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RESEARCH ARTICLE

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Synopsis of New World Sigalphinae (Hymenoptera, Braconidae) with the description of two new species and a key to genera

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Abstract

We describe and illustrate *Paphanus paloi* **sp. n.**, first generic record for Brazil, and *Minanga patriciamadrigalae*, first generic record for Costa Rica. We present illustrated keys for the New World genera of Sigalphinae, and the New World species of *Paphanus* and *Minanga*. *Minanga patriciamadrigalae* **sp. n.** was reared from caterpillars of *Chloropteryx* nordicariaDHJ01 (Geometridae).

Keywords

Neotropical, taxonomy, Costa Rica, Brazil, *Minanga*, *Paphanus*

Introduction

Though rarely collected, members of Sigalphinae Blanchard, 1845 are worldwide in distribution (van Achterberg 1985; Iqbal and Austin 2002; Sharkey 2004; Tan et al. 2010; Sharkey and Braet 2012; Braet 2014). The subfamily includes eight genera

(*Acampsis* Wesmael, 1835; *Aposigalphus* van Achterberg & Austin, 1992; *Malasigalphus* van Achterberg & Austin, 1992; *Minanga* Cameron, 1906; *Notosigalphus* van Achterberg & Austin, 1992; *Paphanus* van Achterberg & Riedel, 2009; *Pselaphanus* Szépligeti, 1902; and *Sigalphus* Latreille, 1802) with fewer than 50 described species, all of which are presumably koinobiont endoparasitoids of Lepidoptera larvae (Yu et al. 2016).

Shaw and Quicke (2000) presented a detailed description of the biology and immature stages of *Acampsis alternipes* (Nees). Their major findings include the following. Eggs are placed in host ganglia; early instars are attacked; the first parasitoid instar is polypodiform; and the final instar larvae feed externally on the host.

Sigalphus bicolor is reported as a gregarious, multivoltine parasitoid of *Acronicta clarescens* Cuenée (Noctuidae); first instar larvae are parasitized, and parasitoid cocoons are spun within the host cocoon (Cushman 1913). *Sigalphus romeroi* Sharkey from Costa Rica and *S. irrorator* (Fabricius) from the Palearctic are solitary endoparasitoids of Noctuidae (Sharkey and Janzen 1995) that cut a pupal chamber into rotten wood, in which the parasitoid spins its cocoon that looks much like that of *Minanga* (Fig. 3B). Yu et al. (2016) list all host records for members of Sigalphinae taken from the literature. Some of these may be erroneous from the source. Here we elucidate the biology of a species of *Minanga* for the first time.

Methods

Morphological terms

Metasomal median tergites are abbreviated as follows, T1 = metasomal median tergite 1, T2 = metasomal median tergite 2. T2–3 = metasomal median syntergite 2+3. Morphological terms used can be found in the Hymenoptera Anatomy Ontology (HAO) (Yoder et al. 2010). To find definitions for any structure search for the term at http://glossary.hymao.org.

Museum acronyms

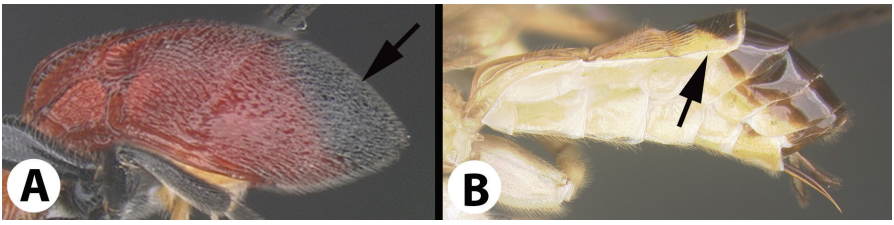
DCBU	Collection of the Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, São Carlos, SP, Brazil.
NHMUK	The Natural History Museum, London, UK;
HNHM	Hungarian Natural History Museum, Budapest, Hungary.
NBCN	Naturalis Biodiversity Center, Leiden, The Netherlands.
MNHN	Muséum National d'Histoire Naturelle, Paris, France.
HIC	The Hymenoptera Institute, 116 Franklin Ave., Redlands, California 92373, USA.
EMUS	The Entomological Museum of Utah State University, Logan, Utah, USA.

Taxonomy

Key to new world genera of Sigalphinae

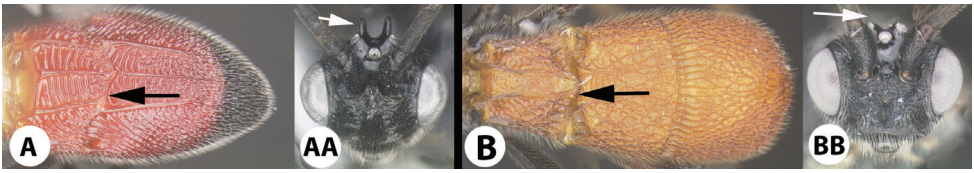
1 **A.** Metasomal terga 1–3 forming carapace covering entire metasoma, tergum 4 not visible dorsally **2**

– **B.** Metasomal terga 1–3 not forming carapace covering entire metasoma, tergum 4 visible dorsally **4**



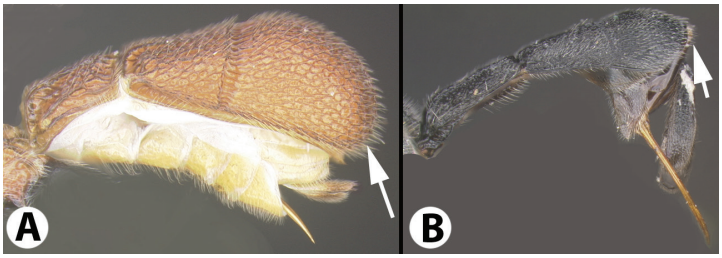
2 **A** Joint between terga 1 and 2 fused, not articulating. **AA.** Head with a pair of horns posterior to lateral ocelli or posterior to genae ..... ***Minanga*** **Cameron, 1906**

– **B.** Joint between terga 1 and 2 articulating, not fused. **BB.** Head lacking horns **3**



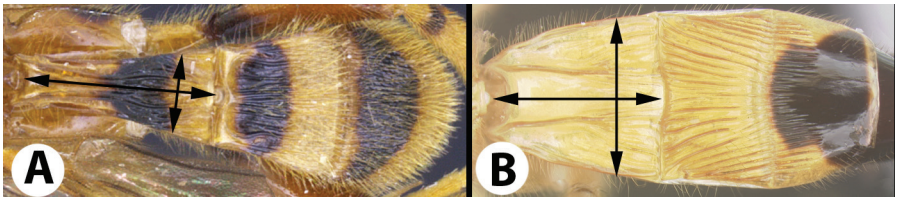
3 **A.** Third tergum curved ventrad such that it is visible in posterior view ..... ***Sigalphus*** **Latreille, 1802**

– **B.** Third tergum not curved ventrad, comparatively flat and not visible in posterior view ..... ***Acampsis*** **Wesmael, 1835**



4 **A.** Median tergite 1 much longer than wide; third median tergite completely sculptured..... ***Paphanus***

– **B.** Median tergite 1 almost as long as wide; third median tergite smooth in the posterior half..... ***Pselaphanus* Szépligeti, 1902**



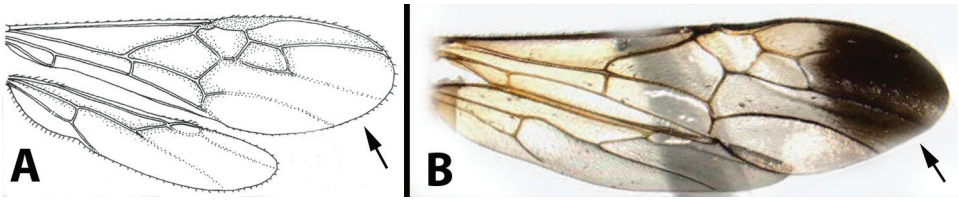
***Paphanus* van Achterberg & Riedel, 2009**

Van Achterberg and Riedel (2009) proposed the genus *Paphanus* (Sigalphinae: Pselaphanini) and described one species, *P. drechseli* van Achterberg & Riedel, 2009, from Paraguay. Subsequently, *P. priscillae* Braet, 2014 was described from French Guiana. Studying the material collected in the Northwest of São Paulo State, Brazil, we found many specimens of a third species of *Paphanus*. This is the first record of the genus in Brazil. The biology of members of *Paphanus* is unknown, although they can be presumed to be koinobiont endoparasitoids of lepidopteran larvae.

Key to species of the genus *Paphanus* van Achterberg

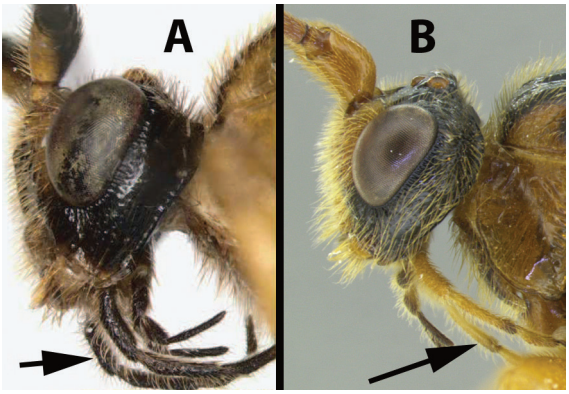
1 **A.** Fore wing lacking an infuscate apex (Image modified from van Achterberg and Riedel 2009) ..... ***P. drechseli***

– **B.** Fore wing with a distinctly infuscate apex (Image modified from Braet, 2014)..... **2**



2 **A.** maxillary palpi entirely black. (Image modified from Braet 2014) .... ***P. priscillae***

– **B.** Maxillary palpi mostly yellow..... ***P. paloi* sp.n.**



***Paphanus paloi* Penteado-Dias, sp. n.**
http://zoobank.org/E17B3A96-C6FD-41D1-A444-F9BB538A0C2A
Fig. 1A–F

Diagnosis. Lengths: body 10.0 mm, fore wing 9.3 mm. The following characters separate this species from *P. drechseli* van Achterberg & Riedel, 2009: fore wing membrane yellowish, infuscate apically, not infuscate near veins and hind wing yellowish with vein 1-M sinuous. The following characters separate this species from *P. priscillae* Braet, 2014: hind coxa yellowish with darkened patch, 1-cu-a of fore wing postfurcal, scutellum not protruding in lateral view and length of first tergite more than 1.8 times the apical width. The following characters separate this species from both *P. drechseli* and *P. priscillae*: face largely rugulose, notauli crenulate.

Male. Unknown

Hosts. Unknown.

Type material. ***Holotype***: ♀ (DCBU 264287), "Magda, SP, Brasil, Fazenda São Francisco BIOTA NOROESTE, 05.XII.2007, Malaise trap, S20 28'25 W50 17'36", F. Noll coll.. ***Paratypes***: 50 ♀♀ (DCBU 264297 – DCBU 264346) same data as holotype except date, 16.X.2007. Same data as holotype, 9 ♀♀ (DCBU 264288 to DCBU 264296), 2 ♀♀ (NHMUK), 2 ♀♀ (HNHM), 2 ♀♀ (NBCN), 2 ♀♀ (HIC), 2♀♀ (EMUS), 3 ♀♀ (MNHN).

Etymology. Named in memoriam of our friend, Haroldo Palo Jr., for his work as a photographer and naturalist.

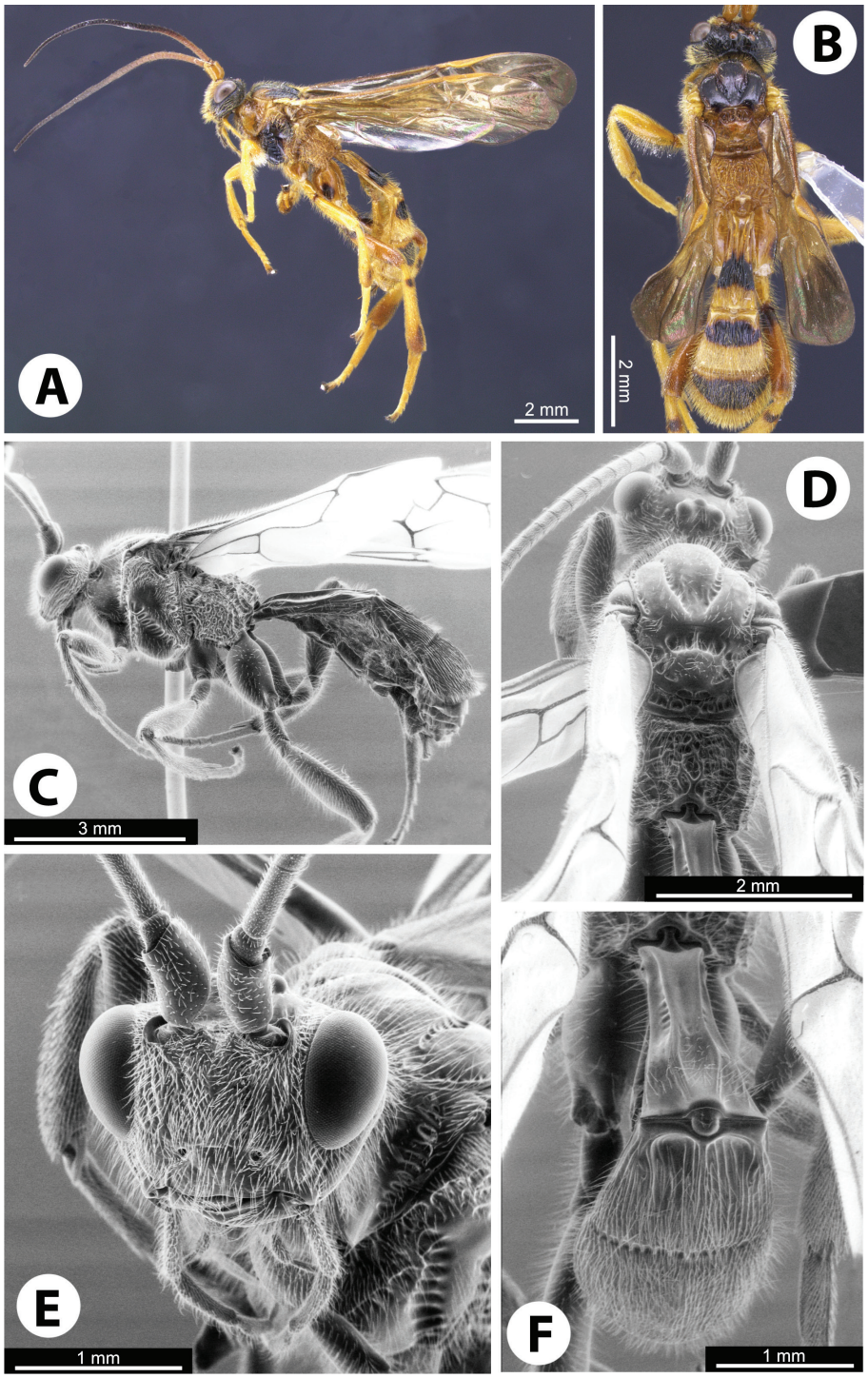


Figure 1. *Paphanus paloi*: **A** Lateral habitus **B** Dorsal habitus **C** Lateral habitus **D** Dorsal head and mesosoma **E** Anterior head **F** Dorsal metasoma.

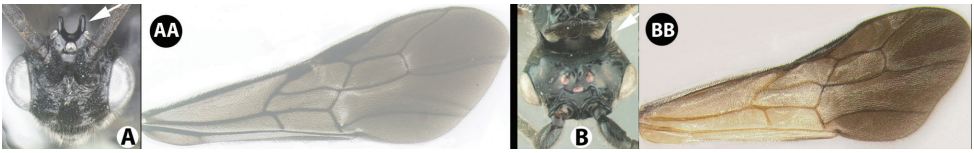
Minanga Cameron, 1906

Minanga includes 11 species. Three species, including the species proposed here, are found in the New World, whilst the remaining are in the Oriental and Afrotropical realms. Before this account, no hosts or life-history information were known for members of the genus.

Key to New World species of *Minanga*

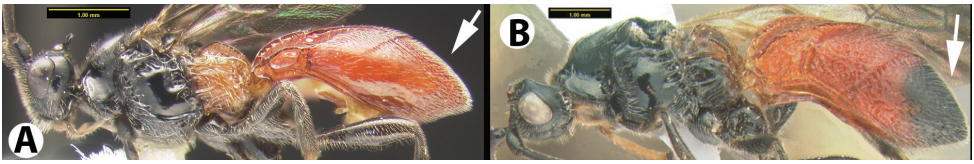
1 **A.** Horns of head directed dorsally and situated directly posterior to lateral ocelli. **AA.** Fore wing entirely infuscate **2**

– **B.** Horns of head directed posteriorly and situated on lateral occiput. **BB.** Fore wing yellow basally, infuscate apically..... ***Minanga angelus***



2 **A.** Metasoma completely reddish-orange..... ***Minanga achterbergi***

– **B.** Metasoma melanic apically..... ***Minanga patriciamadrigalae* sp. n.**



***Minanga patriciamadrigalae* Sharkey, sp.n.**
http://zoobank.org/74324E0B-7051-4E8F-A76A-FFED7F67BE64
Figs 2 A–F, 3B

Diagnosis (male and female). Similar to *M. achterbergi* but easily separated with the following character states and those in the key. Anterolateral areas of metasomal carapace: smooth in *M. achterbergi*, rugose in *M. patriciamadrigalae*. Medial longitudinal carinae of propodeum: absent in *M. achterbergi*, present in *M. patriciamadrigalae*. Number of depressions in scutellar sulcus: two in *M. achterbergi*, four in *M. patriciamadrigalae*. Medial longitudinal carinae of T2: absent in *M. achterbergi*, present in *M. patriciamadrigalae*. Body length: 5.2 mm. *M. achterbergi*, 7.5 mm. *M. patriciamadrigalae*. Metapleuron color: orange in *M. achterbergi*, melanic in *M. patriciamadrigalae*.

Host/Biology. There are hundreds of species of thin "green twig" species of Geometridae in Area de Conservación Guanacaste (Janzen and Hallwachs 2016), and

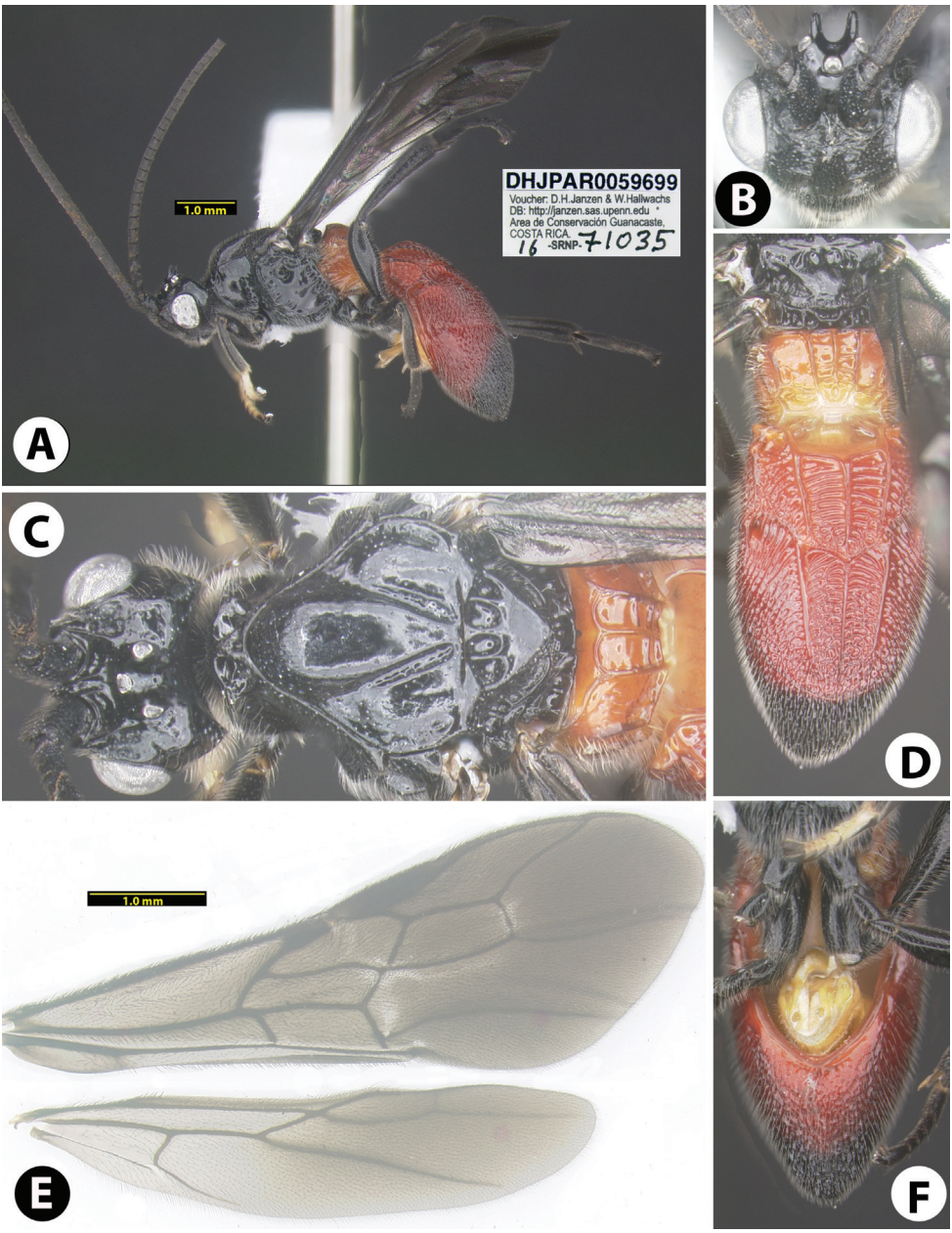


Figure 2. *Minanga patriciamadrigalae*, holotype male **A** Lateral habitus **B** Anterodorsal view of head **C** Dorsal head and mesosoma **D** Dorsal scutellum, propodeum, and metasoma **E** Wings **F** Ventral metasoma.

the host of *M. patriciamadrigalae* (15-SRNP-70988-DHJ727460.jpg) is one of them. As is the case with many of this life form of geometrid caterpillar, its cocoon is merely a flap of lightly-silked green leaf; the larva of *M. patriciamadrigalae* emerges from the prepupal caterpillar in this flimsy cocoon and spins its own rust-colored ovoid tough

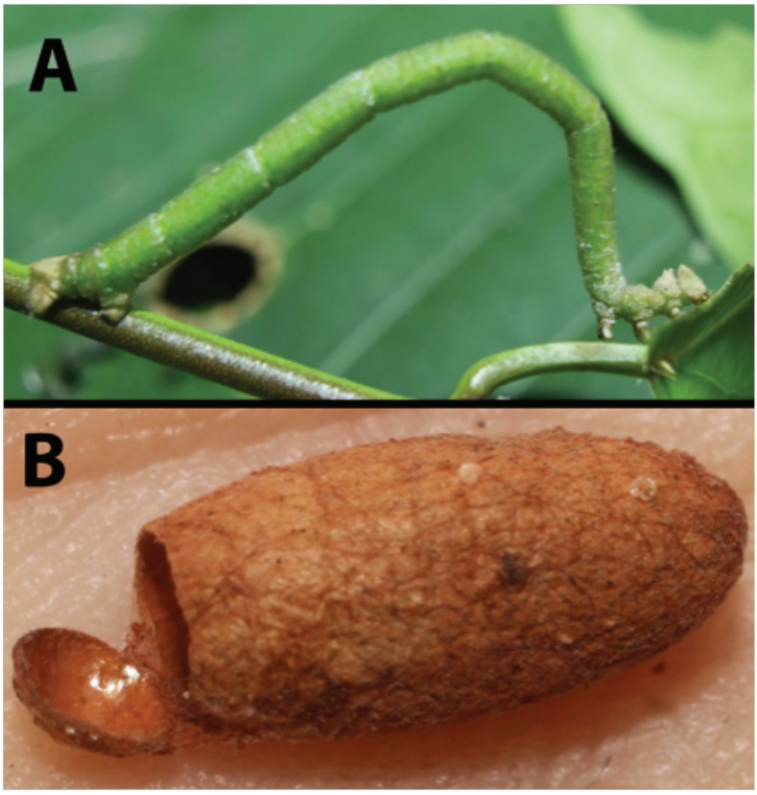


Figure 3. A *Chloropteryx* nordicariaDHJ01, host caterpillar of *Minanga patriciamadrigalae* **B** Cocoon of *Minanga patriciamadrigalae*.

cocoon inside the geometrid's cocoon (Fig. 3B). The duration of the wasp cocoon in its rain forest habitat is about 15 days. The host *Chloropteryx* nordicariaDHJ01 feeds on just one species of herbaceous vine Asclepiadaceae (*Blepharodendron mucronatum*). There have been 7 rearings of wild-caught caterpillars over two years, 2 of which had been parasitized. What have been identified as *Chloropteryx nordicaria* (Schaus, 1901), based on their very similar morphological appearance, are in fact two species as demonstrated by their very different DNA barcodes; *Chloropteryx* nordicariaDHJ02 has only been taken with light traps in the same forest and to date the caterpillar has not been located.

Since adults of both species of "*Chloropteryx nordicaria*" occur in ACG early secondary succession, moist rain forest at mid-elevations, year-round, it is not surprising that the caterpillars have been found in May, July and October. Both species are probably multivoltine, as are likely their parasitoids as well.

Etymology. *Minanga patriciamadrigalae* is named for Sra. Patricia Madrigal Cordero, Vice-Ministra of the Ministerio de Ambiente y Energía (MINAE) of Costa Rica, in recognition of her facilitation of the mutualism between Area de Conservación Guanacaste of MINAE and the Instituto Costarricense de Electricidad (ICE) in 2019–2020.

Material Examined. Holotype male, Costa Rica, Area de Conservación Guanacaste, Guanacaste, Sector Pitilla, Coneja, 415 m., latitude: 11.01525, longitude: -85.3977, Dinia Martinez, reared from a caterpillar of *Chloropteryx* nordicariaDHJ01 (Geometridae) (Fig. 3A) feeding on *Blepharodon mucronatum* (Asclepiadaceae), host collection date = 14.vii.2016, host prepupal on 07/19/2016, parasitoid eclosion date = 9.viii.2016, parasitoid voucher = DHJPAR0059699, from deceased caterpillar voucher 16-SRNP-71035 (EMUS). Paratype female, same data as holotype except: eclosion date is 08/09/2016 and caterpillar was prepupal on 22.vii.2016, parasitoid voucher = DHJPAR0059700, from caterpillar voucher 16-SRNP-71036 (HIC).

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SHORT COMMUNICATION

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First host record and morphological notes on the rare Chilean wasp *Vervoortihelcon scaramozzinoi* van Achterberg (Hymenoptera, Braconidae, Helconinae, Vervoortihelconini)

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Abstract

The first host record for *Vervoortihelcon scaramozzinoi* van Achterberg, 1998 is presented and additional notes on its morphology are provided and illustrated. The species is recorded as parasitizing the cerambycid beetle *Stenorhopalus rubiginus* in *Podocarpus* L'Hér ex Pers (Podocarpaceae). The metasomal carapace is shown to be sexually dimorphic and comprising 4 tergites in females but 5 in males. Some aspects of metasomal sculpture are reported as being variable and others highly consistent between individuals and differing somewhat from the original description.

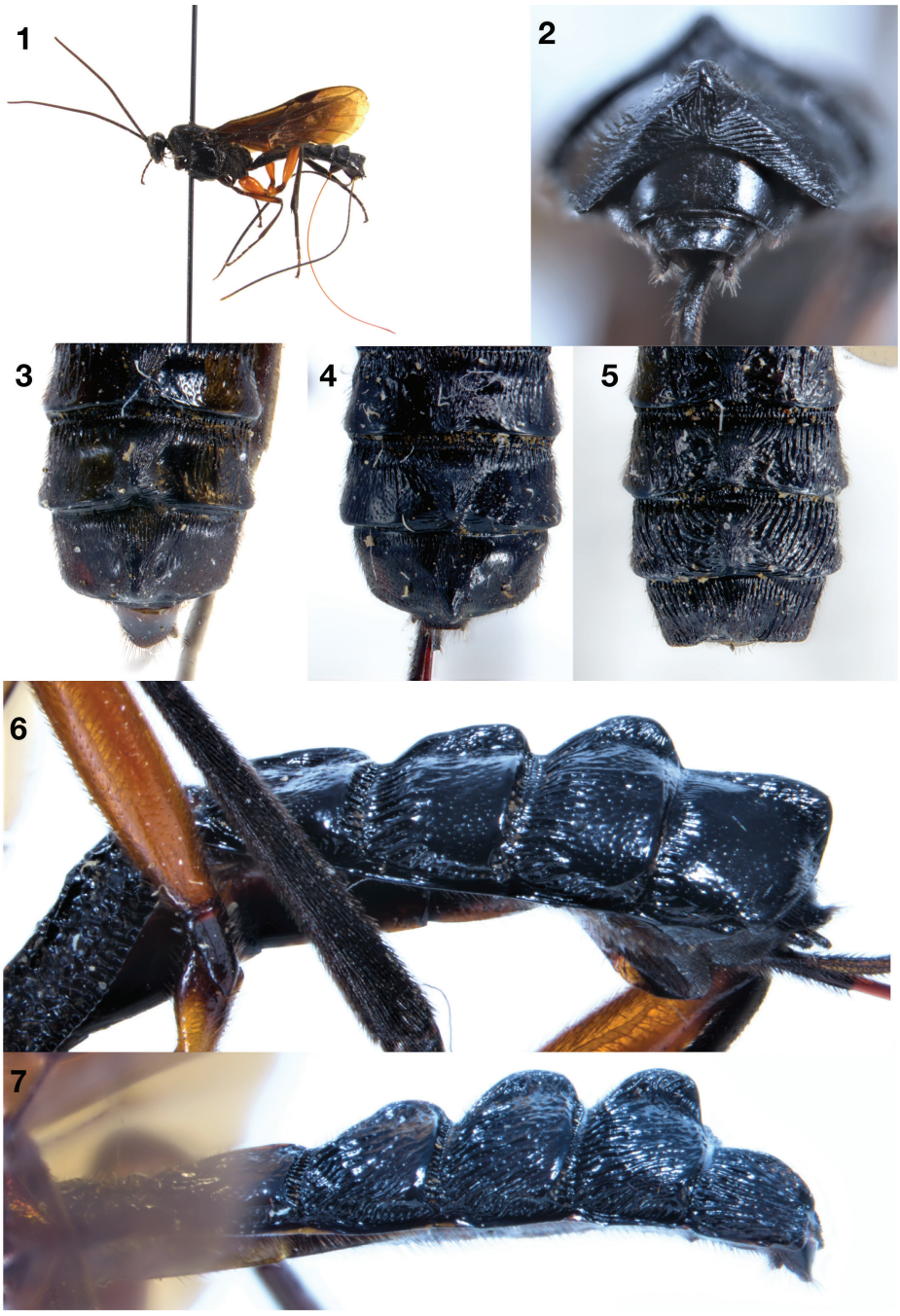
Keywords

Braconidae, Helconinae, Chile, sexual dimorphism, sculpture, host record, Stenorhopalus, Coleoptera

Introduction

The genus *Vervoortihelcon* van Achterberg, 1998, was until now known from only the female holotype and male paratype of its type species, *V. scaramozzinoi* van Achterberg (fig. 1 in van Achterberg 1998), both from Valdivia, Sto Domingo, Valdivia Province, Chile. Van Achterberg placed *Vervoortihelcon* in a new subtribe, the Vervoortihelconina in the Helconini because of the fused and strongly sculptured metasomal tergites 2–5. With the elevation of Helconini to subfamily status (Sharanowski et al. 2011), Chen and van Achterberg (2019) now give this group full tribe status, i.e. Vervoortihelconini. Neither of the type specimens has any associated biological data. Here we report on 12 further specimens of *V. scaramozzinoi* discovered in the New Zealand Arthropod Collection, Auckland, New Zealand. These comprise 6 males and 5 females from Bio Bio Region of Chile, probably sent to E.S. Gourlay (a New Zealand entomologist), and then bequeathed to the New Zealand Arthropod Collection in 1970. These specimens have no further data but do provide additional morphological data. The material additionally includes, one male specimen from Llanquihue, Rio Pescado, in the Los Lagos Region, dated 28 Nov. 1983 which was collected by G. Kuschel with the following notation "reared ex *Platynocera* sp. in *Podocarpus rubigenus*". *Platynocera* is now regarded as a junior synonym of *Stenorhopalus*, a cerambycid beetle in the subfamily Necydalinae, tribe Necydalini. G. Kuschel was a world renown coleopterist who generated many interesting rearing records (e.g. Quicke et al. 2019) especially in Chile and New Zealand.

Morphological notes. The additional specimens now available allow us to comment on some aspects of structure and sculpture not apparent from the original description and illustrations of the type specimens of *V. scaramozzinoi*. The metasomal syntergite of the female comprises metasomal tergites 2–5 as in the original description, with the 6th tergum smooth and retracted under the 5th (Figs 1, 6). However, in males the syntergite comprises five segments, tergites 2–6 are strongly sculptures and completely fused (Figs 4, 7). In addition there is variation in the degree of metasomal sculpturation, especially of the posterior tergites. Among females, some individuals have the 5th tergite coursely striated as in the type specimens (Fig. 3), others have it almost smooth with only a weak indication of longitudinal striation (Fig. 4). In males the 6th metasomal tergite is always strongly longitudinally sculptured (Figs 5, 7). In both sexes tergites 3–5 have a strong medial-posterior 'tubercle' which is more or less posteriorly blunt and sculptured (Figs 6, 7) but the male 6th tergite lacks a tubercle (Figs 5, 7). Van Achterberg (1998: figs 9, 14) illustrates the sculpture of the posterior faces of the 5th metasomal tubercles of male and female *V. scaramozzinoi* respectively. All our specimens differ somewhat from these illustrations in that in both sexes the sculpture comprises a medio-dorsal lozenge-shaped area with more or less vertically orientated fine sculpture, and lateral areas with fine parallel, more or less horizontal striation (Fig. 2).



Figures 1–7. *Vervoortihelcon scaramozzinoi* morphological features. **1** habitus **2** posterior aspect of apex of female 5th metasomal tergite showing largely sub-parallel lateral pattern of striation **3** metasomal tergites 3–5 of female with relatively strong sculpture **4** metasomal tergites 3–5 of female with reduced sculpture **5** metasomal tergites 3–6 of male **6** female metasoma, lateral view showing fused tergites 2–5 **7** male metasoma, lateral view showing fused tergites 2–6.

Acknowledgements

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SHORT COMMUNICATION

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

Validation of *Distatrix pandora* Grinter, 2009 (Hymenoptera: Braconidae, Microgastrinae)

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Grinter et al. (2009) described six new Neotropical species of *Distatrix* Mason (Hymenoptera: Braconidae, Microgastrinae), including *D. pandora* Grinter, which was described as a parasitoid reared from *Eois nympha* and related caterpillars (Geometridae) feeding on *Piper cenocladium* (Piperaceae) in Central America. In the "Material examined" section of the paper, the depositories of the topotypic portion of the type series (including the holotype) were provided, but the precise depository location of the holotype was inadvertently left unspecified. As such, under Article 16.4.2 of the International Code of Zoological Nomenclature, the name *Distratrix pandora* can be considered unavailable. The purpose of this note is to correct the oversight in Grinter et al. (2009) – the holotype depository is provided below – and to make the name *D. pandora* formally available.

Distatrix pandora Grinter, sp. n.

Distratrix pandora Grinter, 2009 (Grinter et al. 2009: 13–15; figs. 14–19, 23, 27, 28, 32, 34).
Unavailable name.

Material examined. Holotype female: PANAMA: Barro Colorado Island. 9°09'N, 90°51'W, artificial island made up of 15 km^2 of lowland moist forest located in the

Panama Canal (Gatun Lake), 2003. Holotype deposited in **MIUP** (Museo de Invertebrados Graham Bell Fairchild, Universidad de Panamá). Paratypes: 9 females, 10 males, similar data except emergence and pupation dates. 9 females, 10 males, similar data except 2004. 1 female, similar data except 11 June 2001. 1 male, 1 female, similar data except 22 July 2003. 3 females, 3 males, similar data except 23 July 2003. 1 female, similar data except 25 June 2005. (MIUP, INHS and CAS collections). 3 females, 7 males: COSTA RICA: Heredia Prov., La Selva Biological Reserve, located at 100m on the Caribbean slope, 10°26'N 83°59'W (Hartshorn and Hammel 1994, http://www.ots.duke.edu/en/laselva/intro.shtml). 1 male, ECUADOR: Napo Prov., Yanayacu Biol. Station 80% primary forest, montane wet forest at 2100m 0°42'01.33"S, 77°44'00.00"W, 16 March 2002. 1 male, similar data except 3 June 2001.

Hosts. (Fig. 14) Single holotype female reared from *Eois nympha* (Geometridae) feeding on *Piper cenocladum* C. DC. (Piperaceae). All other host data from Costa Rica and Panama similar except locality, pupation and eclosion times. Two male specimens from Ecuador reared from an undetermined Geometridae.

Diagnosis. This species is almost identical to *Distatrix teapae* (Nixon), both in morphology and coloration, sharing with it and *D. solanae* Whitfield, *D. xanadon*, *pitillaensis* and *D. belliger* (Wilkinson) the enlarged eyes (females only at least in *D. pandora*). With *D. teapae* and at least *D. maia* (Nixon), *D. formosus* (Wesmael), *D. loretta*, *xanadon*, *vigilis*, *pitillaensis* (but not *D. belliger*), it shares a modified distal front tarsomere, which is excavated apically on the ventral side and bears a strongly curved modified spine.

This new species differs from *D. teapae* in having an overall smaller body size, darker coloration. In addition, the hypopygium of the new species appears to be wider medially, more so than immediately anteriad sternum (in *D. teapae* the hypopygium gradually tapers towards anterior apex (Fig. 21)). The new species also is conspicuously setiferous along entire width of ventral sclera, whereas in *D. teapae* the setae are constricted to the ventral third of specimen.

The distances between the ocellus and eye margins, as well as the flagellomere distances are slightly larger by about half than that of *D. teapae*. *D. pandora* also shares with at least herein described species, a very large lateral metapleural pit, which appears to be reduced in *D. teapae* (Fig. 25). Mesopleural sternaulus is also nearly absent in *D. teapae*. Metasomal tergum II with median area defined by grooves diverging at an angle roughly 90°, whereas in *D. teapae* the angle is greater than 120° (Fig. 20).

D. pandora appears to be a specialist on *Eois* caterpillars, however specific level interactions are undetermined.

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RESEARCH ARTICLE

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A new species of *Platygaster* (Hymenoptera, Platygastroidea) from India with an unusual antenna

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Abstract

A new species, *Platygaster harpagoceras* Popovici & Veenakumari, is described from India. The most unusual features of this species are the acuminate shape of the last antennomere and the 9-merous antenna in both sexes. The male and female are described and illustrated with brightfield and scanning electron microscopy. We provide a comparative analysis of the acuminate distal antennomere in the superfamily Chalcidoidea, including several genera of Pteromalidae (*Callitula* Spinola, *Homoporus* Thomson, *Norbanus* Walker, *Rhaphitelus* Walker).

Keywords

taxonomy, new species, Platygastridae, SEM

Introduction

The "mega-genus" *Platygaster* Latreille is apparently one of the most speciose genera of Platygastroidea, but its taxonomy is presently in a state of confusion. With around 640 described species (various contributors 2018) and few taxonomically reliable external characters, *Platygaster* offers a unique challenge to any specialist dealing

with the taxonomy of Platygastroidea. In some *Platygaster* species, previous authors have found a high degree of variability. For example, in *P. depressiventris* Thomson the size of the last tergite varies significantly, which Huggert (1974) considered "an ecological adaptation" for different "local populations" and in *P. mainensis* MacGown & Osgood were found three distinct "phena", which included one wingless specimen among more than 50 fully winged specimens (MacGown and Osgood 1971). Most "classical" species of *Platygaster* were described from Western Europe, so describing a new species using material from outside this geographical area may reveal additional problems as a consequence of geographical variation. Because of this, only very characteristic species can be described as new without a thorough revision and, preferably, when both sexes are available.

There are no comprehensive modern reviews of *Platygaster* for any region of the world. The species of "classical" authors were reviewed as follows: the species of Haliday and Walker preserved in the National Museum of Ireland and in the British Museum by Vlug (1984); the species of Zetterstedt and Thomson in Lund University, Sweden (Biological Museum – Entomological collections) by Buhl (1995), and the species of Förster by Buhl (1996). To study *Platygaster*, students have to use the monograph of Kieffer (1926) or some identification keys concerning local faunas, e.g. Buhl (1999) for species of Fennoscandia and Denmark, or Buhl (2006) for the species of Denmark.

Material and methods

The material described in this paper is deposited in the collection of the Hungarian Natural History Museum (Budapest) and in the National Bureau of Agricultural Insect Resources, Bengaluru.

Photographs were produced using a Leica DFC-500 camera on a Leica 205A stereomicroscope (facilities of the Integrated Centre of Environmental Science Studies in the North East Region – CERNESIM, from the Faculty of Biology, Iași, Romania), using the illumination protocol described in Fusu and Polaszek (2017) and Popovici et al. (2019). Single montage images were produced from image stacks with Zerene Stacker (Zerene Systems LLC, http://www.zerenesystems.com/) using the PMax algorithm. For SEM, the dried specimen was mounted on a double adhesive tape, coated with gold and imaged with a VEGA TESCAN SEM (WD=6.0146 mm; HV=30.00 kV).

Morphological terminology follows Masner (1979, 1980), Masner and Huggert (1989), and Mikó et al. (2007). The terminology of surface sculpturing follows Harris (1979).

Abbreviations:

HNHM	Hungarian Natural History Museum (Budapest, Hungary)
BMNH	The Natural History Museum (London, UK)
NBAIR	National Bureau of Agricultural Insect Resources (Bengaluru, India)

Results

We place the newly described species in *Platygaster* based on the following characters: number of maxillary palpal sclerites: 2; number of labial palpal sclerites: one; malar sulcus: absent; malar striae: absent; distal two antennomeres: not tightly joined; lateral compression of mesosoma: absent; notauli: obviously converging to the scutoscutellar suture; spines or tuft of hairs on the mesoscutellum: absent; foamy structure of propodeum: absent; lateral propodeal carinae: separated (MacGown 1979, Austin and Field 1997); tibial spur formula: 1:2:2 (MacGown 1979); forewing venation: absent; number of apparent terga (female): 6; number of apparent terga (male): 7; horn of T1: absent.

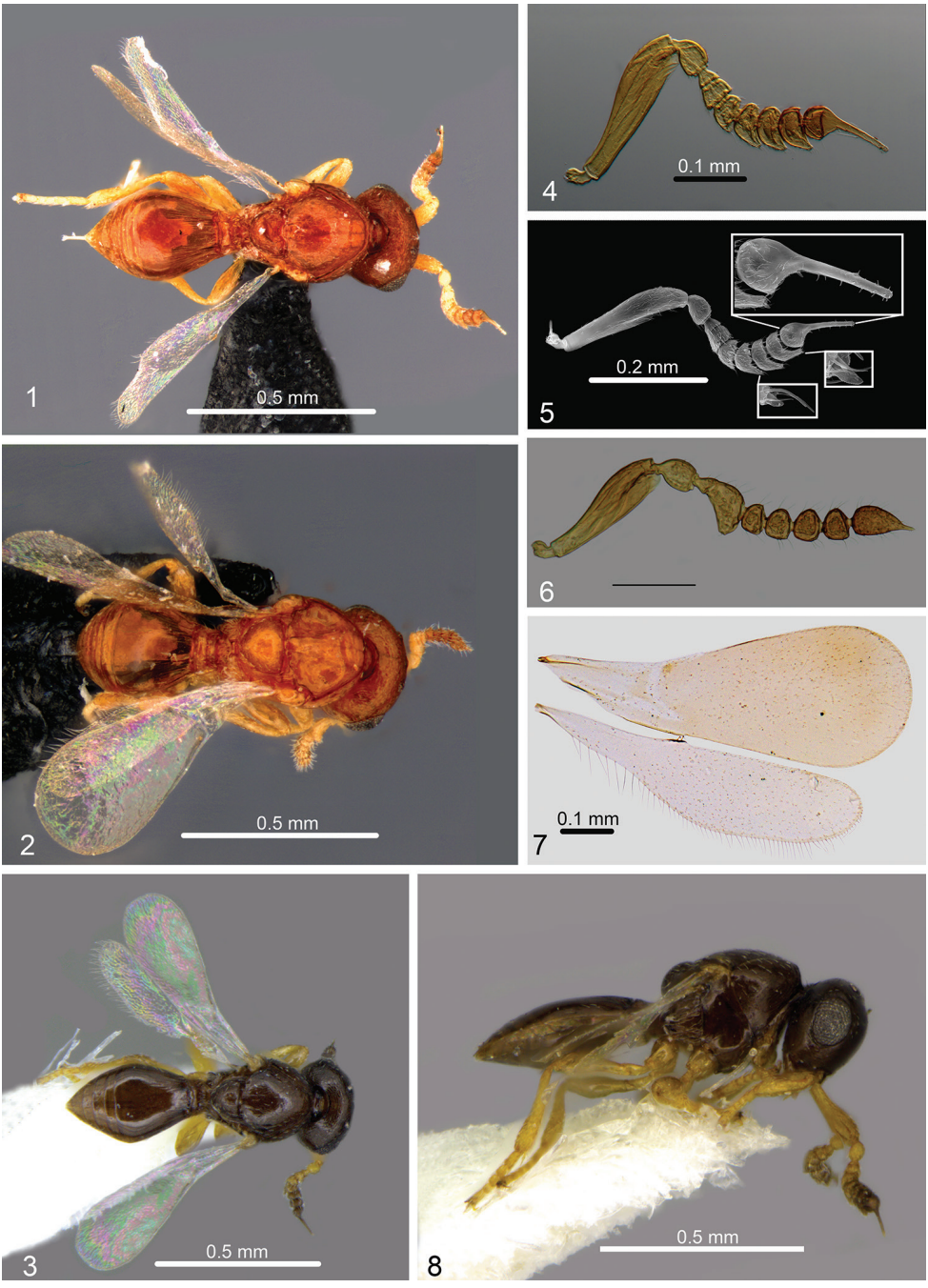
Platygaster harpagoceras Popovici & Veenakumari

Description. Female (Figs 1, 3, 8–10). Colour (Figs 3, 8): Head, mesosoma, and metasoma dark brown with uneven patches of black, posterior tergites paler than anterior tergites; black band above occipital carina; legs and tegula yellowish brown; A1–A4 yellowish brown, A5–A6 slightly darker than preceding antennomeres, A7–A9 blackish brown; mandibles yellowish brown. The lighter colour of the specimens pictured in Figs 1 and 2, compared with the specimen in Figs 3 and 8, is attributed to the older age of these specimens.

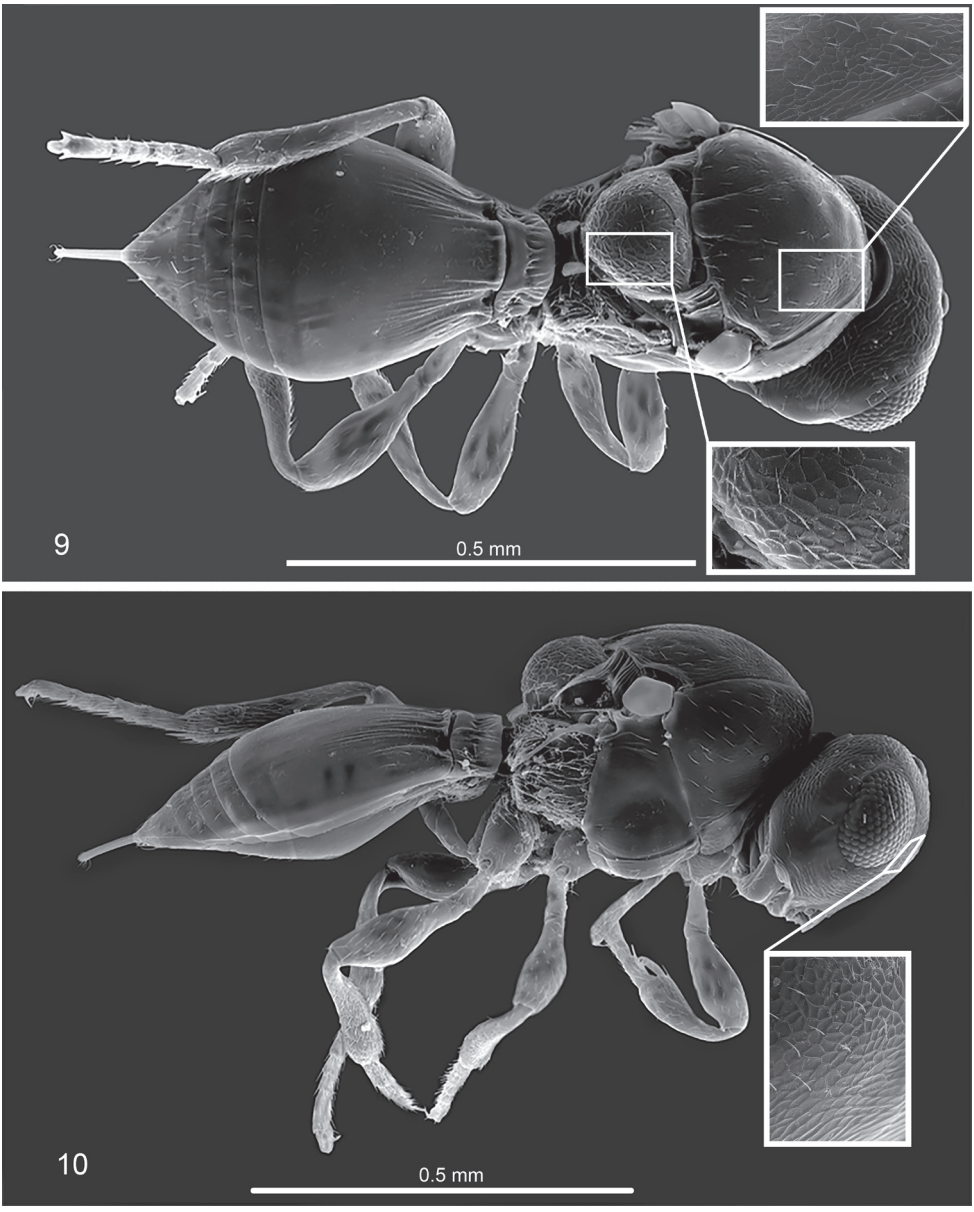
Head. Shape of head in dorsal view: subellipsoidal, 1.8–1.9 times as wide as long; occipital carina: present, not crenulate, weak; sculpture of posterior vertex: coriaceous-imbricate, transversally arranged; pilosity of posterior vertex: sparse, short setae, generally in two transverse rows; sculpture of temple: coriaceous-imbricate; hyperoccipital carina: absent; sculpture of interocellar area: coriaceous-imbricate to reticulate; ratio OOL/OD: OOL 2.3–2.5 times as long as OD; OOL/POL/LOL: 1:2:1; sculpture of frons: reticulate, but above toruli similar to sculpture of posterior vertex; IOS/EH: IOS longer than EH (IOS 1.8–1.9 times as long as EH); setation of eyes: short, with fine scattered hairs (visible at 70 X magnification); interantennal process: not prominent, concave; width of interantennal process: about equal to diameter of torulus; mandible: bidentate.

Antenna (Figs 4, 5). Number of female antennomeres: 9; number of clavomeres: 4; abrupt clava: absent; compact clava: absent; sensillar formula (A9–A6): 1:1:1:1; A6–A8: distinctly projecting anteroventrally, resembling a tooth; A9: distinctly acuminate.

Dorsal mesosoma (Figs 1, 3, 9). Pronotum: distinctly visible; pronotal shoulders: not enlarged; epomial carina: well developed; cervical pronotal area: weakly concave; setation of cervical pronotal area: absent; sculpture of mesoscutum: finely imbricate-coriaceous; sculpture of mesoscutellum: reticulate; antero-admedian line: absent; parapsidal line: absent; notauli: abbreviate, superficial, convergent posteriorly; mid lobe on posterior margin of mesoscutum: extending onto mesoscutellum; transaxillar and axillular carinae: fused, the resulting carina clearly visible; posterior mesoscutellar rim: not distinct; metanotum: narrow, smooth; metascutellum: not visible dorsally,



Figures 1–8. *Platygaster harpagoceras*: **1** Holotype female (dorsal) stored in HNHM (Budapest) **2** Paratype male (dorsal) stored in HNHM (Budapest) **3** Paratype female (dorsal), stored in NBAIR (Bengaluru) **4** Female antenna-light microscopy **5** Female antenna (SEM) **6** Male antenna-light microscopy **7** Fore and hind wing **8** Paratype female (lateral), stored in NBAIR (Bengaluru).



Figures 9, 10. *Platygaster harpagoceras*, female (SEM): **9** Habitus, dorsal **10** Habitus, lateral.

covered by mesoscutellum; setation of propodeum: long, dense laterally, absent medially; lateral propodeal carinae: distinct, parallel; metasomal depression: narrow; propodeal spiracle: clearly visible.

Lateral mesosoma (Figs 8, 10). Transverse pronotal sulcus: weak, glabrous; lateral propleural area: weakly convex; sculpture of lateral propleural area: uniform imbricate-coriaceous; setation of lateral propleural area: some sparse setae on the dorsal half;

transaxillar carina, in lateral view: with numerous longitudinal striae; mesopleural depression: weakly indicated; transepisternal line: weakly indicated; transepisternal line: almost transverse, deep, and sharply incised, nearly parallel with mesopleural carina; sculpture of mesopleuron: absent, except for sparse striae dorsally; setation of mesopleuron: absent; sculpture of metapleuron: absent; setation of metapleuron: relatively dense, present throughout; metapleural carina: prominent, well developed; metapleural pit: not visible; metapleural sulcus: not visible.

Fore wing (Fig. 7).Venation: absent; colour of fore wing: faintly infuscate; setation of fore wing: short, sparse microtrichia; fore wing length/width ratio: 2.6 times as long as wide; marginal fringe of fore wing: absent. Hind wing (Fig. 7).Venation: absent; colour of hind wing: hardly infuscated; number of hamuli: 2; setation of hind wing: rare, sparse microtrichia; hind wing length/width ratio: 4.3–4.4 times as long as wide; marginal fringe of hind wing: short, almost 0.2 times as long as hind wing width.

Metasoma. Length of metasoma: shorter than head and mesosoma combined; shape of metasoma in lateral view: convex dorsally; number of visible tergites: 6; shape of T1: trapezoidal; anterior pits of T1: clearly visible; sculpture of T1: medially costate, costae longer laterally; setation of T1: sparse laterally, absent on medial sculptured area; the largest tergite: T2; anterior pits of T2: present; setation of T2: few setae on lateral T2; sculpture of anterior T2: smooth between anterior pits, laterally longitudinally striate); length of striae on T2: surpassing the middle of T2; sculpture of T3–T6: absent; setation of T3–T5: sparse (~ 12–14 setae), in a single transverse row; laterotergites: present, distinct; setation of S1: present throughout; setation of S2: absent; setation of S3–S6: sparse to absent.

Male (Fig. 2): similar to female, differing in the structure of antenna (Fig. 6), with A4 longer than A3, A5–A8 having almost the same shape and size, and metasoma consisting of 7 visible tergites with a rounded apex.

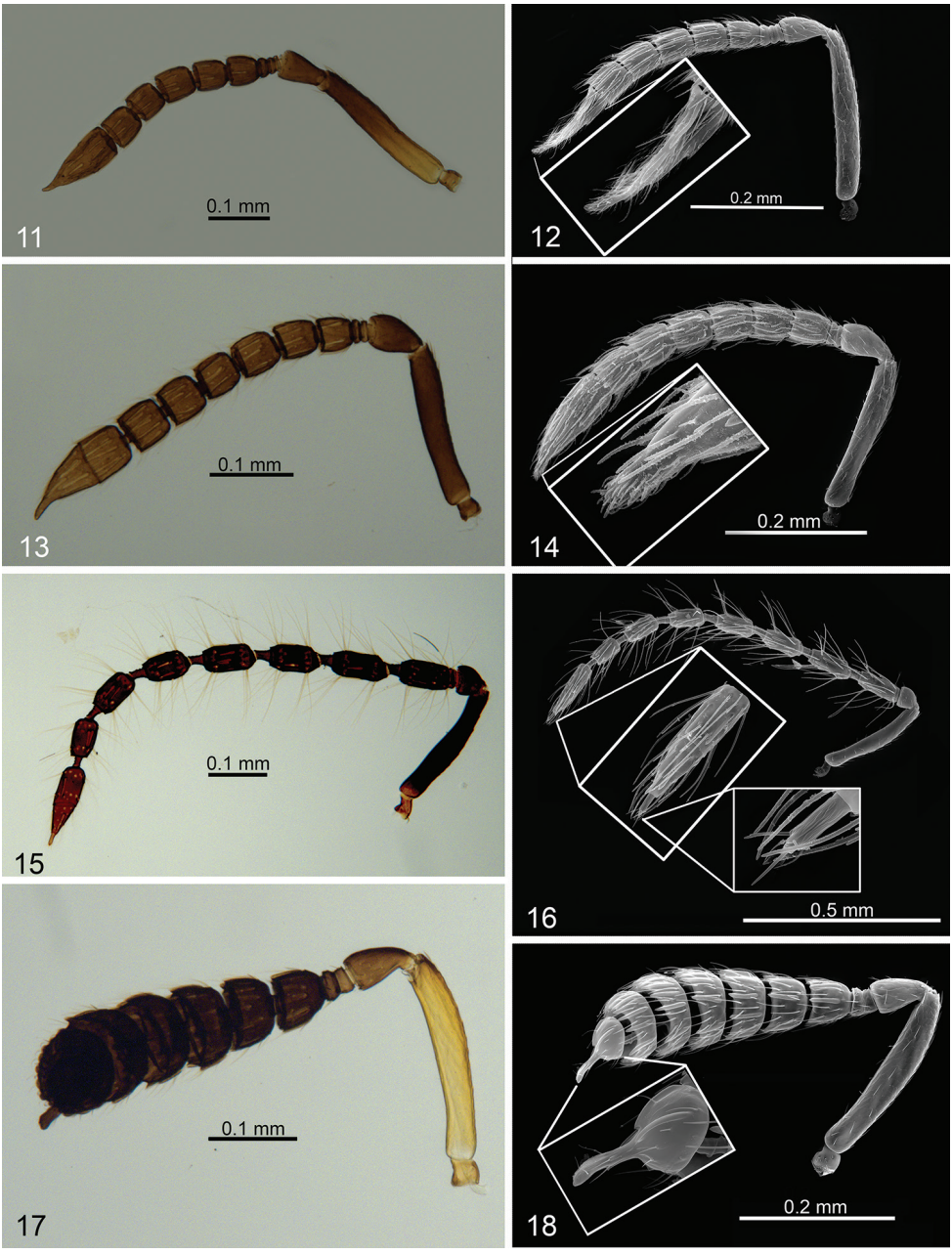
Diagnosis. In *Platygaster*, a 9-merous antenna is known only from *P. harpagoceras* and *P. novemarticulata* Buhl, 2009. *Platygaster harpagoceras* can be distinguished by the acuminate A9, A6–A8 transverse, A6–A8 with anteroventral projection, frons reticulate, and longitudinal striae on T2 surpassing the middle of T2.

Etymology. The epithet "harpagoceras" given to this species refers to the characteristic antenna (gr. "harpagos" – hook, and gr. "kéras" – horn).

Material examined. Holotype: 1 female, "India, Orissa Jajpur-Keonjahr Dists., Daitari, 28.xii.1966, leg. Topál" (Deposited in HNHM); Paratypes: 1 male and 1 female the same data as the holotype (HNHM); 1 female, India, Tamil Nadu, Kanyakumari, Manalodai, 11.25220°N, 78.69680°E, yellow pan trap, 13.v.2013, leg. A. Rameshkumar (NBAIR).

Discussion

The generic limits of *Platygaster* Latreille have not been clearly established and the genus is characterized primarily by the absence of characters that define other genera. There are no studies regarding the phylogeny of the genus *Platygaster*, and its monophyly is dubious, considering its high degree of morphological heterogeneity. Detailed study of the distribution of morphological characters throughout *Platygaster* is badly



Figures 11–18. Antenna (light and SEM microscopy): **11, 12** *Callitula* **13, 14** *Homoporus* **15, 16** *Norbanus* **17, 18** *Rhaphitelus*.

needed to infer monophyletic lineages that may be treated as separate genera and to construct a classification that is navigable at the species-level.

For comparison, we analyzed the acuminate shape of the last antennomere in the superfamily Chalcidoidea, where this trait is fairly common in members of several

families, including Pteromalidae and Eulophidae. In Pteromalidae, common genera, such as *Callitula* Spinola (Figs 11, 12), *Homoporus* Thomson (Figs 13, 14), *Norbanus* Walker (Figs 15, 16), and *Rhaphitelus* Walker (Figs 17, 18), have the distal antennomere spiculated in both sexes (Lotfalizadeh 2015, Mitroiu 2015).

Spiculated antennae are an extreme case of acuminate antennae, where the apical part of the clava distinctly narrows into a terminal projection of various lengths. In Pteromalidae a 'terminal button' (Heraty et al. 2012), which could be regarded as the 4th clavomere, is common in many genera. Whether the spicula represents the terminal button was investigated using SEM images. No suture has been observed in the antenna of the investigated genera, but in the male antenna of *Norbanus* it is evident that the spicula is distinct from the last antennomere. Hence, it is possible that the 4th clavomere of the female antenna is fused to the previous one, at least in Pteromalidae.

Another possibility we investigated was that this terminal structure represents a large sensillum. Tselikh (2010) and Zeiri et al. (2015) state that the female antenna of *Rhaphitelus* has a 'baculiform sensillum' at its apex. However, our SEM images of the female clava of *Rhaphitelus* show the presence of setae on the terminal structure and no discontinuity with the rest of the clava, which suggests the first is part of the antennomere and not a distinct structure. This is also true for the other pteromalid genera, and for *P. harpagoceras*.

Of all previously mentioned pteromalid genera, the clava of *P. harpagoceras* is most similar to that of *Rhaphitelus*, where the male also displays a much reduced spicula compared with the female. Pteromalidae and *Platygaster* are only distantly related, so this character state is clearly a convergence. This seems to be the case within Pteromalidae as well, except for *Norbanus* and *Homoporus*, which are probably closely related based on other features. The function of this particular shape of the distal antennomere is not known.

The anteroventral projections of A6–A8 are another peculiarity of *P. harpagoceras* and are similar to the projections of A7 and A8 in some species of *Allotropa* Förster (Sceliotrachelinae). Similar projections on A6–A9 were illustrated by Buhl (2001) in *Platygaster dilata* Buhl, which has 10 antennomeres, but A10 is not acuminate.

P. harpagoceras is the second known species of *Platygaster* having 9-merous antennae. As Buhl (2009) states, it is not necessary to erect a new genus for these species, at least not at the present level of study, taking into account that among platygastrids there are some genera containing species with 9 or 10 antennomeres (e.g. *Fidiobia* Ashmead, *Metanopedias* Brues). We consider the reduced number of antennomeres and the peculiar morphology of the apical antennomere to be apomorphic characters. Erecting new genera for species with striking apomorphies have to be carefully decided as it could be "detrimental to the construction of a natural classification if it renders other taxa paraphyletic" (Talamas and Buffington 2014).

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SHORT COMMUNICATION

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First detection of the samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae), in Canada

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Abstract

We report the first detection of *Trissolcus japonicus*, an exotic Asian egg parasitoid and the primary candidate for classical biological control of the invasive brown marmorated stink bug, *Halyomorpha halys*, in Canada. Twenty-eight *Trissolcus japonicus* emerged from an *H. halys* egg mass from a site heavily infested by *H. halys* in Chilliwack, British Columbia, in 2018. This egg mass was deployed and retrieved as part of ongoing sentinel egg mass surveys for natural enemies of *H. halys* from 2017–2018 in coastal and interior British Columbia (total of 1,496 egg clusters at 16 sites). The identification of *T. japonicus* was based on biology (high levels of successful emergence from *H. halys* eggs), morphology, and mitochondrial DNA sequences. *Trissolcus japonicus* was not detected at any other survey sites in 2017–2018; however, three species of indigenous egg parasitoids were found attending or emerging from *H. halys* egg masses at low levels (<4%) at several sites. The origin of the detected *T. japonicus*, the extent of its establishment in British Columbia, and its ultimate impact on *H. halys* populations remain to be determined. Nonetheless, the detection of this exotic biological control agent in Canada concurrently with regulatory review of its intentional importation and release is emblematic of the current uncertainty around regulatory control on the movement of biological control agents across borders.

Keywords
adventive establishment, classical biological control, brown marmorated stink bug, *Halyomorpha halys*

Introduction

Classical (= importation) biological control of invasive pests, where natural enemies are imported and intentionally introduced from a pest's area of origin, involves years of research to assess risks and benefits of proposed introductions, followed by regulatory approval (Bigler et al. 2006, Cock et al. 2016, Heimpel and Cock 2018). However, there is increasing recognition that unintentional introductions of natural enemies are probably common, introducing a high level of uncertainty to the regulatory process for biological control introductions (Mason et al. 2017a).

The samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), has become a prominent case study for the establishment of a candidate biological control agent outside of its native range prior to a decision by regulatory authorities on the appropriateness of release (Servick 2018). Its host, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), also native to Asia, is an invasive alien pest that has caused extensive economic damage and increases in insecticide use in a wide range of crops in areas of the United States and Europe where it has become established (Leskey and Nielsen 2018). *Halyomorpha halys* is also a nuisance pest in human dwellings. A classical biological control program for *H. halys* was initiated in the USA and *T. japonicus* was identified as the most promising candidate for introduction based on high parasitism rates of *H. halys* (typically ~60–90%) in Asia (Qiu et al. 2007, Yang et al. 2009, Zhang et al. 2017). However, in 2014, while non-target host range testing was still underway, a population of this parasitoid was recovered in nature from sentinel egg masses in Maryland, USA, indicating that it had been introduced accidentally (Talamas et al. 2015a). Other adventive populations were found in Oregon and Washington State (USA) in 2015 (Milnes et al. 2016, Hedstrom et al. 2017). More adventive populations of *T. japonicus* in both the Pacific Northwest and the northeastern USA continue to be discovered (stopbmsb.org), and intentional redistributions are now taking place within some states (K.A. Hoelmer, personal communication). Unexpectedly, in 2017 and 2018, adventive populations of *T. japonicus* were also detected in Switzerland (Stahl et al. 2018) and Italy (Sabbatini et al. 2018), suggesting that like its host, *T. japonicus* is becoming a "global invader".

Trissolcus japonicus has not been detected previously in Canada, where *H. halys* populations have established recently (Gariepy et al. 2014, Abram et al. 2017a). Thus, intentional introduction of *T. japonicus* to control *H. halys* populations in Canada would require regulatory approval based on review of a petition for release (Mason et al. 2017b). Here we report the detection of *T. japonicus* in British Columbia, Canada, representing another remarkable instance where this parasitoid has arrived in a country before a regulatory decision has been made regarding the appropriateness of its introduction.

From May to September in each of 2017 and 2018, a total of 1,496 *H. halys* sentinel egg masses (= 41,351 eggs) were set out at 16 field sites in coastal and interior British Columbia where large, established breeding populations of *H. halys* are present (Table 1). Eggs were then retrieved to measure parasitism levels and parasitoid species composition. All sentinel sites were in urban, suburban, and backyard settings with mixed woody and herbaceous vegetation. *Halyomorpha halys* egg masses, laid on Reemay® polyester fabric (Avintiv, USA), were collected from *H. halys* laboratory colonies within 24 hours of being laid and either placed in the field the same day or stored at 10 °C to delay development for up to a week before they were deployed. The fabric substrate holding the eggs was stapled to the undersides of the leaves of wide variety of host plants infested by *H. halys*, mostly woody trees (e.g. *Prunus* spp., *Davidia* spp., *Acer* spp., *Gleditsia* spp., *Ailanthus* spp., *Sorbus* spp.) and shrubs (e.g., *Rubus* sp., *Mahonia* spp., *Symphoricarpos* spp., *Rosa* spp.). Sentinel egg masses were retrieved from the field within four days, before the emergence of *H. halys* nymphs. Parasitoids found attending egg masses at recovery (indicating post-oviposition brood guarding behavior; see Abram et al. 2014, Cornelius et al. 2016) were also collected. Egg masses were then kept in Petri dishes (50 mm diameter, 9 mm depth) under ambient laboratory conditions to assess parasitoid emergence. Attending and emerging egg parasitoids were preserved in 95% EtOH, then point-mounted and identified to species using the key of Talamas et al. (2015b). Finally, all egg masses were dissected under a stereomicroscope to verify that non-emerged eggs did not contain parasitoids.

While three species (26 total individuals) of indigenous egg parasitoids [*Trissolcus euschisti* (Ashmead), *Trissolcus cosmopeplae* (Gahan), and *Telenomus podisi* (Ashmead) (Hymenoptera: Scelionidae)] were found attending *H. halys* sentinel egg masses upon recovery, successful emergence of parasitoids from *H. halys* eggs was rare (Table 1). Of these species, only *T. euschisti* successfully emerged. Less than one fourth of the eggs in each of these masses were parasitized successfully (average of 22.5 ± 1.4%; mean ± SE, n = 6), and emerging parasitoids produced few or no offspring when subsequently offered *H. halys* eggs in the laboratory. These findings are consistent with past surveys and laboratory trials in other areas of North America and Europe showing that attack of *H. halys* egg masses by indigenous egg parasitoids is probably common (Gariepy et al. 2018), but their offspring are usually unable to complete development successfully (Abram et al. 2014, Haye et al. 2015, reviewed in Abram et al. 2017b). In contrast, all 28 eggs (100%) of one egg cluster deployed at a site highly infested by *H. halys* in Chilliwack, BC on August 23, 2018 were parasitized, and emerging offspring completely parasitized a number of *H. halys* egg masses offered in the laboratory with >90% successful offspring emergence.

Specimens were identified to species using the key to Nearctic *Trissolcus* by Talamas et al. (2015b) and are fully congruent with the concept of *T. japonicus* presented by Talamas et al. (2015b, 2017). Specifically, the presence of 4 clypeal setae (Fig. 1A) and well-defined episternal foveae that extend from the postacetabular sulcus to the mesopleural pit (Fig. 1B) unambiguously separate *T. japonicus* from the Nearctic fauna. Additionally, the absence of rugae on the mesoscutum (Fig. 1C) and the absence of

Table 1. Locations of field sites for sentinel egg mass surveys, the number of sentinel *H. halys* egg masses set out and retrieved, and the parasitoid species found attending and emerging from *H. halys* egg masses.

Site name (GPS coordinates)	Year(s) surveyed[a]	Total # sentinel egg clusters (total # eggs)	% egg clusters with parasitoid emergence[b]	Parasitoid species emerging from eggs (% of parasitized egg clusters)	Parasitoid species found attending egg clusters (total number)[c]
Chilliwack #1 (49.158°N, -122.003°W)	2017, 2018	313 (8,642)	0.64%	*T. euschisti* (50%); *T. japonicus* (50%)	*T. euschisti* (4); *T. podisi* (3)
Chilliwack #2 (49.159°N, -121.997°W)	2017	55 (1,426)	0.00%	–	*T. euschisti* (1)
Chilliwack #3 (49.192°N, -121.931°W)	2018	186 (5,182)	0.00%	–	–
Rosedale (49.184°N, -121.800°W)	2017	63 (1,647)	0.00%	–	*T. podisi* (1)
Abbotsford (49.003°N, -122.264°W)	2017, 2018	217 (6,004)	0.00%	–	*T. euschisti* (2); *T. podisi*(1)
Langley (49.122°N, -122.657°W)	2017	10 (308)	0.00%	–	–
Kelowna #1 (49.885°N, -119.485°W)	2018	76 (2,128)	1.31%	*T. euschisti* (100%)	*T. euschisti* (1)
Kelowna #2 (49.880°N, -119.485°W)	2018	78 (2,172)	1.28%	*T. euschisti* (100%)	*T. euschisti* (4)
Kelowna #3 (49.872°N, -119.490°W)	2018	76 (2,123)	0.00%	–	–
Kelowna #4 (49.885°N, -119.457°W)	2018	76 (2,096)	0.00%	–	*T. euschisti*(1)
Kelowna #5 (49.882°N, -119.484°W)	2018	75 (2,086)	0.00%	–	*T. euschisti* (3); *T. cosmopeplae* (1)[d]
Kelowna #6 (49.869°N,-119.486°W)	2018	66 (1,845)	1.51%	*T. euschisti* (100%)	*T. euschisti*(1)
Kelowna #7 (49.894°N, -119.405°W)	2018	60 (1,684)	3.33%	*T. euschisti* (100%)	*T. euschisti* (2)
Kelowna #8 (49.879°N, -119.484°W)	2018	60 (1,692)	0.00%	–	*T. podisi* (1)
Kelowna #9 (49.881°N, -119.484°W)	2018	60 (1,662)	0.00%	–	–
Kelowna #10 (49.868°N, -119.494°W)	2018	25 (654)	0.00%	–	–
TOTAL		1,496 (41,351)	0.47%	–	–

[a] For sites that were surveyed in both years, results are pooled.

[b] Percentage of egg clusters from which at least one parasitoid emerged.

[c] Attending parasitoids were found resting on top of the egg cluster at the time of collection, probably indicating post-oviposition brood guarding behaviour.

[d] This represents the first published record of *T. cosmopeplae* in British Columbia

a smooth area below the median ocellus (Fig. 1A) confirm that it is neither of the Palearctic species closest to *T. japonicus*, *T. kozlovi* and *T. plautiae* (Talamas et al. 2017). Voucher specimens are deposited in the Florida State Collection of Arthropods and the Canadian National Collection of Insects (Table 2). The collection data, including host associations, for all voucher specimens are deposited in the Hymenoptera Online Database (hol.osu.edu).

DNA was extracted from 5 specimens using a chelex DNA extraction protocol, and the universal primers LCO-1490 and HCO-2198 (Folmer et al. 1994) were used for amplification and sequencing of the DNA barcode region of the Cytochrome Oxidase I (COI) gene (as described by Gariepy et al. 2014). All 5 specimens yielded identical COI sequences of 643-bp in length, and a representative sequence was submitted to GenBank (Accession number: MK188349) and uploaded to the North American Scelionidae DNA barcode database (project NSCEL) available on the Barcode of Life Datasystems (BOLD, http://www.boldsystems.org). In comparison to public sequences available in the NSCEL database, these specimens shared 100% se-

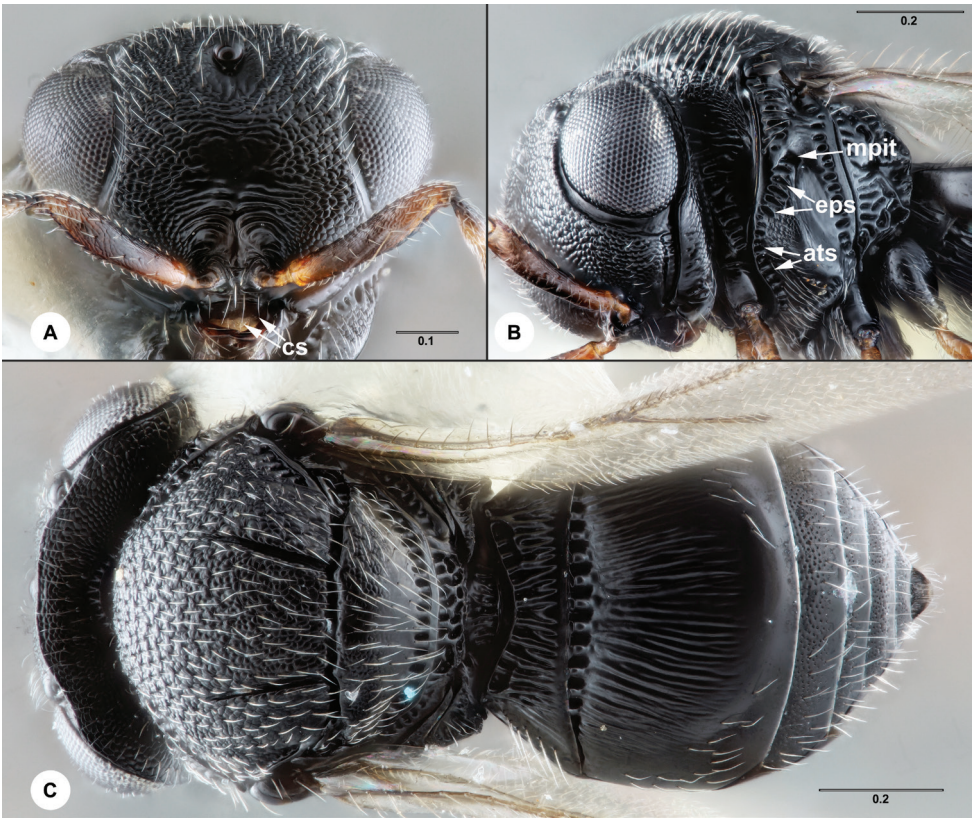

Figure 1. *Trissolcus japonicus* female (FSCA 00033107) from Chilliwack, British Columbia: **A** head, anterior view, cs: clypeal setae **B** head and mesosoma, anterolateral view, ats: postacetabular sulcus, eps: episternal foveae, mpit: mesopleural pit **C** head, mesosoma, metasoma, dorsal view. Scale bars in millimeters.

quence similarity with voucher *T. japonicus* collected from established populations in the USA, and shared 99–100% sequence similarity with voucher specimens collected in Asia. The small amount of variation among *T. japonicus* specimens is likely due to intraspecific variation between individuals collected from geographically distinct regions (e.g., Stahl et al. 2018).

This detection of *T. japonicus* in Canada occurred while a petition for the release of this biological control agent was under review by the Canadian Food Inspection Agency (CFIA), the national regulatory authority in Canada. We are not aware of any historical cases where this has occurred, in Canada or elsewhere, and its implications for the prospects of intentionally importing and releasing *T. japonicus* in Canada remain to be seen. It is important to note that because *T. japonicus* has been detected only at a single site in one year, we cannot yet definitively conclude that this species is established in Canada. However, given the relative proximity (<400km) of the closest known established populations in Washington State (Milnes et al. 2016, stopbmsb.org), it is plausible that this detection is indicative of a range expansion of adventive

Table 2. Collecting unit identifiers and institutions where voucher specimens are deposited.

Species	Collecting Unit Identifier	Institution
Trissolcus cosmopeplae	FSCA 00033197–FSCA 00033201	Canadian National Collection of Insects
	FSCA 00033202–FSCA 00033206	Florida State Collection of Arthropods
Trissolcus euschisti	FSCA 00033177–FSCA 00033181	Canadian National Collection of Insects
	FSCA 00033182–FSCA 00033186	Florida State Collection of Arthropods
Trissolcus japonicus	FSCA 00033110–FSCA 00033111	Canadian National Collection of Insects
	FSCA 00033107–FSCA 00033109	Florida State Collection of Arthropods
Telenomus podisi	FSCA 00033187–FSCA 00033191	Canadian National Collection of Insects
	FSCA 00033192–FSCA 00033196	Florida State Collection of Arthropods

T. japonicus populations, and that the parasitoid is in the early phases of establishment. Very low parasitism levels, as we observed here, were also characteristic of the initial detections in other areas where adventive *T. japonicus* populations have since been confirmed and are spreading (Talamas et al. 2015a, Milnes et al. 2016). In addition, conservative climate suitability modeling has predicted that several areas of southern Canada, including British Columbia, are suitable for *T. japonicus* establishment, survival, and reproduction (Avila and Charles 2018). Continuing field surveys and extensive phylogeographic analyses using microsatellite DNA markers are underway to track the establishment and biological control impact of *T. japonicus* in Canada, and to reconstruct potential pathways of introduction.

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The attraction of *Tremex apicalis* (Hymenoptera, Siricidae, Tremecinae) and its parasitoid *Ibalia japonica* (Hymenoptera, Ibaliidae) to the fungus *Cerrena unicolor*

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Abstract

Woodwasps (Hymenoptera: Siricidae) are saproxylic insects and a common forest pest. Siricid woodwasps are classified into two subfamilies: Siricinae and Tremecinae. All known symbiotic fungi of Siricinae are in the genus *Amylostereum* Boidin while some species of Tremecinae have been observed to have a relationship with the fungus *Cerrena unicolor* (Bull.) Murrill. Previous studies about the host searching behavior of woodwasps and their parasitoids have focused primarily on the subfamily Siricinae.

We analyzed the role of *C. unicolor* volatiles on the host searching behavior of *Tremex apicalis* Matsumura (Hymenoptera: Siricidae: Tremecinae) and its parasitoid *Ibalia* (*Tremibalia*) *japonica* Matsumura

(Hymenoptera: Ibaliidae). The results of an olfactory response experiment indicated that the females of *T. apicalis* and its parasitoid find their respective hosts using volatiles from *C. unicolor*. Using DNA barcode, we identified basidiocarps on the trees infested with *T. apicalis*. The basidiocarps were all white-rot fungi that cause sapwood decay, including *C. unicolor*. Two additional species that we identified belonged to genera closely related to *C. unicolor*.

Woodwasp species are known to carry symbiotic fungi in a pair of specialized sacs called mycangia. Notably we found that mycangia-like structures were absent in the abdomens of *T. apicalis* females. To the best of our knowledge, *Xeris spectrum* (Linnaeus) (Hymenoptera: Siricidae) is the only reported example of woodwasp species that do not contain symbiotic fungi in their bodies.

Our results suggested that: (1) *T. apicalis* females search for host wood that is already infected with sapwood decaying fungus using volatile compounds; (2) *T. apicalis'* female parasitoid also uses volatile compounds from fungus to locate wood that is infested with its potential host.

Keywords
Woodwasp, horntail, host searching, mycangia, saproxylic insect, *Tremibalia*, Y-tube

Introduction

Saproxylic insects, like woodwasps in the family Siricidae and their parasitoids, locate suitable host wood/host insect-infested wood in their environment to increase their reproductive success (Feldhaar and Schauer 2018; Hilszczański 2018; Ulyshen and Šobotník 2018). Siricidae consists of two subfamilies: Siricinae and Tremecinae. Siricinae infest coniferous trees and Tremecinae infest broad-leaved trees (Morgan 1968; Schiff et al. 2012). Like most saproxylic insects, woodwasps are unable to digest structural polysaccharides such as lignocellulosic compounds because they lack the necessary cellulolytic enzymes (Fukuda and Hijii 1997; Slippers et al. 2012).

Many woodwasp species carry fungal symbionts in their mycangia. Symbiotic fungi are transferred to host wood during oviposition and hatched larvae feed on the fungus-infected wood. To date, all known symbiotic fungi of Siricinae are in the genus *Amylostereum* Boidin. A limited example of Tremecinae woodwasps demonstrates that this subfamily is associated with fungus *Cerrena unicolor* (Bull.) Murrill (Stillwell 1967; Tabata and Abe 1995; Pazoutova and Srutka 2007; Kuramitsu et al. 2016).

Siricinae woodwasps use semiochemicals emitted by trees to locate host wood. For example, Siricinae species of *Sirex*, *Urocerus* and *Xeris* (Hymenoptera: Siricidae: Siricinae) are attracted to monoterpene hydrocarbons from host pine trees (Sato and Maeto 2006; Matsumoto and Sato 2007; Coyle et al. 2012; Matsumoto and Sato 2012; Erbilgin et al. 2017; Kües et al. 2018). European woodwasp, *Sirex noctilio* Fabricius, is similarly attracted to the volatiles from its fungal symbiont, *Amylostereum areolatum* (Chaillet ex Fr.) Boidin (Fernández Ajó et al. 2015).

Like *S. noctilio*, egg-larval or larval endoparasitoids of Siricinae, *Ibalia* (*Ibalia*) spp. (Hymenoptera: Ibaliidae) locate their hosts using the symbiotic fungi volatiles of their hosts (Madden 1968; Spradbery 1974; Martínez et al. 2006; Jofré et al. 2016; Kües et al. 2018; Table 1). For example, *Ibalia* (*I.*) *leucospoides*, a parasitoid of woodwasp *S. noctilio*, is attracted to the volatiles from their fungal symbiont (Martínez et al. 2006;

Table 1. Relationship of Ibaliid parasitoids and their host.

Ibaliid parasitoids	Host woodwasps	Host trees of host woodwasps	Symbiotic fungi of host woodwasps	References*
Ibalia (*Ibalia*) spp.	Siricinae	Coniferous trees	*Amylostereum* spp.	1, 2, 3, 4, 5
Ibalia (*Tremibalia*) spp.	Tremecinae	Broad-leaved trees	*Cerrena unicolor*	1, 2, 3, 4, 6, 7, 8

* 1 Nordlander and Liu (1994), 2 Choi et al. (2013), 3 Morgan (1968), 4 Schiff et al. (2012), 5 Tabata et al. (2012), 6 Stillwell (1967), 7 Pazoutova and Srutka (2007), 8 Tabata and Abe (1995).

Pietrantuono et al. 2012). Parasitoid wasps exhibit an antennal palpating and ovipositor probing response to discs of fungus impregnated agar (Spradbery 1974).

Information about the host wood/host insect searching behavior of Tremecinae and their parasitoids, *Ibalia* (*Tremibalia*) spp., is limited. The information available focuses on the attraction of *Tremex columba* (Linnaeus) (Hymenoptera: Siricidae: Tremecinae) to the wood volatile α/β-pinen (Coyle et al. 2012).

C. unicolor is the only known fungal symbiont of Tremecinae based on previous studies of *Tremex* spp. (Hymenoptera: Siricidae: Tremecinae) (Stillwell 1967; Tabata and Abe 1995; Pazoutova and Srutka 2007; Table 1). Basidiocarps which had the morphology of *C. unicolor* were present on wood infested by *Tremex apicalis* Matsumura (Kuramitsu et al. 2016). Whether or not *C. unicolor* is a symbiotic fungi of *T. apicalis* is not yet determined. To clarify the interaction between *T. apicalis* and *C. unicolor*: (1) we dissected the abdomens of *T. apicalis* females to isolate their mycangia, (2) analyzed the role of *C. unicolor* volatiles on *T. apicalis*' behavior, (3) identified basidiocarps on *T. apicalis* infested trees.

Also, we hypothesized that *Ibalia* (*T.*) *japonica* Matsumura, a parasitoid of *T. apicalis*, uses volatiles from *C. unicolor* to locate trees with potential host woodwasps. To test this hypothesis, we investigated the role of fungus volatiles on the host searching behavior of parasitoid *I. japonica* under laboratory conditions.

Materials and methods

Site of study and host trees

Our field survey and insect collection was conducted at Tsukuba Experimental Forest, University of Tsukuba (36°07'10"N; 140°05'50"E (DMS), ca. 25 m a.s.l.), Tsukuba, Ibaraki Prefecture, Honshu, Japan. We found four *T. apicalis* infested trees belonging to different families from 2016 to 2018 (Table 2). All *T. apicalis*, parasitoids and basidiocarps used for dissection, observation and behavioral experiments were collected from these four trees.

Observations on the abdominal organs of female *T. apicalis*

Twenty *T. apicalis* females were collected from the aforementioned trees (Table 2). *T. apicalis* females were killed using ethyl acetate. The dissection method was based on

Table 2. Host trees from which woodwasps, parasitoids and basidiocarps were collected.

Tree no.	Tree species	Diameter at breast height	Emergence of *T. apicalis* / *I. japonica*			Year of basidiocarp collection
			2016	2017	2018	
1*	*Swida macrophylla* (Wall.) (Cornales: Cornaceae)	40 cm	yes / yes	yes / no	no / no	2016
2	*Euptelea polyandra* Sieb. et Zucc. (Ranunculales: Eupteleaceae)	19 cm	no / no	yes / yes	yes / yes	2018
3	*Fraxinus spaethiana* Lingelsh (Scrophulariales: Oleaceae)	23 cm	–**	yes / yes	no / no	no fungi
4	*Magnolia liliiflora* Desr. (Magnoliales: Magnoliaceae)	44 cm	–**	yes / yes	yes / yes	2018

* This tree was the same tree that Kuramitsu et al. (2016) studied.
** These trees were not observed in 2016.

previous studies (Thomsen and Harding 2011; Li et al. 2015). The abdomen was removed using micro scissors under a stereomicroscope (Leica MZ12). The dorsal plates were removed from the abdomen. A female *Tremex longicollis* Konow, which is known to have the mycangia (Tabata and Abe 1995), was also dissected using the same method. The *T. longicollis* female was collected in Yokohama, Japan on November 12, 2017.

Extracting DNA from basidiocarps and PCR amplification of fungal ribosomal DNA

Collected basidiocarp surfaces were removed to avoid potential contamination. The samples were then ground into a fine powder using a pestle, mortar and liquid nitrogen. Fungal DNA was extracted from the powdered samples by using DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's instructions. Each extracted DNA sample was used as a PCR template to amplify an Internal Transcribed Spacer (ITS) region by using KOD FX Neo (Toyobo) following the manufacturer's instructions. Reactions were performed with 25 μl mixture containing KOD FX Neo, 2×buffer for KOD FX Neo, 2 μM dNTP, 0.3 μM of each primer. Primers used to amplify fungal ITS region were ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White et al. 1990). PCR reactions were performed in the following order: 94 °C for 2 minutes, followed by 40 cycles of 98 °C for 10 seconds, 50 °C for 30 seconds, and 68 °C for 90 seconds. PCR products around 650 baseparis were purified using a QIAquick Gel Extraction Kit (QIAGEN).

Sequencing and molecular identification

Sequence reactions were performed with BigDye Terminator v3.1 (Thermo Fisher Scientific) followed by purification using BigDye Xterminator (Thermo Fisher Scientific). The Sanger method was applied to determine DNA sequence of ITS region using

Applied Biosystems 3130 (Gene Research Center, University of Tsukuba) and commercial sequencing services (Macrogen Japan and Eurofins Genomics). Fungi species were identified using the UNITE database (http://unite.ut.ee) (Kõljalg et al. 2013). ITS sequences were deposited to Genbank (accession numbers are: *Cerrena unicolor*, MH645754; *Daedaleopsis confragosa*, MH645755; *Trametes hirsuta*, MH645756).

Fungal culture disc preparation

Potato Dextrose Agar (PDA) medium (Nissui) was prepared by following the manufacturer's protocol. *C. unicolor* was obtained from the Genebank Project (National Agricultural Research Organization). For behavioral experiments, fungal cultures were inoculated with a PDA medium in a 9-cm petri dish for two weeks at 25 °C.

Olfactory responses of *T. apicalis* and its parasitoid *I. japonica*

To obtain newly emerged *T. apicalis* and *I. japonica*, we cut down woodwasp infested *E. polyandra* (Table 2, tree no. 2) and *M. liliiflora* (Table 2, tree no. 4) on November 11 and October 4, 2017, respectively. The wood was stored outside until May 2018. Newly emerged *T. apicalis* and *I. japonica* were collected upon emergence. Live adults of each species was stored in plastic containers (16 × 28 × 17 cm). All insects were allowed to mate and feed on a solution of sugar and water (30% w/w) for 24–48 hours before behavioral experiments. The olfactory preference of *T. apicalis* and *I. japonica* was examined with a Y-tube bioassay (Fig. 1a) in the laboratory (25 °C ± 1.1 °C). Arms of a glass Y-tube olfactometer (common arm 20 cm, arms 20 cm, diameter 3 cm) were connected to glass jars (17 cm × 12.5 cm) respectively. Each glass jar was connected to an activated charcoal filter. The Y-tube olfactometer was connected to an electric vacuum pump (KNF, Germany) through a flow meter. The arm side of the Y-tube olfactometer was inclined upward at 5-degrees using a metal stand (Fig. 1b). The airflow from outside passed through the charcoal, the glass jar and into the olfactometer. The flow rate of the pump was set at 1.5 L/min.

A single male or female woodwasp or parasitoid was introduced into the starting point of the Y-tube olfactometer. Its behavior was observed for maximum of 15 minutes. We recorded the choice of the wasp if it reached the finish line. If it did not make a choice within 15 minutes, the trial was recorded as, "no choice." A total 43 *T. apicalis* (20 females and 23 males) and 57 *I. japonica* (25 females and 32 males) were tested. To avoid bias in the experimental setup, the positions of the two odors sources were exchanged after testing five woodwasps or parasitoids. Odor sources were renewed after testing five woodwasps or parasitoids. Using a binomial test, we determined the preference of both *T. apicalis* and *I. japonica* between volatiles from fungal disks and control disks.

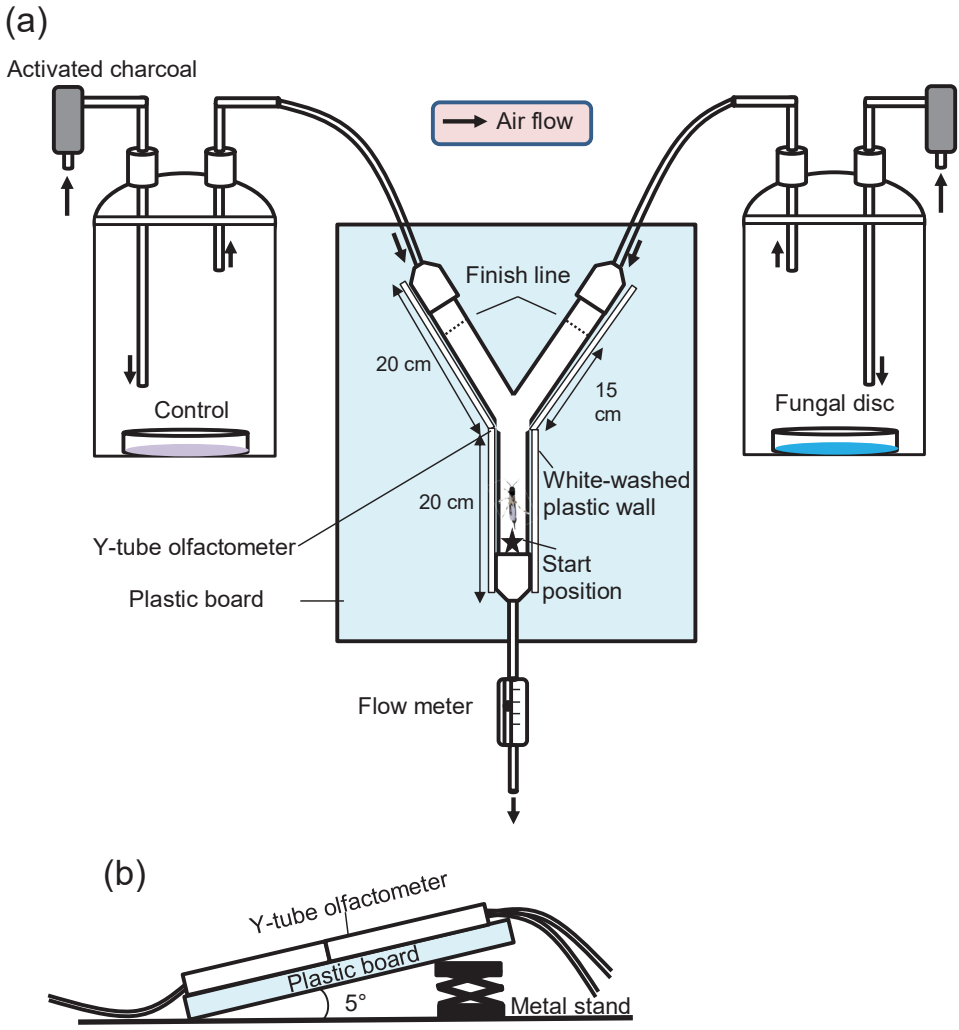

Figure 1. Set-up of the Y-tube olfactometer used to test *T. apicalis* and *I. japonica* attraction to volatiles from a fungal disc of *C. unicolor* in a top view (**a**) and in a side view (**b**) of the Y-tube olfactometer.

Results

Female *T. apicalis* abdominal organs

Abdomens of the female *T. apicalis* had ovaries that contained on average 96 eggs (Fig. 2a). A pair of mucus glands, which looked like large whitish sacs, was highly developed (Fig. 2a). A single mucus reservoir connected to the basement of the ovipositor was detected under the mucus glands (Fig 2b). The reservoir contained a sticky transparent fluid.

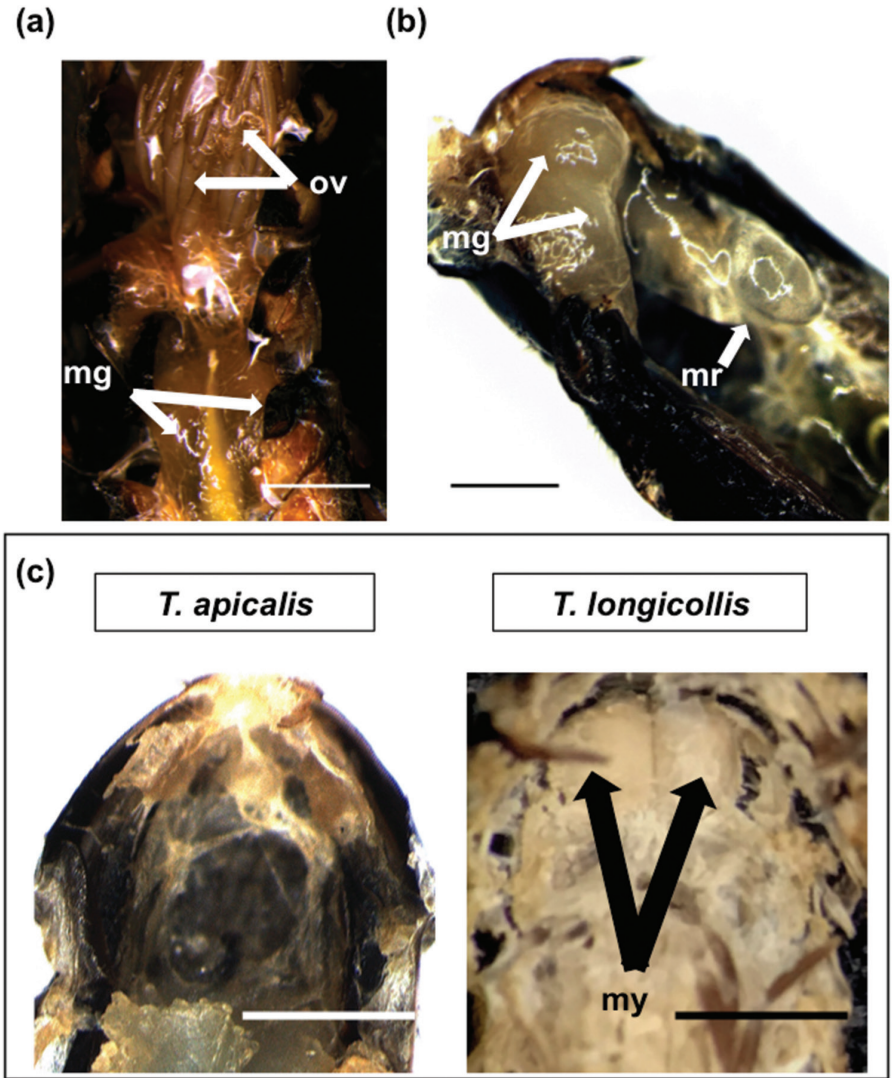

Figure 2. Abdominal organs of female woodwasps. **a** Ventral view of the dissected abdomen of *T. apicalis*. Typical internal organs, ovaries and mucus glands were easily detected. **b** Upper left diagonal view of the dissected abdomen of *T. apicalis*. A mucus reservoir was detected under the mucus glands. **c** Comparative view of dissected region of abdomens close to the ovipositor. In *T. longicollis*, the mycangia are recognized as greyish balls located close to the basement of ovipositor. There were no such sac-like structures in *T. apicalis*. Abbreviations; my: mycangia, mr: mucus reservoir, mg: mucus gland, ov: ovary. Scale bar: 2 mm.

We could not locate mycangia-like structures in the abdomen of the female *T. apicalis* while other anatomical features were nearly identical to other woodwasps. There were no visible sac-like structures located behind the base of the ovipositor where *T. longicollis* has clearly identifiable mycangia (Fig. 2c).

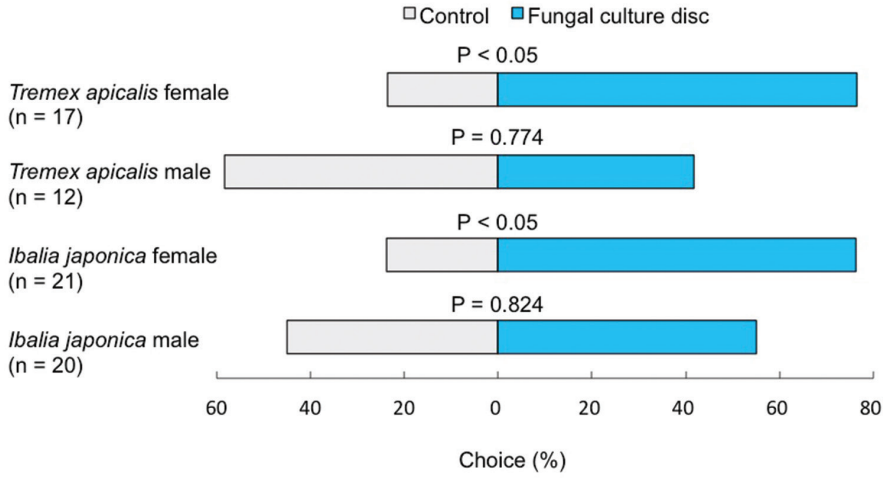

Figure 3. Percent choice by *T. apicalis* and *I. japonica* in arms of Y-tube olfactometer with the volatiles from PDA discs (control) vs. fungal culture discs.

Identification of basidiocarps on woodwasp infested trees

We performed DNA barcoding for basidiocarps found on *T. apicalis* infested trees using ITS region. The results showed that the ITS region from basidiocarps on: (1) *S. macrophylla* had a 99.67% match with *C. unicolor*; (2) *E. polyandra* had a 99.81% match to *Daedaleopsis confragosa* (Bolton) J.Schröt.; and (3) *M. liliiflora* was identical to *Trametes hirsuta* (Wulfen) Lloyd. All the fungus species we identified belong to the family Polyporaceae.

Olfactory responses of *T. apicalis* and its parasitoid *I. japonica*

In the Y-tube bioassay, 85.0% (n = 20) of *T. apicalis* females, 52.2% (n = 23) of its males, 84.0% (n = 25) of *I. japonica* females and 62.5 % (n = 32) of males chose between the volatiles from fungal and control disks. Females of *T. apicalis* woodwasps (76.5 %, n = 17, $P < 0.05$) and *I. japonica* (76.2%, n = 21, $P < 0.05$) preferred volatiles from the fungal disk to the control disks (Fig. 3). In contrast, males of both *T. apicalis* (41.7%, n = 12, $P = 0.77$) and *I. japonica* (55.0%, n = 20, $P = 0.82$) did not display a statistically significant preference for either fungal or control disks.

Discussion

T. apicalis females without mycangia feed opportunistically on rotten wood

Subfamily Siricinae has a close relationship with the genus *Amylostereum* (Schiff et al. 2012; Tabata et al. 2012). For subfamily Tremecinae, *C. unicolor* is only known

symbiotic fungus for many *Tremex* woodwasps (Stillwell 1967; Tabata and Abe 1995; Pazoutova and Srutka 2007). Basidiocarps on *T. apicalis* infested large-leaf dogwood tree (*Swida macrophylla*) were *C. unicolor*. Notably, we could not identify any apparent mycangia-like structures inside the female *T. apicalis* (Fig. 2).

Basidiocarps on other *T. apicalis* infested trees were members of the family Polyporaceae, inclusive of *C. unicolor*. These species are white-rot fungi that cause sap wood decay (Enebak and Blanchette 1989; Stajić et al. 2017; Račko et al. 2018). Our observations suggest that *T. apicalis*, which lacks identifiable mycangia, inhabits host wood that is already infected by wood decaying fungi.

Relationship between *C. unicolor* and *T. apicalis*

The female *T. apicalis'* preference for *C. unicolor* suggests that it uses volatiles from the fungus to locate suitable host wood. This strategy would be similar to female *Xeris spectrum* (Hymenoptera: Siricidae), whose mycangia is also absent and who use the odor from fungi *Amylostereum* to locate host wood (Fukada and Hijii 1997; Matsumoto and Sato 2012).

While mycangia carrying woodwasps *Sirex nitobei* Matsumura and *Urocerus japonicus* Smith form specific relationships with a particular *Amylostereum* fungus, mycangia-less *X. spectrum* can utilize more than one particular species. For example, the *Amylostereum* fungi species has a well-documented relationship with *Sirex nitobei* and *Urocerus japonicas* respectively (Fukuda and Hijii 1997).

It is possible that *T. apicalis* employs similar strategies that take advantage of their lack of mycongia. This may provide woodwasps without mycongia with an evolutionary advantage that increases their overall chances for reproduction and survival.

Parasitoid strategies

The Siricinae parasitoid *I. leucospoides* locates its host using volatile cues from symbiotic fungi (Martínez et al. 2006; Pietrantuono et al. 2012). In our study, we demonstrated that *I. japonica* is attracted to volatile compounds from *C. unicolor* even though this fungus is not a symbiont carried in *T. apicalis*. In contrast, *I. japonica*'s potential host, *T. longicollis*, has symbiotic relationship with *C. unicolor* (Tabata and Abe 1995; Watanabe et al. 2018). In order to locate potential hosts, *Ibalia* parasitoids seem to exploit olfactory cues not only from symbiotic fungi but also fungi species that live outside of the host.

To the best of our knowledge, this is the first report that connects white rot wood decaying fungus, Tremecinae woodwasp species and its parasitoid via volatile compounds.

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RESEARCH ARTICLE

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Review of the ant genus *Anochetus* Mayr, 1861 (Hymenoptera, Formicidae) from China, with revival of the valid status of *Anochetus gracilis*

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Abstract

Eight species of the ant genus *Anochetus* are recognized in China: *A. graeffei* Mayr, *A. lanyuensis* Leong et al., *A. longus* **sp. n.**, *A. madaraszi* Mayr, *A. mixtus* Radchenko, *A. medogensis* **sp. n.**, *A. risii* Forel, *A. subcoecus* Forel. *A. taiwaniensis* Terayama, 1989 is proposed as a junior synonym of *A. risii* Forel, 1900. *Anochetus gracilis* Karavaiev, 1925 is restored from synonymy of *A. risii*. A key based on the worker caste is provided for the Chinese species.

Keywords

Formicidae, *Anochetus*, new species, new record, China

Introduction

The genus *Anochetus* belongs to the tribe Ponerini, and constitutes as the sister group to *Odontomachus* (Bolton 2018, Schmidt 2013). A total of 113 extant species and 8 fossil ones have so far been known in the world (Bolton 2018), and the genus is especially

species-rich in the tropical and subtropical regions, except for a few species extending into temperate areas (Brown 1978). The first comprehensive revision of the genus was provided by Brown (1978), in which 12 new species were described, one genus-level and 38 species-level synonyms were solved, and 22 species groups were proposed. After his outstanding work, a series of studies further contributed to our understanding of the genus *Anochetus* and especially in the last decade. Eight fossil species are described from Dominican (Baroni Urbani 1980, De Andrade 1994, Mackay 1991). Twenty-eight species are described from five zoogeographic regions: eleven in the Malagasy Region (Fisher and Smith 2008, Shattuck and Slipinska 2012), four in the Neotropical Region (Lattke 1987, González-Campero and Elizalde 2008, Feitosa et al. 2012), three in the Palaearctic Region (Terayama 1996, Kugler and Ionescu 2007, Sharaf et al. 2017), and five from the Indo-Australian Region and Oriental Region (Terayama 1989, Nuril Aida and Idris 2011, Zettel 2012, Bharti and Wachkoo 2013, Marathe and Priyadarsanan 2016, Leong et al. 2018), respectively. Besides, *A. ruginotus* Stitz, 1925 was revived from synonymy of *A. graeffei* Mayr, 1870 by Zettel (2012), and *A. yunnanensis* Wang, 1993 was proposed as junior synonym with *A. mixtus* Radchenko, 1993 by Satria et al. (2017).

The Chinese fauna of the genus was treated by Forel (1900), Wheeler (1928, 1930), Terayama (1989, 2009), Radchenko (1993), Wang (1993), Wu and Wang (1995), Tang et al. (1995) , Xu et al. (1998, 1999), Xu (1999, 2002), Zhou (2001), Hua (2006), Lyu (2008), Zhang and Hou (2009), Satria et al. (2017) and Leong et al. (2018). Despite all taxonomic treatments mentioned above, only six species are confirmed to occur in China. However, the species diversity of *Anochetus* in China seems to be far from well-known if considering the complexity and heterogeneity of geography and climate throughout the country.

In this paper, two new species are described, *Anochetus longus* sp. n. and *A. medogensis* sp. n., while *A. madaraszi* is newly recorded from China. *Anochetus taiwaniensis* Terayama, 1989 is proposed as a junior synonym of *A. risii* Forel, 1900, whereas *A. gracilis* Karavaiev, 1925 is revived from synonymy of *A. risii*. A key to Chinese species based on the worker caste is also provided.

Materials and methods

The holotypes, paratypes and all non-type specimens examined are deposited or will be deposited in the following institutions.

GXNU Insect Collection, Guangxi Normal University, Guilin, Guangxi, China.
SWFU Insect Collection, Southwest Forestry University, Kunming, Yunnan Province, China.
IZCAS Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Images of the type specimens available on the AntWeb (http:// www.antweb.org) were examined. The specimens are examined with a Leica M205A stereomicroscope.

High-quality multifocused montage images were produced with Leica DFC 450 digital imaging system and Leica Application Suite V4.3 software. All measurements are given in millimeters. Standard measurements and indices are mostly as defined by Bolton (1975).

CI Cephalic index = HW × 100 / HL.
DPI Dorsal petiole index = DPW × 100 / PL.
DPW Maximum width of petiole in dorsal view.
ED Maximum diameter of eye.
HL Straight-line length of head in perfect full-face view, measured from the mid-point of the anterior clypeal margin to the midpoint of the posterior margin. In species where one or both of these margins are concave, the measurement is taken from the mid-point of a transverse line that spans the apices of the projecting portions.
HW Maximum width of head in full-face view, excluding the eyes.
LPI Lateral petiole index = PH × 100 / PL.
MSL Diagonal length of the mesosoma in lateral view, measured from the point at which the pronotum meets the cervical shield to the posterior basal angle of the metapleuron.
PH Height of petiole measured in lateral view from the apex of the ventral (subpetiolar) process vertically to a line intersecting the dorsalmost point of the node.
PL Length of petiole measured in lateral view from the anterior process to the posteriormost point of the tergite, where it surrounds the gastral articulation.
PW Maximum width of pronotum measured in dorsal view.
SI Scape index = SL × 100 / HW.
SL Straight-line length of the antennal scape, excluding the basal constriction or neck.
TL Total outstretched length of the individual, from the mandibular apex to the gastral apex.

Key to Chinese species of *Anochetus* based on the worker caste

1 Apical portion of mandible with three strong teeth; inner mandibular margin without denticles except preapical tooth (Figs 1B–3B, 4B, 10B) **2**
– Apical portion of mandible only with two distinct teeth; inner mandibular margin with some distinct denticles (Figs 5B–7B)..... **6**
2 Scape surpassing to posterior corner of head by about its 1/7 length (Fig. 3A); large species (TL ≥ 8.35); mesosoma comparatively thinner (Fig. 3D) .... ***A. longus* sp. n.**
– Scape not or just reaching to posterior corner of head (Figs 1A–2A, 4A, 10A); small species (TL ≤ 6.0); mesosoma comparatively stouter (Figs 1D–2D, 4D, 10D)..... **3**

3 The maximum diameter of eye narrower than the width of scape at it midlength (Fig. 10A); the junction between propodeal dorsum and declivity forming a pair of denticles laterally (Fig. 10D); pronotum smooth and shining (Fig. 10C) ..... ***A. subcoecus***

– The maximum diameter of eye broader than the width of scape at it midlength (Figs 1A–2A, 4A); the junction between propodeal dorsum and declivity rounded, not forming a pair of dents laterally (Figs 1D–2D, 4D); pronotum strongly rugose (Figs 1C–2C, 4C) **4**

4 Pronotal disc longitudinally striate (Fig. 2C); posterior half of pronotum to mesonotum straight (Fig. 2D); dorsal margin of petiolar node concave in anterior view ..... ***A. lanyuensis***

– Pronotal disc irregularly striate (Figs 1C, 4C); posterior one third of pronotum to mesonotum weakly convex or weakly concave at metanotal groove (Figs 1D, 4D); dorsal margin of petiolar node weakly convex in anterior view **5**

5 Head slightly longer than broad (CI 95); mesonotum transversely striate; petiole thick, twice as high as long (Fig. 4D) ..... ***A. madaraszi***

– Head distinctly longer than broad (CI 89); mesonotum transversely retirugose; petiole thin, 3.7 times as high as long (Fig. 1D) ..... ***A. graeffei***

6 Whole pronotum coarsely rugose (Fig. 6C) ..... ***A. mixtus***

– Middle portion of pronotum smooth and shining (Figs 5C, 7C) **7**

7 In full-face view, inner mandibular margin with about 11 denticles, preapical five denticles equal-sized; scapes with sparse suberect hairs; mesonotum with a strong transverse ridge anteriorly, situated higher than pronotum; metapleuron striate (Fig. 5) ..... ***A. medogensis* sp. n.**

– In full-face view, inner mandibular margin with 5–8 denticles, preapical denticle distinctly larger than the others; scapes with abundant decumbent hairs; mesonotum without anterior transverse ridge, metapleuron smooth and shining (Fig. 7) ..... ***A. risii***

Taxonomic account of Chinese species of *Anochetus*

Anochetus graeffei Mayr, 1870

Fig. 1

Anochetus graeffei Mayr, 1870: 961. [Lectotype worker images examined, AntWeb, CASENT0915887, photos by Harald Bruckner].

Anochetus punctiventris Mayr, 1879: 659. Synonymized by Wilson, 1959: 507. [Syntype worker images examined, AntWeb, CASENT0915888, photos by Harald Bruckner].

Anochetus rudis Emery, 1889: 499. Synonymized by Brown, 1978: 577. [Syntype worker images examined, AntWeb, CASENT0903978, photos by Zach Lieberman].

Anochetus punctiventris subsp. *oceanicus* Emery, 1897: 597. Synonymized by Wilson, 1959: 507. [Syntype worker images examined, AntWeb, CASENT0903977, photos by Zach Lieberman].

Non-type material examined. 5 workers, CHINA, Hainan, Qiongzhong County, Mt. Limushan, 19.17N, 109.71E, 681 m, 03.IV.2016, leg. Zhilin Chen, No. G160161; 4 workers, 1 male and 1 queen from the same colony, CHINA, Guangxi, Chongzuo City, Zuozhou Village, Pairutun, 22.56N, 107.41E, 222 m, 11.VI.2016, leg. Zhilin Chen, No. G160056; 2 workers, CHINA, Guangxi, Shangsi County, Hongqi Forest Farm, 03.VII.2001, leg. Shanyi Zhou, No. G010062.

Workers. TL 4.40–4.91, HL 1.09–1.13, HW 0.98–1.00, CI 86–89, SL 0.80–0.83, SI 82–84, ED 0.14–0.17, PW 0.58, MSL 1.35, PL 0.27–0.28, PH 0.46–0.48, DPW 0.27–0.29, LPI 57–58, DPI 99–100 (n = 5).

In full-face view head longer than broad, posterior margin strongly concave. Mandibles linear, gradually broadened apically; inner margin without denticles; apical portion with three distinct teeth. Antennae 12-segmented; scapes just reaching to posterior corners of head. Eyes moderate-size, maximum diameter equal to the width of mandible at its base.

In lateral view mesosoma stout, dorsal outline of pronotum convex and gradually sloping anteriorly. Promesonotal suture indistinct dorsally and laterally. Dorsal outline of mesonotum and propodeum almost straight, posterolateral corners of propodeum forming obtuse triangle. Metanotal groove broadly impressed. Petiole thin, distinctly higher than long, narrowing dorsally, anterior margin straight, posterior margin distinctly convex; subpetiolar process developed, anterior margin straight, posteroventral margin rounded. Dorsal margin of petiole weakly convex in anterior view.

Frons and anterior part of vertex longitudinally striate; remainder of head smooth and shining. Frontal lobes weakly striate. Mesosoma irregularly rugose, rugae on pronotal disc inverted U-shaped rugae, metapleuron rugose, sides and declivity of propodeum transversely rugose. Petiolar node smooth and shiny except basal area faintly striate. Gaster smooth and shining, except distinctly punctate first gastral tergite.

Dorsum of body with abundant suberect to subdecumbent hairs and decumbent pubescence, hairs on cephalic dorsum sparse; scapes and tibiae with dense decumbent pubescence. Body reddish brown to brown; antennae, legs and petiole yellowish brown.

Recognition. The species is similar to *A. lanyuensis* Leong et al., 2018, *A. validus* Bharti et Wachkoo, 2013 and *A. victoriae* Shattuck et Slipinska, 2012, but well separated from them by the following characters: dorsal outline of pronotum gradually sloping anteriorly, not forming a straight outline with mesonotum and propodeum; pronotal disc with dense inverted U-shaped rugae; scapes just reaching to posterior corner of head.

Distribution. Known from southern India east through SE Asia to Australia and onwards to the Cook Islands (Shattuck and Slipinska 2012). In China, the species is distributed in Fujian, Guangxi, Hainan and Yunnan.

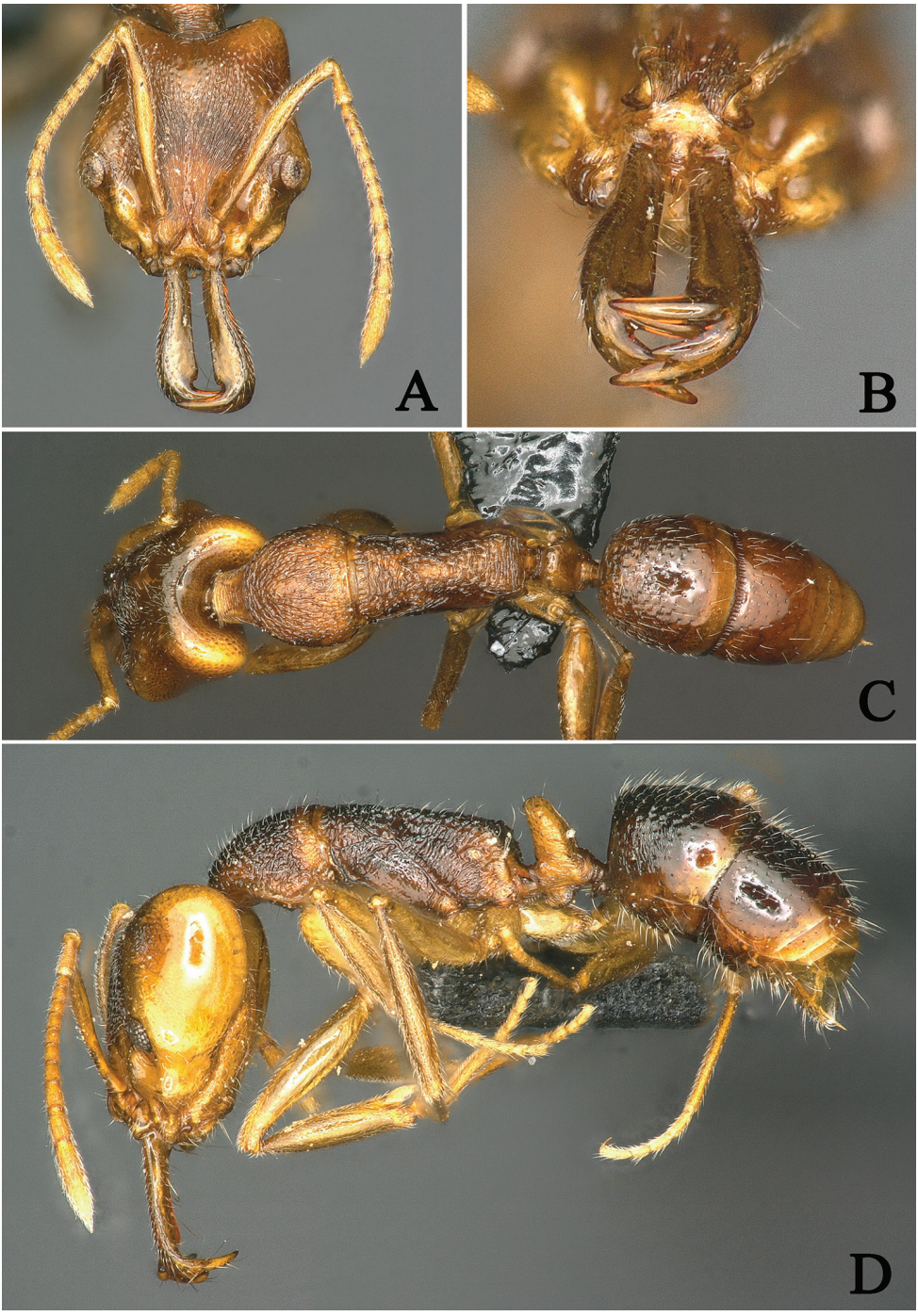

Figure 1. *Anochetus graeffei* worker (No. G160161). **A** head in full-face view **B** mandible in anterior view **C** body in dorsal view **D** body in lateral view.

***Anochetus lanyuensis* Leong, Tsai, Terayama, Shiao & Lin, 2018**
Fig. 2

Anochetus lanyuensis Leong, Tsai, Terayama, Shiao & Lin, 2018: 125. [Holotype worker images examined, cited from Leong et al., 2018, photos by Chi-Man Leong].

Workers. TL5.33–5.84, HL 1.23–1.32, HW 1.16–1.23, CI 93–98, SL 1.00–1.09, SI 82–89, ED 0.16–0.21, PW 0.65–0.70, MSL 1.54–1.72, PL 0.27–0.32, PH 0.52–0.61 (n=7) (After Leong et al., 2018).

In full-face view head longer than broad, posterior margin strongly concave. Mandibles linear, gradually broadened apically; inner margin without denticles; apical portion with three distinct teeth. Antennae 12-segmented; scapes not exceeding to posterior corners of head. Eyes moderate-size.

In lateral view mesosoma stout, dorsal outline of posterior half of pronotum and mesonotum straight. Posterolateral corners of propodeum obtusely angulate. Petiole thin, distinctly higher than long, with acute triangular tip; anterior margin straight, posterior margin distinct convex; subpetiolar process developed with subtriangular ventral margin. Dorsal margin of petiole weakly concave in anterior view.

Frons and anterior part of vertex longitudinally striate extending to posterior lobes of head, remainder of head smooth and shining. Clypeus smooth and shining. Pronotum longitudinally striate. Mesonotum and propodeum transversely striate. mesopleuron smooth and shining. Petiolar node smooth and shiny except basal area faintly striate. Gaster smooth and shining.

Dorsum of body with abundant erect to suberect hairs and abundant decumbent pubescence; scapes and tibiae with scattered subdecumbent hairs dense pubescence. Body reddish brown; antennae, legs and petiole yellowish brown.

Recognition. The species is similar to *A. ruginotus* Stitz, 1925, but can be distinguished from the latter by the following characters: eyes larger with 12 ommatidia along the maximum diameter; dorsal outline of mesosoma straight.

Distribution. Known only from the type locality – Orchid Island of Taiwan.

***Anochetus longus* sp. n.**
http://zoobank.org/ED24B483-30F4-4B90-AE97-359ABBE644C8
Fig. 3

Type material. Holotype worker, CHINA, Guangxi, Fanchenggang City, Fulong Village, 22.VI.2015, leg. Zhilin Chen, No. G150887; 3 paratype workers from the same colony. [holotype worker and 1 paratype worker are deposited in the Insect Collection, Guangxi Normal University, Guilin, China (GXNU); 1 paratype worker will be deposited in the Insect Collection, Southwest Forestry University, Kunming, Yunnan Province, China (SWFU); 1 paratype worker will be deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZCAS)].

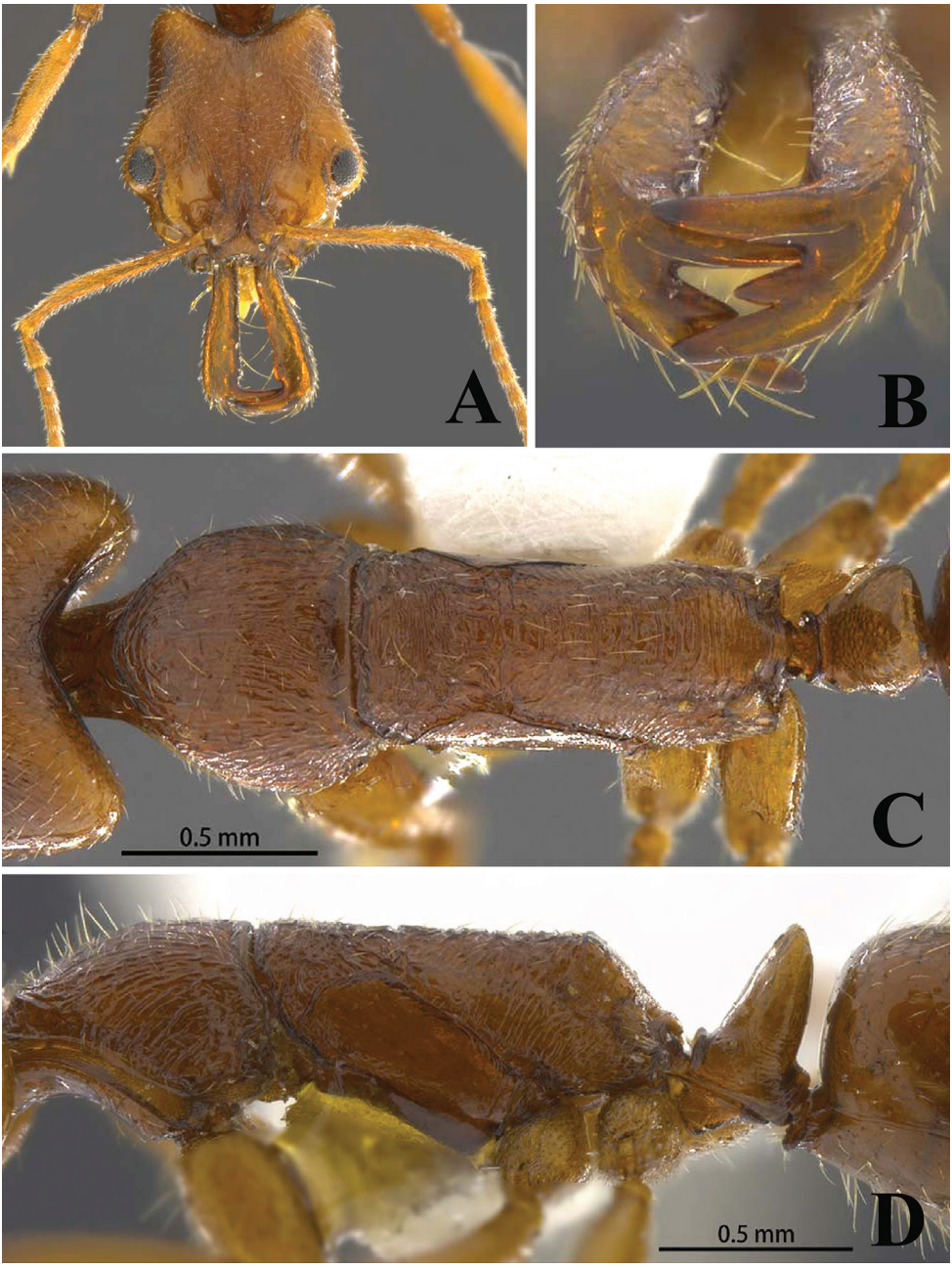

Figure 2. *Anochetus lanyuensis* holotype worker (Cited from Leong et al., 2018). **A** head in full-face view **B** mandible in anterior view **C** mesosoma in dorsal view **D** mesosoma in lateral view.

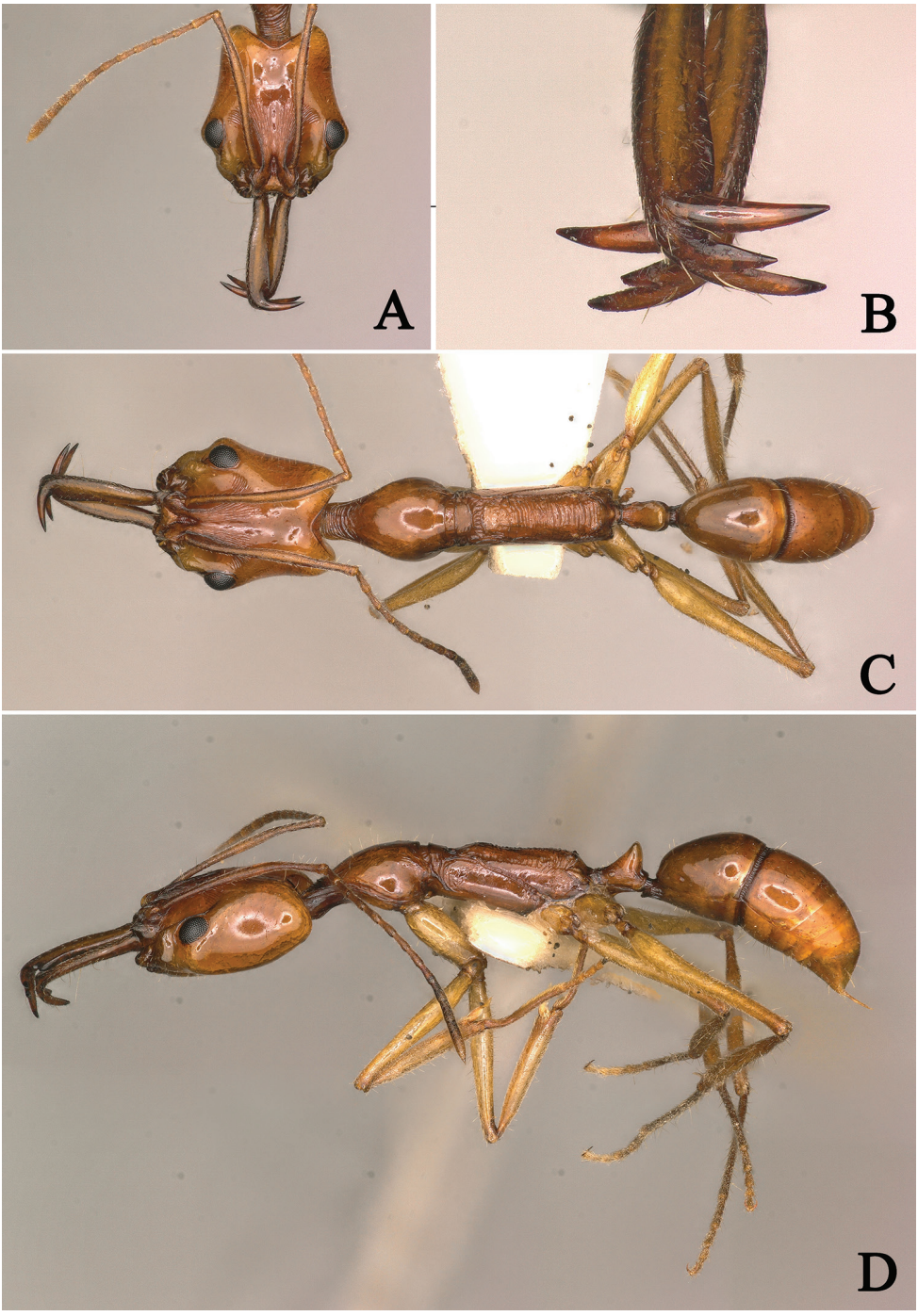

Figure 3. *Anochetus longus* holotype worker (No. G150887). **A** head in full-face view **B** mandible in anterodorsal view **C** body in dorsal view **D** body in lateral view.

Holotype worker. TL 8.56, HL 1.88, HW 1.59, CI 85, SL 1.80, SI 113, ED 0.33, PW 0.88, MSL 2.70, PL 0.54, PH 0.61, DPW 0.34, LPI 113, DPI 63.

In full-face view head longer than broad, posterior margin strongly concave. Mandibles linear, gradually broadened apically; inner margin without denticles, apical portion with three distinct teeth. Anterior margin of clypeus gently concave. Antennae 12-segmented; scapes long, surpassing to posterior corners of head by about 1/7 of its length. Maximum diameter of eye wider than apical width of mandible.

In lateral view mesosoma slender, pronotum weakly convex. Promesonotal suture narrowly weakly notched. Mesonotum short and sloping posteriorly. Mesonotum groove widely depressed. Propodeal dorsum weakly concave, posterolateral corners of propodeum forming a pair of small teeth. Petiole triangular and stout, anterior margin slightly concave, posterior margin convex, anterior margin longer than posterior margin, dorsal apex acutely angulate; subpetiolar process developed, subtriangular.

Frons with striae running in a fan shape, remainder of head smooth and shining. Mesosoma smooth and shining, pronotum with transverse striae on pronotal neck, dorsa of mesonotum and propodeum transversely striate. Petiolar node smooth and shiny except lower half area faintly striate. Gaster smooth and shining.

Dorsum of body with scattered erect hairs; mandibles with abundant subdecumbent pubescence; scapes and tibiae with sparse suberect hairs and dense decumbent pubescence. Body yellowish brown; legs brownish yellow.

Paratype workers. TL 8.35–8.59, HL 1.88–1.89, HW 1.59, CI 84–85, SL 1.80–1.83, SI 113–115, ED 0.33–0.35, PW 0.85–0.88, MSL 2.63–2.70, PL 0.53–0.54, PH 0.59–0.61, DPW 0.34–0.36, LPI 110–113, DPI 63–65 (n = 3). As holotype.

Recognition. The new species is similar to *A. rufus* (Jerdon, 1851), but well separated from the latter by the following characters: sides of pronotum smooth and shining; metanotal groove widely depressed; junction of propodeal dorsum and declivity forming a pair of small lateral teeth; the constriction between AIII and AIV with short ridges.

The new species is also similar *A. agilis* Emery, 1901, but well separated from the latter by the following characters: mesonotum smooth and shining; junction of propodeal dorsum and declivity forming a pair of small lateral teeth; petiolar node narrowly pointed at apex; the constriction between AIII and AIV with short ridges; body yellowish brown.

Distribution. Known only from the type-locality Fangchenggang of Guangxi in China.

Anochetus madaraszi Mayr, 1897

Fig. 4

Anochetus madaraszi Mayr, 1897: 424. [Syntype worker images examined, AntWeb, CASENT0915893, photos by Anna Pal].

Non-type material examined. 3 workers, CHINA, Yunan, XishuangbannaPrefecture, Mengyang Town, Mengyang, 05.X.2016, leg. Chaotai Wei, No. G160654;

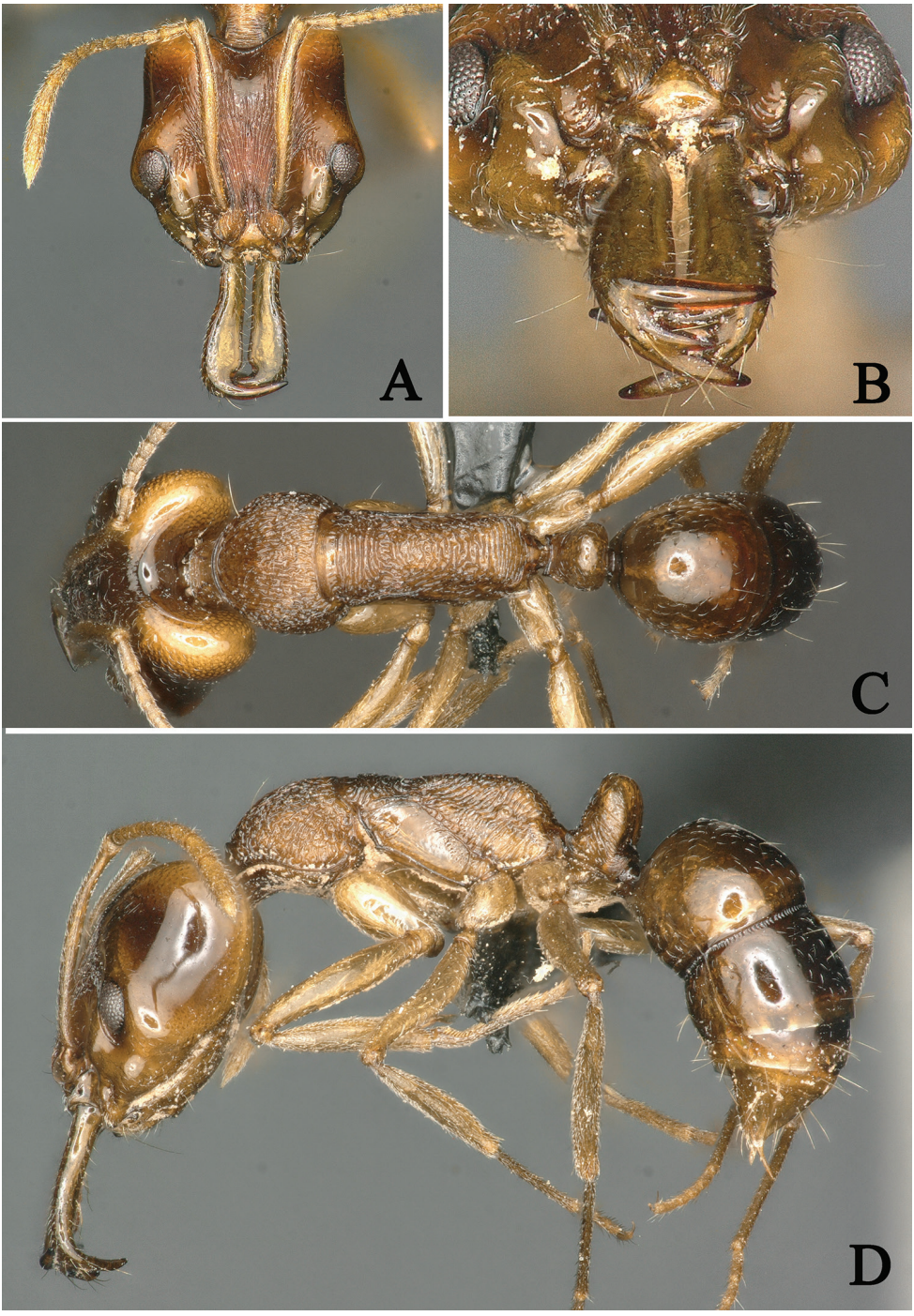

Figure 4. *Anochetus madaraszi* worker (No. G160654). **A** head in full-face view **B** mandible in anterior view **C** body in dorsal view **D** body in lateral view.

4 workers, CHINA, Guangxi, Longzhou Country, Nonggang, 13.VII.2013, leg. Liwei Liang, No. G130988.

Workers. TL 5.12–5.43, HL 1.35–1.39, HW 1.22–1.27, CI 90–92, SL 1.01–1.04, SI 82–84, ED 0.23–0.25, PW 0.69–0.73, MSL 1.53–1.57, PL 0.031–0.33, PH 0.57–0.58, DPW 0.32–0.33, LPI 174–176, DPI 101–103 (n = 5).

In full-face view head slightly longer than broad, posterior margin strongly concave. Mandibles linear, gradually broadened apically; inner margin without denticles; apical portion with three distinct teeth. Antennae 12-segmented; scapes just reaching to posterior corners of head. Eyes large, maximum diameter wider than basal width of scape.

In lateral view mesosoma stout; pronotum convex. Promesonotal suture weakly concave dorsally. Metanotal groove in lateral view faintly impressed. Dorsum of propodeum straight, posterodorsal coner rounded. Petiole thick, distinctly higher than long, narrowing dorsally, dorsal apex narrowly rounded; anterior margin straight, posterior margin distinctly convex; subpetiolar process developed, subtriangular.

Central dorsum of head and frontal lobes longitudinally striate, remainder of head smooth and shining. Pronotum and propodeal dorsum irregularly rugose. Mesonotum and propodeal declivity transversely striate. Propodeal sides obliquely striate. Mesopleuron smooth and shining. Petiole smooth and shining, basal area obliquely striate. Gaster smooth and shining.

Body dorsum with scattered suberect hairs and sparse decumbent pubescence; scapes and tibiae with dense decumbent pubescence. Body blackish brown to brown; antennae, legs yellowish brown.

Recognition. *A. madaraszi* is similar to *A. graeffei* Mayr, 1870, but can be separated from the latter by the following characters: head slight longer than broad (CI 90–92); mesonotum with transversely striate; petiolar node distinctly thick; first gastral tergite smooth and shining.

Distribution. Known from Bangladesh, India, Sri Lanka, China: Guangxi and Yunnan (**new record**).

***Anochetus medogensis* sp. n.**

http://zoobank.org/CED5A5B2-9900-4D7B-9055-3FC254A13F26

Fig. 5

Type material. Holotype worker, CHINA, Tibet, Modog Country, Beibeng Town, Beibeng Village, 29.25N, 95.19E, 840 m, 28.VIII.2016, leg. Zhilin Chen, No. G160581; 9 paratype workers from the same colony. [Holotype worker and 5 paratype worker are deposited in the Insect Collection, Guangxi Normal University, Guilin, China (GXNU); 2 paratype workers will be deposited in the Insect Collection, Southwest Forestry University, Kunming, Yunnan Province, China (SWFU); 2 paratype workers will be deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZCAS)].

Holotype worker. TL7.86, HL 1.89, HW 1.65, CI 87, SL 1.68, SI 102, ED 0.23, PW 0.92, MSL 2.52, PL 0.47, PH 0.67, DPW 0.36, LPI 143, DPI 76.

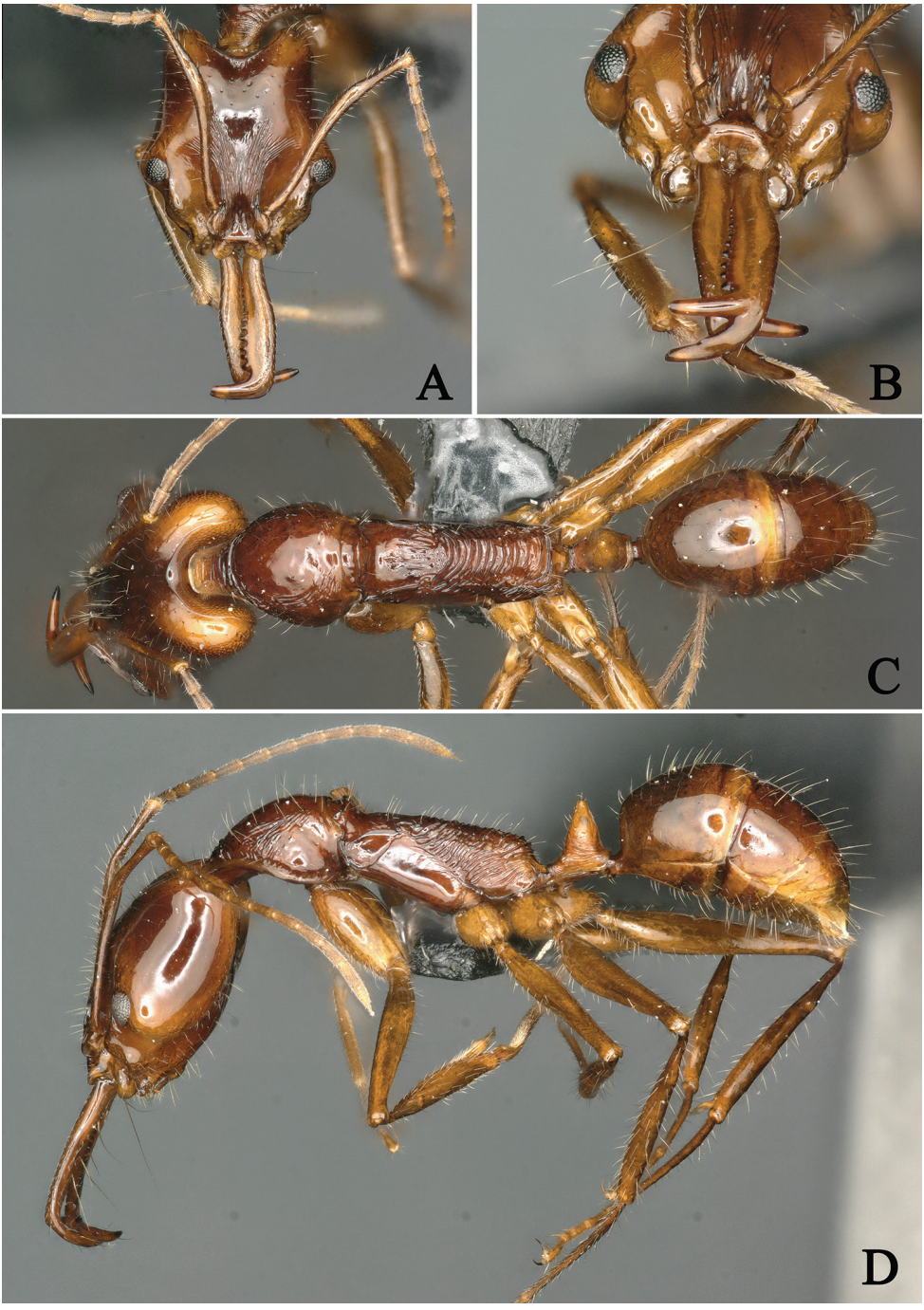

Figure 5. *Anochetus medogensis* holotype worker (No. G160654). **A** head in full-face view **B** mandible in anterior view **C** body in dorsal view **D** body in lateral view.

In full-face view head longer than broad, posterior margin strongly concave. Mandibles linear, gradually broadened apically; inner margin with 11 denticles; apical five denticles equally sized, remaining denticles gradually decreasing in size towards base; apical portion with three distinct teeth. Clypeus with concave anterior margin, lateral portion forming two round lobes above the base of mandibles. Antennae 12-segmented; scapes long, surpassing to posterior corners of head by 1/10 of its length. Eyes moderately large; maximum diameter of eye equal to the basal width of mandibles.

In lateral view mesosoma slender; pronotum moderately convex. Promesonotal suture narrowly notched. Mesonotum with a transverse ridge anteriorly. Metanotal groove deeply depressed. Dorsum of propodeum almost straight, sloping posteriorly; posterodorsal coner of propodeum narrowly rounded. Petiole cone-shaped, with a pointed dorsal apex, anterior margin slightly longer than posterior margin; subpetiolar process developed, directed posteroventrally as a distinct hook.

Frons longitudinally to obliquely striate posteriorly; remainder of head smooth and shining. Mesosoma smooth and shining, upper portion of lateral pronotum longitudinally striate, metathorax and propodeum obliquely rugose. Petiolar node smooth and shining, basal area faintly striate. Gaster smooth and shining.

Body dorsum with abundant erect to suberect hairs and sparse decumbent pubescence; scapes with sparse suberect hairs and abundant subdecumbent pubescence, tibiae with abundant suberect hairs and sparse decumbent pubescence. Body reddish brown; head, mandibles and antennae blackish brown; legs yellowish brown.

Paratype workers. TL7.35–7.89, HL 1.68–1.89, HW 1.44–1.65, CI 85–87, SL 1.49–1.68, SI 102–103, ED 0.19–0.23, PW 0.83–0.92, MSL 2.21–2.52, PL 0.42–0.47, PH 0.62–0.67, DPW 0.28–0.36, LPI 143–150, DPI 68–76. (n = 9). As holotype.

Recognition. The new species is similar to *A. princeps* Emery, 1884, but can be distinguished from the later by the following characters: scapes surpassing to posterior corners of head by 1/10 of its length; petiole cone-shaped, with a pointed dorsal apex.

Distribution. Known only from the type-locality Medog of China.

Anochetus mixtus Radchenko, 1993

Fig. 6

Anochetus mixtus Radchenko, 1993: 77. [Holotype and paratype worker images examined, AntWeb, CASENT0917205 & SEMUT20160608A, photos by Rijal Satria & Kate Martynova, respectively].

Anochetus yunnanensis Wang, M. 1993: 226. Synonymized by Satria, Viet & Eguchi, 2017: 4. [Holotype and paratype workers examined].

Non-type material examined. 20 workers, CHINA, Guangxi, Fangchenggang City, Mt. Shiwandashan, 21.81N, 107.95E, 450 m, 24.VI.2014, leg. Zhilin Chen, No. G140095; 10 workers and 1 male, CHINA, Hainan, Ledong Country, Jianfengling, 18.74N, 108.84E, 969 m, 09. IV.2016, leg. Zhilin Chen, No. G15049.

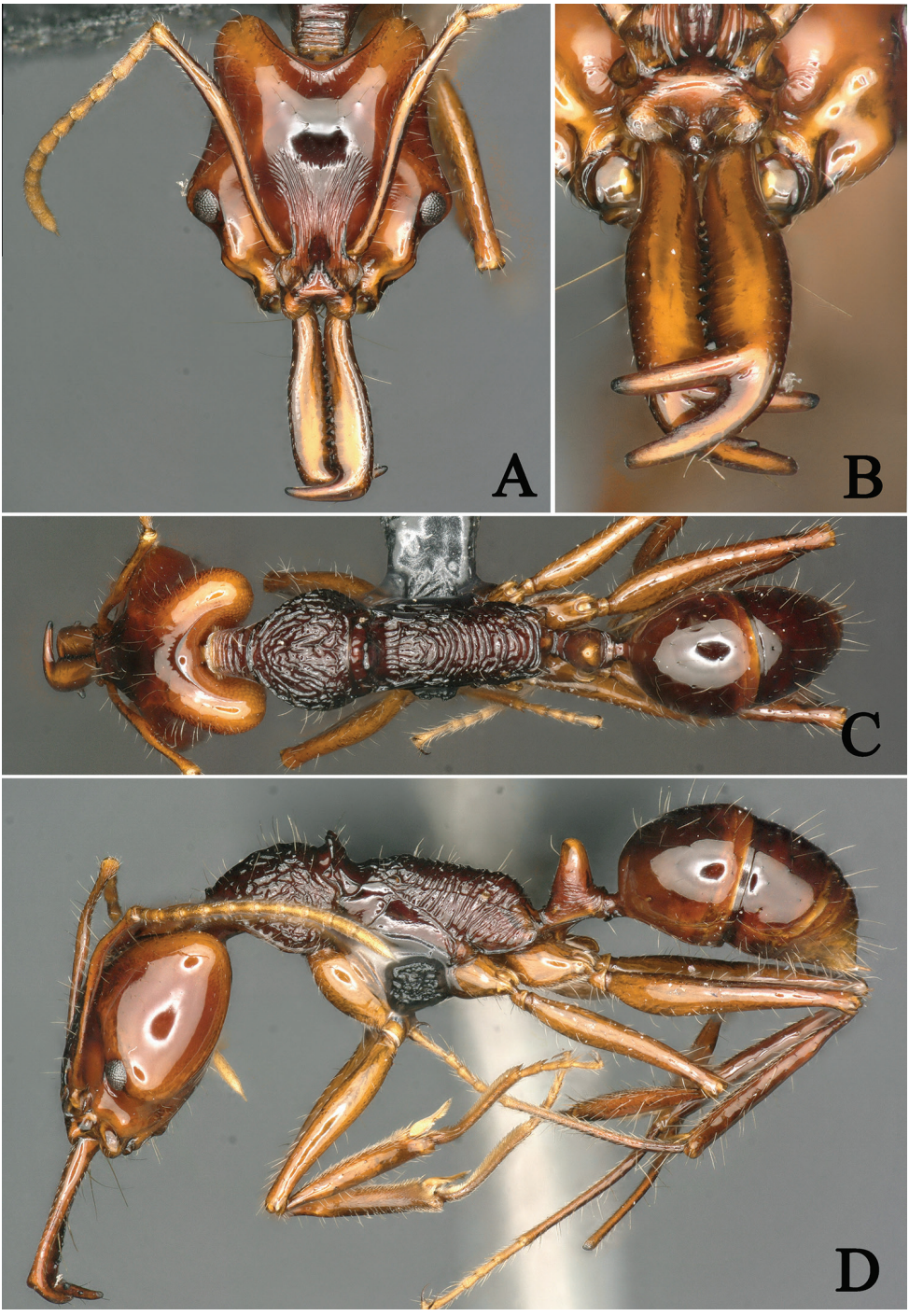

Figure 6. *Anochetus mixtus* worker (No. G140095). **A** head in full-face view **B** mandible in anterior view **C** body in dorsal view **D** body in lateral view.

Workers. TL 8.41–8.76, HL 2.11–2.14, HW 1.87–1.90, CI 89–91, SL 1.88–1.92, SI 100–101, ED 0.24–0.25, PW 1.05–1.06, MSL 2.68–2.73, PL 0.53, PH 0.83, DPW 0.40, LPI 157, DPI 75 (n=5).

In full-face view head longer than broad, posterior margin strongly concave. Mandibles linear, gradually broadened apically; inner margin with 9–10 denticles; apical portion with two distinct large teeth, sometimes with 1 small additional denticle on the midlength of the ventral tooth. Antennae 12-segmented, scapes just surpassing to posterior corners of head. Eyes moderately large, maximum diameter equal to the basal width of mandibles.

In lateral view mesosoma stout, pronotum moderately convex. Promesonotal suture narrowly nothed. Mesonotum with a high transverse ridge anteriorly. Metanotal groove deeply impressed. Dorsum of propodeum straight, sloping posteriorly; posterodorsal coner of propodeum rounded. Petiole roughly cone-shaped and weakly inclined posteriorly, with a blunt and rounded dorsal apex, anterior margin almost straight, posterior margin weak convex; subpetiolar process subtriangular, directed anteroventrally.

Frons longitudinally to obliquely striate posteriorly; frontal lobes weakly striate, remainder of head smooth and shining. Pronotum irregularly rugose. Dorsum of mesonotum with longitudinal rugae, mesopleuron smooth and shining. Metathorax and propodeum obliquely rugose. Petiole smooth and shining, basal area faintly striate. Gaster smooth and shining. Body dorsum with sparse suberect hairs and sparse decumbent pubescence; scapes with scattered suberect hairs and abundant subdecumbent pubescence, tibiae with sparse suberect hairs and sparse decumbent pubescence. Body reddish brown; antennae and legs yellowish brown.

Recognition. *A. mixtus* is similar to *A. rugosus* (Smith, 1857), but can be distinguished from the latter by the following characters: vertex smooth and shining; upper half of petiolar node smooth and shining, basal half weakly striate.

Distribution. Known from Vietnam and China: Yunnan, Guangxi and Hainan.

Anochetus risii Forel, 1900

Figs 7–9

Anochetus risii Forel, 1900: 60. [Syntype worker images examined, AntWeb, CASENT0907411, photos by Will Ericson].

Anochetus taiwaniensis Terayama, 1989: 26. [Two paratype worker images examined, AntWeb, CASENT0915167 & CASENT0902440, photos by Will Ericson]. **Syn. n.**

Non-type material examined. 1 worker, CHINA, Guangxi, Jinzhongshan Natural Reserve, 7.VIII.2014, leg. Zhilin Chen, No. G140447; 5 workers, CHINA, Guangxi, Beiliu City, Liuma Town, Sanhemaoping, 22. IV.2015, leg. Zhilin Chen; 1 worker, CHINA, Guangdong, Yingde City, Shimentai, 14.VIII.2000, leg. Jianhua Huang; 1 worker, CHINA, Guangdong, Nankunshan, 11.VII.2009, leg. Xinbing Yan; 2 workers, CHINA, Guangxi, Nonggang, 14.X.2007, leg. Chang Lin, No. G070219; 1 worker, CHINA, Hunan, Anhua Country, Hongyan, 14.VII.2004, leg. Jianhua Huang.

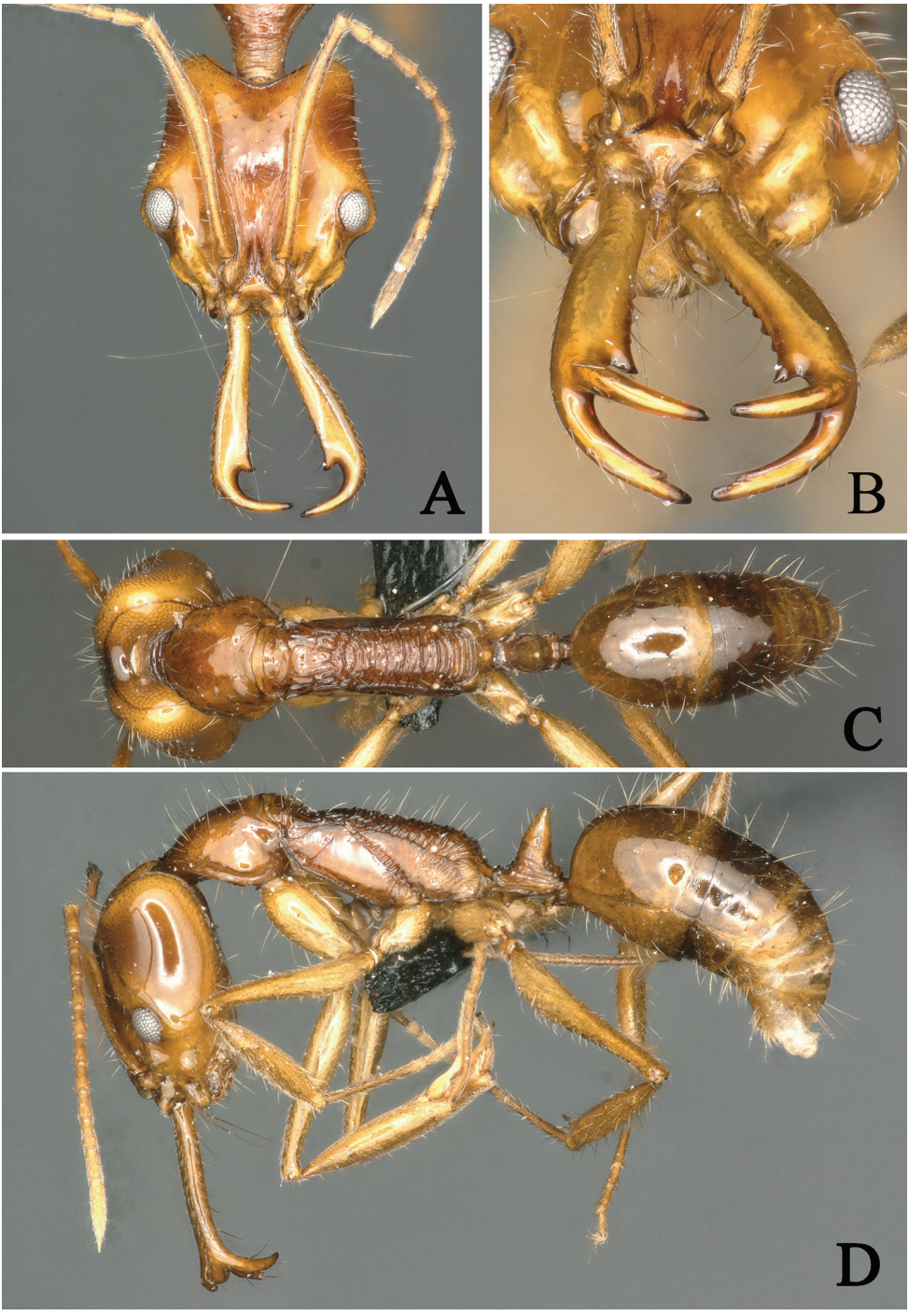

Figure 7. *Anochetus risii* worker (No. G140447). **A** head in full-face view **B** mandible in anterior view **C** body in dorsal view **D** body in lateral view.

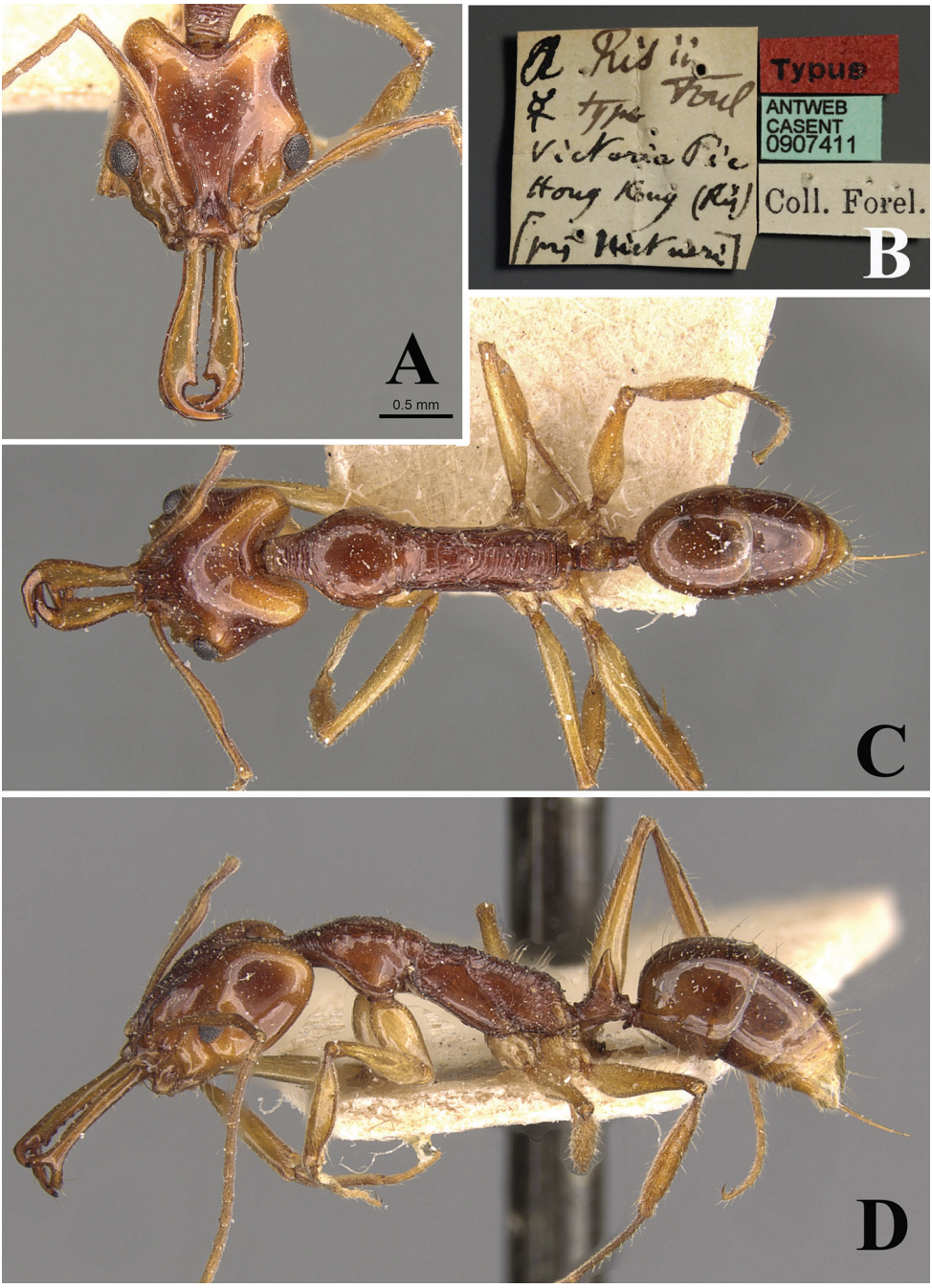

Figure 8. *Anochetus risii* worker (Syntype, AntWeb, CASENT0907411, photos by Will Ericson). **A** head in full-face view **B** labels **C** body in dorsal view **D** body in lateral view.

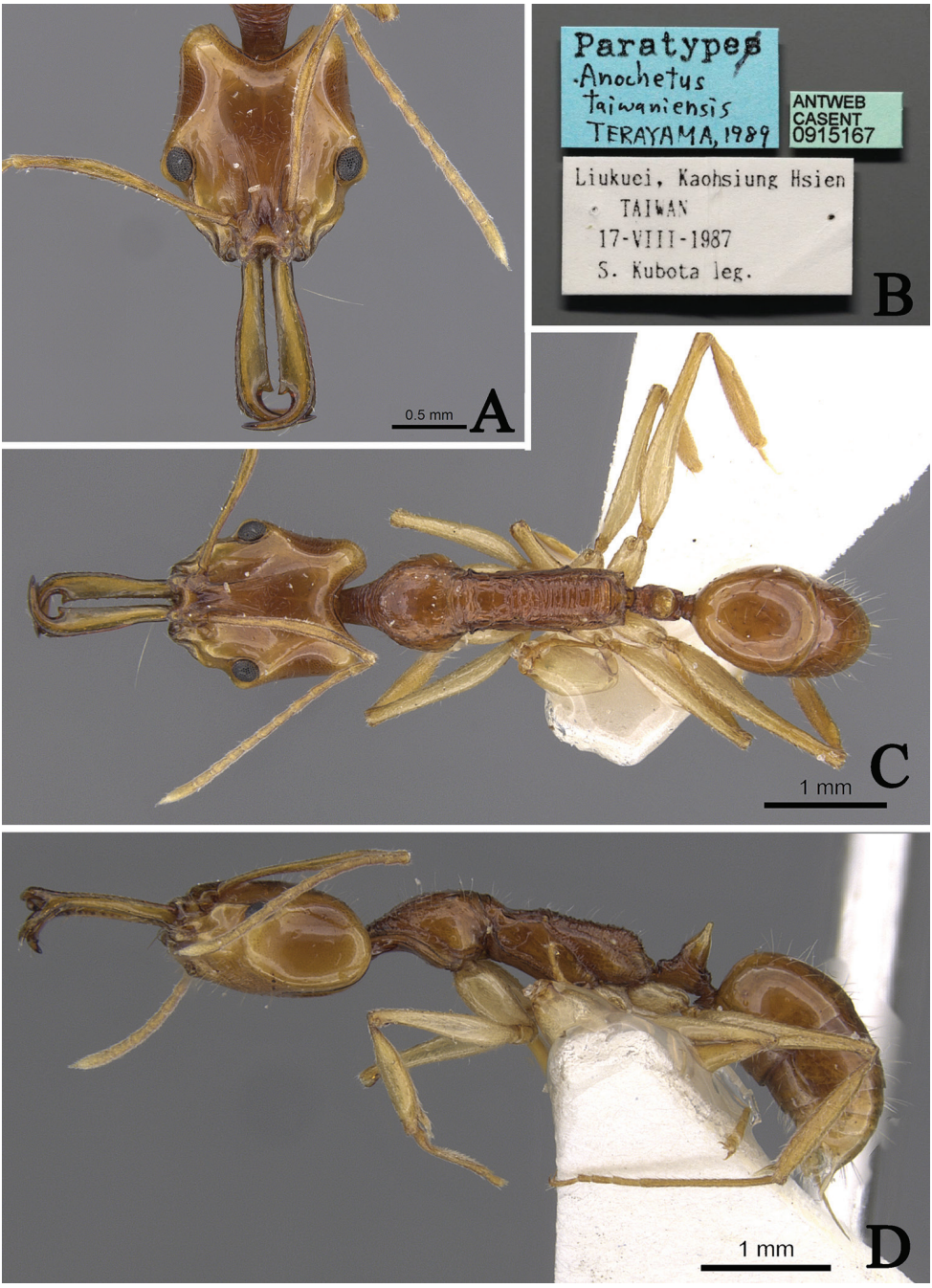

Figure 9. *Anochetus taiwaniensis*, syn. n. (Paratype, AntWeb, CASENT0915167, photos by Will Ericson). **A** head in full-face view **B** labels **C** body in dorsal view **D** body in lateral view.

Workers. TL 5.12–5.34, HL 1.67–1.69, HW 1.44–1.46, CI 85–87, SL 1.55–1.57, SI 106–108, ED 0.11–0.12, PW 0.80–0.82, MSL 2.33–2.36, PL 0.37–0.39, PH 0.74–0.76, DPW 0.30–0.32, LPI 202–207, DPI 83–85 (n = 5).

In full-face view head longer than broad, posterior margin strongly concave. Mandibles linear, gradually broadened apically; inner margin with several denticles, gradually decreasing in size towards base; apical portion with three distinct teeth. Antennae 12-segmented; scapes surpassing to posterior corners of head by about 1/5 of its length. Eyes large, maximum diameter of eye wider than the basal width of mandibles.

In lateral view mesosoma slender. Pronotum weakly convex. Promesonotal suture shallowly impressed. Mesonotum weakly convex, sloping posteriorly. Metanotal groove deeply concave. Dorsum of propodeum almost straight, posterodorsal corner bluntly angled. Petiole cone-shaped and slightly inclined posteriorly, with a pointed dorsal apex, anterior margin weakly convex, posterior margin almost straight; subpetiolar process triangular, directed posteroventrally.

Frons longitudinally to obliquely striate posteriorly; frontal lobes and clypeus weakly striate, remainder of head smooth and shining. Mesosoma smooth and shining, propodeum and lower part of metapleuron obliquely rugose. Sometimes sides of pronotum weakly longitudinal rugose. Petiole smooth and shining, basal area weakly striate. Gaster smooth and shining. Body dorsum with abundant erect to suberect hairs and dense decumbent pubescence; scapes and tibiae with scattered suberect hairs and dense decumbent pubescence. Body blackish brown; antennae and legs yellowish brown.

Recognition and discussion. After detailed comparison of type worker images of *A. risii* (CASENT0907411) and *A. taiwaniensis* (CASENT0902440 & CASENT0915167), we noticed that both species have completely consistent characters in head, mandibles, antennae, eyes, mesosoma, petiole and measurement range. Terayama (1989) pointed out that *A. taiwanensis* is distinguished from *A. risii* by "broader mandibular shafts and small denticles of the dorsal inner margin of the mandible in the former". However, we are unable to recognize these differences. The only difference between them is that *A. taiwaniensis* has the pronotum weakly rugose laterally. Consequently, we consider it safe to propose *A. taiwaniensis* as a junior synonym of *A. rissi* here.

Distribution. Known from Vietnam, Indonesia and Southern China.

Anochetus subcoecus Forel, 1912

Fig. 10

Anochetus subcoecus Forel, 1912. [Type worker images examined, AntWeb, FOCOL0345, photos by Christiana Klingenberg].

Non-type material examined. 5 workers, CHINA, Tibet, Medog Country, Medog Village, 29.32N, 95.34E, 1230 m, 27.VIII.2016, leg. Zhilin Chen, No. G160632; 1 worker, CHINA, Guangxi, Fangchenggang City, Mt. Shiwandashan, 21.18N, 107.95E, 450 m, 24.VI.2014, leg. Zhilin Chen, No. G140055.

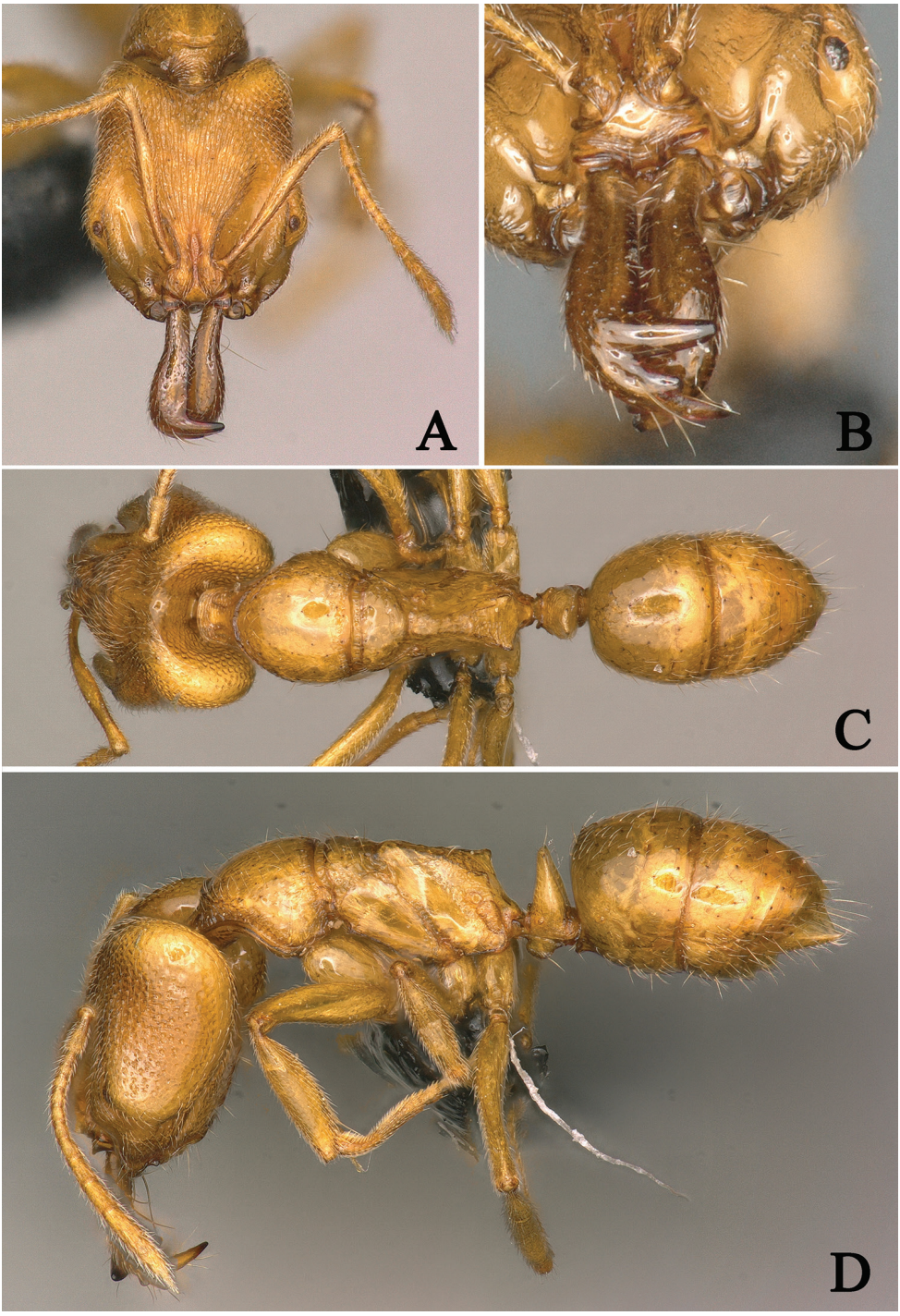

Figure 10. *Anochetus subcoecus* worker (No. G160632). **A** head in full-face view **B** mandible in anterior view **C** body in dorsal view **D** body in lateral view.

Workers. TL 4.90–5.34, HL 1.05–1.11, HW 0.94–0.97, CI 85–87, SL 0.80–0.83, SI 82–84, ED 0.08–0.09, PW 0.54–0.56, MSL 1.16–1.19, PL 0.20–0.21, PH 0.44–0.45, DPW 0.24–0.26, LPI 246–250, DPI 118–121 (n = 5).

In full-face view head longer than broad, posterior margin strongly concave. Mandibles linear, gradually broadened apically; inner margin without denticles; apical portion with three distinct teeth. Antennae 12-segmented; scapes short, not reaching to posterior corner of head. Eyes very small.

In lateral view mesosoma stout. Pronotum moderately convex. Promesonotal suture narrowly impressed. Dorsal margin of mesonotum nearly straight, weakly sloping posteriorly. Metanotal groove weakly concave. Dorsum of propodeum almost straight, posterodorsal corner with a pair of short blunt teeth. Petiole thin and erect, long triangle shaped, narrowing apically, anterior margin straight, posterior margin weakly convex, dorsal margin acute; subpetiolar process developed, nearly rectangular and angled ventrally.

Frons and vertex longitudinally striate, remainder of head punctate, frontal lobes weakly striate, clypeus and antennal scrobes smooth and shining. Mesosoma, petiole and gaster smooth and shining, propodeum weakly punctate.

Dorsum of head with scattered suberect hairs and dense subdecumbent pubescence; dorsa of mesosoma and gaster with abundant suberect hairs and abundant decumbent pubescence; scapes and tibiae with dense subdecumbent dense subdecumbent pubescence. Body yellowish brown.

Recognition. This species can be easily separated from other named species of this genus by its very small eyes and distinct propodeal teeth.

Distribution. Known from China (Taiwan, Guangxi, Yunnan, Tibet).

Revival of the valid status of *Anochetus gracilis*

***Anochetus gracilis* Karavaiev, 1925, stat. n.**
Fig. 11

Anochetus gracilis Karavaiev, 1925: 286. Junior synonym of *Anochetus risii*: Brown, 1978: 558.

Material examined. Two syntype worker images examined, AntWeb, CASENT0916847 & CASENT0915166, photos by Will Ericson.

Recognition and discussion. After observation of the syntype worker images of *A. risii* (Fig. 8) and *A. gracilis* (Fig. 11) from AntWeb, we find that there are clear differences between them: 1) inner margin of mandibles has no denticles in the worker of *A. gracilis*, but possesses several distinct denticles in the worker of *A. risii*; 2) the maximum diameter of eye is much larger than the maximum width of mandible in the worker of *A. gracilis*, but smaller than or just equal to maximum width of mandible in the worker of *A. risii*; 3) pronotal sides distinctly striate in the worker of *A. gracilis*,

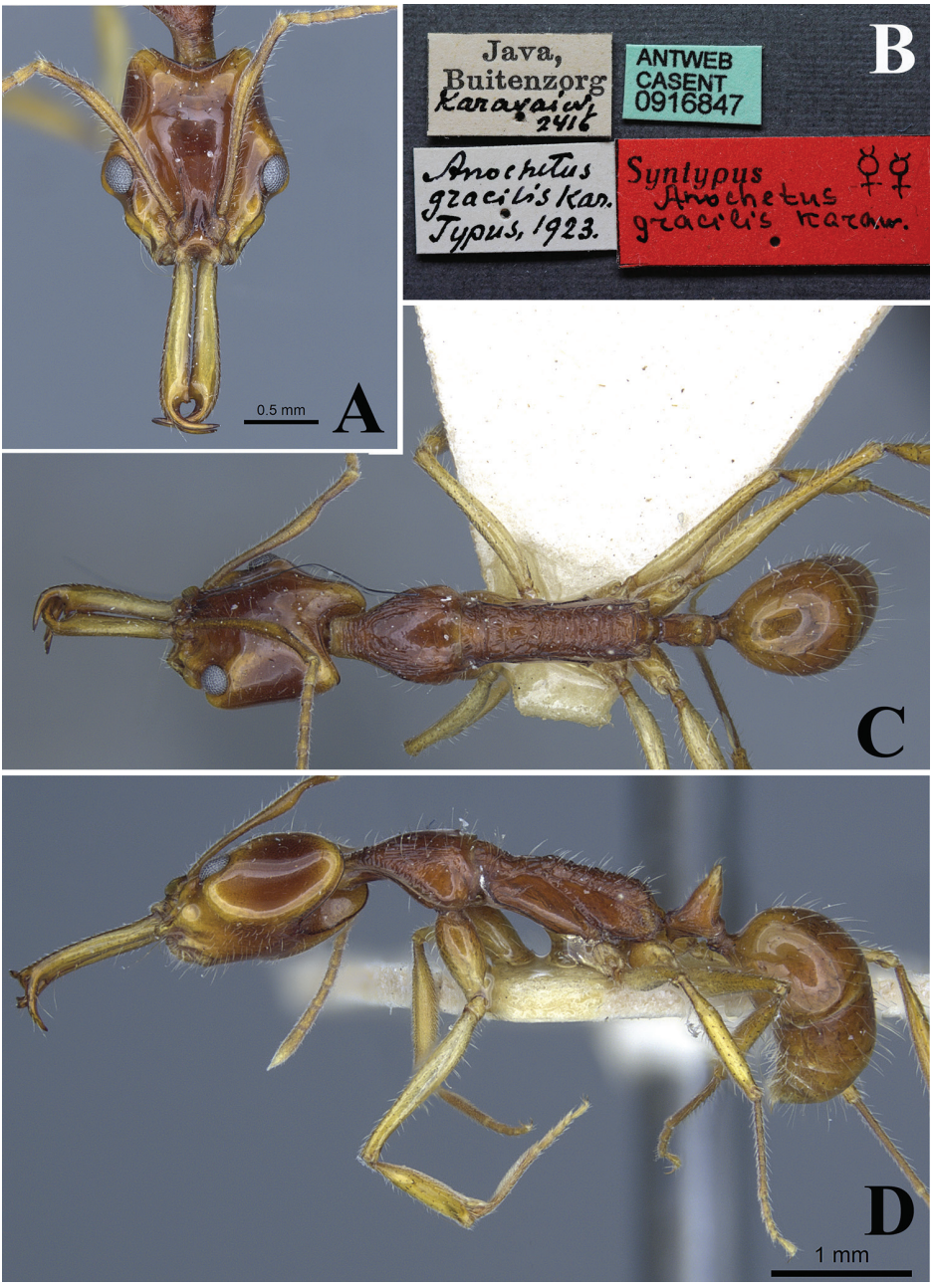

Figure 11. *Anochetus gracilis* worker (Syntype, AntWeb, CASENT0916847, photos by Will Kate Martynova). **A** head in full-face view **B** labels **C** body in dorsal view **D** body in lateral view.

but smooth and shining in the worker of *A. risii*. Therefore, the status of *A. gracilis* is restored from the synonymy of *A. risii* here.

Distribution. Known from Indonesia (Java).

Acknowledgements

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SHORT COMMUNICATION

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Bumble bees (Hymenoptera: Apidae: *Bombus terrestris*) collecting honeydew from the giant willow aphid (Hemiptera: Aphididae)

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Abstract

Only rarely have bumble bees (*Bombus*) been observed collecting honeydew from aphids (Aphididae) feeding on phloem sap. This behavior may be rare because the percentage of sugar in honeydew egested from aphids is generally well below the sugar concentration in floral nectars preferred by bumble bees. Nonetheless, in August 2018, near St. Buryan, Penzance, Cornwall, UK (56.0602°N; -5.6034°W) we observed large numbers of wild *Bombus terrestris* (Linnaeus) collecting honeydew from a colony of the giant willow aphid *Tuberolachnus salignus* Gmelin feeding on the stems of the willow *Salix alba*. Unlike aphid-tending ants, who glean fresh honeydew directly from the aphid anal opening, the bumble bees were collecting honeydew from leaf litter below the aphid colony. We hypothesized that honeydew collected from exposed ground surfaces was more concentrated due to evaporation under ambient conditions than that released directly from the anus (fresh honeydew). We thus monitored sugar concentrations of fresh honeydew and compared them with the concentrations of the crop contents of worker bumble bees foraging from the leaf litter. Our data show that the concentration of sugar in fresh honeydew was as much as 10% w/w lower than that collected from leaf surfaces, as measured from the crop contents of foragers. The unusually hot, dry weather in Cornwall may have enhanced evaporative concentration of honeydew while restricting floral nectar sources, thus favoring honeydew collection by *B. terrestris*, a generalist bumble bee forager.

Keywords

Tuberolachnus salignus, *Salix alba*, solute concentrations, foraging behavior

Introduction

Most bee species depend on carbohydrates, primarily sucrose, glucose and fructose (Baker and Baker 1983), and diverse amino acids (Baker and Baker 1986) collected from floral nectar to fuel foraging flights and to feed larval offspring. On occasion bumble bees (*Bombus*) have been observed collecting honeydew exudates of aphids (Aphididae) (Brian 1957; Morse 1982; Wagner and Cameron 1985; Batra 1993; Bishop 1994). Honeydew foraging is well known in ants, many of which have evolved mutualistic interactions with aphids (Way 1963; Letourneau and Choe 1987; Völkl et al. 1999; Offenberg 2001), and honey bees (Crane and Walker 1985), and has been recorded recently in diverse solitary bees (Meiners et al. 2017) collecting from scale insects (Coccidae), but has rarely been reported in bumble bees (Batra, 1993). This could be because some honeydews are well below the sugar concentrations preferred by nectar-foraging bumble bees, which is 40-65% w/w (Harder 1986; Bailes et al. 2018). Honeydew egested from the giant willow aphid *Tuberolachnus salignus* Gmelin, for instance, ranges from only 0-20% solutes w/w (Mittler 1958).

Nonetheless, in Aug 2018 we observed large numbers of *Bombus terrestris* gleaning honeydew from surfaces below an aphid colony feeding on willow (*Salix*) in Southwest Cornwall, UK. The rarity of this behavior in bumble bees suggested that abnormal environmental conditions might be involved as a result of an unusually dry summer. Drought and relatively high temperature conditions are known to reduce total sugar content in nectar – three times less in some flower species – leading to decreased flower visitation rates by bumble bees (Descamps et al. 2018). Increased ambient temperature and water stress could thus cause bumble bees to become opportunistic foragers, taking advantage of non-floral sugar sources.

Honeydew can be collected either directly from the anus of aphids (common in ants) or after falling onto surfaces below an aphid colony (Douglas 2006). When fallen droplets of honeydew are exposed to dry air, their solute concentration is expected to increase by evaporation (Douglas 2006; Corbet et al. 1979). Given the unusually dry conditions in Cornwall during the summer of 2018, we examined whether dehydrated honeydew, relative to the freshly egested product, provided a sugar-rich food source for bumble bees.

Methods

In early August 2018, during an extended period of unusually hot, dry weather, we heard loud buzzing in the vicinity of a willow shrub growing in a cultivated flower garden at Boskenna Farm, St. Buryan, Penzance, Cornwall, UK (56.0602° N; -5.6034° W). On Aug 6, from dawn to dusk (0500-2130 BST), we observed *B. terrestris* (Linnaeus) females and males collecting honeydew that rained down approximately 1.2 m onto leaf litter directly beneath a colony of the cosmopolitan (Blackman and Eastop 2006) giant willow aphid *T. salignus* Gmelin feeding on the stems of *Salix alba* Linnaeus (Fig. 1A). The flowers in the garden included *Verbena bonariensis* Linnaeus

Figure 1. A Giant willow aphids (*Tuberolachnus salignus*) on a branch of *Salix alba*; larger aphids range from 5.1–5.7 mm **B** *Bombus terrestris* collecting honeydew from leaf litter beneath several branches bearing the aphids **C** holding a plastic plate beneath the aphid colonies to collect honeydew droplets **D** collecting honeydew droplets from the plate using a 5 µl glass capillary tube **E** compressing a worker bumble bee to force regurgitation of crop contents onto the plate **F** using a refractometer to measure solute concentration of honeydew.

and other flowering species in low abundance. The area surrounding the garden was predominantly arable farmland with hedgerows. A second *S. alba* shrub in the garden was also infested with *T. salignus*. To determine whether bumble bees were gleaning honeydew that was more concentrated than the fresh exudate released by the aphids, we monitored solute concentrations of fresh T. *salignus* honeydew over the course of the day and compared them with the concentrations in the crops of worker bumble bees collecting honeydew from the leaf litter below the aphid colony.

At 4-hr intervals during the day (5:45 am, 9:45 am, 1:55 pm, 5:45 pm and 9:15 pm British Summer Time) we made spot counts of all bumble bees on the ground within a ~1.2 m^2 area underneath the aphid colony (Fig. 1B). During each of the 5 sampling periods, we collected fresh aphid honeydew onto a 12 cm x 15 cm unwettable plastic tray held 10-15 cm beneath the highest density patches of aphids (Fig. 1C). Droplets of honeydew falling onto the tray were quickly collected into 5 µl glass capillary tubes (Fig. 1D). We measured total solute concentration (%w/w) in the honeydew with a hand refractometer (Fig. 1F) modified for low volumes (Bellingham and Stanley, Tunbridge Wells, UK). The results are expressed as the equivalent percent sucrose, g solute per 100 g solution (Corbet 2003; Descamps et al. 2018). We were unable to get a clear concentration reading of the fresh honeydew for the first time interval (5:45 am) but obtained a reading 8 am, between the first and second recording intervals.

During each of the five time intervals (when bees were foraging), we collected a random sample of 5-6 live bees foraging on the honeydew using 15-cm long forceps. They were kept cool on ice and taken indoors for measurement of solute concentration in the crop. To measure crop solute concentration, we placed each worker bee onto a clean unwettable 12 x 15 cm plastic plate, holding the bee in place with a foam plunger (Fig. 1E). We applied light pressure to the abdomen, causing regurgitation of the crop contents, which we collected immediately into a 5 µl capillary tube. We used the same hand refractometer to measure solute concentration as that used to measure the fresh honeydew. We excluded any collected males from measurement because they do not regurgitate crop contents. Bees were taken back to the field site and released after sampling. For some time periods all the bees randomly collected were males and thus no crop content data are recorded.

Aphids were identified using Dixon and Thieme (2007), the bumble bees using Prys-Jones and Corbet (2011), and the willow using Meikle (1984). Latitude and longitude coordinates were obtained using the GPS map coordinates function in Google Maps on an iphone.

Results

The total number of *B. terrestris* (workers and males) gleaning honeydew from the surfaces of leaf litter changed through the day, with the largest number of bees arriving early, and numbers declining through the day (Fig. 2A); by 2115 h, when it was dark, no bees were seen. Of 54 bumble bees recorded, only one was collecting honeydew along a willow stem; the others collected from the leaf litter.

Solute concentrations in honeydew egested freshly by the aphids were consistently low, ranging from 12% in early morning to 18% in the afternoon and 16% by sunset (Fig. 2B). The solute concentrations in bumble bee crops were nearly always higher than the fresh honeydew solute concentrations, ranging from 16% to 28% (Fig. 2B).

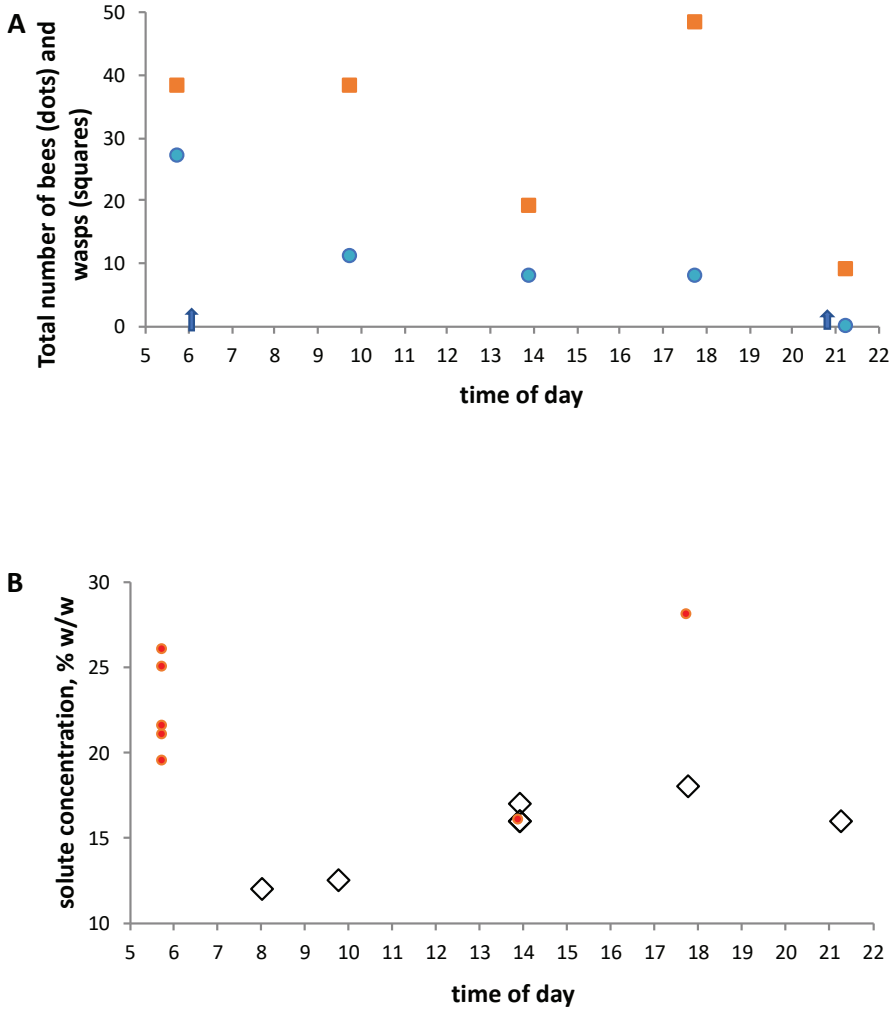

Figure 2. A Diurnal changes in total numbers of bees (blue dots) and wasps (red squares) **B** solute concentration in fresh honeydew (diamonds) and bumble bee crop contents (red dots). Arrows show times of sunrise and sunset.

Discussion

Why were so many *B. terrestris* workers collecting aphid honeydew on this occasion? Our finding that bumble bee crop contents were more concentrated than fresh honeydew indicates that bumble bees were not solely collecting the very dilute fresh honeydew. The abnormal environmental conditions at the time likely played a part in this rarely observed behavior. The summer of 2018 was the hottest in England since records

began, and Cornwall was under significant drought stress, with no significant rainfall from June to August. It is therefore likely that food and water resources from flowers were much reduced, affecting pollinator attractiveness. Bumble bees could enhance their food reward by collecting the more concentrated honeydew. The high temperatures and low relative humidity would cause rapid evaporative concentration of fallen honeydew droplets, perhaps raising the solute concentration to a level acceptable to bumble bees. Their crops may also have contained sugar solutions from other sources. It is notable that Batra's (1993) observations of bumble bees collecting honeydew were made in unusually hot, dry weather, and the egested honeydew had evaporated to dryness.

While we did not quantify the amount of honeydew falling onto the leaf litter, it was audible as it rained down onto the dry leaves in the early morning when fewer bees were buzzing. The honeydew droplets were between 3 and 5 µl, as estimated when drawing droplets into 5 µl capillary tubes. Multiple droplets per second fell from the aphids onto the plastic tray held beneath the colony. As the aphids continued to feed and release honeydew from dawn to dusk, they provided a reward bonanza for the bumble bees at this time.

Global climate change leading to drought and temperature stress has led to multiple reports that wildflowers important to bees experience reduced nectar production with lower sugar quantity (Waser and Price 2016; Descamps et al. 2018; Phillips et al. 2018); heat- and water-stressed flowers are visited less frequently as food rewards are negatively impacted (Descamps et al 2018; Phillips et al. 2018). Under such environmental conditions, bumble bees can modify their foraging behavior to search for alternative sugar sources (Cartar 2004; Dreisig 2012; Fowler et al. 2016). Increased temperature and water stress could, therefore, cause bumble bees to become opportunistic foragers, taking advantage of non-floral sources of sugar, such as concentrated honeydew deposits. This may be a more general phenomenon in bees, as Meiners et al. (2018) found that 42 species of native bees in California become opportunistic foragers on scale insect secretions at times of low floral availability.

Bombus terrestris, a short tongued bumble bee, was the sole bumble bee species seen collecting honeydew at our site, even though several other species are common in the area, including the long-tongued *B. pascuorum* (Scopoli). This might be explained by the fact that *B. terrestris* (subgenus *Bombus*) is a generalist bumble bee, similar to other members of the subgenus *Bombus sensu stricto*, and preadapted to foraging on a wide array of food sources. In fact, two of only three published observations of bumble bees collecting aphid honeydew in North America (Morse 1982; Batra 1993; Wagner and Cameron 1985) pertain to *B. terricola* Kirby (subgenus *Bombus*). Species of this subgenus appear predisposed to search widely for diverse food sources (Walther-Hellwig and Frankl 2000), which may explain their ecological success across much of western Europe. Another possible reason for the concentration of *B. terrestris* at the site is that a *B. terrestris* colony was nesting in a stone retaining wall several m from the aphid infestation. The aphid colony could therefore be reached with little effort in terms of flight energy. We do not know the nesting origins of the individuals observed in our study.

No other bees, such as honey bees or solitary bees, collected honeydew at the site, nor did we see any ants, although it has been reported that *T. salignus* colonies in the UK are often tended by ants (Paul 1974; Sopow et al 2017). The wasp *Vespula germanica* (Fabricius) was, however, collecting honeydew in relatively large numbers at the site. They were 2-6 times more abundant than the bees (Fig. 2A) and tended to collect honeydew from the willow stems in the vicinity of the aphids, although some collected from the leaf litter. Wasps were actively foraging throughout the day, as well as at dawn and dusk, earlier and later than the bumble bees, suggesting they may perform better under low light conditions.

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RESEARCH ARTICLE

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Effect of season and behavioral activity on the hypopharyngeal glands of three honey bee *Apis mellifera* L. races under stressful climatic conditions of central Saudi Arabia

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Abstract

Honey production gains are needed to deal with high demand in Saudi Arabia. The honey bee races are facing stressful hot-arid weather conditions that can affect different aspects of physiology and behavior. The hypopharyngeal glands (HPGs) of honey bees have prominent roles in various social behaviors through their secretions. The measurement of acini size and lipofuscin accumulation indicates the changes in HPGs in response to different factors including weather and behavioral castes. This research aimed to reveal how natural harsh environment of summer and winter can shape the HPGs in foragers and nurses of an indigenous bee race (*Apis mellifera jemenitica* Ruttner) in comparison with two exotic bee races (*Apis mellifera carnica* Pollmann and *Apis mellifera ligustica* Spinola). This study presents new information of significant differences in the HPGs of two behavioral castes (nurses and foragers) of indigenous and exotic bee races under harsh natural environmental conditions. HPGs of foragers have significantly higher lipofuscin accumulation and smaller acini size than nurse bees in all tested races during summer and winter seasons. A strong inverse correlation was found between acini size and lipofuscin accumulation in each race in both seasons. Smaller acini size and lipofuscin accumulation were detected

in the HPGs of indigenous bees (foragers and nurses) than exotic bee races during both seasons. The acini size and lipofuscin accumulation were similar between exotic bee races but higher than that of the indigenous bee race.

Keywords
Acini, honey bee races, hypopharyngeal glands, lipofuscin

Introduction

Beekeeping has been practiced in the Arabian Peninsula for centuries. It is an important aspect in the agriculture sector of Saudi Arabia with an additional objective to increase the income of Saudi beekeepers (Alqarni et al. 2014). Three prominent bee races are commonly domesticated in Saudi Arabia. Approximately 70–80% of bee colonies are of indigenous bees and 20–30% of total colonies include exotic European bees races (Alqarni et al. 2011).

Apis mellifera jemenitica Ruttner (AMJ) is an indigenous bee race that is widely used for honey production throughout the region (Alqarni 2006; Ruttner 1976). This race is well adapted to the local harsh environmental conditions (Alqarni et al. 2011; Iqbal et al. 2018a). In Saudi Arabia, the summer temperature is extremely high and often exceeds 45 °C, and the humidity is very low; in winter, the temperature can fall below 0 °C. (Ali et al. 2017; Alqarni 2006; Alqarni et al. 2011; Iqbal et al. 2018b). *Apis mellifera carnica* Pollmann (AMC) and *Apis mellifera ligustica* Spinola (AML) are exotic bee races imported annually by the beekeepers to boost agricultural production (Alqarni et al. 2014). However, exotic races face many challenges when adapting to the local hot arid environment and have higher mortality rates during the summer than indigenous races (Alattal and AlGhamdi 2015).

Hypopharyngeal glands (HPGs) in honey bees are age-dependent paired glands that are only observed in the heads of worker bees and are associated with various social behaviors via different secretions (Liu et al. 2013; Ueno et al. 2015). Each gland consists of small oval bodies (acini) that are linked to axial or terminal secretory ducts. HPGs become fully developed in young workers (6–13 days old) with large functional secreting acini (Lass and Crailsheim 1996; Rahman et al. 2014). However, these glands degenerate when the bee initiates foraging behavior outside the colony (Robinson 1992).

The activity of the HPGs is mainly dependent on the acini size, which changes with age to express age-polytheism in honey bees (Deseyn and Billen 2005; Johnson 2010; Robinson 1992). These glands exhibit change in their activities from synthesizing major royal jelly proteins in younger nurse bees to synthesis of carbohydrate metabolizing enzymes α-glucosidase in forager bees (Kubo et al. 1996; Ueno et al. 2015). Royal jelly is a protein-based food fed to larvae and to produce healthy queens (Knecht and Kaatz 1990; Ohashi et al. 1999). These glands are also vulnerable to various stresses, such as starvation, heat, and Varroa infestation, which may result in the reduction and degeneration of the glands (Khalil 1992; Yousef et al. 2014).

Lipofuscin are undegradable lipid-protein granules enclosed in a single membrane in the HPGs, and are considered a marker of cellular aging, functional decline and mortality (Fonseca et al. 2005; Hsu and Chan 2013). Lipofuscin granules are linked to chronological age and it increases in the body as the organism ages (Munch et al. 2013b). In honey bees, lipofuscin is a good indicator for studying physiological age (Hsieh and Hsu 2011).

Beekeepers in Saudi Arabia are spending large amounts of money for importing exotic bee races because the population of indigenous bees is too scarce and honey production is too low to meet the increasing demands of the local market (Al-Ghamdi et al. 2017). Nevertheless, the exotic bee races face high mortality due to harsh climatic conditions of central Saudi Arabia. The population of indigenous bees (AMJ) is successful and more heat tolerant than the exotic bee races (AMC and AML) (Abou-Shaara et al. 2012; Alqarni et al. 2014). Therefore, the comparison of HPGs among different bee races could help us to know that which race could have less aging in summer and winter and how it can be improved to avoid early or high mortality. Moreover, this study will aid in future investigations for improvement of HPGs potentially with food in heat tolerant indigenous bee race to get high brood rearing and even royal production in natural harsh climatic conditions of Saudi Arabia. This study unveils the status of HPGs in indigenous bee race in comparison with exotic bee races. We aimed to find out the differential changes in the HPGs (acini size and lipofuscin accumulation) of nurse and forager bees of different bee races during natural summer and winter harsh environment of Saudi Arabia.

Materials and methods

Bee colonies and sample collection

The bee colonies of the indigenous race *A. m. jemenitica* (AMJ) and exotic races *A. m. carnica* (AMC) and *A. m. ligustica* (AML) were maintained at an agricultural farm of King Saud University (KSU), Riyadh, Saudi Arabia (24.7296° N; 46.6101° E and 558 m altitude), during 2015. The indigenous bee race was obtained from reliable local sources. Pure queens of *A. m. carnica* and *A. m. ligustica* were imported from Egypt and Jordan, respectively. These imported queens were subsequently used to raise the colonies of the exotic bee races. One strong colony of each races was selected for the experiments. The bee samples were taken from the same colony during summer and winter seasons.

The selected colonies of each race were of equal estimated colony strengths containing 5 frame bees, 3 frame brood and 2 frame of food. The plenty of flora and flowers of different plants such as *Prosopis* spp., *Eucalyptus* spp., *Ocimum basilicum* L., *Brassica rapa* L., *Eruca sativa* Mill. and some wild flowers were available in the field during the experiments. The colonies were kept healthy without any chemical treatment.

Fifty newly emerged bees of each race from cells were marked with different paint colors of Uni-Paint® (yellow and white) to determine their ages at the time of collec-

tion. Direct marking was done to keep the new emerged bees in their natural environment, no cages were used. Nurse bees were collected at 11 days, whereas foragers were collected 25 days after emergence. The separate experiments were performed during the peak summer (later June: 27–40 °C, 9.5–10% relative humidity) and winter (December: 15–20 °C, 35–38% relative humidity) seasons. A thermo-hygrometer (HANNA, HI93640N, Europe) was used to record the meteorological factors.

Preparation of bee samples

The heads of ten marked nurse or forager bees were dissected with a sharp blade in the Melittology Research Lab, KSU. The HPGs were removed with fine forceps and fixed overnight in 4% formaldehyde in phosphate buffered saline (PBS, pH 7.5) at 4 °C per the protocol of Munch et al. (2013b). Then, the glands were washed three times in PBS and further incubated overnight at 40 °C with 30% glycerol in PBS. Before mounting, the glands were again incubated for 2 h at 40 °C with 50% glycerol in PBS. Slides were prepared from the samples in mounting medium (50% glycerol in PBS). The corners of the slides were locked with nail polish for long-term storage to avoid desiccation. The slides were also incubated for drying at 40 °C for one week.

Measurement of acini size

The morphological measurements and images of the acini size were recorded from mounted specimens using a 10× objective on a stereomicroscope (M165C, Leica, Germany) with a camera (DP71, Olympus). The maximum length (L) and width (W) of ten acini per slide were taken from different image locations for each individual bee (Al-Ghamdi et al. 2011a; 2011b). Two hundred acini were measured for each bee race (100 for nurses and 100 for foragers) (Figure 1). The acinal surface area (SA) was calculated using the following formula (Maurizio 1954):

$$\text{Acinal surface area} = \pi \times ((a \times b) / 2),$$

where a = maximum length, b = maximum width, and $\pi = 3.14$.

Analyses of lipofuscin accumulation

Lipofuscin was identified by its granular appearance in the acini of HPGs for nurse and forager bees. Ten lipofuscin accumulation areas representing one individual bee were measured in one slide. Two hundred lipofuscin accumulation areas were measured for each bee race (100 for nurses and 100 for foragers). Images were taken using a 40× oil immersion objective on a Zeiss laser confocal microscope (Carl Zeiss, AG-Germany).

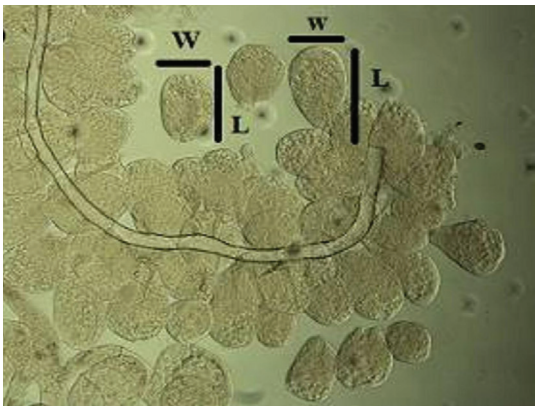

Figure 1. Microscopic measurement of acini length and width in the hypopharyngeal glands of honey bees. **L** length **W** width (Image at 20× magnification).

The longer wavelength spectrum (561 nm with emissions from 570–650 nm) was used for imaging because lipofuscin is only visible in this spectrum (Munch et al. 2013b). The laser power and detector sensitivity were kept constant for all images. The images were processed, and different regions of interest were selected to determine the lipofuscin accumulation percentage.

Data analysis

Data were examined for normality and homogeneity using the Kolmogorov-Smirnov test and Levene's test, respectively. The means were tested using an analysis of variance and subsequently separated using the LSD test at $p \leq 0.05$. The SPSS 22.0 program was used for the statistical analyses.

Results

Measurement of acini size in HPGs

Selected images of the acini of the nurses and foragers of the three bee races are shown in Figure 2. Figure 3 shows that nurse bees have larger HPG acini size measurements than forager bees of all races (indigenous = AMJ; exotic = AMC, AML) during the summer and winter seasons. Thus, irrespective of bee race and season, nurses have significantly larger acini than forager bees.

Figure 4 represents the comparison among indigenous (AMJ) and exotic bee races (AMC and AML). The acini size (L, W, and SA) was smaller in foragers and nurses of the indigenous bees than exotic bee races during the summer and winter seasons. In

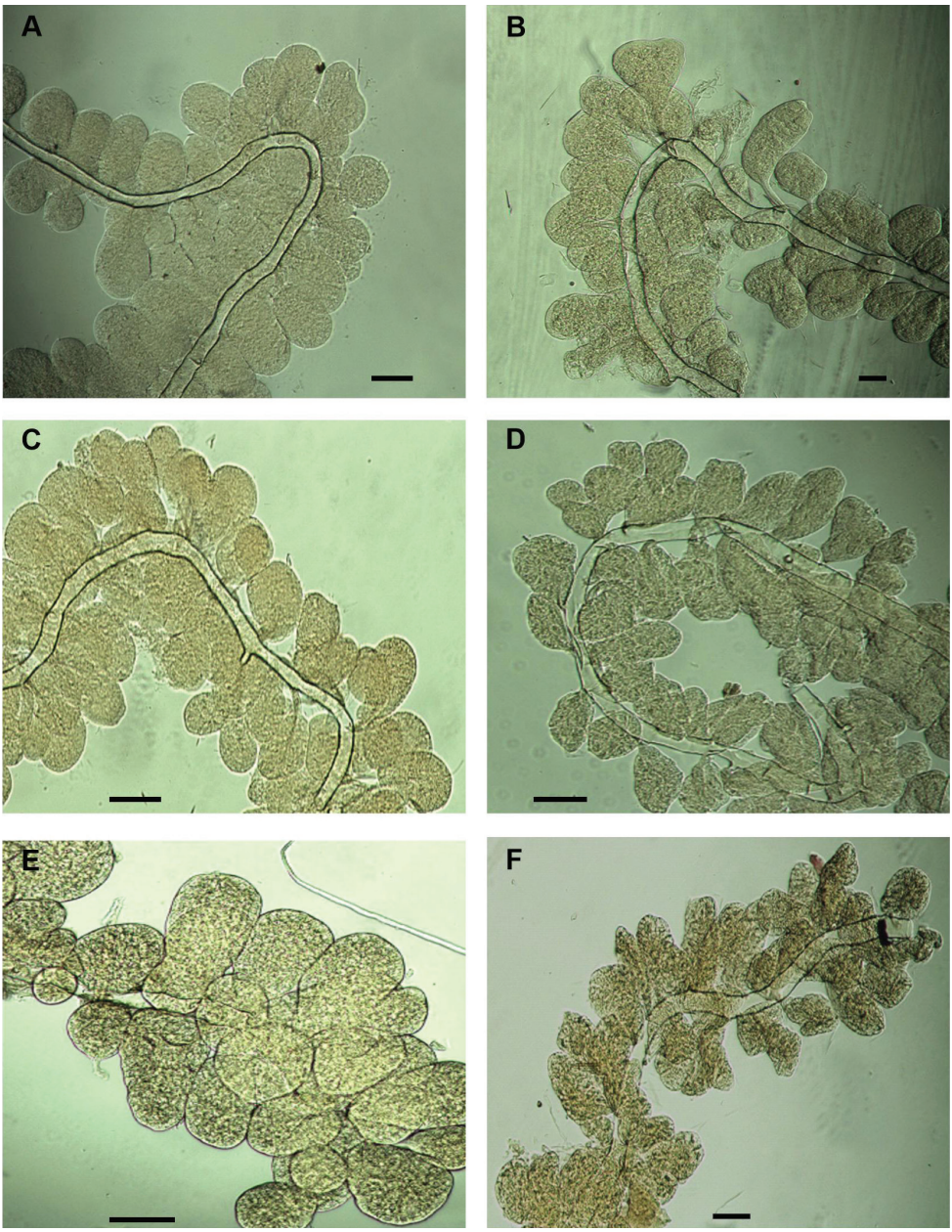

Figure 2. Acini in the hypopharyngeal glands of the nurse and forager bees. **A** *A. m. carnica* nurse **B** *A. m. carnica* forager **C** *A. m. jemenitica* nurse **D** *A. m. jemenitica* forager **E** *A. m. ligustica* nurse **F** *A. m. ligustica* forager. Scale bars: 6.2 mm (**A**), 4.2 mm (**B**), 7.6 mm (**C**), 7.9 mm (**D**), 9.8 mm (**E**), 5.6 mm (**F**). (Images at 20× magnification).

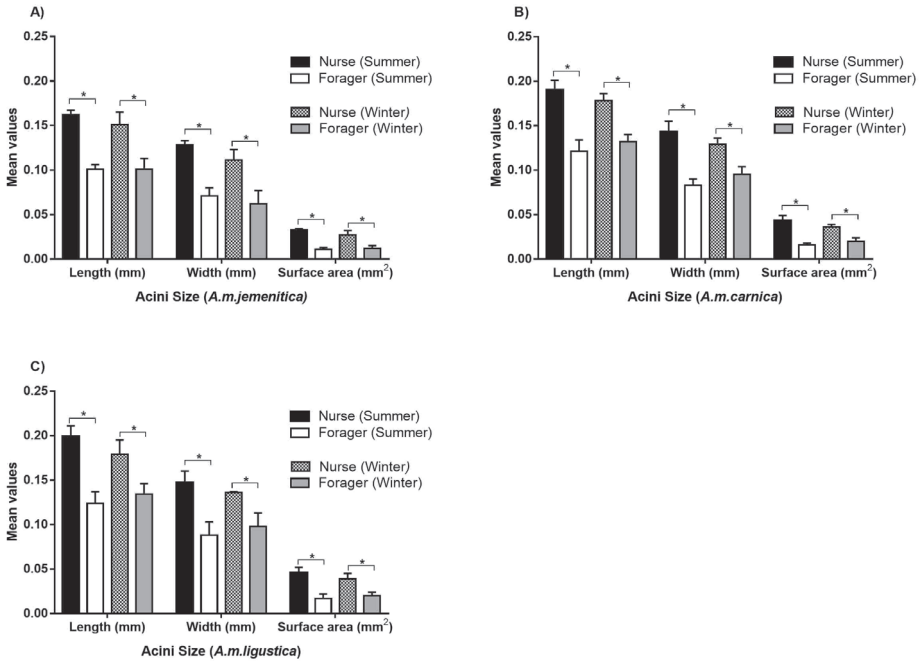

Figure 3. Acini sizes (length, width, and surface area) of nurse and forager bees. **A** *A. m. jemenitica* **B** *A. m. carnica* **C** *A. m. ligustica*. Nurse bees (summer & winter) had significantly larger acini than forager (summer & winter) bees in the three races. Asterisks (*) in the graphs represent the significant differences between the groups (LSD test at $p \leq 0.05$).

addition, significant differences were not observed between the two exotic races (AMC and AML) with respect to acini size.

The mean acini measurements in the AMJ nurses and foragers during the summer were as follows: L (0.162 mm and 0.101 mm, respectively), W (0.128 mm and 0.071 mm, respectively) and SA (0.033 mm^2 and 0.011 mm^2, respectively). Similarly, the acini sizes of the AMJ nurses and foragers during the winter were as follows: L (0.151 mm and 0.101 mm, respectively), W (0.111 mm and 0.062 mm, respectively) and SA (0.027 mm^2 and 0.012 mm^2, respectively). The sequence of bee races based on the three acini size parameters (L, W, and SA) was *A. m. ligustica* > *A. m. carnica* > *A. m. jemenitica* in both foragers and nurse bees (Table 1).

Measurements of lipofuscin accumulation in HPGs

The microscopic lipofuscin accumulation granules in the HPGs of different bee races are shown in Figure 5. The foragers in all bee races (AMJ, AMC, and AML) presented

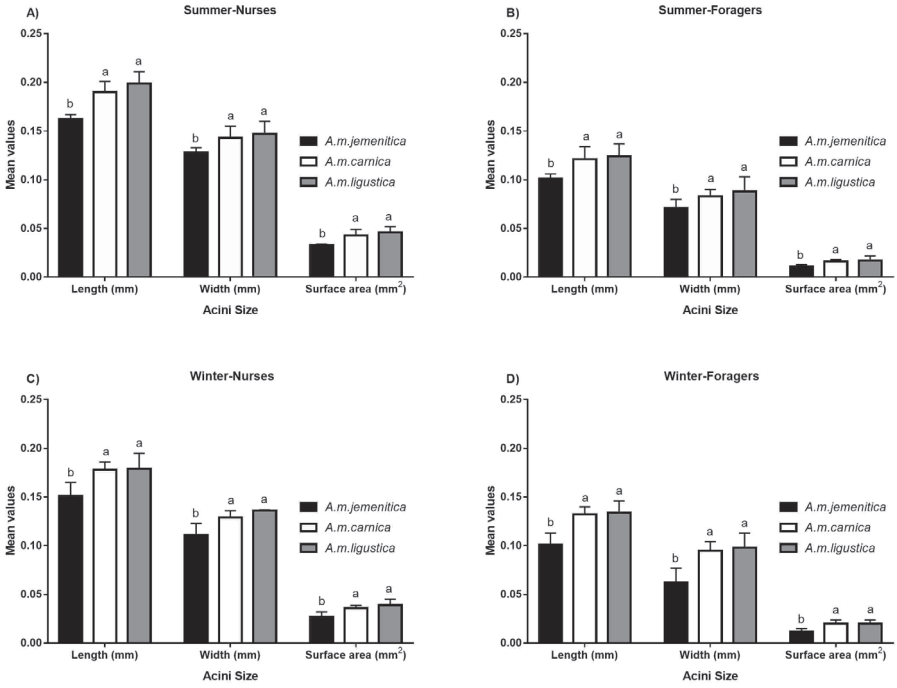

Figure 4. Inter-race comparison of acini size (length, width, and surface area) among the HPGs of *A. mellifera* **A** summer nurse **B** summer forager **C** winter nurse **D** winter forager. Graph bars headed by the same letter represent non-significant differences between the groups (LSD test at $p \leq 0.05$).

a significantly higher lipofuscin accumulation percentage than nurse bees of the same race during the summer and winter seasons (Figure 6). Therefore, irrespective of bee race and season, foragers have significantly higher lipofuscin accumulation than nurse bees.

The comparison among indigenous (AMJ) and exotic bee races (AMC and AML) with respect to lipofuscin accumulation is shown in Figure 7. Nurses and foragers of AMJ presented a significantly lower lipofuscin accumulation than the nurses and foragers of exotic bee races (AMC and AML). Moreover, summer foragers of all races have higher lipofuscin accumulation than winter foragers. No significant differences were found in lipofuscin accumulation in the nurse bees of any race in either season (Figure 8).

In summer, the lipofuscin accumulation values in the nurse bees were AMJ = 12.33%, AMC = 14.13%, and AML = 15.57% and in the forager bees were AMJ = 45.43%, AMC = 56.27%, and AML = 58.23%. In winter bees, the lipofuscin accumulation values in the nurse bees were AMJ = 11.10%, AMC = 12.20% and AML = 14.80% and in the forager bees were AMJ = 40.50%, AMC = 46.00%, and AML = 46.33% (Table 1).

The acini size and lipofuscin accumulation were inversely correlated with each other in the nurses and foragers of all bee races. The larger acini size in the nurse bees was correlated with a lower lipofuscin accumulation, and the smaller acini sizes in the foragers was correlated with higher lipofuscin accumulation (Table 2).

Table 1. Measurements of acini size and lipofuscin accumulation percentages in the hypopharyngeal glands of honey bees.

Season	Bee race	Acini size (mean ± SD)						Lipofuscin accumulation (%)	
		Length (mm)		Width (mm)		Surface area (mm²)			
		Nurses	Foragers	Nurses	Foragers	Nurses	Foragers	Nurses	Foragers
Summer	*A. m. jemenitica*	0.162±0.005	0.101±0.005	0.128±0.005	0.071±0.009	0.033±0.001	0.011±0.002	12.33	45.43
	A. m. carnica	0.190±0.011	0.121±0.013	0.143±0.012	0.083±0.007	0.043±0.006	0.016±0.002	14.13	56.27
	A. m. ligustica	0.199±0.012	0.124±0.013	0.147±0.013	0.088±0.015	0.046±0.006	0.017±0.005	15.57	58.23
Winter	*A. m. jemenitica*	0.151±0.014	0.101±0.012	0.111±0.012	0.062±0.015	0.027±0.005	0.012±0.003	11.10	40.50
	A. m. carnica	0.178±0.008	0.132±0.008	0.129±0.007	0.095±0.009	0.036±0.003	0.020±0.004	12.20	46.00
	A. m. ligustica	0.179±0.016	0.134±0.012	0.136±0.001	0.098±0.015	0.039±0.006	0.020±0.004	14.80	46.33

Table 2. Summary table for the inverse correlation between acini size and lipofuscin accumulation in the three bee races. Larger acini corresponded to lower lipofuscin accumulation and vice versa. **L** Length **W** width **SA** Surface area **HPGs** Hypopharyngeal glands. The arrow direction indicates the increase or decrease in size or accumulation percentage.

	Correlation index in the hypopharyngeal glands		
Bee race	Age polytheism	Acini size (L, W, SA)	Lipofuscin accumulation (%)
A. m. jemenitica, *A. m. carnica*, *A. m. ligustica*	Nurses	↑	↓
	Foragers	↓	↑

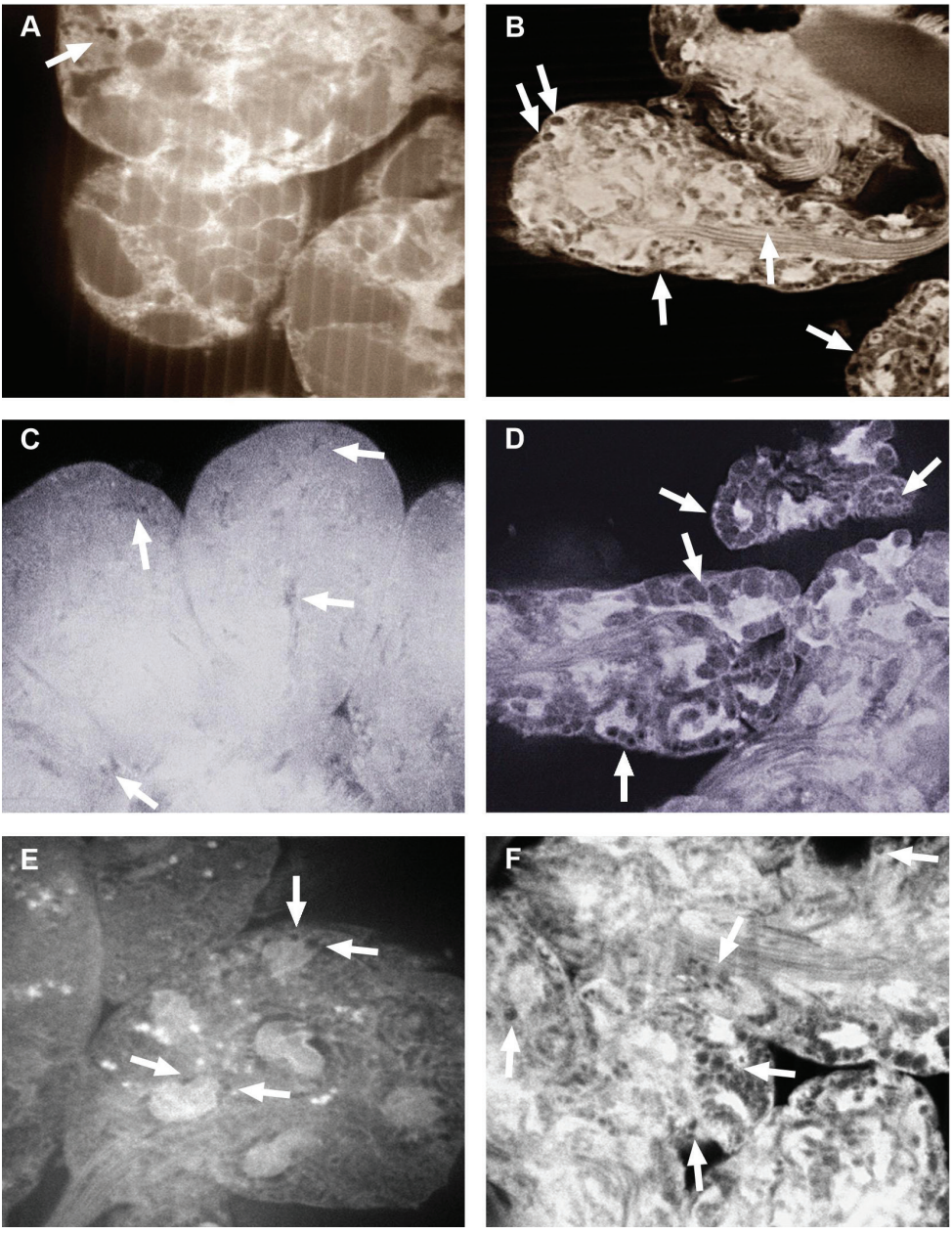

Figure 5. Lipofuscin accumulation (black granular structures: arrows) in the hypopharyngeal glands of nurse and forager bees **A** *A. m. carnica* nurse **B** *A. m. carnica* forager **C** *A. m. jemenitica* nurse **D** *A. m. jemenitica* forager **E** *A. m. ligustica* nurse **F** *A. m. ligustica* forager. (Images at 400× magnification).

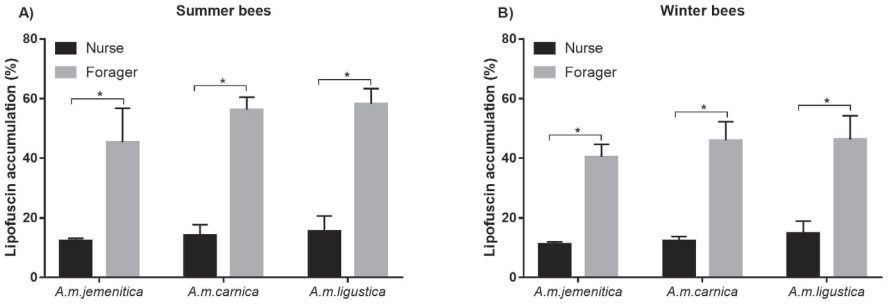

Figure 6. Comparisons between nurse and forager bees in the accumulation of lipofuscin in hypopharyngeal glands. **A** summer bees **B** winter bees. Asterisks (*) in the graph represent significant differences between the groups (LSD test at $p \leq 0.05$).

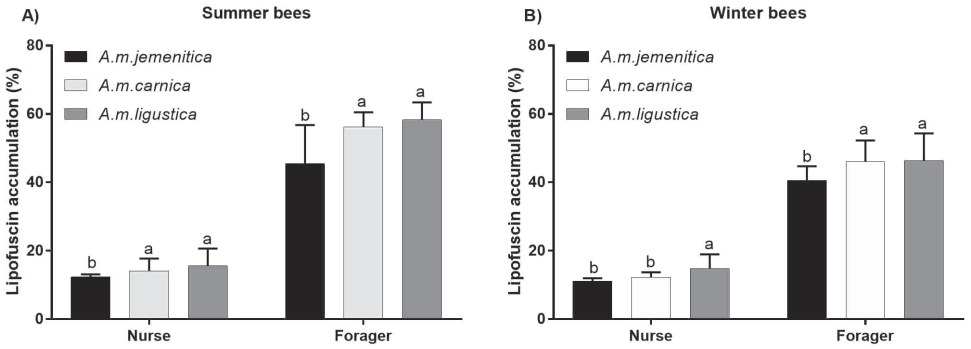

Figure 7. Inter-race comparison of lipofuscin accumulation **A** summer bees **B** winter bees. Graph bars headed by the same letter represent non-significant differences between the groups (LSD test at $p \leq 0.05$).

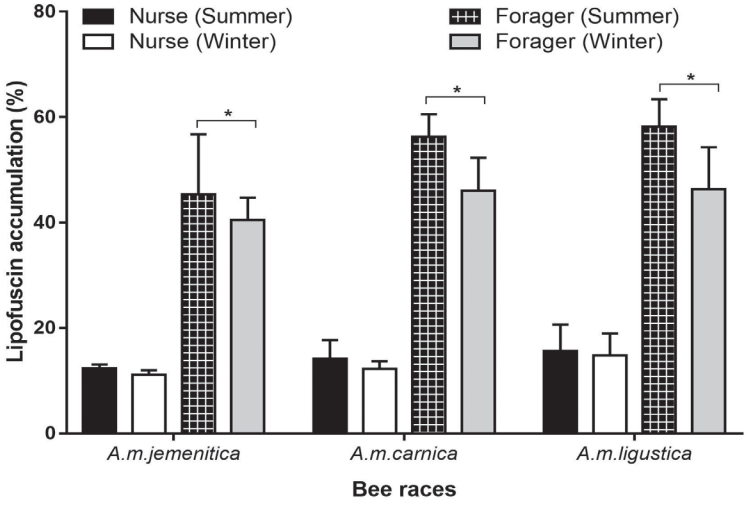

Figure 8. Seasonal variations in lipofuscin accumulation between summer and winter bees of the same race. Asterisks (*) in the graph represent significant differences between the groups (LSD test at $p \leq 0.05$).

Discussion

Acini size

Our results showed that acini size is linked with honey bee age-specific tasks. Nurse bees have significantly larger acini (L, W, and SA) than forager bees in all races (AMJ, AMC, and AML). These results are partially in accordance with a study by Škerl and Gregorc (2015), who reported large acini diameters in AMC nurse bees. Suwannapong et al. (2010) also found larger glands in nurses than foragers and guards in *A. cerana* Fabricius and *A. mellifera* Linnaeus. We also found that foragers have a smaller gland size, which is consistent with the findings of Ohashi et al. (2000). Therefore, all bee races exhibited identical stereotypic age-specific acini size patterns between nurses and foragers, even in the arid climate of Saudi Arabia. Moreover, age-polytheism in honey bees indicates that young nurse bees feed young bees and have larger acini, whereas older forager bees no longer feed the brood and thus present gland atrophy with smaller acini (Costa and da Cruz-Landim 2005; Deseyn and Billen 2005).

Acini size is positively correlated with HPG activity and its secretion. The larger acini in young nurses corresponds to higher HPG activity because of the production of royal jelly (RJ) for larvae. However, the reduced acini size in foragers results in decreased HPG activity (Brouwers 1982; Hrassnigg and Crailsheim 1998). The development and size of HPGs is positively linked with pollen and diet consumption (Al-Ghamdi et al. 2011a; Hrassnigg and Crailsheim 1998; Johnson 2010; Naiem et al. 1999), and nurse bees with larger glands may consume more pollen for preparing nutrients than foragers (Crailsheim 1991). In addition, the summer foragers are exposed to extreme heat stress during summer that might be a reason for less activity of HPGs than those of the nurses that remain inside the colony. The winter bees have hypertrophied HPGs due to the small number of larvae and activity reduction during winter (Škerl and Gregorc 2015). The reduced HPGs exhibit low protein synthesis rate, and secretions are presumed to be stored for the spring season until the young brood is present (Huang 1990).

Furthermore, the acini size (L, W, and SA) was smaller in indigenous bees (AMJ) than exotic bees (AMC and AML). Both nurses and forager bees (winter and summer) presented similar differences among the bee races. These findings are partially in accordance with previous studies (Al-Ghamdi 2006; Al-Ghamdi et al. 2011b), where HPG development and acini size were compared among nurse workers in one season. The present study provides a complete comparison of acini size in nurse and forager bees during the summer and winter seasons. Zeng et al. (1990) found a significant difference in acini size and number between two bee species, with *A. mellifera* presenting longer acini with more RJ secretion than *A. cerana*. In contrast, considerable differences in the size and histochemical structures of HPGs were not found between *A. mellifera* L. and *A. cerana* Fab. bee species (Suwannapong et al. 2010). However, these two studies are not comparable with ours due to taxonomic differences. The present study revealed no significant differences in acini size between the two exotic bee races AMC and AML.

Many factors, such as bee race, body size, brood pheromones, ecological factors, starvation, heat stress, Varroa infestation, diseases, and pesticides could play an important role in acini size in bee races (De Smet et al. 2017; Khalil 1992; Le Conte et al. 2001; Yousef et al. 2014). However, the tested colonies in this study were kept free from infestations, pesticides, and starvation. Therefore, the differences might be attributable to the other listed factors. Indigenous bees (AMJ) have significant difference in morphological characters such as smaller body size, wings and legs length than exotic bees (AMC and AML) (Alattal et al. 2014; Alqarni 1995; Alqarni et al. 2011), which could potentially explain the observed differences in acini size between the indigenous and exotic bee races. Other factors, such as geographical isolation, food preference and food consumption among different bee races, may also contribute to differences in HPG development (Alqarni 2006; Cheng 2001).

Lipofuscin accumulation

In the present study, cellular senescence was observed by measuring the difference in lipofuscin accumulation in HPGs. Forager bees possessed higher lipofuscin accumulation than younger nurse bees, which is in line with the findings of Munch et al. (2013b), who reported high lipofuscin accumulation in older bees.

Lipofuscin accumulation is also known to be related to the onset of senescence and is dependent on foraging age (Heidem 2013; Munch et al. 2013a). Increased lipofuscin accumulation suggests that the organism is aging (Gray and Woulfe 2005), and such accumulations may have a significant impact on biological functions (Hsu and Chan 2013). In this study, acini size and lipofuscin accumulation showed a strong inverse correlation, with a larger acini size corresponding to lower lipofuscin accumulation in nurse and forager bees. This inverse correlation is consistent with Munch et al. (2013b). During summer, foragers are more exposed to high temperature and water stress than in winter. Thus, summer foragers might exert more energy to cope with these harsh circumstances, thereby leading to a relatively high lipofuscin accumulation compared with that of winter foragers (Figure 6).

Furthermore, indigenous bees (AMJ) showed less lipofuscin accumulation than exotic races (AMC, AML). The possible explanation may be the geographical isolation and ecological adaptations of indigenous honey bees to the local environment in the Arabian Peninsula and the different body size of these bees (Abou-Shaara et al. 2012; Alqarni et al. 2011). These bees may have evolved physiological processes to decelerate the accumulation of lipofuscin. Indigenous bees are known for their significantly lower weight loss, higher heat tolerance and greater foraging activity than AMC and AML (Alqarni 2006; Alqarni et al. 2014), and these observed features could be related to the increased adaptation of AMJ to the arid zone environmental conditions.

This study suggests the need to further investigate the association of HPGs, lipofuscin accumulation, royal jelly traits and brood quality in indigenous bees in different geographical regions of Saudi Arabia via new technologies, such as genetic characteri-

zation. It will also urge the researchers to explore the associated facts underlying the physiology of glands development with food and behavior especially in successful and heat tolerant indigenous bees of Saudi Arabia. Likewise, it will be also important to compare the acini size and lipofuscin accumulation in the other cephalic worker secretory hind brain glands. Detecting gene expression in the HPGs of indigenous bees (AMJ) compared with that of exotic bee races (AMC and AML) could be important for further understanding the mechanism of foraging and nursing behavior in the climatic conditions of Saudi Arabia.

Collectively, nurse bees presented significantly larger acini and less lipofuscin accumulation than foragers. Consequently, a strong inverse correlation was observed between acini size and lipofuscin accumulation. Inter-race comparisons showed that indigenous bees possess smaller acini and less lipofuscin accumulation in the HPGs than exotic bees during summer and winter.

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