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Revision of the neotropical genus *Sendaphne* Nixon (Hymenoptera, Braconidae, Microgastrinae)

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Abstract

The Neotropical genus of parasitoid wasps *Sendaphne* (Hymenoptera, Braconidae, Microgastrinae) is revised and the following six new species are described, all authored by Fernández-Triana and Whitfield: *anitae*, *bennetti*, *broadi*, *dianariaspennae*, *penteadodiasae*, and *rogerblancoi*. The greatest species richness is found in northern South America, but the genus extends north to 23° N in Mexico. Most species have been collected in rainforest below altitudes of 900 m, with only a few species found in cloud forests up to 1900 m. Nothing is known of the host caterpillars for these parasitoid wasps.

Keywords

Sendaphne, Microgastrinae, Neotropics, Area de Conservación Guanacaste, taxonomic revision, parasitoid wasps, DNA barcoding

Introduction

The genus *Sendaphne* was described by Nixon (1965) to accommodate two Neotropical species with a number of distinctive features: a very long and bilobate glossa, slender body, extensive yellow coloration, mostly smooth mesosoma and metasoma, enlarged hypopygium, and very long and curved ovipositor. Penteado-Dias (1995) described two additional species, and Scatolini and Penteado-Dias (1999) added another one. Four of the five species described so far are from Brazil, with the exception being *Sendaphne sulmo* Nixon (1965), recorded from southeastern Mexico. No host is known for *Sendaphne*, although the related genus *Promicrogaster* Brues & Richardson, 1913, has been reared from hosts living in bracket fungi (Mason 1981), among other concealed hosts.

In spite of the rather unique morphological traits, the validity of *Sendaphne* as a distinct genus has been questioned even by the same author who described it (Nixon 1965: 204), and it might eventually be determined to be a synonym of the more abundant and diverse *Promicrogaster*. However, Mason (1981) considered the differences as sufficient to maintain them as two genera. That decision has been followed by all subsequent authors and, without a comprehensive phylogenetic study of Microgastrinae, we agree that is better to keep it that way at present. The morphological and molecular analysis of Whitfield et al. (2002), although not conclusive, also found those two genera to be closely related. An anticipated revision of *Promicrogaster* may help to elucidate the limits of these two genera in the future.

As part of comprehensive studies on the fauna of Microgastrinae from Area de Conservación Guanacaste (ACG), northwestern Costa Rica (e.g., Fernández-Triana et al. 2014 and references cited there) we found a new species from that area. The Canadian National Collection of Insects (CNC) in Ottawa, Canada and the NSF-funded "Insect Survey of a Hyperdiverse Country: Colombia" projects led by M. J. Sharkey, B. V. Brown and the Humboldt Institute (Colombia) also contained additional undescribed species from Central and South America. All these new species are described below, altogether with a key to all known, previously described species of *Sendaphne*.

Methods

Among Microgastrinae, *Sendaphne* is one of the most rarely collected genera, and is poorly represented in collections. This study is based on 73 specimens from four sources: 38 Neotropical specimens deposited in the CNC, 18 specimens from Colombia (Humboldt Institute), 10 specimens from the ACG inventory, four specimens from Unité d'Entomologie fonctionnelle et évolutive, Gembloux Agro-Bio Tech, Université de Liège (FUSAGx) and three specimens from the Natural History Museum, London, England (BMNH).

All species previously described were deposited in the Universidade Federal de São Carlos, São Carlos, São Paulo, Brazil (DCBU), Universidade Federal do Paraná,

Curitiba, Paraná, Brazil (UFPR), or in the BMNH. We did not examine the holotypes of those species; however, their original descriptions and illustrations are sufficiently detailed to allow us to describe the new species with confidence.

Morphological terms and measurements of structures are mostly as used by Mason (1981), Huber and Sharkey (1993), Whitfield (1997), Karlsson and Ronquist (2012), and Fernández-Triana et al. (2014). Because the ovipositor in *Sendaphne* is strongly curved, its length was difficult to measure accurately; the ovipositor length measurements provided for each new species are only intended as an approximation. In any case, the ovipositor and its sheaths are some of the longest observed in any Microgastrinae genera; they are usually two times longer than the metatibia length.

Descriptions of the new species are based on the study of all available female specimens, so as to reflect intraspecific variation, but always include data from the holotype. As an exception, two new species only known from males are described below because they were sufficiently distinct to be distinguished from all others; the males of those two species may be identified by the key, but males of most other species may not be readily identified unless associated with females via rearing or molecular data.

The descriptions include 17 characters that are commonly used in describing Microgastrinae (e.g., body measurements such as length of body and fore wing, ovipositor sheath; and also color of particular body areas). Those descriptions are complemented with extensive color photos of every species. Geographic distribution is also provided in the key as supplementary information to aid the morphological identification of species, though we recognize that with time the current known geographic distribution may eventually become obsolete.

Photos were taken with a Keyence VHX-1000 Digital Microscope, using a lens with a range of 13–130×. Multiple images through the focal plane were taken of a structure and these were combined to produce a single in-focus image, using the software associated with the Keyence System.

Together with morphological studies, we also analyzed DNA barcodes (the 5' region of the cytochrome *c* oxidase I (CO1) gene, Hebert et al. 2003) whenever available. DNA barcodes for all ACG inventory *Sendaphne* specimens were obtained using DNA extracts prepared from single legs using a glass fibre protocol (Ivanova et al. 2006). Briefly, total genomic DNA was re-suspended in 30 µl of dH2O, and a 658-bp region near the 5' terminus of the CO1 gene was amplified using standard primers (LepF1–LepR1) following established protocols (Smith et al. 2006, 2007, 2008). If the initial 658 bp amplification was unsuccessful, smaller sequences were generated using internal primers. If each amplification worked a composite sequence was generated, however in cases where only one read amplified, this shorter sequence was used. All information for the sequences associated with each individual specimen can be retrieved from the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007) using the following public DOI: http://dx.doi.org/10.5883/DS-SENDAPH. A Neighbor-Joining tree based on Kimura 2-parameter distances of all described species of *Sendaphne* with DNA barcodes available in BOLD was also generated (Suppl. material 2).

Results

Six new species of *Sendaphne* are described below, increasing the total known species from five to 11. We are aware of potential additional new species in the CNC collection, from Costa Rica (but not ACG), Ecuador, and Brazil. However, they are not described here because each is only represented by a single specimen and they are not sufficiently distinct to warrant description without further specimens and evidence.

Sendaphne is a Neotropical genus. To date it is most abundant and diverse in South America (eight species), while Central America has three species. It extends from 23° N in central Mexico (Durango) to 27° S in Paraguay (Pirapo) and southern Brazil (Santa Catarina). Most of the species have been collected in rain forests, at altitudes between 100 m and 900 m. However, a few species have been found only in cloud forests between 1,450 m and 1,900 m. The specimens collected at higher altitudes have darker coloration (especially on mesosoma and metasoma) than those found in the lowlands.

An interesting result of our morphological study was the relation between body and fore wing lengths. Body proportions in Microgastrinae have not been explored in detail, but in most genera and species with available data the fore wing length tends to be slightly longer than the body length (usually by 0.1–0.2 mm). In the specimens of *Sendaphne* described here, the body length was longer than the fore wing length (usually by 0.2–0.4 mm). The main reason is the long and slender body form (rather than exceptionally short wings), and an unusually enlarged and extended hypopygium.

Observations of COI barcodes for *Sendaphne*

Of 46 specimens sampled (Suppl. material 1), we recovered sequences for only 24 specimens. Only three sequences were over 600 base pairs, while the rest were mostly minibarcodes of 102–390 base pairs each. However, six out of the 11 known species now have some molecular data associated (Suppl. material 2). Many of the initial amplifications from ACG were characterised by unintended amplification of the endosymbiont bacteria *Wolbachia* that were amplified in the first round LepF1/LepR1. These sequences were not mistaken for the wasp DNA (Smith et al. 2012) and are retained on BOLD in the trace files. The standard strategy of amplifying two smaller and overlapping regions secondarily was then followed (as the first round was considered a 'failure'). In this case the 5' amplification (LepF1/C_ANTMR1D) worked and the 3' amplification (RonMWASPdeg_t1 - LepR1) failed. Thus many of the ACG *Sendaphne* are ~260–280bp in length. Finally, regarding the CNC specimens, these were, on average, 35 years old. In those cases, greater success with smaller amplifications compared to larger amplifications is to be expected (Hajibabaei et al 2006). In 2010 and 2011 when these specimens were submitted to the Biodiversity Institute of Ontario (Guelph) for DNA barcoding, they only had the primers generating minibarcode (smaller amplicons) used for PCR. The longer amplicon generating primer pairs were not attempted on these specimens.

Genus *Sendaphne*

Sendaphne Nixon, 1965: 203.

Diagnosis. Glossa elongate and bilobate (Figs 2, 9, 34, 48, 58, 70). Lateral face of scutellum with polished area (=lunules) occupying most of the lateral face (Figs 5, 12, 19, 28, 44, 49, 56). Propodeum usually smooth and without carina (exceptionally having sparse punctures and few rugae on the nucha) (Figs 7, 13, 19, 28, 33, 44, 49, 60, 74). Metacoxa very long, about the same length as metafemur length and metatibia length (Figs 8, 13, 15, 29, 50, 54, 61, 68, 74). Mediotergite 1 strongly narrowing towards posterior margin (Figs 4, 14, 19, 50, 51). Mediotergite 2 subtriangular, much longer medially than its width at its anterior margin (and usually also longer medially than its width at posterior margin). Ovipositor very long for a microgastrine wasp (two times longer than metatibia length) and strongly curved (Figs 1, 20, 35, 53, 54, 57, 64, 68); apex of ovipositor usually not sinuate (exceptionally with very slight sinuation). Fore wing with very wide first discal cell, and with small areolet (Figs 3, 17, 17, 24, 39, 55, 62, 69) (areolet sometimes not well-defined because veins 3RSa and r-m are spectral, as in Figs 10, 31, 47). Body color often mostly yellow to orange (with a few exceptions from species collected at higher altitudes, which have head, mesosoma and parts of metasoma dark brown to black). Body length longer than the fore wing length, usually by 0.2–0.4 mm. Within Microgastrinae, *Sendaphne* can only be confused with *Promicrogaster*, but the later has a more transverse mediotergite 2, apex of ovipositor clearly sinuate, and propodeum usually with more sculpture and carination present.

Key to *Sendaphne* species

[This key is intended for female specimens, although two species are only known from males, and in those cases the key accommodates them. Generally, males tend to have darker coloration than the females, especially on the metasoma].

1 Head and mesosoma entirely dark brown to black (Figs 23, 24, 27, 28, 37–44, 68–75), metasoma entirely dark brown (Figs 69, 74, 75) or at most with mediotergites 1–2 orange-yellow (as in Figs 24, 25, 28, 39, 44) [all specimens collected above 1,460 m, in cloud forests]..... **2**

– Color variable but much lighter than previous couplet, ***if*** head entirely dark brown to black, then mesosoma entirely or mostly yellow, orange or reddish-yellow, ***and*** metasoma at most with brown bands on posterior margins of mediotergites 3+ [all specimens collected below 900 m, in rainforests] **4**

2(1) Tegula, metacoxa, and mediotergites 1–2 dark brown to black (Figs 69, 73–75); body very slender, metasoma longer than combined length of head and mesosoma (Fig. 68) [Costa Rica] ....***Sendaphne rogerblancoi*** **Fernández-Triana & Whitfield, sp. n.**

– Tegula, metacoxa and mediotergites 1–2 yellow to reddish-yellow (Figs 23–28, 37–40, 42–44); body less slender, metasoma shorter than combined length of head and mesosoma (Figs 23, 37) [Ecuador, Mexico] **3**

3(2) Mediotergite 1 length 6.0 × its width at posterior margin, mediotergite 2 length 1.5 × its width at posterior margin (Figs 24, 25, 28); first discal cell 2.0 × as wide as high (Fig. 24) [Ecuador] ..... ***Sendaphne broadi* Fernández-Triana & Whitfield, sp. n.**

– Mediotergite 1 length 4.0 × its width at posterior margin, mediotergite 2 length 1.0 × its width at posterior margin (Figs 39, 42, 44); first discal cell 1.3 × as wide as high (Fig. 39) [Mexico] ..... ***Sendaphne bennetti* Fernández-Triana & Whitfield, sp. n.**

4(1) Head yellow to reddish-yellow **5**

– Head dark brown to black **7**

5(4) Mesosoma with dark brown areas on anteromesoscutum and mesopleuron (Figs 54, 56, 58); dark brown coloration on posterior margin of mediotergite 3 and most of mediotergites 4–6 (Figs 54, 55, 57, 59) [Brazil, Paraguay] ..... ***Sendaphne paranaensis* Scatolini & Penteado-Dias, 1999**

– Mesosoma uniformly orange-yellow to reddish-yellow; metasoma either entirely yellow or with brown bands on posterior margin of mediotergites 4–6 ..... **6**

6(5) Metasoma with brown bands on posterior margin of mediotergites 4–6 (Figs 50–53) [Brazil, French Guiana, Peru] ..... ***Sendaphne olearus* Nixon, 1965**

– Metasoma entirely yellow (Figs 31, 32, 35, 36) [Brazil, Ecuador, French Guiana] ..... ***Sendaphne jatai* Penteado-Dias, 1995**

7(4) Female metasoma either with extensive dark brown coloration on tergites 3+ or with some narrow brown bands on posterior margin of mediotergites 5–7 (Figs 63, 64) **8**

– Female metasoma entirely yellow (Figs 3, 4, 7, 18, 19, 22) **9**

8(7) Metasoma with dark brown bands on posterior margin of mediotergites 3–6 and mediotergite 7 entirely dark brown to black; fore wing vein 1Cu-a much shorter than vein 1Cu-b; T1 10.0 × as long as width at posterior margin; T1 2.0 × as long as T2; metacoxa 1.1 × as long as metafemur ..... ***Sendaphne brasilianus* Penteado-Dias, 1995**

– Metasoma with some narrow brown bands on posterior margin of mediotergites 5–7 (Figs 63, 64); fore wing veins 1Cu-a and 1Cu-b subequal; T1 6.0 × as long as width at posterior margin; T1 1.4 × as long as T2; metacoxa 0.9–1.0 × as long as metafemur ..... ***Sendaphne penteadodiasae* Fernández-Triana & Whitfield, sp. n.**

9(7) Fore wing r and 2RS not clearly distinct from each other (Fig. 3); mediotergite 2 length 1.0 × its width at posterior margin (Figs 3, 4, 7); face centrally orange (Fig. 2); ovipositor 1.8–1.9 × as long as metatibia [Ecuador] ..... ***Sendaphne anitae* Fernández-Triana & Whitfield, sp. n.**

– Fore wing with veins r and 2RS clearly distinct from each other, and meeting at a sharp angle (not clearly visible in Fig. 17); mediotergite 2 length 1.8–2.0

× its width at posterior margin (Figs 18, 19); face entirely dark brown to black (Fig. 16); ovipositor 2.0–2.2 × as long as metatibia [Brazil, Colombia, or Mexico] **10**

10(9) Distance between anatomical line tangent to posterior margin of anterior ocellus and anterior margin of posterior ocelli 0.5 × diameter of anterior ocelli (Fig. 77); ocular–ocellar line 2.5 × as long as posterior ocellus diameter; interocellar distance 1.6 × as long as posterior ocellus diameter; T1 relatively narrower medially, T1 width at half length of tergite clearly less than width at anterior margin, and 1.5 × as wide as width at posterior margin (Fig. 78); T2 1.8 × as long as wide [Mexico] ..... ***Sendaphne sulmo*** **Nixon, 1965**

– Distance between anatomical line tangent to posterior margin of anterior ocellus and anterior margin of posterior ocelli 0.2–0.3 × diameter of anterior ocelli (partially visible in Fig. 16); ocular–ocellar line 2.1–2.4 × as long as posterior ocellus diameter; interocellar distance 1.2–1.4 × as long as posterior ocellus diameter; T1 relatively wider medially, T1 width at half length of tergite about same as width at anterior margin, and at least 2.0 × as wide as width at posterior margin (Figs 18, 19); T2 2.0 × as long as wide [Brazil, Colombia] .... ***Sendaphne dianariaspennae*** **Fernández-Triana & Whitfield, sp. n.**

Taxonomic treatment of species, in alphabetical order

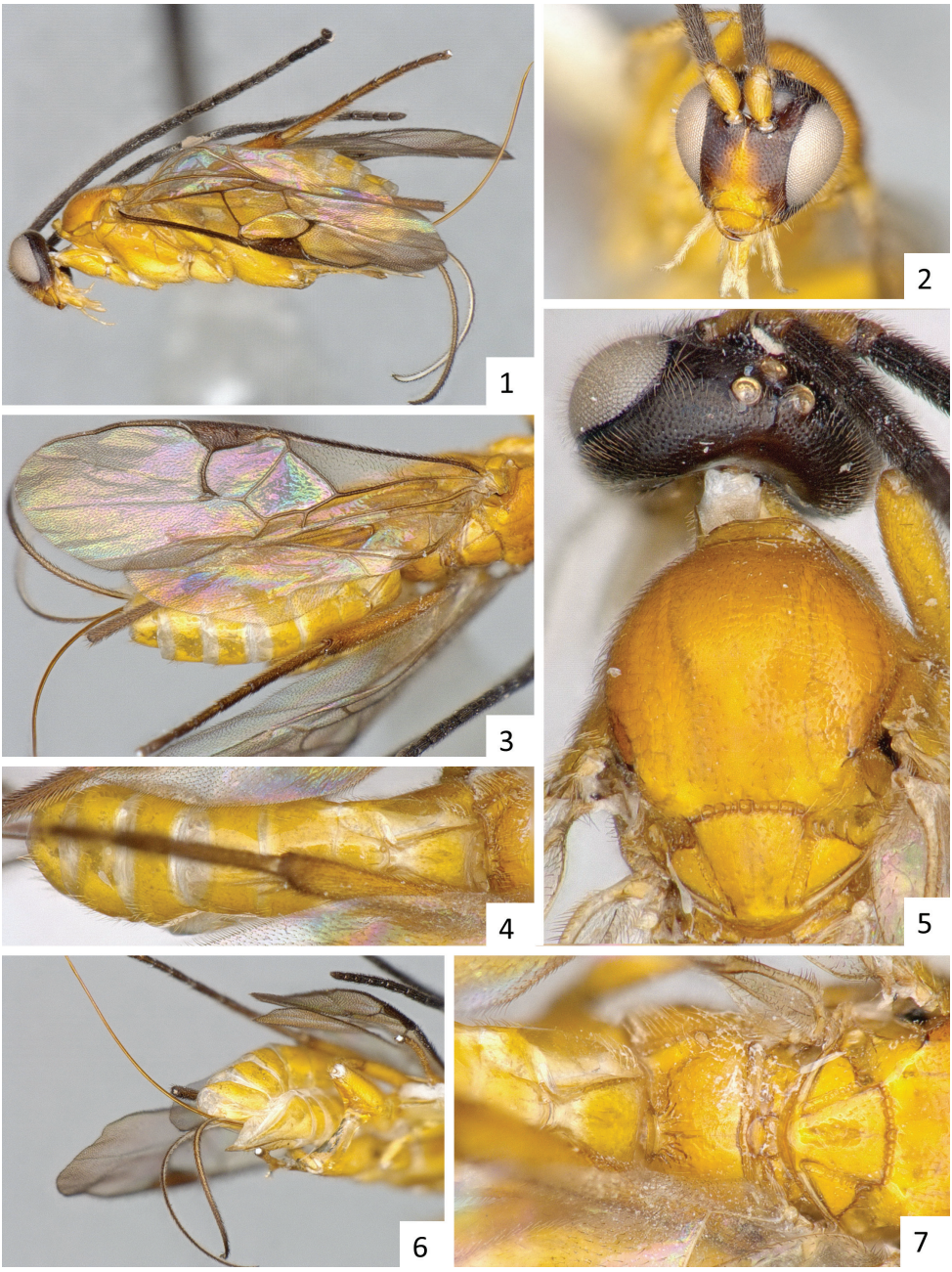
Sendaphne anitae **Fernández-Triana & Whitfield, sp. n.**
http://zoobank.org/5A29AD35-D955-44B1-B1C3-64182FD0E55B
Figs 1–7

Holotype. Female, CNC. ECUADOR, Napo, Reventador; 11.iii.1983; coll. L. Huggert. DNA Voucher code: CNCHYM 07027.

Paratypes. 4 ♀ (CNC), same locality and collecting date than holotype. Two of the specimens also have DNA Voucher codes: CNCHYM 07026, CNCHYM 07028.

Diagnosis. This species is morphological similar to *Sendaphne dianariaspennae* and *S. sulmo* but differs from those species in the shape and angle of junction of veins r and 2RS, shape of mediotergite 2, ovipositor length, and head coloration (orange-yellow on clypeus and face centrally in *anitae*, head entirely dark brown to black in *dianariaspennae* and *sulmo*).

Description. Head color: dark brown to black, except for orange-yellow on clypeus and face centrally. Mesosoma color: orange-yellow. Tegula color: orange-yellow. Metasoma color (dorsally): yellow. Metacoxa color: yellow. Anatomical line tangent to posterior margin of anterior ocellus crossing very slightly (less than 0.01 mm) above anterior margin of posterior ocelli. Ocular–ocellar line: 0.20 mm. Interocellar distance: 0.09 mm. Posterior ocellus diameter: 0.09 mm. Body length: 4.0–4.5 mm. Fore wing length: 3.6–4.1 mm. Ovipositor length: 2.4–2.6 mm. Metacoxa length: 1.3 mm. Metafemur length: 1.2 mm. Metatibia length: 1.3–1.4 mm. T1 length/width at



Figures 1–7. *Sendaphne anitae*, female holotype. **1** Habitus, lateral view **2** Head, frontal view **3** Fore wing **4** Metasoma, dorsal view **5** Head and mesosoma (partially) lateral view **6** Metasoma, ventro-lateral view **7** Mesosoma and metasoma (partially) dorsal view.

posterior margin: 0.6 mm/0.15 mm. T2 length/width at posterior margin: 0.26–0.30 mm/0.3 mm.

Distribution. Only known from the type locality in Ecuador.

Molecular data. No DNA was recovered from the three specimens sampled.

Etymology. Named after Ana María (Anita) Fernández Galliano, daughter of the senior author, for being such a joyful and wonderful person.

***Sendaphne bennetti* Fernández-Triana & Whitfield, sp. n.**

http://zoobank.org/FB93B9D4-E93C-4939-9740-9C6901154A60

Figs 37–44

Holotype. Male, CNC. MEXICO, Durango, 39km W of La Ciudad, 2120m; 2.vii.1964; coll. WRM Mason. DNA Voucher code: CNCHYM 07032.

Diagnosis. This species is morphologically similar to *Sendaphne broadi* from Ecuador, but *S. bennetti* has a wider mediotergite 1 (length 4.0 × its width at posterior margin vs 6.0 × in *broadi*), a less transverse first discal cell in the fore wing (1.3 × as wide as high vs 2.0 ×), and a geographical distribution far apart.

Description. Head color: black. Mesosoma color: black. Tegula color: yellow. Metasoma color (dorsally): mediotergites 1, 2 and anterior half of 3 yellowish-red, rest dark brown to black. Metacoxa color: yellow. Anatomical line tangent to posterior margin of anterior ocellus crossing beneath anterior margin of posterior ocelli. Ocular–ocellar line: 0.21 mm. Interocellar distance: 0.14 mm. Posterior ocellus diameter: 0.09 mm. Body length: 4.1 mm. Fore wing length: 4.0 mm. Metacoxa length: 1.1 mm. Metafemur length: 1.2 mm. Metatibia length: 1.4 mm. T1 length/width at posterior margin: 0.60 mm/0.12 mm. T2 length/width at posterior margin: 0.35 mm/0.35 mm.

Distribution. Only known from the type locality in Mexico.

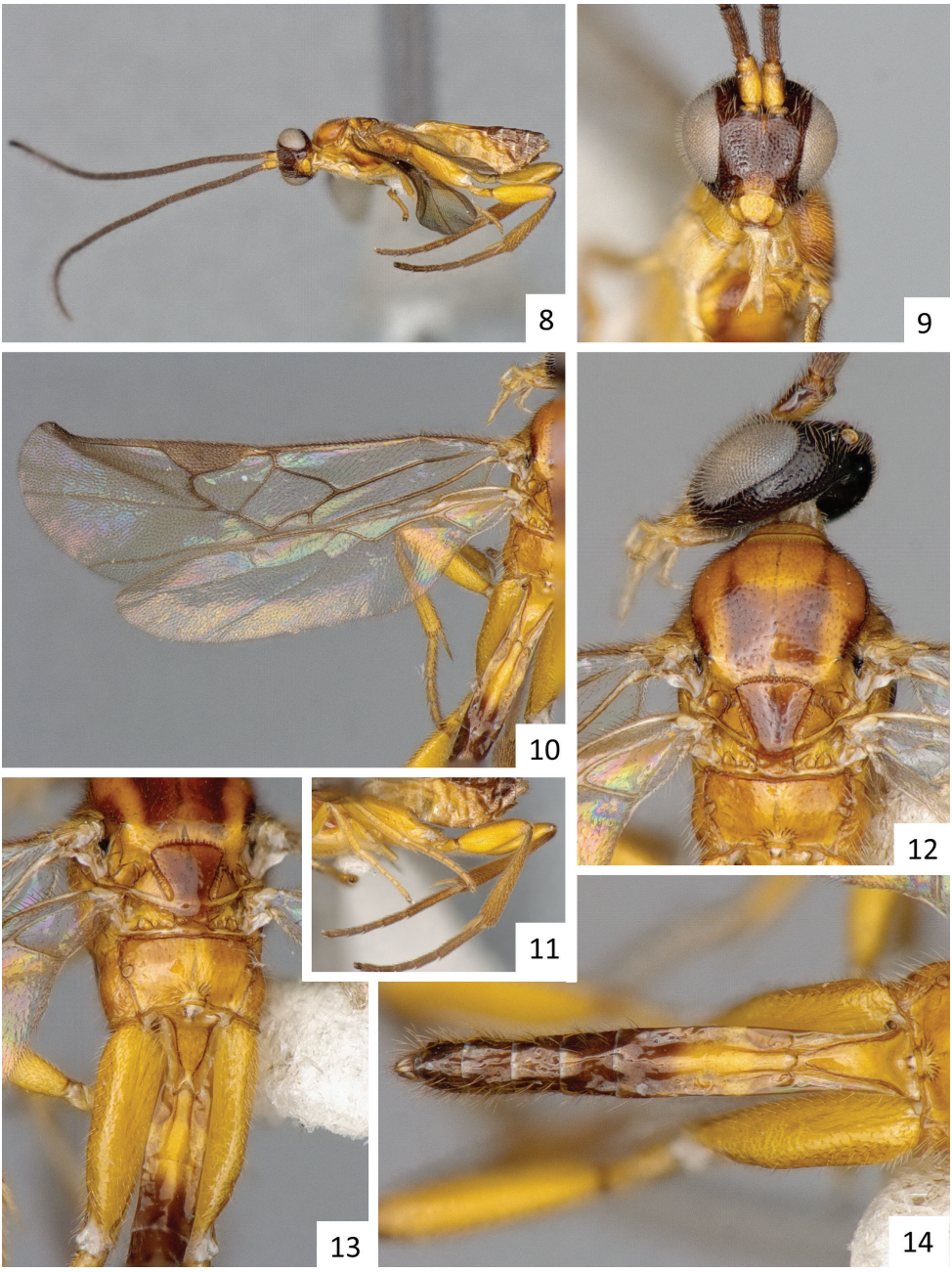
Molecular data. No DNA could be recovered from the specimen sampled.

Comments. Even though only one male specimen is known, it is sufficiently distinctive to warrant description. This species is the northernmost known distribution of the genus *Sendaphne*.

Etymology. Named after Dr. Andrew Bennett of the Canadian National Collection, Ottawa, and Canadian expert on Ichneumonidae, in appreciation for his support and encouragement to study braconid wasps.

***Sendaphne brasilianus* Penteado-Dias, 1995: 251.**

Holotype. Female, DCBU. BRAZIL. Distrito Federal, Brasília, xii-1981, Malaise trap (not examined).



Figures 8–14. *Sendaphne sulmo*, male specimen from Mexico. **8** Habitus, lateral view **9** Head, frontal view **10** Fore wing **11** Meso- and metasoma (partially), lateral view, and hind legs **12** Head and mesosoma, dorsal view **13** Meso- and metasoma (partially), dorsal view **14** Metasoma, dorsal view.

Diagnosis. This species is morphologically similar to *Sendaphne penteadodiasae* but it has a slightly different color pattern, fore wing vein 1Cu-a much shorter than vein 1Cu-b (subequal in *penteadodiasae*), much longer and narrow T1, and slightly longer metacoxa.

Distribution. Only known from the type locality in Brazil.

Molecular data. No specimen is known to have been sampled for DNA.

Comments. We could not study a specimen of this species, but the original description is sufficiently detailed for recognition, including several line drawings of the metasoma, fore wing, tip of antenna and hind leg (Penteado-Dias 1995).

***Sendaphne broadi* Fernández-Triana & Whitfield, sp. n.**
http://zoobank.org/B0C5F4FA-6171-4DFC-93B3-293898C2E94B
Figs 23–28

Holotype. Male, CNC. ECUADOR, Napo, 5km S of Baeza, 1,700 m; 13.ii.1983; coll. Masner & Sharkey. DNA Voucher code: CNCH3323.

Paratypes. 2 ♂ (CNC). One specimen with same locality and collecting date than holotype, the other collected at 1900 m on 9.ii.1983. DNA Voucher codes: CNCH3322 and CNCH3324.

Diagnosis. This species is morphologically similar to *Sendaphne bennetti* from Mexico, but *S. broadi* has a narrower mediotergite 1 (length 6.0 × its width at posterior margin vs 4.0 × in *bennetti*), a more transverse first discal cell in the fore wing (2.0 × as wide as high vs 1.3 ×), and a geographical distribution far apart.

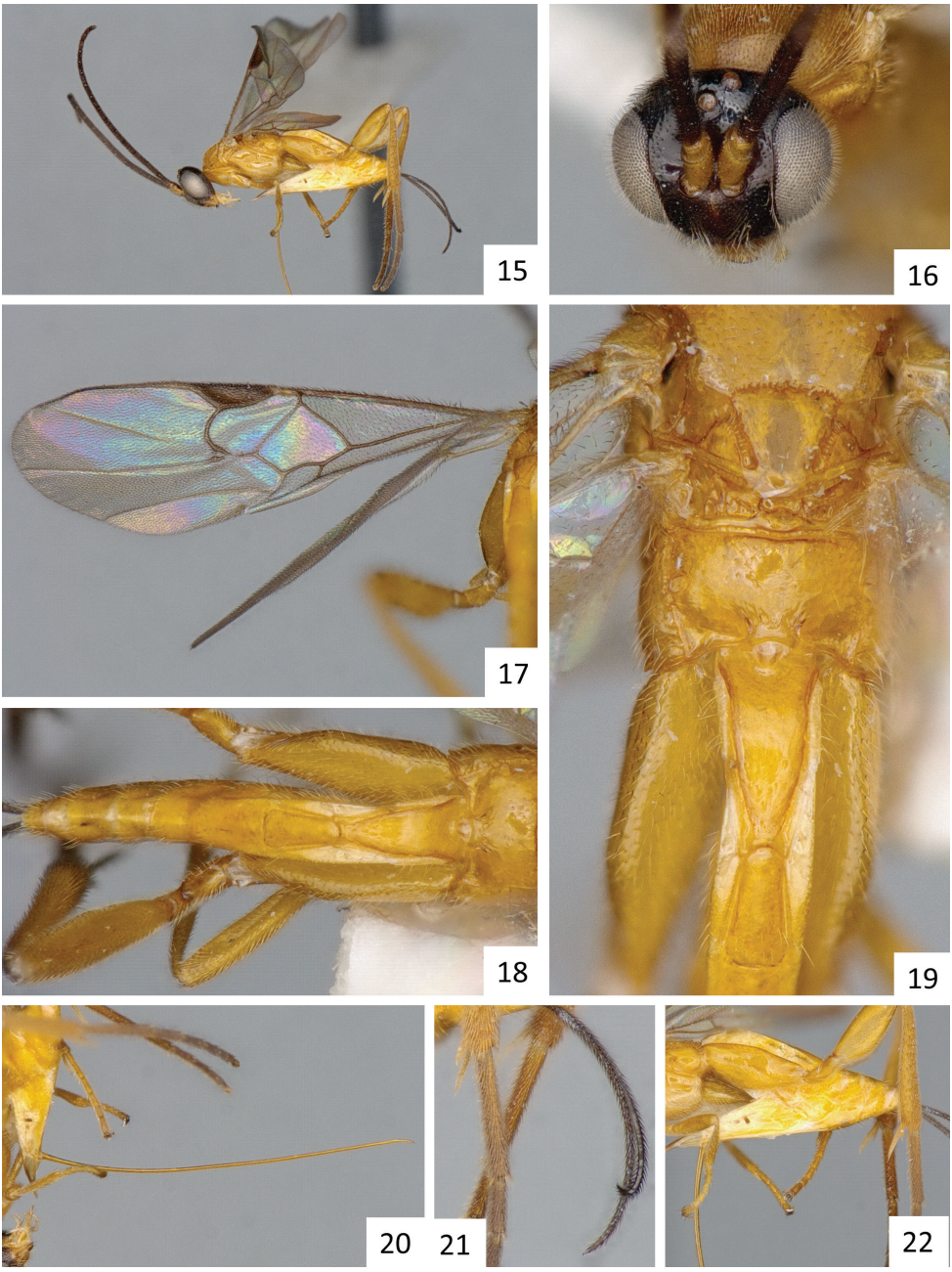
Description. Head color: dark brown. Mesosoma color: dark brown. Tegula color: yellow. Metasoma color (dorsally): mediotergites 1–2 yellowish-brown, rest brown to dark brown. Metacoxa color: yellow. Anatomical line tangent to posterior margin of anterior ocellus crossing very slightly (less than 0.01 mm) above anterior margin of posterior ocelli. Ocular–ocellar line: 0.17 mm. Interocellar distance: 0.09 mm. Posterior ocellus diameter: 0.07 mm. Body length: 3.5–3.7 mm. Fore wing length: 3.3–3.5 mm. Metacoxa length: 1.1 mm. Metafemur length: 1.2 mm. Metatibia length: 1.4 mm. T1 length/width at posterior margin: 0.45 mm/0.06–0.07 mm. T2 length/width at posterior margin: 0.25 mm/0.20 mm.

Distribution. Ecuador.

Molecular data. No DNA could be recovered from the three specimens sampled.

Comments. Even though only male specimens of this species are known, they are sufficiently distinctive to warrant description.

Etymology. Named after Dr. Gavin Broad, of the Natural History Museum, London, England, and British expert on Ichneumonidae, in appreciation for his support over the years, including sharing pictures of and facilitating access to type material deposited in London.



Figures 15–22. *Sendaphne dianariaspennae*, female holotype. **15** Habitus, lateral view **16** Head, frontal view (some inclination downwards) **17** Fore wing **18** Propodeum and metasoma, dorsal view **19** Meso- and metasoma (partially), dorsal view. 20–22: Details of the ovipositor, ovipositor sheaths, and hypopygium.

***Sendaphne dianariaspennae* Fernández-Triana & Whitfield, sp. n.**
http://zoobank.org/423FC0AA-4087-414C-B242-856191CFF5AD
Figs 15–22

Holotype. Female, CNC. BRAZIL, Rio de Janeiro, Mangaratiba; i.1976; coll. M. Alvarenga. DNA Voucher code: CNCHYM 07025.

Paratypes. 3 ♀, 1 ♂ (CNC), Brazil, same locality and date than holotype. 1 ♀ (CNC), Brazil, Nova Teutonia, 27°11'S, 52°23'W, 300–500m, 21.iii.1961, coll. F. Plaumann. 2 ♂ (CNC), Brazil, Guanabara and Represa Rio Grande, i.1969 and i.1972. 1 ♂ (CNC), Brazil, Caruaru, Pernambuco, v.1972. 8 ♀, 10 ♂ (Humboldt Institute), Colombia, Magdalena, PNN Tayrona Zaino, 50m, 11°20'N, 74°2'W, specimens collected between 13.v.2000 and 30.viii.2000, coll. R. Henriquez.

Diagnosis. This species is morphological similar to *Sendaphne sulmo* but differs in the smaller, less separated ocelli (which form a lower triangle, compared to higher triangle in *sulmo*), T1 relatively wider medially, and T2 relatively slender than *sulmo*. The geographical distribution of the two species is more than 2,000 km apart.

Description. Head color: dark brown to black. Mesosoma color: orange-yellow. Tegula color: orange-yellow. Metasoma color (dorsally): yellow. Metacoxa color: yellow. Anatomical line tangent to posterior margin of anterior ocellus crossing slightly (0.01–0.02 mm) above anterior margin of posterior ocelli. Ocular–ocellar line: 0.17 mm. Interocellar distance: 0.10 mm. Posterior ocellus diameter: 0.07–0.08 mm. Body length: 3.2–3.5 mm. Fore wing length: 3.0–3.2 mm. Ovipositor length: 2.3–3.0 mm. Metacoxa length: 0.90–0.95 mm. Metafemur length: 0.90 mm. Metatibia length: 1.12–1.35 mm. T1 length/width at posterior margin: 0.45–0.50 mm/0.05–0.06 mm. T2 length/width at posterior margin: 0.35 mm/0.18–0.20 mm.

Distribution. Brazil and Colombia.

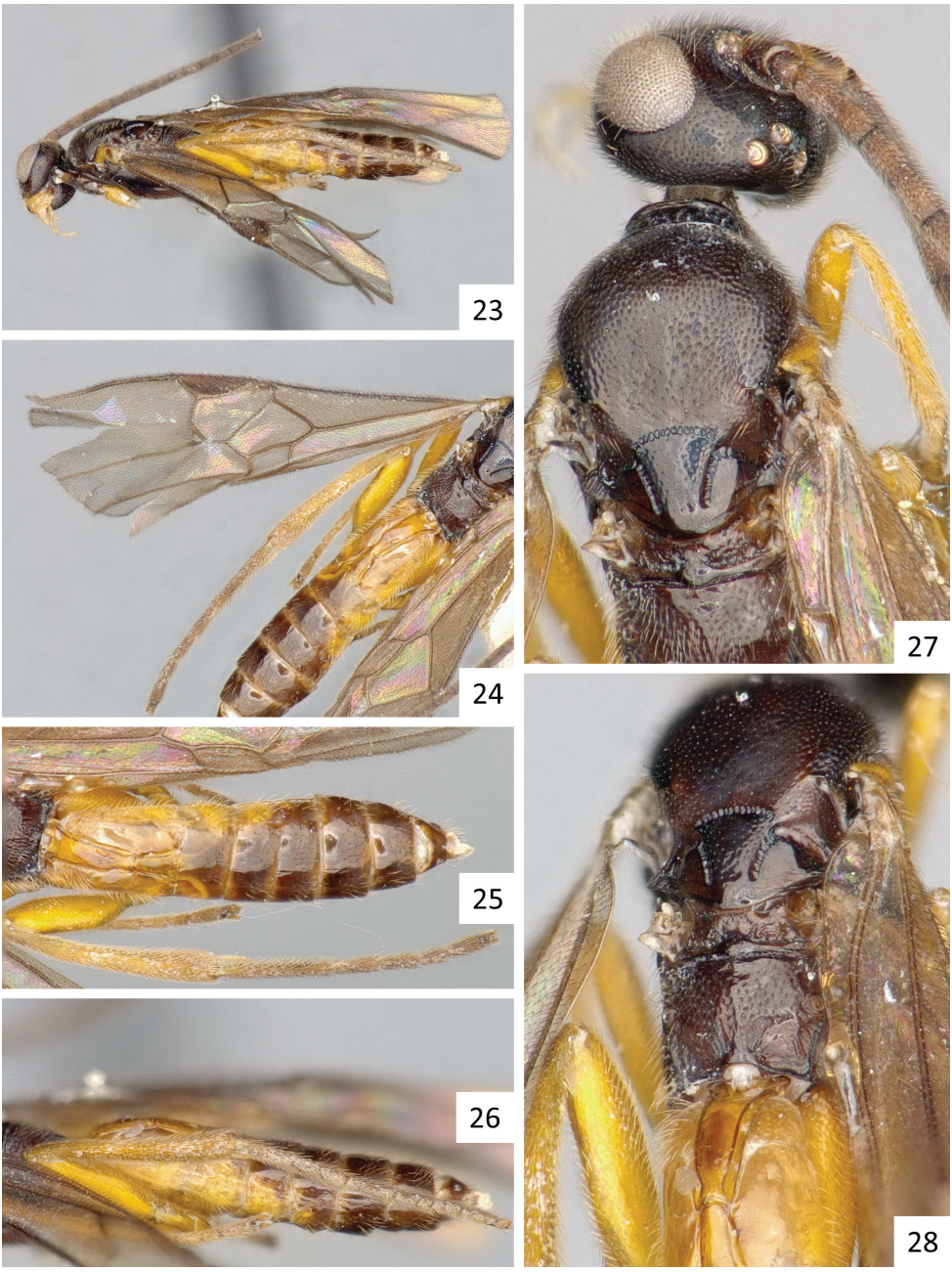
Molecular data. Four of the paratypes from Brazil (DNA Voucher codes: CNCHYM 07023, CNCHYM 07024 and CNCHYM 07040) as well as the holotype were sampled for DNA. Only one of the paratypes (CNCHYM 07040) rendered a minibarcode of 103 base pairs.

Comments. The Brazilian specimens of *S. dianariaspennae* were collected between January and March, while the Colombian specimens were collected between May and August.

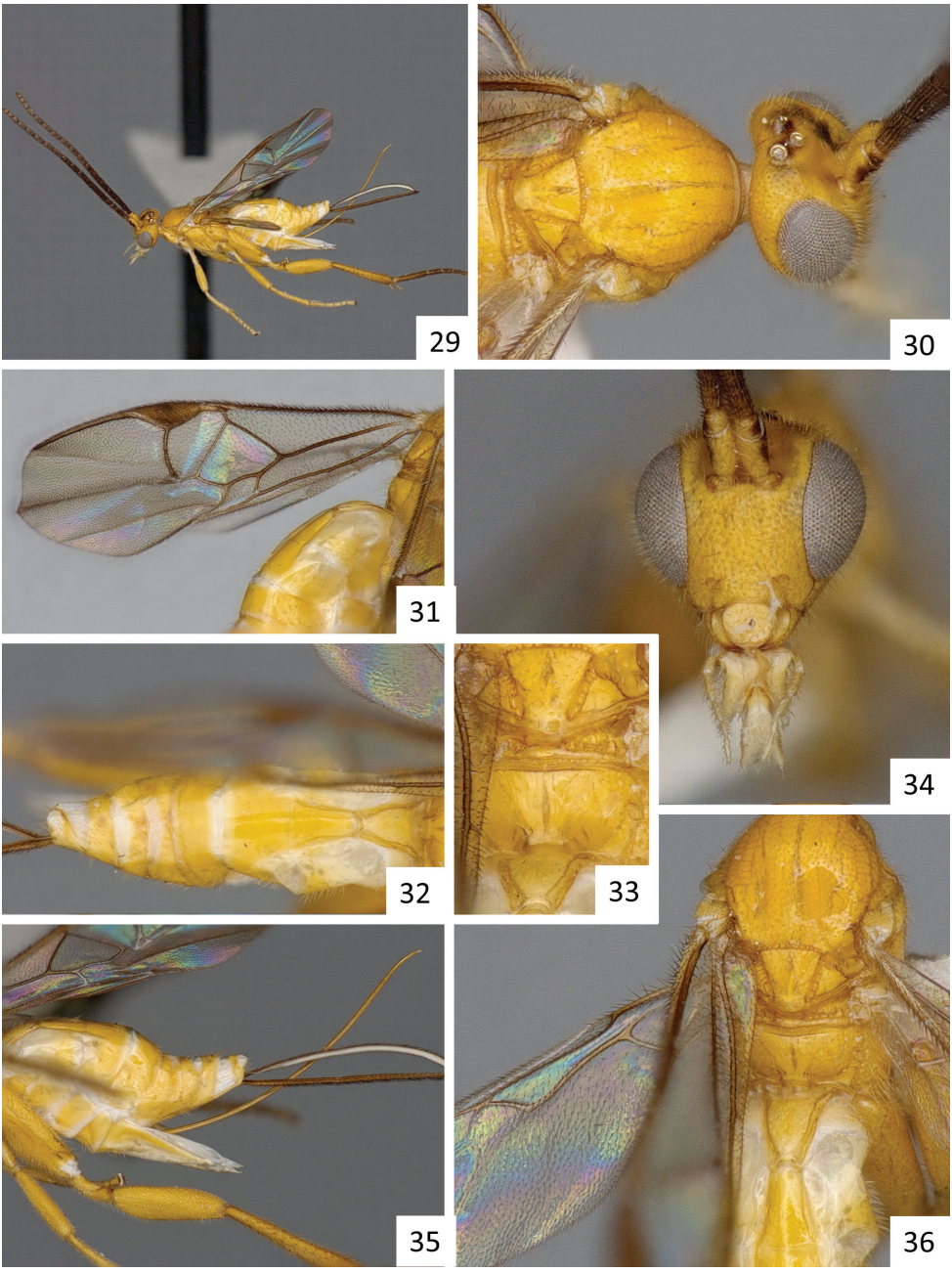
Etymology. Named after Diana Carolina Arias-Penna (Colombia), in recognition of her blossoming career studying neotropical Braconidae, especially species from South America.

***Sendaphne jatai* Penteado-Dias, 1995: 252.**
Figs 29–36

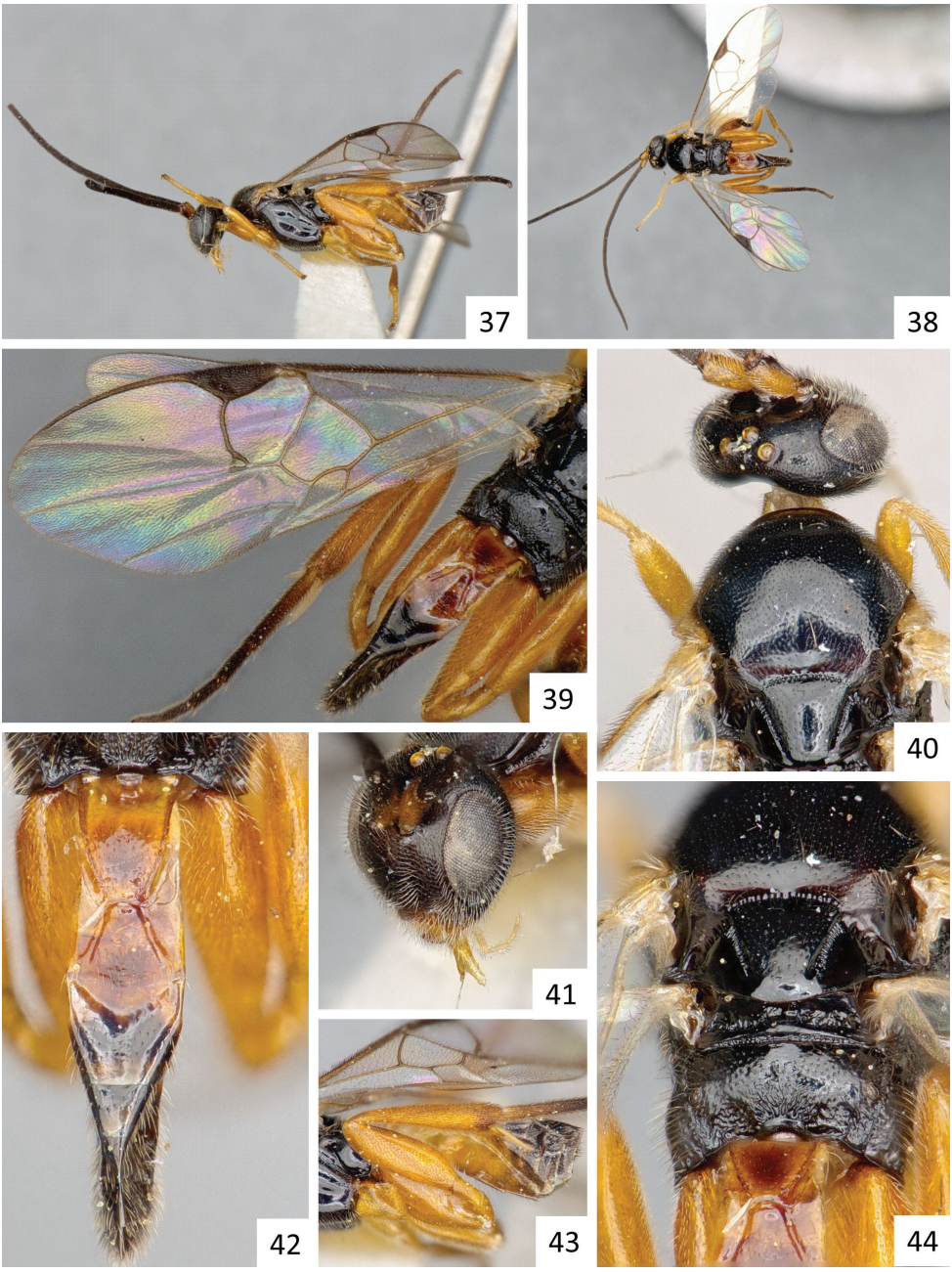
Holotype. Female, DCBU. BRAZIL. Sao Paulo, Reserva Ecológica do Jataí, gallery forest of Mogi River; 24.v.1991; sweeping (not examined).



Figures 23–28. *Sendaphne broadi*, male holotype. **23** Habitus, lateral view **24** Fore wing **25** Metasoma, dorsal view **26** Metasoma, lateral view **27** Head and mesosoma (partially), dorsal view **28** Mesosoma and T1-T2, dorsal view.



Figures 29–36. *Sendaphne jatai*, female specimen from Brazil. **29** Habitus, lateral view **30** Head and mesosoma (partially), dorso-lateral view **31** Fore wing **32** Metasoma, dorsal view **33** Scutellar disc, propodeum and T1 (partially), dorsal view **34** Head, frontal view **35** Metasoma, lateral view **36** Mesosoma and metasoma (partially), dorsal view.



Figures 37–44. *Sendaphne bennetti*, male holotype. **37** Habitus, lateral view **38** Habitus, dorsal view **39** Fore wing **40** Head and mesosoma (partially), dorsal view **41** Head, fronto-lateral view **42** Metasoma, dorsal view **43** Metasoma, lateral view **44** Scutellar disc, propodeum and T1-T2 (partially), dorsal view.

Specimens examined. 1 ♀ (CNC), Brazil, Mato Grosso, Sinop, x.1974, coll. M. Alvarenga, DNA Voucher code: CNCH3320. 1 ♀, 1 ♂ (CNC), Ecuador, Pichincha, 47 km S of Santo Domingo, Rio Palenque, 200m, 18–30.v.1975 and 22-31.vii.1976, coll. S. & J. Peck, DNA Voucher codes: CNCHYM 07034 and CNCHYM 07035. 1 ♀ (FUSAGx), French Guiana, Saul, Crique popote, Mont Belvédere, 3°36'N, 53°10'W, ii.2001, coll. J. Tarin.

Diagnosis. This is the only species where female specimens have the body entirely yellow.

Distribution. Brazil, Ecuador, French Guiana.

Molecular data. The two specimens from Ecuador rendered minibarcodes of 102 base pairs (CNCHYM 07034) and 164 base pairs (CNCHYM 07035).

Comments. The male specimen from Ecuador has some dark bands on mediotergites 5–7, but otherwise is similar to the original description.

***Sendaphne olearus* Nixon, 1965: 204.**
Figs 45–53, 79–80

Holotype. Female, BMNH. BRAZIL, Nova Teutonia, 2.iii.1937 (not examined).

Specimens examined. 1 ♀ (CNC), Brazil, Nova Teutonia, 27°11'S, 52°23'W, 300-500m, 2.iv.1966, DNA Voucher code: CNCHYM 07019. 3 ♂ (FUSAGx), French Guiana, Kaw Mountain, Patawa, 4°32'42.20"N, 52°09'09.19"W, v.1999 and viii.1999, Malaise trap. 1 ♀ (BMNH) Peru, Loreto, Estacion Jenaro Herrera, 4°53'55.0"S, 73°39'00.4"W, 121m, 13–23.i.2011, D. Karlsson & N. Dale-Skey, BMNH(E) 2011-72, BMNH(E)#1249978.

Diagnosis. This species is similar to *Sendaphne jatai*, both having the lightest (i.e., more yellowish) coloration among all known species within the genus. *S. olearus* has brown bands on posterior margin of mediotergites 4–6, and its body coloration is generally more yellow-reddish (*jatai* body color is entirely yellow).

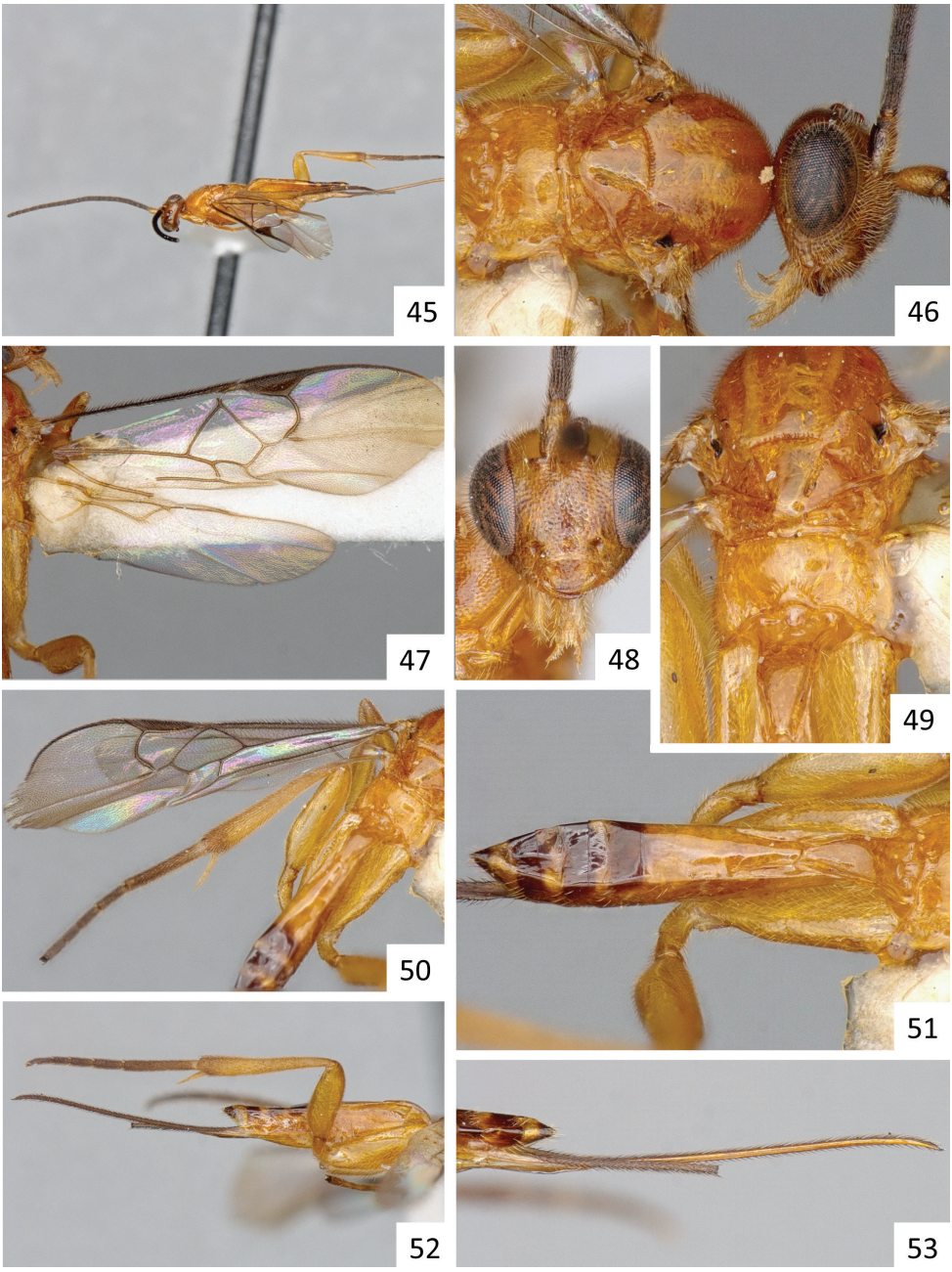
Distribution. Brazil, French Guiana, Peru.

Molecular data. The CNC specimen from the type locality in Brazil (CNCHYM 07019) rendered a minibarcode of 164 base pairs.

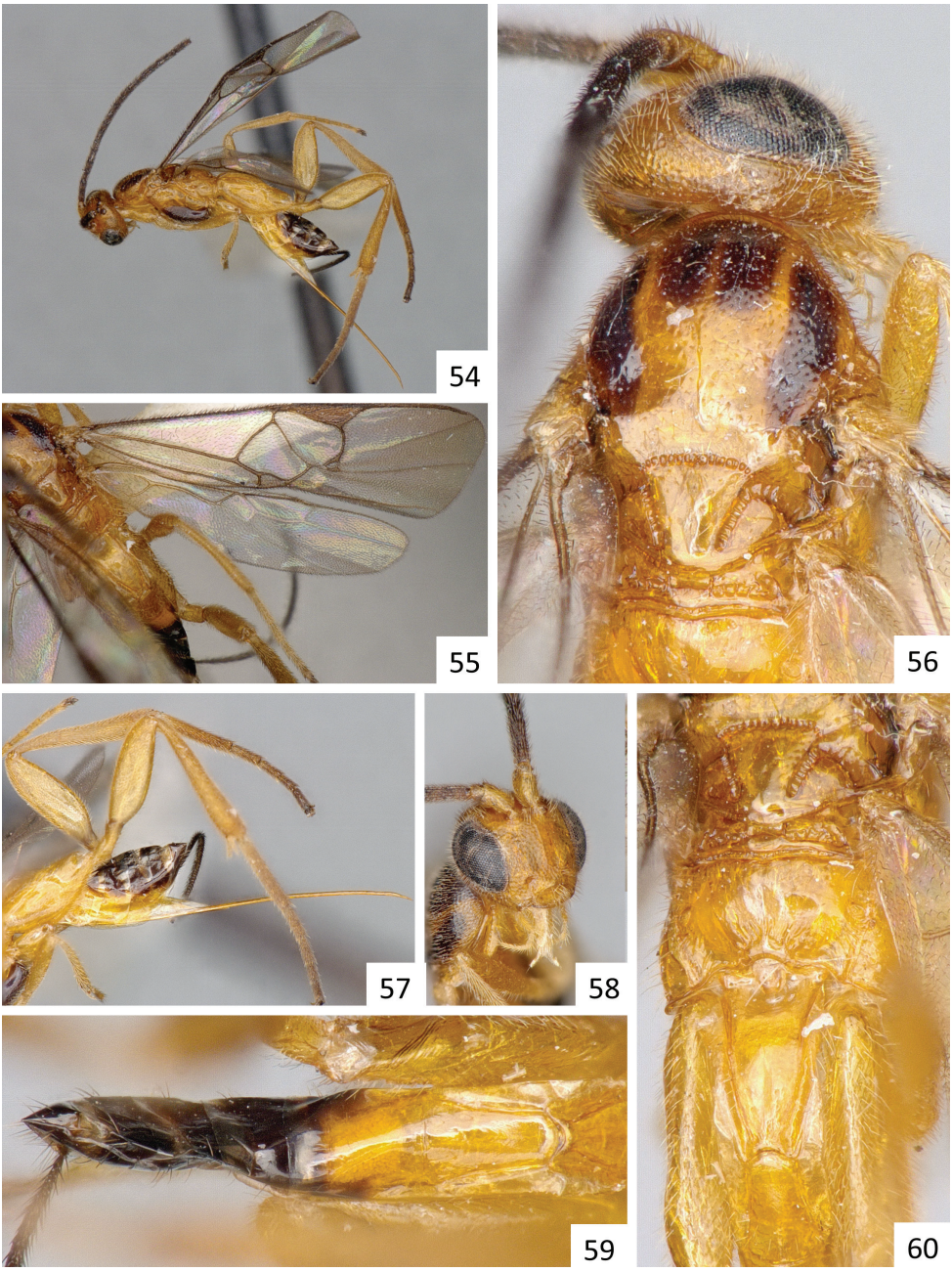
Comments. We could not see the holotype of this species, but the original description is adequately detailed for discrimination. We examined one female from the type locality (collected 30 years after the holotype female), the only known male specimens (from French Guiana), which were discussed in Braet (2006), and the first known specimen from Peru.

***Sendaphne paranaensis* Scatolini & Penteado-Dias, 1999: 53.**
Figs 54–60

Holotype. Female, UFPR. BRAZIL, Reserva Biologica Samuel Klabin, Malaise trap (not examined).



Figures 45–53. *Sendaphne olearus*, female specimen from Brazil. **45** Habitus, lateral view **46** Head and mesosoma, lateral view **47** Fore wing **48** Head, frontal view **49** Mesosoma and T1, dorsal view **50** Mesosoma and metasoma (partially), dorsal view **51** Metasoma, dorsal view **52** Metasoma, lateral view **53** Details of the ovipositor and ovipositor sheaths.



Figures 54–60. *Sendaphne paranaensis*, female specimen from Brazil. **54** Habitus, lateral view **55** Fore wing **56** Head and mesosoma (partially), dorsal view **57** Metasoma and legs, lateral view **58** Head, frontal view **59** Metasoma (partially), dorsal view **60** Scutellar disc, propodeum and T1-T2 (partially), dorsal view.

Specimens examined. 1 ♀ (CNC), Brazil, Rio de Janeiro, Guanabara, Silva Jardim, iii.1974, DNA Voucher code: CNCHYM 07022. 1 ♂ (CNC), Brazil, Espirito Santo, Castello, xi.1976, DNA Voucher code: CNCHYM 07039. 2 ♂ (CNC), Paraguay, Pirapo, 1–3.i.1972, DNA Voucher codes: CNCHYM 07037, and CNCHYM 07038.

Other material mentioned in the original description. Almost 70 specimens (females and males) from Brazil, Paraná, Telêmaco Borba, collecting dates between viii.1986 and iii.1987.

Diagnosis. *S. paranaensis* is one of only three *Sendaphne* species with head entirely yellow (or yellow-orange). It differs from the other two species (*S. jatai* and *S. olearus*) in having dark brown areas on the anteromesoscutum and mesopleuron, and mediotergites 4+ entirely black.

Distribution. Brazil, Paraguay.

Molecular data. Of the four specimens in the CNC sampled for DNA, only one (CNCHYM 07037) rendered a minibarcode of 164 base pairs.

Comments. The male specimens from Paraguay are much darker in coloration, but similar variation is mentioned in the original description for the male paratypes from Brazil (Scatolini and Penteado-Dias 1999: 53).

***Sendaphne penteadodiasae* Fernández-Triana & Whitfield, sp. n.**
http://zoobank.org/7FDD9815-D39B-49EE-9645-749C02277F24
Figs 61–67

Holotype. Female, CNC. BRAZIL, Campina Grande, near Curitiba, 15.ii.1966, coll. H. & M. Townes. DNA Voucher code: CNCHYM 07020.

Paratype. 1 ♂, same locality as holotype, 10.ii.1966. DNA Voucher code: CNCHYM 07021.

Diagnosis. This species is morphologically similar to *Sendaphne brasilianus* but it has a slightly different color pattern, fore wing vein 1Cu-a subequal to vein 1Cu-b (much shorter in *brasilianus*), shorter and wider T1, and slightly shorter metacoxa.

Description. Head color: dark brown to black. Mesosoma color: orange-yellow. Tegula color: orange-yellow. Metasoma color (dorsally): mostly orange-yellow, with some narrow brown bands on posterior margin of mediotergites 5–7. Metacoxa color: yellow. Anatomical line tangent to posterior margin of anterior ocellus crossing very slightly (less than 0.01 mm) above anterior margin of posterior ocelli. Ocular–ocellar line: 0.12 mm. Interocellar distance: 0.10 mm. Posterior ocellus diameter: 0.08 mm. Body length: 4.2 mm. Fore wing length: 3.9 mm. Ovipositor length: 2.5 mm. Metacoxa length: 1.20 mm. Metafemur length: 1.25 mm. Metatibia length: 1.11 mm. T1 length/width at posterior margin: 0.35 mm/0.06 mm. T2 length/width at posterior margin: 0.25 mm/0.12 mm.

Distribution. Only known from the type locality in Brazil.

Molecular data. No DNA could be recovered from the two specimens sampled.

Comments. The specimens of this species (housed in the CNC) were previously identified by W.R.M. Mason as "*Sendaphne sulmo*". However, the morphological differences from the original description of *Sendaphne sulmo* (see key and diagnosis above), and the disparate geographical distribution allows us to consider the Brazilian and Mexican specimens as separate species.

Etymology. Named after Dr. Angélica Maria Penteado Martins-Dias (Brazil), in recognition of her career studying Braconidae, and also for her work describing most of the previously known species of *Sendaphne*.

***Sendaphne rogerblancoi* Fernández-Triana & Whitfield, sp. n.**
http://zoobank.org/09ABE6F1-4EFC-43B5-BE10-5631E671B76E
Figs 68–75

Holotype. Female, CNC. COSTA RICA, Guanacaste, Area de Conservación Guanacaste, Sector Cacao, Sendero Cima, 1,460 m, 10.93328, -85.45729; 18.xii.2008; coll. D.H. Janzen & W. Hallwachs. DNA Voucher code: DHJPAR0031465.

Paratypes. 4 ♀, 5♀ (BMNH, CNC, INBio, NMNH), same locality as holotype. Collecting dates are mostly on December 2008, with one record each in November 2008, March 2009, and April 2009. All specimens are from one Malaise trap at the very peak of Volcán Cacao.

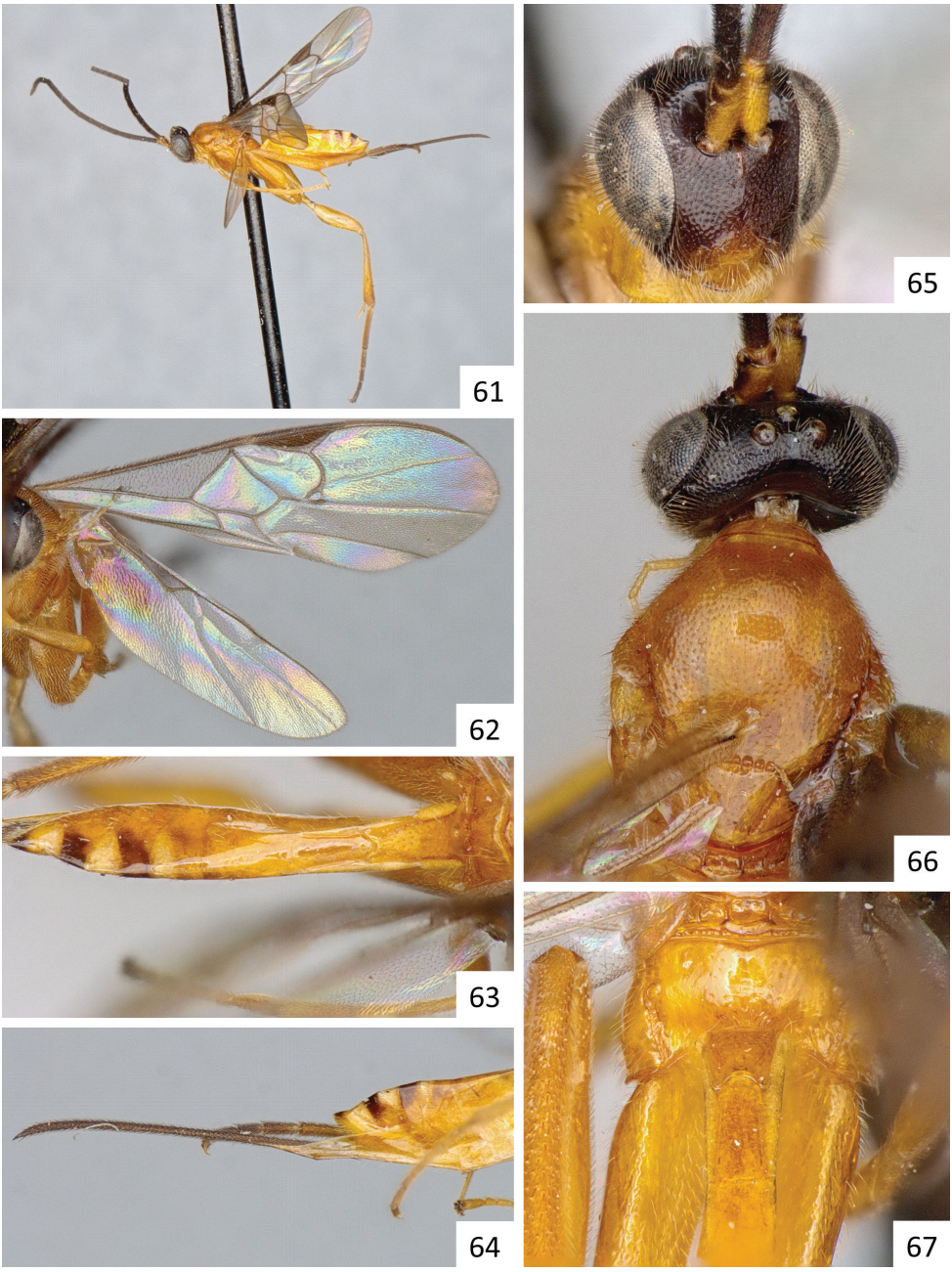
Diagnosis. This is the most distinctive species of *Sendaphne* based on coloration (head, mesosoma, metasoma, and metacoxa black), shape of first discal cell, and narrow mediotergite 1.

Description. Head color: black. Mesosoma color: black. Tegula color: dark brown. Metasoma color (dorsally): black. Metacoxa color: dark brown to black. Anatomical line tangent to posterior margin of anterior ocellus crossing very slightly (less than 0.01 mm) above anterior margin of posterior ocelli. Ocular–ocellar line: 0.17–0.18 mm. Interocellar distance: 0.09 mm. Posterior ocellus diameter: 0.08 mm. Body length: 4.3–4.6 mm. Fore wing length: 4.2–4.3 mm. Ovipositor length: 3.8–4.2 mm. Metacoxa length: 1.10–1.11 mm. Metafemur length: 0.85–0.90 mm. Metatibia length: 1.5–1.6 mm. T1 length/width at posterior margin: 0.80–0.85 mm/0.15–0.16 mm. T2 length/width at posterior margin: 0.45 mm/0.25 mm.

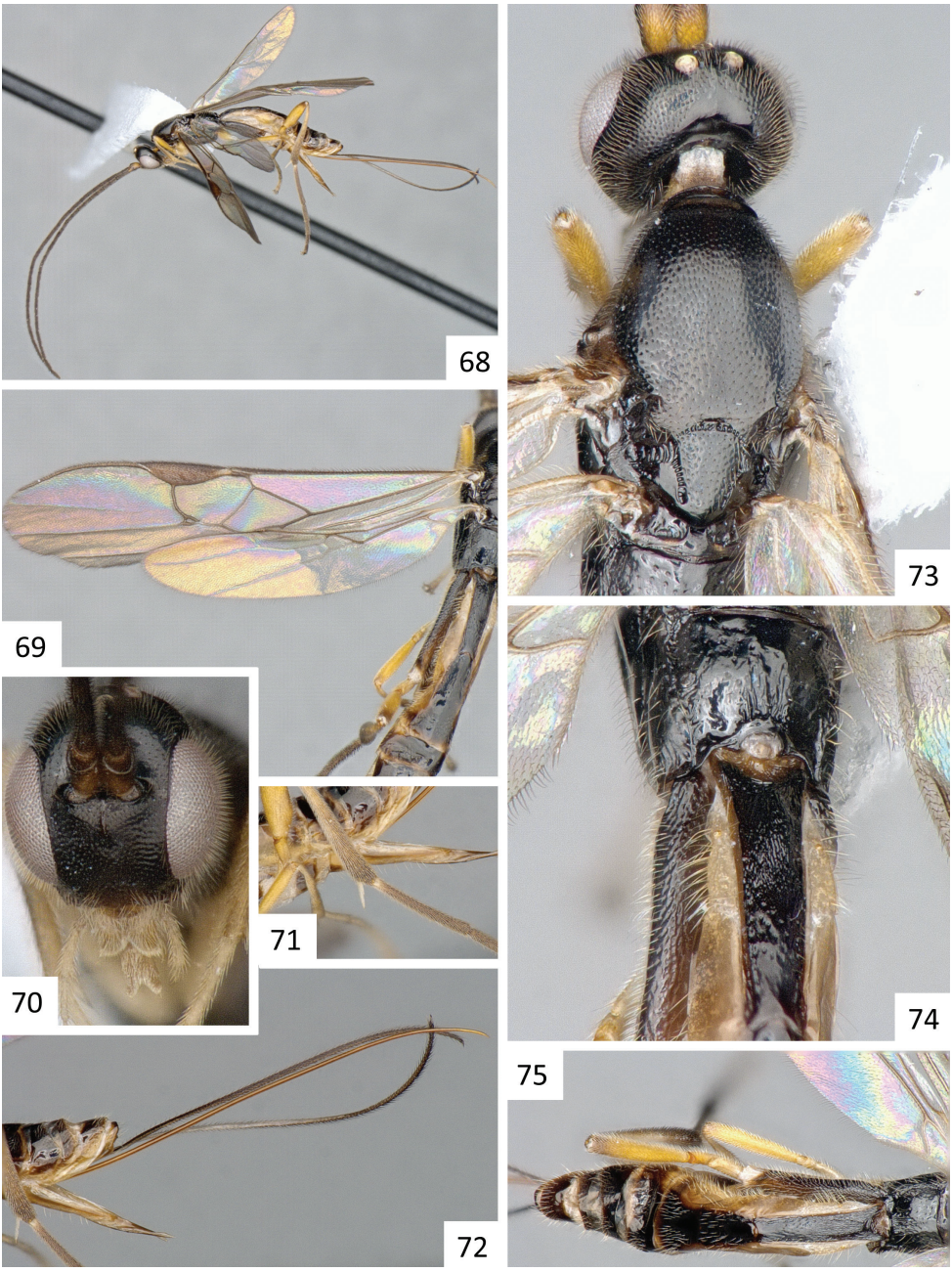
Distribution. Only the summit cloud forest at 1,450 m on Volcán Cacao, northwestern Costa Rica.

Molecular data. In BOLD there are data for 16 specimens of this species (the holotype, the paratypes and other specimens that we could not examine) which rendered partial barcodes, most of them from 260 to 390 base pairs. Only the holotype (DNA Voucher code: DHJPAR0031465) had a longer barcode (633 base pairs).

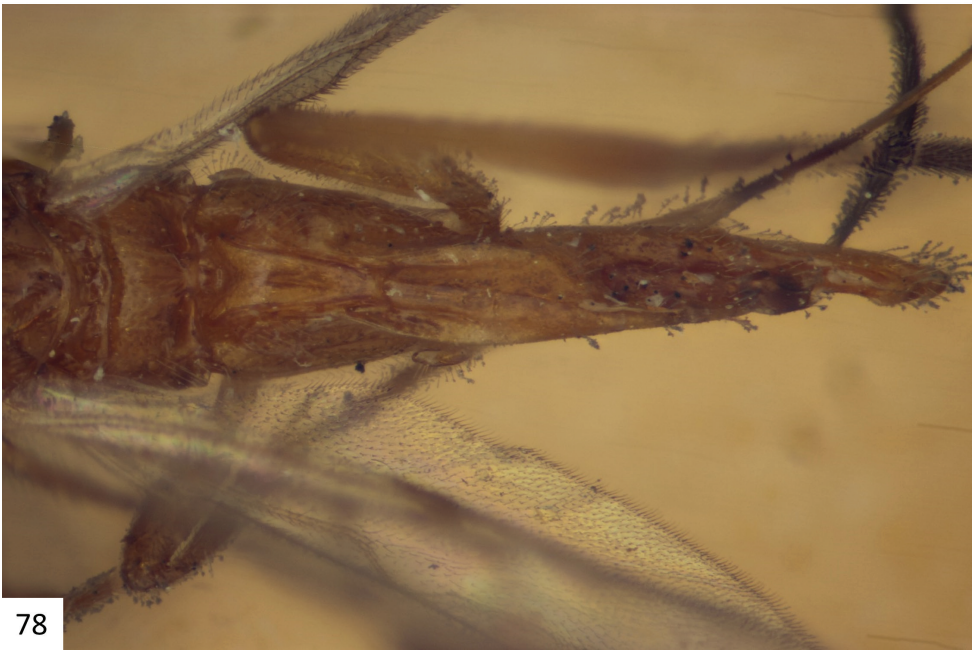
Etymology. This unique species, the only *Sendaphne* known from Costa Rica so far, is named after Sr. Roger Blanco Segura, of Area de Conservación Guanacaste (ACG), northwestern Costa Rica, in recognition of his 3+ decades of intense care and management of ACG in an enormous variety of circumstances and for a very large array of purposes.



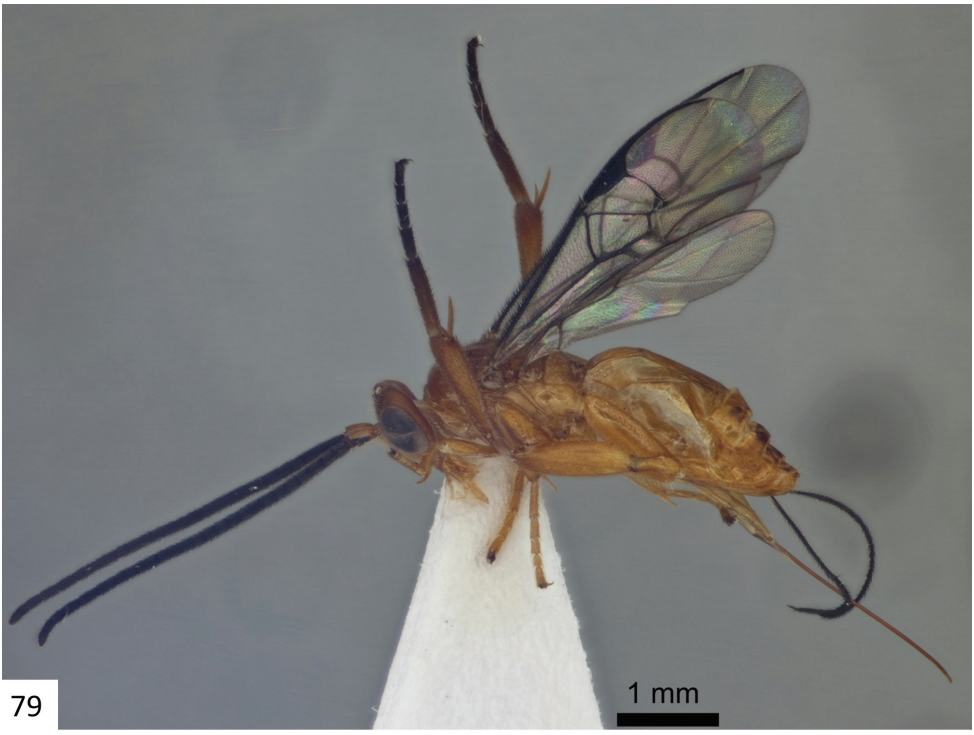
Figures 61–67. *Sendaphne penteadodiasae*, female holotype. **61** Habitus, lateral view **62** Fore wing **63** Metasoma, dorsal view **64** Ovipositor and hypopygium, lateral view **65** Head, frontal view **66** Head and mesosoma (partially), dorsal view **67** Propodeum, T1-T2 (partially), dorsal view.

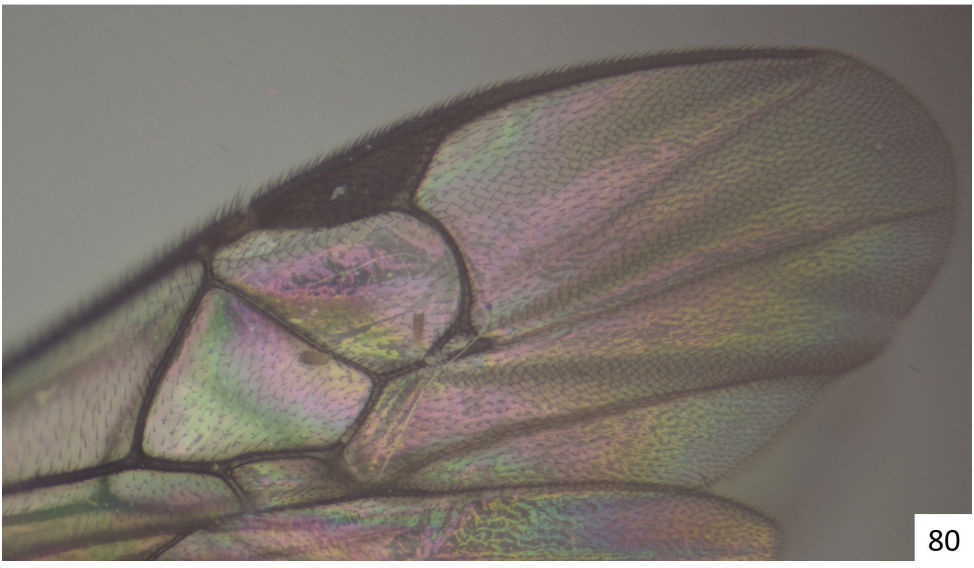


Figures 68–75. *Sendaphne rogerblancoi*, female holotype. **68** Habitus, lateral view **69** Fore wing **70** Head, frontal view **71** Hypopygium and hind legs (partially), lateral view **72** Ovipositor, ovipositor sheaths, and hypopygium, lateral view **73** Head and mesosoma (partially), lateral view **74** Propodeum and T1, dorsal view **75** Propodeum and mesosoma, dorsal view.



Figures 76–78. *Sendaphne sulmo*, female holotype. **76** Habitus, lateral view **77** Head and mesosoma (partially), dorsal view **78** Propodeum and metasoma, dorsal view.





Figures 79–80. *Sendaphne olearus*, female specimen from Peru. **79** Habitus, lateral view **80** Detail of the fore wing.

***Sendaphne sulmo* Nixon, 1965: 204.**
Figs 8–14, 76–78

Holotype. Female, BMNH. MEXICO, Tabasco, Teapa (not examined).

Specimens examined. 3 ♂ (CNC). Mexico, Chiapas, 16°58'N, 91°47'W, 560 m; (collecting dates: 6.ix.1978, 28.x.1978, 8–11.xi.1978); coll. J. Rawlins. DNA Voucher codes: CNCHYM 07030 CNCHYM 07031 and CNCHYM 07033.

Diagnosis. This is the only known species of *Sendaphne* with a higher ocellar triangle (i.e., anatomical line tangent to posterior margin of anterior ocellus crosses far above anterior margin of posterior ocelli). The distance between anatomical line tangent to posterior margin of anterior ocellus and anterior margin of posterior ocelli is 0.5 × the diameter of anterior ocelli (Fig. 77), while for all other known species of *Sendaphne* it is usually 0.1–0.3 ×.

Distribution. Only known from lowlands (100–560 m) of Mexico.

Comments. We could only study some photos of the holotype (Figs 76–78) and the original description which included a line drawing of the metasoma (Nixon 1965: 207, Figure 255). The drawing shows a T1 slightly narrower medially than the photos of the actual holotype reveal, but the rest of the original description is in agreement with the photos we examined. The males mentioned above are, however, different from the female holotype in having a darker anteromesoscutum, scutellar disc, and metasoma (Figs 8–14), and also the ocellar triangle is not as elevated. Lacking more specimens to examine (especially females), we have refrained from considering those male specimens as a different species because the two localities are not too far apart and males are known to be darker in other species of *Sendaphne*. If more material becomes available for study in the future, the status of those specimens may be clarified.

Molecular data. The three male specimens sampled for DNA rendered minibarcodes of 103 base pairs each.

Acknowledgements

We emphatically and gratefully acknowledge the support of the ACG parataxonomist team in finding and rearing these caterpillars, their parasitoids and their hyperparasitoids, and Area de Conservacion Guanacaste (ACG) for preserving the forests in which they live, and the Guanacaste Dry Forest Conservation Fund, the Wege Foundation, the International Conservation Fund of Canada, the JRS Biodiversity Foundation, Jessie Hill, Steve Stroud, Permian Global, and the University of Pennsylvania for funding portions of the research. Several Colombian specimens were collected during a project supported by NSF grant DEB 0205982 awarded to M. J. Sharkey and B. V Brown. This study was also supported by NSF DEB 0515699 to DHJ and by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant to MAS. Laboratory analyses of these sequences were funded by the Government of Canada through Genome Canada and the Ontario Genomics Institute (2008-0GI-

ICI-03), and by BOLD/iBOL of the Biodiversity Institute of Ontario and University of Guelph. JFT thanks Yves Braet (Belgium) for making specimens from French Guiana available for this study and Gavin Broad (BMNH) for sending pictures of the specimens housed in London. The suggestions from two anonymous reviewers and the editor considerably improved the final version of this paper.

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Supplementary material 1

Details of *Sendaphne* specimens with sequences in BOLD.

Authors: Jose L. Fernandez-Triana, James B. Whitfield, M. Alex Smith, Winnie Hallwachs, Daniel H. Janzen

Data type: species data

Explanation note: The Excel file contains details on locality, sampling date, collectors, museum codes, sequence length, and other data related to *Sendaphne* specimens.

Supplementary material 2

Data for phylogenetic analysis.

Authors: Jose L. Fernandez-Triana, James B. Whitfield, M. Alex Smith, Winnie Hallwachs, Daniel H. Janzen

Data type: sequence data

Explanation note: Neighbor-Joining tree based on Kimura 2-parameter distances of all described species of Sendaphne with DNA barcodes available. Sequence data from the Barcode of Life Data Systems (http://www.boldsystems.org/). For every sequence the species name, specimen code, length of sequence (in base pairs), and country/province or country/state is shown.

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doi: 10.3897/JHR.41.8099
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RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
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A new, endemic genus of Anomaloninae (Hymenoptera, Ichneumonidae) from St Helena

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Abstract

A new genus, *Helenanomalon* **gen. n.**, of the ichneumonid subfamily Anomaloninae is described for two newly described species, *Helenanomalon ashmolei* **sp. n.** and *H. bonapartei* **sp. n.** *Helenanomalon* species are endemic to the remote South Atlantic island of St Helena, where they are the only known anomalonines. Unusually amongst anomalonines, there seems to be pronounced sexual dimorphism. The possible affinities of *Helenanomalon* are discussed.

Keywords

Taxonomy, new genus, new species, south Atlantic, UK overseas territory

Introduction

The remote, southern Atlantic island of St Helena (a UK overseas territory) has, unsurprisingly, a very small fauna of endemic Hymenoptera. Being 1,960 km from the nearest landmass (Africa), only a few Ichneumonoidea have colonised the island under their own steam, although a variety of tramp species have been inadvertently introduced. The invertebrate fauna of two key areas of St Helena was recently catalogued by Ashmole and Ashmole (2004) and Mendel et al. (2008), with the latter report based on extensive material collected in the Central Peaks area of the island in 2005–2006. Amongst the five species of Ichneumonidae collected in that survey were two male

specimens of the subfamily Anomaloninae that could not be identified to genus. There are also two published mentions of an undescribed anomalonine genus from St Helena. A short note by Ian Gauld, in his revision of the genera of Anomaloninae (Gauld, 1976), on an undescribed genus from St Helena, led to the rediscovery of a further male specimen in the Natural History Museum (hereafter BMNH) collection. Anomaloninae specimens collected in the Eastern Arid Area of the island by the Belgian expeditions of 1965–1967 are mentioned in a report on the ichneumonoid fauna of St Helena by Decelle (1976) as having been identified as a new genus by Henry Townes. The two females are deposited in Musée de l'Afrique Centrale (Tervuren) (hereafter RMCA) and were labelled as a 'new genus of Gravenhorstiini' by Henry Townes in 1972. These five specimens seem to be the only specimens of Anomaloninae to have been collected on St Helena.

Assigning the St Helena anomalonines to a genus, or defining a new genus to accommodate them, has proved problematic. Gauld's (1976) analysis of anomalonine relationships informed his redefinitions of the genera but was based on phenetic analysis of morphological characters. I had hoped to reanalyse Gauld's data using cladistic methods, and to include the St Helena specimens, as a test of their generic status. However, Gauld did not include a character matrix in his paper, stating that the data had been deposited in the BMNH library. Unfortunately, there is no trace of any such documents in either the library or the archives of BMNH. Given the information provided by Gauld in his generic revision, it is impossible to reconstruct his character matrix so a new genus is proposed here on the basis of character combinations that seem to preclude its placement in any described genus, and some apomorphic characters that attest to its long evolutionary independence from other lineages. I.D. Gauld and H.K. Townes had, probably independently, concluded that the males seen by Gauld and the females seen by Townes represented an undescribed genus. The males and females of this new genus are dissimilar in numerous ways, necessitating the description of two separate species.

Materials and methods

Specimens are deposited in the Natural History Museum, London (BMNH) and the Musée de l'Afrique Centrale (Tervuren) (RMCA). Originally, three males were deposited in BMNH and two females in RMCA. Through the kind cooperation of Eliane de Coninck, curator at RMCA, one of each sex were exchanged so that both institutes now hold both sexes and both species.

Morphological terminology follows Gauld (1991). The format of the generic description follows that of Gauld (1976), for ease of comparison with other anomalonine genera, including numerous indices that Gauld (1976) employed. Photographs were taken using a Canon EOS 450D digital camera attached to a Leica MZ12 stereomicroscope. Partially focused photographs were combined using Helicon Focus software.

Results

Taxonomy

***Helenanomalon* gen. n.**
http://zoobank.org/BAC8F2BC-DF2C-4B4F-A29A-6063811E4697

Type species. *Helenanomalon bonapartei* sp. n.

Description. Eye surface with short hairs, shorter than spaces between them; eyes convergent ventrally; occipital carina complete, dorsally closer to rear ocellus than diameter of ocellus, ventrally reaching mandible base separate from hypostomal carina (difficult to see on the female specimens of *H. ashmolei* sp. n., cannot confirm for both species); frons simple, lacking median longitudinal or transverse carinae. Antenna long, potentially sexually dimorphic, simple in ♂ of *H. bonapartei* sp. n., very short and distinctly clubbed in ♀ of *H. ashmolei* sp. n., with subapical flagellomeres shorter and wider than basal flagellomeres (Fig. 6); flagellum lacking white band; scape from 1.4–2.3× pedicel, truncate at shallow angle. Clypeus truncate (Fig. 3), edge thin and slightly outcurved; mandible strongly narrowed, slightly twisted, with upper tooth 2.0–2.6× lower tooth. Maxillary palp with five segments, labial palp with four (in *H. bonapartei* sp. n., cannot confirm for *H. ashmolei* sp. n.), apical palpomere very small. [Cardo not dissected out.]

Pronotum rather short dorsally with narrow, sharply defined transverse furrow, anterior edge of furrow continuing ventrally as carina close to and parallelling anterior edge of pronotum; posterior, dorsal corner of pronotum not covering spiracular sclerite; lower anterior corner lacking tooth, rounded anteriorly. Mesoscutum steeply rounded anteriorly without an apical concavity. Notauli present or absent; transverse suture of mesoscutum absent, transverse furrows absent. Epicnemial carina reaching to about lower ⅓ of mesopleuron, dorsally distant from anterior margin of mesopleuron, medio-ventrally weakly raised; sternaulus absent; only short, median section of posterior transverse carina of mesosternum present.

Fore coxa smooth; fore tibial spur with long comb of macrotrichia on inner surface with membranous flange posterior to comb. Mid tibia with two spurs. Hind trochanter dorsally 2.0–3.0× as long as trochantellus, ventrally 1.0×; hind tarsi of ♂ not swollen; hind tarsal claws abruptly curved, fully pectinate (Fig. 5a), or weakly curved with only basal pecten (Fig. 5b).

Fore wing with *Rs* straight; 2*rs-m* basal to 2*m-cu*; 1*m-cu* and *Cu*1*a* basally separate (Fig. 9a,b). 1st subdiscal cell approximately parallel-sided. Hind wing with 6 (♀) or 9–10 (♂) hamuli on vein *R*1; distal abscissa of *Cu*1 present, but not joining *cu-a*+basal section of *Cu*1 (nervellus) (Fig. 9a), or absent (Fig. 9c).

Petiolar index:
PI (distance from anterior margin of 1st metasomal spiracle to base of 1st tergite / distance from posterior margin of spiracle to apex of 1st tergite) = 2.19–2.68.

Fore wing indices:
CI (length of *Cu*1 between 1*m-cu* and *Cu*1*a* / length of *Cu*1*b* between *Cu*1*a* and 1*A* = 0.82–1.27;
BI (shortest distance between *Cu*1 and 1*A* at distal end of 1st subdiscal cell / shortest distance between *Cu*1 and 1*A* at proximal end of 1st subdiscal cell) = 1.09–1.13;
DBI (length of *Cu*1between *cu-a* and 1*m-cu* / length of 1*m-cu* between *Cu*1 and 2+3*rs-m*) = 0.49–0.51;
ICI (length of 2+3*rs-m* / length of *M* between 2+3*rs-m* and 2*m-cu*) = 0.60–1.23;
MI (length of *Rs* / length of *Rs*+2*r*) = 1.60–1.89.

Hind wing index:
RI (length of *Rs* between *R*1 and 1*r-m* / length of 1*r-m* between *Rs* and *M*) = 1.25–1.38.

Propodeum reticulate, with longitudinal, median depression; spiracle about 1.6× as long as broad; apex of propodeum extending about 0.3–0.4× length of hind coxa. Metasoma elongate.

♀ genitalia. Ovipositor sheath 1.2× length of tergite 3; ovipositor lacking dorsal notch but pre-apically markedly swollen, higher and wider here than basal or apical sections, apical 0.3 of dorsal valves of ovipositor very narrow, slightly decurved (Fig. 7).

♂ genitalia. Metasomal tergites 8+9 (syntergum of Gauld 1976) entire; 8th sternite roundly produced posteriorly; parameres (=gonosquamae) approximately rectangular; gonolacinia smoothly curved, with median tooth; distivolsella not wholly visible in available specimens; aedeagus in profile distally swollen, convex dorsally with apical, dorsal projection, ventrally membranous with whole membranous area covered with minute spinules, not laterally expanded (Fig. 8).

Etymology. Named after the type locality, St Helena, and the type genus of the subfamily, *Anomalon*.

Remarks. The two available female specimens of *H. ashmolei* sp. n. are, in part, encrusted with glue and dirt so it is not possible to see all of the characters that can be observed in the male specimens of *H. bonapartei* sp. n. Sexual dimorphism seems to be pronounced in this genus, although each species is known from one sex only; see discussion below on the species status of specimens.

Helenanomalon bonapartei sp. n.

http://zoobank.org/302F71B2-114A-41D1-896D-66C0E57B84A5
Figs 1, 3a, 4a, 5a, 8, 9a

Description. Male. Fore wing length 6.7–7.9 mm in ♂. Antenna with 33 (1 specimen)–36 (2 specimens) flagellomeres; all flagellomeres longer than wide and about equal width; antennal flagellum 1.2× length of fore wing (Fig. 1); scape 2.3× pedicel; distance between eyes ventrally c.0.8× distance dorsally (Fig. 3a); mandible more weakly

Figure 1. Holotype ♂ of *Helenanomalon bonapartei* sp. n.; scale bar = 10 mm.

twisted than in *H. ashmolei* sp. n., upper tooth 2.0× lower tooth. Head and mesosoma covered in closely spaced, silvery setae, but sparser than in *H. ashmolei* sp. n. (Fig. 4a); metasoma, beyond first tergite, covered in slightly sparser setae; notauli strongly im-

pressed and narrow medially but faint on anterior slope and posteriorly disappearing in rugosity (Fig. 4a). Head rugose, matt, pronotum rugose-punctate laterally, dorsally punctate, mesoscutum punctate, rugose posteriorly, mesopleuron punctate, transversely striate dorsally, mesosoma with shiny interspaces between obvious punctures. Hind trochanter dorsally 3.0× as long as trochantellus, ventrally 1.0×; hind tarsal claws abruptly curved, fully pectinate (Fig. 5a). First sternite without convexity sub-basally. Fore wing (Fig. 9a) with *cu-a* slightly distal to *Rs&M*; hind wing with 9–10 hamuli on vein *R*1; distal abscissa of *Cu*1 with distal section reaching wing membrane, proximally distant from *cu-a*+basal section of *Cu*1 (nervellus). Petiolar index 2.68; fore wing indices: CI 0.82; BI 1.09; DBI 0.49; ICI 1.23; MI 1.60; hind wing index: RI 1.25.

Colour. Red, paler than in *H. ashmolei* sp. n.; face, inner orbits, underside of scape bright yellow; frons and antennal flagellum black; mesosoma red with black on middle of median lobes of mesoscutum, mesoscutum posteriorly, mesopleuron dorsally, ventrally, mesosternum, propodeum dorsally, metasternum, upper division of metapleuron, postscutellum. Scutellum (mesoscutellum) black anteriorly, medially, ivory or yellow posteriorly and laterally. Following parts ivory: palps, pronotum dorso-posteriorly, ventro-posteriorly, propleural lobe, subalar prominence, fore and mid coxae and trochanters, fore trochantellus, mid trochantellus ventrally, hind trochanter and trochantellus ventrally. Hind coxa apically, dorsally, trochanter and trochantellus, femur basally, tibia and tarsus dark brown/black. First and second metasomal tergites black, remainder of metasoma and legs red.

Female: unknown.

Material examined. Holotype ♂ 'St Helena: High Peak Malaise trap: xii.2005–1.2006 S15°58.7' W5°44.0' c.752m UTM:02/068 18 82/1903', 'St Helena Peaks Project N.P. Ashmole, M. Ashmole, H. Mendel & E. Thorpe BMNH(E) 2006-9', specimen number BMNH(E) #1022370 (BMNH).

Paratypes: 1 ♂ 'St Helena: Peak Dale gumwoods: 24.i.2006: off foliage (2096)', 'St Helena Peaks Project N.P. Ashmole, M. Ashmole, H. Mendel & E. Thorpe BMNH(E) 2006-9' (RMCA); 1♂ 'St Helena Knollcombes 16.x.1957 C.R. Wallace', specimen number BMNH(E) #1022371 (BMNH).

Etymology. Named after the most famous inhabitant of St Helena, Napoleon Bonaparte, who was exiled there from 1815 until his death in 1821.

Helenanomalon ashmolei sp. n.

http://zoobank.org/EECE67E9-2D77-4903-9559-9CD0EB8FF929
Figs 2, 3b, 4b, 5b, 6, 7, 9b,c

Description. Female. Fore wing length 3.6–3.7 mm. Antenna with 16–17 flagellomeres, apical two flagellomeres of partly fused but still with obvious division between them, apical flagellomere 3.7× penultimate flagellomere, penultimate 6 flagellomeres of antenna as wide as or wider than long, apical flagellomere at widest 1.8× as wide

Figure 2. Holotype ♀ of *Helenanomalon ashmolei* sp. n.

as first flagellomere; antennal flagellum 0.6× fore wing (Fig. 2); scape 1.4× pedicel; distance between eyes ventrally c.0.7× distance dorsally (Fig. 3b); mandible more strongly twisted than in *H. bonapartei* sp. n., upper tooth 2.6× lower tooth. Head and

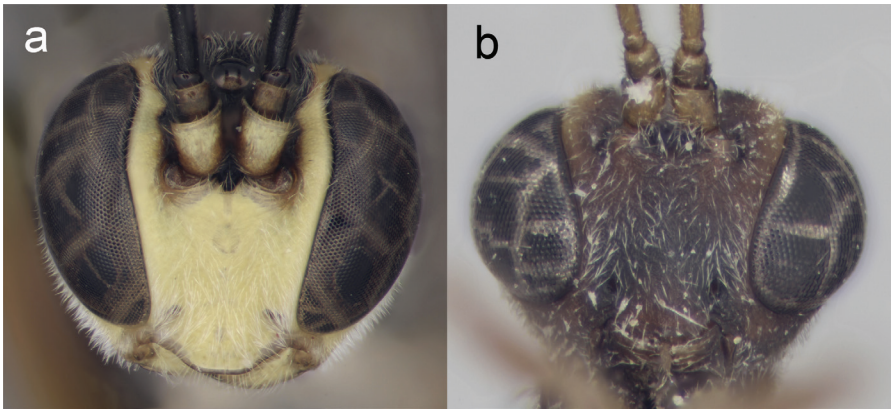

Figure 3. Face of **a** holotype ♂ of *H. bonapartei* sp. n. and **b** holotype ♀ of *H. ashmolei* sp. n.

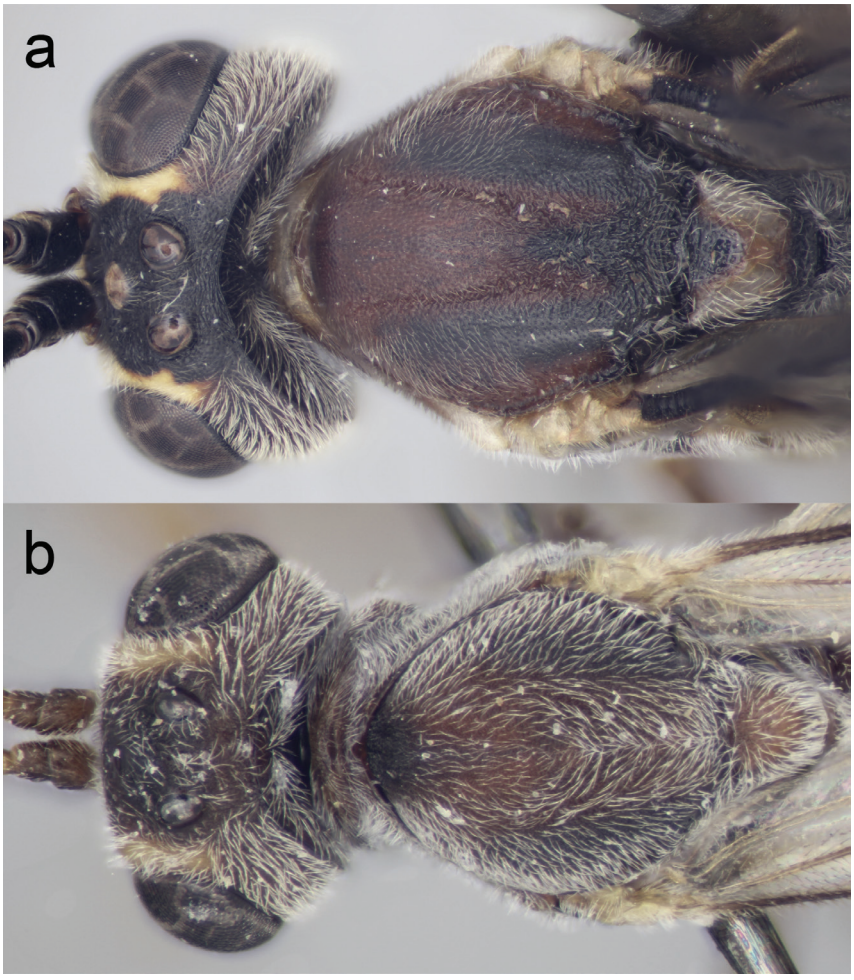

Figure 4. Mesoscutum of **a** holotype ♂ of *H. bonapartei* sp. n. and **b** holotype ♀ of *H. ashmolei* sp. n.

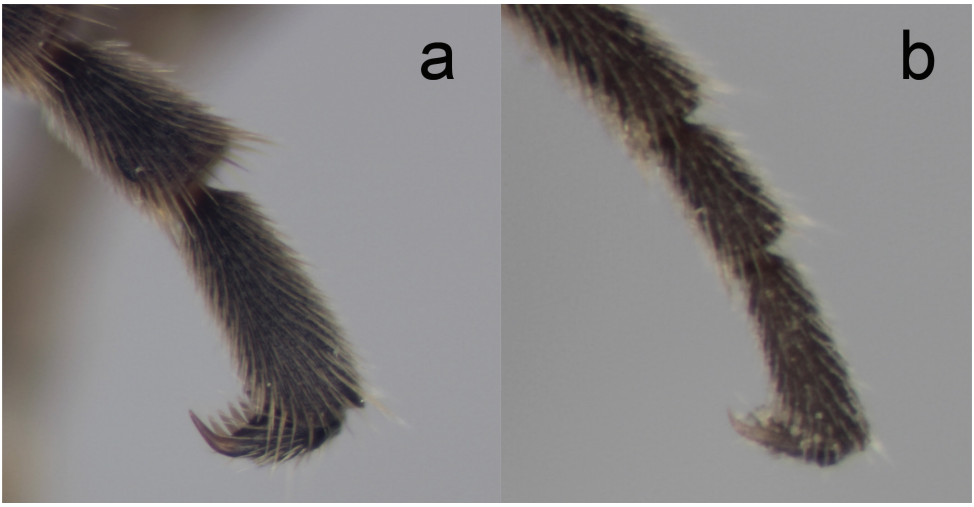

Figure 5. Hind tarsal claw of **a** holotype ♂ of *H. bonapartei* sp. n. and **b** holotype ♀ of *H. ashmolei* sp. n.

Figure 6. Antenna of holotype ♀ of *H. ashmolei* sp. n.

mesosoma covered in closely spaced, silvery setae, denser than in *H. bonapartei* sp. n. (Fig. 4b); metasoma, beyond first tergite, covered in slightly sparser setae; notauli absent (Fig. 4b), no rugose area on mesoscutum. Head and mesosoma predominantly of granulate appearance, rugose, matt, impunctate. Hind trochanter dorsally 2.0× as long as trochantellus, ventrally 1.0×; hind tarsal claws weakly curved with only basal pecten (Fig. 5b). First metasomal sternite with marked convexity sub-basally. Fore wing (Fig. 9b) with *cu-a* slightly proximal to *Rs&M*; hind wing (Fig. 9c) with 6 hamuli on vein *R*1; distal abscissa of *Cu*1 absent but with slight angulation on nervellus (*cu-a*+ *Cu*1). Petiolar index 2.19; fore wing indices: CI 1.27; BI 1.13; DBI 0.51; ICI 0.60; MI 1.89; hind wing index: RI 1.38.

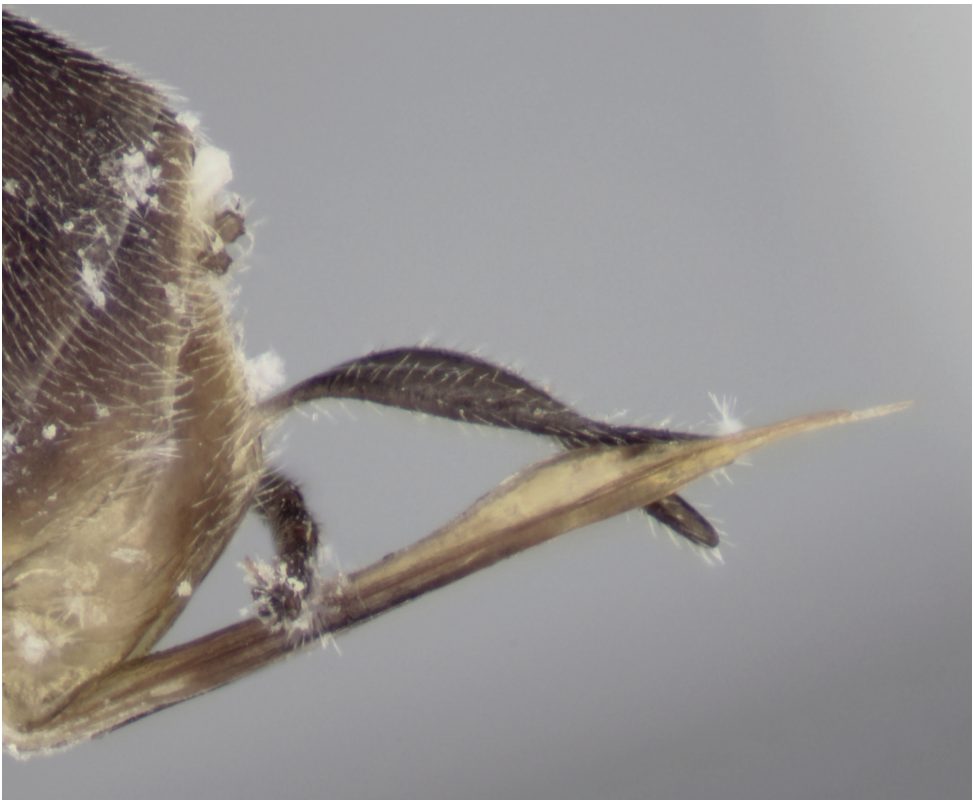

Figure 7. Ovipositor of holotype ♀ (RMCA) of *H. ashmolei* sp. n.

Colour. Basically dark red; fore and mid legs and hind femur paler red; extreme base of hind femur, apex of hind trochanter, yellow; antenna basally paler red; inner orbits from antennal sockets to hind ocelli dull yellow; metasoma extensively orange-red in holotype ♀, darker red in paratype ♀.

Male: unknown.

Material examined. Holotype ♀ 'Sainte-Hélène: Est Prosperous Bay Plain 800-900 ft. 5.II.1967', 'Coll. Mus. Tervuren Seconde Mission Zoologique à Sainte-Hélène J. Decelle, N. et J. Leleup' (RMCA).

Paratype ♀ same data except 1000-1100 ft, 29.IV.1967, specimen number BMNH(E) #1022369 (BMNH).

Etymology. Named after Philip and Myrtle Ashmole, who have done so much to encourage the study and conservation of the unique fauna of St Helena.

Discussion

Only six species of Ichneumonidae have been recorded from St Helena, namely *Diadegma mollipla* (Holmgren), *Diplazon laetatorius* (Fabricius), the endemic subspe-

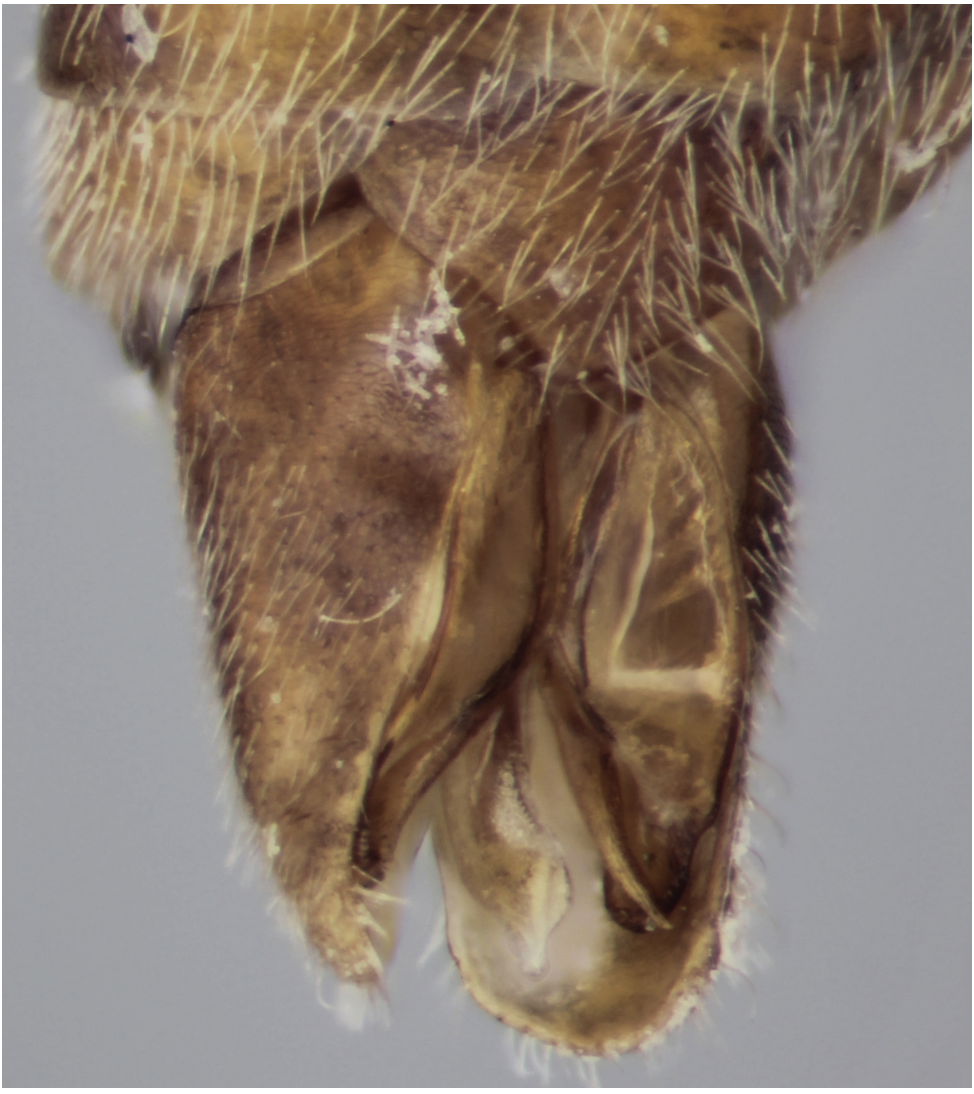

Figure 8. Genitalia of paratype ♂ (RMCA) of *H. bonapartei* sp. n.

cies *Echthromorpha agrestoria atrata* Holmgren, the endemic species *Netelia insulicola* (Morley), and now *Helenanomalon ashmolei* sp. n. and *H. bonapartei* sp. n. (note that Yu et al. (2012) mistakenly list *Diadegma semiclausum* (Hellén) from St Helena, citing Wagener et al. (2006), who actually only record *D. mollipla* from the island). It seems surprising that there is an endemic genus on this island, given that two of the other ichneumonids (*Diadegma mollipla* and *Diplazon laetatorius*) are widespread, probably introduced, associates of agriculture and the other two are closely related to extralimital species (*Netelia insulicola* to African species and *E. agrestoria atrata* is currently classified as a subspecies of the wide-ranging, polytypic *E. agrestoria* (Swederus)). However,

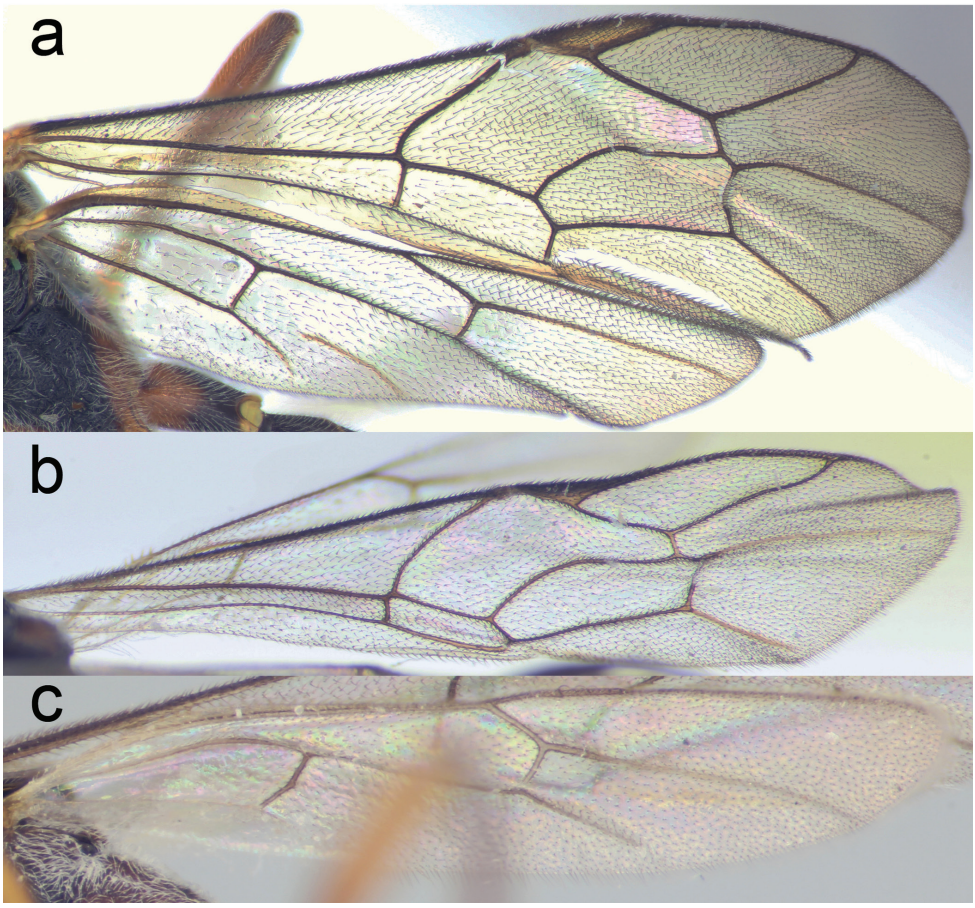

Figure 9. Wings of *Helenanomalon* species **a** fore and hind wings of paratype ♂ (BMNH) of *H. bonapartei* sp. n. **b** fore wing of holotype ♀ of *H. ashmolei* sp. n. **c** hind wing of *H. ashmolei* sp. n.

the Central Peaks and Prosperous Bay areas of St Helena are home to other endemic genera of insects and these are clearly areas of special habitat that have allowed the evolution of distinct lineages in great isolation. It should also be noted that the anomalonine fauna of Africa is poorly known, with few species described; the St Helenan insect fauna shows most affinity with the African fauna (Mendel et al. 2008) and the closest relatives of *Helenanomalon* may well be found there.

With one species known only from females and the other only from males, there must remain some doubt as to whether these are really separate species. Given the small anomalonine fauna likely to be present on St Helena, one hypothesis is that these specimens represent the two sexes of the same species, displaying a more extreme sexual dimorphism than is otherwise known within Anomaloninae. In this scenario, females show striking adaptations for locating and accessing hosts, whilst males have rather more generalised morphology. Development in different sizes of hosts will, of course, produce different sizes of adult ichneumonids, even in koinobiont endopara-

sitoids such as anomalonines, and the two females collected so far, much smaller than the males, may simply have developed in rather smaller hosts than the males that have been collected; anomalonines are rarely host-specific, usually accepting hosts across rather taxonomically wide host ranges within particular parasitoid searching niches (e.g., Shaw et al. 2009). However, I favour the alternative hypothesis, that these are separate species. In favour of this are the differences in wing venation; the well-developed notauli in the male but not the female; the larger size of the males, when often it is the females that are larger in ichneumonids; and the fact that the two sexes were collected in different areas of the island, in different habitats (the females in the more arid Prosperous Bay, the males in the wetter Central Peaks area). The sexes do share numerous characters and I am convinced that they are congeneric. The fauna of St Helena has now been better surveyed than that of many remote islands, nevertheless, further collecting should produce more specimens of this genus on St Helena and confirm or refute the taxonomy proposed here. Fresh material would obviously be very useful for DNA sequencing.

An investigation of the evolutionary relationships of *Helenanomalon* would be rewarding in potentially shedding light on the origins of this geographically isolated genus. The classification of Anomaloninae as a whole would certainly repay a detailed phylogenetic study. In Gauld's (1976) key to genera of Anomaloninae, males of *Helenanomalon bonapartei* will fail to travel properly from couplet nine, because the clypeus is truncate apically and the mesoscutum evenly rounded but the notauli are present and strong. Females, and males if the distal abscissa of hind wing vein *Cu*1 is regarded as missing (at couplet five), will reach couplet 40, where they will key to *Habronyx* (*Camposcopus*), but by ignoring the fact that the clypeus does not have a median tooth. Several characters preclude the classification of these specimens in *Habronyx*, such as the apically truncate clypeus, vestigial transverse carina of the mesosternum and the undivided male metasomal tergites 8+9. The aedeagus most closely resembles that of *Habronyx* (*Habronyx*) *australasiae* (Morley), as illustrated by Gauld (1976). The main difficulty faced in trying to place this taxon in Gauld's scheme of relationships is that Gauld's (1976) proposed set of relationships is based on percentage similarity (this work preceded the widespread acceptance of cladistic methods in phylogeny reconstruction) and not on character distributions. In the absence of clades defined by apomorphies we can only suggest that *Helenanomalon* seems to be related to *Trichomma* or *Habronyx*. The former hypothesis is based on overall similarity, including, in some species of *Trichomma*, the lack of a clypeal tooth and the distal abscissa of hind wing vein *Cu*1. *Helenanomalon* differs most obviously in the short setae on the eye and shorter ovipositor than in *Trichomma*. The strong notauli (in the male only though) and various trends, including the short epicnemial caria and short, sparse setae on the surface of the eye, are present in a few species of *Habronyx s.l.*

In several features, such as lack of a clypeal tooth and the form of the epicnemial carina, *Helenanomalon* resembles *Habronyx* (*Austranomalon*) but differs in the truncate, simple clypeus (when truncate in *Austranomalon* then a clypeal tooth is present); the aedeagus has a well-developed, apical, crest-like area; and possible sexual dimor-

phism in tarsal claws, which is the opposite to the condition found in *Austranomalon*, i.e. the male claws in *Helenanomalon* are strongly curved and pectinate, those of the known females weakly curved and scarcely pectinate. Females of *Helenanomalon* have strikingly short antennae, very unusual for an anomalonine, although this autapomorphy does not help place the genus. There are only small differences between some of the genera of Anomaloninae whilst a few genera as currently defined seem to encompass more variation between species classified in the same genus than is found between other genera. *Habronyx* is a good example of the latter, polythetic genus.

The most distinctive feature of *Helenanomalon*, that should render females instantly recognisable, is the short, clavate antennal flagellum of the female (but it is not known if this is true for the unknown female of *H. bonapartei* sp. n.). Males are less easily recognised but have a unique combination of characters, including the apically truncate clypeus, lacking a tooth; the strongly curved, completely pectinate tarsal claws; short, sparse setae on the eye surface; and the incomplete posterior transverse carina of the mesosternum. Females lack notauli and have only basally pectinate claws. Another potential characteristic of the genus is pronounced sexual dimorphism.

Acknowledgements

Thank you to Howard Mendel and Philip and Myrtle Ashmole for collecting a wealth of insect specimens from St Helena, which prompted me to describe this genus. I am grateful to Eliane de Coninck and Stéphane Hanot for the loan of specimens from Tervuren and for agreeing to the exchange of a female and male between institutes. Harry Taylor at BMNH took the lovely photograph of the holotype male of *H. bonapartei*. I am grateful to Heinz Schnee and Seraina Klopfstein for their helpful reviews of the manuscript, particularly their opinions on the number of species represented by these few specimens.

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RESEARCH ARTICLE

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Dinotrema cavernicola sp. n. (Hymenoptera, Braconidae, Alysiinae), a new species of the genus *Dinotrema* Foerster from caves of Spain

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Abstract

Dinotrema cavernicola **sp. n.** was collected in two caves in Spain. This is the first *Dinotrema* species known to occur in caves. This new species is described and compared to *D. affine* (Fischer, 1973) and *D. collybiae* Munk & Peris-Felipo, 2014, species sharing a mid-longitudinal carina on the propodeum.

Keywords

Alysiinae, *Dinotrema*, taxonomy, new species, caves, Diptera

Introduction

Braconidae is the second largest family of Hymenoptera belonging to the superfamily Ichneumonoidea and with nearly 20,000 valid species around the world (Yu et al. 2012). Nearly all species of Braconidae are primary parasitoids of predominantly immature stages of Lepidoptera, Coleoptera and Diptera (Sharkey 1993).

The Alysiinae is a conspicuously diverse subfamily within the Braconidae (Dolphin and Quicke 2001) with 2,000 described species (Yu et al. 2012) separated in two large and polymorphic tribes Alysiini and Dacnusini (Shenefelt 1974). Species of Alysiini are parasitoids of a wide variety of cyclorrhaphous Diptera, mainly in humid habitats and ephemeral substrata (Wharton 2002). In contrast, Dacnusini are almost exclusively specialised on leaf- and stem-miner hosts, predominantly of the families Agromyzidae, Ephydridae and Chloropidae (Griffiths 1964, Wharton 2002).

Dinotrema Foerster, 1862 is one of the largest genera within the tribe Alysiini (van Achterberg 1988). It comprises many dozens of species described from the Palaearctic Region and mainly from Western Europe (Fischer 1972; van Achterberg 1988; Tobias 2003, 2004a, 2004b, 2006), but numerous Palaearctic species remain as yet unknown. Increasing our knowledge of this genus, several papers have been published by the two first authors (Peris-Felipo and Belokobylskij 2013; Peris-Felipo et al. 2013a, 2013b, 2013c, 2013d, 2014) and a monograph with a revision of the Western Palaearctic *Dinotrema* species will be published soon (Peris-Felipo et al. 2014). An arrangement of *Dinotrema* species in morphological groups was suggested by Fischer (1972) and later, on the basis of more diverse material, further developed by Tobias (2003, 2004a, 2006).

We describe in this paper *Dinotrema cavernicola* sp. n., and include it in the group of *Dinotrema* with a complete median longitudinal carina of the propodeum. This is the first record of a *Dinotrema* species collected in caves.

Materials and methods

The speleology group of Villacarrillo (Grupo de Espeleología de Villacarillo – GEV) has been conducting intensive fieldwork in caves of Jaén Province (Spain) (Fig. 1). From 2001 up to now many caves have been studied, but in just two caves ("Sistema de la Murcielaguina" and "Sima de la Colada") some braconids were captured.

The "Sistema de la Murcielaguina" is located in Cerro de Hornos (38°12'59.35"N, 002°42'37.13"W) at an altitude of 1085 m. The maximum depth of the cave system is 80 m and is more than 4,000 m long (Fig. 2).

The "Sima de la Colada" is located in La Hoya de Herrera (38°11'11.13"N, 002°46'45.83"W) at an altitude of 864 m. The maximum depth of the cave is 65 m and the cave is 352 m long (Fig. 3).

Both caves are located in the Natural Park and Biosphere reserve of Sierras de Cazorla, Segura and las Villas. Part of it is covered with Mediterranean forest containing *Pinus*, *Quercus*, *Cistus*, *Rosmarinus* and *Thymus* species, among others. The climate is warm temperate with moderate temperatures throughout the year (12–18 °C).

Samples were collected by pit-fall traps. All traps are baited with beer, salt and cheese or sobrassada (sausage). In Murcielaguina, specimens were captured in one trap located at 35 m depth, in complete darkness and with an average temperatures of 15 °C and 78% humidity. In Sima de la Colada, specimens were collected in two traps at 65 m depth with average an temperature of 18 °C and 80% humidity.

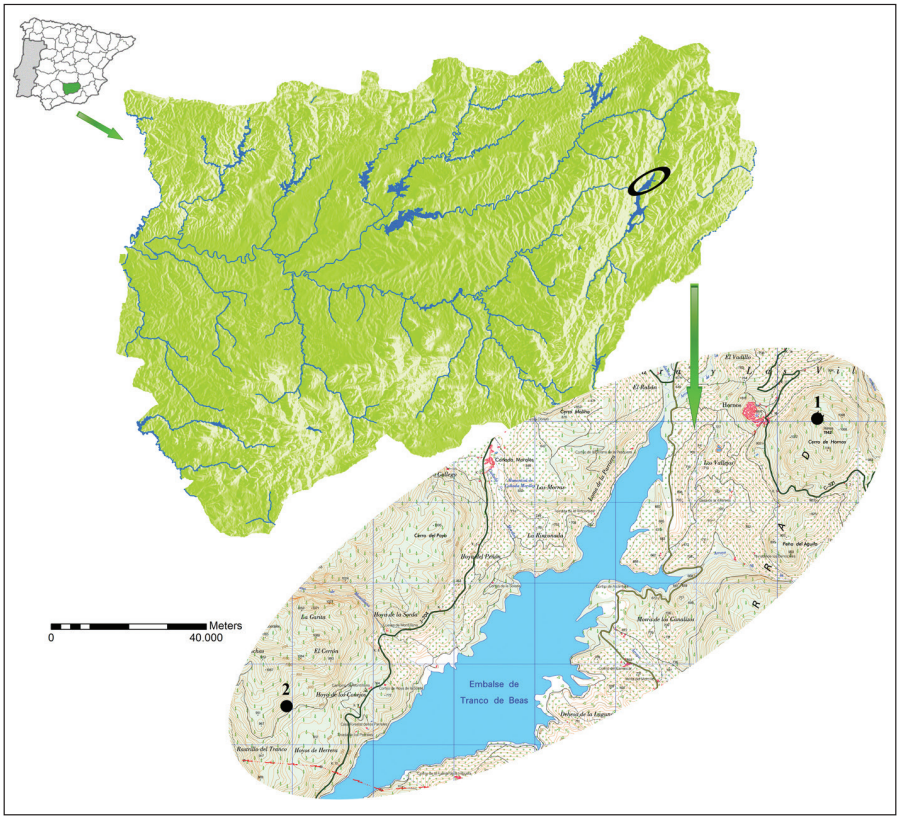

Figure 1. Distribution of studied caves. Caves numbers: **1** Sistema de la Murcielaguina **2** Sima de la Colada.

Figure 2. Map of the Sistema de la Murcielaguina with a red dot where specimens were captured.

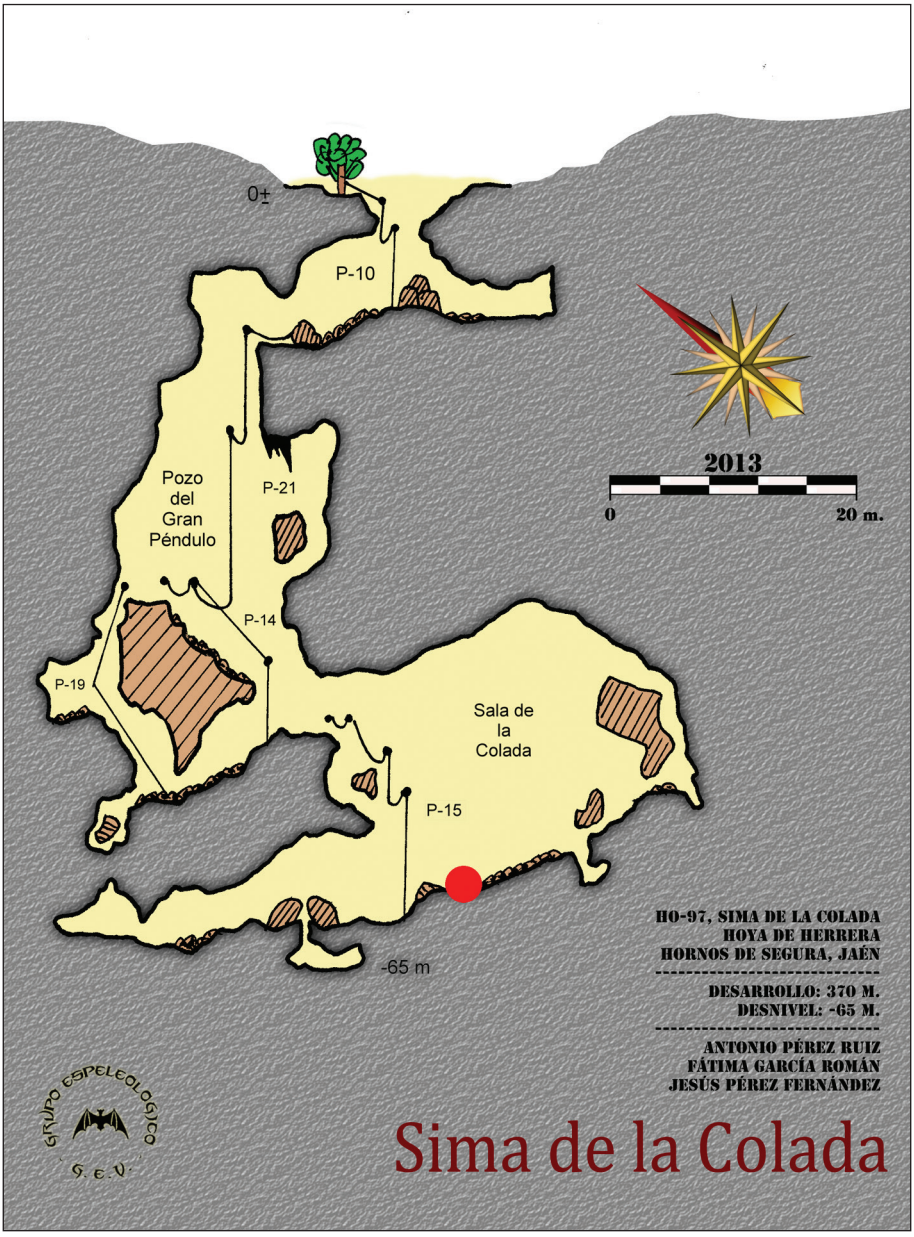

Figure 3. Map of the Sima de la Colada with a red dot where specimens were captured.

Specimens were collected during the summer of 2014 and each cave was visited every two weeks.

For terminology of morphological features and sculpture, measurements and wing venation nomenclature, see Sharkey and Wharton (1997) and HAO (Hymenoptera Anatomy Ontology Portal: http://portal.hymao.org/) (Yoder et al. 2010). To follow

the identification keys by Peris-Felipo et al. (2014), the following differences in terminology should be kept in mind (terms in Peris-Felipo et al. (2014) second):

Gena: temples.
Anterior tentorial pit: paraclypeal fovea.
Mesoscutal midpit: mesoscutal pit.
Scutoscutellar sulcus: prescutellar depression.
Mesopectus: mesopleuron.
Mesepimeral sulcus: posterior mesopleural furrow.
Marginal cell: radial cell.
Nervulus: vein cu-a.
Second submarginal cell: brachial cell.

Type specimens are deposited in the Entomological Collection at the University of Valencia (Valencia, Spain; ENV), in the Grupo de Espeleología de Villacarillo (Jaén, Spain; GEV), and in the Zoological Institute RAS (St Petersburg, Russia; ZISP).

Taxonomic part

***Dinotrema cavernicola* Peris-Felipo, sp. n.**
http://zoobank.org/29ED3F14-03B1-45A4-B499-864B53805AEF
Figs 4–5

Type material. Holotype: female, Spain, Jaén Province, Cerro de Hornos, Sistema de la Murcielaguina, pit-fall trap, - 35 m, 15.vi.2014 (GEV leg.) (ENV). Paratypes: 2 females, same data as holotype (ENV, ZISP); 3 females, Spain, Jaén Province, La Hoya de Herrera, Sima de La Colada, pitfall trap, - 65 m, 17.viii.2014 (GEV leg.) (ENV, GEV, ZISP).

Description. Female.

Head. In dorsal view, 1.7 times as wide as long, 1.3 times as wide as mesoscutum (variation 1.2–1.3 times), smooth, with gena rounded behind eyes. Eye in lateral view 1.5 times as high as wide and 1.2 times as wide as gena. POL about as long as OD; OOL 2.3 times OD. Face 1.5 times as wide as high and covered completely by numerous setae; inner margins of eyes subparallel. Clypeus 2.3 times as wide as high, slightly curved ventrally. Anterior tentorial pit short, not reaching halfway between clypeus and eye. Mandible weakly widened towards apex, 1.6 times as long as its maximum width (variation 1.5–1.6 times). Upper tooth medium sized, wide, shorter than lower and middle tooth. Middle tooth rather small, slightly longer than upper tooth, wide basally and pointed apically. Lower tooth short, wide, rounded. Antenna 21-segmented, 1.1 times as long as body. Scape 2.7 times as long as pedicel. First flagellar segment 4.1 times as long as its apical width (variation 4.1–4.2 times), 1.4 times as long as second segment; second segment 2.5 times as long as its maximum width. Third to twentieth flagellar segments 2.3–2.5 times, and twenty-first segment 2.6 times as long as wide.

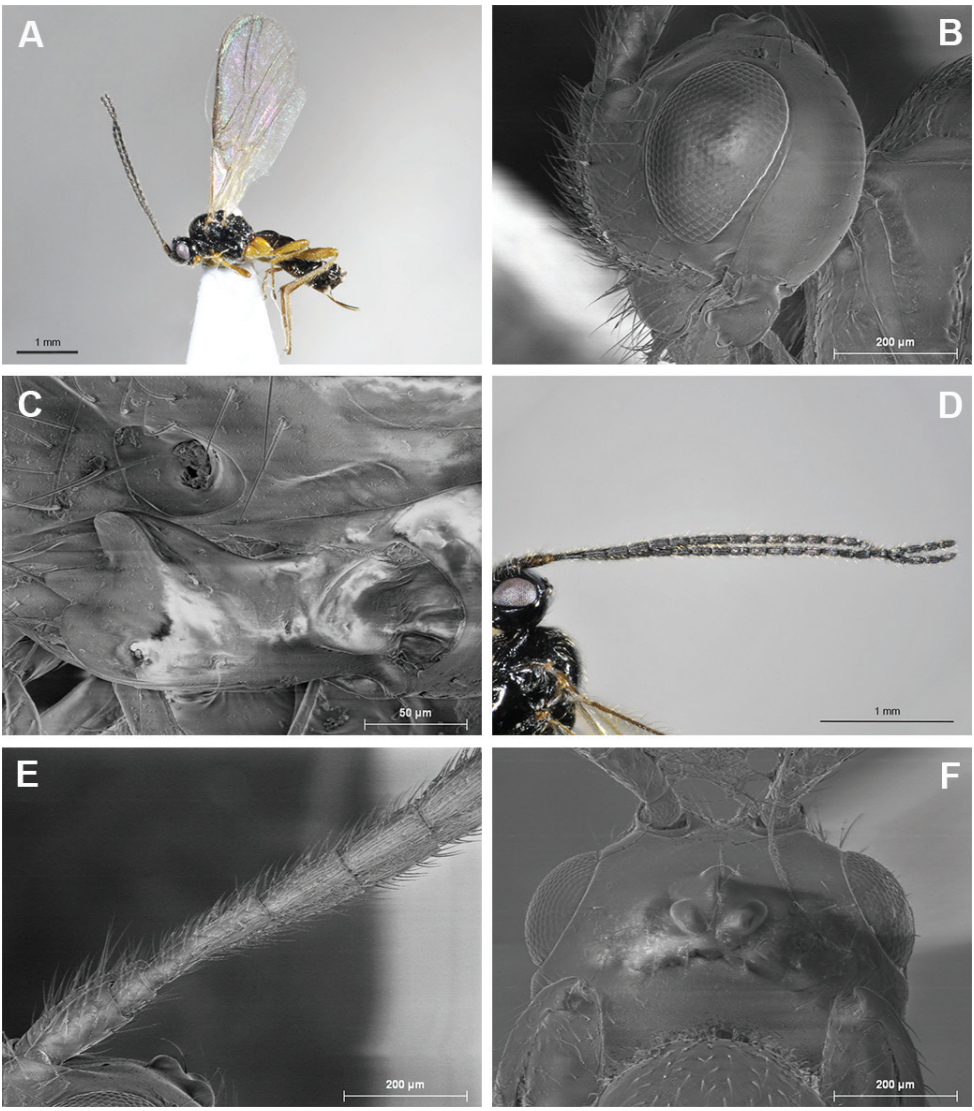

Figure 4. *Dinotrema cavernicola* sp. n. (female). A Habitus, lateral view **B** Head, lateral view **C** Mandible **D** Antenna **E** Basal segments of antenna **F** Head, dorsal view.

Mesosoma. In lateral view, 1.1 times as long as high. Mesoscutum (dorsal view) 0.9 times as long as its maximum width, with numerous setae located on middle part of mesoscutum. Notauli mainly absent on vertical surface of mesoscutum. Mesoscutal midpit present and elongate. Scutoscutellar sulcus smooth, without lateral carinae. Precoxal sulcus present, not reaching anterior and posterior margins of mesopectus. Mesepimeral sulcus smooth. Propodeum mainly smooth, median longitudinal carina complete, with several short transverse carinae crossing median carina but not reaching lateral edge of propodeum. Propodeal spiracles relatively small.

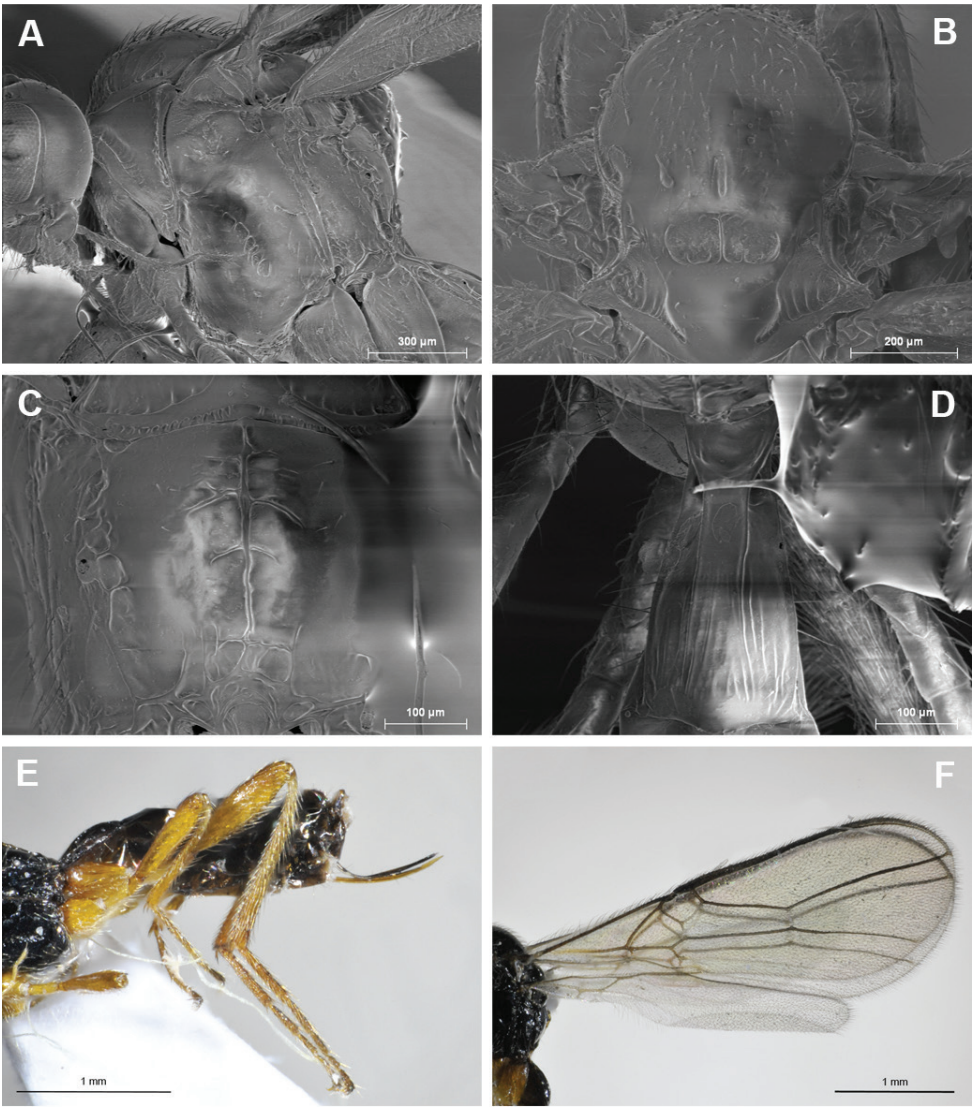

Figure 5. *Dinotrema cavernicola* sp. n. (female). **A** Mesosoma, lateral view **B** Mesonotum, dorsal view **C** Propodeum, dorsal view **D** First metasomal tergite **E** Metasoma, hind leg and ovipositor, lateral view **F** Fore and hind wings.

Wings. Length of fore wing 2.4 times its maximum width (variation 2.4–2.5 mm). Vein r1 present and sclerotised. Marginal cell reaching apex of wing, 5.0 times as long as its maximum width. Nervulus distinctly postfurcal. Second submarginal cell closed distally, 2.7 times as long as its maximum width (variation 2.6–2.7 times). Hind wing 5.4 times as long as its maximum width (variation 5.3–5.4 times).

Legs. Hind femur 4.0 times as long as its maximum width (variation 3.9–4.0 times). Hind tibia weakly widened to apex, 9.2 times as long as its maximum subapical

width, 1.1 times as long as hind tarsus. First segment of hind tarsus 1.7 times as long as second segment.

Metasoma. Long. First tergite weakly widened towards apex, twice as long as its apical width, entirely striate. Ovipositor 1.6 times as long as first tergite, 0.5 times as long as metasoma, 0.9 times as long as hind femur.

Colour. Body, scape, flagellar segments and pterostigma dark brown. Legs, mandible and pedicel yellowish brown. Wings hyaline.

Length. Body 2.7 mm (variation 2.6–2.8 mm); fore wing 3.4 mm (variation 3.4–3.5 mm).

Male. Unknown.

Etymology. Named "cavernicola" because it inhabits caves.

Comparative diagnosis. According to the key by Peris-Felipo et al. (2014), this new species is similar to *D. affine* (Fischer, 1973) and *D. collybiae* Munk & Peris-Felipo, 2014, both belonging to the species group with a complete median longitudinal carina on the propodeum. *Dinotrema cavernicola* sp. n. differs from *D. affine* and *D. collybiae* in having the first flagellar segment 4.1–4.2 times as long as wide (3.5 times in *D. affine* and 3.2 times in *D. collybiae*), middle flagellar segments 2.3–2.5 times as long as wide (1.6 times in *D. affine* and 1.4–1.7 times in *D. collybiae*), metasoma long (short in *D. affine* and *D. collybiae*), POL about as long as OD (1.5 times in *D. affine* and 1.4 times in *D. collybiae*); OOL 2.3 times OD (3.0 times in *D. affine* and 2.0 times in *D. collybiae*), and ovipositor 1.6 times as long as first tergite (0.6 times in *D. affine* and 1.2 times in *D. collybiae*).

Remarks. Specimens belonging to the families Phoridae and Heleomyzidae (Diptera) were captured in the same traps as *Dinotrema cavernicola* sp. n. Among Phoridae, the species *Megaselia rufipes* (Meigen, 1804) and *M. tenebricola* Schmitz, 1934 were abundantly captured (determination by Dr. Henry L. Disney, UK). It is interesting *M. rufipes* was already recorded as a host of some Braconidae taxa, such as *Orthostigma pumilum* (Nees) (Scott 1920; Lundbeck 1922; Achterberg 1988), *Aspilota* sp. near *nervosa* (Schmitz, 1938) and *Dinotrema lineola* (Thomson) (Mostovski 2001), as well as *Platygaster aegeus* Walker (Platygastridae) (Morley 1934). Also, *Heteromyza atricornis* Meigen, 1830 (Heleomyzidae) was abundantly captured during samples (determination by Dr. Miguel Carles-Tolrá, Barcelona, Spain).

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We wish to thank the staff of Consejería de Medio Ambiente de la Junta de Andalucía and the Natural Park and Biosphere reserve of Sierras de Cazorla, Segura and Las Villas for their help during this study. Also, we are thankful to the members of GEV (Grupo de Espeleología de Villacarrillo) for their great work in increasing the knowledge of fauna caves over the past 13 years, and this year celebrates the group's 35th anniversary.

We are very thankful Dr H.L. Disney (UK) and Dr Miguel Carles-Tolrá (Barcelona, Spain) (Heleomyzidae) for determination of the flies.

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RESEARCH ARTICLE

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Zig-zagging across Central Europe: recent range extension, dispersal speed and larval hosts of *Aproceros leucopoda* (Hymenoptera, Argidae) in Germany

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Abstract

Aproceros leucopoda, the zig-zag sawfly, an invasive pest of elms (*Ulmus* spp.), was found in two separate areas of Germany through July 2014, i.e., a northern area including the states of Berlin, Brandenburg, Mecklenburg-West Pomerania, Saxony and Saxony-Anhalt, and a southern area in Bavaria. A speed of self-dispersal of 45–90 km/yr has been calculated from earlier and present records. Observations of *A. leucopoda* in Belgium and the Netherlands during 2013, which are 360–610 km distant from records in Germany of that year, are interpreted as resulting from human-mediated jump dispersal. Larvae, feeding traces and cocoons were frequently found on the native elm species *U. minor* and *U. glabra*, whereas none could be detected on *U. laevis*. Other occurrences were often on Resista® elms, causing severe defoliation in a recent planting. New host plant records for *A. leucopoda* are: *U. minor* 'Webbiana', *U. minor* var. *suberosa* , and the Resista® cultivars *U.* 'New Horizon', *U.* 'Regal' and *U.* 'Rebona'. The future dispersal of *A. leucopoda* throughout most of Germany is expected, because at least *U. glabra* and *U. minor* are widespread in this country.

Keywords

Argidae, *Aproceros leucopoda*, zig-zag sawfly, invasive species, pest species, *Ulmus laevis*, *Ulmus* Resista® hybrids, distribution in Germany, self-dispersal, human-mediated jump dispersal

Introduction

The invasive zig-zag sawfly *Aproceros leucopoda* Takeuchi, 1939, is of East Asian origin and was first found in Europe in 2003 (Blank et al. 2010). Its larvae feed on elms (*Ulmus* spp.) and sometimes severely defoliate their hosts. By 2009, the known European distribution of *A. leucopoda* extended from eastern Ukraine to Austria and from Poland to Romania. Since then, it has also been found in northern Italy (Zandigiacomo et al. 2011), Slovenia (de Groot et al. 2012), Croatia (Matošević 2012), Moldova (Timuş et al. 2008, misidentified as *Arge* sp.) and widely in European Russia from Rostov-on-Don to Moscow (Artokhin et al. 2012, Anonymous 2013). In Germany, it had been recorded only from the extreme southeast of Bavaria (Kraus et al. 2012) but not from the eastern states close to the Polish border, although the records of *A. leucopoda* from Poland made in 2003 are amongst the first from Europe. In 2013, *A. leucopoda* was observed near northwestern Germany in Belgium (Boevé 2014) and in the Netherlands (Mol and Vonk 2013).

Aproceros leucopoda is classified in the Argidae, which comprise roughly 70 species in Europe and about 900 worldwide (Taeger et al. 2006, 2010). In Europe, a few species of *Arge* have been reported to occur as pests of ornamental plants and forest trees (e.g., Pschorn-Walcher 1982, Taeger et al. 1998). Outbreaks of *Arge pullata* (Zaddach, 1859) may occur on birch trees. When the trees grow in pastures, farm livestock can be poisoned after ingestion of larvae (Brummerstedt et al. 1987, Thamsborg et al. 1987, Kannan et al. 1988, Hara and Shinohara 2008). Similar to *Aproceros leucopoda* in its association with elms as the larval hosts, is *Arge captiva* (F. Smith, 1874) (Shinohara et al. 2009). It became an invasive pest after its accidental introduction to Kazakhstan, where the larvae have severely damaged Dwarf Elm trees (*U. pumila*) planted in the new capital Astana (Blank et al. 2011).

To effect an early warning, in 2011 *A. leucopoda* was placed on the EPPO Alert List, which displays information on pest organisms that possibly represent a risk to the European and Mediterranean EPPO member countries (EPPO 2014). The Federal Research Centre for Cultivated Plants of Germany assessed the phytosanitary risk attached to this invasive species as high (Schrader and Schröder 2013). As a result of the monitoring of *A. leucopoda* in Germany, we report here on the recent range extension into large areas of Germany, map the current distribution in this country and provide new information on host plant choice. The current distribution pattern is discussed and the speed of the range extension is estimated.

Methods

Since 2009, A.D. Liston [ADL] and S.M. Blank [SMB] have paid special attention to elms in eastern Brandenburg, in the expectation that *Aproceros leucopoda* would become established in this region. Searches were made for feeding traces, larvae, cocoons and imagines (Figs 1–2). These were described and illustrated in detail by Blank et al.

(2010). Following the first observations in eastern Brandenburg in May 2013, the frequency of searches was increased and the area in Germany that was searched was extended, covering initially Br [Berlin and Brandenburg], Ni [Lower Saxony, Niedersachsen], Sn [Saxony], St [Saxony-Anhalt] (abbreviations of the German states as used in the checklist of the sawflies of Germany, Blank et al. 2001). In early October 2013, SMB checked the western distribution of *A. leucopoda* while on a round-trip through By [Bavaria], BW [Baden-Wurttemberg], He [Hesse], Ni, NW [North Rhine-Westphalia] and SH [Schleswig-Holstein]. Observations of E. Jansen [EJ] during 2012–2013 primarily covered BW, Sn and St. T. Köhler [TK] contributed observations from Br and MV [Mecklenburg-West Pomerania] in 2013–2014, T. Pfannenstill [TP] and B. Zimmer [BZ] from southern Br in 2013–2014, and A. Taeger [AT] from St in 2014. Usually, presence and absence of *A. leucopoda* on a study site was noted by ADL, EJ, SMB, TK, but only presence was recorded by AT, BZ and TP. To determine presence or absence, elms on randomly chosen sites were screened for feeding traces, larvae, cocoons or imagines from late May to early October. The time spent searching for *A. leucopoda* depended on patch size of an individual elm stand, usually varying from 2–5 minutes for small bushes to 5–10 minutes for large trees. Usually, the number of signs of *A. leucopoda* was noted for a patch, but sometimes the search was stopped as soon as a single sign was detected. ADL, BZ, SMB and TK also determined the elm species or cultivar on the study site. The nomenclature of *Ulmus* taxa follows Mackenthun (2010). Unless attributed to other recorders, the observations were made by the authors. Voucher specimens of larvae and imagines from some localities as well as extensive photographic documentation of occurrences are held at the Senckenberg Deutsches Entomologisches Institut. Statistical analysis of the observation data follows Zöfel (1988).

As a contribution towards encouragement of 'citizen science', an attempt was made to mobilize additional potential observers by placing an illustrated description of *A. leucopoda* and its distinctive feeding traces in the German popular press, with an appeal that observations be reported to the Senckenberg Deutsches Entomologisches Institut in Müncheberg (Bartel 2013). Records sent by citizen scientists as photos or herbarium vouchers were collected and re-identified by ADL and SMB.

For the outbreak area in Schlieben, Brandenburg, N. Neuenfeldt and TP assessed the density of specimens hibernating in cocoons in the ground. Samples of soil and leaf litter were taken from below three elm trees in December 2013. Each sample was from a 5–10 cm deep plot of ca 0.40 m² area. Cocoons of *A. leucopoda* were extracted from a mixed sample of ca 1.8 kg soil and litter and stored outdoors in a tent until imagines emerged.

The distribution map was prepared from a draft map produced by Carto Fauna-Flora 1.2 (Barbier and Rasmont 1996), and subsequently enhanced with Adobe Photoshop® and Corel Draw®. Countries outside Germany with records of *A. leucopoda* are labelled with the international vehicle registration codes. German states are labelled with the acronyms explained above. Distribution data for *Ulmus glabra* and *U. minor* were obtained from FLORKART (BfN and NetPhyD 2013). Free use of these data for the purpose of scientific analyses is licensed under the provisions of Creative

 Only data sets with unambiguous identifications and observation dates later than 1979 were used to display the contemporary distribution of these elms, which have disappeared in some regions of Germany since 1980.

To estimate the speed of annual range extension it seems prudent to compare a number of estimates based on different hypothetical dispersal scenarios. Based on the known records, we measured the distance, *A*, between the earliest records from Hungary and Poland (Blank et al. 2010) and the most distant observation sites in Germany; *B*, between the closest neighbouring sites, where *A. leucopoda* was recorded within the period of a few years; *C*, the minimum and maximum distances between the records in Belgium (Boevé 2014) and the Netherlands (Mol and Vonk 2013) and the closest observation sites in Germany known in 2013. The annual dispersal speed was calculated from these distances and the related years of observation.

Results

Distribution of *A. leucopoda* in Germany

Through mid-July 2014 *Aproceros leucopoda* was recorded from the easterly German federal states of Berlin (recorded from 8 study sites), Brandenburg (39), Mecklenburg-West Pomerania (1), Saxony (5) and Saxony-Anhalt (2) and also from southeast Bavaria (5). These numbers also include data published by Kraus et al. (2012) and Sobczyk and Nuss (2014). At most localities, the presence of *A. leucopoda* was revealed by the conspicuous 'zig-zag' feeding traces (Fig. 1) made by the young larvae. However, older larvae or cocoons also drew attention to its presence, particularly later in the year. Only very few imagines were observed in the field, although several were reared from cocoons collected from the undersides of elm leaves.

The data on the presence and absence of *A. leucopoda* throughout Germany and in the neighbouring countries are mapped in Fig. 3. *Aproceros leucopoda* was not found on 140 study sites, which are distributed in the above mentioned as well as in more western federal states of Germany (Fig. 3, blue crosses). The record by Pimpl (2014) for the Erzgebirge in Saxony is based on a misidentification of *Cladius rufipes* Serville, 1823 (Tenthredinidae, re-identification by SMB). Currently, the following additional records for Germany exist (sorted by federal state and 'Landkreis', a subdivision of a German federal state):

Bavaria: Landkreis Deggendorf: Niederalteich NW 3 km, rest area 'Seebach' on highway A3, 48.788°N, 13.011°E, 315 m alt., 07.08.2013, 3 larvae and feeding traces, *Ulmus* sp. Landkreis Freyung-Grafenau: Irlesberg S 700 m, along federal highway B12, 48.720°N, 13.531°E, 425 m alt., 07.08.2013, 1 larva and feeding traces, *U. minor*. Landkreis Regensburg: Wörth SE, rest area 'Tiefenthal' on highway A3, 48.993°N, 12.420°E, 335 m alt., 07.08.2013, 1 larva and 5 feeding traces, *U.* sp.

Berlin: Friedrichshagen, environs of S-train station, 52.456°N, 13.625°E, 20.05.2013, numerous, partly late instar larvae, *U. glabra*; Kreuzberg, Columbiadamm,

Figure 1. Feeding traces, with young larvae of *Aproceros leucopoda*, on leaf of *Ulmus minor*, Forstbotanischer Garten in Eberswalde (Brandenburg). Photo: SDEI/Liston.

52.483°N, 13.401°E, 29.8.2013, feeding traces, *U.* 'New Horizon' (planted in 2007–2009); Lichterfelde, Botanic Garden, Arboretum, 52.453°N, 13.305°E, 24.07.2013, feeding traces, *U. davidiana* var. *japonica*, *U. minor* 'Webbiana' and *U.* sp. (not *U. laevis*); Lichterfelde, Botanic Garden, Balkan section, 52.453°N, 13.305°E, 24.07.2013, feeding traces, *U. minor* 'suberosa'; Pankow, Märchenweg, along Fließgraben, 52.576°N, 13.475°E, 10.08.2013, 1 feeding trace, *U. minor*; Pankow, Treskowstraße, 52.561°N, 13.429°E, 11.08.2013, 2 feeding traces, *U.* 'Rebona'; Wedding, intersection of Tegeler Straße and Lynarstraße, 52.540°N, 13.358°E, 24.07.2013, feeding traces, *U. pumila* var. *arborea*; Wedding, Utrechter Straße, 52.548°N, 13.355°E, 14.8.2013, feeding traces, *U. pumila* var. *arborea*.

Brandenburg: Landkreis Barnim: Biesenthal ESE, Grüntal, Feldgehölz, 52.740°N, 13.728°E, 16.07.2013, feeding traces, *U. minor*; Eberswalde, Forstbotanischer Garten, 52.825°N, 13.791°E, 30 m alt., 23.06.2013, 30 larvae, *U. minor*. Landkreis Dahme-Spreewald: Brusendorf NW, rest area 'Am Fichtenplan' on highway A10, 52.315°N, 13.497°E, 50 m alt., 07.08.2013, 3 feeding traces, *Ulmus* cultivar with smooth leaves, slender crown form. Landkreis Elbe-Elster: Kolochau, federal highway B87 in direction of Herzberg, 51.716°N, 13.281°E, 27.08.2013, numerous larvae, feeding traces and cocoons, massive infestation, *U.* 'Resista' cultivar; Schlieben in direction of Herzberg, bicycle path from Kolochau for 3 km length, 51.727°N, 13.312°E, 29.07.2013, 3 reared ♀, numerous

larvae, feeding traces and cocoons, massive infestation, *U.* 'New Horizon'. Stadtkreis Frankfurt/Oder: Frankfurt/Oder, Leipziger Straße, Südring Center, 52.328°N, 14.521°E, 05.09.2013, 2 cocoons and feeding traces, 1–5% damage observed on 39 of ca 100 *U.* 'Resista' trees. Landkreis Havelland: Märkisch Luch SW, 52.560°N, 12.602°E, 30 m alt., 10.08.2013, 1 eonymph, *U.* cultivar planted as alley along street; Tremmen, 52.533°N, 12.8167°E, 06.09.2013, weak infestation on *U.* 'New Horizon' (planted 2010) and *U.* 'Rebona' (planted 2009). Landkreis Märkisch-Oderland: Gabow N, 52.820°N, 14.071°E, 14.07.2013, 4 larvae, *U. minor*; Hoppegarten E, Berliner Chaussee, 52.496°N, 14.058°E, 20.05.2013, 15 leaves with feeding traces, 10 early instar larvae, *U. glabra*; Jahnsfelde, 52.507°N, 14.228°E, 19.05.2013, 6 leaves with feeding traces, 3 early instar larvae, *U. glabra*; Müncheberg, car-park of Netto supermarket, 52.506°N, 14.133°E, 18.07.2013, feeding traces, *U.* cultivar ('Columella'?); Müncheberg, railway station, 52.524°N, 14.102°E, 04.07.2013, 3 feeding traces, *U. glabra*; Müncheberg, Waschbanksee, 52.502°N, 14.139°E, 14.07.2013, 5 feeding traces, cultivated *U.* cultivar ('Sapporo Autumn Gold', 'New Horizon' or 'Rebona'?); same site, 15.07.2013, 2 larvae, cultivated *U.* sp.; Müncheberg, ZALF campus, 52.515°N, 14.115°E, 07.06.2013, 1♀ swept from *U. glabra*; same site, 04.07.2013, 2 larvae, *U. glabra*; same site, 20.06.2014, 4 larvae, *U. glabra*; Podelzig, entrance of road to railway station, 52.482°N, 14.538°E, 30.07.2013, 10 feeding traces, *U. minor*; Steinhöfel, alley, 52.400°N, 14.167°E, 30.08.2013, feeding traces, *U.* 'Resista' (planted ca 2008); Waldsieversdorf 2 km SW, road in direction of Rotes Luch, 52.523°N, 14.039°E, 14.08.2013, 1 feeding trace, *U. glabra*. Landkreis Oberhavel: Borgsdorf, S of church, 52.704°N, 13.248°E, 25.06.2014, feeding traces, *U. minor*; Borgsdorf, W of quarry pond, 52.704°N, 13.226°E, 25.06.2014, *U. glabra*; Liebenberg, Fichten, rest area of federal highway B167, 52.890°N, 13.267°E, 14.07.2013, 1 feeding trace, *U. glabra*; Oranienburg, Berliner Straße, 52.723°N, 13.250°E, 26.07.2013, feeding traces, *U. glabra*; Oranienburg, Berliner Straße, in front of Poliklinik, 52.742°N, 13.239°E, 23.07.2013, feeding traces, *U.* 'New Horizon'; Oranienburg, Holbeinstraße, 52.725°N, 13.248°E, 20.07.2013, 3 leaves with feeding traces, *U. minor* (2 m high shoots growing from roots); Oranienburg, Idenstraße, 52.771°N, 13.249°E, 23.07.2013, larva and several feeding traces, *U.* 'Regal'; Zehlendorf, W and S of clay pit, 52.799°N, 13.380°E, 20.07.2013, 1 cocoon, *U. minor* (*U. glabra* and *U. laevis* growing nearby not infested). Landkreis Oberspreewald-Lausitz: Calau SW 6 km, rest area on highway A13, 51.700°N, 13.899°E, 135 m alt., 01.08.2013, feeding traces, two *Ulmus* cultivars (possibly sorts of Resista due to the narrowly cone-shaped crown). Landkreis Oder-Spree: Beeskow, alley, 52.174°N, 14.247°E, 30.08.2013, feeding traces, *U.* 'Resista' (planted 2007); Fangschleuse, S of railway station, 52.402°N, 13.825°E, 20.05.2013, 2 leaves with feeding traces, *U. glabra*; Kagel, along road L 323, 52.467°N, 13.917°E, 04.09.2013, 2 young larvae and feeding traces, *U.* 'Resista' (planted 2010). Landkreis Teltow-Fläming: Ahrensdorf near Ludwigsfelde, 52.317°N, 13.200°E, 01.10.2013, feeding traces, *U.* 'Rebona', observed by K. Langner; Ahrensdorf, alley along street K 7220, 52.195°N, 13.172°E, 02.09.2013, heavy infestation, of 22 *Ulmus* trees (planted ca 2008), late instar larvae still present on 01.10.; Blankenfelde SW 4 km, rest area on highway A10, 52.308°N, 13.369°E, 30.06.2013, 4 feeding traces of early instar larvae, *U. glabra*; Dahlewitz, Friedhofsweg, 52.319°N, 13.436°E, 7.9.2013, feeding traces

on 2 leaves, *U. minor*; Löwendorf, Märtensmühle, 52.204°N, 13.184°E, 27.08.2013, larvae and feeding traces, *U.* sp.; Zossen-Neuhof, Cottbuser Straße, 52.144°N, 13.479°E, 29.08.2013, 2 feeding traces, *U.* sp. Landkreis Uckermark: Prenzlau, Seelübber Weg, 53.299°N, 13.879°E, 11.08.2013, 3 feeding traces, *U. glabra*; Schönermark, railway station, 53.106°N, 14.033°E, 11.08.2013, 2 feeding traces, *U. glabra*.

Mecklenburg-West Pomerania: Landkreis Mecklenburgische Seenplatte: Neustrelitz, intersection of Strelitzer Straße and Bürgerhorststraße, 53.357°N, 13.072°E, 27.08.2013, feeding traces, *U.* 'New Horizon'.

Saxony: Landkreis Leipzig: Leipzig, Leipziger Auen, Weiße Brücke, 51.303°N, 12.356°E, 125 m alt., 27.06.2014, 2 feeding traces, *U.* sp.; Leipzig-Rückmarsdorf, Bienitz, 51.353°N, 12.252°E, 120 m alt., 22.06.2014, 3 feeding traces, *U.* sp. Landkreis Meißen: Wildberg, 51.100°N, 13.588°E, 120 m alt., 01.08.2013, 2 larvae, *U.* sp. (*glabra* or *minor*). Landkreis Nordsachsen: Kathewitz, nature reserve 'Alte Elbe Kathewitz', 51.516°N, 13.111°E, 85 m alt., 22.07.2014, more than 50 *Ulmus* controlled, but only 2 feeding traces of early instar larvae found; Schkeuditz, nature reserve 'Luppeaue', 51.381°N, 12.252°E, 100 m alt, 13.06.2014, 1♀ and 2 feeding traces, *U.* sp.

Saxony-Anhalt: Landkreis Aschersleben-Staßfurt: Westeregeln, 51.960°N, 11.386°E, 06.07.2014, feeding traces, *U.* sp. Landkreis Saalkreis: Sietzsch E, rest area 'Kapellenberg' on highway A9, 51.492°N, 12.204°E, 110 m alt., 07.08.2013, 2 feeding traces, *U. minor*.

The altitudinal range of the 66 study sites in Germany where *A. leucopoda* was observed, varies from 20–425 m above sea level (elevation determined using Google Earth where no original data were available). 51 observations were made below 100 m altitude. These comprise most of the data from Berlin, Brandenburg, Mecklenburg-West Pomerania and Saxony-Anhalt. All five records from Bavaria were at over 300 m altitude. In Austria, *A. leucopoda* was found between 160–580 m altitude (Blank et al. 2010; E. Altenhofer, unpublished data).

The press release by Senckenberg, requesting observations of *A. leucopoda* from citizen scientists (Bartel 2013), was published in more than 300 print and online media primarily in Germany, but also in neighbouring German-speaking countries. During the following months, 23 persons responded by contacting ADL and SMB. Observations by three persons (among them TK and TP) were actually of *A. leucopoda*, whereas those of 20 persons related to other insects, plant species other than elms, or were ambiguous. As a result of the press release we were able to include 17 additional sites in this study, including the report of the severe outbreak in Schlieben and the most northern occurrence in Mecklenburg-West Pomerania, together with observations made on 13 elm species and cultivars, of which five were previously unrecorded as hosts.

Infestation of elm species and cultivars

At several localities all three *Ulmus* species which are autochthonous to Germany were present. Where *A. leucopoda* was found at such places, it was more abundant on *U. minor*

Table 1. Infestation of elm species, varieties and cultivars by *Aproceros leucopoda*: Number of study sites in Germany with [+] or without [–] larvae, larval feeding traces or cocoons attached to the tree. Observations from 2011–2014. Only unambiguously identified elm species, varieties and cultivars are listed. The cultivars 'New Horizon', 'Rebona' and 'Regal' belong to the 'Resista' series.

Species or sort of elm	+	–
Ulmus crassifolia		1
Ulmus davidiana var. *japonica*	1	
Ulmus glabra	12	36
Ulmus glabra 'Pendula'		1
Ulmus × *hollandica*		1
Ulmus laevis		21
Ulmus lamellosa		1
Ulmus minor	11	8
Ulmus minor var. *suberosa*	1	
Ulmus minor 'Webbiana'	1	
Ulmus minor 'Wredei'		1
Ulmus multinervis		1
Ulmus 'New Horizon'	5	
Ulmus parvifolia		1
Ulmus pumila var. *arborea*	2	2
Ulmus 'Rebona'	2	
Ulmus 'Regal'	1	2
Ulmus 'Resista'	6	2

than on *U. glabra*. Also the total number of observation sites with infestations on *U. minor* is significantly higher than that on *U. glabra* (chi-squared test, n = 71 records including varieties and cultivars [Table 1], $\chi^2 = 7.79$, $p < 0{,}01$). Despite careful searches of *U. laevis*, no feeding traces or larvae were found. A very few feeding traces were seen on a single elm labelled "*U. laevis*" in Berlin Botanic Garden growing near other specimens under the same name which bore no traces. However, the tree with the feeding traces possessed some characters which are not typical for *U. laevis*, so there is doubt about its identity.

Aproceros leucopoda was found on several occasions feeding on Resista® elms. These cultivars have not previously been recorded as hosts. No clear pattern of difference in abundance of *A. leucopoda* was observed amongst these cultivars, of which three were identified as *U.* 'New Horizon', *U.* 'Regal' and *U.* 'Rebona'. *Ulmus minor* 'Webbiana' and *U. minor* var. *suberosa* were recorded as new hosts for *A. leucopoda*, and *U. davidiana* var. *japonica* (previous records only from Japan) and *U. pumila* var. *arborea* (previous records only from Hungary) were confirmed as hosts (Table 1). No signs of feeding were found on *U. crassifolia*, *U. glabra* 'Pendula', *U. lamellosa*, *U.* × *hollandica*, *U. multinervis* and *U. parvifolia*, nor on *Zelkova* species (Ulmaceae) partly growing close to *Ulmus*.

At most localities, comparatively low population levels were found, with insignificant damage to the hosts. Elms at such localities were at the edges of woodland or within areas of human settlement, i.e., in more or less sheltered positions. Serious

Figure 2. Feeding damage caused by *Aproceros leucopoda* on planted *Ulmus* 'Resista' during an outbreak between Schlieben and Kolochau (Brandenburg). Photo: LELF/Pfannenstill.

defoliation has so far been recorded in Germany only at Schlieben (Brandenburg, Landkreis Elbe-Elster). Surrounded by open agricultural areas, 235 trees of *U.* 'New Horizon' were planted here as a three kilometre long avenue along a road and a bicycle path in 2012. The trees originated from a nursery in the state of Schleswig-Holstein. *Aproceros leucopoda* could neither be observed in this particular nursery (A. Frers, personal communication) nor at other sites in this state. This outbreak was first detected in 2013 but the infestation was much less severe in 2014. In 2013, damage was unevenly distributed within the plantation. Many trees suffered severe damage, ranging from partial defoliation of twigs to defoliation of most of the crown (Fig. 2). 35 solid-walled

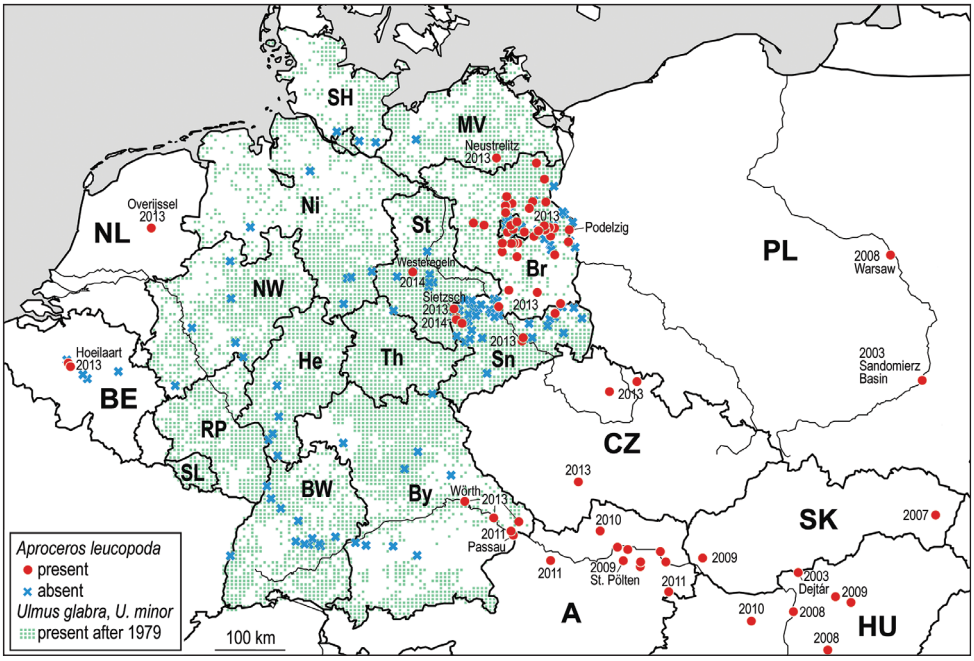

Figure 3. Distribution of *Aproceros leucopoda* in Germany, through 15.07.2014. Modified from Blank et al. (2009), including additional data for Austria (own data, Kraus et al. 2011), Belgium (Boevé 2013), the Czech Republic (own data, Jurášková et al. 2014), Germany (own data, Kraus et al. 2012, Pimpl 2014, Sobczyk and Nuss 2014), Hungary (Haris 2010) and the Netherlands (Mol and Vonk 2013). Distribution of *Ulmus glabra* and *U. minor* in Germany after BfN and NetPhyD (2013). German states are labelled with the abbreviations used in the checklist of the sawflies of Germany (Blank et al. 2001): Br – Brandenburg and Berlin, By – Bavaria, BW – Baden-Wurttemberg, He – Hesse, MV – Mecklenburg-West Pomerania, Ni – Lower Saxony (Niedersachsen), NW – North Rhine-Westphalia, RP – Rhineland-Palatinate, SH – Schleswig-Holstein, SL – Saarland, Sn – Saxony, St – Saxony-Anhalt, Th – Thuringia. Countries outside Germany where *A. leucopoda* is recorded are labelled with the international vehicle registration codes. Graphics: SDEI/Blank.

cocoons were separated from a mixed sample of soil and litter taken from below three elm trees. A density of ca 29 cocoons/m² for overwintering specimens is calculated for this outbreak site. In spring 2014, eight females emerged between April 8–25 under outdoor conditions. A less severe outbreak occurred in Frankfurt/Oder (Brandenburg) in 2013, where 1–5% defoliation was observed on 39 of ca 100 *U.* 'Resista' trees.

Dispersal distances and invasion speed of *Aproceros leucopoda*

In Europe *A. leucopoda* was first found in 2003 in the Sandomierz Basin of southeastern Poland and in Dejtár, northern Hungary (Blank et al. 2010). The following estimates of the invasion speed are based on the distances between presumed sources of subsequent

dispersal represented by the earliest recorded Hungarian and Polish localities and the sites most distant from these where *A. leucopoda* has been observed in Germany:

- Sandomierz Basin (2003) to the most western recorded site, Westeregeln in Saxony-Anhalt (2014): distance 740 km, invasion speed ca 70 km/yr;
- Sandomierz Basin (2003) to the most northern recorded site, Neustrelitz in Mecklenburg-West Pomerania (2013): distance 660 km, invasion speed ca 65 km/yr;
- Dejtár (2003) to the most southeastern recorded site, Passau in Bavaria (2011) (Kraus et al. 2012): distance 440 km, invasion speed ca 55 km/yr.

In the following cases the annual speed of invasion is calculated from the distance between the closest neighbouring sites, where *A. leucopoda* was recorded only a few years later:

- Warsaw in Poland (2008) (Blank et al. 2010) to the closest recorded German site, Podelzig in eastern Brandenburg (2013): distance 450 km, invasion speed ca 90 km/yr;
- St. Pölten in Austria (2009) (Blank et al. 2010) to the most southeastern recorded German site, Passau in Bavaria (2011): distance 170 km, invasion speed ca 85 km/yr;
- from the most southern recorded Bavarian site near Passau (2011) to the most northern recorded Bavarian site, near Wörth (2013): distance 90 km, invasion speed ca 45 km/yr.

In 2013 *A. leucopoda* was observed for the first time in Belgium (Boevé 2014) and the Netherlands (Mol and Vonk 2013). The following are the minimum and maximum distances between these sites and the closest German records known in 2013:

- Overijssel in the Netherlands to Sietzsch in Saxony-Anhalt, Germany: 360 km;
- Hoeilaart in Belgium to Wörth in Bavaria, Germany: 610 km.

Discussion and conclusions

Among the native elm species of Germany, *Aproceros leucopoda* clearly prefers *Ulmus glabra* and *U. minor* as larval hosts (Table 1), since no feeding has been observed on *U. laevis*. On a mixed stand of all three elm species in Lower Austria (Traismauer), *U. laevis* showed a very low infestation even during an outbreak of *A. leucopoda* (E. Altenhofer, personal observation). A number of additional elm species and cultivars must now be considered to be larval hosts. These were introduced to Germany as ornamental trees in horticulture, for afforestation, or planted in botanical gardens for scientific purposes. Feeding could not so far be observed on seven of the taxa listed in Table 1. However, in these cases, it would be premature to conclude that they are not potential hosts, because each of these species and cultivars was studied at only a single site.

Some of the Resista® hybrid elms now prove to be suitable hosts. These cultivars, of complex hybrid parentage, were created to meet the demand for elms which are resistant to the fungal diseases commonly referred to collectively as "Dutch elm disease". *Ulmus* 'New Horizon' and *U.* 'Regal' are at present respectively the most often and the second most often planted elms of this sort in Germany (Mackenthun 2010). They are usually planted in settings where they are highly visible to the public, such as along roads and in city parks. Aside from possible effects on the health of the affected trees, defoliation by *A. leucopoda* will impair their aesthetic value. That 'New Horizon' and 'Regal' are acceptable hosts to *A. leucopoda* is not surprising, because *U. pumila* and *U. japonica* figure prominently in the parentage of both (Mackenthun 2010). *Ulmus pumila* is known to be highly susceptible to attack by *A. leucopoda* (e.g., Wu 2006, Blank et al. 2010, Cao et al. 2011), whilst *U. japonica* is a known host in Japan (Blank et al. 2010) and recorded here for the first time as a host in Europe.

The range of *A. leucopoda* in Germany currently comprises two separate areas (Fig. 3). The northern one extends from southern Mecklenburg-West Pomerania in the north to central Saxony in the south and from central Saxony-Anhalt in the west to the Polish border in the east. The second, southern area covers part of southeast Bavaria. These two large areas of occurrence do not at present appear to be confluent. The distribution of *U. glabra*, one of the two preferred native elm species, covers the low mountain ranges of central Germany (BfN and NetPhyD 2013). Although autochthonous *U. glabra* and *U. minor* have disappeared in several regions (BfN and NetPhyD 2013), their combined, largely continuous distribution throughout Germany (Fig. 3) provides the opportunity for *A. leucopoda* to spread further into more westerly parts of the country.

The northern distribution area of *A. leucopoda* in Germany is mostly within the North German Plain, which in the south is delimited by the low mountain ranges of central Germany. Judging from available distribution data (Blank et al. 2010, Mol and Vonk 2013, Boevé 2014, Sobczyk and Nuss 2014, Juraskova et al. 2014, present data), *A. leucopoda* has been recorded only rarely above 400 m altitude in central Europe. From Austria into Bavaria, *A. leucopoda* has spread along the valley of the river Danube, where *A. leucopoda* was found at a maximum altitude of 580 m near Zwettel, Lower Austria (E. Altenhofer, personal communication). The Alps to the south and the Bohemian Massif to the north of the valley possibly work as barriers. Future observations may reveal whether the low mountain ranges of central Germany will slow down or restrict the dispersal of *A. leucopoda*, despite the general presence there of at least *U. glabra*.

Apart from range expansion through self-dispersal of imagines, it seems likely that individuals can sometimes be accidentally transported by human agency over much greater distances with road, rail, air and canal traffic (Blank et al. 2010). Trade by nurseries of infested plants throughout a wide geographic area might also have a significant impact, but we could not observe such an event involving *A. leucopoda*. The occurrences in Belgium (Boevé 2014) and in the Netherlands (Mol and Vonk 2013) seem likely to belong in the category of human-mediated jump dispersal (Suarez et al.

2001), because the gap of 360–610 km between these western records and the records in eastern Germany was unsuccessfully searched by us for signs of *A. leucopoda* in 2012 and 2013 (Fig. 3). Contrarily, the two observed distribution areas in Bavaria and in the northeastern German states should be explained by self-dispersal of *A. leucopoda* originating from the neighbouring countries Austria, Poland and possibly the Czech Republic, although human-mediated jump dispersal cannot be ruled out as a component within self-dispersal. Records made during consecutive years in Austria indicate a stepwise spread in a westerly direction (Fig. 3). Although comparable data from western Poland are missing, we suppose that self-dispersal also took place here, because the speeds of annual dispersal calculated from the distance between the Polish and the eastern German records (65–90 km/yr) are similar to speeds calculated from Austrian, Hungarian and Bavarian records (45–85 km/yr). The common distribution area of the preferred native larval hosts, *U. glabra* and *U. minor*, is more or less continuous from Germany to Hungary and to Poland (Meusel et al. 1965, BfN and NetPhyD 2013), which has facilitated the self-dispersal of *A. leucopoda* in a westerly direction.

The 2013 observations from southeastern Bavaria indicate an apparently limited speed of range extension (45 km/yr) in that area since *A. leucopoda* was first recorded near Passau in 2011 (Kraus et al. 2012), although the total of study sites in Bavaria is comparatively low. Contrastingly, range expansion into northeastern Germany seems to have been both more rapid and extensive. At the majority of German localities, a low number of feeding traces made by young larvae were often the only signs that the species was present, perhaps indicating that colonisation had recently taken place. The inconspicuous damage may also have been a reason for the low number of replies by citizen scientists in response to our press release in 2013 (Bartel 2013). If the spread of *A. leucopoda* into eastern Germany is nevertheless assumed to have depended purely on natural dispersal of imagines, the distance (ca 220 km) between the most westerly recorded locality (Westeregeln) and the Polish border (Podelzig) indicates that the first female may already have arrived in northeastern Germany two to five years before 2013.

Very little is known about the dispersal ability of sawflies and horntails (Hymenoptera 'Symphyta') in general, or the time scales and distances associated with the spread of individual species undergoing range expansion. The speed of annual range expansion resulting from self-dispersal of 45–90 km/yr estimated here for *A. leucopoda* lies within the range known for a few sawflies and for other insects. *Nematus oligospilus* Förster, 1854 (Tenthredinidae), a sawfly species invasive to New Zealand with larvae feeding on willow, extended its range through the North Island at 300 km/yr (Charles and Allan 2000; ca 165 km/yr measured as a straight line). In South America Ovruski and Fidalgo (1991) observed a range expansion of 300 km/yr also for *N. desantisi* D.R. Smith, 1983, which might be conspecific with *N. oligospilus* from New Zealand (Koch and Smith 2000). Ovruski and Fidalgo (1991) supposed passive transport to be a possible component of the dispersal. *Gilpinia hercyniae* (Hartig, 1837) (Diprionidae) is invasive to the Nearctic, where its larvae damage spruce trees (*Picea* spp.). The speed of range expansion in Canada is given as 50 km/yr (CABI 2014). The average speed for *Sirex noctilio* Fabricius, 1793 (Siricidae), a woodwasp invasive in South America, has

been estimated as 30–50 km/yr (Yemshanov et al. 2009). Smith (1996) summarized distribution data for the invasive Asian woodwasp *Eriotremex formosanus* (Matsumura, 1912), which in the United States was first observed in southern Georgia and in northern Florida in 1974. *E. formosana* was found 15 years later in southeastern Virginia, about 740 km distant from these earliest sites (ca 50 km/yr) and 16 years later in eastern Texas, 990 km distant (ca 60 km/yr). However, *S. noctilio* and *E. formosanus* differ greatly from the *Aproceros*, *Gilpinia* and *Nematus* species in several ways, e.g., in the large body and the larval feeding habit inside solid wood. *Sirex noctilio* reproduces sexually and usually produces at most one generation per year (Eichhorn 1982), whereas *E. formosanus* is apparently parthenogenetic in the US and supposedly has two generations per year, based on phenological data (Smith 1996). Compared to the range expansion of 30–60 km/yr resulting from self-dispersal of these woodwasps, not even the highest estimated speed of 90 km/yr for *A. leucopoda* seems excessive, because several life traits of *A. leucopoda* promote rapid dispersal: it reproduces parthenogenetically, has up to four generations per year, and the larvae are external feeders on leaves (Blank et al. 2010), which appear more nutritious than wood infested by fungi. Among insects other than Hymenoptera, Brown et al. (2008) calculated an invasion speed of 58–145 km/yr for *Harmonia axyridis* (Pallas, 1771) (Coleoptera, Coccinellidae) in Europe. The considerably higher speed of 500 km/yr estimated for this species in South Africa (Stals 2010) probably includes jump-dispersal events. The speed of range expansion in *Cameraria ohridella* Deschka & Dimić, 1986 (Lepidoptera, Gracilariidae) in central and western Europe was estimated to be 60–70 km/yr based on the dates of first observations in European countries (Šerfová and Laštůvka 2001), although these authors also cite much lower speeds for other moths. For *C. ohridella* both active dispersal and passive transport by wind and man were considered to play a role.

The rapid range expansion of *A. leucopoda* throughout Europe predicted by Blank et al. (2010) is evidently taking place, and likely to progress. Although occurrences in Germany have so far seldom resulted in severe defoliation, this may only be because population levels still have not peaked. The considerable differences in climate between the European territories which it has already colonised, together with the altitudinal range inhabited by the species, suggest that it should easily be able to spread through most of Europe where elms grow (Meusel et al. 1965). Whether it is possible in the long-term to exclude it from territories such as the British Isles or Scandinavia, which may be adequately protected by the sea from self-dispersal events, remains to be seen.

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RESEARCH ARTICLE

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Nesting biology of an Oriental carpenter bee, *Xylocopa* (*Biluna*) *nasalis* Westwood, 1838, in Thailand (Hymenoptera, Apidae, Xylocopinae)

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Abstract

The biological study of wild non-*Apis* bees can provide useful information that may help with the pollination of food crops and native plants in areas where the keeping of honey bee colonies is restricted or affected by CCD. Here, we describe the nesting biology of the Oriental large carpenter bee, *Xylocopa* (*Biluna*) *nasalis* Westwood, 1838. An aggregation of more than 80+ bamboo nests of *X. nasalis* was discovered in Suan Pheung district, Ratch Buri province, Thailand on the 25th of May 2012. We collected 27 nests from the site to dissect, measure the external and internal nest architecture, and analyze the pollen composition of the pollen masses. *X. nasalis* constructs linear unbranched nests with nest entrance mostly located at the open-end of the bamboo culms. The nest length and the branch diameter of the nest entrance (excluding nesting edge) are 25.40 ± 6.95 cm and 17.94 ± 6.00 mm, and the maximum number of provisioned cells is 8. A biased sex ratio of 8♀: 1♂ is reported, with up to 7 adults inhabiting in a single nest. 29 pollen types were identified from 14 pollen masses using an acetolysis method and visualization under both light microscope and scanning electron microscope. 13 pollen types were considered as major pollen sources (contribute ≥ 1% in total pollen volume); however, only 10 can be identified to family and generic levels. The dominant pollen sources are of the families Elaeagnaceae (*Elaeagnus* cf. *latifolia*), Euphorbiaceae (*Croton*), Fabaceae (*Senna siamea* and *Cassia*), Fagaceae (*Lithocarpus* and *Castanopsis*), and Lythraceae (*Trapa*) which are mostly native to the region of Southeast Asia. The nesting architectural details should prove to be beneficial to beekeepers and researchers who are interested in trapping and studying *X. nasalis*, and the polylectic behavior of *X. nasalis* can be highly valuable for future crop pollination strategies, particularly for plants that require sonication of their poricidal anthers.

Keywords
Carpenter bee, nesting biology, Thailand, bamboo, pollen

Introduction

Because of the declining honey bee population worldwide resulting from the condition known as Colony Collapse Disorder (CCD; Oldroyd 2007, van Engeldorp et al. 2008, Ratnieks and Carreck 2010), the use of widespread pesticides (Hopwood et al. 2012), climate changes (Bartomeus et al. 2011), and the increase in monotonous agricultural landscapes that reduce the biodiversity and the availability of foods for bees, the study of wild and/or domesticated non-*Apis* bees can provide useful information for complementary bee species that may help with the pollination of food crops in areas where keeping of honey bees colonies are being affected or restricted (Chagnon et al. 1993, Wilmer et al. 1994, Javorek et al. 2002, Hoehn et al. 2008, Brittain et al. 2013). Until now, only a handful of non-*Apis* bee species have been used extensively in agriculture, e.g., *Bombus terrestris* (Linnaeus, 1758), *Megachile rotundata* (Fabricius, 1787), *Nomia melanderi* Cockerell, 1906, *Osmia rufa* (Linnaeus, 1758), and some stingless bee species (Westerkamp and Gottsberger 2000, Hogendoorn et al. 2006, Greenleaf and Kremen 2006, Slaa et al. 2006, Hoehn et al. 2008). These bees have been shown to be effective pollinators, as good as, if not better than, honey bees on certain crop plants (Greenleaf and Kremen 2006).

The large carpenter bees of the genus *Xylocopa* Latreille, 1802 (Hymenoptera; Apoidea) have recently received attention due to their pollination capabilities. The use of large carpenter bees to assist with pollination of greenhouse tomatoes and honeydew melons in Australia and Israel has been reported (Hogendoorn et al. 2000, Sadeh et al. 2007, Keasar 2010). In Brazil, where passion fruits are one of the main exported fruit crops of the nation, studies of using *X.* (*Neoxylocopa*) *grisescens* Lepeletier, 1841 and *X.* (*N.*) *frontalis* (Olivier, 1789) to pollinate the flowers, instead of using manual labor, have shown promising results in increasing the fruit sets and quality and reducing the production costs (Junqueira et al. 2012, Yamamoto et al. 2012).

Carpenter bees can be found throughout the tropical and subtropical parts of the world (Hurd and Moure 1963, Gerling et al. 1989). These are large and robust bees that many novices regularly confuse with bumble bees (*Bombus*) due to their similar sizes and shapes. There are currently ca. 470 species described with 32 subgenera recognized in a single genus (Michener 2007, Ascher and Pickering 2013). Most *Xylocopa* species are known to excavate their nests in dead or decaying woods, with the exception of the subgenus *Proxylocopa* Hedicke, 1938 which excavates nests in the soil (Gottlieb et al. 2005). There are two main types of nests among the wood-nesting *Xylocopa* species: (1) unbranched or linear nests in which the tunnel runs in the same direction as the nest entrance or at most with a single right angle corner from the nest entrance

and (2) branched nests which consist of at least two tunnels or more although with only one nest entrance (Gerling et al. 1989).

One subgenus of Oriental *Xylocopa*, *Biluna* Maa, 1938, comprises five to nine species (Michener 2007, Ascher and Pickering 2013). Its distribution ranges from India and Sri Lanka to Southeast Asia and Japan. Species of *Biluna* are only known to construct unbranched nest in bamboo culms (Maa 1946, Maeta et al. 1985; Hurd and Moure 1963). *Xylocopa* (*Biluna*) *nasalis* Westwood, 1838, is a species commonly found throughout Southeast Asia. It superficially resembles the sympatric species *X.* (*Mesotrichia*) *latipes* Drury, 1773 and *X.* (*M.*) *tenuiscapa* Westwood, 1840 because of the presence of black pubescence on the mesosoma and their large size (21–35 cm in length). Males of *Biluna* lacks both a basitibial plate and a spine on the outer apex of the hind tibia, while the females have a dense mat of short setae on the middle tibia and lack an apical middle tibial spine. The behavior, biology and natural history of *X. nasalis*, is poorly known even though it is commonly found throughout rural and agricultural areas in Southeast Asia. Boontop et al. (2008) briefly described the nesting biology of *X. nasalis* studied in Kasetsart University, Kamphaengsaen Campus, Nakhonpathom province, Thailand, though their account lacks many nest architectural details and, importantly, the palynological data on plant food sources. Here, we extend Boontop et al. (2008)'s work via reporting the finding of a nest aggregation of *X. nasalis* in Suan Pheung district, Ratch Buri province (~100 km southeast of Nakhonpathom province), Thailand, along with details of other nest architectural components, its floral preferences, and some behavioral observations at the nest entrance. We anticipate that by providing such detailed nesting biology and pollen food sources of a local large carpenter bee from an area with poorly known mellitological data (such as Southeast Asia), it will stimulate interest and provide practical information for local bee keepers and bee researchers to consider *Xylocopa* to be an important native pollinator for certain crops and endemic plants in the near future.

Methods

Nesting site and nest dissections

We discovered a nesting aggregation of *Xylocopa nasalis* in a makeshift roof structure (Figure 1; 80+ nests) made from bamboo culms (tribe Bambuseae) at a local restaurant in Suan Pheung district, Ratch Buri province, Thailand (13°33'32.4138"N and 99°21'32.3202"E) on the 25th of May 2012. The collecting of the nests was done in the early month of the Monsoon season in Thailand, when flowers were abundantly blooming. Almost 95% of the bamboo culms were occupied by *X. nasalis*. We also observed some unidentified megachilids using some of the bamboo culms for nesting as well. The bamboo culms ranged in size of outer diameter ≈ from15.80 to 29.39 mm and 3.00 to 3.25 m in length (n = 27) and were situated approximately 2.50 m above the ground. We collected 27 nests (with all of the bee inhabitants) by plugging the nest

Figure 1. Nesting habitat of *X. nasalis*; A nesting habitat of *X. nasalis* on a makeshift roof of a restaurant in Suan Pheung district, Ratch Buri province, Thailand. The red arrows indicate locations where the bamboo culms were arranged ca. 2.50 m above the ground (**1a** and **1b**). At the nest entrance, the female of *X. nasalis* was dehydrating the nectar previously foraged (**1c**).

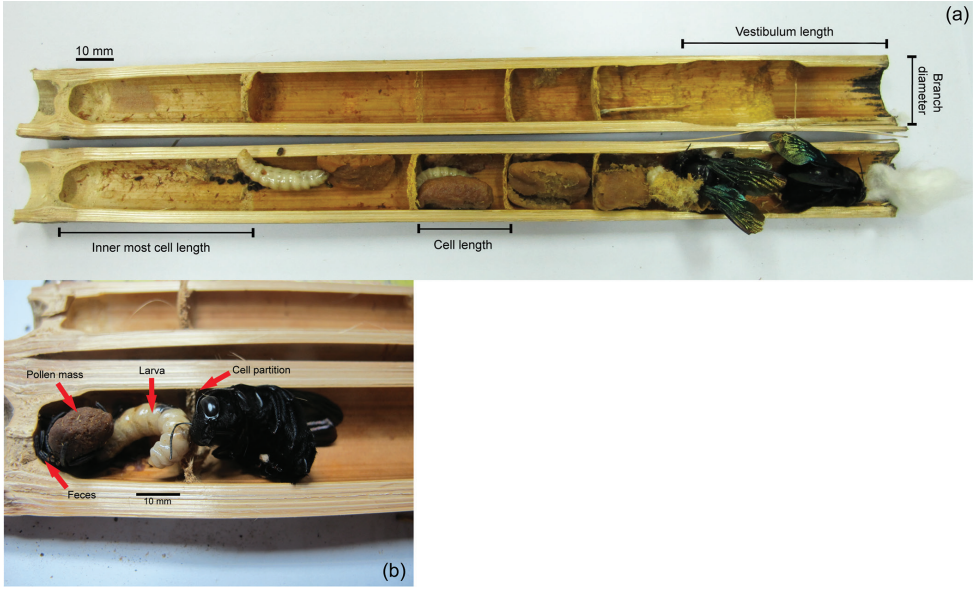

Figure 2. Nesting architecture of *X. nasalis*; Dissected nests of *X. nasalis* revealing the nest structure inside the bamboo culm and its residents. Measurements of the nest parameters are shown in Table 1. The diameters of the nests (excluding the nest thickness) were measured at the nest entrance, followed by the vestibulum (antechamber) length, cell length, and the inner most cell length, respectively (**2a**). Cells containing larvae with pollen masses and their feces were collected and weighted (**2b**).

entrances with cotton balls and sealing them with duct tape. Nests were brought back to the Department of Biology, Chulalongkorn University, Thailand, and preserved in a -20 °C freezer for later dissection. We dissected each nest and recorded the following

data: measurements of the external and internal nest structures using a vernier caliper and tape measurement (Figure 2), numbers of individuals at different life-stages, numbers of provisioned cells, fresh weights of the pollen balls in each cell, feces' weights, and sex ratio of adults. Since the data on *X. nasalis* cell lengths cannot be assumed to be drawn from any given probability distribution, the non-parametric Kruskal-Wallis test was employed to test whether there are differences among the average cell lengths of 54 cells measured; the Mann-Whitney U test was used to test the difference between the average inner most cell length of all 27 nests and the average cell lengths. The two statistical tests were performed using the program SPSS ver. 20.0 (IBM Corp. 2001). Fourteen pollen balls from six nests were collected for pollen analysis. All remaining nests were sketched and photographed. All specimens (including eggs, larvae, and pupae) were preserved in 95% Ethanol and deposited at the Natural History Museum of Chulalongkorn University, Bangkok, Thailand for future genetic analyses.

Pollen analyses

For pollen analysis, we employed the acetolysis method (Erdtman 1960) with a modification at the end of the process where we re-suspended the decanted pollen samples in a benzene solution and topped up with silicone oil in a vial. The pollen-benzene solution was air-dried for one week or until the benzene solution was completely evaporated leaving only the pollen samples preserved in silicone oil for examination under light microscope (Olympus CH-BI45-2). For preparing the pollen pictures to be captured and identified under a scanning electron microscope (Hitachi Tabletop Microscope TM-100), an additional step was performed before pollen samples were re-suspended in benzene solution: 70% ethanol was added and the pollens were mixed in the solution, the pollen was pelleted by centrifugation, and the ethanol was then discarded (we sometimes repeated additional steps with 95% and absolute ethanol).

Before counting the pollen grains, we mixed the vial containing pollen grains submerged in silicone oil to obtain a homogenous pollen suspension. Ten drops of the pollen suspension were removed and placed on microscopic slides and each aliquot was spread to an area of ca. 30 × 30 mm. Three slides per pollen mass were used for the examination. We counted 300 pollen grains for each slide, which provide a total pollen count of 900 grains from a single pollen mass. Since there is no published exhaustive key for the pollens endemic to western Thailand, we were limited in resources to accurately identify most pollens to the specific level. We followed the pollen identification guides from various authors whose works were on the melittopalynology of the Asian Tropics, i.e., Huang (1972), Tissot et al. (1994), Nagamitsu et al. (1999), and Jongjitvimol and Wattanachaiyingcharoen (2006), which allowed us to identify most pollen types to family and genus. We also identified the plant food sources in the area of western Thailand using published botanical keys provided by Hanum and van der Maesen (1977), Gardner et al. (2000), Smitinand (2001), and Phengklai (2006), to

corroborate with our pollen data. The plant classification system of the Angiosperm Phylogeny Group (2009) was followed.

Since pollens are diverse in their shapes and sizes, to accurately identify which pollen type contributes the most to the bee diets, one should not depend only on the most number of grain counts alone. Buchmann and O'Rourke (1991) suggested weighing the volume of pollens with the percentage of the pollen counts to achieve a more reliable estimation of the type of pollens that contribute to the pollen masses. To obtain the volumes of each pollen type, we measured the longitudinal axis (p) and equatorial axis (e) lengths from 30 grains of each pollen type then calculated the mean values. Pollen dimensions are categorized into two types: spherical and elliptical forms. The following formulas were used for the calculation of the pollen volumes: spherical form = $1/6\pi p^3$ and elliptical form = $1/6\pi e^2 p$. Contributing pollen types were subjectively categorized into two groups – the "major" and "minor" pollen sources based on their percentage of total volume. The major pollen sources are defined as contributing in *X. nasalis* diet ≥ 1% of the total pollen volume, whereas minor pollen sources are those that are accounted < 1% of the total pollen volume.

We also observed some behaviors exhibited by the bees on the day before we collected the bamboo nests. These behaviors were related to their nesting habits and are briefly discussed in the next section.

Results

Nest architecture and contents

Nests of *Xylocopa nasalis* nest are strictly unbranched. The provisioned cells are separated by partitions made from bamboo particles excavated by the founding female. All of the nest entrances are located at the end of the bamboo culms, except for a couple of nests that the bees excavated from the undersides. A summary of nest architectural details is provided in Table 1. The average total nest length (including the vestibulum (antechamber) length) is 38.35 cm. The average nest length (measured from the nest entrance to the end of the innermost cell) is ca. 25.40 ± 6.95 cm. The mean branch diameter of the nests (excluding nest thickness) is 17.94 ± 6.00 mm. The number of cells per nest ranged from 0–8 cells with an average cell number around 3 per nest. There is a difference in terms of the average individual cell lengths among cells from 27 nests (χ^2 = 28.11, p = 0.021), though the significance value is fairly weak. On the contrary, the innermost cell lengths were tested to be strongly different from other cells (U < 0.0001, p<0.0001). The average number of individual adult bees found per nest ranged between 1 and 7 individuals (mean ± s. d.: 3.24 ± 1.90) with a sex-ratio bias of 7.98♀: 1♂. We found the average number of pupae and post-defecating larvae: larvae: eggs as follow 1.15: 0.69: 0.04; however, we did not find any nest that had all life stages of the bees present at once. Three of the 27 nests contained eggs; the mean fresh weight of their

Table 1. Nesting structure measurements of *X. nasalis*; Summary of the measurements of nesting architecture of *X. nasalis* (n = 27) from Suan Pheung district, Ratch Buri province, Thailand (13°33' 32.4138"N and 99°21'32.3202"E).

Nest Characters	Ranges (Mean ± S.D.)	Max.	Min.
Vestibulum length (cm)	12.95 ± 67.80	31.00	5.04
Nest length (cm)	25.40 ± 6.95	36.25	10.00
Number of cells / nest	2.83 ± 2.55	8	0
Inner most cell length* (mm)	32.75 ± 11.06	55.30	15.00
Individual cell length (except *) (mm)	23.25 ± 3.88	41.00	17.00
Branch diameter at nest entrance (mm)	17.94 ± 6.00	30.70	11.00
Nest thickness (mm; measured at the entrance)	4.66 ± 0.79	6.80	3.30
Partition thickness (mm)	0.88 ± 0.27	1.60	0.50
Pollen weight / cell (g) (n = 3)	1.37 ± 0.13	1.52	1.20
Feces' weight / cells (g) (n = 6)	0.24 ± 0.23	0.86	0.01
Number of adult individuals	3.24 ± 1.90	7	1
Number of female adults	3.19 ± 2.04	7	1
Number of male adults	0.40 ± 0.70	2	0
Number of pupa and post-defecating larva	1.15 ± 2.41	7	0
Number of larva	0.69 ± 1.38	5	0
Number of eggs	0.04 ± 0.19	3	0

unconsumed pollen masses was 1.37±0.13 g (n = 3). The average weight of the feces in the cells of post-defecating larvae averaged 0.24 ± 0.23 g (n = 6).

Pollen analyses

A total of 29 pollen types were identified from the 14 pollen masses. We were able to identify pollen grains from 13 families, including 12 identifiable genera (Table 2). For three of the 13 plant families – Anacardiaceae, Araceae, and Cyperaceae – generic level identification could not be confirmed. Brief descriptions of the 14 unidentified pollen types are also given in Table 2. We consider 13 pollen types as "major" pollen sources, whereas the other 16 pollen types are considered as "minor" pollen sources based on their percentage total volumes of the diets (Table 2, Figure 3).

For the 13 pollen types classified as major pollen sources, we were able to identify 10 pollen types to their generic level and 2 of these to species (*Elaeagnus* cf. *latifolia* Linnaeus and *Senna siamea* (Lam.) Irwin et Bradley). These include the family Acanthaceae (*Thunbergia*; 2.35%), Anacardiaceae (genus unknown; 4.68%), Elaeagnaceae (*E.* cf. *latifolia*; 12.88%), Euphorbiaceae (*Croton*; 14.95%), Fabaceae (*Cassia*; 12.17% and *S. siamea*; 12.91%), Fagaceae (*Lithocarpus*; 7.65% and *Castanopsis*; 3.22%), Lythraceae (*Trapa*; 13.36%), and Theaceae (*Schima*; 6.42%). Three pollen types (all are < 3% of total pollen volume) remain unidentified at any level (under "Unknowns" in

Table 2. Pollen type amount and volume foraged by *X. nasalis*; Percentage pollen grain count and percentage pollen volume frequently encountered from 14 pollen masses collected by *X. nasalis* from 6 nests. For each pollen mass, 900 pollens were counted (total of 12,600 pollen grains).

Family Genus/Species	Grains counted	Approximate geometric figure of pollen	p	e	v	Percentage of pollen grains*	Total pollen volume by taxon** ($\times 10^{-7}$ cm^3)	Percentage of total pollen volume***
FAGACEAE								
Lithocarpus	3 310	Elliptic	17.13	23.02	4.71	26.43	155.57	7.65
Castanopsis	1 724	Elliptic	15.05	22.01	3.8	13.77	65.51	3.22
FABACEAE								
Senna siamea	1 045	Elliptic	30.09	40.11	25.12	8.35	262.5	12.91
Cassia	751	Elliptic	32.53	44.09	32.93	6	247.3	12.17
ELAEAGNACEAE								
Elaeagnus cf. latifolia	1 857	Elliptic	22.14	34.9	14.1	14.83	261.84	12.88
LYTHRACEAE								
Trapa	655	Elliptic	33.04	49.1	41.47	5.23	271.63	13.36
THEACEAE								
Schima	576	Elliptic	30.17	37.98	22.67	4.6	130.58	6.42
ANACARDIACEAE	369	Elliptic	34.93	37.57	25.76	2.95	95.05	4.68
EUPHORBIACEAE								
Croton	349	Sphere	55.01	NA	87.07	2.8	303.87	14.95
JUNGLANDACEAE								
Engelhardtia	111	Elliptic	20.87	19.23	3.97	0.87	4.41	0.22
ARACEAE	63	Elliptic	27.44	42.56	25.99	0.5	16.37	0.81
RHAMNACEAE								
Ziziphus	60	Sphere	27.51	25.02	4.19	0.48	2.51	0.12
ACANTHACEAE								
Thunbergia	48	Sphere	57.5	NA	99.5	0.38	47.76	2.35
CAPRIFOLIACEAE								
Sambucus	37	Elliptic	19.98	30.03	9.42	0.3	3.49	0.17
CYPERACEAE	13	Half sphere	25.00	NA	4.09	0.1	0.53	0.03

Family Genus/Species	Grains counted	Approximate geometric figure of pollen	p	e	v	Percentage of pollen grains*	Total pollen volume by taxon** (× 10^{-7} cm^3)	Percentage of total pollen volume***
UNKNOWNS								
Triangular, tripolate	595	Elliptic	27.44	25.08	8.99	4.75	53.49	2.63
Irregular shape, inaperture	368	Half sphere	20.1	NA	2.09	2.94	7.69	0.38
Monolete	226	Elliptic	24.98	42.57	23.63	1.8	53.4	2.63
Three furrows, triangular, tricoplate	185	Elliptic	20.03	22.51	4.71	1.48	8.71	0.43
Three furrows, tricoplate	180	Sphere	35.02	NA	22.44	1.44	40.39	1.99
Oblate, two pores fused, monolete	78							
Inaperture								
Triangular, inaperture								
Triporate								
Triangular, tripolate								
Fenestrated								
Inaperture								
Oblate, triangular, fenestrated								
Three bladders, vesiculate								
	12 600					100	2032.6	100

p: mean longitudinal axis (µm); e: mean equatorial axis (µm); v: mean individual grain volume (× 10^{-9} cm^3)

* Percentage of pollen grains was calculated excluding the 78 unknowns pollen grains (thus the total number of grain for calculation was 12,522)

** Total pollen volume by taxon was calculated by multiplying the number of pollen grains by the mean individual grain volume

*** Percentage of total pollen volume was calculated excluding the 78 unknowns pollen grain volumes (thus the total pollen volume was 2032.60 × 10^{-7} cm^3)

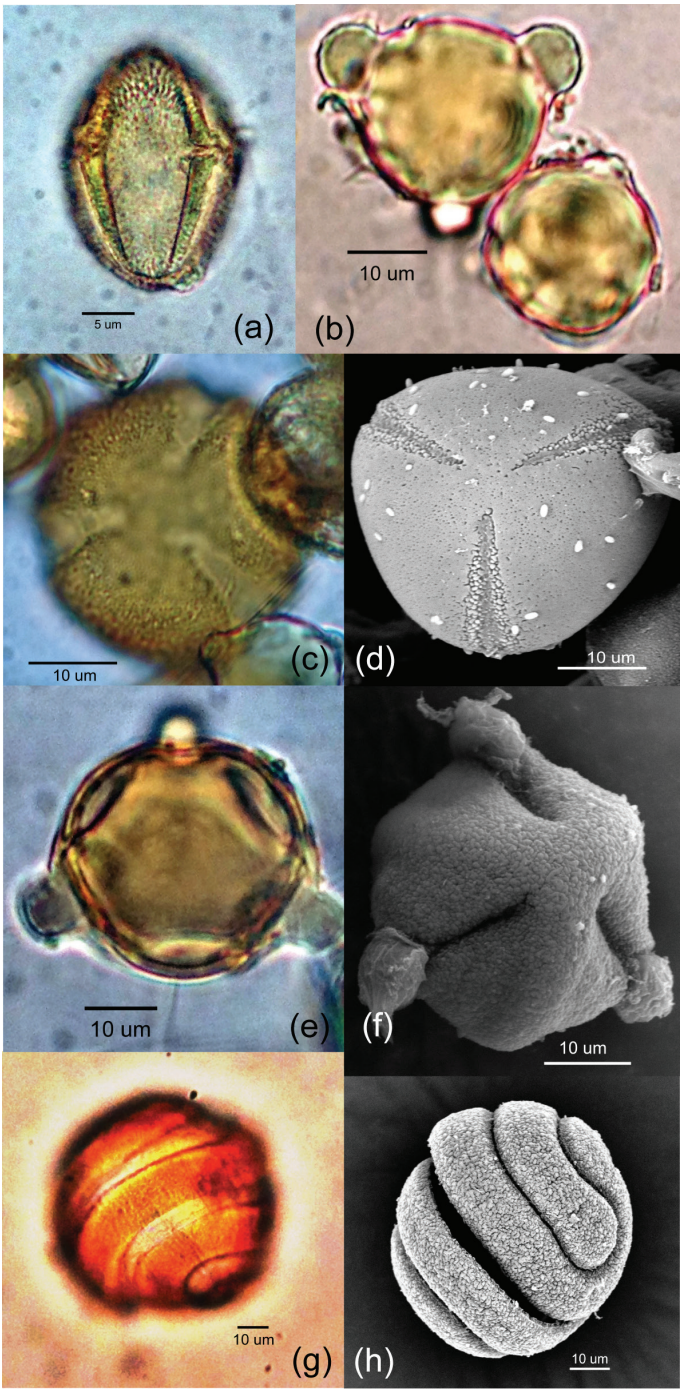

Figure 3. Pollen grains collected by *X. nasalis*; Some representations of pollens collected from pollen masses of *X. nasalis*. The "major" pollen sources: Fagaceae, *Castanopsis* sp. (**3a**); Elaeagnaceae, *Elaeagnus* cf. *latifolia* (**3b**); Fabaceae, *Cassia* sp. (**3c** and **3d**), *Senna siamea* (**3e** and **3f**); Acanthaceae, *Thunbergia* (**3g** and **3h**).

Table 3. Dominant pollens foraged by *X. nasalis*; Dominant pollen types from 14 pollen masses determined by the highest percentage pollen grain count and percentage of pollen volume (sequential order starting from the pollen mass number in the inner most cell (#1) proceeding to the nest entrance).

Nest Number/ Pollen Mass number	Family: Genus/Species	Percentage of pollen grains	Percentage of pollen volume
1/1	FAGACEAE: *Lithocarpus*	83.3	52.6
1/2	FAGACEAE: *Lithocarpus*	70.1	54.5
1/3	FAGACEAE: *Castanopsis*	55.5	15.7
	FABACEAE: *S. siamea*	22.1	41.5
1/4	ELAEAGNACEAE: *E.* cf. *latifolia*	56.4	87.7
1/5	ELAEAGNACEAE: *E.* cf. *latifolia*	76.5	65.1
2/1	ANACARDIACEAE	33.6	49.9
3/1	ELAEAGNACEAE: *E.* cf. *latifolia*	31.1	50
3/2	FAGACEAE: *Lithocarpus*	63.8	54.9
4/1	FABACEAE: *S. siamea*	47.0	73.8
4/2	ARACEAE	11.3	23.5
4/3	EUPHORBIACEAE: *Croton*	18.2	63.6
5/1	LYTHRACEAE: *Trapa*	49.6	74.8
5/2	FAGACEAE: *Lithocarpus*	45.5	37.3
	THEACEAE: *Schima*	14.1	55.7
6/1	FAGACEAE: *Lithocarpus*	45.2	12.2
	EUPHORBIACEAE: *Croton*	8.4	42.1

Table 2). Minor pollen sources that can be identified are of the families Araceae (genus unknown; 0.81%), Caprifoliaceae (*Sambucus*; 0.17%), Cyperaceae (genus unknown; 0.03%), Juglandaceae (*Engelhardtia*; 0.22%), and Rhamnaceae (*Ziziphus*; 0.12%); whereas nine minor pollen types could not be identified.

The dominant pollen types based on both the highest percentage pollen type amount and total percentage of pollen volumes for each of the 14 pollen masses is displayed in Table 3. Eight different families of plants were found to be the dominant contributor to the 14 pollen masses based on the highest total percentage of pollen volumes – Anacardiaceae (genus unknown), Araceae (genus unknown), Elaeagnaceae (*Elaeagnus* cf. *latifolia*), Euphorbiaceae (*Croton*), Fabaceae (*Senna siamea*), Fagaceae (*Lithocarpus*), Lythraceae (*Trapa*), and Theaceae (*Schima*).

Not only does *Xylocopa nasalis* display polylecty as indicated by results of the pollen analyses in its foraging behavior, but each female also exhibited a broad host plant range when foraging for pollen. Table 4 shows a foraging female that utilized 13 different pollen types to construct 5 pollen masses in a single nest, with the dominant pollen source for each pollen mass changing over time, e.g., Cells 1 and 2 are dominated by *Lithocarpus*, whereas Cell 4 and 5 are dominated by *Elaeagnus* cf. *latifolia*. *Castanopsis* pollens are found throughout all five pollen masses.

We also observed some notable nest-entrance behaviors by the bees. Competition for nests seemed to be very high at the nest site despite the abundance of available bam-

Table 4. Pollen composition from a single nest of *X. nasalis*; Pollen composition from a single nest (nest #1; Table 3) of *X. nasalis*. **P** represents a percentage of the given pollen grains in a pollen mass, whereas **V** represents a percentage of the pollen volume. Cell numbers are arranged from as in Table 3.

Family:	**Cell 1**		**Cell 2**		**Cell 3**		**Cell 4**		**Cell 5**	
Genus/Species	**P**	**V**	**P**	**V**	**P**	**V**	**P**	**V**	**P**	**V**
FAGACEAE										
Castanopsis	5.3	2.7	17.8	11.1	55.5	15.7	29.3	11.5	12.9	2.9
Lithocarpus	83.3	52.6	70.1	54.5	-	-	-	-	-	-
FABACEAE										
Cassia	9.8	42.6	-	-	14.2	34.4	-	-	-	-
S. siamea	-	-	-	-	22.1	41.5	-	-	-	-
ELAEAGNACEAE										
E. cf. *latifolia*	-	-	-	-	-	-	56.4	87.7	76.5	65.1
CAPRIFOLIACEAE										
Sambucus	1.6	2.1	-	-	2.5	1.7	-	-	-	-
THEACEAE										
Schima	-	-	5.3	19.5	-	-	-	-	-	-
ACANTHACEAE										
Thunbergia	-	-	-	-	-	-	-	-	5.4	31.2
TRAPACEAE										
Trapa	-	-	2.1	14	2	6.1	-	-	-	-
UNKNOWNS										
Triangular and tripolate	-	-	2.6	0.5	1.0	<0.1	-	-	-	-
Triangular and inaperture	-	-	2.1	0.4	-	-	-	-	-	-
Three furrows triangular and tricoplate	-	-	-	-	1.2	<0.1	7.6	0.5	-	-
Three furrows and tricoplate	-	-	-	-	2.5	0.5	6.7	0.3	-	-
Irregular and inaperture	-	-	-	-	-	-	-	-	5.2	0.8

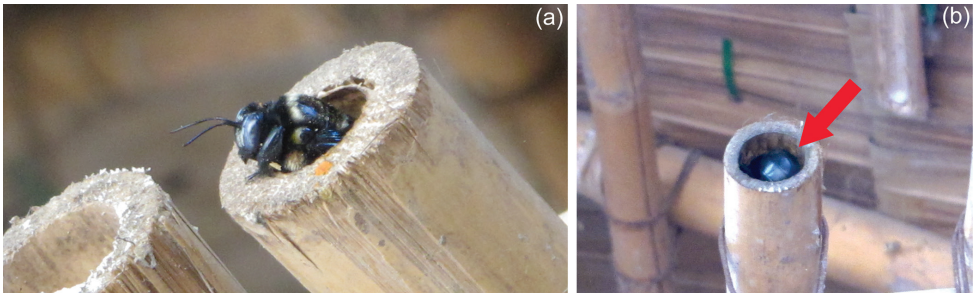

Figure 4. Nest defending postures of *X. nasalis*; Defending posture tactics performed by females *X. nasalis* to repel other conspecifics in the aggregated nesting site. The bee blocking the entrance via protruding her head out from the nest entrance (**4a**). Guarding the entrance by using the dorsal side of her metasoma to block the invaders (**4b**).

boo culms. Two defending posture tactics were observed. The most common defense posture is that of a female blocking the entrance with her head (Figure 4a), although sometimes we observed a female bees using her metasoma to block the nest entrance (Figure 4b).

Discussion

Boontop et al. (2008) provided a brief nest architectural description of 20 *Xylocopa nasalis* communal nests collected from Nakhon Pathom province ~100 km southeast of our collecting site, though they only reported the nest total lengths (described as "Internode length"), size of the nest entrances, diameter of the bamboo nests, and the sex ratio. Here, we also reported additional detailed nest characteristics that were undescribed previously. The total nest length averaged at 38.35 cm, whereas Boontop et al. (2008) found theirs to be 32.63 cm. The branch diameters of the nests are also similar between our work and that of the previous authors, 17.94 and 15.60 mm, respectively; however, the sex ratio between female and male bees from this observation is about twice to what was earlier described (8♀: 1♂ vs. 4♀: 1♂). The difference in the number of female to male bees can be explained by the collecting date, which may correspond to a later period of colony development, where most of the sister bees have emerged and stay together inside the nest, whereas male bees may have departed right after emerging from their cocoons or there is a sex ratio bias in egg-laying by the mothers. Observations on such activity are needed to test these hypotheses about the skew sex ratio in the nest. The three unconsumed pollen masses have an average fresh weight of 1.37±0.13 g compared to 1.16 and 1.09 g in *X.* (*Ctenoxylocopa*) *sulcatipes* Maa, 1970 and *X.* (*Koptortosoma*) *pubescens* Spinola, 1838, species found in the desert area of the Middle East (Gerling et al. 1989).

Maeta et al. (1985) reported finding of a nesting aggregation of another *Biluna* species, *Xylocopa tranquebarorum tranquebarorum*, in Szechungchi near Henchun, Taiwan. This *Biluna* species also nested in bamboo culms though the nest entrances were excavated exclusively from the underside (the authors found only five nests in successive internodes of a single culm. In contrast to the previous finding, we found the nest entrance of *X. nasalis* to be mostly at the end of the bamboo culms, but we also observed that a couple of the nest entrances were on the underside of the culms excavated by the bees as well. This observation suggests that both *X. nasalis* and *X. tranquebarorum tranquebarorum* can excavate nest entrance from the underside of the bamboo culms, which might be a behavior shared by members of the subgenus *Biluna*. This tedious nest entrance excavation of a smooth and hard surface such as the sides of bamboo culm may be explained by the compensation that the bees will receive after the initial perforation of the culm with the omission of the need for later heavily burrowing (Iwata 1938). However, if the ends of the bamboo culms are open and

exposed, the bees may choose not to allocate their energy in the excavation of the undersides of the culms as seen in this study.

Xylocopa nasalis is polylectic with a diverse group of pollens collected. This is consistent with the described foraging behaviors of other carpenter bee species (Hurd and Moure 1963, Gerling et al. 1989, Burgett et al. 2005). Interestingly, the family Fagaceae, particularly of the genera *Lithocarpus* and *Castanopsis*, constitutes abundant pollen sources for *X. nasalis*. Both pollen types can be accounted for 26.43% and 13.77% , respectively, in terms of total grains count; however, their pollen sizes are twice or thrice smaller than other pollen types found in this study (Table 2), thus the total percentage pollen volumes contribute to the bee diets are rendered to only 7.65% and 3.22%. *Lithocarpus* and *Castanopsis* are evergreen genera of large shrubs and trees that can reach more than 20 m in height. Records show that there are 56 species of *Lithocarpus* and 33 species of *Castanopsis* indigenous to Thailand (Phengklai 2006), though there is no available information pertaining to a reliable identification of the pollen species of both plant genera in the area of study. Many species of the lowland *Lithocarpus* and *Castanopsis* flowers bloom abundantly during the beginning of the dry season to the end of monsoon period (March–October), which corresponds to the time of our collecting. It is known that species of both *Lithocarpus* and *Castanopsis* are pollinated via insects (Nixon 1989, Manos and Steele 1997, Manos et al. 2001), therefore it is evident that we should consider *X. nasalis* as one of the important pollinators for genera of large endemic trees that constitute the deciduous and evergreen landscape in the area of central Thailand. In addition, Burgett et al. (2005) reported the importance of *Lithocarpus* and *Castanopsis* pollens as two of the top food sources of the night-flying carpenter bee *X.* (*Nyctomellita*) *tranqueberica* (Fabricius, 1804) as well. They suggested that these pollen types serve as the primary pollen sources for *X. tranqueberica* found in northern Thailand, second to the introduced plant species of *Casuarina* Linnaeus, 1753, which is heavily planted throughout Thailand for reforestation (Burgett et al. 2005).

Another group of large trees that also benefit from *Xylocopa nasalis* visitation is *Senna siamea* (Fabaceae), and other related but unidentified species in the genus *Cassia*. *Senna siamea* is an indigenous evergreen tree found throughout Thailand and other neighboring countries in South and Southeast Asia; locals use its leaves mainly for consumption; it is seldom used as fodder for animals and intercropping. The flowering period of this species is documented to be during March to September or otherwise year round, if the hot and humid weather permitted (Hanum and van der Maesen 1997, Sosef et al. 1998). Both *S. siamea* and *Cassia* have poricidal anthers, which require a sonication or "buzz-pollination" from floral visitors to extract pollen from their anthers and thus eventually affect pollination (Buchmann and Hurley 1978, Buchmann 1983). Visitations by carpenter bees, which are known for their abilities to vibrate their thoraces at the pores of the flowers' anthers to release the pollens (King et al. 1996, King and Buchmann 2003), are crucial for the reproductive successes in these plant genera. *Xylocopa nasalis* may as well be an important pollinator of this group of large trees in this area.

From the analyses of the pollen volume, we found that *Croton* (Euphorbiaceae) contributes the highest volume (14.95%). It is important to note that though this genus was found for only 2.80% of the total pollen count (Table 2), the relatively large size of *Croton* makes it become one of the most important food sources for *Xylocopa nasalis*. In Thailand, there are about 30 species of *Croton* (Chayamarit and Welzen 2005). The genus has a reputation of containing biomedical-active compounds such as alkaloids and terpenoids (Rizk, 1987) that have potential values to the pharmaceutical industries.

One genus of an annual floating-leaved aquatic plant is also frequently visited by *Xylocopa nasalis*. The pollens of water chestnut of the genus *Trapa* (Lythraceae) contribute 13.36% of the total pollen volume in the bee diets. Smitinand (2001) listed only three *Trapa* species in Thailand: *T. bicornis* Osbeck, 1771, *T. incisa* Siebold & Zuccarini, 1845, and *T. natans* Linnaeus, 1753. However, the taxonomy of *Trapa* is still in flux (Kadano 1987; Cook 1996; Takano and Kadano 2005), and a thorough survey of this common aquatic plant in Thailand is needed, since the fruits of *T. bicornis* are one of the important food crops in Thailand. Identification of *Trapa* pollens to specific level can provide important information regarding which species *X. nasalis* visit and pollinate.

Lastly, the main shrub species that *X. nasalis* visits for pollen is *Elaeagnus* cf. *latifolia* (Elaeagnaceae), a prominent shrub that has a native range in northern Thailand, although it can be found throughout the country due to its high adaptability to various soil conditions and habitats (Smitinand 2001). Other minor pollen-providing plants that can be identified in this work such as *Sambucus* and *Ziziphus*, which are possibly introduced into the area as ornamental plants, and which contribute less than 1% of the pollen volume in a given pollen mass.

Our observations of nest-defending by resident females are consistent with the nest defending postures reported in *Xylocopa sulcatipes* and *X. pubescens* in Israel (Gerling et al. 1989) and in *X.* (*Ctenoxylocopa*) *fenestrata* (Fabricius, 1798) in India (Kapil and Dhaliwal 1968a), though whether the guarding females are the progeny of the founding female still needs to be investigated in *X. nasalis*.

Conclusion

In summary, our observations and dissections of *Xylocopa nasalis* nests agree with known reports of other *Xylocopa* species (Hurd 1958, Hurd and Moure 1960, 1961, 1963, Kapil and Dhaliwal 1968a, 1968b, Michener 1974, Mordechai et al. 1978, Gerling et al. 1989, Boontop et al. 2008). The *X. nasalis* nest is strictly unbranched. The provisioned cells are separated via partitions made from bamboo particles excavated by the founding female. The nesting architectural details provided within this work should prove to be of beneficial to beekeepers and researchers who are interested in trapping and studying *X. nasalis*. For further genetic and social behavioral studies, we found that in a given nest, sister bees can tolerate and live inside the same nest with up to 7 individuals along with their mother. Kinship analyses using molecular mark-

ers such as microsatellite DNA will reveal interesting details pertaining to the social structure in a single nest and the population structure of the bees living communally in the same vicinity in Ratch Buri province, Thailand (W. Hongjamrassilp and N. Warrit (unpublished data)). As for the pollens foraged by *X. nasalis*, the broad host plants range can be highly beneficial for many crop pollinations, particularly for plants that require the "buzz" pollination method by their pollinators (Keasar 2010). Flower constancy and other related pollination studies are required for further justification of using *X. nasalis* as future potential pollinator for agricultural and forest plants in Southeast Asia.

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Polygyny and strong genetic structuring within an isolated population of the wood ant *Formica rufa*

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Abstract

Social structuring of populations within some *Formica* species exhibits considerable variation going from monodomous and monogynous populations to polydomous, polygynous populations. The wood ant species *Formica rufa* appears to be mainly monodomous and monogynous throughout most of its distribution area in central and northern Europe. Only occasionally it was mentioned that *F. rufa* can have both polygynous and monogynous colonies in the same geographical region. We studied an isolated polydomous *F. rufa* population in a deciduous mixed forest in the north-west of Belgium. The level of polydomy within the colonies varied from monodomous to 11 nests per colony. Our genetic analysis of eight variable microsatellites suggest an oligo- to polygynous structure for at least the major part of the sampled nests. Relatedness amongst nest mate workers varies considerable within the population and colonies but confirms in general a polygynous structure. Additionally high genetic diversity (e.g. up to 8 out of 11 alleles per nest for the most variable locus) and high within nest genetic variance (93%) indicate that multiple queens contribute to the gene pool of workers of the same nest. Moreover significant genetic structuring among colonies indicates that gene flow between colonies is restricted and that exchange of workers between colonies is very limited. Finally we explain how possible factors as budding and the absence of *Serviformica* can explain the differences in genetic structure within this polygynous *F. rufa* population.

Keywords

Formica rufa, genetic differentiation, polygyny, budding, *Serviformica*, habitat fragmentation

Introduction

Within the wood ant genus *Formica* s. str., monogynous colonies can only be founded when young and newly emerged queens disperse over long distances to find suitable conditions to start a new colony (Gösswald 1952, Rosengren and Pamilo 1983, Rosengren et al. 1993, Mabelis 1994). Moreover queens of the *Formica rufa* group are incapable of independently founding a colony. Hence, to establish a new colony, a queen must be accepted into a nest of a species belonging to the subgenus *Serviformica* (in Western Europe generally *F. fusca*), because she is unable to raise her own brood. High dispersal risks and low independent colony-founding success of individual wood ant queens are expected to promote a polygynous situation wherein daughter queens are adopted into the queens nest. This strategy is often accompanied by budding of the colony, whereby mated females of polygynous populations leave their natal nests with workers, and found a new nest in the vicinity of the ancestral nest. In particular when these queens have mated with males originating from outside the colony, such colony reproduction may strongly reduce the intranest relatedness within an ant population and even lead to multicolonial populations comprising several colonies each with several nest units that may or may not exchange workers.

Some western and northern European populations of mound building and other *Formica* species exhibit considerable intraspecific variation in colony founding and social structure within one region [for *Formica exsecta* (Seppä et al. 2004); *Formica truncorum* (Bargum et al. 2004); *Formica aquilonia* (Pamilo et al. 2005); *Formica lugubris* (Mäki-Petäys et al. 2005) and *Formica selysi* (Chapuisat et al. 2004)]. In some extreme cases, even "unicolonial" populations or supercolonies (Helanterä et al. 2009), where boundaries between populations are virtually absent, have been observed within the genus *Formica* (Chapuisat et al. 2005, Holzer et al. 2006, 2009, Bezděčková and Bezděčka 2011).

Shifts in the social structure of wood ant species are most commonly observed between populations living in nearby woodland patches that differ in management, vegetation characteristics or degree of isolation (Gyllenstrand and Seppä 2004). As these differences in social structure are frequently associated with a marked phenotypic divergence in morphological traits of the queen (i.e. the polygyny syndrome *sensu* Keller 1993), local adaptation to changing environmental conditions is frequently considered to be a main factor that allows intraspecific variation in social structure of wood ants (Seppä and Pamilo 1995, Mäki-Petäys et al. 2005, Holzer et al. 2006, Sorvari and Hakkarainen 2007a,b).

During the last decade, only a few studies reported extreme differences in social structure within a single ant population (Pirk et al. 2001, Chapuisat et al. 2004, Bargum et al. 2007, Kümmerli and Keller 2007, Saapunki et al. 2008). This persistence of sympatric social divergence within ant populations still remains poorly understood.

The social structure of *Formica rufa*, a wood ant species presumed to be monogynous throughout its distribution area, was studied in detail only once so far. Gyllenstrand et al. (2004) concluded that not all studied *F. rufa* colonies in Sweden are monogynous and

that their estimates of worker relatedness suggest a mix of polyandrous single queens nests and nests with a few coexisting queens. Indeed, other studies also mention that *F. rufa* might be very exceptionally polygynous (Seifert 1991, Seifert et al. 2010, Bezděčková and Bezděčka 2011) but genetic studies at colony level are lacking. In some regions of northern Belgium, both monodomous and oligodomous populations of the presumed monogynous species *Formica rufa* occur in highly fragmented, small forest habitats embedded within an agricultural matrix (Dekoninck et al. 2010). In this hostile matrix it is unlikely that dispersing young queens will encounter remote and isolated, suitable forest fragments. If habitat patches are too distant from each other to be bridged by dispersing queens, long distance dispersal will not be favoured (Rosengren et al. 1993, Mabelis 1994, Höfener et al. 1996). Consequently, even if dispersal is only successful within these suitable small patches, this may in the long term result in extinction of *Serviformica* ants due to the repeated parasitism by young queens and competition from neighbouring ant nests (Mabelis 1984, Czechowski and Vepsäläinen 1999, Czechowski and Markó 2006, Dekoninck et al. 2010). At one particular site in this wood ant hostile agricultural matrix, colonies of a *F. rufa* population vary extremely in degree of polydomy; multiple queens are often observed in a single nest and observations of nuptial flights and *Serviformica* are lacking. These preliminary observations suggest that this population might be another example of a shift in social organization of a wood ant, and in particular in a wood ant species that appears to be mainly monodomous and monogynous throughout most of its distribution area (Seifert et al. 2010).

Here in this study, our first aim was to infer by means of microsatellites if *F. rufa* shifted its social structure towards polygyny in this hostile fragmented forest complex. Second, we investigated the genetic structure of the population and explain its consequences. Furthermore, we analysed the variation in number of queens and number of interconnected nests in this population by relating intranest relatedness with the degree of polydomy and its persistence over multiple years. Finally we discussed the impact of budding and the lack of *Serviformica* in this context.

Material and methods

Study area

The *Formica rufa* population included in this study is situated in the forest of the Sixtusbossen at Poperinge-Vleteren (south of Western Flanders, Belgium) (Fig. 1). Former observations (Loones et al. 2008) showed that colonies within this populations vary in degree of polydomy and that multiple queens are often observed in a single nest, which suggests that multiple queens are present within most nests. New nests are primarily formed by budding from existing ones. Nuptial flights are short ranged and probably only performed by males. Copulations were only observed in the immediate vicinity of, and on the nest mounds. A relatively unique feature of the population is that *Serviformica* species, which are necessary for independent colony founding in *F. rufa*, have

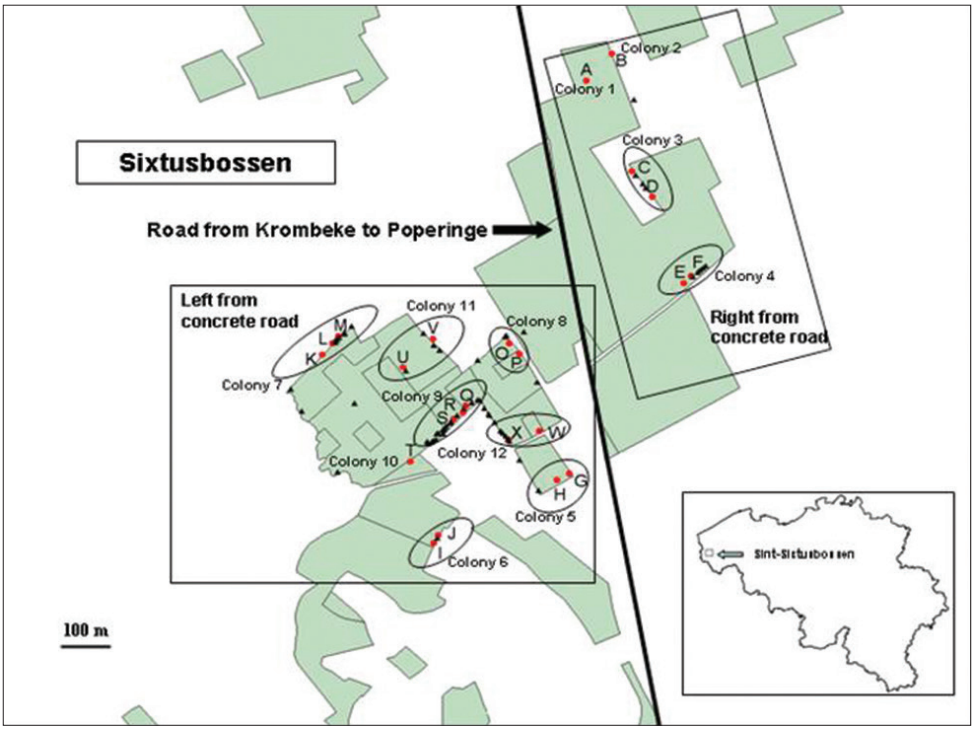

Figure 1. All nests in the woodland patches of the Sixtusbossen in 2005–2006. Red ● are nests that have been sampled for genotyping, ▲ are the positions of the remaining nests present during the time of sampling.

never been found in the forest stands of the Sixtusbossen (Loones et al. 2008) and the closest known *Serviformica* population is located at least 15 km away from the Sixtusbossen (Dekoninck et al. 2003).

Sampling design

Workers were sampled at different hierarchical levels to investigate the social structure and degree of genetic differentiation (Fig. 1). The isolated set of forest fragments that cover the Sixtusbossen was considered as the entire population. Within this population, between 20 and 37 workers were sampled from 23 separate nest mounds during spring 2005. The distance between two nests ranged from 5.7 m (nest O to nest P) to 1200 m (nest B to nest I).

Spatial separation and detailed observations during the last 10 years (Loones et al. 2008) allowed us to group nests into at least 12 colonies (C1–C12). Nests are considered as belonging to the same colony if historical observations revealed that they originated from budding from a former single nest. Colonies were further defined as nest aggregations based on spatial separation caused by the different forest patches

and forest edges and by following interconnecting trails. When a colony consisted of multiple nests, two or three nests were sampled.

Monitoring and detailed mapping of the nests during the past ten years (Loones et al. 2008) revealed that in one of the larger fragments, the number of nests increased from 29 in 1996 to 52 in 2006.

We also investigated temporal variation in genetic structure and relatedness by resampling two monodomous nests in the summer of 2009 (nest J and S) shortly after they budded and produced one and three daughternests respectively. For comparison, we also included nest I, which remained monodomous during these five consecutive years.

Molecular analysis

All sampled workers were stored in 97% ethanol until DNA extraction. DNA was extracted from the legs of adult workers in 200 µl 6% Chelex (Biorad, Instagene Matrix™) and 10 µl proteinase K (Qiagen), incubated for two hours at 55 °C and subsequently for 15 min at 97 °C. Extracted DNA was kept frozen at -20 °C.

Specimens were genotyped with 8 microsatellite loci originally designed for *F. exsecta*: FE13, FE19, FE37, FE38 (Gyllenstrand et al. 2002) and for *F. paralugubris*: FL12, FL20, FL21 and FL29 (Chapuisat 1996).

Polymerase chain reactions (PCR) were carried out in 10 µl volumes. The PCR-mix for both FE13, FE19 and FE37 contained: 0.5 µl DNA, 1× PCR buffer (Qiagen), 1× Q-solution (Qiagen), 0.5 mM $MgCl_2$ (Qiagen), 100 µM dNTP (Fermentas), 0.4 µM forward and reverse primer and 0.5 U *Taq* polymerase (Qiagen). The other primers (FE38, FL12, FL20, FL21 en FL29) were used in a multiplex with 0.5 µM F&R FL20-primer, 0.2 µM F&R for the 4 other loci and 1× MP Master Mix (Qiagen). We repeated the samples that did not amplify the first time by adding 0.16 mg/ml BSA (100×) to the PCR mix and using only 0.25 µl DNA.

PCR amplification was performed under the following cycling conditions: initial denaturing at 94 °C for 3 min followed by 35 cycles of denaturing at 94 °C for 45 s, annealing at 50 °C (FE13, FE19,and FE37) or at 55 °C (FE38, FL12, FL20, FL21 and FL29) for 45 s and extension for 1 min at 72 °C followed by a last extension step of 10 min at 72 °C. Products were resolved and visualized by capillary electrophoresis on a SCE 9610 genetic analyzer (Spectrumedix) and using the Genospectrum 3.0.0 Software.

Data analysis

The GENEPOP software package (Raymond and Rousset 1995) was used to calculate observed heterozygosity (H_O), expected heterozygosity (H_E), and test for Hardy-Weinberg equilibrium and population differentiation (Goudet et al. 1996). FSTAT was also used to calculate fixation index (F_{ST}) between pairs of colonies.

For each nest, we extracted information about (i) the degree of polygyny, (ii) the degree of genetic differentiation at the different hierarchical levels, (iii) relatedness amongst worker nestmates and (iv) population viscosity. Besides relatedness amongst nest mate workers, we used an alternative method to infer the number of queens that contributed in worker reproduction. This was done by identifying the absolute minimum number of queens (hereafter called AMQ) necessary to result in the observed worker genotypes per colony. This was performed by first assuming that only one single queen founded the colony, without restrictions on the degree of polyandry. If the given genotype data per colony did not fit with this assumption, there was evidence that the workers originated from at least a second queen and so forth. Although we realized that the number of queens obtained by this method clearly underestimates the effective number of reproducing queens as different queens can have identical alleles, it does not falsely reject the null hypothesis of monogyny.

When polygynous nests recruit their own daughters as new reproductives and relatedness between nestmate queens equals that among workers (r), the effective mean number of queens per colony or nest (hereafter called Qn) is a function of relatedness: $Qn = (1 + 2/m - r) / 3r$ where m is the effective paternity (Pamilo 1993, Seppä 1994, Gyllenstrand et al. 2004). For a typical *Formica rufa* nest, the effective paternity has been estimated to be 1.47 (Boomsma and Sundström 1998) giving the expected relatedness of 0.59 among the single-queen brood; a relatedness of 0.1 for oligogyne (Qn = 7) brood and 0.0079 for very polygynous brood (Qn = 100).

The genetic relatedness among individuals within a nest was estimated by means of the relatedness estimator developed by Queller and Goodnight (1989) with the program GenAlEx6 (Peakall and Smouse 2006). Relatedness records the degree of shared genetic material between individuals of the same nest with respect to randomly taken individuals from the total population.

Testing the correlation between relatedness and level of polydomy was performed by means of an exact Spearman rank order correlation (StatXact v.5). To avoid pseudoreplication of nests within a colony, only the average intranest relatedness per colony was used (n = 12).

Patterns of genetic differentiation between nests (N) and colonies (C) were first investigated by means of visual inspection of a principal component analysis (PCA). This PCA was performed on average allele frequencies per nest with the programme GenAlEx6 (Peakall and Smouse 2006). Genetic structure of the population was further investigated using Wright's *F*-statistics and average F_{IN} (among individuals within nests), F_{NC} (among nests within colonies) and F_{CT} (among colonies within the population) were calculated. The significance of these *F*-statistics was tested by comparing the observed values against the null distribution as obtained by random permutation. The total genetic variation was partitioned according to these different hierarchical levels by means of Analysis of Molecular variance (AMOVA) with the program Arlequin version 3.0 (Excoffier et al. 2005).

Limited dispersal of individuals from their birth place results in genetic viscosity (Hamilton 1964). This induces genetic differentiation between geographically distant groups and consequently increases relatedness among neighbours. Genetic differentia-

tion in relation to geographic distance was investigated with a Mantel test (Liedloff 1999). This relationship was compared between nests from the same colony as well as between nests from different colonies. Comparing both relationships reveals whether the expected lower genetic differentiation between nests from the same colony are merely due to their limited geographic distance or due to the direct effect of an increased genetic relatedness of the budded nest.

Results

Hardy-Weinberg equilibrium and genetic diversity

We did not find significant differences between observed and expected genotype frequencies and hence no deviation from the Hardy-Weinberg equilibrium. Genetic diversity, calculated as expected heterozygosity (H_E) at the nest level, ranged from 0.296 in the monodomous colony A up to 0.557 in a nest from a polydomous colony. The number of alleles per locus ranged from 1 (several loci) to 8 (FL20) at the nest level and from 3 (FE19) tot 11 (FL20) for the total population. When focussing on the most diverse locus FL20, most nests contained more than 50% of the total number of alleles observed in the total population (11).

Polygyny or monogyny estimation based on the AMQ, Qn and genetic relatedness

For the majority of the investigated colonies, the observed worker genotypes did not match with reproduction by a single queen based on AMQ (Table 1). For only five out of 23 nests, no clear evidence for polygyny could be observed. Worker genotypes from 14 nests originated at least from two different queens and for 4 nests even from three different queens. When assuming that this is the true number of queens that gave rise to these worker genotypes, the obtained levels of polyandry were unrealistically high (for example nest A, 1 queen would have mated with at least 4 different males) and hence indicates that these numbers clearly underestimate the effective number of reproductive queens within a single nest.

Relatedness estimates differed substantially among nests within the population and even within colonies and ranged from 0.49 to -0.218. Negative relatedness estimates indicates that individuals are more different than average individuals in the population. These values represent most likely random variation of estimates which are close to 0. With this estimation of relatedness we calculated Qn. In general, this parameter confirmed the polygyn levels seen with AMQ, as for most of the nests the Qn indicate a polygynous structure. Furthermore, three nests (nest A, B and P) of which the AMQ suggested they might be monogynous nests, had a Qn between 1 and 2. Unfortanatly, we could not calculate Qn for each nest and this for two resaons. (i) For nest C, the relatedness estimate approaches zero and consequently the estimate of Qn approaches

Table 1. The number of workers analysed for each nest and the number of the colony, the maximum number of allels per locus, the level of polydomy within the colony, the absolute minimum number of queens (AMQ), relatedness (r) and the estimated queen number according to Boomsma and Südström (1998) for all genotyped nests.

Nest (subpop)	N workers	Max number of alleles[1]	Level of Polydomy	AMQ	r	Queen number
A(1)	20	6 (55%)	1	1	0.493±0.060	1.26
B(2)	20	4 (36%)	1	1	0.395±0.044	1.66
C(3)	30	8 (73%)	5	2	0.009±0.047	87.09
D(3)	22	5 (45%)	5	1	0.242±0.043	2.92
E(4)	34	7 (64%)	7	3	0.035±0.035	22.15
F(4)	20	6 (55%)	7	3	0.068±0.057	11.24
G(5)	20	8 (73%)	3	2	0.089±0.057	8.51
H(5)	20	8 (73%)	3	2	0.160±0.061	4.58
I(6)	37	7 (64%)	3	2	0.037±0.028	20.93
J(6)	20	6 (55%)	3	2	-0.068±0.080	NA
K(7)	20	7 (64%)	10	2	0.351±0.046	1.91
L(7)	20	6 (55%)	10	1	0.290±0.052	2.38
M(8)	24	6 (55%)	10	2	0.038±0.058	20.37
O(8)	20	8 (73%)	3	2	0.085±0.047	8.92
P(8)	20	4 (36%)	3	1	0.445±0.056	1.43
Q(9)	20	7 (64%)	11	2	-0.149±0.076	NA
R(9)	20	8 (73%)	11	2	-0.012±0.063	NA
S(9)	20	8 (73%)	11	3	-0.033±0.069	NA
T(10)	19	6 (55%)	1	2	0.164±0.051	4.46
U(11)	20	5 (45%)	6	2	-0.218±0.080	NA
V(11)	20	5 (45%)	6	2	0.062±0.053	12.36
W(12)	20	7 (64%)	6	2	-0.149±0.056	NA
X(12)	20	8 (73%)	6	3	0.124±0.061	6.01

[1] = the % of total number alleles for this locus in the population

infinite. (ii) Due to negative relatedness estimates, Qn could not be retrieved for six other nests. Therefore, they are all marked with NA in Table 1. However, this still means that the estimate of Qn is very large (or approaches infinite).

Relatedness versus level of polydomy

The correlation between the average intranest relatedness per colony (n = 12) and the level of polydomy was significantly negative (r_S = -0.61, p = 0.04, Fig. 2), indicating that monodomous colonies had a significantly higher relatedness. Although the average relatedness of highly polydomous colonies approached zero, relatedness of some nests within these highly polydomous colonies was much higher, suggestiong lack of random mating.

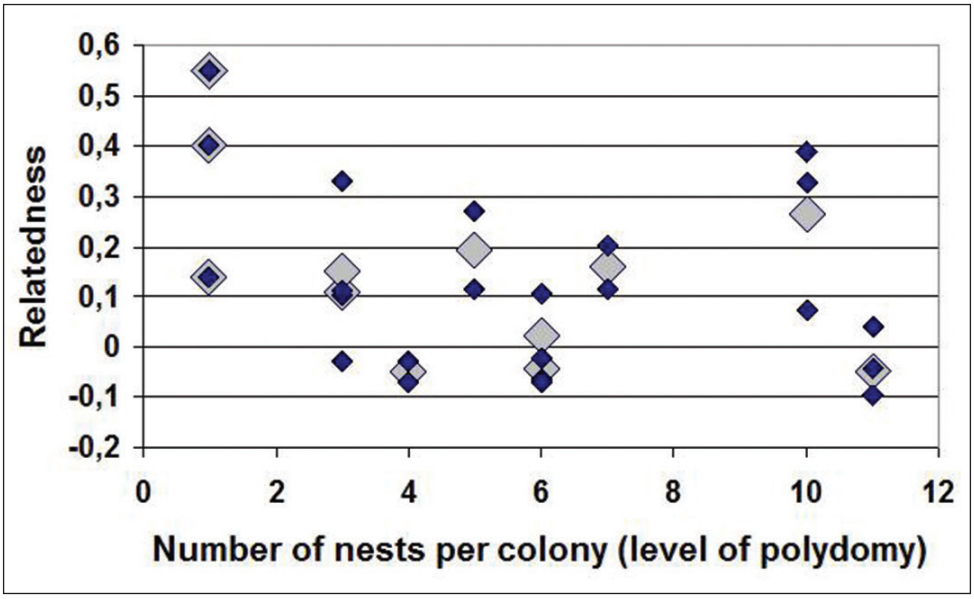

Figure 2. Correlation between the relatedness and the level of polydomy. Large grey symbols are the average intranest relatedness per colony, smaller dark symbols are estimated genetic intranest relatedness per nest.

Genetic structuring

Visual inspection of the PCA revealed, in general, that nests from the same colony (e.g. C11) were often more similar in allele frequency compared to nests from different colonies (Fig. 3). However, substantial genetic variation in allele frequency still remained among nests within a colony, suggesting their independent genetic structure (e.g. nests from C4 and C7).

Hierarchical analyses of variance indicated that the major part of the total genetic variation (93.5%) was found within nests. Genetic variation among nests within colonies was low (F_{NC} = 0.027) and explained 1.72% of the total genetic variation. This estimate was significantly higher ($p < 0.0001$) than expected from random mating among nests members within a colony. At the highest hierarchical level, differentiation among colonies was higher (F_{CT} = 0.077 and $p<0.0001$) and contributed to 4.88% of the total genetic variation. The within nest inbreeding coefficient F_{IN} was estimated as –0.004 and not significantly different from zero ($p = 0.5$).

To investigate whether the lower differentiation among nests within colonies is merely an effect of larger distances between nests of different colonies, we compared the relationship between Nei's genetic distance and geographic distance of pairs of intracolonial nests and pairs of intercolonial nests (Fig. 4). For intracolonial nest comparisons, the Manteltest showed no obvious relationship between genetic distance and geographic distance (all exept one genetic distance below 0.1). This pattern contrasted

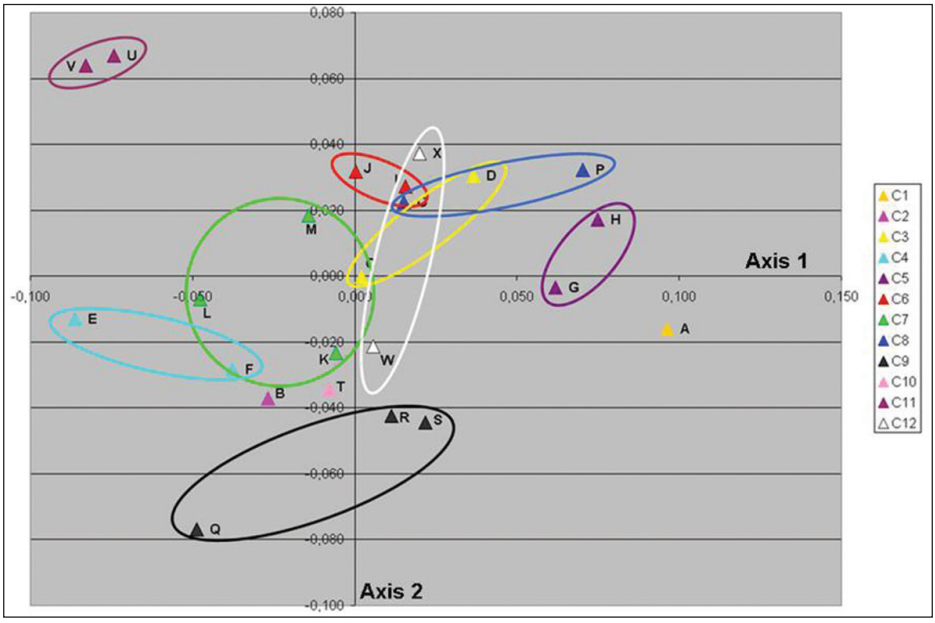

Figure 3. Genetic structure of the *Formica rufa* population at Sixtusbossen as revealed by Principal Component Analysis of the allele frequencies per nest. Nests with the same colour originate from the same colony. First and second PCA axes explained 36.5% and 20% of the total among nest genetic variation, respectively.

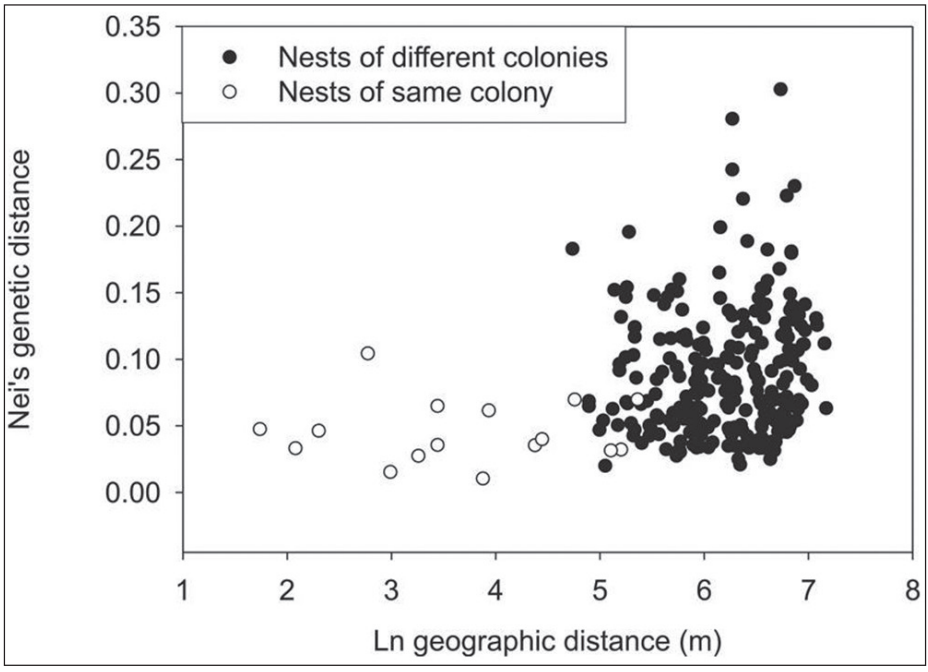

Figure 4. Manteltest showing the relationship between the Nei genetic distance and the geographical distance Ln(m).

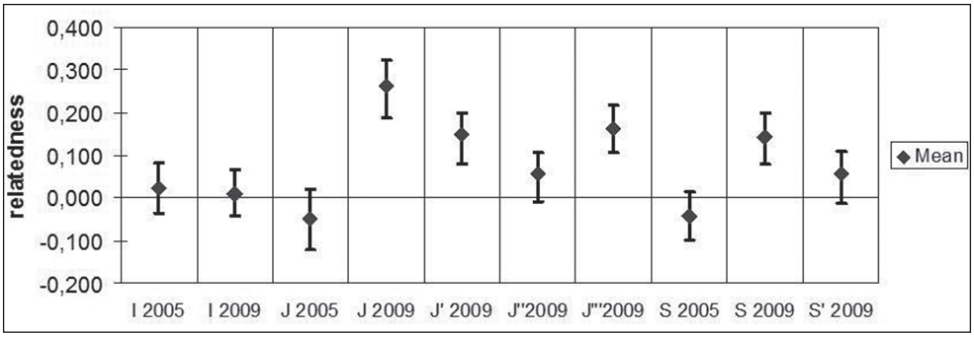

Figure 5. Mean relatedness values of the nests sampled in 2005 and 2009 and their daughternests in 2009.

with the intercolonial nest comparisons, wherein nests located at a comparable geographic distance (about 200 m) as intercolonial nests, often showed a genetic distance higher than 0.10.

The impact off budding on relatedness

The relatedness of the mother nests that had budded between 2005 and 2009 (nest J and S) has increased significantly ($p < 0.001$) in 2009 (respectively from -0.050 to 0.262 and from -0.042 to 0.142; Fig. 5). Moreover the relatedness of the daughter nests J', J", J"' and S' also show a positive relatedness that is higher than the relatedness of the mothernest in 2005. The relatedness of nest I where no budding was observed in 2009 has decreased.

Discussion

In this forest complex where opportunities for independent colony founding are virtually absent, our results demonstrated that the presumed monogynous species *Formica rufa* has shifted its social structure towards polygyny for at least a part of the population. This polygynous social structure results in a strong reduction of the intra-nest relatedness and the majority of the colonies showed relatedness values that were only marginally higher than zero. The average intra-nest relatedness of worker nestmates in the population was 0.129 ± 0.014, suggesting weak polygyny (Rosengren et al. 1993). This loss of genetic identity within colonies was confirmed by the distribution of the genetic variation, wherein almost all of the genetic variation within the population (93.5%) was observed within colonies. Remarkably, some colonies still attained high relatedness values that are primarily observed among workers of monodomous colonies. The number of different alleles in these colonies is still considerably higher than expected under a single queen – single male mating scenario. This suggests that the

high relatedness observed in these monodomous colonies is most likely due to adoption of daughter queens from inside the colony.

The effective number of queens per colony can be inferred from estimates of the relatedness of workers within a colony (Pamilo 1985, Queller 1993, Seppä 1994, Keller 1995) and here it ranges from 1 to infinite. This estimator however relies on strong assumptions about the degree of polyandry and population structuring, which could not be validated in this study. A high relatedness within a nest or colony for example does not necessarily imply a low absolute number of queens. If all reproducing queens have similar genotypes this results in high relatedness and hence estimates of a low number of queens. Therefore we used a second estimator AMQ. Although we did not intend to estimate the effective number of breeding queens per nest (according to Rosengren et al. (1993) this can usually not be determined because of the large nest volume) based upon AMQ, it can be expected that most, if not all nests of this population, are polygynous. Our approach only revealed the absolute minimum number of queens required to reconstruct the observed worker genotypes per nest. Assuming that this equals the true number of reproducing queens that gave rise to the observed worker genotypes would however imply a strong degree of polyandry. In nests categorised as monogynous based on AMQ, up to six different alleles could be observed for the most polymorphous locus. In this case, a single queen should have mated with at least four different males. However the effective paternity has been estimated to be 1.47 for a typical *F. rufa* (Boomsma and Sundström 1998). Although polyandry has been observed and assumed for this species (Gyllenstrand et al. 2004), here assumed levels of polyandry would be unrealistically high. It is therefore much more likely that for nests where we could not provide unequivocal evidence for a polygynous colony structure, multiple reproducing queens generated the genotyped workers sampled at each nest.

The observed multicolonial genetic structure in this study seems to be identical to that observed in other wood ants such as *Formica polyctena* (Beye et al. 1997), *F. lugubris* (Gyllenstrand and Seppa 2003) and *Formica pratensis* (Beye et al. 1998).

We found a significant negative correlation between the level of polydomy and the relatedness amongst nest mate workers per nest and per colony. Interestingly, almost all sampled monodomous colonies showed relatedness estimates that were higher than 0 while polydomous colonies were on average characterised by lower relatedness estimates that, in most cases, did not differ from zero. Nevertheless, some nests within these polydomous colonies exhibited relatedness estimates that are larger than 0. Our data from the sampling and analyses of 2005 indeed point in the direction that budding probably occurs when relatedness among workers drops due to immigration of extra-nest males or queens. In such cases, nests of polydomous colonies with high relatedness could be recently budded nests. A restricted data set of nests resampled in 2009 suggests this. Seppä (2008) mentions in this context that if the number of reproducing queens becomes extremely high or the queens originate from very different genetic pools, the relatedness amongst queens and nest workers becomes 0, and this induces budding. However to confirm this, a more profound and longer-term sampling and identical follow-up of the *Formica rufa* population in Westvleteren is necessary.

In ant species that have a unicolonial population structure, each nest contains numerous queens, are interconnected and individuals move freely between nests (Chapuisat et al. 2005, Holzer et al. 2009). Moreover the nestmate relatedness values are often indistinguishable from zero and unicoloniality is often associated with low overall genetic variability. Elsewhere in Europe, *Formica* species which follow a sessile life history in a stable habitat, indeed have the potential to develop unicoloniality as was confirmed by Chapuisat et al. (2005) and Elias et al. (2005). Our study suggests that *F. rufa* has the potential to do so when a population is hosted in a isolated forest complex without *Serviformica* in the near environment. In the small forest patches at Westvleteren long-term domination of long-lived resources (tending of Homoptera) leads to habitat saturation so that the colony might increase continuously. However, in such cases independent colony foundation becomes increasingly difficult because of a lack of territory and food. Furthermore, independent colony foudation is completely impossible because of lack of *Serviformica*. This situation promotes the reacceptance of queens by the maternal colony or maybe, in some cases, even nests of nearby colonies and the establishment of networks of nests originating from budding as is typical for unicolonial ant populations (Jackson 2007). However in the field aggression and wood ant wars were sometimes observed. These wars were as described for *F. polyctena* by Mabelis (1979) and can be very intense. These wars can even be between very nearby nests from the same and sometimes from different colonies. This latter aspect is lacking in real unicolonial ant populations (Jackson 2007) or almost lacking (Holzer et al. 2006). Unicoloniality suggests limited queen dispersal and free adult worker dispersal. In general free adult worker dispersal between different colonies is not observed in Westvleteren. Probably this can be confirmed in the near future with mark-recapture measures and a comparative study of workers and eggs of summer- and winternests of several colonies (cf. Elias et al. 2005). We conclude that in Westvleteren we have a multicolonial population structure of extended family-based nests.

Conclusions and further research

Most colonies and nest of this population of *Formica rufa* appear to be polygynous. Moreover our genetic analyses suggest the presence of genetic structuring in the Westvleteren population. The allelic diversity was high compared to that found at the same loci in other monogynous wood ant populations elsewhere in Belgium (Flanders). Further research on a large geographic scale by extensive genetic sampling of mono- and polydomous *F. rufa* populations in Flanders (e.g. near Bruges Dekoninck et al. 2010) could explain whether the rarely reported polygynous structure of *F. rufa* as in Westvleteren can indeed be attributed to fragmentation and/or lack of *Serviformica* ants like *Formica fusca*. Our results indicate that more detailed temporal analyses of relatedness at the nest and colony levels for this and other wood ant species, will teach us more about the driving factors that might induce a shift in social structure (here from monogynous to oligogynous and polygynous) and variation within one population.

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Nest architecture and colony composition of communally nesting *Spilomena socialis* sp. n. (Hymenoptera, Crabronidae, Pemphredoninae) from peninsular Malaysia

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Abstract

Communal nesting, rare in the crabronid wasps, has been recorded for various species in the Spilomenina clade of the Pemphredoninae. A new communally nesting species, *Spilomena socialis,* is described from peninsular Malaysia where it nested on buildings at Bukit Fraser. The nest consists of a group of closely spaced clusters of vertically oriented cells attached to walls, and is constructed of tiny pieces of vegetal and mineral origin, parts of insects, and fungal hyphae bound together by silk secreted from each female wasp's abdominal gland. Nests contained up to 39 cells (average 10.4 cells, N = 35). Nest entrances were at the upper end of the cells and were protected on one side by a "roof". Cells constructed side-by-side have their roofs connected to form a tube that allowed access to all the cells. Nests were inhabited by 1–13 females (average 4.3 females per nest, N = 21) and 0–4 males, the overall sex ratio being 0.22. Ovarian development among the females in a nest varied. In 8 of 20 nests with 3 or more females only one female had developed ovaries, but female size (measured as head width) did not correlate with ovarian development. Cells are apparently progressively provisioned with thrips, and are often re-used. Adult females cooperatively defend the nests against intruders.

Keywords

Social wasp, ovarian development, sociality, thrips

Introduction

In the superfamily Apoidea, which includes the apoid wasps of the family Crabronidae, nesting habits vary from strictly solitary to eusocial. The majority of the more than 8000 described crabronid species are characterized by solitary nesting habits but some members of one clade in the subfamily Pemphredoninae, the Spilomenina, consisting of the genera *Arpactophilus*, *Microstigmus*, *Spilomena*, and *Xysma*, have evolved relatively complex social behavior (Matthews 1991).

Reproductive division of labor has so far only been ascertained in the Neotropical *Microstigmus comes* Krombein, where nests are usually founded by solitary females and relatedness between female colony members varies from 0.6 to 0.7 indicating the presence of mother-daughter associations with single-mated foundresses (Matthews 1968, Ross and Matthews 1989a and b). Females of *M. nigrophthalmus* Melo perform trophallactic exchanges, practice oophagy and differ in their activities on the nest (Melo and Campos 1993, Lucas et al. 2011). However, ovarian development was comparable in all colony females. In the Australian *Arpactophilus mimi* Matthews and Naumann, an average of three females occupied and progressively provisioned the nest, but displayed no differences in ovarian development (Matthews and Naumann 1989).

Most of the 80+ species of the cosmopolitan genus *Spilomena* nest in preexisting cavities in twigs, decayed wood and structural timber and prey on thrips, psyllids, aphids or coccids (Bohart and Menke 1976). However, communal nests have been reported in a few species. An Australian ground-nesting species, *S. subterranea,* McCorquodale and Naumann, had an average of 2.5 females with similarly developed ovaries per nest (McCorquodale and Naumann 1988). In an undetermined species from Costa Rica, West Eberhard (1977) reported a nest with ten females differing widely in size but all having comparable ovarian development and practicing progressive provisioning of the larvae. Some nests of another undetermined *Spilomena* species from southeastern Brazil contained as many as four females (Carvalho and Zucchi 1989).

We here describe a new species of communally nesting *Spilomena* found in the Central Mountain Range of Peninsular Malaysia and present information on its nest structure and composition, and other features of the biology.

Methods

Nests of *Spilomena socialis* were found at Bukit Fraser (1600 m; 03°42.77'N, 101°46.32'E) a mountain resort at the higher elevations of the Central Range of Peninsular Malaysia, in the state of Pahang. Entire nests were collected in February and March in both 2004 and 2007. For each colony all adults present were captured and the nest cells were removed from the substrate. Eggs, larvae and pupae were recorded for each nest. Adults and immature brood were preserved in 70% ethanol. Some nests were mounted on pieces of hard cardboard and others preserved in 70% ethanol.

Adult females were dissected to determine ovarian development, classified from 0 to 3 (maximum development = mature egg ready to be laid) according to their relative size. We photographed the head of each female present in each colony and used the open source software ImageJ to measure the maximum width of the head (reported in pixels).

Brief ethological observations were performed on some colonies, and simple experiments were conducted in an attempt to ascertain the defensive reactions of the adults against ants.

To better describe the relationship among small clusters of nests, and the use of different nests by wasps, we built an association network in which two nests were considered "associated" when a wasp passed from one nest to the other, or visited two nests consecutively. The resulting graph was a weighted and directed network in which each nest represents a node and their associations the edges. Degree (i.e., the sum of the strength of all edges connected to a node) was the only centrality measure calculated for each node. The analysis was based on two days of video observation of five adjacent nests, from 0900 to 1900 h. The graph (network) was obtained using NET-DRAW 2.097.

Digital images were captured using Microptics Digital Lab equipment. All statistical analyses were performed using the statistical program SPSS® 13.0 for Windows®.

Results

Taxonomy

***Spilomena socialis* Matthews, sp. n.**
http://zoobank.org/45A90318-7457-4F52-9BAD-754E80415D7F
Figs 1–5

Holotype. Female, 03°42.77'N, 101°46.32'E MALAYSIA: Pahang State, Bukit Fraser, Feb. 2007, 1600 m, S. Turillazzi (The Natural History Museum, London, UK [BMNH]).

Paratypes: 3 females, 3 males, same data as holotype (in The Natural History Museum (BMNH), one male, U.S. National Museum, Washington, DC (USNM), one male, one female, Australian National Insect Collection, Canberra, Australia (ANIC), one male, one female, University of Georgia, Athens, Fattig Museum), one female.

Female. Body length, 4 mm.

Head. (Figs 2–3). Transverse, globular, eyes slightly convergent dorsally; Vertex finely, distinctly coriareous; setigerous punctures minute; single elongate seta just in front of each lateral ocellus; occipital carina lacking; frontal carina extending about 1/2 distance to median ocellus; gena longitudinally finely striate ventrally, becoming smooth dorsally; clypeus broadly emarginate, faintly striate near orbits; malar space distinctly less than diameter of median ocellus (9:16); labrum deeply notched medially; frons distinctly protuberant, evenly convex; antennal sockets separated from clypeal

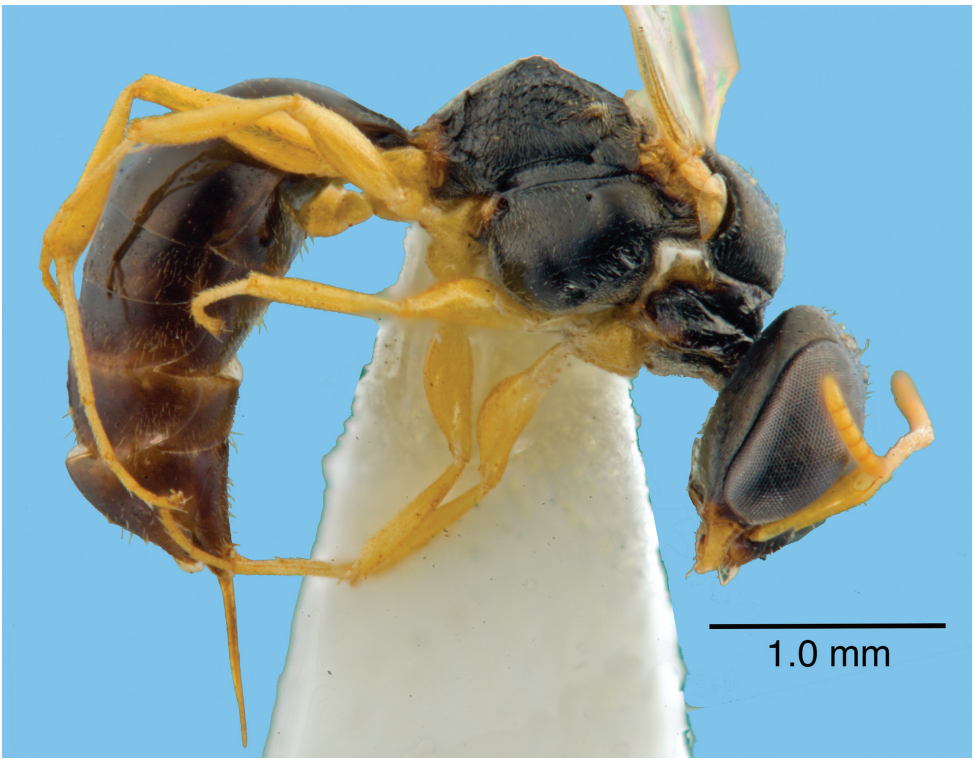

Figure 1. *Spilomena socialis* sp. n., holotype female, lateral habitus.

margin by about their diameter, and from eyes by twice their diameter; mandibles bidentate, outer tooth distinctly longer than inner tooth. *Antenna*. Scape about 6 times longer than maximum width, about equal in length to pedicel plus the first 5 flagellomeres; pedicel, first and second flagellomeres subequal in length; last flagellomere elongate, about twice as long as penultimate flagellomere.

Mesosoma. (Figs 1 and 2). Pronotal carina well defined, straight. Notauli distinct, punctate-crenulate, reaching nearly to center of scutum; scutum, scutellum, metanotum, and mesopleuron predominantly coriareous with minute setigerous punctures; scutum with narrow crenulate furrow along lateral margin above wing insertion; scutellum anteriorly with a broad transverse crenulate furrow; episternal sulcus incomplete, weakly aerolate; Posterior-lateral corners of propodeal hind face each with a small but distinct tooth-like turbercle; propodeal dorsum areolate with a network of coarse ridges.

Gaster. Tergum I basally longitudinally striate, apically smooth and shining; tergites II-VII shining, faintly scaly reticulate and sparsely clothed with short, erect setae; dense brush of short setae at apex of Tergite VI.

Forewing. (Fig. 4). Marginal cell distally acute; two submarginal cells; lm-cu vein proximal to bifurcation of Rs + M. M absent beyond lr-m. Stigma L:W = 8:3; hind wing with cu-a straight, not appendiculate.

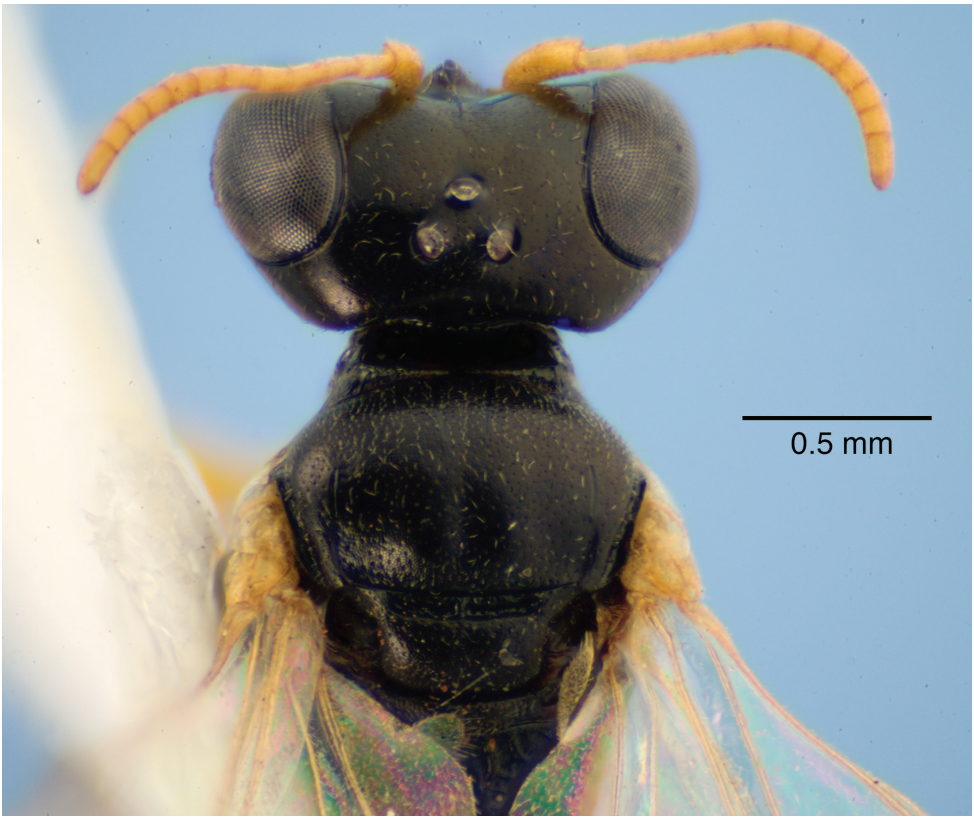

Figure 2. *Spilomena socialis* sp. n., holotype female, head and mesonotum dorsal view.

Color. (Figs 1–3). Body predominantly black, non-metallic, gaster dark brown. Antennae, palps, mandibles, tegula, and legs (including coxae) uniformly yellow; apical margins of clypeus and pronotum yellow; wing veins yellow, becoming brown distally beyond stigma; mandible tips and stigma brown; pronotal lobes with cream spot on posterior portion. Setal brush on tergite VII white.

Male. Similar to female, except body color uniformly dark brown and clypeus and frons with extensive cream colored maculations (Fig. 5); mandibles cream colored, tips light brown; antennae, palps, pronotal lobes, legs and wing veins colored as in female.

Remarks. The presence of two submarginal cells, the entirely yellow clypeal margin, and the pair of elongate ocellar setae readily distinguish this species from *S. obliterata* Turner (1914), the only other *Spilomena* species described from Malaysia.

Biology

Nest sites. Nests of *Spilomena socialis* were found on the vertical walls of buildings and, in particular, along the grooves of white pillars of cement recreational gazeboes

Figure 3. *Spilomena socialis* sp. n., holotype female, head frontal view.

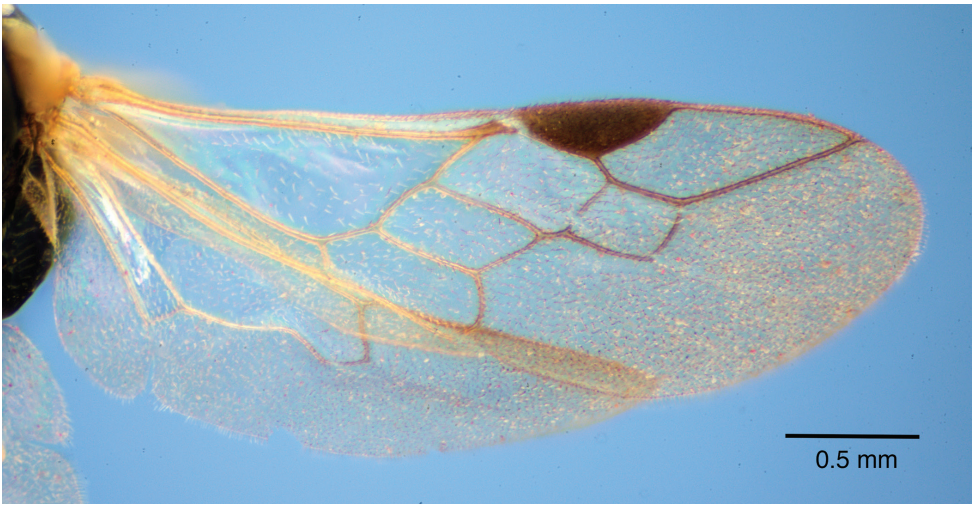

Figure 4. *Spilomena socialis* sp. n., holotype female, fore and hind wing.

scattered along the roads of the resort. This substrate makes nests, usually dark brown, highly visible to a human observer; evidently nests are well camouflaged on natural substrates as we never found any except those on artificial nest sites. It is also possible that this species is ecologically associated with pine trees as nidification sites were all in close proximity to these plants.

We found both active colonies and abandoned nests in studies made during January-February of 2004 and 2007 (Table 1).

Table 1. Characteristics of 32 active nests of *Spilomena socialis* collected at Bukit Fraser in January-February of 2004 and 2007.

Nest	Females	Males	Cells	Larvae	Pupae	Collected	Year
1	5	4	17	0	3	night	2004
2	6	1	23	3	6	night	2004
3	1	0	3	0	0	night	2004
4	3	0	8	1	1	night	2004
5	3	1	6	0	5	night	2004
6	8	2	12	3	3	night	2004
7	8	0	20	4	2	night	2004
8	8	0	26	2	2	night	2004
9	6	1	14	4	2	night	2004
10	1	0	3	1	0	night	2004
11	4	0	7	2	2	night	2004
12	8	2	21	10	1	night	2004
13	4	2	7	2	5	night	2004
14	1	0	14	0	4	day	2004
15	4	0	14	0	0	day	2004
16	2	1	15	2	2	day	2004
17	2	1	3	0	0	day	2004
18	2	0	5	0	0	day	2004
19	4	3	23	3	7	night	2007
20	11	4	35	5	1	night	2007
21	5	0	13	1	3	night	2007
22	3	1	23	3	3	night	2007
23	7	0	39	5	4	night	2007
24	6	0	15	3	0	night	2007
25	6	4	35	6	11	night	2007
26	8	1	32	2	7	night	2007
27	5	0	19	0	11	day	2007
28	3	0	14	2	4	day	2007
29	3	0	30	4	11	day	2007
30	7	1	23	3	3	day	2007
31	3	0	7	2	3	day	2007
32	6	0	14	4	5	day	2007

Nest architecture. The nest of *Spilomena socialis* has a distinctive architecture. It consists of cylindrical cells attached to a vertical plane substratum. Active nests (Fig. 6) contained from 3–39 cells (average = 10.39, N = 32, see Table 1). The substratum forms one wall of each cell. Cells are placed vertically, one beside the other, forming clusters, each containing up to seven cells (usually five). The cell opening is always situated at the upper end and is covered by a hood-like roof that protects the entrance from one side when the cell is isolated (Fig. 7). When contiguous cells form a cluster, the roofs of each cell merge to form a common tube connecting the entrances of all the cells, becoming

Figure 5. *Spilomena socialis* sp. n., male head, frontal view.

Figure 6. The shape of the individual cells of *Spilomena socialis* can be appreciated in this 6-celled nest. Note the female on the top right cell. Scale bar is 5 mm long.

also a shelter for the adult individuals of the colony (Figs 7, 8) Each cell is securely attached to the substratum and clusters of cells have never been found superimposed. Nests often consist of multiple closely spaced cell clusters and can also be quite close to each other (Fig. 9). Older, mostly abandoned nests, can be quite extensive (Fig. 10).

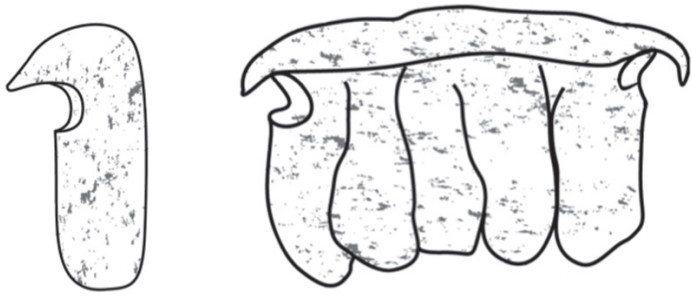

Figure 7. A sketch of a single cell (left) and a nest of *Spilomena socialis* consisting of a cluster of 5 cells showing the nest entrances and hood-like roof which becomes a connecting tube when there are multiple cells.

Figure 8. A *Spilomena socialis* nest composed of clusters of various sized groups of cells showing the connecting tubes formed by the merger of the individual cell roofs. Scale bar is 5 mm long.

Construction material consists of pieces of vegetal and mineral origin, parts of insects, and fungal hyphae (Fig. 11), all bound together by silk threads secreted by the wasps' abdominal glands. Females that returned with nest material were observed to affix it to the nest using repeated back and forth movements of their ventrally bent abdomens. Some cells are apparently re-used as old nests were noted to often have a few active cells. Nests can persist for extended periods as shown by various abandoned nests and by algae growth covering some active nests (Fig. 12).

Number of adults. The total number of adults found in nests collected after dark varied from 1 to 15 (N = 21 colonies) (females 1–11, males 0–4) (Table 1). For other

Figure 9. Four contiguous nests of *Spilomena socialis* composed of different numbers of cells. Scale bar is 10 mm long.

Figure 10. An abandoned group of *Spilomena socialis* nests consisting of various sized clusters of cells. Scale bar is 5 mm long.

Figure 11. Glossy pieces of materials of different origins constitute the material of this 5-celled nest. Scale bar is 5 mm long.

Figure 12. A nest of *Spilomena socialis* covered by green algae. Note adult female at left center. Scale bar is 5 mm long.

colonies (N = 11) we collected adults at different times of the day (females 1–7, males 0–1). Females present in the nests collected at night were more numerous than in those collected during the day (x = 5.47 vs 3.45, Mann Whitney U = 59.5, P = 0.025). However there was no difference in the total number of individuals (or females) collected on nests at night or day if this was normalized for the cell number of each nest.

Number of females (and total number of adults) was highly correlated with the number of nest cells (N = 21 colonies collected at night; Spearman ϱ = 0.669,

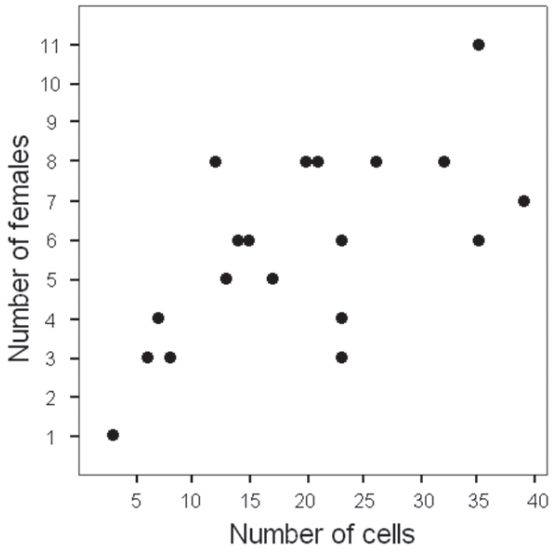

Figure 13. Total number of females in relation to number of nest cells in the colonies collected at night (N = 21).

P = 0.0009) (Fig.13) but no significant correlation was found between the number of females and the number of males collected in the colonies. Sex ratio overall averaged 0.18, (0.22 in colonies collected at night (N = 21) and 0.10 in colonies collected during the day (N = 11)) but differences were not statistically significant (Mann Whitney U test = 81, N.S.)

Immature brood and prey. As in many other species of the genus, *Spilomena socialis* preys on small thrips. *Spilomena socialis* probably practices progressive provisioning of the larvae since we did not find clusters of prey stored in the cells.

Eggs were only rarely found in the nests and it could not be ascertained if they had been laid in empty cells or attached to a prey. Twenty-eight nests contained larvae or pupae and 4 nests contained no brood (Table 1). On average 35.9% (N = 32) or 41.1% (N = 28, when we excluded the 4 empty nests) of cells in a nest were occupied by larvae at various stages of development (range 0–10) and by pupae (range 0–11). In 14 nests out of 28 containing immature brood, the number of pupae was greater than that of the larvae; in 7 nests they were the same and in 7 the larvae were more than the pupae. Total number of immature brood (L+P) was positively correlated with the total number of females on a nest (N = 32, Spearman ϱ = 0.507, P = 0.003). However considering only the nests with brood the correlation was not significant (N = 28, Spearman ϱ = 0.35, P = 0.063). Mean number of brood (L+P) per female was 1.15 (SD = 0.69, N = 21) in the colonies collected at night.

Female ovarian development and head width. We dissected a total of 124 females from 25 colonies. Twenty-nine of them had very small ovaries (ovarian development = 0), 37 females had ovarian development = 1, 28 females = 2, and 30 females = 3. The number of females with maximal ovarian development (= 3) in a colony was positively

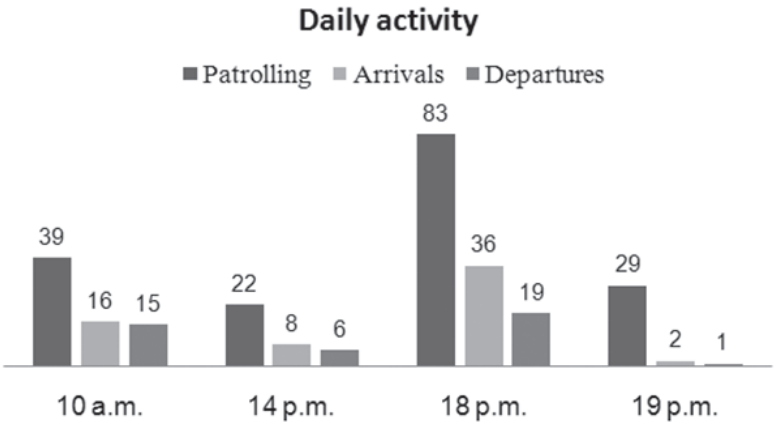

Figure 14. Activity observed in a small cluster of five nests of *Spilomena socialis* at selected times during the day.

correlated with the total number of larvae and pupae found in the nest (Spearman $\varrho = 0.6558$, P = 0.0042, N = 18)). However, the number of females with the maximum ovarian index did not increase significantly with the total number of females present in the colony (Spearman $\varrho = 0.3019$, P = 0.1, N = 25). In 8 out of the 20 colonies with three or more females only one of the females showed the maximal ovarian development. This was particularly evident in the three colonies with 8 females each.

In the 8 colonies for which we measured the maximum head width of the females present (mean female head width = 1224.10 pixels ± 68.09 (sd), N = 48), we did not find any significant relationship between this parameter and the respective ovarian development (Spearman $\varrho = 0.16$, P = 0.28, N = 48). Reanalyzing excluding the females with an ovarian index of 0 (assuming these to be very young individuals) was again insignificant (Spearman $\varrho = 0.267$, P = 0.18, N = 8).

Behavioral observations. We performed only limited ethological observations owing to the difficulty of individually marking wasps due to their small size. In two nests where we succeeded, we observed particular females patrolling (a female from nest 6, for example, emerged from the tunnel of a cell group every now and then, walked over the surface of the entire nest and then reentered the tunnel again). In nest 7 two marked females shared the periodic patrolling of the left and right part of the nest.

When we placed small unidentified ants on a nest we observed active defense of the colony by different females. These females attacked and repelled ants wandering on the cells, and then rested on guard head facing out at the entrance of cell connecting tubes. A male, in contrast, flew away at the first contact with an ant. Ants walked on the nests without showing any sign of repellence, suggesting that there is no chemical or mechanical protection of the nest of the type found in various social Vespidae (see references in the review by Smith et al. 2001).

Daily activity. The activity of the members belonging to the small cluster of 5 nests we videotaped was markedly different during different daylight hours (Fig. 14).

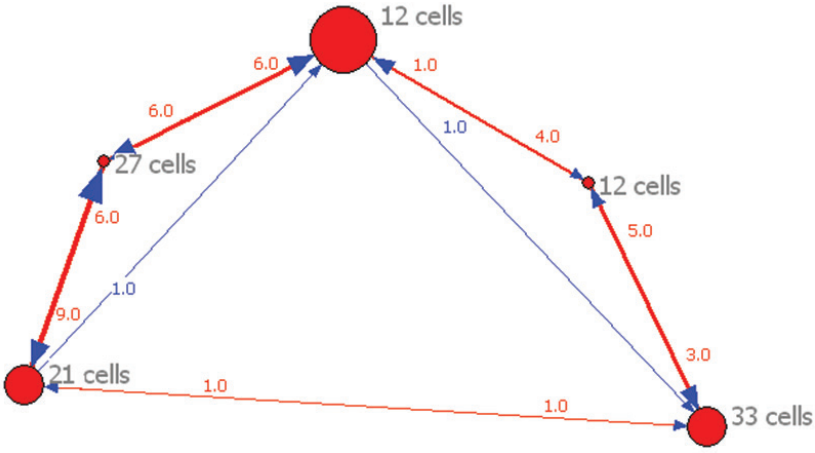

Figure 15. Accumulated association network for five nests in a small cluster. Each node represents a nest and lines represent movements of wasps between connected nodes (Blue lines: uni-directional shift in the direction of the arrow; red lines: shift in both directions; associated numbers on the lines refer to the number of movements). The size of a node is proportional to that nest's degree centrality (summed rate of links with other nests, where a link is represented by a shifting wasp). The spatial position of each node (nest) mirrors the real position of nests in the cluster.

In particular, in late afternoon (18.00 h) there was an evident increase in patrolling activity, probably because many females also returned to the nest at that time. At sunset (19.00 h) when nearly all had returned and no more were departing, patrolling activity decreased as well. Patrolling thus seems to be a behavior aimed at defending the nest from intruders (perhaps also from conspecifics belonging to different nest clusters).

In the observed 5 nest cluster, individuals often moved from nest to nest during the day, suggesting that these nests were communal and shared by the same individuals. As shown in Fig.15, the size of a nest (i.e., the number of cells) did not influence the probability of being a more visited nest. In contrast, individuals moved more between adjacent nests than between far nests. The nest at the center of the cluster was the smallest (12 cells) but also the nest with the higher degree value (i.e., the size of the node in Fig. 15) meaning that it was the most connected to others, being visited by wasps from all other nests). Overall, however, position in the cluster did not influence the amount of visits a nest received.

Discussion

Spilomena socialis colonies are comparable to those of several other species of the subtribe Spilomenina. For example, the maximum number of females (11) is similar to that reported for other species (13 in *Microstigmus comes* (Matthews 1991), 6 for *M. nigrophtalmus* (Melo 2000, Lucas et al. 2011), 26 for *M.* sp. (West-Eberhard 1977),

10 in *Arpactophilus mimi* (Matthews and Naumann 1989), *Spilomena subterranea* (McCorquodale and Naumann 1988), and a Costa Rican *Spilomena* sp. (West-Eberhard 1977)). Their colonies also display a well-defined, strongly female-biased sex ratio; males while regularly present in the nests, appear to contribute very little to the colony life.

Some females in a colony display fully developed ovaries, as occurs in *M. nigriphtalmus*, but in more than a third of the colonies examined with at least three females only one possessed mature eggs. However, it was not possible to demonstrate a reproductive division of labor between the various females in a nest, as occurs in *M. comes*, because other females in the colonies display at least some ovarian development. Whether wasp age is the key factor in ovarian development, and whether age correlates with epicuticular chemical profiles as occurs in some species of Stenogastrinae wasps (Turillazzi et al. 2004, Bridge and Field 2007, Baracchi et al. 2010), must await future studies. Unlike the situation in *M. comes* (where egg laying females are significantly larger than the population average) no size difference between females with fully developed and undeveloped ovaries was found in *S. socialis*.

Despite the relatively limited behavioral observations it appears that females practice progressive provisioning and cooperate in defense of the nest, patrolling the cells and attacking approaching ants and conspecific intruders. In contrast, males seem not to take part in the nest defense as was reported in *M. nigrophtalmus* (Lucas et al. 2011). Progressive provisioning is also reported for *M. nigrophtalmus* (Melo 1992, 2000), while *M. comes* mass provisions its cells (Matthews 1968). Patrolling and provisioning behaviors peak during morning and late afternoon similar to activity profiles reported for other tropical wasps like hover wasps (Turillazzi 1988, Baracchi et al. 2009, 2013).

In *S. socialis,* nests are composed of a series of cells clustered on flat substrata. The 'invention' of the tube connecting nest cells permits access to the immature brood. However, this architecture not only gives adults the possibility to monitor and guard groups of cells, but also provides a place where they can rest and interact with other adult individuals. The connecting tube may also constitute a barrier to further social evolution by physically limiting the space available for adult interactions. Other than patrolling, no interactions among these wasps have been observed outside of the nest or in the area surrounding the nest clusters.

In no case were cells found superimposed on others, a design that is more energetically expensive to create, due to the greater amounts of material needed (Jeanne 1975). Examples of nests composed of cells attached to flat substrata can be found also in Stenogastrinae wasps. In the genus *Liostenogaster*, for example, *L. topographica* Turillazzi presents a quite peculiar nest architecture with cells arranged along ribs of material (Baracchi et al. 2009), while in *L. vechti* Turillazzi, a series of contiguous cells form rings or brackets with their openings facing a central area where the adults rest (Turillazzi 1988). In both cases, however, adult individuals are not separated by architectural barriers and all the cells of the nest can be directly accessed by any member of the colony.

Construction of a nest has always been regarded as an important (and probably obligatory) characteristic of social insects (Hansell 1996). However, the vast majority of crabronids also construct nests, but are not communal or social. Thus there must be other

factors that predispose evolution toward communal living in species like *S. socialis*. Unless preexisting cavities such as hollow twigs are 'rented' for nests, there is the need to acquire, transport, and assemble suitable nest building materials. This in turn creates opportunities both for cooperation and for making more complex nests. The latter is greatly facilitated through the use of silk to bind and shape nest materials. Thus the possession of silk-producing glands on the 6th metasomal tergite of females, unique to the Spilomenina (Melo 1997), may be a key preadaptation facilitating the evolution of communal behavior in this clade (Matthews 1991, Melo 2000). That nest architecture can strongly influence social wasp evolution, is generally accepted (Jeanne 1975, Wenzel 1991, Matthews 1991, Hansell 1996), and this aspect of *S. socialis* biology merits further investigation.

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