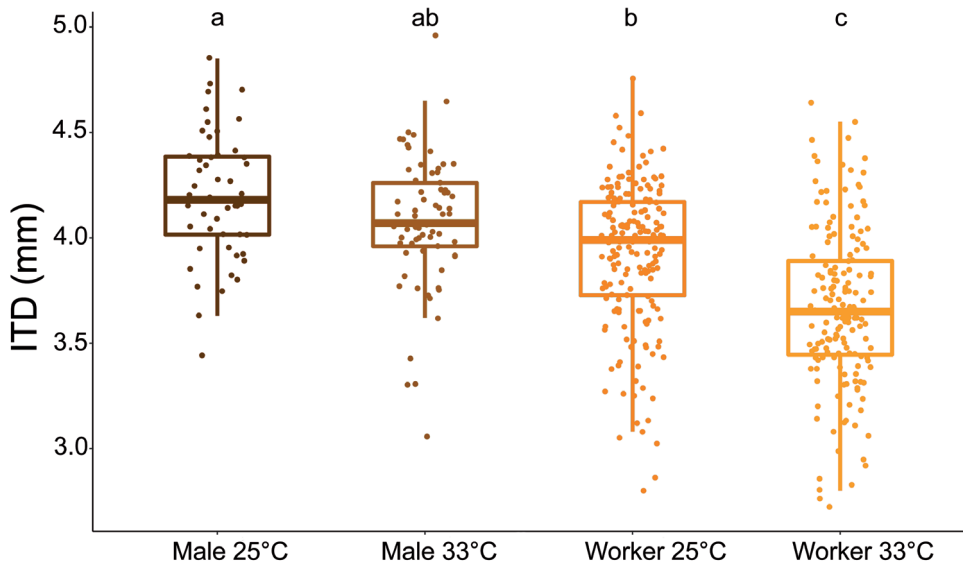


Figure 2. The impact of developmental temperature on bumblebee body size. Letters at the top of the boxplots indicate significant differences when the letters are different.

(Duchenne et al. 2020), notably bumblebee queens (Pawlikowski et al. 2020). If our results in controlled conditions can be translated the field, and if the queen production of social species also advances, we may observe an increase of voltinism (*i.e.* number of generations per year) with increasing temperature, as has already been observed in some Mediterranean regions (Rasmont et al. 2005).

As with previous studies (Atkinson 1994; Angilletta and Dunham 2003), we observed a decrease of body size at the higher developmental temperature. Among ectotherms, the decrease of body size in warmer developmental temperatures is known as the temperature-size rule and is a widespread plastic response in controlled conditions. However, as bumblebees are partly endotherms, they have the potential to buffer these changes to some extent though behavioural adaptations, such as fanning. Very little work has been done to specifically assess the impact of rearing temperature on bee body size. In one of the only studies on the topic, Gérard et al. (2018) found that, at high temperature, the centroid size of the wings (*i.e.* a proxy of wing size, often correlated with body size) was smaller, although the measurements in this study were only done on males and in very small colonies containing only few workers and some males. Thus, in contrast to our study, these colonies did not have the possibility to thermoregulate. Our results suggest that the effect of higher developmental temperatures was not balanced by the thermoregulation of workers, leading to the smaller body size of emerging workers. This decrease of body size could have potential adverse effects on the food-collecting capabilities of a colony, as it is known that bee body size can be positively correlated to foraging distance (Greenleaf et al. 2007; Kendall et al. 2019) and the mass of the pollen load (O'Neill et al. 2011; Murua 2020).

Conclusion

Our study brings us one step closer to understanding the impact that global warming may have on bumblebee colony production and individual body size. Our findings contribute to the existing body of evidence that higher developmental temperatures lead to a higher production of queens with earlier emergence times. This may represent an emergency-state of the colony where stressful conditions induced by high temperature, leading to increased fanning of workers and to a higher/earlier investment in reproduction and the success of further generations. Further studies should first try to replicate this experiment, as few studies have focussed on the impact of heat stress on life history traits, but also because working with full colonies is time-consuming and makes it difficult to have many replicates in the same experimental session. Finally, further research should also focus on how this interplay between colony production and body size could ultimately affect the efficiency with which a colony can collect resources and pollinate.

Acknowledgments

This work was supported by an Interdisciplinary Research Environment Grant from the Swedish Research Council (grant number 2018-06238). MGe was also supported by a Visiting Postdoctoral Researcher Grant from the Wenner-Gren Foundation.

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First record of the bee genus *Bathanthidium* (*Bathanthidium* s. str.) Mavromoustakis (Hymenoptera: Megachilidae) from Vietnam: Description of a new species and a key to species

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Academic editor: Jack Neff | Received 5 September 2021 | Accepted 19 October 2021 | Published 30 December 2021

<http://zoobank.org/03725F23-6D56-4E73-AE11-16B8F53E4880>

Citation: Tran NT, Truong LX, Ljubomirov T, Nguyen LTP (2021) First record of the bee genus *Bathanthidium* (*Bathanthidium* s. str.) Mavromoustakis (Hymenoptera: Megachilidae) from Vietnam: Description of a new species and a key to species. Journal of Hymenoptera Research 88: 51–60. <https://doi.org/10.3897/jhr.88.73969>

Abstract

The little-known bee genus *Bathanthidium* Mavromoustakis, 1953 of the family Megachilidae is reported for the first time from Vietnam. A new species, *Bathanthidium* (*Bathanthidium* s. str.) *paco* Tran & Nguyen, **sp. nov.** from Hoa Binh province is described and illustrated. A nest of the new species is reported from a wooden house. An updated identification key to all known species of the subgenus *Bathanthidium* s. str. is also provided.

Keywords

Apoidea, Anthidiini, identification key, morphology, taxonomy

Introduction

Bathanthidium is a small genus of anthidiine bees occurring in eastern Asia (Michener 2007; Ascher and Pickering 2021). It is an uncommonly collected genus with most species known from a small number of specimens and some species lack associated

males. Currently, the genus consists of 12 species belonging to three subgenera: subgenus *Bathanthidium* s. str. Mavromoustakis, 1953 with three species, *Manthidium* Pasteels, 1969 with five species and *Stenanthidiellum* Pasteels, 1968 with four species (Niu et al. 2019). China is considered the distribution center of the genus because almost all its species occur in this country (Niu et al. 2019).

In Vietnam, knowledge concerning the taxonomy of the genus *Bathanthidium* has been extremely limited as the country has been under sampled for bees. We here report this genus for the first time for the Vietnamese fauna, with a species belonging to subgenus *Bathanthidium* s. str. described as new to science. A nest site of the new species is also described. In addition, an updated key to all recognized species of the subgenus *Bathanthidium* s. str. is provided.

Materials and methods

The materials examined in the present study are deposited in the Hymenopteran collection of the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam. The adult morphological and color characters were examined with a Nikon SMZ745 stereomicroscope. Images were photographed with a Nikon SMZ800N digital stereo microscope, and with ILCE-5000L/WAP2 digital camera attached on that. Photographs were combined with Helicon Focus 7 software to achieve focus stacked images. Finally, all files were processed with Adobe Photoshop CS6.

The morphological terminology used in the description follows Michener (2007).

Measurements of body parts follows Niu et al. (2004): **Body length**: measured from the base of antennal socket to the apex of metasoma (in dorsal view), **head length**: measured from the apico-median margin of clypeus to the upper margin of vertex (in frontal view), **head width**: measured at the widest point of the head across the compound eyes (in frontal view), **eye width**: the greatest width of eye (in lateral view), **genal width**: the greatest width of the gena (in lateral view), **mesosomal width**: measured between the outer rims of the tegulae (in dorsal view).

The abbreviations S and T (1, 2, 3...) refer to numbered metasomal sterna and terga, respectively.

Results

Genus *Bathanthidium* Mavromoustakis, 1953

Subgenus *Bathanthidium* s. str.: *Dianthidium* (*Bathanthidium*) Mavromoustakis, 1953: 837. Type species: *Dianthidium bifoveolatum* Alfken, 1937, by original designation.

Diagnosis. According to Michener (2007) and Niu et al. (2012), the diagnosis of the genus *Bathanthidium* is as follows: Small to moderate-sized species. Body all black or

black with yellow markings, with the yellow bands broken on anterior terga; epistomal suture generally straight; medially divided, smooth-floored mesoscuto-mesoscutoscutellar sulcus; preoccipital ridge not carinate; omaular carina absent; pronotal lobe with carina weak or absent; scutellum rounded or medially emarginated, in profile rounded or at least not sharp and not overhanging metanotum; propodeum with a fovea behind spiracle; 2nd m-cu slightly exceeding the apical margin of 2nd submarginal cell; arolia present and large; median S4 of both sexes with pectinate hairs; S5 of the male with pectinate hairs; T6 of the male simple; T7 of the male ranges from simple to trilobed, with the median lobe longest; front coxa of the male normal, without spine.

***Bathanthidium* (*Bathanthidium* s. str.) *paco* Tran & Nguyen, sp. nov.**

<http://zoobank.org/3769E63C-29F2-4552-8431-620E056400CE>

Figs 1A–H, 2A–E, 3A–I

Material examined. *Holotype*: VIETNAM: ♀, Hoa Binh, Mai Chau, Pa Co, Hang Kia – Pa Co Natural Reserve, 20°44'36"N, 104°53'42"E, alt. 1340 m, Nest#VN-HB-2020-Bath-01, 24 Jun. 2020, Nguyen LTP, Nguyen CQ, Tran NT & Mai TV leg., deposited in IEBR. *Paratypes*: VIETNAM: 2 ♂, same data as holotype, deposited in IEBR.

Diagnosis. Based on the presence of fovea behind propodeal spiracle rounded and delimited by strong carina, the new species belongs to subgenus *Bathanthidium* s. str.

This species can be distinguished from all other known species of subgenus

Bathanthidium s. str. by the following combination of characters: in the both sexes, mandible widened apically, the lowest tooth slightly rounded; in female, clypeus and mandible entirely black, paraocular area with thin, yellowish-orange marking extending along the inner margin to the top of the eye, scutellum entirely black, T1 with lateral small, dull orange markings, T2–T5 basally with yellowish-orange bands, T1–T2 apically with dull orange bands, the rest of T3–T5 with dull orange to dirty yellow bands, T6 entirely black; in male, T7 of trilobed, median lobe longest and broadest, lateral lobe short, acute and curved inwards, male genitalia large, forked gonostylus with two unequally branches, penis valves with blade shape.

Description. Female (Figs 1, 2). *Holotype*: Body length 8.5 mm, fore wing length 8.0 mm, head width 2.1 mm, and mesosomal width 3.8 mm.

Head: In frontal view subcircular, approximately 1.2 times as wide as long (Fig. 1A), gena about 0.7 times as wide as eye (Fig. 1G). Mandible widened apically, with 4 teeth, gradually elongate from the first tooth to the fourth tooth, the lowest tooth slightly rounded (Fig. 1B). Clypeus broader than long. Supraclypeal area and clypeus slightly convex.

Mesosoma: Fore wing with two submarginal cells, the first sub-equal in length to second, basal vein nearly straight and meeting Cu vein at acute angle, 2nd m-cu vein exceeding apical margin of the second submarginal cell, stigma nearly twice as long as broad (Fig. 1E). Outer margin of axilla slightly curved. Scutellum convex, with two foveae at basal margin, each fovea about four times as wide as long and with api-

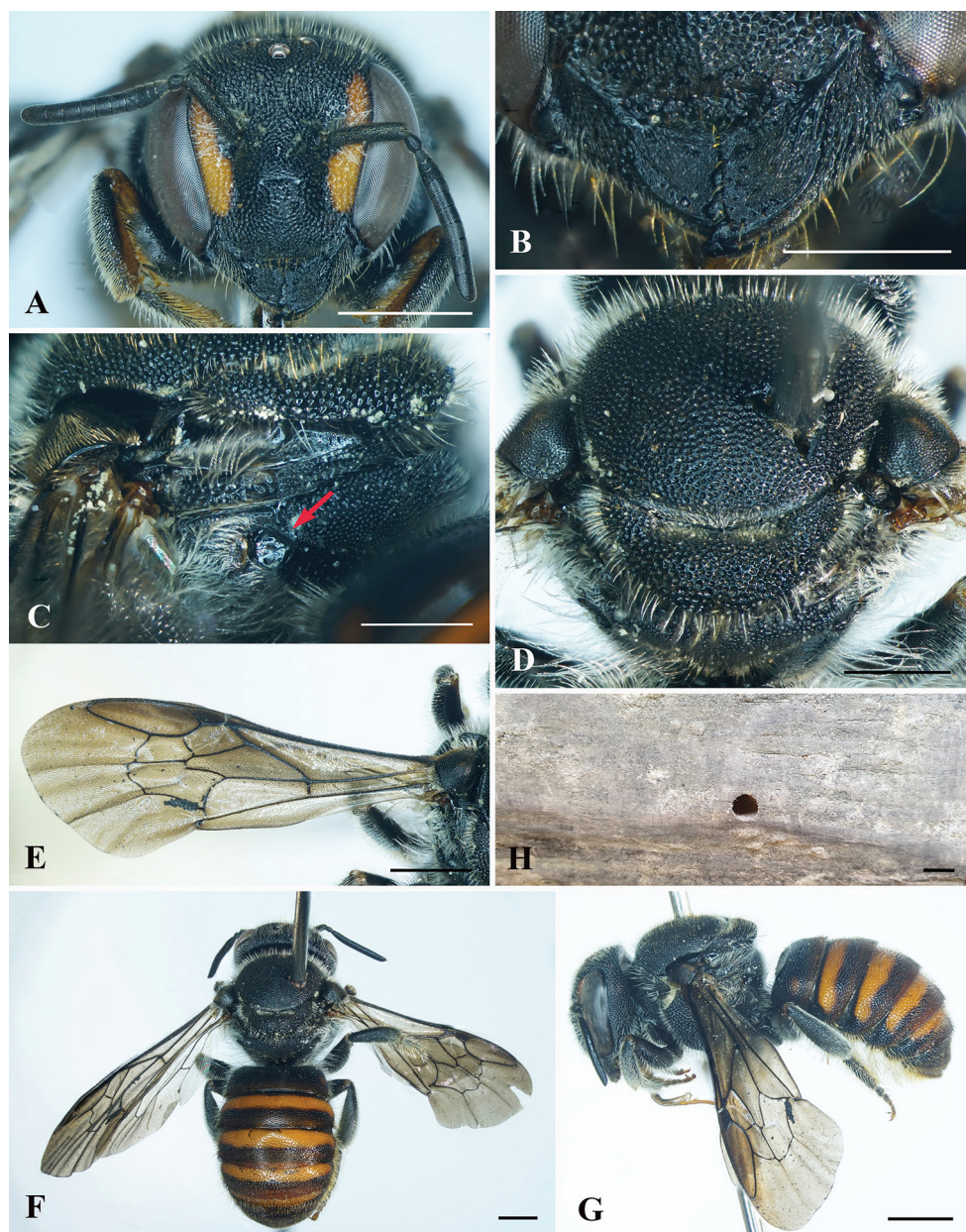


Figure 1. A–H *Bathanthidium* (*Bathanthidium* s. str.) *paco* Tran & Nguyen, sp. nov., holotype, female **A** head in frontal view **B** mandible in frontal view **C** propodeum in lateral view, showing the fovea behind propodeal spiracle **D** mesoscutum and scutellum in dorsal view **E** fore wing in dorsal view **F** habitus in dorsal view **G** habitus in lateral view **H** nest entrance. Scale bars: 5 mm (**H**); 1 mm (**A, C, E, F, G**); 0.5 mm (**B, D**).

cal margin slightly emarginated medially (Fig. 1D). Fovea behind propodeal spiracle rounded, delimited by strong carina, basal zone of propodeum distinct laterally, with regular and shiny pits (Fig. 1C).



Figure 2. A–E *Bathanthidium* (*Bathanthidium* s. str.) *paco* Tran & Nguyen, sp. nov., holotype, female **A** middle tibia in dorso-lateral view, showing the tibial spine **B** distitarsus of hind leg in frontal view, showing the arolium **C** metasoma in lateral view **D** T6 in dorsal view **E** mesoscutum and scutellum in dorsal view. Scale bars: 1 mm (**C, D, E**); 0.5 mm (**A, B**).

Metasoma: T1 with carina on basal margin, T6 with margin round apically (Fig. 2C–D). T6 with large, rough, dense punctures on basal haft and with small, extremely dense punctures on remain part. Fore and middle tibiae in dorso-lateral view with a short spine (Fig. 2A), hind tibia without a spine. Arolia present (Fig. 2B). Pygidial plate absent.

Punctuation. Clypeus with large, rough and dense punctures, except the apex with small punctures; frons, vertex, and gena with round, large and dense punctures. Mesoscutum with round and dense punctures, size of the punctures smaller and shallower than that on frons. Scutellum with round and dense punctures, size of punctures uneven. T1–T5 with round, small and sparse punctures on basal half and smaller and denser punctures on remain part.

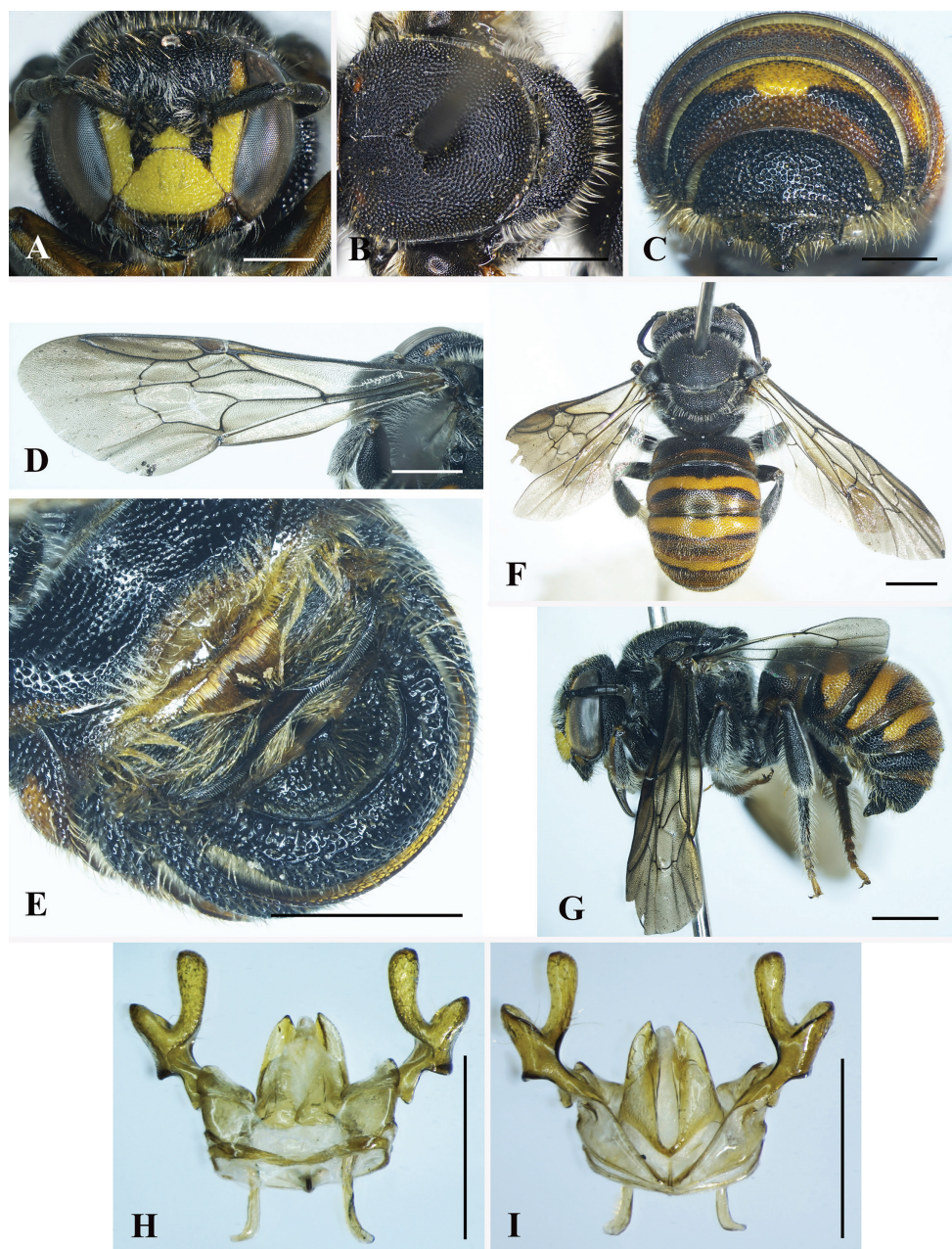


Figure 3. **A–I** *Bathanthidium* (*Bathanthidium* s. str.) *paco* Tran & Nguyen, sp. nov., paratype, male **A** head in frontal view **B** mesoscutum and scutellum in dorsal view **C** T4–7 in dorsal view **D** fore wing in dorsal view **E** abdomen in latero-ventral view **F** habitus in dorsal view **G** habitus in lateral view **H** genitalia in dorsal view **I** genitalia in ventral view. Scale bars: 1mm (**A, B, D, E, F, G**); 0.5 mm (**C, H, I**).

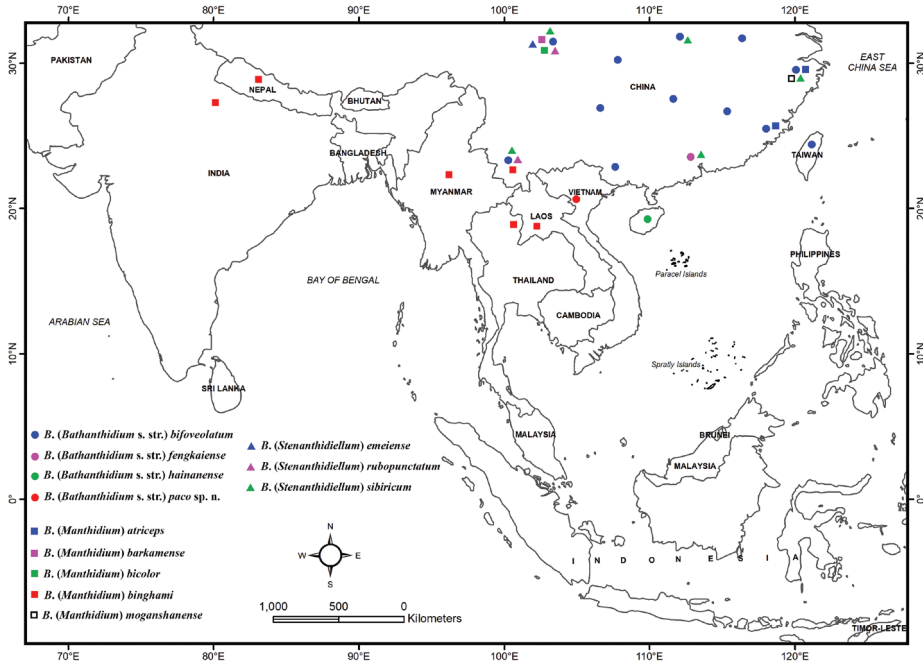


Figure 4. Distribution map of *Bathanthidium* species in the Indomalayan region.

Pubescence. Clypeus, paraocular area and frons with sparse, short and pale white hairs. Mandible with extremely short, brownish-yellow hairs except for some longer hairs at outer margin. Vertex, mesoscutum, scutellum with short, brownish-yellow hairs. S2–S6 with scopa.

Colour. Paraocular area with thin, yellowish-orange marking extending along the inner margin to the top of the eye (Fig. 1). T1 with lateral small, dull orange markings, T2–T5 basally with yellowish-orange bands, T1–T2 apically with dull orange bands, the rest of T3–T5 with dull orange to dirty yellow bands, T6 entirely black (Fig. 2C–E). Fore and middle femora apically, fore tibia in inside view with dull orange.

Male (Fig. 3). Body length 9–9.2 mm, forewing length 8.5–8.7 mm. Head about 1.2 times as wide as long, gena about 0.7 times as wide as eye.

Most characteristics of the male are similar to those of the female, except for the following:

Metasoma: T7 trilobed, median lobe longest and largest, lateral lobe short, acute and curved inwards (Fig. 3C). Male genitalia large, gonostylus forked with two unequal branches, penis valve with blade shape (Fig. 3H–I).

Colour. Clypeus yellow, supraclypeal area with small yellow marking, paraocular area with thin, long and yellow to yellowish-orange marking extending along the inner margin to the top of the eye (Fig. 3A). Mandible basally with dull yellow marking. Gena with yellowish-orange markings near the apical margin of the eye, mesoscutum latero-anteriorly with small yellowish-orange spots (sometimes

absent), scutellum entirely black (Fig. 3B). Inner surface of fore femur apically, fore tibia, fore basitarsus, middle femur apically, and middle tibia with dull orange markings. T1 with lateral small, light yellowish-orange markings; T2 medially with light yellowish-orange bands, T3–T5 basally with light yellowish-orange bands; T1, T2, T5 apically with dirty yellow bands; most of the rest of T3 and T4 with dirty yellow bands; T6 and T7 entirely black (Fig. 3C, F–G). Fore and middle femora apically, fore tibia in inside view with dull orange.

Pubescence. The median part of S3 orange, apical margin of S2–S3 with pale white to dull orange feather combs, S4 shiny on basal half and with short, dense, pale white hairs on remain part and simple hair comb in apical margin, S5 shiny on basal half and short, sparser, pale yellow hairs on remaining part with black, serrated papillae at apical margin (Fig. 3E).

Remarks. This species is most similar to *B. (Bathanthidium) bifoveolatum* (Alfken, 1937), but can be easily distinguished from the later by the mandible with the lowest tooth slightly rounded in both sexes (mandible with the lowest tooth acute in both sexes of *B. bifoveolatum*); clypeus entirely black in female (clypeus laterally with yellow markings in female of *B. bifoveolatum*); mandible basally with dull yellow marking in male (mandible with yellow marking extending from base to nearly top in male of *B. bifoveolatum*); mesoscutum latero-anteriorly with small faint yellowish-orange spots or absent, scutellum, pronotal lobe black in both sexes (mesoscutum anteriorly and scutellum apically with thin and long yellow marking interrupted medially; pronotal lobe with yellow marking basally in both sexes of *B. bifoveolatum*); T2 with yellowish-orange entirely bands, not interrupted medially in both sexes (T2 laterally with yellow markings in both sexes of *B. bifoveolatum*).

Nest biology. A nest was discovered in a wooden plank in the door of a house, the entrance hole of the nest with a diameter of about 4 mm (Fig. 1H). A female specimen was observed and collected when flying out from the nest. Male specimens were collected in the garden behind the wooden house.

Distribution. Northern Vietnam (Hoa Binh province).

Etymology. The specific name refers to the type locality, Pa Co commune in Hoa Binh province, it is treated as a noun in apposition.

An updated key to the species of the subgenus *Bathanthidium* s. str.

(Based on Niu et al. 2019, males of *B. fengkaiense* Niu & Zhu, 2019 and *B. hainanense* Niu, Wu & Zhu, 2012 are unknown)

- 1 Flagellum 10–segmented, female2
- Flagellum 11–segmented, male5
- 2 Paraocular area with thin and long yellow or yellowish-orange marking extending along the inner margin to the top of the eye.....3
- Paraocular area near ventral extreme with a small sub-triangle or sub-rectangle dull yellow marking.....4

- 3 Mandible with the lowest tooth acute (see fig. 34 in Niu et al. 2012). Clypeus laterally with yellow markings (see fig. 31 in Niu et al. 2012), mesoscutum anteriorly and scutellum apically with thin yellow marking interrupted medially (see fig. 29 in Niu et al. 2012) ***B. (Bathanthidium s. str.) bifoveolatum* (Alfken, 1937)**
- Mandible with the lowest tooth slightly rounded (Fig. 1B). Clypeus, mesoscutum and scutellum entirely black (Fig. 1A, D) ***B. (Bathanthidium s. str.) paco*, sp. nov.**
- 4 T2 entirely black, T3–T5 with yellow bands, the band on T3 obviously interrupted medially (see fig. 2d in Niu et al. 2019); mandible with the lowest tooth acute, distance between each two of the three basal teeth short and concave (see fig. 1e in Niu et al. 2019) ***B. (Bathanthidium s. str.) fengkaiense* Niu & Zhu, 2019**
- T2 with a pair of small, stripe-like yellow markings medially, T3–T5 with broader yellow bands, not interrupted medially (see fig. 12 in Niu et al. 2012); mandible with the lowest tooth slightly rounded, distance between each two of the three basal teeth longer and lesser concave (see fig. 5 in Niu et al. 2012) ***B. (Bathanthidium s. str.) hainanense* Niu, Wu & Zhu, 2012**
- 5 Mandible with yellow marking extending from base to nearly top (see fig. 39 in Niu et al. 2012). Mesoscutum anteriorly and scutellum apically with thin yellow marking interrupted medially (see fig. 36 in Niu et al. 2012) ***B. (Bathanthidium s. str.) bifoveolatum* (Alfken, 1937)**
- Mandible basally with dull yellow marking (Fig. 3A). Mesoscutum latero-anteriorly with small, yellowish-orange markings or absent, scutellum entirely black (Fig. 3B) ***B. (Bathanthidium s. str.) paco*, sp. nov.**

Discussion

Bathanthidium has a primarily Indomalayan distribution with almost all the described species (except *B. malaisei* from South Korea, and depending on whether or not one considers *B. emeiense* and *B. bicolor* to occur in Indomalaya) occurring in this biogeographic realm, the majority having been reported from southern China. The discovery of *B. paco* in northern Vietnam is both consistent with this pattern and extends the range of *Bathanthidium*.

Very little is known of the biology of *Bathanthidium*. The only description of a nest of any *Bathanthidium* is that of Romankova (1988) who observed *B. (Stenanthidiellum) malaisei* (Popov) (as *Lasianthidium malaisei* (Popov)) constructing linear series of resin cells in preexisting cavities in wood. This is consistent with the observation that anthidiines with three of four mandibular teeth (like *Bathanthidium*) typically construct their nests with resin (Michener 2007: 492). Michener (2007: 512) also noted that the slender body-form of members of *Bathanthidium* s. str. and *Stenanthidiellum*

suggested that, like other similarly shaped megachilids, they probably nest in narrow, preexisting cavities in wood. Our discovery of a female of *B. paco* emerging from a narrow hole in a wood plank is consistent with this suggestion and is the first observation of a probable nest site of any *Bathanthidium* s. str. species.

Acknowledgements

The authors would like to thank the subject editor, Jack Neff, and an anonymous reviewer for their valuable comments to improve the draft quality. We are also sincerely thank the Director (Mr. Luong Thanh Nguyen) and staffs (Mr. Khanh Tien Nguyen and Mr. Cong Van Bui) of Hang Kia – Pa Co Natural Reserve for providing support and permission. The present study was supported by the Vietnam Academy of Science and Technology under two grants (ĐLTE00.04/22-23 and QTBG01.01/21-22).

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Azteca ants repair damage to their *Cecropia* host plants

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Academic editor: F. Hita Garcia | Received 27 September 2021 | Accepted 7 December 2021 | Published 30 December 2021

<http://zoobank.org/BDCEBC79-F401-44FB-99F2-542B96B2478A>

Citation: Wcislo A, Graham X, Stevens S, Toppe JE, Wcislo L, Wcislo WT (2021) *Azteca* ants repair damage to their *Cecropia* host plants. Journal of Hymenoptera Research 88: 61–70. <https://doi.org/10.3897/jhr.88.75855>

Abstract

Some *Azteca* ants are well-known symbionts that defend their *Cecropia* host plants against herbivory, although there is considerable variability in behavior among colonies, conditions, and species. In exchange, ants receive food, and also shelter within the plants' internodes. Here we demonstrate that ants repair damage to the host plant when their brood is directly threatened. Using comminuted plant fibers and an unidentified binding liquid (probably plant sap) ants generally began patching holes in the tree trunk immediately, and significantly reduced the size of the hole 2.5 hours after it was created, and they generally completed the repairs within 24 hours.

Keywords

Ant-plant interactions, mutualism, nest construction, nest repair

Introduction

In mutualistic symbioses one partner is expected to be sensitive to danger to another partner if any necessary services (e.g., providing food) are threatened, but to be largely indifferent if not (e.g., Bronstein 2015). Some animals live in shelters made by their symbionts, such as snapping shrimp living within corals (e.g., Chak and Rubenstein

2019), and ants within plants (e.g., Janzen 1967; Longino 1991a; Davidson 2005). In many ant-plant symbioses the ants are provided with food and shelter in exchange for guarding the plants against herbivores, and plants ensure against cheating by linking the food they produce (e.g., Mullerian bodies) to the numbers of surviving leaves (e.g., Davidson 2005). These rewards benefit both partners, given widespread and intense pressure from herbivores, especially on young leaves (e.g., Coley and Barone 1996). But what happens when damage to the plant compromises the ants' shelter instead of its food production?

Some species of *Azteca* ants live in a permanent symbiosis with *Cecropia* plants: these mutualistic ants aggressively defend their host plants against herbivores, and in exchange the plants provide them with food and shelter (Longino 1999a; Davidson 2005; Marting et al. 2018a, b). The ants prevent damage to their hosts' leaves, because the ants' food is produced at the base of the petioles (e.g., Bischof et al. 2013) and the undersides of the leaves (e.g., Folgarait and Davidson 1995). Worker ants also open and maintain small (~1 mm) entrance holes in each internode that they occupy (Fig. 1a).

One of us (AW) used a sling shot to shoot a clay ball (9 mm diameter) at high velocity through an upper internode of a large *Cecropia* tree, making clean entry and exit wounds. Within 24 hours both holes were nearly sealed. This anecdotal observation suggested that the ants may also provide an additional benefit in repairing structural damage to their host plant. We tested the hypothesis that symbiotic *Azteca alfari* ants repair breaches into the interior of symbiotic *Cecropia* host plants that provide them shelter.

Materials and methods

Study area

Observations and experiments were made during the rainy season from June through November 2020. We opportunistically selected smaller trees growing near the edge of disturbed secondary-growth tropical forest (Bosque Urbano de Cárdenas), or located in backyards within the community of Cárdenas, Ancón, Republic of Panamá (~8°59'22"N, 79°34'10"W). Movement and logistics were constrained due to public health restrictions during a pandemic.

Data collection

The size of an *Azteca* ant colony is related to the diameter of the plant stem, and taller trees have more internodes (Marting et al. 2018a). As trees grow the colonies shift their domiciles and broods upward, and abandon lower internodes (*op. cit.*). A DeWalt portable electric drill with a 6.4 mm bit was used to drill a hole into each of $N = 22$ *Cecropia* trees that were all inhabited by *Azteca alfari* ants (Fig. 1b). In all cases the hole was drilled in the center of the internode at heights < 2 m from the ground; we selected internodes as high up as we could reach. Anecdotal preliminary observations

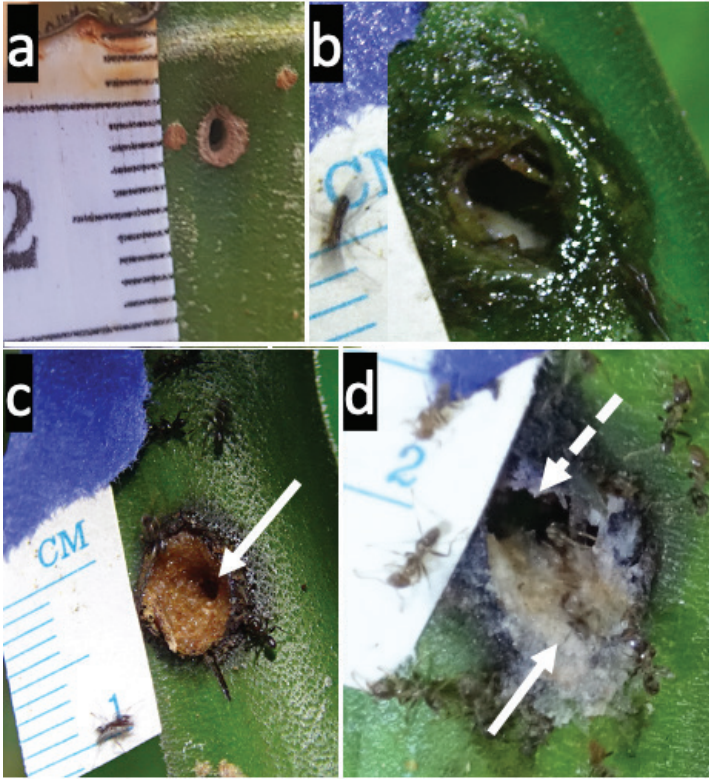


Figure 1. Natural and experimentally made holes in the walls of *Cecropia* stems, exposing the interior of the internode, and repair patches applied by ants (scale bars, mm) **a** a natural entrance to an internode opened and maintained by the ants **b** a newly drilled 6.4 mm hole in a stem (diameter of seventh internode from terminal bud = 19 mm, of 23 internodes total) **c** rapid reduction in hole diameter after 2.5 hours (arrow points to the remaining hole) **d** slower reduction in hole diameter after 24 hrs (dashed arrow points to the remaining hole), with a light-colored patch over half the hole (solid arrow) (diameter of sixth internode from terminal bud = 1 cm, of 50 internodes total).

suggested that ants tended not to respond when holes were drilled into internodes near the base of very large trees, so we used smaller trees, haphazardly selected. We measured the diameter at breast height (DBH), and counted the number of internodes from the terminal bud to where one hole was drilled. To assess whether plants are able to heal themselves in the time frame of our study, we drilled holes in two small myrmecophytic *Cecropia* stems (~1 cm DBH) that had not been colonized yet (not included in quantitative analyses).

Photographs of drilled holes each included a mm scale bar, and were taken with a hand-held Samsung Galaxy S8+, which introduced some non-systematic imprecision (e.g., minor deviations in the angle of the photograph, or a slightly out-of-focus scale bar). Photographs were taken immediately after the hole was drilled (Fig. 1b), after 2.5 hrs (Fig. 1c), and after 24 hrs (Fig. 1d, Fig. 2). These photographs were used to

assess the extent to which the holes were sealed by measuring the diameter of the opening. Additionally, we took photographs of natural entrance holes from $N = 8$ trees to determine the diameter of openings that the ants create and maintain (Fig. 1a). Photographs for figures, and Suppl. material 1 and 2, were recorded with a Sony Handycam FDR-AXP35 camera (20.6 MP resolution). Photographs were not digitally manipulated, except for cropping.

Data analyses

Hole diameters were measured from photographs using ImageJ 1.53. Statistical tests are indicated in the Results and were done using packages in R 4.0.3 and R Studio 1.3.1093 (RStudio Team 2020). Many variables did not meet assumptions of normality (Shapiro-Wilk tests) (statistical results given in Text Suppl. material 3), so non-parametric paired statistics were used to test differences in medians. Mean values are given with their standard deviations, and for all paired comparisons $N = 22$. Voucher specimens: Voucher specimens of the ants are deposited in the dry reference collection of the Smithsonian Tropical Research Institute, and were identified as *Azteca alfari* using Longino (1991b).

Results

Cecropia spp. trees in our sample were relatively young and small (mean number of internodes from terminal bud to drill site = 41.7 ± 31.5 ; mean DBH = $3.0 \text{ cm} \pm 0.77$). Each internode had a natural entrance with mean diameter = $0.76 \text{ mm} \pm 0.27$, $N = 8$) (Fig. 1a).

Once a hole is opened *A. alfari* ants reacted to the disturbance as described in Marting et al. (2018b), running to the area of the wound. In cases where holes were drilled into internodes that contained brood near the top of small saplings, ants exited with brood and transported them to adjacent internodes (see Suppl. material 1 and 2). The materials used to patch the drill hole are undetermined plant fibers bound by a liquid, presumably plant sap that often was visible around the wound, especially in internodes where the tissue was still green, which continued to ooze after it was sealed by the ants (Fig. 2b). We were unable to determine if ants added any glandular secretions to the patches. Usually several to ~ 10 ants worked on repairing the hole from both outside and within the stem (see Suppl. material 1 and 2), but ants were not marked so the total size of the repair force is unknown. No ants carried foreign material (e.g., resin) to the repair site (based on ~ 4 hours video observations). Ants carried small pieces of plant material and patched the hole from inside and outside the nest; in the latter case, ants exited from an adjacent internode with building materials (see Suppl. material 1 and 2). Ants applied their pulp to the hole working inwards around the entire circle (Fig. 1c, d). Necrotic tissue around the perimeter of the wound quickly turned black, which was especially conspicuous in small stems that lacked ants (Fig. 2d; these stems were not included in quantitative analyses). Once the hole was sealed (Fig. 2a) they continued to apply plant materials to fill it in

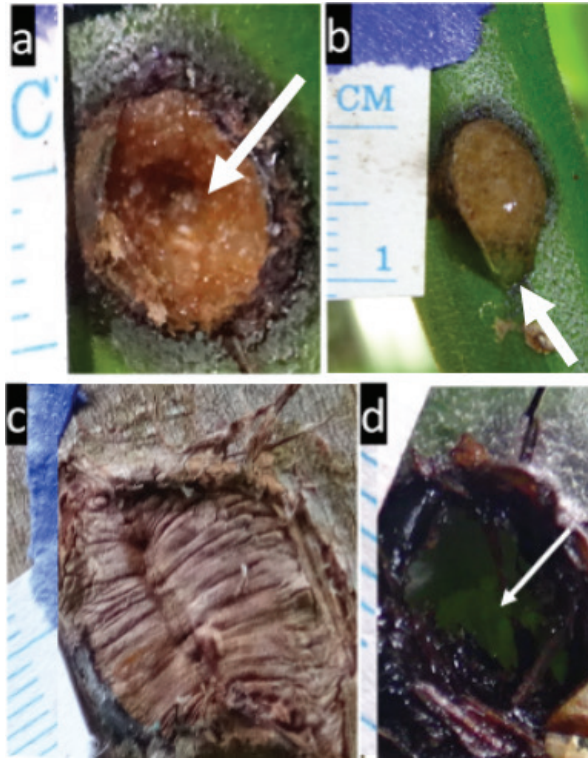


Figure 2. *Azteca* ant and *Cecropia* plant responses to wounds in the stems **a** a sealed hole after 24 hours, but not yet filled in to the stem surface (arrow) **b** a fully patched and filled-in hole after 24 hrs, oozing sap from the ant-sealed wound (arrow) **c** a natural plant scar surrounding a 6.4 mm hole that was fully sealed by the ants, approximately 5 months later **d** a hole in a plant without ants after 24 hrs, showing the green wall of the opposite side of the stem (arrow).

completely (Fig. 2b). Eventually the plant formed its own scar tissue, which differs in color, texture, and durability (Fig. 2c). The initial diameter of the opening (median drill hole diameter = 6.4 mm) was significantly reduced by the ants after 2.5 hrs (median hole diameter = 5.9 mm) (Fig. 1c) (Wilcoxon signed rank test with continuity correction, $V = 213.5$, $P = 0.002$, $N = 22$; Fig. 3). After 24 hrs ants further reduced the diameter of the opening (median = 4.0 mm) (Figs 1d, 2a, 2b) (Wilcoxon signed rank test with continuity correction, $V = 244$, $P < 0.001$, $N = 22$), or these holes were completely sealed ($N = 4$).

Using a cut-off value approximately twice the value of natural entrances to inter-nodes, colonies were scored as having worked to repair the damage if the difference between the initial drill hole diameter and the final diameter was > 1.5 . With this criterion, slightly more than half the holes were sealed or were only minimally repaired ($N = 14$ of 22; exact binomial test, $P = 0.286$). In at least one case a hole that was categorized as “open” after 24 hrs was sealed after 72 hrs (JET personal observation), but we did not gather temporal data beyond 24 hrs due to pandemic movement restrictions (see Methods). In those trees in which ants did respond, using the above crite-

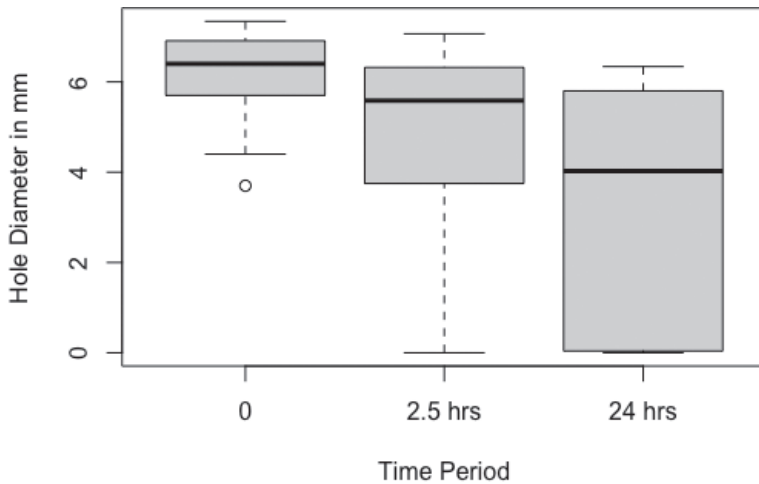


Figure 3. Boxplot of hole diameters 2.5 hours and 24 hours after a hole was initially drilled into a *Cecropia* stem.

rion, there was no significant association with work effort (as measured by a reduction in the diameter of the hole) and the number of internodes from the top of the tree to the drilled internode (Spearman's rank correlation between number of nodes from the top and hole diameter after 24 hours: $\rho = 0.2252$, $S = 352.5$, $P = 0.4387$).

Discussion

Azteca alfari ants tend to repair structural damage to their host plants, with variation among colonies. Behavioral personalities of *Azteca* colonies vary considerably (Marting et al. 2018a,b), but we do not know if repair behavior maps to those behavior types. The reasoning behind hole repair behavior could be because a hole in the wall exposes the ants' vulnerable immature stages to external pathogens, predators, or changes in other environmental parameters (e.g., relative humidity). The importance of such protection is evidenced by the fact that *Azteca* foundresses plug the prostomal entrances right after they open a hole to colonize a new *Cecropia* stem (Mayer et al. 2018; see references in Valverde & Hanson 2011; for a video of this behavior by P. Marting [pers. comm.], see <https://www.youtube.com/watch?v=fhNJJJE5zZOA>).

A change in the internal environment probably explains why ants evacuated brood from compromised internodes. Many organisms that build physical shelters for themselves or their offspring will regularly repair them when damaged (Madeiros et al. 2016; Farji-Brener and Tadey 2016; Downing 1992), as do humans (e.g., Divringi et al. 2019). But here we demonstrate that ants will work to repair a living structure, using plant material from inside the stem. Light-colored parenchyma cells are used by foundress queens to seal the entrance hole they make when colonizing a new plant (references in Valverde and Hanson 2011), which may be the light-colored patch material seen in Fig. 1d.

In a symbiosis, repairing the hole hypothetically would allow the plant to conserve energy on repairing wounds, but this benefit may be unlikely because eventually the trees form natural scars around the wounds using their own defenses (Fig. 2c; see Vasyukova et al. 2011). Ant repair behavior may be unlikely to benefit the plant directly, and thus the behavior may differ from microbial symbionts that facilitate wound healing in their hosts, which is beneficial to both (Poutahidis et al. 2013). However, we could not exclude the possibility that ants apply glandular secretions, which in some ant species have known antimicrobial activity (e.g., Fernández-Marín et al. 2015). If so, plants would benefit from the ants' behavior. We cannot explain why approximately half of the colonies repaired the damage and the others did not or did so minimally, but additional sources of uncontrolled variables would include plant age and colony size, and species of *Cecropia*.

Anecdotal observations of holes drilled into upper internodes of very small saplings (DBH < 1 cm) suggest that damage to internodes containing brood is rapidly repaired and closed within 5 hours, but first brood were evacuated (WTW, pers. obs.). Conversely, extensive damage is not repaired: clay balls shot through upper internodes in small stems with thin walls caused extensive damage, leaving frayed entry and exit wounds that were not repaired (AW, WTW pers. obs.). We hypothesize that colonies that did not respond in a short time frame had their broods far up in the tree, such that minor damage to a long-abandoned internode represents minimal risk for them, but we did not sacrifice the trees and ants, nor had access to a hydraulic lift to study colonies in larger trees to test it.

Sling shots wielded by teen-agers are a novel threat to the ants' shelter, but in nature *Bradypus* sloths are frequent visitors to *Cecropia* trees (Leigh 2002), and their sharp toenails sometimes pierce the wood (Peter Marting, personal communication, 2020), as would those of silky anteaters (*Cyclopes*). These openings likely would be smaller than the size of our experimental holes. These results of home-repair behavior in a symbiont reveal a new level of attention by the ants to their host plants. The ants not only behave in ways to minimize damage to their hosts, but when damage does occur, they actively work to fix it, albeit for their own benefit.

Acknowledgements

We greatly thank the Cárdenas police patrols for allowing us to work safely outdoors during the early days of a pandemic, and tolerating our activity during severe restrictions on movement. We also thank Panama's Ministry of the Environment for providing permits, and the Smithsonian Tropical Research Institute for supplies and administrative support, especially Taisha Parris who helped make arrangements for the first five authors to participate in the Smithsonian Institution's Behind-the-Scenes Volunteer Program. We thank Peter Marting, Hermógenes Fernández-Marín, Alejandro Farji-Brener, Sabrina Amador, and several anonymous reviewers for comments and criticisms that helped improve the manuscript; Donna Conlon provided helpful suggestions and logistical support throughout the study.

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Supplementary material I

Video S1

Authors: Alex Wcislo, Xavier Graham, Stan Stevens, Johannes Ehoulé Toppe, Lucas Wcislo, William T. Wcislo

Data type: Mp4 file.

Explanation note: Azteca ant repair behavior approximately 15 min after a 6.4 mm hole was drilled into an internode near the terminal bud; recorded with a 20.6 MP Sony Handycam FDR-AXP35 (normal speed). Video edited with OpenShot Video Editor 2.5.1.

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Link: <https://doi.org/10.3897/jhr.88.75855.suppl1>

Supplementary material 2

Video S2

Authors: Alex Wcislo, Xavier Graham, Stan Stevens, Johannes Ehoulé Toppe, Lucas Wcislo, William T. Wcislo

Data type: Mp4 file.

Explanation note: Azteca ant repair behavior approximately 2.5 hr after a 6.4 mm hole was drilled into an internode near the terminal bud; recorded with a 20.6 MP Sony Handycam FDR-AXP35 (playback speed, 4X speed). Video edited with OpenShot Video Editor 2.5.1.

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Link: <https://doi.org/10.3897/jhr.88.75855.suppl2>

Supplementary material 3

Azteca ants repair damage to their *Cecropia* host plants

Authors: Alex Wcislo, Xavier Graham, Stan Stevens, Johannes Ehoulé Toppe, Lucas Wcislo, William T. Wcislo

Data type: Docx file.

Explanation note: The following statistical tests Statistical tests were done using a package in R4.0.3 and RStudio 1.3.1093 (RStudio Team 2020), to test whether data met assumptions for normality.

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Link: <https://doi.org/10.3897/jhr.88.75855.suppl3>

Description of a new species of *Foenatopus* Smith (Hymenoptera, Stephanidae), with a key to the species from Vietnam

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Academic editor: Michael Ohl | Received 12 October 2021 | Accepted 6 November 2021 | Published 30 December 2021

<http://zoobank.org/FB4A46CC-E23D-4752-AF53-652B2FCD5943>

Citation: Ge S-X, Ren L-L, Tan J-L (2021) Description of a new species of *Foenatopus* Smith (Hymenoptera, Stephanidae), with a key to the species from Vietnam. Journal of Hymenoptera Research 88: 71–83. <https://doi.org/10.3897/jhr.88.76421>

Abstract

Foenatopus meridionalis Ge & Tan, **sp. nov.**, as the first species of the genus *Foenatopus* Smith, 1861 discovered from southern Vietnam, is reported and illustrated in detail. The key to the species of *Foenatopus* from Vietnam is compiled. A distribution map of the Vietnamese species is provided.

Keywords

new species, parasitoids, crown wasps, southern Vietnam

Introduction

The cosmopolitan family Stephanidae Leach, 1815, consisting of 365 extant species (van Achterberg 2002; Aguiar 2004, 2006; Aguiar and Jennings 2005; van Achterberg and Quicke 2006; Aguiar et al. 2010; Hong et al. 2010, 2011; Tan et al. 2015a, 2015b, 2018; Chen et al. 2016; Moghaddam et al. 2019; Gupta and Gawas 2020;

Binoy et al. 2020; Ge et al. 2021) are generally parasitoids of xylophagous insect larvae, including species of Coleoptera and Hymenoptera (Chao 1964; Taylor 1967; Kirk 1975; Königsman 1978; van Achterberg 2002; Aguiar 2004). The genus *Foenatopus* Smith, 1861 (Hymenoptera: Stephanidae) is the largest genus in the family that contains about 50% species of the family (Aguiar et al. 2010; Binoy et al. 2020). *Foenatopus* can be found in the tropical and subtropical areas of the Afrotropical, Neotropical, Palearctic and Oriental regions (Hong et al. 2011; van Achterberg, 2002). Biological information is almost lacking. Up to now, only four species are known from Vietnam, i.e. *Foenatopus annulitarsus* Enderlein, 1913, *Foenatopus chinensis* (Elliott, 1919), *Foenatopus flavidentatus* (Enderlein, 1913), *Foenatopus ruficollis* (Enderlein, 1913), which are also recorded from China. Here we report the fifth Vietnamese species, which is the first species discovered from southern Vietnam. A key to all species from Vietnam is provided.

Materials and methods

The descriptions, measurements, and figures were made using a ZEISS AxioZoom V16 microscope with a ZEISS AxioCam 503 color camera. Photographs were combined using the ZEN 2.3 (blue edition). Morphological nomenclature follows van Achterberg (2002) including the abbreviations for the wing venation. The types are deposited in the College of Forest Protection, Beijing Forestry University (BFU), China.

Taxonomy

Genus *Foenatopus* Smith, 1861

Foenatopus Smith, 1861: 58. Type species (by monotypy): *Stephanus indicus* Westwood, 1841.

Diastephanus Enderlein, 1905: 473. Type species: *Stephanus flavomaculatus* Enderlein, 1901. Synonymized by Benoit, 1956.

Neostephanus Kieffer, 1904: 1–4. Type species (by monotypy): *Neostephanus alluaudi* Kieffer, 1904. Synonymized by Narendran et al. (2001).

Diagnosis. Small to medium size. Temple always with pale yellowish streak behind eye. Neck emarginate anteriorly, finely striate, pronotal fold absent; reduced venation with vein 2-CU1 of fore wing always less developed, but sometimes complete; veins 2-SR and 2-SR+M of fore wing absent; vein 1-SR absent or faintly developed; hind tibia distinctly narrowed and compressed basally, outer side usually without fine oblique striae; hind tarsus with three tarsomeres in the female, while five in the male.

Distribution. Afrotropical, Neotropical, Palearctic and Oriental.

Key to species of the genus *Foenatopus* Smith from Vietnam

- 1 Ovipositor sheath with ivory sub-apical band..... 2
- Ovipositor sheath completely blackish or with brownish sub-apical band 3
- 2 Large ventral tooth of hind femur ivory *F. flavidentatus* (Enderlein, 1913)
- Large ventral tooth of hind femur blackish [The male with frons entirely bright yellow; hind femur with the third basal tooth acutely developed]
..... *F. chinensis* (Elliott, 1919)
- 3 Pterostigma relatively short and wide, obtuse apically; hind femur tridentate, ventral tooth blackish to partly ivory; propodeum completely covered with rather large and dense reticulate-foveolae 4
- Pterostigma comparatively long and narrow, somewhat subparallel-sided, acute apically; hind femur bidentate, large ventral tooth ivory; propodeum covered with sparse, shallow and circular foveolae, and with a relatively smooth area anteriorly [The male with a large reddish-brown spot developed in the middle part of the hind femur, the third tooth of the hind femur moderately developed].....
..... *F. meridionalis* Ge & Tan, sp. nov.
- 4 Vertex with 4 carinae between ocelli; vein 2-CU1 of fore wing distinct, 0.7–1.1 × as long as vein cu-a [male with frons completely yellowish; vein 2-CU1 of the fore wing 0.3 × as long as vein cu-a] *F. annulitarsus* Enderlein, 1913
- Vertex with 3 carinae between ocelli; vein 2-CU1 of fore wing short or absent [male unknown]..... *F. ruficollis* (Enderlein, 1913)

Foenatopus meridionalis Ge & Tan, sp. nov.

<http://zoobank.org/FDB28C76-5980-4253-A039-43D556C3F480>

Figs 1–31

Material examined. **Holotype**, ♀ (BFU), Vietnam: Binh Thuan, Huyen Thuan Bac, Dong Tien, 108°2.382'E, 11°12.912'N, VI.2020, leg. Local collector; **Paratypes**, 3♂ (BFU), Vietnam: Binh Thuan, Huyen Thuan Bac, Dong Tien, 108°2.382'E, 11°12.912'N, VI.2020, leg. Local collector.

Diagnosis. Head transverse in dorsal view and slightly elliptical in lateral view; frons completely yellowish-brown without streaks; vertex coarsely transversely carinate rugose with three distinct carinae between ocelli; propodeum shallowly circularo-foveolate with a relatively smooth coriaceous area anteriorly (the anterior coriaceous area concave more deeply in the male than in the female); pterostigma translucent with dark brown margins, long and narrow, subparallel-sided; vein 2-CU1 extremely short; hind femur with 2 ivory large teeth ventrally and with a blackish tooth obtusely developed basally; ovipositor sheath without sub-apical band.

Description. **Holotype. Female.** The length of body (except ovipositor sheath) 10.5 mm; forewing 6.4 mm long, 1.4 mm wide; the length of ovipositor sheath 9.6 mm.

Head. Antenna with 28 flagellomeres; frons finely and transversely rugose (Fig. 1); three anterior coronal teeth large and lobe-shaped, while the posterior two relatively small and wide; the ocular triangular area with three coarse transverse rugae; vertex finely transversely rugose anteriorly, with coarse and slightly curved rugae reaching occipital carina; temple smooth and shiny, roundly contracts behind eyes (Fig. 2).

Mesosoma. Pronotum moderately robust and largely coriaceous; neck anteriorly deeply emarginated, with several transverse ridges (Fig. 4), neck at about the same level of the middle part of pronotum postero-dorsally; pronotal fold absent; middle part of pronotum finely coarsely striate; posterior pronotum distinctly elevated and transversely rugose; mesoscutum with finely reticulate striate anteriorly and posterior half coarsely; scutellum smooth and with foveolae laterally (Fig. 5); propodeum completely with shallow, circular foveolae, with a relatively smooth coriaceous area anteriorly (Fig. 6).

Wings. Fore wing: hyaline (Fig. 7); vein 2-CU1 weakly developed, $0.25 \times$ as long as vein cu-a; pterostigma subparallel-sided, elongate and acute apically, $2.5 \times$ as long as vein r and $11.0 \times$ as wide as its maximum width; vein SR1 $2.1 \times$ as long as vein r; vein SR1 and vein r obtusely angled; vein r ends $0.13 \times$ length of pterostigma behind level of apex of pterostigma.

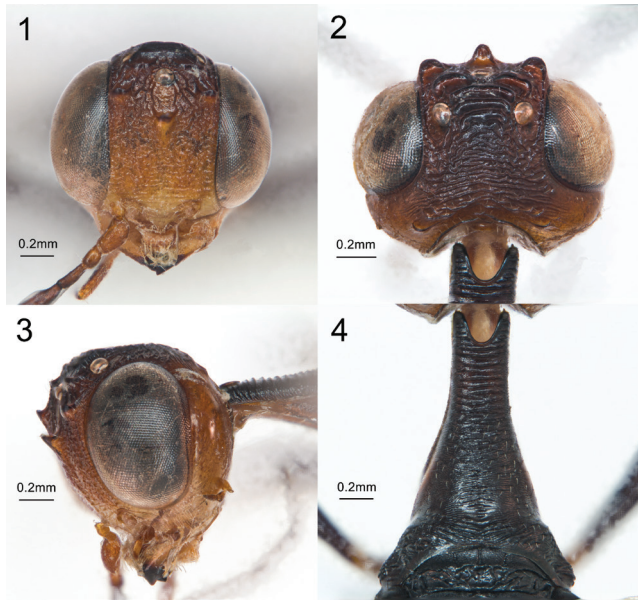
Legs. Hind coxa transversely striate, dilated sub-apically (Fig. 8); hind femur densely reticulate, with two ivory large ventral teeth and with a blackish tooth rather obtuse developed basally (Fig. 9); hind tibia coriaceous, $1.25 \times$ as long as hind femur; basal narrow part of hind tibia $1.1 \times$ as long as widened part, inner side of widened part basally distinctly V-shaped depressed, apically setose (Fig. 10); basitarsus $4.7 \times$ as long as wide, with dense and bristle setae ventrally (Fig. 11).

Metasoma. Tergite I (T_I) finely transversely striate (Fig. 12), $ca\ 7.4 \times$ as long as its maximum width, $2.25 \times$ as long as T_{II}; basal one fifth of T_{II} striate, and the remaining tergites largely shiny, smooth or weakly aciculate (Fig. 13); pygidial area indistinctly differentiated in color, and truncate apically (Fig. 14); ovipositor sheath completely black, and $ca\ 0.9 \times$ as long as body length (Fig. 15).

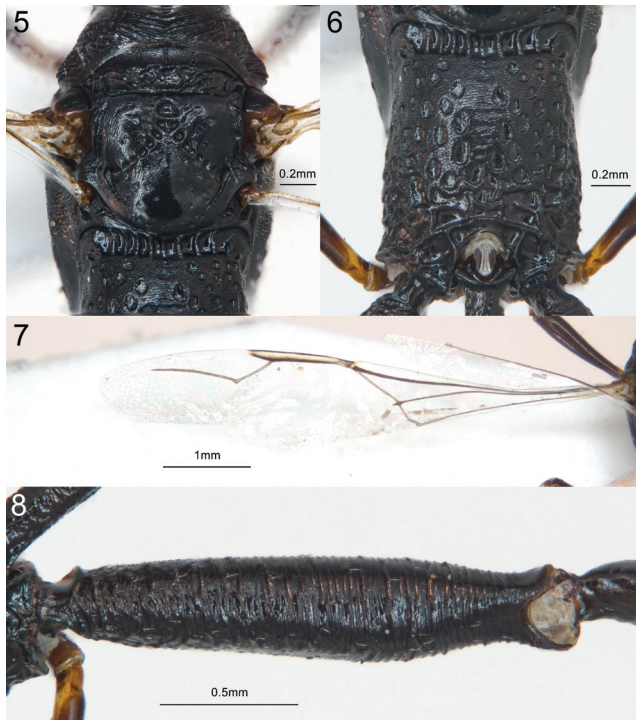
Colour. Brownish to blackish; frons completely yellowish-brown without streaks (Fig. 1), temple ventrally yellowish-brown along compound eye; wing membrane hyaline (Fig. 7), wing veins brownish; pterostigma translucent with dark brown margins; pronotum, mesoscutum and propodeum blackish; prosternum brownish; hind femur, hind tibia and metasoma largely blackish to blackish-brown; large ventral tooth of hind femur ivory (Fig. 9); ovipositor sheath completely blackish without whitish sub-apical band (Fig. 15).

Paratypes. Male. The length of body 9.5–11.4 mm; the length of forewing 4.9–5.7 mm.

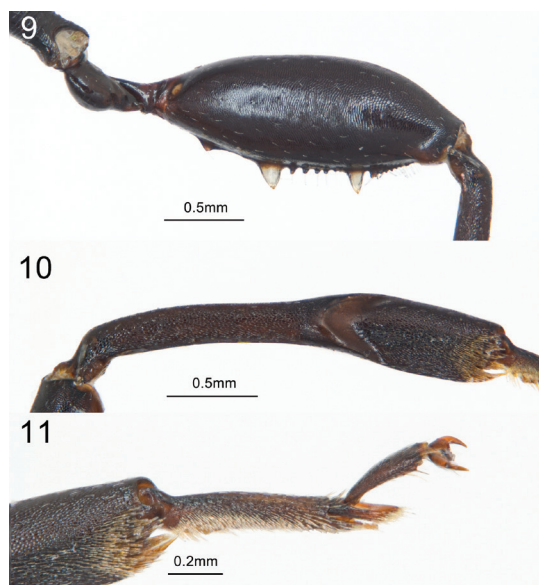
Resemble to female but differs as follows: fore legs and mid legs brown; a large reddish-brown spot developed in the middle part of hind femur; the blackish tooth on the basal part of hind femur comparatively more developed; tergite I $ca\ 3.2$ – $3.37 \times$ as tergite II and 0.92 – $1.05 \times$ as remainder of metasoma; tergite III reddish-brown in dorsal view, and tergite II to tergite IV brownish to reddish-brown in ventral view;



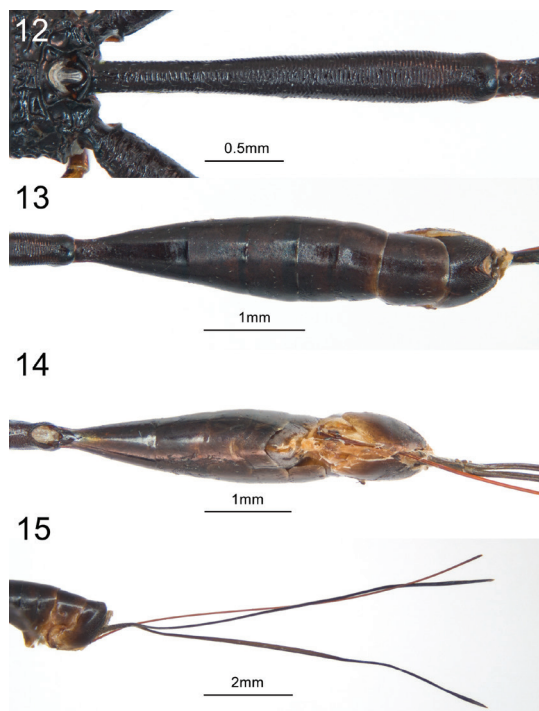
Figures 1–4. *Foenatopus meridionalis* Ge & Tan, sp. nov. Holotype ♀. **1** Head, frontal view; **2** Head, dorsal view; **3** Head, lateral view; **4** Pronotum, dorsal view.



Figures 5–8. *Foenatopus meridionalis* Ge & Tan, sp. nov. Holotype ♀. **5** Mesoscutum and scutellum, dorsal view; **6** Propodeum, dorsal view; **7** Fore Wing; **8** Hind coxa, lateral view.



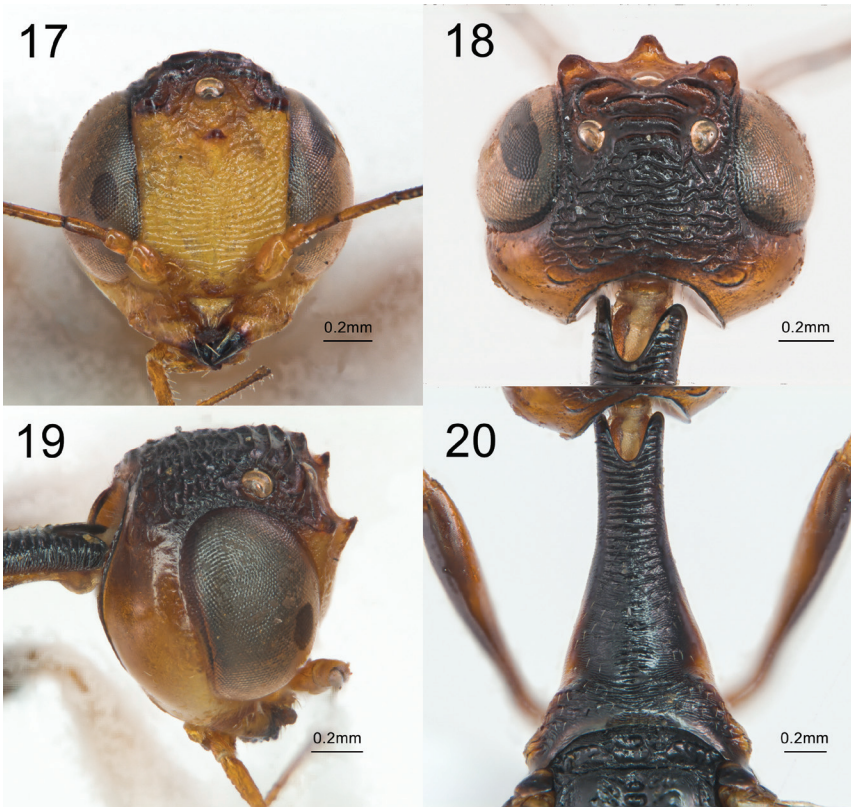
Figures 9–11. *Foenatopus meridionalis* Ge & Tan, sp. nov. Holotype ♀. **9** Hind femur, lateral view; **10** Hind tibia, lateral view; **11** Hind tarsi, lateral view.



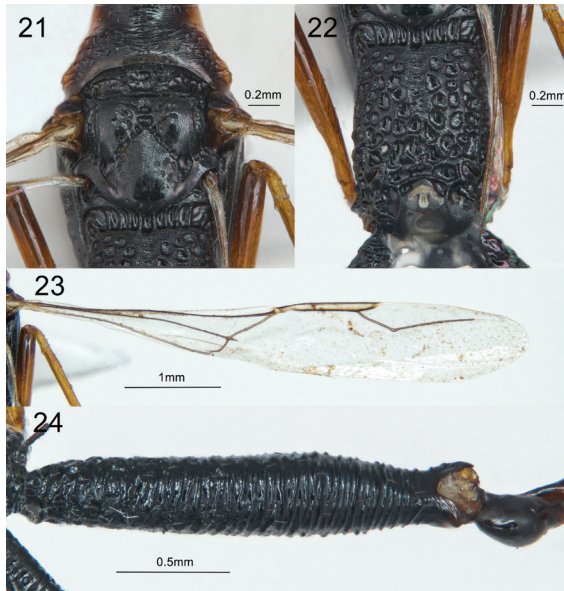
Figures 12–15. *Foenatopus meridionalis* Ge & Tan, sp. nov. Holotype ♀. **12** Tergite I, dorsal view; **13** Metasoma (except tergite I), dorsal view; **14** Metasoma (except tergite I), ventral view; **15** ovipositor and sheath, lateral view.



Figure 16. *Foenatopus meridionalis* Ge & Tan, sp. nov. Holotype ♀, dorsal habitus.



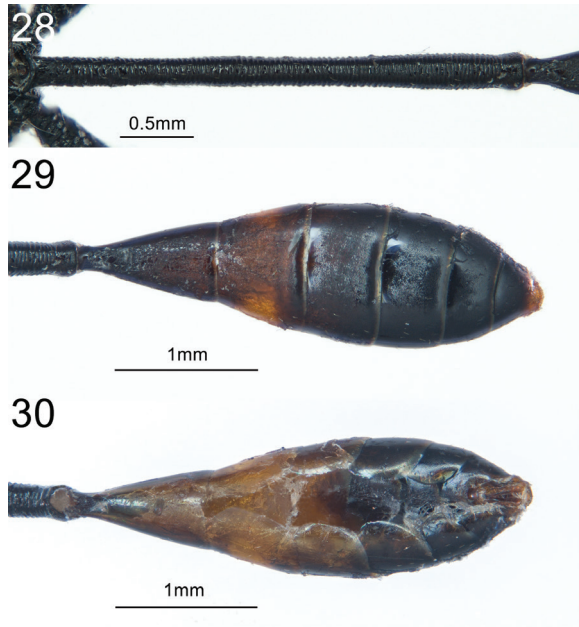
Figures 17–20. *Foenatopus meridionalis* Ge & Tan, sp. nov. Paratype ♂. **17** Head, frontal view; **18** Head, dorsal view; **19** Head, lateral view; **20** Pronotum, dorsal view.



Figures 21–24. *Foenatopus meridionalis* Ge & Tan, sp. nov. Paratype ♂. **21** Mesoscutum and scutellum, dorsal view; **22** Propodeum, dorsal view; **23** Fore Wing; **24** Hind coxa, lateral view.



Figures 25–27. *Foenatopus meridionalis* Ge & Tan, sp. nov. Paratype ♂. **25** Hind femur, lateral view; **26** Hind tibia, lateral view; **27** Hind tarsi, lateral view.



Figures 28–30. *Foenatopus meridionalis* Ge & Tan, sp. nov. Paratype ♂. **28** Tergite I, dorsal view; **29** Metasoma (except tergite I), dorsal view; **30** Metasoma (except tergite I), ventral view.



Figure 31. *Foenatopus meridionalis* Ge & Tan, sp. nov. Paratype ♂, dorsal habitus.

forewing 4.9–5.7 mm; vein 2-CU1 weakly developed, $0.21\text{--}0.3 \times$ as long as vein cu-a; pterostigma subparallel-sided, elongate and acute apically, $1.74\text{--}2.35 \times$ as long as vein r and $8.6\text{--}9.6 \times$ as wide as its maximum width; vein SR1 $1.74\text{--}2.06 \times$ as long as vein r; vein SR1 and vein r obtusely angled; vein r ends $0.24\text{--}0.26 \times$ length of pterostigma behind level of apex of pterostigma.

Etymology. We name the new species as “*meridionalis*” (Latin for south) for the type locality is in the southern part of Vietnam.

Distribution. Vietnam.

Biology. Collected in June. Host is unknown.

Note. The new species runs to *F. flavidentatus* in the key to Chinese species by Hong et al. (2011) in having the base of anterior tooth of corona yellowish brown; teeth of hind femur ivory and a less developed vein r on fore wing. However, the new species differs from *F. flavidentatus* in lacking the ivory sub-apical band of ovipositor sheath; propodeum with a relatively smooth coriaceous area anteriorly and an indistinctly differentiated pygidial area (pygidial impression in *F. flavidentatus* deep and reverse V-shaped). This new species runs to *F. sudhae* (Narendran & Sureshan, 2003) in the key to Indian species by Binoy et al. (2020) but it differs from *F. sudhae* in having 3 carinae between ocelli of the vertex and a distinct median longitudinal grove on

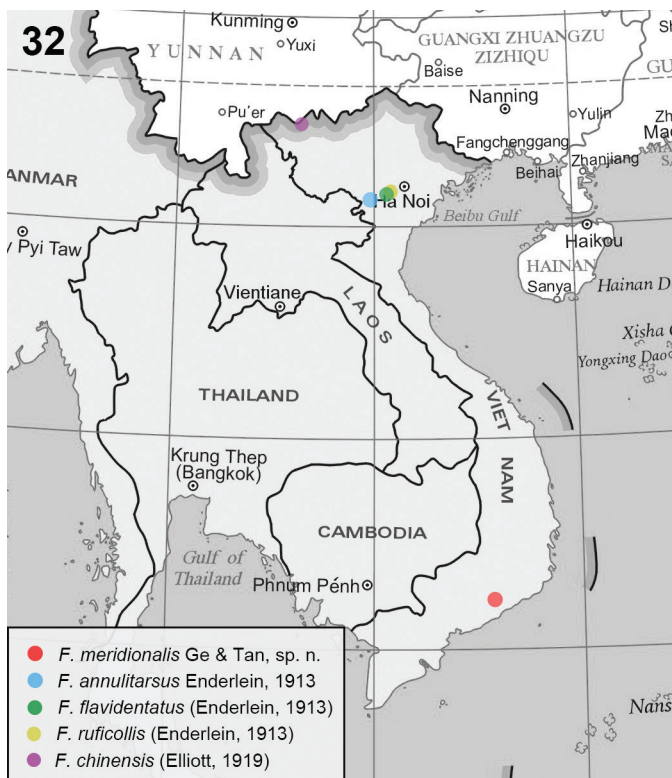


Figure 32. Distribution map of *Foenatopus* species from Vietnam (map of Vietnam from: <http://bzdt.ch.mnr.gov.cn/>)

posterior half of mesoscutum. The new species is also similar to *F. quadridens*, a species from Luang Prabang, Laos, in having posterior half of the pronotum distinctly striate, the ovipositor sheath completely blackish and a coarsely sculptured frons, but it can be easily distinguished by the two robust and ivory teeth on the hind femur (*F. quadridens* has 4 medium to large blackish teeth) and a slightly curved vein SR1 on the fore wing (more straight in *F. quadridens*).

Discussion

Except the new species described in this paper, all of the former recorded species in *Foenatopus* from Vietnam were collected in northern Vietnam (Fig. 32) and China (Hong et al. 2010, 2011). As the fusion area between Gondwana and Malaya, Vietnam is a country with mega-biodiversity and the Indochina Peninsula also serves as a bridge area between the East Asian continent and the Malay Archipelago, thus reflects an integrated fauna composition and characteristics. However, only a few species of Stephanidae have been described from Vietnam and its adjacent areas, and more undescribed species can be expected.

Acknowledgements

The research was supported jointly by the National Natural Science Foundation of China (NSFC, No. 31201732, 31572300, 31872263) and Beijing's Science and Technology Planning Project "Z201100008020001".

We thank Prof Shi-Xiang Zong (Beijing Forestry University, Beijing) and Dr Tao Li (General Station of Forest and Grassland Pest Management, National Forestry and Grassland Administration, Shenyang) for their great support for this study.

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First record of the *Sulia glaesaria* Simutnik, 2015 (Hymenoptera, Chalcidoidea, Encyrtidae) from Rovno amber

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Academic editor: Petr Janšta | Received 29 September 2021 | Accepted 13 October 2021 | Published 30 December 2021

<http://zoobank.org/4DC92DF2-AD5D-410B-A63D-7AC53D3C13C7>

Citation: Simutnik SA, Perkovsky EE, Khomych MR, Vasilenko DV (2021) First record of the *Sulia glaesaria* Simutnik, 2015 (Hymenoptera, Chalcidoidea, Encyrtidae) from Rovno amber. Journal of Hymenoptera Research 88: 85–102. <https://doi.org/10.3897/jhr.88.75941>

Abstract

Sulia glaesaria Simutnik, 2015 (Chalcidoidea, Encyrtidae), originally described from late Eocene Danish amber, is reported in coeval Rovno amber. A revised diagnosis of this genus is provided based on the new specimen and high-resolution photomicrographs of the holotype. Some character states, such as a short radicle, clypeus with long lateral margins, the presence of a strigil and basitarsal comb, spur vein of the hind wing, costal cell of hind wing along entire marginal vein with single line of long setae, and almost vertical syntergum with abruptly reflexed extension apically are reported in this species for the first time.

Keywords

Acropleural sulcus, acropleuron, Danish amber, Eocene, fossil Encyrtidae, syntergum

Introduction

Our research on several amber collections shows that the Encyrtidae is one of the most widespread families of Chalcidoidea in Eocene ambers (Simutnik 2001, 2002, 2007, 2015a, 2020; Simutnik and Perkovsky 2017, 2018a, b, c, 2020; Simutnik et al. 2014, 2021) along with Mymaridae, Aphelinidae and Trichogrammatidae. Most extant families of Chalcidoidea, including Tanaostigmatidae s.s., are, however, still unknown from the late Eocene. The large and distinctive chalcidoid wasp *Leptoomus janzeni* Gibson, 2008 from Baltic amber was previously attributed to Tanaostigmatidae s.l. along with the extant genus *Cynipencyrtus* Ishii, (1928) (Gibson 2008, 2009), but Heraty et al. (2013) subsequently treated *Cynipencyrtus* as its own family, Cynipencyrtidae Trjapitzin, 1973. A second, well preserved female specimen of *L. janzeni* was then reported from Rovno amber by Simutnik et al. (2020a). Some of its character states, in particular a bare and flat prepectus, suggest that a separate family should likely also be established for *Leptoomus*.

Sulia glaesaria Simutnik, 2015 was described from late Eocene Danish amber (Simutnik 2015b) and recently discovered in Rovno amber. Using a Leica Z16 APO stereomicroscope allowed revision of the diagnosis of this extinct genus.

The earliest known Encyrtidae include one female and four males ascribed to five different genera from middle Eocene Sakhalinian amber (Simutnik 2014, 2015b, 2020; Simutnik et al. 2021). They differ significantly from modern and late Eocene European amber encyrtids.

Material and methods

Ukrainian Rovno amber (33.9–37.8 Mya) is coeval with Baltic and Danish ambers from which *L. janzeni* and *S. glaesaria* were described.

The localities and composition of the Rovno amber fauna were recently characterized in a series of reviews by Perkovsky et al. (2010), Perkovsky (2018), Martynova et al. (2019) and Mitov et al. (2021). Including new Pristocerinae and Epyrinae (Bethyilidae) (Colombo et al. 2021a, b), *Formica* (Formicidae) (Radchenko et al. 2021) and the species reported here, 141 species of Hymenoptera are now known from Rovno amber, with 66 (47%) in common with Baltic amber.

Nearly all studied Rovno amber inclusions from Rovno were collected from Klesov and the Horyn River Basin (Perkovsky et al. 2010; Perkovsky 2017; Kirichenko-Babko et al. 2021; Sukhomlyn et al. 2021; Mitov et al. 2021 and references therein) except those of new collections from the former Vladimirets and Zarechnoye districts of the Rovno region and Manevichi district of the Volyn region (basins of Styr, Veselukha and Stokhod rivers). These new collections (mostly from Kuchotskaya Volya, Voronki and Velyki Telkovichi) reveal dozens new species of hymenopterans, isopterans, blattodeans, hemipterans, coleopterans, trichopterans, neuropterans and raphidiopterans (listed in Tshernyshev and Perkovsky (2021), with additions in Legalov et al. (2019,

2021), Gifka et al. (2021), Melnitsky et al. (2021), Jałoszyński and Perkovsky (2021), Golub et al. (2021) as well as species previously recorded from Baltic amber (Perkovsky and Olmi 2018; Martynova et al. 2019; Mamontov et al. 2020; Simutnik et al. 2020) or Baltic and Bitterfeld ambers (Radchenko and Perkovsky 2018, 2020).

Danish amber is much understudied (Nadein et al. 2016). Its Hymenoptera was briefly reviewed by Simutnik et al. (2021). *Sulia glaesaria* is the first of four Danish encyrtid genera (Simutnik 2015b; Simutnik and Perkovsky 2017, 2018a, b; Simutnik et al. 2020b, 2021) shared with another amber. Only two *Dolichoderus* (Formicidae) species were previously known exclusively from Rovno and Danish amber, and *Fallomyrma transversa* Dlussky et Radchenko – from Rovno, Bitterfeld and Danish amber (Perkovsky 2018).

The amber piece containing the new specimen of *S. glaesaria* was found near the village of Voronki in the Vladimirets District (basin of Styr River), Rovno Region, Ukraine. It is housed in the collection of the Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine, Kiev (SIZK). Danish amber containing the holotype is reddish and not very transparent. The sample from Rovno amber is yellow and clear (Fig. 1A). The Rovno amber specimen is distinguished by a darker coloration of the body, antennae, legs, wing venation, and by having slightly infusate, brownish, rather than hyaline forewings with narrow darkening ventral marginal vein and parastigma, although this may be an artefact of differing amber preservation. It is smaller (1.7 mm vs 1.9 mm of holotype) but no differences were found in the proportions of its body parts.

The condition of the Rovno specimen changed over time since it was initially studied, in one month (7.03.2021–13.04.2021) the air layer spreading over almost the entire mesoscutum (Fig. 5A, B), though since then until 7.07.2021 the extent of the air layer has not changed.

Photographs were taken using Leica Z16 APO stereomicroscope equipped with a Leica DFC 450 camera and processed with LAS Core and Adobe Photoshop software (brightness and contrast only).

Terminology and abbreviations follow Sharkov (1985), Gibson (1997), and Heraty et al. (2013). For the emended diagnosis, we used the characters listed by Noyes and Hayat (1994) in which the character states were polarized through comparison with those of the Tanaostigmatidae; diagnosis of Tanaostigmatidae by Gibson (1989); the detailed analysis of the morphology of *L. janzeni* and comparison it with that of *Cynipencyrtus* Ishii, 1928 (Cynipencyrtidae), Encyrtidae, Eupelmidae, and Tanaostigmatidae (Gibson 2008); and lists of synapomorphies for the Chalcidoidea families of the clade E given by Heraty et al. (2013).

The following abbreviations are used in the text and plates of illustrations: **ac** = acropleuron; **acs** = acropleural sulcus; **ae** = arched elevation of Mt6; **btc** = basitarsal comb; **c** = cercus; **cers** = cercal setae; **cly** = clypeus; **cs** = covering setae of the linea calva; **cuf** = cubital fold; **F1, F2, etc.** = funicular segments 1, 2, etc.; **hyp** = hypopygium; **lc** = linea calva; **LOL** = minimum distance between the anterior ocellus and a posterior ocellus; **ls** = line of setae along marginal vein of hind wing; **mps** = multiporous

plate sensilla; **Mt1, Mt2, etc.** = metasomal terga, numbering starts from petiole (Mt1); **OOL** = minimum distance between an eye margin and the adjacent posterior ocellus; **OCL** = minimum distance between a posterior ocellus and the occipital margin; **opo** = outer plates of ovipositor; **pl3** = metapleuron; **POL** = minimum distance between the posterior ocelli; **pre** = prepectus; **r** = radicle; **sp** = spiracle; **spf** = spur fold; **spv** = spur vein; **st** = strigil; **syn** = syntergum; **v1+v2** = ovipositor stylet; **v3** = ovipositor sheath.

SIZK = I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine (Kyiv); **ZMUC** = Zoological Museum of the University of Copenhagen.

Results

Taxonomy

Chalcidoidea Latreille, 1817

Family Encyrtidae Walker, 1837

Genus *Sulia* Simutnik, 2015

Type species. *Sulia glaesaria* Simutnik, 2015 (Simutnik 2015b, 2020).

Material examined. *Holotype*, ZMUC, 5–1–1961, ♀, Børge Mortensen, Danish amber, late Eocene. The inclusion is in a reddish and not very transparent piece of amber in a shape of an almost rectangular parallelepiped (ca. 6 × 5 × 4 mm) (Fig. 1A). All body parts are preserved. Syninclusions absent.

New material: SIZK, no. L-139, 1♀, vicinities of Voronki village, Vladimirets District, Rovno Region, Ukraine; Rovno amber; late Eocene. The inclusion is in a yellow and clear piece of amber in a shape of triangular prism (ca. 20 × 16 × 15 × 7 mm) (Fig. 4A). All body parts are preserved. Syninclusions are absent.

Diagnosis (emended). Body robust, barrel-shaped; clypeus setose, with long lateral margins and sharp ventrolateral angles; scape with apicoventral depression; funicle cylindrical; dorsoapical part of mesopleuron with convex differentiated region ventral to tegula delineated by sulcus; linea calva without filum spinosum, but with well-developed line of long setae along basal margin of dorsal surface; costal cell of hind wing with single line of long setae along the entire marginal vein; spur vein or fold originating from the marginal venation of the hind wing present but visible only at some angles; strigil and basitarsal comb present; gaster barrel-shaped, syntergum rather vertical with abruptly reflexed extension apically; cerci only slightly advanced toward gastral base; apex of hypopygium far from reaching apex of gaster; outer plates of ovipositor narrow, crescent-shaped in lateral view.

Redescription. Female. Largest known fossil encyrtid, body length 1.7–1.9 mm, not flattened, robust, barrel-shaped.

Coloration. Head and body black, but appear silvery where surrounded by thin layer of air (only in these places a shallow reticular sculpture visible, Figs 2C, 3B, 4B, D, E, 5A, B, D). Scape black with lighter apex (holotype) or completely black (Rovno

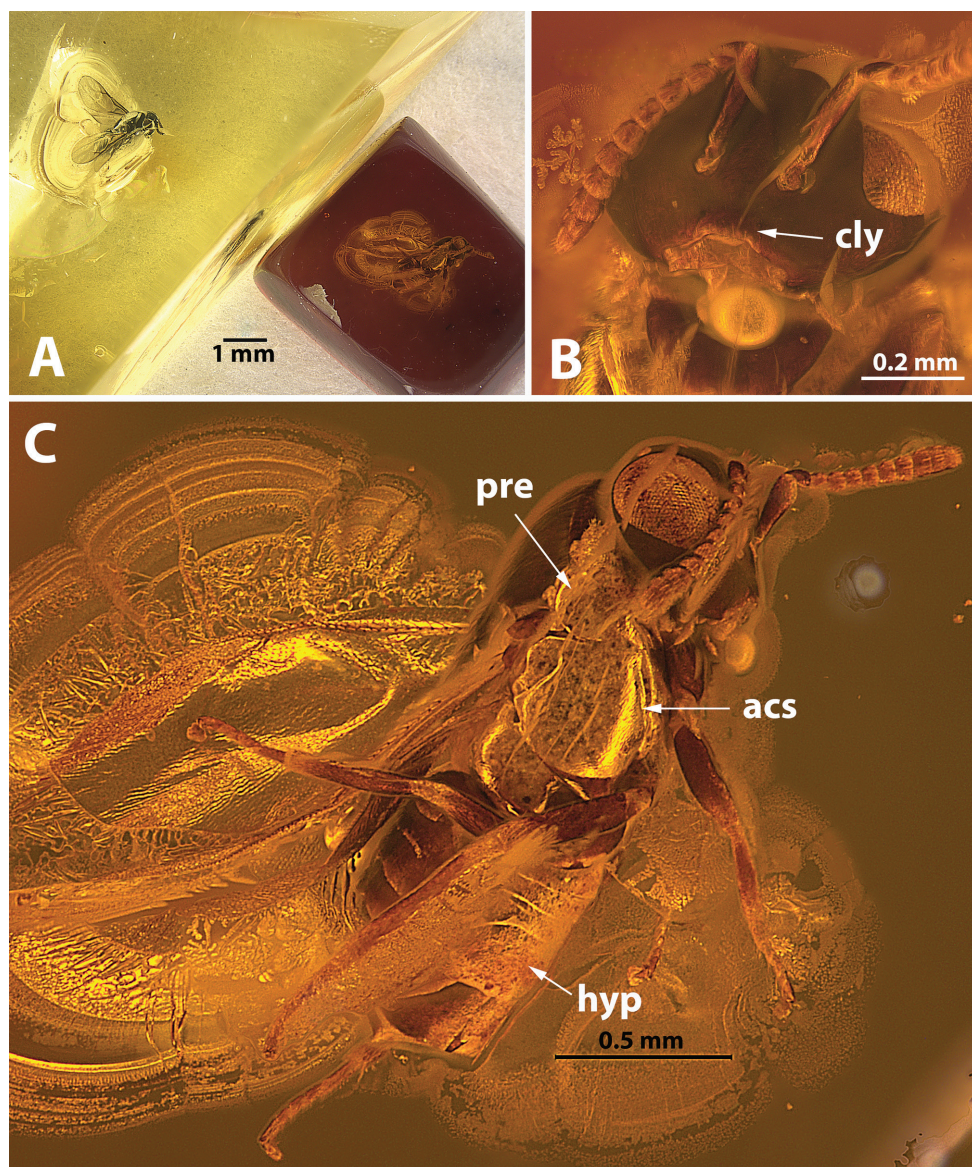


Figure 1. *Sulia glaesaria*, females **A** pieces of amber containing the inclusions: holotype in Danish amber, deposited in ZMUK (red) and new specimen from Rovno amber, deposited in SIZK (yellow) **B** head, holotype, ventrofrontal **C** habitus, holotype, right lateral (acs – acropleurul sulcus, cly – clypeus; hyp – hypopygium, pre – prepectus).

specimen); pedicel black; all segments of funicle from pale yellow (holotype) to entirely black (Rovno specimen); tegulae dark, legs of holotype lighter than those of Rovno specimen. Differences in color most likely an artefact of preservation.

Head. Lenticular, as wide as thorax, broader than long, frontovertex curved to posterior ocelli; broadly rounded in frontal view, not vaulted above eyes; eyes bare, without

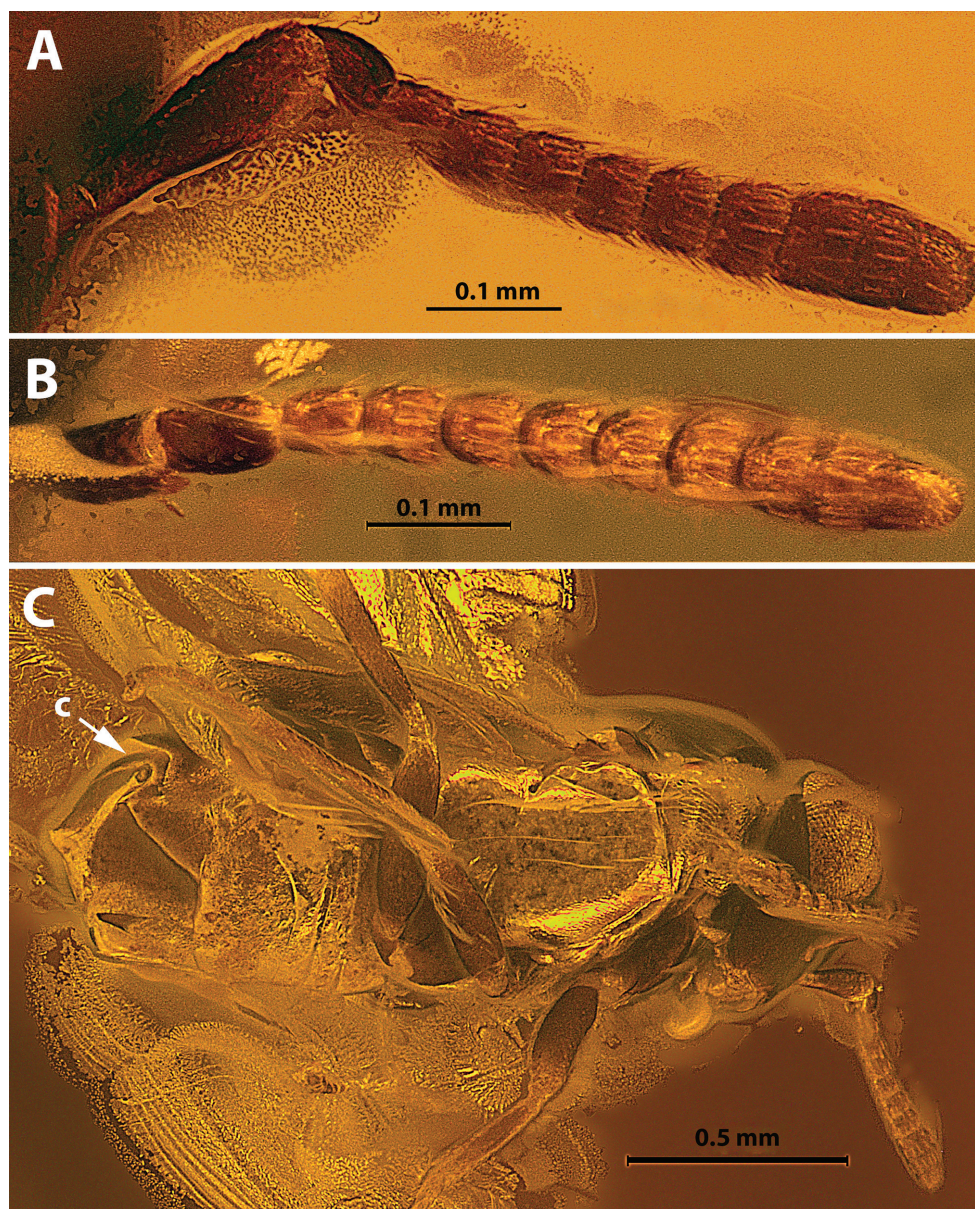


Figure 2. *S. glaesaria*, female, holotype **A** antenna lateral **B** antenna dorsal **C** habitus, right posterolateral (c – cercus).

visible microtrichia, inner orbits parallel over much of height but ventrally divergent (Figs 1B, 3C, 5D); frontovertex broader than long, minimum distance between eyes about $0.4\times$ head width; ocelli normal in size, forming about 100° angle; OOL about equal to posterior ocellar diameter (Figs 3C, 5B); OOL:POL:LOL:OCL about 1.5:6:3.5:0.8; occipital margin sharp; eye reaching occipital margin; antennal scrobe deep, not extended

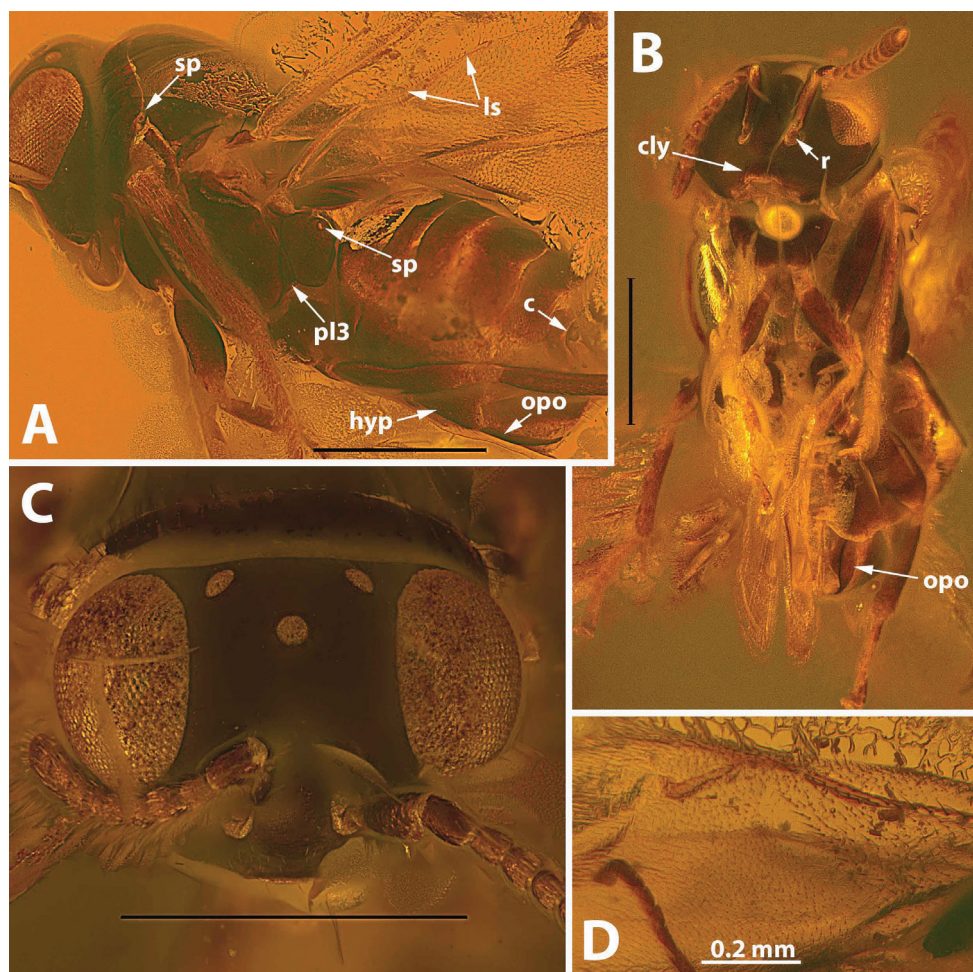


Figure 3. *S. glaesaria*, female, holotype **A** habitus, left lateral **B** habitus, ventral **C** head, frontal **D** forewing venation (cly – clypeus, hyp – hypopygium, ls – line of setae, opo – outer plates of ovipositor, pl3 – metapleuron, r – radicle, sp – spiracle) number of mandibular teeth not visible; maxillary palpi 4-segmented; labial palpi 3-segmented (Fig. 5D). Scale bars: 0.5 mm (**A–C**).

to anterior ocellus, in dorsal view anterior ocellus at equal distance from occiput margin and from margin of scrobal depression; interantennal projection small but visible in lateral view (Figs 1C, 2C); toruli located at level of lower margin of eyes (Figs 1B, 5D); distance between toruli equal to distance between lower margin of torulus and oral margin, about twice width of torulus, about $0.3\times$ length of malar space; malar space with complete malar sulcus (Fig. 5D), slightly shorter than height of eye, about equal to width of mouth; clypeus widely emarginated with long lateral margins, about twice as broad as long, with sharp ventrolateral angles, and with row of setae (Figs 1B, 5D); mandibles wide (Figs 1B, 5D),

Antenna. Geniculate, 11-segmented, without differentiated anelli, with 6 funicular segments and with 3-segmented clava; radicle short, about $2\text{--}2.5\times$ as long as broad

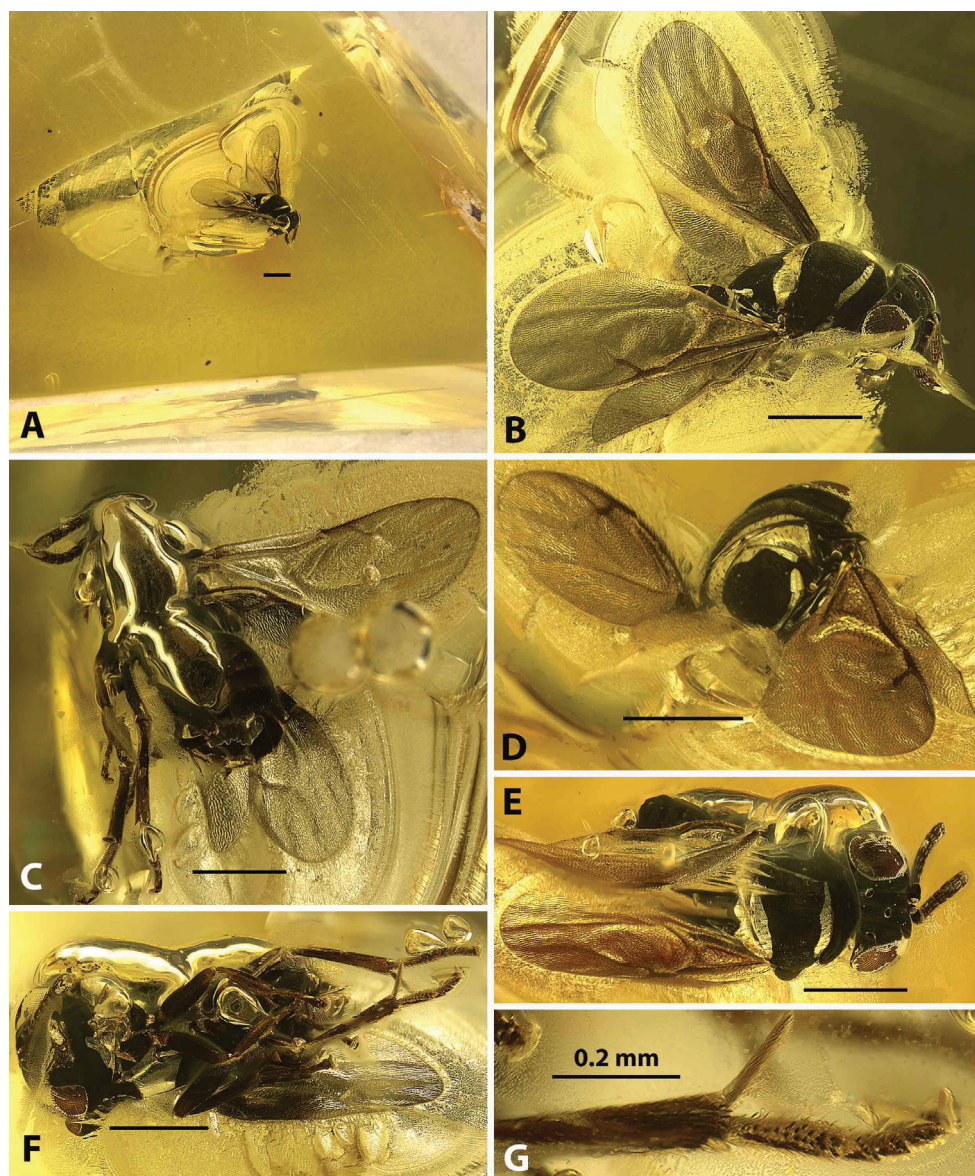


Figure 4. *S. glaesaria*, female, new Rovno specimen **A** general view in piece of amber **B** dorsolateral **C** lateral **D** posterodorsal **E** dorsal **F** ventral **G** apex of mid leg. Scale bars: 0.5 mm (**A–F**).

(Fig. 1B); scape with apicoventral depression (Figs 2A, 3C), $2.7\times$ as long as broad, widest at apex, flattened; pedicel conical, slightly shorter than first two funicular segments combined, longer than any segment of funicle; funicle cylindrical, F1–F3 longer than broad, F4–F6 almost square; all segments of funicle and clava with mps; clava as long as F4–F6 combined, without oblique truncation (Fig. 2A, B).

Mesosoma. Pronotum almost vertical, but in dorsal view with very narrow transverse dorsal surface (Figs 1A, 3A, C), without medial line; mesoscutum not

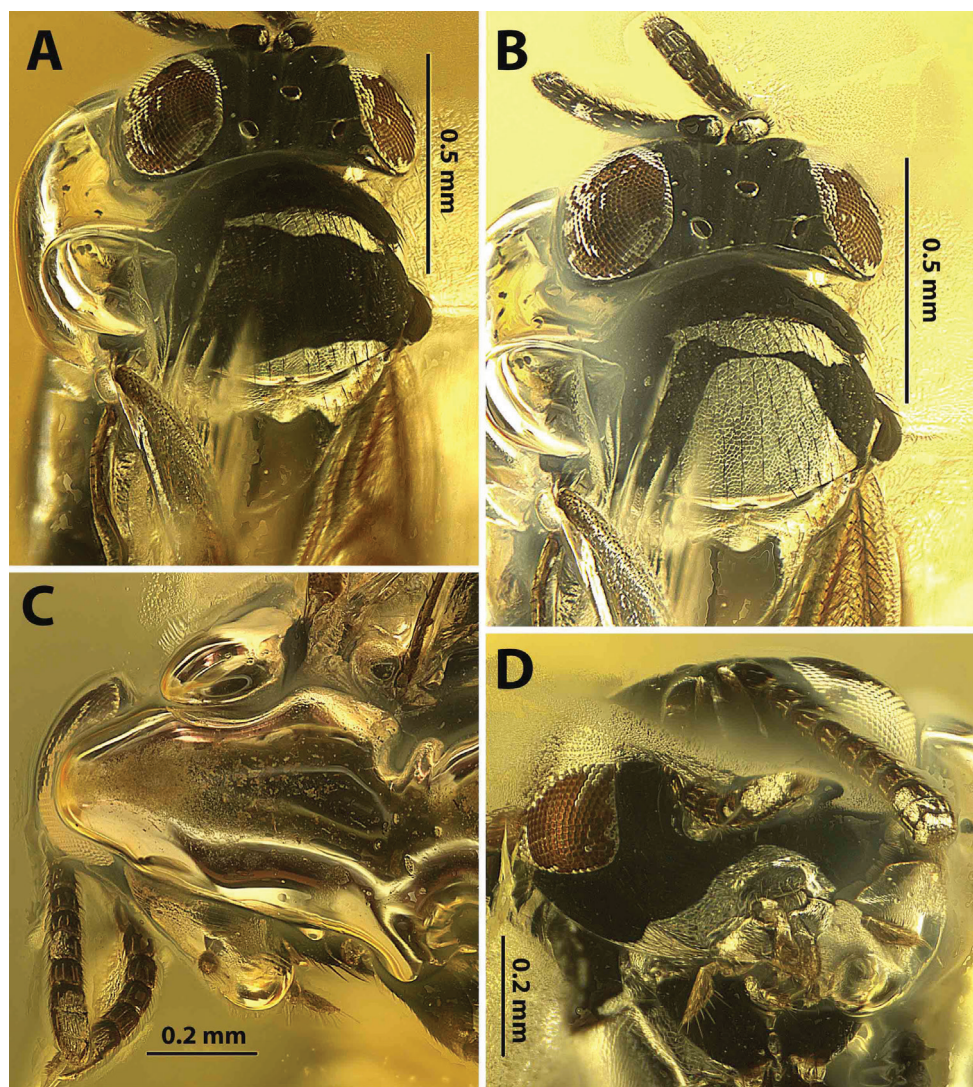


Figure 5. *S. glaesaria*, female, Rovno specimen **A** head, mesosoma, dorsal **B** head, mesosoma with layer of air spread over mesoscutum, dorsal **C** head, antennae lateral **D** head, clypeus, mandibles, frontal.

conspicuously wider than pronotum, broader than long, polygonally reticulate with sparse punctures, with posterior edge transverse, straight; mesoscutum articulated with scutellar-axillar complex only laterally, with edges of sclerites narrowly separated medially (Rovno specimen Figs 4D, 5A, B), connected by slender, delineated anterior portion of scutellar-axillar complex; mesothoracic spiracle open, not concealed beneath pronotum (Fig. 3A); notauli absent, axillae transverse-triangular with antero-medial angles contiguous (Figs 4D, 5B); scutellum as broad as long, slightly convex, sculptured as mesoscutum and clothed in long, stout, black setae; prepectus large, bare, flat, with anterior margin not extend anterior to mesothoracic spiracle, posterior

margin extended to base of tegula (Figs 1C, 2C, 3A); acropleuron convex, bare, with longitudinal reticulate sculpture, short, in lateral view its length equal to height (Figs 1C, 2C, 3C), with distinct acropleural sulcus (Figs 1C, 2C) extended anteriorly from above base of mesocoxa toward posteroventral angle of prepectus; dorsoapical part of mesopleuron with small, convex differentiated region ventral to tegula, also delineated by distinct sulcus (Figs 1C, 2C, 3A), visible in holotype only; metapleuron triangular, narrow, without visible setation (Fig. 3A: pl3); propodeum bare, with large, polygonally reticulate lateral parts, and circular spiracle (Fig. 3A); mid coxa inserted level with mid line of mesopleuron (Fig. 2C), in ventral view with mesosternum transverse-rectangular and abutting bases of coxae, without a membranous area anterior to each coxal base; mesotarsal pegs arranged only along anteroventral margin of tarsus and coxae cannot rotate anteriorly out of their fossae (Fig. 4F).

Wings. Fully developed. Fore wing with basal cell uniformly setose; costal cell broad; submarginal vein with single line of long setae, parastigma with two lines of shorter setae; cubital vein present as non-pigmented but distinct fold (Fig. 7B, cuf); linea calva almost entire, closed posteriorly by only one seta along cubital fold, without filum spinosum, but with well-developed line of long setae along basal margin of dorsal surface (Figs 3D, 7A, B: cs); parastigma only slightly widened, hyaline break (unpigmented area) present (Fig. 7B, C); marginal vein long, about 5 times as long as broad; stigmal vein slightly longer than marginal, with long narrow uncus and uncal sensilla; postmarginal vein twice as long as stigmal vein, enlarged seta marking apex of postmarginal vein of fore wing absent; setae of marginal fringe short. Hind wing relatively narrow; submarginal and marginal veins swollen, submarginal vein with single line of setae; costal cell narrow, membrane of cell along entire marginal vein with single line of long setae (Figs 3A, 6C, 7F, E: ls); spur vein originating from marginal venation present as differentiated hyaline (holotype) or pigmented (Rovno specimen Figs 6C: spf; 7C–E: spv) process or fold (visible only at some angles); apex of marginal vein with 3 hamuli.

Legs. Relatively short, stout; all coxae large, with polygonal reticulate sculpture (only visible under layer of air, Figs 4D, 5B); protibia with long, curved, bifurcate calcar, with strong, straight, socketed seta and three stout setae along anteroapical margin (Fig. 6A); strigil and basitarsal comb present (Fig. 6A); tarsi 5-segmented; mesotibia with thick mesotibial spur, about as long as mesobasitarsus (Figs 4G, 6B), and with row of pegs along anteroapical edge (Fig. 4G); ventral surface of mesobasitarsus and each next tarsomere with row of setae and irregular pattern of pegs along anteroventral edge (Fig. 6B); metatibia with two spurs.

Gaster. Sculpture of gaster not visible, apical margins of Mt2–Mt5 straight, parallel; Mt6 with a small arched elevation medially (Figs 8B–D, 9A: ae); Mt7 U-like between cerci, almost vertical in both specimens but, perhaps, is an artefact of preservation resulting from metasoma being abnormally inflated due to decomposition effects; Mt8, Mt9 fused as syntergum; syntergum also almost vertical except for abruptly reflexed extension apically (Figs 1A, 2C, 4C, 8A–D, 9A), setose and with two longer setae apicolaterally; cercus with long straight setae (Fig. 9A: cers), cerci only slightly

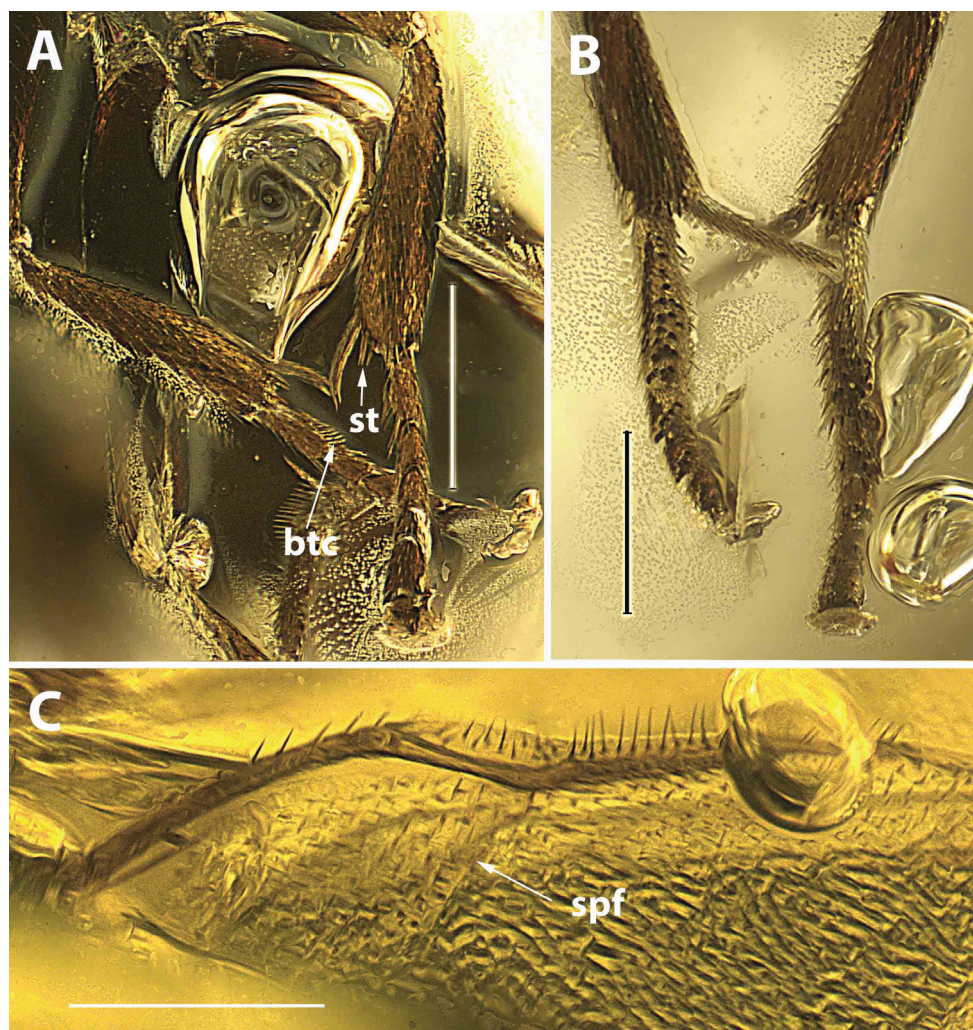


Figure 6. *S. glaesaria*, female, Rovno specimen **A** front legs, ventral **B** apices of mid legs **C** venation of hind wing. (btc – basitarsal comb, spf – spur fold, st – strigil). Scale bars: 0.2 mm.

advanced toward gastral base (Figs 2C, 8A–D), but gaster with elongate membranous band posterior to each cercus (Fig. 8A–C) differentiating dorsal surface of syntergum from slender lateral portion recurved around cercus; paratergites not visible; apex of hypopygium far from reaching apex of gaster (Figs 1C, 2C, 8A–C: hyp); ovipositor not extended beyond apex of ultimate tergum; only apical parts of ovipositor sheaths visible in ventrolateral and posterior views (Figs 8D, 9A: v3); membranous bands posterior to cerci subdivide the dorsal surface of syntergum from narrow, crescent-shaped in lateral view outer plates of ovipositor (Figs 3A, B, 8A, C, D, 9A: opo).

Male. Unknown.

Genus composition. Type species only.

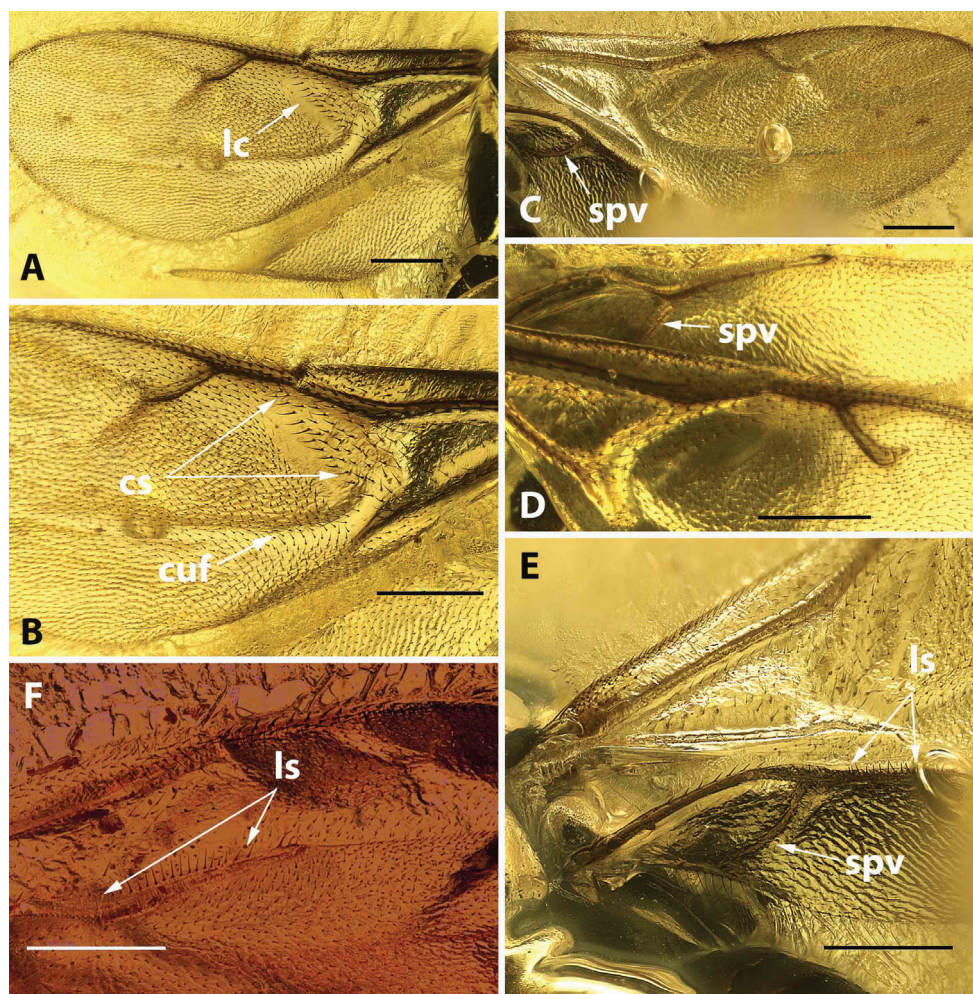


Figure 7. *S. glaesaria*, females, wings **A–E** Rovno specimen **F** holotype from Danish amber **A** left pair of wings dorsal **B** forewing venation, dorsal **C** left pair of wings, ventral **D** right pair of wings, venation, dorsal **E** left pair of wings, hind wing venation, ventral **F** wings venation, ventral. (cs – covering setae, cuf – cubital fold, lc – linea calva, ls – line of setae, spv – spur vein). Scale bars: 0.2 mm.

Biology. Unknown.

Comments. The **radicle** of many recent encyrtids is very long, four times or more as long as broad. This character state is included in lists of synapomorphies of extant Encyrtidae by Heraty et al. 2013, but there are many exceptions, among both extant Encyrtinae and Tetracneminae. The radicle of all known fossil Encyrtidae, including *S. glaesaria*, is also no more than twice as long as broad.

The **forewing venation** of *S. glaesaria* is typical for fossil Encyrtidae. In modern encyrtids, the most similar venation of the forewings occurs, e.g., in the genera *Savzdargia* Trjapitzin, 1979, *Ericydnus* Walker, 1837, *Mira* Schellenberg, 1803, and *Moraviella* Hoffer, 1954 (Tetracneminae). All veins are long, including the

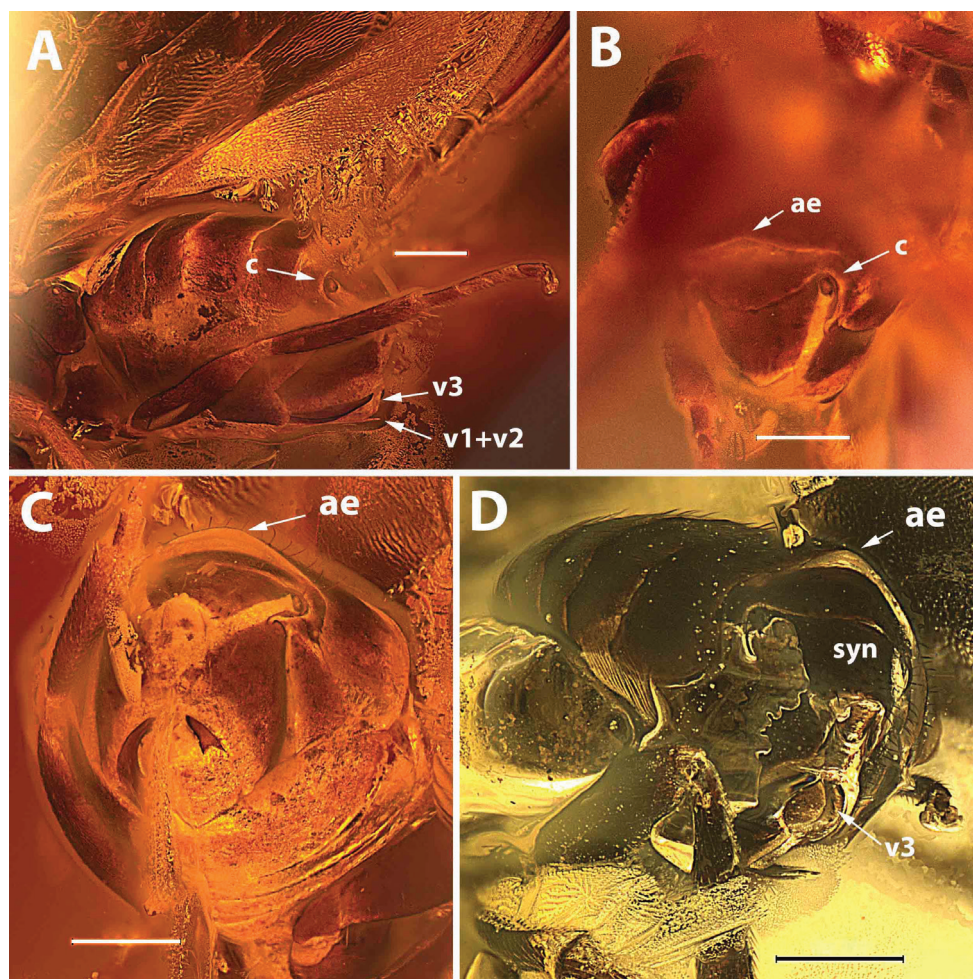


Figure 8. *S. glaesaria*, females, metasoma **A–C** holotype **D** Rovno specimen **A** lateral **B** dorsal **C** posteroventral **D** posterolateral. (ae – arch elevation of Mt6, c – cercus, syn – syntergum, v1+v2 – ovipositor stylet, v3 – ovipositor sheath). Scale bars: 0.2 mm.

marginal. The linea calva has a line of long setae along its basal margin (Fig. 7B: cs), **covering setae** sensu Sharkov (1985). When the wings are folded, the anterior margin of one abuts this row of setae. This structure is present in most extant Encyrtinae, some extant Tetracneminae, and in all known earliest, middle Eocene encyrtids. Late Eocene encyrtids have been reported both with and without covering setae (Simutnik 2020).

In the hind wing of *S. glaesaria*, the costal cell has a membrane with a single **line of long setae** along the entire marginal vein as in Tanaostigmatidae. A similar character state is rarely present in extant Encyrtidae, e.g., in *Exoristobia* Ashmead, 1904. The fossil *E. sugonjaevi* also has this line of setae, but it differs from *S. glaesaria* by the hypopygium extending past the apex of horizontal syntergum.

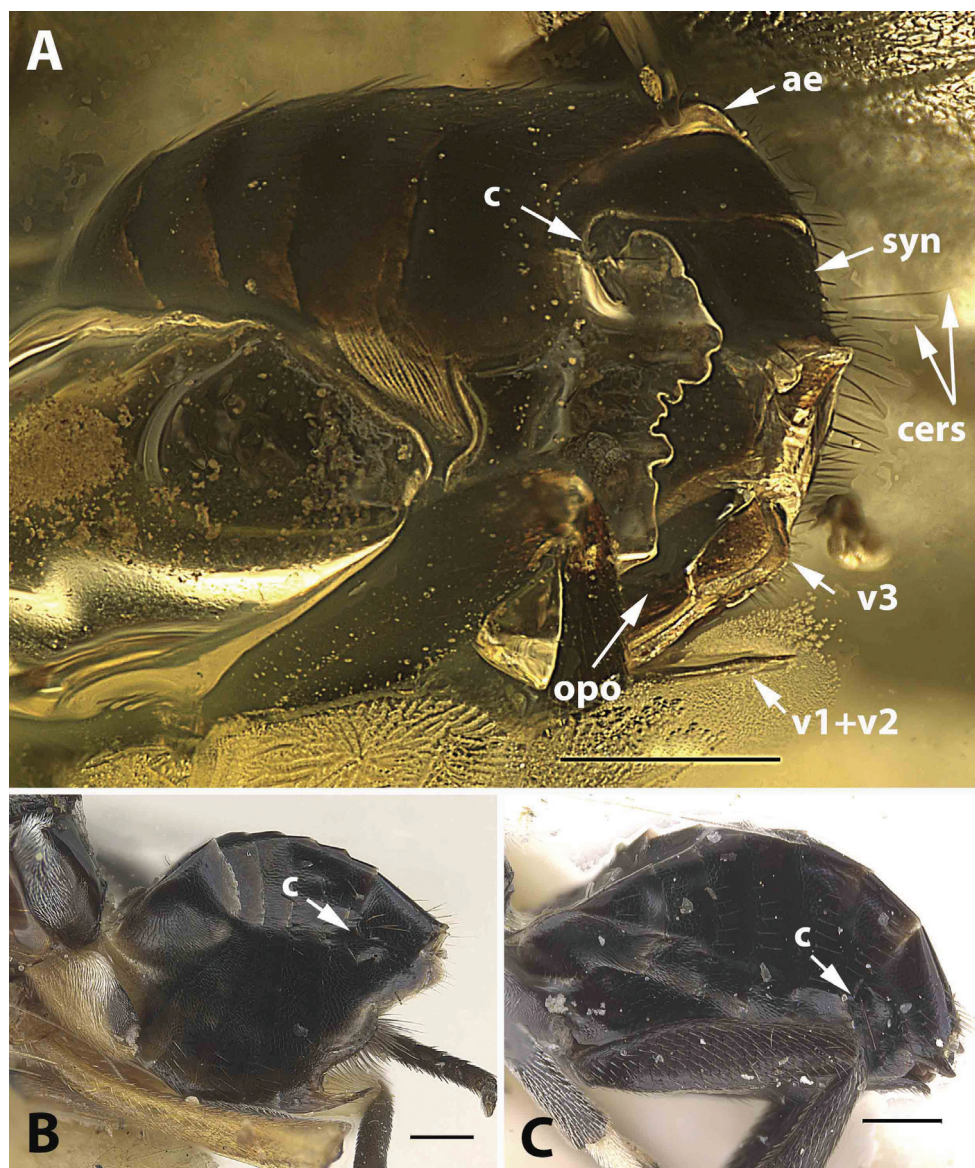


Figure 9. Metasoma, lateral **A** *S. glaesaria*, female, Rovno specimen **B, C** *Prionomastix morio* (Dalman, 1820) **B** female **C** male. (ae – arched elevation of Mt6, c – cercus, cers – cercal setae, syn – syntergum, v1+v2 – ovipositor stylet, v3 – ovipositor sheath). Scale bars: 0.2 mm.

A **spur vein** or fold originating from the marginal venation of the hind wing is visible in *S. glaesaria* (Fig. 7C–E: spv) similar to that found in Tanaostigmatidae, *Lep-toomus*, some Pteromalidae, and Eupelmidae (Simutnik et al. 2020a). In *S. glaesaria*, however, the spur vein is present rather as a fold, visible only at some angles.

The both specimens of *S. glaesaria* have almost **vertical syntergum** (Figs 2A, 9A). Perhaps, it is an artefact of preservation resulting from metasoma being abnormally

inflated due to decomposition effect. But, abruptly reflexed extension at the apex of syntergum (Figs 2A, 9A) indicates that it can actually be somewhat vertical in living specimens. The almost vertical syntergum is very rare in extant Encyrtidae. A similar structure is known, e.g., in *Prionomastix* Mayr, 1876 (Encyrtinae) (Fig. 9B, C), and *S. glaesaria* shares this character with many of members of Tanaostigmatidae.

The taxonomic position of the *Sulia* is retain unplaced within the Encyrtidae. Its biology is also unknown. Nevertheless, a large size of *S. glaesaria*, its barrel-shaped habitus, short and high acropleuron, structure of protibial apex, presence of spur vein and line of long setae along the entire marginal vein of hind wings, and supposedly almost vertical syntergum somewhat resemble those of members of Tanaostigmatidae and may indicate convergent adaptation to the plant galling.

Acknowledgements

We are grateful to Gary A.P. Gibson (Canadian National Collection of Insects and Arachnids) and James Woolley (Texas A&M University) for discussion and valued comments; Lars Vilhelmsen (Zoological Museum of University of Copenhagen) kindly arranged a loan of specimens; Bruce Archibald (Simon Fraser University, Burnaby, Canada) for valued comments and editing the English. The authors are thankful to the editor Petr Janšta.

This work was supported by grant NRFU No. 2020/02/0369 (to S.A. Simutnik).

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Two new species of *Cymodusa* Holmgren (Hymenoptera, Ichneumonidae) with a key to species known from China and Oriental region

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Academic editor: Gavin Broad | Received 16 September 2021 | Accepted 18 October 2021 | Published 30 December 2021

<http://zoobank.org/C7C29787-CD57-43B0-BC1D-02DF5B13B12D>

Citation: Li T, Chang G-B, Yang Z-H, Sun S-P, Tian Y, Sheng M-L (2021) Two new species of *Cymodusa* Holmgren (Hymenoptera, Ichneumonidae) with a key to species known from China and Oriental region. Journal of Hymenoptera Research 88: 103–114. <https://doi.org/10.3897/jhr.88.75304>

Abstract

Two new species of genus *Cymodusa* Holmgren, 1859, *C. culaica* Sheng, Li & Sun, **sp.nov.** collected from Culaishan Natural Reserve, Shandong province and *C. melana* Sheng, Li & Sun, **sp.nov.** collected from Guiyang and Fanjingshan National Natural Reserve, Guizhou province, are described and illustrated. A taxonomic key to the species of *Cymodusa* from China and the Oriental region is provided.

Keywords

Campopleginae, new species, Key, taxonomy

Introduction

Cymodusa Holmgren, 1859 (Hymenoptera, Ichneumonidae, Campopleginae) comprises 43 species (Yu et al. 2016; Watanabe 2020), of which seven are from the Oriental region (two of them occur in the Eastern Palearctic region, one also occurs in the Western Palearctic) (Holmgren 1859; Cameron 1905; Uchida 1956; Gupta & Gupta

1974), 18 from the Eastern Palaearctic region (seven occur in the Western Palaearctic region, one also occurs in the Western Palaearctic and Nearctic regions) (Uchida 1956; Aubert 1974; Sawoniewicz 1978; Dbar 1984, 1985; Choi et al. 2013; Yu et al. 2016; Watanabe 2020), 18 from the Western Palaearctic region (Dbar 1984; 1985, Kolarov & Yurtcan 2008; Yu et al. 2016), ten from the Nearctic (Sanborne 1986, 1990) and two from the Neotropical region, which also occur in the Nearctic (Sanborne 1986). Prior to the present study, two species, *C. josephi* Gupta & Gupta, 1974 and *C. orientalis* Uchida, 1956, have been recorded from China (Gupta & Gupta 1974; Dbar 1985).

The Oriental species of *Cymodusa* Holmgren and a key to the known species from this region were reported by Gupta & Gupta (1974). The Palaearctic species of *Cymodusa* were revised by Dbar (1984, 1985). A key to species from the Russian Far East was provided by Khalaim & Kasparyan (2007). The Korean species were reported by Choi et al. (2013). Watanabe (2020) revised the Japanese species of *Cymodusa*, and synonymized *C. aenigma* Dbar, 1985 with *C. orientalis* Uchida, 1956.

Ten host species of *Cymodusa*, mainly belonging to families Crambidae, Gelechiidae, Geometridae, Erebiidae, Pyralidae, Yponomeutidae, have been recorded (Meyer 1935; Talhouk 1961; Pisica & Petcu 1972; Dbar 1984; Kusigemati 1976; Heckford & Sterling 2005; Lozan et al. 2012).

In this paper two new species of *Cymodusa* and a key to known species from China and the Oriental region are reported.

Material and methods

Specimens were collected by interception traps (IT) (Li et al. 2012) in Culaishan Natural Reserve, Tai'an, Shandong Province; Panlongshan, Wudang, Guiyang and Fanjingshan National Natural Reserve, Guizhou Province, China.

Images were taken using a Leica M205A stereomicroscope with LAS Montage MultiFocus. Morphological terminology is based on Broad et al. (2018). All specimens are deposited in the Insect Museum, General Station of Forest and Grassland Pest Management, National Forestry and Grassland Administration, P. R. China.

Taxonomy

Cymodusa Holmgren, 1859

Cymodusa Holmgren, 1859:327. Type-species: *Cymodusa leucocera* Holmgren.

Diagnosis. Eyes with setae. Inner margins of eyes weakly indented opposite antennal socket. Face strongly convergent ventrally (especially in female). Mandible short, lower edge with a narrow flange, or as a high carina; upper tooth usually as long as lower

tooth. Malar space very short, or absolutely wanting in female. Posterior transverse carina of mesosternum complete. Propodeal spiracle circular. Glymma absent. Ovipositor stout, compressed, almost straight.

Key to the species known from China and the Oriental region

- 1 Hind wing nervellus not intercepted, discoidella absent **2**
- Hind wing nervellus intercepted, discoidella present, usually unpigmented **4**
- 2 Malar space $0.25\text{--}0.26\times$ basal width of mandible. Anterior tentorial pit distinctly distant from eyes. Metasomal tergites entirely black. Proximal flagellomeres white ***C. antennator* Holmgren, 1860**
- Malar space at most $0.15\times$ basal width of mandible. Anterior tentorial pit almost obscured. At least part of metasomal tergites red to brownish red. Flagellomeres almost entirely black **3**
- 3 Area basalis (Fig. 7) elongate, at least $2.5\times$ as long as anterior width. Antenna with 24–25 flagellomeres. Hind leg except tarsus yellowish to reddish brown ***C. culaiica* Sheng, Li & Sun, sp.nov.**
- Area basalis relatively short, at most $2.0\times$ as long as anterior width. Antenna with 29–34 flagellomeres. Hind leg mostly brownish black ***C. orientalis* Uchida, 1956**
- 4 Area basalis, area superomedia and area petiolaris of propodeum completely confluent. Areolet receiving 2m-cu distinctly basal of its middle. Fourth tergite entirely red. Tegula yellowish white. Hind coxa black ***C. dravida* Gupta & Gupta, 1974**
- At least area basalis distinctly separated from area superomedia by transverse carina. Areolet receiving 2m-cu almost at its middle. Fourth tergite entirely or partly black. Tegula and hind coxa with different coloration **5**
- 5 Area basalis of propodeum with lateral carinae parallel, $3.0\times$ as long as wide. Area externa finely rugose. Hind coxa and all metasomal tergites black ***C. taprobanicum* (Cameron, 1905)**
- Area basalis of propodeum at most $2.0\times$ as long as wide, more or less convergent. Area externa with different sculpture. Hind coxa or metasomal tergites (except *C. melana*) not entirely black **6**
- 6 Malar space $0.25\text{--}0.35\times$ basal width of mandible. Postocellar line $0.9\text{--}1.0\times$ as long as ocular-ocellar line. Hind basitarsus $2\times$ as long as hind tibial spur. Hind coxa and femur black ***C. shiva* Gupta & Gupta, 1974**
- Malar space wanting, or at most $0.15\times$ basal width of mandible. Postocellar line at least $1.2\times$ as long as ocular-ocellar line. Hind basitarsus at least $2.3\times$ as long as hind tibial spur. Hind coxa and femur (except *C. melana*) not entirely black **7**
- 7 Tergite 2 granulose in anterior portion, posterior portion and subsequent tergites subpolished. Tergites 2–4 with posterior transverse red bands ***C. santoshae* Gupta & Gupta, 1974**
- Tergites 2 and 3 granulose. Metasomal tergites entirely or almost entirely black **8**

- 8 Dorsal median portion of occipital carina evenly arched. Lateral carinae of area basalis convergent posteriorly, 2× as long as wide. Tergite 2 as long as tergite 1. 1cu-a opposite M&RS. Claw pectinate subbasally. Posterior margin of tergite 2 red *C. josephi* Gupta & Gupta, 1974
- Dorsal median portion of occipital carina (Fig. 14) angled. Lateral carinae of area basalis slightly divergent posteriorly, 1.2–1.3× as long as wide. Second tergite shorter than first. 1cu-a basal of M&RS. Claw almost simple. All tergites entirely black. *C. melana* Sheng, Li & Sun, sp.nov.

***Cymodusa culaiica* Sheng, Li & Sun, sp.nov.**

<http://zoobank.org/79D8F06D-76CC-42CF-A4ED-51676D39CD21>

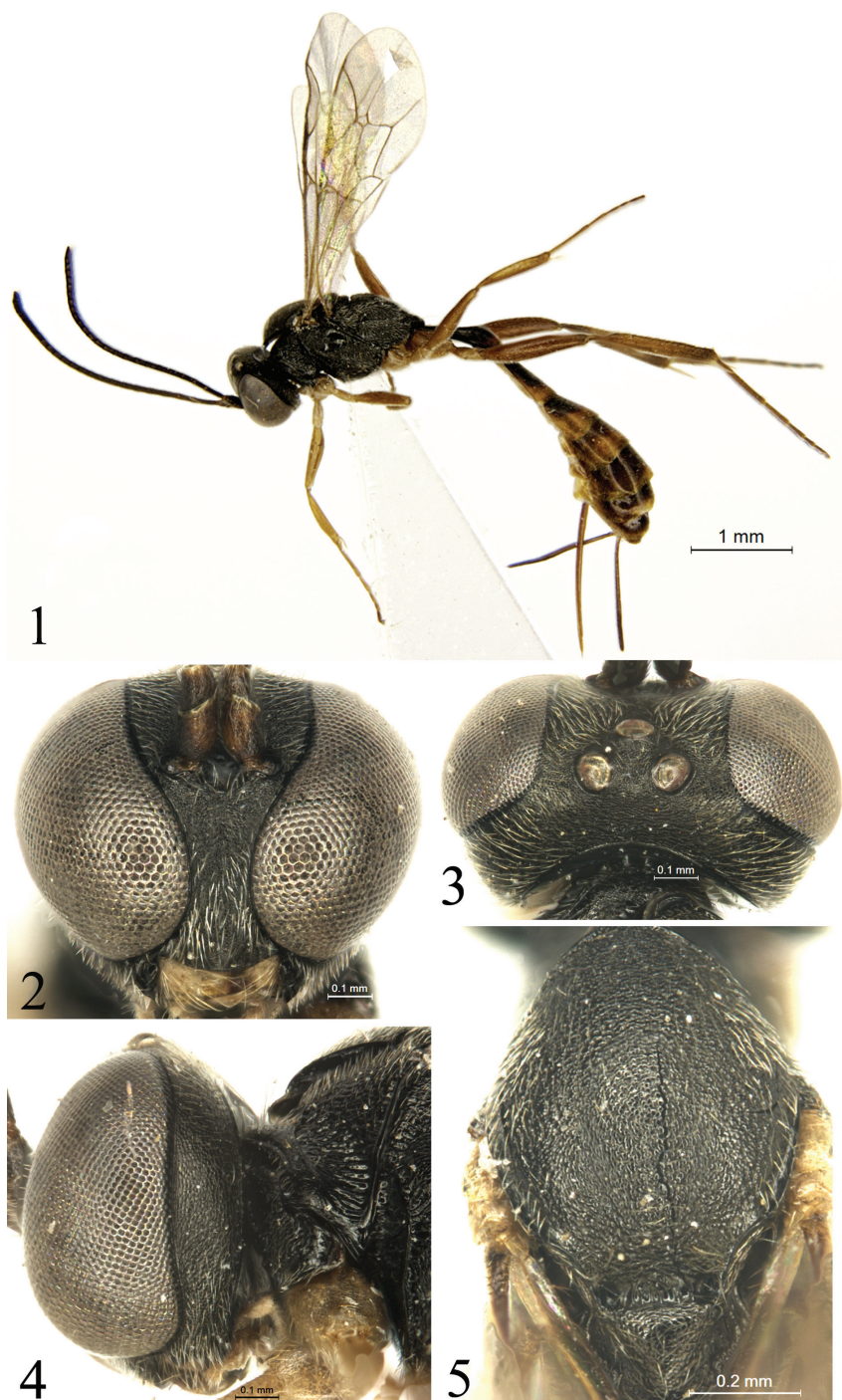
Figures 1–9

Diagnosis. Head, mesosoma and all tergites coriaceous. Anterior tentorial pit obscure, against eye. Areolet sessile (Fig. 1). Nervellus not intercepted. Area basalis of propodeum (Fig. 7) elongate, 2.5–2.7× as long as anterior width, 3.6× as long as posterior width; lateromedian longitudinal carinae from base of area superomedia evenly strongly divergent posteriorly. Legs almost entirely yellowish brown. Posterior 0.5–0.8 of tergites 3 and 4 reddish brown.

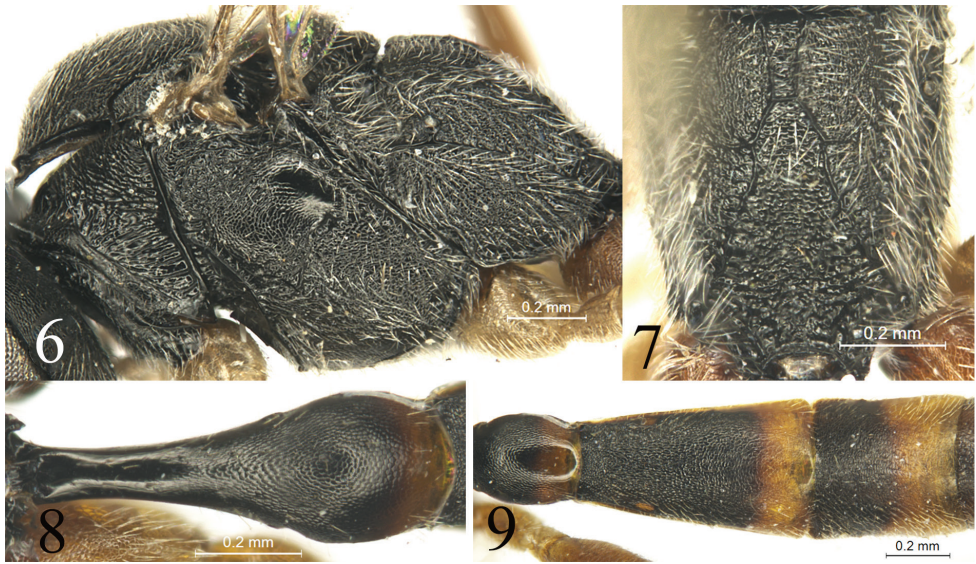
Description. Body length 4.0–5.0 mm. Fore wing length 2.2–2.5 mm. Ovipositor sheath 0.8–1.0 mm.

Head. Head with coriaceous surface. Eye (Figs 2–4) large. Median portion of inner margins of eyes (Fig. 2) slightly concave near antennal sockets. Eye with weak, short setae. Face and clypeus with dense gray white setae. Face strongly convergent ventrally, maximum width beneath antennal socket approximately 2.5× as long as minimum width; sublateral portion of upper margin, beneath antennal socket, distinctly raised. Clypeus slightly convex medially, apical margin slightly convex forward. Anterior tentorial pit small, obscure, against eye. Mandible with fine indistinct punctures; lower tooth as long as upper tooth. Malar space very narrow, almost absent. Gena, vertex and frons (Figs 3, 4) with dense gray setae. Gena strongly convergent backward. Posterior portion of vertex behind hind margin of stemmaticum steeply oblique. Stemmaticum raised. Postocellar line 1.1–1.2× as long as ocular-ocellar line. Frons almost flat, lower portion with fine indistinct wrinkles. Antenna with 24–25 flagellomeres; ratio of length from first to fifth flagellomeres: 1.1:0.7:0.7:0.7:0.6. Occipital carina (Fig. 3) complete, dorsal portion evenly arched.

Mesosoma. Lateral concavity of pronotum (Figs 4, 6) with dense strong oblique wrinkles; dorsoposterior portion rough, with short indistinct transverse wrinkles. Epomia present. Mesoscutum (Fig. 5) evenly convex. Notaulus indistinct. Scuto-scutellar groove with distinct longitudinal carinae. Scutellum slightly convex, with dense thin setae. Postscutellum strongly narrowed anteriorly, anterolaterally with small pit. Lower half of mesopleuron (Fig. 6) slightly convex, with short indistinct wrinkles; speculum slightly convex, relatively small, smooth, shiny. Epicnemial carina strong, upper end al-



Figures 1–5. *Cymodusa culaica* Sheng, Li & Sun, sp. nov. Holotype. Female **1** habitus, lateral view **2** head, anterior view **3** head, dorsal view **4** head and pronotum, lateral view **5** mesoscutum and scutellum, dorsal view.



Figures 6–9. *Cymodusa culaiica* Sheng, Li & Sun, sp. nov. Holotype. Female **6** mesosoma, lateral view **7** propodeum, dorsal view **8** first tergite, dorsal view **9** postpetiole and terga 2 and 3, dorsal view.

most reaching front edge of mesopleuron, about 0.6 distance to subtegular ridge. Metapleuron evenly convex; juxtacoxal carina absent; submetapleural carina complete. Hind femur $5.0\times$ as long as its maximum width. Ratio of length of hind tarsomeres from first to fifth: $3.2:1.5:1.0:0.6:0.7$. First tarsomere $2.2\times$ as long as length of longer spur. Claw small, weakly pectinate. Wings slightly gray, hyaline. Fore wing with vein 1cu-a almost opposite M&RS. Areolet sessile, receiving vein 2m-cu approximately $0.45\times$ distance from vein 2rs-m to 3rs-m, 2rs-m slightly longer than 3rs-m; postnervulus intercepted almost at middle. Hind wing with basal portion of M+CU distinctly arched; nervellus not intercepted, almost vertical. Propodeum (Fig. 7) evenly convex, apical portion evenly oblique; area basalis separated from area superomedia by distinct carina, slightly convergent posteriorly, $2.5\text{--}2.7\times$ as long as anterior width, $3.6\times$ as long as posterior width; lateromedian longitudinal carinae from base of area superomedia evenly strongly divergent posteriorly; areas superomedia and petiolaris completely confluent, with short, indistinct transverse wrinkles; costula present. Propodeal spiracle small, circular.

Metasoma. First tergite (Fig. 8) $2.6\text{--}2.8\times$ as long as apical width, basal half smooth, shiny, apical half shagreened; postpetiole distinctly convex, subapical portion widened; spiracle small, located approximately at apical 0.3. Tergites 2–3 (Fig. 9) slightly rough, posterior portions with indistinct fine punctures. Second tergite elongate, slightly widened posteriorly, $1.7\text{--}1.8\times$ as long as posterior width. Third tergite with parallel sides, $1.0\text{--}1.1\times$ as long as posterior width. Tergites 4 to 5 widened posteriorly. Ovipositor sheath almost ($0.98\times$) as long as hind tibia. Ovipositor compressed, with sharp dorsal notch.

Coloration (Fig. 1). Black, except for the following: ventral profile of base of antenna brownish. Mandible except teeth, maxillary palpi, labial palpi and tegula yellow.

Legs yellowish brown, except fore and middle coxae and ventral profiles of trochanters brownish yellow; apical portion of hind tibia slightly black brown. Pterostigma and veins blackish brown. Metasomal tergites brownish black, posterior portions of tergites 1, 2, 6 and 7, posterior 0.5–0.8 of tergites 3 to 5 reddish brown.

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

Material examined. *Holotype*: China • ♀; Shandong, Chashankou, Culaishan Natural Reserve, Tai'an; 2.VI.2018; IT by Tao Zhao. *Paratypes*: China • 13 ♀♀; same data as for holotype except 26.V.–9.VI.2018.

Distribution. China.

Differential diagnosis. The new species is similar to *C. orientalis* Uchida, 1956, but can be distinguished easily from the preceding key.

***Cymodusa melana* Sheng, Li & Sun, sp.nov.**

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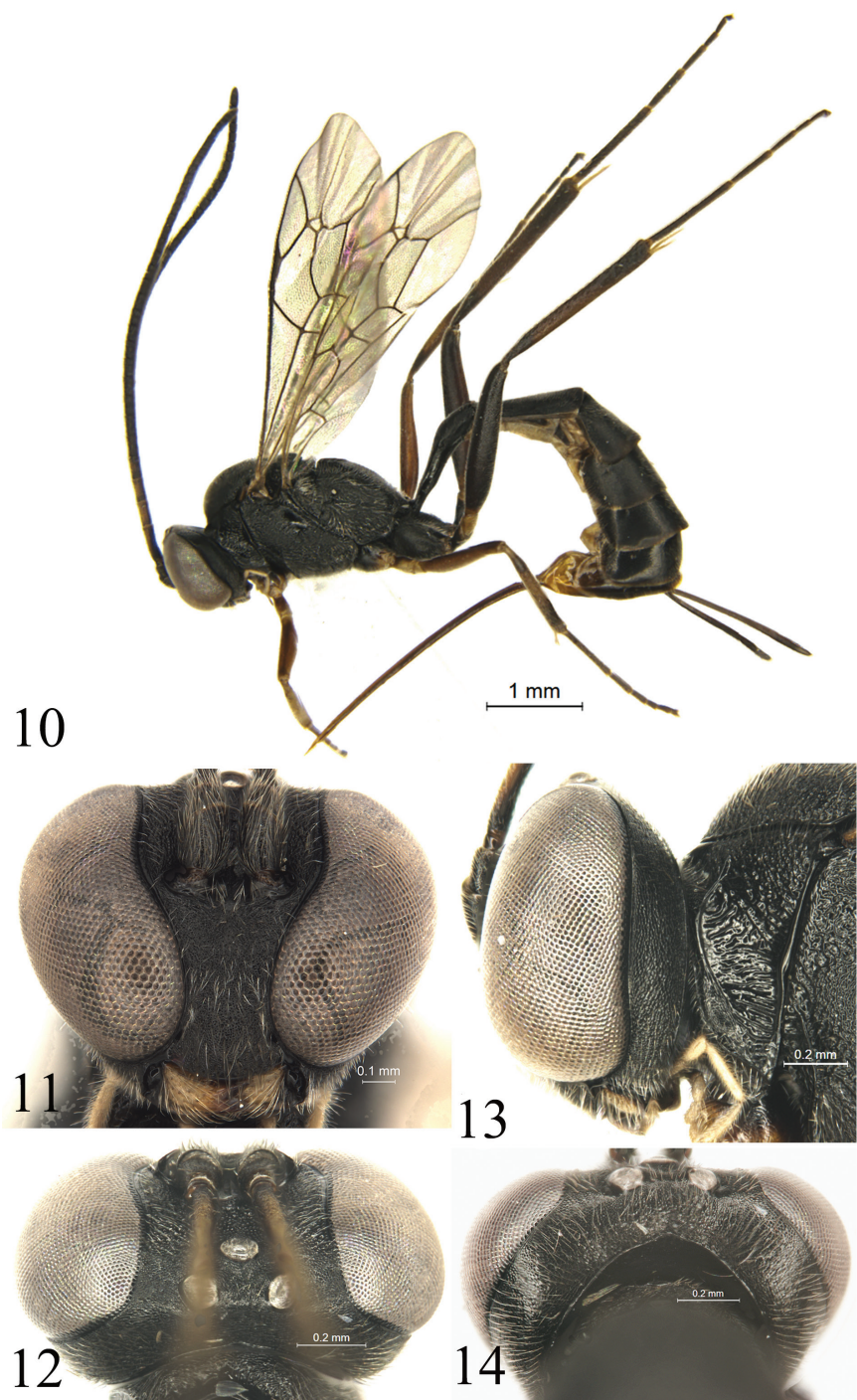
Figures 10–20

Diagnosis. Dorsal median portion of occipital carina (Fig. 14) angled. Anterior tentorial pit distinct, against eye. Postocellar line approximately 1.2× as long as ocular-ocellar line. Lateral carinae of area basalis almost parallel, 1.2–1.3× as long as wide. Lateromedian longitudinal carinae distinctly angled in level of posterior transverse carina. Tergites 2 (Fig. 19) elongate, 1.7–1.8× as long as posterior width. Posteromedian portions of tergites 6–7 (Fig. 20) distinctly concave. Head, mesosoma, all metasomal tergites and hind leg almost entirely black.

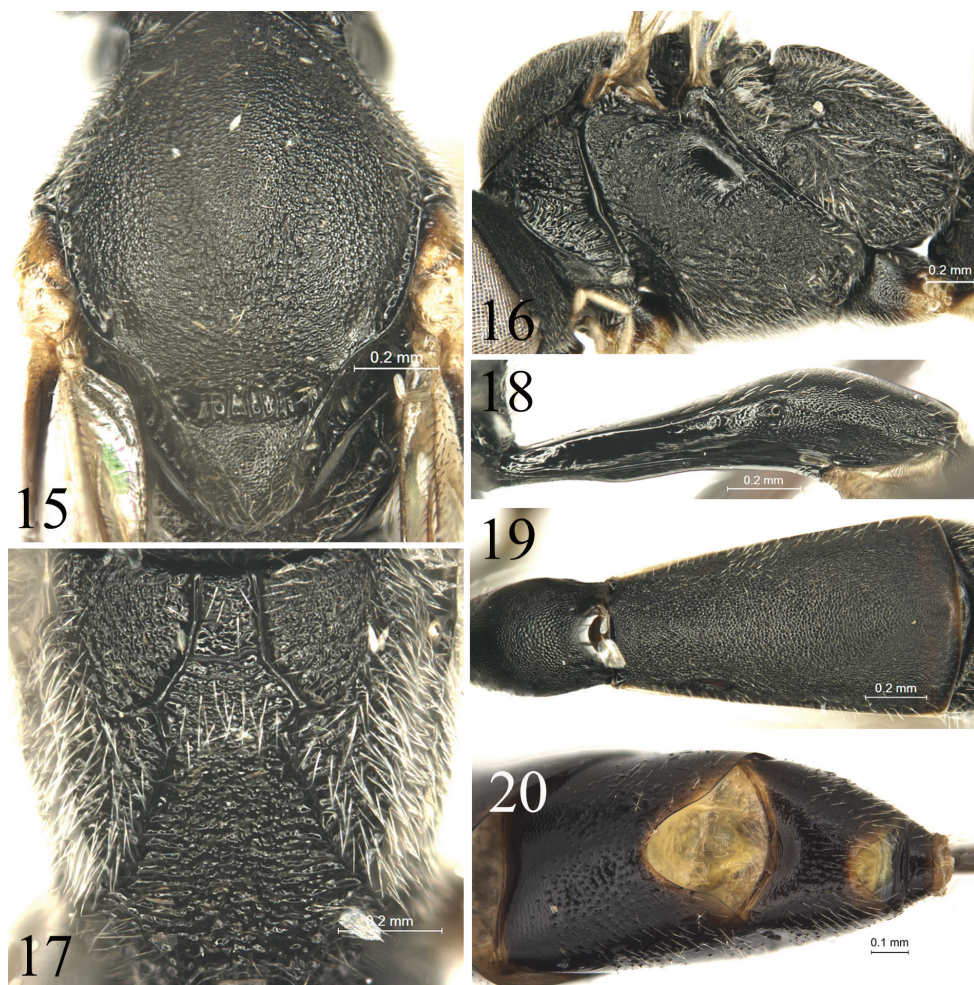
Description. Body length 7.0–7.5 mm. Fore wing length 3.5–4.0 mm. Ovipositor sheath 1.2–1.5 mm.

Head. Eye with weak, short setae, inner margins (Fig. 11) distinctly concave near antennal sockets. Face and clypeus roughly shagreened. Face strongly convergent ventrally, maximum width beneath antennal socket approximately 1.8× as long as minimum width, dorsal margin concave medially. Clypeus with dense fine punctures, evenly convex, apical margin evenly convex forward. Anterior tentorial pit small, distinct, against eye. Mandible with dense yellowish white setae and sparse fine punctures; lower tooth almost as long as upper tooth. Malar space about 0.1× as long as basal width of mandible. Gena (Figs 12, 13, 14) shagreened, lower portion with dense delicate yellowish white setae, upper portion strongly convergent backward. Vertex finely shagreened, posterior portion behind hind margin of stemmaticum steeply oblique. Postocellar line approximately 1.2× as long as ocular-ocellar line. Frons almost flat, shagreened, median portion with fine indistinct transverse wrinkles. Antenna with 32 flagellomeres; ratio of length from first to fifth flagellomeres: 2.0:1.3:1.2:1.2:1.1. Occipital carina (Fig. 14) complete, dorsal median portion angulated.

Mesosoma. Lateral concavity of pronotum (Figs 13, 16) with dense strong oblique wrinkles; dorsoposterior portion roughly shagreened. Epomia long and strong. Mesoscutum (Fig. 15) evenly convex, with leathery texture, lateral portion



Figures 10–14. *Cymodusa melana* Sheng, Li & Sun, sp.nov. Holotype. Female **10** habitus, lateral view **11** head, anterior view **12** head, dorsal view **13** head and pronotum, lateral view **14** head, dorsoposterior view.



Figures 15–20. *Cymodusa melana* Sheng, Li & Sun, sp. nov. Holotype. Female **15** mesoscutum and scutellum, dorsal view **16** mesosoma, lateral view **17** propodeum, dorsal view **18** first tergite, lateral view **19** postpetiole and tergite 2, dorsal view **20** (Paratype) Posterior portion of metasoma, dorsal view.

with fine distinct punctures, distance between punctures 0.2–1.5× as long as one diameter of puncture; posterior portion with short indistinct transverse wrinkles. Notaulus indistinct. Scuto-scutellar groove with 6–7 distinct longitudinal carinae. Scutellum finely shagreened, basal 0.25 with lateral carina. Postscutellum transversely convex, anterior portion depressed transversely, anterolateral with small pit. Lower half of mesopleuron (Fig. 16) slightly convex; median portion in front of speculum with distinct oblique wrinkles; beneath subregular ridge with short indistinct transverse wrinkles. Speculum almost smooth, shining. Epicnemial carina strong, upper end almost reaching front edge of mesopleuron, about 0.7 distance to subregular ridge. Metapleuron evenly convex, with dense grayish white setae;

juxtacoxal carina absent; submetapleural carina complete. Hind femur 5.7–6.0× as long as its maximum width. Ratio of length of hind tarsomeres from first to fifth: 5.5:2.7:1.7:1.0:1.0. First tarsomere 2.4× as long as length of longer spur. Claw small, pectinate basally. Wings slightly brownish, hyaline. Fore wing with vein 1cu-a slightly basal of M&RS. Areolet sessile, receiving vein 2m-cu approximately at middle, 2rs-m approximately as long as 3rs-m; postnervulus intercepted at middle. Hind wing with basal portion of M+CU distinctly arched; nervellus almost vertical, intercepted at lower 0.25; final abscissa of CU unpigmented. Propodeum (Fig. 17) roughly shagreened, lateral portion with dense gray setae; lateromedian longitudinal carinae strong, distinctly angled in level of posterior transverse carina; area basalis separated from area superomedia by weak carina, lateral sides slightly convergent anteriorly or parallel, 1.6–1.7× as long as anterior width, 1.2–1.3 × as long as posterior width; areas superomedia slightly wider than long; areas superomedia and petiolaris completely confluent, with short, indistinct transverse wrinkles; costula present. Propodeal spiracle small, circular.

Metasoma. First tergite (Fig. 18) 3.2× as long as apical width; petiole smooth, shiny, lateral side slightly longitudinally concave; postpetiole (Fig. 19) distinctly convex, shagreened; spiracle small, weakly convex, located approximately at apical 0.35. Tergites 2 (Fig. 19) with texture as postpetiole, elongate, slightly evenly widened posteriorly, 1.7–1.8× as long as posterior width. Tergite 3 with parallel sides, 1.3× as long as posterior width. Fourth and subsequent tergites compressed. Posteromedian portions of tergites 6–7 (Fig. 20) distinctly concave. Ovipositor sheath 1.2× as long as hind tibia. Apical portion of ovipositor weakly compressed, with sharp dorsal notch.

Coloration (Fig. 10). Black, except for the following: ventral profile of base of antenna reddish brown. Mandible except teeth, maxillary palpi, labial palpi yellow. Tegula, fore and middle legs reddish brown, except coxae black and ventral profiles of trochanters darkish brown. Hind trochantellus yellow. Base of hind femur reddish brown. Pterostigma and veins blackish brown.

Etymology. The specific name is derived from the body and hind leg almost entirely black.

Material examined. Holotype: China • ♀; Guizhou, Lengjiaba, 840 m, Fanjingshan National Natural Reserve, Jiangkou; 24.VI.2019; IT by Zhen-Hai Yang. **Paratypes:** China • 1♀; same data as for holotype. • 1♀; same data as for holotype except 23.IX.2019. China • 2♀♀; Guizhou, Panlongshan, 1179 m, Wudang, Guiyang; 24.VI.2019; IT by Zai-Hua Yang.

Distribution. China.

Differential diagnosis. The new species is similar to *C. taprobanica* (Cameron, 1905), but can be distinguished from the latter by the following combination of characters: areolet sessile; area basalis of propodeum at most 1.7× as long as maximum width; area superomedia wider than length; hind leg almost entirely black. *Cymodusa taprobanica* (Cameron): areolet petiolate; area basalis of propodeum about 3.0× as long as wide; area superomedia longer than width; hind leg partly black.

Discussion

According to Watanabe's report (Watanabe, 2020), the area basalis of the propodeum of *Cymodusa orientalis* Uchida, 1956 shows strong variation: slightly to strongly convergent posteriorly, even triangular (also see Dbar, 1985). The original description of *C. josephi* stated that the hind wing nervellus is intercepted; in *C. orientalis* the hind wing nervellus is not intercepted; and the character of area basalis of propodeum is within the range of *C. orientalis*. The specimens in our collections, collected from Guangxi, Guizhou and Jiangxi, located at the north border of Oriental region, and Shandong and Sichuan, located at the south border of the Eastern Palaearctic region, almost entirely with same characters, except some of them have the nervellus obscurely intercepted, the remainder with the nervellus not intercepted. For a decision, the types of *C. josephi* Gupta & Gupta, 1974, *C. orientalis* Uchida, 1956, *C. aenigma* Dbar, 1985 and more similar material should be studied in the future, or more accurate assessments will be helped by molecular methods.

Acknowledgements

The authors are deeply grateful to Drs Gavin Broad (Department of Life Sciences, the Natural History Museum, London, UK), Kyohei Watanabe (Kanagawa Prefectural Museum of Natural History, Kanagawa, Japan) and Matthias Riedel (Zoologische Staatssammlung München, München, Germany) for reviewing this manuscript. The authors also thank Dr. Kyohei Watanabe for sending some type photos of *Cymodusa orientalis* Uchida, 1956, and Yang Li, Tao Zhao (Culaishan Forest Farm, Tai'an, Shandong, China) and Zhen-Hai Yang (Fanjingshan National Natural Reserve, Jiangkou, Guizhou, China) for their help in the course of exploration in Shandong and Guizhou Provinces. This research was supported by National Animal Collection Resource Center, China and by the National Natural Science Foundation of China (NSFC, No. 31501887, No. 31110103062).

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First record in Africa of the parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) on tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) from tomato fields in Algeria

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Academic editor: Elijah Talamas | Received 14 September 2021 | Accepted 19 October 2021 | Published 30 December 2021

<http://zoobank.org/17C95222-AF04-49B0-A979-E18BE0EB4891>

Citation: Krache F, Boualem M, Fernandez-Triana J, Bass A, Arnó J, Benourad F (2021) First record in Africa of the parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) on tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) from tomato fields in Algeria. Journal of Hymenoptera Research 88: 115–131. <https://doi.org/10.3897/jhr.88.75279>

Abstract

The Neotropical parasitoid wasp *Dolichogenidea gelechiidivoris* (Marsh, 1975) (Hymenoptera: Braconidae), one of the most important biocontrol agents of the South American tomato pinworm *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is reported for the first time from Africa, from tomato grown in open fields and greenhouses in several regions of Algeria. Color photos of specimens from Algeria, Spain and South America, as well as the holotype and one paratype are provided. Morphological and molecular details to better characterize and recognize the species are also provided. We speculate that *D. gelechiidivoris* arrived accidentally to Algeria from Spain, where it has recently been reported. The consequences for future biocontrol projects against *T. absoluta* in Africa are discussed.

Keywords

Tuta absoluta, Afrotropical region, Microgastrinae, tomato, biological control, species diagnosis, DNA barcoding, accidental introduction

Introduction

The past decade has seen Africa invaded by the tomato pinworm *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), an important pest native to South America that causes severe crop losses when not controlled. The first reported record of the pest out of its continent of origin was from Spain in 2006 (Urbaneja et al. 2007). To date, the pest insect has spread to almost the entire African continent at lightning speed (Biondi et al. 2018; Tarusikirwa et al. 2020), with devastating effects, notably, on small-scale tomato farming (Mansour et al. 2018). The first record of *T. absoluta* in Algeria was in March 2008, on tomatoes in open fields in Achaacha, Mostaganem (EPPO 2008; Guenaoui 2008). Since then, it quickly spread to all tomato-producing areas in the country. The larvae of *T. absoluta* can damage between 80% and 100% of tomato in open fields (Desneux et al. 2010). Larvae are hidden inside leaves, stems and fruits, where they feed, and are therefore protected from most insecticides (Cocco et al. 2015), resulting in repeated pesticide treatments and the development of pest resistance (Roditakis et al. 2018). Thus, integrated pest management (IPM) strategies have been developed, including augmentation and conservation biological control (Biondi et al. 2018; Mansour et al. 2018).

Biological control is often considered one of the most economical and environmentally sustainable means of managing native as well as exotic pests and crop diseases (van Lenteren et al. 2018). When compared to other biological control agents (i.e., predators and entomopathogenic organisms), insect parasitoids commonly show greater host search efficiency and host specificity, traits that are considered important in preventing non-target host attacks (e.g., Daane et al. 2016; Hardwick et al. 2016; Kenis et al. 2017; Cascone et al. 2018; Thancharoen et al. 2018; Salas Gervassio et al. 2019). Thus, biological control against *T. absoluta* using insect parasitoids is a potentially successful control tool (Han et al. 2019).

In Africa (Nigeria, Tunisia, Algeria and Morocco), the parasitoid complex of *T. absoluta* that has so far been recorded through field monitoring and surveys include the larval parasitoids *Dolichogenideia appellator* (Telenga, 1949) and several species in the genera *Apanteles* Foerster and *Bracon* Fabricius (Hymenoptera: Braconidae) as well as egg parasitoids in the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) (Oke et al. 2016; Biondi et al. 2018; Mansour et al. 2018). In Algeria, the Mostaganem area showed the presence of seven parasitoids species: *Necremnus tutae* Ribes & Bernardo, 2015 (Hymenoptera: Eulophidae), which was the most abundant species, *Hyposoter didymator* (Thunberg, 1822) (Hymenoptera: Ichneumonidae), *Neochrysocharis* sp., *Sympiesis* sp., *Diglyphus isaea* (Walker, 1838) (Hymenoptera: Eulophidae), *Bracon* sp. and *Trichogramma* sp. (Boualem et al. 2012; Gebiola et al. 2015).

Among Braconidae subfamilies, Microgastrinae is a critically important group of parasitoid wasps estimated to include 17,000–50,000 species worldwide (Rodriguez et al. 2013; Fernandez-Triana et al. 2020), exclusively parasitizing larvae of Lepidoptera (caterpillars of butterflies and moths) across nearly the full spectrum of host taxa and terrestrial habitats. Microgastrinae are one of the most important groups in the biological control of agricultural and forestry lepidopterous pests worldwide (Whitfield 1997).

The microgastrine species *Dolichogenidea gelechiidivoris* (Marsh, 1975), originating from the Neotropics, is a koinobiont solitary endoparasitoid of *T. absoluta* and a few other closely related species (Marsh 1975; Yu et al. 2016). *Dolichogenidea gelechiidivoris* was originally described from specimens collected in Colombia, Chile, and Peru (Marsh 1975). Specimens were released in the USA for the control of *Phthorimaea operculella* (Zeller, 1873) (Lepidoptera: Gelechiidae) (Marsh 1975) and the species has also been considered as a biocontrol agent of *Keiferia lycopersicella* (Walshingham, 1897) (Lepidoptera: Gelechiidae) (Marsh 1975; Palacios & Cisneros 1995; Mujica & Kroschel 2013). Recently, *D. gelechiidivoris* has been reported as parasitoid of *T. absoluta* in Spain (Denis et al. 2021), where the wasp was considered as unintentionally introduced from the Neotropics along with the lepidopteran pest.

The International Centre of Insect Physiology and Ecology (icipe) in Kenya, in collaboration with the International Potato Center (CIP) in Peru, also identified *D. gelechiidivoris* as an effective endemic natural enemy of *T. absoluta* in their native range (Peruvian central highlands) and imported specimens of the parasitoid wasp into Kenya from Peru in 2017 for studies of its feasibility as a candidate for classical biological control of *T. absoluta* in Africa (Aigbedion-Atalor et al. 2020). The results of these studies, under laboratory conditions, indicated the potential of *D. gelechiidivoris*. However, as far as we know, there has not been any intentional release of this parasitoid wasp in agricultural fields in Africa.

In this paper we present the first record of *D. gelechiidivoris* from Africa, recovered as a parasitoid of *T. absoluta* in tomato grown in open fields and greenhouses in Algeria. We also provide morphological and molecular information to better diagnose this parasitoid wasp species and discuss the significance of its finding for future biocontrol projects against *T. absoluta* in Africa.

Methods

In December 2020, one species of Microgastrinae was reared from larvae of *T. absoluta* collected in tomato fields of the municipality of Stidia, along the Bay of Arzew and 15 km SW from the city of Mostaganem. The locality is characterized by a mild Mediterranean climate. The same wasp species was later found in greenhouses growing tomatoes in other municipalities of Mostaganem province (Table 1 and Fig. 1), including both coastal (Hadjadj, Mazagran, Hassi Mamèche, Stidia) and interior regions (Ain Tâdles, Sidi Ali).

The identification of the parasitoid wasp species was made by one of the authors (JFT) in the Canadian National Collection of Insects (CNC), Ottawa, by comparison with vouchered South American specimens of *D. gelechiidivoris* housed in the CNC, which included one paratype and other authenticated specimens from South America. The holotype (deposited in the National Museum of Natural History, Washington DC, United States) and additional samples from Catalonia (Northeast of Spain) were also examined and photographed. The identified specimens from Algeria are deposited in the CNC, Canada and the University Abdelhamid Ibn Badis of Mostaganem, Algeria.

Table 1. Collecting sites of specimens of *Dolichogenidea gelechiidivoris* parasitizing *Tuta absoluta* in Algeria.

Site	Collection dates	Field characteristics
Stidia	16/12/2020; 01/05/2021	Open fields and greenhouses
Mazaghran	21/04/2021	Greenhouses
Hassi Mameche	26/04/2021	Greenhouses
Hadjadj	26/05/2021	Greenhouses
Ain Tadles	05/06/2021	Greenhouses
Sidi Ali	28/06/2021	Greenhouses

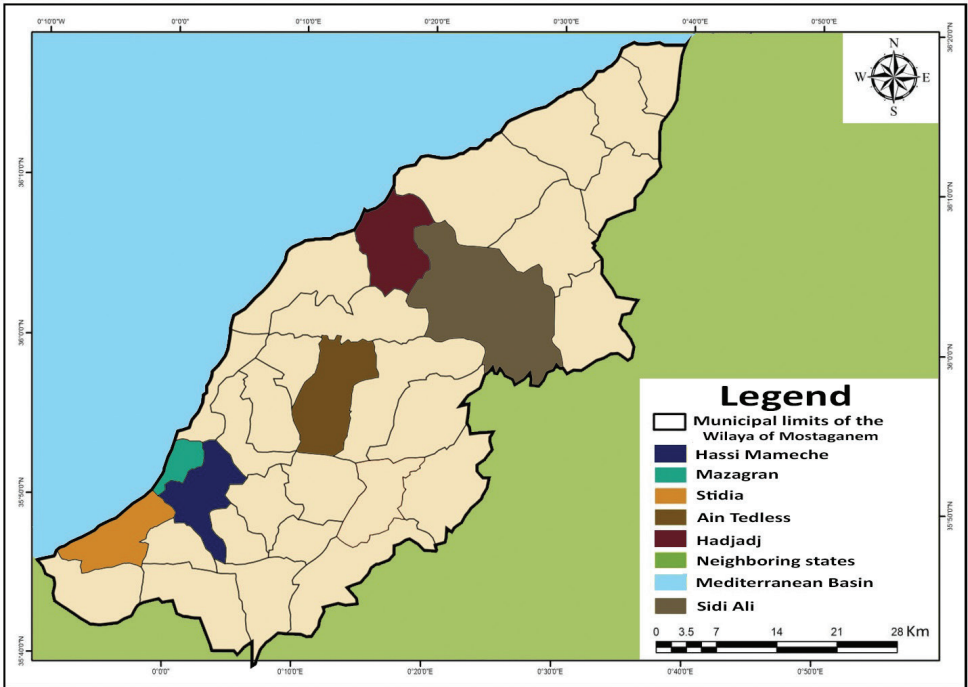


Figure 1. Map of the municipality of Stidia, along the Bay of Arzew and 15 km SW from Mostaganem, Algeria.

For morphological terms we follow several published references (see details in Fernandez-Triana et al. 2020) as well as the Hymenoptera Anatomy Ontology (HAO) website (<http://portal.hymao.org/projects/32/public/ontology/>).

Photographs were taken with a Keyence VHX-1000 Digital Microscope, using lenses with a range of 10–130×. Multiple images were taken of a structure through the focal plane and then combined to produce a single in-focus image using the software associated with the Keyence System. Plates were prepared using Microsoft PowerPoint 2010 and later saved as .tiff files.

DNA barcodes were obtained from specimens collected in Algeria and Spain. DNA was extracted destructively from the hind leg of 4 specimens with the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). Modifications to the manufacturer’s protocol from Moreau (2014) and Cruaud et al. (2019) were incorporated into the

extraction. The 658 base pairs (bp) barcoding region of the mitochondrial COI gene was amplified on an Eppendorf MasterCycler Pro S in reactions containing 2 µl 2.5 mM dNTPs, 2 µl 25 mM MgCl₂, 2.5 µl 10 X Taq Buffer, 1 µl of each 10 µM universal primer LCO1490 and HCO2198 (Folmer et al. 1994), 0.2 µl of ExTaq HS DNA polymerase (Takara Bio USA, Madison, WI), 1–4 µl of DNA template, and ddH₂O to 25 µl. The amplification program was as follows: 95 °C for 1 minute, 35 cycles of denaturation (95 °C for 15 s), annealing (49 °C for 15 s), extension (72 °C for 45 s), and a final extension at 72 °C for 4 minutes. Amplification success was confirmed by visualization on a 1% gel. Samples that successfully amplified were cleaned with ExoSAP-IT (PE Applied Biosystems, Foster City, CA, USA). Cycle sequencing was completed with the BigDye Terminator v3.1 Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA, USA) in 10 µl reactions. Samples were purified and sequenced at the Agriculture & Agri-Food Canada Eastern Cereal and Oilseed Research Centre Core Sequencing Facility (Ottawa, ON, Canada) on a 3500xl DNA Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). Chromatograms were trimmed, aligned, and assembled with Geneious Prime v2020.0.4. Assembled sequences were compared to available sequences for similarity.

Sequences were deposited in the Barcode of Life Data Systems (BOLD; <http://www.boldsystems.org>). A dataset containing all available sequences of *D. gelechiidivoris* (ours and others previously obtained from specimens in South America) was created in BOLD and assigned a doi for future reference ([dx.doi.org/10.5883/DS-DOLIGELE](https://doi.org/10.5883/DS-DOLIGELE)). A Neighbour joining (NJ) tree with the 16 sequences of the species over 600 base pairs (bp) was built using BOLD standard parameters.

Results and discussion

The parasitoid wasp specimens reared in Algeria from *T. absoluta* on tomato were identified as *D. gelechiidivoris*, based on the original description (Marsh, 1975) and comparison with the species holotype, one paratype and other material deposited in the CNC (Figs 2–6). The Algerian specimens have slightly darker hind legs (Figs 6, 7) but otherwise match the rest of the material studied.

Additional corroboration of the species identity came from Algerian specimens from which DNA barcodes were obtained and were a perfect match to specimens from South America and Spain (Fig. 8).

A total of 25 species of *Dolichogenidea* have been so far recorded from Africa (Fernandez-Triana et al. 2020), but many more remain undescribed. There has never been a taxonomic review of the genus in the continent, although some species, at that time under the genus *Apanteles*, were treated by Wilkinson (1932) and Nixon (1965), and the faunas of the Arabian Peninsula and Réunion Island have been partially studied recently (Rousse & Gupta 2013; Fernandez-Triana & van Achterberg 2017).

At present, there are no keys to separate African species of *Dolichogenidea*, but the following combination of morphological characters should suffice to characterize and

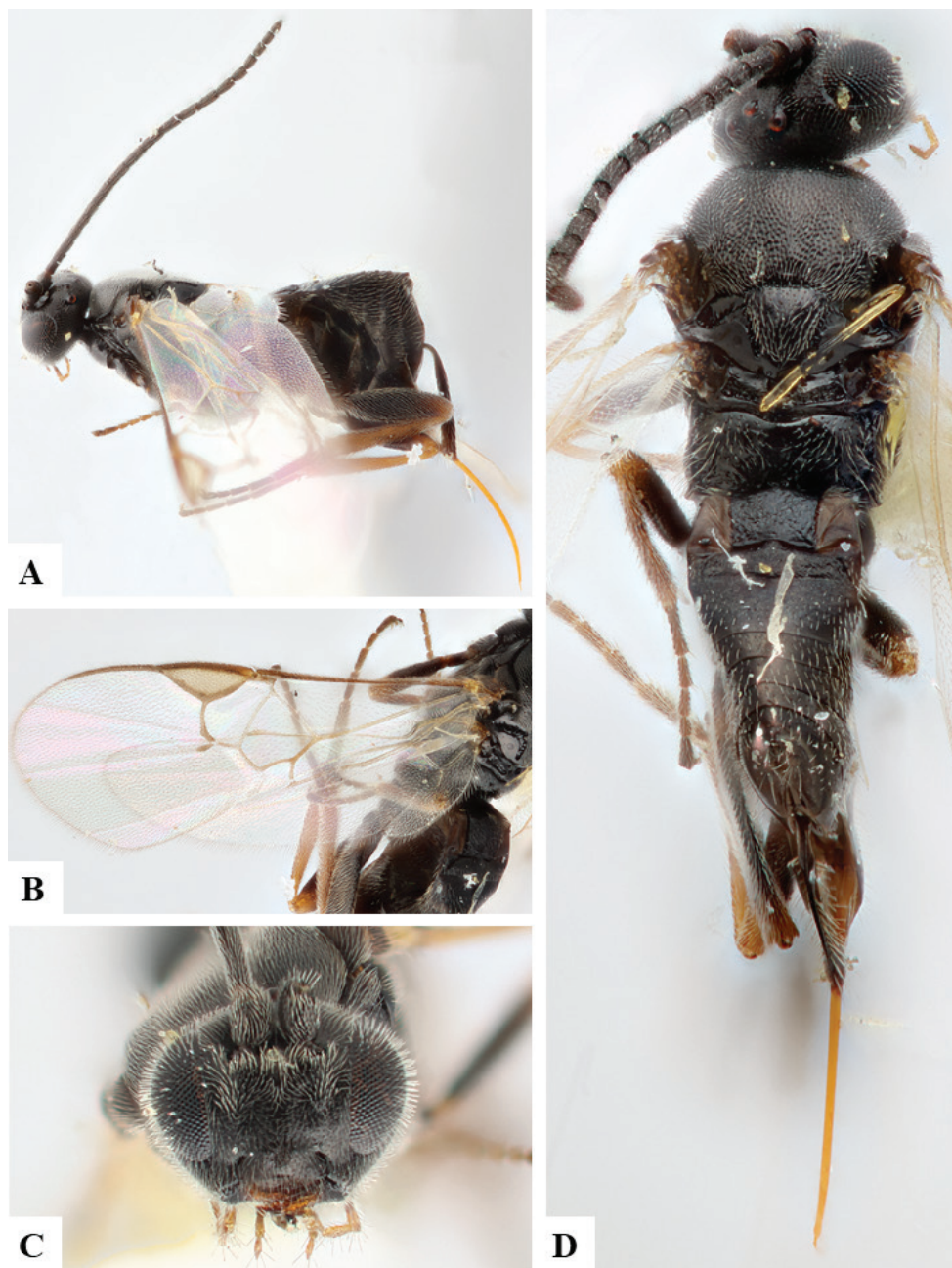


Figure 2. Female holotype of *Dolichogenidea gelechiidivoris*, Colombia, voucher code USNMMENT00831760.

unequivocally recognize *D. gelechiidivoris* among all other described species of the genus in Africa: 1) antenna shorter than body; 2) anteromesoscutum mostly shiny, covered with white setae and with relatively sparse but evenly distributed and well-marked

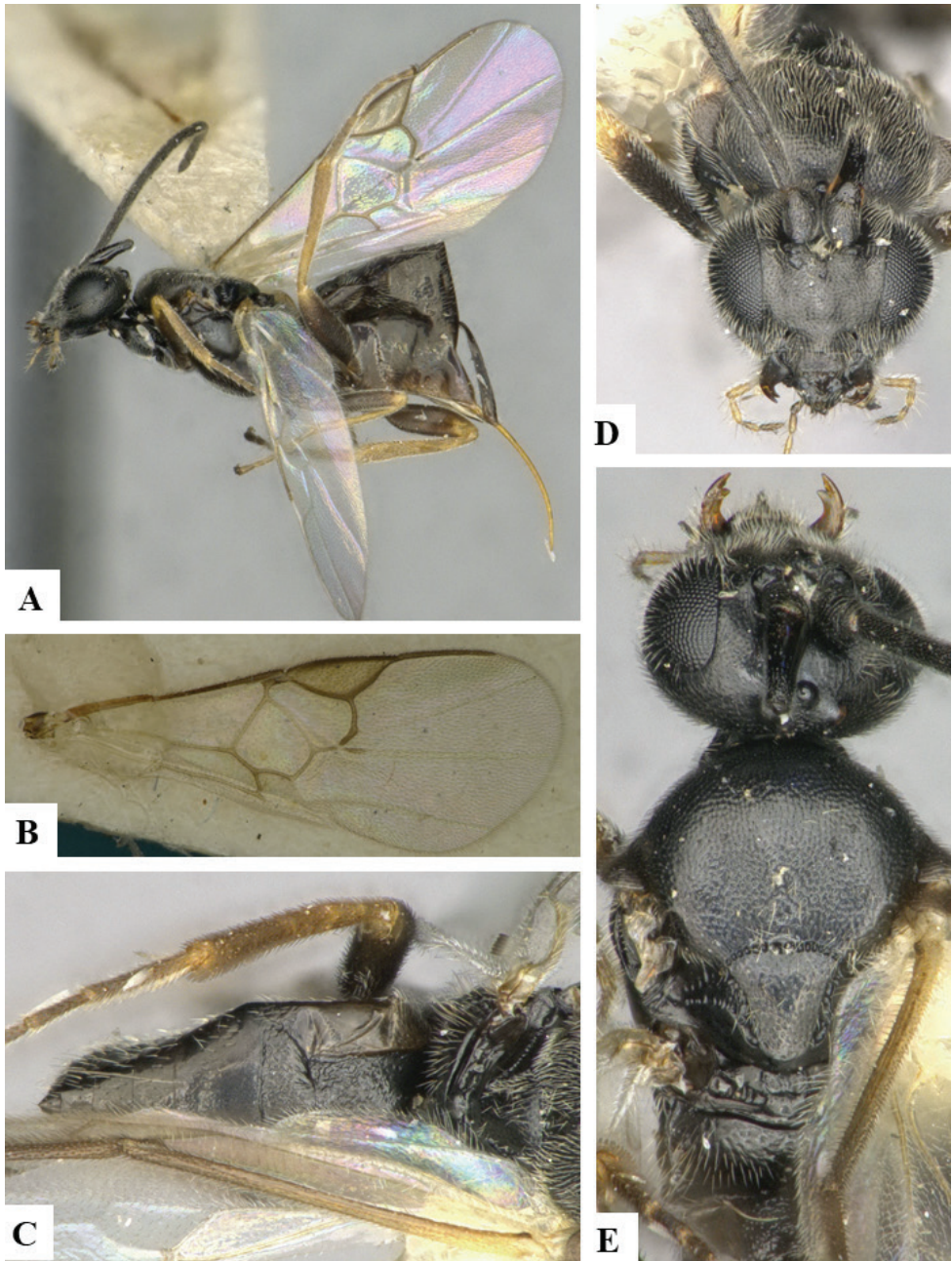


Figure 3. Female paratype of *Dolichogenidea gelechiidivoris*, Colombia, voucher code CNC678061.

punctures; 3) mesoscutellar disc shiny with punctures that are comparatively shallower and more sparse than in anteromesoscutum; 4) polished area (lunules) of the lateral side of the axillar complex about half the height of lateral side; 5) propodeum mostly

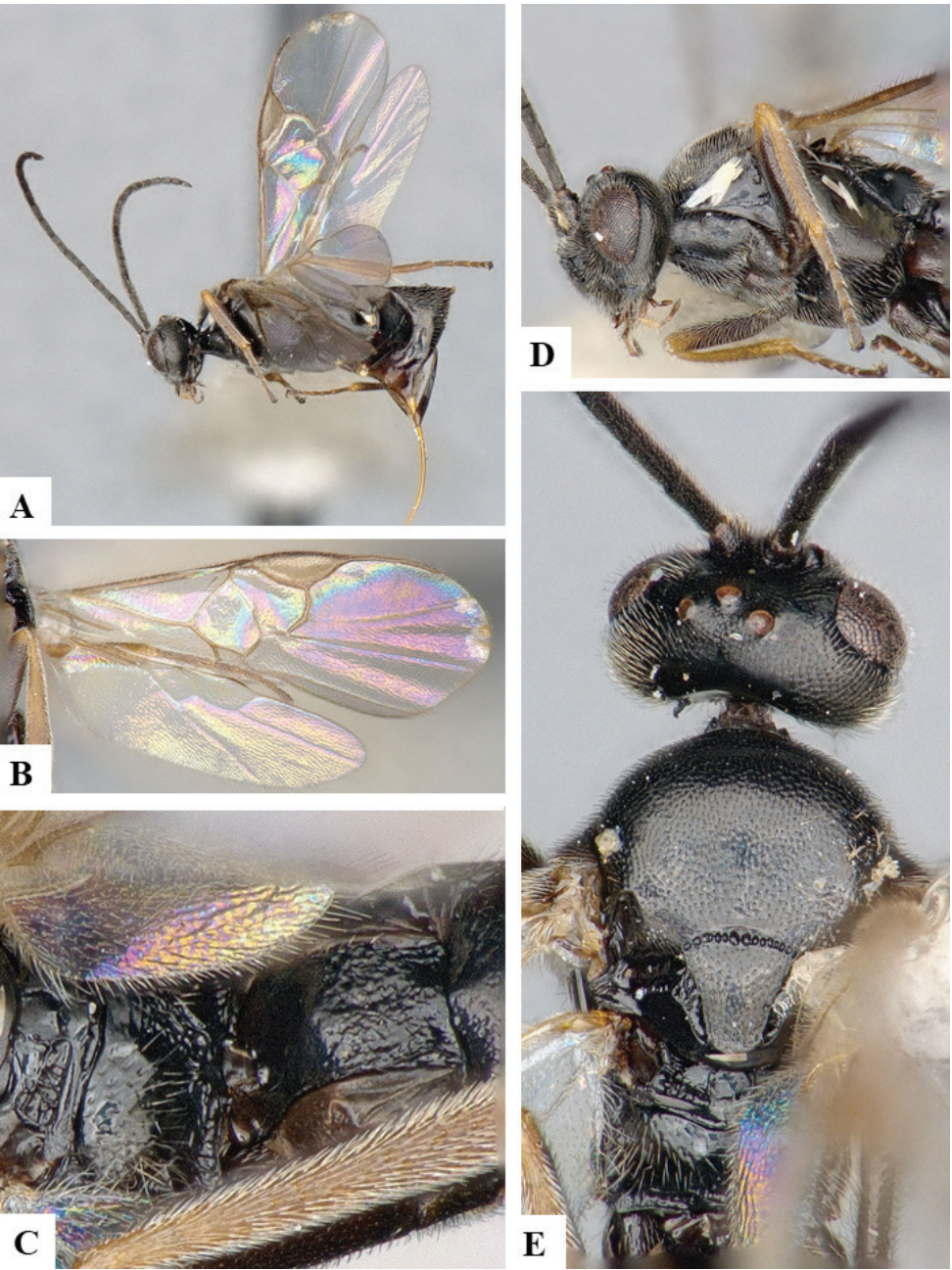


Figure 4. Female paratype of *Dolichogenidea gelechiidivoris*, Colombia, voucher code CNCHYM 01043.

smooth and shiny on anterior half, posterior half rugose medially, with rugae near nucha and forming lower part of an areola; 6) metasoma relatively short and slightly compressed laterally; 7) first mediotergite (T1) mostly parallel-sided, but slightly nar-

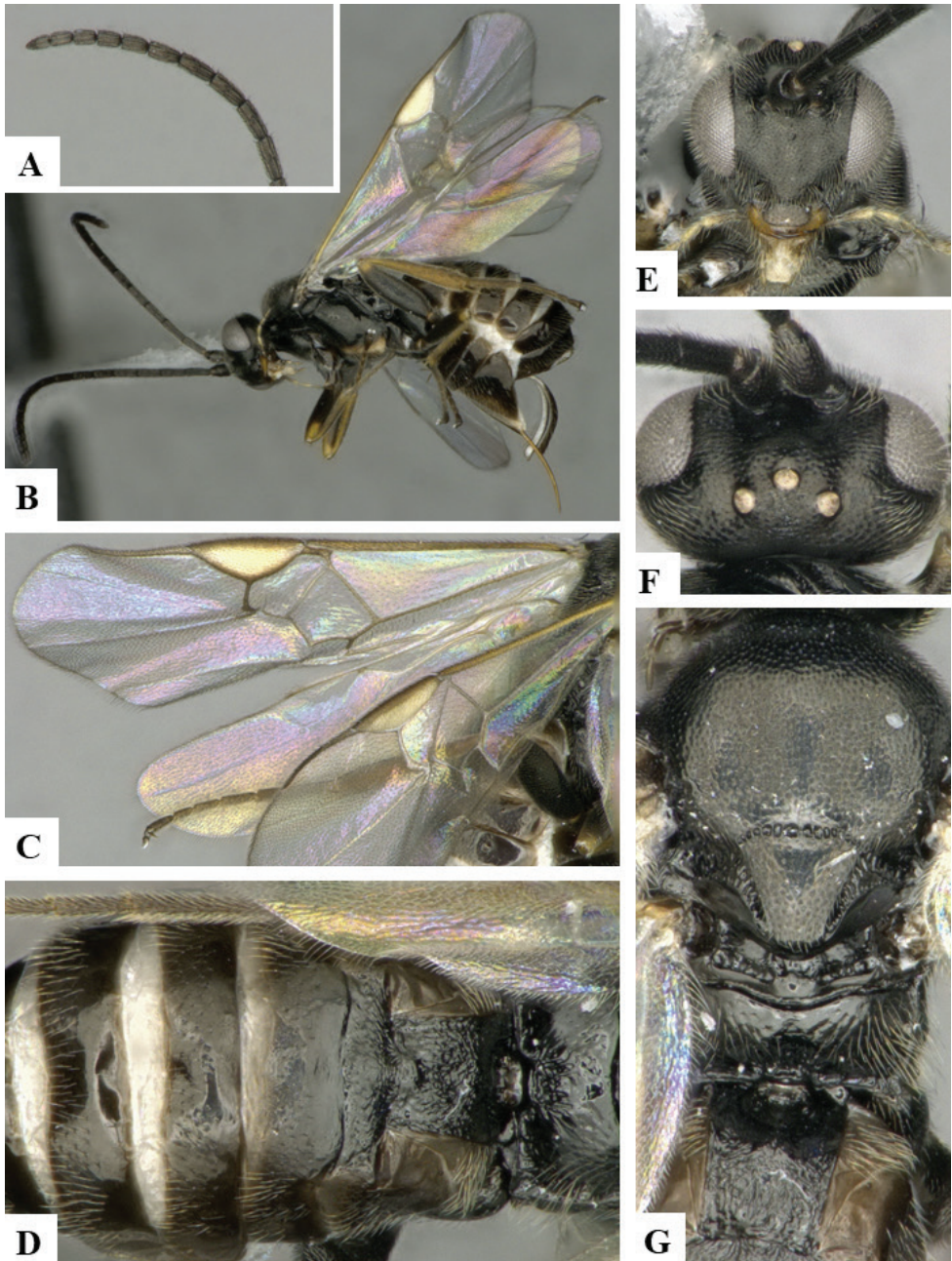


Figure 5. Female specimen of *Dolichogenidea gelechiidivoris*, Spain, voucher code CNC1196542.

rowing near posterior margin; 8) T1 smooth on anterior half, rugose on posterior half; 9) second mediotergite (T2) relatively transverse, its width at posterior margin more than 3.0× its medial length, with lateral sides oblique and posterior margin sinuate

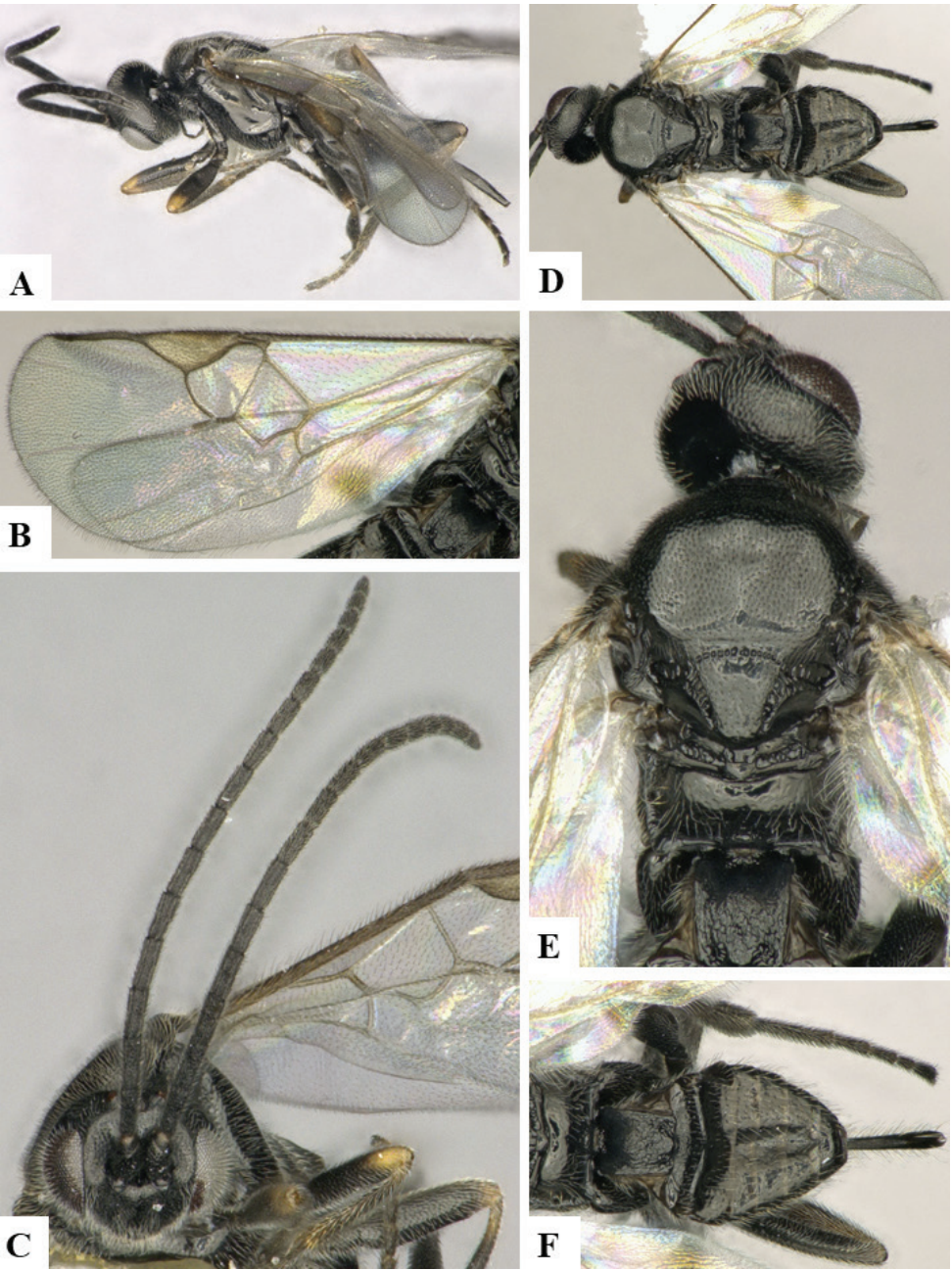


Figure 6. Female specimen of *Dolichogenidea gelechiidivoris*, Algeria, voucher code CNC1196948.

(shape of T2 varies slightly and it is sometimes less transverse in some specimens from all geographical areas examined); 10) T2 mostly sculptured, with small area smooth along posterior margin; 11) ovipositor sheaths about same length (or slightly shorter)

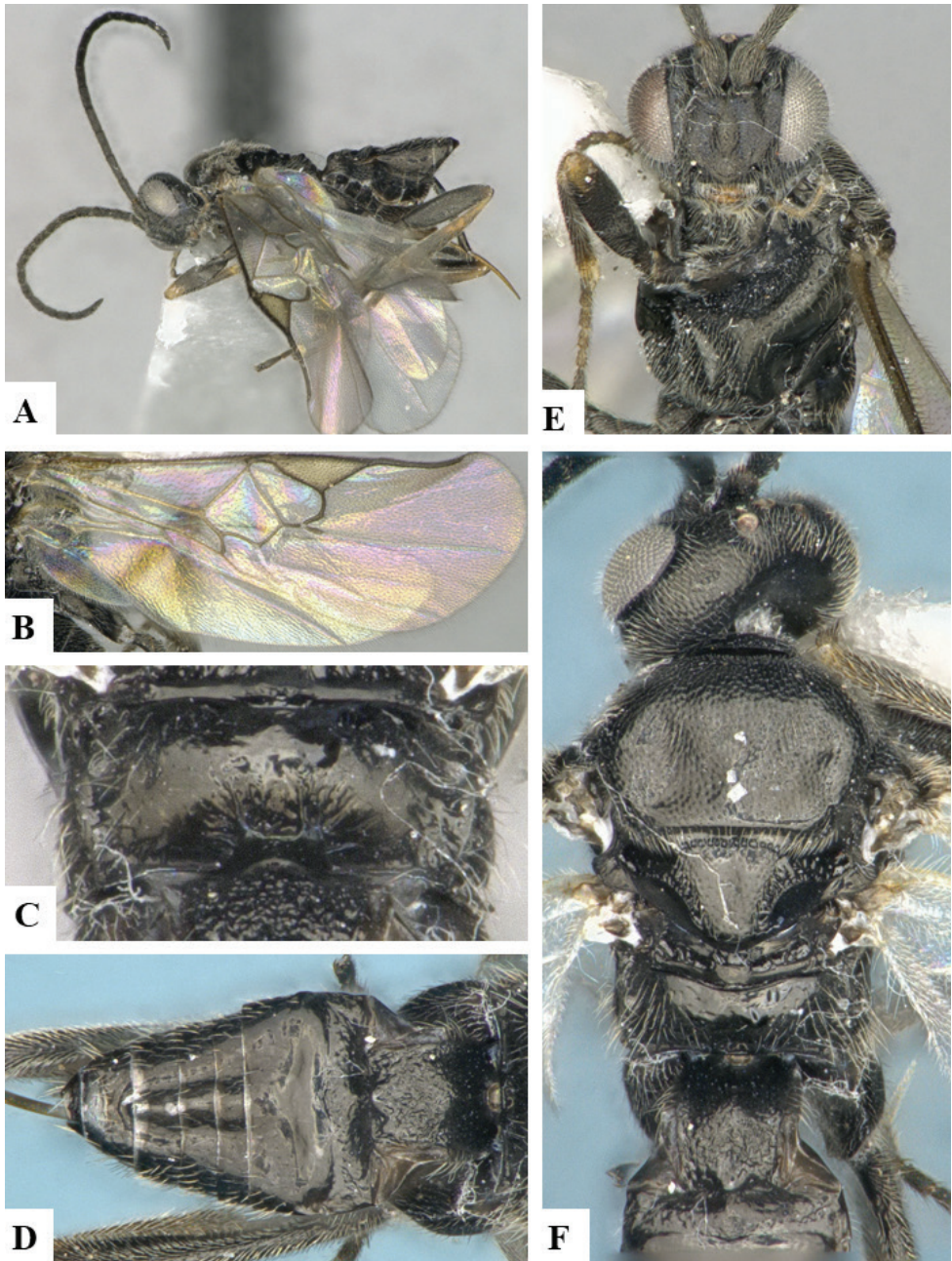


Figure 7. Female specimen of *Dolichogenidea gelechiidivoris*, Algeria, voucher code CNC1180035.

than metatibia length, ovipositor evenly down curved; 12) fore wing with pterostigma relatively thick, light coloured (pale yellow-brown) but with thin brown margins; 13) fore wing vein R1 comparatively short, its length about the same than pterostigma

length, but less than $2.5\times$ the distance between its end and end of vein 3RS; 14) most veins in wings either light brown to yellow-brown or transparent; 15) legs mostly dark brown to black but with posterior 0.1–0.2 of femora and most of tibiae yellow to yellow brown (specimens from Algeria and some specimens from Spain we have examined have darker tibiae, which can be mostly dark brown to black); 16) body color mostly black, including tegula and humeral complex.

Especially, the following characters are useful to recognize the species: the sculpture of propodeum; sculpture and shape of T1 and T2; color and shape of pterostigma; short vein R1; relative short antenna and metasoma relatively compressed laterally. Among the specimens included in the following study, the morphological variation was relatively minor (with some specimens having slightly darker legs and slightly less transverse T2 shape), thus the species is fairly recognizable and uniform.

Dolichogenidea appellator (Telenga, 1949) is the only other species of *Dolichogenidea* from Africa which has been recorded parasitizing *Tuta absoluta* in the continent (Idriss et al. 2018; Mansour et al. 2018). It can be clearly distinguished from *D. gelechiidivoris* because of different sculpture of first two metasomal segments (T1 and T2 are entirely smooth); fore wing pterostigma mostly light brown with a white or transparent spot on anterior 0.2–0.3; tegula (entirely) and humeral complex (partially) yellow; profemur (entirely) and mesofemur (anterior half) pale (yellow to light brown-yellow); and longer antenna, as long as body length.

From a molecular perspective, *D. gelechiidivoris* is also clearly distinctive and diagnosable. The specimens with available data in BOLD come from six different countries, Algeria, Chile, Colombia, Peru, Spain and Venezuela. They comprise 19 sequences, with 16 of them over 600 bp (15 representing full barcodes). Out of the three short sequences (107–164 bp) two are especially important as they were retrieved from paratype specimens housed in the CNC (voucher codes: CNCHYM 01042 and CNCHYM 01043, see details in dataset dx.doi.org/10.5883/DS-DOLIGELE); those paratype sequences match very well with the full barcodes. There were no differences between specimens from South America, Spain or Algeria (Fig. 8); the only exception was a specimen from Chile (voucher code WAM 0495) which had one base pair different (0.16%) compared to all other sequences. The Barcode Index Number (BIN, see more details on the BIN concept in Ratnasingham and Hebert 2013) assigned to *D. gelechiidivoris* is BOLD:AAM4042. That BIN has 31 bp (4.72%) of difference with the closest available BIN in BOLD (BOLD:AAZ9580) which comprises a single, unidentified specimen of *Dolichogenidea* specimen from California, USA; therefore making the identification of *D. gelechiidivoris* using DNA barcoding fairly easy and very reliable (http://www.boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:AAM4042).

Although South American specimens of *D. gelechiidivoris*, from Peruvian central highlands, were brought to Kenya in 2017 by researchers at icipe, they have only been studied in laboratory conditions for potential classical biological control of *T. absoluta* (Aigbedion-Atalor et al. 2020). Based on available reports and scientific publications, *D. gelechiidivoris* has not yet been released into field conditions in Africa. Thus, our finding of the parasitoid wasp species in open tomato fields and greenhouses in Algeria



Figure 8. Neighbour joining (NJ) tree with all available sequences of *Dolichogenidea gelechiidivoris* over 600 base pairs. For every specimen details of the voucher code, country of origin and total of base pairs is shown in the corresponding branch.

is the first report of individuals of this species naturally occurring in Africa. Its presence is unlikely to be related to the efforts being undertaken in Kenya; rather, it is more likely that the species arrived in northern Africa from Spain, where it had been recently found (Denis et al. 2021). In any case, the introduction seems to be accidental, probably following its host (*T. absoluta*), as it is supposed to have been the case in Spain.

The documentation in this paper of *D. gelechiidivoris* already established in Africa (at least in northern areas of the continent) is very relevant for the future of biological control of *T. absoluta* in the continent. Since this wasp species occurred spontaneously in tomato crops but autonomous spreading to isolated fields seems limited, it is possible to recommend its mass rearing and augmentation release for its rapid establishment in other areas, and to include its management in combination with other biocontrol agents in the IPM programs of *T. absoluta* in the region.

Acknowledgments

We are grateful for the excellent comments and suggestions provided by the reviewers Kaoru Maeto, Ilgoo Kang and Sergey Belokobyl'skij as well as the editor Elijah Talamas, which considerably improve the final version of this paper. The work of JFT and AB was supported by Project J-002276 “Systematics of beneficial arthropods in support of resilient agroecosystems”, from Agriculture and Agri-Food Canada. JA was supported by Horta.Net and the CERCA Programmes of the Generalitat de Catalunya.

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The genus *Orionis* Shaw (Hymenoptera, Braconidae, Euphorinae) in the Old World

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Academic editor: Jose Fernandez-Triana | Received 5 October 2021 | Accepted 23 October 2021 | Published 30 December 2021

<http://zoobank.org/26EF8AFA-B7B8-4B86-BEF2-805DAF35B7A8>

Citation: Broad GR, Stigenberg J (2021) The genus *Orionis* Shaw (Hymenoptera, Braconidae, Euphorinae) in the Old World. Journal of Hymenoptera Research 88: 133–145. <https://doi.org/10.3897/jhr.88.76177>

Abstract

The euphorine braconid genus *Orionis* Shaw, 1987 is found to be more diverse in the Old World than had previously been recognised. *Orionis* was regarded previously as largely Neotropical, with one Oriental species (*Orionis orientalis* Shimbori & Shaw, 2016) known from Thailand, but we recognise an additional three species from the Oriental and Palearctic regions. Three species of Euphorinae are transferred to *Orionis* Shaw, 1987 and are new combinations: *Orionis coxator* (Belokobylskij, 1995), **comb. nov.**, *Orionis erratus* (Chen & van Achterberg, 1997), **comb. nov.**, and *Orionis flavifacies* (Belokobylskij, 2000), **comb. nov.** Previously known from the Far Eastern Palearctic, *O. coxator* has surprisingly been found in Europe, in Belgium, England and the Netherlands. The inclusion of these species in *Orionis*, whereas most previous species have been described from the Neotropics, is justified by Bayesian analysis of the D2 region of 28S, Cytochrome Oxidase I barcode sequences, and morphology.

Keywords

Bayesian, new record, parasitoid, phylogeny, taxonomy

Introduction

On 29th September 2020, GRB caught a braconid wasp (Hymenoptera: Braconidae) at an actinic light in his garden in South-east England (Fig. 1). It seemed to be either a species of *Meteorus* Haliday or *Perilitus* Nees but was atypical for either placement. Perplexed as to what this could be, and sure it was nothing he had seen before, GRB sent a

leg to JS for barcode sequencing. The resulting species identification was '*Perilitus*' *coxator* Belokobylskij, 1995, with high probability, but the implications were interesting, as this and other similar species described from China ('*Perilitus*' *erratus* (Chen & van Achterberg, 1997)) and Russia ('*Perilitus*' *flavifacies* Belokobylskij, 2000), are clearly atypical for *Perilitus*, which prompted us to re-evaluate the generic classification of these species.

Material and methods

Specimens and images

Institutional abbreviations:

NHMUK Natural History Museum, London, UK;

ZISP Zoological Institute of the Russian Academy of Sciences, St Petersburg, Russia;

NHRS Swedish Museum of Natural History, Stockholm, Sweden.

The English specimen (NHMUK014425411) was collected at a white sheet with a 15W actinic bulb. The right hind leg was removed from the dry, card-pointed specimen for sequencing. Photos were taken with a Canon SLR EOS 5DSR with either a 65 mm macro lens or a Mitutoyo 10 × lens in combination with a 70–130 mm macro lens, mounted on a copy stand with an automated Z-stepper; images were aligned using Helicon Focus software version 6.6.1.

Morphological terminology follows van Achterberg (1993).

DNA extraction and sequencing

DNA was extracted from the hind leg using the Thermo Labsystems KingFisher extraction robot at the NHRS laboratory facility. The standard DNA barcode fragment of the mitochondrial cytochrome oxidase I (CO1) gene was obtained using primers by Folmer et al. (1994). Ready-To-Go PCR beads were used (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; Zaldívar-Riverón et al. 2006; Sharanowski et al. 2011; Stigenberg and Ronquist 2011), but latterly mainly in combination with other, particular nuclear, genes. In addition to the CO1 gene we also utilised the nuclear 28S D2 fragment (see Stigenberg and Ronquist 2011). PCR products were purified with EXO1 and FastAP. The product was sequenced using both the forward and reverse primers. Sequences were assembled and edited using Geneious Pro v.9.1.8. The Voseq v.1.7.4 (Peña and Malm 2012) database was used for storing voucher and DNA sequence data. BLAST analysis was performed to identify similar sequences within the NCBI database.

Phylogeny reconstruction

Bayesian Inference analyses were performed using MrBayes 3.2.7a (Ronquist et al. 2012). All BI analyses were performed through the Cipres web portal for phylogenetic analysis (Miller et al. 2010) (<http://www.phylo.org>). The protein coding CO1 was partitioned after codon positions 1+2 and 3, the 28S dataset was not partitioned. The Bayesian analysis was performed by running four MCMC chains for 10 million generations, sampling post-burn-in trees every 1000 generations. A CO1 analysis including 60 taxa as well as a combined CO1+28S analysis including 65 taxa were run. The datasets included an assortment of members of the Euphorinae tribe Perilitini with an ichneumonid and distant as well as closer braconids as outgroups. GenBank accession numbers for the *Orionis* species are shown in Table 1.

Results

In all our BI analyses we recovered the genus *Orionis* as monophyletic with 100% support. The reverse strand of CO1 was not successfully amplified for the English specimen (Euph_220), but the forward strand (GenBank ID: MW401798) was of good quality and on a BLAST search matched with '*Perilitus*' *coxator* with 96.7% similarity. The specimen was compared with the original descriptions of *P. coxator* (Belokobylskij 1995) and the apparently similar *P. erratus* (Chen and van Achterberg 1997); Sergey Belokobylskij then kindly compared images of the English specimen with specimens of *P. coxator* in ZISP. Bayesian analyses of CO1 barcode sequences as well as combined CO1+28S analyses of Perilitini species, including the sequence generated for this study, resulted in a topology (Fig. 2; the full phylogenies are presented in Suppl. material 1, 2) largely congruent with that of Stigenberg et al. (2015), with a monophyletic *Orionis*, including '*Perilitus coxator*' and '*P. flavifacies* Belokobylskij, 2000 alongside *O. eximius* (Muesebeck, 1955), the type species of *Orionis* (Shaw 1987), and *O. orientalis* Shimbori & Shaw, 2016. Based on morphological synapomorphies (see Discussion) and the results of the phylogenetic analyses, *Orionis coxator* (Belokobylskij, 1995) and *Orionis flavifacies* (Belokobylskij, 2000) are both new combinations.

The original description (Chen and van Achterberg 1997), including very useful illustrations, clearly shows that *Meteorus erratus* shares the synapomorphies of *Orionis*, and *Orionis erratus* (Chen and van Achterberg, 1997) is a new combination.

Table 1. GenBank accession numbers for *Orionis* sequences.

Specimen	COI	28S	Country
JS10_00511 <i>P. coxator</i>	KJ591484	KJ591277	Russia
JS10_00526 <i>P. coxator</i>	MZ021572	KJ591278	Russia
Euph_220 <i>P. coxator</i>	MW401798	MW600657	UK
JS10_00510 <i>P. flavifacies</i>	KJ591486	KJ591279	Russia
AB097 <i>O. eximius</i>	KJ591480	KJ591272	Costa Rica
GB_orionis <i>O. eximius</i>	-	AJ302824	unknown
AB101 <i>Orionis orientalis</i>	-	KJ591273	Thailand

Building on Shaw (1987) and Bortoni et al. (2016), we propose that *Orionis* can be diagnosed by the following combination of character states: frons with rugose or strigose sculpture (Fig. 3c); eyes strongly convergent ventrally (Fig. 3a); first metasomal segment long and slender, lacking dorsope or laterope, curved in lateral view, with spiracles around or not far posterior to the mid-length (Fig. 3c); propodeum strongly convex, with distinct dorsal and posterior faces of about equal length and often clearly separated by distinct bend/angulation (Figs 1, 3c).

However, two or three of those characters can be found in combination in some *Perilitus* species and the separation of these two genera is not entirely clear-cut. The key to Old World *Orionis* species presented here is based largely on the literature, and additional species might be expected.

Key to Old World species of *Orionis*

- 1 Face yellow; propodeum with rounded division between dorsal and posterior faces.....**2**
- Face black; propodeum with division between dorsal and posterior faces almost a right angle**3**
- 2 Pronotum and propleuron yellow; first metasomal tergite dorsally weakly rugose [Thailand] ***O. orientalis* Shaw & Shimbori**
- Pronotum and propleuron black; first metasomal tergite dorsally entirely striate [Eastern Palearctic] ***O. flavifacies* (Belokobylskij)**
- 3 Ovipositor sheath $1.4\text{--}1.6 \times$ length of the first tergite; hind coxa dorsally with some curved striae [Palearctic] ***O. coxator* (Belokobylskij)**
- Ovipositor sheath about $2.0 \times$ length of the first tergite; hind coxa dorsally rugose-punctate [China and Korea]..... ***O. erratus* (Chen & van Achterberg)**

Old World species of *Orionis*

Bortoni et al. (2016) revised the species of *Orionis* known to them, which included four Neotropical species and one from Thailand. Including the species transferred here to *Orionis*, the genus is now represented by equal numbers of described species in the Old and New Worlds. None has a recorded host.

Taxonomy

Genus *Orionis* Shaw, 1987

Type species. *Perilitus eximius* Muesebeck, 1955 by original designation.

***Orionis coxator* (Belokobylskij, 1995), new comb.**

Perilitus coxator Belokobylskij, 1995.

Comments. Described from Russia, Primorskiy Territory (Belokobylskij 1995), subsequently recorded from Korea (Ku et al. 2001). Russian (Primorskiy Territory) specimen sequenced by Stigenberg et al. (2015). The record of '*Perilitus erratus*' from the Netherlands (van Achterberg et al. 2020) probably refers to this species.

Diagnosis. Mostly black, head with dorsal reddish patches; propodeum with posterior and dorsal faces angled about 100° relative to each other; hind coxa dorsally with curved striae; first metasomal tergite weakly striate medially (Figs 1, 3).

Specimens examined. ENGLAND • 1♀; Kent, Tonbridge; 51.186N, 0.287E; 29th September 2020; actinic light; G.R. Broad; NHMUK014425411; new record for the United Kingdom.

***Orionis erratus* (Chen & van Achterberg, 1997), new comb.**

Meteorus erratus Chen & van Achterberg, 1997

Perilitus erratus: Belokobylskij (2000b)

Comments. Described from Oriental and Palaearctic Provinces of China: Guizhou, Liaoning and Yunnan (Chen and van Achterberg 1997); recorded from Korea by Papp



Figure 1. Lateral habitus of *Orionis coxator* (NHMUK014425411). Scale bar: 2 mm.

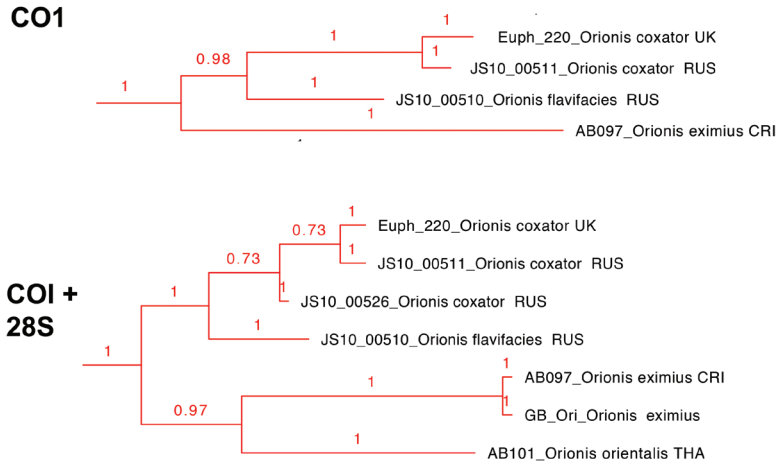


Figure 2. Extract from Bayesian phylogeny of Perilitini: relationships of *Orionis* species based on CO1 and CO1+28S analyses. Numbers on branches are posterior probabilities.

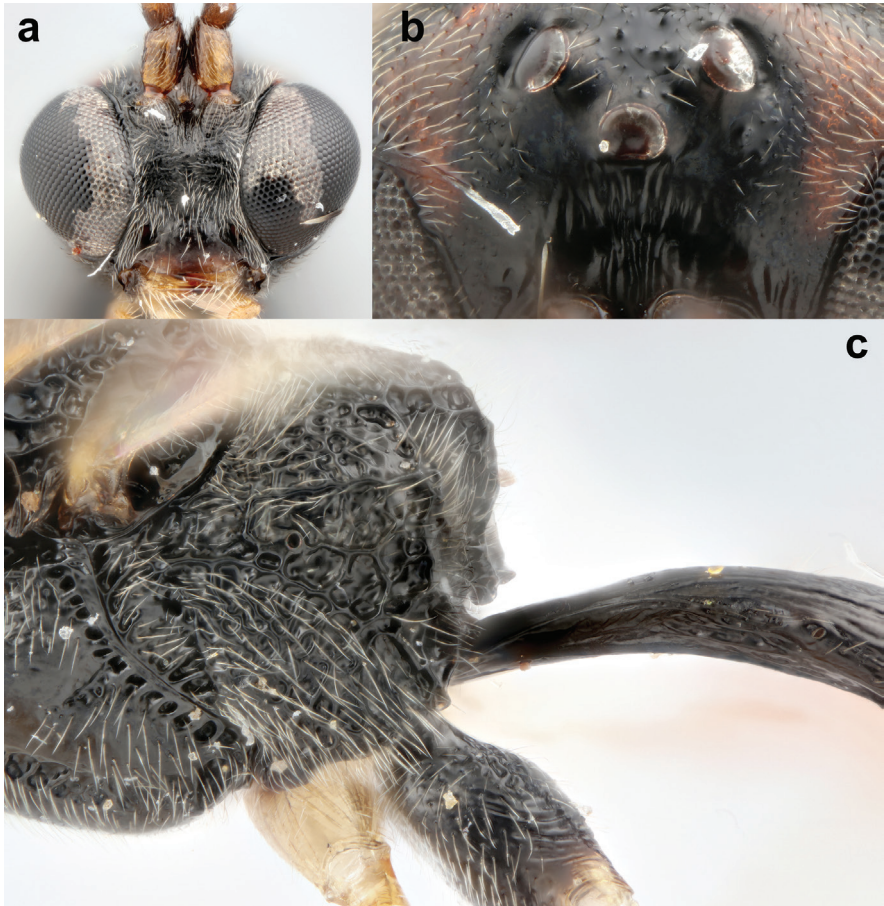


Figure 3. Details of *Orionis coxator* (NHMUK014425411) **a** head in frontal view **b** frons and upper head **c** propodeum, first metasomal segment and hind coxa in dorsolateral view.

(2003) and probably erroneously from the Netherlands by van Achterberg et al. (2020) (see Discussion).

Diagnosis. Mostly black, head with dorsal reddish patches and clypeus reddish brown; scuto-scutellar groove with median carina and several carinulae in pits; propodeum with posterior and dorsal faces angled about 90° relative to each other; hind coxa dorsally rugose-punctate; first metasomal tergite with weak rugae medially.

***Orionis flavifacies* (Belokobylskij, 2000), new comb.**

Perilitus flavifacies Belokobylskij, 2000

Comments. Described from Russia, Primorskiy Territory (Belokobylskij 2000a); recorded from Korea by Papp (2003) based on an identification by Belokobylskij. Russian (Primorskiy Territory) specimen sequenced by Stigenberg et al. (2015).

Diagnosis. Mostly black, head largely yellowish-brown; propodeum with rather rounded division between posterior and dorsal faces; hind coxa dorsally with curved striae; first metasomal tergite largely striate.

***Orionis orientalis* Shaw & Shimbori, 2016**

Comments. Described from Thailand.

Diagnosis. Largely black, head with face and clypeus yellowish-brown, pronotum and propleuron yellowish-brown; propodeum with rather rounded division between posterior and dorsal faces; hind coxa largely unsculptured dorsally; first metasomal tergite largely weakly rugulose.

Discussion

Distribution and ecology of *Orionis coxator*

While this work was in preparation, van Achterberg et al. (2020) reported the occurrence of a very similar specimen in Amsterdam, the Netherlands, which they identified as *Perilitus erratus*. Subsequent posts on Facebook showed that similar specimens have been found in Belgium and Germany recently and a further UK specimen came to light on Facebook (England, Hampshire, New Forest, October 2021; Colin Easton). We strongly suspect that all these specimens belong to the same species and that, as van Achterberg et al. (2020) surmised, this is a recent introduction from the Far East. However, we offer a different perspective on the identification. The Dutch specimen illustrated by van Achterberg et al. (2020) has curved dorsal striae on the hind coxa, identifying this as *Orionis coxator*. Given that two of the authors also described *Perilitus erratus* as a new species (Chen and van Achterberg 1997), perhaps the hind coxa is variable within

the species and these names are synonymous. We have not examined the holotypes of *Perilitus erratus* or *Perilitus coxator* and are relying on the (very informative) original descriptions; should these names prove to be synonymous, *Orionis coxator* has priority.

Whether *Orionis coxator* is native to Europe is probably unknowable, although circumstantial evidence, mainly that these distinctive wasps have never been found in Europe before, strongly suggests that there has been an accidental introduction and perhaps a rapid range expansion within Europe. However, other ichneumonoids have been discovered in Europe following their description in the Russian Far East and these massive range discontinuities have been assumed to represent spot samples from an extensive and undocumented range; for example, the diplazontine ichneumonid, *Episemura diodon* Kasparyan & Manukyan, 1987 (Klopfstein 2014). The distribution of *Orionis* species is rather similar to that of another ichneumonoid genus that was poorly known until recently; *Rodrigama* Gauld, 1991 (Ichneumonidae, Poemeniinae) comprises a few known species, with the type species described from Costa Rica (Gauld 1991) then additional species being discovered in the Oriental and Eastern Palaearctic regions (Matsumoto and Broad 2011; Choi and Lee 2020) and one species in the Western Palaearctic (Broad and Kuslitzky 2019). There are similar examples of apparently huge range discontinuities in Braconidae, for example *Proclithrophorus* Tobias & Belokobylskij, 1981, with the type species (*Proclithrophorus mandibularis* Tobias & Belokobylskij, 1981) described from the southern Russian Far East (Tobias and Belokobylskij 1981) and a second species (*P. genalis* Vikberg & Koponen, 2001), of very similar morphology, subsequently described from Finland (Vikberg & Koponen 2001). Or *Colastes (Pseudophanomeris) pilosus* Belokobylskij, 1984, known from the Palaearctic Far East, recently being found in Ukraine and the Czech Republic (Belokobylskij 2019). Some parasitoid species suddenly become numerous and then suddenly disappear again. A good example in Britain is *Phrudus badensis* Hilpert, 1987, described from Germany (Hilpert 1987), collected in Britain in good numbers (Shaw 1991) and then apparently disappearing again (M.R. Shaw pers. comm.). *Phrudus badensis* closely resembles *Phrudus longius* Chiu, 1987, described from Taiwan (Chiu and Wong 1987). The lack of known hosts for most of these ichneumonoids hinders interpretation of their distribution.

Orionis eximius is associated with unknown hosts on *Lantana camara* L. (Verbenaceae) (Shaw 1987). *Lantana camara* does not survive outdoors in the cool temperate climate of NW Europe, although other ornamental Verbenaceae do. The as yet unknown hosts of *Orionis* are presumably Coleoptera, as with other Perilitini (Shaw 1985; Stigenberg et al. 2015). The large eyes of *Orionis coxator*, and the fact some specimens have been caught at light (as have some *O. flavifacies*; Belokobylskij 2000a), suggest it is at least partly nocturnal.

Composition of the genus *Orionis*

Orionis is diagnosed by a combination of characters, more than one of which can be present in species classified in *Perilitus*. For example, *Perilitus mylloceri* (Wilkinson, 1929) (originally described in *Dinocampus* Förster, 1863) shares a narrow, ventral-



Figure 4. *Perilitus mylloceri* paratype female, propodeum and first metasomal segment.

ly fused first metasomal segment, strongly convex propodeum (Fig. 4) and bulging, ventrally convergent eyes with *Orionis*. However, we consider this species best placed in *Perilitus*, as the spiracles of the first metasomal tergite are at the posterior third and this segment is virtually straight until the postpetiole. An unidentified or undescribed African species of *Perilitus* in NHMUK has a strongly angled propodeum, very similar to some *Orionis*, and ventrally convergent eyes (Fig. 5). In this case, the first metasomal segment is more typical of *Perilitus*, being only $2.5 \times$ as long as posteriorly wide, with the spiracles at the posterior third of the tergite and the sides of the first tergite ventrally distinctly separated. Nevertheless, *Perilitus* as currently defined is probably not monophyletic (Stigenberg et al. 2015; Suppl. material 1, 2) and some of these species might be better classified elsewhere, perhaps in an expanded concept of *Orionis*. Figure 6 compares the propodeum and first metasomal segment of *O. coxator* and *Perilitus rutilus* (Nees, 1811), the type species of *Perilitus*.

Chen and van Achterberg (1997) described *erratus* in the genus *Meteorus*, despite the lack of vein *rs-m* (i.e., the second submarginal cell is open). GRB's first thought was that the English specimen of *O. coxator* could be an aberrant *Meteorus*. The structure of the first metasomal segment and the strongly convergent eyes are both more similar to some *Meteorus* species than they are to *Perilitus*. The first metasomal segment of these Old World species is not as long and slender as in the type species of *Orionis*, *O. eximius* (Muesebeck), which has hindered the correct identification of the Old World species. Bortoni et al. (2016) had recognised *Orionis* as occurring in the Old World, with the description of *O. orientalis* from Thailand, and pointed out the close relationship to certain '*Perilitus*' species in Stigenberg et al.'s (2015) phylogeny. It took a chance discovery of *O. coxator* on a different continent to join the dots and more accurately describe the diversity of *Orionis* species.



Figure 5. Unidentified *Perilitus* species from Cameroon (NHMUK014425412). Scale bar: 1 mm.

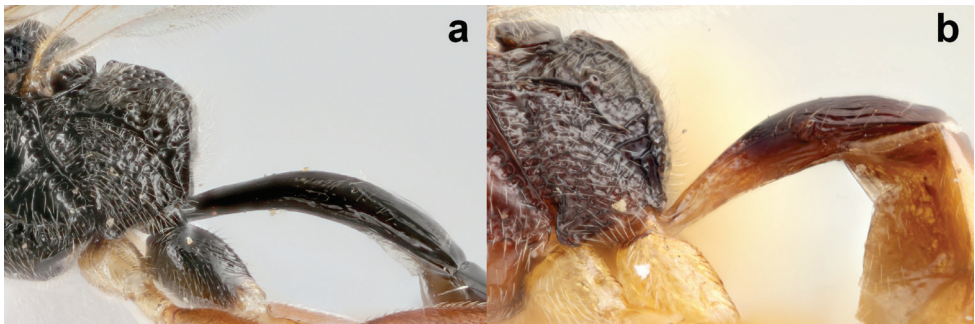


Figure 6. Comparison of propodeum and first metasomal segment of **a** *Orionis coxator* and **b** *Perilitus rutilus*.

Acknowledgements

We are grateful to Sergey Belokobylskij for checking the identity of the *Orionis coxator* specimen and to Mark Shaw for his thoughts on apparent range discontinuities in parasitoid wasps. Mark and Sergey also provided very helpful reviews of the manuscript.

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Supplementary material 1

Bayesian analysis of Perilitini based on CO1

Authors: Gavin R. Broad, Julia Stigenberg

Data type: Phylogenetic.

Explanation note: Bayesian analysis of selected Perilitini (Braconidae, Euphorinae) based on CO1 gene.

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Link: <https://doi.org/10.3897/jhr.88.76177.suppl1>

Supplementary material 2

Bayesian analysis of Perilitini based on CO1 and 28S

Authors: Gavin R. Broad, Julia Stigenberg

Data type: Phylogenetic.

Explanation note: Bayesian analysis of selected Perilitini (Braconidae, Eurhorinae) based on combined analysis of CO1 and 28S.

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Corrigendum: Description of *Kavayva*, gen. nov., (Chalcidoidea, Eurytomidae) and two new species associated with *Guarea* (Meliaceae), and a review of New World eurytomids associated with seeds. Journal of Hymenoptera Research 86: 101–121. <https://doi.org/10.3897/jhr.86.71309>

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Academic editor: Petr Janšta | Received 4 November 2021 | Accepted 5 November 2021 | Published 30 December 2021

<http://zoobank.org/05EA29C7-90B3-4D47-B6C2-71D4861FFCAA>

Citation: Zhang YM, Gates MW, Silvestre R, Scarpa M (2021) Corrigendum: Description of *Kavayva*, gen. nov., (Chalcidoidea, Eurytomidae) and two new species associated with *Guarea* (Meliaceae), and a review of New World eurytomids associated with seeds. Journal of Hymenoptera Research 86: 101–121. <https://doi.org/10.3897/jhr.86.71309>. Journal of Hymenoptera Research 88: 147–148. <https://doi.org/10.3897/jhr.88.77628>

In a paper about the description of *Kavayva*, a new genus of Eurytomidae (Zhang et al. 2021), the indication of repositories for the type specimens were missing. We regret this omission, and provide the missing information below.

MUSM	Natural History Museum of the San Marcos University, Lima, Peru
UFGD	Museum of Biodiversity of the Federal University of Grande Dourados, Dourados-MS, Brazil
USNM	United States National Museum of Natural History, Washington, D.C., USA

***Kavayva bodoquenensis* Zhang, Silvestre & Gates, sp. nov.**

Holotype female deposited at USNM.

Paratypes deposited at USNM [12F, 13M] and UFGD [8F, 3M].

***Kavayva davidsmithi* Zhang & Gates, sp. nov.**

Holotype female deposited at MUSM.

Paratypes deposited at USNM [1F, 1M].

Acknowledgements

We thank John Noyes and Natalie Dale-Skey for reaching out to us regarding the omission.

Reference

Zhang YM, Gates MW, Silvestre R, Scarpa M (2021) Description of *Kavayva*, gen. nov., (Chalcidoidea, Eurytomidae) and two new species associated with *Guarea* (Meliaceae), and a review of New World eurytomids associated with seeds. Journal of Hymenoptera Research 86: 101–121. <https://doi.org/10.3897/jhr.86.71309>