

# Revision of the European species of *Euplectrus* Westwood (Hymenoptera, Eulophidae), with a key to European species of Euplectrini

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## Abstract

The European species of *Euplectrus* Westwood are comprehensively treated for the first time, using a combination of morphological and DNA data (CO1, the barcode). Ten species are included, seven already described: *E. bicolor* (Swederus), *E. flavipes* (Fonscolombe), *E. intactus* Walker, *E. liparidis* Ferrière, *E. maculiventris* Westwood, *E. nigriceps* Ferrière, *E. phthorimacae* Ferrière, and three new species: *E. carinifer* **sp. n.**, *E. geometricida* **sp. n.**, *E. pallidigaster* **sp. n.** To stabilize the nomenclature a neotype is designated for *E. bicolor*. *Euplectrus intactus* is removed from synonymy under *E. bicolor* and *E. nigriceps* from synonymy under *E. platyhyphenae* Howard, and both are re-established as valid species. Several host records are given and presented in a table, and new geographical records are introduced for previously described species. All host records are from exposed Lepidoptera caterpillars and the dominant host groups are Geometridae and Noctuidae. A key including all European species of tribe Euplectrini is presented. Prior to this paper the identification of European *Euplectrus* species has been difficult and misidentifications have probably been common. Existing host and geographical records in the literature must therefore be treated with great care.

## Keywords

Neotype designation, reevaluation of synonymized species, new species, new host records, new geographical records, gregarious ectoparasitoids, cocoon spinning, Geometridae, Noctuidae, *Platyplectrus*, *Metaplectrus*

## Introduction

Species of the genus *Euplectrus* are found all over the world, with most species occurring in tropical parts (e.g. Hansson et al. 2015). This genus currently includes 199 species, but only five of them are recorded from Europe (Noyes 2018). Even though it is a very small genus in Europe the confusion resulting from misidentifications of specimens, and the unresolved taxonomy and nomenclature on species level, have resulted in many erroneous geographical and biological records in the literature. Most European records, geographical as well as biological, have been attributed to *E. bicolor* Swederus, this is in spite of the unclear identity of this species (very short and bland original description and type material missing – see below). The results presented in this paper necessitate a check, and probable re-evaluation of several records in the literature regarding European *Euplectrus*. Species of *Euplectrus* are parasitoids of Lepidoptera caterpillars, some of which cause damage to cultivated plants, and the accurate identification of the parasitoids on these pests is essential for successful biological control efforts.

The first more comprehensive study of *Euplectrus* in Europe was by Ferrière (1941) who included four species: *E. bicolor*, *E. nigriceps*, *E. cacoeciae*, *E. phthorimaeae*, the three latter were described in the paper. Graham (1963) made an effort to sort out the British fauna of *Euplectrus* but due to unresolved morphological variation he expressed uncertainty regarding the identity of some names. He included three species, *E. intactus* Walker, *E. maculiventris* Westwood, and *E. nigriceps* in a key, but due to total lack of information for the type material of *E. nigriceps* he had doubts about the geographical status of that species. In addition, he mentioned two species: *E. bicolor* with uncertain identity because of lack of type material, and *E. flavipes* (Fonscolombe) that obviously did not occur in Britain but was regarded as a distinct species by Graham. Bouček and Askew (1968) synonymized *E. maculiventris* and *E. intactus* with *E. bicolor*. Zhu and Huang (2002) re-evaluated the status of *E. maculiventris* and removed it from synonymy, and later (2003) included *E. bicolor*, *E. flavipes* and *E. maculiventris* in their study of *Euplectrus* from China, and added *E. liparidis* Ferrière to the list of European species, a species that previously was known only from North Africa.

Due to previous difficulties with the identification of the species and the somewhat unclear nomenclatural situation only distributional and biological information for specimens examined here will be included in this paper.

## Methods

### Imaging

The SEM micrographs are from uncoated specimens and were done with a Hitachi SU 3500, using a backscatter detector. The colour images were made using a Canon camera equipment including an EOS 70D body, MP E-65 macro lens, and macro twin lite MT-24 EX. The camera was attached to a Cognisys stackshot macrorail system. The picture stacking was done with Helicon Focus version 6 software.

## DNA sequencing

For DNA extraction, whole specimens were sent to the Canadian Centre for DNA Barcoding (CCDB) in Guelph, Canada, for DNA extraction and barcode sequencing, and subsequent recovery of vouchers for preparation and morphological study. A complete list of voucher specimens included in the revision is given in Suppl. material S1. DNA extraction, PCR amplification, and sequencing were conducted at the Canadian Centre for DNA Barcoding (CCDB) using standardised high-throughput protocols (Ivanova et al. 2006, deWaard et al. 2008, <http://www.ibolproject.org/resources.php>). The 658 bp target region, starting from the 5' end of the mitochondrial cytochrome c oxidase I (COI) gene, includes the DNA barcode region of the animal kingdom (Hebert et al. 2003). The DNA extracts are stored at the CCDB. Specimens that were successfully sequenced are listed in Suppl. material S1. All specimen data are accessible in BOLD as a single citable dataset ([dx.doi.org/10.5883/DS-EUPLEUR](http://dx.doi.org/10.5883/DS-EUPLEUR)). The data include collecting locality, geographic coordinates, elevation, collector, one or more digital images, identifier, and voucher depository. Sequence data can be obtained through BOLD and include a detailed LIMS report, primer information, and access to trace files. The sequences are also available on GenBank (for accession numbers see Suppl. material S1).

## Data analysis

Sequence divergence statistics were calculated using the Kimura two parameter model of sequence evolution (Kimura 1980). Barcode Index Numbers (BINs) were assigned by the BOLD system, representing globally unique identifiers for clusters of sequences that correspond closely to biological species (Ratnasingham and Hebert 2013). For BIN assignment, a minimum sequence length of 500 bp is required, and sequences between 300 and 500 bp can join an existing BIN but will not create or split BINs. In the present study, BINs were used to delineate Molecular Operational Taxonomic Units (MOTUs) prior to a detailed taxonomic study based on morphological characters. Sequences were aligned using the BOLD Aligner (amino acid-based hidden Markov models). The analyses are based on sequences with a minimum length of 500 bp and <1% ambiguous bases. Genetic distances and summary statistics were calculated using analytical tools in BOLD and are given as mean and maximum pairwise distances for intraspecific variation and as minimum pairwise distances for interspecific variations.

## Abbreviations of morphological terms (Figs 3–6)

DO = largest diameter of one posterior ocellus; HE = height of eye in frontal view; HH = height of head; LC = length of scape; LP = length of petiole; LT = length of hind tarsus; LT1–4 = length of first–fourth tarsomere on hind leg; MS = malar space; OOL = the distance between eye and posterior ocellus; PM = length of postmarginal vein; POL = the distance between posterior ocelli; POO = the distance between posterior ocelli and oc-

cipital margin; ST = length of stigmal vein; TS1 = length of longest hind tibial spur; TS2 = length of shortest hind tibial spur; WE = width of eye; WF = width of frons, in frontal view the largest distance between eyes; WH = width of head, measured across the widest part; WM = width of mouth opening; WP = width of petiole, measured across widest part; WS = width of scape, measured across widest part. Lower face as defined by Gibson (1997), i.e. the part below an imaginary line from eye to eye touching ventral edge of toruli.

### Museum acronyms

MZH	Finnish Museum of Natural History, Zoological Museum, Helsinki, Finland.
MZLU	Museum of Biology (Entomology), Lund University, Lund, Sweden.
NHM	the Natural History Museum, London, United Kingdom.
NHRS	the Natural History Museum, Stockholm, Sweden.
OUMNH	Oxford University Museum of Natural History, United Kingdom.
SMTP	Swedish Malaise Trap Project, Station Linné, Ölands Skogsby, Sweden.
VV	private collection of Veli Vikberg, Turenki, Finland.
ZSM	Zoologische Staatssammlung München, Germany.

### Results

#### Key to the European species of Euplectrini (i.e. Eulophinae species with 1–2 long spurs at apex of hind tibia)

- 1 Scutellum without lateral grooves (e.g. Fig. 48) (genus *Euplectrus*) ..... 2
- Scutellum with lateral grooves (Figs 57, 58) ..... 13
- 2 Head completely dark (Figs 44, 45) ..... *Euplectrus nigriceps* Ferrière
- Head with at least clypeal area pale (e.g. Figs 10, 27) ..... 3
- 3 Entire frons below level of toruli, including part below eye, white to yellowish-white, in males pale area reaches the eye and usually covers part of frons lateral to toruli (e.g. Figs 14, 31), in female pale area leaves at most a narrow dark stripe close to eyes (e.g. Fig. 30) ..... 4
- Frons below toruli with pale area smaller (e.g. Figs 9, 10), pale area never reaches eyes – in some males it almost reaches eyes but leaves a dark stripe close to eyes (Fig. 12) and never reaches up on surface lateral to toruli ..... 7
- 4 Female ..... 5
- Male ..... 6
- 5 Without groove between dorsellum and scutellum (Fig. 37); female gaster with pale area in anterior part as a round spot about as wide as ½ the width of gaster (Fig. 37) ..... *Euplectrus liparidis* Ferrière (♂ unknown)
- With a groove between dorsellum and scutellum (Fig. 52); pale area on female gaster with anterior ½ narrow, narrower than width of petiole, and with posterior

- ½ expanding and as wide as ½ the width of gaster (Fig. 19) .....  
 ..... *Euplectrus geometricida* sp. n.
- 6 Scape 2.8–3.0× (mean = 2.86, n=10) as long as wide (Fig. 14) .....  
 ..... *Euplectrus maculiventris* Westwood
- Scape 2.0–2.5× (mean = 2.24, n=10) as long as wide (Fig. 15) .....  
 ..... *Euplectrus geometricida* sp. n.
- 7 Midlobe of mesoscutum with a distinct and complete median carina (Figs 51, 53), carina sometimes weak or missing close to posterior margin of pronotum and sometimes replaced by a groove close to scutellum; female gaster with apex pale (Figs 22, 35) ..... **8**
- Median carina on midlobe of mesoscutum missing (e.g. Fig. 49) or incomplete (e.g. Fig. 48); female gaster with apex dark (Fig. 8) or pale (Figs 25, 26) ..... **9**
- 8 Female with pale area in anterior ½ of first gastral tergite large and with narrow brown margins, margins about ½ as wide as width of petiole (Fig. 35); male gaster with pale area reaching margin in posterior part thus interrupting lateral brown margin (Fig. 13) ..... *Euplectrus flavipes* (Fonscolombe) (♀, ♂)
- Female with pale area in anterior ½ of first gastral tergite smaller and with wider brown margins, margins on average at least as wide as width of petiole (Fig. 22); male gaster with evenly wide brown uninterrupted margins in anterior ½ (Fig. 16) ..... *Euplectrus carinifer* sp. n. (♀, ♂)
- 9 Female gaster dark yellowish-brown with anterolateral corners dark brown (Fig. 25), in some specimens also with a dark brown round spot posteromedially (Fig. 26); male scutellum with weak and predominantly ±isodiametric meshes ..... *Euplectrus pallidigaster* sp. n. (♀, ♂)
- Female gaster predominantly dark brown (Figs 8, 29, 32); male scutellum with ± elongate meshes, thus appearing more striate than in alternate ..... **10**
- 10 Posterior part of midlobe of mesoscutum wide (Figs 49, 56), ratio width base of midlobe (a)/width base of one sidelobe (b) = 0.80±0.048 (females), 0.80±0.054 (males); midlobe of mesoscutum without median line indicated (Figs 49, 56) ....  
 ..... *Euplectrus intactus* Walker (♀, ♂)
- Posterior part of midlobe of mesoscutum narrow (e.g. Figs 48, 55), ratio width base of midlobe (a)/width base of one sidelobe (b) = 0.57±0.070 (females), 0.55±0.070 (males); median line on midlobe of mesoscutum usually indicated by a median groove or median carina in posterior ½ (Figs 48, 55), sometimes by a weak carina in anterior ½, in some specimens median line indicated just through a change in the reticulation ..... **11**
- 11 Female with pale area on lower frons with upper-lateral part drawn out towards eye and almost reaches eye (Fig. 30); scutellum with isodiametric meshes (Fig. 50) (males key out under couplet 6) ..... *Euplectrus maculiventris* Westwood
- Female with pale area on lower frons not drawn out laterally (Fig. 10), scutellum with elongate or isodiametric meshes, or males ..... **12**

- 12 Reticulation on posterior part of scutellum with elongate meshes, this part of scutellum thus appearing striate (Fig. 48) ..... *Euplectrus bicolor* (Swederus) (♀, ♂)
- Reticulation on posterior part of scutellum with ±isodiametric meshes (Fig. 40)..... *E. phthorimaeae* Ferrière (♀, ♂)
- 13 Hind tibia with one spur at apex.....*Metaplectrus szepligetii* (Erdős) (♀, ♂)
- Hind tibia with two spurs at apex (as in Fig. 36) (genus *Platyplectrus*) ..... 14
- 14 Scutellum smooth and shiny, without reticulation (Fig. 57) ..... 15
- Scutellum reticulate (Fig. 58) ..... 16
- 15 Petiole about twice as long as wide; flagellomeres long in both sexes, e.g. funicular 2&3 in female both 1.3× as long as wide (Fig. 62), in male 1.1 and 1.3× respectively.....*Platyplectrus laeviscuta* (Thomson) (♀, ♂)
- Petiole transverse; flagellomeres short in both sexes, e.g. funicular 2&3 in female both 0.8× as long as wide (Fig. 61), in male 1.0× (Erdős 1966).....*Platyplectrus bouceki* (Erdős) (♀, ♂)
- 16 Occipital margin with a sharp carina (Fig. 59) .....*Platyplectrus pannonica* (Erdős) (♀, ♂)
- Occipital margin rounded (Fig. 60) .....*Platyplectrus chlorocephala* (Nees) (♀, ♂)

**Genus *Euplectrus* Westwood**

*Euplectrus* Westwood, 1832:128. Type species *E. maculiventris* Westwood, 1832:128, by monotypy.

*Diplectron* Dahlbom, 1857:292. Type species *Pteromalus bicolor* Swederus, 1795:204, designated by Gahan and Fagan 1923:46. Synonymized by Dalla Torre 1898:74.

*Pachyscapha* Howard, 1897:159. Type species *P. insularis* Howard, 1897:159, by monotypy. Synonymized by Ferrière 1941:38.

*Rekabia* Cameron, 1904:65. Type species *R. testaceipes* Cameron, 1904:66, by monotypy. Synonymized by Kerrich 1974:636.

*Heteroscapus* Brèthes, 1918:9. Type species *H. ronnai* Brèthes, 1918:10, by monotypy. Synonymized by De Santis 1980:153.

*Heteroscapiscus* Ghesquière, 1946:370. Replacement name for *Heteroscapus* Brèthes.

**Biology**

Species of *Euplectrus* are all parasitoids on Lepidoptera caterpillars that live exposed on their food plant and their life-history is very interesting, including some unique features (e.g. Hansson et al. 2015). Prior to egg-laying the female wasp injects a substance that prohibits further ecdysis by the host caterpillar – moulting would shed the parasitoid

eggs/larvae that are attached to the cuticle. The female wasp then lays her eggs on the host, usually several per host and if the host is large several hundred eggs may be deposited, but occasionally if the host is small, only one egg per caterpillar is laid. The eggs are anchored in the cuticle of the caterpillar, usually on the dorsum. When the wasp larvae hatch they will remain attached to the same spot where the eggs were anchored (Fig. 1), using the egg shell as a pad to which they attach themselves to the host and they are difficult to scrape off. After completed development the wasp larvae pupate on, under or beside the (now) dead host, but before they pupate they spin a loose cocoon (Fig. 2), a feature that is unique within the entire Chalcidoidea. The spin is produced by modified Malpighian tubules and is secreted through the anal opening. The adult wasps emerge after a few days, the number of days depend on the ambient temperature and *Euplectrus* species.

## Species treatments

### *Euplectrus geometricida* sp. n.

<http://zoobank.org/FD397708-4CE5-470E-B979-FC5A90FAABBF>

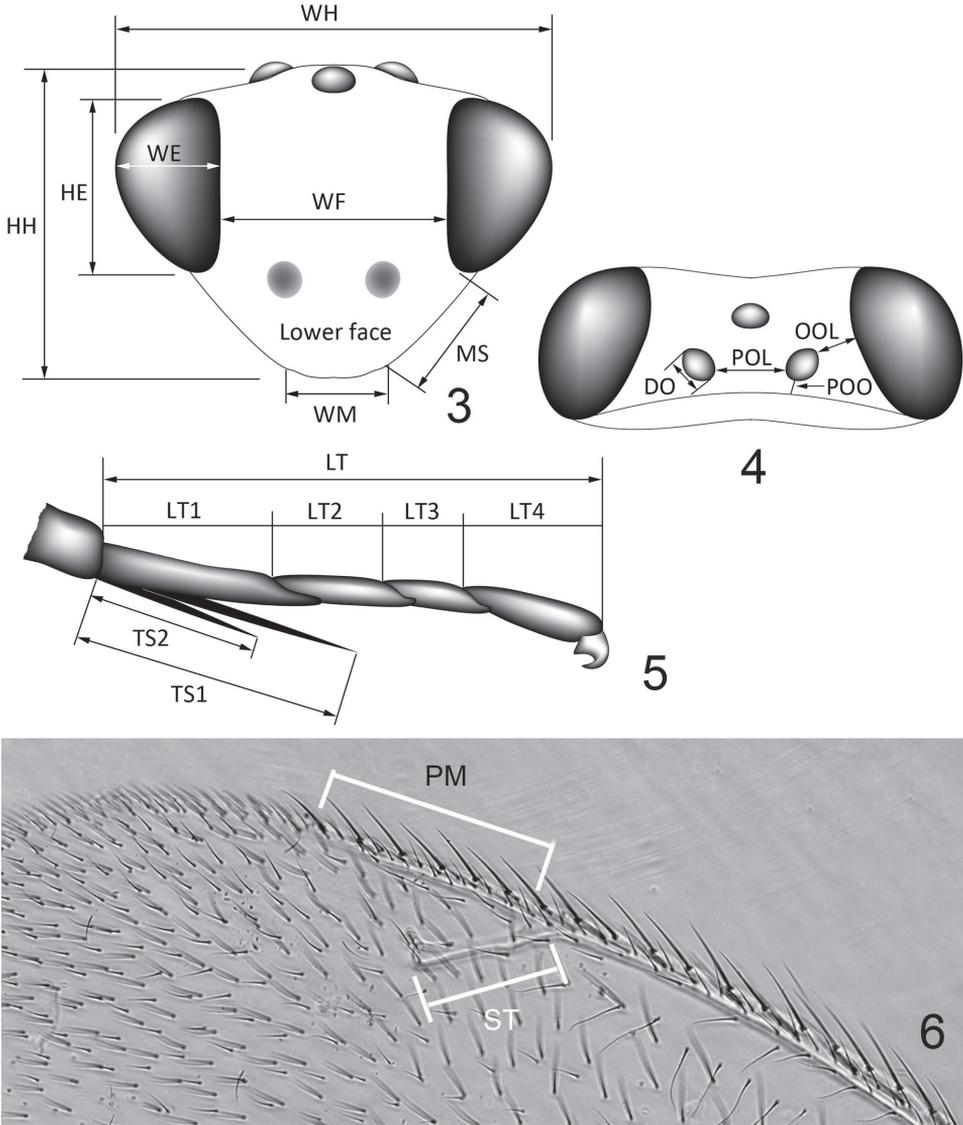
Figures 15, 17–19, 52

**Material.** Holotype female labeled “CZECH REPUBLIC: Mikulcice, 48,808N, 17,094E, 169m, 21-May-2013, M. Volf, Sample BC-ZSM-HYM-23872-E10, CO1-5p 652(0)bp BOLD: ACU3230”, from *Colotois pennaria* on *Carpinus betulus*, in ZSM. Paratypes (133♀ 88♂): **CZECH REPUBLIC** (38♀ 54♂, in MZLU, NHM, ZSM): 1♀ 2♂ with same label data as holotype; following from same locality as holotype but collected 22-May-2013 from *Agriopis marginaria* on *C. betulus* (1♀), 23-May-2013 from *A. marginaria* on *C. betulus* (2♀ 1♂), 27-May-2013 from *Operophtera brumata* on *C. betulus* (2♂), 29-May-2013 from *Alsophila aescularia* on *C. betulus* (2♀), 31-May-2013 from *C. pennaria* on *C. betulus* (1♂), *A. aescularia* on *C. betulus* (1♂), 01-June-2013 from *A. aescularia* on *C. betulus* (1♀); “CZECH REPUBLIC: obora Soutok, Lanzhot, 48,69N, 16,945E, 165m, 08-May-2014, P. Drozd”, from *A. marginaria* on *C. betulus* (13♀ 16♂), *A. aurantiaria* on *C. betulus* (7♀ 2♂), *A. aurantiaria* on *Tilia cordata* (1♀), *Epirrita dilutata* on *C. betulus* (3♀), *Phigalia pilosaria* on *C. betulus* (2♀ 2♂), unidentified Geometridae on *C. betulus* (2♀ 18♂); following from same locality as previous but collected 16-May-2013, from *Agriopis* sp. on *C. betulus* (1♂), 19-May-2013, from unidentified larva on *C. betulus* (2♂), *O. brumata* on *C. betulus* (2♂), 04-May-2014, from *Phigalia pilosaria* on *Acer campestre* (2♂), 05-May-2014, from *A. marginaria* on *C. betulus* (1♀), *O. brumata* on *Quercus cerris* (1♀ 2♂), 09-May-2014, from *A. aurantiaria* on *T. cordata* (1♀). **FINLAND** (44♀ 7♂, in MZH, MZLU): 1♀ “FINLAND, Ab: Lohjan kunta, 6693:334, 21.6.1991, M. Koponen leg.”; 1♀ “FENNIA: EH, Hämeenlinna, 1978, P. Somerma leg.”; 2♀ “SUOMI: EH, Janakkala, 6754:369, 19.8.1979, M. Koponen leg.”; 1♂ “SUOMI: EH, Janakkala, 6760:369, 24.8.1980, M. Koponen leg.”; 1♀ “SUOMI: EH, Lammi, 6773:394, 19.9.1982, M. Koponen leg.”; 1♀ “SUOMI:



**Figures 1–2.** *Euplectrus bicolor* (Swederus): **1** larvae on caterpillar of *Orthosia* sp. (Lepidoptera: Noctuidae) **2** pupae with spin threads. Photo courtesy Špela Modic.

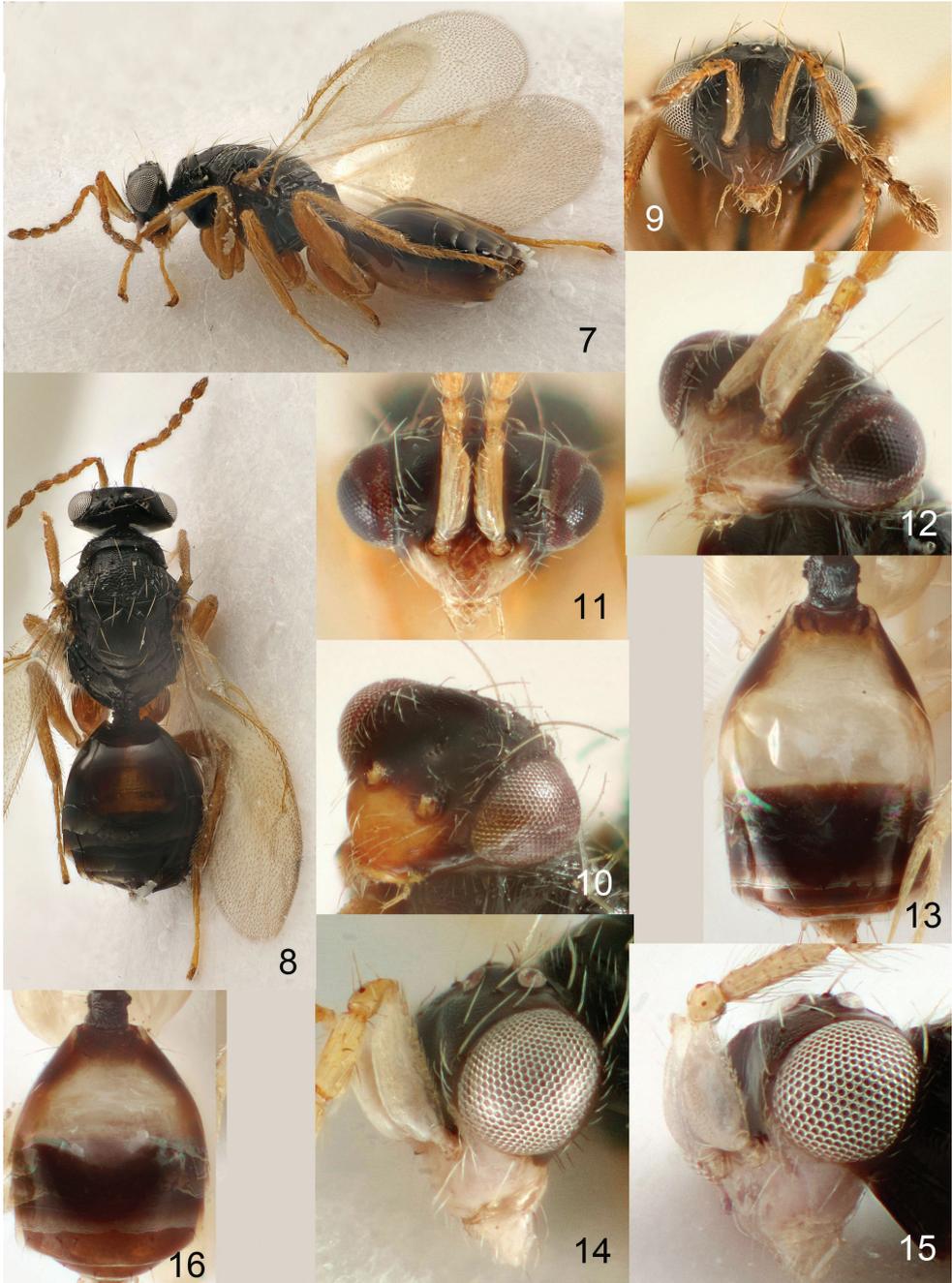
ES, Mikkelin mlk. 6830:501, 7.8.1989, M. Koponen leg.”; 3♀ “SUOMI: ES, Ristiina, 6830:502, 5.8.1978, M. Koponen leg.”; 1♂ “SUOMI: ES, Ristiina, 6826:502, 2.8.1981, M. Koponen leg.”; 1♀ “SUOMI: KP, Vimpeli, 7015:346, 18.8.1982, M. Koponen leg.”; 1♀ “SUOMI: PH, Rautalampi, 6961:484, 17.7.1983, M. Koponen leg.”; 1♀ “SUOMI: PP, Pello, 7248:372, 5.7.1982, M. Koponen leg.”; 1♀ “SUOMI: PP, Yli-Ii, 7248:444, 21.7.1980, M. Koponen leg.”; 1♀ “SUOMI: PP, Ii, 7249:424, 11.8.1982, M. Koponen leg.”; 1♀ “FINLAND, Sa, Ristiina, 6824:503, 10.7.1993, M. Koponen leg.”; 1♀ “SUOMI: U. Helsinki, 6682:383, 6.9.1980, M. Koponen leg.”; 1♀ “SUOMI: U. Hyvinkää, 6716.374, 2.6.1979, M. Koponen leg.”; following from same locality as previous but collected 14.v.1983 (1♀), 19.vi.1983 (1♀), 31.vii.1984 (1♀); 1♀ “SUOMI: U. Nurmijärvi, 6718:381, 17.6.1979, M. Koponen leg.”; 1♀ “SUOMI: U. Nurmijärvi, 6712:370, 11.6.1989, M. Koponen leg.”; 1♀ “SUOMI: U. Nurmijärvi, 8713:386, 28.7.1989, M. Koponen leg.”; 1♂ “SUOMI: U. Nurmijärvi, 6710:381, 1.9.1993, M. Koponen leg.”; 1♂ “SUOMI: V, Vihti, 6717:366, 17.8.1975, M. Koponen leg.”; 1♀ ”Nystad, Hellén” ”29.7.1923”; 1♀ ”Nystad, Hellén” ”26.8.1914”; 1♀ ”Nystad, Hellén” ”25.8.1923”; 1♀ ”FENNIA, Hammarland, 3.8.1953, W. Hellén”; 1♀ ”Sibbo, Norrkulla, 8-11.8.57, Hellén”; 1♀ ”Suomi V Turku, 10.10.1949, E.K. Lahtiperä leg.”; 1♀ ”Kivinebb, K. Ehnberg”; 1♀ ”Fennia, Bobäck, Hellén”; 1♀ ”Fennia, Luumäki, Hellén”; 1♀ ”Fennia, Oa, Maxmo, 4-14.6.46, Hellén”; 1♀ ”Hauho, Hiiriniemi, 14.8.1935, Hellén”; 1♀ ”Hauho, 1935, Hellén”; 1♀ ”Keuru, 14.8.1928, Hellén”; 2♀ ”Terijoki, 25.8.1927, Hellén”; 1♀ ”Parikkala, Laurila, 15.7.1940, Hellén”; 1♀ ”Parikkala, Hellén”; 1♀ ”Vammeljoki, 4.8.1927, Hellén”; 1♀ ”Fennia, Taipalsaari, Hellén”; 1♂ ”P. Pirkkala, Grönb-lom, 11/7 1913”; 1♀ ”Finland, AI, Lemland, Flaka, 1956, Vaselius”; 1♂ ”Fennia, Lemland, 5.8.53, W. Hellén”; 1♂ ”Fennia, Nurmes, 21.7.1939, A. Saarinen”; 1♀ ”Tvärminne, Storå, Storkärret, medio 7.35, e.l. Agr. subtr”, from *Agrotis segetum*. **FRANCE** (20♀ 12♂, in NHM, leg. M.W.R. de V. Graham): 2♂ “FRANCE: Auvergne, nr. Les Essards (oakwood), 7.viii.1973”; 4♀ “FRANCE: Auvergne, S. bank of river Rhone, nr Essards, 7.viii.1973”; 1♀ “FRANCE: B. du Rhone, Fonscolombe (1),



**Figures 3–6.** *Euplectrus* terminology, schematic illustrations: **3** head in frontal view **4** vertex **5** apex of hind leg with tibial spurs and tarsus **6** part of left forewing. For explanation of abbreviations see text above “Abbreviations of morphological terms”.

24.vii.1984”; following from same locality but collected 2.vii.1980 (1♂), 21.vi.1982 (1♂), 10.viii.1983 (1♂), 10.vii.1986 (2♀), 14.viii.1986 (1♂); 1♀ “FRANCE: B. du Rhone, Rognes, 13.vii.1978”; 1♀ “FRANCE: B. du Rhone, nr. Rognes, 24.vii.1974”; 3♀ 4♂ “FRANCE: Drôme, Le Poet-en-Percip (2), 21.viii.1990”; 1♀ “FRANCE: Drôme, Montagne de Bluye, 18.vii.1974”; 1♂ “FRANCE: Haute-Loire, nr. Lubilhac, 20.vii.1977”; 1♀ “FRANCE: Provence, Alpes de Haute, 2km N of Reillane, 21.vii.1978”; 1♀ “FRANCE: Provence, Alpes de Haute, nr. Reillane, 17.vii.1978”;

1♂ “FRANCE: Var: St. Tropez, 6.vi.1980, Bouček”; 1♀ “FRANCE: Vaucluse, nr Bèdoïn (2), 13.vii.1980”, 1♀ from same locality but collected 19.vii.1983; 1♀ “FRANCE: Vaucluse, nr Col de Murs, 27.vii.1975”; 1♀ “FRANCE: Vaucluse, Mt. Ventoux, Massif des Cèdres, 25.viii.1975”; 1♀ “FRANCE: Vaucluse, Sérignan, 29.6.1977”. **ITALY** (1♂): “PIEMONTE, Castel, D.Bosca, 12.viii.1954, A. Goidanich leg” (NHM). **THE NETHERLANDS** (1♀): “NETHERLANDS, Gelderland, Ede, Edense Heide, 52.056N, 5.7E, 30m, 09-Aug-2005”, from *Anarta myrtilli* (ZSM). **POLAND** (3♂): “POLAND, Dybki, Mazovia, 52.7333N. 21.7166E, 108m, 19-May-2016, M. Shaw” (ZSM). **ROMANIA** (1♀): “Romania: Iași, Bârnova Forest, nr Slobozia, 47°00'41"N, 27°36'11"E, 290m, 4.vii.2011, J.S.Noyes” (NHM). **SWEDEN** (20♀ 10♂): 1♀ “Bohuslän, Ljung, Lyckorna, Bo Tjeder, 25/6 -46” (MZLU); 1♀ “Lycksele Lappmark, Vilan, 20.7.1981, K.-J. Hedqvist” (NHM); 2♀ “Lycksele Lappmark, Tärna, 3.8.1956, K.-J. Hedqvist” (NHM); 1♂ “Norrbotten, Haparanda, 23.7.66, A. Sundholm” (MZLU); 1♂ “Skåne, Åhus, 14.7.1957, leg. K.-J. Hedqvist” (NHM); 1♀ 5♂ (one male without head) on same pin “Skåne, Vånga, utkl. 1/7 06” (MZLU); 1♀ “S: Småland, Växjö, Åryd, 3.VII.1989, leg. R. Danielsson (DAYS)” (MZLU); 1♀ “Södermanland, Röm., 1937, A.J.” (MZLU); 1♀ “SWEDEN: Södermanland, Haninge kn, Tyresta, 59.107N, 18.138E, 18.vi.2004, SMTP” (SMTP); 1♀ “SWEDEN: Södermanland, Haninge kn, Tyresta, Urskogsslingan, 59.10N, 18.14E, 28.1–28.iv.2004, SMTP” (SMTP); 1♀ “Södermanland, Tungelsta, 13.6.1957, B. Hansson”, from *Angerona prunaria* (NHM); 1♂ “Runmarö, Geometrid, 8.1916” “F.N.m” [=Frithiof Nordström] (MZLU); 1♀ “SWEDEN: Uppland, Älvkarleby kn, Båtfors, 60°27'38.3"N, 17°19'4.1"E, 26.viii-9.ix.2003, SMTP” (SMTP); 1♀ “Uppland, Frösunda, 21.x.1977, leg. K.-J. Hedqvist” (NHM), following from same locality but collected 24.8.1973 (1♂), 29.7.1977 (1♂) (NHM); 1♀ 1♂ “Uppland, Älvkarleby, Komossen, Kl. 15.6.1981, Nils Ryrholm” “Ur larv av *Cleora repandata*”, from *Alcis repandata* (NHM); 1♀ “SWEDEN: Värmland, Ransäter, Rudstorp, 59.46N, 13.28E, 7–15.vii.2005, SMTP” (SMTP); 1♀ “SWEDEN: Värmland, Munkfors kn, Ransäter, Ransberg herrgård, 59.47N, 13.25E, 12.ix.2005–19.ii.2006, SMTP” (SMTP); 1♀ “SWEDEN: Västerbotten, Vindelns kn, Svartbergets försökspark, 64.138N, 19.471E, 22.ix.2003, SMTP” (SMTP); 1♀ “SWEDEN: Västerbotten, Vindelns kn, Kulbäckslidens trail park, 64°09'16.2"N, 19°35'35.5"E, 1–22.ix.2003, SMTP” (SMTP); 1♀ “Västerbotten., Hällnäs, 20.9.1956, K.-J. Hedqvist” (NHM); 1♀ “Ångermanland, Mjällom, 28.7.1989, K.-J. Hedqvist” (NHM); 1♀ “Sweden: Öland, Jordtorpsåsen, Kvarnbackarna, 56°40'44.3"N, 13°34'51.0"E, 27.vii.2015, C. Hansson” (MZLU); 1♀ “Östergötland., Ändebol, 1–4.9.1955, K.-J. Hedqvist” (NHM). **SWITZERLAND** (1♀): “Helvetia, umgb Aarau, 25.v.1926” (NHM). **UNITED KINGDOM** (9♀ 1♂, in NHM, leg. M.W.R. de V. Graham): 2♀ “ENGLAND: Berks., Wytham Wood (2), 1.ix.1959”, following from same locality but collected 18.vii.1958 (2♀), 25.vii.1958 (3♀); 1♀ “ENGLAND: Bucks., Hell Coppice (5), nr Oakley, 24.vi.1958”; 1♂ “ENGLAND: Cambs., Peterborough, Castor Hanglands, NNR”; 1♀ “ENGLAND: Oxon, Otmoor (3), 12.viii.1955”.



**Figures 7–16.** *Euplectrus* spp.: **7–12** *E. bicolor* (Swederus) **7–9** neotype, female **7** habitus lateral **8** habitus dorsal **9** head frontal **10** non-type female, head front-lateral **11** head frontal, male **12** head front-lateral, male **13** *E. flavipes* (Fonscolombe), gaster dorsal, male **14–15** head including scape lateral, male **14** *E. maculiventris* Westwood **15** *E. geometricida* sp. n. **16** *E. carinifer* sp. n., gaster dorsal, male.

**Diagnosis.** Entire frons below level of toruli white to yellowish white, including part below eye (Figs 17, 18), some specimens with a very narrow dark stripe close to eyes; male scape 2.0–2.3× as long as wide; reticulation on median part of scutellum with elongate meshes (Fig. 52); with a groove between scutellum and dorsellum (Fig. 52).

**Description** (holotype female). Length of body 2.4 mm, female paratypes 1.9–2.4 mm. Antenna with scape yellowish-white with apical  $\frac{1}{3}$  yellowish-brown, pedicel and flagellum yellowish-brown. Mandibles and palpi yellowish-white. Head black, lower face yellowish-white, reaching to eyes and also below eyes (Fig. 17). Frons smooth, medially with a reticulate band reaching from eye to eye; close to eyes with scattered setae in lower  $\frac{1}{2}$  (Fig. 17). Vertex smooth and shiny. Occipital margin with a carina behind ocellar triangle.

Mesosoma black and shiny; midlobe with raised and strong reticulation, meshes isodiametric, midline on midlobe of mesoscutum indicated by a carina (Fig. 52), carina sometimes replaced by a change in the reticulation in posterior  $\frac{1}{3}$ . Scutellum 1.0× as long as wide; with strong reticulation, meshes elongate (Fig. 52). Dorsellum with a deep groove along anterior margin (Fig. 52), groove medially 0.2× as long as length of dorsellum. Propodeum smooth and shiny (Fig. 52); anteromedially with a semicircular cup that is strongly raised in posterior part; propodeal callus with 19 setae. Legs yellowish-white, except yellowish-brown hind coxa and hind femur. Forewing: costal cell with two rows of setae on ventral surface, and margin with six setae close to marginal vein; with 15 admarginal setae.

Gaster dark brown with an inverted T-shaped white area in anterior  $\frac{1}{2}$  (Fig. 19).

Ratios. HE/MS/WM = 1.8/1.0/1.1; POL/OOL/POO = 9.7/4.3/1.0; OOL/DO = 1.4; WE/WF/WH/HH = 1.0/2.1/3.9/2.8; WH/WT = 1.0; PM/ST = 1.8; TS1/TS2/LT/LT1/LT2/LT3/LT4 = 4.6/3.1/7.4/2.4/1.4/1.0/1.9; LP/WP = 0.9; MM/LG = 1.3.

Male. Length of body 1.7–2.4 mm. Scape white, 2.0–2.3× as long as wide, widest medially, with sensory pores along entire ventral margin, sensory area white. Gaster with anterior  $\frac{1}{2}$ – $\frac{2}{3}$  white with narrow dark brown margins, posterior part dark brown. Otherwise similar to female.

Ratios. LC/WS = 2.0–2.3, LP/WP = 1.0.

**Hosts. Geometridae:** *Agriopsis aurantiaria* (Hübner) on *Carpinus betulus* and *Tilia cordata*, *Agriopsis marginaria* (Fabricius) on *C. betulus*, *Alcis repandata* (L.), *Alsophila aescularia* (Denis & Schiffermüller) on *C. betulus*, *Angerona prunaria* (L.), *Colotois penaria* (L.) on *C. betulus*, *Epirrita dilutata* (Denis & Schiffermüller) on *C. betulus*, *Maccaria brunneata* (Thunberg) on *Vaccinium myrtillus*, *Operophtera brumata* (L.) on *C. betulus* and *Quercus cerris*, *Phigalia pilosaria* (Denis & Schiffermüller) on *Acer campestre* and *C. betulus*. **Noctuidae:** *Agrotis segetum* (Denis & Schiffermüller), *Anarta myrtilli* (L.), *Orthosia opima* (Hübner) on *Vaccinium* sp.

**Distribution.** Czech Republic, Finland, France, Italy, the Netherlands, Poland, Romania, Sweden, Switzerland, United Kingdom.

**Etymology.** Named after preferred target group, geometrid caterpillars. From the Latin suffix *-cida*, which means “killer”.

**Genetic data.** The species exhibits high levels of intraspecific variation, with a maximum of 11.6%, but a clear separation from the next neighbour, with a distance of 10.8% to the next neighbour species, *E. carinifer* (Fig. 63). The populations of the species segregate into several subclusters, each with its own Barcode Index Number. Four of the six subclusters were recorded from one country (Czech Republic) whereas two were recorded in two or more countries (Suppl. material S1). The presence of several distinct subclusters within the species indicates the presence of more than one species, but morphological analysis did not reveal any reliable characters for separating the species.

***Euplectrus carinifer* sp. n.**

<http://zoobank.org/4D4F531F-2F74-4D5C-9FD8-F8A466716695>

Figures 16, 20–22, 53

**Material.** Holotype female labelled “CZECH REPUBLIC: Mikulcice, 48.808N, 17.094E, 169m, 21-May-2013, M. Volf, Sample BC-ZSM-HYM-27734-H05, CO1-5p 591 (0)bp BOLD: ACU2970”, from *Amphipyra pyramidea* on *Carpinus betulus*, in ZSM. Paratypes (2♀ 6♂): 1♂ with same label data as holotype (ZSM); following from same locality as holotype but collected 22-May-2013, from *Orthosia cruda* on *C. betulus* (2♂, MZLU, NHM), *Perigrapha munda* on *C. betulus* (2♀, MZLU, NHM), 24-May-2013 from *P. munda* on *C. betulus* (2♂, ZSM); 1♂ “CZECH REPUBLIC: obora Soutok, Lanzhot, 48,69N, 16,945E, 165m, 16-May-2013, P. Drozd”, from *Carcina quercana* on *Acer campestre* (ZSM); 3♀ “NETHERLANDS ZH Delft 30-VIII-2009 e.l., leg S. Wegh, ex *Autographa gamma*” (ZSM).

**Diagnosis.** Frons below level of toruli with pale area not extending laterally to the eye but with a wide dark stripe between pale area and eye, in both sexes (Figs 20, 21); midlobe of mesoscutum with a complete median carina (Fig. 53); with a narrow groove between scutellum and dorsellum (Fig. 53); female gaster with wide brown margins (Fig. 22).

**Description** (holotype female). Length of body 2.0 mm, female paratypes 2.1–2.2 mm. Antennal scape yellowish-white with apical ½ yellowish-brown, pedicel and flagellomeres yellowish-brown. Mandibles and palpi yellowish-white. Head black with yellowish-brown clypeal area, pale area does not extend to eyes (Fig. 20). Frons smooth except a reticulate band closer to anterior ocellus than to toruli reaching from eye to eye, close to eyes with two rows of setae (Fig. 20). Vertex smooth and shiny. Occipital margin with a carina behind ocellar triangle.

Mesosoma black and shiny; midlobe with raised and strong reticulation, meshes ± isodiametric, midlobe of mesoscutum with a complete median carina (Fig. 53). Scutellum 1.0× as long as wide; with engraved reticulation, meshes small and isodiametric in median part and larger and elongate in lateral part, except smooth and shiny posterior margin (Fig. 53). Dorsellum with a narrow groove along anterior margin (Fig. 53), groove medially 0.3× as long as length of dorsellum. Propodeum smooth and shiny (Fig. 53); anteromedially with a triangular cup that is strongly raised in posterior part; propodeal callus with

12 setae. Legs pale yellowish-brown. Forewing: costal cell with two rows of setae on ventral surface, and margin with four setae close to marginal vein; with 14 admarginal setae.

Gaster dark brown, anterior ½ with a wide white stripe medially, stripe 2× as wide as width of petiole and expanding in posterior part, and with apex pale (Fig. 22).

Ratios. HE/MS/WM = 2.1/1.0/1.0; POL/OOL/POO = 6.3/2.9/1.0; OOL/DO = 1.4; WE/WF/WH/HH = 1.0/2.4/4.2/3.2; WH/WT = 1.0; PM/ST = 1.3; TS1/TS2/LT/LT1/LT2/LT3/LT4 = 3.5/2.2/7.2/2.0/1.6/1.0/2.0; LP/WP = 1.0; MM/LG = 1.2.

Male. Length of body 1.8–2.0 mm. Scape slightly enlarged, widest medially, with sensory pores along entire ventral margin. Similar to female except gaster with anterior ½ white with dark brown lateral margins, posterior ½ dark brown (Fig. 16).

Ratios. LC/WS = 2.8–3.0, LP/WP = 1.0.

**Hosts. Noctuidae:** *Amphipyra pyramidea* (L.) on *Carpinus betulus*, *Autographa gamma* (L.), *Orthosia cruda* (Denis & Schiffermüller) on *C. betulus*, *Perigrapha munda* (Denis & Schiffermüller) on *C. betulus*. **Depressariidae:** *Carcina quercana* (Fabricius) on *Acer campestre*.

**Distribution.** Czech Republic, the Netherlands.

**Etymology.** Named after complete median carina on midlobe of mesoscutum. From the Latin *carina* (=keel) and the suffix *-fer* (=carry).

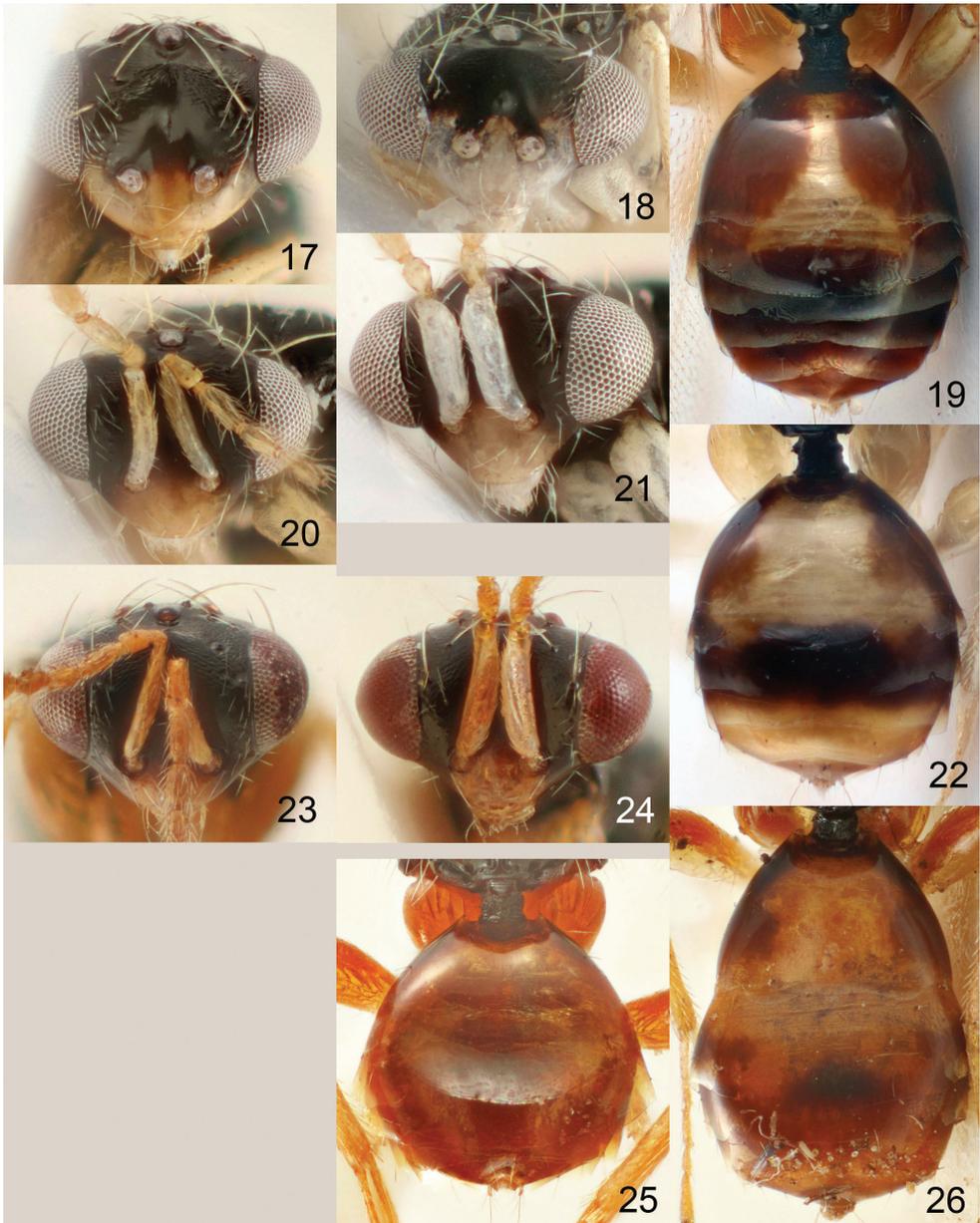
**Genetic data.** The species consists of several subclusters, each assigned a different BIN by the BOLD system (Fig. 63) and a maximum intraspecific variation of 8.7%. Three of the subclusters occur in the Czech Republic, whereas the fourth was recorded from the Netherlands (Suppl. material S1). The high levels of intraspecific variation suggest the presence of more than one species, but in absence of reliable morphological characters for separating the MOTUs the populations are treated as a single species until more material from other regions and additional genetic data will allow a more thorough examination of the species status of each of the populations.

### *Euplectrus pallidigaster* sp. n.

<http://zoobank.org/B368575A-16F2-4C75-ADFB-25C61DD122B9>

Figures 23–26, 54

**Material.** Holotype female labelled "FRANCE: B du Rhone [possibly meaning "Bouches-du-Rhone], Fonscolombe, 20.vii.1979", in NHM. Paratypes (20♀ 8♂): **FRANCE** (17♀ 8♂, in NHM, MZLU, leg. M.W.R. de V. Graham): following from same locality as holotype but collected 13.vii.1984 (1♀), 1.viii.1984 (1♀), 24.vi.1986 (1♀), 1.vii.1986 (1♀), 10.vii.1986 (1♀), 17.vii.1986 (1♀); 4♀ "FRANCE: Drome, Le Poet-en-Percip, (2), 3.viii.1990", 1♂ from same locality but collected 21.viii.1990; 1♀ 2♂ "FRANCE: Gard, Causse de Blandas, (2), 8.viii.1984"; 1♀ "FRANCE: Gard, Estezargues, nr. Remoulins, on elm leaf, 23.vi.1977"; 1♂ "FRANCE: Vaucluse, Beauregard, nr Gigondas, 19.ix.1989"; 1♀ "FRANCE: Vaucluse, nr Bèdoin, (1), 20.vii.1981", following from same locality but collected 11.vii.1980 (1♀), 15.vii.1981 (2♂), 22.vii.1981 (1♀), 28.vii.1981 (1♂); 2♀ "FRANCE: Vaucluse, Mt Ventoux, Col de Perrache, (2), 6.viii.1979"; 1♂ "FRANCE: Vaucluse, Mt Ventoux, about Rte. de Per-



**Figures 17–26.** *Euplectrus* spp.: **17–19** *E. geometricida* sp. n., paratypes **17** head frontal, female **18** head frontal, male **19** gaster dorsal, female **20–22** *E. carinifer* sp. n., paratypes **20** head frontal, female **21** head frontal, male **22** gaster dorsal, female **23–26** *E. pallidigaster* sp. n., paratypes **23** head frontal, female **24** head frontal, male **25** gaster dorsal, female **26** gaster dorsal, female.

rache, (3), 11.viii.1976". **ITALY** (2♀): 1♀ "ITALY, Malcesine, 200–300m, 23.4.1977, leg. V. Vikberg" (MZH); 1♀ "ITALIA: Varazze, nr Genova, 4.IX.71, Bouček" (NHM). **SPAIN** (1♀): "SPAIN: Barcelona, Calella d. Costa, VI.1971, Bouček" (NHM).

**Diagnosis.** Frons with clypeal area yellowish-brown (Figs 23, 24); scutellum with engraved reticulation, meshes  $\pm$ isodiametric (Fig. 54); without a groove between scutellum and dorsellum (Fig. 54); female gaster dark yellowish-brown with anterolateral corners dark (Fig. 25), in some specimens also with a round black spot in medio-posterior part (Fig. 26).

**Description** (holotype female). Length of body 1.9 mm, female paratypes 1.6–2.6 mm. Antenna yellowish-brown, flagellomeres 4–6 slightly darker than scape and pedicel. Mandibles and palpi yellowish-white. Head black and shiny, with clypeal area yellowish-brown (Fig. 23). Face and median part of frons smooth, remaining part of frons with weak reticulation, close to eyes with two rows of setae (Fig. 23). Vertex smooth and shiny. Occipital margin with a carina behind ocellar triangle.

Mesosoma black and shiny; midlobe with raised and strong reticulation, meshes slightly transverse, midlobe of mesoscutum with a weak median groove in posterior  $\frac{1}{3}$  (Fig. 54). Scutellum 1.0 $\times$  as long as wide; with engraved reticulation, meshes slightly elongate, except smooth and shiny posterior margin (Fig. 54). Without groove between scutellum and dorsellum (Fig. 54). Propodeum smooth and shiny medially, with weak reticulation laterally (Fig. 54); anteromedially with a semicircular cup that is raised in posterior part; propodeal callus with 10 setae. Legs yellowish-brown. Forewing: costal cell with two irregular rows of setae on ventral surface, and margin with five setae close to marginal vein; with 13 admarginal setae.

Gaster circular, dark yellowish-brown with anterolateral corners dark brown (Fig. 25).

Ratios. HE/MS/WM = 2.1/1.2/1.0; POL/OOL/POO = 8.0/4.3/1.0; OOL/DO = 1.6; WE/WF/WH/HH = 1.0/2.4/4.2/3.1; WH/WT = 1.0; PM/ST = 1.7; TS1/TS2/LT/LT1/LT2/LT3/LT4 = 4.1/2.9/6.9/2.3/1.4/1.0/1.3; LP/WP = 0.8; MM/LG = 1.3.

**Variation.** Several female paratypes have a dark round spot posteromedially on gaster (Fig. 26).

Male. Length of body 1.7–2.5 mm. Scape slightly enlarged, widest medially, with sensory pores along entire ventral margin. Similar to female except scutellum with  $\pm$ isodiametric meshes, and gaster with posterior  $\frac{1}{2}$  dark brown.

Ratios. LC/WS = 2.7–2.9, LP/WP = 1.0–1.1.

**Hosts.** Unknown.

**Distribution.** France, Italy, Spain.

**Etymology.** From the Latin *pallidus*, meaning pale, referring to the predominantly pale gaster in female.

**Genetic data.** No specimens of the species were available for genetic analysis.

### *Euplectrus bicolor* (Swederus)

Figures 1–2, 7–12, 48, 55

*Pteromalus bicolor* Swederus, 1795:204. Neotype female, **designated here**, in MZLU. Combined to *Eulophus* by Walker (1839:173), and to *Euplectrus* by Haliday (1844:297).

*Elachertus albiventris* Spinola, 1811:151. Combined to *Eulophus* by Haliday (1842:plate J) and to *Euplectrus* by Walker (1872b:112); synonymized with *E. bicolor* by Bouček and Askew (1968:15).

**Material.** Type material: Neotype female labelled “Sweden: Skåne, Kranke, Ekskogen, 55°41'10.3N, 13°27'40.2E, 5.vii.2015, C. Hansson”, ”BC-ZSM-HYM-25460-C11” in MZLU. Additional material (440♀ 306♂): Finland: 13♀ 8♂ (VV), 163♀ 125♂ (MZH, MZLU), this material includes 1♀ 1♂ from *Eugraphe subrosea*, 1♀ 4♂ from *Agrotis* sp., 4♀ 14♂ from *Orthosia opima*, 1♀ from *Xylina* sp., 4♀ 2♂ from a “polyphagous noctuid”; France: 18♀ 4♂ (NHM); Greece: 1♀ (MZLU); Hungary: 1♂ (MZLU); Norway: 1♀ 1♂ (VV); Slovenia 6♂ (MZLU), this material includes 6♂ from *Orthosia* sp. on raspberries (*Rubus idaeus*); Sweden: 179♀ 211♂ (MZLU, NHM, ZSM); United Kingdom: 65♀ 54♂ (NHM), this material includes 6♀ from *Diarsia mendica*, 7♀ 14♂ from *Mamestra brassicae*, 9♀ 26♂ from *Polia hepatica*, 14♀ 8♂ from *Polia nebulosa*. Detailed geographic information of all barcoded specimens is listed in Suppl. material S1.

**Diagnosis.** Frons below level of toruli with pale area not extending laterally to the eye but with a dark stripe between pale area and eye, in the female dark area is wider (Figs 9, 10) than in the male (Figs 11, 12); midline on midlobe of mesoscutum usually indicated by either a median carina (Fig. 48) or a median groove in posterior ½, in some specimens midline indicated just through a change in the reticulation; posterior part of midlobe mesoscutum narrow (Figs 48, 55), ratio width base of midlobe (a)/width base of one sidelobe (b) =  $0.57 \pm 0.070$  (female),  $0.55 \pm 0.070$  (male), width base of midlobe/width base of entire mesoscutum =  $0.22 \pm 0.019$  (female),  $0.21 \pm 0.019$  (male), n = 10 for female and male respectively.

**Description** (neotype). Length of body 2.8 mm (2.0–3.1 mm in additional material). Antenna with scape yellowish-brown with dorsal edge pale brown, pedicel and flagellomeres 1+2 yellowish-brown, flagellomeres 3–6 pale brown. Mandibles and palpi yellowish-brown. Head black and shiny, lower face with median part yellowish-brown reaching laterally to level of outer edge of toruli (Figs 9, 10). Frons smooth except a reticulate band closer to anterior ocellus than to toruli, reaching from eye to eye, close to eyes with two rows of setae (Fig. 9). Vertex smooth and shiny. Occipital margin with a carina behind ocellar triangle.

Mesosoma black and shiny; midlobe with raised and strong reticulation, meshes isodiametric, midline on midlobe of mesoscutum usually indicated by either a median carina (Fig. 48) or a median groove in posterior ½, in some specimens midline indicated just through a change in the reticulation. Scutellum 0.9× as long as wide; with engraved reticulation, meshes elongate, except smooth and shiny posterior margin (Fig. 48). Dorsellum with a very narrow groove along anterior margin (Fig. 48), groove medially 0.1× as long as length of dorsellum. Propodeum smooth and shiny medially, with very weak reticulation laterally (Fig. 48); anteromedially with strongly raised triangular cup in posterior part; propodeal callus with 17 setae. Legs yellowish-brown. Forewing: costal cell with two rows of setae on ventral surface, and margin with four setae close to marginal vein; with 17 admarginal setae.

Gaster dark brown with a yellowish-brown spot in anteromedian part (Fig. 8).

Ratios. HE/MS/WM = 1.8/1.0/1.1; POL/OOL/POO = 9.7/5.3/1.0; OOL/DO = 1.5; WE/WF/WH/HH = 1.0/3.0/5.3/3.6; WH/WT = 1.0; PM/ST = 1.6; TS1/TS2/LT/LT1/LT2/LT3/LT4 = 3.6/2.3/2.3/1.3/1.0/1.8; LP/WP = 0.8; MM/LG = 0.9.

Male. Length of body 1.7–2.4 mm. Scape slightly enlarged, widest medially, with sensory pores along entire ventral margin. Similar to female except wider pale clypeal area (Figs 11, 12), wider scape, longer petiole.

Ratios. LC/WS = 3.1–3.4, LP/WP = 1.0–1.2.

**Hosts.** *Agrotis* sp., *Diarsia mendica* (Fabricius), *Eugraphe subrosea* (Stephens), *Mamestra brassicae* (L.), *Orthosia opima* (Hübner), *Orthosia* sp. on raspberries (*Rubus idaeus*), *Polia hepatica* (Clerck), *Polia nebulosa* (Hufnagel), *Xylina* sp., a “polyphagous noctuid”. All records are from caterpillars of the Noctuidae.

**Distribution.** Sweden (Swederus 1795), Finland, France, Greece, Hungary, Norway, Slovenia, United Kingdom (new/confirmed records).

**Remarks.** Neotype designation: the original type material for *E. bicolor* is lost (Graham 1963). Presumably it was originally in the Natural History Museum in Stockholm (Sweden), but cannot be found there. When Swederus described *E. bicolor* he was very parsimonious with information, which was as usual at that time. The description is very short and fits any European species of *Euplectrus*. Biological and geographical information were not included. Swederus was working in Sweden but made scientific trips to several European countries (Waldeck 2018) and it is difficult to be sure from where he had the material forming the base for the description. However, since Swederus was Swedish it is probable that he had access to Swedish material. Therefore, the neotype is selected from Swedish material, and it is selected from material belonging to the species that appears to be the most common in this country. The neotype has a DNA barcode of 621 bp and belongs to one of the two haplotypes that were found within the species (Fig. 63).

**Genetic data.** Genetically analysed specimens of *E. bicolor* exhibited comparatively high levels of intraspecific variation (maximum 6.5%) but with a distinct gap to the nearest neighbours (*E. intactus*, 10.9% and *E. carinifer*, 10.2%) (Fig. 63). The analysed specimens, all from Sweden, fall into two genetic clusters that occur sympatrically (Suppl. material S2). The absence of morphological characters to separate the two haplotypes does not preclude the possibility that *E. bicolor* consists of two or more species, but analysis of material of other populations and ideally additional (nuclear) gene regions will be required to clarify the status of each population.

### *Euplectrus flavipes* (Fonscolombe)

Figures 13, 33–35, 51

*Spalangia flavipes* Fonscolombe, 1832:299. Lectotype female, designated by Bouček (1970:88) in OUMNH, examined. Combined to *Euplectrus* by Fonscolombe (1840:192).

*Euplectrus cacoeciae* Ferrière, 1941:42. Holotype female in NHM, examined. Synonymized with *E. flavipes* by Bouček (1970:88).

**Material** (74♀ 39♂). Bosnia/Hercegovina: 1♀ (NHM); Cyprus: 19♀ 11♂ (NHM), this material includes 1♀ from *Spodoptera litura*, 6♀ 4♂ from *Spodoptera exigua*, 6♀ ♂ from *Heliothis* sp. on alfalfa (*Medicago sativa*), 22♀ ♂ from *Plusia* sp. on alfalfa; Czech Republic: 20♀ 14♂ (MZLU, ZSM), this material includes 13♀ 8♂ from *Alsophila aescularia* on *Carpinus betulus* and 2♀ and *Populus alba*, 1♂ from *Carcina quercana* on *C. betulus*, 1♂ from *Cosmia trapezina* on *C. betulus*, 3♀ from *Cyclophora annularia* on *Acer campestre*; France: 15♀ 9♂ (NHM, ZSM), this material includes 1♀ 2♂ from *Alsophila aescularia*, 1♀ from *Colobochyla salicalis*, 1♀ 1♂ from unknown caterpillar on *Sonchus* sp; Greece: 1♀ (MZLU); Hungary: 1♀ (MZLU); Italy: 1♀ (NHM); Macedonia: 1♀ (NHM); Romania: 6♀ (MZLU; NHM); Serbia: 3♀ (NHM); Slovenia: 2♀ 1♂ (MZLU, NHM), this material includes 1♀ from unidentified noctuid caterpillar on tomato; Spain: 1♀ 2♂ (NHM); Switzerland: 1♀ 1♂ (MZLU), this material includes 1♀ 1♂ from *Mamestra brassicae*; Turkey: 2♀ 1♂ (NHM), this material includes 2♀ 1♂ from *Plusia* sp. Detailed geographic information of all barcoded specimens is listed in Suppl. material S1.

**Diagnosis.** Frons below level of toruli with pale area not extending laterally to the eye but with a wide dark stripe between pale area and eye in both sexes (Figs 33, 34); midlobe of mesoscutum with a complete median carina (Fig. 51); female with pale area in anterior ½ of first gastral tergite large and with narrow brown margins, margins about ½ as wide as width of petiole (Fig. 35); male gaster with pale part in anterior ½ reaching margin in posterior part, thus interrupting the dark lateral margins of the gaster (Fig. 13); female gaster with apex pale (Fig. 35).

**Hosts. Tortricidae:** *Archips rosana* (L.) (Ferrière 1941). **New/confirmed records: Depressariidae:** *Carcina quercana* (Fabricius) on *Carpinus betulus*. **Erebidae:** *Colobochyla salicalis* (Denis & Schiffermüller); **Geometridae:** *Alsophila aescularia* (Denis & Schiffermüller) on *C. betulus* and *Populus alba*; *Cyclophora annularia* (Fabricius) on *Acer campestre*; **Noctuidae:** *Cosmia trapezina* (L.) on *C. betulus*; *Heliothis* sp.; *Mamestra brassicae* (L.); *Plusia* sp.; *Spodoptera exigua* Hübner; *Spodoptera litura* Fabricius, unidentified noctuid caterpillar on tomato (*Solanum lycopersicum*).

**Distribution.** Bulgaria (Ferrière 1941), France (Fonscolombe 1832), Bosnia/Hercegovina, Cyprus, Czech Republic, Greece, Hungary, Italy, Macedonia, Romania, Serbia, Slovenia, Spain, Switzerland, Turkey (new/confirmed records). This species seems to occur only in Central and South Europe, but not in northern Europe. Hedqvist (2003) listed *E. flavipes* from Sweden, from the province of Södermanland. One of us (CH) has seen this material in coll. Hedqvist (now in NHM), a reared series consisting of 15 females and four males and they are misidentified. Instead these specimens belong to *E. bicolor*.

**Genetic data.** *Euplectrus flavipes* consists of eight subclusters, each of which was assigned a different BIN by the BOLD system (Fig. 63). The subclustering coincides with a higher than usual intraspecific variation of 5.3%. Geographic subclustering in this species is prominent and four of the haplotypes have been recorded from a single country only, whereas the other haplotypes occurred in two or more countries. In

Romania, three haplotypes, each with a different BIN, represented by four specimens, were recorded (Suppl. materials S1, S2). The analysed specimens do not represent the full distributional range of the species, and it is therefore to be expected that more haplotypes are present in the species. A more comprehensive sampling regime and analysis of more material and additional gene regions is required to assess the status the genetically different populations of *E. flavipes*.

### ***Euplectrus intactus* Walker, revised status**

Figures 27–29, 49, 56

*Euplectrus intactus* Walker, 1872a:102. Lectotype female in NHM, examined. Synonymized with *E. bicolor* by Bouček & Askew, (1968: 15).

**Material** (26♀ 18♂). Belgium: 3♀ 3♂ (ZSM), this material includes 3♀ 3♂ from *Pieris rapae*; France: 1♀ 2♂ (NHM), this material includes 2♂ from a caterpillar on *Artemisia vulgaris*; Hungary: 2♀ 9♂ (MZLU, NHM); Romania: 1♀ (NHM); Sweden: 7♀ 1♂ (MZLU, NHM); United Kingdom: 12♀ 5♂ (NHM), this material includes 1♀ from *Noctua comes* on *Corylus avellana*.

**Diagnosis.** Frons below level of toruli with pale area not extending laterally to the eye but with a wide dark stripe between pale area and eye in both sexes (Figs 27, 28); midlobe of mesoscutum usually without median groove or carina (Fig. 49, 56) (sometimes with a weak median groove at very base); posterior part of midlobe mesoscutum wide (Fig. 56), ratio width base of midlobe (a)/width base of sidelobe (b) =  $0.80 \pm 0.048$  (female),  $0.80 \pm 0.054$  (male), width base of midlobe/width base of entire mesoscutum =  $0.29 \pm 0.018$  (female),  $0.28 \pm 0.014$  (male), n=9 for female and male respectively. Very similar to *E. bicolor*, distinguished from this species by the wide posterior part of midlobe of mesoscutum.

**Hosts. Noctuidae:** *Noctua comes* Hübner on *Corylus avellana*. **Pieridae:** *Pieris rapae* (L.). From an unidentified caterpillar on *Artemisia vulgaris*.

**Distribution.** France (Corsica) (Walker 1872a), Belgium, Hungary, Romania, Sweden, United Kingdom (new/confirmed records).

**Remarks.** *Euplectrus intactus* was synonymized with *E. bicolor* by Bouček and Askew (1968), but DNA-data and a renewed analysis of the morphology both support that these are different species.

**Genetic data.** Barcoded specimens of *Euplectrus intactus* exhibited an intraspecific variation of 6.9% and a pronounced geographic subclustering (NJ-tree, Suppl. material S2). One of the nine different haplotypes in *E. intactus* was recorded from four countries (specimens with BIN BOLD:ACR7308 from Hungary and Romania, Sweden and the UK, Fig. 63). The other eight haplotypes were recorded from a single country each. More than one haplotype occurs in Sweden (four haplotypes), Belgium (two haplotypes), and Hungary (three haplotypes). As in the other *Euplectrus* species with high haplotype divergence, a broader sampling from different populations and geographic regions is required to clarify the status of different haplotypes of the species.



**Figures 27–35.** *Euplectrus* spp.: **27–29** *E. intactus* (Walker) **27** head frontal, female **28** head frontal, male **29** gaster dorsal, female **30–32** *E. maculiventris* Westwood **30** head frontal, female **31** head frontal, male **32** gaster dorsal, female **33–35** *E. flavipes* (Fonscolombe) **33** head frontal, female **34** head frontal, male **35** gaster dorsal, female.

### *Euplectrus liparidis* Ferrière

Figures 36–38

*Euplectrus liparidis* Ferrière, 1941:43. Holotype female in NHM, examined.

**Diagnosis.** Female with frons below level of toruli completely pale, pale area reaching from eye to eye (Fig. 38); midlobe of mesoscutum without median groove or carina

(Fig. 37); reticulation on scutellum with elongate meshes (Fig. 37); without groove between dorsellum and scutellum (Fig. 37); female gaster with pale area in anterior part as a round spot about as wide as  $\frac{1}{2}$  the width of gaster (Fig. 37).

**Hosts.** From larva of *Lymantria dispar* (L.) (Erebidae) (Ferrière 1941). This host is odd as the larvae are hairy, whereas larvae of all other hosts accounted for in this article are naked.

**Distribution.** Algeria (Ferrière 1941), Czech Republic & Italy (Zhu and Huang 2003).

**Genetic data.** No specimens of the species were available for genetic analysis.

### *Euplectrus maculiventris* Westwood

Figures 14, 30–32, 50

*Euplectrus maculiventris* Westwood, 1832:128. Lectotype female in OUMNH, examined. Combined to *Eulophus* by Haliday (1842:plateJ); synonymized with *E. bicolor* by Bouček and Askew (1968:14); revalidated by Zhu and Huang (2002:134).

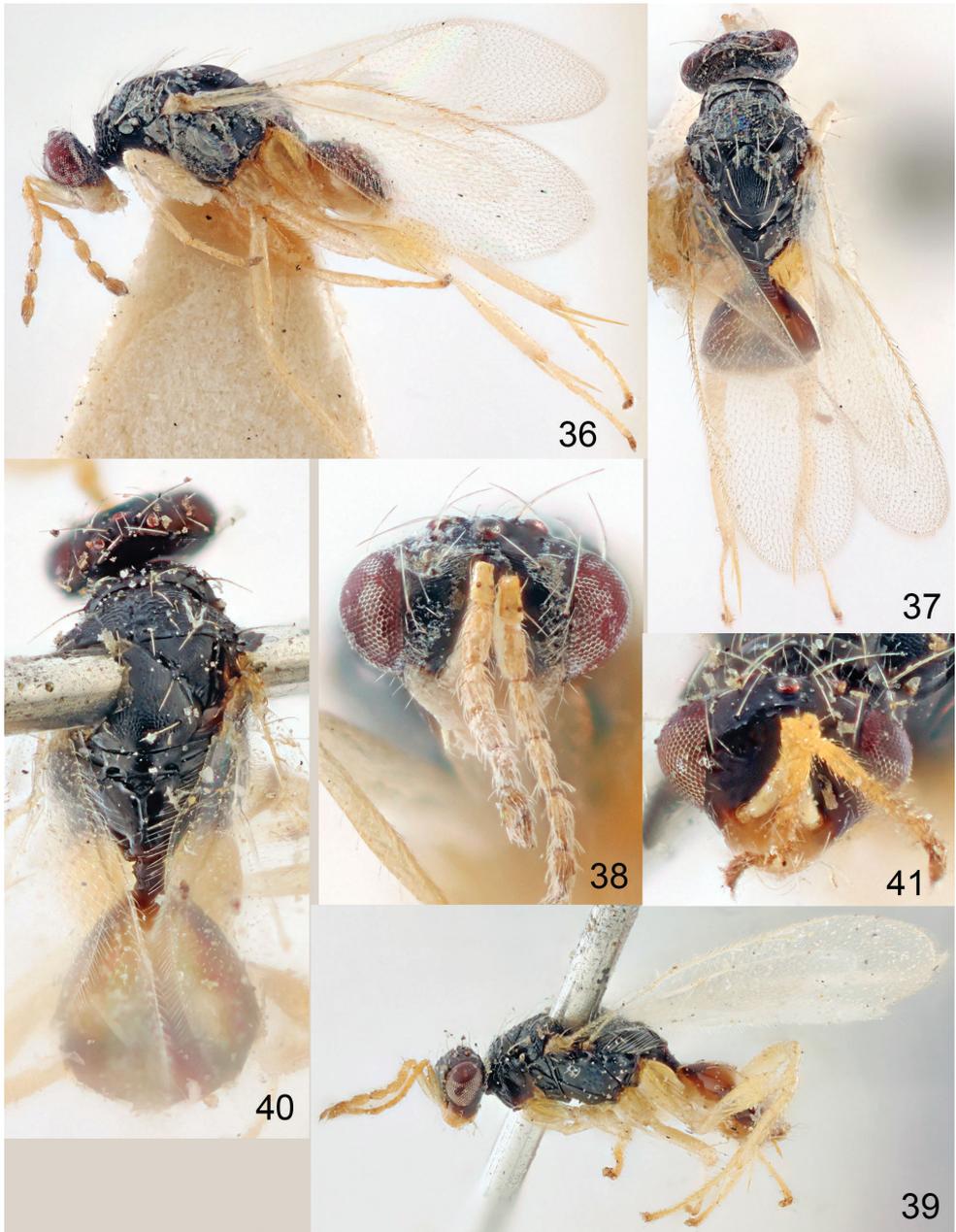
**Material** (249♀ 200♂). Finland: 15♀ 8♂ (VV), 153♀ 121♂ (MZH, MZLU, ZSM), this material includes 11♀ 4♂ from *Agrotis segetum*, 5♀ from *Eurois occulta*, 4♀ from *Lithomoia solidaginis*; France: 22♀ 9♂ (NHM); Spain: 1♀ 2♂ (MZLU, NHM); Sweden: 35♀ 43♂ (MZLU, NHM), this material includes 6♂ from *Mniotype satura*; United Kingdom: 23♀ 17♂ (NHM).

**Diagnosis.** Female with clypeal area pale, and with pale colour drawn out towards eye and almost reaches eye but with part below the eye dark (Fig. 30), male with entire frons below level of toruli pale and with parts lateral to toruli pale (Fig. 31); female with reticulation on scutellum with isodiametric meshes (Fig. 50), reticulation usually strong but there is some variation in this and some specimens have weak reticulation; male scape 2.8–3.0× (mean 2.86, n=10) as long as wide (Fig. 14). Similar to *E. bicolor*, including the narrow posterior part of midlobe of mesoscutum, but can be distinguished from this species through the colour of lower face (both sexes) – pale area confined to clypeal region in *E. bicolor*, and through the reticulation on the scutellum (females only) – meshes elongate in *E. bicolor*. Males are similar to males of *E. geometricida*, but can be distinguished through the relatively long and slender scape.

**Hosts.** *Agrotis segetum* (Denis & Schiffermüller), *Eurois occulta* (L.), *Lithomoia solidaginis* (Hübner), *Mniotype satura* (Denis & Schiffermüller). All records are from caterpillars of the Noctuidae.

**Distribution.** United Kingdom (Westwood 1832), Finland, France, Spain, Sweden (new/confirmed records).

**Genetic data.** The single barcoded specimen of *E. maculiventris* was assigned a distinct BIN and shows a distance of 11.3% to the next neighbour species, *E. bicolor* (Fig. 63).



**Figures 36–41.** *Euplectrus* spp.: **36–38** *E. liparidis* Ferrière, holotype female **36** habitus lateral **37** habitus dorsal **38** head frontal **39–41** *E. phthorimaeae* Ferrière, holotype female **39** habitus lateral **40** habitus dorsal **41** head frontal.

***Euplectrus nigriceps* Ferrière revised status**

Figures 42–46

*Euplectrus nigriceps* Ferrière, 1941:42. Holotype female in NHM, examined. Synonymized with *E. platyhypenae* Howard by Bouček and Graham (1978).

**Material** (1♀). Sweden: Öland, Ismantorp, 56°44'45.8N, 16°38'29.0E, 30.vi.2014, C. Hansson (MZLU).

**Diagnosis.** Head including clypeal area completely black (Figs 44, 45); midlobe of mesoscutum with a complete median carina (Figs 43, 46); scutellum shiny, with very weak reticulation (Figs 43, 46).

**Hosts.** Unknown.

**Distribution.** Sweden (new record). This is the only geographical record for the species as the type material did not have information about collecting locality.

**Remarks.** *Euplectrus nigriceps* was described from two females and one male that stood together with British material of *E. bicolor* in NHM, but all three specimens lacked locality information (Ferrière 1941). In spite of this lack of information *E. nigriceps* was regarded as European by Ferrière. Graham (1963) included *E. nigriceps* in an addition to the British species of the Eulophidae, but because of the lack of information on the type specimens he expressed doubts about the record. Later Bouček and Graham (1978) synonymised *E. nigriceps* with *E. platyhypenae*, a species found in the Nearctic and Neotropical regions (Hansson et al. 2015). These two species are very similar, e.g. through the completely dark head, complete median carina on midlobe of mesoscutum, and the shiny scutellum with very weak reticulation. But they also differ. The female gaster is different: in *E. platyhypenae* the gaster is pale with narrow dark lateral margins (Fig. 47), but in *E. nigriceps* it is dark brown in posterior ½, in anterior ½ pale with wide dark lateral margins (Fig. 46).

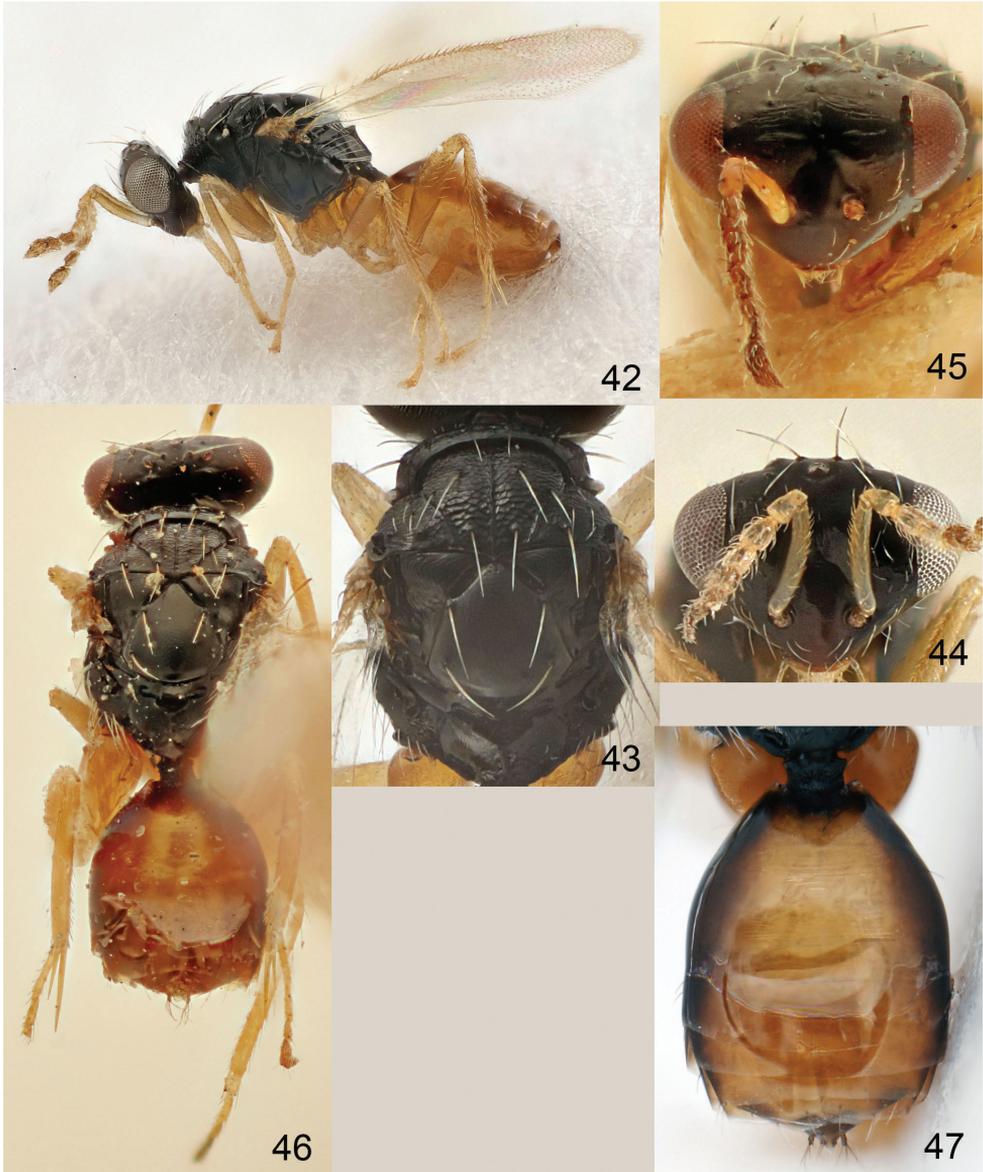
**Genetic data.** The single genetically examined specimen of *E. nigriceps* (BC-ZSM-HYM-29751-A01) has a distance of 11.4% to the next neighbour species, *E. bicolor*.

***Euplectrus phthorimaeae* Ferrière**

Figures 39–41

*Euplectrus phthorimaeae* Ferrière, 1941:42–43. Holotype female in NHM, examined.

**Diagnosis.** Female with frons below level of toruli with pale area not extending laterally to the eye but with a wide dark stripe between pale area and eye (Fig. 41); midline on midlobe of mesoscutum indicated by a median groove in posterior ½ (Fig. 40); posterior part of midlobe of mesoscutum narrow as in *E. bicolor*; scutellum with isodiametric meshes in posterior part (Fig. 40).

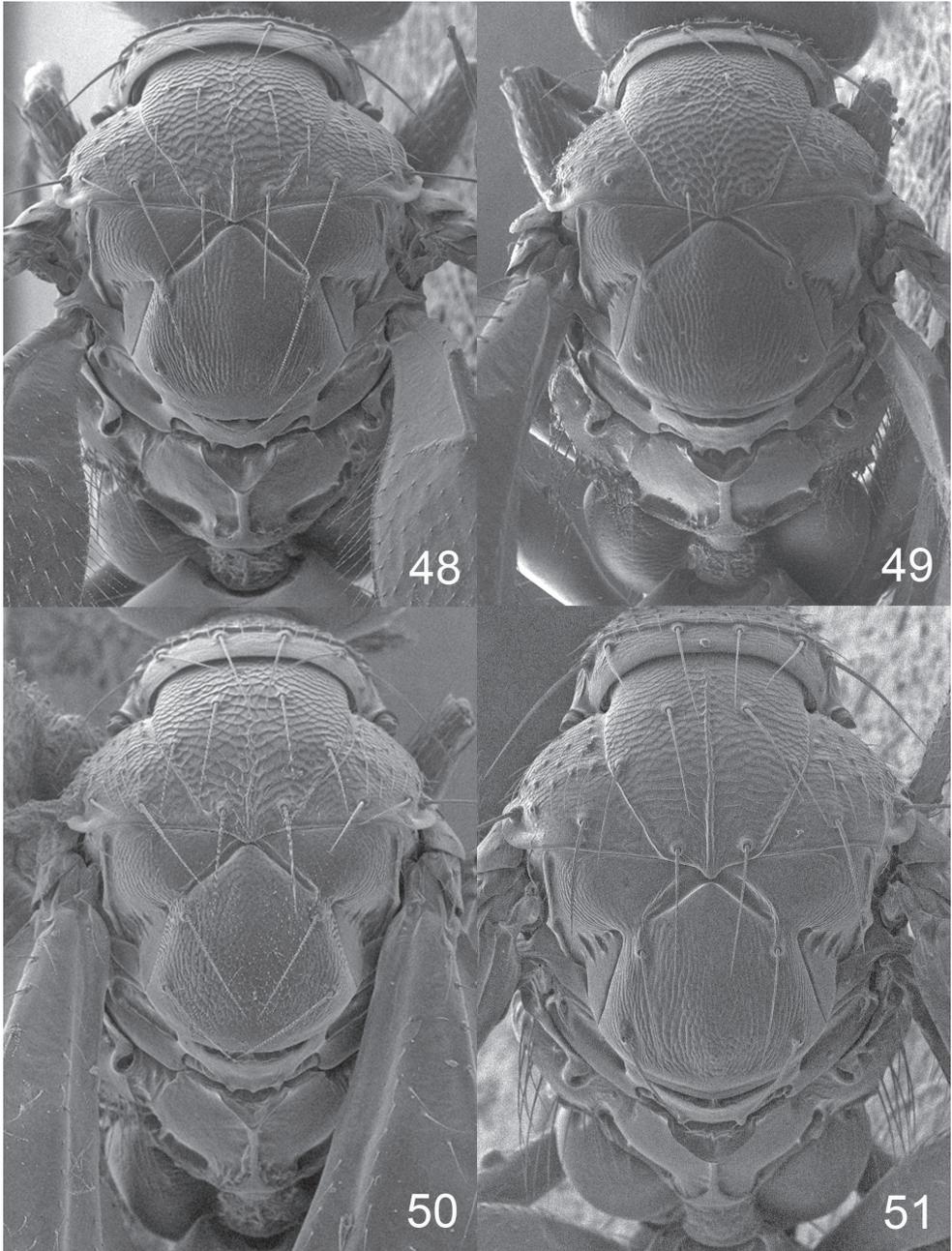


**Figures 42–47.** *Euplectrus* spp.: **42–46** *E. nigriceps* Ferrière, female **42–44** non-type specimens from Sweden **42** habitus lateral **43** thoracic dorsum **44** head frontal **45–46** holotype **45** head frontal **46** habitus dorsal **47** *E. platyhypenae* Howard, gaster dorsal, female specimen from Costa Rica.

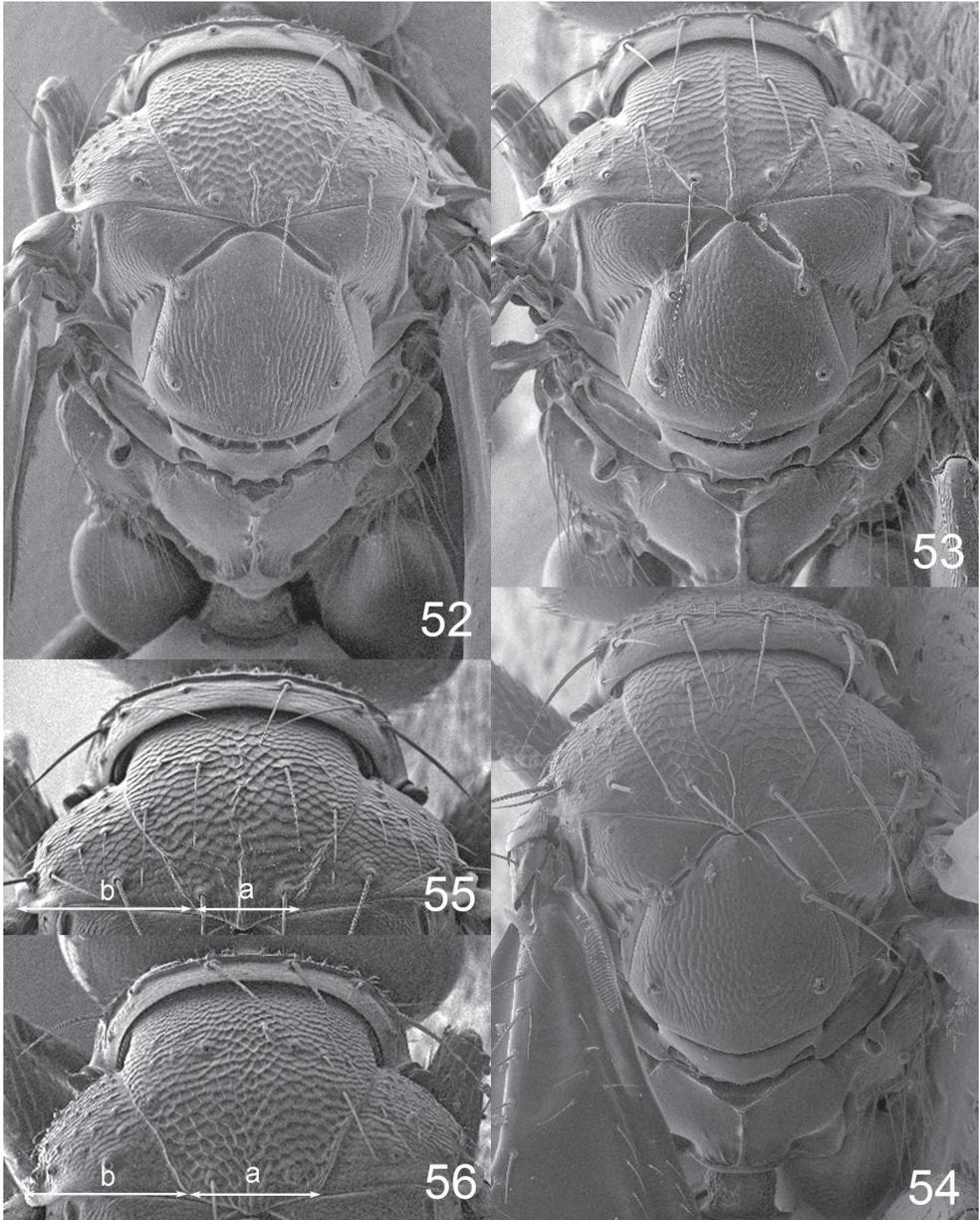
**Hosts.** *Phthorimaea operculella* (Zeller) (Gelechiidae) (Ferrière 1941).

**Distribution.** Cyprus & Israel (Ferrière 1941).

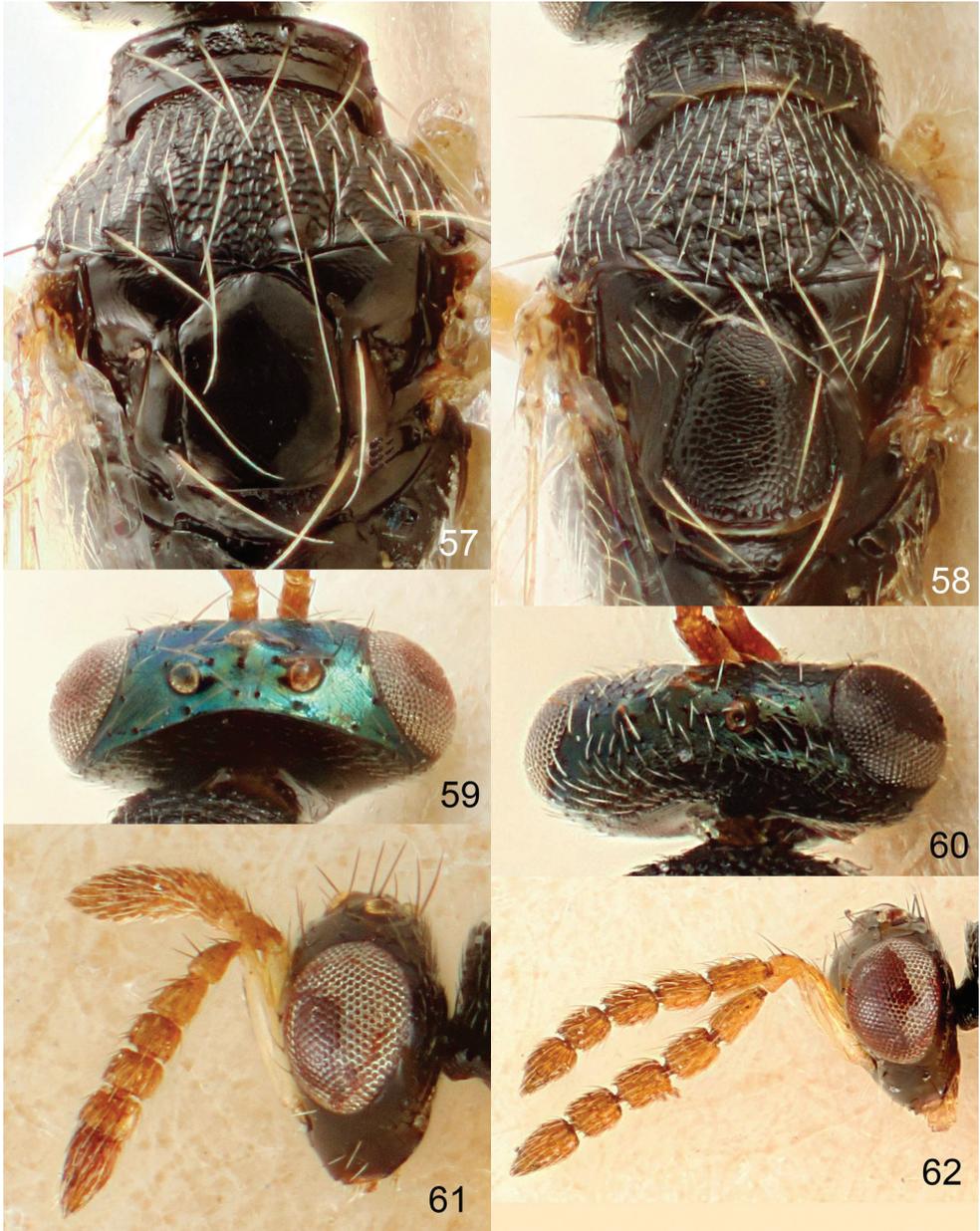
**Genetic data.** No specimens of the species were available for genetic analysis.



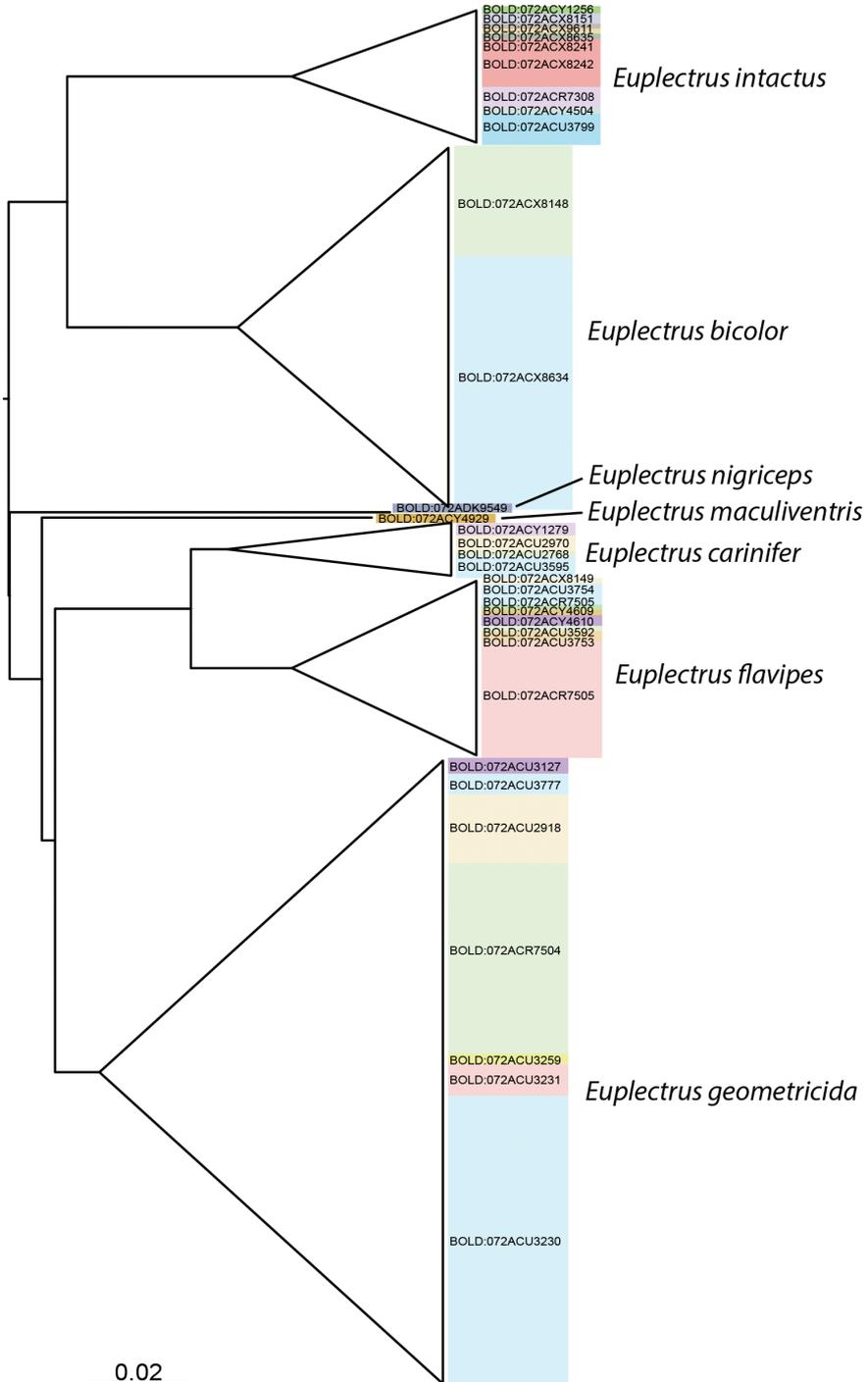
**Figures 48–51.** *Euplectrus* spp., mesosoma dorsal, female: **48** *E. bicolor* (Swederus) **49** *E. intactus* (Walker) **50** *E. maculiventris* Westwood **51** *E. flavipes* (Fonscolombe).



**Figures 52–56.** *Euplectrus* spp. **52–54** mesosoma dorsal, female **52** *E. geometricida* sp. n. **53** *E. carinifer* sp. n. **54** *E. pallidigaster* sp. n. **55–56** mesoscutum, female, a = width of base of midlobe, b = width of base of sidelobe **55** *E. bicolor* (Swederus) **56** *E. intactus* (Walker).



**Figures 57–62.** *Platyplectrus* spp., females: **57–58** mesoscutum and scutellum, dorsal **57** *P. laeviscuta* (Thomson) **58** *P. pannonica* Erdős **59–60** vertex **59** *P. pannonica* **60** *P. chlorocephala* (Nees) **61–62** head including antenna, lateral **61** *P. bouceki* (Erdős) **62** *P. laeviscuta*.



**Figure 63.** Neighbour-joining tree of *Euplectrus*, with colours indicating different Barcode Index Numbers (BINs). For a fully resolved tree with additional terminal taxa information, including sequence length and country of origin, see Suppl. material S2.

## Discussion

### Analysis of molecular and morphological data

*Euplectrus* is a cosmopolitan group currently including 203 species and because of the interspecific morphological similarity the species are easily recognizable as belonging to *Euplectrus*. Members of *Euplectrus* thus seem to be very conservative regarding the evolution of morphological features. This similarity between species frequently causes problems when specimens are identified using morphological features. And yet the intra- and interspecific variation of DNA barcode sequences are, compared to most other groups of insects, very large – at least among some of the European species treated here. Consider for instance *E. bicolor* and *E. intactus*, two morphologically very similar species, almost identical, that prior to this article were regarded as one species. They can be separated by just one morphological character and yet the minimum genetic distance between them using data from CO1 is 10.9%, which compared to species from other insect groups is a very large gap. Both species also exhibit large intraspecific variation in CO1, 6.5% in *E. bicolor* (including two sympatric subclusters) and 6.9% in *E. intactus* (including nine subclusters, each with a different BIN). The large intraspecific variations of the COI barcode fragment, also present in some other species treated here, may indicate the presence of more species in the material included. However, pending more sampling from different populations and geographical regions, and further analyses using more gene regions, we prefer to regard as species only those that show consistent morphological differences, no matter how small these differences may be. Considering the high intraspecific variation within most species and the virtual absence of diagnostic characters it seems mandatory for accurate identification of *Euplectrus* to provide DNA barcodes with each identification at species level.

### Hosts

The hosts presented in this article (Table 1) are all from information given on pins of the *Euplectrus* specimens examined. This is a potential source of error as we cannot verify the identity of these host names. Nevertheless, we accept these as they are presented, with the nomenclature updated. Using this, probably very incomplete information on host range for each species, following can be speculated upon.

The host range for European *Euplectrus* species where we have been able to examine a larger material (Table 1) seems to be extensive, and hosts from either Noctuidae or Geometridae are the dominant target groups. Apart from having wide host spectra, species can also overlap in their host preferences. Sometimes two *Euplectrus* species have been recorded from the same host species. *Euplectrus geometricida* seems to favour geometrids and share this host group with *E. flavipes*. Both species also parasitize noctuids, which is also the target group for *E. bicolor* and *E. maculiventris*. *Euplectrus flavipes*, a species that probably only occurs in Central and South Europe, has been recorded from four Lepidoptera families, including geometrids and noctuids. This wide host spectrum

**Table 1.** Host records for examined specimens of *Euplectrus* (host records in the literature are not included due to uncertainty regarding previous identifications of *Euplectrus* species).

Host	<i>Euplectrus</i> species
<b>Depressariidae:</b>	
<i>Carcina quercana</i> (Fabricius)	<i>E. carinifer</i> , <i>E. flavipes</i>
<b>Erebidae:</b>	
<i>Colobochyla salicalis</i> (D. & S.)	<i>E. flavipes</i>
<i>Lymantria dispar</i> (L.)	<i>E. liparidis</i>
<b>Gelechiidae:</b>	
<i>Phthorimaea operculella</i> (Zeller)	<i>E. phthorimaeae</i>
<b>Geometridae:</b>	
<i>Agriopsis aurantiaria</i> (Hübner)	<i>E. geometricida</i>
<i>Agriopsis marginaria</i> (Fabricius)	<i>E. geometricida</i>
<i>Alcis repandata</i> (L.)	<i>E. geometricida</i>
<i>Alsophila aescularia</i> (D. & S.)	<i>E. flavipes</i> , <i>E. geometricida</i>
<i>Angerona prunaria</i> (L.)	<i>E. geometricida</i>
<i>Cyclophora annularia</i> (Fabricius)	<i>E. flavipes</i>
<i>Colotois pennaria</i> (L.)	<i>E. geometricida</i>
<i>Epirrita dilutata</i> (D. & S.)	<i>E. geometricida</i>
<i>Macaria brunneata</i> (Thunberg)	<i>E. geometricida</i>
<i>Operophtera brumata</i> (L.)	<i>E. geometricida</i>
<i>Phigalia pilusaria</i> (D. & S.)	<i>E. geometricida</i>
<b>Noctuidae:</b>	
<i>Agrotis segetum</i> (D. & S.)	<i>E. geometricida</i> , <i>E. maculiventris</i>
<i>Agrotis</i> sp.	<i>E. bicolor</i>
<i>Amphipyra pyramidea</i> (L.)	<i>E. carinifer</i>
<i>Anarta myrtilli</i> (L.)	<i>E. geometricida</i>
<i>Autographa gamma</i> (L.)	<i>E. carinifer</i>
<i>Cosmia trapezina</i> (L.)	<i>E. flavipes</i>
<i>Diarsia mendica</i> (Fabr.)	<i>E. bicolor</i>
<i>Eugraphe subrosea</i> (Stephens)	<i>E. bicolor</i>
<i>Eurois occulta</i> (L.)	<i>E. maculiventris</i>
<i>Heliothis</i> sp. on <i>Medicago sativa</i>	<i>E. flavipes</i>
<i>Lithomoia solidaginis</i> (Hübner)	<i>E. maculiventris</i>
<i>Mamestra brassicae</i> (L.)	<i>E. bicolor</i> , <i>E. flavipes</i>
<i>Mniotype satura</i> (D. & S.)	<i>E. maculiventris</i>
<i>Noctua comes</i> Hübner	<i>E. intactus</i>
<i>Orthosia cruda</i> (D. & S.)	<i>E. carinifer</i>
<i>Orthosia opima</i> (Hübner)	<i>E. bicolor</i> , <i>E. geometricida</i>
<i>Orthosia</i> sp.	<i>E. bicolor</i>
<i>Perigrappa munda</i> (D. & S.)	<i>E. carinifer</i>
<i>Plusia</i> sp. on <i>Medicago sativa</i>	<i>E. flavipes</i>
<i>Polia hepatica</i> (Clerck)	<i>E. bicolor</i>
<i>Polia nebulosa</i> (Hüfnagel)	<i>E. bicolor</i>
<i>Spodoptera exigua</i> Hübner	<i>E. flavipes</i>
<i>Spodoptera litura</i> Fabricius	<i>E. flavipes</i>
<i>Xylina</i> sp.	<i>E. bicolor</i>
<b>Pieridae:</b>	
<i>Pieris rapae</i> (L.)	<i>E. intactus</i>
<b>Tortricidae:</b>	
<i>Archips rosana</i> (L.)	<i>E. flavipes</i>

and host overlap in European *Euplectrus* species is in stark contrast to the situation in some tropical areas, as presented by Hansson et al. (2015). Analysing the *Euplectrus* fauna in the Área de Conservación Guanacaste, a comparatively small area (1470 km<sup>2</sup>) in north-western Costa Rica, they recognized 75 *Euplectrus* species, diagnosed through data from both the morphology of the adult wasps and from CO1, i.e. the same set of characters we have used for European species in this article. The species were either (usually) host-specific to a particular species of caterpillar or (occasionally) to a particular life-form of caterpillar within a genus or family, and those usually feeding on a narrow range of food plants. Host overlap between species were rare, occasionally two *Euplectrus* species parasitized the same host species. The differences between tropical and temperate patterns of diversity in *Euplectrus* are in conformity with other groups of parasitic wasps (e.g. Fernandez-Triana et al. 2014, Smith et al. 2008). The results found here for European species compared to the results for tropical species accounted for in Hansson et al. (2015) suggest that a more refined niche separation in tropical areas is at least part of the explanation for the much higher species diversity in such areas.

The host larvae presented here are with one exception naked. The exception is *Lymantria dispar*, the only known host for *E. liparidis*, which has distinctly hairy caterpillars in all stages. The presence of hairs presents a mechanical obstacle, and the lack of such hairs is possibly a prerequisite for the female wasp to walk about on the host and enabling her to anchor her eggs in the cuticle of the host. Either the record for *E. liparidis* is a mistake, or females of this species use a different approach when laying eggs on the host. Apart from the host name very little is known about the biology for this species.

## Acknowledgements

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## Supplementary material 1

### List of voucher specimens with species of *Euplectrus*

Authors: Christer Hansson, Stefan Schmidt

Data type: specimens data

Explanation note: List of voucher specimens with species of *Euplectrus*, specimen ID, country of origin, Barcode Index Number (BIN), GenBank accession number and host information for reared specimens.

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Link: <https://doi.org/10.3897/jhr.67.28810.suppl1>

## Supplementary material 2

### BOLD TaxonID Tree

Authors: Christer Hansson, Stefan Schmidt

Data type: phylogenetic data

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# Two Asian egg parasitoids of *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) emerge in northern Italy: *Trissolcus mitsukurii* (Ashmead) and *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae)

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## Abstract

*Halyomorpha halys* (Stål) is a severe agricultural pest that is spreading worldwide from its original distribution in Asia. Egg parasitoids from Asia, which play a key role in the population dynamics of *H. halys*, are following its host along global pathways. We present the first records of *Trissolcus mitsukurii* in Europe, and of *Trissolcus japonicus* in Italy. Both discoveries were made in northern Italy, where *H. halys* is widely present and has reached extremely high population densities in some areas. Given the availability of their host, the distributions and populations of these exotic egg parasitoids are expected to expand, even in the absence of human intervention.

## Keywords

Egg parasitoid, brown marmorated stink bug, exotic species

## Introduction

The invasive stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), also known as the brown marmorated stink bug, is now a cosmopolitan pest. From its native range in East Asia, it first became established in North America in the mid-1990s (Hoebeke and Carter 2003), followed by Europe in the mid-2000s (Wermelinger et al. 2008), and is now established in South America (Faúndez and Rider 2017). It has been intercepted numerous times in New Zealand but has not yet established a permanent population there (Ormsby 2018). *Halyomorpha halys* is a highly polyphagous pest, and severe damages are recorded on forest, urban and agricultural trees, and on the fruits and seeds of crops and horticultural plants (Lee et al. 2013, Leskey and Nielsen 2018). Worldwide, the control of *H. halys* currently relies mainly on pesticides (Kuhar and Kamminga 2017). However, because only broad-spectrum pesticides are effective, beneficial insects are also killed and integrated pest management programs are negatively impacted. Thus, much attention has been given to alternative methods for a long-term solution, including biological control. Among natural enemies of *H. halys*, considerable emphasis has been placed on egg parasitoids, which appear to be the most effective enemies in the native Asian range (Yang et al. 2009, Lee 2015). Several studies explored the ability of parasitoids to attack *H. halys* eggs, both in its native distribution and in the newly invaded areas worldwide (Haye et al. 2015, Talamas et al. 2015, Roversi et al. 2016, Herlihy et al. 2016, Abram et al. 2017, Dieckhoff et al. 2017). Presently, only Asian native species of egg parasitoids appear to be promising candidates as biological control agents, primarily *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) (Zhang et al. 2017).

The spread of *H. halys* has also provided an invasion opportunity for its parasitoids. In 2015 *T. japonicus*, commonly known as the samurai wasp, was detected in the eastern United States (Talamas et al. 2015), followed shortly by discovery of a second, independently established population in the Pacific Northwest (Milnes et al. 2016). In 2017 and again in 2018, *T. japonicus* was recorded in Switzerland, its first reported recovery in Europe (Stahl et al. 2018). We present here the discovery of two adventive populations of exotic parasitoids, *T. mitsukurii* (Ashmead) and *T. japonicus*, that are parasitizing *H. halys* eggs in the wild in northern Italy.

## Methods

### Field survey and recovery of *Trissolcus mitsukurii* in northeastern Italy

During the 2018 brown marmorated stink bug monitoring campaign in fruit orchards in the Region of Friuli-Venezia-Giulia (northeastern Italy), personnel of ERSA on August 7, and jointly with personnel of CREA on August 8, noted the presence of darkly colored *H. halys* egg masses, which is an indicator of parasitism (see Results for site descriptions). During the surveys, egg masses of other stink bug

species were also collected when found. A portion of the egg masses collected in the field were reared in climatic chambers (26 °C, 65%RH, 16:8 L:D) until adult parasitoids emerged. For further study, adults were kept alive in glass tubes and provided with pure honey droplets as food. The remaining field collected egg masses were reared in a laboratory room in Petri dishes until parasitoids emerged and specimens were stored in ethanol for further studies. All emerged specimens were counted, identified to species and sexed.

### Field survey and recovery of *Trissolcus japonicus* in northwestern Italy

During routine research activities of CREA personnel on July 27 and August 3, 2018, several *H. halys* egg masses with dark coloration were observed on *Acer campestre* L. trees in a parking lot near Lodi, a site close to the town of Milan (see Results for site description). All collected egg masses were reared in climatic chambers as described previously, and adult parasitoids were kept alive in rearing tubes with pure honey for further studies. Emerged specimens were counted, identified to species and sexed.

Reared specimens of *Trissolcus* were identified using the key to Palearctic *Trissolcus* provided in Talamas et al. (2017). Image Z-stacks were taken with a Canon EOS 80D camera attached to an Olympus BX51 compound scope and a Macropod imaging system from Macroscopic Solutions and were rendered using Helicon Focus (Helisoft). Voucher specimens are deposited in the Florida State Collection of Arthropods (Gainesville, FL), the USDA-ARS European Biological Control Laboratory (EBCL) (Montpellier, France), and CREA-DC (Florence, Italy). Collection data associated with a subset of these specimens are deposited in the Hymenoptera Online Database (hol.osu.edu). Morphological terminology follows Mikó et al (2007).

### Character annotations

ats	postacetabular sulcus (Fig. 7)
cs	clypeal setae (Fig. 9)
eps	episternal foveae (Fig. 7)
hoc	hyperoccipital carina (Figs 3, 6, 8)
lo	lateral ocellus (Figs 3, 6)
lpt1	lateral setal patch on mediotergite of T1 (Fig. 3)
mp	mesopleural pit (Fig. 7)
ms	malar sulcus (Figs 4, 7)
mtps	metapleural sulcus (Fig. 2)
not	notauli (Fig. 8)
of	orbital furrow (Figs 4, 7)
pof	preocellar furrow (Fig. 9)
slt1	setal patch on laterotergite of T1 (Fig. 3)

## Molecular analysis

### DNA extraction, PCR amplification and sequencing

Following their morphological examination, all specimens were preserved in 95% ethanol and shipped to EBCL. Tables 3, 4 list the specimens and voucher information included in the analysis. Prior to DNA extraction, individual specimens were bathed three times at room temperature in molecular grade water for five minutes. Genomic DNA was nondestructively isolated from the entire specimen using the Qia-gen DNeasy kit (Hilden, Germany) as published in Taekul et al. (2014) with minor modification. In Step 7, the elution buffer, warmed to 55 °C, was allowed to sit on the membrane during 15 min before centrifugation. The collected flow-through was reloaded onto the spin column to increase the DNA yield. A negative control (no insect tissue) was included in each extraction to detect potential contamination. The barcode region of the mitochondrial Cytochrome Oxidase Subunit I (*COI*) was amplified using the universal barcoding primers LCO1490 (5'-GGTCAACAAATCATAAA-GATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Amplification and barcode editing and analysis were done as described in Ganjisaffar et al. (2018). All sequences generated from this study and those from our custom barcode database are deposited in Genbank (Tables 3 and 4) and all residual DNAs are archived at EBCL (Tables 3 and 4). The sequences obtained were compared with sequences present in Genbank by similarity search using the Basic Local Alignment Search Tool (<http://www.ncbi.nlm.nih.gov/BLASTn>).

Although tracing the source of the Italian populations was not the scope of the present study, we used this barcode approach to provide better insights into the mitochondrial diversity of these two *Trissolcus* species, identify and exclude Asian populations that were highly divergent from Italian populations, and tentatively find similar populations. Of note, *T. mitsukurii* barcodes are poorly represented in Genbank database and BOLD as there is only one record published by Mita et al. (2014). For this study we had access to an unpublished EBCL database of 12 barcodes of *T. mitsukurii* collected in China, Korea and Japan and obtained within the framework of an ongoing USDA biocontrol program. Two sequences generated from the present study were aligned using Clustal W with the sequence from Mita et al. (2014) and the 12 sequences of our custom database.

For *T. japonicus*, the five sequences generated from this study were aligned with Clustal W with all barcode sequences retrieved from Genbank and BOLD (which contained some sequences not present in Genbank). Only sequences from Asian and Swiss samples were included in the dataset. For both taxa, the phylogenetic relationships among haplotypes were depicted using statistical parsimony in TCS as implemented in PopART (Leigh and Bryant 2015). This approach enabled us to display the geographical distribution of all haplotypes.

## Field surveys

### *Trissolcus mitsukurii* recoveries in northeastern Italy

Site descriptions for recovery sites are given in Table 1. A total of 31 *H. halys* egg masses were collected at three survey sites in northeastern Italy on August 7–8, 2018 (Fig. 1, Table 2). Of these, 4 egg masses were not parasitized, and nymphs hatched at a rate of 98.23%; 25 egg masses were parasitized by *T. mitsukurii*; and 2 egg masses were parasitized by *Anastatus bifasciatus* (Geoffroy) (Eupelmidae) and a species of Pteromalidae. From the 25 egg masses parasitized by *T. mitsukurii*, a total of 424 adults emerged of which 91.75% were females. Depending on locality, the parasitism rate of eggs within single egg masses ranged from 32.14% to 100% and number of parasitized egg masses out of the total number of egg masses detected ranged among sites from 50.00% to 84.21%.

During surveys at the Codroipo site, an egg mass of a predatory stink bug belonging to the subfamily of Asopinae was collected which was also parasitized by *T. mitsukurii*. From this egg mass, 83.87% of the eggs (31 eggs in total) were parasitized (emergence rate 96.15%; 80.00% females); only one egg of the cluster produced a stink bug nymph, and four apparently unparasitized eggs did not hatch.

### *Trissolcus japonicus* findings in northwestern Italy

The recovery site of *T. japonicus* in northwestern Italy was located at 45.3031N, 9.4794E (Fig. 1). Parasitized egg masses were found on *Acer campestre* L. trees in a parking lot surrounded by a multi-host patchy landscape (crop fields, uncultivated fields, hedgerows), and industrial and urban areas with ornamental plants.

**Table 1.** Descriptions of *Trissolcus mitsukurii* recovery sites in northeastern Italy.

Locality	Coordinates	Main culture (plant species and management)	Surrounding cultures	Surrounding environment
Cordenons site 1	46.0089N, 12.6824E	Kiwi orchard ( <i>Actinidia chinensis</i> ), organic farming	Vineyards, maize and soybean crops managed by integrated pest management	Hedgerows, apple and kiwi orchards, vineyards, maize and soybean fields
Cordenons site 2	46.0082N, 12.6713E	Hedgerow ( <i>Robinia pseudoacacia</i> )	Apple orchard, vineyards, maize and soybean crops managed by integrated pest management	Hedgerows, apple and kiwi orchards, vineyards, maize and soybean fields
Codroipo	45.9675N, 13.0251E	Kiwi orchard ( <i>Actinidia deliciosa</i> ), integrated pest management	Apple and pear orchards, vineyards, maize and soybean crops managed by integrated pest management	Hedgerows, apple and pear orchards, vineyards, maize and soybean fields

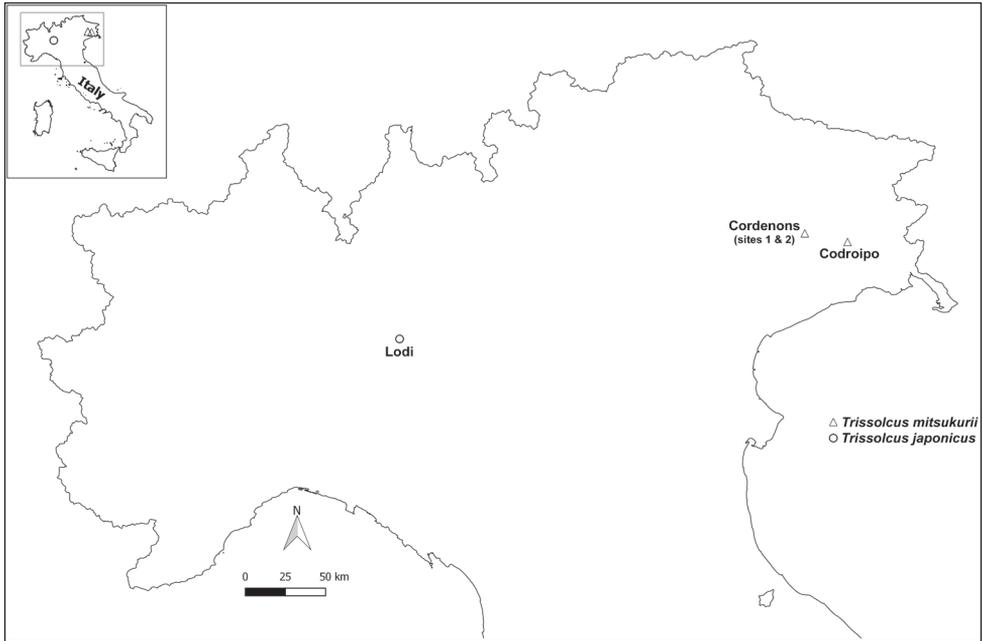


Figure 1. Recovery sites of *Trissolcus mitsukurii* and *Trissolcus japonicus* in northern Italy.

Table 2. Parasitism of *Halyomorpha halys* eggs by *Trissolcus mitsukurii* collected in field surveys of August 7–8, 2018, in northeastern Italy.

Site	n. of parasitized egg masses/total egg masses detected	Mean n. of eggs/egg mass <sup>a</sup>	Parasitized egg masses				Unparasitized egg masses
			Mean % of parasitized eggs/egg mass <sup>a</sup>	Mean emergence rate (%) <sup>a</sup>	Mean sex ratio (% of females) <sup>a</sup>	Mean % of hatched/unhatched eggs/egg mass	
Cordenons site 1	16:19	28.00	87.04	94.22	92.31	0.00:12.74	100% (3)
		(27–29)	(32.14–100)	(57.89–100)	(88.99–100)		
Cordenons site 2	1:2*	21.50	100	100	92.86	0.00:0.00	not assessed (0)
		(15–28)					
Codroipo	8:10*	28.00	92.89	90.30	90.44	0.00:3.59	100% (1)
		(27–29)	(78.57–100)	(65.38–100)	(84.21–95.45)		

\* one egg mass was entirely parasitized by other parasitoids. <sup>a</sup> minimum and maximum values in parentheses

Field collections were made on July 27 and August 3, 2018, and a total of 45 *H. halys* egg masses were collected (with a mean of 25.77 eggs/egg mass). On July 27, only one egg mass was detected and was found to be successfully parasitized by *T. japonicus*, with the emergence of 8 specimens, all of which were males. Among the 44 egg masses collected on August 3, 21 egg masses were not parasitized and eggs hatched into nymphs with a mean rate of 82.44%, and 22 egg masses were parasitized by *A. bifasciatus*. Only

**Table 3.** Sampling Information, GenBank Accession numbers and haplotypes for *Trissolcus mitsukurii* included in this study.

Collection code and Sex	Country	Site	Year of Collection, Name of Collector <sup>b</sup>	Host	GenBank Accession Number	Barcode Haplotype (617bp)	Isolate (EBCL)
FSCA00033071, ♀	Italy	Cordenons, Friuli-Venezia-Giulia	2018, IB, LB & GM	<i>Halyomorpha halys</i>	MK097189 (this study)	H5	Tsp270
FSCA00033072, ♀	Italy	Cordenons, Friuli-Venezia-Giulia	2018, IB, LB & GM	<i>H. halys</i>	MK097190 (this study)	H5	Tsp269
Tm1-EBCL <sup>a</sup> , ♂	Japan	Tsukuba, (NARO) <sup>c</sup>	2007, KH	<i>H. halys</i>	MK097191 (this study)	H1	Tm1
USNMENT01197989, ♀	South Korea	Gochang	2015, KH	unidentified host eggs (not <i>H. halys</i> )	MK097192 (this study)	H3	Tsp202
USNMENT01197242, ♀	South Korea	Jeju	2012, ET& IM	na	MK097193 (this study)	H4	Tsp233
USNMENT01197243, ♀	South Korea	Jeju	2012, ET& IM	na	MK097194 (this study)	H4	Tsp234
USNMENT01197244, ♀	South Korea	Jeju	2012, ET& IM	na	MK097195 (this study)	H4	Tsp235
USNMENT00977533, ♀	China	Yunnan Prov., Kunming	2013, KH	<i>Erthesina fullo</i>	MK097196 (this study)	H2	Tsp39
USNMENT01059335, ♀	China	Yunnan Prov., Kunming	2013, KH	<i>E. fullo</i>	MK097197 (this study)	H2	Tsp60
USNMENT01197294, ♀	Japan	Tsukuba, (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MK097198 (this study)	H1	Tsp149
USNMENT01197295, ♀	Japan	Tsukuba, (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MK097199 (this study)	H1	Tsp150
Tsp151- EBCL <sup>a</sup> , na	Japan	Tsukuba, (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MK097200 (this study)	H1	Tsp151
Tsp152- EBCL <sup>a</sup> , na	Japan	Tsukuba, (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MK097201 (this study)	H1	Tsp152
Tsp153- EBCL <sup>a</sup> , na	Japan	Tsukuba, (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MK097202 (this study)	H1	Tsp153
na, na	Japan	Fukuoka	na, na	<i>Nezara viridula</i>	AB971831 (Mita et al. 2014)	H1	

<sup>a</sup> EBCL DNA collection. <sup>b</sup> name of collectors: IB: Iris Bernardinelli, LB: Luca Benvenuto, GM: Giorgio Malossini, KH: Kim Hoelmer, ET: Elijah Talamas, IM: Istvan Mikó. <sup>c</sup> NARO: National Agriculture and Food Research Organization.

one egg mass collected on August 3 was parasitized by *T. japonicus*. All eggs that were apparently parasitized (35.71% of eggs in the mass) produced adult parasitoids (9 females and 2 males), 46.43% of eggs were unhatched and 17.86% of the eggs hatched into stink bug nymphs. Ninety percent of the emerged *T. japonicus* were females.

## Taxonomy

### *Trissolcus mitsukurii*

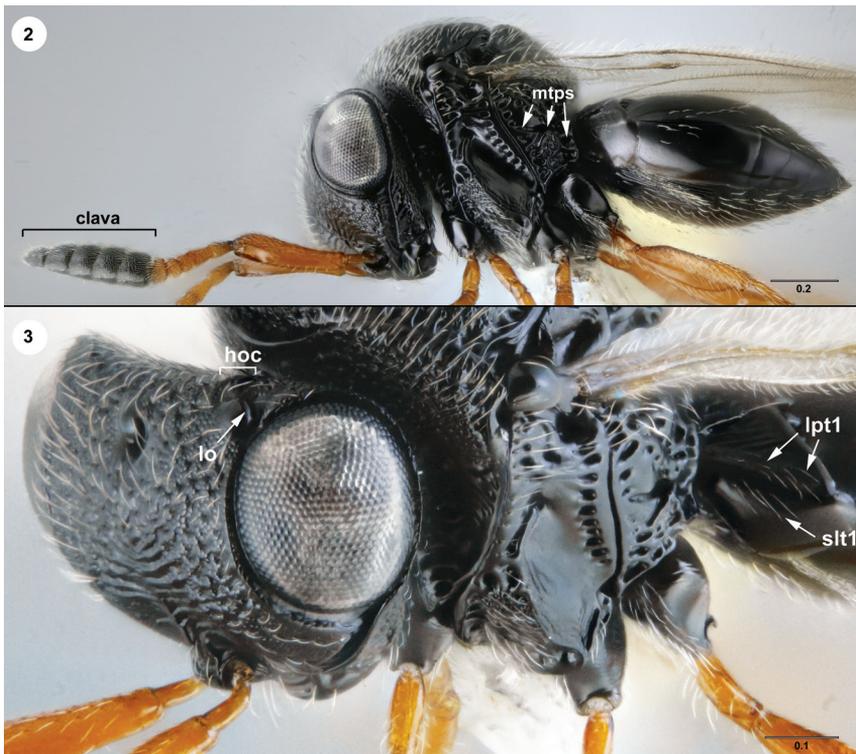
*Trissolcus mitsukurii* is a straightforward species to identify and is separated early in the key to Palearctic *Trissolcus* (Talamas et al. 2017). We thus do not consider it necessary to produce an updated key to European *Trissolcus* that includes this species, and instead

**Table 4.** Sampling Information, GenBank Accession numbers and haplotype for *Trissolcus japonicus* included in this study.

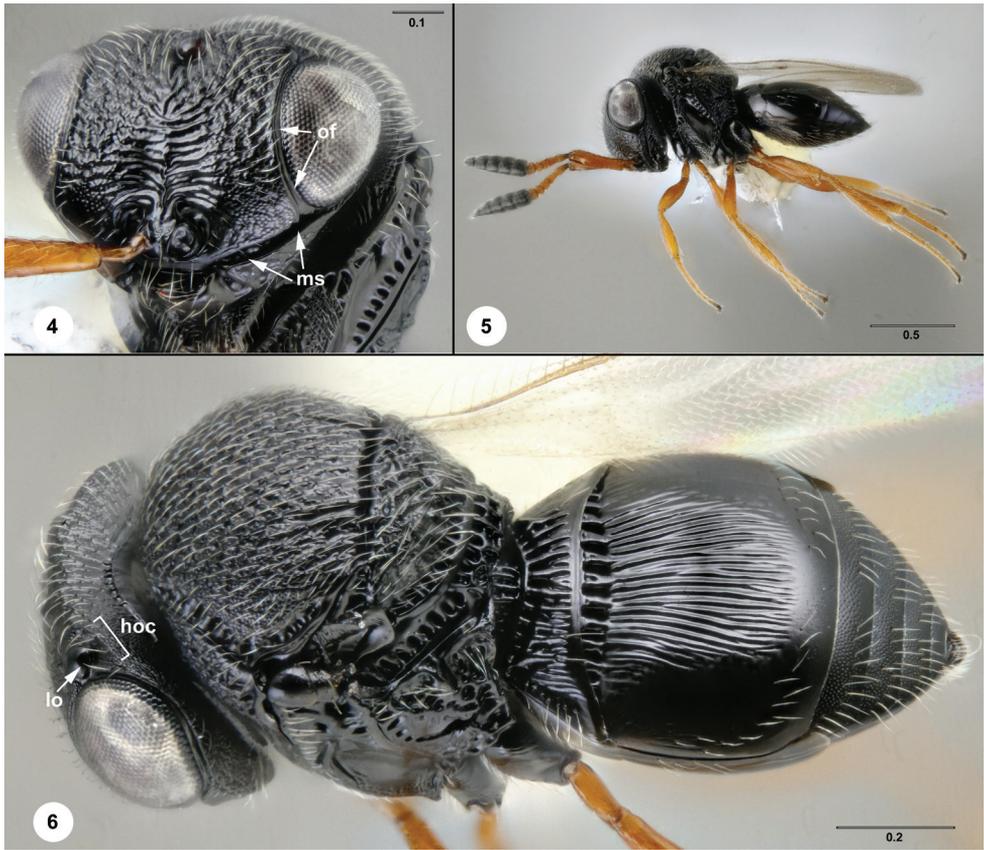
Collection code and Sex	Country	Site	Year of Collection, Name of Collector <sup>b</sup>	Host	GenBank Accession Number/ Bold Accession number	Barcode Haplotype (373bp)	Isolate (EBCL)
FSCA 00033060, ♀	Italy	Lodi, Lombardy	2018, PFR	<i>Halyomorpha halys</i>	MK097184 (this study)	H1	Tj406
FSCA 00033065, ♀	Italy	Lodi, Lombardy	2018, PFR	<i>H. halys</i>	MK097185 (this study)	H1	Tj408
FSCA0033097, ♂	Italy	Lodi, Lombardy	2018, PFR	<i>H. halys</i>	MK097186 (this study)	H1	Tj421
FSCA0033098, ♂	Italy	Lodi, Lombardy	2018, PFR	<i>H. halys</i>	MK097187 (this study)	H1	Tj422
FSCA0033096, ♂	Italy	Lodi, Lombardy	2018, PFR	<i>H. halys</i>	MK097188 (this study)	H1	Tj423
GBIFCH00543446, ♀	Switzerland	Ticino	2017, JS	<i>H. halys</i>	MH919753 (Stahl et al., 2018)	H1	Tj388
GBIFCH00543447, ♀	Switzerland	Ticino	2017, JS	<i>H. halys</i>	MH919754 (Stahl et al., 2018)	H1	Tj389
GBIFCH00543448, ♀	Switzerland	Ticino	2017, JS	<i>H. halys</i>	MH919755 (Stahl et al., 2018)	H1	Tj390
GBIFCH00543449, ♂	Switzerland	Ticino	2017, JS	<i>H. halys</i>	MH919756 (Stahl et al., 2018)	H1	Tj391
GBIFCH00543450, ♂	Switzerland	Ticino	2017, JS	<i>H. halys</i>	MH919757 (Stahl et al., 2018)	H1	Tj392
GBIFCH00543451, ♂	Switzerland	Ticino	2017, JS	<i>H. halys</i>	MH919758 (Stahl et al., 2018)	H1	Tj393
Tsp77- EBCL <sup>a</sup> , na	Japan	Tsukuba (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MH919744 (Bon et al., unpublished)	H1	Tsp77
Tsp78- EBCL <sup>a</sup> , na	Japan	Tsukuba (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MH919745 (Bon et al., unpublished)	H1	Tsp78
Tsp79- EBCL <sup>a</sup> , na	Japan	Tsukuba (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MH919746 (Bon et al., unpublished)	H1	Tsp79
Tsp88- EBCL <sup>a</sup> , na	Japan	Tsukuba (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MH919747 (Bon et al., unpublished)	H1	Tsp88
Tsp90-EBCL <sup>a</sup> , na	Japan	Tsukuba (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MH919748 (Bon et al., unpublished)	H1	Tsp90
Tsp91-EBCL <sup>a</sup> , na	Japan	Tsukuba (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MH919749 (Bon et al., unpublished)	H1	Tsp91
Tsp93- EBCL <sup>a</sup> , na	Japan	Tsukuba (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	MH919750 (Bon et al., unpublished)	H1	Tsp93
Tsp226-EBCL <sup>a</sup> , ♀	Japan	Kanagawa	2015, KH	<i>Plautia stali</i>	MH919752 (Bon et al., unpublished)	H1	Tsp226
na, ♀	Japan	Kanagawa	2012, TM	<i>P. stali</i>	AB847131-32,36 (Matsuo et al., 2014)	H1	
na, ♀	Japan	Fukuoka	2012, KM	<i>P. stali</i>	AB847144-145 (Matsuo et al., 2014)	H2	
na, ♀	Japan	Fukuoka	2012, KM	<i>H. halys</i>	AB908179-182 (Matsuo et al., 2014)	H2	
na, na	Japan	na	na, na	na	AB894834-35, AB894838-39 (Matsuo, K. and Hirose, Y., unpublished)	H2	
na, ♀	Japan	Fukuoka	2012, KM	<i>P. stali</i>	AB847129,130, 137,143,146 (Matsuo et al., 2014)	H3	
na, na	Japan	na	na, na	na	AB894836,837,840,841 (Matsuo, K. & Hirose, Y. (unpublished),	H3	
na, na	Japan	Kanagawa	na, na	<i>P. stali</i>	AB971832 (Mita et al., 2014)	H1	
na, na	China			<i>H. halys</i>	KF303518.1 (Garipey et al., 2014)	H7	
USNMENT01059340, ♀	China	Langfang	2012, KH	<i>E. fullo</i>	/ NSCEL009-18 (Garipey unpublished)	H4	Tsp61
USNMENT01197300, ♀	China	Kunming	2014, KH	<i>E. fullo</i>	/NSCEL010-18 Garipey unpublished)	H5	Tsp155
Tsp1-EBCL <sup>a</sup> , na	Japan	Tsukuba (NARO) <sup>c</sup>	2012, KH	<i>H. halys</i>	/NSCEL011-18	H1	Tsp1

Collection code and Sex	Country	Site	Year of Collection, Name of Collector <sup>b</sup>	Host	GenBank Accession Number/ Bold Accession number	Barcode Haplotype (EBCL) (373bp)	Isolate (EBCL)
USNMENT00977534, ♀	S. Korea	Jirisan Park	2013, KH	<i>H. halys</i>	/NSCEL012-18 Gariepy unpublished)	H6	Tsp53
Tj1-EBCL <sup>a</sup> , na	China	Hebei	2012, KH	<i>H. halys</i>	/NSCEL013-18 Gariepy unpublished)	H7	Tj1
Trj2-EBCL <sup>a</sup> , na	China	Hebei	2012, KH	<i>H. halys</i>	/NSCEL014-18 Gariepy unpublished)	H7	Trj2
USNMENT01197806, ♀	Japan	Kanagawa	2015, KH	<i>H. halys</i>	/NSCEL017-18 Gariepy unpublished)	H1	Tsp223
USNMENT01197320, ♀	South Korea	Seoul	2014, KH	<i>H. halys</i>	/NSCEL018-18 Gariepy unpublished)	H7	Tsp175
na, na	China	Hebei	2012, TH	<i>H. halys</i>	/PPENT028-12 Gariepy unpublished)	H7	
na, na	China	Hebei	2012, TH	<i>H. halys</i>	/PPENT029-12 Gariepy unpublished)	H7	
na, na	China	Hebei	2012, TH	<i>H. halys</i>	/PPENT030-12 Gariepy unpublished)	H7	
na, na	China	Hebei	2012, TH	<i>H. halys</i>	/PPENT031-12 Gariepy unpublished)	H7	
na, na	China	Hebei	2012, TH	<i>H. halys</i>	/PPENT032-12 Gariepy unpublished)	H7	

<sup>a</sup> EBCL DNA collection. <sup>b</sup> name of collector: PFR: Pio Federico Roversi, JS: Judith Stahl, KH: Kim Hoelmer, KM: Kazunori Matsuo, TM: Toshiharu Mita, TH: Tim Haye. <sup>c</sup> NARO: National Agriculture and Food Research Organization.



**Figures 2–3.** *Trissolcus mitsukurii*, female (FSCA 00033025) **2** head, mesosoma, metasoma, lateral view **3** head, mesosoma, metasoma, anterolateral view. Scale bars in millimeters.

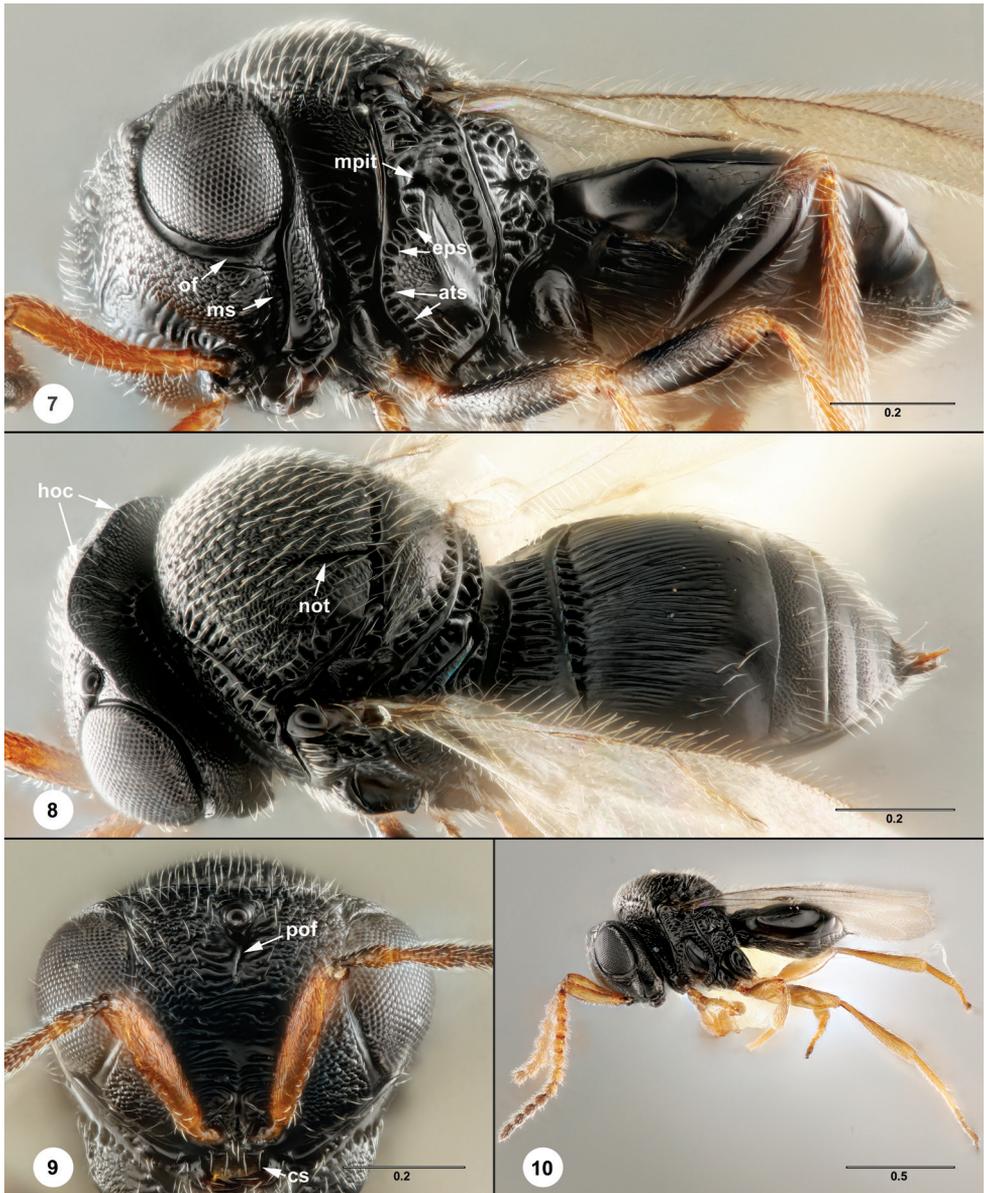


**Figures 4–6.** *Trissolcus mitsukurii*, female (FSCA 00033025) **4** head, anterior view **5** habitus, lateral view **6** head, mesosoma, metasoma, dorsolateral view. Scale bars in millimeters.

we provide the following diagnosis, with references to illustrations, that will be helpful for distinguishing *T. mitsukurii* from the European fauna: the clava of the female antenna is 5-merous, and the clavomeres are distinctly larger and darker than preceding antennomeres (Fig. 2); the orbital furrow is expanded where it intersects the malar sulcus (Fig. 4); the hyperoccipital carina is often present directly posterior to the lateral ocellus, but is absent between the ocelli (Figs 3, 6); the metapleuron is without setae below the metapleural sulcus (Fig. 3); a setal patch is present on the first laterotergite of the metasoma (slt1, Fig. 3). Care should be taken not to confuse the setal patch of the laterotergite with the setal patch on the mediotergite (lpt1, Fig. 3).

### *Trissolcus japonicus*

The key to European *Trissolcus* in Talamas et al. (2017) included *T. japonicus* to provide identification of this species given the possibility that it might become estab-



**Figures 7–10.** *Trissolcus japonicus* **7** female (FSCA 00033063), head, mesosoma, metasoma, ventrolateral view **8** female (FSCA 00033063), head, mesosoma, metasoma, dorsolateral view **9** female (FSCA 00033063) head, anterior view **10** male (FSCA 00033095), habitus, lateral view. Scale bars in millimeters.

lished in Europe. The specimens from northern Italy are fully congruent with the concept of this species presented by Talamas et al. (2017). *Trissolcus japonicus* can be separated from other species of European *Trissolcus* by the following diagnosis: four clypeal setae are present below the antennal insertions (Fig. 9); microsculpture is pre-

sent throughout the frons (Fig. 9); the orbital furrow is expanded at its intersection with the malar sulcus (Fig. 7); the hyperoccipital carina is complete (Fig. 8); episternal foveae extend from the postacetabular sulcus to the mesopleural pit (Fig. 7); and the mesoscutum is without oblique rugae between the notauli (Fig. 8). The precellar furrow, which extends ventrally from the median ocellus (Fig. 9), is a useful character for confirming the identity of *T. japonicus*, but it is not always present, and exhibits the greatest variability in males.

### *Anastatus bifasciatus*

Specimens of *A. bifasciatus* were identified by GSP using the keys of Kalina (1981) and Askew and Nieves-Aldrey (2014) and the identification was confirmed by Dr. Lucian Fusu (University of Iasi, Romania) who compared them with authoritatively identified specimens.

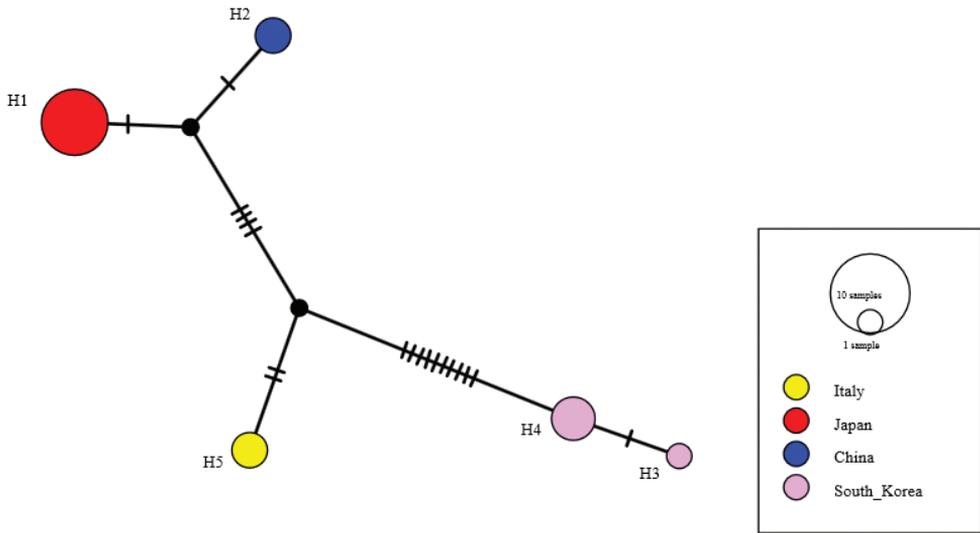
## Molecular identification

### *Trissolcus mitsukurii*

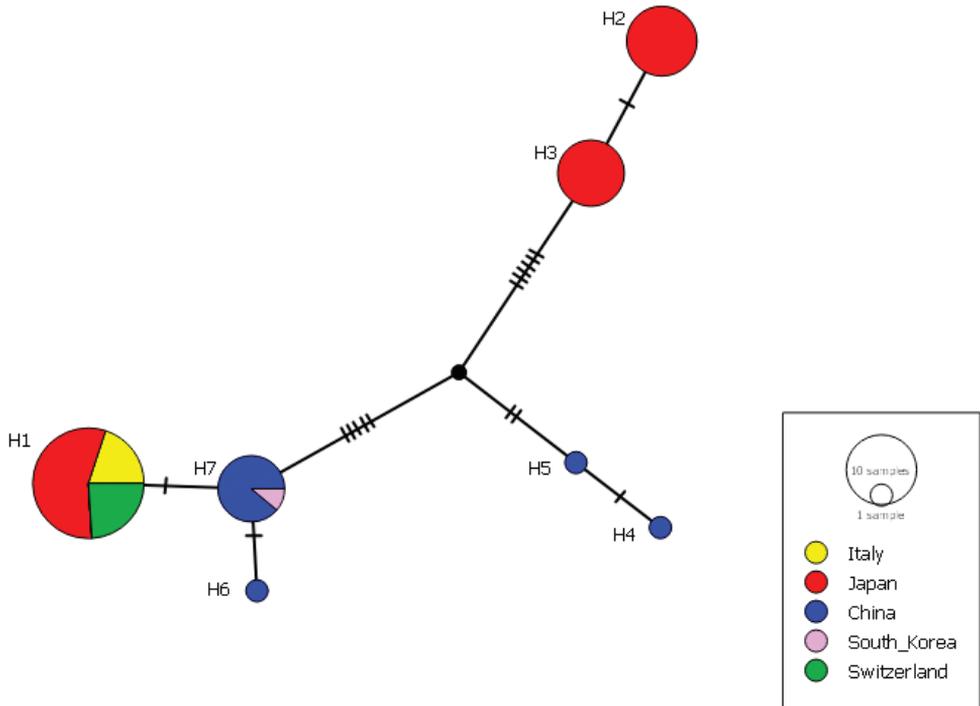
The two voucher specimens recovered from the field in Cordenons (site 1) in the region of Friuli-Venezia-Giulia yielded a similar barcode sequence of 666-bp in length. A BLAST search showed the best similarity score (99%) of this barcode sequence with *T. mitsukurii* (Accession No. AB971831). From the final alignment of 617-bp of 15 *T. mitsukurii* barcodes, a total of five haplotypes (denoted H1-H5) were recovered (Table 3). The haplotype H5 found in Italy is a new haplotype, as it did not match any haplotype found so far in Asia (Fig. 11, Table 3). From the network analysis, H5 differed by seven substitutions from the two closest haplotypes H1 and H2 from Japan and China respectively (Fig. 11).

### *Trissolcus japonicus*

The five voucher specimens recovered from the field in Lodi yielded a unique barcode sequence of 666-bp in length. A BLAST search showed the best similarity score (100%) of this barcode sequence with *T. japonicus* (Accession No. AB971832). From the final alignment of 373-bp of 60 *T. japonicus* barcodes, a total of seven haplotypes (denoted H1 to H7) were recovered (Table 4). The haplotype H1 found in Italy was also found in Tsukuba and Kanagawa in Japan, and in Switzerland (Fig. 12, Table 4). H1 is also the predominant haplotype found in the specimens collected in Japan (45%). Samples from China were the most diverse, displaying 4 haplotypes (H4, H5, H6, H7), although they represent only 22% of the total sampling. From the network analysis, H7 from China and South Korea differed only by one substitution from the haplotype



**Figure 11.** *COI* haplotype network of the *Trissolcus mitsukurii* analyzed in this study. Each circle corresponds to one haplotype; circle size gives the proportion of individuals belonging to the haplotype. The color inside each circle represents the geographical origin. Numbers correspond to the haplotype numbers. Hatch marks symbolize the number of mutations between haplotypes.



**Figure 12.** *COI* haplotype network of the *Trissolcus japonicus* analyzed in this study. Each circle corresponds to one haplotype; circle size gives the proportion of individuals belonging to the haplotype. The color inside each circle represents the geographical origin. Numbers correspond to the haplotype numbers. Hatch marks symbolise the number of mutations between haplotypes.

H1 (Fig. 12). Although our haplotype analysis evidenced a best match of the Italian populations to Japanese and Swiss populations so far, we cannot entirely exclude that the haplotype H1 may also be present in unsampled populations of other parts of Asia where *T. japonicus* is present. Tracing the source of an introduction depends on the availability of information about population structure and may require analysis of more than one locus. To this end, a more comprehensive phylogeography study is underway which includes the *COI* barcode and microsatellite loci recently developed de novo in *T. japonicus*.

## Discussion

Since the first detection of adventive *T. japonicus* in 2015, additional recoveries in the USA have shown that the adventive populations have established and are spreading. Given that *T. japonicus* occurs throughout the range of *H. halys* in its native range of eastern Asia, one potential outcome of the discovery of *T. japonicus* in Italy is that it will also establish and spread wherever *H. halys* has established in this region. Its recent discovery in the Ticino region of Switzerland (Stahl et al. 2018) lends support to this possibility.

*Trissolcus mitsukurii* is widespread in Asia, and its distribution extends to the southern limit of eastern Australia (Johnson 1991). Barcode sequences of Australian specimens of *T. mitsukurii* are not yet available and this region must be considered as a possible source of the Italian population. Of note, *T. mitsukurii* was introduced into Hawaii in 1966 as a biological control agent of *Nezara viridula* (L.) but apparently did not become established (Davis and Krauss 1967). This species has otherwise not been reported as an adventive parasitoid outside of its native range, but it is conceivable that its distribution will follow that of *H. halys*, as has occurred with *T. japonicus*. Continued surveys throughout the region will be needed to document their establishment and dispersal, in addition to determining their impact on *H. halys* populations and their interaction with native natural enemies in the region.

The phenomenon of parasitoids following in the footsteps of their invasive hosts has become a growing trend, particularly with species of *Trissolcus* Ashmead. In addition to discoveries of *T. japonicus* and *T. mitsukurii* in the invaded range of *H. halys*, an adventive population of *T. hyalinipennis* Rajmohana & Narendran was found in California, USA, parasitizing eggs of the invasive bagrada bug, *Bagrada hilaris* (Burmeister) (Ganjisaffar et al. 2018). In each of these cases, the discovery was made through the cooperative effort of scientists working in the disciplines of biological control, taxonomy, and biological diversity, highlighting the synergy and necessity of collaboration.

Expansive ranges have recently been documented in platygastroid wasps that are not known to be of agricultural significance (Masner et al. 2009, Oliveira and Schoeninger 2017, Popovici et al. 2018), but are charismatic and thus more easily recognized. As parasitoids are increasingly examined in the context of the world fauna, we expect to discover many more widespread species.

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# Siblicidal behaviour by larvae of the gregarious parasitoid *Cotesia vanessae*

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## Abstract

Contrasting life histories distinguish solitary from gregarious parasitoids. Females of solitary species typically lay one egg in a host; when more than one parasitoid is present in the host, larvae will kill their rivals so that only one parasitoid completes development. Females of gregarious species typically lay multiple eggs in the same host with the resultant larvae co-existing to complete development. Here we provide an unusual report of siblicide by larvae of a gregarious parasitoid; i.e., *Cotesia vanessae* (Reinhard) (Hymenoptera: Braconidae) developing in noctuid caterpillars (Lepidoptera). Siblicidal behaviour has not previously been reported with larvae of gregarious Braconidae. We speculate that this behaviour reflects a trade-off between the finite amount of resources within the host available for larval development, and selection to optimize use of these resources. ‘Flooding’ the host with eggs allows the female to use the finite resources of the host to their fullest extent, regardless of host size. This strategy also may allow the female to overwhelm the host’s immune system to enhance survival of her progeny in otherwise marginal host species. It further may enhance the ability of the female’s progeny to competitively exclude the larvae of conspecific females or larvae of other parasitoid species co-occurring in the host. Siblicide allows for self-regulation of brood size when host resources are insufficient to support egg-to-adult development of all eggs initially laid in the host.

## Keywords

*Trichoplusia ni*, competitive exclusion, siblicide, host-parasitoid interaction, Braconidae, Noctuidae

A finite amount of resources are available within a host to support parasitoid development. Gregarious parasitoids typically lay multiple eggs per host, whereas solitary parasitoids typically lay one egg per host. Resource competition arises when a parasitoid lays multiple eggs in the host during one ovipositional bout, when conspecific gregarious or solitary parasitoids parasitize the same individual host multiple times (superparasitism), or when multiple species oviposit in the same individual host (multiparasitism).

Competition can take various forms along a continuum defined by extremes of scramble competition versus contest competition (Nicholson 1954). In the former, the resource is more or less equally partitioned among all contestants. They all may survive when there is a surfeit of resources, but all may die when resources are limiting. In the latter, resource distribution is unequal with ‘winners’ and ‘losers’. Solitary parasitoids display an extreme form of contest competition. In cases of superparasitism, they will kill even siblings (siblicide) with only one parasitoid surviving to complete development (Mayhew and van Alphen 1999; Pexton and Mayhew 2002). In contrast, gregarious parasitoids tend towards scramble competition. They are physically tolerant of each other with multiple individuals surviving to emerge from the host (Dorn and Beckage 2007) and siblicide is rare (but see Bartlett and Ball (1964), Bartlett and Medved (1966), and Grbić et al. (1992)).

Here we report high rates of siblicide among larvae of the gregarious parasitoid *Cotesia vanessae* (Reinhard) (Hymenoptera: Braconidae). Comprised of both sexual and parthenogenetic populations (Stefanescu et al. 2012), this species parasitizes caterpillars of certain Nymphalini (Nymphalidae) and Noctuidae (Lepidoptera) (Hervet 2017). Females lay multiple eggs in a single oviposition bout that usually lasts only a few seconds. The developing larvae feed on their host’s haemolymph and fat tissues before egressing en masse from the host’s sides prior to pupating (Hervet et al. 2014). We have observed anywhere from one to 250 parasitoids emerging from one individual host, and averages of between 32 and 148 adult parasitoids produced per host, depending upon the host species (Hervet 2017).

In previous experiments using a parthenogenetic population, we assessed a range of lepidopteran species as potential hosts for *C. vanessae* by parasitizing early 4<sup>th</sup> instar caterpillars. Female *C. vanessae* were placed in contact with the caterpillar until a single bout of oviposition was observed. The parasitized caterpillars were then reared on an artificial diet (Hervet et al. 2016) until parasitoid emergence. Depending upon the lepidopteran species, none, some, or many parasitoids emerged. Caterpillars without parasitoid emergence either died or completed development (see Hervet (2017) for full details).

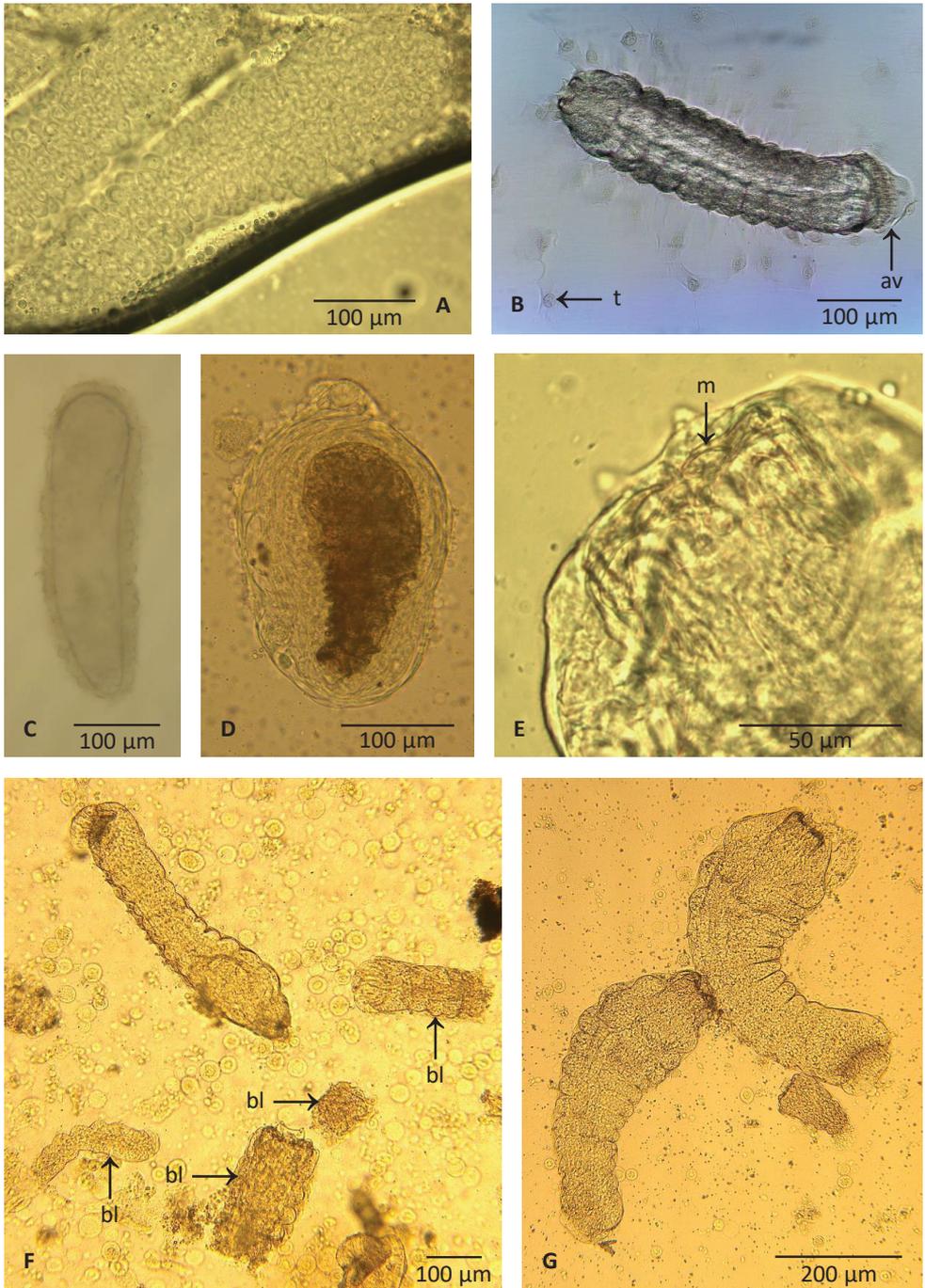
To better understand the factors that affect parasitoid emergence, we dissected parasitized caterpillars of three species. Dissections were performed up to 11 days after exposure to parasitism. For these dissections, the head and the last rear abdominal segments of each caterpillar were excised and the digestive system removed. Haemolymph was then squeezed from the hemocoel onto a microscope slide, diluted with one drop of saline solution, topped with a coverslip, and examined using a compound light microscope (400×). Some caterpillars dissected immediately after parasitism had large fat bodies that clouded the haemolymph and hindered observations. Thereafter, cater-

pillars were held for 2 days without food prior to dissections. This method allowed for easy viewing of egg and larvae stages (Fig. 1).

The species selected for dissections were cabbage looper (*Trichoplusia ni* (Hübner)) ( $n = 20$ ), true armyworm (*Mythimna unipuncta* (Haworth)) ( $> 10$ ), and the corn earworm (*Helicoverpa zea* (Boddie)) (Lepidoptera: Noctuidae) ( $n = 17$ ). Our previous experiments identified these species to be 'good', 'medium' and 'poor' hosts, respectively (Hervet 2017). For example, exposure of 30 *T. ni* caterpillars to parasitism resulted in 26 individuals (87%) dying from parasitism and producing an average of 95  $F_1$  adult *C. vanessae*. The remaining 4 *T. ni* died as larvae without parasitoid emergence. Exposure of 30 *M. unipuncta* caterpillars to parasitism resulted in 6 individuals (20%) dying from parasitism and producing an average of 76  $F_1$  adult *C. vanessae*. Most (70%) of the remaining *M. unipunctata* caterpillars survived, and some (10%) died as larvae without parasitoid emergence. Exposure of 75 *H. zea* caterpillars to parasitism resulted in three individuals (4%) dying from parasitism and producing an average of 24  $F_1$  adult *C. vanessae*. Most (75%) of the remaining *H. zea* survived and many (21%) died without completing development.

Our observations on the dissected caterpillars showed both teratocyte production and encapsulation to affect the survival of immature *C. vanessae* (Fig. 1). In each of the three lepidopteran species, eggs were readily detected to document the act of oviposition. Newly laid eggs were similar to those displayed in Yu et al. (2008). Five days after parasitism, teratocytes were observed associated with eggs and neonate larvae in *T. ni* (Fig. 1A, B). Teratocytes are cells produced by the extraembryonic egg membrane and released into the host at the time of egg hatch. They have trophic, immunosuppressive, hormonal, and antimicrobial functions that enhance parasitoid survival (Dahlman 1990). Eggs oviposited into the 'poor host' *H. zea* were encapsulated by host hemocytes within four days (Fig. 1C). Many of these eggs hatched, but the neonate larvae did not develop further; i.e., caterpillars dissected after 11 days contained numerous dead first-instar larvae encapsulated by hemocytes (Fig. 1D). Only one of 17 *H. zea* contained a few live parasitoid larvae in its hemocoel among many encapsulated dead ones. The large size of these surviving larvae indicated that they had successfully evaded host's immune defenses. Encapsulation by the host's hemocytes is a common immune response by lepidopteran larvae to parasitism (Lavine and Strand 2002). Dissection of the 'good host' *T. ni* showed that almost all eggs had hatched within 5 days with no signs of encapsulation. At 11 days there were still a few unhatched eggs, indicating that some eggs failed to hatch, though these eggs were not encapsulated.

Unexpectedly, caterpillars of all three lepidopteran species contained many dead first instar parasitoids that were bisected in the transverse plane (Fig. 1F). Observations of larvae physically attacking each other (Fig. 1G) identify these deaths as an outcome of rival combat. To our knowledge, siblicide in hymenopteran parasitoids previously only has been observed for solitary species (Balduf 1926; Mayhew and van Alphen 1999; Pexton and Mayhew 2002; Salt 1961; Willard 1920; Yu et al. 2008) and for a few species of gregarious Encyrtidae (Bartlett and Ball 1964; Bartlett and Medved 1966; Grbić et al. 1992). Therefore, these observations provide the first evidence of



**Figure 1.** Immature stages of *Cotesia vanessae* in caterpillars of three lepidopteran species. **A** Egg (parts of adjacent eggs visible on left and top sides), with visible extraembryonic membrane made of large cells that will become teratocytes (within *Trichoplusia ni*, five days post-oviposition)

larval siblicide by a gregarious braconid parasitoid. Depending upon whether the larvae of the solitary species use their mandibles to pierce or chew their rivals, rivals are killed by either puncturing or what appears to be messily chewing (Salt 1961; Yu et al. 2008). Larvae of the gregarious *C. vanessae* use their mandibles for chewing, which they seemed to use with an acquired efficiency to neatly transect their rivals. It may be that siblicide is common in other gregarious species of *Cotesia*, but has gone unobserved. Future studies to examine immature parasitoids *in situ* will help clarify this matter. As documented with other gregarious *Cotesia* spp. (Magdaraog et al. 2016), first instar *C. vanessae* have reduced mandibles (Fig. 1E), are vesiculate, and do not have a caudal appendage (Fig. 1).

Gregariousness has evolved multiple times from solitary ancestors within the genus *Cotesia* and in other parasitoid taxa, typically accompanied by a loss of antagonistic behaviour toward siblings and a loss of motility (Mayhew 1998; Michel-Salzat and Whitfield 2004). This negative association between antagonistic behaviour toward siblings and gregariousness is common across parasitoid taxa (Dorn and Beckage 2007), and likely reflects kin selection for tolerance among close relatives (Godfray 1987). The apparent exception to this general rule exhibited by *C. vanessae* therefore seems particularly noteworthy.

Motility of first instar solitary species is typically enabled by a caudal appendage (Harvey et al. 2013). These larvae need to move within the host to find contestants in order to kill them because the hosts of these species generally do not have sufficient resources to enable the development of multiple individuals. The lack of a caudal appendage in first instar *C. vanessae* (Fig. 1) suggests that gregarious development in this species was enabled by a loss of motility. Indeed, previous work suggests that the evolution from solitary to gregarious development in hymenopteran parasitoids is more likely to evolve through reduced larval motility than through reduced sibling rivalry, because the loss of larval motility is less stringent than that for tolerance of other larvae (Boivin and Van Baaren 2000; Pexton et al. 2003).

The laying of supernumerary eggs combined with reduced mobility of antagonistic first instars means that for *C. vanessae* it is not the mother wasp but its offspring that adjust brood size according to the available resources. The existence of this parent-offspring conflict could mean that *C. vanessae* larvae still have to evolve kin tolerance, which would enable female wasps to lay fewer eggs into hosts, all of which could survive to maturity,

**Figure 1.** *Continued.* **B** Neonate larva with teratocytes (t) (within *T. ni*, five days post-oviposition). Head on the left, anal vesicle (av) on the right, thoracic and first 7 abdominal segments each partly surrounded on their dorsal and lateral sides by a row of cuticular spines projecting backward **C** Egg becoming encapsulated by hemocytes (within *Helicoverpa zea*, four days post-oviposition) **D** Encapsulated first-instar larva (within *H. zea*, eleven days post-oviposition) **E** Front of head (within *T. ni*, eight days post-oviposition). Microscope focused on mandibles (m) **F** First-instar larva (on its side), with four pieces of bisected larvae (bl) nearby (within *Mythimna unipuncta*, seven days post-parasitism) **G** Larva biting a sibling, with fore-half of bisected larva nearby (centre right) (within *M. unipuncta*, seven days post-oviposition). (Photo credit: Photo B by S. Harris, Agriculture and Agri-Food Canada, Saskatoon, SK; all other photos by V.A.D. Hervet.)

but it may also be that this strategy confers an advantage to this species. Laying hundreds of eggs in the same individual in one oviposition bout will generally optimize the use of available resources, regardless of host size. On one hand, large clutches enable the development of large broods in those hosts that allow for it. On the other hand, siblicide reduces scramble competition and acts as a safety mechanism to prevent the death of the whole brood in hosts that do not have sufficient resources for the development of all eggs laid (Hardy et al. 1992). A large number of fighting larvae may further enhance the ability of the female's progeny to competitively exclude the larvae of conspecific females or larvae of other parasitoid species co-occurring in the host (Giron et al. 2004; Harvey et al. 2000). In addition, a greater number of parasitoids within a host may increase their ability to overwhelm the host's immune system (van Alphen and Visser 1990), therefore enabling larvae to complete development in otherwise marginal host species.

It is interesting to speculate on the factors that could trigger larval siblicide. Presumably these include physical or chemical cues associated with the immature parasitoids. Arguably, the likelihood of physical contact or the concentration of chemical cues would be greater in a 'good' host (e.g., *T. ni*) than in a 'poor' host (e.g., *H. zea*); the immune system of the latter killing many first instars. We also note that our observations are based on a parthenogenetic population of *C. vanessae* for which all immatures within the host are essentially clones. Comparison of siblicide behaviour between parthenogenetic versus bisexual populations might prove enlightening. For example, what are the implications for larval siblicide on adult sex ratios in host species of different quality?

*Cotesia vanessae* has a broad range of lepidopteran hosts within the Noctuidae and Nymphalidae, including many species that are important economic pests of crops (Hervet 2017). Understanding features of host-parasitoid interactions, including larval siblicide within the host, has practical implication for potential use of *C. vanessae* as a biocontrol agent to control these pests. Thus, our unanticipated discovery of siblicidal behaviour is not only relevant for studying basic questions of life history, but also has consequences for applied ecology.

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# Beta diversity of four braconid subfamilies (Braconidae, Agathidinae, Braconinae, Doryctinae and Macrocentrinae) of the Ria Lagartos Biosphere reserve in Yucatan, Mexico, with some considerations on biological habits

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## Abstract

The species diversity composition and phenological behaviour of four braconid subfamilies (Hymenoptera: Braconidae: Agathidinae, Braconinae, Doryctinae and Macrocentrinae) were monitored in three vegetation communities (dune vegetation, tropical deciduous forest and savannah) of the Ria Lagartos Biosphere Reserve (RLBR) in Yucatan, Mexico. Braconid wasps were collected with Malaise traps every 15 days over one year (June 2008 to June 2009). A total of 2,476 specimens were inventoried comprising 233 species and 63 genera. The composition of braconids and their lifestyles differed among the three vegetation communities studied. Doryctinae was the most diverse and abundant subfamily in RLBR (40

genera, 145 species, 990 specimens) and the tropical deciduous forest recorded the maximum abundance and diversity ( $H' = 4.1$ ; alpha value = 1.059), with 61 exclusive species. Phenological sequence indicates an influence of the rainy season in braconid diversity, but its effects differed among braconid subfamilies and among the vegetation communities. Finally, the importance of the RBRL as a conservation site for this hymenopterous wasp is discussed.

### Keywords

Neotropical, braconid wasps, abundance, conservation

### Introduction

Parasitic Hymenoptera are extremely rich in species of terrestrial ecosystems, especially in tropical areas, and they are a group with a principally parasitoid lifestyle (Noyes 1989, Shaw and Hochberg 2001). Their sensitivity to environmental perturbations makes them good indicators of diversity and environmental stability (Delfín-González and Burgos 2000, Shaw and Hochberg 2001).

Braconidae is the second most diverse family in Parasitic Hymenoptera with about 40,000 species, although its richness is estimated at approximately 100,000 (Hanson and Gauld 2006). Specimens vary in size from 1 to 30 mm. The family is cosmopolitan (Gauld and Bolton 1988, Hanson and Gauld 2006) and members are parasitoids of other insects, mainly Diptera, Coleoptera, Lepidoptera and some Heteroptera (Askew 1971, Gillot 2005). Certain braconid females, called Idiobiont, feed on the host immediately, preventing further development, while others, called Koinobiont, allow the host to continue to reach maturity at a delayed rate. In both types of parasitism, the result is the same: death and potential reduction of future host populations (Quicke 1997). In a few cases, another feeding habit reported in braconids is phytophagy as gall formers (Marsh 1991, Infante et al. 1995, Wharton and Hanson 2005, Penteadó-Dias and de Carvalho 2008, Chavarria et al. 2009, Centrella and Shaw 2010, 2013).

Knowledge of the diversity of braconids in Mexico is limited, with only a fraction of the potential species identified (Figuroa-De la Rosa et al. 2003), and few studies characterize the faunal composition of any site (Chay-Hernández et al. 2006, Pérez-Urbina et al. 2011, Ruiz-Guerra et al. 2015). In contrast, the composition and phenology of Braconidae has been documented in several regions of Brazil (Cirelli and Penteadó-Dias 2003a, b, Scatolini and Penteadó-Dias 2003, Barbieri and Penteadó 2012, Souza et al. 2012).

The Ria Lagartos Biosphere Reserve (RLRB) in Yucatán, Mexico, belongs to the Yucatan Peninsula biogeographical province, characterized by areas not exceeding 200 m. altitude with a marine climatic influence. This biogeographic province includes ecosystems that are considered the most threatened globally and have only been the focus of a few faunistic studies (Ramírez-Barahona et al. 2009).

The diversity of Braconidae can be used as an indicator of environmental quality in tropical regions (Delfín-González and Burgos 2000, Barbieri and Penteadó 2012), however this has never been evaluated in terms of Neotropical Mexican fauna. This

research is the first undertaking of Mexican origin to document the diversity, relative abundance and annual distribution of adult activity of four braconid subfamilies in a region, employing a systematic sampling of three vegetative communities.

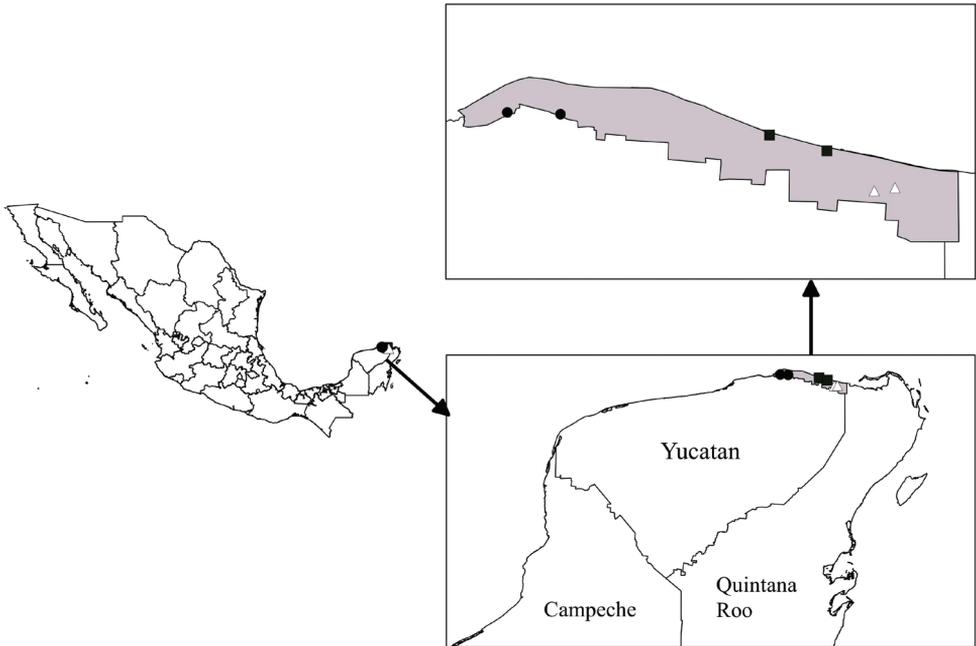
## Materials and methods

### Study area

The Ria Lagartos Biosphere Reserve is in the state of Yucatan, in the Southern Mexico (21°37'29.56"N and 21°23'00.96"N; 88°14'33.35"W and 87°30'50.67"W; 60,347.82 hectares, 100 masl) (Fig. 1). The biosphere is bordered on the north by the Gulf of Mexico; on the south by the municipalities of Tizimin, Rio Lagartos and San Felipe; on the west by the Dzilam State Reserve; and on the east by the Yum Balam Wildlife Protection Area. The climate in most of the reserve is very dry. Temperatures are homogeneous and range between 23 to 27 °C (Arriaga et al. 2002, CONANP 2007, CNA 2006). From June to October, the region receives 62% of the total annual rainfall, with the remaining 38% occurring during the dry season (November to May). The region also experiences between October and February a strong cold northeasterly wind which blows along the shore of the Gulf of Mexico called Norte. (Cuevas-Jiménez and Euán-Ávila 2009). Coastal dune vegetation is integrated with tropical xerophyte species, small and succulent big palms, as *Acanthocereus tetragonus* (L.), *Agave sisalana* Perrine, *A. vivipara* L., *Coccoloba uvifera* (L.)L., *Coccothrinax readii* H.J.Quero, *Pseudophoenix sargentii* H.Wendl. ex Sarg., *Opuntia dillenii* (Ker Gawl.) Haw., and *Thrinax radiata* Lodd. Ex Schult. & Schult.f. The dominant plants in the tropical deciduous forest are chandelier-shaped and succulent plants, mainly *Cephalocereus gaumeri* Britton & Rose, *Nopalea gaumeri* Britton & Rose, *Pterocereus gaumeri* (Britton & Rose) Th. acDoug. & Miranda, *Stenocereus griseus* (Hwa.) Buxb. This type of vegetation is expands from central to eastern areas of Ria Lagartos. Savannah are extensive areas covered with a mixed association of *Cladium* sp., *Phragmites australis* (Cav.) Trin. Ex Steud., and *Typha* sp. (INE 1999, CONANP 2007).

### Specimen collection

A systematic sampling was taken from the three dominant types of vegetation on the reserve: tropical deciduous forest, savannah and dune vegetation. Cauich-Kumul et al. (2012) describe the ecological and botanical characteristics of each vegetation mentioned above. Among each type of vegetation, two sites were selected, each approximately two hectares in size. Two bidirectional Malaise traps (Townes 1972) were placed in the center of each area to prevent edge effects (Fig. 1). The traps (12 in total) collected samples for 365 days, from June 2008 to June 2009, and the collecting pots were replaced every two weeks (Gonzalez-Moreno and Bordera 2011).



**Figure 1.** Ria Lagartos Biosphere Reserve, Yucatan, Mexico; black dots= savannah sites; black squares= dune sites; white triangles= tropical deciduous forest sites).

The braconid wasps were stored and handled in accordance with the curatorial standards proposed by Wharton et al. (1998). The taxonomic identification of the specimens was determined using specialized literature (Sharkey 1990, Berta de Fernandez 1998, Marsh 2002, Leathers and Sharkey 2006, Sarmiento-Monroy 2006) and verified by comparison with specimens held at the Colección Entomológica Regional (CER-UADY) and the Hymenoptera Institute (University of Kentucky). The identified specimens were then deposited at CER-UADY.

## Data analysis

Richness was characterized by the number of species found (Moreno and Halffter 2001) and using rarefaction curves (Jiménez-Valverde and Hortal 2003). The Shannon-Wiener index ( $H'$ ) was used to assess diversity and evenness (E) of the four braconid subfamilies in each vegetation type, based on richness and abundance data (Halffter et al. 2001, Feinsinger 2003). The Solow test (1993) was employed to detect differences in braconid diversity among habitats, using Species Diversity and Richness 3.02 software (Henderson and Seaby 2002). To simultaneously compare patterns of species abundance and diversity among vegetation types, rank-abundance curves (Magurran 1988, Feinsinger 2003) were created. Beta diversity (spatial  $\beta$ ) was calculated using the index of complementarity (Price 1984). This index highlights the difference in the spe-

cies list from two habitats or communities as a percentage, to determine if the replacement of species is linked to factors such as the distance between habitats, vegetation structure or environmental heterogeneity (Colwell and Coddington 1994, Halffter and Moreno 2005, Pineda et al. 2005). Phenological graphs evaluated braconid flight patterns, with reference to the wet (June to August) and dry (March-May) seasons.

Classification of braconid biological host development strategies (koinobiont, idiobiont, phytophage) was followed according to Harvey et al. (2013). Reported information were used for the taxa identified (Shaw and Huddleston 1991, Wharton et al. 1998, Marsh 2002).

## Results

### Species richness

2,476 specimens of the four subfamilies were collected and classified under 63 genera and 233 species; 77 taxa were determined as morphospecies; 29 of these lack taxonomic keys; 15 are new species (Table 1). The subfamily with the highest number of species and specimens was Doryctinae, 145 and 990, respectively; Agathidinae exhibited the second highest, with 39 species and 942 specimens. The two subfamilies constitute 78.9% of all species and 78% of all specimens collected in the RLBR (Table 1).

The genus with the highest number of species was *Heterospilus* (18), followed by *Ecpylus* (17), *Bracon* (16), *Allorhogas* and *Macrocentrus* (10). Together these genera represented 30.4% of the species collected. The remaining 61 genera were represented by one to seven species. The tropical deciduous forest represented 39.2% of the specimens (168 species). Savannah and dune vegetation presented similar abundance and species richness (761 and 116; 745 and 120, respectively) (Table 2, 3).

Rarefaction curves do not reach an asymptote (Fig. 2), indicating that there are many uncollected species. Tropical deciduous forest composition could potentially be more diverse, and dune vegetation and savannah share similar numbers of species.

The tropical deciduous forest had the highest diversity value, according to the Shannon-Wiener index, while the lowest value was collected from savannah vegetation (Table 4). The Solow diversity contrast test (Delta values) demonstrated that the tropical deciduous forest is statistically more diverse than the savannah and dune vegetation; and the dune vegetation is more diverse than the savannah vegetation ( $\alpha < 0.05$ ) (Table 4).

### Tropical deciduous forest community

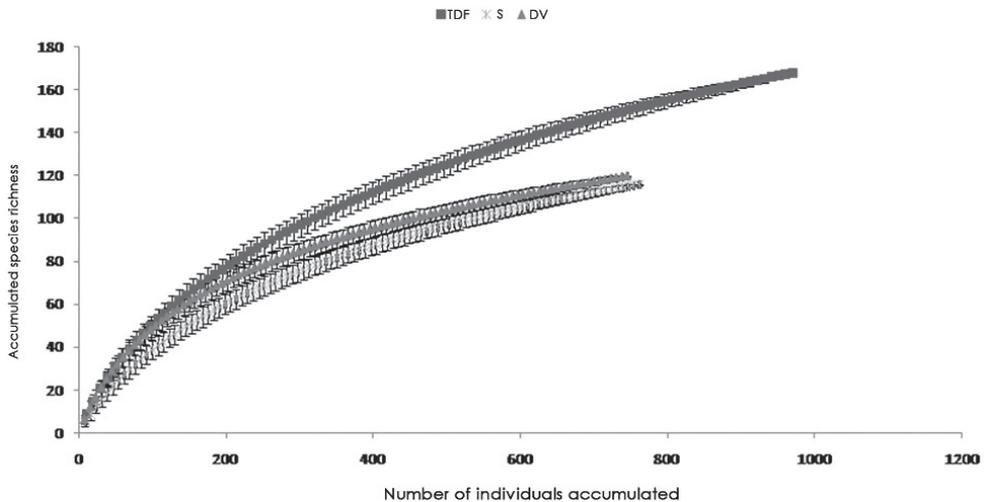
This community was the richest in number of species and had the greatest equality value (Table 2, 3, 4). The most abundant species in this vegetation were: *Alabagrus albispina* (Cameron), with 139 specimens; *Macrocentrus* sp8, with 103 individuals; and *Heterospilus* sp17, with 34 specimens (Fig. 3). *Alabagrus albispina* and *Macrocentrus*

**Table 1.** Braconidae richness and abundance in the RLBR, Yucatan, Mexico.

Subfamily	Generic richness	Species richness	Abundance
Agathidinae	14	39	942
Braconinae	5	31	222
Doryctinae	40	145	990
Macrocentrinae	4	18	322
Total	63	233	2476

**Table 2.** Braconidae richness and abundance in three vegetation communities in the RLBR, Yucatan, Mexico.

Plant community	Subfamilies	Genera	Species	Specimens
Dune Vegetation	4	42	120	745
Savannah	4	44	116	761
Tropical Deciduous Forest	4	89	168	970

**Figure 2.** Species accumulation and rarefaction curves in three vegetation communities in the Ria Lagartos Biosphere Reserve, Yucatan, Mexico (TDF= Tropical Deciduous Forest, S= Savannah and DV= Dune Vegetation).

sp8 provide most of the abundance for this area (10%). The idiobiont strategy predominated, with 115 species, principally Doryctinae. Koinobionts were represented by 42 species. This type of community had the highest number of potential phytophagous species, with 11 species of *Allorhogas* (Table 3).

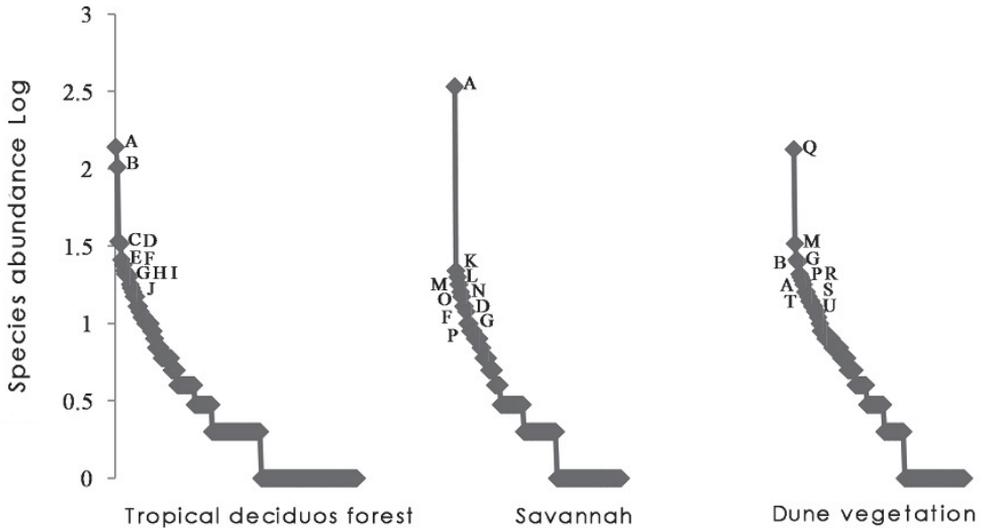
### Savannah community

This vegetation type was the least equitable community studied, but the second with highest abundance, with a total of 761 specimens collected. The dominant species

**Table 3.** Life strategies and habitats for Braconidae genera collected in Ria Lagartos reserve, Yucatan, Mexico.

Subfamily	Genus	Host development strategy			Habitat		
		Idiobiont	Koinobiont	Phytophagous	Dune	Savannah	Tropical deciduous forest
Agathidinae	<i>Alabagrus</i>		X		X	X	X
	<i>Amputoearinus</i>		X		X		
	<i>Cremnops</i>		X				X
	<i>Zacremnops</i>		X			X	X
	<i>Zelomorpha</i>		X		X	X	X
Braconinae	<i>Atanycolus</i>	X			X	X	X
	<i>Bracon</i>	X			X	X	X
	<i>Compsobraconoides</i>	X			X		X
	<i>Digonogastra</i>	X			X	X	X
Doryctinae	<i>Acrophamus</i>	X			X	X	X
	<i>Allorhogas</i>			X	X	X	X
	<i>Caigangia</i>	X			X	X	X
	<i>Callibormius</i>	X			X	X	X
	<i>Coiba</i>	X			X	X	X
	<i>Curtisella</i>	X				X	
	<i>Ecphylus</i>	X			X	X	X
	<i>Glyptocolastes</i>	X			X	X	X
	<i>Gymnobracon</i>	X					X
	<i>Hansonorum</i>	X				X	X
	<i>Hecabolus</i>	X				X	
	<i>Heterospathius</i>	X				X	X
	<i>Janzenia</i>	X			X		
	<i>Johnsonius</i>	X				X	X
	<i>Labania</i>				X		X
	<i>Leluthia</i>	X			X	X	X
<i>Masonius</i>	X				X	X	
<i>Notiospathius</i>	X		X	X	X	X	
Doryctinae	<i>Odontobracon</i>	X			X	X	X
	<i>Pedinotus</i>	X					X
	<i>Pioscelus</i>	X			X		X
	<i>Platydoryctes</i>	X			X	X	X
	<i>Psenobolus</i>				X	X	X
	<i>Rhaconotus</i>	X			X	X	X
	<i>Spathius</i>	X			X		
	<i>Stenocorse</i>	X		X	X		X
Macrocentrinae	<i>Austrozele</i>		X			X	
	<i>Dolichozele</i>		X				X
	<i>Hymenochaoinia</i>		X		X	X	X
	<i>Macrocentrus</i>		X		X	X	X

were *A. albispina*, with 340 individuals, followed by *Lytopilus* sp2, with 22 specimens (Fig. 3). Idiobiont species were more species-rich than koinobionts, with 77 and 32 species, respectively. The number of potential phytophagous species was equal to that one found among dune vegetation (7) (Table 3).



**Figure 3.** Braconidae rank/abundance curves in three vegetation communities in the RLBR (only the most abundant species are included). A= *Alabagrus albispina*, B= *Macrocentrus* sp8, E= *Macrocentrus* sp2, S=*Macrocentrus* sp6, C= *Heterospilus* sp17, D= *Heterospilus* sp2, F= *Heterospilus* sp12, G= *Heterospilus* sp8, H= *Heterospilus* sp3, I= *Heterospilus* sp4, J= *Heterospilus* sp6, N= *Heterospilus* sp1, K= *Lytopilus* sp2; L= *Bracon* sp4, O= *Bracon* sp2, M= *Zelomorpha arizonensis*, P= *Zelomorpha lenisterna*, Q= *Cremnops ferrugineus*, R= *Cremnops melanoptera*, T= *Coiba woldai* and U= *Zacremnops cressoni*.

### Dune vegetation community

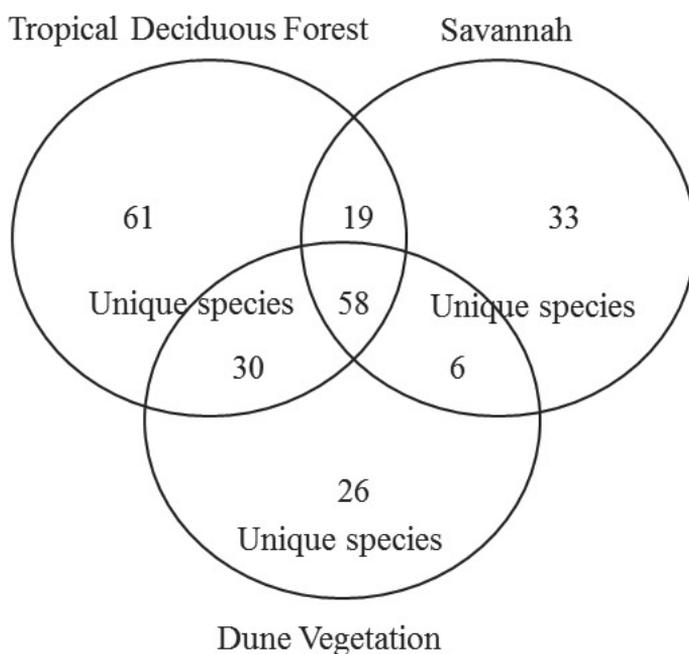
This vegetation community had richness and equity values similar to that of tropical deciduous forest (Table 4), but it was less abundant in specimens (Table 2). The dominant species were *Cremnops ferrugineus* (Cameron) and *Zelomorpha arizonensis* Ashmead, which provided the highest abundance recorded for this area, with 134 and 33 specimens, respectively (Fig. 3). The composition of life history strategies was similar to that reported for savannah vegetation, with 29 koinobiont and 84 idiobiont species. The number of potential phytophagous species was also similar to that observed in the savannah (7 species) (Table 3).

### Beta diversity ( $\beta$ )

The results obtained through the index of complementarity indicate that the tropical deciduous forest and the savannah had the highest value, with 83% and 77 species shared. The second highest value was for tropical deciduous forest and dune vegetation at 80%, sharing 88 species. Finally, for the savannah and dune vegetation the complementarity index was 69% with 64 shared species (Fig. 4). Overall, the values calculated represent relatively high beta diversity for Braconidae in the RLBR.

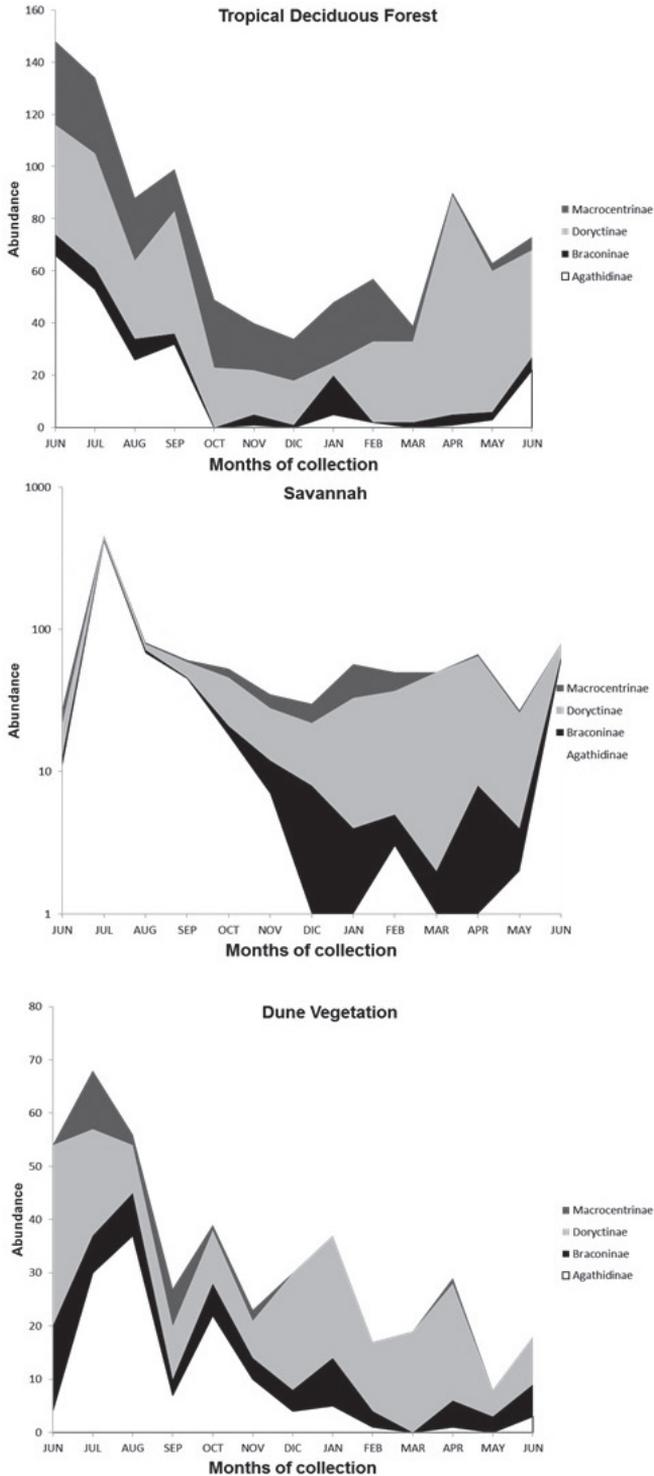
**Table 4.** Diversity indexes and number of braconid species collected according biological host development strategies and three vegetation communities in the RLBR.

	Tropical deciduous forest	Savannah	Dune vegetation
Shannon-Wiener ( $H$ )	4.1	3.1	3.9
Delta ( $\alpha < 0.05$ )	1.059	0.144	0.915
Koinobiont	42	32	29
Idiobiont	115	77	84
Phytophagous	11	7	7

**Figure 4.** Exclusive and shared Braconidae (Insecta: Hymenoptera) species in three vegetation communities in the RLBR, Yucatan, Mexico.

### Braconidae phenology

Braconidae wasps are active throughout the year, but the number of species and individuals varied across the collection period. 73 species were collected in the rainy season, while 64 were collected during the driest months. June was the month with the highest species richness during the rainy season (66 species), while April exhibited the highest species richness during the dry season (57 species). In November and December (*Nortes* season), braconid activity was low, with only 34 species collected and a maximum richness reported in November with just 26 species. *Amazondoryctes bicolor* Barbalho



**Figure 5.** Phenology of four Braconidae subfamilies (Agathidinae, Braconinae, Doryctinae and Macrocentrinae) in three vegetation communities in the RBRL, Yucatan, Mexico.

and Pentead-Dias, *Coiba woldai* Marsh, *Hansonorum carolinae* Marsh, *Odontobracon janzeni* Marsh, *O. nigra* Marsh, *O. nigriceps* Cameron, *Rhaconotus chrysochaitus* Marsh and *R. rugosus* Marsh, were collected throughout the year. Except in the tropical deciduous forest, Agathidinae was abundant in all the vegetation zones, peaking in June (tropical deciduous forest), July (savannah) and August (dune vegetation) (Figure 5). In the tropical deciduous forest, Doryctine abundance peaked in June. Agathidinae was the subfamily least represented in the tropical deciduous forest. Macrocentrinae abundance was highest in the dune vegetation and in the tropical deciduous forest.

## Discussion

The 233-species recorded in this study exceed the diversity and abundance reported by other authors for a single ecosystem; Whitfield and Lewis (1999) mentioned 23 subfamilies, 84 genera and 251 morphospecies in Arkansas, USA; Idris and Hasmawati (2002) reported 19 subfamilies and 95 morphospecies in disturbed Sengalor forests of Malaysia. In Brazil, the number of subfamilies recorded from one locality varies widely, from 10–17 subfamilies, 19–56 genera, but without a clear definition of the species collected (Scatolini and Pentead-Dias 2003, Barbieri and Pentead 2012, Souza et al. 2012). In Mexico, the only comparable study is from Chay-Hernández et al. (2006), who collected 21 subfamilies, 84 genera and 342 species in cultivated areas in Yucatan; and Pérez-Urbina et al. (2011), with 25 subfamilies, 130 genera and 156 species in a locality from Tamaulipas. The latter study showed that braconids exceeded the diversity of its sister group, Ichneumonidae, in the same locality. González-Moreno and Bordera (2012) recorded 148 Ichneumonidae species for the RLBR. Ruiz-Guerra et al. (2015) recorded 65 species and 15 subfamilies in a region of Veracruz. Our results suggest that Yucatan State has the highest number of braconid species and genera in the country.

The greater diversity of braconid wasps reported in this study, as compared to other studies, is most likely a result of using 12 Malaise traps to take samples, which is the best method for catching ichneumonoids (Papp 1999, Fraser et al. 2008). Replicas of sampling sites were also made. Species richness of Ichneumonoidea is underestimated globally, and in Neotropical environments the systematic use of Malaise trapping is increasing our knowledge of its diversity (Sääksjärvi et al. 2004, Cauch-Kumul et al. 2012, González-Moreno and Bordera 2012).

With the results obtained in this work, the community of Braconidae in Yucatan is becoming the most identified in Mexico, in terms of diversity and taxonomic classification, even with many taxa still to describe (López-Martínez et al. 2011, Cauch-Kumul et al. 2014).

## Diversity of Braconidae in the three vegetation sites sampled

The braconid subfamily alpha diversity reported here may be the result of plant complexity and the resulting diversity of host availability. Tropical deciduous forests in the Yucatan

have up to 103 different species of woody plants in a small area (0.1 hectare) (Gutierrez et al. 2011). By example, a large proportion of palm genera and subfamilies reported from Mexico are in Yucatan tropical deciduous forests (Alvarado-Segura et al. 2012).

In RLBR Doryctinae had the highest abundance and species richness. This is the second most diverse subfamily of Braconidae, with at least 200 genera in the Neotropics (Marsh 2002). In the present study we collected 145 species belonging to 40 genera (Table 1), representing 20.5% of all Neotropical doryctine genera. The subfamily exhibits a broad host range primarily on larval Coleoptera (Bruchidae, Bostrichidae, Buprestidae, Cerambycidae, Curculionidae, Proterrhinidae), but also on Lepidoptera (Brachodidae, Crambidae, Gelechiidae, Phycitidae, Pyralidae and Pyraustidae) (van Achterberg and Shaw 2010), and rarely Symphyta (Xiphydriidae) and Embioptera (Belokobylskij et al. 2004). Phytophagy is another biological habit registered for doryctines (Marsh 1991, Infante et al. 1995, Pentead-Dias and de Carvalho 2008, Chavarria et al. 2009, Centrella and Shaw 2010). This variety of habits provides Doryctinae with numerous possibilities to exploit biological resources in different ecosystems.

In contrast, the low abundance and diversity of Braconinae may be a consequence of the distribution of its subfamily. Although it is cosmopolitan, it is more diverse in the Old-World tropics (Pentead-Dias et al. 2007).

With respect to the diversity between habitats, the greatest similarity occurred in the tropical deciduous forest and dune vegetation communities (88 species in common). We suggest that the low  $\beta$  diversity found in the studied area may be the result of a higher proportion of generalist idiobionts species (Askew and Shaw 1986). The changes in the spatial structure, such as patch types or an increase in patch isolation, could modify the capacity of these organisms to disperse. Likely, the generalist idiobiont species cannot disperse effectively because of a change in structure, suffering decreases in regional population sizes (Fahrig and Merriam 1994). In addition, the three ecosystems have completely different vegetation cover that could impact the species diversity (CONANP 2007).

### **Life strategies: koinobionts, idiobionts and phytophagous**

Molecular evidence suggests that the common ancestor of Ichneumonoidea was a concealed host idiobiont parasitoid (Belshaw and Quicke 2002). Koinobiosis is considered a more derived characteristic than idiobiosis in Braconidae, despite the high percentage of koinobiont species (Wharton 1993, Kasparian 1996). The specialization of the koinobiont species in the subfamilies studied in the RLBR is common and is possibly due to the strong selection pressure which led them to modify and develop certain characteristics to maintain these high abundance levels over time (Thompson 1994).

The idiobiont life strategy (generalists) was consistent among the four subfamilies and in all three types of vegetation studied. It was present in greater abundance in the tropical deciduous forest (49%). In this habitat *A. albispina* had the greatest number of individuals. The koinobiont life strategy had the highest proportion of species in the tropical de-

ciduous forest (18%) and in the dune vegetation (12%) (Table 3). However, our findings cannot explain the influence of vegetation type on the braconid biological traits distribution. Perhaps, the inclusion of highly diverse koinobiont subfamilies like Microgastrinae, Alysiinae and Opiinae could provide more clarity on this topic. Based on previous works (Owen and Owen 1974, Rathcke and Price 1976, Janzen 1981, Hawkins 1990), koinobionts species richness is lower in the tropics, but richness of generalist idiobiont species exhibit high values. However, Askew and Shaw (1986), Wharton (1993) and Hawkins (1994) argue that idiobionts species are less abundant in the tropics. Chay (1999), Cicero (2002), González (2002), Burgos (2003) and Chay-Hernández et al. (2006), mention that koinobionts species increase according to the degree of habitat disturbance, which can be compared to the study of Restello and Penteadó-Dias (2006) in Brazil, where they conclude that braconids are more abundant in areas with some degree of modification.

Phytophagy has been a well-known phenomenon identified in Braconidae since 1989, when Macêdo and Monteiro reported an undetermined species of *Allorhogas* as a consumer of Leguminosae seeds. Phytophagous braconids primarily produce galls on stems and fruits, from feeding on seeds or fruits, and feed on species of Araceae, Burseraceae, Fabaceae, Melastomataceae, Mimosaceae, Moraceae, Proteaceae, Rubiaceae and Solanaceae. The phytophagic life strategy occurs primarily in the Neotropics, in species of *Allorhogas*, *Bracon*, *Monitoriella* and *Psenobolus* (Marsh, 1991, Infante et al. 1995, Ramirez and Marsh 1996, Marsh et al. 2000, Marsh 2002, Flores et al. 2005, Centrella and Shaw 2010, Martinez et al. 2011, Perioto et al. 2011, Shimbori et al. 2011, Centrella and Shaw 2013). The number of potential phytophagous species collected in the RLBR are represented in 75% of the genera known with this biological trait and indicate a future need to determine the interactions between host plants and phytophagous braconids, its abundance and its role in the vegetation composition.

### **Braconidae phenology**

The largest number of individuals and species (1050 and 73, respectively) was collected during the rainy season (June-July), a result which correlates with that reported by Falcó et al. (2006); while the lower specimen abundance and species-richness occurred in the dry season (February-March), with 233 individuals and 57 species. Rain favors the profusion of plants and its associated phytophagous and xylophagous insects, which are the potential hosts on which parasitoids depend (Falcó et al. 2006). The rainy season is also when foliage, fruits and seeds provide niches and nutrients for the development of herbivore species.

Our results do not match those reported by González (2002), who concluded that Braconidae communities reach higher abundances in the dry season. Rain is considered a factor that negatively affects the movement of small Hymenoptera species (Speight et al. 1999). Evidently, this was not the case in our study, as similar abundances or small sized species of the four Braconidae subfamilies were collected in both dry and rainy seasons.

The seasonality of koinobiont braconids is determined by the phenology of their hosts (Memmott et al. 1994, Wolda 1988). Idiobionts typically have a wider host

range (Sharkey 1993) and are not necessarily synchronized with the life cycle of any one host, but rather by abiotic factors (Memmot et al. 1994). Random patterns might be expected over time.

### **Ría Lagartos Biosphere Reserve significance**

The RLBR is the only nesting site of the pink flamingo (*Phoenicopterus ruber* L. *ruber*) in Mexico (Fraga 2006), with a high fish species-richness in the coastal area (Peralta-Meixueiro and Vega-Cendejas 2011) and a growing reported insect richness (González-Moreno and Bordera 2011, 2012, Cauich-Kumul et al. 2014). Cué-Bär et al. (2006) granted the RLBR and other Mexican areas with priority conservation status, based on the unique composition of tree species. Floral richness has a direct impact on the Hymenoptera richness, which has been demonstrated in the sister group of Braconidae, Ichneumonidae (Sääksjärvi et al. 2006).

### **Conclusion**

Ria Lagartos Biosphere Reserves with distinctive vegetation types support a vast diversity of four braconid wasp subfamilies (Agathidinae, Braconinae, Doryctinae, Macrocentrinae) in Yucatan, Mexico. It benefits one of the most diverse and abundant braconid subfamilies, Doryctinae, which recorded 40 genera, 145 species, and 990 specimens. Such abundance of species allows us to hypothesize about the existence of several parasitism relationships and the existence of many unknown hosts.

### **Acknowledgments**

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## Supplementary material I

### Life strategies and habitats for Braconidae species collected in Ria Lagartos Biosphere Reserve, Yucatan, Mexico

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Data type: species data

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# The unknown followers: Discovery of a new species of *Sycobia* Walker (Hymenoptera: Epichrysomallinae) associated with *Ficus benjamina* L. (Moraceae) in the Neotropical region

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## Abstract

Biotic invasion in mutualistic communities is of particular interest due to the possible establishment of new relationships with native species. *Ficus* species are widely cultivated as ornamental plants, and they host specific communities of chalcid wasps that are strictly associated with the fig inflorescences. Some introduced fig species are capable of establishing new relationships with the local fig wasps, and fig wasp species may also be concomitantly introduced with their host plants. *Ficus benjamina* L. is widely cultivated across the world, but the associated fig wasps are not reported outside of the species native range. We describe for the first time a non-pollinating fig wasp associated with *F. benjamina* inflorescences outside its native distribution. *Sycobia hodites* Farache & Rasplus, **sp. n.** is the third known species of the genus and was recorded in populations of *F. benjamina* introduced in the Neotropical region throughout several

localities in Argentina, Brazil and Colombia. *Sycobia* is a gall-inducing non-pollinating fig wasp genus associated with fig trees in the Oriental and Australasian regions. This species competes with pollinators for oviposition sites and may hinder the future establishment of the native pollinator of *F. benjamina*, *Eupristina koningsbergeri* Grandi, 1916 in the New World. However, the occurrence of a gall inducing species in this host plant may open ecological opportunities for the establishment of species belonging to other trophic levels such as cleptoparasite and parasitoid wasps.

### Keywords

biocontrol, Chalcidoidea, dispersal, invasive species, parasitoid

### Introduction

Biotic invasions are altering the world's natural communities at an unprecedented level, and they impose a global challenge for the conservation of biodiversity and the maintenance of ecological communities (Mack et al. 2000; Simberloff et al. 2013; Vitousek et al. 1997). The role of biological invasions in symbiotic communities is of particular interest, since introduced plants and phytophagous organisms may establish new relationships with native species and change the community structure. Generalism and specialism have a central role in the capacity of establishment in invasive species. In the case of plants, groups with specialized pollination syndromes are less likely to reproduce in foreign environments than generalist species that are pollinated by widely distributed species (Richardson et al. 2000).

*Ficus* establishes mutualistic relationships with pollinating agaonid wasps that develop in their inflorescences. These wasps induce galls in fig inflorescences. Several other lineages of chalcid wasps utilize fig inflorescences as oviposition sites without pollinating (but see Jousselin et al. 2001), and they may induce galls or develop asinquilines or parasitoids, sometimes hyperparasitoids, of other galling wasps (Kjellberg et al. 2005). Several fig species are widely used as ornamental plants, as examples we can cite *F. benjamina* L., *F. benghalensis* L., *F. lyrata* Roxb., *F. macrophylla* Desf. ex Pers., *F. microcarpa* L. f., *F. pumila* L., *F. religiosa* L., and *F. rubiginosa* Desf. ex Vent. (Dehgan 1998; Ibarra-Manríquez et al. 2012). These trees are cultivated in urban areas and roadsides in several (sub)tropical but also temperate countries. As they depend on their specific pollinators to reproduce, they cannot propagate sexually outside their original range of distribution in the absence of their obligate pollinators. Nevertheless, the pollinators of some *Ficus* species have been accidentally or deliberately introduced in several regions of the globe (Beardsley 1998; Ramírez and Montero 1988; Vianna-Filho et al. 2017) and some of these fig species may become invasive. In a few cases, wasp species associated with local figs may colonize the newly introduced fig trees, develop inside figs and pollinate (Ramírez and Montero 1988; van Noort et al. 2013). Non-pollinating fig wasps also often establish outside their native ranges on their native host-plant. These species are usually referred as 'followers' whereas some local species that colonize introduced fig species are referred as 'colonists' (van Noort et al. 2013; Wang et al. 2015a).

Within the Neotropical region several species of pollinating and non-pollinating fig wasps are associated with the oriental *F. microcarpa* (Farache et al. 2009; Figueiredo

and Motta-Júnior 1986; Figueiredo and Motta-Júnior 1993; Figueiredo et al. 1995; Ramírez and Montero 1988). To the contrary, only the pollinator (i.e., *Platyscapa quadriceps* Mayr, 1885) of *F. religiosa* has been observed (Vianna-Filho et al. 2017). Until now, there was no register of Old-World fig wasps associated with *F. benjamina*, another widely ornamental cultivated species, in the Neotropical region. Indeed, there is no report of Old-World fig wasps associated with *F. benjamina* outside its native range. However, a native Neotropical fig wasp species has been recorded developing in *F. benjamina* figs in Costa Rica (Ramírez and Montero 1988).

Epichrysomallinae are distributed throughout the Old-World tropics and the group is particularly diverse in the Oriental region (Bouček 1988; Rasplus et al. 2003). They are naturally absent from the Neotropical region except for species of *Odontofroggattia* Ishii, 1934 introduced with their host plant (*F. microcarpa*) (Farache et al. 2009; Rasplus and Soldati 2005; Wang et al. 2015a). They induce large galls inside the figs either in the flowers or in the fig wall. A few species are known to induce galls on fig leaves (Beardsley and Rasplus 2001) or on fig twigs (Ferrière 1929). Their ovipositor does not protrude beyond the metasoma as in many non-pollinating fig wasps, but is coiled inside the metasoma (Galil and Copland 1981). Epichrysomallinae exhibit several degrees of sexual dimorphism. Possibly due to their small brood size, most species have only winged males. Brachypterous and apterous males are only known in three genera, namely *Neosycophila* Grandi, 1923, *Lachaisea* Rasplus, 2003 and *Camarothorax* Mayr, 1906 (Bouček 1988; Joseph 1961; Vincent and Compton 1992)(Bouček 1988; Joseph 1961; Vincent and Compton 1992)(Bouček 1988; Joseph 1961; Vincent and Compton 1992) .

About 49 species names are associated with 19 genera of Epichrysomallinae (van Noort and Rasplus 2018), but the circumscription of the genera is still unclear, and the subfamily requires a thorough taxonomic revision. Among the Epichrysomallinae genera, *Sycobia* Walker, 1871 is closely related to *Odontofroggattia* (Feng and Huang 2010). Both genera are characterized by the absence of external occipital carina; notauli reaching the transscutal articulation outside of scutoscuteellar sutures; absence of submarginal vein; and presence of only 5 or 6 funicular segments. *Sycobia* can be separated from *Odontofroggattia* by its pentamerous tarsi (tetramerous in *Odontofroggattia*, the species cited as pentamerous by Bouček (1988) is probably a misidentification of a female *Sycobia*); antennal formula 11163, with presence of an anellus, sometimes subquadrate (11063 or 11053 in *Odontofroggattia*, no anellus); the shape of the male head, which is elongated or subquadrate (subcircular in *Odontofroggattia*). Two species of *Sycobia* are described from India, namely *S. bethyloides* Walker, 1871 and *S. mathewi* Joseph, 1956 (Joseph 1956). At least one undescribed species is also cited to be associated with *F. benjamina* in China (Niu et al. 2009) and many undescribed species are known from South-East Asia (Rasplus J.-Y. pers. comm.).

Here we report the first occurrence of *Sycobia* outside its native range. The species discovered is new to science and is probably associated with *F. benjamina* (section *Conosycea*) somewhere in its large native distribution, which extends from India to Australia. *Sycobia hodites* Farache & Rasplus, sp. n. is described and characters to distinguish it from congeneric wasps are given. The distribution of this new species over the Americas and its abundance in fig syconia is also reported on.

## Methods

### Specimen sampling and localities analyzed

After observing the presence of fig wasp galls in *Ficus benjamina* in Rio de Janeiro/Brazil, fig samples were collected between August 2016 to August 2018 in several localities in Argentina (Misiones), Brazil (states of Acre, Bahia, Distrito Federal, Pará, Rio de Janeiro, Rio Grande do Norte, Roraima, Santa Catarina and São Paulo) and Colombia (Valle del Cauca).

Near ripe figs were collected and left in netting bags until wasp emergence, after 2–3 days. Wasps were then killed with ethyl acetate and conserved in 70% ethanol. In each sampling site, 10–30 figs were dissected in order to quantify the number of *Sycobia* galls. As only one fig wasp specimen emerged from a gall, the number of galls may correspond to the number of wasps developing in the figs.

For one tree in Ribeirão Preto city/Brazil, figs were individualized in plastic specimen jars until the wasps emerged, then the number of wasps and total fig flowers were quantified in order to evaluate (1) the percentage of fig flowers occupied by wasps and (2) the sex ratio and the proportion of winged and wingless males observed in an individual fig.

Distribution maps for the occurrence of *Sycobia hodites* sp. n. and for samples of *F. benjamina* that did not show any wasp galls, as well as abundance exploratory data analyses were performed using R v3.4.2 (R Core Team 2018) and the packages ggplot2 (Wickham 2009), ggsm (Baquero 2017) and rnatuarearth (South 2017).

### Specimen preparation and description

Wasp specimens were dried using a Critical Point Drier (BALTEC CPD 0300) and card-mounted following Noyes (1982). A few specimens were mounted on grey triangular cards in order to facilitate imaging. Acronyms for specimen depositories are as following:

<b>CBGP</b>	Centre de Biologie pour la Gestion des Populations, Montferrier-sur-Lez, France;
<b>MUSENUV</b>	Universidad del Valle Museum – Museo de Entomología de la Universidad del Valle;
<b>RPSP</b>	Universidade de São Paulo, Ribeirão Preto, Brazil.

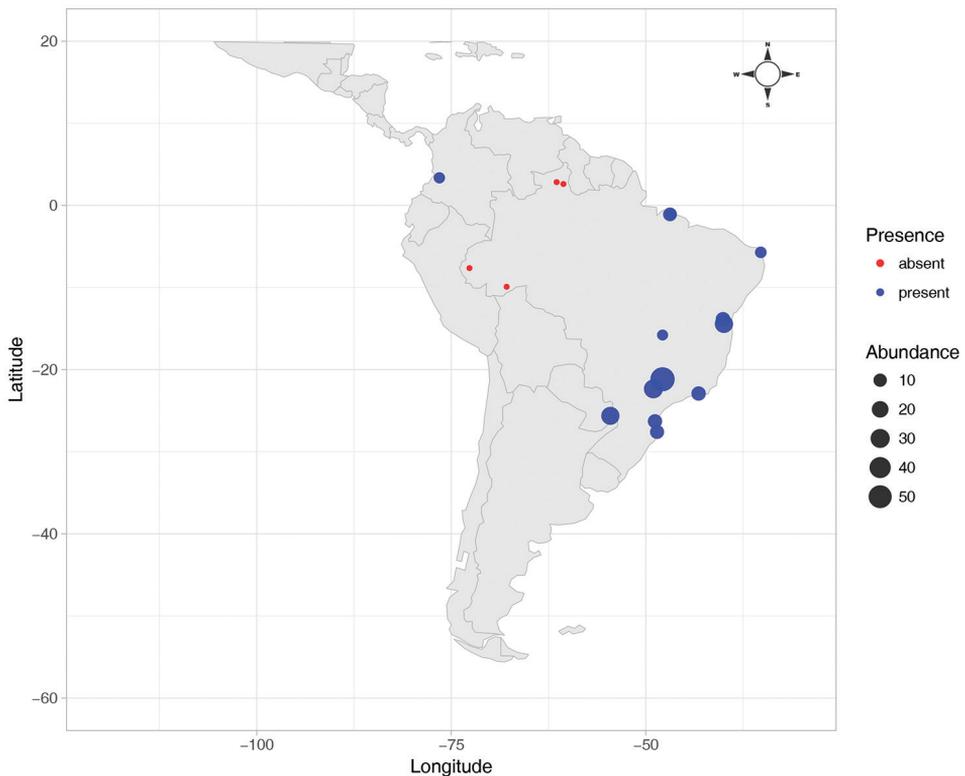
Images of specimens were produced using a Keyence VHX-5000 digital microscope. Some specimens were dissected, mounted and sputter-coated with gold for scanning electron microscopy (SEM), which was performed with a JEOL JCM-6000Plus microscope. Images were then edited in Pixelmator v.3.7. Measurements were taken using ImageJ (Abràmoff et al. 2004).

## Results

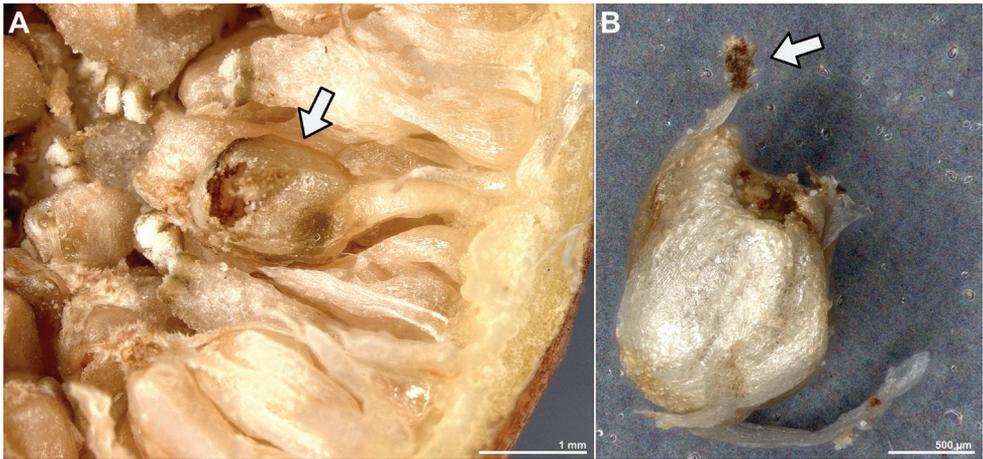
### Sampling and bionomics

*Sycobia hodites* Farache & Rasplus, sp. n. was reared from figs in Argentina, South, Southeastern, North and Northeastern Brazil, and in Colombia, but was not observed in fig samples of *F. benjamina* from Acre and Roraima (Fig. 1), suggesting that *Sycobia hodites* may still be absent in central and western Amazon. Each gall of *Sycobia* was induced in a single fig pistillate flower, as remnants of flower stigmata could be observed on the galls (Fig. 2). The larva develops at the expense of the induced gall tissues.

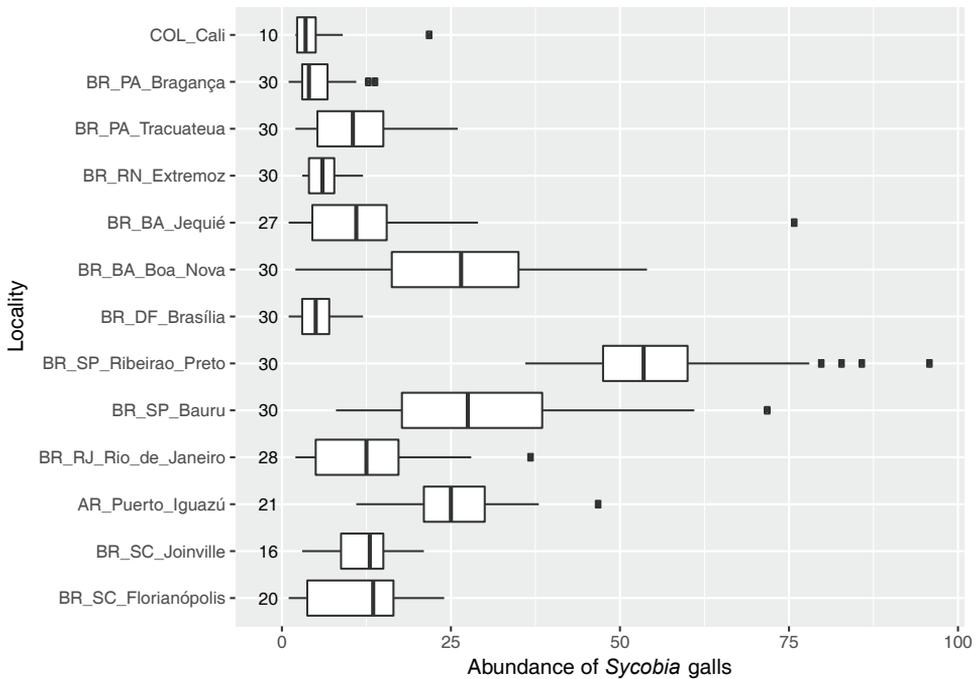
Figs of *F. benjamina* were  $1.12 \pm 0.07$  cm long (measured from ostiole – peduncle insertion)  $\times$   $0.99 \pm 0.05$  cm wide (Mean  $\pm$  SD,  $N = 30$  figs) and shown an average of 359.5 flowers per fig (Table 1). The average number of *Sycobia* wasps per fig ranged from 5.07 (Brazil, PA) to 57 (Brazil, SP; Fig. 3). *Sycobia* wasps occupied an average of 18% of the available female flowers.



**Figure 1.** *Sycobia hodites* Farache & Rasplus, sp. n., Sampling. Blue circles represent abundances where the species was sampled, whereas red circles represents localities where *F. benjamina* was sampled but no evidence of galls were found. Made with natural earth (naturalearthdata.com).



**Figure 2.** A Detail of galls induced by *Sycobia hodites* Farache & Rasplus, sp. n. arrow indicates the gall. B Arrow indicates remains of flower stigma in the gall.



**Figure 3.** Average abundance of *Sycobia hodites* Farache & Rasplus, sp. n. galls in *Ficus benjamina*. The number of galls was calculated when *Sycobia* was present in the figs. Localities sorted by latitude. Countries: AR = Argentina, BR = Brazil, COL = Colombia; Brazilian States: BA = Bahia, DF = Distrito Federal, PA = Pará, RJ = Rio de Janeiro, RN = Rio Grande do Norte, SC = Santa Catarina, SP = São Paulo. Numbers on the left side of the plot indicate sample sizes.

**Table 1.** Number of flowers and wasps in figs sampled in Ribeirão Preto. N = number of figs, m = males, f = females, wm = winged males.

Character	Mean $\pm$ SD	N
Pistillate flowers	359.5 $\pm$ 44.87	10
Staminate flowers	44.3 $\pm$ 8.37	10
Female wasps	37.73 $\pm$ 11.76	30
Winged males	1.2 $\pm$ 1.13	20
Brachypterous males	18.07 $\pm$ 6.02	30
Sex ratio (m/(m + f))	0.34 $\pm$ 0.08	30
Winged-male sex ratio [wm/(m + f)]	0.022 $\pm$ 0.024	30

Most males were brachypterous. Winged males were comparatively less frequent. Sex ratio was female biased, with males representing only 34% of the total wasp offspring (Table 1). The winged males were commonly observed copulating with the females, even inside figs, whereas no mating was observed with brachypterous males.

## Systematic position and species description

### Family Pteromalidae Dalman, 1820

### Subfamily Epichrysomallinae Hill & Rieck, 1967

### *Sycobia* Walker, 1871 [Type species: *Sycobia bethyloides* Walker, 1871]

### *Sycobia hodites* Farache & Rasplus, sp. n.

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Figs 4–7

**Etymology.** The name is derived from the Greek “οδοιπόρος”, which means traveler/wayfarer.

**Type material.** Holotype: ♀ BRAZIL, São Paulo, Ribeirão Preto, Jardim Recreio, -21.1754° -47.8579°, 560m elev., 23.viii.2016 ex *Ficus benjamina*, n° RASP00417, Pereira, RAS leg. USP\_RPSP 00003485 (RPSP). *Paratypes:* BRAZIL, same information as holotype. (19 ♀♀, 1 winged ♂, 3 brachypterous ♂♂, RPSP; 7 ♀♀, 3 winged ♂♂, 6 brachypterous ♂♂, CBGP); Ribeirão Preto, Jardim Recreio, -21.1754° -47.8579°, 560m elev. 29.v.2017, n° RASP00434, Pereira, RAS leg. (12 ♀♀, 4 winged ♂♂, 8 brachypterous ♂♂, RPSP).

**Non-type specimens.** BRAZIL, Rio de Janeiro, Rio de Janeiro, Horto Botânico, Museu Nacional, -22.9083° -43.2247°, 15m elev., 23.vii.2016, ex *Ficus benjamina*, n° RASP00422, Pereira, Cecília B. leg. (2 ♀♀, 1 winged ♂, 5 brachypterous ♂♂, RPSP); Santa Catarina, Florianópolis, -27.5901° -48.5619°, 15m elev., 19.ii.2017, ex *Ficus benjamina*, n° RASP00431, Koschnitzke, C leg. (3 brachypterous ♂♂, RPSP);

COLOMBIA, Valle del Cauca, Cali, La Leonera, 3.4519° -76.6558°, 1930 m elev, 12.x.2016 ex *Ficus benjamina*, Cardona W. leg (29 ♀♀, 2 winged ♂♂, 39 brachypterous ♂♂, MUSENUV).

**Species description. *Diagnosis:*** Female body color mostly yellow. Anellus transverse, very short (less than 0.2× the first flagellomere length). Clypeal margin sinuate, without distinct lobes. Propodeum with a median longitudinal crest reaching the posterior part of the sclerite and with two broad furrows, parallel to the anterior margin of propodeum. Male petiole short, inconspicuous.

**Female** (Figs 4, 5). Body (head + mesosoma + metasoma) length 2.1 mm. Body color mostly yellow. Mandibles and oral margin darker, mostly brown. Mesosoma in dorsal view yellow to orange. Metasoma yellow to orange, dorsally brown to black.

**Head.** Transverse, 1.2–1.4× as wide as high. Face and vertex sculpture mostly engraved, with sparse pilosity. Scrobal depression shallow, smooth. Supraclypeal area mostly smooth. Clypeal margin sinuate, without distinct lobes. Clypeus with two pairs of setae. Distance between toruli ca 0.6× torulus diameter. Distance from torulus to oral margin 0.6× distance from torulus to median ocellus; antennae inserted above the level of the ventral margins of the compound eyes and below the median region of compound eyes. POL 1.8× OOL. Mandibles bidentate. Malar sulcus absent. Antennal formula 11163. Scape 2.5× as long as pedicel. Anellus transverse, very short (less than 0.2× the first flagellomere length). Funicular segments transverse, 0.6–0.8× as long as wide, each of them bearing a single row of sensilla. First funicular segment with 6–7 longitudinal sensilla, the remaining funicular segments with 8–10 longitudinal sensilla in each. Claval segments fused, each one bearing a single row of sensilla

**Mesosoma.** Pronotum sculpture smooth to engraved. Prosternum transverse, nearly 1.5× as wide as long, with smooth to engraved sculpture. Mesoscutum sculpture smooth to engraved, median region transversally engraved, strigose. Notauli becoming faint near transscutal articulation. Axilla transverse in dorsal view; both axillae almost touching near the median region of transscutal articulation; sculpture smooth to engraved. Mesoscutellum sculpture mostly smooth, sculpture smoother than in mesoscutum and axillae. Mesoscutellum with 2 pairs of long setae, inserted in the sublateral and postero-lateral margin of the sclerite. Mesepisternum and mesepimeron fused, with engraved sculpture. Metascutellum inconspicuous. Propodeum with a median longitudinal crest reaching the posterior part of the sclerite. Anterior portion of propodeum with two broad furrows, parallel to the anterior margin of propodeum. Propodeum sculpture engraved to coriaceous (“scale-like”). Posterior region of propodeum with longitudinal striae. Propodeal spiracles 1× its diameter distant from propodeal anterior margin; anterolateral margin of propodeal spiracles covered by a rounded expansion of propodeal tergite. Wings hyaline, with sparse pilosity; wing margin moderately pilose. Marginal vein virtually as long as stigmal vein. Postmarginal vein very short, nearly absent. Stigmal vein almost forming a right angle with marginal vein. Hind tibia 1.1× as long as hind coxa. Metabasitarsus (MtT1) 3 times as long as MtT2.

**Metasoma.** With 6 apparent tergites; tergites 3 to 6 each showing a row of elongated setae. Ovipositor sheaths inconspicuous, not protruding from the metasoma.

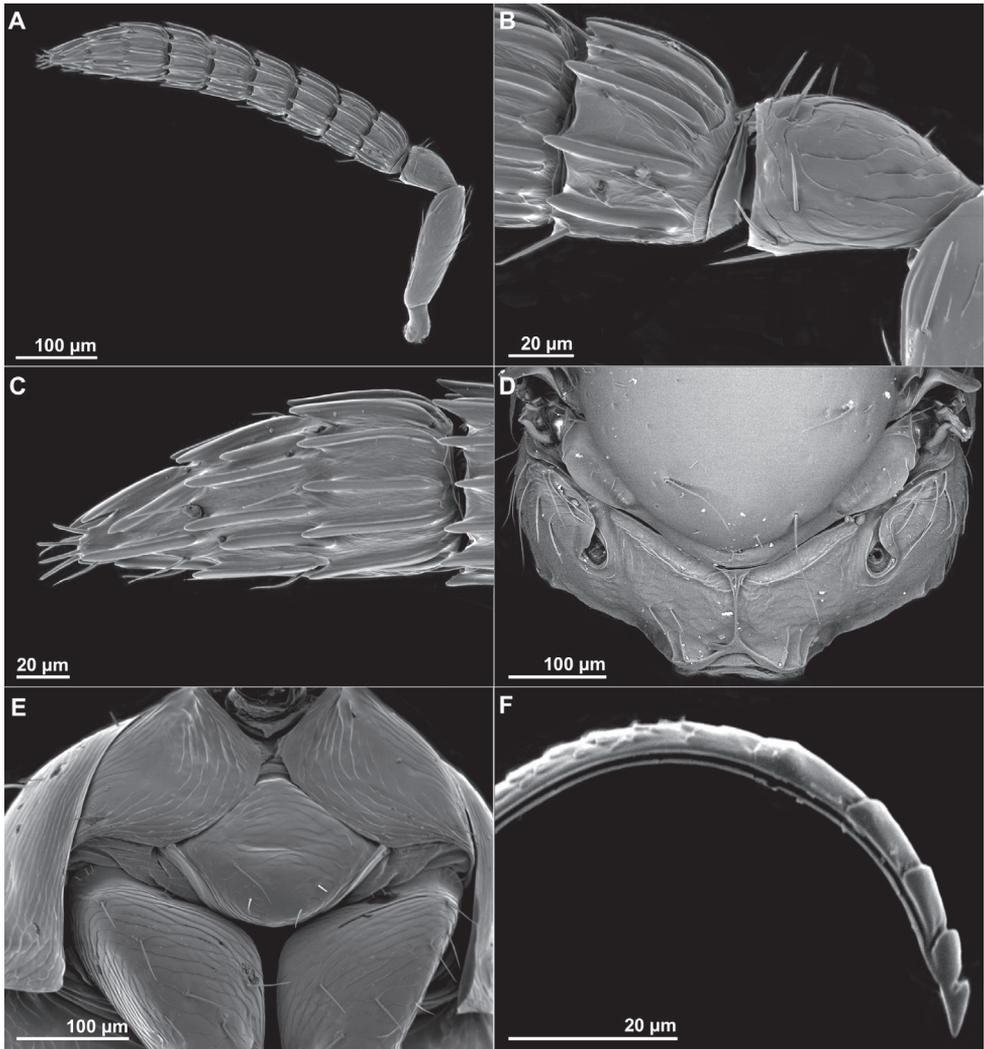


**Figure 4.** *Sycobia hodites* Farache & Rasplus, sp. n. Female. **A** Habitus, lateral view **B** Head, frontal view **C** Mesosoma, lateral view **D** Mesosoma, dorsal view **E** Wings and detail of venation.

Ovipositor teeth of homogeneous height; ovipositor with scale-like projections located just before the ovipositor teeth.

**Measurements** ( $\mu\text{m}$ ). Posterior ocellar distance 133; ocellocular distance 88; lateral ocellar line 67. Antennomeres length): scape 159; pedicel 63; anellus 10; F(unicle)1 52; F2 48; F3 54; F4 52; F5 53; F6 55; clava 146. Forecoxa length 233; forefemur 303; foretibia 303; foretarsomere (FoT): FoT1 75; FoT2 39; FoT3 40; FoT4 22; FoT5 43. Metacoxa length 366; metafemur 384; metatibia 412; metatarsomeres (MtT): MtT1 208; MtT2 63; MtT3 50; MtT4 34; MtT5 52.

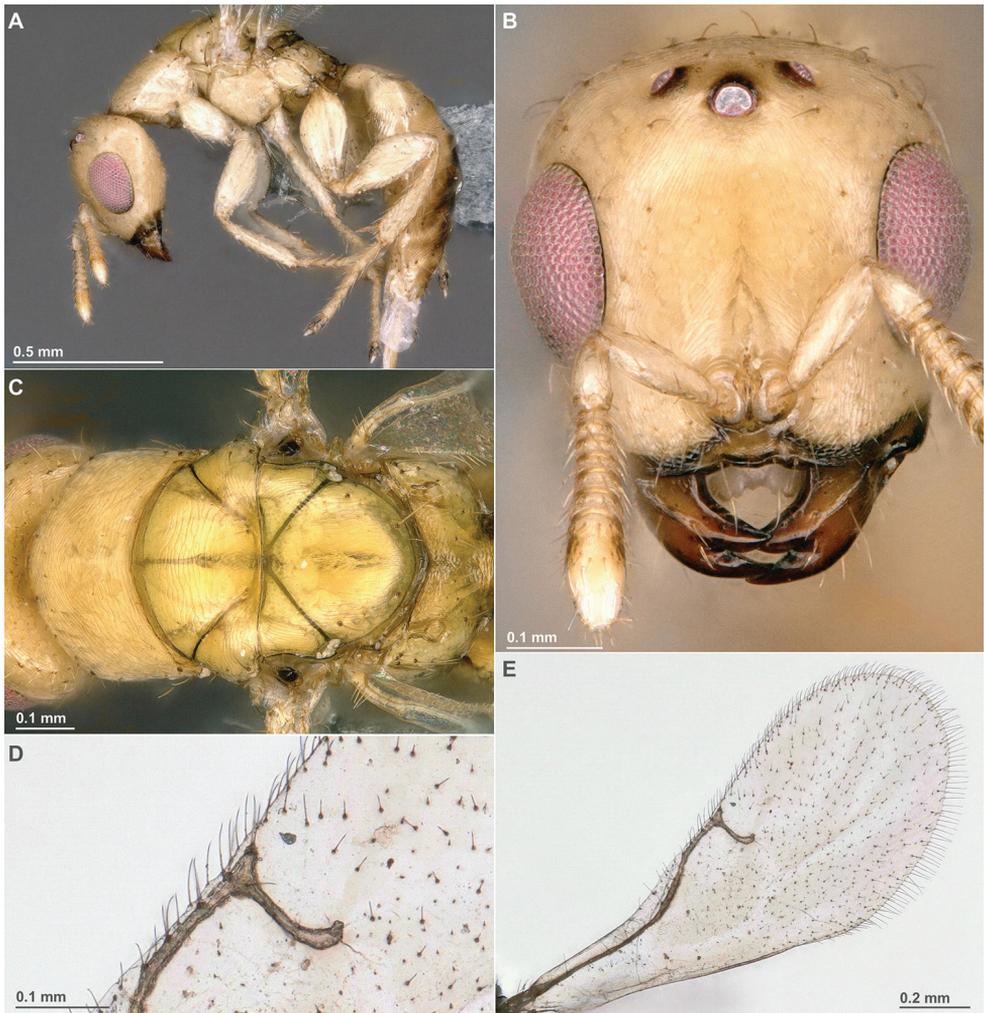
**Winged male** (Fig. 6). Mostly similar to female. Body length 2 mm. Body color mostly pale yellow. **Head.** Mandibles and oral margin brown. Metasoma dorsally brown. Head square to transverse,  $1.1\times$  as wide as high. Toruli contiguous. Mandibles



**Figure 5.** *Sycobia hodites* Farache & Rasplus, sp. n. Female, sem. **A** Antenna **B** Close-up of the pedicel, anellus and fl **C** Clava **D** Propodeum in dorsal view **E** Prosternum **F** Ovipositor.

elongated, larger than in females. Distance from torulus to oral margin  $0.6\times$  distance from torulus to median ocellus. Ocelli well developed. POL  $1.0\text{--}1.1\times$  OOL. Funicular segments transverse, less than  $0.5\times$  as long as wide. Pronotum elongated, nearly  $0.5\times$  as long as wide in dorsal view. Propodeum without a median longitudinal crest or furrows. Wings slightly infusate.

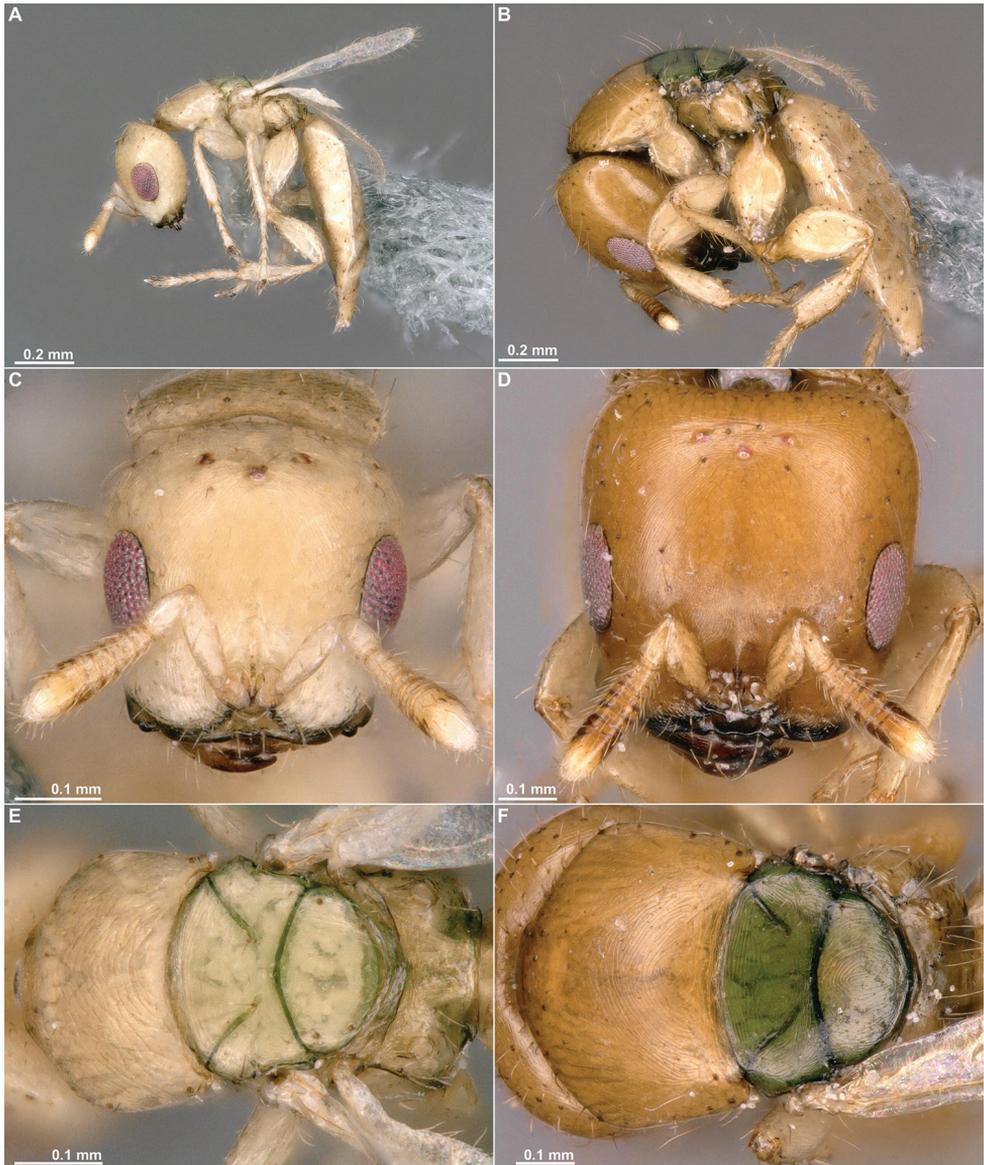
**Brachypterous male** (Fig. 7). Showing two more or less discrete forms. Body length 1.5 mm (pale form) to 2.0 mm (yellow-orange form). Body color from pale yellow to yellow orange. Mesothorax slightly green. Head nearly square. Eyes and ocelli reduced compared to winged forms. Toruli contiguous. POL  $0.6\text{--}1.0\times$  OOL.



**Figure 6.** *Sycobia hodites* Farache & Rasplus, sp. n. Winged male. **A** Habitus, lateral view **B** Head, frontal view **C** Mesosoma, dorsal view **D** Detail of wing venation **E** Forewing.

Mandibles and oral margin brown. Funicular segments transverse, less than  $0.5\times$  as long as wide. Pronotum elongated, nearly  $0.6\text{--}0.7\times$  as long as wide in dorsal view. Notauli incomplete. Propodeum without a median longitudinal crest or furrows, sometimes highly setose.

**Comparative comments.** *Sycobia hodites* Farache & Rasplus sp. n. differs from other congeneric species by the following characters. Females: (1) antennae inserted above the level of the ventral margins of the compound eyes and below the median region of compound eyes – inserted at the level of the ventral margins of the compound eyes in *S. mathewi*; (2) anelli transverse and inconspicuous in females – nearly square in *S. mathewi*; (3) anterior portion of propodeum with two



**Figure 7.** *Sycobia hodites* Farache & Rasplus, sp. n. Brachypterous males. **A, C, E** Small form **B, D, F** Large form **A, B** Habitus, lateral view **C, D** Head, frontal view **E, F** Mesosoma, dorsal view.

broad furrows, parallel to the anterior margin of propodeum – furrows absent in *S. bethyloides* and *S. mathewi*. Males: (4) mandibles short, not protruding from the head – mandibles long and scythe-like in *S. bethyloides* (5) funicular segments transverse, funicle near as long as scape – funicle segments nearly square (6) pronotum shorter than wide in dorsal view – much longer than wide in *S. bethyloides* and *S. mathewi*; (7) petiole short, inconspicuous – longer than wide and conspicuous in *S. bethyloides* and *S. mathewi*.

## Discussion

*Ficus benjamina* is widely cultivated around the world as an ornamental plant, yet few cases of fig wasp colonists (*i.e.*, wasps associated with other local fig trees) are known for this species. There is a single record where a *Pegoscapus* species, native to the Neotropics, was registered associated with *F. benjamina* introduced in Costa Rica (Ramírez and Montero 1988). Cases of fig wasp followers for this *Ficus* species have never been reported. This contrasts with *F. microcarpa*, which also belongs to section *Conosycea* and is widely introduced over the world. This species exhibits a diverse community of fig wasp followers and colonists (Wang et al. 2015a). The community of wasps associated with fig inflorescences is highly diverse and often poorly known (Bouček 1988), even for widely introduced species, which imposes difficulties in tracing biological invasions. Indeed we can only infer the probable origin of *Sycobia hodites*, which is probably native to Asia or Australasia (Darwell et al. 2018).

*Sycobia hodites* belongs to the guild of large gall inducers (Darwell et al. 2018), however its ovipositor structure is not consistent with other large sized species that induce galls in the fig receptacle (*e.g.*, *Anidarnes*, *Idarnes* group *incertus*, *Conidarnes* and *Pseudidarnes*, Elias et al. 2018), which lack teeth on their ovipositors. Conversely the ovipositor of *S. hodites* bears homogeneously-sized terminal teeth, compatible with the ovipositor structure of species that induce galls in the ovaries of pistillate flowers (*e.g.*, *Idarnes* group *flavicollis* and some *Sycophaga* species (Elias et al. 2018)). Indeed, the gall of *S. hodites* keeps the remains of the floral stigma (Fig. 2B), showing that it developed from a differentiated pistillate flower. This finding highlights that one guild (*i.e.*, large gall inducers) can in fact encompass different oviposition strategies. This is important for understanding the ecological interactions among fig trees and their associated wasps, as gallers that use receptacle tissues, and those that use flower ovaries have a distinct impact on fig tree reproduction, and consequently on the fig-wasp mutualism.

*Sycobia hodites* is one of the very few species of Epichrysomallinae known to have polymorphic males (Joseph 1961; Vincent and Compton 1992) and represents the first record of brachypterous males for the genus *Sycobia*. In fig wasps, sexual selection on male mating opportunities favored winged males in species with small broods and wingless males in species with large broods (Cook et al. 1997; Cruaud et al. 2011). The large brood size observed in some of our *S. hodites* samplings corroborated this hypothesis and may explain the high proportion of brachypterous males observed. Further investigation is nevertheless needed to confirm this hypothesis, and *S. hodites* appears an ideal model to test it.

*Sycobia hodites* has not been recorded in recent samplings of fig wasps from native Neotropical figs in Brazil (Farache, unpublished data). In fact, large gall inducers are relatively specific to their hosts (Farache et al. 2018) and therefore cross-occurrence between wasp species in different fig sections may be unlikely. The ~140 *Ficus* species native to the Neotropical region belong to sections *Americanae* and *Pharmacosycea*, whereas *Ficus benjamina* belongs to section *Conosycea*, native to Asia, Australasia and Madagascar (Berg 1989). The potential of this invasive wasp species to induce galls in Neotropical fig species should be evaluated experimentally in order to test the potential of interference in native *Ficus*-associated communities.

As *S. hodites* is a gall making fig wasp, its presence may contribute to prevent *F. benjamina* becoming invasive if the pollinator of *F. benjamina*, *Eupristina koningsbergeri* Grandi, 1916, colonizes the Neotropical region in the near future. Indeed, a species of *Meselatus* Girault, 1922, another epichrysomalline fig wasp associated with *F. microcarpa*, appears to be an excellent candidate for biocontrol of this invasive fig (Wang et al. 2015b). However, the presence of *S. hodites* opens the opportunity for the establishment of species belonging to other trophic levels such as cleptoparasite and parasitoid wasps, having, therefore, a potential future impact on the community of non-pollinating fig wasps associated with *F. benjamina*.

## Conclusions

*Sycobia hodites* sp. n. associated with *Ficus benjamina* is described here. Since neither Epichrysomallinae nor its host are native to the New World, this species can be considered as an invasive species. It is remarkable that this species was described based on specimens collected outside its probable native range, which is an indication of the great need of taxonomic studies in Chalcidoidea. The species is a gall inducer and a competitor that may encumber a future establishment of the pollinator of *F. benjamina* in the New World and prevent its natural reproduction.

## Acknowledgements

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## Supplementary material I

### Occurrence of *Sycobia hodites* in studied localities

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Data type: occurrences

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Link: <https://doi.org/10.3897/jhr.67.29733.suppl1>

# Discovery of the nest of the yellow jacket *Vespula structor* (Smith) (Hymenoptera, Vespidae) from China with description of its immature stages

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<http://zoobank.org/8A3B1D6F-8C07-4AD4-8655-074C6EB0B5F8>

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## Abstract

Data on the nest structure, morphology of all stages and behaviour have contributed to the phylogenetic and taxonomic studies of social vespids. Two underground nests of *Vespula structor* (Hymenoptera, Vespidae, Vespinae) were discovered in China. For the first time, the morphology of all the stages, and the nests are described in detail and illustrated. It is additional evidence supporting the recent conclusion that synonymized *Vespula gongshanensis* with *V. structor*. The status of *Vespula structor* within both the genus *Vespula* and the *vulgaris*-group are briefly discussed.

## Keywords

larval instars, nest structure, morphology, Vespinae

## Introduction

The yellow jacket genus *Vespula* Thomson (Hymenoptera: Vespidae: Vespinae) consists of 25 valid species, of which 12 species occur in China (Carpenter and Kojima 1997, Archer 2012, Kimsey and Carpenter 2012, Tan et al. 2015, Kumar and

Carpenter 2018). As a highly-developed eusocial wasp group, most species are widely distributed, and probably as a result, most species are subject to considerable colour variation (Archer 1989; Carpenter and Kojima 1997; Carpenter et al. 2011; Tan et al. 2015). The three castes (queen, drone and worker) of these eusocial wasps enlarge the spectrum of variation found within a species. In addition, its three castes, as well as the nest architectural and the larval characteristics, are important for phylogenetics and taxonomy of the genus (Yamane 1976, Kojima 1998, Wenzel 1998, Tan et al. 2013, 2014, 2015, 2017, Tan et al. 2018). Unfortunately, seven species (i.e., *Vespula arisana*, *V. austriaca*, *V. kingdonwardi*, *V. koreensis*, *V. nursei*, *V. orbata*, *V. structor*) are reported from China based on limited material. Among them, *Vespula structor* was originally described (only the female) as *Vespa structor* (Smith). Bingham (1897) briefly described the three castes. Yamane et al. (1980) provided the facial and gastral colour patterns of the female. The label data indicate that *V. structor* occurs in mountainous regions between 900–3700m (Archer 2008). Since then, no more information (i.e., immature stages, nest structure, or a clear illustration of genitalia which is the most important identification characteristic) has been added (Das and Gupta 1989, Archer 1989, 2012, Tan et al. 2015, Dorji et al. 2017).

In 2005, *Vespula gongshanensis* Dong, 2005 was described from Yunnan and collected at 2950 m altitude. Carpenter et al. (2011) considered it a synonym of *Vespula rufa* (Linnaeus) based on the original description. Tan et al. (2015) synonymized it with *V. structor* after studying the type series. Unfortunately, the available material is too limited to draw clear conclusions, considering the variation observed, it is expected that additional data will corroborate our provisional results.

During our field work in the Qinling (Shaanxi, China), two underground nests of *V. structor* were discovered in secondary scrubland. The envelope was complete and the combs were still undergoing enlargement. All castes and larval stages were collected in two nests, and they provided the additional information on nest biology, castes and all larval stages of *V. structor* documented in this paper.

## Material and methods

Two underground nests of *V. structor* were discovered in Ankang (Xunyangba, 33°18'N; 108°19'E, altitude 1481 m) and Baoji (Jialing River source, 34°08'N 106°33'E, altitude 1558 m). The specimens were collected either together with the nest or by hand net. The nest was excavated carefully, wrapped in a woven bag and placed in a plastic case to carry it back to the laboratory. Adults were picked off the nests after the bag had been 15 minutes in a freezer to avoid stinging. Samples were kept in 70% ethanol before preparation. Mature larvae and pupae were preserved in 2.5% glutaraldehyde and then transferred to 70% ethanol for later observation. The nests were kept in plastic boxes in the refrigerator at 4 °C.

For the descriptions and measurements, an Olympus SZX11 stereomicroscope was used and the photographs were taken with a Keyence VHX-5000 digital microscope.

The photographs of the nest and combs were taken using a Canon SX60 HS. For scanning electron microscopy, the larvae were fixed for 8 hours in a 2.5% glutaraldehyde solution buffered with phosphate (pH 7.2). They were then dehydrated in a graded ethanol series (30%, 50%, 70%, 90%, 100%, 100%), placed in isoamylacetate twice for 30 minutes each time, dried in a CO<sub>2</sub> critical-point dryer, sputter-coated with gold and observed with a Hitachi S3400N scanning electron microscope at 15 kV.

Morphological terminology follows Archer (1989, 2012), Wenzel (1998) and Tan et al. (2015). The following main abbreviations are used in the text: APOL = antero-postocellar line; HW = head width; OMS = oculo-malar space; OOL = postocellar-ocular line; PBHL = postocellar back of head line; POL = postocellar line. Other abbreviations used in the figures are explained in the legends. The length of the body is measured from the anterior margin of the head to the apex of the second metasomal tergum. The specimens examined are preserved in the Hymenoptera Collection at the College of Life Sciences, Northwest University, Xi'an, China (NWUX).

## Results

### Nesting sites and nest structure

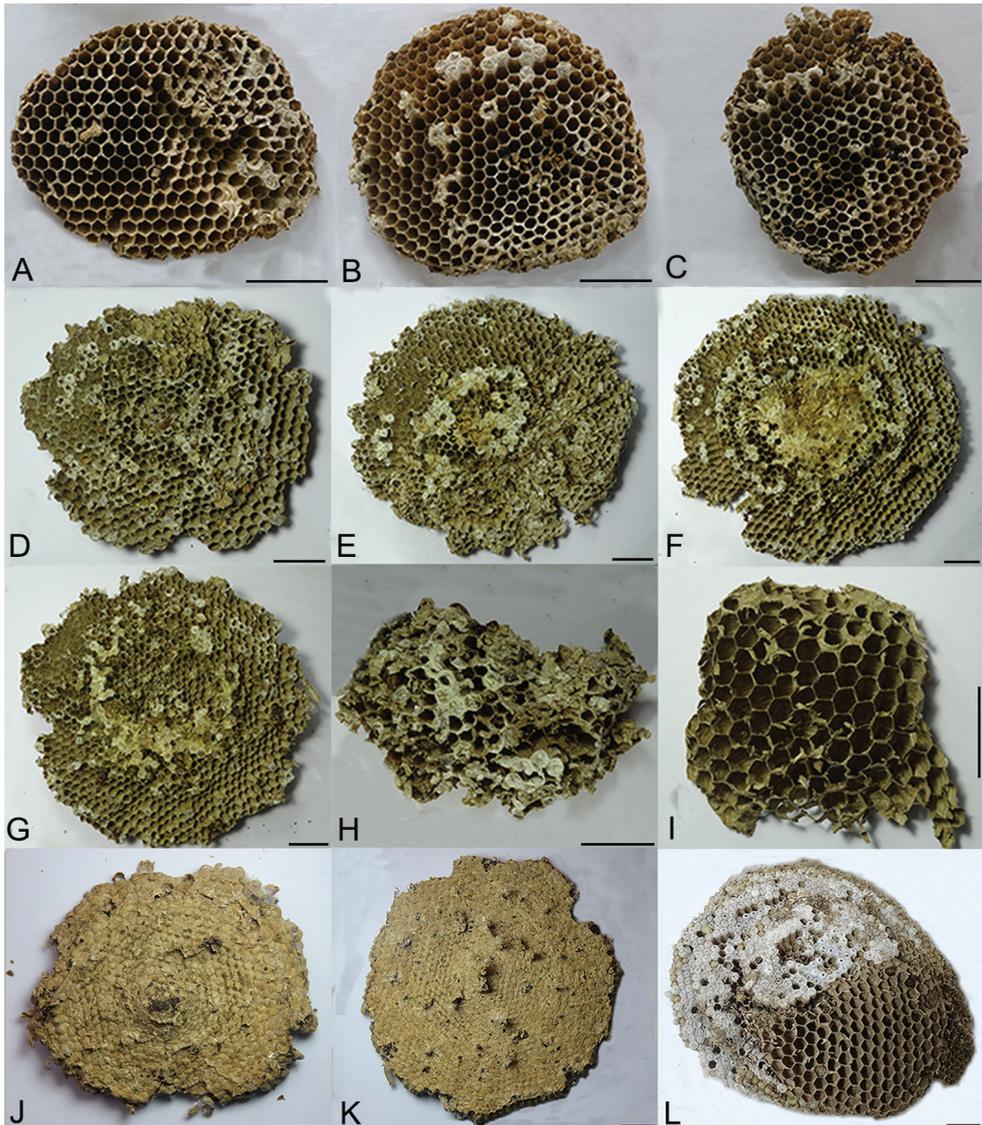
Both nests of *Vespula structor* were found buried underground in the secondary scrub-land at an altitude of about 1500 m. The nest was connected to the surface by a narrow channel of about 10 cm long. The envelope of the nest is tan and scaly with wavy parallel lines (Figure 1A, C). The combs were attached to each other by a large buttressed sheet centrally with many narrow auxiliary pedicels. The distance between the combs was about 4.5 mm (Figure 1D, E).

The nest from Xunyangba was a developing colony with many workers, uncertain number of males and one queen. Due to the nest's having been burned by locals before it was excavated, it was less developed and had only three layers of combs with about 1100 cells in total. Each comb is almost circular and surrounds the central mainstay, where a slight swelling is apparent. The combs are nearly flat but the uppermost one bends upwards rather steeply toward its edge (Figure 1B). Diameters of the combs (measured from top down) were 7.6 cm, 9.1 cm and 8.2 cm, respectively (Figure 2A-C). Unfortunately, the envelope was mostly broken; therefore, it is unknown how the uppermost comb was connected to the envelope. The second comb was attached to the one above by a buttressed sheet about 2.1 cm wide centrally. The lowest comb had only a narrow central pedicel and most cells were devoid of immature stages. The distance between two layers was approx. 4.5 mm (Figure 1B). Cells were hexagonal and mostly regularly arranged with the average diameter (between opposite sides) of 4–5 mm, usually 4.5 mm ( $n = 30$ ). Cells were variable in length and in the degree to which cocoons extended outside, 6–8 mm ( $n = 30$ ) high at the periphery without cocoon and increased as the stage advanced. Cells had been capped by silken cocoons 9–11 mm high, usually 10 mm (measured without cocoon,  $n = 30$ ).



**Figure 1.** *Vespula structor* (Smith). **A–B** Nest from Xunyangba **C–F** Nest from Baoji: **C** Site of nest; combs of nest **D–F** combs of nest, lateral view.

The nest from Baoji housed a developing colony with many workers, males and one queen. The nest consisted of six combs with about 220 large cells and 3500 small cells (Figure 2L). Diameters of the combs (measured from top down) were 12 cm, 15 cm, 17 cm, 15 cm, 8.5 cm and 6.5 cm, respectively (Figure 2D–I). Each layer was similar to those of the other nest described previously except for differences in cell distribution. The upper comb was connected to the inner top of the envelope broadly by a central buttressed sheet with diameter of 2.5 cm and many narrow-marginal pedicels completely connected with the inner combs of envelope (Figure 2J). The second through fifth layers were attached to the respective one above by many thinner buttressed peduncles, which were widened centrally (Figure 2K). The lowest comb was



**Figure 2.** *Vespula structor* (Smith). **A–C** Three layers of nest (from Xunyangba) **D–I** Six layers of nest (from Baoji) **J** one central buttressed sheet and many thinner auxiliary pedicels on the first comb **K** many thinner auxiliary pedicels on the third comb **L** different diameters of cell, vertical view. Scale bar: 2 cm.

devoid of immature stages; obviously only large cells with the diameter (between opposite sides) about 7.0 mm were present (Figure 2L). The other combs having a diameter (between opposite sides) of approx. 4.5 mm. Three stages (egg, larvae, pupa) were visible according to the cyclic arrangement of the successive cocoons of the preliminary five layers (Figure 1F). Most cells were capped by silken cocoons and these capped cells were 1.0–1.3 cm high. Cocoon caps of the third and fourth combs are slightly larger than those of the upper combs, protruding about 0.5 cm beyond the cell mouth.

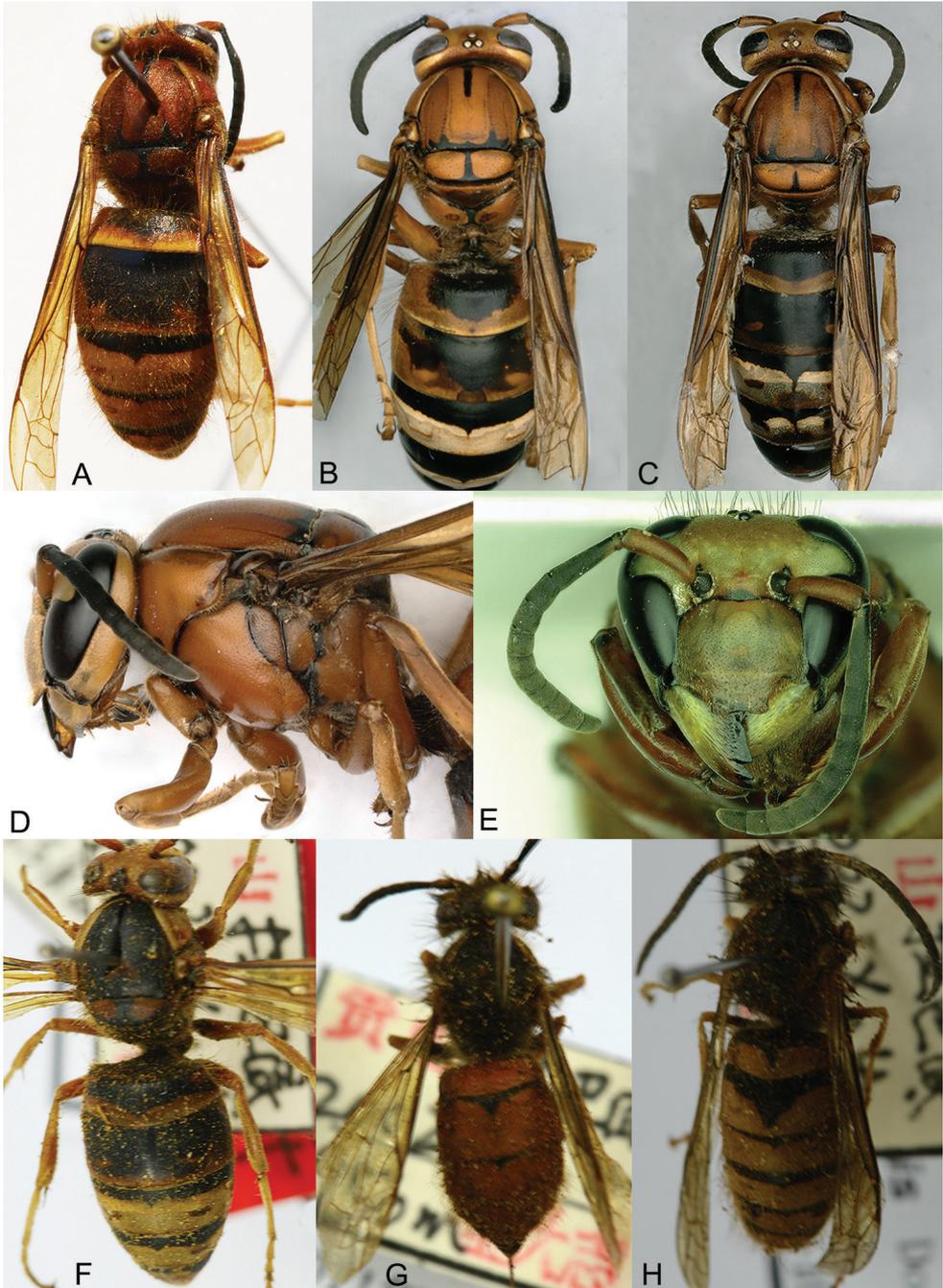
## Adults

**Diagnosis.** This species can be identified by the combination of the following characteristics: ocular sinus yellow or brown with a ventral black marking; clypeus yellow or brown without a black central mark; apical lateral angles of the clypeus semicircular; gena with a continuous yellow band not interrupted by black markings; oculo-malar space short; pronotum punctate and without rugae; first metasomal tergum (T I) yellow with long yellow setae and without a median black mark; T II–V black produced angularly medio-posteriorly; the last tergum almost entirely black; propodeum smooth with fine punctures; male genitalia: shaft of aedeagus narrow and its apex spoon-shaped with a backwardly directed pointed barb on each side below apical spoon-shaped region.

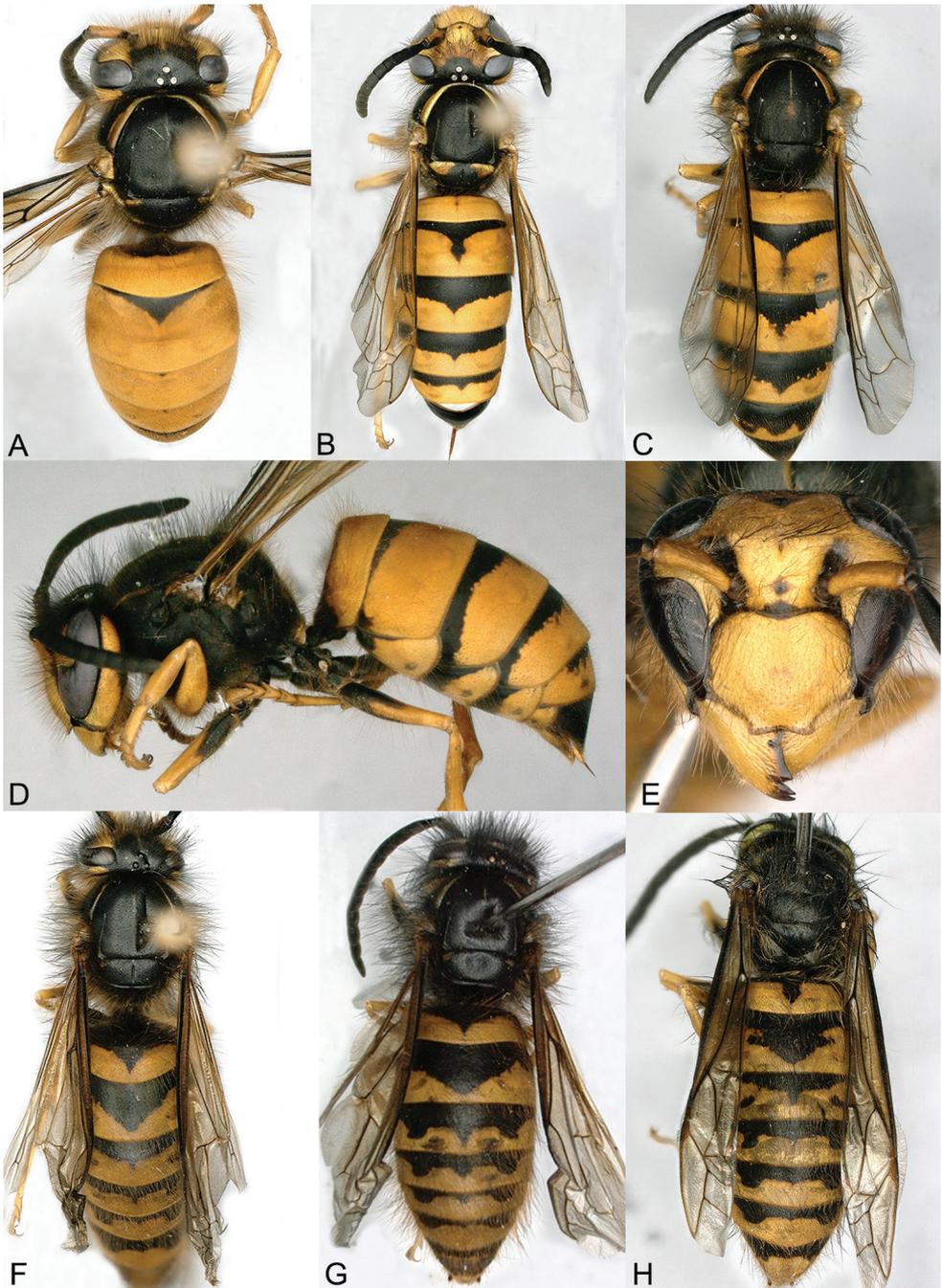
**Queen** (Figure 3A–E). ♀<sub>q</sub> ( $n = 2$ ), length of body (measured from the anterior margin of the head to the end of the second metasomal tergum (T II)) about 13.4 mm; fore wing length about 15.6 mm; width of metanotum (including tegula) about 4.0 mm; hind wing about 10.0 mm long and about 2.0 mm wide; width of mesoscutum (including tegulae) about 5.6 mm; vertex brown or yellow with erect black setae (Figure 3E); HW/OMS ratio about 15.6; head, mesosoma and legs generally yellowish brown with few black markings; anterior and posterior margins of pronotum, tegulae, lateral angles of mesoscutellum and postscutellum reddish yellow. T I–IV black basally with an vague brown transverse band subapically and narrowly yellowish brown apically, respectively; the subapical band of T I and T II irregularly expand laterally; T III and T IV produced angularly medially; T V–VI entire black with terminal brown marking (Figure 3A–C).

**Worker** (Figure 4A–E). ♀<sub>w</sub>, length of body (measured in same way as the queen,  $n = 10$ ) about 8.9 mm; fore wing length about 9.3 mm; width of metanotum (including tegula) about 3.4 mm; HW/OMS ratio 15; POL/APOL (postocellar line/antero postocellar line) = 1.1. POL/PBHL (postocellar back of headline) = 0.8. Head extensively yellow or orange; vertex black with two yellow spots or narrow stripes posteriorly; temple black or mostly so (Figure 4D, E); mesosoma black except metanotum with two small yellow spots anteriorly; declivous part of metasomal T I entirely black or black with orange; horizontal part entirely orange; basal at most 1/3 of T II–V black produced angularly medio-posteriorly while apical 2/3 orange; one shallow spot on each side of T II–V (sometimes invisible); T VI entirely black (Figure 4A–C). The colour of metasomal sternum paler than of tergum, black basally with two black spots laterally and terminal sternum nearly entirely orange yellow.

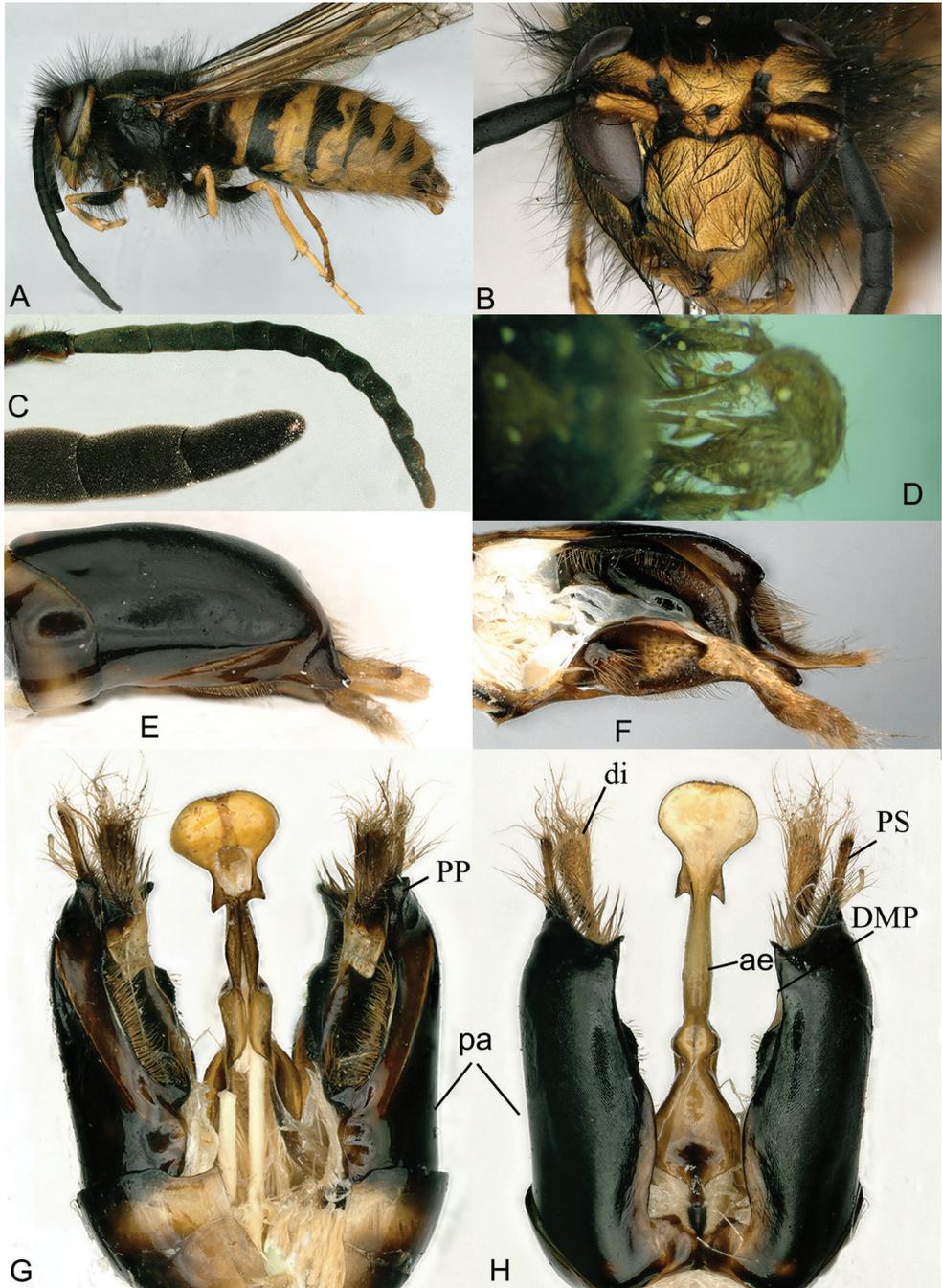
**Male** (Figures 4F–H, 5A–H). ♂, length of body (measured in the same way as the queen,  $n = 5$ ) about 10.0 mm, fore wing length about 12.4 mm; width of metanotum (including tegula) about 3.8 mm; mesosoma entirely intense black except for bright yellow posterior margin of pronotum; T I–VI black area produced angularly medially; T III–VI sometimes produced more semi-circularly medio-posteriorly; T VII black with apex yellow (Figure 4F–H); black area of metasomal sternum produced semi-circularly medio-posteriorly.



**Figure 3.** *Vespula structor* (Smith). **A–E** Queen (♀q): **A** Habitus (from Ningxia), dorsal view **B** habitus (from Baoji, Shaanxi), dorsal view **C** habitus (from Xunyangba, Shaanxi) **D** head and mesosoma, lateral view **E** head, anterior view. **F–H** *Vespula gongshanensis* (type series from Yunnan) **F** Queen (♀q), habitus, dorsal view **G** worker (♀w), habitus, dorsal view **H** male (♂), habitus, dorsal view.



**Figure 4.** *Vespula structor* (Smith). **A–E** Worker (♀): **A–C** Dorsal view, showing variation of colour pattern **D** habitus, lateral view **E** head, anterior view. **F–H** Male (♂), dorsal view, showing variation of colour pattern.



**Figure 5.** *Vespula structor* (Smith), male ( $\sigma$ ). **A** Body, lateral view **B** habitus, anterior view **C** right antenna and terminal seven segments of antenna **D** metasomal apex of paratype of *Vespula gongshanensis* (from Yunnan), showing part of aedeagus **E** genitalia, lateral view **F** paramere, inner view **G** genitalia, ventral view **H** ibid, dorsal view. Abbreviations: ae, aedeagus; di, digitus; pa, paramere; DMP, dorsal margin of paramere; PP, parameral process; PS, parameral spine.

*Genitalia.* Parameres (pa) in dorsal view smoothly curved inwards; dorsal margin of paramere (DMP) inwards projecting and with long setae; ventral terminal process columnar apically; in ventral view paramere distinctly straight projecting inwards at their one fourth of length basally; dorsal terminal process (parameral process, PP) flattened, pointed apically, shortly forwards and inwards without setae; in lateral view ventral margin depressed medially (Figure 5E); parameral spine (PS) ends in round structure with long setae dorsally, extending beyond parameral processes (PP); volsella arises from inner wall of each paramere, not visible in ventral view of gonostipes (Figure 5F); shaft of aedeagus narrow and its apex spoon-shaped with a backwardly directed pointed barb (elongate and triangularly shaped) on each side subapically (Figure 5G, H).

## Distribution

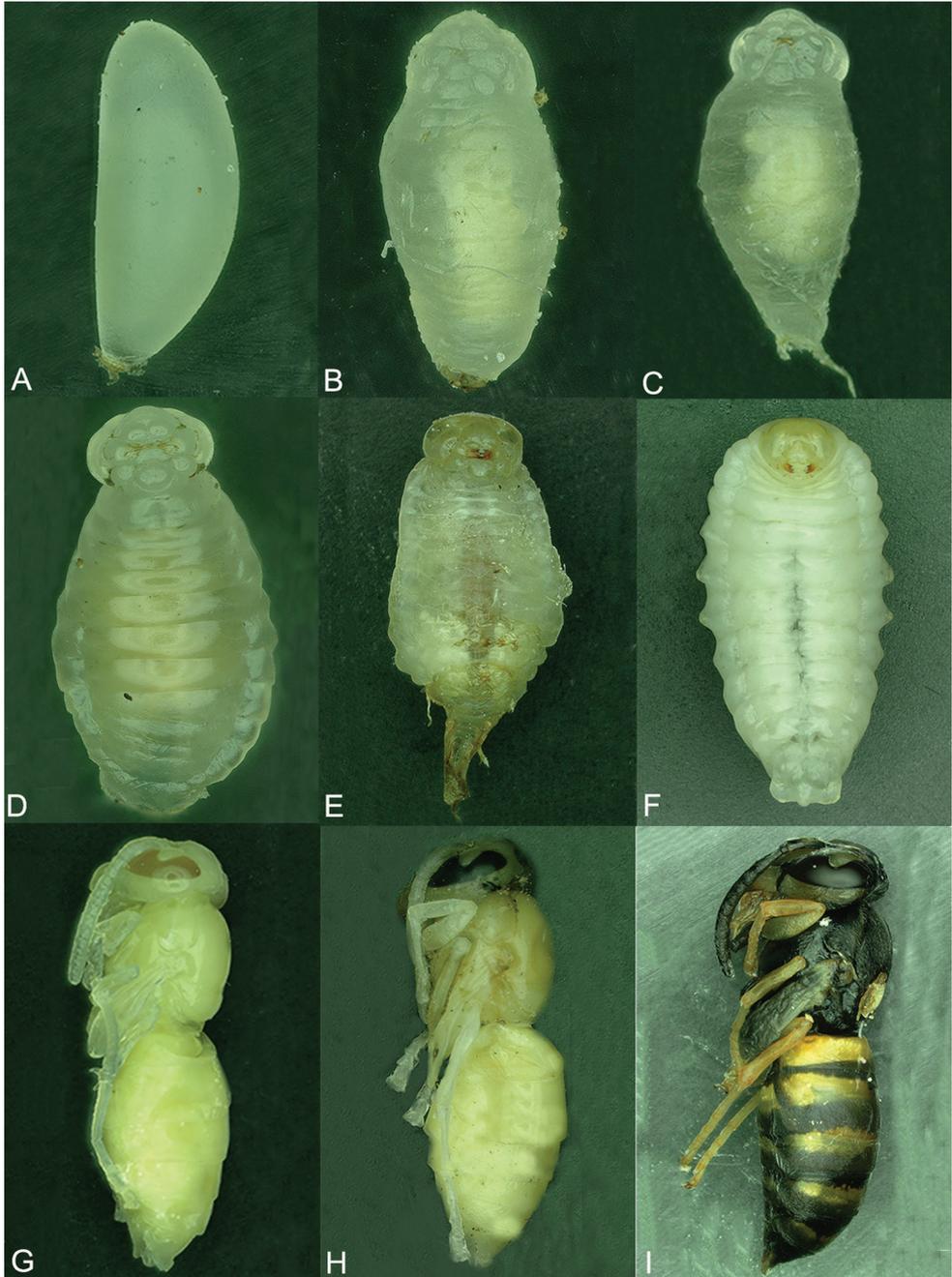
China (Henan, Shaanxi, Gansu, Ningxia, Sichuan, Yunnan, Tibet); India; Nepal; Burma; Laos; Bhutan.

## Immature stages

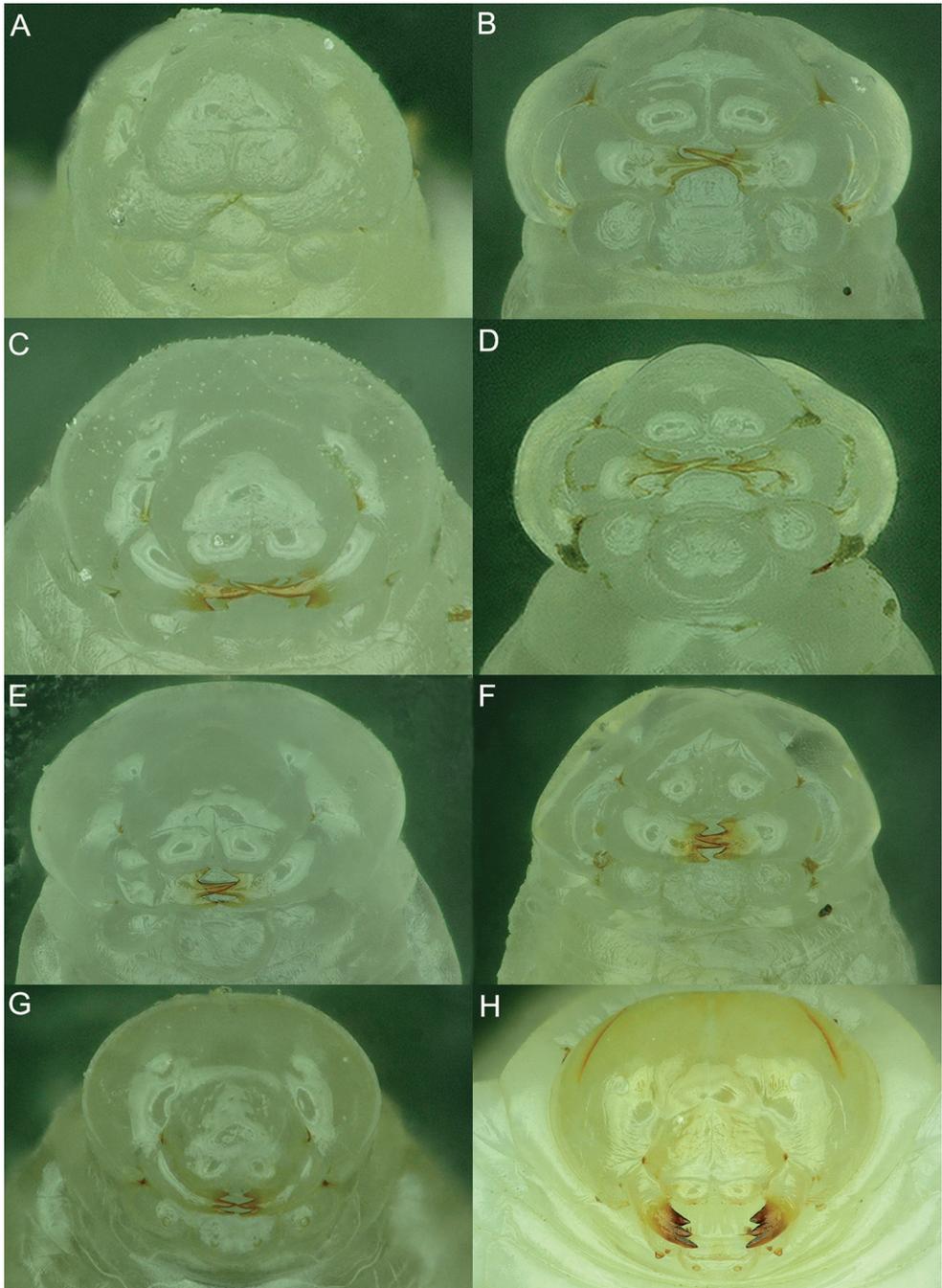
*Egg.* The egg is white, elongate oval, approx. 2.1 mm long, its maximum width 0.9 mm, a little wider at one end, slightly curved, and is generally attached by its smaller end to the inner side wall of cell basally, adhering to the wall by a gummy substance on its surface. The egg chorion is membranous; its surface is soft and smooth without any sculpture (Figure 6A).

*Larvae.* The larval body is milky-white and soft except for the chitinized head; it consists of three thoracic and ten abdominal segments, with no constriction between thorax and abdomen (Figure 6B–F).

The structure of the head in the younger larvae is generally the same as that in the mature larvae. Distinct differences in the shape of the mandible are present in each instar (Figure 7A–H). Observations of the changes in mandibular shape and body size of *V. stractor* larvae showed that there are five larval instars. In the first instar larvae, the average body length is approx. 1.75 mm and the cranial width is approx. 0.42 mm; the clypeus, labrum and mandibular teeth show a dim transparent outline; the mandibles are apically pointed and have only one tooth; the abdominal segments have shallow lines and the terminal segment is connected to the cell (Figures 6B, 7A). In the second larval instar, the average body length is approx. 2.33 mm and the cranial width approx. 0.85 mm; the mandibular tooth has three teeth; the two smaller teeth are slightly pointed and the middle tooth is chitinized and strongly pointed apically; the outline of the clypeus, labrum are clearer (Figures 6C, 7B). In the third larval instar, the average body length is approx. 2.48 mm and the cranial width approx. 0.98 mm; the inner and outer teeth are chitinized and strongly pointed apically, and are smaller than the middle tooth (Figure 7C–F); the abdominal segments show a distinct curved



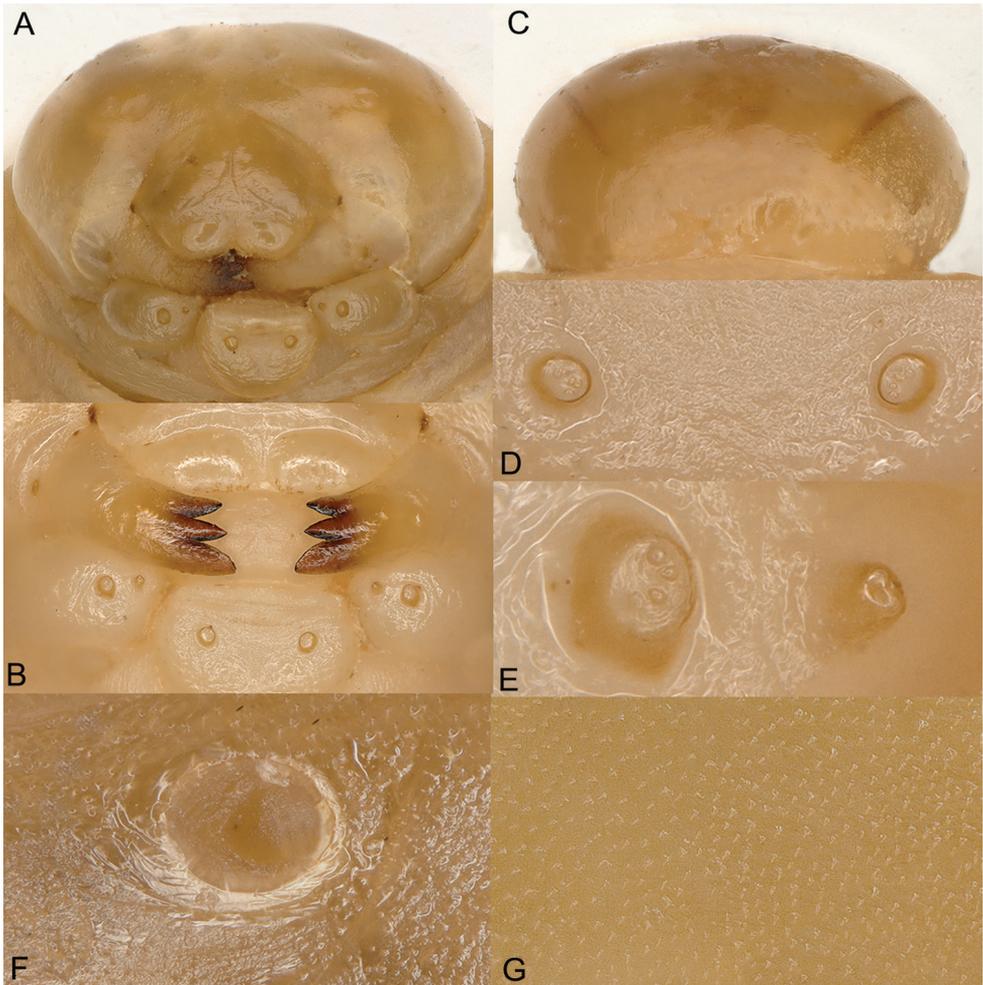
**Figure 6.** *Vespula structor* (Smith), immature stages. **A–I** Ventral view of immature stages. **A** Egg **B** first larvae instar **C** second instar **D** third instar **E** fourth instar **F** fifth instar **G, H** pupa, lateral view **I** immature worker.



**Figure 7.** *Vespula structor* (Smith), development of mandible. **A** Fifth instar **B** second instar **C–F** third instar **G** fourth instar **H** fifth instar.

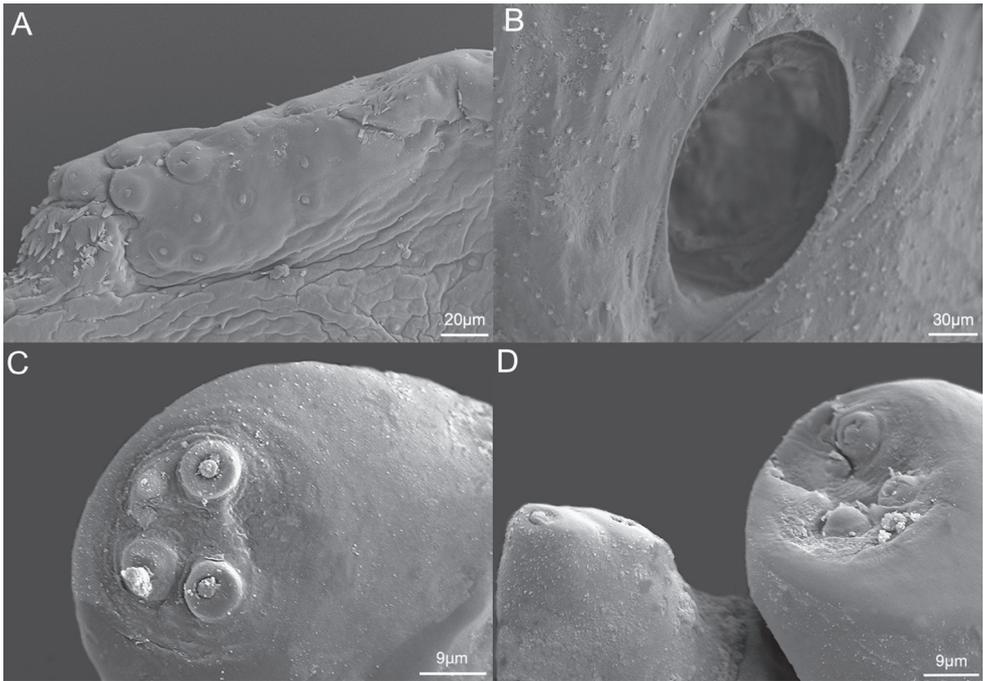
surface (Figure 6D). These stages still have the egg shell attached to the body. The cement by which the queen attaches the egg to the side of the cell thus acts as the point of attachment for the first to third instars. The larvae hold themselves onto the egg shell by means of a viscid secretion. After the ecdysis to the fourth larval instar, the average body length is approx. 5.99 mm and the cranial width approx. 1.95 mm; the larvae can hold their position in the cells by means of their pleural lobes, dorsal ridges and attachment and can easily move in their cells (Figure 6E). In this instar, the larvae now re-orientate themselves, and they rotate through 180°, from the fixed, outward facing position of the earlier instars, to face inwards towards the axis of the comb. The fourth larval instar is not free from the last ecdysis and remains connected to the cell by skin of the ecdysis (Figure 6E). The fourth instar is similar to the early fifth instar, except that the mandibular teeth are of almost the same size and less chitinized (Figure 7G). The final larval instar grows very rapidly and fills the cell completely, the average body length is approx. 10.03 mm and the cranial width approx. 2.14 mm; the dorsal ridges and pleural lobes become less obvious and the larvae become more rounded and less annulated in appearance (Figures 6F, 7H).

*Mature larvae.* Cranium nearly yellowish and some parts brown. Margin of parietal band, apical part of mandible, margins of maxillary and labial palpi and galea brown; mandibular teeth dark brown; margins of spiracles pale orange brown (Figure 8A–F). Cranium. Widest part of cranium in frontal view above level of line joining antennae, about 1.1 times as wide as high, laterally slightly sinuate near ventral margin (Figure 8A); in profile posterior margin nearly straight; integument with scattered minute punctures, each bearing minute setae (Figure 8G); part of gena (or temple) along its latero-posterior margin with sparse minute setae and remainder smooth. Ecdysial sulcus distinct. Outer one-third of parietal band reticulate (Figure 8C). Antenna small, separated from anterior tentorial pit by distance about 2.2 diameter of antenna, with three minute campaniform sensilla arranged in a triangle; frontoclypeal suture well sclerotized. Gena ventrally with sparse spicules; clypeus nearly half as wide as maximum width of cranium with few punctures and about 65 short setae (Figure 8A). Labrum narrowed at junction with clypeus, with many small punctures bearing 32 minute setae on average and 9 small conical papillae along two lateral margins (Figure 8B). Each side of palate with three small yellowish brown patches bearing more than 15 relatively large sensilla; the medio-ventral margin with about 11 conical papillae; dense minute spicules present in median part and ventro-lateral area (Figure 9A). Mandible well chitinized, especially in its concave apical part, with three apical teeth (I, II and III, counted from below) of which tooth III nearly truncate apically; tooth I markedly projecting as compared with tooth II and tooth III (Figure 8B). Maxilla with scattered short setae; upper surface and apex with spicules; four apical sensilla (sometimes three) on maxillary palpus and two apical sensilla on galea were observed, respectively (Figures 8E, 9D), area between labial palpi and spinneret with spicules; spinneret similar or slightly longer than the distance between the two labial palpi and surrounded by dense spicules; each labial palp with four apical sensilla and a single seta behind palpus (Figures 8D, 9C).



**Figure 8.** *Vespula structor* (Smith), mature larvae. **A** Head, anterior view **B** part of mouth parts, showing maxillary palp, galea, labial palpi and spinneret **C** head, dorsal view **D** labial palpi with four apical sensilla **E** maxillary palp with four apical sensilla (sometimes three) **F** spiracle **G** cuticle integument with scattered, short setae and dense minute spicules.

*Thorax and abdomen.* The cuticle integument with scattered, short setae and minute spicules (Figure 8G); terminal segment with two projections dorsally. Setae on venter of second to ninth abdominal segments confined to anterior half of each segments; setae on dorsum arranged in a transverse, median band on each segment; spicules on third to ninth abdominal segments becoming sparser towards posterior segment; The abdomen has 10 pairs of spiracles and first, second, ninth and tenth spiracle nearly equal in size, third a little smaller than first one; fourth to eight spiracles larger than third one; atrial wall of spiracle with sparse minute spicules; processes at perimeter of primary tracheal opening simple. Collar process of spiracle sort, even rudimentary (Figures 8F, 9B).



**Figure 9.** Ultrastructures of *Vespula structor* (Smith) (scanning electron micrographs). **A** Left half of labrum, ventral view, showing minute setae and conical papillae **B** spiracle **C** labial palpi with four apical sensilla **D** maxillary palp with three apical sensilla (sometimes four), galea with two apical sensilla.

*Pupa.* Average length of body approx. 16.0 mm and 5.5 mm wide ( $n = 5$ ); pupa exarate, creamy-white; compound eye colour changes gradually from translucent pinkish-brown to black brown; mandible tips yellowish brown. Similar to adults in appearance; wing pads extending approximately to the posterior margin of the first metasomal segment (Figure 6G–I).

## Discussion

The genus *Vespula* consists of 26 species which are divided into four groups, i.e. *rufa*-, *squamosa*-, *vulgaris*- and *koreensis*-group (Carpenter 1991). *Vespula structor* belongs to the *vulgaris*-group (comprised of 10 species) which are characterized in the male by having the shaft of aedeagus narrow and its apex spoon-shaped, which differs from the strap found in the in the *V. rufa*, *V. squamosa*, *V. koreensis* (Yamane et al. 1980, Carpenter and Perera 2006, Archer 2008, 2012, 2016, Tan et al. 2015). Although the three castes of *Vespula structor* has been briefly described, detailed illustration of the genitalia and the colour pattern were still lacking (Yamane et al. 1980, Archer 1989, 2012, Das and Gupta 1989, Dorji et al. 2017). This study is the first to show the clear

characteristics of all the life stages and its various. Compared within *vulgaris*- groups, *Vespula structor* subtly differs from others on the shape of the small backwardly directed pointed barb on each side below the apical spoon-shaped region of the aedeagus which is consistent with the male specimens of *Vespula gongshanensis* (checked by TJL) and strongly supports the result of Tan et al. (2015).

The male is similar to the worker, but show more black marking on metasomal tergum and the light-colour parts of the body. There are some distinct differences in the colouration of the queen. According to Bingham (1897) the head and metasoma are yellow; the mesosoma is black and the femora anteriorly with a broad black streak basally. Our result is agreement with the pictured queen in Tan et al. (2015): the mesosoma is reddish yellow and the metasoma is more extensively black. The colour variation maybe due to the altitude. Our nests were collected about 1500 m. The queen of Bingham (1897) was collected at 8000ft (2438 m) in Sikkim (India). The same colour pattern is shown in *V. gongshanensis* collected at 2950 m. The colour pattern of *V. gongshanensis* (both of male and female) agrees with the colour pattern we observed (Figure 3G correspond Figure 4A; Figure 3H correspond Figure 4F). The shape of the aedeagus agrees with that of our sample (Figures 5D, H). The shaft of the aedeagus is narrow and its apex spoon-shaped with the subapical barb elongate and triangularly shaped. The digitus is large, extending nearly as far as the level of the parameral spine (Figures 5D, H). Obviously, it supports the synonymy of *V. gongshanensis* with *V. structor*.

Before this study, there were only ten species (*Vespula atropilosa*, *V. consobrina*, *V. flaviceps*, *V. flavopilosa*, *V. germanica*, *V. maculifrons*, *V. pennsylvanica*, *V. rufa*, *V. shidai*, *V. vulgaris*) known from their immature stages (Wheeler and Wheeler 1976, Yamane 1976, Matsuura and Yamane 1990, Kojima 1998, Archer 2012). For the first time, the immature stages of *V. structor* are reported here. Compared with its known congeners, there are subtle differences in the palate and in the processes at the perimeter of the primary tracheal opening of the spiracles. The developmental process of the mandible fits well with other known species within the *vulgaris*- groups (Potter 1964, Yamane 1976). Additionally, the colour pictures which show the development of mandible are the first illustrated firstly in this genus.

Potter (1964) and Edwards (1980) reported that the abdomen got free from egg shell in the third instar larvae, but the tip of abdomen is not separated from the skin of the previous instar. This is also found in *Vespa bicolor* Fabricius, and *Vespa mocsaryana* du Buysson (Tan et al. 2015). But this is uncertain according to Matsuura & Yamane (1990). They rarely found the fourth instar larvae dropping from the cell as sometimes happens with the fifth instar larvae. The fourth instar larvae may be connected within the cell. In our study, the fourth instar sometimes is not free from the skin of the previous instar (Figure 6E). This result corroborates Matsuura & Yamane's (1990) conclusion.

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# Probable marking behavior of *Cerceris clypeata* (Philanthinae, Crabronidae, Hymenoptera)

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## Abstract

Males of *Cerceris clypeata* appear to mark grass stems and other plant parts with secretions from cephalic glands by placing the head and tip of the abdomen on the plant as they walk up the stem or around the leaf of the marked plant. Behavior of this sort has rarely been recorded for *Cerceris* but is common in the Philanthinae including some members of the Cercerini, which includes *Cerceris*. Males of *C. clypeata* very occasionally defended their small marking site against intruders but no nests or females were observed during the study. The abdomen dragging behavior of *C. clypeata* and another member of the genus suggest that marking behavior is ancestral for the subfamily.

## Keywords

evolution of marking, male behavior, territoriality

## Introduction

Marking behavior or abdomen dragging (we shall use the terms interchangeably), in which males walk up or down plant stems apparently depositing a sex-attractant pheromone from glands prominent only in the male (Weiss et al. 2017), is well known in *Philanthus* (Alcock 1975a, 1975b, McDaniel et al. 1987, Evans and O'Neill 1988, 1991, Gwynne 1980, O'Neill 1983). However, males belonging to the genus *Cerceris*, which is

also placed in the Philanthinae, have only been recorded abdomen dragging once in the literature, based on a brief observation involving a single individual of *C. nigrescens* (Evans and O'Neill 1985). Other species of *Cerceris* have been observed nonaggressively patrolling areas (particularly those with flowering plants or near nests with emerging females) in search of receptive females (Alcock and Gamboa 1974, Elliott 1984, Evans 2000). Two European species of *Cerceris* have very distinctive cephalic glands that differ from those exhibited by members of the genus *Philanthus* suggesting that male *Cerceris* do not generally engage in the marking behavior of other Philanthinae (Weiss et al. 2017). Here we report on observations of *C. clypeata* over a period of several days in which several males regularly appeared to mark grass stems in a hayfield in northern Virginia. Male behavior of this very small species of *Cerceris* has not been reported previously although female nesting behavior has been described by Matthews and Matthews (2005).

## Materials and methods

When a single male of *C. clypeata* was seen apparently marking grass stems in a hayfield in the middle of the day on 16 July 2018, the site was inspected at midday on 21 following days. However, a male or males were only present for eight of these days (rainy or cloudy days were apparently unacceptable to the wasps although they were also absent on five sunny, warm days). The area in which males were found was located at the edge of a hayfield on the farm at 38°52'27.22"N, 77°54'17.12"W. The grasses there were introduced species, including Johnson grass (*Sorghum halepense*).

Five males were given distinctive color marks on the thorax with blue, white and pink Uniposca pencil-pens in order to attempt to follow known individuals at the marking sites.

## Results

From one to three males occurred at the hayfield location during the 8 days when at least one male was present. During this time, a total of 5 sites were occupied at one time or another with the males flying out and returning to spots within a meter of their previous perches. Of the 5 painted males, one returned soon after application of the thoracic paint dot and for three consecutive days, one returned soon after being marked and for two consecutive days, one returned for a single day, and two did not return after being marked. The arrival of the first male at any site took place no earlier than 1111 E.D.T. and the duration of occupation of a site could last as long as slightly more than three hours, although some individuals were present for less than an hour.

When males first arrived, abdomen dragging behavior was frequent in which the male walked generally upward along a grass stem or leaf with the head and the tip of the abdomen touching the substrate (see Figure 1, Alcock 1975b). As time passed marking behavior became less frequent. Thus on 29 July when a male marked pink thorax on 27 July was watched from 1151 to 1406 E.D.T, the male flew out from its



**Figure 1.** A male of *C. clypeata* on his perch, a grass stem, at the site where he dragged his abdomen in the farm hayfield.

perch (Figure 1) and spent a few seconds flying through the site before returning to the perch or shifting to a new perch a few centimeters distant. The male did so without marking 16 times in the first half hour while apparently marking grass stems after its departure from its perch on 19 occasions; the same male left its perch without marking 42 times in the last half hour of observation and marked only 5 times. Likewise, on 6 August when a male with a blue thoracic dot (added on 5 August) was watched between 1111 and 1317 E.D.T. the wasp left its perch without marking 67 times in the first half hour while marking 34 times; the same male flew from its perch without abdomen dragging 43 times in the last half hour while marking only 10 times. Occasionally the male marked several different plant parts during one flight out from its perch.

Only three interactions between *C. clypeata* were seen during the study, two involving a resident male that pursued another individual presumed to be a conspecific male for a short distance and one that resulted in a brief grapple on the ground between the resident and the other wasp. No nests of the wasp were found near the marking sites and no copulations were observed.

## Discussion

The behavior of the male *C. clypeata* was very similar to that of other philanthine wasps seen apparently marking plant stems and leaves at certain restricted locations that they

often defended from other males (Evans and O'Neill 1988, Kroiss et al. 2010). Males walked along stems in the middle of the day (as is true for many species of *Philanthus*, see Evans and O'Neill 1988) with their head and tip of abdomen lowered to touch the plant, presumably brushing the stem or leaf with a pheromone scent produced by cephalic glands to attract females (Evans and O'Neill 1991, Kroiss et al. 2010). Abdomen-dragging occurs more often early in the daily residence of a male rather than later in *P. basilaris* (O'Neill 1983) as in *C. clypeata*. Male tenure at marking sites is often short, lasting only a few days at most (Alcock 1975b, O'Neill 1983). Copulations are rarely, if ever, seen in any marking species (Alcock 1975b, O'Neill 1983, this study), even in those species that mark territories near nesting females (Gwynne 1980).

The occurrence of abdomen dragging by at least two species of *Cerceris* and several others in the Cercerini (especially *Eucerceris* spp.) (Alcock 1975a, Evans and O'Neill 1985) suggest that this behavior is widespread in the Philanthinae and perhaps is ancestral in this group. The fact that abdomen dragging is absent in many species of *Cerceris* is indicative of the loss of the application of a sex pheromone in some of the Cercerini, in addition to the modification of the application brushes found in many members of this group (Evans and O'Neill 1985) and changes in the cephalic glands that produce the sex attractant pheromone (Weiss et al. 2017).

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