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Review of the genus *Plutothrix* Förster, 1856 (Hymenoptera, Pteromalidae) with a key to Palaearctic species

Ekaterina V. Tselikh[1], Gergely Várkonyi[2], Natalie Dale-Skey[3]

1 *Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia* **2** *Finnish Environment Institute, Biodiversity Centre, Lentiirantie 342B, FI-88900 Kuhmo, Finland* **3** *Natural History Museum, London, UK*

Corresponding authors: Ekaterina V. Tselikh (tselikhk@gmail.com), Gergely Várkonyi (gergely.varkonyi@syke.fi), Natalie Dale-Skey (n.dale-skey@nhm.ac.uk)

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Abstract

The species of *Plutothrix* Förster, 1856 are reviewed. *Plutothrix gribanovi*, **sp. nov.**, is described from Russia, *P. longigaster*, **sp. nov.**, and *P. zerovae*, **sp. nov.**, are described from Finland and Russia. The male of *P. canariensis* Hedqvist, 1974 is described for the first time. The species *Plutothrix transdanuviana* (Erdös, 1946), **syn. nov.**, is synonymized under *Seladerma antennatum* (Walker, 1833). The following new records are reported: *Plutothrix nudicoxa* Graham, 1993 and *P. perelegans* Graham, 1993 from Finland, *P. obtusiclava* Graham, 1993 and *P. zhangyieensis* Yang, 1996 from Russia, and *P. perelegans* Graham, 1993 from Ukraine. An identification key to females of all Palaearctic species of *Plutothrix* is provided.

Keywords

Fauna, key, new species, parasitoids, Pteromalinae, taxonomy

Introduction

The pteromalid genus *Plutothrix* (type species *Plutothrix foersteri* Mayr, 1904) belongs to the family Pteromalidae, subfamily Pteromalinae. Up to now, it comprised twentyeight species worldwide (Noyes 2019). However, this figure also includes the species *Plutothrix transdanuviana* (Erdős, 1946), syn. nov.,which was examined in

the present study and identified as *Seladerma antennatum* (Walker, 1833). Fifteen of the known species, *P. bicolorata* (Spinola), *P. canariensis* Hedqvist, *P. coelius* (Walker), *P. kuboi* Kamijo, *P. kusigematii* Kamijo, *P. narendrani* Kamijo, *P. nudicoxa* Graham, *P. obtusiclava* Graham, *P. pallidiclava* Graham, *P. perelegans* Graham, *P. pilicoxa* Graham, *P. rugosa* Kamijo, *P. scrobicula* Kamijo, *P. trifasciata* (Thomson) and *P. zhangyieensis* Yang, inhabit the Palaearctic region (Spinola 1811; Walker 1839; Thomson 1878; Graham 1969, 1993; Hedqvist 1974; Yang 1996; Kamijo 2004; Noyes 2019). Ten species, *P. aerata* Heydon, *P. ascita* Heydon, *P. ceonotalis* Heydon, *P. glareosa* Heydon, *P. ligyptera* Heydon, *P. pilosiclava* Heydon, *P. recula* Heydon, *P. smithi* Heydon, *P. ungutta* (Girault) and *P. unguttella* Heydon, are distributed in the Nearctic region (Heydon 1997; Noyes 2019). Only a single species, *P. acuminata* (Thomson), has a Holarctic distribution (Heydon 1997; Noyes 2019). One extinct species, *Plutothrix minutissima* Meunier, 1905 was described from Zanzibar copal (Meunier 1905).

Unfortunately, the biology is unknown for most of the species, but available records suggest they are mostly primary parasitoids of coleopterans in the families Anobiidae, Ciidae, Curculionidae and dipterans in the family Platypezidae (Graham 1969; Herting 1973; Yang 1996; Heydon 1997; Noyes 2019).

The aim of this work is to describe new species of *Plutothrix* from Finland and Russia. An identification key to females of all Palaearctic species of *Plutothrix* is also provided.

Material and methods

The material used in this review is deposited in the Hymenoptera collections of the Finnish Natural History Museum, Helsinki, Finland (**ZMUH**), Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (**ZISP**), Natural History Museum, London, United Kingdom (**NHMUK**), Zoological Museum of the Lund University, Lund, Sweden (**LUZN**), Hungarian Natural History Museum, Budapest, Hungary (**HNHM**), Naturalis Biodiversity Center, Leiden, Netherlands (**NBC**), Ehime University Museum, Matsuyama, Japan (**EUM**), Entomological Laboratory of Hokkaido University, Sapporo, Japan (**EIHU**), Yeungnam University, Gyeongsan, South Korea (**YNU**), Northwestern College of Forestry, Yangling Shaanxi, People's Republic of China (**NWCF**).

Morphological terminology, including sculpture and wing venation nomenclature, follows Bouček and Rasplus (1991) and Gibson (1997). The flagellum consists of two anelli, the funiculus composed of six funicular segments, and the clava. The following abbreviations are used: **POL** – posterior ocellar line, the minimum distance between the posterior ocelli; **OOL** – ocello–ocular line, the minimum distance between a posterior ocellus and compound eye; **C1–C3** – claval segments; **PST** – parastigma; **M** – marginal vein; **S** – stigmal vein; **PM** – postmarginal vein; **F1–F6** – funicular segments; **Mt2–Mt8** – metasomal tergites (Mt1 – petiole). The scape is measured without the radicle; the pedicel is measured in lateral view. The distance between the clypeal lower

margin and the toruli is measured from the lower margins of the toruli. Eye height is measured as maximum diameter, eye length as minimum diameter. The mesosoma and metasoma are measured in lateral view, the latter including the ovipositor sheaths.

Observations were made using MC-2 ZOOM and Leica MZ16 stereomicroscopes, and images were acquired using a combination of Olympus SZX 10 stereomicroscope and digital camera EOS 70D, Micromed 3 microscope and digital camera ToupCam UCMOS 5.1MP (ZISP specimens), and a Canon 5DsR camera + Mitutoyo 10× lens or Canon MPE ultra macro lens (65 mm), Canon MT-24ex flash and Cognysis Stackshot, and Helicon remote software (NHMUK specimens). The acquired images were then processed with Helicon Focus.

Taxonomy

Plutothrix Förster, 1856

Plutothrix Förster, 1856: 46. Type species by subsequent monotypy *Plutothrix foersteri* Mayr, 1904.

Anoglyphis Förster, 1878: 49. Type species by original designation *Anoglyphis nubilosa* Förster, 1878. Synonymy by Kerrich and Graham (1957: 296).

Diagnosis. Clypeal margin with angular median tooth (*e.g.*, Figs 4, 37, 43, 75); tentorial pits indistinct; antennal formula 11263; male antennae with distinct adpressed setae (*e.g.*, Figs 13, 27); pronotum with collar margin carinate; notauli complete (*e.g.*, Figs 8, 15, 24, 29, 40, 72); prepectus bare and smooth or coriaceous sculpture (*e.g.*, Figs 17, 45, 67); scutellum with distinct frenal area (*e.g.*, Figs 8, 15, 24, 29, 40, 72); fore wing with speculum reaching cubital line (*e.g.*, Figs 3, 5, 12, 16, 22, 31, 34, 39, 50, 58, 66, 73, 76, 82); petiole inconspicuous; (Graham 1969; Bouček and Rasplus 1991).

Distribution. Palaearctic, Nearctic.

Key to Palaearctic species of *Plutothrix* Förster based on females

1 Clava yellow (Fig. 62). Propodeum with costula (Fig. 61). Hind tibia with median white ring (Figs 60, 63) ..... ***P. pallidiclava* Graham**

– Clava brown or black (*e.g.*, Figs 6, 11, 26, 32, 33, 41, 49, 55, 57, 65, 69, 80, 92). Propodeum without costula (*e.g.*, Figs 2, 8, 15, 24, 46, 86, 88). Hind tibia without median white ring (*e.g.*, Figs 1, 9, 19, 30, 36, 38, 48, 77, 84, 87) **2**

2 Fore wing with three or four fuscous clouds (*e.g.*, Figs 12, 22, 82) **3**

– Fore wing with two fuscous clouds (*e.g.*, Figs 5, 50, 53, 66, 68), or with one fuscous cloud touching stigma (*e.g.*, Figs 16, 31, 94), or hyaline (*e.g.*, Figs 3, 34, 39, 58, 73, 76, 85) **5**

3 M as long as PM (Fig. 12). Dorsellum reticulate. Basal cell setose (Fig. 12) .. .. ***P. canariensis*** **Hedqvist**

– M 0.73–0.90× as long as PM (*e.g.*, Figs 22, 82). Dorsellum alutaceous (*e.g.*, Figs 24, 80). Basal cell bare (Fig. 22) or with some setae on upper part (Fig. 82)..... **4**

4 Basal cell with some hairs on upper part (Fig. 82). Cubital vein setose (Fig. 82). Scutellum srtrongly convex (Fig. 81). Metasoma 3.70–3.80× as long as broad, basal part brown with metallic violet and coppery lustre, Mt8 1.72–1.80× as long as broad (Fig. 83) ..... ***P. trifasciata*** **(Thomson)**

– Basal cell bare (Fig. 22). Cubital vein bare (Fig. 22). Scutellum less convex (Fig. 23). Metasoma 4.47–5.15× as long as broad, basal part yellowish-brown, Mt8 1.90–2.10× as long as broad (Fig. 25) ..... ***P. gribanovi*** **sp. nov.**

5 Fore wing with two fuscous clouds, one median and the other subapical (*e.g.*, Figs 5, 50, 53, 66, 68)..... **6**

– Fore wing with one fuscous cloud touching stigma (*e.g.*, Figs 16, 31, 94) or hyaline(*e.g.*, Figs 3, 34, 39, 58, 73, 76, 85)..... **10**

6 Combined length of pedicel and flagellum 1.35–1.45× breadth of head. Clava with micropilosity on C3 or rarely C3 and small part of C2 (Fig. 6). Fore wing with S slightly curved (Fig. 5) ..... ***P. bicolorata*** **(Spinola)**

– Combined length of pedicel and flagellum 1.20–1.30× breadth of head. Clava with micropilosity on C3, C2 and sometimes distal half C1 (*e.g.*, Figs 49, 65). Fore wing with S straight (*e.g.*, Figs 50, 53, 66, 68)..... **7**

7 Hind coxa dorsally and ventrally thickly setose, (*e.g.*, Fig. 67). Clava with micropilosity on C3, C2 and distal half C1 (*e.g.*, Fig. 49)..... **8**

– Hind coxa with some setae dorsally but far less than ventrally (*e.g.*, Fig. 54). Clava with micropilosity on C3 and C2 (*e.g.*, Fig. 65)..... **9**

8 Scutellum with deep median furrow (Fig. 51). Pedicel and anelli yellowish (Fig. 49). Stigma 1.50–1.55× as long as broad (Fig. 50)..... ..... ***P. narendrani*** **Kamijo**

– Scutellum without deep median furrow (Fig. 70). Pedicel and anelli brown (Figs 68, 70). Stigma 2.50–2.60× as long as broad (Fig. 68)..... ..... ***P. pilicoxa*** **Graham**

9 Antenna with F1 2.30–2.80× as long as broad, F2 1.70–2.00× as long as broad (Fig. 65). Head dark metallic blue (Fig. 64). Mt2 and Mt3 yellowish-brown or red (Fig. 64)..... ***P. perelegans*** **Graham**

– Antenna with F1 2.00–2.15× as long as broad, F2 1.50–1.60× as long as broad (Fig. 55). Head dark metallic green with diffuse coppery lustre (Figs 52, 53). Mt2 and Mt3 dark brown (Fig. 52) ..... ***P. nudicoxa*** **Graham**

10 Fore wing with PST longer than M (Fig. 58). Antenna with clava obtuse apically, C3 short and thickly setose (Fig. 57) ..... ***P. obtusiclava*** **Graham**

– Fore wing with PST shorter than or as long as M (*e.g.*, Figs 3, 16, 31, 34, 39, 73, 76, 85, 94). Antenna with clava acute, C3 not short and not thickly setose (*e.g.*, Figs 32, 33, 41, 92)..... **11**

11 Metapleuron alutaceous, upper mesepimeron with lower part alutaceous, upper part smooth (*e.g.*, Fig. 45). Metasoma 1.65–1.97× as long as head plus mesosoma (*e.g.*, Figs 36, 38, 40, 71). Mt8 2.90–5.25× as long as broad (*e.g.*, Figs 35, 42, 71) **12**

– Metapleuron reticulate, upper mesepimeron reticulate or smooth, upper part smooth (*e.g.*, Fig. 91). Metasoma 1.15–1.50× as long as head plus mesosoma (*e.g.*, Figs 1, 14, 29, 78, 84, 87). Mt8 1.26–2.50× as long as broad (*e.g.*, Figs 14, 29, 78, 93) **14**

12 Combined length of pedicel and flagellum 1.06–1.15× breadth of head. Scutellum irregular rugose (Fig. 72). Fore wing with PST 0.9–1.0× as long as M (Fig. 73) ..... ***P. rugosa* Kamijo**

– Combined length of pedicel and flagellum 1.30–1.57× breadth of head. Scutellum reticulate (*e.g.*, Figs 35, 46). Fore wing with PST 0.65–0.70× as long as M (*e.g.*, Figs 34, 39) **13**

13 Antenna with F1 2.50–2.80× as long as broad, with 4–5 rows of sensilla (Fig. 33). Fore wing with M 1.70–1.80× as long as S (Fig. 34). All coxae metallic green with diffuse coppery lustre, all femora dark (Fig. 36). Mt8 2.90–3.50× as long as broad (Fig. 35) ..... ***P. kusigematii* Kamijo**

– Antenna with F1 2.15–2.35× as long as broad, with 3 rows of sensilla (Fig. 41). Fore wing with M 2.00–2.30× as long as S (Fig. 39). All coxae yellowish-brown, all femora yellow (Fig. 38). Mt8 4.30–5.25× as long as broad (Fig. 42) ..... ***P. longigaster* sp. nov.**

14 Antenna with F1 2.20–2.45× as long as broad (*e.g.*, Figs 32, 92). Mt8 1.85–2.50× as long as broad (*e.g.*, Figs 29, 93) **15**

– Antenna with F1 2.00–2.10× as long as broad (*e.g.*, Figs 15, 75, 84). Mt8 1.26–1.75× as long as broad (*e.g.*, Figs 14, 78) **16**

15 Scutellum coarsely reticulate (Fig. 29). Fore wing with elongate stigma (Fig. 31). Clava rounded (Fig. 32). Metasoma 3.20–3.70× as long as broad (Fig. 29), Mt8 1.84–2.0× as long as broad (Fig. 29) ..... ***P. kuboi* Kamijo**

– Scutellum finely reticulate (Fig. 88). Stigma less elongate (Fig. 94). Clava acute (Fig. 92). Metasoma 4.30–6.25× as long as broad (Fig. 93), Mt8 2.20–2.50× as long as broad (Fig. 93) ..... ***P. zerovae* sp. nov.**

16 Frenum of scutellum coarsely reticulate (Fig. 15). Metapleuron strongly reticulate (Fig. 17). Fore wing with one fuscous cloud touching stigma (Fig. 16) ..... ***P. coelius* (Walker)**

– Frenum of scutellum finely reticulate (*e.g.*, Figs 2, 78, 86). Metapleuron weakly reticulate (*e.g.*, Fig. 84). Fore wing immaculate (*e.g.*, Figs 3, 76, 85) **17**

17 Head black (Fig. 75). Clypeus with blunt tooth (Fig. 75). Basal cell of fore wing with dense pubescence (Fig. 76). Propodeum alutaceous ..... ***P. scrobicula* Kamijo**

– Head metallic green or dark green with diffuse coppery lustre (*e.g.*, Figs 4, 84). Clypeus with sharp tooth (*e.g.*, Fig. 4). Basal cell of fore wing with sparse pubescence (*e.g.*, Figs 3, 85). Propodeum smooth (*e.g.*, Figs 2, 86) **18**

18 Fore wing with M 1.55–1.60× as long as S (Fig. 3). Eye height 3.00–3.10× as long as malar space. Scutellum finely reticulate, dorsellum smooth (Fig. 2)... ..... ***P. acuminata*** **(Thomson)**

– Fore wing with M 1.80–1.85× as long as S (Fig. 85). Eye height 2.10–2.16× as long as malar space. Scutellum coarsely reticulate, dorsellum alutaceous (Fig. 86).....***P. zhangyieensis*** **Yang**

Plutothrix acuminata **(Thomson, 1878)**
Figs 1–4

Trigonoderus acuminatus Thomson, 1878: 13. Lectotype female (LUZN, not examined) designated by Kerrich and Graham 1957: 297.

Plutothrix cisae Hedqvist, 1966: 194. Holotype female missing (Forshage et al. 2016). Synonymy by Graham (1993: 117).

Material examined. ***Paratype*** female (ZMUH): "Suomi [Finland], EH, Luopioinen, 7.8.1963, E. Kangas", "Paratypus *Plutothrix cisae* Hedqvist, 1966". ***Other material*****:** Finland (in ZMUH): 1 female, **Ta** [biogeographical province Tavastia australis], Aitolahti, 22.VII.1932, coll. V. Saarinen, *Plutothrix acuminata* (Thom.) det. Koponen 2009. Russia (all in ZISP): **Belgorod Prov**., 1 female, 1 male, 10 km S Novy Oskol City, 50°40.683'N, 37°48.551'E, 15.VIII.2020, coll. S. Belokobylskij and O. Kosheleva.

Distribution. Belgium, Canada, Croatia, Czech Republic, Finland, France, Hungary, Netherlands, Russia (European part), Slovakia, Spain, Sweden, United Kingdom (Noyes 2019; Tselikh 2019).

Biology. Primary parasitoid of *Cis boleti* (Scopoli, 1763) (Coleoptera, Ciidae) in tree fungus Graham (1969), and *Platypeza* sp. (Diptera, Platypezidae) (Heydon 1997).

Plutothrix bicolorata **(Spinola, 1808)**
Figs 5–8

Diplolepis bicolorata Spinola, 1808: 221–222. Type specimens probably lost (Graham 1993: 117).

Plutothrix bicolorata (Spinola, 1808) new combination in Graham 1993: 116–117.

Pteromalus invenustus Walker, 1836: 11. Lectotype male (NHMUK, not examined). Designated by Kerrich and Graham (1957: 294). Synonymy by Graham (1993: 116).

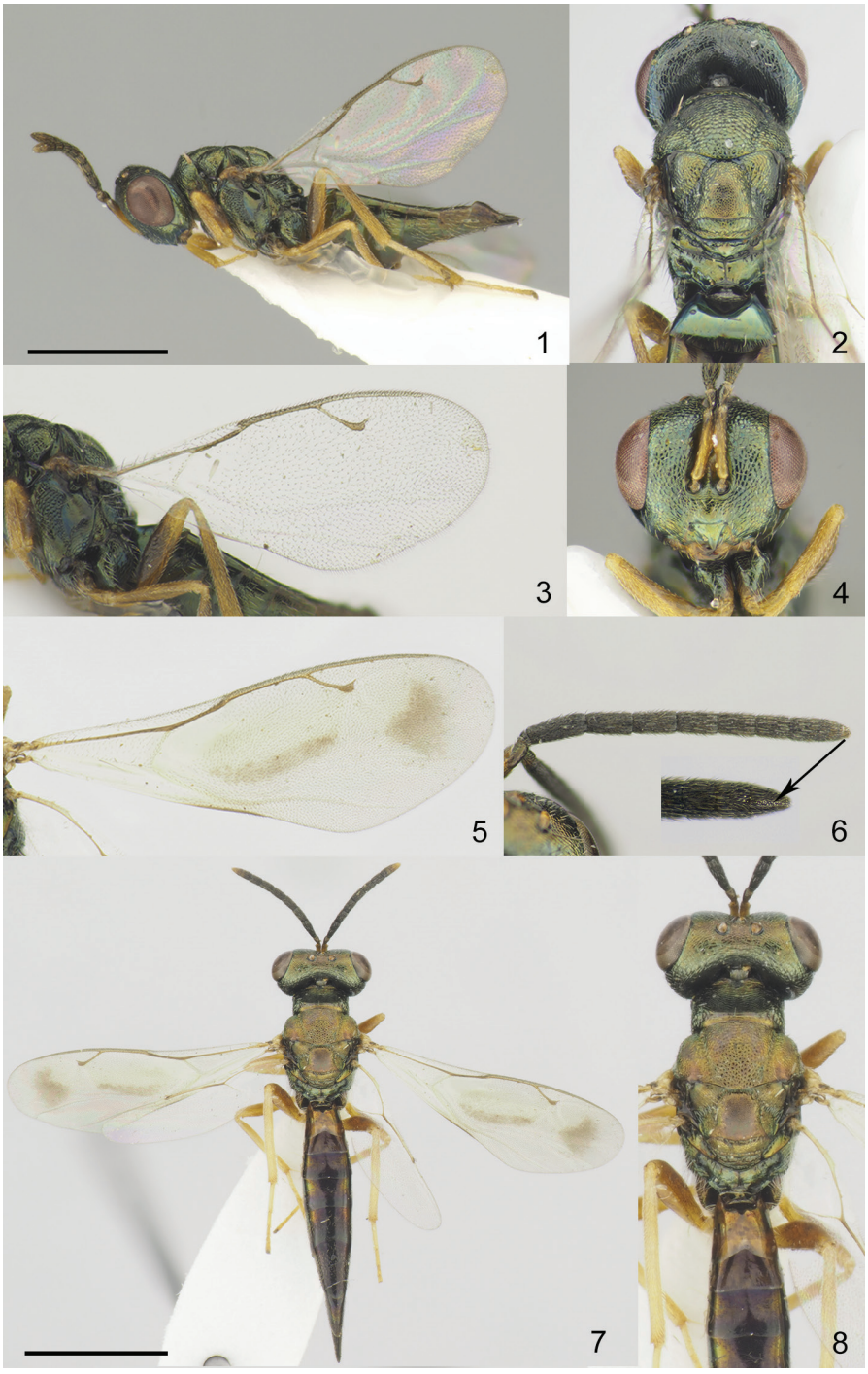
Pteromalus praepileus Walker, 1836: 12. Lectotype female (NHMUK, not examined). Designated by Kerrich and Graham (1957: 295). Synonymy by Graham (1993: 116).

Pteromalus scenicus Walker, 1836: 10. Lectotype female (NHMUK, not examined). Designated by Kerrich and Graham (1957: 294). Synonymy by Graham (1993: 116–117).

Trigonoderus apicalis Thomson, 1878: 12–13. Lectotype female (LUZN, not examined). Designated by Kerrich and Graham (1957: 294). Synonymy by Graham (1993: 116).

Trigonoderus vittiger Thomson, 1878: 12. Lectotype female (LUZN, not examined). Designated by Kerrich and Graham (1957: 294). Synonymy by Graham (1993: 116).

Material examined. ***Other material*:** Finland (all in ZMUH): **A** [Alandia], 1 female, "Lemland, Nordman, 5375, coll. Nordman", "*Plutothrix scenicus* (Walk.) det. Hedqvist, 1973"; **Ab** [Regio aboënsis], 1 female, "Finland, 669:25, Sauvo, Karuna, 23.VII–9.VIII.2000, Malaise trap, coll. R. Jussila", "*Plutothrix bicolorata* (Spinola) det. Tselikh 2021"; 1 female, "Finland, 669370:323763, V, Parainen, Malaise 1A, 19.07–02.08.2020, coll. S. Väänänen, J. Paukkunen", "*Plutothrix bicolorata* (Spinola) det. Tselikh 2021"; 1 female, 1 male, "Äppelö, E. Ölund", "*Plutothrix bicolorata* (Spinola) det. Tselikh 2021"; **N** [Nylandia], 1 female, "Borgå, Fennia, Hellén", "*P. scenicus* Thom. Det. Kerrich 1956", "*Plutothrix bicolorata* (Spin.) det. Koponen 09". Russia (all in ZISP): **Smolensk Prov.**, 1 female, 1 male, near Smolensk City, 54°49'10"N, 32°05'09"E, 23.VIII.2020, coll. E. Tselikh; **Belgorod Prov.**, 13 females, 17 males, Roven'skii Distr., Roven'ki Vill., "Roven'ki Nature Park," "Aydar," 49°59'01"N, 38°53'23"E, 12–13.VIII.2020, coll. S. Belokobylskij, K. Fadeev and E. Tselikh; 8 females, 5 males, Novooskolskii Distr., 10 km S of Novy Oskol City, "Belogorie" Reserve, "Stenki Izgor'ya," 50°40'41"N, 37°48'33"E, 15.VIII.2020, coll. S. Belokobylskij and O. Kosheleva; 15 females, 32 males, Borisovskii Distr., Borisovka Vill., "Belogorie" Reserve, "Les na Vorskle," 50°36'34"N, 35°58'55"E, 17.VIII.2020, coll. S. Belokobylskij, K. Fadeev and O. Kosheleva; 16 females, 45 males, Borisovka Vill., "Melkiy les", 55°39'20"N, 36°00.38'E, coll. S. Belokobylskij, K. Fadeev, O. Kosheleva and E. Tselikh; **Voronezh Prov.**, 20 females, 24 males, Bogucharskii Distr., 20 km SW of Boguchar City, "Khripunskaya Steppe," 49°35'58"N, 40°23'56"E, 8–9.VIII.2020, coll. S. Belokobylskij, O. Kosheleva, E. Tselikh; 6 females, Kantemirovskii Distr., 20 km SW of Rossosh' City, Zhilino Vill., 49°49'58"N, 39°19'48"E, 10–11.VIII.2020, coll. S. Belokobylskij, O. Kosheleva and E. Tselikh; **Krasnodar Reg.**, 4 females, Sochi City, Lazarevskoe, 27.V.1979, 18.VI.1979 coll. V. Tobias; 3 females, 1 male, Sochi City, Soloniki Vill., 20.X.1980, coll. V. Tobias; 1 female, Goryachij kluch City, Kesukh River, 44°26'19"N, 39°01'52"E, 25.VIII.2015, coll. D. Rachin and E. Tselikh; 4 females, 1 male, Sochi City, "Mamedova Shchel", 43°57'20"N, 39°18'39"E, 28.VII.2020, coll. S. Belokobylskij, K. Fadeev and E. Tselikh; 3 females, 4 males, 5 km SEE Aderbeevka Vill., 44°37'30"N, 38°09'16"E, 26.VII.2020, coll. E. Tselikh; 3 females, 1 male, Sochi City, Kalezh Vill., 44°00'25"N, 39°22'03"E, 30.VII.2020, coll. O. Kosheleva and



Figures 1–8. *Plutothrix acuminata* (Thomson, 1878), non-type female (**1–4**) **1** body, lateral view **2** head and mesosoma, dorsal view **3** fore wing **4** head, frontal view. *Plutothrix bicolorata* (Spinola, 1808), non-type female (**5–8**) **5** fore wing **6** antenna **7** body, dorsal view **8** head, mesosoma and part of metasoma, dorsal view. Scale bars: 0.8 mm (**1**); 2.1 mm (**7**).

E. Tselikh; **Adygea Rep.**, 1 female, Guzeripl' Vill., Kavkazsky Reserve, 21.VI.1976, coll. D. Kasparyan; **Karachay-Cherkess Rep.**, 1 female, 1 male, Teberda, 8.VI.1976, coll. D. Kasparyan; **Ingushetia Rep.**, 1 female, 14 km E Verkhny Alkun Vill., 10.VI.1972, coll. D. Kasparyan.

Distribution. Belgium, Croatia, Czech Republic, Finland, Germany, Hungary, Italy, Moldova, Netherlands, Romania, Russia (European part), Serbia, Slovakia, Spain, Sweden, Switzerland, United Kingdom (Noyes 2019; Tselikh 2019).

Biology. Primary parasitoid of *Anobium punctatum* (De Geer, 1774) and *Ernobius abietis* (Fabricius, 1792) (Coleoptera, Anobiidae) (Graham 1969).

Plutothrix canariensis Hedqvist, 1974

Figs 9–13

Plutothrix canariensis Hedqvist, 1974: 26–28. Holotype female (NHMUK, examined).

Description. Male. Body length 2.60 mm. Fore wing length 2.40 mm.

Head, mesosoma, metasoma Mt2 metallic green with diffuse coppery lustre, Mt3–Mt6 yellowish-brown, Mt7–Mt8 brown. Antenna with scape yellowish-brown, pedicel and flagellum brown. Fore and hind coxa basally metallic green with diffuse coppery lustre, apically yellowish-brown, mid coxa yellowish-brown; all femora, tibiae, and tarsi yellowish-brown. Fore wing with brownish tint and fuscous cloud touching stigma, venation yellowish-brown.

Head in dorsal view 2.20× as broad as long and 1.36× as broad as mesoscutum; in frontal view 1.40× as broad as high. POL as long as OOL. Eye height 1.25× eye length and 2.35× as long as malar space. Distance between antennal toruli and lower margin of clypeus 0.92× distance between antennal toruli and median ocellus. Antenna with scape 0.80× as long as eye height and as long as eye length; pedicel 1.80× as long as broad and 0.37× as long as F1; combined length of pedicel and flagellum 2.36× breadth of head; F1 4.80× as long as broad, F3–F6 longer than broad; clava 4.50× as long as broad.

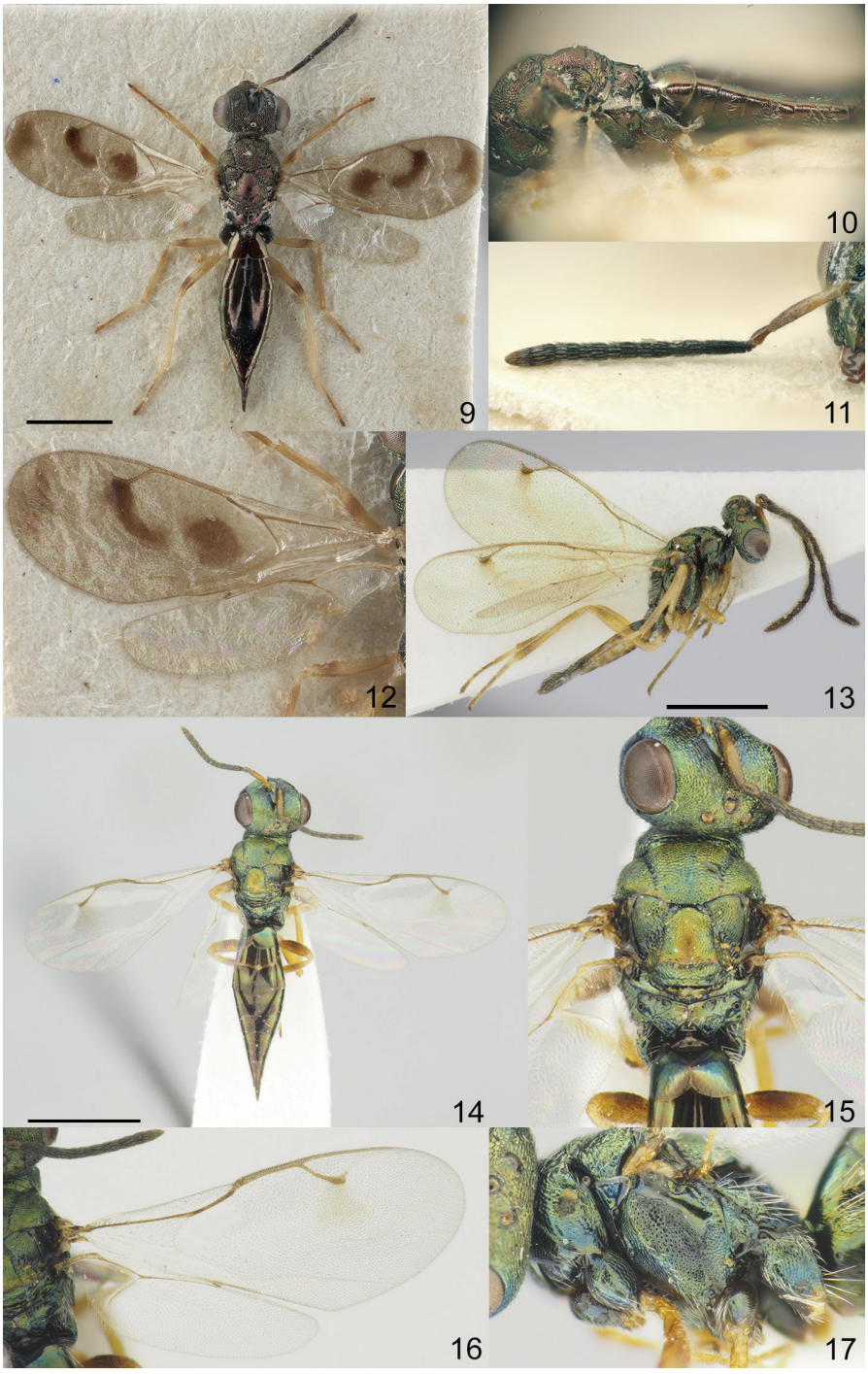
Mesosoma 1.87× as long as broad. Scutellum finely reticulate, 1.2× as long as broad. Propodeum without nucha, 0.65× as long as scutellum; median carina present. Metapleuron alutaceous, upper mesepimeron smooth. Fore wing 2.40× as long as maximum width; basal cell, cubital vein, basal vein setose; speculum closed; PST 0.43× as long as M, M 0.97× as long as PM and 2.30× as long as S.

Metasoma 2.60× as long as broad, 1.15× as long as mesosoma and 0.89× as long as mesosoma and head.

Material examined. ***Holotype*** female (NHMUK): "Tenerife IV 1967 leg. T. Palm", "HOLOTYPUS *Plutothrix canariensis* ♀ sp.n. K.J. Hedqvist det. 1970", "Hedqvist coll. BMNH(E) 2011-27", "HOLOTYPE", "B.M. TYPE HYM 5.4754", NHMUK 013457290. ***Other material*:** Spain, Canary Islands (all in ZMUH): 1 female, 1 male, Tenerife, Los Silos Monte del Aqua, 16.XI.2000, coll. M. Koponen.

Distribution. Canary Islands.

Biology. Unknown.



Figures 9–17. *Plutothrix canariensis* Hedqvist, 1974, holotype female (**9–12**), non-type male (**13**) **9** body, dorsal view **10** mesosoma and metasoma, lateral view **11** antenna **12** wings **13** body, lateral view. *Plutothrix coelius* (Walker, 1839), non-type female (**14–17**) **14** body, dorsal view **15** head and mesosoma, dorsal view **16** wings **17** mesosoma, lateral view. Scale bars: 1.05 mm (**9**); 0.85 mm (**13**); 1.3 mm (**14**).

***Plutothrix coelius* (Walker, 1839)**
Figs 14–17

Anoglyphis nubilosa Förster, 1878: 49. Type female (possibly in Berlin University Museum, not examined). Synonymy by Kerrich and Graham (1957: 296).

Pteromalus britannicus Morley, 1910: 47 (*n.n. pro coelius* Walker 1848 *non* 1839). Lectotype female (not located in NHMUK collection). Designated by Graham (1969:105). Synonymy by Graham (1969: 105).

Pteromalus coelius Walker, 1839: 272. Lectotype female (NHMUK, not examined). Designated by Kerrich and Graham (1957: 297).

Pteromalus eleuthera Walker, 1848: 193. Lectotype female (NHMUK, not examined). Designated by Kerrich and Graham (1957: 298). Synonymy by Kerrich and Graham (1957: 296).

Material examined. ***Other material*:** Finland (all in ZMUH): **Ab**, 1 female, Nystad, M. Hellén, 1841, *Plutothrix coelius* (Walker) det. M. Koponen 2009; 1 female, Suomi, V, Karjalohja, 16.07.1964, coll. Jonny Perkiömäki, *Plutothrix coelius* (Walker) det. M. Koponen 1982; 1 female, Nystad, M. Hellén, 609, *Plutothrix coelius* (Walker) det. Tselikh, 2021; **Ka** [Karelia australis], 1 female, Fennia, Virolahti, 671:53, 16–21.07.1974, coll. V.J. Karvonen, *Plutothrix coelius* (Walker) det. M. Koponen; 1 female, Fennia, Vehkalahti, 01.07.1961, leg. E. Valkeila, *Plutothrix scenicus* (Walker) det. E. Valkeila, *Plutothrix coelius* (Walker) det. Tselikh 2021; **St** [Satakunta], 1 female, Suomi, Suoniemi, 28.06.1947, *Plutothrix coelius* (Walker) det. Tselikh 2021; **Ta**, 1 female, Tampere, Grönblom, 15.05.57, coll. Th. Grönblom, *Plutothrix coelius* (Walker) det. M. Koponen; 1 female, Pirkkala, Grönblom, coll. Th. Grönblom, *Trigonoderus acuminatus* Thomson det. Hellén, *Plutothrix coelius* (Walker) det. M. Koponen; 1 female, Fennia, Hämeenlinna, 67736, 26.06.1970, coll. Erkki Valkeila, *Plutothrix coelius* (Walker) det. M. Koponen 1982; 1 female, Fennia, Hämeenlinna, 67736, 27.06.1971, coll. Erkki Valkeila, *Plutothrix coelius* (Walk.) det. Valkeila; 1 female, Fennia, Pälkäne, 680:35, e.l.1976, leg. Esko Saarela, *Plutothrix coelius* (Walker) det. M. Koponen; **Sa** [Savonia australis], 1 female, Suomi, Mikkelin mlk., 6830:501, 22.06.1979, leg. M. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen; 1 female, Suomi, Mikkelin mlk., 6830:501, 10.07.1981, leg. M. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen; 1 female, Suomi, ES, Ristiina, 6826:502, 06.07.1947, leg. M. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen; 1 female, Suomi, Mikkelin mlk, 6830:501, 12.07.1981, leg. M. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen; 2 females, Finland, Sa, Mikkeli, 6835:3503, 12.06.2011, leg. M. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen 2011; 1 female, Suomi, Mikkelin mlk, 6830:501, 17.08.1976, leg. M. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen; 1 female, Finland, Ristiina, 6826:502, 25.07.1985, leg. M. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen; 1 female, Suomi, Mikkelin mlk, 6830:501, 30.06.1975, leg. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen; 1 female, Suomi, ES, Ristiina, 6826:502, 24.06.1976, leg. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen; 2 females, Finland, 6826:502, Ristiina, 22.07.1996, leg. M. Koponen, *Plutothrix coelius*

(Walker) det. Tselikh 2021; **Oa** [Ostrobottnia australis], 1 female, Helsinki, Nordman, 427, *Plutothrix coelius* (Walker) det. M. Koponen; **Kb** [Karelia borealis], 1 female, Suomi, PK, Tohmajärvi, 6908:660, 22.07.1982, leg. M. Koponen, *Plutothrix coelius* (Walker) det. M. Koponen, 1982. Russia (all in ZISP): **Novgorod Prov.**, 2 females, Tychkino, 20 km NW Pestovo Vill., 25.VI.1991, coll. V. Tobias; **Altai Rep.**, 1 female, 20 km SE Onguday Vill., 16.VII.2007, coll. S. Belokobylskij; **Kamchatka Reg.**, 1 female, 8 km S Kozyrevsk Vill., 16.VIII.1985, coll. D. Kasparyan; 1 male, Kozyrevsk Vill., 17.VIII.1985, coll. S. Belokobylskij.

Distribution. Belgium, Croatia, Finland, Germany, Hungary, Moldova, Netherlands, Norway, Russia (European part of Russia, Western Siberia, Russian Far East), Romania, Sweden, United Kingdom (Noyes 2019; Tselikh 2019).

Biology. Primary parasitoid of coleopterans *Anobium punctatum* (De Geer, 1774) (Anobiidae) Graham (1969) and *Xylechinus pilosus* (Ratzeburg, 1837) (Curculionidae) (Herting 1973).

Plutothrix gribanovi Tselikh, Várkonyi & Dale-Skey, sp. nov.

https://zoobank.org/78437246-CA0E-40DF-9503-72FBD29DC951
Figs 18–28

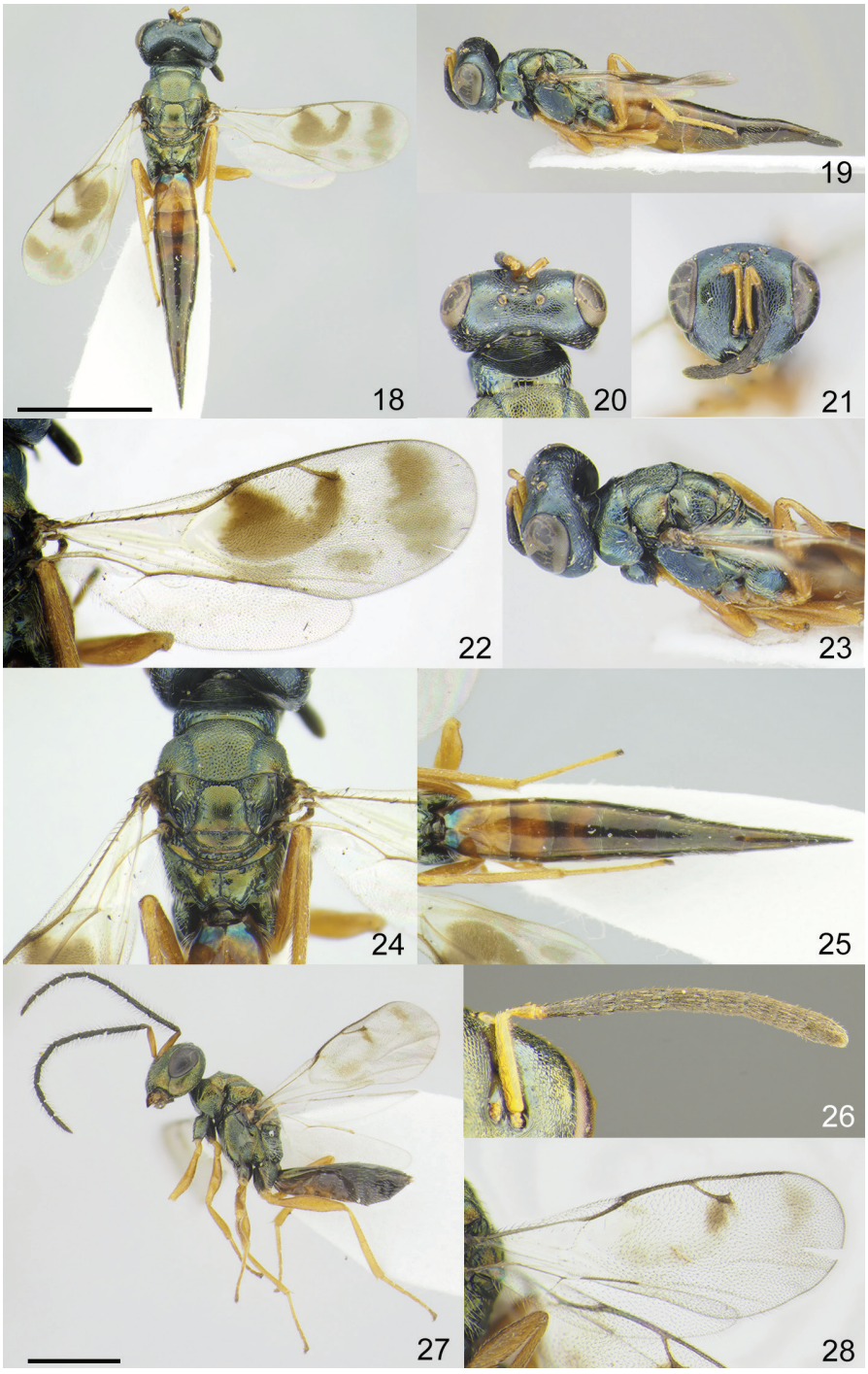
Comparison. *Plutothrix gribanovi* is similar to *P. canariensis* Hedqvist and *P. trifasciata* (Thomson); the differences between these species are given in the key.

Description. Female. Body length 4.90–7.20 mm. Fore wing length 3.00–3.90 mm.

Head dark metallic blue; mesosoma metallic green with diffuse coppery lustre; metasoma with Mt2 basally metallic bluish-green, apically yellowish-brown, Mt3, Mt4, sometimes Mt5 yellowish-brown, Mt6–Mt8 dark brown; ovipositor sheath black. Antenna with scape and pedicel yellow, flagellum brown. All coxae metallic green with diffuse coppery lustre; all femora, tibiae and tarsi yellow except last segment yellowish-brown. Fore wing with three or four fuscous clouds, venation yellowish-brown.

Head in dorsal view 2.10–2.20× as broad as long and 1.25–1.35× as broad as mesoscutum; in frontal view 1.30–1.40× as broad as high. POL 0.96–1.00× OOL. Eye height 1.42–1.45× eye length and 2.60–2.70× as long as malar space. Distance between antennal toruli and lower margin of clypeus 0.75–0.76× distance between antennal toruli and median ocellus. Antenna with scape 0.80–0.85× as long as eye height and 1.15–1.20× as long as eye length; pedicel 2.00–2.16× as long as broad and 0.46–0.52× as long as F1; combined length of pedicel and flagellum 1.33× breadth of head; F1 2.85–3.10× as long as broad, F3–F6 longer than broad; clava 2.35–2.44× as long as broad.

Mesosoma 1.65–1.70× as long as broad. Scutellum finely reticulate, 1.0–1.05× as long as broad. Propodeum without nucha, 0.86–0.90× as long as scutellum; median carina present; sculpture alutaceous. Metapleuron alutaceous, upper mesepimeron smooth. Fore wing 2.67–2.85× as long as maximum width; basal cell bare; cubital vein bare; basal vein setose; speculum partly open; PST 0.53–0.66× as long as M, M 0.78–0.80× as long as PM and 1.68–1.70× as long as S.



Figures 18–28. *Plutothrix gribanovi* sp. nov., holotype female (**18–26**), paratype male (**27, 28**) **18** body, dorsal view **19** body, lateral view **20** head, dorsal view **21** head, frontal view **22** wings **23** head and mesosoma, dorso-lateral view **24** mesosoma, dorsal view **25** metasoma, dorsal view **26** antenna **27** body, lateral view **28** fore wing. Scale bars: 2.1 mm (**18**), 1.1 mm (**27**).

Metasoma 4.47–5.15× as long as broad, 1.90–2.02× as long as mesosoma and 1.45–1.48× as long as mesosoma and head; Mt2 deeply emarginate medially, Mt8 1.90–2.10× as long as broad. Ovipositor sheath projecting beyond apex of metasoma.

Male. Body length 3.1–4.0 mm. Fore wing length 2.7–3.1 mm.

Head metallic green with diffuse coppery lustre; metasoma brown with diffuse coppery or metallic green lustre. Fore wing with four fuscous clouds, venation yellowish-brown.

Head in dorsal view 1.20–1.31× as broad as mesoscutum. Eye height 1.50–1.60× eye length and 2.10–2.30× as long as malar space. Distance between antennal toruli and lower margin of clypeus 1.28–1.40× distance between antennal toruli and median ocellus. Pedicel 1.60–1.63× as long as broad and 0.30–0.32× as long as F1; combined length of pedicel and flagellum 2.34× breadth of head; F1 5.60–7.00× as long as broad; clava 4.66× as long as broad.

Metasoma 4.60–5.30× as long as broad, 1.12–1.16× as long as mesosoma and 0.86–0.87× as long as mesosoma and head. Otherwise, similar to female.

Etymology. The species is named in honour of the senior author's brother, Sergej Gribanov.

Material examined. ***Holotype*** female (deposited in ZISP): "RUSSIA, **Altai Rep.**, 30 km S Kuray, 31.VII.2007, coll. A. Khalaim".

Paratypes (ZISP): Russia: **Krasnodar Reg.**, 1 male, Sochi City, Lazarevskoe, 28.V.1974, coll. Tobias; 1 female, same locality, 3–6.VI.1974, coll. V. Tobias; 3 females, same locality, 14–26.VI.1979, coll. Tobias; 1 female, Sochi City, Golovinka, 9.IV.1975, coll. V. Tobias; 1 female, Sochi City, Lazarevskoe, Polkovnich'ya balka, 43°53'48"N, 39°21'18"E, 31.VII.2020, coll. Tselikh; 1 male, Sochi City, Mamedova shchel', 43°57'11"N, 39°18'27"E, 29.VII.2020, coll. K. Fadeev.

Distribution. Russia (European part, Western Siberia).

Biology. Unknown.

Plutothrix kuboi Kamijo, 2004

Figs 29–32

Plutothrix kuboi Kamijo, 2004: 297–299. Holotype female (EIHU, examined).

Material examined. ***Holotype*** female (EIHU): Japan: **Kanagawa Pref.**, "Kanazawa-ku, Yokohama, Honshu, 19.iii.1995, coll. K. Kubo", "Holotype *Plutothrix kuboi* Kamijo". ***Paratype*** female (EIHU): **Kanagawa Pref.**, "Nishi tanzawa, 16.V.1993, coll. K. Kubo", "Paratype *Plutothrix kuboi* Kamijo". ***Other material*:** Japan (EUM): **Shimane Pref.**, 1 female, Honshu, Hirose Town, Nogi-gun, 8.IV.1980, coll. Seiyama. Russia (ZISP): **Kamchatka Reg.**, 1 female, Mil'kovo Town, 7.VIII.1985, coll. S. Belokobylskij.

Distribution. Russia (Far East), Japan.

Biology. Unknown.

***Plutothrix kusigematii* Kamijo, 2004**
Figs 33–37

Plutothrix kusigematii Kamijo, 2004: 299–300. Holotype female (EIHU, examined).

Material examined. ***Holotype*** female (EIHU): Japan: **Hokkaido Pref.**, "Sapporo, Hokkaido, 13.ix.1968, coll. K. Kusigemati", "Holotype *Plutothrix kusigematii* Kamijo". ***Paratype*** female (EIHU): **Kanagawa Pref.**, "Japan: Ishikawa, Fujisawa, 11.V.2001, coll. I. Waki", "Paratype *Plutothrix kusigematii* Kamijo". ***Other material*:** Russia (all in ZISP): **Sakhalin Prov.**, 1 female, Kunashir, Severyanka River, 24–28.IX.2013, coll. Yu. Sundukov; **Tyumenskaya Prov.**, 2 females, Tyumen' City, Andreevskoe Lake, 57°01'13"N, 65°46'16"E, 9.VII.2021, coll. S Belokobylskij and E. Tselikh.

Distribution. Russia (Eastern Siberia, Far East), Japan.

Biology. Unknown.

***Plutothrix longigaster* Tselikh, Várkonyi & Dale-Skey, sp. nov.**
https://zoobank.org/BF2FF3E5-AA81-4A27-819F-A449D531B887
Figs 38–46

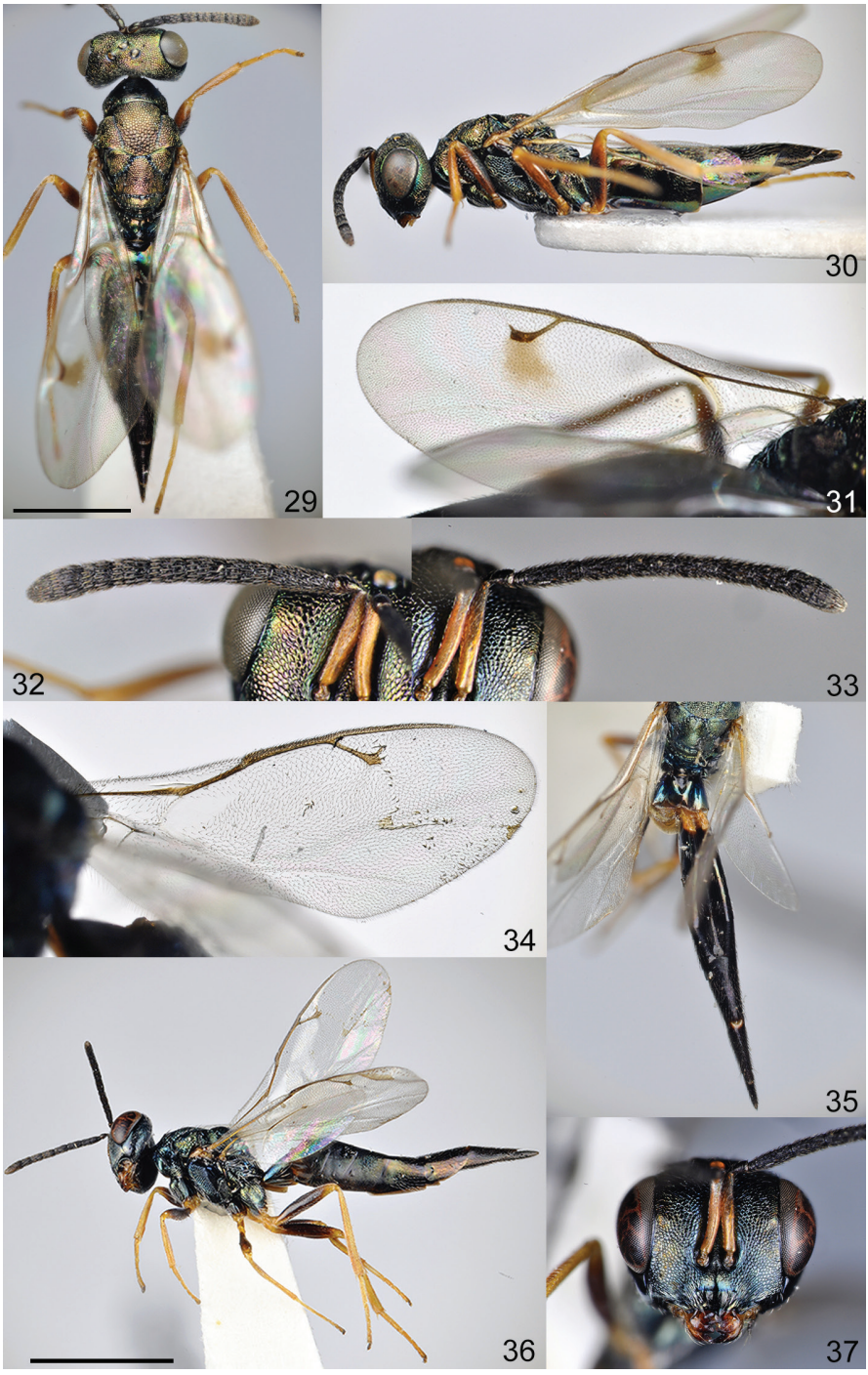
Comparison. *Plutothrix longigaster* is similar to *P. kusigematii* Kamijo and *P. rugosa* Kamijo; the differences between these species are given in the key.

Description. Female. Body length 5.10–6.70 mm. Fore wing length 3.20–3.80 mm.

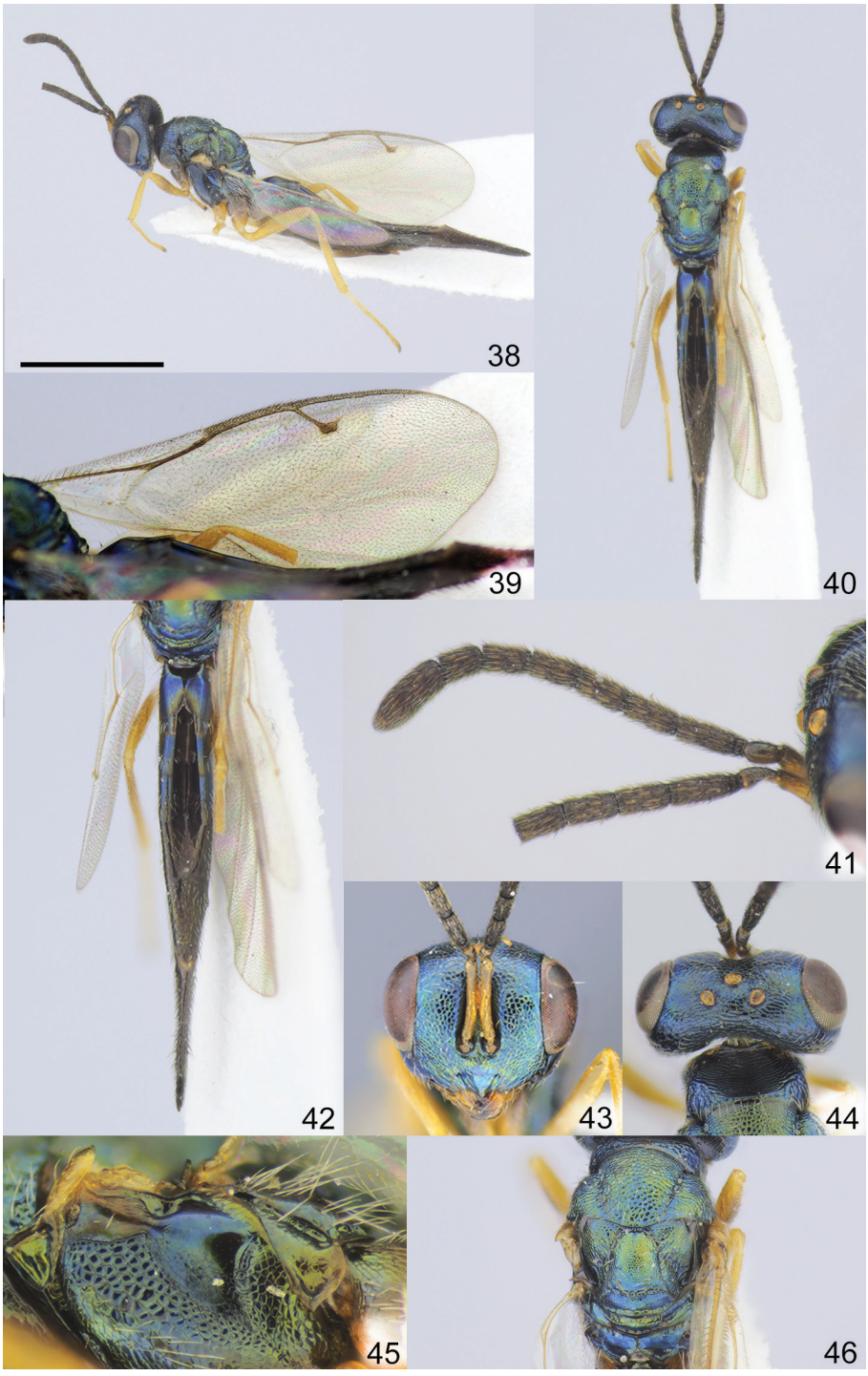
Head, mesosoma and Mt2–Mt4 metallic bluish-green with diffuse coppery lustre; Mt5–Mt8 brown; ovipositor sheath black. Antenna with scape yellowish-brown, pedicel and flagellum brown. Fore and hind coxae yellowish-brown, mid coxa yellow; all femora, tibiae and tarsi yellow except last segment yellowish-brown. Fore wing with light brownish tint, venation yellowish-brown.

Head in dorsal view 2.10–2.17× as broad as long and 1.25–1.30× as broad as mesoscutum; in frontal view 1.05–1.20× as broad as high. POL 0.89–1.05× OOL. Eye height 1.38–1.47× eye length and 2.80–3.00× as long as malar space. Distance between antennal toruli and lower margin of clypeus 0.6× distance between antennal toruli and median ocellus. Antenna with scape 0.80–0.85× as long as eye height and 1.15–1.20× as long as eye length; pedicel 1.70–1.95× as long as broad and 0.55–0.65× as long as F1; combined length of pedicel and flagellum 1.40–1.57× breadth of head; F1 2.15–2.35× as long as broad, F3–F6 longer than broad; clava 2.70–2.90× as long as broad.

Mesosoma 1.65–1.70× as long as broad. Scutellum weakly and finely reticulate, 1.00–1.05× as long as broad. Propodeum without nucha, 0.40–0.55× as long as scutellum; median carina present; sculpture smooth. Metapleuron alutaceous, upper mesepimeron with lower part alutaceous, upper part smooth. Fore wing 2.95–3.25× as long as maximum width; basal cell, cubital vein and basal vein setose; speculum closed; PST 0.66–0.68× as long as M, M 0.75–0.80× as long as PM and 2.0–2.2× as long as S.



Figures 29–37. *Plutothrix kuboi* Kamijo, 2004, paratype female (**29–32**) **29** body, dorsal view **30** body, lateral view **31** fore wing **32** antenna. *Plutothrix kusigematii* Kamijo, 2004, paratype female (**33–37**) **33** antenna **34** fore wing **35** metasoma, dorsal view **36** body, lateral view **37** head, frontal view. Scale bars: 1.25 mm (**29**); 2.5 mm (**36**).



Figures 38–46. *Plutothrix longigaster* sp. nov., holotype female (**38–46**) **38** body, lateral view **39** fore wing **40** body, dorsal view **41** antenna **42** metasoma, dorsal view **43** head, frontal view **44** head, dorsal view **45** mesosoma, lateral view **46** mesosoma, dorsal view. Scale bar: 1.7 mm (**38**).

Metasoma 8.50–9.00× as long as broad, 2.50–2.70× as long as mesosoma and 1.84–1.94× as long as mesosoma and head; Mt2 deeply emarginate medially, Mt8 4.40–5.25× as long as broad. Ovipositor sheath projecting beyond apex of metasoma.

Male unknown.

Etymology. The name of the species refers to its long gaster (metasoma); noun in apposition.

Material examined. ***Holotype*** female (deposited in ZISP): Russia: **Altai Rep.**, Chemal Vill., 20.VII.2007, coll. S Belokobylskij. ***Paratypes*** 1 female, (ZISP): Russia: **Amur Reg.**, 10 km E Arkhary, Arkhara River, 24.VII.2003, coll. S Belokobylskij; 1 female (ZMUH): Finland: **Kb**, Ilomantsi, 20.VII.1865, on *Populus tremula*, coll. Woldstedt, 2466.

Distribution. Finland (single West Palaearctic record of this species), Russia (Western Siberia, Far East).

Biology. Unknown.

Plutothrix narendrani **Kamijo, 2004**

Figs 47–51

Plutothrix narendrani Kamijo, 2004: 300–302. Holotype female, (EIHU, examined).

Material examined. ***Holotype*** female (EIHU): Japan: **Hokkaido Pref.**, "Jozankei, Sapporo, Hokkaido, 20.vi.1967, coll. K. Kusigemati", "Holotype *Plutothrix narendrani* Kamijo". ***Paratype*** female (EIHU): "JAPAN: **Hokkaido Pref.**, Jozankei, 20.VI.1967, coll. K. Kusigemati", "Paratype *Plutothrix narendrani* Kamijo"; male (EIHU): "**Hokkaido Pref.**, Sapporo, 21.V.1967, coll. K. Kusigemati", "Paratype *Plutothrix narendrani* Kamijo". ***Other material*****:** Russia (all in ZISP): **Sakhalin Prov.**, 1 female, Kunashir, Alekhino Vill., 11–13.VI.1973, coll. D. Kasparyan; 1 male, Kunashir, Tret'yakovo Vill., 29.VII.2011, coll. D. Rachin and E. Tselikh; 1 female, Kunashir, Stolbchatiy, 01.VIII.2011, coll. D. Rachin and E. Tselikh; 1 female, Kunashir, Ivanovskiy Cape, 17–20.IX.2013, coll. Yu. Sundukov.

Distribution. Russia (Far East), Japan.

Biology. Unknown.

Plutothrix nudicoxa **Graham, 1993**

Figs 52–55

Plutothrix nudicoxa Graham, 1993: 115. Holotype female (NHMUK, examined).

Material examined. ***Holotype*** female (NHMUK): "CROATIA: Krapina. Prunus 22.7.1909 Hensch", "Holotype", "*Plutothrix nudicoxa* sp. n. M. de V. Graham det. 1993", "B.M. TYPE HYM 5.3682", "NHMUK 013457265". ***Other material*****:** Finland (ZMUH): 1 female, "**Ab**, Nystad, Hellén, 13", "*P. scenicus vittiger* Thom. Det. Kerrich 1956", "*Plutothrix bicolorata* (Spin.) det. Koponen 09", "*Plutothrix nudicoxa* Graham det. Tselikh, 2021".

Figures 47–55. *Plutothrix narendrani* Kamijo, 2004, paratype female (**47–51**) **47** body, dorsal view **48** body, lateral view **49** antenna **50** fore wing **51** scutellum, dorsal view. *Plutothrix nudicoxa* Graham, 1993, holotype female (**52–55**) **52** body, lateral view **53** body, dorsal view **54** hind coxa **55** antenna. Scale bars: 1.4 mm (**47**); 1.8 mm (**53**).

Distribution. Croatia, Czech Republic, Finland (**new record**), United Kingdom (Graham 1993; Noyes 2019).

Biology. Unknown.

Plutothrix obtusiclava Graham, 1993

Figs 56–59

Plutothrix obtusiclava Graham, 1993: 116. Holotype female (NHMUK, not examined).

Material examined. ***Other material*:** Russia (ZISP): **Voronezh Prov.**, 1 female, Khopersky Reserve, VI.1969, coll. T. Gur'yanova.

Distribution. England, Switzerland, Russia (**new record**) (Graham 1993; Noyes 2019).

Biology. Unknown.

Plutothrix pallidiclava Graham, 1993

Figs 60–63

Plutothrix pallidiclava Graham, 1993: 112–114. Holotype female, (NBC, examined).

Material examined. ***Holotype*** female (NBC): Greece: "Ellàs Lésvos A.C. & W. N. Ellis", "3 km NW Ayia Paraskevi, 7.XI.1973", "*Plutothrix pallidiclava* sp.n. M. de V. Graham det. 1993 HOLOTYPE", "collective Zoölogisch Museum Amsterdam", "ZMA.INS. 5107052".

Distribution. Greece.

Biology. Unknown.

Plutothrix perelegans Graham, 1993

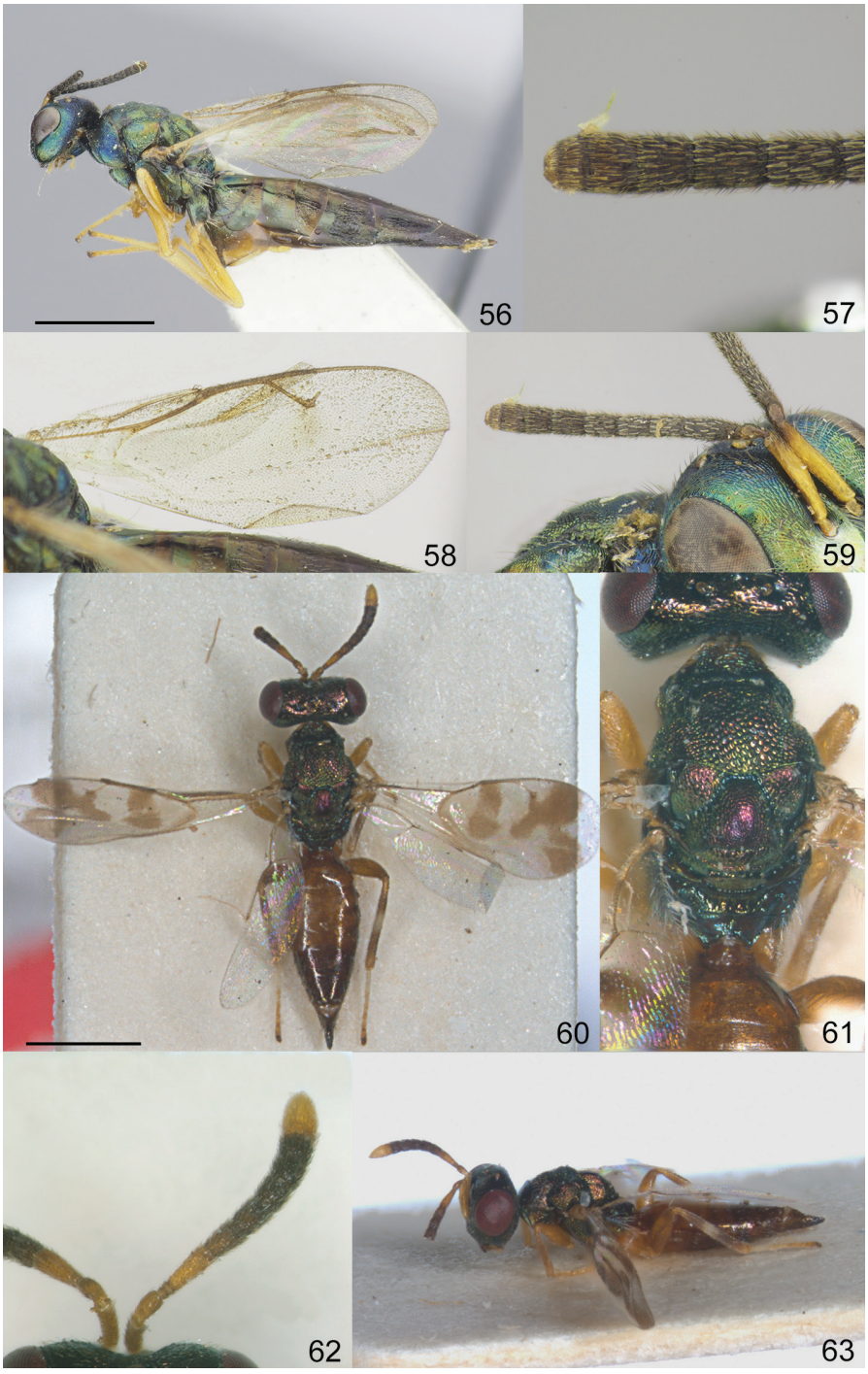
Figs 64–66

Plutothrix perelegans Graham, 1993: 112–114. Holotype female (NHMUK, not examined).

Material examined. ***Other material*:** Finland (all in ZMUH): **A**, 1 female, "Föglö, Hellén, 2123", "*P. scenicus vittiger* Thom. Det. Kerrich 1956", "*Plutothrix bicolorata* (Spin.) det. Koponen 09"; "*Plutothrix perelegans* Graham det. Tselikh 2021"; **N**, 1 female, "Suomi, U, Espoo, 6684:363, 8.8.1965, leg. M. Koponen", "*Plutothrix scenicus* (Walk.) det. M. Koponen 1975"; "*Plutothrix perelegans* Graham det. Tselikh 2021". Ukraine (ZISP): **Khar'kov Prov.**, 1 female, Kupyansk City, 5.VIII.1897, coll. Yaroshevsky.

Distribution. Austria, Croatia, Finland (**new record**), France, Sweden, Ukraine (**new record**) (Graham 1993; Noyes 2019).

Biology. Unknown.



Figures 56–63. *Plutothrix obtusiclava* Graham, 1993, non-type female (**56–59**) **56** body, lateral view **57** antennal clava **58** fore wing **59** antenna. *Plutothrix pallidiclava* Graham, 1993, holotype female (**60–63**) **60** body, dorsal view **61** mesosoma, dorsal view **62** antenna **63** body, lateral view. Scale bars: 1.9 mm (**56**); 1.0 mm (**60**).

***Plutothrix pilicoxa* Graham, 1993**
Figs 67–70

Plutothrix pilicoxa Graham, 1993: 115–116. Holotype female (NHMUK, examined).

Material examined. ***Holotype*** female (NHMUK): "FRANCE: Vaucluse nr. Bédoin (1), 29.5.1985, M. de V. Graham", "*Plutothrix pilicoxa* sp. n. Graham det. 1993 HOLOTYPE", "Holotype", "B.M. TYPE HYM 5.3683", "NHMUK 013457266". ***Other material*:** Russia (all in ZISP): **Belgorod Prov.**, 1 female, 1 male, Borisovskii Distr., Borisovka Vill., "Belogorie" Reserve, "Les na Vorskle," 50°36'34"N, 35°58'55"E, 17.VIII.2020, coll. K. Fadeev; **Krasnodar Reg.**, 1 female, Sochi City, Lazarevskoe, 18.VI.1979, coll. Tobias; 3 females, 2 males, Sochi City, "Mamedova Shchel", 43°57'20"N, 39°18'39"E, 28.VII.2020, coll. S. Belokobylskij and E. Tselikh; 2 females, 2 males, Kalezh Vill., Ashe River, 44°01'25"N, 39°22'03"E, 30.VII.2020, coll. O. Kosheleva and E. Tselikh.

Distribution. France, Russia.

Biology. Unknown.

***Plutothrix rugosa* Kamijo, 2004**
Figs 71–73

Plutothrix rugosa Kamijo, 2004: 303–304. Holotype female (EIHU, examined).

Material examined. ***Holotype*** female (EIHU): "JAPAN: **Tokyo**, Higashiyamato", "3.VI.1994, K. Kamijo", "Holotype *Plutothrix rugosa* Kamijo". ***Other material*:** Japan (EUM): **Ehime**, 1 female, Shikoku, Sugitate, 25.IV.1959, coll. M. Sato. Russia (all in ZISP): **Primorskii Reg.**, 1 female, 20 km SE Spassk-Dal'ny, Evseevka Vill., 09.VI.1989, coll. S Belokobylskij; 1 female, 1 male, 40 km NE, Dukhovskoe Vill., 01.VIII.1996, coll. S Belokobylskij.

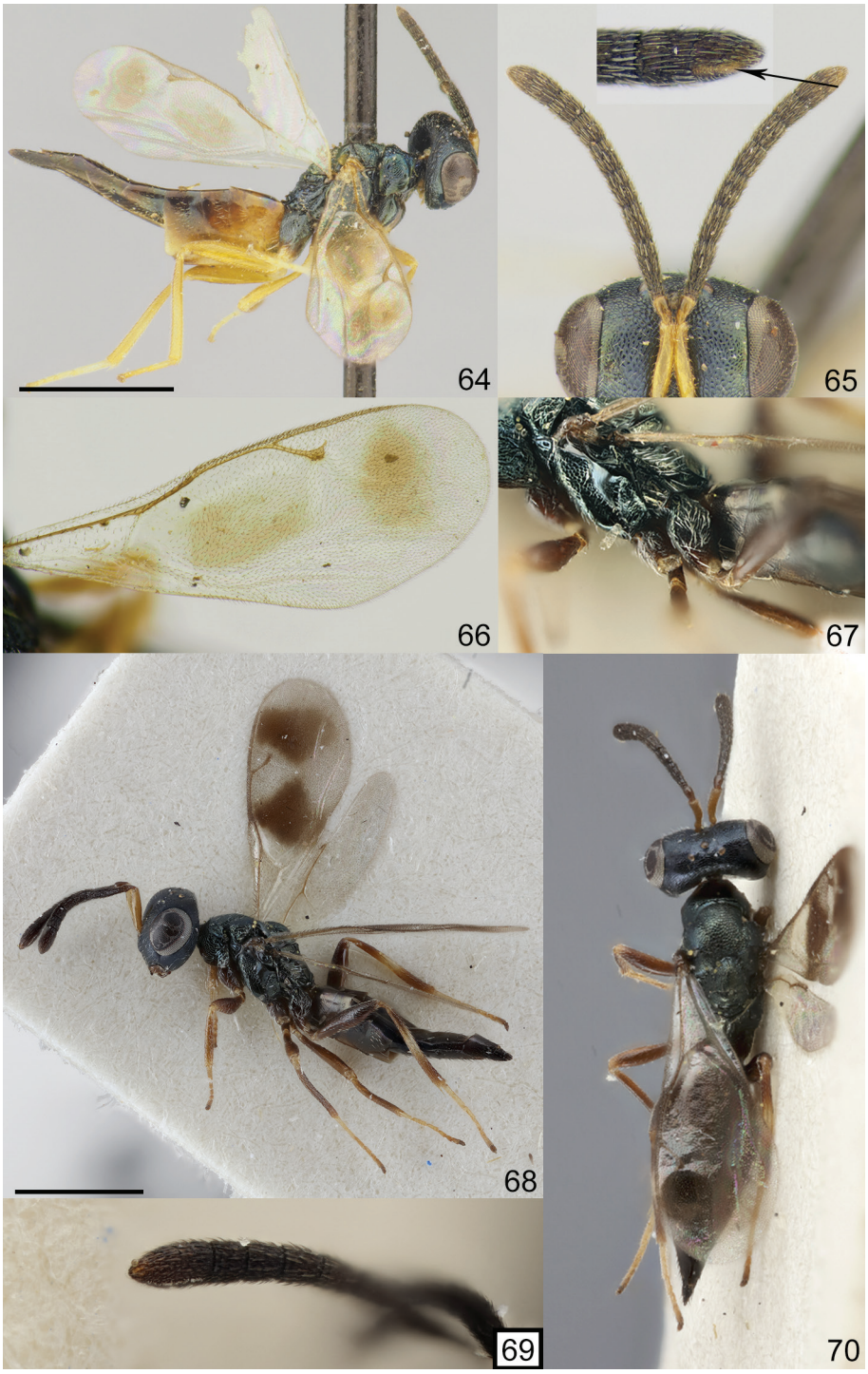
Distribution. Russia (Far East), Japan.

Biology. Unknown.

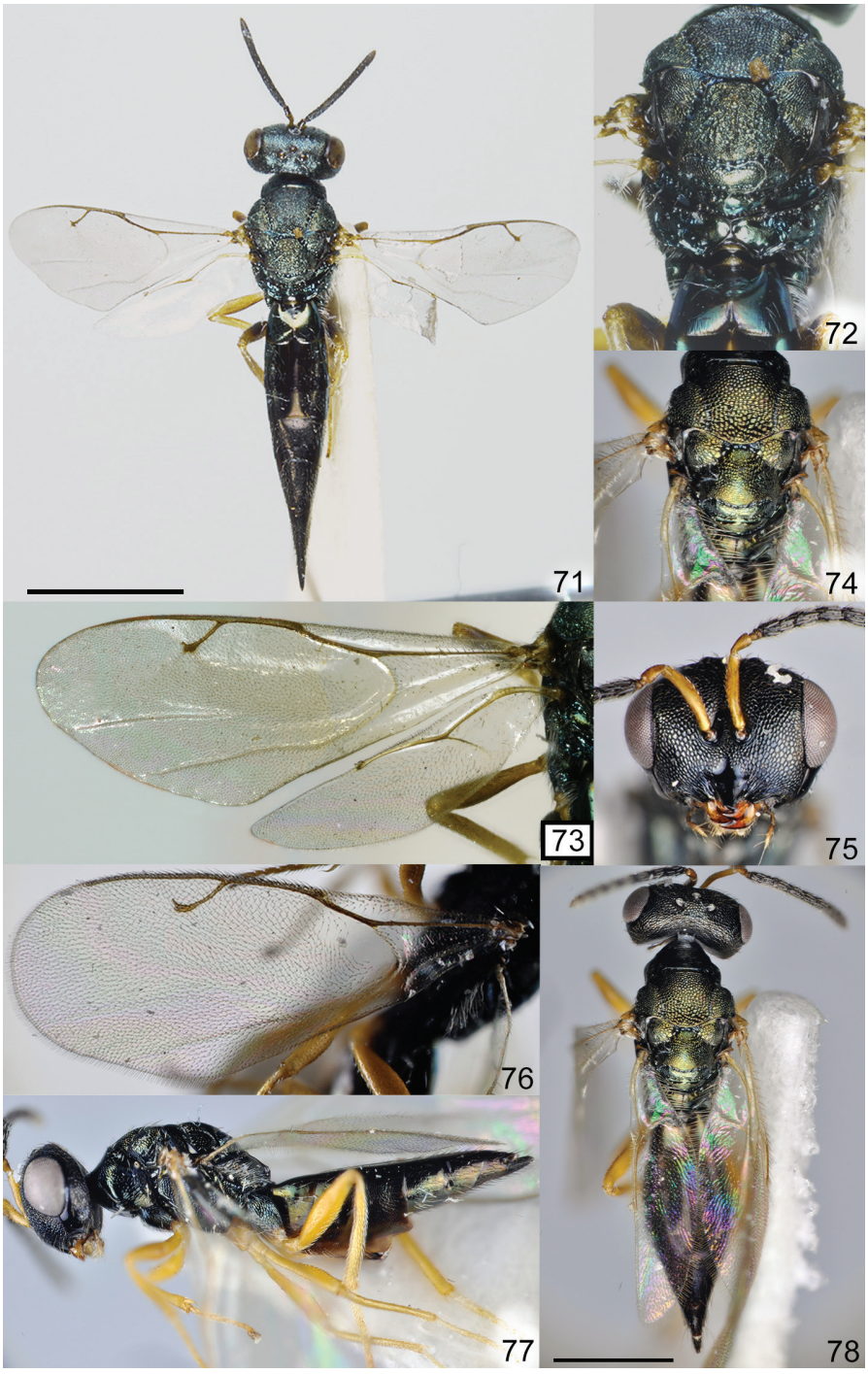
***Plutothrix scrobicula* Kamijo, 2004**
Figs 74–78

Plutothrix scrobicula Kamijo, 2004: 306–307. Holotype female (EIHU, examined).

Material examined. ***Holotype*** female (EIHU): "JAPAN: **Ehime Pref.**, Koya-yama, Oda-miyama, Oda-cho, Shikoku, 2.viii.1994, E. Yamamoto", "Holotype *Plutothrix scrobicula* Kamijo". ***Paratype*** female (EIHU): "JAPAN: Kyushu, Mt. Hikosan Soeda, Fukuoka, 5.VIII.1992, E. Ikeda leg.", "Paratype *Plutothrix scrobicula* Kamijo". ***Other material*:** Russia (all in ZISP): **Primorskii Reg.**, 1 female, Spassk-Dal'ny Town,



Figures 64–70. *Plutothrix perelegans* Graham, 1993, non-type female (**64–66**) **64** body, lateral view **65** antenna **66** fore wing. *Plutothrix pilicoxa* Graham, 1993, holotype female (**67–70**) **67** hind coxa **68** body, lateral view **69** antennal clava **70** body, dorsal view. Scale bars: 1.4 mm (**64**); 1.2 mm (**68**).



Figures 71–78. *Plutothrix rugosa* Kamijo, 2004, holotype female (**71–73**) **71** body, dorsal view **72** mesosoma, dorsal view **73** wings. *Plutothrix scrobicula* Kamijo, 2004, paratype female (**74–78**) **74** mesosoma, dorsal view **75** head, frontal view **76** fore wing **77** body, lateral view **78** body, dorsal view. Scale bars: 2.7 mm (**71**); 1.0 mm (**78**).

17.VIII.1993, coll. S Belokobylskij; 1 male, same locality, 08.VIII.1996, coll. S Belokobylskij. South Korea (YNU): **GW**, 1 female, Wonju-si, Socho-myeon, Hakgongri, Mt. Chiak, 37°22'18"N, 128°03'02"E, 20.VI–19.VII.2013, coll. J.W. Lee.

Distribution. Russia (Far East), South Korea, Japan.

Biology. Unknown.

***Plutothrix transdanuviana* (Erdős, 1946), syn. nov.**
Figs 95–97

Anoglyphis transdanuviana Erdős, 1946: 158. Lectotype female (HNHM, examined).

Material examined. ***Lectotype*** female (HNHM): Hungary: "Kőszegi h. 1944.V.22. dr. Erdős", "Hym. Typ. No. 5742 Mus. Budapest", "Cotypus", "♀", "Alnus glutinosa L.", "Lectotypus *Anoglyphis transdanuviana* Erd. 946", "*Seladerma antennatum* (Walk.)"; "Kőszegi h 1944.VI.26. dr. Erdős", "Hym. Typ. No. 5743 Mus. Budapest", "Cotypus", "♂", "rét", "Paralectotypus *Anoglyphis transdanuviana* Erd. 946".

Distribution. Hungary.

Biology. Unknown.

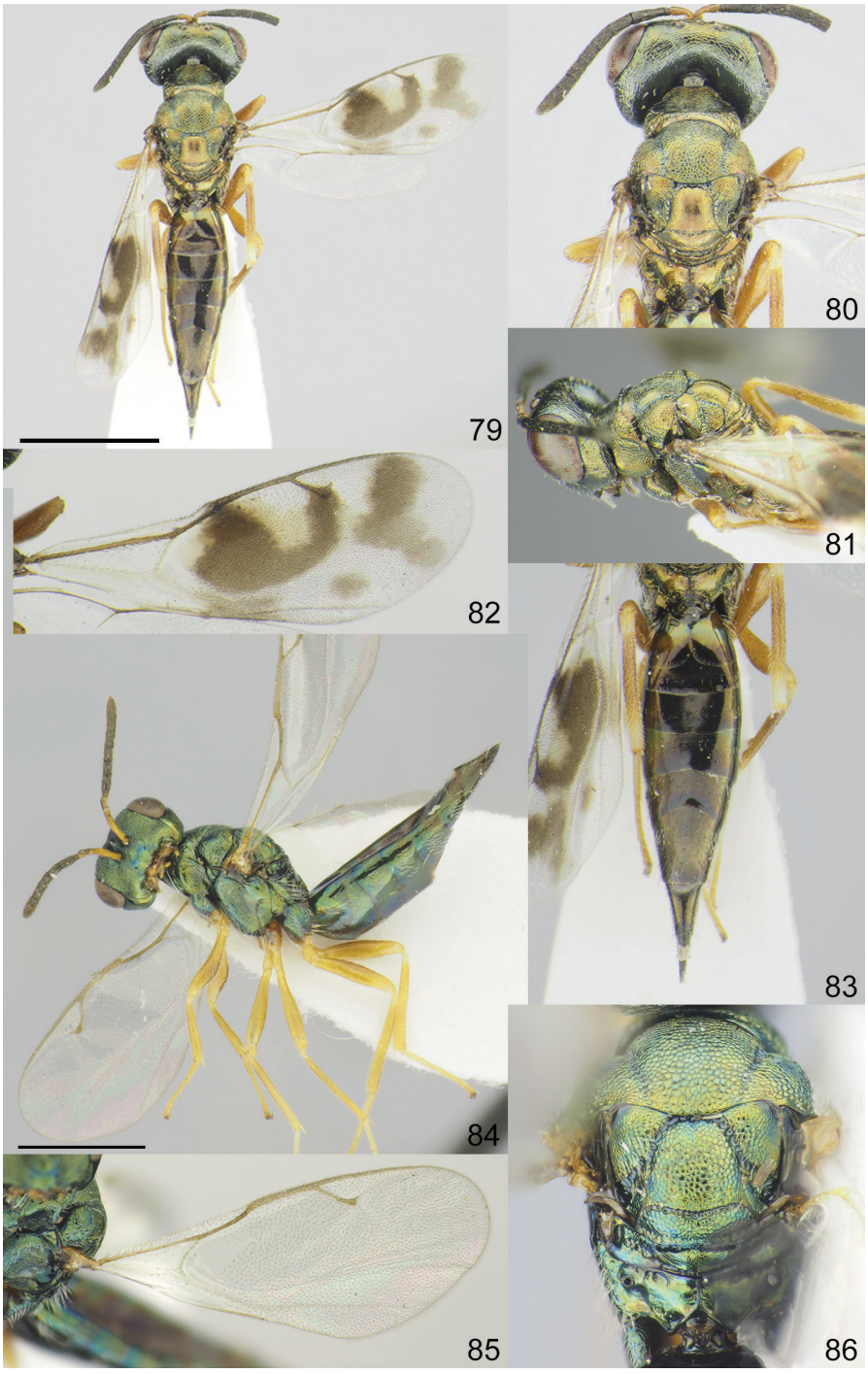
Remarks. Erdős (1946) described the species *Anoglyphis transdanuviana* Erdős from the Kőszegi Hills, West Hungary. Kerrich et Graham (1957) synonymized the genus *Anoglyphis* Förster, 1878 with *Plutothrix* Förster, 1856. As a result, *A. transdanuviana* was transferred to the genus *Plutothrix*. Subsequently, Graham (1969) suggested that *A. transdanuviana* might belong to *Seladerma* Walker, 1834. Examination of the types showed they belong to the species *Seladerma antennatum* (Walker) based on the following character states. Head 2.05× as long as broad. Clypeal margin with asymmetric teeth. Funicular segments of antenna with one row of sensilla. Mesosoma with complete notauli. Fore wing with speculum, basal vein incomplete, PM longer than M. Propodeum 0.37× as long as scutellum. Petiole transverse. Metasoma longer than mesosoma and head, 2.33× as long as broad. *Plutothrix transdanuviana* (Erdős, 1946) is thus hereby synonymized under *Seladerma antennatum* (Walker, 1833).

***Plutothrix trifasciata* (Thomson, 1878)**
Figs 79–83

Plutothrix foersteri Mayr, 1904: 586. Male type, lost. Synonymy by Ferrière and Novitzky (1955: 31).

Trigonoderus trifasciatus Thomson, 1878: 11. Lectotype female (LUZN, examined). Designated by Kerrich and Graham (1957: 293).

Material examined. ***Lectotype*** female (LUZN): Sweden: "Fardhem 3.Jli 41", "*Trigonoderus trifasciatus* Thoms. LECTOTYPE G.J. Kerrich & M.W. Graham 1955", "Type", "TYPE NO. 134:1 Pteromalidae Zool. Mus. Lund Sweden". ***Other material*:**



Figures 79–86. *Plutothrix trifasciata* (Thomson, 1878), non-type female (**79–83**) **79** body, dorsal view **80** head and mesosoma, dorsal view **81** head and mesosoma, lateral view **82** fore wing **83** metasoma, dorsal view. *Plutothrix zhangyieensis* Yang, 1996, non-type female (**84–86**) **84** body, lateral view **85** fore wing **86** mesosoma, dorsal view. Scale bars: 1.6 mm (**79**); 1.0 mm (**84**).

Finland (all in ZMUH): 1 female, **Ab**, "669370:323763, V, Parainen, Malaise 1A, 19.07–02.08.2020, leg. S. Väänänen, J. Paukkunen", "*Plutothrix trifasciata* (Thomson) det. Tselikh 2021"; **Ta**, 1 female, "Fennia, Ta, Vanaja, 25.07.1957, leg. Valkeila", "*Plutothrix trifasciata* (Thomson) det. Valkeila"; **Sa**, 1 female, "Fennia, ES, Joutseno, Marttila raidat, 676950: 58854, 05.08.2012, leg. M. Raekunnas", "*Plutothrix trifasciatus* det. M. Koponen"; 1 female, "Finland, 669370:323763, V, Parainen, Malaise 1A, 19.07–02.08.2020, leg. S. Väänänen, J. Paukkunen", "*Plutothrix trifasciata* (Thomson) det. Tselikh 2021»;1 female, "154, Fennia, Snappert., 15.07.1933, Klingstedt, coll. Klingstedt", "*Plutothrix trifasciata* (Thomson) det. Tselikh 2021"; **Om** [Ostrobottnia media], 1 female, "Fennia, Pyhäjärvi, 27.07.1957, leg. V. Vikberg", "*Plutothrix trifasciatus* Ths, det. V. Vikberg". Moldova (all in ZISP): 2 females, "Bendery City, 08.VI.1974, coll. D. Kasparyan". Russia (all in ZISP): **Voronezh Prov.**, 1 female, Saval'skoe forestry, 08.VI.1954, coll. V. Stark; 1 female, 20 km SW of Rossosh' City, Zhilino Vill., 49°49'58"N, 39°19'48"E, 11.VIII.2020, coll. Tselikh; **Kostroma Prov.**, 1 female, Vasil'evskoe Vill., 21.VII.1935, coll. V. Gussakovsky; **Samara Prov.**, 1 female, Krasny Yar Vill., 53°31'23"N, 50°22'28"E, 23.VIII.2020, coll. K. Samartsev; **Krasnodar Reg.**, 1 female, Kubanskaya Vill. 20.VI.1933, coll. Shestakov; **Orenburg Prov.**, 2 females, Kondurovka Vill., 07.VIII.2021, coll. K. Fadeev; **Primorskii Reg.**, 1 female, Novokachalinsk Vill., Khanka Lake, 04–07.VIII.2006, coll. S Belokobylskij. Tajikistan (ZISP): 1 female, "Kondara, 30.V.1939, coll. V. Gussakovsky". South Korea (all in YNU): **GB** [Gyeongsangbuk-do], 1 female, Dalseo-gu, Daegok-dong, Daegu Arboretum, 35°48'3.26"N, 128°31'15.3"E, 12.IX.–4.X.2012, coll. S.G. Gang; 1 male, Gyeongsan-si, Daehak-ro 280, Yeungnam Univ., 35°49'30"N, 128°45'39"E, 30.VII.–25.X.2013, coll. J.W. Lee; 1 female, Cheongdo-gun, Gakbuk-myeon, Namsan-ri, 15.IX.–21.X.2013, J.W. Lee; **GN** [Gyeongsangnam-do], 1 female, Jinju-si, Ibanseong-myeon, Daecheon-ri, Gyeongsangnam-do, For. Env. Res. Inst., 35°9'39.7"N, 128°17'41.3"E, 16.IX.–1.X.2013, coll. J.H. Hwang; **GW** [Gangwon-do], 1 female, Wonju-si, Heungeop-myeon, Maeji-ri 234, Yonsei University, 5–26.IX.2014, coll. H.Y. Han; 1 female, Seoul, Dongdaemun-gu, Cheongnyangni-dong, 29.VIII.–05.IX.2005, coll. W.L. Choi.

Distribution. Croatia, Czech Republic, Denmark, Finland, Germany, Hungary, Kazakhstan, Korea, South, Lithuania, Moldova, Netherlands, Romania, Russia, Slovakia, Spain, Sweden, United Kingdom (Noyes 2019; Tselikh 2019).

Biology. Unknown.

***Plutothrix zerovae* Tselikh, Várkonyi & Dale-Skey, sp. nov.**

https://zoobank.org/EBE2A581-F111-4BBE-9343-BDB7A4663E91

Figs 87–94

Comparison. *Plutothrix zerovae* is similar to *P. kuboi* Kamijo, 2004; the differences between these species are given in the key.

Description. Female. Body length 5.60–6.50 mm. Fore wing length 4.10–4.50 mm.

Head, mesosoma and Mt2–Mt6 metallic bluish-green with diffuse coppery lustre, Mt8 brown; ovipositor sheath black. Antenna with scape yellow, pedicel yellowish-

brown, flagellum brown. All coxae metallic green with diffuse coppery lustre; all femora, tibiae and tarsi yellow except last segment yellowish-brown. Fore wing hyaline with one fuscous cloud touching stigma, venation yellowish-brown.

Head in dorsal view 2.10–2.26× as broad as long and 1.16–1.19× as broad as mesoscutum; in frontal view 1.24–1.26× as broad as high. POL 0.90–1.00× OOL. Eye height 1.43–1.46× eye length and 2.10–2.22× as long as malar space. Distance between antennal toruli and lower margin of clypeus 0.58–0.65× distance between antennal toruli and median ocellus. Antenna with scape 0.85–0.89× as long as eye height and 1.20–1.30× as long as eye length; pedicel 2.00–2.07× as long as broad and 0.72–0.80× as long as F1; combined length of pedicel and flagellum 1.12–1.18× breadth of head; F1 2.20–2.35× as long as broad, F3–F6 longer than broad; clava 2.45–2.60× as long as broad.

Mesosoma 1.55–1.60× as long as broad. Scutellum finely reticulate, 1.18–1.20× as long as broad. Propodeum without nucha, 0.86–0.90× as long as scutellum; median carina present; sculpture weakly reticulate. Metapleuron reticulate, upper mesepimeron alutaceous. Fore wing 2.50–2.70× as long as maximum width; basal cell, cubital vein, basal vein setose; speculum closed; PST 0.75–0.86× as long as M, M 0.74–0.76× as long as PM and 1.80–1.93× as long as S.

Metasoma 4.30–4.40× as long as broad, 1.80–1.95× as long as mesosoma and 1.45–1.56× as long as mesosoma and head; Mt2 emarginate medially, Mt8 2.20–2.50× as long as broad. Ovipositor sheath projecting beyond apex of metasoma.

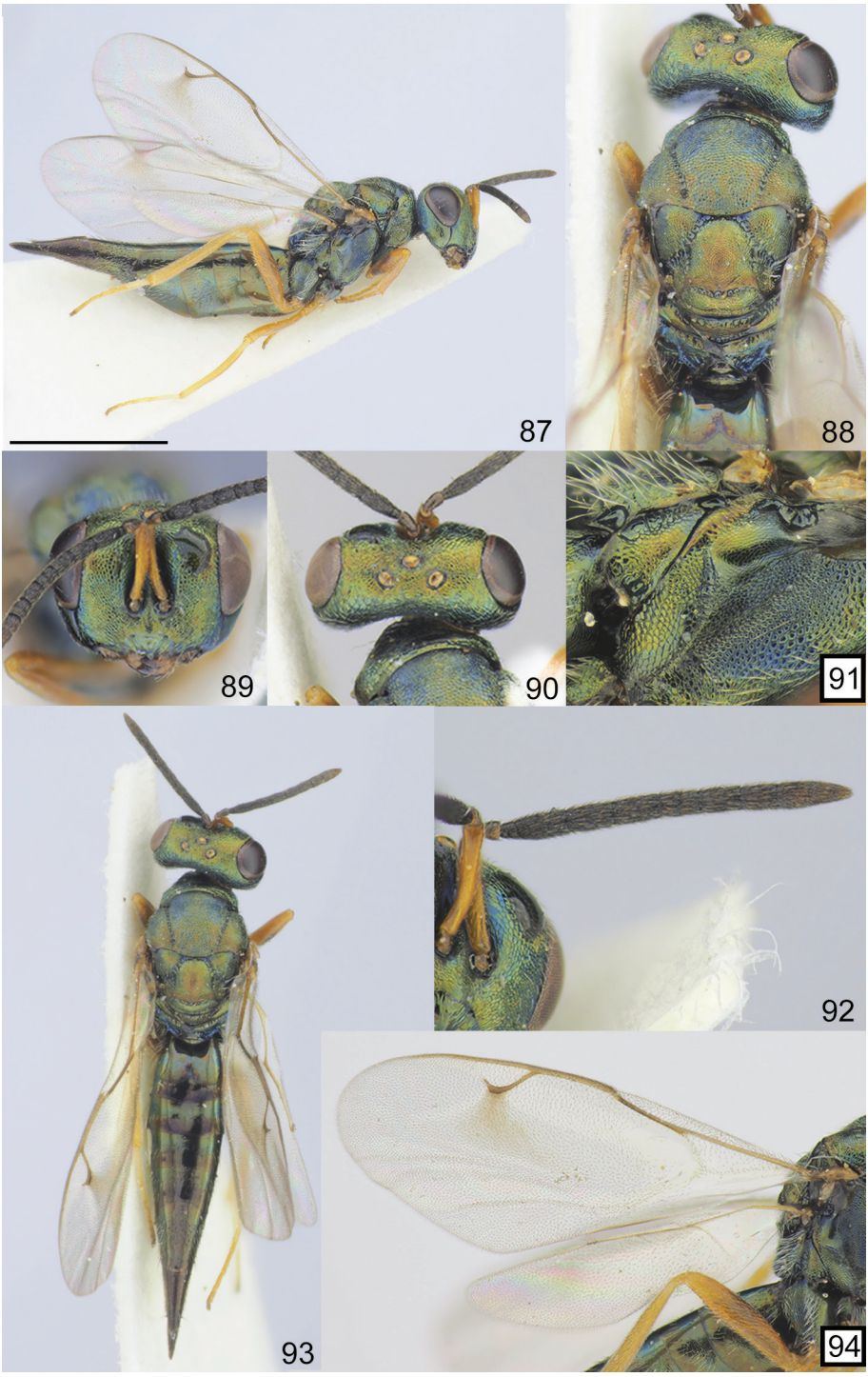
Male unknown.

Etymology. The species is named in honour of the prominent entomologist, Dr M.D. Zerova (1934–2021), an expert on Eurytomidae, Torymidae and Ormyridae (Hymenoptera).

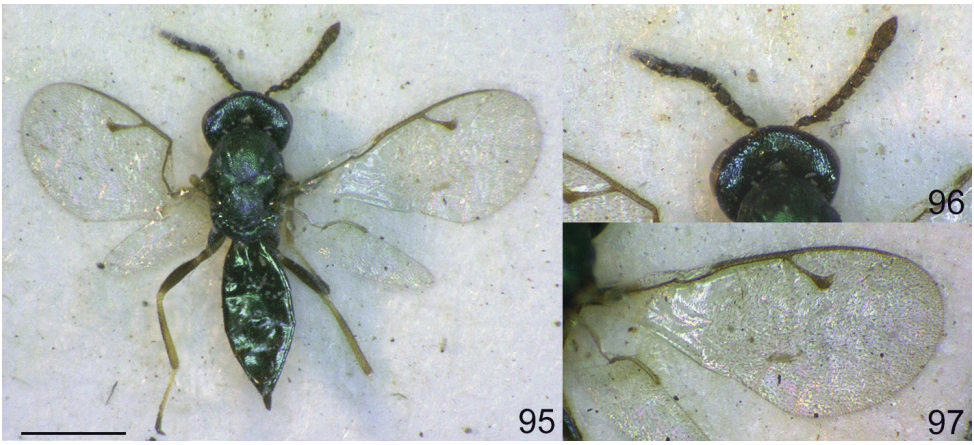
Material examined. ***Holotype*** female (ZMUH): Finland: "Suomi, ES, Mikkelin mlk., 6830:501, 05.07.1987, leg. M. Koponen", "*Plutothrix coelius* (Walker) det. M. Koponen", "Holotype *Plutothrix zerovae* sp.n. Tselikh". ***Paratypes.*** Finland (all in ZMUH): **N**, 1 female, "Suomi, Kauniainen, 09.07.1946, leg. A. Saarinen"; **Ta**, 1 female, "Suomi, EH, Luopioinen, 07.08.1976, leg. E. Kangas"; 1 female, "Suomi, EH, Luopioinen, 21.07.1956, leg. E. Kangas", "*Plutothrix coelius* (Walker) det. M. Koponen"; 1 female, "Finland, Loppi, 03.07.1937, leg. C. Ahnger"; **Sa**, 1 female, "Finland, Ristiina, 6826:502, 28.06.1992, leg. M. Koponen", "*Plutothrix coelius* (Walker) det. M. Koponen"; 1 female, "Suomi, ES, Ristiina, 6826:502, 08.07.1978, leg. M. Koponen", "*Plutothrix coelius* (Walker) det. M. Koponen"; 1 female, "Suomi, ES, Mikkelin mlk, 6830:501, 03.09.1974, leg. M. Koponen", "*Plutothrix coelius* (Walker) det. M. Koponen"; 1 female, "Finland, Ristiina, 6826:502, 03.07.1995, leg. M. Koponen"; **Tb**, 1 female, "Jyväskylä, Hellén, 208"; **Kb**, 1 female, "Suomi, PK, Tohmajärvi, 6908:660, 18.07.1982, leg. M. Koponen". Russia: (ZMUH) **Leningrad Prov.**, 1 female, "Viipuri, Linnaniemi, 610, MUS., ZOOL. UNIV. TURKU"; (ZISP) **Smolensk Prov.**, 1 female, near Smolensk City, 54°49'01"N, 32°04'50"E, 22.VIII.2020, coll. S Belokobylskij.

Distribution. Finland, Russia (European part of Russia).

Biology. Unknown.



Figures 87–94. *Plutothrix zerovae* sp. nov., holotype female (**87–94**) **87** body, lateral view **88** head and mesosoma, dorsal view **89** head, frontal view **90** head, dorsal view **91** mesosoma, lateral view **92** antenna **93** body, dorsal view **94** wings. Scale bar: 2.1 mm (**87**).



Figures 95–97. *Plutothrix transdanuviana* (Erdős, 1946) syn. nov. to *Seladerma antennatum* (Walker, 1833), holotype female (**95–97**) **95** body, dorsal view **96** head and antenna, dorsal view **94** fore wing. Scale bar: 0.75 mm (**95**).

Plutothrix zhangyieensis Yang, 1996

Figs 84–86

Plutothrix zhangyieensis Yang, 1996: 125–127. Holotype female (NWCF, not examined).

Material examined. ***Other material*:** Russia (all in ZISP): **Primorskii Reg.**, Vladivostok City, Okeanskaya, 30.VII.2001, coll. S. Belokobylskij; 1 female, Lazovsky Reserve, Proselochny, 11.VII.2008, coll. A. Khalaim; **Kuril Islands**, Kunashir, Alekhino Vill., 30–31.VII.1981, coll. S. Belokobylskij.

Distribution. People's Republic of China (Gansu), Russia (Far East) (**new record**) (Yang 1996).

Biology. Primary parasitoid of *Polygraphus poligraphus* (Linnaeus, 1758) (Coleoptera, Curculionidae) (Yang 1996).

Conclusion

The present study considerably supplements our knowledge of the genus *Plutothrix* Förster. After the inclusion of the three new species (*Plutothrix gribanovi*, sp. nov., *P. longigaster*, sp. nov., and *P. zerovae*, sp. nov.) from the Palaearctic region described in this paper, and the exclusion of *P. transdanuviana*, syn. nov., the genus *Plutothrix* now consists of thirty valid species.

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SHORT COMMUNICATION

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Cecinothofagus Nieves-Aldrey & Liljeblad (Hymenoptera, Cynipidae) is likely an endoparasitoid of the gall-maker genus *Aditrochus* Rübsaamen (Hymenoptera, Pteromalidae)

Jean-Yves Rasplus[1], José-Luis Nieves-Aldrey[2], Astrid Cruaud[1]

1 *CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Université de Montpellier, Montpellier, France* **2** *Museo Nacional de Ciencias Naturales (CSIC), José Gutiérrez Abascal 2, 28006 Madrid, Spain*

Corresponding author: Jean-Yves Rasplus (Jean-Yves.Rasplus@inrae.fr)

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Abstract

Paraulax Kieffer and *Cecinothofagus* Nieves-Aldrey & Liljeblad (Cynipidae: Paraulacini) were long supposed to be gall-makers on southern beeches (*Nothofagus*, Nothofagaceae). Dissections of galls on *Nothofagus* Blume, suggested that *Cecinothofagus* could be instead either endoparasitoid or inquiline of *Aditrochus* larva (Chalcidoidea). We sequenced the universal *COI* barcode and Ultra-Conserved Elements (UCEs) from young larvae of *Aditrochus* collected from galls on *Nothofagus* and highlighted that one of them also contained DNA from *Cecinothofagus ibarrai* Nieves-Aldrey & Liljeblad. So far, when galls attributed to *Aditrochus* were dissected in early development stages they all contained only a single larva and no remains of other larvae. Conversely, when *Cecinothofagus ibarrai* was reared from galls on *Nothofagus*, remains of the host larva were observed inside the larval chamber. Altogether, biological observations and molecular results suggest that *Cecinothofagus ibarrai* is likely an endoparasitoid of *Adritrochus*. This result confirms the tribe Paraulacini as being entomophagous and supports the hypothesis of an ancestral parasitoid lifestyle for Cynipoidea.

Keywords

Biology, Cynipoidea, Chalcidoidea, *Nothofagus*

Introduction

Paraulacini is a tribe of Cynipidae that contains two closely related genera, *Paraulax* Kieffer and *Cecinothofagus* Nieves-Aldrey & Liljeblad (Nieves-Aldrey et al. 2009). Unlike most Cynipidae that are found in the Northern Hemisphere, the six species described in Paraulacini occur in southern South-America (Argentina and Chile; Nieves-Aldrey et al. 2009). Our lack of biological knowledge on Paraulacini would have been anecdotal if the tribe was not recovered sister to all other Cynipoidea in a recent phylogenomic hypothesis proposed by Blaimer et al. (2020). Acquiring knowledge on their biology has thus become crucial to accurately infer the ancestral lifestyle of Cynipoidea.

Paraulax and *Cecinothofagus* were long supposed to be gall-makers on southern beeches (*Nothofagus*, Nothofagaceae) (Dalla Torre and Kieffer 1910; De Santis et al. 1993; Ronquist 1999; Csóka et al. 2005), probably by analogy with the Cynipidae of the northern hemisphere that induce gall on many plant lineages (Ronquist 1999). As of today, the biology of *Paraulax* remains unknown. Dissections of galls on *Nothofagus* suggested that *Cecinothofagus* could be instead either endoparasitoid or inquiline of larva of *Aditrochus* Rübsaamen (Chalcidoidea) (Nieves-Aldrey et al. 2009). Along with the genera *Espinosa* Gahan and *Plastobelyta* Kieffer, *Aditrochus* belongs to the tribe Melanosomellini (Pteromalidae, Ormocerinae) that only occurs in southern South America (Bouček 1988; De Santis et al. 1993). As for Paraulacini, the biology of *Aditrochus* is poorly known. *Aditrochus* is indeed supposed to be a gall-maker (Bouček 1988), while *Espinosa* and *Plastobelyta* have been considered inquilines or parasitoids of gall-makers (Bouček 1988).

In the course of a project to infer the tree of life of Chalcidoidea, we sequenced the universal *COI* barcode and Ultra-Conserved Elements (UCEs) from larvae of *Aditrochus* and highlighted that one of them contained DNA from another species that was identified as *Cecinothofagus ibarrai*. We discuss the implication of such result in the light of biological data to infer the most likely trophic relationships between *Aditrochus* and *Cecinothofagus* (Fig. 1).

Methods

Sampling, morphological identification and DNA extraction

Two morphologically identical larvae of a rare gall inducer *Aditrochus coihuensis* Ovruski, 1993 were extracted from two galls sampled on *Nothofagus dombeyi* (Mirb.) Ørst by JLNA [Ensenada to PN Vicente Perez Rosales, 24.xi.2013, Nieves J.L. leg.]. These larvae were databased in the collection of CBGP (Centre de Biologie pour la Gestion des Populations) and in our storage of DNA under the numbers JRAS07470_0103 and JRAS07470_0104. Larvae were independently identified as belonging to Chalcidoidea by JYR on the basis of head morphology, structure of the labrum and head chaetotaxy. DNA was extracted from the two larvae using the Qiagen DNeasy Blood and Tissue kit. A slightly modified manufacturer's protocol was used to increase DNA yield (Cruaud et al. 2019). Extractions were conducted without destruction of the larvae.

Figure 1. Adults of *Aditrochus coihuensis* (above) and *Cecinothofagus ibarrai* wasps (under) showing their different size. Photograph J.L. Nieves-Aldrey.

DNA barcoding

The DNA extracted from each larva was amplified with a 2 step PCR approach targeting *COI* (universal barcode fragment) following the protocol detailed in Cruaud et al. (2017). Two overlapping fragments [FC and BR (Shokralla et al. 2015)] were amplified and sequenced on a Illumina MiSeq System (2*250 bp) together with other insects (mostly Coleoptera). Importantly, no Cynipoidea were included in the experiment. Raw data were analysed following Cruaud et al. (2017). Briefly, adapter trimming and selection of high-quality paired reads was performed with Trimmomatic (Bolger et al. 2014); paired reads were merged with FLASH (Magoc and Salzberg 2011); clustering of sequences was performed with SWARM (d=1) (Mahé et al. 2015) after dereplication with VSEARCH (Rognes et al. 2016). Only consensus sequences obtained from clusters with more than 5 identical sequences were retained for downstream analyses.

Non-coding sequences as well as sequences of endosymbionts and bioaresols were removed. FC and BR fragments that passed through all quality controls were assembled in Geneious 11.1.4 (https://www.geneious.com) to get full-length *COI* barcodes (658 bp).

Hybrid capture of UCEs

The two DNA extracts were then used to capture about 1,400 UCEs with the 2,749 RNA probes designed by Faircloth et al. (2015) and using the protocol detailed in Cruaud et al. (2019). Extracts were included in a larger capture experiment (N samples = 96) that was sequenced on a Illumina MiSeq system, but again no Cynipoidea were included (only Chalcidoidea). Reads were analysed following Cruaud et al. (2019). Briefly, adapter trimming and selection of high-quality paired reads was performed with Trimmomatic (Bolger et al. 2014); paired reads were merged with FLASH (Magoc and Salzberg 2011) and demultiplexing was performed with a custom script (Cruaud et al. 2019). Assembly into contigs was performed with CAP3 (Huang and Madan 1999) and contigs were aligned with Lastz (Harris 2007) to the set of reference UCEs assembled from probes.

Phylogenetic inference

Small taxa sets were assembled to assess the phylogenetic placement of the *COI* or UCE sequences obtained from the two *Aditrochus* larvae (Table 1). Alignment of *COI* sequences and individual UCEs was done with MAFFT-linsi (Katoh and Standley 2013). Alignment cleaning of individual UCEs was performed using SEQTOOLS (Mirarab et al. 2014): positions with more than 10% gaps and sequences with more than 25% gaps were removed. Three rounds of Treeshrink with b=10 (Mai and Mirarab 2018) were also performed on individual UCE trees to remove abnormally long branches. Trees were built with IQ-TREE 2.0.6 (Minh et al. 2020) from the *COI* data set and from the concatenated UCE data set (no partition) with best fit models selected by ModelFinder (BIC criterion) (Kalyaanamoorthy et al. 2017). FreeRate models with up to ten categories of rates were included in tests for the UCE data set, but only common substitution models were tested for *COI*. The candidate tree set for all tree searches was composed of 98 parsimony trees + 1 BIONJ tree and only the 20 best initial trees were retained for NNI search. Statistical support of nodes was assessed with ultrafast bootstrap (UFBoot) (Minh et al. 2013) with a minimum correlation coefficient set to 0.99 and 1,000 replicates of SH-aLRT tests (Guindon et al. 2010).

Results

DNA barcoding

Only one *COI* sequence was obtained from the first larva (JRAS07470_0103; BR only; 88 sequences in the SWARM cluster). For the second larva (JRAS07470_0104), the exact same sequence was obtained (BR only; 6 sequences in the SWARM cluster) but,

in addition, another sequence that had a positive match on NCBI with *Cecinothofagus ibarrai* Nieves-Aldrey & Liljeblad, 2009 (100% identity; query cover 91%) was also found (FC+BR with, respectively, 127 and 240 sequences in the SWARM clusters). This second sequence corresponds exactly to the sequences of *Cecinothofagus ibarrai* deposited in Genbank by the describers, which cross validated both sequences. Sequences obtained from the two larvae were analysed with Genbank sequences (Table 1) to produce the phylogenetic tree shown in Fig. 2a (best fit model = TIM+F+G4).

Capture of UCEs

For a large number of reference UCEs, two contigs instead of one were recovered in the second larva of *Aditrochus* (JRAS07470_0104). These contigs were blasted against a subset of 400 UCEs that were successfully captured from both *Cecinothofagus ibarrai* (by Blaimer, et al. 2020) and the first larva. 712 contigs retrieved from the second larva had a hit for 392 of these 400 UCEs. For 62 UCEs (on 392), contigs had a hit only with *Aditrochus*; for 17 UCEs, contigs had a hit only with *C. ibarrai* and for the remaining 313 UCEs two contigs were present in the second larva that either matched with *Aditrochus* or *C. ibarrai*. The phylogenetic tree obtained from a larger set of taxa (n=12; Table 1) and 310 UCEs (90% complete matrix; 91,607 bp) is shown in Fig. 2b (best fit model = GTR+F+I+G4).

Table 1. Taxa included in phylogenetic analyses.

Classification	Species	Accession *COI* / UCEs	Source *COI* /UCEs	Nb UCEs (after Treeshrink)
CHAL: Pteromalidae: Ormocerinae	*Aditrochus coihuensis* [JRAS07470_0103 larva1]	OP539441 / SAMN31038493	This study	266
CHAL: Pteromalidae: Ormocerinae	*Aditrochus coihuensis* [JRAS07470_0104 larva2]	OP539442 / SAMN31038494	This study	246
CHAL: Pteromalidae: Ormocerinae	*Espinosa nothofagi*	n.a. /SAMN31038496	n.a. /This study	191
CHAL: Pteromalidae: Epichrysomallinae	*Odontofroggatia* sp.	HM770633 /n.a.	Cruaud et al. 2011 /n.a.	n.a.
CHAL: Ormyridae	*Ormyrus rosae*	KM561583 /n.a.	Unpublished /n.a.	n.a.
CYNI: Cynipidae: Paraulacini	*Cecinothofagus ibarrai*	FJ998298 / SAMN15608859	Nieves-Aldrey et al. 2009 / Blaimer et al. 2020	266
CYNI: Cynipidae: Paraulacini	*Cecinothofagus ibarrai* [endoparasitoid of JRAS07470_0104]	OP539440 / SAMN31038494	This study	248
CYNI: Cynipidae: Cynipini	*Disholcaspis lasia*	n.a. /SAMN06672405	n.a. /Branstetter et al. 2017	268
CYNI: Cynipidae: Cynipini	*Disholcaspis quercusmamma*	n.a. /SAMN06672406	n.a. /Branstetter et al. 2017	275
CYNI: Ibaliidae	*Ibalia anceps*	DQ012641 / SAMN06672424	Unpublished /Branstetter et al. 2017	275
CYNI: Figitidae: Eucoilinae	*Leptopilina japonica*	MK268803 / SAMN15608914	Unpublished /Blaimer et al. 2020	149
CYNI: Cynipidae: Pediaspidini	*Pediaspis aceris*	AY368929 / SAMN15608898	Nylander et al. 2004 / Blaimer et al. 2020	96
CYNI: Cynipidae: Diastrophini	*Periclistus brandtii*	KF936633 / SAMN31038495	Malm and Nyman 2015 / This study	264
CYNI: Figitidae: Aspicerinae	*Prosaspicera sp.*	n.a. /SAMN06672413	n.a. /Branstetter et al. 2017	265

CHAL= Chalcidoidea; CYNI=Cynipoidae.

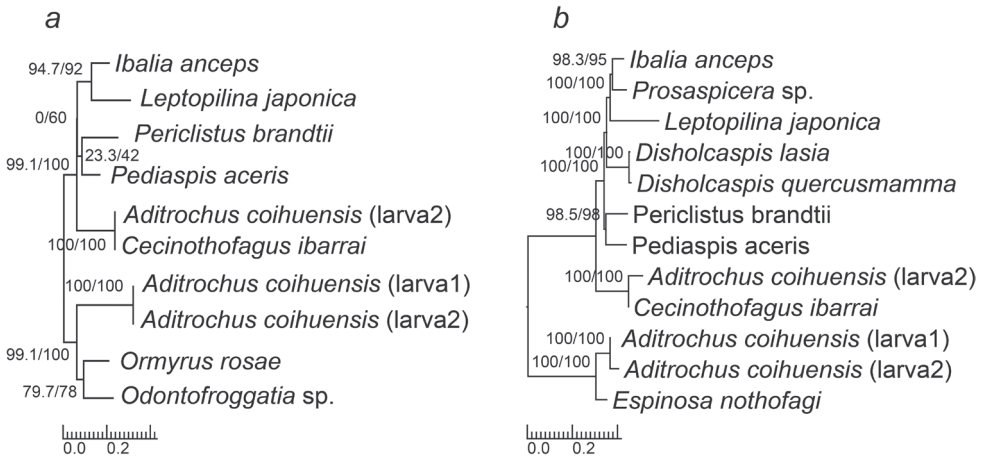

Figure 2. Phylogenetic trees. **a** *COI* barcode tree **b** UCE tree (obtained from the concatenation of 310 UCEs after 2 rounds of treeshrink on each individual UCEs). Statistical support (SHaLRT/UFBoot) are shown at nodes. Accession numbers for sequences used in the analyses are listed in Table 1.

Discussion

Cynipids reared from galls on *Nothofagus* (*Paraulax* and *Cecinothophagus*) have long been supposed to be gall inducers (Dalla Torre and Kieffer 1910; De Santis et al. 1993; Ronquist 1999; Csóka et al. 2005). Gall dissection by Nieves-Aldrey et al. (2009) (see also Figs 3, 4) suggested that species of *Cecinothofagus* were instead parasitoids or lethal inquilines within galls induced by species of *Aditrochus*.

Larvae assigned to *Aditrochus* were observed by one of us (JLNA) in dozens of dissected galls collected on *Nothofagus* species in Chile in field campaigns from the years 2005, 2006, 2012, 2013 and 2014. In all cases, the galls dissected in early development stages contained only a single larva occupying the central larval chamber in the gall (Fig. 3). Furthermore, no remains of other larvae were present which confirmed that the larvae were gall inducers and not parasitoids. Here we confirm that these larvae belong to Chalcidoidea on the basis of morphology and both DNA barcoding and sequencing of UCEs. Although the biology of Melanosomellini is poorly known (only half of the 30 genera have reliable host records; Noyes 2019), most of them are considered to be gall makers (Noble 1941). *Trichilogaster acaciaelongifoliae* (Froggatt, 1892) has even been used to control the invasive *Acacia longifolia* (Andr.) Willd. in South Africa. Melanosomellini are associated with eight plant families that originated in the southern hemisphere: Myrtaceae (7 genera), Fabaceae Mimosoideae (5), Fagales [Nothofagaceae (3) and Casuarinaceae (2)], Malvales [Malvaceae (2) and Elaeocarpaceae (1)], Celastraceae (1) and Apocynaceae (1). However, *Brachyscelidiphaga* appears to be an inquiline in galls of *Apiomorpha* Rübsaamen (Hemiptera, Eriococcidae) on *Eucalyptus* L'Hér. (Bouček 1988). Therefore, our results are in agreement with the most common biology found in Melanosomellini.

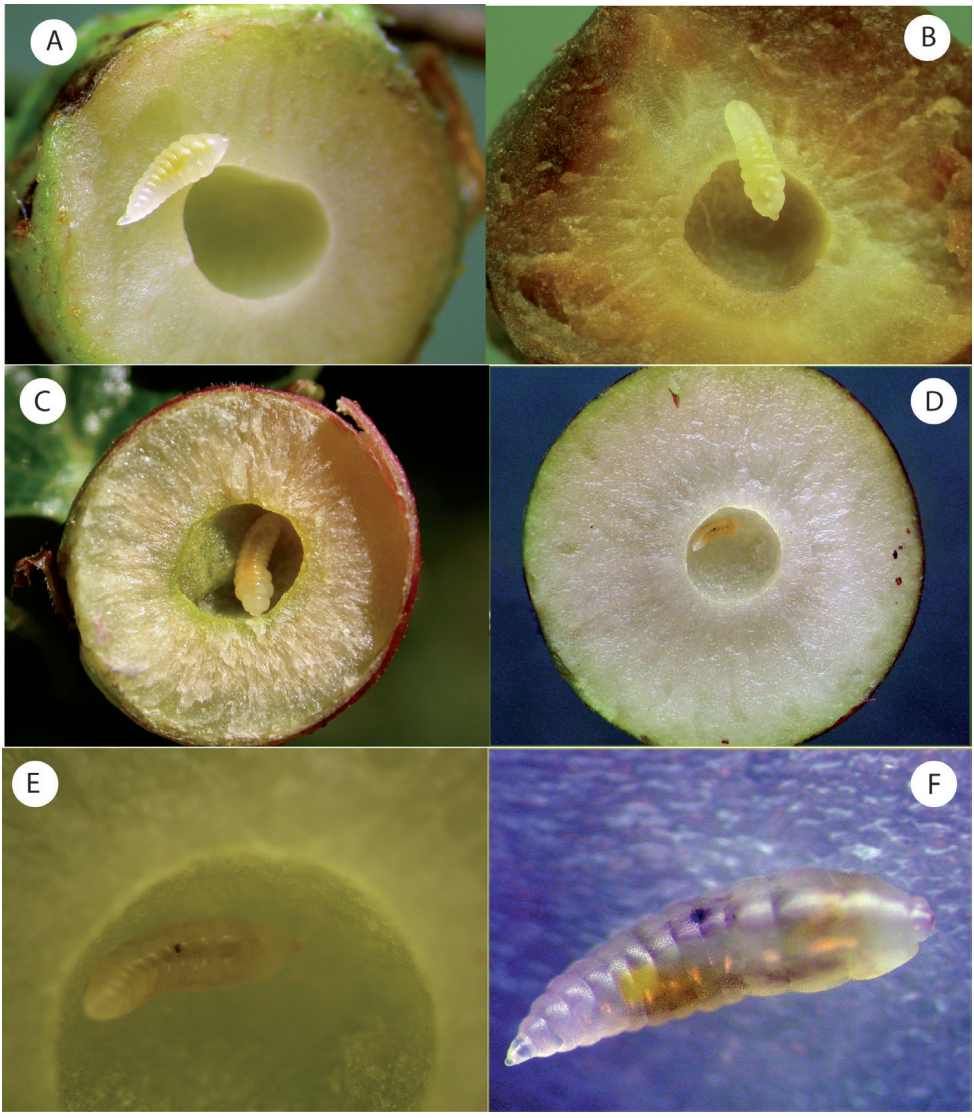

Figure 3. Cross section of galls of *Aditrochus* species on *Nothofagus* showing the central larval chamber and the gall inducer *Aditrochus* larva (note the absence of remains of other larvae inside the chamber). **A, B** *Aditrochus coihuensis* **C** *Aditrochus fagicolus* **D–F** *Aditrochus coihuensis* larva paralyzed by an endoparasitoid (likely *Cecinothofagus ibarrai*). Photographs J.L. Nieves-Aldrey.

Conversely, when *Cecinothofagus ibarrai* was reared from galls on *Nothofagus*, remains of the host larva were observed inside the larval chamber (Fig. 4). Here, we show that one larva of *A. coihuensis* (JRAS07470_0104) also hosted the DNA of *Cecinothofagus ibarrai*. From all these results, we can conclude that *Cecinothofagus ibarrai* was likely an endoparasitoid of this larva. This result confirms that the early

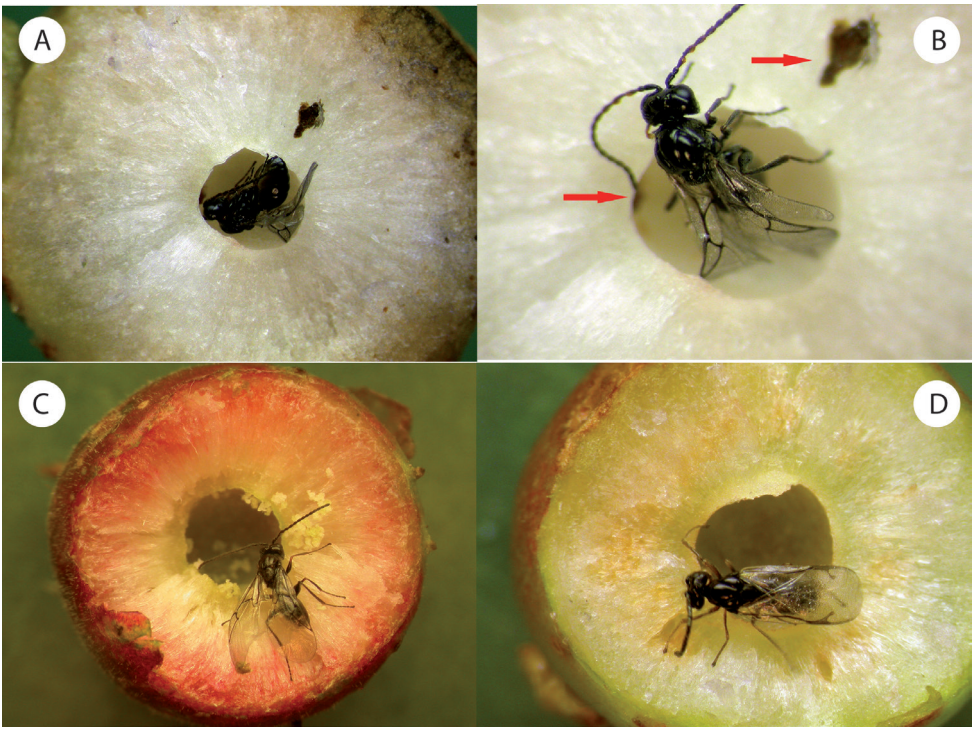

Figure 4. Cross sections of galls of *Aditrochus* species on *Nothofagus* showing emergences of the gall inducer *Aditrochus* adult and the endoparasitoid *Cecinothofagus* adult **A, B** *Cecinothofagus ibarrai* (Cynipidae) (note the remains of the host larva inside the larval chamber) **C** *Cecinothofagus ibarrai* emerged from a gall of *Aditrochus gnirensis* on *Nothofagus antarctica* **D** Adult *Aditrochus coihuensis* emerged from its gall on *Nothofagus dombeyi*. Red arrows show the remains of the host larva. Photographs J.L. Nieves-Aldrey.

evolution of cynipoids may be entomophagous in nature (Blaimer et al. 2020). In this study where the ancestral lifestyle of Cynipoidea was estimated to be either inquiline or parasitoid, our results may contribute to remove ambiguity.

To conclude, our study demonstrated that the usual trophic interactions observed in northern hemisphere on Fagaceae (cynipids are gall makers and pteromalids are parasitoids) is reversed in the southern hemisphere on Fagaceae (pteromalids are gall makers and cynipids are parasitoids or inquilines) … … a bit like water drains the other way Down Under!

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RESEARCH ARTICLE

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Meteorus lucianae sp. nov. (Hymenoptera, Braconidae), a new parasitoid of the bud borer *Crocidosema aporema* (Lepidoptera, Tortricidae)

Luis Felipe Ventura de Almeida[1], Angélica Maria Penteado-Dias[1]

1 *Universidade Federal de São Carlos, Departamento de Ecologia e Biologia Evolutiva, Rod. Washington Luiz Km 235, São Carlos, SP, Brazil*

Corresponding author: Luis Felipe Ventura de Almeida (almeidalfvd@gmail.com)

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Abstract

Crocidosema aporema is a Neotropical Tortricidae moth that feeds on several wild and cultivated Fabaceae, and has a potential to cause economic damages. A new parasitoid wasp belonging to the genus *Meteorus* (Hymenoptera, Braconidae), which has been reared from *C. aporema* feeding on soybean in Brazil, is described and illustrated. A checklist of parasitoids previously recorded for *C. aporema* is provided.

Keywords

biological control, Brazil, *Epinotia*, taxonomy

Introduction

Crocidosema (=*Epinotia*) *aporema* (Walshingham) is a borer moth belonging to the family Tortricidae. It is widespread across Central and South America and can feed on several wild and cultivated Fabaceae, including alfalfa, broad bean, clover, common bean, lupin, melilot, peanut and soybean (Sanchez and Pereyra 2008). *Crocidosema aporema* is multivoltine and remains active year around, producing four to five generations (Sanchez et al. 1997).

This species can be of economic importance for several crops. *Crocidosema aporema* has received particular attention as a pest of soybean, due to the importance of this crop in South America, where this insect can become a relevant problem especially in colder regions such as Argentina and the south of Brazil (Sanchez et al. 1997; Hoffmann-Campo et al. 2000). It is also considered one of the few insects regularly causing damage to fodder leguminosae in Uruguay, especially due to the impact to seed production (Alzugaray 2004).

The control of *C. aporema* using agrochemicals can be a twofold problem, first due to its borer behavior, which reduces the effectiveness of contact pesticides, second because *C. aporema* is more likely to impact crop productivity when present during the flowering stage, thus the use of insecticides during this critical phase could also affect pollinators (Foerster et al. 1983; Alzugaray 2004). Furthermore, the conspicuous plant damages caused by *C. aporema*, even when occurring below the economic threshold, are known to precipitate the use of insecticides by farmers and consequently increase economic and ecological costs (Bueno and Sosa-Gómez 2021). *Crocidosema aporema* has also been recorded developing on Bt soybean expressing the cry1Ac gene, indicating the existence of populations that have acquired some level of resistance to this particular protein (Bueno and Sosa-Gómez 2021).

Meteorus Haliday is a genus of endoparasitoid wasps belonging to the family Braconidae, it has a cosmopolitan distribution and around 350 described species (Yu et al. 2016). The genus has been recorded parasitizing a wide range of Lepidotera larvae, including several of economic relevance (Shaw 1997). The biology of *M. pulchricornis*, for instance, has been extensively studied and the species has been considered a potential biocontrol agent for major insect pests such as *Helicoverpa* spp. and *Spodoptera* spp. (Maeto 2018).

Biological control agents acting naturally or under an integrated pest management system could help maintain a more sustainable and productive cropping (Bortolotto et al. 2015). In this context, the present work aims to contribute to this goal by describing a novel parasitoid of *C. aporema* belonging to the genus *Meteorus*.

Materials and methods

The studied material is deposited at "Coleção Taxonômica do Departamento de Ecologia e Biologia Evolutiva da UFSCar" (DCBU), São Carlos, Brazil. Each examined specimen was given a unique collection catalog number (e.g. DCBU00000). Morphological terminology follows Sharkey and Wharton (1997), microsculpture terminology follows Harris (1979), measurements are taken as proposed by Aguirre et al. (2015). The description is based on the holotype and the variation found in paratypes is presented in parenthesis.

Images were obtained using a Leica DFC295 camera attached to a Leica M165C stereomicroscope and stacked with the Leica Application suite software v3.7.0. Pictures were later processed using Adobe Photoshop.

Results

***Meteorus lucianae* sp. nov.**
https://zoobank.org/2F7FAD13-B6A2-43F2-826C-18CF368A034C
Figs 1–7

Diagnosis. Dorsope absent; mandibles twisted; occipital carina complete; eyes large and convergent; head height 1.35–1.65 × eye height; face maximum width 1.24–1.55 × its minimum width; malar space length 0.40–0.62 × mandible width basally; ovipositor length 1.93–2.53 × first tergite length; ventral borders of T1 touching for a short distance or almost touching.

Description. ***Body length*:** 3.36 (2.93–4.05) mm.

***Color*:** Antenna dark brown with scape and pedicel yellow; head mostly yellow, frons and vertex medially black (sometimes frons mostly black, except by yellow patches around eyes); propleuron yellow; pronotum yellow ventrally, black dorsally; mesonotum black; mesopleuron dark brown-black with a yellow area on posterior margin (Fig. 1); metanotum dark brown; metapleuron dorsally dark brown, ventrally yellowish; propodeum black, with posterior margin yellow; prothoracic and mesothoracic legs yellow with telotarsus brown; metathoracic legs yellow with tibia and tarsus brown; T1 basal half brown, apical half black (T1 basally yellow, apically light brown); T2 and T7–T8 yellow, T3–T6 dark brown (Fig. 6); sterna yellow; wings hyaline.

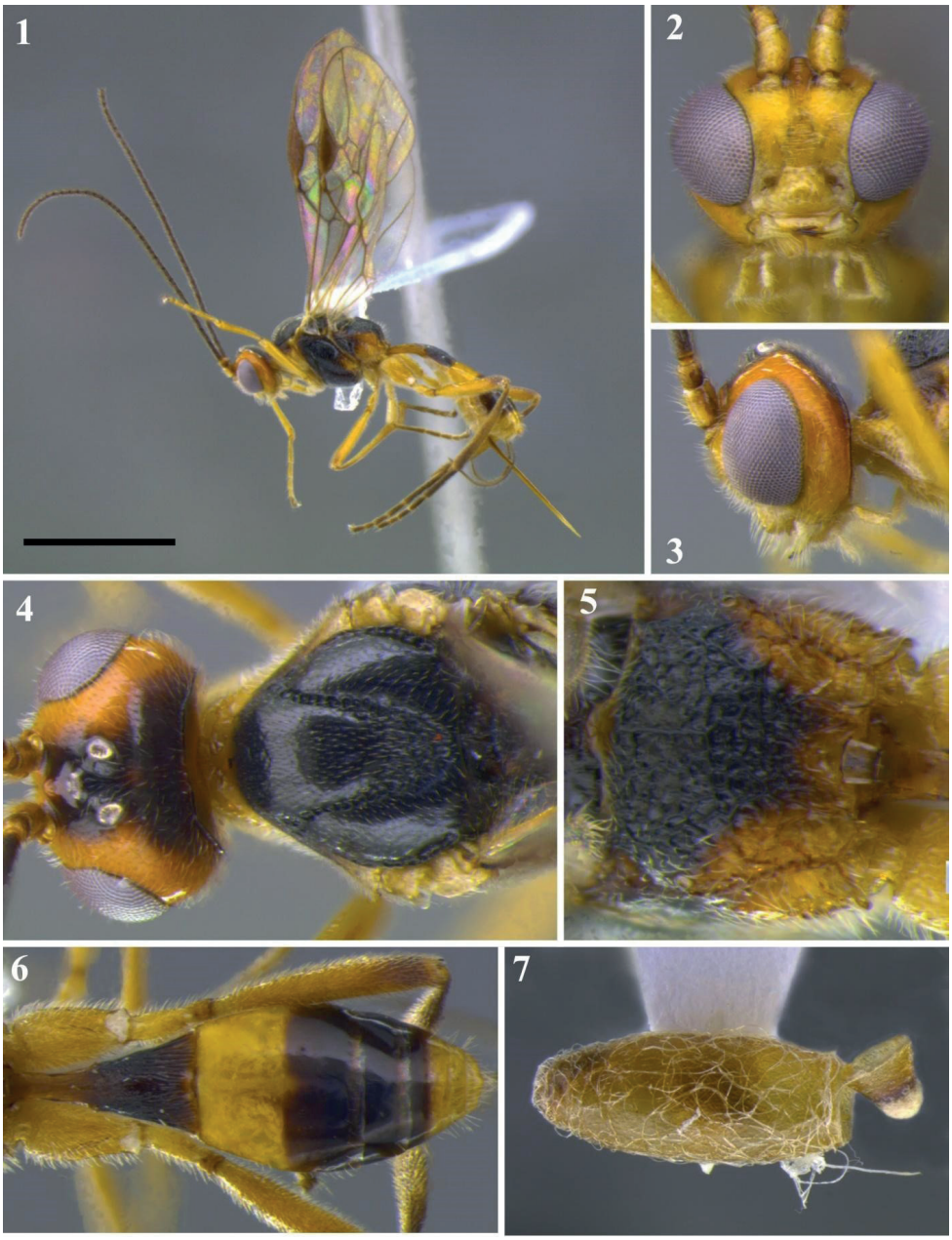
***Head*:** Antenna with 28 (27–29) flagellomeres; mandibles twisted; eyes not protuberant; occipital carina complete; vertex in dorsal view descending vertically behind the lateral ocelli; frons smooth, with a protuberance medially; face smooth, with a rugose area below the insertion of the antenna; clypeus rugulose with long hairs (Fig. 3); head width 1.27 (1.17–1.27) × its height; head height 1.35 (1.35–1.65) × eye height; face maximum width 1.44 (1.24–1.55) × its minimum width; face minimum width 1.25 (0.95–1.25) × clypeus width; minimum face width 1.04 (0.95–1.18) × face height; malar space length 0.60 (0.40–0.62) × mandible width basally; gena length 0.53 (0.40–0.59) × eye length in dorsal view; ocellus-ocular distance 1.37 (1.33–1.68) × ocellar diameter; ocellar diameter 0.67 (0.54–0.73) × posterior ocellar line (Fig. 4).

***Wings*:** Fore wing: length 2.93 (2.93–3.75) mm; vein m-cu postfurcal; length of vein r 0.42 (0.33–0.64) × vein 3Rsa; vein 3Rsa 0.67 (0.67–1.00) × length of vein r-m. Hind wing: vein 1M 1.51 (1.26–2.00) × length of vein cu-a; length of vein 1M 1.00 (1.00–1.78) × length of vein r-m.

***Mesosoma*:** Height 0.68 (0.65–0.68) × its length; propleuron smooth; pronotum mostly rugulose, carinate medially; central lobe of mesoscutum smooth; notauli distinctive and rugose, mesonotal lobes well defined (Fig. 4); scutelar sulcus with five (or three) carinae; mesopleuron smooth with a rugose area near tegula; precoxal sulcus long, carinate-rugose; metapleuron rugose; propodeum areolate-rugose without longitudinal or transversal carinae (longitudinal carinae visible in some specimens).

***Legs*:** Tarsal claw simple; hind coxa rugulose.

***Metasoma*:** Dorsope absent; first tergite with basal half smooth, apical half rugulose medially and costate laterally; remaining tergites smooth and shining; ventral



Figures 1–7. *Meteorus lucianae* sp. nov. **1** Lateral habitus **2** head, frontal view **3** head, lateral view **4** head and mesonotum, dorsal view **5** propodeum, dorsal view **6** metasoma, dorsal view **7** cocoon. Scale bar: 2 mm (**1**).

borders of first tergite touching for a short distance distally (or almost touching); ovipositor length 2.53 (1.93–2.53) × first tergite length (Fig. 1).

***Cocoon*:** Length 4.25 mm; width 1.53 mm; mostly honey–brown, translucent, and slightly covered by loose silk; apex cap protruding, whitish, and bordered by a dark ring (Fig. 7).

Examined material. ***Holotype*** Brazil • Female; Paraná, Lapa, Fazenda experimental IAPAR; 03 Feb.2016; A. C. Dudczak & A.M. Borba leg; DCBU 478005.

Paratypes Brazil• 1 Female; Idem holotype, except; 25 Feb. 2016.• 3 Females; Minas Gerais, Poços de Caldas, Sitio da Ferradura; 21°47'03"S, 46°37'23"W; 19 Apr. 2007; A. E. de Carvalho leg.; Malaise Trap; DCBU 09311, DCBU 09298 and DCBU 09294. • 1 Female; Idem previous, except,13 Dec.2007; DCBU 09899.• 1 Female; Rio de Janeiro, Itatiaia, Parque Nacional do Itatiaia; 22°26'01"S, 44°36'49"W; 30 May. 2014; R.F. Monteiro leg.; Malaise Trap; DCBU 78978.•1 Female; São Paulo, Campos do Jordão, Parque estadual de Campos do Jordão; 22°39'43"S, 45°27'2.8"W; 06 Nov. 2010; A. S. Soares leg.; Malaise Trap; DCBU 09112. • 1 Female; Sao Paulo, São Carlos, Fazenda Canchim; 31 Aug. 1983; A.S. Soares leg.; DCBU 478004 • 1 Female; São Paulo, Ribeirão Grande, Parque Estadual Intervales; 24°16'28"S, 48°25'19"W; 22 Nov. 2010; N.W. Perioto leg.; DCBU06906.• 1 Female; Minas Gerais, Bom Repouso, Serra dos Garcias; 22°29'25"S, 46°11'25"W; 17 Oct. 2009; I. F. Melo leg.; DCBU39826.

Additionally to the type series 87 specimens are deposited at DCBU (See Suppl. material 1 for detailed records).

Biology. The holotype of *Meteorus lucianae* sp. nov. was reared as a solitary parasitoid of *Crocidosema aporema* (Lepidoptera: Tortricidae) collected in soybean.

Distribution. Brazil (Paraná, Minas Gerais, São Paulo, Rio de Janeiro).

Etymology. *Meteorus lucianae* sp. nov. is named in honor of Luciana Bueno dos Reis Fernandes, recognizing the extensive technical support provided to the INCT Hympar Lab at the Federal University of São Carlos.

Discussion

Meteorus lucianae sp. nov. is morphologically most similar to *M. pseudodimidiatus* Zitani and would be identified as such in the key to Neotropical *Meteorus* presented in Aguirre et al. (2015). Nevertheless the new species can be separated from *M. pseudodimidiatus* especially by the differences on head and eye shape. This can be most easily identified by the ratio between the malar space length and the mandible width, which is 0.40–0.62 × in *M. lucianae* sp. nov., while 0.80–1.50 × in *M. pseudodimidiatus*. The new species also resembles *M. dimidiatus* (Cresson) but can be recognized by having a complete occipital carina and a shorter ovipositor, with its ovipositor length being 1.93–2.53 × the first tergite length (while *M. dimidiatus* has an incomplete carina and ovipositor length 2.60–2.80 × first tergite length).

When compared to the Paleartic fauna, *M. lucianae* sp. nov. is most similar to *M. tenellus* Marshall. Most notably these species share the lack of dorsope, a narrow

face, strongly twisted mandibles, frons with a median protuberance and ovipositor usually at least 2 times longer than the first tergite. Those shared morphological characteristics suggest that the new species could also belong to "Clade IIA" retrieved in the phylogenetic analysis presented in Stigenberg and Ronquist (2011). Similarly to the new species described here, *M. tenellus* and the closely related *M. cinctellus* (Spinola) have both been recorded utilizing Tortricidae hosts (Yu et al. 2016).

Currently the only known host association for the new species is the one here presented (*C. aporema* feeding on soybean). Nonetheless, it is noteworthy that several specimens studied were obtained from Malaise traps and were recorded in areas not typically associated with soybean production, including conservation areas. Is thus likely that in those areas the species is either utilizing *C. aporema* feeding in other Fabaceae or a different host species.

The genus *Meteorus* had not been recorded as a parasitoid of *C. aporema*, and in the Neotropics the use of Tortricidade as host is unusual in *Meteorus* (Aguirre et al. 2015). In contrast, several parasitoids from other taxonomic groups have been recorded using it as host, most belonging to the Hymenoptera superfamilies Chalcidoidea and Ichneumonoidea (Table 1).

The use of biological control agents such as parasitoids should be considered in managing pest insects as it represents a more sustainable alternative to the currently employed practices (Baker et al. 2020). Further investigation into this parasitoid autecology would be relevant to elucidate the factors affecting their natural occurrence. This in turn could be a relevant aid on conservation biological control programs, as well as providing new options for the integrated management of *C. aporema*.

Table 1. List of previously recorded parasitoids of *Crocidosema aporema* (Lepidoptera, Tortricidae).

Parasitoid	Reference
Order Hymenoptera	
Agathis sp. (Braconidae)	(Foerster and Calderón 1977)
Apanteles piceotrichosus Blanchard (Braconidae)	(Molinari and Monetti 1997)
Bassus sp. (Braconidae)	(Liljesthrom and Rojas-Fajardo 2005)
Bracon sp. (Braconidae)	(Liljesthrom and Rojas-Fajardo 2005)
Campoletis perdistinctus Viereck (Ichneumonidae)	(Sanchez and Pereyra 2008)
C. grioti Blanchard (Ichneumonidae)	(Molinari and Monetti 1997)
Chelonus sp. (Braconidae)	(Panizzi and Correa-Ferreira 1997)
Cotesia lesbiae (Blanchard) (Braconidae)	(Molinari and Monetti 1997)
Encarsia porter Mercet (Aphelinidae)	(Rojas 1968)
Goniozus nigrifemur Ashmead (Bethylidae)	(Panizzi and Correa-Ferreira 1997)
Itoplectis niobe Schrottky (Ichneumonidae)	(Zerbino and Alzugaray 1991)
Spilochalcis sp. (Chalcididae)	(De Santis and Monetti 2008)
Trathala sp. (Ichneumonidae)	(Liljesthrom and Rojas-Fajardo 2005)
Trichogrammatoidea bactrae Nagaraja (Trichogrammatidae)	(Whu and Valdivieso 1999)
Trichogramma pretiosum Riley (Trichogrammatidae)	(Basso et al. 2006)
T. brasiliensis (Ashmead) (Trichogrammatidae)	(Sanchez and Pereyra 2008)
Order Diptera	
Carcelia sp. (Tachinidae)	(Callohuari et al. 2018)
Eucelatoria australis Townsend (Tachinidae)	(Sanchez and Pereyra 2008)
Nemorilla ruficornis (Thompson) (Tachinidae)	(Panizzi and Correa-Ferreira 1997)

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Supplementary material 1

Table S1

Author: Luis Felipe Ventura de Almeida

Data type: Ocurrences.

Explanation note: Spreadsheet with ocurrence data of the examined material, deposited at DCBU (Coleção taxonomica do departmento do departamento de ecologia e biologia evolutiva da ufscar, Sao Carlos, Brazil).

Link: https://doi.org/10.3897/jhr.93.94621.suppl1

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Host-specific demography of *Utetes anastrephae* (Hymenoptera, Braconidae), a native parasitoid of *Anastrepha* spp. fruit flies (Diptera, Tephritidae)

María Dina Estrada-Marroquín[1], Jorge Cancino[2], Daniel Sánchez[1], Pablo Montoya[2], Pablo Liedo[1]

1 *El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km. 2.5, Tapachula, Chiapas, 30700 Mexico*
2 *Programa Moscas de la Fruta, SENASICA-SADER, Camino a los Cacaotales S/N, Metapa de Domínguez, Chiapas, 30680 Mexico*

Corresponding author: María Dina Estrada-Marroquín (maria.estrada@estudianteposgrado.ecosur.mx)

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Abstract

The braconid *Utetes anastrephae* (Viereck, 1913) (Hymenoptera: Braconidae) is a larva-pupal parasitoid of fruit flies of the genus *Anastrepha* Schiner, commonly associated with *Anastrepha obliqua* (Macquart, 1835) (Diptera: Tephritidae), the most important pest of mango (*Mangifera indica* L., 1753) in Mexico. This parasitoid was established in a laboratory colony using larvae of *Anastrepha ludens* (Loew, 1873) as host. Here we describe a demographic study to compare the reproductive and population parameters of this parasitoid reared on *A. obliqua* and *A. ludens* under laboratory conditions. Two *U. anastrephae* cohorts of 30 individual pairs each were set up, one was reared on *A. obliqua* larvae and the other one on *A. ludens*. Every day, 30 third instar larvae of each host species were exposed to an adult pair through the lifespan of the female. Daily mortality and fecundity were recorded. Life tables were constructed and sex ratios, parasitism rates, survival, reproductive and population parameters were estimated. Higher survival of *U. anastrephae* females was observed in females from *A. obliqua* (mean live expectancy of 22.4 days), but higher fecundity and parasitism occurred in females from *A. ludens* (net fecundity of 62.61 daughters/female and 16.72% parasitism rate). The intrinsic rate of increase (r = 0.128 and r = 0.134 for *A. obliqua* and *A. ludens* respectively), mean generation time (27.88 and 28.30 days) and population doubling time (5.42 and 5.16 days) were similar in both cohorts, as well as the sex ratio (73 and 69% of females). These results suggest that *A. ludens* as host increase the production rates; however, any one of these two species could be used as host for mass rearing purposes.

Keywords
biocontrol, fecundity, intrinsic rate of increase, life table, mass rearing, parasitism, survival

Introduction

The use of native parasitoids for the management of *Anastrepha* fruit flies has been a subject of discussion, since these species would be used in an environment where fruit flies generally have a higher rate of natural increase (Vargas et al. 2002; Aluja et al. 2009). However, given the good results obtained with augmentative releases of *Diachasmimorpha longicaudata* (Ashmead, 1905) in different environments (e.g., Montoya et al. 2000; Montoya et al. 2007; Montoya et al. 2016; Cancino et al. 2019a), the potential for native species for the control of *Anastrepha* fruit flies is worth to explore.

Utetes anastrephae (Viereck, 1913) (Hymenoptera: Braconidae) is a koinobiont, solitary endoparasitoid (Ovruski et al. 2000) native to the Neotropics that parasitizes larva-pupa of fruit flies. It is found parasitizing *Anastrepha obliqua* (Macquart, 1835) (Diptera: Tephritidae) feeding upon *Spondias* spp. (Anacardiaceae), and in minor frequency in other species such as *Anastrepha alveata* Stone, 1942 and *Anastrepha fraterculus* (Wiedemann, 1830) (Aluja et al. 1990; Hernández-Ortíz et al. 1994; López et al. 1999). This parasitoid competes successfully with other native braconid parasitoid species, such as *Doryctobracon areolatus* (Szépligeti, 1911), *Doryctobracon crawfordi* (Viereck, 1911), and *Opius hirtus* Fischer, 1963; it even competes with the introduced *D. longicaudata*, showing a remarkable capacity for conspecific discrimination and heterospecific intrinsic competition in previously parasitized larvae (Aluja et al. 2013; Ayala et al. 2018; Murillo et al. 2018).

The use of a parasitoid species for augmentative biological control applications requires the development of methods for mass production of good quality individuals. One essential element is the selection of an adequate host species (Eitam et al. 2003; Cancino et al. 2009). Although *A. ludens* is not considered a preferred host for *U. anastrephae*, it can be used as an alternative host because it is a species relatively easy to rear, being a high-quality host that is currently used to produce massively *D. longicaudata* (Orozco-Dávila et al. 2017; Cancino et al. 2020). Under laboratory conditions *U. anastrephae* successfully parasitizes 5–8 days old *A. obliqua* larvae (Poncio et al. 2018) and 7–8 days old *A. ludens* larvae (Aluja et al. 2009; Cancino et al. 2009).

Knowledge of the demography of parasitoids, in addition to allowing a better understanding of their biology, allows us to compare the effect of different hosts and make mass rearing more efficient (Bellows et al. 1992; Carey and Roach 2020; Ganjisaffar and Perring 2020). For example, the intrinsic rate of natural increase (r) is a population parameter described as the potential growth of a population (Jervis and Copland 1996) and can be used as an indicator of the capacity of a parasitoid species to suppress or regulate the target pest population (Vargas et al. 2002; Stark et al. 2004). This parameter combines both the survival and reproduction of a population and allows

comparison among different species of parasitoids or when species are evaluated under different environmental conditions (Núñez-Campero et al. 2014; Gonçalves et al. 2018; Fernandes et al. 2021).

Our previous trials, trying to establish a colony of *U. anastrephae* using *A. obliqua* larvae as host, were unsuccessful, despite being considered its natural host. Here, we used a strain of *U. anastrephae* reared on *A. ludens* larvae as host, applying the concept of factitious host used for *Trichogramma* spp. mass rearing (Iranipour et al. 2010; Gowda et al. 2021). Our hypothesis was that the demographic parameters of *U. anastrephae* would be affected by the host species used for the development of their offspring. Therefore, our aim was to determine the effect of two different hosts, *A. obliqua* (the preferred host in nature) and *A. ludens* (the host used in laboratory rearing), on the survival, reproductive and population parameters of *U. anastrephae*. Our results improved our understanding of the performance of *U. anastrephae* reared on both host species and indicate that both can be used for mass rearing it as a biocontrol agent of *Anastrepha* fruit flies.

Materials and methods

Biological material

The study was carried out at the Laboratory of Biological Control, of the Programa Moscafrut (SENASICA-SADER) in Metapa de Domínguez, Chiapas, Mexico. *Utetes anastrephae* specimens were obtained from a laboratory colony maintained using *A. ludens* larvae as hosts. This colony was established with specimens of *U. anastrephae* emerged from larvae of *A. obliqua* developed in tropical plum trees (*Spondias mombin* L). After three unsuccessful attempts using *A. obliqua* as host, we decided to use *A. ludens* as alternative host. This strategy was successful in terms of colonization and the current colony has ≈ 250 generations under laboratory mass rearing conditions. The larvae of both *A. ludens* and *A. obliqua* were obtained from the mass reared colonies maintained at the Moscafrut facility (Orozco-Dávila et al. 2017). All experiments were carried out under laboratory conditions at 26 ± 0.5 °C, 70 ± 10% relative humidity, and a 12:12 h L:D photoperiod.

Oviposition period, parasitism rate and adults sex ratio using two species of host larvae

Two cohorts of *U. anastrephae* of 30 pairs (♀, ♂) each were set up. Individual pairs of newly emerged adults were placed in 25 × 11 × 13 cm plastic cages. They were provided with water and honey throughout the experiment. One cohort was exposed to *A. obliqua* and the other one to *A. ludens*. Each pair was daily provided with 30 larvae of the corresponding species along the lifespan of each female. The larvae were exposed in parasitization units consisting in 5 cm diameter × 0.2 cm height Petri dish bottoms,

mixed with larval diet, and covered with tricot fabric clothe fastened with an elastic band. The surface of the parasitizing unit was smeared with ripe guava pulp to attract the parasitoids.

Parasitization units were exposed 4 h every day. Then the larvae with diet were placed in 6 cm diameter × 4 cm height plastic containers. Three days later the larvae were carefully sorted out from the diet with entomological forceps and returned to the same container but now with humid vermiculite as a pupation substrate. The pupae were maintained in humid vermiculite for 14 days at 26 ± 0.5 °C and 60–80% RH. Subsequently, the pupae were removed from the vermiculite and kept in these same conditions until emergence.

The number of dead parasitoids and their sex was recorded daily to estimate sex-specific survival. The number of flies and parasitoids emerged by sex were also recorded every day. Pupae that did not emerge were dissected to investigate the presence of parasitoids or flies. The oviposition period was determined based on the emergence of parasitoids per day. The percentage of parasitism was obtained by dividing the number of emerged parasitoids by the number of exposed larvae, multiplied by 100, as well as the percentage of accumulated parasitism (daily sum of parasitism). The sex ratio of the parasitoids was estimated by dividing the number of females by the sum of females and males and was expressed as the proportion of females.

Life tables and reproductive and population demographic parameters

To know the survival of the immature stages, 400 larvae of each host species were exposed to two separate groups of 30 couples of five-day old *U. anastrephae* adults; from each host species 20 subsamples of 20 larvae were obtained, and each subsample was dissected daily to know the number of immatures. For life table construction we used the mean egg to adult developmental time and percent survival for each host species.

With the mortality and fecundity data, the corresponding life tables were elaborated, following methods described by Carey (1993), and Carey and Roach (2020). Survival curves were estimated with the proportion of live females per day (l_x), that is the number of live females at age x between the original number of the cohort ($l_x = N_x / N_0$).

In addition, the following reproductive parameters were estimated: gross and net fecundity rates, mean daily offspring production, and mean age for gross and net fecundity. The population demographic parameters were net reproductive rate (R_o), intrinsic rate of increase (r) using Newton's method based on the formula $r_1 = r_0 - f(r)/f'(r)$, finite rate of increase (λ), mean generation time (T), and doubling time (DT).

Data analysis

The experimental design was completely randomized with two treatments (hosts) and 30 replicates, considering each pair of parasitoids as an experimental unit. The data were tested for normality by means of Anderson-Darling test, and for homogeneity of vari-

ances with the Bartlett and Fligner-Policello tests. The pre-oviposition and reproductive periods were compared by means of t-student and Mann-Whitney test, respectively.

Sex ratio and percent parasitism were analysed using a generalized lineal model (GLM) with quasibinomial response, whereas fecundity (offspring per female) was a GLM with negative binomial response. The link-log function was used in each model and a likelihood ratio test was applied to test for the effect of the treatments. Survival curves for females and males were compared using the Log-rank test. A significance level of .05 was used for all statistical tests. All analyses were carried out using the statistical software R v4.0.5 (R Development Core Team 2021).

Results

Immatures developmental time and survival

The mean developmental time from egg to adult was 19 days for both host species. Survival of immatures was 68.37% in *A. ludens* and 57.5% in *A. obliqua*. These data were used to construct the life tables and estimation of demographic parameters.

Oviposition period, parasitism, and sex ratios

The onset of oviposition occurred from the first day of female adult life (first 24 h) for both cohorts. The average female matured on the third day, and it ranged from 1 to 11 days in *A. obliqua* and from 1 to 13 days in *A. ludens*; the pre-oviposition period did not show significant differences (W = 368, p = .9506) between species. Within the reproductive period, the cohort exposed to *A. obliqua* lasted on average (± SD) 13.5 ± 4.99 days with a range of 1 to 21 days, while the cohort exposed to *A. ludens* lasted 11.5 ± 6.11 days with a range of 1 to 22 days. The difference in the reproductive period of both treatments was not significant (t (2) = -1.2899, p = .2028).

The percentage of days in which females produced at least one offspring was 65.7% and 86.1% for *A. obliqua* and *A. ludens*, respectively. This means that the cohort parasitizing *A. ludens* larvae produced more offspring in a shorter time. Fecundity (offspring per female) was significantly higher in females from *A. ludens* (χ^2 (1) = 15.551, p < .001). The maximum number of offspring per female was 191 with a mean (± SD) of 91.83 ± 67.77 individuals per female. For those exposed to *A. obliqua* larvae, the maximum offspring per female was 149 with a mean of 82.33 ± 41.87 individuals (Fig. 1).

The cohort using *A. obliqua* larvae as a host reached its maximum reproductive peak between four and six days, and by day seven 1241 offspring (50.24%) had been produced. In the case of females that parasitized *A. ludens*, the reproductive peak occurred between five and seven days of age, and by day eight they had produced 54% (1507 individuals) of their total offspring (Fig. 2).

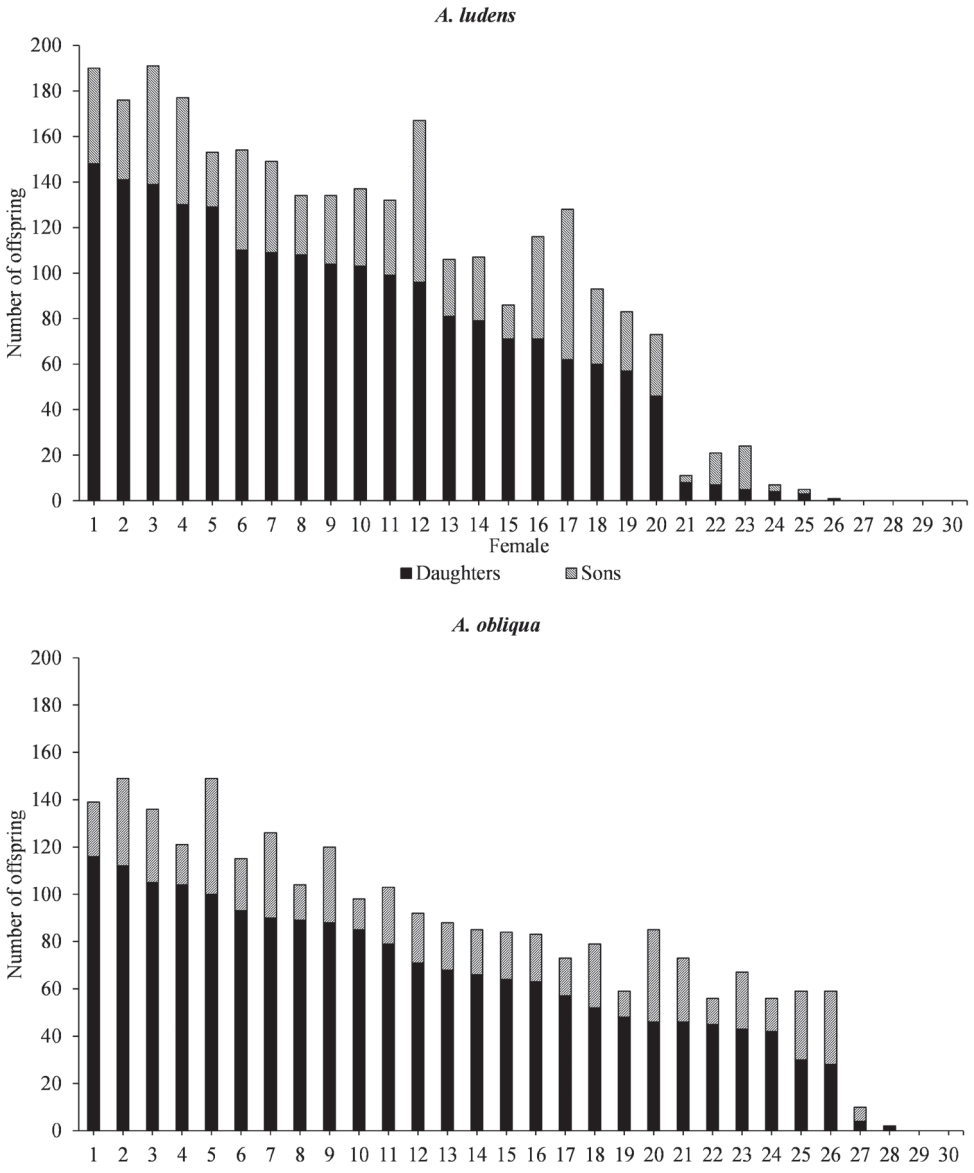

Figure 1. Total offspring and sex ratio produced by each female, in both cohorts studied.

Average percentage (± SD) of total parasitism was higher (χ^2 (1) = 4.4137, p = .0357) in *A. ludens* larvae (16.72 ± 11.56%), than in *A. obliqua* (13.04 ± 9.69%, Fig. 3). Offspring sex ratio was biased towards females (around 70%) (χ^2 (1) = 0.98385, p = .3213) and this was observed through the whole females life span in both species (χ^2 (1) = 0.00014, p = .9904). In *A. obliqua* a proportion (± SD) of 0.73 ± 0.13 females was observed. In *A. ludens* it was 0.69 ± 0.16.

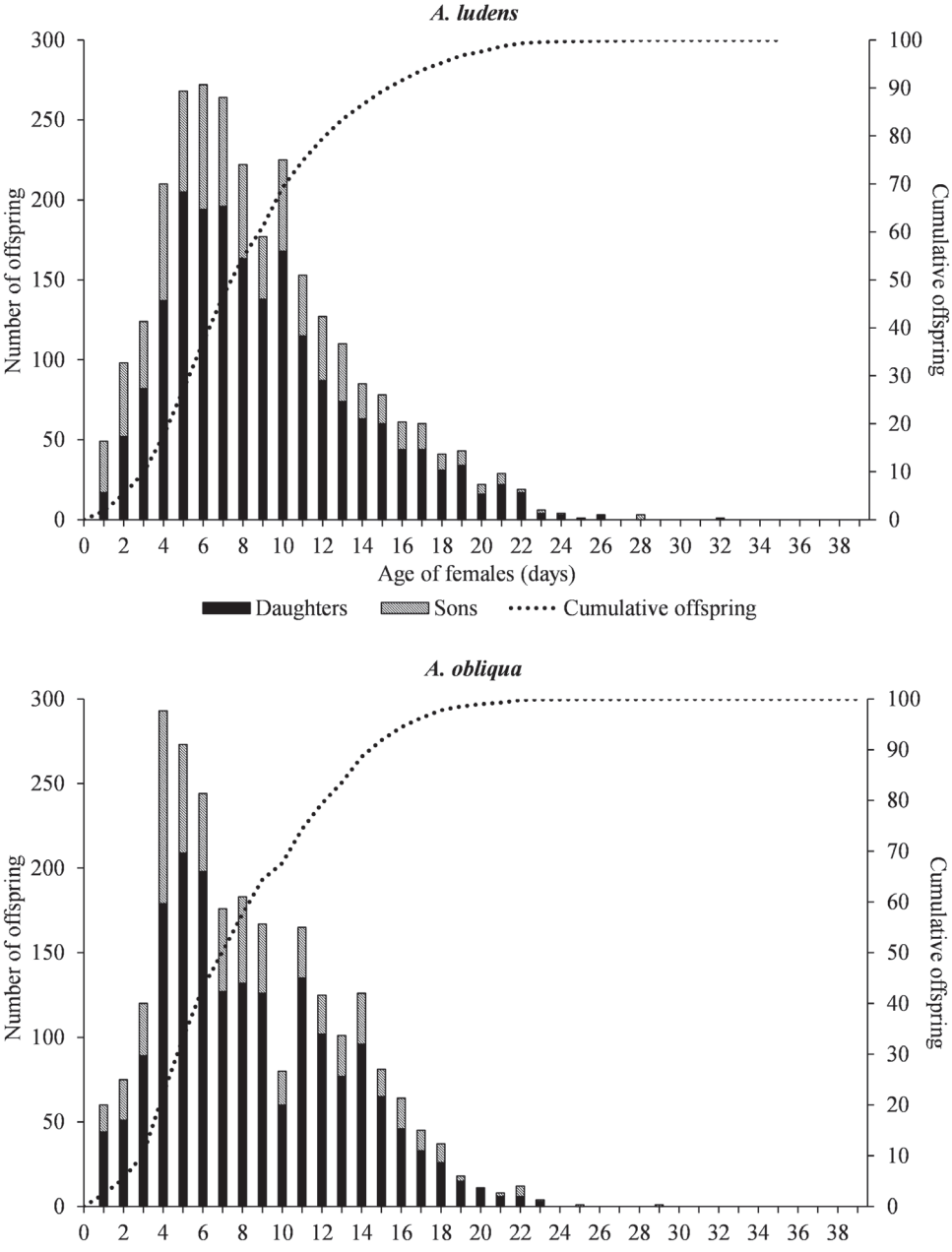

Figure 2. Daily offspring and sex ratio of *U. anastrephae* emerged from *A. obliqua* and *A. ludens* larvae as hosts and their respective cumulative frequency.

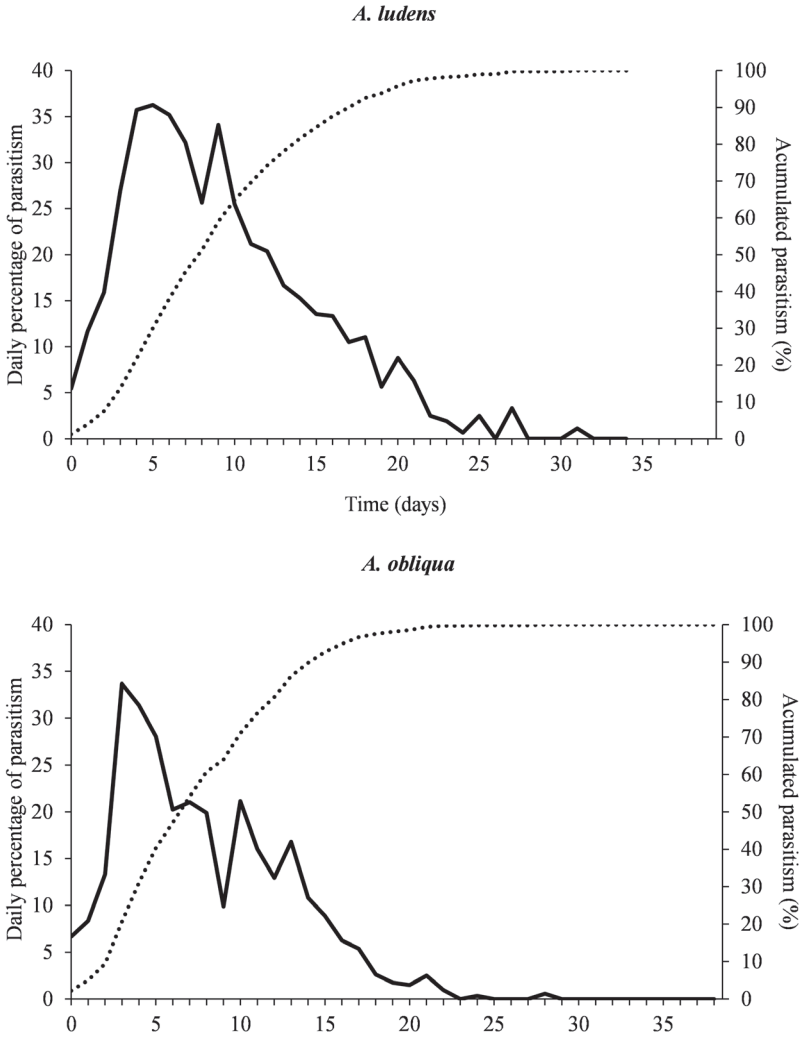

Figure 3. Daily percentage of parasitism of *U. anastrephae* in *A. obliqua* and *A. ludens* as hosts.

Survival, reproduction, and population demographic parameters

Female survival was different (Log-Rank χ^2 (1) = 4.6, p = .03) between the two cohorts.

Females parasitizing *A. obliqua* larvae showed greater survival than those parasitizing *A. ludens* larvae with a mean longevity of 22.93 ± 8.37 (mean ± SD) and 16.93 ± 9.67 days, respectively (Fig. 4). In both cohorts, males lived less than females, without statistically significant difference in their survival (Log-Rank χ^2 (1) = 0.82, p = .4). Males in the cohort with *A. obliqua* had a mean longevity of 13.33 ± 7.59 days and 15.63 ± 7.22 days with *A. ludens*.

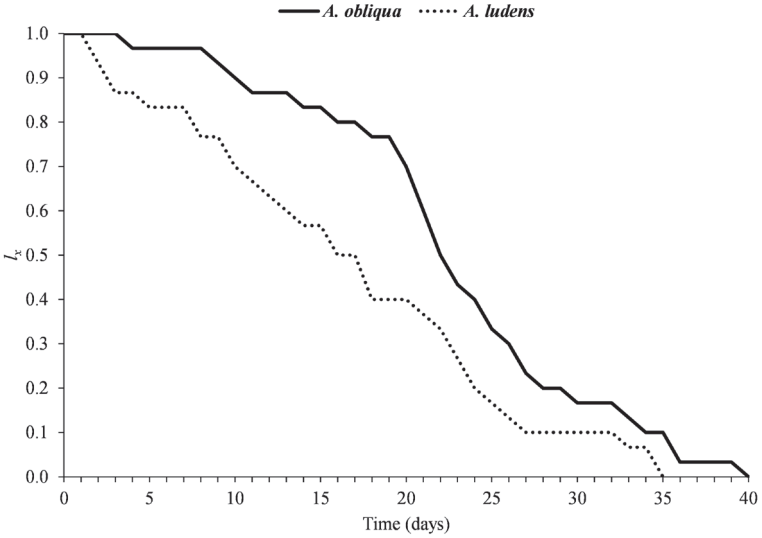

Figure 4. Survival curves of *U. anastrephae* females parasitizing larvae of *A. obliqua* and *A. ludens* as hosts for the respective offspring development.

Reproductive rates were greater for parasitoids using *A. ludens* larvae as hosts than those using *A. obliqua*. The trajectories of net fecundity for both cohorts are shown in Fig. 5. Table 1 shows the gross and net fecundity rates, the daily mean of offspring production and the mean age for gross and net fecundity for the two cohorts. Regarding the population demographic parameters (Table 2), while the net reproductive rate (R_0) was higher in *U. anastrephae* females that parasitized *A. ludens* larvae, all other population parameters were very similar for the two cohorts. The intrinsic rate of increase was similar (≈ 0.13) between the two cohorts.

Discussion

It was interesting to find that *U. anastrephae* could develop equally successfully in both hosts, one of them being its most frequent natural host (*A. obliqua*), and the other its host in the laboratory rearing colony (*A. ludens*). *Anastrepha ludens* has been reported as the natural host of *U. anastrephae* very rarely (Montoya et al. 2016). However, since most studies on natural parasitism of fruit fly parasitoids have generally been directed to the same fruits of specific interest (Aluja et al. 1990; López et al. 1999; García et al. 2020); it is possible that other fruits that have not been inspected are hosting *A. ludens* parasitized by *U. anastrephae*. Likewise, there may be confusion regarding the emergence of *U. anastrephae* from *A. obliqua* when it could also emerge from *A. ludens*, since parasitoids have been detected in mango, where both species of flies are present (Aluja et al. 1990). However, this assumption needs to be investigated.

Table 1. Reproductive parameters of *U. anastrephae* with larvae of *A. obliqua* and *A. ludens* as hosts.

Reproductive parameters	Host	
	A. obliqua	***A. ludens***
Gross fecundity rate	91.06	136.26
Net fecundity rate	47.50	62.61
Mean daily production	1.68	2.23
Mean age gross fecundity	28.90	30.37
Mean age net fecundity	28.41	28.84

Table 2. Population demographic parameters of *U. anastrephae* with larvae of *A. obliqua* and *A. ludens*.

Population parameters	Host	
	A. obliqua	***A. ludens***
Net reproductive rate (R_0)	35.31	44.80
Mean generation time (T)	27.88	28.30
Intrinsic rate of increase (r)	0.128	0.134
Finite rate of increase (λ)	1.14	1.14
Doubling time (DT)	5.42	5.16
Adult life expectancy (e_x)	22.4	16.4

The higher reproductive rates found when *A. ludens* larvae were the host, compared to *A. obliqua*, can be attributed to three factors: 1) the effect of host switch, 2) the quality of the host, and/or 3) the immunological response. It is known that host switching may adversely affect the fitness of parasitoid species during the very first generations in a new host, although in subsequent generations their performance can improve (Zenil et al. 2004; Jones et al. 2015; Poncio et al. 2016). When the parasitoid *D. longicaudata* was previously maintained on *A. fraterculus* larvae, the adults parasitized more on this host than when *Ceratitis capitata* larvae (Wiedemann, 1824) were offered as an alternative host (Ovruski et al. 2011; Rohr et al. 2019). Something similar was observed with *Fopius arisanus* (Sonan, 1932) reared on *C. capitata* larvae for 28 generations. When adults were exposed to *Anastrepha* species larvae, the percentage of adult emergence was lower than in those exposed to *C. capitata* larvae (Zenil et al. 2004).

The use of alternative (factitious) hosts for parasitoid rearing has been an important technique (Pluke and Leibee 2006). When parasitoid colonization with the native host is a difficult, or expensive process, the use of this factitious host becomes an option (Boycheva et al. 2019). As mentioned above, *U. anastrephae* colonization with *A. obliqua* as host was an ineffective process. The use of *A. ludens* larvae as factitious host represented a good option.

Regarding the immune response of *A. obliqua* to parasitoids, it has been reported that its larva possesses 5–6 types of haemocytes that generate a strong immune response (phagocytosis and production of reactive oxygen species) (Silva et al. 2002; Gómez-Alonso et al. 2022). The presence of these haemocytes resulted in the melanization and encapsulation of the first immature stages of parasitoids (Silva et al. 2002; Cancino et al. 2022), which has not been observed in *A. ludens* (Poncio et al. 2016; Cancino et al.

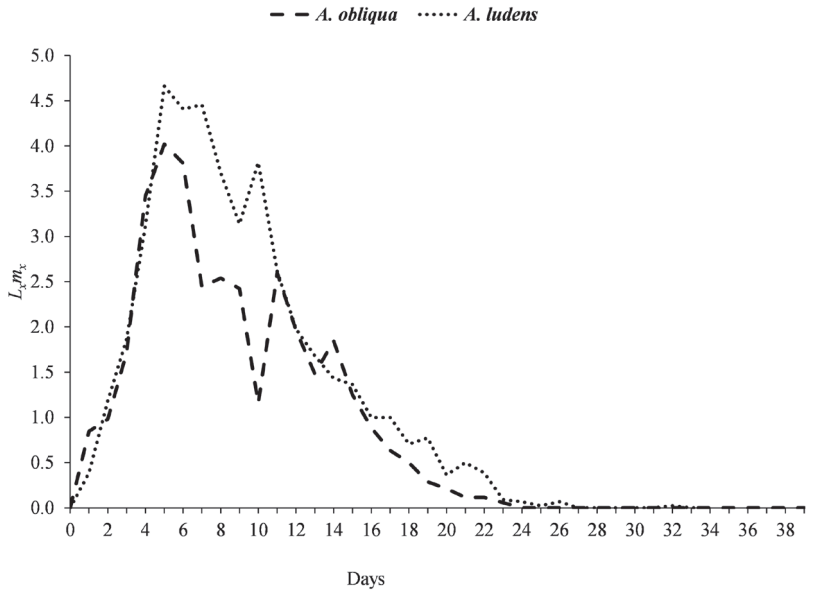

Figure 5. Daily results of net maternity ($L_x m_x$) of *U. anastrephae* with offspring daughters emerged from *A. obliqua* and *A. ludens*.

2020). This high immunity response of *A. obliqua* larva represents an important factor in increasing parasitoid mortality during development.

Host quality could be another factor. *Anastrepha ludens* larvae are larger in size than the *A. obliqua* ones. Under mass-rearing conditions, the mean pupal weight is 20 mg for *A. obliqua* and 24 mg for *A. ludens* (Orozco-Dávila et al. 2017). Usually, larger size hosts are preferred by parasitoids, showing an increase in their fecundity (Brodeur and Boivin 2004; Cohen et al. 2005; Gao et al. 2016). Another reason may be the better adaptation and more stability of *A. ludens* to mass rearing conditions. Compared with *A. obliqua*, it has been, easier to colonize and maintain under laboratory conditions (Orozco-Dávila et al. 2017; Aceituno-Medina et al. 2020).

The lower survival of the parasitoids exposed to *A. ludens* larvae can be explained by the cost of reproduction, the higher the fecundity, the lower the longevity. Since the net reproductive rate and the intrinsic rate of increase were higher for parasitoids reared on *A. ludens* than those reared on *A. obliqua* (Table 2), this trade-off can be considered convenient in terms of fitness.

The demographic parameters we found here were like those reported by Vargas et al (2002) with other larval-pupal braconid endoparasitoid reared on different hosts. For example, the intrinsic rate of increase for *D. longicaudata* and *Psyttalia incisi* (Silvestri, 1916) was r = 0.12 and 0.10, respectively, both reared on *Bactrocera dorsalis* larvae (Hendell, 1912). This suggests that *U. anastrephae* has the potential to be used as a biocontrol agent, as it has growth rates like other parasitoid species that have been used for the control of fruit flies.

The intrinsic growth rate we found here with *A. ludens* as a host (r = 0.134) was 2-fold greater than that reported by Aluja et al. (2009) using the same host when they were colonizing this species for laboratory rearing (r = 0.079). This means that the *U. anastrephae* strain tested here has adapted to this alternative host species, and it can be used for mass production. The sex ratio is a fundamental aspect for biological control, since the females are the ones that attack the host and contribute to population growth. A female-biased sex ratio would be most desirable (Montoya et al. 2013; Nurkomar et al. 2021). Here we found that the sex ratio was female-biased, about 70% female (≈ 3:1) for both hosts used. This proportion coincides with that obtained by Poncio et al. (2018), with a percentage of 65% using *A. obliqua* larvae as hosts. This proportion is adequate for the purposes of augmentative biological control.

The reason why under natural conditions *U. anastrephae* is commonly associated to *A. obliqua* could be the size of the fruit species used by the fruit fly species (Hernández-Ortíz et al. 1994; López et al. 1999). *Utetes anastrephae* has a short ovipositor that might be strongly adapted to small fruits. In general, *A. obliqua* infest fruits that are smaller in size (*Spondias* spp., *Psidium guajava* L.) than those commonly infested by *A. ludens* (*Citrus* spp., *Casimiroa edulis* La Llave & Lex). Then, in large fruits the host larvae might be out of reach for *U. anastrephae*. Also, semiochemicals emitted by fruits infested by *A. obliqua* (usually small) can determine this preference (Aluja et al. 2013). Another possibility, derived from our results, will be to use *A. obliqua* as a host for several generations, expecting that reproductive and population parameters could increase. However, the greater difficulty and cost of producing *A. obliqua* should be considered.

This demographic analysis of *U. anastrephae* comparing two hosts indicates that *A. ludens* can be used as a suitable host for mass production, although releases of parasitoids be strategically targeted to control *A. obliqua*. Biological control of *A. obliqua* in non-commercial hosts could be a strategy to prevent the movement of populations from these hosts to fruit orchards (i.e., mango orchards), where fruits are grown for commercial purpose (Cancino et al. 2019b; Montoya et al. 2000). *Anastrepha obliqua* is a major pest of mango in the Americas and is highly desirable to have a biocontrol alternative to minimize its damage (Cancino et al. 2019b; Ruiz-Arce et al. 2019).

The information generated here can be useful for decision making on the use of native parasitoids in augmentative biological control and new proposals to complement or improve current strategies for managing *Anastrepha* fruit flies. It would be interesting to know the behaviour of *U. anastrephae* reared in *A. ludens* larvae, on host preference in the presence of these two host species studied here, both in the laboratory and under field conditions.

Conclusion

This study provides information about the potential use of the native parasitoid *U. anastrephae* in augmentative biocontrol programs against *A. obliqua* fruit flies. Our results show that both, *A. obliqua* and *A. ludens* larvae can be used as hosts for mass

rearing purposes. Although *A. ludens* is not a common natural host, it can be used as a factitious host, considering the higher fecundity rate observed and considering that *A. ludens* is easier to mass produce than *A. obliqua* (Orozco-Dávila et al. 2017).

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A new fossil euphorine genus and species (Hymenoptera, Braconidae) with the longest known ovipositor from Dominican amber

Sergey A. Belokobylskij[1], Tomáš Hovorka[2,3]

1 *Zoological Institute of the Russian Academy of Sciences, St Petersburg 199034, Russia* **2** *Department of Entomology, National Museum, Cirkusová 1740, Prague 19300, Czech Republic* **3** *Department of Zoology, Charles University, Viničná 1594, Prague 12800, Czech Republic*

Corresponding author: Tomáš Hovorka (hovorkarl@gmail.com)

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Abstract

A new genus and species of the braconid subfamily Euphorinae, *Palaeorionis longicaudis* **gen. et sp. nov.**, is described and illustrated from Miocene Dominican amber. This new genus is characterised by the long and tube-shaped petiole, presence of both radiomedial (2-SR and r-m) veins on the infuscate fore wing and long ovipositor.

Keywords

Aridelus, description, fossil, Miocene, *Orionis*, ovipositor, petiole, *Stenothremma*

Introduction

The subfamily Euphorinae is one of the most diverse groups of koinobiont parasitoids of the family Braconidae, which are known to attack larval, nymphal, and adult stages of hosts from the insect orders Orthoptera, Hemiptera, Psocoptera, Raphidioptera, Neuroptera, Coleoptera, Lepidoptera and Hymenoptera (Tobias 1965, 1966; Shaw 1985; Yu et al. 2016).

Most of the known fossil euphorine taxa have been described from amber (Brues 1933, 1937, 1939; Tobias 1987), though a few of its representatives are known from compression fossils (Belokobylskij 2014). Aside from the relatively common fossil parasitoids of coleopteran and lepidopteran larvae from the genus *Meteorus* Haliday, 1835 of the tribe Meteorini, parasitoids of hemipteran nymphs and coleopteran and hymenopteran adults have been also discovered. There are species of the genus *Leiophron* Nees, 1819 (*Euphorus* Nees, 1834) (Euphorini) parasitods of the hemipteran nymphs, the genera *Pygostolus* Haliday, 1833 (Pygostolini), *Parasyrrhizus* Brues, 1933 (Centistini), *Microctonus* Wesmael, 1835, and perhaps *Onychoura* Brues, 1933 and *Meteorites* Brues, 1939 (Perilitini) (Brues 1933, 1937, 1939) are known to parasitize coleopteran and hymenopteran hosts. The extinct genus *Elasmosomites* Brues, 1933 actually belongs to the isolated euphorine tribe Neoneurini, whose members are parasitoids of the ant workers (Brues 1933; Belokobylskij et al. 2021). Additionally, two peculiar genera that are only known from the Baltic amber have unknown biologies, namely *Oncometeorus* Tobias, 1987 from the monotypic tribe Oncometeorini and *Prosyntretus* Tobias, 1987 from the Prosyntretini (Tobias 1987).

This study provides an illustrated description of the female of a new euphorine genus and species from the Miocene Dominican amber that is characterised by the long tube-shape petiolate first metasomal tergite and long ovipositor.

Methods

Dominican amber (Lower Miocene age; 20–15 Ma) is the fossilized resin of the leguminose tree *Hymenaea protera* Poinar, being mostly transparent and often containing a high number of fossil inclusions, and it has been collected in various sites within the Dominican Republic (Iturralde and Macphee 1996; Rasnitsyn and Quicke 2002).

During the present study, fossil braconid specimen were examined using a Leica M205 C stereomicroscope (Microsystems, Wetzlar, Germany). Photographs were obtained using a Keyence VHX-5000 (Mechelen, Belgium) digital microscope under suitable magnifications. Subsequent image processing was performed using Helicon Focus Pro 7 software. Final plates were prepared in Adobe Photoshop CS6.

The terminology employed for morphological features, sculpture and body measurements follows Belokobylskij and Maeto (2009). Wing venation nomenclature also follows Belokobylskij and Maeto (2009), with the terminology of van Achterberg (1993) shown in parentheses.

The material used for this study is deposited in the collection of the Stuttgart Museum of Natural History, Germany (**SMNS**).

Results

Systematic part

Class Insecta Linnaeus, 1758
Order Hymenoptera Linnaeus, 1758
Family Braconidae Nees, 1811
Subfamily Euphorinae Foerster, 1863

Genus *Palaeorionis* gen. nov.
https://zoobank.org/3DBBCBF8-D9A1-455C-857A-F76B18E09778
Figs 1, 2

Type species. *Palaeorionis longicaudis* gen. et sp. nov., by present designation and monotypy.

Etymology. Named after "palaeo" (Greek for "ancient") and the generic name of its similar extant genus, *Orionis*, which belongs to the subfamily Euphorinae. Gender: masculine.

Description. ***Head*** (Fig. 1B, C) weakly transverse. Ocelli rather large and distinctly convex. Frons weakly convex. Eyes large, elongate-oval, glabrous. Face distinctly convex. Malar space very short; malar suture perhaps absent. Clypeus complete, distinctly convex (lateral view); hypoclypeal depression absent. Occipital carina distinct laterally, perhaps widely interrupted dorsally (Fig. 1B). Mandibles relatively small. Maxillary palpus very long (Fig. 1C), perhaps 6-segmented (medial segments hidden by mesosoma), its apical segment very long and slender, almost 25.0 times longer than its maximum width. Labial palpus short, with 4 segments, third segment very small, tiny, subglobular; apical (fourth) segment longest, knife-shaped, narrowed towards apex.

Antenna (Fig. 1A, C) long, slender, filiform, about 33-segmented. Scapus rather short and wide. Pedicel relatively small. First flagellar segment subcylindrical, without any transformations, much longer than its apical width, about as long as second segment. Apical segment pointed apically, but without spine.

Mesosoma (Fig. 1C, D, E). Sides of pronotum rugose upper and areolate below. Mesoscutum perhaps smooth, narrowly reticulate laterally. Notauli present, perhaps almost complete and shallow especially posteriorly. Scutellum distinctly convex. Prepectal carina present, sharp and distinct. Mesopleuron mainly smooth. Precoxal sulcus present, long, not deep, curved, distinctly crenulate-reticulate. Metascutum without dorsal tooth (lateral view). Propodeum dorsally almost straight in basal two-thirds, distinctly oblique sloped, starting from basal third, without lateral tubercles (in lateral view).

Wings (Fig. 1G). Fore wing rather narrow, pterostigma long and rather narrow. Radial (marginal) cell weakly shortened, narrow, about 4.5 times longer than its maximum width. Metacarpus (1-R1) 1.2 times longer than pterostigma. First medial abscissa (1-SR+M) present and curved. Present both radiomedial veins (2-SR and r-m). Second radiomedial (submarginal) cell short, pentagonal. Discoidal (first discal) cell not petiolate anteriorly, sessile. Recurrent vein (m-cu) postfurcal, subparallel to basal vein (1-M). First

mediocubital vein (M+CU1) well sclerotised and distinctly sinuate. Nervulus (cu-a) postfurcal. Brachial (first subdiscal) cell open posteriorly; brachial vein (CU1b) absent. Transverse anal veins (1a and 2a) absent. Hind wing. Submedial (subbasal) cell short. First abscissa of mediocubital vein (M+CU) distinctly shorter than second abscissa (1-M).

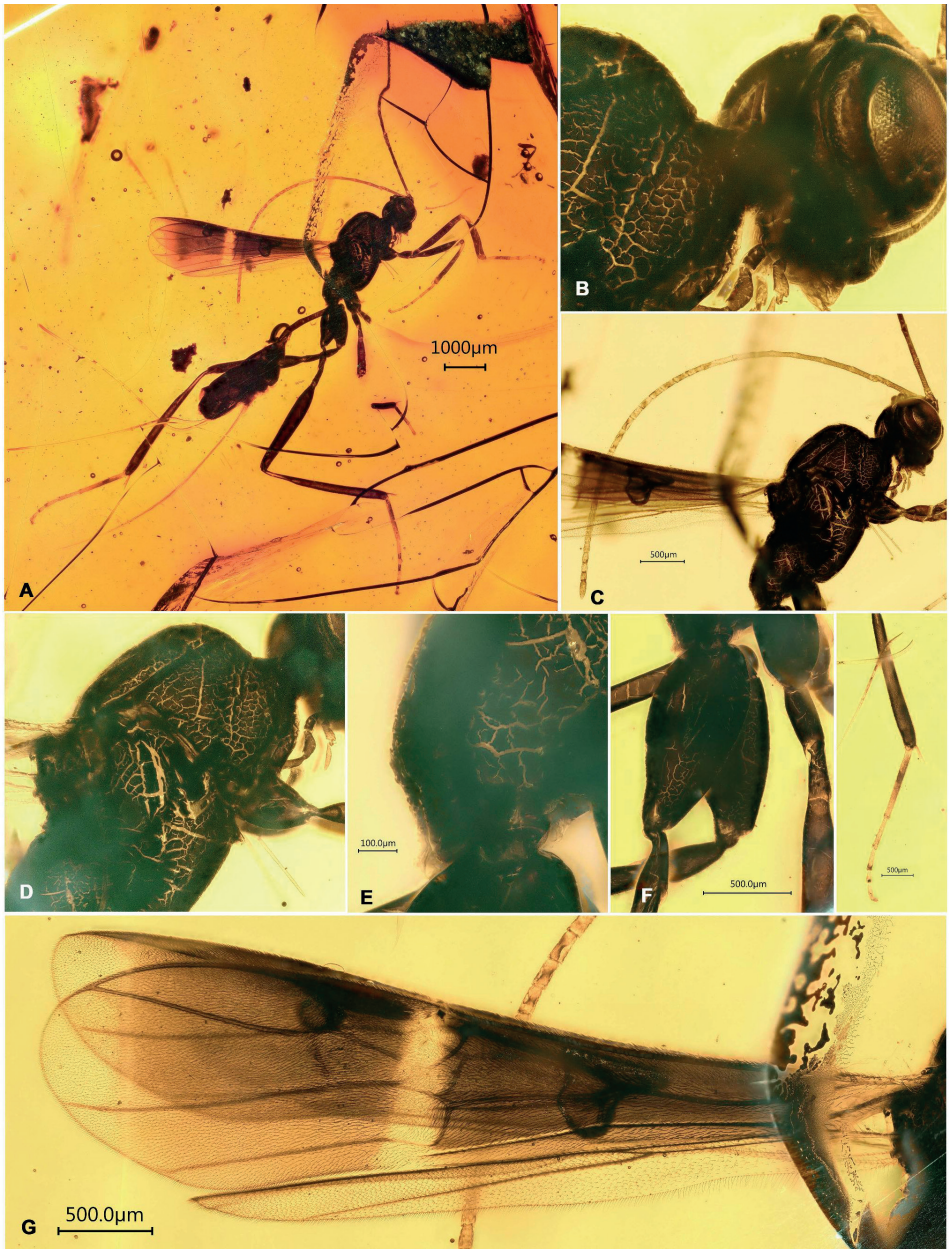

Figure 1. *Palaeorionis longicaudis* gen. et sp. nov. (holotype, female) **A** habitus, lateral view **B** head, latero-posterior view **C** head, antenna and mesosoma, lateral view **D** mesosoma without propodeum, lateral view **E** propodeum, lateral view **F** hind coxa **G** wings.

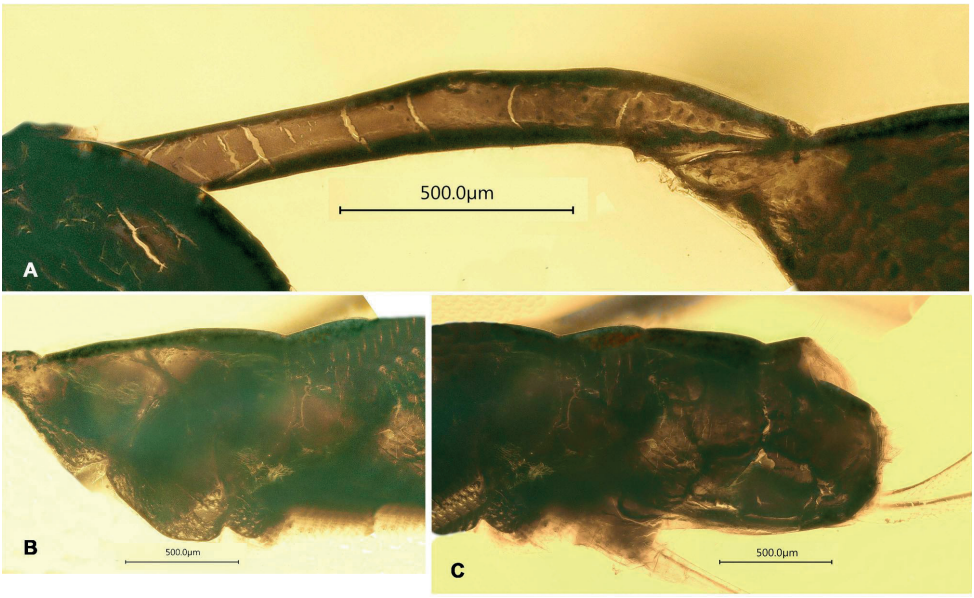

Figure 2. *Palaeorionis longicaudis* gen. et sp. nov. (holotype, female) **A** petiole, lateral view **B** anterior half of metasoma without petiole (gaster), lateral view **C** posterior half of metasoma, lateral view.

Legs (Fig. 1A, F) slender and very long. Hind coxa elongate, without ventro-basal tubercle, as long as propodeum. Hind femur long and slender, 0.8 times as long as hind tibia. Hind tibia narrow basally, distinctly widened in apical 0.8. Hind tibial spurs relatively short, 0.3 times as long as hind basitarsus. Hind basitarsus about 0.8 times as long as second-fifth segments combined. Tarsal claw small and simple.

Metasoma (Figs 1A, 2) elongate, compressed behind petiole, entirely smooth, segments behind third one distinctly exposed posteriorly. First metasomal tergite very narrow entirely, fused ventrally almost entirely, tubular, smooth dorsally, with spiracles situated behind middle of petiole, without dorsope and laterope; 0.6 times as long as mesosoma and metasoma behind petiole. Suture between second and third tergites absent. Laterotergites (epipleura) of all segments not separated. Ovipositor long, weakly curved, compressed basally. Ovipositor sheath 1.2 times longer than the body length, almost twice longer than mesosoma, 1.1 times longer than fore wing (Fig. 1A).

Comparative diagnosis. *Palaeorionis* gen nov. is characterised by a long and tube-like petiole resembling a similar structure in some extant Euphorinae genera, especially *Aridelus* Marshall, 1887, *Chrysopophthorus* Goidanich, 1948, *Orionis* Shaw, 1987, *Stenothremma* Shaw, 1984, and *Wesmaelia* Foerster, 1863.

Palaeorionis gen. nov. differs from *Orionis* Shaw by having the last segment of the maxillary palpus very long and narrow (shorter and thicker in *Orionis*), discoidal (discal) cell of infuscate fore wing sessile (petiolate in hyaline fore wing in *Orionis*), second radiomedial vein (r-m) present (absent in *Orionis*), mediocubital vein (M+CU1) sinuate (straight in *Orionis*), brachial (subdiscal) cell long and rather narrow (short and

wide in *Orionis*), petiole of metasoma smooth and without any carinae (at least partly sculptured and with lateral carinae in *Orionis*), and ovipositor sheath longer than metasoma (distinctly shorter in *Orionis*).

Palaeorionis gen nov. differs from *Aridelus* Marshall by having the last segment of maxillary palpus very long and narrow (shorter and thicker in *Aridelus*), mesosoma relatively long (short in *Aridelus*), hind coxa distinctly elongate-oval (shortly oval in *Aridelus*), mesosoma without areolate sculpture (entirely areolate in *Aridelus*), mediocubital vein (M+CU1) of fore wing sinuate (straight in *Aridelus*), hind femur relatively wide (narrow in *Aridelus*), metasoma rather compressed and with distinctly exposed apical segments (not compressed and retracted apical segments as in *Aridelus*), and ovipositor sheath longer than metasoma (very short and usually concealed inside of the metasoma in *Aridelus*).

The newly described genus also differs from *Stenothremma* Shaw by the last segment of the maxillary palpus very long and narrow (shorter and thicker in *Stenothremma*), mesosoma relatively long (short in *Stenothremma*), hind coxa distinctly elongate-oval (subglobal in *Stenothremma*), body without granulate sculpture (head, mesosoma and petiole densely granulate in *Stenothremma*), discoidal (discal) cell of infuscate fore wing sessile (petiolate in hyaline fore wing in *Stenothremma*), second radiomedial vein (r-m) present (often absent in *Stenothremma*), mediocubital vein (M+CU1) sinuate (straight in *Stenothremma*), brachial (subdiscal) cell long and rather narrow (short and wide in *Stenothremma*), petiole of metasoma smooth (petiole mainly granulate in *Stenothremma*), and ovipositor sheath longer than metasoma (distinctly shorter in *Stenothremma*).

The differences from the extant genera *Wesmaelia* and *Orionis* are summarized in Table 1.

Table 1. The differences between the Palaeorionis gen nov. and two similar recent genera (*Wesmaelia* Foerster and *Chrysopophthorus* Goidanich).

Genus Character	*Palaeorionis* gen. nov.	*Wesmaelia*	*Chrysopophthorus*
1. Last segment of the maxillary palpus	very long and narrow	shorter and thicker	shorter and thicker
2. Pedicel of antenna	distinctly enlarged, more than half as long as scape	short, much less than half of the length of scape	distinctly enlarged, about half as long as scape
3. Mesosoma	relatively long	short	short
4. Colour of fore wing	infuscate	hyaline	hyaline
5. Second radiomedial vein (r-m) of fore wing	present	absent	present
6. Mediocubital vein (M+CU1) of fore wing	sinuate	straight	straight
7. Discoidal (discal) cell of fore wing	sessile	petiolate	petiolate
8. Hind coxa	distinctly elongate-oval	weakly oval	weakly oval
9. Hind femur	widened	narrow	narrow
10. Metasoma	rather compressed and with distinctly exposed apical segments	not compressed and with retracted apical segments	not compressed and with retracted apical segments
11. Ovipositor sheath	longer than metasoma	very short and usually concealed inside of metasoma	distinctly shorter than metasoma

Between the known fossil Euphorinae genera, *Palaeorionis* gen nov. is similar to *Onychoura* Brues, 1933 (with type species *O. petiolata* Brues, 1933) and *Meteorites* Brues, 1939 (with type species *M. inopinata* Brues, 1939), both from Baltic amber. This new genus differs from *Onychoura* by having malar area short (very long in *Onychoura*), mesosoma relatively elongated (very short in *Onychoura*), notauli present (perhaps absent in *Onychoura*), propodeum long (very short in *Onychoura*), radial (marginal) cell of fore wing weakly shortened (strongly shortened in *Onychoura*), recurrent vein (m-cu) distinctly postfurcal (interstitial in *Onychoura*), petiole of metasoma not swollen (swollen in *Onychoura*), and ovipositor longer than metasoma and without apical hook (distinctly shorter and with very slender apical hook in *Onychoura*). *Palaeorionis* gen nov. distinctly differs from *Meteorites* Brues by the last segment of maxillary palpus very long and narrow (much shorter and thicker in *Meteorites*), antenna long, about 33-segmented (short, 13–14-segmented in *Meteorites*), mesosoma relatively long, about twice longer than height (short, about as long as height in *Meteorites*), second radiomedial vein (r-m) of fore wing present (absent in *Meteorites*), nervulus (cu-a) and recurrent (m-cu) veins distinctly postfurcal (almost interstitial in *Meteorites*), petiole of metasoma not widened distally and almost straight (widened distally and distinct evenly curved in *Meteorites*), and ovipositor longer than metasoma and almost straight (distinctly shorter and strongly arcuate in *Meteorites*).

***Palaeorionis longicaudis* sp. nov.**

https://zoobank.org/42C8A588-A16D-4E8E-A1FC-8C304CE98D78

Figs 1, 2

Type material. *Holotype*: Female, preserved in Lower Miocene Dominican amber (20–15 Ma), deposited in SMNS under collection number Do-2886-D. Well preserved, complete parasitoid inside amber piece (50 × 40 × 20 mm).

Description. Female. Body length 7.7 mm; fore wing length 4.6 mm.

***Head*:** Head not depressed, relatively high. Occiput at least weakly concave. Temple rather short. Transverse diameter of eye 3.7 times longer than temple (lateral view). Eye large, about 1.5 times as high as broad (lateral view). Malar suture perhaps absent. Malar space short. Clypeus without lower flange. Mandible rather short. Fourth segment of labial palpi the longest, 4.5 times longer than its maximum width, 1.7 times longer than second segment.

***Antenna*:** First flagellar segment subcylindrical, 6.2 times longer than its apical width, as long as second segment; second segment 5.5 times longer than its apical with. Submedial segments about 2.5 times longer than their width. Penultimate segments short, 1.2–1.3 times longer than its width, 0.4 times as long as apical segment.

***Mesosoma*:** Mesosoma relatively long, not depressed, its length about 2.0 times height. Neck of prothorax short. Mesoscutum highly and convex-roundly elevated above pronotum, its median lobe convex, weakly protruding forward, perhaps without anterolateral corners. Prescutellar depression (scutellar sulcus) invisible. Subalar depression shallow and sculptured. Mesopleuron widely smooth; metapleuron rugose.

***Wings*:** Fore wing narrow, 3.9 times longer than its maximum width. Pterostigma about 5.0 times longer than width. Radial vein (r) arising behind middle of pterostigma, from basal 0.6. First (r) and second (3RSa) radial abscissae forming very obtuse angle. Second radial abscissa (3RSa) almost equal to first abscissa (r), 0.1 times as long as the straight third abscissa (3RSb), 0.2 times as long as the almost straight first radiomedial vein (2RS). Second radiomedial (submarginal) cell relatively narrow and short, 1.6 times longer than its maximum width, 0.6 times as long as the wide brachial (first subdiscal) cell. Brachial (first subdiscal) cell almost straight anteriorly. First medial abscissa ((RS+M) a) slightly curved. Recurrent vein (1m-cu) almost 0.5 times as long as first radiomedial vein (2RS), 0.3 times as long as basal vein (1M). Discoidal (first discal) cell rather long, 3.2 times longer than its maximum width. Nervulus (1cu-a) postfurcal, almost 2.0 times longer than distance from basal (1M) vein and nervulus (1cu-a). Parallel vein (2CUb) arising from posterior 0.2 of apical margin of brachial (second subdiscal) cell. Brachial (second subdiscal) cell long and wide. Hind wing relatively narrow. Radial (marginal) cell weakly widened basally and narrowed apically, without additional transverse vein (r). Nervellus (cu-a) present, oblique. Submedial (subbasal) cell short. First abscissa of mediocubital vein (M+CU) 0.6 times as long as second abscissa (M).

***Legs*:** Fore trochanter almost twice longer than trochantellus. Fore tarsus almost as long as fore tibia. Hind coxa 2.0 times longer than maximum width, 0.7 times as long as petiole. Hind femur 6.5 times longer than width. Hind tarsus slender, 0.8 times as long as hind tibia. Second segment of hind tarsus 0.5 times as long as basitarsus, almost 2.0 times longer than fifth segment (without pretarsus).

***Metasoma*:** Metasoma 1.2 times longer than head and mesosoma combined. First metasomal tergite 9.6 times longer than medial high (at spiracles level), tergite ventrally fused in basal 0.8. Lateral suture between second and third tergites present, but dorsal suture absent. Second and third tergites combined 0.7 times as long as following tergites. Hypopygium short, obtuse distally, strongly retracted below under metasoma, almost glabrous.

***Sculpture*:** Vertex and temple mainly smooth. Hind coxa and femur smooth. Metasoma entirely smooth. Hind tibia with rather dense and short semi-erect setae, its length 0.2–0.3 times maximum width of tibia.

***Colour*:** Body almost entirely black or dark brown. Antenna mainly light brown. Labial palpi light brown; maxillary palpi dark reddish brown, but at least apical segment brownish yellow. Legs mainly dark brown, all tibiae basally yellow at short distance. Ovipositor sheaths light brown, infuscate apically. Fore wing almost entirely distinctly infuscate, paler basally and apically, with distinct hyaline transverse stripe under base of pterostigma. Pterostigma mainly dark brown, pale brown in basal fifth.

Male. Unknown.

Etymology. Named from Latin “longus” (= long) and “caudus” (= tail, ovipositor) because this taxon has the longest known ovipositor of all fossil Euphorinae taxa.

Discussion

The fossil braconid taxa from the subfamily Euphorinae are relatively common in the Paleogene in comparison to the members of some other braconid subfamilies. They were often attributed as representatives of extant genera, however various of these fossil taxa actually belong to peculiar extinct taxa, namely *Elasmosomites* Brues, *Meteorites* Brues, *Oncometeorus* Tobias, *Onychoura* Brues, *Parasyrrhizus* Brues and *Prosyntretus* Tobias (Brues 1933, 1937, 1939; Tobias 1987, Belokobylskij 2014, Belokobylskij et al. 2021). These euphorine genera were recorded from the inclusions found in the Baltic and Canadian ambers (Brues 1933, 1939, 1937; Tobias 1987) and were placed in the tribes Meteorini, Oncometeorini, Perilitini, Centistini, Euphorini, Prosyntretini and Neoneurini.

The tubular petiole of the mesosoma is practically unknown from previously recorded fossil euphorine taxa. In this situation, the genus *Palaeorionis* gen nov., is the first extinct genus having such long tube-shaped petiole. Perhaps only *Onychoura* Brues, 1933 from Baltic amber possessed a petiole with similar structure, but it is much shorter and thickened towards the apex. The new genus is also well characterised by the distinctly infuscate fore wing having submedially only a single narrow transverse subhyaline stripe, very long ovipositor (much longer than metasoma), and distinctly thickened tibia of the hind leg. This character combination is unknown in all previously recorded amber euphorine genera.

The host of *Palaeorionis longicaudis* gen. et sp. nov. is unclear. However, based on available characters such as a long ovipositor and presence of both radiomedial veins (2-SR and r-m) in the fore wing this genus likely belongs to the tribe Meteorini, whose members are known to be endoparasitoids of coleopteran and lepidopteran larvae. Regarding its long ovipositor, this species might have been a parasitoid of some concealed hosts such as wood-boring beetles.

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RESEARCH ARTICLE

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Biological notes on *Rhysipolis taiwanicus* Belokobylskij (Hymenoptera, Braconidae, Rhysipolinae)

Cornelis van Achterberg[1], Clive Siu-Ki Lau[2]

1 *Naturalis Biodiversity Center, P.O. 9517, 2300 RA Leiden, Netherlands* **2** *Agriculture, Fisheries and Conservation Department, Hong Kong Special Administrative Region, Hong Kong, China*

Corresponding author: Cornelis van Achterberg (kees@vanachterberg.org)

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Abstract

Data on the cocoons and possibly host of *Rhysipolis taiwanicus* Belokobylskij, 1988 (Braconidae, Rhysipolinae) are presented for the first time. Their peculiar cocoons found on the upper surface of a leaf of *Rhaphiolepis indica* (L.) Lindl. are described and illustrated. The species is new for Hong Kong and the second record after its description from Taiwan.

Keywords

Cocoons, Hong Kong, hosts, Indian hawthorn - *Rhaphiolepis indica*, koinobiont ectoparasitoid, new record

Introduction

On 19 April 2022 the junior author discovered six enigmatic cocoons (Figs 1–6) on the upper surface of a leaf of Indian hawthorn (*Rhaphiolepis indica* (L.) Lindl., an evergreen shrub in the family Rosaceae. The leaf was one of a few Indian hawthorn

leaves collected four days earlier on Hong Kong Island for intended subsequent rearing of an immature stick insect (*Phraortes stomphax* (Westwood, 1859)) that fed on the shrub. Synchronized hatching of six tiny wasps (of ca. 3.2 mm body length; Fig. 12) was observed 9 days later on 28 April 2022 (Figs 7–11). The wasps (Figs 12–15) were identified by the senior author as *Rhysipolis taiwanicus* Belokobylskij, 1988 (Braconidae, Rhysipolinae). Rhysipolinae is a small subfamily of koinobiont ectoparasitoids of lepidopteran larvae (Shaw 1983). According to the most recent phylogenomic research the group is the basal lineage of the rogadinoid subcomplex and the Leuriniinae should be included (Jasso-Martínez et al. 2022a, 2022b). From the East Palaearctic and northern Oriental regions are 13 species of *Rhysipolis* Foerster known, which can be identified with the key by Zhang et al. (2016).

Materials and methods

About five leaves were collected of Indian hawthorn (*Rhaphiolepis indica* (L.) Lindl.) growing along Mount Parker Road midway between Hong Kong Country Trail and Quarry Pass Pavilion inside the Tai Tam (Quarry Bay Extension) Country Park on 15 April 2022. The GPS coordinates are 22°16'10.6"N (22.269599) and 114°12'41.8"E (114.211612). The six cocoons all on one leave were kept at ambient temperature varying between 23.7 to 25 °C and the wasps emerged on 28 April 2022. Two specimens were sent to the senior author, prepared and deposited in the Naturalis Biodiversity Center (Leiden, Netherlands) and the remaining four specimens are deposited in the Shatin Plant Quarantine Station, (Hong Kong, China).

For the morphological terminology used in this paper see van Achterberg (1988, 1993). The cocoons were examined and measured by the junior author with a Leica M205C stereomicroscope. Photos were taken using a Leica DFC450 digital camera mounted to a Leica M205C stereomicroscope. Each photo was produced by taking 10–50 digital images at different focal planes and combining them into a sharp composite image using the Leica Applicadtion Suite multifocus software v.4.13. Photographic images of adult wasp were edited using Adobe Photoshop to hide the insect pinning.

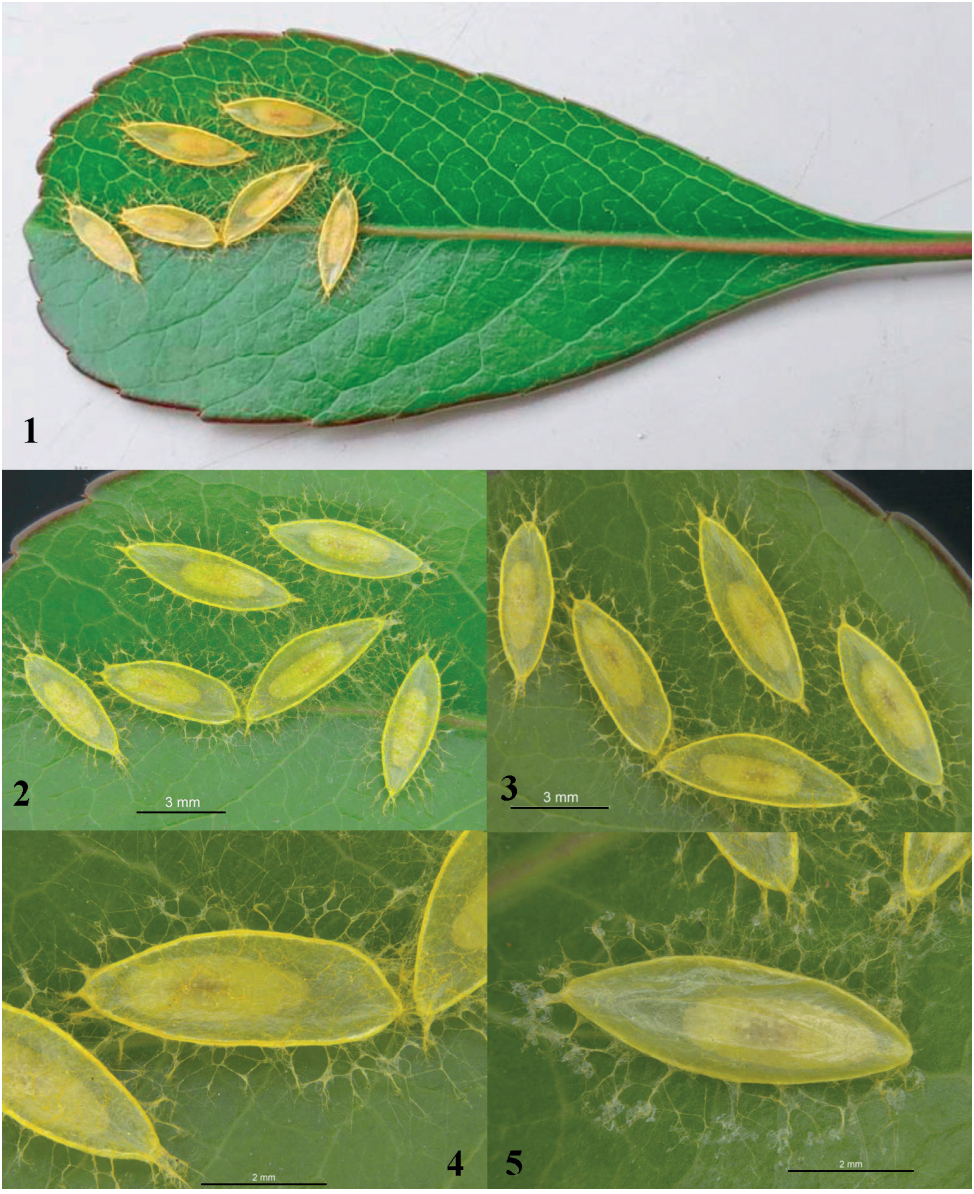
Rhysipolis taiwanicus Belokobylskij, 1988

Figs 1–15

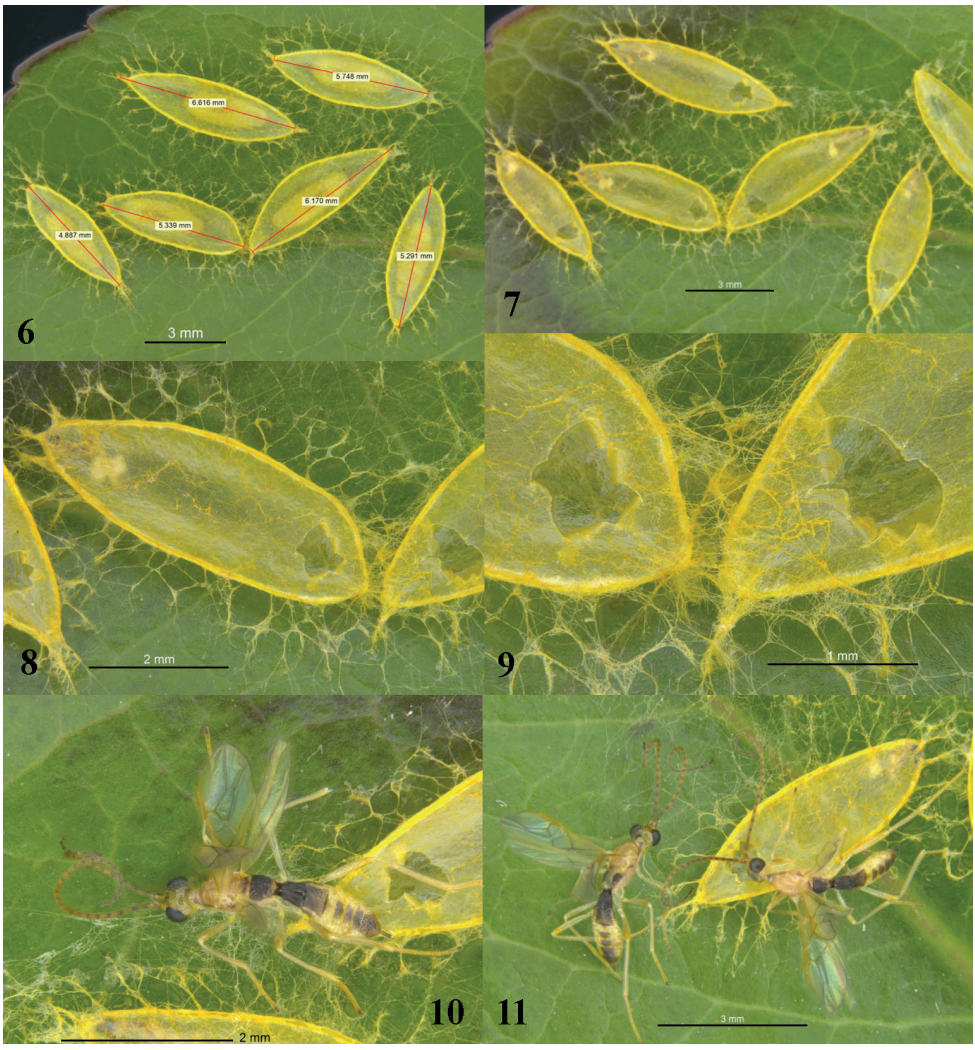
Notes. *Rhysipolis taiwanicus* Belokobylskij is a rarely collected species known from Taiwan and Vietnam (Belokobylskij 1988; Long and Belokobylskij 2004). It can be easily differentiated from similar species by the small stemmaticum and ocelli, the glabrous middle lobe of the mesoscutum, the strongly receding temples behind the eyes and the subglobular head (Zhang et al. 2016). The stemmaticum is situ-

ated comparatively close to the antennal sockets (Fig. 15). The body length of the imagines is 3.2–4.0 mm (Belokobylskij 1988; this paper) and are slender with long straight antennae when alive but the antennae are curled up after death (Figs 10–12) as in most Rhysipolinae.

Biology. The bright yellow cocoons were on the upper side of the leaf and appeared to naked eyes as little fried eggs (Fig. 1). At closer look, they resembled elongated



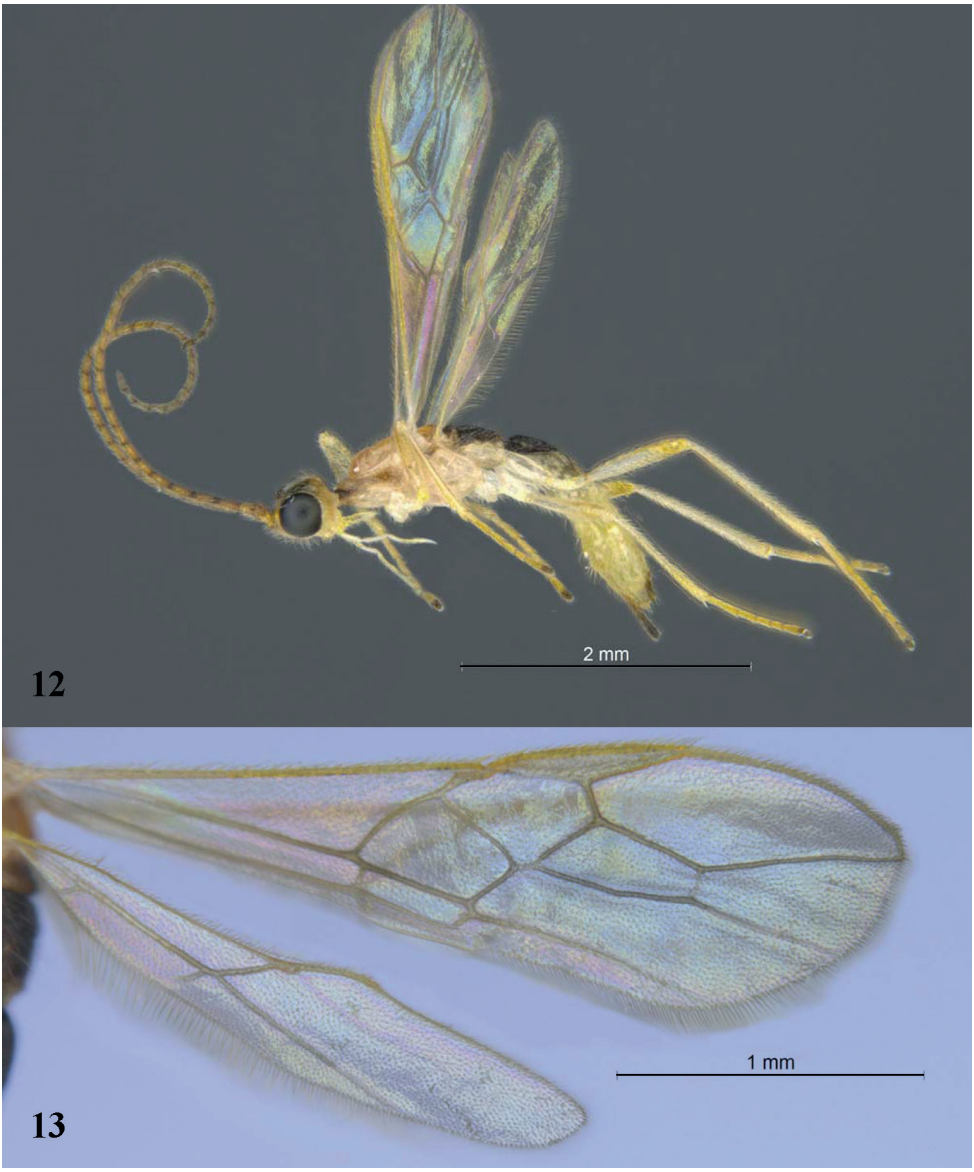
Figures 1–5. Cocoons of *Rhysipolis taiwanicus* Belokobylskij on *Rhaphiolepis indica* (L.) Lindl.



Figures 6–11. Cocoons of *Rhysipolis taiwanicus* Belokobylskij on *Rhaphiolepis indica* (L.) Lindl. **6** sizes of cocoons **7–9** cocoons after hatching **10–11** hatched wasps near cocoons.

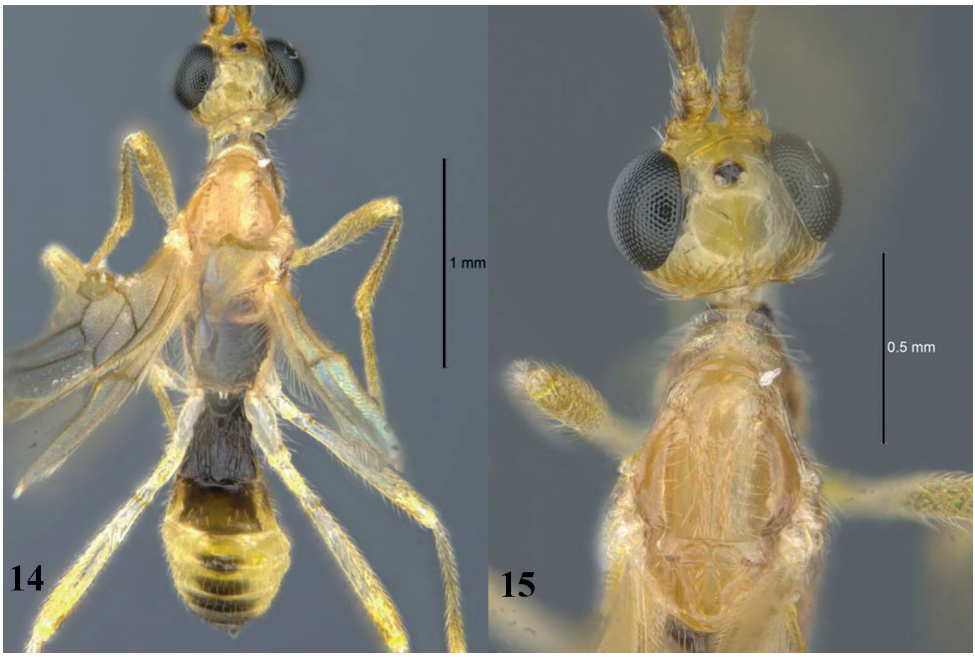
trampolines fixed to a leaf by silken threads (Figs 2–5). All cocoons were found at the distal part of the leaf (Fig. 1) and the average size was 5.67 ±0.63 mm long (Fig. 6). The wasps inside the cocoons were showing obvious movement (Figs 3–5) before hatching simultaneously 13 days after the collecting of the leaves.

Checking for small lepidopterans occurring on *Rhaphiolepis indica* seems to be the best possible tactic to discover the unknown host of *Rhysipolis taiwanicus*. *Rhaphiolepis indica* is one of the most common shrubs on hillsides in Hong Kong. So far seven species of caterpillars are known to feed on this plant (Table 1). Given the recorded



Figures 12–13. *Rhysipolis taiwanicus* Belokobylskij. **12** habitus, lateral **13** wings.

hosts of *Rhysipolis* species are mainly leaf-mining microlepidopterans belonging to the Gracillariidae and to a much lower degree to Gelechiidae, Psychidae and Pyralidae (Yu et al. 2016; Zhang et al. 2016), it may worth to have a close look at *Dichomeris ochthophora* Meyrick, 1936 (Li et al. 2010) in due course to investigate if it could be the unknown host of *Rhysipolis taiwanicus* Belokobylskij. A second choice would be *Chalioides kondonis* Kondo, 1922.



Figures 14–15. *Rhysipolis taiwanicus* Belokobylskij. **14** habitus, dorsal **15** detail of head and mesosoma, dorsal.

Table 1. Lepidoptera associated with *Rhaphiolepis indica* (L.) Lindl.

Species	Family: Subfamily	Reference
Caeneressa diaphana (Kollar, 1848)	Erebidae: Arctiinae	Personal record of junior author
Chalioides kondonis Kondo, 1922	Psychidae: Psychinae	Personal record of junior author
Delias pasithoe pasithoe (L., 1767)	Pieridae: Pierinae	Personal record of junior author
Dichomeris ochthophora Meyrick, 1936	Gelechiidae: Dichomeridinae	Li et al. 2010. Host plant was cited as *Rhaphiolepis umbellata* (Thunb.) Makino which is a synonym of *Rhaphiolepis indica* (L.) Lindl.
Nygmia plana (Walker, 1855)	Erebidae: Lymantriinae	Personal record of junior author
Remelana jangala mudra (Fruhstorfer, 1907)	Lycaenidae: Theclinae	Personal record of junior author
Zeuzera coffeae Nietner, 1861	Cossidae: Zeuzerinae	Pun and Batalha 1997

Discussion

The minute ocelli are an indication that *R. taiwanicus* is a day-active species and may be found searching for the host caterpillars during day time. According to the known host relationships of *Rhysipolis* species it is considered likely that *Rhysipolis taiwanicus* emerged from *Dichomeris ochthophora* Meyrick or *Chalioides kondonis* Kondo. The reason of the peculiar attachment of the cocoons is unclear, but it might be an adaptation to drain off water.

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RESEARCH ARTICLE

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Elevation gradient affects the distribution and host utilisation of *Zatypota anomala* (Hymenoptera, Ichneumonidae) associated with mesh web weaving spiders (Araneae, Dictynidae)

Stanislav Korenko[1], Jakub Sýkora[1], Ľudmila Černecká[2], Peter Gajdoš[3], Pavol Purgat[3], Ján Černecký[3], Kamil Holý[4], Petr Heneberg[5], Ingi Agnarsson[6,7]

1 *Department of Agroecology and Crop Production, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha – Suchdol, Czech Republic* **2** *Institute of Forest Ecology, Slovak Academy of Sciences, Štúrova 2, 96053 Zvolen, Slovakia* **3** *Institute of Landscape Ecology, Bratislava, Branch Nitra, Slovak Academy of Sciences, Akademicka 2, POB-23B, 949 01 Nitra, Slovakia* **4** *Crop Research Institute, Drnovská 507, 161 06 Prague 6, Ruzyně, Czech Republic* **5** *Third Faculty of Medicine, Charles University, Prague, Czech Republic* **6** *Department of Entomology, National Museum of Natural History, Washington, DC 20560, USA* **7** *Faculty of Life and Environmental Sciences, University of Iceland, Sturlugata 7, 102 Reykjavik, Iceland*

Corresponding author: Stanislav Korenko (korenko@af.czu.cz)

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Abstract

The spatial distribution of parasitoids is closely linked to the distribution and ecological requirements of their hosts. Several studies have documented changes in the fauna composition of parasitoids in response to elevation, but data on parasitoids associated with spiders are missing. The koinobiont ichneumonid wasp *Zatypota anomala* is strictly specialised on spiders of the genus *Dictyna* (Dictynidae) in Europe. We examined the distribution of spiders of the family Dictynidae in forest ecotones in central Europe across a broad elevation gradient (110–1466 m a.s.l.). We checked the spiders for parasitism by *Z. anomala*. It was most abundant at the mid-elevations (median 712 m a.s.l., range 179–870 m a.s.l.). We identified four dictynid spider species as *Z. anomala* hosts. These were *Dictyna arundinacea*, *Dictyna uncinata*, *Nigma flavescens*, and *Nigma walckenaeri*. All four species and the genus *Nigma* were recorded as hosts for the first time. The parasitoids

strongly preferred juvenile instars of their hosts. The body length differed between parasitised *Dictyna* and *Nigma* spiders (medians: 1.95 mm and 2.55 mm, respectively). The distribution of *Dictyna* and *Nigma* spiders overlapped along the elevation gradient, but parasitism incidence significantly differed between spider genera along the elevation gradient. *Nigma* was parasitized at lower elevations between 179–254 m a.s.l. and *Dictyna* at higher elevations between 361–870 m a.s.l. The phenology of *Z. anomala* is closely tied to the univoltine life strategy of its host spiders. The parasitoid female oviposits in autumn, and its offspring overwinter as larvae on the host, reach adulthood during spring, and pass the summer as an adult.

Keywords

altitude, Darwin wasp, Ephialtini, host-parasitoid interaction, host range, host shift, host specificity, *Polysphincta* group of genera

Introduction

The effect of elevational gradient on species assemblages remains a central theme of biogeography and ecology (Körner 2007; Nogueira et al. 2021). Elevation effects on distribution ranges were well studied in both spiders (e.g., Chatzaki et al. 2005; Bowden and Buddle 2010; Foord and Dippenaar-Schoeman 2016; Mammola et al. 2021) and parasitoids (e.g., Péré et al. 2013; Corcos et al. 2018). Elevational gradients may occur within small geographical distances. Thus, they help assess community response to climate change and other environmental factors (Axmacher and Fiedler 2008; McCain 2009; Sundqvist et al. 2013; Chamberlain et al. 2016; Corcos et al. 2018). Published data on elevation effects on parasitoid richness and abundance appear to conflict in several studies (e.g., Veijalainen et al. 2014; Perillo et al. 2017); however, these conflicts might be resolvable if other ecological variables besides elevation are taken into consideration.

As some parasitoids may be specialised on hosts living at higher elevations, species-specific data are needed, particularly for associations where specific hosts are present at various elevations. Parasitism rates can differ with elevation and latitude (Virtanen and Neuvonen 1999; Hodkinson 2005). The meta-analysis by Péré et al. (2013) revealed that parasitism rates usually decrease as elevation increases. Some parasitoids use multiple hosts, including ichneumonid wasps, which we focus on here. Several studies have already quantified parasitoids associated with multiple hosts along elevation gradients (e.g., Hodkinson 2005; Corcos et al. 2018; Libra et al. 2019). However, studies of parasitoids associated with spiders which belong to the *Polysphincta* group of genera (usually called polysphinctines) are still missing.

Spiders of the family Dictynidae are aerial web builders producing 3D tangle webs on tree canopies, shrubs, or higher vegetation (Foelix 2011). Its genera, *Dictyna*, *Lathys*, and *Nigma*, are abundant in central Europe, share a similar ecological niche, and often co-exist in the canopy (e.g., Kůrka et al. 2015; CAS 2022; SARAS 2022). The only parasitoid that is known to be associated with Dictynidae is the here-studied *Zatypota anomala* (Holmgren) (Fitton et al. 1988). *Zatypota anomala*, distributed across the Holarctic, is known to be associated with Dictynidae – specifically, with the genus *Mallos* in North America (Vincent 1979) and with the genus *Dictyna* in Europe (Fitton

et al. 1988; Gauld and Dubois 2006; Miller et al. 2013; Korenko 2016). This wasp is also the only known parasitoid able to oviposit on cribellate spiders, whose webs contain hackled threads. This kind of silk requires the spider to comb the threads to a hackle (calamistrum), which are understood to attach and adhere to insects by van-der-Waals forces (Hawthorn and Opell 2002, 2003). The cribellate web is highly adhesive, attaches to prey, and deters attacks by predators and parasitoids (e.g., Foelix 2011).

In the present study, we aimed to explore elevation gradient effects on the parasitism of Dictynidae by the ichneumonid wasp parasitoid *Z. anomala*. We hypothesised that the composition of the dictynid spider community differs with elevation and that this may reflect elevation preferences in its parasitoid.

Materials and methods

Communities of spiders from the family Dictynidae (potential hosts of *Z. anomala*) were studied in 227 localities in 39 orographic units in Czechia and Slovakia at elevations ranging from 110 to 1466 m a.s.l. in the years 2016–2021 (Fig. 1). One to three samplings were performed at each locality. Spiders were collected by beating tree canopies (between 30 and 250 cm above the ground), with a square-shaped beating net (1-m^2 area) placed beneath. Parasitoid larvae were identified visually on spiders in the collected samples. The parasitised spiders were placed individually into tubes and

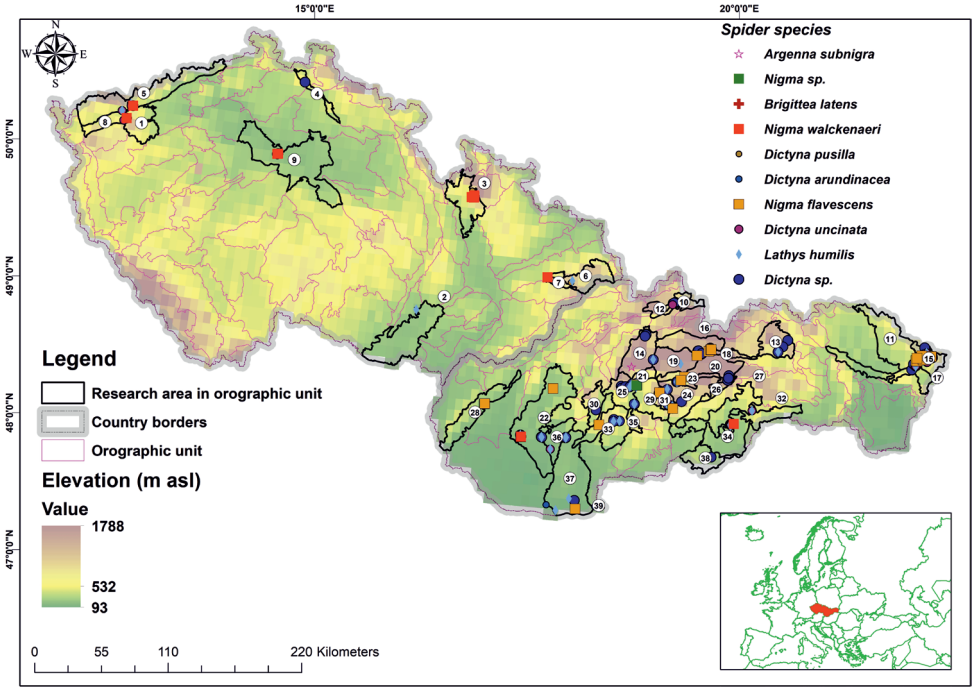

Figure 1. Map of studied localities and distributions of collected potential hosts (spiders of the family Dictynidae).

kept for wasp rearing and identification in the laboratory. Parasitised spiders that were not identified directly in the field were put in 70% ethanol together with unparasitised spiders, and all the collected individuals were analysed in detail in the laboratory. The parasitism rate was calculated as the sum of parasitized spiders relative to the number of examined spiders. Spiders were identified to species using Nentwig et al. (2022), and parasitoids were identified to species using Fitton et al. (1988) and Zwakhals (2006).

Statistical analyses

The two-tailed Kruskal-Wallis test and Dunn's multiple comparison test as Multiple Choice Test (MCT) were used to reveal differences in the distribution of dictynid species/genera along the elevation gradient. The two-tailed Mann-Whitney U test was used to reveal differences in the body size of parasitised dictynid spiders and differences in parasitoid distribution along the elevation gradient between those reared from *Dictyna* and *Nigma*. The calculations were performed in GraphPad InStat v. 3.06. Data are shown as medians and 1Q and 3Q of the Interquartile Range (IQR) unless stated otherwise.

We used the Arc GIS 10.3 environment for the spatial presentation of the association of the distribution of *Dictyna* and *Nigma* and their parasitoid *Z. anomala* with elevation. Part of the spatial analysis was dedicated to the distribution of host species and their parasitised specimens within the orographic units. Orographic units represent functional ecological units, each defined by elevation and by ecological, climatic, and geological conditions.

Results

Distribution of dictynid spider hosts

The examination of tree and shrub branches led to the collection of 2,332 individuals of dictynid spiders. They represented eight species that belonged to five genera, namely *Argenna*, *Brigittea*, *Dictyna*, *Lathys*, and *Nigma* (Table 1). Their distributions along the elevation

Table 1. Total number of collected dictynid spiders (potential hosts), their distribution along the elevation gradient, and parasitism by *Zatypota anomala*. Median and (1Q–3Q) of IQR are shown; data are presented in mm. N – number of collected spiders; p – number of parasitized spiders.

Spider species	N	Distribution	p
Argenna subnigra (O. P.-C.)	5	138(138–263)	0
Brigittea latens (Fabricius)	3	124(121–288)	0
Dictyna arundinacea (Linnaeus)	240	747(712–760)	5
Dictyna pusilla Thorell	61	870(870–870)	0
Dictyna uncinata Thorell	319	318(273–570)	14
Dictyna sp.	808	708 (439–760)	44
Lathys humilis (Blackwall)	742	572 (205–672)	0
Nigma flavescens (Walckenaer)	111	439(305–760)	1
Nigma walckenaeri (Roewer)	38	231(183–405)	8
Nigma sp.	5	431(431–441)	0
Total	2332	663(273–755)	72

gradient differed significantly among species (Kruskal-Wallis one-way ANOVA on ranks $H = 356$, $d_f = 7$, $p < .0001$, Table 2) and among genera (Kruskal-Wallis one-way ANOVA on ranks $H = 56$, $d_f = 2$, $p < .001$, Table 2). The genus *Dictyna* occurred at elevations with a median of 710 (361–760) m a.s.l. Its distribution differed significantly from *Lathys* and *Nigma*. *Nigma* preferred elevations with a median of 406 (288–754) m a.s.l., which was significantly lower than for *Dictyna* (MCT, $p < .01$, Table 2). *Lathys* was, like *Dictyna*, distributed across a relatively wide range of elevations, with a median of 572 (205–675) m a.s.l. that was significantly lower than that for *Dictina* (MCT, $p < .001$, Table 2). The rare species *Argenna subnigra* (O. Pickard-Cambridge) and *Brigittea latens* (Fabricius) were not included in the analyses because only five and three individuals were collected, respectively.

Table 2. Differences in elevation distribution between dictynid species and genera. Dunn's Multiply Comparisons Test. "z" means Mean Rand Differences. The rare species *A. subnigra* and *B. latens* were not included in the analysis. Spider individuals identified only to genus level were not included in the species analysis. The rare genera *Argenna* and *Brigittea* were not included in the genera analysis.

Comparison	*z*	*p*-value
Species		
D. arundinacea vs. *D. pusilla*	-287.87	< 0.001
D. arundinacea vs. *D. uncinata*	388.28	< 0.001
D. arundinacea vs. *L. humilis*	381.49	< 0.001
D. arundinacea vs. *N. flavescens*	250.46	< 0.001
D. arundinacea vs. *N. walckenaeri*	662.22	< 0.001
D. pusilla vs. *D. uncinata*	676.15	< 0.001
D. pusilla vs. *L. humilis*	669.36	< 0.001
D. pusilla vs. *N. flavescens*	538.34	< 0.001
D. pusilla vs. N. walckenaeri	950.09	< 0.001
D. uncinata vs. *L. humilis*	-6.788	ns
D. uncinata vs. *N. flavescens*	-137.82	ns
D. uncinata vs. *N. walckenaeri*	273.94	< 0.01
L. humilis vs. *N. flavescens*	-131.03	< 0.05
L. humilis vs. *N. walckenaeri*	280.72	< 0.01
N. flavescens vs. *N. walckenaeri*	411.75	< 0.001
Genera		
Dictyna vs. *Lathys*	192.87	< 0.001
Dictyna vs. *Nigma*	164.37	< 0.01
Lathys vs. *Nigma*	-28,50	ns

Host specificity and host trait preferences

Of the eight collected dictynid species, four species of spiders from the genera *Dictyna* and *Nigma* were documented as hosts (Table 1). All four spider species are documented as hosts of *Z. anomala* for the first time. Except for one adult spider female of *Dictyna uncinata*, all parasitised spiders (N = 72) were in the juvenile or subadult developmental stage. The average body length of the parasitised spider was 2.08 (1.92–2.60) mm. We found a significant difference in size between parasitized *Dictyna* (1.95 (1.83–2.09) mm) and *Nigma* spiders (2.55 (2.45–2.60) mm) (Mann-Whitney test, $U = 8.5$, $U' = 127.5$, $p < .001$). The body sizes of potential hosts (juvenile dictynid

Table 3. Body size of potential hosts and body size of parasitised spiders. Median and (1Q–3Q) of IQR are shown in mm. Different letters beside juvenile body sizes denote significant differences in body size analysed by Dunn's multiple comparison test.

	Dictyna	*Nigma*	*Lathys*
Female	2.55 (2.3–2.6)	3.0 (2.6–3.2)	2.45 (2.4–2.5)
Male	2.41 (2.3–2.5)	2.6 (2.51–2.73)	No data
Juvenile	1.92 (1.78–2.14)[b]	2.5 (2.3–2.7)[a]	2.0 (1.8–2.0)[b]
Parasitised	1.95 (1.84–2.09)	2.55 (2.45–2.6)	No record

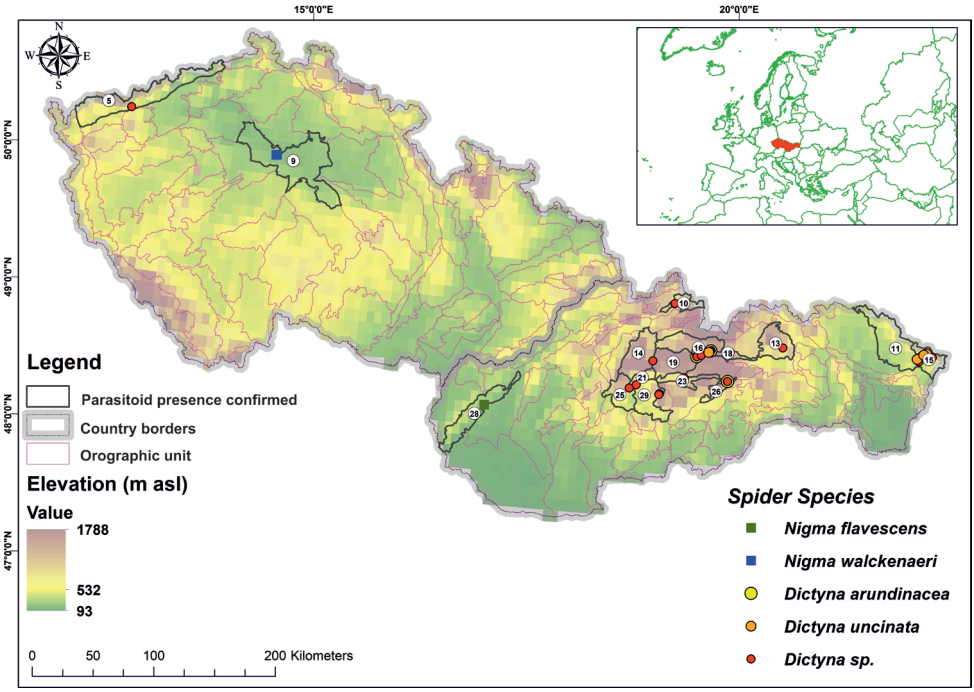

Figure 2. Map of the distribution of *Z. anomala* in association with different dictynid hosts.

spiders) differed among genera (Table 3); *Nigma* spiders were significantly larger than *Dictyna* and *Lathys* spiders (Kruskal-Wallis one-way ANOVA on ranks $H = 57$, $d_f = 2$, $p < .001$).

Host preferences change with elevation gradient

The parasitoid *Z. anomala* was present in 16 (33 localities) out of the 39 orographic units (227 localities) where accepted hosts (the genera *Nigma* and/or *Dictyna*) occurred in the examined spider community (Fig. 2). The parasitoid preferred sites with an intermediate elevation of 712 (486–765) m a.s.l. These elevations were inhabited by both accepted genera of spider hosts – specifically, by several species of the genus *Dictyna* and by *N. flavescens* (Fig. 2).

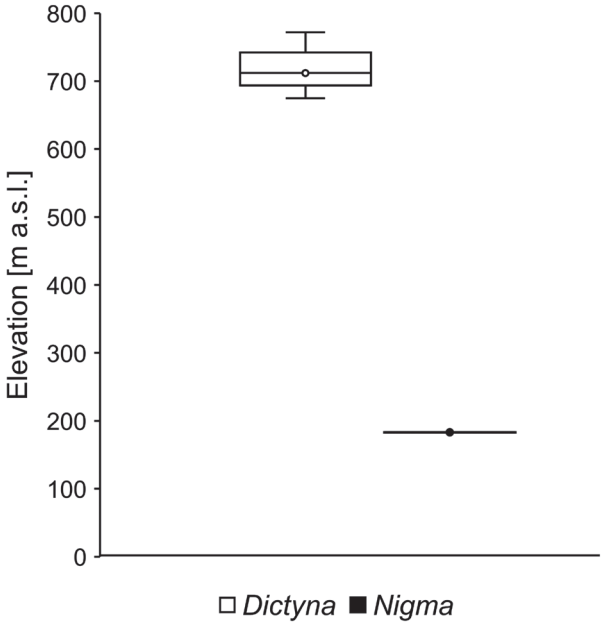

Figure 3. Differences in elevation where *Nigma* and *Dictyna* were parasitised. Bars and whiskers indicate median values, and Q1 and Q3 of IQR.

However, elevation differed significantly between localities where *Nigma* spp. were parasitised (183 (183–183) m a.s.l) and where *Dictyna* spp. were parasitized (712 (675–772) m a.s.l.) (Mann-Whitney test, $U = 0$, $U' = 567$, $p < .001$, Fig. 3). Parasitised *Nigma* spiders only occurred in three localities, and 70% of parasitised *Nigma* (N = 9) were collected in one locality at 183 m a.s.l.

Phenology

Dictynid spiders were parasitised by *Z. anomala* in high numbers in early spring and autumn. The parasitoid overwintered as larvae of the second instar and reached adulthood the following spring. Summer is expected to be the period of adult flight. Adult *Z. anomala* attacks the next generation of juvenile hosts in autumn, when the spiders have grown to a body size suitable for oviposition (Fig. 4). *Z. anomala* is thus univoltine and appears to be strictly dependent on the phenology of its dictynid hosts.

Discussion

Zatypota anomala presumably possess phenotypic plasticity, which enables it to attack spiders from different genera at different elevations. Although these accepted taxa differ in some morphological and behavioural traits, their ecological position in the local

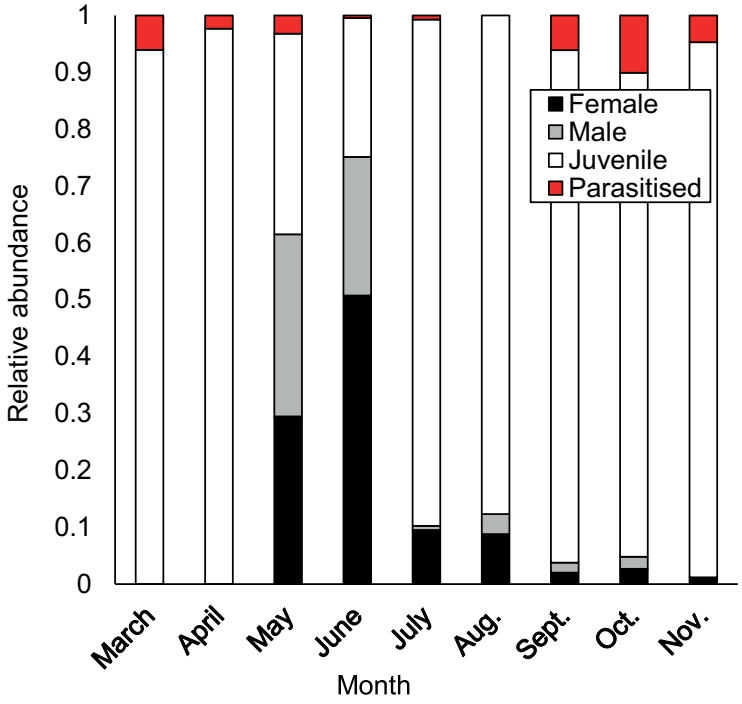

Figure 4. Seasonal occurrence of each developmental stage of dictynid hosts (genera *Dictyna* and *Nigma*) and parasitism incidence of *Z. anomala* in the years 2016–2021.

community is very similar across continents (e.g., Europe vs. North America) and along the elevation gradient. We found that *Z. anomala* attacked at least four species from the genera *Dictyna* and *Nigma*. Former host records of *Z. anomala* from Europe were mostly identified only to genus level as *Dictyna* spp.; only *Dictyna pusilla* Thorell was identified as a specific host species by Sedivy (1963) and Korenko (2017). Sparse records of hosts in North America include only the genera *Mallos* (Vincent 1979) and *Emblyna* (Howard 1888). It is interesting that several species of the genus *Dictyna* (including *D. arundinacea*, which is an accepted host in Europe) and one *Nigma* species are also present in North America (WSC 2022) but have not so far been recorded as hosts of *Z. anomala* there.

We found that *Z. anomala* accepted both *Dictyna* and *Nigma* spiders in Europe, but we never found both genera parasitised in the same locality. *Zatypota anomala* constantly attacked spider species which were highly abundant locally. Our results suggest that locally dominant dictynid species were easily available and consequently became preferred hosts. Local preference for the dominant host might also be imprinted onto the local population by hatching from the dominant host over several generations. In other parasitoids, it is known that offspring prefer to develop in/on the host in/on which their mother developed (Uller 2008; Stillwell and Fox 2009; Wolf and Wade 2009; Jones et al. 2015).

Polysphinctine wasps strictly associated with spiders possess high host specificity, even monophagy (e.g., Eberhard and Gonzaga 2019). *Zatypota anomala*, like some other

polysphinctine wasps, retains the ability to switch hosts within the group of closely-related spider taxa. We found that the host range of *Z. anomala* includes several genera across the Holarctic. In Europe, it attacks the ecologically similar genera *Dictyna* and *Nigma*. However, *Z. anomala* did not attack the ecologically similar and abundant genus *Lathys*. An explanation of this rejection of *Lathys* as host is still unavailable. Host size is of high importance for host acceptance by a parasitoid. *Zatypota anomala* parasitised spiders of the genus *Dictyna*, which are smaller than the parasitized *Nigma* species. In *Nigma*, only a few of the smallest individuals (below 2.70 mm) were parasitised, resembling in size the parasitised *Dictyna* spiders. The size of juvenile *Lathys* was very similar to that of parasitised *Dictyna* individuals, yet, the acceptance of spiders of the genus *Lathys* was not documented, despite the examination of 742 *Lathys* individuals. Thus, in this case, it appears that body size is not a sufficient reason for the parasitoid *Z. anomala* to avoid *Lathys*.

We found that *Z. anomala* preferred mid-elevations, around 700 m a.s.l., but we also found it in smaller numbers at lower elevations and not at all at higher elevations over 800 m a.s.l. *Zatypota anomala* is strictly associated with spiders from the family Dictynidae, which were rarely collected at elevations over 800 m a.s.l. The absence and low abundance of dictynid spiders in localities at higher elevations seem to indicate the upper limit of the parasitoid distribution on the elevation gradient. On the basis of the collected data, we assume the occurrence of host substitution in *Z. anomala*, where spiders of the genus *Dictyna* were parasitised in localities at middle elevations, and spiders of the genus *Nigma* were attacked in localities at lower elevations.

Parasitoid phenology is synchronised with host phenology. This synchronisation influences many traits of the parasitoid life history, including the number of generations per year (e.g., Godfray 1994). We found that *Zatypota anomala* is univoltine, and its phenology completely differs from that of *Z. percontatoria* and *Z. albicoxa*. Adult wasps emerge from overwintered juvenile or subadult dictynid spiders in the early spring, and flying female wasps must live until late summer and autumn, when dictynid spiders of the new generation grow to a suitable body size and females can successfully oviposit on them. In contrast, *Z. percontatoria* has several generations per year, since it accepts different host species that are available with a suitable body size at different times of the year. Therefore, the female can lay eggs on different hosts throughout most of the year (Korenko et al. 2016). *Zatypota albicoxa* (Walker) is also plurivoltine in Japan, having up to four generations annually due to the all-year availability of juvenile spiders (Tanaka 2007; Takasuka and Tanaka 2013).

The most important limitation on the acceptance of spider hosts by polysphinctines is the suitability of their size – specifically, the body size ratio between parasitoid and host (e.g., Korenko et al. 2011). *Zatypota anomala* prefers medium-sized juvenile spiders. However, the phenology of its spider hosts provides juvenile spiders of suitable body size for oviposition only in late summer and autumn. Therefore, females must overcome the hot summer period and oviposit when suitable spiders of the new generation become available. *Zatypota anomala* females are not documented to overwinter, so we suggest that females cannot oviposit on the spring spider population although there are juvenile spiders of suitable body length.

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RESEARCH ARTICLE

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Contribution to the taxonomy of the *Pseudepipona* subgenus *Deuterepipona* Blüthgen, 1951 (Hymenoptera, Vespidae, Eumeninae) from Central Asia, with the description of four new species

Alexander V. Fateryga[1], Maxim Yu. Proshchalykin[2]

1 *T.I. Vyazemsky Karadag Scientific Station – Nature Reserve of RAS – Branch of A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Nauki Str. 24, Kurortnoye, 298188 Feodosiya, Russia* **2** *Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences, 100-let Vladivostoku Ave. 159, 690022 Vladivostok, Russia*

Corresponding author: Alexander V. Fateryga (fater_84@list.ru)

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Abstract

Four new species are described: *Pseudepipona* (*Deuterepipona*) *kostylevi* Fateryga, **sp. nov.** (Turkmenistan, Uzbekistan, Kazakhstan), *P.* (*D.*) *nikolayi* Fateryga, **sp. nov.** (Kazakhstan), *P.* (*D.*) *popovi* Fateryga, **sp. nov.** (Turkmenistan), and *P.* (*D.*) *vladimiri* Fateryga, **sp. nov.** (Kazakhstan). A new synonymy is proposed: *Pseudepipona* (*Deuterepipona*) *superba* (Morawitz, 1867) = *P.* (*D.*) *tricolor* Gusenleitner, 1976, **syn. nov.** Lectotypes are designated for two species: *Pseudepipona* (*Deuterepipona*) *herzi* (Morawitz, 1895) and *P.* (*D.*) *superba*. Two species, both not occurring in the region under study, are transferred to the subgenus *Deuterepipona* from the nominotypical one: *Pseudepipona* (*Deuterepipona*) *priesneri* Gusenleitner, 1970 and *P.* (*D.*) *pseudominuta* Gusenleitner, 1971. An identification key to all six species of the subgenus *Deuterepipona* from Central Asia is provided.

Keywords

Eumenine wasps, new synonymy, Palaearctic region, solitary wasps

Introduction

Pseudepipona de Saussure, 1856 is a genus of solitary wasps in the subfamily Eumeninae with 39 hitherto described species. Most of them occur in the Palaearctic region except two Afrotropical species; one species is also found in India and another one is present in both the Palaearctic and Nearctic region (Carpenter et al. 2010; Girish Kumar et al. 2017; Kim 2020; Bai et al. 2021; Fateryga 2022). The genus *Pseudepipona* includes two subgenera: *Deuterepipona* Blüthgen, 1951 and *Pseudepipona s. str.* Seven Palaearctic species were hitherto recognized in the subgenus *Deuterepipona* (Giordani Soika 1970; van der Vecht and Fischer 1972; Fateryga et al. 2017). The bionomics of the genus were recently summarized by Fateryga (2022); those of the subgenus *Deuterepipona* are unknown. Two species of the subgenus *Deuterepipona* have been reported from Central Asia: *Pseudepipona herzi* (Morawitz, 1895), distributed in Israel, Turkmenistan, Kyrgyzstan, Kazakhstan, Mongolia, and China (Kurzenko 1977; Oehlke 2012), and *P. tricolor* Gusenleitner, 1976, distributed in Russia, Iran, and Kazakhstan (Dvořák and Castro 2007; Gusenleitner 2013).

In this paper, four new species of *Pseudepipona* (*Deuterepipona*) are described from Central Asia and one species is synonymized; lectotypes of two species are designated. Two species, both not occurring in the region under study, are transferred to the subgenus *Deuterepipona* from the nominotypical one. As a result, 42 species of the genus *Pseudepipona* are currently recognized: 12 in *Deuterepipona* and 30 in *Pseudepipona s. str.*

Materials and methods

The material for the present study was mainly from the collection of the Federal Scientific Center of the East Asia Terrestrial Biodiversity of the Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia [**FSCV**]. Type material was also studied in the Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia [**ZISP**]. Some additional type specimens were examined by photos from the collections of the Naturalis Biodiversity Center, Leiden, The Netherlands, and the Stuttgart State Museum of Natural History, Stuttgart, Germany.

Photographs of the specimens were taken with a Canon EOS 550D digital camera and a Yongnuo YN-14EX macro flash attached to an Olympus SZ60 stereomicroscope. Multifocus images were created from stacks of photographs using CombineZP software. The final illustrations were postprocessed for sharpness, contrast, and brightness using Adobe Photoshop CS2 software. Male genitalia were extracted after re-softening the specimens and were then boiled in 10% NaOH for 5 min. After that, they were rinsed in 80% ethanol and only then stored and studied in glycerin.

Species delimitation is based on the external morphology and the structure of the male genitalia. No subspecies based on different coloration are recognized within species according to Carpenter (1987) and Fateryga et al. (2021).

In morphological descriptions, the letter "F" refers to antennal flagellomeres, the letter "T" to metasomal terga, and the letter "S" to metasomal sterna.

Taxonomy

Genus *Pseudepipona* de Saussure, 1856

Pseudepipona de Saussure, 1856: 309; type species: *Odynerus herrichii* de Saussure, 1856, by monotypy.
Leptepipona Blüthgen, 1951: 194; type species: *Vespa tripunctata* Fabricius, 1787, by original designation; synonymized by van der Vecht and Fischer 1972: 82–83.
Metepipona Blüthgen, 1951: 193; type species: *Odynerus peculiaris* Morawitz, 1895, by original designation; synonymized by van der Vecht and Fischer 1972: 82–83.
Trichepipona Blüthgen, 1951: 193; type species: *Odynerus lativentris* de Saussure, 1855, by original designation; synonymized by van der Vecht and Fischer 1972: 82–83.

Diagnosis. Female clypeus with a narrow apical margin; labial palpus with four segments; male antenna hooked apically; female vertex with a single fovea; anterior face of the pronotum without distinct foveae; pretegular carina present; tegula broad, without deep large punctures, protruding posteriorly adjoining the parategula but not surpassing it; second submarginal cell of the forewing with acute basal angle; axillary fossa relatively broad, not slit-like; propodeum with a transverse carina between the dorsal and posterior surfaces; propodeal valvula mono-lamellate; propodeal orifice broadly rounded dorsally; T1 not petiolate, somewhat narrower than T2, without a transverse carina; both T1 and T2 without an apical lamella.

Subgenera and species included. Twelve species are currently recognized in the subgenus *Deuterepipona* Blüthgen, 1951 and 30 species in the subgenus *Pseudepipona s. str.*

Subgenus *Deuterepipona* Blüthgen, 1951

Deuterepipona Blüthgen, 1951: 194. Type species: *Odynerus ionius* de Saussure, 1855, by original designation.

Diagnosis. Male mandible without a notch between the basal and preapical teeth; pronotal carina usually forming obtuse or rounded angle laterally.

Species included. *Pseudepipona ankarensis* Giordani Soika, 1970 (Turkey), *P. herzi* (Morawitz, 1895) (Israel, Turkmenistan, Kyrgyzstan, Kazakhstan, Mongolia, China), *P. inexspectata* (Blüthgen, 1955) (Italy), *P. ionia* (de Saussure, 1855) (Bulgaria, Greece, Turkey, Syria), *P. kostylevi* Fateryga, sp. nov. (Turkmenistan, Uzbekistan, Kazakhstan), *P. nikolayi* Fateryga, sp. nov. (Kazakhstan), *P. niveopicta* Giordani Soika, 1970 (Russia, Turkey), *P. popovi* Fateryga, sp. nov. (Turkmenistan), *P. priesneri* Gusenleitner, 1970 (Saudi Arabia, Iran), *P. pseudominuta* Gusenleitner, 1971 (Turkey, Israel), *P. superba* (Morawitz, 1867) (Russia, ?Azerbaijan, Iran, Kazakhstan), and *P. vladimiri* Fateryga, sp. nov. (Kazakhstan).

Remarks. *Pseudepipona priesneri* and *P. pseudominuta* are transferred to the subgenus *Deuterepipona* from the nominotypical one (see below).

Key to the species of the subgenus *Deuterepipona* from Central Asia

The male of *Pseudepipona nikolayi* sp. nov. not known.

1 Females **2**

– Males **7**

2 Epicnemial carina obsolete; transverse carina of propodeum indistinct at center; S2 without longitudinal furrow at base (Fig. 5D); clypeus dull, with small and sparse shallow punctures and microsculpture (Fig. 5C); cephalic fovea weakly developed; body black with orange pattern, wings strongly fuscous ..... ***P. nikolayi* Fateryga, sp. nov.**

– Epicnemial carina distinct; transverse carina of propodeum complete; S2 with at least weakly developed longitudinal furrow at base (see Fig. 7H); clypeus with larger and deeper punctures, sometimes with shining interstices (Figs 1D, 2B, D, F, 4C, 6C, 7D, 8C, 9C); cephalic fovea variable; coloration variable **3**

3 Wings completely transparent; clypeus with coarse punctures and shining interstices, black (Fig. 4C); pronotal carina forming blunt angle laterally (Fig. 4A); cephalic fovea well developed; longitudinal furrow at base of S2 distinct; body black with whitish pattern ..... ***P. kostylevi* Fateryga, sp. nov.**

– Wings fuscous at least in marginal cell; clypeus with less coarse punctures, interstices often dull, with variable coloration (Figs 1D, 2B, D, F, 6C, 7D, 8C, 9C); pronotal carina either forming blunt angle (Figs 1A, 7A, 8A, 9A) or rounded laterally (Fig. 6A); cephalic fovea variable; longitudinal furrow at base of S2 variable; coloration variable **4**

4 Pronotal carina rounded laterally (Fig. 6A); cephalic fovea nearly indistinct; clypeus with shining interstices, black or with light central spot in distal half (Fig. 6C); longitudinal furrow at base of S2 rather weakly developed; body black with pale yellow pattern ..... ***P. popovi* Fateryga, sp. nov.**

– Pronotal carina forming blunt angle laterally (Figs 1A, 7A, 8A, 9A); cephalic fovea at least weakly developed; clypeus with rather dull interstices, with various color from entirely black or reddish to entirely yellow or yellow with black central spot in distal half but not black with light central spot in distal half (Figs 1D, 2B, D, F, 7D, 8C, 9C); longitudinal furrow at base of S2 variable; coloration variable **5**

5 Cephalic fovea weakly developed; longitudinal furrow at base of S2 weakly developed; basal part of flagellum (F1–F3) ferruginous dorsally; S2 completely yellow; S3–S6 with yellow apical bands or central spots; clypeus completely yellow (Fig. 9C) ..... ***P. vladimiri* Fateryga, sp. nov.**

– Cephalic fovea well developed; longitudinal furrow at base of S2 distinct; entire flagellum black dorsally; S2 black with light apical band; S3–S6 mostly black or just S3 with lateral spots; clypeus with variable coloration (Figs 1D, 2B, D, F, 7D, 8C) **6**

6 Body black with either yellow or orange-yellow pattern (Figs 1A, B, 2A, C, E); clypeus from black to yellow (Figs 1D, 2B, D, F) ..... ***P. herzi* (Morawitz, 1895)**

– Body black with both whitish and reddish pattern (Figs 7A, B, 8A, B); clypeus from black to reddish (Figs 7D, 8C) ..... ***P. superba* (Morawitz, 1867)**

7 Wings completely transparent; clypeus with very shallow apical emargination (Fig. 4F); F11 small, hardly reaching apical margin of F8 (Fig. 4G); median expansion of aedeagus comparatively narrow (Fig. 3B); ventral lobe of aedeagus in lateral view saddle-shaped, with distinctly emarginated ventral side (Fig. 3G); pronotal carina forming blunt angle laterally (Fig. 4E); body black with whitish and whitish-yellow pattern..... ***P. kostylevi* Fateryga, sp. nov.**

– Wings fuscous at least in marginal cell; clypeus with more deeply emarginated apical margin (Figs 1E, 6E, 7E, 9F); F11 larger, fully reaching apical margin or even middle of F8 (Figs 1H, 6H, 7I, 9G); median expansion of aedeagus variable (Fig. 3A, C–E); ventral lobe of aedeagus in lateral view variable, with not emarginated or just very slightly emarginated ventral side (Fig. 3F, H–J); pronotal carina either forming blunt angle (Figs 1F, 7F, 9E) or rounded laterally (Fig. 6F); coloration variable..... **8**

8 Pronotal carina rounded laterally (Fig. 6F); clypeus with shining interstices (Fig. 6E); F11 robust (Fig. 6H); median expansion of aedeagus broad (Fig. 3C); ventral lobe of aedeagus in lateral view rather triangle-shaped, gradually narrowing towards apex (Fig. 3H); body black with pale yellow pattern..... ***P. popovi* Fateryga, sp. nov.**

– Pronotal carina forming blunt angle laterally (Figs 1F, 7F, 9E); clypeus with rather dull interstices (Figs 1E, 7E, 9F); F11 variable but less robust (Figs 1H, 7I, 9G); median expansion of aedeagus variable (Fig. 3A, D, E); ventral lobe of aedeagus in lateral view rather trapezoidal (Fig. 3F, I, G); coloration variable..... **9**

9 F11 very slender (Fig. 9G); clypeus with acute apical teeth (Fig. 9F); median expansion of aedeagus broad (Fig. 3E); ventral lobe of aedeagus small (Fig. 3J); basal part of flagellum (F1–F2) ferruginous dorsally; S2 nearly completely yellow; S3–S6 with yellow apical bands.... ***P. vladimiri* Fateryga, sp. nov.**

– F11 less slender (Figs 1H, 7I); clypeus with rather blunt apical teeth (Figs 1E, 7E); median expansion of aedeagus comparatively narrow (Fig. 3A, D); ventral lobe of aedeagus much larger (Fig. 3F, I); entire flagellum black dorsally; S2 black with light apical band; S3–S6 mostly black or just S3 with lateral spots **10**

10 Body black with either yellow or orange-yellow pattern (Fig. 1F, G) ..... ***P. herzi* (Morawitz, 1895)**

– Body black with both whitish and reddish pattern (Fig. 7F, G) ..... ***P. superba* (Morawitz, 1867)**

***Pseudepipona herzi* (Morawitz, 1895)**
Figs 1A–H, 2A–F, 3A, F

Odynerus herzi Morawitz, 1895: 471–473, ♀ ♂; type locality "Transcaspia: Sumbar" [Turkmenistan].
Pseudepipona herzi herzi: van der Vecht & Fischer, 1972: 87.
? *Odynerus kozlovi* Kostylev, 1937: 222, ♀ ♂; type locality: in Russian "Уургин-худук" [Uurgin-khuduk] and in French "Mongolie, Alachan" [China: Inner Mongolia].
Pseudepipona herzi kozlovi: van der Vecht & Fischer, 1972: 87.
? *Deuterepipona herzi enslini* Blüthgen, 1955: 28–29, ♂; type locality: "Jericho" [Israel].
Pseudepipona herzi enslini: van der Vecht & Fischer, 1972: 86.

Lectotype (designated here). Turkmenistan: "Sumbar", 1 ♀, leg. O. Herz [ZISP] (Fig. 1A–D).

Paralectotype. Turkmenistan: "Sumbar", 1 ♂, leg. O. Herz [ZISP] (Fig. 1E–H).

Distribution. Israel, Turkmenistan, Kyrgyzstan, Kazakhstan, Mongolia, China (van der Vecht and Fischer 1972; Kurzenko 1977; Oehlke 2012).

Remarks. This species is very variable in coloration. Females from Southern Kazakhstan (Fig. 2A, B) differ from the typical form (Fig. 1A, B, D) by the absence of the spot on the dorsal mesepisternum and a completely black clypeus while females from Southeastern Kazakhstan (Fig. 2C, D) differ from the typical form by a completely yellow clypeus. Females from Eastern Kazakhstan (*P. herzi kozlovi*, Fig. 2E, F) differ from all other forms by an orange-yellow pattern instead of a pure yellow. No differences were found in the male genitalia between specimens from Turkmenistan and Southern Kazakhstan while males of the form from Eastern Kazakhstan were not examined, nor were males of *P. herzi enslini*. Both subspecies are probably conspecific with *P. herzi* but need further study.

***Pseudepipona kostylevi* Fateryga, sp. nov.**
https://zoobank.org/97E2833D-AFD1-4705-B413-905DA10D9B77
Figs 3B, G, 4A–H

Holotype. Turkmenistan: "Туркмения, Ахчакуйма, NW Казанджика" [Akhcha-Kuyma, NW Gazandjyk (currently Bereket)], 3.VI.1976, 1 ♀, leg. N.V. Kurzenko [FSCV] (Fig. 4A–D).

Paratypes. Turkmenistan: "Туркмения, Ахчакуйма, NW Казанджика" [Akhcha-Kuyma, NW Gazandjyk (currently Bereket)], 2.VI.1976, 1 ♀, leg. N.V. Kurzenko [FSCV]; ibid., 3.VI.1976, 1 ♀, leg. N.V. Kurzenko [ZISP]; "Туркмения, Ахча-Куйма, 30 км СЗ Казанджика" [Akhcha-Kuyma, 30 km NW Gazandjyk (currently Bereket)], 1.VI.1985, 1 ♀ (specimen without metasoma), leg. A.S. Lelej [FSCV]. Uzbekistan: "Узбекистан, окр. Бухары" [vicinity of Bukhara], 25.V.1972, 3 ♀ (one specimen without left wings), leg. V.L. Kazenas [FSCV]; "Узбекистан, 53 км зап. Бухары" [53

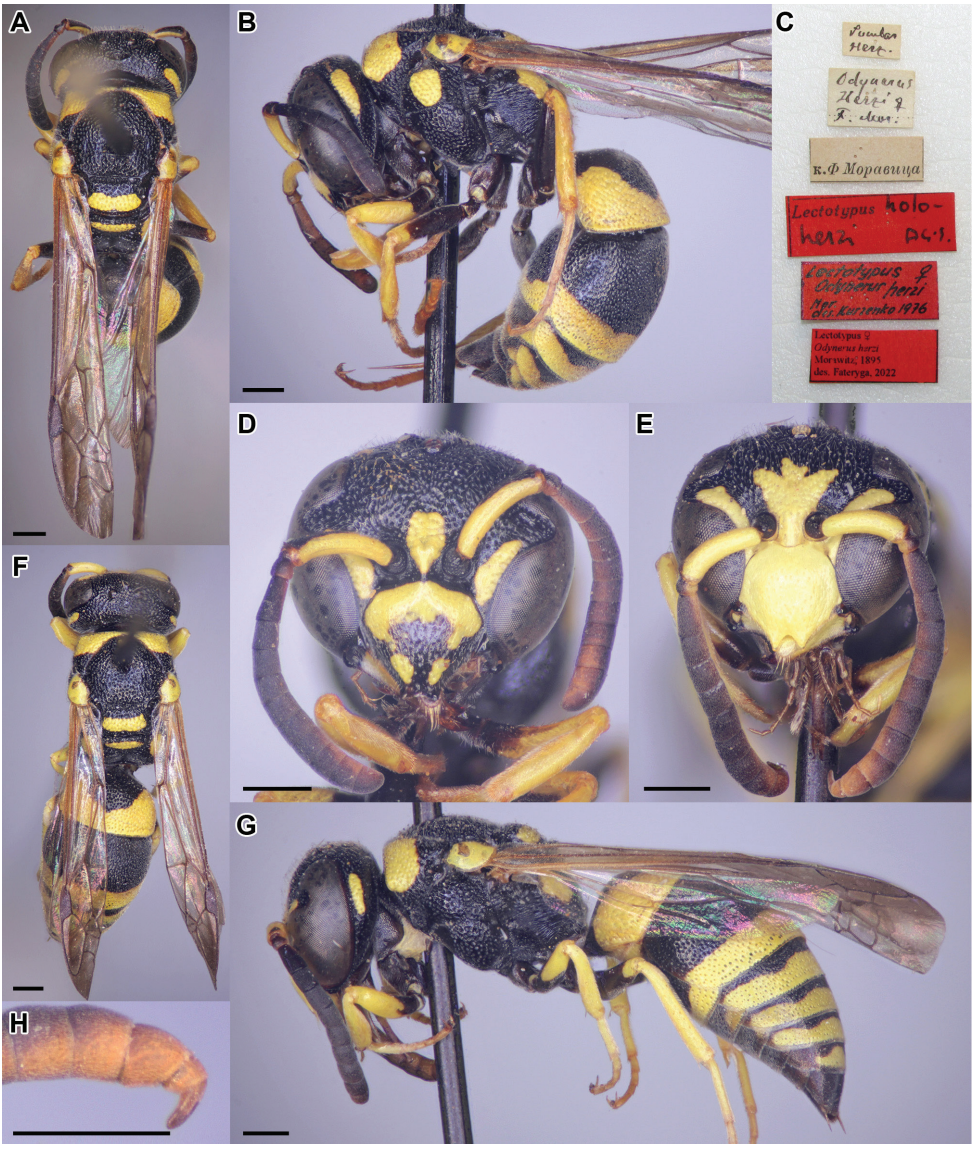

Figure 1. *Pseudepipona herzi* (Morawitz, 1895) **A–D** ♀, lectotype (Turkmenistan) **E–H** ♂, paralectotype (Turkmenistan) **A, F** habitus in dorsal view **B, G** habitus in lateral view **C** labels **D, E** head in frontal view **H** apex of antenna. Scale bars 0.5 mm.

km W Bukhara], 22.V.1973, 1 ♀, leg. V.L. Kazenas [FSCV]. Kazakhstan: Kyzylorda Province: "Казахстан, 10 км ЮЗ Чардары" [10 km SW Shardara], 19.V.1979, 1 ♀ (specimen without metasoma), leg. V.L. Kazenas [FSCV]; "120 км N Кзыл-Орды. оз. Карамолла" [120 km N Kyzylorda, Kara-Molla Lake], 23.V.1973, 2 ♂ (one specimen without metasoma), leg. N.V. Kurzenko [FSCV] (Fig. 4E–H).

Figure 2. Females of *Pseudepipona herzi* (Morawitz, 1895) **A, B** Kazakhstan (Turkestan Province) **C, D** Kazakhstan (Almaty Province) **E, F** Kazakhstan (Pavlodar Province) **A, C, E** habitus in lateral view **B, D, F** head in frontal view. Scale bars: 0.5 mm.

Diagnosis. The new species can be easily recognized among other representatives of the subgenus *Deuterepipona* by completely transparent wings, coarse punctures on clypeus, a shallow apical emargination of the clypeus in the male, a small F11 in the male, a saddle-shaped ventral lobe of the male aedeagus, and a whitish pattern (see Key).

Description. Female. Body length (from head to apical margin of tergum 2) 6.5–7.0 mm; fore wing length 6.0 mm.

Head about 1.1× as wide as long in frontal view. Clypeus as wide as long; its apical emargination very shallow, about 0.2× as deep as wide, taking 1/4 of clypeal width, apical teeth blunt. Cephalic fovea shallow but well developed, as broad as

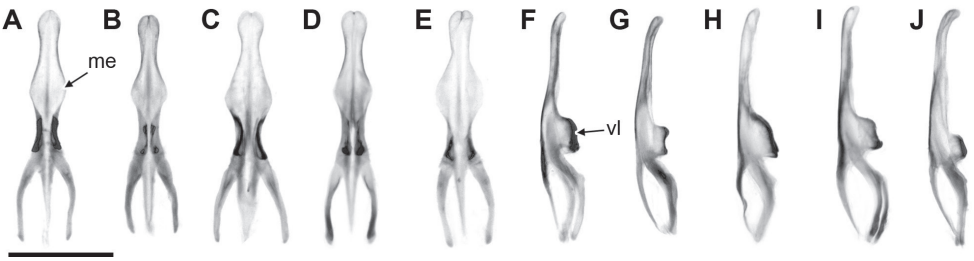

Figure 3. Aedeagi of *Pseudepipona* spp. **A, F** *P. herzi* (Morawitz, 1895) **B, G** *P. kostylevi* Fateryga, sp. nov. **C, H** *P. popovi* Fateryga, sp. nov. **D, I** *P. superba* (Morawitz, 1867) **E, J** *P. vladimiri* Fateryga, sp. nov. **A–E** dorsal view (**me** = median expansion) **F–J** lateral view (**vl** = ventral lobe). Scale bar 0.5 mm.

distance between lateral ocelli; distance between lateral ocellus and occiput 1.1× as distance between lateral ocelli. Pronotal carina well developed, forming blunt angle at anterolateral corner of pronotum. Epicnemial carina developed. Scutellum and metanotum convex. Propodeum with distinct carina between shelf and concavity, carina forming rectangularly rounded projection in lateral view. Propodeal valvula mono-lamellate, evenly rounded. T1 1.8× as wide as long in dorsal view, bluntly roundly angled in lateral view. T2 evenly convex in lateral view. S2 in lateral view rather flattened, roundly elevated at base, in ventral view with distinct longitudinal furrow at base.

Clypeus with coarse dense punctures, interstices approximately equal to puncture diameter, shining. Frons and vertex with punctures coarser than those on clypeus, interstices usually less than puncture diameter; punctures on gena slightly smaller and sparser. Pronotum dorsally with punctures similar to those on vertex; lateral part of pronotum with denser and smaller punctures and dull interstices with distinct microsculpture. Sculpture on scutum coarser than that on dorsal surface of pronotum, interstices usually less than puncture diameter. Tegula nearly smooth, with few minute punctures. Punctures on mesepisternum, scutellum, and metanotum similar in size to those on dorsal surface of pronotum, interstices usually approximately equal to puncture diameter except whitish parts where they exceed puncture diameter. Mesepimeron with coarse punctures forming longitudinal rows. Metapleuron dull, weakly longitudinally rugose. Dorsal and dorsolateral surfaces of propodeum with shallow irregular, indistinct but coarse punctures similar in size to those on metanotum. Lateral surface of propodeum longitudinally rugose, more distinctly than metapleuron, without punctures. Propodeal concavity transversally rugose. T1 and T2 with dense coarse punctures similar to those on frons and vertex, interstices usually less than puncture diameter except apical bands where punctures are smaller. T3–T5 with sparser and smaller punctures. T6 mostly with microsculpture only. Sculpture of S1 similar to that of lateral part of T1. Basal part of S2 before transverse furrow dull, with microsculpture only. Sculpture of distal part of S2 after transverse furrow and S3–S6 as that of corresponding terga but interstices larger and microsculpture more distinct.

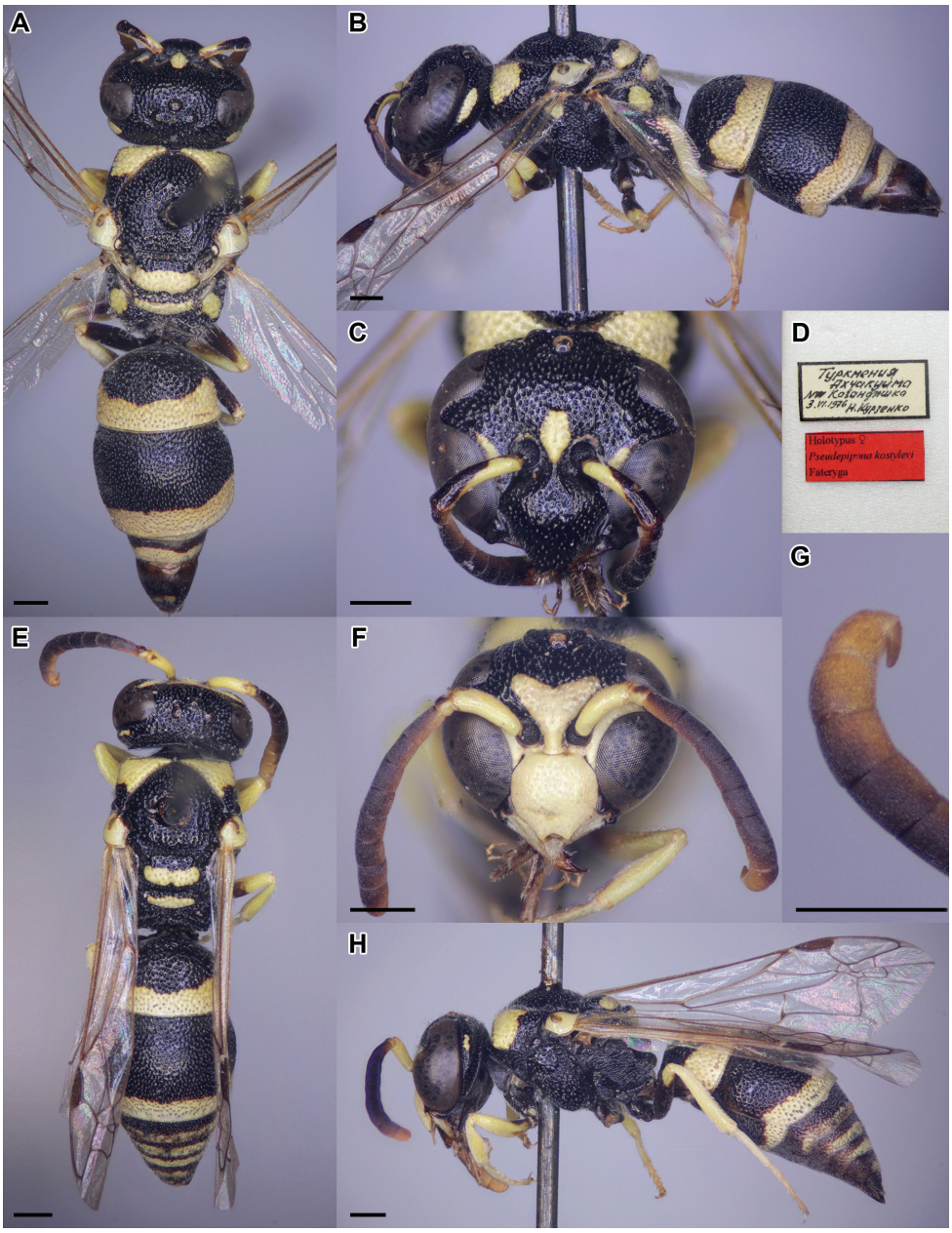

Figure 4. *Pseudepipona kostylevi* Fateryga, sp. nov. **A–D** ♀, holotype (Turkmenistan) **E–H** ♂, paratype (Kazakhstan: Kyzylorda Province) **A, E** habitus in dorsal view **B, H** habitus in lateral view **C, F** head in frontal view **D** labels **G** apex of antenna. Scale bars: 0.5 mm.

Setation weakly developed. Frons, vertex, dorsal surface of pronotum, scutum, and tarsi with sparse setae less in length than diameter of scapus at base. Posterior margin of gena with very short setae equal in length to puncture diameter on gena. Most other parts of body bare or with very minute setae.

Basal color black. The following parts whitish: spot on frons between antennal sockets, anterior and lower faces of scapus, small band along inner margin of eye from clypeus to ocular sinus, small spot on gena, large lateral spots on dorsal surface of pronotum, spot on dorsal mesepisternum, tegula and parategula, bands on scutellum and metanotum, lateral spots on propodeum, front leg from middle of femur onwards, middle leg from apex of femur onwards, hind leg from tibia onwards, apical bands on T1 and T2 enlarged laterally, apical bands on T3–T4, spot on T6, apical band on S2, apical spots laterally on S3. Ventral side of flagellum ferruginous. Wings transparent, without infuscation.

Male. Body length (from head to apical margin of T2) 5.5 mm; fore wing length 5.0 mm.

Structure as in female but clypeus with apical emargination taking about 1/3 of clypeal width. F11 rather acute, straight, and small, narrowing towards apex, hardly reaching apical margin of F8. Cuspis without the dorsal process typical of some species in the nominotypical subgenus (see Fateryga 2022). Aedeagus as in Fig. 3B, G, median expansion comparatively narrow, ventral lobe in lateral view saddle-shaped, with distinctly emarginated ventral side.

Sculpture similar to that in female but punctures on clypeus shallower. T6 and S6 punctate similarly to previous segments. T7 and S7+8 mostly with microsculpture only.

Setae as in female.

Coloration mostly as in female but mandible, labrum, and clypeus whitish-yellow, spot on frons and band along inner margin of eye larger, entire scapus and ventral side of pedicel whitish-yellow, all legs whitish-yellow from femur; whitish spots on dorsal mesepisternum and propodeum reduced. Entire F10 and F11 ferruginous. T7 and S7+8 black.

Etymology. The new species is named after the Soviet entomologist Georg Kostylev, also known as Yuriy A. Kostylev (1889–1942), in recognition of his great contribution to the systematics of Central Asian Vespidae.

Distribution. Turkmenistan, Uzbekistan, Kazakhstan (Kyzylorda Province).

***Pseudepipona nikolayi* Fateryga, sp. nov.**

https://zoobank.org/2803327D-95C6-49E7-A085-70A87A422D97

Fig. 5A–E

Holotype. Kazakhstan. Karaganda Province: "Бетпак-Дала, ср. теч. р. Сары-Су" [Betpak-Dala, middle reaches of Sary-Su River], 19.V.1973, 1 ♀, leg. N.V. Kurzenko [FSCV] (Fig. 5A–E).

Diagnosis. The new species can be easily recognized among other representatives of the subgenus *Deuterepipona* by an obsolete epicnemial carina, the transverse carina of the propodeum indistinct at center, the absence of a longitudinal furrow at the base of S2, a fine punctation of the clypeus with a distinct microsculpture, and an orange pattern (see Key).

Description. Female. Body length (from head to apical margin of tergum 2) 6.0 mm; fore wing length 5.5 mm.

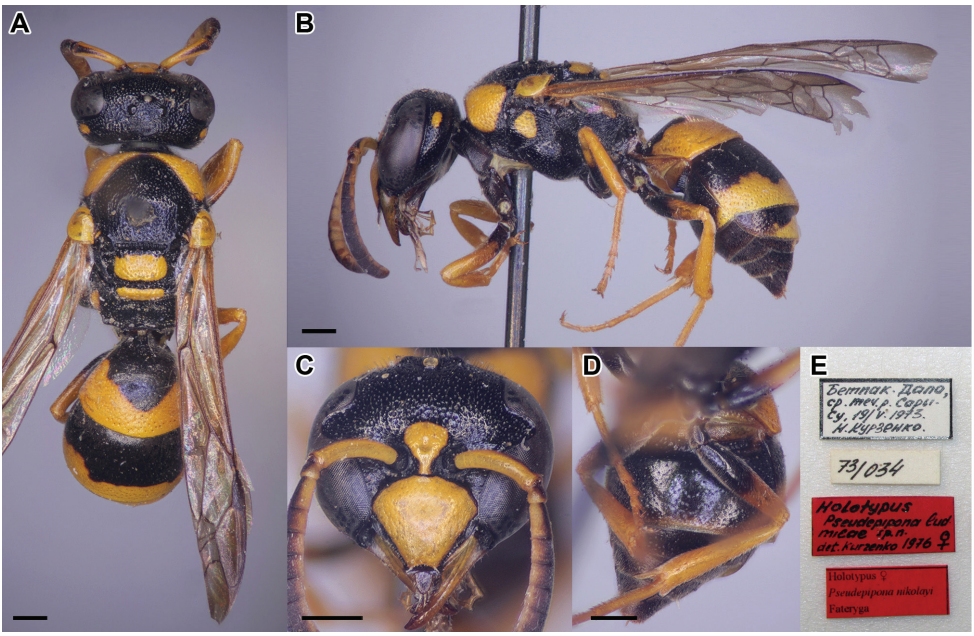

Figure 5. *Pseudepipona nikolayi* Fateryga, sp. nov., ♀, holotype **A** habitus in dorsal view **B** habitus in lateral view **C** head in frontal view **D** metasoma in ventral view **E** labels. Scale bars 0.5 mm.

Head about 1.1× as wide as long in frontal view. Clypeus about 1.1× as wide as long in frontal view, its apical margin nearly truncated, taking nearly 1/3 of clypeal width, apical teeth blunt. Cephalic fovea shallow and weakly developed, less broad than distance between lateral ocelli; distance between lateral ocellus and occiput nearly 1.3× as distance between lateral ocelli. Pronotal carina forming small blunt angle at anterolateral corner of pronotum. Epicnemial carina obsolete. Scutellum and metanotum convex. Propodeum with carina between shelf and concavity indistinct at center; laterally this carina forming acute projection. Propodeal valvula mono-lamellate, rounded apically. T1 2.0× as wide as long in dorsal view, rather evenly rounded in lateral view. T2 evenly convex in lateral view. S2 in lateral view convex, evenly rounded, in ventral view without longitudinal furrow at base.

Clypeus dull, with small and sparse shallow punctures, interstices significantly exceeding puncture diameter, with microsculpture. Frons and vertex with deep dense punctures, interstices reaching puncture diameter; punctures on gena slightly smaller and sparser. Pronotum dorsally with punctures similar to those on gena; lateral part of pronotum with sparse shallow punctures and dull interstices with distinct microsculpture. Sculpture on scutum coarser than that on dorsal surface of pronotum, similar to that on frons and vertex or somewhat sparser, interstices with evident micropunctures. Tegula nearly smooth, with few minute punctures. Dorsal mesepisternum dull, sparsely punctate and longitudinally rugose. Ventral mesepisternum, scutellum, and metanotum with sparse punctures; interstices shining, reaching several puncture diameters.

Mesepimeron, metapleuron, and lateral surface of propodeum longitudinally rugose, dull. Dorsal and dorsolateral surfaces of propodeum and propodeal concavity dull, indistinctly transversally rugose. T1–T5 with sparse small punctures similar to those on clypeus but much deeper, interstices reaching several puncture diameters, with distinct microsculpture. T6 mostly with microsculpture only. S1 with dense coarse punctures, interstices less than puncture diameter. Basal part of S2 before transverse furrow dull, with microsculpture only. Sculpture of distal part of S2 after transverse furrow and S3–S6 mostly as that of corresponding terga.

Frons and vertex with somewhat hooked or wavy setae reaching in length diameter of scapus at apex. Dorsal surface of pronotum, propleuron, and legs with shorter and mostly straight setae. Posterior margin of gena with very short setae reaching in length diameter of first labial palpomere at base. Most other parts of body bare or with very minute setae.

Basal color black. The following parts orange: clypeus, spot on frons between antennal sockets, anterior and lower faces of scapus, small spot on gena, most part of pronotum, spot on dorsal mesepisternum, tegula and parategula, bands on scutellum and metanotum, small lateral spots on propodeum, legs from middle of femur onwards, apical bands on T1 and T2 enlarged laterally, apical band on T3, apical spot at center of T4 and T5, apical spots laterally on S2. Mandible and ventral side of pedicel and flagellum ferruginous. Wings strongly fuscous.

Male. Unknown.

Etymology. The new species is named after the Soviet and Russian entomologist Nikolay V. Kurzenko, the collector of the holotype, in recognition of his great contribution to the systematics of the eumenine wasps of the USSR; this species was recognized by him but not described.

Distribution. Kazakhstan (Karaganda Province).

***Pseudepipona popovi* Fateryga, sp. nov.**

https://zoobank.org/D09BFAEA-ED17-4605-850F-BD0B0E30248D

Figs 3C, H, 6A–H

Holotype. Turkmenistan: "Туркмения, Бадхызский запов. Кызыл-жар" [Badhyz Nature Reserve, Kyzyl-Zhar], 16.V.1976, 1 ♀, leg. N.V. Kurzenko [FSCV] (Fig. 6A–D).

Paratypes. Turkmenistan: "Туркмения, Бадхызский запов. Кызыл-жар" [Badhyz Nature Reserve, Kyzyl-Zhar], 16.V.1976, 1 ♂, leg. N.V. Kurzenko [FSCV] (Fig. 6E–H); ibid., 17.V.1976, 2 ♀, leg. N.V. Kurzenko [FSCV, ZISP].

Diagnosis. The new species can be easily recognized among other representatives of the subgenus *Deuterepipona* by the pronotal carina rounded laterally, a nearly indistinct cephalic fovea in the female, a robust F11 in the male, and a triangle-shaped ventral lobe of the male aedeagus (see Key).

Description. Female. Body length (from head to apical margin of tergum 2) 6.0–7.0 mm; fore wing length 5.5–6.0 mm.

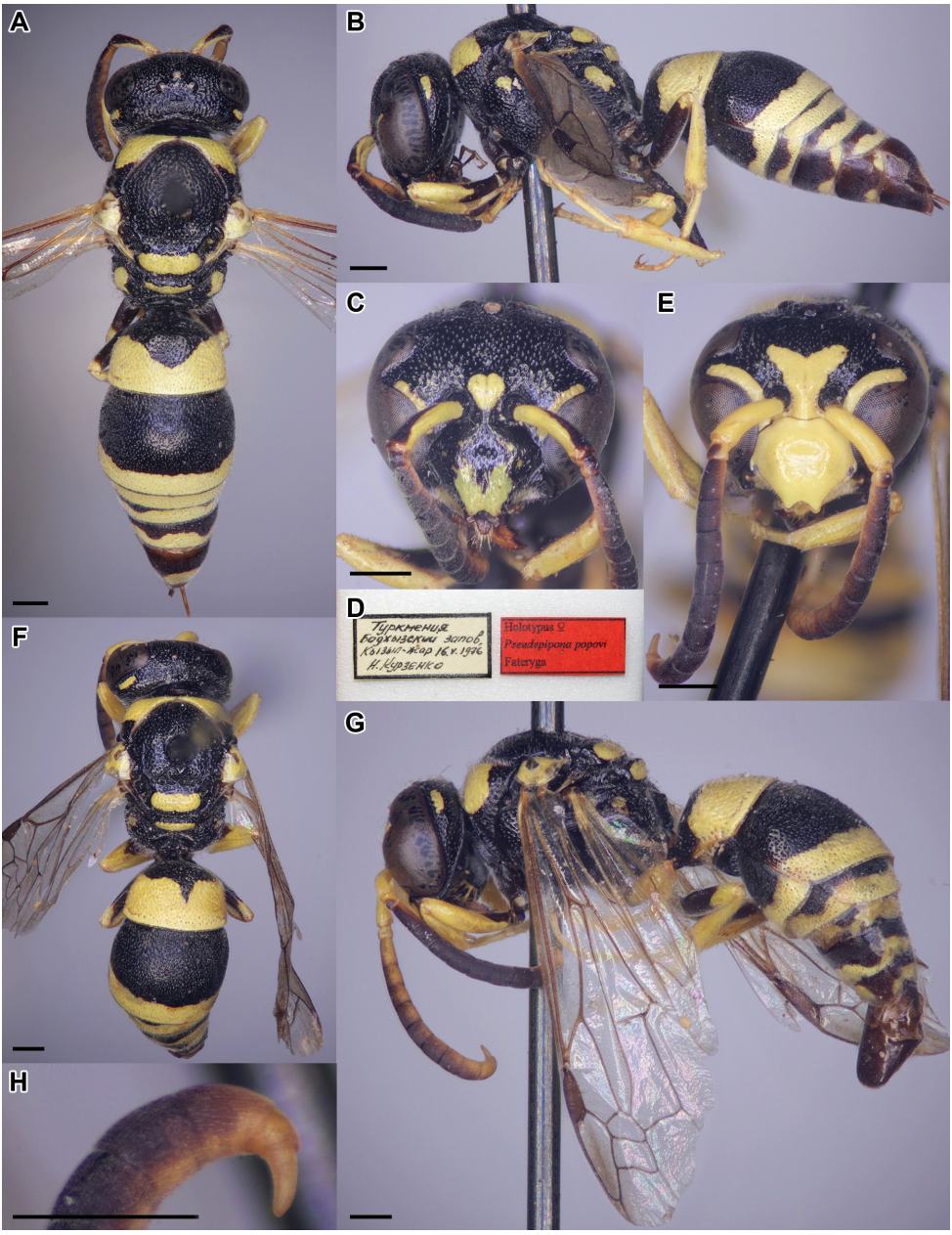

Figure 6. *Pseudepipona popovi* Fateryga, sp. nov. **A–D** ♀, holotype **E–H** ♂, paratype **A, F** habitus in dorsal view **B, G** habitus in lateral view **C, E** head in frontal view **D** labels **H** apex of antenna. Scale bars: 0.5 mm.

Head about 1.1× as wide as long in frontal view. Clypeus as wide as long; its apical emargination shallow, about 0.2× as deep as wide, taking 1/4 of clypeal width, apical teeth blunt. Cephalic fovea nearly indistinct; distance between lateral ocellus

and occiput 1.2× as distance between lateral ocelli. Pronotal carina weakly developed, pronotum rounded at anterolateral corner. Epicnemial carina developed. Scutellum convex, slightly impressed at center; metanotum convex. Propodeum with distinct carina between shelf and concavity, carina forming rectangularly rounded projection in lateral view. Propodeal valvula mono-lamellate, evenly rounded. T1 2.0× as wide as long in dorsal view, bluntly roundly angled in lateral view. T2 evenly convex in lateral view. S2 in lateral view rather flattened, roundly elevated at base, in ventral view with weakly developed longitudinal furrow at base.

Clypeus with sparse fine punctures, interstices exceeding puncture diameter, shining. Frons and vertex with punctures much denser and coarser than those on clypeus, interstices reaching puncture diameter; punctures on gena slightly smaller and sparser. Pronotum dorsally with punctures similar to those on gena; lateral part of pronotum rather longitudinally wrinkled, with interstices dull due to microsculpture. Sculpture on scutum coarser than that on frons and vertex, interstices reaching puncture diameter; punctures sometimes form longitudinal rows, especially posteriorly. Tegula nearly smooth, with few minute punctures. Punctures on mesepisternum similar in size and density to those on scutum. Punctures on scutellum similar in size to those on dorsal surface of pronotum but interstices larger, reach several puncture diameters, shining; metanotum with similar punctures in proximal half and nearly smooth distally. Mesepimeron with coarse punctures similar in size to those on mesepisternum but interstices narrower, sharp. Metapleuron longitudinally rugose, with microsculpture but slightly shining. Dorsal and dorsolateral surfaces of propodeum with shallow irregular, indistinct but coarse punctures. Lateral surface of propodeum longitudinally rugose, dull. Propodeal concavity transversally rugose. T1 and T2 with deep sparse punctures, larger on black parts and smaller on pale yellow parts, interstices reaching several puncture diameter, with distinct microsculpture. T3–T5 with somewhat sparser and smaller punctures. T6 mostly with microsculpture only. Sculpture of S1 similar to that of lateral part of T1. Basal part of S2 before transverse furrow dull, with microsculpture only. Sculpture of distal part of S2 after transverse furrow similar to that of T2 but interstices larger and more shining. Sculpture of S3–S6 as that of corresponding terga but interstices larger and microsculpture more distinct.

Frons and vertex with sparse pale setae equal in length to diameter of scapus at apex. Mesosoma dorsally with setae equal in length to approximately 2/3 of those on frons. Posterior margin of gena, tarsi, S1 and S2 with setae approximately two times shorter than those on dorsal mesosoma. Most other parts of body bare or with very short appressed setae.

Basal color black. The following parts pale yellow: distal part of clypeus (but clypeus entirely black in one specimen), spot on frons between antennal sockets, anterior and lower faces of scapus, small band along inner margins of eye from clypeus to ocular sinus, small spot on gena, most part of dorsal surface of pronotum, spot on dorsal mesepisternum, tegula and sometimes parategula (black in two specimens), bands on scutellum and metanotum, spot on dorsolateral surface of propodeum, front leg from middle of femur onwards, middle leg from apex of femur onwards, hind leg from tibia

onwards, apical band on T1 enlarged laterally, apical bands on T2–T4, spot on T6, apical bands on S1–S4, apical spot on S5. Ventral side of pedicel and flagellum ferruginous. Wings slightly but evidently fuscous, particularly in marginal cell.

Male. Body length (from head to apical margin of T2) 6.0 mm; fore wing length 5.5 mm.

Structure as in female but clypeus 1.2× as wide as long, with apical emargination 0.3× as deep as wide, taking distinctly more than 1/4 of clypeal width. F11 robust, slightly curved, and rather long, slightly narrowing towards apex, fully reaching apical margin of F8. Cuspis without the dorsal process typical of some species in the nominotypical subgenus (see Fateryga 2022). Aedeagus as in Fig. 6C, H, median expansion broad, ventral lobe in lateral view triangle-shaped, gradually narrowing towards apex.

Sculpture similar to that in female but punctures on clypeus finer and sparser. T6 and S6 punctate similarly to previous segments. T7 and S7+8 mostly with microsculpture only.

Setae as in female.

Coloration mostly as in female but mandible, labrum, clypeus, entire scapus and ventral side of pedicel pale yellow, spot on frons and band along inner margin of eye larger, all legs pale yellow from femur; spots on dorsal mesepisternum and propodeum reduced. Entire F10 and F11 ferruginous. T7 with pale yellow spot, S7+8 black.

Etymology. The new species is named after the Soviet entomologist Vladimir B. Popov (1902–1960), a corresponding member of the Academy of Sciences of the USSR, in recognition of his great contribution to the knowledge of Central Asian Hymenoptera.

Distribution. Turkmenistan.

Pseudepipona superba (Morawitz, 1867)

Figs 3D, I, 7A–I, 8A–D

Odynerus superbus Morawitz, 1867: 121–122, ♀ ♂; type locality: "Gouvernement von Saratow" [Russia].

Odynerus hyalinipennis André, 1884: 745–746, ♀; type locality: "Sarepta" [Russia: Volgograd Province]; synonymized by Blüthgen, 1942: 65.

Pseudepipona superba: Blüthgen, 1942: 65.

Pseudepipona tricolor Gusenleitner, 1976: 115–116, ♀ ♂; type locality: "Daghestan, Cmapomepera" [Russia: Dagestan; "Cmapomepera" is a misread word "Staroterechnoye" actually written in Cyrillic as "Старотеречное"], syn. nov.

Lectotype (designated here). Russia. Volgograd Province: "Sarepta", 1 ♀ [ZISP] (Fig. 7A–D).

Distribution. Russia, ?Azerbaijan, Iran, Kazakhstan (Dvořák and Castro 2007; Gusenleitner 2013; Fateryga et al. 2017).

Remarks. Examination of the photos of the holotype (Fig. 8A–D) and the paratype of *P. tricolor* from the Naturalis Biodiversity Center in Leiden revealed no significant

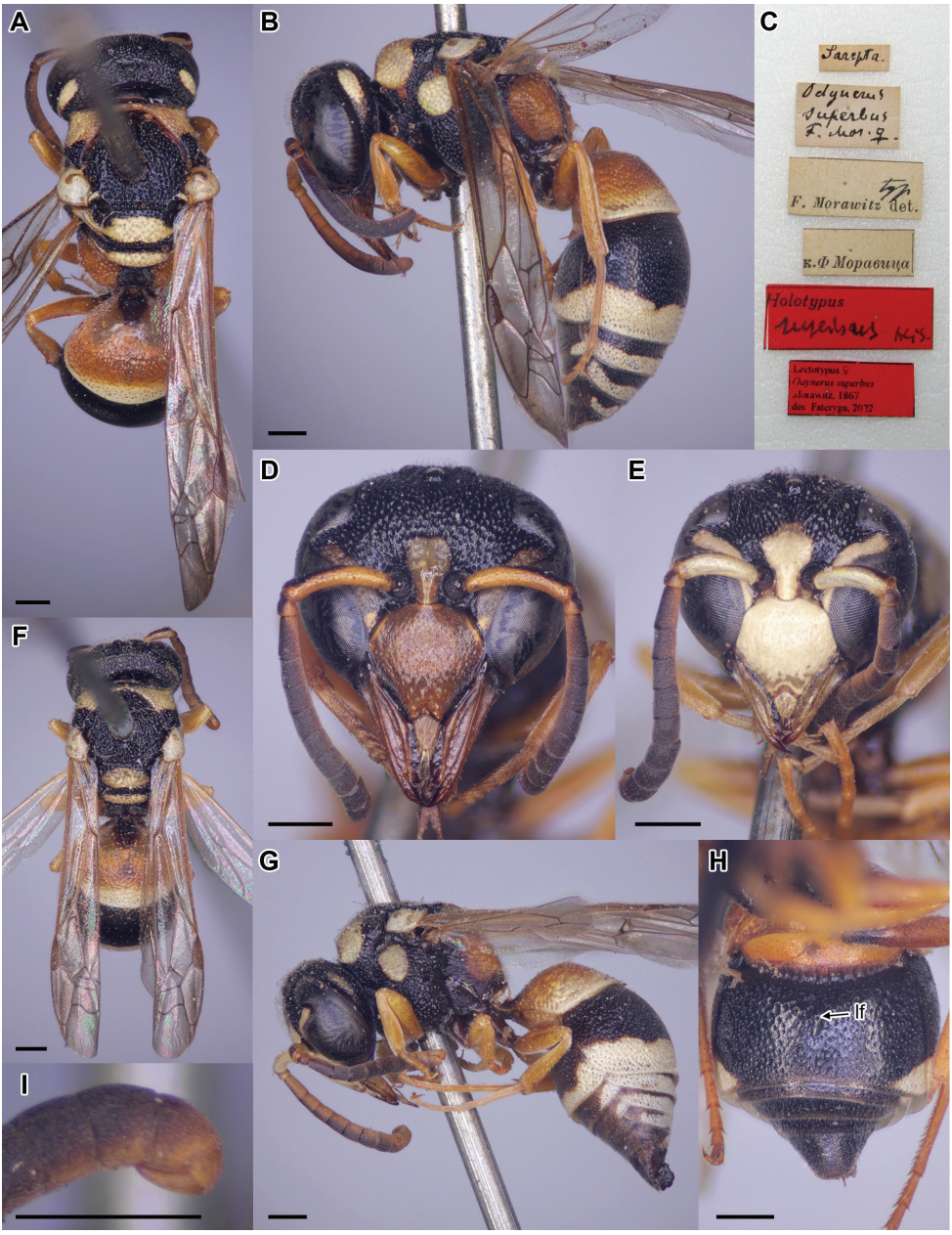

Figure 7. *Pseudepipona superba* (Morawitz, 1867) **A–D** ♀, lectotype (Russia: Volgograd Province) **E–G, I** ♂ (Russia: Crimea) **H** ♀ (Russia: Volgograd Province) **A, F** habitus in dorsal view **B, G** habitus in lateral view **C** labels **D, E** head in frontal view **H** metasoma in ventral view (**lf** = longitudinal furrow) **I** apex of antenna. Scale bars: 0.5 mm.

differences between this species and *P. superba* besides the coloration. Although the lectotype of *P. superba* has a reddish clypeus (Fig. 7D), the clypeus of all other specimens examined (including the ones from the type locality) is either entirely black or with

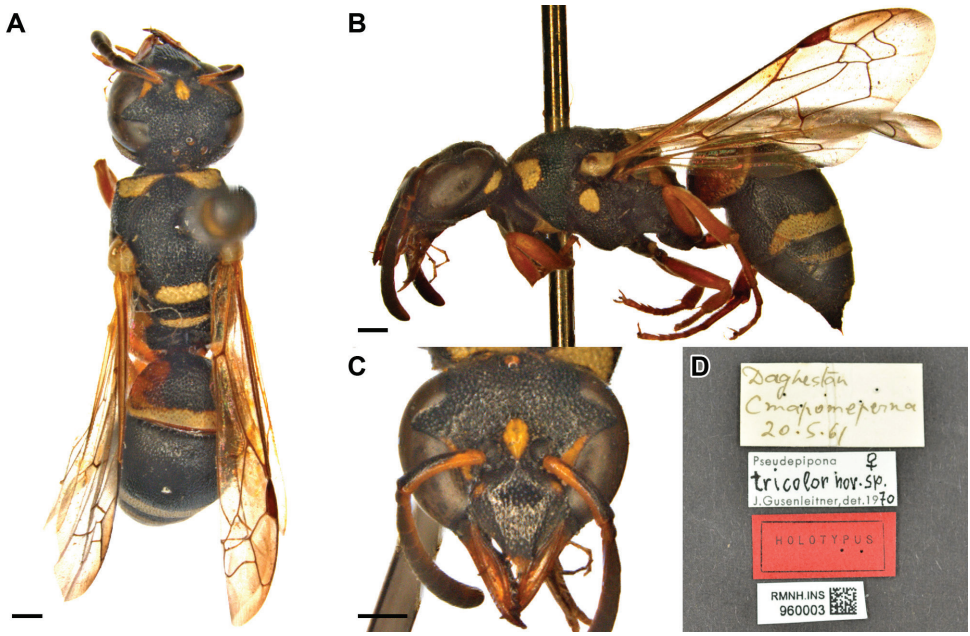

Figure 8. *Pseudepipona superba* (Morawitz, 1867), ♀, holotype of *P. tricolor* Gusenleitner, 1976 (Russia: Dagestan) **A** habitus in dorsal view **B** habitus in lateral view **C** head in frontal view **D** labels. Scale bars 0.5 mm. Photos by Frederique Bakker.

basal reddish spots laterally as corresponds to *P. tricolor*. *Pseudepipona superba* is closely related to *P. herzi* and these two species are distributed allopatrically. The differences between them are mainly in the coloration.

***Pseudepipona vladimiri* Fateryga, sp. nov.**
https://zoobank.org/C50B9D36-D1BB-4DD3-A79E-FB0CB3D8081D
Figs 3E, J, 9A–H

Holotype. Kazakhstan. Karaganda Province: "Казахстан, окр. г. Балхаш" [vicinity of Balkhash], 2.VI.1974, 1 ♀, leg. V.L. Kazenas [FSCV] (Fig. 9A–D).

Paratypes. Kazakhstan. Karaganda Province: "Казахстан, окр. г. Балхаш" [vicinity of Balkhash], 2.VI.1974, 5 ♂, leg. V.L. Kazenas [4 ♂ FSCV, 1 ♂ ZISP] (Fig. 9E–H).

Diagnosis. The new species can be easily recognized among other representatives of the subgenus *Deuterepipona* by the ferruginous coloration of the basal part of the flagellum dorsally, a very extensive yellow pattern of the body, acute apical teeth of the clypeus in the male, a long and slender F11 in the male, and a small ventral lobe of the male aedeagus (see Key).

Description. Female. Body length (from head to apical margin of tergum 2) 6.0 mm; fore wing length 5.5 mm.

Head about 1.1× as wide as long in frontal view. Clypeus as wide as long; its apical emargination shallow, about 0.2× as deep as wide, taking somewhat less than 1/4 of clypeal width, apical teeth rather rectangular. Cephalic fovea shallow and weakly developed, as broad as distance between lateral ocelli; distance between lateral ocellus and occiput 1.4× as distance between lateral ocelli. Pronotal carina well developed, forming blunt angle at anterolateral corner of pronotum. Epicnemial carina developed. Scutellum and metanotum convex. Propodeum with distinct carina between shelf and concavity, carina forming rectangularly rounded projection in lateral view. Propodeal valvula mono-lamellate, rounded apically. T1 1.5× as wide as long in dorsal view, bluntly roundly angled in lateral view. T2 evenly convex in lateral view. S2 in lateral view rather flattened, slightly roundly elevated at base, in ventral view with weakly developed longitudinal furrow at base.

Clypeus with sparse punctures sometimes forming longitudinal rows, interstices exceeding puncture diameter, rather dull. Frons and vertex with punctures coarser than those on clypeus, interstices reaching puncture diameter; punctures on gena slightly smaller and sparser. Pronotum dorsally with punctures similar to those on frons and vertex; lateral part of pronotum with dense indistinct sculpture, dull. Sculpture on scutum coarser than that on dorsal surface of pronotum, interstices usually less than puncture diameter. Tegula nearly smooth, with few minute punctures. Punctures on dorsal mesepisternum, scutellum, and metanotum similar in size to those on dorsal surface of pronotum, interstices usually approximately equal to puncture diameter. Ventral mesepisternum punctate rather similarly to scutum. Mesepimeron with coarse punctures forming longitudinal rows. Metapleuron and lateral portion of propodeum dull, longitudinally rugose. Dorsal and dorsolateral surfaces of propodeum with shallow irregular and coarse reticulate sculpture, interstices much less than puncture diameter. Propodeal concavity transversally rugose. T1 and T2 with dense coarse punctures, interstices reaching puncture diameter, with distinct microsculpture; punctures become smaller and sparser towards apical parts of terga. T3–T5 with sparser and smaller punctures. T6 mostly with microsculpture only. S1 with shallow irregular and coarse reticulate sculpture. Basal part of S2 before transverse furrow dull, with microsculpture only. Sculpture of distal part of S2 after transverse furrow and S3–S6 as that of corresponding terga.

Setation weakly developed. Frons and posterior margin of gena with sparse setae reaching in length 1/2 diameter of scapus at base. Most other parts of body bare or with very minute setae.

Black with extensive yellow pattern: clypeus, large spot on frons, entire scapus, band along inner margin of eye from clypeus to ocular sinus, large spot on gena, nearly entire pronotum, large spot on dorsal mesepisternum, spot on ventral mesepisternum, tegula and parategula, nearly entire scutellum and metanotum, lateral spots on propodeum, all legs, nearly entire T1–T6 except basal black areas, entire S1 and S2, nearly entire S3–S6 except basal black areas. Mandible, labrum, ventral side of flagellum, entire pedicel, and entire F1–F3 ferruginous. Wings fuscous, particularly in marginal cell.

Male. Body length (from head to apical margin of T2) 5.0–6.0 mm; fore wing length 5.0–5.5 mm.

Structure as in female but clypeus with apical emargination 0.5× as deep as wide, taking more than 1/4 of clypeal width, apical teeth acute. F11 very slender, slightly

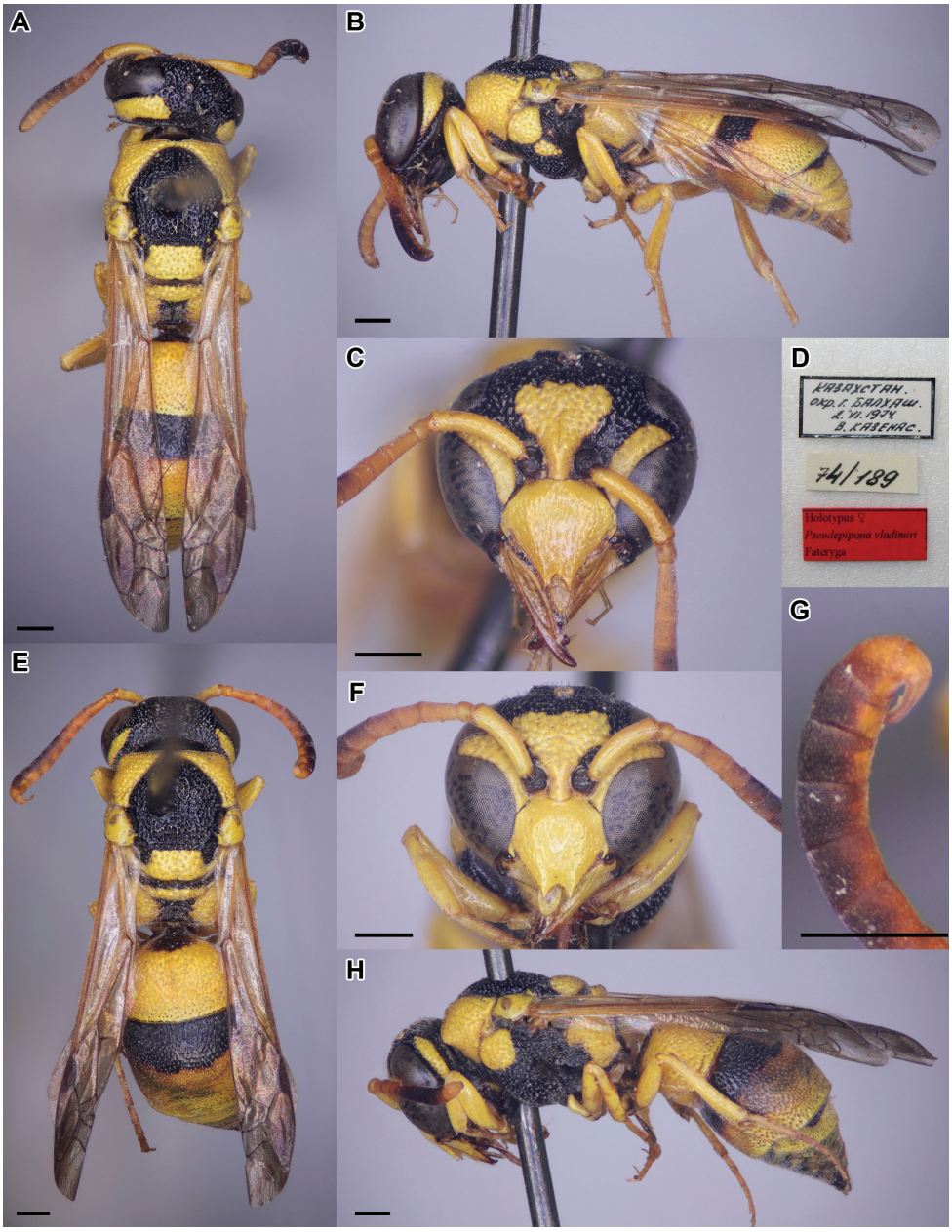

Figure 9. *Pseudepipona vladimiri* Fateryga, sp. nov. **A–D** ♀, holotype **E–H** ♂, paratype **A, E** habitus in dorsal view **B, H** habitus in lateral view **C, F** head in frontal view **D** labels **G** apex of antenna. Scale bars: 0.5 mm.

curved, and long, narrowing towards apex, reaching middle of F8. Cuspis without the dorsal process typical of some species in the nominotypical subgenus (see Fateryga 2022). Aedeagus as in Fig. 3E, J, median expansion broad, ventral lobe in lateral view trapezoidal and comparatively small.

Sculpture similar to that in female but punctures on clypeus not forming longitudinal rows. T6 and S6 punctate similarly to previous segments. T7 and S7+8 mostly with microsculpture only.

Setae as in female.

Coloration mostly as in female but mandible and labrum yellow. Spot on ventral mesepisternum reduced. Entire F10 and F11 ferruginous but F3 darkened dorsally. T7 mostly yellow; S7+8 black.

Etymology. The new species is named after the Soviet and Kazakh entomologist Vladimir L. Kazenas, the collector of the type series.

Distribution. Kazakhstan (Karaganda Province).

Remarks on two extralimital species

Pseudepipona priesneri Gusenleitner, 1970

Pseudepipona priesneri Gusenleitner *in* Blüthgen and Gusenleitner 1970: 5, 10–11, ♀♂; type locality: "Jarrahi Ufergebiet, 18 km nordöstl. Shadegan, Khuzistan" [Iran].

Distribution. Saudi Arabia, Iran (Blüthgen and Gusenleitner 1970).

Remarks. This species was described without indication of the subgenus and thus was then placed in the nominotypical one by default. However, *P. priesneri* fits the diagnosis of the subgenus *Deuterepipona*. Particularly, the male mandible is without a notch between the basal and preapical teeth (Blüthgen and Gusenleitner 1970). Photos of a female and a male paratypes of this species from Iran (Stuttgart State Museum of Natural History, Stuttgart, Germany) were examined to confirm this. It is also of note that *P. priesneri* is very closely related (or may be even conspecific) to *P. herzi*; the differences between them are only in the coloration. Therefore, a further study of the specimens (not photos) is necessary to confirm the taxonomic independence of *P. priesneri*.

Pseudepipona pseudominuta Gusenleitner, 1971

Pseudepipona pseudominuta Gusenleitner *in* Bytinski-Salz and Gusenleitner 1971: 295, ♀; type locality: "Israel, Jericho".

Distribution. Turkey, Israel (Gusenleitner 2013).

Remarks. *Pseudepipona pseudominuta* was also described without indication of the subgenus and thus was then placed in the nominotypical one by default. However, this species is "very similar to *P. niveopicta*" (Bytinski-Salz and Gusenleitner 1971) and the latter taxon has been already transferred to the subgenus *Deuterepipona* by Fateryga et al. (2017). A female and a male of *P. pseudominuta* from Turkey (FSCV) were examined to confirm that it fits the diagnosis of the subgenus *Deuterepipona*. Particularly, the male mandible is without a notch between the basal and preapical teeth.

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Yulia V. Astafurova (Saint Petersburg, Russia) loaned to us the specimens from ZISP. Leopoldo Castro (Teruel, Spain) provided some comparative photos from his collection. Frederique Bakker (Leiden, The Netherlands) kindly provided photos of the type material of *P. tricolor*. Tanja Schweizer (Stuttgart, Germany) kindly provided photos of the type material of *P. priesneri*. Ting-Jing Li (Chongqing, China), James M. Carpenter (New York, USA), and especially Leopoldo Castro provided helpful suggestions to improve the first version of this paper.

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SHORT COMMUNICATION

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Nesting of the keyhole wasp *Pachodynerus nasidens* (Latreille, 1812) (Vespidae, Eumeninae) in a nest of a paper wasp (Vespidae, Polistinae)

Gabriel de Castro Jacques[1], Wellington Donizet Ferreira[2,3], Paola Aparecida Moura[4], Gabriel Teofilo-Guedes[5], Marcos Magalhães de Souza[4]

1 *Instituto Federal de Educação, Ciência e Tecnologia de Minas Gerais - IFMG, Campus Bambuí, Varginha Farm, Bambuí/Medeiros Highway - Km 05, Bambuí, Minas Gerais, Brazil* **2** *Laboratório de Sistemática e Biologia de Insetos, Departamento de Biologia, Universidade Federal de Lavras - UFLA, Lavras, Minas Gerais, Brazil* **3** *Universidade do Estado de Minas Gerais - UEMG Campus Divinópolis, Divinópolis, Minas Gerais, Brazil* **4** *Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas - IFSULDEMINAS, Campus Inconfidentes, Tiradentes Square, 416, Inconfidentes, Minas Gerais, Brazil* **5** *Instituto de Geociências, Universidade Estadual de Campinas - UNICAMP, Zeferino Vaz University City, Barão Geraldo, Campinas, São Paulo, Brazil*

Corresponding author: Gabriel de Castro Jacques (gabriel.jacques@ifmg.edu.br)

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Abstract

Potter wasps (Hymenoptera, Vespidae, Eumeninae) adopt different substrates for nesting, including other wasp nests. Nevertheless, such behavior rarely occurs with abandoned nests of the paper wasps (Hymenoptera, Vespidae, Polistinae). In this study, we report the occurrence involving the nesting of a potter wasp on a paper wasp's nest. Such a record occurred in November 2021 in a segment of a deciduous forest, at Mata Seca State Park, Southeast Brazil. An abandoned Polistinae nest was found, with 14 cells sealed with mud, from which four male *Pachodynerus nasidens* individuals emerged. This record of *P. nasidens* reusing a Polistinae's nest increases our knowledge of Eumeninae nesting strategies and on possible associations between different groups of vespid wasps.

Keywords

Neotropical wasps, nest abandonment, nesting strategy, social wasp

Introduction

The Vespidae family includes around 5300 species (Piekarski et al. 2018), which present diverse nesting and social behaviors (Iwata 1976; Cowan 1991). Among Neotropical vespids, two taxa stand out in this regard: Eumeninae, with 3404 species (Piekarski et al. 2018), usually builds their nests with mud and are commonly classified as solitary wasps (Hermes et al. 2014); and Polistinae, with 1003 species (Piekarski et al. 2018), uses macerated cellulose for nesting and presents eusocial behavior (Somavilla and Carpenter 2021). Several ecosystem services are attributed to these insects, including floral visiting (Pires et al. 2022) and biological control of agricultural pests (Jacques et al. 2020), which demonstrates their ecological and economic importance (Brock et al. 2021).

Records of other insects reusing nests of Polistinae wasps are scarce (Bakar et al. 2015). For example, Pinto (2005), recorded solitary bee *Tetrapedia diversipes* Klug, 1810 reusing nests of *Polistes*, affirming that nests without an envelope were more propitious for reuse. Rau (1928) also reported the reuse of *Polistes* nests by species of Apoidea: *Trypoxylon* Latreille, 1796 and *Osmia* Panzer, 1806. There are also reports of the use of abandoned Polistinae nests by solitary wasps of the subfamily Eumeninae (Rau, 1944), who recorded the reuse of old Polistes nests by *Euodynerus foraminatus* (de Saussure, 1853) and *Paracinstrocerus fulvipes* (de Saussure 1856). Here, we add information to this ecological condition, with a report of the occurrence of the species of Eumeninae *Pachodynerus nasidens* (Latreille, 1812) reusing a Polistinae nest, recorded in a Neotropical seasonal forest, in Southeast Brazil.

Methods

We made this observation on November 30th, 2021, at the beginning of the rainy season, in the Mata Seca State Park, an Integral Protection Conservation Unit, situated in the municipalities of Manga and Itacarambi, the northern part of the State of Minas Gerais, Brazil (14°52'0"S, 43°59'58"W). This region houses deciduous forest remnants, the phytophysiognomy of the Atlantic Rainforest Domain. More than 50% of arboreous species are deciduous, characterized by the intensive loss of leaves in response to the two defined seasons of the year: rainy (spring/summer) and dry (fall/winter) (Belém et al. 2021).

We carried out the photographic record in the field, with a digital camera Nikon 60x Optical Zoom Wide. The nest was collected and stored in a glass recipient topped with a net, allowing gas exchange with the external environment and avoiding escape by the insects which would emerge from the occupied cells. Nest observations were performed daily until the imagoes emerged, at the Zoology Laboratory, in the Federal Institute of Education, Science, and Technology of South Minas Gerais – IFSULDEMINAS, Campus Inconfidentes.

To identify the genus of Polistinae that would have produced the nest, we adopted the dichotomous key of Barbosa et al. (2021) and compared the nest with others stored

in the IFSULDEMINAS entomological collection. The nest length and diameter were measured, in addition to the dimensions of the collected specimens, using a pachymeter, 0.2 mm precise. The number and relative position of both empty and sealed cells were also registered. To identify the individuals that emerged from the occupied reused cells, a stereoscopic microscope Leica S8 APO was used and the dichotomous key of Carpenter and Garcete-Barrett (2002 [2003]) at the genus level, and the dichotomic criteria presented by Willink and Roig-Alsina (1998), at the specific level, were employed.

Results

We found one Polistinae nest, built on the plant species *Quiabentia zehntneri* (Britton & Rose) Britton & Rose (Cactaceae). This nest is of the gymnodomous type (Richards and Richards 1951; Barbosa et al. 2021), without a protection envelope, 154.2 mm in length (pedicel to the basis), and 40 mm in width. It contained a total of 223 counted cells, 14 of which were sealed with mud, indicating the probable presence of solitary wasps (Fig. 1). Four of the sealed cells belonged to the medium portion of the nest, and the other 10 cells, to the superior portion, 20 mm from the pedicel.

Two weeks after the field collection, on December 14th, 2021, four male individuals of *Pachodynerus nasidens* (Latreille, 1812) emerged from the cells at the nest medium portion. These cells were situated around 60 mm above the basis, with an average diameter of 6.2 mm. *P. nasidens* sealed the cells' entrance and underlaid their bottom, having emerged one adult individual for each cell.

Individuals emerging from these cells presented average measurements of 7.395 mm in body length, 2.68 mm in mesosoma width, and 3.485 mm in T2 width. These male individuals presented average dimensions, relatively smaller than those presented by *P. nasidens* females deposited in the Entomological Collection of Federal University of Lavras (CEUFLA) (9.03 mm in body length, 3.78 mm in mesosoma width, 3.485 mm in T2 width). The other 10 sealed cells presented an average diameter of 5.74 mm; we did not observe emergence from them until May 1st, 2022.

Discussion

Nests completely built by *P. nasidens* are rare. This species commonly acts as an inquiline, in cavities made by humans (House et al. 2020) or in abandoned nests of other hymenopterans (Freeman and Jayasingh 1975; Matthews and González 2004). *P. nasidens* also build mud structures in the ground and fixed to plants (Carpenter 1986). The use of abandoned cavities in cells of a Polistinae nest seems to be one more case of the species' plasticity in nesting behavior.

Pachodynerus nasidens nesting peaks occur in months of higher temperature and humidity throughout the year (House et al. 2020). The ideal temperature for egg development ranges from 26 to 31 °C (Jayasing and Taffe 1982), while higher humidity

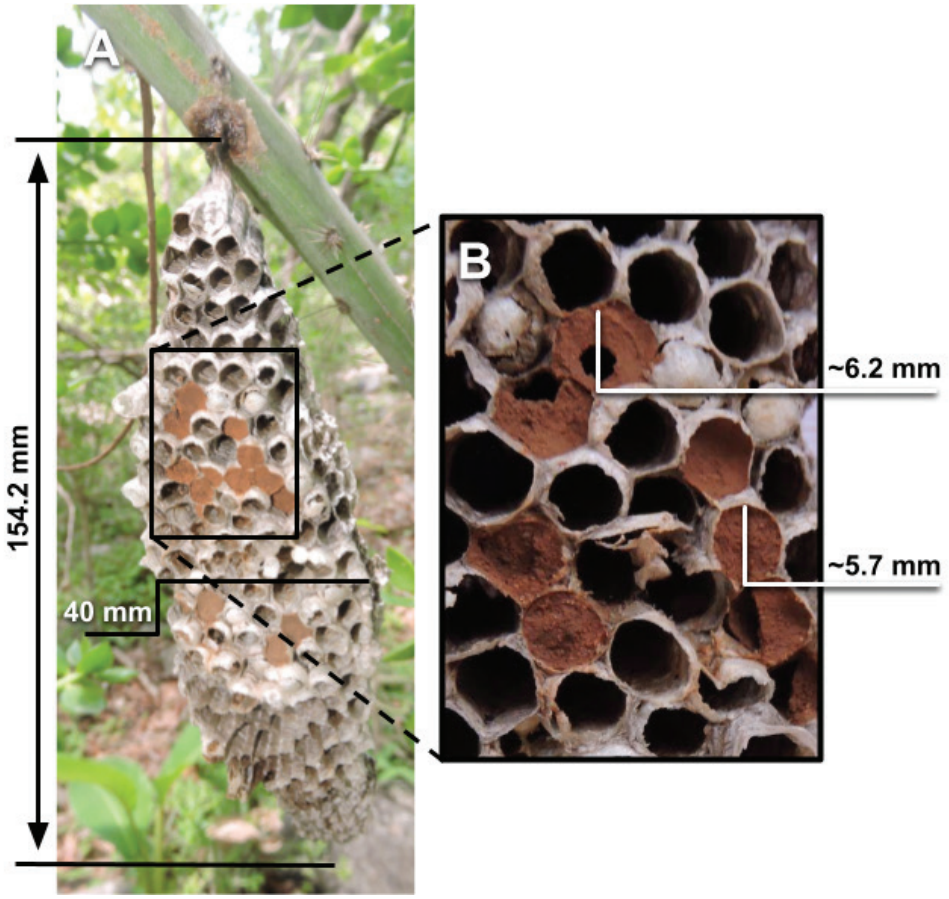

Figure 1. Polistinae's abandoned nest **A** with sealed cells occupied **B** by juveniles of the *Pachodynerus nasidens* solitary species (Vespidae, Eumeninae).

ensures the availability of water and mud for nest building (Freeman and Jayasingh 1975), explaining our record in the hot and humid season.

The nesting preference of *P. nasidens* is for cavities with openings of 6 to 9 mm in diameter (Bequaert 1948; Oliveira-Nascimento and Garófalo 2014), which explains the use of Polistinae cells, which have 6.2 mm in average diameter. The exclusive emergence of male individuals, smaller than females from the same collection, may be related to the cell size of the Polistinae nest, which may be too small for females' development. In this species, feminine eggs are usually elongated in comparison with masculine ones. Additionally, female immatures develop more slowly and need more food, consequently needing a bigger physical space for development (Jayasingh 1980; Jayasingh and Taffe 1982).

The nest herein described probably belonged to *Polistes versicolor* (Olivier, 1791). According to the dichotomous key presented by Barbosa et al. (2021), we conclude that this nest was produced by *Polistes* social wasp genus. Close to the abandoned nest, we found nests of *P. versicolor*, including one being built on *Q. zehntneri*

(Moura et al. 2022). In another study (Jacques et al. in press) the Polistinae diversity on social wasps from the Mata Seca State Park is presented, in which only nests of this species of the genus *Polistes* genus were found.

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SHORT COMMUNICATION

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The first recorded occurrence of the Asian hornet (*Vespa velutina*) in Ireland, genetic evidence for a continued single invasion across Europe

Eileen Dillane[1], Rachel Hayden[1], Aidan O'Hanlon[2], Fidelma Butler[1], Simon Harrison[1]

1 *School of Biological, Earth & Environmental Sciences, Environmental Research Institute, University College Cork, Distillery Fields, North Mall, Cork, Ireland* **2** *National Museum of Ireland, Merrion St., Dublin 2, Ireland*

Corresponding author: Eileen Dillane (e.dillane@ucc.ie)

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Abstract

The first record of the Asian/yellow-legged hornet (*Vespa velutina*) in Ireland was reported in April 2021, when a single female individual was discovered in Dublin. *Vespa velutina* has been present in mainland Europe since 2004 and in the UK since 2016 and poses an enormous threat to European apiculture and bee-mediated pollination services. Three mitochondrial genes were sequenced from the Irish specimen to determine whether the specimen originated from the established European population or signified a new point of entry from its native range in China. Additionally, specimens from Portugal, Spain, France, Germany, and the Channel Islands were sequenced at these three genes to build on previous studies which have asserted, based solely on Cytochrome Oxidase 1 (COI) analysis, that the entire range of *V. velutina* in Europe represents a single invasion which has proliferated since the first record in France. Further data were retrieved from GenBank for comparison. Results reveal that the mtDNA lineage observed in Dublin is the same as that seen throughout Europe, and therefore the arrival of this species in Ireland likely represents a further spread of the ongoing European invasion.

Keywords

Asian hornet, haplotype, invasive, Ireland, mtDNA

Introduction

The discovery of an Asian hornet, also known as the yellow-legged hornet (*Vespa velutina*, Lepeletier, 1836) in Dublin in April 2021 (https://biodiversityireland.ie/asian-hornet-alert/) has raised concern among beekeepers and biologists in Ireland due to the threat posed to apiculture through predation on domestic European honey bees (*Apis mellifera*) and other important pollinators (Monceau et al. 2014). *V.velutina* is native to South-East Asia, where it hunts a range of pollinating insects, and has been observed, when introduced, to be extremely successful in colonising new areas (e.g. Villemant et al. 2011; Monceau et al. 2014; Arca et al. 2015). Since the first record of *V. velutina* in France in 2004 it has become widespread throughout continental Europe and Jersey Island (Lopez et al. 2011; Villemant et al. 2011; Monceau et al. 2014; Arca et al. 2015; Robinet et al. 2016; Robinet et al. 2018; Husemann et al. 2020a; Laurino et al. 2020) while in England there have been 21 confirmed sightings (12 nests) since 2016 - (https://www.gov.uk/government/publications/asian-hornet-uk-sightings/asian-hornet-sightings-2020). Given that the species is expanding its range at an estimated 75 and 82 km/year in mainland Europe (Robinet et al. 2016), it is critical from an Irish perspective to determine the path of this potential invasion and gain an understanding of dispersal dynamics.

Previous studies addressing the provenance of Asian hornets in Europe have focused on the Cytochrome oxidase subunit I (COI or COX1) gene within the mitochondrial DNA (mtDNA) genome. However, the reliance on a single, relatively slow evolving/mutating gene may result in an oversimplification of invasion dynamics. Jeong et al. (2021) considered this and utilised sequence data from four other genes to track the invasion of Asian Hornets in South Korea. Analysing specimens from 11 Korean and 2 Japanese localities revealed that all individuals had identical sequences at COI, CytB (cytochrome B) and lrRNA (16S rRNA), which could have been interpreted as a single invasion. However, two intergenic spacer (IGS) sequences displayed substantially more variability indicative of multiple entry sites for *V. velutina*, independent of the southeast region, which had previously been considered as the sole entry point of the invasion.

We analysed sequences at three mtDNA genes; COI, IGS2 and IGS3 in the specimen recovered from Dublin, Ireland. Due to a paucity of sequence data (other than COI) for Asian hornets in Europe, we also sought samples from continental Europe and the island of Jersey, with which to compare data from this 'Irish' hornet. We further sourced available sequence data from GenBank for comparison.

Materials and methods

Samples and laboratory analysis

The *V. velutina* specimen was deposited with the entomological collections in the National Museum of Ireland (specimen number NMINH:2021.2.1) immediately after it's discovery. A single middle leg was removed from the pinned specimen (Fig. 1) and

Figure 1. Female *V. velutina* specimen from Dublin, Ireland (museum specimen no. NMINH:2021.2.1). Note the absence of the middle left leg, which was removed below the coxa for DNA extraction.

stored in ethanol for subsequent DNA extraction. Additional whole specimens from across Europe were provided by researchers from Portugal (4), Spain (3), France (3), Germany (3), and Jersey Island (3).

DNA extraction was carried out using DNeasy Blood and Tissue Kit (Qiagen). Three mtDNA genes were chosen for analysis; cytochrome oxidase I (COI), Intergenic spacer region 2 (IGS2) which spans *COII-trnK-trnD-ATP8* and Intergenic spacer region 3 (IGS3) spanning *trnR-trnN-trnE– trnS*$_1$*-trnF*. COI primers were from Folmer et al. (1994) and amplified a product of 707 bp. Primers for IGS1 and IGS2 were from Jeong et al. (2021) and these amplified products of approximately 390 bp and 700 bp respectively.

PCRs were performed in 20 µl volumes consisting of 10 µl of 2× Plain Combi PP Master Mix (Top-Bio), 1 µM each of forward and reverse primers and 10–50 ng of DNA. PCR cycling conditions were as follows; an initial denaturation step of 3 minutes at 95 °C was followed by 40 cycles of 95 °C for 30 seconds, 48 °C for 30 seconds and 72 °C for 1 minute, with final extension step of 72 °C for 5 minutes. Electrophoresis of PCR products was performed on 1% agarose and products were excised and purified using a QIAquick Gel Extraction Kit (QIAGEN). Sequencing was performed with the forward primer for each locus using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were purified using the EDTA-ethanol precipitation method described in the sequencing kit handbook and were run on an ABI3500XL DNA analyser.

Raw sequence data was examined using Chromas software 2.6.6.(Technelysium Pty Ltd) to resolve ambiguous calls. 5' and 3' ends of sequences were trimmed to the first shared nucleotides as gaps in sequences would be incorrectly interpreted as genetic distance during analysis rather than nucleotides that failed to be sequenced.

Sequence analysis

All sequences were used in BLAST searches which served two purposes; firstly to confirm species and secondly so that search results could be downloaded for downstream alignments and analysis.

Each locus was analysed separately. Sequences were trimmed at either end to the point where full alignments could be performed and compared with publicly available data from other studies. Alignments were performed using the Clustal Omega multiple sequence alignment tool (https://www.ebi.ac.uk/Tools/msa/clustalo/) to determine whether the Dublin specimen was of the same sequence composition as those we analysed from around Europe, and also to compare with sequence data available on GenBank from other studies

Results

Sequencing of all three genes was successful for *the V.velutina specimen* discovered in Ireland. Success varied for the additional samples provided from Europe, but in all cases at least one hornet from each country was sequenced at each gene.

COI

For CO1, 557 bp of sequence were resolved for at least one individual from each area sampled. This sequence was identical in all of the specimens we examined. The sequence from the Dublin hornet was deposited on GenBank (Accession number OP437698). Using this sequence as a BLAST query revealed that this individual was also identical to all other sequenced and deposited samples from previous studies on Asian hornets in Europe, and therefore, like those, is most closely related to *V.velutina* inhabiting eastern China.

IGS2

In the case of IGS2, we resolved 435 bp of sequence in the Dublin specimen (GenBank accession number OP537231). This was also identical across all of the European origin specimens we sequenced. There are only two other available sequences for this gene in Europe, one from France (OU525148.1) and another from the Channel Islands (AP018461.1, Takahashi et al. 2019), both of which were also 100% match (both of these sequences formed part of full mtDNA genome sequences). There are upwards of 200 other sequences from studies in Asia (native and non-native range) which differ from the European specimens, mostly in a short tandem repeat (AT) region. The number of repeats observed in this region varied from four (in all European specimens examined) to between 5 and 13 in Asia (Korea, Japan, Indonesia, China). Two other point mutations (SNPs) were also observed between European samples and the Asian range.

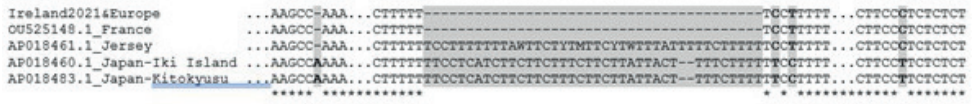

Figure 2. Summary figure of polymorphisms observed among IGS3 sequences. `…' denotes areas of homologous sequence not included for brevity (AP018461, AP018460, AP018483 from Takehashi et al 2019; OU525148 from *Vespa velutina* genome assembly – sample provided by Seirian Sumner in collaboration with the Sanger 25 Genomes Project and Vertebrate Genomes Project (http: //vertebrategenomesproject.org)).

IGS3

The IGS3 locus was successfully sequenced to a length of 700 bp in the Dublin specimen (GenBank accession number OP537232) and from all countries that we looked at. This sequence was identical in all hornets provided for this investigation. As with IGS2, there were just two sequences available from Europe for comparison (from the same studies). The French sample was identical to our specimens at this gene, but the Channel Islands sequence differed significantly in that there was a region of 37 bp present therein which was not seen in any of our European samples (Fig. 2), and that region was consistent with the Iki Island sample which was sequenced in that study (Takehashi et al 2019). There were however three additional single nucleotide polymorphisms and one single base pair deletion at which the Jersey Island sample was consistent with European samples and different from Iki Island. In the context of sequences available from throughout Asia (approximately 60), the 37 bp region in question was present in all cases, suggesting that the source population for Asian hornets in Europe has not yet been fully sequenced.

Discussion

Our analysis of three mtDNA loci is more comprehensive than has been previously undertaken within the range of invasive *V. veluntina* in Europe and suggests firstly that contemporary populations of Asian hornets in Europe are of a single phylogenetic lineage, and secondly that the Irish specimen is likely to have found its way (anthropogenically) to Dublin from Europe or Britain . Our study builds on earlier work (e.g. Arca et al. 2015; Budge et al. 2017; Granato et al. 2019; Husemann et al. 2020b; Jones et al. 2020; Quaresma et al. 2022) which utilised the cytochrome oxidase I gene and microsatellites to demonstrate a single invasion of *V.velutina* to Europe. The inclusion of additional mtDNA loci described in Jeong et al (2021) further indicates that, unlike the situation in Japan and Korea where multiple points of entry were detected, contemporary (2021) Asian hornet specimens collected across Europe, and the individual discovered in Ireland represent a common invasion history for hornets thus-far discovered in Europe, Britain and Ireland.

COI in our study specimens matched all available European sequences from other studies (Arca et al. 2015; Budge et al. 2017; Granato et al. 2019; Husemann et al. 2020b). In the case of IGS2 and IGS3, only two specimens in Europe had previously

been sequenced, one specimen from France (OU525148.1) which was identical in composition, and another from Jersey (Channel Islands, AP018461.1, Takehashi et al. 2019), which was characterised by a longer sequence length with a 37 bp insertion within IGS3, consistent with specimens from Japan and Korea (Jeong et al 2021), and indeed all previously sequenced samples from Asia currently available on GenBank. This specimen had been collected in Jersey Island in 2017. It would be worthwhile to analyse more individuals from a wider range of locations and dates particularly in the Channel Islands, France and the UK to determine if this polymorphism might be detected again.

The finding that the Irish specimen and those studied from Europe are likely to represent a single invasion (founder) event is not unexpected. Arca et al (2015) looked at microsatellite DNA alongside COI haplotypes and found that the French (and by extension European) invasion was descended from a population of *V.velutina* from eastern China, and that the introduction of a single queen fertilized by several males was the most likely scenario. Studies of nests discovered in Britain (Budge et al (2017), Jones et al (2020)) found even lower levels of genetic variability than had been observed in French and Korean specimens by Arca et al. (2015) with each nest containing only a subset of the alleles seen in French hornets, suggesting that incursions from mainland Europe are responsible for the presence of Asian hornets in England. We conclude that the discovery of the hornet in Dublin is also the result of an incursion from either mainland Europe or Britain, rather than an independent invasion from a source within the native East Asian distribution of this species. Further sequencing of individuals from the Zhejiang and Yunnan areas of Eastern China would be useful to determine if the IGS3 haplotype observed in Europe is present there, which would go further towards confirming whether that region was indeed the source of the European invasion.

Given the proximity of Ireland to Britain and continental Europe, and the existing trade links, it is prudent to expect further occasional anthropogenic introductions of *V. velutina*. Whether the species succeeds in establishing a self-sustaining population in Ireland is less certain. It is possible that climatic conditions in Ireland may prevent the establishment of a viable population, however the recent arrival, for example, of the Saxon wasp (*Dolichovespula saxonica* (Fabricius, 1793)) in the East of Ireland (Finch and Finch 2020) demonstrates the possibility of non-native social Vespidae to reach Ireland naturally from established populations in Britain, and highlights the need for vigilance from Irish authorities around the situation with *V. velutina*.

Finally, our results, along with those of other groups, suggest that the entire population of *V.velutina* in Europe, now potentially numbering many millions of individuals, are descended from a single mated queen arriving from China some 15–20 years ago. This demonstrates the potential for alien insects to become invasive pests via accidental imports of only very few, or single, individuals, and also the potential for biological control mechanisms, given the very low genetic diversity inherent in such populations.

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Description of the nest of the pollen wasp *Celonites jousseaumei* Du Buysson, 1906 (Hymenoptera, Vespidae, Masarinae) with a new host association of the cuckoo wasp *Spintharina innesi* (Du Buysson, 1894) (Hymenoptera, Chrysididae)

Volker Mauss[1], Christophe Praz[2], Andreas Müller[3], Rainer Prosi[4], Paolo Rosa[5]

1 *Staatliches Museum für Naturkunde, Abt. Entomologie, Rosenstein 1, D-70191 Stuttgart, Germany* **2** *University of Neuchâtel, Institute of Biology, Emile-Argand 11, 2000 Neuchâtel, Switzerland* **3** *ETH Zürich, Institute of Agricultural Sciences, Biocommunication and Entomology, Schmelzbergstraße 9/LFO, CH-8092 Zürich, Switzerland* **4** *Lerchenstraße 81, D-74564 Crailsheim, Germany* **5** *Laboratory of Zoology, Institute of Biosciences, University of Mons, Place du Parc, 20, 7000 Mons, Belgium*

Corresponding author: Volker Mauss (volker.mauss@gmx.de)

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Abstract

Two nests of *Celonites jousseaumei* are described in detail from the Antiatlas in Morocco. The nests consisted of two or three linearly arranged earthen brood cells that were attached to the almost vertical surface of medium sized stones. The brood cell provisions consisted exclusively of *Heliotropium* pollen (Boraginaceae). Species affiliation of developmental stages by DNA barcoding revealed that one of the brood cells contained a pupa of *Spintharina innesi* in a cocoon.

Keywords

brood parasitism, COI-5P, nest construction, Palaearctic

Introduction

The knowledge about nest architecture of the more than 40 Palaearctic species of the pollen wasp genus *Celonites* is fragmentary. Detailed nest descriptions are only available for *Celonites abbreviatus* (Villers, 1789) (Ferton 1901, 1910; Bellmann 1984, 1995), *Celonites tauricus* Kostylev, 1935 (Mauss et al. 2016) and *Celonites fischeri* Spinola, 1838 (Mauss and Müller 2014), which are all members of the *C. abbreviatus*-group of the subgenus *Celonites* s.str. In contrast, information about nesting in the subgenus *Eucelonites* Richards, 1962 is not available despite a short note by Richards (1962: 224) concerning two specimens of *Celonites* (*Eucelonites*) *jousseaumei senegalensis* Richards, 1962 from Bambey, Senegal that "had pinned with them some mud cells which from their shape must have been attached end to end, longitudinally, on a plant-stem. The cells were approximately 9.0 mm long and 3.5 mm in diameter". During a field trip to Morocco in 2019 two nests of *Celonites* (*Eucelonites*) *jousseaumei* were discovered that are described in the present paper.

Materials and methods

The nests were found and dissected on the 19th of April 2019. Outer cell dimensions were measured using a calliper rule (accuracy 0.1 mm), inner cell dimensions were reconstructed from macro photos. All brood cells were dissected with fine tweezers (Dumont INOX No. 5 Biologie, No. 7 and spring steel tweezers) using a combination of two reading glasses that provided a sufficient magnification. Photos were taken with a Canon EOS 70D or 80D camera with a 50 mm or 100 mm macro lens (scale up to 1:1, resolution 20 or 24 mega pixel) and macro flash-lights. Dry specimens of all *Celonites* species were labelled with an individual, serial database number (dbM = database Mauss) printed on the determination label and placed in the collection of Volker Mauss (*C. jousseaumei* 1♂ dbM 5402 1♀ dbM 5386; *C. pictus* 5♀ dbM 5385, 5398–5401). Specimens of all plant species that were visited by pollen wasps were collected and preserved dried. The material was placed in the herbarium of the State Museum of Natural History in Stuttgart (Herbarium STU).

Pollen samples from brood cell provisions were prepared using the method outlined by Westrich and Schmidt (1986). The content of each provision was distributed over two or three slide preparations. The different pollen types were ascertained under a light microscope at magnifications of 400× and determined to generic level with the aid of a reference collection. For each slide all pollen grains were determined along three randomly chosen lines transversal to the cover glass.

1♀ *Celonites jousseaumei* (dbM 5421 [BOLD process ID: CECYP002-20]) and 1♀ *Celonites pictus* (dbM 5422 [CECYP004-20]) from the nest locality, as well as the pupa from cell B2 [CECYP001-20] and the larva from cell N1 [CECYP003-20] from the brood cells were preserved in 96% pure ethanol for DNA barcoding. For

further reference, the barcoding fragment of the mitochondrial gene was sequenced from 1♂ (dbM 4320 [AIMEJ011-20]), 4♀ (dbM 4319 [AIMEJ010-20], dbM 5590 [CECYP035-22], dbM 5592 [CECYP036-22], dbM 5594 [CECYP037-22]) of *C. jousseaumei*, and 1♂ (dbM 5117 [AIMEJ013-20]), 1♀ (dbM 4322 [AIMEJ012-20]) of *Celonites afer* from different localities in Morocco. To facilitate identification of possible cuckoo wasp nest parasites, we barcoded dry specimens of six chrysidid wasps morphologically close to *Spintharina* that had been collected at four localities in Morocco between 2011 and 2019 identified as *Spintharina procuprata* (Linsenmaier, 1959) [CECYP022-22], *Spintharina innesi* (du Buysson, 1894) [CECYP023-22, CECYP024-22, CECYP025-22, CECYP026-22] and *Chrysis patruela* Linsenmaier, 1999 [CECYP027-22]. Species identification was based on morphological characters of the imagines. DNA barcoding followed standard methods of DNA extraction from a single leg of dry specimens or specimens collected and stored in 96% pure ethanol. The barcoding fragment of the gene Cytochrome Oxidase subunit 1 (COI-5P) was amplified in PCR using the universal primers LepF and LepR (Hebert et al. 2004). Sequencing was performed bi-directionally using the same primers and the resulting chromatograms were edited in Geneious 6.0.6 (Kearse et al. 2012). DNA barcoding of the additional reference specimens that had not been collected at the nest site was accomplished by AIM Advanced Identification Methods GmbH Leipzig. All nucleotide sequences were uploaded and analysed using the BOLD database (https://www.boldsystems.org). Another four public COI-5P sequences of two taxa of *Spintharina* were added from the BOLD database. Genetic distances were computed using Kimura 2-parameter (K2P) distance model in a test version of Paup 4.0 (Swofford 2002) kindly provided by D. Swofford. Finally, a species ID tree was computed using the following parameters: distance model Kimura 2 Parameter; pairwise deletion of positions containing gaps and missing data; minimum complete overlap 0 bp; alignment with BOLD Aligner (Amino Acid based HMM); individual nucleotide sequence length varied from 252 to 675 bp.

Results and discussion

Locality

Two nests of *Celonites jousseaumei* were found at Ait Daoud (WGS 84: 29°36.977'N, 08°59.009'W), 15 km south of Tafraout in the Antiatlas in Morocco, situated at a height of 1140 m a.s.l. The climate of the area is arid with a mean annual precipitation of 235 mm and a mean annual temperature of 16.6 °C (data from Tafraout, AM ONLINE Project). The habitat consisted of a richly flowering roadside (Fig. 1b) with adjacent former terraces of almond orchards that were left fallow since approximately ten years because of increasing aridity (Fig. 1a). The stony area was heavily grazed and somewhat polluted with rubbish, i.e. along little dry drainage channels.

Figure 1. a, b habitat of *Celonites jousseaumei* at Ait Daoud, 15 km south of Tafraout, Morocco **a** nest site **b** foraging area **c** exterior view of nest B **d, e** stones used as base for nests of *C. jousseaumei* **d** nest N **e** nest B.

In the area *Celonites jousseaumei* and *C. pictus* were recorded. Imagines of both species were exclusively observed to visit flowers of *Heliotropium crispum* Desf. (number of sightings at *H. crispum* flowers: *C. jousseaumei* 1♂ 3♀, *C. pictus* 66♀), although other plants were flowering at the site, for example *Cladanthus arabicus* (L.) Cass., *Centaurea calcitrapa* L., *Pallenis spinosa* (L.) Cass., *Senecio glaucus* subsp. *coronopifolius* (Maire) C. Alexander (all Asteraceae), *Echium horridum* Batt. (Boraginaceae), *Lotus* sp. (Fabaceae) and *Convolvulus trabutianus* Schweinf. & Muschl. (Convolvulaceae).

Nest site

Both nests were situated at the steep edge of a 0.5 m high terrace with an exposure of 70° to the north (ENE) (Fig. 1a). The site was approximately 10 m away from large patches of *Heliotropium crispum* flowering on the embankment of the road (Fig. 1b). Both nests were attached to almost vertical surfaces of medium sized stones (Table 1, Fig. 1d, e), 6 cm and 29 cm respectively above the foot of the terrace. The distance between the nests was 75 cm.

Nest structure

The nests were made of fine clayey soil with a small proportion of tiny stones and consisted of 2 or 3 cells (Table 1). The cells were arranged in a longitudinal row in which each subsequent cell abutted with its basal end onto the apical end of the completed preceding cell. The resulting almost vertical or diagonal linear construction was 15.5 mm or 20.9 mm long, with the apical ends of the cells oriented downward (Figs 1c, 2a). An additional nest covering was not present. This exclusively linear arrangement of the brood cells is in congruence with the fragmentary nest description of *Celonites jousseaumei senegalensis* by Richards (1962). Linearly arranged brood cells were also observed in a few nests of *C. abbreviatus* (Lichtenstein 1869) and *C. fischeri* (Mauss & Müller, 2014), but in these nests at least a single cell was also attached longitudinally to the others in addition. Moreover, in most nests of *C. abbreviatus* (Bellmann 1984) and *C. fischeri* (Mauss & Müller, 2014) the cells are only attached longitudinally to each other, which is also the case in all of the few known nests of other members of the *C. abbreviatus*-group, in particular *C. tauricus* (Mauss et al. 2016) and *C. mayeti* Richards, 1962 (Lichtenstein 1875). Therefore, the observed exclusively linear arrangement of the brood cells of *C. jousseaumei* is of note.

Table 1. Parameters of two nests of *Celonites jousseaumei* recorded at Ait Daoud, Morocco.

Nest	Condition	Height above ground (cm) [1]	Orientation to the North (°)	Nest substrate	Σ cells	Contact between adjacent cells	Nest covering
B	sealed, current season	29	70	stone (base 18×23 cm, height 10 cm)	2	linear	absent
N	sealed, current season	6	0	stone (base 17×7 cm, height 10 cm)	3	linear	absent

[1]measured from the lowest part of the nest to the foot of the terrace.

The brood cells were cylindrical with almost parallel sides, rounded at the basal and truncate at the apical end (Fig. 2b, c). The median dimensions of the cells were: outer length 7.3 mm (n = 5), outer diameter 3.6 mm (n = 5), inner length 6.3 mm (n = 4), inner diameter at the cell opening 3.4 mm (n = 4) (Table 2). The outer cell surface showed a distinct "fish scale" pattern (Figs 1c, 2a) while the inner surface was smooth (Fig. 2c). Towards the stone it was attached to, the cell wall was not completely constructed resulting in a few spots in the median axis where the surface of the stone formed the boundary of the cell (Fig. 2c). The outer cell wall continued between adjacent cells laterally covering the small hollow space between them that resulted from the existence of two separate cell walls that is the transversal apical wall of the basal cell and the rounded basal wall of the apical cell (Fig. 2b, c). At the apical end of the nest the outer wall of the last cell was produced into a small rim protruding the apical transversal cell wall by 2.1 mm (Fig. 2b). The wall of the rim had one or two deep notches and was slightly bent outwards (Fig. 1c). Within this short tube-like entrance, the nest was sealed by a circular, curved, circa 0.2–0.3 mm thick mud plug (Fig. 2b). This nest seal was positioned about 0.2 mm above the apical wall of the last cell and 1 mm inwards from the edge of the nest opening resembling the bottom of a new cell in form, position and structure.

Brood cell content

The content of the brood cells is summarized in Table 2. The provision consisted of a yellowish-white, viscous pollen mass with shining surface forming a pollen loaf. The surface of the loaf was characteristically papillated (Fig. 2b) so that it barely touched the cell walls. Between the apical end of the pollen loaf and the apical wall of the cell remained a hollow space measuring approximately 0.8–0.9 mm (Fig. 2b). Inwards the pollen mass became more sticky and rather liquid, so that it could not be removed as a whole with a pair of tweezers. The provision in the cells of both nests consisted exclusively of *Heliotropium* pollen indicating narrow oligolecty (sensu Müller and Kuhlmann 2008) of *C. jousseaumei* at this locality. Both eggs of *Celonites jousseaumei* were whitish and curved. Each egg was situated on top of the provision close to the basal end of the cell indicating that it was laid by the female prior to brood cell provisioning (Fig. 2b). Small remnants of membranous material attached to one pole of each egg suggested that the eggs had initially been attached to the wall. The small larva from cell N1 was also situated basally on top of the pollen mass, where it fed on the provision (Fig. 2b).

Species identification

The COI-5P gene sequence of the larva from brood cell N1 was 100% identical (distance of 0) to the sequence of a *Celonites jousseaumei* female [CECYP037-22] collected northeast of Sidi Ifni at a distance of approximately 100 km from the nest site. Four additional specimens of *C. jousseaumei* from Morocco were sequenced; the average within-species genetic distance among these specimens was 0.51% (minimum 0, maximum 1.19). The

Figure 2. Structure of *Celonites jousseaumei* nest N on 19th of April: **a** exterior view **b** brood cells opened in longitudinal direction **c** cell content removed. (N1–N3 = brood cell numbers; aw = apical cell wall; bw = basal cell wall; e = egg; hs = hollow space below pollen loaf; l = larva; nr = nest rim; ns = nest seal; p = pollen loaf; pa = papilla on surface of pollen loaf; s = bare stone surface; measurements and cell content summarized in Table 2).

sequence of the larva from brood cell N1 to *C. pictus* [CECYP004-20] was 24.25%. In the distance-based tree (Fig. 3), the larva N1 clusters together with the imagines of *C. jousseaumei*. Therefore, it can be concluded that the recorded nests belong to *C. jousseaumei*. This is in congruence with the cell dimensions that are too small for *C. pictus* but match the size of *C. jousseaumei* (median total length of females 7.46 mm (n = 8) in *C. pictus* versus 5.75 mm (n = 7) in *C. jousseaumei*).

Table 2. Measurements and condition of brood cells from two nests of *Celonites jousseaumei* recorded at Ait Daoud, Morocco.

Nest	Cell No.	Condition	Outer cell length (mm)	Outer cell width (mm)	Inner cell length (mm)	Inner cell width (mm)	Content	Species affiliation	Pollen type composition of provision
B	B1	sealed	7.3	3.5	5.8	2.8	yellowish-white pollen loaf, dead dry larva		exclusively *Heliotropium*[1]
	B2	sealed	8.2	3.6			pupa in light yellowish cocoon	*Spintharina innesi*	
N	N1	sealed	7.1	3.6	6.4	3.3	yellowish-white pollen loaf, small larva feeding on basal end of provision	*Celonites jousseaumei*	exclusively *Heliotropium*[1]
	N2	sealed	7.0	4.0	6.2	3.4	yellowish-white pollen loaf, curved egg basally on top of provision		exclusively *Heliotropium*[1]
	N3	sealed	7.9	3.7	7.3	3.4	yellowish-white pollen loaf, curved egg basally on top of provision		exclusively *Heliotropium*[1]

[1]at most very rarely with single pollen grains of other plant taxa.

The pupa from brood cell B2 turned out to belong to a species of Chrysididae, as the obtained COI-5P gene sequence of the pupa closely matched the public sequences of *Spintharina versicolor* Spinola, 1808 within BOLD database. However, the identity of the sequences was only 92%, indicating that the pupa belongs to a different but related species. After additional sequencing of the reference chrysidid specimens from Morocco, a match was found between the pupa and a specimen of *Spintharina innesi* collected in Tizourgane [CECYP026-22] (Fig. 3). Three additional specimens of *Spintharina innesi* were sequenced; the average within-species genetic distance was 0.71% (minimum 0.33, maximum 1.31). The genetic distances between the pupa from brood cell B2 and *Spintharina procuprata* or *Chrysis patruela* were 17.20% and 12.13%, respectively. Therefore, it can be concluded that *Celonites jousseaumei* is a host of *Spintharina innesi*. Host-parasite associations of *Spintharina* with *Celonites* seem to be common. In Europe *Spintharina versicolor* has been recorded as a brood parasite of *Celonites abbreviatus* (Blüthgen 1961; Erlandsson 1972), while in the Afrotropical Region *Spintharina arnoldi* (Brauns, 1928) and *Spintharina bispinosa* (Mocsáry, 1902) were reared from brood cells of two different *Celonites* species (Brauns 1913; Gess 1996). Moreover, Pauli et al. (2019) found a close phylogenetic relationship between *Spintharina* and the Nearctic *Chrysurissa*, which also appear to parasitize exclusively pollen wasps. This phylogenetic placement indicates that both genera are descendants of a last common ancestor which may already have exploited pollen wasps as hosts, suggesting an old and exclusive association with pollen wasps in this clade. However, the host specificity of numerous *Spintharina* species is poorly known and it remains to be shown whether *Spintharina innesi* is restricted to *C. jousseaumei* or if it may also parasitize brood cells of other species of *Celonites* or even of other pollen wasp genera. Conversely, there are at least three other species of *Spintharina* recorded from Morocco that are all suitable in size to fit the brood cells of *C. jousseaumei* (cf. Linsenmaier 1999, classified as species of the *Chrysis versicolor*-group), so that this pollen wasp may be parasitized by more than one *Spintharina* species.

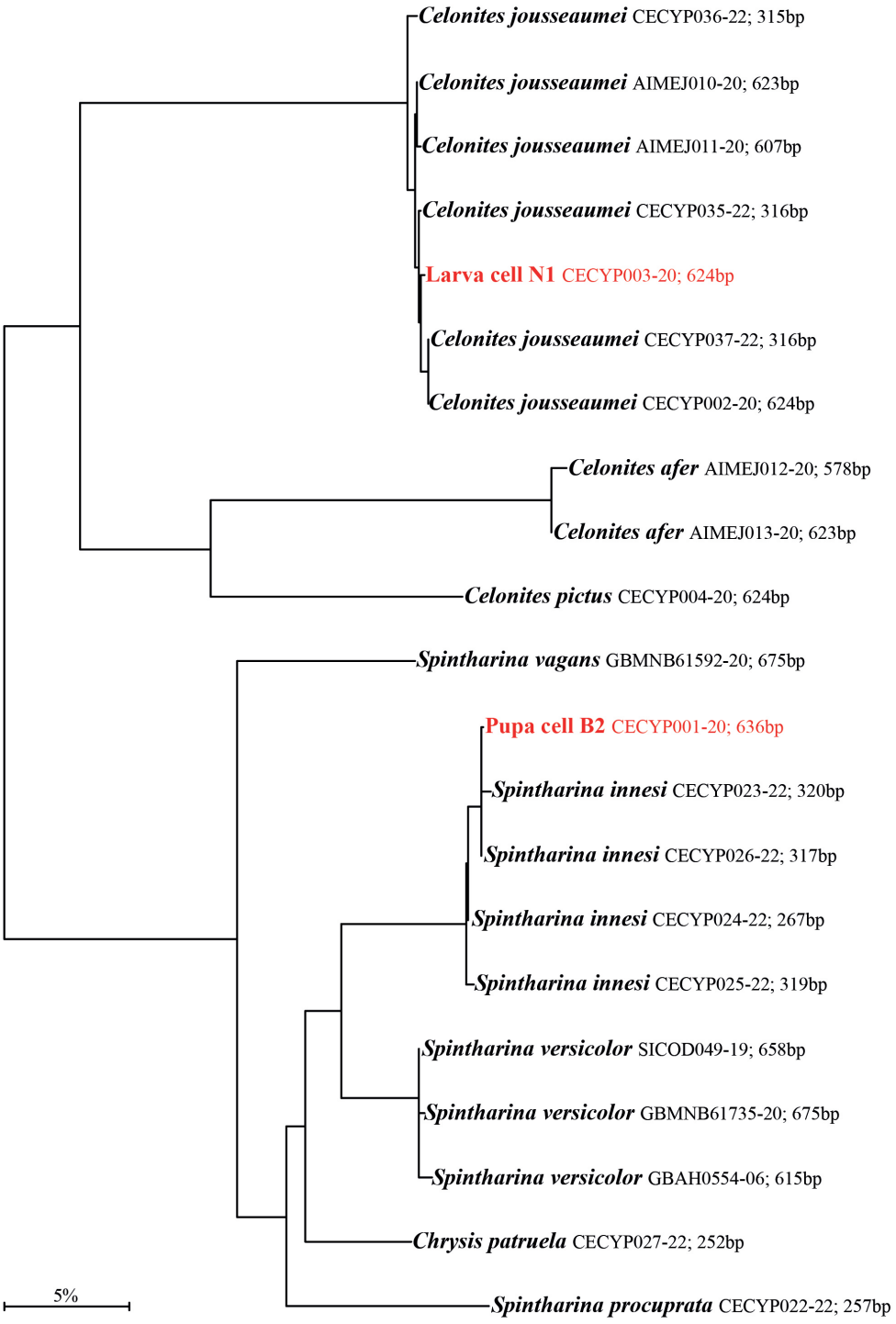

Figure 3. Neighbour joining tree of COI-5P sequences of 9 imagines of *Celonites* (Masarinae) from Morocco, 10 imagines of *Spintharina* (Chrysididae) and 2 immature stages collected from brood cells of two *Celonites* nests from Morocco (see text for details).

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RESEARCH ARTICLE

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Three new species and one new country record of velvet ants (Hymenoptera, Mutillidae) from Thailand

Narit Thaochan[1], Kevin A. Williams[2], Kodeeyah Thoawan[1], Tadsanai Jeenthong[3], Wisut Sittichaya[1]

1 *Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University, Songkhla, 90110, Thailand* **2** *Plant Pest Diagnostics Center, California Department of Food & Agriculture, 3294 Meadowview Road, Sacramento, CA 95832, USA* **3** *Office of Natural Science Research, National Science Museum, 39 Moo 3, Khlong 5, Khlong Luang, Pathum Thani, 12120, Thailand*

Corresponding author: Wisut Sittichaya (wisut.s@psu.ac.th)

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Abstract

Three new species of velvet ants known from females are here described: *Mickelomyrme leleji* Sittichaya & Williams, **sp. nov.**, *Nordeniella dokbua* Sittichaya & Williams, **sp. nov.**, and *Smicromyrme songkhwae* Sittichaya & Williams, **sp. nov.** One additional species is newly recorded from Thailand: *Bischoffitilla selangorensis* (Pagden). Synoptic list of Mutillidae in Thailand Natural History Museum with new records is given.

Keywords

Diversity, Mutillidae, new record, new species, Oriental region, taxonomy

Introduction

In Thailand, studies of velvet ants (Mutillidae) are rare and a systematic nation-wide survey is still crucial to study the diversity of these wasps in the country. Before 2019, Thai velvet ants were only discussed in catalogs or revisions of various genera in the Oriental Region (Lelej and Krombein 1999; Lelej 2005; Lelej et al. 2016, 2017; Okayasu et al. 2018, 2021a, b, c) and 33 species were recorded from Thailand (25 known from

females). The first survey focused on Thailand was written by Williams et al. (2019) and treated female specimens in southern Thailand. The result of that project raised the number of Thai species from 33 to 61. Since then, three additional Thai species were recognized in the genus *Andreimyrme* Lelej, 2005 (Okayasu et al. 2021b), raising the count to 64 species. In the present paper, we examined 83 pinned specimens from the Thailand Natural History Museum, National Science Museum resulting from multiple collecting events mostly from Northern and Northeastern Thailand. Three species new to science and one new country record were recognized, raising the number of species recorded from Thailand to 68.

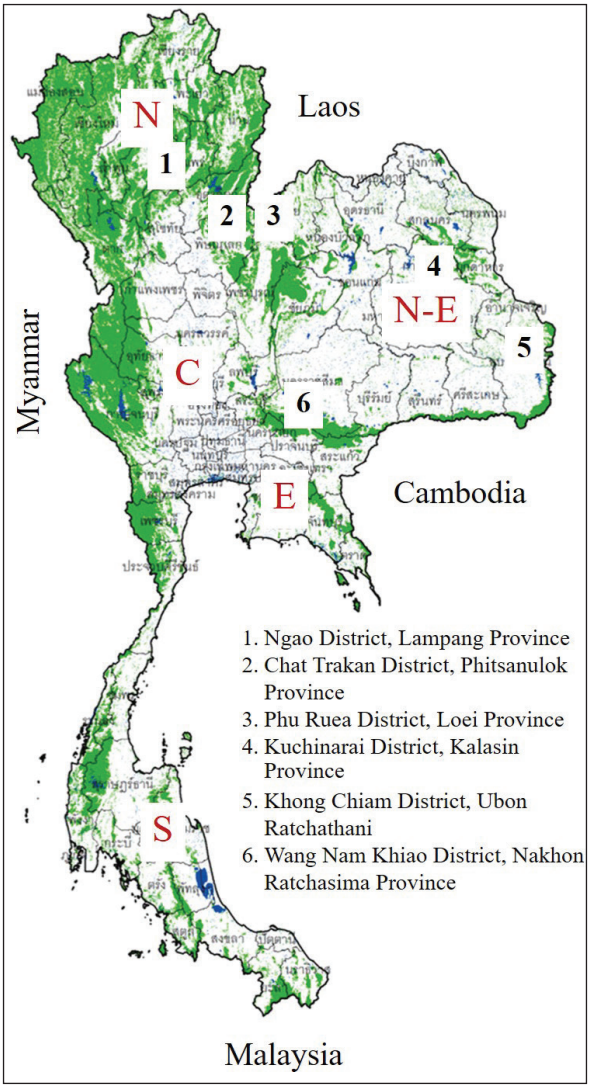

Figure 1. Specimen collecting localities, the ordinal numbers in accordance with the sequence of locations in the text. N=Northern region, N-E=North-Eastern region, C=Central region; E=Eastern region, S=Southern region. Source: map modified from a Royal Forest Department of Thailand map.

Material and methods

The specimens were collected from different localities in Thailand (Fig. 1) with hand collecting or honey baiting. Specimens were then observed with Leica S9-D, Leica S8 APO Leica, or Leica M165C stereomicroscopes (Leica Microsystems Pte Ltd, Germany). The habitus of specimens were photographed using Canon 6D digital camera with a Canon MP-E 65mm Macro Photo Lens, magnified with 2×-extender tube (Canon, Tokyo, Japan) and StackShot-Macrorail (Cognisys Inc, MichiganI, USA). The photos were then combined with Helicon Focus 6.8.0. (Helicon Soft, Ukraine), all photos were improved with Adobe Photoshop CS6 (Adobe Systems, California, USA). The terminology mostly follows Hymenoptera Anatomy Ontology (2013) and the taxonomic characters and type specimen measurements follow the methodology used in Williams et al. (2019).

Abbreviations

CSCA California State Collection of Arthropods, Sacramento, California, USA.
THNHM Natural History Museum of the National Science Museum, Pathum Thani, Thailand.
PSUC Prince of Songkhla University Collection, Hat Yai, Songkhla, Thailand.

Results

Taxonomic treatment

Family Mutillidae Latreille, 1802
Subfamily Myrmillinae Bischoff, 1920
Genus *Bischoffitilla* Lelej, 2002

***Bischoffitilla selangorensis* (Pagden, 1934)**
Fig 2

Squamuloltilla selangorensis Pagden, 1934: 452.
Bischoffitilla selangorensis (Padgen). Comb.n. Lelej, 2002: 127.

Material examined. *Holotype*: ♀, Malaysia, *Selangor*, Bukit Kutu, 31.I.1930 (BMNH).

Other material. New to Thailand, *Lampang Province*, Ngao District, Tham Pha Thai NP., 20. XII.2001, S. Hasin leg., (1♀, THNHM); *Loei Province*, Phu Ruea District, Waranya Resort 17.460 –101.355, 25–26.III.2019, K. Williams, S. Puttasok, K. Thoawan, R. Malee and N. Thaochan leg., (4♀ CSCA); 19.I.1999, W. Jaitrong leg. (1♀ THNHM); *Nakhon Ratchasima Province*, Wang Nam Khiao District, 09.V.2001, W. Jaitrong and T. Jeentong leg. (1♀, THNHM).

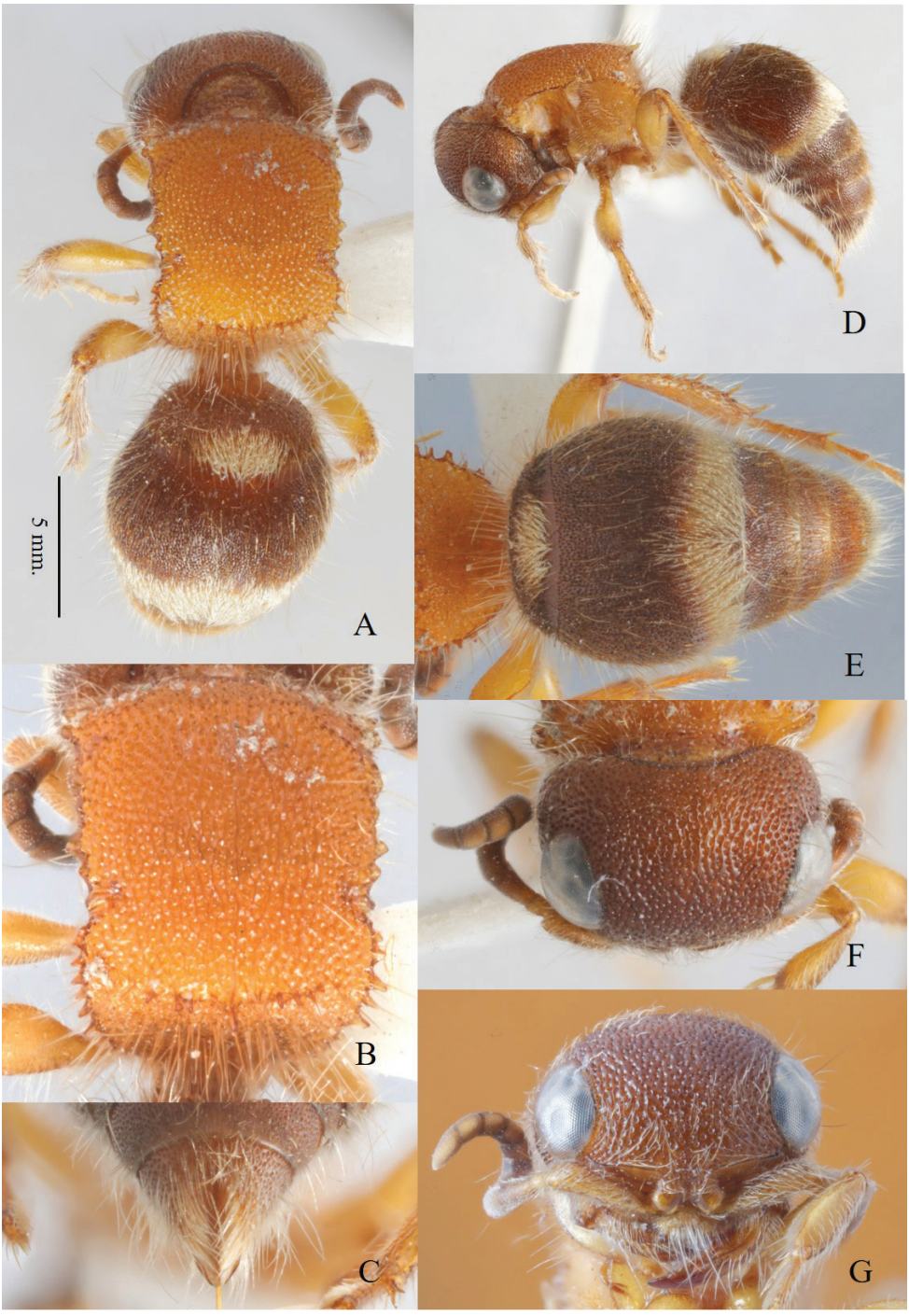

Figure 2. *Bischoffitilla selangorensis*, ♀, Thailand **A** dorsal view **B** mesosoma dorsum **C** pygidium **D** lateral view **E** metasomal terga **F** vertex **G** frons and clypeus.

Diagnosis. Female. This species can be separated from other Thai species by having the posterior propodeal surface with uniformly dense small punctures. The following combination of characters are also useful for diagnosis: genal carina short with weak anterior tooth; mesopleural lamella longer than flagellar width, apically rounded; lateral margins of mesosomal dorsum margined with wavy multidentate carina; dorsoposterior propodeal row with median tooth distinct, larger than lateral teeth; T3–5 with brown or black appressed setae and interspersed sparse erect blackish and white to yellowish setae. Body length 5.0–6.2 mm.

Male. Unknown.

Variation. The head and metasoma cuticle colors vary from reddish-brown to black. The appressed metasomal setae vary from brown to black. The cuticle beneath the whitish setal markings of T1 and T2 vary from mostly brown or black to entirely bright whitish-yellow. The size and number of apparent teeth along the dorso-lateral pronotal and mesonotal margins vary. The height of the spines on the dorsoposterior propodeal row varies and the number of spines varies from three to five on each side. The leg color varies from entirely pale orange-brown to mostly dark brown.

Male. Unknown.

Distribution. Malaysia (Selangor); Thailand (Loei, Lampang, Nakhon Ratchasima).

Remarks. This species is newly recorded from Thailand. In the key to Thai females from Williams et al. (2019), *B. selangorensis* terminates at couplet 4. It can be differentiated from *B. lamellata* and the other species by having T3 without a distinct band or mesal patch of whitish setae; rather, T3 has sparse interspersed blackish and pale yellow or whitish setae. Unlike the other Thai species, the posterior propodeal surface has uniformly dense small punctures; the other Thai species have the posterior propodeal surface widely areolate, becoming smooth ventrally.

Subfamily Mutillinae Latreille, 1802
Tribe Smicromyrmini Bischoff, 1920
Genus *Mickelomyrme* Lelej, 1995

***Mickelomyrme leleji* Sittichaya & Williams, sp. nov.**
https://zoobank.org/53BF13DA-3D42-4AA5-AE12-766BE0F2FE05
Fig. 3

Material examined. *Holotype*: ♀, Thailand, *Ubon Ratchathani Province*, Khong Chiam District, Khao Phan Bok, Mekong river, 140 msl., N152708.33 E1053545.96, 09.ii.2016. W. Jaitrong leg. (THNHM). ***Paratype*:** ♀, Thailand, *Loei Province*, Phu Ruea District, Waranya Resort 17.460–101.355, 25–26.III.2019, K. Williams, S. Puttasok, K. Thoawan, R. Malee and N. Thaochan leg. (1♀ CSCA).

Diagnosis. Female. This species can be separated from other *Mickelomyrme* by the unique metasomal setal pattern: the T2 posterior fringe is has a mesal patch of white setae and T3–5 have the setae mostly whitish. The following characters are also diagnostic: the vertex has sparse whitish setae; the mesosoma is uniformly orange-brown

without distinct whitish setal patches; the scutellar scale is transversely arcuate, ~6 punctures wide, with transverse wavy carinae anterior and posterior to the scale; the T2 disc is convex and the cuticle is uniformly blackish beneath the white setal spots; and the pygidium is elongate triangular with most striae continuous nearly to the apical margin. Body length 3.5–4.5 mm. **Male.** Unknown.

Description. Female. Body length 3.5 mm. ***Coloration*.** Head dark brown except antennal tubercle, malar space, clypeus, mandibular bases, scape and pedicel bases apparently paler brown. Mesosoma orange-brown. Legs brown except pro-, meso- and metacoxae paler. Metasoma dark brown except T1 orange-brown, S1–6 paler brown. Body setae generally sparse except T3–4 apically. Setal color generally whitish, except mesosoma dorsum and T2 aside from setal patches covered with dark-brown appressed setae; T2 disc with three large white ovate setal patches; T2 fringe white mesally; T3–6 setae almost entirely whitish. ***Head*.** Width behind eye subequal to mesosoma width. Frons, vertex, and gena punctures small, widely separated. Mandible apex apparently unidentate. Clypeus with transverse entire carina carina, basomedial portion triangulate narrow with a small, median tubercle. Antennal scrobe without dorsal carina. Antennal tubercle smooth. Genal carina obliterated. F1 1.6× pedicel length, F2 1.2× pedicel length. ***Mesosoma*.** Length 1.4× width. Dorsum with shallow hexagonal punctures, punctures on apical onefifth very shallow obscure, deeper and more prominent posteriorly. Side of mesosoma smooth and shining, upper portion sparsely covered with shorter setae, lower portion with dense white long setae. Mesopleural lamella absent. Humeral carina weakly developed and obliterated dorsally. Ratio of width of humeral angle, anterior spiracle, narrowest point of mesonotum, propodeal spiracle, and widest point of propodeum 46:52:44:46:48. Scutellar scale transversely arcuate, ~6 punctures wide, with transverse wavy carinae anterior and posterior to scale. Posterior propodeal face; upper portion areolate, lower portion shagreened without punctures. Lateral and posterior propodeal faces not separated by carina. Metatibio-tarsal ratio 39:20:12:9:6:6. ***Metasoma*.** T1–5 with small dense punctures. S1 with simple longitudinal carina. T2 felt line 0.56× T2 total length. T6 with elongate triangular pygidial plate, with sub-parallel striae (~14 near base), mostly reaching apex. S6 posterior margin narrowly emarginate.

Male. Unknown.

Variation. The paratype is 4.5 mm in length and has slightly a wider patch of blackish setae mesally on T5.

Distribution. Thailand (Loei, Ubon Ratchathani)

Etymology. The name honors Arkady S. Lelej, who described the genus and for his great contributions to modern velvet-ant taxonomy.

Remarks. In the key to female velvet ants in southern Thailand (Williams et al. 2019), this species terminates at couplet 17 because the metasomal coloration does not match any of the species. It terminates at couplet 2 in the key to East Asian *Mickelomyrme* species (Lelej 1996) for the same reason. Structurally, this species seems most similar to *M. kinguri* Williams in Williams et al,. 2019 or *M. puttasoki* Williams in Williams et al. 2019, based on the distinct pygidial striae. The pygidial shape is somewhat intermediate

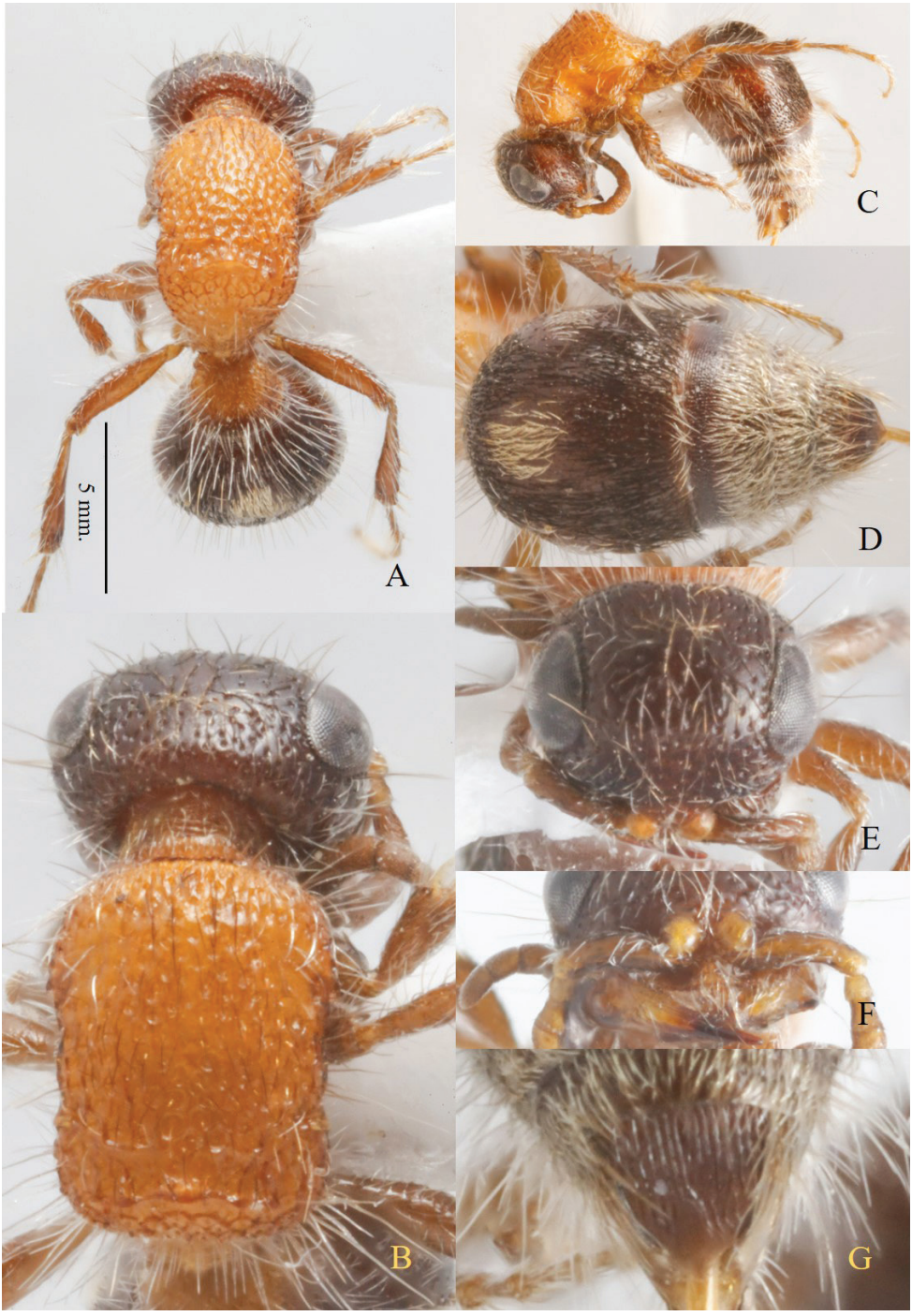

Figure 3. *Mickelomyrme leleji* sp.nov., holotype, female **A** dorsal view **B** mesosoma dorsum **C** lateral view **D** metasomal terga **E** vertex **F** clypeus **G** pygidium.

between those species, being elongate triangular (broadly triangular in *M. puttasoki*, elongate ovate in *M. kinguri*). Unlike those species, the mesonotum is uniformly orange-brown (pronotum largely blackened in *M. kinguri* and *M. puttasoki*); the mesonotum lacks distinct white setal patches (present in *M. kinguri* and *M. puttasoki*), the T2 disc patches are composed of concolorous whitish setae (mesal T2 disc patch yellowish, orange, or absent in *M. kinguri* and *M. puttasoki*), and the T2 fringe has a whitish mesal setal patch (T2 fringe entirely black in *M. kinguri* and *M. puttasoki*). Additionally, unlike *M. kinguri*, the head is dark brown (pale orange-brown in *M. kinguri*) and, unlike *M. puttasoki*, the pygidial striae are sub-parallel (striae posteriorly divergent in *M. puttasoki*).

Genus *Nordeniella* Lelej, 2005

Nordeniella dokbua Sittichaya & Williams, sp. nov.

https://zoobank.org/71FB56B1-41DC-4FA9-A135-7FF053678485
Fig. 4

Material examined. ***Holotype*****:** ♀, Thailand, *Ubonratchatthani Province*, Sirinthon district, 18.VII. 2002. W. Jaitrong. (THNHM). ***Paratypes*** 4♀, Thailand, *Nakhon Rathasima Province*, 5.7 km N Muak Lek, 14.717 –101.188, 29.III.2019, K. Williams, S. Puttasok, K. Thoawan, R. Malee and N. Thaochan (2♀ CSCA); *Saraburi Province*, Mai Ngerm Thong Resort, 7 km NW Muak Lek, 14.711 –101.165, 15.III.2019, K. Williams, S. Puttasok, K. Thoawan, R. Malee and N. Thaochan (2♀, CSCA).

Diagnosis. Female. This species can be recognized by the following combination of characters: head and metasoma with cuticle black; mesosoma with cuticle orange-brown; propodeum clearly wider than pronotum; T2 disc with large mesal spot of whitish setae basally. Body length 4.0–5.5 mm.

Male. Unknown.

Description. Female. Body length 5.9 mm. ***Coloration.*** Head black, except antennal tubercle, mandible, scape, and pedicel largely orange-brown. Mesosoma entirely orange-brown, except legs moderately darkened apically. Metasoma black, except S1 and T1 basally orange-brown and T2–3 obscurely yellow-brown beneath white setal markings. Body setae generally sparse and silvery, except vertex and mesosomal dorsum with scattered silver and erect blackish setae; T2 disc, T4, and T5 setae dense black; and T2 basomedial spot, T2 apex, T3 entirely, and T6 basal tuft with dense whitish silver setae. ***Head.*** Width behind eye 1.15× pronotal width. Frons, vertex, and gena punctures dense to confluent. Mandible apex tridentate. Clypeus with transverse truncate lamella; basomedial portion convex, densely punctate, with obscure longitudinal carina basally. Antennal scrobe with dorsal carina. Antennal tubercle shagreened with a few scattered punctures. Genal carina weakly defined, forming raised tooth with hypostomal carina. F1 1.4× pedicel length, F2 1.4× pedicel length. ***Mesosoma.*** Length 1.1× width (width measured at propodeum). Dorsum of mesosoma with coarse confluent punctures. Side of mesosoma with scattered micropunctures with short setae, ventral portion of meso- and metapleuron areolate, posterior portion of propodeal

Figure 4. *Nordeniella dokbua* sp.nov., holotype, female **A** dorsal view **B** posterior propodeal face and metasoma dorsum **C** lateral view **D** mesosoma **E** frons and vertex **F** clypeus **G** pygidium.

side with obscure reticulations. Mesopleural lamella absent. Humeral carina distinct, arcuate. Ratio of width of humeral angle, anterior spiracle, midpoint of mesonotum, propodeal spiracle, and widest point of propodeum 60:69:70:73:75. Scutellar scale obliterated. Posterior propodeal face areolate. Lateral and posterior propodeal faces separated by interrupted wavy carina. Metatibio-tarsal ratio 79:33:23:17:13:11. ***Metasoma.*** Terga 1–5 with small dense punctures, sparser on T1, confluent on T2. S1 with long simple longitudinal carina. T2 felt line 0.25× T2 total length. T6 convex, mostly smooth. S6 posterior margin bidentate.

Male. Unknown.

Distribution. Thailand (Nakhon Ratchasima, Saraburi Provinces and Ubonratthani province).

Etymology. This name refers to an old name and meaning for Ubonratthani province (*dokbua*=water lily), the holotype specimen locality. Treat as a noun in apposition.

Remarks. In the key to female velvet ants in southern Thailand (Williams et al. 2019), this species keys out to *N. maleeae* Williams in Williams et al. 2019, the only other *Nordeniella* species known from the eastern Oriental region. *Nordeniella dokbua* can be separated from that species by having the propodeum clearly wider than the pronotum (mesosoma equally wide throughout its length in *N. maleeae*). These two Thai species can be separated from the known Indian and Sri Lankan *Nordeniella* females by having the head black, the head is reddish in western Oriental *Nordeniella* (see André 1894, 1907; Turner 1911). These Thai species can be separated from the Australasian N. sumbawaensis Okayasu, 2022 by having the basomesal clypeal carina indistinct and restricted to the basal portion and the metasoma dark brown to black; N. sumbawaensis has the basomesal clypeal carina distinct and continuous to the anterior margin, and the metasoma with obscure metallic blue lustre (Okayasu 2022).

Genus *Smicromyrme* Thomson, 1870

Smicromyrme songkhwae Sittichaya & Williams, sp. nov.

https://zoobank.org/601D3E34-F438-4908-BD33-660110C129A6
Fig. 5

Material examined. ***Holotype*** ♀, Thailand, *Phitsanulok Province*, Chat Trakan District, Phu Soi Dao National Park, Dry evergreen forest, 21.IV.2002. W. Jaitrong. (THNHM).

Diagnosis. Female. This species can be recognized by the following combination of characters: the mesosoma is longer than wide; the scutellar scale is transversely arcuate, ~6 punctures wide; the T2 disc has a single mesal spot; the T2 posterior fringe and T3 are covered with whitish setae above lighter yellow-brown cuticle; the pygidium is elongate ovate with ~10 weakly incurved striae mostly ending before pygidial midpoint. Small species, 2.9 mm. long.

Male. Unknown.

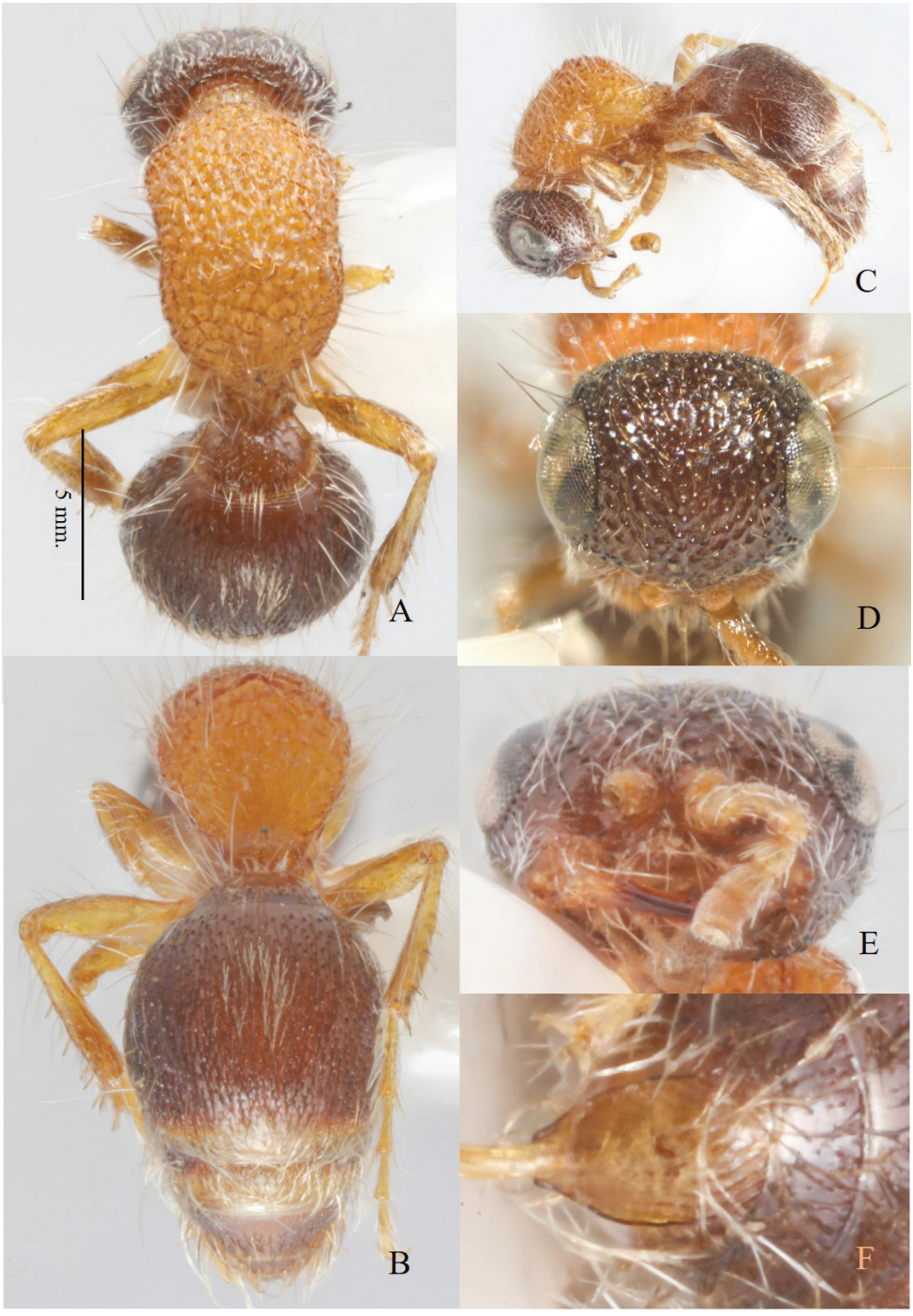

Figure 5. *Smicromyrme songkhwae* sp.nov., holotype, female **A** dorsal view **B** posterior propodeal face and metasoma dorsum **C** lateral view **D** frons and vertex **E** clypeus **F** pygidium

Description. Female. Body length 2.9 mm. ***Coloration.*** Head dark brown except frons, base of mandible, clypeus, and antenna yellowish brown; mesosoma orange-brown; legs yellowish brown, darker marginally and apically; metasoma dark brown, somewhat paler ventrally, except T1 and S1 orange-brown, and T2 posterior fringe and T3 mostly yellow brown. Body setae generally sparse and silvery, except appressed setae on T2and T4–5 setae blackish, and T2 basomedial spot, T2 apical margin, and T3 entirely whitish. ***Head.*** Width behind eye 1.13× mesosoma width. Frons, vertex, and gena punctures tightly confluent. Frons surface rugose, forming transverse wavy carinules. Mandible apex apparently unidentate. Clypeus with obscure transverse carina; basomedial portion with weak flat tubercle. Antennal scrobe without dorsal carina. Antennal tubercle with weak punctures. Genal carina obscure, not reaching hypostomal carina. F1 1.0× pedicel length, F2 1.15× pedicel length. ***Mesosoma.*** Length 1.05× width. Dorsum of mesosoma with small coarse confluent punctures. Mesopleural lamella absent. Humeral carina present, weakly developed. Ratio of width of humeral angle, anterior spiracle, narrowest point of mesonotum, propodeal spiracle, and widest point of propodeum 38:40:37:38:39. Scutellar scale transversely arcuate, ~6 punctures wide, forming transverse isosceles carina in posterior view. Posterior propodeal face with upper portion areolate, lower portion shagreened without punctures. Lateral and posterior propodeal faces not separated by carina. Metatibiotarsal ratio 34:18:11:9:7:6. ***Metasoma.*** T1–5 with small dense punctures, sparser on T1. S1 without longitudinal carina. T2 felt line 0.40× T2 total length. T6 with long ovate pygidial plate, with ~10 laterally incurved striae mostly ending before pygidial midpoint. S6 posterior margin bidentate.

Male. Unknown.

Distribution. Thailand (Phitsanulok Province).

Etymology. This name refers to an old name for Phitsanulok Province (*song*=two in the Thai numeral system and *khwae*=tributary), the type specimen locality. Treat as a noun in apposition.

Remarks. In the key to female velvet ants in southern Thailand (Williams et al. 2019), this species terminates at couplet 25 with *S. helarctos* Williams in Williams et al. 2019) and *S. borkenti* Williams in Williams et al. 2019; currently *Andreimyrme borkenti*, see Okayasu et al. 2021). Unlike *A. borkenti*, the scutellar scale is wide and the pygidial plate is widest mesally. Furthermore, this species does not belong in *Andreimyrme* based on the unidentate mandible, unarmed prementum, and wide distinct scutellar scale (Okayasu et al. 2021b).

Based on similarities in the scutellar scale, pygidial shape, and light brown cuticle of the T2 fringe and T3, this species is apparently closely related to *S. helarctos*. Unlike that species, *S. songkhwae* has the mesosoma uniformly orange-brown (blackened laterally in *S. helarctos*) and longer than wide (as wide as long in *S. helarctos*). Additionally, the pygidial striae in *S. songkhwae* are fainter and restricted to the anterior half of the pygidial plate (pygidial striae distinct and extending beyond midpoint in *S. helarctos*).

Discussion

The four species discussed above are known from females only. In fact, of the 68 species known from Thailand, 49 (~72%) are known from females only; another 11 (~16%) are known from both sexes, while eight (~12%) are known from males only (Lelej 2005; Williams et al. 2019; Okayasu et al. 2021b). Based on these data, male velvet ants in Thailand are much more poorly understood than females.

Of the 83 specimens housed in the Thailand Natural History Museum (Table 1), seven belonged to the four species discussed above. Fifty-eight specimens were identified to species that are already known from Thailand, mainly species that also occur in southern Thailand and were treated in Williams et al. (2019). The remaining 24 specimens could not be reliably identified to species, because they were unassociated males in genera that have not been adequately revised (20 specimens) or females that were potentially conspecific with other Oriental species (4 specimens). Without molecular data or structural comparisons of larger series with both sexes, we refrain from describing these forms as new or attributing them to already recognized taxa. Altogether, 20 new Region records and 47 new Province records were found in the material of the Thailand Natural History Museum (Table 1).

Table 1. Synoptic list of velvet ants in the Thailand Natural History Museum. Asterisks (*) represent new distribution records for the Region or Province.

Species	Sex	Thai-Distribution
Andreimyrme borkenti (Williams in Williams et al. 2019)	f	E*: Chachoengsao*
Andreimyrme substriolata (Chen, 1957)	f	NE*: Mukdahan*
Bischoffitilla perakensis (Pagden, 1934)	f	C: Pathum Thani*
Bischoffitilla selangorensis (Pagden, 1934)	f	N*: Chaing Mai*, Lampang*; NE*: Loei*, Nakhon Ratchasima*
Bischoffitilla cf. mammalifera (Chen, 1957)	m	S: Nakhon Si Thammarat
Cockerellidia sohmi (Cockerell, 1928)	m	E*: Chachoengsao*
Ctenotilla guangdongensis Lelej, 1992	f	NE: Nakhon Ratchasima*
Eotrogaspidia oryzae (Pagden, 1934)	f	C: Pathum Thani*
Eotrogaspidia auroguttata (Smith, 1855)	f	W*: Tak*; C: Pathum Thani*
Krombeinidia cf. subfossata (Chen, 1957)	m	E: Chonburi; S: Nakhon Si Thammarat
Mickelomyrme isora (Cameron, 1900)	m	N: Chiang Mai
Mickelomyrme leleji Sittichaya & Williams, sp. nov.	f	NE: Ubon Ratchathani
Mickelomyrme pusillaeformis (Hammer, 1962)	f	N: Chiang Mai
Mickelomyrme sp.	m	E: Chonburi
Mutilla harmandi André, 1898	f	NE: Kalasin*; S*: Nakhon Si Thammarat *
Nemka conjugenda (Magretti, 1892)	f	N: Lampang*; NE*: Ubon Ratchathani *
Nemka cf. conjugenda (Magretti, 1892)	f/m	NE: Ubon Ratchathani
Nordeniella dokbua Sittichaya & Williams, sp. nov.	f	NE: Ubon Ratchathani
Odontomutilla cf. haematocephala (André, 1896)	m	S: Nakhon Si Thammarat
Odontomutilla sp.	m	E: Chachoengsao
Orientidia manleyi Williams in Williams et al. 2019	f	NE*: Nakhon Ratchasima *; E*: Chonburi*
Orientidia thoawanae Williams in Williams et al. 2019	f	E*: Chonburi*

Species	Sex	Thai-Distribution
Orientilla vietnamica Lelej, 1979	f/m	E*: Chonburi*
Physetopoda thai Lelej, 1995	f	N: Chaing Mai; C: Saraburi*; E: Chachoengsao*
Promecilla decora (Smith, 1879)	f	E: Chonburi*
Promecidia cf. birmanica (de Dalla Torre, 1897)	m	NE: Nakhon Ratchasima; E: Chachoengsao, Trad
Sinotilla cyaneiventris (André, 1896)	f	C: Pathum Than*
Smicromyrme songkhwae Sittichaya & Williams, sp. nov.	f	N: Phitsanuklok
Smicromyrme triguttatus Mickel, 1933	f	N: Chiangrai*; NE*: Nakhon Ratchasima *
Smicromyrme cf. dardanus (Smith, 1857)	m	NE: Nakhon Ratchasima
Trogaspidia fuscipennis (Fabricius, 1804)	f	NE*: Burirum*
Trogaspidia pagdeni (Mickel, 1933)	m	NE*: Burirum*, Kalasin*; W*: Tak*; E: Chonburi*, Rayong*; S: Nakhon Si Thammarat*
Trogaspidia pittsi Williams in Williams et al. 2019	f	N: Mae Hong Son *
Trogaspidia wilsoni Williams in Williams et al. 2019	f/m	E*: Trad*; NE*: Burirum*, Chaiyaphum*
Trogaspidia cf. rhea (Mickel, 1933)	f	N: Lampang
Trogaspidia sp.	m	E: Sa Kaeo
Wallacidia oculata (Fabricius, 1804)	m	N: Lampang; E: Chachoengsao*
	f	N: Chaing Mai, W*: Tak*; NE: Burirum; E: Chachoengsao*; S: Phuket*
Zeugomutilla pycnopyga Chen, 1957	m	NE*: Chachoengsao*
Zeugomutilla saepes (Chen, 1957)	f	E: Sa Kaeo *; NE: Chachoengsao*

Note. See Fig. 1 for the acronym of Thailand parts: N – northern, N-E – north-eastern, C – central, E – eastern, S – southern.

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RESEARCH ARTICLE

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Leioproctus zephyr Prendergast (Hymenoptera, Colletidae, *Leioproctus*), an oligoletic new bee species with a distinctive clypeus

Kit S. Prendergast[1]

1 *School of Molecular and Life Sciences, Curtin University, Kent Street, Bentley, Perth Western Australia, 6102, Australia*

Corresponding author: Kit S. Prendergast (kit.prendergast21@gmail.com)

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Abstract

A new species *Leioproctus zephyr* (Hymenoptera: Colletidae) is described from both sexes. *Leioproctus zephyr* **sp. nov.** is remarkable in featuring a large longitudinal ridge on the clypeus. This diagnostic morphological feature present in both sexes, along with various other distinctive characters including the male genitalia, female hind-tibial spur, and glossa morphology, clearly distinguish this species from all other *Leioproctus*. Along with these unique traits, *L. zephyr* cannot be classified into any of the existing subgenera of *Leioproctus*, sharing some, but not all, of the characters of the subgenera *Ceratocolletes*, *Charicolletes*, *Protomorpha* and *Odontocolletes*. DNA barcoding with the CO1 gene confirmed the sexes belonged to the same species and it did not match any previously barcoded species. This species is restricted to native vegetation remnants in the southwest Western Australian biodiversity hotspot, and is highly specialised, foraging only on a few species in the genus *Jacksonia* (Fabaceae). The unusual clypeus may be an adaptation for foraging on the keeled papilionaceous flowers. The limited number of sites this species has been collected from and its oligolectic diet suggest *L. zephyr* should be considered to be a species of conservation concern. Further taxonomic research is required to determine the phylogenetic position of this unusual *Leioproctus*.

Keywords

Australia, biodiversity hotspot, colletid, DNA barcoding, new species, specialist

Introduction

The genus *Leioproctus* Smith 1853 (Colletidae Lepeletier 1841), as currently described, is a highly diverse, speciose taxon (Almeida and Daforth 2009; Engel and Gonzalez 2022). In Australia, it is distributed throughout most of the continent, and the most recent guide to native bees of Australia divides this genus into 24 subgenera, with 193 named species (Houston, 2018), yet with hundreds awaiting description (Houston, personal comm.). New species are continually being described (e.g., Batley and Popic 2013; Leijs et al. 2018).

The systematics of *Leioproctus* requires clarification (Packer 2006; Almeida et al. 2019). A phylogeny combining morphological and molecular data of currently recognised subgenera is yet forthcoming. Even *Leioproctus* s. str. appears to be paraphyletic based on molecular phylogenies (Almeida and Danforth 2009; Almeida et al. 2019). In the magnum opus on the classification of native bees, Michener (2007) drew attention to how the sub-genera of Australian *Leioproctus* can be ill-defined, with partial intergradation among some taxa, as evident in the last revision of seven subgenera of *Leioproctus* (Maynard, 2013), A comprehensive and more thorough understanding of the Australian subgenera is hampered by the sheer diversity of many unusual species that are undescribed, and even of those that are described, many are described from only one sex. It is clear that an updated classification for the Australian Neopasiphaeinae is needed (Almeida et al. 2019; Engel and Gonzalez 2022). A new species, with a distinctive clypeus, that does not fit neatly into an existing subgenus is described, including its CO1 barcode, and data on its restrictive foraging and distribution range. This description will contribute to documenting and describing the diversity of Australian Neopasiphaeinae.

Materials and methods

Specimens involved in the description were collected by the author with an entomological sweep-net (bag mesh size 0.9 × 0.3mm, Australian Entomological Supplies Pty Ltd) during surveys to sample native bee assemblages in residential gardens and bushland remnants within the urbanised region of the southwest Western Australian biodiversity hotspot (Prendergast et al. 2022; Suppl. material 1). The unusual appearance of this species and an inability to key the species out to subgeneric or species level from published keys led the author to contact Dr Terry Houston of the WA Museum to inquire whether he had seen this species before. This species was confirmed to be undescribed, lodged in the WA Museum and catalogued as *Leioproctus* (*Protomorpha*?) F188/M173. Further consultation with Dr Glynn Maynard who undertook the most recent revision of Australian *Leioproctus* (in part, Maynard 2013) confirmed that these specimens did not match described species.

Standard melittological terminology is used to describe the morphology (Michener, 2007). The following standard acronyms are used (following Michener (2007), Houston (1990) and Leijs et al. (2018)): **HL** head length; **HW** head width; **AOD** antennocular distance; **IAD** interantennal distance; **OOD** ocellocular distance; **OAD** ocelloantennal distance; metasomal sterna and terga are denoted **S**[segment number] and **T**[segment number], and flagellomeres are denoted **F**.

Following Packer (2006), the relative diameter and spacing for punctures (sculpture) are denoted by d and i, respectively. Other types of surface sculpturing follow Houston (Houston, 1975), as used in (Leijs et al. 2018). Measurements of key morphological features and relative head measurements were made on five specimens of each sex and averaged, and given in millimetres (Suppl. material 2). Specimens were observed with a Leica M205 C stereomicroscope, and measurements were made on high-resolution images taken with the same stereomicroscope and using the Leica auto-montage image stacking software. Images of key features were taken using a Nikon camera with Passport and Helicon image stacking software.

A sample (hind femur) of the female and male type and allotype were submitted to BOLD (Barcode of Life Database) for DNA barcoding using the cytochrome c oxidase subunit 1 (CO1) gene. The DNA barcode sequence, and other specimen information associated, can be accessed in BOLD via: as part of the Australasian and Pacific bee fauna Project (MSAPB): http://www.boldsystems.org/index.php/MAS_Management_DataConsole?codes=MSAPB.

The sequences were obtained from Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph, Guelph, Ontario, Canada. Standard DNA sequencing protocols were carried out by CCDB (available online at: http://www.ccdb.ca/resources.php), using the PCR primers LepF1/LepR1. The barcoded vouchers are housed at the Museum of Western Australia. BOLD delineates molecular operational taxonomic units (MOTUs), which typically are in close concordance with species delineations based on traditional methods (Schmidt et al. 2015). The barcode index number (BIN) (Ratnasingham and Hebert 2013) is automatically assigned to a MOTU, which is incorporated into BOLD.

To ascertain the position of this species in relation to other *Leioproctus* and infer its placement within one of the described subgenera, a Taxon ID tree was created in BOLD using all specimens in the AUSBS project. The Taxon ID tree procedure uses varied distance metrics to generate a neighbour-joining (NJ) tree based on nucleotide similarity in the barcoded COI gene. Sequence alignment is automatically handled, with the Kimura 2 Parameter as the default distance model.

Taxonomy

Family Colletidae Lepeletier, 1841
Subfamily Neopasiphaeinae

Genus *Leioproctus* Smith, 1853

Type species. *Leioproctus imitatus* Smith, 1853.

Leioproctus zephyr sp. nov. can be assigned to the genus *Leioproctus* based on the following diagnostic features: facial fovea broad, moderately impressed; mandibles with only one subapical tooth, with the rutellum the largest and longest; labrum more than three times as wide as it is long; stigma well-developed, tapering apically to marginal

vein, well within the marginal cell; propodeum with sloping, subhorizontal basal zone; inner hind tibial spur of the female pectinate, not crowded; basitibial plate of the female well-defined.

This species cannot be clearly assigned to the currently recognised subgenera of *Leioproctus*. Although this species shares various features of the two species currently assigned to the subgenus *Ceratocolletes* Michener, 1965, *L. zephyr* diverges in details of the hind-tibial spurs, propodeum, and male genitalia, and whilst it shares similarity in the clypeus morphology of *L.* (*Ceratocolletes*) *antennatus* Smith, 1879, it lacks the modified antennae of the male. The species also shares some diagnostic characters of *Protomorpha* Rayment, 1959, *Charicolletes* Maynard, 2013, and *Odontocolletes* Maynard, 1997, such as the malar space absent; strong punctures on the dorsal surface of the mesosoma with smooth interspaces; terga with pale apical hair bands; flagellum short, middle segments mostly broader than long or scarcely longer than broad; clypeus and supraclypeal area not flat, usually punctate, suture separating the m distinct; S7 of the male has two apical lobes. However, it lacks other diagnostic features, and has features unique to it and absent in these subgenera. On this basis, *L. zephyr* cannot be confidently assigned to any of the current subgenera of *Leioproctus.* This species may represent a new subgenus of *Leioproctus*, however but a revision of these subgenera and species currently assigned to them is required.

Leioproctus zephyr **Prendergast, sp. nov.**
https://zoobank.org/7C496A48-0D63-43AF-802A-9B8C5B144BF8
Figs 1–7 (female), 8–15 (male)

Material examined. ***Holotype*** female, allotype male, 60 additional male ***paratypes*** and 52 female paratypes : Australia, Western Australia.

Type-locality. Australia, Western Australia: Western Australia, Star Swamp; 31.8575°S, 115.7602°E; alt. *ca.* 11 m, Banksia woodland, collected with an entomological sweepnet, foraging on *Jacksonia sericea*, 16 Dec 2017, K. Prendergast.

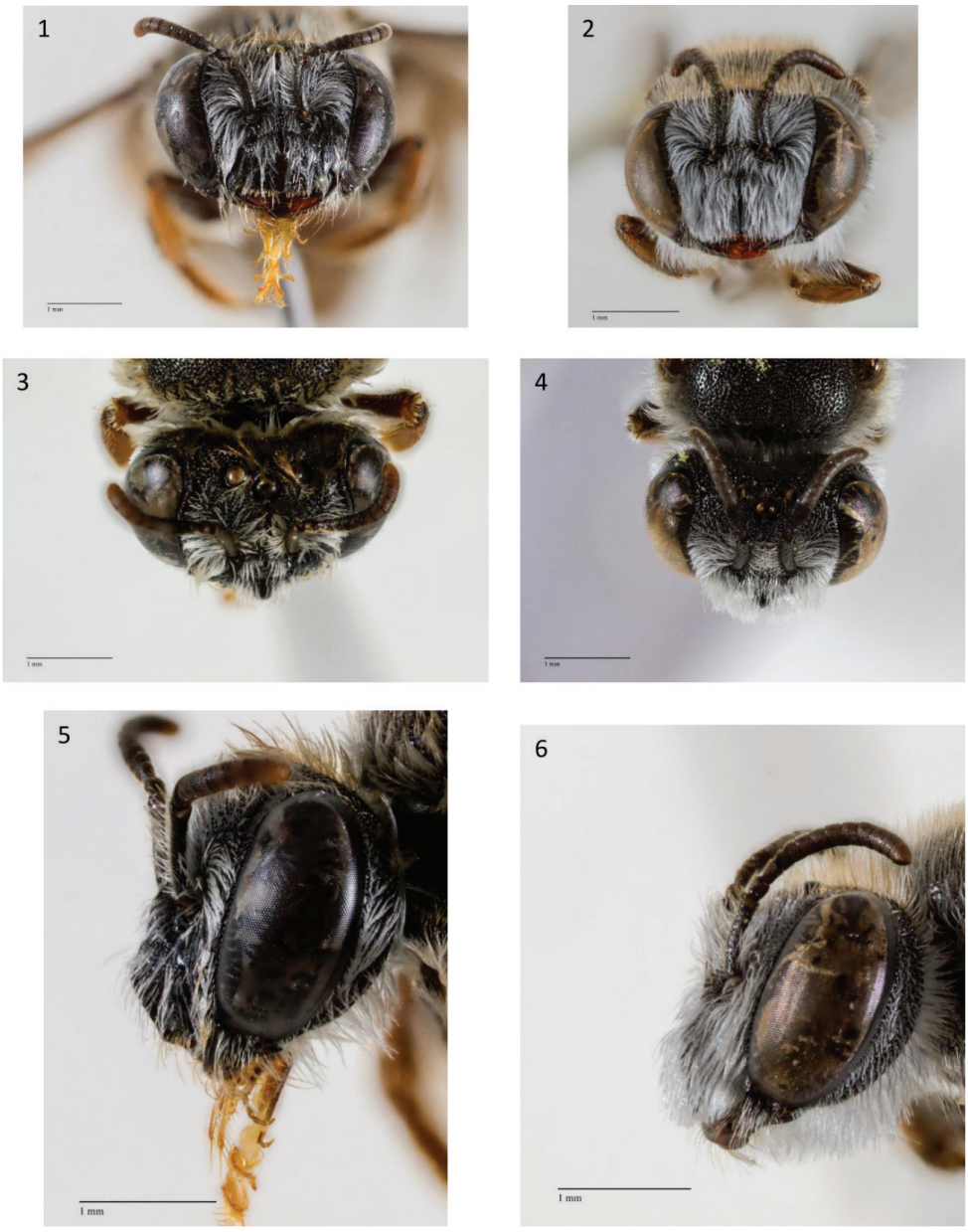
Type-specimen. Holotype female, pinned, with the printed label: "WA: Western Australia, Star Swamp 31.8575°S, 115.7602°E 16/12/2017 Sweepnet AM 0003436 K. S. Prendergast" (WAM).

Type material. ***Holotype*** Australia • 1 ♀, holotype; Western Australia, Western Australia, Star Swamp; 31.8575°S, 115.7602°E; alt. *ca.* 11 m; 16 Dec. 2017; K. S. Prendergast leg.; sweepnet; KSP code 003436. BOLD DNA barcode: BOLD:AEC1713 (WAM).

Other material. ***Allotype*** Australia • 1 ♂; Western Australia, Star Swamp; 31.8575°S, 115.7602°E; alt. ca. 11 m; 3 Dec. 2016; K. S. Prendergast leg.; sweepnet; KSP code 000261. BOLD DNA barcode: BOLD:AEC1713 (WAM).

Paratypes. Paratypes listed in Suppl. material 1. Paratype used in description of male S7, S8 and genital capsule: • 1 ♂ same data as for allotype.

All specimens were collected with an entomological sweep-net by K. Prendergast (Suppl. material 1).



Figures 1–6. *Leioproctus zephyr* sp. nov., emphasising the protruberant clypeus. Frontal view **1** female **2** male; dorsal view **3** female **4** male; lateral view **5** female **6** male. Photographs by N. Tatarnic.

The holotype, allotype and paratype specimens are bequeathed to the Western Australian Museum.

Diagnosis. *Leioproctus zephyr* is distinguished from all other species of the genus in that both sexes are easily distinguished by the presence of a large medial ridge extend-

ing the length of the clypeus with a large, prominent protuberance on the upper half (Figs 1–6). Females are unique in having a pectinate inner hind tibial spur featuring a blunt apex (Fig. 14). Male genitalia are also unique in S7 with two broad, flat apical lobes orientated laterally, fringed with hair, with particularly long hairs on the apical edge; posterior lobes of S7 extended laterally with broad, flat flanges (Fig. 22); S8 with large lateral lobes extending beyond the breadth of the apical process; apical process broad, somewhat narrowed towards base, and hirsute, with apex expanded, rounded and membranous (Fig. 28). The glossa of both sexes are also distinctive, being more bifurcated than is typical for most Australian *Leioproctus.* Additionally, in *L. zephyr*, labial and maxillary palps are comparatively short, as they do not reach the base of the prementum or apex of paraglossa, respectively; this contrasts with most *Leioproctus* where the labial and maxillary palps extend just beyond apex of the glossa..

Description. Female (Figs 7–14):

***Dimensions*:** Total body length 6.2 mm, HW 2.2 mm, ITD 1.6 mm (variation: total body length 6.0–6.9 mm, HW 2.1–2.2 mm, ITD 1.5–1.6 mm (n = 5)).

***Colouration*:** Non-metallic black; integument of head black; facial protuberance black, but sometimes with reddish tinge tip of protuberance; mesosoma black; terga and sternum black apically through to brown on posterior margin; apical impressed area of T1 brown; T6 and pygidial plate brown; legs and tarsi brown; wings dusky, semi-opaque very dark brown with wing veins very dark brown; scape and flagellum black except for F10, and part of F9, mandibles black basally, rest mostly testaceous, except apex black.

***Pubescence*:** White pubescence on face around antennal sockets covering paraocular area and gena, sides of thorax; sparser setae on supraclypeal area, and each side medial carina along the transverse portion of the epistomal suture. Short, fine sparse pale orange hairs on vertex, mesosomal dorsum (mesoscutum, scutellum, and propodeum), thicker, longer on metanotum; thick dense cream hairs on pronotal lobe; sparse long pale brown hairs on T3 and T4 on lower half, incomplete medially; on T5 gold-brown hairs very dense; prepygidial fimbria thick, dense pale brown hairs either side of pygidial plate. Apical fringe of long gold-brown hairs towards sides of S1-S6. Shorter orange hairs on legs, longer white hairs on posterior margin of forefemur. Hairs on forelegs long and dense, especially on basitarsus; midtarsal hairs branching in a V-pattern. Pubescence never obscuring integument below.

***Sculpture*:** Head, mesoscutum, and scutellum with large, deep, close punctures i=1d; punctures open, sparse on clypeus i=5d, except impunctate on median carina; antennal scape fine, close punctures i=1d; metanotum and propodeum with small, close punctures; propodeal triangle with deep, sparse punctures apically i=3d, lower propodeal triangle imbricated (Fig. 11); terga with shallow, minute, close punctures i=1d; fore-, mid- and hind- femur, tarsus and basitarsus with longitudinal, large, irregular striae i=1d.

***Structure*: *head*:** face wider than long (1.6×); ocelloccipital area weakly concave; mouthparts distinctive: galea large and strongly bifurcate, each fork reaching just above the base of the mentum and with long, golden hairs; mentum and prementum

Figures 7–14. *Leioproctus zephyr* sp. nov., female. **7** Head, frontal view **8** Lateral habitus **9** ventral view **10** dorsal view **11** propodeal triangle **12** pygidial plate **13** basitarsal plate **14** inner hindtibial spur. Scale bars: 1 mm (**1–5**); 0.5 mm (**6–7**). Photographs by K.S. Prendergast.

approximately equal in length; maxillary palpus extremely short, not reaching base of prementum and labial palps short, not reaching apex of paraglossa; paraglossa large, triangular; glossa strongly bifurcate, more so than in most Australian *Leioproctus*, with a long, dense apical fringe; clypeus convex, broader than long, with a medial longitudinal ridge and distinct protuberance in middle of upper half, protuberance triangular in profile, apex above clypeal midlength and almost one quarter length of head, with smaller protuberance at base of median ridge; clypeus lateral to this medial ridge and below epistomal suture convex; supraclypeal area elevated, surface concave, somewhat triangular; frontal line continuous with median ridge strongest at level of antennal sockets, extending to the medial ocellus; compound eyes slightly more convergent

below; malar space absent; mandibles bidentate, with the preapical tooth being approximately half length of rutellum; mandibles with acetabular and condylar grooves, outer and condylar ridge absent; facial fovea impressed, smooth, from lower tangent of lateral ocelli extending to level with lower tangent of antennal sockets, forming a triangular shape, broadest at level just below median ocellus, impression deepest adjacent to eye; gena ca. 0.4× as wide as compound eye viewed laterally; scape not attaining median ocellus; F1 length>width, F2-F10 length<width, tip of antennae slightly pointed.

Head measurements: HW 2.14 mm; eye width in profile 0.61 mm; gena width 0.22 mm; eye length 1.25 mm; HL 1.38 mm; clypeus length 0.63 mm; LOD 1.11 mm; UOD 1.20 mm; clypeoantennal distance 0.07 mm; IAD 0.38 mm; IOD 0.38 mm; OOD 0.29 mm; AOD 0.47 mm; OAD distance 0.33 mm (variation: HW 2.08 – 2.15 mm; eye width in profile 0.52–0.62 mm; gena width 0.18–0.26 mm; eye length 1.18–1.26 mm; HL 1.37–1.55 mm; clypeus length 0.46–0.63 mm; LOD 0.45–1.11 mm; UOD 1.14–1.22 mm; clypeoantennal distance 0.15–0.18 mm; IAD 0.36–0.39 mm; IOD 0.31–0.38 mm; OOD 0.30–0.38 mm; AOD 0.47–0.66 mm; OAD distance 0.32–0.40 mm, n = 5).

Relative head measurements: UOD:LOD 1.23; OOD:IOD 0.93; clypeus:HL 0.35.

***Mesosoma*:** overall mesosoma length 2.12 mm; pronotal collar absent; ITD 1.60 mm; mesoscutum length 1.60 mm; mesoscutum width 1.52 mm; metanotum length 0.18 mm; propodeum length 0.41 mm (variation: overall mesosoma length 1.89–2.12 ± 0.03 mm; pronotal collar absent; ITD 1.54–1.61 mm; mesoscutum length 1.00–1.57 mm; mesoscutum width 1.46–1.60 mm; metanotum length 0.14–0.20 mm; propodeum length 0.31–0.50 mm, n = 5).

Forewing with three submarginal cells, with second sub-marginal cell much shorter than the first and third. Propodeal triangle with strong carina, almost vertical.

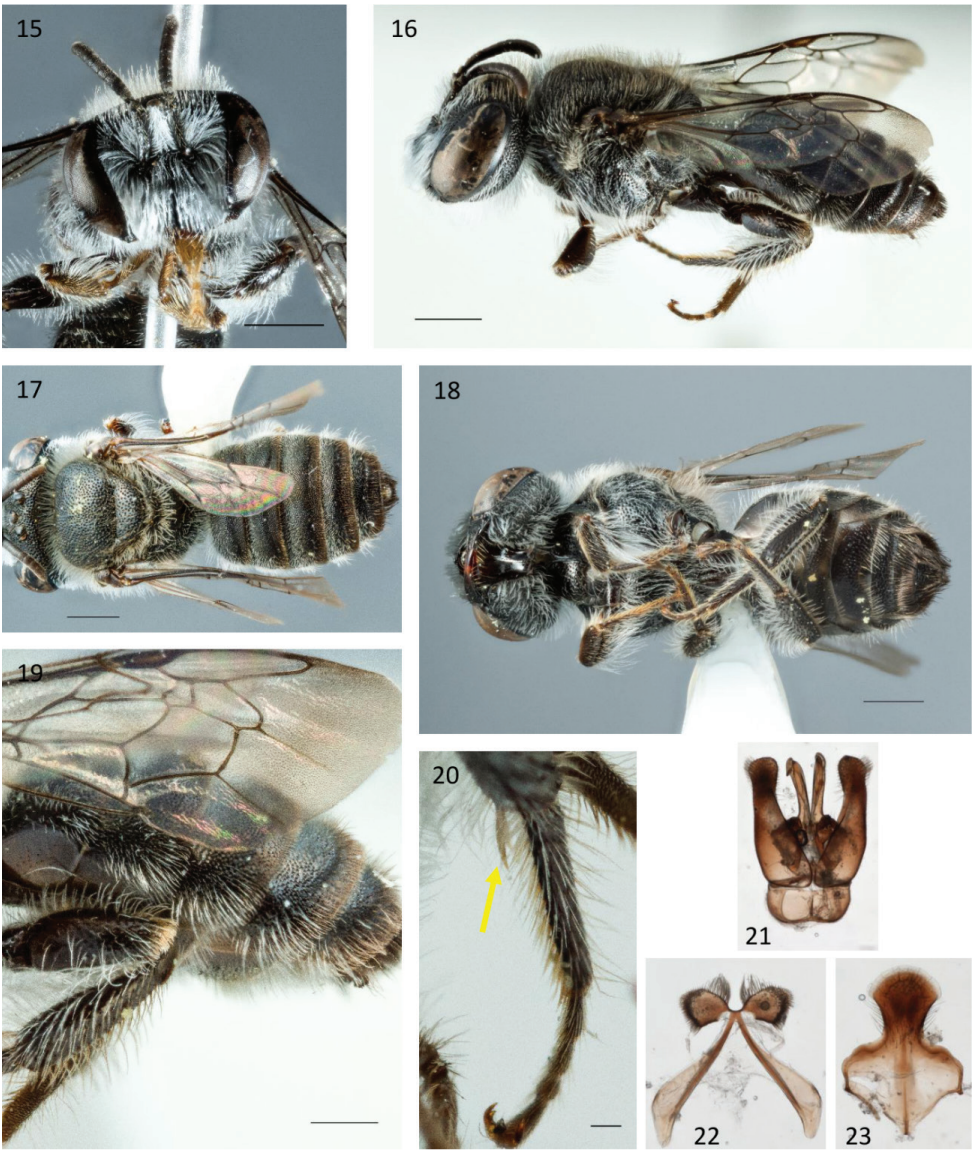
Relative mesosomal structure measurements: mesoscutum length:breadth 0.84; scutellum:mesoscutum 0.28; metanotum:scutellum 0.53.

***Legs*:** tarsal claws on all legs simple; basitibial plate approximately one-quarter as long as basitarsus, oval, concave, covered with dense short orange hairs (Fig. 13); metatibial spur long, almost straight, outer spur with small, dense serrations, inner spur pectinate with four teeth on basal half of the spur, decreasing in length from base to apex, the second tooth from the base thickest, apex of spur rounded (Fig. 14).

***Wings*:** stigma approximately half the length of the marginal cell; marginal cell with apex rounded, curved away from costal wing margin by approximately two vein widths; basal vein slightly curved and at approximately 45° to costal wing margin; three submarginal cells, first longest, and second shortest; first recurrent vein slightly basal to first submarginal cross-vein; jugal lobe of hind wing approximately one-quarter as long as vannal lobe, reaches cu-a vein.

***Metasoma*:** overall metasoma length 3.1 mm (variation: 3.15 ± 0.116 mm); metasoma longer than mesosoma (metasoma:mesosoma 1.55); T1 declivous surface concave with longitudinal medial groove just below point of concavity; anterior declivous surface longer than dorsal horizontal portion; metasoma broadest at second segment, width 1.98mm (variation 1.97 ± 0.014 mm); pygidial plate well-developed, smooth.

Male (Figs 17–23):



Figures 15–23. *Leioproctus zephyr* sp. nov, male **15** head, frontal view **16** lateral habitus **17** dorsal view **18** ventral view **19** forewing vein structure **20** hindleg showing hindtibial spurs **21** male genital capsule (dorsal view) **22** S7 (ventral view) **23** S8 (ventral view). Scale bars: 1 mm. Photographs by K. S. Prendergast, diagrams by K. S. Prendergast.

***Dimensions*:** Total body length 5.01–5.71 mm, HW 1.07–1.97 mm, ITD 1.30–1.41 mm (n = 5).

***Colouration*:** integument black except for foreleg basitarsus which is orange-brown; antennal scape black, flagellomeres 1 and 2 black, flagellomere 3 partly black and partly brown, and flagellomeres 4–11 brown; mandibles black with orange-brown tips; tergites black with posterior margin brown.

***Pubescence*:** Pubescence on face much thicker than female, hairs cover entire head except for carina and protuberance on clypeus; very short, sparse hairs on basal margin of clypeus; pubescence on pronotal lobes not as thick as female; long white hairs on tarsi of fore and mid legs. Orange-brown short hairs on vertex and dorsal region of mesosoma, as in female, but much shorter and sparser, whereas white hairs on metanotum, propodeum, and metepisternum are longer, and feathery; very short brown hairs emerging along posterior region of each tergite, and longer white hairs from the anterior and laterally on each tergite; fringe of white hairs from sternites 1–5, very thick and black-tipped on T6; wings same as female.

***Sculpture*:** similar to female, except legs only have sparse, small punctures.

***Structure – head*:** prominent medial carina on the clypeus with a prominent protuberance on upper half of clypeus, extent of protuberance from face relatively more pronounced than in the female with length of protuberance:length of head 0.29; gena ca. 0.49× as wide as compound eye viewed laterally; eyes converging somewhat below; UOD:LOD 1.21; mandibles similar to female; facial fovea most depressed near eye, narrower than in female oblong in shape.

***Head measurements*:** HW 1.07–1.97 mm; eye width in profile 0.52–0.59 mm; gena width 0.26–0.33 mm; eye length 1.07–1.17 mm; HL 1.14–1.44 mm; clypeus length 0.49–0.57 mm; LOD 0.88–0.96 mm; UOD 1.07–1.15 mm; clypeoantennal distance 0.10–0.17 mm; IAD 0.30–0.33 mm; IOD 0.39–0.34 mm; OOD 0.24–0.30 mm; OAD 0.36–0.51 mm; AOD 0.27–0.29 mm (n = 5).

***Relative head measurements*:** UOD:LOD 1.21; OOD:IOD 0.82; clypeus:HL 0.41.

***Mesosoma*:** overall mesosoma length 1.71–1.92 mm; pronotal collar absent; ITD 1.30–1.41 mm; mesoscutum length 0.82–1.80 mm; mesoscutum width 1.24–1.41 mm; metanotum length 0.12–0.19 mm; propodeum length 0.27–0.46 mm (n = 5).

***Relative mesasomal structure measurements*:** mesoscutum length:breadth 1.02; scutellum:mesoscutum 0.28; metanotum:scutellum 0.43.

***Structure – legs*:** tarsal claws simple. Pair of almost straight hind tibial spurs. Inner-spur slightly longer, thicker than outer-spur.

***Structure*: *metasoma*:** metasoma longer than mesosoma, less so than female (metasoma:mesosoma 1.24); broadest at second segment, S7 two broad, flat apical lobes orientated laterally, fringed with hair, with particularly long hairs on the apical edge; posterior lobes of S7 extended laterally with broad, flat flanges, >3× length of apical lobes (Fig. 22); S8 with large lateral lobes extending beyond the breadth of the apical process; apical process broad, somewhat narrowed towards base, and hirsute, with apex expanded, rounded and membranous (Fig. 23); penis valves slightly longer than gonostylus and about half the width of the gonostylus; apex of gonostylus hirsute and rounded; gonobase about half as long as wide, with each half curved to look like a bum (Fig. 21).

Etymology. The species is named after the author's beloved Maremma dog, Zephyr. The name "zephyr" is proposed as a noun in apposition.

Distribution. Southwest Western Australia (Fig. 24).

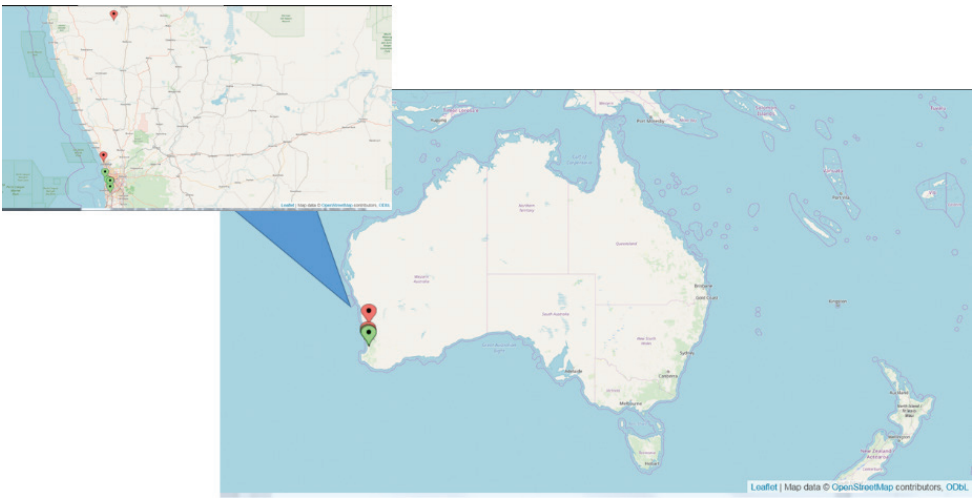


Figure 24. Map of Australia showing sites where specimens of *Leioproctus zephyr* sp. nov. has been collected, with close-up of locations. Green locations: collection localities by the author in 2016–18; red localities: collection locations by T. F. Houston 1979, 1992, 1996, 1997. Refer to Table 1 for further information. Map produced via the online program MapCustomizer: https://www.mapcustomizer.com/.

Ecology. Months collected: Dec – Jan. Earliest collection date by the author 3-Dec 2016, latest collection date 8-Jan 2017. The latest date collected was 29-Jan 1979. **Floral visitation:** Most visitation records have been from *Jacksonia sericea* Bentham (Fabaceae) (Suppl. material 1). The species has previously been collected mainly from *J. sericea*, with three records of bees visiting *J. eremodendron* E. Pritz, and one record of a bee visiting *J. horrida* (de Candolle) (however, based on how the distribution of *J. horrida* does not extend north to where the bee was collected, this is likely a misattribution and this collection record was also from *J. sericea* (Western Australian Herbarium 2022) (Suppl. material 1).

Conservation status. The species has only been collected at six sites, all of which are in parks or reserves (Fig. 24, Suppl. material 1). Recent systematic surveys across twenty-one sites over an area of ca. 300 km^2 revealed the species to only occupy four of these. On the basis of all known records to date, the total area of occupancy is ca. 40 km^2, and this habitat is fragmented by urban development. The species has also been collected at one other site within this region, as well as another site widely separated from the others some 200 km north. The species is presumably oligolectic on a small number of *Jacksonia* species, with the two main confirmed hosts also having a narrow distribution restricted to the Swan Coastal Plain (Western Australian Herbarium 2022) As no nests have been recorded, its nesting requirements are unknown, other than that it would be a ground-nesting species (Almeida, 2008). All populations however were recorded on the well-drained and weathered sandy soils of the Swan Coastal Plain (MacArthur, 2004), and thus it may be a psammophile. As a ground-nesting bee, it is sensitive to destruction of nesting habitat due to road-building and development that leads to impervious surfaces.

Under the IUCN Red List criteria, criteria A, C and E cannot be assessed as there is no ongoing monitoring; however, based on criteria B: Geographic range in the form of either B1 (extent of occurrence) OR B2 (area of occupancy) OR both, it may be considered to be vulnerable to extinction in that: Extent of occurrence is estimated to be less than 20,000 km^2, and estimates indicate habitat in which it has been recorded is severely fragmented or known to exist at no more than 10 locations (IUCN, 2012).

DNA barcoding. DNA barcoding confirmed that that male and female specimens collected were the same species, with both the male and three specimens which were successfully sequenced receiving the BOLD BIN number BOLD:AEC1713 (http://www.boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:AEC1713). A tree of sequences generated from the MSAPB sequences (involving a total of 4136 specimens of 169 Australian bee species) places this species in an undefined group with four other *Leioproctus* species, all of which include species that do not appear to have been scientifically described.

Discussion

This new *Leioproctus* species is highly distinctive in its morphology. It does not conform to any of the subgenera in the latest revision (Maynard, 2014). This morphological distinctiveness of this species was supported from DNA barcoding studies. This species was in a cluster with four other species (none of which appear to have been formally described), with an average distance of about 15%. The closest species from an NJ tree based on sequenced species is an undescribed *Leioproctus* (*Leioproctus sp*. "CH13"). It appears that the ridge is an autapomorphy, as none of the species in this clade have a ridge on the clypeus or a blunt thick apex of the hind tibial spur. Dissections of genitalia of the males revealed the S7 is comparatively simple for *L. zephyr*, being more complex in these other species. The only distinctive trait of *L. zephyr* shared with these other species is the short, robust S8 (R. Leijs, personal communication, 2020). Although sequencing with a single gene is insufficient to accurately represent evolutionary relationships, on the basis of these results it appears that clypeal protuberances can be homoplastic and represent convergent evolution in *L. zephyr* and *Leioproctus* (*Ceratocolletes*).

In the WA Museum collection database, Houston tentatively placed this undescribed species in the subgenus *L.* (*Protomorpha*), however although this species exhibits some features characteristic of this subgenus (namely terga with pale apical hair bands; flagellum short, middle segments mostly broader than long or scarcely longer than broad; clypeus and supraclypeal area not flat, usually punctate, suture separating them distinct), other key features of *Protomorpha*, are lacking, including: females with striate pygidial plate (pygidial plate lacks any ornamentation or sculpturing); males with hind tibia and basitarsus elaborately expanded (no elaborations on these leg segments, tibia only slightly broader than is typical for male *Leioproctus*, no expansion of the basitarsus); males with robust body like that of females (although robust compared with some *Leioproctus* subgenera, female is distinctly more robust than the male); S7 of male with

two large apical lobes (lobes, although present, are greatly reduced); mandibles simple, sharply pointed, without preapical tooth (mandibles broad, blunt, with preapical tooth); propodeum shorter than metanotum (propodeum is longer than metanotum). Similarly, this species exhibits features of *Odontocolletes* (which has features that are consistent with most of the major external features of *Protomorpha*), including the malar space absent, strong punctures on the dorsal surface of the mesosoma with smooth interspaces; it is also from the same geographic region as the majority of *L.* (*Odontocolletes*) species (Michener 2007; Maynard 2013). However, it lacks other key diagnostic traits, including red terga lacking apical hair bands and the diagnostic feature of *Odontocolletes* of a large, blunt, median tubercle on the metanotum (Michener 2007; Maynard 2014). And whilst like these subgenera, S7 of the male has two apical lobes, these wouldn't be considered "large", as is the case for *Protomorpha* and *Odontocolletes.* It also features some similarities with *Charicolletes* as described by Maynard (2013), including impressed facial fovea, strong punctures, and short antennal scapes. However, it is not metallic like *Charicolletes*, nor does it have a median metanotum tubercle. The morphologies of S7 and S8 of *L. zephyr* do not match those of the taxa illustrated in Maynard (2013). Whilst a number of taxa have two broad, flat apical lobes oriented laterally, few are as short in relation to the ventral processes, nor are they the same shape, as that of *L. zephyr*. Interestingly, S7 of *L. zephyr* is most similar to that of *Goniocolletes parvus* Maynard, 2013, however S8 and the genital capsule are morphologically dissimilar (compare with fig. 217–219 in Maynard (2013)). The genital morphology also bears some similarity to *L.* (*Exleycolletes*) *argentifrons* Smith, 1979 and *L.* (*Leioproctus*) *macmillani* Houston, 1991, but the ventral lobes are not as long in relation to the apical lobes, and distribution of hairs on the apical lobes are dissimilar, and the apical portion of S8 is narrower (see Maynard 2013, figs 48–49, 121–122). This species shares features with the subgenus *Ceratocolletes*, and appears to be most closely related to this genus, in being a stout-bodied, strongly punctate colletid; surface sculpture, on the metasoma in particular, having small, strong punctures with clearly defined, polished interspaces; the second to fourth metasomal terga in females and second to fifth metasomal terga in males with white, apical bands; malar space absent, and, notably, the clypeus with narrow, longitudinal, median, glabrous area (obscured by hair in males) (Maynard, 1993). However, unlike *Ceratocolletes* there is no distinct horizontal basal area on the propodeum and the basal area is not rounded onto the vertical area, and the propodeum in punctured, rather than smooth; in addition the scape does not attain the level of the median ocellus (Maynard 1993; Michener 2007). In the key to subgenera of *Leioproctus* with three submarginal cells by Maynard (2014), *Ceratocolletes* is separated along with *Lamprocolletes* and *L. opaculus* Cockerell 1929 from all other subgenera in having the jugal lobe of the hindwing not reaching cu-a. In *L. zephyr*, the tip of the jugal lobe extends just past the cu-a vein. The inner hind tibial spur of the female is also distinctly blunt and, unlike *Ceratocolletes*, does not have 11 long, fine teeth (Maynard 1993), instead having three blunt prongs only on the proximal section of the spur (see Fig. 7). The female's pygidial plate is also not narrow and convex (Maynard 2014). The current species lacks the diagnostic paired lateral lobes on the male seventh sternum, and instead has only a single lateral lobe, and

other features of the genitalia exhibit degrees of difference from *Ceratocolletes*. Only two species are currently recognised as belonging to *Ceratocolletes*. *L.* (*Ceratocolletes*) *xanthosus* Maynard 1993 has been collected from two areas in eastern Australia, and differs from the current species in the above differences related to the subgenus, as well as yellow colouration, golden hair in the males, and although the clypeus of the female is convex with a median ridge, it is not strongly protuberant (Maynard, 1993). The other species, *Leioproctus* (*Ceratocolletes*) *antennatus* also occurs only in southwest WA like *L. zephyr* and the apical hair bands on the terga in the female are incomplete medially (although these are also incomplete in the male of *L. zephyr*). However, in addition to the above differences at the subgeneric level, and like *L.* (*Ceratocolletes*) *xanthosus*, the antennae have yellow colouration, and males of *L.* (*Ceratocolletes*) *antennatus* have antennae with the apical segment expanded and flattened, in contrast to the unmodified antennae of *L. zephyr*. This unusual modification of the male's antennae however is an autapomorphy and is absent in *L.* (*Ceratocolletes*) *xanthosus.* In Michener (2007)'s description of this subgenus, he notes that hind legs of the male are incrassate, trochanters toothed, tibiae bent, and tibial spurs reduced in size-features absent in *L. zephyr*, however it is noted that these features of the male's hindlegs are only in one of the species, but which of the two species is not mentioned, and the description of *Ceratocolletes* by Maynard (1993) does not include these features. Although the first description of *Ceratocolletes* included a medially protuberant clypeus as a diagnostic feature of this subgenus (Michener, 1965), Maynard (1993), in placing *L. xanthosus* into *Ceratocolletes*, suggested that this feature was no longer subgenerically significant.

Like *L. zephyr*, both *Ceratocolletes* have only been collected on Fabaceae: *Pultanaea* spp. for *L.* (*Ceratocolletes*) *xanthosus*, and as with *L. zephyr*, *L.* (*Ceratocolletes*) *antennatus* have been recorded exclusively foraging on *Jacksonia* (Houston, 2000).

Looking at its phylogenetic relationships and cladistics groupings based on the Taxon ID Tree functionality in BOLD, the dendrogram generated from sequencing using the neighbour joining algorithm was not able to resolve its subgeneric grouping. Rather, it suggests that *L. zephyr* belongs to a distinct clade with a number of other undescribed *Leioproctus* (Mark Stevens, Remko Leijs, pers. comm. March 2022). The closest scientifically-described species were *Leioproctus conospermi* Houston 1989 (Supporting Information3), – an oligolectic species that features highly modified features as adaptations for foraging on the host, *Conospermum* (Houston, 1989), and *Leioproctus excubitor* Houston 1991, which has highly modified antennae in the male (Houston, 2018). Both of these species are currently placed in the subgenus *Leioproctus.* As such, the various features outlined above that *L. zerphyr* shares with various other subgenera (*Protomorpha* and *Cladocerapis*) are not taxonomically informative. It should be noted that phylogenetic analyses involving more than just the CO1 gene are required to further elucidate the taxonomic placement of *L. zephyr*. In particular, the dendrogram using just the CO1 gene is a phenetic result, used for illustrative purposes, and is merely suggestive but is not a reliable phylogenetic estimate; a rigorous phylogeny using more genes and sophisticated phylogenetic analyses is recommended (Ramírez et al. 2010; Trunz et al. 2016; Packer and Ruz 2017).

The remarkable feature about this new species is its highly distinct clypeus, featuring the medial ridge and protuberance, which is unusual for *Leioproctus* (Maynard, 2014). Only the monotypic subgenus *Colletopsis* Michener 1965, and the two species in *L.* (*Ceratocolletes*) feature a median ridge on the clypeus, which is especially pronounced in *L. zephyr*. This feature invites speculation about its evolution and function. Present in both sexes, it is unlikely to be due to sexual selection (although there is the possibility of mutual sexual selection acting on this feature). The male is relatively robust and broad metasoma compared with some *Leioproctus* males, but still relatively slimmer than that of the female. Patterns of sexual dimorphism vary across bees, and the relative size of the sexes can be considered to be informative about the relative sexual and natural selective forces acting up on the species. The relatively larger size of the female suggests that *L. zephyr* is non-territorial (Alcock and Houston 1996; Paxton 2005). The protuberance, although present in both species, is slightly larger relative to the bee's head in the male (length of protuberance extending from clypeus in profile relative to head length 0.22 for the female compared with 0.29 for the male; see Supporting Information2), which may suggest a role for this protuberance in sexual selection. No instances of mating or nesting behaviour were observed but would be insightful for future studies.

The raised ridge and protuberance may be a point for muscle attachment of the mandibles (Grimaldi et al. 2005). X-ray micro-computed tomography (micro-CT) scans would shed light on whether this hypothesis has support. Another intriguing possibility is that this feature serves as a wedge to open up the keel of *Jacksonia* flowers. As a papilionaceous flower, the flowers of *J. sericea* have their fertile organs, and thus pollen and nectar "hidden" by a keel comprised of two ventral petals (Córdoba and Cocucci 2011). In order to access the floral rewards, pollinators must push down on the keel and the lateral petals of papillionate flowers (Córdoba and Cocucci 2011). This requires some force (Córdoba and Cocucci 2011), and it may be that the clypeus with its prominent protuberance of this specialised *Leioproctus* is used to wedge open the keel of its host flower. Although involving glossa or leg modifications rather than clypeus structure, other cases of unusual bee morphological structures have been linked to adaptations for accessing floral rewards in flowers that have limited access to flower visitors (e.g. Houston 1983; Pauw et al. 2017). Observations of other bee species have revealed behaviours that involve using the head and mouthparts to push or force themselves into flowers that have petal morphologies limiting access (Packer, 2004), including those with keeled flower parts (e.g. Westerkamp 1993; Raju and Rao 2006; Amaral-Neto et al. 2015). Although foraging observations were made in the field, the speed at which the bees foraged on flowers precluded being able to discern whether they performed this behaviour; specialised video-cameras recording this specie's foraging behaviour and analysed in slow-motion play-back would be able to evaluate support for this hypothesised function.

Leioproctus zephyr has an extremely limited range of flowers it will forage on, namely a subset of species within the genus *Jacksonia* (Suppl. material 1). This contrasts with most *Leioproctus* species, which are often highly polylectic (Maynard, 2014). A notable exception in this region of SWWA are three species that are specialised on

Conospermum (Proteaceae) (Houston, 1989). In this region where collections were made, most *Leioproctus* I collected or have observed have been recorded foraging on a range of Myrtaceae (*Corymbia*, *Callistemon*, *Eucalyptus*). The only other occurrence of a *Leioproctus* on a plant in the family Fabaceae was on *Acacia* (previously classified as a distinct family, Mimosaceae) (Maynard, 2014). Despite other *Jacksonia* (*J. sternbergiana* Bentham and *J. furcellata* Bonplande & de Candolle co-flowering, often in abundance, at sites where *L. zephyr* was collected, no specimens were ever observed foraging on these other related species.

The reason for this specialisation can only be speculated. It is unlikely to be due to avoiding competition, as *J. sericea* is frequently visited by *Megachile* Latreille 1802, a genus which is more typically associated with Fabaceae (Houston 2000; Prendergast and Ollerton 2021).

Leioproctus zephyr also appears to have a limited season of activity covering only two months in summer (December to January). The species was not observed after early January in the more recent collections by the author. Although the latest date the species has been collected was the end of January (January 19th), this was a single collection forty years ago. As temperatures have risen by almost 1 °C over the last century, and rainfall has declines of 15% since the mid-70s, and it may be that climate change (Climate Council 2014) means that temperatures are now too hot for this species to remain active at this later collection date (Prendergast, 2022). Climate change may post a threat to *L. zephyr* by causing mismatches between the phenology of the emergence of the bee and its host plant (Hughes 2003; Pyke et al. 2016; Schleuning et al. 2016; Settele et al. 2016; Prendergast 2022).

This new species appears to be restricted to native vegetation reserves in the southwest Western Australian biodiversity hotspot (refer to Fig. 3). The only other population the species has been collected from is Pinjarrega Nature Reserve, almost 200 km^2 away from the other sites and thus well outside the flight range of the species (Zurbuchen et al. 2010). Whether other populations exist in the intervening region is unknown, however as *J. sericea* occurs in the intervening area, targeted surveys are recommended. It is also unknown whether the species still persists at this location, as it has been over two decades since the three specimens have been collected from this location. If it were to become extirpated, re-colonisation is therefore unlikely.

Comprehensive surveys that I conducted over 10 months failed to record this species in any residential gardens, which can be attributed to the lack of suitable foraging resources. Even at bushland remnants were *Jacksonia sericea* was flowering, this did not guarantee the presence of this species: for example, Piney Lakes Reserve has *J. sericea* patches, and is approximately only 4 km away from Wireless Hill where this species was recorded, yet no records were made at Piney Lakes. *L. zephyrus* was also not recorded on *J. sericea* at other bushland remnants surveyed in the City of Bayswater in 2020–22 (Prendergast 2021, 2022b). This region has become highly fragmented due to urbanisation and the associated loss of natural habitat for road and urban development. Other studies outside of Australia have also found that specialist

species are underrepresented in small, isolated fragments in urbanised areas (Cane et al. 2006; Pauw 2007). Given the dependence of this new species on native vegetation remnants with *J. sericea*, efforts much be made to protect any native bushland that remains with these plants, and encourage bushland restoration initiative to plants patches of *J. sericea* to promote connectivity and increase the overall habitat area for this remarkable species.

There has been no formal conservation status of *Leioproctus zephyr*, but some recommendations can be made based on information regarding its distribution, phenology, habitat, and resource associations. 18 specimens have been collected by T. Houston along with 96 by the author (Suppl. material 1). The majority however (two thirds of all specimens) have been collected from a single site, Kings Park (Suppl. material 1). Moreover, it is unknown whether the species still exists at two of the sites surveyed by T. Houston, especially the site where the species was first collected (Neerabup National Park) in 1979, which is a great distance from where the other populations occur. *L. zephyr* is locally abundant at Kings Park, which is a large, intact area of remnant bushland that is under strong conservation legislation as an A-class Reserve, and is well-managed by the Government of Western Australia's Kings Park and Botanic Gardens Authority, with two-thirds of this 400.6ha park being protected as managed bushland (Botanic Gardens and Parks Authority 2017). One of the sites where *L. zephyr* was recently collected is marked to be undergoing partial destruction to make way for urban development (Young, 2018); ongoing urbanisation of the matrix surrounding sites may also affect populations through edge effects, by increasing isolation, and preventing metapopulation dynamics.

Further surveys during December and January in areas where *Jacksonia sericea* is flowering are required to establish this species extent of occurrence. Ongoing monitoring is also required to detect any population trends. Preservation of *J. sericea* is of utmost importance for this species.

Conclusion

Native bees are suffering from a major taxonomic crisis, and without a scientific name, understanding their distribution, abundance, and conservation status is a challenge, which is contributing to the poor state of conservation of invertebrates, including in a megadiverse country like Australia (Braby, 2018). Describing and naming this *Leioproctus* will enable it to receive conservation attention, as well as serve as a springboard for further taxonomic work on the diverse *Leioproctus* in Australia. This species is moreover morphologically distinct, featuring a modified clypeus, is oligolectic, and appears restricted to a few locations in the southwest Western Australian biodiversity hotspot. DNA barcoding has reinforced its distinct position and offers inspiration for further research into the taxonomy and systematics of Australian native bees and Hymenoptera at large.

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No ethics approval was required.

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Supplementary material 1

Details of specimens of *Leioproctus zephyrus*
Authors: Kit S. Prendergast
Data type: COL
Explanation note: Collection details of specimens of *Leioproctus zephyr* sp. nov.

Link: https://doi.org/10.3897/jhr.93.85685.suppl1

Supplementary material 2

Morphological measurements
Authors: Kit S. Prendergast
Data type: excel file
Explanation note: Morphological measurements of specimens

Link: https://doi.org/10.3897/jhr.93.85685.suppl2

Supplementary material 3

Taxon ID tree
Authors: Kit S. Prendergast
Data type: png file
Explanation note: Taxon ID tree showing the relationship between *Leioproctus zephyr* sp. nov. in relation to other barcoded specimens generated in BOLD using the CO1 gene. Tree created in FigTree (http://tree.bio.ed.ac.uk/software/figtree/)

Link: https://doi.org/10.3897/jhr.93.85685.suppl3

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Double-blind validation of alternative wild bee identification techniques: DNA metabarcoding and *in vivo* determination in the field

Fernanda Herrera-Mesías[1,2*], Christopher Bause[1*], Sophie Ogan[3], Hannah Burger[4], Manfred Ayasse[4], Alexander M. Weigand[2], Thomas Eltz[1]

1 *Department of Animal Ecology, Evolution and Biodiversity, Ruhr-Universität, Bochum, Germany* **2** *Musée national d'histoire naturelle de Luxembourg, Luxembourg* **3** *Department of Biogeography, Trier University, Trier, Germany* **4** *Institute of Evolutionary Ecology and Conservation Genomics, Ulm University, Ulm, Germany*

Corresponding author: Thomas Eltz (thomas.eltz@rub.de)

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Abstract

Over the past few decades, several investigations around the globe have reported alarming declines in the abundance and diversity of bee species. The success of effective conservation strategies targeting these important pollinators relies heavily on accurate biodiversity assessments. The shortage of taxonomic experts and the escalation of the ongoing biodiversity crisis call for the development of alternative identification tools to implement efficient monitoring programs. The validation of such techniques is crucial to ensure that they provide results comparable to those of traditional morphotaxonomy. Here we performed two double-blind experiments to evaluate the accuracy of a pair of new techniques used for wild bee identification: DNA metabarcoding and *in vivo* identification in the field. The methods were tested on sets of wild bees from Germany and their results compared against evaluations done by panels of bee experts using traditional morphotaxonomy. On average the congruency of species identification between metabarcoding and morphotaxonomy was 88.98% across samples (N = 10), while *in vivo* identification and morphotaxonomy were 91.81% congruent (N = 7) for bees considered feasible for *in vivo* identification in the field. Traditional morphotaxonomy showed similar congruencies when compared to itself: 93.65% in the metabarcoding study and 92.96% in the *in vivo* study. Overall, these results support both new methods as viable alternatives to traditional microscopy-based assessment, with neither method being error-free. Metabarcoding provides a suitable option to analyze large numbers of specimens in the absence of highly

* These authors contributed equally to the article.

trained taxonomic experts, while *in vivo* identification is recommended for repeated long-term monitoring, and when working in areas where the sampling of individuals could threaten local populations of endangered wild bee species. Further research is still needed to explore the potential of both techniques for conservation management and wildlife monitoring, as well as to overcome their current limitations as taxonomic tools.

Keywords
Apiformes, conservation, molecular taxonomic tools, morphotaxonomy, non-lethal identification

Introduction

Wild bees (Hymenoptera, Anthophila) are insect pollinators that are both ecologically important and of remarkable economic interest (Brown and Paxton 2009; Papanikolaou et al. 2017). As such, they are a key component of the global biodiversity, providing ecosystem services to wild flowering plants and commercially grown crops (Potts et al. 2010). Their services have a direct impact on food production. Not only do 75% of the world food crops benefit from insect-mediated pollination, mostly performed by bees, but it is estimated that about 42% of the leading crops grown for direct human consumption are pollinated by at least one wild bee species (Klein et al. 2007; Potts et al. 2010).

The recent decline of wild bees and other major insect groups in several regions of the world has become a matter of global concern among conservation biologists and the general public (Biesmeijer et al. 2006; Potts et al. 2015; Hallmann et al. 2017; Wagner 2020). The underlying causes for this decline are variable and still under investigation, but habitat loss and fragmentation, as well as agricultural pesticides and climate change, are mentioned as major drivers (Winfree et al. 2009; Hofmann et al. 2018; Meeus et al. 2018).

To preserve wild bee biodiversity, conservation initiatives adapted to the habitat requirements of local bee communities must be implemented (Müller et al. 2006; Brown and Paxton 2009; Henry and Rodet 2018; Ganser et al. 2021). The success of these conservation efforts relies heavily on accurate taxonomic information. Detailed knowledge regarding local species composition is key to selecting adequate strategies for habitat management and preservation (Ji et al. 2013).

Despite its importance, reliable taxonomic information is rather incomplete in several regions of the world. Even in Central Europe, the population trend of most wild bee species remains unknown (Potts et al. 2010; Gueuning et al. 2019). An estimate of 1,101 species in Europe (56.7% of the total) are classified as "data deficient" according to the European Red List of Bees, indicating a lack of scientific information to assess extinction risk (Nieto et al. 2014). Changes in regional bee fauna are poorly understood due to the lack of long-term insect monitoring programs, but there is evidence of local decline in species richness and community composition shifts (Hallmann et

al. 2017; Hofmann et al. 2018; Rollin et al. 2020). In Germany, about half of the occurring 550+ species of wild bees are categorized as threatened, based on Red List evaluations (Westrich et al. 2011; Schneider 2018; Vereecken 2018; Westrich 2019; Hofmann and Renner 2020). Conservation projects aiming to protect local wild bee populations must first retrieve accurate taxonomic information regarding which species are present in the area of interest, applying reliable taxonomic tools.

It is a common procedure in wild bee monitoring to collect adult specimens in the field via active methods such as targeted sweep netting, or passive sampling using devices like pan traps or vane traps (Roulston et al. 2007; Westphal et al. 2008; Falk 2016; Prendergast et al. 2020). The collected specimens are pinned, labeled and prepared for identification using a stereo microscope and morphological keys (Westrich 2019). Identification of pinned specimens based on morphological traits ("*PIN*") is the current gold standard for bee inventories. However, there are situations when *PIN* has shortcomings, especially (1) in the context of multi-replicate inventories over large spatial scales that are prone to exceed the available funds for or the capacity of classical morphological identification (Yu et al. 2012; Lebuhn et al. 2013; Gueuning et al. 2019), (2) for reduced-impact bee monitoring in areas where collecting/killing all individuals would risk exterminating local populations of rare species (Gezon et al. 2015) and (3) in cases of challenging morpho-identification (i.e. cryptic species complexes) (Schmidt et al. 2015). While these three challenges arise from quite different aspects of *PIN*, they are all serious concerns that are intensively discussed among wild bee experts (VDI-Richtlinie 4340-1, 2021).

The accuracy of *PIN* relies strongly on the experience of the taxonomist because it can be extraordinarily complex, as diagnostic traits can vary substantially between regions, localities, or even within local populations. Traits, especially coloration and vestiture, can even vary for a given individual bee over the flight season (Falk 2016). While in some taxonomic groups traits are well differentiated, in others the character states overlap and identifications require evaluation of combinations of traits, making unambiguous classification challenging even for trained experts (Michener 2000). In some bee genera reliable identification requires access to an established reference collection, a resource that is not always available (Gibbs et al. 2013). Due to these challenges, reliable *PIN* of large numbers of specimens is costly and may be precluded by the limited availability of trained taxonomic experts (Hopkins and Freckleton 2002; Engel et al. 2021).

DNA-based monitoring methods and molecular identification pipelines have great potential to assist *PIN* in wild bee inventorying (Gueuning et al. 2019). DNA metabarcoding is a molecular identification technique that relies on PCR primers for mass-amplification of taxonomically informative gene regions from bulk samples, combining high throughput sequencing (HTS) and parallel DNA-based species identification using bioinformatic tools to compile taxonomic lists up to species level (Ji et al. 2013; Brandon-Mong et al. 2015). It represents an upscaling to traditional Sanger sequencing DNA barcodes, as it allows the analysis of thousands of specimens simultaneously, assessing biodiversity rapidly and cost-efficiently (Yu et al. 2012), regardless of the life

stage of the specimens or their sex. Also, it provides an objective way to discriminate cryptic sibling species (Elbrecht and Leese 2015).

Despite their advantages, metabarcoding approaches are not free of technical limitations and flaws. Several investigations have reported that it is generally not possible to retrieve taxon abundance data because final read numbers are heavily affected by species amplification efficiency (i.e. primer bias; Zhou et al. 2013; Elbrecht and Leese 2015; Gueuning et al. 2019; Piñol et al. 2019). Moreover, results can be affected by other error sources leading to false positives (e.g. environmental contaminations), false negatives (e.g. gaps in the barcode reference libraries and significant biomass differences of specimens) or to discrepancies with traditional taxonomic outcomes (hybridization and shared barcodes among more recently diverged species) (Sheffield et al. 2009; Clarke et al. 2014; Schmidt et al. 2015; Elbrecht and Leese 2015; Weigand et al. 2019; Zinger et al. 2019). Therefore, the performance of metabarcoding approaches targeting wild bees must be cross-validated to ensure that robust data is produced for its use in conservation biology.

In the present study we test the accuracy of a customized metabarcoding pipeline ("*DNA*") incorporating a voucher-saving work-flow targeting Central European wild bees (Herrera-Mesías et al. submitted).

Both *PIN* and *DNA* metabarcoding of bulk samples, are invasive techniques in the sense that they remove specimens from the population, thereby reducing local population size and potentially endangering local population survival. Only very few studies are dealing with effects of such lethal sampling methods on population development. Even though Gezon et al. (2015) found no evidence for harmful effects of repeated, lethal sampling of bees, this might still be an important factor for species with very small population size or, in case of traps being used, for species that are particularly attracted to the type of trap (e.g. colored vane traps, Gibbs et al. 2017). To minimize such potential effects, Schindler et al. (2013) proposed a set of low-impact monitoring rules, which has been further developed in the BienABest project (www.bienabest.de) aiming to safeguard the ecosystem service of pollination and to enhance wild bee diversity in agricultural landscapes. The method, which has already been used in bee surveys within BienABest (Neumüller et al. 2020, 2021), has been elaborated in detail by VDI-Richtlinie 4340-1 (2021). It relies on identifying the majority of encountered bee specimens alive in the field, either by on-sight observation (e.g., on flowers) or by capture, brief confinement and immediate release following identification. The method is abbreviated as *IVI* in the present article (for *in vivo* identification). *IVI* is aimed to reduce negative impacts on the entire bee community, but in particular on species that are vulnerable and can be recognized with reasonable certainty directly in the field. It is also thought to improve data quality for long-term bee monitoring by reducing the effects of monitoring itself on the results, i.e. in case of repeated sampling in the same restricted bee habitats. Even more than *PIN*, *IVI* relies on trained and experienced bee experts that are capable of identifying many bee species directly in the field, without microscope and without consulting a reference collection, solely assisted by hand-net, observation jar, magnifying glass and identification keys.

Thus, for this study, double-blind experiments were performed to evaluate the accuracy of two alternative taxonomic identification techniques used on wild bees,

DNA metabarcoding of bulk samples ("*DNA*"), and *in vivo* identification ("*IVI*"). We compared the output of both methods against the evaluation of a panel of wild bee experts to determine similarities and discrepancies between the new approaches and traditional morphotaxonomy based on dry-pinned specimens ("*PIN*").

Materials and methods

DNA - Wild bee sampling and double-blind approach

To evaluate the metabarcoding pipeline described in Herrera-Mesías et al. (submitted) a total of 230 wild bee specimens were used. The samples were collected by S.O. and a field assistant using hand nets during 10 sampling events from 27 April to 22 July in 2020 in 7 different sites distributed across the Federal State of Rhineland-Palatinate (Germany). The netted bees were killed with ethyl acetate and immediately stored under cool conditions. From the end of the field day until the pinning of the individuals, all samples were stored frozen to prevent possible degradation of the DNA. Bees were pinned (males with genitals pulled out) and labeled by the end of the field season. For DNA extraction one complete midleg of each individual was removed using fire-sterilized tweezers and transferred to 2 mL Eppendorf tubes. After processing the bees of a sampling event, all surfaces and tools, i.e., tweezers, were sterilized to exclude cross contamination. The legs were pooled per sampling event, the pooled samples labeled with integers 1 through 10 by S.O. and shipped to the Zoology Department of the Musée national d'histoire naturelle Luxembourg, where further molecular analysis ("*DNA*") was conducted by F.H-M. and A.W. without specific knowledge of sites or specimens.

The pinned voucher specimens were shipped to two internationally recognized wild bee experts, both with over 15 years of experience in wild bee faunistics and taxonomy, who were asked to identify them to species level ("*PIN*"). Both experts consented the use of their identifications for the double-blind evaluation of the metabarcoding approach. During the laboratory analysis, the team processing the pooled leg samples had no access to the voucher specimens nor any of their metadata information or the evaluations done by the experts. The wild bee experts never met each other, and their taxon lists were handled by a third party (T.E.) until the DNA pipeline output was completed. The voucher specimens are deposited in the MNHNL invertebrate dry collection for long-term storage and curation (MNHNL127130-127359).

DNA - Metabarcoding pipeline

For the metabarcoding pipeline, a two-step PCR protocol using fusion primers based on Elbrecht and Steinke (2019) was used. The tags used for the second PCR are described in Elbrecht and Leese (2017). The laboratory protocols of Weigand and Herrera-Mesías (2020) were used for DNA extraction, as well as for the first PCR. For the

second PCR, 1 µl of the amplicon (without cleanup) was used as a template and the reaction volume was modified to a final volume of 50 µl. Both PCRs were run on an Eppendorf Mastercycler nexus eco Thermocycler using programs based on Elbrecht and Steinke (2019) and described in Herrera-Mesías et al. (submitted).

To increase the data robustness and the probability of detecting low biomass specimens, a PCR replicate strategy was followed. Two replicates of each sample plus one positive control (i.e. a mock community of known wild bee community composition) were included in the final setup. The success of both PCR replicates was verified by electrophoresis and their amplicons were purified with a NucleoSpin Gel and PCR Clean-up kit (Macherey-NagelTM). The DNA concentrations of the purified products were measured and equimolarly pooled into the final library (27.42 µl, 48.47 ng/µl). The clean library was sequenced on one lane of an Illumina MiSeq System (2x250 bp) at the Luxembourg Centre for Systems Biomedicine (Belval, Luxembourg).

The resulting DNA metabarcoding sequence data was processed using the JAMP R package (https://github.com/VascoElbrecht/JAMP), with the settings and supplementary tools described in Herrera-Mesías et al. (submitted). Taxonomic sorting was performed by comparing the resulting OTU fasta files against sequences stored in the Barcode of Life Data system (BOLD; Ratnasingham and Hebert 2007) using BOLDigger (Buchner and Leese 2020). As the team performing the bioinformatic analysis was blind to any metadata regarding the potential species composition of the samples, the default thresholds of BOLDigger were considered to find the best fitting hit for OTU taxonomic identification: at least a match of 85% for identification to the level of order, 90% to the level of family, 95% to the level of genus and 98% to the level of species.

The resulting data were pruned using TaxonTableTools (Macher et al. 2021) to remove all non-Hymenoptera OTUs, as well as Hymenoptera OTUs present in only one PCR replicate. Finally, the taxon name assignation of the filtered data was manually reviewed and partly modified from the original BOLD output by A.W. (blind to *PIN* results) to comply with current taxonomic nomenclature, thus creating a curated taxon list (Suppl. material 1). Only Hymenoptera OTUs present in both replicates with read numbers above 0.01% of abundance for each replicate and identified to species level were included in the final curated table. If a species was represented by multiple OTUs in the dataset, the results were collapsed into a single species entry.

To maintain double-blindness between *DNA* and *PIN*, the curated table was sent to T.E. who cross-tabulated identification results for each sample for a first comparison. Only then were the results made available to the rest of the team for numerical analysis. To allow comparison among the output of both approaches, the curated taxon list was transformed into a presence/absence table and combined with the results of the morphological approach.

IVI - Wild bee sampling and double-blind approach

To test the accuracy of *in vivo* determination of wild bees in the field, one of the authors (C.B.) accompanied bee monitorers during wild bee surveys within the "BienABest" project. Surveys took place from April to September 2020 at nine different sites

throughout Germany and were conducted by a total of seven trained bee monitorers, whose experience in bee faunistics and taxonomy varied from some to many years. The monitorers used a reduced-impact monitoring method that includes *in vivo* identification (*IVI*) of encountered wild bees along variable transect walks (Neumüller et al. 2020, 2021, VDI-Richtlinie 4340-1 2021). Bees were either identified by the monitorer "on sight" when no closer scrutiny was deemed necessary, or were captured and identified with the help of an observation jar and a magnifier (ID method "capture"). Bees that could still not be identified *in vivo* were killed for later identification under the microscope. Overall, a total of 552 bee individuals were encountered by the seven monitorers during the surveys, of which 56 individuals (10.14%) were deemed impossible to identify in the field. The remaining 496 individuals were identified alive "on sight" or following "capture" by the monitorer. Of those, 210 individuals (42.34%) were consecutively collected by C.B. and stored in pre-labeled vials for later validations (see below). The remaining 287 individuals either could not be captured or were excluded from the evaluation because they represented species that had already been identified three times by an individual monitorer. This exclusion rule treated sexes separately, i.e., the maximum number of *IVI* individuals evaluated per species and monitorer was six (three females and three males).

The 210 bees to be included in the laboratory evaluation of *IVI* were killed with ethyl acetate or by freezing, and pinned by C.B. Furthermore, genitalia of male specimens were extracted and fixed outside the metasoma if required for species identification. The pinned specimens were re-labeled with a unique number code to omit information about date, locality or any other detail that would violate the anonymity of the monitorers.

The pinned bees were first identified by one internationally recognized wild bee expert with many years of experience in bee faunistics, morphotaxonomy and systematics (EXP data set) who worked under the knowledge that the identifications would later be used for *IVI* evaluation. Consecutively the specimens were sent to four other recognized wild bee experts, with several to many years of experience in bee morphotaxonomy, for independent identification (*PIN*). These experts were paid at rates typical for freelance work and were also aware that their work was part of a scientific investigation. To reconcile all discrepancies of identifications between the EXP data set and *PIN*, these were consecutively discussed in detail with the respective *PIN*-experts. Based on these discussions, and taking into account COI barcodes of two critical bee individuals (see Suppl. material 2 for laboratory protocol), a consensus list (CON data set) was established that represents our most objective assignment of true species affiliation. The voucher specimens are deposited in the collection of the Department for Evolutionary Ecology and Conservation Genomics at the University of Ulm.

For data analysis, the whole data set of wild bee IDs was divided into seven bee sets, each representing the identifications made by one individual monitorer, enabling us to analyze discrepancies between *IVI* and *PIN* across monitorers, and to contrast them with the discrepancy among *PIN* identifications for the same sets of bees. Additionally, a comparison to the consensus list showed the percentage of correctly identified bees per *IVI* and *PIN* expert.

Similarity analysis (*DNA* and *IVI*)

To further analyze the congruency and discrepancy of identification within and among *DNA* and *PIN*, and within and among *IVI* and *PIN*, we calculated Bray-Curtis similarities based on presence/absence taxon tables (*DNA* evaluation) or quantitative taxon tables (*IVI* evaluation) using the PRIMER-E software (version 6.1.6; Clarke and Gorley 2001), which was also used to plot dendrograms (hierarchical cluster analysis, complete linkage) based on the calculated similarity matrices.

Results

DNA - Evaluation of metabarcoding

After trimming and quality filtering, 2,874,629 high quality reads from the original 4,395,456 read pairs were retained (Short Read Archive bioproject: PRJNA876388). About 67.8% of the 1,447,238 original unassigned reads corresponded to PhiX. A total of 17.27% of the original 278 OTUs detected in the dataset were discarded after filtering based on a 0.01% read abundance threshold, remaining 230 OTUs for further analysis. 480 chimeras were discarded as well during clustering. After comparison against the BOLD systems database and replicate consistency analysis with Taxon Table Tools,146 OTU consistently found across replicates were preliminary identified as Hymenoptera taxa to various levels of taxonomic resolution (Suppl. material 3). After filtering, data merging and curation, 91 distinct taxonomic units representing detected wild bee species and species groups were included in the final curated table comparing *DNA* with *PIN* (Suppl. material 4).

The number of taxonomic units detected by *DNA* in individual samples varied between 11 and 22. All the species intentionally pooled in the mock community sample (positive control) were detected. From the ten samples considered in the analysis, only one (S2) presented a perfect congruence between the metabarcoding results ("*DNA*") and the evaluations of both taxonomic wild bee experts ("*PIN*1" and "*PIN*6"), based on the values of the Bray-Curtis index and the visual analysis of the dendrogram (Fig. 1). Two more DNA-based species lists were identical to the *PIN*1 expert results (S7, S9), none to *PIN*6. In six of the remaining samples, the DNA pipeline outcome was grouped closer to *PIN*1 on a terminal branch, with higher similarity than the resulting one from the comparison of the results of both experts. In three samples (S1, S3 and S8), the results of both *PIN* experts were more similar with each other than with the results of the DNA pipeline. When results are considered within the same sample, the Bray-Curtis similarity was 80% or higher among all three methods, with the lowest similarity observed between the pipeline and both experts in S3.

Across samples, the average congruency between the *DNA* and *PIN* ("*PIN*av") was 88.98% (Table 1). The mean congruency within *PIN* was slightly higher (93.65%). When *PIN* identifications were considered separately, the results of *DNA* were in better agreement with the evaluation of the first expert ("*PIN*1") than with the second one

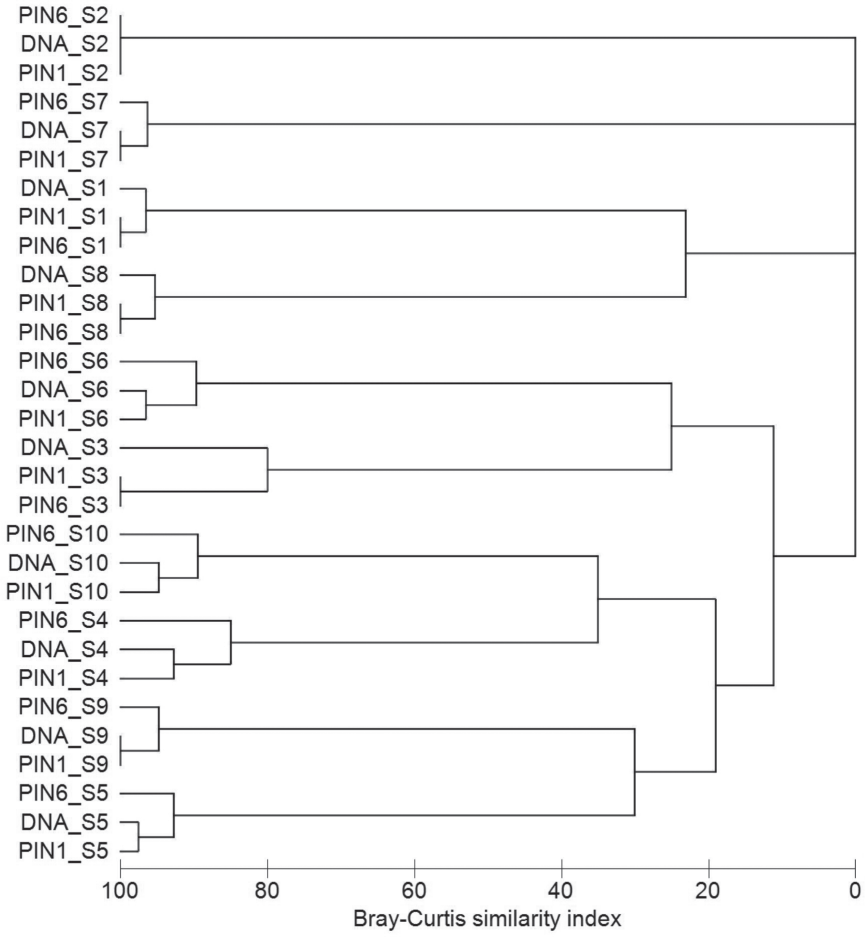

Figure 1. Dendrogram based on Bray-Curtis index to illustrate congruency of wild bee identification among the metabarcoding pipeline (*DNA*) and two *PIN* experts. X-axis shows index values expressed as percentage Bray-Curtis similarity.

Table 1. Percentage congruency of taxon lists resulting from *DNA* and *PIN* across samples. DNA x PIN1, DNA x PIN6 and PIN1 x PIN6: Percentages are calculated based only on the wild bee taxa detected by the methods considered in each pairwise comparison. DNA x PINav: Average of pairwise congruency between *DNA* and both *PIN* experts. Mean congruency across samples and standard deviations (SD) are also given. N = number of bee individuals in each set.

	Bee Set (=Sample)											
Comparison	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**Mean congruency (%)**	**SD**
	N=25	**N=14**	**N=21**	**N=28**	**N=30**	**N=27**	**N=17**	**N=14**	**N=27**	**N=27**		
DNA x PIN1	93.33	100.00	66.67	90.48	95.24	93.33	100.00	90.91	100.00	90.00	92.00	9.75
DNA x PIN6	93.33	100.00	66.67	77.27	86.36	81.25	92.86	90.91	90.00	80.95	85.96	9.63
DNA x PINav	93.33	100.00	66.67	83.87	90.80	87.29	96.43	90.91	95.00	85.48	88.98	9.30
PIN1 x PIN6	100.00	100.00	100.00	85.71	90.48	87.50	92.86	100.00	90.00	90.00	93.65	5.77

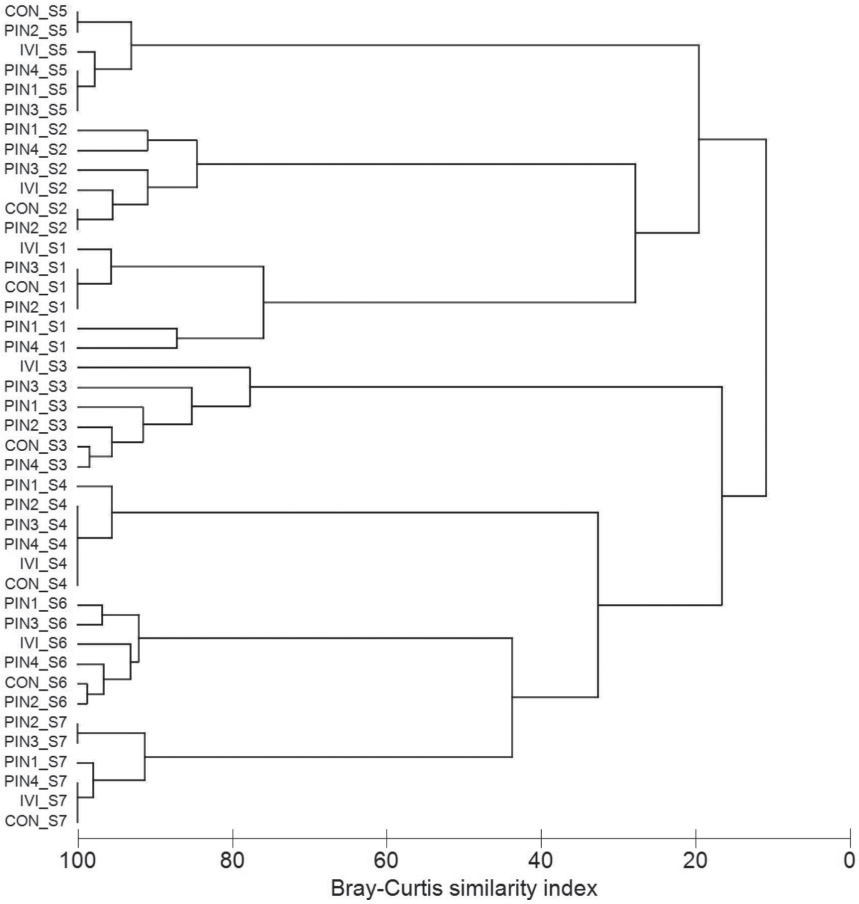

Figure 2. Dendrogram based on Bray-Curtis similarity index to illustrate congruency of wild bee identification among *IVI* and *PIN* experts. X-axis shows index values expressed as percentage Bray-Curtis similarity. *PIN* experts 1 to 4 are the same among the seven bee sets, whereas the *IVI* expert is different for each bee set.

Table 2. Percentage congruency of taxon lists resulting from *IVI* and *PIN* in comparison to each other and a curated consensus list (CON) for each of the seven bee sets. IVI x PIN: Average of pairwise congruencies between one *IVI* expert and the four *PIN* experts. PIN x PIN: Average of pairwise congruencies between the four *PIN* experts. IVI x CON: Congruency between one *IVI* expert and the consensus list. PIN x CON: Average of congruencies between each of the four PIN experts and the consensus list. Grand means and standard deviations (SD) across bee sets are also given. N = number of bee individuals in each set.

	Bee Set (=Sample)								
Comparison	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**Mean congruency (%)**	**SD**
	N=21	**N=29**	**N=39**	**N=19**	**N=24**	**N=46**	**N=30**		
IVI x PIN	86.90	92.24	80.77	98.68	94.79	93.48	95.83	91.81	5.62
PIN x PIN	85.71	91.38	90.60	97.37	97.92	93.84	93.89	92.96	3.90
IVI x CON	95.24	96.55	84.62	100.00	91.67	97.83	100.00	95.13	5.06
PIN x CON	90.48	93.97	94.87	98.68	96.88	95.65	95.83	95.19	2.38

("*PIN*6") or with their average outcome. The highest disagreement between *DNA* and *PIN* was observed in S3, where *DNA* detected five additional species and missed one identified by both *PIN* experts, reaching a congruency of only 66.67%.

IVI – Evaluation of *in vivo* identification

The total sample size of evaluated bees was reduced from originally 210 bees to 208 bees due to critical damage in two specimens caused by repeated shipping. The number of identified bees per monitorer/bee set varied from 19 to 46 bee individuals. Fig. 2 shows the similarity of species identifications by *IVI*, *PIN* and the CON data set based on the Bray-Curtis similarity index. The greatest congruency was found in bee set 4, in which the monitorer (*IVI*4) and three out of four *PIN* experts as well as the CON data set produced a perfectly identical taxon list. In bee set S2, S3 and S6 identification results differed at least slightly among the consulted *IVI* and *PIN* experts. The largest discrepancies were found in bee sets S1 and S3 (86.90% and 80.77%, respectively).

Averaged across bee sets, there was a taxon list congruency between *IVI* and *PIN* of 91.81%. *PIN* results among themselves showed an average taxon list congruency of 92.96% (Table 2). Overall, and in comparison with the CON dataset, the average percentage of correctly identified bee individuals was 95.13% for *IVI* and 95.19% for *PIN* (see Table 2). Apparent misidentifications of *IVI* and *PIN* experts appeared mainly within bee genera *Andrena*, *Bombus*, *Halictus* and *Megachile*. In addition, some bee individuals of *Lasioglossum* spp. were misidentified by *PIN* experts (Suppl. material 5).

Discussion

The performed double-blind validations demonstrated that error rates of the evaluated novel methods were of a similar (low) order of magnitude as compared to traditional morphotaxonomy, suggesting they represent valid alternatives for wild bee monitoring. In addition, we found that neither of the methods, traditional pinning, *in vivo* identification or DNA metabarcoding, were error free. In the following we shed light on the types of errors that occurred and discuss strengths and weaknesses of the respective methods. To our knowledge, this is the first double-blind study to evaluate per-sample accuracy of wild bee identification within and across methods. Even if previous studies have compared the congruency of diverse identification techniques used in wild monitoring against traditional morphotaxonomic outcomes (Tang et al. 2015; Gueuning et al. 2019), this is the first experiment to date that has been explicitly designed to control the bias resulting from the exchange of preliminary taxonomic information among the different participants, thus to ensure that the results are based purely on the detection capacity of each identification technique.

Evaluation of DNA metabarcoding in comparison with morphotaxonomy

The overall congruency found between the metabarcoding pipeline (*DNA*) and morphological identification results (*PIN*) on a per-sample basis analysis (88.98% mean congruency) agrees well with previous findings reported by Gueuning et al. (2019). In their study, based on a multi locality setting in Switzerland, over 90% of the traditionally identified morphospecies were also detected by DNA metabarcoding.

Despite the high overall similarity of the results obtained by *DNA* and *PIN* in our study, 26 cases of disagreement were present (Suppl. material 4), which are worth further discussion: In 12 cases, the molecular results support the assessment of one morphotaxonomic expert against the other, resolving conflicting morphological evaluations. Incongruence between *DNA* and both *PIN* experts can partially be explained by unclear species delimitation. There is a historical controversy regarding whether *Andrena ovatula* and *Andrena albofasciata* should be consider as one or two species (Westrich et al. 2011; Schmidt et al. 2015; Praz et al. 2022). In our study, the metabarcoding pipeline supported the presence of *A. albofasciata* against *A. ovatula* in S4 and S10, in opposition to the morphological analysis, but was in agreement with both *PIN* experts regarding detecting only *A. ovatula* in S5. As *DNA* recognized these taxa as two separate OTUs in our dataset based on a 97% genetic similarity threshold, this suggests the presence of a second species, potentially overlooked by *PIN*, within what has been traditionally considered *Andrena ovatula sensu lato*. These results are in agreement with recent analyses that have resolved the controversy by consistently demonstrating the existence of two distinct species within the complex, *A. ovatula* and *A. afzeliella* (Kirby, 1802) (=*A. albofasciata*), based on molecular, morphological and ecological evidence (Praz et al. 2022). Therefore, the nomenclature of DNA barcodes currently available in BOLD should be updated accordingly to match this new taxonomic consensus, further improving the detection capacity of molecular approaches.

Further research on cryptic diversity following a similar approach would contribute to reach final conclusions regarding the status of similarly challenging species complexes, such as the *Halictus simplex*-complex. Although our dataset pooled species within this complex into one entity for the overall comparisons, *DNA* was able to precisely identify *H. langobardicus* regardless of the sex of the individual, whereas *PIN* was only able to assign a species-level annotation to males (Suppl. material 4).

Given that the genetic results of controversial species complexes involve an additional level of analysis (Schmidt et al. 2015), a sufficient number of validated DNA reference barcodes should be a pre-requirement to perform metabarcoding on taxonomically problematic sibling species. Whenever possible, barcodes from local specimens reliably identified by known taxonomic experts should be preferred as reference material, thus to reach accurate interpretations.

Another factor potentially affecting the congruency of metabarcoding results with morphological analysis is environmental contamination. For example, in seven cases the pipeline detected additional wild bee species to the ones reported by the taxonomic experts. Five false positive detections were found in S3 (*Bombus lapidarius*, *Bombus*

pascuorum, *Andrena cineraria*, *Chelostoma florisomne* and *Dasypoda hirtipes*), one in *S5* (*Halictus confusus*) and one in S8 (*Melecta luctuosa*). The additional species in S3 correspond to easily identifiable wild bees and three of them were completely absent in the whole wild bee set, which means that they cannot have been overlooked by *PIN*. Most likely, DNA traces from an outside source are likely responsible for these additional findings. Carry-over DNA from other specimens in the field, the sampling containers, or from specimen handling before DNA extraction represents a more likely explanation than cross-contamination in the laboratory as no other bees were being processed within the laboratory premises at the time of the double-blind experiment. The same situation may explain the presence of *H. confusus* in S5 and of *M. luctuosa* in S8. Tag-switching as an alternative explanation for the false positive results of species generally present in the overall data set seems unlikely, as tag combinations with high Levenshtein distances (=>3) were chosen to avoid the artificial generation of existing tag combinations given the sequencing platform used (Salipante et al. 2014; Elbrecht and Steinke 2019).

False positives and false negatives are known drawbacks affecting taxonomic assessment results originating from PCR-based high throughput sequencing techniques, potentially leading to taxonomic biases such as "biodiversity inflation" (Zhou et al. 2013; Tang et al. 2015; Gueuning et al. 2019). Identifying contaminants in wild bee metabarcoding datasets can be hard, because amplification bias may result in false positives, with read numbers equal or higher than the read numbers of true positives (Tang et al. 2015). Even if the false positive found in S8 had fewer reads than any true positive within the sample, their numbers were still over the defined threshold and similar to the read numbers of true positives found in other samples (see Suppl. material 3). Strategies that boost data robustness, such as increasing the number of PCR replicates of the same biological sample (Alberdi et al. 2018; Weigand and Macher 2018) or adjusting the value of filtering thresholds during bioinformatic pruning may be helpful to separate out potential false positives.

Finally, three false negatives were also found in the metabarcoding dataset (*Sphecodes gibbus* in S1, *Lasioglossum pauxillum* in S3 and *Melecta albifrons* in S6). In this case, insufficient sequencing depth seems a more likely explanation than obscurity due to primer bias, as all missing species show low primer-template mismatch with the selected primer pair (Herrera-Mesías et al. submitted). In the experiment, the sequencing run produced fewer overall read numbers than the ones reported by similar works (13.8 million reads in Gueuning, et al. (2019); 11.7 million in Herrera-Mesías et al. (submitted)). Compared to the 47,471 average reads per community of Gueuning et al. 2019, the average number of reads per sample replicate obtained in the double-blind experiment was almost three fold higher (134,340 reads after trimming and quality filtering). However, it was less than a third of the 460,074 average reads per replicate included in the final dataset of Herrera-Mesías et al. (submitted). Therefore, insufficient sequencing depth may have negatively affected specimens of low biomass represented by single individuals in certain sample mixtures. This seems to be the case for *L. pauxillum* in S3. The species presented 12 reads in the first replicate (threshold

of 14 reads) of the sample and 20 reads in the second (threshold of 14 reads), just barely below the 0.01% inclusion threshold (Suppl. material 3). Adjusting the pooling scheme of the library considering different criteria and additional metadata regarding the sample in question (i.e., final DNA concentration in relation to the number of specimens for each bulk sample, size sorting, etc.) may help to reduce the likelihood of false negatives.

Despite the lack of a perfect match with the expert evaluations, the results of the DNA metabarcoding pipeline are similar enough to be advised as a viable alternative to microscopy-based assessment, especially when considering its high congruency to the *PIN*1 results. Moreover, this approach offers several advantages for broad-scale assessments in the context of conservation biology projects, when large quantities of wild bees may be challenging and costly to identify (Lebuhn et al. 2013; Creedy et al. 2020). The number of specimens here analyzed could be increased 10-fold without substantially rising laboratory expenses, work effort, or compromising the quality of results. However, increasing the number of samples can also reduce the number of sequences per replicate, potentially increasing the risk of false negatives. Therefore, each analysis must consider the desired sequencing depth per sample as well as the performance of the platform selected to determine the maximum number of samples that can be pooled on the same run (Elbrecht and Steinke 2019).

Finally, DNA metabarcoding presents a crucial limitation for wild bee monitoring purposes, as it should only be used for qualitative assessment. An alternative molecular, cost-effective but specimen-based solution allowing qualitative results can be offered by high-throughput or next-generation sequencing DNA barcoding (Creedy et al. 2019; Gueuning et al 2019).

Evaluation of In-Vivo Identification

We found that *IVI* of bee individuals considered feasible for alive determination in the field by the monitorer led to similar rates of correct identification as *PIN*, i.e., 95% as judged post-hoc based on the curated consensus list (CON). This may seem surprising, because *IVI* took place in the field without a dissecting microscope. For a better understanding of the results, it is necessary to look more closely at the different error sources that led to incongruencies between the expert identifications.

First, biased expectations appeared to have caused misidentifications especially in *IVI*, where monitorers had knowledge of local bee communities from previous visits. This kind of mistake seems to have generated several cases of incorrect bumblebee identification. For example, in case of BBV86 and BBV98 (see Suppl. material 5) a similar but more noteworthy species was chosen instead of the abundant *Bombus lapidarius*. In another case a female *Megachile leachella* (BBV188) was confused with *Megachile pilidens*. Whereas *M. leachella* was not previously known to occur in the locality, the similar *M. pilidens* had been expected from previous encounters (pers. comm. of monitorer with C.B.).

In contrast, *PIN* appears to have been more susceptible to mistakes like misplaced entries in excel sheets or mix-up of specimens. Such errors were suggested by unlikely misidentifications as in BBV42, a worker bumblebee *Bombus lapidarius* that had been identified as *Halictus subauratus*, a bee that could not be more different. In addition, some errors arose from biases in the used identification keys or reference collections. For example, the popular (and generally very good) identification key for bumblebees by Mauss (1987) does not cover the full range of (corbicula hair) color variability of *Bombus humilis*. Its use by *PIN* experts was associated with repeated misidentification of *Bombus humilis* workers as *Bombus ruderarius*, for which reddish corbicula hair is a well known trait (BBV13, BBV14, BBV16, pers. comm. with C.B.). The alternative distinctive trait (shape of labrum bottom edge) given by the key was not considered by the experts and another evident characteristic of the specimens (bright facial hair; untypical for *B. ruderarius*) was neither explicitly treated by the key nor noticed by the experts. Biased reference collections appeared to have caused other errors in *PIN*. For example, the expert who incorrectly identified a female of *Megachile maritima* (BBV169) as *Megachile willughbiella* did so based on divergent reference material collected from populations outside of Germany (pers. comm. with C.B.). In discussions with C.B., some *PIN* experts stated their insufficient experience with species outside of their region of expertise as a possible source of error.

Due to the design of the study there might be a number of intrinsic biases that could have increased the accuracy of *IVI* relative to *PIN*. First, *IVI* experts had a free choice regarding which of the encountered bees they considered feasible for *IVI* (during evaluated monitorings approximately 90% of individuals were considered feasible for *IVI*, a rate that corresponds well with *IVI* rates during regular BienABest monitorings; BienABest project, unpublished results). Thus, they could directly influence the sample of bees/identifications that was being evaluated. In addition, *IVI* experts were very aware of being evaluated, and were constantly reminded of the fact by the presence of C.B. who collected their *IVI* bees. *PIN* experts, while also having been informed that their results will be used in a double-blind evaluation, did not work under close observation. This discrepancy in experienced scrutiny could have led to different likelihoods of careless mistakes.

The relationship between the amount of experience of the expert and the accuracy of identification results is less than clear. All experts included in this study (*IVI* and *PIN*, also for the *DNA* comparison) were recognized experts of bee morphotaxonomy with at least some years, but mostly many years, of experience. If there was a difference at all, the amount of experience was slightly higher and less variable among *PIN* than among *IVI* experts. The *IVI* monitorer considered least experienced did indeed deliver the least accurate identification result of only 84.6% in comparison to the consensus list. However, the respective bee set (S3) was also the one that had the lowest congruency among *PIN* experts (90.6%), suggesting that the set was difficult.

In general, *IVI* as conducted within the BienABest project yielded accurate identifications in nineteen out of twenty bees (95%). It needs to be emphasized that such accuracy can only be achieved by highly trained experts, a resource that is in short

supply (Drew 2011) and needs to be replenished by concerted efforts of universities, NGOs, national authorities and funding agencies. Probably, *IVI* will remain limited to a certain part of bee diversity that is feasible for *IVI*. Exactly how large this part is, is a matter of debate. According to a list ("Ampelliste") prepared by experts during their work on the VDI-Richtlinie 4340-1 (2021) just about 50% of females of German bee species can currently be identified alive. In the male sex the percentage is considered to be even lower (30%). It remains to be seen if this percentage can be increased in the future with the help of digital tools that allow scrutiny of additional taxonomical characters. Currently, such a tool is being developed within the BienABest project for identification of 300 bee species of Central Europe via smartphone app, which includes high quality pictures to guide reliable identification under field conditions.

There is a controversial debate on whether the use of *IVI* is in fact necessary and desirable for wild bee monitoring (Gezon et al. 2015). Generally, the effect of invasive sampling on insect populations, and bees in particular, has not been well studied (Packer and Darla-West 2021). We are aware of only one study that was dedicated to assess the effect on wild bee communities: Gezon et al. (2015) found no negative effects of several years of bi-weekly pan trapping and netting on bee communities in the Rocky Mountains (Colorado, USA). However, the study was conducted in large tracts of near natural habitat, and it is questionable whether the results can be transferred to the degraded and fragmented bee habitats in Central Europe (e.g., Steffan-Dewenter et al. 2006). It seems plausible to assume that repetitive removal of reproductive individuals can affect local populations of already endangered species, especially in solitary bees which are characterized by low reproductive rates and which often demonstrate a highly localized distribution (Westrich 2019). This is supported by at least one study that used colored vane traps and found conspicuous declines of attracted species in one locality (Gibbs et al. 2017). Depending on locality and monitoring design, *IVI* may be the way of erring on the safe side.

On reference specimens

IVI and most DNA metabarcoding approaches relying on bulk samples might have another disadvantage, as both strategies usually do not deposit extensive reference material. A reference collection for future comparison is often a legal requirement or at least important to judge about spatio-temporal patterns of individual species in times of changing taxonomies, e.g. within species complexes. Voucher specimens are also relevant in case upcoming taxonomic methods require biomaterial or morphometric data to address open taxonomic questions, or for educational purposes (Lister et al. 2011; Monfils et al. 2017; Kharouba et al. 2019), or to validate particularly noteworthy findings. Moreover, voucher specimens stored in local natural history collections represent an important resource for the construction of future taxonomic lists, including potentially overlooked findings relevant to the development of national conservation strategies (Herrera-Mesías and Weigand 2021). The most common referencing strategy of DNA metabarcoding approaches – if any – is the deposition of DNA vouchers. However, in cases of surprising results, DNA vouchers will make it difficult to further judge about the unexpected results.

In the metabarcoding setup here applied, DNA was extracted from individual legs while the rest of the voucher specimens were archived in the invertebrate collection of the MNHNL. Although this led to an increase in the hands-on-times and costs per sample, it preserves specimens for future conservation studies (Herrera-Mesías and Weigand 2021). Single specimen barcoding or HTS barcoding might also be helpful in the context of wild bee monitoring (Schmidt et al. 2015; Gueuning et al. 2019), especially when abundance data are desirable and total specimen numbers feasible to handle.

Regarding *IVI*, additional documentation could be provided by depositing high-quality images taken from live bees confined in observation jars, as is currently done by some experts. However, this requires appropriate equipment and imposes substantial additional effort during field work. Also, there currently exists no general depository for digital specimens of wild bees.

Conclusion

To our best knowledge, this is the first study to compare the accuracy of alternative taxonomic tools against morphology-based identifications using a double-blind approach. Both DNA metabarcoding and *in vivo* determination in the field presented high overall congruency of their identification results with a traditional microscopy-based assessment performed by morphotaxonomic experts. These results validate the use of these alternative assessment techniques in conservation projects targeting wild bees of Central Europe. The metabarcoding pipeline is recommended for the qualitative analysis of large samples in the absence of taxonomic experts, and for resolving morphotaxonomic problems. However, strategies that boost data robustness are highly advised to control the effect of potential environmental contaminations, false positives, and false negatives. Moreover, metabarcoding data should not be used on its own to estimate quantitative population parameters due to biases in PCR amplification. On the other hand, *in vivo* identification can be used for quantitative assessment. It is advised for long-term monitoring, especially in fragile ecosystems with vulnerable bee populations. It is susceptible to misidentification due to preconceptions and potentially constrained by the experience and availability of monitorers. By concept, *in vivo* identification results in no or fewer deposited reference specimens so that the detection of rare and particularly noteworthy species may be difficult to validate. Generally, all techniques rely heavily on the availability of reference materials such as barcode sequences, voucher specimens, or reference images. Further efforts are needed to address this issue, thus filling the gap of information needed to refine the detection capacity of alternative identification techniques.

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Supplementary material 1

Supplementary file 1

Authors: Fernanda Herrera-Mesías, Christopher Bause, Sophie Ogan, Hannah Burger, Manfred Ayasse, Alexander M. Weigand, Thomas Eltz

Data type: Docx file.

Explanation note: Curations made to merge taxonomic lists resulting from DNA and *PIN* (metabarcoding pipeline part).

Link: https://doi.org/10.3897/jhr.93.86723.suppl1

Supplementary material 2

Supplementary file 2

Authors: Fernanda Herrera-Mesías, Christopher Bause, Sophie Ogan, Hannah Burger, Manfred Ayasse, Alexander M. Weigand, Thomas Eltz

Data type: Docx file.

Explanation note: Barcoding of two critical specimens for the consensus list (CON).

Link: https://doi.org/10.3897/jhr.93.86723.suppl2

Supplementary material 3

Supplementary file 3

Authors: Fernanda Herrera-Mesías, Christopher Bause, Sophie Ogan, Hannah Burger, Manfred Ayasse, Alexander M. Weigand, Thomas Eltz

Data type: Xlsx file.

Explanation note: Raw Hymenoptera metabarcoding data with number of sequence reads as well as information on taxon curation and individual tagging combinations.

Link: https://doi.org/10.3897/jhr.93.86723.suppl3

Supplementary material 4

Supplementary file 4

Authors: Fernanda Herrera-Mesías, Christopher Bause, Sophie Ogan, Hannah Burger, Manfred Ayasse, Alexander M. Weigand, Thomas Eltz

Data type: Docx file.

Explanation note: Identification results of *DNA* and *PIN*.

Link: https://doi.org/10.3897/jhr.93.86723.suppl4

Supplementary material 5

Supplementary file 5

Authors: Fernanda Herrera-Mesías, Christopher Bause, Sophie Ogan, Hannah Burger, Manfred Ayasse, Alexander M. Weigand, Thomas Eltz

Data type: Docx file.

Explanation note: Identification results of *IVI* and *PIN* experts per bee specimen compared to the CONsensus list.

Link: https://doi.org/10.3897/jhr.93.86723.suppl5

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BOOK REVIEW

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Book review: The Bumblebees of the Himalaya – An Identification Guide, by Paul H. Williams

Guillaume Ghisbain[1], Denis Michez[1]

1 *Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Mons, Belgium*

Corresponding author: Guillaume Ghisbain (guillaume.ghisbain@umons.ac.be)

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Presentation of the book

"The Bumblebees of the Himalaya – An Identification Guide", written by Dr. Paul H. Williams and published in May 2022, is a ~200-page guide for the bumblebee fauna of the Himalaya, aiming to provide an up-to-date aid for laboratory identification of all species recorded in the region.

The introduction of the book presents general traits of bumblebees, how to recognize them among other bees, how to catch them in the field, and how to preserve them properly in entomological collections. The biogeographical region of interest – the Himalaya – is also introduced, and a habitat classification for Himalayan bumblebees is proposed and briefly illustrated. The last part of the introduction mostly covers taxonomy, with a concise introduction on what a species is, and importantly with an explanation of the taxonomic concepts followed by the author. An updated checklist of all 62 species present in the Himalaya is provided, including common synonyms present in the literature. Four pages are dedicated to color pattern diagrams formatted to illustrate the distribution of these forms in the studied region, and the type of habitat they are associated with. Bumblebee anatomy is described and illustrated to prepare the reader for the keys that follow, the first being a key to bumblebee subgenera from the studied region.

Then starts the main part of the book ("Systematic account"), that consists in chapters with content organized by subgenera. Every bumblebee subgenus is first briefly described with general notes on its ecology, including habitat requirements, nesting behavior, and type of visited flowers. Following this section are keys for males and females for all Himalayan species belonging to each subgenus. The author then proposes information on every species, including (i) frequently encountered synonyms and type revision, (ii) the color patterns male and female specimens can present, (iii) an illustration of the male genitalia, (iv) the habitat type the species is associated with, (v) maps highlighting the regions in which the species has either been recorded, where the species is likely to be present, or has not yet been recorded from and (vi) notes including taxonomic revisions, accompanied with comments on intraspecific variation. One new species for science is described (*Bombus rainai* Williams), with information on the type series (holotype, paratype) and a formal description. Several species have their status revised and a taxon is synonymized.

General appreciation and scientific appreciation

Bumblebees are the most studied wild bees worldwide (Cameron and Sadd 2020; Ghisbain 2021). Their big body size, colorful appearance, natural abundance and diversity in areas visited by naturalists of the northern hemisphere have made them relatively well represented in museum collections compared to other bees (Wood et al. 2019, 2021). As a direct consequence, scientists were able to gather immense quantities of data about their biogeography, ecology, taxonomy and conservation (Kleijn and Raemakers 2008; Williams and Osborne 2009; Goulson et al. 2011, 2015). Most scientific and naturalist works, however, are still very centered around North America and Europe (Cameron and Sadd 2020; Ghisbain et al. 2020; Rasmont et al. 2021), and Asia (the continent hosting by far the highest species diversity) is still largely overlooked (Williams et al. 2010, 2020).

With more than 40 years of in-depth work on Asian bumblebees, there is no doubt that Dr. Paul H. Williams was the best candidate for writing such a book. Through this publication, the author shares a synthetic, yet critical knowledge on the extremely diversified bumblebee fauna of the Himalaya. Written as a "lockdown project" with no access to museums due to the COVID-19 pandemic, the book is a solid base for building further knowledge on the ecology, taxonomy, and conservation of this strikingly difficult and polymorphic fauna.

General background

The introduction of the work covers most important notions needed for this identification guide to be used properly. As stated by the author himself, the book is "not an introduction to bumblebee general ecology", and therefore the reader must not expect to find there a deep revision. Overall, the introduction is clear, concise and pragmatic, allowing a reader to acquire a sufficient background for properly using the book.

Taxonomy

The taxonomic part is rigorous. The author is aware of how difficult bumblebee taxonomy is, and how complex data analysis and interpretation appear when investigating large, polymorphic species complexes. A positive aspect is that the author provides his vision on how taxonomic work should be conducted to achieve the most robust hypotheses possible. This habit of defining the species concept followed is in line with the author's previous works on subgeneric revision of bumblebees (e.g. Williams et al. 2019, 2020; Williams 2021).

In addition to the discovery and description of a new *Alpigenobombus* species, *Bombus rainai* Williams, the author suggests that some taxa (*Bombus hilaris* (Tkalců), *B. kotzschi* Reinig, *B. sikkimi* Friese, *B. longiceps* Smith) deserve a species status, and justifies this interpretation rigorously based on a combination of morphological and genetic characters (some still to be formally published). Such taxonomic modifications and additions are critical for the implementation of future conservation strategies, and therefore bring a great value to the book. The author took care to thoroughly revise the type material of many taxa, and was careful to document the location of these specimens for future research, which is also immensely appreciated.

One noticeable weakness of the book, however, lies in the fact that the introductory section on taxonomy is very centered around one view of bumblebee taxonomy, rather than a concise, synthetic view of how to approach species delineation in this group of bees. A reader who is not fully aware of the scientific literature about bumblebee taxonomy thus receives only one particular interpretation of how bumblebee taxonomy can be conducted, and only involving the tools that the author is routinely using (*i.e.* morphology and genetic barcodes). Other lines of evidence for species delineation such as the analysis of cuticular hydrocarbons, cephalic labial gland secretions and non semio-chemical tools such as geometric morphometrics are not mentioned, despite their common use (Dehon et al. 2019; Valterová et al. 2019). This is unfortunate as all these approaches are complementary to the author's rigorous view of taxonomy. Such analyses would also help bring key information on the ecology and evolutionary history of the bumblebees of the Himalaya, for which so much is still to discover. As these approaches are not mentioned, the section of the book about the collection and preservation of specimens does not account for their preparation for such analyses. As the addition of such complementary lines of evidence can lead to divergence in opinion on the taxonomic status of some species (*cf.* Williams et al. 2019; Rasmont et al. 2021), we believe that mentioning their existence is essential.

In line with the previous taxonomic works he led (e.g. Williams et al. 2012, 2019, 2020), the author does not recognize subspecies, although he does appreciate the importance of carefully illustrating and documenting the rich intraspecific diversity of bumblebee species. We certainly agree with the author that (i) the use of subspecies in bumblebees can be challenging for some species and (ii) caution should be drawn when extrapolating or inferring (sometimes unmeasured) characteristics of subspecies. Keeping this in mind, we believe that the book could have benefitted from presenting complementary

arguments to balance these points. First, in a purely taxonomic framework, it is relatively uncommon that such ecological extrapolations are done, and the fact that some authors could make unjustified inferences does not constitute an argument against the concept of subspecies itself ; in that case the issue would lie in the authors themselves extrapolating the concept. Second, knowledge from other widely studied insect models (e.g. the Neotropical *Heliconius* butterflies) shows that recognizing infraspecific status can be extremely useful for understanding how evolutionary patterns and processes shape natural diversity (e.g. Flanagan et al. 2004; Baxter et al. 2008; Supple et al. 2013; Arias et al. 2017; Concha et al. 2019). Furthermore, the absence of names from recognizable entities is risky as it makes the knowledge gathered about them unstable across time ("*Nomina si nescis perit cognitio rerum; et nomina si perdas, certe distinctio rerum perditur*", Edward Coke). This is especially important as what we assume today to be infraspecific variation might later appear distinct genetically, semio-chemically, or ecologically when data become available (cf. the case of *B. konradini*, Martinet et al. 2018; and other cases recently presented by the author in his works Williams et al. 2020; Williams 2021). As this scenario is relatively common in bumblebees (many species indeed show a high degree of crypticism: Ghisbain et al. 2020, 2021; Williams 2021), properly naming entities with different phenotypic aspects can be helpful for future research. Finally, because subspecies can be recognized as valid taxonomic entities that can receive conservation measures following the IUCN standards, presenting them in identification guides could be useful to allow their monitoring at local scales. Overall, we would advocate for a more balanced view on the topic of subspecies, above all in a group of insects that represents such a good model to understand phenotypic radiation across space and time.

Ecology and conservation

The author presents information on the ecology of each species, including some information on their habitat. This information is concise and is based on the author's own original observations. These data are greatly appreciated as barely anything is currently known about the habitat requirements of the bumblebees of the Himalaya. It also suggests that further research is strongly needed to investigate more in detail both their habitat and climatic requirements.

With bumblebee conservation currently of global interest and concern (Cameron and Sadd 2020), we feel that the introduction of the book would have benefitted from a broader perspective about this topic, above all given the author's renowned experience on the field (cf. Williams et al. 1996; Williams and Araéjo 2000; Williams and Osborne 2009, among many other important works). Although we acknowledge that barely anything is known about the conservation of the bumblebees in the Himalaya (none has received a proper IUCN conservation status), we believe that the publication of such an important book would also be a great opportunity for raising awareness about how critical conservation is for bumblebees, and how much we need involvement from local communities for avoiding reproducing in the Himalaya the mistakes seen in other regions of the world.

Illustrations

The book has been written during the COVID-19 pandemic, at a time of highly limited access to entomological resources in museums. Despite this, the book is well illustrated. The genitalia of each species are photographed, which can be of great aid (and sometimes critical) for the identification of males in many species. For all taxa, intraspecific variation is illustrated with color diagrams, which are also greatly appreciated given the high degree of polymorphism of the Himalayan fauna. Although the maps can look very synthetic as no individual data points are shown, the idea of the author to highlight where the species could be expected (using a color code) is highly informative, as it can help in further investigation and field trips across the regions.

Conclusion

Overall, the book "The Bumblebees of the Himalaya – An Identification Guide" is an essential contribution to its field. It properly serves its role to document and help in the identification of the strikingly diverse fauna of the Himalaya. Although we regret some short-cuts in the introduction (mostly about taxonomy and conservation), we are certain that the research that will be allowed thanks to this book in the near future will help better understand the remarkable ecology of this fauna.

We sincerely congratulate Dr. Paul H. Williams for his identification guide. With ongoing and incoming work from local scientists and passioned naturalists, we hope that his book will raise interest to study, admire and protect this largely overlooked yet critically important Asian bumblebee hotspot.

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