

A new species of *Grotea* Cresson, the first record of Labeninae (Hymenoptera, Ichneumonidae) in the Greater Antilles

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Academic editor: G. Broad | Received 18 October 2020 | Accepted 23 November 2020 | Published 25 February 2021

<http://zoobank.org/EE363439-72DE-40FF-AD69-7F965041F29B>

Citation: Sandoval M, Santos BF (2021) A new species of *Grotea* Cresson, the first record of Labeninae (Hymenoptera, Ichneumonidae) in the Greater Antilles. *Journal of Hymenoptera Research* 81: 1–8. <https://doi.org/10.3897/jhr.81.59769>

Abstract

Grotea ambarosa **sp. nov.** is described, illustrated and compared to currently described species of the genus. The new species is characterized mainly by having the mesosoma 2.9× as long as high; genal projection distinct and apically subquadrate; pleural carina distinct only anteriorly to anterior transverse carina; mesosoma almost entirely amber-orange and legs with complex dark brown and white marks. This is the first record of any labenine species for the Greater Antilles; the few distribution records elsewhere in the Caribbean Islands are reviewed and discussed in the context of the biogeography of Labeninae.

Keywords

Caribbean Islands, biogeography, Darwin wasp, ichneumonid

Introduction

Labeninae are a relatively small subfamily of ichneumonid wasps, currently with 12 genera and 163 extant species (Yu et al. 2016 and additions in Herrera-Florez 2018 and Herrera-Florez and Pentead-Dias 2019). Genera within Labeninae exhibit a diverse range of morphologies but share the following putative synapomorphies: an

apically truncated vein Sc in the hind wing; a uniformly weakly sclerotized proctodeal membrane; the ventral valve of the ovipositor enclosing the dorsal valve; and a basally lobate ovipositor sheath (Gauld 2000). In addition, species of Labeninae have a distinct areolet on the front wing, cylindrical flagellum, simple tarsal claws, slender hind coxa, and first tergite and sternite usually fused (Townes 1969; Gauld 1991). The subfamily historically has been known to have a mainly “Gondwanan” distribution: almost all species occur in the Australasian and Neotropical regions, with a few species of *Labena* and *Grotea* found in the Nearctic region (Gauld and Wahl 2000).

Grotea Cresson is a moderately diverse genus of labenines characterized by the rather elongate and slender body; clypeus with apical margin distinctly concave; lower part of gena usually strongly raised into a lamella or process; hind wing with about 6–8 basal hamuli on vein C; and sternite 1 extending far past the level of spiracle of tergite 1 (Gauld 2000). Species of *Grotea* are cleptoparasitoids of bees that build nests in hollow twigs, including species of *Ceratina* Latreille, *Megachile* Latreille, *Chilicola* Spinola, and *Manuelia* Vachal (Daly et al. 1967; Packer 2004; Gonzalez and Giraldo 2009). The adult female oviposits through the wall of the twig into the cell, and after eclosing the wasp larva consumes both the bee larva and its pollen reserves, sometimes going through the contents of multiple cells.

The genus currently includes 26 species broadly distributed in the New World, from Canada to southern Chile (Slobodchikoff 1970; Gauld 2000; Herrera-Florez 2014; Herrera-Florez and Pentead-Dias 2019). However, there are no records for the genus, and in fact for any species of Labeninae, from the Greater Antilles. Herein we describe a new species of *Grotea* from the Dominican Republic, providing the first record of the subfamily for this region.

Material and methods

The type series is deposited in the Utah State University Insect Collection (USUC); comparative material for other species of *Grotea* was obtained from USUC as well as the American Museum of Natural History (AMNH); *Instituto Nacional de Pesquisas da Amazônia* (INPA) and the National Museum of Natural History (USNM).

Morphological terminology follows Broad et al. (2018). Proportions of the mesosoma (length vs. height) were calculated by measuring length from the point at which the pronotum meets the cervical shield to the posterior basal angle of the metapleuron (i.e. “Weber’s length”, Weber 1938) against the distance from the midcoxal attachment to the dorsal apex of the scutellum (Fig. 1). Leg surfaces were interpreted as in Aguiar and Gibson (2010). When potentially ambiguous, color names are followed by their respective RGB formula, as determined from digital pictures of the studied specimens, according to procedures described by Aguiar (2005).

Specimens were examined using a Leica Wild Stereo/Dissecting M10 microscope. Images were prepared using a modified version of the EntoVision suite (GTVision, Hagerstown, Maryland), including a Leica Z16 zoom lens attached

to a digital video camera that feeds image data to a desktop computer, with a custom fabricated illumination dome (RK Science Factory, Vitória, Brazil). Stacks of photos were combined in a single image using Zerene Stacker (Zerene Systems, Richland, WA, USA).

Results

Grotea Cresson, 1864

Figs 1–6

Grotea Cresson, 1864:397. Type-species: *Grotea anguina* Cresson (ANSP), by monotypy.

Macrogrotea Brèthes, 1916:84. Type-species: *Pimpla gayi* Spinola *sensu* Brèthes [= *Grotea superba* Schmiedeknecht], by original designation.

Echthroopsis Brèthes, 1916:86. Type-species: *Echthroopsis porter* Brèthes [= *Pimpla gayi* Spinola], by original designation.

Labenidea Rohwer, 1920:413. Type species: *Grotea superba* Schmiedeknecht, by original designation.

Diagnosis. Body slender and elongate, mesosoma in lateral view 2.1–2.9× as long as high. Clypeus with apical margin distinctly concave, exposing labrum; gena close to junction of occipital and hypostomal carinae usually raised into a lobe; hind wing with 6–8 basal hamuli on vein C; metasomal insertion above but close to coxal insertions; sternite 1 extending far past the level of spiracle of tergite 1.

Grotea ambarosa sp. nov.

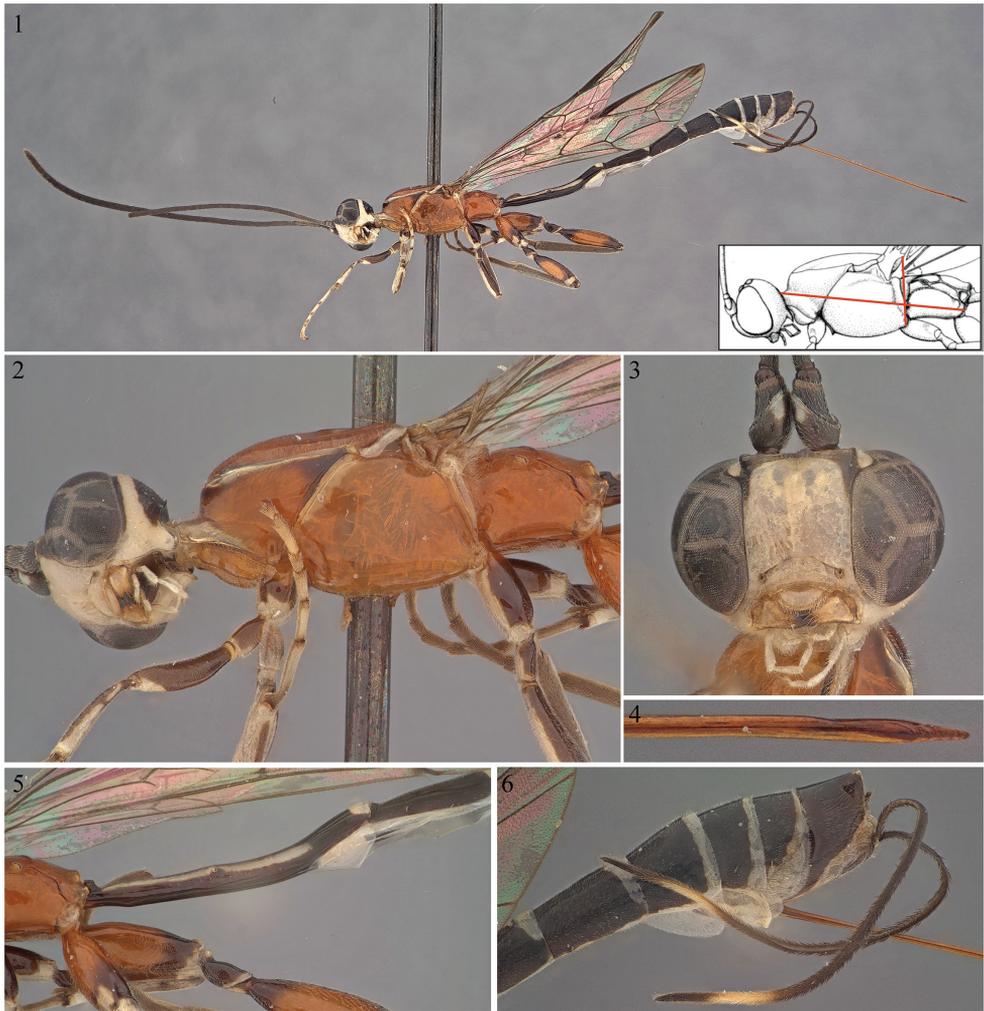
<http://zoobank.org/93FBADC2-EC6D-47F1-B1EF-8ACCD548DD92>

Material Examined. Holotype: DOMINICAN REPUBLIC • ♀; Duarte, 20 km NE San Francisco de Macoris, Loma Quita Espuela; 300 m; VII.1991; L. Masner (USUC). Pinned; left apical tip of antenna missing, otherwise in good condition.

Paratypes: DOMINICAN REPUBLIC • 1 ♀ 1 ♂, same data as holotype (USUC).

Diagnosis. Mesosoma 2.9× as long as high, polished and almost entirely impunctate; genal projection distinct and apically subquadrate; pleural carina distinct only in front of transverse carina; mesosoma almost entirely amber-orange, legs with complex dark brown and white marks.

Description. Forewing 7.7 mm long. **Head.** Head in dorsal view with gena rounded behind eye, occiput lightly pubescent. Posterior ocellus separated from eye by 1.2× its diameter. Supra-clypeal area shiny and shallowly punctate. Groove between clypeus and face strong (Fig. 3). Clypeus 2.8× as wide as long. Occipital carina dorsally slightly raised, sublaterally slightly expanded forming a subtle flange. Gena at junction with hypostomal carina abruptly projected as a lamella with subquadrate apex.



Figures 1–6. *Grotea ambarosa* sp. nov., holotype female **1** lateral habitus; detail showing measurement of length vs. height of mesosoma overlaid onto image from Townes (1970) **2** head and mesosoma, lateral view **3** head, frontal view **4** ovipositor, lateral view **5** first metasomal tergite, lateral view **6** posterior tergites and ovipositor sheath.

Antenna with 40 flagellomeres. First flagellum segment as long as the second and third flagellum segments combined.

Mesosoma. Mesosoma 2.9× as long as high, polished and almost entirely impunctate (Fig. 2). Pronotum centrally uniformly and evenly concave. Epomia absent. Scuto-scutellar groove deeply impressed and smooth; scutellum distinctly convex. Area basalis of propodeum triangular in shape, about 1.5× as long as wide. Spiracles kidney-shaped. Pleural carina distinct only anteriorly to anterior transverse carina. Anterior transverse carina forming a smooth arc. Lateral longitudinal carina distinct from propodeal apex to anterior transverse carina, sinuous and irregular. Area spiracularis and



Figure 7. Diagram showing type locality for *Grotea ambarosa* sp. nov.

area lateralis not fully enclosed. Posterior transverse carina absent. Forewing crossvein 1cu-a arising distinctly distad of M&Rs. Aerolet large and pentagonal, about 1.6× as wide as long.

Metasoma. Tergite 1 very long, 0.85× as long as mesosoma, curved upwards on posterior 0.25 (Fig. 5). Postpetiole only slightly wider than petiole. Exposed portion of ovipositor 4.4 mm, about 2.05× as long as hind tibia.

Color. Mostly amber-orange with black and whitish marks. Head whitish (247,214,160) (Fig. 3); mandible apex, supra-antennal area and occiput except orbital band and posterior 0.3 of dorsal 0.75 of gena glossy black; supra-antennal area just anteriorly to ocelli with brownish spot. Dark grey regions of eyes bordered by light grey pigment. Supraclypeal area white. Antenna entirely dark brown (084,076,060). Mesosoma mostly amber-orange (172,091,046) (Fig. 2). Anterior portion of pronotal collar, narrow bands on dorsal margin of pronotum and anterior margin of mesopleuron, tegula, small spot on postscutellum and dorsal apex of mesepimeron whitish. Posterior 0.2 of scutellum lighter in color than the rest. Fore and mid coxae, all trochanters and femora and fore tibia dark brown (093,070,068) on dorsal and posterior faces, whitish on ventral and anterior faces; fore tarsus whitish with brownish apical marks; hind coxa amber-orange on basal 0.6, dark brown on apical 0.4, with a white apical spot on dorsal face; hind trochanter and trochantellus mostly dark brown, whitish in the articulation; hind femur medially amber-orange, basal and apical fourths dark brown; mid and hind tibiae and tarsi dark brown. Wings hyaline, forewing with a faint brown spot at apex. Tergites mostly blackish; T1 with sublateral white bands on anterior 0.75 and posterior whitish band; T2–3 with triangular whitish mark on posterior apex; T4–6 with posterior whitish bands, medially narrower on T6. Ovipositor sheaths black with subapical pale yellow band (Fig. 6).

Remarks. *Grotea ambarosa* does not run well to any of the species in the keys of Slobodchikoff (1970) or Gauld (2000) and does not correspond either to the species described later on by Herrera-Florez (2014, 2018) and Herrera-Florez and Pentead-Dias (2019). Among described species, it is most similar to *G. paulista* Herrera-Florez and *G. perplexa* Slobodchikoff. The new species can be differentiated from *G. paulista* by the pleural carina posteriorly absent (vs. distinct in *G. paulista*); area basalis 1.5× as long as wide (vs. 3.4×) and ovipositor 2.0× as long as hind tibia (vs 1.5×). The color pattern on the legs is also different, with *G. paulista* having the apex of all femora and of the hind tibia entirely white and hind femur basally amber-brown (vs. dark brown in *G. ambarosa*). Color pattern is also quite different between *G. ambarosa* and *G. perplexa*, which has a distinct whitish mark on antero-dorsal corner of mesopleuron (absent in *G. ambarosa*); fore- and mid legs almost entirely white (vs. extensively marked with dark brown); and hind femur and tibia apically entirely white (vs. dark brown). In addition, *G. perplexa* has a distinct pleural carina (absent in *G. ambarosa*) and the forewing crossvein 1cu-a arising opposite to M&Rs (vs. distinctly distad).

Etymology. The name refers to the striking resemblance of the color of the mesosoma to the rich amber deposits found in the Dominican Republic.

Discussion

This is the first record of *Grotea*, or in fact any labenine genus, in the Greater Antilles (Fig. 7). A few labenine species are recorded from islands in the Atlantic Ocean: *Labena nigra* Rohwer in Bermuda (Rohwer 1920), *L. trilineata* Ashmead in Grenada (Ashmead 1900) and the predominantly South American *G. delicator* Thunberg with records from Trinidad and Tobago (Slobodchikoff 1970) and a nonspecific record to the Leeward Islands (i.e. northern islands of the Lesser Antilles chain, from the Virgin Islands to Guadeloupe; Slobodchikoff 1970).

It has been repeatedly suggested that the Labeninae have originated in Gondwana after its separation from Laurasia, but before the landmasses of South America and Australia split from each other (e.g. Gauld 1983; Wahl 1993; Gauld 2000; Gauld and Wahl 2000; but see Spasojevic et al. 2018). If that is the case, the occurrence of Labeninae in the Greater Antilles would likely be due to overwater dispersal. The occurrence of some species in rather remote landmasses (e.g. Bermuda, or *Certonotus fractinervis* (Vollenhoven) in New Zealand) seems to indicate that labenines can be relatively good dispersers, making their absence from the Greater Antilles until now rather surprising. This study reveals that labenines do indeed occur in this region, suggesting that the lack of labenine species was due to a deficit in collecting and taxonomic studies rather than to a real faunal depletion. We predict that yet other species of Labeninae remain undiscovered in the Greater Antilles.

Acknowledgements

This work was supported by the National Science Foundation (REU Site, EAR-1062692, OCE-1560088) through funding to the NMNH's Natural History Research Experiences (NHRE) program. Special thanks to Gene Hunt and Virginia Power (NMNH) who run this program. Daniell Fernandes (INPA) provided help with literature on distribution records of Labeninae. Seán Brady (NMNH) hosted the first author as an intern at the NMNH and provided logistical help and good advice. David Wahl (USUC) kindly received the second author in a visit to his institution and provided a generous loan of labenine specimens. The authors have declared no competing interests exist.

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A survey of aphid parasitoids and hyperparasitoids (Hymenoptera) on six crops in the Kurdistan Region of Iraq

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Academic editor: J. Fernandez-Triana | Received 18 October 2020 | Accepted 27 January 2021 | Published 25 February 2021

<http://zoobank.org/284290E0-6229-4F44-982B-4CC0E643B44A>

Citation: Bandyan SK, Peters RS, Kadir NB, Ferrer-Suay M, Kirchner WH (2021) A survey of aphid parasitoids and hyperparasitoids (Hymenoptera) on six crops in the Kurdistan Region of Iraq. Journal of Hymenoptera Research 81: 9–21. <https://doi.org/10.3897/jhr.81.59784>

Abstract

In this study, we surveyed aphids and associated parasitoid wasps from six important crop species (wheat, sweet pepper, eggplant, broad bean, watermelon and sorghum), collected at 12 locations in the Kurdistan region of Iraq. A total of eight species of aphids were recorded which were parasitised by eleven species of primary parasitoids belonging to the families Braconidae and Aphelinidae. In addition, four species of hyperparasitoids (in families Encyrtidae, Figitidae, Pteromalidae and Signiphoridae) were recorded. *Aphelinus albipodus* (Hayat & Fatima, 1992), *A. flaviventris* (Kurdjumov, 1913), *A. varipes* (Förster, 1841) (Aphelinidae), *Aphidius rhopalosiphi* (De Stefani, 1902), *A. uzbekistanicus* (Luzhetzki, 1960), (Braconidae) and *Alloxysta arcuata* (Kieffer, 1902) (Figitidae) were recorded in Iraq for the first time. The results represent the first survey of these interactions in this region and form the basis for understanding crop-aphid-parasitoid-hyperparasitoid networks and for future biological control actions.

Keywords

Aphelinidae, Aphididae, Braconidae, biocontrol, pests

Introduction

Aphids (Homoptera: Aphididae) are considered as an economically important group amongst insect pests and attack crops in the Kurdistan region in Iraq, as well as in many other countries. There are around 4700 species of Aphididae worldwide, approximately 450 species have been reported infesting crop plants and almost 100 species have significant economic importance (Blackman and Eastop 2000; Blackman and Eastop 2007). Their economic importance is mainly due to the reduction of both quality and quantity of the crops (Carter et al. 1980), by the aphids feeding on phloem sap, producing honeydew and transmitting over 200 plant viruses (Kennedy et al. 1962; Mill 1989; Blackman and Eastop 2000, 2007; Hogenhout et al. 2008; Talebi et al. 2009). The potato aphid *Macrosiphum euphorbiae* (Thomas, 1878), the melon aphid *Aphis gossypii* (Glover, 1877), the black bean aphid *Aphis fabae* (Scopole, 1763) and the green peach aphid *Myzus persicae* (Sulzer, 1776) are the most common aphid pests in Iraq (Jasman et al. 2016).

Aphids have many natural enemies, including hymenopteran parasitoids which potentially can also be used as biological control agents (Boivin et al. 2012). These parasitoids mainly belong to two taxa: Braconidae: Aphidiinae (Ichneumonoidea) and Aphelinidae (Chalcidoidea). Aphidiinae are solitary endoparasitoid wasps of aphids and play a significant role in reducing aphid populations (Starý 1970, 1988, 2006; Vorley and Wratten 1985; Hagvar and Hofsvang 1991) with more than 505 described species belonging to 38 genera (Žikić et al. 2017), among them only 28 species have been recorded in Iraq (Farahani 2016; Rakhshani et al. 2019). Aphelinidae are a species-rich Hymenoptera family with more than 1000 described species in 43 genera, only seven species belonging to two genera have been recorded in Iraq (Noyes 2020). Many species are solitary koinobiont endoparasitoids of Sternorrhyncha, attack aphids and are used in biological control programmes (Starý 1988; van Lenteren et al. 1997; Wei et al. 2005; Boivin et al. 2012). Hyperparasitoids are secondary parasitoids that attack primary parasitoid wasps, several of these are known to use aphid primary parasitoids and have a huge impact on the dynamics of insect communities (Sullivan and Völkl 1999; Kos et al. 2012).

Despite the economic and ecological importance of this multi-trophic system, few investigations of aphid parasitoids and hyperparasitoids have been conducted in Iraq (Starý 1969; Al-Azawi 1970; Starý and Kaddou 1971). Until now, no research has been conducted on aphid parasitoids in the Kurdistan region in northern Iraq where the arable land of national importance is located and where different types of vegetable crops are grown, among them the most economic important vegetable crops (wheat, sweet pepper, eggplant, broad bean, watermelon and sorghum) (Kurdistan Regional Statistics Office 2012). Most of these crops are attacked by various herbivore insect species including aphids, to date, farmers have relied on the use of pesticides to control these pests (Kurdistan Regional Statistics Office 2012). Biological control could be an environmentally friendly alternative (van Lenteren et al. 2017). The prerequisite for this approach is the knowledge of the

native aphid parasitoids and their hyperparasitoids in this region. Therefore, the purpose of this study was to survey aphids and associated parasitoid and hyperparasitoid wasps from six main crops in the Kurdistan region and to obtain a first understanding of the host plant-aphid-parasitoid-hyperparasitoid networks. These results will then allow us to explore the possibilities of using aphid parasitoids in biological control programmes.

Materials and methods

Sampling sites and collection

Sampling was conducted from April to August 2017 at 12 localities on six crops in the Kurdistan region which is located the northern part of Iraq (Fig. 1), the altitudes range from 430 to 950 m a.s.l.

Samples of plants, including leaves, straws and small branches bearing aphid colonies (consisting of both live and mummified aphids) were collected weekly from wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L.), watermelon (*Citrullus lanatus* L.), green pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), and broad bean (*Vicia faba* L.) (Table 1). Samples were placed in paper bags with labels and transferred to the laboratory for further processing. A total of 100–150 live winged aphids were killed and kept in 75% ethanol and preserved following Eastop and van Emden (1972) for later identification. The remaining aphids (living and mummies) were placed in Petri dishes along with some host plant material. The Petri dishes were kept in the laboratory condition (22.5 °C, 65% relative humidity, 16:8 L:D photoperiod) to rear parasitoids, for at least 14 days post-collection (Kavallieratos et al. 2004). The Petri dishes were examined and the host plant material was exchanged when necessary. The mummies were inspected daily. The emerging parasitoids were transferred into 96% ethanol and kept at -20 °C (Tomanović et al. 2014). The aphids were identified to species level using the key of Blackman and Eastop (2000).

Table 1. Sampled crops species and sampling sites as shown in Fig. 1.

Locations	Coordinates	Crops
Erbil-Grdarasha	36.0444°N, 44.1091°E	Broad bean, Eggplant, Sorghum, Watermelon and Wheat
Erbil-Sablax	36.0442°N, 44.1024°E	Broad bean and Wheat
Erbil-Kalak	36.2574°N, 43.7576°E	Broad bean, Eggplant, Sorghum, Watermelon and Wheat
Drbandi Gomaspan – Field1	36.3027°N, 44.2236°E	Broad bean and Wheat
DarbandiGomaspan – Field2	36.2638°N, 44.3307°E	Broad bean and Wheat
DarbandiGomaspan – Field3	36.2914°N, 44.2540°E	Broad bean and Wheat
Harir – Field1	36.5475°N, 44.3098°E	Sorghum and Wheat
Harir – Field2	36.5290°N, 44.3253°E	Sorghum and Wheat
Harir – Field3	36.5860°N, 44.2862°E	Sweet pepper and Wheat
Choman – Field1	36.5877°N, 44.8039°E	Wheat
Choman – Field2	36.5874°N, 44.8109°E	Wheat
Choman – Field3	36.5836°N, 44.8192°E	Wheat



Figure 1. Study sites in Northern Iraq, Kurdistan region (Red dots).

DNA extraction, amplification and sequencing

Wasp specimens were identified to morphospecies using a NIKON SMZ-1stereomicroscope. Based on morphospecies designation, 192 of the total 737 parasitoid wasp specimens were selected for DNA barcoding.

DNA was isolated using a DNeasy Blood and Tissue Kit and the BioSprint 96 magnetic bead extractor by Qiagen (Hilden, Germany) in accordance with the standard

protocols of the GBOL (German Barcode of Life) for purification of total DNA from animal tissue. Extracted DNA was preserved at 4 °C for the subsequent polymerase chain reaction (PCR). Amplification of a partial fragment of the mitochondrial cytochrome oxidase 1 (COI) gene was performed by PCR using primers: LCO1490-JJ [5'-CHACWAAYCATAAAGATATYGG-3'] and HCO2198-JJ [5'-AWACTTTCVG-GRTGVCC AAARAATCA-3'] (Astrin and Stüben 2008). PCR for the COI gene was carried out in total reaction mixes of 20 µl (2 µl of undiluted DNA template, 0.8 µl of each primer (10 pmol/µl) and standard amounts of the reagents provided with the 'Multiplex PCR' kit from Qiagen.

PCR reactions were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California, USA). The tubes were subjected to the PCR cycle involving two cycles and initial denaturation at 95 °C (15 min), the first cycle set 15 cycles of denaturation at 94 °C (35 s), annealing at 55 °C (-1 °C per cycle) and 90 s and extension at 72 °C (1 min). The second cycle involved 25 cycles of denaturation at 94 °C (35 s), annealing at 40 °C, (90 s) and extension at 72 °C (1 min), followed by a final extension at 72 °C (10 min). PCR products were stored at 4 °C in the short-term (< 1 month) before subsequent processing. Unpurified PCR products were sent for bidirectional Sanger sequencing to BGI (Hong Kong, China). Out of the 192 parasitoid wasp samples processed, 170 samples delivered sequences. Sequences were edited and assembled using Geneious R7.

Critical point drying and mounting the specimens

The specimens were critical point dried with a Leica EM CPD 300 AUTO and card mounted. All specimens are deposited at the Zoological Research Museum Alexander Koenig, Bonn (ZFMK).

For species identification, we followed an integrative approach, using the results from analysing molecular data and identification based on external morphology. First, all sequences were checked against and compared with the Barcode of Life Database (BOLD Systems (www.barcodinglife.org) using the BOLD Blast tool. Then, the sequences were aligned with CLUSTALW in MEGA, version 5.1 (Tamura et al. 2011) and a Neighbour-Joining (NJ) tree was constructed in MEGA V.10 (Suppl. material 1: Fig. S1). Finally, the specimens were morphologically identified using the keys by Japoshvili and Karaca (2009), Japoshvili and Abrantes (2006) and Hayat (1998) (Aphelinidae), Ferrer-Suay et al. (2019) (Figitidae) and Rakhshani et al. (2019) (Aphidiinae) and the species identifications were double-checked or done by Ehsan Rakhshani (Aphidiinae) and George Japoshvili (Aphelinidae). Combining the results of the search against the barcode database, the NJ tree (Suppl. material 1: Fig. S1) and the morphological identification, we assigned species names to all specimens, except for one putative species in *Aphelinus* (see results). One specimen was identified using only morphology, because the barcode generation failed (*Chartocerus* sp., see below).

The 170 sequences are deposited at GenBank with accession numbers MT945966–MT991672 (Suppl. material 2: Table S1).

Results

In this study, 5382 adult and nymph aphids and 737 parasitoids specimens were collected from the six crops in the studied region. A total of eight species of aphids were recorded (Table 2). They were parasitised by seven species of primary parasitoids belonging to Braconidae: Aphidiinae and four species belonging to Aphelinidae. The primary parasitoids are associated with four species of hyperparasitoids, i.e., *Pachyneuron aphidis* (Bouché, 1834) (Chalcidoidea: Pteromalidae), *Syrphophagus aphidivorus* (Mayr, 1876) (Chalcidoidea: Encyrtidae), *Alloxysta arcuata* (Kieffer, 1902) (Cynipoidea: Figitidae) and *Chartocerus* sp. (Chalcidoidea: Signiphoridae).

The following species were recorded from Iraq for the first time:

Aphidiinae (Braconidae)

Aphidius rhopalosiphi (De Stefani, 1902) from wheat and sorghum

Aphidius uzbekistanicus (Luzhetzki, 1960) from wheat

Aphelinidae

Aphelinus albipodus (Hayat & Fatima, 1992) from watermelon

Aphelinus varipes (Förster, 1841) from watermelon and broad bean

Aphelinus flaviventris (Kurdjumov, 1913) from sorghum

Figitidae

Alloxysta arcuata (Kieffer, 1902) from broad bean

Table 2. The trophic associations (host plant-host aphid-primary parasitoid-hyperparasitoid) on six important crop plants in the Kurdistan Region, Iraq.

Crops	Aphids	Primary parasitoids	Hyperparasitoids
Wheat <i>Triticum aestivum</i> (L)	<i>Metopolophium dirhodum</i> (Walker, 1849)	<i>Aphidius matricariae</i> (Haliday, 1834)	None recorded
Poaceae	<i>Rhopalosiphum maidis</i> (Fitch, 1856)	<i>Aphidius rhopalosiphi</i> (De Stefani, 1902)	
	<i>Rhopalosiphum padi</i> (Linnaeus, 1758)	<i>Aphidius uzbekistanicus</i> (Luzhetzki, 1960)	
	<i>Sitobion avenae</i> (Fabricius, 1775)	<i>Diaeretiella rapae</i> (McIntosh, 1855)	
	<i>Schizaphis graminum</i> (Rondani, 1852)		
Sorghum <i>Sorghum bicolor</i> (L) Poaceae	<i>Rhopalosiphum maidis</i>	<i>Aphidius matricariae</i>	<i>Chartocerus</i> sp.
	<i>Schizaphis graminum</i>	<i>Aphidius rhopalosiphi</i>	<i>Pachyneuron aphidis</i> (Bouché, 1834)
		<i>Aphelinus flaviventris</i> (Kurdjumov, 1913)	<i>Syrphophagus aphidivorus</i> (Mayr, 1876)
		<i>Aphelinus</i> sp.	
Watermelon <i>Citrullus lanatus</i> (L) Solanaceae	<i>Aphis fabae</i> (Scopoli, 1763)	<i>Aphidius funebris</i> (Mackauer, 1961)	<i>Pachyneuron aphidis</i>
	<i>Myzus persicae</i> (Sulzer, 1776)	<i>Aphidius matricariae</i>	
		<i>Binodoxys acalephae</i> (Marshall, 1896)	
		<i>Aphelinus albipodus</i> (Hayat & Fatima, 1992)	
		<i>Aphelinus varipes</i> (Förster, 1841)	
Sweet pepper <i>Capsicum annuum</i> (L) Solanaceae	<i>Myzus persicae</i>	<i>Lysiphlebus fabarum</i> (Marshall, 1896)	<i>Pachyneuron aphidis</i>
			<i>Syrphophagus aphidivorus</i>
Eggplant <i>Solanum melongena</i> (L) Solanaceae	<i>Aphis craccivora</i> (Koch, 1854)	<i>Aphidius funebris</i>	<i>Pachyneuron aphidis</i>
	<i>Aphis fabae</i>	<i>Diaeretiella rapae</i>	
Broad bean <i>Vicia faba</i> (L) Fabaceae	<i>Myzus persicae</i>	<i>Lysiphlebus fabarum</i>	
	<i>Aphis craccivora</i>	<i>Binodoxys acalephae</i>	<i>Syrphophagus aphidivorus</i>
	<i>Aphis fabae</i>	<i>Lysiphlebus fabarum</i>	<i>Alloxysta arcuata</i> (Kieffer, 1902)
		<i>Aphelinus varipes</i>	

Discussion

The present study on aphid parasitoids and hyperparasitoids on six economically important crops is the first of its kind in the Kurdistan region of Iraq, even though this region is significant for agriculture in Iraq with a large territory that includes different bio-geographical elements and climatic conditions.

Our evaluation of trophic associations reveals several species that could potentially be considered in the environmentally friendly management of aphid pests.

The results show that the wheat and sorghum plants are infested by important and common cereal crop pest aphid species, i.e., *Metopolophium dirhodum*, *Rhopalosiphum maidis*, *R. padi*, *Sitobion avenae* and *Schizaphis graminum* (Sigsgaard 2002; Praslicka et al. 2003). Watermelon, sweet pepper, eggplant and broad bean plants are infested by known aphid pest species of vegetables, i.e., *Aphis fabae*, *A. craccivora* and *Myzus persicae* (Blackman and Eastop 2007). Even though our study design does not allow for exact aphid species-parasitoid species links, we found that all aphid species were attacked by primary parasitoids. *Aphidius matricariae* was found on three different host plants (wheat, sorghum and watermelon) and *Lysiphlebus fabarum* was also found on three host plants (eggplant, sweet pepper and broad bean), both associated with different aphid species. *Aphidius matricariae* can utilise both aphid tribes Aphidini and Macrosiphini on a wide range of host plants and is used to control *Myzus persicae* in greenhouse crops (Starý and Kaddou 1971; Acheampong et al. 2012; Ghazali et al. 2015; Rakhshani et al. 2019). *Aphidius matricariae* is also known to play an important role in controlling aphids that infest cereal crops (Sigsgaard 2002; Praslicka et al. 2003). *Lysiphlebus fabarum* is a common species and considered as the best biological control agent of *Aphis* spp on various vegetable crops in different habitats (Starý and Kaddou 1971; Kavallieratos et al. 2004; Satar et al. 2019). Both *A. matricariae* and *L. fabarum* are cosmopolitan species, well known biological control agents and commercially sold to control aphids on cereal and vegetable plants (Boivin et al. 2012; Rakhshani et al. 2019).

Aphelinus varipes was found on two crops in our study (watermelon and broad bean). In addition, *Aphelinus albipodus* was found on watermelon, *Aphelinus flaviventris* and another *Aphelinus* species were found on sorghum. *Aphelinus varipes* is an abundant species in Mediterranean countries and Europe (Japoshvili and Abrantes 2006) parasitising a wide variety of aphid species, including *Aphis* spp. (Blackman and Eastop 2000). It has been used commercially in biological control of aphids on vegetable crops in Europe (Yashima and Murai 2013). In the 1990s, *A. varipes* was introduced into many countries and released to control aphids on cereals (Powell and Pell 2007). This species is able to suppress aphids even at low density and is also rarely prone to hyperparasitoidism (Takada and Tatsumi 2002). Although it is recorded here for the first time in Iraq, it might also be a suitable candidate for future biological control studies. *Aphelinus varipes* and *A. albipodus* both belong to the *varipes* species complex, which includes at least five cryptic species (Gokhman et al. 2017). Species in this species complex are difficult to separate from each other by morphology and by DNA barcodes (Chen et al. 2002; Riddick et al. 2019) which is also reflected in our NJ tree in which both species are mixed (Suppl. material 1: Fig. S1). However, they are considered as two distinct species and,

therefore, might also have different host specificity and biology. *Aphidius* and *Aphelinus* species were attacked by four hyperparasitoid species. *Alloxysta arcuata* (Kieffer, 1902) was recorded for the first time in Iraq, and according to (Sampaio et al. 2017), the species of the genus *Alloxysta* are obligatory hyperparasitoids of the species of Braconidae and Aphelinidae. *Syrphophagus aphidivorus* (Mayr, 1876) plays an important role in biological control and parasitises different aphid parasitoids (Roy et al. 2012). It lays eggs in both living and mummified parasitised aphids (Boivin et al. 2012; Kos et al. 2012). *Pachyneuron aphidis* (Bouché, 1834) is a common hyperparasitoid of the Aphidiinae species (Kos et al. 2012) and has already been reported as hampering the biological control of aphid pests by aphidiines (Burgio et al. 1997). In this study, it was recorded from four out of six crop species. Furthermore, a single specimen of *Chartocerus* sp. (Signiphoridae) was recorded. This genus has been reported only once in the southern part of Iraq on mealybug of citrus (Shalaby et al. 1970), and this is the first record associated with aphids in the country. While signiphorids seem not to play an important role as hyperparasitoids of aphid parasitoids, the other three species should be considered when evaluating possible future biological control agents. We could not make quantitative assessments of the parasitoid-hyperparasitoid associations, but our results indicate that, on most crop plants, biological control might be adversely affected by hyperparasitoids. Interestingly, no hyperparasitoids were recorded in samples from wheat, despite a high diversity of aphid and primary parasitoid species. Whether this is an artefact or true absence, needs further investigation. Our results suggest that hyperparasitoids might play a lesser role in this crop than in the other crops sampled. If confirmed, biological control might be easier and more efficient in wheat. Additionally, the possible reasons for this absence of hyperparasitoids would be an interesting research question.

Conclusion

In conclusion, the knowledge across four trophic levels (crops, aphids, primary parasitoids and hyperparasitoids) in economically important crop plants is significant to any future biological control programmes. The primary parasitoids species *Aphidius matricariae* and *Lysiphlebus fabarum* and the newly recorded *Aphelinus varipes* and *A. flaviventris* that are present in the field in the Kurdistan region can be potentially selected and used as biocontrol agents and could become powerful alternatives to pesticides used in this region. However, the efficiency and specificity of these parasitoid species and the effects of native hyperparasitoid will need to be studied further before applying and implementing in biological control programmes. Both, classical biological control using releases and subsequent establishment in the ecosystem as well as inundative application of mass-reared parasitoids should be taken into consideration. In addition to the basic knowledge for possible biological control actions, this study contributes to the still extremely poor knowledge of parasitoid wasps in the study region and also provides a DNA barcode resource for 15 important hymenopteran aphid associates from the Kurdistan region in northern Iraq.

Acknowledgements

This research was granted by the Zoological Research Museum Alexander Koenig and Ruhr University Bochum. We would like to thank Jana Thormann, Björn Rulik, Claudia Etzbauer and Dirk Rohwedder at the Zoological Research Museum Alexander Koenig, Bonn, Germany for lab work, processing sequences and assistance in aphid parasitoids mounting and identification. This study was also supported by the Biology and Biotechnology Department of Ruhr University Bochum and the Department of Plant Protection, Salahaddin University, Kurdistan Region, Iraq. We are thankful to Ehsan Rakhshani and George Japoshvili for their identifications of wasp species. We also thank Mariusz Kanturski (Zoology Research Team, the University of Silesia in Katowice, Poland) for checking and confirming identifications of aphids.

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Supplementary material I

Figure S1. Neighbour joining tree

Authors: Srwa K. Bandyan

Data type: phylogenetic

Explanation note: Neighbour joining tree using p-distance of 170 COI sequences of aphid parasitoids and hyperparasitoid on six crops (658 nucleotide positions). Bootstrap support values (1000 replicates) are shown next to the branches; Branches with less than 50% bootstrap support collapsed.

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Link: <https://doi.org/10.3897/jhr.81.59784.suppl1>

Supplementary material 2

Table S1. List of aphid parasitoids species included in this study

Authors: Srwa K. Bandyan

Data type: list

Explanation note: List of aphid parasitoids species included in this study: taxonomic information, sampling location, sampling date, host species, GenBank accession numbers and ZFMK-TIS-numbers.

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Link: <https://doi.org/10.3897/jhr.81.59784.suppl2>

Supplementary material 3

Alignment sequences

Authors: Srwa K. Bandyan

Data type: DNA sequences

Explanation note: Alignment sequences of aphid parasitoids species in this study.

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Link: <https://doi.org/10.3897/jhr.81.59784.suppl3>

A bilateral gynandromorph of *Discoelius dufourii* (Hymenoptera, Vespidae, Zethinae): morphology and mating behaviour

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Academic editor: Michael Ohl | Received 1 December 2020 | Accepted 26 December 2020 | Published 25 February 2021

<http://zoobank.org/5CD0A1F5-DA29-4523-8721-A9ED1E79AD80>

Citation: Budrienė A, Budrys E, Orlovskytė S (2021) A bilateral gynandromorph of *Discoelius dufourii* (Hymenoptera, Vespidae, Zethinae): morphology and mating behaviour. Journal of Hymenoptera Research 81: 23–41. <https://doi.org/10.3897/jhr.81.61550>

Abstract

A gynandromorph is an organism combining both female- and male-type tissues. While the vast majority of studies have described the morphology of gynandromorphs, their sexual behaviours remain under-explored. We studied a predominantly bilateral gynandromorph specimen of the predatory wasp *Discoelius dufourii* (Hymenoptera: Vespidae: Zethinae) reared from a trap-nest. In addition to describing the morphology, we explored the gynandromorph's response to a conspecific female, comparing it with the behavioural interactions between normal males and females of *D. dufourii* and the phylogenetically related *D. zonalis*. For most specific behavioural elements, males of both *Discoelius* species exhibited a greater frequency and a higher probability of occurrences of the behaviour than did females. However, aggression and plopping behaviours were prevalent in females. The gynandromorphic specimen demonstrated behavioural activities similar to those of a male, including a relatively frequent mate body antennation and an attempt to mount a female. However, it approached the female less frequently in comparison to conspecific normal males, and it did not demonstrate substrate antennation and jumping, typical of *Discoelius* male mating behaviour. Based on the parameters of the nest cell, the probability of the development of the gynandromorphic specimen from an unfertilized (male) egg was higher than the probability of development from a fertilized (female) egg.

Keywords

Aggression, antennation, development, mounting, sex-specific behaviour

Introduction

Gynandromorphy is a developmental abnormality, resulting in chimaeric individuals, which combine patches of both genetically female- and male-type tissues (Narita et al. 2010). Their generation is thought to be associated with the sex-determination systems, and study of this phenomenon broadens our current knowledge of the evolutionary mechanisms driving the diverse sex determination and differentiation systems (Narita et al. 2010; Yang and Abouheif 2011; Dobata et al. 2012; Aamidor et al. 2018).

Among Hymenoptera, females predominantly develop from fertilized diploid eggs that have biparentally inherited genomes, while males develop from haploid unfertilized eggs that contain only the maternally inherited genome (Cook 1993). The single-locus complementary sex determination (sl-CSD) mechanism, based on the complementary sex determiner (*csd*) gene, is well explored and understood, though a few other mechanisms are also known (Heimpel and de Boer 2008).

Gynandromorphs are thought to arise in a number of different ways, of which the most possible cytogenetic mechanisms are either fertilization of only one maternal nucleus in a bi-nucleate egg, or a fusion of two sperm nuclei in a mono-nucleate egg. A combination of these events is also possible (Michez et al. 2009; Narita et al. 2010; Aamidor et al. 2018). The resulting gynandromorph individuals are likely to comprise diploid female tissues of either biparental or paternal (androgenic) origin and haploid male tissues of either maternal (gynogenetic) or paternal origin. Impaternal gynandromorphs arising from unfertilized eggs may also occur, when either one of the two meiotic spindles of the second meiotic division fails to segregate a heterozygous nucleus, or two of the four nuclear products of meiosis fuse to form a heterozygous for the *csd* gene alleles diploid nucleus (Cooper 1959). This nucleus and one of the two haploid meiotic products are believed to undergo cleavage producing a wholly gynogenetic embryo with female-determined diploid nuclei and male-determined haploid nuclei. Such aberrant meiosis, leading to a gynandromorphy, has been thought to be induced by *Wolbachia* (Alphaproteobacteria) or possibly other endosymbionts (Narita et al. 2010). In addition, chromosomal aberrations, eliminations or differences in sl-CSD expression within embryonic tissues during embryogenesis have been proposed to be responsible for the generation of gynandromorphs (Michez et al. 2009). The occurrence of gynandromorphism might be linked with an inbreeding depression (Kinomura and Yamauchi 1994), higher or lower than normal temperatures (Pereira et al. 2004; Kamping et al. 2007; Gjershaug et al. 2016) or fluctuations of other specific environmental factors (Fateryya et al. 2011).

Although rare, gynandromorphs have been regarded as more or less randomly distributed among ants, wasps and bees, probably occurring in most species of Hymenoptera (Cooper 1959). Overall, gynandromorphs are known from at least 29 hymenopteran families (Rosa and Zettel 2018). In some of these, gynandromorphs have been extensively explored and described, e.g. in bees (Wcislo et al. 2004; Michez et al. 2009; Fateryya et al. 2011; Hinojosa-Díaz et al. 2012; Lucia and González 2013; Suzuki et al. 2015) and ants (Jones and Phillips 1985; Kinomura and Yamauchi 1994; Heinze and

Trenkle 1997; Yoshizawa et al. 2009; Gjershaug et al. 2016). Among other Hymenoptera, this phenomenon has been documented less frequently, e.g. in Diprionidae (Martini et al. 1999), Chrysididae (Rosa and Zettel 2018; Rosa 2019) and Eumeninae (Cooper 1959; Turrisi and Borsato 2008). Based on the external morphology, the distribution of male and female characteristics in gynandromorphs can be most commonly categorized into three types: axial (anterior-posterior), bilateral (left-right) or mosaic (patchily distributed) (Wcislo et al. 2004; Kamping et al. 2007; Michez et al. 2009). Most studies have focused on the description of the morphology of the gynandromorphs, while aspects of their reproductive behaviours have remained largely unexplored.

A long-standing problem in the evolutionary biology of gynandromorphs concerns the questions of to what extent do their behaviours deviate from the norm, or what gynandromorphs could reveal about the systems controlling sex-specific behaviours (O'Dell and Kaiser 1997; Hinojosa-Díaz et al. 2012). Developmental biologists and geneticists have long been interested in system analysis of the behaviour using gynandromorphs. An example is the use of gynandromorphs of the parasitic wasp *Habrobracon* as a tool to locate the anatomical foci in the nervous system determining sex-specific steps in their reproductive behaviour (Whiting 1932). Within the social Hymenoptera, it has been revealed that gynandromorphs with various patches of female- and male-type tissues demonstrate diverse levels of abnormalities in sex-specific behaviour (Yoshizawa et al. 2009; Ugajin et al. 2016; Matsuo et al. 2018). Within the solitary Hymenoptera, one study has described the sexual behaviour of a gynandromorphic specimen of *Osmia* (Megachilidae) (Sampson et al. 2010). Yet, the sexual behaviour of gynandromorphs in the solitary vespid wasps has not been investigated.

The objectives of this study were: (1) a description of a gynandromorphic specimen of a solitary predatory wasp *Discoelius dufourii* Lepeletier 1841 (Hymenoptera: Vespidae: Zethinae); (2) an investigation of the behavioural response of the gynandromorph to a conspecific female, comparing it with the behavioural interactions between normal males and females of *D. dufourii* and a related zethine wasp *D. zonalis* Panzer 1801. Based on the nest cell parameters of *D. dufourii* and *D. zonalis*, we also estimated the fertilization probability of the egg that developed into the gynandromorphic specimen.

Materials and methods

Study species

Discoelius dufourii and *D. zonalis* are the only European representatives of the predominantly tropical subfamily Zethinae (Vespidae). In northern Europe, both species are uncommon, they are typical of woodlands in a semi-natural landscape (Budrys et al. 2010).

Females of these univoltine cavity-nesting (xylicolous) mass-provisioning solitary predatory wasps build their nests in pre-existing tubular cavities, e.g. the exit holes of xylophagous beetles. The nest consists of a row of brood cells, built starting with the deepest one and separated by diaphragms made of fragmented and partly crushed leaves. The egg

is attached to the upper internal surface of the brood cell by a thin stalk. After oviposition, the wasp supplies the brood cell with several paralyzed caterpillars or sawfly larvae.

As in other aculeate Hymenoptera, females of *D. dufourii* and *D. zonalis* pre-determine both the sex (by the arrhenotokous haplodiploidy) as well as the body size (by the amount of cell provision) of their offspring. Similarly to most Hymenoptera, females of *D. dufourii* and *D. zonalis* are the larger sex (Budrienė and Budrys 2007). Commonly the mother wasp oviposits the fertilized eggs developing into females in the innermost brood cells of the nest, while the unfertilized male eggs are placed in the cells closer to the entrance of the nesting cavity.

Material

Wasp nests for the study were obtained using small trap-nests: 20–35 internode fragments of dry reed (*Phragmites australis*) stems wrapped into polyethylene or Tetrapack cartons and fixed using sticky tape (Budrys et al. 2010). Most of the material was collected in Alytus (54°32'N, 24°03'E), Anykščiai (55°24'N, 25°16'E), Molėtai (55°08'N, 25°16'E), Pasvalys (55°56'N, 24°17'E), Varėna (54°00'N, 24°25'E) and Vilnius (54°50'N, 24°58'E) districts, Lithuania, in the years 1998–2018. Some material was collected using the same method in Hirya, Belarus (54°39'N, 26°12'E), Koeru, Estonia (58°59'N, 26°04'E), Pychowice, Poland (50°01'N, 19°53'E), Tázlár, Hungary (46°31'N, 19°31'E) and Livenza, Italy (46°02'N, 12°30'E) in the years 2007–2013. After exposure, the trap-nests were collected and the reed stems with nests dissected. The diapausing prepupae were individually placed in plastic containers for hibernation in a climatic chamber at +4 °C. After reactivation, the containers were kept at +25 °C until the adults hatched. After eclosion, the wasps were kept individually in plastic cages (6 cm height × 5 cm diameter) at an ambient temperature (+22–26 °C) with honey solution and water available. In total, 677 nests of the two *Discoelius* species with 2722 brood cells were obtained and studied in 1996–2019. Of these, the sex of the brood could be established in 509 nest cells of *D. dufourii* (235 females, 273 males and 1 gynandromorph) and 1557 nest cells of *D. zonalis* (516 females and 1041 male).

The single gynandromorph specimen of *D. dufourii* was reared from a nest in a reed stem bundle trap-nest exposed from 31 May 1998 to 30 June 1998 on an old wooden building in Varnupys, Lithuania (55°24'N, 25°16'E) (Budrienė 2003). The nest with prepupae was reactivated in a refrigerator from 05 Nov. 1998 to 28 Mar. 1999, with the imago eclosing on 04 May 1999. The specimen was used for a mating experiment on 15 May 1999, when a conspecific female specimen emerged. Afterwards, the gynandromorph specimen was pinned and deposited in the collection of the Nature Research Centre, Vilnius, Lithuania.

Morphology

The external morphological characters of the *D. dufourii* gynandromorph were studied and measurements were taken using a Nikon SMZ800 binocular microscope with an

ocular micrometer (www.nikon.com). Images were obtained using a digital camera Nikon DS-Fi2 connected to the microscope. The images were stacked using software CombineZP by Alan Hadley (alan-hadley.software.informer.com).

Mating behaviour

Mating trials were conducted in 1999–2008, applying a no-choice mating design in which the subjects were presented with a single option, having to choose between this option or nothing (Dougherty 2020). Following the mating protocol described in Budrienė and Budrys (2007), the pairings were observed on a white paper arena under a bell-glass (22 cm height × 22 cm diameter). A portion of the pairing experiments took place on a smaller white paper arena under a transparent plastic cage (12 cm height × 10 cm diameter). Pairing arenas were exposed to daylight, with additional artificial illumination, at a temperature of +27–29 °C in a climatic chamber. A single virgin female was let into the arena and the cage with a single male was placed nearby for a one minute habituation. Afterwards, the male was introduced into the arena. In order to prevent the wasps from responding to odour cues of previous pairings, the arena paper was replaced each time. Observations lasted 30 min, or until copulation or mounting had finished. The behavioural interactions between a normal female and the gynandromorph of *D. dufourii* were observed over 37 min. Unfortunately, it was a single observation of the specimen's behaviour due to the unavailability of other conspecific specimens during the life time of the gynandromorph.

Among the previously identified and described sex-specific behavioural elements (Budrienė and Budrys 2004), the following elements were recorded in the mating experiments with *Discoelius*:

aggression	moving towards the other sex with opened mandibles, sometimes followed by biting;
approaching	walking or flying towards the other sex;
jumping	pouncing flight at short distance;
mate body antennation	moving towards the other sex followed by touching it with the tips of the antennae;
plopping	short flight with deliberate falling on the surface with a plopping sound;
substrate antennation	inspection of the substrate with antennae.

The probability of occurrence of a behavioural element was estimated as the proportion of the experiments where the element was observed at least once. The frequency was estimated as the number of the occurrences of the behavioural element divided by the duration of the mating phase in minutes. We recorded the duration of the following phases of mating: premounting (the period from the start of an experiment until the male mounts the female), mounting (the male taking a parallel position on the dorsum of the female) and postmounting (the male continuing copulation after

released leg-hold, laying or hanging behind the female). In the cases in which the male did not succeed in mounting the female, the duration of the whole experiment was considered as the duration of the premounting phase. Since the studied gynandromorph did not succeed in mounting, the frequency of its behavioural elements was compared with the respective frequencies during the premounting phase of the mating in the experiments with normal males.

Egg fertilization probability

We estimated the probability of whether the gynandromorph had developed from a fertilized or unfertilized egg based on the following four parameters of the brood cell as independent variables: (1) the diameter of the nesting cavity in mm; (2) the depth (distance from the entrance) of the bottom of the brood cell in mm; (3) the serial number of the brood cell, starting from the first (the deepest) one; and (4) the serial number of the brood cell, counting from the last (the outermost) one. The combination of the parameters (3) and (4) reflected the total number of brood cells in a nest. We estimated the significance of these parameters for a female wasp decision to fertilise an egg as a dependent dichotomic variable 1 (mother wasp fertilizes the egg, this thereafter developing into a female brood) versus 0 (mother wasp does not fertilize the egg, this thereafter developing into a male brood), using logit models for both studied *Discoelius* species, *D. dufourii* and *D. zonalis*. We also built discriminant classification functions, separating the brood cells with female and male broods of the two *Discoelius* species, based on the same four cell parameters, and we estimated the posterior probability of egg fertilization.

Statistical methods

To test the significance of sex differences in *D. dufourii* and *D. zonalis* in the probability of the occurrence of the sex-specific behavioural elements, Fisher's exact test was applied. The significance of differences in the frequency of the behavioural elements was assessed using the Wilcoxon signed rank test with continuity correction.

The discriminant equations were built using Statsoft STATISTICA, version 8 (Statsoft, Tulsa, Oklahoma, United States of America). Wilcoxon signed rank test and Fisher exact test were applied using R 4.0.2 (R Core Team 2020).

Results

Morphology of *D. dufourii* gynandromorph

Body length 12.2 mm, right forewing length 9.6 mm, left forewing length 8.9 mm. Most of the right side of the body was female-like, most of the left side of the body was male-like, with the following exceptions: the right side of the frons and clypeus were male, the left side of the frons and clypeus were female (Fig. 1G); the right side of the abdominal



Figure 1. Head of *Discoelius dufourii*, frontal view: ♀ female; G gynandromorph; ♂ male.

segment 8 (7th segment of metasoma) was male-like (Fig. 4G: T8), the left side of the segment was female-like, including three left-side valvules of the sting (Fig. 4G: V1–3); the abdominal segment 9 included two-sided, symmetrical male genitalia.

The general shape of the head was sub-quadratic, more similar to that of a female than to a more rounded head of a male. The vertex and genae were female-like, relatively long and convex, more shiny and less densely punctate than those of a male; vertex before the occipital carina with a smooth shiny medial longitudinal strip, typical of a female. The face was asymmetrical: the sculpture and colouration of the left side of the frons and clypeus were female-like; those of the right side were male-like (Fig. 1G). The left side of the clypeus was slightly longer, more distinctly striate and with a narrow yellow transverse preapical marking, typical of a female; the right side of the clypeus was shorter, with slightly more distinct punctation and with a wide yellow preapical marking, typical of a male. The antennae and mandibles were asymmetrical in the opposite way to the face and the clypeus (Fig. 1G). The left antenna was male: the scape with a complete anteroventral yellow strip; flagellum long, with 11 flagellomeres, of which the apical two forming a hook; flagellomeres 6–10 with smooth pale-brown tyloidea. The right antenna was female: the scape with a small apical dark-yellow spot; flagellum short, with 10 flagellomeres. The left mandible was male: the internal edge subrectangular, with nearly straight apical margin; the anterior surface with a dark-yellow patch. The right mandible was female: the internal edge bidentate (in addition to the two apical teeth); anterior surface dark.

The mesosoma was asymmetrical, with a longer female-like right side and a shorter male-like left side (Fig. 2G). The punctate-rugulose sculpture of the scutum and scutellum was more similar to that of a female. The right side of pronotum was longer, with a shorter obtuse right angle of the pronotal carina, typical of female; the left side of pronotum was shorter, with a longer acute left angle of the pronotal carina (dorsal view), typical of male. The right legs were black, except a dark-yellow spot on the fore tibia; fore and mid tarsi are thick, typical of female. The left legs were typical of a male: black with yellow preapical spots on the mid femur and the hind tibia, and with long yellow mark-

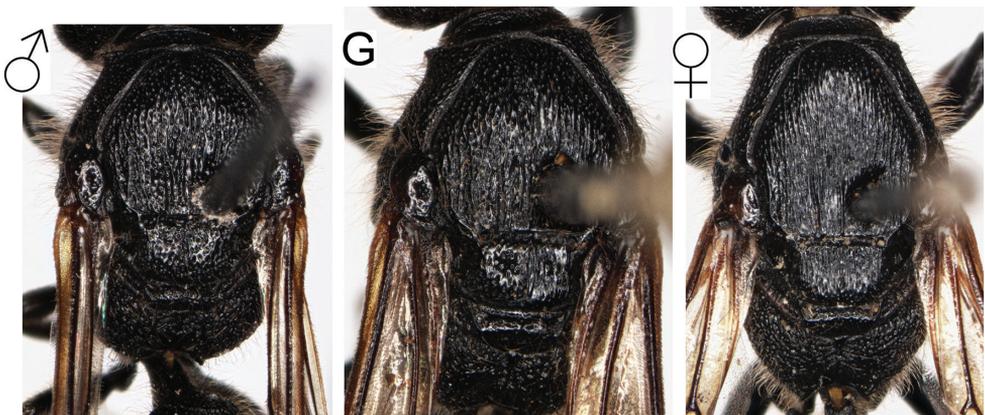


Figure 2. Mesosoma of *Discoelius dufourii*, dorsal view: ♂ male; G gynandromorph; ♀ female.

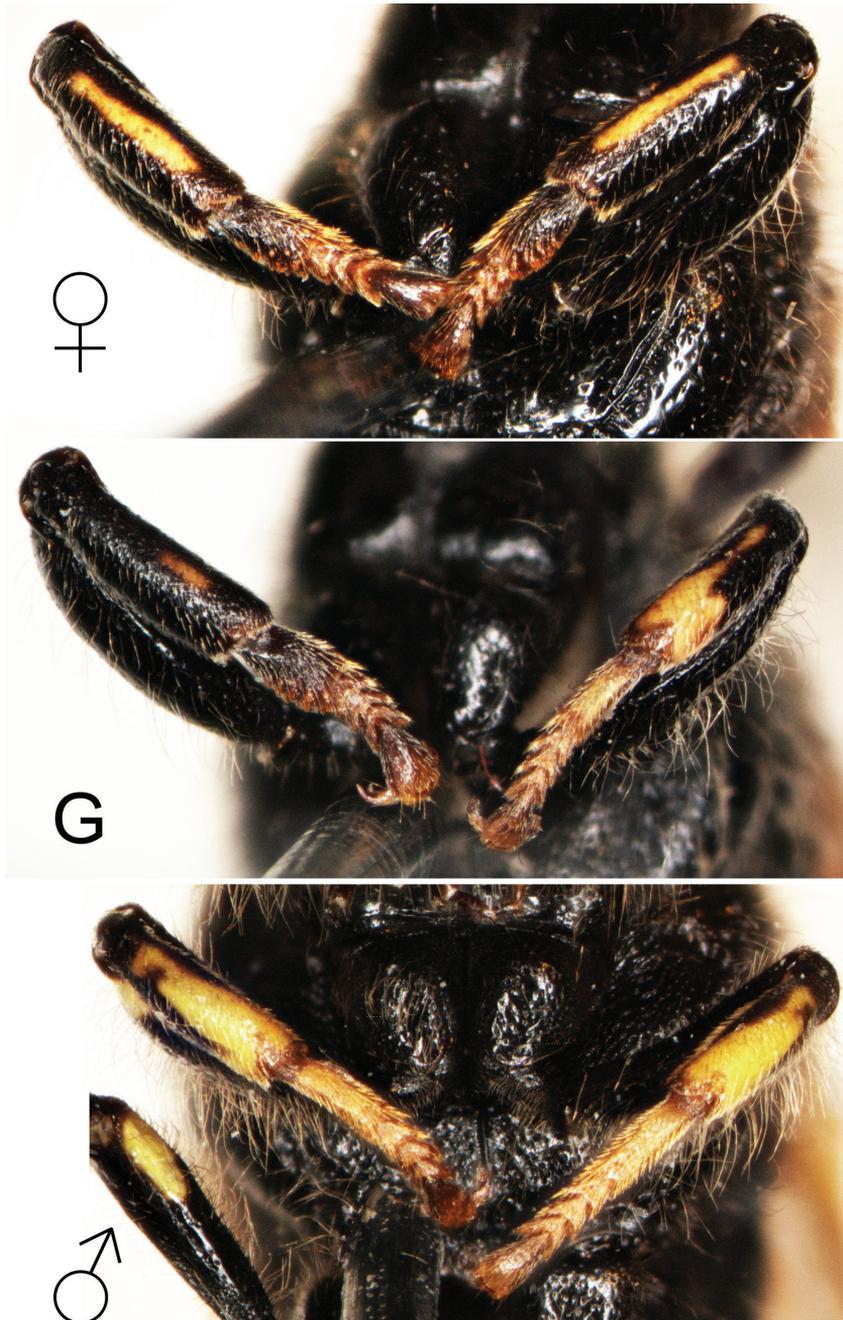


Figure 3. Fore legs of *Discoelius dufourii*, antero-ventral view: ♀ female; G gynandromorph; ♂ male.

ings on the fore and mid tibiae. The left fore and mid tarsi were slimmer and longer, and of a paler colouration than the right ones; the right fore basitarsus was 1.4 times as long as wide, the left fore basitarsus was 2.9 times as long as wide (Fig. 3G); the right mid basitarsus was 2.8 times as long as wide, the left mid basitarsus was 4.1 times as long as wide.

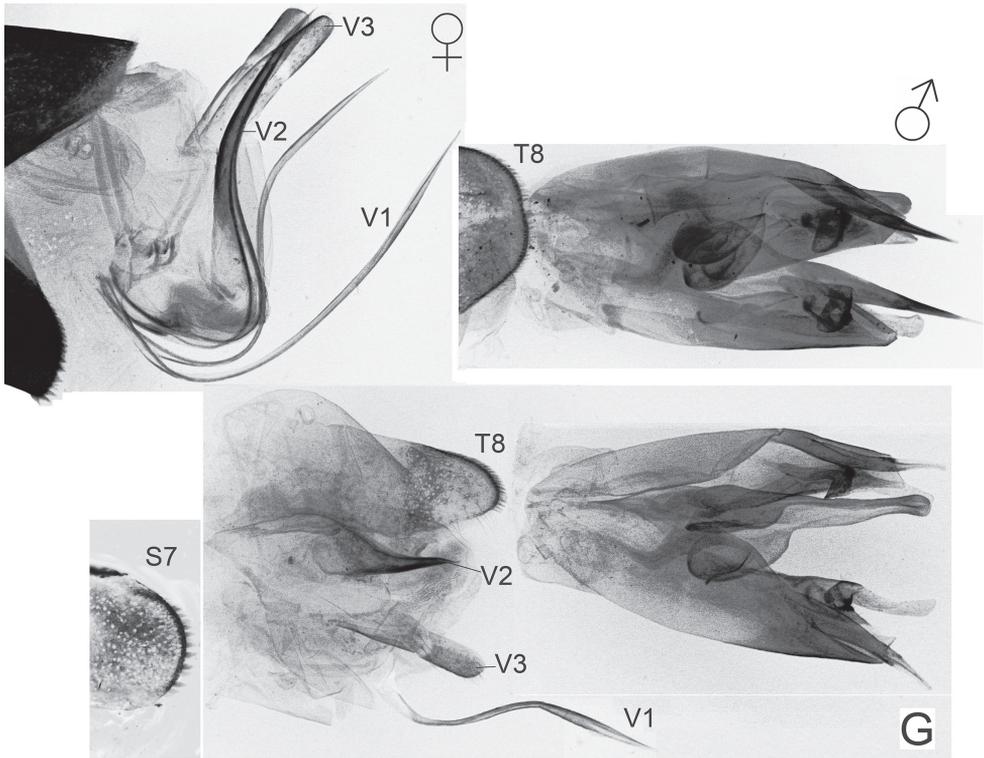


Figure 4. Genitalia of *Discoelius dufourii*, lateral (left side) view: ♀ female; ♂ male; **G** gynandromorph; **T** tergite; **S** sternite; **V** valvula.

The metasoma was nearly symmetrical; an uneven narrow trace of yellow submarginal band on the 3rd tergum, more commonly observed in males, was present on the left side only.

The apical margin of the terminal segment was slightly asymmetrical. The hidden terminal structures included an asymmetrical sclerite with the right side similar to the 7th metasomal (8th abdominal) tergum of a male (Fig. 4G: T8) and the adjacent left side looking like an underdeveloped 2nd valvula of a female sting. The left 1st valvula of the sting was also developed, though its basal structures were a little sclerotised, membranous; posterad of this asymmetrical “half-sting”-like structure, there was a symmetrical male genital capsula present.

The specimen may be considered a predominantly bilateral gynandromorph with half of a female sting and a complete male genital structure.

Behavioural interactions

A typical mating behaviour of *Discoelius dufourii* in experimental conditions consisted of the premounting, mounting and postmounting phases. Mounting occurred in 53 of 59 experiments; it included copulation in 24 observations. The premounting phase (duration 5.83 ± 0.02 minutes, here and hereafter mean \pm SE) included a visual rec-

ognition of the mate and/or chemical recognition of mate traces on the substrate, followed by substrate antennations, with subsequent demonstrations (jumping and plopping), attempts of the male to mount the female and a more or less aggressive rejecting behaviour of the female. In cases of copulation present, the mounting phase included the precopulatory (before insertion of the male genitalia; duration 0.46 ± 0.05 minutes) and the copulatory (duration 0.23 ± 0.08 minutes) sub-phases. When mounted, the male continued the courtship by an antennation of female antennae, presumably spreading a pheromone from the cuticular glands of his tyloidea, present on terminal flagellomeres, along female flagella. The postmounting phase (duration 1.03 ± 0.11 minutes) was observed in 21 of 24 copulations. During the postmounting phase, the female demonstrated a more or less active rejecting behaviour by kicking the male with her hind legs and wriggling her abdomen.

During the mating experiment, the gynandromorph demonstrated a higher intensity of behavioural activities than a female. It approached the female six times. In two instances, the approach was followed by female head and abdomen antennations, while in one instance, it touched the female's body with its mandibles multiple times. In one instance, the gynandromorph attempted to mount the female from flight. The mounting attempt consisted of a brief (approximately 2 sec) grasping of the female's pronotum with forelegs. The female displayed the plopping behaviour, it avoided the gynandromorph by walking away or flying away. In the case when the gynandromorph approached her "face to face", the female reacted with an apparent aggression display. This behaviour started with a motionless position with antennae obliquely straightened forward and to the sides, and a raising of the anterior part of the mesosoma ("alert posture"), and was followed by a biting of the gynandromorph with mandibles.

Of the six behavioural elements, aggression and plopping were prevalent in females of both *D. dufourii* and *D. zonalis*. The probability and frequency of aggression demonstration in females were significantly higher than those in males (Fig. 5d; Table 1). For the plopping, the difference between sexes in frequency was significant in both species, however the probability of occurrence was significantly higher in females of *D. zonalis* only (Fig. 5f; Table 1). The aggression appeared to occur with a greater frequency and a higher probability in both sexes of *D. zonalis* compared to those of *D. dufourii*, sug-

Table 1. The probabilities and frequencies of occurrence of selected sex-specific behavioural elements in *Discoelius dufourii*, *D. zonalis* and the *Discoelius dufourii* gynandromorph. Significant ($p < 0.05$) values in bold; $N = 59$ for *D. dufourii* and $N = 116$ for *D. zonalis*.

Behaviour-relevant element	Sex-specific prevalence	Occurrence in the gynandromorph	Sex differences in frequency, Wilcoxon signed rank test with continuity correction, p value		Sex differences in probability of occurrence, Fisher exact test, p value	
			<i>D. dufourii</i>	<i>D. zonalis</i>	<i>D. dufourii</i>	<i>D. zonalis</i>
approaching	male	yes	0.000	0.001	0.000	0.014
substrate antennation	male	no	0.001	0.000	0.001	0.000
mate body antennation	male	yes	0.174	0.004	0.244	0.003
aggression	female	no	0.003	0.000	0.002	0.000
jumping	male	no	0.560	0.000	0.836	0.006
plopping	female	yes	0.002	0.008	0.432	0.055

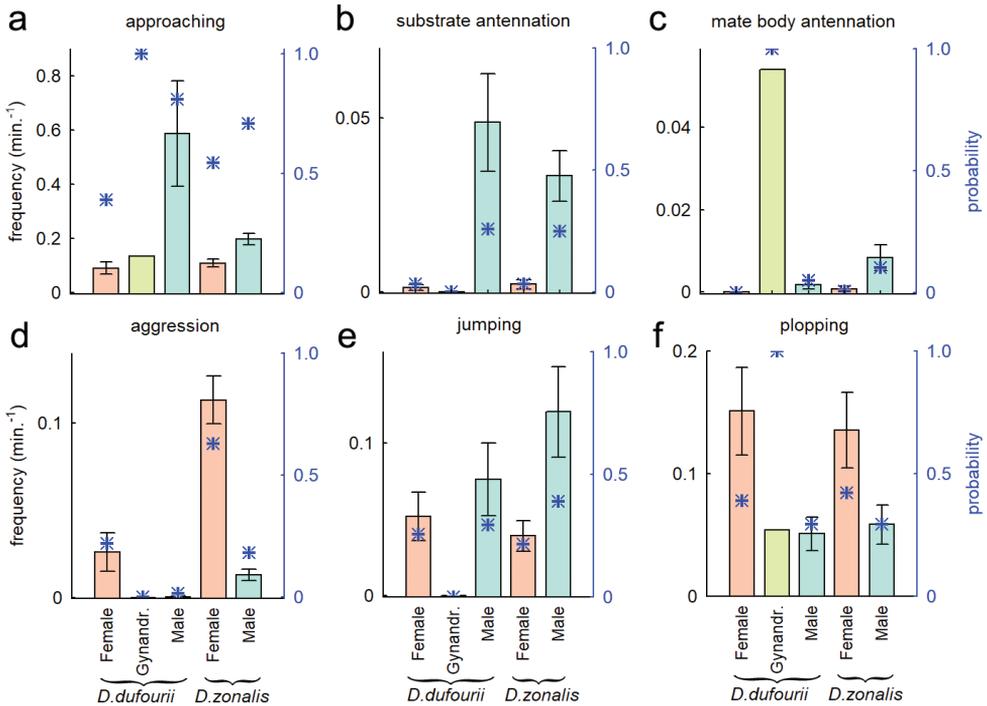


Figure 5. Mean frequency in minutes⁻¹ (bars, left y axis) and probability of presence (blue asterisks, right y axis) of six behavioural elements (a–f) of *Discoelius* females (rose), males (azure) and the gynandromorph (lime green) in mating experiments.

gesting an interspecific difference in the amplitude of this behavioural element. The gynandromorph did not demonstrate any aggression and showed a similar frequency of plopping to the conspecific males, thus behaviourally strikingly resembling a male and contrasting to a female (Fig. 5d, f).

The other four behavioural elements had significantly higher occurrence probability and frequency in *D. zonalis* males in comparison to females (Fig. 5a–c, e; Table 1). In *D. dufourii*, however, only the difference of approaching and substrate antennation between the sexes was significant (Fig. 5a, b; Table 1). The gynandromorph of *D. dufourii* demonstrated a relatively very high frequency of the mate body antennation, typical of male mating behaviour in *Discoelius* (Fig. 5c). However, it did not perform any substrate antennation and jumping, and it approached the female much less frequently in comparison to the conspecific males (Fig. 5b, e).

Gynandromorph's egg fertilization probability

The analysis demonstrated that all four brood cell parameters, namely the nesting cavity diameter, the depth of the brood cell bottom, the serial number of the cell by building sequence and the serial number of the cell counting from the nest entrance, significantly affected the decision of the *Discoelius* mother wasp about the brood sex.

Table 2. Estimated coefficients of logit models describing the probability of egg fertilization (female brood) in brood cells of *Discoelius dufourii* (model log-likelihood -222.7) and *D. zonalis* (model log-likelihood -677.6).

Brood cell parameter	<i>D. dufourii</i>		<i>D. zonalis</i>	
	Estimate±SE	<i>p</i>	Estimate±SE	<i>p</i>
Intercept	-5.22 ± 0.90	0.000	-6.44 ± 0.57	0.000
Diameter (mm)	0.53 ± 0.19	0.006	0.935 ± 0.097	0.000
Depth of cell bottom (mm)	0.013 ± 0.004	0.000	0.006 ± 0.002	0.008
No of cell from the bottom	-0.378 ± 0.089	0.000	-0.538 ± 0.053	0.000
No of cell from the entrance	0.606 ± 0.086	0.000	0.233 ± 0.035	0.000

Table 3. Coefficients of classification functions for egg fertilization (female brood) in brood cells of *Discoelius dufourii* (correct classification: 79.1%; female brood 75.4%, male brood 82.4%) and *D. zonalis* (correct classification: 79.8%; female brood 61.9%, male brood 88.8%).

Brood cell parameter	<i>D. dufourii</i>			<i>D. zonalis</i>		
	Estimate	<i>F</i>	<i>p</i>	Estimate	<i>F</i>	<i>p</i>
Intercept	-5.632			-7.325		
Diameter (mm)	0.532	7.72	0.006	0.936	111.2	0.000
Depth of cell bottom (mm)	0.0161	18.7	0.000	0.009	17.3	0.000
No of cell from the bottom	-0.356	19.8	0.000	-0.423	110.9	0.000
No of cell from the entrance	0.574	64.2	0.000	0.262	54.1	0.000

We obtained logit models of sufficient predictive power (Table 2) and classification functions well separating brood sex (Table 3) for each species.

The gynandromorph specimen emerged from the 3rd brood cell of a 4-cell linear nest, where the 1st (the deepest, innermost) cell contained a female brood, the 2nd cell contained a dead larva of unknown sex and the 4th cell contained a male brood. According to the logit model (log-likelihood -222.7), the predicted probability of the fertilization of the egg that had developed into the gynandromorph was 0.17 ± 0.22 , 95% CI 0.11–0.24. The posterior classification probability of the gynandromorph's egg fertilization, estimated using discriminant function analysis, was 14.7%. Consequently, the brood cell in which the gynandromorph had developed had to contain an unfertilised egg developing into a male brood with a probability of $83 \pm 22\%$ (logit regression model) or a posterior probability of 85.3% (discriminant function).

Discussion

Morphology

The bilateral gynandromorphic individual of *D. dufourii* described in this paper appears to be the first documented case of gynandromorphy in the subfamily Zethinae. There are documented cases of gynandromorphic individuals in ten species of the related vespid subfamily Eumeninae (Turrisi and Borsato 2008). Based on the head picture, an eleventh recorded eumenine gynandromorph belongs to an undescribed *Ancistrocerus* sp. A. (Buck et al. 2008: fig. C13.4).

The studied gynandromorphic specimen of *D. dufourii* represents a specific case among the gynandromorphs in two aspects. First, the bilateral differentiation of the male and female parts (left side male and right side female) was intervened with an opposite differentiation on the head capsula (but not head appendages), where the left side of the frons and clypeus were female and the right side were male (Fig. 1), as well as on the 7–8th abdominal segments, where the left side with a half-sting was female and the right side was male; the 9th segment was fully male with symmetrical genital capsula (Fig. 4). Such morphology implies a higher probability of tissue exchange between the body sides at the anterior and the posterior end of the developing embryo.

The second aspect that must inspire further research is the high (ca. 80–85%) probability that the gynandromorph had developed from an unfertilised (i.e. male) egg. From the dominating viewpoint, gynandromorphs are considered to arise typically from fertilized eggs, i.e. from female brood cells, in the case of cavity-nesting solitary wasps. The available evidence for the emergence of gynandromorphs with several different parental origins, such as the androgenetic and gynogenetic ones, potentially leading to evolutionary novelties, comes from the studies of social Hymenoptera, emphasizing the range of developmental variants that can potentially lead to an evolutionary novelty. It has been hypothesized that some of these developmental variants, for example androgenetic male production, might share the same underlying cytogenetic mechanism with gynandromorphism (Yoshizawa et al. 2009; Dobata et al. 2012; Aamidor et al. 2018). Our study implies that the gynogenetic origin of a gynandromorph is possible in solitary Hymenoptera as well. Taking into account the much lower fecundity of the solitary wasps and bees compared to social Hymenoptera, such cases, based on the successful development of an egg after a failure in meiotic division of germ cells, must be naturally very rare.

The studied gynandromorph had a smaller body size than a typical female and demonstrated a distinct size asymmetry, particularly in the appearance of the thorax (Fig. 2). This observation supports the suggestion that the development of tissues of each sex is self-determining (Cooper 1959) and confirms that, at the same amount of provision, the average relative growth rate in female tissues is higher than in male tissues, as has been observed in an eumenine wasp *Symmorphus allobrogus* (Budrienė et al. 2013).

Behaviour

Our mating experiments with *Discoelius* wasps demonstrated that approaching the mate, substrate antennation, mate body antennation and short flights (“jumping”) were male-specific behavioural elements, while aggression and plopping on the surface were female-specific behaviours, with a greater frequency and higher probability of occurrence compared with the opposite sex (Fig. 5, Table 1). The most distinctive sex-specific behavioural traits were female aggression and male substrate antennation (Fig. 5b, d). We observed interspecific differences in aggression levels between *D. dufourii* and *D. zonalis* in both sexes (Fig. 5d). *Discoelius* females possess strong mandibles used to cut and macerate leaf fragments for nest construction or in threat displays. Using their

mandibles, the females can inflict significant damage on their opponents, cutting their appendages, such as legs and antennae (AB, personal observations).

In our mating experiment, the gynandromorph showed no aggression, demonstrated a similar plopping frequency to conspecific males, intensively antennated the female's body (Fig. 5c, d, f) and attempted to mount the female. Despite the relatively low frequency of approaches to the female and the lack of substrate antennation, which are typical of males, we consider the gynandromorph's behavioural displays as predominantly male-specific and its sexual identity as male.

Among insects, gynandromorphs commonly tend to express a unisexual sex-specific behaviour, either like females or like males. The behaviour may correlate with the phenotype of the abdomen; this regularity was observed in an *Osmia ribifloris biedermannii* gynandromorph with a predominantly female body form, which was courted by normal males (Sampson et al. 2010). Other studies have suggested that gynandromorphs' sexual behaviours may be more strongly influenced by the head (brain) rather than by the abdomen (reproductive organs) (Yoshizawa et al. 2009). Under effect of sex-specific *fruitless* (*fru*) gene products, neurones of the insect brain form sexually dimorphic circuits, regulating sexual behaviours such as courtship and aggression (Watanabe 2019). Matsuo et al. (2018) have analyzed the encephalic and antennal expression patterns of the *fru* gene in a *Bombus ignitus* gynandromorph with a bilaterally dimorphic head and thorax (left side-male, right side-female) and a uniformly masculine gaster. This gynandromorph with left-side-biased expression of male-type *fru* transcripts demonstrated approaching and touching behaviour towards virgin queens, though it rarely attempted to copulate. Such behaviour has been interpreted as an outcome of the difficulty in the olfactory-dependent transition from the male-like approaching behaviour to copulation. In our case of the *D. dufourii* gynandromorph, the absence of substrate antennation contrasting with very high estimates of mate body antennation may suggest an incapability of a male-like antennal response to weak chemical cues left on the substrate by the female. However, in closer proximity, this response might be triggered by stronger chemical cues on the cuticle of the female, thus the mate antennation behaviour could be initiated.

Gynandromorphs may express behaviours that resemble normal sex-biased behavioural patterns in the wild as well. These include attraction to scent traps (Mertins and Coppel 1971; Martini et al. 1999; Giangarelli and Sofia 2011; Silveira et al. 2012), foraging for nectar or pollen (Gordh and Gulmahamad 1975; Michez et al. 2009), nesting activities (Michez et al. 2009) and defence (Silveira et al. 2012).

In some cases, gynandromorphs may express conflicting sex-specific behaviours. For instance, male-specific eucalyptol essence collecting combined with the female-specific stinging have been recorded in a gynandromorphic individual of the euglossine bee *Eulaema atleticana* (Silveira et al. 2012). However, Camargo and Gonçalves (2013) urge caution in predicting the sex-specificity of gynandromorph behaviour in context of scent attraction. Krichilsky et al. (2020) have speculated that in a gynandromorphic bee *Megalopta amoena*, a bilaterally split brain was unable to integrate conflicting sex-specific signalling in relation to the circadian rhythm. In our case of the *D. dufourii* gynandromorph, the female-like low intensity of substrate antennation, approaching to the female and jumping and, at the same time, the male-like high intensity of female

body antennation and the attempt to mount the female may be interpreted as a result of conflicting activities of the female and male parts of the brain.

Despite the predominantly male-specific mating behaviour, the gynandromorph of *D. dufourii* was not able to copulate. Opportunities of gynandromorphs to reproduce are usually limited, resulting in unviable egg production by mated individuals (Melcher 1997). However, the oviposition by a virgin gynandromorphic individual of haploid eggs, developing into males (Melcher 1997) and successful copulations of a gynandromorph with a normal queen, producing normal workers and fertile queens (Yoshizawa 2009), may occasionally happen. The ability of gynandromorphs to successful reproduction could have important consequences enabling a generation of evolutionarily novel body traits. Yang and Abouheif (2011) have proposed that mutations related to gynandromorph-like development may facilitate the evolution of new phenotypes. For instance, the deviant expression of male-like traits (e.g. absence of scopa) in a gynandromorphic bee female may be considered an evolutionary novelty associated with the evolution of brood parasitism (Wcislo et al. 2004; Fateryga et al. 2011).

Acknowledgements

A part of this study was supported by a grant from the Research Council of Lithuania (contract No S-MIP-20-23). The research was carried out using the Open Access to research infrastructure of the Nature Research Centre under the Lithuanian open access network initiative. Authors are grateful to Jos Stratford for help in the drafting of the English language.

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New species of *Hybristodryinus* Engel (Hymenoptera, Dryinidae) from mid-Cretaceous amber of northern Myanmar, with notes on their possible hosts

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Academic editor: M. Ohl | Received 19 August 2020 | Accepted 26 December 2020 | Published 25 February 2021

<http://zoobank.org/6E3EC3E6-F3EF-4AA5-9B2A-10C4D719CE19>

Citation: Olmi M, Chen H-Y, Shih C, Müller P, Capradossi L, Ren D, Perkovsky EE, Guglielmino A (2021) New species of *Hybristodryinus* Engel (Hymenoptera, Dryinidae) from mid-Cretaceous amber of northern Myanmar, with notes on their possible hosts. Journal of Hymenoptera Research 81: 43–55. <https://doi.org/10.3897/jhr.81.57792>

Abstract

Two new species of *Hybristodryinus* Engel, 2005, are described from mid-Cretaceous amber of northern Myanmar: *H. castaneus* **sp. nov.** (based on one male) and *H. zaifui* **sp. nov.** (based on one female). Keys to the females and males of *Hybristodryinus* species are modified to include the two new taxa. A syninclusion, a nymph of Cixitettiginae (Perforissidae), present in the same amber piece containing *H. zaifui*, is studied. This syninclusion, together with the presence of Antennal Dorsal Organs (ADOs) in *Hybristodryinus*, suggests that Perforissidae are possible hosts of *Hybristodryinus*.

Keywords

Chrysoidea, Cixitettiginae, host, keys, Perforissidae, taxonomy

Introduction

Dryinidae (Hymenoptera: Chrysidoidea) are parasitoids and often also predators of leafhoppers, planthoppers and treehoppers (Hemiptera, Auchenorrhyncha) (Guglielmino et al. 2013). They comprise 16 subfamilies, 50 genera and almost 1900 species worldwide (Olmi et al. 2020).

Mid-Cretaceous Kachin (Myanmar) amber (about 99 Ma) is the famous Burmese amber (Lin et al. 2019; Cao et al. 2020; Zhao et al. 2020), different from the recently discovered Campanian Tilin amber (Zheng et al. 2018) and Albian Hkamti amber (Xing and Qiu 2020). Dryinidae from Burmese amber are not common. Lists of the species known from this type of amber, reported by Perkovsky et al. (2019, 2020a) and Tribull et al. (2020), include the following subfamilies and genera: Anteoninae: *Burmanteon* Engel, 2003 (one species); Burmadryininae: *Burmadryinus* Olmi, Xu & Guglielmino, 2014 (one species); Dryininae: *Dryinus* Latreille, 1804 (13 species); *Hybristodryinus* Engel, 2005 (14 species); *Pseudodryinus* Olmi, 1991 (one species); Palaeoanteoninae: *Palaeoanteon* Olmi, 2000 (one species); Raptodryininae: *Raptodryinus* Olmi, Perkovsky, Martynova, Contarini, Bückle & Guglielmino, 2020 (one species).

The extinct genus *Hybristodryinus* is known only from Burmese amber (Perkovsky et al. 2019). With 14 described species, it is the most diverse genus of pincer wasps from that type of amber (Perkovsky et al. 2020a; Tribull et al. 2020).

In this study, we examined a small collection of dryinids from Burmese amber and recognized two additional new species, described below. In addition, we studied a syninclusion, represented by a nymph of a possible host of *Hybristodryinus*.

Material and methods

The descriptions follow the terminology used by Olmi et al. (2019) and Perkovsky et al. (2019). The measurements reported are relative, except for the body length (head to abdominal tip, without the antennae). In the descriptions, POL is the distance between the inner edges of the lateral ocelli; OL is the distance between the inner edges of a lateral ocellus and the median ocellus; OOL is the distance from the outer edge of a lateral ocellus to the compound eye.

The term “metapectal-propodeal complex” is here used in the sense of Kawada et al. (2015). It corresponds to the term “propodeum” sensu Olmi (1984).

In all monographs on Dryinidae (Olmi 1984; Xu et al. 2013; Olmi and Virla 2014; Olmi and Xu 2015; Olmi et al. 2019), different names of the cells and veins of the fore wing were used. They are here used in the sense of Azevedo et al. (2018), and Perkovsky et al. (2019). The correspondence between old and new names is the following (the first name is the old name): median cell = radial cell (R); submedian cell = first cubital cell (1Cu); marginal cell = second radial 1 cell (2R1); stigmal vein = second radial cross & radial sector (2r-rs&Rs); metacarp = poststigmal abscissa of radial 1 (PostabR1). In the text, cells and veins are named by their respective abbreviations, including costal cell (C).

The term “ADOs” (= Antennal Dorsal Organs) is here used in the sense of Riolo et al. (2016). It corresponds to the term “rhinaria” sensu Olmi (1984) and Xu et al. (2013). According to Riolo et al. (2016), ADOs are sensory structures that might mediate the antennal responses to vibratory stimuli. As far as we know, they are present only in the antennae of dryinid females attacking Fulgoromorpha (Olmi 1984). Antennae without ADOs are present mainly in species that are parasitoids of Cicadomorpha.

Because of the nature of the fossils and distortions sometimes caused by artifacts, the word “apparently” is used when describing characters for which there is slight uncertainty about the true condition or where a false impression is obtained at first sight.

The types of all fossil species of *Hybristodryinus* were examined. The type material of the new taxa studied in this paper is deposited in the collections of the Key Lab of Insect Evolution and Environmental Changes, the College of Life Sciences, Capital Normal University, Beijing, China (CNUB) and the Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy (DAF).

Results

Generic placement

The new species described in this paper have been placed in the genus *Hybristodryinus*, because they fit the generic diagnosis reported below.

Genus *Hybristodryinus* Engel, 2005

Hybristodryinus Engel 2005: 486. Type species: *Hybristodryinus resinicolus* Engel 2005, by monotypy and original designation.

Diagnosis of the genus. Female: Macropterous; occipital carina complete; mandible quadridentate, with teeth becoming regularly progressively larger from dorsal to ventral tooth; palpal formula 6/3; antenna without tufts of long hairs; antennal ADOs present; disc of metapectal-propodeal complex with posterior corners strongly projected posteriorly; fore wing with three cells enclosed by pigmented veins (C, R, 1Cu); chela with rudimentary claw; protarsomere 5 less than twice as broad as enlarged claw; enlarged claw shorter than protibia; tibial spurs 1/1/1 or 1/1/2. Male: Macropterous; antenna with scape much broader than pedicel; palpal formula 6/3; occipital carina complete; mandible with four irregular teeth; epicnemium visible, because lateral regions of prothorax not continuous with mesopleura; mesopleuron protruding laterally; fore wing with three cells enclosed by pigmented veins (C, R, 1Cu), 2r-rs&Rs vein and pterostigma; fore wing with PostabR1 slightly shorter than pterostigma; tibial spurs 1/1/2.

***Hybristodryinus castaneus* Olmi, Guglielmino & Chen, sp. nov.**

<http://zoobank.org/5CA10E8A-2DE2-4FD6-B4C4-9D3F580BA92F>

Figure 1

Diagnosis. Male: macropterous, with antennomere 9 about six times as long as broad (Fig. 1E); face without long deep longitudinal furrow (Fig. 1D); posterior margin of vertex not deeply excavated medially (Fig. 1C); lateral ocelli not touching occipital carina; notauli complete, posteriorly separated (Fig. 1C).

Description. Male. Macropterous (Fig. 1A, B); length 1.4 mm. Completely dark brown. Antenna filiform, about as long as body, with setae very short. Scape about as broad as pedicel. Antennomeres in following proportions: 5:4:8:9:9:9:8:8:6:7. Antennomere 9 about six times as long as broad (Fig. 1E). Head with sculpture not visible; face without long deep median longitudinal furrow; mandible with teeth not visible; frontal line not visible; occipital carina complete, laterally not reaching eyes; ocellar ratio: POL:OL:OOL:OPL = 1:1:2:1; temple more than twice as long as OOL (5:2); greatest breadth of lateral ocellus as long as OOL; posterior margin of vertex not excavated medially (Fig. 1C); occiput deeply excavated. Palpal formula 6/3. Mesosoma much longer than head (30:10) and metasoma (30:20). Pronotum with lateral regions not protruding. Mesoscutum shiny, with sculpture not visible, slightly shorter than head (9:10), much longer than mesoscutellum (9:5). Notauli complete, posteriorly separated (Fig. 1C); minimum distance between notauli longer than OOL (3:2). Mesoscutellum shiny, with sculpture not visible. Metanotum and metapectal-propodeal complex not visible. Epicnemium not visible. Mesopleuron not visible. Fore wing hyaline, without dark transverse bands (Fig. 1F). Stigmal vein (2r-rs&Rs) with angle between proximal and distal parts, with distal part much longer than pterostigma (12:8). Second radial 1 cell (2R1) open. Pterostigma much longer than broad (9:2). Metacarpus (PostabR1) slightly shorter than pterostigma (7:9). Fore leg ratio: ? (coxa not visible, because it is hidden by an air bubble): 5 (trochanter): 16 (femur): 12 (tibia): 8 (protarsomere 1): 3 (protarsomere 2): 3 (protarsomere 3): 2 (protarsomere 4): 4 (protarsomere 5). Protrochanter more than four times as long as broad (5:1). Mid leg ratio: 7 (coxa): 4 (trochanter): 17 (femur): 15 (tibia): 9 (mesotarsomere 1): 3 (mesotarsomere 2): 2 (mesotarsomere 3): 1.5 (mesotarsomere 4): 3 (mesotarsomere 5). Mesotrochanter more than twice as long as broad (4:1.5). Hind leg ratio: 8 (coxa): 4 (trochanter): 17 (femur): 21 (tibia): 10 (metatarsomere 1): 4 (metatarsomere 2): 3 (metatarsomere 3): 2 (metatarsomere 4): 3 (metatarsomere 5). Tibial spurs 1/1/2.

Female. Unknown.

Material examined. Holotype: male (DAF3869), MYANMAR: specimen in mid-Cretaceous Burmese amber (about 99 Ma). Obtained from a mine situated in Northern Myanmar, Kachin State, Tanai Township, Hukawng Valley, SW of Tanai City (DAF).

Etymology. The species is named *castaneus* (Latin adjective meaning “brown”) because of its dark brown colour.

Remarks. *H. castaneus* is similar to *H. konbaung* Perkovsky, Olmi, Müller & Martynova, 2019, known from Burmese amber, for the following characters: face



Figure 1. *Hybristodryinus castaneus* sp. nov., male, holotype **A** habitus, dorsal view **B** habitus, ventral view **C** head and mesosoma, dorsal view **D** head and mesosoma, ventral view **E** antenna **F** fore wing.

without a long deep longitudinal furrow, lateral ocelli not touching the occipital carina, posterior margin of the vertex not excised medially, notauli complete and separated posteriorly. However, the antenna of *H. castaneus* is slenderer, with antennomere 9 about six times as long as broad (less slender in *H. konbaung*, with antennomere 9 about twice as long as broad). Perkovsky et al. (2019) indicate “pronotum with lateral regions protruding” among the characters of the generic diagnosis. However, this character is not visible in all known males (as in *H. pyu* Perkovsky, Olmi, Müller & Martynova, 2019) and it is not present in *H. castaneus* sp. nov. (see description above). Except this character, *H. castaneus* meets with all other generic

diagnosis characters of *Hybristodryinus*. Therefore, we update the diagnosis of the genus by excluding this character.

***Hybristodryinus zaifui* Chen, Olmi & Perkovsky, sp. nov.**

<http://zoobank.org/6455A84B-B0A6-42E6-818E-F7B4F6586BE8>

Figures 2, 3

Diagnosis. Female: macropterous, with disc of pronotum shaped as an isosceles triangle; notauli incomplete, reaching about $0.7 \times$ length of mesoscutum (Fig. 2C).

Description. Female. Macropterous (Fig. 2A, B); length 1.8 mm. Apparently completely brown-testaceous. Antenna clavate, apparently almost hairless; antennomeres in following proportions: 6:4:5:4:4:3.5:3.5:3:3:4; antenna about three times as long as head (37:12), reaching metapectal-propodeal disc. ADOs apparently present in antennomeres 6–10 (Fig. 2E). Flagellomeres longer than wide; antennomere 9 about three times as long as broad. Head excavated, apparently unsculptured; clypeus not visible; mandible not visible; face slightly concave; occipital carina complete (Fig. 2C); vertex without two longitudinal keels connecting lateral ocelli to occipital carina; occiput deeply excavated; eye normally bulging; ocellar ratio: POL:OL:OOL:OPL = 1:2:2:1; temple about twice as long as OOL (4:2); greatest breadth of lateral ocellus longer than POL (2:1); frontal line apparently complete; palpal formula probably 6/3 (labial palpus not distinct). Pronotum crossed by anterior deep transverse impression between anterior collar and disc; disc humped, apparently isosceles triangle shaped, not deeply medially longitudinally excavated; posterior collar absent; anterior surface of pronotum not sculptured by longitudinal striae; sculpture of disc not distinct; pronotum shorter than head (6:12); anterior collar of pronotum much shorter than disc (2:4); pronotal tubercle reaching tegula. Epicnemium not visible. Mesoscutum apparently unsculptured, longer than pronotum (10:6). Notauli incomplete, reaching about $0.7 \times$ length of mesoscutum (Fig. 2C). Mesoscutellum apparently unsculptured, shorter than mesoscutum (4:10). Metanotum shorter than mesoscutellum (2:4), with sculpture indistinct. Metapectal-propodeal complex shorter than mesoscutum (5:10), with disc reticulate rugose, with areolae very broad, with posterior corners lamina shaped, strongly projected posteriorly (Fig. 2D); propodeal declivity not visible. Fore wing (Fig. 2F) apparently hyaline, not darkened, with the usual venation of Dryiniinae; pterostigma much longer than broad (10:2); second radial cell (2R1) open; stigmal vein (2r-rs&Rs) regularly curved; fore wing with usual three basal cells (C, R, 1Cu) clearly enclosed by pigmented veins. Hind wing not visible. Fore leg ratio: 7 (coxa): 5 (trochanter): 15 (femur): 9 (tibia): ? (tarsus only partly visible); enlarged claw (Fig. 3A, B) slightly shorter than protarsomere 5; protrochanter very slender and long, with long and slender proximal stalk, broadened after half-way; protrochanter less than seven times as long as broad (5:1); protarsomere 3 produced into hook; rudimentary claw not visible; arolium not visible; enlarged claw apparently with one subapical tooth, with one row of lamellae (number of lamellae uncertain). Protarsomere 5 (Fig. 3B) with inner side not visible; lamellae of protarsomere 5 not visible. Mid leg

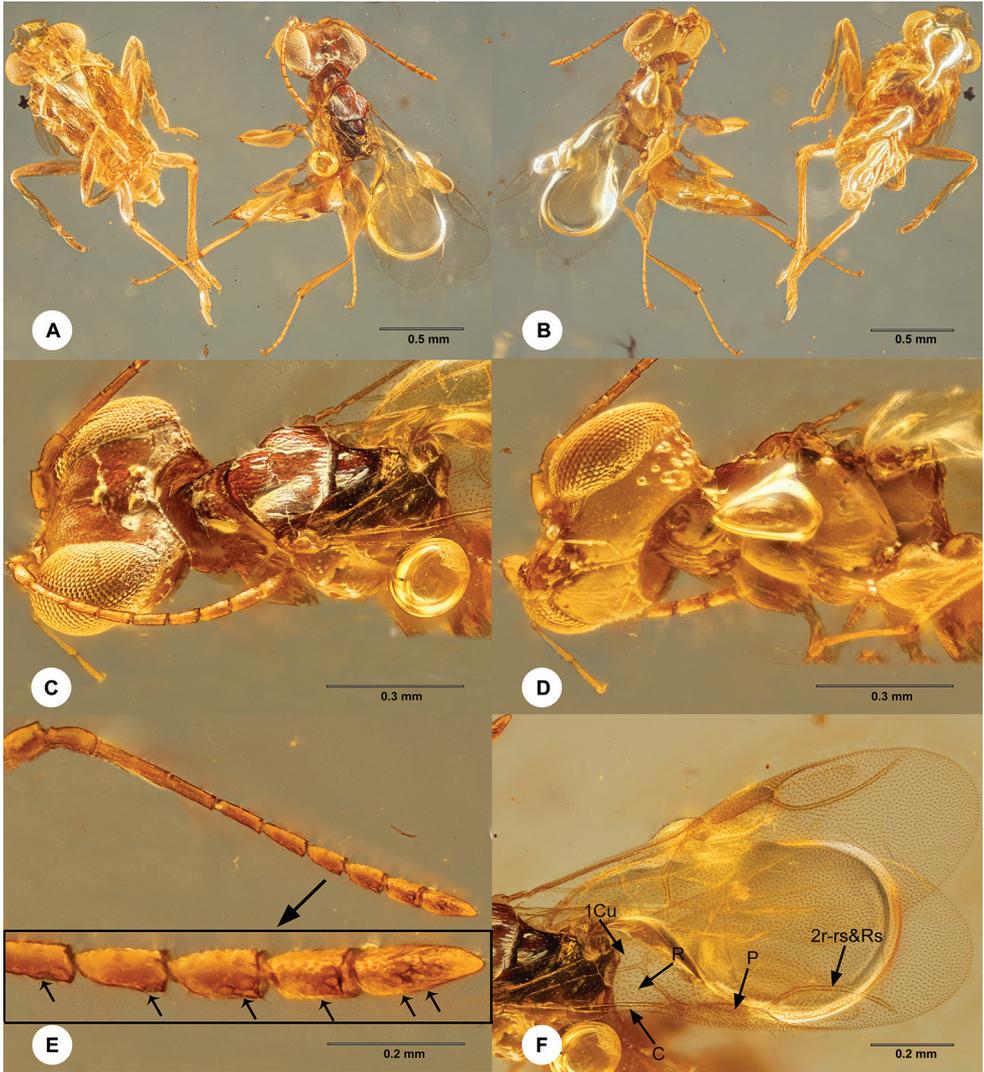


Figure 2. **A** right, *Hybristodryinus zaifui* sp. nov., female, holotype, habitus, latero-dorsal view; left, nymph of Perforissidae (Perforissidae), ventral view **B** left, *Hybristodryinus zaifui* sp. nov., female, holotype, habitus, latero-ventral view; right, nymph of Cixitettiginae, habitus, dorsal view **C, D** *Hybristodryinus zaifui* sp. nov., female, holotype: **C** head and mesosoma, dorsal view **D** head and mesosoma, latero-ventral view **E** antenna, arrows indicate the ADOs in the antennomeres 6–10 (one ADO per antennomere, except two ADOs in antennomere 10) **F** wings, 1Cu = cubital 1 cell, 2r-rs&Rs = stigmal vein, C = costal cell, P = pterostigma, R = radial cell.

ratio: 5 (coxa): 3 (trochanter): 13 (femur): 12 (tibia): 16 (tarsus). Mid trochanter short and broad. Hind leg ratio: 6 (coxa): 3 (trochanter): 18 (femur): 17 (tibia): 21 (tarsus). Metasomal petiole very short. Metasoma (excluding sting (Fig. 3C)) longer than mesosoma (39:27). Tibial spurs 1/1/2.

Male. Unknown.

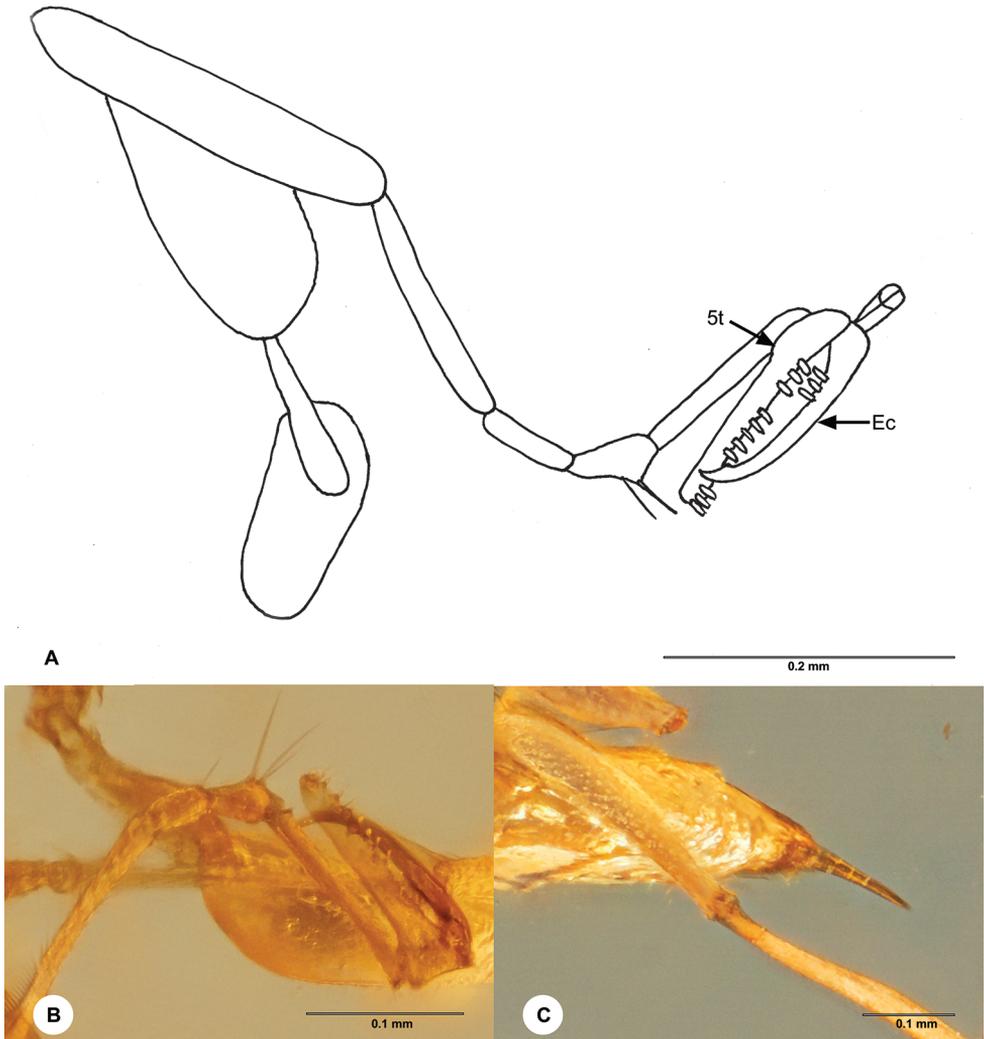


Figure 3. *Hybristodryinus zaifui* sp. nov., female, holotype **A** fore leg, 5t = protarsomere 5, Ec = enlarged claw **B** claw **C** sting.

Material examined. Holotype: female (CNN-HYM-MA 2017087), MYANMAR: specimen in mid-Cretaceous Burmese amber (about 99 Ma). Obtained from a mine situated in Northern Myanmar, Kachin State, Tanai Township, Hukawng Valley, SW of Tanai City (CNUB).

Etymology. The new species is named after the late Prof. Zaifu Xu (South China Agricultural University, Guangzhou, Guangdong, China), for his important contribution to the study of Chinese Dryinidae.

Remarks. For its isosceles triangle shaped pronotal disc, *H. zaifui* is similar to *H. nalae* Perkovsky, Olmi, Müller & Martynova, 2019, *H. concavifrons* Perkovsky, Olmi, Müller & Martynova, 2019, *H. resinicolus* Engel, 2005 and *H. ligulatus* Perko-

vsky, Olmi, Müller & Martynova, 2019. However, *H. zaifui* has incomplete notauli (Fig. 2C), reaching about $0.7 \times$ length of mesoscutum, whereas in the other species the notauli are complete.

Partial keys to species of *Hybristodryinus**

Females

- 1 Disc of pronotum isosceles triangle shaped (fig. 2A in Perkovsky et al. 2019).....2
 – Disc of pronotum with normal shape, not isosceles triangle shaped (fig. 8A in Perkovsky et al. 2019) 6
 2 Notauli incomplete, reaching about $0.7 \times$ length of mesoscutum (Fig. 2C)...
 ***H. zaifui* sp. nov.**
 – Notauli complete, posteriorly separated (fig. 2A in Perkovsky et al. 2019)..... 3
 3 Pronotum with disc deeply excavated longitudinally
 ***H. nalae* Perkovsky, Olmi, Müller & Martynova**
 – Pronotum with disc not deeply excavated longitudinally 4
 4 Enlarged claw with two subapical teeth.....
 ***H. concavifrons* Perkovsky, Olmi, Müller & Martynova**
 – Enlarged claw without subapical teeth (fig. 7C in Perkovsky et al. 2019) 5
 5 Enlarged claw with distal apex sharp ***H. resinicolus* Engel**
 – Enlarged claw with distal apex spoon-shaped (fig. 7C in Perkovsky et al. 2019) ***H. ligulatus* Perkovsky, Olmi, Müller & Martynova**

Males

- 2 Notauli incomplete, reaching about $0.5-0.9 \times$ length of mesoscutum (fig. 5A in Perkovsky et al. 2019) 3
 – Notauli complete, posteriorly separated (fig. 10A in Perkovsky et al. 2019)..... 4
 3 Notauli reaching about $0.5 \times$ length of mesoscutum
 ***H. kayin* Perkovsky, Olmi, Müller & Martynova**
 – Notauli reaching about $0.9 \times$ length of mesoscutum
 ***H. karen* Perkovsky, Olmi, Müller & Martynova**
 4 Lateral ocelli touching occipital carina (fig. 10A in Perkovsky et al. 2019)
 ***H. pyu* Perkovsky, Olmi, Müller & Martynova**
 – Lateral ocelli not touching occipital carina 5
 5 Antenna slenderer, with antennomere 9 about six times as long as broad (Fig. 1E) ***H. castaneus* sp. nov.**
 – Antenna less slender, with antennomere 9 about twice as long as broad
 ***H. konbaung* Perkovsky, Olmi, Müller & Martynova**

* Modified from Perkovsky et al. 2019.

Discussion

Following the descriptions of the above two new taxa, the number of known *Hybristodryinus* species has increased from 14 to 16 (Perkovsky et al. 2019; Tribull et al. 2020; present paper), indicating that this extinct genus of Dryininae is the most diverse from Burmese amber, based on the known records. However, the main problem of this genus is the significant sexual dimorphism between the male and the female, so that it is impossible to associate the opposite sexes based on morphologic characters. This extreme sexual dimorphism is common to almost all pincer wasps (except the subfamily Aphelopinae, whose females are often similar to males, so that the association of the opposite sexes is less difficult). Males and females of extant species can be associated by rearing or DNA analysis, which apparently are not applicable to species in amber. Although males and females of this genus have been assigned to different new species, some of them are possibly the opposite sexes of one species. Therefore, currently there are two separate keys for *Hybristodryinus* species, one for females and one for males.

In the same subfamily Dryininae, *Dryinus* (13 species, see Martynova et al. 2020) is another relatively diverse genus in Burmese amber. However, *Dryinus* is an extant genus and has been reported not only from Burmese amber, but also from Priabonian Baltic, Scandinavian and Rovno amber (nine species, see Martynova et al. 2020; Perkovsky et al. 2020b), amber from upper Cenomanian Taimyr (Nizhnyaya Agapa River, Siberia, one species), Campanian Medicine Hat (Canada, one species) and Middle Miocene (Mexico, one species; Dominican Republic, nine species) (Martynova et al. 2020). In conclusion, there are 34 species of *Dryinus* reported from amber, much more than those of *Hybristodryinus*.

The hosts of *Hybristodryinus* are unknown, whereas the extant *Dryinus* are known to parasitize and prey on nymphs and adults of many Fulgoromorpha families: Acanaloniidae, Cixiidae, Dictyopharidae, Flatidae, Fulgoridae, Issidae, Lophopidae, Ricaniidae and Tropiduchidae (Olmi 1999; Guglielmino et al. 2013).

In Fig. 2A, B, *H. zaifui* is close to a nymph of unidentified Cixitettiginae (Hemiptera, Auchenorrhyncha, Perforissidae), a subfamily of extinct planthoppers known from Cretaceous amber (Barremian Lebanese, Barremian Jordanian, Albian Spanish, Cenomanian Burmese, Santonian Taimyr) and Aptian Bon Tsaagan Marl (Shcherbakov 2007; Petrulevicius et al. 2014; Rasnitsyn et al. 2016; Zhang et al. 2017; Perkovsky and Vasilenko 2019; Luo et al. 2020). Two genera and three species of the subfamily Cixitettiginae were described from Burmese amber (Luo et al. 2020); additional new taxa were reported, but not named (Shcherbakov 2007).

In *Hybristodryinus*, the antenna has the ADOs, which are sensorial structures present in dryinid females parasitizing Fulgoromorpha (Martynova et al. 2020). Perforissidae belong to Fulgoromorpha and therefore they could be hosts of *Hybristodryinus*. As mentioned previously, in mid-Cretaceous Burmese amber the most common dryinids were species of *Dryinus* and *Hybristodryinus*, both with antennal ADOs (Perkovsky et al. 2019; Martynova et al. 2020). *Hybristodryinus* is an extinct genus, whereas *Dryinus* is not extinct. The morphology of these two genera is similar (differences il-

illustrated in Martynova et al. 2020 and Perkovsky et al. 2019) and cannot explain why one genus is extinct and the other is not. It could be hypothesized that *Hybristodryinus* is extinct as their hosts became extinct (Perkovsky et al. 2019). From this point of view, as Perforissidae are extinct planthoppers, they are perfect as one of the hosts of *Hybristodryinus*. Finding of perforissids with a thylacium of dryinids could strengthen the above hypothesis.

The above conclusion contrasts with the hypothesis proposed by Perkovsky et al. (2019) and Martynova et al. (2020), who asserted that Perforissidae are less likely hosts of *Hybristodryinus*, because they are well known from *Baeomorpha* realm (see Perkovsky and Vasilenko 2019 and references therein), where *Hybristodryinus* is unknown, and are scarce from Burmese amber. However, already two genera and three species of Cixitettiginae (Perforissidae), were described from Burmese amber (Luo et al. 2020), indicating that Perforissidae from Burmese amber were more diverse than expected.

Acknowledgments

The authors are grateful to Jacek Szwedo (Laboratory of Evolutionary Entomology, Department of Invertebrate Zoology and Parasitology, Faculty of Biology, University of Gdańsk, Poland) and Dmitry Shcherbakov (A. A. Borissiak Paleontological Institute, Russian Academy of Sciences, Moscow, Russia) for the identification at family and subfamily levels of the nymph of Cixitettiginae (Perforissidae) photographed close to *Hybristodryinus zaifui* in Fig. 2A, B.

This research was carried out in the frame of the MIUR (Italian Ministry for Education, University and Research) initiative “Department of excellence” (Law 232/2016).

Dong Ren was supported by the National Natural Science Foundation of China (No. 31730087), Project of High-level Teachers in Beijing Municipal Universities (IDHT20180518), and Program for Changjiang Scholars and Innovative Research Team in University (IRT-17R75).

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Amiseginae and Cleptinae from northeastern Brazil, with the description of four new species (Hymenoptera, Chrysididae)

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Academic editor: M. Ohl | Received 2 November 2020 | Accepted 3 February 2021 | Published 25 February 2021

<http://zoobank.org/53B8A0C7-8782-4FC5-BDC1-F0EA8DCF0AD4>

Citation: Lucena DAA, Almeida EAB, Zanella FCV (2021) Amiseginae and Cleptinae from northeastern Brazil, with the description of four new species (Hymenoptera, Chrysididae). *Journal of Hymenoptera Research* 81: 57–85. <https://doi.org/10.3897/jhr.81.60048>

Abstract

The diversity of two subfamilies of cuckoo wasps in northeastern Brazil is reviewed. Four new species are described and illustrated: *Amisega boyi* Lucena, **sp. nov.**, *A. sertaneja* Lucena, **sp. nov.**, and *Duckeia dudui* Lucena, **sp. nov.** (Amiseginae), and *Cleptidea nordestina* Lucena, **sp. nov.** (Cleptinae). These new species of *Amisega* and *Duckeia* represent the first records of both genera in the core zone of the Caatinga dry region, and they seem to be endemic to this portion of the region. The other two *Amisega* species previously recorded in northeastern Brazil are restricted to ecotone habitats between Caatinga and Atlantic forest. *Cleptidea nordestina* Lucena, **sp. nov.** (Cleptinae) is the most septentrional record for the *fasciata* species group in South America. With the present contribution, the total recorded diversity of Amiseginae and Cleptinae in northeastern Brazil is represented by the following species: *A. boyi* Lucena, **sp. nov.**, *A. flavipes* Kimsey, 1987, *A. sertaneja* Lucena, **sp. nov.**, *A. similis* Kimsey, 1987, and *D. dudui* Lucena, **sp. nov.** (Amiseginae), and *C. nordestina* Lucena, **sp. nov.** (Cleptinae).

Keywords

Chrysidoida, deciduous forest, diversity, rain forest, semiarid, taxonomy

Introduction

Cuckoo wasps are a species-rich cosmopolitan group of aculeate hymenopterans, currently with more than 2,500 valid species distributed into 94 extant genera and four subfamilies (e.g., taxonomic data modified from Kimsey and Bohart 1991; Aguiar et al. 2013). The great morphological disparity and highly specialized biology exhibited by its representatives are the outcomes of more than 130 Mya of evolution (Lucena and Almeida *in press*). While the Chrysidinae are widespread, being relatively common in almost all kinds of habitats, the other three subfamilies are less frequently collected and seem to have had a diversification-history largely associated with tropical savannah and humid forested environments (Krombein 1983, 1986; Lucena and Almeida *in press*). In Brazil, the pioneering investigation of Adolf Ducke provided a preliminary account of the chrysidid fauna in the country, which was particularly important for the northern (Pará state) (e.g., Ducke 1904, 1907a, 1911a) and northeastern regions of Brazil. Ducke's contributions to the description of the chrysidid fauna in the northeast largely resulted from his expeditions to Ceará state in the early 1900s (e.g., Ducke 1907b, 1908, 1910, 1911b).

Northeastern Brazil corresponds to an area roughly equivalent to Colombia and Ecuador together, totaling 1,552,157 Km² and extending from 07°12' to 48°20'S, and the easternmost point in South America, nearly 34°47' to 48°45'W (IBGE 2019). The largest Brazilian biomes are represented in this region (Fig. 1): Amazon and Atlantic rain forests, Caatinga, and Cerrado. The majority of northeastern Brazil, over 50% of its area, is occupied by a large central longitudinal strip characterized by a tropical semiarid climate: the semiarid region (Pereira-Júnior 2007). The xerophytic deciduous Caatinga vegetation is the predominant phytophysiognomy, and it occupies much of its dry core zone, which is also considered as part of the neotropical seasonally dry forests (Fig. 1A–D) (Pennington et al. 2000; Cardoso da Silva et al. 2017). The Atlantic rainforest covers a narrow eastern stripe near the coast. In the western border, there are transition zones with areas of the Amazon rainforest and Cerrado, the Brazilian savannah. In the highlands of southern Bahia state, there is also a quite distinct highland rupestrian grassland vegetation, the “campos rupestres”, probably older in origin, which was recently recognized as a distinct biogeographical province, namely “Chapada Diamantina” (Colli-Silva et al. 2019). Within the dry Caatinga core region, the hilltop, plateaus and hillside forests (e.g., Serra do Ibiapaba and Chapada do Araripe) harbor distinct biota which contrast with that typically found in the xerophilous caatinga. There are substantial evidences showing that these more humid enclaves (Fig. 1E–H) share elements of biota with the Amazon and Atlantic rainforests, and Cerrado, as exemplified by some taxa of bees (Camargo and Moure 1991), scorpions (Lourenço 2010), lizards (Rodrigues et al. 2002, 2013), and plants (Ribeiro-Silva et al. 2012).

The driest biome, Caatinga, has traditionally been considered to harbor the poorest biodiversity in comparison with more humid neighboring biomes, which also reflected in a relative overlook of its biology. Despite that, this biome has received growing attention from researchers (e.g., Bravo and Calor 2014). Caatinga is now characterized

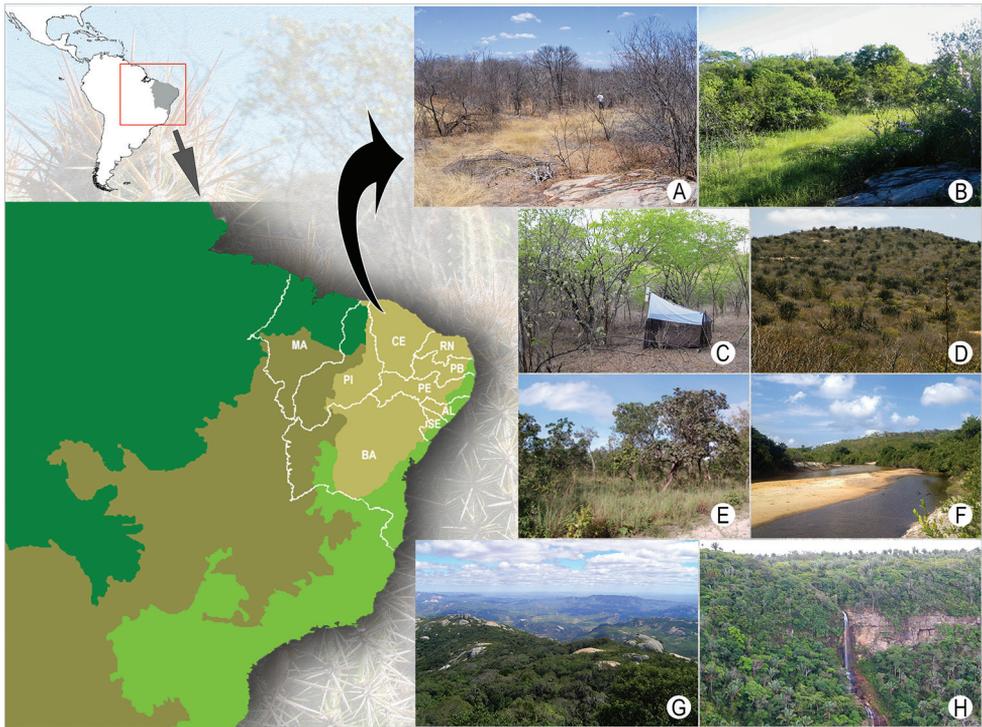


Figure 1. Easternmost biogeographic regions of South America (modified from Morrone 2014)—brown: Chacoan domain (light brown: Caatinga province); light green: Parana/Atlantic forest; dark green: northern Amazonia. Abbreviations for the northeastern Brazilian states are as the follow: Bahia (BA), Sergipe (SE), Alagoas (AL), Pernambuco (PE), Paraíba (PB), Rio Grande do Norte (RN), Ceará (CE), Piauí (PI), and Maranhão (MA). Limits of the Caatinga province in the Chacoan Dominion (dark brown band) indicated by the longitudinal light brown strip. The photographs (right) depict the diversity of habitats in northeastern and Caatinga **A, B** xerophilous deciduous caatinga vegetation during dry (**A**) and rainy (**B**) seasons, ESEC-Seridó, Serra Negra do Norte-RN **B** malaise trap set in a caatinga fragment in Santa Teresinha-PB **D** caatinga vegetation with abundant arboreal cacti, Picuí-PB **E** cerrado vegetation enclave in Caatinga region, Chapada do Araripe, CE **F** amazon rainforest near northwest border of the semiarid region, São Benedito do Rio Preto, MA **G, H** evergreen humid montane enclaves in Caatinga region, Parque Estadual Pico do Jabre, Maturéia, PB (1,197 m), and Parque Nacional de Ubajara, Ubajara, CE (847 m) (**H**).

as quite heterogeneous region, resulting from the interplay between presenting high spatial and historical complexity (Fig. 1A–H) (Cardoso da Silva et al. 2017; Queiroz et al. 2017). It has been suggested that its flora displays higher diversity per area than the Amazon rainforest (Fernandes et al. 2020).

Caatinga is mostly characterized by high temperatures throughout the year, with a marked seasonal climate marked by a strong hydric deficit (Fig. 1A, B). The amount of rainfall varies between 240–1500 mm per year (generally below 800 mm per year in the semiarid region), and it is irregularly distributed, usually concentrated in three consecutive months during austral summer (Prado 2003). The adverse weather conditions imposed by the hydric deficit imply that biota in the Caatinga has significant

tolerance to dehydration. At least part of the fauna may be unique, potentially with a high number of undiscovered endemic taxa remaining to be found.

Herein, we expand the knowledge of Chrysididae fauna in northeastern Brazil by describing and illustrating four new species in the Amiseginae and Cleptinae. We comment on the new species' habitats and discuss some geographic records of previously known taxa from the northeastern region. Additionally, we discuss and update the list of taxa with occurrence in the core region of Caatinga.

Material and methods

Geographic delimitation

For the analyses of geographic occurrence of species into the semiarid limits, we used delimitation presented by Pereira-Júnior (2007), established considering the isohyet of 800 mm or lower per year, the Thornwaite aridity index equal to 0.50, and hydric deficit with drought risk of 60%. The shapefiles of Morrone's (2014) biogeographic dominions (Löwenberg-Neto 2014) were used as reference for the limits of other major natural areas in South America. The main biogeographic areas shown in the Figure 1 correspond to combinations of units of the complex biogeographical regionalization proposed for the Neotropical region by Morrone (2014): the **Amazon rainforest** herein corresponds to Boreal Brazilian Dominion + South Brazilian Dominion + South-eastern Amazonian subregion; the **Atlantic rainforest** corresponds to Parana Dominion; the **Cerrado** and **Caatinga** correspond to provinces in the Chacoan Dominion. We used QGIS Development Team (2020) for combining shapefiles and generating new maps for species distribution. Distribution maps were based on locality records taken from specimen labels and complemented by information in the literature.

Material studied

Most specimens were collected using malaise traps standing in the field for at least two consecutive months (Patu and Mossoró-RN), a whole year (Jequié-BA), and four consecutive years (Santa Teresinha-PB) (detailed descriptions of localities and sampling methods were provided in Lucena et al. 2012; Zanella and Lucena 2014; Fernandes et al. 2014, 2019, 2020; Guedes et al. 2019). We also examined specimens housed in the following institutions: **BME**: Bohart Museum of Entomology, University of California, Davis, California, USA (Lynn S. Kimsey; Steven Heydon); **DZUP**: Coleção Entomológica "Pe. Jesus Santiago Moure", Universidade Federal do Paraná, Curitiba, Brazil (Gabriel A. R. Melo); **INPA**: Coleção Entomológica do Instituto Nacional de Pesquisa da Amazônia, Manaus, Brazil (Márcio L. Oliveira; Thiago Muniz); **MPEG**: Museu Paraense Emílio Goeldi, Universidade Federal do Pará, Belém, Brazil (Orlando Silveira); **MZSP**: Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (Carlos R. F. Brandão; Kelli S. Ramos); **RPSF**: Coleção Entomológica "Prof. J.M.F. Camargo", FFCLRP, Universidade de São Paulo, Ribeirão Preto, Brazil (Eduardo A. B. Almeida);

UFPB: Universidade Federal da Paraíba, João Pessoa, Brazil (Celso F. Martins); **UNI-LA:** Universidade Federal da Integração Latino-Americana, Foz do Iguaçu, Brazil (Fernando C. V. Zanella); **UFES:** Coleção Entomológica da Universidade Federal do Espírito Santo, Vitória, Brazil (Marcelo T. Tavares; Ricieri C. Dall'Orto); **UFMG:** Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil (Fernando A. Silveira; Alessandro Lima); and **USNM:** The National Museum of Natural History, Smithsonian Institution, Washington, USA (Seán G. Brady). Part of the type specimens of taxa described by Kimsey (1987) were originally housed at The American Entomological Institute (**AEI**), Gainesville, Florida, but the AEI collection was recently relocated to Logan, Utah, and became part of the Utah State University, Logan, USA (**USU**) (James Pitts). Finally, some taxonomic decisions were possible by comparisons of specimens with high-resolution images of the holotypes housed in the Canadian National Collection, Ottawa, Canada (**CNC**) (Sophie Cardinal).

Photographs

Specimens were photographed at Laboratório de Biologia Comparada e Abelhas, Departamento de Biologia, FFCLRP/USP, using a Leica DFC425 camera attached to a Leica M205C stereomicroscope, equipped with a Leica FlexiDome lighting diffuser, and light system model Leica LED5000 HDI. Images were improved using Leica application suite software-LAS to combine multiple photos, then enhanced with Adobe Photoshop.

Terminology

The general morphological terminology follows primarily Huber and Sharkey (1993), with few modifications intended to incorporate the terms specifically used for Chrysididae, such as: malar space, malar sulcus, propodeal angle, and scapal basin (Kimsey and Bohart 1991: 41–42). The abbreviations **F**, **S**, and **T** are employed for **flagellomere**, **metasomal sternum**, and **metasomal tergum**, respectively. **MOD** refers to the **median ocellus diameter**. **LID** refers to the **least interocular distance**. **PD** refers to **puncture diameter**. **OL** refers to the minimum distance between the inner margin of the **lateral ocellus** and median ocellus. **OOL** refers to the **minimum distance from the outer margin of lateral ocellus to compound eye**. **POL** refers to the minimum distance between inner margins of **lateral ocelli**. Malar space and subantennal distance refer to the minimum distance between the inferior ocular margin and mandible, and between the inferior margin of torulus and dorsal margin of clypeus. Sculpturing terminology follows Harris (1979).

Taxonomy

Subfamily Amiseginae

The Amiseginae are specialized parasitoids of walking stick eggs (e.g., Costa Lima 1936; Krombein 1957, 1960, 1983; Kimsey and Bohart 1991; Baker 2016). Due to

their relatively small size and cryptic habits, these wasps are uncommon in collections. They are most frequently collected in forested habitats using Malaise traps and yellow pan-traps. Generic revisions with notes on biology, geographic ranges, list of valid species, and illustrated keys were provided by Krombein (1957, 1960, 1983) and Kimsey and Bohart (1991). There are nineteen species and four genera recorded in Brazil (Lucena and Zanella 2016). Still, we can assume that this number is underestimated for the country, especially considering there are vast geographic areas without any significant sampling. In northeastern Brazil, we found two genera and five species. Three of these species are herein described as new and represent the first records of species of this subfamily for the Caatinga region.

***Amisega* Cameron, 1888**

Amisega Cameron, 1888: 457. Type species: *Amisega cuprifrons* Cameron, 1888: 457 (by monotypy).

This genus is one of the largest in the Amiseginae. Currently, *Amisega* includes 24 valid species (Kimsey 1987, 1990, 1993), ranging from southeast Canada, midwestern and southeastern United States, as far west as Arizona, through Mexico, Central America, Chile, and in most South America (e.g., Kimsey and Bohart 1991). Although *Amisega* is one of the most abundant amisegines in collections, its biology is poorly known. Milliron (1950) reported *Amisega kahlii* (Ashmead, 1902) (= *Mesitiopteros*) in North America rearing from eggs of *Diapheromera femorata* (Say, 1824) (Diapheromeridae), and Kimsey (1990) described the flightless *Amisega chilensis* Kimsey, 1990 reared from eggs of *Heteronemia mexicana* Gray, 1835 (= *Bacunculus phyllopus*) (Heteronemiidae). Kimsey (1987) revised the genus, described twelve new species, and presented the most complete discussion for species distinctions to date, later complemented by Kimsey (1990, 1993), and Kimsey and Bohart (1991). In Brazil, eight valid species were previously known to occur from the Atlantic rainforest along east coast, as far north as Pernambuco state, through the Cerrado in the central-west and Amazon rainforest on north. The genus is represented in northeastern Brazil by two previously known species, and two new ones are herein described.

***Amisega boyi* Lucena, sp. nov.**

<http://zoobank.org/6451E71C-2FC6-48EB-96FC-0D513350C858>

Fig. 2

Diagnosis. Vertex and anterolateral border of pronotum with rugose-striate marks; anterior declivity of pronotum without medial pit; dorsum of propodeum polished; scapal basin punctate-rugulose, vaguely cross-ridged; metasoma with distinct purple highlights; legs brown, lighter on pro- and mesotibiae, and tarsomeres; malar space 1.5× MOD. *Amisega boyi* Lucena, sp. nov. most resembles *A. flavipes* Kimsey, 1987

and *A. similis* Kimsey, 1987. This new species can be distinguished from both species based on the following combination of characters: rugose marks on vertex and pronotum (lacking rugose marks on vertex in *A. similis*, and only striatiform punctures on anterior border of pronotum in *A. flavipes*); scapal basin punctate-rugulose, vaguely cross-ridged medially (entirely cross-ridged in *A. flavipes* and *A. similis*); dorsum of propodeum polished and impunctate (dorsum of propodeum smooth and scarcely punctated in *A. flavipes* and *A. similis*); sparse punctation on disc of T2, with more than 1PD apart and medial impunctate polished strip clearly defined (disc of T2 densely punctated in *A. similis*, and medial impunctate polished strip not defined in *A. flavipes*); and purple highlights on metasoma (blue highlights in *A. similis*). The light brown antenna, mandible, and clypeus, and the anterior declivity of pronotum lacking medial pit also help to distinguish this new species from *A. flavipes* (dark brown antenna, mandible, and clypeus, and with well-marked medial pit on anterior border of pronotum); and the greenish blue body coloration contrasts with the light blue pattern of *A. similis*.

Description. Holotype, male. Body length: 2.8 mm (Fig. 2A).

Head: 0.95× wider than high; toruli barely separated, inner margins slightly produced, forming a short lamellar projection; scape long, cylindrical, slightly wider basally, 3.8× longer than its maximum width; F1 length 2.2× breadth, 1.25× longer than F2, F2 1.15× longer than F3; F4 0.9× F3, F5–F10 slightly shorter than F4, F11 acute; lower margin of clypeus evenly round; subantennal distance 0.2× MOD; malar space 1.5× MOD; POL 1.6× OL, 4.3× OOL; inner ocular margin convergent submedially, LID 0.95× scape length; eye height 1.75× breadth.

Mesosoma: anterior declivity of pronotum lacking medial pit, lobe slightly separated from tegula; scutum notaulus barely distinguishable, impressed only basally (Fig. 2D, E); parapsidal line deeply impressed; M with first abscissa gently curved submedially, diverging before 1cu-a; Rs very short, spectral trace gently curved towards costal wing margin; dorsum of propodeum posteriorly limited by discrete transverse groove.

Metasoma: lateral margins of T1–T3 sharp, but not carinate; first metasomal segment with ventral keel; posterior margin of S1 concave, posterior margins of S2–S4 nearly straight.

Coloration: head greenish blue, with greenish highlights on frons, scapal basin, and gena; bluish purple tint on vertex (Fig. 2B); antenna, clypeus, and mandible brown, labrum and mouthparts light brown; mesosoma mainly greenish blue; lateral pronotum, mesopleuron, and metapleuron-propodeum mostly dark brown, extending from half portion to venter, with greenish blue tints on anterior corners; tegula light brown; wing membrane light fuscous, veins brown; legs dark brown, lighter on pro- and mesotibiae and tarsomeres; metasoma mostly brown, with distinct purple highlights on dorsum (e.g., Fig. 2F), venter entirely brown.

Sculpturing: head regularly punctate, punctures becoming clumped among ocelli and near face; vertex with rugose marks marginally (Fig. 2B); scapal basin somewhat rugulose, vaguely cross-ridged medially, with discrete medial groove extending from dorsal border of torulus to midface (Fig. 2C); gena punctate; malar area and clypeus impunctate; scape punctulate dorsally, with broad basal impunctate area; dorsum of

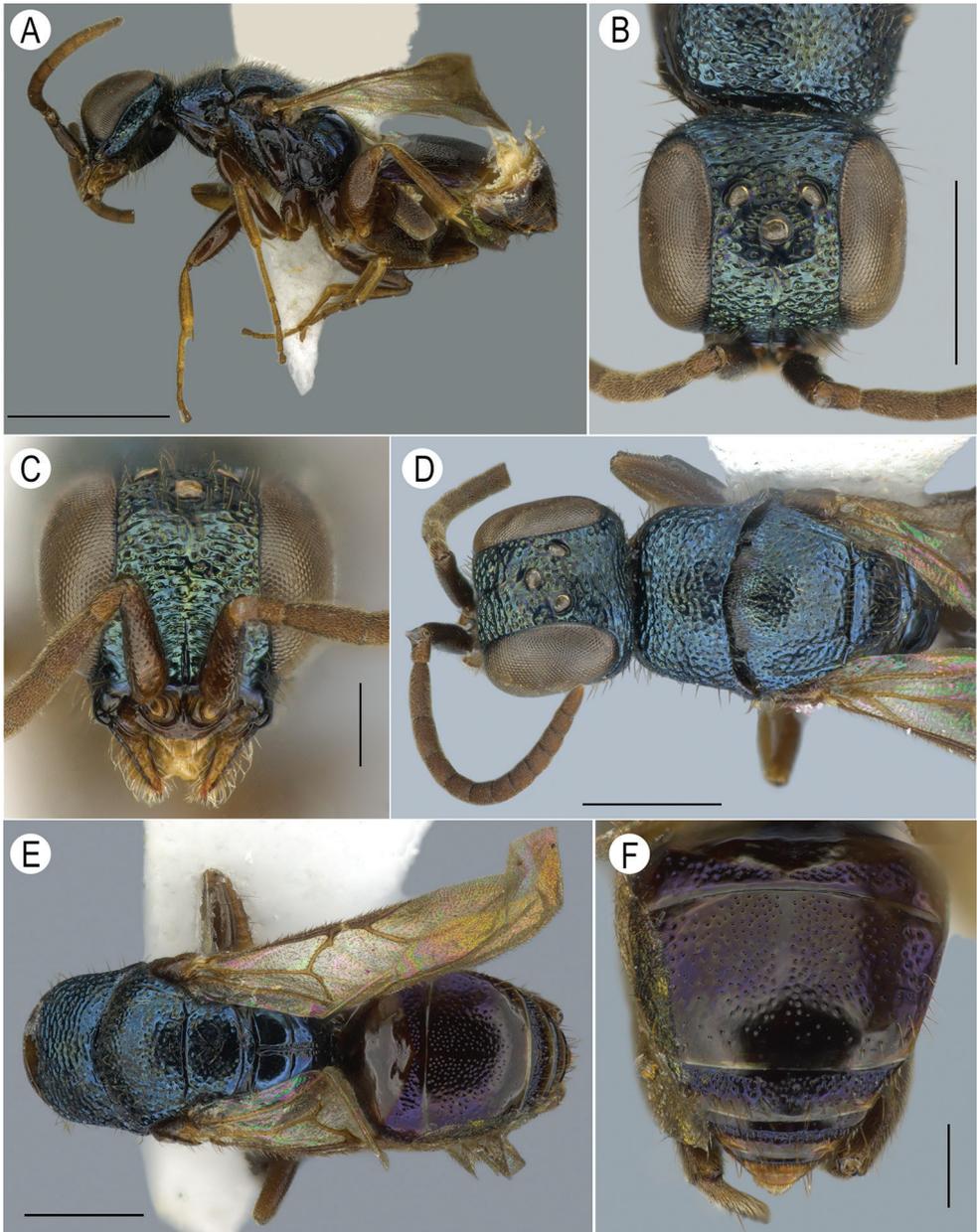


Figure 2. *Amiseqa boyi* Lucena, sp. nov., holotype, male (A–D) A lateral habitus B head and pronotum, dorsal view C head, frontal view D head and mesosoma, dorsal view. Paratype, male (E, F): E dorsal habitus F metasoma, dorsal view. Scale bars: 1 mm (A), 0.5 mm (B, D, E), 0.2 mm (C, F).

mesosoma without distinct striae, except anterolateral margin of pronotum with rugose-striate marks (Fig. 2B, D); scutum and scutellum punctate, sparser posteriorly on scutellum (e.g., Fig. 2E); mesopleuron contiguously punctate anteriorly, with broad

impunctate stripe along posterior margin; metanotum scarcely punctate; propodeum polished on dorsum (e.g., Fig. 2E), posterior declivity with some sparse punctures; anterior border of T1 and most of laterotergite impunctate, marginal punctate stripe running from dorsum to dorsoposterior corner of laterotergite; punctuation on disc of T2 more than 1PD apart (e.g., Fig. 2F); T2–T4 contiguously punctate, becoming clumped on laterotergites, posterior borders of terga with impunctate stripes, S2–S4 densely punctate.

Vestiture: short erect light brown setation on vertex, gena, and frons, with relatively longer and denser setation on occiput; mandible, clypeus and labrum with distinct long gold setation; antenna with short, decumbent, pale setation; eye with sparse microtrichia; dorsum of mesosoma with dense, relatively long, light brown setation; lateral pronotum, posterior border of mesopleuron, dorsum of propodeum, and metapleuron-propodeum, glabrous; wing membrane entirely setose; outer surface of metatibia, and pro- and metafemora, with long dark setae; venter of tarsomeres with abundant irregularly-sized spines; T1–T2 mostly glabrous, except for sparse short setae placed marginally; T3–T5 with marginal stripe of dense long dark setae; S2–S5 abundantly setose.

Female. Unknown.

Material examined. Holotype: BRAZIL • ♂; Rio Grande do Norte, Mossoró, “Faz. Sta. Júlia” [Fazenda Santa Júlia], Malaise 1, caatinga; 05°01'10"S, 37°22'56"W; 14.iv.2008; Fernandes, DRR & cols; RPSP.

• 3♂ paratypes: same data as holotype (1♂ INPA; 2♂ UNILA).

Distribution. BRAZIL, RN: Mossoró.

Comments. The holotype is missing F6–F11 of the left antenna. The metasoma is damaged, with distal segments partly detached and upside down. Two paratypes lack heads, and another paratype has a detached metasoma. This species is only known from Mossoró, Rio Grande do Norte state (sympatric with *A. sertaneja* Lucena, sp. nov., see below), within the semiarid region. All specimens were collected in a Caatinga native vegetation fragment adjacent to a melon crop *Cucumis melo* L. (Cucurbitaceae) (Fernandes et al. 2014).

Etymology. The new species is named after Daniell Fernandes (*Boy*), collector of many specimens used in this study.

Host. Unknown.

Amisega flavipes Kimsey, 1987

Fig. 3

Amisega flavipes Kimsey, 1987: 67. Holotype ♀ (USU: not examined). BRAZIL: Bahia, Encruzilhada.

Material examined. BRAZIL • 1♂ paratype; Bahia, Encruzilhada; xi.1975; M Alvarenga; BME • 1♂; Bahia, Jequié; i.2016; Antunes RCL; RPSP.

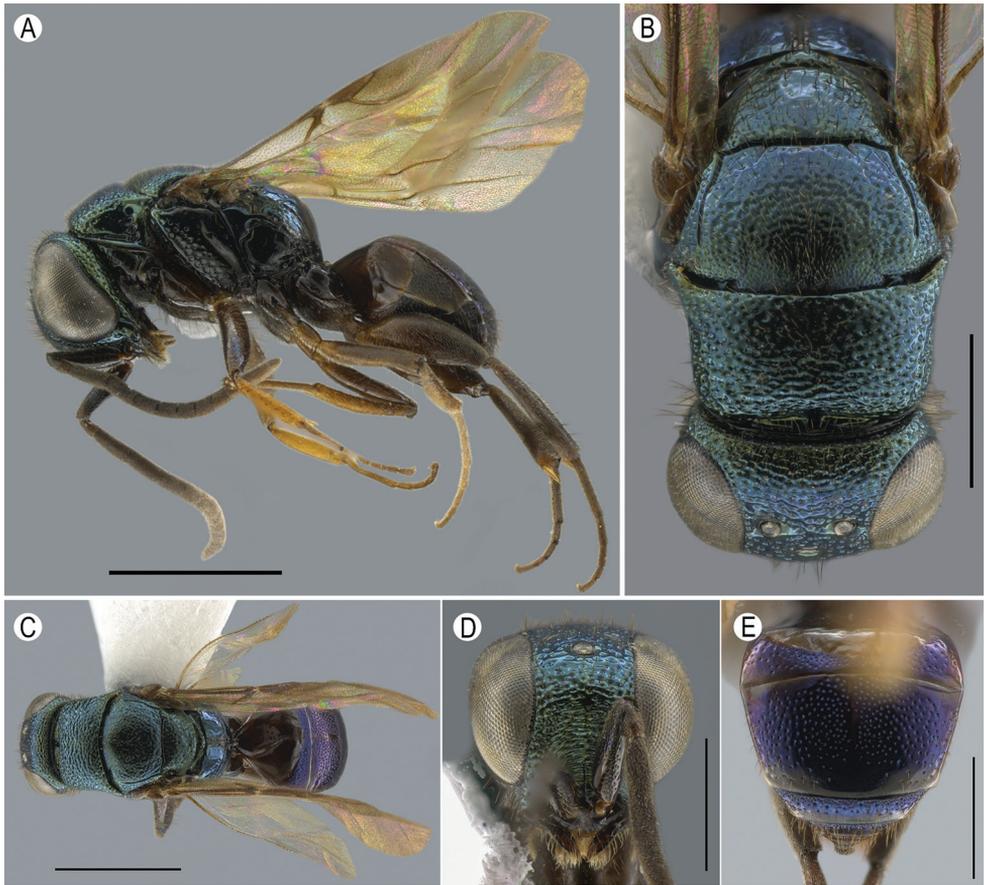


Figure 3. *Amisega flavipes* Kimsey, male (Brazil: Bahia, Jequié) **A** lateral habitus **B** head and mesosoma, antero-dorsal view **C** dorsal habitus **D** head, frontal view **E** metasoma, dorsal view. Scale bars: 1 mm (**A, C**); 0.5 mm (**B, D, E**).

Distribution. BRAZIL, BA: Encruzilhada, Jequié.

Comments. This species can be readily distinguished from other species of the northeastern Brazil by the following combination of characters: scapal basin cross-ridged; dark brown antenna, clypeus, mandible, and metaleg; and lack of distinct striation on vertex and dorsum of mesosoma (Fig. 3C–E). In the original description, Kimsey (1987: 67) cited F1 length $4\times$ breadth, with the male interpreted as identical. The analyzed specimen from Jequié-BA has comparatively shorter F1 (length $2.3\times$ breadth), and a distinct medial pit on anterior declivity of pronotum (not cited in the original description). All other diagnostic characters described for *A. flavipes* are identical with this specimen. This species has been recorded in a transition area between Caatinga and Atlantic rainforest. The Encruzilhada county is in the “Sul-Baiano” highlands, on altitudes above 600 m, with milder climate than usual in the Caatinga (Radambrasil 1983). The new record herein presented

for Jequié is only 207 km north of the previous record, and in spite of having a lower altitude and warmer and drier climate than the type locality, still in the transition zone between the Atlantic rainforest and Caatinga. The taxonomic conclusions about this species are based on the original description and by examination of a male paratype from Bahia: Encruzilhada (BME), and a male recently collected in Bahia: Jequié (RPSP).

Host. Unknown.

***Amisega sertaneja* Lucena, sp. nov.**

<http://zoobank.org/D5531506-E382-4EA2-B2D1-81F9DBA19D3C>

Figs 4, 5

Diagnosis. Dorsum of head and mesosoma densely striate, with sparse punctures inserted among striae; propodeum with rugose marks, becoming irregularly striate posteriorly, posterior declivity with some sparse punctures; lateral pronotal pit sulci-form; scapal basin transversely cross-ridged; metasoma brown, without blue nor purple highlights; legs light brown; malar space $1.7\times$ MOD. This new species can be readily distinguished from other species occurring in northeastern Brazil, *Amisega boyi* Lucena, sp. nov., *Amisega flavipes* Kimsey, and *A. similis* Kimsey, based on densely striate vertex and mesosoma; rugose marks on propodeum; and lack of blue or purple highlights on the metasoma in both genders. Furthermore, *A. sertaneja* sp. nov. has the lateral pronotal pit shallow and sulci-form (pit well-marked in *A. flavipes*), and concolorous light brown legs in both sexes (bicolored in *A. similis*, and dark brown in males of *A. flavipes*). The extensive transverse striation on dorsum of head and mesosoma also resembles *A. striata* Kimsey (Costa Rica). However, *A. sertaneja* sp. nov. is readily distinguished from *A. striata* by its relatively shorter body (3.1 mm *vs.* 4 mm), lack of reddish highlights on the legs, brassy tints dorsally on the body, and the entirely dark brown scape (paler ventrally in *A. striata*).

Description. Holotype, male. Body length: 3.1 mm (Fig. 4A).

Head: $1.2\times$ higher than broad; toruli barely separated, inner margins slightly produced, forming a short lamellar projection (Fig. 4E); scape long, cylindrical, slightly wider basally, $3.6\times$ longer than its maximum width; F1 length $3.1\times$ breadth, $1.3\times$ longer than F2, F2 $1.3\times$ longer than F3; F4 slightly shorter than F3, F5–F10 subequal, progressively shorter; lower margin of clypeus evenly round; subantennal distance $0.4\times$ MOD; malar space $1.7\times$ MOD; OL $1.2\times$ POL, $7\times$ OOL; inner ocular margin convergent submedially, LID $1.1\times$ scape length; eye height $1.4\times$ breadth.

Mesosoma: anterior declivity of pronotum with shallow medial pit, lateral pit near pronotal lobe sulci-form, lobe slightly separated from tegula (Fig. 4C); scutum notaulus barely distinguishable, impressed only basally; parapsidal line deeply impressed; M with first abscissa gently curved submedially, diverging at 1cu-a (e.g., Fig. 5F); Rs very short, spectral trace gently curved towards costal wing margin; dorsal surface of propodeum posteriorly limited by discrete transverse groove (Fig. 4I).

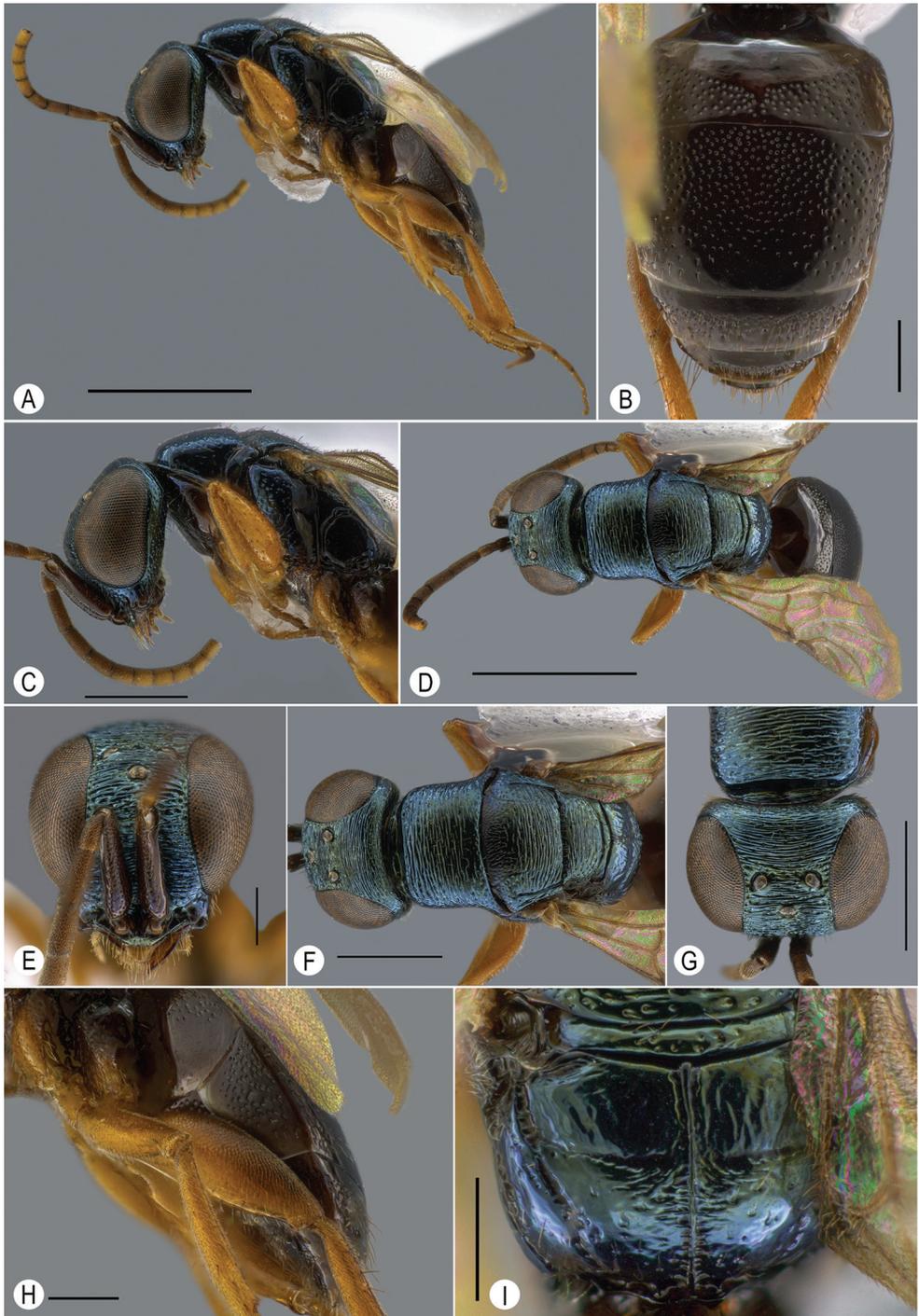


Figure 4. *Amisega sertaneja* Lucena, sp. nov., holotype, male **A** lateral habitus **B** metasoma, dorsal view **C** head and mesosoma, lateral view **D** dorsal habitus **E** head, frontal view **F** head and mesosoma, dorsal view **G** head, dorsal view **H** metasoma, lateral view **I** propodeum, dorsal view. Scale bars: 1 mm (**A**, **D**), 0.5 mm (**F**, **G**), 0.2 mm (**B**, **C**, **E**, **H**, **I**).

Metasoma: lateral margins of T1–T3 sharp, but not carinate (Fig. 4H); first metasomal segment with discrete ventral keel; posterior margin of S1 concave, posterior margins of S2–S4 nearly straight.

Coloration: head dark blue, with greenish highlights on frons, scapal basin, and gena; eye, antenna, and mouthparts light brown; distal flagellomeres light brown; mesosoma mainly dark blue, with greenish highlights dorsally; mesopleuron with ventral light brown tint, posterior margin with dark brown stripe; metapleuron-propodeum dark brown with marginal bluish highlights; tegula light brown; wing membrane light fuscous, veins brown; legs brown, darker on metacoxa; metasoma entirely brown (Fig. 4B).

Sculpturing: frons and vertex heavily striate (Fig. 4G), with sparse punctures inserted among striae; scapal basin densely cross-ridged; gena punctate; clypeus with small punctures on disc, impunctate marginally; scape densely punctulate dorsally, sparser ventrally, with medial polished stripe; dorsum of pronotum, scutum and scutellum densely striate (Fig. 4D, F), with sparse punctures inserted among striae; lateral pronotum with sparse punctures; disc of propleuron impunctate, marginal border with sparse punctures; mesopleuron contiguously punctate, with broad impunctate stripe along posterior margin; disc of metanotum punctate; outer surface of profemur with sparse punctures; dorsum of propodeum with lateral rugose marks (Fig. 4I), becoming irregularly striate marginally, posterior declivity with some sparse punctures; anterior border of T1 and most of laterotergite impunctate and polished, with marginal punctate stripe running from dorsum until dorsoposterior corner of laterotergite; T2–T4, including laterotergites, densely punctate, except by posterior impunctate stripes; S2–S4 densely punctate.

Vestiture: short sparse pale setation on vertex, gena, and frons, with relatively longer and denser setation on lateral border of face and occiput; mandible, clypeus and labrum with distinct long gold setation; antenna with very short, decumbent, pale setation; eye with sparse microtrichia; dorsum of mesosoma with short sparse light brown setation; lateral pronotum, posterior border of mesopleuron, dorsum of propodeum, and metapleuron-propodeum, glabrous; wing membrane entirely setose; outer surface of metatibia, and pro- and metafemora, with long dark setae; venter of tarsomeres with abundant irregularly-sized spines; T1–T2 mostly glabrous, except for sparse short setae placed marginally; T3–T5 with marginal stripe of long dense dark setae; S2–S5 abundantly setose.

Female (Fig. 5). Same as male, except: scape 4.4× longer than its maximum width; F1 length 3.3× breadth, 1.7× longer than F2; LID 0.85× as long as scape; transverse groove on dorsal surface of propodeum barely marked; metasoma dark brown becoming lighter ventrally; T2 with broad impunctate marginal area (Fig. 5C); and denser punctation among striae on dorsum of mesosoma (Fig. 5D, E).

Material examined. Holotype: BRAZIL • ♂; Rio Grande do Norte, Mossoró, “Faz. Sta. Júlia” [Fazenda Santa Júlia], Malaise 1, caatinga; 05°1'10"S, 37°22'56"W; 14.iv.2008; Fernandes, DRR & cols; RPSP • 9 paratypes same data as holotype (2♂1♀ RPSP; 1♂1♀ UNILA; 2♂ DZUP; 1♂1♀ MZSP) • 3 paratypes, same data as holotype, but collected in 06.iii.2007 (1♀ RPSP; 1♀1♂ BME) • 3♂1♀ paratypes; Paraíba, Santa Teresinha, Fazenda Tamanduá, Ciliar A1; 05.iv.2010; Messias KDVS leg.;

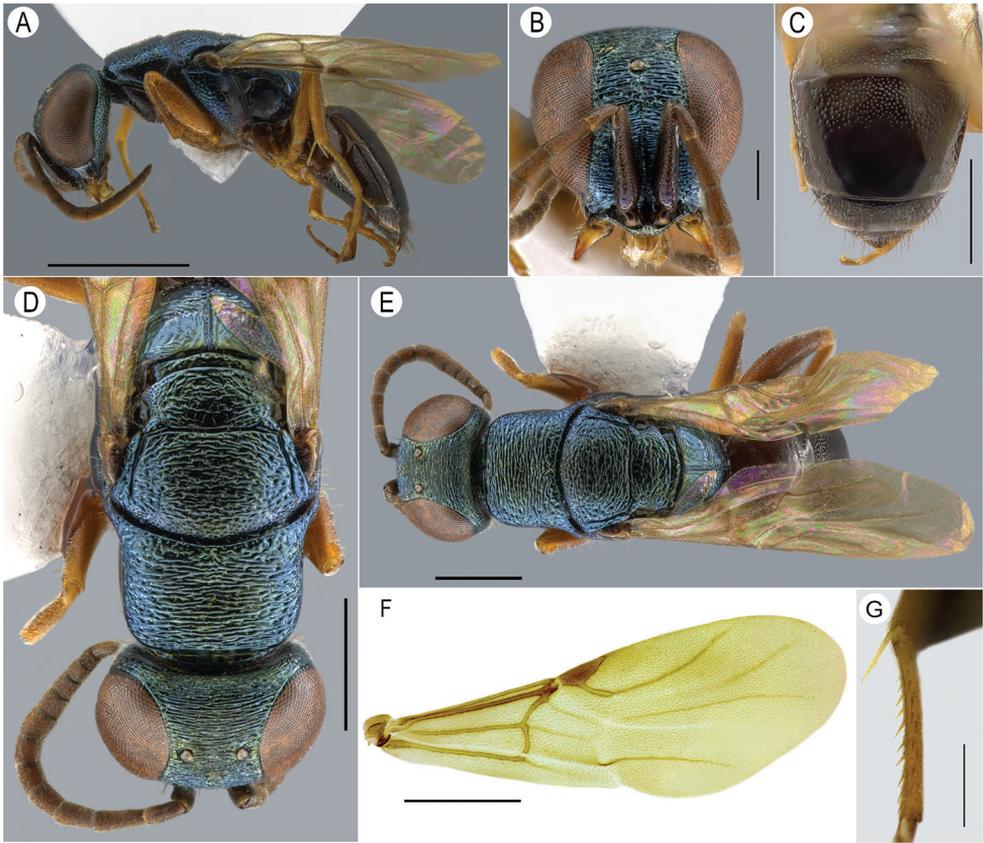


Figure 5. *Amisega sertaneja* Lucena, sp. nov., paratype, female **A** lateral habitus **B** head, frontal view **C** metasoma, dorsal view **D** head and mesosoma, dorsal view **E** dorsal habitus **F** forewing **G** inner margin of metabasitarsus. Scale bars: 1 mm (**A**), 0.5 mm (**C**, **E**, **F**), 0.2 mm (**B**, **G**).

UNILA • 13 paratypes, same data as preceding, except: Reserva B3; 01.iii.2010; 1♂ UFMG; Reserva B1; 01.ii.2010; 1♂ UFMG; Reserva 2; 08.iii.2010; 1♂ MPEG 1♂ UFES 1♂ UFPB; Reserva 3; 07.vi.2010; 1♂ INPA; Reserva A3; 21.iii.2010; 2♀ INPA 2♀ UFPB; Reserva B3; 18.i.2010; 1♀ UFES; Reserva B2; 17.v.2010; 1♀ MPEG; Ciliar; 12.vii.2010; Silva JKS leg.; 1♀ DZUP.

Distribution. BRAZIL, PB: Santa Teresinha; RN: Mossoró.

Comments. The holotype is missing F10–F11. This species is only known from localities in the core zone of the Caatinga dry region. It is sympatric with *A. boyi* Lucena, sp. nov. in Mossoró-RN, which suggests both species are endemic to Caatinga. All specimens of both new species were collected between January to May, which is the period of most intense rainfall in the region.

Variation. Body size: 2.8–3.0 mm; F1 length 2.6–3.3× breadth.

Etymology. The name is a Brazilian gentile adjective for person living in the semi-arid region.

Host. Unknown.

***Amisega similis* Kimsey, 1987**

Amisega similis Kimsey 1987: 70. Holotype ♂ (CNC: examined by photos). BRAZIL: Rio de Janeiro, Guanabara, Represa Rio Grande (presently Rio de Janeiro county).

Material examined. BRAZIL • 1♂ paratype; Pernambuco, Caruaru; iv.1972; M Alvarenga; USNM.

Distribution. BRAZIL, BA: Encruzilhada; MG: Pedra Azul; PE: Caruaru; RJ: Rio de Janeiro and Mangaratiba.

Comments. This species can be distinguished from other species of the northeastern Brazil by its bicolored legs, blue highlights on dorsum of metasoma, and lack of striation on vertex and dorsum of mesosoma. Kimsey (1987) cited AEI as the repository of the holotype, which is actually housed at CNC. The previous record for Pernambuco is now clarified as it came from Caruaru county (originally misspelled as “Carvary” by Kimsey 1987). *Amisega similis* is sympatric with *A. flavipes* in the “Sul-baiano” highlands in southern Bahia, and probably also in northern Minas Gerais state, although *A. similis* clearly also reaches areas in the Atlantic rainforest in Rio de Janeiro state. Therefore, it is possible that neither species occur in the limits of the Caatinga.

Host. Unknown.

Key to species of *Amisega* Cameron from northeastern Brazil

- 1 Vertex and dorsum of mesosoma densely striate (e.g., Fig. 4D, F, G); metasomal terga brownish, without metallic blue or purple highlights (Figs 4B, 5C) ***Amisega sertaneja* Lucena, sp. nov.**
- Vertex and dorsum of mesosoma lacking striae or with only stratiform punctures or rugose marks on vertex and anterolaterally on pronotum (e.g., Fig. 2D); metasomal terga with distinct metallic blue or purple highlights (e.g., Figs 2F, 3E) **2**
- 2 Pronotum without any striae or rugose marks; metasomal terga with metallic blue highlights; malar space 1.3× MOD long; male F1 length 3.5× breadth ***Amisega similis* Kimsey**
- Pronotum with discrete anterolateral stratiform punctation or rugose marks; metasomal terga with metallic purple highlights; malar space 1.5× MOD long; male F1 length shorter than 3× breadth **3**
- 3 Anterior declivity of pronotum lacking distinct medial pit; scapal basin punctate-rugulose, vaguely cross-ridged medially (Fig. 2C); antenna, mandible, and clypeus light brown (Fig. 2C); legs light brown; vertex roughly sculptured, with punctures inserted among discrete striae, punctures 1 PD apart or more (Fig. 2B); T2 with medial impunctate polished strip clearly defined (Fig. 2F) ***Amisega boyi* Lucena, sp. nov.**
- Anterior declivity of pronotum with deep medial pit (Fig. 3B); scapal basin entirely cross-ridged (Fig. 3D); antenna, mandible, and clypeus dark brown (Fig. 3D); legs dark brown; vertex lacking striae, punctures touching to 2 PD apart; T2 with medial impunctate polished strip not defined (Fig. 3E) ***Amisega flavipes* Kimsey**

***Duckeia* Costa Lima, 1936**

Duckeia Costa Lima 1936: 174. Type species: *Duckeia cyanea* Costa Lima 1936: 174 (monotypy).

Duckeia is a rarely collected taxon. Currently, the genus comprises three species, two of them were recorded in Rio de Janeiro state, southeastern Brazil, in an area of Atlantic rainforest, and one species is known from Costa Rica (Costa Lima 1936; Kimsey 1993; Lucena and Zanela 2016). According to Costa Lima (1936), the type series of *Duckeia cyanea* Costa Lima, 1936 was reared from eggs of *Prisopus ohrtmanni* (Lichtenstein, 1802) (Phasmatidae), in Rio de Janeiro, Brazil. Kimsey (1987) described *D. vagabunda* based on specimens reared from quarantine material carried by airplane from an unknown locality of Mexico that arrived in Memphis, Tennessee, 27 March, 1962 (Kimsey 1987: 75). According to Kimsey (1987), the host phasmatid egg was probably *Prisopus berosus* Westwood, 1859 (label record). Some years later, Kimsey (1993) recorded *D. vagabunda* for Costa Rica. For the first time, this genus is reported for the Caatinga in northeastern Brazil, and a new species is described.

***Duckeia dudui* Lucena, sp. nov.**

<http://zoobank.org/04628463-EF89-472B-9756-81DF513D2A6F>

Fig. 6

Diagnosis. Body short, stocky build, not compressed laterally; posterior margin of eye lacking carina; upper gena slightly produced; malar space about half eye height; antennae brown, lighter on distal flagellomeres; legs light brown, becoming yellow in tarsomeres; metasoma with dense appressed punctation on marginal borders; dorsum of metasoma with distinct blue highlights. *Duckeia dudui* sp. nov. closely resembles *D. gracile* Kimsey, 1987. The new species differs from *D. gracile* by the following combination of characters: elongated head, 1.3× higher than broad (0.9× higher than broad in *D. gracile*); long malar space, about half of eye height (one third eye height in *D. gracile*); light brown legs (darker in *D. gracile*); short, sparse pale setation on dorsum of meso- and metasoma (relatively longer and denser setation on dorsum of meso- and metasoma in *D. gracile*); marginal borders of T1–T2 with contiguous punctures (punctation on marginal borders of T1–T2 more than 0.5–1.0 PD in *D. gracile*); dorsum of metasoma with distinct bluish highlights marginally on terga (only T2 with faint bluish tint in *D. gracile*). Furthermore, *D. dudui* sp. nov. has a stocky built and relatively short body, contrasting with the laterally compressed and relatively elongated body of *D. gracile*.

Description. Holotype, female. Body length: 3.4 mm (Fig. 6A).

Head: pyriform, 1.3× higher than broad (Fig. 6B); inner margins of toruli produced, touching medially, forming a short lamellar projection; scape long, cylindrical, slightly wider basally, 4.7× longer than its maximum width; F1 length 2.7× breadth,

2.2× longer than F2, F2 1.6× longer than F3; F4 as long as F2; F3 as long as F5, F5–F10 sub-equal in size, F11 acute apically, slightly longer than F10, F4–F10 slightly flattened ventrally; lower margin of clypeus nearly straight; subantennal distance about 0.5× MOD; malar space 0.4× eye height; OL 1.7× POL, 4× OOL; inner ocular margin distinctly convergent above, LID 0.9× shorter than F1; eye height 1.3× breadth; posterior border of eye slightly elevated, lacking carina or crest; upper gena slightly produced; occiput with pair of shallow foveae (e.g., Fig. 6D).

Mesosoma: pronotum with discrete medial longitudinal sulcus, posterior margin not elevated upon scutum, lateral and posterior pronotal pits well-marked, lobe separated from tegula by about 0.7× tegular diameter (Fig. 6E); scutum with notaulus deeply impressed, strongly convergent posteriorly (Fig. 6C); parapsidal line stronger posteriorly, faintly marked anteriorly; M with first abscissa slightly curved submedially, diverging at 1cu-a (e.g., Fig. 6J); stigma somewhat round, longer than first abscissa of M; Rs short, fading gradually, spectral trace curved towards costal wing margin; outer posterior margin of procoxa carinate; lateral margin of propodeum carinate.

Metasoma: lateral margins of T1–T2 carinate (Fig. 6I), T3 with faintly produced anterolateral carina; S1 with ventral keel; posterior margin concave, posterior margins of S2–S4 slightly convex.

Coloration: head dark brown green, with green highlights on frons, vertex and gena, darker basally at malar area; mouthparts light brown; antenna brown, lighter on pedicel and distal flagellomeres; mesosoma dark green brownish, with bluish purple tints dorsally on propodeum; mesopleuron with ventral and posterior light brown stripe; tegula light brown; wing membrane slightly pale to subhyaline, veins brown; legs light brown, becoming yellowish on tibiae and tarsomeres; metasoma dark brown, metallic blue highlights on dorsum, becoming greenish marginally on T3–T4; disc of T1 and T2 brownish; sterna mostly dark brown, except for marginal bluish highlights on S2.

Sculpturing: head contiguously punctate (Fig. 6B, F); clypeus impunctate; scapal basin densely cross-ridged; scape punctulate dorsally, venter slightly excavated and polished; pronotum, scutum, and scutellum densely punctate (Fig. 6C), punctures comparatively deeper and larger on metanotum; anterior border of pronotum with distinct medial pit, lateral margins with shallower pits, latero-posterior border with hyaline impunctate stripe; propleuron widely impunctate on disc, lateral margins densely punctate; mesopleuron contiguously punctate, with narrow impunctate stripe along posterior margin; outer surface of coxae and femora with shallow and sparse punctation; propodeum densely punctate, except by lateral polished areas beside to the metapostnotal median carina; metasoma densely punctate (Fig. 6G–I), with narrow posterior impunctate stripes on T1–T3; medial line of S2–S3 finely punctulate.

Vestiture: sparse long golden pale setation on vertex, gena and clypeus, frons and face with relatively shorter and sparser setation; flagellomeres with decumbent dense pale setation, sparser and longer on scape and pedicel; eye with sparse microtrichia on upper border; mesosoma setose, except glabrous disc of metapleuron-propodeum; mesosomal dorsum with sparse, pale setation, longer on metanotum; mesopleuron with long dense

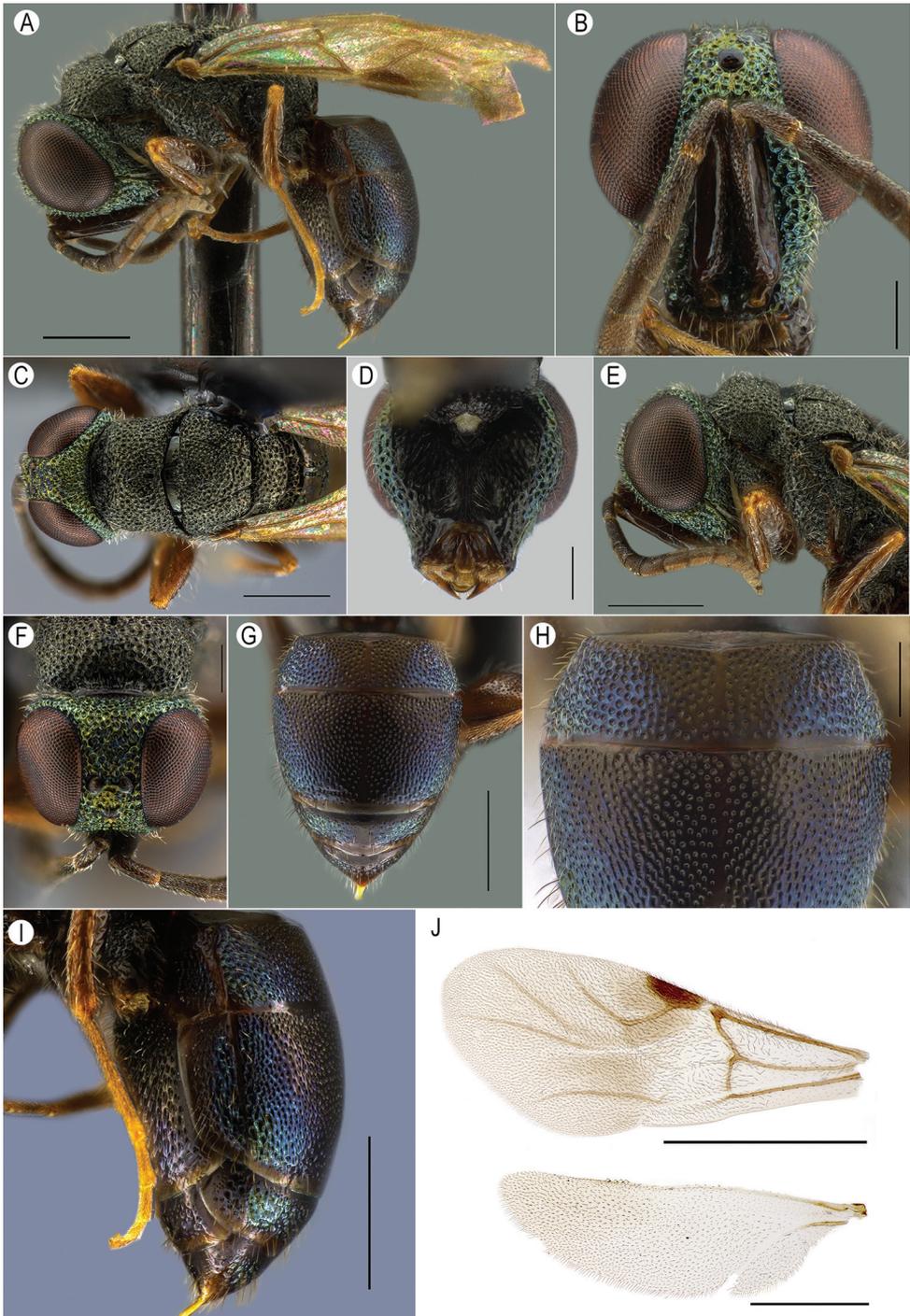


Figure 6. *Duckeia dudui* Lucena, sp. nov., holotype, female **A** lateral habitus **B** head, frontal view **C** head and mesosoma, dorsal view **D** occiput (female paratype) **E** head and mesosoma, lateral view **F** head, dorsal view **G** metasoma, dorsal view **H** amplified view of T1–T2, dorsal view **I** metasoma, lateral view **J** wings (female paratype). Scale bars: 0.5 mm (**A**, **C**, **E**, **G**, **I**), 0.2 mm (**B**, **D**, **F**, **H**, **J**).

pale setation, becoming longer ventrally; wing membrane entirely setose (e.g., Fig. 6J); legs with pale setation, comparatively longer on pro- and metafemora, and meso- and metatibiae; tarsomeres with short, condensed, spine-like, yellow setation; disc of T1 and T2 glabrous, sparse setation marginally, and on laterotergites; T3 and T4 comparatively with longer setation, denser posteriorly, and on laterotergites; S2–S4 entirely setose.

Male. Unknown.

Material examined. Holotype: BRAZIL • ♀; Rio Grande do Norte, Patu, “caatinga-arm. Malaise” [Malaise trap in caatinga]; 06°06'S, 37°37'W; ix.2008; DRR Fernandes & colls; RPSP • 4 paratypes, same data as holotype (1 ♀ MZSP, 1 ♀ DZUP 1 specimen RPSP, 1 specimen UNILA—the latter two could not have their gender determined as detailed in the Comments below).

Distribution. BRAZIL, RN: Patu.

Comments. The holotype is missing part of left metaleg. Two paratypes are poorly conserved, lacking antennae, legs and metasoma, thus, preventing gender determination. This species is only known from Patu county. Specimens were collected in the base of “Serra do Lima” (inselberg), 248 m above sea level (Fernandes et al. 2020), in the core zone of the Caatinga dry region. Interestingly, the specimens were collected in November, which is characterized as part of the dry season in the region.

Etymology. The species is named after Sebastião Antônio de Araújo, *Dudu* (*in memoriam*), grandfather of the first author.

Host. Unknown.

Subfamily Cleptinae

Cleptinae are rarely collected and there are only few specimens housed in Brazilian collections. These wasps are parasitoids of prepupal larvae of Tenthredinoidea sawflies (e.g., Kimsey and Bohart 1991). This subfamily currently includes three extant genera: *Cleptes* Latreille, *Cleptidea* Mocsáry, and *Lustrina* Kurian (Kimsey and Bohart 1991; Móczár 1996b; Rosa et al. 2020). Among these, only *Cleptidea* is recorded in Brazil (Lucena and Zanela 2016).

Cleptidea Mocsáry, 1904

Cleptidea Mocsáry 1904: 567. Type species: *Cleptes aurora* Smith 1874: 452 (by original designation).

This genus is neotropical, occurring mostly in forested warm habitats from southwestern Mexico, through Central America, into South America as far south as northern Argentina (Kimsey 1981, 1986; Kimsey and Bohart 1991). Kimsey (1981, 1986) revised the genus, described eight new species, and provided identification keys and illustrations. Three other species were later described by Móczár (1996a,b) along with an updated key for all species in the genus. Móczár (1996b) also designated a lectotype for *Cleptidea fasciata* (Dalman, 1823) (a dorsal habitus image of the lectotype specimen is available in Rosa and Vårdal 2015) and synonymized *Cleptidea propodealis* Kimsey,

1986 under *C. fasciata*. Currently, the genus includes nineteen valid species, divided into six species groups (*sensu* Kimsey 1986). Seven species and five species groups are known to occur in Brazil (Lucena and Zanela 2016). The genus was first recorded in northeastern Brazil in a preliminary local survey (Lucena et al. 2012). The morphological distinction between that specimen and members of the *fasciata* species group is now evident, and this new species is described.

***Cleptidea nordestina* Lucena, sp. nov.**

<http://zoobank.org/1C3F8F4B-F198-4616-B750-401AE446B901>

Fig. 7

Diagnosis. Head bicolored, mostly dark green extending from vertex until upper half of face, mid face to malar area orange; clypeus raised and square-shaped, with lower margin straight; scutum, scutellum and metanotum testaceous; propodeum entirely black, except for the whitish tip of lateral angle. *Cleptidea nordestina* sp. nov. most resembles *C. fasciata* (Dalman). The new species can be readily distinguished from *C. fasciata* by the following combination of unique characteristics: bicolored head, hyaline marks at body (especially trochanters, pronotum, and propodeal angles), clypeus produced, orange metanotum, and entirely black propodeum.

Description. Holotype, female. Body length: 6.7 mm (Fig. 7A).

Head: 1.2× wider than high; toruli 0.9× MOD separated; scape more or less cylindrical, broader apically, 3.4× longer than its maximum width; F1 length 3.1× breadth, 3.1× longer than F2; F2–F10 sub-equal in size, slightly flattened ventrally, F8–F11 excavated ventrally, F11 acute apically, slightly longer than F10; clypeus protruded medially, squared lobe, with lower margin slightly convex; malar space about 0.6× MOD; ocelli set in compact triangle on vertex, with transverse sulcus linking laterals; posterodorsal border of eye surpassing lateral ocellus dorsal limit; inner ocular margin sub-parallel, slightly convergent above; LID 1.1× eye height; eye height 1.5× breadth; OOL 2.3× OL, 2.6× POL; frons with short longitudinal sulcus below median ocellus briefly interrupted.

Mesosoma: pronotum crossed antero- and submedially by transverse crenulate grooves, longitudinal sulcus culminating in a deep fovea posteriorly (Fig. 7H); posterior margin of pronotum slightly elevated, posterolateral lobe touching tegula; scutum with notaulus deeply impressed, parapsidal line discrete, faintly marked; disc of metanotum slightly convex, marginal areas depressed, posterior margin with pair of small medial pits (Fig. 7G); M with first abscissa more or less straight, diverging at 1cu-a; costal cell wider distally, about 2.2× the maximum width of C; dorsum of metacoxa with distinct longitudinal carina; lateral angle of propodeum short, thumb-like.

Metasoma: posterior margin of S1 distinctly concave medially; posterior margin of S2–S4 slightly convex.

Coloration: head basally orange, extending from malar area to mid face, paler basolaterally, metallic dark green hue extending from upper half of face to vertex and gena (Fig. 7B); scape, pedicel and F1–F2 orange; F3–F11 brownish orange, paler ventrally; pronotum predominantly orange, paler on lobe; disc of scutum testaceous, be-

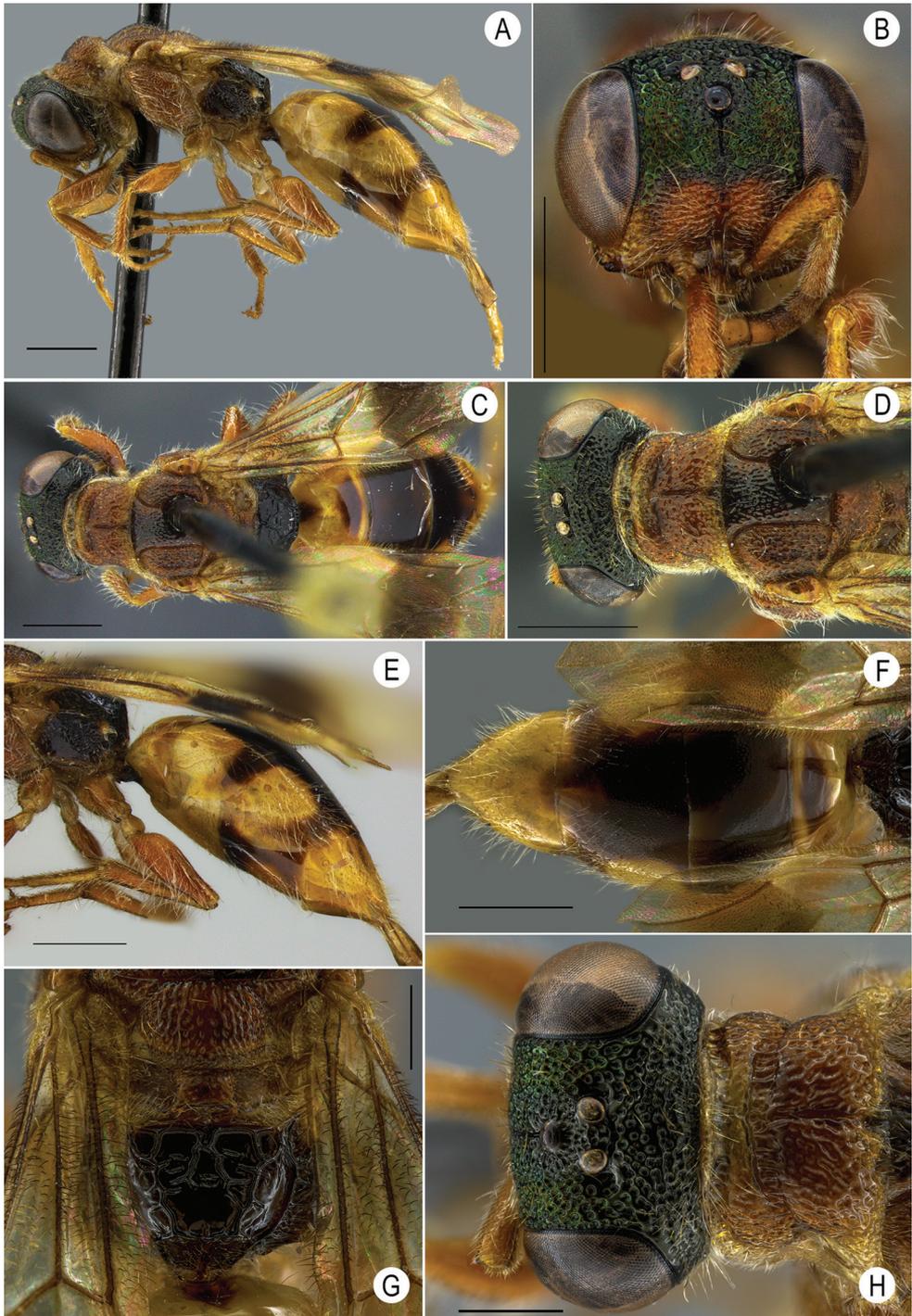


Figure 7. *Cleptidea nordestina* Lucena, sp. nov., holotype, female **A** lateral habitus **B** head, frontal view **C** dorsal habitus **D** head and mesosoma, dorsal view **E** metasoma, lateral view **F** metasoma, dorsal view **G** propodeum, dorsal view **H** head and pronotum, dorsal view. Scale bars: 1 mm (**A–F**), 0.5 mm (**G, H**).

coming gradually orange laterally (Fig. 7C, D); scutellum and metanotum orange; legs yellowish orange, paler on trochanters and ventrally on coxae; tegula orange; wing membrane dusky-hyaline, with broad brownish band submedially, veins brown; metapleuron-propodeum black, tip of lateral angle of propodeum white; metasoma mostly yellowish orange, T1–T3 with broad dark brown bands (Fig. 7E, F); T4 predominantly yellowish; sterna most yellowish orange, anterior S1 and posteromarginal borders of S2–S3 brown; S4 orange.

Sculpturing: head heavily sculptured, densely punctate, rugulose-lacunose, particularly on vertex and frons, becoming sparser on gena, and impunctate basally near torulus and clypeus; pronotum, scutum and scutellum mostly rugulose-lacunose, lobe of pronotum impunctate; mesopleuron faintly costate above, punctate to rugulose-lacunose below; metanotum punctate on disk; femora and tibiae sparsely punctate; dorsum of propodeum heavily carinate forming areolate enclosures, posterior declivity longitudinally crossed by three main carinae, somewhat rugulose in-between; T1 nearly impunctate and polished posteromedially, with sparse tiny lateral punctures; T2 sparsely punctate on disk, marginal areas impunctate; T3–T4 densely punctate on disc, with broad impunctate stripes marginally; S1 and S4 nearly impunctate, with sparse marginal punctures; S2–S3 finely punctate on disk becoming sparser marginally.

Vestiture: head with sparse, long, pale setae on vertex; frons, face, gena and scape with relatively shorter and denser setation; flagellomeres with decumbent dense pale setation; eye with tiny, sparse, sub-erect microtrichia; dorsum of mesosoma with long, sparse, pale setation, longer on venter of mesopleuron; marginal depression of metanotum with patch of pale setae posteriorly; wing membrane entirely setose, with some distinctive, long, erect dark setae inserted proximally; legs with abundant long pale setation, comparatively longer on metaleg; inner surface of tarsi with short, thick setation and short spines irregularly distributed; disc of T1 and T2 glabrous, sparse setation only marginally, T3 and T4 with long dense setae posteriorly, S1 and S4 glabrous, S2 and S3 with long sparse setation; gonapophysis with some erect setae apically.

Male. Unknown.

Host. Unknown.

Material examined. Holotype: BRAZIL • ♀; Bahia, Jequié, *campus* II UESB, Malaise III; 11.v.2007; Silva-Jr. JC & cols; RPSP.

Distribution. BRAZIL, BA: Jequié.

Comments. The holotype is missing distal flagellomeres of the left antenna. The new species is only known from Jequié, located in a transition zone between Atlantic rainforest and Caatinga (Fig. 8C). Three unidentified male specimens from Maranhão state (06.vii.1987, Möericke, UFES; but no collector nor locality data) are also new records of the genus *Cleptidea* for northeastern Brazil. These male specimens are poorly preserved (lacking appendices, altered coloration, and their metasomas are collapsed); thus, we could not assign them to *C. nordestina* sp. nov. confidently or to interpret them as representing yet another new species.

Etymology. The name refers to the region where the new species was collected, and it is a Brazilian gentile adjective for a person native from the northeastern region.

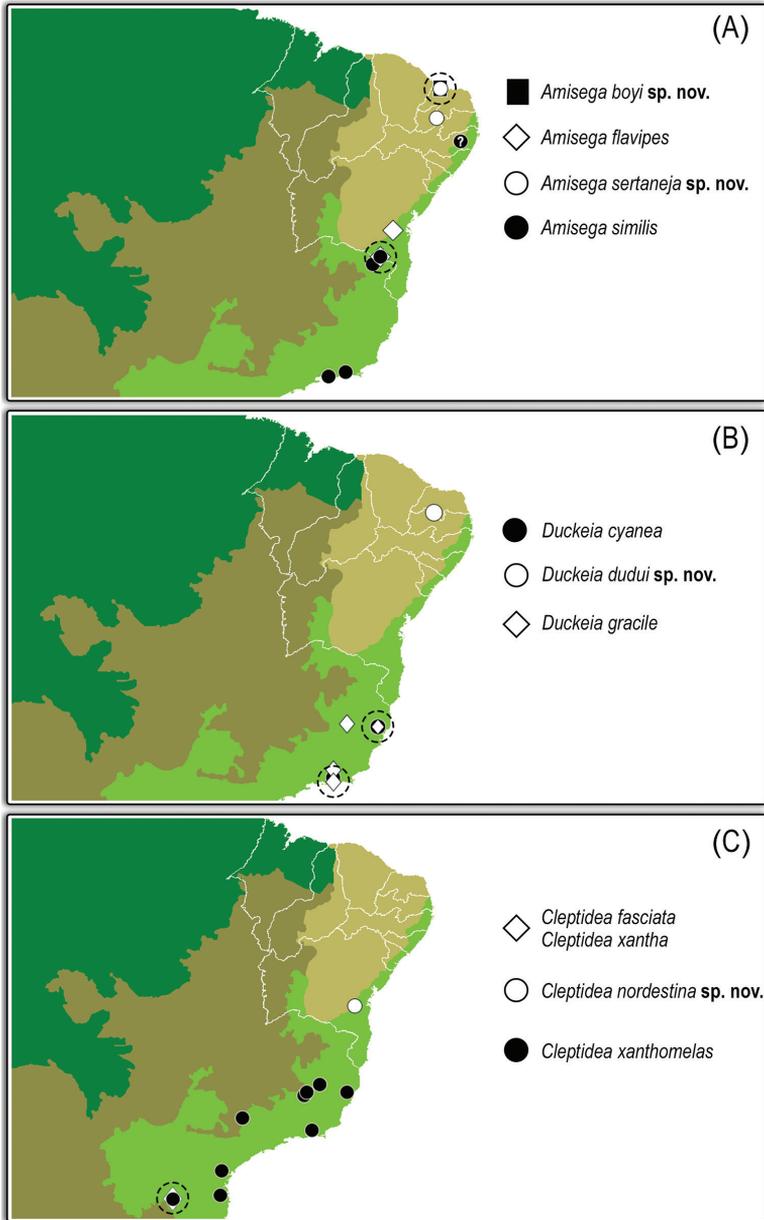


Figure 8. Biogeographic regions of easternmost South America, shown in Fig. 1, and geographical records of species in the genera *Amisegea* and *Duceia* (Chrysididae: Amiseginae), and *Cleptidea* (Chrysididae: Cleptinae). Political division of northeastern Brazilian states is indicated by white lines **A** distribution records of *Amisegea* species (one paratype of *Amisegea similis* Kimsey is signed with a question mark—see Discussion) **B** distribution records of *Duceia* species **C** distribution records of *Cleptidea* species. Some localities harbor more than one species, which are indicated by dashed circles. *Cleptidea fasciata* and *C. xantha* Kimsey, 1986 are only known from the same type locality and both are represented by the same symbol in **C** (see Discussion).

Discussion

Since Kimsey (1987, 1990, 1993), further contributions to the taxonomy of *Amisega* were not published, and consequently, the identities of several taxa remain challenging as new samplings become accessible. Previously to this study, three out of eight valid species known to Brazil had been described by Ducke (1902, 1903) from Amazon rainforest in northern Brazil: *A. aeneiceps* Ducke, 1903, *A. azureus* Ducke, 1903, and *A. mocsaryi* Ducke, 1902. In contrast, *A. semiflava* Kimsey, 1987 is known from localities in the Cerrado of central Brazil, and *A. flavipes* Kimsey, *A. rufilateralis* Kimsey, 1987, *A. similis* Kimsey, and *A. tenebrae* Kimsey, 1987, are known from localities along the eastern coast of South America in the Atlantic rainforest (Kimsey 1987; DAA Lucena *unpubl. data*). The geographical records cited by Kimsey (1987) for *A. similis* are the most disparate among these species, ranging from the southernmost limit of the Atlantic rainforest in Rio de Janeiro state (its type locality) to the northern boundary in the transition zone between the Atlantic rainforest and Caatinga in Caruaru-PE (Fig. 8A). The examination of high-resolution pictures of the holotype of *A. similis* deposited at CNC made it clear that this species is distinct from every new species described in this work. It is possible that the paratypes of *A. similis* from Caruaru-PE represent yet another undescribed species, which should be temporarily interpreted with caution (we have represented with a question mark in the distribution map: Fig. 8A). Future examinations of the complete type series of *A. similis* will prove essential to clear any doubts regarding the taxonomic identity of each specimen.

The present records of *Duckeia* species in quite distant sites in Central America and eastern Brazil without references to intermediate areas suggest a rudimentary knowledge of its diversity. We examined the type material of *D. cyanea* and *D. vagabunda* (USNM), and additional material of *D. cyanea* and *D. gracile* from localities with Atlantic rainforest collected in Espírito Santo state (Laranja da Terra), Minas Gerais state (Marliéria), and Rio de Janeiro state (Nova Iguaçu) (MZSP, UFES) (Fig. 8B). *Duckeia dudui* sp. nov. represents the first record of this genus in a habitat characterized by a seasonally dry forest.

Kimsey (1986) placed four species in the *fasciata* species group in *Cleptidea*, three of them recorded only in Santa Catarina state, southern Brazil (Fig. 8C). The type series of *Cleptidea fasciata* was collected in an unknown locality in Brazil. Móczár (1996b) synonymized *C. propodealis* under *C. fasciata*. The former has only been known from Santa Catarina as well. Previously, Kimsey (1981) had cited *C. fasciata* for Guatemala, Panamá, Peru, and Argentina (Tucumán), but the distribution was later revised and restricted to Brazil and Argentina (Kimsey 1986: 324; Kimsey and Bohart 1991). We had examined specimens housed in the most important Brazilian entomological collections. Still, we only found new records for *C. xanthomelas* (Mocsáry, 1889), which seems to be the most common and abundant species in south-eastern Brazil, occurring mostly in localities of Atlantic forest of the following Brazilian states: Espírito Santo (Santa Teresa), Minas Gerais (Marliéria, Santa Bárbara, and São Gonçalo do Rio Abaixo), Paraná (Campina Grande do Sul), Santa Catarina (Blumenau), and São Paulo (Luis Antônio) (Fig. 8C). In this regard, the new species herein proposed and three

unidentified male specimens from Maranhão represent now the most septentrional records for the *fasciata* species group in South America.

Conclusions

The diversity of Amiseginae and Cleptinae is probably the most underestimated in comparison with other groups of cuckoo wasps in South America. Their relatively small size and peculiar parasitoid lifestyles contribute to these wasps being rarely collected and frequently misidentified as other hymenopterans. The new species herein described in Amiseginae and Cleptinae enhance our understanding of chrysidid diversity in a poorly known Brazilian region. These new findings also reinforce the importance of long-term collecting studies and strategic samplings, which provide precious audited data potentially useful for selecting priority areas for conservation.

Acknowledgements

We are grateful to the aforementioned curators who kindly loaned specimens or allowed us to study specimens in their collections, and to Lynn Kimsey (BME) and James Pitts (USU) for providing valuable information on the whereabouts of some type specimens. We are grateful to Sophie Cardinal (CNC) for providing images of types under her stewardship. We would like to thank Clemens Schindwein (UFMG, Belo Horizonte-MG), Daniell Fernandes (INPA, Manaus-AM), Freddy Bravo (UEFS, Feira de Santana-BA), and Juvenal Cordeiro Silva-Júnior (UESB, Jequié-BA), for donating part of specimens that substantially enriched this research. We would like to thank Bolívar Garcete-Barrett, Paolo Rosa, and one anonymous reviewer for their suggestions to refine this work. FCVZ acknowledges CNPq for supporting the research Project “Diversity, Ecology and Conservation of Hymenoptera in the semiarid region of Northeastern Brazil, with emphasis in bees”, which allowed long-term malaise sampling to be carried out in Santa Teresinha-PB. We are truly grateful to Pierre Landolt (Fazenda Tamanduá, Santa Teresinha-PB) for authorizing the realization of the entomofaunistic inventory carried out on his property. We express our cordial thanks to Emanuelle Brito, Francisca de Araújo, and Gisele Azevedo for kindly allowed us to use their images that illustrated diversity of habitats in Caatinga. We are thankful to Pedro Santos-Neto for collaborating during preliminary studies.

DAAL was supported by scholarships granted by the São Paulo Research Foundation (FAPESP): 2014/12407-0, 2015/12326-3, and 2017/25081-4; and FAPESP grant 2018/09666-5 to EABA. Additional funding for this research came from the CNPq (Brazilian National Council for Scientific and Technological Development) grants 422019/2018-6 and 310111/2019-6 and REFAUNA Program to EABA, and 501850/2009-0 to FCVZ. We are grateful for the support by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* – Brazil (CAPES) – Finance Code 001.

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Four new species of the genus *Carinostigmus* Tsuneki (Hymenoptera, Apoidea, Crabronidae) from Oriental China, with an updated key to the Chinese species

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Academic editor: Michael Ohl | Received 10 December 2020 | Accepted 8 February 2021 | Published 25 February 2021

<http://zoobank.org/6983B007-E436-4C51-B938-D2C00685139F>

Citation: Bashir NH, Li Q, Ma L (2021) Four new species of the genus *Carinostigmus* Tsuneki (Hymenoptera, Apoidea, Crabronidae) from Oriental China, with an updated key to the Chinese species. Journal of Hymenoptera Research 81: 87–107. <https://doi.org/10.3897/jhr.81.61892>

Abstract

The taxonomy of the apoid wasps of the genus *Carinostigmus* from China is studied, with description of four new species, namely *Carinostigmus capiconvergens* Bashir & Ma, **sp. nov.**, *Carinostigmus longivertex* Bashir & Ma, **sp. nov.**, *Carinostigmus rugipunctatus* Bashir & Ma, **sp. nov.**, and *Carinostigmus triangularis* Bashir & Ma, **sp. nov.** An updated key to the Chinese species of *Carinostigmus* is provided.

Keywords

Apoid wasps, identification key, Oriental region, Stigmina, taxonomy

Introduction

Carinostigmus was described by Tsuneki (1954) as subgenus of *Stigmus* Panzer, 1804, and was elevated to genus level by Bohart and Menke (1976). *Carinostigmus* belongs to the subfamily Pemphredoninae and currently comprises of 40 species and one subspecies worldwide. A huge diversity is found in the Oriental region with 23 species, followed by Afrotropical realm with 11 species and one subspecies; two species in this diversity are distributed across Palearctic region and four species are both Palearctic and

Oriental regions (Maidl 1925; Gussakovskij 1934; Tsuneki 1954, 1956, 1966, 1974, 1976, 1977; Bohart and Menke 1976; Krombein 1984; Bashir et al. 2020; Pulawski 2020; Rajan et al. 2020). To date, 13 species have been reported from China (Fig. 1), out of which 10 species are present in Oriental China, and 3 species are found in both Oriental and Palearctic China (Li and Yang 1995; Li and He 2004; Ma et al. 2012, 2018). Recently, three new species of *Carinostigmus*: *C. frontirugatus* Bashir & Ma, *C. latidentatus* Bashir & Ma, and *C. vesulcatus* Bashir & Ma and one new record *C. palawanensis* (Tsuneki) have been described from the Oriental region of China (Bashir et al. 2020).

The current study described four new species of *Carinostigmus* from China as: *C. capiconvergens* sp. nov., *C. longivertex* sp. nov., *C. rugipunctatus* sp. nov., and *C. triangularis* sp. nov. The key by Bashir et al. (2020) to the Chinese species of *Carinostigmus* is updated.

Materials and methods

Institutional abbreviations:

YNAU Insect Collections of Yunnan Agricultural University, Kunming, Yunnan, China;

ZJU Parasitic Hymenoptera Collection of Zhejiang University, Hangzhou, Zhejiang Province, China.

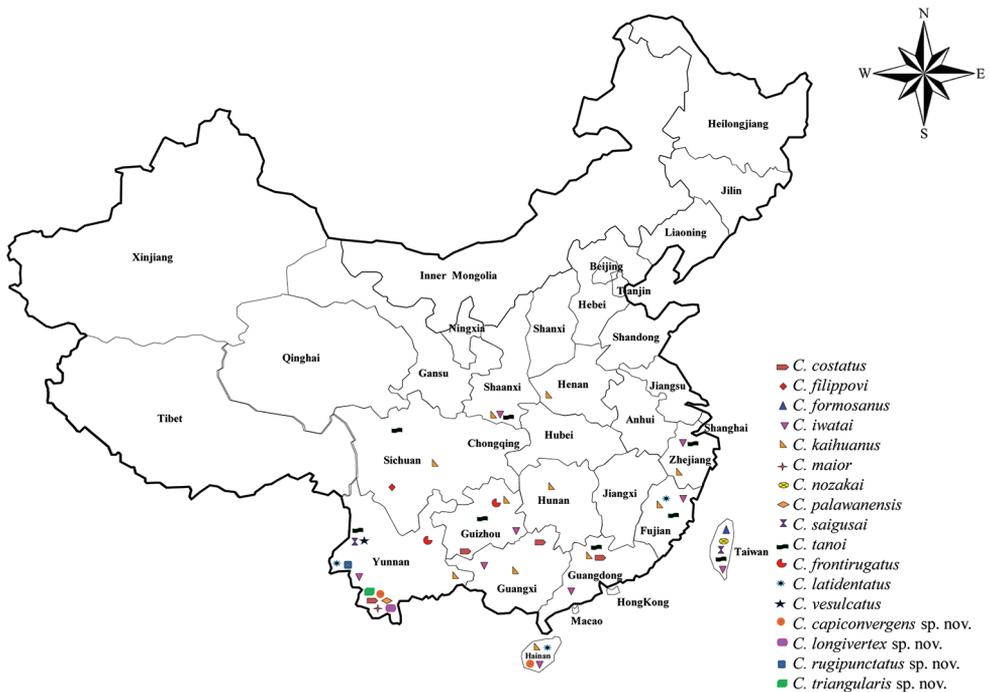


Figure 1. Map showing records of *Carinostigmus* from China (updated from Bashir et al. 2020).

Specimens were examined with an Olympus stereomicroscope (SZ Series) with an ocular micrometer. The images were taken with a Keyence VHX-5000 digital optical microscope (camera with resolution of 1600 × 1200 pixels), and edited with Adobe Photoshop 8.0. Measurements and ratio were acquired using an ocular scale on Olympus stereo microscope SZX2-TR30 at 2× and 5.4× magnification, respectively.

For terminology, we mainly followed Bohart and Menke (1976), Harris (1979), and Bashir et al. (2020). The descriptions are based on the holotypes only, and paratypes displaying some variation are given in square brackets in detail.

Taxonomy

Genus *Carinostigmus* Tsuneki, 1954

Type species. *Stigmus congruus* Walker, 1860; by original designation.

Carinostigmus capiconvergens Bashir & Ma, sp. nov.

<http://zoobank.org/47816309-3CC6-4422-9AA0-859DEFE1FAF6>

Figs 2, 6A, B

Type material. Holotype: ♀, China: Hainan: Jianfengling Mount, 18°41'N, 108°52'E, 6.VI.2007, 900–950 m, No. 200707957, coll. Jingxian Liu (ZJU). **Paratypes:** 1♀, same locality as holotype, 7.VI.2007, No. 200707997; 1♂, same locality and date as holotype, No. 200707955; 1♀, China: Yunnan: Jinghong: Menghai: Bulang Mountain, 21°56'N, 100°26'E, 2018.IV.24–V.17, No. 2019000007, coll. Malaise trap (YNAU).

Diagnosis. This new species can be separated from all other *Carinostigmus* species from China by the following character combinations: free margin of clypeus emarginate; median and lower frons with broad frontal median furrow; upper frons without longitudinal carina; occipital carina broad, distinctly foveolate; admedian line extending to half of scutum length; mesopleuron with longitudinal rugae posteriorly; propodeum with large smooth area medially. It is closely related to *C. iwatai* (Tsuneki, 1954), but differs from it in the following characters (characters of *C. iwatai* in brackets): inter-antennal tubercle short, less than midocellus diameter (inter-antennal tubercle long, more than midocellus diameter); ocellar triangle and vertex behind ocelli impunctate (ocellar triangle and vertex behind ocelli finely punctate); occipital carina broad as Fig. 2B, distinctly foveolate (occipital carina narrow as Fig. 3B, without foveolate); outer and inner orbital furrows narrow as Fig. 2A (outer and inner orbital furrows broad as Fig. 3A); pronotal collar without rugae (pronotal collar with sparse and sturdy rugae); scutum with transverse striations anteriorly (scutum without striations); parapsidal and admedian lines inconspicuous (parapsidal and admedian lines distinct); metanotum with dense, slender longitudinal rugae medially (metanotum without rugae medially); omaulus broad, 1.3× pedicel width (omaulus

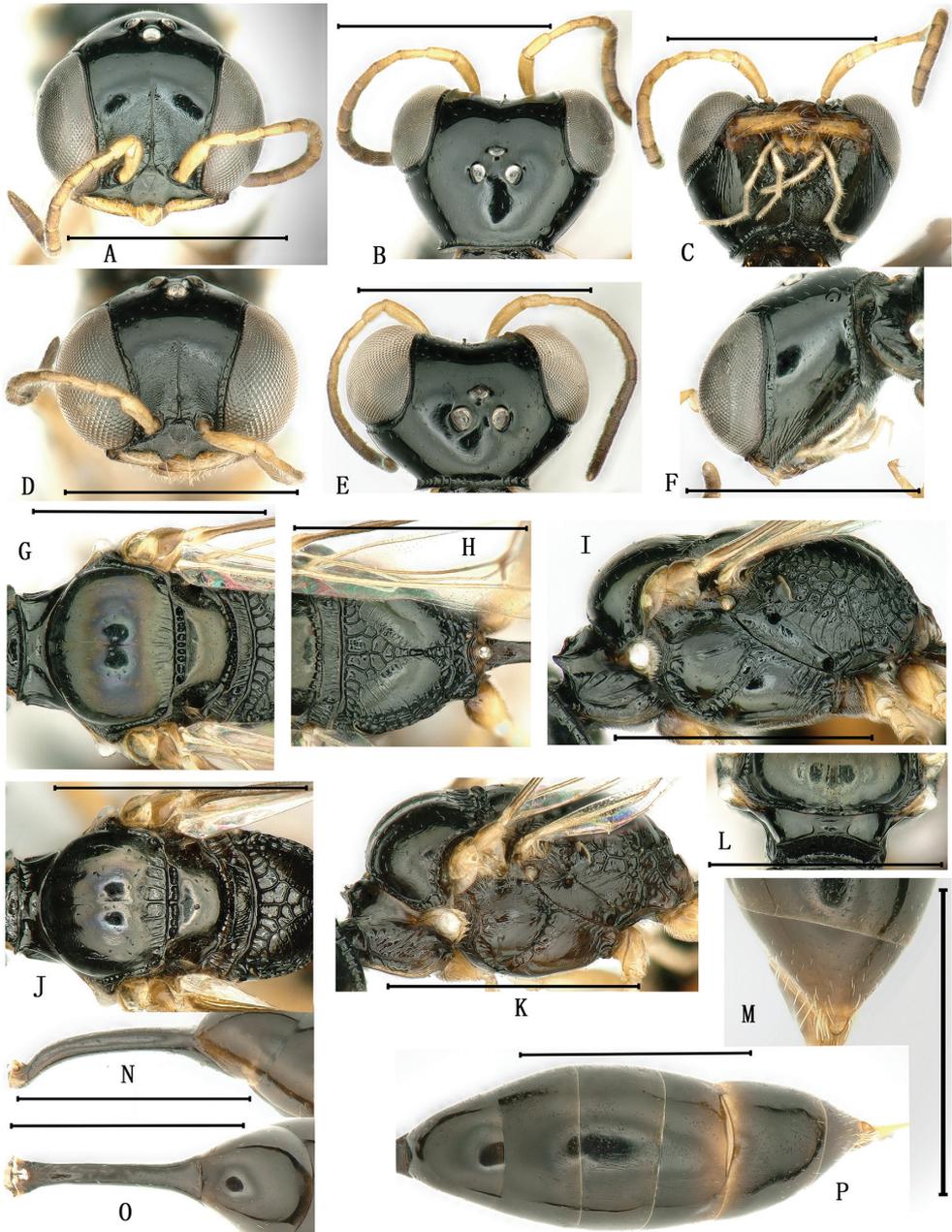


Figure 2. *Carinostigmus capiconvergens* Bashir & Ma, sp. nov. (A–C, F–I, L–P female D, E, J, K male) A, D head, frontal B, E head, dorsal C head, ventral F head, lateral G, J scutum, scutellum and metanotum, dorsal H propodeum, dorso-posterior I, K thorax, lateral L scutum anterior, dorsal M pygidial plate N petiole, lateral O petiole, dorsal P metasoma, dorsal. Scale bars: 1.0 mm.

narrow, 0.8× pedicel width); scrobal suture lacking (scrobal suture present, short as Fig. 4I); propodeum posterior with large smooth area medially (propodeum posterior with small smooth area medially); petiole laterally smooth (petiole laterally with few

weak carinae) and pygidial area smooth, u-shaped (pygidial area punctate medially, oval shaped).

Description. Female (Figs 2A–C, F–I, L–P, 6A): length of body 4.9 [4.5–5.0] mm.

Colouration. Body black and shiny, except for the following: mandible (reddish brown apically), labrum, scape, pedicel, flagellomeres I–IV (remainder reddish brown to dark brown), tegula, fore and mid femur extensively (remainder ivory to yellowish), hindleg (basal one third to half of tibia ivory, remainder dark brown) fulvous; palpi and pronotal lobe ivory; forewing veins dark brown; setae on mandible and margin of clypeus golden.

Head (Fig. 2A–C, F). Mandible tridentate apically; labrum pentagonal, gently round toward apex; clypeus nearly flat, with several coarse punctures medially; free margin of median lobe of clypeus narrowly produced, nearly truncate, with two small lateral teeth, lateral lobe with inconspicuous tooth (Fig. 2A); setae on margin of clypeus and mandible long, longer than labrum length; median and lower frons coriaceous, dull, with broad, deep frontal median furrow, a sturdy frontal median longitudinal carina, inter-antennal tubercle shorter than midocellus diameter; upper frons with several fine punctures, longitudinal carina lacking (Fig. 2A); ocellar triangle area impunctate, nearly flat; vertex behind ocelli impunctate (Fig. 2B); gena with several slender oblique transverse rugae near mandible area (Fig. 2F); lower gena with fine, sparse punctures medially (Fig. 2C); occipital carina broad, distinctly foveolate (Fig. 2B); inner orbital furrow narrow, with inner marginal carina distinct, coarsely foveolate (Fig. 2A); outer orbital furrow narrow, hind marginal carina slender, inconspicuously foveolate (Fig. 2F); length of scape: length of pedicel: length of flagellomere I: width of flagellomere I = 20: 8: 8: 3; maximum head width, dorsally: distance from upper margin of the frons to occipital carina in middle: distance from vertex to clypeal margin in middle = 64: 39: 51; maximum eye length, laterally: maximum eye width, laterally: maximum eye width, frontal view = 40: 22: 18; inner-orbital width, medially: outer-orbital width, medially: occipital carina width, dorsally = 2: 2: 2; maximum width of gena, laterally: distance between antennal sockets, frontal view = 16: 10; distance between inner margins of hind ocelli: distance between outer margin of hind ocellus and nearest inner eye margin: distance between posterior margin of hind ocellus and occipital carina, dorsally = 6: 13: 17.

Mesosoma (Fig. 2G–I, L). Pronotal collar smooth, slightly elevated medially, anterior pronotal ridge strong, lateral angles projected and sharp; width of collar in middle: length of collar in middle = 5: 30; mesoscutum with coarse, sparse punctures, several slender, transverse striations anteriorly (Fig. 2L), posterior area with sparse, strong, parallel ridges (Fig. 2G); admedian line inconspicuous, extending to nearly half of scutum length; notaulus inconspicuous [invisible], extending to anterior $\frac{1}{4}$ of scutum length; parapsidal line weakly impressed; scutellum with fine, sparse punctures; metanotum with dense, slender, longitudinal rugae (Fig. 2G); mesopleuron with several sturdy, short, longitudinal rugae posteriorly, hypoepimeral area with few slender, longitudinal rugae; omaulus and hypersternaulus broad, $1.3\times$ pedicel width, distinctly foveolate, scrobal suture lacking (Fig. 2I); propodeal enclosure shallowly impressed, triangular, with sturdy longitudinal rugae, median area reticulate; propodeum posterior with broad median groove, several fairly slender, oblique, longitudinal rugae anteriorly

[inconspicuously], large smooth area medially, and irregular reticulation posteriorly (Fig. 2H); propodeum laterally with several slender, oblique, longitudinal rugae anteriorly, and irregular, reticulation posteriorly (Fig. 2I).

Metasoma (Fig. 2M–P). Petiole dorsal nearly cylindrical, slightly convex, basal width equal as apical width (Fig. 2O), petiole laterally smooth (Fig. 2N); maximum width of petiole, dorsally: length of petiole, laterally: maximum length of metasomal tergum I, dorsally: maximum width of metasomal tergum I, dorsally = 6: 47: 30: 32; metasomal sterna IV–VI with dense, fine punctures, remaining sterna nearly impunctate (Fig. 2P); pygidial area smooth, depressed, u-shaped, apex truncate (Fig. 2M).

Male (Figs 2D, E, J, K, 6B). Similar to female except length of body 4.5 mm; mandible ivory (reddish brown apically); setae on mandible and margin of clypeus yellowish; mandible bidentate apically; clypeus slightly convex; setae on mandible short, less than labrum length; lower gena with irregular, rugulose medially; outer orbital furrow with hind marginal carina foveolate; length of scape: length of pedicel: length of flagellomere I: width of flagellomere I = 17: 6: 7: 2; maximum head width, dorsally: distance from upper margin of the frons to occipital carina in middle: distance from vertex to clypeal margin in middle = 60: 33: 45; maximum eye length, laterally: maximum eye width, laterally: maximum eye width, frontal view = 35: 20: 19; maximum width of gena, laterally: distance between antennal sockets, frontal view = 14: 7; distance between inner margins of hind ocelli: distance between outer margin of hind ocellus and nearest inner eye margin: distance between posterior margin of hind ocellus and occipital carina, dorsally = 5: 11: 14; width of collar in middle: length of collar in middle = 5: 24; maximum width of petiole, dorsally: length of petiole, laterally: maximum length of metasomal tergum I, dorsally: maximum width of metasomal tergum I, dorsally = 5: 45: 25: 25; sterna III–VI with sparse, fine punctures.

Distribution. China (Hainan, Yunnan).

Etymology. The name, *capiconvergens*, is derived from the Latin words *caput* (= head) and *con-vergo* (= converge), referring to the head from above with temples distinctly convergent posteriorly.

***Carinostigmus longivertex* Bashir & Ma, sp. nov.**

<http://zoobank.org/D5C82AC9-573C-4865-91C5-2D392A3E6EDA>

Figs 3, 6C

Type material. Holotype: ♀, China: Yunnan: Mengla: Wangtianshu Forest Park, 21°34'N, 101°39'E, 1.V.2005, coll. Peng Wang (YNAU). **Paratypes:** 2♀, same data as holotype; 1♀, China: Yunnan: Dehong: Nabang, 24°26'N, 98°35'E, 15.V.2009, No. 201005193, coll. Jie Zeng (YNAU).

Diagnosis. Within Chinese *Carinostigmus*, this new species can be separated by the following character combinations: free margin of clypeus conspicuously produced, slightly emarginated in middle; median and lower frons with shallow frontal median furrow; inter-antennal tubercle distinctly broadened at apex; vertex behind ocelli finely punctate; lower gena coarsely punctate; occipital carina without foveolate; anterior

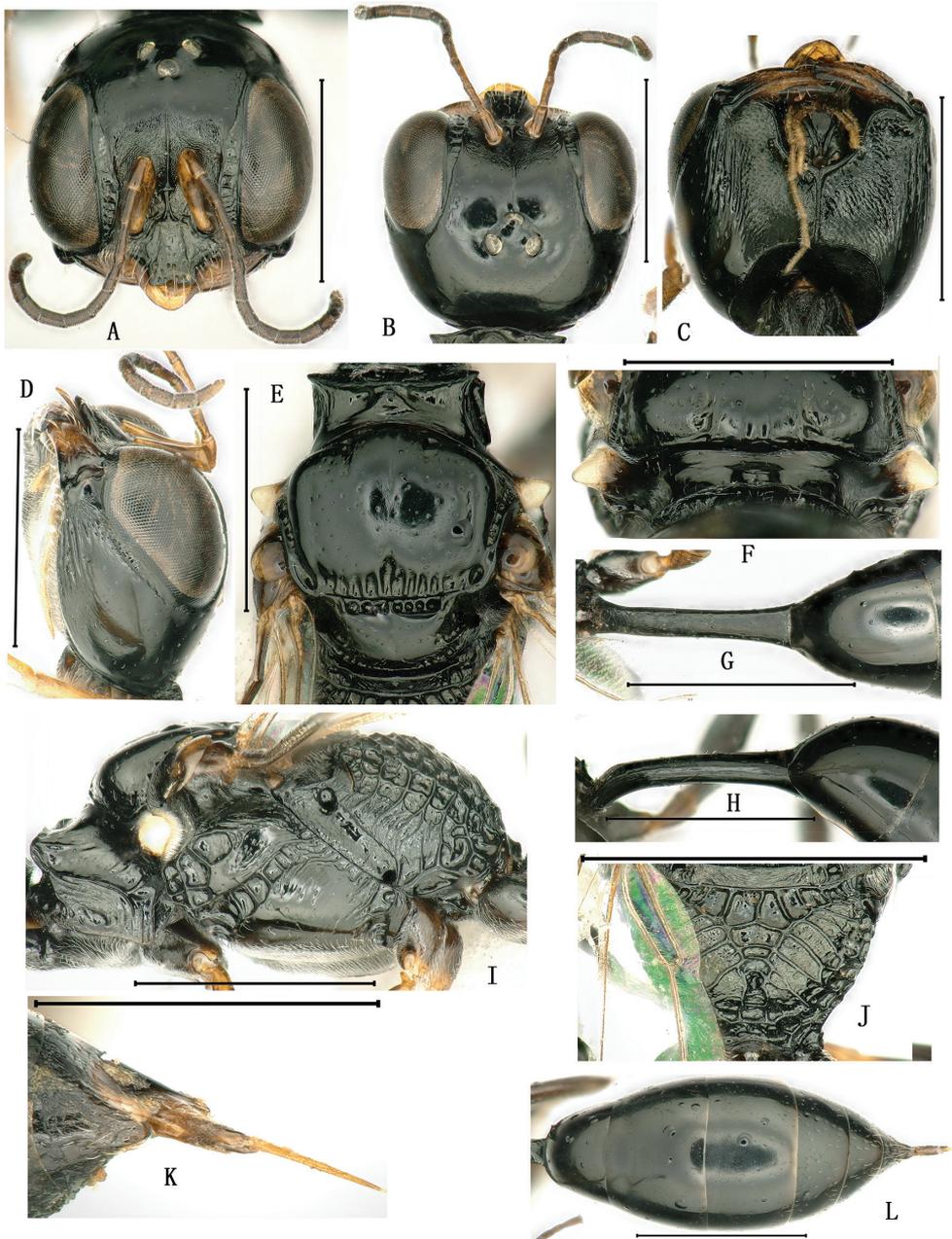


Figure 3. *Carinostigmus longivertex* Bashir & Ma, sp. nov. (female) **A** head, frontal **B** head, dorsal **C** head, ventral **D** head, lateral **E** scutum, scutellum and metanotum, dorsal **F** scutum anterior, dorsal **G** petiole, dorsal **H** petiole, lateral **I** thorax, lateral **J** propodeum, dorso-posterior **K** pygidial plate **L** metasoma, dorsal. Scale bars: 1.0 mm.

pronotal ridge narrowly emarginate in middle; scutellum median line impressed; propodeum with small smooth area medially; basal half of petiole with transverse striations; petiole laterally carinate. *Carinostigmus longivertex* sp. nov. resembles *C. iwatai*

(Tsuneki) 1954, but can be distinguished from it by the following characters (characters of *C. iwatai* in brackets): free margin of clypeus emarginated medially (free margin of clypeus truncate medially); inter-antennal tubercle distinctly broadened at apex as Fig. 3B (inter-antennal tubercle not broad as Fig. 4B); lower gena with coarse, dense punctures mixed with dense, irregular, longitudinal rugae medially (lower gena with fine, sparsely punctured, without rugae); outer orbital furrow narrow as Fig. 3D (outer orbital furrow broad); admedian and parapsidal lines inconspicuous (admedian and parapsidal lines distinct); notaulus deeply grooved (notaulus shallowly grooved); metanotum median area with dense, slender, transverse rugae (metanotum without rugae); omaulus broad, 1.2× pedicel width (omaulus narrow, 0.8× pedicel width); basal half of petiole with dense, slender, irregular transverse striations dorsally (petiole without striations) and pygidial area u-shaped (pygidial area oval shaped).

Description. Female (Figs 3, 6C): length of body 6 [5.7–6.1] mm.

Colouration. Body black and shiny, except for the following: mandible (black basally and apically) and labrum reddish brown; palpi, scape and pedicel fulvous; flagellomeres reddish brown to dark brown; pronotal lobe ivory; tegula and forewing veins dark brown; fore trochanter, base and apex of femur, tibia, tarsi fulvous to reddish brown (remainder dark brown to black); mid trochanter, base and apex of femur, tibia extensively, tarsi fulvous; hind trochanter reddish brown, tibia and tarsus dark brown; setae on mandible and margin of clypeus yellowish.

Head (Fig. 3A–D). Mandible tridentate apically; labrum pentagonal, gently round toward apex; clypeus moderately convex, with sparse, coarse punctures; free margin of clypeus conspicuously produced with two distinctly triangular teeth, slightly emarginated in middle (Fig. 3A); setae on mandible and margin of clypeus long, more than labrum length; median and lower frons coriaceous, with broad, shallow frontal median furrow, a strong frontal median longitudinal carina, inter-antennal tubercle distinctly broadened at apex, long, more than midocellus diameter; upper frons with sparse, fine punctures, longitudinal carina distinct anteriorly, not reaching anterior ocellus (Fig. 3A); ocellar triangle area nearly flat, finely punctate; vertex behind ocelli with fine punctures (Fig. 3B); gena with sparse, coarse punctures and several sturdy, long, oblique transverse rugae (Fig. 3D); lower gena with coarse, dense punctures mixed with dense, irregular, longitudinal rugae medially [weakly] (Fig. 3C); occipital carina narrow, without foveolate (Fig. 3B); inner orbital furrow broad as 0.8× pedicel length, with inner marginal carina distinct, coarsely foveolate (Fig. 3A); outer orbital furrow narrow as 0.5× pedicel length, hind marginal carina inconspicuous, finely foveolate (Fig. 3D); length of scape: length of pedicel: length of flagellomere I: width of flagellomere I = 26: 10: 11: 3; maximum head width, dorsally: distance from upper margin of the frons to occipital carina in middle: distance from vertex to clypeal margin in middle = 82: 57: 65; maximum eye length, laterally: maximum eye width, laterally: maximum eye width, frontal view = 51: 30: 19; inner-orbital width, medially: outer-orbital width, medially: occipital carina width, dorsally = 5: 3: 1; maximum width of gena, laterally: distance between antennal sockets, frontal view = 28: 15; distance between inner margins of hind ocelli: distance between outer margin of hind ocellus

and nearest inner eye margin: distance between posterior margin of hind ocellus and occipital carina, dorsally = 7: 16: 29.

Mesosoma (Fig. 3E, F, I, J). Pronotal collar strongly elevated medially, triangular, with several inconspicuous rugae laterally, anterior pronotal ridge strong, narrowly emarginate in middle, lateral angles strong and distinctly projected (Fig. 3E); width of collar in middle: length of collar in middle = 9: 45; mesoscutum with coarse, sparse punctures, anterior without striations (Fig. 3F), posterior area with several strong parallel ridges, longer in middle than laterally (Fig. 3E); admedian line inconspicuous [sometimes visible], extending to half of scutum length; notaulus deeply grooved, extending to anterior $\frac{1}{4}$ of scutum length (Fig. 3F); parapsidal line weakly impressed; scutellum with fine, sparse punctures, median line impressed; metanotum with several sturdy, longitudinal rugae on lateral area, median area with dense, slender, transverse rugae (Fig. 3E); mesopleuron with coarse, sparse punctures, hypoepimeral and posterior area with slender or sturdy, dense, long longitudinal rugae posteriorly; scrobal suture, omaulus and hypersternaulus broad and distinct, foveolate, scrobal suture short, incomplete (Fig. 3I); propodeal enclosure moderately impressed, triangular, with sturdy longitudinal rugae, median area reticulate; propodeum posterior with broad shallow median groove, sparse, sturdy, oblique longitudinal rugae anteriorly, small smooth area medially, and irregular reticulation posteriorly (Fig. 3J); propodeum laterally with dense or sparse sturdy, oblique, longitudinal rugae anteriorly, and irregular, reticulation posteriorly (Fig. 3I).

Metasoma (Fig. 3G, H, K, L). Petiole dorsal nearly cylindrical, slightly convex and widened toward apex slightly, basal half of petiole with dense, slender, irregular transverse striations (Fig. 3G), petiole laterally with few weak carinae (Fig. 3H); maximum width of petiole, dorsally: length of petiole, laterally: maximum length of metasomal tergum I, dorsally: maximum width of metasomal tergum I, dorsally = 8: 60: 37: 42; metasomal sterna IV–VI with fine, dense punctures, remainder nearly impunctate (Fig. 3L); pygidial area coriaceous, depressed, u-shaped, apex truncate (Fig. 3K).

Male. Unknown.

Distribution. China (Yunnan).

Etymology. The name, *longivertex*, is derived from the Latin words *longus* (= long) and *vertex* (= vertex), referring to the very long vertex, with the ocello-occipital distance (OCD) being four times as long as postocellar distance (POD), 29 : 7. A noun in apposition.

***Carinostigmus rugipunctatus* Bashir & Ma, sp. nov.**

<http://zoobank.org/6C5145C9-D153-4163-9878-7C9B24886E0A>

Figs 4, 6D, E

Type material. **Holotype:** ♀, China: Yunnan: Jinghong: Xishuangbanna National Forest Park, 22°01'N, 100°52'E, 31.VII.2003, 600–1000 m, coll. Qiang Li (YNAU). **Paratypes:** 6♀8♂, same locality and date as holotype, coll. Tingjing Li, Peng Wang (ZJU); 1♂, China: Yunnan: Lincang, 23°53'N, 100°05'E, 3.V.2005, coll. Baoxin Dong; 1♂, China: Yunnan: Ruili, 24°01'N, 97°51'E, 29.IV.1981, coll. Junhua He (YNAU).

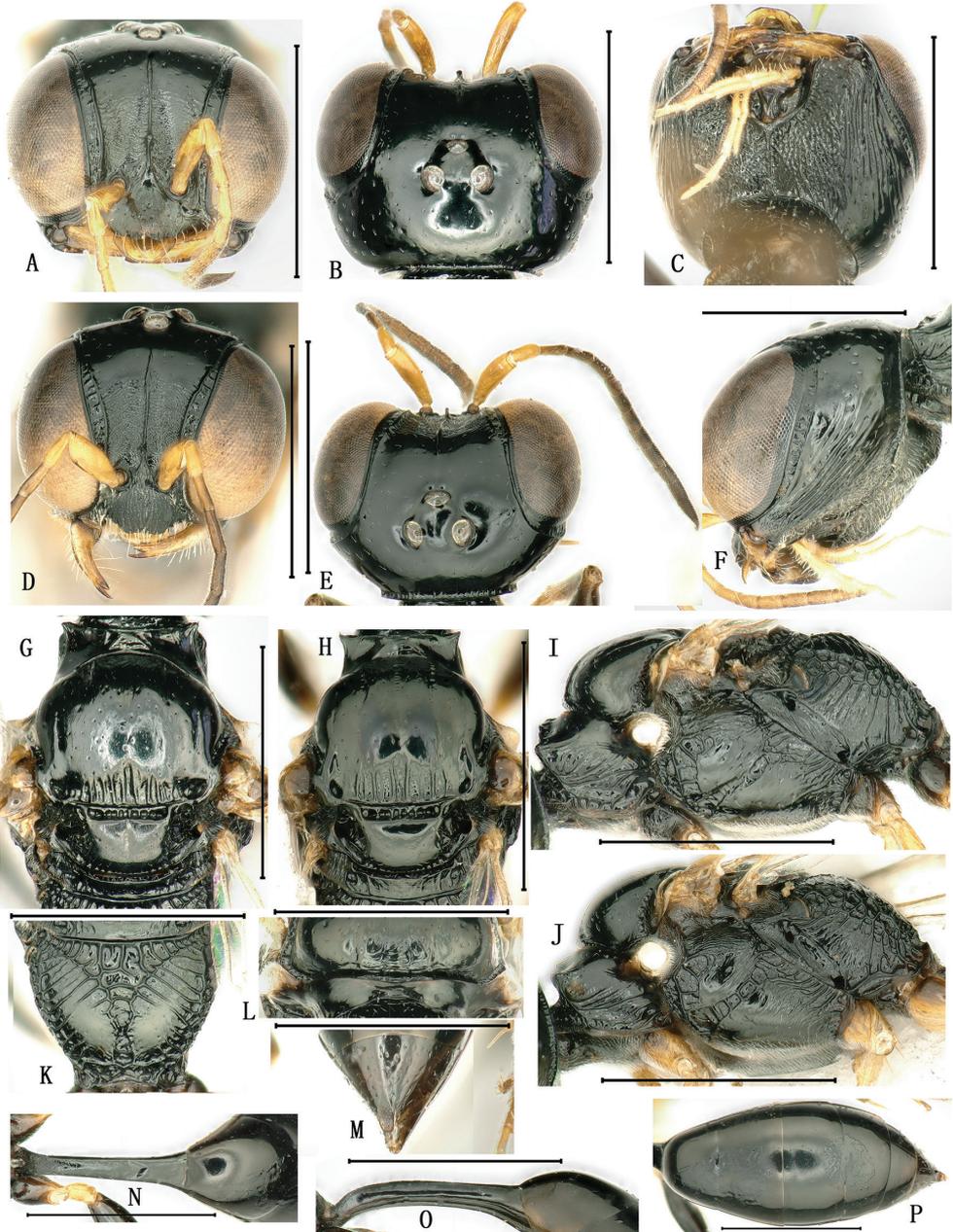


Figure 4. *Carinostigmus rugipunctatus* Bashir & Ma, sp. nov. (A–C, F, G, I, K–P female D, E, H, J male) A, D head, frontal B, E head, dorsal C head, ventral F head, lateral G, H scutum, scutellum and metanotum, dorsal I, J thorax, lateral K propodeum, dorso-posterior L scutum anterior, dorsal M pygidial plate N petiole, dorsal O petiole, lateral P metasoma, dorsal. Scale bars: 1.0 mm.

Diagnosis. This new species can be separated from all other *Carinostigmus* species from China by the following character combinations: mandible reddish brown basally; legs largely fulvous; labrum truncate apically; free margin of median lobe of clypeus

broadly produced; median frons rugulose; occipital carina broad, without foveolate; lateral angles of pronotal collar not projected; metanotum smooth medially; propodeum with large smooth area medially; petiole dorsal with irregularly weak rugae basally; pygidial area depressed. It is closely related to *C. congruus* (Walker) 1860, but differs from it in the following characters (states of *C. congruus* in brackets): inter-antennal tubercle without T-shape at apex (inter-antennal tubercle with T-shaped at apex); inner orbital furrow inconspicuously foveolate (inner orbital furrow distinctly foveolate); pronotal ridge strongly marked (pronotal ridge weakly marked), slightly emarginate in middle (not emarginate in middle); occipital carina broad as Fig. 4B (occipital carina narrow as Fig. 3B); lower gena with dense, sturdy, longitudinal rugae medially (lower gena without rugae medially); notaulus inconspicuously grooved, extending to one third of scutum length (notaulus strongly impressed, extending to anterior $\frac{1}{4}$ of scutum length); propodeum with smaller smooth area than *C. congruus*; petiole dorsal with few irregularly weak rugae basally (petiole dorsal without rugae); petiole laterally smooth (petiole laterally with a few weak carinae or smooth) and pygidial area u-shaped (pygidial area oval shaped).

Description. Female (Figs 4A–C, F, G, I, K–P, 6D): length of body 5.4 [5.2–5.5] mm.

Colouration. Body black and shiny, except for the following: mandible (reddish brown basally and apically), labrum, palpi, scape, pedicel, flagellomere I–III (remainder dark brown) and tegula fulvous; pronotal lobe ivory; forewing veins dark brown; fore trochanter, base and apex of femur, tibia, tarsi yellowish to fulvous (remainder dark brown); mid trochanter, base and apex of femur, tibia extensively, tarsi fulvous (remainder dark brown); hind trochanter, basal one third of tibia fulvous (remainder dark brown); setae on mandible and margin of clypeus golden.

Head (Fig. 4A–C, F). Mandible tridentate apically; labrum pentagonal, truncate apically; clypeus moderately convex, with sparse, fine mixed with coarse punctures; free margin of median lobe of clypeus broadly produced and emarginate apically, with four teeth, median teeth distinct, subquadrate, slightly turnup, lateral teeth small, inconspicuous [sometimes lacking] (Fig. 4A); setae on mandible and margin of clypeus sparse, long, more than labrum length; median and lower frons coriaceous, rugulose on median frons, with broad, shallow frontal median furrow, a sturdy frontal median longitudinal carina, inter-antennal tubercle short, less than midocellus diameter; upper frons with sparse, fine punctures, longitudinal carina distinct anteriorly, not reaching anterior ocellus (Fig. 4A); ocellar triangle area slightly convex, several finely punctate; vertex behind ocelli with sparsely finely punctate (Fig. 4B); gena with dense, slender, long, oblique transverse rugae (Fig. 4F); lower gena with coarse, dense punctures mixed with dense, sturdy, longitudinal rugae medially (Fig. 4C); occipital carina broad, without foveolate (Fig. 4B); inner orbital furrow broad, with inner marginal carina distinct, inconspicuously foveolate (Fig. 4A); outer orbital furrow narrow, hind marginal carina distinct, finely foveolate (Fig. 4F); length of scape: length of pedicel: length of flagellomere I: width of flagellomere I = 21: 7: 9: 3; maximum head width, dorsally: distance from upper margin of the frons to occipital carina in middle: distance from vertex to clypeal margin in middle = 70: 45: 61; maximum eye length, laterally: maximum eye width, laterally: maximum eye width, frontal view = 47: 25: 20; inner-orbital width,

medially: outer-orbital width, medially: occipital carina width, dorsally = 3: 3: 1; maximum width of gena, laterally: distance between antennal sockets, frontal view = 13: 9; distance between inner margins of hind ocelli: distance between outer margin of hind ocellus and nearest inner eye margin: distance between posterior margin of hind ocellus and occipital carina, dorsally = 6: 14: 19.

Mesosoma (Fig. 4G, I, K, L). Pronotal collar moderately elevated medially, anterior pronotal ridge strong, lateral angles blunt and not projected (Fig. 4G); width of collar in middle: length of collar in middle = 5: 35; mesoscutum with fine, sparsely mixed with sparse, coarse punctures, with several, slender, transverse striations anteriorly [striations sometimes lacking] (Fig. 4L), posterior area with sparse, strong parallel ridges (Fig. 4G); admedian line inconspicuous, extending to one third of scutum length; notaulus inconspicuously grooved, extending to one third of scutum length (Fig. 4L); parapsidal line weakly impressed; scutellum with fine, sparse punctures; metanotum with sparse, slender, longitudinal rugae on lateral area, median area smooth (Fig. 4G); mesopleuron with coarse, sparsely punctures, hypopleural and posterior area with slender or sturdy, dense, long, longitudinal rugae posteriorly; scrobal suture, omaulus and hypersternaulus broad and distinct, foveolate, scrobal suture short, incomplete (Fig. 4I); propodeal enclosure shallowly impressed, triangular, with sturdy longitudinal rugae, median area reticulate; propodeum posterior with broad shallow median groove, sparse sturdy, oblique, longitudinal rugae anteriorly, large smooth area medially, and irregular reticulation posteriorly (Fig. 4K); propodeum laterally with several, sturdy, oblique, longitudinal rugae anteriorly and medially, and irregular reticulation posteriorly (Fig. 4I).

Metasoma (Fig. 4M–P). Petiole dorsal slightly convex and widened toward apex slightly, with few irregularly weak rugae basally (Fig. 4N), petiole laterally smooth (Fig. 4O); maximum width of petiole, dorsally: length of petiole, laterally: maximum length of metasomal tergum I, dorsally: maximum width of metasomal tergum I, dorsally = 8: 50: 32: 37; metasomal sterna IV–VI with dense, fine punctures, remainder nearly impunctate (Fig. 4P); pygidial area coriaceous, depressed, u-shaped, apex truncate (Fig. 4M).

Male (Figs 4D, E, H, J, 6E). Similar to female except length of body 5–5.4 mm; labrum and palpi yellowish; tegula dark brown; setae on mandible and margin of clypeus silvery and yellowish, respectively; mandible bidentate apically; labrum subquadrate; clypeus slightly convex, with dense, fine punctures; free margin of clypeus slightly emarginate medially; occipital carina broader than female, distinctly foveolate; length of scape: length of pedicel = 19: 8; maximum head width, dorsally: distance from upper margin of the frons to occipital carina in middle: distance from vertex to clypeal margin in middle = 65: 40: 53; maximum eye length, laterally: maximum eye width, laterally: maximum eye width, frontal view = 43: 28: 21; maximum width of gena, laterally: distance between antennal sockets, frontal view = 11: 7; distance between inner margins of hind ocelli: distance between outer margin of hind ocellus and nearest inner eye margin: distance between posterior margin of hind ocellus and occipital carina, dorsally = 7: 11: 12; width of collar in middle: length of collar in middle = 5: 30; maximum width of petiole, dorsally: length of petiole, laterally: maximum length of metasomal

tergum I, dorsally: maximum width of metasomal tergum I, dorsally = 7: 50: 28: 27; metasomal sterna III–VI with sparse, fine punctures, remaining nearly impunctate.

Distribution. China (Yunnan).

Etymology. The name, *rugipunctatus*, is derived from the Latin words *ruga* (= ruga, wrinkle) and *punctum* (= puncture), referring to the lower gena with coarse punctures mixed with rugae.

***Carinostigmus triangularis* Bashir & Ma, sp. nov.**

<http://zoobank.org/0E13FFC2-B720-4230-BE94-D5F6881C2A51>

Figs 5, 6F

Type material. *Holotype*: ♀, China: Yunnan: Jinghong: Menghai: Bulang Mountain, 21°56'N, 100°26'E, 20.VI.2018–20.VII.2018, No. 2019000381, coll. Malaise trap (YNAU); *Paratypes*: 3♀, same locality as holotype, No. 2019000378, 17.V.2018–20.VI.2018, No. 2019000151, 25.IV.2018–17.V.2018, No. 20190329.

Diagnosis. Differs from Chinese species of *Carinostigmus* by the following features: flagellomeres I–II fulvous; legs largely black; labrum distinctly constricted subapically; free margin of clypeus slightly turnup apically; inter-antennal tubercle longer than midocellus diameter, slightly broadened at apex; lower gena rugose; pronotal collar with transverse striations medially; metanotum with striations medially; petiole dorsal carinate basally and medially; pygidial area elongate. The new species resembles *C. costatus* Krombein 1984, but can be distinguished it by the following characters (characters of *C. costatus* in brackets): labrum distinctly triangular, broader than *C. costatus* (labrum pentagonal, narrower than new species); clypeus moderately convex (clypeus slightly convex); median and lower frons with transverse, irregular rugae medially and laterally (median and lower frons without rugae); inter-antennal tubercle longer than midocellus diameter, without T-shape projection (inter-antennal tubercle shorter than midocellus diameter, with T-shape projection); upper frons longitudinal carina without groove before anterior ocellus (upper frons with a weak groove before anterior ocellus); vertex behind ocelli impunctate (vertex behind ocelli punctate); lower gena with dense rugae near midventral line (lower gena without rugae near midventral line); pronotal collar lateral angles projected (pronotal collar lateral angles blunt); notaulus shallowly grooved, extending to one third of scutum length (notaulus deeply grooved, extending to half of scutum length); parapsidal line distinct (parapsidal line weakly impressed); metanotum with transverse striations medially (metanotum without striations medially); propodeal enclosure without triangular impression (propodeal enclosure with triangular impression); propodeum laterally with densely rugose (propodeum laterally sparsely rugose); petiole sub quadrate dorsally (petiole cylindrical) and pygidial area very elongate (pygidial area not elongate as new species).

Description. Female (Figs 5, 6F): length of body 5.5 [5.2–5.7] mm.

Colouration. Body black and shiny, except for the following: mandible ivory to fulvous (black basally and apically); labrum ivory with dark brown sides; palpi,

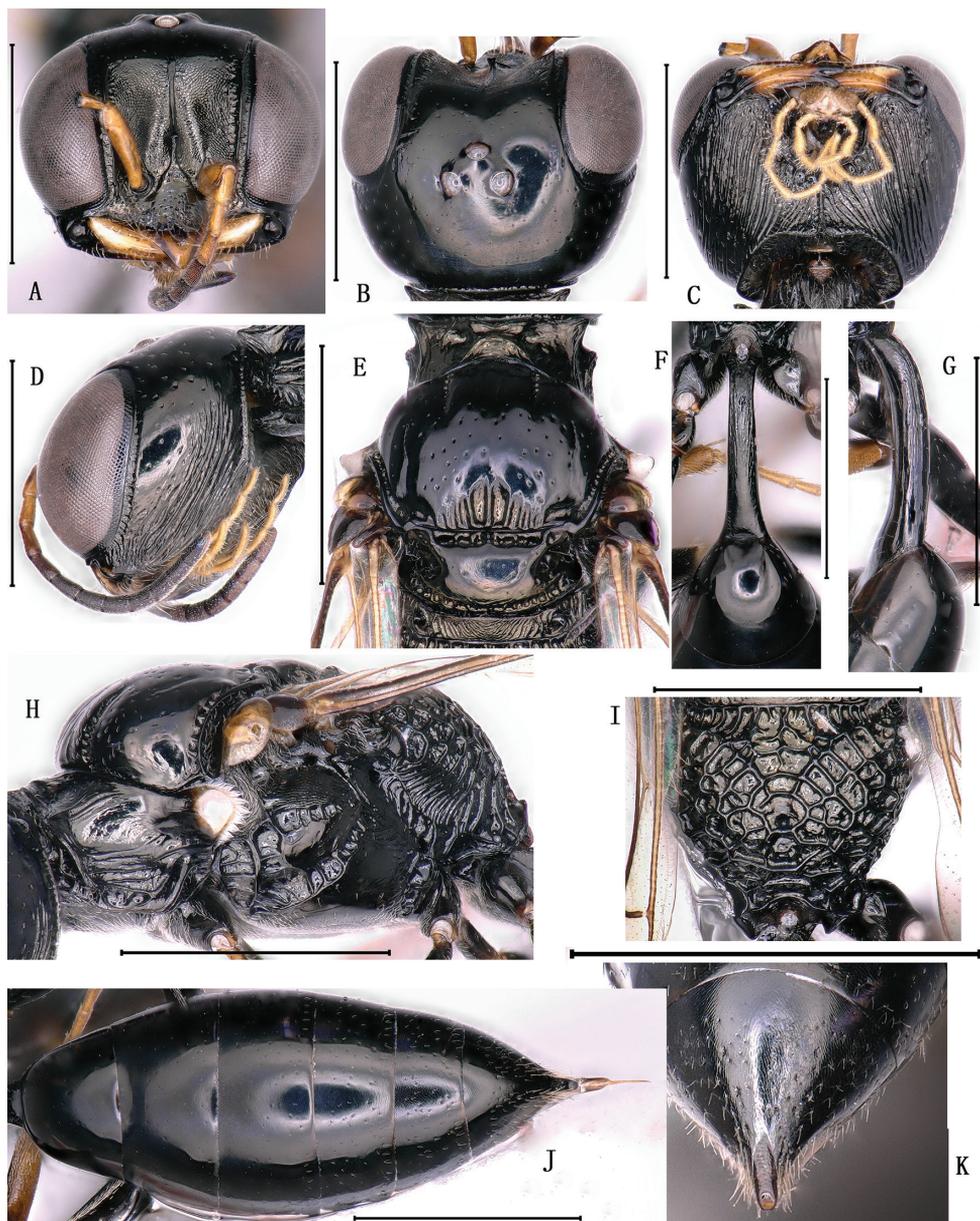


Figure 5. *Carinostigmus triangularis* Bashir & Ma, sp. nov. (female) **A** head, frontal **B** head, dorsal **C** head, ventral **D** head, lateral **E** scutum, scutellum and metanotum, dorsal **F** petiole, dorsal **G** petiole, lateral **H** thorax, lateral **I** propodeum, dorso-posterior **J** metasoma, dorsal **K** pygidial plate. Scale bars: 1.0 mm.

scape, pedicel and flagellomeres I–II (remainder dark brown) fulvous; pronotal lobe white; tegula and forewing veins dark brown; legs black (fore tibia, fore and mid tarsus fulvous, mid tibia and hind tarsus reddish brown); setae on margin of clypeus and mandible yellowish.



Figure 6. **A, B** *Carinostigmus capiconvergens* Bashir & Ma, sp. nov. (**A** female **B** male) **C** *Carinostigmus longivertex* Bashir & Ma sp. nov. (female) **D, E** *Carinostigmus rugipunctatus* Bashir & Ma sp. nov. (**D** female **E** male) **F** *Carinostigmus triangularis* Bashir & Ma, sp. nov. (female) **A–F** lateral view. Scale bars: 1.0 mm.

Head (Fig. 5A–D). Mandible tridentate apically; setae on mandible sparse and long; labrum triangular, round at apex, distinctly constricted subapically; clypeus moderately convex, with sparse, coarse punctures; setae on margin of clypeus sparse and long; free margin of clypeus truncate medially, slightly turnup apically (Fig. 5A); median and lower frons distinctly coriaceous, with transverse, irregular rugae medially and laterally, and with a sturdy frontal median longitudinal carina; inter-antennal tubercle longer than midocellus diameter, slightly broadened at apex; upper frons with sparse, fine punctures, longitudinal carina distinct anteriorly, not reaching anterior ocellus (Fig. 5A); ocellar triangle nearly flat, half mat, with fine, sparse punctures; vertex behind ocelli half mat, nearly impunctate (Fig. 5B); gena with several, sturdy, oblique, transverse rugae medially (Fig. 5D); lower gena with dense, sturdy, regular rugae (Fig. 5C); occipital carina narrow, inconspicuously foveolate (Fig. 5B); inner orbital furrow narrow, with inner marginal carina distinct, inconspicuously foveolate (Fig. 5A); outer orbital furrow narrow, hind marginal carina distinct, inconspicuously foveolate (Fig. 5D); length of scape: length of pedicel: length of flagellomere I: width of flagellomere I = 23: 7: 7: 4; maximum head width, dorsally: distance from upper margin of the frons to occipital carina in middle: distance from vertex to clypeal margin in middle = 75: 48: 56; maximum

eye length, laterally: maximum eye width, laterally: maximum eye width, frontal view = 47: 21: 18; inner-orbital width, medially: outer-orbital width, medially: occipital carina width, dorsally = 3: 2: 1; maximum width of gena, laterally: distance between antennal sockets, frontal view = 28: 14; distance between inner margins of hind ocelli: distance between outer margin of hind ocellus and nearest inner eye margin: distance between posterior margin of hind ocellus and occipital carina, dorsally = 7: 14: 24.

Mesosoma (Fig. 5E, H, I). Pronotal collar strongly elevated medially, with transverse striations medially, anterior pronotal ridge strong, lateral angles sharp and projected (Fig. 5E); width of collar in middle: length of collar in middle = 11: 36; mesoscutum with coarse punctures, sparsely distributed, posterior area with dense, strong parallel ridges; admedian line weakly impressed, extending to anterior ¼ of scutum length; notaulus shallowly grooved and foveolate, extending to one third of scutum length; parapsidal line distinct; scutellum with coarse, sparse punctures; metanotum with sparse, sturdy longitudinal rugae laterally, and dense transverse striations medially (Fig. 5E); mesopleuron with fine, sparsely punctures, hypoepimeral area with dense, irregular weak striations, posterior area with contiguous, long rugae; omaulus broadened, 1.2× pedicel length; hypersternaulus broadened anteriorly, 1.3× pedicel length, narrow apically, 0.5× pedicel length, distinctly foveolate; scrobal suture long, complete (Fig. 5H); propodeal enclosure deeply impressed, sub triangular, with sturdy, longitudinal rugae anteriorly, reticulate medially; propodeum posterior with sturdy, irregular reticulation (Fig. 5I); propodeum laterally with sparse, sturdy, oblique, longitudinal rugae anteriorly, and irregular reticulation posteriorly (Fig. 5H).

Metasoma (Fig. 5F, G, J, K). Petiole dorsal sub quadrate (not cylindrical), carinate basally and medially, basal width of petiole narrower to its apical width (Fig. 5F), petiole laterally with few weak carinae (Fig. 5G); maximum width of petiole, dorsally: length of petiole, laterally: maximum length of metasomal tergum I, dorsally: maximum width of metasomal tergum I, dorsally = 8: 54: 24: 30; metasoma half mat, with fine, sparse punctures (Fig. 5J); pygidial area smooth, very elongate, 1.8× pedicel length (Fig. 5K).

Male. Unknown

Distribution. China (Yunnan).

Etymology. The name, *triangularis*, is derived from the Latin word *triangulus* (= triangle), referring to the triangular shape of the labrum.

Key to the Chinese species of the genus *Carinostigmus* Tsuneki

Note: The key is updated from Bashir et al. (2020). Female of *C. nozakai* Tsuneki, and males of *C. frontirugatus* Bashir & Ma, *C. longivertex* sp. nov. and *C. triangularis* sp. nov. are unknown.

- 1 Ten flagellomeres; metasoma with six segments (Fig. 5J); mandible tridentate apically; female **2**
- Eleven flagellomeres; metasoma with seven segments; mandible bidentate apically (Fig. 4D); male **17**
- 2 Scrobal sulcus present (Fig. 5H) **3**
- Scrobal sulcus absent or inconspicuous (Fig. 2I)..... **11**

- 3 Propodeal posterior extensively covered by reticulated well-marked ridges (Fig. 5I); smooth areas absent..... 4
- Propodeal posterior with median groove, several slender or sturdy longitudinal rugae anteriorly; small or large smooth area medially (Fig. 3J)..... 7
- 4 Frontal line reaching anterior ocellus; petiole smooth dorsally and laterally, without striations (Oriental region)..... *C. formosanus* (Tsuneki)
- Frontal line not reaching anterior ocellus (Fig. 5B); weak transversal or longitudinal striations on petiole dorsal surface (Fig. 5F), few inconspicuous carina or groove on petiole lateral surface (Fig. 5G) 5
- 5 Labrum triangular (Fig. 5A); vertex behind ocelli impunctate (Fig. 5B); propodeal enclosure without triangular impression (Fig. 5I); pygidial area very elongate (Fig. 5K) (Oriental region)..... *C. triangularis* sp. nov.
- Labrum pentagonal (Fig. 2A); vertex behind ocelli finely punctate; propodeal enclosure with triangular impression; pygidial area oval or u-shaped 6
- 6 Inter-antennal tubercle long, equal or more than midocellus diameter; gena punctate ventrally; lateral surface of petiole with two distinct lateral carinae; pygidial area oval shaped (Oriental region) *C. maior* (Maidl)
- Inter-antennal tubercle shorter than midocellus diameter; gena rugate ventrally; lateral surface of petiole with few weak carinae; pygidial area u-shaped (Oriental region) *C. costatus* Krombein
- 7 Free margin of clypeal lobe emarginated 8
- Free margin of clypeal lobe truncate medially..... 10
- 8 Free margin of clypeal lobe deeply emarginated (Oriental region) *C. palawanensis* (Tsuneki)
- Free margin of clypeal lobe slightly emarginated (Figs 3A, 4A)..... 9
- 9 Propodeum posteriorly with small smooth area medially (Fig. 3J); petiole laterally with few weak carinae (Fig. 3H); notaulus deeply grooved (Fig. 3F); occipital carina narrow (Fig. 3B); inter-antennal tubercle distinctly broadened at apex, long (Fig. 3B) (Oriental region) *C. longivertex* sp. nov.
- Propodeum posteriorly with large smooth area medially (Fig. 4K); petiole laterally without carinae, smooth (Fig. 4O); notaulus inconspicuously grooved (Fig. 4L); occipital carina broad (Fig. 4B); inter-antennal tubercle not broad at apex, short (Fig. 4B) (Oriental region) *C. rugipunctatus* sp. nov.
- 10 Pygidial area punctate throughout; omaulus broadened as midtibial width; lower gena with coarse punctures; clypeus moderately convex; several slender transverse striations anteriorly on scutum (Oriental region)..... *C. frontirugatus* Bashir & Ma
- Pygidial area punctate medially; omaulus narrowed; lower gena with fine punctures; clypeus slightly convex; scutum without transverse striations anteriorly (Palearctic and Oriental regions) *C. iwatai* (Tsuneki)
- 11 Posterior surface of propodeum with large smooth area medially; free margin of clypeal lobe nearly truncate or with four teeth medially 12
- Posterior surface of propodeum without large smooth area medially; free margin of clypeal lobe with three distinct teeth medially..... 14

- 12 Labrum wider than long, sub quadrate; free margin of clypeal lobe with four teeth, median lobe broadly produced, with two small inconspicuous lateral teeth, slightly reflexed apically, lateral lobe with a strong tooth on each side (Oriental region) ***C. latidentatus* Bashir & Ma**
- Labrum longer than wide, round toward apex; free margin of clypeal lobe truncate, sinuous, not forming reflexed teeth **13**
- 13 Scutum posterior area with sparse strong parallel ridges (Fig. 2G); ocellar triangle and vertex behind ocelli impunctate (Fig. 2B); outer orbital furrow narrow (Fig. 2F); omaulus and hypersternaulus broadened, distinctly foveolate (Fig. 2I); pygidial area u-shaped (Fig. 2M) (Oriental region) ***C. capiconvergens* sp. nov.**
- Scutum posterior area without parallel ridges; ocellar triangle and vertex behind ocelli finely punctate; outer orbital furrow broad; omaulus and hypersternaulus narrowed, not foveolate; pygidial area oval shaped (Oriental region) ***C. vesulcatus* Bashir & Ma**
- 14 Inter-antennal tubercle short, less than midocellus diameter **15**
- Inter-antennal tubercle long, equal or more than midocellus diameter **16**
- 15 Upper frons with dense, slender striations, impunctate; vertex impunctate (Palearctic and Oriental regions) ***C. filippovi* (Gussakovskij)**
- Upper frons smooth, without striations, with fine punctures; vertex with sparsed, fine punctures (Palearctic and Oriental regions) ***C. tanoi* Tsuneki**
- 16 Pronotal collar with sparsed, inconspicuous rugae laterally; scutum dull, with fine punctures; notaulus deeply grooved and foveolate, extending to one third of scutum length; inner and outer-orbital sulcus broad; lower gena with coarse punctures; upper frons with several, fine punctures, frontal longitudinal carina distinct, reaching anterior ocellus (Palearctic and Oriental regions) ***C. kaihuanus* Li & Yang**
- Pronotal collar smooth, without rugae laterally; scutum shiny, with coarse punctures; notaulus inconspicuous, extending to anterior $\frac{1}{4}$ of scutum length; inner and outer-orbital sulcus narrowed; lower gena impunctate; upper frons impunctate, without frontal longitudinal median carina (Oriental region) ***C. saigusai* (Tsuneki)**
- 17 Scrobal sulcus well-marked, short or long, distinctly foveolate (Fig. 5H) **18**
- Scrobal sulcus absent or very weakly impressed (Fig. 2K) **22**
- 18 Scrobal sulcus long (Fig. 5H); lateral surface of petiole with a groove medially, two distinct lateral carinae or with a groove basally and medially; ocellar triangle area dull, with coarse punctures; free margin of clypeal lobe deeply emarginated medially **19**
- Scrobal sulcus short (Fig. 4J); lateral surface of petiole with a few weak carinae or smooth; ocellar triangle area shiny, with fine punctures; free margin of clypeal lobe slightly emarginated medially **20**
- 19 Gena punctate ventrally; inter-antennal tubercle long, equal or more than midocellus diameter; admedian line inconspicuous; scutellum median line impressed (Oriental region) ***C. maior* (Maidl)**
- Gena rugate ventrally; inter-antennal tubercle short, less than midocellus diameter; admedian line conspicuous; scutellum median line not impressed (Oriental region) ***C. costatus* Krombein**

- 20 Petiole dorsal with few irregularly weak rugae basally (Fig. 4N); clypeus with dense, fine punctures (Fig. 4D); inter-antennal tubercle short (Fig. 4E); notaulus inconspicuously grooved (Fig. 4L) (Oriental region) .. ***C. rugipunctatus* sp. nov.**
- Petiole dorsal without rugae basally, smooth; clypeus with sparsed, coarse punctures; inter-antennal tubercle long; notaulus deeply grooved **21**
- 21 Vertex behind ocelli dull, impunctate; gena smooth; occipital carina narrowed; inner and outer-orbital sulcus narrowed; pronotal collar smooth; metanotum smooth; petiole lateral surface smooth (Oriental region).....
- ***C. formosanus* (Tsuneki)**
- Vertex behind ocelli shiny, with fine punctures; gena with several sturdy oblique transverse rugae near mandible area; occipital carina distinctly broad; inner and outer-orbital sulcus broad; pronotal collar with sparsed sturdy rugae; metanotum with dense sturdy longitudinal rugae laterally, smooth medially; petiole lateral with a few weak carinae (Palearctic and Oriental regions)
- ***C. iwatai* (Tsuneki)**
- 22 Extensive smooth area present on posterior surface of propodeum mesally; free margin of clypeal lobe with two triangular lateral teeth **23**
- Extensive smooth area absent on posterior surface of propodeum mesally; free margin of clypeal lobe with three distinct teeth **25**
- 23 Labrum wider than long; lower gena with fine punctures medially; parapsidal line conspicuous (Oriental region)..... ***C. latidentatus* Bashir & Ma**
- Labrum longer than wide; lower gena with weak striations or rugulose medially; parapsidal line inconspicuous..... **24**
- 24 Ocellar triangle and vertex behind ocelli impunctate (Fig. 2E); outer orbital furrow narrow (Fig. 2F); omaulus and hypersternaulus broad, distinctly foveolate (Fig. 2K); pronotal collar lateral angles sharp and projected (Fig. 2J); notaulus inconspicuous (Fig. 2L) (Oriental region) ***C. capiconvergans* sp. nov.**
- Ocellar triangle and vertex behind ocelli finely punctate; outer orbital furrow broad; omaulus and hypersternaulus narrowed, not foveolate; pronotal collar lateral angles blunt and not projected; notaulus deeply grooved (Oriental region)...
- ***C. vesulcatus* Bashir & Ma**
- 25 Inter-antennal tubercle long, equal or more than midocellus diameter; upper frons frontal carina distinct, not reaching anterior ocellus; inner-orbital sulcus broad (Palearctic and Oriental regions) ***C. kaibuanus* Li & Yang**
- Inter-antennal tubercle short, less than midocellus diameter; upper frons without frontal carina, or if present, clearly reaching anterior ocellus; inner-orbital sulcus narrowed) **26**
- 26 Upper frons with dense, slender striations; vertex impunctate; upper frons frontal longitudinal carina distinct anteriorly, reaching anterior ocellus (Palearctic and Oriental regions)..... ***C. filippovi* (Gussakovskij)**
- Upper frons without striations; vertex with sparsed fine punctures; frontal carina absent on upper frons..... **27**
- 27 Labrum pentagonal, deeply notched at apex; ocellar triangle with fine, sparsed punctures; lower gena with weak striations; occipital carina narrowed; scutum

- with coarsely punctuated; admedian line inconspicuous; parapsidal line conspicuous (Palearctic and Oriental regions)..... *C. tanoi* Tsuneki
- Labrum rounded, without emargination; ocellar triangle impunctate; lower gena smooth; occipital carina broad; scutum with fine punctures; admedian line conspicuous; parapsidal line inconspicuous (Oriental region) *C. nozakai* Tsuneki

Discussion

Tsuneki (1954) was the first to describe *Carinostigmus* species from China: *C. formosanus* and *C. iwatai*, collected from Taiwan and Hainan, respectively. Later on, several Chinese species were discovered: *C. nozakai* and *C. tanoi* from Taiwan (Tsuneki, 1977), and *C. kaihuanus* from Zhejiang province (Li & Yang, 1995). Previous faunistic studies conducted on *Carinostigmus* from China revealed thirteen species (Fig. 1). In this study, four further species: *C. capiconvergens* (Hainan and Yunnan), *C. longivertex* (Yunnan), *C. rugipunctatus* (Yunnan), and *C. triangularis* (Yunnan) are added to the list. The Chinese territory is placed in between two zoogeographical zones, Oriental and Palearctic. Some species of *Carinostigmus* are present in both zones. Most of the species are mainly Oriental (*C. costatus*, *C. filippovi*, *C. frontirugatus*, *C. latidentatus*, *C. formosanus*, *C. maior*, *C. nozakai*, *C. palawanensis*, *C. saigusai*, and *C. vesulcatus*). However, *C. iwatai*, *C. kaihuanus*, and *C. tanoi* are present in both the Palearctic and the Oriental China.

In China, the distribution of *Carinostigmus* species ranges from Shaanxi province (elevation of 400–600 m) to Taiwan and Hainan (elevation of 900–955 m). More species have been recorded from Yunnan province, followed by Taiwan, Guizhou, Hainan, Guangdong, Fujian, Guangxi, Zhejiang, Sichuan, Shaanxi, Henan, and Hunan. *Carinostigmus* was found in the south and southwest of China, where the average annual temperature and precipitation is 12–25 °C and 800–2300 mm.

However, the Chinese fauna of the genus *Carinostigmus* needs further study. Further research is needed to accurately assess distribution patterns of apoid wasps in China and their biology. In particular, taxonomic studies of Oriental China promise to find new, undiscovered species and new country records in *Carinostigmus*.

Acknowledgements

This study was supported by National Natural Science Foundation of China (31760641, 31750002). The authors would like to thank Professor WJ Pulawski for providing literature.

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Comparative ecology of two specialist bees: *Dasypoda visnaga* Rossi, 1790 and *Dasypoda maura* Pérez, 1895 (Hymenoptera, Melittidae)

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Academic editor: Jack Neff | Received 9 November 2020 | Accepted 22 December 2020 | Published 25 February 2021

<http://zoobank.org/555C0B1F-4848-4119-A7E9-97AA1893939F>

Citation: El Abdouni I, Lhomme P, Hamroud L, Wood T, Christmann S, Rasmont P, Michez D (2021) Comparative ecology of two specialist bees: *Dasypoda visnaga* Rossi, 1790 and *Dasypoda maura* Pérez, 1895 (Hymenoptera, Melittidae). Journal of Hymenoptera Research 81: 109–126. <https://doi.org/10.3897/jhr.81.60528>

Abstract

Many wild bee species are declining globally. To design efficient mitigation strategies to slow down or reverse these trends, we urgently need to better understand their basic ecological requirements. In this context, we studied two specialist species for which ecological data are scarce: *Dasypoda visnaga* and *Dasypoda maura*. We provide for the first-time detailed information on their phenologies, morphological traits, floral preferences, and nesting behaviours based on historical data and new samples from Morocco. The flight season of both species extends from late spring to late summer but *D. maura* emerges earlier than *D. visnaga*. Though the two species show different morphological traits, palynological analyses show that *D. visnaga* and *D. maura* females collect almost exclusively pollen from *Scolymus* sp. (Asteraceae). Concerning their nesting behaviour, *D. visnaga* seems to be more gregarious than *D. maura*. Both species build nests in sandy soil that can reach a depth of 80 cm. These ecological observations show that the differences between *D. visnaga* and *D. maura* are minor with regards to habitat requirements.

Keywords

Conservation, floral preferences, habitat requirements, nesting behaviour, phenology, *Scolymus*

Introduction

Worldwide declines in wild bee populations have been reported over the past two decades (Biesmeijer et al. 2006; Burkle et al. 2013; Nieto et al. 2014; Kleijn et al. 2015; Potts et al. 2016; Christmann 2019; Powney et al. 2019). To contain these declines, there is an urgent need to better understand their specific foraging and nesting requirements in order to design efficient mitigation strategies (Müller et al. 2006). Host plant and nesting resource (i.e. materials and substrates) availability are the two principal components driving the structure of wild bee communities (Potts et al. 2003, 2005; Goulson et al. 2015; Razo-León et al. 2018).

Regarding their floral choices, wild bees are usually described as specialists or generalists depending on their foraging strategies. Specialist (or oligolectic) bees exhibit a high fidelity for particular plant taxa of the same botanical family while generalist (or polylectic) bees forage on a wide range of plants from multiple botanical families (Rasmussen et al. 2020). These diverse foraging behaviours influence the composition of bee communities (Scheper et al. 2014) and their conservation. Specialist bees are more affected than generalists by disturbances such as agricultural intensification (Williams et al. 2010) as they are not able to switch to alternative plant resources.

Bees also show a great diversity of nesting behaviours. The majority are ground nesters but some species nest above ground in various substrates such as hollow or pithy stems or abandoned cavities in dead wood or build their nests on open surfaces (Radchenko and Pesenko 1994; Danforth et al. 2019). They can also use various material to build their cells such as mud, pebbles, resin, flower petals, plant leaves, plant hairs, floral oils or secreted building materials (Radchenko 1996; Cane et al. 2007; Danforth et al. 2019). In addition, there are also parasitic (e.g. cuckoo bees) species, which exploit the nest built by their bee host and lay their eggs on the pollen provisions (Michener 2007). They can even exploit the social system of their host in the case of socially parasitic bees (Lhomme and Hines 2019). Within ground-nesting bees, species can show specific nesting site requirements (e.g. soil texture / moisture / compaction, vegetation cover, exposed bare ground) (Potts and Willmer 1997; Sardiñas and Kremen 2014) or nest architecture (e.g. variation in depth and relative position of cells). Nesting resource availability and soil characteristics can therefore greatly affect the composition of bee communities, and 40% of the variation in species abundance pattern can be explained by the availability of nesting resources (Potts et al. 2005). Unfortunately, disturbances like habitat fragmentation, agricultural intensification, pesticide use and tillage can have a negative impact on nesting resources (Williams et al. 2010).

Among the ~20 000 described bee species, melittid bees constitute one of the smallest families (201 species; Michez et al. 2009; Danforth et al. 2013). As they are relatively rare and localised, data on their ecology are scarce. Within this bee family, the genus *Dasygaster* comprises 39 described species (Michez et al. 2004a; Radchenko 2016, 2017; Radchenko et al. 2019). Among them, nine species are recorded in Morocco (Lhomme et al. 2020). *Dasygaster* species are predominantly oligolectic, with the exception of some species such as *Dasygaster crassicornis* Friese which are known to

be polylectic. The subgenera *Dasygaster* s. str. and *Megadasygaster* forage on Asteraceae and Dipsacaceae respectively while *Heterodasygaster* and *Microdasygaster* subgenera visit mainly Cistaceae and Malvaceae (Michez et al. 2004b). Regarding their nesting behaviour, *Dasygaster* species are known to nest in the ground based on studies of three species: *D. argentata* Panzer (as *D. thoracica* Baer) (Celary 2002), *D. braccata* Eversmann (Radchenko 1988), and *D. hirtipes* Fabricius (Müller 1884; Vereecken et al. 2006; Loonstra 2010). Based on the information gathered from these species, we know that after mating, *Dasygaster* females initiate nest construction in sandy soil and then start to collect pollen. They place pollen balls in brood cells and lay an egg on the top. The larvae feed on the pollen ball and do not spin a cocoon. The nests are generally deep and can exceed more than 90 cm in depth (Celary 2002). Some species like *D. hirtipes* build their cells near the main gallery and make tripod-like structures below the pollen balls to reduce contact between the provisions and the cell wall (Müller 1884; Vereecken et al. 2006).

This paper aims to increase our knowledge concerning the ecology of the genus *Dasygaster* focusing on two species observed in Morocco, *Dasygaster maura* Pérez 1895 and *Dasygaster visnaga* Rossi 1790 (Fig. 1). The floral choices and nesting behaviour of these species are poorly documented, so this study aims to describe their phenologies, distribution, host plant preferences, and nesting ecology.

Methods

Data collection

Historical data on distribution, floral choices and phenology were obtained from the database “*Banque de données fauniques Gembloux Mons*”. In total, we gathered information from 839 specimens of *D. visnaga* and 101 specimens of *D. maura*. These records come from different private and institutional collections (Berg, BMNH, Catania, CUI, DWB, Erfurt, FSAGX, Genève, GRUNWALD, ICC, IRSNB, Lausanne, LINSÉNMA, Livory A, MCN, Mendocça Li, University of Mentouri, MNHNP, Munich, NMV, OOLL, RNHL, Schwarz, UMons, UMO, UZMC, VERHOEFF, ZMA; 86% of the data) and literature (14% of data) (Suppl. material 1: Table S1).

We also collected additional specimens in Morocco to study the floral choices and some morphological traits related to the foraging behaviour of both species. Bees were collected using insect nets. They were then killed and separated for identification, trait measurement, and pollen analysis. Specimens are conserved in the collection of the University of Mons.

Phenological, morphological and ecological analyses

Phenological data were obtained from historical records and new Moroccan samples. Initially, records from all years were grouped by month and by country and we

calculated the flight period of each species based on presence/absence of the species in each month. Then, we pooled all data for all countries to estimate the month(s) with the greatest number of observations for each species.

We considered the distance in millimetres between the two insertion points of the wings, the inter-tegular distance (ITD), as a proxy of body size (Cane 1987). We measured the length of the glossa and the prementum as a proxy of tongue length using a Facom 150 mm digital calliper (France, Morangis). These measurements were made from 31 specimens of *D. visnaga* (16 females and 15 males) and 39 specimens of *D. maura* (24 females and 15 males) from Morocco (Suppl. material 1: Table S2).

The floral utilization study of the two species of *Dasygoda* was based on floral visit observations and palynological analyses. The floral records represented 132 specimens, 87 specimens of *D. visnaga* (50 females and 37 males) and 45 specimens of *D. maura* (19 females and 26 males) (Suppl. material 1: Table S3).

Pollen analyses were based on the pollen loads removed from female scopa and the pollen balls sampled within the nest of both species. We analysed pollen from female scopa (three females of *D. maura* and seven for *D. visnaga*) and pollen balls (three pollen balls for *D. maura* and 10 for *D. visnaga*) from specimens newly collected in Morocco. We also used information from historical data presented by Michez et al. (2008), specifically 49 pollen loads of *D. visnaga* (from 34 localities) and 21 of *D. maura* (from 8 localities). Pollen was suspended in water on a microscope slide and allowed to rehydrate. The slide was then heated to evaporate the excess of water. Molten fuchsin jelly was added, and the slide was covered with a coverslip. Pollen grains were identified by light microscopy at a magnification of x400 using a reference collection of West Mediterranean plant species assembled from Iberia and North Africa (TJW *pers. colln.*). Identification to or below genus level in the family Asteraceae is highly challenging, and *Scolymus*-type pollen is characterised by typical Cichorieae shape at the tribal level, and to the group level by the diameter of the grains which measure 45–55 μm . This grain size included the related genera *Cichorium*, *Helminthotheca*, and *Sonchus*. Pollen grains representing less than 2% of the load were assumed to be contamination and neglected (Westrich and Schmidt 1986).

Foraging behaviour was evaluated by measuring visitation rate and time spent per flower (Pesenko et al. 1980; Akter et al. 2017). We examined the time of foraging on *Scolymus hispanicus* L. for the two species at the same site (45 females of *D. visnaga* and 44 females for *D. maura*). To quantify the visitation rate, we counted the number of flowers visited by each female (N = 18 females for each species) and the total time spent foraging. We then calculated the mean number of flowers visited per minute.

Investigation of the nesting architecture of both species was conducted in May (2019, 2020) and July (2019) in two locations in Rabat-Kenitra region. The nests of *D. visnaga* and *D. maura* were excavated in a site located at Douar Oulad Taleb near Maâmora forest (34.1243033°N, -6.5755842°W). The ground was sandy, bare, and exposed to the sun, with plants of *Scolymus* sp. 200 m away. A second nesting site of *D. maura* was investigated in Salé Al-Jadida (34.0226357°N, -6.7495343°W). This site

was moderately vegetated with sandy and compacted soil. The flora included mostly *Scolymus* sp. and *Carduus* sp., but no *Cichorium* sp., *Helminthotheca* sp., or *Sonchus* sp. were recorded. One nest of each species was filled with liquid plaster and left 30 min until the plaster had solidified. This method allowed us to follow the tunnels and reconstruct the nest architecture (Tschinkel 2010). The other nests were excavated to sample pollen balls and larvae. After excavation, different parameters were measured: the distance between neighbouring nests, the width and the height of the tumulus, the length of tunnels, the number of cells and the depth of each cell.

Results

Phenology and distribution

Dasyroda visnaga is distributed in the north of Mediterranean Sea from Portugal to Turkey and in Maghreb (Morocco, Algeria, and Tunisia). *Dasyroda maura* is endemic to Northern Africa (Morocco and Algeria) (Fig. 1). In Morocco, *D. maura* is more widespread than *D. visnaga*, which is found only in coastal parts of the country while *D. maura* is also found in mountainous regions (Rif, Middle and High Atlas).

Records of both species show *D. visnaga* specimens were mainly collected in July (67%) whereas *D. maura* specimens were largely collected in May and June (90%) (Fig. 6). The beginning of the flight season of *D. visnaga* varies between countries, it starts in mid-April in Greece, in May in Morocco, Algeria, Tunisia, Spain and Portugal. It seems to start much later in France and Italy with specimens appearing in June. *Dasyroda maura* flies from April to July in Morocco and has been observed in July in Algeria (Fig. 2).

Morphological traits

Females of *D. visnaga* have the greatest ITD (3.60 ± 0.05 mm) followed by *D. maura* females (3.31 ± 0.02 mm) while the males of the two species have the smallest ITDs (*D. visnaga*: 3.18 ± 0.03 mm; *D. maura*: 3.19 ± 0.02 mm; Fig. 4A). We found a significant difference in ITD between females of both species and between males and females within and between species (Kruskal-Wallis, chi-squared = 40.55, df = 3, $p = 8.122 \cdot 10^{-9}$). No difference was found in ITD between males of both species (Wilcoxon rank-sum test, $p = 1$).

Using a model estimating the foraging range based on body size (Greenleaf et al. 2007), we estimated the foraging distance of each species. The results showed that the estimated mean of foraging distance of *D. visnaga* females is 1.67 ± 0.03 km while *D. maura* females is 1.47 ± 0.01 km.

Tongue length was significantly different between species and sex (Kruskal-Wallis, chi-squared = 36.145, df = 3, $p = 6.977 \cdot 10^{-8}$). *Dasyroda maura* has the longest

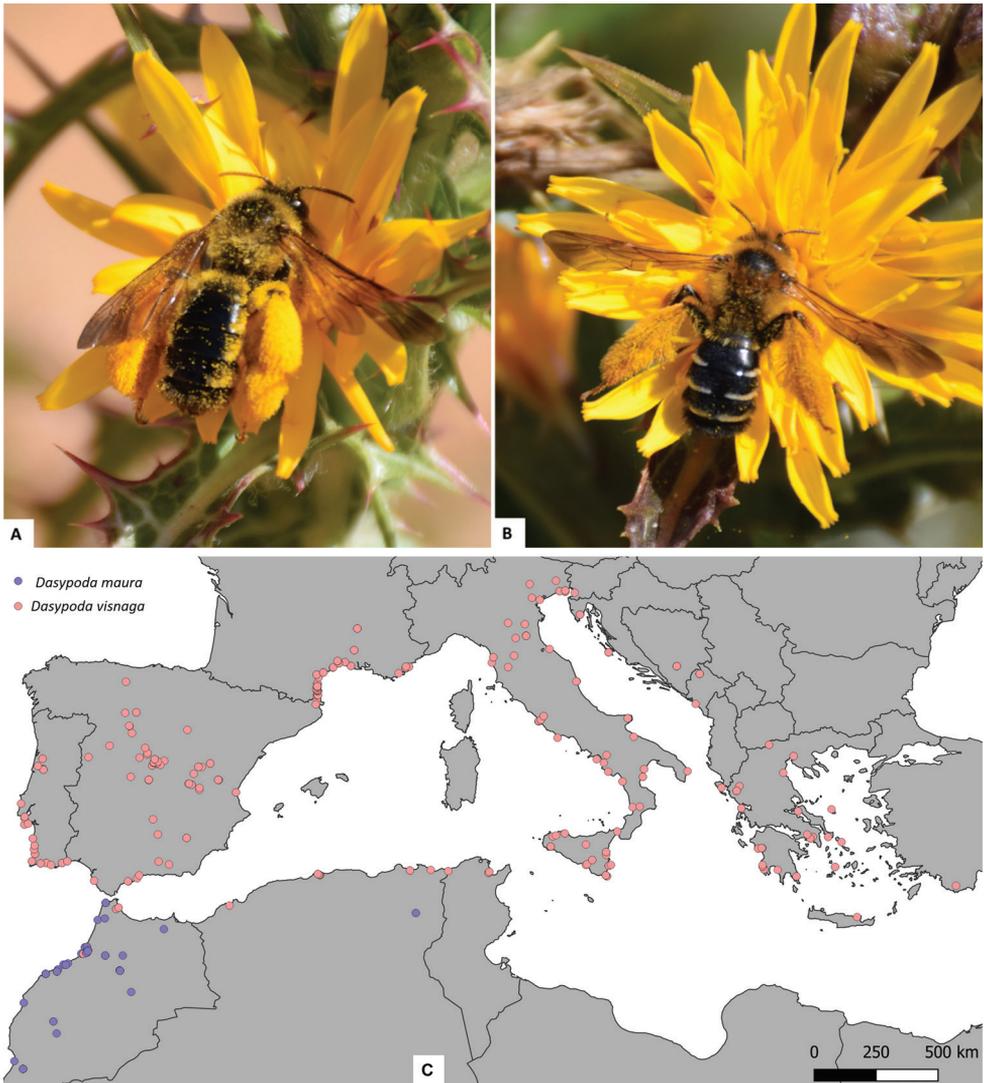


Figure 1. **A** *Dasygaster visnaga* female. Photo by Patrick Lhomme (2020) **B** *Dasygaster maura* female foraging on *Scolymus hispanicus*. Photo by Insafe El Abdouni (2020) **C** geographical distribution of the two species (pink circles = records of *Dasygaster visnaga*; black circles = records of *Dasygaster maura*).

proboscis (female: 4.87 ± 0.019 mm; male: 4.66 ± 0.02 mm) while that of *D. visnaga* is shorter (female: 4.61 ± 0.07 mm; male 4.47 ± 0.04 mm, Fig. 4B).

Floral choices and foraging behaviour

Floral records of *D. visnaga* (50 females and 37 males) and *D. maura* (19 females and 26 males) obtained from historical data and new observations indicate that the most

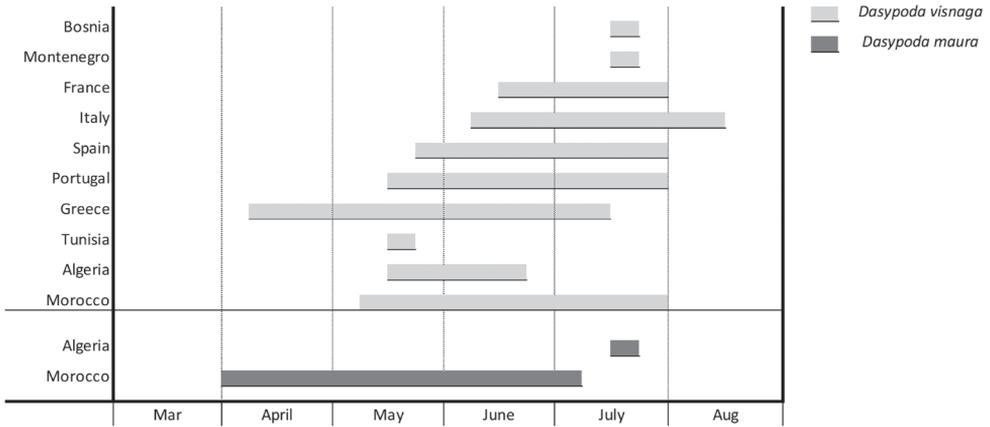


Figure 2. Temporal records of *Dasyroda visnaga* (light grey) and *Dasyroda maura* (dark grey).

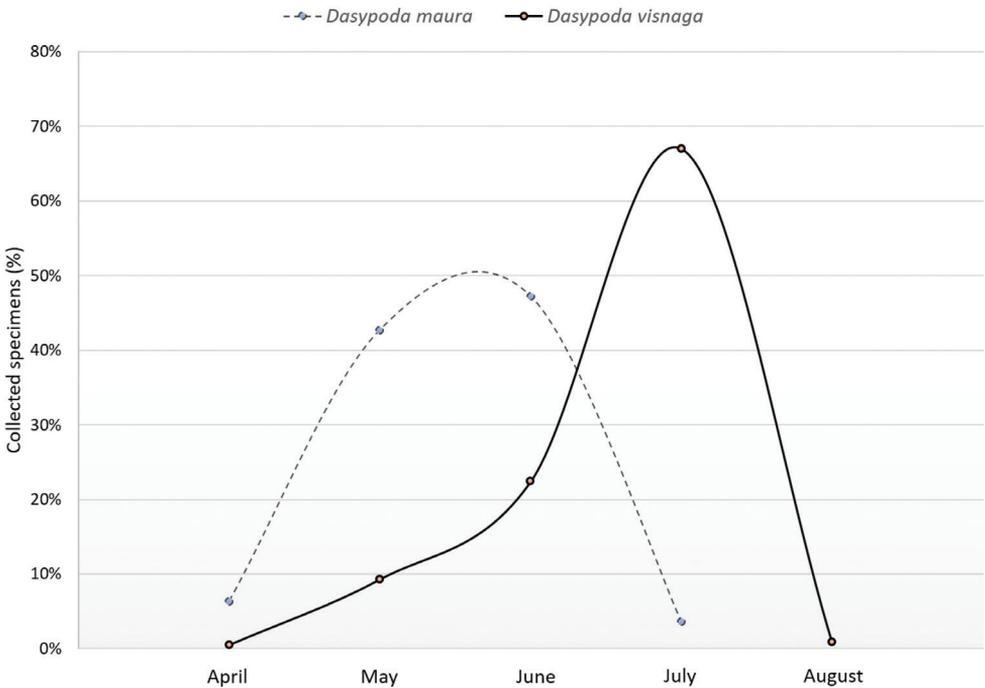


Figure 3. Percentage of collected specimens per month for *Dasyroda maura* (dashed line, N = 110) and *Dasyroda visnaga* (solid line, N = 603) including data from all countries.

visited plant is almost exclusively *Scolymus* sp., except for males of *D. maura* who visit a greater diversity of host plants (Fig. 5). Males of *D. maura* visit a greater diversity of plants than males of *D. visnaga*. The alternative plants visited are *Onopordum* sp. (Asteraceae), *Marrubium vulgare* L. (Lamiaceae), *Echinops spinosus* L. (Asteraceae), and *Scabiosa atropurpurea* L. (Caprifoliaceae).

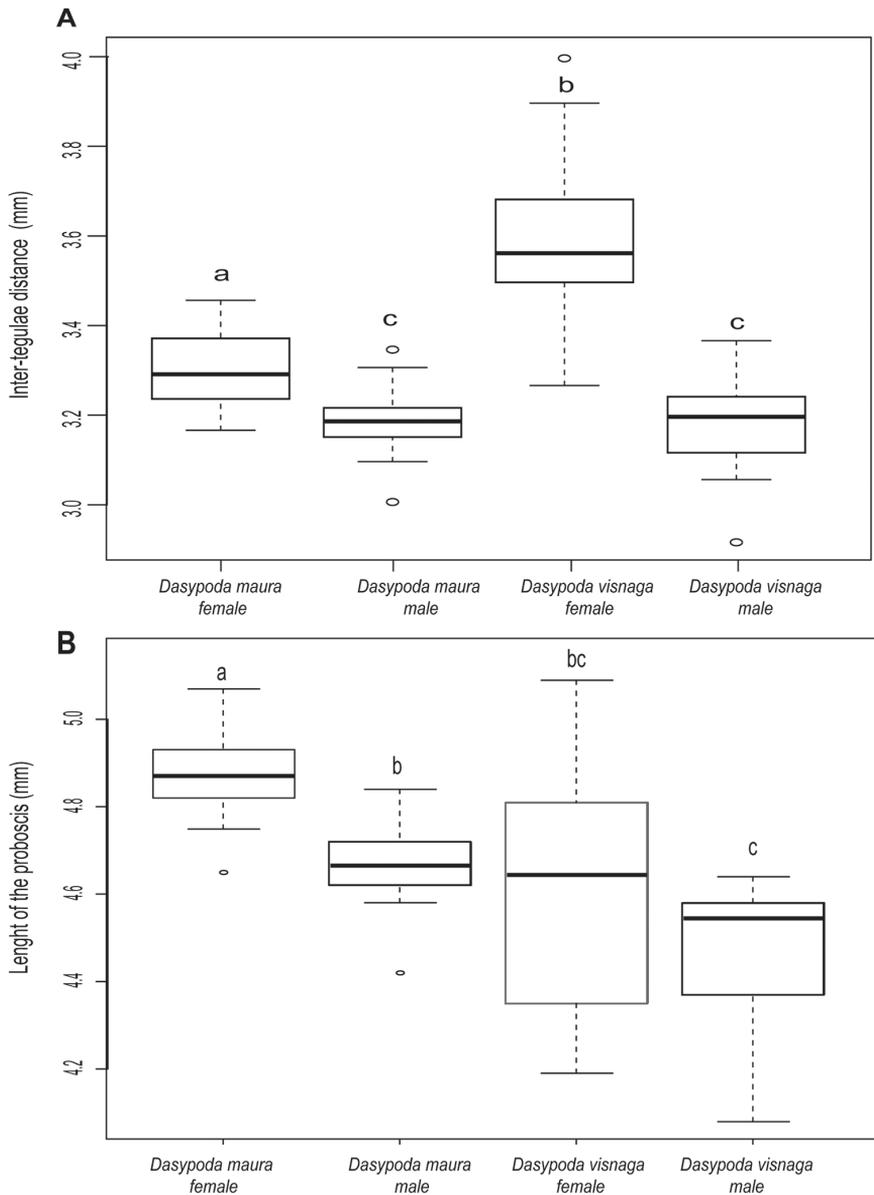


Figure 4. Morphological traits and estimated foraging distance of *Dasypoda visnaga* and *D. maura* **A** inter-tegulae distances **B** lengths of the proboscis. Box plots show the median and 25–75% percentiles. Whiskers show all data excluding outliers. Outliers (circles) are values being more than 1.5 times box length from upper and lower edge of respective box. The different letters indicate significant differences between treatments within experiments (Pairwise comparisons using Wilcoxon rank-sum test, $p < 0.05$).

Palynological analyses revealed that pollen loads of *D. maura* contain only pollen of *Scolymus*-type, while 98% of loads from females of *D. visnaga* did the same. This result was confirmed by pollen analysis of pollen balls found in the nest in Morocco as all pollen balls analysed consisted of *Scolymus*-type pollen. Given the absence of related

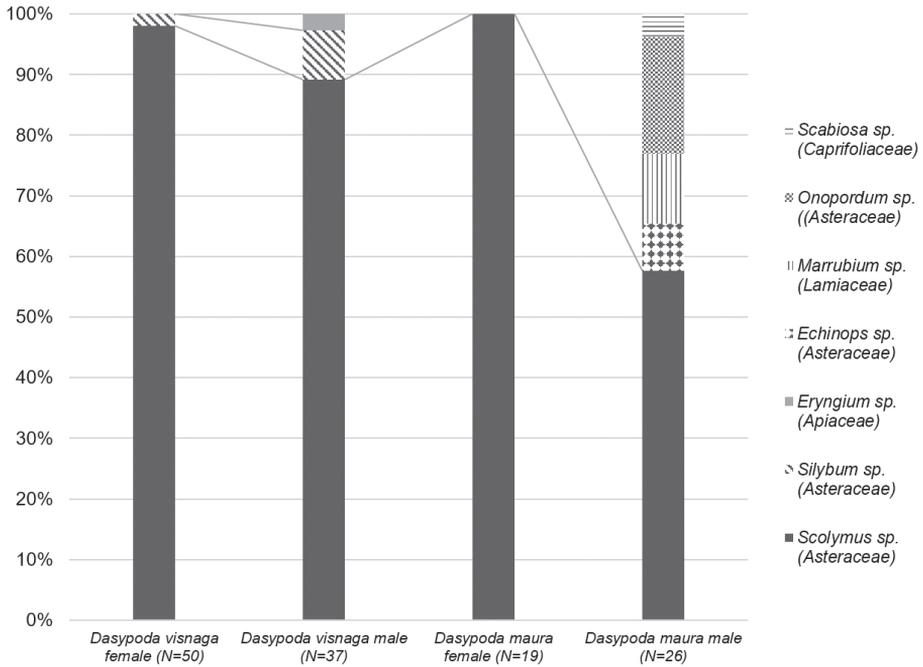


Figure 5. Floral choices of *Dasypoda visnaga* and *D. maura* based on historical and new Moroccan records. N is the number of records for each species and sex..

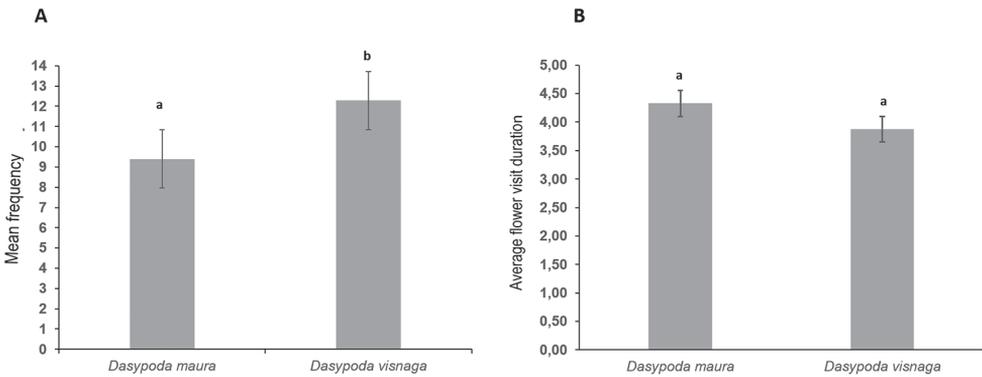


Figure 6. A frequency of floral visits **B** average flower visit duration between *Dasypoda maura* and *Dasypoda visnaga*. The different letters indicate significant differences between species (**A** student t-test, $p < 0.05$ **B** Wilcoxon rank-sum test, $p > 0.05$).

plant genera that produce this pollen type at this study site (see Methods), *Scolymus* plants are highly likely to be the sole pollen source at this locality.

The mean time spent on a flower of *Scolymus hispanicus* L. by *D. visnaga* (4.17 s) and *D. maura* (3.83 s) were similar (Wilcoxon rank-sum test, $p = 0.25$) (Fig. 6B). However, *D. visnaga* visited a higher number of flowers per minute (13 flower/min) than *D. maura* (9 flowers/min) (Fig. 6A).

Nest architecture

Females of *D. visnaga* construct their nests in sandy and non-compacted soil making a heap of sand above the nest entrance called a tumulus (Fig. 7B). This tumulus is built from soil displaced by the female during nest excavation. The diameter of the tumulus

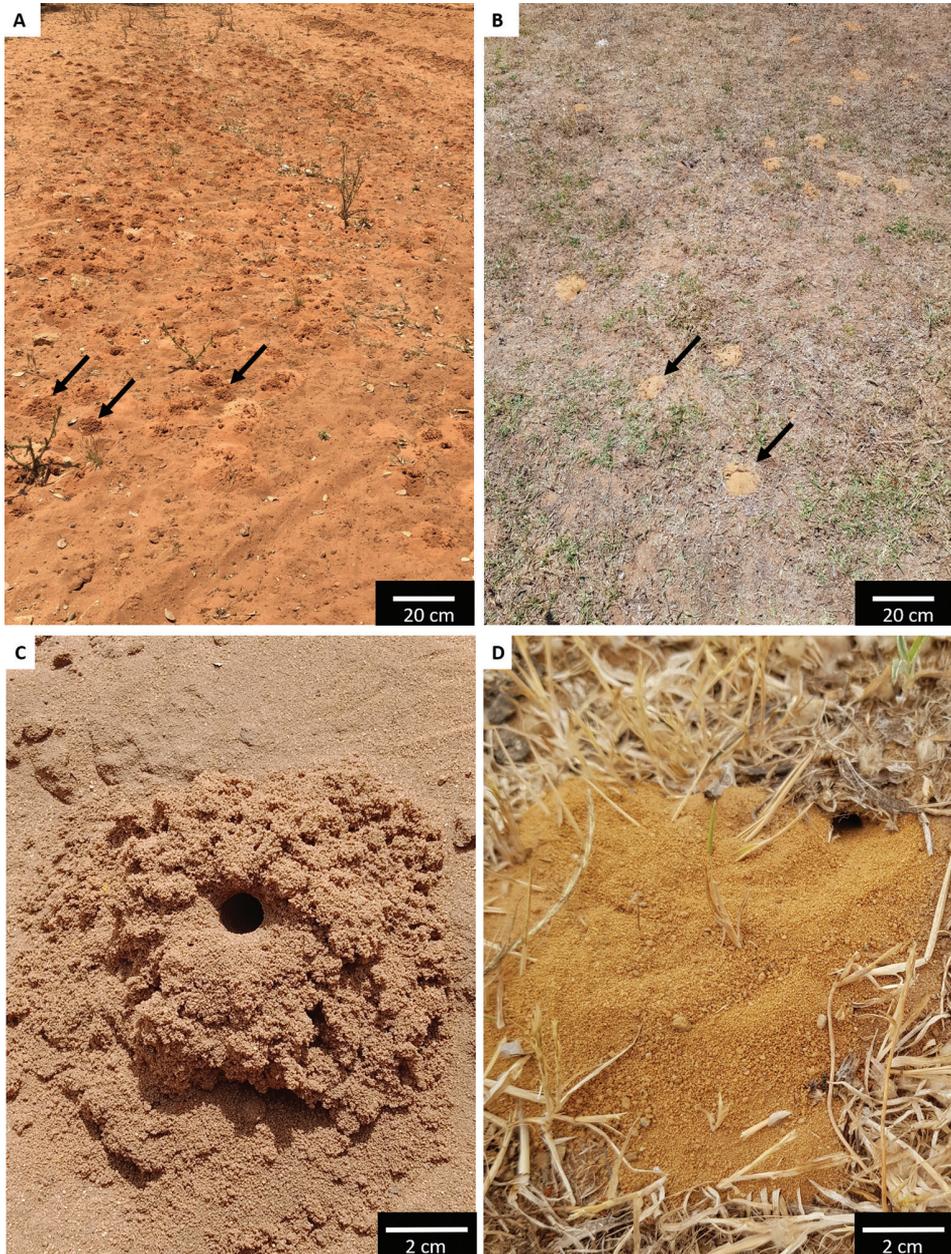


Figure 7. **A, B** nesting sites **C, D** nest entrances **E, F** nest architecture showing main galleries, hypothetical cells, and pollen balls **G, H** larva with pollen balls. Right side: *Dasydota maura*, left side: *Dasydota visnaga*.

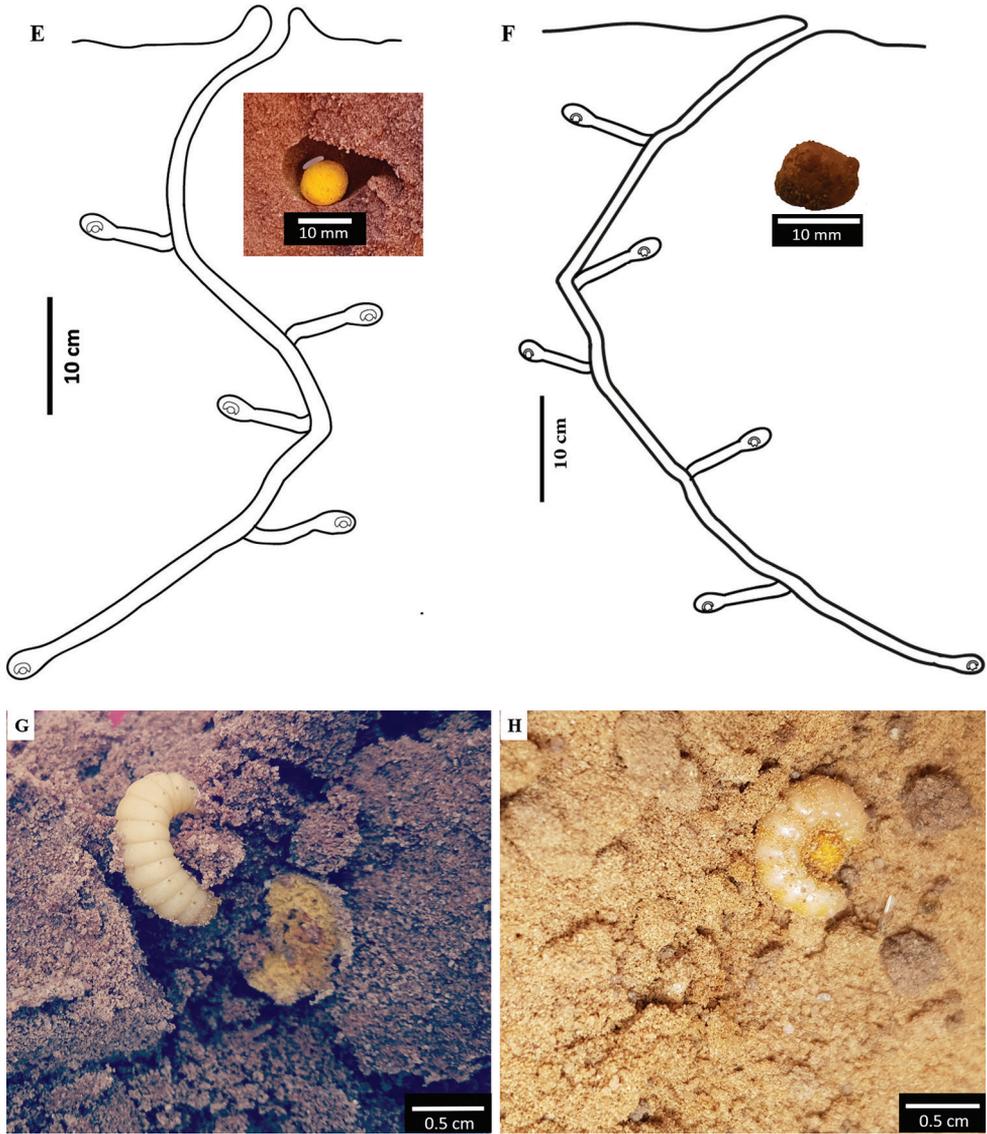


Figure 7. Continued.

ranges from 7 to 10 cm (8.6 ± 1.07 cm) and the height ranges from 1 to 4 cm (2.89 ± 1.34 cm). The main burrow has a diameter of 10 mm, it is oblique on 25 cm and at an angle of 45° then it becomes vertical. The main burrow reaches 80 cm in length (Fig. 7E). The female constructs the brood cells connected to the main burrow by lateral burrows located at different depths. Cells were filled with a spherical pollen ball (without a tripod) with the egg placed on the top (Fig. 7E). After laying the egg, the female closes the cell with an earthen plug and tightly fills the lateral burrow with soil.

Nest entrances of *D. maura* (Fig. 7A) were difficult to find because they do not possess conspicuous tumuli like those of *D. visnaga* (Fig. 7D) and were usually found below

vegetation cover. The diameter of the flat nest tumuli ranges from 12 to 16 cm (13.5 ± 1.17 cm). The distance between nests within an aggregation ranges from 32 cm to 170 cm. The females build their nests also in sandy soil with a low proportion of clay. The main burrow has a diameter of 8 mm and a length of 80 cm. The brood cells are also connected to the main burrow by lateral ones located at different depths and each cell contains a pollen ball with a basal tripod (Fig. 7F). The egg was also laid on the top of the pollen ball.

Discussion

The present study revealed that the two studied *Dasypoda* species have similar ecological requirements with slight differences. *Dasypoda visnaga* is found in most Mediterranean countries, especially in coastal areas. This distribution is probably related to the type of soil as *D. visnaga* nests in non-compacted sand, and its main host plant *Scolymus hispanicus* is often found in coastal areas. *Dasypoda maura* is additionally found inland in Morocco. The soil where *D. maura* nests can be much more compact than the soil where nests of *D. visnaga* were observed. Phenological records in Morocco and other countries showed that *D. visnaga* and *D. maura* are mainly active during late spring and summer (April–August). This period coincides with the flowering of *Scolymus* plants. This suggests the presence of one generation per year like others species of *Dasypoda* (Radchenko and Pesenko 1989; Vereecken et al. 2006).

Morphological data showed that the females of *D. visnaga* have a larger body size than *D. maura*. Following Greenleaf et al. (2007) model, the estimated foraging distance for both species should be large. This distance is close to other large species like *Bombus terrestris* Linnaeus (1500 m) (Osborne et al. 2008) or *Hoplitis adunca* Panzer (1400 m) (Zurbuchen et al. 2010). Consequently, *Dasypoda* species could be less sensitive to disturbances like habitat destruction (De Palma et al. 2015). A slight trend for small species to be more sensitive to land disturbance has been found (Bartomeus et al. 2017), but other studies have shown a positive correlation between body size and sensitivity to agricultural land use (Bartomeus et al. 2013).

Females of the two species have a different tongue length but the same foraging duration on flowers of *Scolymus*. The time spent per flower for *D. visnaga* and *D. maura* (4.2 s and 3.8 s respectively) is higher than for *D. hirtipes* (0.7 s) (Levermann et al. 2000). According to Klumpers et al. (2019), the interaction between the length of a proboscis and the depth of corolla affects the handling time. Insects with a proboscis shorter than nectar tubes spend more time foraging per flower on these flowers and are consequently a less efficient. In this study, the two bee species forage on the same plant species so the time spent foraging does not seem to be affected by tongue length. However we used a different metric and did not investigate the handling time as described by Klumpers et al. (2019) which is the time that an insect takes to extend its proboscis and extract the nectar. Records from historical data and our observations show that *D. visnaga* and *D. maura* have similar floral preferences. They forage mostly on Asteraceae family confirming the position of Michez et al. (2008). Pollen analyses revealed that both species are strict oligoleges of Asteraceae,

and though it cannot be proved definitively with light microscopy, females are highly likely to provision their offspring with pollen of *Scolymus* plants exclusively, thus making them narrow oligolectic. The comparison between male and female choices shows the presence of large differences in floral choices that are known to exist between different bee sexes (Roswell et al. 2019).

Dasygaster maura and *D. visnaga* seem to have nesting behaviour and nest architecture similar to the other *Dasygaster* nests described so far. After emergence and mating, females of *D. visnaga* start to build their nests in sandy soil, similar observations were made for *Dasygaster hirtipes* (Vereecken et al. 2006) and *Dasygaster argentata* (Celary 2002) whereas, *Dasygaster braccata* prefers soil with a high clay content (Radchenko 1988). Females first excavate an oblique burrow for about 25–30 cm. Then, the burrow changes direction and females start to construct cells where they put pollen balls. Pollen balls made by *D. visnaga* do not possess any tripod and were put directly on the soil at the bottom of the cells, which do not have the additional lining that characteristic of many other ground-nesting bees (Fig. 7E) while *D. maura* pollen possess tripods (Fig. 7F), like *D. hirtipes*, another species from the same subgenus (Müller 1884; Vereecken et al. 2006).

Conclusion

This study is the first to compare ecological aspects of two species of *Dasygaster* bees. Both studied species are oligolectic and share many ecological traits. They have very restricted floral preferences and nesting requirements. Future surveys should be performed to better understand their ecology and assess if conservation strategies are needed. The two species were found in agricultural landscape, so these strategies should consider the role of farmers. They should be informed and trained to recognise the bee nests and their host plant to protect them in local areas.

Acknowledgements

This research was funded by the Federal German Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) through the International Climate Initiative (IKI). It was also partly supported by the “Fonds de la Recherche Scientifique – FNRS”, the “Research Foundation of Flanders – FWO” under EOS Project named CLIPS (n°3094785) and by the “Académie de Recherche et d’Enseignement Supérieur (ARES)”.

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Supplementary material I

Tables S1–S3

Authors: Insafe El Abdouni, Patrick Lhomme, Laila Hamroud, Thomas Wood, Stefanie Christmann, Pierre Rasmont, Denis Michez

Data type: Occurrences, morphological traits, floral preferences

Explanation note: **Table S1:** distribution data of *Dasypoda visnaga* and *Dasypoda maura*; **Table S2:** morphological trait and forging distance measurement; **Table S3:** floral preferences of *Dasypoda visnaga* and *Dasypoda maura*.

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Link: <https://doi.org/10.3897/jhr.81.60528.suppl1>

Assessment and conservation status of an endemic bee in a diversity hotspot (Hymenoptera, Melittidae, *Dasyпода*)

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Academic editor: J. Neff | Received 16 November 2020 | Accepted 17 December 2020 | Published 25 February 2021

<http://zoobank.org/666A641C-7B44-4314-8798-7212285B8E8A>

Citation: Ghisbain G, Radchenko VG, Cejas D, Molina FP, Michez D (2021) Assessment and conservation status of an endemic bee in a diversity hotspot (Hymenoptera, Melittidae, *Dasyпода*). Journal of Hymenoptera Research 81: 127–142. <https://doi.org/10.3897/jhr.81.60811>

Abstract

Wild bees represent a global group of highly diversified insect pollinators, nowadays concerning well known for their widespread observed patterns of decline. Amongst them is the genus *Dasyпода*, a widespread Palearctic clade of solitary bees generally poorly represented in entomological collections. Among the 39 accepted species of the genus, 35 are known by both sexes, and a large number of taxa are still known by a low number of specimens. The recently described taxon *Dasyпода* (*Heterodasyпода*) *michezi* Radchenko, 2017 endemic to southern Portugal is just such a case. The species was described from two male specimens, but no female material has been known to date. Here, we provide the first description of the female of *D. michezi*, collected close to the *locus typicus* in southern Portugal along with a series of conspecific males. Sex pairing is proposed based on the sympatry of the male and female specimens and on the similar morphology of non-sexual dimorphic traits and on the barcode of a fragment of Cytochrome Oxidase I. We provide high quality imaging of both sexes of *D. michezi* to help future identification of the species and present a key for all known species of the subgenus *Heterodasyпода*. We finally propose an IUCN status for *D. michezi* and discuss the conservation of such geographically restricted species in the current context of global change.

Keywords

Conservation, *Dasyпода michezi*, endemism, *Heterodasyпода*, Iberia, IUCN, Portugal, taxonomy

Introduction

Wild bees constitute a highly diversified group of pollinating insects, comprising more than 20,000 described species globally (Michener 2007). Approximately a tenth of this global diversity can be found in Europe, one of the most studied places for bee diversity, biogeography and life history in the world (Michez et al. 2019). Concerningly, global changes have triggered widespread population declines across several clades of wild bees, making this group of pollinators widely studied for conservation purposes (e.g. Potts et al. 2010; Nieto et al. 2014). As for all other animals, nevertheless, the effective species-level conservation of bees relies on an unambiguous taxonomic delineation and identification. This principle is well exemplified in Red Lists and large-scale biogeographic studies for which species inventories and occurrence records constitute the starting point for geographic projections and assessments (Nieto et al. 2014; Ras-mont et al. 2015; Ghisbain et al. 2020b).

Amongst the bee families represented in Europe is the Melittidae, a species-poor bee family globally (Michez et al. 2009). Among melittids, the genus *Dasypoda* Latreille is remarkable in its unique morphology, specifically by the presence of well-developed pollen-carrying hair structures (scopae) on the tibia and basitarsus of the hind legs of the females (Michener 2007; Michez et al. 2019). The genus is predominantly distributed in the Palearctic region, except for a single sub-Saharan species (Michez and Pauly 2012). To date, 39 valid species are recognized with 35 of them known from both sexes (Radchenko et al. 2019). Four subgenera are currently recognized within *Dasypoda*, with their centers of diversity found in parts of the Mediterranean region: the Balkans, Morocco and the Iberian Peninsula. Unlike the situation in many groups of wild bees, there continue to be significant additions to the knowledge of the *Dasypoda* fauna of Europe, with the description of new species (Radchenko 2016, 2017) and new records for many countries (Schmid-Egger and Dubitzky 2017; Ghisbain et al. 2018; Shebl 2018; Radchenko et al. 2019, 2020; Wendzonka et al. 2020).

The most recently described species of the genus, *Dasypoda michezi* Radchenko, 2017, is a very localized bee known only from two males collected in two nearby localities in the south-west of Portugal (Radchenko 2017), a biodiversity hotspot for bees (Baldock et al. 2018). The species belongs to the subgenus *Heterodasypoda* Michez, characterized in males by conspicuous morphological autapomorphies, i.e. sternum 7 comprising two large, membranous lateral structures and the genitalia consisting of three distinct lobes, the inner one with a scaly surface (Michez et al. 2004b). The male of *Dasypoda michezi* is distinguishable from its congeners by several features including the diagnostic sculpture and shape of the glossa, galea and sterna. To date however, the female of this taxon has not been described (Radchenko 2017).

In this paper, we combine the use of molecular barcoding and the study of morphological traits to provide the first description of the female of *Dasypoda michezi*. The description is based on two individuals collected along with a longer set of males in the south of Portugal, close to the *locus typicus* of the species. We then assess the conservation status of this overlooked bee species and discuss its conservation as an endemic bee in a diversity hotspot.

Material and methods

Sampling

We examined a series of *Dasyroda* (*Heterodasyroda*) specimens collected in southern Portugal in the years 2005–2006 by M. and E. Howe, following the taxonomic key from Michez et al. (2004a). The morphological characteristics of two females could be identified as those of *Heterodasyroda* but did not correspond to any previously known *Dasyroda* species (following Michez et al. 2004a), while the males could be determined as *Dasyroda michezi* Radchenko following the detailed description provided by Radchenko (2017).

Genetic analyses

We sequenced a fragment of the cytochrome oxidase I (*COI*) gene from these two female specimens and from sympatric males of *Dasyroda michezi*. DNA was extracted from a middle leg of every individual using the Nucleo spin Tissue kit (Macherey-Nagel, Germany) following manufacturer instructions with a lower volume of elution buffer and expanded final incubation times to improve DNA yield. As pinned specimens from old collections (>10 years) can be difficult to sequence (i.e. their DNA has degraded over time and moreover is affected by the different processes of preservation of the collections), it is advisable to amplify small amplicons (<200 bp) (Wandeler et al. 2007). Therefore, modified LEP primers for older samples (LEP-F1/LEP-R2, Hebert et al. 2004) were selected to amplify a fragment of the cytochrome oxidase subunit 1 (*COI*) gene, a region commonly used to study the taxonomic status of animals, and frequently used in bee taxonomy (e.g. Orr et al. 2018; Weissmann et al. 2018; Ghisbain et al. 2020a). After Sanger sequencing (Eurofins Genomics, Spain) and alignment of the cleaned sequences, we ran a neighbor-joining phylogeny with 10,000 bootstraps including *D. michezi* (n = 7, including the two female specimens) along with representatives of each species of *Heterodasyroda*: *D. morotei* Quilis, 1928 (n = 2), *D. pyrotrichia* Förster, 1855 (n = 1), *D. albimana* Pérez, 1905 (n = 3). We chose the slightly more distant *Dasyroda* (*Microdasyroda*) *crassicornis* Friese, 1896 as an outgroup.

Taxonomy

Based on the results of the genetic barcoding (see below), we further examined the morphology of these females in comparison with that of the males and that of the other species of *Heterodasyroda* (i.e. *Dasyroda albimana*, *D. morotei* and *D. pyrotrichia*). High-quality pictures of both sexes of *D. michezi* were taken using a Canon EOS 5DS R (Canon Inc., Tokyo, Japan) camera assembled onto a stereomicroscope Leica M205C (Leica Microsystems, Wetzlar, Germany) with Leica LED5000 HDI illuminator under Helicon Remote 3.9.7.w. software. Photographs were then combined into a single image using Helicon Focus 7.5.6 Pro (Helicon Soft Ltd, Kharkiv, Ukraine) automontage software.

Finally, we established a key to all known species of *Heterodasypoda* following Michez et al. (2004a, b) and the results of the present study. The following abbreviations, after Michener (2007), are used below: T1, T2... = first, second, etc. metasomal terga; S1, S2... = first, second, etc. metasomal sterna; A1, A2... = first, second, etc. antennal segments, pw = puncture width.

Conservation assessment

We assessed an IUCN status for *Dasypoda michezi* following the *Guidelines for using the IUCN Red List Categories and Criteria* V. 14 (<https://www.iucnredlist.org/resources/redlistguidelines>) and the protocol of Nieto et al. (2014). To do so, we measured both the area of occupancy (AOO) and extent of occurrence (EOO) of the species. The AOO is the measure of the area in which a species occurs and corresponds to the sum of the area of grids the species occupies, while the EOO is a measure of the geographic range size of a species and is calculated by drawing a convex hull which is defined as the smallest polygon containing all the sites of occurrence and in which no internal angle exceeds 180 °C.

Results

Genetic analyses

The amplified *COI* fragment of the examined male and female specimens of *Heterodasypoda* collected in southern Portugal were identical, and distinct from all other species of *Heterodasypoda* (Fig. 1A). Given that the males morphologically correspond to the original description of *Dasypoda (Heterodasypoda) michezi* from Radchenko (2017), we can state with confidence that the examined females belong to *D. michezi*. Updated maps of the Western-Mediterranean distributions of the four known species of *Heterodasypoda* are given in Figure 1B, C. New sequences are available on Genbank with the accession numbers MW389319–MW389326; MW401790–MW401795.

First description of the females of *Dasypoda michezi*

Figures 2–14

Note. We describe for the first time the female of *Dasypoda michezi*. Specimens examined during the study are deposited in the entomological collection of the Laboratory of Zoology of the University of Mons (Belgium) and in the Institute for Evolutionary Ecology of the National Academy of Sciences of Ukraine.

Material examined. 2 ♀♀, Portugal, Algarve, Sagres Camp/Heath [37.0249°N, 8.9463°W], 13.IV.2006, leg. M. and E. Howe.

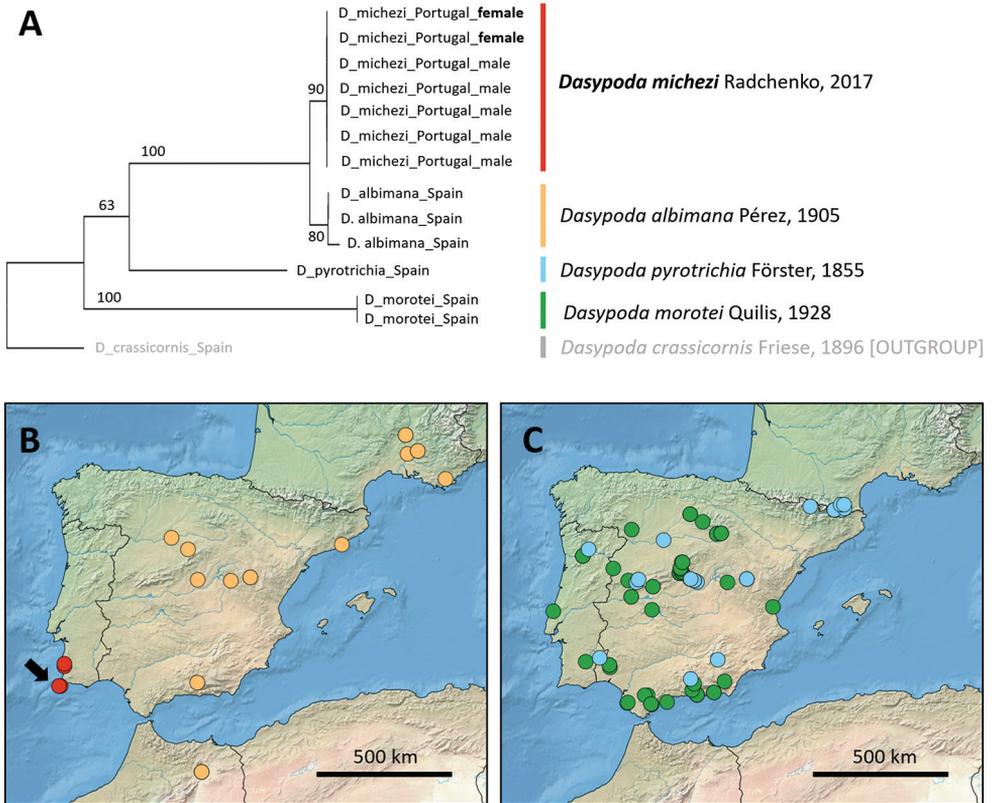


Figure 1. **A** COI phylogeny of the *Heterodasyroda*, highlighting the association of both female and male specimens of *D. michezi* from southern Portugal, with *D. (Microdasyroda) crassicornis* as an outgroup **B, C** West Mediterranean distribution of the four known species of *Heterodasyroda*: **B** records of *D. albimana* (orange) and *D. michezi* (red, new records indicated by the black arrow) **C** records of *D. morotei* (green) and *D. pyrotrichia* (blue). Adapted from Michez et al. (2004) and Radchenko (2017).

Description. Female (Figs 2–15). Body length = 13.5–13.6 mm. **Head (Figs 4–9).** W – 3.55–3.65 mm; H – 3.23–3.25 mm. Face between antennae dull, densely punctured and bearing erect white and dark (brown-black) hairs, proportion of dark hairs greater on lateral parts of face and just below ocellar field. Area in front of ocellae very densely punctured and matt, this puncturing less dense behind ocellar field. Area between lateral ocellae and compound eyes shagreened. Vertex (Fig. 5) with sparse yellow pilosity and dark erect setae. Clypeus densely punctured, punctures less than 1 pw apart, on most of its surface, punctures slightly larger and more separated distally. Medio-distal part of clypeus shiny; distal part with tufts of short white hair mixed with a few darker setae; hairless medially except in some areas with a few white and black setae; basal and lateral parts covered with mixed white and black hairs. Labrum unpunctured and shiny basally; distal part bearing thick orange setae inserted in large punctures. Mandibles black, in some places with



Figures 2–14. Female of *D. michezi* from Algarve, southern Portugal (first description) **2** dorsal view **3** lateral view **4** head (frontal view) **5** vertex and ocelli **6** malar area **7** flagellum and pedicel **8** galea and maxillary palpus **9** glossa and labial palpus inside the galea (ventral view) **10** scutum **11** metanotum and propodeum **12** metasoma (dorsal view) **13** pygidial plate **14** metasoma (ventral view).

dark red shades, bearing long white setae ventrally. Malar space (Fig. 6) shorter than width of mandible at its base, one specimen bearing very short, thick, white, black pilosity. Maxillary palpus and galea subequal. Galea with small tubercles merged

into sinuous lines on most of its surface; margin of galea with bristles all along its length (Fig. 8). Glossa dark with yellow apical bristles. Maxillary palpi approximately 0.75 times as long as length of glossa (Fig. 9). Scape with sparse dark or white setae; surface shiny, sparsely punctate, punctures large. Last article of antennae truncated at apex (Fig. 7).

Mesosoma (Figs 10, 11). W (between tegulae) – 3.15–3.24 mm. Scutum densely punctured medially (Fig. 10), shagreened between punctures, shiny in center, but matt laterally. Small unpunctured, shiny area in center of scutum with starting point of clearly visible carina extending to the most distal part of scutum. Parapsidal lines very thin and long. Scutum with sparse dark pilosity medially, surrounding parts bearing erect dark and yellowish pilosity, proportion of yellowish hairs greater laterally. Scutellum, metanotum and propodeum (Fig. 11) densely punctured and matt, scutellum bearing a majority of erect dark hairs; metanotum and propodeum mostly with yellowish erect setae. Mesosoma ventrally with dense pure-white pilosity ventrally. Tegulae dark brown, slightly shagreened and shiny. Wings transparent, slightly darkened (Figs 2, 3, 15). Forelegs bearing long brown hairs dorsally, protibia and probasitarsus with short orange pilosity ventrally. Mesotibia with yellowish-whitish pilosity ventrally and brown pilosity dorsally. Mesobasitarsus with orange pilosity ventrally, brown pilosity dorsally and whitish pilosity on its outer face. Metatibia and metabasitarsus with whitish pilosity ventrally and brown pilosity dorsally, metabasitarsus bearing orange pilosity on its posterior surface.

Metasoma (Figs 12–14). L – 7.7–7.8 mm; W (at widest point) – 4–4.5 mm. T1 evenly punctate, surface matt, pilosity as on metanotum and propodeum. T2–T4 matt and strongly shagreened, bearing whitish tufts of hair laterally, with very sparse brown pilosity on discs. T5 with denser and longer erect dark pilosity, except lateral part with whitish pilosity as on other terga. Punctures of T5 strong, dense, less than 1 pw apart laterally, but approx. 1 pw medially. Sides of T6 (around pygidial plate) bearing the same dense and long dark pilosity as on T5. Pygidial plate (Fig. 13) hairless, depressed, dark purple, narrowly elongate and bifurcate apically. Sterna thinly shagreened anteriorly with short brown pilosity, strongly punctured posteriorly (Fig. 14).

Additional male material examined. Portugal, Algarve, Sagres Camp/Heath [37.0249°N, 8.9463°W], leg. M and E Howe, 3.IV.2005 (2 ♂♂), 4.IV.2005 (3 ♂♂), 7.IV.2005 (1 ♂) 13.IV.2006 (1 ♂), at the same place and same day as the presently described females; 1 ♂, Portugal, Algarve, Sagres Campsite [same locality, 37.0249°N, 8.9463°W], 10.IV.2006, leg. M and E Howe; 1 ♂, Portugal, Algarve, Cabo de Sao Vicente [37.0227°N, 8.9964°W], 11.IV.2006, leg. M and E Howe. All specimens are deposited in the Laboratory of Zoology (UMons, Belgium).

Phenology and ecology. Flight period. The previous records of *D. michezi* were in late spring (20th–21st May) (Radchenko 2017). Our new records are from earlier in the season (3rd–13th April).

Floral choices. Unfortunately, the labels of the newly recorded specimens do not carry floral records. Some labels state “Heath”, which may refer to the type of vegetation at the collecting sites and not the visited plant (*Erica*).

Key to the species of *Heterodasygoda*

Females

- 1 Pubescence of mesotibia completely white (ventrally and dorsally)..... *Dasygoda albimana*
- Pubescence of mesotibia not entirely white, with at least some yellow or brownish hairs present **2**
- 2 Face with entirely black pilosity. Galea with external surface shiny. Scutum covered with ginger or cream hair. Tegula light orange *Dasygoda pyrotrichia*
- Pilosity of face including at least some lighter hairs. Galea with external surface either matt or shiny. Scutum with dark pilosity in the center, lighter on the sides. Tegula brown **3**
- 3 Mesotibia mostly with plumose white pilosity but including coarse spine-like dark brown hair dorsally (Figs 16–18). Hair band of T4 with sparse, brown pilosity. Ventral part of mesosoma with white pilosity..... *Dasygoda michezi*
- Mesotibia with plumose white pilosity but including coarse spine-like yellow and light brown hair (Figs 19–21). Hair band of T4 wide and dense, medially white and black on the sides. Ventral part of mesosoma with mixed black and white pilosity *Dasygoda morotei*

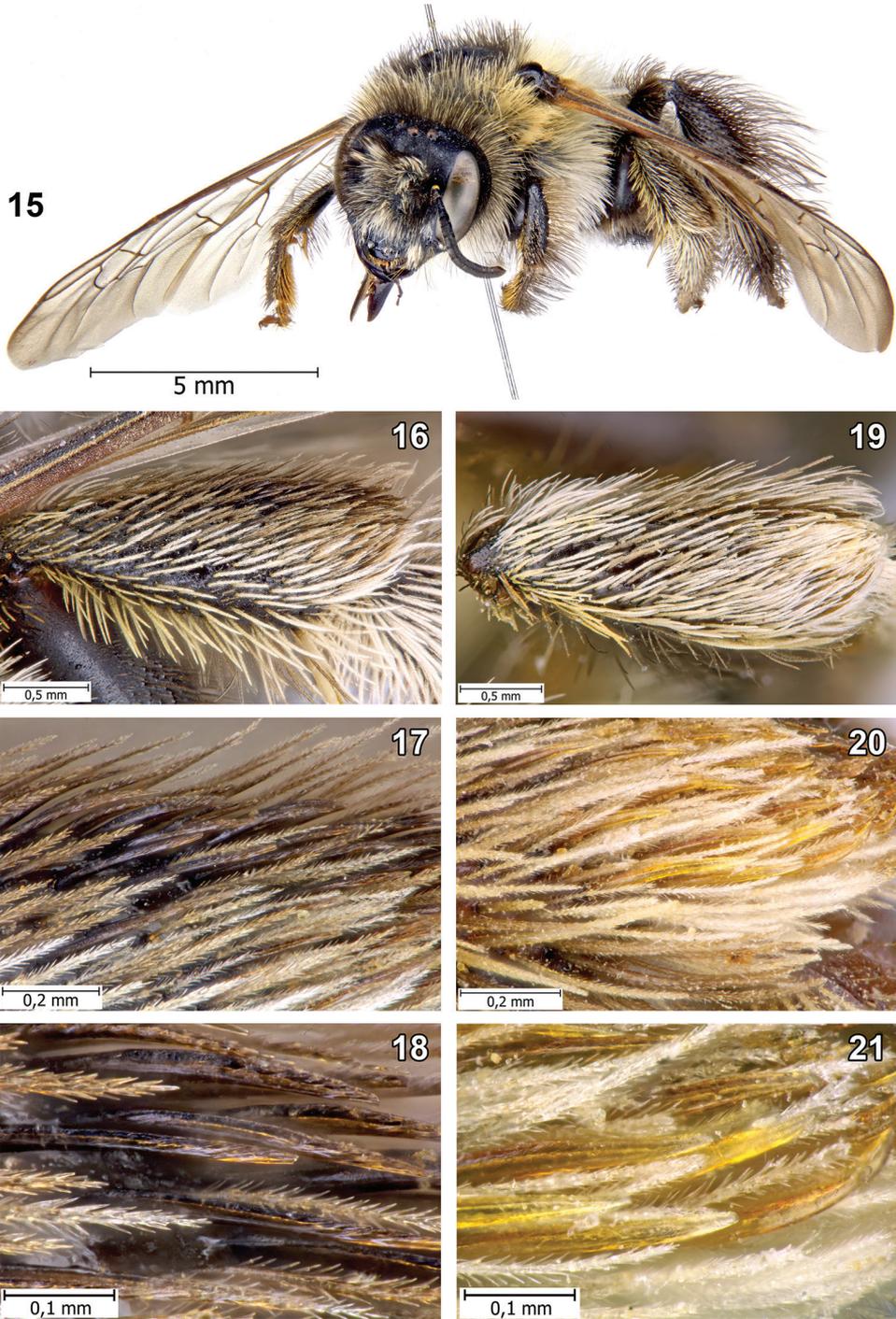
Males

Detailed diagnosis of the males of *Heterodasygoda* is available in Radchenko (2017) and new pictures of are provided in Figures 22–36.

- 1 Clypeus medially completely punctured..... **2**
- Clypeus medially with an unpunctured longitudinal carina **3**
- 2 Galea densely covered with wave-like sculptures (Fig. 28). Glossa six times as long as wide (width taken at its base; Fig. 29) *Dasygoda michezi*
- Galea with weak inconspicuous sculpting. Glossa three times as long as wide (width taken at its base) *Dasygoda albimana*
- 3 A3 at most as long as A4. S6 deeply indented at the apical margin. Base of gonostylar internal lobe as wide as external lobe..... *Dasygoda morotei*
- A3 longer than A4. S6 weakly indented at the apical margin. Base of gonostylar internal lobe wider than the external lobe *Dasygoda pyrotrichia*

Conservation status of *Dasygoda michezi*

The known EOO of *Dasygoda michezi* based on all available records encompasses ~199 km² while its known AOO encompasses 16 km² (based on 2 km × 2 km cell width). Both these measures are associated with the category *Endangered* following the IUCN criteria B1 and B2 and their combination with the condition “a” ([Extent/Habitat] *severely fragmented OR [low] number of locations*) since the bee has only been



Figures 15–21. Comparison of the mesotibia pubescence between *D. michezi* and *D. morotei* **15** female of *D. michezi* (fronto-lateral view; the position of the mesotibia is the same as in Fig. 16) **16–18** pubescence of *D. michezi* mesotibia under different magnifications **19–21** Pubescence of *D. morotei* mesotibia under different magnifications.



Figures 22–36. Male of *D. michezi* from Algarve, southern Portugal **22** dorsal view **23** lateral view **24** head (frontal view) **25** vertex and ocelli **26** malar area **27** antenna **28** galea and maxillary palpus **29** glossa and labial palpus (dorsal view) **30** scutum **31** propodeum **32** metasoma (dorsal view) **33** metasoma (ventral view) **34** S6-8 and genitalia (ventral view) **35** genitalia (lateral view) **36** genitalia (dorsal view).

reported from a very small number of locations. We therefore assess the IUCN Red List criteria of *D. michezi* as *Endangered* with the code B1a+B2a (see discussion).

Discussion

In this work, we provide the first description of the female of *Dasyroda michezi*, a poorly known melittid bee endemic to the south of Portugal, and present a key for both sexes to all known species of the genus *Heterodasyroda*. We finally assess the conservation status of *Dasyroda michezi* as *Endangered* at the European scale following the IUCN protocol. Other known species of the subgenus *Heterodasyroda* are not under such threat. In particular, *D. albimana* is categorized NT, and *D. morotei* and *D. pyrotrichia* LC (Nieto et al. 2014).

Taxonomy

The female of *D. michezi* presents some singular morphological features compared to the rest of the *Heterodasyroda* species, including a clear differentiation in its color pattern from the morphologically closely related *D. albimana*, the most visible being the color of the pilosity of the mid-leg (white in *D. albimana* and dark brown dorsally in *D. michezi*). The species confirms the original combination of morphological characteristics of the female sex proposed by Michez et al. (2004) for the subgenus, namely (i) a clypeus entirely punctured with or without a median carina, (ii) a scopa bicolored, (iii) the disc of T2 with a rather straight marginal line, (iv) pygidial plate glabrous, (v) maxillary palpa and galea of a subequal length, (vi) the margin of the galea with setae along its entire length, (vii) a malar space shorter than the length of the pedicel and (viii) a prefurcal nervulus.

Ecology and conservation

In a context of global declines in bee populations (Potts et al. 2010), the restricted distribution of *Dasyroda michezi* in the south of Portugal ultimately raises questions about its conservation. Having a restricted distribution implies that, if they occur, all or most individuals will experience adverse conditions simultaneously (Gaston 1998) and, because endemic species present by definition a few sites for conservation intervention, they are inherently more vulnerable to extinction in a context of global changes, particularly climate and habitat change. Moreover, the very recent description of the species (in 2017) naturally means that it was not attributed to a conservation status in the Red List of European bees (Nieto et al. 2014). In the latter work, the subfamily Dasypodainae was amongst the most at risk with 25% of its species being threatened. The IUCN status assessed here, *Endangered* with the code B1a + B2a, closely recalls those of the other threatened *Dasyroda* that were all assessed at least with the criterion B2a (accompanied with b[iii]), a threat related to a combination of an overall small AOO, severely fragmented habitats or a low number of locations, and a

continuous decline estimated for the quality of the habitat. These criteria are shared with a large number of other threatened bees in Europe (Nieto et al. 2014), highlighting the need to preserve the most suitable natural habitats for the bee fauna of the continent (Michez et al. 2019; Ghisbain et al. 2020b). The criterion b(iii), associated with a continuous decline of habitat quality, is not appropriate for the status of *D. michezi* given that the whole known distribution of the species is included in a protected area, the Parque Natural do Sudoeste Alentejano e Costa Vicentina. Long-term preservation of such protected habitats is especially critical for bees like *Dasygoda* due to their relatively specialized foraging behaviour (Michez et al. 2008), a characteristic that could make them especially susceptible to changes in their environment as they do not switch to alternative host plants (Scheper et al. 2014). *Dasygoda* bees rely on flower-rich environments, and the subgenus *Heterodasygoda* is mostly associated with Cistaceae and Asteraceae (Michez et al. 2004b, 2008; Ruiz 2013; Özbek 2014) with other records on Lamiaceae and Rosaceae (Michez et al. 2003, 2004b; Ruiz 2013). Radchenko (2017) highlights the association of the males of *D. michezi* with *Cistus* (Cistaceae) pollen, which is consistent with the floral choices of other representatives of the subgenus. Further work is needed to characterize in more detail the floral choices of *Dasygoda michezi* in this National Park and to estimate the population trends of its host plants in its restricted range. With global warming and its associated higher intensity of extreme weather events, such a monitoring of habitat quality is crucial in areas like southern Portugal where heat waves and fires are already frequent.

In addition to the need to characterize the habitats in which *D. michezi* occurs, more observations of the species are also required to precisely delineate the timing of its flight period. *Heterodasygoda* bees are known to fly from late spring to late summer: May–July for *D. albimana* (Michez et al. 2003), mostly May–August for *D. morotei* and *D. pyrotrichia* (Ornosa and Ortiz-Sánchez 1998, 2004; Grace 2010). Our new records combined with those of Radchenko (2017) suggest that the flight period of *Dasygoda michezi* encompasses April and May (late spring). Overall, given the growing evidence that significant phenological shifts and mismatches in plant-pollinator interactions following global warming are occurring (Duchenne et al. 2020; Gérard et al. 2020), further understanding the species phenology and floral associations during their flight period is a key to their effective conservation.

Iberian Peninsula as an understudied hotspot of bee diversity

Although Europe constitutes one of the most extensively studied areas in the world for bees (Michez et al. 2019), the very recent discovery and characterization of *Dasygoda michezi* in Portugal is not surprising. New bee species are still continuously described from the Iberian Peninsula (Müller 2012; Wood and Cross 2017; Kuhlmann and Smit 2018; Smit 2018; Wood et al. 2020a), a geographic area that constitutes a global hotspot not only for bee species diversity (Orr et al. 2020) but also for many other organisms (Gómez and Lunt 2007). In total, over 1000 bee species are known from Spain (Ortiz-Sánchez 2011, 2020) and 722 from Portugal (Baldock et al. 2018; Wood et al. 2020a). This high diversity is partly due to the role that the Peninsula played as a glacial

refugium during the Quaternary (Hewitt 2011), as well as the Mediterranean climate of the region, combined with the striking diversity of habitats (i.e. a combination of meadows, scrublands, mountains, woodlands and coastlines). In particular, the Iberian Peninsula constitutes a center of diversity for both of the *Dasyпода* subgenera *Microdasyпода* and *Heterodasyпода*. The first subgenus includes four known species, with one species endemic to Iberia: *D. iberica* (Michez et al. 2004b; Radchenko et al. 2019). The second includes four species with two endemics: the currently studied *D. michezi* and also *D. morotei* (Michez et al. 2004b; Radchenko 2017). Despite the extremely rich bee diversity of the Iberian Peninsula however, both Spain and Portugal present a very high percentage of species assessed as “data deficient” in the European Red List (Nieto et al. 2014; Wood et al. 2020b). The present case further suggests that additional efforts are encouraged to characterize and monitor the highly diverse bee fauna of these understudied areas. Future projects that focus on understanding the ecology of endemic bee species and delineating more precisely their distributions are encouraged to assess the potential threats that these species could face in the near future.

Acknowledgements

The authors are grateful to Thomas J. Wood for helpful comments on the manuscript. We warmly thank Yulia Astafurova, Ian Cross, Jack Neff and an anonymous reviewer for their constructive comments and advice on the manuscript. The authors thank Michael G. Branstetter for kindly helping confirm the *COI* sequences of *Dasyпода morotei*. The research leading to this publication received funds from the Fonds de la Recherche Scientifique-FNRS (Brussels, Belgium) and “The support of the priority research areas development of Ukraine”, KPKVK 6541230. GG is funded by a F.R.S.-FNRS grant “Aspirant”. DC and DM are partly funded by the F.R.S.-FNRS and the *Fonds Wetenschappelijk Onderzoek* (FWO) under the EOS project CLIPS (n°3094785).

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Simultaneous percussion by the larvae of a stem-nesting solitary bee – a collaborative defence strategy against parasitoid wasps?

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Academic editor: Michael Ohl | Received 23 November 2020 | Accepted 7 February 2021 | Published 25 February 2021

<http://zoobank.org/D10742E1-E988-40C1-ADF6-7F8EC24D6FC4>

Citation: Müller A, Obrist MK (2021) Simultaneous percussion by the larvae of a stem-nesting solitary bee – a collaborative defence strategy against parasitoid wasps? *Journal of Hymenoptera Research* 81: 143–164. <https://doi.org/10.3897/jhr.81.61067>

Abstract

Disturbance sounds to deter antagonists are widespread among insects but have never been recorded for the larvae of bees. Here, we report on the production of disturbance sounds by the postdefecating larva (“prepupa”) of the Palearctic osmiine bee *Hoplitis (Alcidamea) tridentata*, which constructs linear series of brood cells in excavated burrows in pithy plant stems. Upon disturbance, the prepupa produces two types of sounds, one of which can be heard up to a distance of 2–3 m (“stroking sounds”), whereas the other is scarcely audible by bare ear (“tapping sounds”). To produce the stroking sounds, the prepupa rapidly pulls a horseshoe-shaped callosity around the anus one to five times in quick succession over the cocoon wall before it starts to produce tapping sounds by knocking a triangularly shaped callosity on the clypeus against the cocoon wall in long uninterrupted series of one to four knocks per second. Sound analysis revealed that the stroking sounds consist of several syllables, which are very similar to the single syllables of the tapping sounds: both last about 0.5 ms and spread over 40 kHz bandwidth from the audible far into the ultrasonic range. The production of stroking sounds by a prepupa induces other prepupae of the same nest to stroke and/or to tap resulting in a long-lasting and simultaneous albeit unsynchronized percussion by numerous prepupae along the whole nest stem. We hypothesize that these disturbance sounds serve an anti-antagonist function and that they have evolved to disturb the reflectance signals that parasitoid wasps use to localize concealed hosts during vibrational sounding.

Keywords

Anthophila, Apiformes, chorusing behaviour, echolocation, *Hoplocryptus*, ichneumonid wasps, Megachilidae, Osmiini

Introduction

Innumerable insects from many different taxa produce vibrational signals (“sounds” hereafter), which are propagated through air, water or solids and perceived by the recipients with tympanal ears or near-field receptors including sensory sensillae, subgenual organs or the antennae (Gullan and Cranston 2000; Yack and Hoy 2003; Drosopoulos and Claridge 2005). Depending on the insect taxon, these sounds are produced in very different ways encompassing i) vibration of thorax, abdomen or wings, ii) percussion of body parts against each other or against a substrate, iii) deformation of modified cuticular areas by contraction and relaxation of special musculature, iv) expulsion of air through spiracles or proboscis, v) stick-slip movements of adjacent body segments and vi) true stridulation, which involves the movement of two specialized body parts against each other in a regular patterned manner (Ewing 1989; Claridge 2005; Dolle et al. 2018). The majority of these air-, water- or substrate-born insect sounds serve for intraspecific communication, such as species recognition, mate finding, courtship, male competition, recruitment to feeding sites or warning against antagonists.

However, there are other insect sounds, which are not aimed at conspecifics, but which are assumed to be predominantly defensive since they are mainly produced when the insects are disturbed or attacked. In fact, experiments with a species each of ground beetles (Carabidae), tiger beetles (Cicindelidae), water scavenger beetles (Hydrophilidae), scolytid beetles (Scolytidae), mutillid wasps (Mutillidae), cicadas (Cicadidae) and butterflies (Nymphalidae) revealed that such disturbance sounds deter predators like spiders, predatory beetles, mice or birds (Bauer 1976; Smith and Langley 1978; Masters 1979; Lewis and Cane 1990; Olofsson et al. 2012). Three mechanisms are discussed, which might underlie the effect of the disturbance sounds: they may either serve i) to alert the antagonist to the harmfulness of the sound producer (“acoustic aposematism”), ii) to startle the attacker or iii) to render the localization of the sender by parasites or predators more difficult (Masters 1979; Kočárek 2009; Corcoran et al. 2010). Disturbance sounds are widespread among insects and known for example from i) larvae of Blattodea (Blaberidae, Ectobiidae) and Coleoptera (Cerambycidae), ii) pupae of Coleoptera (Scarabaeidae) and Lepidoptera (Arctiidae, Callidulidae, Hesperiiidae, Lycaenidae, Lymantriidae, Noctuidae, Notodontidae, Nymphalidae, Phalaenidae, Pyralidae, Riodinidae, Sphingidae, Zygaenidae) and iii) imagines of Auchenorrhyncha (Cicadidae), Blattodea (Blaberidae), Coleoptera (Cerambycidae, Cicindelidae, Scarabaeidae, Silphidae), Heteroptera (Cydnidae, Reduviidae), Hymenoptera (Apidae, Bradynobaenidae, Mutillidae, Crabronidae, Formicidae, Rhopalosomatidae) and Lepidoptera (Nymphalidae) (Hinton 1945, 1955; Downey 1966; Nelson 1979;

Schal et al. 1982; Tremevan 1985; Tschuch and Brothers 2000; Drosopoulos and Claridge 2005; Kočárek 2009; Olofsson et al. 2012; Dolle et al. 2018).

Sound production for intra- or interspecific communication is rare in bees. It has been observed in the males of several solitary species during courtship and mating (Torchio 1990; Weislo and Buchmann 1995; Toro and Riveros 1998; Conrad et al. 2010) and in several social species of corbiculate Apidae for queen-queen, queen-worker and worker-worker communication, defense or recruitment to food sources (Kirchner and Röschard 1999; Hrnčir et al. 2005). In most cases, these sounds are produced by thoracic or more rarely wing vibrations rather than specialized structures (Tschuch and Brothers 2000). An exception is *Meganomia binghami* (Cockerell) (Melittidae), in which the males produce loud rasping sounds during mating with stridulatory areas on the sterna (Rozen 1977).

Given this rather silent nature of bees, it turned out as a surprise when we recently realized that the postdefecating larvae (“prepupae”) of the stem-nesting osmiine bee *Hoplitis (Alcidamea) tridentata* (Dufour and Perris) (Megachilidae) produce two different types of sounds upon disturbance, one of which is very quiet and difficult to hear by bare ear (“tapping sounds”), whereas the other is loud and well audible (“stroking sounds”). A literature survey revealed that the stroking sounds were already briefly mentioned by Enslin (1925), Malyshev (1937) and Westrich (1989), who describe them as cracking, humming or barking and assume that the prepupae produce them by rapidly contracting their body inside the cocoon.

In this study, we i) describe the prepupal disturbance sounds of *Hoplitis tridentata*, ii) identify the structures that produce them, iii) present the results of experiments on prepupal sound production, iv) examine the prepupae of other osmiine bee species for the presence of sound producing structures similar to those of *H. tridentata* and v) discuss possible functions of the disturbance sounds.

Methods

Bee species

Hoplitis tridentata is a 10–12 mm long Palearctic osmiine bee (Megachilidae, Osmiini), which has a vast distribution ranging from Europe and northern Africa eastwards to Western Siberia and Central Asia (Müller 2020). In Central Europe, the species' flight period lasts from the end of May to mid August (Swiss Bee Team 2000). The females nest in burrows excavated in dead pithy stems (e.g. of *Artemisia*, *Cirsium*, *Cynara*, *Ferula*, *Onopordum*, *Rubus*, *Verbascum*; Figs 1, 2) or – more rarely – in hollow stems (e.g. of *Dipsacus*, *Phragmites*) (Enslin 1925; Malyshev 1937; Westrich 2018; Müller 2020). They enter the stem either through a hole bitten laterally through the stem wall (Fig. 1) or at its broken tip (Fig. 2). The nests usually contain 6–12 and occasionally up to 34 brood cells, which are arranged in a linear series within the maximally 36 cm long nesting burrow (Enslin 1925; Malyshev 1937). Each brood cell is provisioned

with pollen of Fabaceae (e.g. *Lathyrus*, *Lotus*, *Onobrychis*, *Vicia*), sometimes admixed with pollen of *Echium* (Boraginaceae), before a single egg is laid onto the provision and the cell is closed with a wall of masticated green leaves (Westrich 2018; Müller 2020). The egg hatches after a few days and the larva devours the food provision within less than a month, before it spins a cocoon and overwinters as prepupa inside the cocoon (Malyshev 1937; Fig. 3). Pupation and metamorphosis to the adult stage take place in early summer of the following year. In Central Europe, the prepupal stage lasts roughly ten months, i.e. from August to May.

The nests of *Hoplitis tridentata* are attacked by numerous brood parasites (Dufour and Perris 1840; Giraud 1866; Laboulbène 1877; Enslin 1925; Bouček 1952; Le Goff 1997; Baur and Amiet 2000; Schwarz 2007; Westrich 2018, 2020), which act either as cleptoparasites that devour the larval food provisions or as parasitoids that feed on the larvae. The species' known cleptoparasites include the megachilid bees *Stelis minuta* Lepeletier and Audinet-Serville and *S. ornatula* (Klug), the sapygid wasp *Sapyga quinquepunctata* (Fabricius), the gasteruptionid wasp *Gasteruption thomsoni* (Schletterer), the meloid beetle *Zonitis immaculata* (Olivier) and the sarcophagid fly *Miltogramma murinum* Meigen. Known parasitoids are the ichneumonid wasps *Hoplocryptus bellosus* (Curtis) and *H. signatorius* (Gravenhorst), the leucospidid wasps *Leucospis biguetina* Jurine and *L. dorsigera* Fabricius, the chalcidid wasp *Neochalcis osmicida* (Saunders) and the tachinid fly *Triathria setipennis* (Fallén).

Origin of bee material

To obtain nests of *Hoplitis tridentata*, trap nests were positioned in suitable habitats in northern Switzerland (Glattfelden/Zurich) and in southern Switzerland (Kalpetran/Valais) in spring 2020. Each trap nest consisted of a bundle of five dry *Rubus* stems of 50 cm length (Fig. 4). In total, 35 sealed nests were collected in late summer 2020, of which 12 were opened for audio and video recording, examination of larval morphology and experiments on prepupal sound production; the remaining nests – although partly used for other experiments – remained intact and were returned to the field at the end of the study.

Sound recording and analysis

Acoustic recordings were performed with a Batlogger M (Elekon AG, Luzern, Switzerland). This device records wav-files with a sampling rate of 312.5 kHz with 16 bit sampling depth to a SD-memory card. Its microphone shows a relatively flat frequency response (± 5 dB) from the low audio range up to 150 kHz. Recordings of 10.5 s duration were triggered manually at a distance of 2–20 cm between microphone and the nest stem.

The wav-files were analysed with the software Raven Pro 1.6.1 (Center for Conservation Bioacoustics 2019). To calculate temporally well-resolved spectrograms, we used a Blackman window of size 64, overlapping by 90.6%, and zero-buffered the windows for a FFT size of 1024 points. In Raven Pro we designed a band-limited

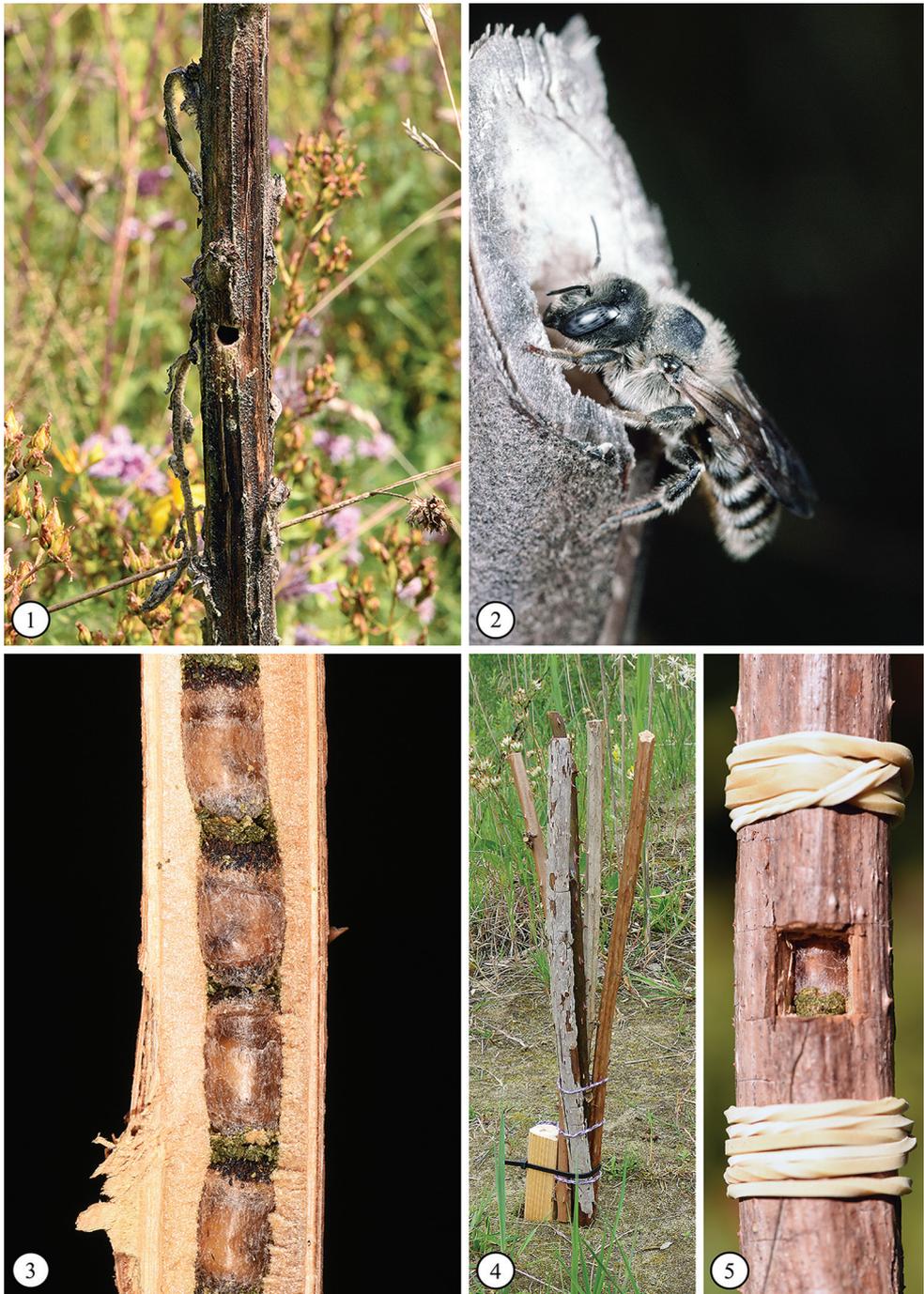
energy detector that was run over 52 high quality recordings of 10.5 s duration each. The detector was iteratively optimized to identify signals of 0.15–4.0 ms duration with a minimum temporal separation of 0.46 ms, searching in the frequency band of 0.1–150 kHz with a SNR threshold of 12 dB and a minimum occupancy of 70%. Spectral and temporal parameters were measured automatically within the detection window. To achieve robust signal measurements from the spectrograms, we used parameters based on temporal and spectral cumulative energy distributions (5%, 95% and differences thereof), which Raven Pro automatically calculates.

Experiments on prepupal sound production

Intended as a first step towards a better understanding of the possible function of the prepupal sounds of *Hoplitis tridentata*, we performed ten experiments (Table 1). For these experiments, we used four types of nests: i) eight trap nest bundles still positioned at their original site on an area of about 40 m² containing one (n = 1), two (n = 3), three (n = 3) and five (n = 1) sealed nests; ii) ten sealed nests with an unknown number of brood cells; iii) four sealed nests (with 2, 4, 9 and 10 prepupae, respectively), which were longitudinally split into two halves, closed with rubber band and opened again for the experiments; and iv) five sealed nests (with 4, 5, 7, 10 and 12 prepupae, respectively), which were longitudinally split into two halves, provided with a single rectangular window exactly corresponding to the position of a cell and closed with rubber band, resulting in one exposed prepupa within its cocoon (Fig. 5) and 3–11 prepupae well protected inside the stem; due to the semitransparent wall of the cocoon, the behaviour of the exposed prepupa inside its cocoon could easily be observed under

Table 1. Description of experiments on prepupal sound production.

Experimental question	Experiment number	Experimental procedure	Nest type
Do the prepupae spontaneously produce sounds?	1	Eight nest bundles were auscultated for 15 min during sunny weather from a short distance.	i
	2	Ten nests were auscultated together during one hour from a short distance.	ii
Which disturbances cause the prepupae to produce sounds?	3	Ten nests were individually subjected to a strong movement by turning the stems five times from a vertical to a horizontal position and back.	ii
	4	Four nests were individually subjected to a strong increase in temperature to 40 °C by irradiating them with a 150 watts infrared heat lamp (Beurer IL21) for 90 sec from a distance of 20 cm.	iii
	5	Ten nests were individually subjected to vibration by holding a vibrating small tuning fork to the stem wall.	ii
	6	Exposed prepupae in five nests were individually subjected to light by illuminating them with a torch for two minutes from a distance of 2 cm.	iv
Is the production of stroking and tapping sounds by a single prepupa linked?	7	In five nests, sound production by the exposed prepupa was recorded after it was stimulated to produce stroking sounds by carefully denting its cocoon wall with a stick.	iv
	8	In ten nests, the duration of the tapping sounds was recorded after the nests were individually turned five times from a vertical to a horizontal position and back.	ii
Do the sounds produced by a prepupa trigger sound production by other prepupae within the same nest?	9	In five well-fixed nests, the exposed prepupa was stimulated to produce stroking sounds by carefully denting its cocoon wall with a stick, before the nests were auscultated for stroking and tapping sounds of other prepupae.	iv
	10	In five nests the exposed prepupa was stimulated to produce tapping sounds by illuminating it with a torch, before the nests were auscultated for stroking and tapping sounds of other prepupae.	iv



Figures 1–5. *Hoplitis tridentata* **1** nest entrance in a dead stem of *Verbascum* **2** female entering her nest at the broken tip of a dead stem of *Verbascum* (photo A. Krebs) **3** linear series of brood cells within a dead stem of *Rubus* each containing a prepupa inside the cocoon **4** trapnest bundle consisting of five 50 cm long stems of *Rubus* **5** experimental nest stem with exposed brood cell.

good light conditions. In all experiments, the stroking sounds were registered by ear, whereas the tapping sounds were perceived with the aid of a bat detector (SSF Bat2, microelectronic Volkmann) set to 30 kHz and held at short distance from the stem. It proved to be impossible to differentiate between the sounds of different prepupae through the stem wall, i.e. to judge whether successive sounds were produced by one or more individuals; to address this inaccuracy, we apply the term stroking or tapping sound “event”, which is defined as sound production by at least one prepupa. The ambient temperature for the field and the lab experiments was 23–25 °C. Experiments 1 and 8 were run once, experiments 2–5 twice and experiments 6, 7, 9 and 10 three times. The results of the different runs were pooled for each experiment.

Sound producing structures in other osmiine bees

To address the question whether the prepupae of other osmiine bees are equipped with similar sound producing structures like *Hoplitis tridentata*, we examined the prepupae of the following eleven Central European species belonging to four genera and ten subgenera obtained from nests collected by the first author in 2020 and by P. Bogusch in the frame of studies on reed gall inhabiting aculeate Hymenoptera (Bogusch et al. 2015): *Chelostoma* (*Chelostoma*) *florisomne* (Linné), *C.* (*Gyrodromella*) *rapunculi* (Lepeletier), *Heriades* (*Heriades*) *truncorum* (Linné), *Hoplitis* (*Alcidamea*) *leucomelana* (Kirby), *H.* (*Hoplitis*) *adunca* (Panzer), *O.* (*Helicosmia*) *caerulescens* (Linné), *O.* (*Hoplosmia*) *spinulosa* (Kirby), *O.* (*Melanosmia*) *uncinata* Gerstäcker, *O.* (*Metallinella*) *brevicornis* (Fabricius), *O.* (*Osmia*) *bicornis* (Linné) and *O.* (*Osmia*) *cornuta* (Latreille). Furthermore, we reviewed the literature on osmiine bee larvae for possible indications that the prepupae possess sound producing structures, such as: i) clypeus projecting over the antennae in lateral view, ii) colour of the clypeus differing from that of the surrounding cuticle, iii) presence of a projecting horseshoe-shaped ridge around the anus, and iv) colour of the area around the anus differing from that of the surrounding cuticle. In total, we checked larval descriptions for 43 species belonging to eight genera and 19 subgenera.

Results

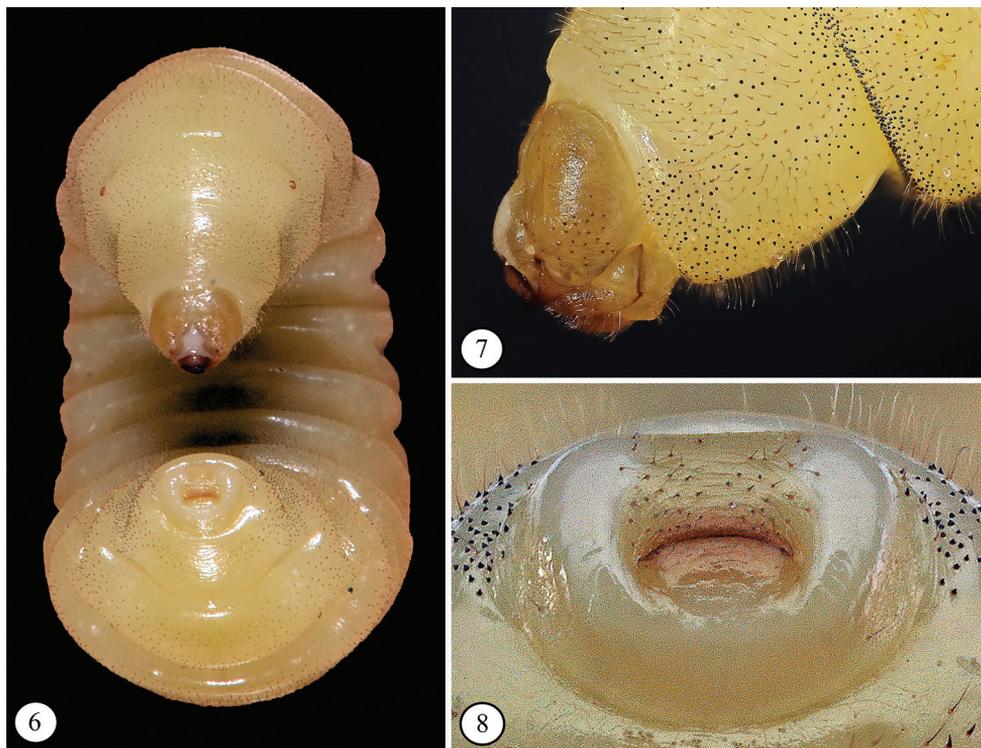
Prepupal sounds

The prepupae of *Hoplitis tridentata* produce two types of sounds, which considerably differ in their intensity. In unopened nest stems and under complete silence, the “tapping sounds” are audible by bare ear only within 10–20 cm (Suppl. material 1), whereas the “stroking sounds” can be heard up to a distance of 2–3 m (Suppl. material 2). The different loudness is also reflected by the strength of the vibrations that can be felt while touching the nest stem: the tapping sounds cannot be felt with the fingertips, whereas the stroking sounds are clearly perceptible.

The prepupae produce the sounds with two projecting body callosities, which are localized on the clypeus and around the anus (Figs 6–8). The clypeal callosity is of roughly triangular shape, while the anal callosity has the form of a horseshoe-shaped ridge, which surrounds the anus. The two callosities are distinctly harder than the surrounding cuticle and stand out by their (snow-)white coloration; in rare cases, the clypeal callosity is completely and the anal callosity partly brownish pigmented.

In larvae that have either started to spin their cocoon or just have finished cocoon construction, the callosities have not yet reached their final functional state: compared with the final state, the clypeal callosity is distinctly softer albeit already white and projecting, while the anal callosity is distinctly softer, still of the same colour as the surrounding cuticle and less projecting. Thus, the callosities seem to reach their functional state only after the prepupae have finalized the cocoon.

The prepupae produce the tapping sounds by knocking the clypeal callosity against the cocoon wall (Fig. 9; Suppl. material 5); each contact with the cocoon wall results in one tapping sound. The tapping sounds are usually produced in long uninterrupted series of one to four knocks per second. To produce the stroking sounds, the prepupae



Figures 6–8. *Hoplitis tridentata* **6** prepupa with a triangularly shaped white callosity on the clypeus and a horseshoe-shaped white callosity around the anus, which both are used for sound production **7** clypeal callosity in profile distinctly projecting over the antennae (photo J. Sommerhalder) **8** anal callosity (photo J. Sommerhalder).

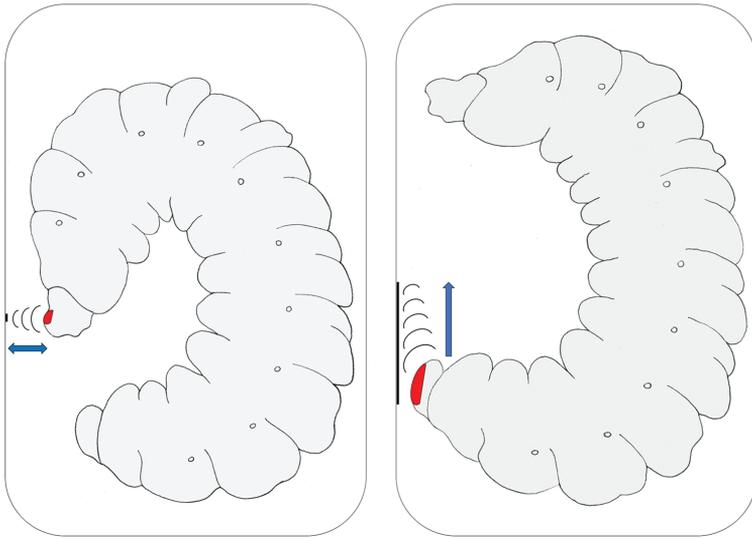


Figure 9. Sound production in prepupae of *Hoplitis tridentata*. The tapping sounds (left) are produced by knocking the clypeal callosity against the cocoon wall and the stroking sounds (right) by rapidly moving the anal callosity over the cocoon wall.

stretch their body and bring the abdominal tip in contact with the cocoon, before the anal callosity is rapidly pulled forward over the cocoon wall (Fig. 9; Suppl. material 6); each pull results in one stroking sound. The stroking sounds are produced one to maximally five times in quick succession and never in long series as the tapping sounds. After stroking, the prepupa invariably starts producing tapping sounds (see Experiments section). Although not substantiated by data, the number of tapping sounds per second and the number of consecutive stroking sounds seem to increase with increasing intensity of the disturbance.

Except for small wart-like protuberances, the inner cocoon wall of *Hoplitis tridentata* lacks special projections such as ridges or teeth, which might help in producing or amplifying the sounds when the prepupae move their anal callosity over the cocoon wall. The wart-like protuberances are unlikely to participate in sound production as their density considerably varies between the cocoons of different individuals, as they are not confined to defined zones of the inner cocoon surface and as similar protuberances also occur in the cocoons of other osmiine bee species (A. Müller, unpublished data). Nevertheless, the cocoon wall might play an important role in sound production going well beyond its function as a mere abutment for the sound producing structures. In fact, even a slight touch of the cocoon wall by the experimenter leads to a crackling sound readily audible both to the unaided ear and by a bat detector set to the ultrasonic range. It would be worthwhile to compare the physical and morphological properties of the cocoon of *H. tridentata* with those of related *H. (Alcidamea)* species, which do not produce sounds. Such a comparison, however, is beyond the scope of this study.

Table 2. Basic temporal and spectral characteristics of the prepupal sounds of *Hoplitis tridentata*. Mean values are given with standard deviation in brackets. In total, 2592 tapping sounds and 148 stroking sounds were measured.

Sound characteristics	Tapping sounds	Stroking sounds
Duration	0.63 (± 0.48) ms	40.6 (± 16.0) ms
Number of syllables	1	15.3 (± 7.2)
Interval between syllables	–	2.8 (± 0.64) ms
Duration of syllable	0.63 (± 0.48) ms	0.48 (± 0.33) ms
Duration of syllable (90% energy sum)	0.46 (± 0.30) kHz	0.38 (± 0.24) kHz
Lower frequency (5% energy sum)	11.91 (± 4.60) kHz	12.07 (± 3.70) kHz
Peak frequency (peak energy)	23.94 (± 9.56) kHz	24.44 (± 8.13) kHz
Upper frequency (95% energy sum)	54.31 (± 13.16) kHz	56.22 (± 11.70) kHz
Bandwidth of syllable (90% energy sum)	42.39 (± 12.24) kHz	44.15 (± 11.64) kHz

Sound characteristics

The tapping sounds are extremely brief lasting less than 1 ms and consist of a single syllable (Table 2, Fig. 10). In contrast, the stroking sounds are considerably longer with a mean duration of 40 ms and consist of an average of 15 syllables (Fig. 11). The single syllables of a stroking sound are separated by a temporal spacing of approximately 2.8 ms and can only be perceived by the human ear after a strong time expansion of $10 \times$ (Suppl. material 3). The composition of the stroking sounds from single syllables suggests that the anal callosity is jerkily rather than evenly moved over the cocoon wall.

The tapping sounds and the single syllables of the stroking sounds show very similar temporal and spectral characteristics (Table 2). Both are very brief and explosive

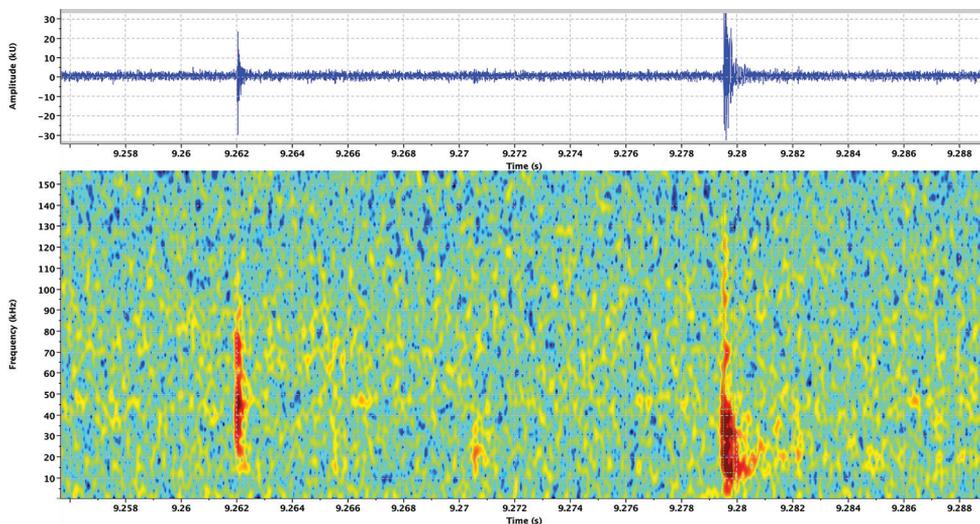


Figure 10. Amplitude plot (above) and spectrogram (below) of two prepupal tapping sounds of *Hoplitis tridentata* originating from two individuals inhabiting the same nest; the sounds were recorded with a batlogger in a distance of 20 cm from the opened nest stem.

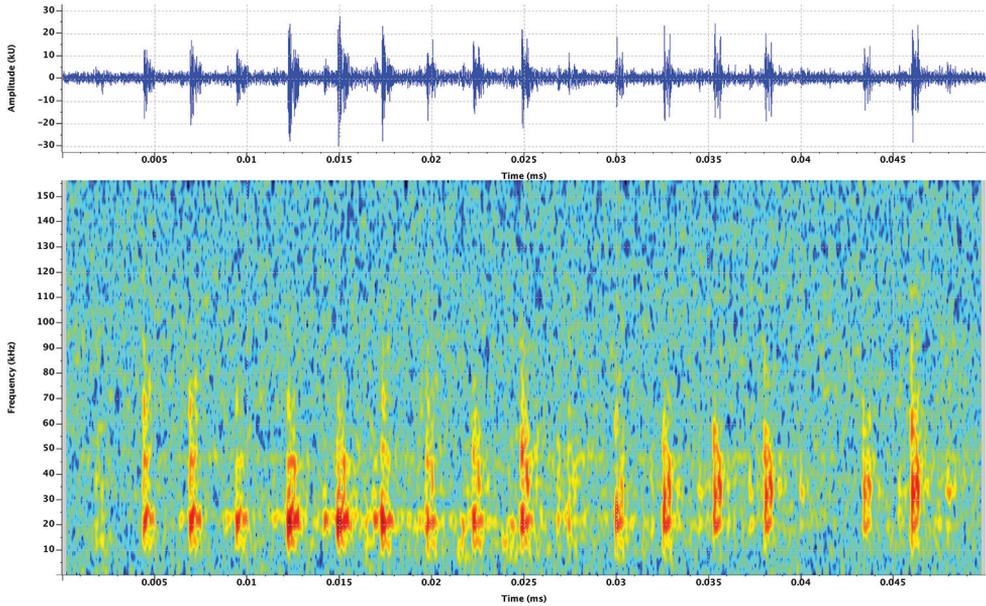


Figure 11. Amplitude plot (above) and spectrogram (below) of a prepupal stroking sound of *Hoplitis tridentata* of 46 ms duration containing 15 syllables; the sounds were recorded with a batlogger in a distance of 20 cm from the opened nest stem.

containing very few non-sinusoidal waves resulting in very broad frequency spectra, which regularly reach into the ultrasonic range, occasionally even above 100 kHz.

Experiments on prepupal sound production

Spontaneous prepupal sounds were absent (experiment 1) or very rare with 0.4 stroking and 0.15 tapping sound events per stem and hour (experiment 2). Movement of the stem and increase in temperature (experiments 3 and 4) stimulated prepupal sounds in every trial ($n = 20$ and $n = 8$, respectively), all of which were both stroking and tapping sound events. Vibration of the stem (experiment 5) stimulated prepupal sounds in 95% of the trials ($n = 20$), of which 32% were both stroking and tapping sound events and 68% only tapping sound events. Exposure to light (experiment 6) stimulated prepupal sounds in 87% of all trials ($n = 15$), of which 8% were both stroking and tapping sound events and 92% only tapping sound events. Stimulated stroking sounds (experiment 7) were followed by tapping sounds by the same prepupa in every trial ($n = 15$). Tapping sound events after the end of a disturbance (experiment 8) lasted on average 21.1 min and ranged from 8.5 min to 36.3 min ($n = 10$). Stimulated stroking sounds (experiment 9) triggered sound production by other prepupae within the nest in 93% of the trials ($n = 15$), of which 71% were both stroking and tapping sound events, 22% only tapping sound events and 7% only stroking sound events. Stimulated tapping sounds (experiment 10) never triggered sound production

by other prepupae within the nest ($n = 15$). In summary, the experiments revealed that i) the prepupae do not or only exceptionally produce sounds spontaneously, ii) the disturbances stimulating sound production are rather unspecific encompassing stem movement and vibration, increase in temperature, exposure to light and denting of the cocoon wall, iii) the tapping sounds and the stroking sounds appear to represent two levels of escalation with the former being produced after a weak disturbance but the latter only after a strong disturbance, iv) the stroking sounds are followed by extended periods of tapping sounds after the disturbance has ended, whereas the stroking sounds stop within 10–15 sec to maximally 30 sec after the end of the disturbance, and v) the stroking sounds trigger sound production by other prepupae of the same nest, which is not the case for the tapping sounds.

Sound producing structures in other osmiine bees

The prepupae of eleven Central European osmiine bee species belonging to four genera and ten subgenera (see Methods section) all lacked clypeal and anal callosities. The descriptions of osmiine bee prepupae in the literature either did not suggest the presence of sound producing structures or proved to be insufficient for a proper assessment (Suppl. material 7). However, a few Nearctic *Hoplitis* (*Alcidamea*) species, such as *H. biscutellae* (Cockerell), *H. hypocrita* (Cockerell) and probably also *H. fulgida* (Cresson), *H. producta* (Cresson) and *H. uvulalis* (Cockerell), were recorded by Rozen and Praz (2016) to possess a “projecting unpigmented ridge ringing anus except for ventral one-quarter”, which corresponds well to the anal callosity of *H. tridentata* and might suggest the presence of sound producing structures also in these species. This suggestion also holds for the Palaearctic species *H. (Alcidamea) acuticornis* (Dufour and Perris), whose prepupae were observed by Enslin (1925) to strongly move upon disturbance exactly as *H. tridentata* does when producing stroking sounds. Interestingly, these six *H. (Alcidamea)* species do not only belong to the same subgenus as *H. tridentata* but also nest in plant stems, either obligatorily, preferentially or regularly (Graenicher 1905; Comstock 1924; Enslin 1925; Hicks 1926, 1934; Rau 1928; Linsley and MacSwain 1943; Michener 1947; Fischer 1955; Hurd and Michener 1955; Medler 1961; Parker and Bohart 1966, 1968; Parker 1975; Clement and Rust 1976; Rust 1980; Tepedino and Parker 1984; Frohlich et al. 1988). In summary, sound producing structures similar to those of *H. tridentata* appear to be absent in most osmiine bee taxa with the possible exception of a few related stem-nesting *H. (Alcidamea)* species in both the Palaearctic and the Nearctic region.

Discussion

The finding that the prepupae of *Hoplitis tridentata* produce well audible sounds is the first record of sound production in larvae of bees and – to the best of our knowledge – also of Hymenoptera. These prepupal sounds are almost exclusively produced after

disturbance, which qualifies them as typical disturbance sounds (Drosopoulos and Claridge 2005). Although disturbance sounds are widespread in insects (see Introduction section), their function has been examined only in a few species, where they were unambiguously found to deter antagonists (Bauer 1976; Smith and Langley 1978; Masters 1979; Lewis and Cane 1990; Olofsson et al. 2012). It is most likely that the prepupal sounds of *H. tridentata* also serve an anti-antagonist function, which would be highly adaptive as the prepupae spend many months within exposed and rather thin-walled plant stems, where they are substantially more susceptible to predators and parasites than the offspring of bee species developing in other substrates, for example in the ground or in dead wood.

The spectrum of potential antagonists affecting *Hoplitis tridentata* in the prepupal stage encompasses two main groups, i.e. vertebrate predators such as birds, which peck open the stems and devour the prepupae, and insect brood parasites such as parasitoid wasps, which insert the eggs through the stem wall and whose larvae feed on the prepupal bodies. These two groups of antagonists perceive vibrational signals differently, i.e. the predators mainly as air-borne sounds and the parasites probably exclusively as substrate-borne vibrations. As the sounds produced by the prepupae of *H. tridentata* are both air-borne and substrate-borne, the quality of the prepupal sounds does not allow us to decide whether the sounds have evolved against vertebrate predators or insect brood parasites. Similarly, the disturbances that stimulated prepupal sound production in the experiments hardly allow any conclusions on the natural triggers of the sounds and suggest that the prepupae react rather unspecifically to any disturbance whether naturally occurring or not. As discussed below, we nevertheless hypothesize that the intended recipients of the prepupal sounds are insect brood parasites, that the sounds act as substrate-borne vibrations, and that the sounds are triggered by the presence of parasites on the nest stem.

Among the three potential mechanisms which might underlie the deterrent effect of disturbance sounds (see Introduction section), acoustic aposematism is highly unlikely to act in *Hoplitis tridentata* because the prepupae are unlikely to be toxic or in any other way dangerous for vertebrate predators and insect brood parasites. It appears also to be improbable that the disturbance sounds have evolved to startle a predator or parasite because the prepupae cannot take advantage of the attacker's short-term confusion for escape as they are enclosed within their cocoons; furthermore, startling vertebrate predators by sound seems to be counteradaptive as the predators might learn to use the sounds to localize nests after they have found that the prepupae are harmless. Instead, we hypothesize that sound production in *H. tridentata* has evolved to render it difficult for parasitoid wasps with a peculiar host-searching strategy to precisely localize the prepupae within the plant stem.

Parasitoid wasps usually localize hidden hosts by scent or vibrations caused by host movement and feeding (Xiaoyi and Zhongqi 2008). However, numerous ichneumonid wasps of several subfamilies as well as orussid wasps employ a special form of echolocation to localize deeply concealed and often immobile larvae, prepupae and pupae of their hosts – a host-searching strategy known as vibrational sounding (Henaut

and Guerdoux 1982; Wäckers et al. 1998; Broad and Quicke 2000; Vilhelmsen et al. 2001; Otten et al. 2002; Fischer et al. 2003; Laurenne et al. 2009). During vibrational sounding, the wasps transmit vibrations through potential host substrate by drumming their modified antennal tips onto the substrate surface and gain information on host occurrence and host position based on the reflected signals, which are perceived by the subgenual organs in the tibiae of all legs (Otten et al. 2002). We suggest that the substrate-born vibrations induced by sound producing prepupae of *H. tridentata* might interfere with the perception of the reflected signals by the echolocating wasps. As the wasps applying vibrational sounding probably have to perceive very small differences in the arrival times of the reflected signals between fore, middle and hind legs to identify the exact position of the host inside the substrate relative to their body (Otten et al. 2002), the vibrations produced by the prepupae of *H. tridentata* might be highly effective in impairing the process of host localization by echolocation.

There are indeed two ichneumonid species of the genus *Hoplocryptus* (Cryptinae) among the known wasp parasitoids of *Hoplitis tridentata* (see Bee species section), which possess strongly modified antennal tips in the females (Laurenne et al. 2009), clearly indicating that these antagonists of *H. tridentata* apply vibrational sounding for host location. Two properties of the sounds produced by the prepupae of *H. tridentata* also support the hypothesis that the prepupal sounds serve to impair host finding by echolocating wasps. First, the tapping sounds were found to be continued for a surprisingly long period of up to more than 30 min after the disturbance has ended; parasitoid wasps often take prolonged walks over the substrate before localizing concealed hosts; thus, prolonged tapping by the prepupae may be a strategy to disturb the wasp's host localization during its surface exploration. Second, the sounds produced by the prepupae after a strong disturbance were found to trigger sound production by other prepupae inhabiting the same nest resulting in a simultaneous albeit unsynchronized percussion by several individuals; such a "chorus" of prepupae tapping from different positions within the nest stem (Suppl. material 4) is expected to reinforce the disturbance effect against echolocating wasps and to expand it along the whole stem, thereby acoustically concealing the exact position of the prepupae within the nest. A similar chorusing behaviour is known from the larvae of the Palaearctic cerambycid beetle *Icosium tomentosum* Lucas, which often develop in groups between wood and the bark of thin dry branches of Cupressaceae and produce disturbance sounds by scraping their strongly sclerotized mandibles against the inner bark surface (Kočárek 2009); the sound produced by the beetle larva of *I. tomentosum* consists of long series of up to eight very short pulses per second, lasts up to three minutes beyond the end of the disturbance and induces sound production by other larvae occupying the same or a nearby branch resulting in a chorus of several simultaneously scraping larvae. The striking similarities between *H. tridentata* and *I. tomentosum* not only with respect to the chorusing behaviour but also with respect to development place, aggregated occurrence of larvae and sound quality suggest that the disturbance sounds of these two unrelated taxa probably have evolved against the very same antagonists. Indeed, the chorusing behaviour of *I. tomentosum* is hypothesized by Kočárek (2009) to be an adaptation to reduce the success of parasitoid wasps and predators in locating their hosts.

The prepupae of *Hoplitis tridentata* produce two types of sounds, which differ in several characteristics. The tapping sounds, which are produced by knocking the clypeal callosity against the cocoon wall, are quiet and require little energy; they are readily induced by a weak disturbance and continued long beyond the end of the disturbance, and they do not trigger sound production by other prepupae. In contrast, the stroking sounds, which are produced by moving the anal callosity over the cocoon wall, are loud and require much energy; they are induced only after a strong disturbance and stop shortly after the disturbance has ended, and they trigger sound production by other prepupae. These different characteristics suggest that the two types of sounds have different functions, which however are most probably linked and work in combination. We envisage the following scenario for the two sounds to work together: when one prepupa perceives the presence of a parasitoid wasp near its cell due for example to vibrations caused by the drumming wasp antennae or by the insertion of the wasp ovipositor, it starts to stroke; the vibrations elicited by this stroking spread through the stem and alert other prepupae, which immediately begin to stroke and/or tap, eventually resulting in a continuing percussion by numerous prepupae along the whole nest stem. Under this scenario, the main function of the tapping sounds is to impair host location by echolocating parasitoid wasps, whereas the main function of the stroking sounds is to induce sound production by alerting other nest inhabitants. As the nest inhabitants are siblings, the simultaneous percussion by the prepupae of *H. tridentata* can be regarded as an extraordinary form of collaboration, which contributes to the inclusive fitness of all individuals within the nest.

Conclusions

The production of sounds by the larvae of bees as reported in this study for the stem-nesting osmiine bee *Hoplitis tridentata* is a new facet in the fascinating biology of solitary bees as is the suspected collaboration against antagonists between siblings inhabiting the same nest. While there is little doubt that the prepupal sounds of *H. tridentata* serve an anti-antagonist function, the assumption that they have evolved to disturb host location by echolocating wasp parasitoids is for now speculative and has to be tested experimentally.

Acknowledgements

P. Bogusch made prepupae of *Hoplitis leucomelana* available for study. G. Delvare and H. Baur informed us about the biology of parasitoid wasps. J. Rozen and J. Cane provided information on prepupae and nests of Nearctic *Hoplitis* (*Alcidamea*) species. J. Sommerhalder made close-up photographs of the prepupal sound producing organs. A. Krebs provided a photo of a *Hoplitis tridentata* female. Comments by the two reviewers J. Cane and J. Neff substantially improved the manuscript.

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Supplementary material 1

Tapping sounds of a single prepupa of *Hoplitis tridentata*

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: The sounds were recorded with a batlogger in a distance of 2 cm from the opened nest stem.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl1>

Supplementary material 2

Four stroking sounds followed by tapping sounds of a single prepupa of *Hoplitis tridentata*

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: The sounds were recorded with a batlogger in a distance of 2 cm from the opened nest stem.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl2>

Supplementary material 3

One stroking sound of a single prepupa of *Hoplitis tridentata* slowed down ten times

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: The sound was recorded with a batlogger in a distance of 20 cm from the opened nest stem.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl3>

Supplementary material 4

Simultaneous percussion by several tapping prepupae of *Hoplitis tridentata* inhabiting the same nest

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: The sounds were recorded with a batlogger in a distance of 20 cm from the opened nest stem.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl4>

Supplementary material 5

Two prepupae of *Hoplitis tridentata* producing tapping sounds

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: Two prepupae of *Hoplitis tridentata* producing tapping sounds by continuously knocking the clypeal callosity against the cocoon wall; the right brood cell contains a cocoon of *Sapyga quinquepunctata*.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl5>

Supplementary material 6

Prepupa of *Hoplitis tridentata* producing stroking sounds

Authors: Andreas Müller, Martin K. Obrist

Data type: multimedia

Explanation note: Prepupa of *Hoplitis tridentata* (left brood cell) producing stroking sounds by rapidly moving the anal callosity five times in quick succession over the cocoon wall; note that the prepupa in the middle brood cell is tapping; the right brood cell contains a cocoon of *Sapyga quinquepunctata*.

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl6>

Supplementary material 7

Literature review on potential sound producing structures in the prepupae of osmiine bee species other than *Hoplitis tridentata*

Authors: Andreas Müller

Data type: species data

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Link: <https://doi.org/10.3897/jhr.81.61067.suppl7>

Missing for almost 100 years: the rare and potentially threatened bee, *Pharohylaeus lactiferus* (Hymenoptera, Colletidae)

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Academic editor: Jack Neff | Received 6 October 2020 | Accepted 4 January 2021 | Published 25 February 2021

<http://zoobank.org/1D8CD33F-25C3-4B87-9F17-D9A151D8FE9B>

Citation: Dorey JB (2021) Missing for almost 100 years: the rare and potentially threatened bee, *Pharohylaeus lactiferus* (Hymenoptera, Colletidae). Journal of Hymenoptera Research 81: 165–180. <https://doi.org/10.3897/jhr.81.59365>

Abstract

The Australian endemic bee, *Pharohylaeus lactiferus* (Colletidae: Hylaeinae) is a rare species that requires conservation assessment. Prior to this study, the last published record of this bee species was from 1923 in Queensland, and nothing was known of its biology. Hence, I aimed to locate extant populations, provide biological information and undertake exploratory analyses relevant to its assessment. *Pharohylaeus lactiferus* was recently rediscovered as a result of extensive sampling of 225 general and 20 targeted sampling sites across New South Wales and Queensland. Collections indicate possible floral and habitat specialisation with specimens only found near Tropical or Sub-Tropical Rainforest and only visiting *Stenocarpus sinuatus* (Proteaceae) and *Brachychiton acerifolius* (Malvaceae), to the exclusion of other available floral resources. Three populations were found by sampling bees visiting these plant species along much of the Australian east coast, suggesting population isolation. GIS analyses used to explore habitat destruction in the Wet Tropics and Central Mackay Coast bioregions indicate susceptibility of Queensland rainforests and *P. lactiferus* populations to bushfires, particularly in the context of a fragmented landscape. Highly fragmented habitat and potential host specialisation might explain the rarity of *P. lactiferus*. Targeted sampling and demographic analyses are likely required to thoroughly assess the status of this species and others like it.

Keywords

Conservation, extinction risk, fragmentation, Hylaeinae, invertebrate conservation, Queensland, wildfire, rainforest

Introduction

The greatest threats to ecosystems and species worldwide are habitat loss, fragmentation and degradation (Vie et al. 2009). Australia has already cleared over 40% of its forests and woodlands since European colonisation, leaving much of the remainder fragmented and degraded (Bradshaw 2012). The vast majority of clearing has occurred on freehold and leasehold land and for animal agriculture (Evans 2016). In particular, Queensland is a contemporary land-clearing hotspot and is responsible for more than half of all land-clearing in Australia over the past four decades (Evans 2016). It is a failing of state and federal government policy and regulation that land clearing in Queensland continues at rates that should be of concern both nationally and internationally (Reside et al. 2017).

Despite the ecological importance of Australian native bees, we know very little about their biology (Batley and Hogendoorn 2009) or conservation status. North Queensland hosts high species richness and endemism (Crisp et al. 2001; Orme et al. 2005; Hurlbert and Jetz 2007) and several bee genera that are found nowhere else in Australia (Houston 2018; Smith 2018). These restricted bee genera include: *Ctenoplectra* Kirby (Apidae: Apinae), *Nomada* Scopoli (Apidae: Nomadinae), *Mellitidia* Guérin-Ménéville (Halictidae: Nomiinae), *Reepenia* Friese (Halictidae: Nomiinae), *Patellapis* Friese (Halictidae: Halictinae) and *Pharohylaesus* Michener (Colletidae: Hylaeinae).

Pharohylaesus has only two described species: *P. papuaensis* Hirashima & Roberts in Papua New Guinea and *P. lactiferus* (Cockerell) in Australia (Houston 1975; Hirashima and Roberts 1986). Both species are relatively large (9–11 mm), robust, mostly black with distinctive white facial and body markings, and have the first three tergal segments enlarged and enclosing the others. The former is known only from two females which were collected on *Syzygium aqueum* (Burm.f.) Alston (Myrtaceae) in 1982 (Hirashima and Roberts 1986). No published records of *P. lactiferus* have been made since the third of January 1923, when three males were collected in the Atherton Tablelands; in May of 1900 a male and a female were collected in Mackay while another female was collected in Kuranda prior to 1910 (Cockerell 1910; Houston 1975). However, the collection localities of these specimens are imprecise and no biological data were recorded.

Due to the dearth of biological information on *P. lactiferus* prior to this study, I aimed to locate extant populations and contribute biological information as part of a broader bee survey. Because of this, much of what follows are exploratory analyses of the potential risks for *P. lactiferus* and suggestions for future research. Hence, I undertook a series of post-hoc analyses in order to provide insights into the biology, ecology and potential extinction risks associated with *P. lactiferus*. I provide insights into the circumstances of the rediscovery of *P. lactiferus* and what is now known of its floral and habitat associations. I also explore spatial data relating to *P. lactiferus* (vegetation association, potential fire risks and occurrences) and my sampling methods (for potential biases). The possible floral and habitat specialisation along with the rarity of *P. lactiferus* raises concerns about its conservation status. I further highlight the need for preservation of remnant vegetation and better arthropod-diversity monitoring, particularly for at-risk and phylogenetically important species.

Methods

Sample locations and methods

I undertook general collections in parts of Queensland and New South Wales in a variety of habitats with most collections made across two sampling periods from December to February 2018–19 and November to December of 2019 (summer). I chose sampling sites by the presence of flowering vegetation on the side of roads and trails. I caught specimens by sweep-netting (up to 13 m from the ground using an 11 m Lito net) off flowering plants, vegetation or potential bee-nesting sites. After collection, I transferred specimens to 99% ethanol and stored them at $-2\text{ }^{\circ}\text{C}$. For each collection event I recorded latitude, longitude and elevation in metres above sea level (m asl) and later checked these to ensure accuracy. I estimated the number of bees for each vial while in the field. Other data that I collected included date, time, collector, sampling effort (in minutes), sampling notes and, where possible, flower species visited and the resource that I used to identify that plant. I undertook general collections between 0525 and 2200 (Suppl. material 2: Table S1). I did not collect any plant vouchers. I identified *Pharohylaeus* using the keys by Houston (1975) and Smith (2018).

I used two initial collection events of *P. lactiferus* to inform targeted sampling (see results). However, I undertook general collections and observations at every site where I found *P. lactiferus* in an attempt to find additional associated plant species. I chose targeted sampling sites haphazardly as target plant species were encountered, generally near the side of roads or hiking trails. I undertook observations of the target plant species, *Stenocarpus sinuatus* (Loudon) Endl. (Proteaceae) and *Brachychiton acerifolius* (A.Cunn. ex G.Don) F.Muell. (Malvaceae), for a minimum of five minutes (maximum of 67 minutes) for each collection event (Suppl. material 2: Table S1). Where I increased sampling time, I did so to collect *P. lactiferus* specimens and to determine their distribution or activity times. I did not undertake a systematic temporal sampling regime; however, I made targeted collections throughout the day (between 0730 and 1751) (Suppl. material 2: Table S1).

Representative materials are stored at the South Australian Museum (SAMA 32-37949, SAMA 32-37950, SAMA 32-40838, SAMA 32-40846, SAMA 32-40847, SAMA 32-40848, SAMA 32-40849).

Data sources and terminology

Historic bee records

I sourced general bee collection data for Australian bioregions from the Atlas of Living Australia (ALA 2019) and overlaid them with Australian bioregion data using *QGIS* version 3.8 (QGIS Development Team 2020). I analysed all data using *R* version 3.6.1 (R Development Core Team 2019) and produced plots using the *R* packages *graphics* and *ggplot2* (Wickham 2016). Because I sampled flowering plants as I encountered them, I also examined potential collection biases (Suppl. material 1: Appendix).

Geographic information system data

I sourced current and pre-European National Vegetation Information System maps from the National Mapping Division (NMD 2003b, a), which defines 85 Major Vegetation Subgroups for Australia (e.g., Tropical or Sub-Tropical Rainforests (TSTRs); Major Vegetation Subgroup 2). NMD (2003b) compiled pre-European maps using the best-available data collected at varying scales, on varying dates and by several organisations. I sourced Interim Biogeographic Regionalisation for Australia maps from the Department of Environment and Energy (DEE 2017), which defines 89 large and distinct bioregions (e.g., the Wet Tropics and Central Mackay Coast).

Results

Sampling

Of the ~3,585 bee specimens that I collected in Queensland over 3,446 sampling-minutes, I collected 694 (19%) in the Wet Tropics and 153 (4%) in the Central Mackay Coast bioregions (Suppl. material 5: Fig. S3; Suppl. material 2: Table S3). In the adjacent bioregions of Cape York Peninsula, Einasleigh Uplands and the Brigalow Belt North, I collected 260 (7%), 453 (13%) and 271 (8%) bee specimens, respectively (Suppl. material 5: Fig. S3). In New South Wales, I collected 2,141 bee specimens over 2,441 sampling-minutes (Suppl. material 2: Table S3). In total I sampled 225 general collection sites; 130 in Queensland and 95 in New South Wales (Suppl. material 2: Table S3).

Following my initial collection of a *P. lactiferus* female on foliage adjacent to Hallorans Hill Conservation Park, Queensland (Atherton; Wet Tropics), I intensified my sampling of flowering plants around the park for a period of three days between the 3rd and 5th of February 2019 (Fig. 1). Subsequently, I collected five males patrolling the flowers of an ~8 m high *S. sinuatus* tree (flowering times range from February to June (Floyd 1978; Foreman 2020)). *Pharohylaenus lactiferus* males patrolled all flowers (5–8 m high). I undertook further sampling of *S. sinuatus* (and other plants) in nearby rainforest patches between the 5th and 19th of February 2019 but I did not collect any further *P. lactiferus* during this period (Fig. 1).

I resampled Hallorans Hill Conservation Park on the 13th of November 2019 and collected *P. lactiferus* foraging on *B. acerifolius* (flowers November to January (Guymer 1988)). I then undertook further sampling on *B. acerifolius* in Queensland and New South Wales between the 13th and 28th of November (Suppl. material 5: Fig. S3). From these collections, I collected four (2♂2♀) *P. lactiferus* near Hallorans Hill Conservation park on *B. acerifolius*. In Kuranda (Wet Tropics) and Eungella (Central Mackay Coast), I collected five (4♂1♀) and seven (3♂4♀) specimens, respectively, on *B. acerifolius* (Fig. 1).

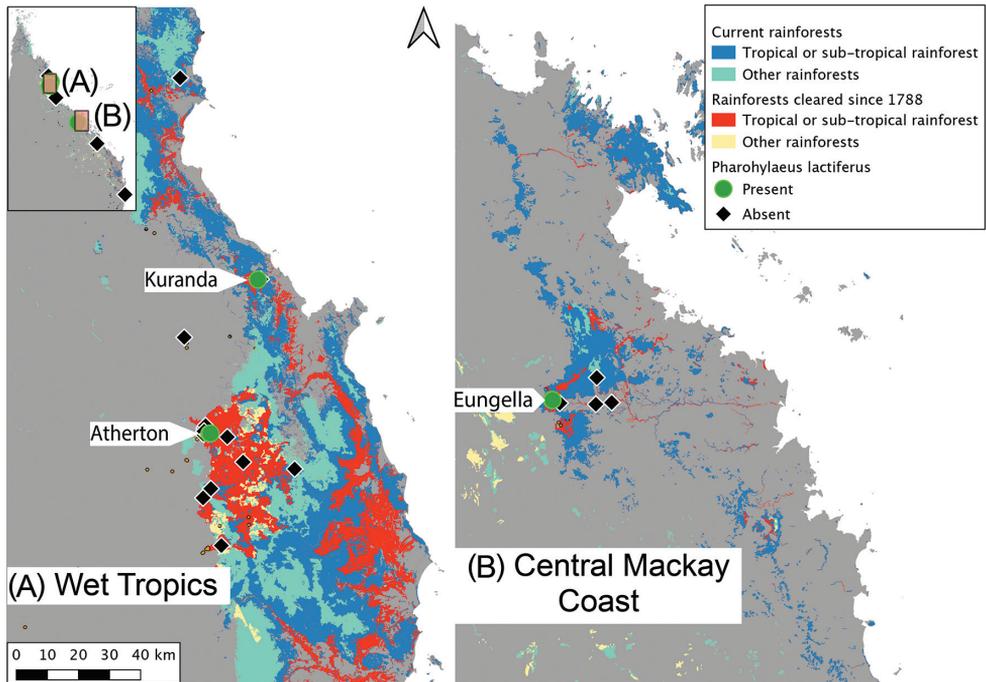


Figure 1. (blues) Current rainforests and (reds) rainforests cleared since European arrival (1788) in the **A** Wet Tropics and the **B** Central Mackay Coast (NMD 2003b, a). Black markers indicate flowering *Stenocarpus sinuatus* or *Brachychiton acerifolius*, sites where no *Pharohyllaenus lactiferus* were detected, and green markers (Kuranda, Hallorans Hill C.P. (Atherton), and Eungella) indicate those where they were present. Inset shows Queensland and northern New South Wales as well as sample locations.

From the sites where I successfully collected *P. lactiferus*, I sampled most plant species that were flowering at the time (and with flowers at or below ~13 m). The plants on which I collected bees – other than *P. lactiferus* – at these sites included: *Alpinia* sp. (Zingiberaceae), Asteraceae sp., *Callicarpa pedunculata* R.Br. (Lamiaceae), *Duranta repens* L. (Verbenaceae), *Leptospermum* sp. (Myrtaceae), *Melicope rubra* (Lauterb. & K.Schum.) T.G.Hartley (Rutaceae), *Parsonsia straminea* (R.Br.) F.Muell. (Apocynaceae), *Senna* sp. (Fabaceae), *Solanum seaforthianum* Andrews (Solanaceae), and *Syzygium* sp. (Myrtaceae) (Suppl. material 2: Table S3). I only identified plants on which I caught bee specimens; thus, this represents a subset of those examined.

I undertook a total of 42 observation events on either *S. sinuatus* or *B. acerifolius*. Ten of my observation events resulted in *P. lactiferus* collections across three sites and 32 of my observation events returned no *P. lactiferus* across 20 sites (Fig. 1). I was only successful in collecting *P. lactiferus* between 351 and 877 m asl and only at three sites in the Atherton, Kuranda and Eungella regions of Queensland (Fig. 1; Suppl. material 2: Table S1). Successful collections were made between 0855 and 1637 (Suppl. material 2: Table S1). Of the 20 sites examined, 15 were within 1 km of TSTRs and

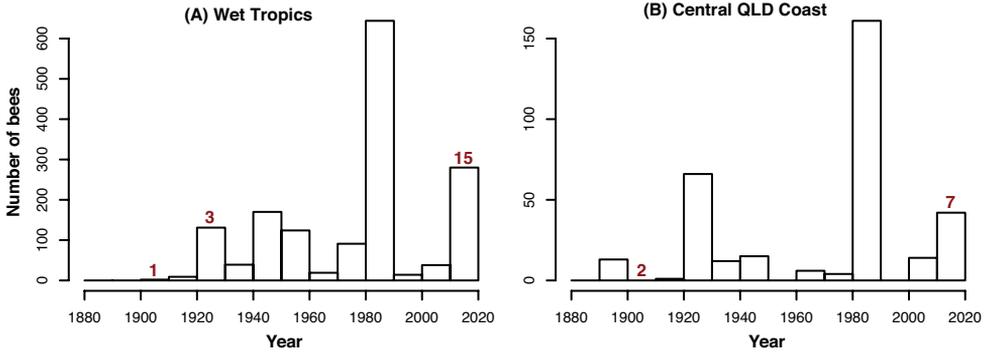


Figure 2. The number of bee specimens recorded on the Atlas of Living Australia for the **A** Wet Tropics and **B** Central Mackay Coast bioregions in ten-year intervals. Numbers above bars indicate *Pharoehylaeus lactiferus* specimen collections.

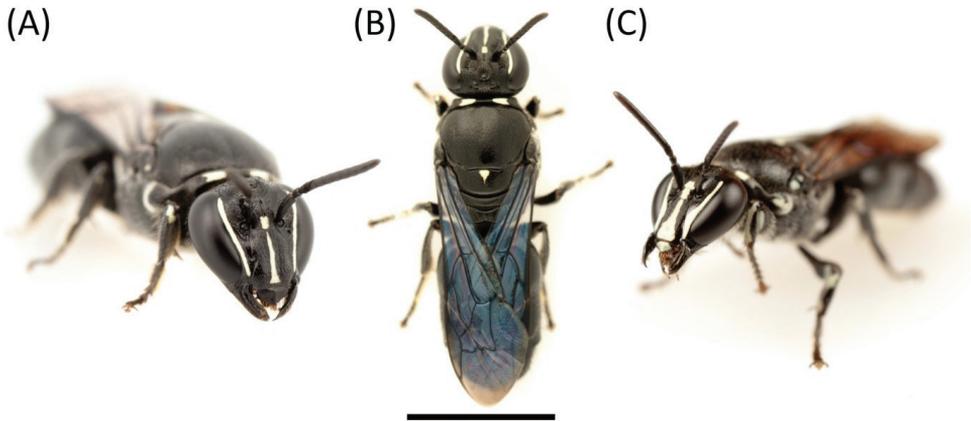


Figure 3. An **A** oblique and **B** dorsal photo of a female and an **C** oblique photo of a male *Pharoehylaeus lactiferus*. Scale bar: 5 mm (**B**). Photos are not to scale.

12 were within 213 m (Suppl. material 2: Table S1). According to Beck et al. (2018)'s Köppen-Geiger climate classification map, *P. lactiferus* occurs in both tropical and sub-tropical climates.

Historic collection data

Historic bee records. The Atlas of Living Australia has a total of 2,198 bee records for the Wet Tropics and 584 for the Central Mackay Coast. Of these records, 637 (29%) in the Wet Tropics and 250 (43%) in the Central Mackay Coast do not include year of collection. Of the records that included year of collection, the Atlas of Living Australia only had 11 of 1,561 and 15 of 334 records that pre-dated 1924 for the Wet Tropics and Central Mackay Coast, respectively (Fig. 2).

Supplementary results summary

I caught significantly more *P. lactiferus* and other bees and spent more time sampling near TSTRs (Suppl. material 1: Appendix; Suppl. material 6: Fig. S4). However, the sum of sampling time was not significantly correlated with the number of *P. lactiferus* caught, suggesting some resilience of data interpretation to bias (Suppl. material 1: Appendix; Suppl. material 6: Fig. S4). *Pharohylaeus lactiferus* was only collected within 213 m of TSTR (Suppl. material 1: Appendix). Tropical or Sub-Tropical Rainforests have undergone habitat destruction and fragmentation since European colonisation and are susceptible to fire (Suppl. material 1: Appendix). In New South Wales and Queensland, most *B. acerifolius* and *S. sinuatus* records occur in rainforests.

Discussion

Despite my extensive non-targeted and targeted sampling as well as bee collection records on the Atlas of Living Australia, *P. lactiferus* records remain rare. Apparent habitat specialisation to TSTRs and few associated floral taxa (*S. sinuatus* and *B. acerifolius*) might explain the rarity of *P. lactiferus*. However, in many cases I found *P. lactiferus* difficult to catch due to the height of the associated plant species (of the trees that I sampled, flowers were between 1 m and 13 m high) and the bees' quick flight (Suppl. material 1: Appendix). It is possible that *P. lactiferus* is a naturally rare species that is not threatened. But, why at least two early collectors sampled *P. lactiferus* on three separate occasions prior to 1924 (Houston 1975) and no published records have been made in the years since, despite a greater sampling effort (Fig. 2), is both unclear and of concern.

The occurrence of host plant species could limit suitable habitat for *P. lactiferus*. For example, the persistence of a *P. lactiferus* population in any one rainforest could require several host plant species to provide food throughout their activity period. From current and historical collections, we know that *P. lactiferus* is active at least between November and May. This could indicate a long flight period, bivoltinism or, like many other tropical bee species (e.g., (ALA 2019; Dorey et al. 2019)), activity could be year-round. Additionally, as many hylaeines nest in preformed holes (Almeida 2008; Houston 2018), *P. lactiferus* might require very specific nesting substrates (Hearn et al. 2019). Nesting substrate could further be limited to certain plant species, and by certain stem-borers that pre-excavate potential nests (Dew and Schwarz 2013; Houston 2018). Habitat destruction and fragmentation might also limit the persistence of the required species in fragments (Suppl. material 1: Appendix). These factors might be particularly relevant to *P. lactiferus*, which was only found within ~200 m of TSTR, suggesting a low foraging and dispersal distance (Suppl. material 1: Appendix).

That bees use *S. sinuatus* and *B. acerifolius* might be unexpected for two primary reasons. Firstly, both plant species exhibit a pollination syndrome that is associated

with birds (e.g., they are bright red) (Nicolson and Van Wyk 1998; Williams and Adam 2010; Shrestha et al. 2013). Bee vision is shifted towards ultraviolet wavelengths and they are thought to not perceive red wavelengths (Dyer et al. 2015); although, this is not always the case (Peitsch et al. 1992) and insect visual perception is complex (Horridge 1998). It is possible that the flowers of *S. sinuatus* and *B. acerifolius* have ultraviolet, or similar, markings or produce olfactory cues that attract bees. That at least ten bee species across eight genera were foraging on *B. acerifolius* could indicate that this plant is not exclusively bird-pollinated (Suppl. material 2: Table S1). Hylaeinae bees were the primary visitors of *B. acerifolius* during observations (Suppl. material 2: Table S1) which could indicate phylogenetically conserved traits that allow the use of flowers that exhibit bird-pollination syndromes (e.g., pollen specialisation or red-shifted vision). Secondly, Guymer (1988) reported that *B. acerifolius* lacks nectaries. While I did not observe bees inside flowers due to the height of trees, I did observe bees ‘drinking’ from flowers of the related *B. populneus* (Schott & Endl.) R.Br., which Guymer (1988) also reports as lacking nectaries. Melittologists might avoid sampling plants that exhibit bird-pollination syndromes and this could bias their collections. The foraging preferences of *P. lactiferus* require further study, likely with a particular focus on plants exhibiting bird-pollination syndromes (e.g., *Alloxylon pinnatum* (Maiden & Betche) P.H. Weston & Crisp, *Castanospermum australe* A.Cunn. ex Mudie or *Erythrina vespertilio* Benth.) or even on canopy-flowering plants in general (Roubik 1993).

In the bioregions that *P. lactiferus* has been found, this major vegetation subgroup has undergone habitat destruction and fragmentation since European colonisation (Suppl. material 1: Appendix) (Bradshaw 2012). Although Queensland’s Wet Tropics have largely been protected from clearing in contemporary times, like much of the state, habitat fragmentation remains a major conservation concern (Tucker 2000). Additionally, three of four rainforest vegetation types (including TSTR) burnt every year between 1988 and 2020 (for which data are available; Suppl. material 1: Appendix). While there was no significant change over time in the area of rainforest burnt during that period, the 2019–2020 bushfire season burnt a greater area than in any year prior for each rainforest type (Suppl. material 1: Appendix).

To monitor and assess the conservation status of each species we require an understanding of their biology and targeted sampling. Data deficiency for rare species raises concerns that other rare or specialist species could become extinct before being discovered, leaving no opportunity to conserve those taxa. We must increase biomonitoring, particularly of diverse invertebrate fauna to assess and protect such taxa worldwide. Additionally, increasing institutional investment to digitise collections would vastly increase the research utility of online databases and potentially allow us to differentiate rare from threatened taxa.

Future research should aim to increase our understanding of the biology, ecology and population genetics of *P. lactiferus*. This work could use targeted seasonal sampling throughout the year at sites where *P. lactiferus* is known to occur, providing insights into phenology and host plant species. Future studies could also use trap-nests at various heights from the ground and targeted searches to uncover nesting requirements and inform conservation management (Roubik 1993; Sutherland et al. 2010).

These data, along with an expanded *a priori* sampling regime, should allow accurate implementation of species distribution models to uncover other potential populations or translocation sites. To determine if *P. lactiferus* is threatened (undergone population declines in the recent past) or simply rare (stable population in the recent past), genetic data could be used to examine past demographies. Additionally, genetic data for each population could allow examination of population isolation. Such research will be invaluable to assess the conservation status of *P. lactiferus* and provide an exemplar for the assessment of other poorly-studied and threatened bee taxa.

Conclusions

Despite extensive sampling undertaken during this study and from publicly available records, *P. lactiferus* remains poorly collected and little is known of its biology. *Pharohylaeus lactiferus* has only been collected on two plant species (*S. sinuatus* and *B. acerifolius*), to the exclusion of other available resources. Thus far, only males have been collected on *S. sinuatus*. These collections might indicate floral specialisation, potentially on plants that exhibit bird-pollination syndromes.

Many of the analyses undertaken here are exploratory and this must be considered when making conclusions. However, it is important for likely issues to be raised in order to inform future research and conservation efforts. To these ends, I make the following remarks. *Pharohylaeus lactiferus* could be a floral- and habitat-specialist bee. The absence of *P. lactiferus* collections since 1923, despite far-greater sampling effort prior to this study, raises concerns about its conservation status. Habitat destruction and fragmentation might have acted synergistically with the floral- and habitat-specialisation of *P. lactiferus* to explain its rarity. However, collection habits of melittologist (e.g., possible avoidance of plants with bird-pollination syndromes) and the height of known associated plants might make possible declines difficult to confirm. Regardless, known populations of *P. lactiferus* remain rare and susceptible to habitat destruction (e.g., from changed land use or stochastic events such as fires; Suppl. material 1: Appendix).

Acknowledgements

I would like to thank Olivia K. Davies, Michael P. Schwarz and Mark I. Stevens for proofing early versions of the manuscript. I further thank Lori Lach, Jack Neff, Terry Houston and four anonymous reviewers for their insightful comments on the manuscript that led to its improvement. I would also like to thank Erinn P. Fagan-Jeffries and Peter Rühr for their company during field work. I would finally like to thank the funders of this research (Holsworth Wildlife Research Endowment, Playford Trust PhD Scholarship and AJ and IM Naylor PhD Scholarship) that support and promote the research of many young scientists. This work was carried out under the Queensland national parks research permit number PTU18-001277-1 and the New South Wales National Parks & Wildlife Service scientific licence number SL102137.

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Supplementary material 1

Appendix

Authors: James B. Dorey

Data type: pdf file

Explanation note: Bee flight observations, collection bias analysis, geographical information systems, and historic associated plant records.

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl1>

Supplementary material 2

Tables S1–S5

Authors: James B. Dorey

Data type: Collection, site, plant and GIS data

Explanation note: **Table S1.** Collection data and notes for both successful and unsuccessful searches for *Pharohylaeus lactiferus* in QLD and north-east NSW, Australia.

Table S2. Atlas of Living Australia data for *Brachychiton acerifolius* and *Stenocarpus sinuatus* by major vegetation subgroup (MVS) number in New South Wales and Queensland. Green highlighted rows indicate rainforest major vegetation subgroups.

Table S3. Collection data from New South Wales and Queensland. **Table S4.** Major vegetation subgroup (MVS) data including MVS number, MVS name. Measurements include total MVS area (km²) and proportions, and sampled MVS area (km²) and proportions. Sum of the number of bees, sample time and the total area of NSW and QLD for each MVS are also included. **Table S5.** Summary of fragmentation statistics for the two bioregions, the *Wet Tropics* and *Central Mackay Coast*.

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Supplementary material 3

Figure S1

Authors: James B. Dorey

Data type: Figure relating to GIS analyses of plant taxa (*Brachychiton acerifolius* and *Stenocarpus sinuatus*) associated with *Pharohylaeus lactiferus*

Explanation note: The number of **A** *Brachychiton acerifolius* and **B** *Stenocarpus sinuatus* in New South Wales (NSW; blue) and Queensland (QLD; maroon) by each major vegetation subgroup (MVS).

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl3>

Supplementary material 4

Figure S2

Authors: James B. Dorey

Data type: The number of each plant taxa (*Brachychiton acerifolius* and *Stenocarpus sinuatus*) associated with *Pharohylaeus lactiferus* by each MVS region and by year of collection.

Explanation note: The number of **A & B** *Brachychiton acerifolius* and **C & D** *Stenocarpus sinuatus* in New South Wales (NSW; blue) and Queensland (QLD; maroon) by each major vegetation subgroup (MVS).

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Supplementary material 5

Figure S3

Authors: James B. Dorey

Data type: GIS data and map of bee collections across New South Wales and Queensland

Explanation note: Heatmap of bee collections by Australian bioregion with the focus bioregions bolded. Points indicate the location of bee samples.

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Supplementary material 6

Figure S4

Authors: James B. Dorey

Data type: Results of collection bias analyses

Explanation note: The sum of **A** number of bees, **B** sample time (mins) and **C** *P. lactiferus* by distance from major vegetations subgroup (MVS) 2 – tropical or sub-tropical rainforest – in 10 km bins. The sum of **E** number of bees, **F** sample time (mins) and **G** *P. lactiferus* by distance from MVS 2 in the first 10 km bin of a, b and c split in 200 m bins. The sum of *P. lactiferus* in **D** 10 km bins and **H** 200 m bins over sampling time (mins) where bin width is 100 minutes.

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl6>

Supplementary material 7

Figure S5

Authors: James B. Dorey

Data type: Sampling effort

Explanation note: Bars show the cumulative **A** number of insects and **B** sampling time by the major vegetation subgroups (MVS) that were sampled in New South Wales (NSW) and Queensland (QLD) (left-most Y-axes).

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl7>

Supplementary material 8

Figure S6

Authors: James B. Dorey

Data type: Wildfire data for Australian rainforests

Explanation note: The area of Major Vegetation Subgroups (MVS) **A** one (cool temperate rainforest), **B** two (tropical or sub-tropical rainforest), **C** six (warm temperate rainforest) and **D** 62 (dry rainforest or vine thickets) burnt by year from 1988 to 2016 and the 2019-20 fire season (red).

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Link: <https://doi.org/10.3897/jhr.81.59365.suppl8>

Corrigenda: An updated checklist of the bees (Hymenoptera, Apoidea, Anthophila) of Pennsylvania, United States of America. Journal of Hymenoptera Research 77: 1–86. <https://doi.org/10.3897/jhr.77.49622>

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Academic editor: M. Ohl | Received 31 December 2020 | Accepted 4 January 2021 | Published 25 February 2021

<http://zoobank.org/F20F8439-60E1-4580-B907-B4731F1F4F2E>

Citation: Kilpatrick SK, Gibbs J, Mikulas MM, Spichiger S-E, Ostiguy N, Biddinger DJ, López-Uribe MM (2021) Corrigenda: An updated checklist of the bees (Hymenoptera, Apoidea, Anthophila) of Pennsylvania, United States of America. *Journal of Hymenoptera Research* 77: 1–86. <https://doi.org/10.3897/jhr.77.49622>. *Journal of Hymenoptera Research* 81: 181–189. <https://doi.org/10.3897/jhr.81.62634>

The recently updated checklist of the bees of Pennsylvania, USA (Kilpatrick et al. 2020) contained several unintentional errors and some specimen records require clarification. We address below: 1) GBIF data retrieval, 2) taxonomy, 3) typographical errors in the text and Suppl. material 10, 4) misattributed county-level records, 5) county-level records for species in both Fig. 3 and Suppl. material 1, 6) data sources that were omitted from the text, and 7) state species record validity. These corrections, additional details, and updated files should be used in combination with the data presented in the original publication.

GBIF data

The specimen data retrieved from GBIF.org on 07 January 2020 (GBIF Occurrence Download <https://doi.org/10.15468/dl.wghcks>) were filtered by “Scientific name”, (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae, Melittidae), “Continent” (North America), “Country or area” (United States of America), and “State province” (PENNSYLVANIA). We have become aware that applying the geography-based terms excluded numerous records from our search results (i.e., a record was excluded if any of these fields were empty/missing when a record was originally submitted to GBIF or if there was a misspelling/variant of the search term in any of these fields). Applying a polygon/shapefile for the state and adjusting “coordinateUncertaintyInMeters”, in place of using geography-based terms, will return all records that contain coordinates within the state, regardless of how complete their other fields are. Combining this search strategy with searches that use a wider variety of geography-based terms – to retrieve records without coordinates or that have been incorrectly georeferenced – can be used to compile a thorough GBIF dataset. However, the addition of records from searches such as these is outside of the scope of the corrigenda.

Checklist taxonomy

The subfamilial classification within Apidae was intended to follow Bossert et al. (2019), however, Anthophorinae was omitted. Thus, all members of Anthophorini were intended to be included in Anthophorinae, not Apinae. Additionally, all members of Emphorini should be classified within Eucerinae, not Apinae.

The species group, *viridatum*, was unintentionally omitted from *Lasioglossum* (*Dialictus*) *dreisbachi* (Mitchell, 1960) in both the text and Suppl. material 1. *Hoplitis* (*Hoplitis*) *anthocopoides* (Schenck, 1853) is in the *adunca* species group, not *Annosmia*–*Hoplitis*. Furthermore, *L. (Sphecodogastra) truncatum* (Robertson, 1901) does not have a species group and is thus not in the *calceatum* group. Suppl. material 1 has been corrected per this information, and to fully reflect the checklist text, group names for six *Lasioglossum* spp. were also added: *cephalotes* group – *L. (Dialictus) cephalotes* (Dalla Torre, 1896); *platyparium* group – *L. (Dialictus) izawsum* Gibbs, 2011, *L. (Dialictus) rozeni* Gibbs, 2011, and *L. (Dialictus) simplex* (Robertson, 1901); *viridatum* group – *L. (Dialictus) georgeickworti* Gibbs, 2011 and *L. (Dialictus) katherineae* Gibbs, 2011.

Literature review records

Typographical and formatting errors that were introduced to Suppl. material 10 when the file was reformatted for publication were corrected.

Checklist legend

Some of the citations of the Legend presented on page 17 were incorrectly formatted and/or had the incorrect year listed. The corrected legend is reprinted in full here for clarity and convenience:

Legend: ¹ = Donovall and vanEngelsdorp (2010); ² = Bartomeus et al. (2013); ³ = Bid-dinger Laboratory Database; ⁴ = Droege Database; ⁵ = Integrated Crop Pollination (ICP) Project: Fleischer Laboratory Database; ⁶ = López-Urbe Laboratory Database; ⁷ = Ma-han et al., in prep; ⁸ = Winfree Laboratory Database; ⁹ = Choate et al. (2018); ¹⁰ = Baker (1975); ¹¹ = Bouseman and LaBerge (1978); ¹² = Broemeling (1988); ¹³ = Cockerell (1908); ¹⁴ = Daly (1973); ¹⁵ = DeBarros (2010); ¹⁶ = Droege et al. (2010); ¹⁷ = Gibbs (2010); ¹⁸ = Gibbs (2011); ¹⁹ = Gibbs and Dathe (2017); ²⁰ = Gibbs et al. (2013); ²¹ = LaBerge (1969); ²² = LaBerge (1971); ²³ = LaBerge (1973); ²⁴ = LaBerge (1977); ²⁵ = LaBerge (1980); ²⁶ = LaBerge (1985); ²⁷ = LaBerge (1987); ²⁸ = LaBerge (1989); ²⁹ = LaBerge and Bouseman (1970); ³⁰ = LaBerge and Ribble (1972); ³¹ = Matteson et al. (2008); ³² = McGinley (1986); ³³ = McGinley (2003); ³⁴ = Mikulas and Barringer (2018); ³⁵ = Milliron (1973a); ³⁶ = Mitchell (1960); ³⁷ = Mitchell (1962); ³⁸ = Onuferko (2017); ³⁹ = Onuferko (2018); ⁴⁰ = Ordway (1966); ⁴¹ = Rehan and Sheffield (2011); ⁴² = Roberts (1972); ⁴³ = Shinn (1967); ⁴⁴ = Sidhu (2013); ⁴⁵ = Stephen (1954); ⁴⁶ = Svensson et al. (1977); ⁴⁷ = Timberlake (1975); ⁴⁸ = AMNH; ⁴⁹ = BugGuide; ⁵⁰ = Swenk (1915); ⁵¹ = PSUB; ⁵² = Rosemary Malfi Insect Collection; ⁵³ = Emily Erickson/BIML.

Checklist species records

Specimen records from Choate et al. (2018) (superscript 9) were misattributed to Erie County, instead of Crawford County. The error affected 103 species, ranging across pages 18 to 63. Of these 103 species, 30 have been recorded in both Crawford and Erie counties, and Erie is considered a new county record for nine species: *Apis* (*Apis*) *mellifera mellifera* Linnaeus, 1758; *Ceratina* (*Zadontomerus*) *strenua* Smith, 1879; *Anthidium* (*Anthidium*) *manicatum manicatum* (Linnaeus, 1758); *Megachile* (*Eutricharaea*) *rotundata* (Fabricius, 1787); *Osmia* (*Melanosmia*) *pumila* Cresson, 1864; *Andrena* (*Andrena*) *tridens* Robertson, 1902; *Halictus* (*Odontalictus*) *ligatus* Say, 1837; *Lasioglossum* (*Dialictus*) *tegulare* (Robertson, 1890); and *Lasioglossum* (*Leuchalictus*) *zonulum* (Smith, 1848). The remaining 73 species have only been recorded in Crawford County, not Erie County.

The county records for each species have been corrected in Suppl. material 1 and Fig. 3. The following additional changes were made to the species records in Suppl. material 1 and Fig. 3, to accurately reflect data presented in the checklist text: *Nomada imbricata* Smith, 1854 – Lancaster County was added; Lackawanna County was removed. *Nomada luteola* Olivier, 1812 – York County was added. *Osmia* (*Melanosmia*)

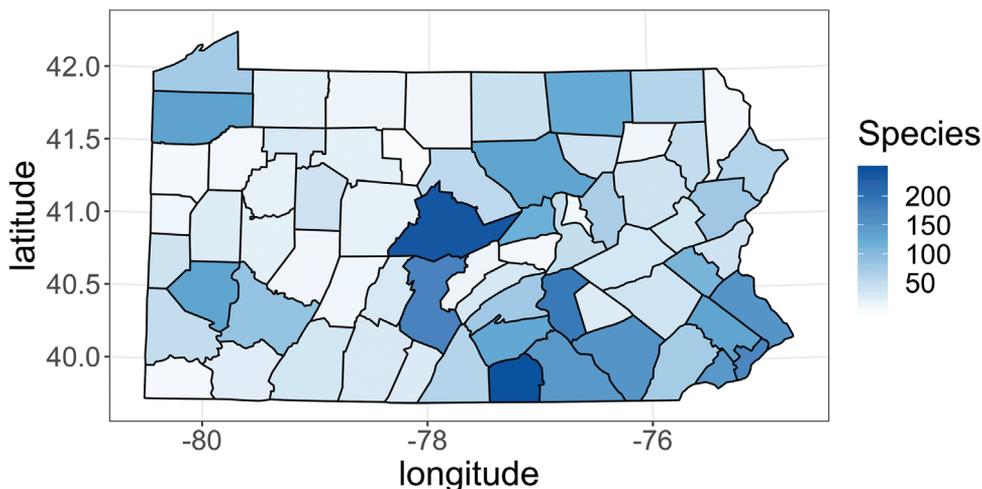


Figure 3. Choropleth map of Pennsylvania specifying bee species richness by county. The greater number of species recorded for a county, the darker blue the county is on the map; lighter-colored counties have fewer species reported from them. The number of species reports for counties ranges from one (Cameron Co.) to 246 (Adams Co.).

pumila Cresson, 1864 – Jefferson County was added; Juniata County was removed. *Andrena (Andrena) cornelli* Viereck, 1907 – Schuylkill County was added.

Sources for county and/or date information reported in the checklist were partially omitted in four species' records in the checklist on pages 37, 47, 49, and 60. The data noted for each of the following species is attributable to the citation for superscript "1": *Hoplitis (Alcidamea) producta producta* (Cresson, 1864) – Butler; *Andrena (Thysandrena) w-scripta* Viereck, 1904 – Potter; *Calliopsis (Calliopsis) andreniformis* Smith, 1853 – Cumberland and Lackawanna; *Sphecodes ranunculi* Robertson, 1897 – Dauphin, Erie, Montgomery, and 14 Jul.

Additional remarks on species record verifications

Several state species records, including the following, were included on the checklist based on external data sources as provided in Kilpatrick et al. (2020). However, they were not independently verified. Since these are uncommon species or reach the limit of their ranges in Pennsylvania, it would be useful to validate these records in the future: *Bombus (Psithyrus) insularis* (Smith, 1861); *Melissodes (Eumelissodes) fumosus* LaBerge, 1961; *Triepeolus rugosus* Mitchell, 1962; *Stelis (Stelis) foederalis* Smith, 1854; *Andrena (Euandrena) phaceliae* Mitchell, 1960; *Andrena (Xiphandrena) mendica* Mitchell, 1960; *Lasioglossum (Dialictus) cephalotes* (Dalla Torre, 1896); *Lasioglossum (Dialictus) marinum* (Crawford, 1904); *Lasioglossum (Dialictus) simplex* (Robertson, 1901); *Lasioglossum (Sphecodogastra) comagenense* (Knerer and Atwood, 1964); *Sphecodes smilacinae* Robertson, 1897; and *Sphecodes solonis* Graenicher, 1911.

Acknowledgements

Thanks again to Beth Choate for graciously sharing specimen data. John (Jack) Neff (Central Texas Melittological Institute) and John Ascher (National University of Singapore) critically reviewed the published article and their feedback contributed to components of the corrigenda. GBIF Secretariats, Daniel Noesgaard and John Waller, reviewed our occurrence download and contributed suggestions for retrieving additional records in future searches. We also thank Emily Erickson, Rosemary Malfi, and T'ai Roulston for their assistance with confirming a few of the specimen records included in the updated checklist.

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Supplementary material I

Bee species of Pennsylvania: taxonomy, collection dates, persistence, and distribution data

Authors: Shelby Kerrin Kilpatrick, Jason Gibbs, Martin M. Mikulas, Sven-Erik Spichiger, Nancy Ostiguy, David J. Biddinger, Margarita M. López-Uribe

Data type: classification, phenology, distribution

Explanation note: The classification, earliest and latest dates of collection in both Donovan and vanEngelsdorp (2010) and Kilpatrick et al. (2020), most recent year of collection/observation in both Donovan and vanEngelsdorp (2010) and Kilpatrick et al. (2020), persistence data, and county-level occurrence data is presented for each of the 437 spp. of bees reported in Pennsylvania. This file has been corrected and updated as described in the text of the Corrigenda. This replacement file for Suppl. material 1 should be used in combination with the data in the original publication (Kilpatrick et al. 2020) and the Corrigenda.

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Link: <https://doi.org/10.3897/jhr.81.62634.suppl1>

Supplementary material 10

Pennsylvania bee species literature review records

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Data type: specimen records

Explanation note: Complete specimen literature record details for species of bees reported in Pennsylvania. Citation details, taxonomic classification (past and updated to current), type of report, occurrence details (both verbatim and transformed), and species notes are presented for 1,283 records. This file has been corrected and updated as described in the text of the Corrigenda. This replacement file for Suppl. material 10 should be used in combination with the data in the original publication (Kilpatrick et al. 2020) and the Corrigenda.

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