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Sawflies from northern Ecuador and a checklist for the country (Hymenoptera: Argidae, Orussidae, Pergidae, Tenthredinidae, Xiphydriidae)

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Abstract

An illustrated list of species of sawflies collected in northern Ecuador, mainly during the end of 2016, is given. *Manaos mulsus* (Konow, 1906), *Ptenos delta* (Malaise, 1957), *Scobina styx* Malaise, 1949 (Argidae), *Stromboceros suppar* Konow, 1903 and *Stromboceros sutilis* Konow, 1903 (Tenthredinidae) are species new for the country. A checklist of species for the country is also provided. Approximately 120 species of Symphyta are known from Ecuador, 25 Argidae, 1 Orussidae, about 40 Pergidae, about 60 Tenthredinidae, and 3 Xiphydriidae.

Keywords

Species list, adult feeding behaviour, host plant, South America

Introduction

As a hotspot of biodiversity, Ecuador includes various environments harbouring many species still to be discovered, especially among the insects (e.g., Heckman 2006). Sawflies known from the country belong to the families Argidae, Orussidae, Pergidae, Tenthredinidae, and

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Xiphydriidae (see checklist and references below). A list of species collected more recently in the southern part of the country was published by Boevé et al. (2016).

Here, an illustrated list is given for sawfly adults from the northern part of Ecuador, which were collected in the frame of a Global Taxonomy Initiative (GTI) project. We also compiled data and list the sawfly species known for the country.

Methods

Sawflies were collected in the north-eastern (province Orellana) and north-western (Pichincha) sides of Ecuador, mainly during November 2016 (Fig. 1) plus a few during March 2015. Nearly all of them were collected as adults using a net and they are stored in ethanol. Field observations include the mention of plants, not necessarily host plants, on which adults were found.

Pictures were taken by J.-L. Boevé with the following cameras: Pentax Optio W10, Nikon Coolpix P300, and Canon EOS 5D Mark III. Since adult specimens are kept in ethanol, they were partly dried to take the pictures, which were mainly intended to illustrate the habitus. Specimens of each species collected in 2015 and 2016 were photographed (Figs 2–22). Screenshots (see Fig. 1e, f, g) were extracted from video sequences taken by Alain Pauly.

The sawfly specimens collected as part of the GTI project are stored in the Royal Belgian Institute of Natural Sciences, Brussels, Belgium (RBINS; J.-L. Boevé collection, with specimen reference codes starting with 'P'), with duplicates that will be located in the Pontificia Universidad Católica del Ecuador, Quito (PUCE). Sawfly specimens from the RBINS, Senckenberg Deutsche Entomologisches Institut, Müncheberg, Germany, and National Museum of Natural History, Washington, D.C., USA were examined. Dates are given by dd.mm.year.

The adult sawflies were identified by D. R. Smith. Identifications of Argidae and Pergidae are based on Smith (1990, 1992). Identifications of Blennocampinae and Selandriinae (Tenthredinidae) are based on Smith (2007) and unpublished manuscripts by DRS.

Results

Family Argidae

***Manaos mammeatus* (Konow, 1906b)**
Fig. 2

Material. Estación científica Yasuní, near río Tiputini, 00°40'S, 076°24'W, 220m, 02.03.2015, P4119.B (1 ♀), leg. T. Delsinne, 14–16.11.2016, on leaf of *Heliconia* sp. (Heliconiaceae), P4217.A, P4220.A, P4220.B, P4222, P4223.C, P4223.D (6 ♀), leg. J.-L. Boevé.

Figure 1. Pictures taken in the field, showing some biotopes and sawfly adults from northern Ecuador. Location **a–g** Yasuni National Park **h** Pacto **i–m** Hacienda San Vicente, Mindo. Leaves of **b** *Anthurium* sp. and **c** *Heliconia* sp., inspected by **d** *Proselandria alvina* and **e–g** probably *Manaos mammeatus*, respectively.

Manaos mulsus (Konow, 1906b)

Fig. 3

Material. Estación científica Yasuní, 00°40'S, 076°27'W, 235, 13.11.2016, on leaf of *Anthurium* sp. (Araceae), P4214.G (1 ♀), 00°33'S, 076°31'W, 260m, 14.11.2016, on leaf of *Heliconia* sp., P4216 (1 ♂), 00°40'S, 076°27'W, 250m, 15.11.2016, on leaf of *Anthurium* sp., P4221.D (1 ♂), leg. J.-L. Boevé.

Note. This is a new record for Ecuador. The species was previously known from Brazil, Peru, and Surinam (Smith 1992).

Figure 1. Continued. **h** Biotope where *Waldheimia* sp. A was common **i** Biotope of pasture (with orchard in the foreground) where, among others **j** *Inea* sp. (P4239.E) and **k** *Waldheimia* sp. A were collected **l** Biotope of forest path **m** with the precise spot where *Stromboceros sutilis* (P4239.G) was collected.

Ptenos delta (Malaise, 1957)

Fig. 4

Material. Estación científica Yasuní, near río Tiputini, 00°40'S, 076°24'W, 220m, 02.03.2015, P4119.C (1 ♀), leg. T. Delsinne.

Note. This is a new record for Ecuador. It was previously known only from Brazil and Peru (as *Hemidineura delta* Smith, 1992).

a **b**

Figure 2. *Manaos mammeatus*, female (P4223.D), body length 6.5 mm. **a** Dorsal view **b** ventral view.

a **b**

Figure 3. *Manaos mulsus*. **a** Female (P4214.G), body length 4.5 mm **b** male (P4221.D), body length 4.5 mm **a** Lateral view **b** dorso-lateral view.

a **b**

Figure 4. *Ptenos delta*, female (P4119.C), body length 6.5 mm. **a** Dorsal view **b** ventral view.

Scobina inculta (Konow, 1906a)

Fig. 5

Material. Estación científica Yasuní, 00°36'S, 076°30'W, 240m, 14.11.2016, by sweeping, P4215.E (1 ♂), leg. J.-L. Boevé.

a **b**

Figure 5. *Scobina inculta*, male (P4215.E), body length 6.5 mm. **a** Dorsal view **b** ventral view.

a **b**

Figure 6. *Scobina notaticollis*, female (P4213), body length 7.5 mm. **a** Dorsal view **b** ventral view.

Scobina notaticollis **(Konow, 1899a)**
Fig. 6

Material. Pompeya, 00°27'S, 076°37'W, 240m, 11.11.2016, flying, P4213 (1 ♀), leg. J.-L. Boevé.

Scobina strophosa **(Konow, 1906a)**
Fig. 7

Material. Nanegal, Reserva Biológica Maquipucana, 00°08'N, 078°38'W, 1300m, 25.11.2016, P4238.A (1 ♀), P4238.F, P4238.H (2 ♂), leg. A. Pauly, J.-L. Boevé; Mindo, Mindo Lindo, 00°01'S, 078°46'W, 1680m, 27.11.2016, flying, P4241.B (1 ♂), leg. J.-L. Boevé.

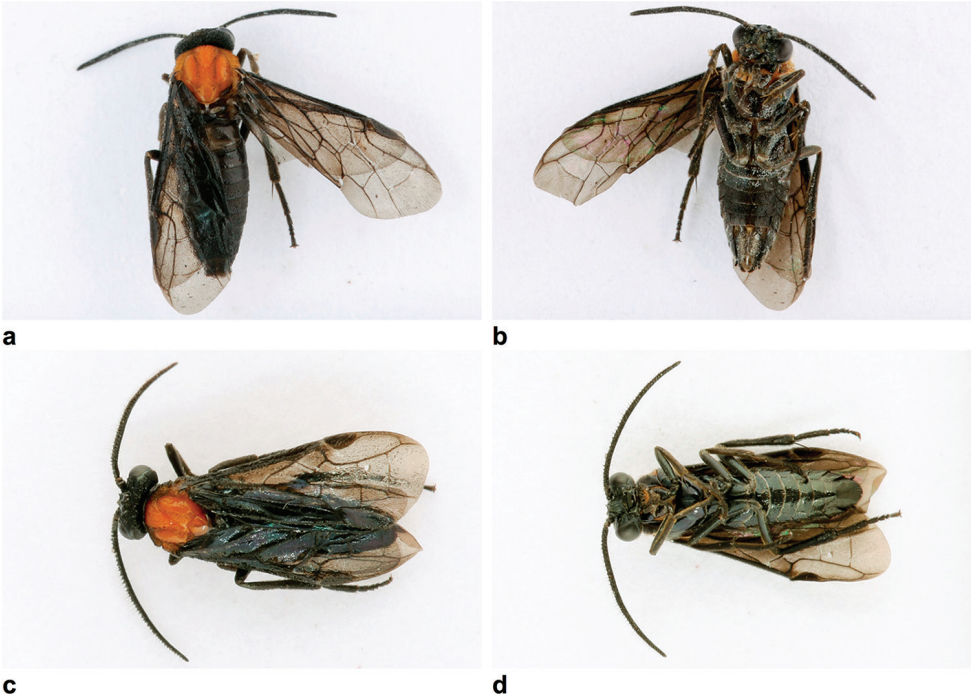

Figure 7. *Scobina strophosa*. **a, b** Female (P4238.A), body length 7.5 mm **c, d** male (P4241.B), body length 7.5 mm. **a, c** Dorsal views **b, d** ventral views.

Scobina styx Malaise, 1949

Fig. 8

Material. Nanegal, Reserva Biológica Maquipucana, 00°08'N, 078°38'W, 1300m, 25.11.2016, P4238.D, P4238.E (2 ♀), P4238.L (1 ♂), leg. A. Pauly, J.-L. Boevé; Mindo, Hacienda San Vicente, 00°03'S, 078°46'W, 1445, 26.11.2016, flying along pasture, P4239.F (1 ♀), leg. J.-L. Boevé.

Note. This is a new record for Ecuador. It was previously known only from northern Argentina (Smith 1992).

Scobina sp.

Fig. 9

Material. Mindo, Hacienda San Vicente, 00°02'S, 078°46'W, 1420m, 23.11.2016, by sweeping along pasture, P4232.C (1 ♂), leg. A. Pauly, J.-L. Boevé.

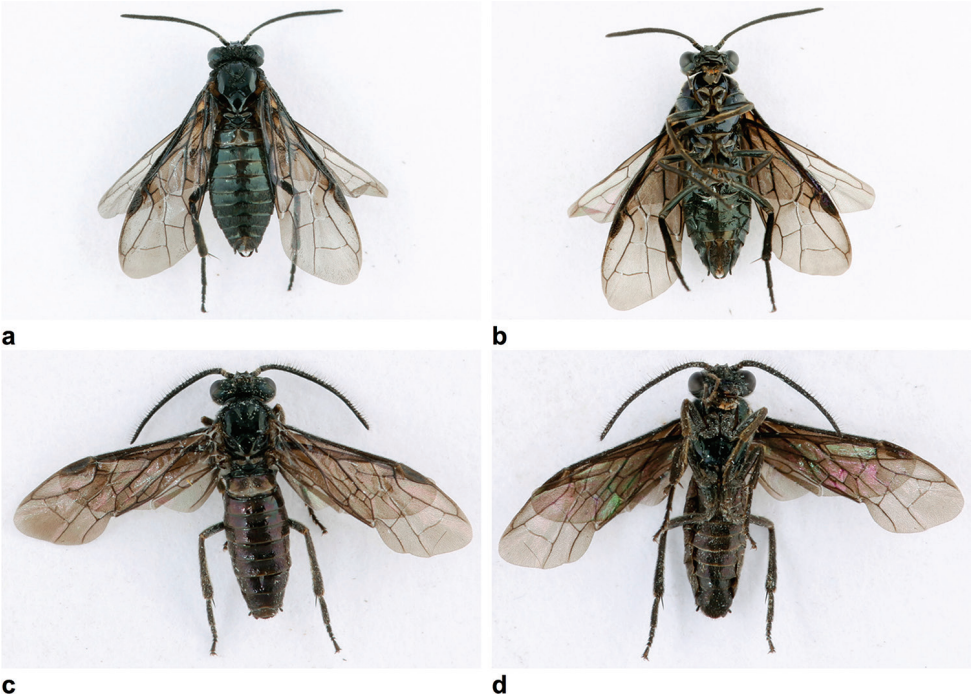

Figure 8. *Scobina styx*. **a, b** Female (P4239.F), body length 7.5 mm **c, d** male (P4238.L), body length 6.0 mm. **a, c** Dorsal views **b, d** ventral views.

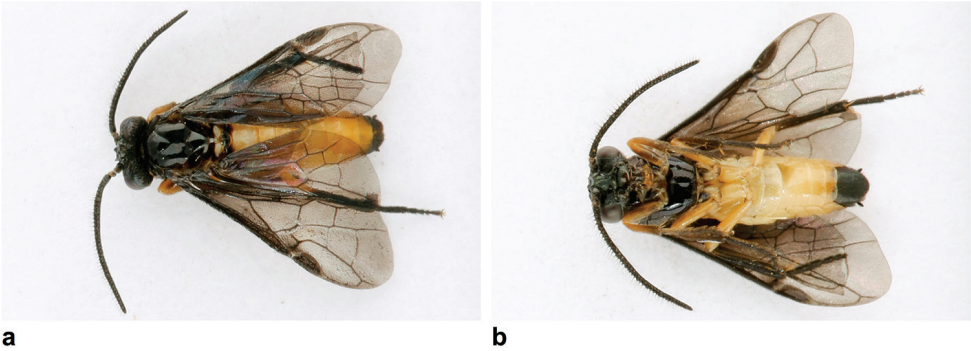

Figure 9. *Scobina* sp., male (P4232.C), body length 6.0 mm. **a** Dorsal view **b** ventral view.

Family Pergidae

Acordulecera spp.

Fig. 10

Material. Estación científica Yasuní, 00°33'S, 076°31'W, 260m, 15.11.2016, on leaf of *Heliconia* sp., P4219.A (1 ♀), leg. J.-L. Boevé; Nanegal, Reserva Biológica Maquipucana, 00°07'N, 078°38'W, 1380m, 25.11.2016, on leaf of Melastomataceae, P4236 (1 ♀), leg. J.-L. Boevé.

a **b**

Figure 10. *Acordulecera* spp. **a** Female (P4219.A), body length 4.5 mm **b** female (P4236), body length 3.5 mm. **a, b** Lateral views.

a **b**

Figure 11. *Decameria* sp., male (P4238.B), body length 8.0 mm. **a** Dorsal view **b** ventral view.

***Decameria* sp.**
Fig. 11

Material. Nanegal, Reserva Biológica Maquipucana, 00°08'N, 078°38'W, 1300m, 25.11.2016, P4238.B (1 ♂), leg. A. Pauly, J.-L. Boevé.

Family Tenthredinidae
Subfamily Blennocampinae

***Waldheimia pallens* (Klug, 1816)**
Fig. 12

Material. Estación científica Yasuní, near río Tiputini, 00°40'S, 076°24'W, 220m, 02.03.2015, P4119.A (1 ♂), leg. T. Delsinne; Estación científica Yasuní, 00°36'S, 076°30'W, 240m, 14.11.2016, by sweeping, P4215.H (1 ♀), P4215.J (1 ♂), leg. A. Pauly, J.-L. Boevé.

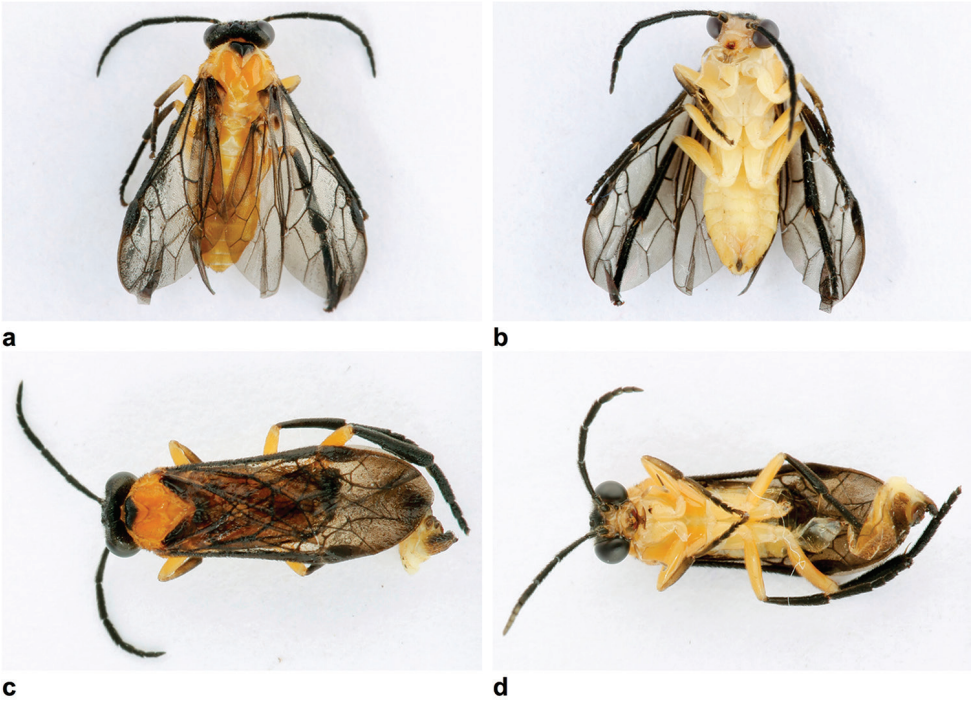

Figure 12. *Waldheimia pallens*. **a, b** Female (P4215.H), body length 8.0 mm **c, d** male (P4215.J), body length unknown. **a, c** Dorsal views **b, d** ventral views.

Waldheimia pellucida Konow, 1904
Fig. 13

Material. Estación científica Yasuní, 00°36'S, 076°30'W, 240m, 14.11.2016, flying above plants of *Rhynchospora* (Cyperaceae), P4215.A, P4215.B (2 ♂), by sweeping, P4215.D (1 ♂), on fern, P4215.F (1 ♂), leg. J.-L. Boevé, by sweeping, P4215.I (1 ♂), leg. A. Pauly, J.-L. Boevé, 00°33'S, 076°31'W, 260m, 15.11.2016, on leaf of *Heliconia* sp., P4218 (1 ♂), leg. J.-L. Boevé.

Waldheimia sp. A
Fig. 14

Material. Mindo, Hacienda San Vicente, 00°02'S, 078°46'W, 1420m, 23.11.2016, on leaf along pasture, P4227.A (1 ♂), 1470m, 23.11.2016, on fern along forest path, P4227.B (1 ♂), leg. J.-L. Boevé, 1480m, 23.11.2016, P4231.A (1 ♂), leg. D. F. Dominguez, J.-L. Boevé, 1420m, 23.11.2016, by sweeping along pasture, P4232.A

a **b**

Figure 13. *Waldheimia pellucida*, male (P4218), body length 6.5 mm. **a** Dorsal view **b** ventral view.

a **b**

Figure 14. *Waldheimia* sp. A, male (P4237.B), body length 5.5 mm. **a** Dorsal view **b** ventral view.

(1 ♂), leg. A. Pauly, J.-L. Boevé; Pacto, 00°09'N, 078°46'W, 1105, 24.11.2016, flying or on leaf, P4233.A, P4233.B, P4233.C, P4233.D, P4233.E, P4233.F (6 ♂), leg. J.-L. Boevé, P4233.G, P4233.H (2 ♂), leg. A. Pauly, J.-L. Boevé; Nanegal, Reserva Biológica Maquipucana, 00°08'N, 078°38'W, 1260m, 25.11.2016, flying above low plants, P4235.A, P4237.B (2 ♂), leg. J.-L. Boevé; Mindo, Hacienda San Vicente, 00°03'S, 078°46'W, 1375, 26.11.2016, flying above and on low plants along pasture, P4239.B, P4239.C (2 ♂), 1415m, 26.11.2016, flying along pasture, P4239.D (1 ♂), leg. J.-L. Boevé.

***Waldheimia* sp.**

Fig. 15

Material. Bellavista Cloud Forest Reserve, 00°02'S, 078°45'W, 1825m, 22.11.2016, on leaf, P4226.C (1 ♂), leg. J.-L. Boevé.

a **b**

Figure 15. *Waldheimia* sp., male (P4226.C), body length 8.0 mm. **a** Dorsal view **b** ventral view.

Subfamily Selandriinae

Adiaclema sp.

Fig. 16

Material. Estación científica Yasuní, near río Tiputini, 00°40'S, 076°24'W, 230m, 14.11.2016, on leaf of *Heliconia* sp., P4217.B (1 ♂), 16.11.2016, on leaf of *Heliconia* sp., P4223.A (1 ♂), leg. J.-L. Boevé.

Bolivius sp.

Fig. 17

Material. Estación científica Yasuní, 00°36'S, 076°30'W, 240m, 14.11.2016, on fern, P4215.C (1 ♂), leg. J.-L. Boevé.

Inea sp.

Fig. 18

Material. Reserva Otongachi, 00°19'S, 078°57'W, 925, 19.11.2016, on fern in forest, P4224 (1 ♀), leg. J.-L. Boevé; Bellavista Cloud Forest Reserve, 00°02'S, 078°44'W, 1945, 22.11.2016, on *Anthurium* sp., P4226.A (1 ♂), 00°02'S, 078°45'W, 1890m, 22.11.2016, on leaf, P4226.B (1 ♂), 00°02'S, 078°45'W, 1820m, 22.11.2016, flying quite frantically, P4226.D (1 ♂), 00°02'S, 078°45'W, 1820m, 22.11.2016, on leaf, P4226.E (1 ♂), 00°02'S, 078°45'W, 1775m, 22.11.2016, flying around a large fern, P4226.F (1 ♀), leg. J.-L. Boevé; Mindo, Hacienda San Vicente, 00°03'S, 078°45'W, 1520m, 23.11.2016, flying around a fern along forest path, P4227.C, P4227.D, P4227.E, P4227.F (4 ♂), 00°03'S, 078°46'W, 1470m, 23.11.2016, on leaf along forest path, P4229 (1 ♀), leg. J.-L.

a **b**

Figure 16. *Adiaclema* sp., male (P4223.A), body length 7.5 mm. **a** Dorsal view **b** ventral view.

a **b**

Figure 17. *Bolivius* sp., male (P4215.C), body length 6.0 mm. **a** Dorsal view **b** ventral view.

Boevé, 00°03'S, 078°46'W, 1500m, 23.11.2016, by sweeping along forest path, P4230.A, P4230.B, P4230.C, P4230.D, P4230.E, P4230.F, P4230.G, P4230.H, P4230.I (9 ♂), leg. A. Pauly, J.-L. Boevé, 00°03'S, 078°46'W, 1480m, 23.11.2016, P4231.B, P4231.C (2 ♂), leg. D. F. Dominguez, J.-L. Boevé, 00°02'S, 078°46'W, 1420m, 23.11.2016, by sweeping along pasture, P4232.B (1 ♂), leg. A. Pauly, J.-L. Boevé; Pacto, Río Toalí, 00°09'N, 078°45'W, 1000m, 24.11.2016, P4234 (1 ♂), leg. A. Pauly, J.-L. Boevé; Nanegal, Reserva Biológica Maquipucana, 00°08'N, 078°38'W, 1260m, 25.11.2016, flying above low plants, P4235.B, P4237.A (2 ♂), leg. J.-L. Boevé, 1300m, 25.11.2016, P4238.G, P4238.I (2 ♀), P4238.J, P4238.K (2 ♂), leg. A. Pauly, J.-L. Boevé; Mindo, Hacienda San Vicente, 00°03'S, 078°46'W, 1375m, 26.11.2016, on leaf along pasture, P4239.A (1 ♂), 1415m, 26.11.2016, on leaf of *Rubus* (Rosaceae) along pasture, P4239.E (1 ♂), leg. J.-L. Boevé; Mindo, Mindo Lindo, 00°01'S, 078°46'W, 1680m, 27.11.2016, flying, P4241.A (1 ♀), P4240.A, P4240.B, P4240.C, P4240.D (4 ♂), leg. J.-L. Boevé, 00°01'S, 078°46'W, 1680m, 27.11.2016, P4241.D (1 ♀), P4241.C, P4241.E, P4241.F, P4241.G (4 ♂), 1635m, 27.11.2016, on leaf (forest clearing), P4241.H, P4241.I (2 ♂), leg.

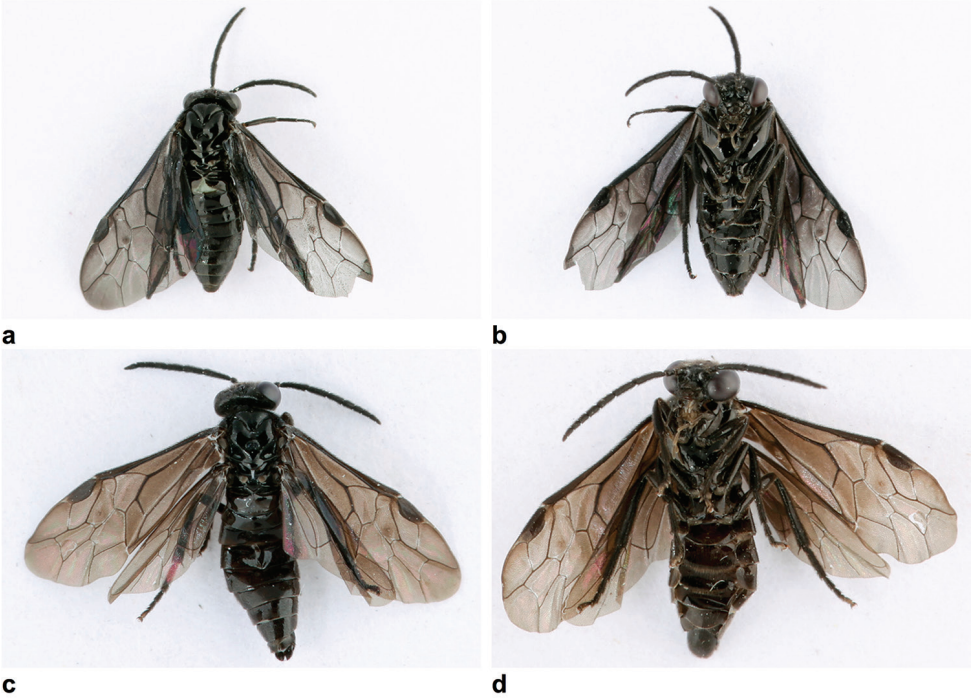

Figure 18. *Inea* sp. **a, b** Female (P4241.A), body length 6.5 mm **c, d** male (P4230.B), body length 6.5 mm. **a, c** Dorsal views **b, d** ventral views.

D. F. Dominguez, J.-L. Boevé, 1680m, 27.11.2016, flying just above the ground, P4242.A (1 ♂), 27.11.2016, by sweeping, P4242.B (1 ♂), 27.11.2016, flying, P4242.C, P4243 (2 ♂), leg. J.-L. Boevé.

Proselandria alvina (Konow, 1899b)

Fig. 19

Material. Estación científica Yasuní, 00°40'S, 076°27'W, 235m, 13.11.2016, on leaf of *Anthurium* sp., P4214.A, P4214.D (2 ♀), P4214.B, P4214.C, P4214.E, P4214.F (4 ♂), 00°36'S, 076°30'W, 240m, 14.11.2016, on fern, P4215.G (1 ♂), 00°40'S, 076°27'W, 250m, 15.11.2016, on leaf of *Anthurium* sp., P4221.A, P4221.C (2 ♀), on leaf of Melastomataceae, P4221.B (1 ♀), near río Tiputini, 00°40'S, 076°24'W, 230m, 16.11.2016, on leaf of *Heliconia* sp., P4223.B (1 ♂), leg. J.-L. Boevé.

Proselandria sp.

Material. Estación científica Yasuní, 00°33'S, 076°31'W, 260m, 15.11.2016, on leaf of Melastomataceae, P4219.B (1 ♂), leg. J.-L. Boevé.

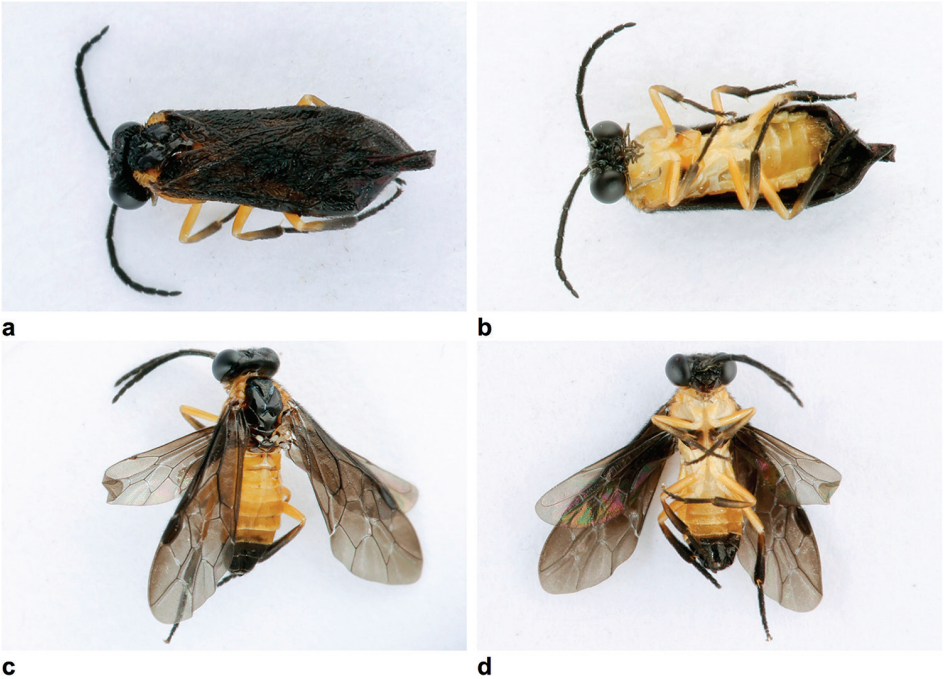

Figure 19. *Proselandria alvina*. **a, b** Female (P4221.A), body length 5.0 mm **c, d** male (P4214.C), body length 6.0 mm. **a, c** Dorsal views **b, d** ventral views.

Stromboceridea albilabris **(Konow, 1885)**
Fig. 20

Material. Reserva Integral Otonga, 00°25'S, 079°00'W, 1950m, 20.11.2016, on leaf (forest), P4225 (1 ♂), leg. J.-L. Boevé.

Stromboceros suppar **Konow, 1903b**
Fig. 21

Material. Nanegal, Reserva Biológica Maquipucana, 00°08'N, 078°38'W, 1300m, 25.11.2016, P4238.C (1 ♀), leg. A. Pauly, J.-L. Boevé.

Note. This is a new record for Ecuador. It was described from Peru.

Stromboceros sutilis **Konow, 1903b**
Fig. 22

Material. Mindo, Hacienda San Vicente, 00°03'S, 078°46'W, 1465m, 26.11.2016, on fern along forest path, P4239.G (1 ♀), leg. J.-L. Boevé.

Note. This is a new record for Ecuador. It was described from Peru.

a **b**

Figure 20. *Stromboceridea albilabris*, male (P4225), body length 7.0 mm. **a** Dorsal view **b** ventral view.

a **b**

Figure 21. *Stromboceros suppar*, female (P4238.C), body length 7.5 mm. **a** Dorsal view **b** ventral view.

a **b**

Figure 22. *Stromboceros sutilis*, female (P4239.G), body length 6.5 mm. **a** Dorsal view **b** ventral view.

Checklist of Ecuador Symphyta

The following checklist (Table 1) reflects the current status of the known Symphyta from Ecuador. The source of the first Ecuador record is given as well as known host plants. The list includes 66 identified species and it mentions the occurrence of about

Table 1. Checklist of Ecuador Symphyta.

Taxon	Occurrence	Host
Family Argidae		
Acrogymnidea udata D.R. Smith, 1992	Described	
Atomacera lobula D.R. Smith, 1992	Described	
Atomacera pubicornis (Fabricius, 1804)	Recorded by Smith (1992)	*Ipomoea* sp. (Convolvulaceae) (Mc Callan 1953, Smith 1992)
Dielocerus fasciatus (Enderlein, 1919)	Described	*Sclerolobium paniculatum* (Fabaceae) (Smith and Adis 1984)
Manaos mammeatus (Konow, 1906b)	Recorded by Smith (1992)	
Manaos mulsus (Konow, 1906b)	New record in this paper	
Manaos toula D.R. Smith, 1992	Described	
Neoptilia liturata (Konow, 1903a)	Described	*Sida rhombifolia* (Malvaceae) (Smith 1992)
Ptenos delta (Malaise, 1957)	New record in this paper	
Ptenos leucopoda (Cameron, 1883)	Recorded by Smith (1992)	
Scobina bolivari (Konow, 1899a)	Recorded by Smith (1992)	
Scobina inculta (Konow, 1906a)	Recorded by Smith (1992)	
Scobina lurida (Klug, 1834)	Recorded by Smith (1992)	
Scobina nigripennis (Enderlein, 1919)	Described	
Scobina notaticollis (Konow, 1899a)	Recorded by Smith (1992)	
Scobina strophosa (Konow, 1906a)	Recorded by Smith (1992)	
Scobina styx Malaise, 1949	New record in this paper	
Scobina terminalis (Klug, 1814)	Recorded by Smith (1992)	
Sericoceros dimidiatus Konow, 1908	Recorded by Smith (1992)	
Sericoceros ecuadoriensis (Enderlein, 1919)	Described	
Sericoceros gibbus (Klug, 1834)	Recorded by Smith (1992)	*Coccoloba* spp. (Polygonaceae) (Smith 1992, Smith and Janzen 2003)
Themos laqueatus (Enderlein, 1919)	Described	
Themos ochreus D.R. Smith, 1992	Described	
Themos semiadusta (Enderlein, 1919)	Described	
Themos surinamensis (Klug, 1814)	Recorded by Smith 1992	*Ceiba pentandra* (Malvaceae), *Thespesia populnea* (Malvaceae) (Smith 1992)
Family Orussidae		
Ophrynopus nigricans (Cameron, 1883)	Recorded by Vilhelmsen and Smith (2002)	
Family Pergidae		
Acordulecera spp.	This is a large genus in the Neotropics. At least 25 or more species probably occur in Ecuador (Smith, unpublished; estimate from material in USNM)	
Aulacomerus ecuadoriensis (Enderlein, 1919)	Described	
Camptoprium sp.	Unidentified species recorded by Schmidt and Smith (2006)	
Decameria carbo (Malaise, 1937)	Described	
Decameria noxua D.R. Smith, 1990	Described	

Taxon	Occurrence	Host
Decameria varipes Cameron, 1883	Recorded by Smith (1990)	
Haplostegus subclavatus Malaise, 1942	Described	
Lagideus kolonus D.R. Smith, 1990	Described	
Lagideus podocarpus D.R. Smith, 2016 (in Boevé et al. 2016)	Described	*Fuchsia vulcanica* (Onagraceae) (Boevé et al. 2016)
Lagideus romius D.R. Smith, 1990	Described	
Perreyia fumipennis (Westwood, 1874)	Recorded by Smith (1990)	
Perreyia nigriceps (Westwood, 1874)	Recorded by Enderlein (1919; as *Perreyia melanopyga* Konow)	
Perreyia picea (Westwood, 1874)	Recorded by Smith (1990)	
Perreyia tropica (Norton, 1869)	Recorded by Rohwer (1921; as *Lophyorides modesta*)	Larvae travel in groups on the ground and feed on dead leaves and probably fungi (Flores et al. 2000)
Perreyiella sp.	Unidentified species recorded by Smith (1990)	
Family Tenthredinidae		
Subfamily Allantinae		
Probleta columbianus (Enderlein, 1920)	Recorded by Smith (2003)	
Subfamily Blennocampinae		
Metapedias torva (Konow, 1899a)	Described	
Metapedias spp.	At least two undescribed species are known (Smith, unpublished)	
Synaptoneura vopida D.R. Smith, 1973	Described	
Waldheimia atripennis (Fabricius, 1804)	Recorded by Boevé et al. (2016)	
Waldheimia erebus (W.F. Kirby, 1882)	Recorded by Boevé et al. (2016)	
Waldheimia galerita Konow, 1904	Described	
Waldheimia ochra (Norton, 1867)	Recorded by Enderlein (1920)	
Waldheimia pallens (Klug, 1816)	Not before recorded (Smith, unpublished)	
Waldheimia pellucida Konow, 1904	Recorded by Enderlein (1920; as *Waldheimia ochreiventris*)	
Waldheimia sulphurea (Fabricius, 1804)	Not before recorded (Smith, unpublished)	
Waldheimia spp.	At least seven or more undescribed species are known (Smith, unpublished)	
Subfamily Nematinae		
Pristiphora fernandezi D.R. Smith, 2003	Recorded by Boevé et al. (2016)	
Subfamily Selandriinae		
Adiaclema blandulum (Enderlein, 1920)	Described	
Adiaclema calvescens Enderlein, 1920	Not before recorded (Smith, unpublished)	
Adiaclema maculipennis (Cameron, 1883)	Not before recorded (Smith, unpublished)	
Adiaclema tetricum (Konow, 1908)	Recorded by Enderlein (1920; as *Stromboceros longicornis*)	

Taxon	Occurrence	Host
Adiaclema spp.	Several undescribed species occur (Smith, unpublished)	
Andeana farcta (Konow, 1900)	Described	
Aneugmenus merida D.R. Smith, 2005	Described	*Pteridium aqulinum* (Dennstaedtiaceae) (Smith 2005, Avila-Nunez et al. 2007)
Aneugmenus sp.	An unidentified species was recorded by Boevé et al. (2016)	
Belea nigripennis (Konow, 1908)	Described	
Bolivius notabilis (Konow, 1899b)	Recorded by Boevé et al. (2016)	
Dochmioglene spp.	Possibly several undescribed species from Ecuador (Smith, unpublished)	
Inea spp.	Probably two or more undescribed species are known (Smith, unpublished)	
Neoanapeptamena nitida (Strand, 1911)	Described	
Neoanapeptamena sp.	Probably an undescribed species (Smith, unpublished)	
Plaumanniana biclinia (Konow, 1899b)	Recorded by Boevé et al. (2016)	
Plaumanniana spp.	Three or four other species occur in Ecuador (Smith, unpublished)	
Proselandria alvina (Konow, 1899b)	Recorded by Boevé et al. (2016)	
Proselandria carminea (Jörgensen, 1913)	Recorded by Enderlein (1920; as *Strongylogaster ecuadoriensis*)	
Stromboceridea albilabris (Konow, 1885)	Recorded by Enderlein (1920)	
Stromboceridea spp.	Possibly two or three undescribed species (Smith, unpublished)	
Tioloma nigrita Strand, 1911	Described	
Family Xiphydriidae		
Derecyrta andrei Konow, 1897	Described	
Derecyrta flavescens D.R. Smith, 2004	Described	
Derecyrta striatifrons Malaise, 1942	Described	

50 unidentified ones. The numbers will undoubtedly increase when certain groups especially such as *Acordulecera* are revised or taxonomic problems resolved. However, many species described from other Andean countries should eventually be found in Ecuador, but these potential species are not listed. "Described" indicates that the species was originally described from Ecuador.

The following species are unplaced Tenthredinidae that have been described from Ecuador, but the correct combination has not yet been published or they are unpublished synonyms of species listed above (Smith, unpublished). Placement and synonymies will be made in papers in preparation.

Waldheimia ochreiventris Enderlein, 1920 (Blennocampinae).

Blennocampa albofemoralis Cameron, 1883; *Hylotoma analis* Fabricius, 1804; *Stromboceros cruralis* Konow, 1899b; *Stromboceros liscivus* Konow, 1908; *Stromboceros suppar* Konow, 1903b; *Stromboceros sutilis* Konow, 1903b; *Stromboceros torquatus* Konow, 1903b; *Stromboceros ustipennis* Konow, 1899b; *Strongylogaster ecuadoriensis* Enderlein, 1920; *Strongylogaster giganteus* Enderlein, 1920; *Strongylogaster longicornis* Enderlein, 1920; *Strongylogaster murcivena* Enderlein, 1920; *Strongylogaster obliquevenosa* Enderlein 1920; *Tioloma rosigenu* Enderlein, 1920 (Selandriinae).

Discussion

Sawfly adults were mainly found in shadowed places. Plants such as *Anthurium* and *Heliconia* have large leaves (Fig. 1), and sawfly adults were often observed running on the leaf surface. Fig. 1e–g illustrate by screenshots of a video sequence that an adult, probably of *Manaos mammeatus*, walked dozens of cm within a relatively short time (ca 20 sec), finally stopping in order to feed (Fig. 1g). Other sawfly species were observed showing the same behaviour of moving rapidly on the surface of leaves, such as *Manaos mulsus*, *Adiaclema* sp., *Waldheimia pellucida*, and *Proselandria alvina*. Since sawfly adults are known to feed on plant (floral and leaf) tissues, sugar sources (nectar and honeydew) as well as insect tissues and faeces (Jervis and Vilhelmsen 2000, Wäckers et al. 2005), it is likely that our observations relate to the uptake of food fragments of plant and/or animal origin. Large plant leaves accumulate such organic particles dropped from the canopy, which may facilitate their uptake by the sawflies.

During the three-week field trip in north-western and north-eastern Ecuador, only a single larva was collected, in Mindo (Hacienda San Vicente, 00°03'S, 078°45'W, 1530m, 23.11.2016, P4228, leg. J.-L. Boevé; Fig. 23). It was found along a forest path (Fig. 1l) and it probably belongs to the pergid genus *Acordulecera*. Their larvae are characterized by lateral sucker-like protuberances on abdominal segments 2–4 or 2–5 and 8 (Smith and Middlekauff 1987), and the collected larva possesses such protuberances (see Fig. 23c). It was feeding at the underside of a leaf of an unidentified plant (height 70 cm; Fig. 23a, b), probably a young tree. On 25.11.2016, the small prepupa (body length 3.5 mm) built a fragile cocoon of white silk appearance (Fig. 23d). The larvae of this genus feed gregariously, which was most probably the case for the larva collected, because it seems not plausible that this single larva consumed such an important leaf part (see Fig. 23a), and because it pupated a couple of days after having been collected (while its rearing was unsuccessful). Thus, it was presumably the last individual of a group.

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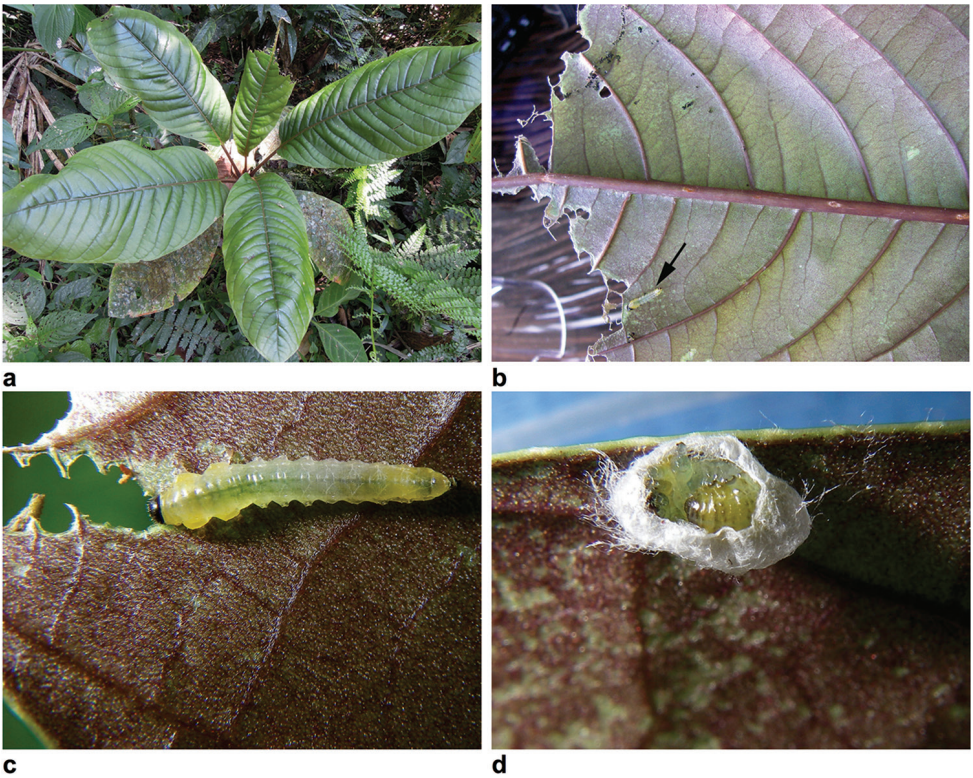

Figure 23. Pictures related to the single larva (P4228) found during a 3-week field trip. **a** Host plant with feeding damage on one of the leaves **b, c** Underside of that leaf occupied with the larva (*arrow*) **d** Cocoon partly damaged, showing the prepupa.

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Seventeen new genera of microgastrine parasitoid wasps (Hymenoptera, Braconidae) from tropical areas of the world

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Abstract

As part of comprehensive studies on the world fauna of microgastrine parasitoid wasps (Hymenoptera: Braconidae) 17 new genera and 29 new species are described from the Afrotropical, Australasian, Neotropical and Oriental regions. The number of extant genera of Microgastrinae is increased by 21% and currently stands at 81. It is anticipated that more genera will be described in the near future, when phylogenetic studies of the group are advanced. The new taxa showcase unusual morphological traits such as atypical head and mouth part modifications, presence of partial occipital and epicnemial carinae, propodeum carination patterns, hind wing venation, trochantellus shape, tarsal claws, sculpture and shape of the first two metasomal tergites, and ovipositor teeth; in some cases, they also represent some of the largest species known in the subfamily. For every new genus putative autapomorphies, morphological diagnostic features, and DNA barcodes (whenever available) are presented, as well as brief discussions of some informal groupings of genera in the subfamily. However, no attempt is made to reassess the phylogeny of the entire Microgastrinae, as that will require more comprehensive analyses beyond the scope of the present work. The following 17 **gen. n.**, authored by Fernandez-Triana, are described: *Agupta*, *Austinicotesia*, *Billmasonius*, *Carlmuesebeckius*, *Gilbertnixonius*, *Janhalacaste*, *Jenopappius*, *Jimwhitfieldius*, *Kotenkosius*, *Markshawius*, *Ohenri*, *Qrocodiledundee*, *Silvaspinosus*, *Tobleronius*, *Ungunicus*, *Ypsilonigaster* and *Zachterbergius*. The following 29 **sp. n.**, authored by Fernandez-Triana and Boudreault, are described: *Agupta danyi*, *Agupta jeanphilippei*, *Agupta raymondi*, *Agupta solangeae*, *Austinicotesia indonesiensis*, *Austinicotesia papuanus*, *Billmasonius cienci*, *Carlmuesebeckius smithsonian*, *Gilbertnixonius biem*, *Janhalacaste danieli*, *Janhalacaste guanacastensis*, *Janhalacaste winnieae*, *Jenopappius magyarmuzeum*, *Jimwhitfieldius jamesi*, *Jimwhitfieldius sydneyae*, *Kotenkosius tricarinatus*, *Markshawius erucidoctus*,

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Markshawius francescae, *Markshawius thailandensis*, *Ohenri gouletorum*, *Qrocodiledundee outbackense*, *Silvaspinosus vespa*, *Tobleronius orientalis*, *Ungunicus vietnamensis*, *Ypsilonigaster naturalis*, *Ypsilonigaster sharkeyi*, *Ypsilonigaster tiger*, *Ypsilonigaster zuparkoi*, and *Zachterbergius tenuitergum*. The following four **comb. n.** are proposed: *Jenopappius niger* (de Saeger, 1944), *Jenopappius aethiopica* (de Saeger, 1944), *Ypsilonigaster bumbana* (de Saeger, 1942), and *Ypsilonigaster pteroloba* (de Saeger, 1944).

Keywords

Microgastrinae, new genera, taxonomy, morphology, DNA barcoding, Afrotropical, Australasian, Neotropical, Oriental

Introduction

The braconid subfamily Microgastrinae is one of the most speciose groups of animals on Earth (Whitfield et al. 2018), with over 2,700 described species at present (Yu et al. 2016). The known diversity of microgastrine wasps has increased dramatically, with almost half of the species being described during the past 50 years (data extracted from Yu et al. 2016). However, the actual species richness of the subfamily has been estimated to be much higher – between 17,000 and 46,000 species (Rodriguez et al. 2013). This means that we likely know just 5–15% of the world's species in this group of parasitoid wasps.

At the generic level the situation is similar, as the number has increased significantly for the past half century, from 20 genera in 1965, to 50 in 1981, and up to 63 genera recognized at present (Nixon 1965, Mason 1981, Whitfield et al. 2018). Still, much remains to be done. Some genera are apparently polyphyletic (e.g., *Diolcogaster*, *Glyptapanteles*, see Mason 1981, Austin and Dangerfield 1992), while others contain such a large number of species that it is almost impossible to work with them (e.g., *Apanteles* has 900–1,200+ described species, depending on the generic concept used, see Fernandez-Triana et al. 2014a, Yu et al. 2016). To complicate things more, many specimens in collections cannot be confidently assigned to any of the currently described genera, with most representing new lineages never dealt with taxonomically.

As a taxonomic category, the genus has special properties that distinguish it from both the less inclusive species group and the more inclusive higher taxonomic levels (Tattersall 2014). It is perhaps the easiest category to pinpoint without further investigation (Anderson 1940, Simpson 1943, Atran 1987, Garbino 2015) and it is considered as one of the most operationally useful taxonomic categories (Allmon 1992, Tattersall 2014, Garbino 2015). Despite its relevance both in taxonomic practice and in communication, the genus as a category is rarely discussed among zoologists in general (Dubois 1988).

This paper describes 17 new genera of Microgastrinae and discusses future steps towards a more resolved classification of the genera in this subfamily. It is a continuation of comprehensive studies on the world fauna of microgastrine parasitoid wasps, and includes species from all tropical regions (Afrotropical, Australasian, Neotropical and Oriental), thereby making the names and morphological concepts available for future phylogenetic studies.

Methods

The specimens studied for this paper are deposited in the California Academy of Sciences, San Francisco, United States (CAS), Canadian National Collection of Insects, Ottawa, Canada (CNC), Muséum National d'Histoire Naturelle, Paris, France (MNHN), Naturalis Biodiversity Centre, Leiden, The Netherlands (RMNH), and Queen Sirikit Botanic Gardens, Mae Rim District, Thailand (QSBG).

Morphological terms and measurements of structures follow those used by Mason (1981), Huber and Sharkey (1993), Whitfield (1997), Karlsson and Ronquist (2012), and Fernandez-Triana et al. (2014a). The abbreviations F2, F3, F14 and F15 refer to antennal flagellomeres 2, 3, 14 and 15; T1, T2, and T3 are used for metasomal mediotergites 1, 2, and 3; and L and W refer to length and width respectively. The fore wing second submarginal cell is mentioned throughout the text as "areolet" for the sake of brevity.

When referring to taxa that are related in a broader sense (e.g., "*Choeras* s.l.") "s.l." is used as an abbreviation of "sensu lato". When referring to taxa that are related in a stricter sense (e.g., "*Sathon* s.str.") "s.str." is used as an abbreviation of "sensu stricto".

The descriptions of the new species contain a general but brief account of color, sculpture, and details on morphological features and ratios commonly used in taxonomic studies of Microgastrinae. Raw measurements of morphological structures (in mm) are also provided, which would allow for additional ratios to be explored in the future. When presenting raw measurements, the holotype value is given first, followed by the range of other specimens between parentheses.

In the species descriptions, the holotype labels are detailed verbatim, with / separating the different lines of each label. For paratypes, specimen information was generated using the CNC database (http://www.cnc-ottawa.ca/taxonomy/TaxonMain.php).

For some specimens, DNA barcodes (the 5' region of the cytochrome c oxidase I (CO1) gene, Hebert et al. 2003) are available. DNA extracts were obtained from single legs using a glass fibre protocol (Ivanova et al. 2006). Total genomic DNA was re-suspended in 30 µl of dH2O, a 658 base pairs (bp) region near the 5' terminus of the CO1 gene was amplified using standard primers (LepF1–LepR1) following established protocols (http://v4.boldsystems.org/index.php), and a composite sequence was generated for all successful amplifications. All information for the sequences associated with each individual specimen barcoded can be retrieved from the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007). We use the Barcode Index Number (BIN) System to discuss species limits, following the BIN concept detailed in Ratnasingham and Hebert (2013). Sequences from the specimens used in this paper were compared with 35,000+ sequences of Microgastrinae available in BOLD as of January 2018.

Photos were taken either with a Keyence VHX-1000 Digital Microscope or with a Leica camera on a Leica M165 C Microscope, using lens with a range of 10–130 ×. Multiple images were taken of a structure through the focal plane and then combined to produce a single in-focus image using the software associated with the Keyence Sys-

tem or, for the images taken with the Leica camera, the Zerene Stacker program (http://zerenesystems.com/cms/stacker). Images were corrected using Adobe Photoshop CS4, the plates were prepared using Microsoft PowerPoint 2010 and later saved as .tiff files.

Results

Systematics

A total of 17 new genera and 29 new species of Microgastrinae are described below. In this paper we do not provide a key to separate the different genera, as a following publication will provide a comprehensive key to all genera of Microgastrinae of the world. However, in the diagnostic description of each new genus we present characters to separate it from the closest (or most similar) taxa, which are sufficient for the time being –as the new genera are very distinct morphologically.

Genera are presented in alphabetical order. A key to species is provided if there are multiple species in the genus, followed by species descriptions, also in alphabetical order.

Agupta Fernandez-Triana, gen. n.

http://zoobank.org/66F44A5A-A630-4C7B-8566-239342221E33

Type species. *Agupta jeanphilippei* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Head relatively elongate. Face, clypeus and labrum with coarse and dense punctures. Face projection between antennal base with median carina. Malar line relatively long. Mouth parts elongate, including bilobate glossa (as in Figs 1B, 2B, 3B, 8B). First few flagellomeres with placodes irregularly distributed (so that at times three rows could be distinguished but other times rows are not clearly defined). Anteromesoscutum relatively long (longer than maximum width). Scutoscutellar sulcus relatively wide and deep, with strong crenulae. Propodeum with strongly raised median carina which has strong lateral carinae radiating across its length (Figs 1E, 2E, 3E, 4F, 5E, 6D, 7E). Fore wing with small, slit-shaped areolet. Fore wing vein (RS+M)b much longer than areolet width (Figs 1C, 3C, 4C, 6C, 7C, 8C). Metacoxa smooth and relatively long (reaching beyond posterior margin of T3). T1 relatively strongly narrowing from anterior margin to half of tergite, then parallel sided up to posterior margin (Figs 1E, 2E, 3D, E, 4E, F, 5E, 6E); T1 anterior half mostly smooth, strongly concave and with central sulcus; posterior half punctured and with a polished area on posterior margin. Hypopygium folded and with several pleats. Ovipositor sheaths setose and about same length as metatibia. Specimens of the genus are among the largest within Microgastrinae (body length and fore wing length almost always 5 mm or more, reaching up to 6.6 mm in the largest specimens).

Putative autapomorphies and potentially related genera. From a morphological perspective, this genus seems to be related to *Choeras* (and several related groups

considered to be part of *Choeras*; e.g., see comments on Austin and Dangerfield 1992 and also Discussion below). From those "*Choeras* s.l." taxa, *Agupta* is unusual because a number of features. The antenna in males (and sometimes in females) has the first few flagellomeres with placodes irregularly distributed in three rows, or no row can be clearly defined. The mouth parts are elongate, including a bilobated glossa. The propodeum has a strongly raised median carina that has small radiating carinae across its length. The shape of T1 is also distinctive (Figs 1C–E, 2E, 3C–E, 4E–F, 5C–E, 6C, E, 7E). The large size of most specimens in *Agupta* is second only to *Larissimus*, which is the largest known Microgastrinae genus (Nixon 1965).

Biology. Host unknown.

Distribution. The known species are found in the Australasian and Oriental regions.

Molecular data. Three of the species described below have DNA barcodes available, corresponding to BINs BOLD:ADE1110 and BOLD:ADE1550. There are at least 25 additional BINs that cluster as a group and likely represent additional species of *Agupta*; however, they are not described in this paper. Overall, the *Agupta* BINs are clearly separated from dozens of other "*Choeras* s.l." sequences in BOLD.

Etymology. The genus name refers to and honors the Indian braconid expert Ankita Gupta in recognition of her significant contributions to the knowledge of Microgastrinae and other parasitoid wasp groups of India. It has been a pleasure to collaborate with Ankita over the past few years and we hope she continues to shine as one of the best Indian taxonomists. The gender of the genus is neuter.

Species. We describe below four new species for the genus. However, as the molecular data suggests, there are probably dozens of additional species awaiting description. The four new species can be separate using the following key.

Key to species

1 Most wing veins golden-yellow, except for pterostigma and veins r, 2RS, 2M and 3RSa (Figs 6C, 7C, 8C) **2**

– All veins dark brown (Figs 1C, 3C, 4C, 5C) **3**

2 Body mostly dark brown, except for white laterotergites 1–3 (Figs 7A, E, 8A) .... ***Agupta solangeae*** **Fernandez-Triana & Boudreault, sp. n.**

– Body lighter coloured, with mesosoma mostly yellow-orange (except for posterior 0.4 of mesopleuron and posterior half of metapleuron which are dark brown), and metasoma with extensive white areas (posterior 0.2–0.3 of T1, T2 and T3 laterally, and most laterotergites) (Figs 6A, C–E)..... ..... ***Agupta raymondi*** **Fernandez-Triana & Boudreault, sp. n**

3 Body mostly dark brown to reddish-brown, including most of legs (Figs 4–5) ....***Agupta jeanphilippei*** **Fernandez-Triana & Boudreault, sp. n**

– Body lighter coloured, with mesosoma yellow-orange and first two pairs of legs mostly yellow-white (Figs 1–3)..... .....***Agupta danyi*** **Fernandez-Triana & Boudreault, sp. n**

***Agupta danyi* Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/01128C76-FE81-41D6-B87C-2CEA6AD446B5
Figs 1, 2, 3

Holotype. Female, Malaysia, RMNH.

Holotype labels. MALAYSIA:SE SABAH/nr Danum Valley Field C./WON1, Mal. trap 5, c 150m/2–23.viii.1987, RMNH'87/C.v.Achterberg &D.Kennedy. Second label: CNC497189.

Holotype locality. MALAYSIA, South East Sabah, near Danum Valley, Field C, 150m.

Paratypes. Malaysia. (1♀ CNC), Sabah, SE Sabah nr Danum Valley Field C., 150m, Malaise trap, 19.iv–22.v.1988, coll. C.v. Achterberg & T. Burghouts, Voucher code: CNC497186; (1♂ RMNH), SE Sabah nr Danum Valley Field C., 150m, Malaise trap, 20.vi–12.vii.1987, coll. C.v. Achterberg & D. Kennedy, Voucher code: CNC497188; (1♂ 1♀ RMNH), near Danum Valley Field C, 150m, Malaise trap, 15.iv–15.v.1987, coll. C.v. Achterberg & D. Kennedy, Voucher codes: CNC924662, CNC924666; (2♀ RMNH), 20.ii–19.iii.1988, coll. C.v. Achterberg & T. Burghouts, Voucher codes: CNC924663, CNC924664; (1♂ RMNH), 22.xi–4.xii.1987, coll. C.v. Achterberg & D. Kennedy, Voucher code: CNC924665; (1♀ RMNH), 23.viii-13.xi.1987, coll. C.v. Achterberg & D. Kennedy, Voucher code: CNC924661; (1♀ RMNH), near Danum Valley, Field C, 150m, Malaise trap, 26.x–22.xi.1987, coll. C.v. Achterberg, Voucher code: CNC924660.

Diagnosis. The dark brown color of wing veins separates this species from *A. solangeae* and *A. raymondi*, both of which have most veins golden-yellow. The lighter color (yellow-orange or yellow-white) of mesosoma and first two pairs of legs will in turn differentiate *A. danyi* from *A. jeanphilippei*, which has body mostly dark brown to reddish-brown.

Description. Female. Head and most of metasoma dorsally dark brown; mesosoma yellow-orange; first two pairs of legs mostly yellow-white, third pair mostly dark brown (except for anterior 0.6 of metatibia yellow-white); scape and pedicel yellow, flagellomeres light to dark brown; wings with veins dark brown. Head relatively elongate. Face, clypeus and labrum with coarse and dense punctures. Face projection between antennal base with median carina. Malar line relatively long. Mouth parts elongate, including bilobate glossa. First few flagellomeres with placodes irregularly distributed (so that at times three rows could be distinguished but other times rows are not clearly defined). Anteromesoscutum relatively long (longer than maximum width). Scutoscutellar sulcus relatively wide and deep, with 4–5 strong crenulae. Propodeum with strongly raised median carina which has strong lateral carinae radiating across its length. Fore wing with small, slit-shaped areolet. Fore wing vein (RS+M)b much longer than areolet width. Metacoxa smooth and relatively long (reaching beyond posterior margin of T3). T1 relatively strongly narrowing from anterior margin to half of tergite, then parallel sided up to posterior margin; anterior half mostly smooth, strongly concave and with central sulcus; posterior half punctured and a polished area on posterior margin. Hypopygium folded and with several pleats. Ovipositor sheaths

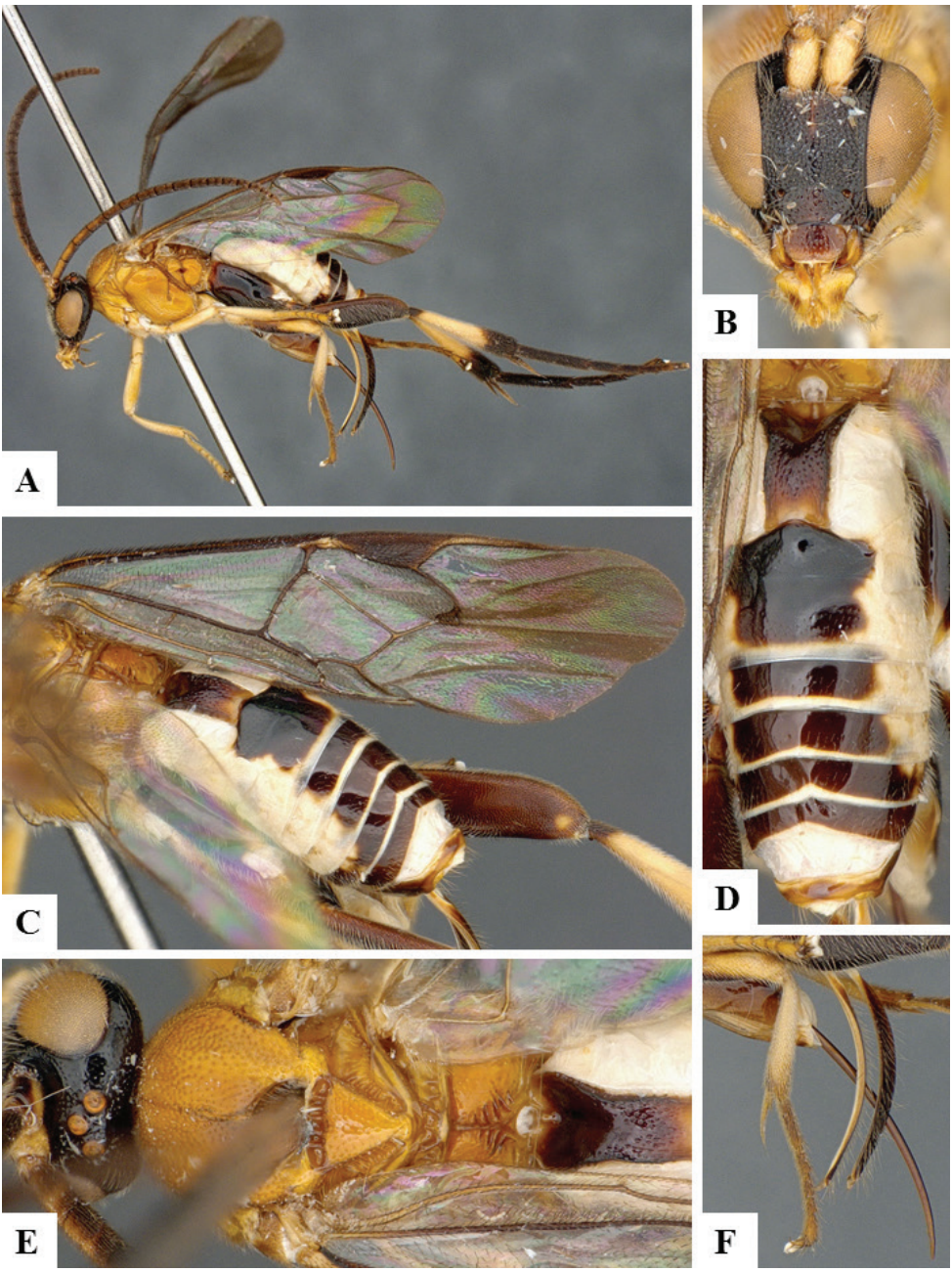


Figure 1. *Agupta danyi* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Metasoma dorsal **E** Head and mesosoma, dorsal **F** Ovipositor and ovipositor sheaths.

setose and about same length of metatibia. **Female body measurements (mm).** F2 L: 0.45 (0.40–0.43); F3 L: 0.43 (0.38–0.41); F14 L: 0.25 (0.22–0.24); F15 L: 0.22 (0.20–0.23); Malar sulcus L: 0.12 (0.12–0.13); Mandible W: 0.23 (0.20–0.23); T1

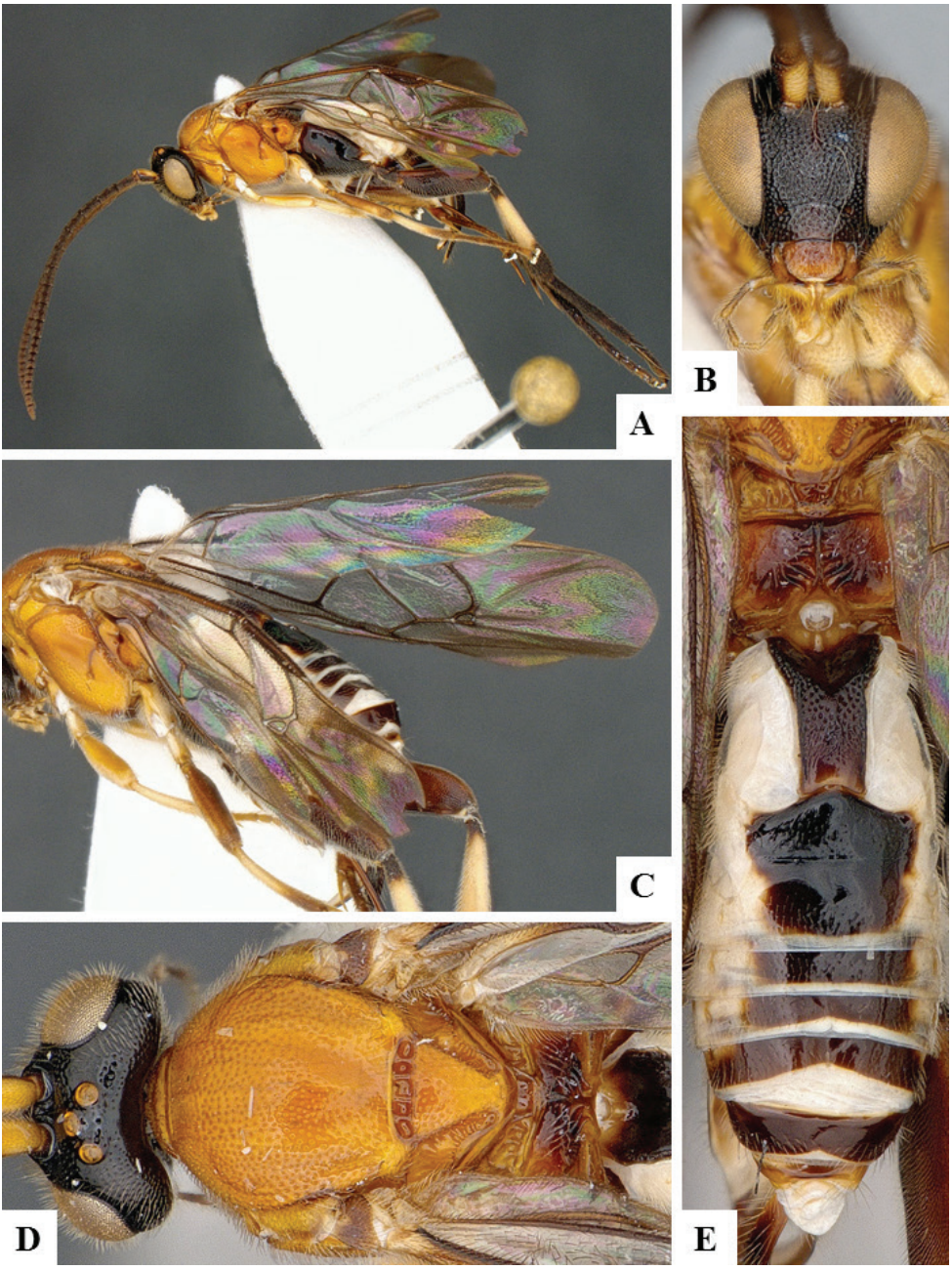


Figure 2. *Agupta danyi* female paratype CNC497186. **A** Habitus **B** Head frontal **C** Fore wing **D** Head and mesosoma, dorsal **E** Propodeum and metasoma, dorsal.

L: 1.05 (0.95–1.06); T1 W at posterior margin: 0.37 (0.33–0.35); T1 Maximum W: 0.61 (0.54–0.65); T2 W at anterior margin: 0.86 (0.79–0.93); T2 W at posterior margin: 0.88 (0.83–0.93); T2 L: 0.37 (0.32–0.38); Metafemur L: 1.63 (1.68–1.76);

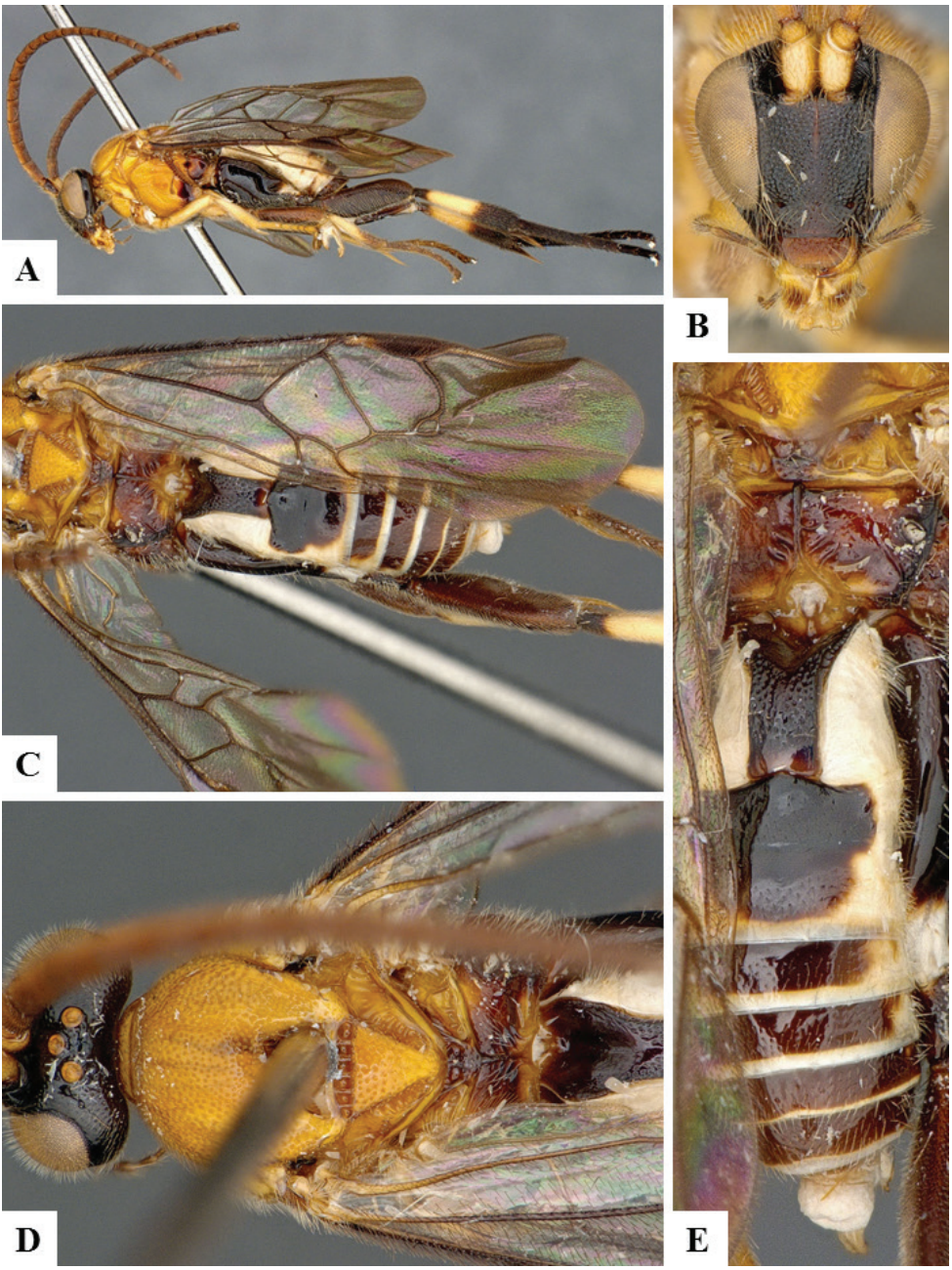


Figure 3. *Agupta danyi* male paratype CNC497188. **A** Habitus **B** Head frontal **C** Fore wing **D** Head and mesosoma, dorsal **E** Propodeum and metasoma, dorsal.

Metafemur W: 0.60 (0.56–0.60); Metatibia L: 2.22 (2.12–2.28); Inner spur L: 0.84 (0.79–0.92); Outer spur L: 0.43 (0.38–0.43); First segment of Metatarsus L: 1.44 (1.34–1.40); Ovipositor sheaths L: 2.17 (2.11–2.52); Body L: 6.19 (5.15–6.00); Fore

wing L: 6.19 (5.40–6.13). Ovipositor sheaths L is approximate for 5 specimens. Fore wing L is approximate for 1 specimen.

Male. As female, but propodeum and metapleuron slightly darker in color. Specimens are also slightly smaller (body and fore wing lengths around 0.7 mm smaller than in female specimens. **Male body measurements (mm).** F2 L: 0.43; F3 L: 0.43; F14 L: 0.38; F15 L: 0.34; Malar sulcus L: 0.13; Mandible W: 0.23; T1 L: 1.00; T1 W at posterior margin: 0.30; T1 maximum W: 0.58; T2 W at anterior margin: 0.80; T2 W at posterior margin: 0.85; T2 L: 0.36; Metafemur L: 1.59; Metafemur W: 0.57; Metatibia L: 71.98; Inner spur L: 0.88; Outer spur L: 0.39; First segment of Metatarsus L: 1.33; Body L: 5.45; Fore wing L: 5.55.

Biology. Host unknown.

Distribution. Malaysia, Sabah.

Molecular data. The holotype (sequence AAHYM352-16 in BOLD) rendered a partial DNA barcode (369 bp) which represents a unique species (when compared with 35,000+ sequences of Microgastrinae available in BOLD).

Etymology. The second author dedicates this species to her husband Dany Girard, as an appreciation for his love, many years of shared magical moments and wonderful trips.

Agupta jeanphilippei **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/7B7701AD-248A-4AF5-BAA4-1778CFFC29C4
Figs 4, 5

Holotype. Female, Malaysia, RMNH.

Holotype labels. MALAYSIA:SE SABAH/nr Danum Valley Field C./WON1, Mal. trap 5, c 150m/20.ii-19.iii.1988, RMNH'89/C.v.Achterberg & T.Burghouts. Second label: CNC497192.

Holotype locality. MALAYSIA, South East Sabah, near Danum Valley Field C, 150m.

Paratypes. Malaysia. (1♂ CNC), Sabah, SE Sabah nr Danum Valley Field C., 150m, Malaise trap, 20–26.iii.1987, coll. C.v. Achterberg, Voucher codes: CNC497191; (1♂1♀ RMNH), near Danum Valley Field C, 150m, Malaise trap, 13.ix-4.x.1987, coll. C.v. Achterberg & D. Kennedy, Voucher codes: CNC924648, CNC924654; (1♂ RMNH), 14–20.iii.1987, coll. C.v. Achterberg, Voucher code: CNC924655; (1♂ RMNH), 20.vi–12.vii.1987, coll. C.v. Achterberg & D. Kennedy, Voucher code: CNC924647; (2♀ RMNH), 22.v–16.vi.1988, coll. C.v. Achterberg & T. Burghouts, Voucher codes: CNC924650, CNC924651; (1♂ RMNH), 24.ii–18.iii.1987, coll. C.v. Achterberg, Voucher code: CNC924653; (1♂ 1♀ RMNH), 140m, Malaise trap, 24.ii-24.iii.1987, coll. C.v. Achterberg, Voucher codes: CNC924649, CNC924652; (4♂ RMNH), 150m, Malaise trap, 26.iii–19.iv.1987, coll. C.v. Achterberg & D. Kennedy, Voucher codes: CNC924646, CNC924656, CNC924658, CNC924659; (1♂ RMNH), 5–26.v.1987, coll. C.v. Achterberg & D. Kennedy, Voucher code: CNC924657.

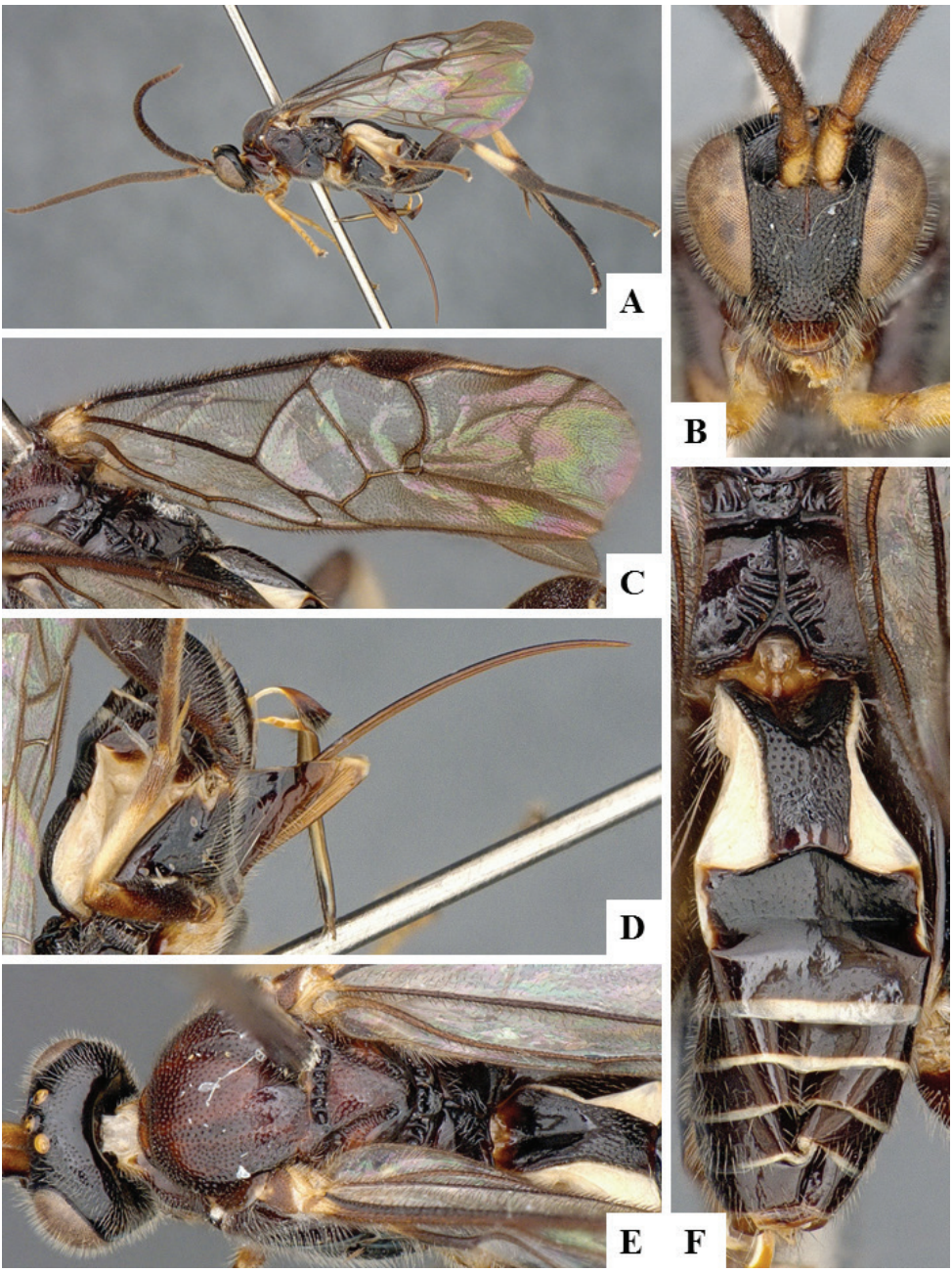


Figure 4. *Agupta jeanphilippei* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Ovipositor and ovipositor sheaths **E** Head and mesosoma, dorsal **F** Propodeum and metasoma, dorsal.

Diagnosis. The dark brown color of wing veins separates this species from *A. solangeae* and *A. raymondi*, both of which have most veins golden-yellow. The body mostly dark brown to reddish-brown will in turn differentiate *A. jeanphilippei* from

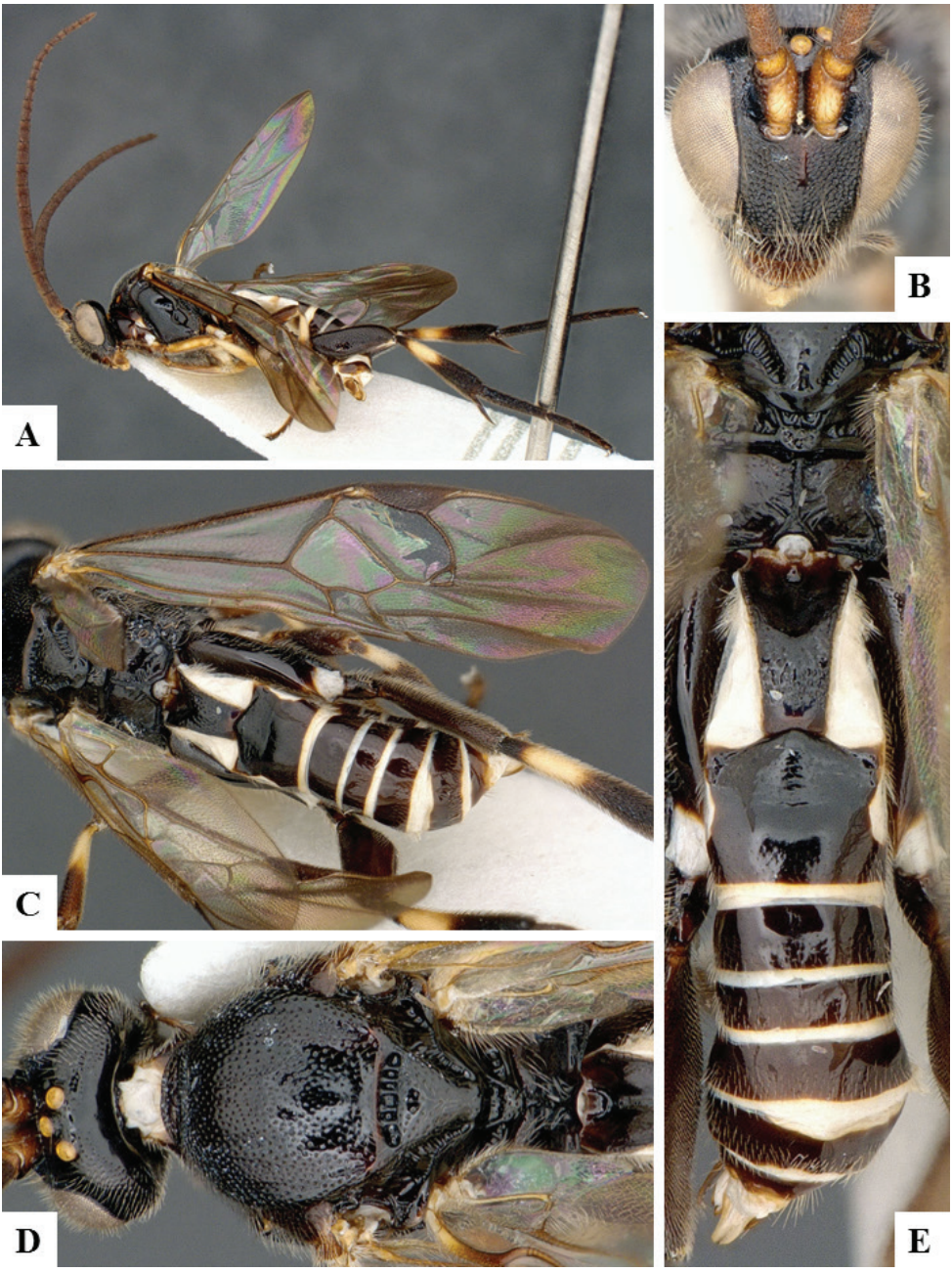


Figure 5. *Agupta jeanphilippei* male paratype CNC497191. **A** Habitus **B** Head frontal **C** Fore wing **D** Head and mesosoma, dorsal **E** Propodeum and metasoma, dorsal.

A. danyi, which has a lighter color (yellow-orange or yellow-white) of mesosoma and first two pairs of legs.

Description. Female. Head and most of metasoma dorsally dark brown to black; mesosoma dark brown to black, except for anteromesoscutum and scutellar disc red-

dish-brown; first pair of legs mostly yellow-orange, second pair mostly brown but with anterior 0.6 of mesotibia white, third pair mostly dark brown to black (except for central yellow-white band on metatibia); scape yellow, pedicel and flagellomeres brown; wings with veins dark brown. Head relatively elongate. Face, clypeus and labrum with coarse and dense punctures. Face projection between antennal base with median carina. Malar line relatively long. Mouth parts elongate, including bilobate glossa. First few flagellomeres with placodes irregularly distributed (so that at times three rows could be distinguished but other times rows are not clearly defined). Anteromesoscutum relatively long (longer than maximum width). Scutoscutellar sulcus relatively wide and deep, with 4–5 strong crenulae. Propodeum with strongly raised median carina which has strong lateral carinae radiating across its length. Fore wing with small, slit-shaped areolet. Fore wing vein (RS+M)b much longer than areolet width. Metacoxa smooth and relatively long (reaching beyond posterior margin of T3). T1 relatively strongly narrowing from anterior margin to half of tergite, then parallel sided up to posterior margin; anterior half mostly smooth, strongly concave and with central sulcus; posterior half punctured and a polished area on posterior margin. Hypopygium folded and with several pleats. Ovipositor sheaths setose and about same length of metatibia. **Female body measurements (mm).** F2 L: 0.42 (0.41–0.42); F3 L: 0.41 (0.38–0.40); F14 L: 0.25 (0.22–0.24); F15 L: 0.23 (0.21–0.22); Malar sulcus L: 0.12 (0.09–0.13); Mandible W: 0.23 (0.20–0.22); T1 L: 1.09 (0.99–1.07); T1 W at posterior margin: 0.33 (0.31–0.37); T1 maximum W: 0.61 (0.59–0.62); T2 W at anterior margin: 0.96 (0.89–1.00); T2 W at posterior margin: 0.95 (0.92–0.97); T2 L: 0.36 (0.33–0.37); Metafemur L: 1.79 (1.67–1.76); Metafemur W: 0.61 (0.58–0.59); Metatibia L: 2.30 (2.14–2.28); Inner spur L: 0.86 (0.81–0.88); Outer spur L: 0.38 (0.40–0.43); First segment of Metatarsus L: 1.45 (1.34–1.46); Ovipositor sheaths L: 2.41 (2.24–2.41); Body L: 5.40 (4.60–6.38); Fore wing L: 6.19 (5.70–6.25). Ovipositor sheaths L is approximate for 4 specimens. Maximum W of T1 is approximate for 1 specimen.

Male. As female, but general body color darker (including most mesosoma black, and smaller central band centrally in metatibia), and pedicel yellow. **Male body measurements (mm).** F2 L: 0.45; F3 L: 0.43; F14 L: 0.36; F15 L: 0.34; Malar sulcus L: 0.13; Mandible W: 0.23; T1 L: 0.98; T1 W at posterior margin: 0.28; T1 maximum W: 0.59; T2 W at anterior margin: 0.86; T2 W at posterior margin: 0.80; T2 L: 0.40; Metafemur L: 1.63; Metafemur W: 0.54; Metatibia L: 1.98; Inner spur L: 0.83; Outer spur L: 0.33; First segment of Metatarsus L: 1.31; Body L: 6.25; Fore wing L: 5.70.

Biology. Host unknown.

Distribution. Malaysia, Sabah.

Molecular data. One male paratype (CNC497191) rendered an almost complete DNA barcode (615 bp), which represents a unique BIN (BOLD:ADE1110), with 5.4% of bp difference compared to the next species in BOLD, which is another *Agupta* species.

Etymology. The second author dedicates this species to her brother Jean-Philippe Boudreault as an appreciation for his love, fun conversations, good laughs and shared memories. Jean-Philippe has been bugging me to have a species named in his honor for over two years now, so here it is!

Agupta raymondi **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/1B965FAB-8A5D-4701-A958-428805DA80E6
Fig. 6

Holotype. Female, Malaysia, RMNH.

Holotype labels. MALAYSIA-SW SABAH/nr Long Pa Sia (West)/c. 1010m, 1–4. IV.1987/Mal. trap 1, RMNH'87/C.v.Achterberg. Second label: CNC497187.

Holotype locality. MALAYSIA, South West Sabah, near Long Pa Sia (West), 1010m.

Diagnosis. The golden-yellow color of most veins separates this species from *A. danyi* and *A. jeanphilippei* (both of which have wing veins dark brown). The lighter colour of body, with mesosoma mostly yellow-orange and metasoma with extensive white areas, will in turn differentiate *A. raymondi* from *A. solangeae* (which has the body mostly dark brown).

Description. Female. Head and most of metasoma dorsally dark brown (except for white on posterior 0.2–0.3 of T1, T2 and T3 laterally, and most laterotergites); mesosoma mostly yellow-orange (except for dark brown on posterior 0.4 of mesopleuron and posterior half of metapleuron); first pair of legs mostly yellow-orange, second and third pairs mostly brown but with anterior 0.6 of mesotibia white; scape and pedicel bright yellow-white, flagellomeres light to dark brown; wings with most veins golden-yellow (except for pterostigma and veins r, 2RS, 2M and 3RSa). Head relatively elongate. Face, clypeus and labrum with coarse and dense punctures. Face projection between antennal base with median carina. Malar line relatively long. Mouth parts elongate, including bilobated glossa. First few flagellomeres with placodes irregularly distributed (so that at times three rows could be distinguished but other times rows are not clearly defined). Anteromesoscutum relatively long (longer than maximum width). Scutoscutellar sulcus relatively wide and deep, with 6 strong crenulae. Propodeum with strongly raised median carina which has strong lateral carinae radiating across its length. Fore wing with small, slit-shaped areolet. Fore wing vein (RS+M)b much longer than areolet width. Metacoxa smooth and relatively long (reaching beyond posterior margin of T3). T1 relatively strongly narrowing from anterior margin to half of tergite, then parallel sided up to posterior margin; anterior half mostly smooth, strongly concave and with central sulcus; posterior half punctured and a polished area on posterior margin. Hypopygium folded and with several pleats. Ovipositor sheaths setose and slightly longer than metatibia length. **Female body measurements (mm).** F2 L: 0.40; F3 L: 0.38; F14 L: 0.22; F15 L: 0.21; Malar sulcus L: 0.13; Mandible W: 0.20; T1 L: 1.01; T1 W at posterior margin: 0.30; T1 maximum W: 0.58; T2 W at anterior margin: 0.75; T2 W at posterior margin: 0.80; T2 L: 0.38; Metafemur L: 1.65; Metafemur W: 0.54; Metatibia L: 2.10; Inner spur L: 0.80; Outer spur L: 0.38; First segment of Metatarsus L: 1.36; Ovipositor sheaths L: 2.37; Body L: 5.70; Fore wing L: 6.19.

Male. Unknown.

Biology. Host unknown.

Distribution. Malaysia, Sabah.

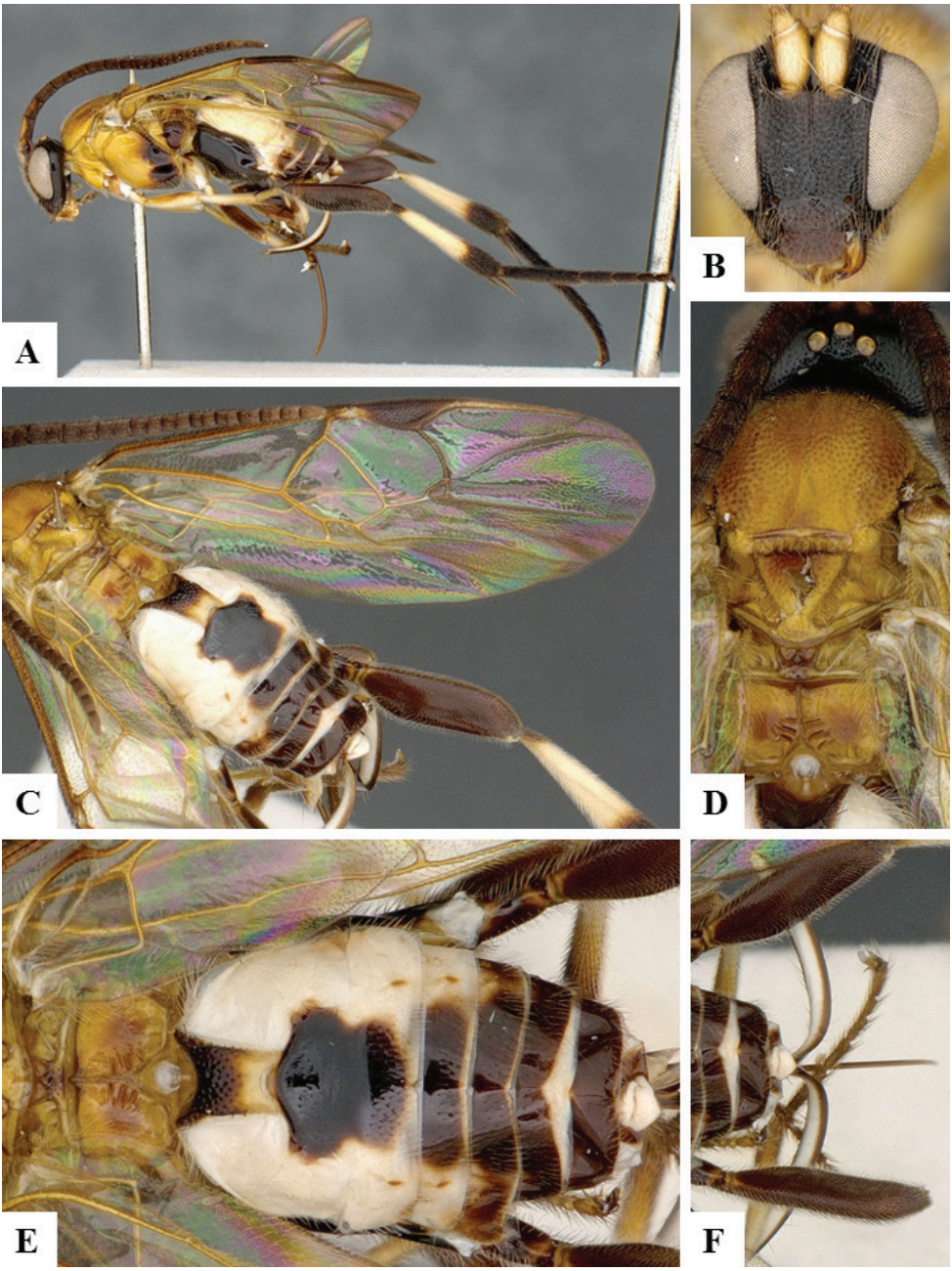


Figure 6. *Agupta raymondi* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Mesosoma dorsal **E** Propodeum and metasoma, dorsal **F** Ovipositor and ovipositor sheaths.

Molecular data. The holotype rendered an almost complete DNA barcode (596 bp), which represents a unique BIN (BOLD:ADE1550), with 5.3% of bp difference compared to the next species in BOLD, which is another *Agupta* species. However,

the sequence is similar to that of the holotype of *A. solangeae*, in spite of clear morphological differences between the two species. It is possible that this situation is a lab contamination, but sequencing of more specimens from both species will be needed to determine whether this is the case or not.

Etymology. The second author dedicates this species to her father Raymond Boudreault, as an appreciation for his love, fun and fascinating conversations, good laughs and tremendous kindness.

Agupta solangeae **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/7C291A9D-6D51-493A-A206-811B6224D834
Figs 7, 8

Holotype. Female, Malaysia, RMNH.

Holotype labels. MALAYSIA-SW SABAH/nr Long Pa Sia (East)/c.1000m, 1–13.IV.1987/Mal. trap 5, RMNH'87/C.v.Achterberg. Second label: CNC497193.

Holotype locality. MALAYSIA, South West Sabah, near Long Pa Sia (East), 1000m.

Paratypes. Malaysia. (1♀ CNC), MALAYSIA: Sabah, SW Sabah nr Long Pa Sia, Payakalaba, 1010m, Malaise trap, 25.xi–1.xii.1987, coll. C.v. Achterberg, Voucher code: CNC497190; (2♀ RMNH), near Long Pa Sia (East), 1000m, Malaise trap, 1–13.iv.1987, coll. C.v. Achterberg, Voucher codes: CNC924643, CNC924644.

Diagnosis. The golden-yellow color of most veins separates this species from *A. danyi* and *A. jeanphilippei* (both of which have wing veins dark brown). The darker body color, mostly dark brown, will in turn differentiate *A. solangeae* from *A. raymondi* (which has a lighter coloured body, with mesosoma mostly yellow-orange and metasoma with extensive white areas).

Description. Body mostly dark brown (except for white laterotergites 1–3); first pair of legs mostly yellow-orange or yellow-brown, second and third pairs mostly brown (except for anterior 0.3 of mesotibia and anterior 0.5 of metatibia white); scape and pedicel yellow-brown, flagellomeres brown; wings with most veins golden-yellow (except for pterostigma and veins r, 2RS, 2M and 3RSa). Head relatively elongate. Face, clypeus and labrum with coarse and dense punctures. Face projection between antennal base with median carina. Malar line relatively long. Mouth parts elongate, including bilobate glossa. First few flagellomeres with placodes irregularly distributed (so that at times three rows could be distinguished but other times rows are not clearly defined). Anteromesoscutum relatively long (longer than maximum width). Scutoscutellar sulcus relatively wide and deep, with 5–6 strong crenulae. Propodeum with strongly raised median carina which has strong lateral carinae radiating across its length. Fore wing with small, slit-shaped areolet. Fore wing vein (RS+M)b much longer than areolet width. Metacoxa smooth and relatively long (reaching beyond posterior margin of T3). T1 relatively strongly narrowing from anterior margin to half of tergite, then parallel sided up to posterior margin; anterior half mostly smooth, strongly concave and with

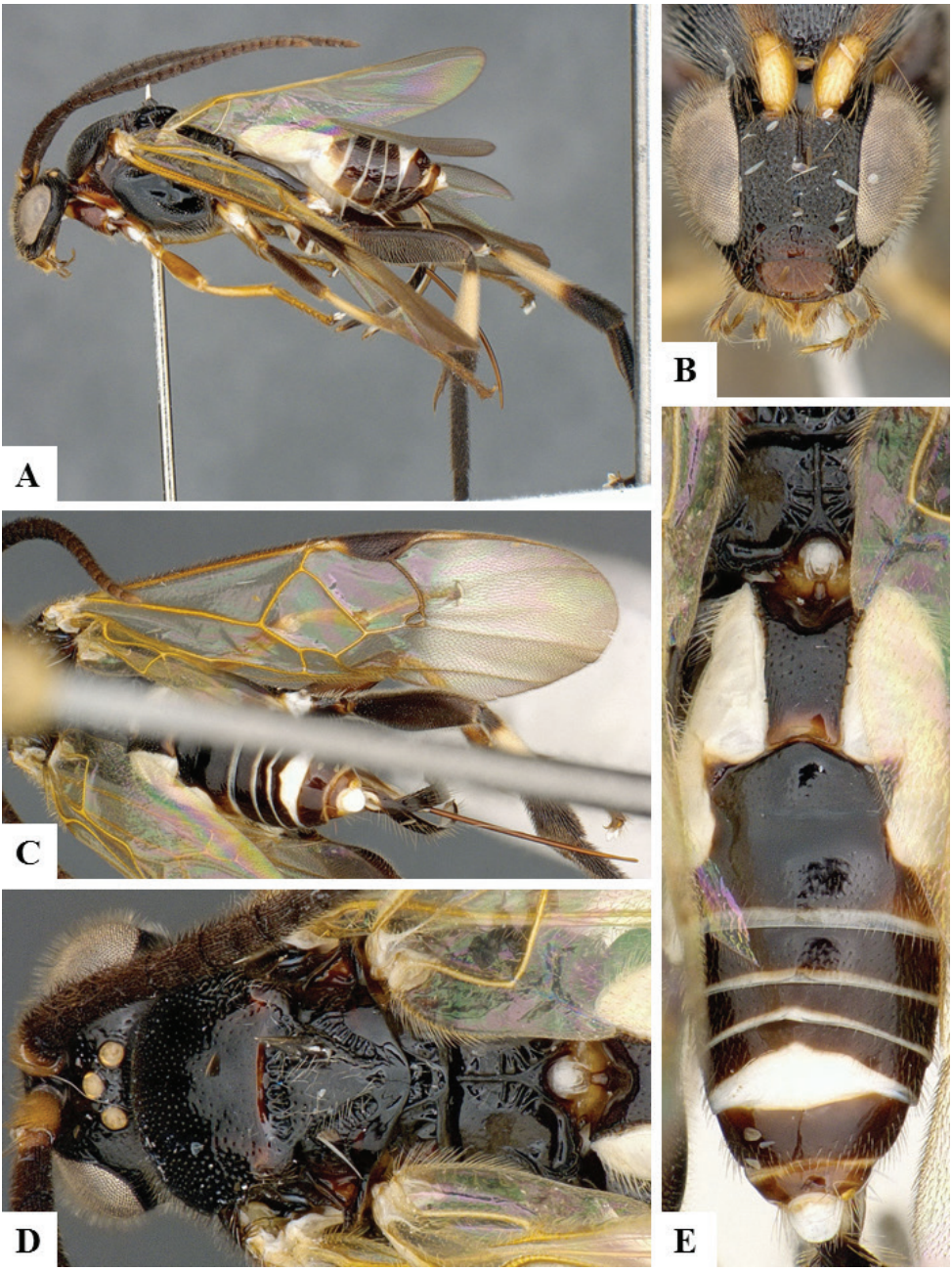


Figure 7. *Agupta solangeae* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Head and mesosoma, dorsal **E** Propodeum and metasoma, dorsal.

central sulcus; posterior half punctured and a polished area on posterior margin. Hypopygium folded and with several pleats. Ovipositor sheaths setose and slightly longer than metatibia length. **Female body measurements (mm).** F2 L: 0.43 (0.40–0.43);

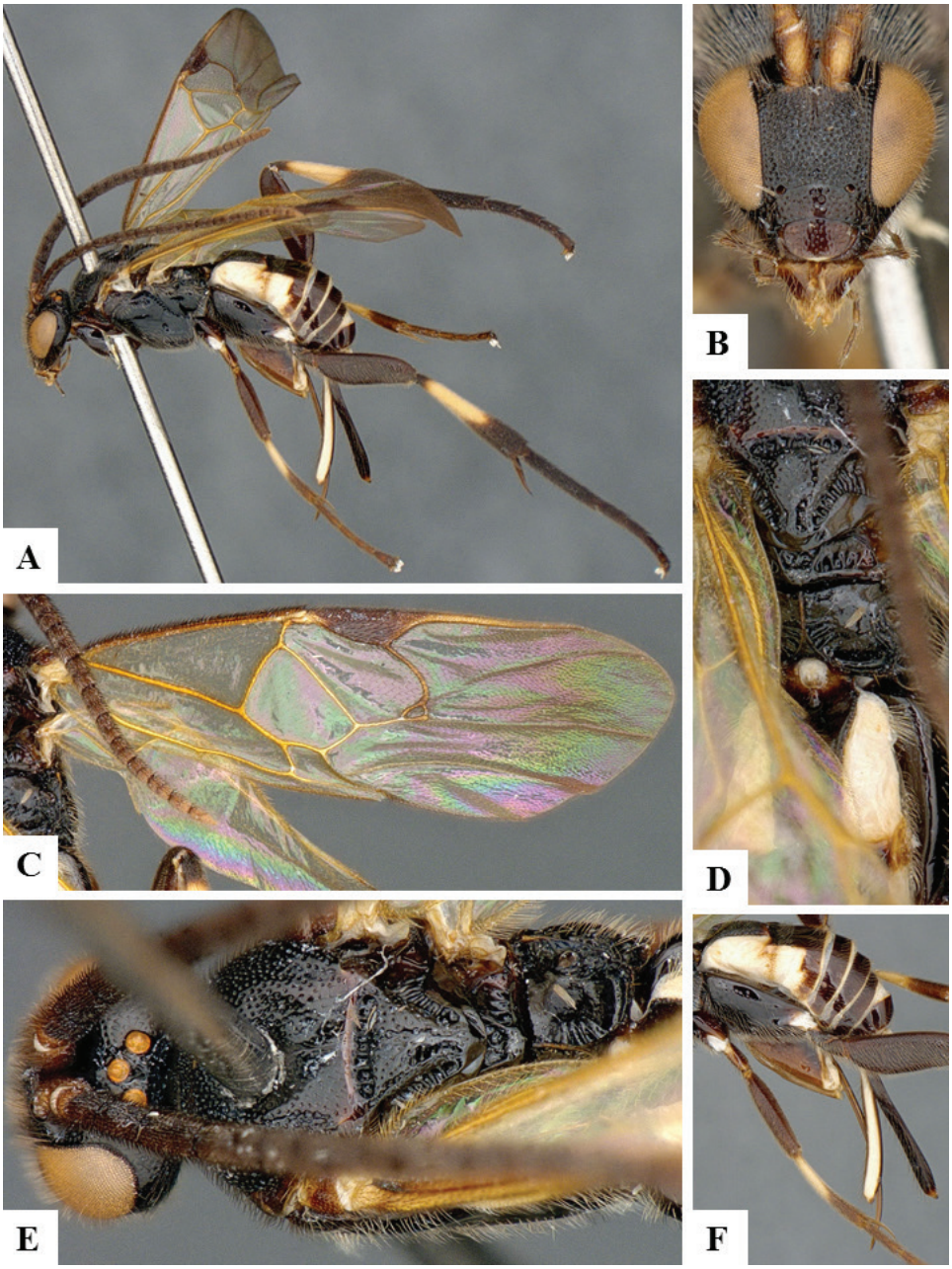


Figure 8. *Agupta solangeae* female paratype CNC497190. **A** Habitus **B** Head frontal **C** Fore wing **D** Propodeum **E** Head and mesosoma, dorsal **F** Ovipositor and ovipositor sheaths.

F3 L: 0.41 (0.37–0.41); F14 L: 0.23 (0.21–0.24); F15 L: 0.21 (0.20–0.22); Malar sulcus L: 0.13 (0.12–0.13); Mandible W: 0.23 (0.20–0.23); T1 L: 1.10 (1.00–1.05); T1 W at posterior margin: 0.33 (0.33–0.36); T1 maximum W: 0.62 (0.57–0.58); T2 W at

anterior margin: 0.83 (0.83–0.89); T2 W at posterior margin: 0.84 (0.81–0.91); T2 L: 0.38 (0.33–0.38); Metafemur L: 1.76 (1.71–1.76); Metafemur W: 0.62 (0.55–0.58); Metatibia L: 2.36 (2.16–2.28); Inner spur L: 0.88 (0.80–0.84); Outer spur L: 0.41 (0.37–0.42); First segment of Metatarsus L: 1.44 (1.43–1.49); Ovipositor sheaths L: 2.62 (2.49–2.59); Body L: 6.63 (5.55–6.25); Fore wing L: 6.56 (5.95–6.06). Ovipositor sheaths L is approximate for 2 specimens. Maximum W of T1, T1 L, T1 W at apex, T2 L, T2 W at base and T2 W at apex are approximate for one specimen.

Male. Unknown.

Biology. Host unknown.

Distribution. Malaysia, Sabah.

Molecular data. The holotype rendered an almost complete DNA barcode (625 bp), which represents a unique BIN (BOLD:ADE1550), with 5.3% of bp difference compared to the next species in BOLD, which is another *Agupta* species. See comments under previous species about similarities of DNA sequences from both species.

Etymology. The second author dedicates this species to her mother Solange Nourry, as an appreciation for her love, nice conversations, great generosity and shared sweet moments.

Austinicotesia **Fernandez-Triana, gen. n.**

http://zoobank.org/CF4FB143-7A9D-4C3E-A24C-7B8B646D0912

Type species. *Austinicotesia indonesiensis* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Head with mouth relatively narrow, resulting in a relatively very large (but rather transverse) malar line (Figs 9B, 10B). Distance between tentorial pits 0.4 × width of head at that same height. Palpi elongate, reaching beyond pronotum when extended (Fig. 9A). Pronotum enlarged dorsally, its median length (on a dorsal view) very large, much longer than width of flagellomeres, and clearly longer than propodeum in most Microgastrinae genera. Pronotum dorsally with a deep central notch and strong punctures on posterior margin (Figs 9H, 10E). Pronotum laterally with only ventral groove present. Anteromesoscutum with relatively deep punctures, each with one seta in the middle (Figs 9H, 10E). Propodeum with strongly defined and raised carinae, delimiting an areola (on posterior half) and a central carina (on anterior half), as well as transverse carinae that fork around spiracles (Figs 9F, 10D, E). Fore wing without areolet, with vein 2RS much longer than vein r. Pterostigma relatively very thin, its length at least 3.5 × its maximum width (Fig. 9D). Hind wing with vein 2r-m absent (Fig. 10C). Hind wing with vannal lobe fully setose. Metacoxa relatively short, not surpassing posterior margin of T2 (Fig. 9A). Metafemur relatively short and thick (Fig. 9A). Metatibia spurs very short, less than 0.3 × length of first segment of metatarsus (Fig. 9A). T1 widening towards posterior margin, and with a strong hump centrally followed by a deep, excavated area which is delimited by strong carinae (Figs 9G, F, 10D, E). Hypopygium uniformly sclerotized. Ovipositor sheaths uniformly setose and clearly shorter than metatibia length (Fig. 9G).

Putative autapomorphies and potentially related genera. From a morphological perspective, this genus could only be confused with *Austrocotesia* (based on similar palpi length, anteromesoscutum sculpture, propodeum carination pattern, hind wing lacking vein 2r-m, and uniformly sclerotized hypopygium). But there are a number of features separating both genera. *Austinicotesia* has a central notch dorsally on pronotum which is almost unique within Microgastrinae (as far as we know it is only present in a couple of *Miropotes* species, see Fernandez-Triana et al. 2014c); fore wing without areolet (areolet present in *Austrocotesia*); fore wing with pterostigma relatively thin and long, 3.5 × as long as wide (pterostigma much less than 3.0 × as long as wide in *Austrocotesia*); fore wing vein 2RS much longer, around 1.5 ×, than vein r (fore wing vein 2RS much shorter, around 0.5 ×, than vein r in *Austrocotesia*); metafemur relatively thick and stout (of more normal proportions in *Austrocotesia*); T1 widening towards posterior margin and with strong hump followed by deeply excavated area and strong carinae (T1 more or less parallel-sided or narrowing towards posterior margin and without hump or excavate area in *Austrocotesia*); and T2 mostly smooth (usually mostly sculptured in *Austrocotesia*). Still, the two genera seem to be related and additional studies, especially molecular, might change in the future our current understanding of these two taxa.

Biology. Host unknown.

Distribution. The known species are found in the Australasian region (Indonesia and Papua New Guinea).

Molecular data. No molecular data available.

Etymology. The genus name refers to and honors the Australian braconid expert Andrew Austin in recognition of his significant contributions to the knowledge of Microgastrinae and other parasitoid wasp groups from Australasia and other regions. The second part of the genus name refers to its putative relationship with *Austrocotesia*. The gender of the genus is neuter.

Species. We describe below two new species for the genus. Three other specimens we saw in collections have some morphological differences, and might represent up to two additional species. However, as the material available to us for study is limited (and the morphological differences are rather subtle) we prefer to consider only two species for the time being. They can be separate using the following key.

Key to species

1 Labrum dark brown; palpi either brown (anterior 1–2 segments of maxillary palp and most segments of labial palp) or yellowish-brown (Fig. 10B); legs mostly dark brown to black (except for yellow-brown protarsus and ventral face of procoxa) (Fig. 10A); wings slightly infumated; anteromesoscutum punctures relatively sparser (separation between punctures 2.0–4.0 × puncture diameter) (Fig. 10E); joining of veins r and 2Rs not angulated (Fig. 10C) [Papua New Guinea, at altitudes of 700–1200 m] .... ***Austinicotesia papuanus*** **Fernandez-Triana & Boudreault, sp. n.**

– Labrum orange; palpi white (Figs 9B, C); all tarsi, mesocoxa, protibia and most of mesotibia yellow-brown (Figs 9A, E); wings hyaline; anteromesoscutum punctures relatively closer (separation between punctures 1.0–2.0 × puncture diameter) (Fig. 9H); joining of veins r and 2Rs strongly angulated (Fig. 9D) [Indonesia island of Halmahera, at 115–175 m]..... .....***Austinicotesia indonesiensis*** **Fernandez-Triana & Boudreault, sp. n.**

Austinicotesia indonesiensis **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/DA8BDB7C-E2CD-40DF-AF41-D44D363BFCCC
Fig. 9

Holotype. Female, Indonesia, RMNH.

Holotype labels. INDONESIA:S Halmahera/20 km S Payahe, Sagutora/Mal. trap 13, c 115 m/18.ii-18.iii.1995/C.v.Achterberg, R. de Vries &/Y.Yasir, RMNH'95. Second label: CNC878550.

Holotype locality. INDONESIA, South Halmahera, 20 km South of Payahe, Sagutora, 115 m.

Paratype. Indonesia. (1♀ CNC) South Halmahera, 20km S Payahe, Sagutora, 175m, Malaise trap, 18.ii-18.iii.1995, coll. C.v. Achterberg, Y. Yasir & R. de Vries, Voucher code: CNC878551.

Diagnosis. *Austinicotesia indonesiensis* body coloration is generally lighter than that of *A. papuanus* (labrum orange, palpi white, with all tarsi, procoxa and mesocoxa, protibia and most of mesotibia yellow-brown) and by having wings hyaline. It also has anteromesoscutum punctures relatively closer (separation between punctures 1.0–2.0 × puncture diameter), and the joining of veins r and 2Rs is strongly angulated.

Description. Female. Head, mesosoma, legs (see below for exceptions) and anterior half of T1 mostly black, rest of metasoma mostly dark or light brown; palpi and apical metatarsomeres white, labrum and anterior metatarsomere light yellow-brown; wings with veins and pterostigma mostly brown to light brown. Head with mouth relatively narrow, resulting in a relatively very large (but rather transverse) malar line. Face shiny but with sparse, uniformly distributed and shallow punctures. Distance between tentorial pits 0.4 × width of head at that same height. Labrum somewhat depressed. Mandibles relatively small. Glossa elongate. Palpi elongate, reaching beyond pronotum when extended. Antenna heavily setose, setae relatively long. Pronotum enlarged dorsally, its median length (on a dorsal view) very large, much longer than width of flagellomeres, and clearly longer than propodeum in most Microgastrinae genera. Pronotum dorsally with a deep central notch and strong punctures on posterior margin. Pronotum laterally with only ventral groove present. Anteromesoscutum with deep punctures, each with one seta in the middle; separation between punctures 1.0–2.0 × puncture diameter. Propodeum with strongly defined and raised carinae, delimiting an areola (on posterior half) and a central carina (on anterior half), as well as transverse carinae that fork around spiracles. Fore wing without areolet, with vein 2RS much

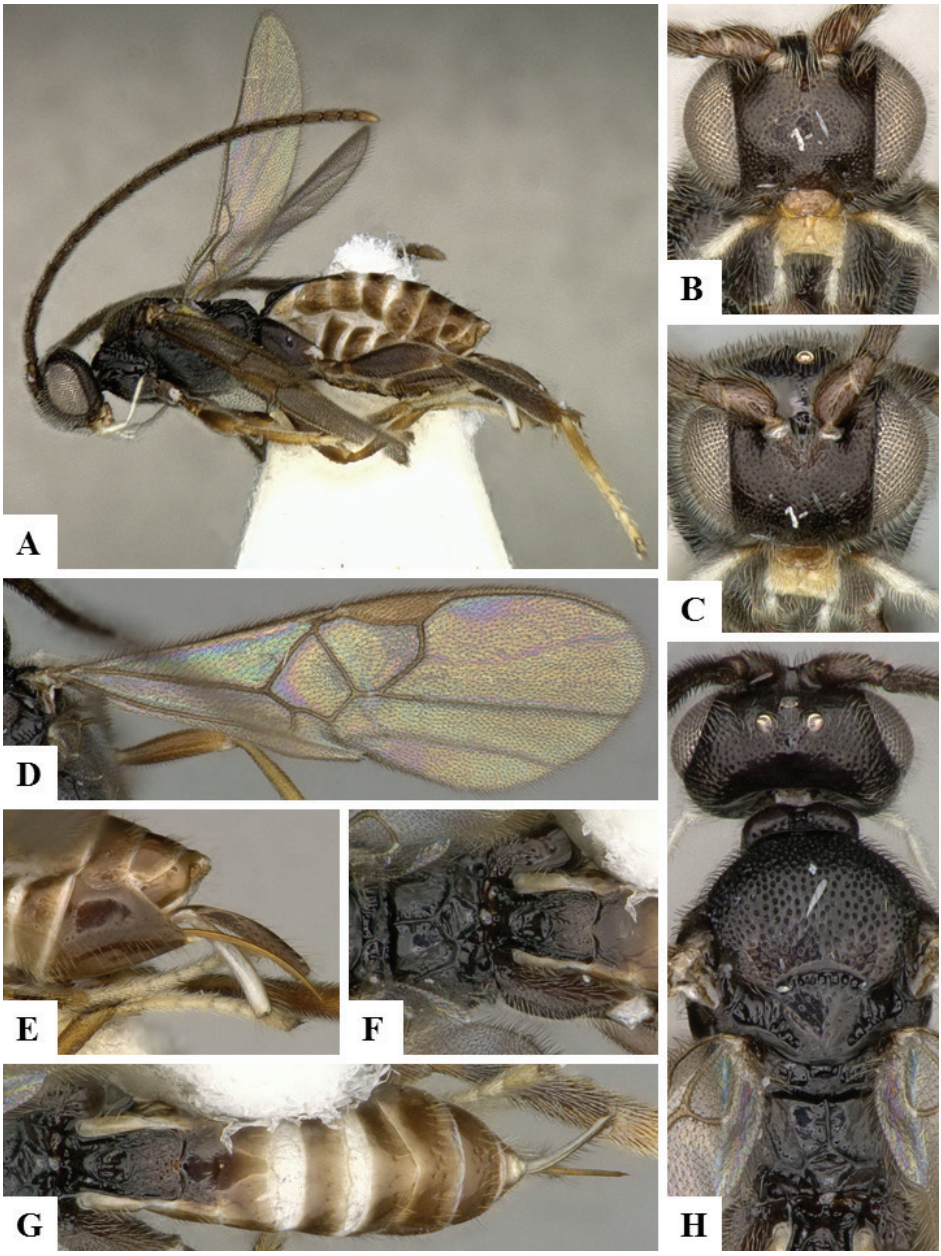


Figure 9. *Austinicotesia indonesiensis* female holotype. **A** Habitus **B** Head fronto-ventral **C** Head frontal **D** Fore wing **E** Ovipositor and ovipositor sheaths **F** Propodeum and tergite 1 **G** Metasoma dorsal **H** Head and mesosoma, dorsal.

longer than vein r (joining of both veins strongly angulated). Pterostigma relatively very thin, its length at least 3.5 × its maximum width. Hind wing with vein 2r-m absent. Hind wing with vannal lobe fully setose. Metacoxa relatively short, not surpassing

posterior margin of T2. Metafemur relatively short (less than 1.3 × as long as metacoxa) and thick (its length 2.7–3.0 × its width). Metatibia spurs very short, less than 0.3 × length of first segment of metatarsus. T1 widening towards posterior margin, and with a strong hump centrally followed by a deep, excavated area which is delimited by strong carinae. T2 mostly smooth, with lateral margins strongly defined. T3+ entirely smooth. Hypopygium uniformly sclerotized. Ovipositor sheaths uniformly setose and clearly shorter than metatibia length. **Body measurements (mm).** F2 L: 0.23 (0.19); F3 L: 0.23 (0.20); F14 L: 0.11; F15 L: 0.10; Malar sulcus L: 0.07 (0.06); Mandible W: 0.08 (0.06); T1 L: 0.39 (0.34); T1 W at posterior margin: 0.18 (0.16); T1 maximum W: 0.21 (0.19); T2 W at anterior margin: 0.21 (0.18); T2 W at posterior margin: 0.20 (0.20); T2 L: 0.12 (0.10); Metafemur L: 0.58 (0.52); Metafemur W: 0.19 (0.19); Metatibia L: 0.89 (0.67); Inner spur L: 0.14 (0.13); Outer spur L: 0.12 (0.12); First segment of Metatarsus L: 0.41 (0.31); Ovipositor sheaths L: 0.54 (0.39); Body L: 2.75 (2.28); Fore wing L: 2.50 (2.22).

Male. Unknown.

Biology. Host unknown.

Distribution. Indonesia.

Molecular data. No molecular data available.

Etymology. Named after the country of the type locality.

Austinicotesia papuanus **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/1377255B-BE1D-432F-9A15-6B8784DB4EB1
Fig. 10

Holotype. Female, Papua New Guinea, MNHN.

Holotype labels. Papua New Guinea./Mount Wilhelm, Plot 3/5.72090°S, 145.27150°E,/1200m, 25–26.X.2012,. Second label: MT, understorey, Philip,/MAL-MW1200C-01/16-/d01, **CNC924668**. Third label: CNC924668.

Holotype locality. PAPUA NEW GUINEA, Mount Wilhelm, Plot 3, 5.72090°S, 145.27150°E, 1200m, understorey.

Paratypes. Papua New Guinea. (1♀ CNC), Madang, Mount Wilhelm, Plot 1, -5.720874, 145.269500, 1200m, understory, Malaise trap, 1–2.xi.2012, coll. Philip, Alois, Novotny & Leponce, Voucher code: CNC924672; (1♂ MNHN), Mount Wilhelm, Plot 3, -5.720903 145.271500, 1200m, understory, Malaise trap, 25–26.x.2012, coll. Philip, Alois, Novotny & Leponce, Voucher code: CNC924674; (1♂ MNHN), -5.732514, 145.256800, 700m, understory, Malaise trap, 28–29.x.2012, coll. Keltim, Uma, Novotny & Leponce, Voucher code: CNC924676; (1♂ MNHN), 3–4.xi.2012, coll. Keltim, Uma, Novotny & Leponce, Voucher code: CNC924669; (1♀ MNHN), 5–6.xi.2012, coll. Keltim, Uma, Novotny & Leponce, Voucher code: CNC924670; (1♀ MNHN), -5.720903, 145.271500, 1200m, understory, Malaise trap, 8–9.xi.2012, coll. Philip, Alois, Novotny & Leponce, Voucher code: CNC924671; (1♂ MNHN), Mount Wilhelm, Plot 4, -5.731961, 145.252200, 700m, understory, Malaise trap,

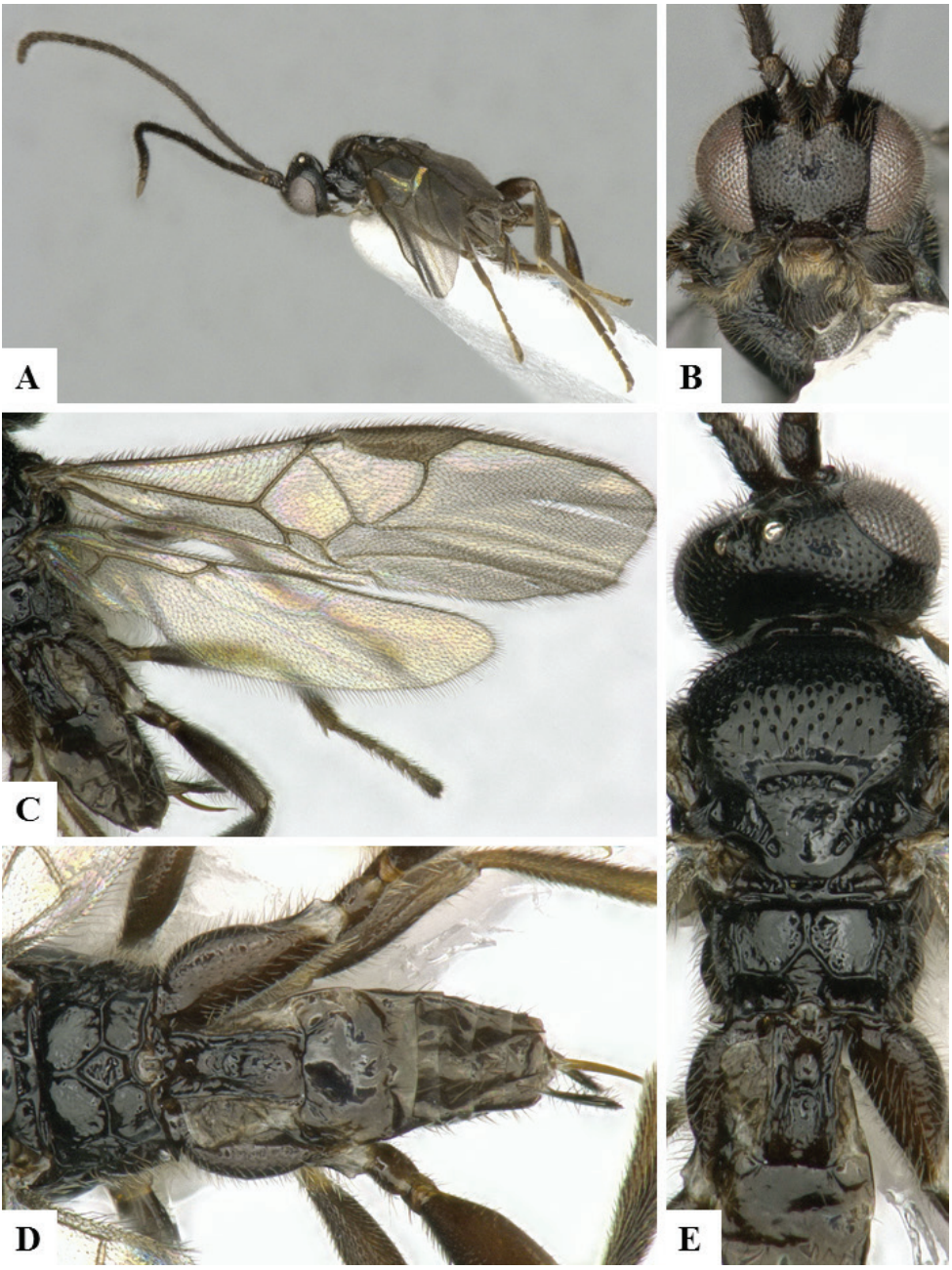


Figure 10. *Austinicotesia papuanus* female holotype. **A** Habitus **B** Head frontal **C** Fore wing and hind wing **D** Propodeum and metasoma, dorsal **E** Head and mesosoma , dorsal.

25–26.x.2012, coll. Keltim, Uma, Novotny & Leponce, Voucher code: CNC924677; (1♂ MNHN), 31.x–1.xi.2012, coll. Keltim, Uma, Novotny & Leponce, Voucher code: CNC924673; (1♂ MNHN), Mount Wilhelm, plot 4, -5.731961 145.252200,

700m, understory, Malaise trap, 25–26.x.2012, coll. Keltim, Uma, Novotny & Leponce, Voucher code: CNC924675.

Diagnosis. *Austinicotesia papuanus* body coloration is generally darker than that of *A. indonesiensis* (labrum, palpi and legs mostly dark brown to black) and by having wings slightly infumated. It also has anteromesoscutum punctures relatively sparser (separation between punctures 2.0–4.0 × puncture diameter), and the joining of veins r and 2Rs is not angulated.

Description. Female. Body mostly dark brown to black (except for yellow-brown protarsus, ventral face of procoxa, and some apical segments of palpi); wings slightly infumated, with veins and pterostigma brown. Head with mouth relatively narrow, resulting in a relatively very large (but rather transverse) malar line. Face shiny but with sparse, uniformly distributed and shallow punctures. Distance between tentorial pits 0.4 × width of head at that same height. Labrum somewhat depressed. Mandibles relatively small. Glossa slightly elongate. Palpi elongate, reaching beyond pronotum when extended. Antenna heavily setose, setae relatively long. Pronotum enlarged dorsally, its median length (on a dorsal view) very large, much longer than width of flagellomeres, and clearly longer than propodeum in most Microgastrinae genera. Pronotum dorsally with a deep central notch and strong punctures on posterior margin. Pronotum laterally with only ventral groove present. Anteromesoscutum with deep punctures, each with one seta in the middle; separation between punctures 2.0–4.0 × puncture diameter. Propodeum with strongly defined and raised carinae, delimiting an areola (on posterior half) and a central carina (on anterior half), as well as transverse carinae that fork around spiracles. Fore wing without areolet, with vein 2RS much longer than vein r (but joining of both veins not angulated). Pterostigma relatively very thin, its length at least 3.5 × its maximum width. Hind wing with vein 2r-m absent. Hind wing with vannal lobe fully setose. Metacoxa relatively short, not surpassing posterior margin of T2. Metafemur relatively short (less than 1.3 × as long as metacoxa) and thick (its length 2.6–2.8 × its width). Metatibia spurs very short, less than 0.3 × length of first segment of metatarsus. T1 more or less parallel-sided, and with a strong hump centrally followed by a deep, excavated area which is delimited by strong carinae. T2 mostly smooth, with lateral margins well defined. T3+ entirely smooth. Hypopygium uniformly sclerotized. Ovipositor sheaths uniformly setose and clearly shorter than metatibia length. **Body measurements (mm).** F2 L: 0.19 (0.20–0.22); F3 L: 0.18 (0.21); F14 L: 0.08 (0.09–0.10); F15 L: 0.08 (0.09); Malar sulcus L: 0.04 (0.05–0.07); Mandible W: 0.07 (0.08–0.11); T1 L: 0.30 (0.32–0.35); T1 W at posterior margin: 0.14 (0.15–0.18); T1 maximum W: 0.14 (0.15–0.18); T2 W at anterior margin: 0.14 (0.16–0.20); T2 W at posterior margin: 0.27 (0.24–0.29); T2 L: 0.11 (0.09–0.16); Metafemur L: 0.50 (0.49–0.63); Metafemur W: 0.18 (0.19–0.23); Metatibia L: 0.69 (0.68–0.81); Inner spur L: 0.13 (0.12–0.16); Outer spur L: 0.09 (0.08–0.13); First segment of Metatarsus L: 0.32 (0.27–0.38); Ovipositor sheaths L: 0.33 (0.38–0.43); Body L: 1.76 (2.08–2.26); Fore wing L: 2.24 (2.22–2.60). Maximum W of T1 is taken at posterior margin of T1 where it is the largest for 4 specimens. T1 L is approximate for 1 specimen. One specimen has no head.

Male. As female.
Biology. Host unknown.
Distribution. Papua New Guinea.
Molecular data. No molecular data available.
Etymology. Named after the country of the type locality.

***Billmasonius* Fernandez-Triana, gen. n.**
http://zoobank.org/516782E6-1FD7-4C25-8E1E-D97380FCB01F

Type species. *Billmasonius cienci* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Head and mesosoma mostly smooth, at most with areas with sparse and shallow punctures. Posteromedian band of scutellum smooth. Propodeum entirely smooth but with partial median carina defined posteriorly (Fig. 11E). Fore wing with small, slit-shaped areolet. Hind wing with vannal lobe entirely setose. Unique T1 shape (better illustrated in Fig. 11D–F), with relatively wide anterior 0.6 and strongly narrowed posterior 0.4, so that widest part of tergite (near anterior margin) is around 4.0 × narrowest width (along posterior 0.4). Anterior 0.6 of T1 mostly desclerotized (only with lateral margins and narrow central strip sclerotized), a totally unique pattern within Microgastrinae. Area surrounding spiracles on laterotergite 2 partially sclerotized and same color than T2, giving the impression of T2 having "three peaks" (the largest and central one being the actual T2, the two smallest and lateral ones being the area surrounding spiracles on laterotergites (better illustrated in Fig. 11E–F). T4-7 with thin desclerotized area medially near posterior margin, giving the appearance of terga being pushed forward medially (Fig. 11E). Hypopygium medially desclerotized, with several pleats. Ovipositor sheaths clearly shorter than metatibia length.

Putative autapomorphies and potentially related genera. The shape and degree of sclerotization of T1 and T2 are unusual among known species of Microgastrinae. A somewhat similar shape of T1 is also found in *Tobleronius*, another genus described below, but the latter genus is completely unrelated (based on characters of the scutellar complex, very different carination pattern of propodeum, shape of T4–T7, and wing venation). *Billmasonius* does not seem to have any close or clear relationship to any described genera in the subfamily.

Biology. Host unknown.

Distribution. The only species known is found in the Oriental region (Thailand).

Molecular data. The DNA barcode of the holotype specimen (BIN BOLD:AAH1264) is very unique, 10.4% different from the closest Microgastrinae sequence in BOLD.

Etymology. The genus name refers to and honors the Canadian braconid expert William R. M. Mason, in recognition of his extraordinary contributions to the knowledge of Microgastrinae and other parasitoid wasps of the world. Although the first author never had the opportunity to meet him, Bill has been an inspiration for many years to continue working on this group. The gender of the genus is neuter.

Species. Only one species is known.

Billmasonius cienci **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/CEDB0B39-9B3C-46BB-A301-8EE672262D99
Fig. 11

Holotype. Female, Thailand, QSBG.

Holotype labels. THAILAND, Chiang Rai,/Doi Luang Nat. Park,/Namptok Champatong, Phayao,/19.217, 99.733, 620 m/CNCH2057. Second label: CNCH2057.

Holotype locality. THAILAND, Chiang Rai Province, Doi Luang National Park, Namptok Champatong, Phayao, 19.217, 99.733, 620 m.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Female. Head and mesosoma dark brown to black; metasoma mostly light brown (but T1 with yellow and white areas, and T2 dark brown); scape and pedicel yellow, flagellomeres brown; legs yellow (except for darker metatarsomeres); wings with veins mostly brown. Head and mesosoma mostly smooth, at most with areas with sparse and shallow punctures. Eyes, on frontal view, slightly convergent ventrally. Scutoscutellar sulcus relatively deep and with seven strong costulae. Posteromedian band of scutellum smooth. Propodeum entirely smooth but with partial median carina defined posteriorly. Fore wing with small, slit-shaped areolet. Hind wing with vannal lobe entirely setose. Unusual T1 shape (better illustrated in Figs 11D–E), with relatively wide anterior 0.6 and strongly narrowed posterior 0.4, so that the widest part of the tergite (near its anterior margin) is around 3.0 × its narrowest width (along its posterior 0.4). Anterior 0.6 of T1 mostly desclerotized (only with lateral margins and narrow central strip sclerotized), a totally unique pattern within Microgastrinae. T2 trapezoidal (subtriangular), its median length 0.3 × its width at posterior margin. Area surrounding spiracles on laterotergite 2 partially sclerotized and same color than T2, giving the impression of T2 having "three peaks" (the largest and central one being the actual T2, the two smallest and lateral ones being the area surrounding spiracles on laterotergites (better illustrated in Figs 11E–F). T4-7 with thin desclerotized area medially near posterior margin, giving the appearance of terga being pushed forward medially (Fig. 11E). Hypopygium medially desclerotized, with several pleats. Ovipositor sheaths 0.7 × metatibia length. **Body measurements (mm).** F2 L: 0.20; F3 L: 0.18; F14 L: 0.09; F15 L: 0.08; Malar sulcus L: 0.04; Mandible W: 0.08; T1 L: 0.38; T1 W at posterior margin: 0.08; T1 maximum W: 0.21; T2 W at anterior margin: 0.05; T2 W at posterior margin: 0.34; T2 L: 0.11; Metafemur L: 0.63; Metafemur W: 0.35; Metatibia L: 0.78; Inner spur L: 0.19; Outer spur L: 0.15; First segment of Metatarsus L: 0.34; Ovipositor sheaths L: 0.58; Body L: 1.89; Fore wing L: 2.26.

Male. Unknown.

Biology. Host unknown.

Distribution. Thailand.

Molecular data. The DNA barcode of the holotype specimen (BIN BOLD:AAH1264) is very unique, 10.4% different from the closest Microgastrinae sequence in BOLD.

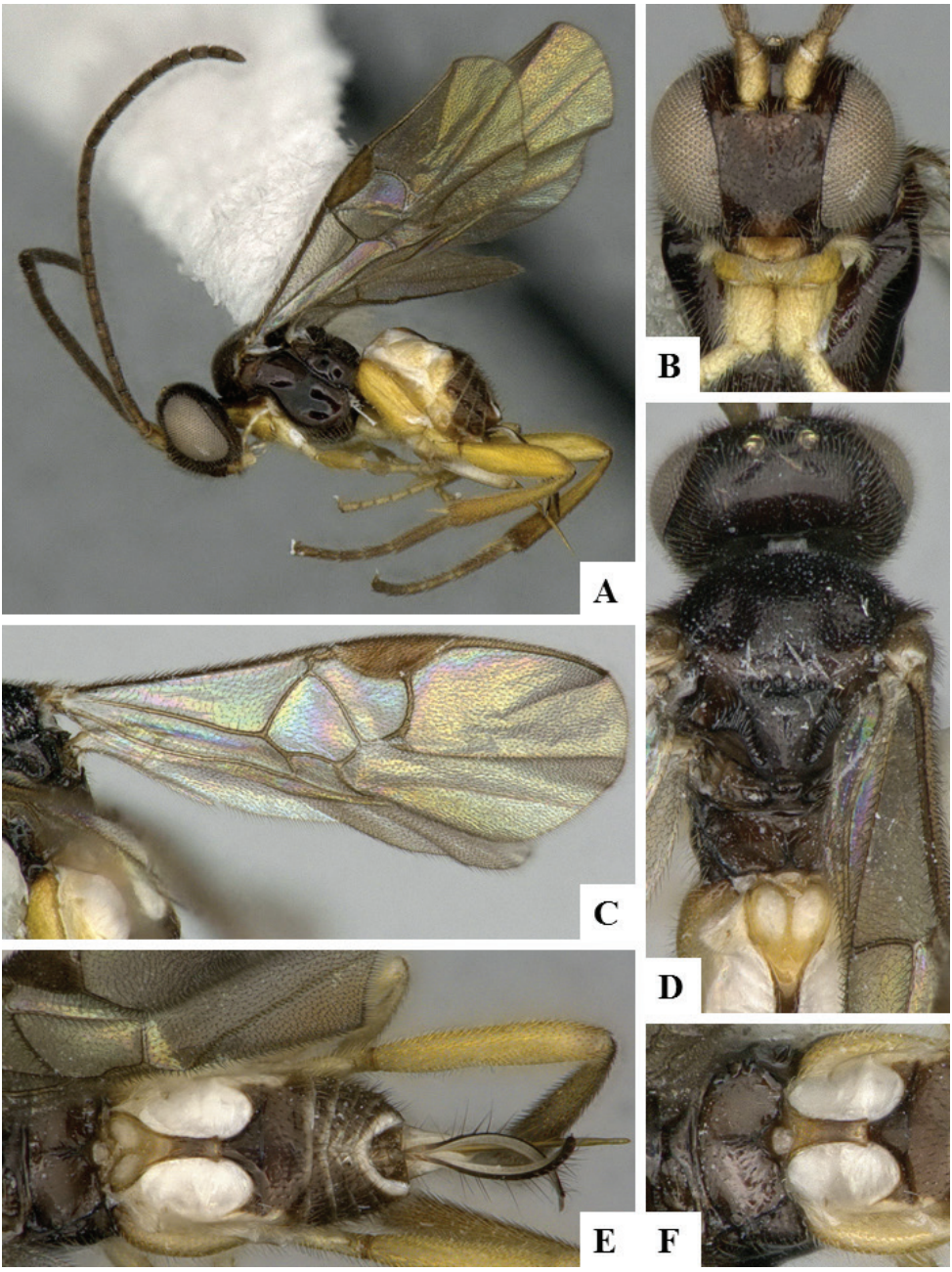


Figure 11. *Billmasonius cienci* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Head, mesosoma and tergite 1, dorsal **E** Metasoma dorsal **F** Propodeum and Tergites 1 to 2.

Etymology. Named after the Canadian National Collection of insects in Ottawa, Canada, in recognition of the outstanding and important collection of 18+ million insect specimens that institution holds, including what is probably the larg-

est and most complete Microgastrinae collection in the world. The acronym "CNC", which is widely used to refer to that institution, is pronounced in English as "Cee-En-Cee", approximately the same as the pronunciation in Latin of the species name "cienci" would be.

***Carlmuesebeckius* Fernandez-Triana, gen. n.**
http://zoobank.org/9D13ED9B-C295-4949-8DB4-6B2D0F71A1D3

Type species. *Carlmuesebeckius smithsonian* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Flagellomeres with three rows of placodes. Pronotum dorsally of normal proportions, not enlarged, its median length (in dorsal view) thinner than width of flagellomeres. Mesosoma, except for propodeum, mostly smooth. Propodeum with areola strongly defined by sharp and raised carinae, transverse carinae forking around big spiracles (partially visible in Fig. 12D, as the bright yellow color of the specimen difficulties the depiction of the carinae in the picture). T1 with longitudinal striae on posterior 0.6, and with a strong and raised median carina for most of its length (Fig. 12E). T2+ smooth. Fore wing without areolet. Hind wing with vannal lobe more or less straight and entirely setose. Tarsal claws pectinate, with two teeth near base. Hypopygium uniformly sclerotized (Fig. 12A). Ovipositor sheaths uniformly setose and clearly shorter than metatibia length. Ovipositor bulging near apex and with two subapical serrate teeth on lower (first) valvulae.

Putative autapomorphies and potentially related genera. Apical part of ovipositor with a node and two ventral teeth in the lower valvae (probably unique within microgastrines, at most similar to ovipositor of *Ohenri*), and T1 with strong and raised median carina on most of its length (also probably unique within the subfamily). Other morphological features are not commonly found within Microgastrinae, and their combination in *Carlmuesebeckius* is highly unusual: flagellomeres with placodes irregularly distributed in three rows (restricted to a few genera, not necessarily related to each other), tarsal claws pectinate (uncommon in the subfamily, although present in a few species from several genera), vannal lobe fully setose, mesosoma mostly smooth, and propodeum fully areolated and with strong carinae forking around spiracles. The relationships of *Carlmuesebeckius* with other genera of Microgastrinae are not clear at present, although some morphological features are related to *Sathon* s.str. and two new genera, *Ohenri* and *Qrocodiledundee*, described below in this paper. *Carlmuesebeckius* is most similar to *Ohenri*, based on antennal placodes distributed in three rows per flagellomere, pectinate tarsal claws, uniformly sclerotized hypopygium and ovipositor with subapical teeth; the carination pattern in the propodeum is also similar in both genera, although in *Carlmuesebeckius* the areola is more complete and better defined, with carinae that are strongly raised. The main differences between these two genera are that *Carlmuesebeckius* does not have an enlarged pronotum dorsally, the vannal lobe is setose, the mesosoma is mostly smooth, and T1 has a median, strongly raised carina

(enlarged pronotum dorsally, setoseless vannal lobe, mostly sculptured mesosoma, and T1 without carinae in *Ohenri*).

Biology. Host unknown.

Distribution. The only known species is found in the Afrotropical region (Madagascar).

Molecular data. No molecular data available.

Etymology. The genus name refers to and honors the American braconid expert Carl F.W. Muesebeck in recognition of his significant contributions to the knowledge of parasitoid wasps of the world. Muesebeck papers on Nearctic Microgastrinae are still a valid source of knowledge, even though some of those papers are almost one hundred years old. The gender of the genus is neuter.

Species. Only one species is known.

Carlmuesebeckius smithsonian **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/52EF765E-DB28-4965-AF5F-81F372D3D693
Fig. 12

Holotype. Female, Madagascar, CAS.

Holotype labels. nrRogezMadag./Mar.'47 900M/C. Lamberton. Second label: New Genus/Det. Ti. Ridge/W.R.M. Mason'81. Third label: CNC924667.

Holotype locality. MADAGASCAR, near Rogez, 900m.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Female. Body color mostly honey-yellow, except for head mostly brown (but with yellow mandibles, labrum, clypeus and face centrally), antenna with scape and pedicel yellow and flagellomeres brown. Wings slightly infumated, with most veins golden-yellow, except for brown pterostigma and fore wing veins R1, r and 2RS. Flagellomeres with three rows of placodes. Head relatively wide, with eyes slightly convergent ventrally and malar line relatively long. Pronotum dorsally of normal proportions, not enlarged, its median length (on a dorsal view) thinner than width of flagellomeres. Mesosoma, except for propodeum, mostly smooth. Propodeum with areola strongly defined by sharp and raised carinae, transverse carinae forking around big spiracles. Fore wing without areolet. Hind wing with vannal lobe more or less straight and entirely setose. Tarsal claws pectinate, with two teeth near base. T1 with longitudinal striae on posterior 0.6, and with a strong and raised median carina for most of its length. T2+ smooth. Hypopygium uniformly sclerotized. Ovipositor sheaths uniformly setose and clearly shorter than metatibia length. Ovipositor bulging near apex and with two subapical serrate teeth on lower (first) valvulae. **Body measurements (mm).** F2 L: 0.46; F3 L: 0.44; Malar sulcus L: 0.09; Mandible W: 0.16; T1 L: 0.75; T1 W at posterior margin: 0.49; T1 maximum W: 0.61; T2 L: 0.23; Metafemur L: 1.56; Metafemur W: 0.44; Metatibia L: 1.90; Inner spur L: 0.65; Outer spur L: 0.33; First segment of Metatarsus L: 1.05; Ovipositor sheaths L: 1.44; Body L: 4.60; Fore wing L: 5.30. T1 L is approximate.

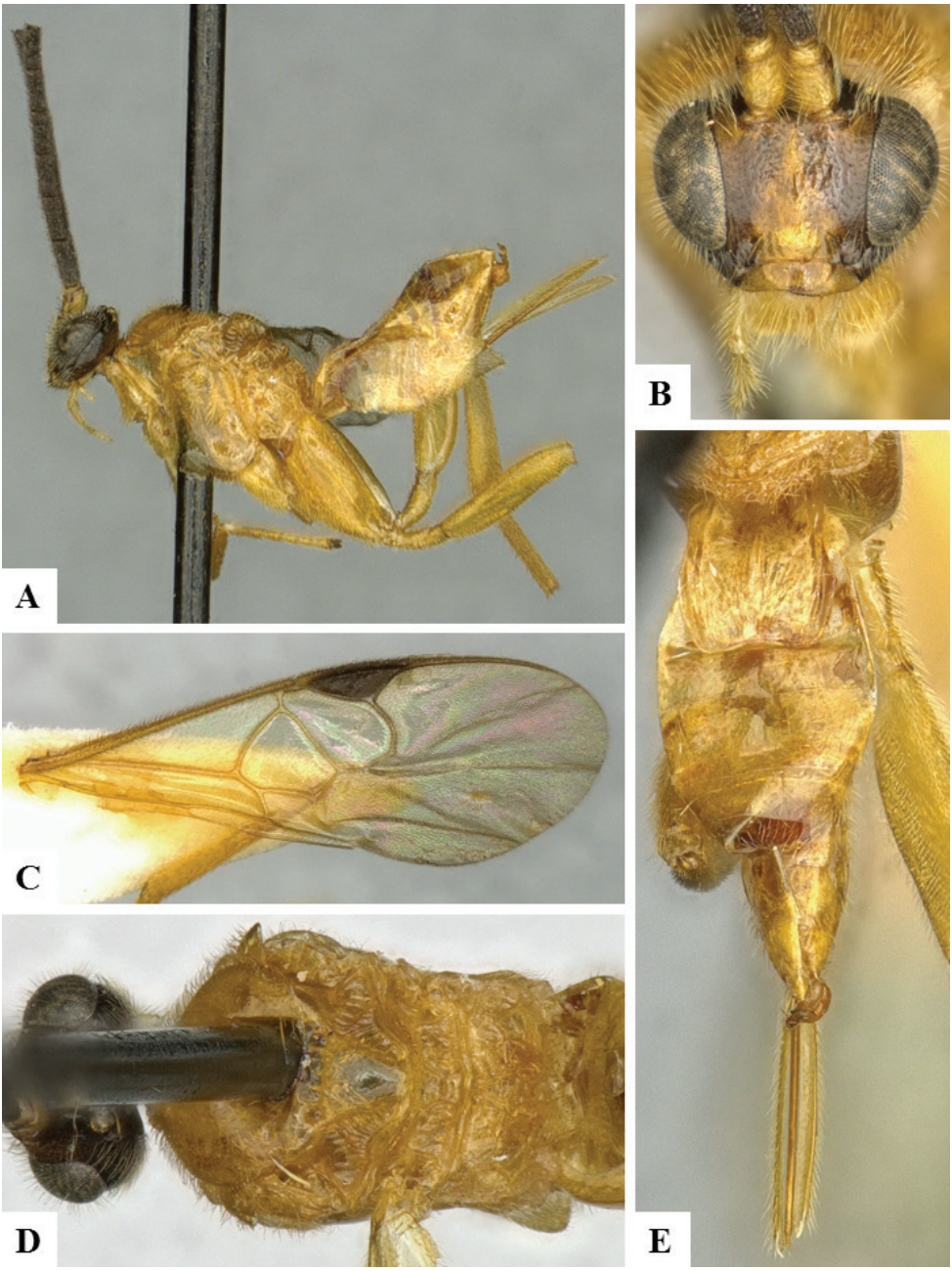


Figure 12. *Carlmuesebeckius smithsonian* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Mesosoma dorsal **E** Metasoma, ovipositor and ovipositor sheaths, dorsal.

Male. Unknown.
Biology. Host unknown.
Distribution. Madagascar.

Molecular data. No molecular data available.

Etymology. Named after the National Museum of Natural History, part of the Smithsonian Institution, Washington, United States, in recognition of the outstanding and important collection of 35+ million insect specimens that institution holds, including one of the largest and most complete Microgastrinae collections in the world.

Gilbertnixonius Fernandez-Triana, gen. n.

http://zoobank.org/6F54346E-3E83-4236-9E4A-2631EE3278C5

Type species. *Gilbertnixonius biem* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Head with relatively large tentorial pits, and very large palps (which reach well into the mesopleuron) (Fig. 13C, D). Occipital carina partially defined. Epicnemial carina partially defined. Mesopleuron and metapleuron strongly sculptured, mostly by transverse striation (Fig. 13D). Anteromesoscutum and scutellar disc mostly sculptured with strong punctures (Fig. 13E). Scutellar disc with sharp carina around margins and slightly protruding posteriorly (Fig. 13D, E). Scutellar disc with rugose band of sculptured postero-medially. Propodeum with median longitudinal and transverse carinae strongly defined (Fig. 13F, G). Fore wing with relatively small, quadrangular areolet (Fig. 13A). Pterostigma mostly white-yellow, except for posterior 0.3 which is light brown. Hind wing with vannal lobe entirely setose. Metacoxa relatively short, not surpassing posterior margin of T2. Metatibia spines relatively short (around 0.3 × length of first segment of metatarsus). T1 with median sulcus on anterior half, posterior half relatively strongly sculptured (Fig. 13G). T2 sub-quadrate, with longitudinal striae (Fig. 13G). Hypopygium relatively short, not extending beyond last tergites. Ovipositor very short, ovipositor sheaths with very few and sparse setae near apex (Fig. 13D).

Putative autapomorphies and potentially related genera. *Gilbertnixonius* belongs to the Microplitini group of genera (sensu Mason 1981). It is the only genus within that group with both longitudinal and transverse carina on propodeum, but without having an areola (*Alloplitis* and the new genus *Tobleronius* described below have those carinae, although sometimes incomplete, but they also have a complete areola). The presence of an epicnemial carina is very unique, as it is only present in Microgastrinae in the unrelated genus *Fornicia* and in some species of *Snellenius* (e.g., Mason 1981, Whitfield et al. 2002, Fernandez-Triana et al. 2015); but *Snellenius* does not have the propodeum carination pattern of *Gilbertnixonius*. The presence of an incomplete occipital carina is a highly unusual feature in Microgastrinae, only shared with at some, or perhaps all, species of *Alloplitis, Philoplits* and *Tobleronius* (see a discussion of that character under the description of *Tobleronius* below, for more details on lineages within Microplitini having a complete or partial occipital carina).

Biology. Host unknown.

Distribution. The only species known is found in the Oriental region (Thailand).

Molecular data. The DNA barcode of the holotype specimen (BIN BOLD:AAZ9883) is very unique, 13.2% different from the closest Microgastrinae sequence in BOLD.

Etymology. The genus name refers to and honors the British braconid expert Gilbert E. J. Nixon in recognition of his significant contributions to the knowledge of parasitoid wasps of the world. Nixon papers on Microgastrinae were of capital importance in the second half of the past century, and paved the way for further studies, including the present one. The gender of the genus is neuter.

Species. Only one species is known.

Gilbertnixonius biem **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/71C28BDE-B211-4AEC-90DE-333F3712AD8D
Fig. 13

Holotype. Female, Thailand, QSBG.

Holotype labels. THAILAND: Suphanburi/Pu Toei Nat. Park/Huai-Tapern, by waterfall/14°58.934'N, 99°19.31'E/Malaise trap, 14–21.2009/P. Wangkum, #3834. Second label: Philoplitis/sp. jft 1. Third label: DNA Voucher/CNCHYM/01950.

Holotype locality. THAILAND: Suphanburi, Pu Toei National Park, Huai-Tapern, by waterfall, 4°58.934'N, 99°19.31'E.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Female. Body mostly dark brown; palpi, scape, pedicel, most of first two pairs of legs, metacoxa, metafemur, first few laterotergites and sternites white or yellow-white; antenna with a subtle banded pattern, with first 10 flagellomeres yellow to light brown, and apical flagellomeres brown; wings hyaline, with most veins light brown, pterostigma mostly white-yellow (except for posterior 0.3 which is light brown). Head with relatively large tentorial pits (which reach well into mesopleuron). Occipital carina defined laterally (not clear in specimen if also defined dorsally). Epicnemial carina partially defined. Meso- and metapleura strongly sculptured, mostly by transverse striation. Anteromesoscutum and scutellar disc mostly sculptured with strong punctures. Scutellar disc with sharp carina around margins and slightly protruding posteriorly. Scutellar disc with rugose band of sculptured postero-medially. Propodeum with median longitudinal and transverse carinae strongly defined. Fore wing with relatively small, quadrangular areolet. Hind wing with vannal lobe entirely setose. Metacoxa relatively short, not surpassing posterior margin of T2. Metatibia spines relatively short (around 0.3 × length of first segment of metatarsus). T1 with median sulcus on anterior half, posterior half relatively strongly sculptured. T2 subquadrate, with longitudinal striae. Hypopygium relatively short, not extending beyond last tergites. Ovipositor very short, ovipositor sheaths with very few and sparse setae near apex.

Body measurements (mm). F2 L: 0.20; F3 L: 0.19; F14 L: 0.10; F15 L: 0.10; Malar sulcus L: 0.09; Mandible W: 0.08; T1 L: 0.32; T1 W at posterior margin: 0.12; T1 maximum W: 0.16; T2 W at anterior margin: 0.13; T2 W at posterior margin: 0.17; T2 L: 0.12; Metafemur L: 0.59; Metafemur W: 0.18; Metatibia L: 0.79; Inner

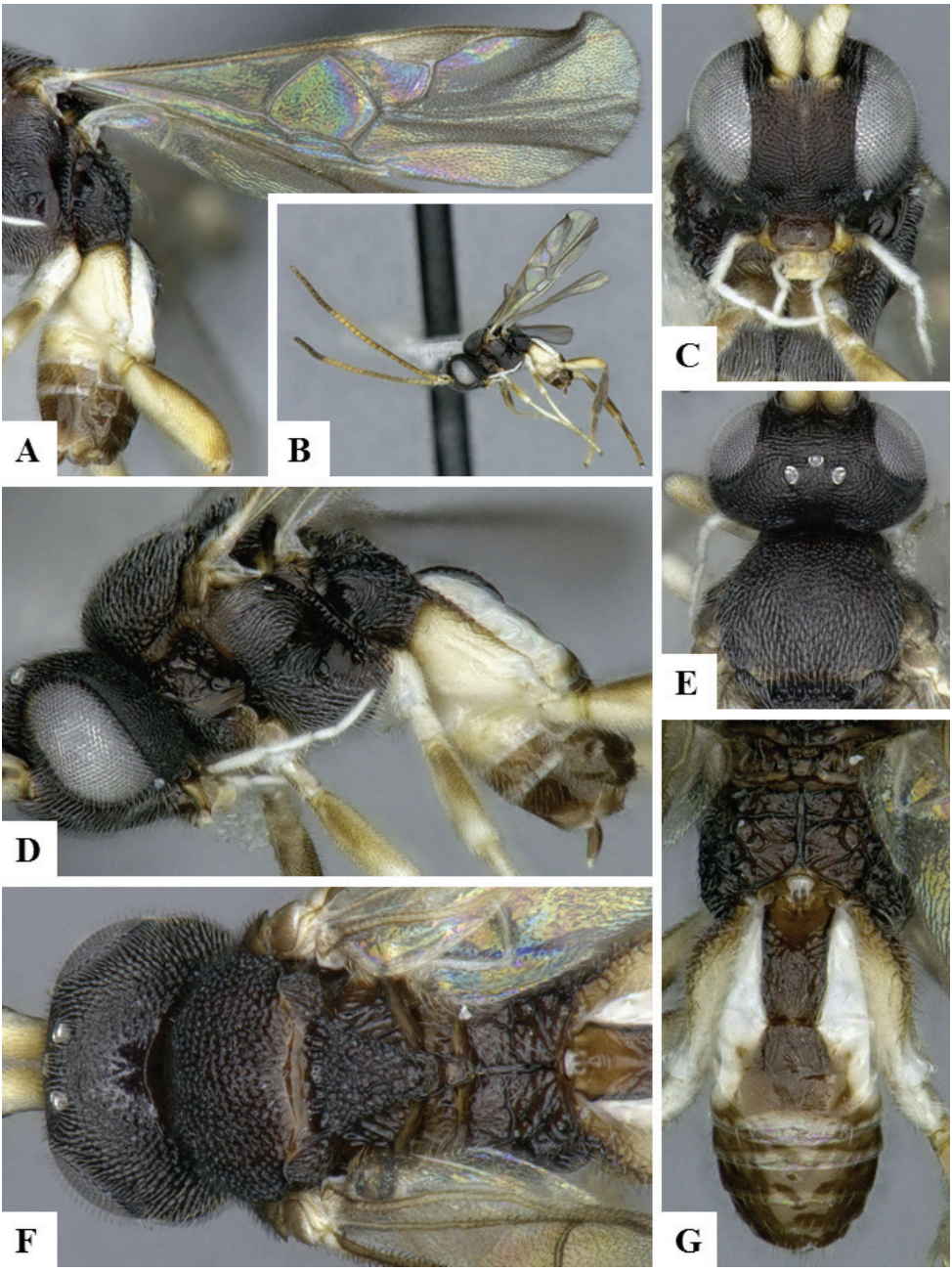


Figure 13. *Gilbertnixonius biem* female holotype. **A** Fore wing **B** Habitus **C** Head frontal **D** Mesosoma and metasoma, lateral **E** Head dorsal **F** Mesosoma dorsal **G** Propodeum and metasoma, dorsal.

spur L: 0.11; Outer spur L: 0.09; First segment of Metatarsus L: 0.32; Ovipositor sheaths L: 0.14; Body L: 2.13; Fore wing L: 2.14.

Male. Unknown.

Biology. Host unknown.

Distribution. Thailand.

Molecular data. The DNA barcode of the holotype specimen (BIN BOLD:AAZ9883) is very unique, 13.2% different from the closest Microgastrinae sequence in BOLD.

Etymology. Named after the Natural History Museum in London (United Kingdom) in recognition of the outstanding and important collection of 34+ million insect specimens that institution holds, including one of the largest and most complete Microgastrinae collection in the world. The old acronym of the Natural History Museum (British Museum until 1992) was commonly referred to as "BM" at the time, which is pronounced in English as "Bee-Em", approximately the same as the pronunciation in Latin of the species name "biem" would be.

***Janhalacaste* Fernandez-Triana, gen. n.**
http://zoobank.org/16ED33E0-79DB-4C46-9012-91E10ED11CDF

Type species. *Janhalacaste winnieae* Fernandez-Triana & Boudreault**,** here designated.

Diagnostic description. Glossa elongate (Fig. 16B). Anteromesoscutum and scutellar disc with relatively deep and close punctures. Posteromedian band of scutellum rugose (Figs 15D, 16D, 17C). Propodeum with complete transverse and longitudinal carinae, and with additional small carinae or striae on most of propodeum surface (Figs 14G, 15G, 16D, F, 17E). Fore wing with small, slit-shaped areolet (as in Fig. 17D). Metacoxa large, surpassing posterior margin of T3 (Figs 14E, 17E). T1 with longitudinal sulcus on anterior 0.6–0.7, posterior 0.3 with two sublateral carinae sharply defined and delimiting a slightly raised area (Figs 14E–G, 17E, F). T2 transverse, with smoother central area, slightly elevated from coarser lateral areas (Figs 14E, F, 16E, G, 17E, F). Hypopygium folded medially and with several pleats. Ovipositor sheaths about same length or slightly shorter than metatibia length.

Putative autapomorphies and potentially related genera. An unusual T1 within Microgastrinae, which has a longitudinal sulcus on the anterior 0.6–0.7 of its length and the posterior 0.3 has two short carinae centrally delimiting a slightly raised area. The propodeum has complete transverse and longitudinal carinae, which is rarely found in Microgastrinae (that trait has also been found in the Old World genera *Beyarslania* and *Neoclarkinella*, and in the Neotropical genus *Prasmodon*, all of which appear distantly related; and also in the more related Neotropical genera *Mariapanteles* and *Pseudapanteles*). Band of rugosity posteromedially on scutellum. Fore wing with very small, slit-shaped areolet. This genus is morphologically similar to *Mariapanteles* but differs in the posteromedian band of the scutellum being rugose, T1 with two carinae on posterior third, and fore wing with an areolet. *Pseudapanteles*, which is morphologically related to *Mariapanteles*, can be separated from *Janhalacaste* by all those features and also by lacking a transverse carina on propodeum.

Biology. Hosts include several species of Depressaridae.

Distribution. The known species are found in the Neotropical region (Costa Rica).

Molecular data. The three species described below have DNA barcodes available, corresponding to the BINs BOLD:AAK9733, BOLD:AAK0117 and BOLD:ACB2460. Overall, the *Janhalacaste* BINs are clearly separate from the rest of Microgastrinae (more than 10% base pairs difference from the closest sequence available in BOLD).

Etymology. The genus name refers to and honors the ecologists Daniel Janzen and Winnie Hallwachs, as well as Area de Conservación Guanacaste (ACG) in northwestern Costa Rica, for the great contributions that both have made to our understanding of Microgastrinae diversity. It is impossible to separate Dan and Winnie from ACG; thus, we are happy and honored to name a new genus of microgastrine wasps after them all. Accordingly, the first part of the genus name is a combination of the first three letters of each researcher's last name ("Jan" from Janzen, "Hal" from Hallwachs), while the second part of the genus name includes the last six letters of the word "Guanacaste". The gender of the genus is neuter.

Species. We describe below three new species for the genus. They can be separated using the following key.

Key to species

1 Metasoma dorsally mostly light yellow (except for posterior 0.2–0.3 of T1 and entire T2, which are dark brown to black; and small, brown spot centrally on T4+) (Fig. 17E, F) ....***Janhalacaste winnieae*** **Fernandez-Triana & Boudreault, sp. n.**

– Metasoma dorsally mostly dark brown to black, at most with anterior 0.6–0.7 of T1 yellow (Figs 14E, 15E, F, 16E) **2**

2 Metacoxa mostly brown, with only dorsal, yellow spot on posterior 0.2 (Fig. 14E); posterior 0.3 of T1 with two sublateral carinae sharply defined (Figs 14E–G) .....***Janhalacaste danieli*** **Fernandez-Triana & Boudreault, sp. n.**

– Metacoxa entirely yellow (Fig. 16A); posterior 0.3 of T1 with two sublateral carinae poorly defined (Figs 15E–G, 16E–G) ....***Janhalacaste guanacastensis*** **Fernandez-Triana & Boudreault, sp. n.**

Janhalacaste danieli **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/68F0E9A2-E009-49AB-A6A9-27844158B620
Fig. 14

Holotype. Male, Costa Rica, CNC.

Holotype labels. COSTA RICA: Guanacaste,/ACG, Sector Pitilla,/Medrano, 380m,/11.01602, -85.38053,/02/06/2012/DHJPAR0049240.

Holotype locality. COSTA RICA, Guanacaste, Area de Conservación Guanacaste, Sector Pitilla, Medrano, 380m, 11.01602, -85.38053.

Diagnosis. This is the only species in the genus with dark (brown) metacoxa.

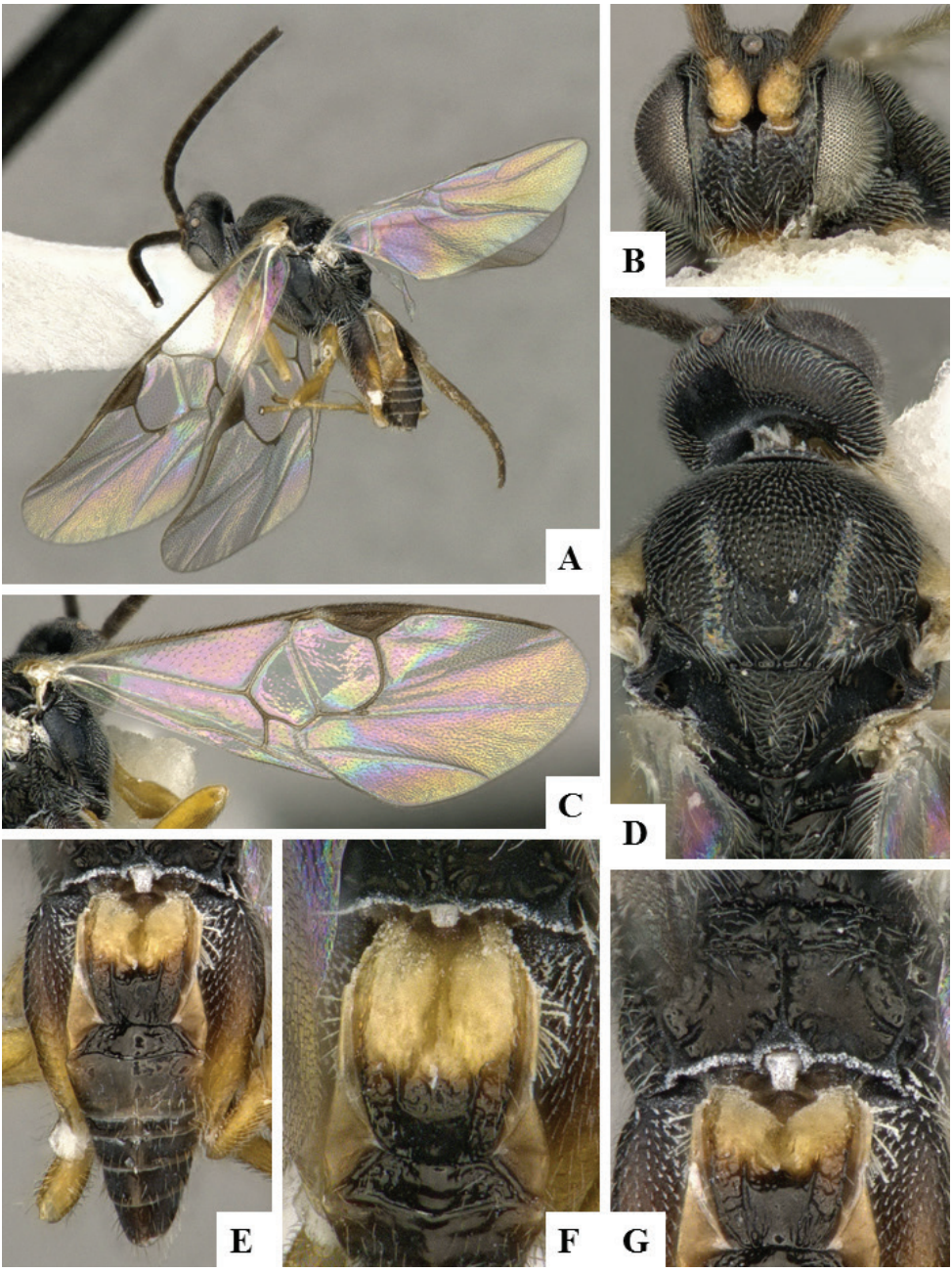


Figure 14. *Janhalacaste danieli* male holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Mesosoma dorsal **E** Metasoma dorsal **F** Tergites 1 to 2 **G** Propodeum.

Description. Male. Head and mesosoma black; metasoma mostly black to dark brown dorsally (except for T1 light yellow on anterior 0.6); metasoma mostly yellow laterally and ventrally; scape and pedicel yellow, flagellomeres mostly dark brown to

black; palpi white; legs mostly yellow (except for metacoxa mostly brown); wings with veins mostly brown. Head, including eyes, mostly covered by relatively long and dense setae (except for smooth, setoseless area, centrally on occiput behind ocelli, Fig. 14D). Anteromesoscutum and scutellar disc with relatively deep and close punctures, and with long, white setae. Posteromedian band of scutellum rugose. Propodeum with complete transverse and longitudinal carinae, and with additional small striae. Fore wing with small, slit-shaped areolet. Metacoxa large, surpassing posterior margin of T3. T1 with longitudinal sulcus on anterior 0.6, posterior 0.3 with two sublateral carinae sharply defined. T2 transverse, with smoother central area, slightly elevated from coarser lateral areas. T2+ with relatively long, sparse, white setae, which are mostly locate laterally on terga.

Female. Unknown.

Biology. Reared from an undetermined species of Depressariidae with the interim name of "elachJanzen01 Janzen131".

Distribution. Costa Rica.

Molecular data. The holotype sequence belongs to BIN BOLD:ACB2460, which has 5.3% of bp differences compared to the next species in BOLD, which is *Janhalacaste winnieae*.

Etymology. Named after Daniel Janzen, in recognition of his extraordinary contributions to the fields of conservation biology, tropical ecology, citizen science and public outreach, and even for helping taxonomists to be better appreciated for what they do. The first author has also been honored to work with Dan on the Microgastrinae fauna of ACG for the past six years and counting.

***Janhalacaste guanacastensis* Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/DF9FC902-DD1C-44A6-A108-4298D4C3D0C0
Figs 15, 16

Holotype. Female, Costa Rica, CNC.

Holotype labels. COSTA RICA: Guanacaste,/ACG, Sector Pitilla,/Medrano, 380m, 09/04/2013,/11.01602, -85.38053,/DHJPAR0054852.

Holotype locality. COSTA RICA, Guanacaste, Area de Conservación Guanacaste, Sector Pitilla, Medrano, 380m, 11.01602, -85.38053.

Paratype. Costa Rica. (1♂ CNC) Brasilia, Piedrona, 11.016200, -85.359000, 340m, 17.iv.2013, Voucher code: DHJPAR0052309.

Diagnosis. This species can be separate from *J. winnieae* because of its almost entirely dark brown metasoma dorsally (almost entirely yellow metasoma dorsally in *J. winnieae*). Its yellow metacoxa in turn separates it from *J. danieli* (which has dark brown metacoxa). *J. guanacastensis* is also the species with the least defined sublateral carinae on posterior 0.3 of T1.

Description. Female. Head and mesosoma black; metasoma mostly black to dark brown dorsally (T1 mostly yellow, except for brown, central spot on poster-

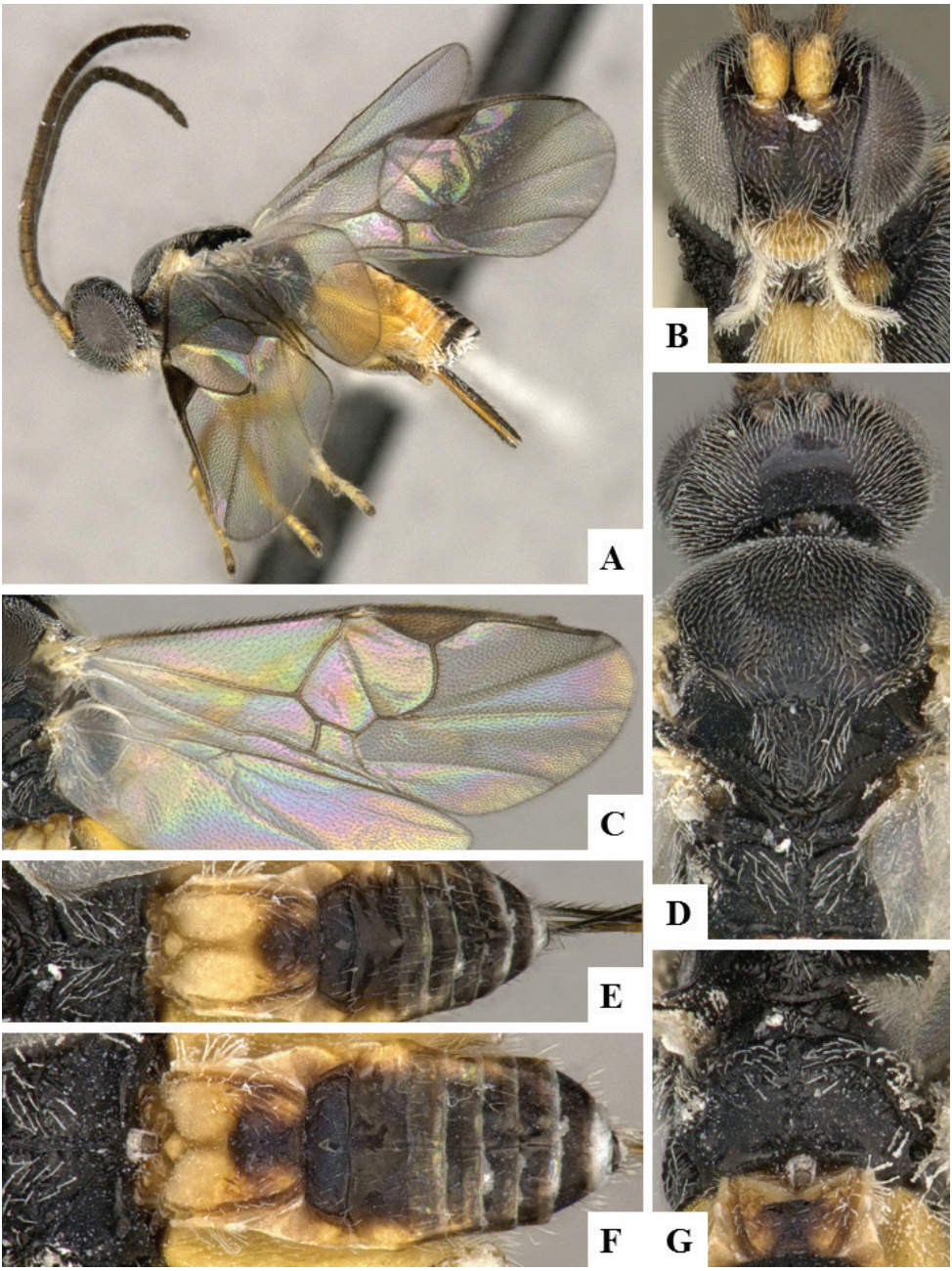


Figure 15. *Janhalacaste guanacastensis* female holotype. **A** Habitus **B** Head frontal **C** Fore wing and hind wing **D** Head and mesosoma, dorsal **E** Metasoma dorsal **F** Tergites 1 to 2 **G** Propodeum.

ior margin; sometimes T3-T6 with small yellow spots laterally); metasoma mostly yellow laterally and ventrally; scape and pedicel yellow, flagellomeres mostly brown; palpi white; legs yellow; wings with veins mostly brown. Head, including

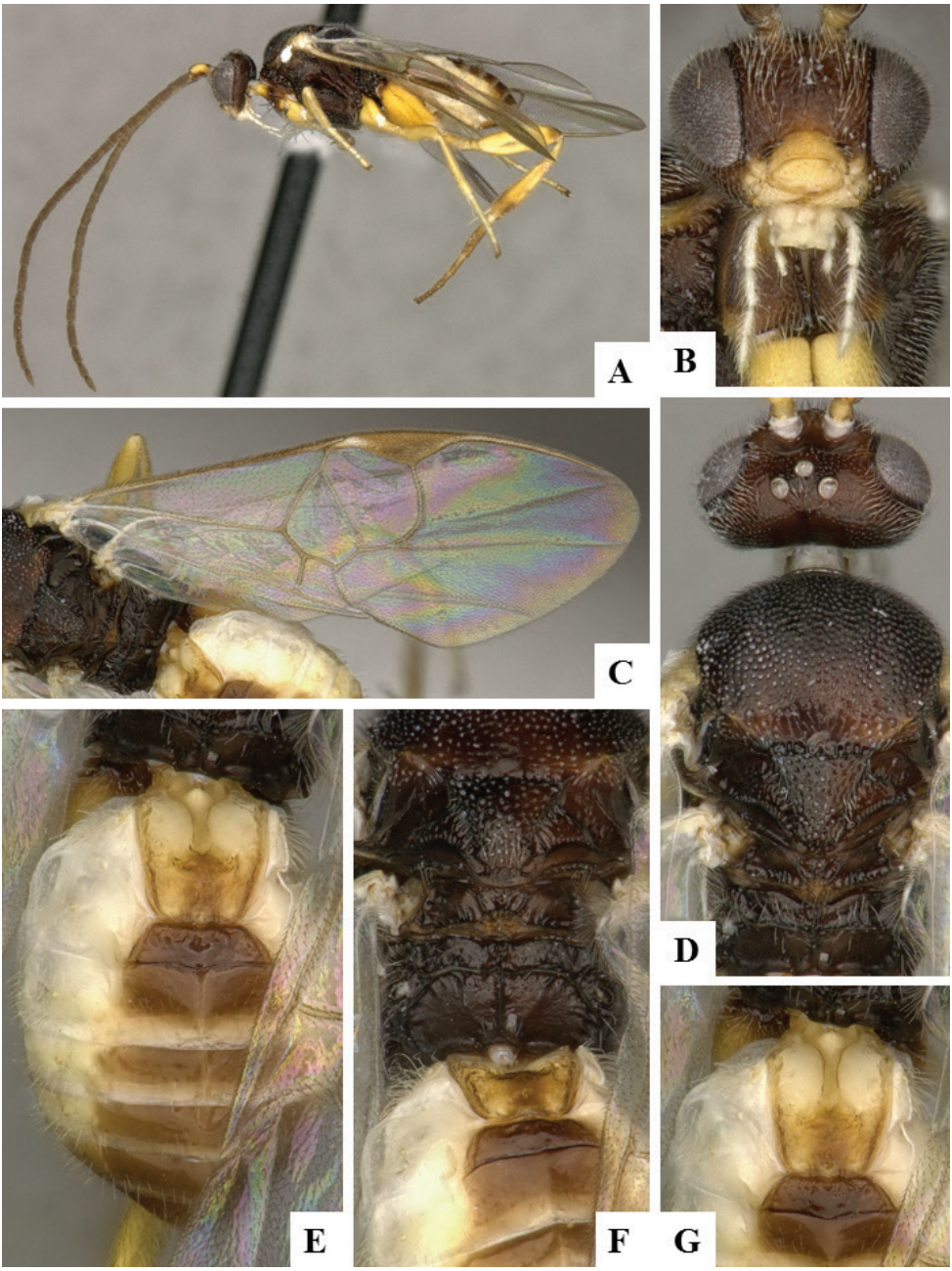


Figure 16. *Janhalacaste guanacastensis* male paratype DHJPAR0052309. **A** Habitus **B** Head frontal **C** Fore wing **D** Head and mesosoma, dorsal **E** Metasoma dorsal **F** Propodeum **G** Tergites 1 and 2.

eyes, mostly covered by relatively long and dense setae (except for smooth, setoseless area, centrally on occiput behind ocelli, Fig. 15D). Anteromesoscutum and scutellar disc with relatively deep and close punctures, and with long, white setae.

Posteromedian band of scutellum rugose. Propodeum with complete transverse and longitudinal carinae, and with additional small striae. Fore wing with small, slit-shaped areolet. Metacoxa large, surpassing posterior margin of T3. T1 with longitudinal sulcus on anterior 0.6–0.7, posterior 0.3 with two sublateral carinae which are barely visible (the latter might be an artifact due to specimen condition). T2 transverse, with smoother central area, slightly elevated from coarser lateral areas. T2+ with relatively long, sparse, white setae, which are mostly locate laterally on terga. Hypopygium folded medially and with several pleats. Ovipositor sheaths shorter than metatibia length. **Female body measurements (mm).** F2 L: 0.23; F3 L: 0.23; F14 L: 0.12; F15 L: 0.11; Malar sulcus L: 0.08; Mandible W: 0.11; T1 L: 0.49; T1 W at posterior margin: 0.18; T1 maximum W: 0.33; T2 W at anterior margin: 0.28; T2 W at posterior margin: 0.37; T2 L: 0.11; Ovipositor sheaths L: 0.82; Body L: 2.70; Fore wing L: 2.85. Malar sulcus L and mandible W are approximate.

Male. As female, but lighter in coloration and less setose. However, those differences might be due to the available specimen being teneral. **Male body measurements (mm).** F2 L: 0.28; F3 L: 0.27; F14 L: 0.16; F15 L: 0.15; Malar sulcus L: 0.08; Mandible W: 0.08; T1 L: 0.52; T1 W at posterior margin: 0.21; T1 maximum W: 0.38; T2 W at anterior margin: 0.27; T2 W at posterior margin: 0.42; T2 L: 0.13; Metafemur L: 0.90; Metafemur W: 0.28; Metatibia L: 1.14; Inner spur L: 0.33; Outer spur L: 0.20; First segment of Metatarsus L: 0.54; Body L: 3.22; Fore wing L: 3.38.

Biology. Reared from five species of Depressariidae: *Antaeotricha* sp. (with specific interim name "Janzen146"), *Filinota* sp. (with specific interim name "Janzen154"), *Stenoma* sp. (with specific interim name "Janzen13"), and two other undetermined species with the interim names of "elachJanzen01 Janzen131" and "elachJanzen01 Janzen861".

Distribution. Costa Rica.

Molecular data. The holotype and paratype sequences belong to BIN BOLD:AAK9733, which has 11.6% of bp differences compared to the next species in BOLD, which is *Janhalacaste danieli*.

Etymology. Named after Area de Conservación Guanacaste, a world icon and example of conservation of tropical ecosystems.

Janhalacaste winnieae **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/65180D52-A805-4EA7-BE66-A47F17914730
Fig. 17

Holotype. Female, Costa Rica, CNC.

Holotype labels. COSTA RICA: Guanacaste,/ACG, Sector Santa Rosa,/Area Administrativa, 295m,/10.83764, -85.61871,/12/25/2008/DHJPAR0031806.

Holotype locality. COSTA RICA, Guanacaste, Area de Conservación Guanacaste, Sector Santa Rosa, Area Administrativa, 295m, 10.83764, -85.61871.

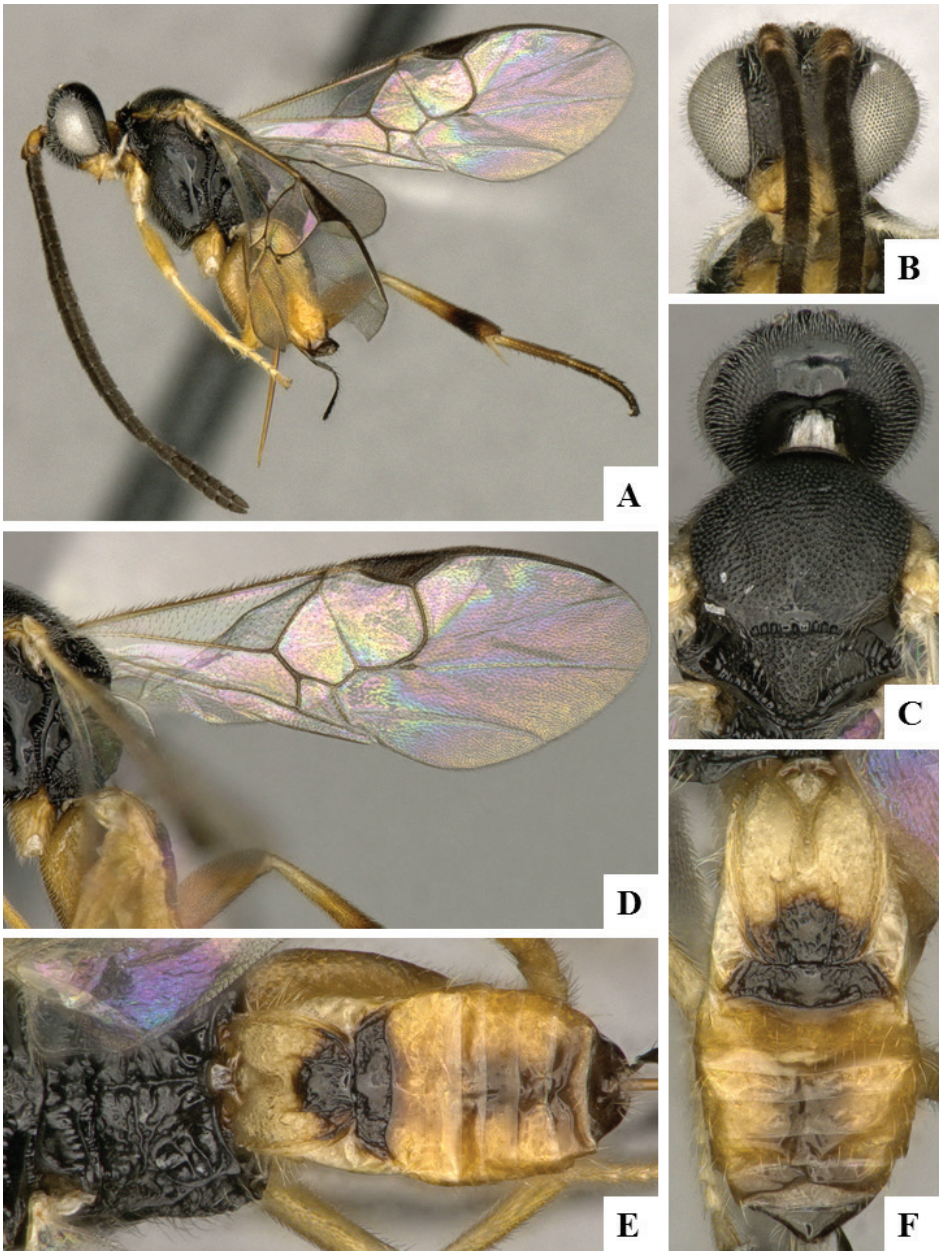


Figure 17. *Janhalacaste winnieae* female holotype. **A** Habitus **B** Head frontal **C** Mesosoma dorsal **D** Fore wing **E** Propodeum **F** Metasoma dorsal.

Paratypes. Costa Rica. (1♀ CNC) Sector Santa Rosa, Area Administrativa, 10.837600, -85.618700, 295m, 25.xii.2008, Voucher code: DHJPAR0031795; (1♂ CNC), Sector Santa Rosa, Bosque San Emilio, 10.843900, -85.613800, 300m, 10.iv.2000, Voucher code: DHJPAR0013312.

Diagnosis. This is the only species in the genus with metasoma mostly yellow dorsally (the two other known species have the metasoma mostly dark brown to black dorsally).

Description. Female. Head and mesosoma black; metasoma mostly light yellow dorsally (except for posterior 0.2–0.3 of T1 and entire T2, which are dark brown to black; and small, brown spot centrally on T4+); metasoma yellow laterally and ventrally; scape and pedicel yellow, flagellomeres mostly dark brown; palpi white; legs mostly yellow (except for posterior 0.4 of metatibia and metatarsus which are dark brown to black); wings with veins mostly brown. Head, including eyes, mostly covered by relatively long and dense setae (except for smooth, setoseless area, centrally on occiput behind ocelli, Fig. 17C). Anteromesoscutum and scutellar disc with relatively deep and close punctures, and with long, white setae. Posteromedian band of scutellum rugose. Propodeum with complete transverse and longitudinal carinae, and with additional small striae. Fore wing with small, slit-shaped areolet. Metacoxa large, surpassing posterior margin of T3. T1 with longitudinal sulcus on anterior 0.6, posterior 0.3 with two sublateral carinae sharply defined. T2 transverse, with smoother central area, slightly elevated from coarser lateral areas. T2+ with relatively long, sparse, white setae, which are mostly locate laterally on terga. Hypopygium folded medially and with several pleats. Ovipositor sheaths shorter than metatibia length. **Body measurements (mm).** F2 L: 0.25 (0.24); F3 L: 0.25 (0.23); F14 L: 0.12 (0.11); F15 L: 0.11 (0.10); Malar sulcus L: 0.05 (0.07); Mandible W: 0.09 (0.07); T1 L: 0.50 (0.50); T1 W at posterior margin: 0.17 (0.18); T1 maximum W: 0.36 (0.35); T2 W at anterior margin: 0.28 (0.27); T2 W at posterior margin: 0.38 (0.40); T2 L: 0.12 (0.10); Metafemur L: 0.89; Metafemur W: 0.26; Metatibia L: 1.13; Inner spur L: 0.33; Outer spur L: 0.17; First segment of Metatarsus L: 0.56; Ovipositor sheaths L: 0.67 (0.90); Body L: 2.83 (2.93); Fore wing L: 3.19 (2.95).

Male. As female.

Biology. Host unknown.

Distribution. Costa Rica.

Molecular data. The sequences of the holotype and two paratypes all belong to BIN BOLD:AAK0117, which has 5.2% of bp differences compared to the next species in BOLD, which is *Janhalacaste danieli*.

Etymology. Named after Winnie Hallwachs, in recognition of her extraordinary contributions to the fields of conservation biology, tropical ecology, citizen science and public outreach, and even for helping taxonomists to be better appreciated for what they do. The first author has also been honored to work with Winnie on the Microgastrinae fauna of ACG for the past six years and counting.

Jenopappius **Fernandez-Triana, gen. n.**

http://zoobank.org/B52BF5A9-BA05-4773-B350-6CA69EEC1C6F

Type species. *Jenopappius magyarmuzeum* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Clypeus relatively small and bulging (Figs 18B, 20B). Tentorial pits relatively large. Notauli marked by coarser sculpture than rest of anteromesoscutum (partially visible in Figs 20F, 21C). Scutoscutellar sulcus deep and with strong crenulae (Figs 18E, 20F, 21C). Propodeum without areola, but with median longitudinal carina obscured by surrounding sculpture (Figs 20F, 21E). Fore wing with four-sided areolet (second submarginal cell). Metacoxa relatively short (not surpassing posterior margin of T2). Metatibial spurs relatively short (less than half length of first segment of metatarsus) (Figs 20A, 21A). T1 longer than wide, mostly sculptured with strong longitudinal striae, but with anteromedian depression (Figs 18F, 20E, 21B, E). T2 rectangular, as long as or longer than T3, with strong longitudinal striation and a central, smooth area (median field) which is slightly more elevated than rest of tergite and it is narrowing towards posterior margin (Figs 18F, 19A, B, 20E, 21B, E). Hypopygium inflexible and not pleated. Ovipositor sheaths very short (Figs 18A, 20A).

Putative autapomorphies and potentially related genera. *Jenopappius* clearly belongs to the Microplitini (sensu Mason 1981). It resembles *Microplitis* but with a strongly sculptured and rectangular T2 and a rather unique pattern of T1. Also, some *Alloplitis* may have somewhat similar sculpture of either T1 or T2 (but the shape of those tergites in that latter genus is very different, and the propodeum is fully areolated with strongly raised carinae). The combination of sculptured propodeum without areola, anteromedian depression of T1, and strong sculpture of T1–T2 are very unusual and will separate *Jenopappius* from any other known genera of Microplitini and indeed Microgastrinae.

Biology. Host unknown.

Distribution. All known species are found in the Afrotropical region (Democratic Republic of Congo, Kenya, Republic of Congo, Rwanda).

Molecular data. A total of 11 DNA barcodes are available, from *Jenopappius magyarmuzeum*. All sequences cluster together in BIN BOLD:AAH1374, and are different by 14.2 % of the closest Microgastrinae in BOLD (based on a Neighbor Joining tree built with 35,000+ Microgastrinae sequences available in BOLD as of January 2018).

Etymology. The genus name refers to and honors the Hungarian braconid expert Jeno Papp, in recognition of his significant contributions to the knowledge of Braconidae of the world, and his work on Palearctic Microgastrinae. The gender of the genus is neuter.

Species. We recognize three different species, two previously described by de Saeger (1944) and a new one described below. They can be separate using the following key.

Key to species

1 T1 narrowing towards posterior margin (T1 width at anterior margin around 1.3 × its width at posterior margin); T2 with raised median area width at its anterior margin as wide as width of T1 at posterior margin, so that lateral margins

of raised area of T2 look like a continuation of lateral margins of T1 (Fig. 19A) [Democratic Republic of Congo] ..... ***Jenopappius niger* (de Saeger, 1944)**

– T1 more or less parallel-side on anterior 0.3–0.4 (at which point there is a small constriction centrally on tergite), then widening towards posterior margin (T1 width at anterior margin around 0.8 × its width at posterior margin); T2 with raised median area width at its anterior margin clearly narrower than width of T1 at posterior margin, so that lateral margins of raised area of T2 do not look like a continuation of lateral margins of T1 (Figs 18F, 19B, 20E, 21B, E) **2**

2 Median raised area on T2 very thin and parallel-sided (Figs 20E, 21B, E); tegula, maxillary and labial palpi brown; all femora dark and most of tibiae brown to black; T2 and T3 yellow-white (Figs 20E, 21B, E) [Republic of Congo] .... ***Jenopappius magyarmuzeum* Fernandez-Triana & Boudreault, sp. n.**

– Median raised area on T2 anteriorly much broader than posteriorly (Figs 18F, 19B); tegula, maxillary and labial palpi yellow; all femora and tibiae mostly yellow (mesofemur and metafemur with narrow brown band dorsally); T2 and T3 brown to black (Fig. 18F) [Democratic Republic of Congo, Kenya, Rwanda] ..... ***Jenopappius aethiopica* (de Saeger, 1944)**

***Jenopappius aethiopica* (de Saeger, 1944), comb. n.**
Figs 18, 19B

Microplitis aethiopica de Saeger, 1944. Original description (de Saeger 1944: 48).
Microplitis aethiopicus de Saeger, 1944. Gender of species name changed (Yu et al. 2016).

Holotype. Female, Democratic Republic of the Congo, RMCA (Musee Royal de l'Afrique Centrale, Tervuren, Belgium). Not examined, but original description checked.

Diagnosis. *J. aethiopica* can be separated from *J. niger* because of shape of T1, and by having raised median area of T2 narrower than width of T1 at posterior margin. It can be distinguished from *J. magyarmuzeum* because it has lightered coloured legs and darker T2 and T3 (darker legs and yellow-white T2 and T3 in *J. magyarmuzeum*).

Biology. Host unknown.

Distribution. Democratic Republic of the Congo, Kenya, Rwanda.

Molecular data. No molecular data available.

Comments. The species records from the Democratic Republic of Congo and Rwanda come from the original description of the species (de Saeger 1944). The record from Kenya (two female specimens) is based on specimens found in the CNC. No molecular data. The Kenyan specimens we examined have the first 2–3 sternites and laterotergites, and the first two pairs of legs mostly yellow (except for tibia and tarsi which are brown). That is slightly lighter coloured compared to the original de-

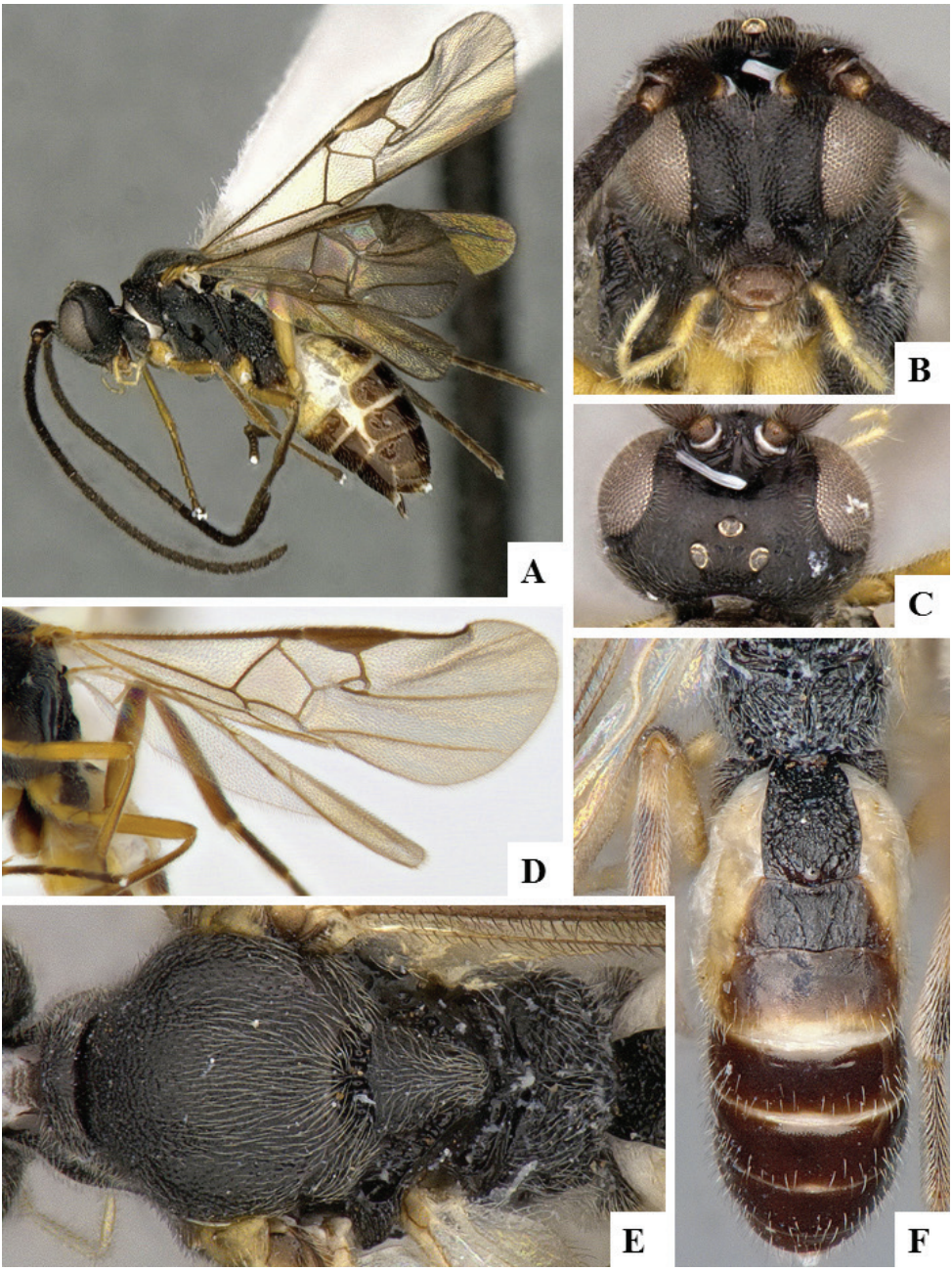


Figure 18. *Jenopappius aethiopica* female non-type specimen CNC878534. **A** Habitus **B** Head frontal **C** Head dorsal **D** Fore wing **E** Mesosoma dorsal **F** Metasoma dorsal.

scription of *J. aethiopica* (where the color of those body parts is described as mostly reddish-yellow or reddish-brown), but we consider those as minor differences and thus keep all examined specimens as part of *J. aethiopica*.

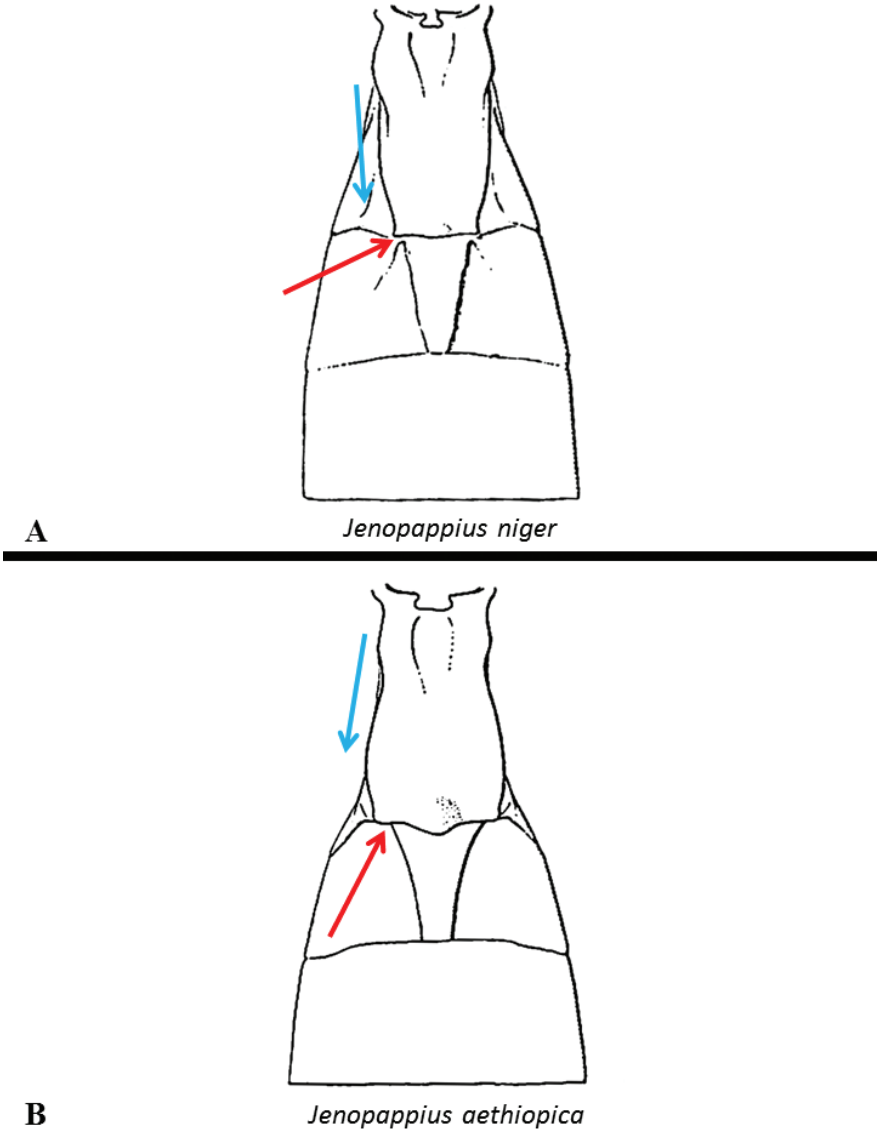


Figure 19. Comparison of tergites 1 and 2 in *Jenopappius niger* (**A**) and *Jenopappius aethiopica* (**B**) based on modified drawings from the original descriptions of the species (de Saeger 1944). Blue arrow shows tergite 1 narrowing (**A**) or widening (**B**) towards posterior margin. Red arrow shows end of lateral margin of tergite 1 and beginning of lateral margin of median area of tergite 2 to be almost continuous (**A**) or end of lateral margin of tergite 1 and beginning of lateral margin of median area of tergite 2 to be clearly separate (**B**).

Jenopappius magyarmuzeum **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/3F430B7B-0A65-476D-8A06-E416F7188682
Figs 20, 21

Holotype. Female, Democratic Republic of the Congo, CNC.

Holotype labels. DEMOCRATIC REPUBLIC OF/THE CONGO. Iboubikro,/ Lesio-Loun Pk,; Pool,/3.27°S, 15.471°E, 340m,. Second label: 25.XI.2088, Braet &/ Sharkey, BIN#BOLD:/AAH1374, **CNCH2795**. Third label: CNCH2795.

Holotype locality. DEMOCRATIC REPUBLIC OF THE CONGO, Iboubikro, Lesio-Louna Park, Pool, 3.27°S, 15.471°E, 340m.

Paratypes. Democratic Republic of the Congo. (2♂ CNC), Iboubikro, Lesio-Loun Pk.; Pool, -3.269931, 15.471100, 340m, 13.x.2008, coll. Braet & Sharkey, Voucher codes: CNCH3029, CNCH3037; (7♂1♀ CNC), -3.270000 15.471000, 340m, 25.xi.2008, coll. Braet & Sharkey, Voucher codes: CNCH2806, CNCH2760, CNCH2768, CNCH2775, CNCH2782, CNCH2787, CNCH2789, CNCH2807; (1♂ CNC), Iboubikro, Lesio-Loun Pk; Pool, -3.162000, 15.283000, 330m, 1.ix.2008, coll. Sharkey & Braet, Voucher code: JMIC0532.

Diagnosis. This is the only known species in the genus with yellow-white T2 and T3. Additionally the shape of T1 would separate it from *J. niger* (Fig. 19A), and the shape of the median raised area on T2 (very thin and parallel-sided) would distinguish it from *J. aethiopica* (which has the raised area on T2 much broader anteriorly than posteriorly).

Description. Female. Head, mesosoma and T1 black, T2–T3 yellow-white, T4+ dark brown; antenna dark brown to black; palpi brown to yellow-brown; most legs dark brown to black (except for profemur and protibia partially yellow-orange); metatibial spurs yellow-white; wings hyaline, most veins brown. Head with relatively large tentorial pits. Clypeus relatively small and bulging. Glossa relatively elongate. Most of head and mesosoma with coarse punctures. Notauli marked by deeper and coarser sculpture. Scutoscutellar sulcus deep and wide, with 4 or more strong crenulae. Scutellar disc with posteromedian band of rugosity. Propodeum strongly sculptured, with irregular pattern of carinae, but a median longitudinal carina clearly defined. Fore wing with four-sided areolet (second submarginal cell). Hind wing with vannal lobe entirely setose. Metacoxa relatively short (not surpassing posterior margin of T2). Metatibial spurs relatively short (less than half length of first segment of metatarsus). T1 mostly coarsely sculptured, with strong longitudinal striae and anteromedian depression. T2 rectangular, as long as or longer than T3, with strong longitudinal striation and a central, smooth area slightly more elevated than rest of tergite (which narrows towards posterior margin). Hypopygium inflexible and not pleated. Ovipositor sheaths very short. **Body measurements (mm).** F2 L: 0.23 (0.24); F3 L: 0.21 (0.23); F14 L: (0.16); Malar sulcus L: 0.10 (0.10); Mandible W: 0.08 (0.11); T1 L: 0.40 (0.38); T1 W at posterior margin: 0.30 (0.33); T1 maximum W: 0.33 (0.33); T2 W at anterior margin: 0.33 (0.34); T2 W at posterior margin: 0.41 (0.43); T2 L: 0.24 (0.24); Metafemur L: 0.72 (0.71); Metafemur W: 0.16 (0.17); Metatibia L: 1.07 (1.04); Inner spur L: 0.13

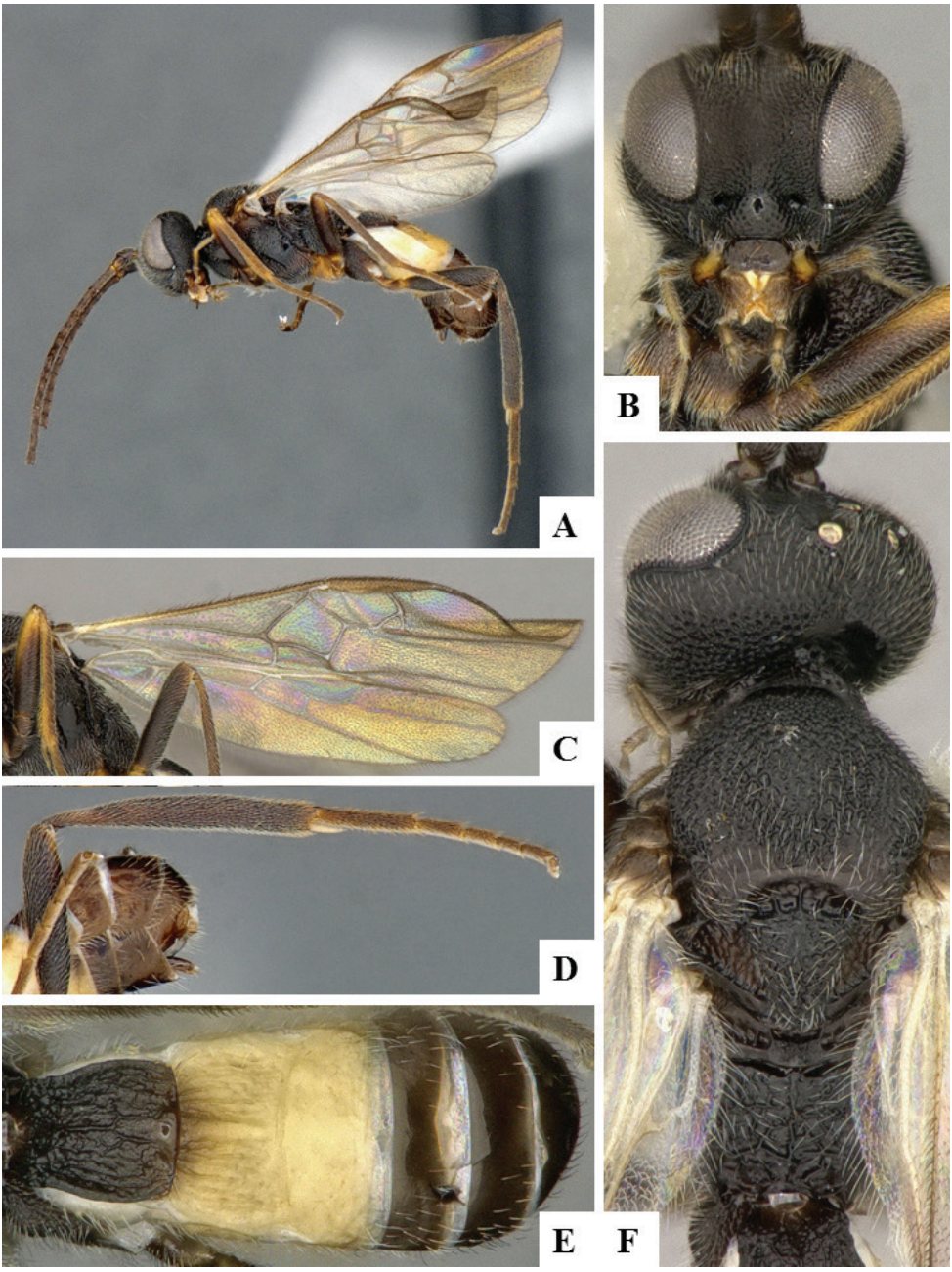


Figure 20. *Jenopappius magyarmuzeum* female holotype. **A** Habitus **B** Head frontal **C** Fore wing and hind wing **D** Ovipositor and hind leg **E** Metasoma dorsal **F** Mesosoma dorsal.

(0.12); Outer spur L: 0.13 (0.13); First segment of Metatarsus L: 0.40 (0.39); Ovipositor sheaths L: 0.13 (0.13); Body L: 2.73 (2.93); Fore wing L: 2.55 (2.53). Maximum W of T1 and T1 W at anterior margin are approximate for one specimen.

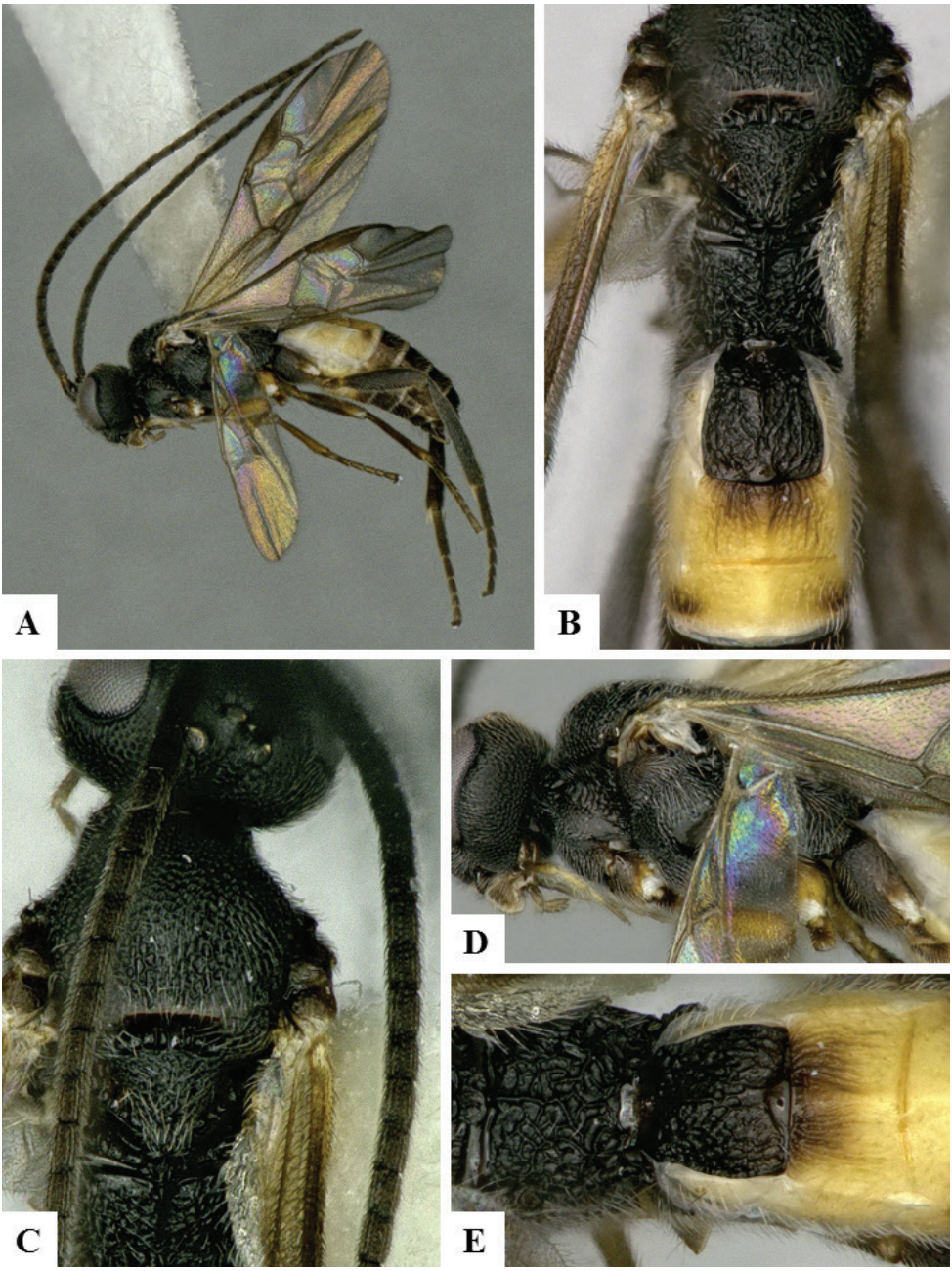


Figure 21. *Jenopappius magyarmuzeum* male paratype CNCH2789. **A** Habitus **B** Tergites 1 to 3 **C** Mesosoma dorsal **D** Mesosoma lateral **E** Propodeum and tergites 1 to 2.

Male. As female.
Biology. Host unknown.
Distribution. Democratic Republic of the Congo.

Molecular data. The holotype and 10 paratype sequences all belong to BIN BOLD:AAH1374, which is 14.2 % different from the closest Microgastrinae in BOLD.

Etymology. Named after the Hungarian Natural History Museum, in recognition of the outstanding and important collection of 8+ million insect specimens that institution holds, including one of the largest and most complete Microgastrinae collections in the world. The species name refers to the first and last words of the Hungarian name of the museum (Magyar Természet-Tudományi Múzeum). Of further significance is that the genus of the new species is itself named after Jeno Papp, who worked in the Hungarian Natural History Museum for many years.

***Jenopappius niger* (de Saeger, 1944)**
Fig. 19A

Microplitis niger de Saeger, 1944. Original description (de Saeger 1944: 46).

Holotype. Female, Democratic Republic of the Congo, RMCA (Musee Royal de l'Afrique Centrale, Tervuren, Belgium). Not examined, but original description checked.

Diagnosis. *J. niger* can be separate from the other known species of the genus because of shape of T1, and by having raised median area of T2 as wide as width of T1 at posterior margin (Fig. 19).

Biology. Host unknown.

Distribution. Democratic Republic of the Congo.

Molecular data. No molecular data available.

Comments. The information about this species was extracted from the original description (de Saeger 1944).

***Jimwhitfieldius* Fernandez-Triana, gen. n.**
http://zoobank.org/E72F425C-78F7-4D0B-B9F1-C0E4751FEC9D

Type species. *Jimwhitfieldius jamesi* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Flagellomere with placodes arranged in three rows (females and males) (Figs 23F, 25G). Head posteriorly with a deep depression, behind occiput (Fig. 26E). Pale spot at base of mandible. Hypostomal carina with a projecting flange. Mesosoma mostly smooth (Figs 22E, 25E, 26E). Propodeum entirely smooth, without any carina (Figs 22D, E). Metatrochantellus with highly unusual shape (better illustrated in Fig. 23I), anteriorly with rounded projections. Relatively very large and thick inner spur in hind leg (0.8 × as long as first segment of metatarsus) (Figs 22A, 23G, J, 24A, D, 25H, 26A). Fore wing with large areolet (Figs 22C, 24A, C, 26C). Hind wing with vannal lone fully setose. Metasoma mostly smooth. Ovipositor extremely short, almost invisible externally (Figs 23H, I, 24A, 26C).

Putative autapomorphies and potentially related genera. The strong depression of the head behind the occiput, the shape of the metatrochantellus, and the length and shape of the inner spur of metatibia are all highly unusual within Microgastrinae. The extremely short ovipositor and ovipositor sheaths are probably the shortest observed in the entire subfamily. The flagellomeres with three rows of placodes are rarely found among some species of a few unrelated Microgastrinae genera. The hypostomal flange is similar to some species of *Prasmodon* (see Fernandez-Triana et al. 2014d), although the two genera are not related at all.

Biology. Host unknown.

Distribution. The known species are found in the Oriental region (Thailand, Vietnam).

Molecular data. A total of 19 sequences representing five BINs, BOLD:AAH1239, BOLD:AAV2073, BOLD:AAV2080, BOLD:AAV2083, and BOLD:ACE5642. Three of those BINs are only know from either one or two male specimens, whereas BOLD:AAH1239 (10 specimens) and BOLD:AAV2073 (5 specimens) are better represented.

Etymology. The genus name refers to and honors the American braconid expert James B. Whitfield, in recognition of his significant contributions to the knowledge of parasitoid wasps of the world, especially Microgastrinae and their associated polydnaviruses. For the past 18 years, Jim has been a mentor for the first author, and his friendship and advice have always been very much appreciated. The gender of the genus is neuter.

Species. All examined specimens are morphologically very similar, with minute differences in coloration (tergites 5+ with or without brown spots) and shape of T2 (more or less broadening towards posterior margin). Based on DNA barcoding, there could be up to 5 different species. However, three of those barcode-species are only represented by one or two male specimens each, and thus are not considered here (they will only be described if more material becomes available in the future). The two species described below differ slightly in morphology, their DNA barcodes have 14–18 bp different (2.1–2.8 %), and are found at different altitudinal ranges. They can be separate using the following key.

Key to species

1 Larger species, body L 3.5–3.8 mm and fore wing L 3.6–4.0 mm; T2 comparatively less strongly broadening towards posterior margin (width at posterior margin 1.8–2.3 × width at anterior margin) and comparatively thinner (length medially 0.9–1.0 × width at posterior margin) [All known specimens collected between 75 and 100 m in southern Thailand and Vietnam] ....***Jimwhitfieldius jamesi*** **Fernandez-Triana & Boudreault, sp. n.**

– Smaller species, body L 2.9–3.0 mm and fore wing L 3.5 mm; T2 comparatively more strongly broadening towards posterior margin (width at posterior margin 3.0 × width at anterior margin), and comparatively less thin (length medially 0.8 × width at posterior margin) [All known specimens collected between 273 and 1,306 m in central Thailand] ....***Jimwhitfieldius sydneyae*** **Fernandez-Triana & Boudreault, sp. n.**

Jimwhitfieldius jamesi **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/9D99AF14-50BC-41EB-94D0-7D3FAC14A7CD
Figs 22, 23, 24, 25

Holotype. Female, Thailand, QSBG.

Holotype labels. Thailand. Trang prov./Khao Chong, Forest/Research Station.,/7.551°N, 99.79°E, 75m. Second label: 1.VIII.2005, D. Lohman,/ BIN#BOLD:AAH1239,/**CNCH2640**. Third label: CNCH2640.

Holotype locality. THAILAND, Trang Province, Khao Chong, Forest Research Station, 7.551°N, 99.79°E, 75m.

Paratypes. Thailand. (1♀ QSBG), Trang, Khao Chong Forest Research Station, 7.550556, 99.789722, 75m, xi.2005, coll. D. Lohman, Voucher code: CNC878555; (3♂ QSBG), Khaochong, 7.561000, 99.886000, 75m, Voucher codes: CNCH2136, CNCH2138, CNCH2139; (1♂ QSBG), Khoa Chong, Forest Research St., 7.551000, 99.790000, 75m, coll. D. Lohman, Voucher code: CNCH2270; (2♂ QSBG), Khoa Chong, 7.551000, 99.790000, 75m, coll. D. Lohman, Voucher codes: CNCH2041, CNCH1994; (3♀ QSBG), Trang, Nayon Khao Chong, 7.561000, 99.886000, 75m, Voucher codes: CNCH2232, CNCH2236, CNCH2239. **Vietnam.** (1♀ CNC), Cat Thien National Park, Dong Nal, 100m, Bird trail, Malaise trap, 15–20.v.2007, coll. C.v. Achterberg&R.de Vries, Voucher code: CNC924047; (4♂ RMNH), Cat Tien National Park, Dong Nai, 100m, Bird trail;Dong Trail, Malaise trap, 1–9.x.2005, coll. C.v. Achterberg&R.de Vries, Voucher codes: CNC924051, CNC924052, CNC924053, CNC924054; (5♂ 2♀ RMNH), Botanical Garden;Eco-trail, Malaise trap, 13–20.v.2007, coll. C.v. Achterberg & R.de Vries, Voucher codes: CNC924028, CNC924033, CNC924050, CNC924034, CNC924035, CNC924036, CNC924037; (3♂ RMNH), Lagerstroemia trail, Malaise trap, 14–20.v.2007, coll. C.v. Achterberg & R.de Vries, Voucher codes: CNC924055, CNC924056, CNC924057; (7♂ RMNH), Bird trail, Malaise trap, 15–20.v.2007, coll. C.v. Achterberg & R.de Vries, Voucher codes: CNC924038, CNC924039, CNC924040, CNC924041, CNC924042, CNC924043, CNC924044; (4♂ RMNH), Dong Trail, Malaise trap, 19–25.iv.2007, coll. Mai Phu Quy & Nguyen Thanh Manh, Voucher codes: CNC924029, CNC924030, CNC924031, CNC924032; (2♂ RMNH), 9–30.iv.2007, coll. M.P. Quiu & N.T. Manh , Voucher codes: CNC924058, CNC924059; (1♂ RMNH), 9.iv-13.v.2007, coll. Mai Phu Quy & Nguyen Thanh Manh, Voucher code: CNC924049; (1♂ RMNH), Dong Trail, Malaise trap, 9.iv-19.v.2007, coll. M.P. Quiu, N.T. Manh & C.V. Achterberg, Voucher code: CNC924048; (2♀ RMNH), Nature reserve, Phong Dien, Thua Thien Hue, 150m, Nature reserve base camp, 15km West Phong My., Malaise trap, 23.iii-6.iv.2001, coll. C.v. Achterberg & R.de Vries, Voucher codes: CNC924026, CNC924027; (2♂ RMNH), Nul Chua National Park, Ninh Thuan, 150m, Northwest part, Malaise trap, 24–30.v.2007, coll. C.v. Achterberg & R.de Vries, Voucher codes: CNC924045, CNC924046.

Diagnosis. The two known species of the genus are very similar morphologically. *J. jamesi* is a larger species (usually its body length is at least 0.5 mm larger than *J. sydneyae*) and T2 is comparatively less broad apically (T2 width at posterior margin

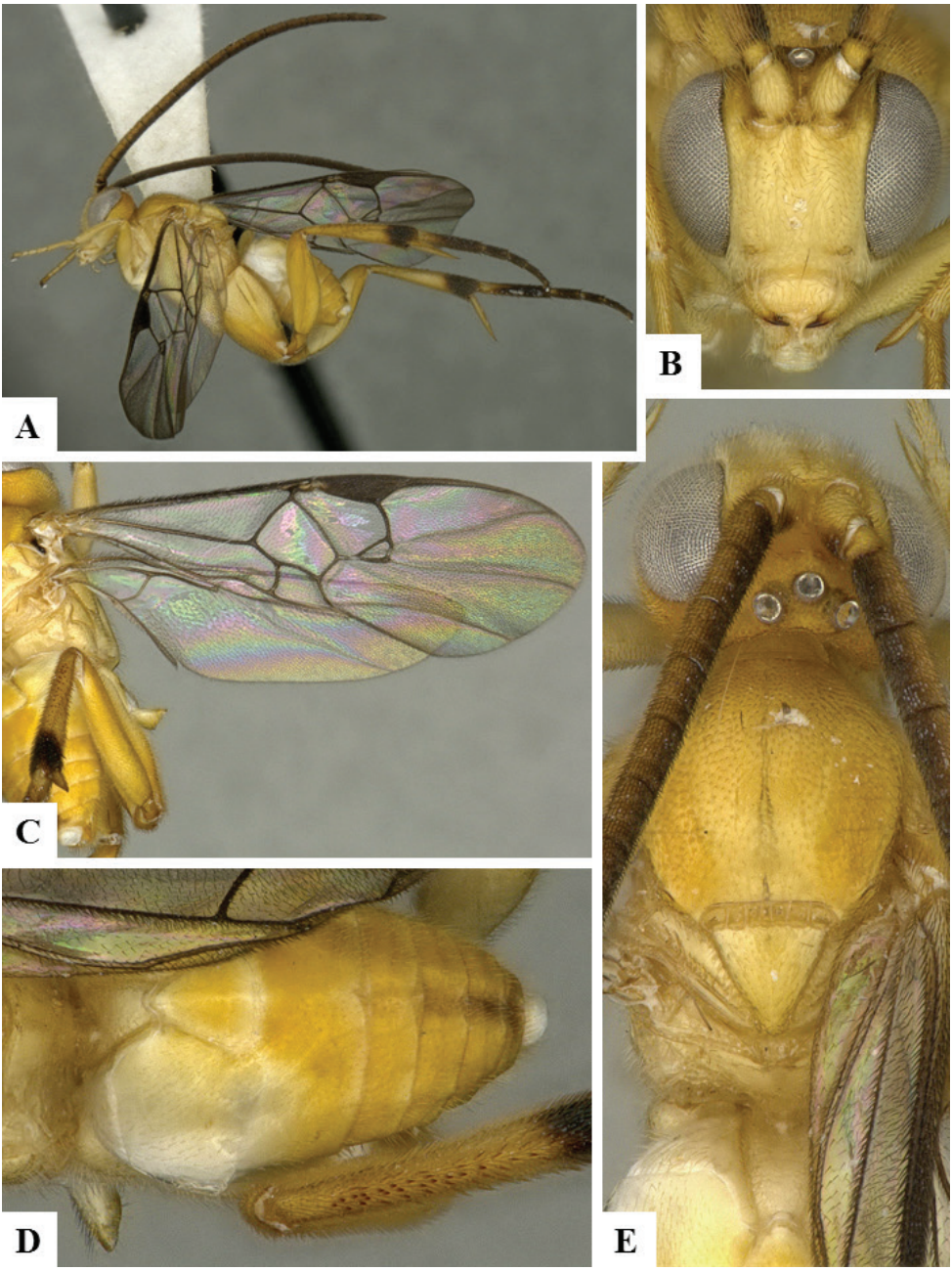


Figure 22. *Jimwhitfieldius jamesi* female holotype. **A** Habitus **B** Head frontal **C** Fore wing and hind wing **D** Metasoma dorsal **E** Head and mesosoma, dorsal.

1.8–2.3 × width at anterior margin, whereas *J. sydneyae* has T2 width at posterior margin 3.0 × width at anterior margin). The known geographical distribution of the two species is also different, with *J. jamesi* found at lower altitudes in southern Thai-

Figure 23. *Jimwhitfieldius jamesi* female holotype. **F** Antennal flagellomeres 1 to 4 **G** Hind leg **H** Hypopygium and ovipositor, lateral **I** Hypopygium and ovipositor, ventral **J** Inner and outer spines of metatibia.

land and Vietnam (75–100 m), whereas all known specimens of *J. sydneyae* have been collected at higher altitudes (273–924 m). DNA barcodes of the two species also have more than 2% of base pair differences.

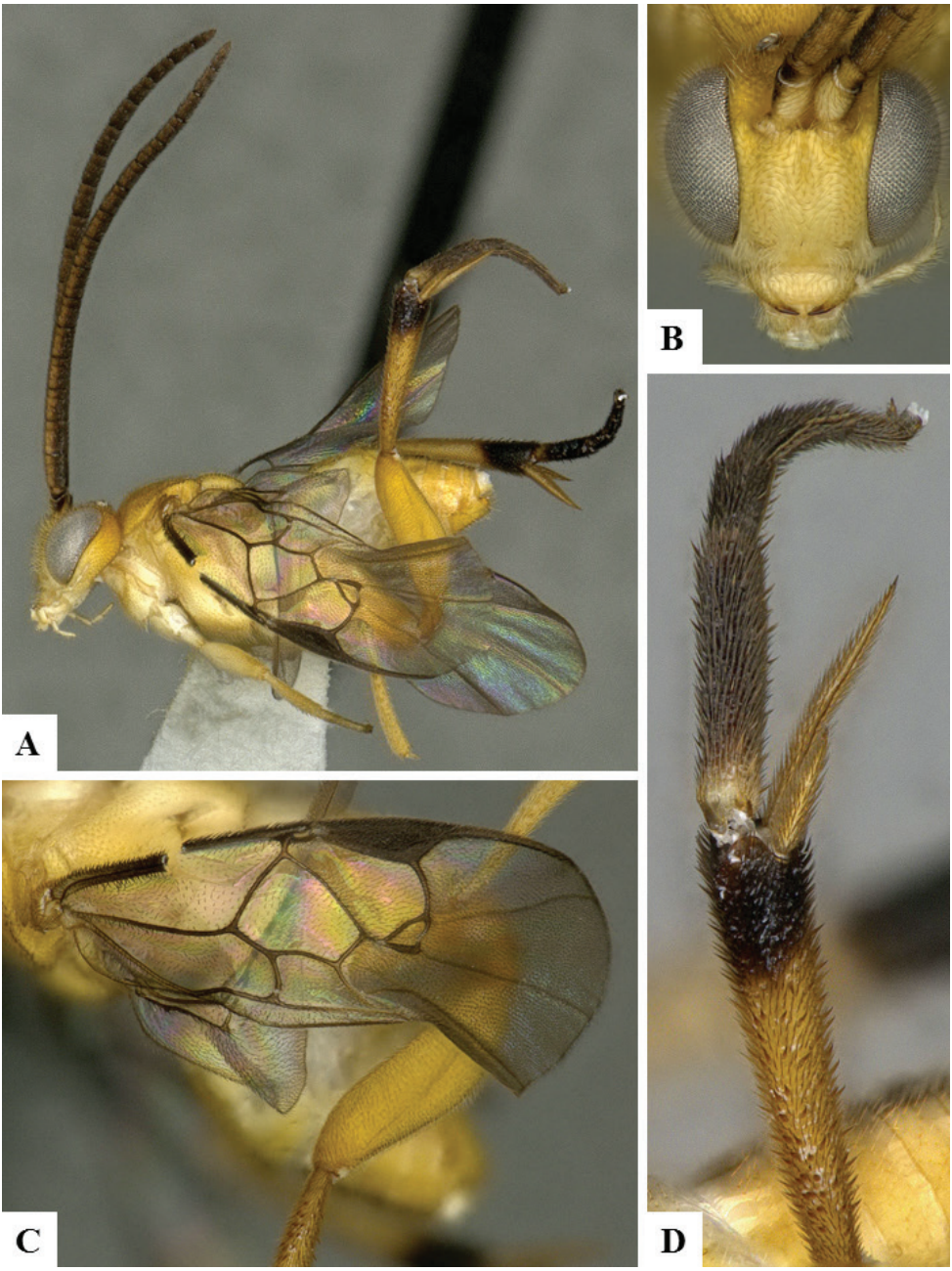


Figure 24. *Jimwhitfieldius jamesi* female paratype CNC878555. **A** Habitus **B** Head frontal **C** Fore wing **D** Inner and outer spines of metatibia.

Description. Female. Body mostly yellow to yellow-white, with only antennae brown (light brown ventrally, dark brown dorsally), posterior 0.2 of metatibia and metatarsus dark brown to black; base of mandible slightly discolored (with paler spot); wings hyaline,

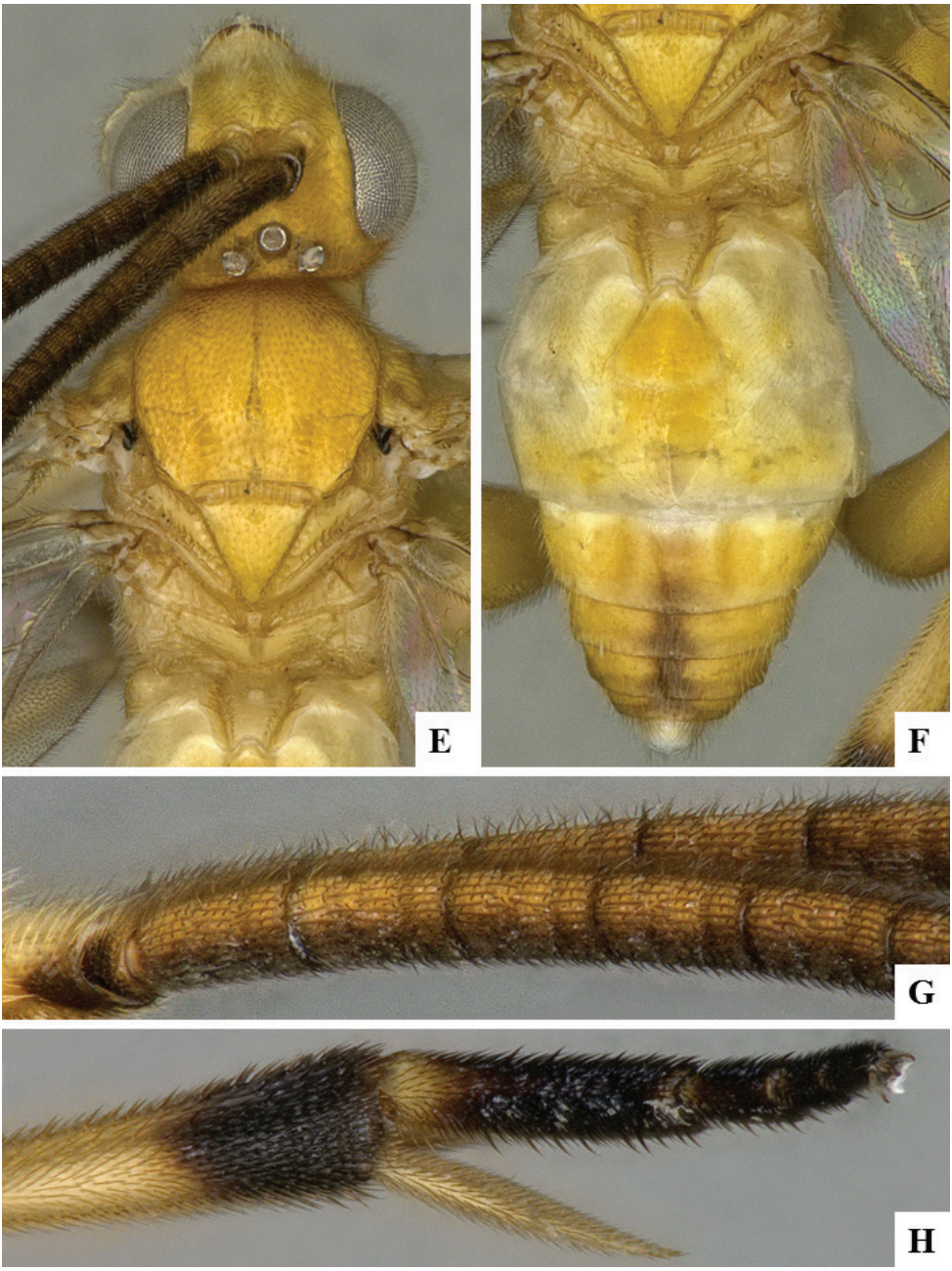


Figure 25. *Jimwhitfieldius jamesi* female paratype CNC878555. **E** Head and mesosoma, dorsal **F** Metasoma dorsal **G** Antennal flagellomeres 1 to 5 **H** Inner spine of metatibia.

veins dark brown. Body mostly smooth, with very shallow and sparse punctures in some areas. Flagellomeres with placodes arranged in three rows. Head posteriorly with a deep depression, behind occiput. Hypostomal carina with projecting flange. Mesosoma mostly

smooth. Scutoscutellar sulcus with some 6 strong crenulae. Propodeum entirely smooth, without any carina. Metatrochantellus with unique shape (better illustrated in Fig. 23I), anteriorly with rounded projections. Relatively very large (0.8 × as long as first segment of metatarsus) and thick inner spur in hind leg. Fore wing with large areolet. Hind wing with vannal lobe fully setose. Metasoma mostly smooth. Ovipositor extremely short, almost invisible externally. **Body measurements (mm).** F2 L: 0.33 (0.29–0.33); F3 L: 0.33 (0.29–0.33); F14 L: 0.29 (0.25–0.29); F15 L: 0.27 (0.23–0.27); Malar sulcus L: 0.10 (0.09–0.10); Mandible W: 0.13 (0.11–0.13); T1 L: 0.53 (0.52–0.54); T1 W at posterior margin: 0.18 (0.13–0.15); T1 maximum W: 0.27 (0.22–0.26); T2 W at anterior margin: 0.15 (0.15–0.18); T2 W at posterior margin: 0.33 (0.30–0.37); T2 L: 0.33 (0.29–0.38); Metafemur L: 1.44 (1.29–1.45); Metafemur W: 0.42 (0.38–0.42); Metatibia L: 1.57 (1.44–1.63); Inner spur L: 0.75 (0.68–0.78); Outer spur L: 0.39 (0.34–0.40); First segment of Metatarsus L: 1.00 (0.91–1.00); Ovipositor sheaths L: 0.08 (0.05–0.07); Body L: 3.84 (3.66–3.92); Fore wing L: 4.08 (3.64–4.08). Maximum W of T1 is taken at anterior margin of T1 for all specimens. T1 L is approximate for 3 specimens and impossible to measure for 2 specimens. Fore wing L is approximate for 2 specimens.

Male. As female.

Biology. Host unknown.

Distribution. Thailand, Vietnam.

Molecular data. The holotype and 9 paratype sequences all belong to BIN BOLD:AAH1239, which is 1.5 % different from the closest Microgastrinae in BOLD (specimens of an undescribed species of *Jimwhitfieldius*).

Etymology. Named after James B. Whitfield in appreciation of the many things the first author has learned from him.

Jimwhitfieldius sydneyae **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/5A4C7912-338D-4A23-B021-550C5A96B875
Figs 26

Holotype. Female, Thailand, QSBG.

Holotype labels. THAILAND: Loei, Phu/Kradueng NP, mixed/deciduous/Elerd, 273m/16.566, 101.49, 19.iii.2008/Thonghuay Phatai/JMIC 0138. Second label: JMIC 0138.

Holotype locality. THAILAND, Loei Province, Phu, Kradueng National Park, mixed deciduous, 273m, 16.566, 101.49.

Paratypes. Thailand. (1♂ CNC), Lampang, Chae Son NP, youth camp/meeting hall, 18.499000, 99.282000, 476m, 16.iii.2008, coll. B. Kwannui & A. Sukpeng, Voucher code: JMIC0284; (1♂ QSBG), Kamphaeng Phet, Mae Wong National Park, Chong Yen, 16.521000, 99.658000, 1306m, 2.iv.2008, coll. C. Piluek, Voucher code: WAM0039; (1♂ QSBG), Thung Salaeng Luang NP, staff house, Gang Sopa waterfall; Phitsanulok, 16.527000, 100.493000, 486m, 7.v.2007, coll. Pongpitak & Sathit, Voucher code: JMIC0165. (1♂ CNC), Mae Wong NP, Chong Yen; Kamphaeng Phet,

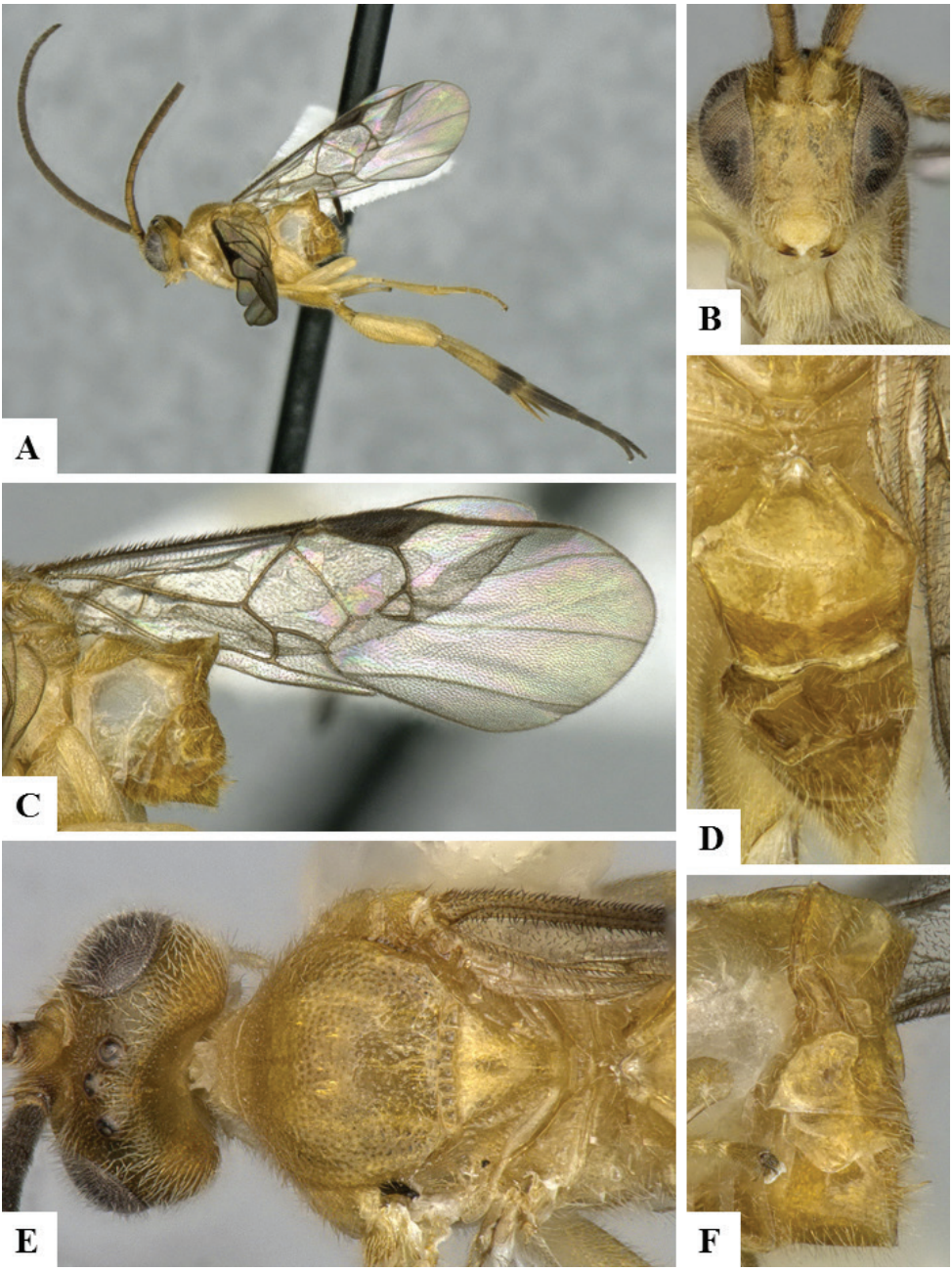


Figure 26. *Jimwhitfieldius sydneyae* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Metasoma dorsal **E** Head and mesosoma, dorsal **F** Hypopygium and ovipositor, lateral.

16.521000, 99.658000, 1306m, 2.iv.2008, coll. C. Piluek, Voucher code: JMIC0026; (1♂ CNC), Phetchabun, Nam Nao NP, Check Point, 16.437000, 101.638000, 924m, 26.v.2007, coll. Noopean Hongyothi, Voucher code: JMIC0258.

Diagnosis. See previous species for comments on how to separate both.

Description. Female. Body mostly yellow to yellow-white, with only antennae brown (light brown ventrally, dark brown dorsally), posterior 0.2 of metatibia and metatarsus dark brown to black; base of mandible slightly discolored (with paler spot); wings hyaline, veins dark brown. Body mostly smooth, with very shallow and sparse punctures in some areas. Flagellomeres with placodes arranged in three rows. Head posteriorly with a deep depression, behind occiput (Fig. 26E). Hypostomal carina with projecting flange. Mesosoma mostly smooth. Scutoscutellar sulcus with some 6 strong crenulae. Propodeum entirely smooth, without any carina. Metatrochantellus with unique shape (see Fig. 23I), anteriorly with rounded projections. Relatively very large (0.8 × as long as first segment of metatarsus) and thick inner spur in hind leg. Fore wing with large areolet. Hind wing with vannal lobe fully setose. Metasoma mostly smooth. Ovipositor extremely short, almost invisible externally. **Body measurements (mm).** F2 L: 0.28; F3 L: 0.28; F14 L: 0.24; F15 L: 0.23; Malar sulcus L: 0.09; Mandible W: 0.10; T1 W at posterior margin: 0.18; T1 maximum W: 0.34; T2 W at anterior margin: 0.18; Metafemur L: 1.19; Metafemur W: 0.36; Metatibia L: 1.31; Inner spur L: 0.67; Outer spur L: 0.36; First segment of Metatarsus L: 0.81; Ovipositor sheaths L: 0.04; Body L: 2.75; Fore wing L: 3.59. Maximum W of T1 is approximate.

Male. As female.

Biology. Host unknown.

Distribution. Thailand.

Molecular data. The holotype and 5 paratype sequences all belong to BIN BOLD:AAV2073, which is 2.3 % different from the closest Microgastrinae in BOLD (specimens of an undescribed species of *Jimwhitfieldius*).

Etymology. Named after Sydney Cameron as appreciation of the very nice moments shared during several visits the first author made to her and Jim in Illinois.

Kotenkosius Fernandez-Triana, gen. n.

http://zoobank.org/0F2736F2-ACC3-405C-98C7-AF92FD8C2BF2

Type species. *Kotenkosius tricarinatus* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Face with slightly coarse punctures. Mesosoma mostly smooth, at most with areas with sparse and shallow punctures. Polished area of lateral face of scutellum (lunules) relatively very small, less than 0.2 height of lateral face (Fig. 27D). Propodeum carination pattern that includes three complete longitudinal carinae (one medially, the other two sublaterally) and a complete transverse carina (subapically), with additional small striae radiating from the median and sublateral carinae (Figs 27D, F); most carinae are strongly defined and raised. Fore wing with relatively large and quadrate areolet (Fig. 27C). Hind wing with vannal lobe entirely setose. Metasomal terga smooth. T1 rectangular, T2 trapezoidal (Figs 27E, F). Hy-

popygium inflexible, without pleats. Ovipositor sheaths setose and less than half the length of the metatibia (Fig. 27A).

Putative autapomorphies and potentially related genera. The carination pattern of propodeum is unique among Microgastrinae. *Kotenkosius* is likely related to *Choeras* s.l. (see Discussion below) but it has a very different propodeum carination and an inflexible, unpleated hypopygium.

Biology. Host unknown.

Distribution. The only species known is found in the Oriental region.

Molecular data. Among the specimens we have been able to study, three have sequences available in BOLD, all corresponding to BIN BOLD:AAV2185. Another three sequences (from specimens we have not seen) are part of that same BIN, suggesting they all belong to the same species. That BIN is far apart from other Microgastrinae with available DNA barcodes (with the exception of BIN BOLD:ADB2437, which seems related to *K. tricarinatus* and could represent a second species of *Kotenkosius*, see more comments on the section "**Species**" below).

Etymology. The genus name refers to and honors the Ukrainian braconid expert Anatoly G. Kotenko, in recognition of his significant contributions to the knowledge of Braconidae, specially his work on East Palearctic Microgastrinae. The gender of the genus is neuter.

Species. Although there are slight differences between specimens from several countries (with some specimens being lighter coloured) we consider them all to be conspecific. Thus, we recognize here only one species, which seems to be rather widespread in the Oriental region (Bangladesh, Malaysia, Taiwan, Thailand, and Vietnam). However, in BOLD there is another BIN (BOLD:ADB2437), which contains five sequences of specimens from Indonesia, which seems closely related to *K. tricarinatus* and thus could represent a second species in this genus. However, we have not seen those specimens nor have access to those sequences and thus cannot conclude on that nor describe that putative second species.

***Kotenkosius tricarinatus* Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/715D9FC0-153C-4A0A-B14E-A3FBDEEAC13F
Figs 27

Holotype. Female, Vietnam, RMNH.

Holotype labels. Mic./760. Second label: VN: Yên Bái, Luc/Yên, Phúc Lợi./Rứng TS 07-X-2003/KH. Đ. LONG. Third label: CNC878543. There is a fourth, red label associated with the holotype specimen. Apparently it was attached as the specimen was considered to be a potential paratype of a *Choeras* species never described. We are not detailing that name here as it is not valid, and the specimen does not belong to *Choeras*, but we are just noting the existence of that fourth label –which we did not remove because the specimen belongs to a different institution than ours.

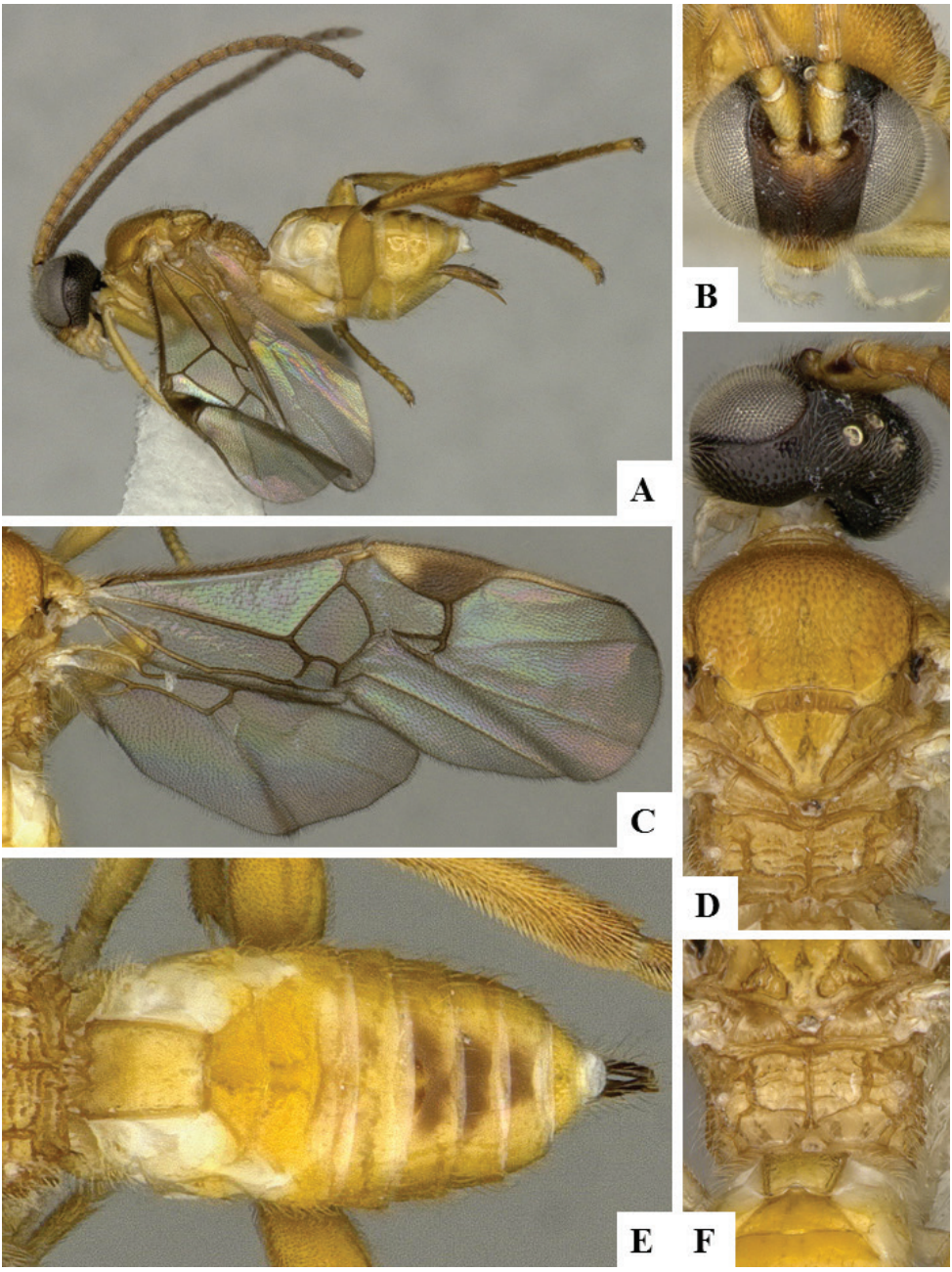


Figure 27. *Kotenkosius tricarinatus* female holotype. **A** Habitus **B** Head frontal **C** Fore wing and hind wing **D** Mesosoma dorsal **E** Metasoma dorsal **F** Propodeum.

Holotype locality. VIETNAM, Yên Bái, LucYên, Phúc Lợi. Rứng.

Paratypes. Malaysia (2♀ RMNH) Sabah, near Long Pa Sia, Payakalaba, 1010m, Malaise trap, 12–13.iv.1987, coll. C.v. Achterberg, Voucher codes: CNC878547,

CNC878548. **Taiwan.** (1♂ CNC) Wushe, 1150m, v.1983, coll. H. Townes, Voucher code: CNC878549. **Thailand.** (1♂ QSBG) Chiang Mai, Doi Chiang Dao WS Nature Trail, 19.404633, 98.921850, 491m, pan trap, 1–2.x.2007, coll. Songkran & Apichart, Voucher code: CNC878546; (1♀ QSBG) Kaeng Krachan NP, km 16/road/stream 2; Phet Buri, 12.481000, 99.267000, 320m, 22.vi.2009, coll. Sirichai, Voucher code: JMIC0181; (1♀ QSBG) Kaeng Krachan NP, km 33/helipad; Phet Buri, 12.502000, 99.207000, 735m, 4.v.2009, coll. Sirichai, Voucher code: JMIC0038; (1♀ QSBG) 8.vi.2009, coll. Sirichai, Voucher code: JMIC0193. **Vietnam.** (1♂ RMNH) Hã Giang, Vi Xuyên Dao Duc. Rung TS, 20.x.2006, coll. KH. D. Long, Voucher code: CNC878544; (1♂ RMNH) Viet Try, Thuong Cuu, near Thanh Son, 20.983333, 105.133333, 300 to 400m, 12–16.x.1999, coll. R. de Vries, Voucher code: CNC878545.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Female. Body color mostly yellow, except for dark brown to black head and T5-7 sometimes with brown marks centrally. Antenna yellow to light brown-yellow. Legs yellow except for dark brown spot on anterior 0.1 of metatibia, and brown metatarsus. Wings veins mostly brown, except for yellow-white vein R1 on fore wing and pterostigma with yellow-white spot on anterior 0.3. Face with slightly coarse punctures. Mesosoma mostly smooth, at most with areas with sparse and shallow punctures. Polished area of lateral face of scutellum (lunules) relatively very small, less than 0.2 height of lateral face. Propodeum carination pattern that includes three complete longitudinal carinae (one medially, the other two sublaterally) and a complete transverse carina (subapically), with additional small striae radiating from the median and sublateral carinae (Fig. 27D, F); most carinae are strongly defined and raised. Fore wing with relatively large and quadrate areolet. Hind wing with vannal lobe entirely setose. Metatibia with relatively strong spines (peg-like) on dorsal surface, which are darker than metatibia color. Metasomal terga smooth. T1 rectangular, T2 trapezoidal. Hypopygium inflexible, without pleats. Ovipositor sheaths setose and less than half the length of the metatibia. **Body measurements (mm).** F2 L: 0.24 (0.25–0.26); F3 L: 0.23 (0.25–0.26); F14 L: 0.13 (0.14–0.17); F15 L: 0.12 (0.11–0.13); Malar sulcus L: 0.06 (0.07); Mandible W: 0.10 (0.09); T1 L: 0.44 (0.43–0.48); T1 W at posterior margin: 0.19 (0.21); T1 maximum W: 0.27 (0.27); T2 W at anterior margin: 0.20 (0.18–0.20); T2 W at posterior margin: 0.38 (0.35–0.36); T2 L: 0.16 (0.14–0.16); Metafemur L: 0.76 (0.80–0.83); Metafemur W: 0.25 (0.26–0.28); Metatibia L: 1.03 (1.03–1.07); Inner spur L: 0.23 (0.26–0.28); Outer spur L: 0.14 (0.18); First segment of Metatarsus L: 0.45 (0.48–0.50); Ovipositor sheaths L: 0.46 (0.50–0.53); Body L: 3.06 (2.45–3.03); Fore wing L: 3.06 (3.19–3.28). T2 W at posterior margin is approximate for 1 specimen.

Male. As female.

Biology. Host unknown.

Distribution. Bangladesh, Malaysia, Taiwan, Thailand, Vietnam.

Molecular data. Three paratypes with available sequences belong to BIN BOLD:AAV2185, which is 6.7 % different from the closest Microgastrinae sequence in BOLD (another putative, undescribed species of *Kotenkosius*, but we have not been able to see specimens from that BIN).

Etymology. From Latin "trēs" (meaning "three") and "carina" (meaning "keel"), referring to the three longitudinal carinae found on the propodeum.

Comments. The record of this species from Bangladesh is based on a sequence recorded in BOLD which matches the sequences of the paratypes; however, we have not seen that specimen and thus cannot confirm unequivocally its identity. A second species of *Kotenkosius* seems to be revealed in BOLD (BIN BOLD:ADB2437), based on how similar the sequences are; however, we have not seen specimens from that BIN and thus cannot conclude on that.

Markshawius Fernandez-Triana, gen. n.

http://zoobank.org/AAB8DED0-1B31-4ACF-8590-9C73F4048991

Type species. *Markshawius erucidoctus* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Female head elongate and strongly concave posteriorly, modified to be tightly appressed to and follow the contour of anterior margin of pronotum (pronotum also concave). Upper margin of face produced dorsally between the antennal insertions into a triangular flange (Figs 28B, 29B, 30B, 31B). Face looking almost depressed, and with very strong sculpture including transverse striae and punctures (Figs 28B, 29B, 30B, 31B). Frons very elongate, with ocelli clearly much higher than normally found in Microgastrinae. Frons with strong excavation at antennal base –better appreciated on a lateral view of the head (Figs 29C, 31E). Antenna very short (much shorter than body length, usually shorter than the combined length of head and mesosoma), with all flagellomeres but first with a single row of placodes (Figs 28A, E, 29A, 30A, 31A). Pronotum only with lower sulcus (which is sometimes barely visible). Propodeum with median carina clearly visible on posterior half (sometimes that carina looks divided, giving the impression of actually being the posterior half of a very thin areola). Propodeum sometimes with transverse rugosity medially, including a poorly and partially defined transverse carina (Figs 28D, F, 29F, G, 30E, F, 31G, H). Fore wing with large, four-sided areolet (Figs 28C, 29D, 30D, 31D). Legs in general short and stout, especially metafemur (Figs 28A, 29A, 30A, 31A). T1 with unusual, very distinctive shape: in some species being extremely long and thin (T1 length at least 6.0 × its width centrally) (Figs 29E–G, 30D, E), in other species very thin on anterior 0.3–0.4, then strongly widening towards posterior margin (width at posterior margin around 3.0 × its width centrally) (Figs 28D, F, 31F, G). T2 either trapezoidal and with lateral margins strongly sculptured, or subtriangular and with lateral margins less sculptured (Figs 28D, F, 29E–G, 30D, E, 31F, G). Ovipositor sheaths almost without setae (with only very few, small setae near apex that are usually invisible at less than 100 × of magnification), ovipositor strongly narrowing toward apex, where it looks almost needle-like.

Putative autapomorphies and potentially related genera. The carination pattern of propodeum is unique among Microgastrinae. The two known shapes of T1 are also highly unusual. All species of *Protomicroplitis* and *Wilkinsonellus*, and some species of *Apanteles*, *Diolcogaster* and *Venanides* have very long and thin T1; however, they have a strong median sulcus on T1 (*Diolcogaster*, *Protomicroplitis* and *Wilkinsonellus*) or are completely unrelated genera with many different and distinguishing features compared to *Markshawius* (*Apanteles* and *Venanides*). The shape of the head is similarly shared with a few species of other genera (e.g., *Diolcogaster* and, to a lesser extent also some species of *Cotesia*, *Keylimepie* and *Venanides*). All of those genera, except for *Diolcogaster*, are unrelated to *Markshawius*, suggesting that trait likely evolved independently several times within Microgastrinae parasitizing stem borers.

Biology. Hosts are unknown at present. However, it is here hypothesized that the modification of head and pronotum serves the purpose of facilitating entering into or egressing from narrow tunnels where the caterpillar hosts live, and those hosts most likely are stem borers, perhaps from the Lepidoptera superfamily Pyraloidea.

Distribution. All known species are found in the Oriental region (Thailand, Vietnam).

Molecular data. Only one sequence available (a complete barcode), but it is very unique, 11.2 % different than next Microgastrinae sequence available in BOLD.

Etymology. The genus name refers to and honors the British braconid expert Mark Shaw, in recognition of his outstanding contributions to the knowledge of Hymenoptera, especially host/parasitoid biology. Throughout the years, Mark has been a mentor, dear friend, and an inspiration for the first author to continue his work with parasitoid wasps. The gender of the genus is neuter.

Comments. The species described below have two different sculpture patterns of propodeum, as well as two different shapes of T1. Future studies may find that those species are better placed in separate genera, but due to the paucity of specimens we prefer to keep them all within one single genus for the time being.

Species. We recognize three different species, all new and described below. They can be separate using the following key.

Key to species 6

1 T1 entirely parallel-sided and extremely long and thin, its length at least 6.0 × its width; T2 subtriangular and with lateral margins less sculptured (Figs 29E–G, 30D, E); propodeum without transverse rugosity medially and without any defined transverse carina (Figs 29F, 30E, F) [Thailand, Vietnam]..... .....***Markshawius francescae*** **Fernandez-Triana & Boudreault, sp. n.**

– T1 thin and parallel-sided on anterior 0.2–0.3, then widening towards posterior margin so that width at posterior margin is up to 3.0 × its width centrally (Figs 28D, F, 31F, G); T2 trapezoidal and with lateral margins strongly sculptured; propodeum with transverse rugosity medially, including a poorly and partially defined transverse carina (Figs 28D, F, 31G, H) **2**

2 Relatively larger size (fore wing L 2.2 mm); fore wing with vein R1 pale (light yellow), in contrast with brown pterostigma; relatively broader pterostigma, its length 2.3 × its maximum width (Fig. 28C); scutoscutellar sulcus with at least ten, well defined crenulae (Fig. 28F); metanotum with five costulae on each side; widest part of T1 (around posterior 0.6) wider than T2 width at anterior margin (Figs 28D, F) [Northern Vietnam] ....***Markshawius erucidoctus*** **Fernandez-Triana & Boudreault, sp. n.**

– Relatively smaller size (fore wing L 1.6 mm); fore wing with vein R1 brown, same color than pterostigma; relatively narrower pterostigma, its length 2.6 × its maximum width (Fig. 31D); scutoscutellar sulcus with around seven, less defined crenulae (Fig. 31H); metanotum with three costulae on each side; widest part of T1 (around posterior 0.6) same width than T2 width at its anterior margin (Fig. 31F) [Southern Thailand]..... ..... ***Markshawius thailandensis*** **Fernandez-Triana & Boudreault, sp. n.**

Markshawius erucidoctus **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/53CBBBDD-9ABF-4F9F-8CBB-259DA909F5F6
Fig. 28

Holotype. Female, Vietnam, RMNH.

Holotype labels. N. VIETNAM: Ninh Binh/Cuc Phuong N.P., nr entrance/c225 m, 15.iv-1.v.2000, Mal. tr. II/Mai Phu Quv, RMNH'00. Second label: CNC878536.

Holotype locality. VIETNAM, Ninh Binh Province, Cuc Phuong National Park, near entrance, 225m.

Diagnosis. The shape of T1 and sculpture of propodeum clearly separate *M. erucidoctus* from *M. francescae* (see under that species for further details). As for the other species, *M. erucidotus* is a larger species than *M. thailandensis* (fore wing L 2.2 mm versus 1.6 mm), has fore wing vein R1 light yellow (R1 brown in *M. thailandensis*), has a broader pterostigma and more defined crenulae on scutoscutellar sulcus, and the widest part of T1 is wider than T2 width at anterior margin (widest part of T1 same width than T2 width at anterior margin in *M. thailandensis*).

Description. Female. Body color mostly brown; face partially reddish-brown; palpi yellow-white; labrum, mandible, scape, pedicel, and most of legs (except for metacoxa, posterior 0.1 of metatibia and metatarsus which are brown) orange-yellow; flagellomeres brown; tegulae and humeral complex, most laterotergites and sternites yellow-white to yellow-brown; wings hyaline, veins mostly brown. Head elongate and strongly concave posteriorly, modified to be tightly appressed to and follow the contour of anterior margin of pronotum (pronotum also concave). Upper margin of face produced dorsally between the antennal insertions into a triangular flange. Face looking almost depressed, and with very strong sculpture including transverse striae and punctures. Frons very elongate, with ocelli clearly much higher than normally found in Microgastrinae. Frons with strong excavation at antennal base –better appreciated on a

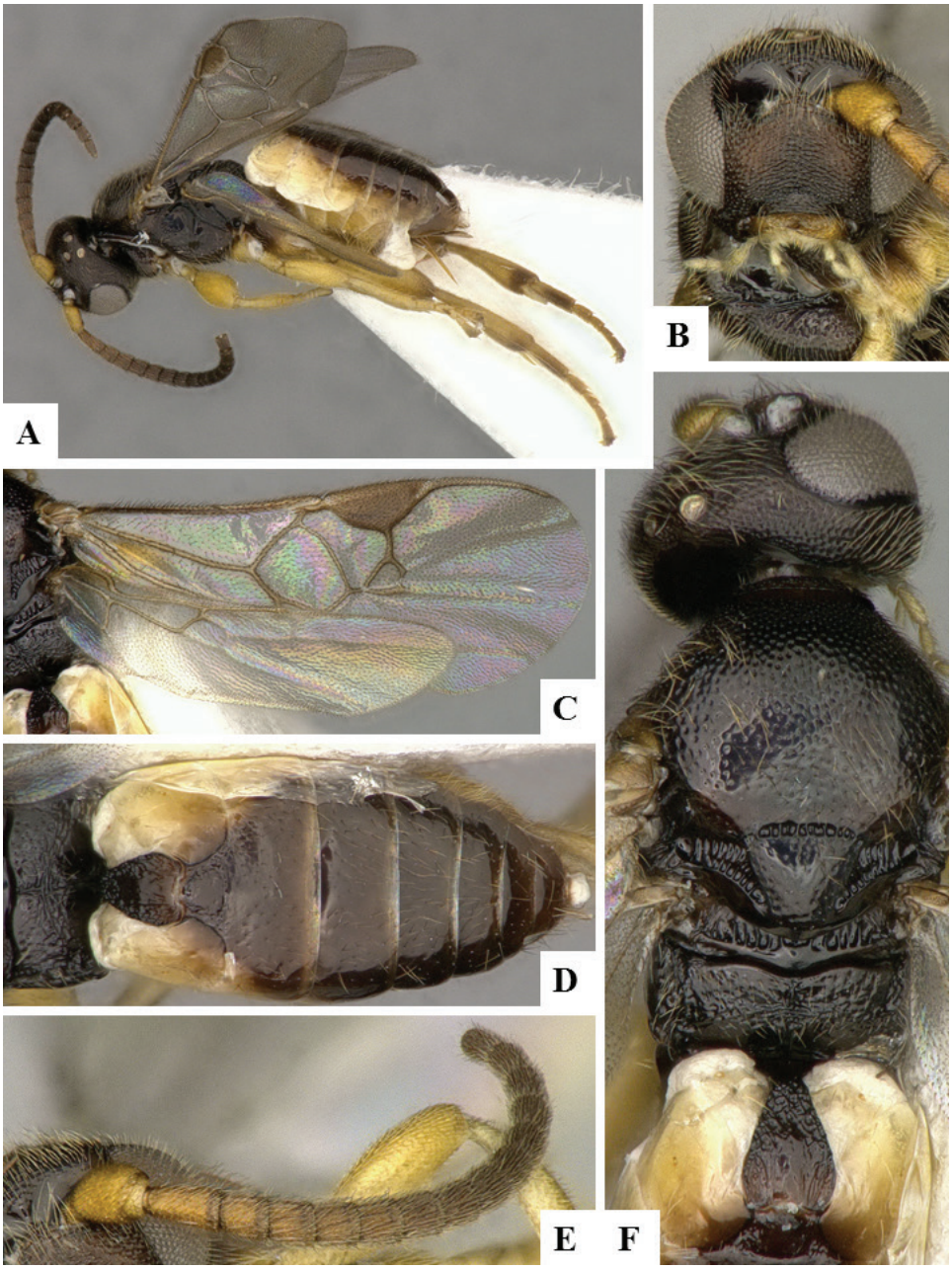


Figure 28. *Markshawius erucidoctus* female holotype. **A** Habitus **B** Head frontal **C** Fore wing and hind wing **D** Metasoma dorsal **E** Antenna **F** Mesosoma dorsal.

lateral view of the head. Antenna very short (shorter than the combined length of head and mesosoma), with all flagellomeres but first with a single row of placodes. Pronotum only with lower sulcus. Propodeum with median longitudinal carina clearly visible

on posterior half and with transverse rugosity medially, including a partially defined transverse carina. Propodeum with different sculpture, anterior area with punctures, posterior area mostly smooth. Fore wing with relatively large, four-sided areolet. Legs in general short and stout, especially metafemur. T1 very thin on anterior 0.3–0.4, then strongly widening towards posterior margin (width at posterior margin around 3.0 × its width centrally). T2 trapezoidal and with lateral margins strongly sculptured. Ovipositor sheaths almost without setae (with only very few, small setae near apex that are usually invisible at less than 100 × of magnification), ovipositor strongly narrowing toward apex, where it looks almost needle-like. **Body measurements (mm).** F2 L: 0.10; F3 L: 0.08; F14 L: 0.08; Malar sulcus L: 0.05; Mandible W: 0.12; T1 L: 0.40; T1 W at posterior margin: 0.16; T1 maximum W: 0.19; T2 W at anterior margin: 0.14; T2 W at posterior margin: 0.23; T2 L: 0.12; Metafemur L: 0.59; Metafemur W: 0.28; Metatibia L: 0.70; Inner spur L: 0.20; Outer spur L: 0.15; First segment of Metatarsus L: 0.27; Ovipositor sheaths L: 0.25; Body L: 2.45; Fore wing L: 2.40. T1 L and mandible W are approximate.

Male. Unknown.

Biology. Host unknown.

Distribution. Vietnam.

Molecular data. No molecular data available.

Etymology. From Latin "eruca" ("caterpillar") and "doctus" ("learned", "skilled", "erudite"), referring to a person with considerable knowledge about caterpillars. This species is dedicated to my dear friend and mentor Mark Shaw, the most knowledgeable researcher on caterpillar/parasitoid biology that I have ever met. He is indeed "the master" of the caterpillars and their parasitoids.

Markshawius francescae **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/76830621-3C39-4955-A8FA-28FF0A7CAA35
Figs 29, 30

Holotype. Female, Thailand, QSBG.

Holotype labels. Thailand. Chiang Mai/Montha Tarn Water Fall,/18.81560°N, 98.92910°E,/700m, **CNCH2123**. Second label: CNCH2123.

Holotype locality. THAILAND, Chiang Mai, Montha Tarn Water Fall, 18.81560°N, 98.92910°E, 700m.

Paratypes. Nepal. (1♀ CNC), Kathmandu, Godavari, 1828m, Malaise trap, 13–17.viii.1967, coll. Canadian Expedition, Voucher code: CNC878538; (1♀ CNC), 7–12.viii.1967, coll. Canadian Expedition, Voucher code: CNC878539; (1♀ CNC), Kathmandu, Pulchauki, 2011m, Malaise trap, 21–27.vii.1967, coll. Canadian Expedition, Voucher code: CNC878537. **Thailand.** (1♂ QSBG), Lampang, Chae Son NP, Doi Laan, unit 1, 18.508000, 99.217000, 1413m, 14.viii.2007, coll. B. Kwannui & A. Sukpeng, Voucher code: JMIC0496; (2♂ CNC, QSBG), Mae Wong NP, Chong Yen; Kamphaeng Phet, 16.521000, 99.658000, 1306m, 28.iv.2008, coll. C. Piluek,

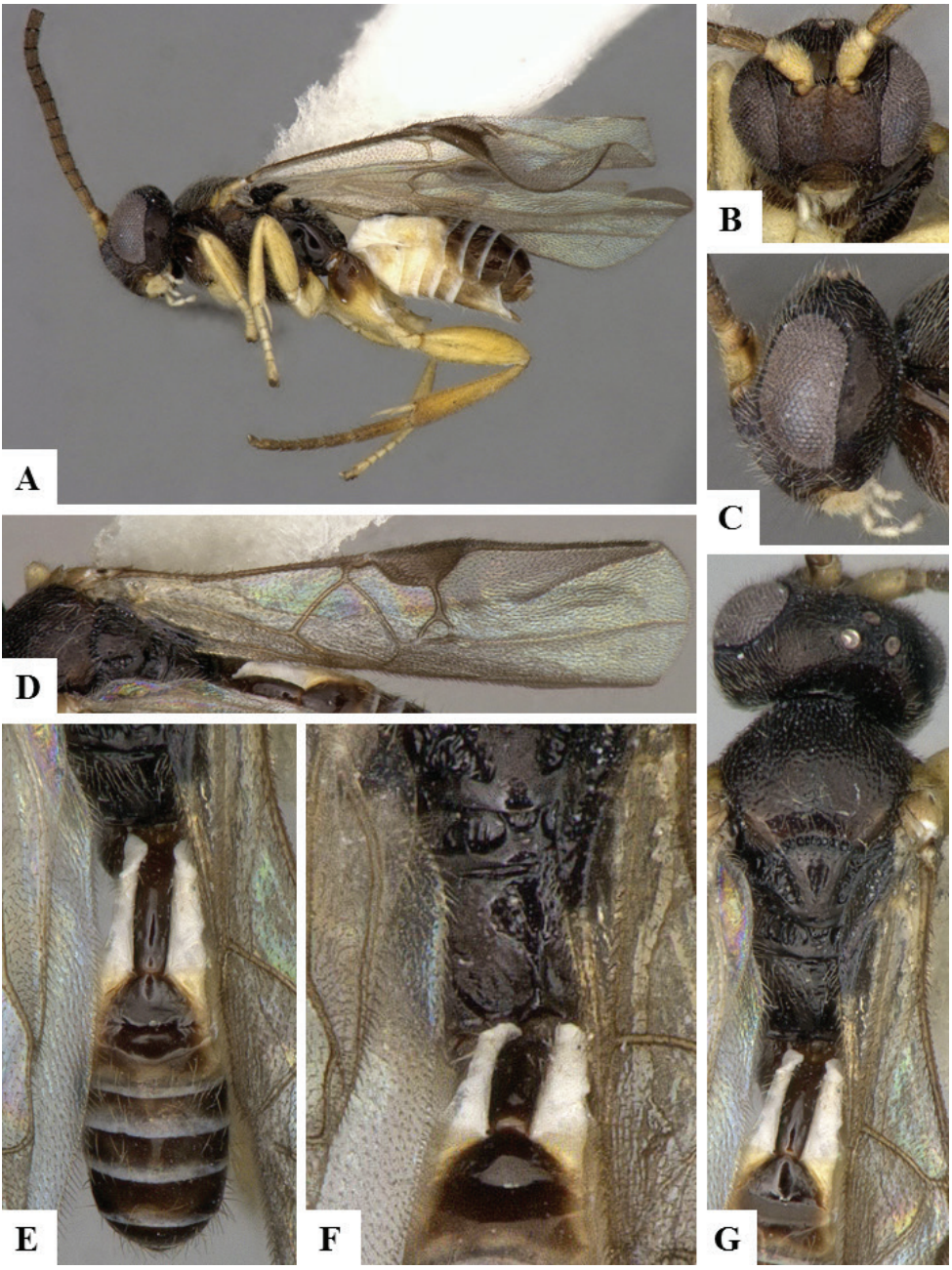


Figure 29. *Markshawius francescae* female holotype. **A** Habitus **B** Head frontal **C** Head lateral **D** Fore wing **E** Metasoma dorsal **F** Propodeum **G** Mesosoma dorsal.

Voucher codes: JMIC0144, JMIC0146; (1♂ QSBG), Chiang Mai, Montha Tarn Water Fall, 18.815600, 98.929100, 700m, Voucher code: CNCH2122; (2♀ CNC, QSBG), Trang, Nayong Khaochong, 7.561, 99.886; 7.561000, 99.886000, 75m,

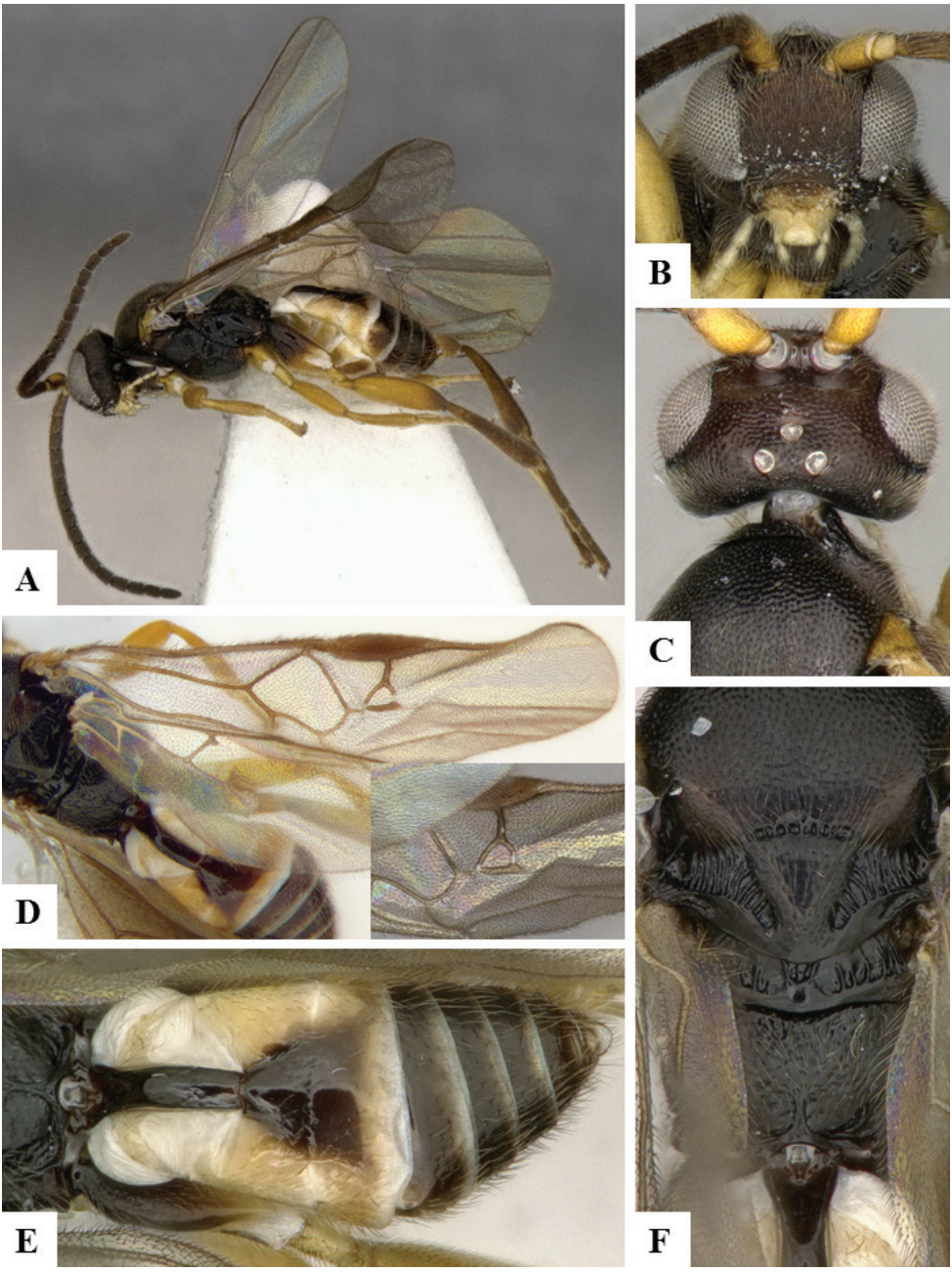


Figure 30. *Markshawius francescae* female paratype CNC878540. **A** Habitus **B** Head frontal **C** Head dorsal **D** Fore wing, inset: details of the areolet **E** Metasoma dorsal **F** Mesosoma dorsal.

Voucher codes: CNCH1584, CNCH2146. **Vietnam.** (1♂ RMNH), Ninh Binh, Cuc Phuong National Park, near centre, 225m, 15–27.v.2000, coll. Mai Phu Quv, Voucher code: CNC878541; (1♂ RMNH), Hoa Binh, Pa Co Hang Kia Nature Reserve,

20.743611, 104.938889, 1045m, Malaise trap, 8–23.x.2009, coll. C.v. Achterberg & R. de Vries, Voucher code: CNC878542; (1♀ RMNH), Tonkin, Hoang Lien National Reserve, 15km W Sa Pa, 1900m, Malaise trap, 15–21.x.1999, coll. C.v. Achterberg, Voucher code: CNC878540.

Diagnosis. The shape of T1 (entirely parallel-sided and extremely long and thin, its length at least 6.0 × its width) as well as sculpture of propodeum (without transverse rugosity medially and without any defined transverse carina) clearly separate this species from the other known species in the genus.

Description. Female. Body color mostly dark brown to black; palpi, labrum and mandible yellow-white; scape, pedicel, tegulae and humeral complex, and most of legs (except for anterior 0.5 of metacoxa and metatarsus which are brown) yellow; flagellomeres brown; most laterotergites and sternites yellow-white to yellow-brown; wings hyaline, veins mostly brown. Head elongate and strongly concave posteriorly, modified to be tightly appressed to and follow the contour of anterior margin of pronotum (pronotum also concave). Upper margin of face produced dorsally between the antennal insertions into a triangular flange. Face looking almost depressed, and with very strong sculpture including transverse striae and punctures. Frons very elongate, with ocelli clearly much higher than normally found in Microgastrinae. Frons with strong excavation at antennal base –better appreciated on a lateral view of the head. Antenna shorter than body (but slightly longer than the combined length of head and mesosoma), with all flagellomeres short, with a single row of placodes or two very small rows that look almost like one. Pronotum only with lower sulcus. Propodeum mostly smooth, with median longitudinal carina clearly visible on posterior half. Fore wing with relatively large, four-sided areolet. Legs in general short and stout, especially metafemur. T1 extremely long and thin (T1 L at least 6.0 × its width centrally). T2 subtriangular and with lateral margins less sculptured. Ovipositor sheaths almost without setae (with only very few, small setae near apex that are usually invisible at less than 100x of magnification), ovipositor strongly narrowing toward apex, where it looks almost needle-like. **Body measurements (mm).** F2 L: 0.09 (0.09–0.13); F3 L: 0.08 (0.09–0.12); F14 L: 0.08 (0.08–0.10); F15 L: 0.08 (0.08–0.10); Malar sulcus L: 0.05 (0.06–0.08); Mandible W: 0.07 (0.08); T1 L: 0.29 (0.34–0.42); T1 W at posterior margin: 0.05 (0.07); T1 maximum W: 0.07 (0.08); T2 W at anterior margin: 0.05 (0.07–0.09); T2 W at posterior margin: 0.17 (0.22–0.24); T2 L: 0.10 (0.11–0.15); Metafemur L: 0.47 (0.58–0.65); Metafemur W: 0.16 (0.19–0.21); Metatibia L: 0.59 (0.71–0.76); Inner spur L: 0.17 (0.18–0.26); Outer spur L: 0.14 (0.14–0.17); First segment of Metatarsus L: 0.26 (0.32–0.35); Ovipositor sheaths L: 0.10 (0.22–0.27); Body L: 1.87 (2.00–2.43); Fore wing L: 1.84 (2.30–2.53). Mandible W is approximate for 1 specimen.

Male. As female.

Biology. Host unknown.

Distribution. Nepal, Thailand, Vietnam.

Molecular data. Only for one of the paratypes (JMIC 0146) there is a 125bp sequence available in BOLD, but it is too short to place the species within the context of other Microgastrinae.

Etymology. Named after Francesca Shaw, in appreciation of her kindness and for being such a wonderful host to the first author when he was visiting the Shaw family in 2013.

***Markshawius thailandensis* Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/C3B9ECFF-EECC-4445-9E86-589EA15392BA
Fig. 31

Holotype. Female, Thailand, QSBG.

Holotype labels. Thailand. Trang Prov./Ampuh Nayon Khao/Chong, 7.561°N, 99.886°E, 75m,/BIN#BOLD:AAH1292/**CNCH2216**. Second label: CNCH2216.

Holotype locality. THAILAND, Trang Province, Ampuh Nayon Khao Chong, 7.561°N, 99.886°E, 75m.

Diagnosis. The shape of T1 and sculpture of propodeum clearly separate *M. thailandensis* from *M. francescae* (see under that species for further details). As for the other species, *M. thailandensis* is a smaller species than *M. erucidoctus* (fore wing L 1.6 mm versus 2.2 mm), has fore wing vein R1 brown (R1 light yellow in *M. erucidoctus*), has a narrower pterostigma and less defined crenulae on scutoscutellar sulcus, and the widest part of T1 is the same width than T2 width at anterior margin (widest part of T1 wider than T2 width at anterior margin in *M. erucidoctus*).

Description. Female. Body color mostly brown; face mostly reddish-brown; palpi yellow-white; labrum, mandible, scape, pedicel, and most of legs (except for brown metacoxa) yellow to yellow-orange; flagellomeres brown-yellow; tegulae and humeral complex yellow-white; most laterotergites and sternites yellow; wings hyaline, veins mostly brown. Head elongate and strongly concave posteriorly, modified to be tightly appressed to and follow the contour of anterior margin of pronotum (pronotum also concave). Upper margin of face produced dorsally between the antennal insertions into a triangular flange. Face looking almost depressed, and with very strong sculpture including transverse striae and punctures. Frons very elongate, with ocelli clearly much higher than normally found in Microgastrinae. Frons with strong excavation at antennal base –better appreciated on a lateral view of the head. Antenna very short (shorter than the combined length of head and mesosoma), with all flagellomeres but first with a single row of placodes. Pronotum only with lower sulcus. Propodeum with median longitudinal carina clearly visible on posterior half (carina looks divided, giving the impression of actually being the posterior half of a very thin areola) and with transverse rugosity medially, including a partially defined transverse carina. Propodeum (apart from carinae and rugosity) mostly smooth, at most with scattered and shallow punctures on anterior half. Fore wing with relatively large, four-sided areolet. Legs in general short and stout, especially metafemur. T1 very thin on anterior 0.3–0.4, then strongly widening towards posterior margin (width at posterior margin around 3.0 × its width centrally). T2 trapezoidal and with lateral margins strongly sculptured.

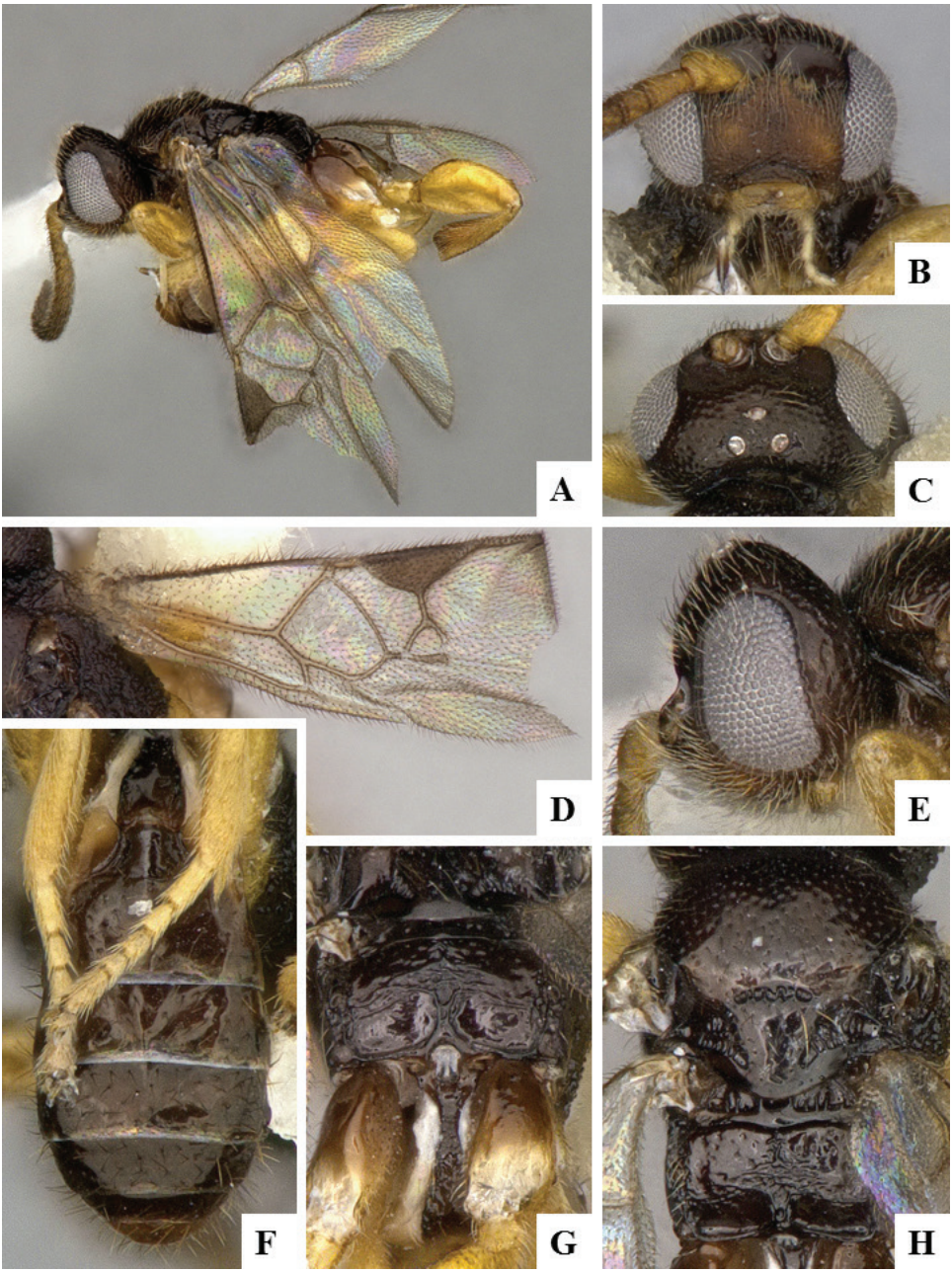


Figure 31. *Markshawius thailandensis* female holotype. **A** Habitus **B** Head frontal **C** Head dorsal **D** Fore wing **E** Head lateral **F** Metasoma dorsal **G** Propodeum **H** Mesosoma dorsal.

Ovipositor sheaths almost without setae (with only very few, small setae near apex that are usually invisible at less than 100 × of magnification), ovipositor strongly narrowing toward apex, where it looks almost needle-like. **Body measurements (mm).** F2 L:

0.08; F3 L: 0.08; F14 L: 0.07; Malar sulcus L: 0.07; Mandible W: 0.08; T1 L: 0.29; T1 W at posterior margin: 0.11; T1 maximum W: 0.12; T2 W at anterior margin: 0.09; T2 W at posterior margin: 0.17; T2 L: 0.11; Metafemur L: 0.47; Metafemur W: 0.22; Metatibia L: 0.52; Ovipositor sheaths L: 0.09; Body L: 1.94. T1 L is approximate. Fore wing is curved and ripped so wasn't measured.

Male. Unknown.

Biology. Host unknown.

Distribution. Thailand.

Molecular data. The holotype sequence represents BIN BOLD:AAH1292, which is 11.2 % different from the closest Microgastrinae sequence in BOLD.

Etymology. Named after the country of the type locality.

***Ohenri* Fernandez-Triana, gen. n.**

http://zoobank.org/D8DAE664-BAA0-45CB-AB07-2EA3F3C31184

Type species. *Ohenri gouletorum* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Antenna with placodes irregularly distributed in three and up to four rows. Pronotum enlarged dorsally, its median length (on a dorsal view) very large, much larger than width of flagellomeres, and clearly larger than propodeum in most Microgastrinae genera (Fig. 32F). Pronotum with dorsal and ventral sulci. Most of mesosoma sculptured with relatively deep, close punctures. Propodeum with median carina clearly defined on anterior 0.6, and then obscured by partially defined areola on posterior 0.4 (Figs 32E, F). Fore wing without areolet. Hind wing with vannal lobe concave and without setae. Tarsal claws pectinate, with two large teeth near base of claw. T1 and T2 dull, T3+ mostly smooth (Fig. 32E). Hypopygium uniformly sclerotized and sharply pointed apically (Fig. 32A). Ovipositor sheaths uniformly setose and clearly shorter than metatibia length. Ovipositor with four subapical serrate teeth on lower (first) valvulae (Fig. 32D).

Putative autapomorphies and potentially related genera. Pronotum enlarged dorsally (shared with *Qrocodiledundee*, see below under description of that genus). The subapical teeth in the lower valvulae of ovipositor are very unusual in Microgastrinae (although not unique to *Ohenri*), as are the antennal placodes irregularly distributed, the large teeth on tarsal claws, and the propodeum with a combination of a median carina and partially defined areola. The relationships of *Ohenri* with other genera of Microgastrinae are not clear at present, although some morphological features are related to *Sathon* s. str. and two new genera, *Carlmuesebeckius* and *Qrocodiledundee*, also described in this paper.

Biology. Host unknown.

Distribution. The only known species is found in the Afrotropical region (Nigeria).

Molecular data. No molecular data available.

Etymology. The genus name refers to and honors the Canadian braconid expert Henri Goulet, a dear friend, colleague and mentor for many years. The letter "O"

added to the beginning of the genus name also plays with words, to loosely refer to the chocolate brand "Oh Henry!" –an indirect mention to Henri's fondness for sweet treats. The gender of the genus is neuter.

Species. Only one species is known.

Ohenri gouletorum **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/6AD07253-FCAC-44EF-ACAC-FE1061DAD1B0
Fig. 32

Holotype. Female, Nigeria, CNC.

Holotype labels. Ibadan, NIGERIA/Aug. 27 1962/D. C. Eidt. Second label: CNC878552. Third label: validus group/Det./W.R.M.Mason78.

Holotype locality. NIGERIA, Ibadan.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Female. Head and mesosoma black; metasoma, palpi, legs (except for metatarsus which is brown), tegula and humeral complex, yellow; mandible, labrum, and most of clypeus orange; antenna dark brown; wings hyaline, most veins brown, pterostigma brown with small spot at base. Antenna with placodes irregularly distributed in three and up to four rows. Pronotum enlarged dorsally, its median length (on a dorsal view) very large, much longer than width of flagellomeres, and clearly longer than propodeum in most Microgastrinae genera. Pronotum with dorsal and ventral sulcus. Most of mesosoma, including anteromesoscutum, scutellar disc, most of mesopleuron and propodeum, with relatively deep, close punctures. Propodeum with median carina clearly defined on anterior 0.6, and then obscured by partially defined areola on posterior 0.4. Fore wing without areolet. Hind wing with vannal lobe concave and without setae. Tarsal claws pectinate, with two large teeth near base of claw. T1 and T2 dull, T3+ mostly smooth. Hypopygium uniformly sclerotized and sharply pointed apically. Ovipositor sheaths uniformly setose and shorter than metatibia length. Ovipositor with four subapical serrate teeth on lower (first) valvulae. **Body measurements (mm).** F2 L: 0.43; F3 L: 0.43; Malar sulcus L: 0.09; Mandible W: 0.18; T1 L: 0.53; T1 W at posterior margin: 0.52; T1 maximum W: 0.58; T2 W at anterior margin: 0.79; T2 W at posterior margin: 0.75; T2 L: 0.28; Metafemur L: 1.08; Metafemur W: 0.43; Metatibia L: 1.45; Inner spur L: 0.48; Outer spur L: 0.27; First segment of Metatarsus L: 0.71; Ovipositor sheaths L: 1.08; Body L: 4.56; Fore wing L: 4.32. T2 W at anterior margin is approximate.

Male. Unknown.

Biology. Host unknown.

Distribution. Nigeria.

Molecular data. No molecular data available.

Etymology. Named after Henri Goulet's family, in recognition of the support they have always given to both authors over the past 15 years.

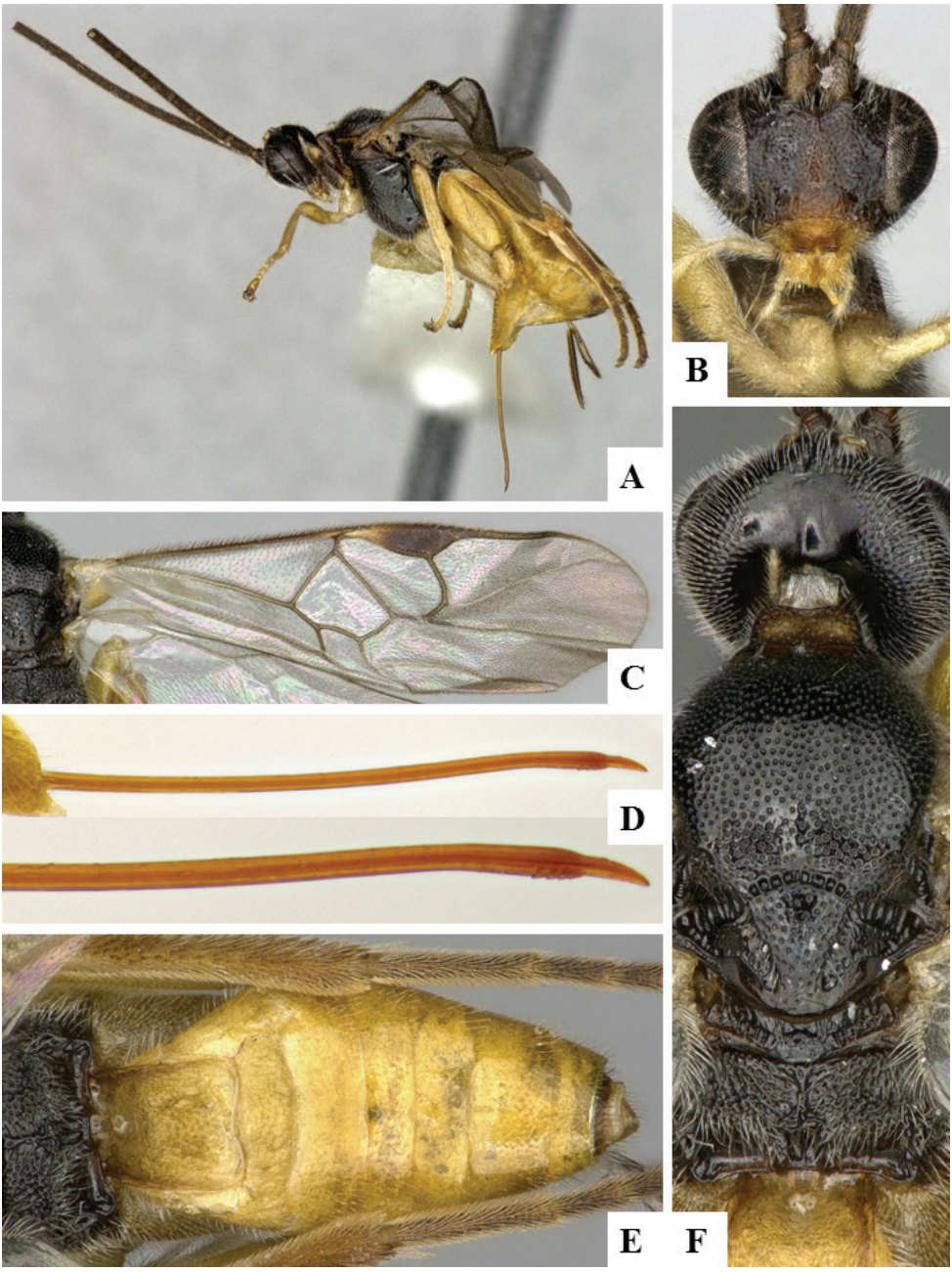


Figure 32. *Ohenri gouletarum* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Ovipositor and details of ovipositor tip **E** Metasoma dorsal **F** Mesosoma dorsal.

Qrocodiledundee **Fernandez-Triana, gen. n.**
http://zoobank.org/4CA43FAC-80EF-4A45-80A8-59CFDDCF7482

Type species. *Qrocodiledundee outbackense* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Head with eyes relatively small, with relatively large malar line, and with gena bulging behind eyes (Fig. 33A, C). Flagellomeres with two rows of placodes. Mesosoma relatively flattened dorso-ventrally. Pronotum enlarged dorsally, its median length (on a dorsal view) very large, much larger than width of flagellomeres, and clearly larger than propodeum in most Microgastrinae genera (Fig. 33C). Pronotum with dorsal and ventral sulcus. Anteromesoscutum with relatively deep and close punctures centrally, smooth anteriorly, laterally and posteriorly (Fig. 33G). Scutellar disc and most of mesopleuron smooth, metapleuron with coarse sculpture on posterior half. Propodeum with an apophysis laterally, near posterior margin (Fig. 33D–G), which looks like a small tubercle. Propodeum with median carina clearly defined on anterior 0.6, and then obscured by partially defined areola on posterior 0.4 (Figs 33F, G). Fore wing without areolet. Hind wing with vannal lobe straight and entirely setose. Metafemur relatively very small and thick, 2.0 × as long as its maximum width. Tarsal claws simple. T1 and T2 dull, T3+ mostly smooth. T2 relatively enlarged, almost as long as T3 (Fig. 33D, F).

Putative autapomorphies and potentially related genera. This new genus shares with *Ohenri* the pronotum enlarged dorsally and propodeum with a median carina and partially defined areola. However, *Qrocodiledundee* has flagellomeres with two rows of placodes, simple tarsal claws, and setose vannal lobe (flagellomeres with 3–4 rows of placodes, pectinate tarsal claws and setoseless vannal lobe in *Ohenri*). *Qrocodiledundee* can be easily recognized on the account of its propodeal apophysis, unique among Microgastrinae, as well as its flattened mesosoma and short and stout metafemur. The relationships of *Qrocodiledundee* with other genera of Microgastrinae are not clear at present, although some morphological features are related to *Sathon* and *Carlmuesebeckius* and *Ohenri* (the latter two also described in this paper).

Biology. Host unknown.

Distribution. The only known species is found in the Australasian region (Australia).

Molecular data. No molecular data available.

Etymology. Named after the iconic Australian movie "Crocodile Dundee", one of the favorite movies of the first author (who at one point was even nicknamed as that because, as with the main character of the movie, he also caught crocodiles and was bitten by one). The first letter of the name is changed to a "Q" to guarantee the uniqueness of the name and avoid potential homonyms. The gender of the genus is neuter.

Species. Only one species is known.

Qrocodiledundee outbackense **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/70264176-BE35-4792-B8DD-01B5CD2FA012
Fig. 33

Holotype. Male, Australia, CNC.

Holotype labels. Normanton/Australia/Mar. 9–20. Second label: CNC878553.

Holotype locality. AUSTRALIA, Normanton.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Male. Body almost entirely orange-yellow, except for small black spot on axillar complex; wings infumated, veins brown. Head relatively wide, with eyes relatively small, relatively large malar line, and gena bulging behind eyes. Flagellomeres with two rows of placodes. Mesosoma relatively flattened dorso-ventrally, in lateral view its length about twice its height. Pronotum enlarged dorsally, its median length (on a dorsal view) very large, much longer than width of flagellomeres, and clearly longer than propodeum in most Microgastrinae genera. Pronotum with dorsal and ventral sulcus. Anteromesoscutum with relatively deep and close punctures centrally, smooth anteriorly, laterally and posteriorly. Scutellar disc and most of mesopleuron smooth, metapleuron with coarse sculpture on posterior half. Propodeum with an apophysis laterally, near posterior margin (Fig. 33D–G), which looks like a small tubercle. Propodeum with median carina clearly defined on anterior 0.6, and then obscured by partially defined areola on posterior 0.4. Fore wing without areolet. Hind wing with vannal lobe straight and entirely setose. Metafemur relatively very small and thick, 2.4 × as long as its maximum width. Tarsal claws simple. T1 and T2 dull, T3+ mostly smooth. T2 relatively enlarged, almost as long as T3. **Body measurements (mm).** F2 L: 0.45; F3 L: 0.43; F14 L: 0.33; F15 L: 0.27; Malar sulcus L: 0.10; Mandible W: 0.12; T1 L: 0.63; T1 W at posterior margin: 0.68; T1 maximum W: 0.68; T2 W at anterior margin: 0.76; T2 W at posterior margin: 0.83; T2 L: 0.42; Metafemur L: 0.86; Metafemur W: 0.35; Metatibia L: 1.43; Inner spur L: 0.29; Outer spur L: 0.23; First segment of Metatarsus L: 0.58; Body L: 4.48; Fore wing L: 4.52. T2 W at posterior margin is approximate. Maximum W of T1 is taken at the posterior margin of T1.

Female. Unknown.

Biology. Host unknown.

Distribution. Australia.

Molecular data. No molecular data available.

Etymology. Named after the Outback, the vast and remote interior of Australia where the holotype specimen was collected. It also happens to be that the Outback is an important part of the "Crocodile Dundee" movie.

Silvaspinosus **Fernandez-Triana, gen. n.**
http://zoobank.org/B458FBB2-30CF-4EC2-BD75-E2FB30810DFC

Type species. *Silvaspinosus vespa* Fernandez-Triana & Boudreault, here designated.

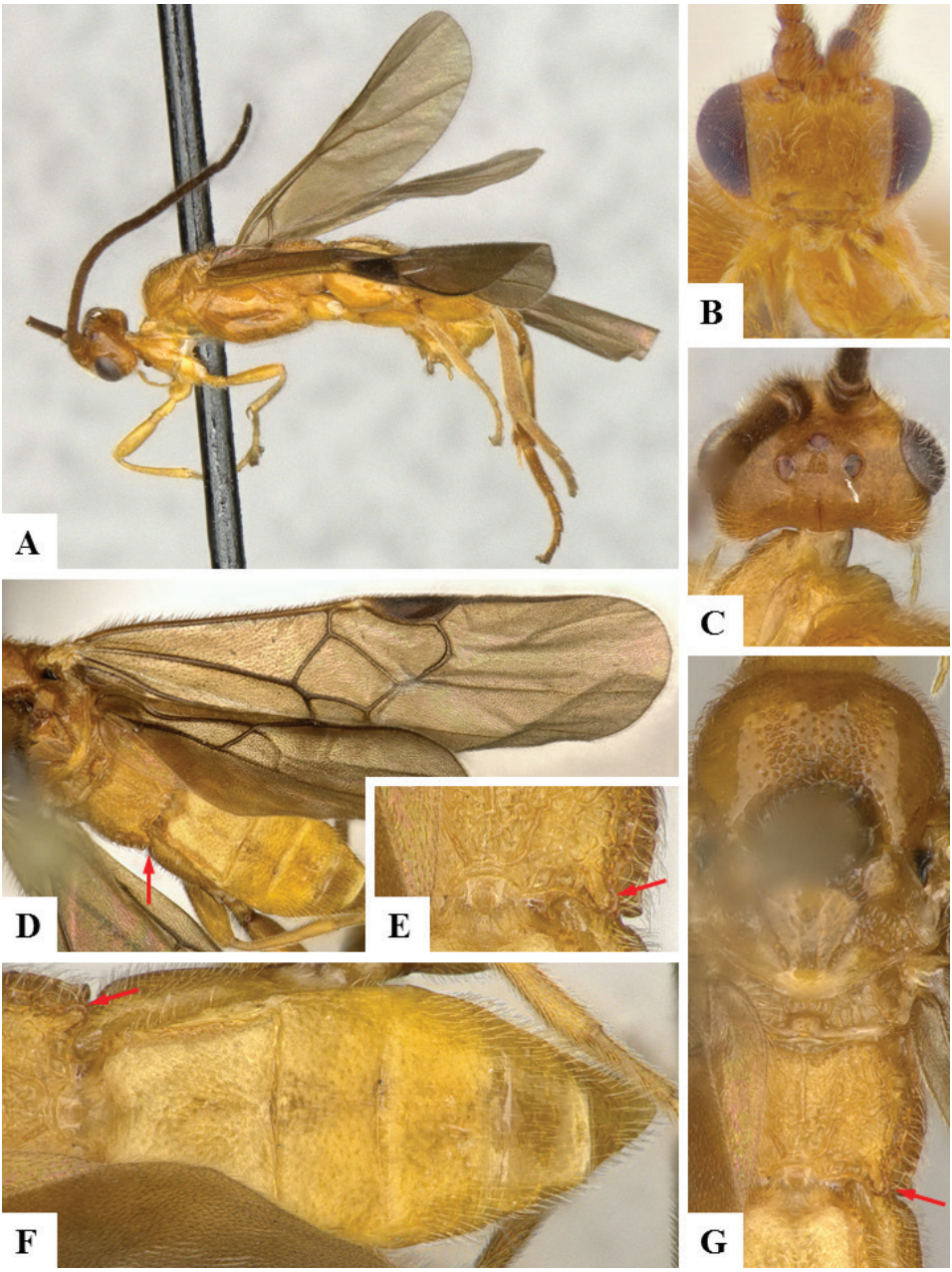


Figure 33. *Qrocodiledundee outbackense* male holotype. **A** Habitus **B** Head frontal **C** Head dorsal **D** Fore wing **E** Pronotum **F** Metasoma dorsal **G** Mesosoma dorsal. Red arrow shows the propodeal apophysis.

Diagnostic description. Clypeus extremely long and thin (Figs 34B, 35B, F). Malar line extremely short, almost nonexistent (0.01 mm long). Mandible base separate from head by a desclerotized area that looks almost like an opening (Figs 34B, 35F).

Mandibles relatively stout and large (Figs 34B, 35F). Tentorial pits relatively very large (Figs 34B, 35F). Anteromesoscutum mostly smooth, with shallow and sparse punctures (Fig. 34F). Notauli not indicated by sculpture. Scutellar disc without posteromedian band of rugosity (Figs 34F, G, 35E). Propodeum mostly with rugose sculpture and with median longitudinal carina complete (Figs 34G, 35D). Fore wing with large, quadrangular areolet (Figs 34C, 35C). Fore tarsus with a curved, spine-like seta. Metacoxa relatively short (its length not surpassing posterior margin of T2), metatibial spurs relatively short (less than half the length of first segment of metatarsus). T1 smooth and without median longitudinal sulcus (Fig. 34E). T2 smooth and with central area slightly raised and poorly defined from lateral areas by weak sulcus (Figs 34E, 35D).

Putative autapomorphies and potentially related genera. The shape of clypeus, and mandible separation from head by desclerotized area are unique among Microgastrinae. *Silvaspinosus* seems to belong to the Microplitini group of genera (sensu Mason 1981), based on the relatively short metacoxa and metatibial spurs, fore wing with large areolet, as well as its DNA barcode sequence (see below under "**Molecular data**"). However, the spine-like seta on the fore tarsus and the absence of a median band of rugosity on the posterior margin of the scutellar disc would be unique and distinctive among Microplitini (those features tend to be present in some species of a few genera within Cotesini (sensu Mason 1981)).

Biology. Host unknown.

Distribution. The only known species is found in the Afrotropical region (Madagascar).

Molecular data. One of the female paratypes (CNCH3044) rendered a partial barcode (427bp), which is 8.3% different from the closest Microgastrinae (several *Microplitis* species).

Etymology. From "silva" (in Latin "forest") and "spinosus" (in Latin "spinous, thorny"), referring to the famed Madagascar spiny forests, where the wasp is found, apparently as an endemic taxon from that ecoregion. The gender of the genus is neuter.

Comments. This genus seems to be related to the *Microplitis* group of genera (Microplitini sensu Mason 1981), based on fore wing areolet size, metacoxa size, length of metatibia spurs, and shape of T2. However, other characters are highly unusual (shape of clypeus) or not previously known from Microplitini (spine on fore tarsus). The spiny forests of Madagascar are considered by the World Wide Fund for Nature (WWF) as one of the "Global 200" ecoregions, a list that includes those areas of the planet with higher value and priority for conservation. Thus, the description of this new genus and species of Microgastrinae wasp as endemic to those forests reinforces the unique biodiversity values of that region.

Species. Only one species is known. We have seen four additional male specimens which have a different and lighter coloration pattern, and might represent a different species, but because they are no associated females, we prefer not to describe them for the time being.

Silvaspinosus vespa **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/A20E7075-8C1A-49A4-B071-3D45E5F7CA2A
Figs 34, 35

Holotype. Female, Madagascar, CAS.

Holotype labels. Madagascar. Toliara/Province: Vohidava/Forest, 88.9 km N/ Amboasary, 24.40556°S. Second label: 46.287778°E, 500m,/6–8.XII.2006, MT, B. L./Fisher et al, BLF15694, **CNC649545**.

Holotype locality. MADAGASCAR, Toliara Province: Vohidava Forest, 88.9 km North of Amboasary, 24.40556°S, 46.287778°E, 500m.

Paratypes. Madagascar. (1♀ CNC), Toliara Province, Beza Mahafaly Reserve, -22.840500, 44.731200, 165m, 24.xii.2002, coll. R. Harin`Hala, Voucher code: CNCH3044; (1♂ CAS), Tulear Province, Andohahela National Park, Ihazofotsy Parcelle III, -24.830833, 46.536167, 80m, dry spiny forest, Malaise trap, 23.ix-4.x.2003, coll. M. Irwin, R. Harin'Hala & F. Parker, Voucher code: CASENT8402170; (3♂ CAS, CNC), Tulear Tsimanampetsotsa National Park, Mitoho Forest, across trail at base of escarpment, -24.048500, 43.752333, 120m, Dense dry forest, Malaise trap, 23–31.i.2009, coll. M. Irwin & R. Harin'Hala, Voucher codes: CASENT8402171, CASENT8402172, CASENT8402173; (1♀ 4♂ CAS, CNC), Toliara Province, Vohidava Forest, 88.9 km N Amboasary, -24.240556 46.287778, 500m, MT, 6–8.xii.2006, coll. B. L. Fisher et al, Voucher codes: CNC649540, CNC649542, CNC649543, CNC649544, CNC649546.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Female. Head and mesosoma mostly black, mesosoma mostly dark brown, except for T1 light brown; clypeus, labrum and flagellomeres dark brown; mandibles orange; scape and pedicel yellow-brown; palpi usually mostly white (except for labial palpi 1–2 dark brown), but some specimens with darker palpi (mostly dark brown); legs mostly dark brown (except for protibial, protarsus, mesotibia and mesotarsus which are orange-yellow or yellow-white, and small white spot on anterior 0.1 or less of all tibiae); metatibial spurs yellow-white; wings slightly infumated on apical half, veins brown but parastigma yellow-white. Clypeus extremely long and thin. Malar line extremely short, almost nonexistent (0.01 mm or less long). Mandible base separate from head by a desclerotized area that looks like an opening. Mandibles relatively stout and large. Tentorial pits relatively very large. Anteromesoscutum mostly smooth, with shallow and sparse punctures. Notauli not indicated by sculpture. Scutellar disc without posteromedian band of rugosity. Propodeum mostly with rugose sculpture, with median longitudinal carina complete. Fore wing with large, quadrangular areolet (second submarginal cell). Fore tarsus with a curved, spine-like seta. Metacoxa relatively short (its length not surpassing posterior margin of T2), metatibial spurs relatively short (less than half the length of first segment of metatarsus). T1 smooth and without median longitudinal sulcus. T2 smooth and with central area slightly raised and poorly defined

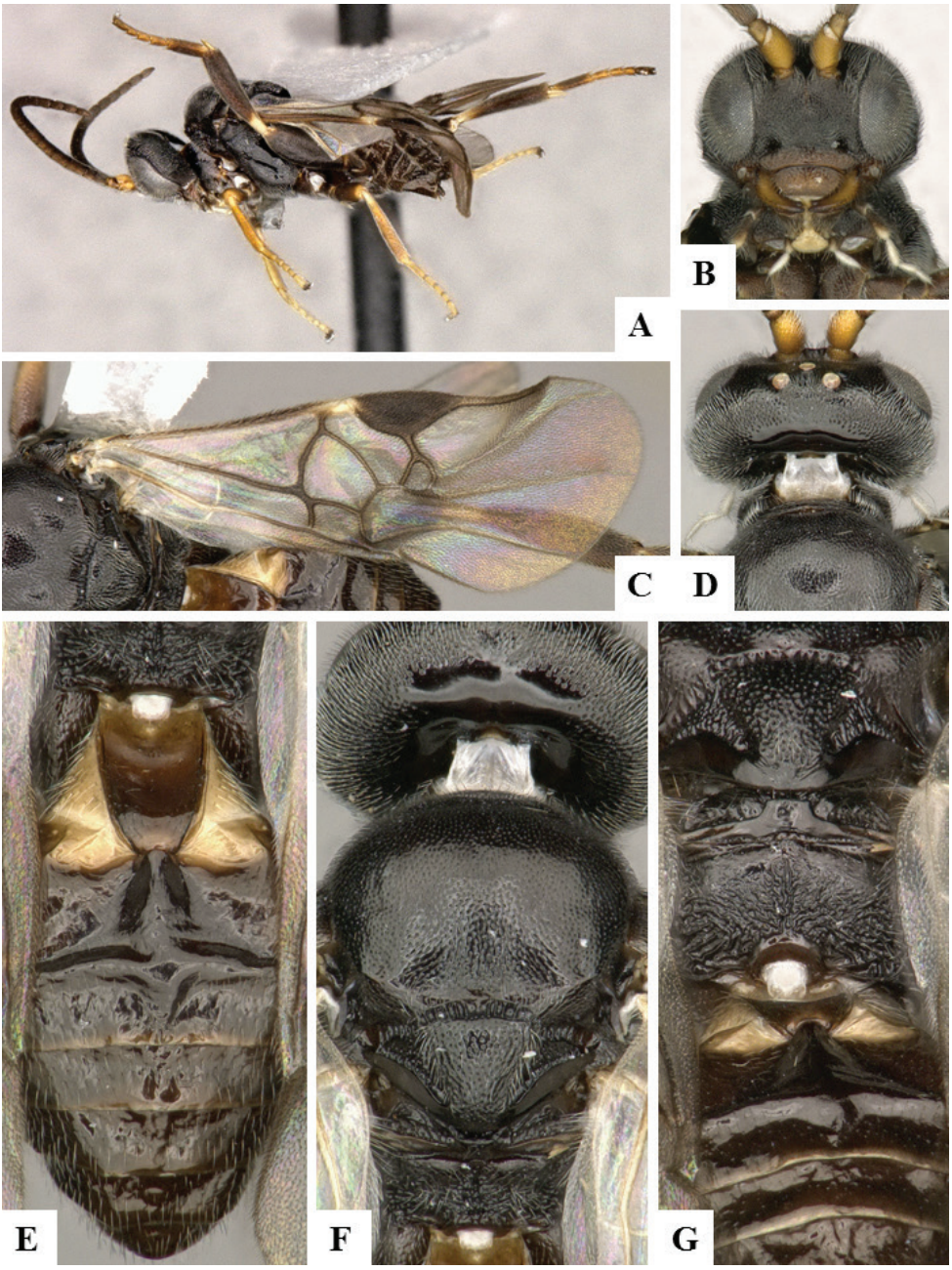


Figure 34. *Silvaspinosus vespa* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Head dorsal **E** Metasoma dorsal **F** Mesosoma dorsal **F** Propodeum.

from lateral areas by weak sulcus. T3+ smooth and with sparse, relatively long setae. Hypopygium relatively short, not extending beyond last tergites. Ovipositor sheaths mostly smooth and very short, 0.14 × metatibia length. **Body measurements (mm).** F2 L: 0.24

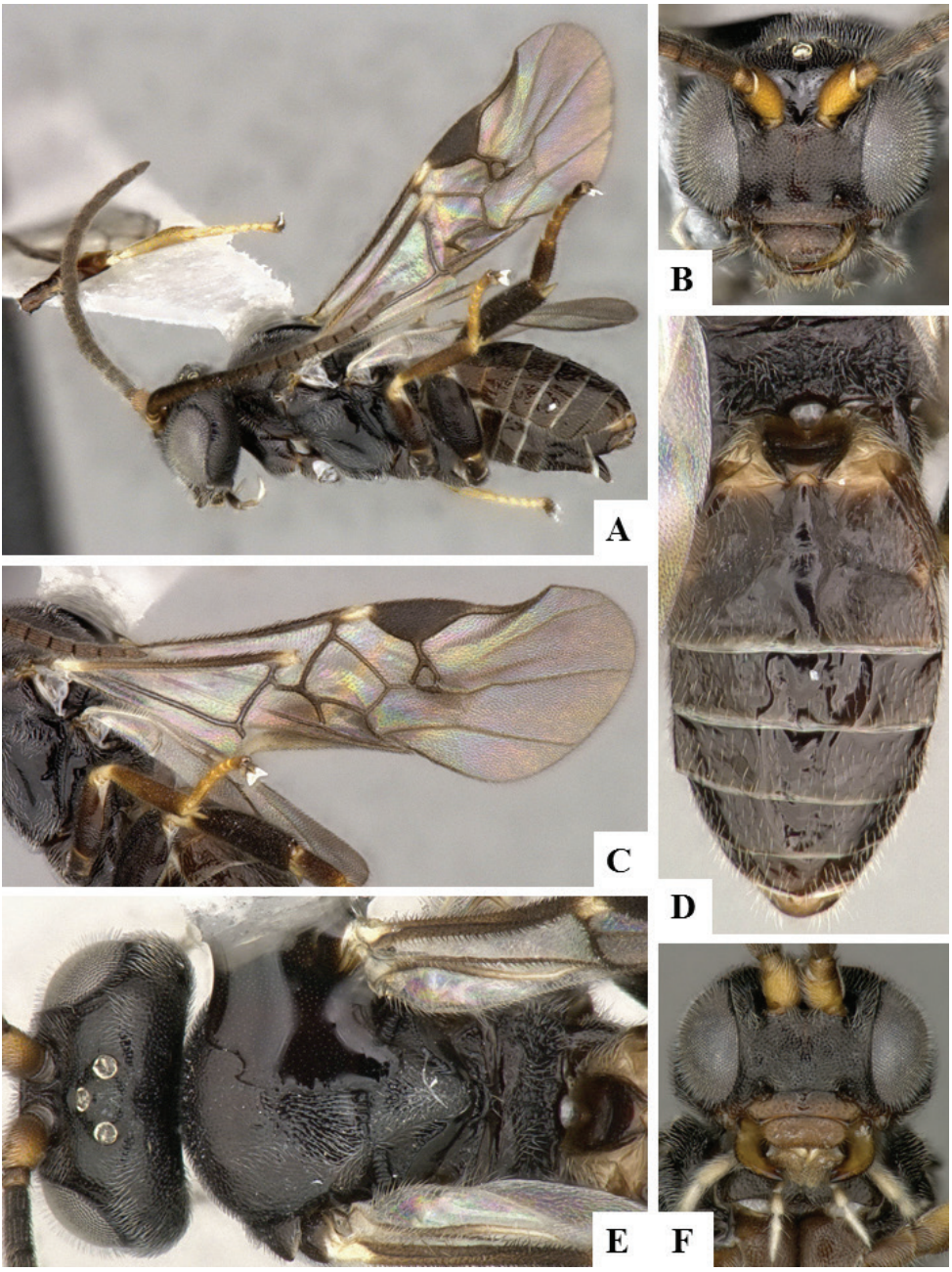


Figure 35. *Silvaspinosus vespa* female CNC644540 (**A–E**) and male CASENT8402171 (**F**) paratypes. **A** Habitus **B** Head frontal **C** Fore wing **D** Metasoma dorsal **E** Head and mesosoma, dorsal **F** Head frontal, male.

(0.21); F3 L: 0.23 (0.21); F14 L: 0.15 (0.13); F15 L: 0.15 (0.13); Malar sulcus L: 0.01 (0.03); Mandible W: 0.21 (0.21); T1 L: 0.51 (0.38); T1 W at posterior margin: 0.10 (0.10); T1 maximum W: 0.28 (0.23); T2 W at anterior margin: 0.60 (0.49); T2 W at

posterior margin: 0.75 (0.68); T2 L: 0.25 (0.23); Metafemur L: 0.83 (0.79); Metafemur W: 0.33 (0.29); Metatibia L: 1.00 (1.00); Inner spur L: 0.18 (0.15); Outer spur L: 0.18 (0.18); First segment of Metatarsus L: 0.39 (0.38); Ovipositor sheaths L: 0.14 (0.18); Body L: 3.31 (3.19); Fore wing L: 2.83 (2.58). T1 L is approximate for 1 specimen.

Male. As female.

Biology. Host unknown.

Distribution. Madagascar, apparently restricted to the Spiny Forest ecoregion, also known as Madagascar spiny thickets (sensu https://www.worldwildlife.org/ecoregions/at1311).

Molecular data. One of the female paratypes (CNCH3044) rendered a partial barcode (427bp), which is 8.3% different from the closest Microgastrinae (several *Microplitis* species).

Etymology. From Latin "vespa" (meaning "wasp"), referring to the species being a parasitoid wasp. It also intends to play with the generic name (which means "spiny forest") thus producing the combined name of "wasp of the spiny forest" for the species.

Tobleronius **Fernandez-Triana, gen. n.**

http://zoobank.org/448E6E18-CF74-4E77-A1F0-E599CB1549BD

Type species. *Tobleronius orientalis* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Head with relatively large tentorial pits and palpi (Fig. 36B). Traces of an occipital carina latero-dorsally (scarsely visible in Fig. 36D). Flagellomeres with two rows of placodes. Scutoscutellar sulcus relatively wide and deep, with 4–6 strongly defined crenulae. Scutellar disc with coarse and slightly raised posteromedian band of rugosity (Figs 36D, 37E). Propodeum with complete areola and incomplete transversal carina (Figs 36D, F, 37D–F). Fore wing with large and quadrate areolet (Figs 36C, 37C). Metacoxa relatively long, extending to the posterior margin of T3 (Fig. 37A). T1 shape relatively unique (better illustrated in Figs 36E, F, 37D–F), with much wider anterior 0.6–0.7 and strongly narrowed posterior 0.3, so that widest part of tergite (near anterior margin) is around 4.0 × narrowest width (on posterior margin). T1 anterior 0.6–0.7 desclerotized and slightly concave. T2 very long and thin, although slightly widening towards posterior margin. Area surrounding spiracles on laterotergite 2 partially sclerotized and same color than T2, giving the impression of T2 having "three peaks" (the largest and central one being the actual T2, the two smallest and lateral ones being the area surrounding spiracles on laterotergites; better illustrated in Figs 36E, F, 37D, F).

Putative autapomorphies and potentially related genera. *Tobleronius* belongs to the Microplitini (sensu Mason 1981) group of genera, and seems to be mostly related to *Alloplitis*. It can be distinguished by all other genera within that group by the unusual shape and lack of sculpture of T1 and T2, and the relatively long metacoxa (which reaches to the posterior margin of T3, unlike most Microplitini, where metacoxa length almost always is shorter than the combined length of T1 and T2). The

carination pattern of the propodeum is also highly unusual, as in Microplitini only *Alloplitis* has a complete areola and complete transverse carina; *Tobleronius* has a complete areola but the transverse carina is incomplete.

An important character to analyze in future studies of Microgastrinae phylogeny is that the back of the head of *Tobleronius* shows traces of an occipital carina laterodorsally. Until now all Microgastrinae had been considered to lack an occipital carina. In this paper we have described two genera with at least partial occipital carina (*Gilbertnixonius* and *Tobleronius*). But even among previously described genera of Microplitini there are additional examples. We have found, upon further examination of specimens in the CNC, that most (perhaps all) species of *Philoplitis* have an occipital carina. That feature was unfortunately overlooked by all authors until now: Nixon (1965) when describing the genus, Mason (1981) when discussing its position within the subfamily, Whitfield et al. (2002) when reassessing Microgastrinae phylogeny based on morphological and molecular data, and Fernandez-Triana & Goulet (2009) in the most recent revision of the genus. We also examined all specimens of *Alloplitis* in the CNC and found that at least some species show traces of an occipital carina, in a similar way to what is found in *Tobleronius*. It now seems clear that at least some lineages within Microplitini have an occipital carina, or at least traces of it.

Biology. Host unknown.

Distribution. The only known species is found in the Oriental region (Thailand, Vietnam).

Molecular data. Three sequences are currently available, two almost complete (601 and 614 bp) and one partial (497 bp). They represent in BOLD two closely related BINS (BOLD:ADE3103 and BOLD:ADE4131), which are 3% different between each other, but are far apart from any other sequence (based on a Neighbor Joining tree built with 35,000+ Microgastrinae sequences available in BOLD as of January 2018).

Etymology. The name refers to the chocolate brand "Toblerone", one of the favourites of the first author. The shape of T2 looks like one of the triangles that compose Toblerone bars (if one has enough imagination and love for chocolate!). Here is hoping that someday a wasp-shaped chocolate bar is produced. The gender of the genus is neuter.

Species. Only one species is recognized at present. However, the molecular differences (see above) as well as slight morphological differences between specimens from Thailand and Vietnam suggest that they could actually represent two different species. But because only three specimens were available for study, we prefer to keep them as one species for the time being.

Tobleronius orientalis **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/7C67F8DB-6CBC-42A4-A02B-ABBC8102DC0C
Figs 36, 37

Holotype. Male, Vietnam, RMNH.

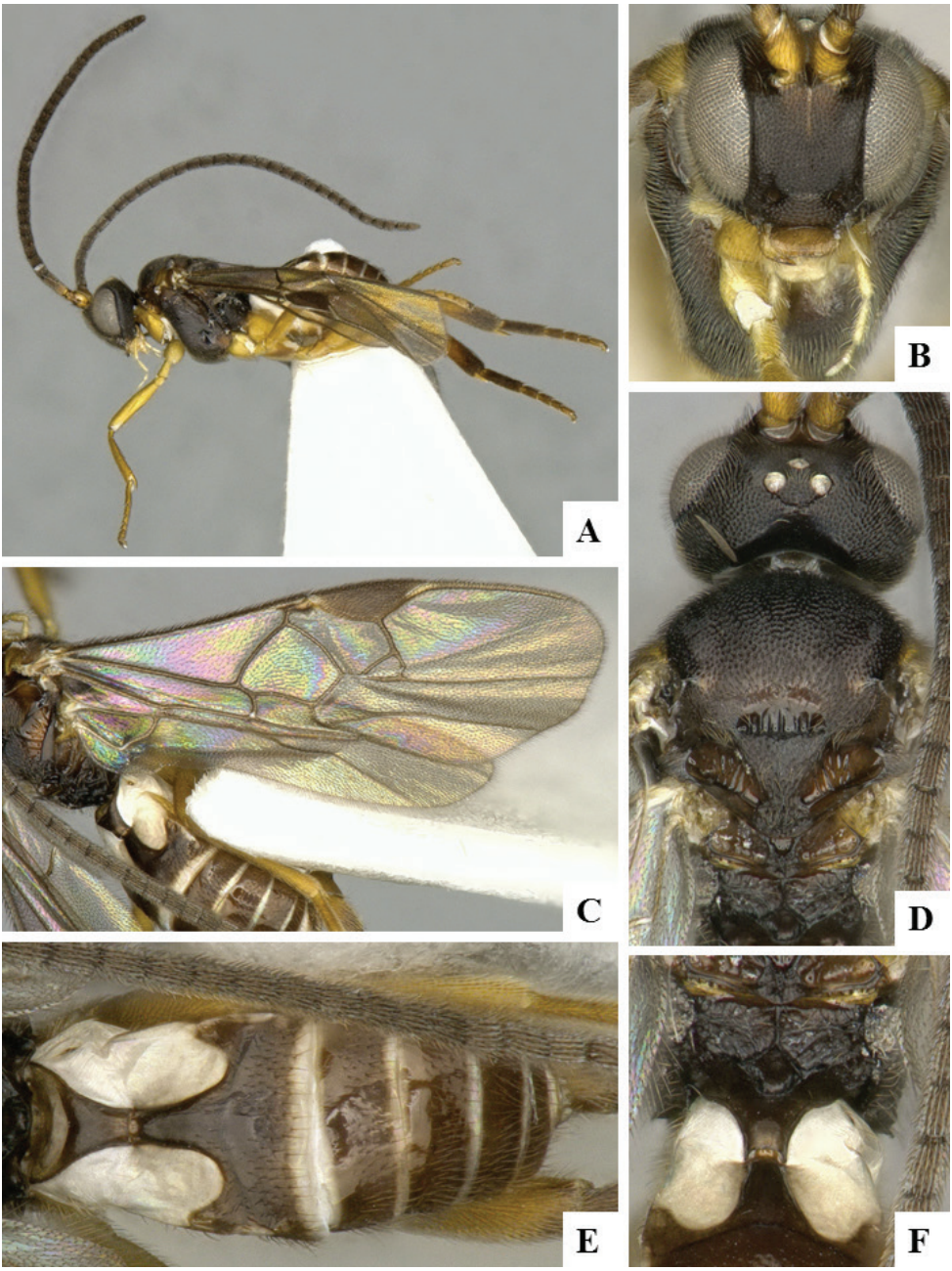


Figure 36. *Tobleronius orientalis* male holotype. **A** Habitus **B** Head frontal **C** Fore wing and hind wing **D** Head and mesosoma, dorsal **E** Metasoma dorsal **F** Propodeum.

Holotype labels. Vietnam. North Vietnam./Hoa Binh Pa Co Hang Kia/N.R., 20.743611°N, 104.938889°E, 1045m,/ MT, C.V./Achterberg & R. de Vries,/ **CNC521393**, 9–23.X.2009. Second label: **N. VIETNAM: Hoa Binh**/Pa Co Hang

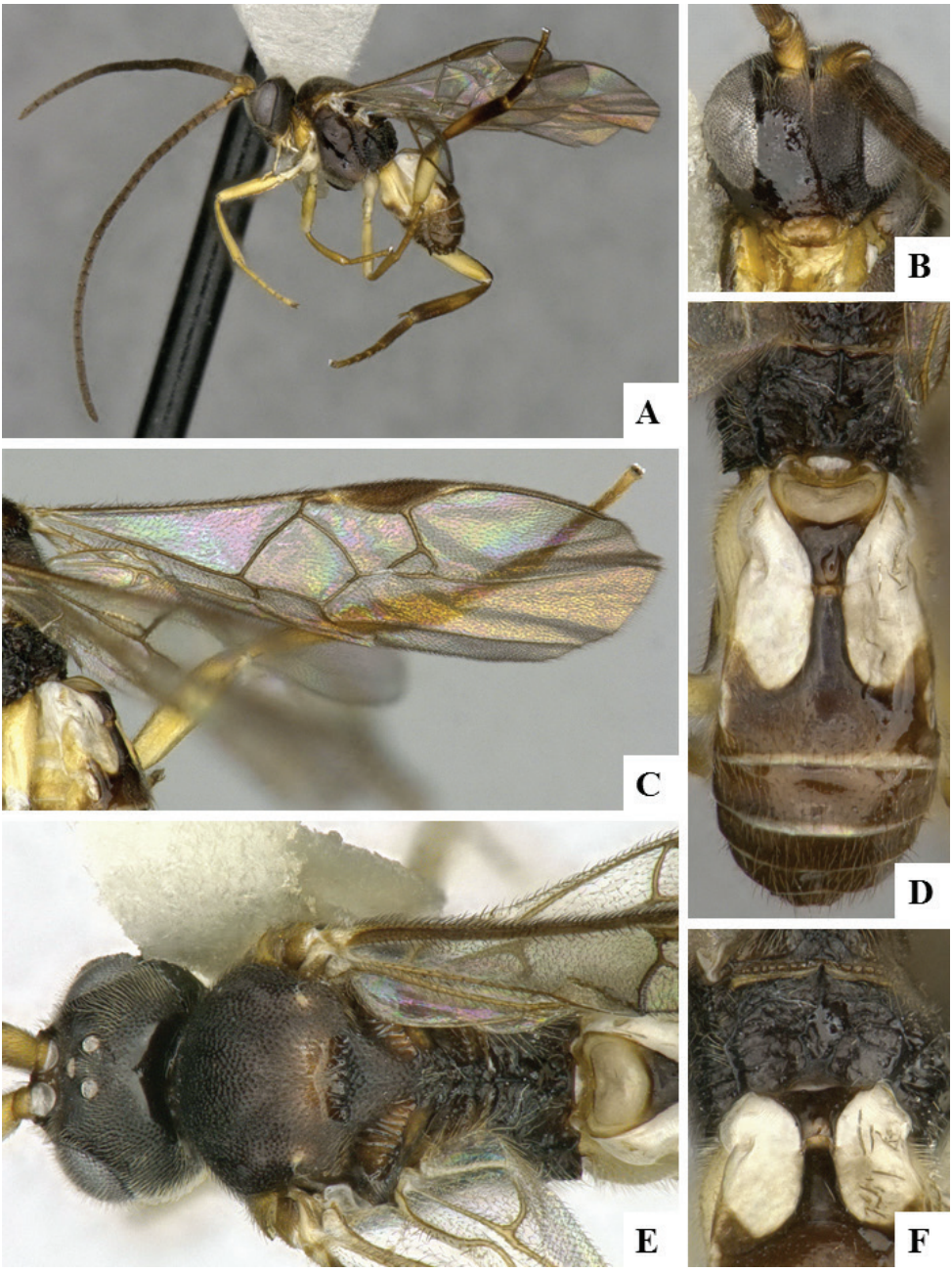


Figure 37. *Tobleronius orientalis* male paratype CNC521929. **A** Habitus **B** Head frontal **C** Fore wing **D** Metasoma dorsal **E** Head and mesosoma, dorsal **F** Propodeum.

Kia N.R., 1045m/N22°44'37", E104°56'20", 9–23.x.2009, Mal. tr. 6, RMNH'09/ C.v.Achterberg & R. de Vries.

Holotype locality. VIETNAM, Hoa Binh Pa Co Hang Kia Nature Reserve, 20.743611°N, 104.938889°E, 1045m.

Paratypes. Thailand. (1♂ QSBG), Chiang Mai, Huai Nam Dang NP Visitor center, 19.313383, 98.606800, pan trap, 30.ix-1.x.2007, coll. Anuchart & Thawatchai, Voucher code: CNC521929. **Vietnam.** (1♂ CNC), North Vietnam. Hoa Binh Pa Co Hang Kia N.R., 20.745000, 104.892778, Malaise trap, 24.x.2009, coll. C.V. Achterberg & R. de Vries, Voucher code: CNC521392.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Male. Body mostly dark brown; palpi and anterior 0.6–0.7 of T1 white-yellow; mandibles, scape, pedicel and most of legs (except for posterior 0.2–0.3 of metatibia and metatarsus which are brown) yellow; anterior laterotergites and sternites white; flagellomeres brown; wings hyaline, most veins brown. Head with relatively large tentorial pits and palpi. Traces of an occipital carina latero-dorsally. Flagellomeres with two rows of placodes. Scutoscutellar sulcus relatively wide and deep, with 4–6 strongly defined crenulae. Scutellar disc with coarse and slightly raised posteromedian band of rugosity. Propodeum with complete areola and incomplete transversal carina. Fore wing with large and quadrate areolet. Metacoxa relatively long, extending to the posterior margin of T3. T1 shape relatively unique (better illustrated in Figs 36E, F, 37D–F), with much wider anterior 0.6–0.7 and strongly narrowed posterior 0.3, so that widest part of tergite (near anterior margin) is around 4.0 × narrowest width (on posterior margin). T1 anterior 0.6–0.7 desclerotized and slightly concave. T2 very long and thin, although slightly widening towards posterior margin. Area surrounding spiracles on laterotergite 2 partially sclerotized and same color than T2, giving the impression of T2 having "three peaks" (the largest and central one being the actual T2, the two smallest and lateral ones being the area surrounding spiracles on laterotergites; better illustrated in Figs 36E, F, 37D, F). T3+ smooth and with sparse, relatively long setae. **Body measurements (mm).** F2 L: 0.26; F3 L: 0.28; F14 L: 0.23; F15 L: 0.23; Malar sulcus L: 0.08; Mandible W: 0.09; T1 L: 0.36; T1 W at posterior margin: 0.08; T1 maximum W: 0.30; T2 W at anterior margin: 0.08; T2 W at posterior margin: 0.49; T2 L: 0.25; Metafemur L: 0.83; Metafemur W: 0.23; Metatibia L: 1.07; Inner spur L: 0.20; Outer spur L: 0.18; First segment of Metatarsus L: 0.42; Body L: 2.97; Fore wing L: 2.90. T1 L is approximate.

Female. Unknown.

Biology. Host unknown.

Distribution. Thailand, Vietnam.

Molecular data. The holotype and one paratype (CNC521392) rendered almost complete barcodes (601 and 614 bp respectively), whereas for the other paratype a partial sequence (497 bp) was also available. Those sequences represent in BOLD two closely related BINS (BOLD:ADE3103 and BOLD:ADE4131), which are 3% different. As explained in the genus description, for the time being we prefer to consider all those specimens as belonging to the same species, although barcodes suggest they could actually represent two different species.

Etymology. The species refer to the species distribution in the Oriental region.

***Ungunicus* Fernandez-Triana, gen. n.**
http://zoobank.org/A6FF0FFC-7A34-4D94-9222-13F788ECF12A

Type species. *Ungunicus vietnamensis* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Body mostly smooth, with few, scattered, mostly shallow punctures. Flagellomeres with two rows of placodes. Pronotum with dorsal and ventral sulcus. Scutoscutellar sulcus relatively narrow but with numerous crenulae (Fig. 38E). Scutellar disc smooth, without posteromedian band of rugosity (Fig. 38E). Propodeum mostly smooth, with strongly defined median longitudinal carina and a few short carinae radiating from median one (Fig. 38E). Fore wing with quadrangular areolet (Fig. 38C). Hind wing with vannal lobe entirely setose. Metacoxa reaching to the posterior margin of T3. Last segment of tarsi relatively large, with small setae or spine (peg-like) on apical half, near the claws (Fig. 39I). Tarsal claws unique in Microgastrinae (better seen in Fig. 39F–I), with a very large basal tooth (longer than tarsal claw apex), and a median lobe (with setae arising from its margin, which seems slightly bilobate). T1 with central sulcus on anterior half, T2+ smooth (Fig. 38D, E). Ovipositor short but relatively thick and strongly curved downwards (Fig. 38A). Ovipositor sheaths with few, sparse, but relatively long setae.

Putative autapomorphies and potentially related genera. *Ungunicus* seems to be related to some species of *Diolcogaster* (sharing with it the ovipositor shape, ovipositor sheaths with setae, and T1 with medium sulcus; but differing in the mostly smooth body, lack of posteromedian band of rugosity on scutellar disc, and shape of tarsal claws) and *Rasivalva* (sharing with it the relatively smooth body and absence of a posteromedian band of rugosity on scutellar disc; but differing in having relatively long setae on ovipositor sheaths and shape of tarsal claws). The tarsal claws are truly unique within Microgastrinae, and serve as the main diagnostic character as well as the main putative autapomorphy.

Biology. Host unknown.

Distribution. The only known species is found in the Oriental region (Vietnam).

Molecular data. Both the holotype and paratype rendered almost full barcode sequences, representing BIN BOLD:ADE2636, which is different by 8.7% of the closest Microgastrinae sequences currently available in BOLD.

Etymology. From "ungu" (in Latin "claw", "hoof", "nail") and "unicus" (in Latin "unique"), referring to the highly unusual and remarkable structure of the tarsal claws found in this genus. The gender of the genus is neuter.

Species. Only one species is known.

***Ungunicus vietnamensis* Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/25BEA67D-744A-455D-8A49-9FD27E2179B1
Figs 38, 39

Holotype. Female, Vietnam, RMNH.

Holotype labels. Vietnam, Hoa Binh Pa/Co Hang Kia N.R./20.743058°N, 104.895833°E,/1319m, 10–24.X.2009,/MT, C. V. Achterberg & R./de Vries, **CNC521412**.

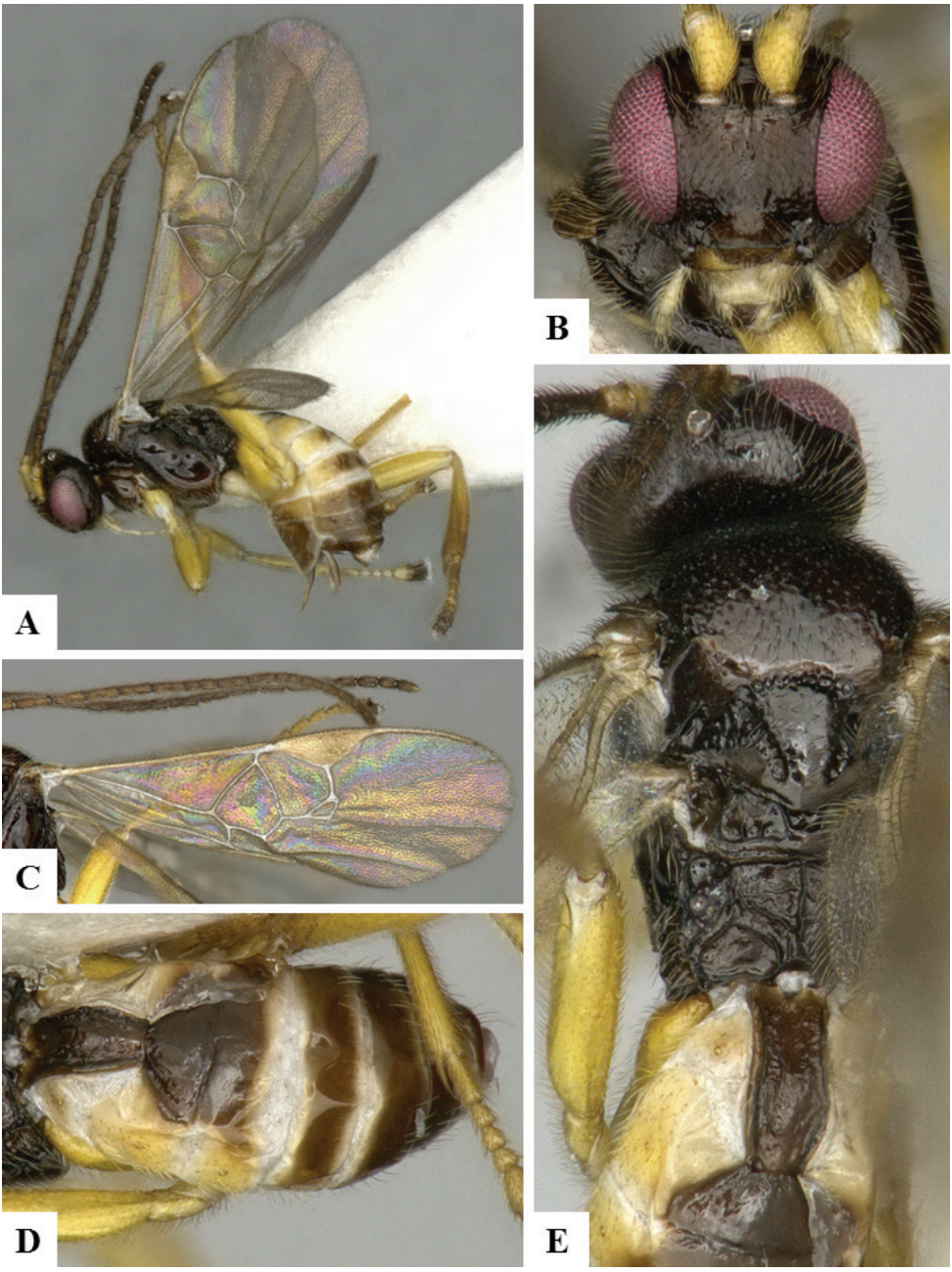


Figure 38. *Ungunicus vietnamensis* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Metasoma dorsal **E** Head and mesosoma, dorsal.

Second label: **N. VIETNAM: Hoa Binh**/Pa Co Hang Kia N.R., 1319m/N20°44'35", E104°53'45", 10–24.x.2009, Mal. tr. 14, RMNH'09/C. v. Achterberg & R. de Vries.

Holotype locality. VIETNAM, Hoa Binh Province, Pa Co Hang Kia Nature Reserve, 20.743058°N, 104.895833°E, 1319m.

Figure 39. *Ungunicus vietnamensis* female holotype, details of metatarsus and claws. **F** Dorsal **G** Ventral **H** Ventro-lateral **I** Last segment of metatarsus and claws, ventro-lateral.

Paratype. Vietnam. (1♀ CNC), same locality than holotype, Voucher code: CNC521411.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Female. Head and mesosoma dark brown to black; metasoma mostly brown, with anterior four laterotergites and sternites white or yellow; palpi, mandibles, scape, pedicel and most of legs (except for metatibia and metatarsus which are slightly darker) yellow; flagellomeres brown; wings hyaline, most veins white, pterostigma light brown-yellow. Body mostly smooth, with few, scattered, mostly shallow punctures. Flagellomeres with two rows of placodes. Pronotum with dorsal and ventral sulcus. Scutoscutellar sulcus relatively narrow but with numerous crenulae. Scutellar disc smooth, without posteromedian band of rugosity. Propodeum mostly smooth, with strongly defined median longitudinal carina and a few short carinae radiating from median one. Fore wing with quadrangular areolet. Hind wing with vannal lobe entirely setose. Metacoxa reaching to the posterior margin of T3. Last segment of tarsi relatively large, with small setae or spine (peg-like) on apical half, near the claws. Tarsal claws unique in Microgastrinae (better seen in Figs 39F-I), with a very large basal tooth (longer than tarsal claw apex), and a median lobe (with setae arising from its margin, which seems slightly bilobate). T1 with central sulcus on anterior half, T2+ smooth. Ovipositor short but relatively thick and strongly curved downwards. Ovipositor sheaths with few, sparse, but relatively long setae. **Body measurements (mm).** F2 L: 0.19 (0.20); F3 L: 0.18 (0.18); F14 L: 0.11 (0.11); F15 L: 0.11 (0.10); Malar sulcus L: 0.06 (0.06); Mandible W: 0.08 (0.10); T1 L: 0.36 (0.33); T1 W at posterior margin: 0.09 (0.12); T1 maximum W: 0.14 (0.16); T2 W at anterior margin: 0.11 (0.13); T2 W at posterior margin: 0.38 (0.33); T2 L: 0.17 (0.17); Metafemur L: 0.65 (0.67); Metafemur W: 0.18 (0.18); Metatibia L: 0.83 (0.85); Inner spur L: 0.13 (0.15); Outer spur L: 0.12 (0.14); First segment of Metatarsus L: 0.29 (0.30); Ovipositor sheaths L: 0.26 (0.26); Body L: 2.06 (2.20); Fore wing L: 2.45 (2.55). Maximum W of T1 and T2 W at posterior margin are approximate for 2 specimens. T1 L, T1 W at posterior margin and T2 W at anterior margin are approximate for 1 specimen.

Male. Unknown.

Biology. Host unknown.

Distribution. Vietnam.

Molecular data. Both the holotype and paratype rendered almost full barcode sequences (629 and 632 bp), representing BIN BOLD:ADE2636, which is 8.7% different from the closest Microgastrinae sequences currently available in BOLD.

Etymology. Named after the country of the type locality.

Ypsilonigaster Fernandez-Triana, gen. n.

http://zoobank.org/9C952BD1-1664-4EA7-8D80-23B1677E63EA

Type species. *Ypsilonigaster tiger* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Face with strong sulcus medially near antennal base. Scutellar disc flat, entirely smooth, and shiny (Figs 41D, 43E, 44C, E, 45G). Propodeum mostly smooth but with strong median carina (Figs 41D, 43E, 44E). Fore wing with small, slit-shaped areolet. Metatibia with short, stout spines dorsally. T1 divided in three areas by a strong sulcus shaped as an inverted "Y" (Figs 40B, 41E, F, 42A, 43D,

E, 44B–C, E, 45D, H). Hypopygium unfolded and inflexible (Figs 41A, 42C, 44A). Ovipositor relatively strongly curved downwards (Figs 41A, 42C, 44A). Ovipositor sheaths thoroughly covered by setae (Fig. 44A).

Putative autapomorphies and potentially related genera. *Ypsilonigaster* has T1 divided in three areas by a strong sulcus shaped as an inverted "Y", a unique feature within Microgastrinae. *Ypsilonigaster* seems to be related to other Old World genera with strong median carina on propodeum, fore wing areolet, and relatively long ovipositor sheaths (e.g., *Choeras* s.l., see Discussion below), but differs from most of those genera by having an unfolded and inflexible hypopygium.

Biology. Host unknown.

Distribution. All known species are found in the Old World tropics (Afrotropical and Oriental regions).

Molecular data. Two DNA barcodes are available, both very distant from any other Microgastrinae sequence available in BOLD (8–10% of base pair differences). However, those two sequences (which were obtained from two different species and belong to BINs BOLD:AAV2124 and BOLD:ABY3660) which are also very different from each other and cluster very separate (based on a Neighbor Joining tree built with 35,000+ Microgastrinae sequences available in BOLD as of January 2018).

Etymology. From "Ypsilon" (in several languages an alternative form or synonym of the ancient Greek letter "Upsilon", which is depicted as a "Y") and "gaster" (in Greek "stomach" or "abdomen", also used for the metasoma in Hymenoptera), referring to the Y-shaped sulcus in the first tergite of metasoma that characterizes this genus. The gender of the genus is neuter.

Species. We recognize at least six different species, four of them new and described below. They can be separate using the following key.

Key to species of *Ypsilonigaster*

1 Tegula (yellow) and humeral complex (dark brown) differently coloured; body with striking contrast of four different colors between areas (yellow on head, front legs, and anterior half of mesosoma; black on posterior half of mesosoma and hind legs; white on T1, parts of T2/T3, some laterotergites and metatibial spines; brown on antenna, middle legs and most of metasoma) (Figs 44A–E) [Thailand] ..... ***Ypsilonigaster tiger*** **Fernandez-Triana & Boudreault, sp. n.**

– Tegula and humeral complex of same coloration (either yellow or light brown); body mostly uniformly colored (either white-yellow, orange-yellow, red-yellow or brown-black), without striking color contrast between areas (at most with parts of mesosoma and/or metasoma slightly darker than surrounding areas) [Africa and Malaysia] **2**

2 Body mostly red-yellow or dark brown or black; wings infumated or partially so **3**

– Body mostly yellow to yellow-white; wings hyaline **4**

3 Mesosoma, tegula, metatibia and metatarsus red-yellow; T1 with anterior and posterior halves of similar width (with a slight constriction around half length of tergite) (Fig. 40B); fore wing with veins r and 2RS meeting in a more acute angle (Fig. 40A) [Democratic Republic of the Congo] ....***Ypsilonigaster bumbana*** **(de Saeger, 1942)**

– Mesosoma, tegula, metatibia and metatarsus dark brown to black; T1 with anterior half clearly wider than posterior half (Fig. 42A); fore wing with veins r and 2RS meeting in a more rounded angle (Fig. 42B) [Democratic Republic of the Congo] .....***Ypsilonigaster pteroloba*** **(de Saeger, 1944)**

4 Mesosoma T1 not significantly narrowing towards posterior margin, its width at anterior and posterior margins about the same (Figs 43D, E); T1 with rather coarse sculpture on posterior 0.3 (Fig. 43E); T3 and T4 mostly brown (Figs 43A, D) [Republic of Congo]..... .....***Ypsilonigaster sharkeyi*** **Fernandez-Triana & Boudreault, sp. n.**

– T1 rather strongly narrowing towards posterior margin, its width at anterior margin at least 1.2 × its width at posterior margin; T1 smooth; T3 and T4 yellow (Figs 41E, F, 45D, H) **5**

5 Body color mostly white-yellow; tegula and humeral complex yellow (Fig. 45A–H) [Madagascar] .... ***Ypsilonigaster zuparkoi*** **Fernandez-Triana & Boudreault, sp. n.**

– Body color darker, mostly yellow with back of head and anteromesoscutum orange to orange-brown, and tergites 5+ mostly brown; tegula and humeral complex brown (Figs 41A-F) [Malaysia] .... ***Ypsilonigaster naturalis*** **Fernandez-Triana & Boudreault, sp. n.**

Ypsilonigaster bumbana **(de Saeger, 1942), comb. n.**
Fig. 40

Microgaster bumbana de Saeger, 1942. Original description (de Saeger 1942: 332).

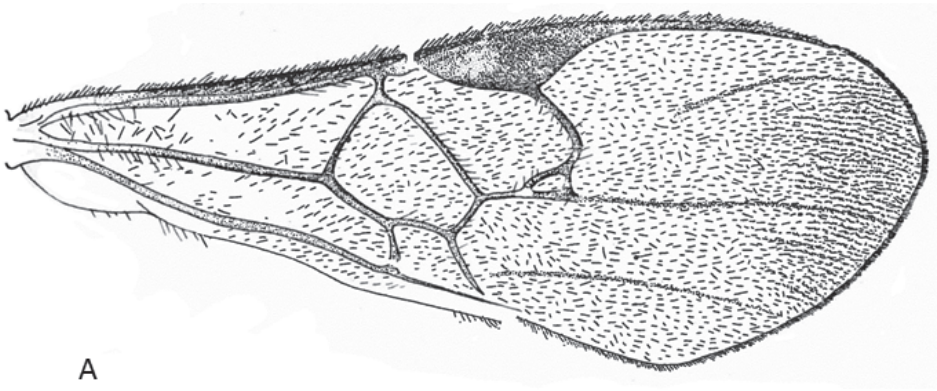
Holotype. Female, Democratic Republic of the Congo, RMCA (Musee Royal de l'Afrique Centrale, Tervuren, Belgium). Not examined, but original description checked.

Diagnosis. *Y. bumbana* can be separate from all known species of *Ypsilonigaster* (except for *Y. pteroloba*) based on its darker body color (red-yellow) and infumated wings (all other species are mostly yellow or white-yellow, or have body with striking contrast of four different colors between areas, and all have hyaline wings). *Y. bumbana* can in turn be differentiated from *Y. pteroloba* because it has a mostly red-yellow body color, a less constricted T1 and the fore wing veins r and 2RS join in a more acute angle (compare Figs 40A, B with Figs 42A, B).

Biology. Host unknown.

Distribution. Democratic Republic of the Congo.

Molecular data. No molecular data available.



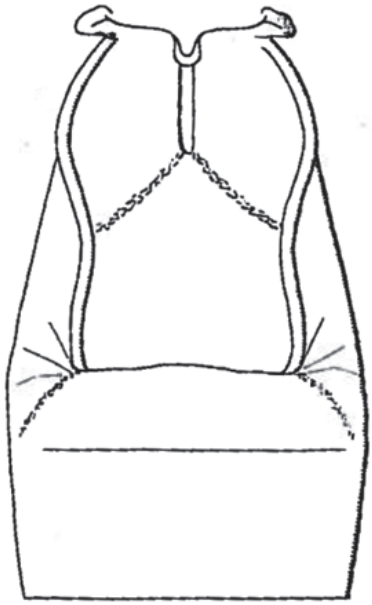


Figure 40. *Ypsilonigaster bumbana* holotype based on modified drawings from the original descriptions of the species (de Saeger 1942). **A** Fore wing **B** Tergites 1 to 3.

Ypsilonigaster naturalis **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/FC36191B-29DE-421E-8A43-B6E486348290
Fig. 41

Holotype. Female, Malaysia, RMNH.

Holotype labels. MALAYSIA: SE.SABAH/nr Danum Valley Field C./WON1, Mal. trap 5, c.150m/5–26.x.1987, RMNH'87/C.v.Achterberg &D.Kennedy. Second label: CNC878528.

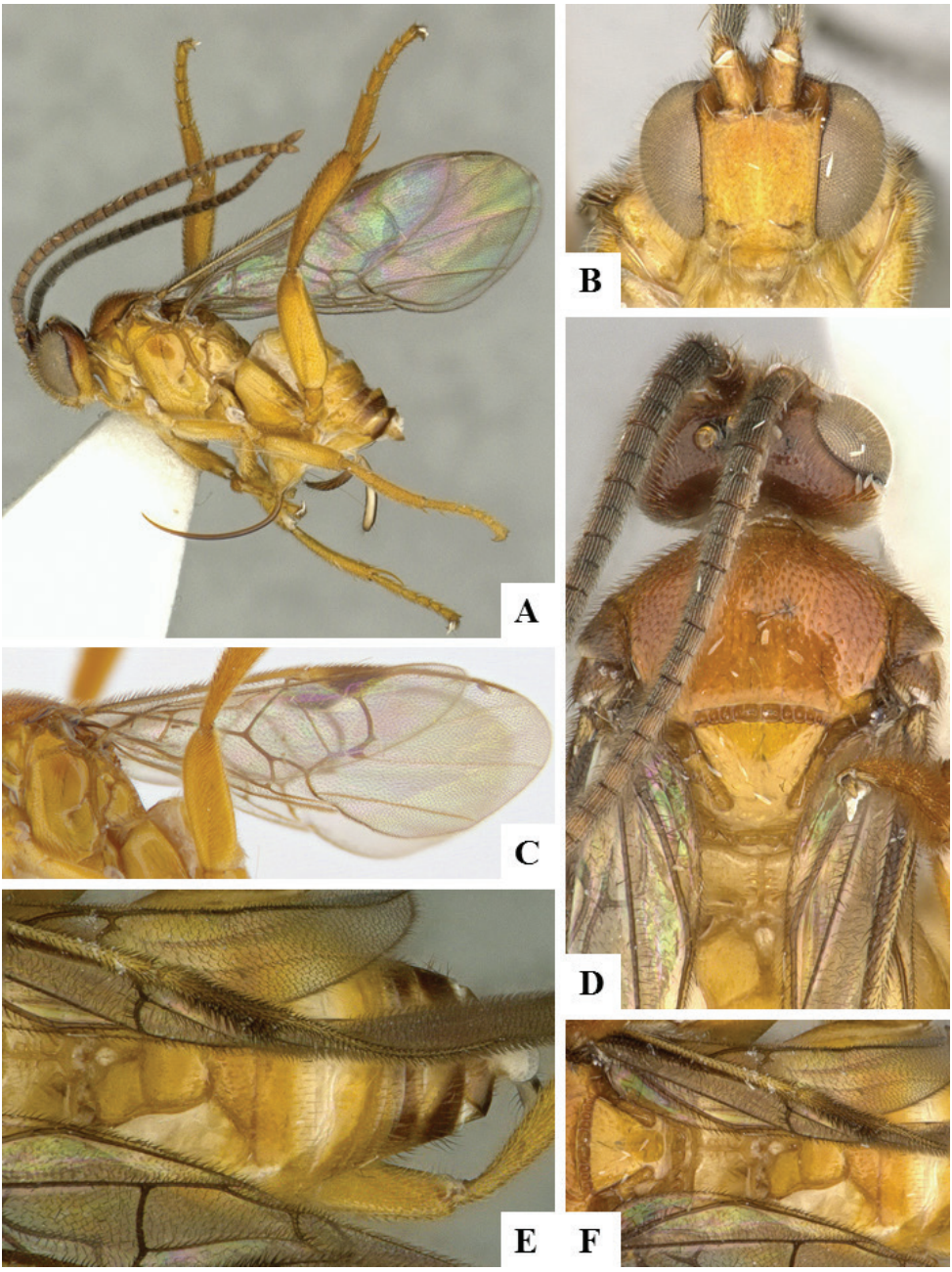


Figure 41. *Ypsilonigaster naturalis* female holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Head and mesosoma, dorsal **E** Metasoma dorsal **F** Propodeum.

Holotype locality. MALAYSIA, South-East Sabah, near Danum Valley, Field C., 150m.

Paratypes. Malaysia. (3♀ RMNH, CNC), same locality than holotype, Voucher codes: CNC878529, CNC878530, CNC878531.

Diagnosis. The combination of body colour mostly yellow (but with back of head and anteromesoscutum orange to orange-brown; antenna, tegula and humeral complex brown; and tergites 5+ mostly brown) and T1 sculpture and shape (T1 smooth and strongly narrowing towards posterior margin, its width at anterior margin at least 1.2 × its width at posterior margin) are enough to separate *Y. naturalis* from all other known species in the genus.

Description. Body colour mostly yellow (but with back of head and anteromesoscutum orange to orange-brown; antenna, tegula and humeral complex brown; and tergites 5+ mostly brown). Body mostly smooth (including most of propodeum, entire scutellar disc, and most tergites except for T2 which is coarsely sculptured), anteromesoscutum sparsely punctate. Scutoscutellar sulcus with 10 crenulae. Lunules relatively low (around 0.3 × height of lateral face of scutellum). Propodeum with strongly raised, median carina. Fore wing with small, slit-shaped areolet. Hind wing with more or less straight vannal lobe which is uniformly setose. Metafemur L 3.10–3.14 × its W. Metatibial inner spur L 1.55–1.88 × metatibia outer spur L; metatibia inner spur 0.61–0.71 × first segment of metatarsus L. T1 divided in three areas by a strong sulcus shaped as an inverted "Y"; T1 L 1.70–1.88 × T1 width at posterior margin. T2 subtriangular; T2 width at posterior margin 3.28–4.21 × T2 L. Ovipositor sheaths uniformly setose and 0.65–0.67 × as long as metatibia length. **Body measurements (mm).** F2 L: 0.28 (0.28); F3 L: 0.27 (0.27); F14 L: 0.21 (0.21); F15 L: 0.18 (0.18–0.19); Malar sulcus L: 0.08 (0.08); Mandible W: 0.12 (0.12–0.13); T1 L: 0.64 (0.67–0.68); T1 W at posterior margin: 0.36 (0.36–0.39); T1 maximum W: 0.59 (0.58–0.63); T2 W at anterior margin: 0.38 (0.34–0.38); T2 W at posterior margin: 0.68 (0.67–0.68); T2 L: 0.21 (0.16–0.19); Metafemur L: 1.16 (1.14–1.15); Metafemur W: 0.38 (0.37); Metatibia L: 1.36 (1.33–1.39); Inner spur L: 0.38 (0.41); Outer spur L: 0.24 (0.22–0.25); First segment of Metatarsus L: 0.62 (0.58); Ovipositor sheaths L: 0.88 (0.88–0.93); Body L: 3.80 (3.64–3.96); Fore wing L: 3.92 (3.64–3.92). Maximum W of T1 and T2 W at anterior margin are approximate for 2 specimens. T1 L is approximate for 3 specimens. T1 W at posterior margin and T2 W at posterior margin are approximate for 1 specimen.

Male. Unknown.

Biology. Host unknown.

Distribution. Only known from the type locality in Malaysia.

Molecular data. No molecular data available.

Etymology. Named after the Naturalis Biodiversity Center in Leiden (the Netherlands) in recognition of the outstanding and important collection of 18+ million insect specimens that institution holds, including one of the largest and most complete Microgastrinae collection in the world.

Ypsilonigaster pteroloba **(de Saeger, 1944), comb. n.**
Fig. 42

Microgaster pteroloba de Saeger, 1944. Original description (de Saeger 1944: 65).

Holotype. Female, Democratic Republic of the Congo, RMCA (Musée Royal de l'Afrique Centrale, Tervuren, Belgium). Not examined, but original description checked.

Diagnosis. *Y. pteroloba* can be separate from all known species of *Ypsilonigaster* (except for *Y. bumbana*) based on its darker body color (dark brown to black) and infumated wings (all other species are mostly yellow or white-yellow, or have body with striking contrast of four different colors between areas, and all have hyaline wings). *Y. pteroloba* can in turn be differentiated from *Y. bumbana* because the later has a mostly red-yellow body color, a less constricted T1 and the fore wing veins r and 2RS join in a more acute angle (compare Figs 40A, B with Fig. 42A, B).

Biology. Host unknown.

Distribution. Democratic Republic of the Congo.

Molecular data. No molecular data available.

Ypsilonigaster sharkeyi **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/BFA95BCD-7299-4559-964A-706D0C681D09
Fig. 43

Holotype. Male, Republic of the Congo, CNC.

Holotype labels. REPUBLIC OF THE CONGO:/Pool, Abio Lesio-Loun Pk,/330m, 09.xi.2008/3°06.020'S, 015°31.440'E/Sharkey & Braet. Second label: JMIC 0517.

Holotype locality. REPUBLIC OF THE CONGO, Abio-Lesio Louna Park, 3°06.020'S, 015°31.440'E, pool, 330m.

Diagnosis. *Ypsilonigaster sharkeyi* can be distinguished from all other known species in the genus due to the unique sculpture pattern and shape of T1 (T1 width at anterior and posterior margins about the same, and with rather coarse sculpture on posterior 0.3).

Description. Female unknown. Male. Body colour mostly yellow (but with antenna brown and tergites 3+ mostly brown). Body mostly smooth (including most of propodeum, entire scutellar disc, and most tergites except for posterior 0.3 of T1 and T2 which are coarsely sculptured), anteromesoscutum sparsely punctate. Scutoscutellar sulcus with 7 crenulae. Lunules relatively low (around 0.25 × height of lateral face of scutellum). Propodeum with strongly raised, median carina. Fore wing with small, slit-shaped areolet. Hind wing with vannal lobe slightly concave centrally, and without setae on that central area. Metafemur L 3.42 × its W. Metatibial inner spur L 1.65 × metatibia outer spur L; metatibia inner spur 0.55 × first segment of metatarsus L. T1

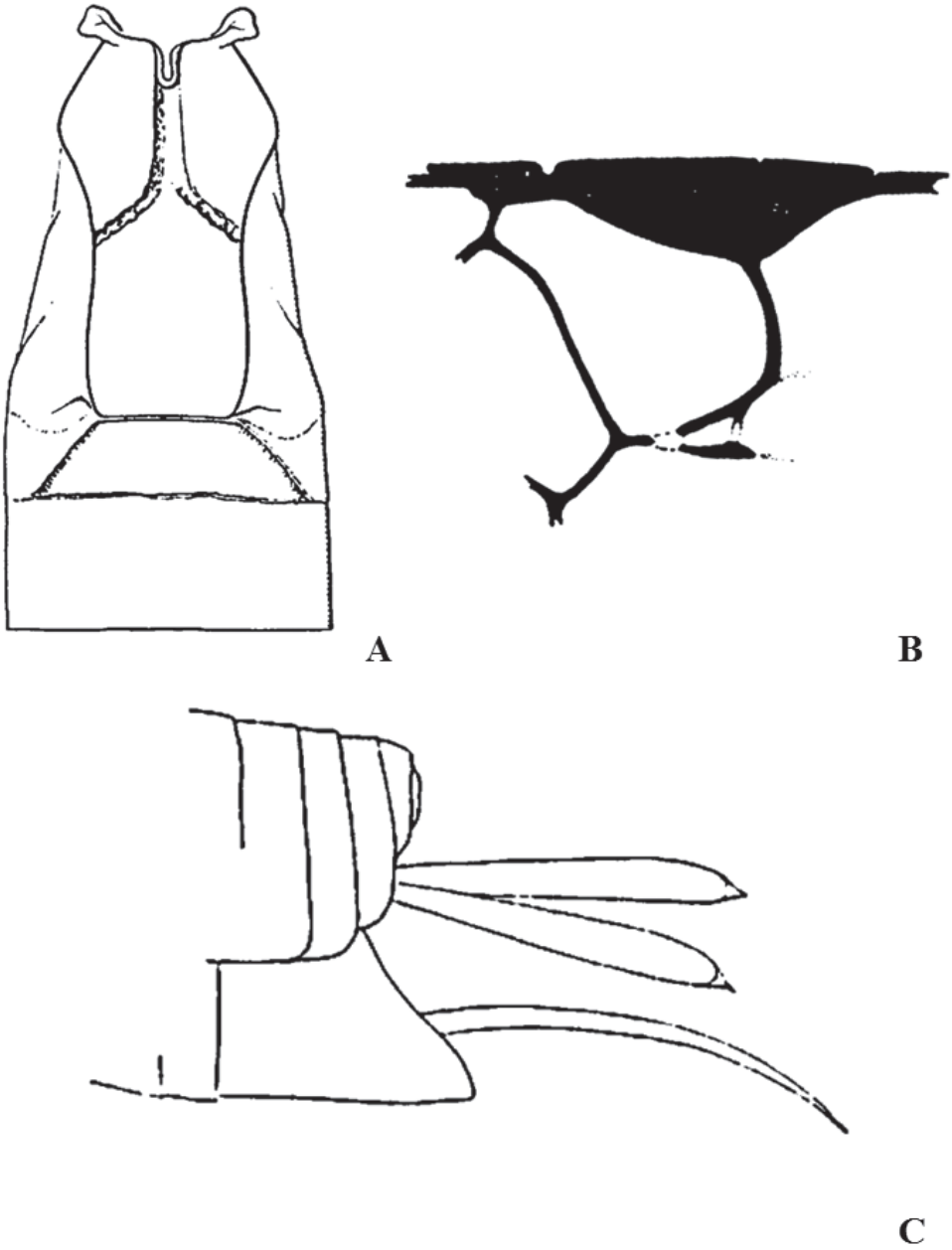


Figure 42. *Ypsilonigaster pteroloba* holotype based on modified drawings from the original descriptions of the species (de Saeger 1944). **A** Tergites 1 to 3 **B** Fore wing details of pterostigma and nearby veins **C** Hypopygium, ovipositor and ovipositor sheaths.

divided in three areas by a strong sulcus shaped as an inverted "Y"; T1 L 1.38 × T1 W at posterior margin. T2 subtriangular; T2 width at posterior margin 3.05 × T2 L **Body measurements (mm).** F2 L: 0.25; F3 L: 0.26; F14 L: 0.23; F15 L: 0.21; Malar

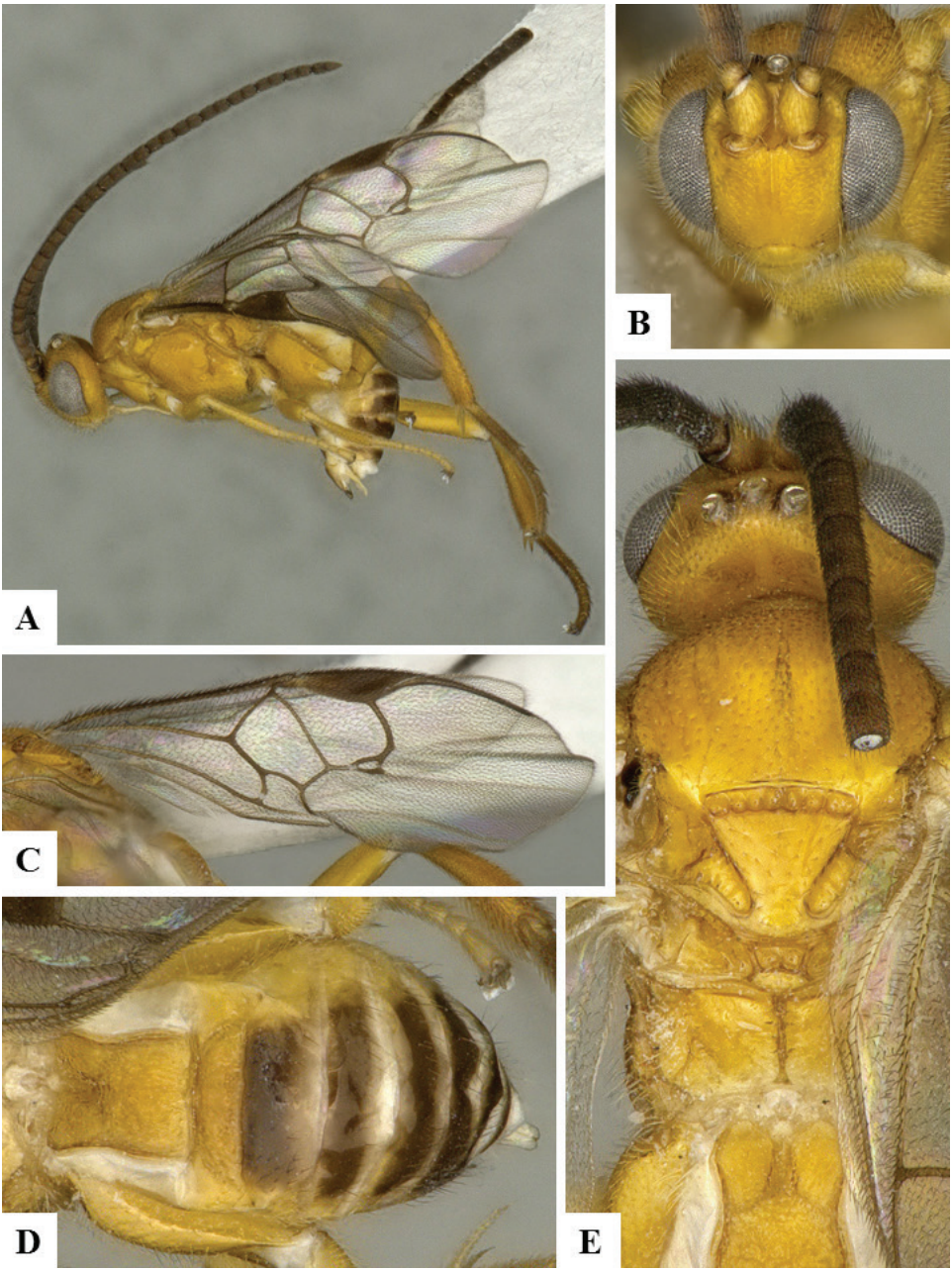


Figure 43. *Ypsilonigaster sharkeyi* male holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Metasoma dorsal **E** Head and mesosoma, dorsal.

sulcus L: 0.08; Mandible W: 0.10; T1 L: 0.46; T1 W at posterior margin: 0.33; T1 maximum W: 0.33; T2 W at anterior margin: 0.26; T2 W at posterior margin: 0.46; T2 L: 0.15; Metafemur L: 0.88; Metafemur W: 0.26; Metatibia L: 1.07; Inner spur L:

0.28; Outer spur L: 0.17; First segment of Metatarsus L: 0.50; Body L: 2.63; Fore wing L: 3.00. Mandible W is approximate.

Biology. Host unknown.

Distribution. Only known from the type locality in southeastern Republic of the Congo.

Molecular data. The holotype rendered an almost complete DNA barcode (621 bp), which represents BIN BOLD:AAV2124. That sequence is 8.02% different from the closest Microgastrinae in BOLD.

Etymology. Named after Michael Sharkey, in recognition of his significant contributions to the knowledge of parasitoid wasps, and also for sending the first author valuable specimens –some of which were studied and are part of this paper.

Ypsilonigaster tiger **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/992B6BED-FA80-4C70-837F-5CC16A23004F
Fig. 44

Holotype. Female, Thailand, QSBG.

Holotype labels. THAILAND Chiang Mai/Huai Nam Dang NP Guest House/19°18.803'N, 98°36.395'E/Malaise trap 31.x-7.xi.2007/Anuchart & Thawatchai leg. T5553. Second label: JMIC 0536.

Holotype locality. THAILAND, Chiang Mai Province, Huai Nam Dang National Park Guest House, 19°18.803'N,t 98°36.395'E.

Diagnosis. This species is very distinctive due to its unusual coloration pattern, which includes contrasting areas in white, yellow, brown and black. It also has the shortest ovipositor sheaths and the longest fore wing among the known species in the genus.

Description. Body with striking contrast of four different colors between areas (yellow on head, front legs, and anterior half of mesosoma; black on posterior half of mesosoma and hind legs; white on T1, parts of T2/T3, some laterotergites and metatibial spines; brown on antenna, middle legs and most of metasoma). Tegula (yellow) and humeral complex (dark brown) differently coloured. Body mostly smooth, including propodeum, entire scutellar disc, and all tergites (but anteromesoscutum with shallow punctures all over except for notauli). Scutoscutellar sulcus with 9–10 crenulae. Lunules relatively normal (around 0.4 × height of lateral face of scutellum). Propodeum with strongly raised, median carina. Fore wing with small, slit-shaped areolet. Hind wing with more or less straight vannal lobe which is uniformly setose. Metafemur L 2.98 × its W. Metatibial inner spur L 1.79 × metatibia outer spur L; metatibia inner spur 0.62 × first segment of metatarsus L. T1 divided in three areas by a strong sulcus shaped as an inverted "Y"; T1 L 2.53 × T1 width at posterior margin. T2 subtriangular; T2 width at posterior margin 3.0 × T2 L. Ovipositor sheaths uniformly setose and 0.48 × as long as metatibia length. **Body measurements (mm).** F2 L: 0.33; F3 L: 0.32; F14 L: 0.24; F15 L: 0.21; Malar sulcus L: 0.08; Mandible

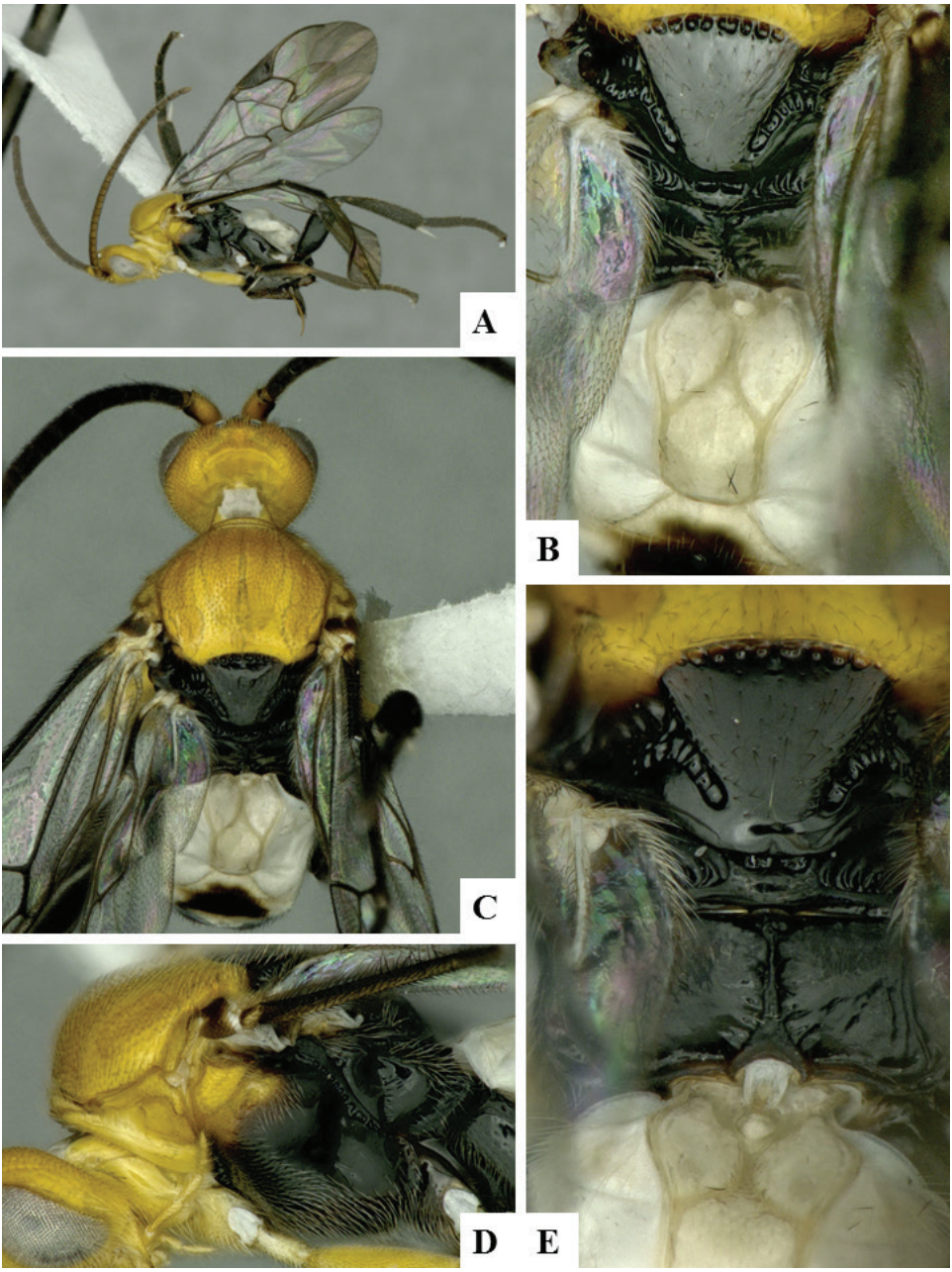


Figure 44. *Ypsilonigaster tiger* female holotype. **A** Habitus **B** Tergite 1 **C** Mesosoma dorsal **D** Mesosoma lateral **E** Propodeum.

W: 0.13; T1 L: 0.76; T1 W at posterior margin: 0.30; T1 maximum W: 0.54; T2 W at anterior margin: 0.23; T2 W at posterior margin: 0.63; T2 L: 0.21; Metafemur L: 1.28; Metafemur W: 0.43; Metatibia L: 1.52; Inner spur L: 0.43; Outer spur L: 0.24;

First segment of Metatarsus L: 0.69; Ovipositor sheaths L: 0.73; Body L: 3.84; Fore wing L: 4.60. Fore wing L is approximate.

Male. Unknown.

Biology. Host unknown.

Distribution. Only known from the type locality in northern Thailand.

Molecular data. The holotype rendered an almost complete DNA barcode (622 bp), which represents BIN BOLD:ABY3660, a unique sequence that is 9.86% different from the closest Microgastrinae in BOLD.

Etymology. Named after the Thailand Inventory Group for Entomological Research (TIGER), a collaborative project between the Queen Sirikit Botanical Garden and the National Parks, Wildlife and Plant Conservation Department with the goal of conducting inventories of insect biodiversity in Thailand (see also: http://www.sharkeylab.org/tiger/). All specimens of Thailand studied for this paper came from those inventories, and will be deposited in the QSBG for future reference.

Ypsilonigaster zuparkoi **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/247A6525-F2B4-4774-8D6A-DB99F2110572
Fig. 45

Holotype. Male, Madagascar, CAS.

Holotype labels. MADAGASCAR: Majunga/Ambatofolaka, Namoroka/53km from Soalala 3km N/Vitanandro village. Second label: 30.xi-9.xii.2007, elev. 400ft/16°28.4'S, 45°23.48'E, Calif./Acad. Of Sciences coll: Irwin,/Harin'Hala, malaise trap in/dense dry forest, MG040A-10. Third label: CNC878532.

Holotype locality. MADAGASCAR, Majunga, Ambatofolaka, Namoroka, 53 km from Soalala, 3km North of Vitanandro village, 16°28.4'S, 45°23.48'E, 400ft, dense dry forest.

Paratypes. Madagascar. (1♂ CNC), same locality than holotype, Voucher code: CNC878533.

Diagnosis. The combination of body colour mostly white-yellow and T1 sculpture and shape (T1 smooth and strongly narrowing towards posterior margin, its width at anterior margin at least 1.2 × its width at posterior margin) are enough to separate *Y. zuparkoi* from all other known species in the genus.

Description. Female unknown. Male. Body colour mostly white-yellow (only ventral sides of scape and F1–F2 brown). Body mostly smooth (including most of propodeum, entire scutellar disc, and most tergites except for T2 which is slightly duller), anteromesoscutum sparsely punctate. Scutoscutellar sulcus with 10 crenulae. Lunules relatively low (around 0.25 × height of lateral face of scutellum). Propodeum with strongly raised, median carina. Fore wing with small, slit-shaped areolet. Hind wing with more or less straight vannal lobe which is uniformly setose. Metafemur L 2.66–2.78 × its W. Metatibial inner spur L 1.59–1.67 × metatibia outer spur L; metatibia inner spur 0.58–0.64 × first segment of metatarsus L. T1 divided in three areas by

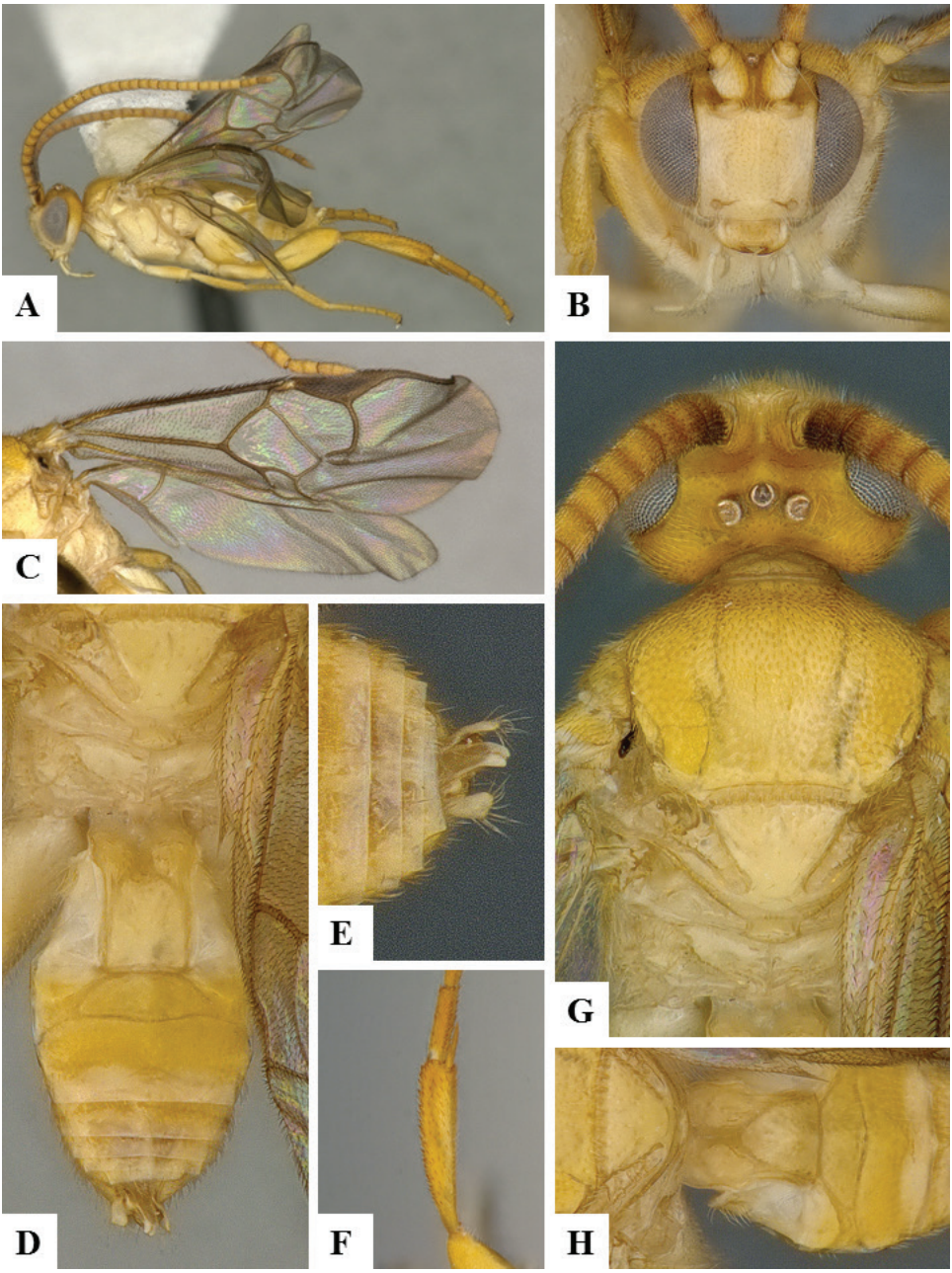


Figure 45. *Ypsilonigaster zuparkoi* male holotype. **A** Habitus **B** Head frontal **C** Fore wing and hind wing **D** Metasoma dorsal **E** Genitalia **F** Metatibia **G** Head and mesosoma, dorsal **H** Tergites 1 to 2.

a strong sulcus shaped as an inverted "Y"; T1 L 2.00–2.41 × T1 width at posterior margin. T2 subtriangular; T2 width at posterior margin 2.95–3.47 × T2 L. **Body measurements (mm).** F2 L: 0.26 (0.26); F3 L: 0.26 (0.26); F14 L: 0.22 (0.23); F15 L:

0.21 (0.21); Malar sulcus L: 0.08 (0.09); Mandible W: 0.13 (0.10); T1 L: 0.50 (0.54); T1 W at posterior margin: 0.25 (0.23); T1 maximum W: 0.33 (0.46); T2 W at anterior margin: 0.23 (0.18); T2 W at posterior margin: 0.49 (0.55); T2 L: 0.17 (0.16); Metafemur L: 0.93 (0.98); Metafemur W: 0.33 (0.37); Metatibia L: 1.04 (1.08); Inner spur L: 0.29 (0.29); Outer spur L: 0.18 (0.18); First segment of Metatarsus L: 0.46 (0.50); Body L: 3.03 (3.28); Fore wing L: 2.75 (2.90).

Female. Unknown.

Biology. Host unknown.

Distribution. Madagascar.

Molecular data. No molecular data available.

Etymology. Named after Robert Zuparko, in recognition of his significant contributions to the knowledge of parasitoid wasps, and also for sending the first author valuable specimens –some of which were studied and are part of this paper.

Zachterbergius Fernandez-Triana, gen. n.

http://zoobank.org/144D32AA-7B76-483E-A0F4-9159CDDD60D5

Type species. *Zachterbergius tenuitergum* Fernandez-Triana & Boudreault, here designated.

Diagnostic description. Labial palpi very long, extending to mesopleuron (Fig. 46A, B). Upper margin of face produced dorsally between the antennal insertions into a small triangular flange which has a median carina (Fig. 46B). Scape relatively very transverse (Fig. 46B). Flagellomere with two rows of placodes. Polished band of scutellum interrupted medially by band of rugosity (Figs 46E, F, 47B). Propodeum with clearly defined median carina and partially defined transverse carina and apical part of an areola (Figs 46E, F, 47A, B). Fore wing with quadrangulate areolet (Fig. 46C). T1 with broad depression on anterior half (Figs 46D, 47A, B). T2 longest and thinnest of Microgastrinae (T2 L 4.0 × its width at base and apex, T2 0.7–0.8 × as long as T1 L, T2 around 1.5 × as long as T3 L) (Figs 46D, E, 47A, B).

Putative autapomorphies and potentially related genera. The relationships of *Zachterbergius* with other genera of Microgastrinae are not clear at present. The length of T2 is unique among known species of Microgastrinae. The propodeum carination pattern is uncommon in the subfamily, as are the scape shape and elongate labial palpi. The available barcode sequence is also very different from all other known barcodes within the subfamily.

Biology. Host unknown.

Distribution. The only known species is found in the Oriental region (Thailand).

Molecular data. A single sequence is available, representing BIN BOLD:AAV2126, which is 15.6% different than the closest sequence available in BOLD.

Etymology. The genus name refers to and honors the Dutch braconid expert Kees van Achterberg, in recognition of his significant contributions to the knowledge of Braconidae of the world, as well as other Hymenoptera groups. Over the years Kees has been a dear friend, mentor and colleague of the first author, and has kindly supported

his work on Microgastrinae. The letter "Z" was added at the beginning of the name to guarantee the uniqueness of the name and avoid potential homonyms -due to the large number of taxa named after Kees van Achterberg. The gender of the genus is neuter.

Species. Only one species is known.

Zachterbergius tenuitergum **Fernandez-Triana & Boudreault, sp. n.**
http://zoobank.org/C317CE9A-DF37-4A5B-90CC-32D9C09D74D3
Figs 46, 47

Holotype. Male, Thailand, QSBG.

Holotype labels. THAILAND: Chiang Mai huai/Nam Dang NP Helipad/19°18.33'N, 98°36.289'E/30.xii.2007–7.i.2008/Anuchart leg. T5597. Second label: CNC878554.

Holotype locality. THAILAND, Chiang Mai Province, Huai Nam Dang National Park, Helipad, 19°18.33'N, 98°36.289'E.

Paratype. Thailand. (1♂ QSBG), Chiang Mai, Huai Nam Dang NP, visitor center, 19.188000, 98.364000, 28.x.2007, coll. Anuchart, Thawatchai, Voucher code: JMIC0538.

Diagnosis. This is the only known species in the genus so far, thus the generic diagnosis works as the species diagnosis as well.

Description. Male. Body mostly brown to dark brown; palpi and first few laterotergites and sternites white; scape, pedicel and labrum yellow-white; flagellomeres light brown; propleuron and pronotum yellow-orange (darker in pronotum); legs mostly yellow-white (except for posterior 0.3 of metatibia and metatarsus which are brown); wings hyaline, most veins brown, pterostigma brown with pale spot on anterior 0.2. Labial palpi very long, extending to mesopleuron. Upper margin of face produced dorsally between the antennal insertions into a small triangular flange which has a median carina. Scape relatively very transverse (Fig. 46B). Flagellomere with two rows of placodes. Polished band of scutellum interrupted medially by band of rugosity. Propodeum with clearly defined median carina and partially defined transverse carina and apical part of an areola. Fore wing with quadrangulate areolet. T1 with broad depression on anterior half. T2 longest and thinnest of Microgastrinae (T2 L 4.0 × its width at base and apex, T2 0.7–0.8 × as long as T1 L, T2 around 1.5 × as long as T3 L). **Body measurements (mm).** F2 L: 0.29 (0.30); F3 L: 0.28 (0.29); F14 L: 0.24 (0.26); F15 L: 0.23 (0.25); Malar sulcus L: 0.08 (0.09); Mandible W: 0.08 (0.08); T1 L: 0.33 (0.33); T1 W at posterior margin: 0.09 (0.08); T1 maximum W: 0.24 (0.26); T2 W at anterior margin: 0.05 (0.06); T2 W at posterior margin: 0.16 (0.23); T2 L: 0.27 (0.30); Metafemur L: 0.88 (0.89); Metafemur W: 0.19 (0.20); Metatibia L: 1.10 (1.10); Inner spur L: 0.25 (0.26); Outer spur L: 0.21 (0.22); First segment of Metatarsus L: 0.53 (0.52); Body L: 2.80 (2.75); Fore wing L: 2.90 (3.00).

Female. Unknown.

Biology. Host unknown.

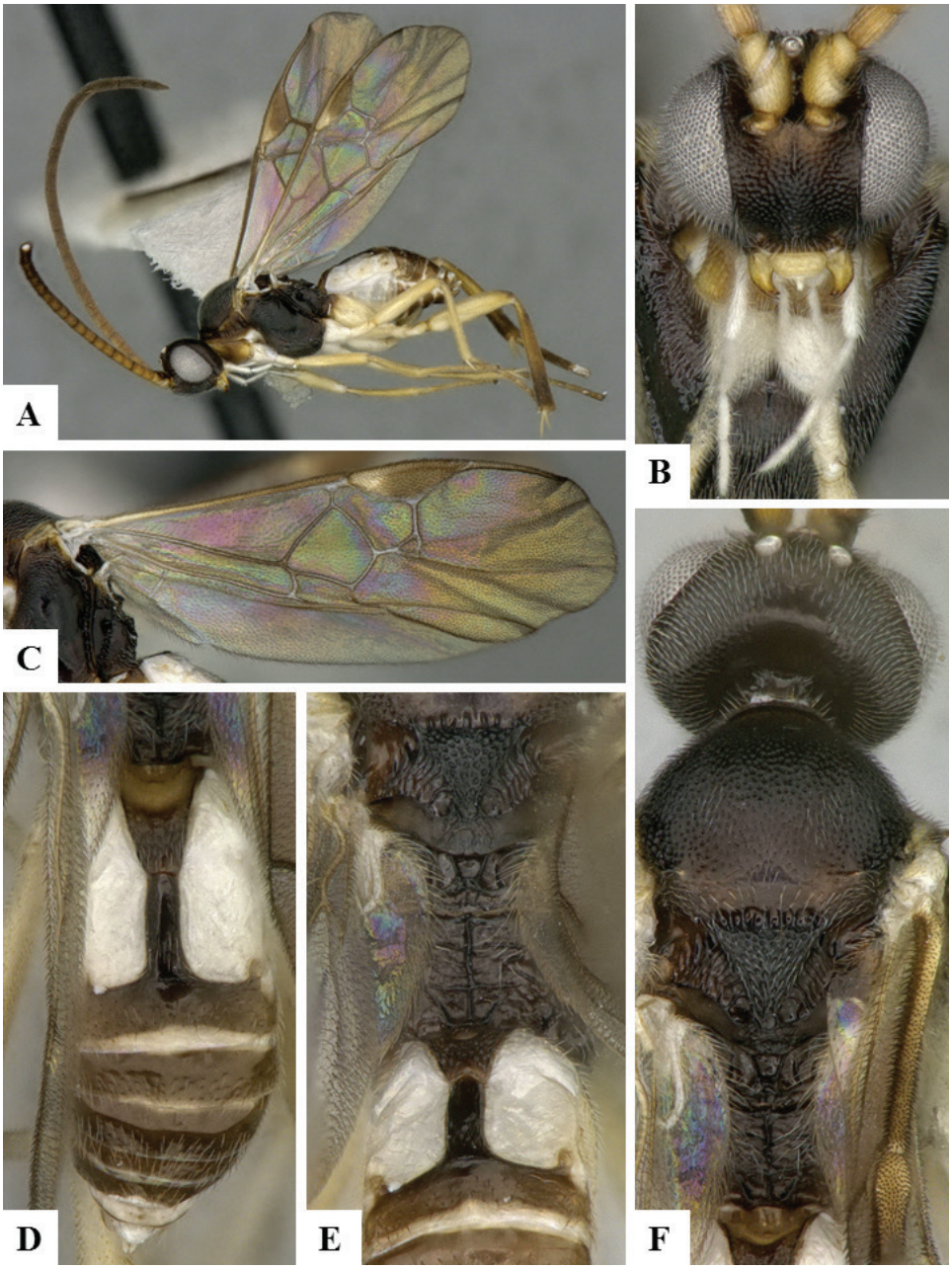


Figure 46. *Zachterbergius tenuitergum* male holotype. **A** Habitus **B** Head frontal **C** Fore wing **D** Metasoma dorsal **E** Propodeum **F** Head and mesosoma, dorsal.

Distribution. Thailand.

Molecular data. A single sequence was obtained from the paratype, representing BIN BOLD:AAV2126, which is 15.6% different than the closest sequence available in BOLD.

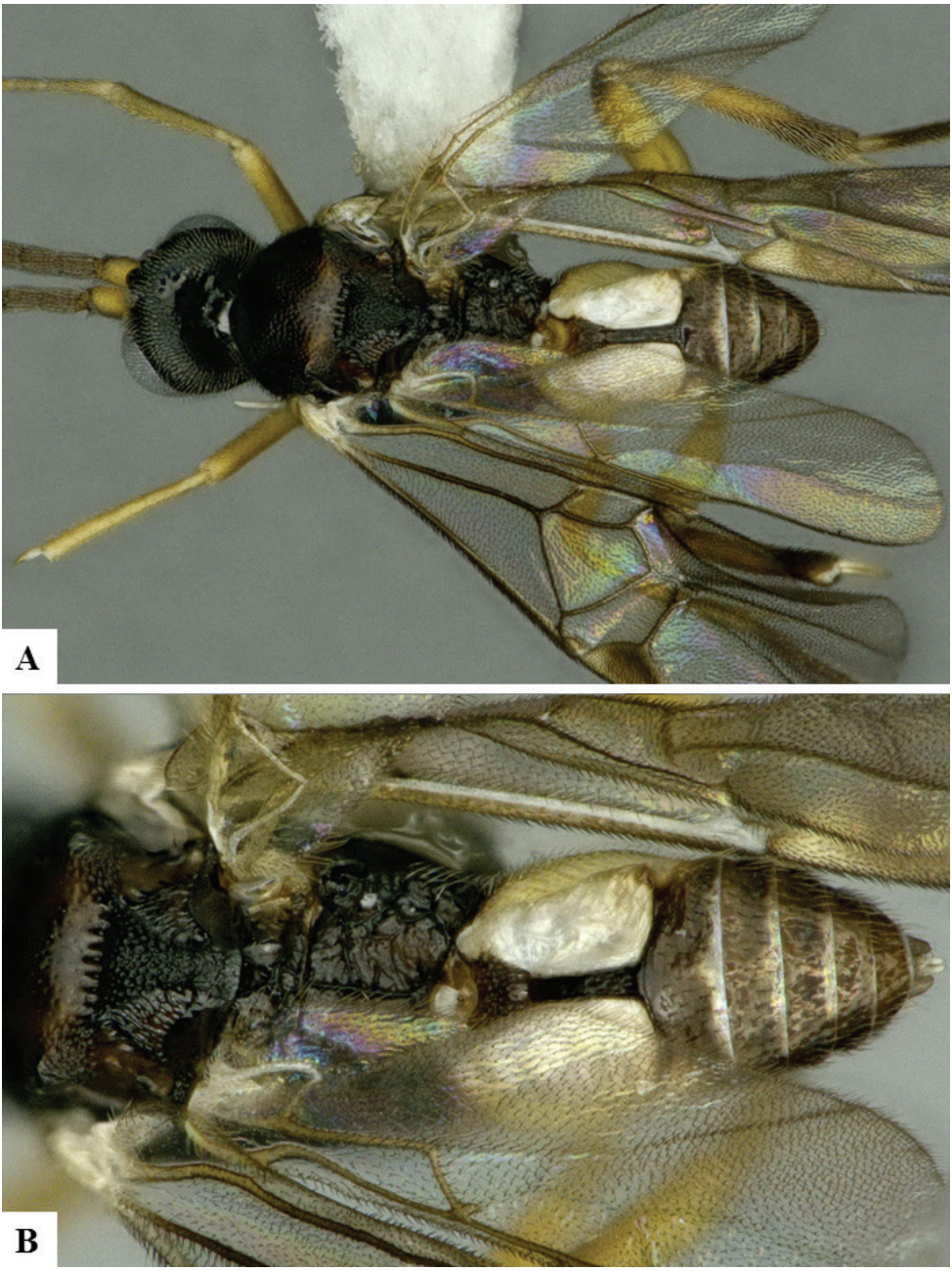


Figure 47. *Zachterbergius tenuitergum* male paratype JMIC 0538. **A** Habitus dorsal **B** Mesosoma and metasoma, dorsal.

Etymology. From "tenuis" (in Latin "thin"), and "tergum" (in Latin "back", also used as the dorsal/upper portion of an arthropod segment), referring the very thin second tergite of metasoma.

Discussion

When considering the classification of braconid wasps, it is interesting to note the disparity of treatments among the different groups. For example, and based on the information currently available (Table 1), there is an average of almost 20 described species per described genus (spp/genus) in Braconidae; however, the variation is very significant. Among the eight most diverse subfamilies, all of which have at least 1,200 described species each, Microgastrinae has 45 spp/genus (or 34 spp/genus when adding the results of the present paper). But even after our results are accounted for, that ratio still ranks as the third highest among Braconidae, almost 2.5 × the average for the entire family and considerably higher than other subfamilies with similar species diversity (e.g., 4.5 × more than in Doryctinae, 2.0 × more than Alysiinae). Although the spp/genus ratio does not necessarily have to be the same between different groups, the data suggest that the current generic framework for Microgastrinae is still insufficient and more work is needed.

Mason (1981) provided the first comprehensive and more explicit phylogenetic analysis of the subfamily Microgastrine, including its arrangement within six tribes and 50 genera (23 of which were newly described by him in that paper). Subsequent papers have found that the monophyly of those tribes is not well supported (e.g., Walker et al. 1990, Maeto 1996, Whitfield et al. 2002, van Achterberg 2003), and thus at present many authors do not follow any tribal arrangement within the subfamily. However, Mason's generic concepts, with a few exceptions, have been widely accepted by most authors (but see van Achterberg 2003, Fernandez-Triana et al. 2014, and Whitfield et al. 2018 for different opinions and further discussion on the topic). The history of Microgastrine systematics and classification was recently summarized by Whitfield et al. (2018), although they only listed 63 genera, as they missed a new genus described around the same time (Xiong et al. 2017). Since 1981, one of Mason's genera has been synonymized (Whitfield 2006) and 15 new genera have been proposed, for a total of 64 valid genera previous to the present paper.

We described in this paper 17 new genera from all tropical regions of the world (Afrotropical, Australasian, Neotropical and Oriental). Thus, the total of extant genera of Microgastrinae stands now at 81 (Table 2). Although our results have increased the number of genera worldwide by 21 %, we are aware of additional genera that remain undescribed in collections. We expect that the actual diversity in the subfamily will probably be around one hundred genera.

For every new genus described above, putative autapomorphies, morphological diagnostic features (to separate the new taxa from their potentially most closely related genera), and molecular data (DNA barcodes, whenever available) were presented. However, no attempt is made in this paper to reassess the phylogeny of Microgastrinae, nor it is possible to conclude at present on generic limits or relationships between different taxa. An improved and updated phylogeny of the subfamily is still years in the making and will require more comprehensive analyses, including revised morphological studies (reassessing characters previously used that were wrongly coded, and adding

Table 1. Diversity of species and genera of the family Braconidae and its eight most diverse subfamilies. To provide a fair comparison all data is based on the same source (Yu et al. 2016), even though numbers for some groups have slightly changed for the past two years. For Microgastrinae, the values between parentheses include the new data from the present paper.

Subfamily	# of described Species	# of described Genera	Species/Genus ratio
BRACONIDAE	21,221	1,103	19.2
Braconinae	3,052	189	16.1
Microgastrinae	2,715 (2,759)	60 (81)	45.2 (34.1)
Alysiinae	2,442	107	22.8
Opiinae	2,063	39	52.9
Doryctinae	2,045	196	10.4
Cheloninae	1,523	23	66.2
Euphorinae	1,270	59	21.5
Agathidinae	1,213	52	23.3

some overlooked in the past), a larger molecular dataset (including many more genes), and biological data (roughly half of the described species of Microgastrinae have some host data associated, but there has never been any attempt to critically revise that information, much less to analyze it at a world scale). In spite of these difficulties, we provide below a few comments which we hope will be useful for future studies of Microgastrinae phylogeny.

Among the newly described genera, four (*Gilbertnixonius*, *Jenopappius*, *Silvaspinosus* and *Tobleronius*) are clearly part of the Microplitini (sensu Mason 1981). This is one the best defined groups of genera within Microgastrinae, and most likely to be monophyletic. Until now, it included four genera (*Alloplitis*, *Microplitis*, *Philoplitis* and *Snellenius*) and thus our results double that total. Except for the very diverse and cosmopolitan *Microplitis*, and the moderately diverse and mostly pantropical *Snellenius* (these two genera comprising more than 95% of the described species of Microplitini), the other six genera are found in the Oriental or Afrotropical regions, perhaps an indication that the origin of this clade was in the Old World tropics. Microplitini is characterized by relatively large tentorial pits, head mostly coarsely sculptured, stemmaticum usually very well defined and slightly to strongly raised from surrounding areas, anteromesoscutum and scutellar disc usually coarsely sculptured, notauli almost always defined (often very clearly), propodeum always sculptured and with several strongly defined carinae, fore wing with areolet (which is usually large), relatively small metacoxa, short metatibial spurs, first metasomal tergite with median longitudinal sulcus, hypopygium inflexible and relatively short, ovipositor sheaths with few setae mostly limited to apex, and ovipositor almost always very short (scarsely or not at all projecting beyond apex of hypopygium).

A partial to complete occipital carina is reported for Microgastrinae for the first time in this paper, and was found in at least members of four different genera (*Alloplitis*, *Gilbertnixonius*, *Philoplitis* and *Tobleronius*). A partial to complete epicnemial carina is present in at least two genera (*Snellenius* and *Gilbertnixonius*), the only other

Table 2. Updated list of 81 extant genera of Microgastrinae worldwide. Based on Whitfield et al. (2018), with the addition of Xiong et al. (2017) and the new genera described in the present paper.

Agupta Fernandez-Triana, 2018	*Glyptapanteles* Ashmead, 1904	*Philoplitis* Nixon, 1965
Alloplitis Nixon, 1965	*Hygroplitis* Thomson, 1895	*Pholetesor* Mason, 1981
Alphomelon Mason, 1981	*Hypomicrogaster* Ashmead, 1898	*Prasmodon* Nixon, 1965
Apanteles Foerster, 1863	*Iconella* Mason, 1981	*Promicrogaster* Brues & Richardson, 1913
Austinicotesia Fernandez-Triana, 2018	*Illidops* Mason, 1981	*Protapanteles* Ashmead, 1898
Austrocotesia Austin & Dangerfield, 1992	*Janhalacaste* Fernandez-Triana, 2018	*Protomicroplitis* Ashmead, 1898
Beyarslania Kocak & Kemal, 2009	*Jenopappius* Fernandez-Triana, 2018	*Pseudapanteles* Ashmead, 1898
Billmasonius Fernandez-Triana, 2018	*Jimwhitfieldius* Fernandez-Triana, 2018	*Pseudofornicia* van Achterberg, 2015
Buluka de Saeger, 1948	*Keylimepie* Fernandez-Triana, 2016	*Pseudovenanides* Xiao & You, 2002
Carlmuesebeckius Fernandez-Triana, 2018	*Kiwigaster* Fernandez-Triana, Ward & Whitfield, 2011	*Qrocodiledundee* Fernandez-Triana, 2018
Chaoa Luo & You, 2004	*Kotenkosius* Fernandez-Triana, 2018	*Rasivalva* Mason, 1981
Choeras Mason, 1981	*Larissimus* Nixon, 1965	*Rhygoplitis* Mason, 1981
Clarkinella Mason, 1981	*Lathrapanteles* Williams, 1985	*Sathon* Mason, 1981
Cotesia Cameron, 1891	*Mariapanteles* Whitfield & Fernandez-Triana, 2012	*Semionis* Nixon, 1965
Cuneogaster Choi & Whitfield, 2006	*Markshawius* Fernandez-Triana, 2018	*Sendaphne* Nixon, 1965
Dasylagon Muesebeck, 1958	*Microgaster* Latreille, 1804	*Shireplitis* Fernandez-Triana & Ward, 2011
Deuterixys Mason, 1981	*Microplitis* Foerster, 1863	*Silvaspinosus* Fernandez-Triana, 2018
Diolcogaster Ashmead, 1900	*Miropotes* Nixon, 1965	*Snellenius* Westwood, 1882
Distatrix Mason, 1981	*Napamus* Papp, 1993	*Tobleronius* Fernandez-Triana, 2018
Dodogaster Rousse, 2013	*Neoclarkinella* Rema & Narendran, 1996	*Ungunicus* Fernandez-Triana, 2018
Dolichogenidea Viereck, 1911	*Nyereria* Mason, 1981	*Venanides* Mason, 1981
Eripnopelta Xiong, van Achterberg & Chen, 2017	*Ohenri* Fernandez-Triana, 2018	*Venanus* Mason, 1981
Exix Mason, 1981	*Papanteles* Mason, 1981	*Wilkinsonellus* Mason, 1981
Exoryza Mason, 1981	*Parapanteles* Ashmead, 1900	*Xanthapanteles* Whitfield, 1995
Exulonyx Mason, 1981	*Parenion* Nixon, 1965	*Xanthomicrogaster* Cameron, 1911
Fornicia Brullé, 1846	*Paroplitis* Mason, 1981	*Ypsilonigaster* Fernandez-Triana, 2018
Gilbertnixonius Fernandez-Triana, 2018	*Pelicope* Mason, 1981	*Zachterbergius* Fernandez-Triana, 2018

genus of Microgastrinae with that carina being the unrelated and very specialized *Fornicia*. The presence, in some members of Microplitini, of the occipital and epicnemial carinae is further indication of the uniqueness of this group within the subfamily.

In spite of Microplitini being relatively well defined and relatively unique within Microgastrinae, its relationship with the rest of the subfamily is not clear at present. Some

results from previous studies have suggested that Microplitini is a basal group within Microgastrinae (e.g., Mason 1981, Dowton and Austin 1998, Quicke et al. 2004). Indeed, in other subfamilies of Braconidae, the presence of occipital and epicnemial carinae has been interpreted as plesiomorphic (e.g., Quicke and van Achterberg 1990 and references there, but also see counterarguments in Wharton et al. 1992). However, data from other studies, mostly based on biology, suggest the opposite (e.g., Shaw and Huddleston 1991, Austin and Dangerfield 1993, Whitfield et al. 2002). All verified host data (information only available for *Microplitis* and *Snellenius*) show that this group only parasitizes the most apomorphic groups of Lepidoptera, Noctuoidea and Bombycoidea (e.g., host data compiled in Fernandez-Triana et al. 2015, Yu et al. 2016). The wasp larvae are mostly haemolymph feeders and their cocoons are very specialized (e.g., Shaw and Huddleston 1991, Quicke et al. 2004), both characters being considered as derived as compared to the presumably plesiomorphic Apantelini and Cotesiini (sensu Mason 1981). More research will be needed to clarify the position of Microplitini within the subfamily.

Four other new genera being described in this paper (*Agupta*, *Kotenkosius*, *Ohenri* and *Ypsilonigaster*) are presumably related to *Choeras* s.l. It has long been proposed that *Choeras* represents a paraphyletic assemble (e.g., Williams 1988), or may even be polyphyletic (e.g., Austin and Dangerfield 1992), although the limits of that genus and related ones are not well understood at present. Based on the described species (and also unpublished data from collections), *Choeras* should probably be redefined, in a stricter sense, to include mainly Holartic taxa. The tropical species of *Choeras* s.l. are very numerous and seem to represent several lineages; they are probably better placed within other genera (including the new taxa described above as well as additional new genera to be proposed in the future).

Three new genera (*Jimwhitfieldius*, *Markshawius*, and *Ungunicus*) clearly belong to what Mason (1981) named as Cotesini –although that tribe is clearly not monophyletic. *Ungunicus* is likely related to *Diolcogaster*, whereas the relationships of *Jimwhitfieldius* and *Markshawius* within Cotesiini are more difficult to assess. The three genera all have rather unique morphological features within the entire Microgastrinae (such as very large metatibial spines and unique trochantellus shape in *Jimwhitfieldius*, propodeum carination pattern and shape of T1 and T2 in *Markshawius*, and very unique tarsal claws in *Ungunicus*).

The new genus *Austinicotesia* is clearly related to *Austrocotesia*, as both lack vein 2r-m in the hind wing, a feature very rarely found in Microgastrinae (the only other genus to lack that vein is *Miropotes*) (e.g., see Austin and Dangerfield 1992). Although there are some morphological features that seem to indicate they are different (see above under description of *Austinicotesia*), additional studies, especially further analysis of molecular data, might change in the future our current understanding of those two genera.

The genus *Janhalacaste* is clearly related to *Mariapanteles*, and to a lesser extent to *Pseudapanteles*. These three genera comprise mostly Neotropical taxa, with only a few species of *Pseudapanteles* reaching the Neartic (Fernandez-Triana et al. 2014b and unpublished data).

Four of the newly described genera (*Billmasonius*, *Carlmuesebecki*, *Qrocodiledundee* and *Zachterbergius*) are difficult to relate at present with any known group of Micro-

gastrinae. Their rather bizarre morphological features, and the fact that the available barcoding sequences are very different from all other microgastrines with available barcodes in BOLD, prevent us to even propose preliminary relationships with any other genus within the subfamily.

Although an updated and more comprehensive phylogeny of Microgastrinae is probably years ahead, we hope the present paper contributes toward that goal by describing a significant number of new taxa and making them available for future studies.

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Description of four new species of *Eadya* (Hymenoptera, Braconidae), parasitoids of the *Eucalyptus* Tortoise Beetle (*Paropsis charybdis*) and other *Eucalyptus* defoliating leaf beetles

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Abstract

Eucalyptus L'Héritier, 1789 (Myrtales: Myrtaceae) plantations are a global economic resource with a wide array of uses. As this forestry crop grows in popularity around the world, the exotic introduction of pests such as the leaf beetles belonging to the genera *Paropsis* Oliver, 1807 and *Paropsisterna* Motschulsky, 1860 increases in frequency. These pest introductions have spurred a need to understand the natural enemies of these pests for use in classical biological control programs. One such enemy, *Eadya paropsidis* Huddleston & Short, 1978 (Hymenoptera: Braconidae), has shown potential as a biological control agent against *Paropsis charybdis*, an exotic pest of New Zealand *Eucalyptus* plantations. However, observations made by biocontrol researchers have raised concerns that *E. paropsidis* is a complex of cryptic species. A comprehensive large-scale phylogenetic study utilizing both host and molecular data (Peixoto et al. 2018), as well as a morphological multivariate ratio analysis, was utilized to ensure accurate delimitation of the species of *Eadya*. Here we formally describe the three new species (*Eadya annleckieae* Ridenbaugh, 2018, **sp. n.**, *Eadya daenerys* Ridenbaugh, 2018, **sp. n.**, *Eadya spitzer* Ridenbaugh, 2018, **sp. n.**), and one additional new species discovered in the Australian National Insect Collection (*Eadya duncan* Ridenbaugh, 2018, **sp. n.**). All distributions and host associations for *Eadya* are listed as well as a redescription of the originally described *E. paropsidis* and *E. falcata*. An illustrated key to all known species is included to assist biological control researchers. The value of citizen science observations is discussed, along with the need for a further understanding of mainland *Eadya* populations given the recent spread of paropsine pests. Finally, we discuss the subfamilial placement of *Eadya*, and suggest it belongs within Euphorinae based on morphological characters.

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Keywords
Parasitoid wasps, Taxonomy, DNA Barcoding, Morphometrics, Biological Control, Tasmania, New South Wales, Victoria, Euphorinae, Eucalyptus

Introduction

Although native to Australia, the cultivation of production and trade of goods derived from *Eucalyptus* L'Héritier, 1789 (Myrtales: Myrtaceae) is a massive global industry. The largest subdivision of this industry is the *Eucalyptus* oil market (Coppen 2003). *Eucalyptus* oil is a coveted aromatic/medicinal product with major producers in Australia, Brazil, Chile, China, India, Portugal, Spain, and South Africa (Coppen 2003). Between 1991 and 2000, China alone exported 32,244 tons of *Eucalyptus* oil, valued at $108 million USD (Coppen 2003). *Eucalyptus* is also one of the most important sources of commercial cellulose fiber for Asia, the Mediterranean, southern Africa, and South America (Paine et al. 2011). In North America, *Eucalyptus* is most often cultivated for use as ornamental plants (Paine et al. 2011), but has also been evaluated in the southern United States as a potential source of energy (Gonzalez et al. 2011).

Species of *Paropsis* Oliver, 1807 and *Paropsisterna* Motschulsky, 1860 are endemic Australian leaf-beetles (Coleoptera: Chrysomelidae: Chrysomelinae) that feed upon the leaves and shoots of *Eucalyptus*. These beetles have been known to cause serious damage to *Eucalyptus* plantations both within (de Little 1989; Nahrung 2004) and outside (Millar et al. 2009; Lin et al. 2017) of their native Australian range. Invasive paropsine beetles have recently become established in New Zealand (Rogan 2016), Ireland (Reid and de Little 2013), California (von Ellenrieder 2003), and South Carolina (Clemson University Extension 2012). Continued global expansion of the *Eucalyptus* industry will likely result in further incursions of invasive paropsine beetles, necessitating an understanding of their native natural enemies that could be utilized in classical biological control. The suite of predators and parasitoids that attack paropsine beetles in Australia is not well known. Additionally, the taxonomy of the beetles themselves has been in flux (Peixoto et al. 2018), with the most recent revision based solely on morphological characters (Reid 2006). Further revisions are needed using molecular characters to understand the identity and origin of the beetles themselves.

Larval endoparasitic wasps in the genus *Eadya* Huddleston & Short, 1978 (Hymenoptera: Braconidae) have great potential as biocontrol agents for invasive paropsines. Classical biological control studies have begun for *Eadya* from Tasmania to control the Eucalyptus Tortoise Beetle, *Paropsis charybdis* Stål, 1860 (Withers et al. 2012; Withers et al. 2013; Peixoto et al. 2018), a defoliating pest of *Eucalyptus nitens* (Deane & Maiden, 1899) plantations. The presence of possible cryptic species of *Eadya* spurred a large-scale molecular phylogenetic study on Tasmanian species of *Eadya* (Peixoto et al. 2018). This comprehensive study, a collaboration between biocontrol researchers and taxonomists, utilized a combination of molecular and host data taken from multiple locations over six years to reveal three new species of *Eadya* (*Eadya annleckieae* Ridenbaugh, sp. n.,

Eadya daenerys Ridenbaugh, sp. n., *Eadya spitzer* Ridenbaugh, sp. n.). *Eadya daenerys* sp. n. (referred to as Eadya sp.3 in Peixoto et al. 2018), is now the focus for importation into New Zealand to control *P. charybdis*.

In this paper, we formally describe these three new species discovered from Peixoto et al. (2018) using all available data, including newly collected morphological data. *Eadya paropsidis* and *E. daenerys* sp. n. are the two cryptic species that spurred the molecular phylogenetic paper of Peixoto et al. (2018). We redescribe *E. paropsidis* and use a multivariate ratio analysis to ensure these species can be accurately diagnosed. A fourth new species, *E. duncan* sp. n. was discovered from the Australian National Insect Collection (ANIC) and is also described using morphology. All known host records for all species of *Eadya* are listed so these records are available in the event of further paropsine introductions around the world. Furthermore, a well-illustrated key to *E. paropsidis* and all new and known species is provided to facilitate identification by applied researchers along with a discussion of the potential for species of *Eadya* as biological control agents. Finally, based on morphology, we suggest that *Eadya* belongs within Euphorinae, as originally placed by Huddleston and Short (1978) and not Helconinae as recovered in a one gene molecular analysis (Belshaw and Quicke 2002).

Methods

We utilized material collected from Peixoto et al. (2018), and additional museum specimens. Type specimens were deposited in the following institutions: the Australian National Insect Collection (ANIC), the American Entomological Institute (A.E.I.), and the University of Central Florida Collection of Arthropods (UCFC). All material examined and locations of deposition are listed in Suppl. material 1. Depositions of holotypes and paratypes are also listed in the descriptions, in brackets, under Type material. Terminology for morphology follows that of Sharkey and Wharton (1997) and the Hymenoptera Anatomy Ontology project (Yoder et al. 2010), while terminology for sculpture follows that of Harris (1979).

A molecular diagnostic key was created using the barcoding region (Hebert et al. 2003) of *Cytochrome c oxidase subunit 1* (*COI*) sequences obtained from Peixoto et al. (2018) under GenBank accession numbers KX99052–KX990220, and MH107809–MH107817. Sequences were translated and hand aligned in Bioedit v.7.1.3 (Hall 1999). As there were no indels in the sequence, alignment was achieved using the reading frame as a guide. Diagnostic molecular characters are listed with reference to their amino acid position on the complete *COI* reference gene of *Apis mellifera mellifera* Linnaeus, 1758 (GenBank ref AHY80993.1). Positions are listed in parenthesis followed by the corresponding diagnostic molecular characters. Species that are polymorphic at these codon sites have all observed amino acids for a given position listed in brackets.

Photographs were taken using a Canon 7D Mark II with the following lenses: MP-E 65mm 1–5× Canon macro lens, and a M Plan Apo 10× Mitutoyo objective mounted onto the EF Telephoto 70–200mm Canon zoom lens. For lighting, the MT-24EX Macro Twin Lite Canon Flash was used in conjunction with a custom made

Table 1. Abbreviations and definitions of the 8 morphological characters used for the morphometrics analysis of *Eadya paropsidis* and *Eadya daenerys*.

Abbreviation	Character name	Definition	Magnification (*E. paropsidis*)	Magnification (*E. daenerys*)
LOL	Lateral Ocellar Line	The shortest distance between the median and lateral ocellus, dorsal view (Fig. 16B)	100×	100×
OOL	Ocular Ocellar Line	The shortest distance between the lateral ocellus and the eye, dorsal view (Fig. 16B)	100×	100×
POL	Posterior Ocellar Line	The shortest distance between the lateral ocelli, dorsal view (Fig. 16B)	100x	100×
oci.l	Occipital Ocellar Line	The shortest distance from the posterior edge of the lateral ocellus at a 90° angle to the occipital carinae, dorsal view (Fig. 16B)	100×	100×
gsp.l	Genal Space	Length of the genal space taken midway between the dorsal and ventral margins of the eye from the posterior edge at a 90° angle to the occipital carinae, lateral view (see Fig. 2D, Zhang et al. 2017)	100×	100×
mlr.l	Malar Space	Length of the malar space taken from the posterior margin of the eye to the base of the mandible, anterior view (Fig. 16A)	100×	100×
hea.b	Head breadth	Greatest breadth of head, dorsal view (see Fig. 2B, Zhang et al. 2017)	50×	50×
mt1.b	Metasomal tergite 1 breadth	Greatest breadth of metasomal tergite 1 at the posterior margin, dorsal view (see Fig. 2F, Zhang et al. 2017)	50×	100×

diffuser. Multiple images were taken of each specimen and compiled into a single image using Zerene Stacker 1.04 (Zerene Systems LLC.). Scale bars were added using ImageJ 1.51 (Schneider et al. 2012). Images were edited using Adobe Photoshop Creative Cloud and Adobe Lightroom Creative Cloud (Adobe Systems Inc.). Figures were prepared using Adobe Illustrator Creative Cloud (Adobe Systems Inc.).

Of the four species supported by the molecular data presented in Peixoto et al. (2018), *E. paropsidis* and *E. daenerys* sp. n. were examined using a morphometric multivariate ratio analysis due to their cryptic morphology. For this study, species were grouped based on molecular operational taxonomic units (MOTUs) in accordance with the results of Peixoto et al. (2018). To test the validity of the MOTUs, a series of shape principal component analyses (PCAs) were performed to determine if variation was due to shape or allometric in nature. A shape PCA analysis was chosen to avoid bias towards one group or another, as an assignment to species was not required (László et al. 2013). A series of 20 female specimens, eight *E. paropsidis* and 16 *E. daenerys* sp. n. were selected based upon the number of female specimens available and the condition of those specimens (see Suppl. material 1). Female specimens were used exclusively as most type specimens are female, and to eliminate any variation that may be attributed to sexual dimorphism.

The characters evaluated in this study were as follows: Lateral ocellar line (LOL), ocular ocellar line (OOL), posterior ocellar line (POL), occipital ocellar line (oci.l), genal

space (gsp.l), malar space (mlr.l), head breadth (hea.b), and metasomal tergite 1 breadth (mt1.b). The definition of these characters and how they were measured can be found in Table 1 and are depicted in Fig. 16. For application of the PCA ratio spectrum, characters furthest from each other show the most variation and are ideal for diagnosing species, whereas those closest together account for very little variation and should be avoided (Baur and Leuenberger 2011; László et al. 2013). The allometry ratio spectrum can be applied in a similar manner, with characters closer together being favored as they are less allometric (Baur and Leuenberger 2011; László et al. 2013). Character measurements were recorded as the average of three measurements taken using a Nikon SNZ18 stereomicroscope with an ocular micrometer. The morphometrical analysis (Baur and Leuenberger 2011) was applied in R (R Core Team 2016) as outlined in Baur et al. (2014) using code modified by Zhang et al. (2017). The data and R script files for this analysis can be obtained from figshare (https://figshare.com, DOI: 10.6084/m9.figshare.6022259).

The host, *Paropsisterna variicollis** (Chapuis, 1877) is listed with an asterisk within descriptions due to the uncertainty surrounding the taxonomic validity of this species with respect to *Pst. obovata* (Chapuis, 1877) and *Pst. cloelia* (Stål, 1860). For a detailed discussion on the taxonomic uncertainty of this species, see Peixoto et al. (2018).

Results

Morphometrics analysis

Separating most species of *Eadya* was relatively straightforward using morphological characters (see Key to Species of *Eadya* below). However, *E. paropsidis* and *E. daenerys* sp. n. presented only size differences morphologically, with the latter species being smaller, even though they were well supported phylogenetic species based on molecular data (Peixoto et al. 2018). To examine if there were any usable morphological characters to discriminate these species, we performed a multivariate ratio analysis. The first and second shape PC were the only ones that were informative, accounting for 83.9% of the variation observed (Fig. 1A). From these two shape PCAs separation of the species was recovered from the first principal component, but not the second. Isometric size, defined by Baur and Leuenberger (2011) as the geometric mean of all body measurements, was plotted against the first principal component (Fig. 1B). A correlation between shape and size was observed, indicating that the differences in measured ratios between the two species are due to size and not shape (Fig. 1B).

A PCA and allometry ratio spectrum were generated to determine which characters were the best for delimiting the two cryptic species. The most discerning ratios according to the first principal component were *LOL:mlr.l*, *LOL:mt1.b*, and *LOL:gsp.l* (Fig. 1C). According to the allometry ratio spectrum, the ratios *LOL:gsp.l*, *LOL:mlr.l*, and *LOL:mt1.b* were the most allometric between the two groups (Fig. 1D). As the characters corresponding to the separation of these species were also the characters displaying the greatest degree of allometric variation, the variation between these species is due primarily to differences in size and not shape (László et al. 2013).

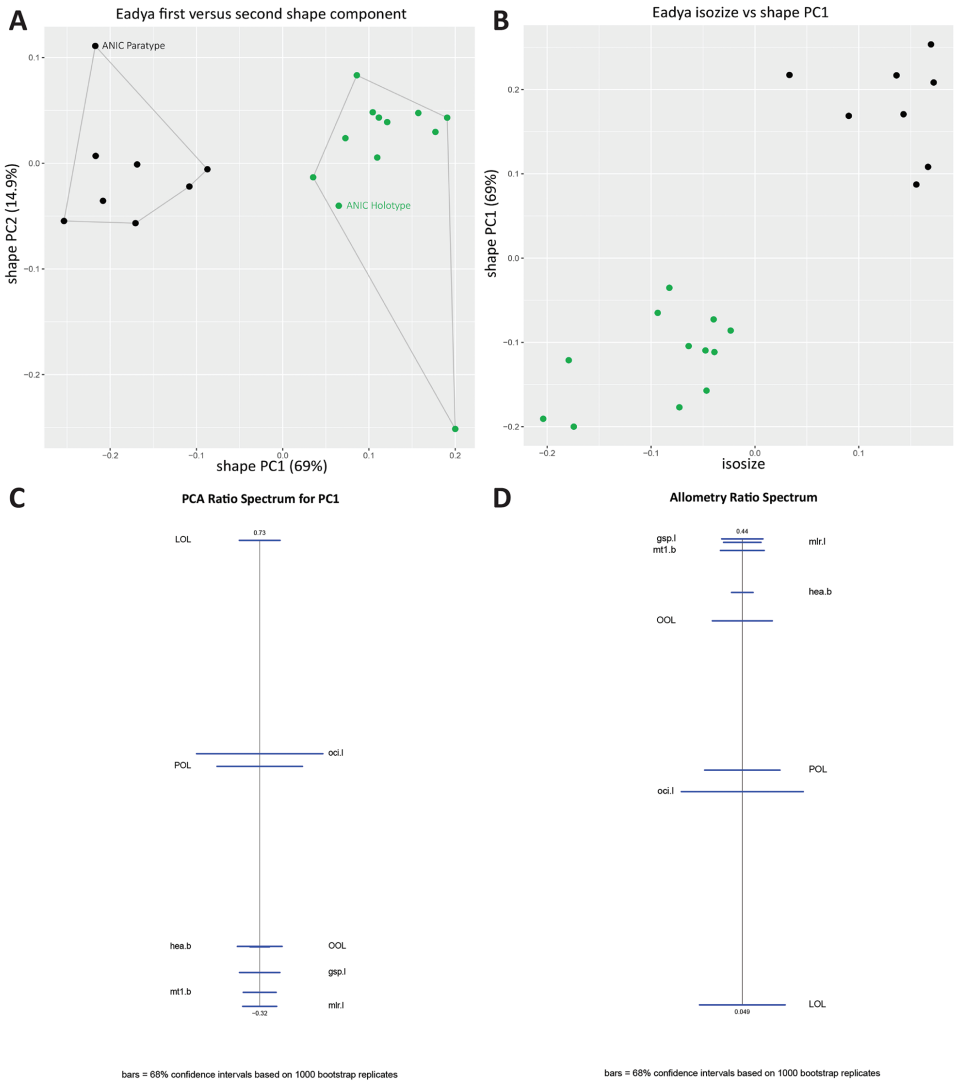

Figure 1. Multivariate morphometric ratio analysis of female specimens of *Eadya paropsidis*, and *Eadya daenerys* Ridenbaugh, sp. n. **A** Scatterplot of the first shape principal component plotted against the second shape principal component. Black - *Eadya paropsidis*, Green - *Eadya daenerys* sp. n. **B** Scatterplot of isosize plotted against the first shape principal component. Black - *Eadya paropsidis*, Green - *Eadya daenerys* sp. n. **C** Ratio spectrum for the first principal component with horizontal bars representing 68% confidence based on 1000 bootstrap replicates **D** Allometry ratio spectrum with horizontal bars representing 68% confidence based on 1000 bootstrap replicates.

When applied to *E. paropsidis* and *E. daenerys* sp. n., the morphometrical analysis only supported one species, contrasting with the results of Peixoto et al. (2018). These results indicate that the two species are truly cryptic, as the molecular and ecological data strongly supported the separation of these two species (Peixoto et al. 2018). With

this in mind, the four new species of *Eadya* are formally described using morphological and molecular characters, while purposely avoiding ratios to account for the allometric variation observed between *E. paropsidis* and *E. daenerys* sp. n.

Taxonomic descriptions

Eadya can be recognized from other braconid genera by the following combination of characteristics: head large, subcubic and as wide as thorax, clypeus flat, labrum flat, interantennal carina present; forewing with r-m crossvein present, 3RSb curved and meeting R1a before apex of wing, and 2cu-a absent; metasoma petiolate.

Eadya annleckieae **Ridenbaugh, sp. n.**

http://zoobank.org/150ABAF3-37F2-405C-A86B-5768BEF6D68A
Figs 2A–C; 3A–E

Diagnosis. *Eadya annleckieae* sp. n. can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged across ventral margin, without medial tubercles (Fig. 3A); frons with weak inter-antennal carinae and lateral carina with a faint elevated ridge wrapping around the antennal socket (Fig. 3A, B); occipital carina simple (Fig. 3B); occiput normal; notaulus wide and rugulose (Fig. 3C); scutellar sulcus divided into two distinct foveae with rugulose sculpturing along the posterior margins (Fig. 3C); sternaulus rugulose (Fig. 3D); propodeum rounded in appearance from lateral angle, without transverse carinae (Fig. 3E), and not creating a distinct posterior face when viewed laterally; propodeal spiracle circular; head black except for mandible orange with base black and apex ferruginous, maxillary and labial palp orange (Figs 2A; 3A), antenna dark brown (Figs 3C); pronotum black (Figs 2B; 3B); propleuron black (Fig. 3D); hindwing hyaline with dark brown veins (Fig. 2C); legs orange except for hind tibia dark orange with apex black (Fig. 2A); amino acid sequence (112–118) LRRLTNI (Fig. 15).

Description. Female. Body length 6.46mm. Ovipositor length 1.72mm.

Color. Head black except for mandible orange with base black and apex ferruginous, maxillary and labial palp orange, and antenna dark brown (Figs 2A; 3A, C); prothorax black (Fig. 2A); mesoscutum black (Fig. 2B); mesopleuron black with the dorsal posterior margin orange (Fig. 3D); scutellum black except for the posterior margin directly behind the scutellar sulcus orange (Fig. 2B); sternum black; metathorax orange (Fig. 2A); forewing and hindwing hyaline with dark brown veins (Fig. 2C); legs orange except for hind tibia dark orange with apex black (Fig. 2A, B); abdomen orange except for ovipositor sheath brown (Fig. 2A, B).

Head. Clypeus simple, punctate and pubescent, flanged across ventral margin, without medial tubercles (Fig. 3A); mandibles overlapping, dorsal and ventral teeth of equal length (Fig. 3A); face densely punctate, pubescent (Fig. 3A); frons rugulose, with

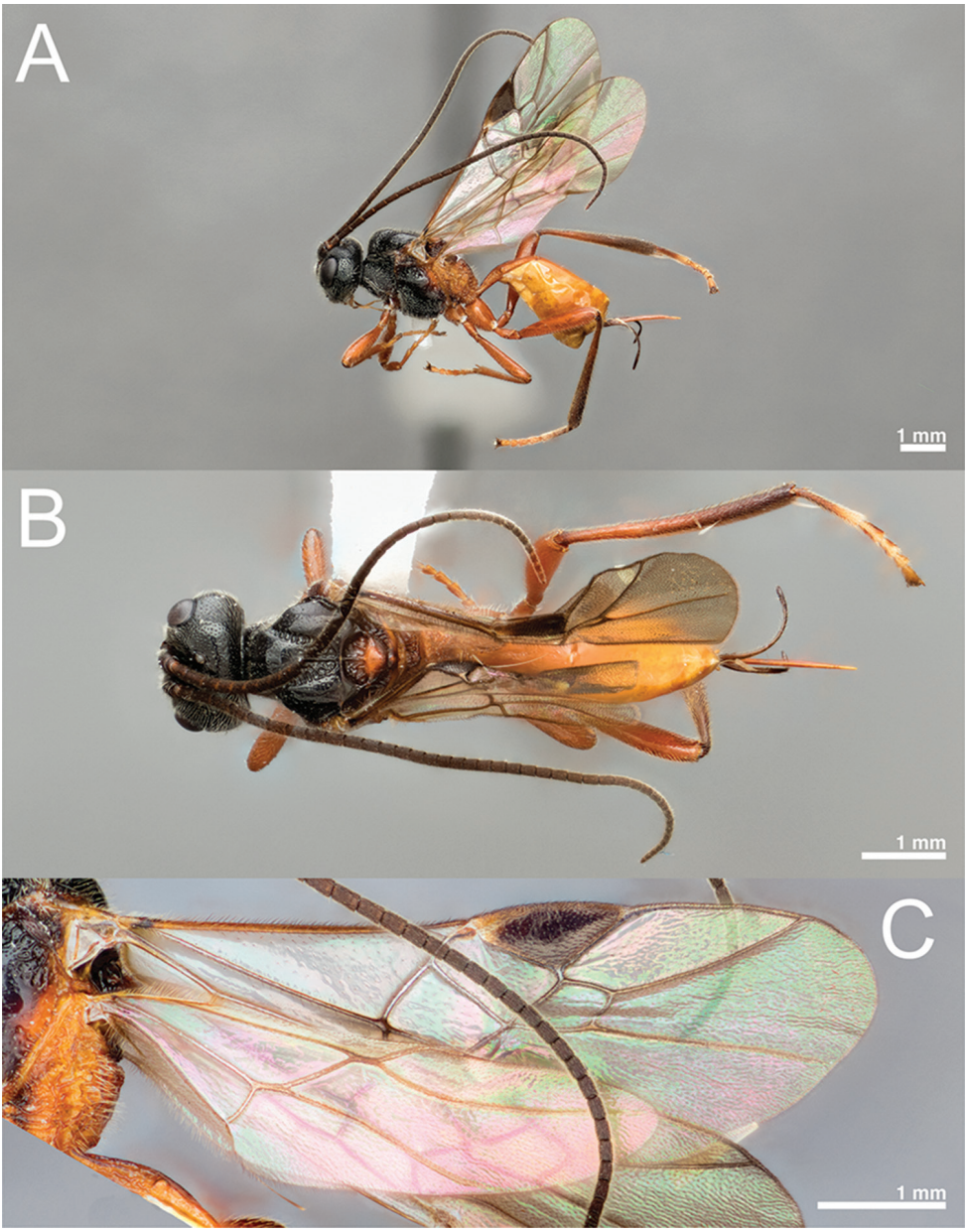

Figure 2. *Eadya annleckieae* Ridenbaugh, sp. n. holotype. **A** Lateral habitus **B** Dorsal habitus **C** Fore and hindwing. All scale bars are 1 mm in length.

a weak inter-antennal carinae and with lateral carinae with a faint elevated ridge wrapping around the antennal socket (Fig. 3A, B); vertex punctate and pubescent (Fig. 3B); occipital carina simple (Fig. 3B), reaching the hypostomal carina; hypostomal carina simple, not strongly flanged, meeting the mandible at the mandibular condyle; occiput smooth, normal.

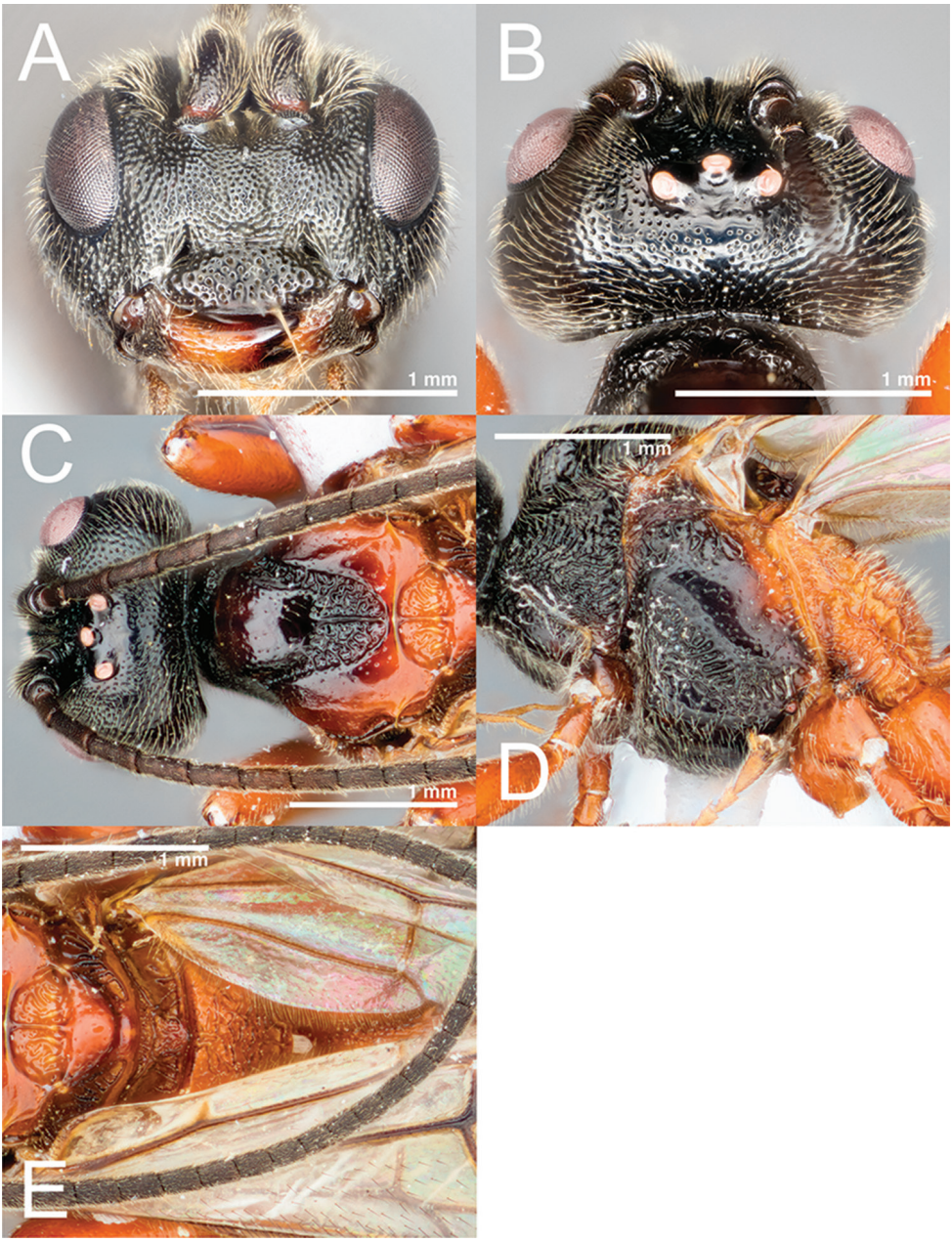

Figure 3. *Eadya annleckieae* Ridenbaugh, sp. n. holotype. **A** Head, frontal view **B** Head, dorsal view **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view. All scale bars are 1 mm in length.

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronobe present, covered in rugulose sculpturing (Fig. 3C, D); mesoscutum with posterior half of median mesonotal lobe rugulose, a distinct longitudinal carinae extending from the posterior margin to about the middle of the lobe (Figs 2B; 3C); notaulus wide and rugulose

(Figs 2B; 3C); scutellar sulcus divided into two distinct foveae with rugulose sculpturing along the posterior margins (Figs 2B; 3E); sternaulus rugulose (Fig. 3D); propodeum rugose, covered in setae but not pubescent, rounded in appearance from lateral angle, without transverse carina and not creating a distinct posterior face when viewed laterally (Figs 2A; 3D, E); propodeal spiracle circular; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent (Fig. 2A, B); tarsal claws simple.

Forewing. r-m sinuous (Fig. 2C).

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal surface smooth, lateral surface punctate with associated setae; ovipositor straight (Fig. 2A).

Male. Same as female.

Host. *Paropsisterna nobilitata* (Erichson, 1842), *Paropsisterna variicollis** , *Paropsisterna selmani* Reid & de Little, 2013, *Paropsis charybdis*.

Variations. Paratype with propleuron black except for lateral posterior margin orange; mesoscutum orange except for the median mesonotal lobe black with the anterior margin and lateral mesonotal lobes ferrunginous (Fig. 3C); mesopleuron orange except for the sternaulus and ventral margins black; scutellum orange (Fig. 3C, E); legs orange except for apex of hind tibia black and hind tarsus with tarsomere 1 yellow and white at apex, tarsomeres 2–4 white, and tarsomere 5 yellow; abdomen orange except for lateral margins of metasomal sternites 3–6 brown, the second and third to last metasomal tergites with two light brown spots near the anterior margin. Some of this variation may be the result of the DNA extraction process.

Diagnostic molecular characters. Amino acid positions (22–27) MWAGIL; (32–34) SII; (41–46) SRGSLL; (54) R; (67–73) MVMPVIM; (81) I; (90) I; (95–98) MNNM; (104–109) LPSLFI; (112–118) LRRLTNI; (126) I; (133–139) GGRHSGV; (143–144) VA; (150) I; (157) [I or K]; (167–169) FNM; (172–191) NGIAVDRVTLFRWSVKITAF (Fig. 13).

Distribution. Tasmania.

Etymology. This species is named in honor of the science fiction author, Ann Leckie by the second author (EB).

Remarks. This species is referred to as Eadya sp.1 in Peixoto et al. (2018). The UCFC paratype is in poor shape due to the DNA extraction process. The flange of the inter-antennal carinae is difficult to see in the images (Fig. 3A, B), but is clear when viewing the specimens, provided the antennae are separated enough.

Type material. Holotype, Female (ANIC), "Ellendale, TAS, Female, 21a, 10 Dec 2014, D. Satchell". Paratype, Female (ANIC), "Moina, TAS, S41°29.5' E152°04.7', *Paropsis charybdis* sentinel, Emerged 2 Jan 2013, G.R. Allen, E127", "DNA voucher BJS196", GenBank accession numbers KX031361, KX99032, and KX990052. Paratype, Male (UCFC), "The Lea, TAS, #12, *Eadya paropsidis* cocoon (brown). Emerged from *Pst. variicollis**, 4 Dec 2014, UCFC 0 567 827", "DNA voucher BJS501", GenBank accession number KX990216.

***Eadya daenerys* Ridenbaugh, 2018, sp. n.**
http://zoobank.org/38860F10-4E44-4C6A-A396-51364FB71F09
Figs 4A–C; 5A–F

Diagnosis. *Eadya daenerys* sp. n. can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged along ventral margin, with two medial tubercles projecting outward (Fig. 5A); frons with inter-antennal and lateral carinae flanged (Fig 5B); occipital carina simple (Fig. 5B); occiput normal; notaulus crenulate (Fig. 5C); scutellar sulcus divided into many deep pits by longitudinal carinae (Fig. 5C); sternaulus crenulate (Fig. 5D); propodeum rounded in appearance from lateral angle (Figs 4A; 5D), without transverse carina (Fig. 5E, F), and not creating a distinct posterior face when viewed laterally; propodeal spiracle circular; head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 5A, B); pronotum black except for anterior dorsal margin orange (Figs 4A, 5B); propleuron orange; hindwing infuscate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 4C); legs black (Fig. 4A, B); amino acid sequence (112–118) IRNFIGA (Fig. 15).

Description. Female. Body Length 5.77mm. Ovipositor Length 0.82mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black (Figs 4A, B; 5A, B); pronotum black except for anterior dorsal margin orange (Figs 4A, 5B); propleuron orange; mesothorax black (Figs 4A, B; 5C, D); metathorax black (Figs 4A, B; 5E, F); forewing infuscate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 4C); hindwing infuscate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 4C); legs black (Figs 4A, B); abdomen black except ovipositor orange (Fig. 4A).

Head. Clypeus simple, smooth with scattered setae, flanged at ventral margin, with two medial tubercles projecting outward (Fig. 5A); mandibles overlapping, dorsal tooth longer than ventral (Fig. 5A); face finely punctate with associated setae (Fig. 5A); frons rugose, inter-antennal and lateral carinae flanged, starting at the toruli and reaching the ocellar triangle (Fig. 5A, B); vertex smooth with scattered setae (Fig. 5B); occipital carina simple (See arrow, Fig. 5B), reaching the hypostomal carina; hypostomal carina strongly flanged, reaching the mandible and bending around to the mandibular condyle; occiput smooth, normal (Fig. 5B).

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronope absent, smooth except for a crenulate line extending laterally and rugulose sculpturing along the lateral posterior margin (Fig. 5B); mesoscutum with median mesonotal lobe smooth (Fig. 5C); notaulus crenulate (Fig. 5C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 5C); sternaulus crenulate (Fig. 5D); propodeum rugose and pubescent, rounded in appearance from lateral angle, without transverse carina and not creating a distinct posterior face when viewed laterally (Figs 4A; 5D, E, F); propodeal spiracle circular; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent (Fig. 4A, B); tarsal claws simple.

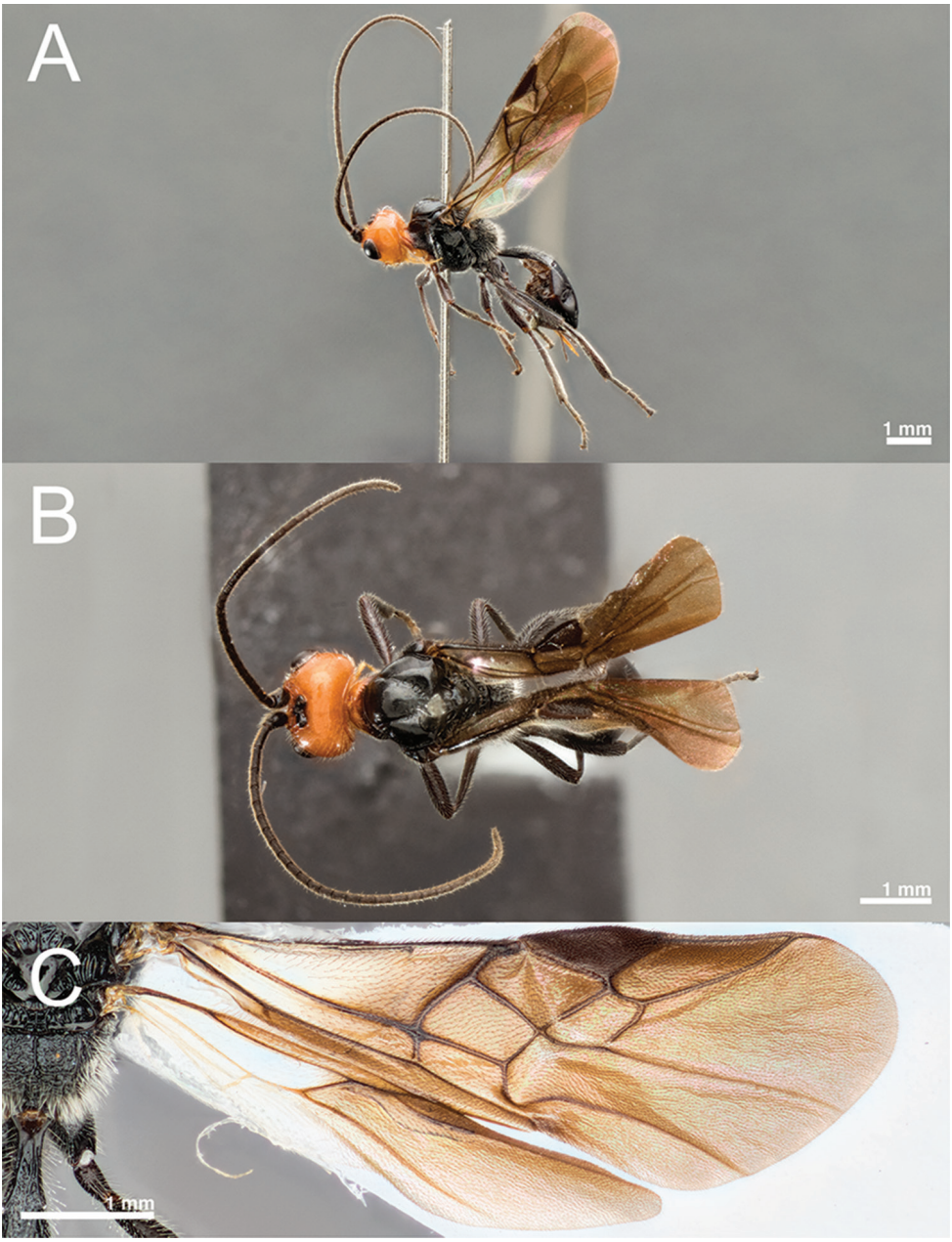

Figure 4. *Eadya daenerys* Ridenbaugh, sp. n. **A** Lateral habitus, holotype **B** Dorsal habitus, holotype **C** Fore and hindwing, paratype. All scale bars are 1mm in length.

Forewing. r-m curved slightly towards stigma before reaching the junction of 3RSa and 3RSb (Fig. 4C).

Hindwing. R1a with three hamuli.

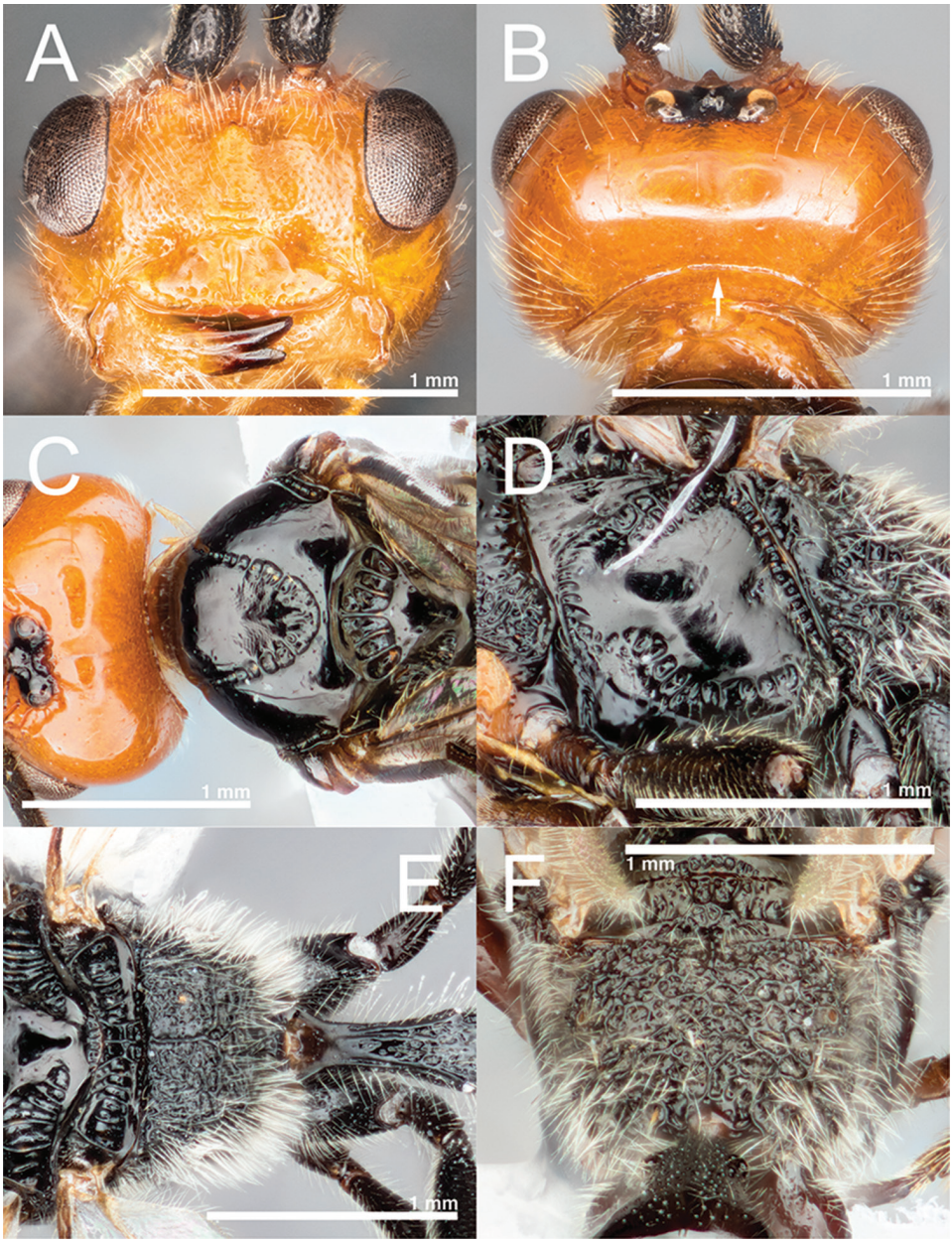

Figure 5. *Eadya daenerys* Ridenbaugh, sp. n. paratype. **A** Head, frontal view **B** Head, dorsal view, arrow indicating simple occipital carinae **C** Head and mesoscutum, dorsal view, paratype **D** Mesopleuron, lateral view, paratype **E** Propodeum, dorsal view **F** Propodeum, posterio-dorsal view. All scale bars are 1mm in length.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae (Fig. 5E); ovipositor straight (Fig. 4A).

Male. Same as female.

Host. *Paropsisterna agricola* (Chapuis, 1877), *Paropsisterna bimaculata* (Olivier, 1807), *Paropsisterna nobilitata*, *Paropsis charybdis*.

Diagnostic molecular characters. Amino acid positions (22–27) [M or R] WSGII; (32–34) RVL; (41–46) ILGRLL; (54) S; (67–73) IVIPIII; (81) I; (90) I; (95–98) INNI; (104–109) PPSL[I or V]L; (112–118) IRNFIGA; (126) I; (133–139) NLSHRGV; (143–144) [V or I]S; (150) L; (157) I; (167–169) INI; (172–191) LGLSYDNISLLVWSVNITAI (Fig. 15).

Distribution. Australian Capital Territory, New South Wales, Tasmania.

Etymology. This species is named for Daenerys Stormborn of House Targaryen, the First of Her Name, Queen of the Andals and the First Men, Protector of the Seven Kingdoms, the Mother of Dragons, Khaleesi of the Great Grass Sea, the Unburnt, the Breaker of Chains, from the literary series *A Song of Ice and Fire* by George R.R. Martin, as well as the television series *Game of Thrones* on Home Box Office (HBO). This is a noun in apposition to the generic name in order to retain integrity of the fictional character name Daenerys.

Remarks. This species is referred to as *Eadya* sp.3 in Peixoto et al. (2018).

Type material. Holotype, Female (ANIC), "Frankford, TAS, Female, 3 Dec 2001, A.D. Rice, NT#5, Pin#8". Paratype, Female (ANIC), "The Creel, Kosciusko, NSW, 8 Nov 1961, E.F. Riek, A35, Aust. Nat. Ins. Coll.". Paratype, Female (ANIC), "Canberra, ACT, 19 Nov 1958, E.F. Riek, A34, Aust. Nat. Ins. Coll.". Paratype, Female (ANIC), "Canberra, ACT, 26 Nov 1959, E.F. Riek, Aust. Nat. Ins. Coll.". Paratype, Female (ANIC), "Canberra, ACT, 26 Nov 1959, E.F. Riek, Aust. Nat. Ins. Coll.". Paratype, Male (ANIC), "Canberra, ACT, 30 Nov 1959, E.F. Riek, Aust. Nat. Ins. Coll.". Paratype, Female (ANIC), "Canberra, ACT, 18 Nov 1960, E.F. Riek, Aust. Nat. Ins. Coll.". Paratype, Male (ANIC), "Canberra, ACT, 24 Nov 1960, E.F. Riek, A35, Aust. Nat. Ins. Coll.". Paratype, Female (ANIC), Black Mt., F.C.T, 10 XI 30, W. Broce, Aust. Nat. Ins. Coll.". Paratype, Female (UCFC), "Frankford, TAS, 2 Jan 2002, Malaise Trap, A.D. Rice, MT6, UCFC 0 567 735". Paratype, Female (UCFC), "Frankford, TAS, Female, 19 Nov 2001, A.D. Rice, NT#5, Pin #5, UCFC 0 567 736". Paratype, Female (UCFC), "Frankford, TAS, Female, 3 Dec 2001, A.D. Rice, NT#5, Pin #9, UCFC 0 567 737". Paratype, Female (UCFC), "Frankford, TAS, Female, 3 Dec 2001, A.D. Rice, NT#5, Pin #10, UCFC 0 567 738". Paratype, Female (UCFC), "Frankford, TAS, Female, 19 Nov 2001, A.D. Rice NT#5, Pin #7, UCFC 0 567 741". Paratype. Female (A.E.I.). "King William Range, I. 8-23, Tasmania, A.E.I. Sep/05". Paratype, Male (A.E.I.), "Runnymede,TAS, 24 Nov 2015, 42 38'13.3"S 147 33'53.8"E, Malaise trap, G.R. Allen, Male, MTM2". Paratype, Male (A.E.I.), "Ellendale, TAS, Male, 14 Dec 2015, D. Satchell, EM2". Paratype, Male (A.E.I.), "Runnymede, TAS, 24 Nov 2015, 42 38'13.3"S 147 33'53.8"E, Malaise trap G.R. Allen, Male, MTM1". Paratype, Female (A.E.I.), "Frankford, TAS, Female, 27 Nov 2000, A.D. Rice, Em Trap #1, Pin #3".

Non-type material. See Suppl. material 1.

***Eadya duncan* Ridenbaugh, sp. n.**
http://zoobank.org/10EA7B5B-E6F6-49BA-BCBD-A1388D5B5390
Figs 6A–C, 7A–E

Diagnosis. *Eadya duncan* sp. n. can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged at ventral margin, with two medial tubercles projecting outward (Fig. 7A); frons with inter-antennal and lateral carina strongly flanged (Fig. 7B); occipital carina simple (Fig. 7B); occiput concave; notaulus narrow and impressed towards anterior margins of mesoscutum, crenulate at apex (Fig. 7C); scutellar sulcus divided into two distinct foveae with short longitudinal carinae ending before reaching anterior margin (Fig. 7C); propodeum not rounded in appearance from lateral angle (Fig. 6A), with transverse carina creating a distinct posterior face when viewed laterally; propodeal spiracle elliptical; head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 7A, B); prothorax orange (Figs 6A, 7C); hindwing infuscate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 6C); legs black except for fore coxa and trochanter orange, fore femur dark orange (Fig 6A).

Description. Male. Body length 6.37mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black (Figs 6A, B; 7A, B); prothorax orange (Figs 6A, B; 7B); mesothorax orange (Figs 6A, B; 7B, C); propodeum black except for medial posterior margin at the insertion of metasomal tergite 1 orange (Figs 6A, B; 7B); metapleuron black; forewing infuscate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 6C); hindwing infuscate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 6C); legs black except for fore coxa and trochanter orange, fore femur dark orange; abdomen black (Fig. 6A, B).

Head. Clypeus simple, smooth with scattered setae, flanged at ventral margin, with two medial tubercles projecting outward (Fig. 7A); mandibles overlapping, dorsal tooth longer than ventral (Fig. 7A); face finely punctate with associated setae (Fig. 7A); frons rugulose, inter-antennal and lateral carina strongly flanged, starting at the toruli and reaching the ocellar triangle (Fig. 7A, B); vertex smooth with scattered setae (Fig. 7B); occipital carina simple, reaching hypostomal carina (Fig. 7B); hypostomal carina strongly flanged, meeting the mandible and bending around to the mandibular condyle; occiput smooth, normal.

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronope absent, smooth except for a faint crenulate line extending laterally and rugulose sculpturing along the lateral posterior margin (Fig. 7B); mesoscutum with median mesonotal lobe smooth (Fig. 7C); notaulus impressed towards anterior margins of mesoscutum, crenulate at apex (Fig. 7C); scutellar sulcus divided into two distinct foveae with short longitudinal carinae ending before reaching anterior margin (Fig. 7C); sternaulus crenulate (Fig. 7D); propodeum rugose and pubescent, not rounded in appearance from lateral angle, with transverse carina creating a distinct posterior face (Fig. 6A); propodeal

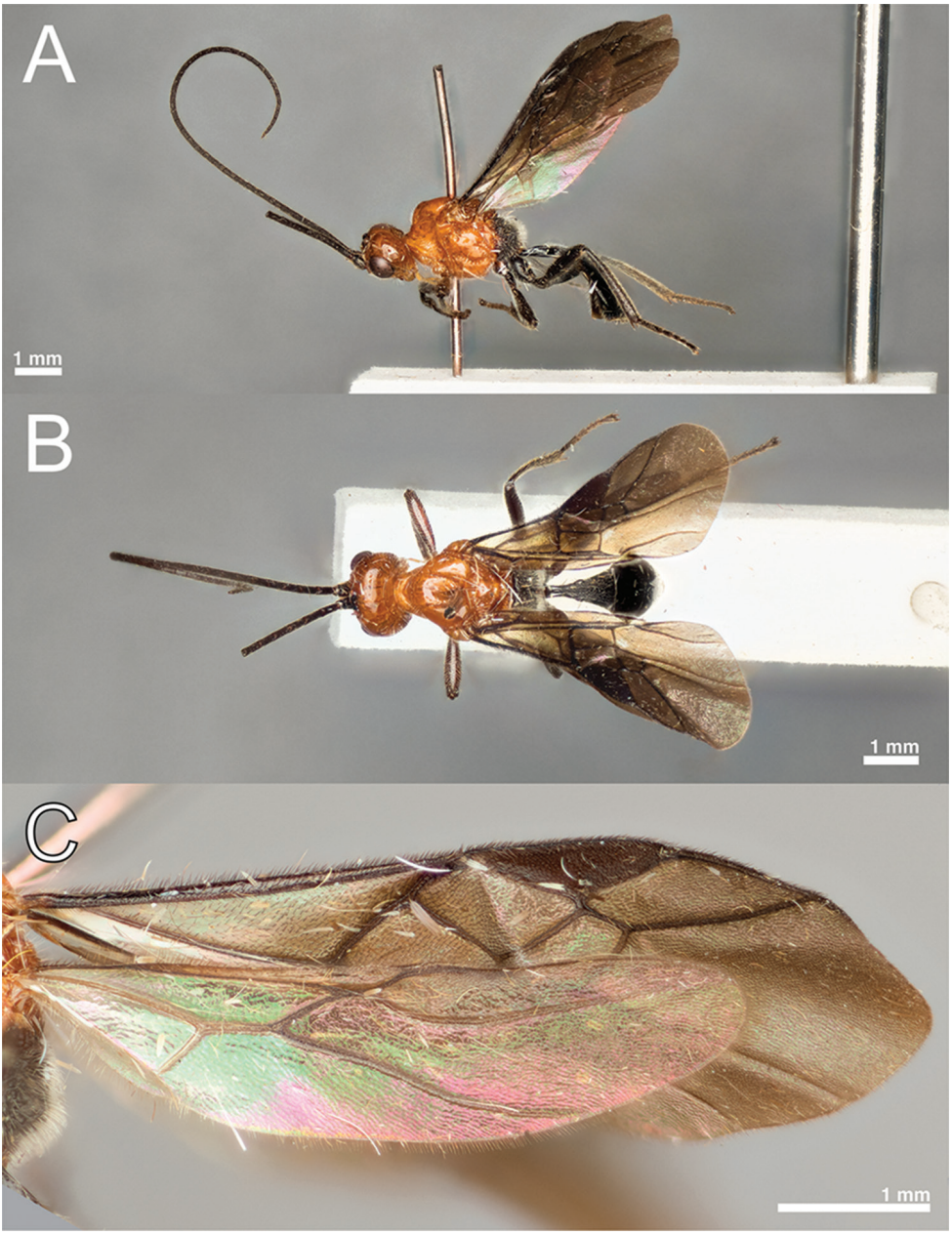

Figure 6. *Eadya duncan* Ridenbaugh, sp. n. holotype. **A** Lateral habitus **B** Dorsal habitus **C** Fore and hind wing. All scale bars are 1mm in length.

spiracle elliptical; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent (Fig. 6A, B); tarsal claws simple.

Forewing. r-m curved slightly towards stigma before reaching the junction of 3RSa and 3RSb (Fig. 6C).

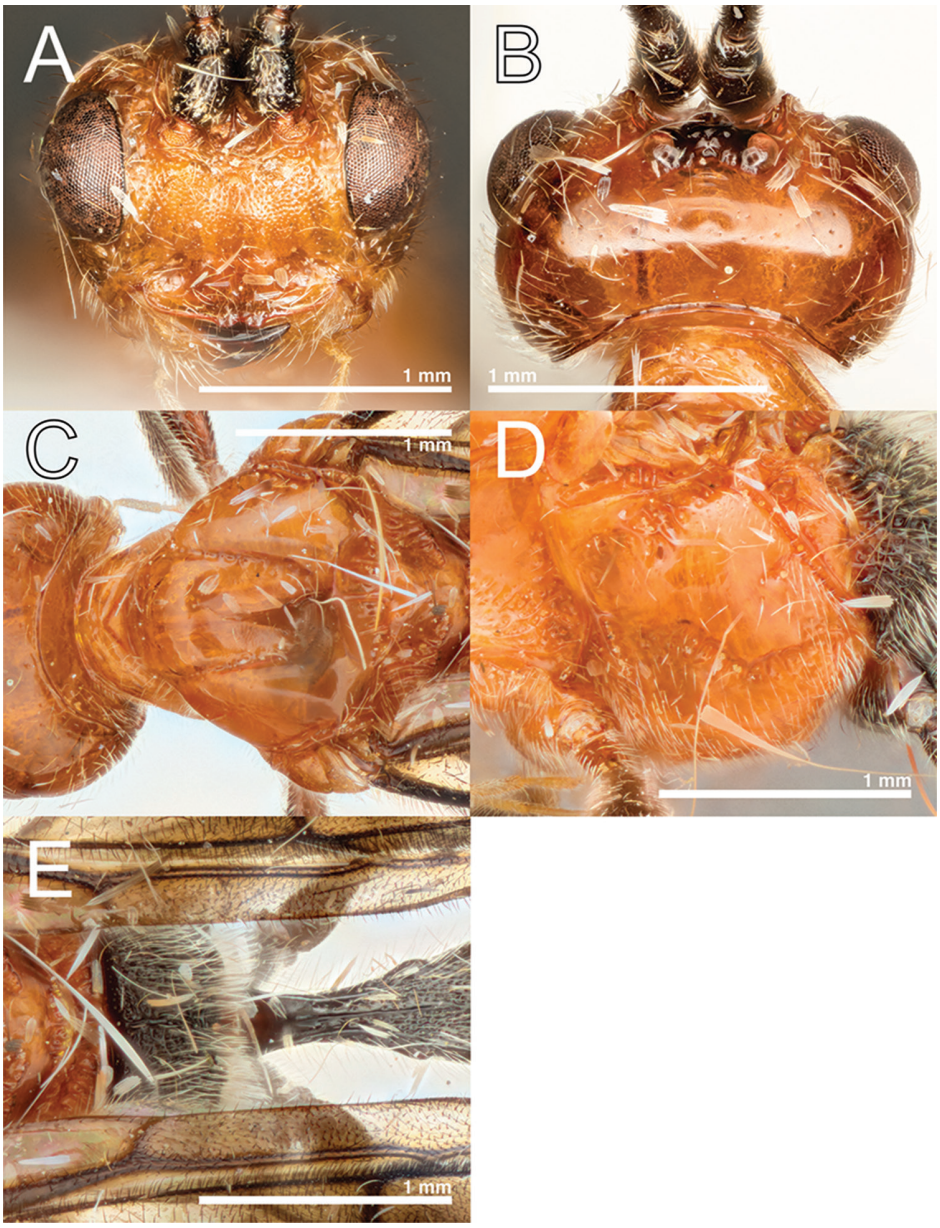

Figure 7. *Eadya duncan* Ridenbaugh, sp. n. holotype. **A** Head, frontal view **B** Head, dorsal view **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view. All scale bars are 1mm in length.

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae (Fig. 7E); ovipositor straight.

Female. Unknown.

Host. Unknown.

Distribution. New South Wales, Victoria (see discussion).

Etymology. This epithet is named in honor of the senior author's (BJS) sister in law, Julie Brant nee Duncan, who is an Australian-born beauty. This is a noun in apposition to the generic name in order to retain integrity of the surname Duncan.

Remarks. The holotype for this species was identified as a species of *Eadya* by Huddleston in 1977 and deposited at ANIC, but was not listed as material examined in the original description of *Eadya*. The flange of the inter-antennal carinae is difficult to see in the images (Fig. 7A, B), but is clear when viewing the specimen, provided the antennae are separated enough.

Type material. Holotype, Male (ANIC), "Upper Kangaroo Valley, NSW, 24 Nov 1960, E.F. Riek, A44, Aust. Nat. Ins. Coll.".

Eadya falcata Huddleston & Short, 1978

Figs 8A–C; 9A–E

Diagnosis. *Eadya falcata* can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged at ventral margin, with two medial tubercles projecting outward (Fig. 9A); frons with inter-antennal and lateral carina flanged (Fig. 9A, B); occipital carinae simple (Fig. 9B); occiput normal (Fig. 9B); notaulus impressed towards anterior margin of mesoscutum, crenulate at apex (Fig. 9C); scutellar sulcus divided into two distinct foveae with short longitudinal carina ending before reaching anterior margin (Fig. 9C, E); sternaulus crenulate (Fig. 9D); propodeum rounded in appearance from lateral angle, without transverse carina, and not creating a distinct posterior face when viewed laterally (Fig. 8A); propodeal spiracle elliptical; head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 9A, B); pronotum orange expect for lateral posterior margins black (Figs 8A; 9B, C); propleuron orange; hindwing infuscate with dark brown veins except for anal, basal, discal, and subbasal cells hyaline (Fig. 8C); legs black except for foreleg orange with femur, tibia, and tarsus black (Fig. 8A, B).

Description. Female. Body Length 5.26mm. Ovipositor Length 1.80mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black; pronotum orange expect for lateral posterior margins black (Fig. 9A, B); propleuron orange; mesothorax black (Figs 8A, B; 9C, D); metathorax black (Figs 8A, B; 9D, E); forewing infuscate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 8C); hindwing infuscate with dark brown veins except for anal, basal, discal, and subbasal cells hyaline (Fig. 8C); legs black except for foreleg orange with femur, tibia, and tarsus black (Fig. 8A, B); abdomen black except for ovipositor orange (Fig. 8A).

Head. Clypeus simple, smooth with scattered setae, flanged at ventral margin, with two medial tubercles projecting outward (Fig. 9A); mandibles overlapping, dorsal tooth longer than ventral (Fig. 9A); face finely punctate with associated setae (Fig. 9A); frons smooth, inter-antennal and lateral carina flanged, starting at the toruli and reaching

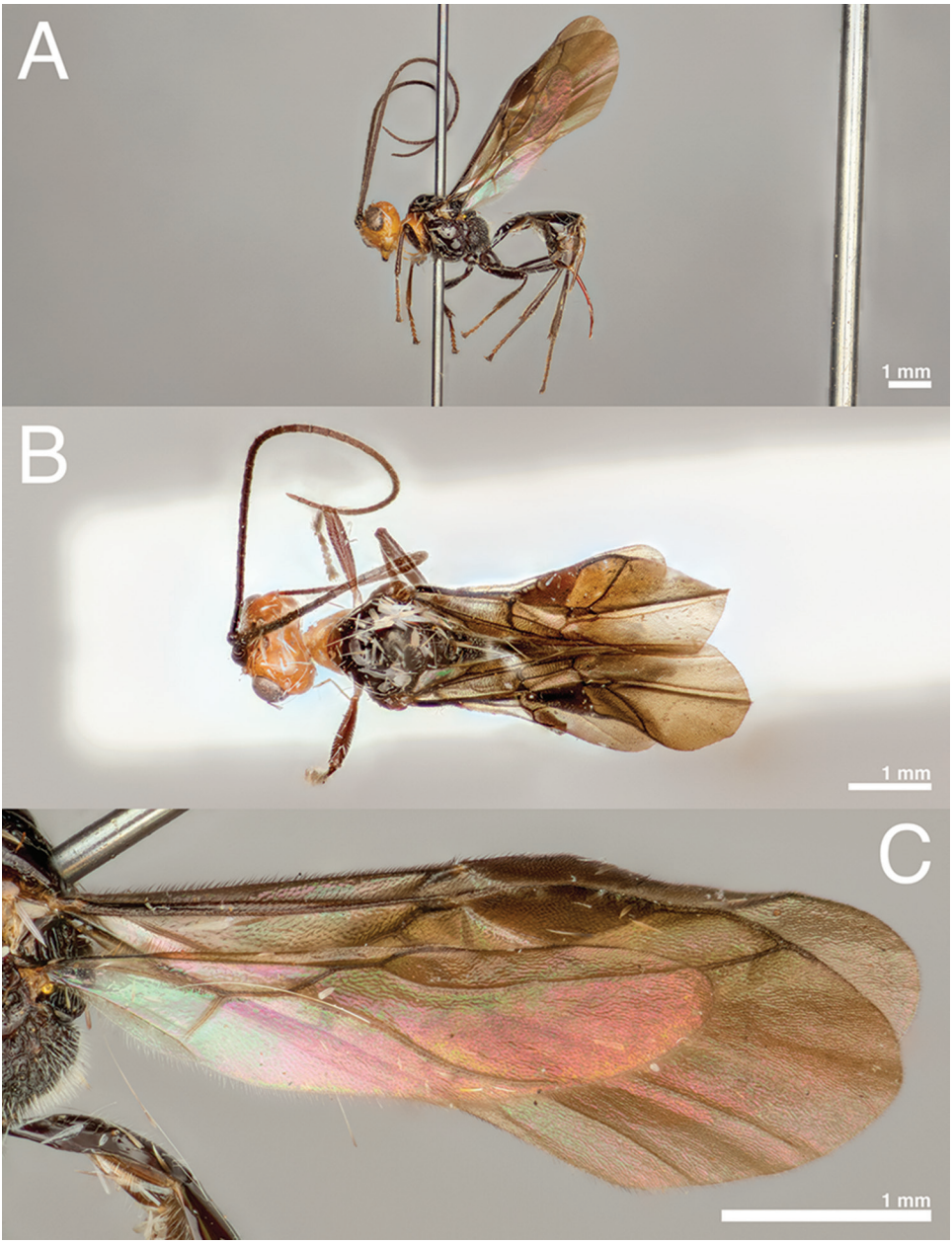

Figure 8. *Eadya falcata* holotype. **A** Lateral habitus **B** Dorsal habitus **C** Fore and hindwing. All scale bars are 1mm in length.

the ocellar triangle (Fig. 9A, B); vertex smooth with scattered setae (Fig. 9B); occipital carina simple, reaching hypostomal carina (Fig. 9B); hypostomal carina strongly flanged, meeting the mandible and bending around to the mandibular condyle; occiput smooth, normal.

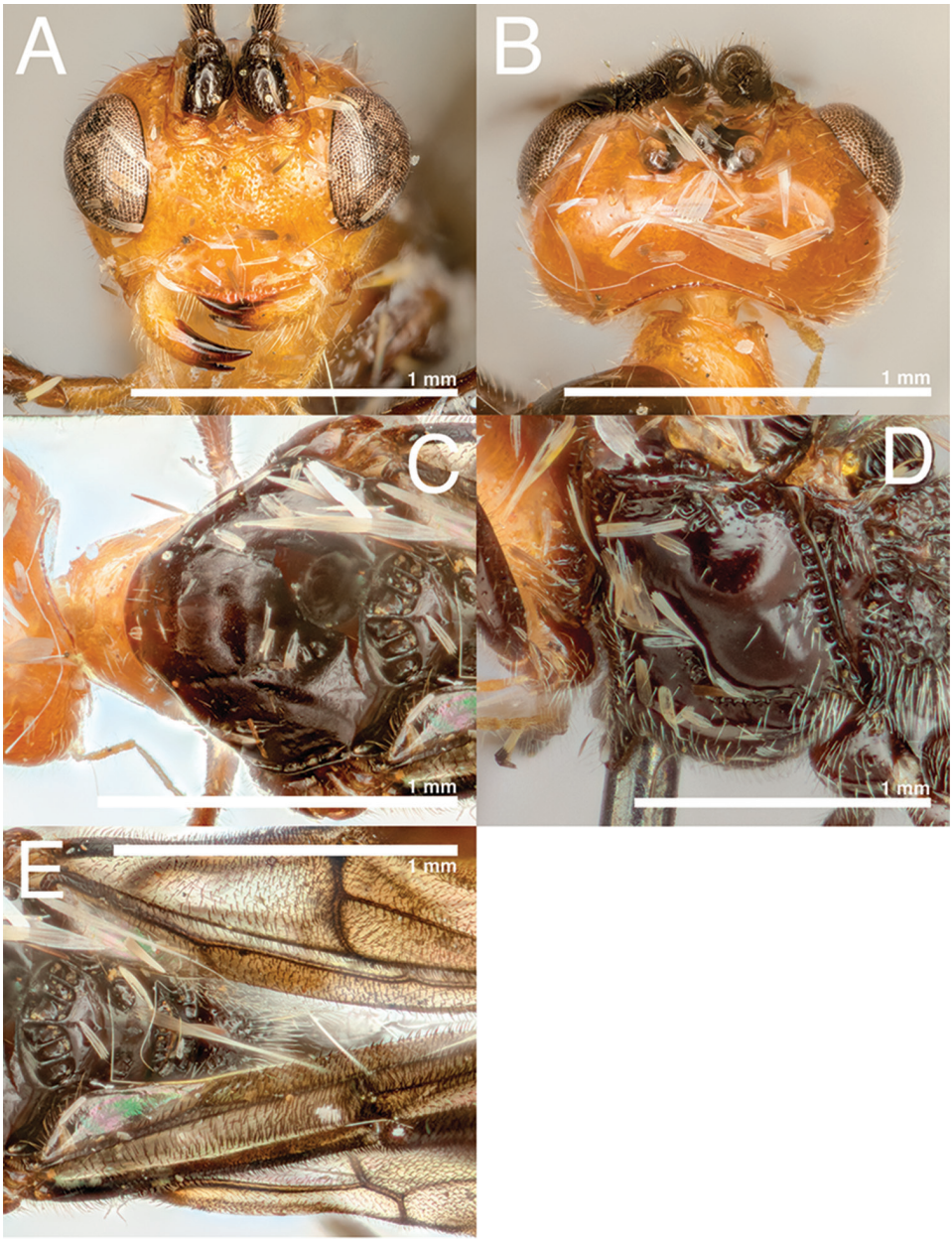

Figure 9. *Eadya falcata* holotype. **A** Head, frontal view **B** Head, dorsal view, arrow pointing to emarginate occipital carinae **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view.

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronope absent, smooth (Fig. 9B); mesoscutum with median mesonotal lobe smooth (Fig. 9C); notaulus impressed towards anterior margin of mesoscutum, crenulate at apex (Fig. 9C);

scutellar sulcus divided into two distinct foveae with short longitudinal carinae ending before reaching anterior margin (Fig. 9C, E); sternaulus crenulate (Fig. 9D); propodeum rugose and pubescent (Fig. 9E), rounded in appearance from lateral angle (Fig. 8A), without transverse carinae and not creating a distinct posterior face when viewed laterally; propodeal spiracle elliptical; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent; tarsal claws simple (Fig. 8A, B).

Forewing. r-m curved slightly towards stigma before reaching the junction of 3Rsa and 3RSb.

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae; ovipositor curved downward (Fig. 8A).

Male. Same as female.

Host. Unknown.

Variations. Paratype with foreleg coxa orange and trochanter, trochantellus, femur, tibia, and tarsus black.

Distribution. Western Australia.

Remarks. The crenulation at the apex of the notaulus is difficult to see in the holotype due to damage caused by pinning (Fig. 9C). However, this character is much better preserved in the paratype.

Type material examined. Holotype, Female (ANIC), "18 miles W. of Mogumber, WA. 13 April 1968, I.F.B Common & M.S. Upton, 039, Eadya falcata, Female, Holotype, det. T.Huddleston, 1977, Aust. Nat. Ins. Coll.". Paratype, Male (ANIC), "18 miles W. of Mogumber, WA. 13 April 1968, I.F.B Common & M.S. Upton, Eadya falcata, Male, Paratype, det. T.Huddleston, 1977, Aust. Nat. Ins. Coll.".

Eadya paropsidis Huddleston & Short, 1978

Figs 10A–C; 11A–F

Diagnosis. *Eadya paropsidis* can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged at ventral margin, with two medial tubercles projecting outward (Fig. 11A); frons with inter-antennal and lateral carina strongly flanged (Fig. 11B); occipital carina emarginate (Fig. 11B); occiput strongly concave; notaulus crenulate (Fig. 11C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 11C); sternaulus crenulate (Fig. 11D); propodeum not rounded in appearance from lateral angle (Fig. 10A), with transverse carina creating a distinct posterior face (Fig. 11E, F) when viewed laterally; propodeal spiracle circular; head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 11A, B); pronotum orange except for lateral posterior margins black (Figs 10A, 11B); propleuron orange; hindwing infuscate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 10C); legs black except for foreleg orange with tibia dark orange medially

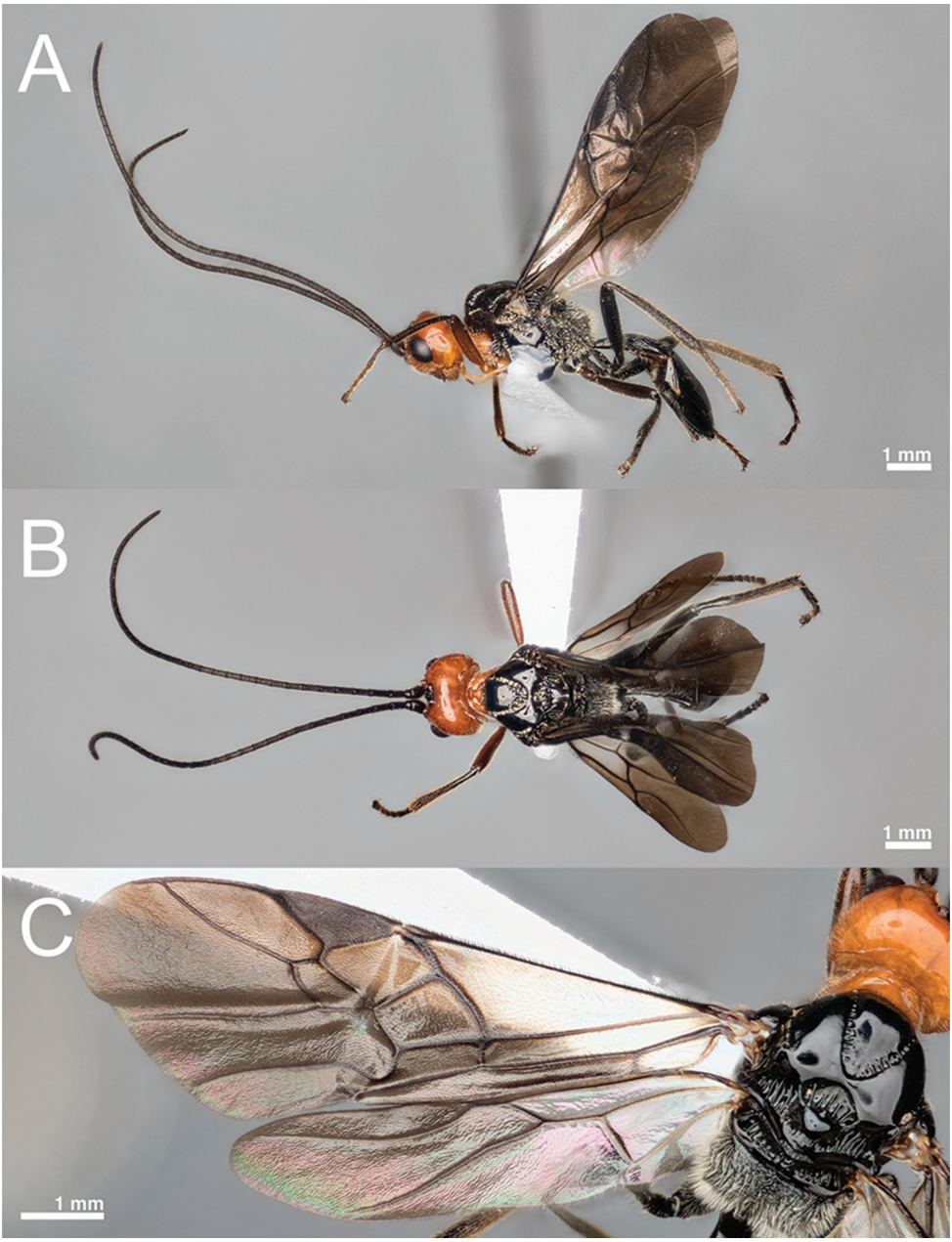

Figure 10. *Eadya paropsidis*. **A** Lateral habitus **B** Dorsal habitus **C** Fore and hindwing. All scale bars are 1mm in length.

and anterior and posterior apices brown, tarsi black (Fig. 10A); amino acid sequence (112–118) TRNFIGI (Fig. 15).

Description. Female. Body Length 6.29mm. Ovipositor Length 1.08mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black (Figs 10A, B; 11A, B); pronotum orange except for lateral posterior margins black (Figs 10A, B; 11A, B); propleuron orange; mesothorax black (Figs 10A, B; 11C, D); metathorax black (Figs 10A, B; 11D, E, F); forewing infuscate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 10C); hindwing infuscate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 10C); legs black except for foreleg orange with tibia dark orange medially and anterior and posterior apices brown, tarsi black (Fig. 10A, B); abdomen black except for ovipositor orange (Fig. 10A, B).

Head. Clypeus simple, smooth with scattered setae, flanged at ventral margin, with two medial tubercles projecting outward (Fig. 11A); mandibles overlapping, dorsal and ventral teeth of equal length (Fig. 11A); face finely punctate with associated setae (Fig. 11A); frons rugulose, inter-antennal and lateral carina strongly flanged, starting at the toruli and reaching the ocellar triangle (Fig. 11A, B); vertex smooth with scattered setae (Fig. 11B); occipital carinae emarginate (See arrow, Fig. 11B), reaching hypostomal carina; hypostomal carina strongly flanged, meeting the mandible and bending around to the mandibular condyle; occiput smooth, strongly concave (Fig. 11B, see arrow).

Mesosoma. Pronotum exposed in dorsal view, pronope and subpronope absent, smooth except for a faint crenulate line extending laterally and rugulose sculpturing along the lateral posterior margin (Fig. 11B, C); mesoscutum with rugulose sculpturing along the posterior margin of median mesonotal lobe (Fig. 11C); notaulus crenulate (Fig. 11C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 11C); sternaulus crenulate (Fig. 11D); propodeum rugose and pubescent, not rounded in appearance from lateral angle, with transverse carina (see arrows, Fig. 11F) creating a distinct posterior face when viewed laterally (Figs 10A; 11E, F); propodeal spiracle circular; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent (Fig. 10A, B); tarsal claws simple.

Forewing. r-m curved slightly towards stigma before reaching the junction of 3RSa and 3RSb (Fig. 10C).

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae (Fig. 9E); ovipositor straight.

Male. Same as female.

Host. *Paropsis atomaria* Olivier, 1807, *Paropsis tasmanica* Baly, 1866, *Paropsis charybdis*.

Diagnostic molecular characters. (22–27) MWSGII; (32–34) SVL; (41–46) ILGRLI; (54) S; (67–73) IVIPIII; (81) V; (90) M; (95–98) INNI; (104–109) PPSLIL; (112–118) TRNFIGI; (126) I; (133–139) NLRHRGI; (143–144) IS; (150) L; (157) M; (167–169) INI; (172–191) LGLNYDNISLLVWSVNITAI (Fig. 15).

Distribution. Australian Capital Territory, Victoria, New South Wales, Tasmania.

Type material examined. Holotype, Female (ANIC), "Canberra, A.C.T., Em. 1. 1. 58 cx, host larva coll. 4. 1. 57. Parasite of Paropsis reticulata. C.I.E. COLL. NO. 18079. Eadya paropsidis Holotype det. T. Huddleston, 1977. ANIC Database No. 32

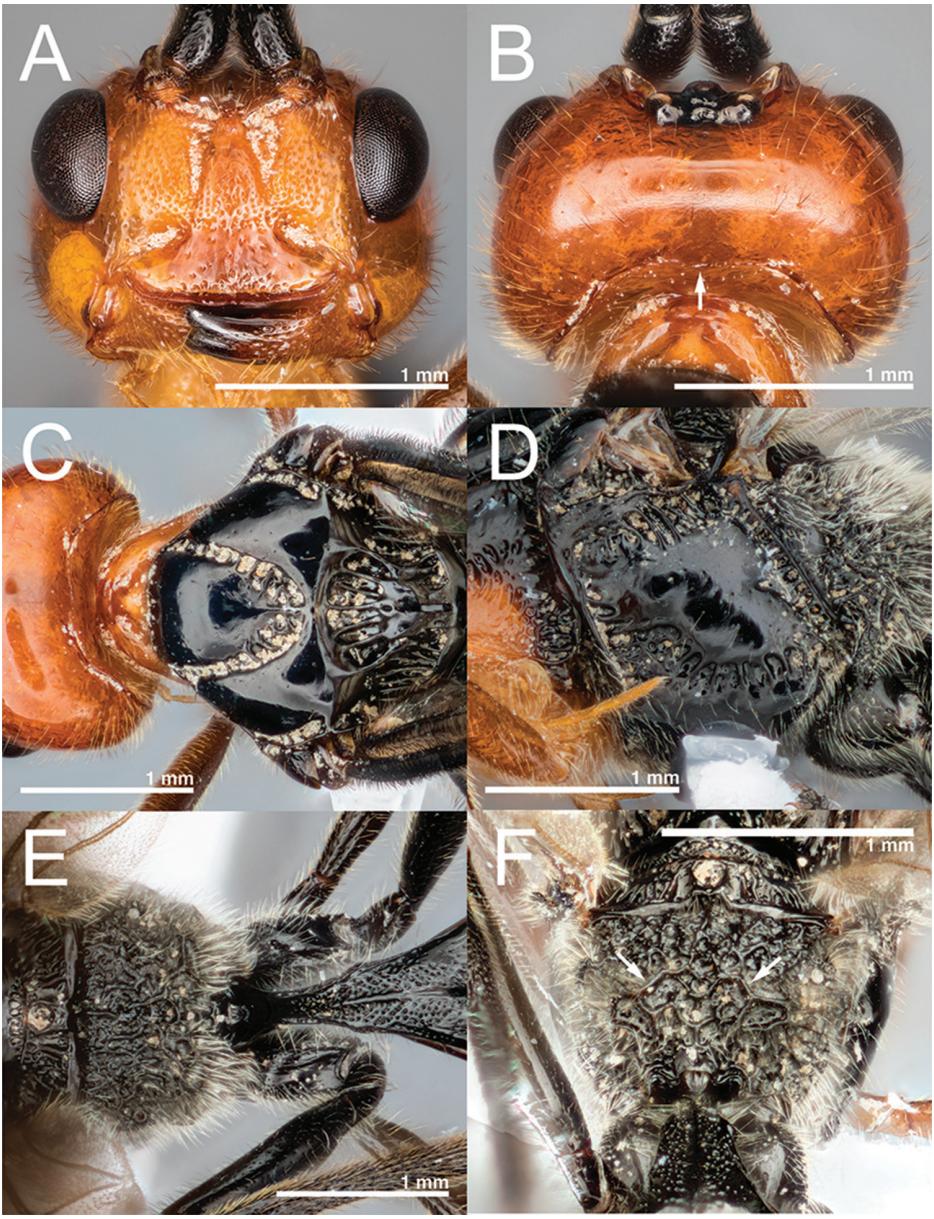

Figure 11. *Eadya paropsidis*. **A** Head, frontal view **B** Head, dorsal view, arrow pointing to emarginate occipital carinae **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view **F** Propodeum, dorsal view, with arrows indicating transverse carinae. All scale bars are 1mm in length.

111891". Paratype, Female (ANIC), "Canberra, A.C.T., 1. 10. 1957, Dissected from cocoon, Parasite of *Paropsis reticulata*, CIE COLL No 18079. *Eadya paropsidis* Paratype Female det T. Huddleston, 1977, Aust. Nat. Ins. Coll.".

Non-type material examined. See Suppl. material 1.

***Eadya spitzer* Ridenbaugh, sp. n.**
http://zoobank.org/68DF4AF7-FA6A-48A4-9305-CC8D6D4EECF7
Figs 12A–C; 13A–C; 14A–E

Diagnosis. *Eadya spitzer* sp. n. can be distinguished from all other members of *Eadya* by the following combination of characters: Clypeus flanged at ventral margin, with two medial tubercles projecting outward (Fig. 14A); frons with inter-antennal and lateral carina flanged (Fig. 14B); occipital carina simple (Fig. 14B); occiput simple; notaulus impressed towards anterior margin of mesoscutum, foveate at apex (Fig. 14C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 14C); sternaulus crenulate (Fig. 14D); propodeum rounded in appearance from lateral angle (Fig. 13A), without transverse carinae (Fig. 14E), and not creating a distinct posterior face when viewed laterally; propodeal spiracle circular; head orange except for antenna, apex of mandible, and ocellar triangle black, median of clypeus brown (Figs 14A, B); prothorax orange (Figs 12A, 13A, 14B); hindwing infuscate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 13C); legs black except for fore coxa and trochanter orange (Fig. 13A); amino acid sequence (112–118) IRNFIGM (Fig. 15).

Description. Female. Body length without abdomen 3.30mm. Abdomen 2.86mm. Ovipositor 1.17mm.

Color. Head orange except for antenna, apex of mandible, and ocellar triangle black (Figs 12A, B; 13A, B; 14A, B), median of clypeus brown; prothorax orange (Figs 12A, B; 13A, B; 14A, B, C); mesoscutum orange (Figs 12A, B; 13A, B; 14A, B); mesopleuron black except for anterior dorsal margin orange (Figs 13A; 14D); metathorax black (Figs 12B; 13A, B; 14D, E); forewing infuscate with dark brown veins except for anal, basal, and subbasal cells hyaline (Fig. 13C); hindwing infuscate with dark brown veins except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 13C); legs black except for fore coxa and trochanter orange (Figs 12A; 13A, B); abdomen black except for ovipositor orange (Figs 12C; 13A).

Head. Clypeus simple, smooth with scattered setae, flanged as ventral margin, with two medial tubercles projecting outward (Fig. 14A); mandibles overlapping, dorsal tooth longer than ventral (Fig. 14A); face finely punctate with associated setae (Fig. 14A); frons rugose, inter-antennal and lateral carina flanged, starting at the toruli and reaching the ocellar triangle (Fig. 14A, B); vertex smooth with scattered setae (Fig. 14B); occipital carina simple (Fig. 14B), reaching the hypostomal carina; hypostomal carina strongly flanged, reaching the mandible and bending around to the mandibular condyle; occiput smooth, normal (Fig. 14B).

Mesosoma. Pronotum exposed in dorsal view (Fig 14B, C); pronope absent, subpronope absent, smooth except for a faint crenulate line extending laterally and rugulose sculpturing along the lateral posterior margin (Fig. 14B); mesoscutum with median mesonotal lobe smooth (Fig. 14C); notaulus impressed towards anterior margin of mesoscutum, foveate at apex (Fig. 14C); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 14C); sternaulus crenulate (Fig. 14D); propo-

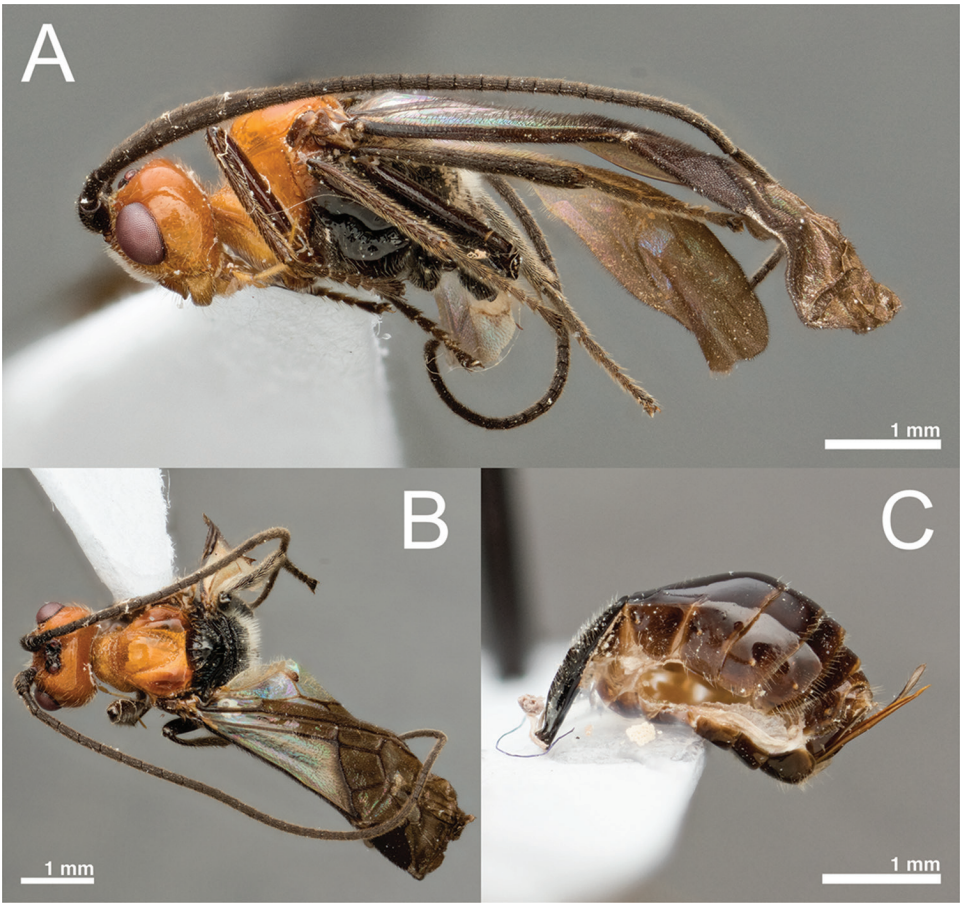

Figure 12. *Eadya spitzer* Ridenbaugh, sp. n. holotype. **A** Lateral habitus **B** Dorsal habitus **C** Metasoma, lateral view. All scale bars are 1mm in length.

deum rugose and pubescent, rounded in appearance from lateral angle, without transverse carinae and not creating a distinct posterior face when viewed laterally (Figs 13A; 14E); propodeal spiracle circular; coxa, trochanter, trochantellus, and femur covered in setae, tibia and tarsus pubescent; tarsal claws simple (Figs 12A; 13A, B).

Forewing. r-m curved slightly towards stigma before reaching the junction of 3RSa and 3RSb (Fig. 13C).

Hindwing. R1a with three hamuli.

Metasoma. Metasomal tergite 1 petiolate, spiracle protruding as a tubercle at about the middle of the segment, dorsal and lateral surface punctate with associated setae (Fig. 14E); ovipositor straight (Figs 12C; 13A).

Male. Unknown.

Host. *Paropsis charybdis*, *Paropsis aegrota elliotti* Selman, 1983.

Variations. Paratype with clypeus orange (Fig. 14A). This variation may be the result of the DNA extraction process of the Holotype.

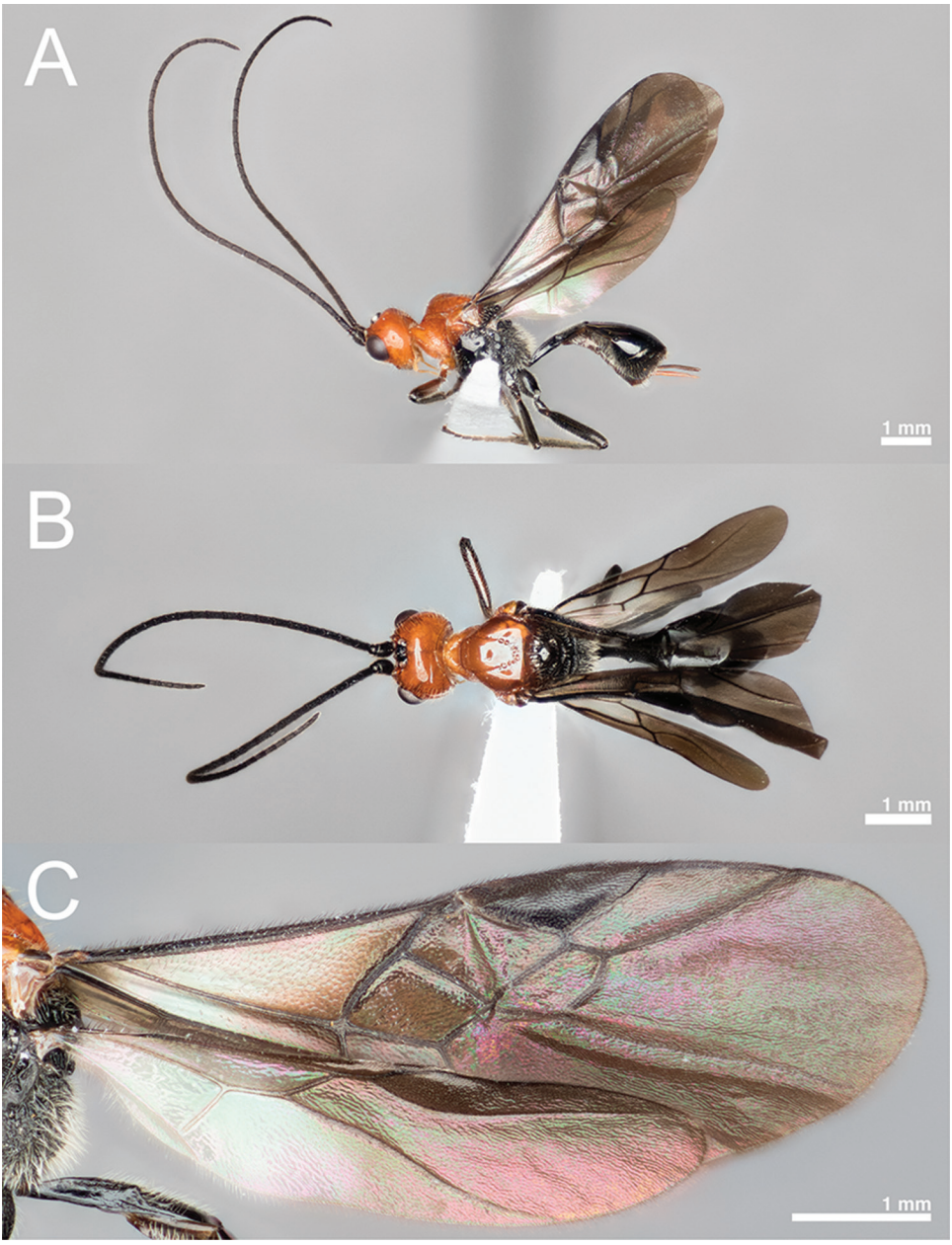

Figure 13. *Eadya spitzer* Ridenbaugh, sp. n. paratype. **A** Lateral habitus **B** Dorsal habitus **C** Metasoma, lateral view. All scale bars are 1mm in length.

Diagnostic molecular characters. (22–27) IWSGII; (32–34) SVL; (41–46) [M or K]LGRLL; (54) S; (67–73) IVIPIII; (81) I; (90) MM; (95–98) INNI; (104–109) PPSLIL; (112–118) IRNFIGM; (126) M; (133–139) NLRHRGI; (143–144) MS; (150) L; (157) I; (167–169) INI; (172–191) LGLNYDNISLLVWSVNITAI (Fig. 15).

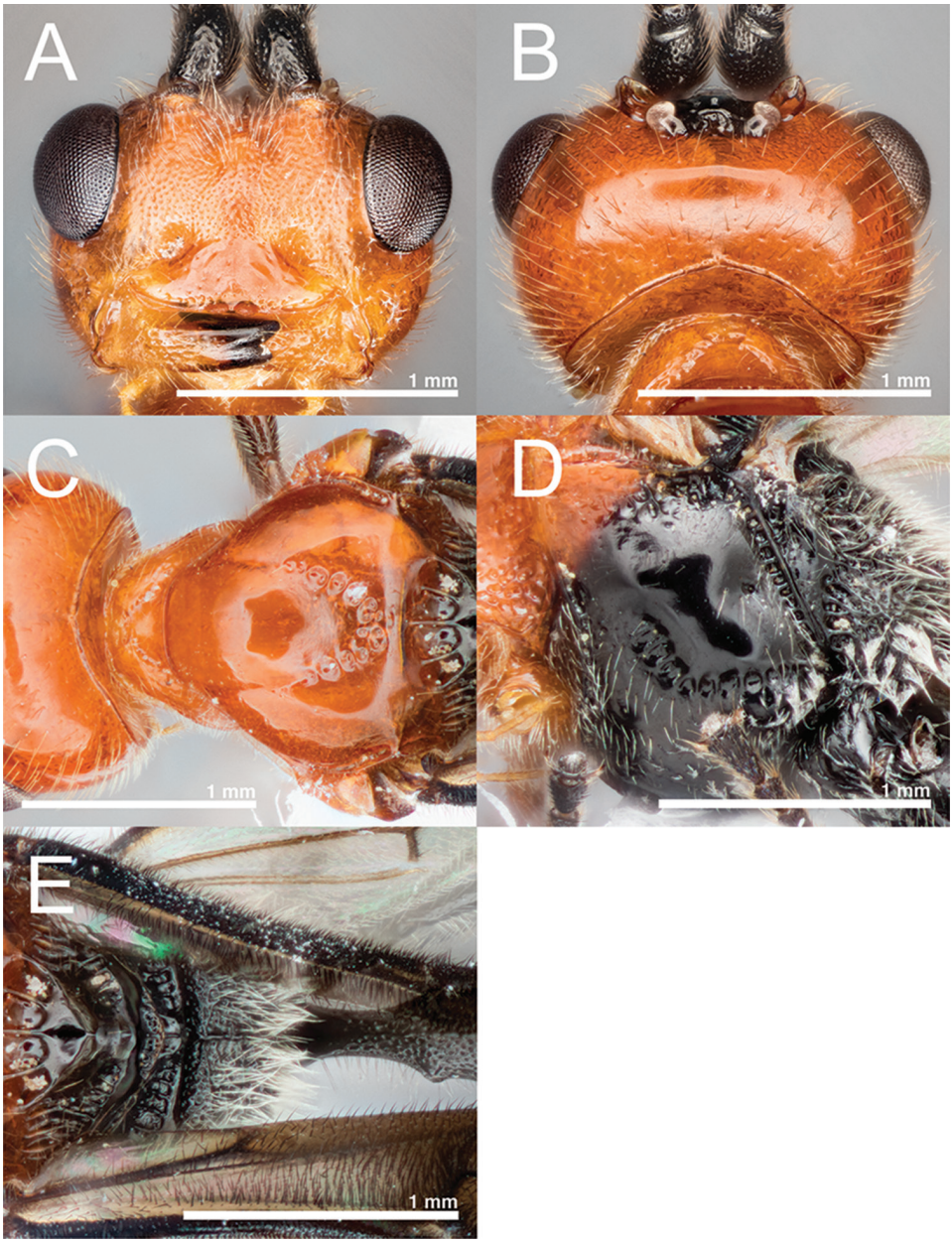

Figure 14. *Eadya spitzer* Ridenbaugh, sp. n. paratype. **A** Head, frontal view **B** Head, dorsal view **C** Head and mesoscutum, dorsal view **D** Mesopleuron, lateral view **E** Propodeum, dorsal view.

Distribution. Tasmania.

Etymology. This species is named in honor of Edwin Spitzer, the first author's (RDR) late grandfather. This is a noun in apposition to the generic name in order to retain integrity of the surname Spitzer.

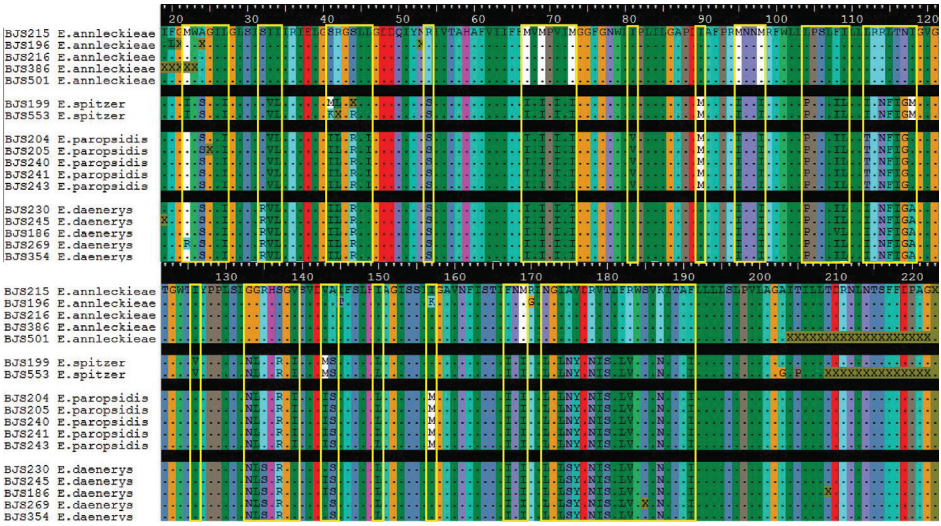

Figure 15. Cytochrome c oxidase subunit 1 amino acid sequences from Peixoto et al. (2018). Boxes indicate diagnostic molecular characters. For each sequence a unique corresponding DNA voucher code is listed as BJS followed by a number.

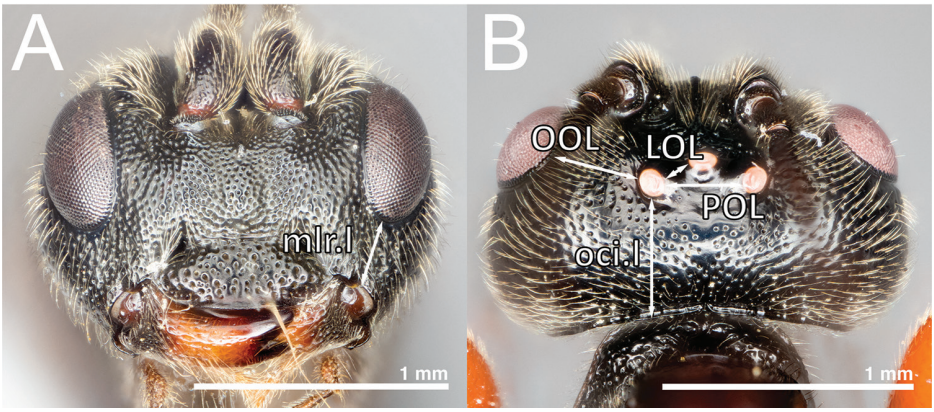

Figure 16. Characters used in the morphometric analysis. **A** Frontal view of the head illustrating the morphometric character malar space (mlr.l), *Eadya annleckieae* Ridenbaugh, sp. n. paratype **B** Dorsal view of the head illustrating the morphometric characters lateral ocellar line (LOL), ocular ocellar line (OOL), posterior ocellar line (POL), and occipital ocellar line (oci.l), *Eadya annleckieae* Ridenbaugh, sp. n. paratype. All scale bars are 1mm in length.

Remarks. The paratype is for this series is badly damaged, missing both antennae, all six legs, and the abdomen excluding metasomal tergite 1. However, the specimen was photographed before destruction and can be seen in Figures 13A–C and 14A–E. This species is referred to as Eadya sp.2 in Peixoto et al. (2018).

Type material. Holotype, Female (ANIC), "The Lea, TAS, 11 Dec 2012, Emerged 26 Dec 2012, G.R. Allen, Field collected in *P. charybdis*, E135", "BJS 199", GenBank

accession numbers KX989902, and MH107810. Paratype, Female (ANIC), "Runnymede Site #1, TAS, 13 Dec 2015, 42°38'11.1"S, 147°33'54.7"E, Flying adult, D. Satchell, Female".

Key to the species of *Eadya*

1 Propodeum with transverse carinae (See arrows, Fig. 11F) creating a distinct posterior face when viewed from the lateral angle (Fig. 10A).....**2**

– Propodeum without transverse carinae (Fig. 5F), rounded in appearance when viewed from the lateral angle (Fig. 4A) **3**

2 Occipital carinae simple (See arrow, Fig. 5B); propodeal spiracles elliptical; mesothorax orange (Fig. 7C, D) .....***E. duncan* Ridenbaugh, sp. n.**

– Occipital carinae emarginate (See arrow, Fig. 11B); propodeal spiracles circular; mesothorax black (Fig. 11C, D)..... ***E. paropsidis* Huddleston & Short, 1978**

3 Notaulus impressed towards anterior margin of mesoscutum, crenulate at apex (Fig. 9C); propodeal spiracles elliptical; hindwing infuscate except for anal, basal, discal, and subbasal cells hyaline (Fig. 8C); ovipositor downcurved (Fig. 8A); Distribution: Western Australia ...***E. falcata* Huddleston & Short, 1978**

– Notaulus rugulose (Fig. 3C), crenulate (Fig. 5C), or impressed towards anterior margin of mesoscutum and foveate at apex (Fig. 14A); propodeal spiracles circular; hindwing either completely hyaline (Fig. 2C) or infuscate except for anal, basal, subbasal, and anterior half of discal cells hyaline (Fig. 13C); ovipositor straight (Fig. 13A); Distribution: Australian Capital Territory, New South Wales, Tasmania **4**

4 Head black (Fig. 3A, B); sternaulus rugulose (Fig. 3D); scutellar sulcus divided into two distinct foveae with rugulose sculpturing along the posterior margins (Fig. 3C).....***E. annleckieae* Ridenbaugh, sp. n.**

– Head orange except for antenna, apex of mandible, and ocellar triangle black (Fig. 14A, B); sternaulus crenulate (Fig. 14D); scutellar sulcus divided into many deep pits by ridge like longitudinal carinae (Fig. 14C).....**5**

5 Pronotum orange (Fig. 12A); mesoscutum orange (Fig. 14C); legs black except for fore coxa and trochanter orange; notaulus impressed towards anterior margins of mesoscutum, foveate at apex (Fig. 14C) .....***E. spitzer* Ridenbaugh, sp. n.**

– Pronotum black except for anterior dorsal margin orange (Figs 4A, 5B) mesoscutum black (Fig, 5C); legs black; notaulus crenulate (Fig. 5C) ....***E. daenerys* Ridenbaugh, sp. n.**

Discussion

With the description of the four new species described here, the distribution of *Eadya* has expanded to include Tasmania, the Australian Capital Territory, New South Wales,

Victoria, and Western Australia. As Peixoto et al.'s (2018) study was limited to Tasmania, much is still unknown about mainland populations of *Eadya*. Of the six species of *Eadya* now known, two (*E. annleckieae* sp. n. and *E. spitzer* sp. n.) are known solely from Tasmania. This may not be an accurate distribution given our limited knowledge of mainland *Eadya* and because both *E. paropsidis* and *E. daenerys* sp. n. have been recorded from both Tasmania and mainland Australia.

Interestingly, knowledge on *Eadya* distribution has grown from a citizen science observation. Citizen science initiatives are a valuable, yet underutilized, resource for biodiversity research which can survey large geographical areas over extended periods of time (Silvertown 2009; Theobald et al. 2015). In November of 2012, a series of photos taken in Melbourne depicting a wasp stinging beetle larvae and labeled "? *Eadya paropsidis*" was uploaded to ProjectNoah.org (Ridgway 2012). The photos were tagged with the following description:

"A small (7mm) wasp with an orange head, thorax and first pair of legs. The rest of the wasp was black. The larvae being parasitized were those of the eucalyptus leaf beetle (*Paropsis atomaria*), probably the 2nd instar".

Although the image quality and detail was not sufficient to positively identify the beetle larvae, the images of the wasp coupled with the contributor's description matches that of *E. duncan* sp. n., and represents a new distribution record. With this observation, the distribution of *E. duncan* sp. n. is expanded to include Victoria, AUS in addition to New South Wales, AUS. Thus, citizen science observations can be invaluable for expanding knowledge on species and provides additional collecting localities for future research into this relatively unknown species.

Host records for *Eadya* outside of Tasmania are incomplete as well, with only *E. paropsidis* recorded from *Paropsis atomaria* (synonym *P. reticulata*) in the Australian Capital Territory and New South Wales (Huddleston and Short 1978). Again this may not represent the entire complement of possible hosts for *E. paropsidis* given the plastic nature of host usage in *Eadya* (Peixoto et al. 2018). Thus, there may be more host associations to be discovered with focused sampling and careful rearing. *Eadya daenerys* sp. n. from Tasmania has been considered as a potential biocontrol agent for *Paropsis charybdis* in New Zealand (Withers et al. 2012), and continues to be a promising candidate (Peixoto et al. 2018). With two mainland species of *Paropsisterna* (*Pst. m-fuscum* and *Pst. variicollis**) recently introduced as pests outside of Australia (von Ellenreider 2003; Paine et al. 2011; Clemson University Extension 2012; Rogan 2016), establishing accurate host records for *Eadya* could prove beneficial for future biocontrol efforts.

Much is still unknown about the species of *Eadya*, but as the popularity of *Eucalyptus* grows internationally as an ornamental landscape and forestry product (Paine et al. 2011), and with it the number of invasive pests, future biocontrol programs may look to *Eadya* for classical biological control. Although Peixoto et al. (2018) has added much to our understanding, further research into the biology of *Eadya* is required, with a particular focus on the host associations and distributions of mainland Australian populations. The sooner this research can be completed the more likely rapid

measures can be taken to control additional incursions of paropsine beetles in new countries and regions.

Finally, it is prudent to discuss the subfamily placement of *Eadya*. In the original description, Huddleston and Short (1978) placed *Eadya* within Euphorinae, but without much justification. Shaw (1985) in his analysis of Euphorinae relationships, agreed that *Eadya* belonged within Euphorinae, likely as a basal member because *Eadya* has a complete second submarginal cell (r-m cross vein present) and a long ovipositor, similar to *Meteorus* (a long suspected basal taxon of Euphorinae (Stigenberg et al. 2015). In a subsequent molecular phylogenetic analysis, based on 28S (D2-D3) rDNA, Belshaw and Quicke (2002) recovered *Eadya* within the Helconoid complex, sister to species of Diospilini (Brachistinae - following Sharanowski et al. 2011). They erected the tribe Eadyini within Helconinae to accommodate this aberrant taxon. The presence of an inter-antennal carina is shared among *Eadya* as well as several members of Helconinae (*sensu stricto* - following Sharanowski et al. 2011) providing some morphological evidence for this placement. However, *Eadya* attacks exposed leaf-feeding beetle larvae, not concealed xylophagous beetle larvae as do species of Helconinae *s.s.* Further, the morphological characters of *Eadya* are far more consistent with placement in Euphorinae (Shaw 1985; 1997) than Helconinae, and include: forewing vein 2cu-a absent; forewing vein 3RS curved, reaching the costa and therefore creating a small marginal cell; and a petiolate metasoma. Further, *Eadya COI* sequences share the greatest similarity to other Euphorines based on BLAST searches (Peixoto et al. 2018). Thus, the presence of an inter-antennal carina is likely convergent with members of Helconinae. We suggest that *Eadya* is indeed a member of Euphorinae, and forthcoming molecular phylogenetic analyses (Stigenburg, unpublished data; Sharanowski, unpublished data) will formally test that assertion.

Conclusions

Three new species from the genus *Eadya* are described (*Eadya annleckieae* Ridenbaugh, sp. n., *Eadya daenerys* Ridenbaugh, sp. n., *Eadya spitzer* Ridenbaugh, sp. n.) based upon the results of Peixoto et al. (2018), along with a fourth new species discovered in the Australian National Insect Collection (*Eadya duncan* Ridenbaugh, sp. n.). In addition to these descriptions, the distribution of *Eadya* is expanded from the Australian Capital Territory, New South Wales, and Western Australia, to include Tasmania and Victoria. Host records for all newly described species are listed along with two new host records for *Eadya paropsidis* (*Paropsis tasmanica* Baly, 1866, and *Paropsis charybdis* Stål, 1860). Finally, based upon several morphological characters (forewing vein 2cu-a absent; forewing vein 3RS curved, reaching the costa and therefore creating a small marginal cell; and a petiolate metasoma) and *COI* sequences presented in Peixoto et al. (2018), we suggest the placement of *Eadya* within the subfamily Euphorinae.

Acknowledgments

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Supplementary material 1

Table S1

Authors: Ryan D. Ridenbaugh, Erin Barbeau, Barbara J. Sharanowski

Data type: species data

Explanation note: List of all materials examined along with collecting localities, its type designation and location of deposition, and associated DNA voucher number or unique identifier. *Eadya annleckieae* Ridenbaugh, sp. n. is referred to as Eadya sp.1, *Eadya spitzer* Ridenbaugh, sp. n. is referred to as Eadya sp.2, and *Eadya daenerys* Ridenbaugh, sp. n. is referred to as Eadya sp.3.

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Link: https://doi.org/10.3897/jhr.64.24282.suppl1

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RESEARCH ARTICLE

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The International Society of Hymenopterists

Three new species of *Dolichogenidea* Viereck (Hymenoptera, Braconidae, Microgastrinae) from Australia with exceptionally long ovipositors

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http://zoobank.org/0E6D1EC0-08EF-4B95-BF43-BEA599728432

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Abstract

The subfamily Microgastrinae contains an extraordinarily rich diversity of parasitoid wasps which parasitise larval lepidopterans. The Australian fauna has generally been poorly studied, particularly for the very speciose genera. One such genus is *Dolichogenidea* Vierek, which in Australia is known from only six described species. Here we describe three new species of *Dolichogenidea* from Australia, which are distinguished by possessing extremely long ovipositors compared with the typical form for the genus. These are *D. finchi* Fagan-Jeffries & Austin, **sp. n.**, *D. mediocaudata* Fagan-Jeffries & Austin, **sp. n.**, and *D. xenomorph* Fagan-Jeffries & Austin, **sp. n.** In describing these new species we also discuss relationships within the genus, and the diversity and biology of the Australian fauna.

Keywords

Microgastrinae, *Dolichogenidea*, parasitoid, ovipositor

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Introduction

The subfamily Microgastrinae are agriculturally and environmentally important as endoparasitoid wasps of larval lepidopterans. There are currently over 2700 species described worldwide (Yu et al. 2016), with estimates from cytochrome c oxidase subunit I (*COI*) DNA barcoding suggesting this could be as little as 6% of the true global diversity (Whitfield et al. 2018, Rodriguez et al. 2013). The subfamily comprises 81 genera (Fernandez-Triana and Boudreault in review), several of which are very large, including *Dolichogenidea* Viereck, with over 180 described species (Yu et al. 2016). This genus was initially described as a subgenus of *Apanteles* Foerster (Viereck 1911) for the placement of his new species *D. banksi* because of its elongated genae. Subsequently, it was treated as one or more species-groups of *Apanteles* sensu lato (Wilkinson 1928, Nixon 1965), but was then raised to genus level by Mason (1981) in his reclassification of the large and polyphyletic *Apanteles* sensu lato, which previously contained the majority of described microgastrine species. Mason's (1981) concept of *Dolichogenidea* included three of Nixon's (1965) species-groups; the *ultor*-group, the *laevigata*-group, and the *longipalpis*-group. Whilst Mason (1981) proposed several characters to distinguish *Dolichogenidea* from *Apanteles* sensu stricto, including *Dolichogenidea* having 'punctures of the mesonotum typically distinctly separated and never breaking into aciculations posterolaterally', many species have strongly reduced cuticular sculpturing, making the punctation characters unusable for placement in the correct genus. Analysis of thousands of specimens by Fernández-Triana et al. (2014) suggests that "the only reliable character is the number and density of setae fringing on the median portion of the vannal lobe" of the hind wing. *Dolichogenidea* has a convex to almost straight vannal lobe, which is uniformly fringed by setae, while in *Apanteles* sensu stricto the vannal lobe is strongly concave to almost straight and is lacking setae at the midlength. This lack of setae may be partial (i.e. there may be some small and sparse setae on the lobe) or total (i.e. no setae at all). The two genera are also generally resolved as separate monophyletic clades using molecular data and thus are distinguishable in DNA barcoding studies (Fagan-Jeffries et al. in press, Smith et al. 2013).

The Australasian members of *Dolichogenidea* were reviewed by Austin and Dangerfield (1992) and currently the genus contains six species from Australia: *D. biroi* (Szépligeti, 1905), *D. eucalypti* (Austin and Allen, 1989), *D. hyposidrae* (Wilkinson, 1928), *D. lipsis* (Nixon, 1967), *D. miris* (Nixon, 1967), and *D. tasmanica* (Cameron, 1912). Long-term sorting of microgastrines in Australian collections and a recent large barcoding study (Fagan-Jeffries et al. in press) have revealed several remarkable specimens belonging to three species of *Dolichogenidea* that possess extremely long ovipositors. We here describe these species as a contribution to on-going studies on Australian members of the genus.

Materials and methods

Terms for general morphology follow Fernández-Triana et al. (2014) who combined traditional microgastrine morphological terms, such as those used by Mason (1981), with the standards introduced in the Hymenoptera Anatomy Ontology (HAO) pro-

ject (Yoder et al. 2010). Terms for sculpturing follow Eady (1968). The following acronyms and abbreviations are used throughout the paper: T1, T2, T3 for the first, second and third mediotergites, respectively; S1–3 for the first, second and third sternites; ACT, Australian Capital Territory; NSW, New South Wales; Qld, Queensland; Vic, Victoria; WA, Western Australia. The following abbreviations are used for collections: **ANIC**, Australian National Insect Collection, Canberra; **WAM**, Western Australian Museum, Perth. We define colour as either pale (white, cream or pale yellow), orange, or dark (brown or black).

Taxonomy

Dolichogenidea Viereck

Dolichogenidea Viereck, 191 1: 173 (as a subgenus of *Apanteles* Foerster s.1.). Type species, by original designation, *Apanteles* (*Dolichogenidea*) *banksi* Viereck. Generic status by Mason 1981: 34. See Shenefelt (1972) for bibliographic history and Mason (1981) for discussion of relationships.

Diagnosis. Fore wing areolet (second submarginal cell) absent (i.e. vein r-m absent); hind wing vannal lobe convex to almost straight and uniformly fringed by setae; propodeum often with a complete areola, sometimes areola reduced with at least posterior diverging carinae present, rarely with these carinae completely absent; metasoma with T2 variable in shape, but usually rectangular or subrectangular; hypopygium membranous mid-ventrally and expandable (sometimes folded inwards and hidden by laterotergites in dead specimens); ovipositor protruding from posterior metasoma, usually as long as or longer than length of metatibia.

Remarks. In resurrecting *Dolichogenidea,* Mason (1981) allocated three of Nixon's (1965) species-groups to the genus: the *ultor*-, *laevigatus*-, and *longipalpis*-groups. The *longipalpis*-group was erected by Nixon for a single European species, *D. longipalpis* (Reinhard, 1880), which has unusually long mouthpart palps. The *ultor*-group was defined by Nixon (1965) for those species with a complete or partially complete propodeal areola, and the *laevigatus*-group for species with the areola represented only by two basal diverging carinae, or the propodeum virtually completely devoid of carinae. However, there are numerous species that represent intermediates between these conditions, and Mason (1981) was instrumental in recognising that there were likely to be independent pathways for reduction and eventual loss of the areola (Whitfield et al. 2018). Hence, it is very likely that neither the presence of a propodeal areola or its loss define monophyletic groups. This said, the three species described here most closely resemble the condition found in classic '*laevigatus*-group species', having a smooth and shiny propodeum, a transverse T2 (rather than triangular) and an ovipositor much longer than the metatibia.

Identification of the species described here. *Dolichogenidea* is highly speciose and there are large numbers of undescribed species in Australia. Austin and Dangerfield (1992) estimated that fauna to be 50–70 species. However, it may be much larger

than this given that a recent DNA barcoding study of Australian microgastrines recognised 236 species from 525 individuals, 42 of which belonged to *Dolichogenidea* (Fagan-Jeffries et al. in press). Given this considerable number of additional species in *Dolichogenidea*, it is pointless to present a key to the described fauna; rather we provide the characters that distinguish the three species treated here from the six described species, as follows: the absence of a conspicuous white blotch on the gena separates the three species from *D. lipsis*, *D. biroi*, and *D. tasmanica*; *D. hyposidrae* and *D. eucalypti* both have ovipositors significantly shorter than the metatibia and a clearly defined propodeal areola, whilst the species described here all have ovipositors significantly longer than the metatibia and a propodeal areola only indicated at most by short posterior diverging carinae; *D. miris* is separated by the presence of a partially defined areola with lateral costula, and a shorter T2 with strong rugose sculpturing, differing from the smooth or almost smooth T2 of the three new species here. In addition, the lengths of the ovipositor and sheaths of all undescribed *Dolichogenidea* we have seen in Australian collections do not exceed approximately 1.5 × that of the metatibia, compared with 1.8–4.2 × for the three new species.

The newly described species appear to be quite rare, although two are widespread (Fig. 1). After considerable collecting effort and searching of both pinned and ethanol museum material from all major Australian collections, only 14 specimens have been located.

***Dolichogenidea finchi* Fagan-Jeffries & Austin, sp. n.**
http://zoobank.org/CDDB476E-FE7F-4404-AE6D-5764C44ACE9F
Figure 2

Material examined. Holotype ♀: AUSTRALIA, WA, Kariijini NP, Kariijini Dr, 22.5716°S, 118.3072°E; 19-25/iv/2003, C Lambkin & T Weir, malaise in open *Eucalyptus* grassland, 814 m (WAM: E94085). Paratypes: ♀ WA, Kariijini NP, Weano Gorge Rd, 22°21'19"S, 118°15'00"E; 25/iv/2003 - 15/v/2003, C Lambkin & T Weir, malaise in grassy dry creek *Eucalyptus-Acacia* scrub, 695 m (WAM: E94086); ♀ NSW, Wilcannia, 23/xi/1949, E.F. Riek (ANIC: #32 130282); ♀ QLD, Binna Burra, Lamington National Park, 29/v/1966, Z. Liepa, at light (ANIC: #32 130284); ♀ QLD, Brisbane, vi/1904, RCL Perkins (NHM: #NHMUK010880682); ♀ QLD, 3.5 km SW by S of Mt Baird, 15.10°S, 145.07°E; 3-5/v/1981, I.D. Nauman, ex. ethanol, collected at light (ANIC: #32 130286); ♀ Vic, 18 km NW by N Omeo, 28/ii/1980, J.C. Cardale, ex alcohol (ANIC: #32 130283); ♀ WA, Millstream, 26/x/1970, J.C. Cardale (ANIC: #32 130285); ♀ WA, 21 km E by N Yellowdine, 10/x/1981, I.D. Naumann, J.C. Cardale, ex ethanol, on *Eucalyptus* flowers (WAM: E94087); ♀ WA, 1 km NNW of Eucla Pass, 31.40°S 128.52°E, 20/v/1984, E.S. Nielson, E.D. Edwards (WAM: E94088).

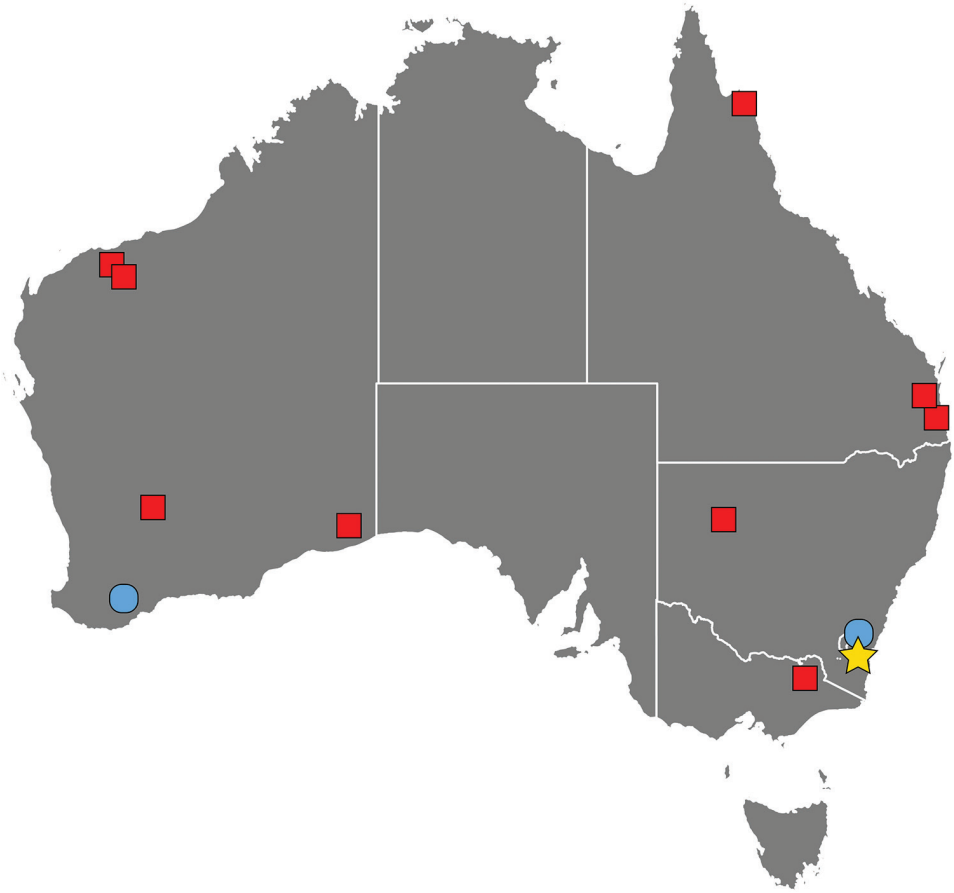

Figure 1. Known distributions of *D. xenomorph* (blue circles) *D. finchi* (red squares) and *D. mediocaudata* (yellow star).

Diagnosis. *Dolichogenidea finchi* can be separated from *D. mediocaudata* by having a longer ovipositor, smoother T1, and more consistent pale orange colouration of the legs; and from *D. xenomorph* by absence of a strong sculpturing pattern on the propodeum (Fig. 2d) and lighter colouration of the lateral metasoma (Fig. 2b).

Description. (Female). Colour. Head and body dark; tergites dark, T3 sometimes orange on lateral thirds (Fig. 2a); S1-3 paler than posterior sternites; antenna dark; coxae (pro-, meso-, metacoxa): dark, dark, dark; femora (pro-, meso-, metafemur): pale/orange, pale/orange, pale/orange; tibiae (pro-, meso-, metatibia): pale/orange, pale/orange, pale/orange anteriorly and subtly darker at basitarsus boundary; tegula and humeral complex pale; pterostigma dark, often with subtle to distinct pale patch at proximal end; fore wing veins pale proximally transitioning to dark distal to pterostigma.

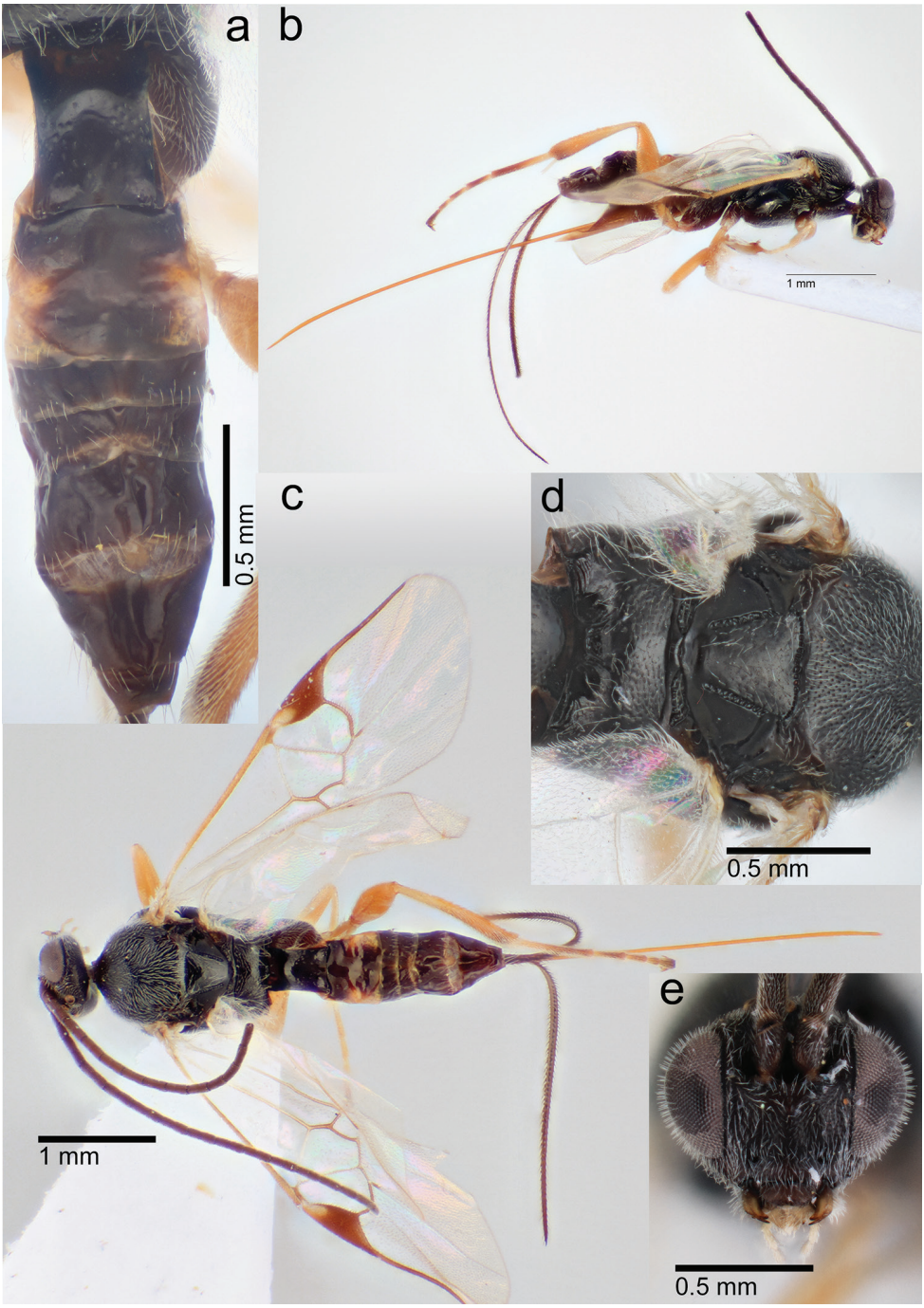

Figure 2. *Dolichogenidea finchi* (holotype): **a** metasoma **b** lateral habitus **c** dorsal habitus **d** mesosoma **e** head.

Head. Antennae slightly shorter than body length; body length (head to apex of metasoma): 3.4–4.4 mm; ocular–ocellar line/posterior ocellus diameter: 1.4–1.9; interocellar distance/posterior ocellus diameter: 1.3–2.3.

Mesosoma. Anteromesoscutum densely and evenly punctate; mesoscutellar disc mostly smooth and shining with sparse punctures mostly associated with setae, lateral faces of the mesoscutellum normally smooth and shining to lunules but sometimes with a distinct line of pits or with subtle area of sculpturing posterior to lunules; number of pits in scutoscutellar sulcus: varies from 12 to 22; maximum height of mesoscutellum lunules/maximum height of lateral face of mesoscutellum 0.7–0.8. Propodeum with sparse punctures associated with setae, areola only indicated by smoother area in centre of propodeum and short carinae diverging from centre posterior margin of propodeum.

Fore wing length 3.2–4 mm; length of veins r/2RS 1.5-2.2; length of veins 2RS/2M 1.0–1.7; length of veins 2M/(RS+M)b 0.5-0.8; pterostigma length/width 2.6–3.1.

Legs. Metatibia inner spur length/metabasitarsus length 0.2–0.4.

Metasoma. T1 length/width at posterior margin 1.2–1.8; T1 shape broad, rectangular, almost parallel-sided; T1 mostly smooth with sparse punctures associated with short setae on lateral sides of posterior half; T2 width at posterior margin/length 2.1–3.1; T2 sculpture smooth and shiny, few shallow punctures associated with setae; T2/T3 boundary indistinct and sinuate. T3 smooth and shiny, at least twice as long as T2; hypopygium large with lateral creases, ovipositor sheath length/metatibial length 2.9–3.9.

Male. Unknown.

COI Genbank accession numbers. MH138733 (Holotype) MH138940 (Paratype WAM: E94086)

Remarks. It is possible that if more specimens become available and are amenable to DNA sequencing, *D. finchi*, as described here, will turn out to be a species complex of several closely related species. There is variation in several morphological characters such as subtle differences in the length and shape of the metanotum, the colour of T3, and length of the ovipositor in relation to the metatibia. However, with so few specimens and a lack of molecular data we feel it is more practical at this stage to treat them as one variable species. Further, the *COI* sequences of the two specimens, sequenced as part of a parallel study (Fagan-Jeffries et al. in press), are 2.5% divergent, which is above the 2% divergence of the *COI* barcoding region threshold considered to delimit species of microgastrines in 95% of cases (Smith et al. 2013).

Etymology. This species is named for the late grandfather of one of us (EFJ), Alexander Finch, who was a sheep pastoralist near the town of Wilcannia, the locality for one of the paratypes.

Distribution. This species occurs widely across the continent (Fig. 1) and is recorded from WA, Qld, Vic and NSW.

Host. Whilst the host for this species has not been recorded, two specimens were collected in association with *Eucalyptus*. As *D. xenomorph* is the parasitoid of a larva feeding on *Eucalyptus*, it is a strong possibility that *D. finchi* also parasitises a *Eucalyptus*-associated lepidopteran.

Dolichogenidea mediocaudata **Fagan-Jeffries & Austin, sp. n.**
http://zoobank.org/8AD0F877-7CBD-4B6C-82EE-C77F58B6EE4E
Figure 3

Material examined. Holotype ♀: AUSTRALIA, NSW, 8 miles ESE of Nimmitable 3600ft, emerged 03/xii/1969, I.F.B. Common & J. Cusbert, L19. Larva tying leaves on fallen dead branch of *Eucalyptus pauciflora* (ANIC: #32 130288).

Diagnosis. This species can be separated from *D. finchi* and *D. xenomorph* by having a shorter ovipositor (Fig. 3a) and deeper sculpturing on both the propodeum and T1 (Fig. 3e), and presence of distinct dark colouration on the distal half of the metatibia.

Description. (Female). Colour. Head and body dark other than S1-3 which are distinctly paler than posterior sternites; antenna dark; coxae (pro-, meso-, metacoxa): dark, dark, dark; femora (pro-, meso-, metafemur): pale, dark, dark; tibiae (pro-, meso-, metatibia): pale, pale, pale anteriorly, posterior half distinctly darker; tegula and humeral complex pale; pterostigma dark; fore wing veins pale proximally transitioning to dark distally.

Head. Antennae slightly shorter than body length; body length (head to apex of metasoma): 3 mm; ocular–ocellar line/posterior ocellus diameter: 2.2; interocellar distance/posterior ocellus diameter: 1.9.

Mesosoma. Anteromesoscutum densely and evenly punctate, no punctures at posterior margin; mesoscutellar disc mostly smooth and shining with sparse punctures mostly associated with setae, lateral faces of the mesoscutellum smooth and shining but with a distinct line of pits posterior to lunules; number of pits in scutoscutellar sulcus: varies from 12–13; maximum height of mesoscutellum lunules/maximum height of lateral face of mesoscutellum 0.7. Propodeum with deep non-uniform punctures, posterior half with rugose sculpturing, areola only indicated by a central depression and short carinae diverging from centre posterior margin of propodeum.

Fore wing length 2.7 mm; length of veins r/2RS 1.3; length of veins 2RS/2M 1.8; length of veins 2M/(RS+M)b 0.6; pterostigma length/width 2.8.

Legs. Metatibia inner spur length/metabasitarsus length 0.4.

Metasoma. T1 length/width at posterior margin 1.6; T1 shape broad, rectangular, almost parallel-sided; T1 with rugose sculpturing and sparse punctures over most of length; T2 width at posterior margin/length 2.0; T2 sculpture smooth and shiny, few shallow punctures associated with setae; T2/T3 boundary indistinct and sinuate. T3 smooth and shiny, at least twice as long as T2; hypopygium large with lateral creases, ovipositor sheath length/metatibial length 1.8.

Male. Unknown.

Etymology. This species is named for the length of the ovipositor, which appears to be intermediate between most *Dolichogenidea* and the extremely long ovipositors of *D. xenomorph* and *D. finchi*.

Distribution. This species is only known from the holotype collected near Nimmitable in south-eastern NSW.

Host. This specimen was reared from a lepidopteran larva tying leaves together on a dead branch of *Eucalyptus pauciflora*.

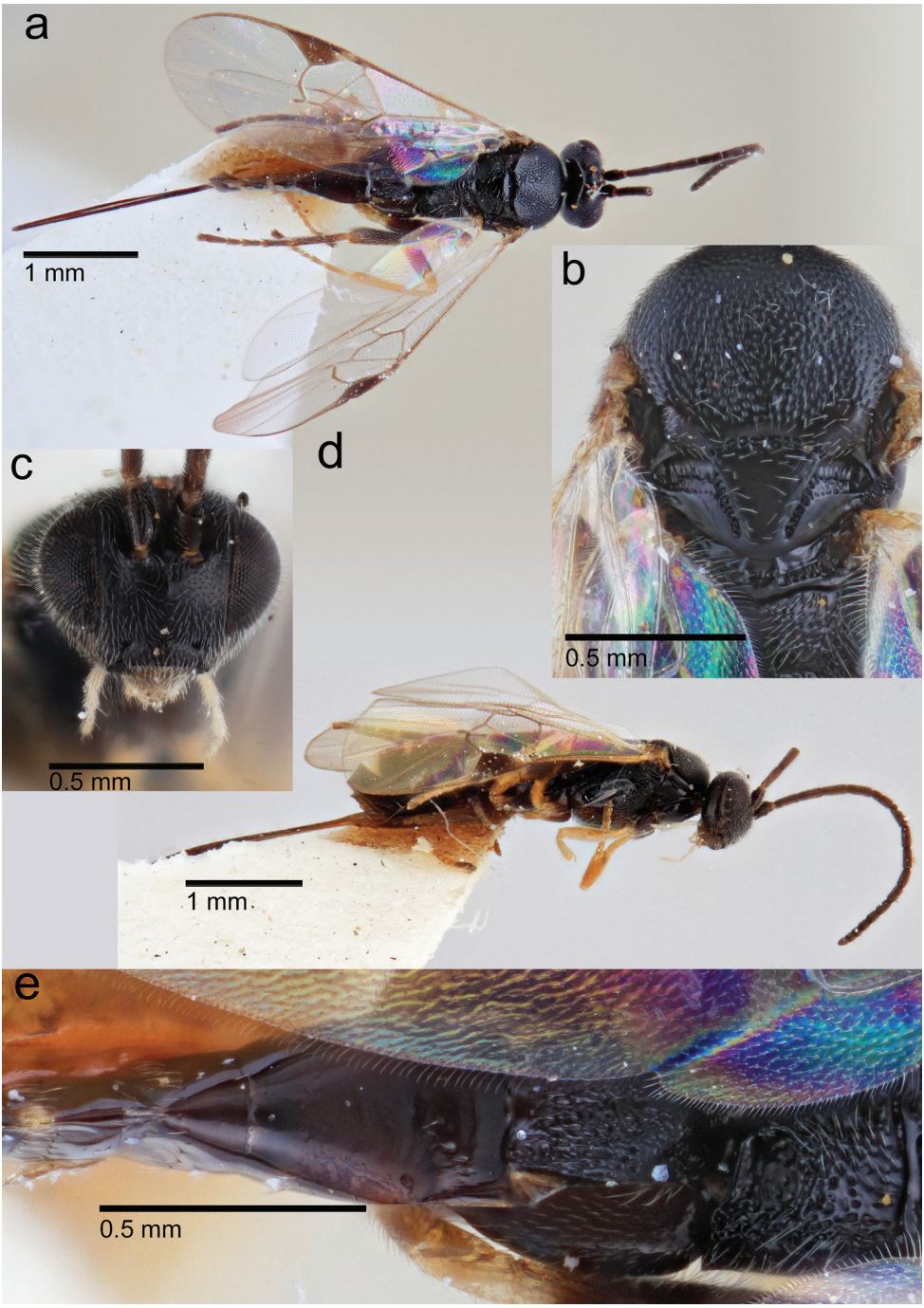

Figure 3. *Dolichogenidea mediocaudata* (holotype): **a** dorsal habitus **b** anteromesoscutum, mesoscutellum and metanotum **c** head **d** lateral habitus **e** propodeum and tergites.

Dolichogenidea xenomorph **Fagan-Jeffries & Austin, sp. n.**
http://zoobank.org/F7E2A57E-8F65-45F3-9752-C3165DD513DC
Figure 4

Material examined. Holotype ♀: AUSTRALIA, NSW, 2.7 km NE of Queanbeyan, emerged 28/x/1979, I.F.B. Common, ex *Ocystola euanthes* Meyr (ANIC: #32 130289). Paratype ♀: same data as holotype (ANIC: #32 130290). Other material ♀: AUSTRALIA, WA, Stirling National Park, 22/ix/1965, E. Britton, U. Baker (ANIC: #32 130287).

Diagnosis. *Diolichogenidea xenomorph* can be separated from *D. mediocaudata* by having a longer ovipositor, smoother T1, and lighter, more consistent colouration of the femora and tibiae. The species is very similar to *D. finchi*, but can be separated by the stronger sculpturing pattern on the propodeum (Fig. 4d) and darker colouration of the lateral metasoma (Fig. 4b).

Description. (Female). Colour. Head and body dark, including tergites and sternites; antenna dark; coxae (pro-, meso-, metacoxa): dark, dark, dark; femora (pro-, meso-, metafemur): orange, orange, dark to orange; tibiae (pro-, meso-, metatibia): orange, orange, orange; tegula and humeral complex orange; pterostigma dark; fore wing veins pale proximally transitioning to dark distally.

Head. Antennae slightly longer than body length; body length (head to apex of metasoma): 4 mm; ocular–ocellar line/posterior ocellus diameter: 1.8–2.1; interocellar distance/posterior ocellus diameter: 1.7–2.5.

Mesosoma. Anteromesoscutum densely and evenly punctate; mesoscutellar disc mostly smooth and shining with sparse punctures mostly associated with setae, lateral faces of mesoscutellum with anterior shallow sculpturing posterior to lunules (Fig. 4c); number of pits in scutoscutellar sulcus: 16; maximum height of mesoscutellum lunules/maximum height of lateral face of mesoscutellum 0.7–0.8. Propodeum with sparse punctures associated with setae, areola only indicated by smoother area in centre of propodeum and short carinae diverging from centre posterior margin of propodeum. Propodeum with rugose sculpturing in posterior half.

Fore wing length 4.3–4.4 mm; length of veins r/2RS 1.3–1.9; length of veins 2RS/2M 1.1–1.2; length of veins 2M/(RS+M)b 0.8–1; pterostigma length/width 2.6–3.

Legs. Metatibia inner spur length/metabasitarsus length 0.3–0.4.

Metasoma. T1 length/width at posterior margin 1.1–1.4; T1 shape broad, rectangular, almost parallel-sided; T1 mostly smooth with sparse punctures associated with short setae on lateral sides of posterior half; T2 width at posterior margin/length 4; T2 sculpture smooth and shiny, few shallow punctures associated with setae; T2/T3 boundary indistinct and sinuate. T3 smooth and shiny, at least twice as long as T2; hypopygium large with lateral creases, ovipositor sheath length/metatibial length 3.7–4.2.

Male. Unknown.

Remarks. The specimen from WA is here assigned to this species, but excluded from the type series due to its disjunct distribution which is also outside the known

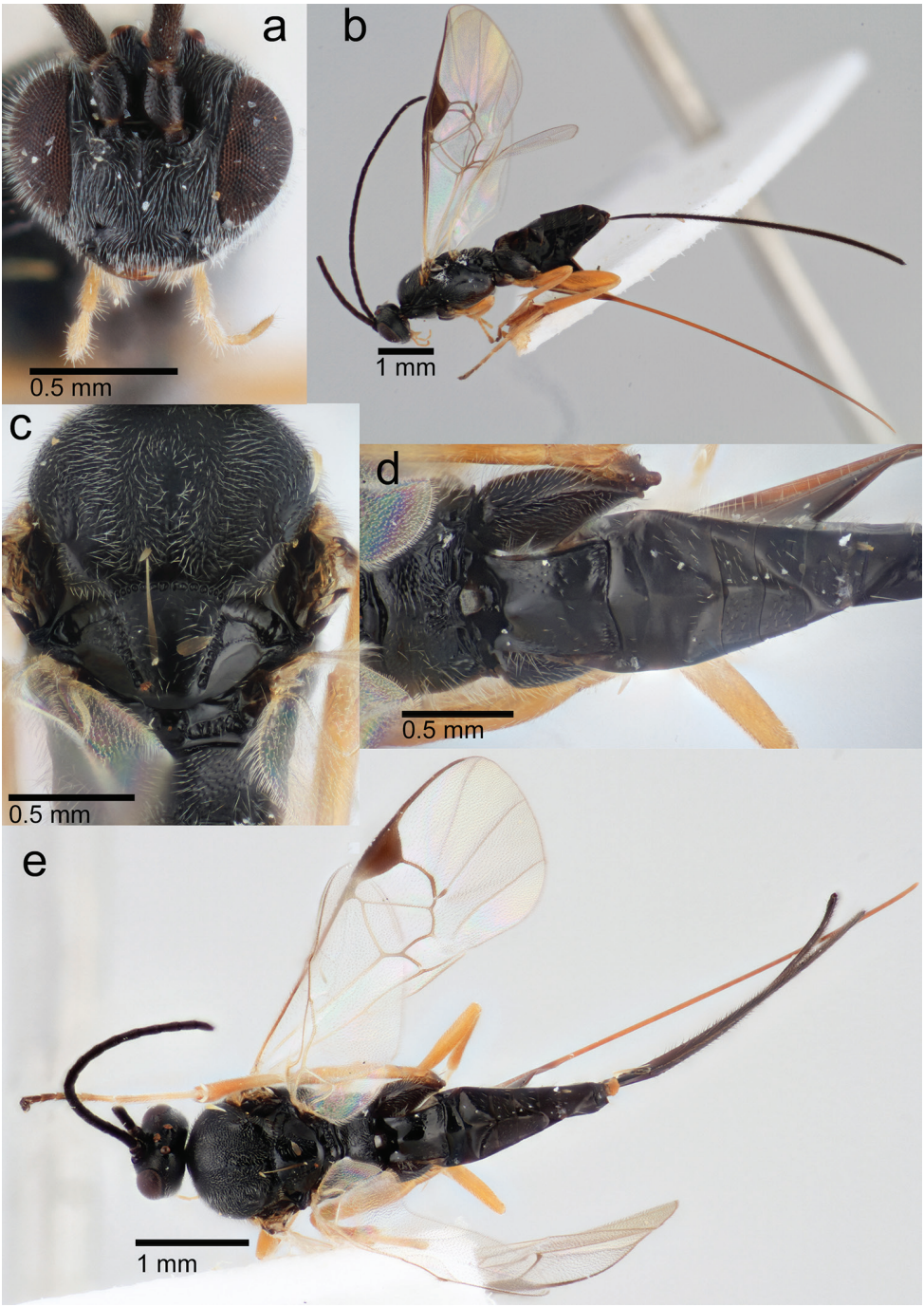

Figure 4. *Dolichogenidea xenomorph*: **a** head (paratype) **b** lateral habitus (paratype) **c** anteromesoscutum, mesoscutellum and metanotum (holotype) **d** propodeum and tergites (holotype) **e** dorsal habitus (holotype).

range of the host species. However, other species of the host genus are known from WA, but we take a more conservative approach until further specimens and host data become available.

Etymology. This species is named for the fictional creature from the movie franchise 'Alien', which reportedly was inspired by the lifecycle of parasitic wasps. The name of the fictional creature comes from the Greek 'xeno' (strange) and 'morphe' (form) which is also appropriate, considering the remarkably long ovipositor of this species compared to other members of the genus. The species name is a noun in apposition.

Distribution. Recorded from NSW and south-western WA.

Host. Reared from *Antipterna euanthes* (Meyrick, 1885) (Oecophoridae), a species in which the larvae fold over the tip of a *Eucalyptus* leaf and continue developing even after the leaf is shed from the tree (Common 1994). This lepidopteran species is recorded from ACT, NSW and Vic, however the genus extends into eastern Qld, Tasmania, and south-western WA (Common 1994). The holotype and paratype of *D. xenomorph* have the same locality and host information. Whether they emerged singularly from two host larvae collected on the same date, or were gregarious in the one host is unknown.

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New internal primers targeting short fragments of the mitochondrial COI region for archival specimens from the subfamily Aphidiinae (Hymenoptera, Braconidae)

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Abstract

Archival specimens are a great resource for molecular research in population biology, taxonomy and conservation. A primary goal for researchers is to preserve specimens from collections by improving non-invasive methods for DNA extraction and to achieve successful amplification of the short fragments of a target gene in the event of DNA fragmentation. We tested the suitability of a noninvasive method of DNA extraction and amplification of the barcoding region of the mitochondrial gene cytochrome c oxidase subunit I from archival specimens of aphid parasitoids belonging to the genera *Aphidius*, *Lysiphlebus* and *Praon* (Aphidiinae, Braconidae, Hymenoptera). Using a commercial kit as a noninvasive method, we successfully extracted DNA from dry 7 to 41 year old samples of 26 different parasitoid species. However, amplification of the barcoding region failed using the standard primer pair LCO1490/HCO2198. In order to reconstruct DNA barcodes we designed internal genus-specific degenerative primers and a new amplification protocol to target the short fragments within the mitochondrial region. Novel primers were designed using as a template the reference sequences from congeners retrieved from the public database. The combination of standard primers with internal primers, in direct and nested amplification reactions, produced short overlapping subsequences, concatenated to recover long barcoding sequences. Additional analyses also confirmed that primers initially designed for *Aphidius*, *Lysiphlebus* and *Praon* can be combined in a mixture, and successfully used to obtain short fragments of disintegrated DNA from archival specimens of several other braconid species from the genera *Ephedrus* and *Monoctonus*.

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Keywords
COI, archival specimens, *Aphidius*, *Ephedrus*, *Lysiphlebus*, *Monoctonus*, *Praon*, short fragments

Introduction

The DNA from an archival species is an important source of data in the areas of population genetics, conservation, taxonomy and phylogeny. In the past researchers were in conflict between the maintenance of specimens undamaged and their use in molecular analyses, which created a strong limitation for studies on museum specimens, in particular studies with rare or extinct species, or those restricted to one or a few individuals collected many years ago (Gilbert et al. 2007; Mandrioli 2008). However, archival DNA study is now a rapidly developing area of research due to the continual improvements of molecular tools with which it is possible to recover DNA information from museum specimens and dry remains, without damaging the material.

Insects are a group where these tools have received increasing attention and noninvasive techniques have been developed and used for a variety of orders (Gilbert et al. 2007; Andersen and Mills 2012). Noninvasive methods of DNA extraction from dried specimens are important in order to preserve the quality of museum specimens. Unfortunately, not all specimens contain DNA of suitable quality and in the right amount for conclusive genetic studies. Successful amplification depends on post-mortem processes of DNA degradation, which can cause miscoding lesions or physical destruction of the DNA molecule (Rizzi et al. 2012). Degradation of DNA consequently produces methodological difficulties in amplification and sequencing of the target region, processes that are limited by the small quantity of template DNA and recovery of short fragments. Besides natural processes of disintegration, another factor that makes archival specimens difficult to work with is the preservation methodology, which can over time result in DNA damage (Dillon et al. 1996; Burrell et al. 2015). In the case of parasitic Hymenoptera, Andersen and Mills (2012) determined that age was a significant factor for successful sequencing, while size and DNA concentration did not influence the amplification of the targeted nuclear and mitochondrial genes.

Parasitoid Hymenoptera are a taxonomically challenging group under frequent revision, making them a group of great interest for retrieval of genetic information from museum specimens (Andersen and Mills 2012). Among parasitoids that have been intensively surveyed by taxonomists and ecologists are aphid endoparasitoids from the subfamily Aphidiinae (Braconidae, Hymenoptera). They are distributed worldwide, closely following the distribution of their aphid hosts (Starý 1988). As solitary endoparasitoids, Aphidiinae are one of the most important natural enemies of aphids and can effectively regulate their populations (Hågvar and Hofsvang 1991). They have been commercially produced and released as classical biological control agents of aphids in many regions and have achieved significant results in diverse agroecosystems. The most important genera of aphid parasitoids used in biological control are *Aphidius* Nees, 1818; *Diaeretiella* Starý, 1960; *Ephedrus* Haliday, 1833 and *Praon* Haliday, 1833 (Boivin et al. 2012).

The subfamily Aphidiinae is a diverse group with many cryptic species complexes, and reliable identification is therefore of key importance for their use as biological control agents.

This study included aphid parasitoids belonging to the common aphidiine genera *Aphidius*, *Lysiphlebus* Förster, 1862 and *Praon*. Identification based on morphology has often been shown to be inadequate in distinguishing the species of these genera due to the limited number of valid discriminatory morphological characters, as well as their high variation on the intraspecific level (Pungerl 1983; Kavallieratos et al. 2005, 2010; Tomanović et al. 2003, 2004). Furthermore, several species have confusing taxonomic histories and are in need of revision. In fact, over the last two decades these genera have been constantly rearranged on the basis of new morphological characters and more recently obtained molecular data as well.

Mitochondrial barcoding region of the cytochrome oxidase c subunit I (COI) had been used to reconstruct phylogenetic relationships within the genera (Jafari-Ahmadabadi et al. 2011), and examine the phylogenetic affinity and diversity of Aphidiinae from different geographical regions (Lenin 2015). In addition, it has successfully detected immature stages of parasitoids inside their aphid hosts, e.g., *Lysiphlebus testaceipes* Cresson, 1880 inside its host *Aphis fabae* Scopoli, 1763 (Traugott and Symondson 2008). Either solely or in combination with morphometric methods, the barcoding method was routinely applied in revisiting and resolving the taxonomic status of many species complexes. For example, three species - *Aphidius colemani* Viereck, 1912; *A. platensis* Brèthes, 1913, and *A. transcaspicus* Telenga, 1958- were distinguished within the *A. colemani* group (Tomanović et al. 2014); three species - *A. rubi* Starý, 1962; *A. silvaticus* Starý, 1962, and *A. urticae* Haliday, 1834 were re-described within the *A. urticae* group (Jamhour et al. 2016); two new species - *Praon longicaudus* Tomanović & Starý, 2014 and *P. sambuci* Tomanović & Starý, 2014 - were described within the species complex *Praon abjectum* Haliday, 1833 (Mitrovski et al. 2013); the species status of *P. dorsale* Haliday, 1833; *P. longicorne* Marshall, 1896; *P. volucre* Haliday, 1833, and *P. yomenae* Takada, 1968 was confirmed and a new species, viz., *Praon staticobii* Tomanović & Petrović, 2014 was described within the *Praon dorsale-yomenae* s. str. group (Mitrovski et al. 2014). Apart from taxonomic revisions, the barcoding marker was successfully used to discover new allochthonous species accidentally introduced into new habitats, such as the invasive species *Lysiphlebus orientalis* Starý & Rakhshani, 2010 (Petrović et al. 2013) and *Aphidius ericaphidis* Pike & Starý, 2011 (Petrović et al. 2017).

Considering that these parasitoids are important for fundamental taxonomic and conservation research, as well as being potential biological control agents in aphid management programs, it would be of great value to investigate the possibility of recovering barcoding fragments of COI from museum specimens. Thus, the main objectives of this study were as follows: i) DNA extraction from dry archival specimens belonging to the genera *Aphidius*, *Lysiphlebus* and *Praon* using a noninvasive method; ii) PCR amplification of several short and overlapping fragments within the barcoding region of cytochrome c oxidase subunit I, iii) traditional Sanger sequencing and alignment of

different short overlapping fragments and concatenation to recover longer target barcoding region of mitochondrial DNA and iv) testing the suitability of novel primers for targeting barcodes in archival specimens of other braconid species.

Material and methods

Analyses included species from three different genera of aphid parasitoids, viz., *Aphidius*, *Praon* and *Lysiphlebus*. In total 45 specimens were submitted to molecular analyses, including 11 species of *Aphidius*, nine of *Lysiphlebus* and six of *Praon*, killed and preserved in dry condition from 7 to 41 years prior to DNA extraction (Table 1). Additionally, in order to test the suitability of these primers in amplification of other parasitiods we chose four species from the genus *Monoctonus* Haliday, 1833 and four of *Ephedrus* Haliday, 1833, all dry material up to 31 year old (Table 1).

Table 1. The list of analyzed species from the genera *Aphidius*, *Lysiphlebus*, *Praon*, *Ephedrus*, *Monoctonus* with designated aphid host/plant associations and geographic origin.

Sample code	Parasitoid species	Country of origin	Sampling year/ age of samples*	Host plant	Aphid host	Specimen condition **
AF1	*Aphidius tanacetarius*	Serbia	2011/7	*Tanacetum vulgare*	*Metopeurum fuscoviridae*	F
AF2	*Aphidius sussi*	Montenegro	2005/13	*Aconitum toxicum*	*Delphiniobium junackianum*	F
AF 3	*Aphidius sonchi*	Serbia	2010/8	*Sonchus arvensis*	*Hyperomyzus lactucae*	F
AF4	*Aphidius linosiphonis*	Montenegro	2011/7	*Galium* sp.	*Linosiphon* sp.	F
AF5	*Aphidius ribis*	Montenegro	2011/7	*Ribes petreum*	*Cryptomyzus* sp.	F
AD1	*Aphidius funebris*	Serbia	1998/20	*Crepis* sp.	*Uroleucon* sp.	D
AD2	*Aphidius absinthii*	Serbia	2001/17	*Artemisia vulgaris*	*Macrosiphoniella* sp.	D
AD3	*Aphidius sussi*	Serbia	1998/20	*Aconitum toxicum*	*Delphiniobium junackianum*	D
AD4	*Aphidius ervi*	Slovenia	2009/9	*Triticum aestivum*	*Sitobion avenae*	D
AD5	*Aphidius eadyi*	Russia	2007/11	*Pisum sativum*		D
AD6	*Aphidius eglanteriae*	Serbia	1996/22	*Rosa* sp.	*Chaetosiphon* sp.	D
AD7, AD8	*Aphidius avenae*	Montenegro	2000/18	*Salix retusa*		D
AD9	*Aphidius sussi*	Serbia	2000/18	*Aconitum pentheri*	*Delphiniobium junackianum*	D
AD10	*Aphidius arvensis*	Iran	2010/8	*Inula* sp.	*Aphis sargasi*	D
AD11	*Aphidius erysimi*	Czech Republic	1999/19	*Erisymum* sp.	*Pseudobrevicoryne erysimi*	D
AD12	*Aphidius eglanteriae*	Serbia	1998/20	*Thalictrum elatum*	*Longicaudus trirhodus*	D
AD13	*Aphidius smithi*	United States	1977/41	*Medicago sativa*	*Acyrthosiphon pisum*	D

Sample code	Parasitoid species	Country of origin	Sampling year/ age of samples*	Host plant	Aphid host	Specimen condition **
AD14	*Aphidius eadyi*	Iran	1977/41	*Medicago sava*	*Acyrthosiphon pisum*	D
AD15	*Aphidius banksae*	Israel	1979/39	*Medicago sativa*	*Acyrthosiphon pisum*	D
PF1	*Praon volucre*	Iran	2009/9	*Sonchus oleraceus*	*Uroleucon sonchi*	F
PF2	*Praon dorsale*	Serbia	2010/8	*Corylus avelana*		F
PF3	*Praon abjectum*	Serbia	2011/7	*Thallium aquile*	*L. trialeurodes*	F
PD1	*Praon longicorne*	Montenegro	2009/9	*Geranium robertianum*	*Aphis malvae*	D
PD2	*Praon dorsale*	Montenegro	2006/12	*Filipendula ulmaria*	*Macrosiphum cholodkovskyi*	D
PD3	*Praon longicorne*	Serbia	2006/12	*Rubus* sp.	*Macrosiphum funestum*	D
PD4	*Praon yomenae*	Montenegro	2009/9	*Rubus* sp.		D
PD5	*Praon yomenae*	Iran	2009/9	*Acroptilon repens*	*Uroleucon* sp.	D
PD6	*Praon longicorne*	Czech Republic	2008/10	*Rubus* sp.	*Macrosiphum funestum*	D
PD7	*Praon spinosum*	Croatia	2005/13	*Carex nigra*	*Thripsaphis verrucosa*	D
PD8	*Praon spinosum*	Croatia	2009/9	*Carex* sp.	*Thripsaphis verrucosa*	D
PD9, PD10, PD11	*Praon longicorne*	Czech Republic	1998/20	*Urtica dioica*	*Microlophium carnosum*	D
PD12	*Praon barbatum*	Serbia	2011/7	*Medicago sativa*	*Acyrthosiphon pisum*	D
PD13	*Praon necans*	Serbia	2005/12	*Typha* sp.	*Rhopalosiphum nymphaeae*	D
PD14, PD15	*Praon yomenae*	Japan	2002/16	*Hemerocallis fulva*	*Indomegoura indica*	D
LF1	*Lysiphlebus hirticornis*	Serbia	2011/7	*Tanacetum vulgare*	*Metopeurum fuscoviridae*	F
LF2	*Lysiphlebus cardui*	Serbia	2010/8	*Cirsium arvense*	*Aphis fabae cirsicanthoides*	F
LF3	*Lysiphlebus fabarum*	Serbia	2009/9	*Cirsium arvense*	*Aphis fabae cirsicanthoides*	F
LD1	*Lysiphlebus hirticornis*	Serbia	2011/7	*Tanacetum vulgare*	*Metopeurum fuscoviridae*	D
LD2	*Lysiphlebus cardui*	Serbia	2010/8	*Cirsium arvense*	*Aphis fabae cirsicanthoides*	D
LD3	*Lysiphlebus fabarum*	Serbia	2009/9	*Cirsium arvense*	*Aphis fabae cirsicanthoides*	D
LD4	*Lysiphlebus testaceipes*	Italy	2006/12	*Hedera helix*	*Aphis hederae*	D
LD5	*Lysiphlebus testaceipes*	France	2006/12	*Rubus fruticosus*	*Aphis ruborum*	D
LD6	*Lysiphlebus testaceipes*	Costa Rica	2000/18	*Eugenia wilsonii*	*Toxoptera aurantii*	D
LD7	*Lysiphlebus fritzmuelleri*	Serbia	2006/12	*Vicia cracca*	*Aphis craccae*	D
LD8	*Lysiphlebus confusus*	Iran	2005/13	*Verbascum* sp.	*Aphis verbasci*	D
LD9, LD10	*Lysiphlebus desertorum*	Iran	2005/13	*Achillea millefolium*	*Protaphis* sp.	D

Sample code	Parasitoid species	Country of origin	Sampling year/ age of samples*	Host plant	Aphid host	Specimen condition **
LD11, LD12	*Lysiphlebus fabarum*	Iran	2005/13	*Tragopogon pratensis*	*Brachycaudus tragopogonis*	D
LD13	*Lysiphlebus alpinus*	Serbia	1996/22	*Daucus carota*	*Semiaphis dauci*	D
LD14	*Lysiphlebus melandriicola*	Chech Republic	1998/20	*Carduus* sp.	*Brachycaudus cardui*	D
LD15	*Lysiphlebus fabarum*	Iran	2005/13	*Tragopogon pratensis*	*Brachycaudus tragopogonis*	D
ED1	*Ephedrus laevicollis*	Serbia	2000/18	*Rosa* sp.	*Chaetosiphon* sp.	D
ED2	*Ephedrus plagiator*	Montenegro	2004/14	*Lonicera xylosteum*	*Hyadaphis* sp.	D
ED3	*Ephedrus validus*	Finland	1987/31			D
ED4	*Ephedrus koponeni*	Finland	1987/31			D
MD1	*Monoctonus paulensis*	Canada	2005/13	*Capsicum annuum*	*Myzus persicae*	D
MD2	*Monoctonus allisoni*	USA	2001/17	*Delphinium galucum*	*Nasonovia* (*Eokakimia*) *wahinkae*	D
MD3	*Monoctonus washingtonensis*	USA	1992/26	*Triticum* sp.	*Rhopalosiphum padi*	D
MD4	*Monoctonus leclanthi*	Montenegro	2002/16	*Aconitum toxicum*	*Delphiniobium junackianum*	D

* number of years the specimens were kept dry in collections prior to DNA extraction

**Specimen condition: (F) fresh refers to specimens kept after collection in 96% ethanol; (D) dry are specimens which were kept dry in collections, pinned or glued to cardboard

DNA extraction

Dry specimens were carefully removed from the card points so that they could be remounted afterwards if the specimens are holotypes. The whole specimens were used for DNA extraction using the QIAGEN Dneasy Blood and Tissue Kit. In the case of parasitoid specimens used as a control, they were preserved in 96% ethanol prior to extraction. Whole specimens were placed in 2 ml Eppendorf tubes with proteinase K and ATL buffer. After incubation overnight at 56 °C insect specimens were removed from the buffer, rinsed with 96% ethanol several times, air-dried and put back in the collection. The remaining solution was treated according to the manufacturer's instructions.

PCR amplification

The first step was an attempt to amplify a barcoding region of mitochondrial gene cytochrome c oxidase subunit I from dry material using the standard primer pair LCO1490/HCO2198 (Folmer et al. 1994). Each PCR reaction was carried out in a volume of 20µl, including: 1µl of extracted DNA, 11.8 µl H_20, 2 µl High Yield Reac-

tion Buffer A with 1xMg, 1.8 µl of $MgCl_2$ 2.25 mM, 1.2 µl of dNTP 0.6 mM, 1µl LCO1490 0.5 µM, 1µl HCO2198 0.5 µM, 0.2 µl DNA polymerase 0.05U/µl. The amplification protocol included : i) initial denaturation at 95 °C for 5 min; ii) 35 cycles of 1 min at 94 °C, 1 min at 54 °C and 30 sec at 72 °C and iii) final extension at 72 °C for 7 min. Products were visualized on agarose gel.

Due to DNA fragmentation in dry specimens, internal degenerative primers were designed to amplify overlaping short fragments of COI through direct and nested PCR, which could thereafter be aligned to a longer barcoding sequence (Fig. 1). Reference COI sequences of parasitoids retrieved from the GenBank (www.ncbi.nlm.nih.gov/Genbank) were used as a template to design primers for dry material of the genera *Aphidius*, *Praon* and *Lysiphlebus* (Table 2). They were aligned and manually searched for shared conservative regions on which to place the newly designed primers.

The initial idea was to divide the barcoding fragment of COI obtained with LCO1490/HCO2198 into three overlapping subsequences, around 260 bp, 270 bp and 280 bp long respectively, and the primers designed for this were marked as for direct PCR. Furthermore, additional internal primers were designed within these three subsequences to amplify even shorter fragments through nested PCR (Fig. 1).

The genus-specific degenerative primers were used in combination with standard primers LCO1490 and HCO2198 (Fig. 1). Finally, the position of internal primers allowed diverse combinations and targeting of overlapping fragments of different length and position. Due to the shared conservative sites in COI sequences, it was possible for primers initially designed for *Aphidius* species to be also used in amplification of short fragments in combination with primers specifically designed for *Lysiphlebus* species (Aph1Rn, Aph2Fd, Aph3Rn) and for dry *Praon* specimens as well (Aph2Fn) (Fig. 1).

Prior to testing their suitability for amplification of short fragments from dry samples, the designed primers were initially tested on control specimens preserved in

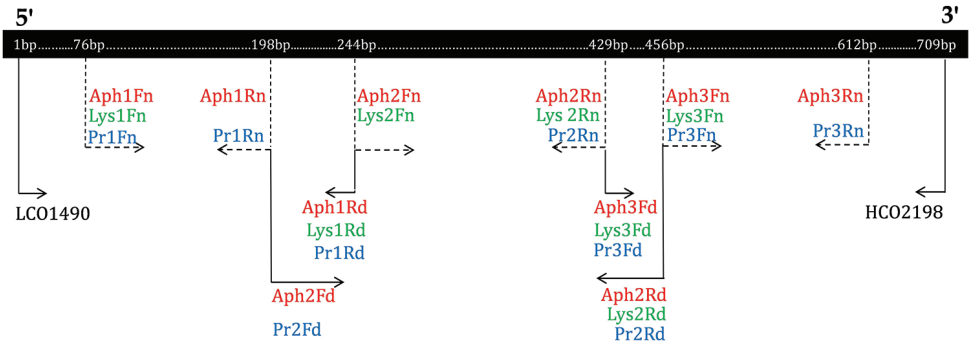

Figure 1. Position of internal degenerative primers within the barcoding region of COI. *Aphidius* - specific primers: Aph1Fn, Aph1Rn, Aph1Rd, Aph2Fd, Aph2Fn, Aph2Rn, Aph2Rd, Aph3Fd, Aph3Fn and Aph3Rn; *Lysiphlebus* - specific primers: Lys1Fn, Lys1Rd, Lys2Fn, Lys2Rn, Lys2Rd, Lys3Fd and Lys3Fn; *Praon* - specific primers: Pr1Fn, Pr1Rn, Pr1Rd, Pr2Fd, Pr2Rn, Pr2Rd, Pr3Fd, Pr3Fn and Pr3Rn. Arrows refer to the direction of the primers, forward or reverse. The exact position of internal primers is designated in comparison to the first nucleotide of the forward LCO1490 primer sequence (5' GGTCAACAAATCATAAAGATATTGG 3').

Table 2. The list of reference *Aphidiinae* species obtained from GenBank and used in designing the genus-specific primers.

Parasitoid species	Accession number
Aphidius matricariae	JN620563
Aphidius urticae	JN620590
Aphidius sonchi	JN620589
Aphidius rhopalosiphi	JN164779
Aphidius ervi	JQ723411
Aphidius microlophii	JN620566
Aphidius uzbekistanicus	JN164751
Aphidius funebris	JN620561
Aphidius rosae	JN620582
Aphidius eadyi	JN620551
Aphidius salicis	JN620585
Aphidius ribis	JN620579
Aphidius colemani	KJ615362
Aphidius transcaspicus	KJ615375
Lysiphlebus testaceipes	HQ599569
Lysiphlebus orientalis	KC237736
Lysiphlebus hirticornis	HQ724540
Lysiphlebus fabarum	JQ723416
Lysiphlebus cardui	JN620640
Lysiphlebus confusus	KM408535
Praon barbatum	JN620671
Praon yomenae	JN620693
Praon gallicum	JN620680
Praon abjectum	KC128671
Praon dorsale	KC128677
Praon exsoletum	KJ848478

96% ethanol. In total, five *Aphidius* species were submitted to initial testing (samples AF1-AF5; Table 1). Three following primer combinations were confirmed successful in direct PCR reactions: i) LCO1490/Aph1Rd, ii) Aph2Fd/Aph2Rd and iii) Aph-3Fd/HCO2198 (Fig. 2). Three species from the genus *Praon* were used for test trials (samples PF1- *P. volucre*, PF2- *P. dorsale* and PF3- *P. abjectum*; Table 1). Three individual analyses were conducted: 1. LCO1490/Pr1Rd; 2. Pr2Fd/Pr2Rd; and 3. Pr3Fd/HCO2198. All of the products with fresh samples were visualized (Fig. 3). *Lysiphlebus hirticornis* Mackauer, 1960 (LF1), *L. cardui* Marshall, 1896 (LF2) and *L. fabarum* Marshall, 1896 (LF3) were included in the initial trials (Table 1). The four following primer combinations were confirmed suitable: 1) LCO1490/Lys1Rd; 2) Aph2Fd/Lys2Rd; 3) Pr2Fd/Lys2Rd; and 4) Lys3Fd/ HCO2198 (Fig. 5).

After confirmation of their suitability, the new primers were then used in trials with dry specimens. Products of PCR were obtained in 40 µl volumes. In the direct PCR reac-

Table 3. The list of primers designed for the genera *Aphidius*, *Lysiphlebus* and *Praon* to amplify short fragments of COI barcoding region from dry specimens through direct and nested PCR analyses.

Parasitoid group	Primer name*	5 ' 3' primer sequence**	Primer direction
Aphidius	Aph1Rd	GRGGRAAAGCYATATCAGGAG	reverse
Aphidius	Aph1Fn	TAAGWTTATTAATTCGWATRGA	forward
Aphidius	Aph1Rn	CAATTWCCAAATCCWCCAATTAT	reverse
Aphidius	Aph2Fd	ATAATTGGWGGATTTGGWAATTG	forward
Aphidius	Aph2Rd	GTWCTAATAAAATTAATWGCWCC	reverse
Aphidius	Aph2Fn	CTCCTGATATRGCTTTYCCYC	forward
Aphidius	Aph2Rn	GADGAAATHCCTGCTAAATG	reverse
Aphidius	Aph3Fd	CATTTAGCWGGDATTTCYTC	forward
Aphidius	Aph3Fn	GGAGCWATTAATTTTATTAGWAC	forward
Aphidius	Aph3Rn	GTAGTATTTAARTTWCGATC	reverse
Lysiphlebus	Lys1Rd	GAGGAAAAGCYATATCWGGAG	reverse
Lysiphlebus	Lys1Fn	TAAGWTTAATTATTCGWATRGA	forward
Lysiphlebus	Lys2Rd	GTWCTAATAAAATTAATTGCHCC	reverse
Lysiphlebus	Lys 2Fn	CTCCWGATATRGCTTTTCCTC	forward
Lysiphlebus	Lys 2Rn	GAWGAAATACCWGCTAAATG	reverse
Lysiphlebus	Lys3Fd	CATTTAGCWGGDATTTCWTC	forward
Lysiphlebus	Lys3Fn	GGDGCAATTAATTTTATTAGWAC	forward
Praon	Pr1Rd	GAGGRAAAGCTATATCAGGAG	reverse
Praon	Pr1Fn	AAGWGATCAAATTTAYAATAG	forward
Praon	Pr1Rn	CAATTWCCAAAYCCWCCAATTAT	reverse
Praon	Pr2Fd	ATAATTGGAGGRTTTGGWAATTG	forward
Praon	Pr2Rd	GTTGWAATAAAATTAATWGCYCC	reverse
Praon	Pr2Rn	CATTTAGCWGGTATTTCWTC	reverse
Praon	Pr3Fd	CATTTRGCTGGWATTTCYTC	forward
Praon	Pr3Fn	GGAGCWATTAATTTTATTWC	forward
Praon	Pr3Rn	GTWGTATTTAWATTTCGATC	reverse

*the last letter in the primer's name refers to PCR reaction: *d*-direct and *n*-nested

**degenerative base designation/actual base coded: R or - A, or - G; Y or -C or - T; W or -A, or - T.

tion, 4 µl of extracted DNA was added into 36 µl of mix, following the recipe described for the LCO1490/HCO2198 primer pair. In nested PCR, 0.25 µl of a product from direct PCR was added into 39.75 µl of mix. The following protocol was developed for direct and nested PCR: i) initial denaturation at 95 °C for 5 min; ii) 37 cycles of 1 min at 95 °C, 1 min at 54 °C, and 30 sec at 72 °C; and iii) final extension at 72 °C for 7 min.

Amplified COI fragments were sequenced in both directions using an automated equipment (Macrogen Inc, Seoul, South Korea). Overlapping short fragments of the barcoding region were manually edited in FINCHTV ver.1.4.0 (www.geospiza.com), concatenated to obtain longer sequences and aligned using the CLUSTAL *W* program integrated in MEGA5 (Tamura et al. 2011). A Maximum likelihood tree was constructed using the MEGA5 software, with 500 bootstrap replicates performed to assess the branch support. The evolutionary distances were computed using the Tamura-Nei

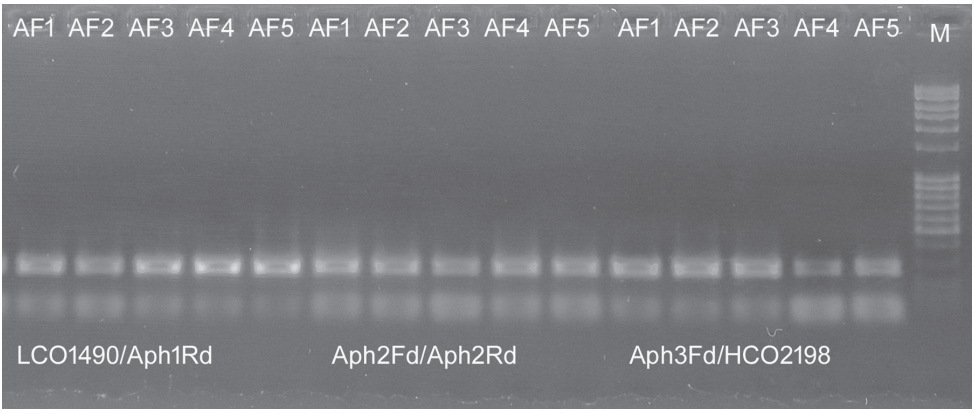

Figure 2. Agarose gel visualizing the products of direct PCR in initial trials testing the novel primers with fresh *Aphidius* samples. Three direct PCR reactions were conducted with the following primer pairs: **1** LCO1490/Aph1Rd **2** Aph2Fd/Aph2Rd; and **3** Aph3Fd/HCO2198. The species included in trials were: AF1- *A. tanacetarius*, AF2- *A. sussi*, AF3- *A. sonchi*, AF4- *A. linosiphonis* and AF5- *A. ribis*. M – marker.

method (Tamura and Nei, 1993). Phylogenetic analyses included the sequenced barcodes recovered from archival parasitoid specimens combined with the reference COI sequences of Aphidiinae from GenBank.

Results

Initial trials with dry specimens using standard primer pair for the COI barcoding region LCO1490/HCO2198 failed to give products. Thereafter, 15 dry specimens of 11 different *Aphidius* species (*A. absinthii* Marshall, 1896; *A. arvensis* Starý, 1960; *A. avenae* Haliday, 1834; *A. banksae* Kittel, 2016; *A. eadyi* Subba Rao and Sharma, 1959; *A. eglanteriae* Haliday, 1834; *A. erysimi* Starý, 1960; *A. funebris* Mackauer, 1961; *A. ervi* Haliday, 1834; *A. smithi* Subba Rao and Sharma, 1959; *A. sussi*) were submitted to molecular analyses (Table 1). Insects had been killed and stored dry in collections for 8 to 41 years prior to DNA extraction. The same three combinations of standard and degenerative primers previously confirmed as suitable in the test trials with fresh material were used with dry samples AD1-AD15 as well. Direct PCR produced amplicons in all three combinations for samples AD1 to AD6, while in the cases of samples AD7 to AD15 no product was visualized. The products from direct PCR with primer pair LCO1490/Aph1Rd were submitted to two independent nested reactions with primers LCO1490/Aph1Rn and Aph1Fn/Aph1Rd; from direct PCR with primers Aph2Fd/Aph2Rd to nested reactions with Aph2Fd/Aph2Rn and Aph2Fn/Aph2Rd; and products obtained with Aph3Fd/HCO2198 were included in nested trials with the primers Aph3Fd/Aph3Rn and Aph3Fn/HCO2198. In all six individual nested reactions short fragments of the barcoding region were amplified successfully and visualized for all of the tested samples.

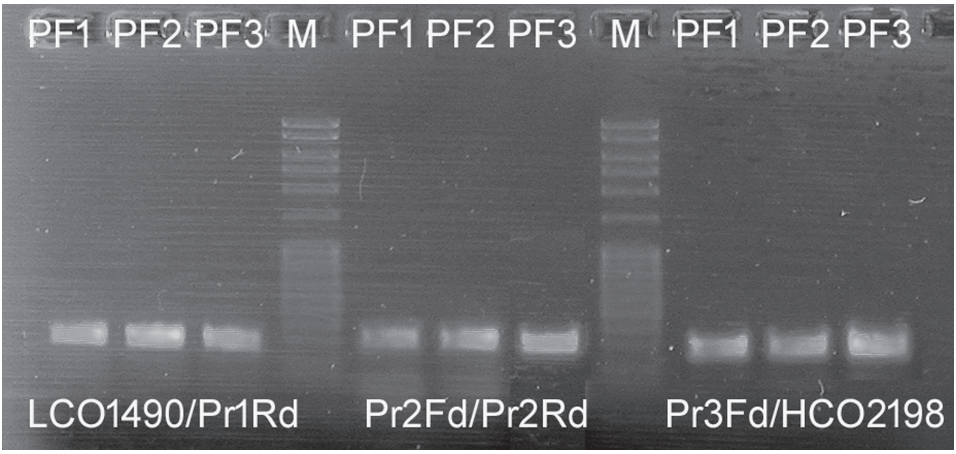

Figure 3. Agarose gel visualizing the products of direct PCR in initial trials testing the novel primers with fresh *Praon* samples. Three direct PCR reactions were conducted with primer pairs: 1. LCO1490/Pr1Rd, 2. Pr2Fd/Pr2Rd, 3. Pr3Fd/HCO2198. The species included in trials are PF1- *P. volucre*, PF2- *P. dorsale*, PF3- *P. abjectum*; M – marker.

In total 15 specimens of eight *Praon* species preserved dry for 7 to 20 years prior to DNA extraction were analysed (Table 1). We attempted to retrieve short overlapping fragments of COI barcodes from dry samples PD1-PD15 through the same three direct amplifications as with the fresh material. In analyses with primers targeting the first fragment of the barcoding sequence, all products were obtained. In the second and third reactions short fragments of barcode were amplified in samples PD1-PD11 and PD13, while no product was visualized for samples PD12, PD14 and PD15. The same methodological approach was applied here, namely using the products from direct PCR as a template for secondary nested trials. The amplicons of samples PD12, PD14 and PD15 from the trial with primer pair Pr2Fd/Pr2Rd were processed further in two nested reactions with combinations Pr2Fd/Pr2Rn and Aph2Fn/Pr2Rd, while the products of direct PCR with Pr3Fd/HCO2198 were processed in secondary analyses using the combinations Pr3Fd/Pr3Rn and Pr3Fn/HCO2198. Subsequent analyses successfully targeted short fragments within the subsequences of the barcoding region in all four nested test trials (Fig. 4).

The novel primers were tested on *Lysiphlebus alpinus* Starý, 1971; *L. confusus* Tremblay & Eady, 1978; *L. desertorum* Starý, 1965; *L. fabarum*; *L. fritzmuelleri* Mackauer, 1960; *L. hirticornis*; *L. melandriicola* Starý, 1961; *L. testaceipes*), stored dry in collections for 7 to 22 years. Three separate analyses were conducted using the primer combinations confirmed as suitable with fresh material. Amplicons were visualized in the first direct analysis with the LCO1490/Lys1Rd combination for samples LD1-LD7 and LD10-LD15. No products were visible for samples LD8 and LD9 which were further processed in nested trials with LCO1490/Lys1Rn and Lys1Fn/Lys1Rd. Products of the direct PCR conducted with the primer combination Aph2Fd/Lys2Rd were obtained in all samples except LD8, LD9 and LD12 which were thereafter processed in nested analyses with 1. Aph2Fd/Lys2Rn; and 2. Lys2Fn/Lys2Rd. In the third direct

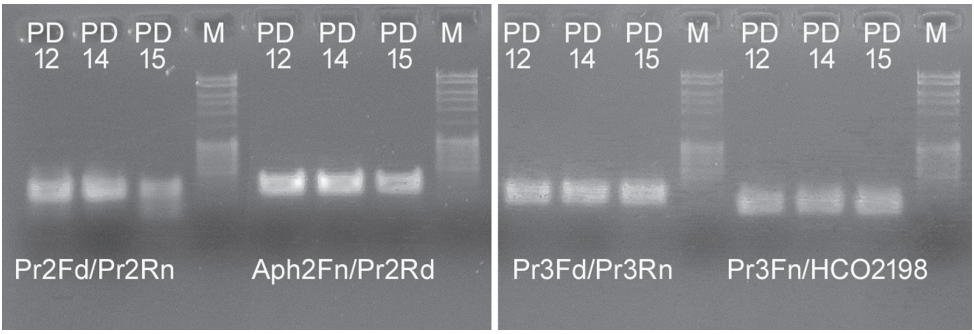

Figure 4. Agarose gel visualizing the products of nested trials with products of direct PCR for samples PD12 - *P. barbatum*, PD14 - *P. yomenae*, and PD15 - *P. yomenae*. The products from PCR with Pr2Fd/Pr2Rd were submitted to secondary nested trials with primer pairs Pr2Fd/Pr2Rn and Aph2Fn/Pr2Rd. Amplicons obtained with Pr3Fd/HCO2198 were used as the template for nested reactions with Pr3Fd/Pr3Rn and Pr3Fn/HCO2198.

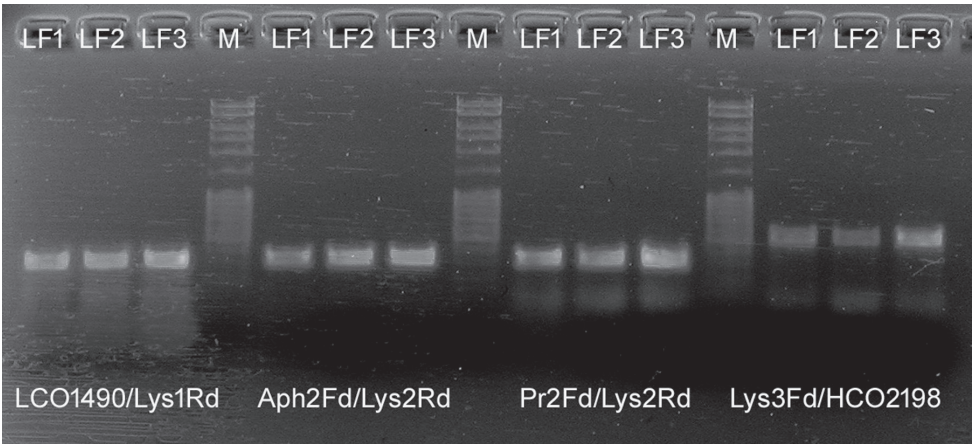

Figure 5. Agarose gel visualizing the products of direct PCR in initial trials testing the novel primers with fresh *Lysiphlebus* samples. Tested combinations of primers were: 1) LCO1490/Lys1Rd; 2) Aph2Fd/Lys2Rd; 3) Pr2Fd/Lys2Rd; and 4) Lys3Fd/HCO2198. The species included in trials were: LF1 - *L. hirticornis*; LF2 - *L. cardui*; and LF3 - *L. fabarum*; M – marker.

PCR trial, amplicons were visualized in all analyzed specimens besides LD8, LD9 and LD13 which were further submitted to analyses with primers 1. Lys3Fd/Aph3Rn; and 2. Lys3Fn/HCO2198. We obtained products in all nested trials (Fig. 6).

Our research covers different taxonomically challenging Aphidiinae, for which reason we tested suitability of the newly designed primers on several other archival specimens from the genera *Monoctonus* and *Ephedrus*. In order to preserve the limited amount of DNA obtained from dry specimens, we avoided blind PCR trials as well as testing of all possible combinations by doing initial alignment of barcode sequences of fresh material (unpublished data) and degenerative primers (Table 4). According to the alignment we chose the primers best suited to target the species of interest.

Table 4. Comparison of barcode fragments of COI for *Monoctonus* and *Ephedrus* with degenerative primers sequences.

Degenerative primer	Difference in base pair substitutions (bp)	
	***Monoctonus* sp.**	***Ephedrus* sp.**
Aph1Rd	0–2 bp	4–6 bp
AphF1n	2–5 bp	0–3 bp
Aph1Rn	0–4 bp	0–4 bp
Aph2Fd	0–4 bp	2–3 bp
Aph2Rd	0–2 bp	2–5 bp
Aph2Fn	0–2 bp	4–7 bp
Aph2Rn	1–3 bp	2–5 bp
Aph3Fd	0–3 bp	1–4 bp
Aph3Fn	0–2 bp	4–7 bp
Aph3Rn	0–1 bp	1–4 bp
Lys1Rd	0–2 bp	4–5 bp
Lys1Fn	0–4 bp	0–4 bp
Lys2Rd	0–1 bp	1–2 bp
Lys2Fn	0–2 bp	5–7 bp
Lys2Rn	1–4 bp	0–5 bp
Lys3Fd	0–3 bp	1–3 bp
Lys3Fn	0–1 bp	5–7 bp
Pr1Rd	0–3 bp	3–5 bp
Pr1Fn	1–4 bp	4–6 bp
Pr1Rn	0–4 bp	1–3 bp
Pr2Fd	0–4 bp	0–2 bp
Pr2Rd	1–2 bp	0–1 bp
Pr2Rn	1–4 bp	0–4 bp
Pr3Fd	0–4 bp	0–4 bp
Pr3Fn	1–3 bp	4–7 bp
Pr3Rn	0–2 bp	0–3 bp

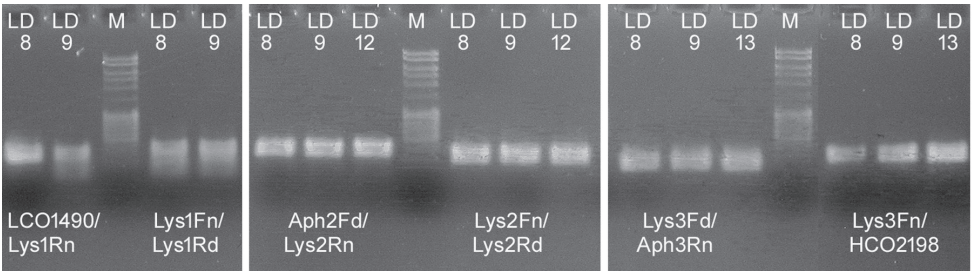

Figure 6. Agarose gel visualizing the products of nested trials with products of direct PCR for samples LD8 – *L. confusus*, LD9 – *L. desertorum*; LD12 – *L. fabarum*; and LD13 – *L. alpinus*. The products of LD8 and LD9 from PCR with LCO1490/Lys1Rd were submitted to secondary reactions combining two primer pairs, viz., 1. LCO1490/Lys1Rn; and 2. Lys1Fn/Lys1Rd. Amplicons of LD8, LD9 and LD12 obtained with Aph2Fd/Lys2Rd were submitted to secondary nested trials with primer pairs Aph2Fd/Lys2Rn and Lys2Fn/Lys2Rd. Products from direct PCR with Lys3Fd/HCO2198 were used as the template for nested reactions with Lys3Fd/Aph3Rn and Lys3Fn/HCO2198.

In the case of *Ephedrus* species, we chose two combinations for direct PCR, i.e., 1. LCO1490/Pr2Rd, and 2. Aph3Fd/HCO2198. Four species preserved in dry condition for 14 to 31 years in collections were included in the test trials, viz., *E. plagiator* Nees, 1811 (ED1); *E. laevicollis* Thomson, 1895 (ED2); *E. validus* Haliday, 1833 (ED3); and *E. koponeni* Halme, 1992 (ED4) (Table 1). Amplicons of both targeted fragments were visualized on gel for specimens ED1, ED3, and ED4, while in the case of the ED2 sample a PCR product was visible only with primer pair Aph3Fd/HCO2198. Products of the ED2 were subjected to separate nested reactions with primer pair LCO1490/Pr1Rd and Pr2Fd/Pr2Rd. Both short fragments of the barcode were successfully amplified and concatenated with the third subsequence obtained in direct PCR to retrieve a longer barcode fragment of COI.

Dry specimens of the following four *Monoctonus* species preserved for 13 to 26 years were subjected to PCR analyses: *M. paulensis* (Ashmead) (MD1); *M. allisoni* Pike and Starý, 2003 (MD2); *M. washingtonensis* Pike and Starý, 1995 (MD3); and *M. leclanthi* Tomanović and Starý, 2002 (MD4). The same approach was repeated as with *Ephedrus*, i.e., barcoding sequences of fresh material were aligned and analysed for primers suitability prior to molecular analyses (Table 4). The final choice fell on three combinations in direct PCR to retrieve three overlapping short fragments within the barcoding COI fragment: 1. LCO1490/Aph1Rd; 2. Pr2Fd/Lys2Rd; and 3. Pr3Fd/HCO2198. The final results show that the tested combinations of standard and degenerative primers successfully amplified all three short subsequences in all tested *Monoctonus* species.

The overall results of combining different primers in direct and secondary nested reactions are summarized in Fig. 7.

Short fragments of the COI barcodes obtained from direct and nested PCR analyses of the following samples were deposited in the GenBank: AD4 - *A. ervi* (MG991997), AD7 - *A. avenae* (MG991998), AD10 - *A. arvensis* (MG991999), LD1 - *L. hirticornis* (MG992000), LD4 - *L. testaceipes* (MG992001), LD7 - *L. fritzmuelleri* (MG992002), PD2 - *P. dorsale* (MG992003), PD5 - *P. yomenae* (MG992004), ED2 - *E. plagiator* (MG991993), ED4 - *E. koponeni* (MG991992), MD1 - *M. paulensis* (MG991996), MD2 - *M. allisoni* (MG991995), MD3 - *M. washingtonensis* (MG991994). Several reference COI sequences from different Aphidiinae species were obtained from the public database and used with the archival material for tree construction. A total of 31 barcoding sequences were aligned, trimmed to the same length and submitted to phylogenetic analysis. A Maximum likelihood tree shows evident clustering of congeneric species in separate lineages with substantial bootstrap support (Fig. 8), confirming the quality of COI barcoding sequences retrieved from archival parasitoids specimens by targeting the short overlapping fragments with newly designed primers.

Discussion and conclusion

The barcoding method has shown to be a useful tool in discriminating parasitoid species from the five Aphidiinae genera studied, enabling further research on their biodiversity

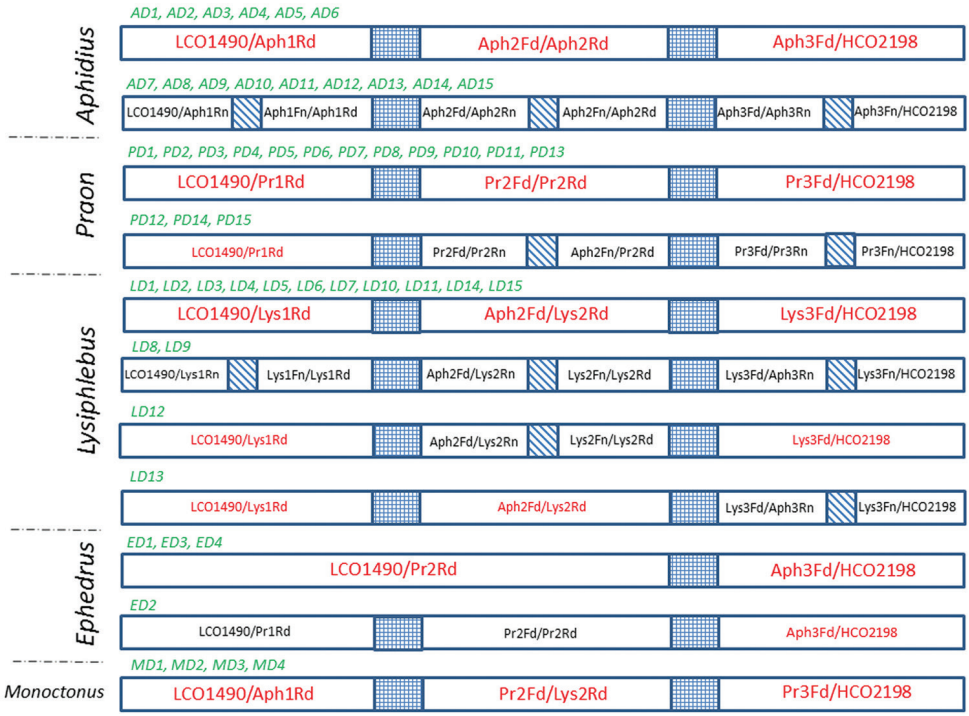

Figure 7. Scheme with overview of PCR attempts to recover the barcoding region of cytochrome c oxidase subunit I with novel primers from archival specimens from the genera *Aphidius*, *Praon*, *Lysiphlebus*, *Ephedrus* and *Monoctonus*. Primer pairs coloured red were used in direct PCR; black coloured primers were used in secondary nested reactions. Positions where short fragments within the subsequences overlap are marked with a pattern.

and phylogeny. The results presented here indicate the possibility of testing many other different combinations of primers in future research on archival specimens with the expectation of achieving success in retrieving the targeted subsequences. The position of the newly designed primers was evidently well chosen, targeting sites conservative enough to permit their multiple uses on a much wider spectrum of museum material than initially planned.

Similar to the results obtained by Andersen and Mills (2012), in our study age was apparently a limiting factor for successful amplification with the newly designed internal primers. On the other hand, the starting point in this study was awareness that museum specimens are not always available, or that the type material is sometimes restricted to a single specimen, etc., and thus cannot be manipulated in numerous trials. For this reason, blank PCR products were always further processed through secondary analyses with additional internal primers. This assumption was confirmed to be the basis of a good methodological approach with substantial success.

The results presented above refer only to combination of primers randomly selected to test their suitability in retrieving the barcoding region from *Ephedrus* and *Monoctonus* species. Without the need for further expenditure of limited DNA sources, the

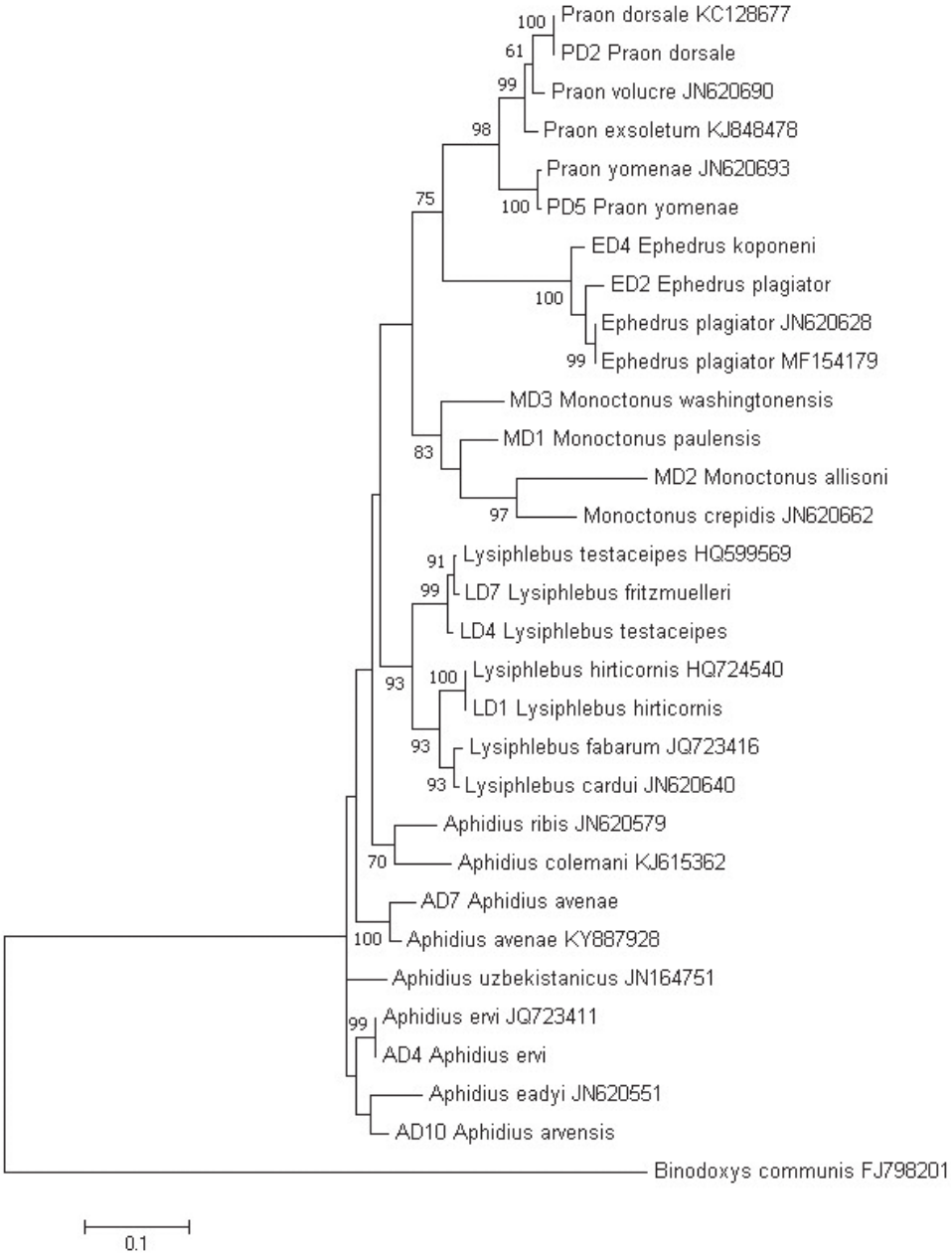

Figure 8. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood is shown. There were a total of 568 positions in the final dataset. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The percentage of replicate trees >50% in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.

here presented overview of nucleotide differences between the barcodes of parasitoids and information about primers clearly indicate that quite a few other combinations can be tested with the expectation of successfully retrieving short fragments.

Many benefits of using novel primers in conservation genetics and phylogeny studies are recognized, above all, the possibility of analyzing archival material of Aphidiinae parasitoids with unresolved taxonomic status. To date there have been many phylogenetic studies with different hypotheses about the origin and classification of certain taxa. Many examples in the literature show the importance of an integrative approach combining molecular and morphological data in taxonomic, phylogenetic and conservation studies, but even when using such an approach, researchers are quite often left with open questions. In view of the many confronting opinions held by different groups of authors, we can assume that the involvement of archival remains of Aphidiinae in molecular analyses will prove to be of great usefulness by yielding results enabling us to resolve the problems of phylogenetic relationships and the taxonomic recognition of different parasitoid groups.

It can be predicted that the herein described method of retrieving the barcoding region in parasitoids will take on increasing importance by making it possible to include not only extinct species preserved in museums, but also endemic or rare species under threat of extinction as well. Good examples of parasitoid species with potential risk of extinction are various associations of aphid hosts/parasitoids whose distribution are restricted to habitats under constant anthropogenic pressure of degradation such as the wetlands (Tomanović et al. 2012).

Modern genomic research opened complex questions exceeding the capacity of traditional DNA sequencing technologies. The Next-generation sequencing has revolutionized the biological sciences allowing us to study biological systems at higher level. In the light of an ongoing rapid progress in the field of modern sequencing technologies, newly designed primers could meet the demands in terms of depth of information in studying genomics of different Aphidiinae by delivering an insight into DNA variation of the target mitochondrial region.

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